Validation guide

HyClone™ cell culture media

cytiva

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Introduction: HyClone™ Cell Culture Products

Overview

Cytiva is a global manufacturer of HyClone™ sera, liquid and powdered media, and process liquids, to support cell-based research and the production of biopharmaceuticals. Our global presence is supported by manufacturing facilities in Logan, USA; Pasching, Austria; and Singapore; and by serum processing sites in Australia and New Zealand. Our manufacturing operations are fully compliant with current good manufacturing practices (cGMP) 21 CFR 820, ISO 9001, and ISO 13485.

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Validation overview

2.1. Introduction

The bioprocessing industry relies upon liquid and dry powder cell culture media, process liquids, reagents, water for injection (WFI) quality water, and process water for biopharmaceutical production. To make sure that the stringent requirements of biopharmaceutical manufacturers are met, Cytiva develops and implements validated processes and procedures for the manufacture of our products.

2.2. Scope

This validation guide summarizes the qualification and validation of our facilities, equipment and manufacturing processes. Qualification and validation procedures that pertain to WFI quality water, process water, animal origin and non-animal origin components and segregation, medium designations, quality control, product stability, packaging, risk mitigation, and regulatory compliance are also covered. As of the date of this publication the data is current and correct.

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Medium design qualification

3.1. Design control

Medium design qualification encompasses the process of ensuring that new medium products meet specific design requirements. This process involves design control and risk analysis in cell culture medium development and ensures that quality and reliability are designed into every product we manufacture.

Design control applies to new product development process of standard products. Its purpose is to establish and promote an effective new product development process and to make sure that designs of new and changed products meet specified requirements. Design and development inputs are established based upon the intended use of the product.

These inputs include functional, performance, and safety requirements; applicable regulatory requirements; characteristics of similar designs; other requirements needed for design and development; and anticipated risk management requirements. The inputs are reviewed for adequacy to make sure that they are complete, unambiguous, and not in conflict with each other. Design and development verification testing is performed to demonstrate that the new product meets the established criteria. This verification documents how the product design and development outputs meet the product design and development inputs.

3.2. Risk analysis

Risk analysis is intended to serve as a design tool by providing a means for ensuring a robust product or process design. Risk analysis is performed in the following situations:

- During the up-front design phase of a product
- During the up-front design phase of a manufacturing process
- During tasks that require engineering changes
- During product or process failure investigations
- During a periodic review

To serve the needs of all groups engaged in risk analysis, a failure mode and effects analysis (FMEA) approach is used. For the product being analyzed, a design FMEA is used. The FMEA ranks the severity of each potential effect in accordance with the following scale:

- **Negligible**: a noticeable but insignificant anomaly
- **Marginal**: a flaw of minor concern to the customer
- **Slightly** critical: a product flaw that does not affect product performance
- **Critical**: a product flaw that decreases performance
- **Catastrophic**: a product flaw that results in death, injury, leak, sterility breach, or loss of the customer’s product

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Medium operational qualification

Medium operational qualification ensures that products meet design and process requirements, and the qualification process consists of process validation, process control, and risk management.

4.1. Process validation

Process validation is a defined program that, in combination with routine production methods and quality control, provides documentation that a system is performing as intended, and the product conforms to its predetermined specifications. Validation management provides a standardized approach to the qualification of utilities and equipment, as well as the validation of products and production processes.

4.2. Process control

Process control creates manufacturing and testing specifications and procedures that ensure manufactured products are consistently adequate for intended use, including appropriate documentation.

4.3. Risk management

The risk management program is based upon principles found in ANSI/AAMI/ISO 14971, Medical devices — application of risk management to medical devices and activities within a quality management system, GHTF/SG3/N15R8. The objective of risk management is not to eliminate all risk, but to reduce risk to an acceptable level while maintaining feasibility and functionality. The following definitions and standards are applied to risk management:

- **Harm**: physical injury or damage to the health of people, damage to property, or damage to the environment (ANSI/AAMI/ISO 14971:2007 Definitions)
- **Hazard**: potential source of harm (ANSI/AAMI/ISO 14971:2007 Definitions)
- **Residual risk**: risk remaining after risk control measures have been taken (ANSI/AAMI/ISO 14971:2007 Definitions)
- **Risk**: combination of the probability of occurrence of harm and the severity of that harm (ANSI/AAMI/ISO 14971:2007 Definitions)
- **Risk analysis**: systematic use of available information to identify hazards and to estimate the risk (ANSI/AAMI/ISO 14971:2007 Definitions)
- **Risk assessment**: overall process comprising a risk analysis and a risk evaluation (ANSI/AAMI/ISO 14971:2007 Definitions)
- **Risk control**: process in which decisions are made and measures are implemented in order to reduce or maintain risks within specified levels (ANSI/AAMI/ISO 14971:2007 Definitions)
- **Risk evaluation**: process of comparing the estimated risk against given risk criteria in order to determine the acceptability of the risk (ANSI/AAMI/ISO 14971:2007 Definitions)
- **Risk management**: systematic application of management policies, procedures, and practices to the tasks of analyzing, evaluating, controlling, and monitoring risk (ANSI/AAMI/ISO 14971:2007 Definitions)
- **Risk management file**: set of records and other documents that are produced by risk management (ANSI/AAMI/ISO 14971:2007 Definitions)

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Facility and equipment qualification

5.1. Facility qualification

Production buildings are designed according to specifications that facilitate the manufacture of high-quality products in controlled environments. To make sure that this goal is met, an engineering building qualification book is maintained for each production building. The contents of the engineering building qualification book include building specifications, engineering change orders, building qualification documents, supporting documents and information, building drawings, manuals, purchase orders, and relevant correspondence.

5.2. Production equipment qualification

Production equipment is designed according to specifications that ensure the manufacture of high quality products in controlled environments. To make sure this goal is met, an equipment qualification life cycle model is followed for each production system. The documentation includes equipment specifications, engineering change orders, equipment qualification documents, equipment qualification sign-off documents, supporting documents and information, risk analysis, work instruction document references, preventive maintenance references, calibration requirements, drawings, manuals, purchase orders, and other relevant correspondence.

For each production system, the following protocols have been established:

- An installation qualification (IQ) protocol that documents the production system is installed correctly
- An operational qualification (OQ) protocol that documents the production system or process is capable of operating in accordance with the specifications and requirements
- A performance qualification (PQ) protocol that documents the production system operates according to predetermined specifications under normal operating conditions

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Powdered medium manufacturing validation

6.1. Process qualification

Powdered medium facilities and equipment are used to produce and package products in compliance with Cytiva's approved procedures and cGMP. The processes for formulating, milling, and blending dry powdered media have been qualified by testing batches to predetermined standards that include particle size, osmolality, pH, phenol red, mean value of chemical components, and mean value of amino acid components. The qualification protocol ensures that the processes used to produce dry powdered media result in quality products that meet predetermined specifications.

6.2. Segregation

Pin mill powdered medium operations are segregated from ball mill powdered medium operations. The pin mill powdered medium production area is completely animal-derived component-free (ADCF). The ADCF production area includes rooms for gowning, formulating, continuous pin milling, blending, drying, cold storage, and ambient temperature storage. Equipment includes a weight verification system, scales, pin mill, blenders, micronizer, vacuum assist transfer system, particle size analyzer, packaging equipment, testing equipment, and dust collection system.

Ball mill powdered medium operations are segregated from pin mill powdered medium operations. The non-ADCF ball mill production area includes rooms for gowning, formulating, ball milling, blending, drying, cold storage, and ambient temperature storage. Equipment includes a weight verification system, scales, jar mills, ball mills, blender, dust collection system, packaging equipment, and testing equipment.

6.3. Formulation

Powdered medium components are weighed, measured, and formulated in accordance with finished product specifications. The weight verification system is fully integrated throughout both the ADCF and non-ADCF production areas, and is validated twice per year.

6.4. Milling

The pin milling process relies upon the force of impact between medium components and milling pins to reduce the particle size of medium components. The pin milling process produces uniform particles that meet specified statistical standards for range and mean particle size.

The ball milling process grinds components to the desired particle size through the action of milling stones. The ball milling process produces uniform particles that meet specified statistical standards for range and mean particle size.

A model medium is used to annually validate particle size uniformity from both milling processes. In addition, every production batch of dry powdered medium is tested for particle size uniformity. Particle size uniformity testing standards include comparison of mean particle diameter, and passage through a sieve of specific pore diameter.

6.5. Blending

The ADCF blending process transfers dry powdered medium components from the pin mill to a blender. At the completion of the pin milling process, the blender homogenizes the medium components into a uniform mixture.

The non-ADCF blending process transfers sublots of medium components from ball mills to a blender, and the blender homogenizes the medium components into a uniform mixture.

The annual validation of dry powdered medium blending is based upon various batch sizes. The ability of each blender to produce a homogeneous batch is challenged with 13% and 100% volume capacities. Acceptance criteria are based upon the mean particle size distribution and phenol red concentration at each sampling location, and by moisture content.

6.6. Cleaning

Powdered medium production equipment is cleaned by following approved and validated procedures:

Cleaning of equipment used in the pin mill process takes place before each lot of different part numbers or after 72 hours has expired since the last cleaning date. For different lots of the same part number, pin mill equipment may be used up to three times without cleaning if production takes place within a 72 hour time period. The cleaning solution is 2% NaOH, and the rinse solutions include process water and WFI quality water for removal of any residual product or cleaning agent. Conductivity testing is performed on the final WFI quality rinse water, and rinsing continues if necessary until the conductivity of the final rinse water is below the maximum specification.

Cleaning of equipment used in the ball mill process takes place at least 72 hours prior to use, and the equipment may be used up to five times without cleaning if used for different lots of the same part number within a 72 hour time period. Cleaning and rinse solutions include 3% hydrogen peroxide, process water or WFI quality water, and the final rinse solution is WFI quality water for removal of any residual product or cleaning agent. Conductivity testing is performed on the final WFI quality rinse water, and rinsing continues if necessary until the conductivity of the final rinse water is below the maximum specification.

The process for cleaning dry powdered medium production equipment is validated annually by testing the final rinse water for phenol red, protein, bioburden, endotoxin, conductivity, pH, and total organic carbon.

6.7. Containers

Dry powdered media is packaged in high-density polyethylene (HDPE) bottles, buckets and drums, and in Powdertainer™ II single-use containers. The ability of each container to prevent moisture from entering the powdered medium is validated by placing them in a humidified incubator at a predetermined temperature and length of time. Each incubated container is compared with a non-incubated control container, and the acceptance criteria require that the moisture content of the powdered medium in the incubated container does not exceed the moisture content of the powdered media in the control container.

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Liquid medium manufacturing validation

7.1. Process qualification

Liquid product facilities and equipment are used to produce and package products in compliance with approved Cytiva procedures and cGMP. The processes for formulating sterile filtered liquid products have been qualified by testing batches to predetermined standards that include osmolality, pH, mean value of chemical components, mean value of lipid components, and mean value of amino acid components. The qualification protocol ensures the processes used to manufacture liquid products result in quality products that meet predetermined specifications.

7.2. Production flow

The liquid medium production process proceeds as follows:

- **Incoming staging**
  Prior to production, all of the necessary packaging and filling supplies are staged, prepared, and set up in the appropriate areas.
- **Cleaning and steaming**
  Each piece of equipment to be used in conjunction with a production batch, such as tanks, blenders, pumps, hoses, and filling manifolds, are cleaned prior to each production lot. After the filling manifolds have been cleaned, the single-use containers are steamed onto the specified manifolds.
- **Formulation**
  Production personnel verify that the equipment being used for each production lot has been properly cleaned and steamed. The relevant process control document is consulted to make sure all necessary packaging, filling supplies, dry powder media are available, and that the correct volume of water is in the mixing tank.
- **Filling**
  Production personnel verify that the filters, filling equipment, and single-use containers have been properly prepared for filling. The filling operation commences when the formulation process has been completed and the equipment has been properly prepared.
- **Finish packaging**
  When the filling process is complete, production personnel move the finished product into the packaging area, where the product is packaged, labeled, and prepared for storage in a warehouse quarantine area. After release testing has been completed, the product is moved into an appropriate warehouse area pending shipment to the customer.

7.3. Clean room certification

Liquid production takes place within ISO14644 certified clean room environments. Clean room testing parameters include room air changes per hour, room-to-room differential pressure, high-efficiency particulate air (HEPA) filter integrity, and non-viable particle counts.

7.4. Segregation of manufacturing

The liquid production facility is segregated into two separate areas, including a general production area and a single-use disposable area. The general production area utilizes stainless steel process vessels, and the single-use area uses disposable product contact surfaces. The use of disposable product contact surfaces enables the production of ADCF liquids, and eliminates the potential for batch-to-batch cross contamination.

7.5. Formulation

The components for liquid media are weighed, measured, and formulated in accordance with finished product specifications. The weight verification system is fully integrated throughout both the large volume and small volume production areas, and is validated twice per year.

7.6. Single-use disposables

Disposables used in the single-use manufacturing process include powder feed bags, tank liners, associated tubing and line sets, and disposable filters. All disposables are tested according to established criteria.

7.7. Mixing

Homogeneity of liquid medium products results from the use of validated powdered medium manufacturing processes, solubility, and an effective mixing process. Annual validations are performed on stainless steel tanks and single-use disposable systems. Liquid samples are obtained from the top, middle, and base of each mixing vessel and compared with a control to determine uniformity of the formulation. The acceptance criteria are based upon the comparison of sample results with control results.

7.8. Sterile filtration

Sterile filtration of liquids is accomplished through proprietary filtration schemes based upon volume, type of liquid product, and the type of filtration specified. Multiple filters are used in each filtration scheme to achieve 0.1 um filtration or 0.2 um filtration. Integrity testing of filters used in liquid manufacturing have methods that are not all used at the same time, but includes forward-flow testing, bubble-point testing, forward-flow followed by bubble-point testing, and diffusion-flow testing. Integrity testing of filters follows standardized and documented procedures according to filter type.

The final filter in the liquid manufacturing process is a sterilizing grade filter validated by the manufacturer to retain a challenge of at least 10^7 colony forming units (CFU) per cm^2 of *Brevundimonas diminuta*, in accordance with "FDA guidelines on sterile products produced by aseptic processing (1987)".

7.9. Liquid product filling

The ability of production personnel, equipment, and associated procedures to aseptically fill liquid medium containers is validated twice per year. A complete range of filled plastic bottles and single-use containers are tested for sterility, and positive controls are used to confirm test results. A complete range of filled plastic bottles and single-use containers are tested for weight and volume, to confirm that the fill-by-weight system is providing the required volume of liquid medium for each container's configuration.

Single-use containers, which are tested by the manufacturer and used for the containment of sterile-filtered liquids, are tested for tensile strength, burst strength, leak detection and hydrostatic pressure.

7.10. Cleaning

Liquid production equipment is cleaned with 2% NaOH and rinsed with WFI quality water to remove any residual product or cleaning agent. The process for cleaning liquid medium production equipment is validated annually by testing the final rinse water for phenol red, protein, bioburden, endotoxin, conductivity, pH, and total organic carbon.

7.11. Sterilization

Liquid filling manifolds are steam-in-place sterilized, and the sterilization cycle is validated annually. To validate the effectiveness of the steam-in-place sterilization procedure, spore strips containing at least 1.0×10^6 CFU *Geobacillus stearothermophilus* are placed with thermocouples throughout the liquid medium filling system in accordance with USP <1035>.

7.12. Packaging containers

Liquid products are packaged in polyester terephthalate glycol (PETG) and polyethylene terephthalate (PETE) bottles, and in single-use containers. PETE and PETG bottles are purchased gamma irradiated from the manufacturer. Single-use containers are irradiated at a minimum of 25 kGy. This dose is validated quarterly according to AAMI/ANSI/ISO guidelines.

The integrity of each container enclosure is validated by filling the container with tryptic soy broth (TSB), and immersing the closed container in a log phase suspension of Serratia marcescens for at least 30 min. Subsequently, the container is incubated to determine sterility of the contents.

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WFI quality water and process water

8.1. Manufacturing process

WFI quality water and process water are produced in a sequential purification process that converts high-quality source water into ultrapure WFI quality water or process water. Whereas bioburden reduction in WFI quality water is accomplished by means of a recirculating hot loop process and distillation, bioburden reduction in process water is accomplished by filtration.

8.2. Testing

WFI quality water and process water in the system are continually monitored at set intervals, and pass United States Pharmacopoeia (USP) and/or European Pharmacopoeia (EP) standards. Current WFI quality water standards include the following:

- Not for parenteral use
- Purify by distillation/reverse osmosis
- Endotoxin less than 0.25 EU/mL
- Conductivity less than five microseconds/cm at 25 ± 1°C
- Particulate meets USP <788>
- Sterility meets USP <71> and/or EP 2.6.1
- EP test for acidity or alkalinity
- EP test for ammonium
- EP test for calcium and magnesium
- EP test for chlorides
- EP test for residue on evaporation
- EP test for sulfate
- EP and USP tests for oxidizable substances
- EP and USP tests for pH
- Prepared from source water complying with US National Primary Drinking Water Regulations, or comparable regulations of EP or Japanese Pharmacopoeia (JP)

The action limits we have imposed for our system are 4 CFU/100 mL for microbial bioburden and 0.06 EU/mL for endotoxin. WFI quality water is filtered through sterile 0.1 µm filters and may be used in the following applications.

- To produce liquid products such as media, buffers, or other process fluids as part of a batch process
- Filled directly into PETE or PETG bottles for customer purchase and use
- Filled directly into large volume bags through a manifold system for customer purchase and use

WFI quality water packaged in either bottles or single-use containers is an aseptic fill process. WFI quality water is filled into presterilized containers, either directly from the loop system and filtration scheme or off the loop via a pooling tank for immediate fill through a filtration scheme. When a single-use container is filled, the fill line is sealed before the single-use container is severed from the manifold.

8.3. Limitations

WFI quality water packaged in large volumes with the intended use of “FOR FURTHER MANUFACTURING” does not meet the USP definition and intended use of packaged sterile water for injection. Therefore, it is not intended to be used as a parenteral or injectable product.

09

Quality control

9.1. Standardized testing

A number of standardized tests are regularly performed to validate medium components, products and systems, including the following:

- pH testing is performed in accordance with USP <791> and/or EP 2.2.3. pH testing is monitored and adjusted as needed throughout the production process.
- Osmolality testing is performed in accordance with USP <785> and/or EP 2.2.35. Osmolality is monitored throughout the production process.
- Endotoxin testing is performed in accordance with USP <85> and/ or EP2.6.14. The Limulus amebocyte lysate (LAL) assay is validated for each product type to demonstrate assay sensitivity and inhibition/enhancement. Inhibition/ enhancement testing is completed and minimum detection limits are determined for each product type.
- Sterility testing is performed in accordance with USP <71> and/or EP 2.6.1. The membrane sterility test is used as an end product release test for process liquids.

9.2. Raw material qualification

Because HyClone products are used in the biopharmaceutical industry, our standard procedure is to specify USP or National Formulary (NF) raw material grades where possible. Multicompendial grades such as EP or JP might also be specified. For those raw materials that do not have pharmacopoeia monographs established, we specify chemicals that meet Food Chemical Codex (FCC), American Chemical Society (ACS), or other applicable grades. Any other chemicals must meet minimum requirements established by our Quality Department.

Before any lots are produced, all raw ingredients are controlled and confirmed via our bar code verification system.

WFI quality water or process water is used in the manufacture of liquid media and process liquids. All plastic product contact materials meet requirements for USP Biological Class VI testing and physiochemical testing.

9.3. Incoming inspection

Inspection is performed on all incoming components by Quality Control. The certificate of analysis that is provided by the supplier is compared with the approved chemical product specifications and with any compendial reference (USP, NF, FCC, EP, JP, etc.). The sampling plan is defined in each component specification.

Examples of quantitative and qualitative tests that may be performed include the following:

Qualitative tests

- Fourier transform infrared spectroscopy (FTIR)
- USP (assay)
- Inductively coupled plasma (ICP)
- Gel electrophoresis
- Solubility
- Cytotoxicity
- Growth promotion
- Mycoplasma
- Virus
- Trypsinization

Quantitative tests

- Moisture
- Endotoxin
- Bioburden
- Hemoglobin
- pH

9.4. Testing of powdered media

The quality control (QC) testing of dry powdered media is performed on every manufacturing batch in accordance with established product specifications. The specific QC tests include appearance, pH, osmolality, endotoxin, moisture, bioburden, growth promotion, and amino acid analysis. The results are recorded in the product lot history record. If all results meet the established product specifications, the lot history record is forwarded to Quality Assurance (QA) for product review and release. If any result does not meet the established product specifications, an out of specification test results procedure is followed.

9.5. Testing of liquid media

The QC testing of liquid media is performed on every manufacturing batch in accordance with established product specifications. Samples are collected intermittently throughout the given production run and submitted for QC testing. The specific QC tests include appearance, pH, osmolality, endotoxin, sterility, growth promotion, and amino acid analysis. The results are recorded in the product lot history record. If all results meet the established product specifications, the lot history record is forwarded to QA for product review and release. If any result does not meet the established product specifications, an out of specification test results procedure is followed.

9.6. Creation of certificate of analysis (CoA)

The CoA is generated to document product specifications evaluated in the QC testing process. These specifications include test results for specifications such as product appearance, pH, osmolality, sterility, endotoxin, and growth promotion. Any results pertaining to nutritional deficiency, cytotoxicity or morphological aberrations observed in cell culture can also be included in the CoA. The customer may specify unique tests to be included in the CoA for custom formulations. A completed CoA is generated for every lot number, maintained by Cytiva, and is included when the product is shipped. The CoA is also maintained on www.cytiva.com/hyclone. CoA examples are shown in Section 16 of this document.

9.7. Product correction and removal

We have established a procedure whereby we maintain records of all corrections and removals in accordance with the provisions of ISO and cGMP.

9.8. Component secure sourcing policy

To ensure secure component sourcing, Cytiva has implemented the following processes, depending on the nature of the component:

Chemical materials are evaluated for suitability based on specifications, manufacturing method, starting materials, and manufacturing site. For plant and recombinant materials, genetically modified origin (GMO) is evaluated. Water processing and purity is ensured. Disposable components of sourced single-use liquid management systems are tested for cytotoxicity, extractables, and leachables. Resins are specified. An aggressive supplier evaluation program is in place for all components.

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Stability

10.1. Shelf Life

The shelf life of dry powdered and liquid media is initially established on the basis of accelerated shelf life studies, or on the basis of shelf life data for similar formulations. After the initial shelf life has been established for a given formulation, ongoing validation of product stability is performed. Additional stability testing can take place for existing formulations if there is a change in the manufacturing process, manufacturing equipment, or raw materials.

Stability testing criteria can include solubility, appearance, pH, osmolality, bioburden, moisture content, growth promotion, cytotoxicity, and endotoxin.

10.2 Stability during shipment

Stability testing, which is only for dry powdered media, is designed to mimic temperature extremes that are encountered during product shipment. Examples of temperature extremes that are tested include 37°C and -20°C. The 37°C testing is intended to mimic temperature extremes that might be encountered during surface shipments in summer months. The -20°C testing is intended to mimic temperature extremes that might be encountered during surface shipment in winter months or during air shipment.

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Packaging

11.1. Medium containers

Powdered media are packaged in HDPE bottles, in rigid HDPE pails and drums that are lined with poly bags, and in Powdertainer II single-use containers.

Liquid media and buffer products are packaged in PETE and PETG bottles, and in single-use containers that are supported by corrugated box packaging, HDPE pails, HDPE drums, or palletized bins.

Some of the test standards and methods performed by the supplier are used to validate the physical and chemical properties and the biocompatibility of single-use containers and components are listed below:

American Society for Testing and Materials

- **ASTM D882**: standard test method for tensile properties of thin plastic sheeting
- **ASTM D1003**: standard test method for haze and luminous transmittance of transparent plastics
- **ASTM D3985**: standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor
- **ASTM E1640**: standard test method for assignment of the glass transmission temperature by dynamic mechanical analysis
- **ASTM F1249**: standard test method for water vapor transmission rate (WVTR) through plastic film and sheeting using a modulated infrared sensor

Puncture resistance

Puncture resistance is measured using an Instron™ tensile test machine equipped with a 6-inch diameter annular fixture. The total force absorbed by the film during puncture is measured.

Seal integrity

Routine seal integrity testing is performed on representative single-use container samples to make sure that functional strength requirements are met. These tests include seam peel, hydrostatic, and leak/burst testing.

ADCF contact surface

The status of ADCF single-use containers and product contact materials is as follows:

Plastic materials might contain additives used as release agents or as anti-slip or anti-block processing aids produced from bovine tallow. These substances (such as calcium stearate) are incorporated into resins to facilitate the production and processing of the resin material. While certain plastic contact surfaces like filters, connectors, tank liners, and tubing will have limited exposure time to HyClone products, the greatest surface-to-product contact areas and product residence times will be with the chamber films. Cytiva offers two film options for long term storage of the product, both are ADCF.

We provided animal origin questionnaires to our plastic component suppliers and incorporate the response information into our QA database. Additionally, we are working with key filter and other plastic suppliers to encourage them to move to ADCF plastics where possible.

Biological compatibility

Several test standards are available to show biocompatibility, including the USP, ISO, EP, and JP.

Chemical compatibility

The film, being the largest component of any flexible container system, warrants particular attention in the selection process. Single-use containers are made of several film types with polyethylene (PE) product contact layers that demonstrate chemical compatibility in a variety of bioprocessing applications. Chemical compatibility data are obtained from resin suppliers, technical publications, and field and in-house testing. Ratings are based on physical test results after exposure to a specific environment for a specific period of time.

Leachables and extractables

Film used in single-use containers has been tested to identify and quantify compounds that might migrate from the single-use container into extraction solutions.

11.2. Shipping containers

Packaging specifications for packaged products weighing 150 pounds (68 kg) or less conform to the International Safe Transit Association (ISTA) 2 Series tests. ISTA 2 Series tests are a combination of basic test elements from ISTA 1 Series tests (non-simulation integrity performance testing) and advanced test elements from ISTA 3 Series tests (general simulation performance testing). ISTA 2 Series tests challenge the capability of the package and product to withstand transport hazards. However, these tests only simulate some actual transport hazards, and do not necessarily comply with carrier packaging regulations.

Unitized (palletized) products

Packaging specifications for unitized loads of the same product conform to the International Safe Transit Association (ISTA) 1 Series tests. A unitized load is defined as one or more products or packaged products, typically on a skid or pallet, but always secured together or restrained for distribution as a single load. Examples include a stretch wrapped pallet load of individual containers, and a polypropylene or stainless steel rigid bin designed to ship 100 to 1000 L of liquid.

ISTA 1 Series tests comprise the most basic category of performance tests. ISTA 1 Series tests challenge the capability of the package and product to withstand transport hazards. However, these tests only simulate some actual transport hazards, and do not necessarily comply with carrier packaging regulations.

UN rated packaging

Packaging specifications for products shipped in UN rated drums meet the following UN ratings:

- UN 1H2/Y250/S (solids)
- UN 1H2/Y1.2/100 (liquids)

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Medium designations

12.1. Chemically defined media (CDM)

Designation of a medium as chemically defined indicates that the formulation has been manufactured with raw materials whose compositions are known and precisely quantifiable. Each raw material is a purified component, and exact amounts are added during the manufacturing process. The formulation contains no hydrolysates or animal serum.

12.2. ADCF media

Designation of a medium as ADCF indicates that the formulation has been manufactured with raw material components that are of non-animal origin.

12.3. Serum-free media (SFM)

Designation of a medium as SFM indicates that the medium does not contain serum. Depending upon the formulation, SFM may or may not include components of animal origin. The formulation may or may not be chemically defined or ADCF as described above.

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Animal origin, non-animal origin, segregation

13.1. Animal origin definition

The current working definition of animal origin is a substance derived directly from animal tissue.

13.2. Raw materials of animal origin

Some of our medium and buffer formulations include raw materials that are directly of animal origin. With these raw materials, we undertake to document supplier certifications of the animal species, tissue of origin, country of origin, and animal origin material adventitious agent risk reduction steps. Copies of the European Directorate for the Quality of Medicines & HealthCare (EDQM) certificates of suitability are obtained where applicable. We provide animal origin surveys to our raw material suppliers and input the response information into our QA database.

13.3. ADCF raw materials

For those raw materials that are ADCF, we obtain certification from our raw material suppliers that the raw materials are of non-animal origin or ADCF. Suppliers of ADCF components have either responded to questionnaires or provided specification documents in order to certify that these components are not of animal origin. We document supplier certification of the non-animal source of origin, the method of manufacture, the country of origin and the manufacturing location (country). We provide material origin surveys to our raw material suppliers and input the response information into our QA database.

13.4. Incidental contact substances in certain raw materials

It is recognized that certain raw materials can be derived from non-animal sources such as microbial fermentation. Some of these might utilize animal origin peptides or other components in fermentation or purification processes. However, these are incidental contact materials and are not the original source of derivation. In these limited cases, we document supplier certifications of source, country of origin, and risk reduction steps relating to the incidental contact substances.

13.5. ADCF status of packaging and product contact materials

Plastic materials can contain additives used as release agents or as anti-slip or anti-block processing aids produced from bovine tallow. These substances (such as calcium stearate) are incorporated into resins to facilitate the production and processing of the resin material. While certain plastic contact surfaces like filters, connectors, tank liners, and tubing will have limited exposure time to our products, the greatest surface-to-product contact areas and product resident times will be with the chamber surfaces. We provide animal origin questionnaires to our plastic component suppliers and incorporate the response information into our QA database. Additionally, we are working with key filter and other plastic suppliers to encourage them to move to ADCF plastics where feasible.

13.6. ADCF component segregation

ADCF components for use in dry powdered or liquid medium production are received and transferred to the appropriate controlled component storage area. From the controlled component storage area, ADCF components are moved into segregated ADCF production areas where they are sequentially formulated, mixed, filtered, filled, and labeled. Segregation includes the separation of air handling systems in powdered medium production areas, and the use of disposables in liquid medium production. After QC testing and QA review, the finished ADCF products are transferred to the appropriate controlled finished goods storage area, and then shipped to customer.

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Risk mitigation

To enable an uninterrupted supply of products, we have developed and implemented procedures that anticipate and address our production reliability, risk assessment and mitigation, and crisis management. These documented procedures anticipate and minimize interruption to key business processes, production facilities, and distribution sites ensuring the continuity of supply to customers.

Our business continuity plan document addresses an organization-level response to emergencies of sufficient magnitude that can cause a significant disruption to normal business operation functioning.

A categorical description of business units and their activities stratified by level of importance with minimal staffing levels and recovery time objectives is addressed in our “Essential business activities” document. Our “Emergency response plan” document is a set of comprehensive response procedures to be implemented in the event of workplace emergencies that include medical emergencies, fires or explosions, power disruptions, hazardous material spills, chemical spills, infectious disease pandemics, and natural or man-made disasters. All our employees are trained for emergency response according to the “Desktop emergency guide”.

All raw materials used in the manufacture of media, process liquids, and reagents are evaluated for a variety of supply chain factors according to our component and service risk evaluation procedure. Where applicable, supply agreements are established on unique, single-sourced raw materials. Additionally, R&D, supply chain, and QC/QA resources are employed to identify additional vendors. For high-use raw materials, minimum/maximum inventory levels are managed on an ongoing basis.

We have the ability to utilize our qualified vendors for components used in all media, process liquids, and reagents, or to use client-specified vendors for custom media, process liquids, and reagents for that particular client through our supplier certification procedure. Metrics are in place within our Supply Chain department, and our qualified vendors are graded quarterly.

We continue to review and refine our contingency strategies to meet the needs of our customers. Each refinement enhances our ability to serve our customers. Please direct questions concerning our contingency strategies to your local sales representative.

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Regulatory

We register annually with the U.S. Food and Drug Administration (FDA) as a Class I medical device establishment. The intended use statements and promotional materials define the regulatory status of HyClone products and verbal claims associated with them. Media are exempt from premarket notification requirements (21 CFR 864). Media may be used as a component in the manufacture of a device or drug that requires licensing. It is the responsibility of the device or drug manufacturer to determine if serum or cell culture medium is suitable for their application.

As a manufacturer of medical devices, our facilities are inspected by the U.S. Food and Drug Administration for compliance with cGMP as listed in 21 CFR 820 Chapter 1, Quality systems regulation.

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Certificate of analysis examples

HyClone

SAMPLE CERTIFICATE OF ANALYSIS

Product: RPMI-1640 MEDIUM
+ 2.05 mM L -Glutamine
− Sodium Bicarbonate
(+ 2.0 g/L)

Lot #: AAG204085 **Catalog #:** SH30011 **Expiration Date:** JUL/2018

Test	Specification	Units	Results
Appearance	White to Off -White Powder		White to Off -White Powder
Solubility at 1X Conc.	Clear Solution		Clear Solution
Moisture	≤ 2.00	%	0.43
pH without $NaHCO_3$	7.5 - 8.5		8.3
Osmolality without $NaHCO_3$	220 - 245	mOsm/kg	227
Endotoxin	≤ 1.0	EU/mL	< 0.1
Growth Promotion			Satisfactory

Cell growth was assessed over a minimum of three subculture generations. Cell cultures are observed fo r evidence of nutritional deficiency, cytotoxicity, or morphological aberrations. The product is tested in parallel with a control lot.

Cell Lines Used: CHO -K1 Cells

Quality Control Department **Date**

Cytiva
HyClone Laboratories, Inc.
925 West 1800 South Logan, Utah 84321 USA T 435 792 8000 F 435 792 8011

cytiva

HyClone

SAMPLE CERTIFICATE OF ANALYSIS

Product: DMEM/LOW GLUCOSE
+ 4.00 mM L -Glutamine
+ 1000 mg/L Glucose
+ 110 mg/L Sodium Pyruvate

Lot #: AAE202830 **Catalog #:** SH30021 **Expiration Date:** MAY/2016

Test	Specification	Units	Results
Appearance	Clear reddish solution		Clear reddish solution
pH	7.0 - 7.4		7.2
Osmolality	290 - 320	mOsm/kg	318
Sterility Testing			
Bacteria and Fungi	No Growth		No Growth
Endotoxin	≤ 1.0	EU/mL	<0.1
Growth Promotion			Satisfactory

Cell growth was assessed over a minimum of three subculture generations. Cell cultures are observed for evidence of nutritional deficiency, cytotoxicity, or morpholog ical aberrations. The product is tested in parallel with a control lot.

Cell Line Used: BHK -21 Cells

Quality Control Department **Date**

Cytiva
HyClone Laboratories, Inc.
925 West 1800 South Logan , Utah 84321 USA T 435 792 8000 F 435 792 8011

cytiva

HyClone

SAMPLE CERTIFICATE OF ANALYSIS

Product: SFM4CHO™
– L-Glutamine
+ 2.2 g/L Sodium Bicarbonate

Lot #: AAH206036 **Catalog #:** SH30548 **Expiration Date:** AUG/2016

Test	Specification	Units	Results
Appearance	Clear solution		Clear solution
pH	7.0 – 7.4		7.1
Osmolality	280 - 320	mOsm/kg	308
Endotoxin	≤ 10	EU/mL	<0.1
Sterility Testing Bacteria a nd Fungi	No growth		No growth
Growth Promotion			Satisfactory

Cell growth was assessed over a minimum of three subculture generations. Cell cultures are observed for evidence of nutritional deficienc y, cytotoxicity, or morphological aberrations.

Cell Lines used: Suspension CHO -K1 Cells

Quality Control Department Signature **Date**

Cytiva
HyClone Laboratories, Inc.
925 West 1800 South Logan, Utah 84321 USA T 435 792 8000 F 435 792 8011

cytiva

cytiva.com/hyclone

For local office contact information, visit cytiva.com/contact

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