VITROLOGY IS NOW PART OF SGS, THE WORLD'S LEADING INSPECTION, VERIFICATION, TESTING AND CERTIFICATION COMPANY.

Biosafety Considerations for Cell Based Viral Vaccines

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WHEN YOU NEED TO BE SURE

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Presentation Outline:

- Sources of adventitious contamination in biological material
- Safety concerns relating to virus based therapeutics
- Why validated test methods are of interest to the Regulatory Authorities
- Cell bank and virus vaccine characterisation – tests and methods

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An MHRA accredited contract testing company providing GMP and /or GLP drug development services to the global biotechnology industry, to comply with the current requirements of the international regulatory authorities.

Testing data supports products in clinical evaluation and marketed under commercial licence

APPROVAL OF A CELL BASED VACCINE

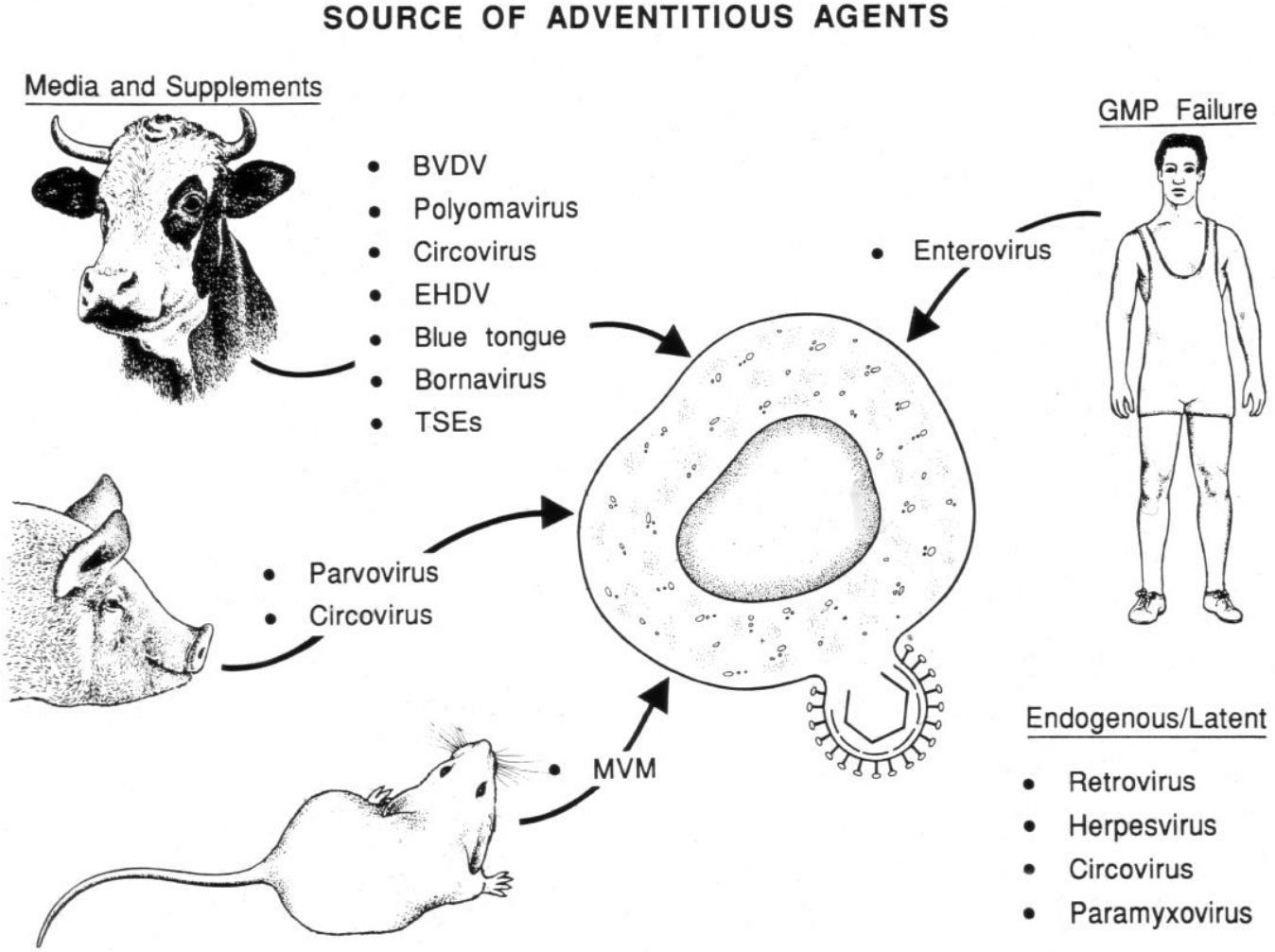
For Marketing Approval

- **Safety**
- **Efficacy**
- **Consistency**

Investigational Medicinal Product (IMP) for clinical trials

- **Safety**
- **Efficacy**
  - **Toxicological**
  - **Biological**
- **Consistency**

ADVENTITIOUS AGENTS: CELL CULTURE DERIVED PRODUCTS

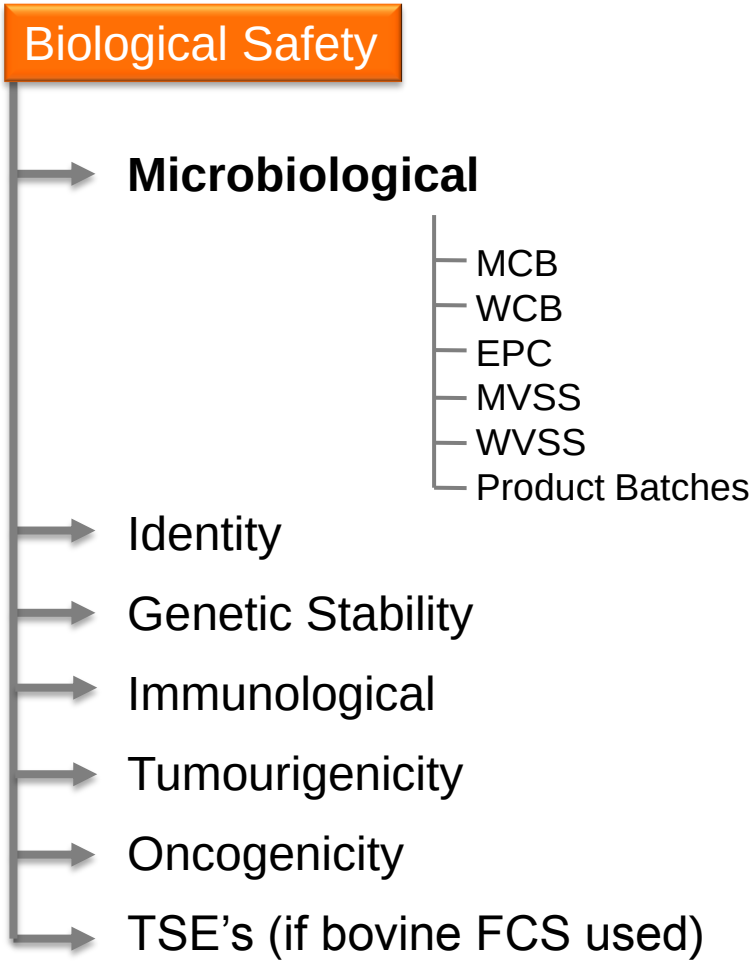


SAFETY CONCERNS RELATING TO VIRUS BASED THERAPEUTICS

Biological Safety

- Microbiological
- Identity
- Genetic Stability
- Immunological
- Tumourigenicity
- Oncogenicity
- TSE's (history & if bovine FCS used)

SAFETY CONCERNS RELATING TO VIRUS BASED THERAPEUTICS



SAFETY CONCERNS RELATING TO VIRUS BASED THERAPEUTICS

- **Biological Safety**
  - **Microbiological** (MCB / WCB / EPC / Control cells / MVSS / WVSS /Batches)
    - Sterility
    - Mycoplasma
    - Virus
      - Species Specific
      - Retrovirus
      - Adventitious
  - Identity
  - Genetic Stability
  - Immunological
  - Tumourigenicity
  - Oncogenicity
  - TSE's (if bovine FCS used)

SAFETY CONCERNS RELATING TO VIRUS BASED THERAPEUTICS

Biological Safety

- Microbiological
- **Identity**
  - Species & contamination (by Isoenzyme)
  - DNA fingerprinting
  - Karyology – unlikely only diploid cells
- Genetic Stability
- Immunological
- Tumourigenicity
- Oncogenicity
- TSE's (if bovine FCS used)

SAFETY CONCERNS RELATING TO VIRUS BASED THERAPEUTICS

Biological Safety

- Microbiological
- Identity
- **Genetic Stability**
  - Sequence (only if recombinant virus)
  - Restriction mapping (only if recombinant virus)
- Immunological
- Tumourigenicity
- Oncogenicity
- TSE's (if bovine FCS used)

SAFETY CONCERNS RELATING TO VIRUS BASED THERAPEUTICS

- **Biological Safety**
  - Microbiological
  - Identity
  - Genetic Stability
  - **Immunological**
    - Autoimmune response
    - Neutralising antibody status of patients
  - Tumourigenicity
  - Oncogenicity
  - TSE's (if bovine FCS used)

SAFETY CONCERNS RELATING TO VIRUS BASED THERAPEUTICS

Biological Safety

- Microbiological
- Identity
- Genetic Stability
- Immunological
- **Tumourigenicity**
  - Not expected for known tumourigenic lines HEK 293/PER.C6/A549
- Oncogenicity
- TSE's (if bovine FCS used)

SAFETY CONCERNS RELATING TO VIRUS BASED THERAPEUTICS

Biological Safety

- Microbiological
- Identity
- Genetic Stability
- Immunological
- Tumourigenicity
- **Oncogenicity**
  - Define acceptable levels of host cell DNA & proteins
  - HCD Size
- TSE's (if bovine FCS used)

SAFETY CONCERNS RELATING TO VIRUS BASED THERAPEUTICS

Biological Safety

- Microbiological
- Identity
- Genetic Stability
- Immunological
- Tumourigenicity
- Oncogenicity
- **TSE's (history)**
  - Adapt to serum free cultivation where possible
  - Sourcing of FCS, if essential

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Testing of

- Cell banks and raw materials
- Cell derived harvests and final product
- Viral seeds and harvests
- Animal tissue and cell derived products

For manufacture of

- Monoclonal antibodies
- Recombinant protein
- Viral vaccine
- Cell therapies
- Gene therapies

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Assay Systems Require:

- Protocol (Development, Validation, Study)
- Method SOP(s) (Development, Validation, Study)
- Data Record
- Final Report or (Development, Validation, Study)
- Summary Report/C of A (Development, Validation, Study)

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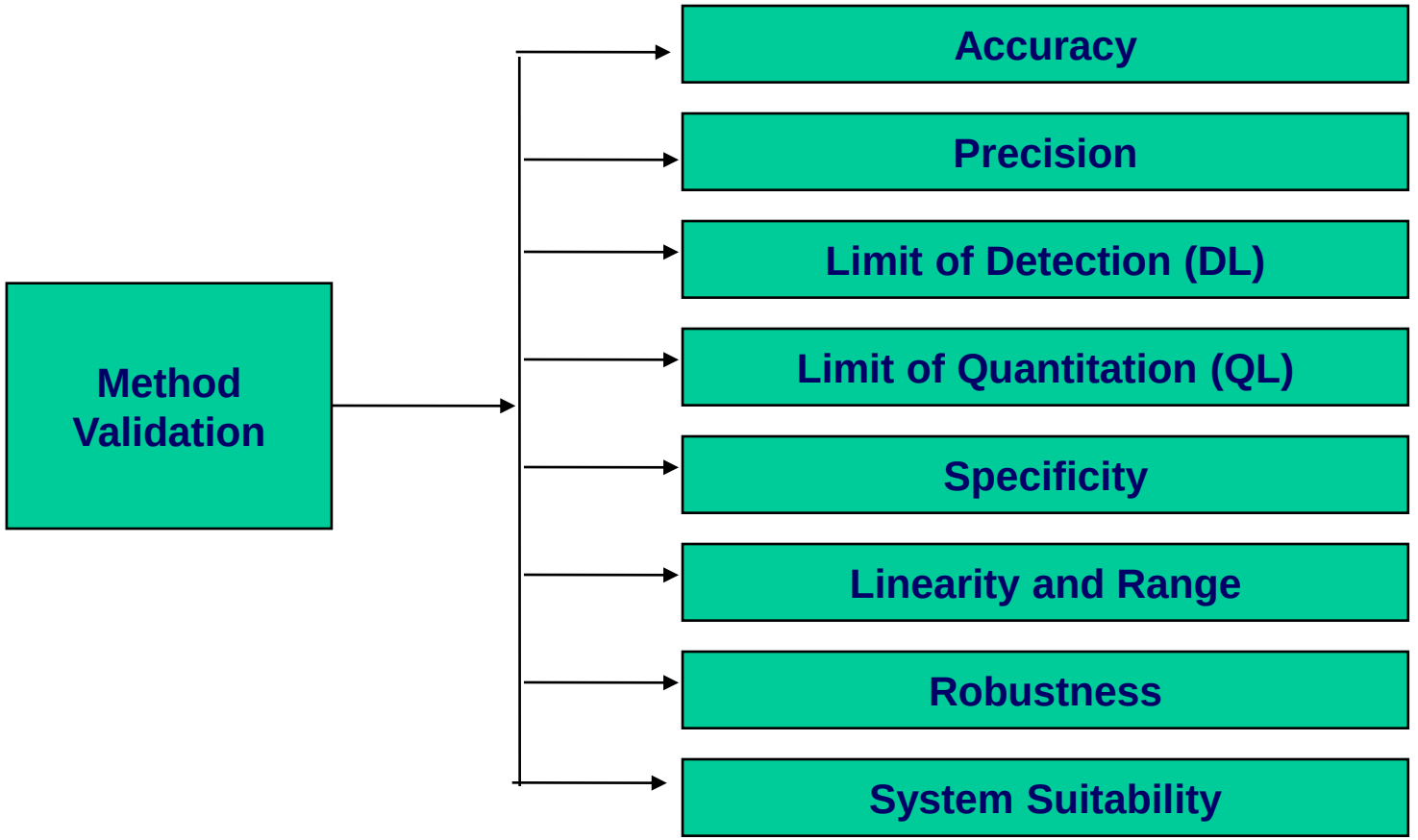
Validation of methods to meet ICH guidelines Q2(R1)



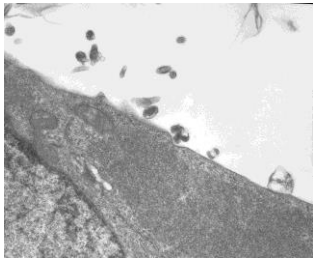
Importance of Assay Validation Documents

- **FDA Guidance for Industry: Analytical Procedures and Methods Validation; Chemistry, Manufacturing and Controls Documentation**
  - Validated procedures should be used for in-process, release, acceptance, and stability testing

- **EMEA Guideline on virus safety evaluation of biotechnological IMP**
  - For Phase I/II clinical trials, the suitability of the analytical methods used for viral testing should be demonstrated.
  - Results of values found for ICH validation should be available (eg. specificity, linearity, range, accuracy, precision, quantification and detection limit)
  - It is not necessary to provide a full validation report.
  - Phar. Eur. viral tests are not normally required to be re-validated.
  - Note: For Phase III studies a full validation report should be available

Methods used for the Characterization

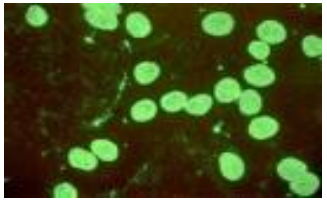
In vivo assays



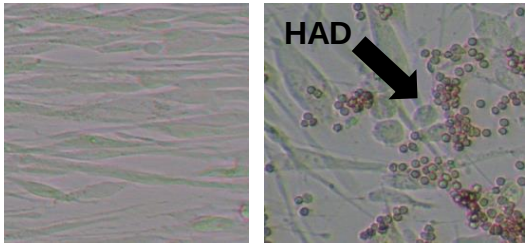
Transmission Electron Microscopy

Sterility

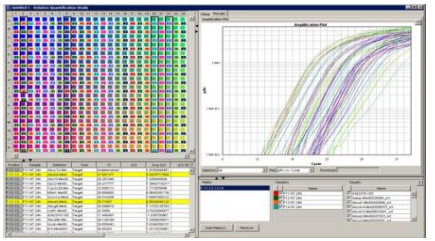
Cell Bank & Viral Vaccine Characterisation



Mycoplasma



In vitro adventitious agent assays



Q-PCR for specific viruses, RT activity

Cell Bank Characterisation (eg. Human)
FDA Vaccine Guidance 2010
Phar. Eur. 5.2.3 & ICH Q5A

Concern	Assay	MCB	WCB	CAL	CC
Identity	DNA fingerprint	+	+	+	
	Isoenzyme	+	+	+	
Microbial	Sterility	+	+	+	+
	Mycoplasma	+	+	+	+
	Mycobacteria	(+)	(+)	(+)	(+)
Adventitious viruses	*In vitro*	+	(+)	+	(+)
	In vivo (including GPigs)	+		+	(+)
Tumourigenicity	Nude mice			+	
Karyology	G-banding	+	+	+	

1 Note If production culture conditions are changed the status of the unprocessed bulk harvest should be confirmed by an *In vivo* assay.

Requirement for Phar. Eur.

Recommended for FDA/CBER Vaccine Guidance.

Master cell bank (MCB)
Working cell bank (WCB)
Cells at limit (CAL) (EOP)
Bulk Harvest (BH)
Control cells (CC)
Alternative stage (+)

Testing of Human Cell Bank continued....

Concern	Assay	MCB	WCB	CAL	CC
Retroviruses	TEM	+		+	
	PERT	+		+	(+)
	[1]HEK 293 Co-cult	(+)		(+)	(+)
*Bovine/Porcine 9CFR	*In vitro*	(+)	(+)	(+)	(+)
*PCV, BPyV, TTV, Hoko, swHepE	PCR	(+)	(+)	(+)	(+)
Human viruses	PCR	(+)	(+)	(+)	

* recommended if previous potential exposure to bovine serum and/or porcine trypsin

[1] Required if a PERT positive is obtained

Requirement for Phar. Eur.

Requirement for FDA/CBER.

Master cell bank (MCB)
Working cell bank (WCB)
Cells at limit (CAL) (EOP)
Bulk Harvest (BH)
Control cells (CC)
Alternative stage (+)

Bovine viruses of concern that may go undetected by 9CFR

Table 3
Viruses with human and bovine host range that are not predicted to be detected by 9CFR test.

Viruses to Consider for Additional Evaluation
Anelloviridae (proposed family)
Torque teno virus TTV[a]
Bornaviridae
Borna disease virus BDV [134]
Bunyaviridae
Cache valley virus CVV [2]
Puumala virus [135]
Caliciviridae
Norovirus [formerly Norwalk agent] [136]
Circoviridae
Bovine circovirus bovCV[a]
Coronaviridae
Bovine torovirus BtoV[a]
Flaviviridae
Louping ill virus[a]
Saint Louis encephalitis virus SLEV[a]
Wesselsbron virus [137]
Hepeviridae
Hepatitis E virus HEV[a]
Papillomaviridae
Bovine papilloma virus BPV[a]
Parvoviridae
Bovine adeno-associated virus BAAV[a]
Bovine hokovirus BHoV[a]
Picornaviridae
Bovine enterovirus BEV-1[a], BEV-2[a]
Seneca valley virus SVV [138]
Polyomaviridae
Bovine polyomavirus BPyV [71, 139]
Poxviridae
Aracatuba virus[a]
Cantagalo virus[a]
Cowpox virus [140]
Pseudocowpox virus PCPV [141, 142]
Reoviridae
Banna virus BAV [82]
Epizootic haemorrhagic disease virus EHDV [143]
Rotavirus [144]
Retroviridae
Bovine foamy virus BFV [145]
Bovine leukemia virus BLV[a]
Togaviridae
Ross River virus RRV [99]

[a] No information found to allow assessment of ability to be detected by 9CFR, hence considered as potential risk.

Marcus- Sekura et al. 2011 (Biologicals)

Porcine viruses of concern that may go undetected by 9CFR

Table 4
Viruses with human and porcine host range that are not predicted to be detected by 9CFR test.

Viruses to Consider for Additional Evaluation	
Anelloviridae (proposed family) Torque teno virus TTV[a]	*Paramyxoviridae* Menangle virus MENV [119]
Caliciviridae Norovirus [formerly Norwalk agent] [136] Sapovirus[a]	*Parvoviridae* Porcine hokovirus PHoV [62]
Circoviridae Porcine circovirus PCV-1 & PCV-2 [146](B.Potts per	*Picornaviridae* Porcine enterovirus PEV-9 PEV-10 [129] Seneca valley virus SVV [138]
Flaviviridae Louping ill virus[133] Powassan virus [147] Wesselsbron virus [137]	*Reoviridae* Banna virus BAV [82] Rotavirus[144]
Hepeviridae Hepatitis E virus HEV[a]	*Retroviridae* Porcine endogenous retrovirus PERV[a]
Herpesviridae Porcine cytomegalovirus PCMV[a]	*Togaviridae* Ross River virus RRV [99]

Note: These are not exhaustive lists, and other viruses related to those listed above may also be of concern.

[a] No information found to allow assessment of ability to be detected by 9CFR, hence considered as potential risk.

PCR available at Vitrology => already being used for large vaccine clients

Marcus- Sekura et al. 2011 (Biologicals)

Origin, Source and History of Cell Line

- Source
  - species, strain, tissue or organ of origin
  - state of health, medical history if human donor

- Cultivation history
  - procedures used in culturing cells
  - procedures used to establish cell line
  - description of genetic manipulation or selection
  - identity, testing for endogenous and adventitious agents
  - constituents of culture medium

- Procedures used
  - cell fusion
  - transfection
  - Selection & colony isolation
  - cloning
  - gene amplification
  - adaptation to culture conditions and media

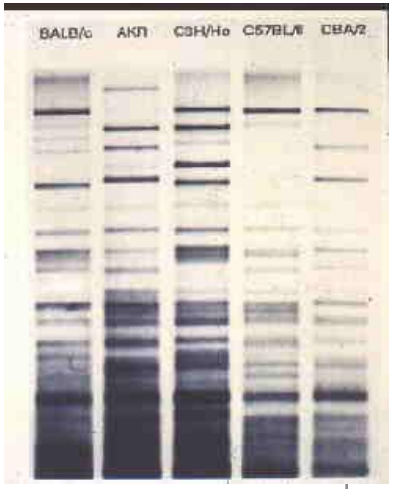
Methods for cell bank identity testing

- Microscopic observation
  - Cell monolayers can show “general” characteristic morphology
  - Does not provide definitive results on the identity
- Isoenzyme analysis
  - Provides identification of the species of origin
  - Technique is referenced in the regulatory guidelines. AuthetiKit™ system (Innovative Chemisty Inc.)
  - Defines a set of enzymes with a characteristic "mobility pattern" for each species

Potential Cell Mixture	Distinguishing Enzymes
NS0 and CHO	PEP B
Human and Vero	AST or MDH
CHO and BHK	MDH
Human and CHO	LDH
Human and NS0	LDH
Insect and Mammal	NP or G6PD

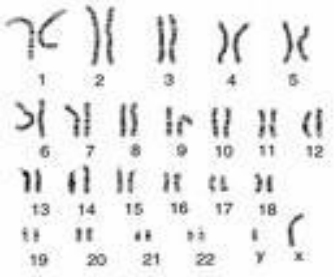
Methods for cell bank identity testing

- DNA fingerprinting or Random Amplified Polymorphic DNA (RAPD)



- Vaccine cell substrate guideline requirement (Ph. Eur. 5.2.3)
- The technique can be used to distinguish between individuals of the same species
- When applied to cell lines, the method is sensitive enough to identify a specific cell line
- For example, can distinguish between human cell lines HEK 293 and A549.

- Karyotypic analysis
  - In general is required when product may contain live cells (eg vaccine)
  - Modal chromosome number and characteristic chromosome markers
  - Useful for the evaluation of novel cell lines
  - Diploid cells should be shown to be diploid
  - Diploid cells have two homologous copies of each chromosome
    - MRC-5, WI-38, FRhL-2 are well characterised diploid cell lines and do not require further qualification
  - Karyology may not be required for highly purified products

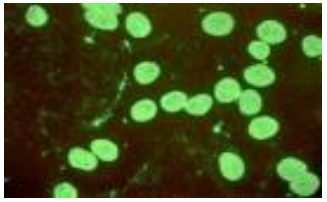


Sterility

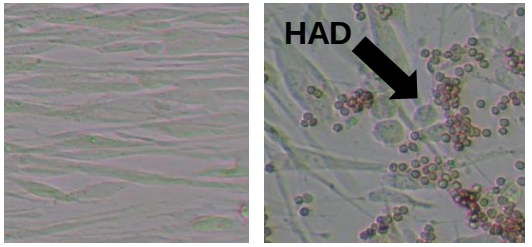
In vivo assays

Transmission Electron Microscopy

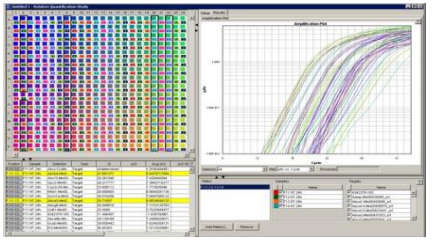
Purity of Cell Banks



Mycoplasma



In vitro adventitious agent assays



Q-PCR for specific viruses, RT activity

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Sterility of cell banks (USP/Phar.Eur/JP)

- Cell and virus banks manufactured under cGMP conditions require sterility testing
- Methods are outlined in the US and EU Pharmacopeias and the 21 CFR 610.12.
- For cell banks test 1% or minimum of 2 containers for MCB and WCB (ICH Q5D recommendation)
- Direct broth inoculation or membrane filtration
- Aerobic and anaerobic incubations, 14 days
- Observation for turbidity (microbial growth)
- Qualification of Test Item by spiking

- Alternative Rapid Microbiological Methods (RMM) if validated may be used on cell therapy/gene therapy products.

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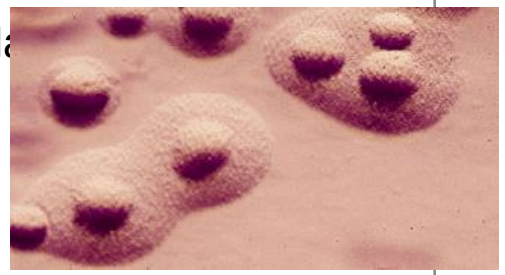
Mycoplasma contamination

- Small extracellular parasite- prokaryote
- Infect a wide variety of hosts (mammals, birds, reptiles, fish, insects, pl
- 5 species cause 95% of the contaminations of cell cultures
- Mycoplasma presence decreases quality or quantity of product
- Mycoplasma alters:
  - Cell function, growth & metabolism
  - Virus propagation and yield
  - Can cause chromosomal aberrations
- Testing according to guidelines
  - USP 63 and Eur Phar 2.6.7 Mycoplasma
  - Important to check for assay inhibitory substances



Mycoplasma colonies on agar
Fried egg apearance

Mycoplasma Detection – Indicator cell, Broth & Agar

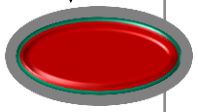
Cell bank

Hoechst stain

1.0 ml → **Vero Cells** → 3-5 days →  **Observe DNA fluorescent staining pattern**

0.2 ml →  Agar plate → 14 days → **Observe for Mycoplasma colonies**

10 ml →  Mycoplasma Broth

- Day 3 →  → 14 days → Day 17
- Day 7 &14 →  → 14 days → Day 21 & 28
- Day 21 →  → 7 days → Day 28

Observe for Mycoplasma colonies

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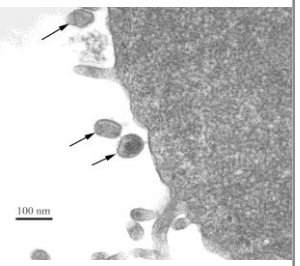
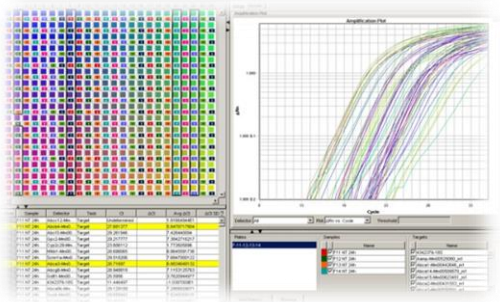
Purity- Adventitious Agent Assays

- Requirement to utilize a number of different assays to detect and wide range of possible contaminants

the “unknown”

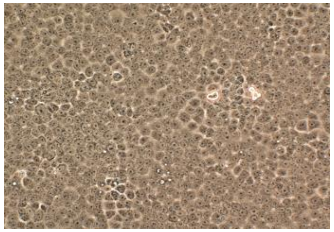


- Broad specificity assays
  - *in vitro* and *in vivo* virus assays,
  - transmission electron microscopy (TEM)
- Assays to detect contaminants associated with specific species
  - rodent, human, bovine, porcine viruses
- Assays to detect retroviruses
  - infectivity assays
  - molecular biology assays (PCR)
  - biochemical assays (reverse transcriptase)
  - morphological assays (electron microscopy)

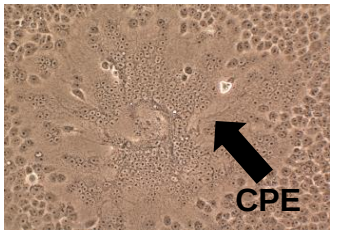


Broad specificity virus detection methods in cells

- Cytopathic Effect (CPE)
  - CPE is a degenerative change in cells
  - CPE may lead to plaque formation
  - not all viruses produce CPE



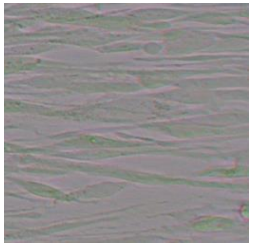
Vero

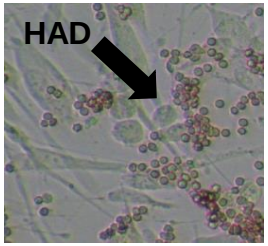


Vero + *Measles virus*

- Haemadsorption (HAD)
  - HAD is a visual method for detecting viruses based on adherence of red blood cells (RBC) to infected cells.
  - Mix of bloods- Guinea pig, Human O, and Chick

MRC-5

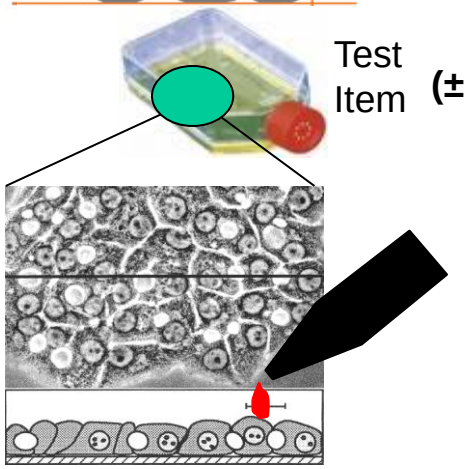




MRC-5 + *Parainfluenza virus*

CPE and HAD are critical methods within the *In vitro* virus detection assay

In Vitro Cell Culture Assay

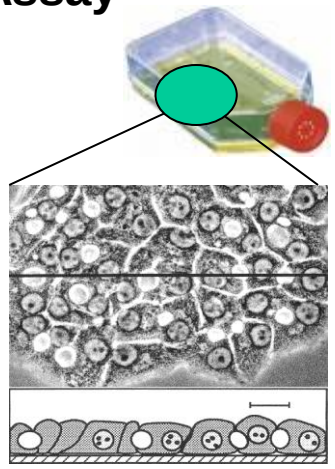


Test Item **(± spike virus)**

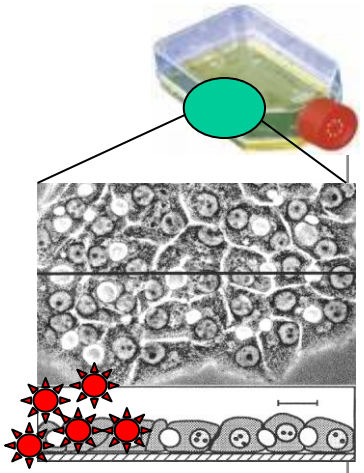
Detector Cell Monolayer

Detector cells passaged at Day 14

28 Days



Detector Cell Monolayer



Infected Detector Cell Monolayer

Common Detector Cells

- MRC-5 (Human)
- Vero (Simian)
- Same Species and Tissue as Cell Bank
  - SP2/0, BHK, CHO, NS0 etc.

- Include Rabbit kidney cells:- for simian cell substrates used in vaccine production (detects Herpes B virus)
- In vitro assays to detect bovine and porcine viruses described in:
  - Title 9 Code Federal Regulations 113.47/51/53

End Point Detection Methods

- Cytopathic Effect - CPE
- Haemadsorption - HAD
- Others
  - Immunofluorescence
  - RTase / PERT assay
  - Electron Microscopy
  - PCR

Not all viruses grow well in cell culture

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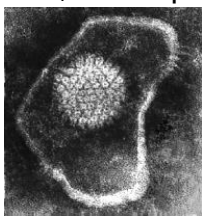
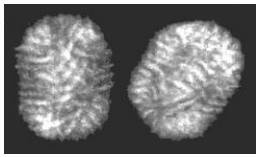
TEM

- detects intracellular virus particles
- rapid morphological detection
- used to indentify “unknown” contaminants
- a test with broad specificity
- may detect viruses that are not detected by *In vitro* or *In vivo* assays
- used to measure retrovirus load in bulk harvest prior to downstream purification

Double stranded
DNA, enveloped

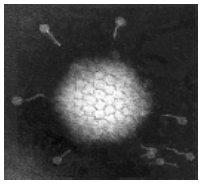


Herpes virus



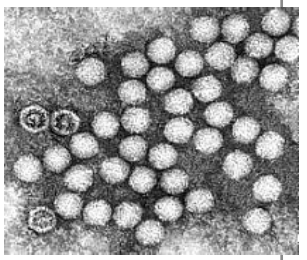
Poxvirus

Double stranded
DNA, unenveloped



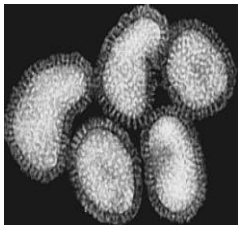
Adenovirus

Single stranded
RNA, unenveloped

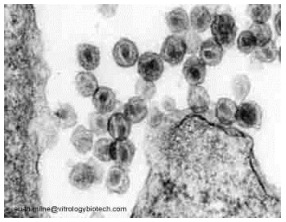


Enterovirus

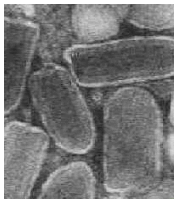
Single stranded
RNA, enveloped



myxoviruses

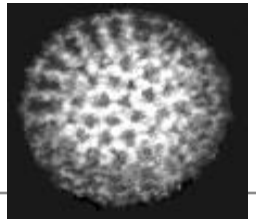


HIV



Rabies

Double stranded
RNA, unenveloped

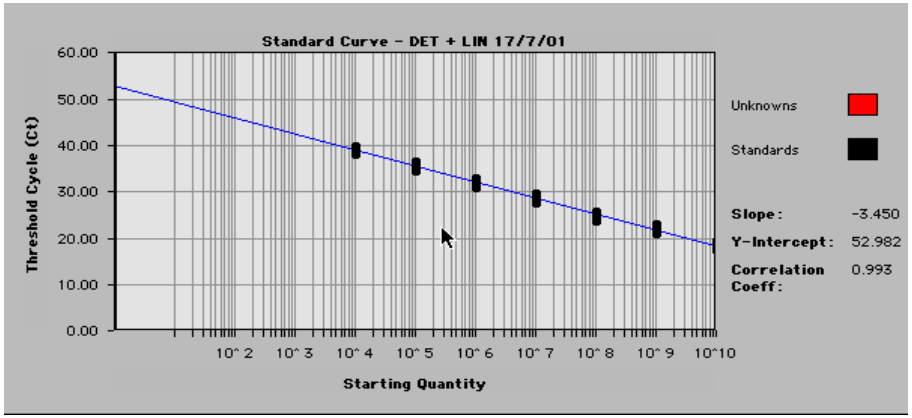


Rotavirus

SGS VITROLOGY

The Product Enhanced Reverse Transcriptase (PERT) Assay – detection and quantitation of Retroviral RT

***Brome Mosiac virus* (BMV) RNA template is converted to cDNA by retroviral RT enzyme if present in a "test article"**



Q-PERT Assay is Quantitative

F-PERT Assay is Qualitative

Measurement of RT Enzyme Activity

F-PERT used for testing vaccines and for end point in retroviral infectivity assays

QPERT used in virus vaccine bulk harvest retroviral load monitoring (CEF).

SGS VITROLOGY

RT Assays (F-PERT) Retrovirus RTase detection by PCR

- Regulatory Guidance
  - Letters to Industry, CBER FDA, 1998 (Vaccines)
  - ICH Q5A (cell therapy, recombinant proteins etc)
  - FDA Guidance Vaccines (2010)
  - Phar. Eur. 5.2.3 Cell Substrates (GT vector and vaccines)
  - WHO TRS 878 (cell substrates for biologicals)

- Extremely sensitive assay for detection of a wide range of retroviral RT activities in cell substrate supernatant, vaccines and gene therapy vectors. **Sensitivity**: <1000 retrovirus particles (500 particles per ml)

- FDA 2010 Vaccine guidance document states that the assay limit of detection should be comparable to published literature (specifically Lovatt *et al*., 1999)

- Assay can sometimes be false positive from normal background cellular DNA polymerase activity → Retroviral infectivity testing (HEK 293 detector) are then used for confirmation

- Assay can also be performed using a Quantitative PERT (Q-PERT)

Assays used to detect Retroviruses

- **Infectivity Assays**
  - **XC plaque:** for the detection of ecotropic murine retrovirus
  - **S+L- focus forming:** for the detection of xenotropic murine retrovirus
  - ***Mus dunni:*** detects all types of murine leukaemia viruses
  - **Co-cultivation:** test sample co-cultured with human or murine cell lines followed by specific end point analysis
- **RTase**
  - PCR-based RT assays (PBRT or PERT)
  - Detects reverse transcriptase activity associated with virus particles
  - Sensitivity: 1000-10,000 retrovirus particles
- **Quantitative PCR**
  - Specific nucleic acid amplification of viral genomes
  - Detects infectious and non-infectious virus
  - Specific, but only detects the specified virus
- **TEM**
  - Sensitivity: -10^5 particles per ml (bulk harvest)
  - Specific for identification of retrovirus type

APPROVAL OF A CELL BASED VACCINE

For Marketing Approval

- **Safety**
- **Efficacy**
- **Consistency**

Investigational Medicinal Product (IMP) for clinical trials

- **Safety**
- **Efficacy**
- **Consistency**

In summary each vaccine should have a case by case, regulated biosafety testing strategy and manufacturing controls at each stages from development

VITROLOGY IS NOW PART OF SGS, THE WORLD'S LEADING INSPECTION, VERIFICATION, TESTING AND CERTIFICATION COMPANY.

Biosafety Considerations for Cell Based Viral Vaccines

Appendix

Margaret Temple
SGS Vitrology
Site Director
Glasgow - UK

WHEN YOU NEED TO BE SURE

SGS

SGS LIFE SCIENCE SERVICES OVERVIEW

VITROLOGY

SGS MEANS EXPERIENCE

- Over 35 years experience
- 1,400 full time staff
- 28 facilities in 15 countries
- Leader with unique international analytical laboratory network
  - across America, Europe, Asia
  - with Centers of Excellence
- Global drug development partner from Molecule to Market
  - Wide-range of laboratory infrastructure, size and diverse testing capabilities matching Biopharmaceutical and Small Molecules needs
- Strong commitment to clinical and laboratory Quality and Operational Excellence
  - Harmonized QMS and Validation & Transfer methods, LIMS

VITROLOGY HISTORY

- Established in April 2007
  - 4 founding members now part of SGS Life Science Services – all former senior employees with Q-One Biotech/BioReliance
  - Dedicated Partnerships with Moredun Foundation (animal), Beckman Coulter Genomics (MPS) and Takara Bio Inc. (Japan)
- 2010-12 – successful re-inspections by MHRA for GLP and GMP compliance.
  - GLP/GMP compliant since 2008
- Acquired by SGS in May 2012
  - "Centre of Excellence for cell bank characterisation & virus testing"

VITROLOGY CORE SERVICES

- GMP Cell Bank & Virus Seeds Characterisation (Vaccines & Gene Therapies)
  - Cell line characterization & safety testing : sterility, mycoplasma, virus, retrovirus
  - Genetic stability studies from MCB, WCB, CAL: gene copy number
- GMP Bulk Harvest Release Testing
  - Sterility, in vitro assays, RVLPs quantification, (EM), QPCR
- Host Cell DNA for Non - GLP (process development) & GMP testing by qPCR
  - 15 years experience in GMP, FDA, EP, ICH compliant validated assays
- GMP Final Product Batch Release Testing
  - HCD & HCP – Endotoxin - Ab.Toxicity - process related impurity testing
- Regulatory Consultancy
  - Expert Report - Custom Assay development & validation