Cell Banking - Master Cell Banks (MCB) and Working Cell Banks (WCB)

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Presentation Overview

- Cell Banking - Definitions, Systems & Considerations
- Guidelines
- Manufacturing and Storage
- Origin, Source and History
- Characterisation and Testing
- Conclusions

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Cell Banking Systems and Definitions

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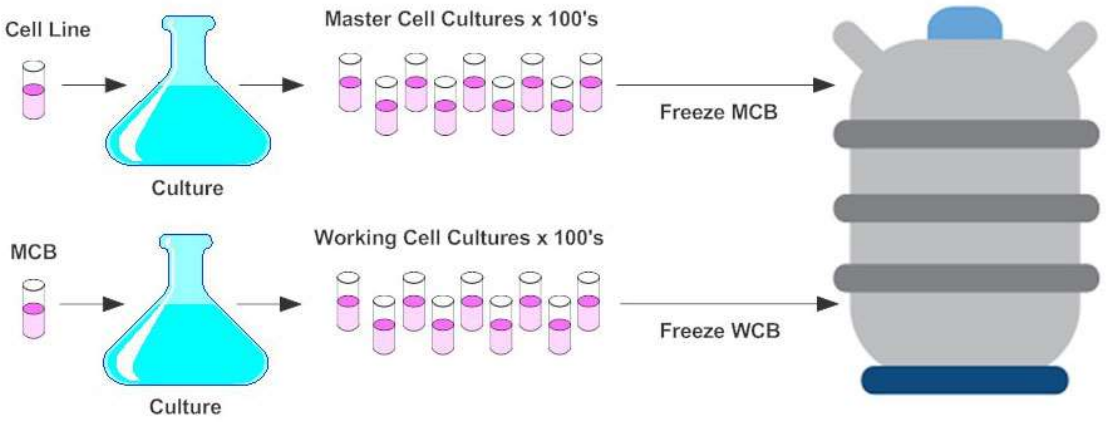
Cell Banking Definitions (ICH)

- Cell Bank:
  - A cell bank is a collection of appropriate containers, whose contents are of uniform composition, stored under defined conditions. Each container represents an aliquot of a single pool of cells
- Pre-seed bank
  - Research bank from the selected clone
- Master Cell Bank (MCB):
  - An aliquot of a single pool of cells which generally has been prepared from the selected cell clone under defined conditions, dispensed into multiple containers and stored under defined conditions. The MCB is used to derive all working cell banks. The testing performed on a new MCB (from a previous initial cell clone, MCB or WCB) should be the same as for the MCB unless justified
- Working Cell Bank (WCB):
  - The Working Cell Bank is prepared from aliquots of a homogeneous suspension of cells obtained from culturing the MCB under defined culture conditions

Conventional Cell Banking + Production System



Some Cell Banking Definitions

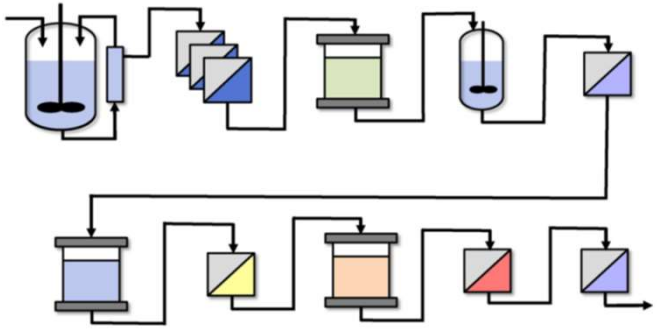
Research Bank

↓

MCB

↓

WCB → Fermentation → DSP (Downstream Processing)



Fermentation ↓ EPC

End of Process Cells (EPC)
Cells at the end of the routine fermentation process

Fermentation ↓ Further cultivation ↓ PPC (CAL)

Post Process Cells (PPC)
Cells at the limit or beyond the production *in vitro* cell age (for validation only)

***In vitro* cell age**
Time or population doublings between thaw of the MCB and harvest of the production fermentation harvest

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Cell Banking - Important Considerations

- They are the initial point of every biotechnological production
  - Cell banks are an *API starting material*
- Production of the intended substance should be consistent in quantity and quality:
  - Show stability and robustness regarding productivity over the whole life cycle of fermentation
- For establishment and maintenance is important to have a characterized common starting source for each production lot - *Major impact in product safety and purity*
  - Qualification of raw materials
    - e.g. media components
  - Characterisation of the cell banks

    Authenticity, purity, stability
  - Process & environment

    IPC, cleaning, virus validation, ..
  - Characterisation of the product

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Guidelines

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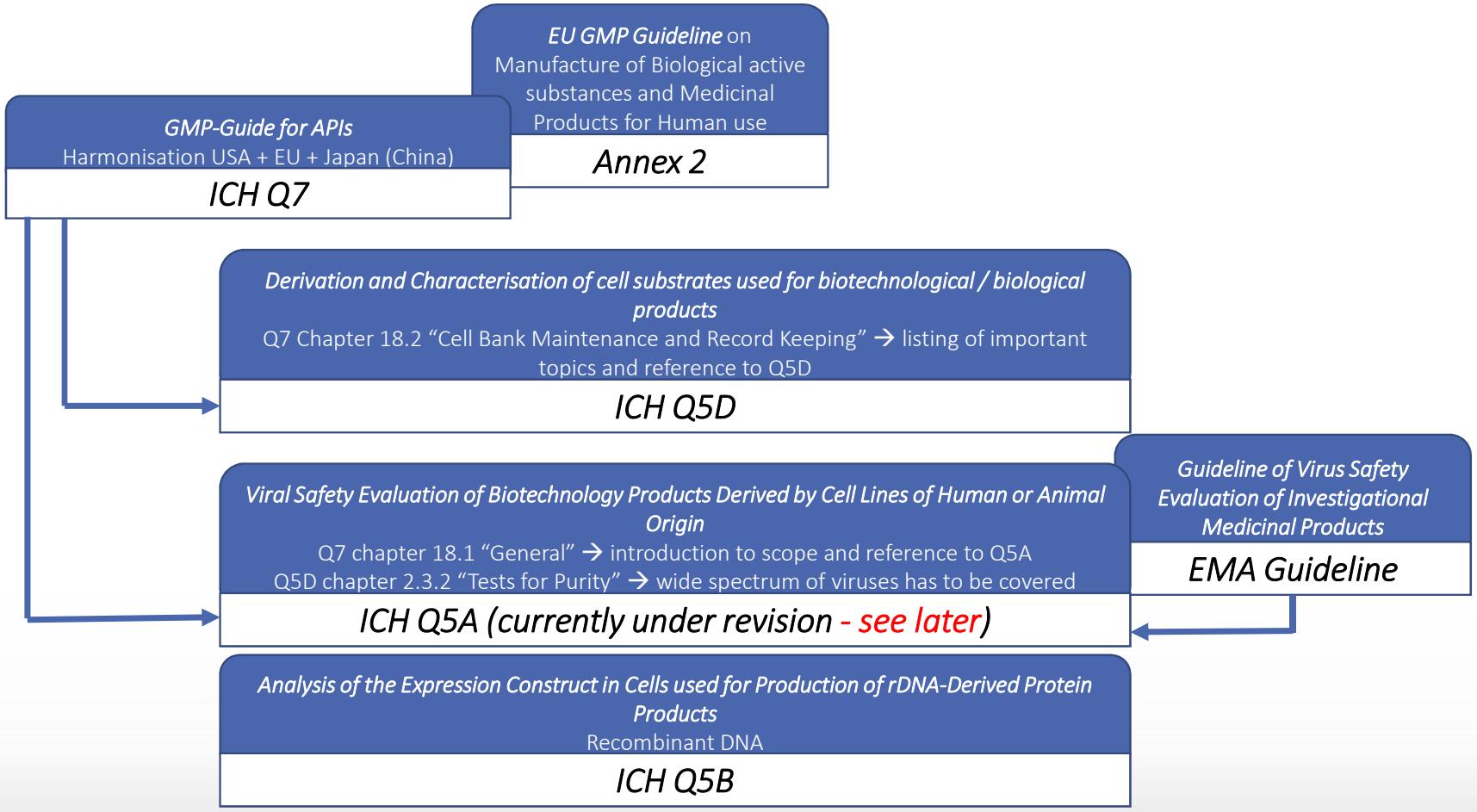
Guidelines Overview



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ICH Q7- Application to API Manufacturing

Type of Manufacturing	Application of this Guide to steps (shown in grey) used in this type of manufacturing				
Chemical Manufacturing	Production of the API Starting Material	Introduction of the API Starting Material into process	Production of Intermediate(s)	Isolation and purification	Physical processing, and packaging
API derived from animal sources	Collection of organ, fluid, or tissue	Cutting, mixing, and/or initial processing	Introduction of the API Starting Material into process	Isolation and purification	Physical processing, and packaging
API extracted from plant sources	Collection of plant	Cutting and initial extraction(s)	Introduction of the API Starting Material into process	Isolation and purification	Physical processing, and packaging
Herbal extracts used as API	Collection of plants	Cutting and initial extraction		Further extraction	Physical processing, and packaging
API consisting of comminuted or powdered herbs	Collection of plants and/or cultivation and harvesting	Cutting/ comminuting			Physical processing, and packaging
Biotechnology: fermentation/ cell culture	Establishment of master cell bank and working cell bank	Maintenance of working cell bank	Cell culture and/or fermentation	Isolation and purification	Physical processing, and packaging
"Classical" Fermentation to produce an API	Establishment of cell bank	Maintenance of the cell bank	Introduction of the cells into fermentation	Isolation and purification	Physical processing, and packaging

Note: Cell banking is considered a step prior to API manufacture (it is an ***API start material***)

For biotechnological API-production
ICH Q5D, Q5A & Q5B

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EU GMPs Annex 2: Application to API Manufacturing

	immunosera,				
2. Virus or bacteria/ fermentation/ cell culture	Viral or bacterial vaccines; enzymes, proteins	Establishment & maintenance of MCB[8], WCB, MVS, WVS	Cell culture and/or fermentation	Inactivation when applicable, isolation and purification	Formulation, filling
3. Biotech-nology - fermentation/ cell culture	Recombinant products, MAb, allergens, vaccines	Establishment & maintenance of MCB and WCB, MSL, WSL	Cell culture and/or fermentation	Isolation, purification, modification	Formulation, filling
4. Animal sources: transgenic	Recombinant proteins,	Master and working transgenic bank	Collection, cutting, mixing, and / or initial processing	Isolation, purification and modification	Formulation, filling
5. Plant sources: transgenic	Recombinant proteins, vaccines, allergen	Master and working transgenic bank	Growing, harvesting[9]	Initial extraction, isolation, purification, modification	Formulation, filling
6.Human sources	Urine derived enzymes, hormones	Collection of fluid[10]	Mixing, and/or initial processing	Isolation and purification	Formulation, filling
7. Human sources	Products from cells tissues	Donation, procurement and testing of starting tissue / cells[11]	Initial processing, isolation and purification.	Cell isolation, culture, purification, combination with non-cellular components.	Formulation, combination, filling

Increasing GMP requirements

Note: GMP compliance is required from establishment and maintenance of MCB onwards

Source: EudraLex: The Rules Governing Medicinal Products in the European Union Volume 4; EU guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use; Annex 2: Manufacture of Biological active substances and Medicinal Products for Human Use

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Manufacturing and Storage

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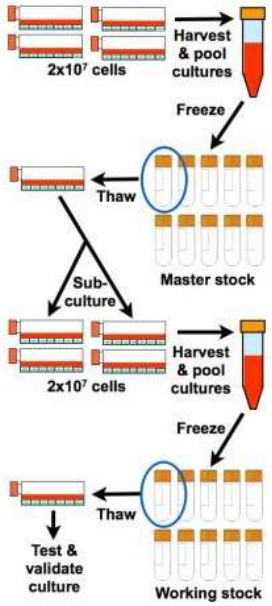
Manufacturing Cell Banks (1)

- Calculate the size of cell banks → estimated product life span
- SOPs → for preparation methods including cryoprotectants and media used
- Avoid contaminations from operators and environment
  - e. g. laminar airflow hood for open process steps; room classification can also be important
- Avoid cross-contamination from other cell banks
  - Handle only one cell bank at a time
- Prepare the cell bank from a single pool to ensure uniformity
  - Same cell density in first and last vial
  - Try to minimise exposure to cryoprotectants (for larger banks ensure that all vials remain at the same temperature to minimise variation between first and last vials)



Manufacturing Cell Banks (2)

- Aliquot in suitable sterilised containers
  - vials, ampoules or other appropriate vessels
- Procedure to trace the containers
  - cold resistant labelling/documentation
- Freeze at a controlled freezing rate
  - Other preservation and storage may be also be suitable
  - e. g. freeze-drying for microbial cells
- Transfer to vapour phase of liquid nitrogen or ultra-low freezers (e.g. <-135°C) for long term storage

Storage of Cell Banks

- Store at appropriate conditions
  - to maintain viability and prevent contamination
- Protect cell banks from fires, power outages and human error to:
  - Guarantee continuous, uninterrupted production
  - Minimise the risk of loss
  - Actions:
    - Limited access for authorised staff
    - Alarm systems
    - Controlled filling of liquid nitrogen systems (automated systems need careful validation and control)
    - Back-up storage → split banks between 2 storage locations
- Traceability of vial
- Continuous Records of storage conditions
- Cell banks should be monitored periodically (interval to be defined by the manufacturer) to demonstrate continued suitability for production (viable cell number/ growth characteristics)

Storage Considerations with Liquid Nitrogen

- Liquid nitrogen can act as a vehicle for transmission of viruses, bacteria and fungi
- Condensation of the atmosphere within the cryotubes creates a vacuum which can draw in liquid nitrogen
- Bone marrow and stem cells harvested from patients undergoing cytotoxic treatment became contaminated with Hepatitis B virus (HBV) as a result of storage in liquid nitrogen and caused the transmission of HBV (Tedder *et al.* 1995)
- Study at Roche: Liquid nitrogen was contaminated with 8 cfu/100ml- mainly *Bacillus*, *Staphylococcus* and *Aspergillus* species;
  - Source: Holger Kavermann; ECA ICH Q7 Course (Nov 2019; Prague- presentation on cell banking)
- Storage of cell banks in the vapor phase needs to be ensured using sealed ampoules
  - Ultra-low freezers offer an alternative that minimises these risks without compromising stability of the cell bank

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Origin, Source, History, Characterisation and Testing

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Potential Sources of Risk

- Donor or Source cells:
  - Inapparent infections - many virus infections have no clinical symptoms *(e.g. endogenous Retroviruses)*
  - Poorly controlled collection or handling procedures - introduction of contaminants
  - Re-activation of latent viruses *(e.g. EBV)*
- Medium components
  - Many culture or differentiation procedures for cells require the use of animal or human derived components:
    - Platelet derived lysate for stem cells (limits now set on the maximum number of donations allowed to be pooled)
    - Bovine serum *(e.g. BVDV, BPyV, ...)*
    - Porcine Trypsin *(e.g. PPV, PCV, ...)*
    - Purified bovine components
    - Recombinant expressed growth or differentiation factors
  - Cell culture medium
    - Even chemically defined medium can theoretically be contaminated; *e.g. MMV, Vesivirus 2117*

Characterisation and Testing

- Characterisation of MCB/WCB should include
  - Tests of identity (*Q5D*)
  - Tests of microbial purity (*Q5D*)
  - Tests of viral purity (*Q5A/EMA*)
  - Cell substrate stability (*Q5B*)
  - [Tests for karyology and tumorigenicity] (*Q5D*)
    - Tumorigenicity is particularly important for vaccine cell lines
  - Analysis of the expression construct (*Q5B*)
  - Suitability for manufacturing (*Annex 2*)

Tests for Virus Purity- Historical Examples

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Year of Contamination	Contaminations (virus / host cell)	Total
1985-1989	Bluetongue / CHO EHDV / CHO	2
1990-1994	Herpesvirus / Primary Monkey Herpesvirus / Vero MMV / CHO (x2) Parainfluenza virus / MRC-5 Reo3 / MRC-5 Simian adenovirus / Primary monkey	7
1995-1999	Cache valley virus / CHO Reovirus / Human primary kidney Vesivirus 2117 / CHO	3
2000-2004	CVV / Unknown (x2) Human adenovirus / HEK293	3
2005-2010	CVV / CHO MMV / CHO (x2) Vesivirus 2117 / CHO (x3)	6
2010-Present	MMV / CHO MMV / BHK-21 PCV-1 / Vero	3
Unknown	MMV / BHK-21 Reovirus / Unknown	2
	Total:	26

Data from Barone et. al.; Nature Biotechnology (2020); Vol 38; pp 563-572

Methods of Virus Detection

- *In vivo* tests
  - Antibody production tests
  - Animal safety tests
- *In vitro* tests
  - Cell culture based
  - Molecular biological methods
    - PCR
    - RT, PERT
    - Next generation sequencing (currently under discussion for inclusion in the next revision of ICH Q5A)
- Electron microscopy
  - For characterisation and quantification of retrovirus like particles
- (Immunoassays)
  - Can be used if justified

Methods of Virus Detection: *In Vivo* Adventitious Agent Tests

- Inoculation of different animal species
  - Adult mice *(not required by EP anymore)*
  - Suckling mice
  - Embryonated hens´eggs
  - (guinea pigs, rabbits)

- Health status of animals is monitored
  - Any indications of ongoing infection require additional investigations to determine the cause

In vivo Methods of Viral Detection: Antibody Production

- Inoculation of virus-free animals (MAP/HAP/RAP)
  - Detection of viruses present in rodent cell lines
  - Mouse (MAP), hamster (HAP) or rat (RAP) Antibody Production test

- Monitoring for the presence of antibodies against specific viruses or elevated liver enzyme
  - Detection by ELISA, IF, HI etc

Methods of Viral Detection: *In Vitro* Cell Culture

- Inoculation of 3 (-4) different cell types
  - Same species/tissue type as continuous cell line
  - Cultures of a human diploid cell line (MRC-5)
  - Another cell line from a different species (monkey kidney cells)
  - Rodent production cells: cell line for detection of MMV (A9/324K)
    - PCR testing for MMV is accepted as an alternative here
- At least two weeks observation period, sub-passage may increase sensitivity
- Culture supernatant is tested for haemagglutination inhibition (HI), cells for haemadsorption (HA), presence of CPE, IF
- Erythrocytes originating from different species (chicken, human, guinea) should be used in parallel

Problem Materials

- Test articles that contain live virus are difficult to test (e.g. vaccines). Potential solutions include:
  - Virus could be neutralised with specific serum/antibody
  - Removal of live virus from test article (e.g. filtration)
  - Cell lines that are non susceptible for this specific virus may be used
  - Appropriate facilities/procedures etc are essential for testing of BSL-3 agents

- Highly cytotoxic Test Articles (e.g. cytokines) may impede testing
  - Sample pre-treatment may be required (e.g. centrifugation, dialysis etc)
  - Effect of sample pre-treatment on assay validity must be evaluated

ICH Q5A Revision 2

New Draft for Public Comment Released 29 September 2022 *(deadline for submitting comments is already passed)*

- The ICH Q5A guidance has been a widely accepted reference document for virus safety for almost 30 years
  - Revised in 1999, it was recognised as needing updating to reflect knowledge gained and the implementation of more modern technologies like PCR and NGS
- The scope of the guideline has now been widened:
  - *This document covers products produced from in vitro cell culture using recombinant DNA technologies such as interferons, monoclonal antibodies, and recombinant subunit vaccines. It also covers products derived from hybridoma cells grown in vivo as ascites: special considerations apply for these products, and Annex 1 contains additional information on testing cells propagated in vivo. The document also applies to certain genetically-engineered viral vectors and viral vector-derived products, which can undergo virus clearance without a negative impact on the product. These products may include viral vectors produced using transient transfection or from a stable cell line, or by infection using a recombinant virus. It also includes viral vector-derived recombinant proteins, for example, baculovirus-expressed Virus-Like Particles (VLPs), protein subunits and nanoparticle-based vaccines and therapeutics. Furthermore, the scope includes Adeno-Associated Virus (AAV) gene therapy vectors that depend on helper viruses such as baculovirus, herpes simplex virus or adenovirus for their production.*

ICH Q5A Revision 2- Time for NGS to Become more Routine?

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Key Points Around NGS/PCR Testing

- *Next Generation Sequencing (NGS) and Nucleic Acid Amplification Techniques (NATs) such as Polymerase Chain Reaction (PCR) may be appropriate for broad and specific virus detection, respectively. The introduction of these tests may be done without a systematic head-to-head comparison with the currently recommended in vitro and in vivo assays. In particular, a head-to-head comparison is not recommended for in vivo assays to meet the intent of the global objective to replace, remove, and refine the use of animals.*

- *Adventitious virus testing should be routinely applied to each unprocessed bulk. This may include in vitro screening assays using several cell lines or broad molecular virus detection methods such as NGS (see Section 3.2). Based on the risk assessment (considering the cell substrate, use of animal-derived raw materials or reagents, and level of virus clearance of the process), the indicator cell cultures should be observed for at least 2 weeks. Detection for specific viruses or families of viruses may also be appropriate to include based on risk assessment (e.g., Minute virus of mice). When appropriate, a PCR or other molecular method may also be selected as rapid test methods can facilitate real-time decision making.*

Quality Control of Raw Materials: Cell banks

- Master Cell Bank (MCB)
  - Testing based on origin
  - Extensive testing for endogenous and non-endogenous viruses
  - General in-vitro and in-vivo tests and specific tests as appropriate

- Working Cell Bank (WCB)
  - Limited testing required

- Cells at the end of production (EOPCB)
  - Testing based on origin
  - Extensive testing for endogenous and non-endogenous viruses
  - General in-vitro and in-vivo tests and specific tests as appropriate

Virus Tests to be Performed once on Cell Banks: Retroviruses and Endogenous Viruses

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	MCB	WCB	EPCB
Infectivity	**Yes**	**No**	**Yes**
Electron Microscopy	**Yes**	**No**	**Yes**
Reverse Transcriptase	**Yes**	**No**	**Yes**
Other virus-Specific tests	**As appropriate**	**No**	**As appropriate**

Virus Tests to be Performed once on Cell Banks: Non-Endogenous and Adventitious Viruses

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	MCB	WCB	EPCB
In vitro	**Yes**	**(No)**	**Yes**
In vivo	**Yes**	**(No)**	**Yes**
Antibody production	**Yes**	**No**	**No**
Other virus-Specific tests	**Yes**	**No**	**No**

Virus Safety in Clinical Trials

- Q5A defines the data requirements for marketing authorisation applications (MAA)
- Extend of viral safety tests on cell banks used in clinical trials was often controversially disputed with the authorities.
- EMA "Guideline on virus safety evaluation of biotechnological investigational medicinal products" provides scientific guidance:
  - Complete testing of the MCB as described in the Q5A prior to initiation of Phase I trial
  - Testing of WCB (if established) in principle as described in the Q5A but: PPC testing is not required if testing on the unprocessed bulk is performed as indicated in Table 1 (see next slide)

Testing for Investigational Medicinal Products (EMA Requirements)?

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- EOPC testing for investigational products is only necessary where there is no testing of the unprocessed bulks
- Testing for unprocessed bulks:

	***In vitro* testing**	**Tests for infectious retroviruses**	***In vivo* testing**
CHO	Yes, all bulks	No	No
NS0 and Sp2/0	Yes, all bulks	Yes, once for given scale	No
All other cell lines	Yes, all bulks	Yes, once for given scale	Yes. Once for given scale

Genetic Stability of the Production Cells

- Performed to ensure that the genetic constructs within the cells are stable throughout the whole production process (Q5B):
  - Sequencing of the transgene (MCB and PPC)
  - Determination of the copy number (MCB and PPC)

- Additionally the suitability for manufacturing should be evaluated:
  - Doubling time of cells
  - Viability
  - Specific productivity for the expressed protein

Summary

- The sourcing, QC testing and validation of virus clearance are the key pillars in the safety tripod for ensuring purity of the cell bank

- Virus safety studies are often a source of questions and delays to product approval:
  - Studies should be appropriately designed to cover all aspects of regulatory guidelines
  - Studies can take a long time (e.g. ~4-6 months) and so should be planned appropriately to avoid delays
  - Many of the tests are standard, but certain products may require optimisation of the tests, e.g.:
    - Live virus vaccines
    - Highly cytotoxic or interfering substances (e.g. Cytokines)
    - Antibody containing matrices