cobetter®
filtration









AVL Center

Application and Validation Laboratory Center

ilac-MRA

CNAS

China National Accreditation Service
for Conformity Assessment
TESTING
CNAS L9495



AVL Center

Through years of effort and continuous improvement, Cobetter Validation Center is committed to providing comprehensive solutions for a wide range of filtration applications. Accredited by the China National Accreditation Service for Conformity Assessment (CNAS) in accordance with ISO/IEC 17025, the Center provides professional validation reports aligned with international standards and the regulatory requirements of target markets, supporting global compliance submissions and market access.

The Validation Center spans 14,000 m² and represents a cumulative investment of more than RMB 200 million. It comprises 13 service branches across three major industries—pharmaceutical, semiconductor, and industrial manufacturing—and a team of more than 600 scientists and engineers. To date, it has provided more than 42,000 pharmaceutical validation reports.

Service Items

- Bacterial Retention Test Validation Services
- Extractables & Leachables (E&L) Study Services
- Chemical Compatibility Validation Services
- Product-Wet Integrity Test Validation Services
- Customized Filtration Solutions
- Filtration Process Optimization Services
- Ultrafiltration Process Optimization & Validation Services
- Single-Use System Solutions & Validation Services
- Particle Distribution & Efficiency Testing
- Contaminant Analysis Services

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Validation Expert Taketomi Hidetoshi, Japanese
Previous Pall Lab Expert for More Than 30 Years





Filter Validation Guide

The critical component in the sterile filtration process is the sterilizing-grade filter. According to "Guidance on Sterile Filtration Technology and Application" and "GMP Guidance for Sterile Medicinal Products"(2023), the confirmation and validation items are summarized in the following table

Criteria	Filter User	Filter Manufacturer	
		Membrane	Device
Bacteria Retention in Water or Saline Lactose Broth (SLB) with Integrity Test Correlation in Water or Solvent	-	Q, L	Q, L
Bacteria Retention in Product with Respective Process	V	-	-
Chemical Compatibility, Effects on Filter Integrity	V	Q	Q
Extractables	E	Q	Q
Leachables	E	-	-
Sterilization Method, Effects on Filter Integrity	E	Q	Q
Integrity Test (Water or Solvent)	E	Q, L	Q, L
Integrity Test (Product)	V	-	-
Toxicity Testing – Bioreactivity Tests	-	Q	Q
USP Bacterial Endotoxin Test	E	-	Q, L
USP Particulate Matter	E	-	Q
USP Nonfiber Release	E	-	Q
Flush Volume	E	-	Q*
Filter Selection/Fit for Purpose	E	-	-
Impact to Product Critical Quality Attributes (CQAs)	E	-	-

L = Filter Manufacturer's Lot Release Criteria
Q = Filter Manufacturer's Qualification
V = Process Validation Testing (Rationale can be provided for scale-down testing)
E = Evaluate the Need for Additional Testing, Document Technical Assessment
+= Fully Assembled Filter with Membrane, Filter Components and Plastic Housing (if applicable)
*= Typically Using Water

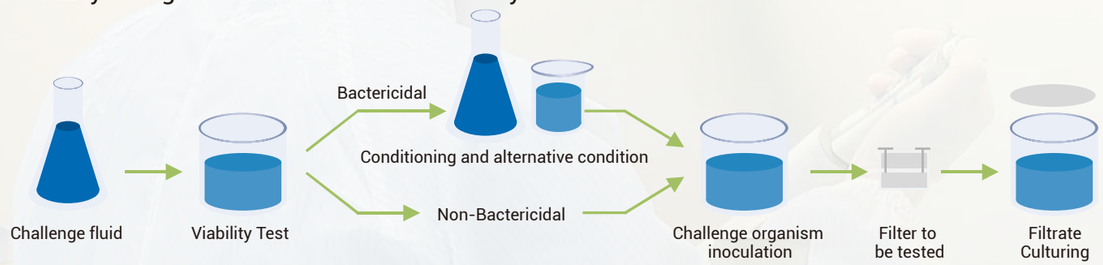
Remarks: L = Batch Release Criteria, Q = Confirm, V = Specific Process Validation,
V* = Acceptable Validation with Membrane Disc or Housing, E = Evaluation Required of the Validation Necessity

	Items	Sterile Filtration	Bioburden Reduction Filtration
Process Validation	Bacterial retention study	Required	Optional
	Chemical compatibility	Required	Required
	Extractables/Leachables	Required	Required
	Adsorption	Required	Required
	Product-wet Integrity test	Optional	Optional

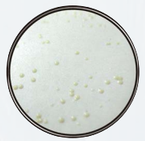
Bacterial Retention Study

- Sterilizing-grade filter——refers to a filter that can retain 10^7 CFU of ***Brevundimonas diminuta*** (ATCC 19146) per square centimeter of effective filtration area under process conditions.
- ***Bacterial Retention Study*** is the only way to confirm if the filter is sterilizing-grade.

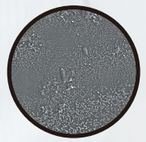
Study Design of Bacterial Retention Study



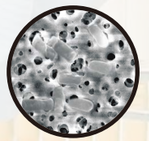
Scanning Electron Microscopy Observation of Bacteria



Colonies of Brevundimonas diminuta
(ATCC19146)



SEM analysis of Brevundimonas diminuta ——5000 times

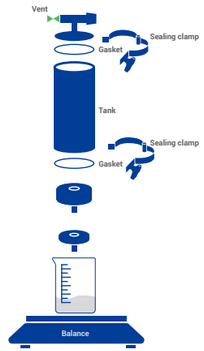


SEM analysis of Brevundimonas diminuta in filter membrane

We start with the science:
How do your products and processes affect the challenge bacteria?
Then we build a test method that provides bacterial challenge results you can rely on.

Viral (Phage) Clearance Validation

- According to PDA Technical Report No. 41, "Virus Filtration," and PDA Technical Report No. 47, "Preparation of Virus Spikes Used for Virus Clearance Studies," bacteriophages are used for the nominal pore size qualification of virus filters. Specifically, bacteriophage PR772 is used to define the PR772-LRF6 rating for 53 nm filters in first-generation large-virus filtration; bacteriophage PP7 is used to define the PP7-LRF4 rating for 20 nm filters in small-virus filtration; and φX174 and MS2 are included as two additional optional virus models.



Gram Staining-
Based Microbial Identification

Processes for the cultivation, purification, clearance, and detection of bacteriophages.

Crude Extract Preparation

10^5 pfu/Plate

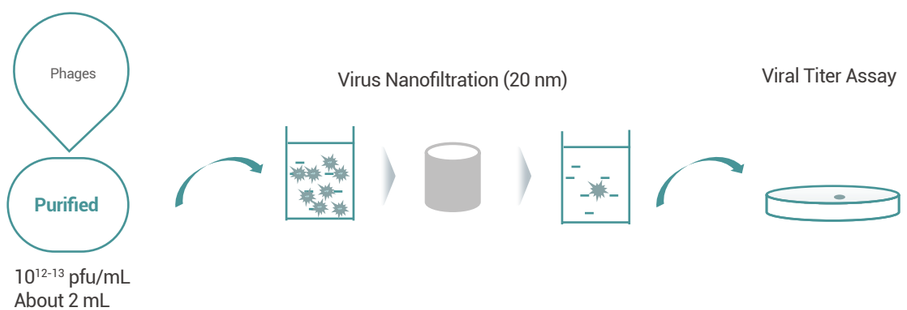
Elution in Phosphate
-Buffered Saline (PBS)

High-Speed Centrifugation

10^{9-11} pfu/mL
About 200 mL



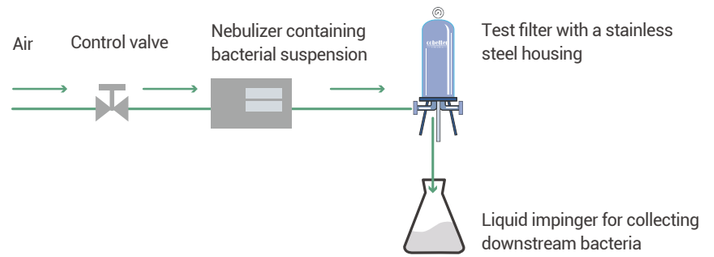
Phage Clearance Validation Process



10^{12-13} pfu/mL
About 2 mL

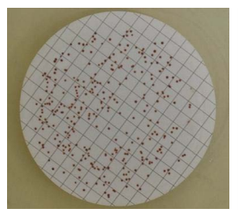
Microbial Aerosol Challenge Test

- Microbial aerosol challenge test is used to simulate the gas-phase filtration process and to verify the removal efficiency of microorganisms, including bacteria and viruses. Aerosol challenge tests commonly employ bacterial strains such as ***Brevundimonas diminuta*** (ATCC 19146), ***Staphylococcus aureus*** (ATCC 6538), bacteriophages ΦX-174, PP7, MS2, and T1, etc.



Microbiological Analysis – Total Viable Count and Microbial Identification

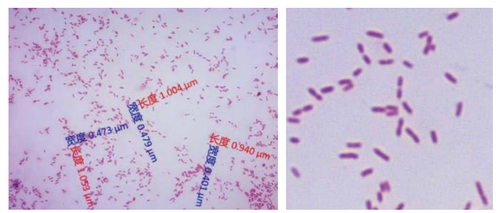
- For high-purity cleaning water used in the electronics and semiconductor industry, total microbial count testing shall be performed, along with microbial identification based on Gram staining. Colony morphology and size shall also be observed.



Total Viable Count
(TVC) Testing



Plate Streaking



Gram Staining-Based Microbial Identification

Based on the actual usage conditions of the customer, appropriate disinfectants are recommended, which generally include acids, bases, oxidizing agents, chelating agents, and surfactants. Disinfection efficacy validation may also be conducted. Test microorganisms typically include Staphylococcus aureus (ATCC 6538, Gram-positive bacteria), Escherichia coli (8099, Gram-negative bacteria), Candida albicans (ATCC 10231, fungi), and organisms cultured from water samples.

Extractables and Leachables

Overview

Extractables

An organic or inorganic chemical entity that is released from a manufacturing component or system into an extraction solution under laboratory conditions. Depending on the specific purpose of the extraction study, these laboratory conditions (e.g., solvent, temperature, stoichiometry, and others) may accelerate or exaggerate the normal conditions of use for a manufacturing component or system.



Leachables

A foreign organic or inorganic chemical entity that is present in a manufactured biopharmaceutical DS, or pharmaceutical or biopharmaceutical DP because it has leached from a component used in the manufacturing system and has persisted through the entire manufacturing process.

Reference	USP <1665>	BPOG
Risk Assessment Dimensions	Time	Exposure Duration
	Temperature	Exposure Temperature
	Solvent	Process Fluid Interaction
	Material properties	Distance along the production stream
	Other: Clearance, clinical use	Dilution Ratio

You may request for a toxicological risk assessment reports.

The methods used by Cobetter

TYPE	Analytical Methods	Abbreviation	Target
Organic	Headspace-Gas Chromatography -Mass Spectrometry	HS-GC-MS	Volatile Substances
	Direct injection-Gas chromatography -Mass Spectrometry	DI-GC-MS	Volatile and Semi-Volatile Substances
	Ultra Performance Liquid Chromatography -Ultraviolet-Mass Spectrometry	UPLC-UV-MS	Semi-Volatile, Non-Volatile Substances
	High Performance Liquid Chromatography	HPLC	Semi-Volatile, Non-Volatile Substances
	Total Organic Carbon	TOC	Total Organic Carbon
	Fourier Transform Infrared Absorption Spectroscopy	FTIR	Non-Volatile Residue
Inorganic	Inductively Coupled Plasma Mass Spectrometry	ICP-MS	Inorganic Elements
Organic/ Inorganic	Non-Volatile Residue	NVR	Non-Volatile Substances
	UV	UV	UV-Absorbing Substances
	pH	pH	Acidic or Alkaline Substances
	Conductivity	Conductivity	Conductive Lons

Extractables and Leachables (E&L) Mass Spectrometry Analysis

Overview

Cobetter Validation Center is equipped with advanced hardware and software systems featuring strong capabilities for unknown compound analysis. These resources enhance confidence in qualitative identification and improve quantitative accuracy, thereby ensuring the safety of single-use systems in pharmaceutical applications.

Professional Staff

With more than 20 experienced mass spectrometry analysts

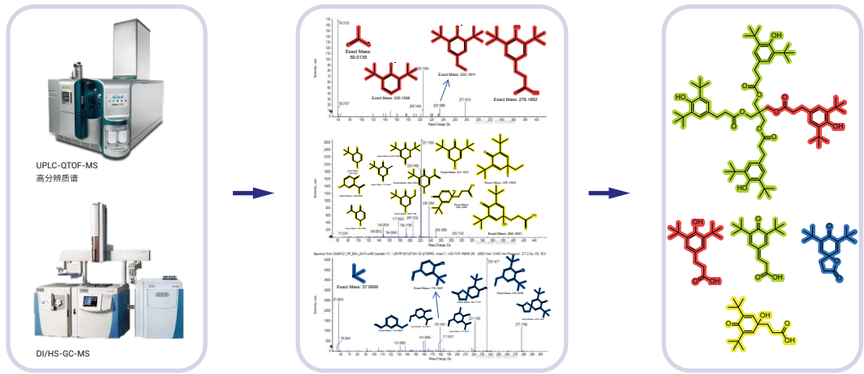
Advanced Instruments

Instruments with high resolution and high sensitivity

Comprehensive Spectral Library

- NIST library with over 40,000 compounds
- Commercial library containing more than 1,000 plastic additives
- Cobetter's in-house library of extractables & leachables containing over 400 compounds

Cobetter Validation Center Strong Unknown Identification Capability



Confirmation of the molecular ion peak in the MS¹ spectrum of unknowns → Library Search or Literature Search → Interpretation and Confirmation of Candidate Compound Spectra

Determine the molecular formula CnHxNyOz and verify the presence of any additional elements. → Search chemistry-specific databases (e.g., ChemSpider, PubChem), perform software matching, and confirm by spectral interpretation. → Obtain authentic standards of candidate compounds to perform final confirmation of structural identification results → Generate the final identification list and incorporate the spectra information of identified compounds into the internal library.

Toxicological Risk Assessment

Overview

- The toxicological risk assessment consists of four stages: Hazard Identification, Dose-Response Assessment, Exposure Assessment, and Risk Characterization. It is typically based on regulatory-accepted risk thresholds to calculate the Permitted Daily Exposure (PDE) value of a compound.

  In cases where literature data is insufficient, Quantitative Structure-Activity Relationship (QSAR) analysis can be conducted for prediction. The AVL is able to perform QSAR assessments using multiple software platforms based on expert rule sets and statistical models. Meanwhile, the toxicological risk assessment team can carry out comprehensive toxicological evaluations and certified with global registered toxicologist qualifications, supporting customers in worldwide submissions.



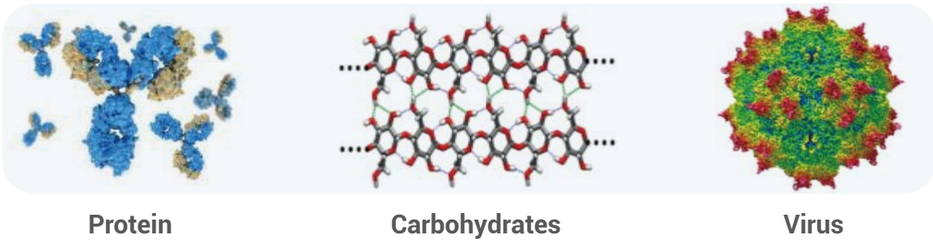
Figure Referances: [1]-[6]

Biomacromolecule Analysis Platform

Overview

- Biomacromolecules are the foundation of life. Biomacromolecule analysis involves the characterization of polymer biomacromolecules, such as proteins, carbohydrates, viruses, lipids, plasmids, mRNA, and even studies at the cellular level. Available techniques include fluorescence assays, photometric methods, gel electrophoresis, capillary electrophoresis, and high-performance liquid chromatography (HPLC). The application of mass spectrometry in biomacromolecule analysis is also becoming increasingly widespread.

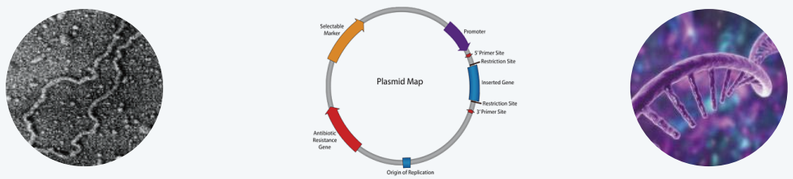
  To this end, Cobetter has established a biomacromolecule analysis platform dedicated to in-depth characterization of recombinant proteins, plasma-derived products, antibodies, vaccines, nucleic acids, and their by-products and impurities during the manufacturing process. This enables us to better support biopharmaceutical customers in addressing challenges related to single-use product applications, as well as solving complex issues arising from the biological products themselves.



Protein **Carbohydrates** **Virus**



Lipid **Cell**



Nucleic acid (plasmid, mRNA)

Figure Referances: [7]-[8]

Pictures sourced from: Inside Precision Medicine, Pixastock, Leica Microsystems, Addgene, ScienceAlert, WhatIsBiotechnology

Cobetter Biomacromolecule Analysis Capabilities

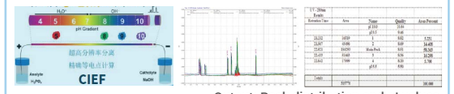
Analytical Instruments	Analytical methods	application
Ultra-high performance liquid chromatography-orbitrap mass spectrometry	Reverse-phase chromatography, Mass spectrometry	Intact molecular weight, Light/Heavy chain molecular weight
		Subunit, Characterization of disulfide bonds
		peptide map, post-translational modification characterization
		Host cell protein quantification
		Nucleic acid fragment quantification and characterization, Viral protein fragment quantification and characterization
		Screening of media components, Semi-quantitative analysis
	Hydrophilic interaction chromatography-mass spectrometry	Glycosylation characterization, polysaccharide quantification
Capillary electrophoresis	Capillary gel electrophoresis	Protein purity, non-glycosylated ratio
	Capillary isoelectric focusing	Isoelectric point determination and charge variant quantification
	Laser-induced fluorescence detection	Purity analysis of plasmid supercoil structure, mRNA integrity analysis, size distribution of residual DNA fragments in host cells, and monoclonal antibody N-glycan analysis
HPLC	Ion exchange chromatography	Charge variant quantification
	Size exclusion chromatography	Molecular size variants
Nucleic acid fragment analyzer	Capillary electrophoresis	Plasmid DNA identification, sequence confirmation, content, purity, supercoiling ratio, mRNA sequence purity, integrity, capping efficiency, poly A tail distribution, and host cell residual DNA fragment size distribution
Electrophoresis	Electrophoresis	Nucleic acid molecular weight, protein molecular weight
Biochemical analyzer	Optical detection	Glucose concentration, IgG concentration, lactic acid concentration
Fully automatic fluorescence analyzer	Fluorescent staining	Cell density,viability,diameter
Microplate Reader	Photoelectric colorimetry	Protein concentration, total nucleic acid concentration, ELISA
Sequencer	Next generation sequencing	Pathogen detection, gene expression levels, transcriptome sequencing
Flow Cytometry	Fluorescence quantification	Cell immunophenotyping analysis, vesicle detection, exosome detection, antibody screening
Fluorescence quantitative PCR instrument	Fluorescence signal quantification	Absolute quantification of nucleic acids (DNA or RNA), gene expression level analysis

Capillary Electrophoresis Analysis



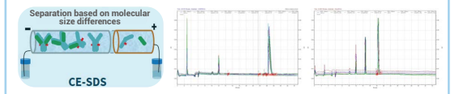
Capillary Electrophoresis

Capillary Isoelectric Focusing (CIEF) – Charge Heterogeneity



Output: Peak distribution and pI values

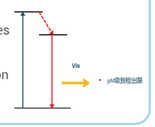
Capillary Gel Electrophoresis (CE-SDS) – Size Heterogeneity



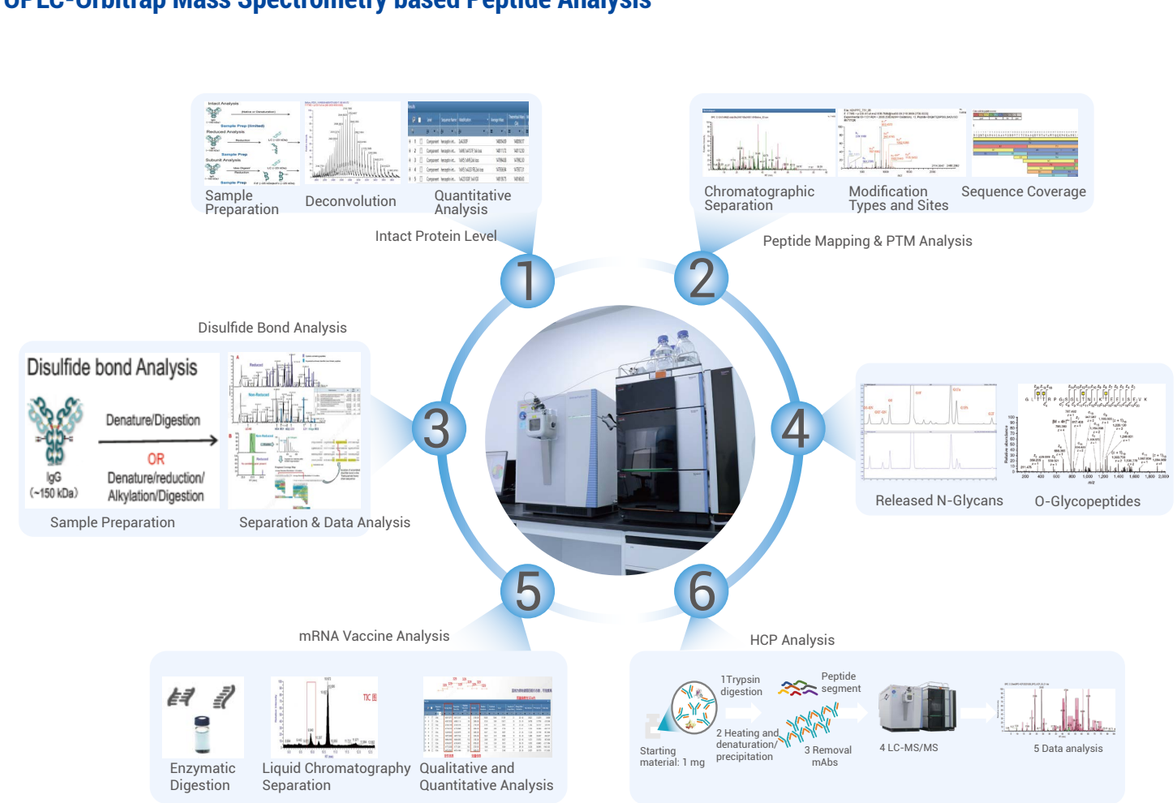
Non-reducing SDS | Reducing SDS profiles

Laser-Induced Fluorescence (LIF) Detector

- Purity analysis of plasmid supercoiled structures
- mRNA integrity assessment
- Host cell residual DNA fragment size distribution
- Monoclonal Antibody N-Glycan Profiling



UPLC-Orbitrap Mass Spectrometry based Peptide Analysis



Chemical Compatibility

In the filter selection stage, we need to verify that the filter is compatible with the process fluid



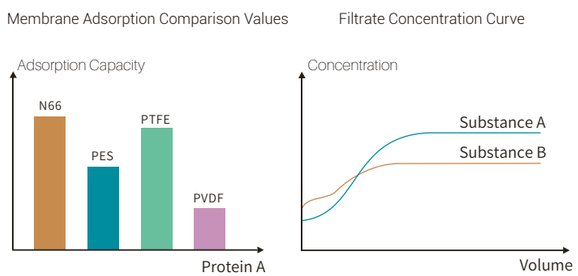
Performance tests and SEM surface-structure analyses allow chemical compatibility to be assessed, facilitating easy and effective filter selection.



Chemical compatibility is the basis of all validation and requires a comprehensive evaluation method.

Product Adsorption Analysis

In the production of the product, it is important to know whether the product adsorption has an impact on our finished products



We can provide professional product absorption test suggestions for pharmaceutical customers.

Product-wet Integrity Test

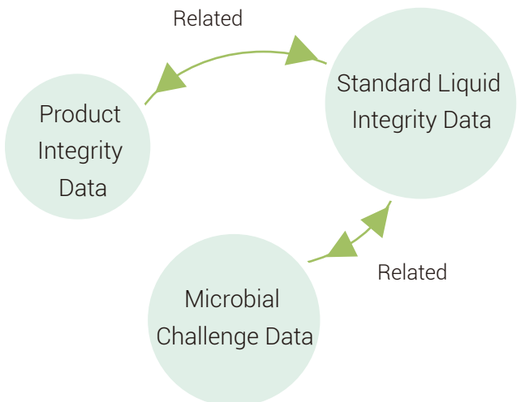
Overview

For sterilizing-grade filters used in liquid filtration, the drug product solution may be the most appropriate wetting fluid in some cases. By comparing integrity test results obtained with water or another solvent with established integrity specifications, and then comparing those results with results for the same filter wetted with the drug product, we can determine product-wet integrity test specification for the filter, including diffusion flow and bubble-point.

PWIT is Suitable for	PWIT is not suitable for
Products of high viscosity	Highly volatile organic solvents
Products containing residue-prone components	Products that are extremely prone to degradate
Products with extreme long flushing durations	Products that are unstable at room temperature

Advantages of Product Wetting:

1. Product-wet integrity test can be performed without extra cleaning steps, and the post-use integrity test is easier to pass.
2. Single-use systems can also be tested against pre-use integrity specifications. For hydrophobic filters, avoid introducing organic reagents that are not production-sourced. For hydrophilic filters, prevent residual moisture from altering the concentration of the product solution.
3. Post-use cleaning in single-use systems requires large waste liquid bags; the entire cleaning step can be avoided if PWIT specification is established.



Only when a correlation between integrity and bacterial retention has been established can integrity testing be used to monitor the filter's sterilizing performance

Neutral Bioassay - Viral Clerance Validation Services
A Third-party Laboratory Platform

Neutral Bioassay Services Overview

Hangzhou Neutral Bioassay Co., Ltd. is an independent, third-party testing organization specializing in virus clearance validation for biopharmaceutical manufacturers. The company has established a comprehensive viral analysis platform, validated its testing methods in accordance with ICH Q2, and implemented a quality system compliant with GLP and GMP. Its experienced, dedicated team helps ensure smooth project execution. To date, Neutral Bio has conducted validation studies for dozens of pharmaceutical companies, and its newly developed NGS and UPB testing platform will further strengthen viral safety assurance.





Independently Operated Laboratory Qualifications
Compliant with FDA/EMA/NMPA Audit Standards

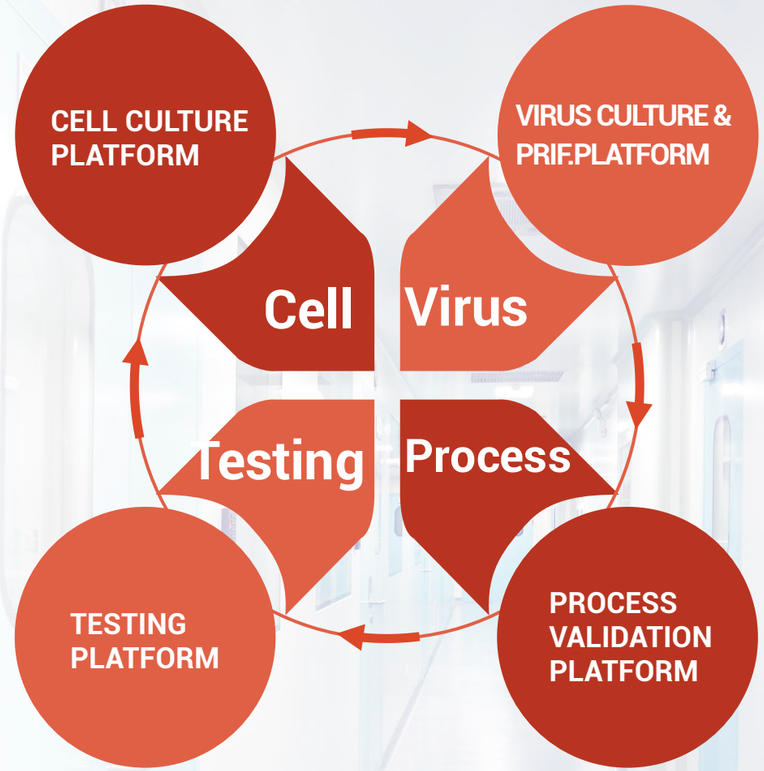
A Third-Party Public Platform
Providing efficient validation services for the biopharmaceutical industry chain

BSL-2 Registered Laboratory

CMA Certified and CNAS Accredited

Compliant with GLP Quality Management Standards
Established a standardized quality management system, including over 600 documents across a four-level system

Vaisala Online System for Real-Time Monitoring of Temperature and CO2 Levels



CELL CULTURE PLATFORM
The cell culture platform supports both suspension and adherent cell lines, including all cells necessary for antibody and blood-derived viral clearance validation, as well as cell lines commonly used in antibody expression research.

TESTING PLATFORM

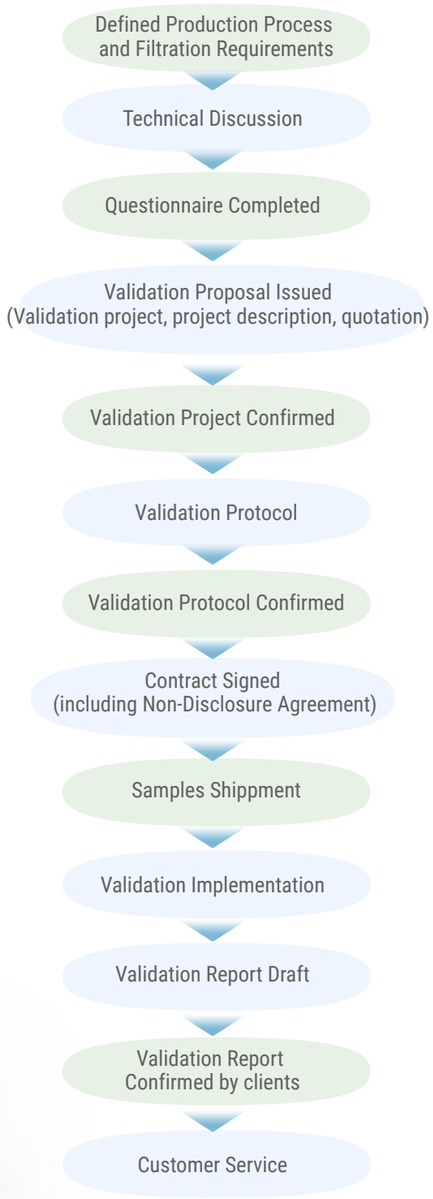
- Molecular Biology: Capable of performing qPCR, PCR, electrophoresis, Western Blot, Southern Blot, Northern Blot, ELISA, and viral vector construction.
- Methods: Validated plaque assays and qPCR quantification-methods.
- NGS Applications: Viral safety testing.

VIRUS CULTURE & PRIF.PLATFORM
Our platform is capable of culturing and purifying viruses commonly used for antibody and blood-derived process validation, producing high-titer and high-purity preparations. Supported viruses include MVM, x-MuLV, PRV, Reo-3, PPV, VSV, EMCV, BVDV, HSV, and others.

PROCESS VALIDATION PLATFORM

- Capable of performing common process validation studies, including low-pH treatment, S/D, chromatography, and nanofiltration for viral clearance validation.
- Filterability Studies: Providing timely support to confirm the viral clearance efficiency of customers' process development, helping them to shorten development timelines.

Validation Procedures



Validation Services & Referencing Guidelines

Service Items	Main Regulations and Guidelines Referenced
Chemical Compatibility	PDA TR 26, Guidance for Sterilization Filtration Technology and Application, Guidance of GMP for Drug - Sterile Medicinal Product
Extractables/Leachables	USP <665> <1665>, BPOG, BPSA, PDA TR 26, Technical Guidelines for Compatibility Studies of Plastic Components Used in the Production of Chemical Drug Injections (Trial), Guidance for Sterilization Filtration Technology and Application, Guidance of GMP for Drug - Sterile Medicinal Product
Bacterial Retention Test	ASTM F838, PDA TR 26, Guidance for Sterilization Filtration Technology and Application, Guidance of GMP for Drug - Sterile Medicinal Product
Product-wet Integrity Test	PDA TR 26, Guidance for Sterilization Filtration Technology and Application, Guidance of GMP for Drug - Sterile Medicinal Product
Adsorption	PDA TR 26, Guidance for Sterilization Filtration Technology and Application, Guidance of GMP for Drug - Sterile Medicinal Product

References

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[7] Sun, Y.; Wang, J.; Li, D.; Cheng, F. The Recent Progress of the Cellulose-Based Antibacterial Hydrogel. *Gels* **2024**, *10* (2), 109.

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Filtration
Separation
Purification

cobetter®
filtration

Please contact us for more information

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