



Chinese Hamster
Ovary (CHO)
Cell Banks Safety
Testing and
Characterization

Simplifying Progress

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Chinese Hamster Ovary (CHO) Cell Banks Safety Testing and Characterization

CHO cells are by far the most commonly used production cell lines in the pharmaceutical industry with a safety profile established over 20 years. The guidelines for the safety and characterisation of CHO production cell lines are harmonised in the International Conference on Harmonisation (ICH) Q5 (R1) "Viral Safety Evaluation of biotechnology products derived from cell lines of human or animal origin" and ICH Q5D "Quality of Biotechnological Products: Derivation and Characterization of Cell Substrates Used for Production of Biotechnological Biologic Products".

Since 2007, Sartorius has performed safety and characterization testing of over 200 Chinese Hamster Ovary (CHO) cell banks. Our scientists have a wealth of experience and knowledge and will advise on the most appropriate and cost-effective testing strategy to meet scientific and regulatory requirements. The package of testing recommended takes into consideration the history of the cell line and raw materials used during the manufacture of the bank.

Sartorius offer a comprehensive GMP, GLP compliant suite of assays to support release of CHO production cell banks for GMP manufacturing, in accordance with ICH Q5 (R1) and ICH Q5D in our MHRA licenced and FDA inspected facilities.

Test	MCB	WCP	EoPC
Identify	+	+	+
Mycoplasma	+	+	+
Sterility	+	+	+
In vitro assays	+	+	+
In vivo assays	+	-	+
Antibody tests	+	-	-
Retrovirus infectivity	+	-	+
Reverse transcriptase	-	-	-
Electron microscopy	+	-	+
Species specific tests	+	-	-

Table 1: Overview of GMP, GLP Testing Requirement

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Master Cell Bank Testing (MCB) and End of Production Cell Bank Testing (EoPC)

Prior to initiation of GMP manufacturing of product, a Master Cell Bank (MCB) must be created. The MCB is a uniformly expanded cell line from a clonal population of cells, homogeneously distributed into cryovials, forming the first expansion of the cell line and requiring extensive characterisation. All Working Cell Banks (WCB) are manufactured from the established MCB. Testing for the presence of microbiological contaminants as well as extensive screening for the presence of both exogenous and endogenous viral contamination should be performed. Testing for the presence of microbiological contaminants as well as extensive screening for the presence of both exogenous and endogenous viral contamination should be performed.

MCB testing is performed once. A similar but slightly reduced testing package is repeated on the End of Production Cell Bank (EoPC) in order to determine that the cells remain unchanged from the original cells, and to detect any low level contamination which may have been present but undetected in the MCB. Testing the EoPC also allows detection of latent infections as well as any contaminants which may have introduced during the production process. Testing of EoPC used in production should be performed prior to submitting a Product Licence Application (PLA). A summary of the testing required for MCB and EoPC is described in Table 2.

Assay Description	Assay Duration (weeks)	Guide Sample Requirements
Microbiological Contaminants (Technical Note T1)		
Sterility test by direct inoculation according to EP USP JP, Matrix Validation and Test	4	1% of cell bank (minimum 2 vials) for test plus 1.5 x for qualification
Test for Mycoplasma according to EP USP (Agar, broth and indicator cells)	6	1 vial
Identify		
DNA barcoding assay for cell line identity	3	1 vial
Adventitious Agents Microbiological Contaminants		
28 day in vitro assay for the detection of adventitious agents (Vero, MRC-5 and CHO detector cell lines)	6	Prepared from Cell Expansion (or 3 vials + 70 mL spent supernatant)
In vivo adventitious agent assay: Viral Safety Evaluation of Biotechnology Products according to ICH Topic Q5A (R1) using Adult Mice, Suckling Mice and Embryonated eggs	8	Prepared from Cell Expansion (or 1×10^7 cells/mL in 23 mL spent supernatant)
Retroviruses (Technical Note T2)		
Detection of murine xenotropic retroviruses by Mus dunni co-cultivation followed by MiCI S+L- assay	6	Live cells required from Cell Expansion
Examination of 200 cell profiles by Transmission Electron Microscopy (TEM)	4	Live cells required from Cell Expansion
Species Specific Viruses		
5 viruses and Mouse Antibody Production Test (MAP), 16 viruses (not required for EoPC)	10	Prepared from Cell Expansion (or 1×10^7 cells/mL in 8 mL spent supernatant)
Bovine and Porcine virus screen by real-time PCR	2	1 vial
Detection of MVM by real-time PCR	2	1 vial
Detection of Vesivirus by real-time PCR	2	1 vial

Table 2: MCB and EoPC testing Refer to Technical Notes 1 – 6

Working Cell Bank (WCB) Testing

A package of limited testing should be performed on the WCB, as the MCB has already been extensively characterised.

In some cases full testing may be performed on the WCB as an alternative to the MCB. An outline of testing recommended on the WCB is shown on Table 3.

Assay Description	Assay Duration (weeks)	Guide Sample Requirements
Microbiological Contaminants (Technical Note T1) Sterility test by direct inoculation (Eu Pharm current edition 2.6.1 sterility, US Pharmacopeia <71>)	4	1% of cell bank (minimum 2 vials)*
Test for mycoplasma (Eu Pharm, current edition 2.6.7. mycoplasmas and USP - NF Mycoplasma test, USP <63> current edition)	6	1 vial
Identify DNA barcoding assay for cell line identity	2	1 vial containing minimum 1×10^7 cells
Adventitious Agents 28 day in vitro assay for the detection of adventitious agents (Vero, MRC-5 and CHO detector cell lines)	6	Prepared from Cell Expansion (or 3 vials + 70 mL supernatant)

Table 3: WCB testing

*or minimum as qualified for the MCB



Genetic Characterization Assays

ICH Guidelines indicate that the stability and integrity of the insertion sequence needs to be characterized to ensure stable production of the correct protein.

Sartorius offers the following genetic stability assays and can advise on the most appropriate approach for your product.

It is recommended that a comparative assessment of MCB, WCB and EoPC is performed in parallel for genetic characterization assays at an appropriate point in your product lifecycle.

Assay Description	Assay Duration (weeks)	Guide Sample Requirements
Genetic Analysis		
Confirmation of sequence of transgene (coding region) by mRNA sequencing	6	1 vial
Confirmation of transgene flanking regions by DNA sequencing	6	1 vial
Design and Validation – Determination of Insert Gene Copy Number by Real-Time PCR	5	-
Test – Determination of Insert Gene Copy Number by Real-Time PCR	2	1 vial
Restriction enzyme analysis by Southern Blot	6	1 vial

Table 4: Genetic Characterization Assays
Refer to Technical Note 7



Integrated Service Offering

As part of our integrated service offering for a complete solution we also offer comprehensive solutions for:

- Cell Line Development
- CHO GMP Cell Banking
- CHO products Testing and characterization
- Biosimilar and New Biological Entity (NBE) analytical characterization
- Custom Assay Design

For further information please refer to the individual brochures for each service area

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Technical Notes

T1.

Qualification of the cell bank sample in the Mycoplasma and Sterility tests is required once on the MCB. For the Sterility test an alternative membrane filtration method can be performed if antibiotics are present in the final freezing mix formulation.

T2.

CHO cell lines contain an endogenous, defective, non-infectious retrovirus. When testing for retrovirus, a number of approaches can be taken and it is not necessary to perform all of the procedures.

- a. The electron microscope is a powerful tool used to visualise retroviral particles (for example budding C-type, R-type and intracisternal A-type particles).
- b. The PERT assay is used to detect enzymatic activity (which could provide a positive result as hamster cells contain an endogenous, defective, non-infectious retrovirus).
- c. Cell based infectivity assays for detection will determine if virus detected is infectious. In the event that infectious virus is detected, cells can be co-cultivated with a human cell line to determine if infection of human cells is possible. As standard, TEM and Infectivity assays would be performed for CHO testing. It is expected that this will provide a positive result for TEM and a negative result for Infectivity. In this instance, PERT is not required. If no retrovirus-like particles are observed by TEM then PERT testing can also be performed. The PERT test is likely to produce a positive result by PERT due to the endogenous virus.

T3.

The HAP test will detect the following viruses; Lymphocytic Choriomeningitis virus (LCMV), Pneumonia Virus of Mice (PVM), Reovirus Type 3, Sendai Virus and Simian virus type 5 (SV5).

T4.

The MAP test will detect the following viruses; Ectromelia Virus; Lymphocytic Choriomeningitis virus, Hantaan Virus, Pneumonia Virus of Mice (PVM), Reovirus Type 3, Mouse Theilers Encephalomyelitis Virus, Lactic Dehydrogenase Virus, Sendai Virus, Minute Virus of Mice (MVM), Mouse Adenovirus, Mouse Cytomegalovirus, Mouse Hepatitis Virus, Mouse Rotavirus, Polyoma Virus, Thymic Virus and K virus.

T5.

If at any point during production or development the parental cells have come into contact with bovine serum or porcine trypsin, then a full testing for a range of bovine and porcine viruses should be performed.

T6.

Cell expansion can be performed on the assumption that cells can be culture in standard conditions using procedures and equipment available at BioOutsource. If there are specialised requirements for your cells, please contact Sartorius to discuss.

T7.

Design will be dependent on whether product consists of a single transgene or multiple inserts (for example antibody heavy and light chain).

References

- (1) ICH Q5A (R1) 'Viral Safety Evaluation of Biotechnology products derived from cell lines of human or animal origin'.
- (2) ICH Q5D 'Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological Biological Products'.

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