HUMAN AND ANIMAL GOOD CELL CULTURE BANKING PRACTICES

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Italy

CELL CULTURE LABORATORY BASED ON:

- Laboratory facilities
- Equipment
- Reagents, media
- Competence of staff
- Human and animal cell cultures
- Quality controls
- Validation techniques
- Records of the different steps
- Standard Operating Procedures (SOPs)

LABORATORY FACILITIES

- For diagnosis and research (Category 2, ACDP)
- For cell therapy, drugs/biologicals "CGMPs"
- Dedicated laboratories
- Proper maintenance duties
- Environmental contamination kept at minimum

EQUIPMENTS AND FACILITIES

- Class II Biological Safety Cabinets
- Liquid nitrogen tanks in a designated area
- Warm (37°C) and cold (+4°C, -20°C) rooms
- Freezers (-80°C)
- Areas for "*general services*"

All the instruments are properly maintained, tested and calibrated

REAGENTS AND MEDIA

- Names of the suppliers and all information for traceability
- Quality controls
  - ✓ pH
  - ✓ Sterility
  - ✓ Traceability label

Two kinds of media:

- Liquid (ready to use)
- Dried, diluted and sterilized

COMPETENCE OF STAFF

- Basic culture technique
- Safety precautions
- Documented training program
- Regular reviews of training improvements
- Provided in-house
- External courses

What types of cells are banked in our centre and what type of quality controls we perform?

HUMAN AND ANIMAL CELL CULTURES

- Primary cells
- Continuous cell lines
- Hybridomas
- Animal mesenchymal stem cells
- Samples from Alzheimer Disease patients

INFORMATIONS:

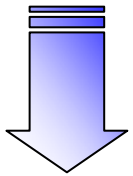
- ✓ Origin and name
- ✓ Identification code
- ✓ Methods for cell manipulation
- ✓ Morphological and culture characteristics
- ✓ Specific data

PRIMARY CELL CULTURES

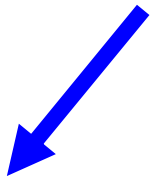
Animal tissues / raw materials

ISOLATION

QUALITY CONTROLS

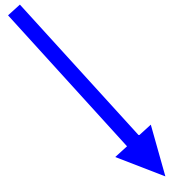


SEEDING

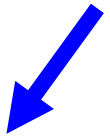


Eubacteria

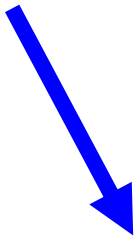
Viruses



Mycoplasma



Storage

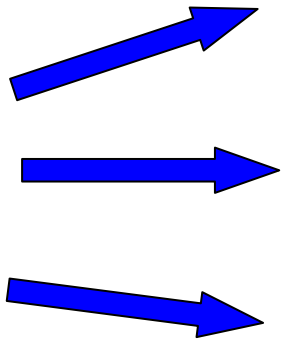


Sub-cultures

ANIMAL MESENCHYMAL STEM CELLS

Isolated from

- Adipose Tissue
- Bone Marrow
- Amnion



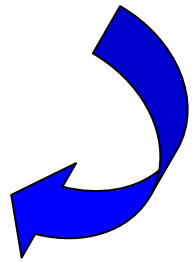
Amplification

Biobanking

Allogenic or autologous transplantation

To repair tendon and cartilage injuries in

equine species



PRIMARY CULTURE DATA RECORD

ORIGIN OF TISSUE						DISAGGREGATION				
Species	Race of strain	Age	Sex	Tissue	Site	Enzyme	Concentration	Duration	Temperature	Solvent
Head of the laboratory: Date:										

SEEDING			MEDIUM					MATRIX COATING
Vassel (n° & type)	Concentration	Volume	Type	Batch n°	Serum (Type & concentration)	Batch n°	Antibiotic (Type & concentration)	Type
Head of the laboratory: Date:								

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RISKS OF CELL CULTURE BANKING

- Cross-contamination
- Pathogen contamination
- Phenotypic and genotypic changes

Correct cell banking:

- A master cell bank
- Several working cell banks

ANIMAL CELL LINES AND REAGENTS

- High risk of contamination by viruses and microorganisms
- Viruses: undetectable as non cytopathogenic *in vitro (circovirus, BVDV, TTV, pestivirus, retroviruses)*
- Sources: SPF animals or controlled raw material
- Proper testing with validated laboratory methods

RISKS OF ANIMAL PRODUCTS

- ✓ Enzymes
- ✓ Foetal calf serum

Selection of the source

- ➢ Country and herd of origin
- ➢ Veterinary certificate
- ➢ Production methods
- ➢ Analytical data
- ➢ Storage and shipping information

RISKS OF ANIMAL PRODUCTS

Bovine Spongiform Encephalopathy (BSE)

Guideline of the European Directive for the Quality of Medicine:

Certificate of Suitability

- Geographical origin of the material
- Part of the material
- Age of animals
- Manufacturing process

Note: brain and nervous tissue - targets

diagnostic tests not for PrPSc detection in serum

QUALITY CONTROLS

According to European Pharmacopeia

Eubacteria

Fungi & Yeasts

Mycoplasma

Viruses

Other parameters

Authentication

Cell biology and Tumorigenicity

Serial amplification

Records

Working cell Bank (WCB)

STERILITY TESTING FOR BACTERIA, FUNGI AND YEASTS:

Cell cultivation in antibiotic-free media	***During routine serial passages***
➢ Inoculation in bacteriological media at different temperatures; 28 days required ➢ HEPA- filtered air in the laboratory and biosafety cabinets ➢ Carried out on the MCB and WCB	Inoculation in other bacteriological media: ➢ Blood agar (37°C/72 h) ➢ Sabouraud medium (30°C/96 h)

STERILITY TEST ACCORDING WITH EUROPEAN PHARMACOPEIA

CELL LINE	PASSAGE N° BATCH N° FREEZING DATA	THYOGLICOLATE MEDIUM BATCH N°	TSB BATCH N°	DATA INOCULUM	TECHNICIAN	FINAL DATA
Head of the laboratory:						

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ROUTINE TEST FOR EUBACTERIA CONTAMINATION BLOOD AGAR BASE AND SABOURAUD AGAR MEDIA

DATA	CELL LINE	PASSAGE	FREEZING DATA	RESULT BLOOD AGAR			RESULT SABOURAUD AGAR			
				DAY 1	DAY 2	DAY 3	DAY 1	DAY 2	DAY 3	DAY 4
Technician:										

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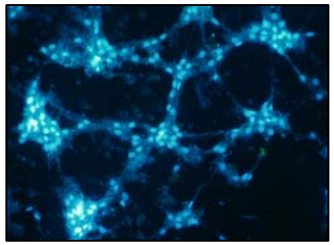
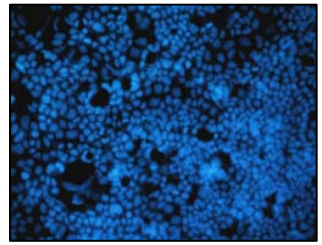
MYCOPLASMA TESTING



Infected cells



Not infected cells

Stringent controls

- Inoculation in sensitive cells (VERO) and staining by Hoechst method
- Isolation in specific growth media
- NAT assay

Rapid tests

- Direct Hoechst staining low sensitivity (10^4 microrganisms/ml not visualized)
- ELISA:

  identification of four species (M. orale, M. arginini, M. hyorhinis, A. laidlawi)

- Detection of mycoplasma metabolites (PCR by RNA hybridisation)

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HOECHST STAINING

TEST DATA	INOCULUM DATA	CELL LINE	PASSAGE NUMBER	FREEZING DATA	RESULTS BACTERIA	RESULTS MIYCOPLASMA	TECHNICIAN	RECORD ON SOFTWARE

Head of the laboratory

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HUMAN AND ANIMAL VIRUSES MORE FREQUENTLY INVESTIGATED

Species	Virus
Bovine	BVD, Polyoma, Retrovirus
Swine	Circovirus, CMV, Parvovirus, Pestivirus, Retrovirus, Hepatitis E
Fowl	Retrovirus, Chick anemia
Human	HIV, HTLV, HBV, HCV, CMV, Parvovirus, HSV, EBV, Papilloma
Monkey	SV 40, Herpes simiae B, Parainfluenza
Mouse	Reovirus, Sendai, Lactic dehydrogenase, lymphocitic choriomeningitis, Reovirus-3, Hepatitis, Minute virus of mice

VIRUSES IN CELL CULTURES

Related to the species and tissue of origin

- Detection by inoculation in:
  - susceptible cell cultures
  - chicken embryos
  - laboratory animals

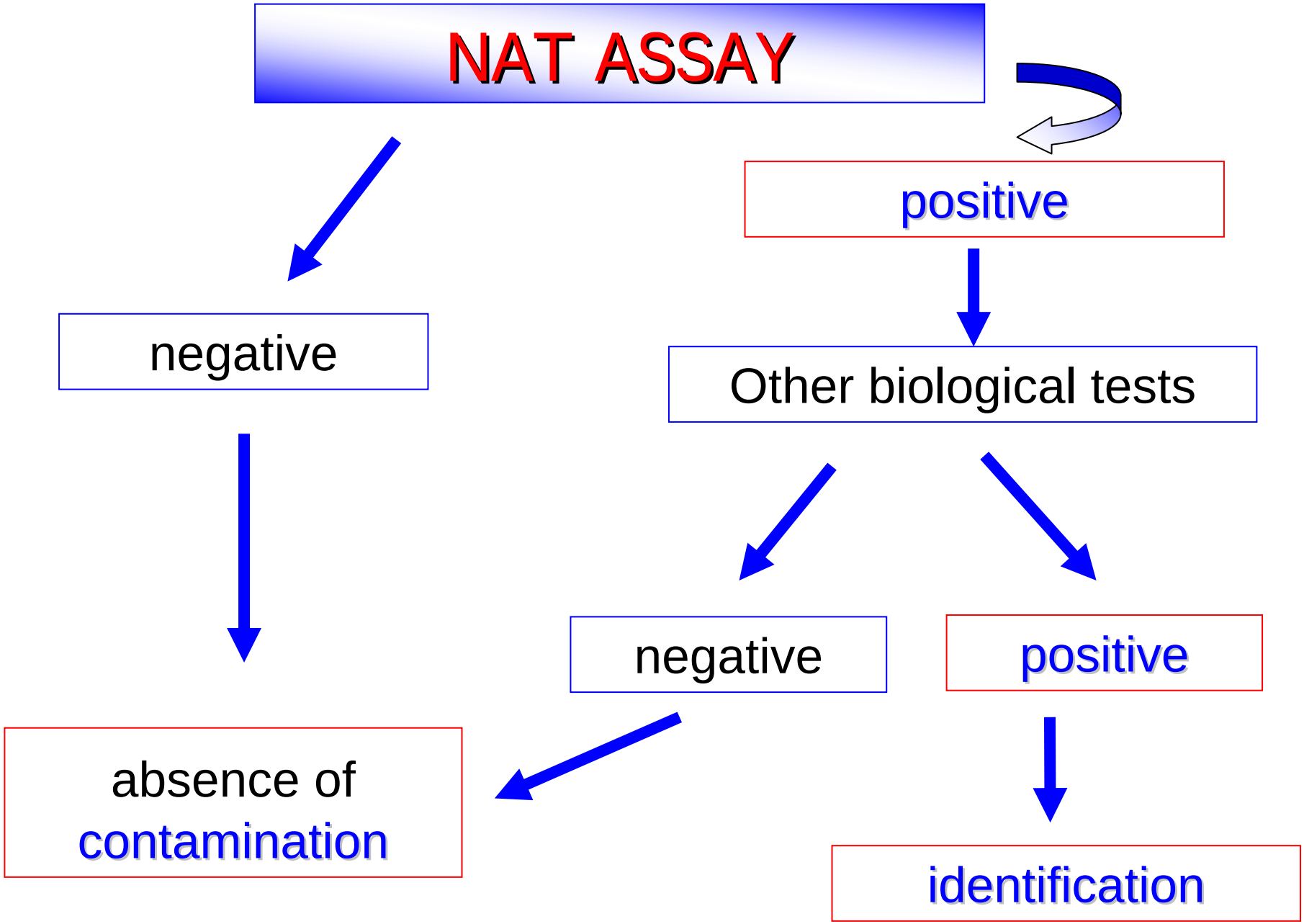
Time consuming and expensive

- Electron microscopy

- Reverse transcriptase assay

- NAT assay
  - ✓sensitive
  - ✓rapid
  - ✓reproducible

No differentiation between alive and inactivated virus


NAT ASSAY
positive
negative
Other biological tests
negative
positive
absence of
contamination
identification

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SUBCULTURE RECORD

CELL LINE		STATUS BEFORE CULTURE				DISSOCIATION		
Cell line	Passage n°	Phase	Morphology	Density	Clarity of medium	Enzyme	Concentration	Duration
Head of the laboratory: Date:								

SEEDING				MEDIUM					MATRIX COATING
Vassel (n° & type)	Cell concentration	Split ratio	Volume	Type	Batch n°	Serum (Type & concentration)	Batch n°	Antibiotic (Type & concentration)	Type
Head of the laboratory: Date:									

TO SUMMARIZE

- Bacteria, fungi and yeasts
- Mycoplasma
- Viruses: exogenous (primary cells) and endogenous

Risks of infection spreading to

other cells / workers

Precautions

- Suitable facilities, processing clearly defined, proper handling during freezing and proper retrieval of vials from liquid nitrogen

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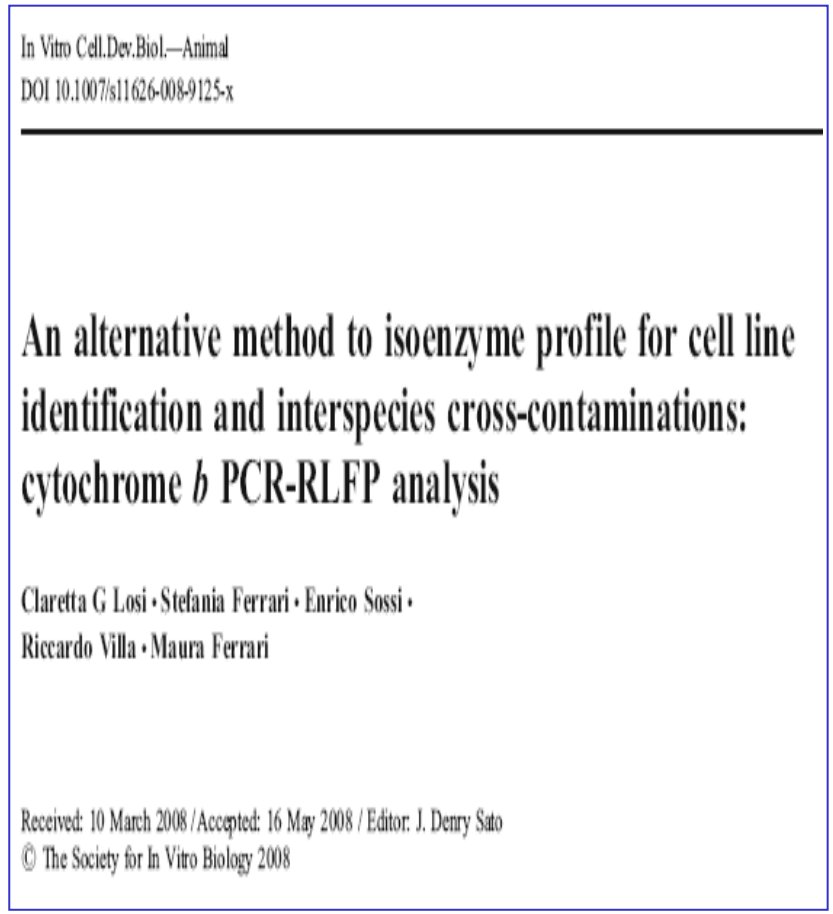
AUTHENTICATION

Identity of cell type:

- ✓ Isoenzyme analysis
- ✓ DNA profile
- ✓ Kariology
- ✓ Mitochondrial DNA analysis



In Vitro Cell.Dev.Biol.—Animal
DOI 10.1007/s11626-008-9125-x

An alternative method to isoenzyme profile for cell line identification and interspecies cross-contaminations: cytochrome *b* PCR-RLFP analysis

Claretta G Losi · Stefania Ferrari · Enrico Sossi · Riccardo Villa · Maura Ferrari

Received: 10 March 2008 / Accepted: 16 May 2008 / Editor: J. Denry Sato

HeLa or contamination with other cells and their current use

Doubtfoul scientific conclusions on the results of studies with these cross-contaminated cells

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CELL BIOLOGY AND TUMORIGENICITY

- Cell substrate stability – at the beginning and end of the intended span of use

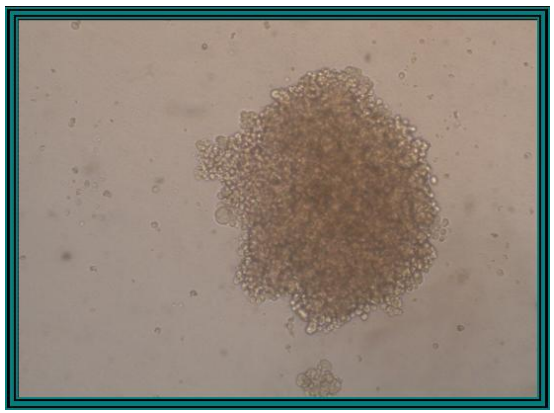
- Karyotyping and life span (cell population doubling) for diploid cells

- Tumorigenicity

In vitro

Transformed

colony



In vivo

Tumor

formation

Quality system: accreditation UNI CEI EN ISO/IEC 17025 ISO 9001/9002

DEFINITIONS

In a cell culture bank

- Quality of a product: conforms to specification
- Quality assurance: based on management procedures, environment and quality control

  to ensure the quality and safety of a product
- Quality controls: based on tests to

  ensure identity, viability, sterility
- They must by performed by

  validated methods

Standard operative procedures (SOPS)

- Maintenance and sterility of all equipment and instruments
- Culture reagents
- Good culture techniques
- Coding and recording system for cryopreserved cells
- Quality control procedures

Records for each process

- Subculture
- Passages
- Batches
- Position in liquid nitrogen
- Quality controls

to

- Reduce risks of contamination or misidentification
- Identify cells

Moreover

- Good and standardised handling procedures (records)
- Seed stock at the first passages after validation and working stocks (records and software)
- Distribution and transfer together with detailed cell line information and handling and storage method

Storage of a validated cell line in a cell culture bank allows to protect against loss and to provide authenticated cells

VALIDATION

a cell line is completely characterised and it is suitable for a specific use

Principles of good cell culture technique similar to good laboratory practice

CONCLUSIONS

The maintenance of high standards is fundamental to all scientific community and manufactures which uses cell cultures

However

Quality standards must be selected for each type of operation

THANKS FOR YOUR KIND ATTENTION