Safety Qualification of Impurities in Biopharmaceutical Drugs

Impurities in Drugs:
Monitoring, Safety and Regulation
The Israel Chapter of PDA

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Food and Drug Administration

Examples of Commonly Used Cell Substrates and Biologics Produced

- CHO (hamster) – recombinant proteins
- NS0 (mouse myeloma) – monoclonal antibodies
- Mouse hybridoma – monoclonal antibodies
- Vero (monkey) – viral vaccines
- MRC-5 (human) – viral vaccines
- 293 (human) – adenoviral vectors for gene therapy
- PER.C6 (human) – adenoviral vectors for gene therapy
- MDCK (canine) – viral vaccines (e.g., Influenza)
- Insect – recombinant proteins

(Microbial expression systems also used – not focus of this presentation)

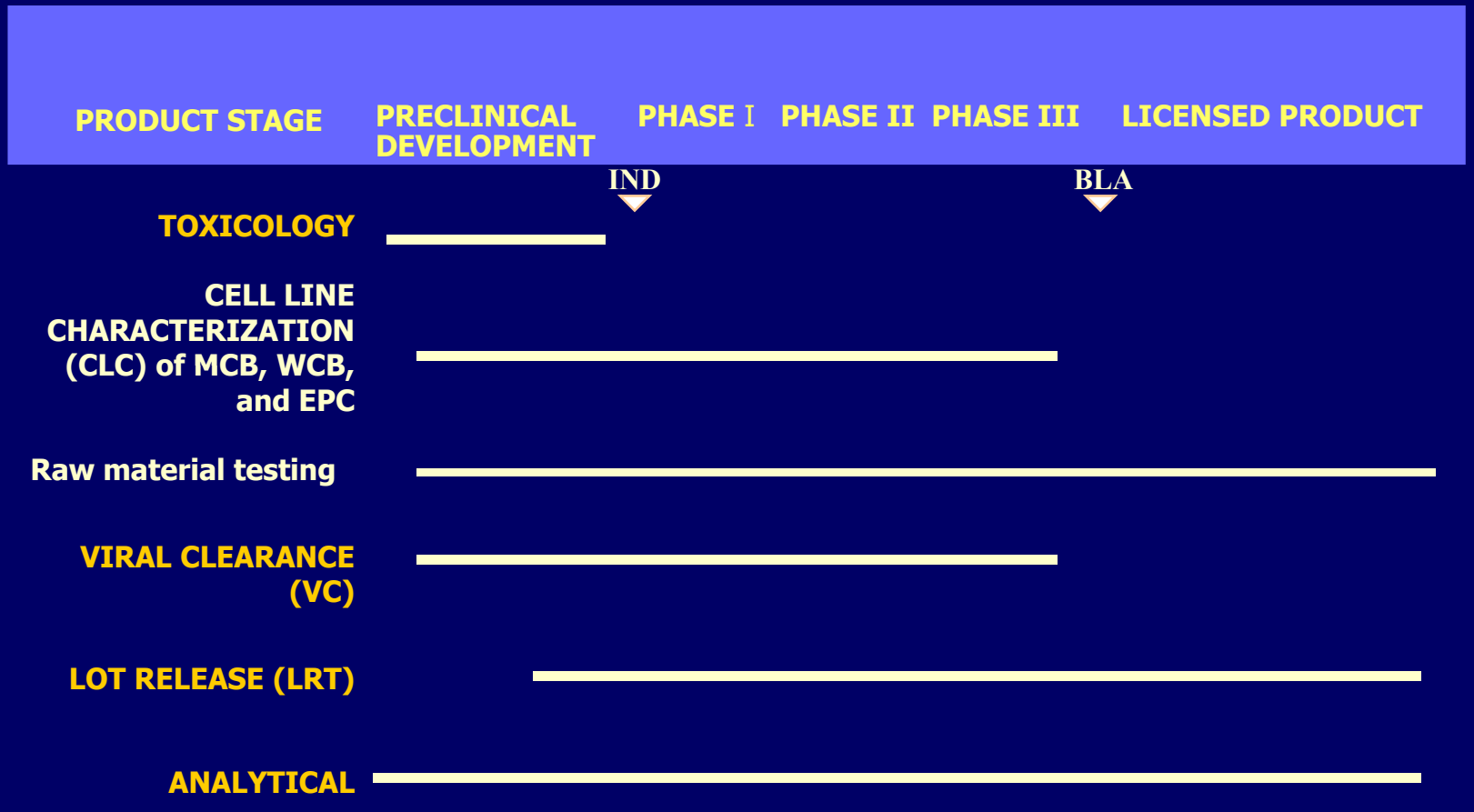
Examples of Adventitious Agent Contamination of Biologics

- SV40 in early poliovirus and adenovirus vaccines
- Avian leukosis virus and Hepatitis B in yellow fever vaccine
- Creutzfeldt-Jakob disease from growth hormone
- Hepatitis B, Hepatitis C, HIV and B19 in blood products
- MMV (Mouse parvovirus) in CHO cells

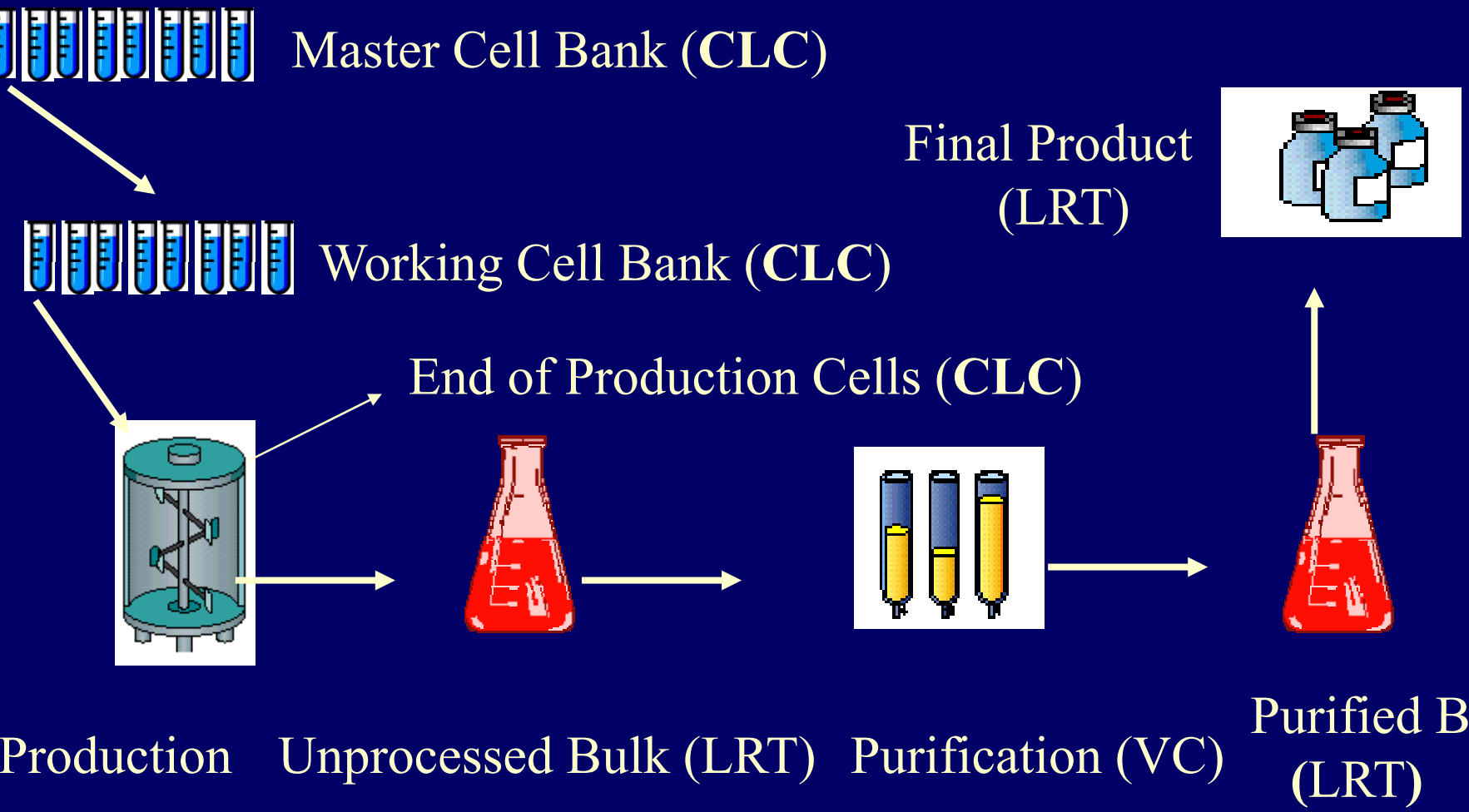
Key Guidance Documents

- *Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993)*
- *ICH Q5D. Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products (1998)*
- *Points to Consider in the Manufacturing and Testing of Monoclonal Antibody Products for Human Use (1997)*
- *ICH Q5A. Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (1998)*
- *Guidance for Human Somatic Cell Therapy & Gene Therapy (1998)*
- *European Pharmacopoeia 5.0, section 5.2.3 – Cell substrates for the production of vaccines for human use (2005)*
- *Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines fo the Prevention and Treatment of Infectious Diseases (FDA, CBER, Draf Guidance, September, 2006)*

Biosafety Testing Across Biologics Spectrum



Biologics Production: Flow and Testing Points



CLC = cell line characterization ; LRT = lot release testing ; VC = viral clearance studies

Key Questions to Determine Appropriate Testing for Cell and Virus Banks

- Is it a FDA or a global submission?
- What is the species of the cells for banking or the cells used for virus production?
- Was serum or trypsin used during the history of the cell line?
- Was cell line exposed to cells or ingredients from other species (e.g., mouse feeder cells and human stem cells)?
- Are the cells grown in medium containing antibiotics or ingredients (e.g., methotrexate) possibly inhibitory or toxic to adventitious agents or to cells in the test system?
- Is the sample matrix osmotically and pH compatible with the test system?
- What is the source/history of the cell line and virus stock?

General Categories of Characterization Testing for Cell Banks and EPC and Virus Banks

- ***Purity*** - Microbial Contaminants
  - Bacteria, Fungi
  - Mycoplasma (Spiroplasma for insect cells and Baculovirus)
  - Viruses : Adventitious, Retroviruses, Endogenous
- ***Identity***
  - Species for cells
- ***Genetic Stability (for transfected cell substrates)***

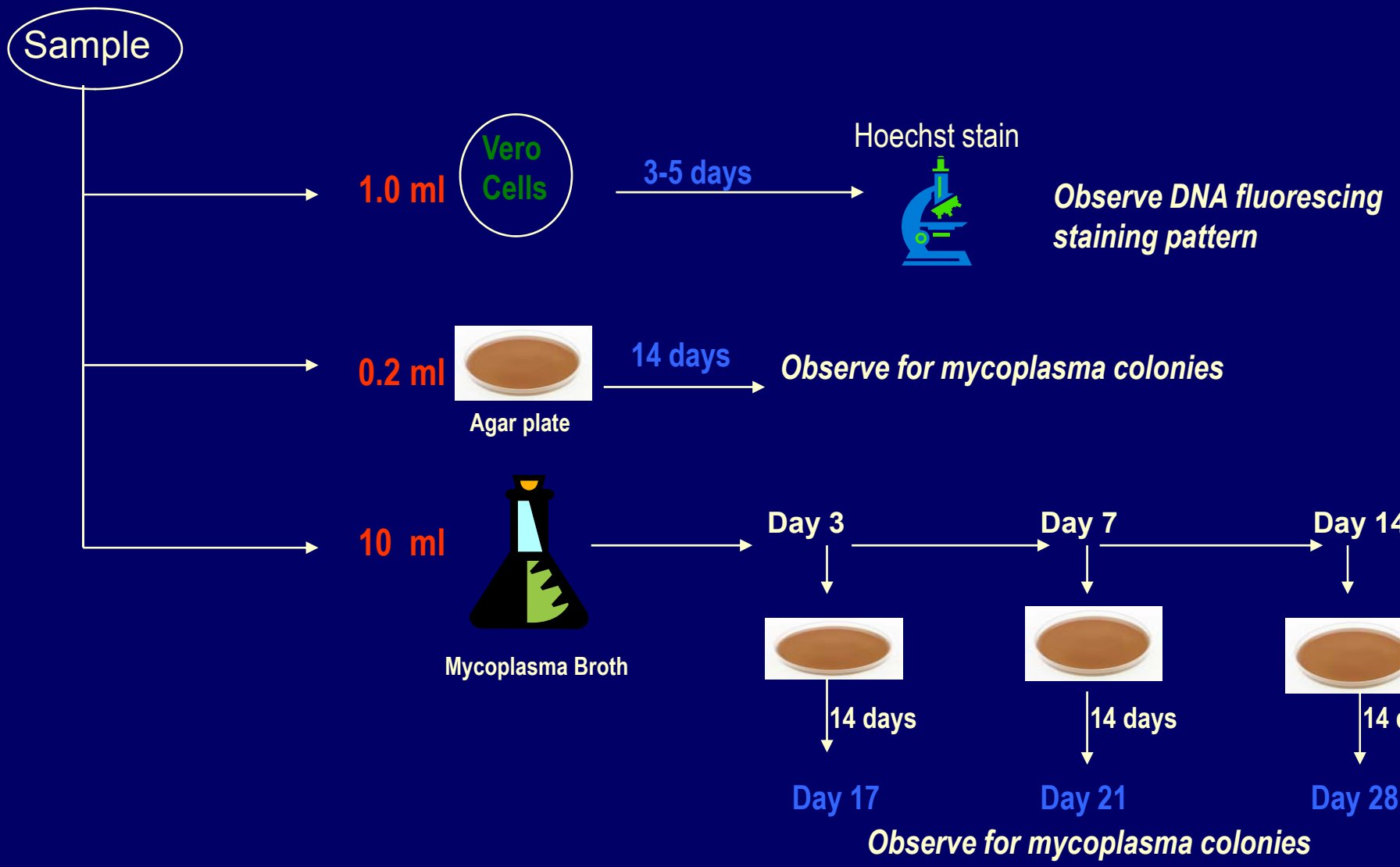
General Testing Categories Performed on MCB, WCB, EPC, and Virus banks

Tests	**MCB/MVB**	**WCB/WVB**	**EPC (CAL)**
Sterility	+	+	+
Mycoplasma	+	+	+
Virus: *-Adventitious* *- Retrovirus* *- Specific (species/virus)*	 + + +	 –/+ – –	 + + –/+
Species identification) *- for cells*	+ (cells)	+ (cells)	+

Characterization Testing of Cell and Virus Bank and EPC: Sterility and Mycoplasma Testing

- **Sterility testing for bacteria and fungi**
  - Follow ICH recommendations – testing performed on contents of individual containers (1% of total bank but not less than two containers or vials).
  - Bacteriostasis and Fungistasis (B&F) assay recommended prior to performing the Sterility assay to assess sample matrix for inhibition.
- **Mycoplasma testing - PTC (28 day assay) or EP (28 day assay)**
  - Mycoplasma agar plate and semi-solid broth bottle procedures for agar cultivable mycoplasma and the VERO cell culture/H stain procedure fo non-agar cultivable mycoplasma
  - Qualification (Mycoplasmastasis) – Stasis assay is not referenced in any FDA regulation or guidance document, while the EP requires it. It i good science to perform a stasis assay to assess the sample matrix fo any inhibitory effect, which could prevent the detection of an adventitious mycoplasma.

28-Day Mycoplasma Culture Method



Characterization Testing of Cell and Virus Banks and EPC: Virus Assays

- ***In vitro* adventitious virus screening assay**
- ***In vivo* adventitious virus screening assay**
- ***In vivo* species-specific assays**
- ***In vitro* virus-specific assays**

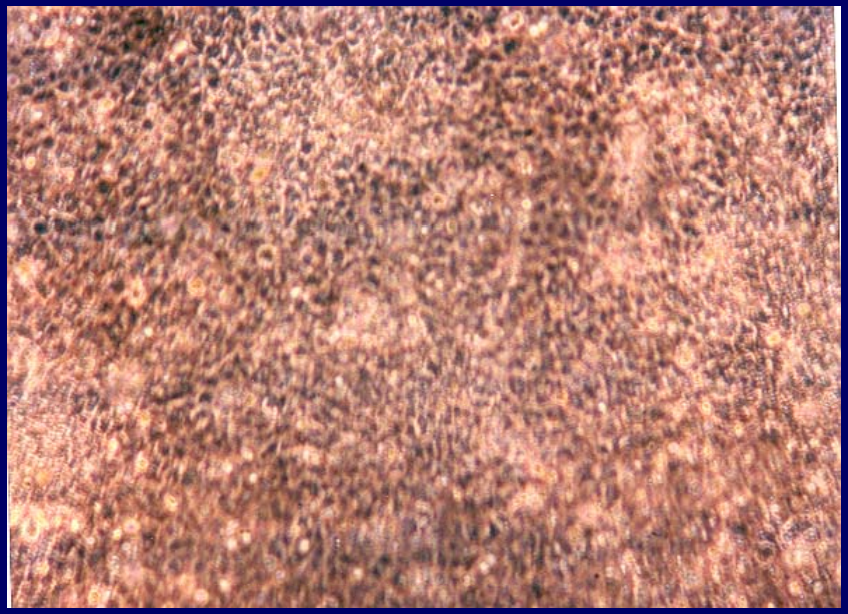
Assay for Adventitious Virus Testing - 14-Day and 28-Day Virus Screens

28-Day Adventitious Virus assay
14-Day Adventitious Virus assay

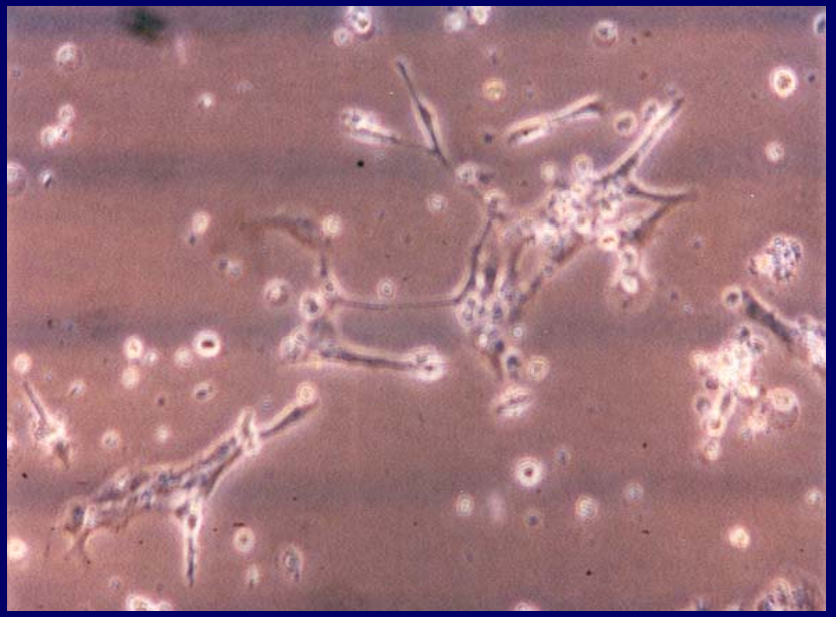
Observations		Observations
Day 0	**Day 14**	**Day 28**
Inoculate 0.5 ml/well	**HA/HAD**	**HA/HAD**
MRC-5, Vero, CHO-K1	**Blind Passage**	**End of study**

(Positive control viruses: MRC-5 = measles; Vero = PI-3; CHO-K1 = SV-5)

Morphological Changes (CPE) in 324K Cells Infected with REO Viru



Control 324K cells
Day 20



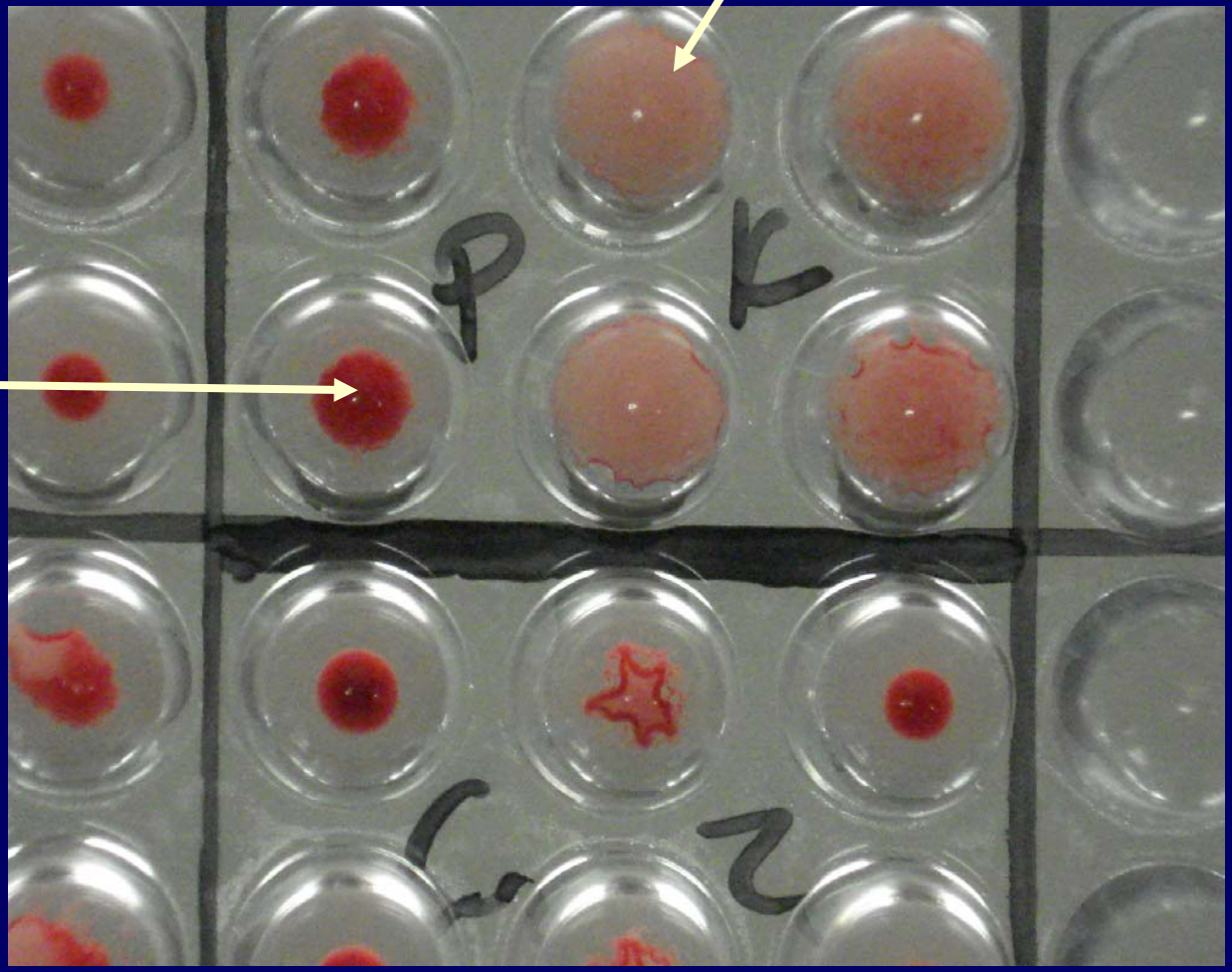
REO-infected 324K cells
Day 20

Hemadsorption (HAD) – Vero cells and Rhesus RBCs

Hemagglutination (HA)

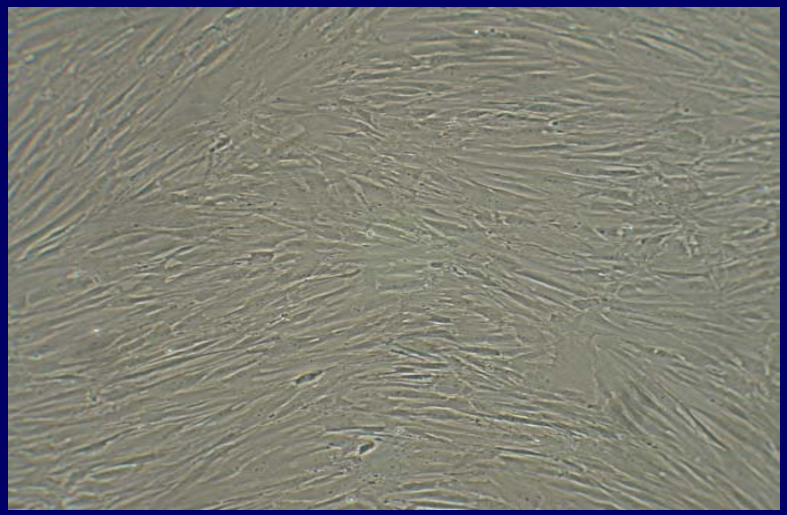
Positive HA result

Negative HA result

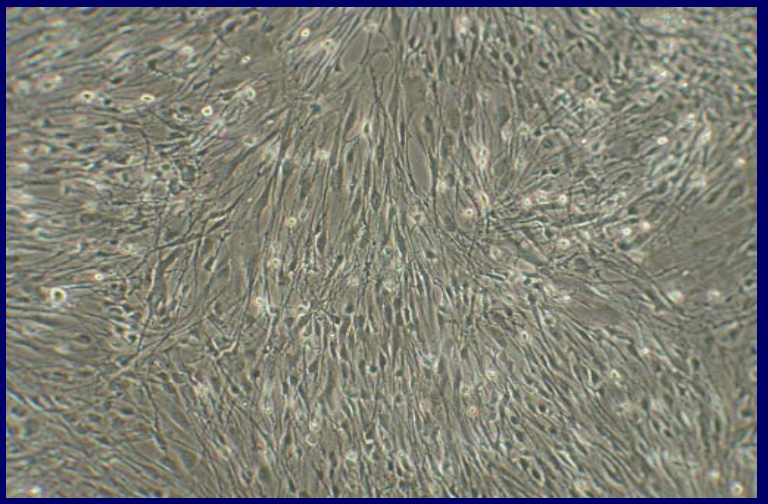


Sample cytotoxicity effect – non-viral CPE

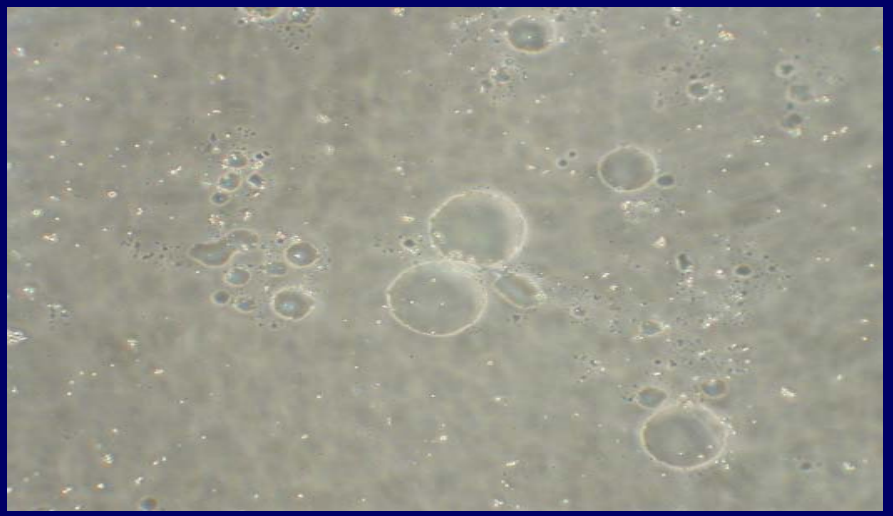
Control MRC-5 Cells



Sample Treated MRC-5 Cells



Viewing plane above cell monolayer



Ascitic fluid sample contains lipids.

Detection of Adventitious Viruses in Biologicals Using Screening Assays - A Very Rare Event

Biological Type	Viruses Detected
-Cell Lines (MCB, WCB, EPC)	**None**
-Gene Therapy Vectors adenovirus	**Replication-competent**
-Monoclonal Antibodies	**None**
-Recombinant Proteins	
➢ **Non-CHO cell process**	**None**
➢ **CHO cell process**	**MMV, REO, Cache Valley Virus**
-Vaccines	**None**

Susceptibility of CHO Cells to Viruses

1) CHO cells have a limited susceptibility to viruses.
2) Not susceptible to the following virus groups: Adenovirus, Coronavirus, Picornavirus (Coxsackie, Rhinovirus), Herpes (HSV 1&2, CMV, VZV), Orthomyxo (Influenza A&B), Togavirus (BVDV)
3) CHO cells are not susceptible to Retroviruses (no infectious retrovirus isolated from CHO cells – to date)
4) CHO cells are susceptible to Reovirus, (1,2,3), Paramyxo (Parainfluenza 1,2,3 and SV-5), Bunya (Cache Valley), Parvo (MMV)

Characterization Testing of Cell and Virus Banks and EPC: *In Vivo* Adventitious and Species-Specific Assays

- *In vivo* adventitious virus assay – General screen using suckling and adult mice and embryonated eggs to reveal viruses that cannot grow in cell cultures- additional species may be used depending on nature and source of cell line (Guinea pigs frequently used for FDA submissions); Endpoint = morbidity and mortality
- Species-specific assays (In vivo tests) – MAP (mouse antibody production) and HAP (hamster antibody production) assays; both MAP and HAP done on hamster MCBs (i.e., CHO) and MAP done on mouse MCBs (e.g., NS0 or mouse hybridoma)

Mouse Antibody Production (MAP) Assay

- **Basis**
  - Detection of murine viruses based upon generation of specific antibody (or serum enzyme) in response to virus infection
- **Procedure**
  - Inoculate cell lysate into mice via intracranial, intranasal, intraperitoneal and *per os* route
  - Test serum for elevated lactic dehydrogenase level after 3 days
  - Bleed mice after 28 days and test for virus-specific antibody by ELISA and/or immunofluorescent staining

Characterization Testing of Cell and Virus Banks and EPC: Virus-Specific Assays

Virus-Specific Assays

- PCR and in vitro infectivity assays – MMV (mouse parvovirus) for CHO cell lines
- Bovine polyoma virus (PCR) expected by EP reviewers; Bovine polyoma infects human and simian cell lines; no evidence for infecting CHO
- PCR panel for simian viruses for simian cell lines such as Vero
- PCR panel for human viruses for human cell substrates

Characterization of Cell and Virus Banks: Standard PCR panel for a Human MCB and Virus Banks Produced in Human Cells

Specific Human Viruses

- HIV 1&2
- HTLV 1&2
- CMV
- EBV
- HAV, HBV, HCV
- HHV-6,7,8
- B19
- SV-40
- Others

Characterization Testing of Cell and Virus Banks: Animal Sourced Materials – Testing Considerations

- Cells exposed to serum or additives derived from animal sources (at any time in its history) should be certified to be free from adventitious agents (i.e., 9CFR bovine virus testing) and BSE (may require re-establishment of cell bank if appropriate serum source documentation is absent).
- If porcine trypsin used in harvesting cells, cells should be tested for adventitious viruses including porcine parvovirus (9 CFR porcine virus testing).
- 9CFR virus screening of FBS and trypsin is part of a good cGMP production program. 9CFR bovine virus test includes PI3 and IBR viruses for EP.
- There is an increasing expectation to account for anti -BVDV antibodies in serum.

In Vitro Assay for Bovine Viruses – 9CFR testing

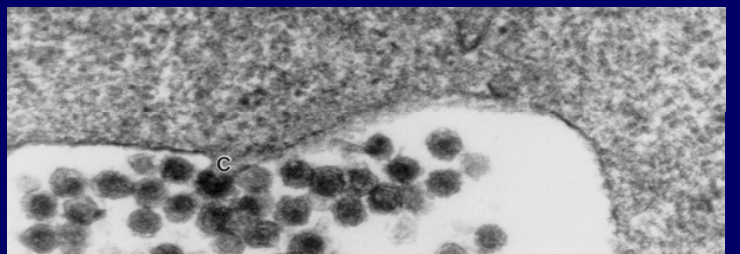
- **Basis**
  - Detection of wide variety of bovine viruses based upon development of cytopathology, hemadsorption of red blood cells, and specific immunofluorescent staining
- **Procedure**
  - Inoculate serum or clarified cell lysate into bovine turbinate (BT) and VERO cells
  - Monitor microscopically for 21 days, subculturing twice
  - Test for hemadsorption
  - Stain fixed cells with anti-bovine virus fluorescein-labeled antibodies

Characterization Testing of Cell Banks and EPC : Retrovirus (RV) - Adventitious and Endogenous

- Mouse and hamster cell lines produce endogenous retrovirus-like particles (Type A and Type C). Mouse cells are inherently capable of producing infectious mouse RV. Positive testing results may be obtained with mouse cell lines (e.g., NS0)
- CHO cell lines express defective RV particles. To date, it has not been shown that hamster cell lines can express infectious RV.
- Cocultivation assays (assess tropism for human cells); Extended S^+L^- focus (mink lung or feline cell) assays; and Extended XC plaque assays detect infectious RV based on amplification in culture followed by appropriate endpoint assays.
- RV infectivity tests not required for murine hybridoma cell lines for FDA submissions.
- Endogenous RVs in porcine tissues (i.e., PERVs) – Concern of regulators for xenotransplantation or devices utilizing porcine cells/tissues.

Characterization Testing of Cell and Virus Banks and EPC : Retrovirus (RV)–Adventitious and Endogenous - continued

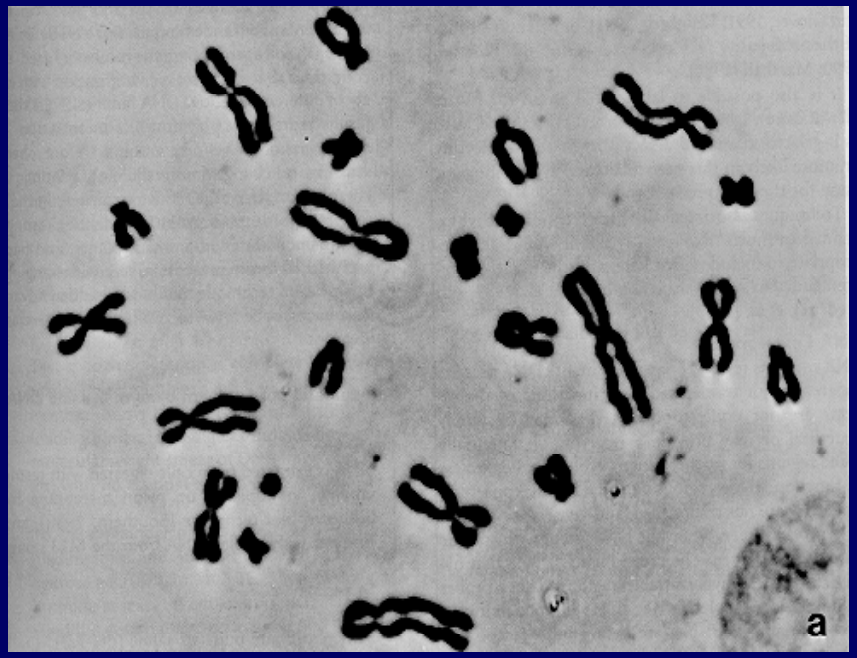
- Reverse transcriptase (RT) assay for detecting RT activity associated with RVs. PERT testing is done on human and simian MCBs and EPCs and not appropriate for rodent MCBs or EPCs. PERT testing is required by the FDA for viral vaccine manufacturers. Samples for RT testing need to be prepared carefully to prevent DNA polymerase contamination, which can result in a false positive.
- For MCB and EPC, Transmission Electron Microscopy (TEM) analysis is performed for detecting the presence of virus-like particles or other microbial agents; to characterize any retrovirus-like particles present; to enumerate the number of particles associated with the cells; and to provide an ultrastructural evaluation of cellular morphology.



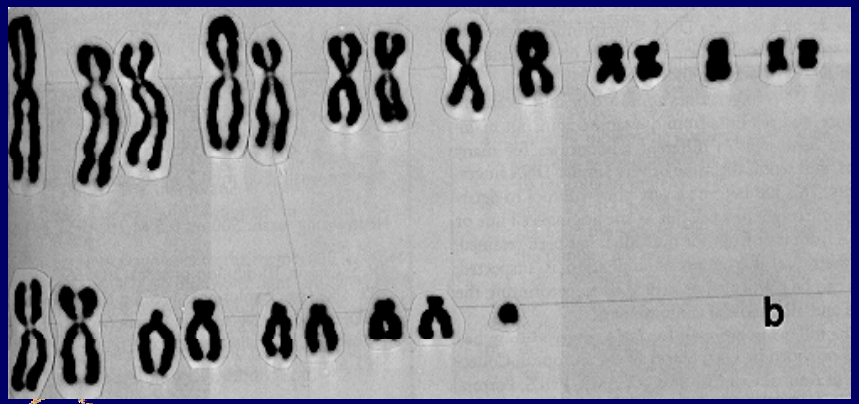
Characterization Testing of Cell Banks and EPC: Species Identification

- Either phenotypic (e.g., isoenzyme analysis) or genotypic (e.g., DNA fingerprinting) methods may be used for most FDA submissions. The EP requires both fingerprinting and another identity test (e.g., isoenzyme).
- Karyology is recommended for new cell lines and for diploid cell lines (not necessary for well characterized cell lines such as MRC-5, WI-38 and FRhl-2 cell lines).
- Perform on MCB, WCB and EPC.

Karyological Analysis

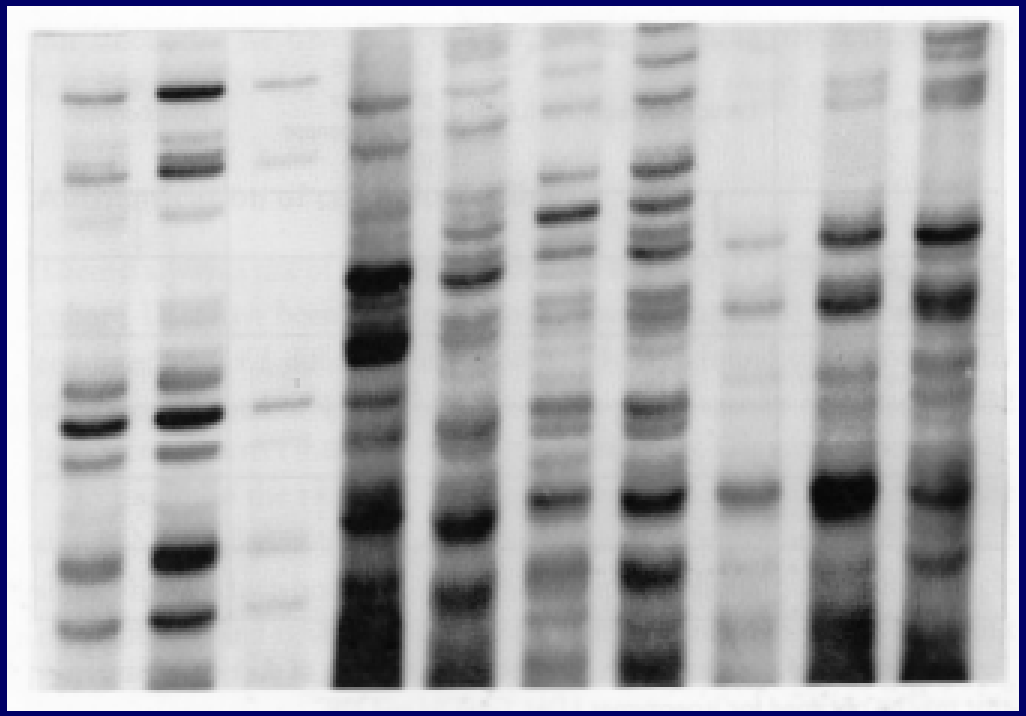


Metaphase spread



Karyotype

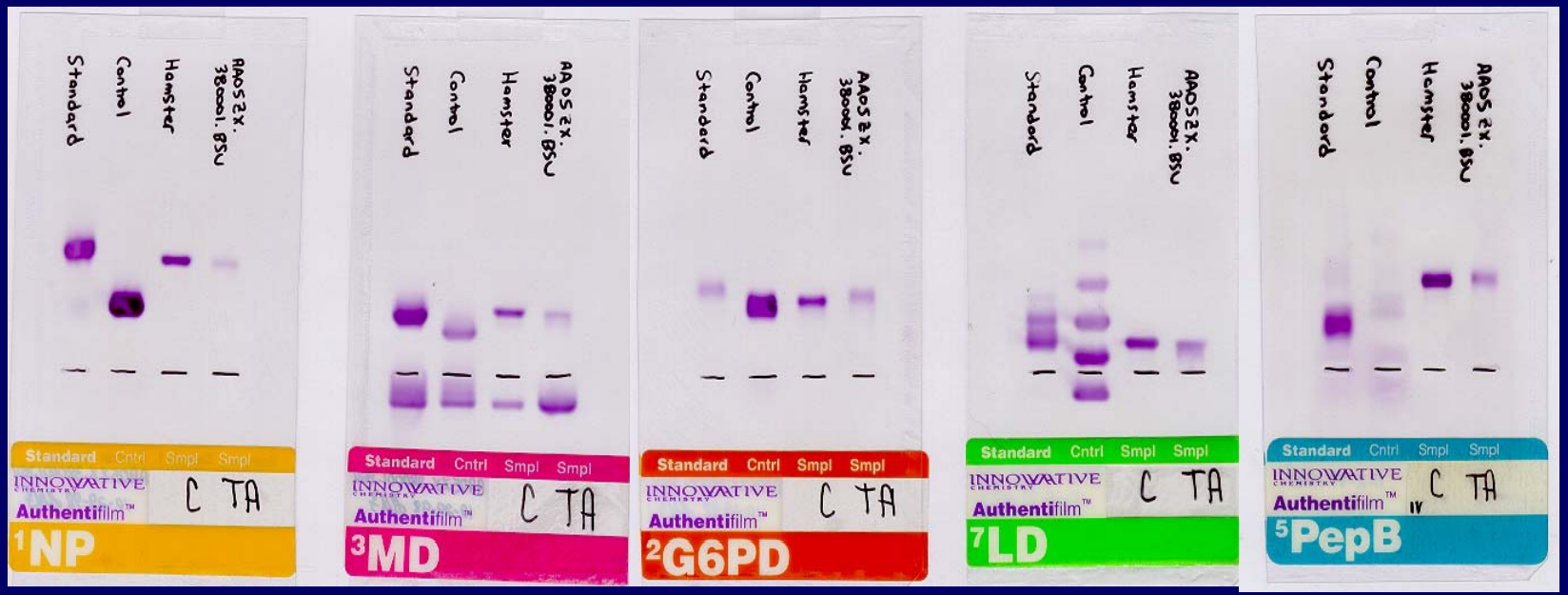
DNA fingerprinting analysis



A A A B C D D E E E

Individual

Isoenzyme analysis



Characterization Testing of Cell Banks and EPC (CAL) : Genetic Stability

- Genetic stability (i.e., determine gene copy #; sequencing of the insert of the MCB and EPC) typically performed during Phase 3 once the process is defined.
- Cells used to synthesize products must be genetically stable enough to produce the expected protein throughout the fermentation period.

Characterization Testing of Cell Banks and EPC (CAL): Genetic Stability

Molecular studies required to show that:

- Correct sequence made and incorporated into host cell
- Structure and copy # maintained to end of production
- Stability of the expression system – in the EPC and at least once in MCB
  - Gene copy # (QPCR)
  - Deletions/Insertions (Southern blotting)
  - Protein produced – can be analyzed at the mRNA level (mRNA sequencing)
  - Number of integration sites (FISH/Southern)
  - mRNA transcript size distribution (Northern blotting)

Lot Release Testing: Unprocessed Bu

TESTING	ASSAY
Microbial	**- B&F and Sterility** **- Mycoplasma**
Adventitious Viruses	**- In Vitro Virus Assay** **- MMV assay (infectivity or PCR) for CHO cells**
Viral Quantitation	**TEM particle count (required for determining average retroviral load; links with VC studies) – three lots tested**

Lot Release Testing Purified Bulk

TESTING	ASSAY
Microbial	**- B&F and Sterility**
Other Contaminants	**Host Cell DNA, Host Cell Protein (Residual testing)**
Analytical Characterization	**- Purity** **- Potency** **- Stability**

Final Filled Product Testing

TESTING	ASSAY
Microbial	**- B&F and Sterility**
Safety	**- Endotoxin: Rabbit Pyrogen or LAL** **- General Safety**
Analytical Characterization	**- Purity** **- Potency** **- Stability**

Conclusion

- *Sponsors need to review carefully their testing plan to assure it meets regulatory expectations..*
- *PreIND meeting is a good time for that discussion.*