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Scientific Report on

"Tuberculosis testing in deer"

Panel on Animal Health and Animal Welfare

(Question No EFSA-Q-2006-179)

Adopted on 30 January 2008

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ABBREVIATIONS:

BCG	*M. bovis* bacille Calmette Guerin
BTB	Blood bovine tuberculosis test
CFU	Colony formatting units
CI	Confidence Interval
CMI	Cell mediated immune response
CULT	Culture
GINT	Gamma-interferon assay
ELISA	Enzyme-linked immunosorbent assay
EFSA	European Food Safety Authority
HIST	Histology
LCT	Lymphocyte transformation test
KOT	Koch's Old Tuberculin
LAM	*M. bovis*-derived lipoarabinomannan-specific immunoglobulin
MAPIA	Multiantigen print immunoassay
MS	EU Member State
MCMC	Monte Carlo Markov Chain
NECR	Necropsy
OIE	World Organization for Animal Health
PCR	Polymerase chain reaction
PPD	Purified protein derivative
PPD-A	Tuberculin PPD from *M. avium*
PPD-B	Tuberculin PPD from *M. bovis*
PWM	Pokeweed mitogen
RT-PCR	Real time - PCR
Se	Sensitivity
OIE SOP	OIE standard operating procedure
Sp	Specificity
SST	Single tuberculin intradermal test
SICCT	Comparative intradermal tuberculin test
TB	Tuberculosis

GLOSSARY:

Deer Tuberculosis: the disease caused by infection in deer with any species within the *'Mycobacterium tuberculosis*-complex. *M. tuberculosis* complex members include *M. tuberculosis*, *M. canetti, M. africanum, M. pinnipedii, M. microti,* Dassie bacillus, *M. caprae, M. bovis* and *M. bovis* BCG (the same definition as for bovine tuberculosis).

Control: a disease control programme is based on a combined system of disease detection/testing and intervention strategies that over a prolonged period of time is employed to reduce the incidence of a specific disease. In this report, the testing of herds for control purposes refers to testing in known infected herds.

Design prevalence: defines the lower limit of a theoretical level of infection in the population which a given surveillance activity would be able to detect with a specified probability.

Eradication: means the elimination of a pathogenic agent from a country or zone (OIE, 2007).

Expert Opinion: qualitative or quantitative information provided by an expert. In this report, experts in TB-testing of deer were requested to indicate the minimum, most likely and maximum value for sensitivity and specificity of diagnostic tests for TB in deer and give a score reflecting their expertise.

Farmed deer herd: a herd of deer being farmed for commercial purposes and surrounded by fences or a barrier in order to prevent entry or exit.

Farmed deer: deer belonging to a farmed deer herd are defined as "farmed deer".

Free compartment: means a compartment (specific animal production line) in which the absence of the animal pathogen causing the disease under consideration has been demonstrated by all requirements specified in this Terrestrial Code for free status being met (OIE, 2007).

Free zone: means a zone in which the absence of the disease under consideration has been demonstrated by the requirements specified in this Terrestrial Code for free status being met. Within the zone and at its borders, appropriate official veterinary control is effectively applied for animals and animal products, and their transportation (OIE, 2007)**.**

Herd[1]: means an animal or group of animals kept on a holding* (within the meaning of Article 2 (b) of Directive 92/102/EEC) as an epidemiological unit; if more than one herd is kept on a holding, each of these herds shall form a distinct unit and shall have the same health status (Article 2 of Directive 64/432/EEC).

Holding[1]: shall mean any establishment, construction or, in the case of an open-air farm, any place in which animals are held, kept or handled (within the meaning of Article 2 (b) of Directive 92/102/EEC).

Official control programme: means a programme which is approved, and managed or supervised by the Veterinary Administration of a country for the purpose of controlling a vector, a pathogen or disease by specific measures applied throughout that country or within a zone or zones of that country (OIE, 2007).

[1] In the present report, "Herd" has been used when it refers to the health status of the animals, as the epidemiological unit. "Holding" has been used when it refers to the farm, premises or agriculture land, and whenever biosecurity measures were considered.

Meta-analysis: statistical methods to combine the effect from different studies into a summary effect estimate. In this report, Bayesian logistic regression models were used to estimate the diagnostic sensitivity and specificity of tests based on a systematic literature review.

Monitoring: means the continuous investigation of a given population or subpopulation, and its environment, to detect changes in the prevalence of a disease or characteristics of a pathogenic agent (OIE, 2007)

Screening: testing of animals not pre-selected based on results of previous tests.

System sensitivity: the ability of the entire surveillance system to correctly identify an infected population (herd, in this case). This is the combined sensitivity of each surveillance system component such as testing and other indirect methods for detecting the presence of infection in the herd.

Surveillance: means the investigation of a given population or subpopulation to detect the presence of a pathogenic agent or disease and measures taken to reduce the disease impact; the frequency and type of surveillance will be determined by the epidemiology of the pathogenic agent or disease, and the desired outputs (OIE, 2007). Disease surveillance implies that some form of directed action will be taken if the data indicate a disease prevalence or incidence above a certain threshold. In the EU legislation the associated actions are not included in the concept of surveillance. In this report, the testing of herds for surveillance purposes refers to testing in herds with no current or recent evidence of infection.

Surveillance system: the combination of all surveillance activities that provide evidence of freedom from, for example, TB in deer.

Surveillance system component: a single surveillance activity (such as herd intradermal testing, or abattoir surveillance).

Test sensitivity: the ability of a test to correctly identify a single infected animal.

BACKGROUND AS PROVIDED BY THE COMMISSION

Articles 14 and 15 of Council Directive 92/65/EEC provide for the possibility to grant additional guarantees regarding the approval of control/eradication programmes or free status attained in relation to diseases listed in Annex B to the same Directive.

So far, such additional guarantees have never been recognised by the Commission for any Member State, nor for any disease/ species.

The guarantees are to be granted when a Member State has introduced for all or part of its territory a compulsory national control programme complying with certain criteria and where it can demonstrate a steady progress towards control and eradication of the disease concerned.

Directive 92/65/EEC covers intra-Community trade and importation of all live animals not covered by other EU Directives. This means that a great number of different species are covered by this Directive. Article 6 lays down the animal health rules for intra Community trade of ungulates which prescribe under which conditions deer can be traded in the EU. In particular, as regards Tuberculosis (TB), the animals must either come from a holding that is officially free of TB in accordance with Directive 64/432/EEC or present a negative result in a tuberculosis (reaction) test. However, pending the harmonisation of such a test and status, the Directive allows the use of national rules. Following the request of a Member State to have its national control programme for TB in deer approved under Article 14 of Directive 92/65/EEC and the consequent request for deer to be declared free of TB under Article 15 of the same Directive, the Commission has been asked to assess this dossier. However, due to the current lack of scientific evidence for the reliability of TB tests validated for deer or other wild species, it is difficult for the Commission to assess this dossier.

It is well known that the intradermal tuberculin test may not be sufficiently accurate if used on deer. This jeopardises the possibility to put in place a science-based control of the disease and establishing a definition of TB free animal/holding/region for deer, which would have major consequences on intra-Community trade in deer and on imports from Third Countries.

Therefore, the scientific opinion should review the knowledge and data available on sensitivity and specificity of current TB tests for use in deer and based on this review identify possible criteria for defining TB free animal/holding/region for deer. The opinion should also indicate options on the possible testing protocols to be followed in order to guarantee that the official TB-free status is properly granted/ maintained/ suspended/ regained.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In view of the above, and in accordance with art. 29 of Regulation (EC) 178/2002, the Commission asks the European Food Safety Authority to issue a scientific opinion on:

1. the suitability of the existing TB tests for deer for the purpose of granting official TB-free status in the framework of Directive 92/65/lEEC;
2. the modalities for the validation of a TB test for deer;
3. a definition, including options for possible testing regimes giving sufficient guarantees for a animal/holding/region to be qualified/ maintained/ regained as officially free from TB infection in deer.

In agreement with the Commission the following clarification was made:

- In this report a "farmed deer herd" is defined as a herd of deer being farmed for commercial purposes and surrounded by fences or a barrier that might prevent entry or exit. Deer belonging to such a herd are defined as "farmed deer".

- This report will focus on farmed deer. The role of wildlife is only considered because of the risk posed to farmed deer. This report will not address the TB status of wildlife.
- Testing of animals imported from third countries should be considered as well as the rules for suspending or withdrawing OTF (officially TB- free) - status.
- It was also agreed that the report would consider the trade in animals between countries, regions and herds that are not free of TB, and specifically the minimum testing requirements that would be required to minimise the risk of TB transmission to deer herds, as well as wildlife and other domestic animals. It is expected that these testing requirements will facilitate efforts in these countries towards future TB freedom.

1. Supportive documents

1.1. Summary of relevant EU Directives and other documents

The key EU legislation on trade in Deer is Directive 92/65/EEC, which covers trade in animal species that are not subject to the animal health requirements laid down in the specific Community acts listed in Annex F to the same Directive.

Member States may not restrict or prohibit intra-community trade in deer for animal health reasons other than those laid down in this Directive.

As regards TB, article 6 to Directive 92/65/EEC refers to the definition of an officially tuberculosis free holding as defined in Directive 64/432/EEC concerning of bovine animals. The definitions, criteria and technical procedures as regards freedom from TB are laid down in Annexes A and B to Directive 64/432/EEC. Where animals are not from an officially TB free holding, it provides testing as an alternative.

However, in the same article it is specified that, as regards the test to be used, pending the harmonisation of the matter, national rules shall continue to apply.

Restrictions to the general rule may fall into two groups:

1. Requirements for trade in deer in the absence of special provisions for one particular State; or
2. Higher level requirements (additional guarantees) for a particular Member State (MS), which have been approved by the EC, as a result of either a control and monitoring program in force or a country having achieved the national or regional status of freedom from infection (Tuberculosis, in this case).

1.2. Summary of relevant OIE Terrestrial Code

The OIE Terrestrial Animal Code (2007) only relates to *M. bovis* in cattle, so is not directly relevant to deer.

1.3. Control measures in the EU

Measures for the control of TB in farmed deer, and strategies applied, seem to vary among MS: from voluntary control on a limited number of farms out of the national farmed deer holdings through to official control programmes covering all such holdings.

2. CONTEXT AND BACKGROUND

2.1. Animal

2.1.1. Deer species to be considered

The deer species described and illustrated below are those that are to be found confined on the commercial deer farms throughout the European Union. Their wild and feral geographic distribution is also noted.

2.1.1.1. Red deer (*Cervus elaphus*)

The 23 geographical subspecies of red deer differ greatly in size. The North American and Caucasian races are the largest and the European races the smallest. In North America, the red deer is called the wapiti or elk, and is designated *Cervus elaphus canadensis*. The Caucasian race *(C. elaphus maral*) is called the Maral.

Description: The coat is a sleek reddish brown in summer and a coarse, greyish brown in winter. The branched antlers, present only in males (stags), are deciduous, being cast at the end of winter and growing back during the following spring and early summer. European males stand 100 to 170 cm at the shoulder and weigh 100-260 kg. The North American wapiti (elk) is bigger and can weigh up to 350 kg. The male Caucasian Red Deer or "Maral" (*Cervus elaphus maral)* is bigger and heavier than the North American wapiti/elk (Cer*vus elaphus canadensis*) and can weigh up to 450 kg.

Due to the fact that the word "Elk" is used for different animal species Table 1 is included to clarify the difference between the species (*Cervus elaphus* and *Alces alces*) involved.

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Table 1 - Clarification on the names applied in different countries for *Cervus elaphus* and *Alces alces*

Distribution: The European red deer is found throughout the temperate forests of Europe, in

Cervus elaphus		*Alces alces*	
North America:	Europe:	North America:	Europe/ Scandinavia:
Wapiti or Elk	Red deer	Moose	UK: Elk Germany: Elch
			Scand: Älg English: Moose

Red deer - Hungary[1)]

Älg – Sweden

1) Photo: János Perényi

Appearance: The animals are generally larger in North America than in Europe for both species

the Caucasus mountains, in Asia Minor, west of the Caspian Sea and in Iran. It is the only species of deer found in Africa where a small population occurs in the Atlas Mountains. Most wild red deer populations in Ireland and Scotland are now considered to be red deer-Sika hybrids. The only pure red deer in the British Isles and Ireland are believed to occur on some islands off the west coast of Scotland.

Cervus elaphus canadensis occurs in North America and Canada where it is called the wapiti or elk. In Europe, the Moose (*Alces alces)* is called the Elk, while in North America, this species is called the "Moose".

Cervus elaphus maral occurs in the Crimean Peninsula, Eastern Turkey, Iran, Armenia, Azerbaijan and the Russian Caucasus.

The Wapiti and the Maral have been introduced into New Zealand deer farms in order to enhance the size and weight of the farmed Red deer races.

Habitat: Habitat is varied, and can include open moorland, woodland, broadleaf and coniferous forests and high montane regions. In North Africa, the red deer lives in semi-desert conditions.

Behaviour: The adult males form herds separate from those of the females (hinds), which also include fawns and adolescents of both sexes. During the 'rutting' season, which takes place in Europe between October and December, each stag establishes a territory into which he lures as many females as possible. In this season the stags become very aggressive against any intruder. During the rut, some stags lose so much condition that they do not have enough bodily resources to survive the following winter. After a gestation of 220-240 days, the hinds give birth, usually to a single fawn. Occasionally twins are born. The fawns have reddish brown coats dappled with white spots. Fawns are suckled for about 6 months and remain with their mother until she gives birth again the following spring.

Lifespan: In captivity, red deer can live to over 20 years of age; however, in the wild, the

maximum lifespan is 9 to 12 years.

2.1.1.2. Fallow deer (*Dama dama*)

Two species of fallow deer are recognised: *Dama dama* and *D. mesopotamica.* The latter is an endangered species and is very rare.

Description: *D. dama* is a medium sized deer with a coat in summer that is reddish brown and speckled with creamy white spots. The belly, lower neck and the insides of the legs are white. In winter, the coat is much darker in colour and the flank spots disappear. Sometimes melanistic fallow deer occur. These have a completely black coat through the year.

The antlers which are only carried by the males (bucks) are deciduous and are shed annually (as in the red deer). In *D. dama*, the antler tips are palmate or flattened, while in *D. mesopotamica* they are pointed. In other respects, the two *Dama* species are similar. The shoulder height is 80 to 110 cms and the weight is 65-85 kg.

Distribution: Fallow deer are now widespread in the wild throughout Europe. It is a popular 'park' deer and is often kept in wildlife parks. This species is widely farmed for venison and has been introduced for this purpose into New Zealand and other countries where it has often escaped and become feral.

D. mesopotamica occurs in the wild in Khuzestan in Iran, and in captivity in Israel and Germany.

Habitat: Fallow deer are grazing animals. Their preferred habitat is mixed, open woodland and open grassland.

Behaviour: Fallow deer are gregarious animals. Females (does) and their young live in small herds, and males (bucks) in separate herds. The fallow deer is not territorial, herds moving freely throughout large home ranges which overlap with those of other herds. In wildlife parks and on deer farms, they become very tame and tolerate close human presence. This is in contrast with their secretive and timid behaviour in the wild. Mating (rutting) takes place in September and the gestation period is 230 days. The doe gives birth to 1-2 fawns which are suckled for about 8 months. The fawns become sexually mature from about one year of age, the males reaching maturity a little later than the females.

Lifespan: Captive fallow deer have a lifespan of 11-15 years. Wild fallow deer rarely reach 7 or 8 years.

2.1.1.3. Sika (*Cervus nippon*)

(Sika is the Japanese word for deer; therefore, it is permissible to omit the word 'deer' from the title)

Description: There is considerable variation in the coat colour of Sika. In summer, typically it is brownish-chestnut with 7 or 8 rows of white spots, and the belly, lower neck and throat are white. In winter, the coat becomes much coarser and darker, and the spots are less visible.

The Sika is a little bigger than fallow deer. The antlers of the stags are deciduous, as in both red and fallow deer. The stags stand about 100 to 130 cm at the shoulder, and weigh about 65-90 kg.

Distribution: In the wild, Sika occur in east Asia from China in the west to Japan and Korea in the east, and from the eastern tip of the former USSR in the north to south eastern China. The species has been widely introduced in Europe as a 'park deer'. Subsequently, it has escaped captivity and there are thought now to be +/- 5000 Sika in the wild throughout Britain, Ireland, Poland, Czech Republic, Denmark, Germany Austria and Russia. Sika have also been widely introduced into, North America and New Zealand where they are now farmed. Some have escaped and are now feral. Sika and red deer readily interbreed in the wild, but surprisingly this does not occur when they are confined together in a park or enclosure.

Habitat: Sika occupy a similar habitat to the red deer, mixed woodland and grassland. They are often maintained in wildlife parks along with both fallow and red deer.

Behaviour: The Sika are social animals and, similar to red deer, live in small herds which vary in structure according to the time of year. The rut takes place between September and November. At the beginning of the rut, animals move to their traditional rutting grounds. Here, the males compete for small breeding territories. The stags in possession of the best territory collect and mate with the greatest number of hinds. After a gestation of 220 days, a single fawn is born in May or June. The fawns remain with their mother until the birth of the next fawn the following spring/summer.

Lifespan: In captivity, Sika have been known to live for 20 years but in the wild they seldom exceed 7 years.

2.1.1.4. Roe deer (*Capreolus capreolus*)

Description: The smooth summer coat is reddish; the winter coat is a dense -grey-brown. Only the male (buck) has antlers and these are shed annually. This deer species is relatively small compared with sika red and fallow deer.

Shoulder height is 65-75 cm and weight is 15-30 kg. The female (doe) is somewhat smaller than the male.

Distribution: The roe deer is native throughout Europe, Asia Minor and the Caspian coastal region. It also occurs in northern Asia and as far east as Siberia. Roe deer are absent from Ireland. Roe deer in the east and north of their range are larger and heavier than those found in southern and western Europe.

Habitat: Roe deer are predominantly forest or woodland deer. They also occur in mountainous regions below the tree line. In cultivated landscapes, roe deer occur widely on arable farmland wherever there are thick hedges and small copses in which to hide.

Behaviour: Although roe deer are crepuscular, i.e. active at twilight, in habit, they also graze throughout the day in large open areas where they can anticipate the approach of danger. They normally live in small family groups consisting of a male (buck) a doe and the young of the year, usually twins. The buck marks his territory with secretions from glands on the forehead, from anal and metacarpal glands and with urine.

Roe deer mate in July and August and the fawns, 1-3 in number, are born in the following May. No matter when fertilisation actually takes place, the embryo does not start to develop until late winter/early spring (delayed implantation). Actual gestation is thus about five months although the time from fertilisation to parturition can be as long as 300 days. The fawns, whose coats are speckled with white spots at birth, remain concealed in thick vegetation for the first 3-5 days, after which they emerge and follow the doe. They suckle for 3-5 months.

Roe deer are often found in extensive deer parks along with other deer species, but they are not usually considered to be farmed for meat. They may, however, be maintained as a 'trophy species' to be sold to visiting sportsmen.

Lifespan: The oldest known wild roe deer reached an age of 14 years. However, the average lifespan seldom exceeds 8 years.

2.1.1.5. Reindeer or caribou (*Rangifer tarandus*)

There are seven subspecies of reindeer/caribou, including three subspecies in Europe and four in North America. In North America and Canada, the reindeer is known as the caribou. Some authorities refer to the wild, free/living species of North America as the "caribou", and to the semi-domesticated animals of Europe/ Asia as the "reindeer". There are believed to be some genetically programmed behavioural differences between caribou and reindeer.

Description: The coat is extremely thick in order to provide insulation against the winter cold. The coat colour ranges from pale cream to a dark brown with the under parts lighter in colour. The coat tends to be lighter in colour during the winter and darker in the summer months. The head is mostly white with a brown muzzle. Male reindeer tend to be larger than the females but size varies considerably with geographic location. These animals possess some adaptations to the cold climate of their habitat, such as a thick layer of fat under the skin that helps to store energy and provides insulation. The hooves are broad and furry, allowing the animals to move quickly over ice and snow.

Reindeer are the only deer species in which both males and females carry antlers. In males, antlers are shed after the rut in November, regrowing the following summer. Females lose their antlers in spring after the young are born.

Reindeer stand 80-120 cm at the shoulder and weigh 70-150 kg. Their weight varies with geographic location and food supply.

Distribution: Reindeer/caribou have a circumpolar distribution and are found across northern Europe, Asia and North America.

Habitat: Reindeer are found in the northern coniferous forests and on the tundra of northern Europe. Caribou occur in the forests of North America/Canada and on the bare, open tundra plains of the arctic far north.

Behaviour: The caribou of North America undertake long migrations. At the beginning of spring, they form into large herds that can number up to tens of thousands. These herds move slowly northwards towards the traditional calving grounds and away from the boreal forests. After calves are born in June, the caribou continue to move north to new feeding grounds on the tundra where they spend the summer. As autumn approaches, the herds reform and the caribou return southwards to find shelter from the harsh winter weather in the forests. The semi-domesticated reindeer of Scandinavia are herded seasonally according to the local

weather conditions. The rut takes place in September and October. Mating is very competitive and the males try to collect and defend a small number of females. The gestation period is 210-240 days and normally only a single calf is born in June. Calves at birth weigh up to 5 kg and have to become mobile very quickly to avoid predators such as wolves and bears. The calves grow quickly and reach 35 kg by October. Sexual maturity is reached from two years of age.

Lifespan: Although the maximum possible lifespan of wild reindeer/caribou is between 10 and 15 years, most animals do not live beyond five years of age. Predation, insects and the severe climate contribute to early mortality.

2.2. The farmed deer industry

Deer have been farmed for centuries in many countries and under a range of farming systems, including farms, extensive ranching conditions, hunting parks, zoological parks and private estates (Mackintosh et al., 2004). Internationally, farmed deer are most common in New Zealand (1.6 million in 2007), China (1 million), Russia (400,000) and the United States (250,000) (Griffin and Mackintosh, 2000). There are an estimated 410,000 farmed deer in Europe (Fletcher, 2004a) raised in a number of Member States, including Germany (mainly red and fallow), the United Kingdom (mainly red and fallow), Ireland (mainly red), Sweden (mainly red and fallow), Denmark (mainly red and fallow), France (mainly red and fallow) and Norway (mainly red) (Griffin and Mackintosh, 2000). A further 2 million reindeer are extensively herded in Canada, Alaska, Siberia, Mongolia and Scandinavia (Griffin and Mackintosh, 2000; Mackintosh et al., 2004; Fletcher, 2004a). It is estimated that there are approximately 6-7 million wild deer in Europe, including red deer (>1 million), roe (5.5 million), fallow (125,000), moose (0.5 million) and reindeer (50,000) (Fletcher, 2004a).

2.2.1. EU country level information

The farmed deer sector is small, in comparison with wild deer harvesting, where animals are kept for venison and trophies. There are substantial regional differences in the farmed deer industry, relating to management, species farmed, methods of slaughter and venison marketing (Fletcher, 2004a).

A survey was conducted of the EU MS through a query distributed to the Zoonoses reporting network, and several other countries, as part of the current review. The results are presented in Tables 2-5. Red and fallow deer are the most numerous farmed deer species in Europe, although there are also sizeable numbers of farmed sika and roe deer. These animals are mainly raised for antlers/trophies, venison and breeding. Between-farm movement of deer is relatively common.

Table 2 - Farmed deer production in EU Member States, based on recent survey results (source: Zoonoses reporting network)

	EU member states																									
	AT	BE	BG	CY	CZ	DE	DK	EE	EL	ES	FI	FR	HU	IE	IT	LT	LU	LV	MT	NL	PL	PT	RO	SE	SI	SK	UK
Farmed deer																										
Red *(Cervus elaphus)*																										
Animals	8,000	2,000			6,000	250									194	510		1,787			1,919			5,097	430		27,000
Farms	400	100			40	15				50		300			16	8		23			31			120	20		220
Wapiti/Elk *(C. elaphus canadensis)*																										
Animals					100																					250
Farms					10																					10
Fallow *(Dama dama)*																										
Animals	30,000	10,000			12,000	7,000									739	173		404			587			18,314	4,000		
Farms	1,200	1,500			150	270						250			95	8		12			4			215	220		
Sika *(Cervus nippon)*																										
Animals	1,000	100			500	250										299										
Farms	50	5			5	15										3										
Roe *(Capreolus capreolus)*																										
Animals		50													187	3		169							10		2,500
Farms		10													16	3		11							4		20
Reindeer *(Rangifer tarandus)*																										
Animals																									5		
Farms																									1		
Total/additional																										
Animals				0[b]			19,000[a]	0[b]						c	45[d]											0[b]	
Farms				0			640	0							666											0	
Purpose																										
Trophy/antlers					Y		Y			Y		Y		Y				Y			Y				Y		
Venison	Y	Y			Y	Y	Y			Y		Y		Y				Y		Y	Y			Y	Y		Y
Breeding					Y		Y			Y						Y									Y		Y
Zoo/tourism		Y						Y																	Y		
Management																										
Game parks					Y		Y			Y		Y															Y
Farms	Y	Y			Y		Y					Y		Y							Y						Y
Gardens/parks		Y			Y		Y			Y																	

a Predominantly red and fallow; b No farmed deer

c Farmed species include red deer and hybrids (~60%), fallow deer (~30%) and sika and hybrids (balance)

c Additional, mixed species

Table 3 - Farmed deer production in some third countries, based on the EFSA recent survey results

	Third countries			
	AU	NO	NZ	US
Farmed deer				
Red *(Cervus elaphus)*				
Animals	37,800	1,100	1.5 mill.	
Farms	240	46	3,750	
Wapiti/Elk *(C. elaphus canadensis)*				
Animals	600		5,000	
Farms	10		85	
Fallow *(Dama dama)*				
Animals	23,000	350	12,000	
Farms	120	6	65	
Sika *(Cervus nippon)*				
Animals	8		1,000	
Farms	1		6	
Roe *(Capreolus capreolus)*				
Animals				
Farms				
Reindeer *(Rangifer tarandus)*				
Animals				
Farms				
Total/additional				
Animals				286,863
Farms				4,901
Purpose				
Trophy/antlers	Y		Y	
Venison	Y		Y	
Breeding			Y	
Zoo/tourism				
Management				
Game parks				
Farms			Y	
Gardens				

a Predominantly red and fallow; b No farmed deer; c Additional, mixed species

Table 4 - Tuberculosis and farmed deer production in EU MS, based on recent survey results (source: Zoonoses reporting network).

	EU member states																									
	AT	BE	BG	CY	CZ	DE	DK	EE	EL	ES	FI	FR	HU	IE	IT	LT	LU	LV	MT	NL	PL	PT	RO	SE	SI	SK	UK
Tuberculosis																											
Livestock[a]																											
TBOF status	Y	Y			Y	Y	Y				Y	Y			part		Y			Y				Y		Y	
EU co-financed prog.									Y	Y				Y	part	Y					Y	Y					part
Farmed deer																											
Infected[b]	N	N		N	N, 1999	N, 2003[c]	N, 1994			Y		N, 1996		Y	N	N		N		N	N			N, 1997	N		Y
TB control																											
On-farm testing										Y				Y										Y			Y
Abattoir surv.	Y	Y		Y	Y	Y	Y			Y		Y		Y		Y		Y		Y	Y			Y	Y		Y
Infected wildlife	Y	N		N	N	Y	N	N		Y		Y		Y	Y	N		N		N	N				N		Y
Animal movement																											
Bet. farms	Occ.	Occ.			Freq.		Freq.			Yes		Rare		Yes				Occ.			Occ.				Occ.		Yes

a Reveriego Gordejo and Vermeerch (2006)
b TB in farmed deer present (Y) or (N) not (N, year recorded)
c Wild deer

Table 5 - Tuberculosis and farmed deer production in some third countries, based on recent survey results

	Third countries			
	AU	NO	NZ	US
Tuberculosis				
Livestock[a]				
TBOF status				
EU co-financed prog.				
Farmed deer				
Infected[b]	N, 1985	N	Y[c]	Y
TB control				
On-farm testing			Y	Y
Abattoir surv.		Y	Y	Y
Infected wildlife	N	N	Y	Y
Animal movement				
Bet. farms		Occ.		Restricted

a Reveriego Gordejo and Vermeerch (2006)
b TB in farmed deer present (Y) or (N) not (N, year recorded)
c Prevalence of TB infected deer herds in NZ reported in 2006/7 - 0.38% (NZ Animal Health Board.)

2.3. TB in farmed deer

2.3.1. Definition

Bovine tuberculosis has previously been defined as 'the disease caused by infection in cattle with any of the mycobacterial species within the *M. tuberculosis*-complex'. In this document, an equivalent definition is used, namely 'the disease caused by infection in deer with any species within the *M. tuberculosis*-complex'. *M. tuberculosis* complex members include *M. tuberculosis, M. canetti, M. africanum, M. pinnipedii, M. microti, Dassie bacillus, M. caprae, M. bovis* and *M. bovis* BCG (Mostowy and Behr, 2005).

2.3.2. History

Bovine tuberculosis has long been recognised in captive and free-living wild deer (Clifton-Hadley and Wilesmith, 1991), and several relevant reviews are available, both from Europe (Clifton-Hadley and Wilesmith, 1991; Fletcher, 2004b) and New Zealand (Griffin and Mackintosh, 2000; Mackintosh et al., 2004; Morris et al., 1994). In New Zealand, bovine tuberculosis was first diagnosed in wild deer in 1956 (presumptive) and 1970 (confirmed), and in farmed deer in 1978. In the early 1980s, tuberculosis was recognised as a serious threat to intensive deer farming in a number of countries, in particular New Zealand (Griffin and Mackintosh, 2000). Within Europe, the tuberculosis situation in deer is variable. In some countries, the disease was found to be introduced by importation of deer e.g Sweden (Bölske et al., 1995) while notably in Norway and Switzerland, infection in deer has not been reported for many years. In contrast, in those countries where disease remains problematic in cattle (particularly the UK and Ireland), infection is also frequently reported in deer (Fletcher, 2004b). In the UK, the industry introduced and funded a deer health scheme in the late 1980s to increase the pool of attested deer herds. In most other European countries, reports of infection are infrequent, suggesting (where surveillance is adequate) that infection is rare.

There has been substantial research in recent years, and very significant advances are being made, leading to improved strategies for disease control.

2.3.3. Current situation

Tuberculosis has been diagnosed in deer in a number of MS, including several with a disease-free cattle population. Deer are inspected for TB at slaughter, and in several MS, testing is also conducted on-farm. Wildlife reservoirs for TB, including badgers, wild deer and wild boar, are present in a number of MS.

Tuberculosis has been reported in a wide range of deer species (Griffin and Mackintosh, 2000). The status of tuberculosis in farmed deer in MS and selected third countries is summarised in Tables 4 and 5, respectively. The source for the introduction of TB in farmed deer could often be traced back to trade of infected deer (e.g Bölske et al. 1995). The TB status of livestock in the EU is summarised by Reviriego Gordejo and Vermeersch (2006).

In deer, the importance of tuberculosis is several-fold. Infected deer can act as a reservoir of infection for livestock and for protected and endangered wildlife species (Fletcher, 2004b). For example, in Ireland equivalent spoligotypes have been identified in cattle, deer and badgers (Costello et al., 1999), highlighting potential cross-infection between these species. In addition, infected wild and farmed deer present a public health hazard, particularly to those handling live animals or carcasses (Clifton-Hadley and Wilesmith, 1991; Fanning and Edwards, 1991; Liss et al., 1994; Nation et al., 1999).

2.3.4. Microbiology

The most significant mycobacterial diseases of free-living, captive and farmed deer are bovine tuberculosis, caused by *M. bovis*, Johne's disease (paratuberculosis) caused by *M. avium* subsp *paratuberculosis*, and avian tuberculosis, caused by *M. avium* subsp *avium* (Mackintosh et al.,

2004). Only the former is a member of the *M. tuberculosis* complex. Infection with *M. tuberculosis* has occasionally been reported in zoos (Griffin et al., 1994; Mackintosh et al., 2004).

Species of mycobacteria other than *M. bovis* may interfere with the histopathological or immunological diagnosis of TB (Griffin and Mackintosh, 2000). In the UK (Fletcher, 2004b) and New Zealand (Griffin and Mackintosh, 2000), *M. avium intracellulare* complex (MAIC) is routinely isolated from a small percentage of animals (particularly fallow and sika deer) with lesions typical of TB. Further, Johne's disease has emerged as a significant disease of red and fallow deer in a number of countries, both in Europe (Fletcher, 2004a) and New Zealand (Griffin and Mackintosh, 2000).

2.3.5. Epidemiology

2.3.5.1. Farmed deer

In contrast to the wild deer situation, farmed deer are considered a maintenance host for tuberculosis, in the absence of infection in other hosts (Ryan et al., 2006). Several authors have suggested that deer are more susceptible to infection with *M. bovis* than cattle (Griffin et al., 1994; Morris et al., 1994; Wahlström et al., 1998). Infection is generally sporadic in farmed deer populations, with a single or few cases (Mackintosh et al., 2000; Fletcher, 2004b). However, severe outbreaks do occasionally occur (Griffin and Mackintosh, 2000). During severe outbreaks, within-herd transmission is rapid (Morris et al., 1994) and up to 50% of animals can be affected, including 10% with severe (generalised) disease (Mackintosh et al., 2004). Transmission is probably influenced by particular grazing patterns (Clifton-Hadley et al., 1991). Disease outbreaks may be related to the presence of one or more animals with generalised infection and active excretion, resulting in significant infection challenge for in-contact cohorts (Griffin et al., 1998). Further, within-herd prevalence may be related to duration of exposure (Griffin and Mackintosh, 2000). Within-herd transmission is likely to be slow in extensively farmed herds in Sweden (Wahlström et al., 1998), where animals are held in large enclosures with very low population densities and little or no individual animal handling (Wahlström et al., 2000).

Figure 1 - Close contact during feeding in deer farming in Argentina.

Photograph by: Juan Pablo Soler

The most probable routes of exposure are via inhalation or ingestion (Wilkins et al., 2003), with the organism initially colonising the oropharyngeal tonsil (Lugton et al., 1997; Lugton et al., 1998; Mackintosh et al., 2004), before spreading to cranial lymph nodes and thoracic tissue (Wilkins et al., 2003). In infected animals, lesion distribution appears to be related to conditions of management. Infection of head lymph nodes (as occurs in New Zealand, Griffin et al., 1994) is most common among free-ranging animals, the thorax among intensively managed animals (O'Reilly et al., 1995) and gut-associated lymphatics among animals with a heavy environmental challenge (Griffin and Mackintosh, 2000). Animals do not exhibit clinical signs until late-stage disease; once clinical signs are observed, animals invariably die within 1-2 weeks (Griffin et al., 1994). It has been suggested that deer are more infectious for other species than cattle (Morris et al., 1994), with infectivity being directly related to the number of bacilli excreted (O'Reilly et al., 1995).

A range of factors affect susceptibility to infection and disease, including age, environment, population density, exposure and genetics (Mackintosh et al., 2004). Young animals (less than 6 months of age) are believed to be particularly susceptible, and may harbour high levels of infectious organisms within their lymphatic tissue without any pathological evidence of disease. Further, these animals may remain negative to routine diagnostic tests (Griffin et al., 1994). Population density and related contact rates play a key role in the maintenance of infection in wild populations of white-tailed deer in Michigan (Corner, 2006). Stressors relating to climate, nutrition, intensive management (Griffin and Mackintosh, 2000) and mating (Parra et al., 2005) are each believed associated with susceptibility and the development of fulminating disease (Griffin et al., 1994). There is a wide range of inherent susceptibility in the farmed deer population (Griffin et al., 1994), and it is now recognised that genetics plays an important role in disease susceptibility. A heritability of 0.48 (+/- 0.23) was measured in a group of farmed red deer (Mackintosh et al., 2000). Among highly susceptible animals, disease development is rapid, leading to generalised disease in as little as 5 months. Further, these animals are likely to be highly infectious, and act as 'super spreaders' at latter stages of disease (Mackintosh et al., 2000). There is no evidence of differences between deer species in TB susceptibility.

2.3.5.2. Wild deer

Although accurate prevalence, incidence and mortality figures for tuberculosis in wild deer are not available, available reports suggest that prevalence of disease is generally less than 5% (Clifton-Hadley and Wilesmith, 1991). Tuberculosis has been reported in wild populations in Canada, Great Britain, Hungary, Ireland, New Zealand, Switzerland, the USA (Griffin and Mackintosh, 2000) and Spain (Hermoso de Mendoza et al., 2006). In most wild deer populations, bovine tuberculosis is a sporadic disease (Mackintosh et al., 2000; Fletcher, 2004b) with deer acting as a spillover host (Griffin and Mackintosh, 2000). Infection in a spillover host will not persist indefinitely unless there is re-infection from another species (Corner, 2005). The likelihood of transmission between wild deer is much lower than in farming situations (Lugton et al., 1998). However, wild deer can also become a maintenance host (that is, infection can persist by intra-species transmission alone; Corner, 2005) when contact rates are high (Ryan et al., 2006). Increasing deer numbers coupled with increasing contact through winter feeding are key contributors to a TB epidemic in wild white-tailed deer (*Odocoileus virginianus*) in Michigan, USA (Corner, 2006; O'Brien et al., 2002; O'Brien et al., 2006). An increase in TB prevalence has been observed in wild boar and red deer in western Spain, possibly also in association with supplementary feeding in winter (Hermoso de Mendoza et al., 2006).

2.3.6. Pathogenesis and host response

In deer, tuberculous lesions are usually found in the lymph nodes draining the nasopharynx, lung or mesenteric tissues, most likely reflecting the different routes of transmission, either by the respiratory route or by oral ingestion (Griffin, 1988). In farmed deer, lesions are most commonly found in the Waldeyer's ring, a ring of lymphoid tissue that encircles the nasopharynx and oropharynx, including the lymphatic tissue of the pharynx, the palatine tonsil, the lingual tonsil, as well as other lymphoid tissue in the area (Beatson, 1984). The tonsils are regarded as the main site of natural infection in deer, but remain free, in most cases, of visible lesions (de Lisle et al., 2001). For this reason, tonsils are often used as the primary site during experimental infections to establish disease mimicking the natural situation (Griffin et al., 2006). Tuberculosis in deer progresses slowly. In deer herds where the annual intradermal test is performed as part of eradication programmes, new cases generally only have minimal signs of infection at post mortem (Griffin et al., 2004). The host defence mechanisms are able to contain further spread of the disease within infected individual. However, even in advanced stages of disease, when the animals are harbouring large numbers of mycobacteria, the animals might appear clinically healthy and disease may remain subclinical and unnoticed (Griffin, 1988).

2.3.7. Pathology

Tuberculosis presents as a spectrum of pathological conditions at post-mortem, ranging from liquefactive abscessation of lymph nodes (more typically associated with acute pyogenic bacterial infection) to classical caseo-granulomatous lesions (Griffin and Mackintosh, 2000). Most commonly, however, tuberculosis presents as a lymphadenitis involving one or more lymph nodes draining the nasopharynx, lung or mesenteric tissue (Griffin et al., 1994). TB lesions in deer are similar to cattle, both grossly and histopathologically, although abscesses in deer tend to have a thinner wall containing pus with multiple bacilli, and minimal calcification or fibrosis. In some deer, sinus tracts develop from diseased lymph nodes in the throat, axilla and groin areas (Beatson, 1985 cited in Griffin and Mackintosh, 2000; Lugton et al., 1998). Given the spectrum of presentations, it is not possible to distinguish infection with *M. bovis* from other mycobacterial infections, nor indeed from acute bacterial infection (Griffin and Mackintosh, 2000). All the mycobacterial species can cause caseous or necrotic lesions, and infection with *M. bovis, M. paratuberculosis* and *M. avium* are also not distinguishable on histopathology (Mackintosh et al., 2004).

2.3.8. Welfare aspects

TB in deer may affect the welfare of infected animals. As well as malaise due to infection and the inflammatory response, infection will also impact on animal welfare according to the specific location of the lesions, e.g. swollen lymph nodes in the throat, axilla and groin may impair swallowing, rumination, and gait, and any swelling of the wall of viscera due to abscessation is likely to cause pain (as does appendicitis in humans). The prevention and control of TB in deer will therefore have a positive impact on welfare. In this report the impact on welfare has been limited to the testing procedures (i.e the mandate for the opinion). Testing strategies can thus have a welfare impact on both infected and uninfected animals, e.g. the restraint required for injection, measuring responses and removal of blood may all cause temporary mild pain and distress.

3. DIAGNOSTIC TESTS FOR TB IN DEER

3.1. General principles

The following information is mainly based on data and experience of tuberculosis in cattle.

The cell mediated immune (CMI) response is the predominant immunological immune

response of the host against mycobacteria and starts immediately after infection. The CMI response is not only a defence mechanism but also responsible for the main sign of tuberculosis, the formation of the characteristic granulomas. At this first phase, measuring the CMI response, either by intradermal test, gamma-interferon assay or histology, provides the main diagnostic tool for tuberculosis. Due to the low bacterial load at this stage of infection, routine culture performed at post-mortem of intradermal test positive animals might result in a culture negative status of the animal and not in a confirmation of infection. However, this should not be regarded as a guarantee that the animal is not infected (Griffin and Buchan, 1994).

The second phase is also called the "anergic" phase because of a loss of detectable reactivity to the tuberculin test. In this phase, the immune response is dominated by the formation of circulating antibodies and correlates with a severe progression of the disease which is characterized by an increasingly large number of mycobacteria. For this reason, tests, like the enzyme-linked immunosorbant assay (ELISA), aimed at detecting antibodies or the direct detection of the bacterium, either by culture or polymerase chain reaction technique (PCR), become the most reliable and sensitive diagnostic tests.

As a consequence, a combination of different tests, representing each category, is often used to diagnose tuberculosis in an animal or herd with an unknown TB status. Furthermore, in herds where the annual intradermal test is performed as part of an eradication programme, few animals with advanced stages of disease will be present. Therefore tests aimed at the detection of the bacterium and/or antibodies will rapidly loose their effective sensitivity and for this reason tests aimed at the detection of the CMI response are likely to become the most important diagnostic tools in long term certification programmes.

When an infected herd is tested at any given moment by any test (or combination of different tests), it is unlikely that all infected animals present in this herd will be detected. This is caused by the fact that it is unlikely that all infected animals are in a stage of infection that would allow their detection (see figure 2) and they will be missed in the first round of testing. As a result, the test will have to be repeated for at least a second time to guarantee a certain degree of freedom.

The interval between successive rounds of testing is dictated by two opposing objectives:

- The interval *should be long enough* to enable infected, but previously test-negative animals to develop a measurable immune response and to minimise the potential for interference of the immune response caused by previous test(s), and
- The interval *should be sufficiently short* to minimise the risk of further within-herd transmission from infected animals.

The purpose of testing will affect decisions regarding testing interval:

- *In control situations* (that is, testing in known infected herds), the test interval should be as short as possible, to maximise the likelihood of early detection of infected animals and mimimise the risk of further within-herd transmission. In cattle, the minimum interval between tests of individual animals is 42 days. In deer, interference (immunosuppression) with previous tests has been reported after an interval of 60 days and no suppression was found at 120 days (Griffin and Cross, 1989), and therefore, longer intervals should be applied.
- *When the purpose of testing is surveillance* (that is, testing in herds with no recent evidence of infection), a longer test interval should be used, to maximise the likelihood that infected animals will have sufficient time to develop a measurable immune response. In cattle, test intervals of 6 to 12 months are routinely applied.

Fortunately, bovine tuberculosis is reported to spread rather slowly within a herd. Therefore certification schemes for bovine tuberculosis are often based on repetitive, annual testing using the comparative intradermal test. The optimal interval for using the intradermal tuberculin test in deer to give sufficient guarantee might have to be established by continuous evaluation of ongoing programmes.

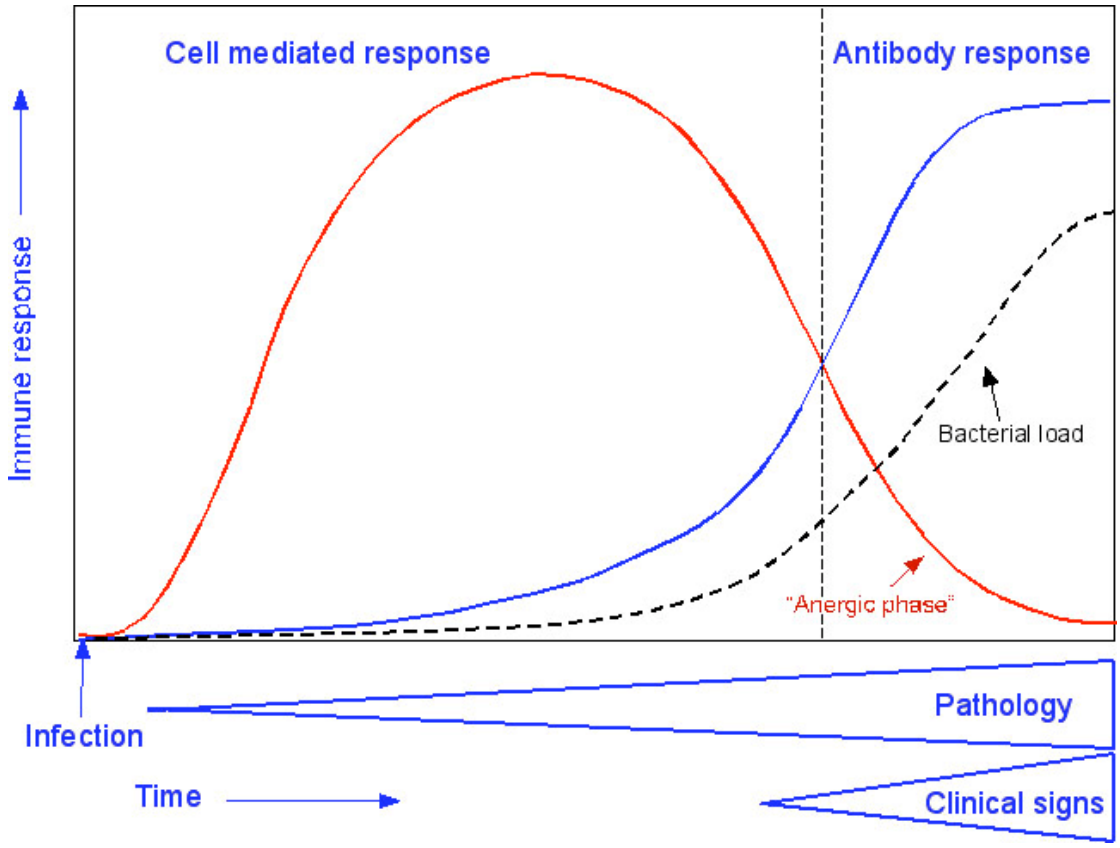


Figure 2 - The different phases of immune response following infection with *M. bovis*, relative to the development of pathological changes (source: Douwe Bakker)

The lessons learned during the first eradication programmes for bovine tuberculosis in the 1950's, lead to the Council Directive 64/432/EEC on animal health problems affecting intra-Community trade in bovine animals and swine. This Directive and its recently amended annexes form the basis for the eradication and control for bovine tuberculosis in livestock in the EU. Freedom from bovine tuberculosis is achieved and guaranteed by repetitive use of the intradermal tuberculin test.

The gamma-interferon assay used in parallel with the tuberculin intradermal test was recently accepted by the EU (Annex B.3. of the above mentioned Directive) in order to maximize the detection of infected animals in a herd or a region with a high prevalence.

Even though, the intradermal tuberculin test and the gamma-interferon assay are the official tests to be used, other test are in use to give additional information on the infection status of the animals being tested. As mentioned above, the ELISA, is often used to detect animals in more advanced stages of disease, and slaughterhouse surveillance remains in place to detect visible lesions caused by tuberculosis after freedom from disease has been obtained.

In addition, a combination of tests may be used for the confirmation of animals with an intradermal positive test or detected at slaughter and consists of a detailed necropsy followed by culture, PCR and (immuno-) histology. This combination of tests is not suitable for surveillance but crucial for confirmation of results from the "official" tests and for their quality control.

Thus far, all eradication and certification schemes for tuberculosis in deer (Griffin et al., XX; Wahlström et al., 2000; Waters et al., XX) are based on the same test and interpretation of the test results are used for bovine tuberculosis in cattle. However, the interpretation of the test results in bovine are not harmonized. In some countries a deer adapted interpretation is applied (Wahlström, 2004).

3.1.1. Combined testing:

To overcome the limitations of moderate test sensitivity or specificity, tests can be used in combination. Two main systems can be used: in series and in parallel. For example, in cattle, testing in series involves retesting skin-test positive animals with the gamma-interferon assay (GINT) to enhance specificity, though with a resulting reduction in sensitivity (animal is positive only if both tests are positive) (Ryan et al., 2000). Testing in parallel involves all animals being tested with both the intradermal test and the GINT to enhance sensitivity, though with a decrease in specificity. In these combined schemes, the GINT can be used at different times post intradermal test, although the effect of this remains unclear. Thus, if specificity needs to be maximised (thereby minimising the likelihood that positive test results could be false-positives), the use of diagnostic tests in series will be more suitable than applying single test interpretation. Parallel testing combining two techniques (intradermal tuberculin test and GINT) has been previously used for the eradication of tuberculosis in goats (Vidal et al., 1995; Liébana et al., 1998) and cattle (González-Llamazares et al., 1999; Aranaz et al., 2006) resulting in gradual reduction in the prevalence of the infection. Liébana et al. (1998) reported elimination of infection after three cycles, and González-Llamazares et al. (1999) reported that all animals were tested negative in 50% of herds after 2 cycles, and in all herds after 4 cycles. The herd with delayed clearance in the last report probably required more cycles because dual infection was present (Aranaz et al., 2006)

Parallel testing has also been applied to deer herds: intensive diagnostic testing was carried out over short time intervals after initial diagnosis of TB in three deer farms (with different prevalences). Whole herd single intradermal tuberculin testing or mid cervical testing (SST) was used as the primary test and a single intradermal comparative cervical test (SICCT), as an ancillary test. In an attempt to enhance TB eradication, ancillary blood tests comprising lymphocyte transformation test (LCT) and ELISA were used in parallel with SST, or as serial tests, to complement intradermal testing. TB could be eradicated from infected farmed deer herds, using currently available TB tests, irrespective of disease prevalence (Griffin et al., 2004).

3.1.2. Practicality for combined tests:

Regarding practicality of the GINT, the first is related with time period from blood extraction to stimulation at the laboratory. Blood samples need to be stimulated within 8 hours of collection because a delay in the processing of samples may affect the sensitivity (Gormley et al., 2004; Rothel et al., 1992; Whipple et al., 2001). Also, some factors have been described to impair the immune response of cattle to the diagnostic test, as the treatment with corticoids (Doherty et al., 1995; Goff, 1996), although the use of a mitogen as positive control has not been considered absolutely necessary in the application of the GINT test (Vordermeier et al., 2001). In this sense, the effect of the stress associated to mustering and handling or should be taken into account.

Also, the performance of the intradermal test and the GINT tests regarding sensitivity and specificity is impaired in cattle with dual mycobacterial infections; and this reduction is more evident in the latter (Aranaz et al., 2006). This fact should require further research as paratuberculosis has also been recognised in farmed deer (de Lisle et al., 1993, Power et al., 1993; Fawcett et al., 1995; Manning et al., 1998; Godfroid et al., 2000; de Lisle et al., 2003).

The information about the effect of the previous intradermal test on the GINT assay is controversial, and interferences of the intradermal test test in the GINT assay have been described (Rothel et al., 1992; Whipple et al., 2001).

3.2. Test evaluation methods

3.2.1. Qualitative evaluation

The group identified key papers and used expert knowledge of the working group to provide general descriptions of each test, taking into account the following issues: definition, description of test, factors influencing Se, factors influencing Sp, repeatability, reproducibility and practicality.

3.2.1.1. Performance

In this report, several characteristics of diagnostic accuracy will be considered, including sensitivity (Se) and specificity (Sp). Se is defined as the probability of a positive test result in an infected animal, and Sp as the probability of a negative test result in an animal that is not infected. The infection, rather than the clinical, status is of primary interest for surveillance purposes because infected but clinically inapparent animals may transmit the disease. The reference method ("gold standard") as well as biological factors such as stage of disease will be taken into account in the assessment of Se and Sp as far as possible depending on the available data. The performance measures will also be considered at the herd-level, whereby herd-level Se refers to the probability of classifying a herd as positive if the herd is truly infected for a given threshold level (≥ 1 infected animal/ herd). Reference will be made to appropriate values for the assumed design prevalence at animal level in an infected herd, and the number of reactors that classify a herd as positive. The design prevalence defines the lower limit of a theoretical level of infection in the population which a given surveillance activity would be able to detect with a specified probability. The herd-level Se and Sp are mathematical functions of the parameters Se and Sp.

Repeatability and reproducibility are measures of test variability within and between laboratories, respectively. Pertinent information on these measures will be reported in a qualitative manner.

3.2.1.2. Practicality

Test practicality was assessed qualitatively (in words) and could include the following aspects:

- robustness (sensitivity of the performance measure to slight procedural changes and/or environmental factors);
- ability to transfer the testing procedure to peripheral smaller laboratories;
- availability of test reagents;
- welfare and animal handling considerations (capturing and invasiveness of testing procedure).

3.2.2. Quantitative evaluation

3.2.2.1. General methodological approach

The main objective was to estimate the sensitivity (Se) and specificity (Sp) of the main tests for TB in deer for the purpose of using this information in quantitative assessments of testing strategies to support claims of TB-free status. Two sources of information have been used: expert opinion and systematic literature review of data. The uncertainty of the expert opinions on Se and Sp for each test was expressed as a distribution derived from minimum, most likely

and maximum values for each parameter. The statistical uncertainty in the estimates of Se and Sp from the literature was expressed as posterior distributions obtained from Bayesian logistic regression models using Monte Carlo Markov Chain (MCMC) techniques (see below). The following rules were used to establish the final estimates of the diagnostic parameters (Se and Sp) for each test depending on the available information.

- In the case that both expert opinion and data from literature were available the expert opinion was used as prior information in the meta-analysis of the literature data. This resulted in a posterior distribution reflecting the uncertainty around the diagnostic parameters given the data from the systematic literature review and the expert opinion.
- In the case that no expert opinion was available the meta-analysis of the literature data was conducted using non-informative priors for the diagnostic parameters. This resulted in a posterior distribution reflecting the uncertainty around the diagnostic parameters given the data from the systematic literature review and no other sources of information
- In the case that no data from literature was available the estimates on sensitivity and specificity were based on expert opinion.

3.2.2.2. Comment on the use of expert opinion:

The advantage of the systematic literature review is that this approach is state-of-art for gathering scientifically sound empirical evidence. However, it was anticipated that the literature data may be limited to some relevant tests and deer species. While differences in the sample size, study design and documentation of results are obvious, it is difficult to address such quality issues in a formal way. There is a risk of publication bias (data on specificity and sensitivity out of expected range may be rejected for publication) and motivational bias (working group or commercial suppliers interested to promote their product). Empirical data can be misleading if no adjustment is made to control for confounding factors. Therefore, the use of a gold standard to define "truly" infected or non-infected animals and the stage of infection (if known) were considered as primary potential confounding factors in the analysis of the data. If too few data are published, there is a chance that they do no represent the "reality". Total reliance on empirical data may result in no data for certain species/test combinations. In this situation, no statistical analysis would be possible.

The advantage of using experts to judge the performance of diagnostic tests is that experts may have access to unpublished information and that their judgement may be informed by their scientific assessments of published or unpublished data, which is not formally documented. The use of expert opinion, on the other hand, introduces several potential biases, such as through selection of experts, cognitive biases, motivational biases or group biases. The procedure followed on the study was aiming at reducing such biases by the selection of experts on the basis of their documented expertise (publications) and avoidance of group-interactions. The experts have also been asked to score their level of expertise for each question item.

3.2.2.3. Expert opinion

3.2.2.3.1 *Participants*

A total of 85 experts were contacted to invite them to participate in the survey. Experts were selected on the following basis:

- Members of the EFSA working group (some with expertise, others just for information and were not expected to respond);
- Scientists experienced in the use of TB tests in deer, nominated by members of the working group;

- Lead authors of relevant papers identified by the members of the working group.

Participants were contacted by email on 27 June 2007 and were provided with:

- Background on the EFSA working group's objectives;
- A request to undertake a quick on-line survey (taking less than 5 minutes);
- A user name and password to log into the questionnaire site;
- Step by step instructions.

A deadline for submissions was set (13th of July, 2007). A follow-up reminder email was sent to those experts who had not responded on 11th of July.

Fifteen of the experts contacted provided answers to one or more questions. A further 14 responded to indicate that they felt they did not have adequate expertise to provide useful information. Another 5 automated responses were received to indicate that the person was out of the office during the period of the survey.

3.2.2.3.2 *Questionnaire*

The aim of the questionnaire was to easily obtain quantitative estimates of the sensitivity and specificity of the tests, as well as a self-assessment of the level of expertise of the participant, and limits for the possible participant uncertainty or test variability.

The opening page provided further instructions. The details related with the "Questionnaire used to collect expert opinion - performance of diagnostic tests for tuberculosis in deer" are explained in Appendix A.

3.2.2.3.3 *Analysis*

The data obtained for each response in relation to each test were: level of expertise; estimated most likely value; minimum possible value; maximum possible value. These were combined to produce a summary distribution of the estimated test performance based on all responses for each test parameter.

For each test, parameter and response combination, the minimum, most-likely and maximum values were used to define an alternative Pert distribution, in which the minimum and maximum values provided by experts were considered as the 2.5th and 97.5th percentile. This has resulted in a slightly more spread-out distribution (increase in uncertainty) and non-zero probability densities over the entire support range between 0 and 100%. This alternative parameterisation was chosen because to have sampled prior values all between 0-100%. The resultant distributions from each response for that test / parameter combination were combined by repeatedly drawing random values from each of the Pert distributions, selecting each response with a probability proportional to the self-assigned level of expertise. This means that a response from a person with a high expertise value would provide more weight in the resultant distribution than one with a low expertise level. This process was repeated over 10,000 iterations, to produce final distributions.

3.2.2.3.4 *Results*

The results are presented below under each individual test.

3.2.2.4. Systematic literature review

A systematic review of literature was carried out to improve reproducibility and minimise selection bias. The procedure was structured in two stages and is described in detail in Appendix B. Briefly, in stage 1, seven online scientific literature databases were searched with a highly sensitive search statement. The title, keywords and abstracts were screened by two

independent reviewers. Based on agreed eligibility criteria, any article that was found relevant by at least one reviewer was ordered for full review. All full papers identified in stage 1 that became available before an agreed deadline were submitted to two (out of 9) independent reviewers for stage 2 review. In stage 2 any discrepant assessments by the two independent reviewers were identified and reported back using an automated report generating system. A series of meetings were held to resolve discrepant assessments between reviewers whereby new ad hoc rules were agreed by the whole working group to maintain consistency throughout the process.

The information extracted from the full papers consisted of 4 variable items related to the source (publication), 6 variables related to the test description and each 11 variables related to any given estimate of a sensitivity and/or specificity, respectively. This has resulted in a hierarchically organised data set in which one publication could be linked to one or more tests and each test described in each publication could be linked to one or more estimates of a sensitivity and specificity.

The target outcome of the systematic literature review is the numerator and denominator of any estimate of a diagnostic sensitivity or specificity. Characteristics of the test (mainly test principle and animal species) and of the study approach (mainly nature of reference population) were considered as covariate information

In the end of Appendix B plots are provided visualizing all sensitivity and specificity estimates with exact binomial confidence intervals extracted from the papers considered in the systematic literature review. In addition funnel plots are provided to check for possible publication bias.

The list of references retained for the systematic literature review has been attached in the end of the report (Chapter 11).

3.2.2.5. Meta-analysis

3.2.2.5.1 *Methods*

The results from the systematic literature review and expert opinion were used to provide estimates of test performance. First, the empirical data for each test were analysed to explore potential confounding factors for Se or Sp using logistic regression analysis. These models had the generic form:

$$\text{logit}(p) = a + bX,$$

where p is the empirical parameter estimate for Se or Sp based on a given number of true results and sample size, X is a set of potential confounding factors observed along with each estimate and a and b are estimated model coefficients.

The potential effects of deer species, region (EU and Russia vs. non-EU), the inclusion of repeated samples (yes vs. no) and obvious bias noted by the reviewers (none vs. "over-estimate" and "under-estimate") were considered only for exploratory reasons. The effect of the gold standard (experimental vs. non-experimental condition) and stage of infection (earlier or later than 6 months) if known were considered for adjusting the estimates of Se and Sp for the more relevant level (non-experimental data from EU or Russia) if the standard logistic regression model indicated a significant effect (p-value for the Wald statistic 0.05 or smaller). The number of confounding factors that could be analysed for each test varied due to the available information.

For each test the diagnostic sensitivity and specificity were summarised based on the available information. Below, for each test and parameter, four different estimates are reported. These estimates were generated as follows:

Estimate 1 (Expert opinion): The information elicited from experts using the online questionnaire was merged for each test and parameter by Monte-Carlo sampling from the specified pert distributions with sampling probabilities proportional to the expert's self assessment of their expertise. The resulting empirical distribution summarises the uncertainty about the parameter in question. For descriptive purpose, these uncertainty distributions were summarised using a point estimate (median value) and 95% interval (2.5th and 97.5% percentile). For two tests (SST and SICCT), information on Se was given for early and late stage of infection. The data were merged (resulting in bimodal distributions) if the empirical literature data provided no statistical evidence for an effect of the stage of infection. This approach was also chosen because experts may have considered this effect explicitly for these two tests and implicitly for other tests without providing an explanation.

Estimate 2 A (Systematic Literature review - unadjusted estimates): All available estimates from the literature review were summarised into a pooled parameter estimate. The latter is defined as the total of all true positive (for Se) or true negative (for Sp) results over the total sample size for all available Se or Sp estimates for the given test. An exact binomial 95% confidence interval was constructed using standard methods. It is noted that this estimate ignores any potential confounding effect of covariate factors and was given for comparative reasons.

Estimate 2 B (Systematic Literature review - adjusted estimates): A Bayesian logistic regression-based estimate of sensitivity or specificity by Markov chain Monte Carlo (MCMC) was established using a non-informative (uniform) prior distribution for the parameter of interest. The model building was conducted using standard logistic regression analysis as described above. The model-based estimates of Se and Sp are based on the Bayesian posterior distributions of these parameters and were adjusted (where possible) to non-experimental conditions regarding the gold standard and, if stage of infection was found significant, separate for early and late stage of infection. The approach resulted in posterior distributions of the parameter of interest, which were summarised using the point estimate (median) and 95% credibility interval (*i.e.* 2.5th and 97.5th percentile of the posterior distribution).

Estimate 3 (Systematic Literature review - adjusted estimates combined with prior expert opinion): A similar Bayesian logistic regression model as above was fitted but empirical distributions (see Estimate 1) based on the expert opinion were used as informative priors. Because in a lot of cases the combined expert opinion was extremely difficult to parameterize (e.g. extremely skewed, bimodal) it was decided to use the empirical distributions of the combined expert opinion straight away as priors. To achieve this discrete prior was specified on 5,000 sampled values from the combined expert opinion.

The final goal of this analysis was to elaborate the quantitative input information required by the model for the surveillance strategies (see Chapter 6). The working group decided that with a decreasing rank of suitability for the purpose of the further quantitative work, the estimates 3, 2B and 1 should be used. That means if both expert opinion and data is available for one parameter, a Bayesian analysis of the data using expert information as prior would be used. Quantitative expert opinion would be used alone in the absence of data. The results below describe for each test the available basis for estimates of sensitivity and specificity.

The values used for the disease freedom model have been highlighted in the tables for each individual test presented below.

3.2.2.5.2 *Validation*

For this report information on the diagnostic performance of tests for TB in deer has been collected using a questionnaire sent to experts in the area and based on a systematic quantitative literature review. For some tests only data but no expert information was available

(LCT, MAPIA, NECR followed by CULT, OTHER tests, SST followed by SICCT, SST followed by ELISA). For most of the tests where both sources of information were available (BTB, SICCT, ELISA, HIST, NECR, PCR/Sp, SST), the final estimate is practically based on the data alone and the expert information is not very influential. This may be due to a high level of uncertainty of the experts in combination with a large empirical basis (large sample sizes) in the published literature. For these tests, the empirical basis could be considered as sufficient. However, the report provided some evidence that, for example, the diagnostic performance of some tests (CULT/Se, ELISA/Se, HIST, LCT/Sp, NECR/Se, SST) may differ among deer species. It should be concluded that further studies should investigate the species effect and other potential confounding factors further. It is also possible that the wide range of possible parameter values given by the expert opinion reflects uncertainty due to the "case definition". Although our analysis has been adjusted for the effect of the gold standard, it could be recommended to use a more uniform gold standard criterion for further validation studies (see 4.2). Another explanation of wide prior distributions elicited from experts is that they convey valuable information about relevant biological variability of these parameters. Further validation studies should aim at generating realistic estimate of Se and Sp under conditions similar to the intended purpose of testing herds/ populations free for TB. The use of latent class analysis to estimate the Se and Sp of two or more tests applied in parallel to the same sample of deer could be considered. This technique does not require the assumption that one of the tests is a perfect gold standard. However, care must be taken to design a study such that the assumptions regarding prevalences in the sampled populations, constant Se and Sp in all populations and independence of tests are justified.

For some tests under investigation (GINT/Se, INSP/Se), the expert prior was confirmed by the literature data. Although these results are in good agreement, the combined evidence may still be quite limited as seen by the wide 95% credibility intervals.

For some tests (CULT/Se, GINT/Sp, INSP/Se, PCR/Se), the prior information provided by experts was relative precise in comparison to the information based on literature data. In these cases it is possible that uncertainty due to a small data basis was mitigated by the use of expert knowledge.

Only expert opinions were available for one test (INSP/Sp). It should not be unrealistic to obtain field data to support this estimation.

It is noted that the OIE has developed a standard operating procedure for the validation of diagnostic tests for purposes related to international trade. These procedures could therefore be recommended as a guideline for the conduct of validation studies for diagnostic tests for TB in deer.

3.3. Test evaluation

3.3.1. Direct Identification of the TB agent

3.3.1.1. Culture (CULT)

3.3.1.1.1 *Qualitative evaluation*

Introduction

Although a presumptive diagnosis of TB infection in a herd or animal may be made by tests while the animal is alive (e.g. intradermal test, gamma interferon, serological tests), and by post-mortem investigation (e.g. meat inspection, histopatology or molecular tests) (Mackintosh et al., 2004), isolation by culture and subsequent identification of tubercle bacilli is usually considered the gold standard for diagnostic confirmation of TB infection in cattle and deer as

reported by several authors (Palmer, 2004; Rohonczy et al., 1996; Rhyan et al., 1992; Griffin et al., 2004; de Lisle et al., 1985).

In some cases the long time required for the mycobacteria to grow can cause undue delays in the implementation of control measures. Sometimes bacterial culture can be unsuccessful despite strong suspicions of TB infection being present. To overcome the problems associated with these false negative tests, other additional diagnostic tests, such as histopathology or molecular tests, should perhaps be considered as confirmatory even though a positive culture result has not been obtained.

Definition

Culture includes isolation of *M. bovis* or other *M. tuberculosis*-complex bacilli and final identification of the isolated strains following standard bacteriological procedures.

Description of test

Although in diagnosing human tuberculosis culture isolation is attempted on clinical samples, such as body fluids, tissue biopsy and exudates, in veterinary medicine culture isolation is not usually performed on these kinds of specimens but on tissues taken from post-mortem examinations.

Tissue samples collected during the meat inspection surveillance programme or during post-mortem examination are submitted to homogenization with a stomacher, pestle and mortar or other equivalent system such as a blender jar (Rohonczy et al., 1996), and decontamination by the use of either an acid or an alkali followed by a neutralization step, according to conventional procedures described by de Lisle et al., (1983), Rohonczy et al., (1996). An additional protocol based on the use of hexadecyl pyridinium chloride as described by Corner and Trajstman (1988) has shown a higher mycobacterial recovery rate (Aranaz et al., 1999).

The suspension is then centrifuged, the supernatant discarded and the sediment inoculated for culture isolation onto egg-based solid media such as Lowenstein–Jensen, Stonebrinks, or Colestos base which can be supplemented by pyruvate, glycerol or both (Palmer, 2004).

The media are incubated for up to 12 weeks at 37°C and examined regularly, e.g. every two weeks, to identify colony formation (Rohonczy et al., 1996). Growth of *M. bovis* generally occurs after 3-6 weeks' incubation (Palmer, 2004) but other members of *M. tuberculosis*-complex, such as *M. microti*, may require a longer incubation time.

Mycobacterial isolation can also be performed using liquid media such as Middlebrook 7H9 modified by adding growth supplements and antibiotics according to the instructions of the supplier. Liquid culture systems are also now being used in several veterinary laboratories (Zanoni et al. 2005; D'Incau et al. 2006, Hines et al., 2006).

Mycobacterial isolates are then identified by molecular tests (Kulski et al., 1995, Niemann et al. 2000, Huard et al., 2003; Cimara et al., 2004), and conventional bacteriological tests as described by Metchock et al., (1999) and by Holt et al., (1986).

Sensitivity

Most of the studies on TB in deer have not evaluated the sensitivity of gold standard itself (culture isolation), but they have compared the other diagnostic techniques with the gold standard (Fitzgerald et al., 2000; Schmitt et al., 1997, Rohonczy et al., 1996).

Moreover sensitivity trials are expensive and can be difficult to manage, considering the need to purchase sufficient animals to provide statistical significance and their management during the trial. For these reasons sensitivity trials are conducted infrequently (Pharo and Livingston, 1997).

Factors influencing Se are:

Selection of specimen: Specimens must be selected on the basis of the presence of typical or suspicious lesions where viable organisms are most likely to be found. Routine culture of *M. bovis* from tissue samples taken from the field is technically demanding and strongly influenced by the sample taken and its quality (Griffin and Buchan, 1994).

Preservation of the specimen: Transportation and storage techniques must ensure that organisms remain viable between collection and culture. The transport time between collection of specimens and performing cultural examination may interfere with successful isolation (Kaneene et al., 2002).

Decontamination of the sample: Different protocols of decontamination have shown diverse mycobacterial recovery rates (Aranaz et al., 1999).

Culture medium: Several studies indicate that liquid culture isolation significantly improves the recovery rate and also reduces the time required for isolation of *M. tuberculosis* from human clinical specimens (Somoskovi et al., 2003; Tortoli et al., 1999; Jayakumar et al., 2001; Whyte et al., 2000). The same performance improvements have been reported for *M. bovis* isolation from animal samples (Zanoni et al., 2005; D'Incau et al., 2006; Hines et al., 2006).

Laboratory variability: the sensitivity of the culture method can vary greatly between laboratories (Griffin et al., 1994).

Identification technique: Biochemical methods of identification can be slow and not sufficiently informative, in particular in discriminating between mycobacteria of the *M. tuberculosis*-complex; therefore, nowadays identification is also performed using molecular protocols (Kulski et al., 1995; Niemann et al. 2000; Huard et al., 2003; Cimara et al., 2004).

Specificity

Since culture isolation is considered the reference method for diagnostic confirmation of TB, it is assumed that the specificity is 100% when isolation is confirmed by standard bacteriological procedures including molecular identification.

Factors influencing Sp are:

Cross-contamination: it has been reported in several hospital laboratories with the consequence of false positive subject identification (de Boer et al., 2002).

Repeatability and reproducibility

There are no data available on repeatability or reproducibility of cultural tests in deer.

Practicality

Cultural examination is performed on samples collected during post-mortem inspection; particular care should be taken in collecting samples, to prevent environmental bacterial contamination. Samples should be submitted to the laboratory within 48 hours, chilling to refrigerator temperature or frozen at –70°C (de Lisle, 2002, Rohonczy et al., 1996).

Cultural isolation must be performed in certified laboratories with the necessary equipment, supplies and trained personnel to conduct mycobacterial culture (Kaneene, 2002). Specialised facilities (e.g. HEPA-filtered air inflow under negative pressure) and methods are required for isolating and identifying mycobacteria. Particular attention must be taken to prevent infection of laboratory staff and to avoid contaminating the samples with environmental bacteria (de Lisle, 2002).

3.3.1.1.2 *Quantitative evaluation*

Sensitivity

Prior expert information about sensitivity of CULT has been provided by 10 experts.

Data from literature comprises of 9 estimates, involving a total number of 177 animals, published in 6 papers (see Table 47 - references with the Ref. Id. 124 181 258 347 354 1007).

Using the literature data, the impact of the following variables: *species, dpi3* and *rep* on sensitivity could be investigated using logistic regression analysis (see Table 55 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 6 - Sensitivity estimates of CULT based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.809	0.402	0.959
Estimate 2A - Systematic Literature review - unadjusted estimates	0.729	0.657	0.793
Estimate 2B - Systematic Literature review - adjusted estimates	0.730	0.662	0.792
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	**0.741**	**0.670**	**0.794**

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

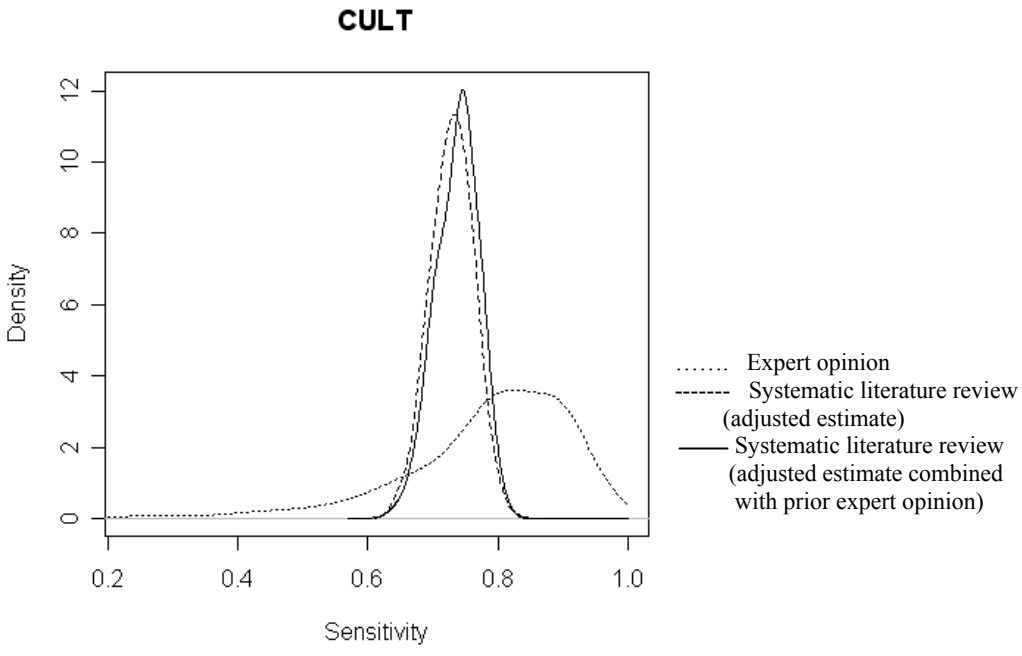


Figure 3 - Probability density distributions representing the information about sensitivity of CULT

Specificity

Prior expert information about specificity of CULT has been provided by 11 experts.

No estimates have been retrieved from literature.

Table 7 - Specificity estimates of CULT based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.973	0.47	0.996
Estimate 2A - Systematic Literature review - unadjusted estimates	NA	NA	NA
Estimate 2B - Systematic Literature review - adjusted estimates	NA	NA	NA
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	NA	NA	NA

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.
NA = not available.

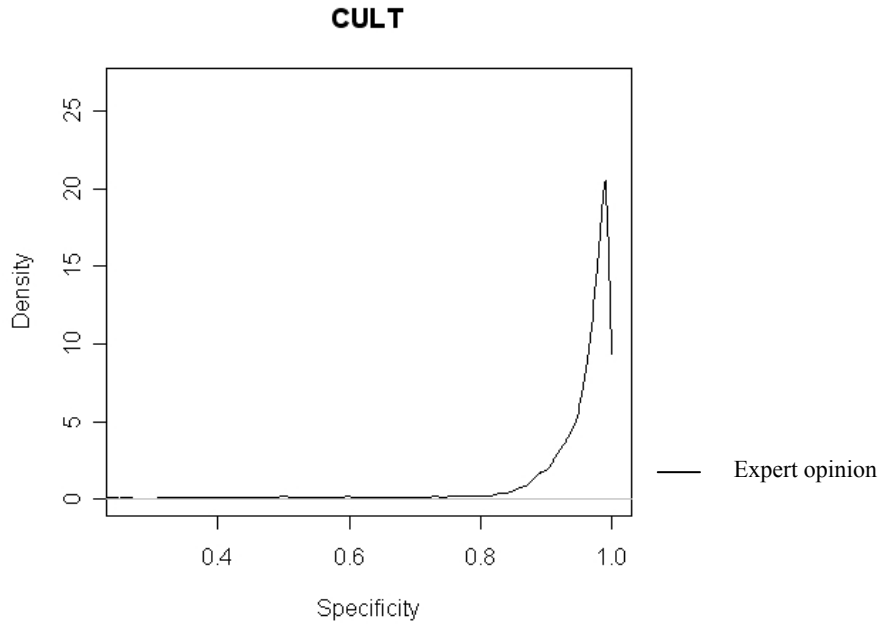


Figure 4 - Probability density distributions representing the information about specificity of CULT.

3.3.1.2. Polymerase chain reaction (PCR)

3.3.1.2.1 *Qualitative evaluation*

Introduction

A number of amplification-based techniques have been developed and widely evaluated for the detection of *M. tuberculosis*-complex in fresh and fixed tissues, as well as in clinical samples (mainly sputum in human patients). Most of the protocols for molecular detection of the TB agent are based on the polymerase chain reaction (PCR) and have been developed "in house".

In the diagnosis of bovine tuberculosis, even if bacterial culture is considered the "gold standard", the use of a PCR test of suspected samples is often applied to speed up detection by several weeks and improve the sensitivity of TB detection.

Definition

Polymerase Chain Reaction is a highly sensitive test that can detect small amounts of DNA or RNA (genetic material) in a blood or tissue sample using an amplification technique that

multiplies the existing DNA/RNA so that it can more easily be detected. It is generally considered a useful tool for post-mortem confirmation of a diagnosis based on macroscopic lesions and histopathological examination (on lymph node/organs). As yet, PCR detection has only been used on a limited number of specimens from live animals, including nasal or pharyngeal swabs, milk, blood, lymph node aspirates, urine, rectal pinch and fecal samples (Mishra et al., 2005; Zumarraga et al., 2005; Vitale et al., 1998). The small number of bacilli present in these clinical specimens is a big limitation for using PCR in a surveillance programme.

Further, PCR is particularly useful as a method to speciate *Mycobacterium* isolates. In some laboratories, PCR is now being used on a routine basis to detect the *M. tuberculosis* group and distinguish it from *M. avium* (Kulski et al. 1995, Godfroid et al. 2005: species-specific identification of *M. bovis, M. avium* subsp. *avium*, and *M. avium* subs. *paratuberculosis* in mixed mycobacterial infections in red deer).

Description of test

At the moment there is no standardized PCR protocol for detecting TB in deer, or indeed in other species. However, a large number of "in house" methods are in use. Different DNA extraction procedures have been developed, generally based on chemical or mechanical lysis coupled with either the use of commercial kits for DNA purification or a sequence-capture step on a specific *M. tuberculosis*-complex region (e.g. the DR region). For molecular detection tests, the most widely used target is the transposable genetic element IS6110, found in mycobacterial species in 1-25 copies. A number of different primer combinations specific for the *M. tuberculosis*-complex (based on the IS6110 sequence) have also been evaluated in deer (Hénault et al. 2006). The PCR based on IS6110 amplification is not specific to *M. bovis* identification, but it could be useful for detecting tuberculosis caused by other highly pathogenic members of this complex such as *M. caprae*, which has also been isolated from bovines and wild animal species and *M. microti* which has been detected in wild boar and requires a longer incubation period than 42 days. Some laboratories amplify the insertion sequence IS1081, present in the *M. bovis* genome in 5-6 copies instead of IS6110 (only 1-3 copies). Other primers have been used, including primers that have amplified sequences from 16S-23S rRNA, genes coding for *M. tuberculosis*-complex specific proteins, such as MPB70, MPB64 and the 38kDa antigen b amongst others.

Different PCR strategies have been developed: standard PCR, touch-down PCR, nested PCR and more recently real-time PCR have been reported by various authors. The Real time PCR technique is considerably simpler and faster with respect to the standard PCR technique and it shows a high degree of specificity, sensitivity and rapidity.

Sensitivity

PCR is a powerful technique that potentially can detect few copies of a specific target in a complex sample.

In the veterinary field, *M. tuberculosis*-complex detection by PCR-test has been reported to have a diagnostic sensitivity of 93% in cattle, using bacteriological culture as the gold standard (Norby at al., 2004) but other authors reported a lower sensitivity (Liebana et al., 1995). However, it has been demonstrated that PCR can detect *M. tuberculosis*-complex organisms in formalin-fixed tissues from culture-negative ruminants (Miller et al., 2002).

Factors influencing Se include:

Nature of specimen: Clinical samples as nasal or pharyngeal swabs, milk, blood, lymph node aspirates, urine, rectal pinch and fecal samples contain a lower number of bacilli compared to necropsy samples such as lymph nodes with typical lesions.

DNA extraction method and PCR strategy: A variety of DNA extraction protocols have been tested, reflecting the complexity of mycobacteria wall structure. The efficiency of these procedures can influence PCR sensitivity. Moreover, nested PCR shows an increased sensitivity compared to standard PCR methods and Real-time PCR is usually considered to have a higher sensitivity due to the fluorogenic probe that permits amplification product detection.

PCR target: If the target is present in the genome with more than one copy (multicopy target), PCR shows a higher sensitivity compared to a single copy target. Some laboratories amplify the insertion sequence IS1081, present in the *M. bovis* genome in 5-6 copies instead of IS6110 (only 1-3 copies). Moreover, the IS6110 PCR has a greater sensitivity when detecting *M. microti* rather than *M. bovis* because of the difference in terms of numbers of copies of the insertion sequence between the two genomes.

Presence of inhibitors: substances present in the samples that inhibit Taq DNA polymerase activity, can cause false negative results. These inhibitors can come either from the specimen itself (*e.g.* heme) or from reagents used during DNA extraction (*e.g.* SDS, urea, ethanol). PCR tests should include internal controls in order to assess the efficacy of each amplification reaction and to identify those samples that are inappropriate for PCR or that require further manipulation to remove inhibitors. Their use will ultimately increase confidence in the reliability of negative results.

Specificity

PCR analytical specificity is usually evaluated by testing the chosen specific primers on mycobacterium species found in the environment or potentially present in animal samples. If the primers are correctly designed on a specific sequence, the analytical specificity should be 100%.

Factors influencing Sp include:

PCR Target: The selection of the target determines the species-specific level of PCR. Because of the high homology between the *M. tuberculosis*-complex members, the PCR target is usually specific for the *M. tuberculosis*-complex (*i.e.* IS6110, IS1081, etc.); alternatively it can be specific for the genus *Mycobacterium* (i.e. 16S rDNA).

Primer design: selection and design of the amplification primers is a critical point for PCR specificity. They have to be chosen to prevent cross-pairing to other sequences and must to be checked with BLAST (basic local alignment search tool) software (http://www.ncbi.nlm.nih.gov/BLAST/), otherwise the risk of misdiagnosis is increased.

Cross contamination: PCR can detect very low numbers of the target sequence. At the same time a very low level of cross contamination can lead to false positivity. Another problem is the occurrence of false-positive reactions caused by contaminating amplicons, since each amplicon can serve as a template for subsequent PCRs. Considerable effort has been devoted to devising ways to limit amplicon carryover.

PCR strategy: The nested PCR shows an increased specificity compared to standard PCR methods but, due to its highly increased sensitivity and the required additional amplification step, sample contamination easily occurs. Real-time PCR is more specific thanks to the third oligo used in this type of reaction. Moreover Real-time allows direct monitoring of amplicon accumulation during the PCR process, and it combines amplification, detection, and quantification in a single step, thereby eliminating the need for post-PCR processing with a subsequent decrease of the risk of amplicon contamination.

Repeatability and reproducibility

There are no data available on repeatability and reproducibility of a PCR test in deer. The test however was evaluated for its reproducibility in the detection of *M. tuberculosis*-complex in humans and such data indicated wide variations in the results (Noordhoek et al. 1996).

Generally, variability in results has been attributed to the low copy number of the target sequence per bacillus combined with a low number of bacilli. Variability has also been attributed to decontamination methods, DNA extraction procedures, techniques for the elimination of polymerase enzyme inhibitors, internal and external controls and procedures for the prevention of cross-contamination.

Practicality

Nowadays, most laboratories are equipped with the specific equipment and trained personnel necessary to perform a PCR test. Special care should be taken to avoid contamination and specific operating procedures should be applied. In addition to internal and external controls, an organization of the workflow based on four rooms would be optimal.

3.3.1.2.2 *Quantitative evaluation*

Sensitivity

Prior expert information about sensitivity of PCR has been provided by 6 experts.

Data from literature comprises of 4 estimates, involving a total number of 72 animals, published in 3 papers (see Table 47 - references with the Ref.Id. 312 476 1001).

Using the literature data, the impact of the following variables: *species, region* and*, bias* on sensitivity could be investigated using logistic regression analysis (see Table 56 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 8 - Sensitivity estimates of PCR based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.815	0.334	0.919
Estimate 2A - Systematic Literature review - unadjusted estimates	0.889	0.793	0.951
Estimate 2B - Systematic Literature review - adjusted estimates	0.894	0.806	0.951
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.869	0.806	0.917

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

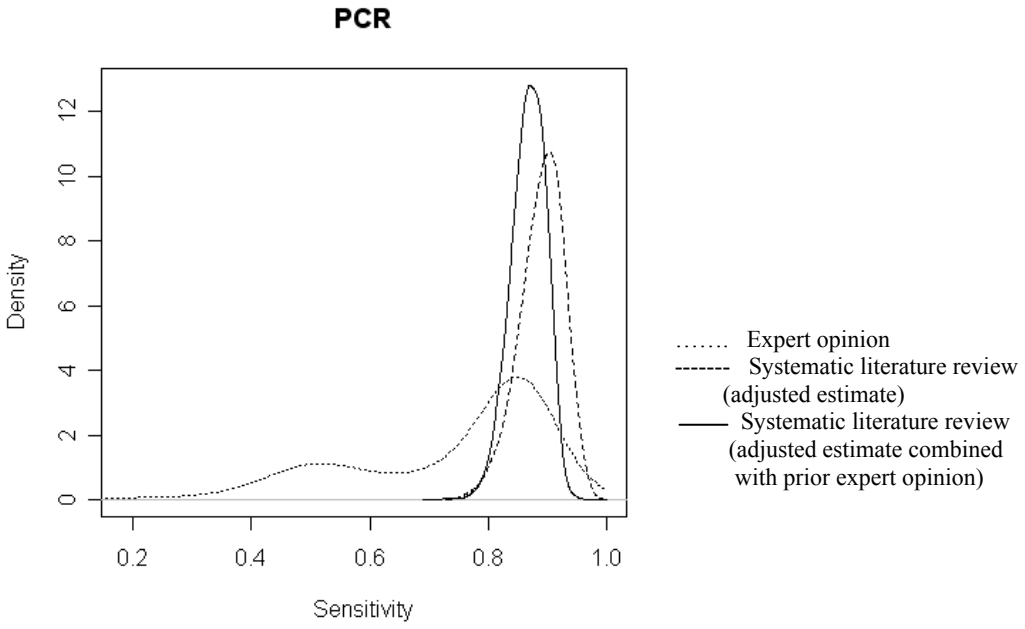


Figure 5 - Probability density distributions representing the information about sensitivity of PCR.

Specificity

Prior expert information about specificity of PCR has been provided by 7 experts.

Data from literature comprises of 1 estimate, involving a total number of 53 animals, published in 1 paper (see Table 47 - references with the Ref.Id. 279).

Using the literature data, it was not possible to investigate the impact of any of the study variables on specificity using logistic regression analysis (see Table 57 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 9 - Specificity estimates of PCR based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.917	0.345	0.999
Estimate 2A - Systematic Literature review - unadjusted estimates	1.000	0.933	1.000
Estimate 2B - Systematic Literature review - adjusted estimates	1.000	0.985	1.000
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.995	0.941	1.000

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.
Only one estimate available from literature

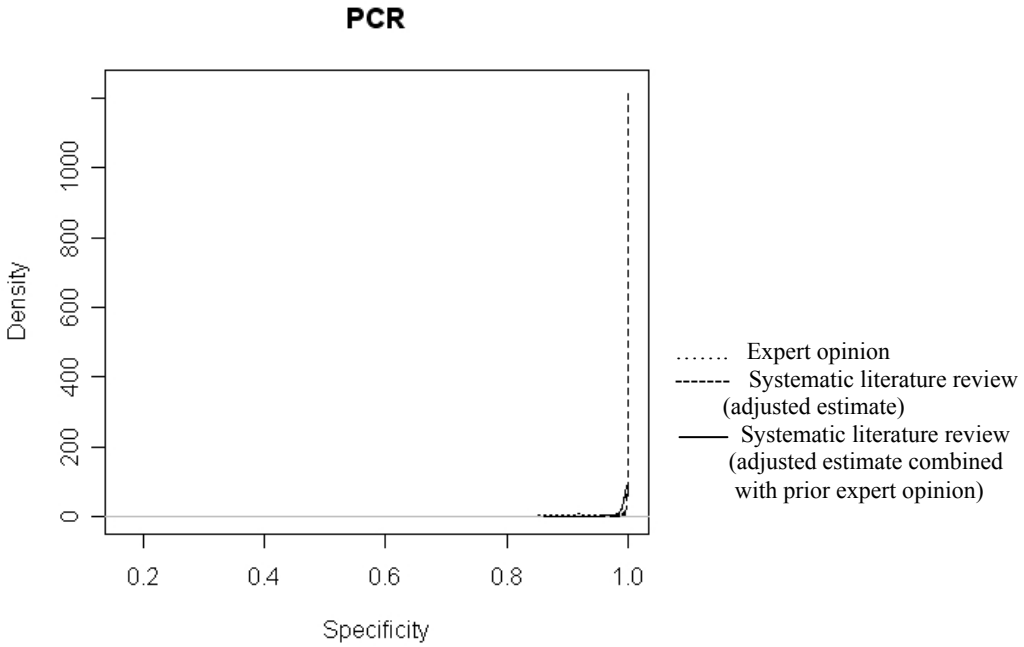


Figure 6 - Probability density distributions representing the information about specificity of PCR.

3.3.2. Indirect Identification of TB agent

Introduction

The indirect identification of tuberculosis in deer is based on the detection of the immune response of the infected animal directed against the infectious agent, *Mycobacterium bovis* or another member of the *M. tuberculosis*-complex. In general, the immune response in the first phase of infection is characterised by a predominant cell mediated immune response (CMI) (see also Figure 3 in Section 3.1). The second phase, also called the "anergic" phase because a loss of a detectable reactivity to the tuberculin tests, resulting in false negatives in the intradermal test and in which the immune response is dominated by the formation of antibodies.

As a result two groups of indirect tests are available for the detection of infection: those detecting a CMI response: the comparative intradermal tuberculin test (SICCT), the single intradermal tuberculin test (SST), the g-IFN-test (GINT) and the lymphocyte proliferation test (LCT) and those detecting antibody responses: the enzyme linked immuno-assay (ELISA) and the more recently developed rapid test (RAPID). Using both groups of tests in parallel obviously has the advantage of offering the possibilities of detecting animals within an infected herd in different stages of infection.

Moreover, as a result of the tuberculin intradermal test, the antibody levels in TB infected animals against will rise sharply within 2-4 weeks after the injection of the PPD's (purified protein derivative). This so called anamnestic rise will therefore result in an improvement of the detection of infected animals using antibody based tests. Further, emphasizing the possibilities of using different diagnostic tests capable of detecting animals in different phases of TB infection (Griffin and Mackintosh; 2000, Waters et al., 2005; see also ELISA). In all of the above tests antigens are used for the indirect detection of the infection, either for the detection of the CMI response or the detection of circulating antibodies. As a consequence, the diagnostic quality of the antigens and their standardization is crucial for the test performance.

3.3.2.1. Antigens

3.3.2.1.1 *Mycobacterial antigens for diagnostic use*

All available indirect diagnostic tests depend on the availability and the use of soluble mycobacterial antigens, either to measure the presence of specific antibodies in the infected host using an ELISA or to stimulate the cell mediated immune response, *in vivo* using the tuberculin intradermal test (SST, SICCT) or *in vitro* using the gamma-interferon test (GINT) or the lymphocyte stimulation test (LCT).

The first mycobacterial antigens were produced by Robert Koch (Koch, 1891) from cell-free, culture filtrates of mycobacterial cultures, concentrated by boiling, as the so-called "Tuberkulose Heilmittel", for the treatment of tuberculosis. These attempts were abandoned, but soon it was shown (Koch, 1891) to be a very useful as diagnostic tool. Koch noted an exaggerated reaction, nowadays known as the "Koch phenomenon", that appeared when living or killed *M. tuberculosis* bacteria were inoculated into the skin of previously infected guinea pigs. Koch also observed a similar reaction when his antigen preparation was injected subcutaneously into humans suspected of being infected with *M. tuberculosis*. Non-infected persons did not display such a response. Therefore it was possible to use this first antigen preparation, or "Koch's Old Tuberculin (KOT)" as it is called, for diagnostic purposes and subsequent investigators introduced this intradermal test or "Mantoux reaction" as a routine diagnostic procedure for tuberculosis in humans as well as in livestock.

3.3.2.1.2 *The PPD - tuberculins*

Even though different variations of KOT, mainly the heat concentrated synthetic medium (HCSM) tuberculin produced from *M. tuberculosis* until 1975, have been in use in MS into the 1960's, already in the 1930's a major improvement in the quality of the antigen preparation was introduced by the work of Florence Seibert (Seibert and Munday, 1932; Seibert, 1934; Seibert and Glenn, 1941).

The work was started as an attempt to identify 'the active principle of tuberculin" using dialysis and different precipitation methods (Seibert and Long, 1926). *Mycobacterium tuberculosis* was cultured on a synthetic medium, heat killed by steam and replacing Koch's hours long boiling method for the concentration of the antigen by an ammonium sulphate precipitation methods resulted in 104 grams of the first (and still available) standard of human tuberculin PPD-Standard or PPD-S (Seibert and Glenn, 1941). Adjusting the precipitation method by using a trichloroacetic (TCA) precipitation method the International Standard for avian PPD was produced from *M. avium* D4ER (Weybridge,1954) and for bovine PPD from *M. bovis* AN5 (Lelystad, 1984). At present all standards are deposited at National Institute for Biological Standards and Control (NIBSC; http://www.nibsc.ac.uk/), from where they can be obtained by National reference laboratories and PPD producers for the standardization and control of the respective PPD's.

The actual production method developed by Seibert in the 30's has not been changed significantly since the 1940's after the introduction of the changes by Green (1946) of e.g. a centrifugation step, allowing a large scale production procedure.

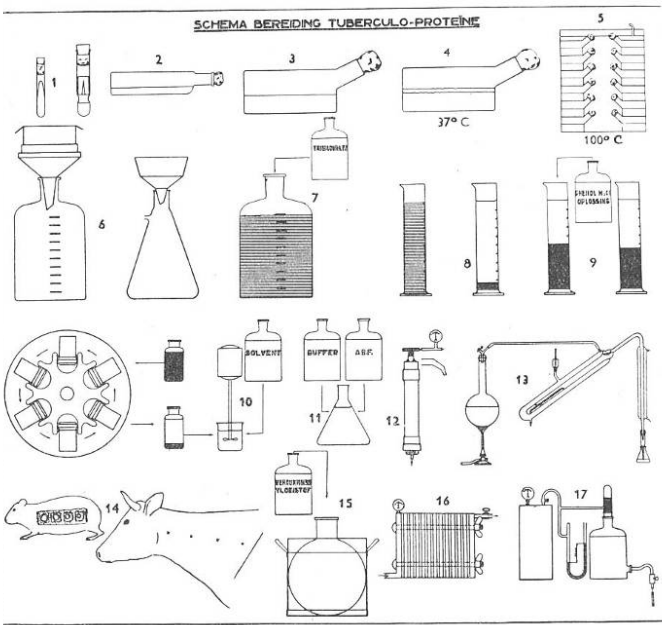


Figure 7 - A schematic representation of the tuberculin production and quality control (1954, CVI, The Netherlands)

A schematic representation of the production (1954) process is given in Figure 7 and remains almost identical worldwide.

One of the remaining differences between producers is the use of either an ammonium sulphate or a trichloroacetic acid precipitation.

Despite an identical production procedure, the bioactivity or potency as measured in the guinea-pig test of the commercially available PPD's is highly variable, in particular for the bovine PPD (Haagsma and Eger, 1997).

For this variability, a number of reasons can be responsible. One of the main reasons could be due to the fact that, because the amount of International Standard PPD is limited, all producers use a local standard derived from this International Standard.

However, because of the biological variation in the guinea pig test, the European Pharmacopoeia allows significant margins in the testing results of the PPD's: for bovine PPD the estimated potency must not be less than 66% and not more than 150% of International Standard, and for avian PPD the estimated potency has to between 50% and 200% of the International Standard. Since the results for the potency testing of the same batch of PPD are variable not only between laboratories, but also highly variable between different guinea pig trials of the same batch in the same laboratory (EU, 1979) , this will have resulted in a large variation between the local standards that are in use. The difference in potency test results can be 100% or higher (EU, 1979). Subsequent standardization of new batches using the above mentioned standards allows for the same large margins and will have the same test variability, resulting in an even larger variation in the final product. The fact that the guinea-pig test show a larger variation than the same test done in cattle (EU, 1979; Haagsma and O'Reilly, 1997) does not account for the large variation observed between batches and is likely to have consequences for use in field situations.

Other than the variability caused by the test procedures, the production strains themselves could be a cause of the problem as well. The European Pharmacopoeia, as well as EU legislation (64/432/EC, and Annex B.2.2.) allows for the use of any *M. bovis* strain for production of bovine PPD as long as the potency test are done in guinea-pigs infected with live *M. bovis* AN5. Within the EU bovine PPD is indeed produced using other strains than *M. bovis* AN5, but

is also tested using this other strain (Bakker, pers. observations)

This combined with the fact that the respective production strains *M. avium* D4ER and *M. bovis* AN5 have been in use since the late 1940's for production. Until the recent introduction of better quality control systems, these strains were transferred onto new media for decades without going back to the original stock, most likely resulting in strains diverged highly from their origin as well as from each other between laboratories and having different expression profiles of their antigens. Identical situations have been described for *M. bovis* BCG, the human TB-vaccine strain (Mostowy et al., 2003) as well as for *M. paratuberculosis* 316F the Johne's disease vaccine strain (Thibault et al., 2007).

3.3.2.1.3 *Other antigens: cell-free extracts, recombinant proteins and synthetic peptides*

Despite the fact that the PPD's are no longer heat concentrated antigens, the bacterial mass from which they have been prepared still has been steam treated for 3 hours to kill the bacteria. In attempts to use less denatured antigens in the immunological tests, several other approaches have been used. Extracts were prepared from live bacteria using different methods (e.g. de Bruyn et al., 1987; Hall and Thoen, 1983) and For biosecurity reasons working with large volumes of live *M. bovis* posed difficulties and most of the work was restricted to the attenuated *M .bovis* BCG (e.g. Harboe et al., 1986).

The introduction of the recombinant DNA technology in the late 1970's and 1980's allowed for the cloning and production of recombinant antigens of mycobacteria. One of the first *M. bovis* specific antigens to be cloned was MPB70 (Radford et al., 1988) a sero-reactive antigen, which is together with its genetic sibling MPB83 (Hewinson et al., 1996) which was discovered later, still one of the most widely used bovis antigen in TB serology.

Since the host immune response is dominated by the cell mediated component, the characterization and cloning of the *M. tuberculosis*-complex secreted antigens ESAT-6 (Sorensen et al., 1995) and CFP10 (Berthet et al., 1998) both having high T-cell reactivity in TB infected individuals. Both are therefore widely used in cattle, non human primates and humans as stimulating agents in the gamma-interferon assays (GINT) used worldwide. Because of the cost involved in the production of such recombinants, their use *in vivo* tests, e.g. the intradermal test, is not at present a viable option.

More recently, a new group of antigens have been introduced: the synthetic peptides. Since recombinant proteins are mostly produced in *E. coli*, they will contain variable amounts of lipopolysaccharide (LPS) or endotoxin (Franken et al., 2000) which can not always be completely removed and which will interfere with immunological tests, in particular the CMI directed ones. Since the production of synthetic peptides is becoming more affordable, they have been introduced as an alternative for recombinant proteins (Cockle et al., 2006) and the first results look very promising.

3.3.2.2. Tests based on the detection of Cell Mediated Immune response

3.3.2.2.1 *Comparative intradermal tuberculin test (SICCT)*

3.3.2.2.1.1 Qualitative evaluation

Introduction

The standard method, and the only primary test prescribed in the EU legislation, for the detection of bovine tuberculosis is the intradermal tuberculin test. In this case the test is performed as a comparative intradermal test using both bovine and avian tuberculin PPD.

Definition

The way the testing is performed, preparations of the PPD tuberculins, the strength or the potency of the PPD's to be used, as well as the interpretation of the results are described for cattle in the EU trade legislation (64/432/EC, and Annex B.2.2.) as well as in the O.I.E. manual (2004) and these guidelines are used for most other animal species as well, including deer.

Description of test

The comparative intradermal tuberculin test or in full, the single intradermal comparative cervical test (SICCT), is performed by injecting 0.1 ml of avian and bovine tuberculin into two different injection sites in the skin of the neck.

The injection sites must be clipped and cleansed. A fold of the skin at each clipped area is measured with calipers and the injection site is marked prior to injection. Because of the very thin skin of cervids, the injection of the dose of tuberculin has to be performed with great care and the use of disposable syringes with a very fine needle (26G) is recommended. The correct injection is confirmed by palpating a small swelling to make sure that the injection has not been sub-cutaneous. The injection sites have to be at least 12-15cm apart, in young animals or when one of the injections was not performed correctly and has to be repeated, the second dose can be applied on the other side of the neck. An excellent description of the test is given by Griffitths (1990).

The potency of the PPD's used should be at least 2000 International Units per dose. In some countries, the same doses are given in mg per ml, in which a dose of 0.1 ml bovine PPD containing 0.1 mg corresponds with 2000 I.U., or for avian PPD a dose of 0.1 ml containing 0.04 or 0.05 mg corresponds with 2000 I.U..

Using calipers, the same person who performed the first measurement, the thickness of the skin-fold at each injection site is measured gain 72 hours later. Clinical signs, such as oedema, exudation, necrosis, pain or inflammation at the injection region should be recorded.

In Figure 8 a standard interpretation of the results of the measurements of the increase in skin thickness is shown.

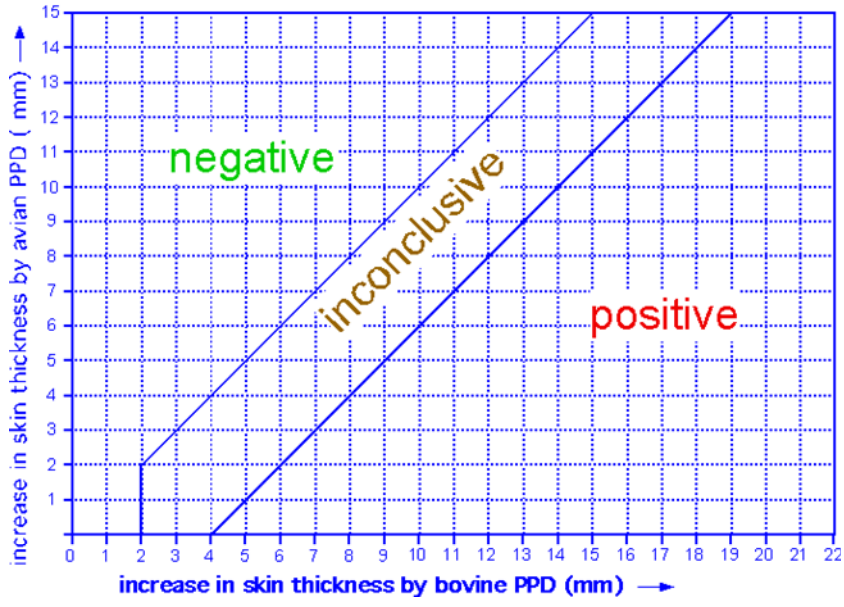


Figure 8 - A standard interpretation in bovines of the comparative tuberculin intradermal test.

Briefly, the reaction in the comparative test is regarded to be positive if the increase in skin

thickness at the bovine injection site is 4 mm greater than the reaction at the avian injection site. The intradermal test is regarded to be inconclusive if the increase in thickness of the skin-fold at the bovine injection site is 1 to 4 mm greater than at the avian injection site and regarded to be negative if the increase in thickness at the bovine injection site is equal or smaller than at the avian injection site. A severe interpretation of the comparative test would mean that the inconclusive animals are removed directly from the herd for slaughter (see Figure 9).

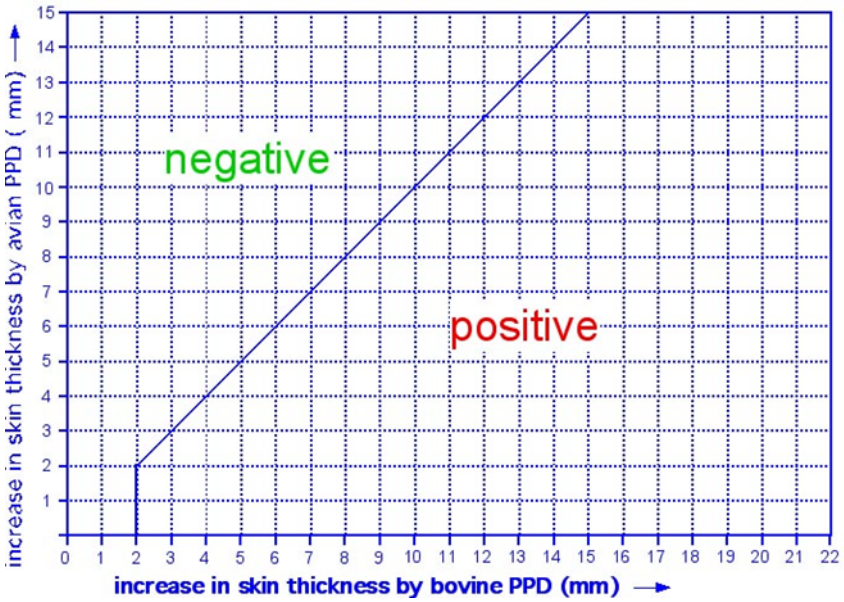


Figure 9 - Severe interpretation in bovines of the comparative tuberculin intradermal test.

In the severe interpretation of the intradermal test, as can be seen in the graph, the outcome of the intradermal test will compensate less for a non-specific or avian sensitization. As a logical result, the sensitivity of the test will increase, but specificity will decrease. Removal of non-specific reactors could be expensive and is therefore only to be used in a final stage of eradication or maintaining freedom of disease. In a holding, known to be infected, this approach will accelerate (re-) gaining freedom of disease.

Otherwise, the inconclusive animals (cattle) should be retested after at least 42 days, if not tested negative, the animals are regarded as positive and removed directly from the herd for slaughter.

However, reports indicate that in contrast to observations with the intradermal test in cattle, the period between repetitive intradermal test in deer should be at least 60 and preferably 90 days to prevent the outcome of the intradermal test being impaired by the previous intradermal test (e.g. Corrin et al., 1987). Also Griffin and Cross (1989) referred to a 60 days period suppression for SICCT. The authors are citing work by Carter et al. (1984, 1985). In the same study no suppression was found at 120 days and this period is suggested as the minimum interval between two tests.

Some specifications from the literature on the performance of SICCT in different deer species:

- Red Deer (*Cervus elaphus*): While the SICCT produces increased levels of specificity, its sensitivity is lower when used under field conditions (Kollias *et al.*, 1982; Beatson *et al.*, 1984: Griffin and Mackintosh, 2000). Whereas the SICCT used under controlled conditions in experimentally infected deer has a sensitivity of >91% when testing intervals are greater than 60 days (Corrin *et al.*, 1993), its use under field conditions has produced sensitivity values ranging from 31% to 90% (Kollias et al., 1982) (Griffiths, 1988) (Stuart et al., 1988). The overall performance of intradermal tuberculin testing appears to be influenced

markedly by the stage of disease. Experience in experimentally infected deer, has shown that intradermal intradermal testing (SST or SICCT) is extremely sensitive in diagnosing infection from six to 24 weeks post-infection (Griffin *et al.*, 1994).

- White-tailed Deer (*Odocoileus virginianus*): The comparative cervical intradermal test for antemortem diagnosis of tuberculosis was done 169 times on 116 different white-tailed deer of known *Mycobacterium bovis* infection status. The sensitivity and specificity were 97 and 81%, respectively. The magnitude of change in skin thickness was significantly greater in deer infected for less than 109 days than in deer infected for more than 109 days (Palmer et al., 2001).
- Reindeer (*Rangifer tarandus*): Identification by comparative cervical intradermal test of reindeer truly infected with *M. bovis* is feasible using current guidelines. It is very sensitive but has low specificity. Use of the modified scattergram for reindeer or the scattergrams for cattle and bison decreased the number of false-positive intradermal test results (Palmer et al., 2006).

Sensitivity

Factors influencing Se:

The sensitivity of SICCT may be markedly influenced by the stage of disease. It may be highly sensitive (80%) within the first 6 months following infection but has reduced sensitivity in chronically diseased animals (Griffin and Mackintosh, 2000).

Specificity

Factors influencing Sp:

The specificity of the SICCT will be influenced by the sensitization of deer following exposure to saprophytic mycobacteria or infection by other mycobacteria *M. avium* (de Lisle and Havill, 1985; Quigley et al., 1997) or, increasingly, by *M. paratuberculosis* (Mackintosh, 1998).The specificity problem is likely to be less than with the SST. However, for cattle the problem has been noticed for e.g. in the Republic of Ireland. To compensate for this non-specific response small changes have been made in the potency of the PPD tuberculins that are being used: a bovine PPD of 3000 I.U. per dose and an avian PPD of 2500 I.U. have been introduced.

Repeatability and reproducibility

There are no data available on repeatability or reproducibility of the comparative intradermal tuberculin test in deer. Is is likely, however, that the reproducibility will depend on the skills and accuracy of the operator including the instrument used for measuring the thickness of the skin.

Practicality

Performing the comparative intradermal tuberculin test requires the animals to be captured and to be immobilised in a so-called crush twice with a 72 hour interval.

However, provided the operators are well-trained, the comparative intradermal test is a test that can be easily standardised for use in a in a field situation.

3.3.2.2.1.2 Quantitative evaluation

Sensitivity

Prior expert information about sensitivity of SICCT has been provided by 7 experts.

Data from literature comprises of 26 estimates, involving a total number of 901 animals,

published in 13 papers (see Table 47 - references with the Ref.Id. 144 180 237 315 344 347 349 352 354 508 1007 1010 1019).

Using the literature data, the impact on sensitivity of the following variables: *species, gs, region, dpi3, rep* and *bias*, could be investigated through logistic regression analysis (see Table 58 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 10 - Sensitivity estimates of SICCT based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.730	0.416	0.940
Estimate 2A - Systematic Literature review - unadjusted estimates	0.859	0.835	0.881
Estimate 2B - Systematic Literature review - adjusted estimates	0.859	0.837	0.882
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.858	0.834	0.880

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

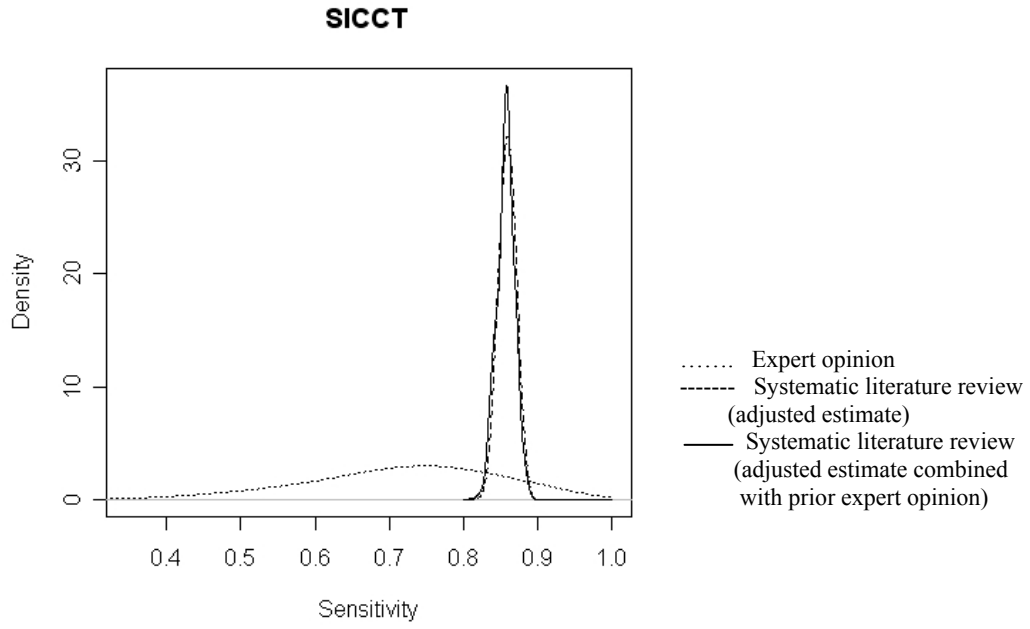


Figure 10 - Probability density distributions representing the information about sensitivity of SICCT.

Specificity

Prior expert information about specificity of SICCT has been provided by 7 experts.

Data from literature comprises of 11 estimates, involving a total number of 3443 animals, published in 7 papers (see Table 47 - references with the Ref.Id. 344 347 349 352 354 1007 1019).

Using the literature data, the impact of the following variables *species, gs* and *rep* on specificity could be investigated using logistic regression analysis (see Table 59 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 11 - Specificity estimates of SICCT based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.938	0.443	0.994
Estimate 2A - Systematic Literature review - unadjusted estimates	0.978	0.972	0.982
Estimate 2B - Systematic Literature review - adjusted estimates	0.978	0.972	0.982
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.977	0.972	0.982

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

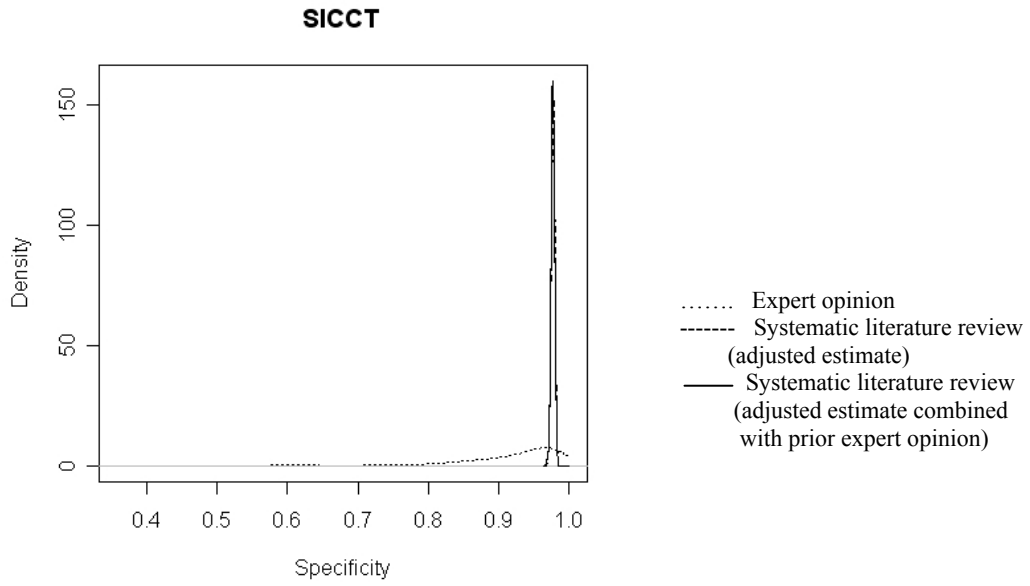


Figure 11 - Probability density distributions representing the information about specificity of SICCT.

3.3.2.2.2 *Single tuberculin intradermal test (SST)*

3.3.2.2.2.1 Qualitative evaluation

Introduction

The standard method, and the only primary test prescribed in the EU legislation, for the detection of bovine tuberculosis in cattle is the intradermal tuberculin test. In this case the test is performed using bovine tuberculin PPD alone.

Definition

The way the testing is performed, preparations of the PPD tuberculins, the strength or the potency of the PPD's to be used, as well as the interpretation of the results are described for cattle in the EU trade legislation (64/432, and Annex B.2.2.) as well as in the O.I.E. manual (2004) and these guidelines are used for most other animal species as well, including deer.

Description of test

The single intradermal tuberculin test or mid cervical test (SST) is performed by intradermal injection of 0.1 ml of bovine PPD tuberculin in the side of the neck.

The injection site must be thoroughly clipped carefully without injuries to the skin and cleansed. A fold of the skin at each clipped area is measured with calipers and the injection site is marked prior to injection. Because of the very thin skin of cervids, the injection of the dose of tuberculin has to be performed with great care and the use of disposable syringes with a fine needle (26G) is recommended. The correct injection is confirmed by palpating a small swelling to make sure that the injection has not been sub-cutaneous. An excellent description of the test is given by Griffitths (1990).

The potency of the PPD used should be at least 2000 International Units per dose. In some countries, the same doses are given in mg per ml, in which a dose of 0.1 ml bovine PPD containing 0.1 mg corresponds with 2000 I.U..

Using calipers, the same person who performed the first measurement, the thickness of the skin-fold at the injection site is measured again 72 hours later. Clinical signs, such as oedema, exudation, necrosis, pain or inflammation at the injection region should be recorded.

As for the SICCT, the interpretation of the results of the SST are described for cattle in the EU trade legislation (64/432/EEC, and additions) as well as in the O.I.E. manual and these guidelines are used for most other animal species as well, including deer.

Briefly: the interpretation of the SST is based on the increase in skin thickness at the injection site of the bovine PPD: if the increase in skin thickness is less than 2 mm and no clinical signs are observed the animal is regarded to be negative. The reaction is regarded to be inconclusive if the increase in skin-fold thickness is between 2 and 4 mm and no clinical signs can be observed. The reaction is positive if the increase in skin-fold thickness is greater than 4 mm or if any of the clinical signs can be observed. The interpretation of the single intradermal test is shown in Figure 12.

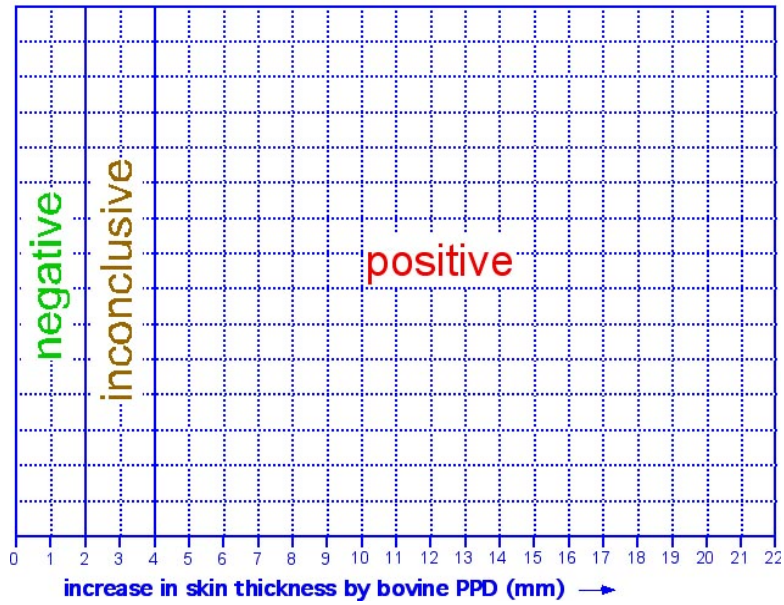


Figure 12 - A standard interpretation of the single intradermal test as used in bovines

Inconclusive animals should be retested after at least 42 days, if not tested negative, the animals are regarded as positive and removed directly from the herd for slaughter. Retesting can be performed by either comparative intradermal test or the single intradermal test according to local regulations.

However, reports indicate that in contrast to observations with the intradermal test in cattle, the period between repetitive intradermal test in deer should be at least 60 and preferably 90 days to prevent the outcome of the intradermal test being impaired by the previous intradermal test

(e.g. Corrin et al., 1987). Also Griffin and Cross (1989) refered to a 60 days period suppression for SICCT. The authors are citing work by Carter et al. (1984, 1985). In the same study no suppression was found at 120 days and this period is suggested as the minimum interval between two tests.

Sometimes a more stringent interpretation is used e.g. in areas at risk, any measurable or palpable reaction is regarded as positive. In areas, close to eradication of bovine tuberculosis or maintaining their TB-free status, e.g. the Netherlands, the single intradermal test is using a high injection dose of 5000 I.U. to remove the last remaining infected animals or rapidly removing the last remaining reactors to maintain the TB-free status, respectively.

Since, the single intradermal test is based on the use of the bovine PPD only, in both the standard and severe interpretation, it has a significantly higher sensitivity than the comparative intradermal test, but at the same time a significantly lower specificity, limiting its use for surveillance programmes.

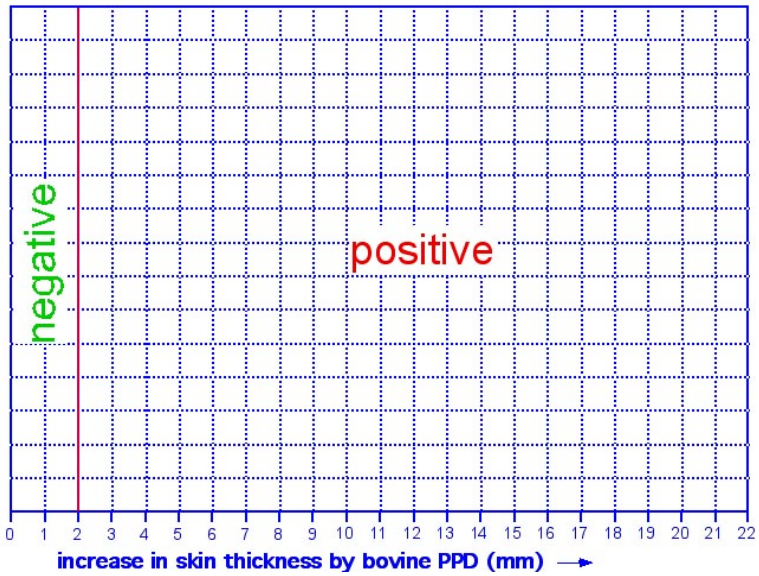


Figure 13 - A severe interpretation of the single intradermal test as used in bovines

The available data mostly originates from New Zealand in the development of diagnostic strategies for the control of outbreaks of bovine tuberculosis in farmed deer.

In these trials the single mid-cervical intradermal test (SST), was initially widely used and was shown to have a sensitivity in red deer (*Cervus elaphus*) of approximately 80% for TB diagnosis in *deer,* but resulted in high numbers of false positive reactions. Therefore the use of the SST was abandoned (Corrin et al., 1987; Griffin and Buchan, 1994; Griffin et al., 2000; Griffin et al., 2004).

Sensitivity

Factors influencing Se:

The sensitivity is markedly influenced by the stage of disease, at a late stage of infection (anergic phase) no measurable CMI response could remain.

Specificity

Factors influencing Sp:

Using the single intradermal intradermal test, major problems will occur due to the poor specificity and low positive predictive value of the test. In New Zealand, more than 80% of

skin-test reactor deer do not show any evidence of infection with *M. bovis* (Carter, 1995).

Sensitization of deer following exposure to saprophytic mycobacteria or infection by other mycobacteria: *M. avium* (de Lisle and Havill, 1985; Quigley et al., 1997) or, increasingly, by *M. paratuberculosis* (Mackintosh, 1998), is most likely the cause of this problem.

Repeatability and reproducibility

There are no data available on repeatability or reproducibility of the single intradermal tuberculin test in deer. Is likely, however, that the reproducibility will depend on the skills and accuracy of the operator including the instrument used for measuring the thickness of the skin (callipers).

Practicality

Performing the single intradermal tuberculin test requires the animals to be captured and to be immobilised in a so-called crush twice with a 72 hour interval. However, provided the operators are well-trained, the single intradermal test is a test that can be easily standardised for use in a in a field situation.

In addition, the test is easier to use than the comparative test, since often no increase in skin thickness is measured: all reactors are regarded as positive.

3.3.2.2.2.2 Quantitative evaluation

Sensitivity

Prior expert information about sensitivity of SST has been provided by 10 experts.

Data from literature comprises of 20 estimates, involving a total number of 1634 animals, published in 11 papers (see Table 47 - references with the Ref.Id. 60 137 166 175 217 258 276 315 1008 1014 1020).

Using the literature data, the impact of the following variables: s*pecies, gs, region, dpi3, rep* and *bias* on sensitivity could be investigated through logistic regression analysis (see Table 60 in Appendix B). The results indicate that the gold standard 'experimental' is negatively associated with Se. The results do not indicate an effect of the stage of infection.

Table 12 - Sensitivity estimates of SST based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.802	0.478	0.926
Estimate 2A - Systematic Literature review - unadjusted estimates	0.823	0.803	0.841
Estimate 2B - Systematic Literature review - adjusted estimates	0.823	0.804	0.840
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.823	0.804	0.840

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

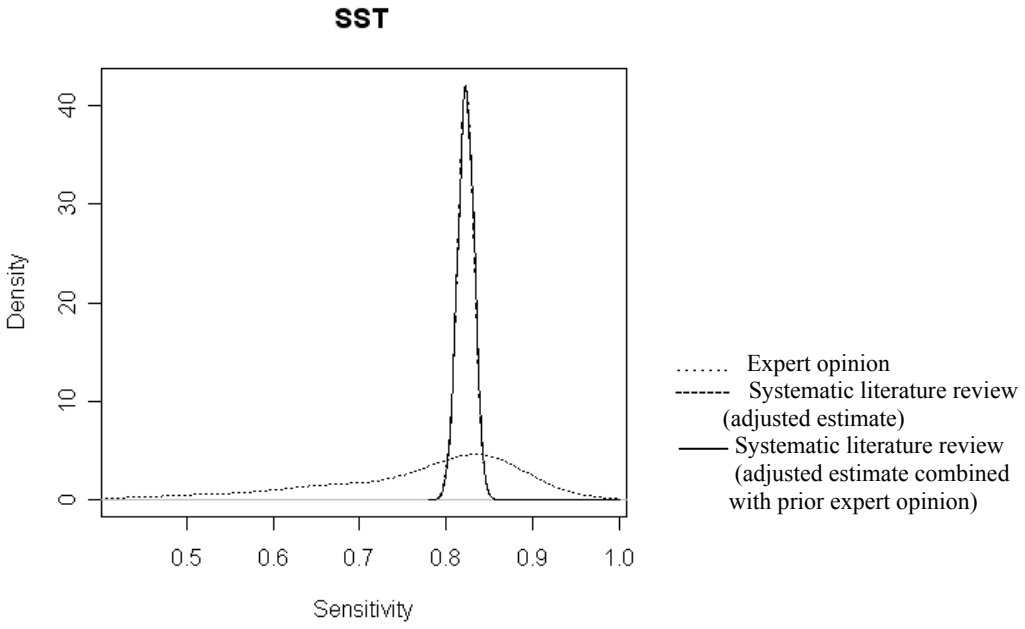


Figure 14 - Probability density distributions representing the information about sensitivity of SST.

Specificity

Prior expert information about specificity of SST has been provided by 5 experts.

Data from literature comprises of 13 estimates, involving a total number of 2277 animals, published in 8 papers (see Table 47 - references with the Ref.Id. 60 166 175 258 274 276 1008 1020).

Using the literature data, the impact of the following variables: *species, gs, region* and *rep* on specificity could be investigated through logistic regression analysis (see Table 61 in Appendix B). The results indicate that the gold standard 'experimental' is negatively associated with Se. The results do not indicate an effect of the stage of infection.

Table 13 - Specificity estimates of SST based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.912	0.271	0.993
Estimate 2A - Systematic Literature review - unadjusted estimates	0.760	0.742	0.777
Estimate 2B - Systematic Literature review - adjusted estimates	0.760	0.742	0.778
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.759	0.744	0.779

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

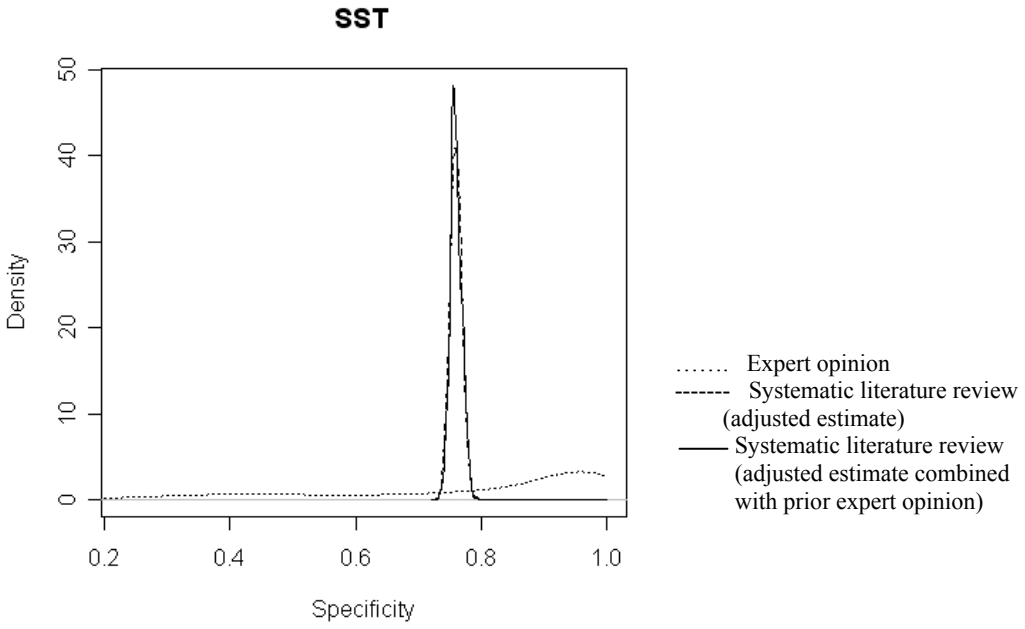


Figure 15 - Probability density distributions representing the information about specificity of SST.

3.3.2.2.3 *Gamma Interferon test (GINT)*

3.3.2.2.3.1 Qualitative evaluation

Introduction

To overcome the problems of intradermal tests sensitivity, Wood et al. described in 1990 the gamma-interferon test (GINT) as an alternative method in the diagnosis of bovine tuberculosis (Rothel et al., 1990; Wood et al., 1990; Wood et al., 1991). The GINT is an *in vitro* immunoassay based in the detection of the specific cell-mediated immune responsiveness and the measurement of the release of gamma-interferon as the indicator of a positive response to *M. bovis* antigen (bovine purified protein derivative - bovine PPD). The GINT is designed for use in specific species and can be detected using enzyme immunoassays (EIA) that are commercially available for several species: for bovines (also used for sheep, goats and red deer), for non-human primates and for cervids.

The development of the GINT during the last decade has been a major advancement in the diagnosis of bovine tuberculosis. The test used in cattle was officially accredited for the diagnosis of bovine tuberculosis in Australia in 1991, and the GINT has been also approved for use in the United States as a complementary test. In the EU countries, the possibility to use the GINT as supplementary for detection of bovine tuberculosis in the official eradication campaigns is regulated by the EU Directive 64/432 EEC amended by the Regulation (EC) 1226/2002 "… *to enable detection of the maximum number of infected and diseased animals in a holding or in a region*…". Thus, the detection of the maximum number of infected animals is achieved by the combination of SICCT and GINT.

Very little is know about performance of the GINT in cervids and most knowledge about the implementation of the technique can only be derived from experiences in cattle or goats.

Recent research on the immune response of deer against *M. bovis* using detection of mRNA or real-time RT-PCR has shown a role of the gamma-interferon. Thus, blood leukocytes from infected white-tailed deer (*Odocoileus virginianus*) expressed more gamma-interferon, interleukin-12p40 (IL-12p40), granulocyte-monocyte colony-stimulating factor, and IL-4

mRNA than did blood leukocytes from uninfected deer (Thacker et al., 2006). The RT-PCR approach was evaluated by monitoring the kinetics of cytokine mRNA synthesis induced by mitogenic and antigenic stimulation of peripheral blood mononuclear cells from *M. bovis*-infected deer (Harrington et al., 2006). The more robust mRNA expression GINT correlated with pathology early in infection. Also the vaccination with live *M. bovis* bacille Calmette Guerin (BCG) elicits a gamma-interferon response, as shown in white-tailed deer; this could be detected beginning 9 days post-vaccination (Waters et al., 2004). These findings indicate that gamma-interferon expression likely plays a role in both protection and pathogenesis (Harrington et al., 2006; Thacker et al., 2006).

Description of test

For a test the blood sample is divided in 4 aliquots that are stimulated with water/PBS (nill or control), avian PPD, bovine PPD and pokeweed mitogen (PWM), a non-specific mitogen used for maximum stimulation of the sample to test for the viability of the lymphocytes.

Therefore a minimum of 4 wells per sample are needed to perform a test. In addition to the use of the respective PPD's, the fact that only minute quantities of antigens are used for stimulation, the format of the GINT allows for the use of more specific recombinant antigens, which would be too expensive for use in a intradermal test.

In an experimental trial to validate the use of the GINT in deer, the production of gamma-interferon in white tailed deer in response to PPDb was tested and was shown to be significantly greater at all time points in samples from *M. bovis*-infected deer (n=20) as compared with uninfected control deer (n=7) (days 90-307 PI). Measurement of gamma-interferon production to PPDb may serve as a useful test for the antemortem diagnosis of tuberculosis in Cervidae (Palmer et al., 2004).

In a follow up study a larger number of different deer species were tested: Fallow deer, elk, and white-tailed deer do not respond very well (only 4% of fallow deer, 20% of elk, 44% of white-tailed deer had responses to PWM exceeding 0.25 D optical density (i.e., PWM stimulation minus no stimulation) indicating an unacceptable level of detection in each of the species. Reindeer do have acceptable responses (91% of reindeer had responses to pokeweed mitogen exceeding 0.25 D optical density). This demonstrates the validity of the gamma-interferon test for detection of TB in reindeer. Further development of the test will be required before use in surveillance programs in white-tailed deer, fallow deer, and elk. (P. Nol, pers. communication, Waters et al., Vet Record, in press)

The ability of the GINT to detect gamma-interferon produced by blood leukocytes in response to mycobacterial antigens from *M. bovis* infected reindeer has been evaluated by Waters et al., 2006. The test was evaluated in a low number (n=13) of reindeer experimentally infected intra-tonsillarly by 105 CFU. The response of infected animals was 3.5-fold higher than those by noninfected reindeer, while responses by infected reindeer to a rESAT-6-CFP-10 fusion protein were also higher and more specific. The findings indicate that gamma-interferon-based tests may prove useful for TB surveillance of white tailed deer (Palmer et al., 2004) and reindeer (Waters et al., 2006). Blood samples from trapped wild deer have been tested in Michigan and results compared to necropsy and culture (O´Brien et al., 2006); however, authors state technical problems and its ineffectiveness.

The most recent publication, although still in press (Harrigton et al.,2007), casts doubts on the use of the GINT, at this stage of development. At the moment it is difficult to assess whether this is caused by the fact that the recognition by the monoclonal antibodies is too stringent or, more likely, by the logistics problems due to sample collection/transport. Given the high homology of the different gamma-interferon molecules between species (see Figure 16) it is highly unlikely that within species a difference in molecules could occur that could be

responsible for the poor reactivity of large groups of animals. There is a high degree of homology between the gamma-interferon molecules of different species as the amino acid sequence shows. Therefore the GINT can be used in a range of different species

Furthermore, the GINT, designed for cattle has been successfully used in red deer as a test for the detection of paratuberculosis or Johne's disease (O'Brien et al., 2006), thus illustrating that handling of the samples/test could be the cause for the observed poor response.

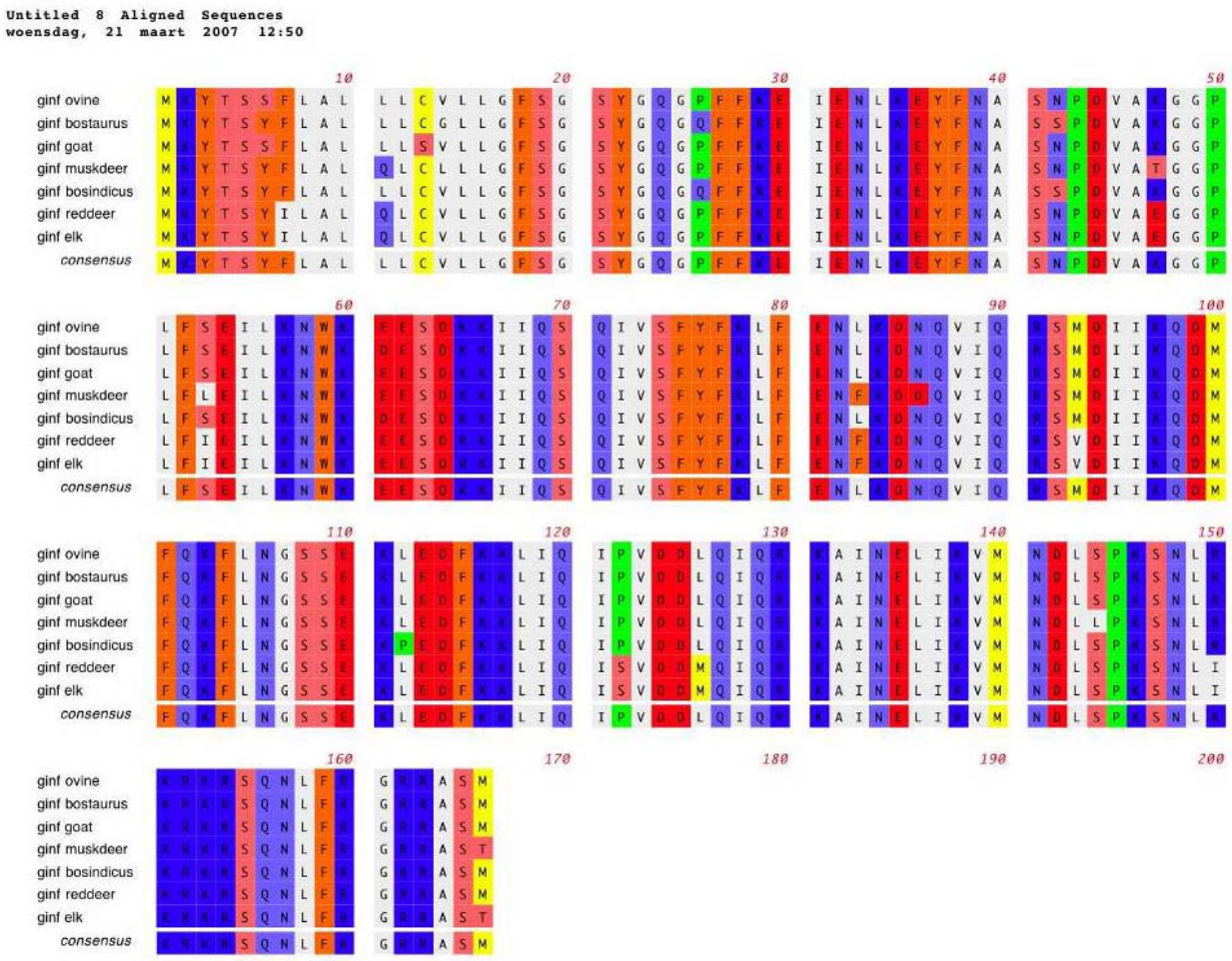


Figure 16 - As the comparison of the amino acid sequence shows, there is a high degree of homology between the gamma interferon molecules of different species (Douwe Bakker)

Sensitivity

Factors influencing Se:

As for the comparative intradermal intradermal test, the sensitivity of gamma-interferon test will be markedly influenced by the stage of disease. It may be highly sensitive within the first 6 months following infection but has reduced sensitivity in chronically diseased animals. The sensitivity could be improved in the future using a combination of specific recombinant antigens for stimulation.

Specificity

Factors influencing Sp:

As for the comparative intradermal intradermal test, sensitization of deer following exposure to saprophytic mycobacteria or infection by other mycobacteria *M. avium* (de Lisle and Havill, 1985; Quigley et al., 1997) or, increasingly, by *M. paratuberculosis* (Mackintosh, 1998) will be reducing the specificity of the test. However the specificity could be improved in the future using a combination of *M. bovis* specific recombinant antigens for stimulation.

Repeatability and reproducibility

There are no data available on repeatability or reproducibility of the gamma- interferon test in deer.

Practicality

A gamma-interferon test for use in deer would mean the availability of a well standardised and easy to use test for the detection of the cell mediated immune response of deer against tuberculosis.

However, as the case for its use in cattle: the limited time (8 hours) between taking the blood sample and performing the test, would be limiting its use in many field situations.

3.3.2.2.3.2 Quantitative evaluation

Sensitivity

Prior expert information about sensitivity of GINT has been provided by 4 experts.

Data from literature comprises of 1 estimate, involving a total number of 91 animals, published in 1 paper (see Table 47 - reference with the Ref.Id. 352).

Using the literature data, it was not possible to investigate the impact of any of the study variables on sensitivity using logistic regression analysis (see Table 62 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 14 - Sensitivity estimates of GINT based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.732	0.245	0.876
Estimate 2A - Systematic Literature review - unadjusted estimates	0.736	0.633	0.823
Estimate 2B - Systematic Literature review - adjusted estimates	0.738	0.640	0.823
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.748	0.661	0.819

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.
Only one estimate available from literature

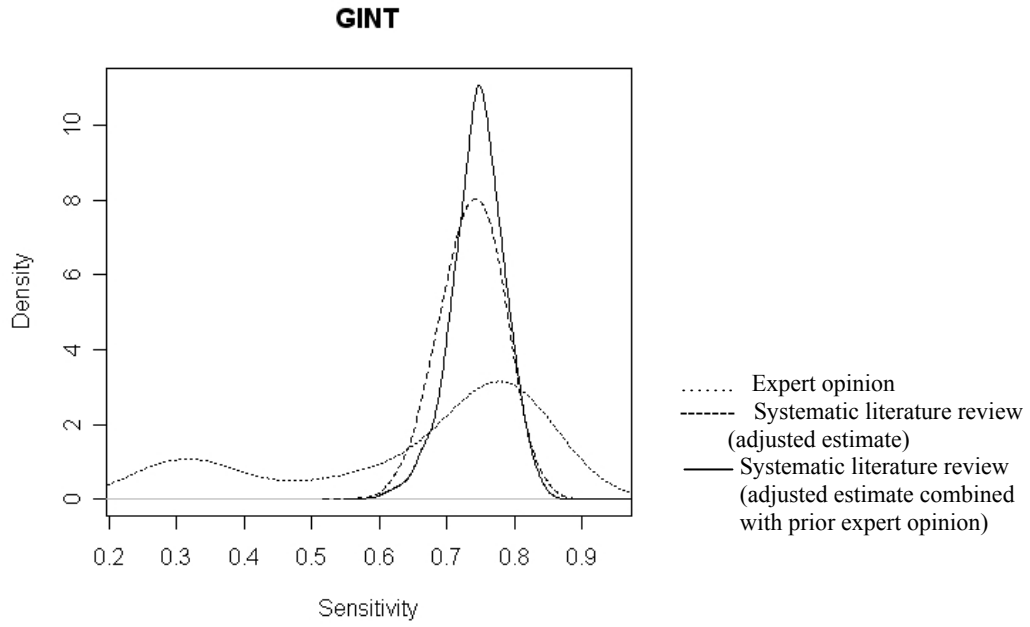


Figure 17 - Probability density distributions representing the information about sensitivity of GINT.

Specificity

Prior expert information about specificity of GINT has been provided by 3 experts.

Data from literature comprises of 1 estimate, involving a total number of 44 animals, published in 1 paper (see Table 47 - references with the Ref.Id. 352).

Using the literature data, it was not possible to investigate the impact of any of the study variables on specificity using logistic regression analysis (see Table 63 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 15 - Specificity estimates of GINT based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.913	0.359	0.976
Estimate 2A - Systematic Literature review - unadjusted estimates	0.977	0.880	0.999
Estimate 2B - Systematic Literature review - adjusted estimates	0.984	0.917	0.999
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.954	0.902	0.980

Estimate, Lower and upper refer to median, 2.5th and 97.5th percentile, respectively.
Only one estimate available from literature

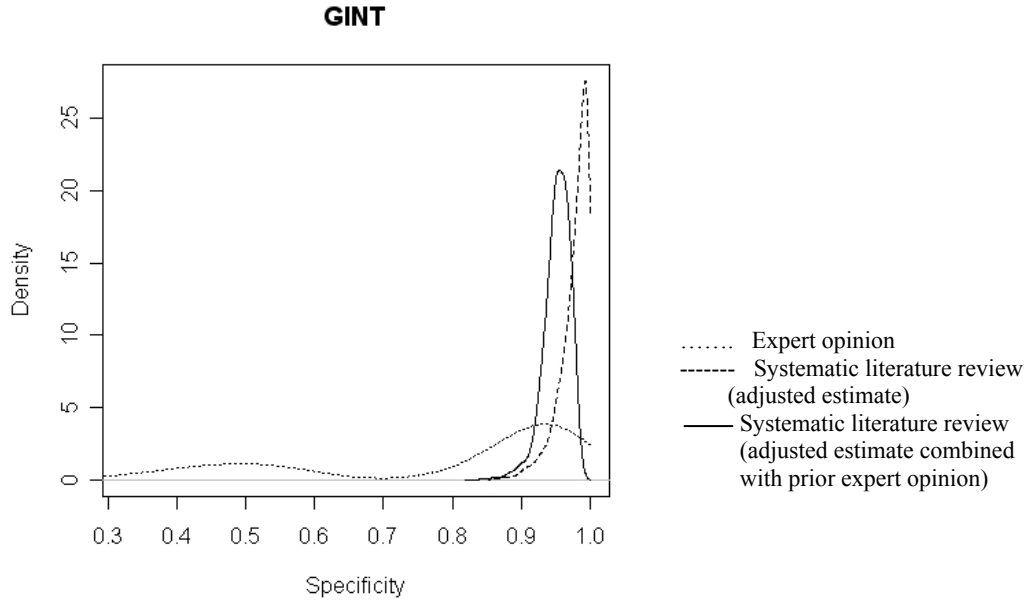


Figure 18 - Probability density distributions representing the information about specificity of GINT.

3.3.2.2.4 *Lymphocyte stimulation (LCT)*

3.3.2.2.4.1 Qualitative evaluation

Introduction

The lymphocyte stimulation test (LCT) compares the reactivity of peripheral blood monocytes, preferably from buffy coat, to stimulation with tuberculin PPD of *M. bovis* (PPD-B) and to tuberculin PPD from *M. avium* (PPD-A). The test has scientific value but is not used for routine diagnosis, because it is time consuming and the logistics and execution are complicated. Therefore, skilled operators are needed (OIE Manual, 2004).

However, since the test is not species specific, unlike the gamma-interferon test, it could be useful for the diagnosis of bovine tuberculosis in wildlife and zoo animals.

Description of test

The test uses lymphocyte proliferation after stimulation with TB specific antigens (PPD's and/or recombinant proteins) to measure sensitization in infected animals. To this purpose buffy coat fractions containing the mononuclear cells are collected. The mononuclear cells are seeded into microtiter plates and cultured for 5-6 days, during which the cells are stimulated

with bovine, avian PPD, pokeweed mitogen. Subsequently, the cells are incubated in the presence of radioactive ^{3}H-thymidine or more recent with the thymidine analog, 5-bromo-2'deoxyuridine (BrdU for 20 hours after which the incorporation of the thymidine into the mononuclear cells is measured by liquid scintillation or monoclonal antibody fluorescence labelling, respectively, and compared with a non-stimulated negative control. All tests are run in triplicates. Results are presented as stimulation indexes (mean stimulated/ mean negative control).

Final outcome of the lymphocyte stimulation test depends on the incorporation of the label in the control samples (nill stimulation and non-specific stimulation using pokeweed) as well as the relative incorporation of the label after stimulation using the respective PPD's. An example of such an interpretation of the radioactive variant of the test resulting in a negative result, a bovine tuberculosis or an avian tuberculosis/ paratuberculosis outcome is given in Figure 19 (Griffin et al., 1994).

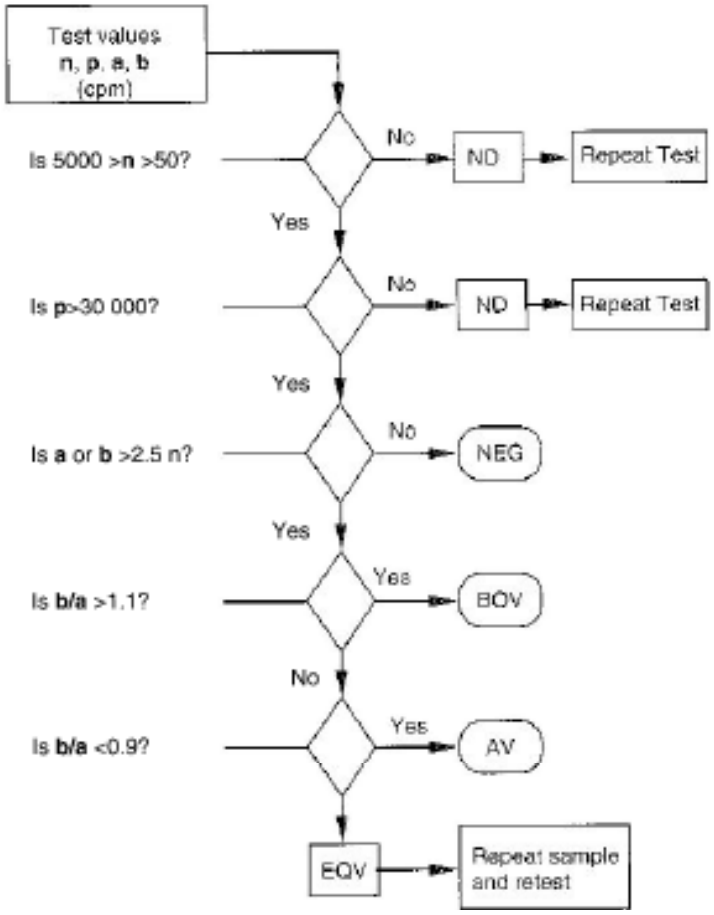


Figure 19 - Flow chart for data validation, discriminant analysis and decisions based on LCT values for TB diagnosis (Griffin et al., 1994)

Sensitivity

Factors influencing Se:

As for the comparative intradermal intradermal test, the sensitivity of lymphocyte stimulation test will be markedly influenced by the stage of disease. It may be highly sensitive within the first 6 months following infection but has reduced sensitivity in chronically diseased animals. The sensitivity could be improved in the future using a combination of specific recombinant antigens for stimulation.

Specificity

Factors influencing Sp:

As for the comparative intradermal intradermal test, sensitization of deer following exposure to saprophytic mycobacteria or infection by other mycobacteria: *M. avium* (de Lisle and Havill, 1985; Quigley et al., 1997) or, increasingly, by *M. paratuberculosis* (Mackintosh, 1998), will be reducing the specificity of the test. However the specificity could be improved in the future using a combination of *M. bovis* specific recombinant antigens for stimulation.

Repeatability and reproducibility

There are no data available on repeatability or reproducibility of the lymphocyte stimulation test in deer.

Practicality

The use of the lymphocyte stimulation test requires a well equipped laboratory and highly trained laboratory personnel and therefore not to be used in large scale certification programmes.

3.3.2.2.4.2 Quantitative evaluation

Sensitivity

Prior expert information about sensitivity of the LCT has been provided by 3 experts.

Data from literature comprises of 9 estimates, involving a total number of 999 animals, published in 5 papers (see Table 47 - references with the Ref.Id. 60 166 175 216 1008).

Using the literature data, the impact of the following variables: *species, rep* and *bias* on sensitivity could be investigated using logistic regression analysis (see Table 64 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 16 - Sensitivity estimates of LCT based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.778	0.568	0.916
Estimate 2A - Systematic Literature review - unadjusted estimates	0.904	0.884	0.921
Estimate 2B - Systematic Literature review - adjusted estimates	0.904	0.885	0.921
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.902	0.883	0.919

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

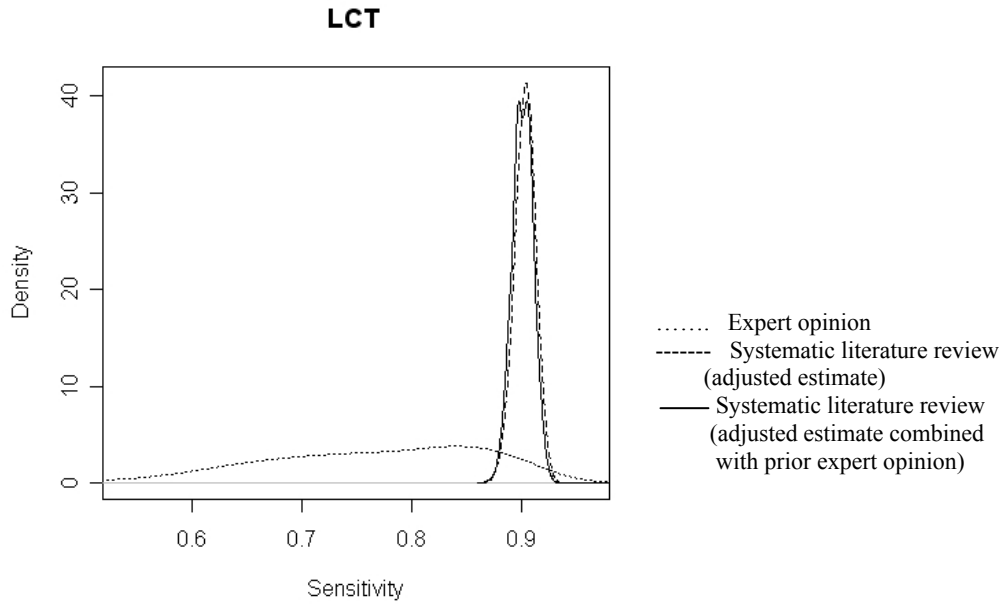


Figure 20 - Probability density distributions representing the information about sensitivity of LCT.

Specificity

Prior expert information about specificity of the LCT has been provided by 2 experts.

Data from literature comprises of 5 estimates, involving a total number of 1371 animals, published in 5 papers (see Table 47 - references with the Ref.Id. 60 166 175 216 1008).

Using the literature data, the impact of the following variables: *species* and *rep* on specificity could be investigated using logistic regression analysis (see Table 65 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 17 - Specificity estimates of LCT based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.969	0.810	0.992
Estimate 2A - Systematic Literature review - unadjusted estimates	0.918	0.903	0.932
Estimate 2B - Systematic Literature review - adjusted estimates	0.918	0.903	0.932
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.908	0.896	0.914

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

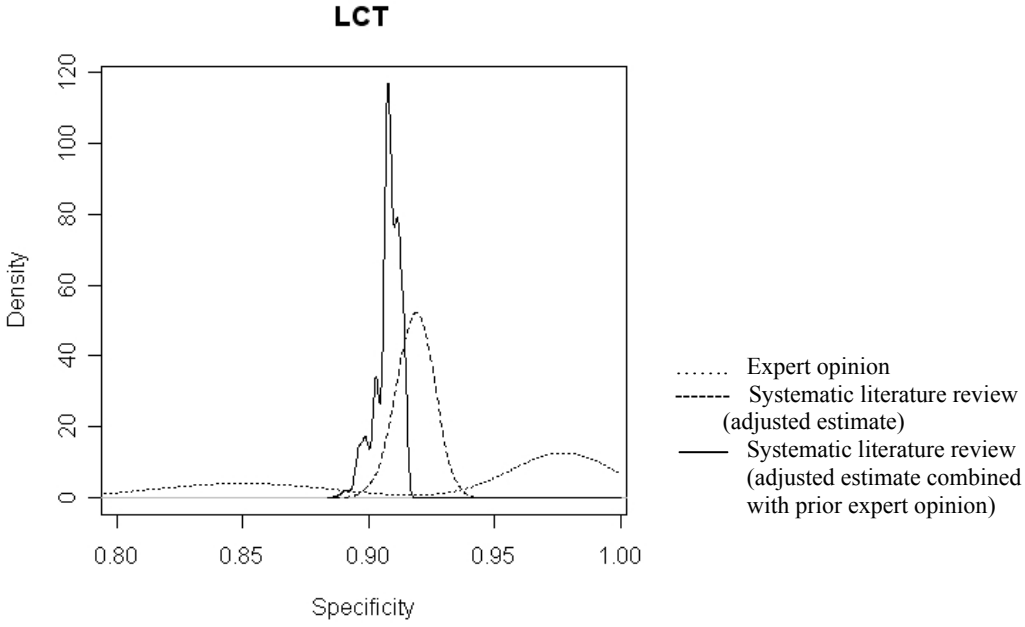


Figure 21 - Probability density distributions representing the information about specificity of LCT.

3.3.2.3. Antibody test

3.3.2.3.1 *Enzyme Linked ImmunoSorbent Assay (ELISA)*

3.3.2.3.1.1 Qualitative evaluation

Introduction

The enzyme linked immunosorbent assay (ELISA) detects circulating antibodies in infected animals.

Microtiter plates are coated with antigens for antibody binding, and then antibody detection is achieved using (for example) peroxidase-labelled secondary antibodies (ProtG or anti-deer IgG1). ELISA vary between laboratories, e.g. in the use of antigen and of microtiter plates. In most cases, the antigen is a local PPD tuberculin, lipoarabinomannan (LAM) or, more recently, more specific recombinant antigens, such as ESAT-6, MPB70 etc. The PPD-based ELISAs are often used in a comparative manner to improve specificity: results obtained from a bovine PPD-ELISA (PPD-B ELISA) are compared with those from an avian PPD-ELISA (PPD-A ELISA). In general, the level of circulating antibodies increases at a late stage of infection, coinciding with an increase in bacterial load. Therefore, on its own, the test is not suitable for early detection of infected animals. However, test performance at earlier stages of infection is improved when the ELISA is used in combination with the intradermal test. The intradermal test leads to a sharp rise in antibody levels (the so-called anaemnestic rise) 2-4 weeks after inoculation (Griffin et al., 2006; Waters et al., 2006).

Description of test

Antibody tests target chronically infected animals with generalized TB. It is these animals that are most likely to be non-reactive (and appear anergic or false negative) to conventional intradermal testing.

Early studies (Sutton et al., 1985) were based on PPD-B ELISA evaluation in naturally and experimentally infected deer. The assay had a sensitivity of approximately 70%, but low

specificity. Based on later work from New Zealand, the ELISA based on PPD-A, PPD-B, and MPB70 had a sensitivity of 46% sensitive when used alone (Griffin et al., 1994), but 74-85% when performed 10 days after the intradermal test in low and high prevalence herds, respectively (Griffin et al., 1994). Therefore, the ELISA test has enhanced sensitivity when used in serial with the intradermal test (Gaborick et al., 1996).

The single intradermal test and ELISA results may be combined, to improve overall test performance. The individual sensitivity of the SST (82%) and ELISA (85%, post-SST) could be increased to 95%, when these tests were interpreted in parallel (Griffin et al., 1994).

In a recent study, 22% of uninfected deer had positive responses (i.e. 0.25 S/P ratio) to *M. bovis*-derived lipoarabinomannan-specific immunoglobulin (LAM), probably as a result of prior exposure to environmental non-tuberculous mycobacteria. Upon infection, either by intratonsillar inoculation or by in-contact exposure, 94% of the deer had a 3-fold or greater response to this ELISA (including 4 deer with pre-existing responses). These responses were detected as early as 36 days post-challenge (Waters et al., 2004).

Sensitivity

Factors influencing Se:

The sensitivity of any antibody assay will be markedly influenced by the stage of disease. These assays are likely to be highly sensitive in chronically diseased animals, or when used in combination with the intradermal test (anaemnestic rise). In the future, the sensitivity of the ELISA may be improved using a combination of specific recombinant antigens.

Specificity

Factors influencing Sp:

The specificity of the ELISA will be influenced by the presence of other mycobacteria within the herd or region. Assay specificity is likely to be increased with increased availability of recombinant antigens or specific synthetic peptides

Repeatability and reproducibility

There are no data available on repeatability or reproducibility of the ELISA test in deer. However, given the nature of the assay, the repeatability and reproducibility are likely to be high.

Practicality

Blood samples can be easily collected when deer are immobilised at the time of the intradermal test. However, deer need to be restrained on a further occasion, several weeks after the single intradermal test has been administered and read, if advantage is to be taken of the anaemnestic rise in antibody levels. The ELISA can be easily standardised within a laboratory. All available ELISA are currently 'homemade', and results from different laboratories and regions are difficult to compare. At the present time, ELISA will have to be validated individually.

3.3.2.3.1.2 Quantitative evaluation

Sensitivity

Prior expert information about sensitivity of ELISA has been provided by 6 experts. A total of 15 estimates were available from the systematic literature review, involving a total number of 1328 animals, and published in 6 papers (see Table 47 - references with the Ref.Id. 166 168 175 258 1008 1020).

Using the literature data, the impact of the following variables: *species, gs, rep* and *bias* on sensitivity could be investigated through logistic regression analysis (see Table 66 in Appendix B). The results indicate that the gold standard 'experimental' is negatively associated with Se. The effect of the stage of infection could not be analysed using the available data.

Table 18 - Sensitivity estimates of ELISA based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.669	0.176	0.957
Estimate 2A - Systematic Literature review - unadjusted estimates	0.767	0.744	0.790
Estimate 2B - Systematic Literature review - adjusted estimates	0.781	0.757	0.804
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.783	0.761	0.806

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

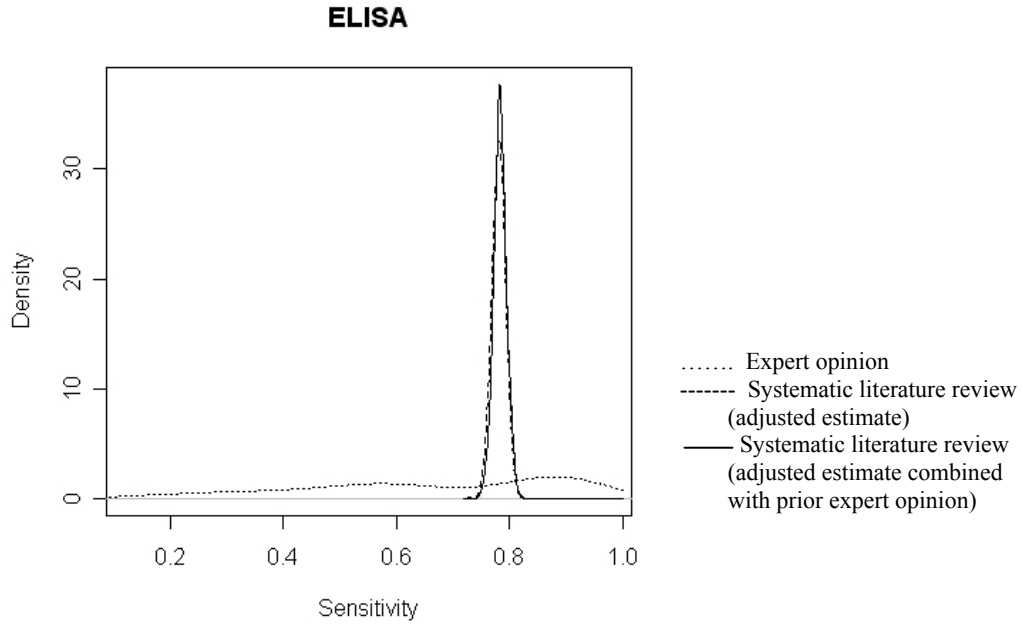


Figure 22 - Probability density distributions representing information about the sensitivity of the ELISA.

Specificity

Prior expert information about specificity of ELISA has been provided by 6 experts. A total of 12 estimates were available from the systematic literature review, involving a total number of 1976 animals, published in 8 papers (see Table 47 - references with the Ref.Id. 166 168 175 258 1003 1008 1012 1020).

Using the literature data, the impact of the following variables: *species, gs* and *rep* on specificity could be investigated using logistic regression analysis (see Table 67 in Appendix B). The results indicate that the gold standard 'experimental' is positively associated with Se. The results do not indicate an effect of the stage of infection.

Table 19 - Specificity estimates of ELISA based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.843	0.212	0.987
Estimate 2A - Systematic Literature review - unadjusted estimates	0.890	0.876	0.904
Estimate 2B - Systematic Literature review - adjusted estimates	0.888	0.873	0.901
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.889	0.873	0.902

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

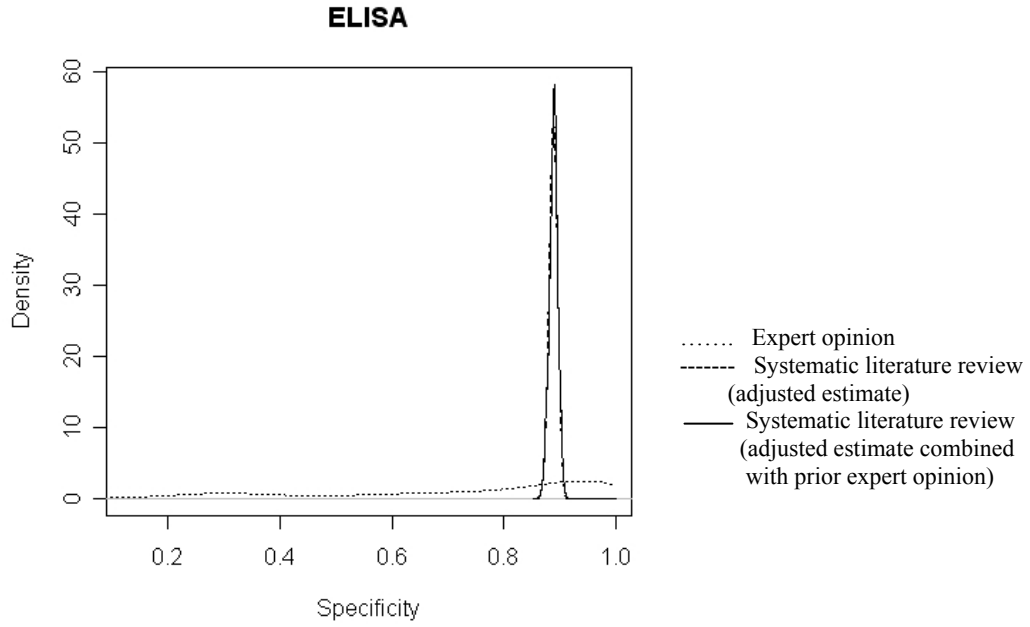


Figure 23 - Probability density distributions representing information about specificity of the ELISA.

3.3.2.3.2 *Rapid test (RAPID)*

3.3.2.3.2.1 Qualitative evaluation

Introduction

The Rapid Test is a novel lateral-flow serological test, for the detection of tuberculosis in humans as well as in a number of animal species. In addition to the rapid test for cervids (for white tail deer, reindeer, and elk), separate tests for non-human primates, cattle, badgers, camelids and exotic species such as elephants have been developed. These tests are all antibody detection assays that use cocktails of selected recombinant antigens of gamma-interferon and *M. tuberculosis*. The rapid tests are currently undergoing validation for USDA licensure.

Description of test

A droplet of serum, plasma, or whole blood is applied to the sample well (S, see Figure 24 below), followed by a few droplets of a dilution buffer. A blue bar at T (test) indicates that the sample is positive and the blue bar at C (control) indicates that the test is valid (see Figure). Results appear within 20 minutes (often within just a few minutes).

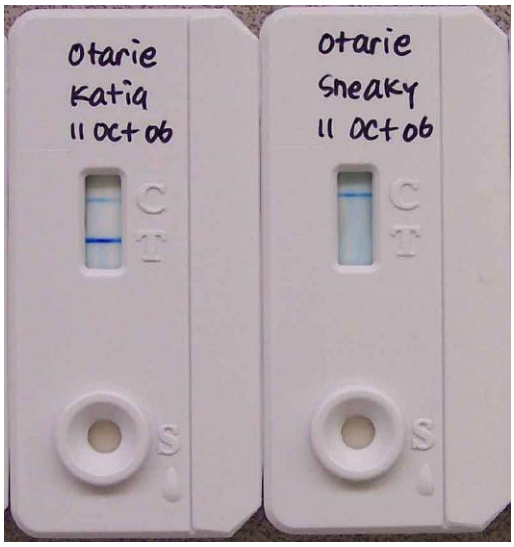


Figure 24 - Example of a Rapid Test: positive sample on the left, negative on the right

Sensitivity

Factors influencing Se:

The sensitivity of the Rapid Test will be markedly influenced by the stage of disease. As with the ELISA, it may be highly sensitive when used in chronically diseased animals or in combination with the intradermal test (anaemnestic rise).

Specificity

Factors influencing Sp:

The specificity of the Rapid Test will be influenced by the presence of other mycobacteria within the herd or region.

Repeatability and reproducibility

Given the simplicity of its design and use, it is likely that repeatability and reproducibility will both be high.

Practicality

The Rapid Test is easy to use. However, the test has not been widely used to date. Quantitative interpretation of the result is also limited.

3.3.2.3.2.2 Quantitative evaluation

Sensitivity

Prior expert information about sensitivity of RAPID has been provided by 1 expert.

No data were available from the systematic literature review.

Table 20 - Sensitivity estimates of RAPID based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.475	0.301	0.601
Estimate 2A - Systematic Literature review - unadjusted estimates	NA	NA	NA
Estimate 2B - Systematic Literature review - adjusted estimates	NA	NA	NA
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	NA	NA	NA

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.
NA = not available.

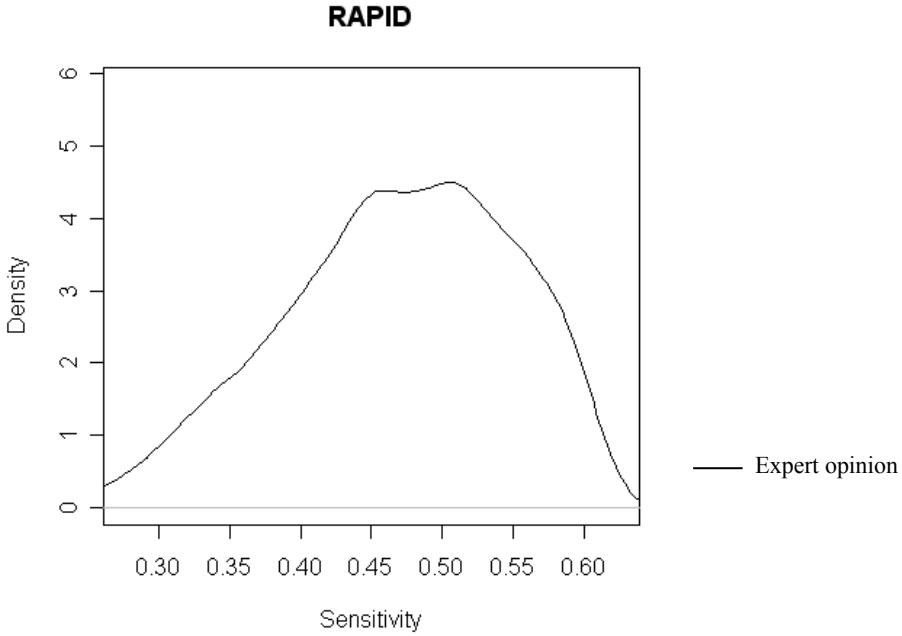


Figure 25 - Probability density distributions representing the information about sensitivity of RAPID.

Specificity

Prior expert information about specificity of RAPID has been provided by 1 expert.

No data were available from the systematic literature review.

Table 21 - Specificity estimates of RAPID based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.802	0.73	0.871
Estimate 2A - Systematic Literature review - unadjusted estimates	NA	NA	NA
Estimate 2B - Systematic Literature review - adjusted estimates	NA	NA	NA
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	NA	NA	NA

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.
NA = not available.

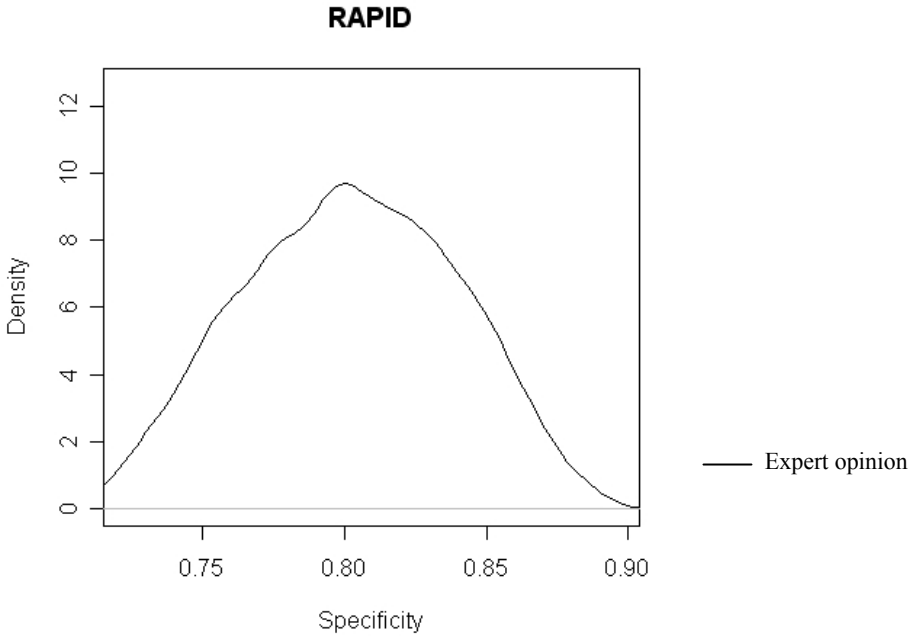


Figure 26 - Probability density distributions representing the information about specificity of RAPID.

3.3.2.3.3 *Multiantigen print immunoassay (MAPIA)*

3.3.2.3.3.1 Qualitative evaluation

Description of test

Currently, antibody detection for tuberculosis is mainly conducted using crude extracts of mycobacteria or PPDs. This presents considerable problems, noting that there are many antigens in common among mycobacteria of interest (*M. tuberculosis*- complex, *M. avium*, *M. paratuberculosis*), as well as among enviromental mycobacteria. For this reason, these diagnostic assays suffer from a lack of specificity.

Recombinant antigens are being examined as a means to improve the specificity of antibody detection assays. However, it must be noted that the antibody repertoire among tuberculous animals is highly diverse. In other words, sera from different animals will react with different antigens (Lyashchenko et al., 1998). Therefore, there has been considerable research on the use of antigen cocktails as a means to cover the diversity of antibody response, thereby increasing both the sensitivity and specificity of these assays. This can be achieved by using either multiple ELISAs or Western blots on nitrocellulose.

Recently, a more standardised method, called multi-antigen print immunoassay (MAPIA), has been developed, based on immobilization of antigens onto nitrocellulose membranes by semi-automated micro-spraying, followed by standard chromogenic development of the reaction. The use of the assay in its present format is limited to research purposes. It has not been adapted to use as a screening test, or in circumstances that require large numbers of samples to be processed.

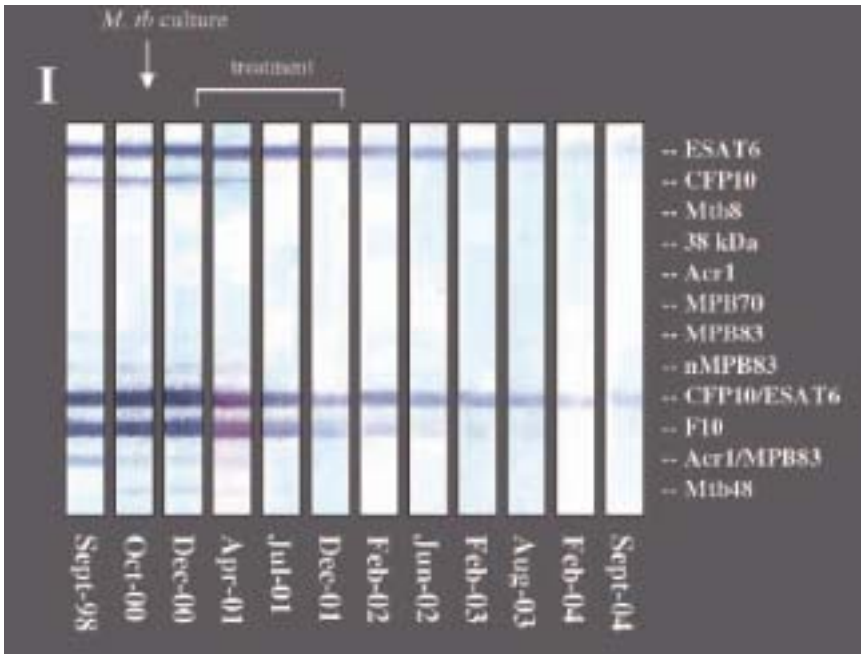


Figure 27 - Example of a MAPIA- immunoblot showing responses to different antigens during the course of TB infection in elephants (adapted from Lyshenko *et al.*, 2006)

Using the MAPIA, responses against individual antigens can be monitored, resulting in a very high specificity. However, use of only a limited number of antigens is possible in this assay; therefore, the sensitivity of the assay will be limited.

3.3.2.3.3.2 Quantitative evaluation

Sensitivity

Prior expert information about sensitivity of the MAPIA is not available.

A total of 13 estimates were available from the systematic literature review, involving a total number of 325 animals, published in 2 papers (see Table 47 - references with the Ref.Id. 1003 1012).

Using the literature data, the impact of the variable *bias* on sensitivity could be investigated using logistic regression analysis (see Table 68 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 22 - Sensitivity estimates of MAPIA based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	NA	NA	NA
Estimate 2A - Systematic Literature review - unadjusted estimates	0.428	0.373	0.483
Estimate 2B - Systematic Literature review - adjusted estimates	0.427	0.375	0.483
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	NA	NA	NA

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.
NA = not available.

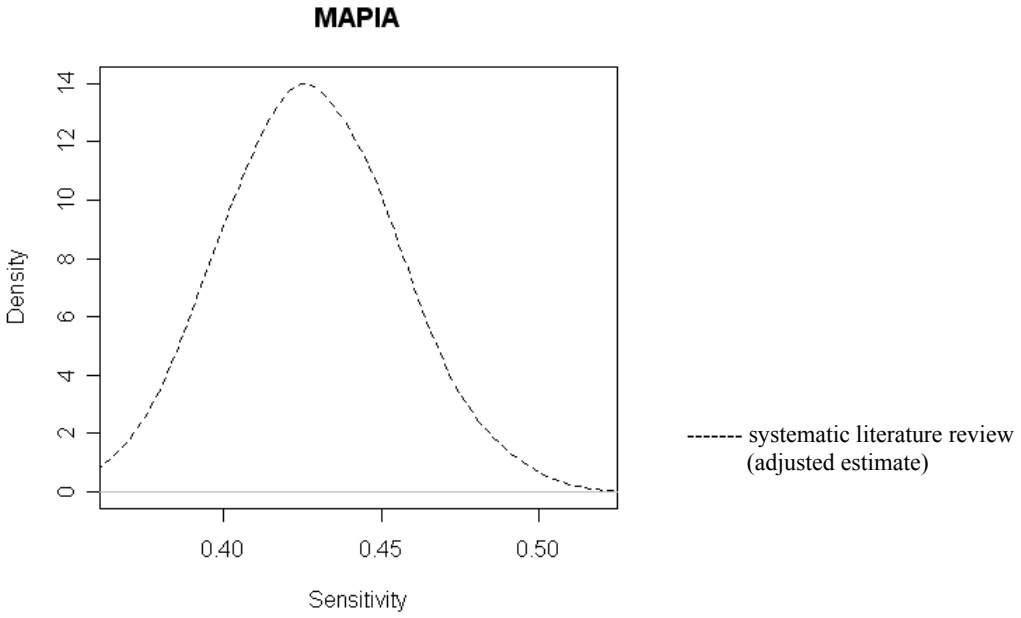


Figure 28 - Probability density distributions representing information about the sensitivity of MAPIA

Specificity

Prior expert information about specificity of the MAPIA is not available.

A total of 12 estimates were available from the systematic literature review, involving a total number of 84 animals, published in 1 paper (see Table 47 - reference with the Ref.Id. 1003).

Using the literature data, it was not possible to investigate the impact of any of the study variables on specificity using logistic regression analysis (see Table 69 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 23 - Specificity estimates of MAPIA based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	NA	NA	NA
Estimate 2A - Systematic Literature review - unadjusted estimates	1	0.957	1
Estimate 2B - Systematic Literature review - adjusted estimates	1	0.988	1
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	NA	NA	NA

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.
NA = not available.

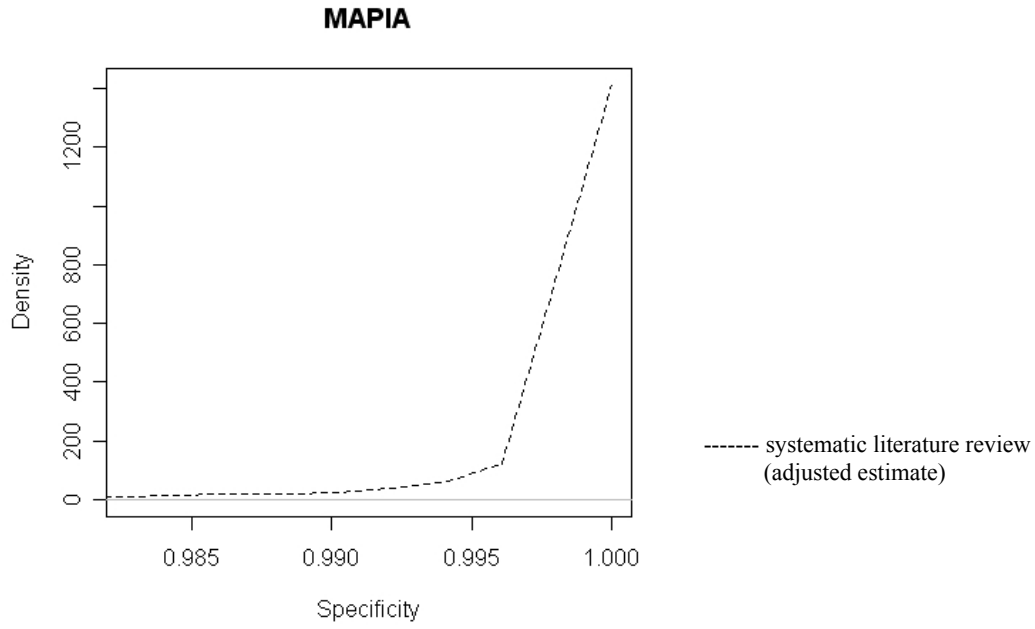


Figure 29 - Probability density distributions representing information about the specificity of MAPIA.

3.3.3. Post mortem diagnosis

3.3.3.1. Necropsy (NECR)

3.3.3.1.1 *Qualitative evaluation*

Introduction

Detailed necropsy has been shown to be a sensitive method for detecting TB-lesions in cattle. In 140 cattle, detailed necropsy detected 85 % of all lesions identified by histological and bacteriological examination (Corner et al., 1990). Similar figures (86%) were obtained by Norby et al. (2004) who considered this estimate as an "upper detection limit" for any slaughter based surveillance system for TB.

The course of tuberculosis in deer is chronic and variable, as are the associated clinical signs and distribution and severity of lesions (Clifton Hadley and Wilesmith, 1991). The macroscopic changes of tuberculosis in deer are similar to those in cattle, comprising granulomatous changes and abscessation in various lymph nodes and internal organs. However, obvious gross lesions are frequently not present in animals with infection confirmed by culture (Kaneene et al., 2002; De Lisle et al., date). Lesions range from a single granuloma in one lymph node to a general anatomic distribution involving many nodes and internal organs (Griffin and Buchan, 1994; de Lisle et al., 2001). However, the lymphoreticular tissues of the head and neck, particularly the tonsil and retropharyngeal lymph nodes are most commonly involved (Lugton et al., 1998; Palmer et al., 2002; O'Brien et al., 2004). Progressive weight loss leading to emaciation may occur but may not be evident, despite extensive pathology.

Although detection of lesions at necropsy is an important tool for monitoring tuberculosis in deer, both farmed and free-living and to support control programmes, it is important to recognise that lesions similar to tuberculosis can be demonstrated by other diseases, such as those caused by other mycobacteria, particularly *M.a. paratuberculosis*, other bacteria and even parasites. Indeed, some authors suggest that all abscesses in deer should be considered as tuberculous until proven otherwise (Beatson, 1985). Consequently, further investigative

approaches are required, as described in other sections of this report.

Definition

A necropsy is the examination and dissection of a carcase to reveal changes produced by diseases or injuries and to deduce potential causes of these changes.

Description of test

The extent of a necropsy for tuberculosis in deer can vary from a complete examination of the carcase to one focusing on specific organs, particularly those of the head and neck. In all cases it is advisable to conduct the necropsy as soon after death as possible. Further, a systematic approach is required as is detailed inspection of the target tissues, including those with a normal appearance, to reveal very small lesions or those deep within the tissue. This inspection is achieved by sectioning the tissue at intervals of a few millimetres. Because tuberculosis lesions in deer are most commonly found in the head and neck lymph nodes, these are often inspected most closely. However, lesions can be found in all lymph nodes of the body, particularly those associated with internal organs (*e.g.* the mesenteric lymph nodes). All granulomatous lesions and abscesses should be considered as potential tuberculosis lesions.

Detailed necropsy has been shown to be a sensitive method for detecting tuberculosis lesions in cattle. For example, in 140 cattle detailed necropsy detected 85% of all lesions identified by histological and bacteriological examination (Corner et al., 1990). Similar figures (86%) were obtained by Norby et al. (2004) who considered this estimate as an "upper detection limit" for any slaughter based surveillance system for bovine tuberculosis. Reported figures for detection of tuberculosis lesions in deer vary from 40% (Whiting and Tessaro, 1994; Kaneene et al., 2002) to 60-70% (Miller et al., 1991; Rhyan et al., 1992).

Sensitivity

Factors influencing Se:

- Some animals may have only a single small lesion or microscopic lesions (often recorded as reactors with no visible lesions);
- Detailed necropsy is an important factor in revealing small or few lesions.
- The stage of the disease;
- The age of the affected animal; and
- The ability of the pathologist to detect and recognise very small lesions deep within tissues.

Specificity

Factors influencing Se:

The lesions induced by bacteria of the *M. tuberculosis*-complex are not pathognomonic. Many organisms can cause granulomata and abscesses.

Repeatability and reproducibility

There are no specific studies in the literature on this aspect. However, formal structured training and particularly that leading to a recognised qualification will provide some assurance that the same type of lesion will be identified reliably by different operators.

Practicality

Pathology is one of the key disciplines in the investigation of animal diseases. It is generally the main tool employed to derive the case definition when a disease appears, particularly a new or newly-emerging disease, and provides the reference and gold standard against which subsequent diagnostic techniques are compared. Although these techniques may replace pathology for many areas of routine diagnosis and surveillance, particularly large volume surveillance, pathology remains a frontline surveillance discipline as it is the only discipline that does not rely on reagents based on prior knowledge of the causal agent.

Training and experience are a very important element in the effectiveness of necropsy as a means to identify deer with suspect lesions. Pathology and necropsy techniques are part of the curriculum for veterinarians and specific training is easily implemented to recognise tuberculosis in deer and collection of samples for further investigation. Obvious lesions are recognised quickly but more complex cases that require a thorough and detailed necropsy are time consuming and more demanding on the operators' experience and expertise. In some countries specific protocols describe e.g. which lymphnodes should be subjected to further examination and culture.

3.3.3.1.2 *Quantitative evaluation*

Sensitivity

Prior expert information about sensitivity of NECR has been provided by 8 experts.

A total of 20 estimates were available from the systematic literature review, involving a total number of 493 animals, published in 18 papers (see Table 47 - references with the Ref.Id. 17 144 156 181 217 315 337 338 347 348 354 393 488 1007 1010 1014 1016 1017).

Using the literature data, the impact of the following variables: *species, gs, region, dpi3, rep* and *bias* on sensitivity could be investigated through logistic regression analysis (see Table 70 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection

Table 24 - Sensitivity estimates of NECR based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.833	0.363	0.978
Estimate 2A - Systematic Literature review - unadjusted estimates	0.834	0.798	0.865
Estimate 2B - Systematic Literature review - adjusted estimates	0.807	0.767	0.844
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.811	0.771	0.847

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

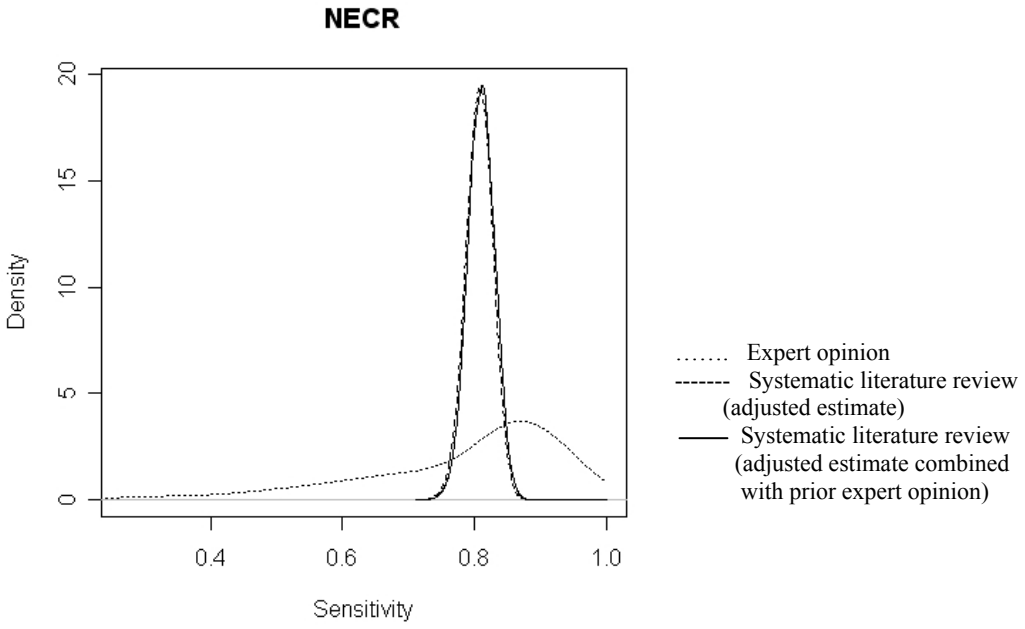


Figure 30 - Probability density distributions representing information about the sensitivity of NECR based on systematic literature review - adjusted estimate (dashed line).

Specificity

Prior expert information about specificity of NECR has been provided by 8 experts.

A total of 5 estimates were available from the systematic literature review, involving a total number of 210 animals, published in 5 papers (see Table 47 - references with the Ref.Id. 17 348 354 488 1007).

Using the literature data, the impact of the following variables: *species* and *rep* on specificity could be investigated using logistic regression analysis (see Table 71 in Appendix B).

Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 25 - Specificity estimates of NECR based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.910	0.366	0.986
Estimate 2A - Systematic Literature review - unadjusted estimates	0.938	0.896	0.967
Estimate 2B - Systematic Literature review - adjusted estimates	0.940	0.900	0.966
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.938	0.903	0.964

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

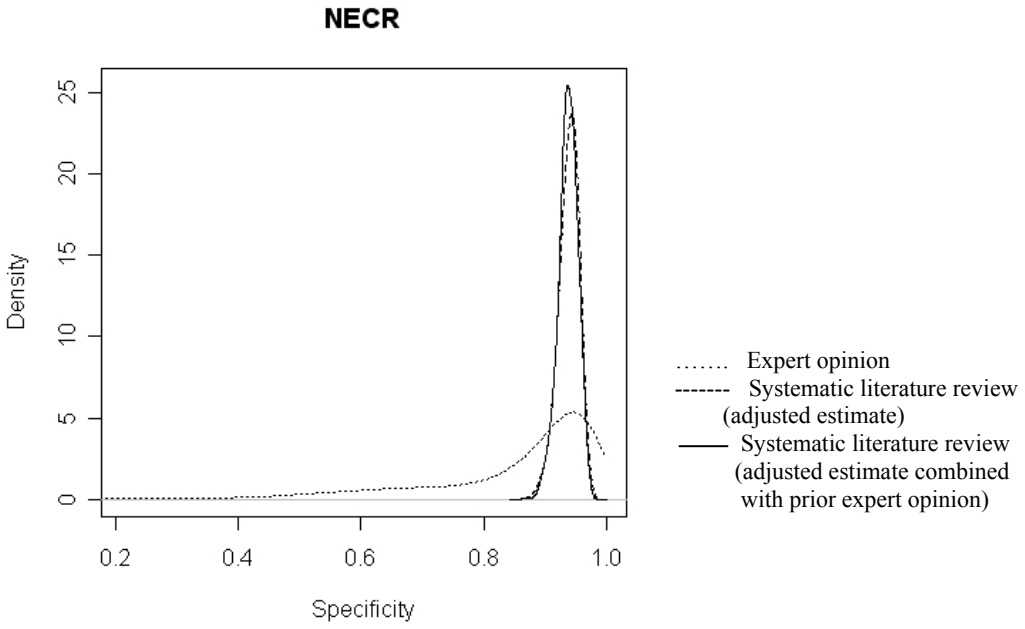


Figure 31 - Probability density distributions representing information about the specificity of NECR.

3.3.3.2. Microscopy/histology (HIST)

3.3.3.2.1 *Qualitative evaluation*

Introduction

Recent advances in tuberculosis testing have enhanced ante-mortem tests for detection of tuberculosis. Nonetheless, post-mortem testing with subsequent histopathological examination, PCR, and culture are central to surveillance and diagnostic efforts. Histological examination is usually carried out on samples where lesions are detected grossly. Ideally, although less common in practice, grossly-normal lymph nodes from reactor animals should also be examined histopathologically, to detect specific microscopic lesions (Schmitt et al., 1997).

Microscopic investigation of lesions detected at meat inspection or necropsy is an important diagnostic method for tuberculosis in deer, as reported by several authors (Fitzgerald et al. 2000; Rohonczy et al., 1996; Rhyan and Saari,1995; Griffin et al., 2004; de Lisle et al., 2002). Focal granulomatous inflammatory lesions (granuloma) in tuberculosis are usually composed of mononuclear lymphocytes, epithelioid macrophages, and multinucleate giant (Langhans) cells, and may show central necrosis and calcification. Although this type of lesion is characteristic for tuberculosis, it may be also found in other infections caused by parasites, bacteria (including *Actinomyces*, *Actinobacillus* and *Nocardia)* or fungi (Clifton-Hadley and Wilesmith, 1991). The presence of acid-fast bacilli (by staining of tissue section using the Ziehl-Neelsen technique) allows a presumptive diagnosis of mycobacteriosis to be made. Histopathology is a useful 'rule-out' method, when the diagnosis of tuberculosis is suspected (de Lisle et al., 2002).

Samples for histopathology and bacteriological culture are generally collected concurrently. Histopathology is a rapid and reliable diagnostic test, for routine use on lesions detected during necropsy or at meat inspection. Alone, histopathology may provide sufficient evidence of disease confirmation for control purposes, even if bacteriologic culture has failed to isolate mycobacteria.

Definition

Microscopy/histology is the study of structure and composition of tissue as examined under a microscope. It includes the use of Ziehl-Neelsen staining and other techniques to identify the presence of acid fast bacilli.

Description of test

Microscopic examination is preceded by an accurate macroscopic inspection by performing transversal cuts every few millimetres across selected tissues. Specimens are placed in 10% neutral buffered formalin; formalin-fixed tissues are then embedded in paraffin wax, cut in 5-6 μm sections, and stained with haematoxylin and eosin (HE) using conventional methods. Additional sections from tissues with lesions suggestive of tuberculosis are stained using the Ziehl-Neelsen method and/or an acridine orange phenylauramine O (AOAO) technique (Rohonczy et al., 1996; Rhyan et al.; 1992, Fitzgerald et al., 2000). Acid-fast staining can be applied to homogenized, digested and concentrated fresh tissues (Kent and Kubica, 1985). A novel technique for the identification of acid-fast bacteria in cytological preparations has been performed in experimentally TB infected white tailed deer *(Odocoileus virginianus)* (Diegel et al., 2003), but it is not reported as a routine diagnostic test.

In the majority of cases, mycobacterial infection is characterized by a local inflammatory response. Initially, leukocytes and macrophages are present. As infection progresses, leukocytes die, resulting in the development of necrosis at the centre of the lesion. The central area of caseous necrosis or mineralization is generally surrounded by inflammatory cells, including neutrophils, epithelioid macrophages, lymphocytes, plasma cells and giant (generally Langhans-type) cells. A typical granulomatous lesion may be present (Skoric et al., 2007, Rhyan and Saari, 1995). Differences between deer species have been noted. In contrast to bovine lesions, those of elk/red deer have scattered peripheral rather than central mineralization, and contain more neutrophilis and fewer giant cells. Fallow deer lesions contain more giant cells, but otherwise are similar to elk/red deer lesions. The abundance of bizarre giant cells is a consistent feature in lesions from sika deer. Due to the wide variation in cervid lesion morphology, tuberculosis should be included in the differential diagnosis whenever granulomatous, necrotizing or suppurative lesions are encountered (Rhyan and Saari, 1995). Also, any microscopic inflammatory disease, such as lymphoid hyperplasia, leukocyte infiltration, necrosis and mineralization, must be considered suggestive of mycobacteriosis (Fitzgerald et al., 2000).

Sensitivity

Factors influencing Se:

Selection of specimen: sensitivity for histopathological evaluation may be affected by sample preparation. In many cases, gross lesions are preferentially selected for mycobacterial culture, rather than for histopathological examination (Fitzgerald et al., 2000).

Preservation of specimen: the samples must be fixed in formalin soon after death in order to maintain the tissue in a suitable condition for subsequent histopathological examination.

Number of bacilli present: a low amount of mycobacteria may give a false negative result to specific staining (i.e. Ziehl-Neelsen stain).

Mixed infection: the presence of granulomata caused by concurrent infection with bacteria other than mycobacteria, such as Actinomyces, could reduce the sensitivity of histopathological evaluation.

Evaluation/interpretation: the interpretation of histopathological lesions may differ between laboratories.

Specificity

Factors influencing Se:

- Presence of mycobacteria other than from the *M. tuberculosis*-complex: tissue lesions caused by other mycobacteria are often indistinguishable from lesions caused from infection with members of the *M. tuberculosis*-complex (de Lisle et al., 2002). Their presence can also contribute to the decrease in specificity of acid-fast staining (Fitzgerald et al., 2000).

Evaluation/interpretation: the interpretation of histopathological lesions may differ between laboratories.

Repeatability and reproducibility

There are no data available on repeatability and reproducibility of microscopy/histology

Practicality

Histological examination is performed on samples collected during post-mortem inspection and it can be carried out in all histopathology laboratories. All statutory diagnostic tests must be performed to ISO17025 standards in accredited laboratories (EU Regulation 882/2004 implemented on 01/06).

Samples should be collected after an accurate macroscopic inspection, and histological sections should be properly cut from formalin-fixed tissue to ensure that samples are suitable for histological examination. In some countries, specific protocols have been developed, including a description of the specific lymph nodes that should be subjected to further examination and culture.

3.3.3.2.2 *Quantitative evaluation*

Sensitivity

Prior expert information about sensitivity of HIST has been provided by 9 experts.

A total of 20 estimates were available from the systematic literature review, involving a total number of 1134 animals, published in 13 papers (see Table 47 - references with the Ref.Id. 17 124 141 144 166 181 217 258 347 354 393 476 1014).

Using the literature data, the impact of the following variables: *species, gs, region, dpi3, rep* and *bias* on sensitivity could be investigated through logistic regression analysis (see Table 72 in Appendix B). The results indicate that the gold standard 'experimental' is positively associated with Se. The results do not indicate an effect of the stage of infection.

Table 26 - Sensitivity estimates of HIST based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.681	0.200	0.965
Estimate 2A - Systematic Literature review - unadjusted estimates	0.881	0.861	0.899
Estimate 2B - Systematic Literature review - adjusted estimates	0.901	0.882	0.918
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.900	0.852	0.891

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

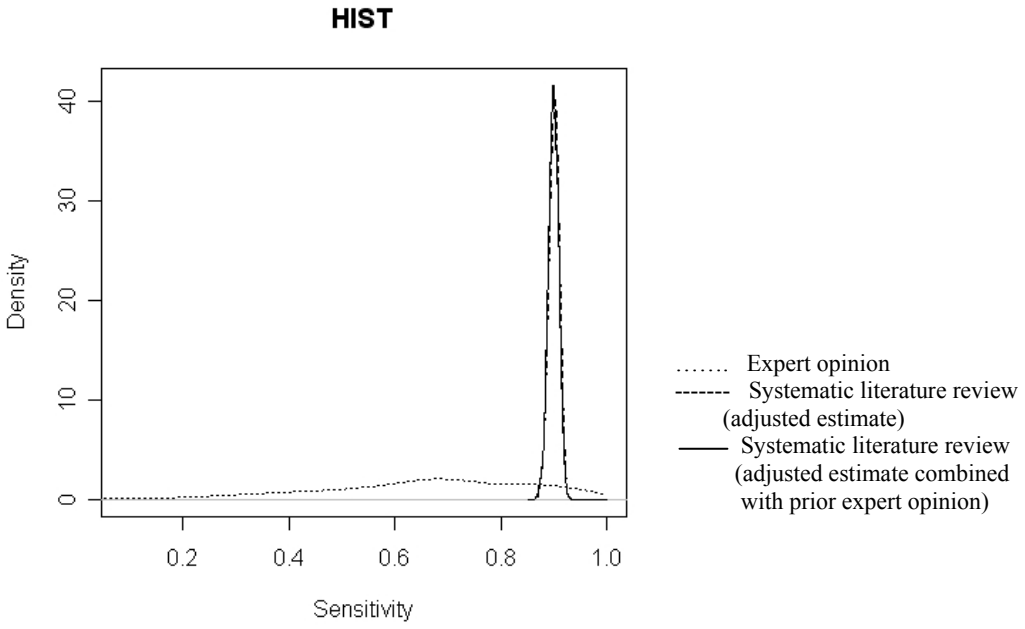


Figure 32 - Probability density distributions representing information about the sensitivity of HIST.

Specificity

Prior expert information about specificity of HIST has been provided by 7 experts.

A total of 5 estimates were available from the systematic literature review, involving a total number of 359 animals, published in 3 papers (see Table 47 - references with the Ref.Id. 17 141 166).

Using the literature data, the impact of the following variables: *species* and *rep* on specificity could be investigated using logistic regression analysis (see Table 73 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection

Table 27 - Specificity estimates of HIST based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.750	0.155	0.970
Estimate 2A - Systematic Literature review - unadjusted estimates	0.802	0.757	0.842
Estimate 2B - Systematic Literature review - adjusted estimates	0.803	0.760	0.842
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.799	0.758	0.837

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

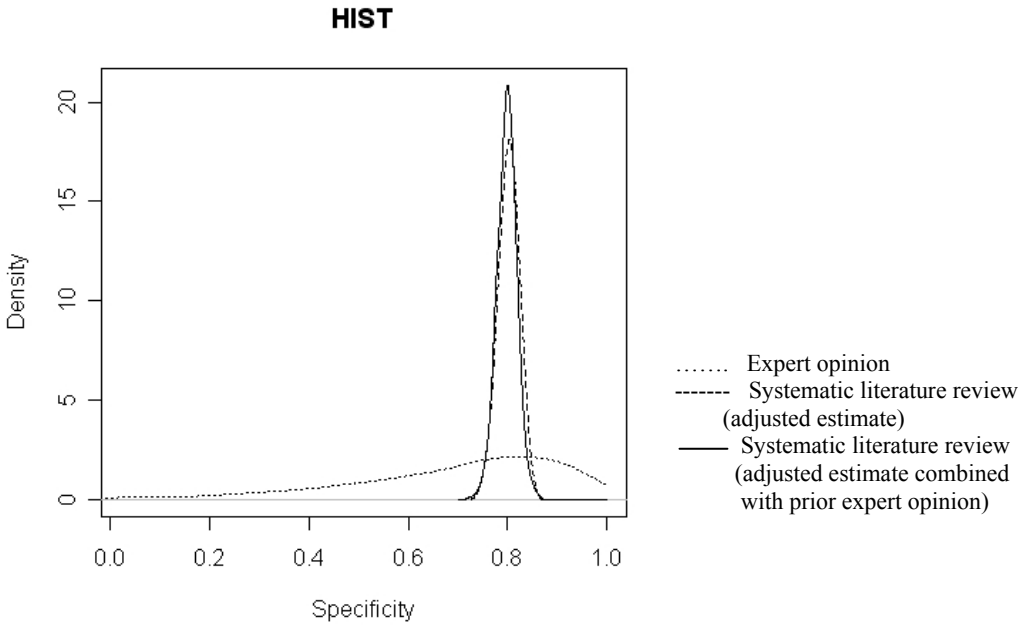


Figure 33 - Probability density distributions representing the information about specificity of HIST.

3.3.3.3. Meat inspection (INSP)

3.3.3.3.1 *Qualitative evaluation*

Introduction

Meat inspection was instigated originally to provide increased assurance to consumers and public health officials that meat and meat products were safe for human consumption. It continues to be used mainly for this purpose but it also can be used for surveillance, either exclusively or to supplement other approaches (Lees, 2004; O'Brien et al., 2006). Meat inspection is particularly useful for surveillance when routine testing of live animals is not possible, for example, in farmed and wild deer (O'Brien et al., 2004; Wahlström and Englund, 2006). Although meat inspection has been advocated as a cost effective means to document freedom from infection after completion of an eradication programme for cattle (Corner et al., 1990), the position in deer is less clear. Routine meat inspection has been reported to identify typical lesions of tuberculosis in less than 50% of culture positive deer (De Lisle et al. (date); Whiting and Tessaro, 1994) i.e. there is a high proportion of deer with no visible lesions, whereas Kaneene et al. (2006) described suspect lesions in <1.5% of deer, all of which were culture negative. Because of the former, some agencies have advocated increasing the submission rate for granulomata to enhance surveillance at slaughter (summarised by Wahlström, 2004; Wahlström and Englund, 2006) or as a means to monitor the quality of meat inspection (EFSA, 2003).

Efficiency of inspection procedure

If abattoir inspection is to be effective, inspectors must show diligence, be well trained, examine the correct tissues and submit identified granulomas for laboratory examination (Cousins, 2001). The efficiency of the surveillance also needs to be evaluated. For example, in the USA, APHIS has identified the need for enhanced surveillance at slaughter (increased submission rate of granulomas) to identify remaining pockets of infection

O´Brien et al. (2004) estimated the sensitivity of the existing bovine tuberculosis surveillance in free-ranging deer performed in the field to be 75%. As could be expected, the sensitivity of meat inspection and necropsy is higher for cattle with more advanced disease. Norby et al. (2004) showed that necropsy detected 80 % of cattle (n = 27) with one lesion and all cattle (n = 16) with two or more lesions. The infection status of these animals had been confirmed using bacteriological culture and/or PCR.

The wide range in the estimates may be due to differences in gold standards, differences in stage of infection in the study population and differences in inspection routine. This highlights the importance of quality assurance of meat inspection.

Definition

Meat inspection is the organised inspection of a slaughtered animal and the organs belonging to it for evidence of disease that would make the meat and/or offals not suitable for human consumption. It is largely a non-destructive process comprising visual inspection and palpation, sometimes supplemented by incision of specified tissues and organs, and additional sites as required, to reveal potential internal lesions or parasites.

Description of test

In most countries, meat inspection is performed in accordance with national regulations. Within the EU, meat inspection has to comply with the conditions laid out in Regulation EC 853/2004. Those applying to deer are described in Annex II, Sections I and III and summarised by Casoli et al., 2005.

Sensitivity

Factors influencing Se:

- As for necropsy (see section 3.3.3.1.)
- If abattoir inspection is to be effective, inspectors must show diligence, be well trained, examine the correct tissues and submit identified suspect lesions for laboratory examination.
- Routine meat inspection is less sensitive than a professional detailed necropsy.

Specificity

Factors influencing Se:

- As for necropsy (see section 3.3.3.1)

Repeatability and reproducibility

There are no specific studies in the literature on this aspect. However, formal structured training (particularly that leading to a recognised qualification), will provide some assurance that the same type of lesion will be identified reliably by different operators.

Practicality

Meat inspection is largely a visual technique based on a systematic examination of a carcase. It therefore is an extremely simple and highly practical technique that can be executed in an abattoir, on-farm or even in the field by hunters. However, an essential element for its effectiveness is the diligence and competence of the inspector, which must be provided by thorough training.

3.3.3.3.2 *Quantitative evaluation*

Sensitivity

Prior expert information about sensitivity of INSP has been provided by 7 experts.

A total of 2 estimates were available from the systematic literature review, involving a total number of 22 animals, published in 2 papers (see Table 47 - references with the Ref.Id. 227 230).

Using the literature data, the impact of the variable *rep* on sensitivity could be investigated using logistic regression analysis (see Table 74 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 28 - Sensitivity estimates of INSP based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.542	0.096	0.868
Estimate 2A - Systematic Literature review - unadjusted estimates	0.636	0.407	0.828
Estimate 2B - Systematic Literature review - adjusted estimates	0.639	0.429	0.816
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.625	0.448	0.777

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

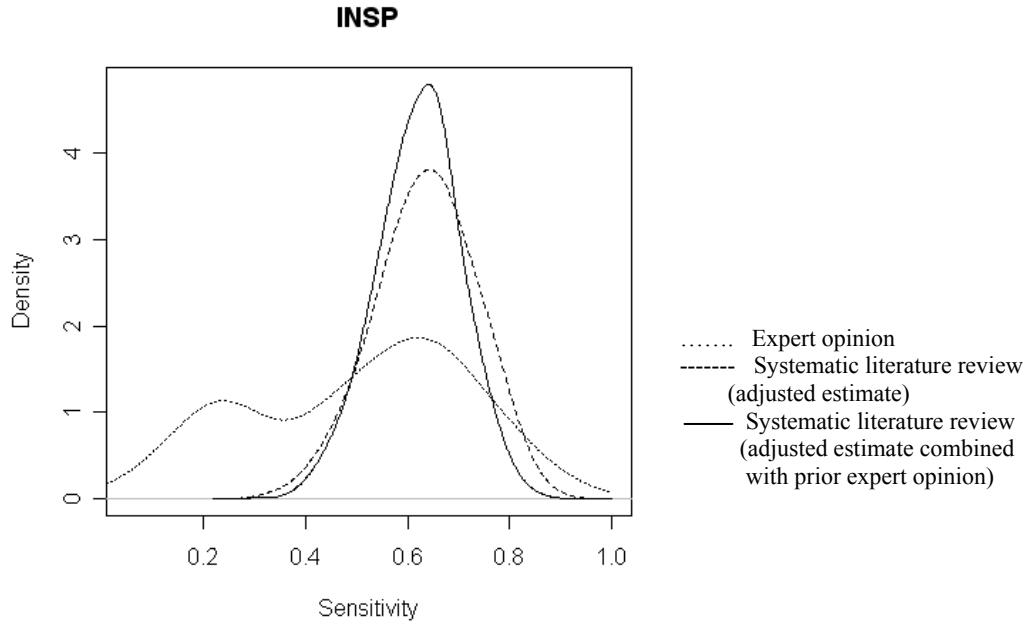


Figure 34 - Probability density distributions representing the information about sensitivity of INSP.

Specificity

Prior expert information about specificity of INSP has been provided by 5 experts.

No data were available from the systematic literature review

Table 29 - Specificity estimates of INSP based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.723	0.153	0.981
Estimate 2A - Systematic Literature review - unadjusted estimates	NA	NA	NA
Estimate 2B - Systematic Literature review - adjusted estimates	NA	NA	NA
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	NA	NA	NA

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.
NA = not available

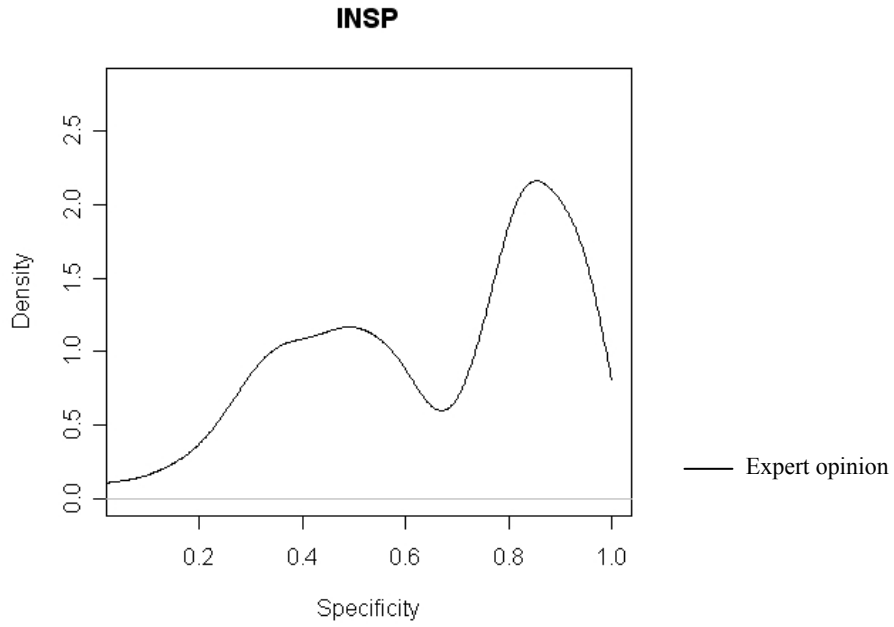


Figure 35 - Probability density distributions representing information about the specificity of INSP based on expert opinion (dotted line).

3.3.4. Combined tests

Research on the use of combined tests to diagnose TB in deer has been an area of research in New Zealand for some years. This work was prompted by concerns among New Zealand deer producers that the SST and SICCT were not adequate to detect TB in farmed deer (Griffin et al., 1994). There have been two direct lines of research, including:

Efforts to minimise the number of false-positive results associated with non-specific mycobacterial reactivity to the SST; and

Efforts to minimise the number of false-negative results associated with anergy in tuberculous animals.

There have been promising results to address these two issues, using the blood test for tuberculosis (BTB) and ELISA, respectively, each approximately 10 days following the initial SST test (Griffin, 1993).

3.3.4.1. Blood bovine tuberculosis test (BTB):

3.3.4.1.1 *Qualitative evaluation*

The blood bovine tuberculosis test or BTB test was developed in the 1980's in New Zealand, based on tests to detect both the cellular and antibody responses. In the literature, all available reports concern the use of the lymphocyte stimulation test (LCT) and the ELISA. However, the LCT is technically demanding, and this test has subsequently been replaced by the comparative intradermal test (SICCT).

An animal is considered positive to the BTB if positive to either the LCT and/or ELISA (Griffin et al., 1994). Therefore, a BTB-negative animal is negative to both tests. The sensitivity of the BTB is enhanced if conducted approximately 10 days after the SST. This is because the intradermal test leads to an anamnestic rise in antibody levels (and therefore improved ELISA sensitivity) 2-4 weeks after inoculation (Griffin et al., 2006, Waters et al., 2006). However, using this approach, there is a need to visit the farm (and restrain each animal) on three occasions (twice for the SICCT, once for serum collection).

3.3.4.1.2 *Quantitative evaluation*

Sensitivity

Prior expert information about sensitivity of BTB has been provided by 3 experts.

A total of 9 estimates were available from the systematic literature review, involving a total number of 886 animals, published in 5 papers (see Table 47 - references with the Ref. Id. 166 168 175 180 1008).

Using the literature data, the impact of the following variables: *species, rep* and *bias* on sensitivity could be investigated using logistic regression analysis (see Table 75 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 30 - Sensitivity estimates of BTB based on different sources of information:

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.901	0.561	0.972
Estimate 2A - Systematic Literature review - unadjusted estimates	0.946	0.929	0.960
Estimate 2B - Systematic Literature review - adjusted estimates	0.946	0.930	0.960
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.945	0.928	0.958

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

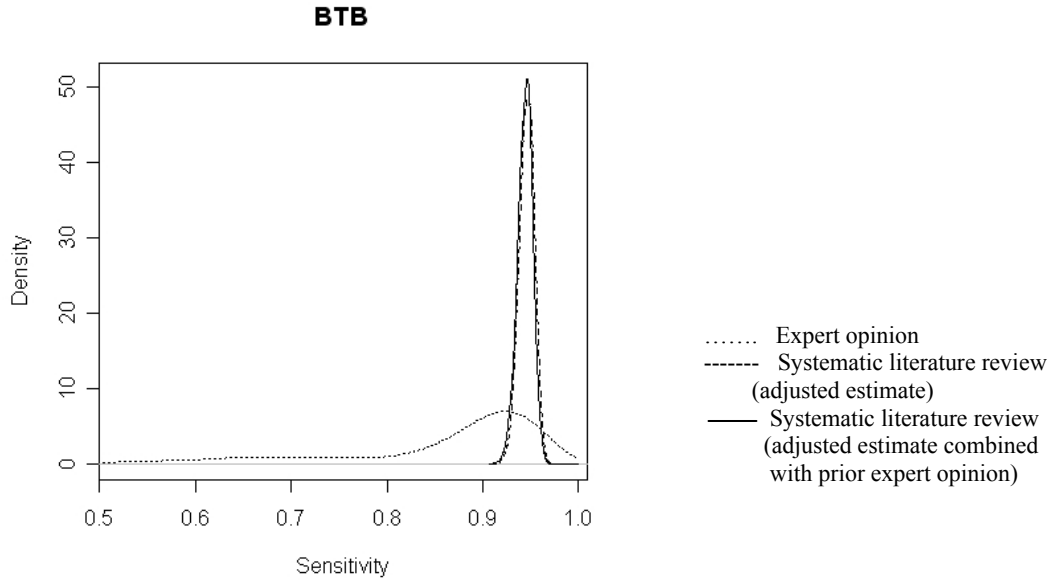


Figure 36 - Probability density distributions representing information about the sensitivity of BTB.

Specificity

Prior expert information about specificity of BTB has been provided by 3 experts.

A total of 4 estimates were available from the systematic literature review, involving a total number of 668 animals, published in 4 papers (see Table 47 - references with the Ref.Id. 166 168 175 1008).

Using the literature data, the impact of the following vaiables: *species* and *rep* on specificity could be investigated using logistic regression analysis (see Table 76 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 31 - Specificity estimates of BTB based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	0.945	0.754	0.992
Estimate 2A - Systematic Literature review - unadjusted estimates	0.982	0.969	0.991
Estimate 2B - Systematic Literature review - adjusted estimates	0.983	0.971	0.991
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	0.981	0.970	0.989

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.

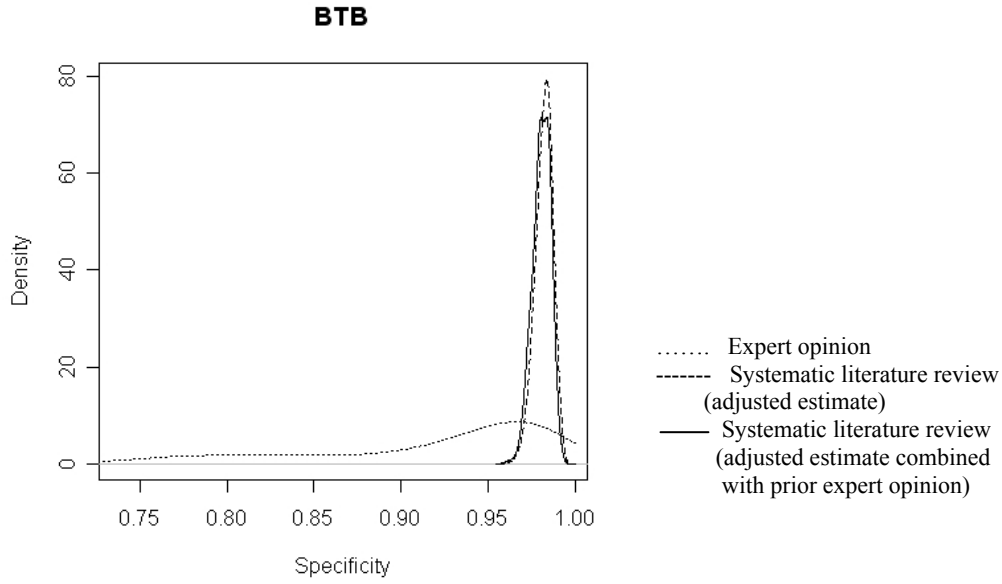


Figure 37 - Probability density distributions representing information about the specificity of BTB.

3.3.4.2. ELISA 10 days after SST (SST_ELISA)

3.3.4.2.1 *Qualitative evaluation*

As indicated previously, the sensitivity of the ELISA is substantially enhanced in the presence of the anamnestic antibody response, 2-4 weeks after the SST. According to Griffin et al. (1994), the sensitivity of the ELISA in deer from a heavily infected herd varied from 45.7% (pre-SST) to 85.3% (post-SST). This test combination is used to identify tuberculous animals that have tested negative to the SST.

3.3.4.2.2 *Quantitative evaluation*

Sensitivity

Prior expert information about sensitivity of the SST_ELISA is not available.

Only one estimate was available from the systematic literature review, involving a total number of 95 animals, published in 1 paper (see Table 47 - reference with the Ref.Id. 1008).

Using the literature data, it was not possible to investigate the impact of any of the study variables on specificity using logistic regression analysis (see Table 77 in Appendix B). Using the available data, it was not possible to analyse the effects of either a gold standard or the stage of infection.

Table 32 - Sensitivity estimates of SST_ELISA based on different sources of information.

	Estimate	Lower	Upper
Estimate 1 - Expert opinion	NA	NA	NA
Estimate 2A - Systematic Literature review - unadjusted estimates	0.853	0.765	0.917
Estimate 2B - Systematic Literature review - adjusted estimates	0.855	0.777	0.916
Estimate 3 - Systematic Literature review - adjusted estimates combined with prior expert opinion	NA	NA	NA

Estimate, Lower and Upper refer to median, 2.5th and 97.5th percentile, respectively.
NA = not available.

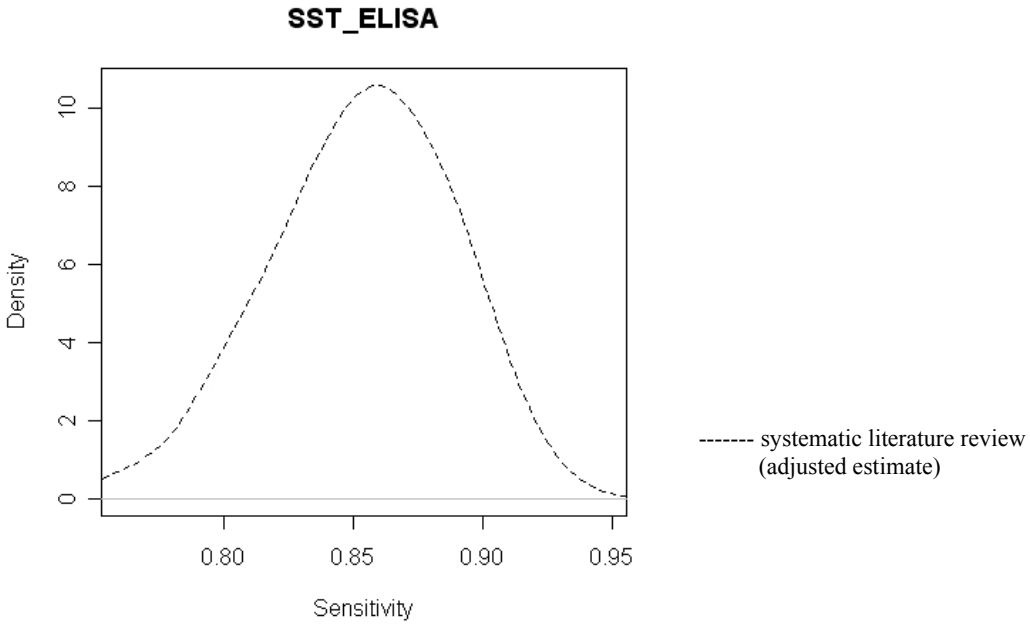


Figure 38 - Probability density distributions representing the information about sensitivity of SST_ELISA based on systematic literature review - adjusted estimate (dashed line).

Specificity

Information about the specificity of the SST_ELISA, either from expert opinion and the systematic literature review, is not available.

3.3.4.3. Tests reviewed but not included in the report

Several additional tests were reviewed, but have not been reported, for a range of reasons:

- There are no reports of recent test development (the 'eye test', 1 paper; immunodiffusion, 1 paper);
- The test is not suitable for screening ('genetic probe to culture', 1 paper);
- The report(s) of test evaluation are limited to a very small number of animals (necropsy/culture, 1 paper; combined SST/SICCT, 1 paper).

3.4. Summary

Table 33- Summary of tests and evaluation of the usefulness of these tests based on the overall biological and practical characteristics [1)]

Test name	Se	CI	Sp	CI	Currently available and useful[2)] for	
					Screening[3)] Yes/No	Confirmation Yes/No
CULT	0.741	0.670 -0.794	0.973	0.47-0.996	N	Y
PCR	0.869	0.806-0.917	0.995	0.941-1.000	N	Y [4,5)]
SICCT	0.858	0.834-0.880	0.977	0.972-0.982	Y	N
SST	0.823	0.804-0.840	0.759	0.744-0.779	Y	N
GINT	0.748	0.661-0.819	0.954	0.902-0.980	N/Y[5)]	N
LCT	0.904	0.885-0.921	0.918	0.903-0.932	N	N
ELISA	0.783	0.761-0.80	0.889	0.873-0.902	N/Y[5)]	N
RAPID	0.475	0.301-0.601	0.802	0.73-0.871	N	N
MAPIA	0.427	0.375-0.483	1.000	0.988-1.000	N	N
NECRO	0.811	0.771-0.847	0.938	0.903-0.964	N	Y [4,6)]
HIST	0.900	0.852-0.891	0.799	0.758-0.837	N	Y [4,6)]
INSP	0.625	0.448-0.777	0.723	0.153-0.981	Y	N
BTB	0.945	0.928-0.958	0.981	0.970-0.989	N	N
SST_ELISA	0.855	0.777-0.916	-	-	N/Y[5)]	N

1) For detailed information see the previous description of each test and for their possible use, in the subsequent chapters
2) In relation to suitability and validation for use in deer
3) Large scale test of live or slaughtered animals for the purpose of surveillance or control
4) Culture required for final confirmation
5) When validated test has the potential for being used in regions or herds where TB prevalence is high
6) For use in pre-selected animals

It is only the two intradermal tuberculin tests (SICCT and SST) that currently are suitable for large scale screening in live animals. These tests are primarily intended for the detection of TB on a herd basis.

When validated for use in deer gamma-interferon assay (GINT) and ELISA could be used in combination with SICCT and SST to maximize the detection of infected animals (in a region or herd with a high prevalence).

Meat inspection (INSP) is currently the only suitable method for surveillance in slaughtered animals and is suitable for use as a complement to other test and control regimes required for maintaining TB free status.

The final confirmation of TB in deer is through the identification of species within the *M. tuberculosis*-complex by culture (CULT).

A combination of different tests studied could also be used for the confirmation of intradermal test positive animals or animals detected at meat inspection and consist primarily of detailed necropsy and histology followed by culture, PCR and (immuno-) histology. These combinations of tests are not suitable for screening but crucial for confirmation of results from official tests and for their quality control.

4. VALIDATION OF DIAGNOSTIC TESTS WITH SPECIAL REFERENCE TO DEER

4.1. General issues

Greiner and Gardner (2000) present detailed guidelines on the validation of diagnostic tests. Challenges faced in the validation of diagnostic tests in deer are not dissimilar to those faced with other diseases and with tuberculosis in other species.

The choice of a reference method (the gold standard) during test validation studies in deer has been problematic. Microbiology is preferred due to its perfect specificity (false-positives will not occur in the absence of cross-contamination). However, the sensitivity of microbiology is imperfect, for a range of reasons as presented previously (section 3.3.1.1). Further, the test is not suitable for use in live animals, and culture results may not be available for 12 weeks. There have been rapid advances in methodology to assess test performance in the absence of a gold standard (so-called latent class models; for example, Enøe et al., 2000, Kostoulas et al., 2006). These methods have been applied to a range of diseases, including infection with *Mycobacterium avium* subspecies *paratuberculosis* [*Map*] (Johne's disease) in cattle (Fosgate et al., 2007) and in sheep and goats (Kostoulas et al., 2006), but not yet with tuberculosis in deer.

The World Organisation for Animal Health (OIE) has issued a standard operating procedure (SOP) for validation of diagnostic tests used in context of international trade with animals (OIE, 2005[1]). According to this SOP, the full validation process includes a documentation of a test's analytical and diagnostic sensitivity and specificity. The diagnostic performance parameters need to be established with regard to the intended purpose of the test such as certification of the free infection/disease status of animals, herds or populations. According to the fitness-for-purpose paradigm, important characteristics of the target population (*e.g.*, vaccination status) need to be considered when study populations are selected for validation. The use of latent class methods is accepted and even foreseen in the structure of the OIE test validation template. To our knowledge, none of the tests considered in this report has been validated according to the OIE SOP.

A "diagnostic test" is usually defined by the triplet:

a) animal species,

b) infectious agent, and

c) test principle ad modification.

Thus, the same TB test applied to different deer species should be considered as different test entities. For some of the tests, our results suggest that sensitivity and/or specificity might differ among deer species (see tables in Appendix B, section D). Moreover, the impact of deer species as well some study design factors (related to the design of the validation study) on the sensitivity and/or specificity could be shown. Therefore, attention should be paid to these sources of variability in the estimation of diagnostic test performance measures.

The estimates of diagnostic sensitivity and specificity (see below) required to inform surveillance strategy models should also be derived from epidemiological evaluation studies in a specific population, under realistic (and reproducible) conditions.

4.2. Estimating sensitivity

The sensitivity of diagnostic tests is heavily influenced by a range of biological factors, including stage of disease, immune status of the host and animal age. Consequently, sensitivity will vary in and among animal populations (Greiner and Gardner, 2000). In deer, as in other

species, tuberculosis is a chronic disease; and a 'spectrum of disease (from early to final-stage) is the norm in a herd with long-standing infection.

Sensitivity estimation has generally been conducted in naturally-infected deer populations (for example, Fitzgerald et al., 2000), for a range of reasons, including our current inability of create a realistic infection model under experimental conditions (Griffin, 1993). In recent years, there have been ongoing improvements in efforts to reproduce tuberculosis experimentally through aerosol delivery, both in cattle and deer (Palmer et al., 2002 a, b, 2003).

4.3. Estimating specificity

Diagnostic specificity varies geographically among different animal populations, due to the presence of cross-reacting organisms (Greiner and Gardner, 2000) such as *M. avium* and other mycobacteria (Griffin and Buchan, 1994). Further, specificity may vary over time during an eradication programme, with the removal of test-positive (both true-positive and false-positive) reactors, resulting in a decrease in prevalence and an increase in specificity (Greiner and Gardner, 2000). Consequently, specificity estimates must be extrapolated between regions with care.

5. DEFINITIONS OF FREEDOM

5.1. General considerations

5.1.1. Concepts of Freedom

The focus in this report is freedom from infection. As with many diseases, but with tuberculosis in particular, freedom from disease in the presence of infection has little or no value in controlling the spread of the agent.

A design prevalence defines the lower limit of a theoretical level of infection in the population which, a given surveillance activity would be able to detect with a specified probability. Consider a situation where a design prevalence of 0.1% is used. The surveillance activity will detect the presence of infection with at least a specified probability (generally 95%), provided infection is present in the population at a prevalence of 0.1% or greater.

In the context of design prevalence, freedom from infection has been interpreted in two subtly different ways:

- The first interpretation is that freedom from infection is an absolute state, that cannot be absolutely proved, but can be disproved by the presence of a single infected animal given the prevalence is above the design level. This means that, even if surveillance has demonstrated that this MS or region is free of infection the prevalence of infection is lower than the design prevalence, the detection of one or more infected animals means that a holding, zone or country cannot claim to be free.
- The second interpretation is that freedom is a relative concept and is measured in terms of the design prevalence. The presence of known infected animals does not invalidate the free status, as long as their prevalence is lower than the design prevalence.

In both cases, it is possible to have infected animals in a nominally disease-free population, but the difference is that, in the second case, these animals have been identified, whereas in the first case, infected animals may be present but have not been identified and are therefore not *known* to be present.

The more 'purist' approach is the former, and this is generally applicable to rapidly moving infection, where even a small proportion of identified infected animals may run the risk of causing a large outbreak. On the other hand, infections such as TB that spread more-slowly it is well recognised that final eradication of the last few cases in a country can be very difficult, and that, as long as adequate control measures continue to be in place, a low number of residual

reactors poses little risk of a significant increase in the level of disease. It is therefore recommended, in the case of TB in deer, that freedom be defined as absence of infection, or, if present, a prevalence of infection lower than a designated design prevalence.

5.1.2. Standards based on data from TB in cattle

In order to provide transparency and equivalency in the area of trade in animals or animal products, it is necessary to establish standards which must be met for trade to be permissible. When dealing with issues of disease freedom, absolute proof of free status is impossible to achieve (especially when using imperfect tests). Standards are therefore established which aim to establish an acceptable probability that the animal or population is free from infection

These standards are usually associated with the acceptable level of the pathogens to die off if they are present in the population. With a disease such as TB this level should be very low due to high resistance and long incubation period for these bacteria.

Standards have been developed in two main ways – input-based and output-based. Input-based standards require a certain amount of activity (testing of animals and application of certain control measures; for instance), on the expectation that an adequate output (confidence in freedom) will be achieved by these inputs. Output-based standards attempt to more directly and quantitatively specify the desired result, potentially allowing a number of different inputs to achieve that result.

The ultimate objective when dealing with trade issues is to determine the probability that an animal or population is free from infection or disease: Pr(D-). Traditionally, quantitative measures of the effectiveness of surveillance activities have been expressed in terms of the sensitivity of the surveillance system, based on an assumed level of infection in the population (design prevalence): Pr(T+|D+). The probability of freedom can be calculated directly from the sensitivity of a surveillance system, using Bayes Theorem, if a prior probability of freedom is available. The key elements required for this calculation are:

a) To calculate sensitivity of the surveillance system:

- The sensitivity of the individual animal test;
- The number of animals tested;
- The design prevalence; and
- Depending on the interpretation of the test results, possibly also:
    - The specificity of the individual animal test; and
    - A threshold number of positive reactors, above which the group will be considered to be infected.

b) To calculate the probability of freedom, based on sensitivity

- Prior probability of infection

Input-based standards may attempt to cater for some of these variables, but generally are not able to capture all of them. As a result, the final probability of freedom when input-based standards are applied may vary significantly from situation to situation. It is therefore recommended that an output-based standard be used in this situation, namely the probability of freedom from infection/disease.

Selection of the value of these standards may be achieved in a number of ways: use of an arbitrary high figure (e.g. 95%, 99% or 99.9%); reference to a country's appropriate level of protection (ALOP); or by comparison with existing equivalent standards.

The later approach may be the most valid, as it reflects currently accepted levels of proof. The existing requirements for demonstration of freedom from TB in cattle in the EC Directives, represent an input-based standard, but may be used to estimate the effective probability of freedom (output) that they are intended to achieve.

Two cases are defined for declaring a cattle holding officially free from TB, based on whether the infection has been eliminated or whether the holding has been assembled from other free holdings.

At the holding level, the EC Directive does not explicitly define the design prevalence. However, the smallest possible design prevalence is one animal per herd. If all animals in the herd are tested, this means that the sensitivity of the herd test is equal to the sensitivity of the individual animal test, regardless of herd size.

The approach is illustrated using data related to the TB intradermal test. The individual animal sensitivity has been modeled as a Pert distribution, with a minimum value of 68%, most likely of 72% and a maximum of 95% (Francis et al., 1978). If it is (conservatively) assumed that nothing is known of the true status of a herd immediately after purported eradication, a value of 50% may be used for the prior probability of freedom. After two rounds of testing with negative results, the posterior probability of freedom is as shown below, with a mean of 94%, and a mode around 93%. This is based on the assumption of a 100 animal herd with a design prevalence of a single infected animal (1%).

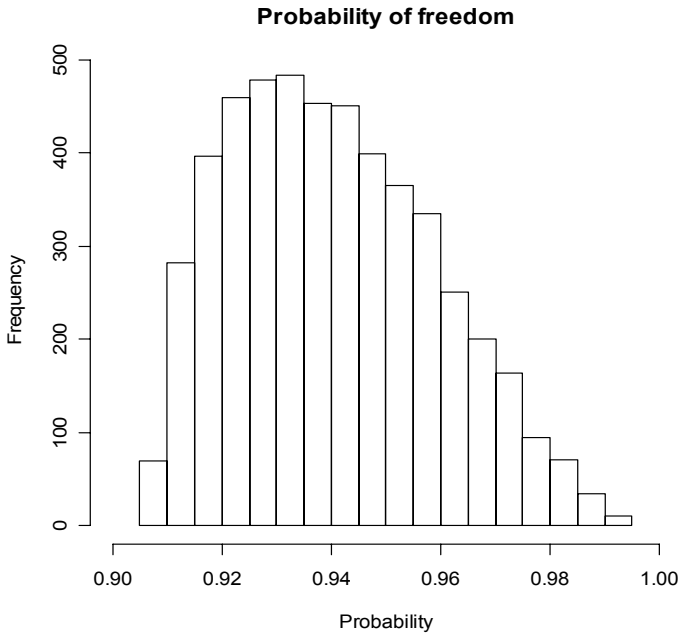


Figure 39 - Distribution of probability of holding freedom in the case of two negative comparative intradermal skin tests being applied to a 100 animal cattle herd from which TB has been recently eradicated.

For holdings assembled from other free holdings, assuming that the holdings had adhered to the biosecurity requirements of the Directive, the probability that the holdings of origin are free should be at least as high as that shown above. A further single test of the assembled holding would result in a probability of freedom as shown in figure 2 below, with a mean of 98.4%.

These calculations are based on the assumption that the consecutive tests of the animals within the herd are independent. As the period between tests is longer than the expected incubation period (infection to becoming a positive reactor on the tuberculin test), this assumption may be considered to be acceptable.

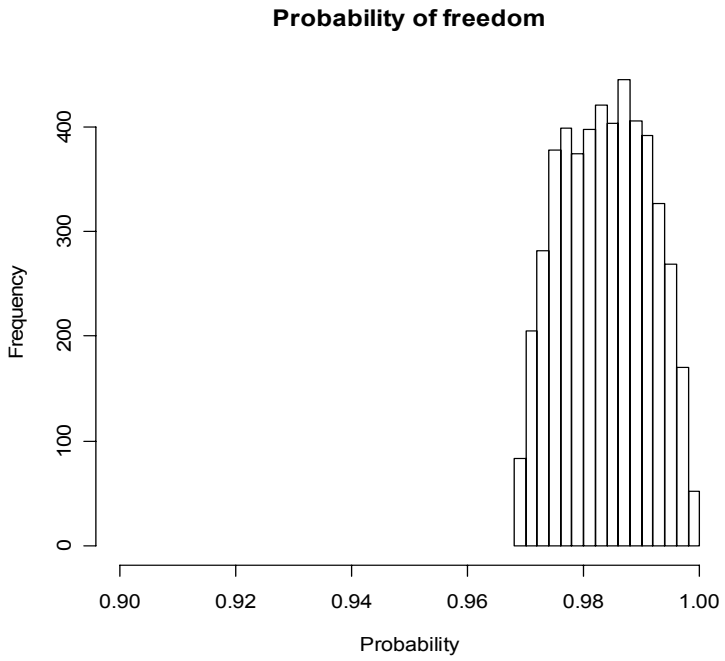


Figure 40 - Distribution of probability of holding freedom in the case of one negative comparative intradermal test being applied to a cattle herd assembled from other "officially" free holdings as presented in Figure 39.

The above analysis indicates that current standards to demonstrate that a holding is free from TB using the comparative skin tuberculin test alone are likely to achieve probabilities of freedom of between 91% and 99.9%, but would be generally in the range of 93% to 99%. This is consistent with the commonly used standards of 95% and 99% but suggests that demanding a probability of freedom of 99.9% would be inconsistent with current practice.

The choice between 95% and 99% as the preferred standard is somewhat arbitrary. However, most countries would view a system which allowed an incorrect classification of 1 holding in 20 to be unacceptable. It is therefore recommended that the standard to be adopted for classifying a holding as officially free from tuberculosis should be that it has a probability of freedom of 99% or greater. This means that in 1 out of 100 holdings can be infected.

5.2. Standards for calculation of probability of freedom in deer holdings

Design prevalence

The design prevalence is the theoretical level of disease present in a population that a surveillance activity would be able to detect. If we assume that, if present, disease would be at a high level in a herd, it would be relatively easy to detect or desired, and the herd-level sensitivity would be high. On the other hand, very low design prevalences mean that the disease is very rare, and many more animals need to be tested to detect the disease. In the case of tuberculosis (in contrast to rapidly transmitted diseases like foot and mouth disease), it is biologically feasible to have a very low prevalence of disease in a herd, so a low design prevalence should be used. Herd-level design prevalence values of 1%, 0.2% or 0.1% are mentioned in Directive 64/432/EEC, but these do not apply at the animal level. Similarly low levels have been applied at the animal level, however there is a logical constraint on the minimum design prevalence, which is that it must represent an integer number of animals. Herd sizes in Europe are generally relatively small, and a design prevalence of, say, 0.1% is only feasible in a herd of 1000 animals. As a result, it is recommended that, for the purposes of analysis of tuberculosis, a standard design prevalence of one infected animal per herd be

adopted. This will result in a variable prevalence (expressed as a percentage), but provide a meaningful value regardless of herd size.

Prior probability of freedom (country, region, zone or compartment)

Calculating the probability of freedom as indicated by a surveillance activity requires the use of Bayes theorem, and therefore needs a prior estimate of the probability of freedom. In the absence of previous evidence, it is recommended that a standard value of 0.5 be used as the prior probability. In contrast, where prior evidence of freedom has been collected, this prior should be used. Evidence that may contribute to the prior probability of freedom includes: The information needs to be summarised for the respective unit under consideration.

- Previous tuberculin herd tests (as described above)
- Other surveillance activities, such as
    - Full herd testing with other tests (e.g. ELISA)
    - Sampling from the herd
    - Abattoir testing
- Biosecurity – any issues which increase the risk of introduction of disease
    - Exposure to potentially infected wildlife
    - Introduction of animals from holdings which are not officially free
    - Other potential sources of infection

5.3. Definitions of freedom from TB in deer at different levels

5.3.1. Animal

Under Article 6 of Directive 92/65/EEC, animals that do not come from an officially free holding may be moved if they come from a holding that has had no recorded case of TB in the last 42 days, and they have tested negative to a single tuberculin intradermal test. The probability that any such animal is truly negative depends on the individual test performance and the prevalence of the disease in the herd of origin. In terms of clinical epidemiology, this probability called the negative predictive value (NPV = Pr(D-|T-). As this provision is for holdings that are not officially free, it may be assumed that the prevalence is greater than zero. The results of simulations based on the following assumptions and based on Se- value from this report, are shown below, and indicate that the probability that an individual animal may be free from infection ranges from 91.09% to 99.76% with a mean of 96.9% and a median of 97.11%.This has not taken into consideration that the Se in the assessment may be overestimated and the true value may therefore be lower.

	Min	Likely	Max
Prevalence	0.01	0.1	0.3

Assumptions: prevalence is stochastic

Sensitivity and specificity of the single skin test were based on the distributions produced by this study.

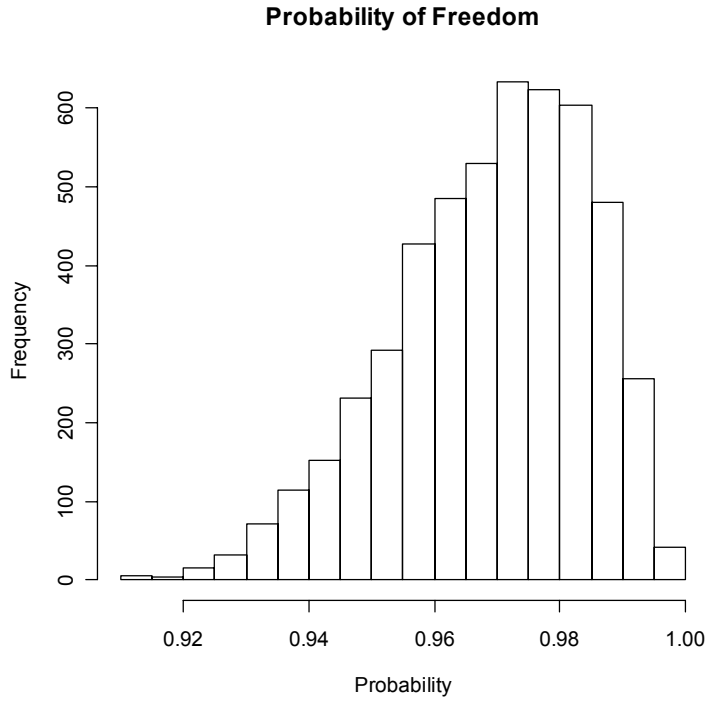


Figure 41 - Distribution of probability of freedom after a single comparative tuberculin scheme of individual animal coming from a non TB free deer holding.

Based on this analysis, it is clear that under many circumstances, the probability that an individual animal is free from disease on the basis of a single tuberculin test does not reach the target probability of 99%. The use of a single test to determine disease status is therefore not considered to be reliable. Instead, the recommended *definition of an officially free animal is an animal that comes from an officially free holding.*

The definition of a free animal used in this document is one that comes from a free holding. This raises the issue of how to deal with movements of an individual or small group of animals, before free holding status has been achieved.

The herd-level design prevalence used in this analysis is a single infected animal. This means that, regardless of herd size, if full herd testing is used, the (minimum) herd level sensitivity is the same as the sensitivity of the test on a single animal. This is because if there is assumed to be only one infected animal in the herd. When all animals are tested the probability of detecting the herd as infected is the same as the probability of detecting that single positive animal as infected. Thus, the assumption of a single animal being infected as minimum level of within-herd prevalence is conservative.

This principle can be extended to very small herds, and (with caution) to the extreme case – a 'herd' of a single animal. It is logical to apply the same principle and requirements to a single animal as have been used at the herd level.

The difficulty with this approach is as follows:

- None of the tests are able to achieve the target 99% sensitivity with a single test neither at the herd nor animal level. Repeat testing is therefore required (a testing interval of 1 year is assumed, although any interval greater that 6 months could be considered).
- Part of the principle of repeat testing over an extended time is the assumption that, if disease were present in just one animal, it would:
    - Develop in that animal over time, to a detectable form, and

- Spread from that animal to others in the group, increasing the probability of detection.

- While the first assumption holds when considering a single animal, the second does not. This decreases our ability to detect disease when using repeated tests on a single animal and an anergic animal may test negative for cell mediated immunity irrespectively the number of tests.

In practice, the movement of animals from non free holdings to free should not be allowed. Movement from non free to non free is considered in Chapter 9.

5.3.2. Holding

As previously discussed, an output-based standard (namely, probability of freedom) is recommended as the basis for the definition of a free holding, as it unambiguous, directly measures the value of interest, and, unlike definitions based on testing protocols or herd sensitivity, can incorporate all relevant factors (such as previous tests and biosecurity). In order to maintain a reasonable level of assurance of freedom, yet at the same time be consistent with existing standards (*i.e.* those established by Directive 64/432/EEC for bovine tuberculosis), it is recommended that the *definition for a deer holding officially free from tuberculosis is one that has a probability of freedom of at least 99%, based on an analysis of the different tests performed on the holding and the biosecurity measures in place to prevent introduction of tuberculosis*. It is acknowledged that this definition may appear more difficult to implement than definitions couched in other terms, however the next section will provide guidance as to the testing regimes that are able to meet this definition.

5.3.3. Country, Zone, Region or Compartment

These groupings represent collections of animals at scales larger than a single holding. Consideration must be given to definitions when using these terms. In all cases, the population of interest must be clear, especially whether it includes wild deer as well as farmed deer. It is recommended that, in this context, all definitions refer only to farmed deer, as previously defined. Recommended definitions therefore include:

5.3.3.1. Country

All farmed deer within a Member State.

5.3.3.2. Zone or Region

In EU legislation "region" is defined in 64/432/EEC (in Article 2 (2) (p)) as "part of a Member State's territory which is at least 2 000 km^2 in area and which is subject to inspection by the competent authorities and includes at least one of the following administrative regions..."

The terms zone and region are both in common use, and under the OIE Terrestrial Animal Code are treated as synonyms (although Zone is used more frequently). It is defined by OIE as a clearly defined part of a country containing an animal subpopulation with a distinct health status with respect to a specific disease for which required surveillance, control and biosecurity measures have been applied for the purpose of international trade.

5.3.3.3. Compartment

The OIE Code (OIE, 2007) defines as compartment as one or more establishments under a common biosecurity management system containing an animal subpopulation with a distinct health status with respect to a specific disease or specific diseases for which required surveillance, control and biosecurity measures have been applied for the purpose of international trade.

5.3.3.4. Summary on the existing / recomended standard definition of freedom at the country/ zone/ region/ compartment level

The OIE Terrestrial Animal Code chapter for Bovine Tuberculosis states that, to qualify as free, in a country, zone or compartment:

"regular and periodic testing of all cattle, water buffalo and wood bison herds has shown that at least 99.8% of the herds and 99.9% of the animals in the country, *zone* or *compartment* have been found free from bovine tuberculosis and the percentage of herds confirmed infected with *M. bovis* has not exceeded 0.1% per year for 3 consecutive years" (OIE, 2007)

In order to remain consistent with existing standards, it is recommended that this definition be used as the basis for a definition of an area free from TB in deer, with appropriate modification.

This definition relates to the discussion of the concept of freedom in section 5.1.1, in that it allows for a country to be considered officially free, while a small percentage of known infected herds still remain.

In this report, an area (country, zone, region or compartment) may thus be considered free from tuberculosis in deer if at least 99.8% of holdings in the area have achieved officially tuberculosis-free status (according to the above definition for a holding), for three consecutive years, given that an official control programme is in place (based on OIE, 2007).

In practice this means that for areas where the total number of deer holdings is less than 500, *all* holdings must be officially free. Where the number of holdings is between 501 and 1000, it is possible to have a single known infected holding, and still be considered as officially free.

Alternatively, an area may be considered free if it is able to demonstrate an equivalent status based on probabilistic methods.

6. ASSESSMENT OF SURVEILLANCE STRATEGIES TO DEMONSTRATE FREEDOM FROM TB IN DEER HOLDINGS

6.1. Overview

A modeling approach was used to assess the ability of a range of testing strategies, applied in a variety of scenarios, to provide adequate proof of freedom from TB in deer. Having determined the required outcome of the surveillance (achieving a probability of freedom from infection ≥ 99%), the aim was to determine which testing strategies are able to meet this required outcome.

The first part of the analysis aimed to determine the required overall surveillance system sensitivity through its various component sensitivities in order to achieve 99% probability of freedom. This is based on repeated testing over time, considering the risk of introduction of disease (section 6.2- 6.4.2):

The approach involved:

- Define the target probability of freedom that represents adequate proof of absence of infection (99%) (section 6.2)
- Assess the ability of ongoing testing in different circumstances to reach this level of proof. This involved calculation of the cumulative probability of freedom over specific time period, considering:
    1) the sensitivity of the surveillance components being used including testing and preventive measures (section 6.3); and
    2) the probability of introduction of new infection (section 6.4).
- This process identified the minimum sensitivity of the surveillance system required to effectively

a) demonstrate freedom within a defined time period (section 6.3.1), and

b) maintain adequate proof of freedom for a specific period of time (section 6.3.2)

- The inputs to the above calculation where then considered separately. The probability of introduction of disease was calculated for a variety of scenarios, considering the risk of introduction through (section 6.4.1 – 6.4.3):

  a) Contact with infected species outside the holding (including wildlife and other non-deer farmed species) (section 6.4.1); and

  b) Introduction of live animals to the holding (section 6.4.2)

- The overall sensitivity of the surveillance was then considered. Surveillance may involve one or more surveillance activities, and it is necessary to consider the combined impact of all surveillance components. A table estimating the overall surveillance system sensitivity was developed based on the combination of two separate surveillance activities. This identified the ranges and combinations of surveillance component sensitivities that are suitable for use in demonstrating freedom from TB infection. (section 6.5)

The second part of the analysis examined combinations of tests and testing strategies to determine if they are able to meet the sensitivity requirements determined in the first part (section 6.6).

6.2. Required probability of freedom

Based on the discussion presented in section 5.3.2, the required probability of freedom ('standard of proof") to be used was set at 99%. This is consistent with the existing EU requirements to demonstrate freedom from TB in cattle holdings (Directive 64/432/EEC for bovine tuberculosis).

6.3. Surveillance requirements

According to Chapter 3, none of the available tests is able to provide adequate evidence of freedom (>99%) in a single test at a single point in time. Repeat testing is therefore required. In theory, an arbitrary interval between tests may be used. However, with TB in deer, retesting at an interval of less than 120 days is unlikely to provide useful results. This is mainly due to the chronicity of infection and the potential for long-term interference to the immune status of deer (resulting in false-negative test results) as a result of earlier testing. For this reason, a test interval of 1 year has been assumed. Some procedures as part of detection of infection (such as meat inspection or post mortem examination of moribund or dead deer) cannot be repeated, and are carried out continuously. For these tests, a cumulative outcome for an analysis period of one year has been used.

6.3.1. Achieving free status

The probability of freedom from infection can be calculated using Bayesian revision, based on:

- The prior probability of infection; and
- The sensitivity of the surveillance.

This would generally refer to events during a single year of surveillance but would include a longer time period at the start of the surveillance effort.

The formula for the posterior probability of freedom is:

Posterior = (1-Prior) / ((1-Prior) + (Prior * (1-Sensitivity))

Where Prior refers to the prior probability of TB in the holding.

Note that this approach assumes that the specificity of the surveillance system is 100%. Clearly the specificity of the individual tests is less than 100%, even when combined with multiple tests. However, the specificity of the *surveillance system* which includes the final conclusion about holding status may be assumed to be 100%. This is because any holding that is found or under suspicion to be infected (which may in fact be a false positive), is no longer a candidate for free status, and is therefore no longer considered.

The prior probability of the holding being infected at the commencement of surveillance is conservatively assumed to be 0.5. If prior information from earlier surveillance is available, this may be analysed year by year to provide an adjusted posterior estimate of the probability of freedom, allowing a possibly much higher value to be used.

When combining the results of surveillance over multiple time periods (t=1,2,…), the posterior from the previous time period ($Post_{t-1}$) can be used as the prior for the given time period ($Prior_t$). However, in this case, it needs to be first adjusted to take into account the probability of introduction of disease during the time period, using the following formula:

PriorP2 = Posterior(P1) + P(Intro) – (PosteriorP1 * P(Intro))

Priort = Postt-1 + P(Intro) – (Postt-1 * P(Intro))

Where P(Intro) is referring to the probability of introduction between period 1 and period 2.

Table 34 presents the number of years of surveillance that would be required for a holding to achieve a probability of freedom of 99%, based on differing surveillance systems sensitivities and risks of introduction of disease.

The following conclusions can be drawn from the results of this table:

- It is only possible to achieve the required probability of freedom in a single year if the sensitivity of surveillance is ≥ 99%. This is effective regardless of the probability of introduction of disease;
- It is possible to achieve the required probability within three years when the risk of introduction is greater than 1% and less than 8%, however, a surveillance system sensitivity of > 90% is required;
- The sensitivity of the surveillance system must be ≥ 80% to achieve the required probability within 3 years, even when the risk of introduction is low (< 0.05%);
- With a surveillance system with low sensitivity (< 80%), requires more than 3 years is necessary to achieve the required probability, and can only be used when the risk of introduction is low (<~1%).

In summary and based on the risk of introduction and as example:

When there is no risk of introduction either from wild life or deer imports:

- Herds will need to be tested annually for at least 2 years using a surveillance system with a Sensitivity of at least 90%;
- Herds will need to be tested annually for at least 9 years using a surveillance system with a Sensitivity of 40%;

When the risk of introduction is low to moderate (0% to 5%):

- Herds will need to be tested annually for at least 2 years using a surveillance system with a sensitivity of at least 93%;
- Herds will need to be tested annually for at least 3 years using a surveillance system with a sensitivity of at least 90%;

When the risk of introduction is moderate to high (5% to10%):

- Herds will need to be tested annually for at least 3 years using a surveillance system with a sensitivity of at least 92%;

- Herds will need to be tested annually for at least 2 years using a surveillance system with a sensitivity of at least 95%.

Table 34 - Years of surveillance required to achieve a probability of freedom of > 99% given varying surveillance sensitivities and probabilities of introduction of disease.

Surveillance Se	P(Intro)																					
	0	**0.001**	**0.002**	**0.003**	**0.004**	**0.005**	**0.01**	**0.015**	**0.02**	**0.025**	**0.03**	**0.035**	**0.04**	**0.05**	**0.06**	**0.07**	**0.08**	**0.09**	**0.1**	**0.2**	**0.3**	**0.4**
0.4	9	10	10	11	11	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	7	7	7	8	8	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.6	6	6	6	6	6	6	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.65	5	5	5	5	5	5	6	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.7	4	4	4	4	4	5	5	5	6	-	-	-	-	-	-	-	-	-	-	-	-	-
0.75	4	4	4	4	4	4	4	4	5	5	-	-	-	-	-	-	-	-	-	-	-	-
0.8	3	3	3	3	3	3	4	4	4	4	4	5	-	-	-	-	-	-	-	-	-	-
0.85	3	3	3	3	3	3	3	3	3	3	3	3	4	4	-	-	-	-	-	-	-	-
0.9	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	-	-	-	-	-
0.91	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	4	-	-	-	-
0.92	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	4	-	-	-
0.93	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	-	-	-
0.94	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	-	-	-
0.95	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	-	-	-
0.96	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	-	-	-
0.97	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	-	-
0.98	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	-
0.99	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

" - " indicates that the time required is either greater than 20 years or that it is not possible to achieve a probability of freedom of 99% based on this combination of surveillance sensitivity and P(intro).

Based on the previous simplifications, the required sensitivity can be calculated from Table 35.

Table 35 - Sensitivity (%) required to achieve 99% probability of freedom from infection based on the number of consecutive years of testing and the annual probability of introduction.

P(Intro) %	1 year	2 years	3 years	4 years	5 years
0	99	90	80	70	65
0.5	99	91	80	75	65
1	99	91	85	75	70
5	99	93	90	85	-
10	99	95	93	92	-
20	99	97	-	-	-

For test combinations with a sensitivity of less than 90%, it is only possible to demonstrate freedom by testing for 3 years or more, but and only when the probability of introduction is less than ~1%.

6.3.2. Maintaining free status

Once a probability of free status of 99% has been achieved, it must then be maintained. The only reason why the probability of freedom would decrease is through the introduction of infection (if the holding had perfect biosecurity, there would be no change in the probability of freedom, and no further testing would be required).

The calculations for the probability of the free status are exactly as described before. However, unlike the calculations above to achieve free status, the prior when maintaining free status is 0.01 (or 1-99%, the probability of freedom) assuming that a holding has acquired the nominal level of 99% in compliance with the appropriate testing strategy. The following table (Table 36) and figure (Figure 41) indicates the minimum annual system sensitivity required to continuously maintain the probability of freedom above 99%.

Table 36 -Indication of the minimum annual system sensitivity (Se) required to continuously maintain the probability of freedom above 99% at different probabilities of introduction of disease (P(Intro)).

P(Intro)	Se
0.001	0.1
0.002	0.18
0.003	0.25
0.004	0.3
0.005	0.35
0.006	0.39
0.007	0.43
0.008	0.46
0.009	0.49
0.01	0.52
0.015	0.62
0.02	0.69
0.025	0.73
0.03	0.77
0.035	0.8
0.04	0.82
0.045	0.84
0.05	0.86
0.06	0.88
0.07	0.9

0.08	0.91
0.09	0.92
0.1	0.93
0.2	0.98
0.3	0.99

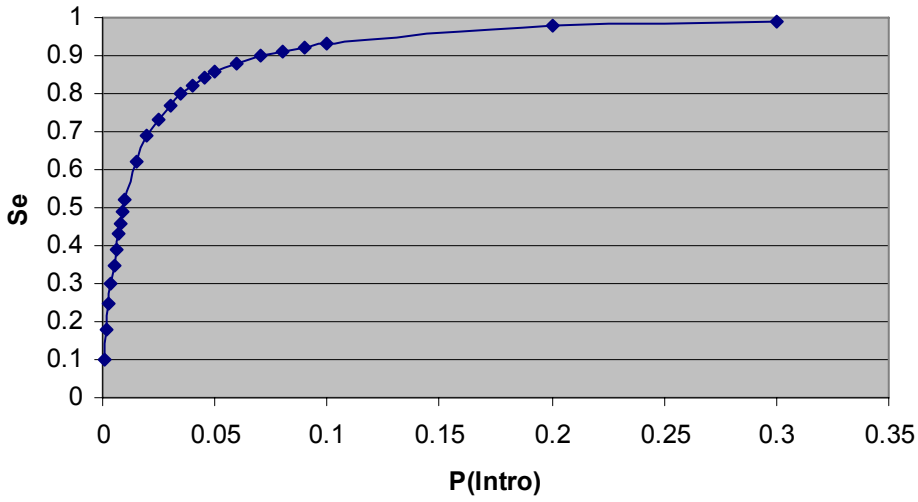
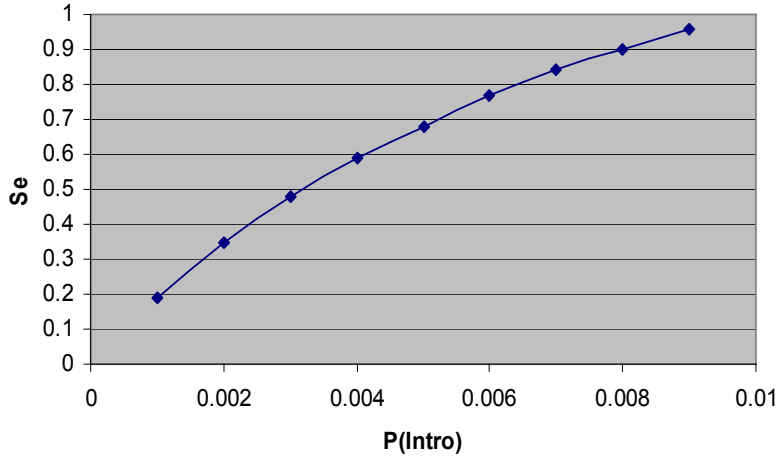


Figure 42 - Indication of the minimum annual system sensitivity (Se) required to continuously maintain the probability of freedom above 99% at different probabilities of introduction of disease (P(Intro)).

In summary:

- Risk of introduction <1%
    - Annual testing with > 50% Se
- Risk of introduction >1% and < 5%
    - Annual testing with >86% Se
- Risk of introduction >5% and <20%
    - Annual testing with >98% Se

When the probability of introduction of disease is very low (<1%) it is possible to maintain a probability of freedom > 99% by testing every second year, as indicated in the table and figure below (Figure 43 and Table 37). It is not possible to achieve this level of proof by testing less frequently than every two years.



P(intro)	Se
0.001	0.19
0.002	0.35
0.003	0.48
0.004	0.59
0.005	0.68
0.006	0.77
0.007	0.84
0.008	0.9
0.009	0.96

Figure 43 and Table 37 - Surveillance sensitivity required to achieve a probability of holding freedom of > 99% when testing every second year (prior probability in first year = 0.01) at different probabilities of introduction of disease (P (Intro)).

6.4. Introduction of infection

As can be seen from the above discussion, the risk of introduction of disease into the holding plays a significant role in determining the amount of surveillance that is required to achieve the required probability of freedom from TB. This analysis considers two separate routes of infection:

- from animals outside the fence (e.g. contact with wildlife (deer, badgers etc), and farmed non-deer species such as cattle)
- from animals inside the fence (introduction of infected deer).

Other routes are possible (e.g. from humans), but are assumed to play an insignificant role when compared to the first two.

6.4.1. Introduction from outside the fence

Little data is available regarding the risks of introduction of TB into deer holdings through contact with wildlife or non-deer farmed species. The following is therefore based on data relating to infections of cattle holdings with TB from badgers as the main wildlife reservoir.

If TB is established in a wildlife population, and the farmed deer have contact with that population, there is a risk of transfer of infection. The following data were used to estimate this risk:

- In Ireland in 2005, it was estimated that 1.75% of all holdings broke down due to infection from wildlife; and
- In England, the proportion of breakdowns ascribed to wildlife was 23.2% (confidence intervals from 12.4% to 32.7%). Assuming an overall holding breakdown rate of 5% (as observed in Ireland), this corresponds to a wildlife-caused breakdown rate of between 0.25% and 2%.

The risk for introduction from non deer farmed species should also be considered. This risk, additional to the introduction from wild life can largely be assumed to be proportional to the TB- status (prevalence) of the region or MS for the holdings under consideration.

Based on these figures, the range of likely values for the risk of introduction of disease from wildlife and non-deer farmed species, as used in this model, are shown below. These values apply to proposed "typical probability values" of zones or regions.

- Wildlife and non-deer farmed species found to be free from infection: 0% (only if justified by surveillance)
- Low prevalence of infection in wildlife and in non-deer farmed species: 0.25% (only if justified by surveillance)
- Moderate prevalence of infection in wildlife and in non-deer farmed specie: 1% (only if justified by surveillance)
- High prevalence of infection in wildlife and in non-deer farmed specie: 2% (default assumption in the absence of surveillance information)

In addition, identified local areas of very high prevalence ('hot spots') will be assumed to have a higher risk of introduction of infection.

- Hot spot: 10%

The numbers presented above related with the risk of introduction of TB from wild life and non deer farmed species are indicative and have been used in the freedom of infection model. It would be important to MS to clarify the risk posed by wild life species.

These risks may be moderated by limiting contact between farmed deer and other potential carrier species (such as wild deer, badgers or farmed cattle). The only practical approach to this is the use of fencing, and this may only be considered as a factor when:

- The fence is able to effectively stop the entry of existing carrier species
- The fence prevents direct contact or indirect contact (contaminated pasture etc) between farmed deer and carrier species (e.g. by using double fencing)

The nature of the fencing depends on the carrier species present. If domestic cattle are the only local carrier species, one cattle-proof fence and one deer proof fence would meet these requirements. If infected wild deer are present, this would require double deer-proof fencing. If badgers are present, specialized badger-proof fencing is required.

When the previous conditions are met, fencing is assumed to decrease the risk of introduction from wildlife to 10% of the risk when no fencing is used (i.e. a 90% reduction in risk).

6.4.2. Introduction through importation of deer into the holding

The risk of introduction of infection into the holding through imports of live deer into the holding is calculated as follows.

$$P(intro) = 1 - (1 - (P * (1 - Se))) \wedge N$$

Where:

P: prevalence of infection in source population

Se: Sensitivity of screening test applied to imported animals

N: number of animals imported

Note that the sensitivity of the screening test is the effective sensitivity. This means that the individual animal sensitivity may be relatively low, but if it is applied as a herd tests (given that all introduced animals originate from one holding), and the imports only permitted if the whole herd tests negative, then the herd level sensitivity can be applied.

It is important to note EU regulations on movement testing in this context. This technical discussion considers the possibility of pre-movement testing (at the farm of origin) as well as post-movement testing (e.g. during a quarantine period at the discretion of the owner of the farm of destination).

Table 38 - Probability of introduction of infection, through importation of live animals from the same holding of origin, with no application of pre-import holding screening tests.

P(holding of origin)	Animals imported									
	1	2	3	4	5	6	7	8	9	10
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.010	0.010	0.020	0.030	0.039	0.049	0.059	0.068	0.077	0.086	0.096
0.020	0.020	0.040	0.059	0.078	0.096	0.114	0.132	0.149	0.166	0.183
0.030	0.030	0.059	0.087	0.115	0.141	0.167	0.192	0.216	0.240	0.263
0.040	0.040	0.078	0.115	0.151	0.185	0.217	0.249	0.279	0.307	0.335
0.050	0.050	0.098	0.143	0.185	0.226	0.265	0.302	0.337	0.370	0.401
0.060	0.060	0.116	0.169	0.219	0.266	0.310	0.352	0.390	0.427	0.461
0.070	0.070	0.135	0.196	0.252	0.304	0.353	0.398	0.440	0.480	0.516
0.080	0.080	0.154	0.221	0.284	0.341	0.394	0.442	0.487	0.528	0.566
0.090	0.090	0.172	0.246	0.314	0.376	0.432	0.483	0.530	0.572	0.611
0.100	0.100	0.190	0.271	0.344	0.410	0.469	0.522	0.570	0.613	0.651
0.150	0.150	0.278	0.386	0.478	0.556	0.623	0.679	0.728	0.768	0.803
0.200	0.200	0.360	0.488	0.590	0.672	0.738	0.790	0.832	0.866	0.893
0.250	0.250	0.438	0.578	0.684	0.763	0.822	0.867	0.900	0.925	0.944
0.300	0.300	0.510	0.657	0.760	0.832	0.882	0.918	0.942	0.960	0.972
0.350	0.350	0.578	0.725	0.821	0.884	0.925	0.951	0.968	0.979	0.987
0.400	0.400	0.640	0.784	0.870	0.922	0.953	0.972	0.983	0.990	0.994
0.450	0.450	0.698	0.834	0.908	0.950	0.972	0.985	0.992	0.995	0.997
0.500	0.500	0.750	0.875	0.938	0.969	0.984	0.992	0.996	0.998	0.999

P – Prevalence

Table 39 - Probability of introduction infection through importation of live animals, from the same holding of origin, following a negative holding screening test with a sensitivity of 50%.

P(holding of origin)	Animals imported									
	1	2	3	4	5	6	7	8	9	10
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.010	0.005	0.010	0.015	0.020	0.025	0.030	0.034	0.039	0.044	0.049
0.020	0.010	0.020	0.030	0.039	0.049	0.059	0.068	0.077	0.086	0.096
0.030	0.015	0.030	0.044	0.059	0.073	0.087	0.100	0.114	0.127	0.140
0.040	0.020	0.040	0.059	0.078	0.096	0.114	0.132	0.149	0.166	0.183
0.050	0.025	0.049	0.073	0.096	0.119	0.141	0.162	0.183	0.204	0.224
0.060	0.030	0.059	0.087	0.115	0.141	0.167	0.192	0.216	0.240	0.263
0.070	0.035	0.069	0.101	0.133	0.163	0.192	0.221	0.248	0.274	0.300
0.080	0.040	0.078	0.115	0.151	0.185	0.217	0.249	0.279	0.307	0.335
0.090	0.045	0.088	0.129	0.168	0.206	0.241	0.276	0.308	0.339	0.369
0.100	0.050	0.098	0.143	0.185	0.226	0.265	0.302	0.337	0.370	0.401
0.150	0.075	0.144	0.209	0.268	0.323	0.374	0.421	0.464	0.504	0.541
0.200	0.100	0.190	0.271	0.344	0.410	0.469	0.522	0.570	0.613	0.651
0.250	0.125	0.234	0.330	0.414	0.487	0.551	0.607	0.656	0.699	0.737
0.300	0.150	0.278	0.386	0.478	0.556	0.623	0.679	0.728	0.768	0.803
0.350	0.175	0.319	0.438	0.537	0.618	0.685	0.740	0.785	0.823	0.854
0.400	0.200	0.360	0.488	0.590	0.672	0.738	0.790	0.832	0.866	0.893
0.450	0.225	0.399	0.535	0.639	0.720	0.783	0.832	0.870	0.899	0.922
0.500	0.250	0.438	0.578	0.684	0.763	0.822	0.867	0.900	0.925	0.944

P – Prevalence

Table 40 - Probability of introduction infection through importation of live animals, from the same holding of origin, following a negative holding screening test with a sensitivity of 90%.

P(holding of origin)	Animals imported									
	1	2	3	4	5	6	7	8	9	10
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.010	0.001	0.002	0.003	0.004	0.005	0.006	0.007	0.008	0.009	0.010
0.020	0.002	0.004	0.006	0.008	0.010	0.012	0.014	0.016	0.018	0.020
0.030	0.003	0.006	0.009	0.012	0.015	0.018	0.021	0.024	0.027	0.030
0.040	0.004	0.008	0.012	0.016	0.020	0.024	0.028	0.032	0.035	0.039
0.050	0.005	0.010	0.015	0.020	0.025	0.030	0.034	0.039	0.044	0.049
0.060	0.006	0.012	0.018	0.024	0.030	0.035	0.041	0.047	0.053	0.058
0.070	0.007	0.014	0.021	0.028	0.035	0.041	0.048	0.055	0.061	0.068
0.080	0.008	0.016	0.024	0.032	0.039	0.047	0.055	0.062	0.070	0.077
0.090	0.009	0.018	0.027	0.036	0.044	0.053	0.061	0.070	0.078	0.086
0.100	0.010	0.020	0.030	0.039	0.049	0.059	0.068	0.077	0.086	0.096
0.150	0.015	0.030	0.044	0.059	0.073	0.087	0.100	0.114	0.127	0.140
0.200	0.020	0.040	0.059	0.078	0.096	0.114	0.132	0.149	0.166	0.183
0.250	0.025	0.049	0.073	0.096	0.119	0.141	0.162	0.183	0.204	0.224
0.300	0.030	0.059	0.087	0.115	0.141	0.167	0.192	0.216	0.240	0.263
0.350	0.035	0.069	0.101	0.133	0.163	0.192	0.221	0.248	0.274	0.300
0.400	0.040	0.078	0.115	0.151	0.185	0.217	0.249	0.279	0.307	0.335
0.450	0.045	0.088	0.129	0.168	0.206	0.241	0.276	0.308	0.339	0.369
0.500	0.050	0.098	0.143	0.185	0.226	0.265	0.302	0.337	0.370	0.401

P – Prevalence

The preceding three tables indicate the risk associated with importing different numbers of animals originating from the same holding and demonstrates that the risk of introduction of

infection is considerably decreased if the originating holding is subjected to a pre-movement testing. The blue shaded areas result in a risk of less than 1%, while the yellow have a risk of less than 10%. As noted in the previous section, a very high level of surveillance is required if the risk of introduction is up to 10%. If the risk of introduction is ≤ 1%, it is more feasible to achieve the required probability.

If the source holding has been demonstrated to be free from disease, the risk of introduction is 0. Otherwise the table below summarises the different risks.

Table 41 - Summarised probability of introduction of infection through different numbers of imported animals [1], different prevalences in the holding of origin and different pre-import herd screening tests.

Screening	Prevalence	1 animal	2 – 5 animals	6 – 10 animals
0% (no testing)	≤ 5%	5%	25%	40%
	>5% and ≤ 10%	10%	40%	65%
50%	≤ 5%	2.5%	12%	25%
	>5% and ≤ 10%	5%	25%	40%
90%	≤ 5%	0.5%	2.5%	5%
	>5% and ≤ 10%	1%	5%	10%

[1] all imports from the same holding of origin

Green shading indicates combinations that result in a probability of introduction ≤5%.

6.4.3. Combined probability of introduction

The probability of introduction of infection into a holding from either imports or wildlife and or domesticated non deer species can be calculated using the following formula:

P(Intro)c = P(Intro)I + P(Intro)W – (P(Intro)I * P(Intro)W)

Where:

P(Intro)c = combined probability of introduction

P(Intro)I = probability of introduction through imports

P(Intro)W= probability of introduction through wildlife

The combined risk of introduction can be calculated from Table 42.

Table 42 - Combined probability of introduction from imports and wildlife.

Probability of Introduction from imports	Probabilities of Introduction from wildlife or other non-deer farmed species						
	Not infected	Fenced[1] /probability of introduction			Unfenced[1] /probability of introduction		
	0.00%	0.03%	0.10%	0.20%	0.25%	1.00%	2.00%
0.0%	0.0%	0.0%	0.1%	0.2%	0.3%	1.0%	2.0%
0.5%	0.5%	0.5%	0.6%	0.7%	0.7%	1.5%	2.5%
1.0%	1.0%	1.0%	1.1%	1.2%	1.2%	2.0%	3.0%
2.5%	2.5%	2.5%	2.6%	2.7%	2.7%	3.5%	4.5%
5.0%	5.0%	5.0%	5.1%	5.2%	5.2%	6.0%	6.9%
10.0%	10.0%	10.0%	10.1%	10.2%	10.2%	10.9%	11.8%
12.0%	12.0%	12.0%	12.1%	12.2%	12.2%	12.9%	13.8%
25.0%	25.0%	25.0%	25.1%	25.2%	25.2%	25.8%	26.5%
40.0%	40.0%	40.0%	40.1%	40.1%	40.2%	40.6%	41.2%
65.0%	65.0%	65.0%	65.0%	65.1%	65.1%	65.4%	65.7%

1) Consider that fence result in a risk reduction to 10% of the risk whitout fence

The probability of introduction from wildlife or other non-deer farmed species in the table above is based on three situations: in the first, there is no infection present in the wildlife (probability = 0%); in the second, infection is present but the farm is fenced; and in the third, unfenced. For the latter one, three typical values (from section 6.4.1) are provided, based on high medium or low density and/or prevalence amongst reservoirs. For the fenced holding, these values are reduced by 90%.

Demonstration of freedom using the available tests is only possible when the probability of introduction is relatively low (below about 10% as shown by the table 42. The areas in the above table where the combined probability of introduction is below 10% have been shaded. When the probability is less than 1% (shaded blue) a wider range of test combinations are available for demonstration of freedom.

6.5. Sensitivity of the surveillance system

6.5.1. Combination of components

Multiple different components of a surveillance system can contribute to the probability of freedom. Table 43 indicates the combined system sensitivity that can be achieved with various combinations, after considering the sensitivity of each system component. These calculations assume that each component is independent. Lack of independence between the system components will decrease the combined sensitivity by varying degrees, depending on the nature of the dependence between the components. In the extreme case, where all the same animals are included in both systems, and tested at around the same time, the contribution of one of the components is effectively reduce to zero. More often, dependence results in a smaller decrease in the combined sensitivity.

Table 43 - Combined sensitivity of two surveillance system components, assuming independence.

	C2															
C1	**0.05**	**0.1**	**0.15**	**0.2**	**0.25**	**0.3**	**0.35**	**0.4**	**0.45**	**0.5**	**0.55**	**0.6**	**0.65**	**0.7**	**0.75**	**0.8**
0.1	0.145	0.190	0.235	0.280	0.325	0.370	0.415	0.460	0.505	0.550	0.595	0.640	0.685	0.730	0.775	0.820
0.2	0.240	0.280	0.320	0.360	0.400	0.440	0.480	0.520	0.560	0.600	0.640	0.680	0.720	0.760	0.800	0.840
0.3	0.335	0.370	0.405	0.440	0.475	0.510	0.545	0.580	0.615	0.650	0.685	0.720	0.755	0.790	0.825	0.860
0.4	0.430	0.460	0.490	0.520	0.550	0.580	0.610	0.640	0.670	0.700	0.730	0.760	0.790	0.820	0.850	0.880
0.5	0.525	0.550	0.575	0.600	0.625	0.650	0.675	0.700	0.725	0.750	0.775	0.800	0.825	0.850	0.875	0.900
0.6	0.620	0.640	0.660	0.680	0.700	0.720	0.740	0.760	0.780	0.800	0.820	0.840	0.860	0.880	0.900	0.920
0.7	0.715	0.730	0.745	0.760	0.775	0.790	0.805	0.820	0.835	0.850	0.865	0.880	0.895	0.910	0.925	0.940
0.8	0.810	0.820	0.830	0.840	0.850	0.860	0.870	0.880	0.890	0.900	0.910	0.920	0.930	0.940	0.950	0.960
0.9	0.905	0.910	0.915	0.920	0.925	0.930	0.935	0.940	0.945	0.950	0.955	0.960	0.965	0.970	0.975	0.980
0.91	0.915	0.919	0.924	0.928	0.933	0.937	0.942	0.946	0.951	0.955	0.960	0.964	0.969	0.973	0.978	0.982
0.92	0.924	0.928	0.932	0.936	0.940	0.944	0.948	0.952	0.956	0.960	0.964	0.968	0.972	0.976	0.980	0.984
0.93	0.934	0.937	0.941	0.944	0.948	0.951	0.955	0.958	0.962	0.965	0.969	0.972	0.976	0.979	0.983	0.986
0.94	0.943	0.946	0.949	0.952	0.955	0.958	0.961	0.964	0.967	0.970	0.973	0.976	0.979	0.982	0.985	0.988
0.95	0.953	0.955	0.958	0.960	0.963	0.965	0.968	0.970	0.973	0.975	0.978	0.980	0.983	0.985	0.988	0.990
0.96	0.962	0.964	0.966	0.968	0.970	0.972	0.974	0.976	0.978	0.980	0.982	0.984	0.986	0.988	0.990	0.992
0.97	0.972	0.973	0.975	0.976	0.978	0.979	0.981	0.982	0.984	0.985	0.987	0.988	0.990	0.991	0.993	0.994
0.98	0.981	0.982	0.983	0.984	0.985	0.986	0.987	0.988	0.989	0.990	0.991	0.992	0.993	0.994	0.995	0.996
0.99	0.991	0.991	0.992	0.992	0.993	0.993	0.994	0.994	0.995	0.995	0.996	0.996	0.997	0.997	0.998	0.998

6.6. Assessment of individual surveillance components

Under the different scenarios described above, surveillance to achieve or maintain a probability of freedom ≥99% requires a system's sensitivity between 35% and 99%. At the lower end (that required to maintain confidence of infection freedom when the risk of introduction is very low), this can be achieved by combining multiple system components, each with sensitivities as low as 30% and 10%. The range of potentially useful sensitivities of each component of the surveillance system therefore ranges from 10% to 99%.

At the upper end of this range, sensitivity achieved by combinations of different tests is shown in the table 44. In this table the performance of different screening tests combined with different confirmatory tests is shown, including screening tests performed on live animals (100% of the herd tested), and those on dead animals (only a proportion of the herd tested). Where only a proportion of the herd is tested, a herd size of 100 animals is used for illustrative purposes. The Relative Risk (RR) indicates the effect of biased or targeted sampling and measures the relative risk of a sampled animal being infected compared to a non-sampled animal (RR=1 when the entire herd is sampled). As the whole herd is not sampled, the following 6 different sampling strategies were considered:

Screening	Sampled	RR
INSP	20%	0.8
NECR	5%	1.5
INSP	20%	1
NECR	5%	1
INSP	10%	1
NECR	2%	1

* RR = Relative risk of tested animals being infected (relative to non-tested)

This is a vast simplification of the possible options but it means:

- It is assumed that 20% or 10% of the herd go for slaughter and meat inspection each year;
- It is assumed that 5% or 2% of the herd die and is necropsied each year;
- It is assumed that animals that are sent to slaughter are either less likely (0.8) or just as likely (1) to be infected as the rest of the herd;
- It is assumed that animals that are sick or die are more likely (1.5) or just as likely (1) to be infected as the rest of the herd.

Due to the variation in test performance, combined sensitivities have been treated as distributions. The figures in the table indicated what proportion of the distribution for a given testing strategy is greater or equal to the target sensitivity shown in the top row. For instance, SST followed by PCR is able to achieve a sensitivity of at least 50% all the time (100%), while it achieves a sensitivity of 70% only 72,5% of the time.

It is recommended that a test or combination of tests be considered as adequate when it is able to meet the target sensitivity at least 80% of the time (values shown in the column on the right).These cases have been highlighted in yellow in the table. Orange highlight indicates that the test meets the target more than 90% of the time.

The overall sensitivity of the surveillance system can be improved when multiple components or testing strategies are combined (assuming independency, Table 45). The figures in the Table 45 indicate the overall sensitivity achieved using the two components. Sensitivities shown are those that would be achieved at least 80% of the time (the values considered for each test were the ones included on the right column of table 44).

Table 44 - Performance of different test combinations and testing strategies

Basic Screening Test	Confirmatory Test	% of herd tested with screening test	RR of tested animals being infected *	Herd-level sensitivity of combined screening and confirmatory tests									Test Se at 80% of the time
				0.1	0.15	0.2	0.5	0.7	0.8	0.85	0.9	0.95	
Whole herd tests on live animals													
SST	PCR	100%	1	100.0%	100.0%	100.0%	100.0%	72.5%	0.0%	0.0%	0.0%	0.0%	69.3%
SST	HIST	100%	1	100.0%	100.0%	100.0%	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	73.1%
SST	CULT	100%	1	100.0%	100.0%	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	58.1%
SST	HIST CULT PCR	100%	1	100.0%	100.0%	100.0%	100.0%	100.0%	98.3%	0.0%	0.0%	0.0%	81.2%
SST	HIST CULT	100%	1	100.0%	100.0%	100.0%	100.0%	100.0%	55.4%	0.0%	0.0%	0.0%	79.3%
SST	CULT PCR	100%	1	100.0%	100.0%	100.0%	100.0%	100.0%	32.2%	0.0%	0.0%	0.0%	78.5%
SICCT	PCR	100%	1	100.0%	100.0%	100.0%	100.0%	94.1%	0.8%	0.0%	0.0%	0.0%	72.2%
SICCT	HIST	100%	1	100.0%	100.0%	100.0%	100.0%	100.0%	1.4%	0.0%	0.0%	0.0%	76.0%
SICCT	CULT	100%	1	100.0%	100.0%	100.0%	100.0%	0.4%	0.0%	0.0%	0.0%	0.0%	60.6%
SICCT	HIST CULT PCR	100%	1	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	66.2%	0.0%	0.0%	84.4%
SICCT	HIST CULT	100%	1	100.0%	100.0%	100.0%	100.0%	100.0%	99.7%	9.3%	0.0%	0.0%	82.4%
SICCT	CULT PCR	100%	1	100.0%	100.0%	100.0%	100.0%	100.0%	97.3%	4.5%	0.0%	0.0%	81.6%
BTB	PCR	100%	1	100.0%	100.0%	100.0%	100.0%	99.9%	76.6%	13.3%	0.0%	0.0%	79.6%
BTB	HIST	100%	1	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	48.3%	0.0%	0.0%	84.0%
BTB	CULT	100%	1	100.0%	100.0%	100.0%	100.0%	48.9%	0.0%	0.0%	0.0%	0.0%	66.8%
BTB	HIST CULT PCR	100%	1	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	10.2%	93.5%
BTB	HIST CULT	100%	1	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	98.5%	0.0%	91.2%
BTB	CULT PCR	100%	1	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	85.2%	0.0%	90.2%
SST_SICCT	PCR	100%	1	100.0%	100.0%	100.0%	73.5%	7.6%	0.2%	0.0%	0.0%	0.0%	47.8%
SST_SICCT	HIST	100%	1	100.0%	100.0%	100.0%	78.9%	11.7%	0.6%	0.0%	0.0%	0.0%	49.6%
SST_SICCT	CULT	100%	1	100.0%	100.0%	100.0%	41.3%	0.1%	0.0%	0.0%	0.0%	0.0%	40.4%
SST_SICCT	HIST CULT PCR	100%	1	100.0%	100.0%	100.0%	90.2%	32.9%	7.5%	2.2%	0.3%	0.0%	54.9%
SST_SICCT	HIST CULT	100%	1	100.0%	100.0%	100.0%	88.3%	26.9%	5.0%	1.1%	0.1%	0.0%	53.7%
SST_SICCT	CULT PCR	100%	1	100.0%	100.0%	100.0%	87.4%	25.3%	4.3%	0.8%	0.0%	0.0%	53.3%

* RR = Relative risk of tested animals being infected (relative to non-tested)

Basic Screening Test	Confirmatory Test	% of herd tested with screening test	RR of tested animals being infected *	Herd-level sensitivity of combined screening and confirmatory tests									Test Se at 80% of the time
				0.1	0.15	0.2	0.5	0.7	0.8	0.85	0.9	0.95	
Slaughtered animals (20% per annum, lower risk (0.8) of being infected)													
INSP	PCR	20%	0.8	23.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	8.0%
INSP	HIST	20%	0.8	32.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	8.4%
INSP	CULT	20%	0.8	1.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	6.8%
INSP	HIST CULT PCR	20%	0.8	63.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	9.3%
INSP	HIST CULT	20%	0.8	56.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	9.1%
INSP	CULT PCR	20%	0.8	54.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	9.0%
Animals killed or found dead (5% per annum, higher risk (1.5) of being infected)													
NECR	PCR	5%	1.5	100.0%	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	23.7%
NECR	HIST	5%	1.5	100.0%	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	25.0%
NECR	CULT	5%	1.5	100.0%	100.0%	80.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	20.0%
NECR	HIST CULT PCR	5%	1.5	100.0%	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	27.7%
NECR	HIST CULT	5%	1.5	100.0%	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	27.1%
NECR	CULT PCR	5%	1.5	100.0%	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	26.8%
Slaughtered animals (20% per annum, equal risk (1) of being infected)													
INSP	PCR	20%	1	71.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	9.6%
INSP	HIST	20%	1	79.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	9.9%
INSP	CULT	20%	1	25.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	8.1%
INSP	HIST CULT PCR	20%	1	92.0%	5.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	11.0%
INSP	HIST CULT	20%	1	89.9%	3.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	10.8%
INSP	CULT PCR	20%	1	89.0%	2.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	10.7%

* RR = Relative risk of tested animals being infected (relative to non-tested)

Basic Screening Test	Confirmatory Test	% of herd tested with screening test	RR of tested animals being infected *	Herd-level sensitivity of combined screening and confirmatory tests									Test Se at 80% of the time
				0.1	0.15	0.2	0.5	0.7	0.8	0.85	0.9	0.95	
Animals killed or found dead (5% per annum, equal risk (1) of being inf ected)													
NECR	PCR	5%	1	100.0%	99.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	16.9%
NECR	HIST	5%	1	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	17.8%
NECR	CULT	5%	1	100.0%	38.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	14.2%
NECR	HIST CULT PCR	5%	1	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	19.9%
NECR	HIST CULT	5%	1	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	19.4%
NECR	CULT PCR	5%	1	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	19.2%
Slaughtered animals (10% per annum, equal risk (1) of being infected)													
INSP	PCR	10%	1	71.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	9.6%
INSP	HIST	10%	1	79.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	9.9%
INSP	CULT	10%	1	25.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	8.1%
INSP	HIST CULT PCR	10%	1	92.0%	5.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	11.0%
INSP	HIST CULT	10%	1	89.9%	3.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	10.8%
INSP	CULT PCR	10%	1	89.0%	2.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	10.7%
Animals killed or found dead (2% per annum, equal risk (1) of being infected)													
NECR	PCR	2%	1	100.0%	99.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	16.9%
NECR	HIST	2%	1	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	17.8%
NECR	CULT	2%	1	100.0%	38.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	14.2%
NECR	HIST CULT PCR	2%	1	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	19.9%
NECR	HIST CULT	2%	1	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	19.4%
NECR	CULT PCR	2%	1	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	19.2%

* RR = Relative risk of tested animals being infected (relative to non-tested)

Table 45 - Sensitivity of detection of TB achieved by using two surveillance components (one based on whole herd testing of live animals, the other based on testing part of the herd using meat inspection or necropsy)

				Component 1: Whole herd tests																					
Component 2: Sample of herd				SST	SST	SST	SST	SST	SST	SICCT	SICCT	SICCT	SICCT	SICCT	SICCT	BTB	BTB	BTB	BTB	BTB	BTB	SST_SICCT	SST_SICCT	SST_SICCT	SST_SICCT	SST_SICCT	SST_SICCT
Screening	Confirmation	% sampled	RR	PCR	HIST	CULT	HIST CULT PCR	HIST CULT	CULT PCR	PCR	HIST	CULT	HIST CULT PCR	HIST CULT	CULT PCR	PCR	HIST	CULT	HIST CULT PCR	HIST CULT	CULT PCR	PCR	HIST	CULT	HIST CULT PCR	HIST CULT	CULT PCR
INSP	PCR	20%	0.8	71.8%	75.2%	61.5%	82.7%	81.0%	80.2%	74.4%	77.9%	63.7%	85.6%	83.8%	83.0%	81.3%	85.3%	69.4%	94.0%	91.9%	91.0%	52.0%	53.6%	45.2%	58.6%	57.4%	57.0%
INSP	HIST	20%	0.8	71.9%	75.3%	61.6%	82.8%	81.0%	80.3%	74.5%	78.0%	63.9%	85.7%	83.9%	83.1%	81.3%	85.3%	69.5%	94.0%	92.0%	91.0%	52.1%	53.8%	45.4%	58.7%	57.6%	57.2%
INSP	CULT	20%	0.8	71.4%	74.9%	61.0%	82.5%	80.7%	79.9%	74.1%	77.6%	63.2%	85.4%	83.6%	82.8%	81.0%	85.1%	69.0%	93.9%	91.8%	90.9%	51.3%	53.0%	44.4%	58.0%	56.8%	56.4%
INSP	HIST CULT PCR	20%	0.8	72.2%	75.6%	62.0%	83.0%	81.2%	80.5%	74.8%	78.2%	64.2%	85.8%	84.1%	83.3%	81.5%	85.5%	69.8%	94.1%	92.0%	91.1%	52.6%	54.3%	45.9%	59.1%	58.0%	57.6%

RR = Relative risk - comparison between sampled population and non sampled population used to determine the effect of targeted sampling

INSP	**HIST CULT**	20%	0.8	72.1%	75.5%	61.9%	82.9%	81.2%	80.4%	74.7%	78.1%	64.1%	85.8%	84.0%	83.2%	81.5%	85.5%	69.8%	94.1%	92.0%	91.1%	52.5%	54.2%	45.8%	59.0%	57.9%	57.5%
INSP	**CULT PCR**	20%	0.8	72.1%	75.5%	61.9%	82.9%	81.2%	80.4%	74.7%	78.1%	64.1%	85.8%	84.0%	83.2%	81.5%	85.4%	69.7%	94.1%	92.0%	91.1%	52.5%	54.1%	45.7%	59.0%	57.8%	57.5%
NECR	**PCR**	5%	1.5	76.6%	79.4%	68.1%	85.7%	84.2%	83.6%	78.8%	81.7%	69.9%	88.1%	86.6%	85.9%	84.5%	87.8%	74.6%	95.0%	93.3%	92.5%	60.2%	61.5%	54.5%	65.6%	64.7%	64.3%
NECR	**HIST**	5%	1.5	77.0%	79.8%	68.6%	85.9%	84.5%	83.9%	79.2%	82.0%	70.4%	88.3%	86.8%	86.2%	84.7%	88.0%	75.1%	95.1%	93.4%	92.7%	60.8%	62.2%	55.3%	66.2%	65.3%	64.9%
NECR	**CULT**	5%	1.5	75.5%	78.5%	66.5%	85.0%	83.5%	82.8%	77.8%	80.8%	68.5%	87.5%	85.9%	85.2%	83.7%	87.2%	73.4%	94.8%	93.0%	92.2%	58.2%	59.7%	52.3%	64.0%	63.0%	62.6%
NECR	**HIST CULT PCR**	5%	1.5	77.8%	80.5%	69.8%	86.4%	85.1%	84.5%	79.9%	82.6%	71.5%	88.7%	87.3%	86.7%	85.3%	88.4%	76.0%	95.3%	93.7%	92.9%	62.3%	63.6%	56.9%	67.4%	66.5%	66.2%
NECR	**HIST CULT**	5%	1.5	77.6%	80.4%	69.5%	86.3%	84.9%	84.3%	79.7%	82.5%	71.2%	88.6%	87.2%	86.5%	85.2%	88.3%	75.8%	95.2%	93.6%	92.9%	61.9%	63.2%	56.5%	67.1%	66.2%	65.9%
NECR	**CULT PCR**	5%	1.5	77.5%	80.3%	69.3%	86.2%	84.9%	84.2%	79.6%	82.4%	71.1%	88.5%	87.1%	86.5%	85.1%	88.3%	75.7%	95.2%	93.6%	92.8%	61.8%	63.1%	56.3%	67.0%	66.1%	65.8%
INSP	**PCR**	20%	1	72.2%	75.6%	62.2%	83.0%	81.3%	80.5%	74.9%	78.3%	64.3%	85.9%	84.1%	83.3%	81.6%	85.5%	69.9%	94.1%	92.1%	91.2%	52.8%	54.4%	46.1%	59.3%	58.1%	57.7%
INSP	**HIST**	20%	1	72.4%	75.7%	62.3%	83.1%	81.4%	80.6%	75.0%	78.4%	64.5%	85.9%	84.2%	83.4%	81.7%	85.6%	70.1%	94.1%	92.1%	91.2%	53.0%	54.6%	46.3%	59.4%	58.3%	57.9%
INSP	**CULT**	20%	1	71.8%	75.2%	61.5%	82.7%	81.0%	80.2%	74.5%	77.9%	63.7%	85.6%	83.8%	83.0%	81.3%	85.3%	69.4%	94.0%	91.9%	91.0%	52.0%	53.7%	45.2%	58.6%	57.4%	57.0%

INSP	**HIST CULT PCR**	20%	1	72.7%	76.0%	62.8%	83.3%	81.6%	80.9%	75.3%	78.6%	64.9%	86.1%	84.4%	83.6%	81.9%	85.8%	70.4%	94.2%	92.2%	91.3%	53.5%	55.1%	47.0%	59.9%	58.8%	58.4%
INSP	**HIST CULT**	20%	1	72.6%	76.0%	62.7%	83.2%	81.5%	80.8%	75.2%	78.6%	64.8%	86.0%	84.3%	83.5%	81.8%	85.7%	70.3%	94.2%	92.2%	91.3%	53.4%	55.0%	46.8%	59.8%	58.7%	58.3%
INSP	**CULT PCR**	20%	1	72.6%	75.9%	62.6%	83.2%	81.5%	80.8%	75.2%	78.5%	64.8%	86.0%	84.3%	83.5%	81.8%	85.7%	70.3%	94.2%	92.2%	91.3%	53.4%	55.0%	46.7%	59.8%	58.6%	58.3%
NECR	**PCR**	5%	1	74.5%	77.6%	65.2%	84.4%	82.8%	82.1%	76.9%	80.0%	67.2%	87.0%	85.4%	84.7%	83.1%	86.7%	72.4%	94.6%	92.7%	91.9%	56.6%	58.1%	50.4%	62.5%	61.5%	61.2%
NECR	**HIST**	5%	1	74.8%	77.9%	65.6%	84.6%	83.0%	82.3%	77.2%	80.3%	67.6%	87.1%	85.6%	84.8%	83.3%	86.9%	72.7%	94.6%	92.8%	92.0%	57.1%	58.6%	51.0%	63.0%	61.9%	61.6%
NECR	**CULT**	5%	1	73.7%	76.9%	64.1%	83.9%	82.2%	81.5%	76.1%	79.4%	66.1%	86.6%	84.9%	84.2%	82.5%	86.3%	71.5%	94.4%	92.5%	91.6%	55.2%	56.7%	48.8%	61.3%	60.2%	59.9%
NECR	**HIST CULT PCR**	5%	1	75.4%	78.4%	66.5%	85.0%	83.4%	82.8%	77.7%	80.8%	68.4%	87.5%	85.9%	85.2%	83.7%	87.2%	73.4%	94.8%	93.0%	92.2%	58.2%	59.6%	52.3%	63.9%	62.9%	62.6%
NECR	**HIST CULT**	5%	1	75.3%	78.3%	66.3%	84.9%	83.3%	82.7%	77.6%	80.6%	68.2%	87.4%	85.8%	85.1%	83.6%	87.1%	73.2%	94.7%	92.9%	92.1%	57.9%	59.4%	52.0%	63.7%	62.7%	62.3%
NECR	**CULT PCR**	5%	1	75.2%	78.2%	66.2%	84.8%	83.3%	82.6%	77.5%	80.6%	68.1%	87.4%	85.8%	85.1%	83.5%	87.1%	73.1%	94.7%	92.9%	92.1%	57.8%	59.2%	51.8%	63.6%	62.6%	62.2%
INSP	**PCR**	10%	1	72.2%	75.6%	62.2%	83.0%	81.3%	80.5%	74.9%	78.3%	64.3%	85.9%	84.1%	83.3%	81.6%	85.5%	69.9%	94.1%	92.1%	91.2%	52.8%	54.4%	46.1%	59.3%	58.1%	57.7%

INSP	**HIST**	10%	1	72.4%	75.7%	62.3%	83.1%	81.4%	80.6%	75.0%	78.4%	64.5%	85.9%	84.2%	83.4%	81.7%	85.6%	70.1%	94.1%	92.1%	91.2%	53.0%	54.6%	46.3%	59.4%	58.3%	57.9%
INSP	**CULT**	10%	1	71.8%	75.2%	61.5%	82.7%	81.0%	80.2%	74.5%	77.9%	63.7%	85.6%	83.8%	83.0%	81.3%	85.3%	69.4%	94.0%	91.9%	91.0%	52.0%	53.7%	45.2%	58.6%	57.4%	57.0%
INSP	**HIST CULT PCR**	10%	1	72.7%	76.0%	62.8%	83.3%	81.6%	80.9%	75.3%	78.6%	64.9%	86.1%	84.4%	83.6%	81.9%	85.8%	70.4%	94.2%	92.2%	91.3%	53.5%	55.1%	47.0%	59.9%	58.8%	58.4%
INSP	**HIST CULT**	10%	1	72.6%	76.0%	62.7%	83.2%	81.5%	80.8%	75.2%	78.6%	64.8%	86.0%	84.3%	83.5%	81.8%	85.7%	70.3%	94.2%	92.2%	91.3%	53.4%	55.0%	46.8%	59.8%	58.7%	58.3%
INSP	**CULT PCR**	10%	1	72.6%	75.9%	62.6%	83.2%	81.5%	80.8%	75.2%	78.5%	64.8%	86.0%	84.3%	83.5%	81.8%	85.7%	70.3%	94.2%	92.2%	91.3%	53.4%	55.0%	46.7%	59.8%	58.6%	58.3%
NECR	**PCR**	2%	1	74.5%	77.6%	65.2%	84.4%	82.8%	82.1%	76.9%	80.0%	67.2%	87.0%	85.4%	84.7%	83.1%	86.7%	72.4%	94.6%	92.7%	91.9%	56.6%	58.1%	50.4%	62.5%	61.5%	61.2%
NECR	**HIST**	2%	1	74.8%	77.9%	65.6%	84.6%	83.0%	82.3%	77.2%	80.3%	67.6%	87.1%	85.6%	84.8%	83.3%	86.9%	72.7%	94.6%	92.8%	92.0%	57.1%	58.6%	51.0%	63.0%	61.9%	61.6%
NECR	**CULT**	2%	1	73.7%	76.9%	64.1%	83.9%	82.2%	81.5%	76.1%	79.4%	66.1%	86.6%	84.9%	84.2%	82.5%	86.3%	71.5%	94.4%	92.5%	91.6%	55.2%	56.7%	48.8%	61.3%	60.2%	59.9%
NECR	**HIST CULT PCR**	2%	1	75.4%	78.4%	66.5%	85.0%	83.4%	82.8%	77.7%	80.8%	68.4%	87.5%	85.9%	85.2%	83.7%	87.2%	73.4%	94.8%	93.0%	92.2%	58.2%	59.6%	52.3%	63.9%	62.9%	62.6%
NECR	**HIST CULT**	2%	1	75.3%	78.3%	66.3%	84.9%	83.3%	82.7%	77.6%	80.6%	68.2%	87.4%	85.8%	85.1%	83.6%	87.1%	73.2%	94.7%	92.9%	92.1%	57.9%	59.4%	52.0%	63.7%	62.7%	62.3%

Summary of currently available practice for screening and confirmation:

- Dead /slaughtered animals:
  - Screening tests:
    - INSP during routine screening of carcasses;
    - NECR in defined situations (but only able to screen a relatively small number of animals at any one time);
  - Confirmatory tests:
    - HIST, CULT, etc;
- Live animals:
  - Screening tests:
    - SST and/or SICCT;
  - Confirmatory tests:
    - HIST, CULT, etc. In most cases should be preceded by a post-mortem examination (NECR).

Therefore, as a screening test, NECR is applicable to the general population (regardless of infection status) but could also be used in a herd/situation where herd-level infection is suspected. As a confirmatory test, it would generally be used in animals with prior knowledge of infection status (i.e., animals killed following an SICCT positive result).

7. PROPOSED TEST REGIMES TO DEMONSTRATION FREEDOM

7.1. General principles

As indicated by the previous analysis, there are a wide range of options that are capable of meeting the requirements to declare a holding officially free from Tuberculosis. However, the options for large scale screening are limited when considering that currently only the two intradermal tests (SICCT and SST) are suitable for that purpose in live animals and meat inspection for screening in slaughtered animals (Chapter 3.4). These tests are combined with tests for verification of TB-suspected cases identified in through the screening.

The basic tests used for verification is necropsy in association with histology, culture and commonly also PCR. Culture is required for final confirmation. The test regimes used for verification are not harmonized among Member States and may not include all those tests, mainly depending on the epidemiological situation and a different need for speeding up the process. However for the verification of TB in officially free holdings it is recommended that harmonized testregimes are used for verification.
Once a holding is found infected a combination of other tests (Chapter 3) can be applied for a possible cleaning up process of the infected holding. This is also applied for final diagnose of holding status if cases identified in the screening can not be fully confirmed, e.g. due to a negative culture in spite of typical lesions and histology.

Necropsy of slaughtered animals (suspected cases identified at meat inspection) or animals killed or found dead with subsequent tests for verification as described above is also recommended screening both during the process of achieving and maintaining freedom.

7.2. Examples

This section provides examples of different suitable testing regimes that can be assessed by the models presented above.

(Example 1) - Achieving freedom:

a) Risk of introduction

Imports:

A farm that has imported three animals from another farm with a prevalence of 1% and applied no pre-import testing to those animals

Result: Probability of introduction in live deer = 3,0% (Table 38)

Wildlife:

Disease is present at a low level in the wildlife, and the farm is fenced.

Result: Low prevalence (0.25% risk), reduced by 90% due to fencing = 0.03% (Table 42)

Combined risk of introduction = 3% (Table 42)

b) Testing:

Tests used are: SICCT + HIST*, CULT – combined Se = 82,4% (> 80% of the time, Table 44)

This test combination is suitable to demonstrate freedom after 4 years of testing (Table 34) (minimum requirement = 80% sensitivity)

Variations:

1) If the risk of introduction were 3.5%, 5 years of testing would be required.

2) If this approach was combined with a second surveillance strategy, it would be possible to demonstrate freedom more quickly. For instance, if all sick or dead animals on the farm were necropsied (and this made up 5% of the population each year) and it was assumed that these animals were 1.5 times as likely to be infected with TB if it were present on the farm, and this test was confirmed by HIST and CULT, the combination of the two surveillance components would achieve an overall sensitivity of 87.2% (Table 45). With this sensitivity it would be possible to demonstrate freedom from disease in 3 years rather than 4 years (table 34).

(Example 2) - Maintaining freedom:

a) Risk of introduction

Imports:

A farm that has imported no animals from another farm

Wildlife:

Disease is present at a high level in the wildlife, and the farm is fenced.

Result: High prevalence 2 % risk, reduced by 90% due to fencing = 0.2%

Combined risk of introduction = 0.2% (Table 42)

b) Testing:

Tests used are: SST and SICCT + PCR* – combined Se = 47,8% (80% of the time)

This test combination is suitable to maintain freedom with annual testing (only 18% Se is required; Table 36)

Variations:

As only 18% sensitivity is required, this may be achieved with a number of other less expensive combinations of tests, such as necropsy of sick or dead animals in at least 2% of the herd each year, confirmed by HIST and CULT (Se = 19.4% - table 44)

8. MOVEMENT OF ANIMALS BETWEEN NON-TB FREE HOLDINGS

Currently, only one MS is applying to be recognised as officially TB-free Therefore, the majority of intra-Community trade will be between countries that do not have official TB-free status (so-called, non-free countries), to which the above-mentioned provisions are not directly applicable. Currently, the sanitary requirements for trade between non-free countries are not harmonised. Some minimum requirements for animals traded in this situation are scientifically desirable/ justifiable to reduce the risk of TB spread between to deer farms, as well as spread to wildlife and the domestic animal population. This would also facilitate efforts towards achieving free status in the future.

8.1. Approach

It may be argued that movement of deer between non-free holdings should only be permitted between holdings which share a similar probability of freedom, or when the holding of origin has a higher probability of freedom than the destination holding. The result of this is that holdings that have undertaken significant testing but have not yet reached free status (but have a high probability of being free) may supply animals to any other holding (other than those that have achieved free status).

The purpose of this is to avoid movements which would result in an increase in the probability that the recipient holding is infected. Unfortunately, this is not possible, as the introduction of any animal with a non-zero probability of being infected will always increase the probability that the recipient holding is infected (even if the increase is very small). It is therefore more appropriate to consider the probability of infection at the individual animal level rather than the herd level, and seek to ensure that movements do not increase the average probability of individual animals being infected.

Holding status probabilities are based on the design prevalence, which, as discussed previously, is one animal per herd in this case. This means that a holding is considered infected if it has just one infected animal. If the within-herd prevalence is very low, the probability that a randomly selected animal from a non-free holding is actually infected is also very low. The key point is that the probability of a herd being infected (having at least one infected animal) and the probability that any particular animal from that herd is infected are very different. When moving a group of animals from a non-free holding, the probability that one or more animal is infected will always be less (and often much less) than the probability that the whole herd is infected, except when the group being moved consists of the whole herd.

8.2. Strategy

A more detailed knowledge of the probability for infection in different holdings would facilitate a detailed approach. However, in absence of such knowledge that would require substantial testing, a simplified approach is recommended through the minimum requirements to test all animals to be moved from untested holdings. Doing so, the risk of introducing TB into recipient holdings is significantly reduced (Table 41).

According to TB-status holdings be divided in the following classes:

- *Officially free* - Holdings that have met the requirements of this document and have been declared officially free (with a probability of freedom of ≥ 99%)
- *Tested* - Holdings that have undergone annual rounds of testing with negative results
- *Untested* - Holdings in which no testing has been conducted

- *Restricted* - Holdings where positive animals have been detected and which are therefore currently restricted (export from these animals is prohibited).

It is suggested that animals may be moved between holdings of the same class (except for restricted holdings, from which no movements are permitted), and from holdings of a higher class to holdings of a lower class. For the tested holdings a further a possible subclassification can be made based upon the number of annual rounds of testing with negative results. This approach considers the single most important factor influencing probability of freedom – the number of consecutive annual negative whole herd tests that have been performed on a holding.

Movement should not be recommended from holdings of a lower class to holdings of a higher class.

No testing should be required for any of the above movements, except for those between untested holdings, in which case each of the animals moved should be tested with the single intradermal or comparative intradermal test (or the ELISA or GINT, when they have been validated).

The testing data in combination with biosecurity can subsequently build up a more informed knowledge on the probability of infection in tested holdings that can be used for a more detailed approach.

These recommendations are summarised in the Table 46:

Table 46 - Suggested strategy for the allowance (Yes/No) of the movement of deer between farmed deer herds of different TB-status

			Destination								
			Officially Free	Tested [1]						Untested	Restricted
				6	5	4	3	2	1		
Source	Officially Free		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No[2]
	Tested[1]	6	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No[2]
		5	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No[2]
		4	No	No	No	Yes	Yes	Yes	Yes	Yes	No[2]
		3	No	No	No	No	Yes	Yes	Yes	Yes	No[2]
		2	No	No	No	No	No	Yes	Yes	Yes	No[2]
		1	No	No	No	No	No	No	Yes	Yes	No[2]
	Untested		No	No	No	No	No	No	No	Yes[3]	No[2]
	Restricted		No	No	No	No	No	No	No	No	No[2]

1) Number (1-6) of whole herd annual tests.
2) According to currently applied principles, otherwise Yes.
3) Should require testing of all animals to be moved.

9. REFERENCES

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APPENDIX A - QUESTIONNAIRE USED TO COLLECT EXPERT OPINION - PERFORMANCE OF DIAGNOSTIC TESTS FOR TUBERCULOSIS IN DEER

Objective

Capturing the experience of experts

Introduction

This survey is being conducted as part of the European Food Safety Authority (EFSA) study into the requirements for declaring populations of deer free from tuberculosis. A number of approaches are being used to investigate the performance of the main tests for tuberculosis in deer. This survey aims to capture the experience of those involved in using different tests.

Instructions

This survey should take **less than 5 minutes** to complete. **Please read these instructions** in order to complete the survey quickly and accurately:

- Log in with the username and password that was sent to you. The log in form is at the bottom of this page, but don't do it yet. Finish reading the instructions first.
- Click on each question about which you feel you are qualified to provide information. You are not required to complete every question, if you don't want to.
- For each test, there are separate questions about *sensitivity* and *specificity*. Check the definitions below to be sure we are all using the same definitions.
- For each question, a new window (or tab) will open. You are required to provide 4 values for each question:
    - Your level of expertise. This is a self assessed ranking of your experience with the test.
    - The most likely value. This is the value (sensitivity or specificity) that, through your experience, you think is the most likely value to be true in most circumstances.
    - The minimum value. If your estimate for the most likely value is wrong, this is the lowest possible value that you would expect. The difference between the most likely and the minimum (and maximum) possible values may be due to variability in the test performance, or your uncertainty about the real values, or both.
    - The maximum value. This is the highest value (sensitivity or specificity) that, through your experience, you think could be correct.
- Enter values by clicking on the yellow part of the scale bar (below) with your mouse.

- When the four values are entered, the numbers will be displayed for you to check, and modify if desired.
- If you are happy with all the values, click 'OK', to return to the list of questions.
- When you have finished the questions (or as many as you wish to do), click 'Finished' to log out.

Definitions

Sensitivity: The proportion of truly infected animals that give a positive result to the test, expressed as a number between 0 and 1. This is also known as the *true positive rate*. This definition is different to the analytical definition of sensitivity, where the term relates to the minimum detectable concentration of the agent.

Specificity: The proportion of truly non-infected animals that give a negative result to the test. This is the *true negative rate*.The web-based questionnaire was designed to be quickly completed using just mouse clicks. After logging in, the user was presented with a list of tests, as shown below:

Questionnaire

There are many factors which influence sensitivity and specificity of these tests. It is difficult to take all these factors into account for regulatory purposes. While recognizing the difficulty of this task, we request that you estimate test performance:

- Under average conditions in a real-life situation (eg as part of an eradication or control program, rather than with experimental infections).
- Based on your own experience with using the test in your own environment
- Describing variation in performance in terms of the minimum and maximum possible values, as well as the most likely value.

You do not have to answer all questions. Only answer questions about test that you have some experience with.

1. **Comparative intradermal tuberculin test**

Sensitivity *In early stage of infection (< 6 months after first being infected)*

Sensitivity *In later stages of infections (> 6 months after first being infected)*

Specificity ☑

2. **Single intradermal tuberculin test**

Sensitivity *In early stage of infection (< 6 months after first being infected)*

Sensitivity *In later stages of infections (> 6 months after first being infected)*

Specificity ☑

3. **Culture (includes subsequent identification using molecular techniques)**

Sensitivity ☑

Specificity ☑

4. **PCR**

Sensitivity ☑

Specificity ☑

5. **Microscopy / Histopathology**

Sensitivity ☑

Specificity ☑

6. **ELISA -** No standardized ELISA exists. Please provide estimates for the realistic performance of a hypothetical optimized ELISA.

Sensitivity ☑

Specificity ☑

7. Lymphocyte Stimulation

Sensitivity ☑

Specificity ☑

8. Gamma Interferon

Sensitivity ☑

Specificity ☑

9. Rapid Test

Sensitivity ☑

Specificity ☑

10. BTB (combined comparative intradermal and ELISA)

Sensitivity ☑

Specificity ☑

11. Necropsy (includes the entire test system of gross necropsy followed by confirmatory tests such as histology and culture)

Sensitivity ☑

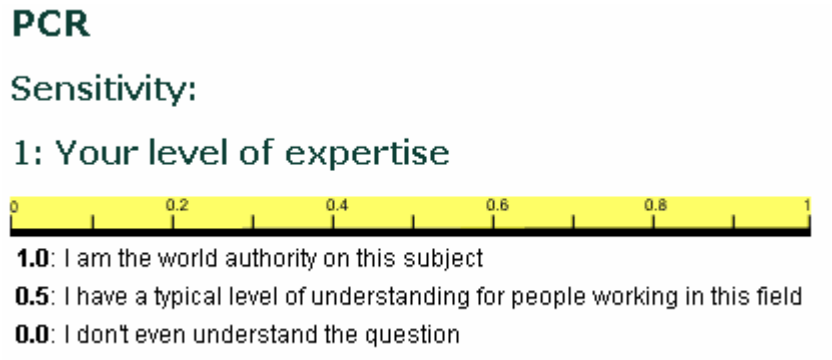
Specificity ☑

12. Meat Inspection (excludes any follow-up testing)

Sensitivity ☑

Specificity ☑

After selecting a test, users were presented with a series of questions, to be answered by clicking in the appropriate place on the scale bar, as shown below:



2: Most likely value

3: Minimum possible value

4: Maximum possible value

5: Review

Confirm or edit the selected values

Expertise:	0.7475
Minuimum:	0.705
Most likely:	0.8
Maximum:	0.86

APPENDIX B - SYSTEMATIC LITERATURE REVIEW

A. THE TWO-STAGE REVIEW

The first stage included a screening of online data bases CAB, Food Science and technology, Web of Science, Biological abstracts, Biosis Previews, Zoological record accessed via a licence of the Federal Institute for Risk Assessment. The search string was:

> TS=((tuberc* OR mycobac* NOT (paratub* or Johne)) AND (cervus OR cervid* OR deer* OR sika OR fallow* OR elk OR moose OR wapiti) AND (test* OR diagn* OR ELISA OR PCR OR lypmpho* OR interferon OR skin* or rapid* OR sens* OR spec* OR accura* OR perfor* OR eval* OR valid* OR detect*))

or any modification as required by the search syntax of the data base. The bibliographic references and abstracts were imported into a bibliographic reference management software. After deletion of duplicates (matched on title, last names authors, periodical) a total of 510 references were randomly assigned to four members of the working group, whereby each reference was allocated to exactly two reviewers. It was noted that these references still contained duplicates due to differences in spelling of author names and periodicals. In these cases, the abstracts were often different for identical articles. The reviewers assessed whether or not a reference should be included in the full review based on the available information (title, keywords and in most cases abstracts). References were included if any information given in the title or abstract indicated that diagnostic performance measures (diagnostic sensitivity, specificity, predictive values, accuracy, validity, detection rate) of any tuberculosis test in deer were reported. No restriction was made as to the nature of the test (including post mortem, histology, inspection, etc), language or date of publication. In case of disagreement among the reviewers, the reference was included in the stage-2 review. It was verified that all references included in the expert's personal literature collection were also included in the retrieved list of references. During stage-1, members of the working group were able to identify additional references to be included in the stage-2 review.

The second stage was based on the complete article review and allowed more elaborated exclusion criteria being applied. A total of 190 references were selected for stage-2 review and subsequently ordered in copy through online access or library service. References that were not accessible as hard copies or electronically within 6 weeks were considered excluded from stage-2 review. The references were randomly allocated to nine different reviewers such that each reference was graded independently by two reviewers for inclusion or rejection. A reference was excluded if one of the following exclusion criteria applied:

Table 48 - Criteria used for exclusion of references

FailureCode	FailureReason
1	not about the subject (validation of TB tests in deer species)
2	not an original report (e.g. duplicated publication or review paper)
3	test is not relevant
4	species not relevant
5	test not standardised, not carried out according to SOP
6	test described insufficiently (no judgement possible)
7	animals vaccinated
8	published before XXXX (NOT IN USE)
9	language other than da, de, en, es, fra, it, nl, pt, ru, sv
10	gold standard is inappropriate
11	no way to read or calculate diagnostic Se or Sp
12	no sample size given
13	failure on multiple criteria

All scorings by experts were done using an MS-EXCEL template which had functionalities for selecting a subset of papers allocated to a reviewer and verification of correct entry of coded information. The EXCEL data sheets from 9 reviewers were automatically compiled and imported into the R-software[2]. Using a programme developed for this purpose, all inconsistencies between two reviewers (i.e. missing or discrepant entry on any variable item) were identified and compiled into a feedback report (R-Sweave and LaTex). Discrepancies between any two reviewers were discussed and resolved. For this purpose, general meetings were held to introduce and demonstrate the general principles of data collection and subsequent meetings were arranged such that each reviewer had the opportunity to discuss with every other reviewer in the presence of a third reviewer. The general principles for data extraction were as follows.

- Three levels of information were considered and collected in different tables: SOURCE (the reference), TEST (the test entity defined by reference number, test principle, the test modification if necessary and the target species) and SENS or SPEC (each estimate of a sensitivity or specificity for the given test entity.
- In case of multiple cut-off values, only the cut-off recommended by the authors was considered. If no such recommendation is given, the working group decided for the most appropriate cut-off value. The guiding rule for this decision was that the sum of the diagnostic sensitivity and specificity is maximised.
- If there were more than two outcome categories (e.g., negative, intermediate, positive), the suspect or intermediate range was considered positive unless otherwise stated in the Standard Operation Procedure for that test;
- In case of experimental infections and multiple estimates of diagnostic sensitivity over the course of infection, all time points were recorded in the data base. For the purpose of summary analysis of sensitivity, only one date, i.e. the sampling date closest to day 20 p.i. was retained;
- In case of tests applied to different matrices or tissues, including necropsy, histology or bacteriological culture of different organs, the unit of diagnosis was the animal. Therefore, a test was considered positive if at least one sample/organ/tissue was scored positive, and negative otherwise ; and
- If values for sensitivity or specificity as reported by the author(s) did not match with the results of recalculation using information in the text or tables, the latter was used for statistical analysis.

B. THE REVIEW DATABASE

The data base consists of four data tables (SOURCE, TEST, SENS and SPEC), each with a corresponding data entry form with all variables are arranged on one page. The link between the tables are indicated as arrows and are usually 1:n links. This means that one entry on the left side can refer to one or more entries on the right side.

[2] R Development Core Team (2007). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

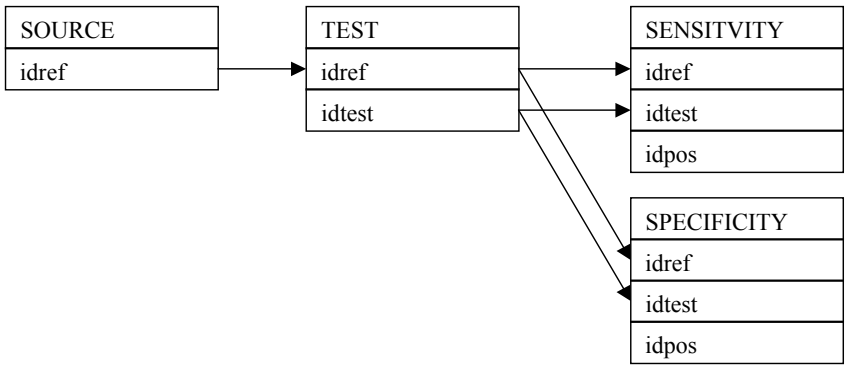


Table 49 - SOURCE: table on the level of one reference

SOURCE		
Variable	**Type**	**Description**
idref	unique counter (automatic)	SOURCE number (unique for each SOURCE)
Reference	text	complete bibliographic reference (uniform style suitable for reporting)
Selected2	numeric	Included in stage-2 review:1=included, 99=excluded
FailureCode	numeric	Reason for exclusion (if Selected2=99); codes see above.

Table 50 - TEST: table on the level of each test reported

Examples: If one SOURCE evaluates one test in one species, one line is reported. If one SOURCE reports one test in three species, three lines are reported, one for each species (because technical details may differ). If one SOURCE reports two tests in two species, four lines are reported (one for each combination test/host species).

TEST		
Variable	**Type**	**Description**
idref*		linked to SOURCE
idtest*	numeric	identification number of the test (unique within database)
testprinciple*	text	_any combination: combination of tests (order reflecting testing strategy, space separated) cst: comparative intradermal test cult: culture, bacteriology elisa: elisa gint: gamma interferon test hist: histology or microscopy insp: meat inspection lct: lymphocyte stimulation test necr: necropsy other: other test pcr: polymerase chain reaction rapid: rapid test sst: single tuberculin intradermal test
testmodification	text	any description, which is required to differentiate between modifications of tests, e.g. application sites of antigen for intradermal test (e.g. PPD, manufacturer)
species*	pick-up list	_any combination: mix of species (order reflecting decreasing percentage of species, space separated) deer: unspecified or other deer species elk: Alces alces: elk, moose (N-Am) fallow: Dama dama: fallow deer other: taxonomically related to deer red: Cervus elaphus: red deer, wapiti, elk (N-Am)

		rein	Rangifer tarandus: reindeer, caribu (N-Am)
		roe	Capreolus capreolus: roe deer
		sika	Cervus nippon: sika
farmed	text	Are the target animals farmed? y=yes, no=no (variable will be considered as a descriptor of the reference population)	
antigentarget	text	free text to describe the target antigen or analyte	
interpret	text	if necessary to explain test modifications due to different cut-off values	

*No missing values allowed.

Table 51 - SENSITIVITY: table on the level of each positive reference group that provides one estimate of sensitivity in a SOURCE

Examples: In one SOURCE, one test may be evaluated for two different groups of infected deer, may be vaccinated and non-vaccinated. Results will be reported in two lines in SENSITIVITY. Or, 20 infected animals may have been checked for antibodies at six time points. Note that in the latter case only one time point will be retained for statistical summary analysis.

SENSITIVITY			
Variable	**Type**	**Description**	
idref*		linked to TEST	
idtest*		linked to TEST	
species*		linked to TEST	
refpos	numeric	number to differentiate different reference populations if required	
criterionpos	text	criterion for selection of animals into the positive reference group:	
		_any combination	combination of tests, order reflecting testing strategy
		b	bacteriology on individual animals
		c	clinical signs individual of animals
		e	epidemiological status of group
		g	group-level diagnosic status
		h	histology on individual animals
		m	meat inspection of individual animals
		o	other criteria for individual animals
		p	post-mortem of individual animals
		t	other test of individual animals
		x	experimental infection
countrypos	text	country of the source population to which the results apply.	
		at	Austria
		au	Australia
		be	Belgium
		ca	Canada
		ch	Switzerland
		cn	China
		de	Germany
		dk	Denmark
		el	Greece
		es	Spain
		fin	Finland
		fr	France
		int	international panel of animals
		irl	Ireland
		is	Iceland
		jp	Japan
		lu	Luxemburg

		mt Malta na not applicable (experimental infection) nl The Netherlands no Norway nz New Zealand pt Portugal ru Russia se Sweden uk United Kingdom us United States of America
dpi	numeric	average number of days (and only days) between infection and testing (usually only known for experimental infection)
repeatpos	text	y= repeated test results from the same individuals are included in the data set for estimating the sensitivity, n= each test result is from one individual animal
mycobact	text	Mycobacterium species if known.
truepos*	numeric	Number of samples tested positive with the new test out of "samplepos"; if this is not given in the paper, please calculate it as "samplepos" times the sensitivity
intpos*	numeric	Number of samples tested intermediate with the new test out of "samplepos"; if this is not given in the paper, please calculate it as "samplepos" times the sensitivity
samplepos*	numeric	Number of samples tested in the group of positive animals to estimate the sensitivity
sensitivity*	numeric	value of the sensitivity (between 0 and 1); only used to double-check data entry; sensitivity= truepos/samplepos.
biaspos	text	indication of direction of bias: u=understimation, o=overestimation, ?=unknown direction of bias
commentpos	text	additional relevant information (potential biases, etc)

*No missing values allowed:

Table 52 - SPECIFICITY: table on the level of each negative reference group that provides one estimate of specificity in a SOURCE

Examples: In one SOURCE, one test may be evaluated for two different groups of non-infected deer, may be two age groups. Results will be reported in two lines in SPECIFICITY.

SPECIFICITY		
Variable	**Type**	**Description**
idref*		linked to TEST
idtest*		linked to TEST
species*		linked to TEST
refneg	numeric	number to differentiate different reference populations if required
criterionneg	text	criterion for selection of animals into the negative reference group: _any combination combination of tests, order reflecting testing strategy b bacteriology on individual animals c absence of clinical signs individual animals d differential diagnosis (cross-reaction or other disease) e epidemiological status of the group g group-level diagnosic status h histology on individual animals m meat inspection of individual animals o other criteria for individual animals p post-mortem of individual animals t other test of individual animals x prior to experimental infection or SPF animals
countryneg	text	**country** (see SENSITIVITY list) of the source population to which the

		specificity applies. **na**= not applicable (for experimental conditions)
crossreact	text	cross-reactive infection if known
repeatneg	text	**y**= repeated test results from the same individuals are included in the data set for estimating the specificity, **n**= each test result is form one individual animal
trueneg*	numeric	**number** of samples tested negative with the new test out of "sampleneg"; if this is not given in the paper, please calculate it as "sampleneg" times the specificity
sampleneg*	numeric	**number** of samples tested in the group of negative animals to estimate the specificity
specificity*	numeric	value of the specificity (between 0 and 1); only used to double-check data entry; specificity= trueneg/sampleneg.
biasneg	text	indication of direction of bias: u=understimation, o=overestimation, ?=unknown direction of bias
commentneg	text	additional relevant information (potential biases, etc)

*No missing values allowed.

C. SENSITIVITY AND SPECIFICITY ESTIMATES

The following section presents:

- Plots of all sensitivity and specificity estimates, with exact binomial confidence intervals
- Funnel plots, to check for possible publication bias.

Table 53 - Available sensitivity estimates of diagnostic tests for deer based on a systematic literature review a)

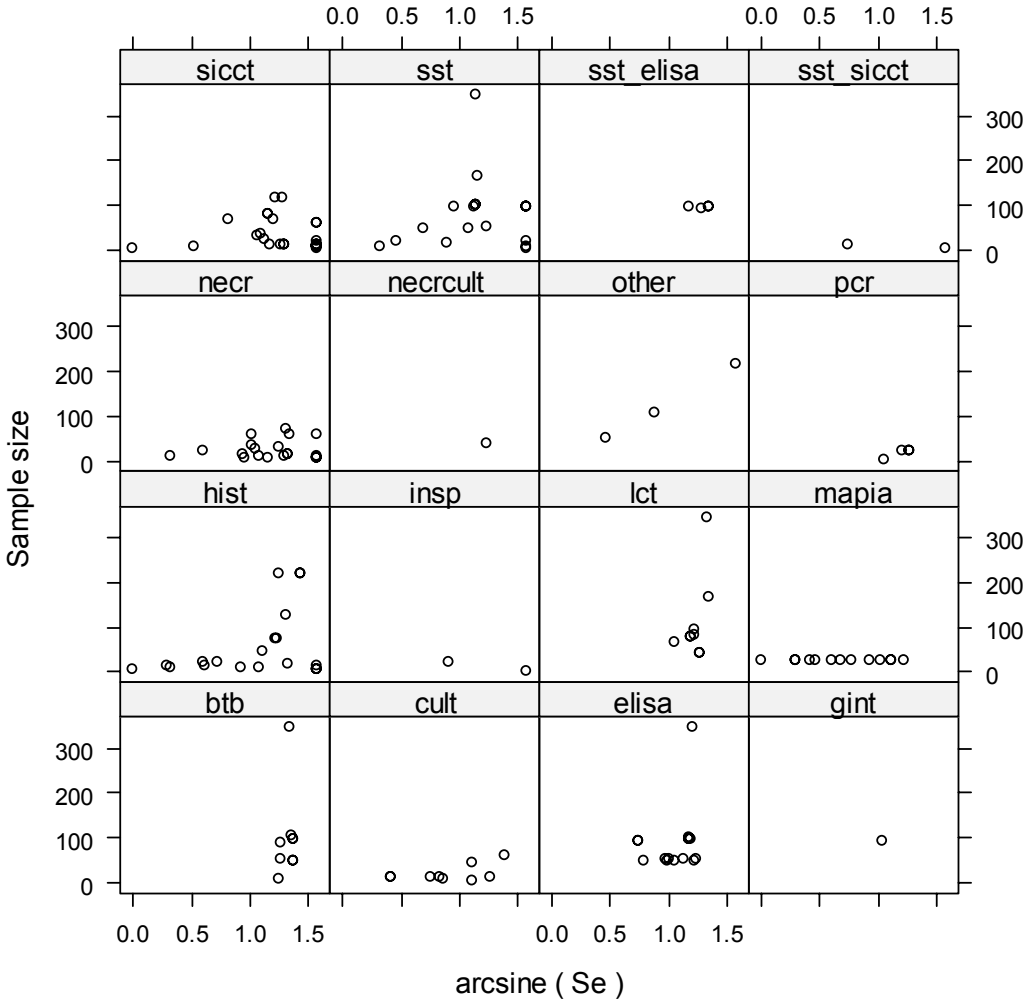
	deer	elk	mixed	red	rein	sika
btb	3	0	0	5	0	0
cst	8	0	6	8	4	0
cult	6	0	0	2	1	0
elisa	8	0	0	7	0	0
gint	1	0	0	0	0	0
hist	9	0	1	7	1	2
insp	2	0	0	0	0	0
lct	1	0	0	8	0	0
mapia	13	0	0	0	0	0
necr	4	0	1	12	1	2
necr cult	0	0	1	0	0	0
other	3	0	0	0	0	0
pcr	2	1	0	1	0	0
sst	10	0	0	8	0	2
sst cst	0	0	0	2	0	0
sst elisa	1	0	0	2	0	0

a) One publication may report more than one estimate. In case of repeated estimates over time, each one estimate below and above 6 months is extracted. See table 50 for species and test abbreviations.

Table 54 - Available specificity estimates of diagnostic tests for deer based on a systematic literature review a)

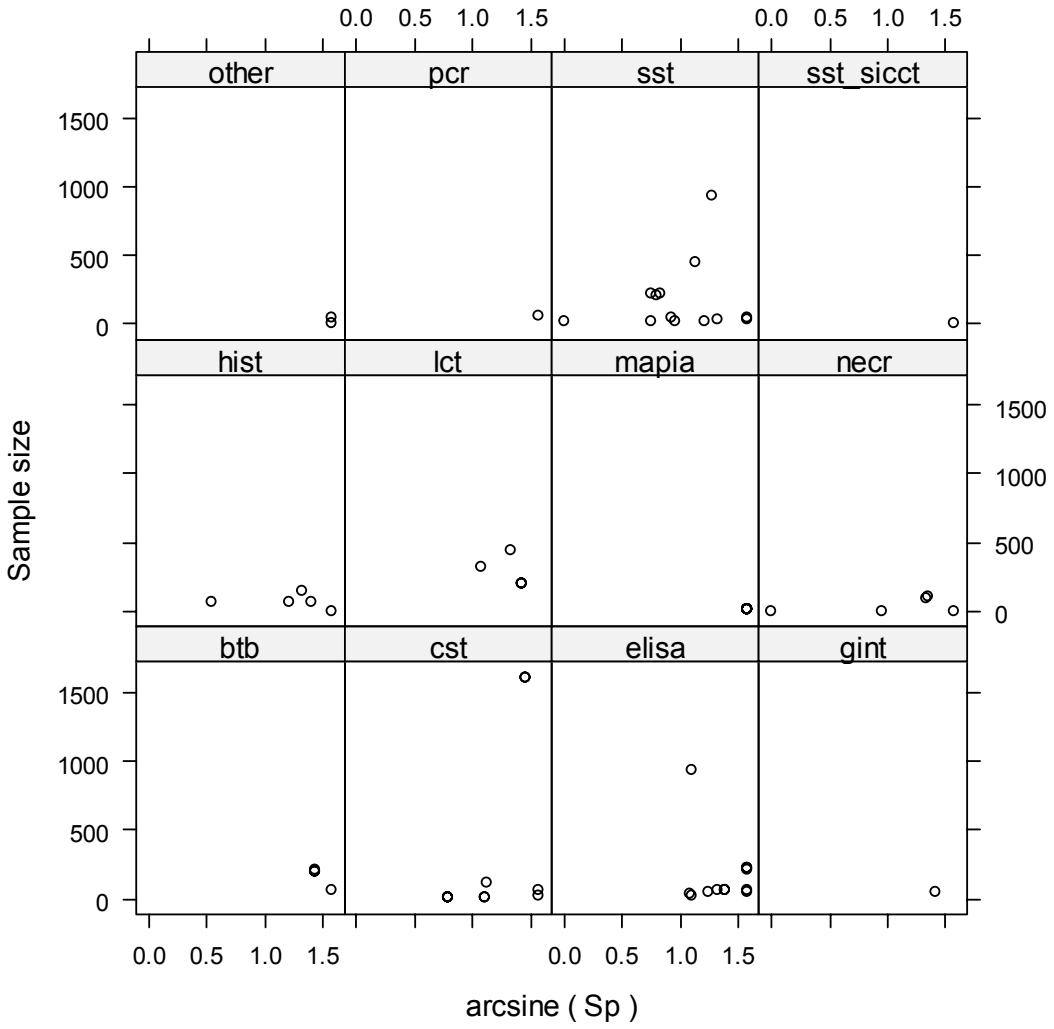
	deer	fallow	mixed	red	rein
btb	2	0	0	2	0
cst	6	0	1	2	2
elisa	10	0	0	2	0
gint	1	0	0	0	0
hist	3	0	0	2	0
lct	1	0	0	4	0
mapia	12	0	0	0	0
necr	1	0	0	3	1
other	2	0	0	0	0
pcr	1	0	0	0	0
sst	8	2	0	3	0
sst cst	0	0	0	1	0

a) One publication may report more than one estimate. See tables 50 for species and test abbreviations.



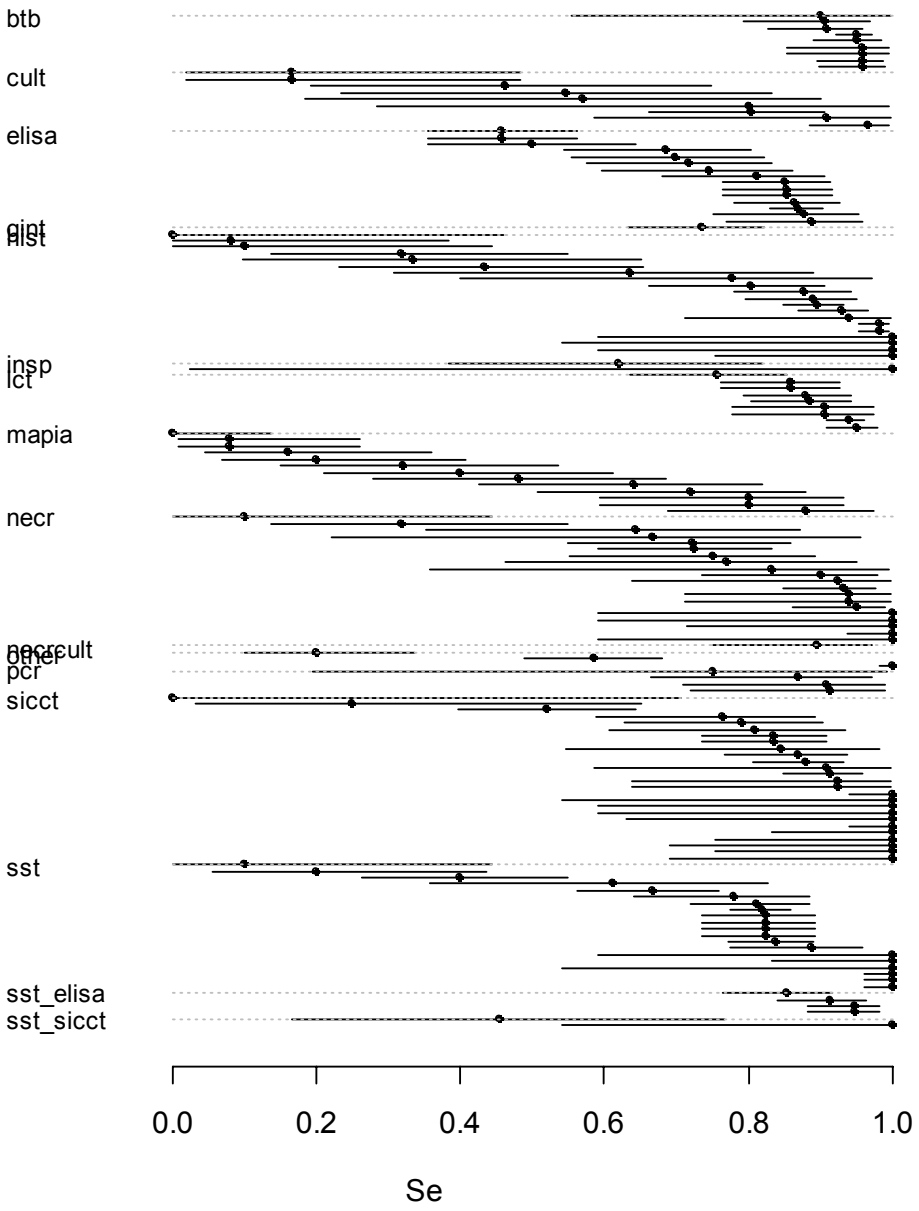
a) All deer species combined. See tables 50 for test abbreviations.

Figure 44 - Funnel plots to explore publication bias for sensitivity of diagnostic tests for deer based on a systematic literature review a)



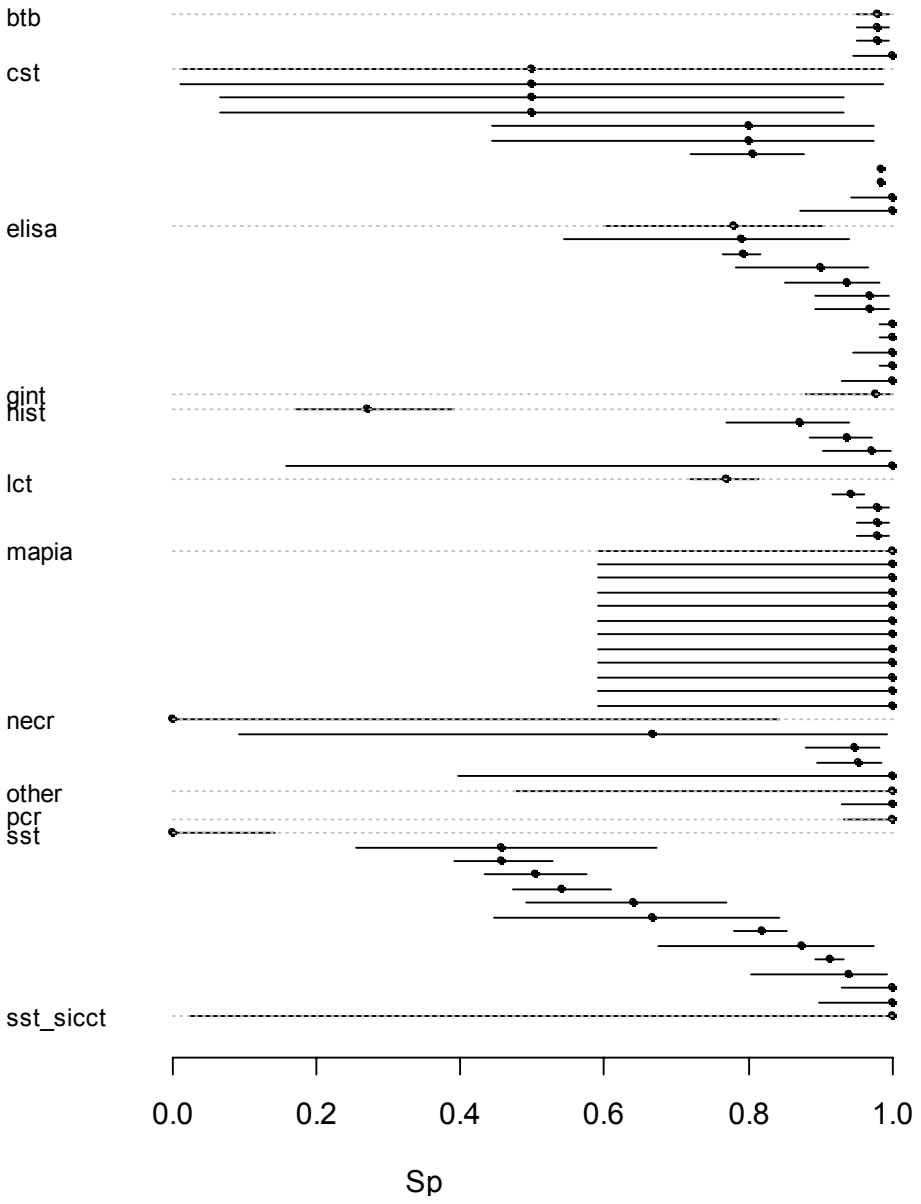
a) All deer species combined. See tables 50 for test abbreviations.

Figure 45 - Funnel plots to explore publication bias for specificity of diagnostic tests for deer based on a systematic literature review a)



a) Estimates are sorted in decreasing order for each test. See table 50 for abbreviation of tests.

Figure 46 - Point estimates and 95% confidence intervals of the sensitivity of diagnostic tests for deer based on a systematic literature review a)



a) Estimates are sorted in decreasing order for each test. See table 50 for abbreviation of tests.

Figure 47 - Point estimates and 95% confidence intervals of the specificity of diagnostic tests for deer based on a systematic literature review a)

D. INVESTIGATION OF CONFOUNDING FACTORS FOR THE SENSITIVITY AND SPECIFICITY OF EACH OF THE TESTS USING LOGISTIC REGRESSION ANALYSIS

The abbreviations used in the following tables

- species (for deer species): see table 47 in Appendix B
- dpi3 (days post infection, 3 categories):
  - 0=not given,
  - 1=less than 6 months,
  - 2=6 months or elder
- rep (sample includes repeated measurements on individual animals):
  - y=yes,
  - n=no

- region (geographical origin of sample):
  - non-EU=nonEU,
  - EU + Russia=EU
- bias (description indicates possible bias):
  - over=overestimation,
  - under=underestimation of Se or Sp
- gs (gold standard):
  - gs=experimental,
  - gns=non-experimental condition

The variable name (e.g. dpi3) has been concatenated to the level (e.g., 1, resulting in dpi31). Another example: repn is the effect of having no replicates in the sample.

Table 55 – Investigation of confounding factors for sensitivity of CULT using logistic regression analysis

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.1957446	0.8081696	-0.2422073	8.086195e-01
speciesred	4.0371861	0.6985901	5.7790486	7.512422e-09
speciesrein	-0.8472979	0.8273595	-1.0240987	3.057886e-01
dpi31	-0.7205462	0.7163366	- 1.0058765	3.144750e-01
repn	1.6094379	0.7176350	2.2426971	2.491635e-02

Table 56 – Investigation of confounding factors for sensitivity of PCR using logistic regression analysis

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	2.1041342	0.4736985	4.4419273	8.915670e-06
specieselk	-1.0055219	1.2480880	-0.8056498	4.204448e-01
speciesred	0.1984509	0.8799945	0.2255139	8.215796e-01

Table 57 – Investigation of confounding factors for specificity of PCR using logistic regression analysis

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	26.68755	51980.71	0.0005134125	0.9995904

Table 58 – Investigation of confounding factors for sensitivity of SICCT using logistic regression analysis

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	5.26377816	2.0856540	2.523802e+00	0.01160932
speciesmixed	-0.05575705	0.4859715	-1.147331e-01	0.90865664
speciesred	-1.14869147	1.0169208	-1.129578e+00	0.25865405
speciesrein	17.54982512	3126.6478523	5.612984e-03	0.99552151
gsx	1.28313924	4452.1405758	2.882073e-04	0.99977004
regionnonEU	-1.31808786	0.9882726	-1.333729e+00	0.18229268
dpi31	0.23514955	4452.1405032	5.281719e-05	0.99995786
repn	-2.66027425	1.3746813	-1.935193e+00	0.05296656
repy	-1.93692723	1.0828766	-1.788687e+00	0.07366526
biasover	2.40464096	1.2140173	1.980730e+00	0.04762151

Table 59 – Investigation of confounding factors for specificity of SICCT using logistic regression analysis

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	4.231583e+00	0.2100237	2.014812e+01	2.795495e-90
speciesmixed	-3.632287e-16	1.1180340	-3.248816e-16	1.000000e+00
speciesred	3.902632e-02	0.2969777	1.314116e-01	8.954497e-01
speciesrein	-1.386294e+00	1.0606602	-1.307011e+00	1.912090e-01
gsx	1.287854e+00	1.0767928	1.196009e+00	2.316929e-01
repn	-2.845288e+00	0.8179914	-3.478384e+00	5.044463e-04
repy	-4.086623e+00	1.1236438	-3.636938e+00	2.758983e-04

Table 60 – Investigation of confounding factors for sensitivity of SST using logistic regression analysis

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.19152157	0.5039225	2.3644935	1.805475e-02
speciesred	-0.93665589	0.4645896	-2.0160929	4.379026e-02
speciessika	-2.73977912	0.6783192	-4.0390708	5.366336e-05
gsx	-2.12449932	0.4974817	-4.2705077	1.950285e-05
regionnonEU	0.15235874	0.4732432	0.3219460	7.474936e-01
dpi31	1.53664295	0.9290044	1.6540749	9.811229e-02
repn	1.14458438	0.4893459	2.3390087	1.933498e-02
biasover	0.05268817	0.2094528	0.2515515	8.013877e-01
biasunder	-1.09982368	0.4934178	-2.2289906	2.581453e-02

Table 61 – Investigation of confounding factors for specificity of SST using logistic regression analysis

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-2.342230	0.2741322	-8.544163	1.294711e-17
speciesfallow	1.134475	0.7272065	1.560045	1.187492e-01
speciesred	-1.694289	0.1368854	-12.377429	3.462572e-35
gsx	-0.845685	0.2853565	-2.963609	3.040543e-03
regionnonEU	2.362033	0.2352542	10.040343	1.013211e-23
repn	2.342230	0.1829803	12.800451	1.630019e-37

Table 62 – Investigation of confounding factors for sensitivity of GINT using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.026639	0.2378908	4.315588	1.591785e-05

Table 63 – Investigation of confounding factors for specificity of GINT using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	3.7612	1.011560	3.718217	0.0002006341

Table 64 – Investigation of confounding factors for sensitivity of LCT using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	2.04475598	0.3204275	6.38133694	1.755486e-10
speciesred	0.25076194	0.3437679	0.72945125	4.657257e-01
biasover	-0.29403793	0.3591881	-0.81861819	4.130043e-01
biasunder	-0.01825064	0.5395773	-0.03382396	9.730175e-01

Table 65 – Investigation of confounding factors for specificity of LCT using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	3.901973	0.5050253	7.726292	1.107243e-14
speciesred	-1.617137	0.5150265	-3.139910	1.689998e-03

Table 66 – Investigation of confounding factors for sensitivity of ELISA using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.8538913	0.2982805	6.2152609	5.123946e-10
speciesred	0.1145086	0.1958145	0.5847812	5.586948e-01
gsx	-0.8292794	0.2618408	-3.1671129	1.539605e-03
repn	-0.6191468	0.3403898	-1.8189347	6.892139e-02
biasover	-0.3889455	0.1695112	-2.2945124	2.176108e-02

Table 67 – Investigation of confounding factors for specificity of ELISA using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	22.0077890	2565.1203277	0.008579632	0.993154528
speciesred	20.4958457	1813.4153824	0.011302345	0.990982226
gsx	0.9201224	0.3549604	2.592183203	0.009536897
repn	-20.4246556	2565.1203288	-0.007962455	0.993646947

Table 68 – Investigation of confounding factors for sensitivity of MAPIA using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.992430	0.6154575	3.237316	0.0012065979
biasover	-2.439742	0.6267371	-3.892769	0.0000991067

Table 69 – Investigation of confounding factors for specificity of MAPIA using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	26.81066	43911.09	0.000610567	0.9995128

Table 70 – Investigation of confounding factors for sensitivity of NECR using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	3.46568839	1.1382134	3.044849344	0.002327969
speciesmixed	-0.78009812	1.5698088	-0.496938287	0.619232571
speciesred	0.09537062	0.4254016	0.224189625	0.822609756
speciesrein	-1.01520243	1.1073069	-0.916821158	0.359236364
speciessika	-2.16864327	0.6715214	-3.229447834	0.001240295
gsx	0.83898933	0.7069629	1.186751551	0.235325634
regionnonEU	-1.00935012	1.0547669	-0.956941395	0.338596836
repn	-1.07615236	0.5347287	-2.012520140	0.044165136
biasover	15.49611881	1689.8201285	0.009170277	0.992683280
biasunder	-0.52004508	0.4094648	-1.270060438	0.204063103

Table 71 – Investigation of confounding factors for specificity of NECR using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	3.015535	0.4580436	6.583510892	4.594669e-11
speciesred	-2.322388	1.3075947	-1.776076108	7.572037e-02
speciesrein	14.500209	4863.5033703	0.002981433	9.976212e-01
repn	1.849579	1.2861155	1.438112697	1.504021e-01

Table 72 – Investigation of confounding factors for sensitivity of HIST using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-20.04347822	2378.0462247	-0.0084285486	0.9932750708
speciesmixed	-19.42050773	4654.3440686	-0.0041725552	0.9966707923
speciesred	1.78973722	0.4623906	3.8706177782	0.0001085599
speciesrein	1.80253744	5020.6528854	0.0003590245	0.9997135399
speciessika	0.01104984	0.6564231	0.0168334046	0.9865695207
gsx	1.67605760	0.5613031	2.9860114453	0.0028264206
dpi31	17.13729918	2378.0460229	0.0072064624	0.9942501247
repn	19.78111395	2378.0461173	0.0083182213	0.9933630962
repy	0.67050565	0.8588449	0.7807063193	0.4349752430
biasover	1.04608709	0.3955599	2.6445728852	0.0081794089

Table 73 – Investigation of confounding factors for specificity of HIST using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	0.8901455	0.1508362	5.901406	3.604167e-09
speciesred	1.8326109	0.3757152	4.877659	1.073521e-06

Table 74 – Investigation of confounding factors for sensitivity of INSP using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	22.56607	48196.14	0.0004682132	0.9996264
repn	-22.08056	48196.14	-0.0004581396	0.9996345

Table 75 - Investigation of confounding factors for sensitivity of BTB using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	3.1354942	0.5107539	6.1389528	8.306722e-10
speciesred	0.6173400	0.4672259	1.3212879	1.864054e-01
repn	-0.8842024	0.6671432	- 1.3253562	1.850530e-01
biasover	0.1068978	0.4942607	0.2162782	8.287709e-01
biasunder	0.2448835	0.7458631	0.3283224	7.426679e-01

Table 76 – Investigation of confounding factors for specificity of BTB using logistic regression analysis.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	3.901973	5.050253e-01	7.7262922100	1.107243e-14
speciesred	-22.992867	5.186626e+04	-0.0004433107	9.996463e-01
repn	22.985262	5.186626e+04	0.0004431641	9.996464e-01

Table 77 – Investigation of confounding factors for sensitivity of SST_ELISA using logistic regression analysis

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.755392	0.2894378	6.064832	1.320914e-09