eurofins | BioPharma Product Testing

Cell Line Characterization and Product Testing for CHO-Derived Products

Cell line characterization (CLC) must be performed on all Master Cell Banks (MCB), working Cell Banks (WCB), and Cells at Limit of *in vitro* age (CAL), or End of Production (EOP) cells in accordance with global regulations. This ensures the identity, purity, and safety of the cells and resulting biologic product.

The regulatory expectations and guidance for characterization testing of cell banks are outlined in various documents from the FDA, EMA, World Health Organization (WHO), and International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Eurofins BioPharma Product Testing's recommendations for cell line characterization, safety evaluation, and product testing for a CHO-derived biopharmaceutical product are summarized in this document. It outlines a testing strategy that should satisfy the latest guidance of global regulatory authorities, including the FDA and EMA.

A global leader in biopharmaceutical laboratory services, Eurofins BioPharma Product Testing provides clients with the tools and support to better manage their biopharmaceutical drug development process. Our vast capacity and extensive capabilities provide the greatest breadth of cGMP-compliant biopharmaceutical services in the industry.

The combination of our cell banking capabilities with our extensive characterization services provide clients with a single-source solution for all cell line, product, and package testing needs.

Why Choose Eurofins BioPharma Product Testing?

We offer capabilities to prepare and characterize a wide variety of mammalian, insect, and avian cell banks, including master, working, research, bioassay, and ready-to-use cell banks.

Our cGMP-compliant facilities include multiple Grade A/B suites with Animal Origin Free options and ISO 7 clean rooms with ISO 5 critical areas designed to meet current FDA/EMA aseptic processing guidelines.

We also offer a comprehensive package of safety testing services in the areas of microbiology, mycoplasma, genetic stability, viral safety, and biochemistry to support our cell banking capabilities. All of these services are provided with strict adherence to cGMP requirements and are designed to fully support the testing of biopharmaceutical products, including the testing of raw materials, cell lines, unprocessed bulk, purified bulk, and final product.

Cell Line Characterization and Product Testing

The MCB is the starting material for the entire GMP production process which makes the on time testing performed on these cells of great importance. MCB and EOP testing require the most extensive testing, including exogenous and endogenous viruses, and microbial contaminants. The EOP/CAL represents the worst case scenario in which endogenous viral contaminants may be amplified. Genetic characterization is performed to ensure identity and the stability of the cells throughout the production process. The MCB genetic profile is compared against the results of the EOP/CAL genetic profile.

WCB testing is performed to ensure that the bank is free from adventitious agents (i.e., bacteria, viruses, mycoplasma, fungi) that may have been

introduced in production of the WCB. Full characterization is not required at this stage although the cell identity should be confirmed.

It may not be necessary to conduct all of the tests listed. We highly recommend a discussion to evaluate those factors that can influence which tests can be omitted or whether additional testing might be required. Sharing the testing strategy with your regulatory reviewer is always highly recommended to ensure regulatory expectations will be satisfied.

CHO-derived Product Testing

Testing for the release of unprocessed bulk (UPB), drug substance (DS), and drug product (DP) are also recommended. Lot release testing of UPB includes assurance of freedom from adventitious virus by *in vitro* screening and retroviral particle quantitation by TEM analysis. Mouse minute virus (MMV) has also been known to contaminant CHO manufacturing process and MMV by PCR testing should be considered for your UPB testing strategy.

As the industry is progressing, and the CHO manufacturing process is maturing, speed has become a substantial influencer in UPB release testing. Regulators are receiving more filings using faster test methods in place of the longer traditional 28 day test methods. For instance, regulatory bodies are seeing the industry move away from the 28 day *in vitro* adventitious agent, 28 day compendial mycoplasma test, and move to a 14 day *in vitro* adventitious agent test (in some cases adding a 4th detector cell line) and the 5 day rapid mycoplasma PCR method. This dynamic shift enables our manufacturing partners to move from a 35 day UPB release to a 21 day UPB release. We always recommend our clients speak with their regulatory reviewer prior to bridging to the shorter assay test methods.

Final product testing should include a final microbial sterility test, residual DNA, and host cell protein testing, as well as key compendial methods such as pH, appearance, osmolality, etc… Protein/mAb concentration will need to be performed, as well as other product specific test methods. Eurofins BioPharma Product Testing has a wide variety of instruments to conduct all compendial and product specific test methods. Phase appropriate validation should be considered for early stage clinical development while a full GMP product/matrix validation should be assessed for late stage and commercialization of any regulatory approved product. Eurofins BioPharma Product Testing can help navigate the early and late stage testing requirements.

Lastly, ICH stability programs will need to be considered to determine shelf life of your product candidate. Eurofins BioPharma Product Testing provides the GMP storage chambers, compendial test methods, and will transfer and validate any product specific test methods to ensure proper stability per ICH timelines.

Master & End of Production Cell Bank Characterization						
	US Sample Volumes			EU Sample Volumes		
Test	Sample Requirement	TAT	Test Code	Sample Requirement	TAT	Test Code
Cell Line Expansion	2 Vials	Varies	QL4WS	1 Vial	Varies	TY046
Sterility Suitability (B&F)*	7 Vials	25 Days	QL1C2	3x amount for sterility test itself	21 Days	TY00I
Sterility GMP Analysis**	1% of cell bank with minimum of 2 vials	25 Days	QL23J	1% of cell bank with minimum of 2 vials	28 Days	GPMC1
Mycoplasmastasis	2x25mL, 4x1.5mL >1x10e^6 cells/mL	35 Days	GPMYK, GPMYL	Use cell lysate from preculture	42 Days	TY00L+TY00M
Mycoplasma GMP Analysis	2x12mL, 2x1.5mL >1x10e^6 cells/mL	35 Days	GPMYM, GPMYN	Use cell lysate from preculture	42 Days	TY00J+TY00K
Identity COI sizing	2x10e^7 cells	21 Days	QL0CJ	5x10^6 cells pelleted	14 Days	TY03S
Identity COI barcoding	2x10e^7 cells	28 Days	QL0FI	N/A	N/A	N/A
in vitro Adventitious Agent CHO Detector Line	7mL Cell Lysate at 1x10e7 cells/mL	42 Days	QL07X	5x10^6 Cell lysate (15 mL)	42 Days	TY01G
in vitro Adventitious Agent MRC-5 Detector Line	7mL Cell Lysate at 1x10e^7 cells/mL	42 Days	QL07V	5x10^6 Cell lysate (15 mL)	42 Days	TY01F
in vitro Adventitious Agent Vero Detector Line	7mL Cell Lysate at 1x10e^7 cells/mL	42 Days	QL07W	5x10^6 Cell lysate (15 mL)	42 Days	TY01F
Optional *in vitro* Adventitious Agent 324K Detector Line	7mL Cell Lysate at 1x10e^7 cells/mL	42 Days	QL07Y	5x10^6 Cell lysate (15 mL)	42 Days	TY01F
in vivo inapparent virus	Cell lysate at 1x10e^7 cells/mL (1x35mL, 1x16mL, 2x6mL, 1x4mL)	56 Days	QL07Z	Cell lysate at 1x10e^7 cells/ mL (51mL)	60 Days	TY04D
HAP	Cell lysate at 1x10e^7 cells/mL (5mL)	65 Days	QL086	1x10^7 cells/ml in spent culture media (10ml) + 5ml culture medium	56 Days	TY05D
MAP	Cell lysate at 1x10e^7 cells/mL (8mL)	65 Days	QL4LC	1x10^7 cells/ml in spent culture media (15ml) + 10ml culture medium	56 Days	TY048
TEM	2x10e^7 cells frozen in cyropreservant****	40 Days	QL4YU	Fixed pellet, 5x10^6 viable cells in TEM-grade Glutaraldehyde	84 Days	TY0A5
Bovine 9CFR	Cell lysate at 1x10e^7 cells/mL (9mL)	35 Days	QL089	5x10^6 Cell lysate (10ml)	35 Days	TY044 + TY045
Porcine 9CFR	Cell lysate at 1x10e^7 cells/mL (8mL)	35 Days	QL0B6	5x10^6 Cell lysate (10ml)	35 Days	TY04F+ TY04G
S+L- Focus Forming	Supernatant (5mL)	35 Days	QL0RV	5x10^6 Cell lysate (5ml)	21 Days	TY0AB
Retrovirus PERT	2x2 mL of supernatant	21 Days	QL0BV	Cell free culture supernatant (2 ml)	14 Days	TY027
Retrovirus Co-Cultivation Mus Dunni	2x10e^7 cells frozen in cyropreservant****	49 Days	QL0BA	1x10^8 live cells	42 Days	TY04R
MMV PCR	2e^6 cells or 2x2 mL cell free material	21 Days	QL0EB	One vial 1x 10^6 cells	14 Days	TY03H
Optional Genetic Characterization						
Copy Number	Cell Pellet at 2x10e^7	21 Days	CSS/MT0006P	One vial 1x10^6 cells	21 Days	Client specific
Restriction Enzyme (Southern)	Cell Pellet 2x10e^7 - 4x10e^7	42 Days	CSS/MT00010	One vial 1x10^6 cells	14 Days	
Sequencing	Cell Pellet/Bank at 2x10e^7 or 1-5 ug purified plasmid DNA	28-42 Days***	QL0V1/MT0003U	One vial 1x10^6 cells	21 Days	

*Sterility B&F assay code QL1C2 will be used if Eurofins generates the GMP cell bank.
**Package integrity testing/vapor hydrogen peroxide ingress assessment may be needed
***Additional time will need to be added to the TAT if Eurofins is to design the primers and probes.
****Cell expansion and sample prep will be performed by Eurofins

Working Cell Bank Characterization

	US Sample Volumes			EU Sample Volumes		
Test	Sample Requirement	TAT	Test Code	Sample Requirement	TAT	Test Code
Cell Line Expansion	2 Vials	Varies	QL4WS	1 Vial	Varies	TY046
Sterility GMP Analysis*	1% of Cell bank (minimum 2 vials)	25 Days	GPMC1	1% of Cell bank (minimum 2 vials)	28 Days	GPMC1
Mycoplasma GMP Analysis	2x12mL, 2x1.5mL >1x10e^6 cells/mL	35 Days	GPMYM, GPMYN	Use cell lysate from preculture	42 Days	TY00J+TY00K
Identity CO1 Sizing	2x10e^7 cells	14 Days	QL0CJ	5x10^6 cell pellet	14 Days	TY03S
in vitro Adventitious Agent CHO Detector Line	7mL Cell Lysate at 1x10e^7 cells/mL	42 Days	QL07X	5x10^6 Cell lysate (15 mL)	42 Days	TY01G
in vitro Adventitious Agent MRC-5 Detector Line	7mL Cell Lysate at 1x10e^7 cells/mL	42 Days	QL07V	5x10^6 Cell lysate (15 mL)	42 Days	TY01F
in vitro Adventitious Agent Vero Detector Line	7mL Cell Lysate at 1x10e^7 cells/mL	42 Days	QL07W	5x10^6 Cell lysate (15 mL)	42 Days	TY01F
Optional *in vitro* Adventitious Agent 324K Detector Line	7mL Cell Lysate at 1x10e^7 cells/mL	42 Days	QL07Y	5x10^6 Cell lysate (15 mL)	42 Days	TY01F
Unprocessed Bulk Testing						
Mycoplasma GMP Analysis**	2x12mL, 2x1.5mL > 1x10e^6 cells/mL	35 Days	GPMYM, GPMYN	25 mL+ 3 mL	42 Days	TY00J+TY00K
in vitro Adventitious Agent CHO Detector Line	7mL of Unprocessed Bulk	35 Days	QL07X	6mL Bulk harvest	42 Days	TY01G
in vitro Adventitious Agent MRC-5 Detector Line	7mL of Unprocessed Bulk	35 Days	QL07V	6mL Bulk harvest	42 Days	TY01F
in vitro Adventitious Agent Vero Detector Line	7mL of Unprocessed Bulk	35 Days	QL07W	6mL Bulk harvest	42 Days	TY01F
Optional *in vitro* Adventitious Agent 324K Detector Line	7mL of Unprocessed Bulk	35 Days	QL07Y	6mL Bulk harvest	42 Days	TY01F
MMV PCR	2x2 mL cell free material	21-28 Days	QL0EB	2x2mL bulk harvest	14 Days	TY03H
TEM***	Unprocessed Bulk (inquire about volume)	70 Days	QL116	10mL Harvest	Varies	TY0A5
Drug Substance/Product Lot Release Testing						
Sterility Suitability (B&F)	Varies by Lot Size	25 Days	GPMC7	Varies by Lot Size (3x amount for sterility test itself)	21 Days	TY00I
Sterility GMP Analysis*	Varies by Lot Size	25 Days	GPMC1	Varies by Lot Size	28 Days	GPMC1
Sterility Subpassage	If required, no additional material required	7 Days	GPMC3	If required, no additional material required	7 Days	TY0A3
pH	800 uL	15 Days	9034951	0.5 ml	7 Days	TY04L
Appearance	3mL	15 Days	9034944	4 ml	7 Days	TY04N
Host Cell Protein	2mL	14 Days	QL0G3	One vial	14 Days	TY022
Residual DNA	4mL	14 Days	QL0GR	One vial	14 Days	TY002
Osmolality, Freezing Point, Vapor Pressure	FP - 300uL VP - 50uL	15 Days	F-9034946 VP-9013122	0.5 ml	7 Days	TY047

*Package integrity testing/vapor hydrogen peroxide ingress assessment may be needed
** For faster UPB release testing, Eurofins also provides 14-day IVAA and 5-day Mycoplasma PCR tests
***TEM is only required on three lots of Unprocessed Bulk
****Cell expansion and sample prep will be performed by Eurofins

Comprehensive GMP Testing Services

Method Development & Validation • Release Testing • Raw Materials Testing
Cell Banking Services • Virology Services • Facility & Process Validation
Chemistry • Biochemistry • Molecular & Cell Biology • Microbiology
Stability Testing & Storage • Primary & Secondary Package Testing

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