BioReliance®

QC testing of cell and gene therapy samples

Steven McDade
Senior Technical Specialist

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Agenda

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1. AAV
2. CAR-T
3. iPSC
4. Insect cell line characterization

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Pharma & Biopharma
Manufacturing &
Testing Services

Viral Risk Mitigation Strategy for gene & cell therapy

Safe sourcing and testing of raw materials

Verify absence of viral contaminants at appropriate Stages

Verify capacity of manufacturing process to remove or inactivate potential viral contaminants

Difficult for many viral vector and cell therapy products

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Safety testing challenges with cell and gene therapies

- Challenging regulatory landscape
- Typically no terminal sterilization process
- Small lot size / limited sample volume
- Limited availability of starting materials for process, product and test method development
- Time constraints for getting therapy to patient

➔ **Rapid and molecular methods address many of these challenges**

Agenda

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AAV

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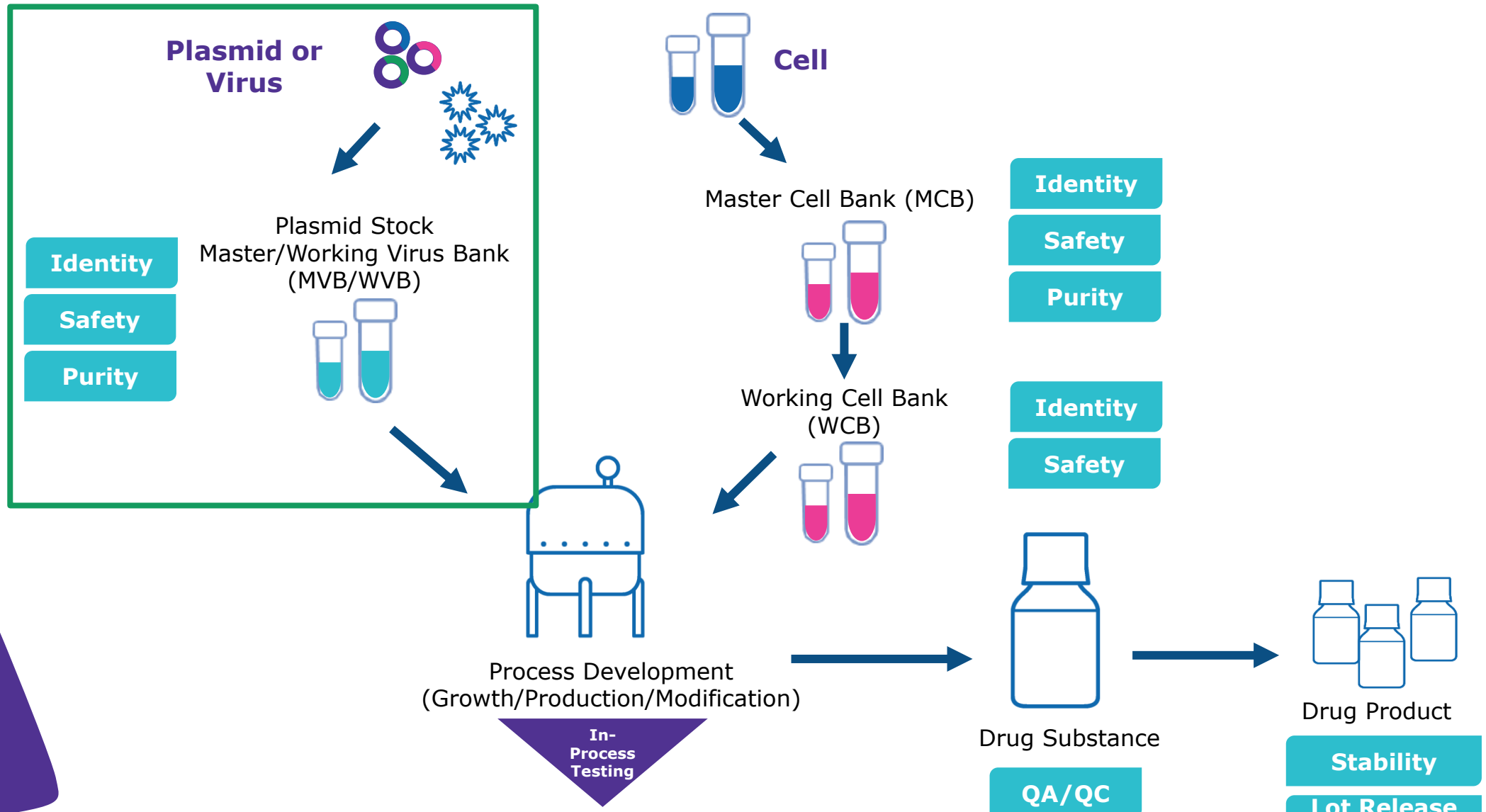
Vector and Cell Safety and Characterization



Key elements of *E.coli* cell bank characterization

Viability

- "Should be capable of maintaining a level of cell viability upon reconstitution which is both consistent and adequate for production use"

Purity

- "The design and performance of specific tests for adventitious microbial agents and adventitious cellular contaminants should take into account the properties of the banked cell, the likely contaminants based upon scientific literature, source, methods and materials used for cultivation and other organisms present in the lab"
- "Visual examination of the characteristics of well isolated colonies is suggested, using several microbiological media"

Identity

- "Analysis of growth on selective media is usually adequate to confirm host cell identity at the species level"
- "Either phenotypic or genotypic characteristics may be used in identity testing"

Stability

- "Consistency of the coding sequence of the expression construct should be verified"
- "Restriction endonuclease mapping or other suitable techniques should be used to analyse the expression construct for copy number, for insertions and deletions"
- "For extrachromosomal expression systems, the percent of host cells retaining the construct should be determined"

Overview of *E.coli* characterization assays

	Assay	Description	Sample requirements per cell bank
Viability	510055GMP.BUK	Determination of cell viability in the sponsor's cell bank	3 vials
Purity	510008Q.BUK	Qualification of purity testing for microbial cell banks	1 vial
	510008GMP.BUK	Purity testing of microbial cell banks: Presence of bacterial and fungal contaminants	1% of the bank (minimum 2 vials)
	510057GMP.BUK	Determination of the purity of the sponsors bacterial strain by Gram's staining	1 vial
	510027GMP.BUK	Detection for the presence of bacteriophage in material derived from a cell bank of E. coli or material used in the propagation of E. coli cultures.	1 vial
	510088GMP.BUK	Detection of lysogenic bacteriophage in test material derived from Escherichia coli cell banks	1 vial
Identity	105058GMP.BUK	Confirmation of the identity of an E. coli strain by RAPD analysis	1 vial
	512002GMP.BUK	Identification of Enterobacteriaceae and other Gram negative rods using the API® 20 Identification system	1 vial
	Select one marker; 512063GMP.BUK 512064GMP.BUK 512084GMP.BUK 512065GMP.BUK 512067GMP.BUK 512078GMP.BUK	Determination of the partial genotype of the sponsor's E. coli; Ampicillin Resistance Kanamycin resistance Chloramphenicol Resistance Tetracycline Resistance Nalidixic Acid Resistance Streptomycin Resistance	1 vial each
	Select one or more; 512069GMP.BUK 512068GMP.BUK 512070GMP.BUK 512072GMP.BUK 512075GMP.BUK 512074GMP.BUK 512071GMP.BUK	Determination of the partial genotype of the sponsor's E. coli; Determination of gal genotype Determination of lac genotype Determination of met genotype Determination of thi genotype Determination of recA-1 genotype Determination of supE genotype Determination of F genotype	1 vial each
Genetic Stability	104030GMP.BUK	Retention of Expression Construct in a Bacterial Cell Bank	1 vial
	106603GMP.BUK	Nucleotide sequence analysis of recombinant plasmid expression vector	2 vials of 5x10e6 or 2x500 µl vials
	104034GMP.BUK	Genetic Stability and Plasmid Identity by restriction enzyme map analysis	1 vial
	107315VAL.BUK	Design and qualification of a quantitative polymerase chain reaction (QPCR) assay for the determination of plasmid copy number	1 vial
	107315GMP.BUK	Determination of Plasmid Copy Number in a Microbial Cell Bank by Quantitative Polymerase Chain Reaction (QPCR)	1 vial

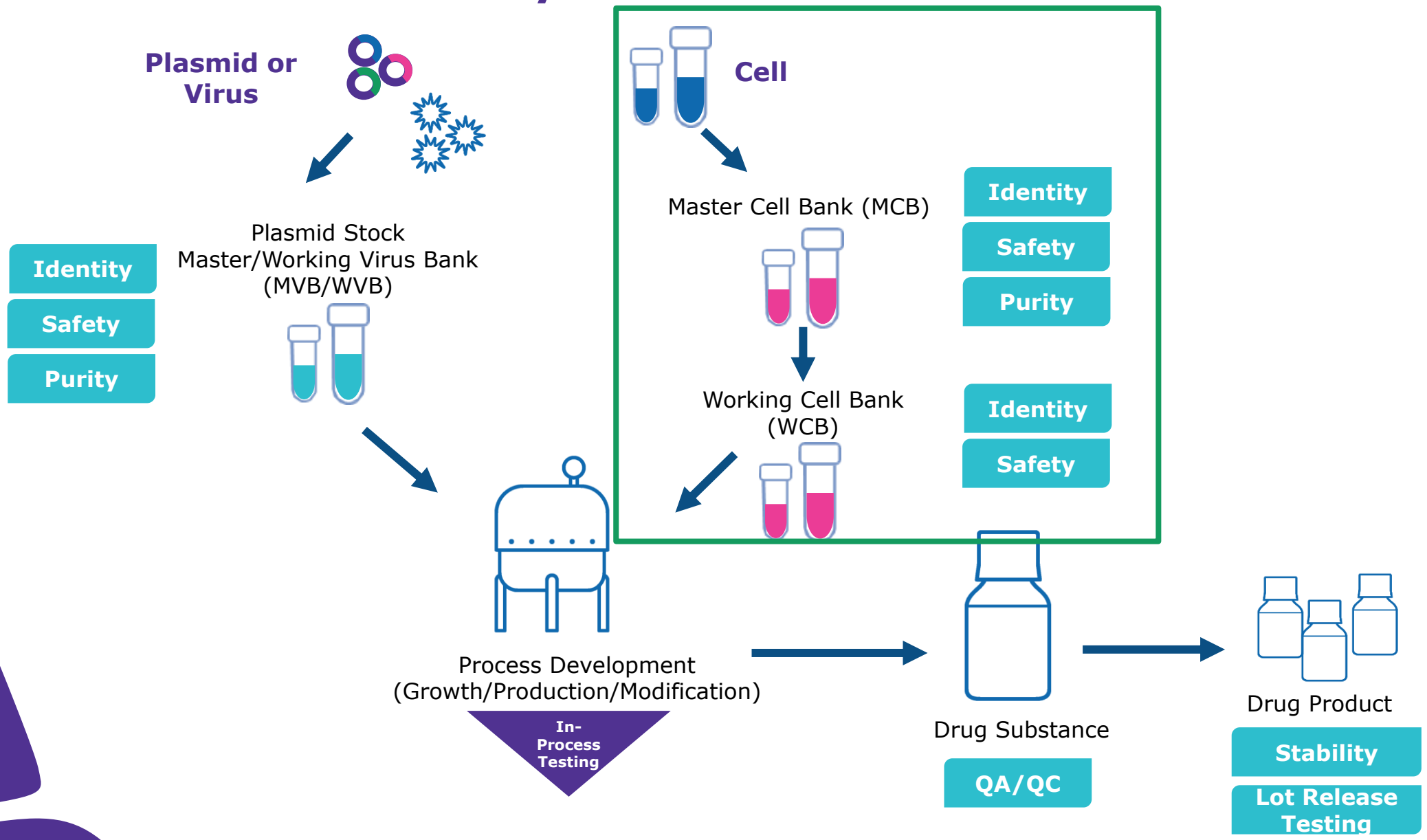
Plasmids used for vector production

EP 5.14 Gene transfer medicinal products for human use

- Plasmid intermediates used for vector production
  - Information needed
    - Identification
    - Source
    - Means of isolation
    - Nucleotide sequence
    - Source and function of promoters, origin of replication, selection marker genes

- Bacterial cell bank used to generate plasmids must comply with the requirements of the “Bacterial cell used for the manufacture of plasmid vectors” section.
- **Plasmids tested for:**
  - Identity: sequencing or PCR
  - DNA concentration
  - DNA forms: HPLC or CE
    - Supercoiled, multimeric, relaxed monomer and linear forms
  - Residual host cell DNA
  - Residual RNA
  - Residual host cell protein
  - Sterility, endotoxin

Vector and Cell Safety and Characterization

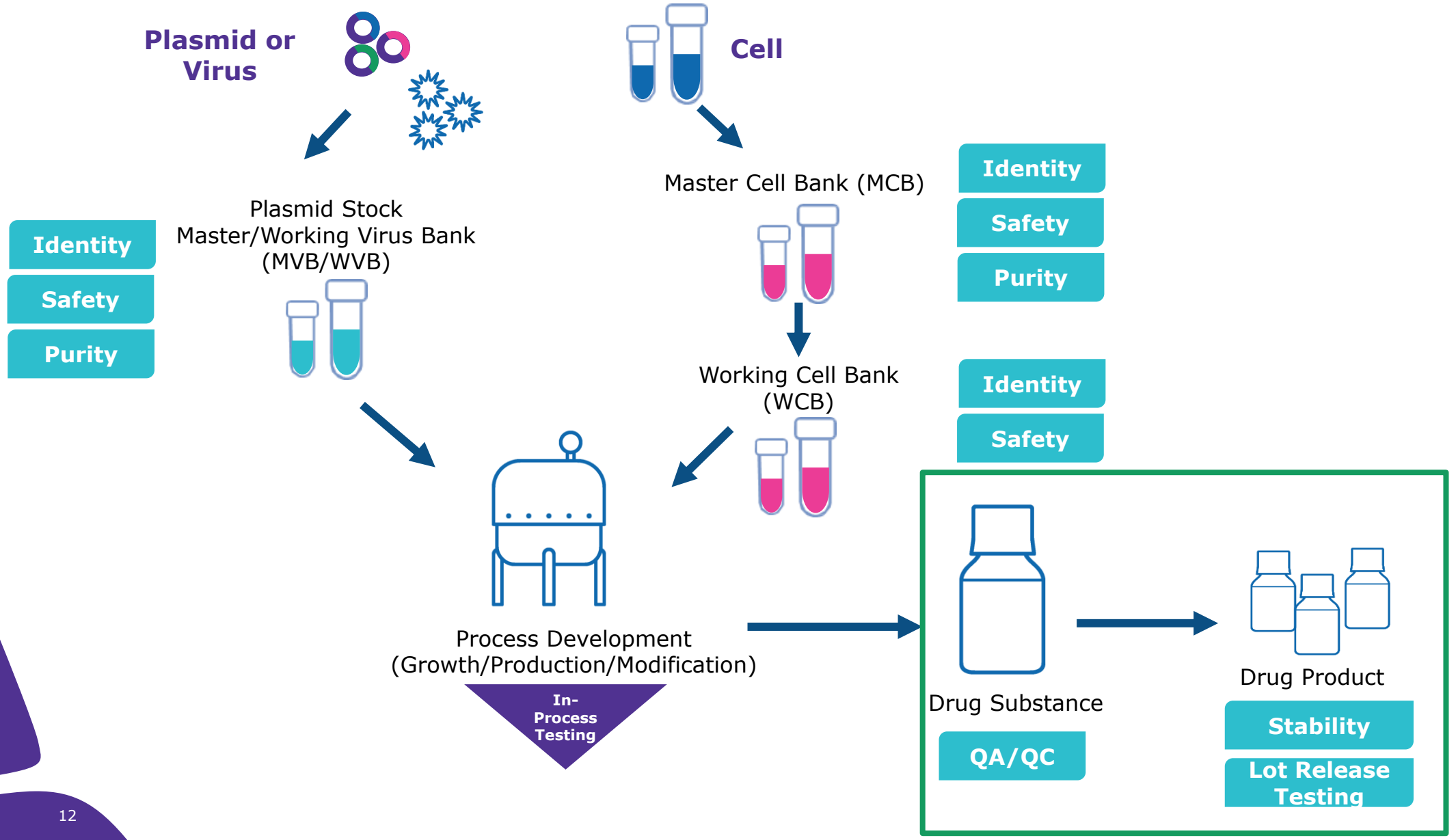


HEK 293 Cell Line Characterization

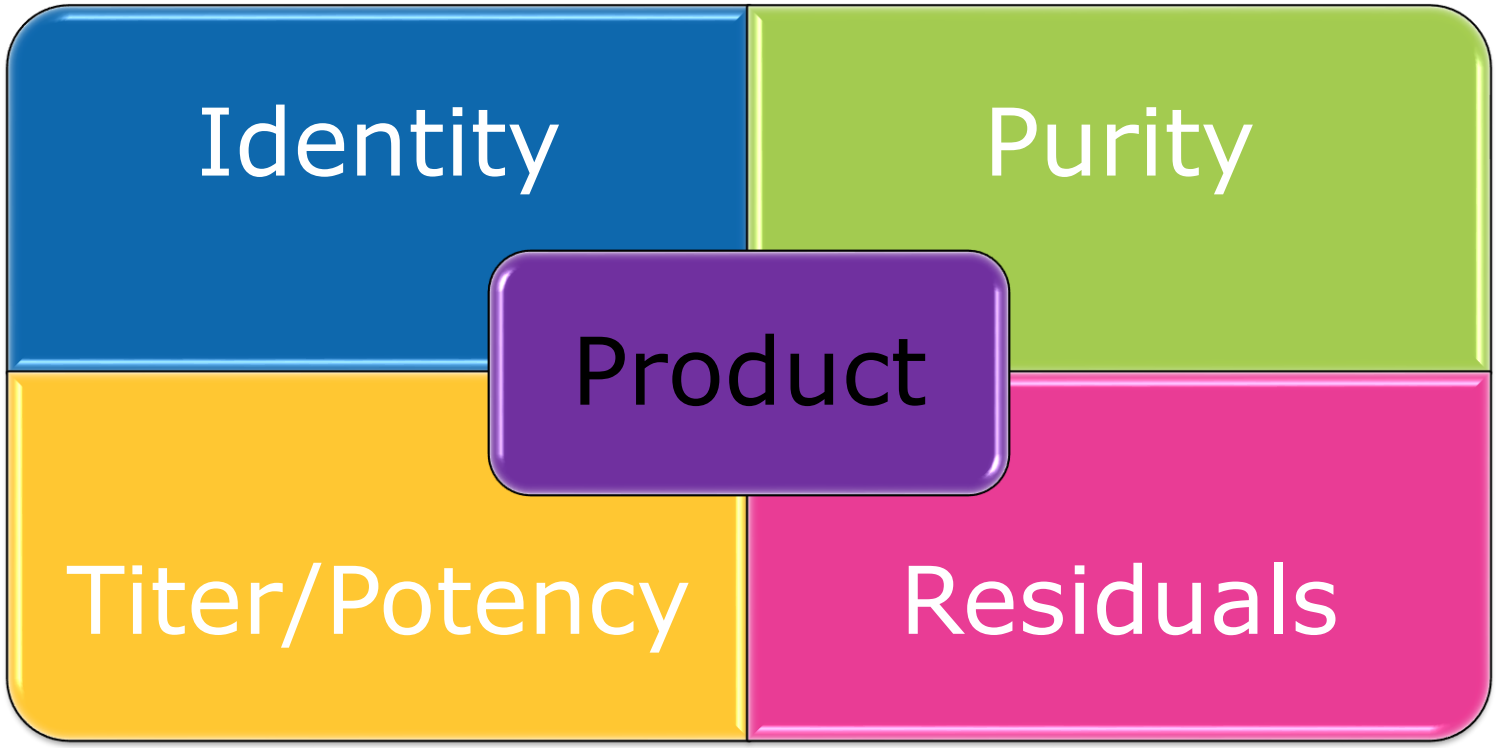
Testing	Assays	MCB	WCB	CAL
Identity	CO1 Barcode Analysis	X	X	X
	Short Tandem Repeat analysis	X	X	X
	Spectral Karyology	X		X
Microbial Detection	Sterility	X	X	X
	Mycoplasma	X	X	X
	Mycobacterium	X	X	X
Virus Detection	In vitro virus assay	X	X	X
	In vivo virus assay	X		X
	TEM	X		X
	QPERT	X		X
	PCR for Human Viruses*	X		X
	Bovine virus assay (9CFR, EMEA, EP)	X		(X)
	Porcine virus assay 9CFR, EMEA, EP)	X		(X)
	PCR for Bovine & Porcine viruses (BPyV, Hep E, Parvovirus, Circovirus)	X		(X)

*HIV 1 & 2, HTLC I & II, HAV, HBV, HCV, CMV, EBV, HHV 6, 7, 8, B19, HSV 1 & 2, human polyoma viruses, Bocavirus, Metapnuemovirus etc

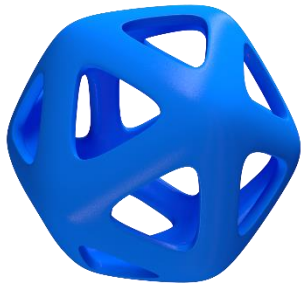
Vector and Cell Safety and Characterization



Characterization of Viral Vectors



Characterization of Virus Vectors - AAV



Identity

- PCR identity assay GOI or promoter √
- AAV serotype ELISAs 2, 5, 8, 9
- AAV Serotype by MS
- Sequencing of vector (NGS) √

Titer

- $TCID_{50}$ infectivity assay platform/custom √
- Viral Particles (ELISA) ELISAs 2, 5, 8, 9 √
- Genomic Particle Count by ddPCR generic promoter/GOI √

Potency of r-AAV expressed protein (Custom)

Purity: Absence of adventitious contaminants

- Sterility traditional and rapid √
- Mycoplasma and spiroplasma culture and PCR √
- *In vitro* assay for adventitious viruses culture √
- Replication competent AAV √
- Size distribution by DLS √
- rcHSV
- Empty/Full (Non GMP)

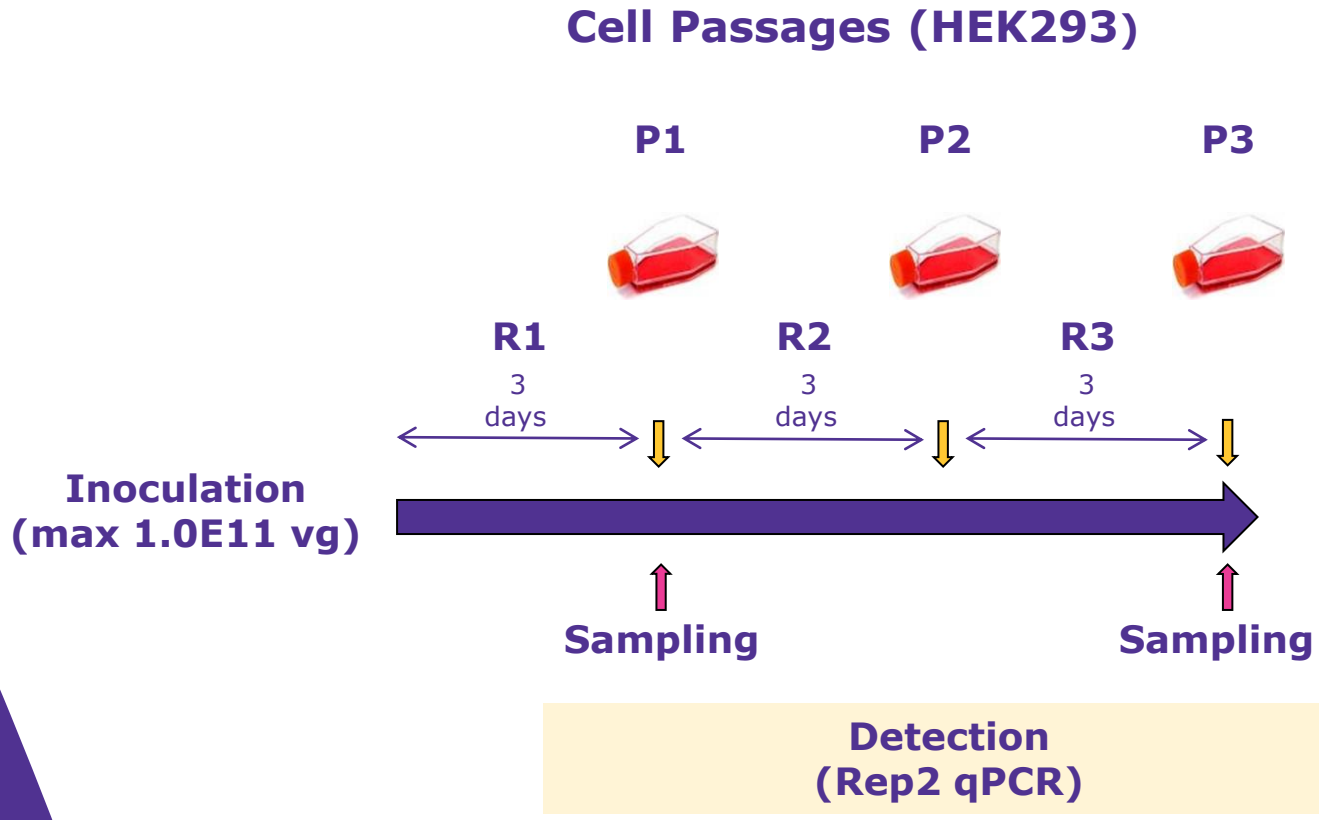
Residuals: Absence of process related contaminants

- PCR for helper viruses or transfected plasmids √ kan/amp/custom
- Host cell DNA √, Host cell proteins √, residual benzonase √, residual BSA √

Detection of replication competent AAV (rcAAV)

003070PSQ.BSV/003070PSQ.BSV

How is the assay performed?



TEST CONDITIONS

PSQ

- Cell Control
- Negative Control
- Test Article D1*
- Test Article D2*
- Test Article D3*
- Positive Control 20 IU
- Test Article D1+ PC 20 IU

GMP

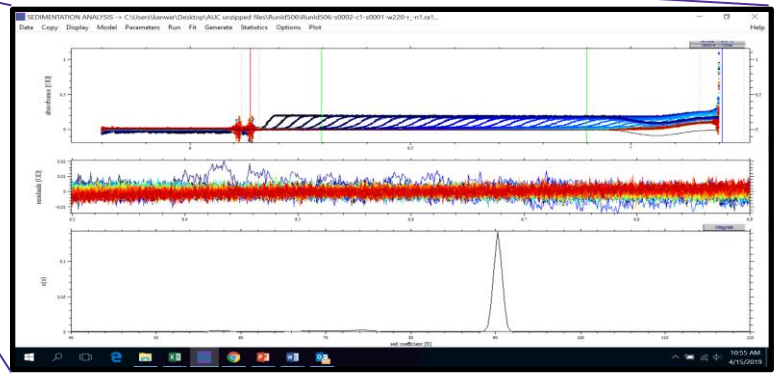
- Cell Control
- Negative Control
- Test Article
- Positive Control 20 IU
- Test Article + PC 20 IU

*** - dilutions (doses) of TA**

Analytical Ultracentrifugation (AUC)

OptimaAUC

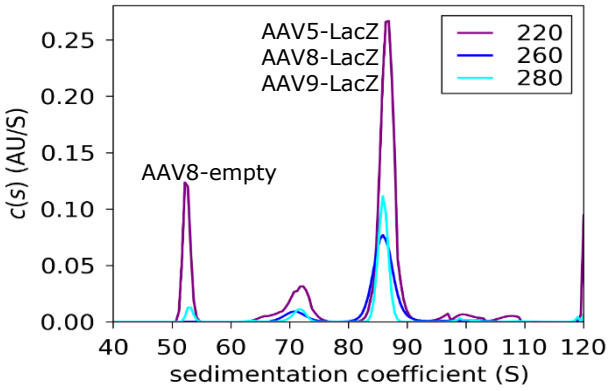
w/AN-50Ti rotor



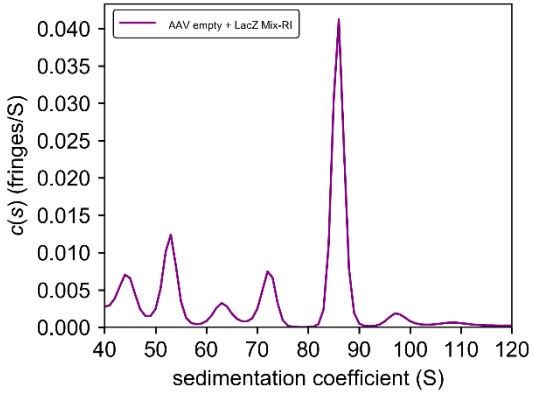
Sedfit analysis software

- Sedimentation velocity (SV) measures how fast macromolecules move in response to centrifugal force
- Moving molecules are scanned simultaneously by 2 independent optical systems:
  - UV Absorbance
  - Rayleigh Interference (RI)detection
- Measuring changes in sedimentation boundary movement provides information about the mass and shape of macromolecules
- Distinctive peaks can be seen and percentage of empty & full capsids is provided in a GMP certificate of analysis

ABSORBANCE



INTERFERENCE



Viral Clearance studies for AAV therapies

- AAV are small, non enveloped viruses
- Focus on inactivation and filtration methods. Target reduction of large, enveloped viruses and medium sized non enveloped viruses
- To date, no specific written guidance about viral clearance for these novel therapies. However if safety can be built in to a process, it should be.
- Clearance steps should be evaluated and be aligned with ICH Q5A expectations for study design. ICH Q5A revision 2 expected to be issued in 2022.
- Viral safety strategy includes prevention, detection, and if possible, removal/inactivation of viruses

Agenda

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CAR-T

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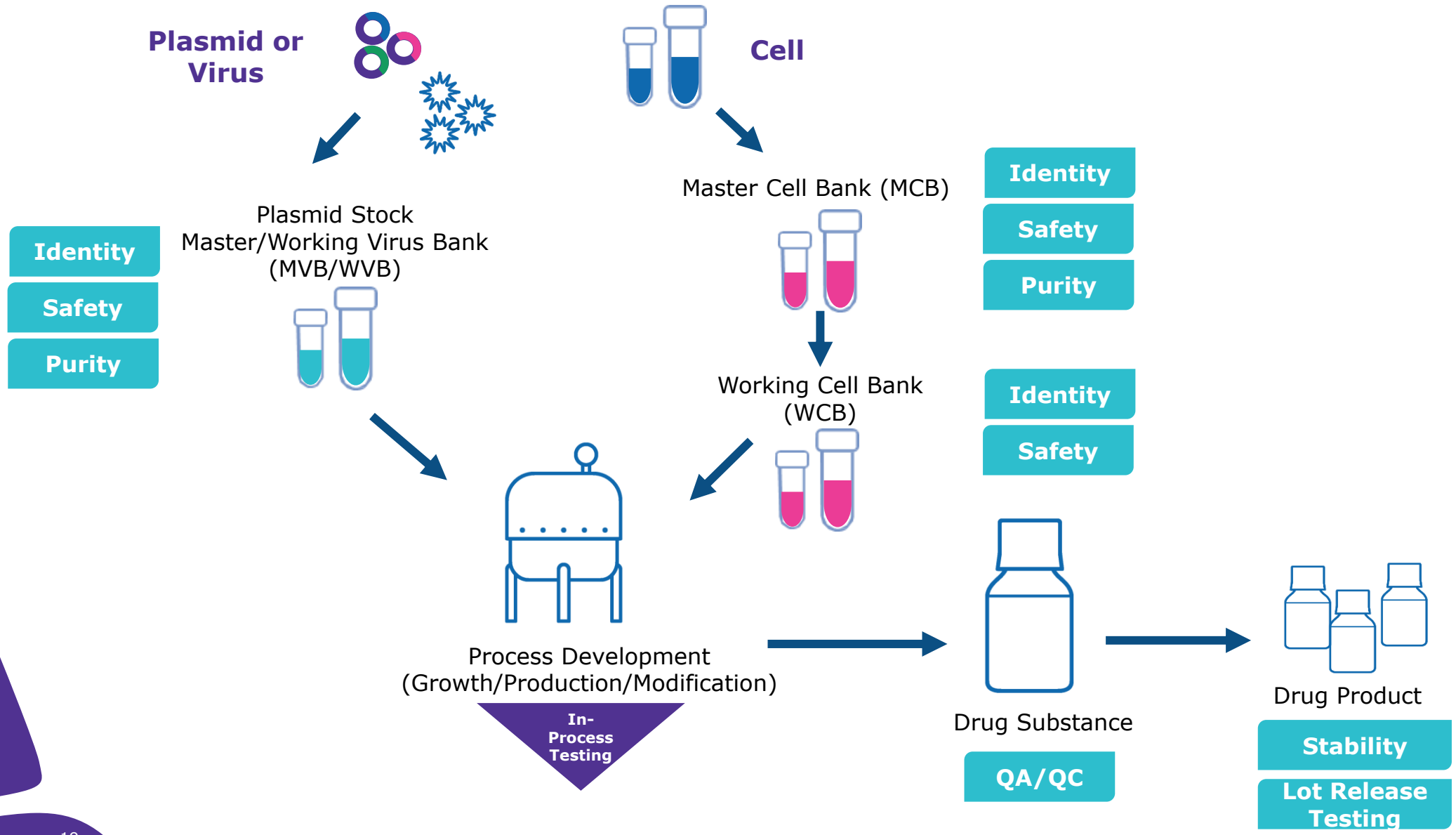
Vector and Cell Safety and Characterization



Characterization of Virus Vectors – Retrovirus & Lentivirus

Lentivirus/Retrovirus

Identity

- PCR identity assay GOI or promoter √
- Provirus sequencing √
- Sequencing of vector (NGS) √

Titer

- Infectivity assay (transduction with provirus quantification or payload expression)
- p24 (LV) √ or p30 (RV) ELISA √
- Genomic Particle Count by ddPCR generic promoter/GOI √

Potency of LV/RV expressed protein (Custom)

Purity: Absence of adventitious contaminants

- Sterility traditional and rapid √
- Mycoplasma and spiroplasma culture and PCR √
- *In vitro* assay for adventitious viruses culture √
- VSV-g PCR √
- Replication competent LV √
- Replication competent RV √

Residuals: Absence of process related contaminants

- PCR for transfected plasmids √ kan/amp/custom
- Host cell DNA √, Host cell proteins √, residual benzonase √, residual BSA √ SV40 T-antigen PCR √

RCR / RCL – Testing volumes

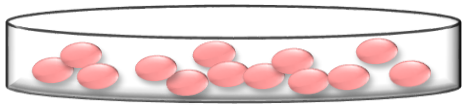
Cells

Production cells, transduced cells and corresponding supernatant

- Cells: 1% of total cells or 1e8 (lesser amount)
- Cell culture supernatant: 5% of total volume

Stable Vector Producing Cells

Master Cell Bank (MCB)



Vector Transduced Cells

Vector Supernatant

- For production volumes < 6 liters
  - 5% of production
- For production volumes > 6 liters
  - Volume should be sufficient to ensure 95% probability of detecting 1RCR per dose equivalent
  - The FDA 2020 RCR guidance document outlines the necessary calculations sponsors should consider to determine volume to be tested.

Assay outline 009133GMP

Day 0

C8166 cell seeding into multiple 175 cm^2 flasks

Day 1

Test Article Inoculation

xN*

Test Article (TA)

Spiked Test Article

TA + 10$TCID_{50}$ HIV-1 Control

Positive Control

10 $TCID_{50}$ HIV-1 control

Negative Control

Media alone

Passages 1 & 2

2-5 days post previous passage

- Observe for CPE

Passage 3

2-5 days post passage 2

- Observe for CPE
- Blind Passage
- Cell free supernatant from all flasks inoculated on to fresh C8166 cells

Passages 4 & 5

2-5 days post previous passage

- Observe for CPE

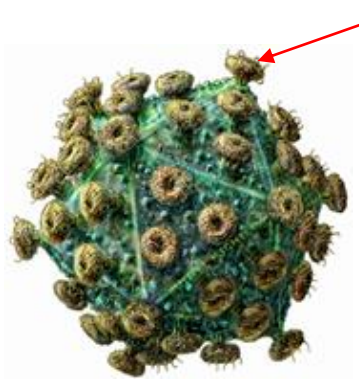
Endpoint Detection

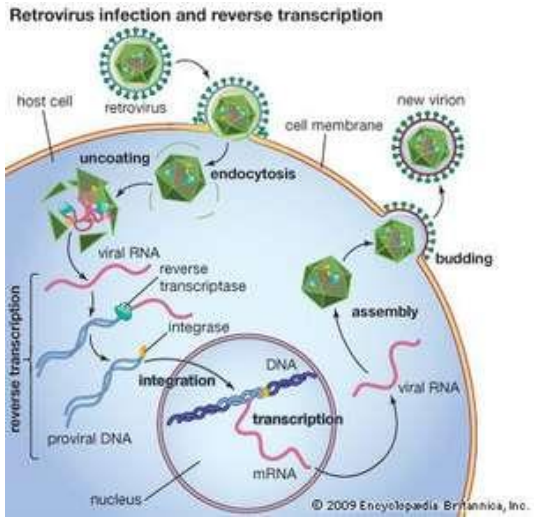
Q-PCR enhanced reverse transcriptase activity (QPERT)

*Number of flasks used for test article is dependent on volume tested and results from RCL pre-study

VSV-G PCR Assay for Lentivirus Vectors

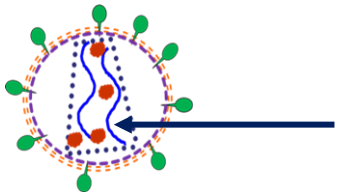
- VSV-G PCR
  - Detects the presence of VSV-G DNA in Proviral DNA:
    - Proviral DNA –virus DNA integrated into the cell genome
    - Lentivirus vectors – only transgene should be integrated into cell DNA
    - Presence of VSV-G DNA may indicate the presence of replication competent virus.
      - Further investigation with cell culture method is necessary
  - For cells that are not cryopreserved prior to administration the FDA allow for the use of a PCR assay for initial detection but the cell based assay must be started in parallel unless they have the ok from the regulators





Genomic Titer

- Measures the number of particle associated vector genome copies.
  - DNA copies for AAV and Adenovirus
  - RNA copies for Retro and Lentivirus



- Measures total virus content (infectious and non infectious)
- Used to guide dosing
- Involves a PCR based method

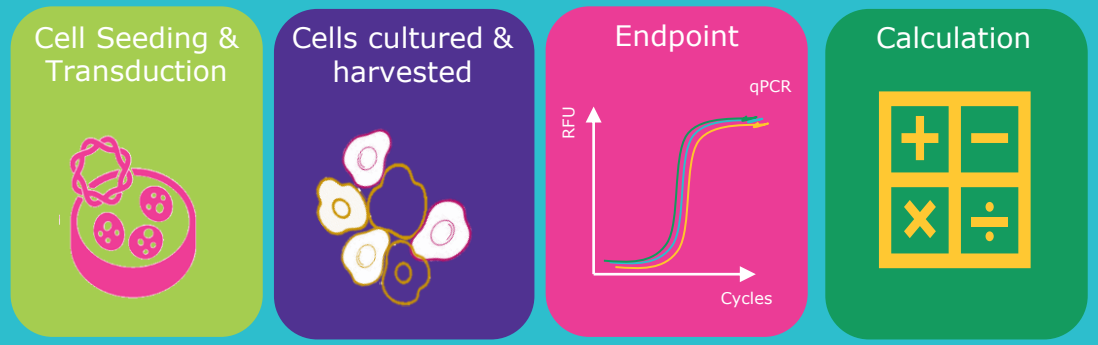
- Droplet Digital PCR – ddPCR
  - Divides the PCR reaction into 20,000 droplets
    - Positive droplet – DNA or RNA present
    - Negative droplet – DNA or RNA not present
  - Result is based on an absolute count of positive and negative droplets
    - No standard curve is needed.
  - Higher accuracy and precision of quantitation than qPCR
    - More robust to lower copy number levels
    - Resolve smaller differences in copy number
    - Higher number of technical replicates (20,000)

Assay	Vector	Target
Genomic Titer DNA Virus	AAV	CMV promoter or gene of interest
Genomic Titer DNA Virus	Adenovirus	E4 or gene of interest
Genomic Titer RNA Virus	Retrovirus	near 5'LTR or gene of interest
Genomic Titer RNA Virus	Lentivirus	5'LTR or gene of interest

Lentiviral Vector Transduction Titer Assay

- Used to understand transduction efficiency and calculate required dose for cell modification (MOI) or effective dose
- Off-the-shelf assay targeting conserved region, independent of GOI
- HT1080 cells selected as they are susceptible to transfection with LVV pseudotyped with VSV-g.
- Using multiple methods to assess titer helps to provide confidence in the quality of the viral prep.
  - **Genomic titer** targets RNAse P in LVV preparations to quantify extracted RNA.
  - P24 **capsid protein** can be identified and quantified using ELISA
  - Next Generation Sequencing (NGS) can also be used to sequence the **viral genome**

HOW?



- Permissive cell line is grown then transduced with the lentiviral vector
- DNA extraction & endpoint analysis using duplex qPCR targeting 5'LTR, with RNase P reference to normalize LV copies
- Calculation to determine transducing units/ml

Next Generation Sequencing assays

Cell Line AAT

- Rapidly identify contaminants (adventitious agent testing (AAT))
- Troubleshoot sources of contamination

Virus AAT

- Provides analysis of samples where neutralising antibody is difficult to obtain
- Assure stock purity

Genetic Integration & Stability

- Integration site assessment
- Genetic stability of Master and End of Production Cell banks
- Rich dataset

Virus ID

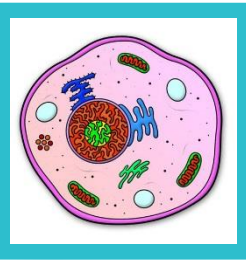
- Confirm genomic identity
- Detect sequence variants and sub-populations

Gene Editing Assessment

- On and off target analysis of gene editing
- Application to cellular therapies

Custom NGS Applications

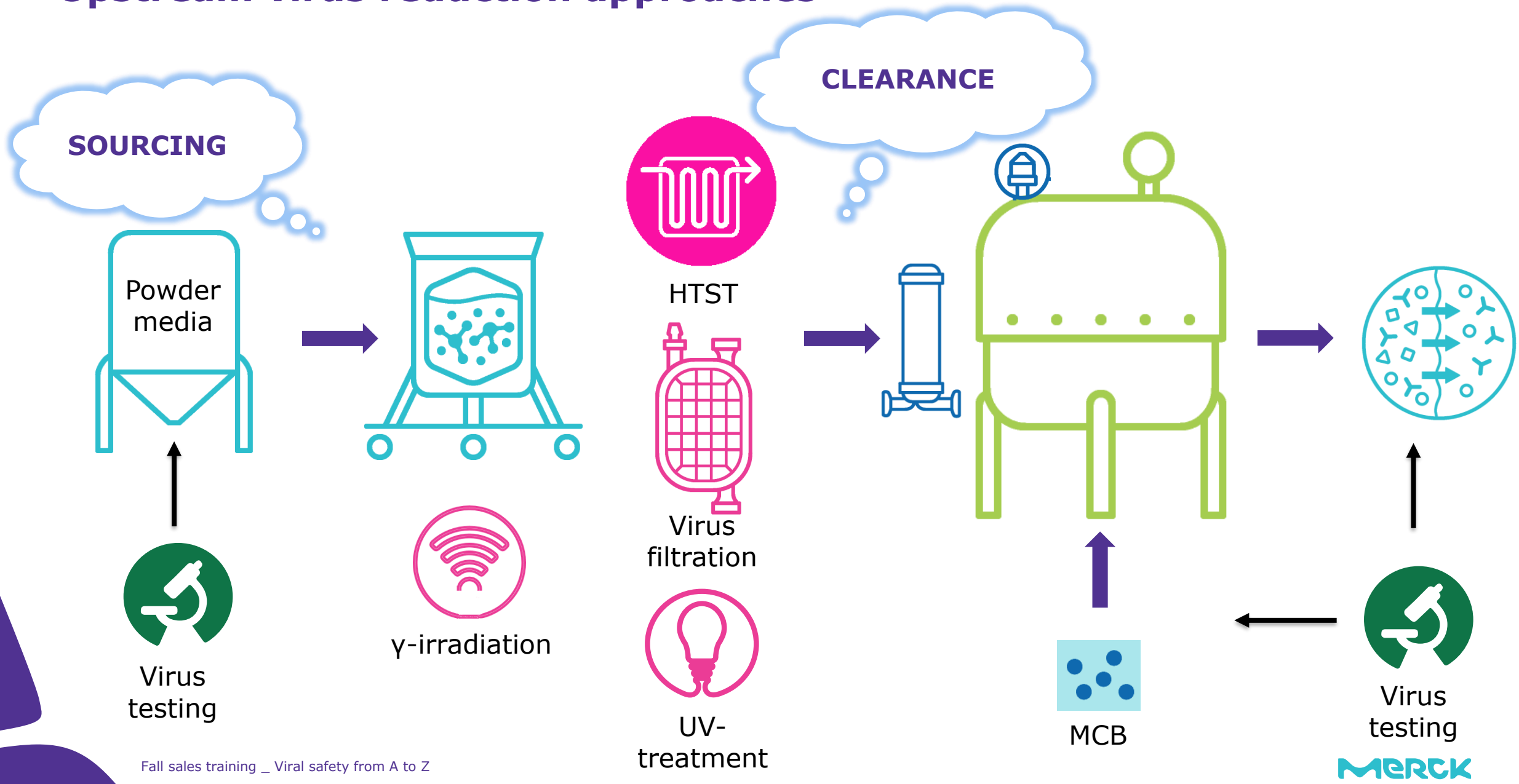
Testing Transfected Patient Cells



Transfected patient cells

- **Purity:** BacT/Alert, Mycoplasma PCR, rcLV (1% of transfected cells or 10e8, whichever is less), VSV-G PCR
- **Insertion site:**
  - NGS for genetic integration and stability including insertion site

Upstream virus reduction approaches



Agenda

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IPSC

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iPSC Line Characterization

Testing	Assays	MCB	WCB	CAL
Identity	CO1 Barcode Analysis	X	X	X
	Short Tandem Repeat analysis	X	X	X
	Spectral Karyology	X		X
Microbial Detection	Sterility	X	X	X
	Mycoplasma	X	X	X
	Mycobacterium	X	X	X
Virus Detection	In vitro virus assay	X	X	X
	In vivo virus assay	X		X
	TEM	X		X
	QPERT	X		X
	PCR for Human Viruses*	X		X
	PCRs for Syphilis, Gonorrhoea, Chlamydia	X		X
	Bovine virus assay (9CFR, EMEA, EP)	X		(X)
	Porcine virus assay 9CFR, EMEA, EP)	X		(X)
	PCR for Bovine & Porcine viruses (BPyV, Hep E, Parvovirus, Circovirus)	X		(X)

*HIV 1 & 2, HTLC I & II, HAV, HBV, HCV, CMV, EBV, HHV 6, 7, 8, B19, HSV 1 & 2, human polyoma viruses, Bocavirus, Metapnuemovirus etc

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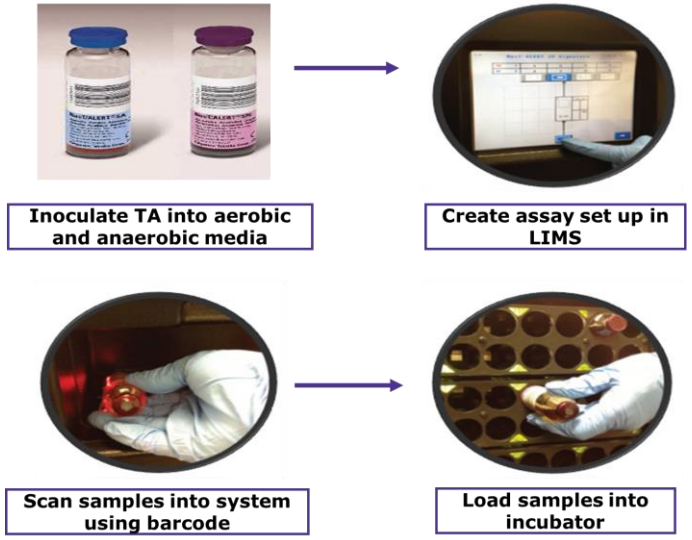
BacT/ALERT 3D System

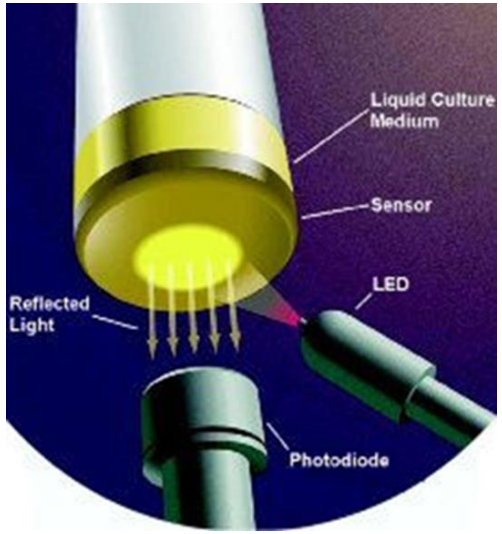
A sensor in the bottom of each broth bottle monitors CO_2 production, which indicates microbial growth.

A light emitting diode (LED) projects light onto the sensor and reflected light is measured by the photodiode.

As more CO_2 is generated, the sensor changes colour due to the change in pH resulting in more light being reflected.

The BacT/ALERT® 3D can detect most common contaminants within 48 to 72 hours.





CO_2 based detection system

Samples are read every 10 minutes

LED illuminates sensor and photodiode collects reflected light

BacT/Alert Assay Comparison

USP/EP/JP (Harmonised method)		EP 2.6.27 (Cellular products)	
510180 (Qualification)	**510185 (Sterility Test)**	**510190 (Qualification)**	**510195 (Sterility Test)**
Testing will be for pharmaceutical products Vials should be shipped frozen		Testing will be for cellular products only Vials should be shipped frozen	
Qualification will be performed using the 6 compendial microorganisms	Test articles inoculated into iAST and iNST media	Qualification will be performed using the 9 microorganisms as stated in EP 2.6.27	Test articles inoculated into iAST and iNST media
B. subtilis, *P. aeruginosa,* *C. albicans,* *A. brasiliensis* *incubated* at 22.5 ± 2.5°C *S. aureus,* *C. sporogenes* incubated at 32.5 ± 2.5°C	iAST broth bottles incubated at 22.5 ± 2.5°C iNST broth bottles incubated at 32.5 ± 2.5°C	*B. subtilis,* *P. aeruginosa,* *C. albicans,* *A. brasiliensis,* *S. aureus,* *C. sporogenes,* *S. pyogenes,* *P. acnes,* *Micrococcus* sp. incubated at 36 ± 1°C	iAST and iNST broth bottles all incubated at 36 ± 1°C

Mycoplasma assay comparison: Cultivation vs QPCR

	
Historical compendial method	Established alternative method
Method largely manual	Automated, reproducible
Detection limit: ≤ 100 Colony Forming Units	Detection limit: 10 Colony Forming Units
15 ml of UBH required	4 ml of UBH required
Assay turnaround time: 28 days	Assay turnaround time: 3 - 7 days

The use of PCR for the detection of mycoplasma, supported by comparability data, has been used within industry for several years. This has included the testing of starting materials through to clinical material for a range of biopharmaceuticals and many submissions have been made to the US and European regulatory authorities over this time.

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Mycobacteria PCR testing

Assay number	Assay title	Target nucleic acid	Gene target region	Negative control	Detection limit
305215GMP.BUK	Detection of Mycobacterium by Real time PCR	DNA	VP1 gene	0.5ug Human placental DNA per reaction	50 copies/reaction

Theoretical specificity of the primer/probe set using blastn analysis.

Species	Species	Species	Species	Species	Species	Total no. GenBank entries detected
*M.abscessus**	*M.caprae*	*M.fuerth*	*M.lentiflavum*	*M.parascrofulaceum*	*M.shinjukuense*	737 Total number of independent species shown here detected with 100 % homology = 168
M.acapulcensis	*M.celatum*	*M.gadium*	*M.lepromatosis*	*M.paraterrae*	*M.shottsii*	
M.aemonae	*M.chelonae**	*M.gastri*	*M.liflandii*	*M.parmense*	*M.simiae*	
*M.africanum**	*M.Chelonae-like organism*	*M.gilvum**	*M.llatzerense*	*M.peregrinum*	*M.simulans*	
M.agri	*M.chesapeaki*	*M.goodii*	*M.madagascariense*		*M.smegmatis**	
M.aichiense	*M.chimaera*	*M.gordonae*	*M.mageritense**	*M.petroleophilum*	*M.sp. 05-1390**	
M.alsiensis	*M.chitae*	*M.haemophilum**	*M.malmoense*	*M.phlei*	*M.sphagni*	
M.alvei	*M.chlorophenolicum*	*M.hassiacum*	*M.manitobense*	*M.phocaicum*	*M.stomatepiae*	
M.anthracenicum	*M.chlorophenolicus*	*M.heckeshornense*	*M.mantenii*	*M.pinnipedii*	*M.sydneyiensis*	
M.aromaticivorans	*M.chubuense**	*M.heidelbergense*	*M.marinum**	*M.porcinum*	*M.szulgai*	
M.arosiense	*M.colombiense**	*M.hiberniae*	*M.marseillense*	*M.poriferae*	*M.terrae*	
M.arupense	*M.coloregonium*	*M.hodleri*	*M.massiliense**	*M.psychrotolerans*	*M.thermoresistibile**	
M.asiaticum	*M.conceptionense*	*M.holsaticum*	*M.microti**	*M.pulveris*	*M.tilburgii*	
M.aubagnense	*M.confluentis*	*M.houstonense*	*M.monacense*	*M.pyrenivorans*	*M.timonense*	
M.aurum	*M.conspicuum*	*M.immunogen*	*M.montefiorense*	*M.ratisbonense*	*M.tokaiense*	
M.austroafricanum	*M.cookii*	*M.immunogenum*	*M.moriokaense*	*M.rhodesiae**	*M.triplex*	
*M.avium**	*M.cosmeticum*	*M.indicus pranii**	*M.mucogenicum*	*M.riyadhense*	*M.tuberculosis**	
M.bacteremicum	*M.crocinum*	*M.insubricum*	*M.murale*	*M.rufum*	*M.tusciae**	
M.barrassiae	*M.diernhoferi*	*M.interjectum*	*M.nebraskense*	*M.rutilum*	*M.ulcerans**	
M.boenickei	*M.duvalii*	*M.intermedium*	*M.neglectum*	*M.sacrum*	*M.vaccae*	
M.bohemicum	*M.elephantis*	*M.intracellulare**	*M.neoaurum*	*M.saskatchewanense*	*M.vanbaalenii**	
M.bolletii	*M.engbaekii*	*M.isoniacini*	*M.neworleansense*	*M.savoniae*	*M.vulneris*	
M.botniense	*M.fallax*	*M.jacuzzii*	*M.nonchromogenicum*	*M.scrofulaceum*	*M.wolinskyi*	
*M.bovis**	*M.farcinogenes*	*M.kansasii**	*M.noviomagense*	*M.senegalense*	*M.xenopi*	
M.brasiliensis	*M.flavescens*	*M.kubicae*	*M.obuense*	*M.seoulense*		
M.brisbanense	*M.florentinum*	*M.kumamotonense*	*M.pallens*	*M.septicum*		
M.brumae	*M.fluoranthenivorans*	*M.kuopiense*	*M.palustre*	*M.setense*		
M.canariasense	*M.fortuitum*	*M.lacticola*	*M.paraffinicum**	*M.shernsii*		
*M.canettii**	*M.frederiksbergense*	*M.lacus*	*M.parafortuitum*	*M.shimoidei*		
Number of further species submissions detected with 100% homology to assay primers and probes (includes *M. sp. JDM601*, sp. KMS*, sp. JLS*, sp. MCS**)						427
Number of uncultured submissions detected with 100% homology to assay primers and probes						968
Total number of submissions detected with 100% homology to assay primers and probes						2132

Ph. Eur. Chapter 5.2.14: Substitution of in vivo method(s) by in vitro methods for the quality of vaccines (Pharmeuropa 28.2; April 2016)

"...Novel sensitive molecular techniques with broad detection capabilities are available, including deep sequencing or high throughput sequencing methods, degenerate PCR for whole virus families or random priming methods..."

Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications

from non-lethal strains. Animals infected during the MAP test with non-virulent strains of LCMV will survive this challenge. If you use alternative methods, such as PCR, you should demonstrate sensitivity comparable to that of the described test.

Agenda

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4

Insect cell line characterization

BioReliance®

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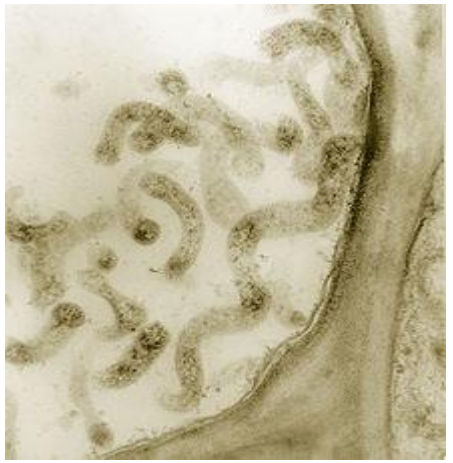
Insect Cell Line Characterization

Testing	Assays	MCB	WCB	CAL
Identity	CO1 Barcode Analysis	X	X	X
Microbial Detection	Sterility	X	X	X
	Mycoplasma	X	X	X
	Spiroplasma	X	X	X
Virus Detection	In vitro virus assay including TEM	X	X	X
	In vivo virus assay including toxicity pre-studies	X		X
	TEM	X		X
	QPERT	X		X
	PCR for Insect Viruses*	X		X
	Bovine virus assay (9CFR, EMEA, EP)	X		(X)
	Porcine virus assay 9CFR, EMEA, EP)	X		(X)
	PCR for Bovine & Porcine viruses (BPyV, Hep E, Parvovirus, Circovirus)	X		(X)

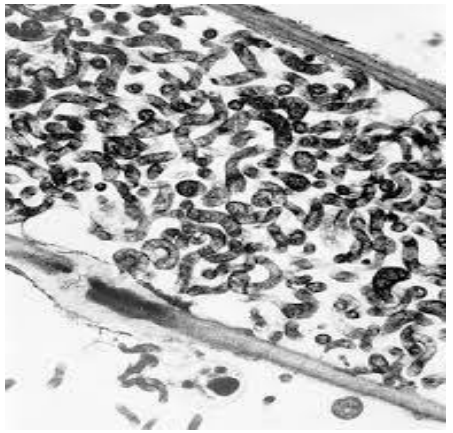
*West Nile Virus, Alpha Nodavirus, Rhabdovirus, St. Louis Encephalitis, Western Equine Encephalitis, Venezuelen Equine Encephalitis, Japanese Encephalitis

Spiroplasma

- Spiroplasma are a type of bacteria without cell walls. They have a parasitic lifestyle and are a disease causing agent in plants, for example leading to deformities in fruit.
  - Grow at ~30C and have a helical morphology



- Many Spiroplasma strains are vertically transmitted endosymbionts of Drosophila species
  - S.poulsonii protects its insect host from parasitic nematodes and parasitoid wasps via ribosome inactivating proteins (similar to Ricin!)



- Limited evidence of pathology in humans, but regulations require testing:
  - FDA PTC 1993 “Biological products made in insect cell lines should be tested for both mycoplasma and spiroplasma contamination”.
  - EP 2.6.7 and USP 63 – Agar and broth cultivation assays, including spiroplasma statis qualification

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Adventitious agent testing (Detector cell culture)

Assay code	003046GMP.BUK	003051GMP.BUK
Duration of assay	28 days	14 days
Detector cell lines	MRC-5, Vero, BHK, SF9	MRC-5, Vero, BHK, SF9, C6/36
Regulatory compliance	FDA 2010, EC CPMP EP 2.6.16 (virus seeds)	EP 5.2.3, WHO TR 878
TEM testing	Insect detector cells only	Insect detector cells only
Heat shock included?	No	Yes (WHO compliance)

- 003046GMP meets US FDA 2010 and EP 2.6.16 vaccine requirements.
- Possible to make a tech spec amendment to have 28 day 003051GMP assay

Toxicity of insect cell media

- Insect cell media can be toxic to suckling mice and embryonated eggs. Recommend pre-studies are performed for:
  - Insect Master Cell Bank (results applied to EPC)
  - Baculovirus Master Virus seed

- GLP compliant assays used for this investigation:
  - 005060.BUK – In vivo pre-study for toxicity using suckling mice
  - 005063.BUK – In vivo pre-study for toxicity and interference using embryonated eggs

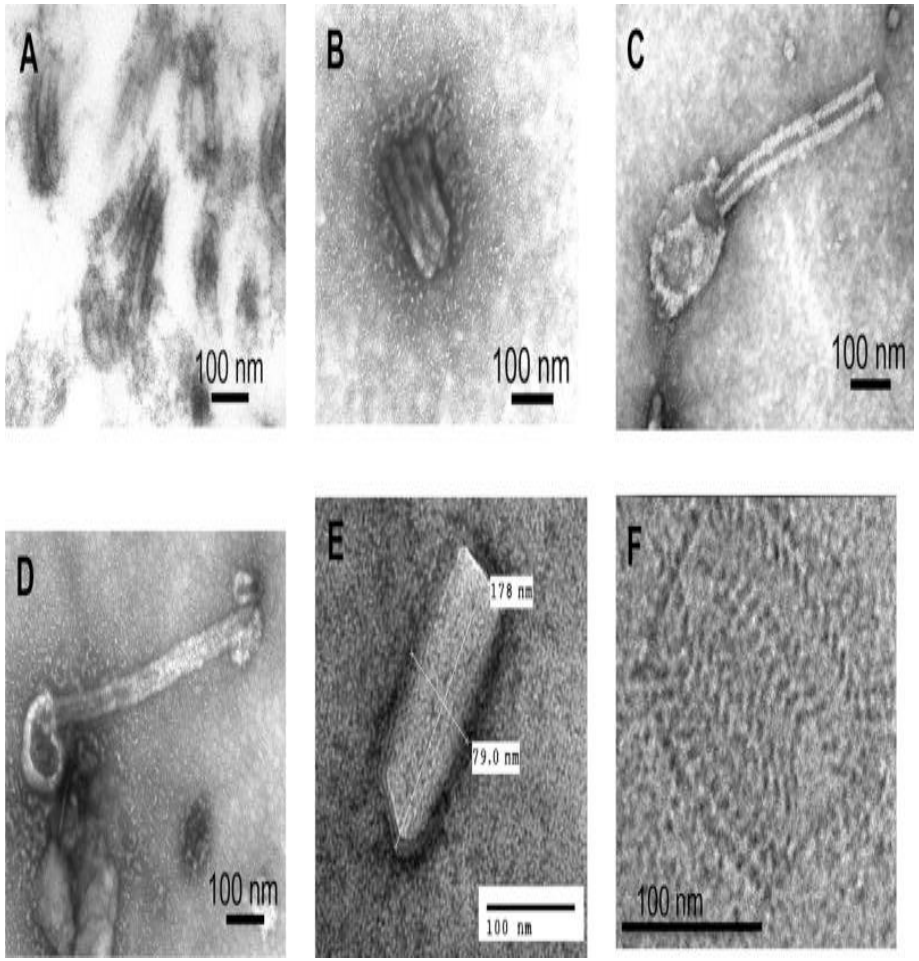
- Pre-studies must be completed before the GMP In vivo assays (005071GMP) can be initiated. Impact on overall timelines for MCB and MVSS characterization.

Rhabdovirus (305600GMP.BSV)

- In 2014, Arifa Khan and colleagues at the FDA identified a novel Rhabdovirus in SF cells. Ma H, Galvin TA, Glasner DR, Shaheduzzaman S, Khan AS. Identification of a novel rhabdovirus in Spodoptera frugiperda cell lines. *J Virol*. 2014;88(12):6576-6585. doi:10.1128/JVI.00780-14

- Detected by Massive Parallel Sequencing (NGS) and subsequently confirmed by TEM.

- CFX platform PCR assay available and is recommended for all SF9 and SF21 cell lines. The presence of Rhabdovirus in other insect cell lines, such as S2, has not been confirmed.



Virus Selection – Viruses of relevance for insect cell lines

Virus	Genome	Envelope	Family	Size (nm)	Resistance To Physical / Chemical Reagents
Baculovirus	RNA	Yes	Baculoviridae	260	Low
Alphanodavirus	RNA	Yes	Nodaviridae	30	Low - Medium
WNV	ssRNA	Yes	Flaviviridae	45-50	Low - Medium
Togavirus	ssRNA	Yes	Togaviridae	65-70	Low - Medium

Virus Selection – Model viruses

Virus	Genome	Envelope	Family	Size (nm)	Resistance To Physical / Chemical Reagents
Baculovirus	RNA	Yes	Baculoviridae	260	Low
WNV	ssRNA	Yes	Flaviviridae	45-50	Low - Medium
Sindbis (Togavirus)	ssRNA	Yes	Togaviridae	65-70	Low - Medium

Thank you!

Steven.mcdade@merckgroup.com
Tel +44 7767 831 277