Biosafety Testing of Vaccines for the Global Market

Dr Martin Wisher
Senior Director, Global Head of Regulatory Affairs
BioReliance UK

DCVMN, New Delhi *November 2013*

BioReliance® by SAFC

Contents

- Introduction to BioReliance
- Human vaccine intermediates
- Regulatory guidelines
- Identity tests
- Cell bank manufacture
- WHO Guidance for cell substrates
- Characterization of cell banks
  - Detection of bacteria, fungi, mycoplasma and viruses
- MVSS and Clinical Lot Testing
- New methods for detecting contaminants
- Emergency response plans

BioReliance® by SAFC

You can rely on us

The world's most trusted provider of biomanufacturing testing services.

Offering GLP and GMP compliant testing services to…

- Pharmaceutical
- Biopharmaceutical
- Vaccine
- Medical Device
- Chemical
- Consumer Health Care Companies
- Animal Health
- Government
- Academia

BioReliance® by SAFC

Sigma-Aldrich At-A-Glance

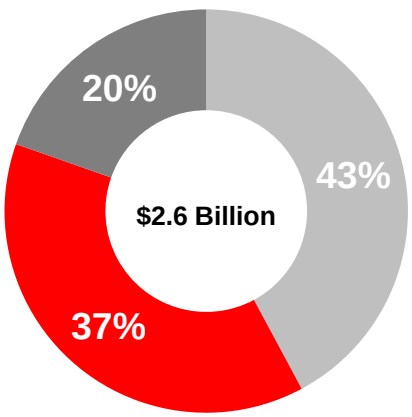
MISSION

Enabling Science to Improve the Quality of Life

VISION

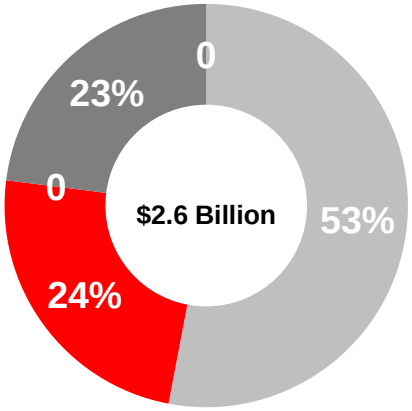
To be the trusted and preeminent global provider to the research laboratory and targeted applied commercial markets

FACTS AND FIGURES



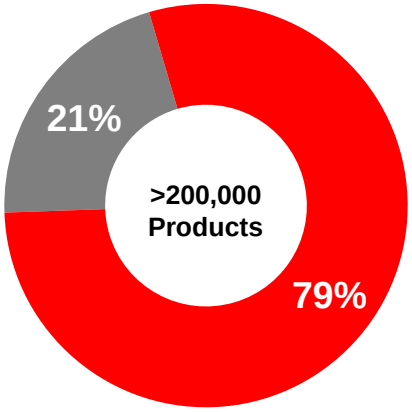
GLOBALLY BALANCED

- United States and Canada
- Europe, Middle East, Africa
- Asia Pacific and Latin America



DIVERSE END-MARKETS

- 53% Research
- 23% Applied
- 24% SAFC Commercial



QUALITY PRODUCTS

- >170,000 Reagents and Chemicals
- 45,000 Laboratory Equipment Items

OUR PEOPLE

~9,000

Employees Worldwide

OUR PLACES

50+ Sales Offices

30+ Distribution Centers

40+ Production/Lab Facilities

BioReliance® by SAFC

A Partner From Research to Commercialization

Critical Raw Materials for Biomanufacturing

Customized Raw Materials

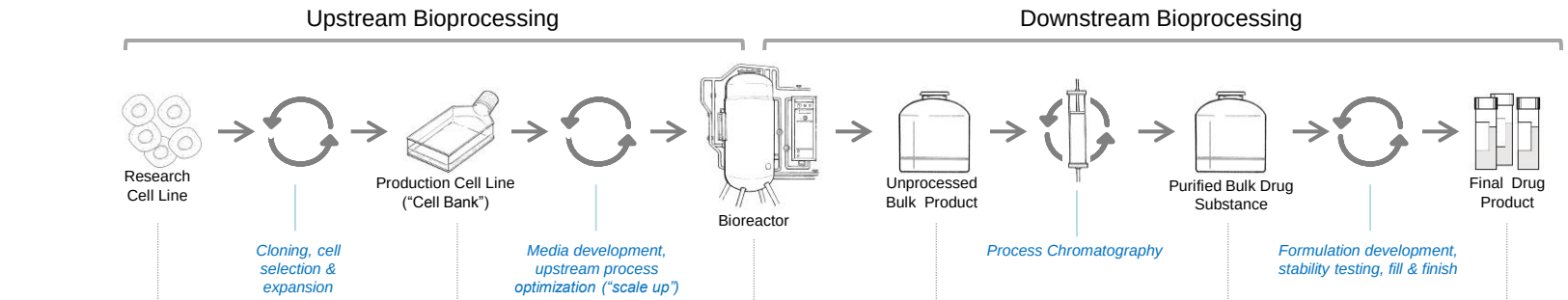
Analytical and Regulatory Services

Contract Manufacturing of APIs and Biologics

BioReliance® by SAFC

SAFC & BioReliance

Complementary Offering for BioPharmaceutical Development & Manufacturing



Raw Material Testing

Lot Release & Biologic Manufacturing Support Testing

Raw Material Manufacturing & Supply

Expression System and Cell Line Development

Contract Biologic Manufacturing

Upstream Process Development

BioReliance Biologics Capabilities

SAFC/BioReliance Capabilities

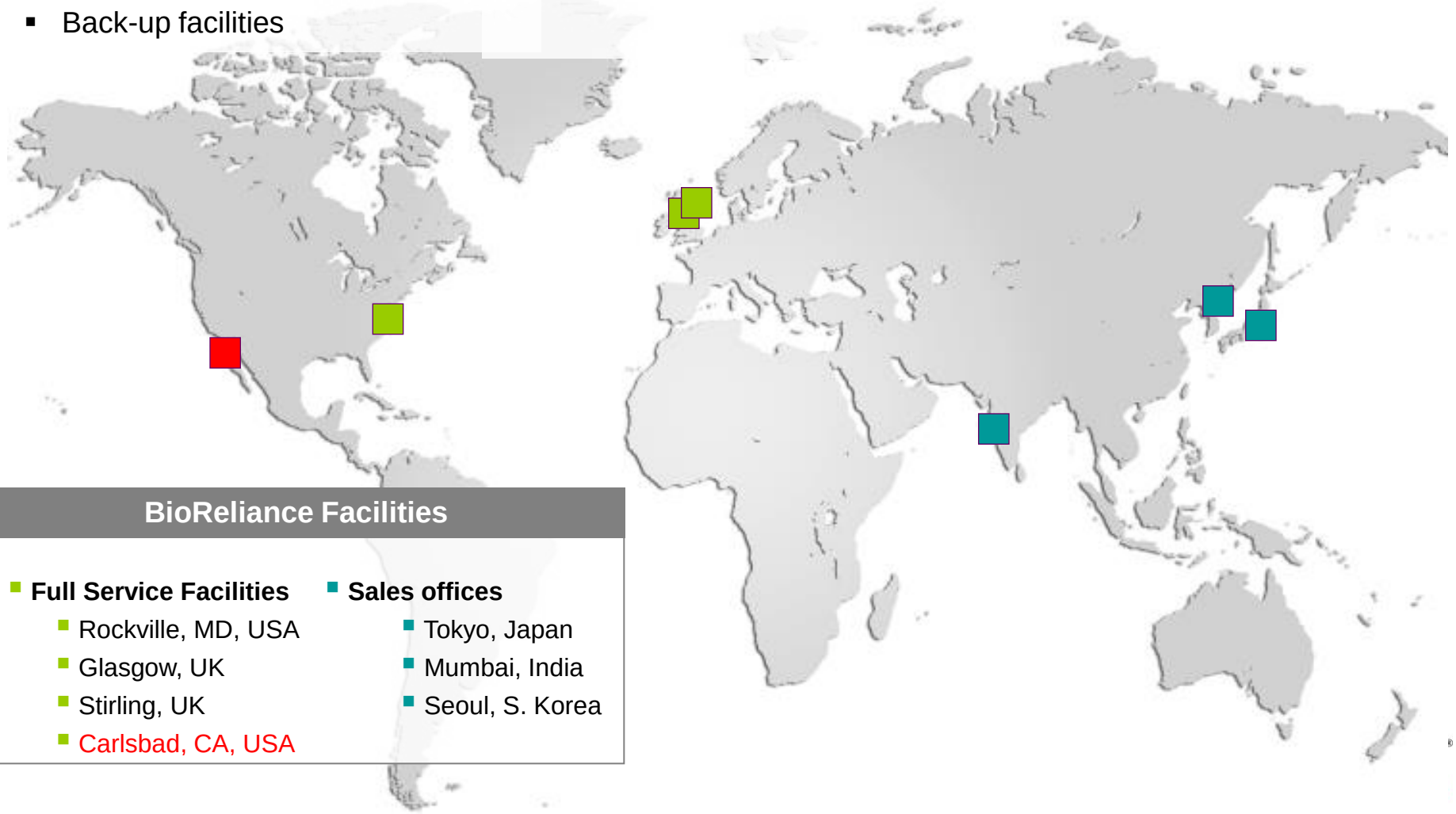
Sigma-Aldrich/SAFC Capabilities

BioReliance® by SAFC

Global presence

Global Services and Support

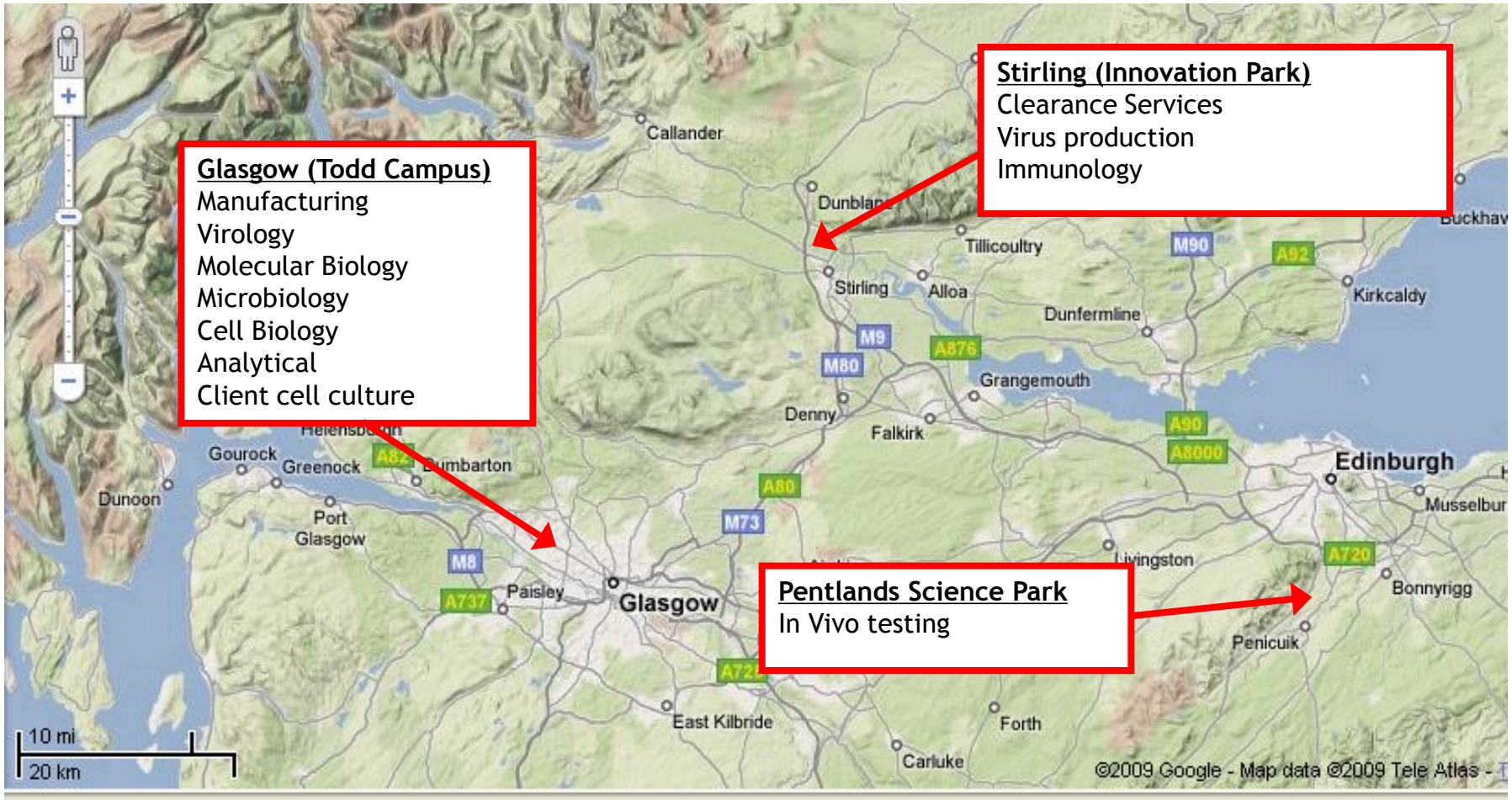
- Global capacity and flexibility
- Back-up facilities
- Centers of excellence



BioReliance Facilities

- **Full Service Facilities**
  - Rockville, MD, USA
  - Glasgow, UK
  - Stirling, UK
  - Carlsbad, CA, USA
- **Sales offices**
  - Tokyo, Japan
  - Mumbai, India
  - Seoul, S. Korea

BioReliance in Scotland



BioReliance® by SAFC

You Can Rely On Us

Proven Track Record

- Serve all of the top 20 Pharma and top 10 Biotech companies. Serve top Vaccine companies
- Successfully supported testing for over 100 licensed products worldwide
- Trusted and collaborative relationships with regulatory agencies around the world
  - FDA, EMEA, MHLW
- Inspected regularly for GMP compliance by US FDA and UK MHRA

BioReliance® by SAFC

BioReliance Client Base

Pharma top 20 companies

Biotech top 10 companies

BioReliance currently performs testing for ~75% of the top Pharma companies and ~90% of the top Biotech companies in the world.

BioReliance® by SAFC

Services for Every Stage

Cell Banking
- Cell & Viral Bank Storage
- Cell & Viral Bank Manufacturing

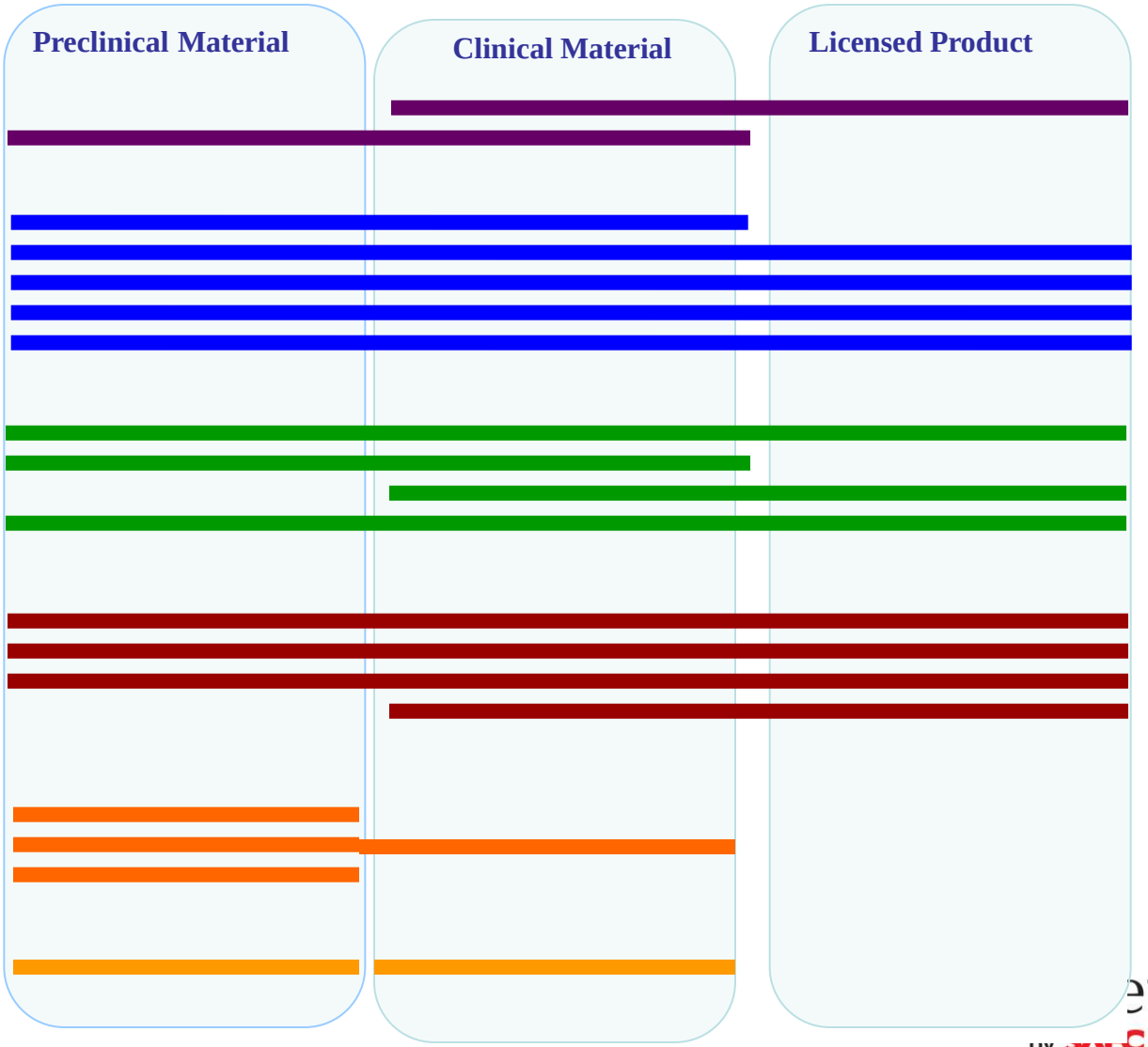
Biosafety Testing
- Cell Line Characterization
- Unprocessed Bulk
- Purified Bulk
- Final Product
- Clinical Trial Support

Analytics
- Stability Testing & Storage
- Method Development & Transfer
- Potency Assays
- Lot Release Testing

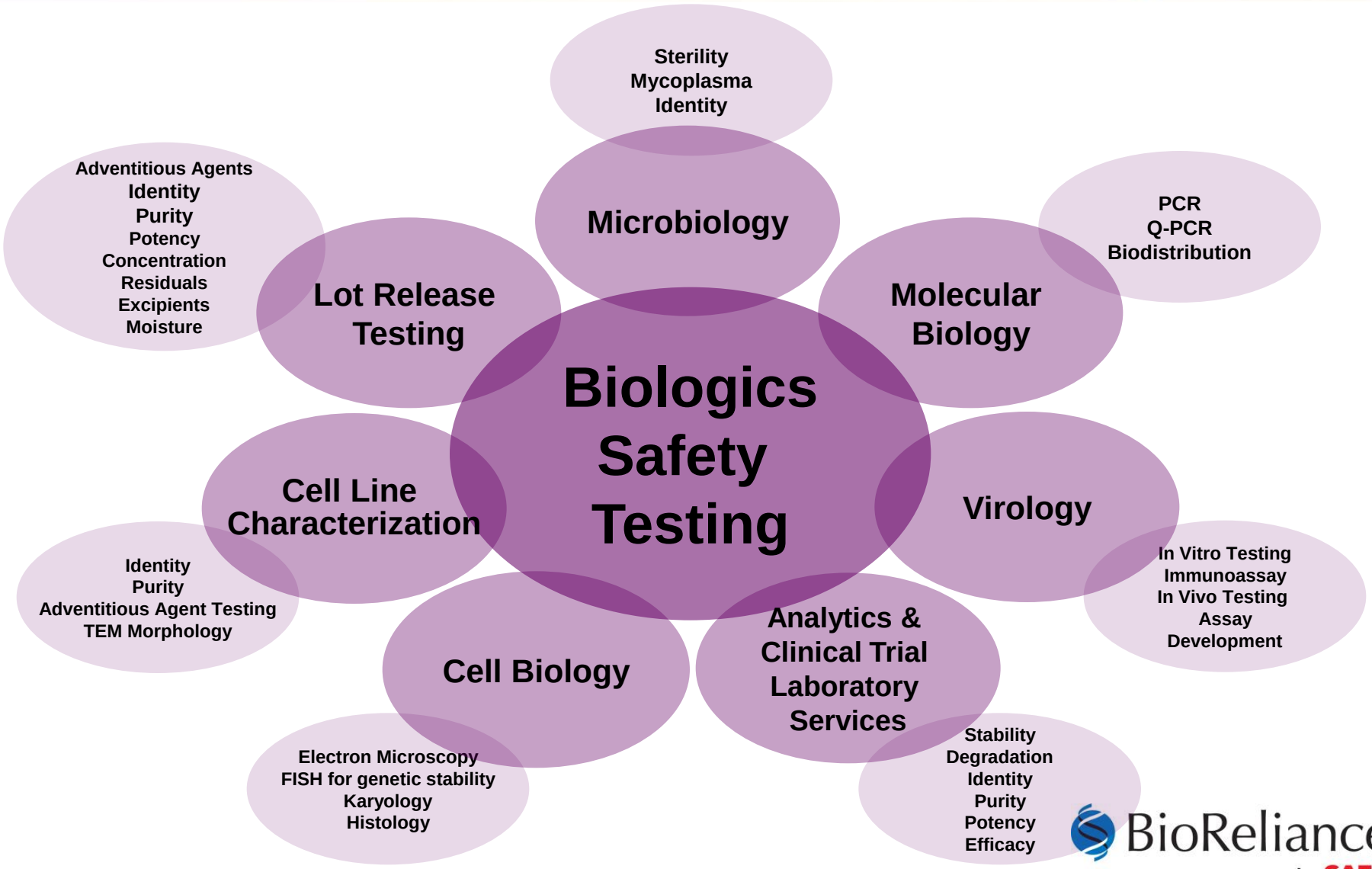
Clearance Studies
- Viral Clearance
- TSE Clearance
- Cleaning Studies
- Chromatography Column Re-use

Toxicology
- Genetic Toxicology
- Mammalian Toxicology
- Molecular and Transgenic Studies

Genomic Services



Biosafety Testing
Laboratory Capabilities



BioReliance® by SAFC

Human Vaccine Intermediates

- Production cell substrate testing
  - MCB, WCB, Maximum use cells
- Master Virus Seed Stock (MVSS)
  - Produced in eggs
  - Produced using reverse genetics
  - Produced using cell culture
- Bulk Virus Harvest
- Control testing
  - Control egg testing
  - Production control cell testing
- Purified bulk product testing
- Virus inactivation and clearance studies

BioReliance® by SAFC

Quality of Medicinal Products

- Quality requirements given in guidance documents and regulations
  - GMP
  - Points to Consider, Notes for Guidance, Guidance for Industry
  - Code of Federal Regulations, European Directives, USP, EP and JP monographs and general chapters
- Guidance generally on requirements for licensed products
  - May not be appropriate for early phases of clinical development
  - Generally lagging behind innovations in product, processes and scientific discoveries
  - Regulations difficult to update
  - Harmonisation

Regulatory Guidelines - Vaccines

- WHO Technical Report Series 878: Requirements for the Use of Animal Cells as *In Vitro* Substrates for the Production of Biologicals, 2010
- US FDA Guidance for Industry: Characterisation and Qualification of Cell Substrates and Other Biological Starting Materials used in Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases, *2010*
- EP 5.2.3 Cell Substrates for the Production of Vaccines for Human Use
- WHO Technical Report Series 927: Recommendations for the production and control of influenza vaccines (inactivated)
- EP 2.6.16 Extraneous agents in vaccines for human use

ICH Documents

- ICH Q5A: Quality of biotechnological products: Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin *(principles apply)*
- ICH Q5B: Quality of biotechnological products: Analysis of the expression construct in cells used for production of rDNA derived protein products *(if vaccine is genetically engineered)*
- ICH Q5C: Quality of biotechnological products: Stability testing of biotechnological / biological products *(principles apply)*
- ICH Q5D: Derivation and characterisation of cell substrates used for production of biotechnological / biological products
- ICH Q2 (R1) Validation of analytical procedures
- *www.ich.org*

Controlling Potential Contaminants

- Selecting and testing raw materials for the absence of contaminants. Production in a controlled environment (GMP)
  - MCB, WCB
  - MVSS, WVSS
  - Media components: bovine serum, porcine trypsin
- Testing the product at appropriate steps of production for absence of contaminants
  - EPC, Production Control Cells
  - Bulk viral harvests
- Assessing the capacity of the production processes to clear viruses
  - Whole virus vaccines difficult to remove contaminating viruses from suspension of viruses
  - More emphasise placed on testing

Assuring Quality of Cell Banks

- Cross contamination with other cell lines is minimised by campaign cell banking under GMP conditions
- Identity testing (isoenzymes, DNA fingerprinting) on MCB, WCB and EOPC (or cells at limit)
- Genetic identity and stability of recombinant cells
  - MCB and Cells at Limit of *In Vitro* Cell Age Used for Production
  - Restriction enzyme analysis
  - Gene copy number
  - Gene sequencing (mRNA and cDNA)
  - FISH analysis

HeLa Cell Contamination

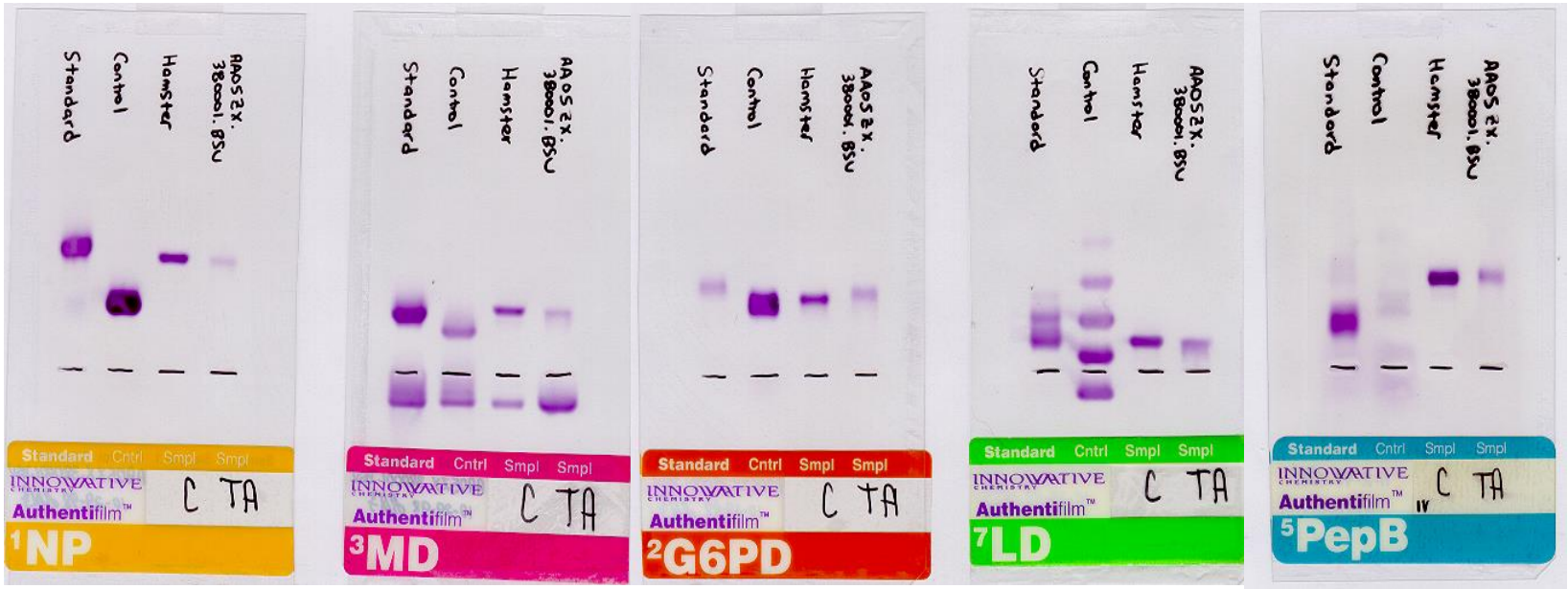
- Nelson- Rees, W A *et al* (1974, 1976) 100 examples of cross contamination of cells
- Gartler, S (1962) 17 out of 18 unique cell lines from ATCC were HeLa
- Markovic, C & Markovic, N (1998), Macleod, R A F (1999) 20% of cell lines are falsely labelled

Cells shown to be HeLa	
'Original' cell name	*'Original tissue designation'*
KB	Oral cancer
Hep-2	Larynx
L132	Embryonic lung
Intestine 407	Intestinal epithelium
Chang liver	Liver

Identity testing of cells

- Isoenzyme analysis: only determines species of cell.
- DNA Fingerprinting (Short Tandem Repeats): can identify cells of the same species
- Karyology: chromosome number and marker chromosomes identify cells of the same or related species
- Cell identity markers indicative of cell type, pluripotency, lineage commitment or terminal differentiation

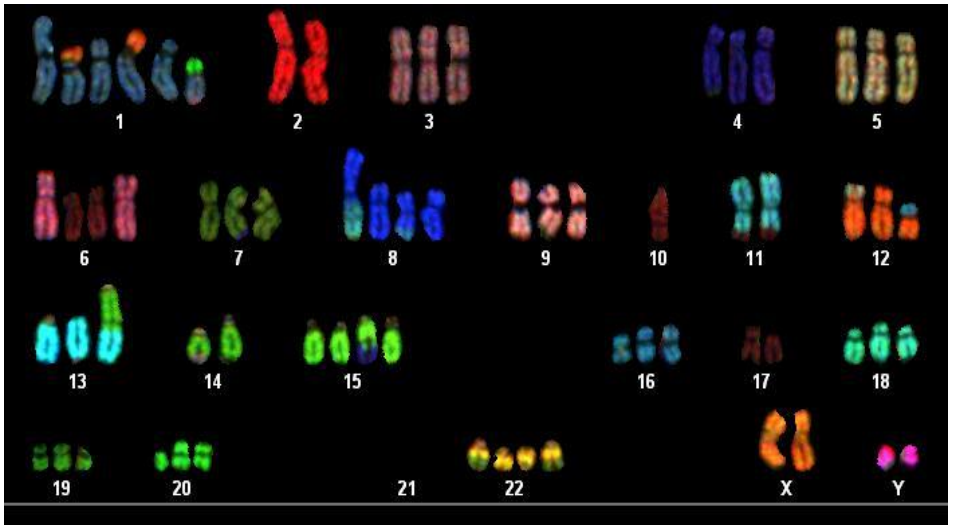
Isoenzyme Analysis

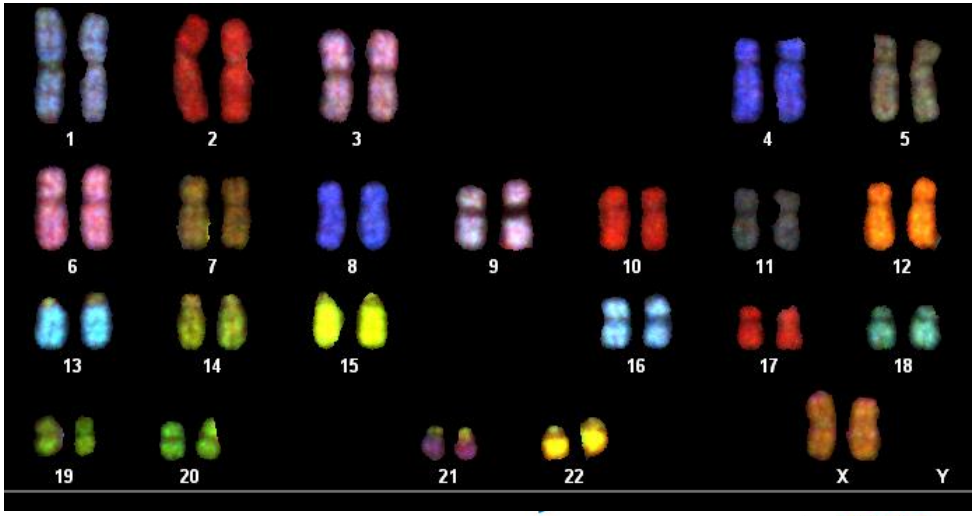


BioReliance® by SAFC

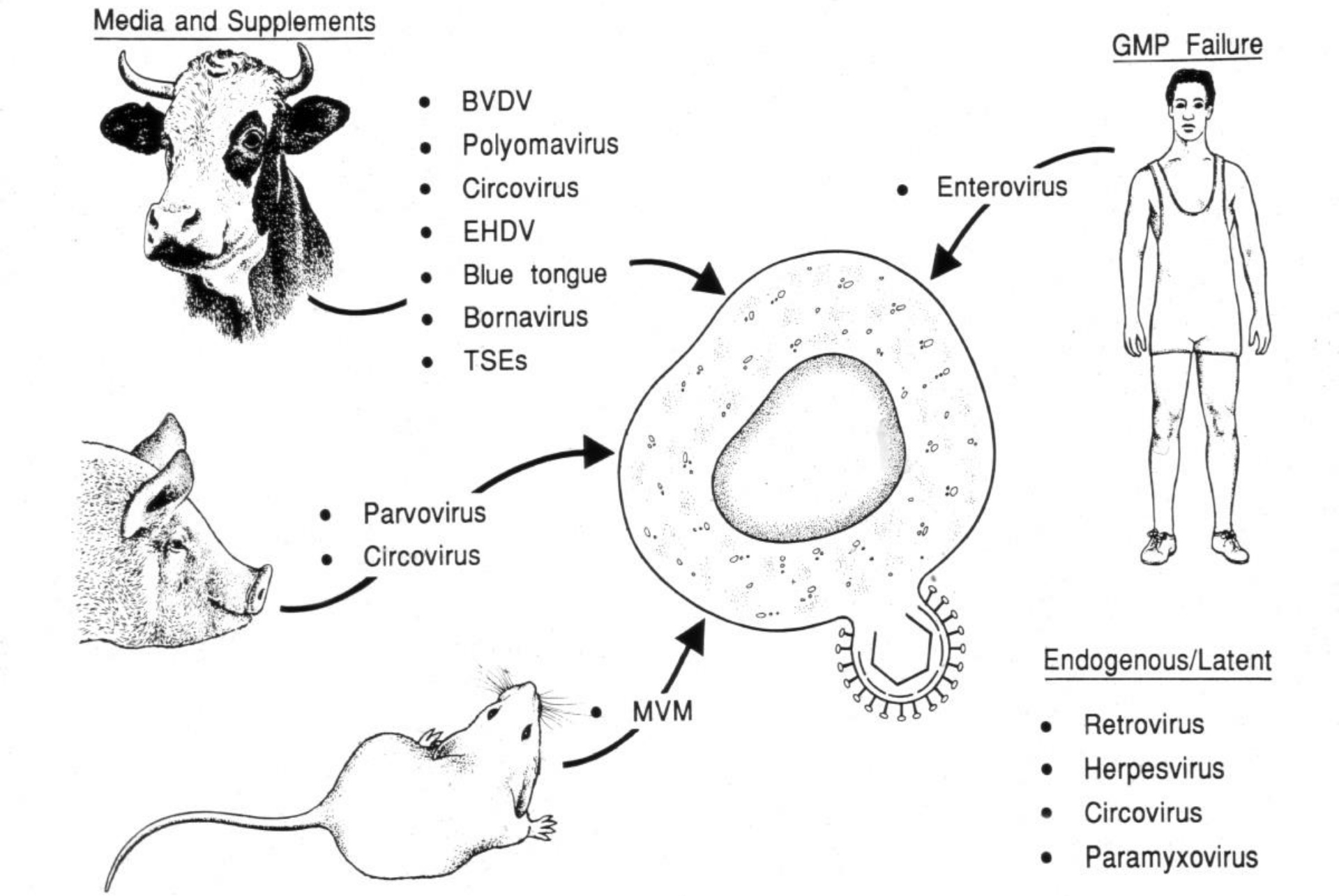
Spectral Karyology (SKY™)

- SKY uses fluorochrome paints across all chromosomes, measured in a single exposure
  - Human, mouse and rat available, BioReliance developing CHO
- High resolution 1 to 2Mbp
- Automated recognition and assembly of karyotypes.
  - Software can “straighten” chromosomes to make comparison with reference standard easier
  - E.g. develop CHO clone specific karyotype from MCB as reference standard





SOURCE OF ADVENTITIOUS AGENTS



Contamination of Cell Lines

Cell Type	Viral Contaminant	Source of Virus
Various	BVDV (non-cytopathic)	Bovine serum
BHK/CHO	Reovirus	Bovine serum
CHO	EHDV	Bovine serum (not screened)
CHO	Cache Valley Virus	Bovine serum (not screened)
CHO	Minute Virus of Mice	Components of media
CHO	Calicivirus 2117	Bovine serum?
Vero	Porcine circovirus	Porcine trypsin
Vero	Bluetongue virus	?
Rhesus monkey kidney	SV40	Primary cell line
Various	SMRV	Other cell lines
Insect Tn5 (High Five)	Nodavirus	Latent infection of cell

Manufacture & Testing of Clinical Material - EU

EU Clinical Trial Directive (2001/20/EC)

- Clinical material must be manufactured to GMP in approved premises
- Applies to IMP manufactured and tested in EU or manufactured outside EU for trials in EU
- Product released by QP
- QC testing during manufacture
  - Starting materials including cell banks and virus banks
  - Bulk harvest, purified drug substance, drug product
- Move from GLP to GMP as quality standard for QC

GMP Testing

- Assay performance assured by:
  - Generic validation (analytical method validation ICH Q2)
  - Verification (compendial assays)
  - Product specific qualification
- Equipment validation
- Control of raw materials and critical reagents
- Appropriate quality systems and procedures
- Documentation
  - Technical specifications, SOPs, work book, report
- Trained personnel

BioReliance® by SAFC

EU GMP Annex 2

- EU Guidelines for GMP for Medicinal Products for Human and Veterinary Use, Annex 2, Manufacture of Biological Active Substances and Medicinal Products for Human Use.
  - Guide applies to establishment and maintenance of MCB, WCB, MVS, WVS. These should be manufactured to GMP and QC testing to GMP. Comes into operation 31 January 2013
  - Level of GMP increases in detail from early to later steps but GMP principles should always be adhered to.
  - “During the establishment of the cell bank, no other living or infectious material (e.g. virus, cell lines or cell strains) should be handled simultaneously in the same area or by the same persons.”
  - “ Following the establishment of cell banks, quarantine and release procedures should be followed. This should include adequate characterization and testing for contaminants.”
  - “Cell banks should be stored and used in such a way as to minimise the risks of contamination (e.g. stored in the vapour phase of liquid nitrogen in sealed containers)”

World Health Organization

WHO Cell Substrates Guidance

- Recommendations for the Evaluation of Animal Cell Cultures as Substrates for the Manufacture of Biological Medicinal Products and for the Characterization of Cell Banks. Final, 2011
- Update to Technical Report Series 878
- Scope
  - Animal cell substrates for the production of biological medicinal products
  - Recombinant products and viral vectors/vaccines
  - Excludes microbial cells and animal cells for cell therapy
- Most up to date guidance document for cell line characterisation
- Drafting committee included members from US FDA, EMA, PMDA

BioReliance® by SAFC

World Health Organization

WHO Cell Substrates Guidance - Contents

- Introduction, historical overview, scope, definitions
- Part A. General recommendations applicable to all types of cell culture production
  - Principles of good cell culture practices
  - Selection of source materials of biological origins
  - Cryopreservation and cell banking
- Part B. Recommendations for the characterization of cell banks of animal cell substrates
  - Identity, stability, sterility, viability, growth characteristics, homogeneity, tumorigenicity, oncogenicity, cytogenetics, microbial agents
  - Testing of new continuous cell substrates (vaccine production)
- Appendices:
  1. Risk assessment in the case of adventitious agent finding
  2. Tests for bovine viruses in serum
  3. Tumorigenicity protocol using athymic nude mice
  4. Oncogenicity protocol for the evaluation of DNA and cell lysates

BioReliance® by SAFC

WHO Cell Substrates Guidance – Specific Points

- Use of Population Doubling Level (PDL) rather than Passage
- Cryopreservation
  - Cooling profile achieved in the cells being frozen should be qualified
  - Number of cells/vial: 5 – 10 x 10^6/vial
  - Antimicrobials should generally not be used
- Stability of cell banks
  - Periodic testing for viability not necessary if continuous monitoring records show no deviation and production runs are successful
  - Banks used < once every 5 years – reconfirm stability every 5 years
  - Thawed cells should have viability > 80%

USP: Cryopreservation of Cells

- US Pharmacopoeia General Chapter 1044 Cryopreservation of Cells
  - Draft chapter in Pharmacopeial Forum 39(2), March 2013. Comments by 31 May
  - Chapter discusses the principles of cryopreservation and particular considerations for:
    - Prefreeze processing and characterization
    - Reagents and containers
    - Addition of cryoprotectant solution
    - Cooling
    - Cryogenic storage, safety and transport
    - Thawing and post-thaw processing and evaluation
  - Cell type- and application-specific information for:
    - Human cell therapy products
    - Haematopoietic stem cells, mesenchymal stem cells, lymphoid cells
    - Human pluripotent stem cell lines
      - Advice for assessing homogeneity, stemness and genetic stability of a culture
    - Cell substrates for recombinant biotechnology products
      - Mammalian, insect and microbial cell lines

BioReliance® by SAFC

Rationale for cell bank testing

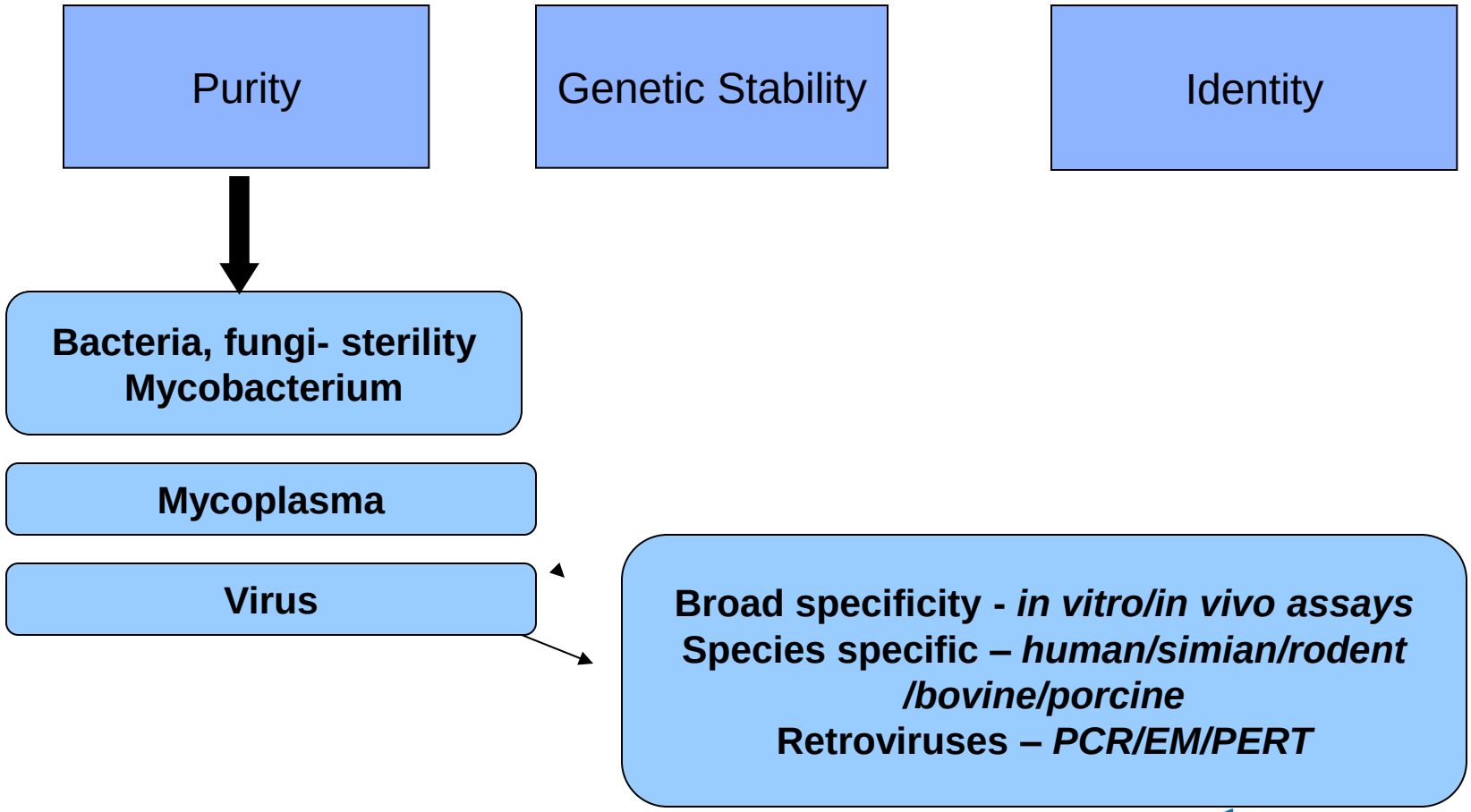
- MCB
  - Starting material for the whole of the production process
  - Full characterisation for microbial and viral contaminants
  - One time testing
- WCB
  - Small number of passages beyond MCB
  - Reduced package of testing
- Maximally expanded cells
  - ‘Worst case’ for amplification of contaminants
  - May not be required to late clinical development
  - Full characterisation, one time testing
- Bulk harvest (BH)
  - Routine QC test for contaminants
  - Rapid tests required

EP and FDA Differences for Vaccine QC

- EP testing strategy (EP 5.2.3) is to perform most testing on cells at the maximum population doubling used in production
  - Minimal testing on MCB and WCB
- US FDA strategy is for full characterisation of MCB, less testing on EOPC and testing of viral bulk harvest.
- Details of some assays differ between EP and FDA
  - *In vitro* virus assays
  - *in vivo* virus assays

Cell Bank Characterisation - MCB

Master Cell Bank (MCB)



BioReliance® by SAFC

Purity - Sterility

- Sterility
  - Pharmacopoeial method
  - Direct inoculation for cells, membrane filtration for final products
  - 14 day assay using two media under aerobic and anaerobic incubations
  - Observation for turbidity
  - For cell banks test 1% of total bank or a minimum of 2 vials (ICH, Q5D recommendation)
  - Bacteriostasis and Fungistasis testing recommended to assess sample matrix for inhibition

Mycoplasma contamination

Organisation	Number of cultures tested	Contamination rates
FDA (1970 – 1990)	20,000	>3000 (15%)
Microbiological Associates (1985 – 1993)	2863	370 (12.9%)
ATCC (1989 – 1994)	5362	752 (14%)
Bionique (reported in 1994)	10,000	1110 (11.1%)
Bionique (reported in 2009)	10,000	679 (6.8%)

Armstrong, S.E. (2010) Biologicals, **38**, 211-213

Mycoplasma assays

- Japanese Pharmacopoeia. ‘Mycoplasma tests for cell substrates used for production of biotechnological /biological products.’
  - Revised, 15th Edition, Supplement 2; October 2009.
- European Pharmacopoeia, 2.6.7. Mycoplasmas
- US FDA (1993) Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals
  - 21 CFR 610.30 (mycoplasma testing of viral vaccines)
- US Pharmacopoeia Edition 33, Chapter 63, Mycoplasma Tests
  - Became official October 1st, 2010
  - Chapter based on EP 2.6.7

BioReliance® by SAFC

28-Day *Mycoplasma* Culture Method

Sample

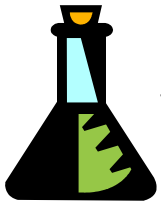
1.0 ml — Vero Cells — 3-5 days → Hoechst stain

Observe DNA fluorescing staining pattern

0.2 ml

Agar plate — 14 days → ***Observe for mycoplasma colonies***

10 ml



Mycoplasma Broth

Day 3 → Day 7 → Day 14

14 days → Day 17

14 days → Day 21

14 days → Day 28

Observe for mycoplasma colonies

BioReliance® by SAFC

EP Mycoplasma Monograph

- ***Nucleic acid amplification method***

- Direct NAT applied to cytotoxic material and where a rapid method is needed
- Cell-culture enrichment followed by NAT
- Methods must be validated for:
  - Specificity particularly for cross reactivity with bacterial genera with close phylogenetic relationship
  - LOD with number of different species
  - LOD shown to detect 10 CFU/ml if NAT an alternative to culture method, 100 CFU/ml if alternative to indicator cell culture method.
  - Require internal controls to verify absence of inhibition
  - If NAT method is to be used instead of present culture method a comparability must be performed

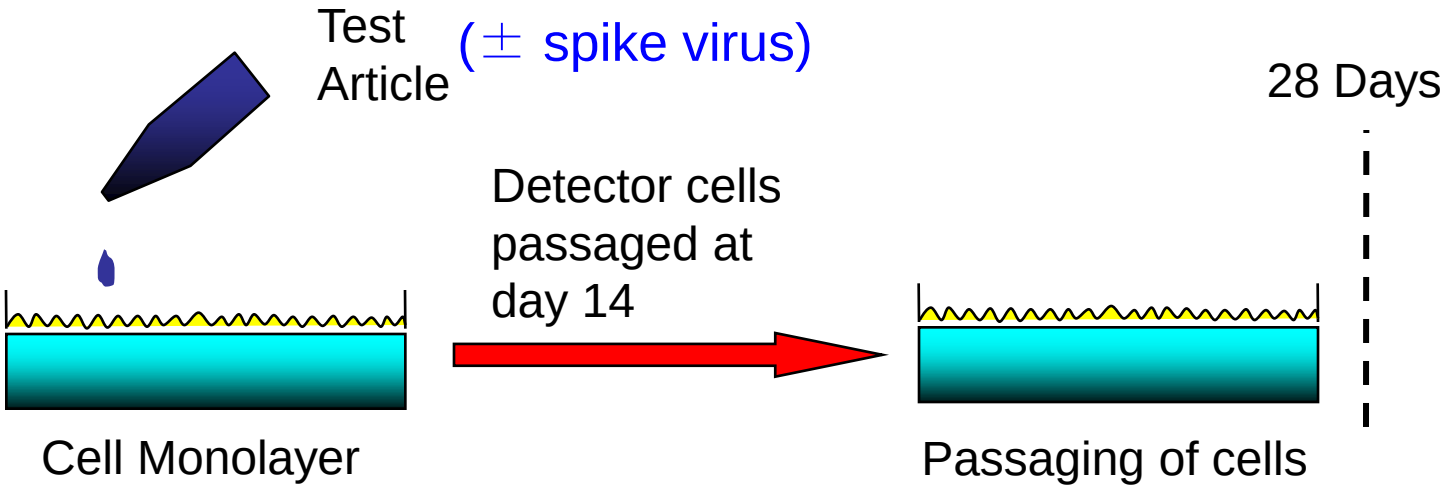
Rationale for virus assays

- To detect an unknown and wide range of possible contaminants need to utilise a number of different assays
- Broad specificity assays
  - *in vitro* and *in vivo* virus assays, electron microscopy
- 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 assays

- *In vitro* virus assay
  - viruses detected by cytopathic effects (CPE), haemadsorption and haemagglutination
  - not all viruses produce CPE
  - can only use a limited number of detector cells
  - not all viruses grow well in tissue culture
- *In vivo* virus assay
  - Inoculation into embryonated eggs (allantoic and yolk sac), suckling mice, adult mice, Guinea pigs
  - viruses detected by morbidity and mortality
  - classical virus isolation methods that may detect viruses that do not grow well in tissue culture
- Electron microscopy
  - detects intracellular virus particles
  - no amplification potential, relatively insensitive

In Vitro Cell Culture Assay - Summary



End Point Detection Methods

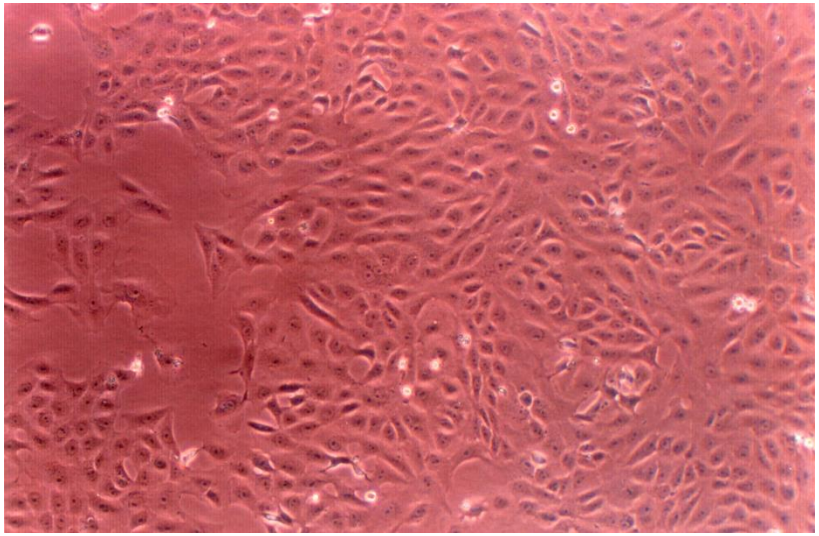
- Cytopathic Effect - CPE
- Haemadsorption - HAD
- Haemagglutination -HA
- Also
  - IF
  - RT / F-PERT
  - EM

Common Detector Cells

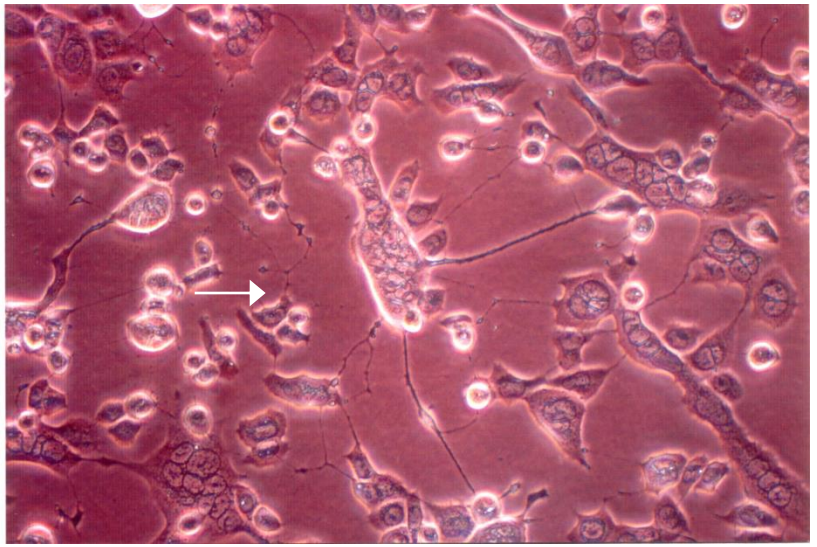
- MRC-5 (Human Diploid)
- Vero (Simian)
- HeLa
- Same Species and Tissue - SP2/0, BHK, CHO, NS0 etc.
- 324K for detection of MVM

BioReliance® by SAFC

Virus-Induced Cytopathic Effect (CPE)



Uninfected Vero cells

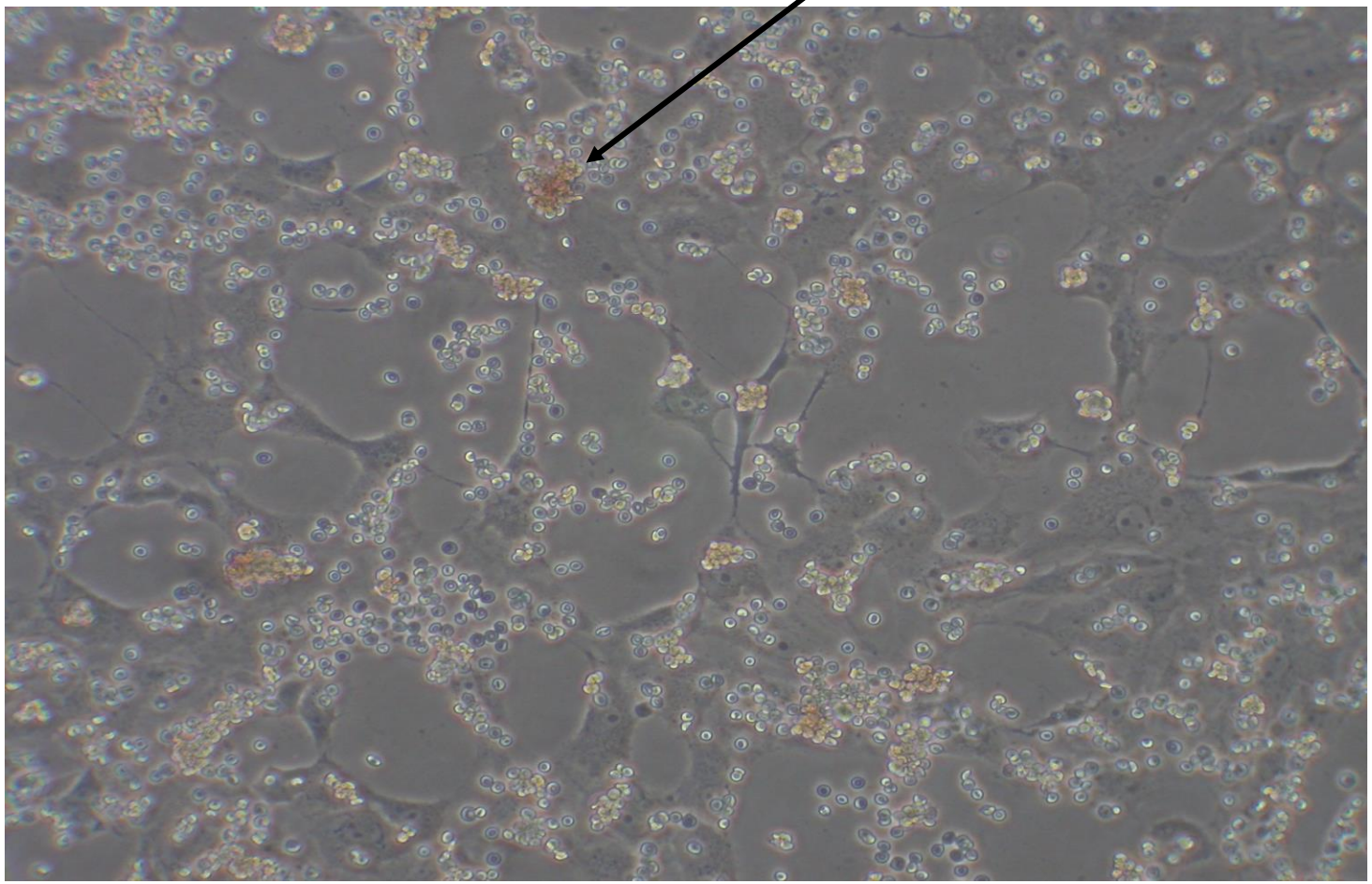


HSV-infected Vero cells

Cell rounding/swelling and syncytia formation visible

BioReliance® by SAFC

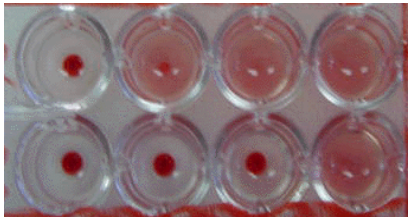
Hemadsorption (HAD) – Vero Cells and Rhesus RBCs



BioReliance® by SAFC

Haemagglutination (HA)

- Supernatants are harvested during the assay to be tested for haemagglutinating viruses such as Influenza and Measles. If virus is present, the haemagglutinin will bind to sialic acid residues of the red blood cells
- The red blood cells are held together in a diffuse matrix. They will not settle and form a pellet

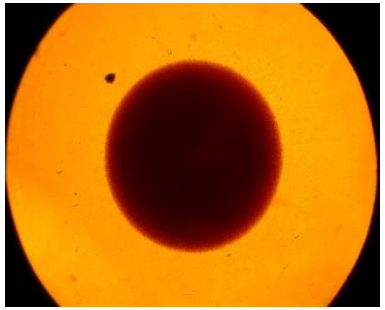


96 well plate

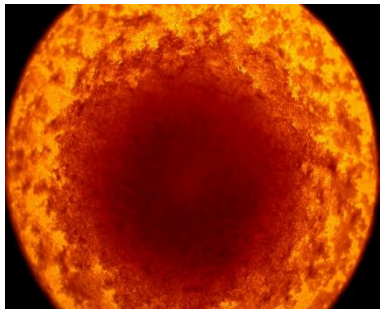
- + + +

- - - +

Results



Negative well



Positive well

BioReliance® by SAFC

In vivo Approaches for Biosafety Evaluation of Cell Banks and Viral Vectors

Detection of extraneous viruses using mice

Inoculate 20 suckling mice
per os (0.01ml)
i.p. (0.1ml)
i.c. (0.01ml)

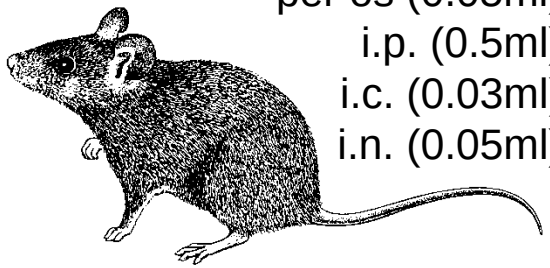
Observe suckling mice for 14 days

Sacrifice and pool organs
Inoculate homogenate into 10 suckling mice

Observe suckling mice for 14 days

Sacrifice

Inoculate 10 adult mice
per os (0.05ml)
i.p. (0.5ml)
i.c. (0.03ml)
i.n. (0.05ml)



Observe mice for 28 days

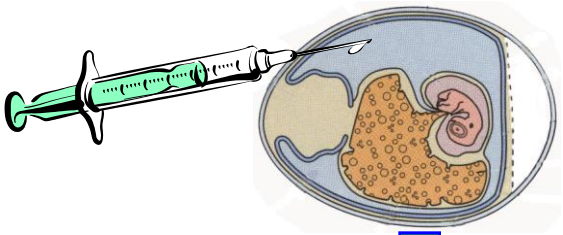
Sacrifice

BioReliance® by SAFC

In vivo Approaches for Biosafety Evaluation of Cell banks and Viral Vectors

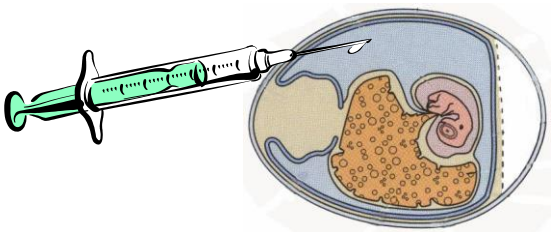
Detection of extraneous viruses using embryonated eggs

10-11 day old embryos inoculated via the allantoic cavity



Incubate for 3 days

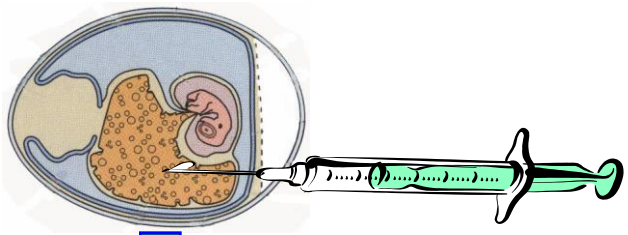
Assess embryos for viability
Test fluids for haemagglutination



Incubate for 3 days

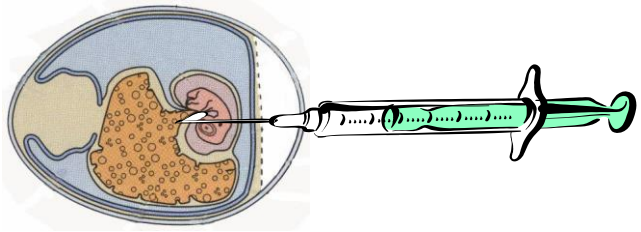
Assess embryos for viability
Test fluids for haemagglutination

6-7 day old embryos inoculated via the yolk sac



Incubate for 9 days

Assess embryos for viability



Incubate for 9 days

Assess embryos for viability

BioReliance® by SAFC

In vivo Virus Assay

- Advantages
  - Broad specificity virus assay
  - Detects viruses that do not grow well in tissue culture
  - Many viruses first isolated using suckling mice: coxsackieviruses type A, SARS, human coronavirus OC43, new human cardiovirus (SAF-V)
  - High sensitivity

- Disadvantages
  - Toxic effects of pluronic acid (serum free media), adenovirus penton proteins
  - Uses animals
  - Little formal validation studies
  - Lengthy assay

BioReliance® by SAFC

Assays for Adventitious Viruses

- Study commissioned by US NIH.
  - Lead investigator Rebecca Sheets
  - Will be published in Vaccine. Data has been seen by FDA and EMA
- Systematically characterises the breadth and sensitivity of the routine adventitious virus tests
  - *In vitro* and *in vivo* assays for inapparent viruses
- Provide regulators and manufacturers with information needed for decision making
- Provide baseline data to serve as basis of comparison for new methods
- Determine ‘value added’ by *in vivo* methods in consideration of 3 R’s policy

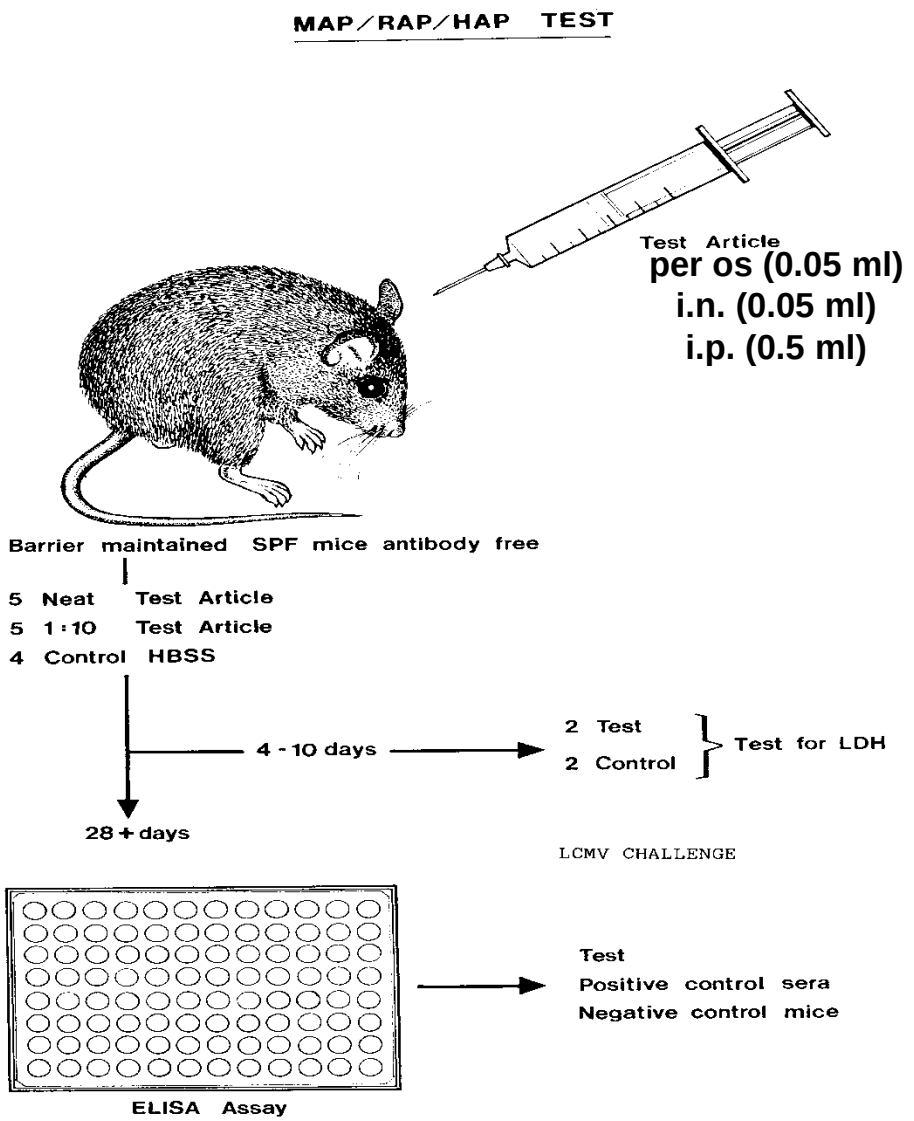
Assays for Adventitious Viruses (2)

- Breath and sensitivity of assays compared using:
  - *In vitro* assay: 3 detector cell lines, 14 day assay and 28 day assay. 11-15 combinations of cells and viruses
  - *In vivo* assay: embryonated eggs, adult mice and suckling mice. 3-6 combinations of virus and test system
- *In vitro* assay:
  - 28 day assay with passage at 14 day more sensitive for all cell/virus combinations than 14 day assay
  - 14 day assay minimum requirement given in US PTC 1993 and 1997
  - US FDA Guidance for cell substrates for vaccine production (2010) and WHO Guidance for cell substrates for biological production (2011) recommend performing a 28 day assay on cell banks
  - US FDA asked manufacturers to justify the use of the 14 day assay rather than performing a 28 day assay for both cell banks and bulk harvests.

Assays for Adventitious Viruses (3)

- *In vivo* assay:
  - Suckling mice: no dilution of virus that passed first passage and was found positive after second passage
  - With the exception of influenza and vesicular stomatitis virus the *in vitro* virus assay was more sensitive than the *in vivo* virus assay. This included Coxsackie A and B viruses
- 3 R's
  - European Directive to reduce the use of animals in pharmaceutical development
  - BioReliance has not detected a positive virus infection with this assay in the last 20 years (> 9000 assays)
  - Can this assay be removed from the standard testing panel?

Antibody Production Assays



BioReliance® by SAFC

Rodent Viruses Detected in MAP Assay

- Human pathogens
  - Hantaan virus, LCMV, Reovirus 3
- Infecting human or primate cells
  - Ectromelia virus
  - Hantaan virus
  - Lactate dehydrogenase elevating (LDV)
  - Lymphocytic choriomeningitis virus (LCMV)
  - Murine minute virus (MMV)
  - Mouse adenovirus (MAV)
  - Mouse cytomegalovirus (MCMV)
  - Epizootic diarrhea of infant mice (EDIM)
  - Pneumonia virus of mice (PVM)
  - Sendai
- No evidence of capacity to infect humans or human/primate cells
  - Mouse K virus, mouse hepatitis virus (MHV), Mouse encephalomyelitis virus type II (GDVII), polyomavirus, mouse thymic virus

Experience with Adventitious Virus Testing

	1981-1992	2004	2006	2007	2008 (UK)
In vitro* virus assay** ***Total	550*	1200	1866	2071	986
% positive	1.5	0.42 (5)	0	0.05 (1)	0.20 (2)
In vivo* virus assay** ***Total	550*	368	449	525	225
% positive	1.2	0	0	0	0
MAP/HAP assays ***Total***	550*	350	381	388	114
% positive	2.2	0	0	0.52 (2)	0

* approx.

BioReliance® by SAFC

Newly Discovered Viruses

- Isolated from human clinical samples
  - Screening of human throat swabs by molecular biology techniques including multiple parallel sequencing
    - Human bocavirus 1, 2 and 3, human polyoma viruses, human cardiovirus, human parvovirus 4 and 5
    - Human bocavirus is 4th most frequent human respiratory virus detected in 20% of children. Also associated with gastroenteritis.
    - Human polyoma viruses
      - Originally JC and BK viruses
      - Now 9 human polyoma viruses identified
  - May not be detected using standard infectivity assays
- Animal viruses infecting human cells
  - LCMV, hepatitis E, bovine herpes virus type IV, bovine polyoma virus, bovine adeno-associated virus, bovine kobuvirus

BioReliance® by SAFC

Characterization of Human Cell Banks

Specific Human Viruses

- HIV 1&2
- HTLV 1&2
- CMV
- EBV
- HAV, HBV, HCV
- HHV-6,7,8
- B19
- Human metapneumovirus
- Human polyoma viruses
  - JC, BK, WU, KI, Merkel cell, HPyV 6,7,9, TSPyV
- Human bocavirus

BioReliance® by SAFC

Bovine and porcine viruses

- EMA and US 9CFR testing recommendations for bovine virus assay
  - Culture indicator cells over 21 days with test serum
  - Cells permissive for a wide range of bovine viruses
    - BT and Vero cells
  - Observe for cpe, HAD and IF staining for specific viruses
    - BVDV, bovine parainfluenza type 3 virus, bovine parvovirus 1, rabies, reovirus 3, IBR, BRSV, blue tongue virus, bovine adenovirus 5, VSV
- US 9 CFR testing recommendations for porcine trypsin
  - Using porcine kidney cells to detect porcine parvovirus
- 9CFR/EP regulated assays do not include recently identified viruses
  - Porcine circovirus, Hepatitis E virus, bovine herpes virus type IV

Testing of Bovine Serum

- EMA Guideline on the use of bovine serum in the manufacture of human biological medicinal products. EMA/CHMP/BWP/457920/2012 rev 1.
  - End of consultation: 31 Dec 2012.
- Revision of June 2003 guidance.
  - 7.3.3. Recommendation for BVDV
    - Level of contamination, if present, should be quantified and must be below the level that has been shown to be effectively inactivated in validation tests for inactivation treatment.
    - If BVDV is detected , the serum must be re-tested for infectious virus after any inactivation step and used only if no infectious virus is detected
  - 7.3.4. Detection of anti-BVDV antibodies
    - A validated test should be employed to detect antibodies and an assessment made of their impact on neutralisation of any infectious BVDV and on virus detection.
    - Removed previous requirement that serum should not have a significant (> 2 logs) inhibitory effect on growth of BVDV

Testing of Bovine Serum (2)

- 7.3.5. Other bovine viruses
  - "Serum suppliers and users should also be aware of emerging bovine viruses and are encouraged to investigate the presence of such agents in bovine serum and take appropriate action to eliminate or reduce the presence of any novel virus in serum."
  - Recently new viruses have been detected by massively parallel sequencing and other techniques that are not detected in classical serum screening assays
    - Bovine parvovirus 2, 3 and 4
    - Bovine adeno-associated virus 2 : can infect wide range of cells including human
    - Bovine herpes virus type 4 : can infect human cells
    - Bovine Norwalk virus
    - Bovine kobuvirus: reported as a contaminant of HeLa cells in 2003

Viruses in Porcine Trypsin

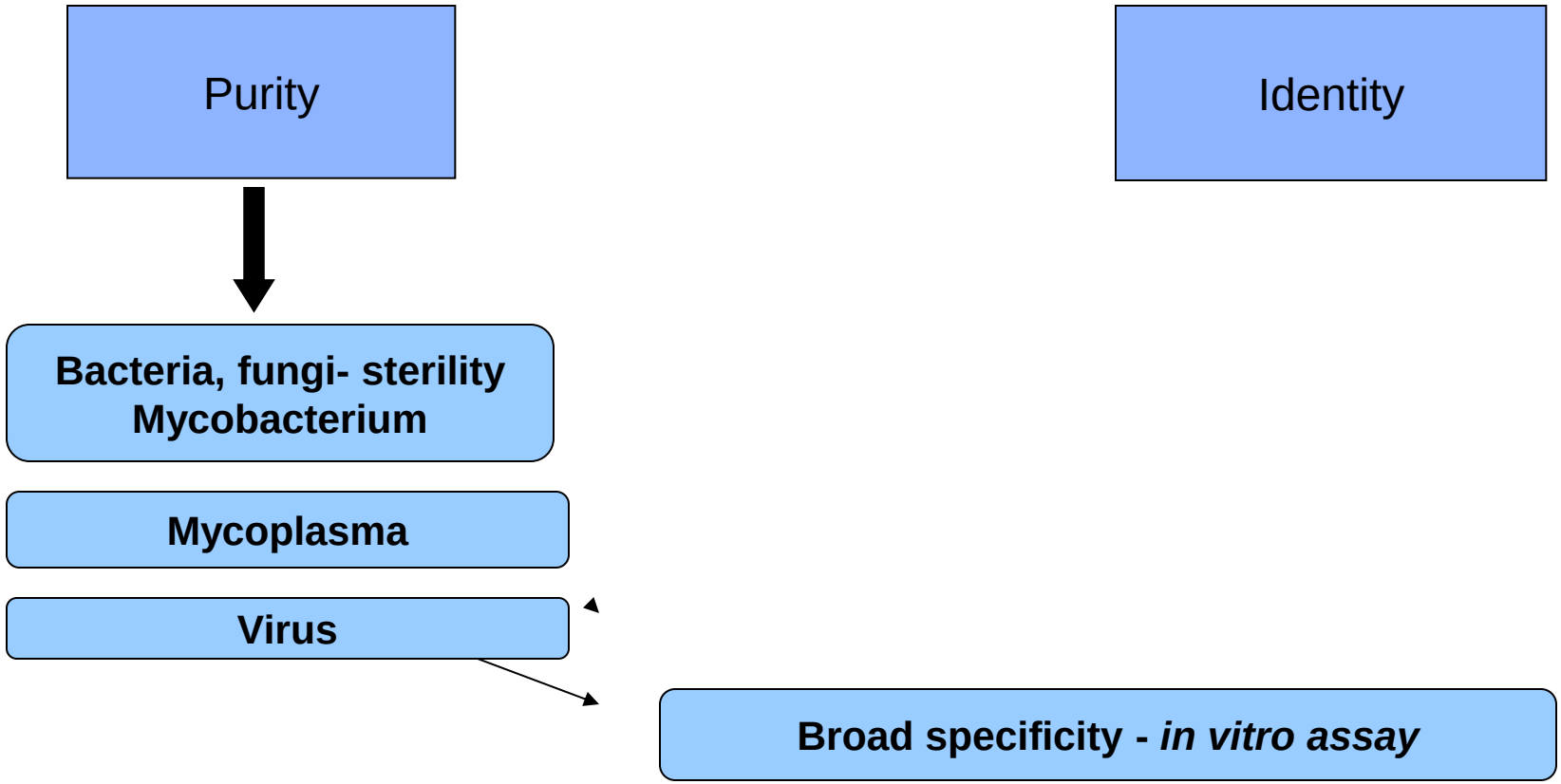
- MP-Seq detected porcine circovirus sequences in Rotavirus vaccines
- EMA Concept paper for a guideline on the quality of porcine trypsin used in the manufacture of human biological medicinal products. EMA/CHMP/BWP/367751/2011 Consultation to 31 Dec 2011
  - Will address:
    - Scope of guideline, types and source of porcine trypsin, manufacture and preparation of batches, tests for identity, purity and suitability for cell culture, testing for adventitious viruses, tests for sterility, virus reduction methods, quality system, certificate of analysis and regulatory implications.

Guidance on Porcine Trypsin

- Guideline on the Use of Porcine Trypsin Used in the Manufacture of Human Biological Medicinal products. EMA/CHMP/BWP/814397/2011
  - Issued for consultation until 31 August 2013
  - Trypsin used in cell culture during manufacture of vaccines, ATMPs and other medicinal products produced by cell culture; trypsin used to activate virus particles; trypsin used as a protein processing reagent.
  - For practical reasons not possible to test individual pancreatic glands before processing
  - Stage where testing should be performed should be clearly defined
  - Tested using primate (Vero) and porcine cells for CPE and HAD
  - Specific tests for viruses not detected in general assay should be considered e.g. Porcine circovirus, Hepatitis E
  - Two virus inactivating/removal steps should be validated
    - Incorporate gamma or UV-C irradiation
  - Validate cleaning processes to minimise batch-to batch contamination

Cell bank Characterisation - WCB

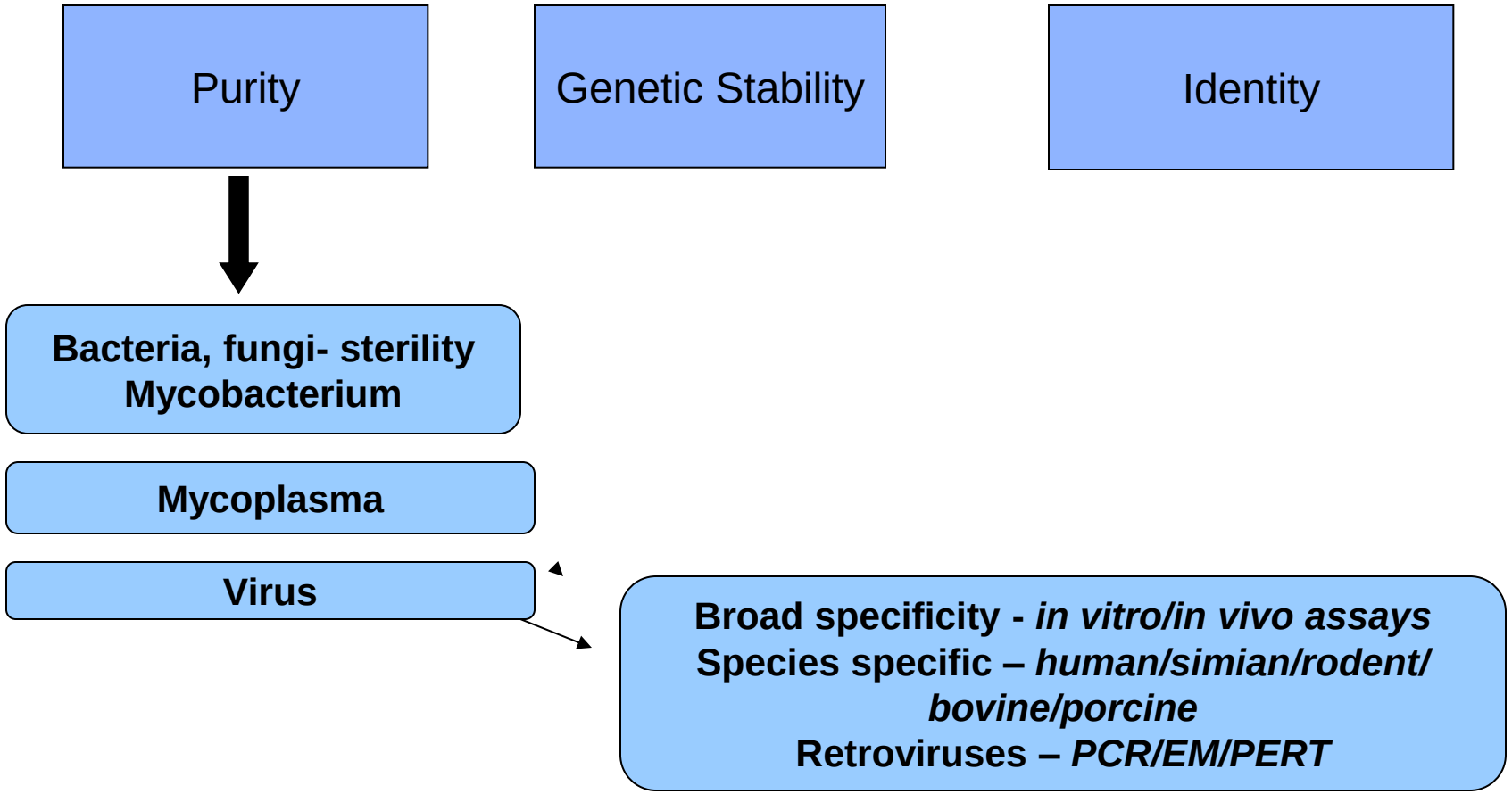
Working Cell Bank (WCB)



BioReliance® by SAFC

Cell bank Characterisation - CAL

Maximum use cells/Cells at Limit of *in vitro* cell age (CAL)



BioReliance® by SAFC

Tumorigenicity

- 'Well characterised' cells (CHO, BHK, NS0, Sp2/0), tumorigenicity testing not required

- Human cells, cells with unknown tumorigenic potential, cells used for viral vector/vaccine production
  - *in vivo* test (e.g. growth in nude mice)
  - for vaccine producers: cell titration to determine tumorigenic potential
  - *in vitro* test (e.g. colony formation in soft agar). Correlation with *in vivo* tests have been imperfect.

- Validate purification process, < 10 ng DNA/human dose

MVSS and Clinical Lot Testing, Viral Clearance Studies

BioReliance® by SAFC

Master Virus Seed Characterisation

- MVSS should be screened fully for adventitious bacteria, fungi, mycoplasma and viruses taking account of the origin and isolation of virus stock
- Neutralising antiserum is required for infectivity assays
  - Should be prepared from a stock that is different from stock used for production and prepared using SPF animals. Human or simian antiserum should not be used.
- Pre-studies are required to ensure neutralisation of virus stocks before testing
- Where neutralising antisera of high enough titre cannot be prepared a panel of PCR assays may be used
- Production control cells (not inoculated with virus) grown in same medium and handled alongside production cells are tested for adventitious mycoplasma and viruses

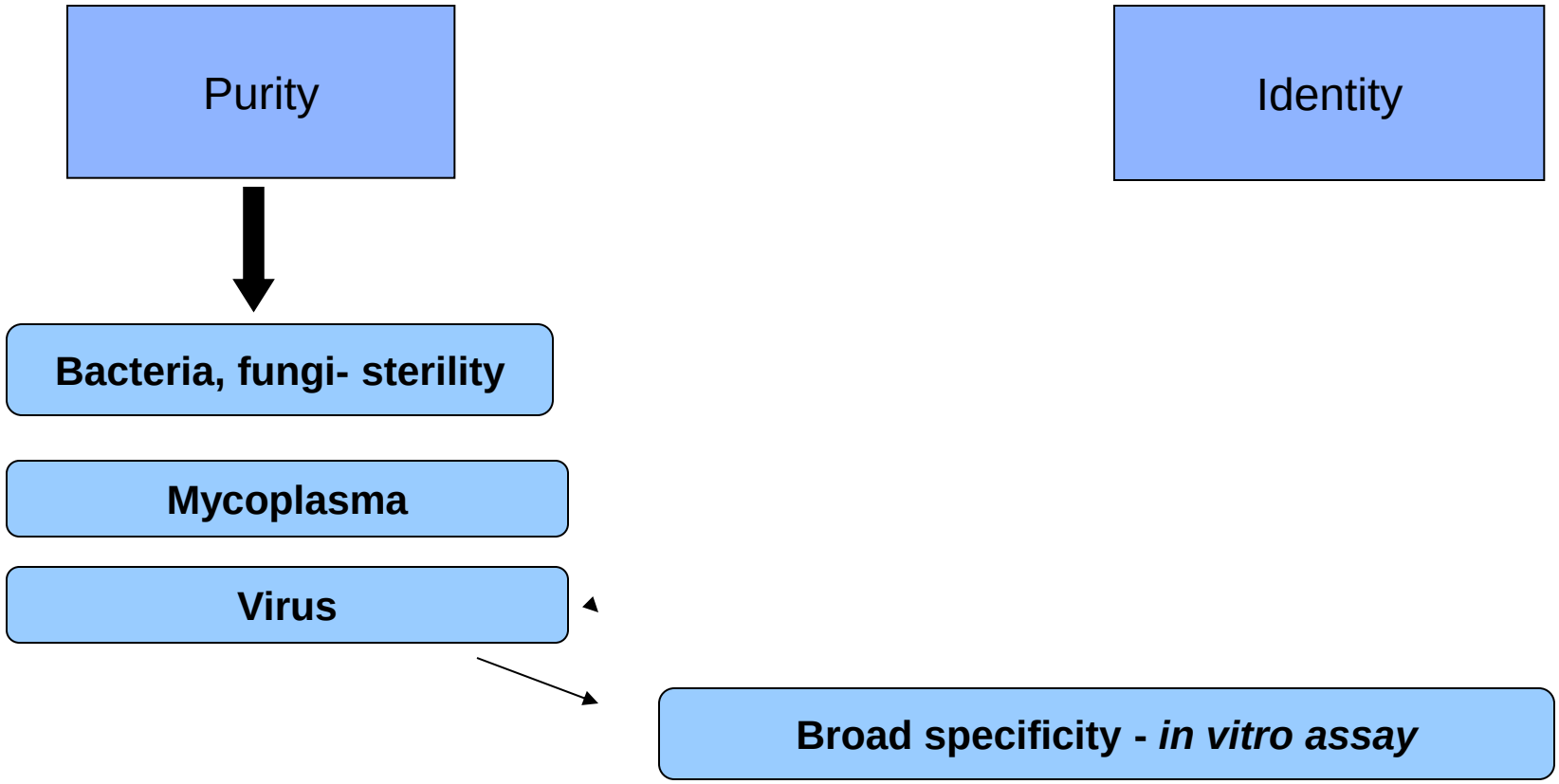
PCR panel of human respiratory viruses

PCR assays for:

- Coronavirus (types 229E and OC43)
- Human respiratory syncytial virus (types A and B)
- Human Parainfluenza virus 1, 2, 3
- Rhinovirus
- Varicella Zoster virus
- Rubella
- Metapneumovirus
- Bocavirus
- Human polyoma viruses

Clinical lot testing

Working Cell Bank (WCB)



BioReliance® by SAFC

Viruses and Transformed Cells

- Novel cell substrates for vaccine production
  - Continuous transformed cells
  - MDCK, Duck fibroblast, A549
- Where mechanism of transformation is not known concern that transformation is due to presence of:
  - Oncogenes
  - Oncogenic viruses
    - Retroviruses, herpesviruses, polyoma viruses, papillomaviruses
    - Latent viruses
- Induction studies to detect latent retroviruses and DNA viruses
- *In vivo* studies to detect oncogenic agents and DNA

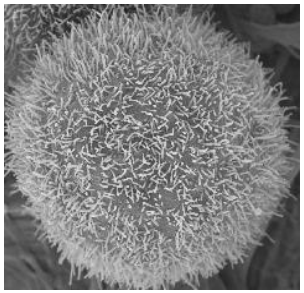
Latent/Occult Viruses

- Cell substrates can be contaminated with latent/occult viruses
  - Retroviruses
    - Alpha retroviruses (ALV)
    - Beta retroviruses (MPMV/SRV)
    - Gamma retroviruses (MLV)
  - RNA viruses
    - LCMV
  - DNA viruses
    - Adeno-Associated Viruses
    - Adenoviruses
    - Hepadenaviruses
    - Herpesviruses
    - Papillomaviruses
    - Polyomaviruses
- Most of these viruses are not detected in standard *in vitro* assays for adventitious viruses

Arifa Khan, CBER, FDA

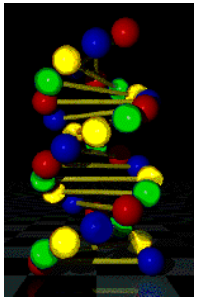
Special Considerations for Continuous Cell Lines

Cells



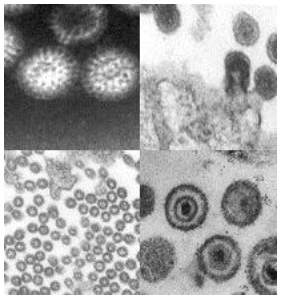
- Demonstrate removal of intact cells
- Demonstrate no capacity for transformation (oncogenicity)

DNA



- Demonstrate lack of oncogenicity
- Demonstrate acceptable DNA removal and/or inactivation
- < 10 ng/dose; <200-400 bp

Adventitious Agents

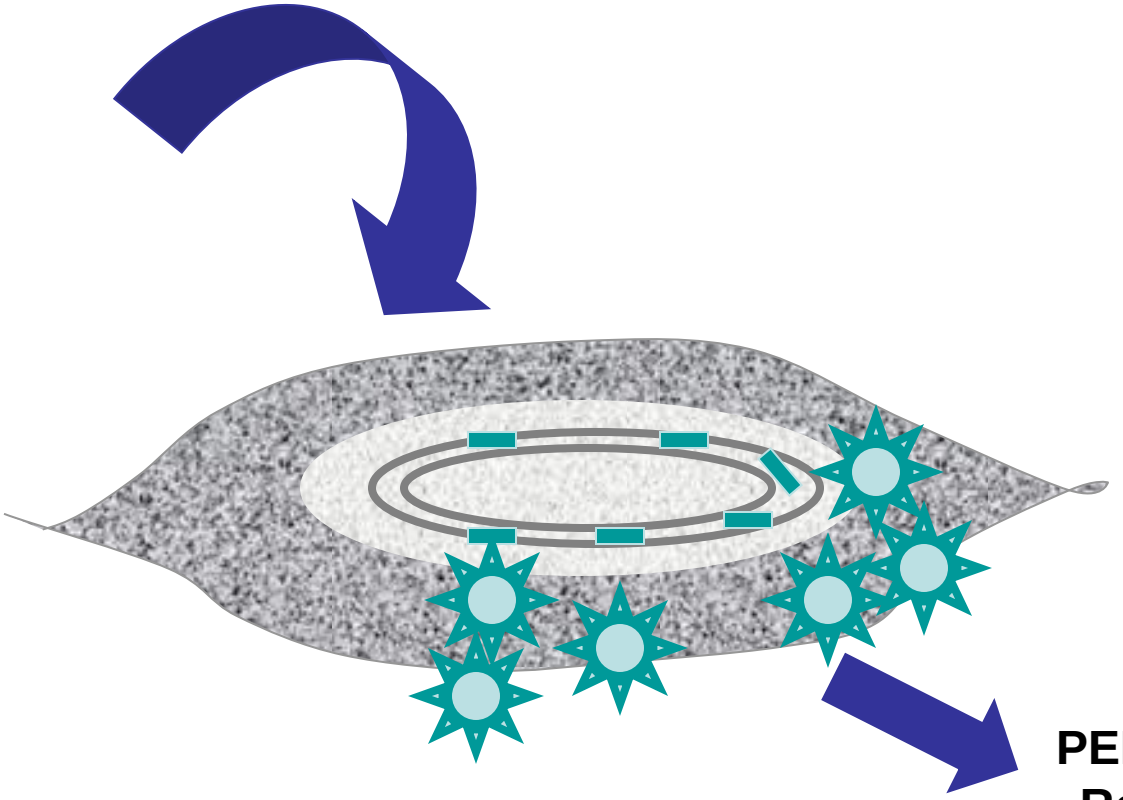


- Demonstrate lack of inherent agents
  - Infectious
  - Latent/occult
  - Oncogenic
- Demonstrate removal and/or inactivation of potential agents

BioReliance® by SAFC

Detection of Endogenous and Latent Viruses

INDUCER
IUdR, AzaC
NaB, TPA



DETECTION ASSAYS
TEM
PERT (Retrovirus)
Redundant PCR
Infectivity / Co-culture

BioReliance® by SAFC

Virus Induction Studies

- BioReliance induction studies have been developed in collaboration with US FDA
  - Protocols reviewed and improved following discussion (Dr Arifa Khan, Office of Vaccines Research and Review, CBER)

- FDA Algorithm for Virus Induction
  - I. Determination of cell growth characteristics
  - II. Drug selection and evaluation
  - III. Detection of induced virus
  - IV. Virus characterisation

BioReliance® by SAFC

Virus Induction Studies (2)

Test cells in exponential growth

I

Treat with inducing agents: IdU and AzaC for retroviruses, NaB and TPA for DNA viruses

I

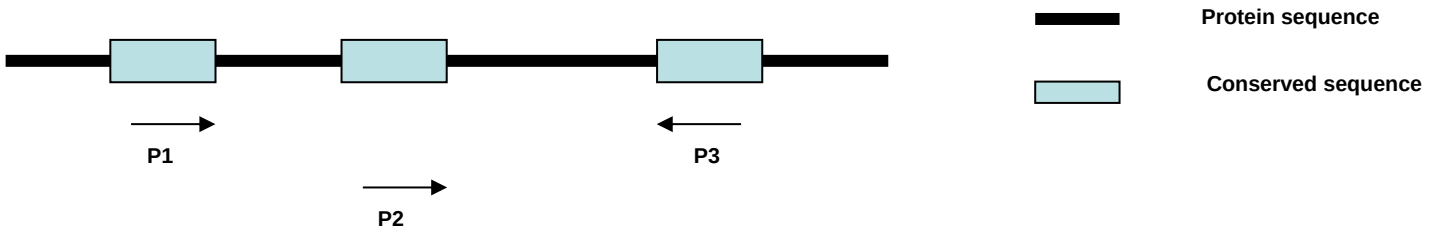
Harvest cells and supernatants for analysis

I	I
Retroviruses	*DNA viruses*
FPERT	Degenerate PCR for herpes & polyoma viruses
TEM	PCR for papillomaviruses
Infectivity (Co-cultivation with PERT AND TEM endpoints)	PCR for adenoviruses
	TEM

BioReliance® by SAFC

Degenerate PCR for latent viruses

- PCR-based method for identifying viruses of a specific family or subfamily based upon conserved proteins (polymerase/glycoproteins)



Conserved regions within a protein family are identified. Degenerate primers (P1, P2, P3) are designed based upon these regions and PCR performed using outer primers (P1 & P3). Semi-nested PCR (using P2 and P3) can then be performed to increase sensitivity.

- Method is non-specific so identification of any virus detected requires further sequencing

BioReliance® by SAFC

Continuous cell substrate characterisation

- Additional assays:
  - Retrovirus and DNA virus induction studies.
  - Tumorigenic potential study
    - Inoculation of dilution series (10^7, 10^5, 10^3, 10^1) of whole cells compared to HeLa cell positive control
    - Inoculated into nude mice and observed for tumour formation for $\geq$ 150 days
  - Oncogenicity studies
    - Inoculation of cell lysate into newborn mice, hamsters and rats. Observation for tumour formation for $\geq$ 150 days

BioReliance® by SAFC

World Health Organization

WHO Cell Substrates Guidance – Insect Cells

- Insect cell line testing
  - *In vitro* assay for adventitious agents
    - Intact and cell lysates from EOPC co-cultivated with 3 different species of insect cells
      - Permissive for growth of arboviruses: mosquito cell line or BHK-21
      - Permissive for a range of insect viruses: Drosophila S2
      - Cells from same species as production cell line
    - Grow at 2 temperatures (37^{o} and 28^{o}C), 14 days. Examine for cpe, HAD, HA
  - TEM
    - Grown at 2 temperatures
  - FPERT and if positive retrovirus infectivity assay
  - PCR for specific viruses that have been reported to contaminate the cell line (eg nodaviruses)
  - Spiroplasma

BioReliance® by SAFC

Microbial Cell Bank Characterisation

Concern	Assay	MCB	WCB	CAL
Viability	Colony count	+	+	+
E.Coli Identity	*E.coli* API	+	+	+
	K12 derivative	+	+	+
	Biochemical markers	+		+
Strain identity	RAPD	+		+
Purity	Absence of bacterial/fungal contaminants	+	+	+
	Bacteriophage	+		+
Stability	Viability & antibiotic resistance	+	+	+
	Genetic stability	+		+

BioReliance® by SAFC

Lot Release Testing

- Lot release testing of final product is dependent on type of vaccine – live, inactivated, subunit
- Antigen concentration (eg Haemagglutinin, Neuraminidase)
- Total protein; antigen : total protein ratio
- Purity: SDS-PAGE
- Test for inactivating agent concentrations (eg formaldehyde)
- Contaminating proteins: ovalbumin, bovine serum albumin
- Host cell DNA (vaccines produced on continuous cell lines)
- pH, appearance, particulates
- Endotoxin
- Sterility
- Potency
  - Immunochemical assay
  - *In vivo* protection assays

Virus Risk Control and Reduction

Viral and microbial safety of biotechnological medicinal products is assured by three/four complimentary approaches:

- Testing of starting materials for viral and microbial contaminants:
  - Cell line, Media components, MVSS
- Treatment of media components to reduce risk of virus contamination
- Testing process intermediates at appropriate steps in the manufacture of the product for contaminating viruses, mycoplasma, bacteria and fungi
  - MCB, WCB, EOPC, bulk harvests
- Assessment of the capacity of downstream process to clear infectious viruses
  - Relevant for inactivated and recombinant vaccines

Viral Clearance Guidelines

- WHO Recommendations for the Production and Control of Influenza Vaccine (Inactivated) TRS 927, Annex 3, 2005
- EMA Guideline on Influenza Vaccines – Quality Module (EMA/CHMP/BWP/310834/2012, Draft, March 2013)
- ICH Topic Q5A. Note for Guidance on Quality of Biotechnological Products: Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin (CPMP/ICH/295/95).
- EMA Note for Guidance on Virus Validation Studies: The design, contribution and interpretation of studies validating the inactivation and removal of viruses (CPMP/BWP/268/95, 1996).
- US FDA Points to Consider in the characterization of cell lines used to produce biologicals, 1993.
- EMA Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products (EMEA/CHMP/BWP/398498)

BioReliance® by SAFC

Clearance Study Goals

- Provide evidence that the manufacturing process will effectively remove/inactivate viruses known to contaminate or which may possibly contaminate the starting materials
- Test the production process for its ability to remove/inactivate any virus in the event that novel or unpredictable contamination occurs
- Should involve a wide range of viruses:
  - Relevant model viruses
  - Specific model viruses
  - Non specific model viruses
- Required by regulatory authorities for products containing materials of animal origin
  - Blood or tissue-derived products
  - Monoclonal antibodies
  - Recombinant proteins from animal cell culture
  - Inactivated vaccines

BioReliance® by SAFC

Virus Spiking Study

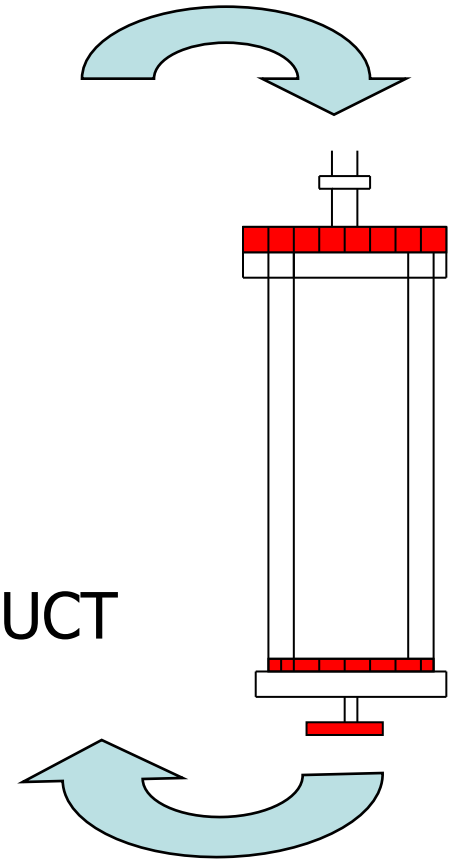
Spike virus into start material = load

|

Collect fractions (*eg* flow-through, eluate, regeneration)

|

Test for infectivity



Reduction: $\frac{\log_{10} \text{ virus [load]}}{\log_{10} \text{ virus [product]}}$

BioReliance® by SAFC

Clearance Studies – Influenza Vaccines

- DSP steps validated for viral inactivation/removal
  - Inactivation steps
    - Formaldehyde
    - Beta propriolactone (BPL)
    - Detergents
  - Removal steps
    - Ion exchange chromatography
    - Virus removing filters

Virus risk mitigation

- Past contamination incidences due to contaminated media components
  - Bovine serum
  - Other components contaminated with MMV
- Good control of suppliers of media
  - Supplier quality agreements, Audit of suppliers
  - What rodent control is there at suppliers of media components?
- Screening of media
  - Limitations in assay sensitivity and amount that can be sampled
  - Spiroplasma contamination of plant peptones used in AOF media
    - WHO recommendation to screen mammalian cells for spiroplasma if plant peptones used
  - MMV never detected in complete media
  - Use media treated using a virus inactivating/removal process
    - UV-C, HTST
    - Virus removing filters

Virus risk mitigation (2)

- Cleaning validation
  - Have cleaning processes been validated to show inactivation of viruses such as MMV?
- Contamination incidence recovery plan
  - Response following discovery of a contaminant
    - Identification of contaminant by MP-Seq
    - Companies have defined recovery plan in place with BioReliance

Virus Inactivation by Gamma Irradiation

Type of raw material	Typsin powder	Typsin liquid	BSA powder	FBS	FBS	FBS
Bovine adenovirus type 3			> 6.0			
BVDV			> 6.5	> 5.1	6	> 6.7
Parainfluenza type 3				> 5.0	6	
IBR				> 4.5	5	> 7.8
Bluetongue virus			> 6.0		> 3	
Bovine/porcine parvovirus	> 3.0	> 5.3	> 6.0	> 4.9	1	> 7.0
Reovirus						> 6.7
Porcine circovirus						1.5
Pseudorabies virus		> 7.0			1	
EMC				> 5.0		
PRRSV	> 4.8					
Feline leukaemia virus					> 3	
Minute virus of mice					2	
Canine adenovirus						> 6.6
SV40				**3.3**		

Nims R *et al* 2011; Plavsic Z *et al* 1998, 2001

BioReliance® by SAFC

Virus inactivation by UV-C

Virus	mJ/cm^2	LRF	Fluid
Feline calicivirus	30	4	Protein solution
Poliovirus	21.5 – 40	4	Waste water
Coxsackievirus	36	4	Serum free media
Hepatitis A	30	4	Factor VIII
Echovirus	33	4	Serum free media
SV40	74	4	Alpha 1 proteinase soln
Reovirus	74	4	Alpha 1 proteinase soln
EMC	75 – 100	6.5/4.7	Factor VIII soln; plasma
VSV	100	4.8	Plasma
Porcine parvovirus	60 – 100	4.8/5/4	Serum, plasma
Bovine parvovirus	100	8	10% serum
Canine parvovirus	50	4.5	10% plasma
MVM	15 - 100	> 6.11 – 6.57	Serum free media*
FMDV	100	8	10% serum
IBR	100	6	10% serum
Adenovirus type 2	100-160	4	Serum free media; water

*Wang et al , 2004; *Weaver & Rosenthal, 2010*

Heat Inactivation of Viruses

Virus	56°C/30 min (serum)	56-60°C/0.25-18h (serum)	HTST (serum) 85°C/0.5 sec	HTST (media) 102°C/10 sec
PPV	< 1	-	< 1.5	3
MVM	-	-	-	> 5.2
BVDV	4.8	6	5	-
IBR	7.2	6	5	-
Reovirus-3	5.5	4.7	5	-
Parainfluenza-3	5.5	-	5	-
Enterovirus	-	5.3	-	-
CAV	6.5	-	-	-
Polio	-	4.7	-	-
CMV	-	5.2	-	-
X-MuLV	-	3.9	-	-

Plavsic, Z (2000); Grun, J et al (1992); Weaver & Rosenthal, (2010)

BioReliance® by SAFC

New Methods for Detecting Contaminants

BioReliance® by SAFC

Limitations of Assays for Adventitious Viruses

- Many different assays are required to detect the large number of different viruses
- Many assays take weeks to complete
- Assays are limited by sensitivity and by specificity
- Infectivity assays not available for all viruses
- PCR assays limited by specificity of primers and probes
- Degenerate/virus family PCR assay design is limited by knowledge of possible contaminant
  - PCRs for human polyoma KI and WU do not detect human Merckle cell polyoma virus

Issues with Detection Assays

- You only find what you are looking for….
  - Screening assays are designed to assess known/past pathogens
    - Source animal surveillance and testing of animal materials by suppliers is limited
    - Screening for specific current/past pathogens
      - Requires assumptions about the type and strain of infectious agent, limiting detection to a small number of known pathogens
      - Ignores emerging, novel viruses
    - Cell line panel used in infectivity assays may not be permissive for novel virus
- You need to routinely update for detection of prevalent infections or use broad based screening methods
  - Vesivirus identified in 2003 as bovine contaminant but found to be widespread in cattle in USA in 2006
  - New bovine viruses identified by MP-Seq.
- *Adapted from K Brorson, US FDA*

BioReliance® by SAFC

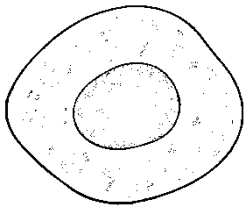
Issues with Detection Assays (2)

- You may not find what is truly there…
  - Sensitivity of assay limits detection
    - All assays have a LOD
    - Sample volume limitations
    - Cell lines may not be permissive for some known or novel viruses
  - Interference/ matrix effects
    - Anti-virus antibodies in FCS used in *in vitro* assays
    - Cytotoxicity of indicator cells
    - Inhibition of PCR assay enzymes

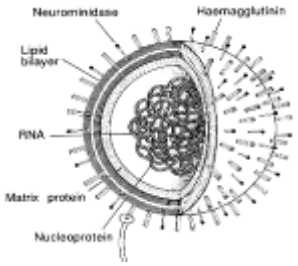
- *Adapted from K Brorson, US FDA*

Technology Options

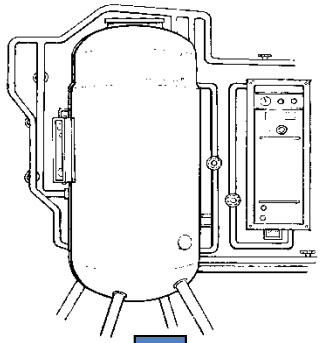
Cell Bank



Virus Seed



Bulk Product



- Greater breadth of detection
- Reduced assay time

- **New technologies:**
  - Massively parallel (deep) sequencing
  - Virochip DNA Array
  - Degenerate PCR: detection by mass spec ID plex or by sequencing

BioReliance® by SAFC

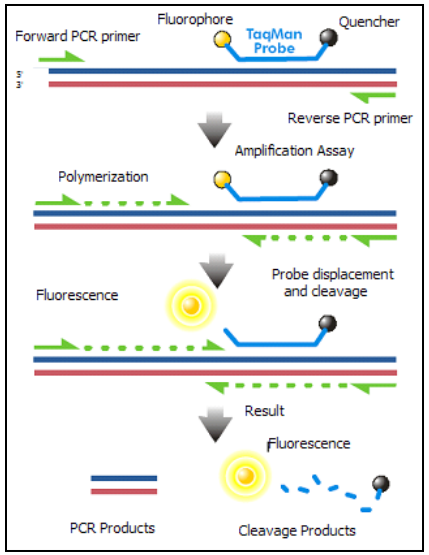
Massively Parallel Sequencing

- Massively Parallel (deep) sequencing is a new method to identify all adventitious viruses, mycoplasma and bacteria and to characterise the state of the transcriptome. At BioReliance we have applied this to:
  - Fermenter contaminations
  - Characterising new vaccine cell substrates
  - Characterising raw materials (plant peptones and fish protein used in media)
  - Characterising virus seeds

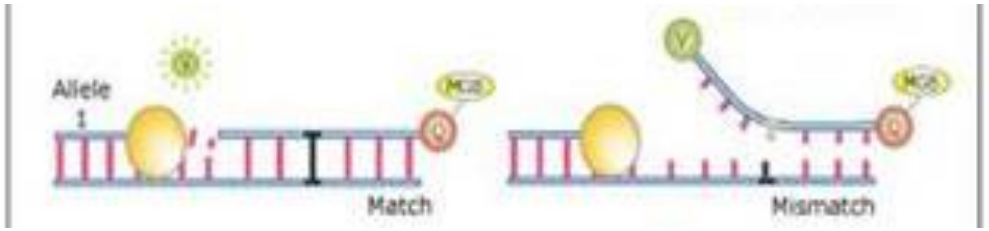
BioReliance® by SAFC

Why not other NAT methods ?

QPCR: You must “know” what you are looking for…



ALIGNMENTS			
Forward		GAAAGACCCCACCATmAGGCT	
Probe		AGCAAGCTAGCTGCAG	
Reverse		AACGCCATTTTGCAAGGC	
Query	1	GAAAGACCCCACCATAAGGCTTAGCAAGCTAGCTGCAGTAACGCCATTTTGCAAGGC	57
Strain 1	7727	.....C.....	7783
Strain 1	7738	.....	7794
Strain 1	7755	.....	7811



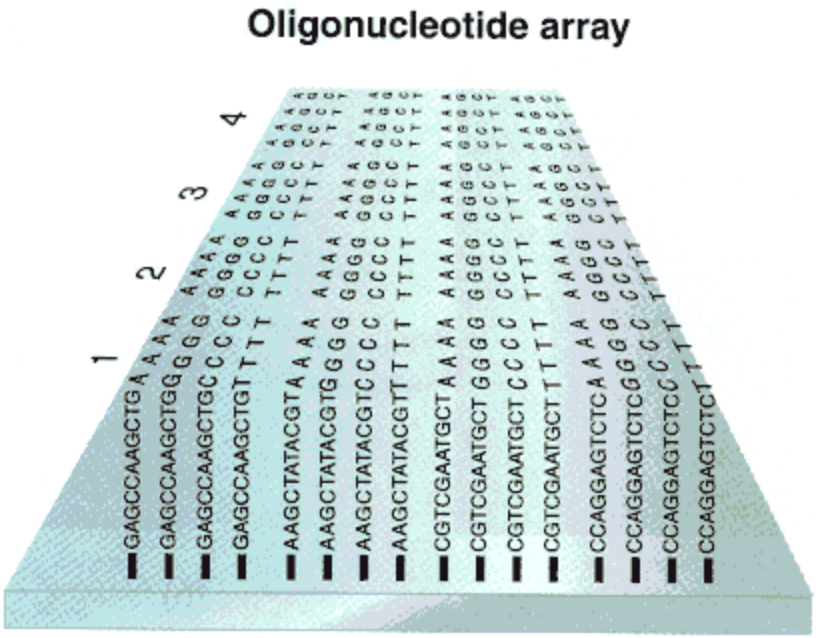
QPCR:
A single unanticipated base change can reduce sensitivity to zero

BioReliance® by SAFC

Why not other NAT methods ?

Oligonucleotide capture arrays:

- Same deal - you must “know” what you are looking for…



BioReliance® by SAFC

Capillary vs. MPS

ABI 3730xl DNA Analyzer

- 96-capillaries simultaneously
- Multiple runs per day
- 3840 samples per day
- 2 Mbases per day

Source: Cloned/PCRed DNA

Roche/454 GS FLX Titanium

- 1 Million wells simultaneously
- 2 – 16 samples per day
- Multiplexing available
- 200-500 Mbases per day

Source: Uncloned nucleic acid

BioReliance® by SAFC

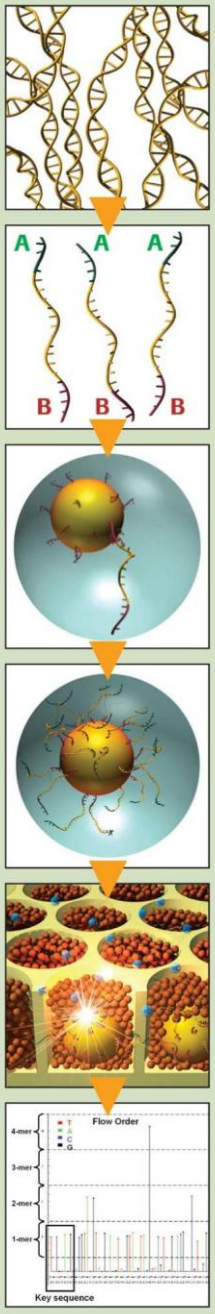
How Does Roche/454 MPS Work?

There are three basic steps:

1) Construction of the library

2) Sequencing on the 454

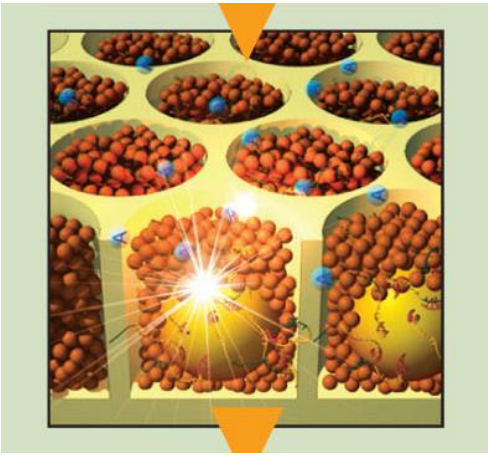
3) Bioinformatics



BioReliance® by SAFC

Roche/454 is “Pyro-Sequencing”

Load the Plate
Run the GS-FLX O/N



Fundamentally, this is “sequencing by synthesis” (enzymatic primer extension).
Two hundred cycles of (T,A,C,G) are flowed over the plate.
When an incorporation is made, pyrophosphate is released.
The pyrophosphate is used to form ATP, which in turn fuels light emission by luciferin.
A CCD camera captures the emission.

BioReliance® by SAFC

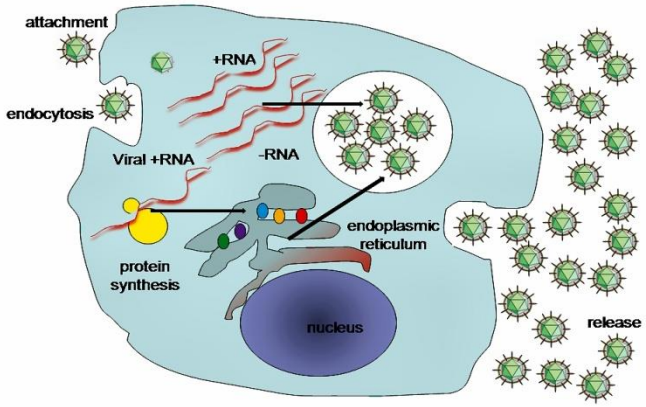
Catching a Virus in a Cell Substrate: Two Parallel Approaches

GENOME or *TRANSCRIPTOME* MP-Seq

CELL- FREE AMPLICON MP-Seq

SUBSTRATE
Total Cellular DNA
Total Cellular RNA

SUBSTRATE and PROCESS
Cell Supernatant
Treat with Nuclease
Concentrate
Extract Capsids
Amplify



High Complexity
Low S/N

Low Complexity
High S/N

DETECTS
Prophage (low S/N)
Fulminant Lytic Infections
Latent Viral Transcripts

DETECTS
All Productive Infections

BioReliance® by SAFC

There is one needle in each image

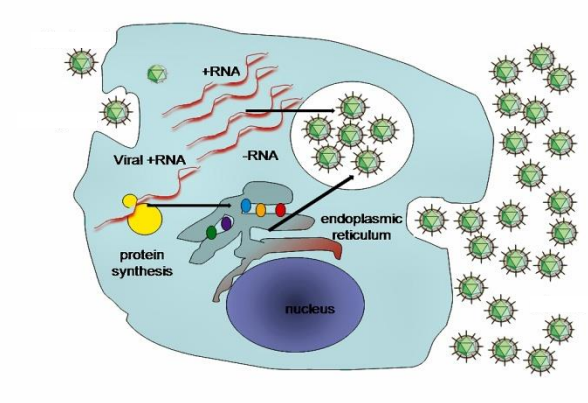
- If you sample a million items
  - You get a million straws

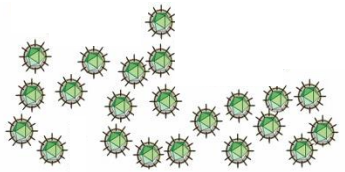
- Remove most of the straw…

- Limit the number of needles…

Use the million reads to the best advantage

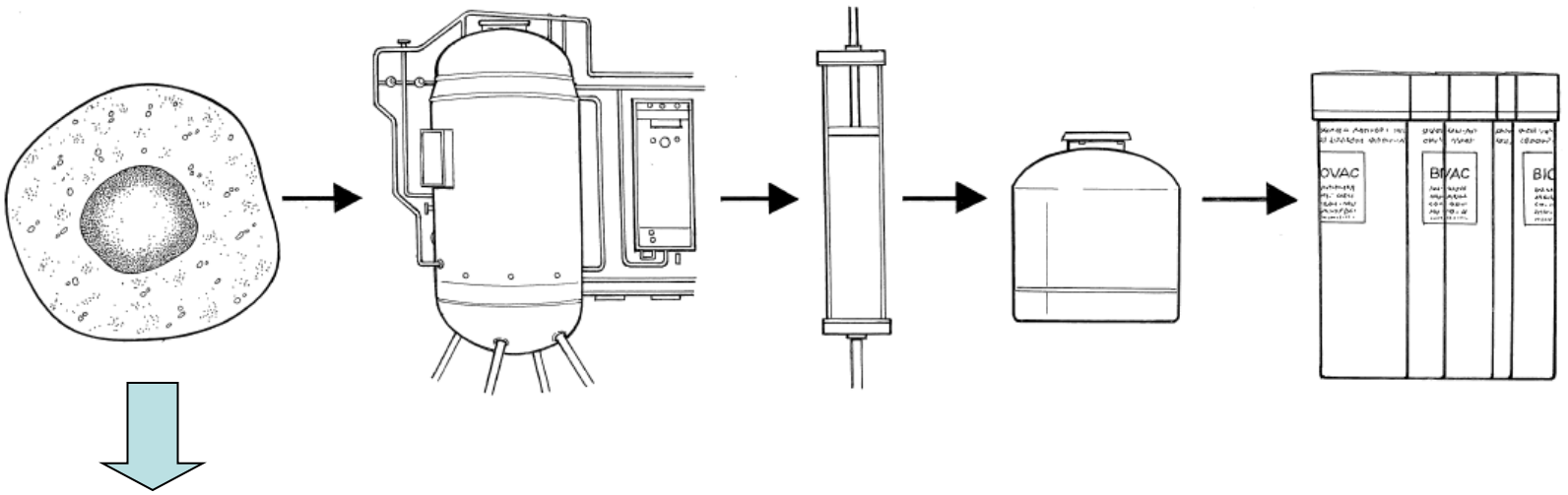
Gain sensitivity by reducing complexity





BioReliance® by SAFC

Transcriptome Analysis Case Studies



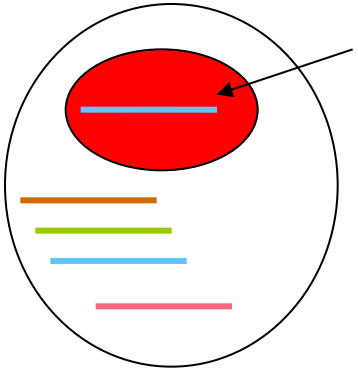
- CHO
- MRC 5
- Vero
- Hi 5
- Sf9

Transcriptome Analysis Is Used to Verify The Safety of Cell banks

BioReliance® by SAFC

Case Study : Screening MRC-5 Cells

Deep Sequencing



Integrated virus sequence

Sequence

Apply Algorithm

Viral sequence

400 million bp of sequence

Reliant Algorithm

E-value 10^{-10} or less

Database: ViralDB was curated from GenBank nucleotide collection. Contains ~1x 10^6 sequences

Remove Ribosomal RNAs & ERVs

Remove Non-viral Sequences

Hits

BioReliance® by SAFC

Algorithm to Filter Hits

E-value 10^{-10} or less

↓

Database: ViralDB was curated from GenBank nucleotide collection. Contains 301,698 sequences

↓

Remove ERVs

↓

Remove Repeats

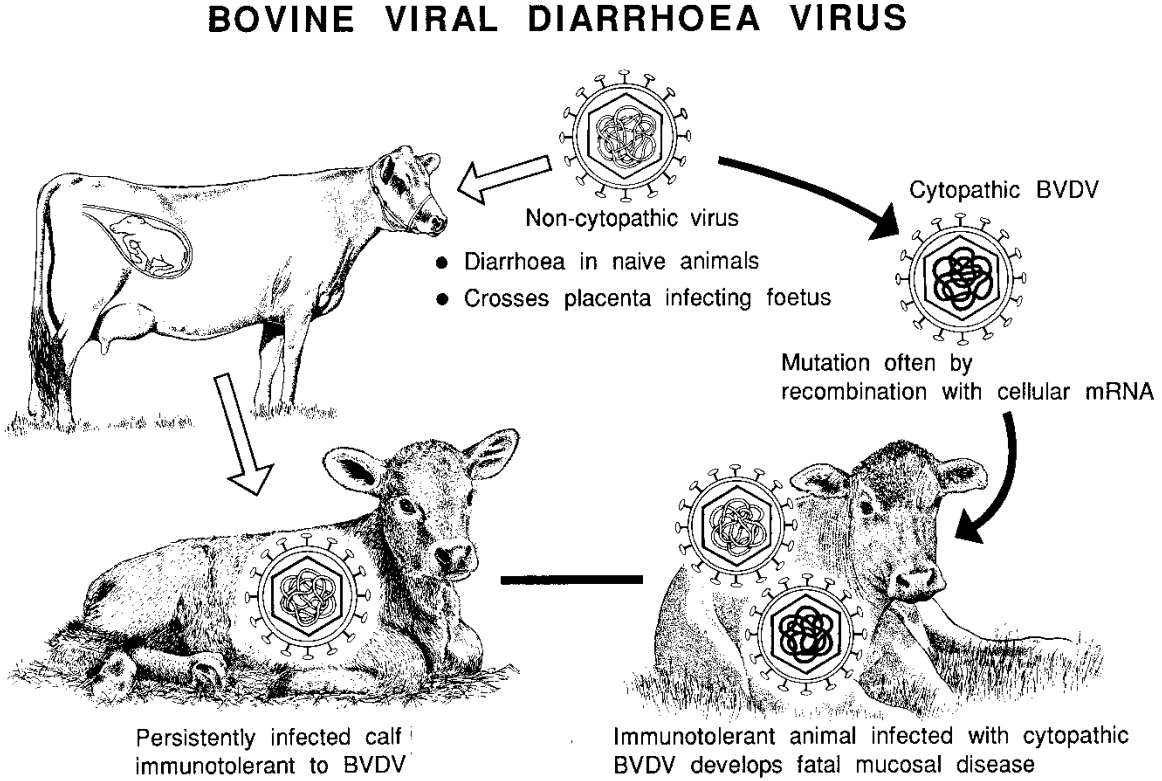
↓

Remove Non-viral Sequences

↓

Hits

False hits: de novo recombination between virus and cellular sequences. e. g. retroviruses and pestiviruses



Algorithm to Filter Hits

E-value 10^{-10} or less

↓

Database: ViralDB was curated from GenBank nucleotide collection. Contains 301,698 sequences

↓

Remove ERVs

↓

Remove Repeats

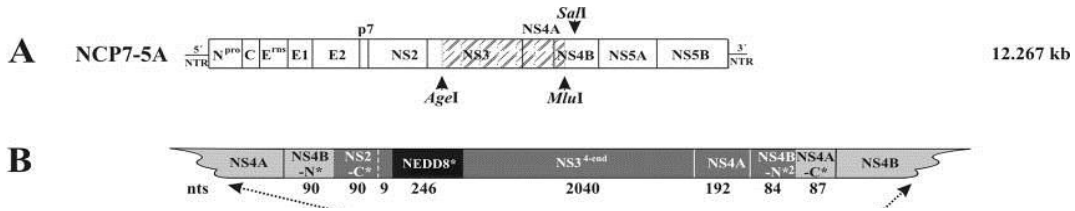
↓

Remove Non-viral Sequences

↓

Hits

False hits: De novo recombination between virus and cellular sequences e.g. retroviruses and pestiviruses

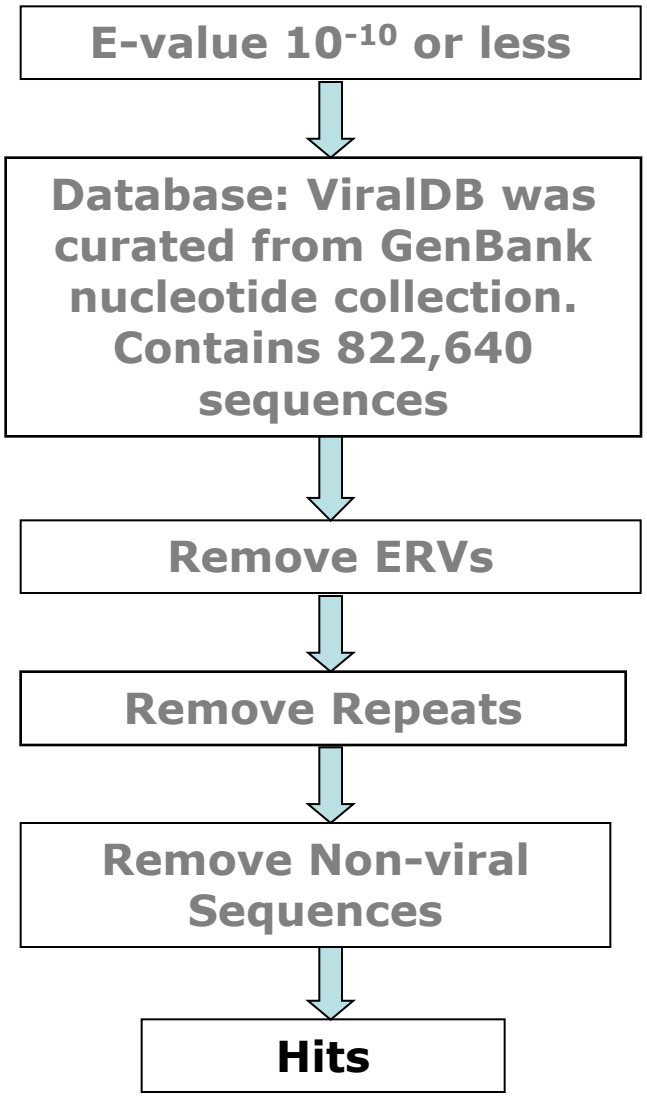


(A) Non-cytopathic BVDV

(B) Cytopathic BVDV has a large duplication of viral sequences together with an insertion encoding part of cellular Nedd8

From Gallei et al. Journal of Virology, November 2005, p. 14261-14270, Vol. 79

MPS Case Study:MRC-5



...

- 195 Million bp
- 746,844 reads
- 5,139 reads BLAST to ViralDB, of these
  - 1691 are HERVs
  - 2326 are false hits to BVDV (ubiquitin or related)
  - Remainder repeats and virokines/viroreceptors
- Control breadth of coverage
  - B2M, GAPD, CGI-119, L37a, CALM2, S11, S13, OAZ1
- .Control sensitivity
  - TUBB ~400c/cell HIT
  - CTBP1 ~300c/cell HIT
  - GOLGA1 ~100c/cell HIT

BioReliance by SAFC

Warning… Statistics ahead

Innovation

Lies, damned lies, and the foundation of modern Science

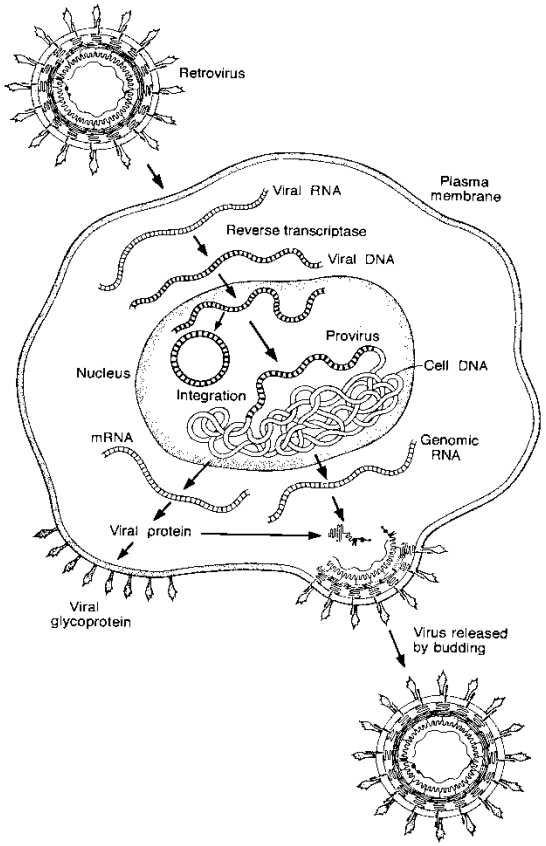
- Imagine mRNA isolated from a population of cells
- Assume messages are roughly the same size
- Approximately 200,000 messages per cell

Unique mRNA to total mRNA ratio	Probability of obtaining at least one AA read (1 PTP)	Probability of obtaining at least one AA read (2 PTP)
1 in 1,000,000	63.21%	86.47%
1 in 800,000	71.35%	91.79%
1 in 600,000	81.11%	96.43%
1 in 500,000	86.47%	98.17%
1 in 400,000	91.79%	99.33%
1 in 300,000	96.43%	99.87%
1 in 200,000	99.33%	100.00%
1 in 100,000	100.00%	100.00%

Very effective detection of single copy transcripts.

Case study : Vero Cell Analysis

- Vero cells analysed before and after treatment with inducing agents for retroviruses (IDU)
- Transcriptome analysis undertaken
- Porcine Circovirus was not detected (and confirmed by specific PCR)

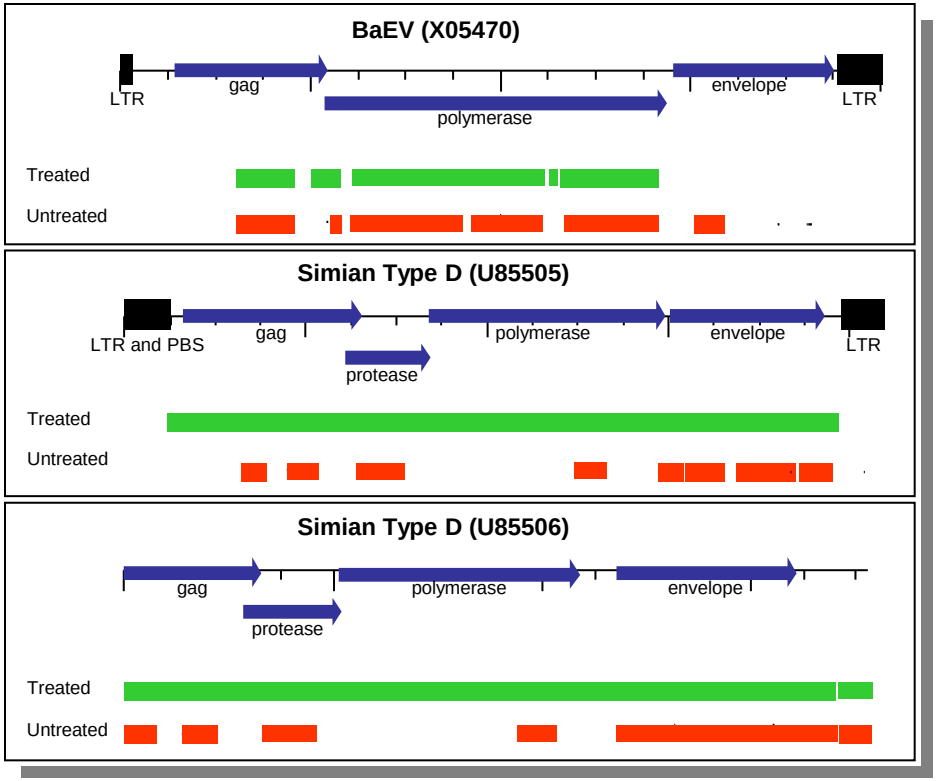


BioReliance® by SAFC

Study 5: Vero expressed retroviruses

Cell Line Characterization

- MP-Seq™ analysis before and after treatment with inducing agents for retroviruses (IDU)
  - Vero cells express betaretrovirus genome(s) that are potentially functional*
- Expression of the full length genome requires induction
  - This virus might pseudotype live **attenuated** flavivirus vaccines produced in Vero cells

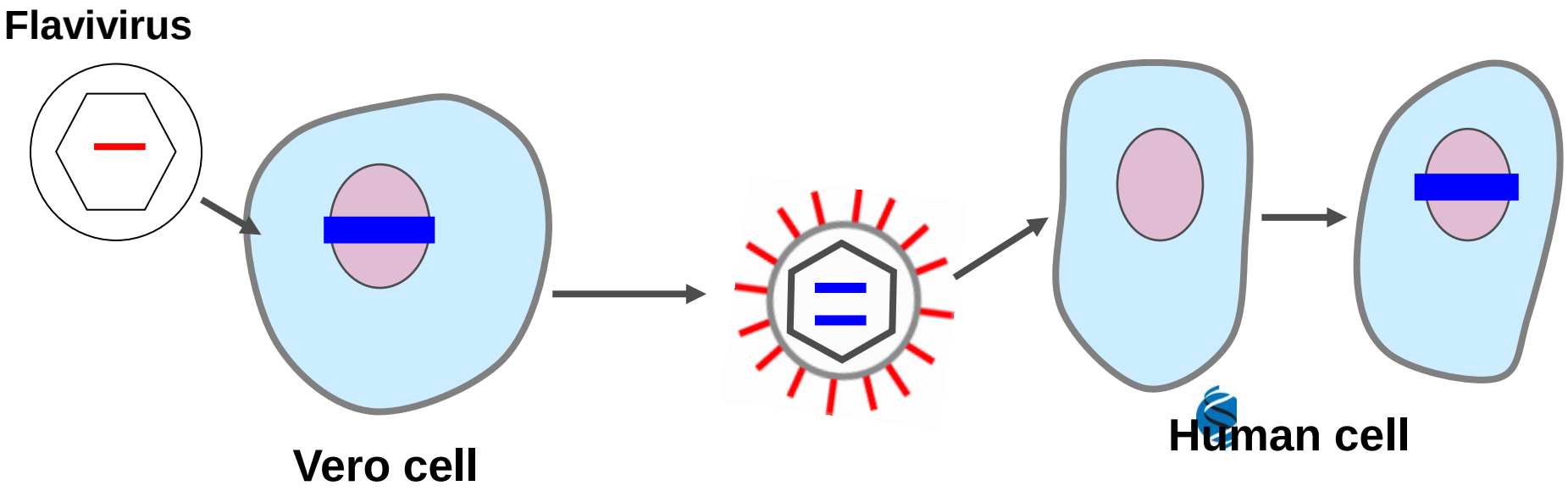


***Onions** et al. Vaccine. 2011 Sep 22;29(41):7117-21*

***Ma** et al. Journal of Virology, July 2011,p. 6579-6588, Vol. 85, No. 13*

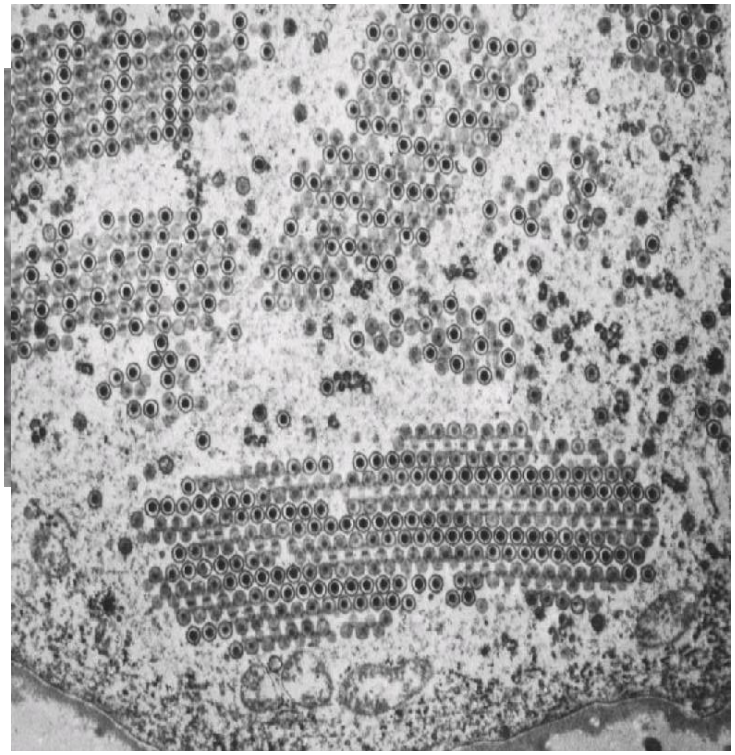
Vero cell betaretrovirus

- Vero cells express betaretrovirus genome that appears to be capable of expressing a functional virus.
- Expression of the full length genome requires induction.
- This virus might be of importance if live attenuated flaviviruses vaccines are produced in Vero cells because of pseudotyping.



Case study 6: Nodavirus contamination of Hi 5 cells

- Virus like particles found in Hi 5 cells
- No cytopathology on the Hi 5 cells
- Subsequently demonstrated to be a nodavirus by PCR
- Nodaviruses not detected in mammals but:
  - paracitio RNA virus can replicate in BHK cells and
  - Nodamura virus is lethal by ic inoculation in mice



Micrographs by George Reid BioReliance

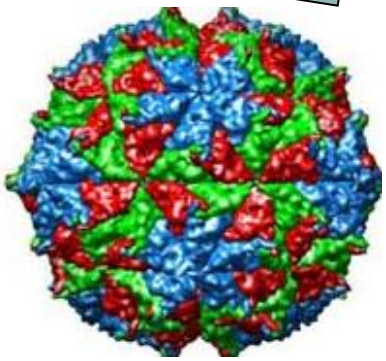
BioReliance® by SAFC

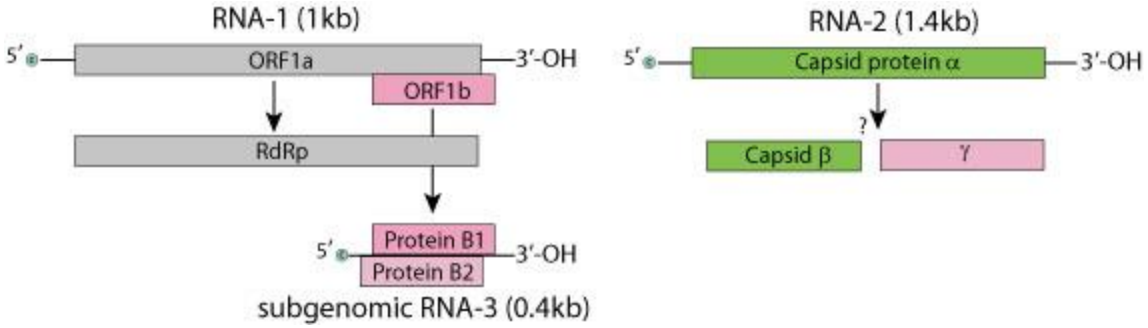
Study 6: High Five™ MP-Seq™

Cell Line Characterization

The Hive Five™ Nodavirus was fully characterized by MP-Seq™

- 1009 hits against nodaviruses
- Complete bipartite genome detected in Hi 5 cells
- Complete viral genome sequence assembled
- Expressed in a subpopulation of cells (1 in 100 cells?)





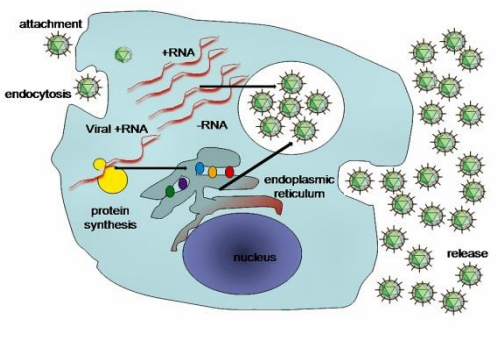
Virus detection from a sub-population of productive cells.

MP-Seq – Case Studies

Raw Material Qualification

Catching a virus in a cell free matrix

Raw Material Qualification



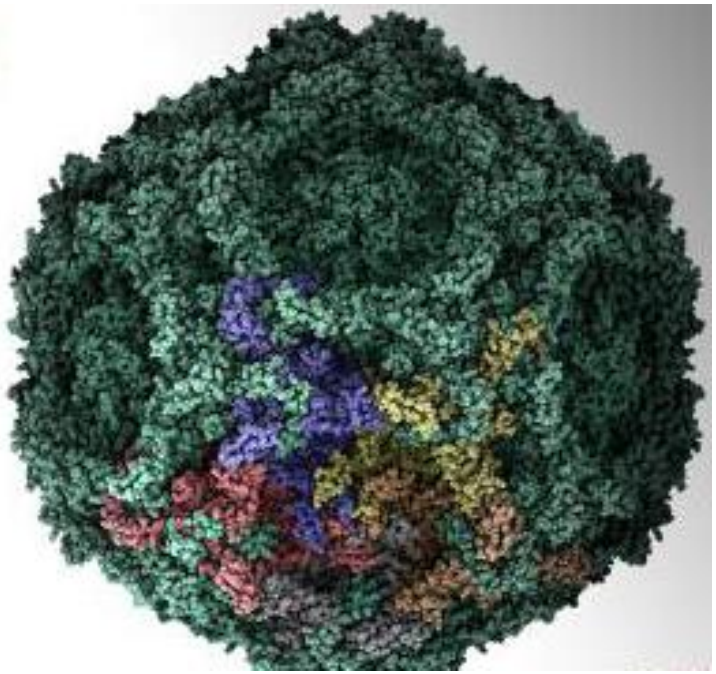
Cell-free Amplicon MP-Seq™

- Substrate
  - Cell-free fluids
- Process
  - Treat with nuclease
  - Concentrate
  - Extract capsid nucleic acids
  - Amplify
- Characteristics
  - Low complexity
  - High S/N
- Detects
  - All productive infections
  - Herd contaminants

Sensitivity: <1000 copies per sample

Case example : New parvovirus contaminant in bovine serum

- We have identified a new parvovirus in bovine serum (BAAV-2) using massively parallel sequencing*
- It is able to infect human cells and cells of other species
- It can establish latent infections. Therefore cells that have been exposed to serum in the past need to be screened.
- It is a dependovirus (AAV) and is likely to be mobilised by adenoviruses and herpesviruses



Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Massively parallel sequencing, a new method for detecting adventitious agents

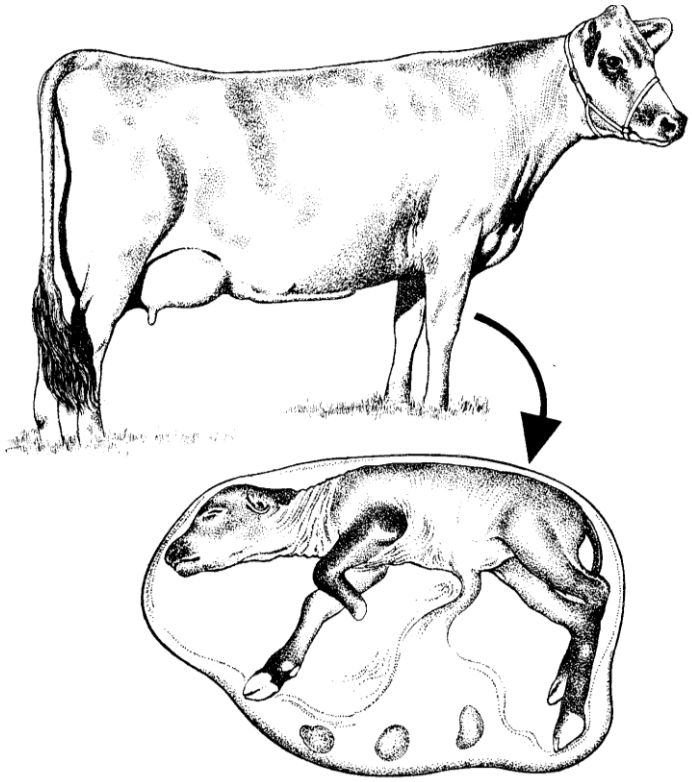
David Onions [a,*], John Kolman [b]

[a] BioReliance, Todd Campus, West of Scotland Science Park, Maryhill Road, Glasgow G20 0XA, UK
[b] BioReliance, 14920 Broschart Road, Rockville, MD 20850-3349, USA

BioReliance® by SAFC

BioReliance survey: commercial serum

Raw Material Qualification

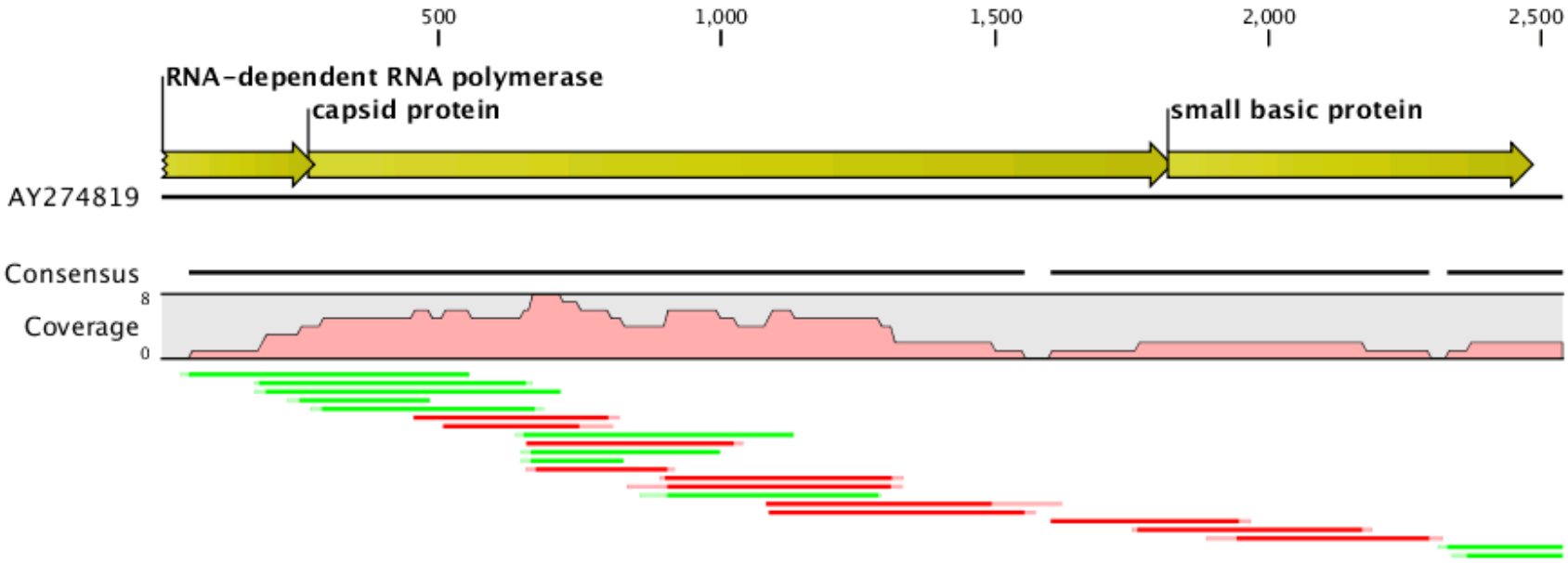


BioReliance MP-Seq Data
(number positive number tested)

Serum	BPV-2	BPV-3	BAAV-2	Fungus
Calf	4/4	2/4	0/4	4/4
FBS	2/6	2/6	1/6	6/6

Bovine Norwalk virus

Raw Material Qualification

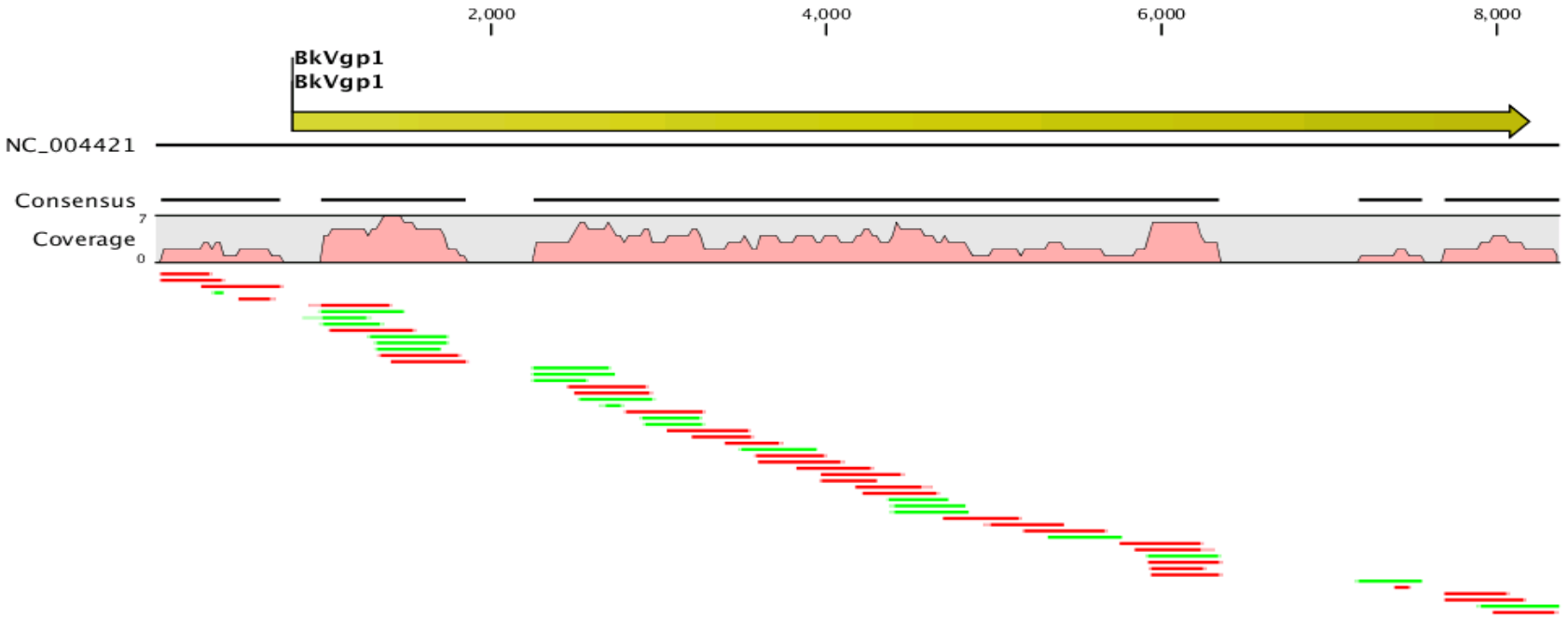


- Bovine Norwalk-like virus (*Norovirus*) detected in 50% of newborn calf sera by MP-Seq™
- Recent serological data indicated bovine strains are transmitted to humans

(***Widdowson*** *et al. J Med Virol. 2005 May;76(1):119-28)*

Bovine Kobuvirus

Raw Material Qualification



- Bovine kobuvirus, a new genotype of Picornavirus, detected in 50% of newborn calf sera by MP-Seq™
- Virus first reported as a contaminant of HeLa cells in 2003

(***Yamishita*** *et al. J Gen Virol. 2003 Nov;84(Pt 11):3069-77*)

Cells exposed to serum should be screened for unknown adventitious agents.

It doesn't affect me, I have an AOF process…

Raw Material Qualification

This is where plant peptones come from

Parvoviruses are shed in feces and are amongst the most resistant viruses in the environment!

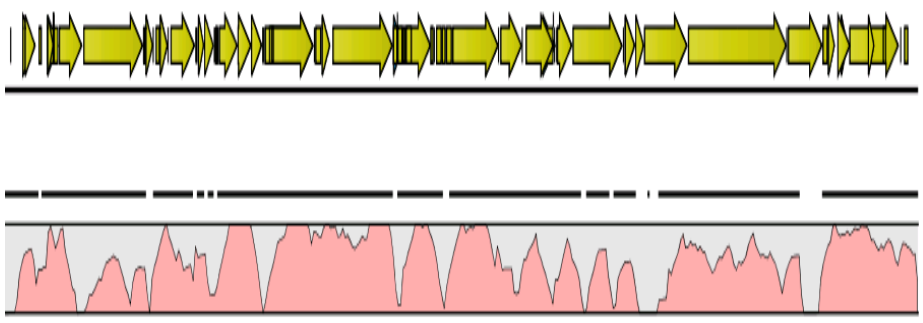
Going forward, should all MCBs be tested by MP-Seq™ for unknown agents?

MP-Seq – Case Studies

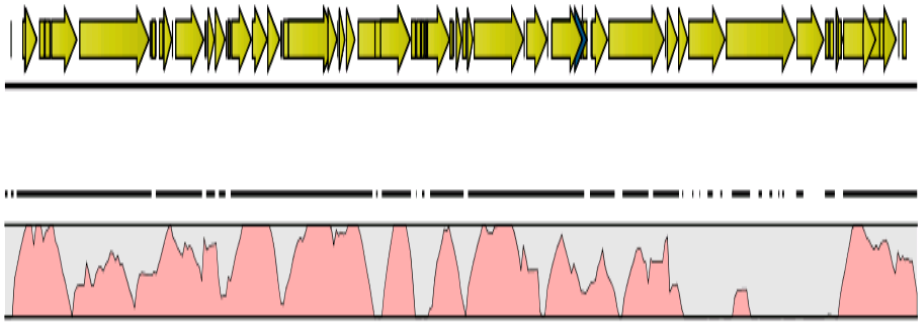
Case Study : *E. coli* Fire

- Catastrophic failure in a fermenter four hours after initiation
- A large proportion of *E. coli* noted to be non-viable
- BioReliance fermenter crash program initiated, including…
  - MP-Seq™ of supernatant / EM / Assays for lytic phage
- MP-Seq™ generated >680,000 reads; average length >400bp
- Identification: novel bacteriophage T7 / T3-like; 80% homology

EU547803; Salmonella phage phiSG-JL2, complete genome; NC_010807



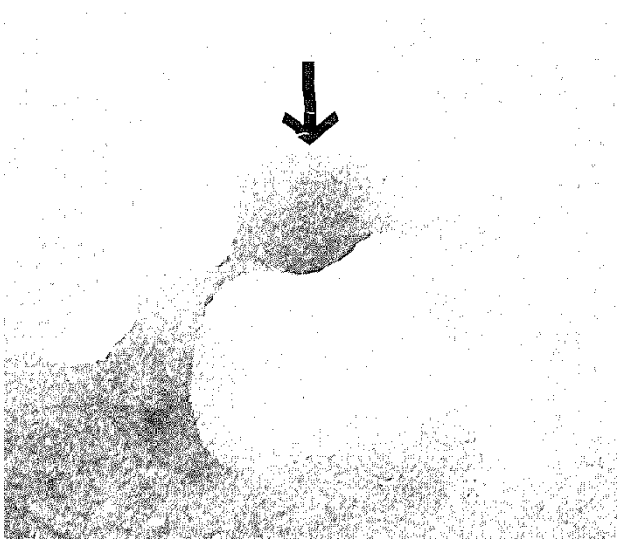
AJ18471; Bacteriophage T3, complete genome; NC_003298

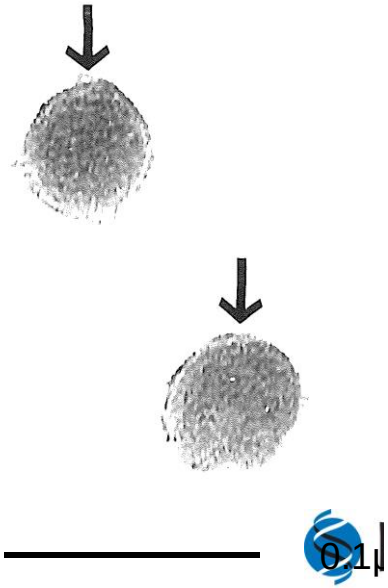


"Fire" agents are never "known" to GenBank. Typically, they are ~80% identical.

Case Study : *E. coli* Fire

  - Follow up actions
- Potential specific PCRs to identify root cause of infection
- Clean down with monitoring of phage sequences by PCR
  - Conclusion
- A new member of the T7 phage family was the cause of the crash
- Phage presence confirmed by EM.





Notes from Three+ Years of Case Studies

- Raw material surveillance
  - Serum is a virus zoo
  - Logical mitigation process
  - MPS AAT for all MCBs?
- Cell Line Characterization
  - Detection of the “usual suspects”
- “Fires” have provided a remarkable observation:
  - New organisms were typically about 80% identical to known organisms
  - These would be undetectable by other NATs and demonstrates the power of MP-Seq™

It is now clear that unknown agents exist that are out of reach of PCRs and arrays.

GMP MP-Seq™ at BioReliance

A validation *tour de force*

- Assay validation
  - 17 modules
- Assay system validation
  - Four start-to-finish strain tests
  - Controls and sensitivity modeling
- Equipment validation
  - Calibration, IOQ, PQ, annual PM
- Computer systems
  - CSV
  - 21 CFR 11
- Authored software validation
  - Software Development Life Cycle SOPs
  - Automated, in-process testing in support of standard QA review

BioReliance® by SAFC

MP-Seq™ assay validation: outline of modules

Extraction	Quantitation	Library construction	MPS

Spike Controls

DNA MPS
Genome ID / Purity
Genetic Stability

Yeast
Virus
Phage
Bacteria
Mammals

DNA
Picogreen®
UV (purity)

Genetic Stability
Custom Lib Prep

Library Prep

RNA MPS
Transcriptome
Genome ID / Purity
AAT

Virus
Mammals

RNA
Quant-iT™
UV (purity)

optional

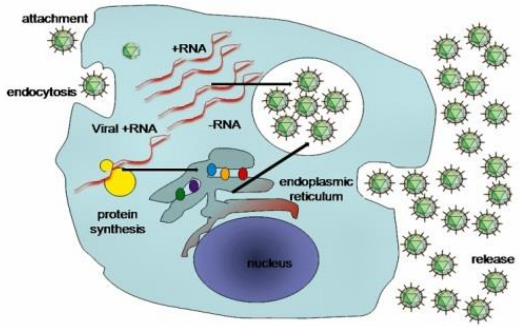
polyA+
Capture

Cell-free MPS
AAT

RNA+DNA

Cell-free MPS
Custom Lib Prep

Library quantitation
↓
emPCR
↓
MPS
↓
BI Algorithms
AAT
Genetic Stability



State of Validation
Development
Pre-Validation
Val in Progress
Val Completed

World Health Organization

WHO Cell Substrates Guidance: MP-Seq

- Recommendations for the Evaluation of Animal Cell Cultures as Substrates for the Manufacture of Biological Medicinal Products and for the Characterization of Cell Banks. Final, 2011
- Update to Technical Report Series 878

- New methods of detecting viruses
  - Transcriptome analysis of cell lines by multiple parallel sequencing (MPS) or degenerate PCR on cell supernatants and MPS
  - “It is probable that application of methods of this type will be expected or required by regulatory agencies in future.”

BioReliance® by SAFC

MP-Seq and US FDA

- Data using MP-Seq for cell line characterisation and contaminant detection has been submitted to FDA
- FDA CBER Vaccines and Related Biological Products Advisory Committee (VRBPAC) Meeting 19th September 2012 on 'Cell lines derived from human tumours for vaccine manufacture
  - "In terms of looking for adventitious agents, it seems to me that the technology (*MP-Seq)* has evolved so that, irrespective of whether the substrate is from a tumour line or some other cell line, you want to use the state-of-the-art technology in order to rule out the presence of adventitious agents." *D Lowy, Director NCI.*
  - "It seems that in the potential use of new technologies (*MP-Seq*), even though there are challenges to the use of new technologies, they have to be embraced and we have to continue to try to learn from them and struggle through that learning curve." *P McInnes, NIH.*

Challenges Resulting from New Detection Technology

- Technologies such as MP-Seq provide ability to detect all contaminants
- Methodology detects viral genomes not infectious virus
- Infectivity assays not currently available for all new viruses detected by these techniques
- What are the consequences of detecting new viruses in raw materials and cell lines used for production?
- How should these new technologies be used?
- As a regulated industry how do we respond to the results generated by this new technology?

WHO Draft Guideline on Assessing a Potential Contaminant Risk

- WHO Guideline on Regulatory Risk Evaluation on Finding an Adventitious Agent in a Marketed Vaccine. Draft, 19th Sept 2013
  - New detection methods such as massively parallel sequencing (MP-Seq) might uncover new adventitious agents in already licensed products (e.g. porcine circovirus in rotavirus vaccines)
  - Document provides an overview of the principles of the scientific assessment of the risk of finding a potential extraneous agent
  - Main areas which should be considered
    - How was the signal found?
    - Where was the signal detected? – The risk associated with the product
    - What exactly was detected? – The risk associated with the agent

Assessing a Potential Contaminant Risk (2)

- How was adventitious agent found?
  - Sensitivity, specificity and validity of the assay.

- Risk associated with the product (Where was it found?)
  - Type of medicinal product
  - Found in starting material, intermediate, final product?
    - How was the agent introduced? What are the results of the root-cause investigation?
  - Is it possible to remove the agent during product purification?
  - Is it possible to inactivate the agent?
  - What is the impact of the route of administration of the product?
  - Environmental risk?

Assessing a Potential Contaminant Risk (3)

- Risk associated with the agent (What exactly was found?)
  - Is the agent a known agent, a member of a known family or a novel agent?
  - Are the nucleic acids that were found free or particle associated?
  - Are the nucleic acids that were found fragments of full-length intact genomes?
  - If associated with particles are the particles infectious?
  - Are the particles infectious for human cells?
  - Is the agent known to be infectious for humans?
  - Does the infectious agent cause disease in humans?
  - Is the agent transmissible from human to human, animal to animal or human to animal?

Risk Mitigation for Viruses in Biotech Products – Emergency Response Plan

- Clear procedures defining process if a contaminant incident occurs
  - Quarantine and labelling of all production materials used in affected process
  - Procedures for clear communication and management of contamination incident
  - Process for identification of contaminant
    - Use MP-Seq
    - Evaluate sequence identification in relation to biological infectivity
  - Procedures to be completed prior to a return to production
    - Are routine cleaning processes adequate?

Questions?

BioReliance® by SAFC

Contact Details

Dr Martin Wisher
Senior Director, Global Head of Regulatory Affairs
martin.wisher@bioreliance.com

BioReliance Ltd
Innovation Park
Hillfoots Road
Stirling, FK9 4NF, UK
www.bioreliance.com

Acknowledgements: John Kolman, David Onions

BioReliance® by SAFC