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Characterization and testing of animal cell substrates used for production of biologicals

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Cell bank systems

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Outline

- Briefly review use of animal cells in production of biotherapeutics and vaccines
- Major concerns
- Concept of Master and Working cell banks
- Virus testing programmes

Biologicals

- Cell banks play a pivotal role in the production of modern biological medicines, including rDNA derived products.
- Many biologicals have been extracted from animal fluids and tissues (including human) (*e.g. antivenoms, heparins, old smallpox vaccine, rabies vaccine made in animal neural tissue)*
- Now biologicals produced in microbial, animal or plant cells (*e.g. vaccines, biotherapeutics*)

Critical Manufacturing Issues in the production of biologicals in cells

- **Production system- type of cells used mammalian / bacterial / yeast / insect / plant**
- Cell culture / fermentation - Batch or continuous production system
- **Genetic stability of cell substrate and microbial seeds**
- **Viral safety issues for mammalian cells**
- Separation , purification , characterization of resulting viral antigen or protein , including glycosylation or other post-translational modifications
- Product / **host cell related impurities** (**including for mammalian cells residual DNA**)
- Emphasis on consistency of production

Biologicals - slight changes in process can have a major impact on clinical performance / safety of the product. Consistency of production critical.

Mammalian Production cells

- **Primary** - taken directly from one or more animals for each production run (e.g. monkey kidney cells)
- **Diploid** – well characterized frozen source of cells which have a finite *in vitro* lifespan and display diploid cytogenetic characteristics. Derived by serial subculture in tissue culture medium of original isolated cells. Generally identical to those of the species from which they were derived (WI-38 : MRC5 cells originally from lung tissue of an aborted human fetus)
- **Continuous** (transformed) – well characterized frozen source of cells with unlimited capacity for population doubling (e.g. CHO cells from Chinese hamster ovaries)

Inactivated and live viral vaccines made using animal cells

- **Polio** - primary monkey kidney cells (directly from clinically healthy animals); human diploid cells (MRC-5 cells), continuous cells (Vero cells)
- **MMR** - avian embryo cell culture (eggs), human diploid cells
- **Rotavirus** - continuous cells (Vero cells)

Biotherapeutics / biosimilars

- **Continuous mammalian cells lines** are the substrates of choice for many rDNA products because they can be transfected and engineered to grow rapidly and produce glycosylated products.
- A range of cells in use - Chinese hamster ovary cells (CHO) , PER.C6, MDCK, etc.
- Bacterial systems (*E. coli*) do not glycosylate the recombinant protein (but still used if glycosylation is not important for product performance)

Three main concerns about mammalian cell substrates

- **Genetic stability** of cell lines
- Contamination of a product by **possibly oncogenic host cell DNA** in products derived from continuous mammalian cells and induction of cancer (**Held up the use of such cells)**
- **Presence of adventitious viruses** - animal cells have the capacity to **propagate viral agents**. In addition, animal cells contain endogenous agents such as retroviruses that may also be of concern.

Genetic stability of cell line

- Primary and diploid cells considered normal cells by existing criteria.
- Diploid cells are non tumorigenic and have a finite life in culture
- Diploid production cells are monitored to show that diploid cytogenetic characteristics are retained on passage until they enter senescence. Avoid genetic drift.
- Diploid cells have a defined maximum population doublings when used in production

Residual host cell DNA

- See an evolution of consensus on recommendations
- In 1986 a WHO Study Group on Cell Substrates was set up to examine the cell substrates issues in great detail, especially to address the residual DNA risk (Report 1987)
- **Concluded no reason to exclude continuous cell lines from production** provided that purification processes reduced DNA to acceptable levels and /or eliminated its biological activity
- On basis of evidence available at the time, Group concluded the risk associated with residual DNA was negligible when amount of DNA is reduced to less that 100 pg per parental dose. **With new data this later revised to 10 ng per dose**.

Adventitious viral agents

- Adventitious viral agents refer to viruses which may contaminate mammalian production cells or are unintentionally introduced into the manufacturing process of a biological.
- The source of these contaminants may lie in the passage history of the production cell line, the raw materials used in culture media to propagate the cells (e.g. in cell banking, in production), in processing the cells (e.g. trypsin), the environment, personnel, equipment or elsewhere)
- From the beginning, production of viral vaccines using primary animal cells involved a strict testing programme for adventitious viral agents
- Cells from wild animals usually show a higher frequency of viral contamination than colony animals

Introduction of Human Diploid cells

- Technology developed which enabled growth and passage in tissue culture medium and cryogenic preservation of cells at low population doubling levels
- Cryopreserved diploid cells could later be expanded to provide production cells for many decades
- **This allows EXTENSIVE testing of cells for adventitious viral agents and for their characteristics before use**; primary cells cannot be as extensively tested.
- **Concept of a Master Cell Bank (MCB) and Working Cell Bank (WCB) system introduced – provides for a standardized source of production cells on a routine basis**
- **Concept now used for all cell lines – advocated best practices (cGMP)**

Master and Working Cell Banks

- **Cell bank:** a collection of appropriate containers whose contents are of uniform composition, stored under defined conditions.
- **MCB** well-characterized cells derived from a cell seed at a specific PDL or passage level, dispensed into multiple containers, cryopreserved and stored frozen under defined conditions.
- **WCB** is derived from one or more containers of the MCB. Used to directly provide cells for the manufacturing process. A newly prepared WCB is appropriately qualified by characterisation and testing. The testing programme less than for MCB

Viral Safety of Biologicals produced in animal cells

- *WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological products and for the characterization of cell banks (2010)* : ICH, national guidance
- Encourages production based on cryopreserved cell banks - exhaustively screened for virus contamination, **and with well documented history**.
- **Control of raw material used in production also critically important – e.g. growth media, trypsin**
- Closed systems for growth of cell culture
- **Testing each cell culture for viral contamination**
- **Validation of viral removal / inactivation by downstream processing** (this possible for rDNA products - unlike live viral vaccines)

For adventitious viruses Belts and braces approach

- Test and qualify the MCB and WCB
- Risk assessment and testing when necessary of raw materials used in production of cell banks and routine cultures
- Routine testing of each production lot for adventitious agents which might have contaminated the system (e.g. infected growth media, trypsin used to release cells)

Validation of virus removal – further step in risk reduction

- When appropriate, validation of purification procedures or viral inactivation procedures should demonstrate adequate reduction of relevant model viruses, with a significant safety factor.
- Provides added assurance product is free of virus contamination
- Usually required for recombinant protein products.
- Inactivation procedures should be shown not to compromise safety and efficacy of product
- **Not possible for live viral vaccines**

Primary Cells

- If they are necessary for the production of a given biological, the frequency of contaminated cell cultures can be significantly reduced by screening source animals for the absence of specific species specific viruses including simian viruses.
- Viruses can be detected by molecular tests such as PCR and by looking for the presence of circulating antibodies to those viruses in source animals.
- The use of animals bred in carefully controlled colonies, especially Specific Pathogen Free colonies, is strongly recommended.
- Nevertheless, as suitable alternative cell substrates become available, primary cells are less likely to be used in the future

Strategies for testing cell banks for microbial agents

- Perform exhaustive testing at the MCB level then more limited testing on the WCB derived from the MCB. Limited testing should focus on those agents that could potentially be introduced during the production of the WCB from the MCB.
- If the number of vials of a MCB is limited, an alternative strategy would be to conduct the more exhaustive testing on the **first WCB** made from that MCB, and to limit testing on the MCB itself.
- End of Production Cells should be characterized once for each commercial production process.
- Testing an Extended Cell Bank serves as further characterization of the MCB or WCB

Testing for adventitious agents in cell banks

- Based on type of cells used (simian, avian, human, rodent, etc.)
- Traditional testing methods now augmented by molecular methods (PCR, Pert Assay, etc.)
- Efforts to identify viruses by testing for viral sequences or other viral markers, especially those not detectable by other means, constitute an important part of the evaluation of cell banks
- Risk assessment should include transmission studies to target cells or animals .
- Generally, contaminated cells should not be used in production (exceptions - CHO/rodent cells express endogenous defective retroviral particles but widely used for production of rDNA products: risk mitigation - show removal during purification)

Traditional testing for adventitious agents-*Tests in animals and eggs*

- Embryonated eggs
- Adult and suckling mice
- Guinea pigs
- Rabbits
- Antibody production tests in mouse, rat or hamster
- Variety of test performed

Traditional testing for adventitious agents - *tests in cell culture*

- Detect a broad array of viruses / viral families
- Decisions about which cell lines to use as **indicator cells** should be guided by the species and passage history of the production cell substrate, taking into consideration the types of viruses to which the cell substrate could potentially have been exposed and thus the viruses one would like to detect by this assay method.
- Examine cells for cytopathic effects (CPEs) after growth of cells with test material.
- Infectivity assays for retroviruses
- Test for haemadsorbing and haemagglutinating viruses

Molecular viral detection methods

- PCR
- PERT (product enhanced assay for reverse transcriptase / retroviruses)
- Detect designated viruses or virus types
- Transmission Electron microscopy
- Deep sequencing - next generation sequencing (may detect agents not previously detected

Characterization and Testing of Cell Banks

- Highly specialized activity
- Needs lots of expertise and infrastructure
- Needs to have and maintain a range of indicator cells and reagents
- Sometimes contracted out to specialized testing organizations

Post approval changes to cell banks

- New MCB from source unrelated to pre MCB material - **may require new application for marketing authorization**
- Adaptation of MCB into new culture medium **- Major**
- New MCB from original clone - **Moderate**
- New WCB - **Minor**
- Some changes may require a GMP inspection or may be reviewed during next routine inspection

(*WHO Guidelines on procedures and data requirements for changes to approved products*: Annex 3 WHO Technical Report Series 1011, 2018)

Good cell culture practice Documenting / traceability of data

- Sourcing, banking and preparing cell cultures,
- Donor should be free of transmissible diseases or diseases of uncertain aetiology
- Authenticity, including identity, provenance and genotypic/phenotypic characteristics of cells
- Absence of contamination with another cell line;
- Absence of microbiological contamination;
- Stability and functional integrity on extended in vitro passage
- Staff training in all cell culture processes is vital to ensure that correct procedures are adhered to under GMP

Final Comments

- The MCB, WCB and cell culture processes are key to consistently producing safe and efficacious biological medicines
- History of production cell banks, handling, growth, monitoring for genetic stability, testing for viral contamination are major issues in the production of biologicals

THANK YOU FOR YOUR ATTENTION