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

CELL BANK AND VIRUS SEED ADVENTITIOUS AGENT DETECTION AND MANAGEMENT FOR VACCINE PRODUCTION

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WHEN YOU NEED TO BE SURE

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Medicines and Healthcare Products Regulatory Agency (MHRA) accredited contract testing company providing GMP and /or GLP drug development services to the global biotechnology industry.

Complies with the current requirements of the international regulatory authorities.

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

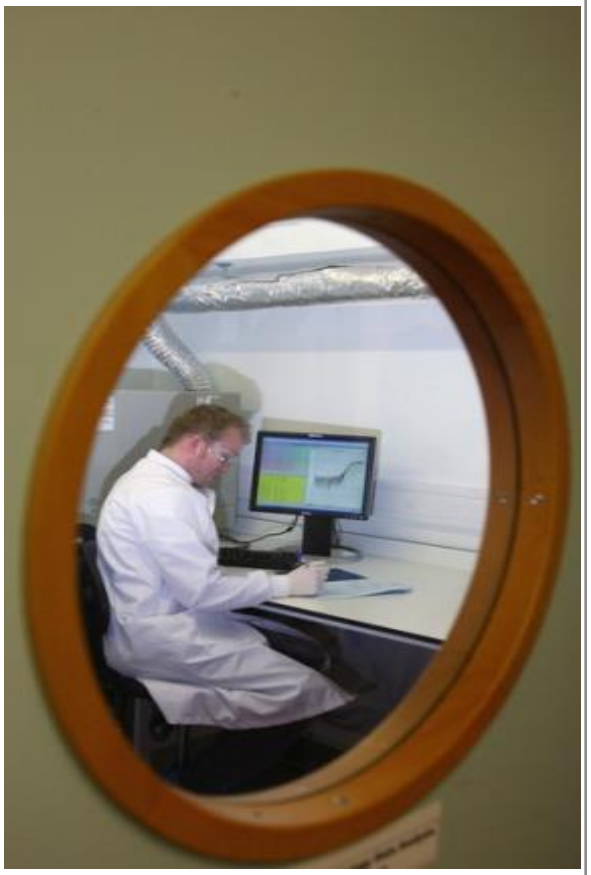
VITROLOGY EXPERIENCE

GLP/cGMP Testing of

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

Supporting manufacture of biologicals

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



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OUTLINE

- SGS and SGS Vitrology – Company overview
- Regulatory Guidance Documents
- Process for a vaccine product & associated testing recommended
- Example of GMP testing services for Vaccine Development process in Vero cells
- Retrovirus Detection
- Assay Validation
- Steps to be considered in event of positive result

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REGULATORY GUIDELINES – ICH

- Tripartite Agreement
  - USA, EU and JP

- **Q5A.** Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin.

- **Q5B.** Analysis of the Expression Construct in Cells used for the Production of rDNA Derived Protein Products.

- **Q5D**. Derivation and Characterisation of Cell Substrates used for Production of Biotechnological / Biological Products.

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REGULATORY GUIDELINES – EU

- European Medicines Agency
  - Production and Quality Control of Monoclonal Antibodies (July 1995)
  - Production and Quality Control of Medicinal Products Derived by Recombinant DNA Technology (December 1994)
  - Guideline on Virus Safety Evaluation of Biotechnological IMP (2008)
  - Guideline on Bovine Serum used to Manufacture Human Biologics (2003)

- European Directorate for the Quality of Medicines & HealthCare

  European Pharmacopoeia (Ph. Eur.)
  - European Pharmacopeia 2.6.16. Tests for extraneous agents in viral vaccines for human use
  - European Pharmacopeia 5.14. Gene transfer medicinal products for human use
  - European Pharmacopeia 5.2.3. Cell substrates for the production of vaccines for human use

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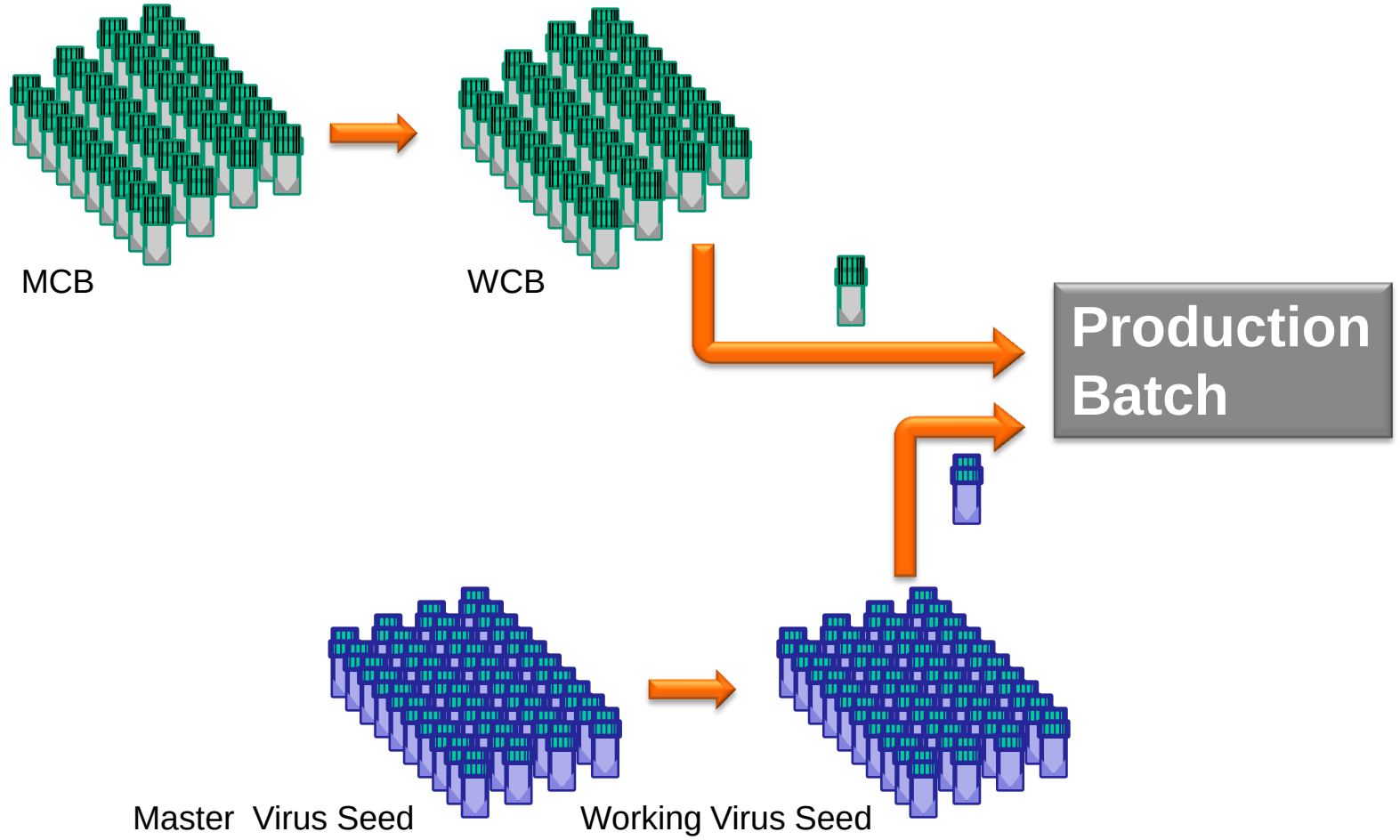
REGULATORY GUIDELINES – USA

- Federal Drug Administration (FDA) and United States Pharmacopoeia (USP)
  - PTC in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (February 1997)
  - PTC in the Characterisation of Cell Lines used to Produce Biologicals (1993)
  - Guidance for human somatic cell therapy and gene therapy (1998, 2008)
  - Guidance for the Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases (2010)
  - **Code of Federal Regulations (CFR)**
    - 9 Part 113 (cell lines and extraneous virus testing)

ENSURING QUALITY AND SAFETY OF BIOLOGIC OR VACCINE PRODUCTS

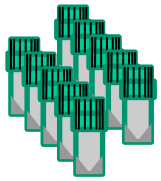
- By Controlling Upstream Manufacturing Process (GMP)
- By Testing Starting & Raw Material (GMP)
  - Cell banks, vaccine seeds, bovine serum, medium components etc.
- By Testing In-process Material and Final Product (GMP)
  - Bulk harvest, purified bulk and final product
- (By Demonstrating Capacity of Manufacturing Process for Removal or Inactivation of potential virus contamination (GLP)
  - Phase I/II and later phase III Viral clearance studies)

VITROLOGY CELL BASED VACCINES PRODUCTION



VITROLOGY PRODUCTION BATCH

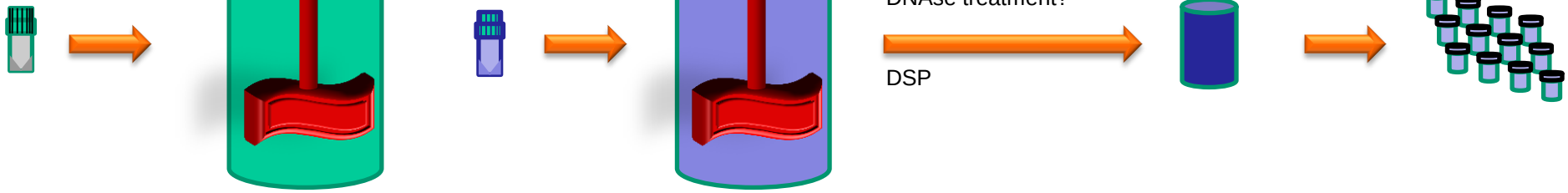
Non-infected - End Of Production Cell Bank (EOPC)



One time only

EUROPEAN EP - Non-infected Control Cells

Europe Each batch



WCB

Bioreactor - Expanded cells

Working Virus Seed

Bioreactor - Bulk Virus Harvest

Bulk Purified Vaccine Harvest

Clinical Batch

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CONSIDERATIONS FOR COMPILING BIOSAFETY STRATEGY

- Countries for clinical trial(s)
- Countries for potential marketing approval
- Intended patient population
- Possible patient populations
  - E.g. skin treatments for diabetic leg ulcers can be used for cosmetic purposes

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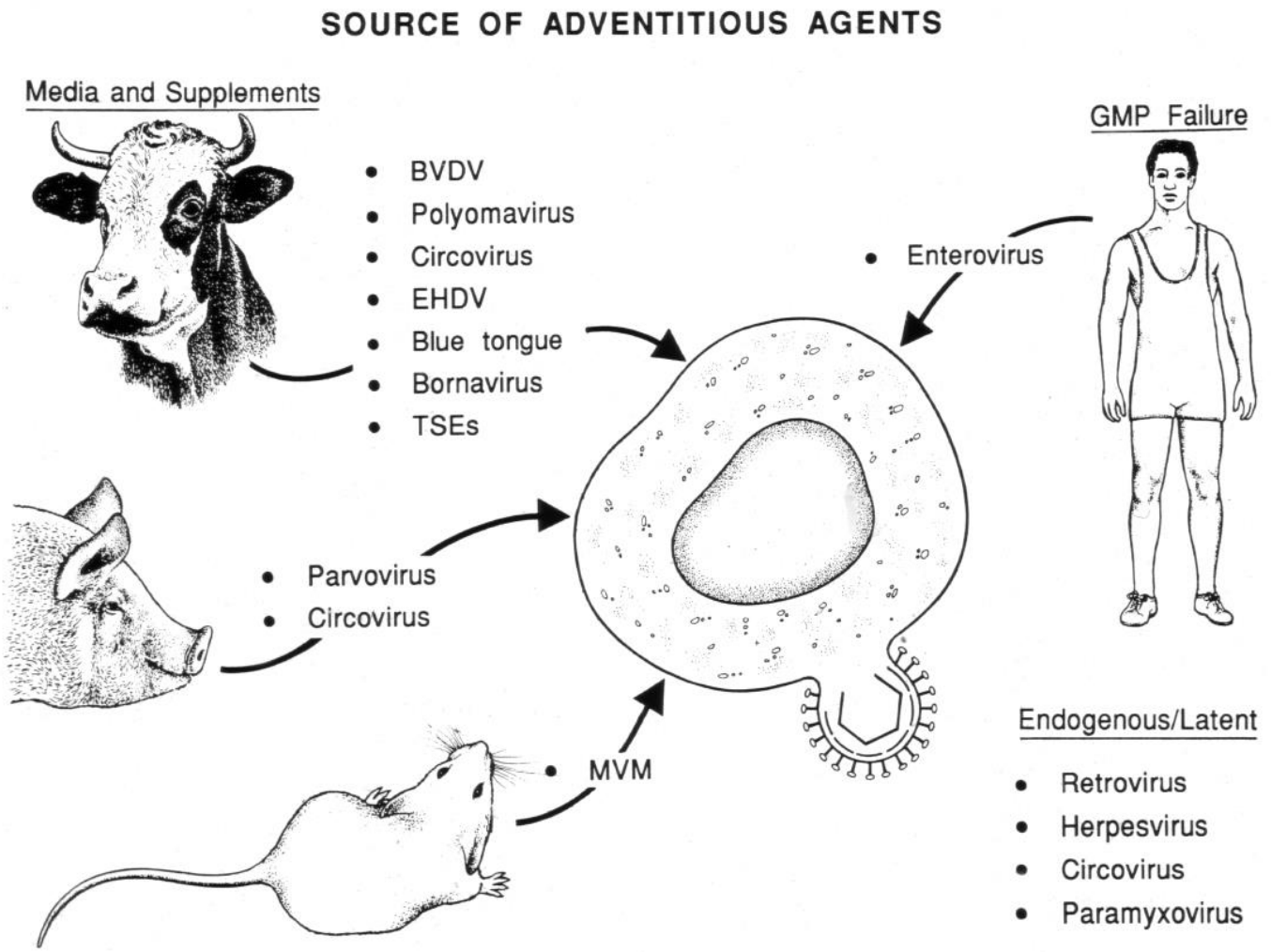
CONSIDERATIONS FOR COMPILING BIOSAFETY STRATEGY CONTINUATION

- Current regulatory authority guidelines and recommendations
  - variations across EU, FDA, ICH and Pharmacopeia
- Trends for future regulatory authority expectations
  - E.g. : detecting HSV8 and polyoma viruses in human derived biologics?
- All possible sources of contamination in the product and product lifecycle

SOURCE OF CONTAMINATION IN THE PRODUCTION OF BIOTECH PRODUCTS

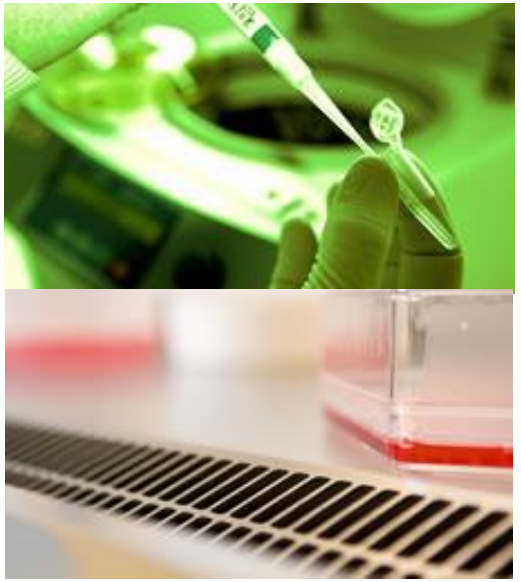


Cell culture derived product

CELL BANK CHARACTERISATION EXAMPLE TEST SCHEDULE

Cell Bank

- Identity
- Genetic stability
- Purity
  - Bacteria, fungi - *sterility*
  - Mycoplasma
  - Viruses
    - Broad specificity - *in vitro/in vivo assays*
    - Retroviruses - *infectivity/EM/RTase/PCR*
    - Species specific - *rodent/human/bovine etc*



VERO CELL BANK CHARACTERIZATION

ASSAY TYPE	MCB	WCB	EOPC (CAL)	COMMENTS
Microbiology				
Sterility + Bacteriostasis	x	x	x	European Pharmacopeia Assay
Mycoplasma + Mycoplasmastasis	x	x	x	European Pharmacopeia and USP Assay
Mycobacterium	x	x	x	European Pharmacopeia Assay
Adventitious Virus				
In vitro (28 day) - MRC-5/Vero/Simian line	x		x	.
In vivo - adult & suckling mice, embryonated eggs, Guinea Pigs	x		x	FDA protocol
Bovine & Porcine *in vitro* to US 9CFR	x			Nearly all cell lines have been exposed to Bovine FCS & Porcine Trypsin in their history
Porcine & Bovine Circovirus	x			Q-PCR
Porcine Hokovirus	x			Q-PCR
Torque Teno Virus (TTV)	x			Q=PCR
Retrovirus				
Transmission EM - 200 Cell Profiles	x		x	
F-PERT	x		x	Should be negative

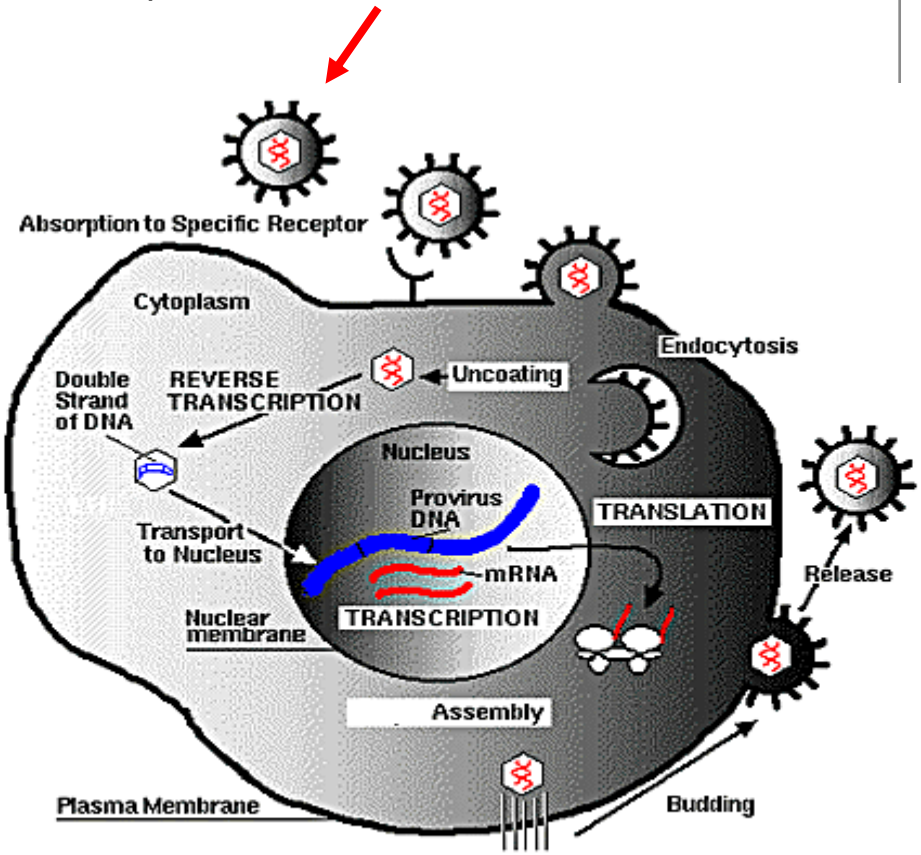
VERO CELL BANK CHARACTERIZATION

ASSAY TYPE	MCB	WCB	EOPC (CAL)	COMMENTS
Species Specific Virus				
Human viruses; HTLV 1&2 / HTLV 1&2 / Hep A, B, C / Herpes 6, 7, 8 / CMV / EBV / SV 40 / B19	x			Q-PCR – Also detects SIV 1&2 / STLV 1&2
Human Adenovirus	x			Q-PCR
Human Erythroviruses	x			Q-PCR – additional B19 strains
Enteroviruses	x			Q-PCR
Simian CMV	x			Q-PCR
Simian Retrovirus	x			Q-PCR
Simian Foamy Virus	x			Q-PCR
Squirrel Monkey Retrovirus (SMRV)	x			Q-PCR
Identity				
DNA Fingerprinting Assay	x	x	x	
Isoenzyme Assay	x	x	x	
Tumourigenicity & Karyology				
Tumourigenicity			x	Cells from the MCB can be expanded to EOPC level in the laboratory
Karyology	x	x	x	Include pre-GMP seed

Detection of Retroviruses

- Do not always show CPE. Can recombine with endogenous retroviral genomes to form new retroviruses.
- Absence of infectious retrovirus must be demonstrated in vaccines
- An area of concern to all global regulatory authorities

Reverse transcriptase (RTase) inside virus particles facilitates genomic integration. Spikes on cell surface for attachment.



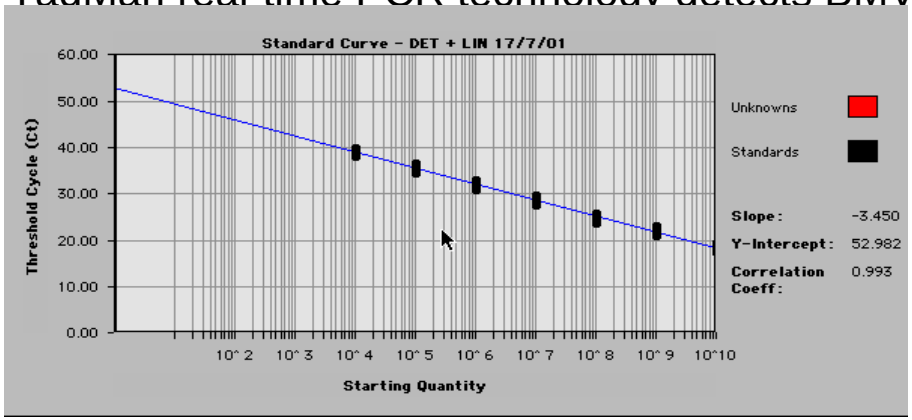
Retrovirus replication

PERT Assays- Detection and Quantitation of Retroviral RT

The Product Enhanced Reverse Transcriptase (PERT) Assay

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

TaqMan real-time PCR technology detects BMV cDNA.



Q-PERT Assay is Quantitative

F-PERT Assay is Qualitative

Measurement of RT Enzyme Activity

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

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

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

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

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

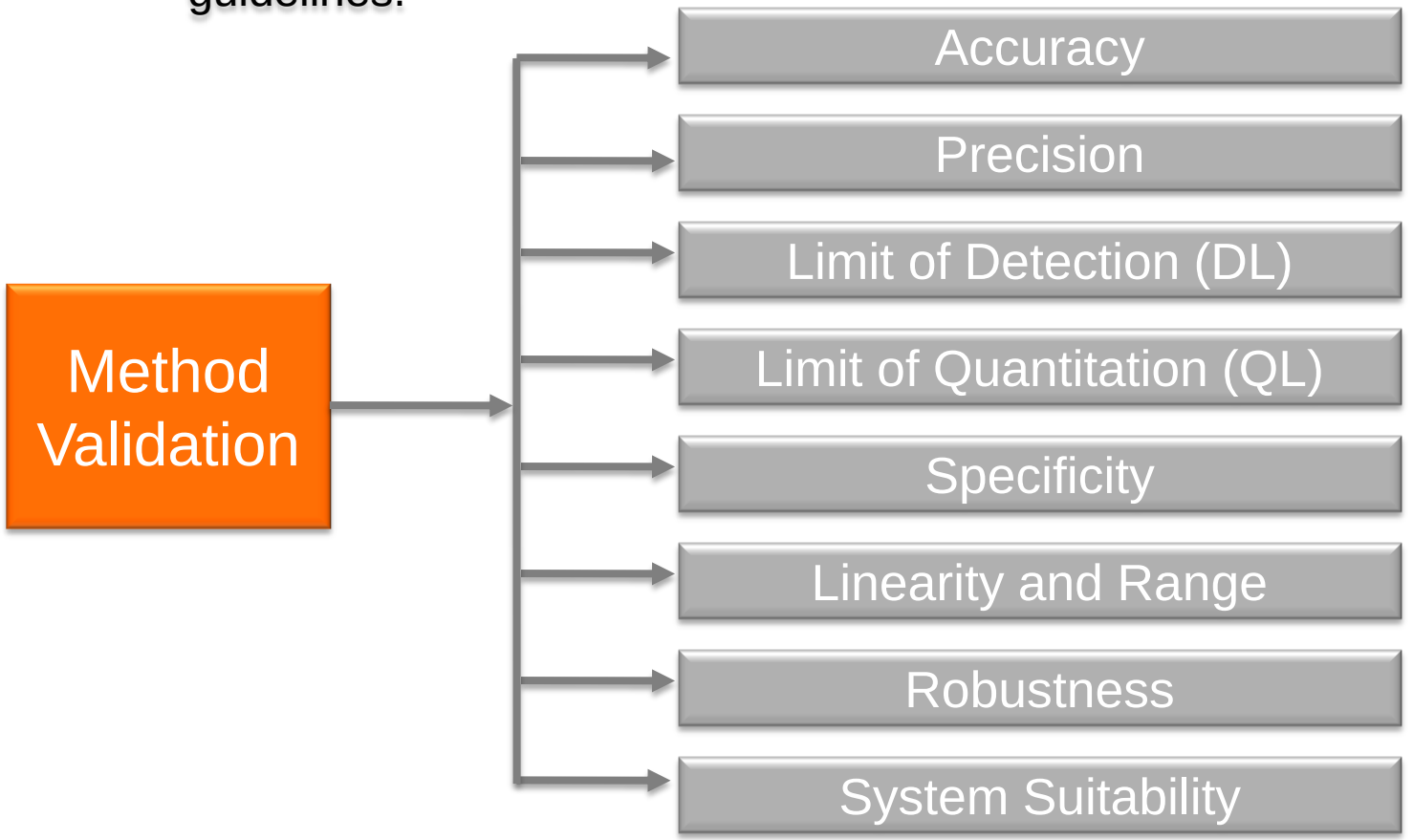
- FDA 2010 Vaccine guidance document states that the assay limit of detection should be comparable to published literature (specifically Lovatt *et al*., 1999)

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

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

VITROLOGY ASSAY VALIDATION

Validating an assay consists of analyzing & verifying the 8 or 9 assay parameters as described in the US pharmacopeia or the ICH guidelines.



ASSAY VALIDATION SUMMARY (AVS) OF CHO HOST CELL DNA TESTING

Vitrology

Assay Validation Summary (AVS) M.8301 Version 01 Quantitation of residual Chinese Hamster Ovary (CHO) host cell DNA by Real Time Polymerase Chain Reaction (Q-PCR)		
Prepared by: SMcFADYEN	**Signature:** SMcFadyen.	**Date:** 08JUN09
Management approved by JOHN BLACK	**Signature:**	**Date:** 08 JUNE 2009
QA approved by TRINA CORBETT	**Signature:** TCorbett	**Date:** 15/6/09

VALIDATION PARAMETER		RESULT OPERATOR 1	RESULT OPERATOR 2	CONCLUSION[1]		
Preliminary Detection Limit (DL)[1] *Lowest concentration with 100% positive amplification (8 replicates)*	DL	50 fg	5 fg	**Preliminary DL[2]**	**50 fg**	
	Mean C_T ± Std Dev	35.52 ± 0.37	38.46 ± 0.78	**Intermediate precision**	Mean C_T ± Std Dev	34.33 ± 1.84
	% CV	1.04	2.04		% CV	5.35
Quantitative Range[1] *Range of concentrations within ± 2 fold of standard value[3]*		50 fg to 50 ng	5 fg to 50 ng	**Assay Quantitative Range**	**50 fg to 50 ng**	
Linearity[1] *R^2 for standard curve > 0.90 (8 replicates)*	R^2	0.99	0.99	**Linearity demonstrated R^2 for standard curve > 0.90 for both operators**		
	Slope	-3.49	-3.58			
	Y-intercept	40.90	40.08			
Quantitation limit (QL)[1] *Lowest concentration with a calculated unknown within ± 2 fold of standard value (24 replicates)*	QL	50 fg	5 fg	**Assay QL**	**50 fg**	
	Mean quantity ± Std Dev	63.2 fg ± 7.65 fg	6.29 fg ± 2.93 fg	**Intermediate precision**	Mean quantity ± Std Dev	65.4 fg ± 8.72fg
	% CV	12.11	46.58		% CV	13.34
95% Cut Off[1] Detection Limit (DL) *Lowest concentration with 95% positive amplification (24 replicates)*	Positive cut-off	50 fg	5 fg	**Positive cut-off (DL)**	**50 fg**	
	Mean C_T ± Std Dev	33.80 ± 0.19	37.06 ± 0.98	**Intermediate precision**	Mean C_T ± Std Dev	33.40 ± 0.46
	% CV	0.56	2.65		% CV	1.37

[1]Conclusion and statistical data for intermediate precision are derived from a combination of operator 1 and 2 data. Note, if the DL, QL, range or positive cut-off values are different for operator 1 and 2, the conclusion is based on the upper value of the 2 data sets.
[2]The assay quantitation limit per ml is 1.8 pg. This is based on a test volume of 6 µl, elution volume of 60 µl and extraction volume of 280 µl.
[3]Quantitative lower range determined from 24 replicates assessment, upper range determined from 8 replicates assessment.

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VIRAL CONTAMINATION EVENTS

- Several documented – MMV in CHO, vesivirus in CHO, polio in Vero derived vaccines, CCV from bovine serum
- Rule out false positives and out of specification events
- Use several methods to identify contaminant – cell based, rapid molecular and microscopy techniques
- Undertake a risk assessment related to final clinical application
- Consult with appropriate regulatory authorities

VITROLOGY APPROVAL OF A CELL BASED VACCINE

For Marketing Approval

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

Investigational Medicinal Product (IMP) for clinical trials

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

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

THANK YOU FOR YOUR ATTENTION

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QUESTIONS ?