Sections in This Document 本文章节

1. Purpose 目的

The purpose of this Pharmaceutical Microbiology Manual (PMM) is to collectively clarify, standardize, and communicate useful analytical procedures that are not specifically addressed in the microbiology methods chapters in the United States Pharmacopeia. In addition, some sections of this manual can serve as a technical reference when conducting microbiological inspections of drug, biotechnology and medical device manufacturers. The contents of this PMM were collaboration between ORS and CDER in order to maximize the efficiency of our analytical results to support CDER's goal to assure the safety and reliability of commercially distributed medical products.

本《药物微生物学手册》(PMM) 的目的是集中澄清、标准化和交流《美国药典》中微生物学方法章节中未具体涉及的有用分析程序。此外，本手册的某些章节可以作为对药品、生物技术和医疗器械生产商进行微生物检验时的技术参考。该 PMM 的内容是 ORS 和 CDER 之间的合作成果，其目的是最大限度地提高我们分析结果的效率，以支持 CDER 确保商业销售医疗产品的安全性和可靠性的目标。

2. Introduction 概述

The Pharmaceutical Microbiology Manual (PMM) evolved from the Sterility Analytical Manual and is a supplement to the United States Pharmacopeia (USP) for pharmaceutical microbiology testing, including antimicrobial effectiveness testing, microbial examination of non-sterile products, sterility testing, bacterial endotoxin testing, particulate matter, device bioburden and environmental monitoring testing. The goal of this manual is to provide an ORS/CDER harmonized framework on the knowledge, methods and tools needed, and to apply the appropriate scientific standards required to assess the safety and efficacy of medical products within ORS testing laboratories. The PMM has expanded to include some rapid screening techniques along with a new section that covers inspectional guidance for microbiologists that conduct team inspections.

《药物微生物学手册》（PMM）由《无菌分析手册》演变而来，是美国药典（USP）对药物微生物学检测的补充，包括抗菌效力检测、非无菌产品微生物检测、无菌检测、细菌内毒素检测、颗粒物、设备生物负载和环境监测测试。本手册的目标是提供 ORS/CDER 双方认可的所需知识、方法和工具框架，并应用所需适当科学标准在 ORS 测试实验室内评估医疗产品的安全性和有效性。PMM 已进行了扩充，其中包括了一些快速筛选技术以及一个新章节，该章节涵盖了进行团队检查的微生物学家的检查指南。

This manual was developed by members of the Pharmaceutical Microbiology Editorial Board and includes individuals with specialized experience and training.

本手册由药物微生物学编辑委员会成员编写，包括具有专业经验和培训的人员。

The instructions in this document are guidelines for ORS analysts. When available, analysts should use procedures and worksheets that are standardized and harmonized across all ORS labs, along with the PMM, when performing analyses related to product testing of pharmaceuticals and medical devices. When changes or deviations are necessary, documentation should be completed per the laboratory's Quality Management System. Generally, these changes should originate from situations such as new products, unusual products, or unique situations.

本文件中的说明是 ORS 分析人员的指南。分析人员在执行与药品和医疗器械产品测试相关的分析时，应使用在所有 ORS 实验室以及 PMM 中标准化和统一的程序和工作表（如有）。当需要修改或有所偏离时，应根据实验室的质量管理体系完成文件记录。一般来说，这些变

化应该源于诸如新产品、异常产品或特殊情况等情况。

This manual was written to reduce compendia method ambiguity and increase standardization between ORS laboratories. By providing clearer instructions to ORS labs, greater transparency can be provided to both industry and the public.

本手册旨在减少药典方法的歧义并提高 ORS 实验室之间的标准化。 通过向 ORS 实验室提供更清晰的说明，可以为行业和公众提供更大的透明度。

However, it should be emphasized that this manual is a supplement and does not replace any information in USP or applicable FDA official guidance references. The PMM does not relieve any person or laboratory from the responsibility of ensuring that the methods being employed from the manual are fit for use, and that all testing is validated and/or verified by the user.

但是，应该强调的是，本手册是一个补充，不能取代 USP 或适用的 FDA 官方指南参考中的任何信息。PMM 不免除任何人或实验室确保手册中采用的方法适合使用以及所有测试均由用户验证和/或验证的责任。

The PMM will continually be revised as newer products, platforms and technologies emerge or any significant scientific gaps are identified with product testing.

随着新产品、平台和技术的出现，或者在产品测试中发现任何重大的科学差距，将持续对 PMM 进行修订。

Reference to any commercial materials, equipment, or process in the PMM does not in any way constitute approval, endorsement, or recommendation by the U.S. Food and Drug Administration.

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5. Chapter 1: Antimicrobial Effectiveness Testing 第一章：抑菌效力测试

Antimicrobial Effectiveness testing is described in USP <51>. Previously this chapter was known as "Preservative Effectiveness Testing". Detailed procedure for the performance of the test can be found in USP <51>.

在 USP<51>中描述了抑菌效力试验，以前这一章被称为"防腐效力试验"。执行测试的详细程序可以在 USP<51>中找到。

A. Media 培养基

For the cultivation of the test organisms, select agar medium that is favorable to the rigorous growth of the respective stock culture. The recommended media are Soybean Casein Digest Agar/Broth and Sabouraud's Dextrose Agar/Broth. Add a suitable inactivator (neutralizer) for the specific

antimicrobial properties in the product to the broth and/or agar media used for the test procedure whenever needed.

为了培养测试微生物，选择有利于相关储备培养菌能严格生长的琼脂培养基。推荐的培养基有大豆胰蛋白消化琼脂/肉汤培养基和沙氏葡萄糖琼脂/肉汤培养基。如果需要，将产品中具有特定抗菌性能的合适灭活剂（中和剂）添加到用于测试程序的肉汤和/或琼脂培养基中。

B. Growth Promotion of the Media 培养基促生长

Media used for testing needs to be tested for growth promotion by inoculating the medium with appropriate microorganisms. It is preferable that test microorganisms be chosen for growth promotion testing (Section D).

用于测试的培养基需要通过用适当微生物的培养基接种来测试促菌生长能力。最好选择测试微生物用于促菌生长能力试验（D 部分）。

Solid media tested for growth promotion is to be set up using the method that will be used to analyze the product (pour plate or spread plate) to determine a microbial plate count (CFU) which must be ≥ 50% of the microorganism inoculum's calculated value.

为了确定微生物平板计数（CFU）必须大于等于（≥）微生物接种菌计算值的 50%，应使用准备用于分析产品的方法（倾注平板法或平板涂布法）制作用于促菌生长能力试验的固体培养基。

C. Suitability of the Counting Method in the Presence of Product 有产品时计数方法的适用性

For all product types, follow current USP methodology in chapter <51>, with the following additional instructions.

所有的产品类型均需遵守现行 USP<51>的方法，和以下附加说明。

Prior to the Antimicrobial Effectiveness testing, determine if any antimicrobial properties exist by performing a Suitability testing utilizing microorganisms used for product testing (section D). Should the Suitability Test fail the results of Suitability test are invalid and will need to be repeated with proper method modification to neutralize the inhibiting property.

在抑菌效力试验之前，使用产品测试的微生物进行适用性试验（D 部分），确定培养基是否存在任何的抑菌特性。如果适用性测试失败，则适用性测试的结果是无效的，应对方法进行适当修改以中和抑菌特性，再重复适用性测试。

If multiple samples of the same product from the same manufacturer (same amount and form) are collected, one sample may be used for method suitability for all the samples collected.

如果收集同一生产商同一产品的多个样品（相同的数量和形式），可仅使用其中一个样品进行方法适用性试验。

D. Test Organisms 检测用菌

All cultures must be no more than 5 passages removed from the original stock culture.

所有培养用菌，从原始菌开始传代不得超过 5 代。

1. *Candida albicans* (ATCC No. 10231) 白色念珠菌
2. *Aspergillus brasiliensis* (ATCC No. 16404) 巴西曲霉
3. *Escherichia coli* (ATCC No. 8739) 大肠杆菌
4. *Pseudomonas aeruginosa* (ATCC No. 9027) 铜绿假单胞菌

5. *Staphylococcus aureus* (ATCC No. 6538) 金黄色葡萄球菌

E. Preparation of Inoculum 接种物的制备

Preparatory to the test, inoculate the surface of the appropriate agar medium from a recently grown stock culture of each of the above test microorganisms. Use Soybean-Casein Digest agar for *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 6538 and incubate at 32.5 ±2.5 °C for 3 – 5 days. Use Sabouraud Dextrose agar for *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 and incubate at 22.5 ± 2.5 °C for 3 – 5 days for *Candida albicans* and 3- 7 days for *Aspergillus brasiliensis*.

准备测试时，从最近生长的每种上述测试微生物的原种培养物中接种至适当的琼脂培养基表面。对大肠杆菌 ATCC 8739、铜绿假单胞菌 ATCC 9027 和金黄色葡萄球菌 ATCC 6538 使用大豆-酪蛋白消化琼脂，并在 32.5 ±2.5 °C 下培养 3 – 5 天。对白色念珠菌 ATCC 10231 和巴西曲霉 ATCC 16404 使用沙氏葡萄糖琼脂，并在 22.5 ±2.5 °C 下培养 3-5 天（白色念珠菌）和 3-7 天（巴西曲霉）。

Harvest the cultures by washing the growth with sterile saline to obtain a microbial count of about $1x10_8$ CFU/mL (see *Microbial Enumeration Tests* <61> and *Tests for Specified Microorganisms* <62>). For the *A. brasiliensis* ATCC 16404 culture, use sterile saline containing 0.05% polysorbate 80.

通过用无菌盐水洗涤生长物来收获培养物，以获得约 $1x10^8$ CFU/mL 的微生物（参见微生物计数测试 <61> 和特定微生物测试 <62>）。对于 A. 巴西曲霉 ATCC 16404 培养，使用含有 0.05% 聚山梨醇酯 80 的无菌盐水。

Alternatively, cultures may be grown in a liquid medium, i.e. Soybean Casein Digest Broth or Sabouraud's Dextrose Broth, (except for the A. brasiliensis ATCC 16404 culture) and harvested by centrifugation, washing and suspending in sterile saline to obtain a count of about 1 X 108 colony forming units (CFU) per mL.

或者，培养物可以在液体培养基中生长，即大豆酪蛋白消化肉汤或沙氏葡萄糖肉汤（A 巴西曲霉 ATCC 16404 培养除外），离心收获，洗涤，置无菌盐水中得 1X 10^8 CFU/ml 的菌悬液。

The estimate of inoculum concentration may be obtained by turbidimetric procedures for the challenge microorganisms and later confirmed by plate count.

接种物浓度的估计值可以通过挑战微生物的比浊法获得，然后通过平板计数确认。

Refrigerate the suspension if not used within 2 hours at 2-8 °C.

如果 2 小时内未使用，则将菌悬液冷藏在 2-8 ℃ 下。

Determine the number of CFU/mL in each suspension using the appropriate media and recovery incubation times to confirm the CFU/mL estimate.

使用适当的培养基和恢复培养时间确定每个悬浮液中的 CFU/mL 数量，以确认 CFU/mL 估算值。

Use bacterial and yeast suspensions within 24 hr. of harvest. The mold preparation may be stored under refrigeration (2-8 °C) for up to 7 days.

细菌和酵母菌悬液在收获后 24 小时内使用。霉菌悬液可在冷藏 (2-8 °C) 下储存长达 7 天。

Note: Alternative commercially available standardized cultures may be used in lieu of in-house prepared cultures.

注意：可使用替代的市售标准化培养物代替自制的培养物。

F. Procedure 方法

The procedure requires that the test be conducted with a suitable volume of product. It is advisable to begin with at least 20 mL of product. Use the original product containers whenever possible or five sterile, capped bacteriological containers of suitable size into which a suitable volume of product has been transferred. If the diluted product exhibits antimicrobial properties, specific neutralizers may need to be incorporated into the diluents or the recovery media. For purposes of testing, products have been divided into four categories:

程序要求用适量产品来进行测试，建议从至少 20mL 的产品开始。尽可能使用原来的产品容器，或将合适体积的产品转移到 5 个合适尺寸的无菌细菌学容器。如果稀释的产品表现出抑菌性能，则可能需要将特定的中和剂掺入稀释剂或恢复培养基中。根据测试的目的，产品分为四类：

Category 1 – Injections, other parenteral including emulsions, otic products, sterile nasal products, and ophthalmic products made with aqueous bases or vehicles.

第一类—注射剂，其他注射剂包括乳剂、耳用产品、无菌鼻用产品和用水溶基质或辅料制成的眼用产品。

Category 2 – Topically used products made with aqueous bases or vehicles, non-sterile nasal products, and emulsions, including those applied to mucous membranes.

第二类—用水溶基质或辅料制成的局部使用的产品，非无菌鼻用产品和乳剂，包括用于粘膜的那些产品。

Category 3 – Oral products other than antacids, made with aqueous bases or vehicles.

第三类—抗酸性以外的口服制剂，用水溶基质或赋形剂制成。

Category 4 – Antacids made with aqueous bases or vehicles.

第四类—用水溶基质或辅料制成的抗酸性产品。

Inoculate each container with one of the prepared and standardized inoculums and mix. The volume of the suspension inoculums used is 0.5% to 1.0% of the volume of the product. The concentration of the test organisms added to the product for Categories 1, 2 and 3 is such that concentration of the test preparation immediately after inoculation is between $1x10_5$ and $1x10_6$ colony forming organisms (CFU) per mL of product. *If no suitable neutralizing agent or method is found and method suitability requires significant dilution, a higher level of inoculum (e.g., 10_7-10_8) may be used so that a 3-log unit reduction can be measured. For category 4 products (antacids) the final concentration of the test organisms is between $1x10^3$ and $1x10^4$ CFU/mL of product.*

每个容器接种一个准备好的标准化接种菌并混合。所用混悬液接种菌的体积为产品体积的 0.5%到 1.0%。加入 1，2 和 3 类产品的测试微生物浓度使得接种后立即测试的制备物浓度在每毫升产品含 1×10^5 至 1×10^6 CFU 之间。加入 4 类产品（抗酸性产品）的最终测试微生物浓度在每毫升产品含 1×10^3 至 1×10^4CFU 之间。

Immediately determine the concentration of viable organisms in each inoculum suspension and calculate the initial concentration of CFU/mL by the plate count method (see *Microbial Enumeration Tests* <61>).

立即测定每个接种混悬液中活菌的浓度，并通过平板计数法计算 CFU/mL 的初始浓度（参见微生物计数测试<61>和特定微生物测试<62>）。

Incubate the inoculated containers between 22.5 ±2.5 ℃ in a controlled environment (incubator) and sample the container at specified intervals. The container sampling intervals include: Category 1 products are sampled at 7, 14, and 28 days and Category 2 – 4 products are sampled at 14 and 28 days. Refer to table 3 within USP <51>. Record any changes in appearance of the product at these intervals. Determine the number of viable microorganisms per mL present at each of these sampling intervals by the plate count method utilizing media with suitable inactivator (neutralizer). Calculate the change in log10 values of the concentration per mL based on the calculated concentration in CFU/mL present at the start of the test for each microorganism at the applicable test intervals and express the changes in terms of log reductions.

在 22.5±2.5 ℃之间的受控环境（培养箱）中培养接种容器，并按规定的时间间隔对容器进行取样。容器取样时间间隔包括：第 1 类产品的取样时间为第 7，14 和 28 天，第 2-4 类产品的取样时间为第 14 和 28 天。请参阅 USP<51>中的表格内容。记录这些时间间隔内产品外观的所有变化。通过使用含有合适灭活剂（中和剂）的平板计数法确定每个取样时间间隔下每毫升产品的活菌数。根据在合适测试间隔的每个微生物开始测试时计算出的 CFU/mL 浓度，来计算每毫升浓度 log10 值的变化，并表示为对数减少的变化。

NOTE: The USP does not require a specific volume of product to be added to each of the five sterile tubes. It is recommended that 20 mL/tube be used to standardize testing for all ORS laboratories.

注：USP 没有要求将特定量的产品分别加入到五个无菌试管中。建议使用 20mL/管对所有 FDA 实验室进行标准化测试。

NOTE: All plate counts should be performed in duplicate (2 plates per dilution), and in a dilution series to detect growth inhibited by the preservative system at the lower dilutions. Carrying the test to the 10^{-3} dilution would be sufficient in most cases to overcome preservative inhibition.

注：所有平板计数应以稀释系列方式一式两份（每个稀释级别 2 个平板），以检测在较低稀释度下由防腐系统抑制微生物生长的情况。大多数情况下在稀释至 10^{-3} 后进行检测足以克服防腐剂的抑制作用。

G. Interpretation 解释

The criteria for microbial effectiveness are met if the specified criteria are met, see table below. No increase is defined as not more than 0.5 log10 unit higher than the previous value measured.

如果上述测试符合规定标准，则也符合微生物有效性的标准，见下表。“没有增加”指的是没有比先前测量值高出 0.5 个 log10 单位。

Category 1 Products 第一类产品	
Bacteria:	Not less than 1.0 log reduction from the initial calculated count at 7 days, not less than 3.0 log reduction from the initial count at 14 days, and no increase from the 14-day count at 28 days.
细菌	7天取样点计数比初始计数减少不少于1.0个对数，14天取样点计数比初始计数不少于3.0个对数，并且在28天取样点的计数从14天后不增加。
Yeast and Molds:	No increase from the initial calculated count at 7, 14, and 28 days.
酵母和霉菌	在7，14和28天取样点的计数从初始计数开始没有增加。
Category 2 Products 第二类产品	
Bacteria:	Not less than a 2.0 log reduction from the initial count at 14 days, and no

	increase from the 14-day count at 28 days.
细菌	14 天取样点计数比初始计数不少于 2.0 个对数，并且在 28 天取样点的计数从 14 天后不增加。
Yeast and Molds:	No increase from the initial calculated count at 14 and 28 days.
酵母和霉菌	在 14 和 28 天取样点的计数从初始计数开始没有增加。
Category 3 Products 第三类产品	
Bacteria:	Not less than a 1.0 log reduction from the initial count at 14 days, and no increase from the 14-day count at 28 days.
细菌	14 天取样点计数比初始计数不少于 1.0 个对数，并且在 28 天取样点的计数从 14 天后不增加。
Yeast and Molds:	No increase from the initial calculated count at 14 and 28 days.
酵母和霉菌	在 14 和 28 天取样点的计数从初始计数开始没有增加。
Category 4 Products 第四类产品	
Bacteria, Yeast and Molds:	No increase from the initial calculated count at 14 and 28 days.
细菌，酵母和霉菌：	在 14 和 28 天取样点的计数从初始计数开始没有增加。

6. Chapter 2: Microbial Examination of Non-Sterile Products 第二章：非无菌药品的微生物检查

This section contains supplemental information for the quantitative enumeration of viable microorganisms and the determination of the absence of specified microorganisms in finished pharmaceutical products and raw materials, previously referred to as Microbial Limits Testing (MLT). The detailed procedures for these tests are not addressed in this PMM chapter because they are found in USP <60> MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: TESTS FOR *BURKHOLDERIA CEPACIA* COMPLEX, USP <61> MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: MICROBIAL ENUMERATION TESTS and <62> MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: TESTS FOR SPECIFIED MICROORGANISMS.

本部分包括活体微生物定量计数和测定成品及原料药中不得检出的特定微生物的补充信息，以前被称为微生物限度试验（MLT）。这些测试的详细程序没有列入本 PMM 中，因为可以在 USP<60>“非无菌药品的微生物检查：洋葱伯克霍尔德菌复合体的检测”、USP<61>“非无菌产品的微生物检查：微生物计数检测”和<62>“非无菌产品的微生物检查：特定微生物检测”中找到。

USP Chapter <60> is a test to detect the presence of *Burkholderia cepacia* species in a substance or preparation. Products for inhalation use or aqueous preparations for oral, oromucosal, cutaneous, or nasal USP <61> describes the methods for enumeration of microorganisms from pharmaceuticals and includes membrane filtration, conventional plate count (including pour-plate method, surface-spread method), and the Most-Probable-Number (MPN).

USP <60> 是检测物质或制剂中是否存在洋葱伯克霍尔德菌的测试。对于用于口腔、口腔粘膜、皮肤或鼻腔的吸入用产品或水性制剂，USP <61> 描述了对药物中微生物计数的方法，包括膜过滤、常规平板计数（包括倾注平板法、表面铺展法）和最可能数 (MPN)法。

USP Chapter <62> describes specific enrichment procedures depending on the target specified microorganism that must be absent, as required by a product monograph. Products which are

insoluble or immiscible in water must be appropriately treated to obtain a suspension suitable for the test procedures. USP <1111> is used to identify organism recommendations for various drug dosage forms. use—are of concern.

USP <62>描述了根据产品专论的要求不得检出的指定目标微生物特定的富集程序。 不溶于水或不溶于水的产品必须经过适当处理以获得适合测试程序的悬液。USP <1111> 用于确定不同用途的各种药物剂型的检测微生物建议。

（译注：上述最后一句英文貌似文理不通，未能理解是何含义。USP<1111>内容为“非无菌药品的微生物检查：原料药和制剂的可接受标准”。）

It is important to note that even though the USP delineates methods for the recovery and identification of specified microorganisms based on monograph requirements, it is normally necessary to determine if any other microorganisms may also be present in the product(s) that may be objectionable and report these microorganisms on worksheets. In many cases, these may be opportunistic or emerging pathogens not targeted for recovery by USP <62>. Identification methods, such as VITEK, should be used to identify any microbes recovered during USP <62> testing. Alternative methods, advanced molecular methods (i.e. PCR, sequencing, etc.) or the use of additional general enrichment agar plates or broth without selective properties, may better suit the screening of test samples. The application of these additional agars or methods may need to be considered based on the target population of the drug or product under analysis and may require a dialogue with the laboratory supervisor for additional instructions.

需要注意的是，即使 USP 根据各论要求描述了特定微生物的回收和鉴定方法，通常也需要确定产品中是否还可能存在任何其他有害微生物，如有则要报告在检验记录中。在许多情况下，可能是凑巧，或者不是作为 USP <62>回收目标的新兴病原体。应使用 VITEK 等鉴别方法来鉴别在 USP <62> 测试期间发现的任何微生物。替代方法、先进的分子方法（即 PCR、测序等）或使用额外的通用富集琼脂平板或无选择性的肉汤，可能更适合测试样品的筛选。这些额外的琼脂或方法的应用可能需要根据被分析的药物或产品的目标人群来考虑，并且可能需要与实验室主管沟通以获得更多指示。

A. Product Storage and Handling 产品存贮和处理

Samples are to be held under the same storage conditions required by the package label or insert.

样品应在包装标签或说明书要求的相同储存条件下保存。

1. Prior to product testing, the exterior of the unit container should be disinfected before transfer to the work station or HEPA filtered laminar flow hood. If the product container is not hermetically sealed do not soak the product container in a disinfection solution which may allow the ingress of bactericidal solution into the product.

   产品测试前，单元容器的外部应在转移到工作站或 HEPA 过滤层流罩之前进行消毒。如果产品容器未密封，请勿将产品容器浸泡在消毒溶液中，否则灭菌溶液可能会渗入产品中。

2. The work area for opening the unit container should be either a HEPA filtered laminar flow hood or an alternate controlled environment to safeguard the exposure of open media and product to either environmental or personnel contamination.

   打开单元容器的工作区应该是一个 HEPA 过滤层流罩或一个替代的受控环境，以保护打开的介质和产品暴露在环境或人员污染中。

3. If the sample is an aqueous based product, the unit(s) should be shaken prior to transfer to work area to maximize microbial dispersement.

   如果样品是水基产品，应在转移到工作区之前摇动装置，以最大限度地分散微生物。

4. All subsequent manipulation of test tubes with product or sub- culturing can be conducted on the laboratory work bench or within a Biological Safety Cabinet (BSC) if filamentous fungi are suspected.

   如果怀疑丝状真菌，可以在实验室工作台上或在生物安全柜 (BSC) 内进行带有产品或传代培养的试管的所有后续操作。

B. Gowning Requirements 更衣要求

When conducting the testing, the analyst should wear a clean lab coat, sterile sleeves and sterile gloves. Gloves should be frequently disinfected especially between opening and handling sample (product) units.

进行检测时，分析人员应穿干净的实验服、无菌护袖和无菌手套。手套应经常消毒，尤其是在打开和处理样品（产品）单元之间。

Depending on the type of Laminar Flow Hood or equipment barriers in a particular laboratory, it might be beneficial to also wear a surgical mask and hair net.

根据特定实验室中层流罩或设备屏障的类型，同时佩戴口罩和发罩可能有好处。

C. Growth Promotion, Indicative and Inhibitory Properties of the Media 培养基的促生长、指示性和抑制特性

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients for growth promotion and where appropriate, for indicative and inhibitory properties, following USP <60>, <61> and <62>. Each chapter provides guidance on test strains to be used for each type of media, refer to Table 1 of USP <60>, <61> and <62>. Ensure that seed-lot cultures used are not more than five passages removed from the original master seed-lot. Test strains suspensions should be used within 2 hours, or within 24 hours if refrigerated between 2 ℃ and 8 ℃. Spore suspensions (A. *brasiliensis*, B. *subtilis, and C. sporogenes)* refrigerated between 2 ℃ and 8 ℃ may be kept for a validated period. If commercially available ready-to-use bacteria or fungal suspension are used, then the manufacturer's instructions should be followed with respect to preparation and storage requirements. Additionally, all bacterial and spore suspensions should be prepared to yield ≤100 CFU. Growth promotion (and suitability test) plates and tubes should not be incubated in the same incubators used for product testing. If this cannot be avoided because of limited space, it is preferable to store the "spiked samples" in the lower half of the incubator below the sample inoculated plates and tubes.

按照 USP <60>、<61> 和<62>对每批预制培养基和每批由脱水培养基或成分制备的培养基进行促进生长试验，并在适当情况下检测指示性和抑菌性。每章都提供了用于每种培养基的测试菌株的指导，请参阅 USP<60>、<61>和 <62>的表 1。确保所用菌从原代开始传代未超过 5 代。测试菌悬液应在 2 小时内使用，如果冷藏在 2-8 ℃ 之间，则应在 24 小时内使用。在 2-8 ℃ 冷藏的孢子悬液（A. brasiliensis 巴西芽孢杆菌、B. subtilis 枯草芽孢杆菌 和 C. sporogenes 生孢梭菌）可以保存一段时间。如果使用市售的即用型细菌或真菌悬液，则应遵守生产商关于制备和储存要求的说明。此外，所有细菌和孢子悬液都应制备为≤100 CFU。促生长试验（和适用性测试）平板和试管不应在用于产品测试的同一培养箱中培养。如果由于空间有限而无法避免这种情况，则最好将"加标样品"存放在培养箱下半部分的样品接种板和试管下方。

D. Suitability of the Test Method 检测方法适用性

Suitability demonstrates that the products tested do not exhibit inhibitory effects on the growth of microorganisms under the conditions of the tests. Although the intent is to perform the suitability test before performing the analysis of the product, it is acceptable to run the product test and the suitability test concurrently. However, it should be noted that if the suitability test is run concurrently with the product test and the suitability test should fail, the results of the product test are invalid and the suitability test as well as the product test will need to be repeated with proper method modification to neutralize the inhibiting property.

适用性表明所测试的产品在测试条件下对微生物生长没有抑制作用。尽管期望在进行产品分析前进行适用性试验，但同时进行产品测试和适用性试验也是可以接受的。但应注意的是，如果同时进行适用性试验和产品测试，适用性试验失败，则产品检测结果是无效的，应对方法进行适当修改，中和抑菌性，然后再重复进行适用性试验和产品检测方法。

Neutralizing agents may be used to neutralize the activity of antimicrobial agents in products, see USP <61> Table 2 for a list of potential neutralizing agents/methods. The appropriate neutralizing agent should be added preferably before sterilization of the media. Include a blank control with neutralizer and without product to demonstrate efficacy and absence of toxicity for microorganisms.

可以使用中和剂中和产品中抑菌剂的活性，见 USP<61>表 2 中所列的中和剂/方法。最好在培养基灭菌之前加入合适的中和剂。用含中和剂但不含产品的空白对照来证明其对微生物的有效性和无毒性。

USP <60>, <61> and <62> describe the suitability tests necessary for each analysis. The correct inoculum of not more than 100 CFU is required in addition to specific incubation temperatures and durations. Ensure that seed- lot cultures used are not more than five passages removed from the original master seed-lot. For in-house prepared test strain suspensions of vegetative bacteria and yeast should be used within 2 hours, or within 24 hours if refrigerated between 2 ℃ and 8 ℃. Spore suspensions (*A. brasiliensis*, *B. subtilis,* and *C. sporogenes*) can be prepared and maintained between 2 ℃ and 8 ℃ for up to seven days. Additionally, all bacterial and spore suspensions should be prepared to yield ≤100 CFU. If commercially available ready-to-use bacteria or fungal suspension are used, then the manufacturer's instructions should be followed with respect to preparation and storage requirements. USP <60>, <61> and <62> require a control which is without test material to be included in the suitability test. The following viable ATCC derived cultures may be used. Please be aware that under the Microbiological Examination of Nonsterile Products chapters <60>, <61> and <62> users are allowed alternative sources of the below listed strains. The organisms below are recommended for ORS use in order to have a consistent and standard worksheet format:

USP<60>、<61>和<62>描述了每项分析所需的适用性试验。除了特定的培养温度和持续时长外，还需要正确接种物不超过 100cfu。要确保所用菌从原代开始传代未超过 5 代。繁殖体细菌和酵母菌的自制测试菌悬液应在 2 小时内使用；如果在 2-8 ℃-冷藏，应在 24 小时内使用。在 2-8 ℃制备冷藏的孢子混悬液（巴西芽孢杆菌，枯草芽孢杆菌和孢子芽孢杆菌）可保持最长 7 天。另外，所有细菌和孢子悬液的制备均应得到≤100 CFU。如果使用的是商业直接使用的细菌或真菌悬液，则应按生产商的指导进行制备和存贮。USP<60>、<61>和<62>要求在适用性试验中放一个不含有测试原料的对照。可以使用以下活 ATCC 衍生培养菌。请注意，根据非无菌药品微生物检查章节<60>、<61>和<62>，用户可以使用下列菌的其它来源。为了获得一致的标准工作表格式，建议 ORS 使用以下微生物：

USP <60>:	*Burkholderia cepacian (ATCC 25416)*	葱头伯克霍尔德菌
	Burkholderia cenocepacia (ATCC BAA-245)	洋葱伯克霍尔德菌
	Burkholderia multivorans (ATCC BAA-247)	多噬伯克霍尔德氏菌
USP <61>:	*Pseudomonas aeruginosa* (ATCC 9027)	绿脓杆菌
	Staphylococcus aureus (ATCC 6538)	金黄色葡萄球菌
	Bacillus subtilis (ATCC 6633)	枯草芽胞杆菌
	Candida albicans (ATCC 10231)	白念珠菌
	Aspergillus brasiliensis (ATCC 16404)	巴西曲霉
USP <62>:	*Pseudomonas aeruginosa* (ATCC 9027)	绿脓杆菌
	Staphylococcus aureus (ATCC 6538)	金黄色葡萄球菌
	Escherichia coli (ATCC 8739)	大肠杆菌
	Salmonella enterica (ATCC 14028)	肠道沙门氏菌
	Candida albicans (ATCC 10231)	白念珠菌
	Clostridium sporogenes (ATCC 11437)	生孢梭菌

USP <60>, <61> and <62> each contain the acceptance criteria for their respective suitability test. USP <60> requires that the BCC microorganisms must be detected with the indicated reactions. Indicated reactions are indicated by the growth of greenish-brown colonies with yellow halos, or white colonies surrounded by a pink-red zone on BSCA. For USP <61> the Results and Interpretation section requires the inoculated product to have a mean count of any of the test organisms not differing by a factor greater than 2 from the control which was without test material. For example, if the control count is 80 CFU the acceptable counts need to be greater or equal to 40 CFU. USP <62> requires the specified microorganisms to be detected with the indicated reactions.

USP <60>、<61> 和 <62> 均包含各自适用性测试的可接受标准。USP <60> 要求必须用指定的反应检测 BCC 微生物。在 BSCA 上有绿褐色菌落的生长与黄色晕圈或白色菌落被粉红色-红色区域包围的生长是指示反应。对于 USP <61>，结果和解释部分要求接种产品的任何测试微生物的平均计数与没有测试原料的对照相差不超过 2 倍。例如，如果控制计数为 80 CFU，则可接受的计数需要大于或等于 40 CFU。 USP <62> 要求用指定的反应检测指定的微生物。

E. Test Procedure 检测方法

Prepare the sample in a manner to achieve a uniform solution or suspension. This is critical because microbial contamination is not evenly dispersed throughout a lot or sample of product. Use conventional mechanical and shaking methods to the extent that original numbers and types of microorganisms are not altered in the product.

制备样品获得均匀溶液或悬液是很重要的，因为微生物污染并没有均匀地分散在整个产品批次或样品中。使用传统的机械和振摇方法达到上述均匀目的，同时不得改变产品中微生物的原始数量和类型。

Use the following general procedures to prepare and handle samples.

使用以下通用程序来制备和处理样品。

1. Analyze samples as soon as possible after receipt. Inspect each unit visually for integrity of primary container and note any irregularities. Do not use the product container if it has been compromised or damaged without supervisor approval. Testing of a compromised or damaged container should be evaluated on a case by case basis. Discuss with supervisor if compromised unit containers need to be tested for forensic purposes (i.e. product tampering).

   在接收之后尽快分析样品。目测每个内包容器完整性，记录异常。如果产品容器受损或不完整，未获得主管批准情况下不要使用。不完整或受损容器的检查应该各案评估。如果不完整单元容器需要进行取证检查（例如，产品篡改）则应与主管讨论。

2. Identify units to be tested with Analyst's initials, date, subsample number, and sample number.

   使用化验员的首字母、日期、子样品编号和样品编号标示准备进行检测的样品单元。

3. Cleanse outer surfaces of sample containers with sterile wipes using a validated effective antimicrobial agent. Place on a disinfected tray or surface in a properly disinfected laminar flow hood or biosafety cabinet. Allow containers to dry.

   采用经过验证的有效抑菌剂，用无菌抹布清洁样品容器外表。放在经过消毒的托盘或放在经过适当消毒的层流罩或生物安全柜表面上，让容器干燥。

4. Aseptically open containers and perform weighing procedures in a laminar flow hood or biological safety cabinet if possible.

   如可能的话，在无菌条件下打开容器，在层流罩中或生物安全柜中称重。

5. Appropriate environmental controls such as air exposure plates should be used in accordance with local quality procedures.

   应根据本地质量程序使用例如空气暴露碟进行适当的环境控制。

6. Appropriate negative controls should be run concurrently with the sample.

   应同时进行适当的空白控制。

F. Interpretation of the Results 检测结果解释

USP <60>

The possible presence of Bcc is indicated by the growth of greenish-brown colonies with yellow halos, or white colonies surrounded by pink-red zone on BCSA. Any growth on BCSA is confirmed by identification tests. Isolates should be identified to genus and, if possible, species using rapid identification kits or DNA sequencing. The product complies with test if no growth is observed or if the confirmatory identification tests are negative.

在 BCSA 上生长出绿褐色菌落和黄色晕圈或白色菌落被粉红色区域包围显示可能存在 Bcc 。BCSA 上的任何菌株生长都应通过鉴别测试来确认。应使用快速鉴定试剂盒或 DNA 测序将

分离株鉴定至属，如有可能，鉴定至种。如果未观察到生长或确认性鉴定测试为阴性，则产品符合测试标准。

USP <61>

Regarding USP <61> the acceptance criterion for microbiological quality as it pertains to quantitative analyses has an allowable variability of the final colony forming units (CFUs). There is a two-fold tolerance in the final results. For example, if the monograph requires a 100 cfu/ml limit, the acceptable upper limit for these results would be 200 cfu/ml. Additional information is included in the "Interpretation of the Results" section of USP <61> that should be read and understood when reviewing quantitative test results.

关于 USP<61>，由于本质上是定量分析，因此其微生物质量的可接受标准允许最终菌落数（CFU）有一定波动范围，最终结果可以有 2 倍的允差。例如，如果各论要求限度为 100 cfu/ml，则其可接受结果上限为 200 cfu/ml。更多信息在 USP<61>的"结果诠释"一节中，在审核定量检测结果时要阅读并理解该内容。

USP <62>

Bile-Tolerant Gram-Negative 胆汁耐受革兰氏阴性

The product complies with the test if there is no growth. Any isolates should be identified to genus and, if possible, species using rapid identification kits or DNA sequencing.

如果没有生长，则产品符合测试标准。应使用快速鉴定试剂盒或 DNA 测序将任何分离株鉴定至属，如果可能，鉴定至种。

Escherichia coli 大肠杆菌

Growth of colonies indicates the possible presence of *E. coli*. This is confirmed by identification test. Isolates should be identified to genus and, if possible, species using rapid identification kits or DNA sequencing. The product complies with the test if no colonies are present or if the identification tests are negative.

菌落的生长表明可能存在大肠杆菌，应通过鉴定试验证实。应使用快速鉴定试剂盒或 DNA 测序将分离株鉴定至属，如有可能，鉴定至种。如果不存在菌落或鉴定测试为阴性，则产品符合测试要求。

Salmonella 沙门氏菌

The possible presence of *Salmonella* is indicated by the growth of well-developed, red colonies, with or without black centers. This is confirmed by identification tests. Isolates should be identified to genus and, if possible, species using rapid identification kits or DNA sequencing. The product complies with the test if no growth is observed or if the confirmatory identification tests are negative.

发育良好，有或没有黑色中心的红色菌落的生长表明可能存在沙门氏菌。应通过鉴定试验证实。应使用快速鉴定试剂盒或 DNA 测序将分离株鉴定至属，如有可能，鉴定至种。如果没有观察到生长或确认性鉴定测试为阴性，则产品符合测试要求。

Pseudomonas aeruginosa 铜绿假单胞菌

Growth of colonies indicates the possible presence of *P. aeruginosa*. This is confirmed by identification test. Isolates should be identified to genus and, if possible, species using rapid identification kits or DNA sequencing. The product complies with the test if no colonies are

present or if the identification tests are negative.

菌落的生长表明可能存在铜绿假单胞菌，应通过鉴定试验证实。应使用快速鉴定试剂盒或 DNA 测序将分离株鉴定至属，如有可能，鉴定至种。如果未检出菌落或鉴定测试为阴性，则产品符合测试要求。

Staphylococcus aureus 金黄色葡萄球菌

The possible presence of *S. aureus is* indicated by the growth of yellow or white colonies surrounded by a yellow zone. This is confirmed by identification tests. Isolates should be identified to genus and, if possible, species using rapid identification kits or DNA sequencing. The product complies with the test if no growth is observed or if the confirmatory identification tests are negative.

黄色或白色菌落被黄色区域包围，表明可能存在金黄色葡萄球菌，应通过鉴别测试证实。应使用快速鉴定试剂盒或 DNA 测序将分离株鉴定至属，如有可能，鉴定至种。如果没有观察到生长或确认性鉴定测试为阴性，则产品符合测试要求。

Clostridia 梭菌

The occurrence of anaerobic growth of rods with or without endospores, giving a negative catalase reaction indicates the presence of *Clostridia.* This is confirmed by identification tests. Isolates should be identified to genus and, if possible, species using rapid identification kits or DNA sequencing. The product complies with the test if no growth is observed or if the confirmatory identification tests are negative.

出现厌氧生长的杆状菌（无论有无内生孢子），过氧化氢酶反应为阴性，则表明存在梭状芽孢杆菌。应通过鉴别试验进行证实。应使用快速鉴定试剂盒或 DNA 测序将分离物鉴定至属，如有可能，鉴定至种。如未观察到生长或确认性鉴别试验为阴性，则产品符合试验要求。

Candida albicans 白色念珠菌

Growth of white colonies may indicate the presence of *C. albicans*. This is confirmed by identification test. Isolates should be identified to genus and, if possible, species using rapid identification kits or DNA sequencing. The product complies with the test if no colonies are present or if the identification tests are negative.

白色菌落的生长可能表明存在白色念珠菌，应通过鉴别试验进行证实。应使用快速鉴定试剂盒或 DNA 测序将分离物鉴定至属，如有可能，鉴定至种。如果不存在菌落或鉴定试验为阴性，则产品符合试验要求。

*Note: Regarding USP <60> and <62>, each isolate should be identified to genus and, if possible, species using rapid identification kits or DNA sequencing.

*注：关于 USP<60>和<62>，应使用快速鉴定试剂盒或 DNA 测序将每个分离物鉴定至属，如有可能，鉴定至种。

7. Chapter 3: Sterility Testing 第三章：无菌检测

A. Method Suitability Test 方法适用性测试

For all product types, follow current USP methodology in <71>, with the following additional instructions.

所有产品类型应遵循<71>中现行 USP 方法和以下附加说明。

In all cases, even if the product does not include a preservative, the product itself may have growth inhibiting properties. All products should undergo a prescribed Method Suitability test.

在所有情况下，即使产品不含防腐剂，产品本身也可能具有生长抑制特性。所有产品应进行规定的方法适用性试验。

Units selected for suitability testing should be subjected to the same disinfection procedure utilized in the sample analysis.

选择进行适用性测试的装置应采用与样品分析相同的消毒程序。

When developing the testing protocol for method suitability the volume of product as well as the concentration of the product should be evaluated such that the highest volume of product and the highest concentration of product should be used for the method suitability testing.

在制定方法适用性测试方案时，应评估产品的体积和浓度，以便使用最高体积的产品和最高浓度的产品进行方法适用性测试。

If multiple samples of the same product from the same manufacturer (same dosage and form) are collected, one sample may be used for method suitability for all the samples collected.

如果从同一生产商（相同剂量和剂型）采集同一产品的多个样品，则一个样品可用于所有采集样品的方法适用性。

1. When to run Method Suitability: 什么时候要做方法适用性

   a. Run the method suitability test prior to conducting the sterility test in accordance with USP requirements under the following conditions:

      以下情况应根据 USP 要求，在执行无菌测试之前进行方法适用性测试：

      i. If insufficient information about the product exists to judge its probable growth inhibiting activity.

         如果用产品信息不足以判断其是否可能具有生长抑制活性

      ii. In all cases, when there is sufficient analytical time available, i.e., survey type samples.

         在任何情况下，如有足够的分析（即调查类别样本）时间

   b. Run the method suitability test concurrently with product sterility tests when time is critical, and problems associated with 1. above have been resolved. However, it should be noted that if the Method Suitability Test is run concurrently with the product sterility test and the Method Suitability Test should fail, the results of the product test are invalid and the Method Suitability Test as well as the product test will need to be repeated with proper method modification to neutralize the inhibiting property.

      如果时间紧急，并且上述问题已得到解决，则可在进行产品无菌测试时同步进

行方法适用性测试。但是应注意的如果方法适用性试验与产品无菌检测同时进行，方法适用性试验失败，则产品检测结果是无效的，需要适当修改方法，中和抑菌性，再重复进行适用性试验和产品检测。

c. If an insufficient amount of product is collected and the analysis is critical, the suitability test can be conducted at the end of the 14-day incubation period. Be sure to use best judgment and maximum neutralization approach when initially conducting the product sterility test. If the suitability results indicate inhibition then the results, if negative, are invalid. However, if the product test results indicate microbial presence and the suitability test shows inhibition, the results are still valid.

如果采集的产品数量不足，但分析至关重要，则可以在 14 天培养其结束时进行适用性试验。在开始进行产品无菌检测时，务必使用最佳判断和最大中和方法。如果适用性试验结果显示有抑制作用，且结果为阴性，则检测结果无效。但如果产品检出微生物，且适用性试验显示有抑制作用，则结果仍有效。

2. Method Suitability Test Procedures 检测方法适用性程序

Method Suitability and positive culture control tests which require the use of viable microorganisms, should be performed outside the clean room or isolator, in a biosafety cabinet or equivalent.

方法适用性试验以及任何其它需要使用活体微生物的阳性培养物对照试验应在洁净室或隔离器之外的生物安全柜或类似设备内进行。

a. Membrane filtration 膜过滤法

i. Pass product fluid through filter membrane. Rinse the membrane with three 100 ml portions (or more if applicable) of specified rinse fluid. Do not exceed a washing cycle of five times 100mL per filter. This step hopefully will neutralize and remove any antimicrobial residue on the filter membrane.

将产品液体经滤膜过滤。用 3 份各 100ml（适用时可更多）指定淋洗液冲洗滤膜。每张滤膜淋洗不得超过 5 次各 100ml。该步骤非常有可能会中和并除去滤膜上的所有抑菌残留物。

ii. Add indicated test organisms in specified numbers (less than 100 CFU) into the last 100 ml rinse fluid used.

在最后 100ml 淋洗液中加入指定数量（不少于 100CFU）的指定测试微生物。

iii. Filter the rinse fluid and aseptically cut the filter membrane into two equal parts, transfer one half into each of two suitable media. If conducting the sterility test using a closed canister system, rinse each canister with the inoculated rinse fluid.

过滤样品液体，在无菌条件下将滤膜对半剪开，将 2 半分别转移至一份适用培养基中。如果是采用封闭式碳罐系统进行无菌测试，则使用接种后的淋洗液淋洗各碳罐。

iv. If the available number of test vessels is insufficient for a complete challenge test for each individual microorganism, then the test organisms may be composited as necessary. However, confirmation of growth for the composited microorganisms will need to be performed.

如果测试容器的可用数量不足以单独完成每个微生物的挑战试验，则测试微生物可以根据需要进行复合。但是在完成培养之后，复合微生物的生长确认将需要通过分离、革兰氏染色和种/属鉴定来证实。

v. Confirm composited microorganisms by Gram stain, microscopic examination, and identification after the completion of incubation.

在完成培养之后，通过革兰氏染色、显微检查和生化鉴别来确认复合微生物。

vi. See step c. below for additional considerations.

更多考量参见以下步骤 c。

b. Direct inoculation: 直接接种法

For direct inoculation, add the test microorganisms to separate test vessels of product and culture media if sufficient product is available. See step c. below for additional considerations.

对于直接接种，如果有足够产品可用，则将测试微生物加入到单独的产品测试用容器和培养基中。更多考量参见以下步骤 c。

c. The following test procedures apply to Direct Inoculation and Membrane Filtration:

以下测试程序适用于直接接种和膜过滤法：

i. Inoculate the same microorganism using the same medium without the product as a positive control.

使用不含产品的相同培养基接种相同的微生物作为阳性对照。

ii. For bacteria and fungi, incubate test vessels according to USP requirements. Ensure that seed-lot cultures used are not more than five passages removed from the original master seed-lot. For in-house prepared test strain suspensions of vegetative bacteria and yeast should be used within 2 hours, or within 24 hours if refrigerated between 2 ℃ and 8 ℃. Spore suspensions (*A. brasiliensis*, *B. subtilis*, and *C. sporogenes*) can be prepared and maintained between 2 ℃ and 8 ℃ for up to seven days. Additionally, all bacterial and spore suspensions should be prepared to yield ≤100 CFU.

对于细菌和真菌，根据 USP 要求接种至检测容器。确保所用种子批培养物不超过原始主种子批的五代。公司自己配制的繁殖性细菌和酵母测试菌悬液应在 2 小时使用；如果冷藏在 2-8 ℃，则应在 24 小时内使用。孢子混悬液（A 巴西芽孢杆菌，B 枯草芽孢杆菌和 C 孢子芽孢杆菌）可在 2-8 ℃制备并保存最长达 7 天。此外，所有的细菌和孢子悬液应制备为≤100CFU。

iii. If growth comparable to that of the positive control vessel without product is obtained, then you may proceed with the sterility test. If comparable visible growth is not obtained, the antimicrobial activity of the product has not been eliminated under the conditions of the test. Modify the test conditions and repeat the Method Suitability test.

如果获得与无产品的阳性对照容器相当的生长，则可进行无菌试验。如果未获得可比的可见生长，则在试验条件下，产品的抗菌活性未被消除，需

修改试验条件并重复方法适用性试验。

d. If product is found to exhibit growth inhibiting activity when determined concurrently with product testing, the sterility test must be repeated using a neutralizing agent (or increase media volume) to modify the conditions in order to eliminate the antimicrobial activity.

如果在产品测试的同时发现产品表现出生长抑制活性，则必须使用中和剂（或增加培养基体积）重复无菌测试，修改条件消除抗菌活性。

e. Cultures used for the method suitability test can be purchased commercially, ready to use, or can be prepared and maintained locally. Either procedure requires quantitative verification of actual CFU's inoculated at time of use.

用于方法适用性试验的培养物可以商业购买即买即用型，也可以就地制备和保存。任何一种程序都需要对使用时接种的实际 CFU 进行定量确认。

B. Sample Analysis 样品分析

1. Sample Containers 样品容器

a. Open the outer sample packaging on a laboratory bench disinfected with a sporicidal antimicrobial agent. Refer to appropriate literature for choosing suitable antimicrobial agents for use in your facility.

在用杀孢子抗菌剂消毒的实验室工作台上打开样品外包装。请参阅适当的文献，以选择合适的抗菌剂在您的设施中使用。

b. Count the number of units received. Compare this number with the number of units collected.

计算收到的单位数。将此数字与采集的单位数进行比较。

c. Inside the clean room preparation area located outside the ISO 5 area (if available) remove all outer packaging from subsample units that will be tested without compromising the sterile integrity of the product. Remove sample units and place them on a tray or cart disinfected with an effective antimicrobial agent.

在 ISO 5 区域外的洁净室准备区域内（如有），移除将要测试的子样品单元的所有外包装，同时不影响产品的无菌完整性。取下样本单元并将其放置在用有效抗菌剂消毒的托盘或推车上。

Note: One or more units can be sacrificed to aid in the determination for how to aseptically remove test material if the number of the units received is sufficient.

注：如果接收的样品件数足够，可使用一件或多件帮助确定如何无菌去除试验材料。

d. Examine all units visually for container closure integrity, for the presence of any foreign matter in the product and other container closure defects. Note findings on analyst's worksheet.

目视检查所有装置的容器密封完整性、产品中是否存在任何异物以及其他容器密封缺陷。在化验员工作表上记录检查结果。

e. If foreign matter is observed within the primary container, discuss with supervisor the

employment of ORS procedure Document ORA-LAB.015 entitled "Screening Protocol for Direct Staining on Products with Appearance of Visible Contamination" (see QMiS for Procedure).

如果在主容器内观察到异物，则与主管讨论是否要使用题为"对出现可见污染的产品进行直接染色的筛选方案"（程序见 QMiS）的 ORS 程序文件 ORA-LAB.015。

2. Sample Identification 样品标识

If sample units are not identified by the collector, the analyst should identify unit with sample #, initials, date, and sub sample # as appropriate for sample traceability. Otherwise, date and initial each unit.

如果采集器未标示采样人，分析员应使用样本号、首字母缩写、日期和子样本号识别样本单位，以便于样本追溯。否则，在每个样本上标示日期和首字母。

3. Unit Container Disinfection 样品单位容器消毒

a. Cleanse the exterior of all product primary containers using antimicrobial/sporicidal agents.

使用抗菌剂/杀孢子剂清洁所有产品内包容器的外部。

Depending on the clean room design, immediately move the sample to the clean room on a disinfected designated stainless- steel cart or place it inside the clean room pass thru for final preparation. If conducting the sterility test in an isolator, place the sample on a designated stainless-steel cart. Allow exposure of the sample to the disinfectant for appropriate time before further handling. All units should be disinfected appropriately. The suggested disinfection procedures can be performed on commonly encountered units as follows:

根据洁净室的设计，立即将样品放在已消毒不锈钢推车上移动到指定的洁净室，或将其放置在洁净室通道内进行最终准备。如果在隔离器中进行无菌测试，则将样品放置在指定的不锈钢推车上。在进一步处理之前，让样品在消毒剂中暴露适当的时间。所有样品单元均要适当消毒。所有装置应进行适当消毒。常见包装建议的消毒程序如下：

i. Ampoules can be wiped with lint free sterile towel/wipes saturated with disinfectant. Ampoules may be soaked in disinfectant/sporicidal following manufacturer's guidance or laboratory SOP.

可用无绒布无菌毛巾/浸透消毒剂的湿巾擦拭安瓿。可按照生产商的指导或实验室 SOP 在消毒剂/杀孢子剂中浸泡安瓿。

ii. Vials should not be soaked due to the possibility of migration of disinfectant under the closure and into the product.

不应浸泡西林瓶，因为消毒剂可能通过封盖处迁移，进入产品

iii. Laminated Tyvek package composed of polyethylene/plastic laminate can be disinfected with sterile towel/wipes soaked in disinfectant. Tyvek portion lightly scrubbed with sterile particle free dry wipe and air dry in a HEPA filtered laminar flow hood before testing.

由聚乙烯/塑料层压板组成的层压 Tyvek 包装可使用浸泡在消毒剂中的无菌毛

巾/湿巾进行消毒。试验前，用无菌无颗粒干布轻轻擦洗 Tyvek 部分，并在 HEPA 过滤的层流罩中晾干。

iv. Paper Packages can be disinfected with UV light if possible. Wipe where applicable with sterile particle free dry wipes and air dry as above.

如有可能，可使用紫外线对纸质包装进行消毒。如适用，使用无菌无颗粒干湿巾擦拭，并如上所述晾干。

b. Number of units and/or amount of product tested: 受测产品单位数和/或数量

Follow the current edition of the USP to determine the correct number of units to be tested and the amount of product to be analyzed from each unit. It is preferable to test the entire contents of each unit if possible. Follow laboratory policy if it requires testing more units than the USP requires.

按照现行版本的 USP 确定要测试的正确件数以及每件要分析的产品数量。如果可能，最好测试每件的全部内容。如果实验室政策要求测试的单元数超过 USP 要求，则遵循实验室政策。

c. If the number of units collected is less than the USP requirements, discuss with the laboratory supervisor before proceeding. Samples collected in a for-cause situation may be analyzed with a number of units less than the USP requirements.

如果所采集的单元数少于 USP 要求，则在执行下步操作之前与实验室主管讨论。有因情形下采集的样本可使用少于 USP 要求的单元数量进行分析。

C. Preparation for the Analysis 分析准备

1. Media and Rinsing Fluid Preparation: 培养基和冲洗液的制备

Follow current USP when preparing media used for sample analysis. Commercially purchased media may also be used for the analysis.

按现行 USP 制备样品分析所用培养基，亦可使用商业购买的培养基。

Both prepared and purchased media must meet the requirements of the USP growth promotion test of aerobes, anaerobes and fungi.

无论是制备的还是购买的培养基均应满足 USP 的需氧菌、厌氧菌和真菌促生长测试要求。

Media used are: 所用培养基为：

a. Fluid Thioglycollate medium (FTM) This medium should be prepared in a suitable container to provide a surface to depth ratio so that not more than the upper half of the medium has undergone a color change indicative of oxygen uptake at the end of the incubation period. If more than the upper third of the medium has acquired a pink color, the medium may be restored once by heating until the pink color disappears. Care should be taken to prevent the ingress of non-sterile air during cooling.

液体巯基乙酸盐培养基（FTM）：该培养基应在适当的容器中制备，以提供表面深度比，从而使培养基的上半部分发生颜色变化，表明在培养期结束时吸氧。如果介质的上三分之一以上呈现粉红色，则可通过加热使介质恢复一次，直到粉红色消失。在冷却过程中，应注意防止非无菌空气进入。

b. Soybean Casein Digest medium (SCD medium) This media must be incubated under

aerobic conditions

大豆酪蛋白消化培养基（SCD 培养基）：此培养基必须在有氧条件下培养

c. Alternative Thioglycollate medium This type of media must be incubated under anaerobic conditions.

替代巯基乙酸盐培养基：这种培养基必须在厌氧条件下培养。

d. Media for Penicillin and Cephalosporin containing drugs. Add sufficient quantity of sterile Beta-lactamase to the media to inactivate the effect of these antibiotics.

青霉素和头孢菌素类药物的培养基：向培养基中添加足量的无菌 β-内酰胺酶，以使这些抗生素的作用失活。

e. Diluting and rinsing fluids. These fluid rinses may be filtered before sterilization to avoid clogging of the filter membrane during testing.

稀释和冲洗液体：这些冲洗液可在灭菌前进行过滤，以避免试验期间堵塞过滤膜。

2. Media storage 培养基存贮

For laboratory prepared media, do not use medium for longer storage period than has been validated.

对于实验室自制培养基，培养基使用时长不要超过经过验证的存贮时长。

For commercially purchased media, follow the manufacturer's recommended storage requirements and expiration date.

对于商业购买的培养基，遵守生产商建议的存贮要求和有效期。

3. Media qualification: 培养基确认

Perform the following tests on the prepared media before use:

在使用培养之前，对已制备的培养基进行以下测试：

a. Sterility: The media batch may be used if the sterilization cycle is validated and monitored with the use of a biological indicator, and the batch passes other quality control testing. Also, if possible, prior to otherwise concurrently, incubate a portion of the media at the specified temperature for 14 days.

无菌测试：如果使用生物指示剂验证和监测灭菌周期，并且该批次通过其他质量控制测试，则可使用该批次培养基。此外，如果可能，在同时进行其他操作之前，将部分培养基在指定温度下培养 14 天。

b. Growth promotion test; follow the current USP using recommended strains of organisms (Table 1, USP <71>). Do not use cultures that are more than five passages removed from the original master seed lot. Commercially prepared and standardized stable suspension cultures of the recommended organisms can also be used. Test strains suspensions of vegetative bacteria or yeast should be used within 2 hours, or within 24 hours if refrigerated between 2 ℃ and 8 ℃. Spore suspensions (*A. brasiliensis*, *B. subtilis*, and *C. sporogenes*) refrigerated between 2 ℃ and 8 ℃ may be kept for a validated period of time. If using commercially prepared organisms, follow the manufacturer's instructions. Additionally, all bacterial and spore suspensions should be prepared to yield ≤100CFU. All bacterial counts must be verified at time of use.

促生长试验；使用现行 USP（表 1，USP<71>）推荐的微生物菌株。不要使用从原始主种子批次中传代五代以上的培养物。也可以使用所推荐微生物的商业制备并标化的稳定悬浮培养物。繁殖体细菌或酵母的试验菌悬液应在 2 小时内使用，如果在 2-8° C 冷藏，则应在 24 小时内使用。在 2-8° C 冷藏的孢子悬浮液（A 巴西芽孢杆菌，B 枯草芽孢杆菌和 C 孢子芽孢杆菌）可保存至经过验证的时长。如果使用商业制备的生物体，应遵守生产商的说明。此外，所制备的所有菌悬液和孢子悬浮液浓度应≤100CFU。使用时必须核查所有细菌数量。

4. Equipment Preparation 设备准备

Analytical equipment and tools used in sterility analysis and suitability should be cleaned and sterilized using a validated sterilization procedure. Commercially purchased equipment and tools should be labeled sterile and accompanied by a certificate of analysis for sterility.

无菌分析和适用性中所用的分析仪器和工具应使用经验证的灭菌程序进行清洁和灭菌。商业购买的设备和工具应贴上无菌标签，并附有无菌分析证书。

D. Clean Room Activities 洁净室活动

1. Gowning 更衣

Personnel are critical to the maintenance of asepsis in the controlled environment. Thorough training in aseptic techniques is required.

人员对于在受控环境中保持无菌至关重要。需要进行彻底的无菌技术培训。

Personnel must maintain high standards each time they deal with sterile product.

人员每次处理无菌产品时必须保持高标准。

a. Personnel gowning qualification should be performed by any analyst that enters the aseptic clean room. Personnel gowning qualification must consist of:

进入无菌洁净室的任何分析员都应进行人员更衣资格确认。人员更衣资格必须包括：

i. Training of gowning techniques by a qualified trainer.

由具备资质的培养师进行更衣技巧培训

ii. Observation of trainee by trainer while gowning.

由培训师在受训者更衣时对其观察

iii. General growth media touch plates utilized to analyze if the trainee gowned correctly without contaminating the sterile outer gown, sterile gloves and sterile head cover.

使用通用接触碟分析受训人员更衣是否正确，不会污染无菌外衣、无菌手套和无菌头套

b. Some consideration should be taken before entering the clean room (see below). Follow applicable specific procedures for the facility.

在进入洁净室之前还应考虑一些问题（见下）。遵守设施内适用的特定程序：

i. Proper gowning immediately prior to entry the clean room is required of all

personnel without exception.

要求所有人员在进入洁净室之前正确更衣，不得例外。

ii. Non-linting clean room scrubs that cover as much skin as possible is the ideal inner-suit to wear before gowning up for an aseptic clean room. Street clothes are not permitted.

最好在穿上无菌洁净室的防护服之前，里面穿上不掉毛的洁净室内衣，覆盖尽可能多的皮肤。不允许穿便装。

iii. Remove jewelry and makeup.

取下首饰并卸妆。

iv. Scrub hands (and arms when possible) before gowning.

更衣前洗手（如有可能，清洗手臂）。

v. Non-shedding sterile uniform components should be used all the time.

应始终使用不掉屑的无菌服配件。

vi. Use aseptic gowning procedure to don sterile uniform components.

按照无菌更衣程序穿戴无菌服配件。

vii. Care should be taken to choose gowning that does not expose any skin to the aseptic clean room environment.

应注意要选择不会将皮肤暴露于无菌洁净室环境的洁净服。

viii. An appropriate sporicidal/disinfectant is used to sanitize the gloves.

应使用适当的杀孢子剂/消毒剂对手套消毒。

ix. If possible, post the gowning procedures in the gowning room or area to help individuals follow the correct order of gowning.

应尽可能在更衣间或区域贴出更衣程序，帮助员工遵守正确的更衣顺序。

x. Should an analyst find it necessary to leave the room, he/she should discard all gowning components and put on new ones upon re-entry.

如果化验员需要离开房间，应丢弃所有洁净服配件，重新进入时要穿戴新洁净服。

xi. If an individual scheduled to enter the clean room for analysis feels sick or has compromised skin, he/she should talk to his/her supervisor to postpone entry into the clean room until fully healed.

如果员工准备进入洁净室进行分析工作但觉得不舒服或皮肤受损，则应告知其主管，到痊愈之后才可进入洁净室。

2. Sample Preparation 样品制备

Repeat disinfection procedure using appropriate disinfectant/sporicidal immediately prior to placing product primary containers in a working certified laminar flow hood. Allow all disinfected containers to completely air dry in the laminar flow hood prior to opening for analysis. Alternatively, if conducting the testing in an isolator, place the disinfected items into the isolator and proceed with the local procedures for the proper decontamination of the interior of the isolator.

使用适当的消毒剂/杀孢子重复消毒程序，然后立即在将产品内包容器放入经过认证的

工作层流罩中。在打开进行分析之前，让所有已消毒的容器在层流罩中完全晾干。或者，如果在隔离器中进行测试，则将已消毒的物品放入隔离器中，并按照当地程序对隔离器内部进行适当的净化。

3. Room Cleaning After Analysis 分析之后房间清洁

   a. Remove inoculated tubes of media and all controls from the analytical area by putting them in the pass-thru or on a stainless- steel cart used for transporting materials in and out of the clean room.

      从分析区域中取出培养基和所有对照的接种管，放在传递窗或放在将物料运输进出清净室的不锈钢推车上。

   b. After analysis, all sample containers, equipment wrap, used equipment and tools are to be removed from the clean room before the analyst exits.

      分析完成后，所有样品容器、设备包装、使用过的设备和工具均要在化验员退出之后送出洁净室

   c. Sample containers used in the analysis should be returned to the original outer containers for storage as part of the reserve sample.

      将分析所用样品容器退回原始外包装中，作为留样的一部分保存

   d. Disinfect working area before exiting the clean room.

      退出洁净室之前对工作区域消毒

4. Clean room disinfection and surface monitoring must be conducted for both aerobic and anaerobic microorganisms on a routine basis. The frequency is to be determined by the local laboratory policy.

   日常必须监测洁净室消毒和表面需氧菌和厌氧菌。监测频次由本地实验室制度确定。

E. Method of Analysis 分析方法

1. Membrane Filtration 膜过滤法

   Follow the current edition of the USP for the amount of sample to be tested.

   样本量执行 USP 现行版本要求。

2. Direct Inoculation 直接接种法

   Follow the current edition of USP for the amount of sample and media to be used. For example: Use 200 ml of each medium when analyzing solid form products. If the membrane filter method is unsuitable, certain liquids may be tested by direct inoculation method.

   使用样品和培养基数量依据现行 USP 确定。例如，分析固体制剂时使用培养基各 200ml。如果不适合使用膜过滤法，可采用直接接种法检测一些液体样品。

3. Devices 装置

   All devices with only the pathways labeled as sterile are to be tested by the pathway with sterile Fluid D and testing the Fluid D via membrane filtration.

   所有仅通道标示为无菌的设备均应采用无菌液体 D 测试该通道，无菌液体 D 采用膜过滤法测试。

4. Incubation of Sterility Test Media 无菌测试培养基的培养

   a. Incubate Fluid Thioglycollate (THIO) at 32.5 ±2.5 ℃. Do not shake or swirl test media during incubation or during examination to minimize aeration of this broth.

      在 32.5±2.5 ℃ 培养液体硫乙醇酸盐 (THIO)。培养或检查期间不要摇晃或旋转测试培养基，以尽量减少肉汤暴露于空气的时间

   b. Incubate Soybean-Casein Digest Broth (SCD) at 22.5 ±2. 5 ℃. Gentle swirling, on occasion is acceptable to increase aeration of media.

      在 22.5 ± 2. 5 ℃ 下培养大豆酪蛋白消化肉汤 (SCD)。有时可以轻轻旋转增加培养基的曝气。

   c. Incubation period for THIO and SCD: THIO 和 SCD 的培养时间

      i. Not less than 14 days except for products sterilized using ionizing radiation. If tubes are not read on day 14 due to holiday or weekend then record the results, even if positive, on the first available day to observe the tubes.

         不少于 14 天，采用离子辐射灭菌的产品除外。如果试管因为节假日在 14 天时未能读取结果，则应在可观察的第一天对试管进行观察，即使是阳性亦要记录结果。

      ii. Additional incubation time may be warranted if the analyst is made aware of sterilization processes other than heat or filtration (e.g. 30 days (at minimum) for products sterilized using ionizing radiation). This is to allow repair of DNA of microorganisms injured by ionizing radiation, if any, that may be present).

         如果化验员知晓灭菌工艺不是湿热或过滤，可以延长培养时间（例如，使用离子辐射灭菌的产品到 30 天（至少）），这样可以修复微生物可能被离子辐射损伤的 DNA（如有）

F. Analysis of Medical Devices (ex. Purified Cotton, Gauze, Sutures and Surgical Dressings) 医疗器械的分析（除净化棉、纱布、缝合线和手术敷料外）

The USP method for analysis of surgical dressing/cotton/gauze (in packages) calls for a minimum quantity of 100 mg, to be tested in each medium. It is recommended that an entire unit shall be tested in each medium for individually packaged single-use articles.

USP 分析手术敷料/棉花/纱布（包装内）的方法要求最少 100 mg，在每种培养基中进行测试。对于单独包装的一次性用品，建议在每种培养基中对整个单元进行测试。

1. Gauze, Purified Cotton, Sutures and Surgical Dressings 纱布、净化棉布、缝合线和外科绷带

   a. Using media containers as large as quart jars analyze entire unit of product.

      使用与夸克瓶一样大的培养基容器分析整个产品单元。

   b. If unit is too large for the container, analyze as much of unit as can be placed in container and covered by the medium.

      如果单元对于容器来说太大，则应尽可能多地分析容器中的单元，并用培养基覆盖。

2. Compositing of Medical Devices 医疗器械的组合

a. Devices may be tested in composites (2 – 4 units/composite) as long as they meet the specifications of Chapter 71 of the current USP with regards to minimum quantity of a test unit and minimum number of units to be tested. All composited units must be the same lot number.

只要设备符合现行 USP < 71 >关于测试单元的最小数量和要测试的单元的最小数量的标准，就可以用组合材料（2 - 4 个单元/复合材料）进行测试。所有组合单位必须是相同的批号。

b. Devices may be composited only if they successfully pass the Method Suitability test. If composited units do not pass Method Suitability test, then the product cannot be composited.

只有成功通过方法适用性测试的设备才能进行组合。如果组合单元未通过方法适用性测试，则产品无法进行组合。

G. Control Systems 控制系统

The objective of a control system is to ensure the sterility, within designated limits, of all items, media, rinsing fluids, and equipment used in a sterility test.

控制系统的目的是确保无菌测试中使用的所有材料、培养基、淋洗液和设备的无菌性在指定限度内。

The control systems which will accompany all sterility analyses are outlined below.

所有无菌分析的控制系统列出如下：

1. System Control 系统控制

A "system control" is used to demonstrate maintenance of sample integrity during all analytical manipulations. Any piece of equipment that comes in contact with the product under analysis, along with any manipulations by the analysts, must be controlled. Thus, all equipment, fluids, and culture media for the "system control" must be handled in a manner which duplicates, as closely as possible, the manipulations of the actual sample being analyzed. All materials used as system controls must be sterilized by the analyzing laboratory. However, the method of sterilization need not be the same as for the product, but they must render the material sterile.

在所有分析操作过程中，“系统控制”用于证明样品完整性的维护。在分析中与产品接触的任何设备，以及分析人员的任何操作都必须加以控制。因此，用于“系统控制”的所有设备、液体和培养基必须与被分析的实际样品尽可能接近的方式进行处理。用于系统控制的所有物料必须通过分析实验室进行灭菌。物料灭菌的方法不必与产品相同，但是必须使物料无菌。

The first choice for the system control is the actual product, if enough test units are available. When complex medical devices must be sacrificed in order to design a suitable sterility test, consider using them for a system control after cleaning, repacking and sterilizing.

如果有足够的测试单元可用，则系统控制的首选是实际产品。为了设计合适的无菌测试而必须牺牲复杂的医疗器械时，在清洁、重新包装和灭菌后，可以考虑将其用于系统控制。

When there are viable alternatives, a product unit should not be sacrificed for use as a system control if this will reduce the number of units available for sterility testing below USP requirements or ORS policy requirements, except as provided in the preceding paragraph. If using a product unit would reduce the subsamples examined below the number required by USP or ORS policy, the analyzing laboratory should prepare a control from other material than a unit of the sample product whenever possible.

如果这样做会降低可用于无菌测试的单元数量，该数量低于 USP 要求或 ORS 法规要求，当有可行的替代方案时，则不应该牺牲产品单元作为系统控制。如果使用产品单元将会使得子样品减少至 USP 或 ORS 法规所要求数量以下，则分析实验室应尽可能使用除样品之外的其他物料进行控制。

a. Membrane Filtration: A filter funnel from the vacuum source connection on each manifold used in the test is used for the system control. Alternatively, if a closed canister system is used to conduct the sterility test a canister set from the same lot used during the analysis should be used for the system control.

膜过滤：测试的每个歧管上离真空源连接最远的过滤漏斗用于系统控制。或者，如果使用封闭罐系统进行无菌测试，则应用分析过程中使用的相同批次的罐进行系统控制。

i. Filterable Materials (liquids, soluble solids, etc.) 可过滤的物料（液体，可溶性固体等）

Use a material similar to the product under test. The control material must be of the same volume, and similarly packaged as the test product. Filter- sterilized and autoclaved Peptone water (USP Fluid A) may be useful for this purpose in many cases.

在测试时使用与产品相似的物料。对照物料必须与测试产品有着相同的体积和相似的包装。在许多情况下，过滤灭菌和高压灭菌的蛋白胨水（USP 流体 A）可用于此目的。

ii. Devices with sterile Fluid Pathway 有无菌液体通道的器械

Use tubing or other containers similarly fitted with needles, valves, connectors, etc., as the product under test. Use USP Fluid D to flush lumens.

使用针孔、阀门、连通器等相似的管子或其他容器作为待测产品。使用 USP 流体 D 冲洗内腔。

b. Materials tested by direct inoculation (devices, insoluble solids, and other non-filterable materials) 直接接种法检测的物料（器械、不可溶固体和其它不可过滤的物料）

Use materials similar in size, shape, and texture, and similarly packaged as product under test. Replicate as nearly as possible pertinent, unusual features that may reflect on the credibility of the sterility test.

使用相似尺寸、形状、材质和相似包装的物料作为待测产品。尽可能复制相关的和不寻常的特征，这些可能反映无菌测试的可信度。

In designing "system controls" for sterility testing, care must be taken to duplicate the sample product for most aspects, as nearly as possible. Be novel and innovative to meet this requirement and make the system control meaningful.

在设计无菌检测的“系统控制”时，必须注意尽可能多的复制样品产品的大部分。以新颖和创新的方式满足这些要求，使系统控制有意义。

2. Equipment Controls 设备控制

   All equipment items used in the analysis listed below will be controlled individually. One item from each autoclave lot of equipment is tested in each medium used in the test. Therefore, for a sample tested in THIO and SCD, one item from each sterilizer load (oven or autoclave) is tested in each medium giving a total of two controls for each forceps, syringe, etc., used in the test.

   下面列出的分析用所有物品要单独控制。试验中使用的每种培养基都测试了每个高压灭菌器中的一个设备。因此，对于在 THIO 和 SCD 中测试的样品，在每个培养基中检测来自每个灭菌器（烘箱或高压灭菌器）装载的一个物品，测试中使用的每个镊子、注射器等总共进行两个控制。

   Forceps 镊子

   Syringes 注射器

   Scissors 剪刀

   Scalpels 手术刀

   Swabs 棉签

   Pipettes 移液管

   Membranes (dry, directly from the package). If membranes are sterilized in place, this control may be omitted. 膜（干燥的，直接取自包装）。如果膜经过原位灭菌，可略去该步控制。

   Hemostats 止血钳

   Other special items that may be required by a specific test. 特定测试可能要求的其它特殊物品。

3. Media and Rinse Fluid Controls 培养基和淋洗液控制

   a. An uninoculated media and rinse fluid control are analyzed to ensure sterility at time of use. 对未接种培养基和淋洗液控制进行分析，以确保使用时的无菌性。

      Alternatively, controls for these materials are accomplished as part of the "system control" for each manifold. This will also include membrane cutters, and other items that contact the product but cannot be individually controlled.

      或者，完成对这些材料的控制，作为每个歧管的“系统控制”的一部分。这还将包括薄膜切割器和其他接触产品但无法单独控制的物品。

4. Environmental Controls 环境控制

   a. Open Media Controls 开放式培养基对照

      Tubes of each medium (THIO and SCD) used in the sterility analysis are exposed to the immediate environment of the test (e.g., laminar flow hood) for the duration of the test. Alternatively, a laboratory may use agar settling plates as detailed in section b.

在无菌性测试中所用的每种培养基（THIO 和 SCD）管在整个检测时间段内均需直接暴露于测试环境中（例如，层流罩）。或者，实验室可使用节 b 中详细说明的琼脂沉淀板。

b. Agar Settling Plates 琼脂沉降碟

Plastic Petri dishes containing an effective non-selective medium (based on test requirements) are exposed in the hood for a period not to exceed four hours during the analysis. After four hours, plates should be replaced to continue monitoring (as appropriate).

含有效非选择性培养的塑料培养皿（根据检测要求）检测期间暴露在层流罩下不应超过 4 小时。4 小时之后，替换进行持续监测（适当时）。

Plates should be incubated for 48 hours at 35 ℃, and an additional 5 days at 25 ℃ in order to detect mold contamination.

平板应在 35 ℃ 培养 48 小时，然后在 25 ℃ 培养 5 天，以检查霉菌污染。

c. Controls within an Isolator 隔离器内的控制

When conducting the sterility test within an isolator, if it has been designed to allow for a connection to an air sampler and particle counter this sampling may be performed for the duration of the sample analysis in lieu of the environmental samples described above. If the isolator is unable to accommodate an air sampler and/or particle counter or the instruments are unavailable the environmental controls described in section a. and b. should be used. Isolator gloves should be examined before and after a testing session to ensure integrity of the gloves were maintained. This examination should be documented. Additionally, prior to each decontamination cycle a leak test of the isolator system must be performed with passing results.

如果在隔离器内进行无菌性测试，其设计可以连接至空气取样器和粒子计数器，可在样品分析期间进行取样，取代上述环境样品。如果隔离器无法放置空气取样器和/或粒子计数器，或者无法获得环境监测仪器，则应使用节 a 和节 b 中所述的环境控制方法。在检测环节前后，均应检查隔离器手套，确保手套的完整性得到维护。该检查应有记录。另外，在每个除污染程序之前，必须对隔离器系统进行泄漏测试，并得到合格结果。

5. Personnel Monitoring 人员监测

Personnel monitoring must be performed after analysts conclude sterility testing and prior to exiting the aseptic clean room. The analyst shall use general media touch plates to monitor the sterile condition of their clean room attire and to ensure aseptic techniques were followed.

化验员在结束无菌性检测之后，退出无菌洁净室之前，必须对人员进行监测。化验员应使用通用培养基接触碟，对其洁净室服装的无菌条件进行监测，确保遵守了无菌技术。

For example, a minimum of five touch plates should be used for the following personnel gowning sites: 例如，以下人员更衣部位最少应使用 5 个接触碟：

RH glove finger tips. 右手手套指尖

LH glove finger tips. 左手手套指尖

Chest 胸部

Left Forearm 左手臂

Right Forearm 右手臂

General media touch plates will be incubated for 5 days at 30-35 ℃.

通用培养基接触碟应在 30-35 ℃ 培养 5 天。

NOTE: The numerical values for personnel monitoring limits and specifications are established on the basis of a review of actual findings within the facility. All isolates are to be identified by local laboratory procedure to ensure that the analyst did not contaminate the sample. Analysts should be sanitizing their gloves throughout the sterility analysis and changing gloves when needed. However, changing gloves prior to performing personnel monitoring is unacceptable. Each laboratory is required to monitor and trend data to ensure compliance and detect any abnormalities.

注：人员监控限值和标准数值是根据对设施内实际数据回顾确定的。所有分离菌株都将通过当地实验室程序进行鉴定，以确保分析人员没有污染样品。化验员应在整个无菌分析过程中对手套进行消毒，并在需要时更换手套。但在执行人员监测之前不得更换手套。每个实验室都需要进行监测并对数据进行趋势分析，以确保合规并发现所有异常情况。

H. Sub-culturing Primary Media 原始培养基的传代培养

Daily observations of primary test media (THIO and SCD) containing product should be performed without unnecessary disturbance. All handling of positive tubes, streaked plates, or subsequent inoculations of additional media will be done outside the clean room. These culture transfers are to be performed within a HEPA filtered biosafety cabinet or equivalent outside the ISO5 area which has been cleansed with an effective sporicidal/disinfectant anti-microbial agent. The analyst should be gowned with at least sterile gloves, sterile sleeves and a mask to minimize any possible cross contamination.

含有产品的原始测试培养基（THIO 和 SCD）的日常观察应该没有不必要的干扰。所有阳性试管、划线平板或其他培养基后续接种的处理将在洁净室外进行。这些培养物转移应在 HEPA 过滤生物安全柜内进行，或者在使用有效的杀孢子剂/抑菌消毒剂进行了清洁的等同 ISO5 区域外执行。分析人员应该至少穿戴无菌手套、无菌护袖和面罩，减少任何可能的交叉污染。

1. Record on Analyst's worksheets the day that the primary isolation media, Fluid Thioglycollate Broth (THIO), or Soybean-Casein Digest Broth (SCD) is turbid and inform supervisor. Streak tubes on the day they first appear positive and again at 14 days to determine the presence of other possible slow-growing (i.e., fungi) microorganisms.

   在化验员工作本上记录初始分离培养基、THIO 或 SCD 混浊的情况并通知主管。当首次发现阳性当天和第 14 天时在试管上划线，以确定是否有其它可能缓慢生长（例如真菌）微生物。

2. Within a HEPA filtered biosafety cabinet or equivalent outside the clean room, streak turbid tubes onto Modified Soybean-Casein Digest Medium [SCD broth + 1.5% agar] (Modified

SCDA) or other non-selective agar plate.

在有 HEPA 过滤的生物案例柜内或等同的洁净室外，将混浊试管划线接种至改良大豆酪蛋白消化培养基【SCD 肉汤+1.5%琼脂】（改良 SCDA）或其它非选择性琼脂平板。

3. All streaked plates are incubated for a period at least as long as required for growth in original isolation media (THIO or SCD) not to exceed seven days.

   所有划线平板均培养原始分离培养基（THIO 或 SCD）生长所要求的相同时长，但不得超过 7 天。

4. Subculturing from Fluid Thioglycollate Broth (THIO) 流体硫乙醇酸盐肉汤（THIO）续培养

   a. Subculture Thioglycollate broth to general medium agar plates in duplicate. Streak two plates; incubate one aerobically, and one anaerobically, each at 32.5 ±2.5 ℃. NOTE: It is suggested to transfer an aliquot of media from close to the bottom of the tube to maximize the recovery of strict anaerobes.

      取双份通用培养基琼脂碟，转种 THIO。划线两碟，一个接种作为需氧，一个接种作为厌氧，分别在 32.5 ±2.5 ℃培养。注：建议从接近试管底部的地方转移一部分培养基，以最大化回收严格厌氧菌。

      i. Note if any growth is observed on the anaerobic plate which differs from growth on the aerobic plate. Pick a single representative colony and perform an aero-tolerance test in order to determine if a strict anaerobe has been recovered. Proceed with identification of any strict anaerobes recovered when isolation is complete.

         注意，如果在厌氧碟中发现有生长，其与需氧碟中的生长是不一样的。挑选单个代表性菌落，进行空气耐受性测试，以确定所回收的是否严格厌氧菌。在分离完成后，进一步对所有严格厌氧菌进行鉴别。

      ii. Note if any growth is observed on aerobic plate and compare to growth on anaerobic plates. Proceed with identification when isolation is complete.

         注意，如果在需氧碟上发现有生长，则应与厌氧碟上的生长情况进行比较。在分离完成之后进一步对其进行鉴别。

5. Subculturing from Soybean Casein Digest broth (SCD) 大豆酪蛋白消化液（SCD）续培养

   a. Sub culture SCD broth to general growth medium and incubate aerobically. Streak one plate; incubate aerobically at 22.5 ±2.5 ℃.

      转接种 SCD 肉汤至通用生长培养基，并在有氧情况下培养。划线转接种一个培养碟，在 22.5 ±2.5 ℃有氧情况下培养。

      i. Note if any growth is observed on general growth medium plate. Proceed with identification when isolation is complete.

         注意，如果在通用生长培养碟中发现生长，则在分离完成后进一步进行鉴别。

      ii. Each organism should be identified to genus and species, if possible, using rapid identification kits or DNA sequencing.

         每种菌均应使用快速鉴定包或 DNA 测序鉴定至种和属（可能时）。

I. Product-Induced Turbidity in Primary Test Media 初始检测培养基中因产品引发的混浊

When product-induced turbidity prevents the confirmation of visual observation of growth, the following instructions apply

如果产品引发的混浊妨碍了对生长情况的目视观察确认，则应执行以下指导：

1. Record "T" for any subsample which is turbid due to product-medium mixture.

   所有因产品培养基混合产生混浊的子样本记录为“T”。

2. On the daily observation page, indicate the meaning of "T" as: "T = product induced turbidity".

   在每日观察页上，记录“T”的含义为“T=产品引发的混浊”。

3. At the end of the initial 14 days of incubation, transfer portions of the medium (not less than 1 ml) to a fresh container of the same medium and then incubate the original and transferred containers for not less than 4 days. Note: Follow the current edition of the USP for any changes concerning subculturing and incubation of turbid samples.

   在最初 14 天培养结束后，转移一部分培养基（不少于 1ml）至相同培养基的新鲜容器，然后接种原始培养基，并培养至少 4 天。注：执行现行 USP 中对混浊样本转种和培养的修改内容。

4. Examine original product inoculated media and the subcultured media for growth daily when possible for not less than 4 days of incubation and record the results on a new daily observation continuation sheet.

   每天检查原始产品接种培养基和转种培养基中的生长情况，可能时不少于 4 天培养期，并将结果记录在新的连续每日观察页上。

8. Chapter 4: Investigating USP Sterility Testing Failure 第四章：USP 无菌检测失败的调查

A. Introduction 概述

When microbial growth is detected in a pharmaceutical or medical device product, the product is considered non-sterile, pending an investigation. Because of the public health importance of a non-sterility finding, preliminary results should be reported by your laboratory management, without delay, to ORS and the appropriate Center (e.g., Office of Compliance/OCTEC).

如果在药品或药械产品上发现微生物生长，则认产品非无菌，需要进行调查。由于非无菌性问题的公众健康重要性，你们实验室管理人员要立即将初步结果报告给给 ORS 和适当的中心（例如，合规办公室/OCTEC）。

Concurrently, a laboratory review should be conducted to answer the following question: Was the result true product contamination or was there a clear laboratory error that caused contamination of the sample during the analysis? The Out-of-Specification (OOS) investigation will review and document whether the test results are based on sound laboratory operation.

同时，实验室要进行回顾，回答以下问题。该结果是否真的产品污染，还是明确的实验室错误导致分析过程中样品受污染？OOS 调查将审核并记录检测结果是否基于合理的实验室操作。

B. Investigations 调查

Whenever a sterility positive occurs, lab supervisors are responsible for starting the investigation immediately. Four factors should be evaluated in the basic investigation:

无论如何，如果发现无菌性呈阳性，实验室主管要负责立即启动调查。在基本调查中应评估 4 个要素：

1. Equipment: 设备

   Determine whether equipment malfunctioned or was not operated properly. If a malfunction occurred, determine whether it was likely to cause the contamination. Determine if monitoring records and any checklists or logs indicate that the ISO 5 environment was in good state of control (serious environmental quality or equipment repair issues) at the time of the sterility test. Be aware of the most likely failure modes in the equipment (e.g., laminar flow hood, glovebox, or isolator) used.

   确定是否设备故障，或操作不当。如果发生故障，确定是否可能导致污染。确定在无菌测试时监测记录和检查清单或日志是否显示 ISO5 环境处于良好的受控状态（严重环境质量或设备维修问题）。了解所用设备（例如，层流罩、手套箱或隔离器）的最可能失效模式。

2. Adherence to Analytical Method:遵守分析方法

   Determine whether there were any anomalies or deviations from the analytical method. Adherence to method should be verified at the time of analysis, and any major breach of sterility test procedure should also be documented at that time. If any method breaches occurred, determine whether it was likely to cause the contamination. Be aware of any possible weaknesses in the test method (e.g., kit, manifold, etc.) used.

确定是否有异常或分析方法偏差。核查分析时是否遵守检验方法，严重违反无菌检测程序的情况亦要即时记录。如果发现违背方法的情况，确定是否有可能导致污染。了解所用检测方法（例如，检测盒，歧管等）中是否有任何可能弱点。

3. Analyst:化验员

   Evaluate the analyst's qualifications, including proficiency, personnel monitoring results, training record, and experience. Also note whether the sterility testing practice of the analyst was observed during this or a recent analysis.

   化验员资格评估，包括专业度、人员监测结果、培训记录和经验。还要注意是否在本次或最近一次分析中对化验员的无菌检测操作进行了观察。

4. Cleanroom and ISO 5 (Class 100) Environmental Conditions: 洁净室和 ISO 5（百级）环境条件

   Determine if disinfection/decontamination of the ISO 5 environment was properly done.

   确定 ISO5 级环境是否已适当消毒/除污染。

   Determine whether there was adverse environmental data. Note that a negative control failure, on its own, is not necessarily cause for invalidating a result. If a negative control was contaminated, consider whether the microbe identified is similar to, or the same as, the sterility test isolate and also consider whether there are other adverse environmental trends.

   确定是否有不良环境数据。注意阴性控制失败其本身并不一定导致结果无效。如果阴性对照受到污染，考虑所鉴别的微生物是否类似或相同于无菌测试分离物，同时考虑是否有其它不良环境趋势。

   It is advisable to summarize this review process in a standard report and maintain a sufficient record to reflect that these areas were investigated. In addition to the four considerations listed above, overall cleanroom design is also an important consideration. There are differences in the construction, configuration, and material flow of laboratory cleanrooms. There may be differences in size, number of rooms, shape, air handling system, pass through autoclaves, gowning room accommodations (sink, HEPA filtration, adequate space, bench, etc.). Proper practices and conditions should be assured by mitigating contamination hazards potentially presented by layout and other design provisions. These include appropriate procedures for room and material disinfection, proper cleanroom uniforms (disposable or reusable), sample preparation area, etc. Cleanroom risks can be mitigated by the design of ISO 5 testing environment. Equipment that provides barrier protection can mitigate risks of the surrounding cleanroom environment.

   建议在标准报告中总结该审核流程，并维护足够的记录，反映这些区域得到调查。除了上述 4 个考量点外，洁净室的总体设计亦是重要的考虑点。实验室洁净室会有不同的结构、参数设置和物流，房间大小数量、形状、空气处理系统、穿墙灭菌器、更衣间配套（水池、HEPA 过滤、足够空间、坐凳等）也可能有差异。应降低因平面布局和其它设计问题带来的污染风险，保证适当的操作和条件，其中包括恰当的房间和物料消毒程序、适当的洁净服（一次性或可重复使用）、样品制备区域等。可通过 ISO5 测试环境的设计降低洁净室风险。提供屏障保护的设备可以降低周围洁净环境的风险。

If an investigation finds that the conduct of the analysis included errors or events that caused the test specimens to be contaminated by the lab environment, the Sterility Test result would be invalid, and the substandard laboratory practice should be corrected to prevent this problem from recurring. For more information on how to judge investigational findings to make this evaluation, see Section XI.C1.and 2 of FDA's guidance on Sterile Drug Products Produced by Aseptic Processing for principles and expectations for investigating a sterility positive.
http://www.fda.gov/downloads/Drugs/.../Guidances/ucm070342.pdf

如果调查发现分析过程有可能导致受试样本被环境污染的错误或事件，则无菌性测试结果可能无效，应纠正不合格的实验室作法，从而防止发生该问题。如何判断调查发现，进行该评价的更多信息参见 FDA 指南《采用无菌工艺生产的无菌药品》中无菌阳性的调查原则和要求。

Also see the current version of USP <71>, which provides some guidance limited on investigations under Interpretation of Data.

亦可参见现行版本 USP<71>，其中提供了一些指南，仅限于数据解释方面的调查。

C. Conclusion 结论

This suggested list of areas and conditions to review should not be considered as comprehensive. Additional areas of review may need to be added based on some of the unique features or procedures employed by individual ORS laboratories.

本建议审核领域和条件清单不应作为完全清单。可能需要基于一些独特属性或各 ORS 实验室所用程序增加其它审核领域。

9. Chapter 5: Bacterial Endotoxin Testing 第五章：细菌内毒素检测

Bacterial endotoxin is a lipopolysaccharide found in the cell membrane of Gram-negative bacteria which may cause adverse events in patients such as, but not limited to fevers, headaches, inflammation, nausea, chills, vomiting, hypotension, lung toxicity, Toxic Anterior Segment Syndrome, abortion or death. Therefore, all sterile drugs, medical devices and combination products must meet bacterial endotoxin specifications. Historically, *in vitro* Limulus Amoebocyte Lysate (LAL) assays have been used to detect and quantify bacterial endotoxin in pharmaceutical products.

细菌内毒素是在革兰氏阴性菌的细胞膜中发现的一种脂多糖，它可能会在患者身上产生不良事件，例如但不仅限于发热、头痛、炎症、恶心、寒战、呕吐、低血压、肺毒性、前节段中毒综合征、流产或死亡。因此所有无菌药品、医疗器械和药械组合产品必须符合细菌内毒素标准。历史上曾经使用体外鲎变形细胞裂解液（LAL）方法检测和定量药品中的细菌内毒素。

NOTE: The Gel-Clot Method is considered the "referee method" per the USP. If there is a disagreement between the photometric turbidimetric or chromogenic methods, the gel-clot method results are reported.

注：根据 USP，凝胶法被认为是“标准方法”。如果其与光度比浊法或显色法之间结果不一致，则报告凝胶法结果。

This chapter of the Sterility Analytical Manual is intended to supplement the methodology procedures found in the USP <85> BACTERIAL ENDOTOXINS TEST and USP <161> MEDICAL DEVICES-BACTERIAL ENDOTOXIN AND PYROGEN TESTS. The USP <85> test is based on a reaction between bacterial endotoxin and the LAL reagent. Requirements for the LAL test includes optimal pH, ionic strength, temperature and incubation time. The USP <85> methods include the gel-clot LAL method and photometric technique methods such as the turbidimetric and chromogenic kinetic LAL methods. The USP <161> chapter describes the bacterial endotoxin extraction procedure for medical devices, if required. After the medical device extraction is performed, the USP <85> chapter is followed to detect and quantify any bacterial endotoxin present in the sample.

无菌分析手册中的该章节意在补充 USP<85>“细菌内毒素检测”和 USP<161>“医疗器械细菌内毒素和热源测试”中的方法学程序。USP<85>的方法是基于细菌内毒素与 LAL 试剂之间的反应。LAL 检测要求包括优化 pH、离子化强度、温度和培养时间。USP<85>方法包括凝胶 LAL 方法和光度技术方法，如浊度和光度动力学 LAL 方法。USP<161>描述了医疗器械的细菌内毒素提取程序（如需要）。在提取之后，按 USP<85>方法检测和定量医疗器械样本中的细菌内毒素。

The Gel-Clot Method is a qualitative assay that detects Gram-negative bacterial endotoxin based upon a reaction between lysate and endotoxin which results in a firm clot formation. For samples with endotoxin, the endotoxin amount present in a test sample is calculated by diluting the sample to determine the assay endpoint where a clot does not form. If no clot forms in the verified dilution from the inhibition and enhancement testing, the sample does not contain detectable endotoxin.

凝胶法是一种定性方法，基于裂解液与内毒素之间的反应形成稳定凝固形态检出革兰氏阴性菌内毒素。对于含有内毒素的样本，检测样本上的内毒素数量是通过稀释样本至不会形成凝胶的测试终点来计算的。如果在抑制和增强检测中经过验证的稀释倍数下未形成凝胶，则认为样本不含有可检出内毒素。

Photometric techniques include the turbidimetric and chromogenic endpoint and kinetic assays. The

assays involve a change in turbidity or color (depending on the assay reagents used) over time for kinetic assays or at the end of the incubation period for endpoint assays. For kinetic assays, the onset time for turbidity or color change is inversely proportional to the concentration of endotoxin in the solution, i.e. more rapid change at higher endotoxin concentrations. An instrument is used to read the changes. The analytical test results are calculated based on the linear relationship between the endotoxin concentration and the turbidity or color development. The standard curve plots the log onset time against the log endotoxin concentration. These assays are rapid and sensitive, allowing large numbers of samples to be assayed quickly. However, the gel-clot method is the reference method per USP and should be used if there are any doubts or disputes, unless otherwise indicated in the monograph for the product tested.

光度技术包括浊度和色度终点和动力学方法。这些方法涉及动力学检测期间或培养结束时浊度或颜色变化（依赖于所用检测试剂）。对于动力学测试，浊度或颜色变化的起始点与溶液中的内毒素浓度成反比，即内毒素浓度越高，变化越快。使用一台仪器读取变化值。根据内毒素浓度与浊度或颜色变化之间的线性关系计算分析结果。标准曲线为开始时间的对数 VS 内毒素浓度的对数。这些分析快速灵敏，可快速分析大量样本。但是根据 USP，凝胶法才是标准方法，如果有质疑或争议，则应使用凝胶法，在被测产品的各论中别有说明者除外。

New microbiologists should review the references at the end of this chapter.

新的微生物学家应参阅本章结尾处的参考文献。

A. Gel-Clot Method 凝胶法

1. Reference Standard Endotoxin and Control Standard Endotoxins 参比标准内毒素和受控标准内毒素

   The potency of the control standard endotoxin (CSE) with respect to the reference standard endotoxin (RSE) is determined by the CSE manufacturer. This information is found in the associated package insert and need not be repeated.

   受控标准内毒素（CSE）相对于参比标准内毒素（RSE）的效价是由生产商确定的。该信息可在相关包装说明书中找到，不需要重复。

   NOTE: Follow manufacturers' recommendations for the storage, reconstitution, and preparation of CSEs, lysates, and other Limulus Amoebocyte Lysate (LAL) reagents. In case of a dispute, final decision is based on results obtained with the USP Endotoxin RSE.

   注：按生产商建议存贮、调配和制备 CSE、裂解液和其它鲎变形细胞裂解液（LAL）试剂。如果有争议，以采用 USP 内毒素 RSE 得到的结果为准。

   The field laboratories are encouraged to mix the reconstituted CSE for at least 5 minutes and for at least 1 minute between dilutions.

   鼓励现场实验室将调配的 CSE 混合至少 5 分钟，每次稀释之间混合至少 1 分钟。

2. Negative Controls 阴性控制

   Run appropriate negative controls with each sample tested. This assures that the equipment and solutions used in the test contain no extraneous detectable endotoxin. This assay requires two negative controls: negative water control and negative system accessory control. The negative water control (blank) consists of the pyrogen-free water (Water for BET or LAL Reagent Water) used in the assay and is tested undiluted. Run a system negative (accessory) control to test the detectable endotoxin level, if any, of

accessories used in the assay (i.e. beaker full of pyrogen- free test tubes stored for an extended period, pipette tips, pipettes, sample tubes, syringes, spatulas, etc.). Rinse all accessories used in the assay with pyrogen-free water and test undiluted prior to completing all testing for a particular sample. If an alternative diluent is used, run an alternative diluent control prior to completing all testing for a particular sample. Record these results on the worksheets.

每个所测样品均进行适当的阴性对照试验。这样能确保检测所用设备和溶液不含有外来可检出内毒素。该检测要有 2 个阴性对照：阴性水对照和阴性系统辅助对照。阴性水对照（空白）含有检测中所用的无热源水（BET 测试用水或 LAL 试剂水），不加稀释直接检测。进行系统阴性（辅助）对照试验检测测试所用辅助用品中（即，放置延长时间装满无热源试管的烧杯、移液头、移液管、取样管、注射针、刮勺等）可检出内毒素水平（如有）。在完成特定样品的所有检测之前，用无热源水淋洗所有检测所用辅助用品，不稀释直接检测。如果使用的是替代稀释剂，则在完成特定样品的所有检测之前使用替代稀释剂进行对照试验。在工作表中记录这些结果。

When using commercially purchased pyrogen-free water for product dilutions, it is recommended to transfer a working volume from the original stock container to an individual pyrogen-free test tube or flask in order to minimize back contamination. Run a negative control for the working volume for each sample run.

如果产品稀释使用的是商业化采购的无热源水，建议从原始存贮容器中转移工作用体积的水至单个无热源试管或烧瓶中，以最大程度减少背景污染。每次检测样品时均要使用工作体积的水进行阴性对照试验。

NOTE: Pyrogen free pipettes, micropipettor tips, test tubes, and other accessories are commercially available.

注：无热源移液管、微量移液头、试管和其它用品均有商业化产品可及。

3. Test for Confirmation of Labeled LAL Reagent Sensitivity 标示鲎试剂灵敏度的确认测试

   Prior to use in the test, the labeled LAL reagent sensitivity must be confirmed. Prepare a control standard endotoxin dilution series having at least four concentrations equivalent to 2 λ, λ, 0.5 λ, and 0.25 λ. Inoculate four replicates from each control standard endotoxin tube with equal amounts of reconstituted lysate per manufacturer's recommendation. Multiple dilution series are not required. The geometric mean of the endpoints must be within the limits of labeled claim. The acceptable variation is one half (0.5 λ) to two times (2 λ) the labeled sensitivity (λ).

   LAL 试剂用于检测之前，必须确认其灵敏度。制备一套标准内毒素对照稀释液，至少有 4 个等同于 2 λ、λ、0.5 λ 和 0.25 λ 的浓度。根据生产商的建议从每个标准内毒素对照管接种出 4 个相同等数量的复溶裂解液。不需要多个稀释系列。终点的等比中数（几何平均数）必须在标称的限度内。可接受变异为标示灵敏度（λ）的 0.5-2 倍（0.5 λ-2 λ）。

4. Inhibition or Enhancement Test/Test for Interfering Factors 干扰因素的抑制或增强测试

   The suitability of the test results for bacterial endotoxin require an adequate demonstration that specimens of the article or of solutions to which the test is to be applied do NOT of themselves inhibit or enhance the reaction or otherwise interfere with the test.

细菌内毒素检测结果的适用性需要充分证明其所检测的物品样本或溶液样本自身不会抑制或增强反应或干扰测试。

USP states to perform this test "on aliquots of the specimen… in which there is no detectable endotoxin". However, this characteristic of the product cannot be ascertained prior to the analysis because the specimens are unknown samples. Because of this limitation, any positive result below the 0.5 lambda level may not be an enhancement trait of the product, but instead a positive reaction due to contamination in the sample. The evidence for this conclusion should be obvious with the results of the assay tubes containing product only.

USP 要求执行该测试"使用一部分样本……其中不应有可检出的内毒素"。但是，产品的这种特性在分析之前是无法保证的，因为样本本身是未知的。由于此局限性，所有低于 0.5 λ 水平的阳性结果可能并不是产品的增强特性，而是由于样品污染引起的阳性反应。有只含有产品的检测试管结果，那么该结论的证据就很明显了。

A large percentage of small volume parenterals appear to be inhibitory to the LAL gel-clot method because of low pH, or some excipient / active component of the product. In order to expedite the neutralization of this interfering trait, determine the lowest product dilution overcoming the interference but still within the Maximum Valid Dilution (MVD). The detailed description of this protocol is delineated in LIB No. 2433 (July 25, 1980), "A condensed procedure for diluting product in determining compatibility with the Limulus Amebocyte Lysate test for endotoxin". In addition, the use of neutralizers such as sodium laurel sulfate or Pyrosperse™ has also been described (see references).

由于其低 pH 值，或产品的有些辅料/活性组份，很大比例的小容量注射剂显示出对 LAL 凝胶方法的抑制性。为了加快对这种干扰特性的中和，要确定克服干扰但仍在最大有效稀释度（MVD）内的最低产品稀释级别。该方案在 LIB No. 2433 (19800725)"在确定与鲎变形细胞裂解物检测内毒素的相容性时稀释产品的浓缩程序"中有详细说明。另外，使用中和剂如月桂基硫酸钠或 Pyrosperse™（参见参考资料）。

LAL manufacturers recommend the test sample to have a pH range of 6.0 to 8.0 for optimal assay performance. Since the lysate is buffered, sample dilutions in pyrogen-free water may be enough to test the sample with the LAL assay. Determine the pH of the sample with the added lysate and document the results. If pH adjustment is needed, use pyrogen-free acid, base or buffers.

LAL 生产商建议检测样本 pH 范围为 6.0-8.0 以优化检测表现。由于鲎试剂被缓冲，样本在无热源水中的稀释级别可能足以使用 LAL 方法检测样本。将鲎试剂加入样本中，检测 pH 值，记录结果。如果需要调整 pH 值，使用无热源的酸、碱或缓冲液。

NOTE: Contact LAL manufacturers for recommendation of commercially available neutralizing buffer to be used with their LAL kits.

注：联系 LAL 生产商获取可用于其 LAL 试剂盒的商业化中和缓冲液的建议。

5. Test Procedure 检测方法

The storage and mixing of samples prior to analysis may affect recovery of endotoxin contamination. Sample (product) bottles should be vigorously shaken prior to analysis,

preferably on a vortex (see reference for supporting evidence for this step). A minimum of 30 seconds to 1 min on the vortex is recommended for each product unit.

样品在分析之前的存贮和混合可能影响内毒素污染的回收。样品（产品）瓶在分析之前应剧烈震摇，最好在涡旋器上震摇（参见参考文献中本步骤支持性证据）。建议每个产品单位在涡旋器上震摇至少 30 秒至 1 分钟。

6. Endotoxin Calculation 内毒素计算

Calculate endotoxin concentration per the USP Bacterial Endotoxins test chapters <85> and <161>. For additional information refer to USP <1085>.

根据 USP 内毒素检测章节<85>和<161>计算内毒素浓度。更多信息参见 USP<1085>。

NOTE: Adjust the final endotoxin value taking into account the volume of the rinse solution used in the extraction procedure.

注：调整最终内毒素值时要考虑提取程序所用淋洗溶液的体积。

7. Compositing Samples 组合样本

The Bacterial Endotoxin test <85> does not directly address the issue of combining product units (compositing/pooling). The risk of unit composites is that one unit (vial, ampoule, etc.) may have bacterial endotoxin contamination at a higher level but the dilution of this one unit with endotoxin-free units of product may reduce the detectable level of endotoxin below the sensitivity of the lysate or dilute the level of endotoxin below the acceptable monograph level. Therefore, **when using a composite format for screening drug products for endotoxin it is important to adjust the MVD calculation to account for this reduced lysate sensitivity**. Secondly, **when compositing is performed for product screening, if a positive result is detected a repeat test is acceptable under the conditions stated by the Interpretation section of the USP chapter**.

细菌内毒素检测<85>并未直接说明组合产品单元（组合/混合）的问题。单元组合的风险是一个单元（西林瓶、安瓿等）可能会有较高水平的细菌内毒素污染，而该单元采用产品的无内毒素单元稀释后可能会将内毒素检出水平降低至鲎灵敏度水平以下，或将内毒素水平稀释至低于可接受的各论水平以下。因此，**如果使用组合形式筛选产品的内毒素，调整该降低的鲎试剂灵敏度 MVD 计算非常重要。**其次，**如果组合是在产品筛选时执行的，如果检出阳性结果，可接受按 USP 章节中的诠释部分所声明条件下重复检测。**

It would be advisable when performing the repeat test from a composite mixture that, if remaining product is available and had been opened aseptically under controlled conditions, the repeat test be performed on the original individual units. It is strongly advised that the individual units be adequately shaken to assure that the endotoxin is re-suspended back into solution before taking the sample test aliquot. **If any of the original individual units fail the USP test at this point, the compendium does not allow any additional repeat testing unless the test can be proven not to be suitable as defined by the USP chapter**.

建议在执行组合混合物的重复检测时，如果有留样，且在受控环境下以无菌方式打开，可采用原受检单元执行重复检测。强烈建议对单个单元进行足够震摇，以确保内毒素在测试取样之前混回至溶液中。**如果所有原始单元均在该点的 USP 检测不**

合格，则药典不允许复验，有 USP 章节证明该检测不适用者除外。

8. Relevant excerpts from Guidance for Industry Pyrogen and Endotoxin testing: Questions and Answers) 热源和内毒素检测问答行业指南节选

Question 1: Can FINISHED product units (vials, ampoules, pre-filled syringes, etc.) be "Pooled" into a composite and screened for bacterial endotoxin?

问 1：成品单元（西林瓶、安瓿、预充填注射针等）可“混合”至组合中并进行筛选后执行细菌内毒素检测吗？

Response 1: 答 1

Yes. With some exceptions (see below), finished drug product units may be pooled into a composite sample and assayed for bacterial endotoxins. The composite sample may be represented by the entire unit or partial aliquots (equal volumes) of finished product containers from one manufactured lot of aqueous-based pharmaceuticals. Pooling would generally be accepted for small-volume parenterals (those with volumes of 100 mL or less) as long as the MVD is adjusted to a proportional, lower value because of the potential for diluting a unit containing harmful levels of endotoxins with other units containing lower, less harmful, levels of endotoxins. This “adjusted MVD” is obtained by dividing the MVD computed for an individual sample by the total number of samples to be pooled. FDA suggests pooling no more than three units per composite in keeping with the concept of testing representative beginning, middle, and end finished product containers. If this reduction in MVD results in an inability to overcome product-related assay interference because of an insufficient dilution, then the samples should be tested individually.

可以。有些例外情况下（见下），成品单元可混合至组合样品中，进行细菌内毒素检测。组合样本可由水基药物的一个生产批次中成品容器的整个单元或其中一部分（等分体积）来代表。只要将 MVD 调整至比例较低的值，样品合并对于小容量（容量小于等于 100ml）注射剂来说通常是可以接受的，因为它有可能用其它含有较低、较少危害水平内毒素的单元稀释含有有害水平内毒素的单元。该“调整后的 MVD”是通过将 MVD 除以被合并的样品总数计算得到的单个样本的值。FDA 建议合并样品不要多于每次组合 3 个单元，以保持检测代表开始、中间和末端成品容器的概念。如果这样降低 MVD 会因为稀释不够而导致无法克服产品相关检测干扰，则应该单独检测各样品。

Finished medical devices may also be pooled into a composite sample and assayed for bacterial endotoxins. Testing for medical devices should be conducted using rinsing/eluting and sampling techniques as described in ISO 10993-1 and ISO 10993-12, as also used for inhibition/enhancement.

成品组合器械可能亦会合并成组合样品进行细菌内毒素检测。器械的检测应该使用 ISO 10993-1 和 ISO 10993-12 所述淋洗/洗脱和取样技术，这也用于抑制/增强。

Sampling can be adjusted for special situations. After a suitable eluate/extract pool is obtained from a finished production lot, this pooled extract should be kept under conditions appropriate for stability until it is tested in duplicate.

取样可根据特殊情形进行调整。在经过适当的洗脱/萃取之后，从一个成品生产批次中获得混合样品，该混合萃取物应该保存在适合于稳定性的条件下，直到制备双样进行检测。

FDA recommends that pooled samples be a composite of aseptically removed aliquots (after at least 30 seconds of vigorous mixing) from each of the product containers. In this way, the original, individual containers will be available for possible retesting in the event the pooled sample displays an OOS result.

FDA 建议混合样品是从每个产品容器中无菌取出的等分试样（剧烈混合至少 30 秒后）的混合物。这样，如果合并样本显示 OOS 结果，原始的单个容器将可用于可能的重新测试。

Some product types should not be pooled. Two examples are drug products that have an initial low MVD (see discussion above of "adjusted MVD") and products that are manufactured as a suspension, because sample aliquot homogeneity may present significant interference issues.

有些产品类型不可混合取样。2 个例子是初始 MVD 较低（参见上述“调整后的 MVD”）的药品和作为混悬液生产的药品，因为样品等分均匀性可能呈现出重大干扰问题。

Question 2: Can INTERMEDIATE (IN-PROCESS) sample aliquots be "pooled" into a composite and screened for bacterial endotoxin?

问 2：中间体样品等分样是否可以“合并”至组合样中用于细菌内毒素筛选？

Response 2: 答 2

FDA does not recommend pooling in-process samples from different in- process stages of the manufacturing process because it may be difficult to ensure the homogeneity of these materials.

FDA 不建议对生产工艺中不同中控步骤的中控样品进行合并，因为可能难以确保这些物料的均一性。

Question 3: Retesting when test failure occurs:

问 3：检测不合格时的复测

Response 3: 答 3：

When conflicting results occur within a test run, the analyst should consult USP Chapter <85>, Gel-Clot Limits Test, Interpretation, for guidance on repeat testing. As specified in Chapter <85>, if the test failure occurred at less than the maximum valid dilution (MVD), the test should be repeated using a greater dilution not exceeding the MVD. A record of this failure should be included in the laboratory results. If a test is performed at the MVD and an out-of- specification (OOS) test result occurs that cannot be attributed to testing error, continue product dilution until the actual endotoxin concentration can be calculated. These results should be recorded on your worksheets.

如果在同一次检测中得到相互矛盾的结果，化验员应遵守 USP<85>“凝胶法限度测试”结果诠释中的复测指导。正如第<85>章所述，如果低于 MVD 时检测结果不合格，应使用不超过 MVD 的较大稀释倍数重复检测。该检测不合格结果记录要放在实验室结果中。如果在 MVD 执行检测并得到不可归因于检测错误的 OOS 结果，则要持续稀释产品直到实际内毒素浓度可计算为止。这些结果均应记录在你的工作表中。

B. Photometric Quantitative Techniques 光度定量技术

Endpoint and kinetic assays are photometric quantitative assays used for the detection of bacterial endotoxins. Endpoint and kinetic assays may utilize turbidimetric or chromogenic formats. Endpoint assays measure endotoxin the increase in turbidity (turbidimetric) or color (chromogenic) at the end of the incubation time.

终点和动力学法是细菌内毒素检测用光度定量分析。终点和动力学分析可使用浊度或色度形式。终点测定法测量内毒素在培养时间结束时浊度（浊度）或颜色（显色）的增加。

This section provides procedural information that can be applied to kinetic assays. The Kinetic Chromogenic and Turbidimetric reagents are commercially available. Assay may be purchased as a kit. A certificate of analysis should be maintained for the control standard endotoxin, Limulus Amebocyte Lysate (LAL), and pyrogen free water used in the assay. Other materials such as pyrogen free pipettes, micropipettor tips, test tubes, and 96- well microplates may be purchased from various vendors. Certificates of analysis indicating these materials are pyrogen-free should also be maintained by the laboratory.

本节提供的程序信息可用于动力学分析方法。动力学色度和浊度试剂可通过商业化获取。分析过程可作为试剂盒采购。控制标准内毒素、检测中所用 LAL 试剂和无热源水的 COA 应保存。其它物料如无热源移液管、微量移液头、试管和 96 阱微生物碟可从不同供应商处采购。实验室亦应保存显示这些物料无热源的 COA。

1. Kinetic Assays: The kinetic bacterial endotoxin detection software is designed to run the following assays.

   动力学分析：动力学细菌内毒素检测软件是设计用于运行以下分析的。

   a. Initial qualification of a lysate/Initial qualification of the testing analyst

      裂解液的初始确认/测试化验员的初始资格确认

   b. RSE/CSE assesses potency of control standard endotoxin (CSE) in terms of reference standard endotoxin (RSE)

      RSE/CSE 评估控制标准内毒素（CSE）相对标准内毒素（RSE）的效价

   c. Test for Interfering Factors

      干扰因子测试

   d. Sample Test

      样品测试

   e. Instrument Calibration Tests

      仪器校正测试

      The initial qualification assay verifies the proficiency of the analyst operating the Kinetic software and equipment. The initial qualification assay may also be used to qualify each new lot of kinetic lysate and control standard endotoxin.

      初始确认分析核查化验员操作动力学软件和设备的熟练程度。初始确认分析亦可用于确认每个新的动力学裂解液和控制标准内毒素批次。

      Requirements of USP <85> must be followed with respect to the number of

endotoxin standards and the number of replicates required for a valid assay.

必须遵守 USP<85>的要求中内毒素标准的数量和有效分析所要求的重复次数。

The RSE/CSE assay may be used to compare the potency of the CSE with the concentration of the RSE. Normally, the RSE/CSE assay does not need to be performed, unless there is reason to believe the values in the manufacturer's certificate of analysis (COA) are not correct.

RSE/CSE 分析可用于比较 CSE 效价和 RSE 浓度。正常情况下，不需要执行 RSE/CSE 分析，有理由相信生产商 COA 中的值不正确者除外。

The test for interfering factors must be run for each sample.

每个样品必须进行干扰因子检测。

The routine assay is designed to test unknown samples for bacterial endotoxins. Samples collected for LAL analysis should be run using the routine assay, after taking the other three assays into consideration.

常规分析是设计用于检测未知样品的细菌内毒素的。采集用于 LAL 分析的样品应该考虑使用其它 3 种分析检测之后再使用日常分析进行检测。

NOTE: Contact LAL manufacturer to qualify the kinetic readers (IQ/OQ/PQ) prior to use. Furthermore, laboratories should follow scheduled preventative maintenance (PM) and/or calibration to ensure the readers perform properly for regulatory sample testing.

注：使用前应联系 LAL 生产商对动力学计数器（IQ/OQ/PQ）进行确认。另外，实验室应该遵守计划的预防性维护（PM）和/或校正计划，确保计数器用于法规样品检测时表现正常。

2. Procedure 方法

Perform the assay according to the instructions that are included with the LAL test kit or LAL lysate, in accordance with USP <85>. Additional instructions may be found in reference 8 listed below.

根据 USP<85>按 LAL 检测试剂盒或 LAL 裂解液中的指导进行分析。在以下参考文献 8 中可找到更多指导。

C. Medical Devices 医疗器械

This section applies to medical devices or assemblies or fluid pathways of medical devices or assemblies (i.e. solid medical devices such as disposable syringes, cartridges, transfusion and infusion assemblies, implants, intravenous catheters, dialysis tubing; liquid medical devices such as saline, heparin and dialysate; and gel medical devices such as demineralized bone matrices and hyaluronic acid devices) that are labeled sterile and nonpyrogenic that are in contact directly or indirectly with the cardiovascular system, lymphatic system or cerebrospinal fluid. Follow USP <161> for testing requirements

本部分适用于医疗器械或其组件或流体通路（即固体医疗器械，如一次性注射器、药筒、输液和输液组件、植入物、静脉导管、透析管；液体医疗器械，如盐水、肝素和透析液；以及凝胶医疗器械，如脱矿骨基质和透明质酸器械），它们被标记为无菌和无热原，与心血管系统、淋巴系统或脑脊液直接或间接接触。遵循 USP <161> 的测试要求

If only the device pathways are labeled as sterile and nonpyrogenic, the device pathways must come in contact with the extraction fluid for the entire course of the extraction per USP<161>.

如果医疗器械只有其通道标示为无菌和无热源，则器械通道必须与萃取液体接触，按 USP<161>执行完整的萃取程序。

Liquid medical devices do not require extractions.

液体医疗器械不要求萃取。

The standard extracting, rinsing and soaking fluid for medical devices is pyrogen-free water. The analytical approach for testing medical devices is covered in USP. If the extraction fluid shows endotoxin interfering results, modification of testing may be necessary depending on the product configuration. Alternate diluents must be verified to not interfere with the endotoxin assay prior to use.

医疗器械的标准萃取、淋洗和浸泡流体为无热源水。检测医疗器械的分析方法在 USP 中写明。如果萃取流体显示出内毒素干扰结果，可能需要根据产品参数设置对检测进行修改。替代稀释剂在使用前必须确认其不会干扰内毒素分析。

NOTE: The rinse or extract volume may be adjusted for the size and configuration of the device. The device or components may be cut into smaller pieces using sterile, nonpyrogenic equipment such as forceps, scissors, wire cutters, etc. prior to the extraction.

注：淋洗或萃取容积可根据医疗器械的尺寸和参数设置进行调整。医疗器械或组份可在萃取前使用无菌无热源设备如镊子、剪刀、剪线钳等切为更小块。

A collaborated method prepared by an ORS laboratory is available for extraction of endotoxin from devices and may be used if necessary. Analytical verification of the final version should be conducted by the responsible laboratory. The ORS laboratory protocol is summarized below for convenience.

ORS 实验室起草了一份协作方法，用于从医疗器械中萃取内毒素，必要时可使用之。应由责任实验室对最终版本的分析方法进行确认。方便起见，将 ORS 实验室方案总结如下：

1. Perform medical device extractions and final endotoxin concentration calculations per USP <161> and USP <1085> Guidelines on the Endotoxins Test.

   根据 USP <161> 和 USP <1085>内毒素测试指南实施医疗器械萃取和最终内毒素浓缩计算。

   Alternatively, if deemed necessary, perform the ORS laboratory modified extraction and analysis of Endotoxin from Medical Devices:

   或者在必要时，实施 ORS 实验室修订后的医疗器械内毒素萃取和分析。

a. Preparation of 1% SLS solution 1%SLS 溶液的制备

Prepare a 1% stock solution by placing one (1) gram of sodium lauryl sulfate (SLS) into a depyrogenated glass flask and add 99 ml of pyrogen-free water. Allow the SLS to fully dissolve. This should be followed by filtration through a 10,000 MW depyrogenation membrane filter into a pyrogen-free glass or plastic container.

向除热源处理的玻璃瓶中加入 1g 月桂基硫酸钠（SLS），再加入 99ml 无热源水，制备

1%贮备溶液。让 SLS 完全溶解。用 10000MW 除热源膜过滤至无热源玻璃或塑料容器中。

b. Equipment needed: Ultrasonic bath with a range of 150 to 480 watts.

所需设备：超声浴，150-480W

c. Extraction procedure

萃取程序

i. Dilute 2mL of 1% SLS stock solution to 20mL (0.1%) using LAL reagent water in a 20 x 150 mm screw-cap tube.

使用 20 x 150 mm 螺旋盖管中的 LAL 试剂水稀释 2ml 的 1%SLS 贮备溶液至 20ml（0.1%）

ii. Dilute 1.5mL of the 0.1% SLS solution to 15 mL (0.01%) using LAL reagent water in a 20 x 150 mm screw-cap tube.

使用 20 x 150 mm 螺旋盖管中的 LAL 试剂水稀释 1.5ml 的 1%SLS 贮备溶液至 15ml（0.01%）

iii. Prepare the appropriate number of tubes (one tube for each device) and one as a negative / system control. Preheat in a water bath to 37 ℃ ±1 ℃.

制备适当数量的试管（每个器械一个试管）和一个阴性/系统对照试管。水浴预热至 37 ℃ ±1 ℃

iv. Aseptically remove the device from its packaging and cut it diagonally into pieces less than 5mm in length. Small metal and plastic pieces such as needles and luer-locks should be tested whole. Pyrogen-free fluid pathways should be flushed with extract solution.

在无菌操作下从包装中取出器械，将其斜切成小于 5mm 的小片。金属和塑料小片如针头和鲁尔锁应整个检测。无热源液体通道应使用萃取液淋洗。

Note: Extract volume may need to be adjusted depending on medical device size.

注：萃取体积可能需要根据医疗器械的尺寸进行调整。

v. Place all pieces into the 20 x 150 mm tube containing 15 mL of preheated (37 ℃ ±1 ℃) 0.01% SLS rinse solution.

将所有小片放入装有 15ml 预热（37 ℃ ±1 ℃）0.01%SLS 淋洗液的 20 x 150 mm 试管。

vi. Vortex the tubes for 30 – 60 seconds or until all pieces of the device are immersed in the rinse solution.

涡旋震摇试管 30-60 秒钟或直到器械的所有小块均浸入淋洗液。

vii. Sonicate the test containers for 60 minutes (wattage range 150 – 480 watts) at 37 ℃ ±1 ℃. Do not sonicate more tubes than can be vortexed within 15 minutes of completion of the sonication. Make sure the water in the sonicator covers the rinse solution in the 20 x 150 mm tubes. Do not allow the water in the sonicator to exceed 38 ℃.

37 ℃ ±1 ℃超声测试容器 60 分钟（功率 150-480 瓦）。在超声处理完成后的 15 分钟内，超声处理的管数不要超过可以涡旋的管数。确保超声装置里面的水覆

盖 20 x 150 mm 试管中的淋洗溶液。不要让超声装置中的水温超过 38 ℃。

viii. Vortex the tubes for 2 minutes. Remove a portion of the eluate (5 – 10 mL) for LAL testing. If the eluates are not tested immediately for endotoxin, they should be refrigerated. All eluates must be tested within 24 hours of extraction. Prior to analysis vortex at least one minute.

涡旋震摇试管 2 分钟。取出一部分用于 LAL 检测洗脱（5-10ml）。如果不能立即检测洗脱液的内毒素，则应冷藏。所有洗脱液必须在 24 小时内进行萃取检测。分析前至少涡旋震摇 1 分钟。

2. Alternative Diluent Examples (Verify prior to use): 替代稀释剂例子（在使用之前核查）

Diluent/Buffer 稀释剂/缓冲液	Interfering Factor 干扰因子
Pyrosperse™	Endotoxin Binding or Masking
Pyrosperse™	内毒素结合或掩蔽
B-G-Blocker®	Enhancement due to β-glucans
B-G-Blocker®	β -葡聚糖可增强
Tris Buffer 50mM solution	Strongly Acidic (pH < 6) or Basic (pH > 8)
Tris 缓冲液 50mM 溶液	强酸性 (pH < 6) 或碱性 (pH > 8)
Magnesium chloride ($MgCL_2$) 10mM solution	Chelating
氯化镁 (MgCL2) 10mM 溶液	螯合
Note: Always use LRW as the diluent for the CSE standard serial dilutions. 注：始终使用 LRW 作为 CSE 标准系列稀释液的稀释剂	

3. Test the sample eluate from the medical device extraction, using the bacterial endotoxins test (BET) assay parameters, procedures, standards, and controls for the gel-clot, kinetic chromogenic or turbidimetric, or endpoint assays, as directed in USP<85> and USP<161>.

按 USP<85>和 USP<161>所示，使用凝胶法、动力学显色或比浊，或终点分析法的细菌内毒素测试（BET）分析参数、方法、标准和对照样品检测从医疗器械萃取的样品洗脱液。

D. Endotoxin References 内毒素参考文献

1. United States Pharmacopeia (USP) Chapter <85> Bacteria Endotoxins Test. Official, Current Version.

USP<85>细菌内毒素测试，正式版本，现行版本

2. USP <161> Medical Devices – Bacterial Endotoxin and Pyrogen Tests, Current Version.

USP <161>医疗器械—细菌内毒素和热源测试，现行版本

3. USP <1085> Guidelines on the Endotoxins Test, Current Version.

USP <1085>内毒素测试指南，现行版本

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10. Chapter 6: Particulate Matter 第六章：颗粒物

This chapter is intended to supplement the methodology procedures found in the USP <788> PARTICULATE MATTER IN INJECTIONS and USP <789> PARTICULATE MATTER IN OPHTHALMIC SOLUTIONS. In addition, USP <1788> DETERMINATION OF PARTICULATE MATTER provides useful guidance for these assays. Where appropriate the laboratory wide procedure, ORA-LAB.019 "HIAC 9703+ Liquid Particle Analyzer" is referenced to address specific requirements for the use of the HIAC instrument.

本章意在补充 USP<788>“注射剂中的颗粒物”和 USP<789>“眼用药水中的颗粒物”中的方法。另外，USP<1788>“颗粒物的检测”为这些方法提供了有用指导。在适当的实验室范围内，参考 ORA-LAB.019“HIAC 9703+ 液体粒子分析仪”来解决使用 HIAC 仪器的具体要求。

Particulate matter consists of mobile, randomly-sourced extraneous substances, other than gas bubbles, that cannot be quantitated by chemical analysis due to the small amount of material that it represents and heterogeneous composition. Injectable solutions, including solutions constituted from sterile solids intended for parenteral use, are essentially free from particulate matter observable on visual inspection. The tests described herein are physical tests performed for the purpose of enumerating sub-visible extraneous particles within specific size ranges.

微粒物质由可移动的、随机来源的外来物质组成，气泡除外，由于其代表的材料量小且组成不均匀，因此无法通过化学分析定量。注射液，包括由用于肠胃外使用的无菌固体构成的溶液，基本上不含肉眼可观察到的颗粒物质。此处描述的测试是为了枚举特定尺寸范围内的不可见外来颗粒而进行的物理测试。

All large-volume injections for single-dose infusion and those small-volume injections for which the monographs or product specifications specify such requirements are subject to the particulate matter limits set forth for the test being applied, unless otherwise specified in the individual monograph or product specification.

除各论或产品质量标准中另有规定外，所有用于单剂量输注的大容量注射剂和那些各论或产品质量标准规定有此类要求的小容量注射剂应符合适用检测项目下颗粒物限度要求。

Not all injection formulations can be examined for particles using the light obscuration method. Any product that is not a pure solution having clarity and viscosity approximating those of water may provide erroneous data when analyzed by the light obscuration counting method. Refer to specific monographs when a question of test applicability occurs. The microscope counting method may be used to analyze such materials. In some instances, the viscosity of a material to be tested may be sufficiently high so as to preclude its analysis by either method. In this event, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

并非所有注射制剂都可以使用遮光法检查颗粒。当通过遮光计数法进行分析时，任何非纯溶液的产品，其透明度和粘度接近于水，都可能提供错误的数据。当出现方法适用性问题时，参见各论。显微镜计数法可用于分析此类材料。在某些情况下，待测材料的粘度可能太高，无法通过任一方法对其进行分析。在这种情况下，如有必要，可以使用适当的稀释剂进行定量稀释降低粘度再进行分析。

A. Light Obscuration Particle Count Test 遮光法颗粒计数测试

The test applies to large-volume injections labeled as containing more than 100 mL and single-

dose or multiple-dose small-volume injections labeled as containing 100 mL or less that are either in solution or in solution constituted from sterile solids, where a test for particulate matter is specified in the individual monograph or drug product specification. It counts suspended particles that are solid or liquid.

该测试适用于标示体积超过 100 mL 的大容量注射剂和标示体积等于或小于 100 mL 的单剂量或多剂量小容量注射剂，它们为溶液形式或由无菌固体调配成溶液，其颗粒物测试在各论或药品质量标准中规定。该测试计算固体或液体的悬浮颗粒。

1. Test Apparatus 测试仪器

   The apparatus is an electronic, liquid-borne particle counting system that uses a light-obscuration sensor with a suitable sample feeding device. Critical operational criteria consist of the following:

   该仪器是一种电子液体颗粒计数系统，它使用带有合适的进样装置的遮光传感器。关键操作标准包括以下内容：

   a. Sensor Concentration Limits 传感器浓度限度

      Use an instrument that has a concentration limit (the maximum number of particles per ml) identified by the manufacturer that is greater than the concentration of particles in the test specimen to be counted.

      使用生产商确定的浓度限（每毫升的最大粒子数）大于待测样本中的粒子浓度的仪器。

   b. Sensor Dynamic Range 传感器动态范围

      The dynamic range of the instrument used (range of sizes of particles that can be accurately sized and counted) must include the smallest particle size to be enumerated in the test articles.

      所用仪器的动态范围（可以精确测量和计数的颗粒尺寸范围）必须包括待测样品中最小颗粒尺寸。

2. Instrument Calibration 仪器校正

   The instrument must be calibrated periodically according to the manufacturer’s recommendation. The following are parameters that should be evaluated as part of the periodic instrument calibration in addition to other tests recommended or routinely performed by the instrument manufacturer.

   必须根据生产商的建议定期校准仪器。除了仪器生产商推荐或常规执行的其他测试之外，以下参数还应作为定期仪器校准的一部分进行评估。

   a. Sample Volume Accuracy 样品容积准确度

      The accuracy of the sample volume must be assessed and found to be within the manufacturer’s recommended range.

      样品容积准确度必须进行评估，并应在生产商建议的范围内。

   b. Sample Flow Rate 样品流速

      Verify that the flow rate is within the manufacturer's specifications for the sensor used.

确认流速在生产商所用传感器质量标准内。

c. Calibration and Resolution of the sensor 传感器的校正和分辨率

Verify that the sensor resolution is within the manufacturer's specifications for spherical particles of known size between 10 µm and 25 µm.

确认传感器分辨率在生产商的 10- 25 µm 之间未知尺寸球形颗粒标准内。

3. Test Environment 检测环境

a. Glassware, closures and other equipment must be suitably cleaned so that the level of particles added has a negligible effect on the outcome of the test.

玻璃仪器、密闭器和其它设备必须适当清洁，使得其引入的颗粒物水平对检测结果的影响可忽略。

Note: Glassware can be purchased in particle free packages which are a suitable particulate free vehicle for compositing liquid samples.

注：可以购买无颗粒包装的玻璃仪器，这是一种适合组合液体样品的无颗粒载体。

b. Perform the test in an environment that does not contribute any significant amount of particulate matter. Preparation of the test specimen, glassware, closers and other equipment should be performed in an environment protected by HEPA filters.

在不会产生任何大量颗粒物的环境中进行测试。样品、玻璃仪器、密闭器和其他设备的制备应在有 HEPA 过滤器保护的环境中进行。

c. The analyst should don non-shedding garments and powder-free gloves throughout the preparation of samples.

在整个样品制备过程中，化验员应穿着不掉屑的衣服和无粉手套。

4. Instrument Start-up and Environment Blank 仪器启动和环境空白

a. Following instrument start-up perform an instrument flush using Milli-Q water or equivalent in accordance with ORA-LAB-.019.

使用 Milli-Q 水或等同者根据 ORA-LAB-.019 按仪器启动程序淋洗仪器。

b. Prior to beginning a test procedure an environment blank must be prepared and tested according to ORA-LAB.019. After passing results have been obtained the instrument can now be used to perform the test procedure.

在开始检测程序之前，必须根据 ORA-LAB.019 制备和检测一份环境空白。在得到合格结果之后，可使用该仪器执行检测程序。

5. Test Procedure 检测程序

a. For containers having volumes of less than 25 mL, test a solution pool of 10 or more units to obtain a volume of no less than 25 mL. Single units of small-volume injections may be tested individually if the individual unit volume is 25 mL or greater.

对于容积小于 25ml 的容器，检测合并了 10 个或更多单元的样品溶液得到不少于 25ml 的样品体积。如果单剂量体积大于等于 25ml，小容量注射剂单个单元亦可单独检测。

b. Prepare the test specimens as described in USP <788> and <789>. Following the preparation of the test specimen the sample can be assayed in accordance with USP <788> and <789> in addition to the procedures found in ORA-LAB.019.

按 USP <788> 和 <789>所述制备检测用样本。如此制备的样品才可按 ORA-LAB.019 中的程序和 USP <788> 和 <789>对样品进行分析。

c. Containers with removable stoppers may be sampled directly by removing the closure. For test specimens that require the contents of the container to be removed for testing withdraw the contents of the container in the normal or customary manner of use, or as instructed in the package labeling. When test specimens are to be pooled remove the closure and empty the contents into a suitably cleaned container (preferably from particle free glassware vendor).

带有可拆卸塞子的容器可以通过移除盖子直接取样。对于需要取出容器内容物进行测试的样品，以正常或惯用的使用方式或包装标签中的说明取出容器中的内容物。当要收集测试样本时，取下盖子并将内容物倒入适当清洁的容器中（最好来自无颗粒玻璃器皿供应商）。

d. Following the completion of the test the instrument will generate a report. The report will include the raw data counts, calculated values and state if the test specimen met the USP limits for the particular test performed.

在完成检测之后，仪器会生成一份报告。该报告会有原始数据计数、计算结果和检测样本是否符合 USP 中颗粒物检测限度的声明。

Note: If the average number of particles exceeds the USP limits the prepared specimen must be tested by the Microscopic Particle Count Test.

注：如果颗粒物平均数超出 USP 限度，则所制备的样本必须采用显微颗粒物计数方法进行检测。

B. Microscopic Particle Count Test 颗粒物显微计数测试

The microscope particulate matter test may be applied to large-volume and small volume parenteral injections and to ophthalmic solution products.

颗粒物显微计数测试可用于大容量和小容量注射剂以及眼用液产品。

The test Apparatus is described in USP <788> with additional information found in USP <1788>.

USP<788>中描述了检测仪器，更多信息参见 USP<1788>。

1. Test Environment and Environmental Blank: 检测环境和环境空白

Refer to Section A.3 for the requirements of the test environment and preparation of the specimen, glassware and equipment used in the assay.

参见节 A.3 中对检测环境和样本制备、检测中所用玻璃仪器及设备的要求。

Prior to initiating the test sequence with a specimen, a blank determination is required and must be carried out according to USP <788>. The environmental blank must meet the requirements set forth in USP <788> in order to initiate testing of the specimen.

在开始样本检测序列之前，应根据 USP<788>进行空白检测。环境空白必须符合

USP<788>中所设定的要求方可开始样本检测。

2. Test Procedure and Interpretation of Data 检测方法和数据解释

   a. For large volume parenterals, single units are tested. For small volume parenterals less than 25 ml in volume, the contents of 10 or more units are combined in a cleaned container; the test solution may be prepared by mixing the contents of a suitable number of vials and diluting 25 ml with particle-free water or with an appropriate particle-free solvent when particle-free water is not suitable. Small volume parenterals having a volume of 25 ml or more may be tested individually.

   对于大容量注射剂，检测的是单个单元。对于体积小于 25ml 的小容量注射剂，会将 10 份或更多单元中的产品合并在一个清洁的容器中，检测溶液可通过混合适合数量小瓶中的产品，并使用无颗粒水或适当的无颗粒溶剂（如果无颗粒水不合适）稀释制备而成。体积大于等于 25ml 的小容量注射剂可单独检测。

   b. Proceed with the test as delineated in USP <788> recording the number of particles that are equal to or greater than 10 µm and the number of particles that are equal to or greater than 25 µm. As an alternative a partial membrane filter count and determination of the total filter count by calculation is allowed. Once a particle count is determined the mean number of particles for the examined specimen is calculated. Note: For test specimens which are covered by USP <789> the number of particles that are equal to or greater than 50 µm must also be counted and a calculated average reported.

   按 USP<788>中所述方法进行检测，记录大于等于 10 µm 的颗粒物数量以及大于等于 25 µm 的颗粒物数量。作为替代方案，允许采用部分膜过滤器计数并通过计算确定总过滤器计数。一旦确定了颗粒物计数结果，则可计算受检样本的平均颗粒物数量。注：对于不在 USP<789>范围内的检测样本，亦可对大于等于 50 µm 的颗粒物计数并报告其平均值。

   c. The calculated data is evaluated according to USP <788> or USP <789> considering the solution type and the container volume.

   根据 USP <788> 或 USP <789>对计算所得数据进行评估，同时考虑溶液类型和容器容积。

11. Chapter 7: Antibiotic Potency Testing 第七章：抗生素效价测定

A. General Information 一般信息

USP <81> Antibiotics- Microbial Assays, is the primary reference for bioassay of human antibiotic potency testing. Prior to testing, the current USP <81> and respective drug monograph should be reviewed for testing requirements, acceptance criteria and applicability. USP <81> lists specific human antibiotics to be tested for microbial potency; this list can change at any time. All other human antibiotics not listed in USP <81> are typically tested using CFR 21 Parts 300-499 or USP HPLC methods. Lastly, animal antibiotics are typically tested using chemistry methods as published in JOAC, AOAC, Laboratory Information Bulletins (LIBs) and/or manufacturer's methodology.

USP <81>【抗生素微生物含量】是人用抗生素效价测定的基本生物含量参考资料。在测定之前，应查阅 USP<81>和相应的药物各论中的检测要求、可接受标准和适用性。USP<81>中列出了需要检测微生物效价的具体人用抗生素，该清单可能会随时间变化。所有 USP<81>中未列出的其它人用抗生素一般采用 CFR 21 节 300-499 或 USP HPLC 方法检测。最后，兽用抗生素一般采用 JOAC、AOAC、实验写到信息公告（LIBS）和/或生产商的方法进行化学方法测定。

Improved manufacturing technology (e.g. purification methods) has evolved potency testing from a simple biological assay to different chemical assays. Most chemical assays are based in the segregation and quantification of antibiotic components through the use of high-performance liquid chromatography (HPLC). However, chemical assays do not demonstrate biological activity and antimicrobial efficacy of a test antibiotic, particularly an antibiotic which may contain numerous active components, each exhibiting different antimicrobial activities. Chemical potency assay does not require the use of a live test microorganism. For the remainder of this chapter, antibiotic potency refers to USP <81> testing only.

改进后的生产技术（例如纯化方法）使得效价测定从简单的生物效价测定发展为不同的化学检测。大多化学分析是基于通过使用高效液相色谱 （HPLC）分离和定量抗生素组份。但是，化学含量测定不能证明测试抗生素的生物活性和抗微生物效力。特别是可能含有许多活性成分的抗生素，每种活性成分表现出不同的抗微生物活性。化学效价测定不需要使用活微生物。本章其它内容中的抗生素效价仅指 USP<81>节。

Antibiotic potency testing is a biological assay whereby varying concentrations of antibiotic are tested against a live microorganism. The resulting biological response is measured and evaluated against a median reference standard [S3] and standard curve [S1], [S2], [S4] and [S5]. The biological response is referred to as antibiotic activity or potency. Antibiotic potency is dependent upon antibiotic-microorganism specificity and is physically expressed by the inability of a microorganism to grow under optimal conditions in the presence of a specific test antibiotic.

抗生素效价测定是一个生物学方法，但抗生素的不同浓度要基于活菌才可以测定。对得到的生物反应进行测量并基于中间标准【S3】和标准曲线【S1】、【S2】、【S3】和【S5】进行评估。生物反应被称为杭生素活性或效价。抗生素效价取决于抑制微生物特异性，采用优化条件下加入待测抗生素后，其对微生物生长的抑制性来表示。

Antibiotic potency testing is a multi-variable test dependent on a variety of factors. Factors may include: 1) Test microorganism growth requirements and inoculum levels 2) Test antibiotic dose and 3) Technical competency in preparation and/or use of equipment, growth media, reagent, test organism and antibiotic standards. Potency testing requires a basic knowledge of laboratory

safety, analytical chemistry, microbiology and aseptic techniques. Potency testing is a manual, multi-step and multi-day process performed with common laboratory equipment. At minimum, two employees are required for preparation and sample setup. Due to the multiple stages of preparation and testing, only qualified (i.e. initial and routine trainings and evaluations) laboratory personnel should be authorized to perform USP <81> testing.

抗生素效价测试是一个多变量测试，取决于许多因素。这些因素包括：（1）测试微生物生长要求和接种水平，（2）待测抗生素剂量，以及（3）制备和/或使用设备、生长培养基、试剂、测试菌和抗生素标准的技术水平。效价测定要求具备基本的实验室安全、分析化学、微生物和无菌技术知识。效价测定是采用一般实验室设备，手工操作的多步骤和多天工作。制备和设置样品至少需要 2 名员工。由于制备和检测步骤多，应该只允许具备资格（即经过初始和常规培训和评估）的实验室人员执行 USP<81>检测工作。

Antibiotic potency testing is performed either by the plate (cylinder-plate or diffusion) or tube (turbidimetric) method. Both plate and tube methods demonstrate measurable levels of growth inhibition. For example, zones of inhibition (ZOIs) are observed and measured during cylinder-plate testing. And, turbidity is observed and measured during tube testing. Growth inhibition measurements are tabulated and integrated into a linear regression curve, resulting in extrapolated antibiotic potency values.

抗生素效价采用碟法（管碟或扩散）或浊度法（浊度计）法测定。碟法和浊度法均会显示出可测量的生长抑制性。例如，在管碟法测试中可观察到抑菌圈（ZOI）。浊度法测定中，观察并测量混浊度。将生长抑制测量结果制表，作线性回归曲线，外推得到抗生素效价值。

Potency is denoted in units (U) or µg of activity and may or may not be exact in equivalence to the µg (weight) of the active compound. The following three reasons may explain this weight-activity discrepancy: 1) activity may be caused by the antibiotic's free base or salt form and activity is denoted in either form 2) the antibiotic may contain similar chemical components but differ in activity or 3) the antibiotic activity is represented by a heterogeneous family of antibiotics and not a single analog.

效价单位为单位（U）或 µg 活性表示，可以准确等同于活性成分的 µg（重量），也可以不等同。以下三种原因可解释为何存在重量-活性差异：（1）活性可能是由抗生素的自由基或者盐引起的，活性可采用任何一种形式表示，（2）抗生素可能含有相似的化学成分，但其活性不同，或者（3）抗生素活性来源于抗生素族多个化学成分，而不是单一化学结构。

An internal quality control is built into each plate and tube tested. Since antibiotics have different listed dosages per label, the test antibiotic/unknown sample is diluted to a known sample concentration ([U3]). The unknown sample [U3] has an equivalent concentration of the median reference standard ([S3]). The median reference standard ([S3]) is the median concentration (i.e. mid-point) of the five-point standard curve ([S1], [S2], [S4] and [S5]). The five- point standard functions as the testable detection range and is prepared from a traceable and within expiry USP Reference Standard (RS).

内部质量控制来源于每个测试平板和试管。由于抗生素在每个标签上都标明不同剂量，检测抗生素/未知样品被稀释为已知样品浓度（【U3】）。未知样品【U3】与中间对照（【S3】）的浓度相同。中值标准品（【S3】）浓度处于 5 点标准浓度曲线（【S1】，【S2】，【S4】和【S5】）中间（即，中点）。5 点标准的作用是建立检测范围，它们

采用可追溯的在效期内的 USP 标准品（RS）制备。

The USP <81> states the following criteria for both the Plate and Tube Methods:

USP <81>对管碟法和浊度法提出了以下标准：

1. The calculated potency of the test antibiotic/unknown sample ([U_3]) must be 80% to 125% of the median reference standard ([S_3]);

   测试抗生素/未知样品（【U3】）的计算效价必须为中值标准品（【S3】）的 80-125%

2. Relative standard deviation for all measured (i.e. millimeters or absorbance) and calculated data (e.g. averages) is NMT 10%; and,

   所有测量值（即，mm 或吸光度）的相对标准偏差和计算所得数据（例如平均值）NMT10%；且

3. Testing is performed in triplicate over a period of three independent test runs

   在 3 个独立的测试运行期间执行一式三份平行样品检测

The USP <81> Plate Method further states:

USP <81>管碟法进一步要求

1. The percentage coefficient of determinations (%R^2) for each standard curve will be NLT 95% (i.e. correlation coefficient of NLT 0.9750); and,

   每条标准曲线的测定系数（%R^2）应 NLT95%（即相关系数 NLT0.9750），且

2. ZOIs for all media reference standard ([S_3] will measure between 14-16 mm).

   所有培养基标准品均有 ZOI（【S3】测量值为 14-16mm）

The USP <81> Tube Method further states:

USP<81>管法还说：

1. The percentage coefficient of determinations (%R^2) for each standard curve will be NLT 90% (i.e. correlation coefficient of NLT 0.950); and,

   每条标准曲线的测定系数（%R2）应 NLT90%（即相关系数 NLT0.950），且

2. Absorbance values of the media reference standard ([S_3]) are predetermined per antibiotic. Refer USP <81> for testing parameters and acceptable data requirements.

   预先确定每种抗生素的中值标准品（【S3】）的吸光度。参考 USP<81>中测试参数和可接受数据要求

B. Equipment 设备

Antibiotic potency testing is a quantitative test dependent upon the preparation and use of growth media, reagents, test organism and antibiotic standards; therefore, Class A volumetric glassware is used in the preparation of the test antibiotic/unknown sample, reference standard and dilutions of the reference standard. Class A glassware is physically labeled either "to deliver" (TD) or "to contain" (TC). An understanding of Class A glassware prior to laboratory use is required. For example, a high viscous liquid (e.g. antibiotic ointment) cannot be delivered from a "to deliver" Class A pipette. "To deliver" Class A pipettes solely rely on gravity to assist in evacuation. In the case of the antibiotic ointment, the ointment would remain in the Class A

pipette at time of evacuation. However, USP <81> states sterile and disposable glassware can also be used for any measurement of test antibiotic/unknown sample, reference standard and dilutions of the reference standard.

抗生素的效价测定是一个定量检测，取决于生长培养基、试剂、试验用菌和抗生素标准品的制备和使用。因此，需使用 A 级容量玻璃器皿配制测试用抗生素/未知样品、标准液和稀释标准品。A 级玻璃仪器上面标有“量出”（TD）或“量入”（TC）。在使用之前，实验室要对 A 级玻璃仪器有所了解。例如，高粘度液体（例如，抗生素膏）无法从 A 级“量出”移液管中排出。A 级“量出”移液管只能依靠重力排出。如果是抗生素膏，在排出时则会残留在 A 级移液管内。当然，USP<81>说在检测抗生素/未知样品、对照品和稀释对照液时，亦可使用一次性无菌玻璃仪器。

Standard laboratory glassware such as beakers, funnels, flasks, roux and 1-2 liter bottles are also required. Sterile and disposable equipment such as test tubes, petri-plates and serological pipettes may be used so long as the use of this equipment does not affect the quantitative aspect of potency testing. Additional equipment includes a stainless steel penicylinders, penicylinder dropper, cuvettes, pipettor and micropipette, pH meter, hot plate, adjustable- temperature water bath, incubator and a manual/automatic plate reader or UV- VIS spectrophotometer.

标准实验室玻璃仪器如烧杯、漏斗、烧瓶、圆颈培养瓶和 1-2 升的瓶子也是必须的。一次性无菌设备，如试管 、培养皿和血清移液管，只要不影响效价检测的定量就可以一直使用。其它设备包括不锈钢瓶、比色皿、血清移液管、单通道微量移液器、pH 计、加热板、可调温水浴锅、培养箱和手动/自动平板计数器或紫外可见分光光度计。

Equipment in direct contact with the test microorganism should be clean (i.e. residue free) and sterile. Residues (e.g. antibiotic or detergent) may interfere with antibiotic potency testing. Methods of equipment cleaning and dry and heat sterilization should have appropriate validation and verification checks to ensure glassware is clean and sterile. See USP <81> for penicylinder cleaning instructions and USP <1051> Cleaning Glass Apparatus, for glassware cleaning instructions.

直接接触测试微生物的设备要进行清洁（即无残留）和灭菌。残留物（例如抗生素或清洁剂）可能会干扰抗生素效价测定。设备清洁和干燥及干热灭菌方法应该进行适当验证和确认检查，确保玻璃仪器的清洁和无菌。参见 USP<81>中青霉素清洁指导和 USP<1051>“清洗玻璃仪器”中的玻璃仪器清洁指导。

Equipment used to provide a unit of measurement should have the appropriate validation and frequent verification checks to ensure reliable and reproducible results; examples of such equipment include a weight scale, pH meter, autoclave, micrometer, manual/automatic plate reader and UV-VIS spectrophotometer. For more information on manual and automatic plate readers, see section titled Antibiotic Potency Testing: Plate Method.

用于对样品进行测量的设备应该有适当的验证和频繁的核查，确保结果可靠和可重复，此类设备的例子包括称重天平、pH 计、灭菌器、微生物计数器、手动/自动读板器和紫外可见光分光计。手动和自动读板器更多信息参见标题为抗生素效价测定章节：管碟法。

Testing can be performed on a laboratory benchtop and does not require setup in a clean room or a laminar flow hood. However, it is important to exercise aseptic technique when working with general growth media and test organisms to prevent cross contamination.

可以在实验室工作台上进行检测，不需要设置洁净间或层流罩。但是，在使用通用生长

培养基和检测微生物时要重视遵守无菌操作技术，防止交叉污染。

When performing the plate method, it is important to use a laboratory bench top that has been checked with a level. The bench top will be used to prepare single and bi-layer agar plates. If the bench top is not level, liquid agar could potentially pool unevenly within a petri plate. Unevenly distributed agar causes uneven antibiotic diffusion. Zones of inhibition formed after diffusion should be uniform in shape (i.e. circular). Irregular shaped ZOIs do not have a diameter that can be measured accurately. Irregular shapes include ovoid, elliptical and/or any shape without a uniform and defined perimeter.

在操作管碟法时，很重要的一点是要使用经过水平检查的实验室操作台。该操作台要用于制备单种双层琼脂平板。如果工作台不平，则液态琼脂倾注在培养皿中可能不平整。琼脂分布不平均会导致抗生素扩散不均匀。扩散后形成的抑菌圈形状应该整齐（即圆形）。不规则的形状 ZOI 没有直径，无法进行准确测量。不规则形状包括卵形、椭圆形和/或任何不整齐无法定义周长的形状。

C. Test Organism, Inoculum Preparation and Standardization 微生物检测，接种准备和标定

Antibiotic potency is dependent upon antibiotic-microorganism specificity. USP <81> identifies specific microorganisms and correlating antibiotics for testing. Prior to use in test, the test microorganism must be characterized as pure and robust. Primary and working cultures must be aseptically prepared and dedicated to preventing contamination of the primary test microorganism. If the primary and/or working culture becomes contaminated, perform a visual inspection and basic microscopy for typical growth characteristics and morphology. Additionally, AOAC approved rapid identity testing methods such as API or VITEK should be performed. Contaminated primary and/or working cultures cannot be used for antibiotic testing; likewise, all antibiotic potency data generated using contaminated cultures will be considered as invalid.

抗生素效价取决于抗生素-微生物特异性。USP <81> 指定了特定微生物和相关测试用抗生素。在用于测试之前，测试微生物必须具有纯净和健壮的特征。原代和工作培养物必须无菌制备并尽可能防止原代测试微生物的污染。如果原代和/或工作培养物被污染，需对典型的生长特征和形态进行目视检查和基本显微镜检查。此外，应使用 AOAC 批准的快速鉴别测试方法，例如 API 或 VITEK。受污染的原代和/或工作培养物不能用于抗生素检测；同样，使用受污染培养物产生的所有抗生素效价数据都将被视为无效。

A test inoculum is prepared from a working culture. Inoculum preparation is a multi-step and multi-day process. Preparation of the inoculum requires a basic knowledge of microbiology, aseptic technique and laboratory safety. Refer to USP <81> Antibiotics- Microbial Assays, for test organism and inoculum preparation. The test inoculum is a solution containing the live test microorganism diluted with a method specific diluent. To be viable, the microorganisms used in the test inoculum must be within 5 passages of the primary test microorganism.

从工作培养物中制备测试接种物。接种物制备过程有多个步骤，需花费数天。接种物的制备需要微生物学、无菌技术和实验室安全的基本知识。请参阅 USP <81> “抗生素微生物学检测”了解测试生物体和接种物的制备。测试用接种物是一种溶液，含有用特定方法稀释剂稀释的测试用活体微生物。为保持其活力，测试接种物中使用的微生物必须在原始测试微生物的 5 代以内。

Verification of the test inoculum is performed prior to sample testing. Verification is a preliminary test which evaluates the potency, purity and robustness of the test inoculum when

challenged against a known median reference standard ([S_3]) and standard curve ([S_1], [S_2], [S_4] and [S_5]). Refer to USP <81> for inoculum verification, testing parameters and acceptable data requirements and Section D, Antibiotic Standard and Sample Solution Preparation. As previously stated, acceptance testing requirements and acceptance criteria are as follows:

在样品测试之前进行测试接种物的验证。验证是一项初步测试，用于评估测试接种物在针对已知中值参考标准 (【S3】) 和标准曲线（【S1】、【S2】、【S4】 和 【S5】）进行挑战时的效价、纯度和稳健性。参阅 USP <81>中有关接种物验证、测试参数和可接受的数据要求以及 D 部分，抗生素标准品和样品溶液制备。如前所述，验收测试要求和验收标准如下：

The USP <81> states the following criteria for both the Plate and Tube Methods:

USP<81>对管碟法和浊度法提出了以下标准：

1. The calculated potency of the test antibiotic/unknown samples ([U_3]) must be 80% to 125% of the median reference standard ([S_3]);

   被测秔生素/未知样品（【U3】）计算所得效价必须为对照标准（【S3】）的 80-125%

2. Relative standard deviation for all measured (i.e. millimeters or absorbance) and calculated data (e.g. averages) is NMT 10%; and,

   所有测量所得（即毫米或吸光度）和计算所得数据（例如平均值）的相对标准偏差不得过 10%，且

3. Testing is performed in triplicate over a period of three independent test runs

   在三次独立检测运行期间执行三次检测

The USP <81> Plate Method further states:

USP<81>管碟法还要求：

1. The percentage coefficient of determinations (%R^2) for each standard curve will be NLT 95% (i.e. correlation coefficient of NLT 0.9750); and,

   每条标准曲线测定值的百分系数（%R^2）不得低于（NLT）95%（即相关系数 NMT0.9750），且

2. ZOIs for all media reference standard ([S_3] will measure between 14-16 mm).

   所有培养基对照品有 ZOI（【S3】测量值为 14-16mm）

The USP <81> Tube Method further states:

USP<81>浊度法还要求

1. The percentage coefficient of determinations (%R^2) for each standard curve will be NLT 90% (i.e. correlation coefficient of NLT 0.950); and,

   每条标准曲线测定值的百分系数（%R^2）不得低于（NLT）90%（即相关系数 NMT0.950），且

2. Absorbance values of the media reference standard ([S_3]) are predetermined per antibiotic. Refer USP <81> for testing parameters and acceptable data requirements.

   预先测定培养基对照标准（【S3】）的单位抗生素吸光度值。参见 USP<81>中检测参数和可接受的数据要求

Primary and working cultures should be identified with the microorganism specie name, ATCC (American Type Culture Collection) number and preparation and expiration date. Recommended storage requirements for primary and working inoculum stock are specified in USP <81>. To reduce assay variability, a primary stock culture of ≤ 14 days should be used to prepare a working stock culture; also, a working stock culture of ≤ 7 days should be used to prepare a test inoculum. The test microorganism bioactivity is known to decrease over time and adjustments to the test inoculum volume may be required to meet testing acceptance criteria (e.g. ZOI diameters between 14-16 mm as exhibited by the median reference standard ([S3]).

原代和工作培养物应标有微生物种类名称、ATCC（美国典型培养物保藏中心）编号以及制备和失效日期。USP <81> 中规定了原代和工作接种物的推荐储存要求。为减少测定变异性，应使用≤14 天的原代贮备培养物来制备工作贮备培养物；此外，应使用≤7 天的工作贮备培养物来制备测试接种物。已知测试微生物的生物活性会随着时间的推移而降低，因此可能需要调整测试接种量以满足测试可接受标准（例如，中值标准品 (【S3】) 显示的 ZOI 直径在 14-16 毫米之间）。

Therefore, to reduce assay variability, it is recommended to use fresh (e.g. ≤ 2 day old) primary and working cultures when possible.

因此，为了降低分析变异，建议尽可能使用新鲜（例如，≤ 2 天）的原代和工作培养物。

D. Antibiotic Reference Standards ([S1] – [S5]) and Unknown Sample (U3]) Preparation 抗生素标准品（【S1】-【S5】）和未知样品（【U3】）制备

Antibiotic potency testing requires the use of a standard curve to test an unknown sample. The reference standard (RS) must be from a verified source such as U.S. Pharmacopeia (USP). Procedures for reference standard preparation can be found in USP <81>. A single RS will be used to prepare a five-point ([S1] – [S5]) standard curve. Typically, the five standard solutions increase in concentration by a ratio of 1:1.25. For example, [S1] – [S5] standards can be represented in test as the following standard concentrations: 6.40 µg/mL, 8.00 µg/mL, 10.0 µg/mL, 12.5 µg/mL and 15.6 µg/mL. See Diagram 1 for further details. Labeling accompanying the RS will contain preparation, storage and expiration information; this information contains specific handling instructions and should be followed in order to achieve reproducible and reliable potency test data. A new standard curve must be prepared each day of inoculum verification (i.e. test plates) and/or each of the three independent test runs.

抗生素效效测试需要使用标准曲线来测试未知样本。标准品 (RS) 必须来自经过验证的来源，例如美国药典 (USP)。 USP <81> 中提供了标准品制备程序，其中使用单个 RS 来制备五点(【S1】 - 【S5】)标准曲线。通常，五种标准溶液的浓度以 1:1.25 的比例递增。例如，【S1】 - 【S5】标准在测试中可以表示为以下标准浓度：6.40µg/mL、8.00µg/mL、10.0 µg/mL、12.5µg/mL 和 15.6 µg/mL。详情请参见图 1。RS 随附的标签包含有制备、储存和有效期信息；此信息包含具体的操作说明，应遵循该说明以获得可重复的可靠效价测试数据。每次接种物确认当天（即测试碟）和/或每次独立测试时，均必须制备新的标准曲线。

Antibiotic potency samples will vary in physical form, dose and administration; examples of differing sample types include tablets, powders, solutions or semi- solids. Regardless of these physical and chemical attributes, the test sample and reference standard must be diluted prior to testing. Each dilution for the test sample and reference standard must be considered. The dilution factor (for each dilution) and the total dilution (for multiple dilutions) are data required

to calculate potency.

抗生素效价检测样品在物理形式、剂量和给药方式上会有所不同；不同样品类型的例子包括片剂、粉末、溶液或半固体。无论这些物理和化学属性如何，在测试前必须稀释测试样品和标准品。必须考虑测试样品和标准品的每次稀释。稀释因子（对于每次稀释）和总稀释倍数（对于多次稀释）是计算效价所需的数据。

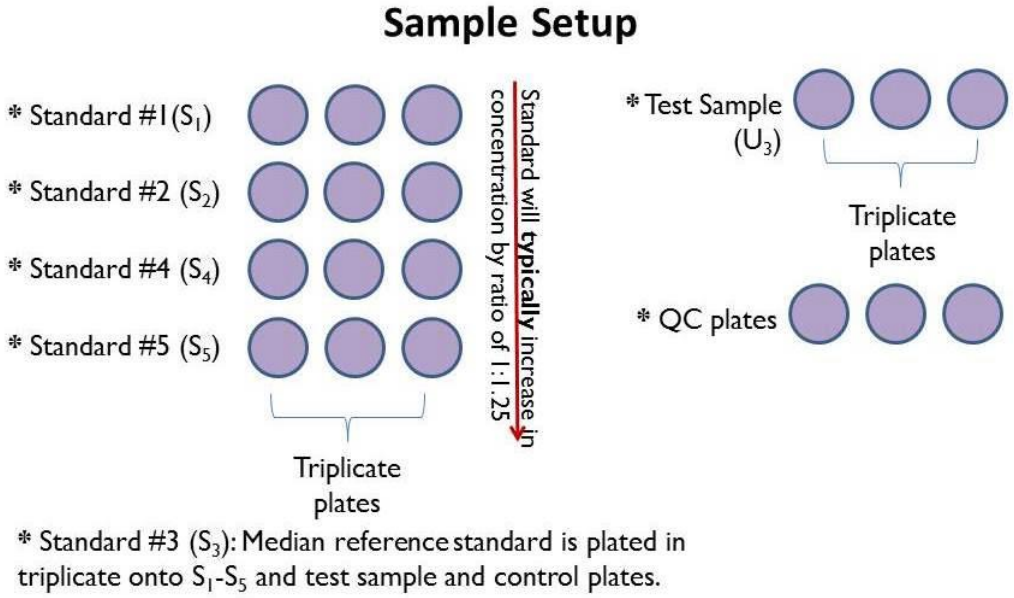


Diagram 1. Example of the Plate Method and placement of the standard curve and unknown sample onto triplicate agar plates. 6/19/17 MBB.

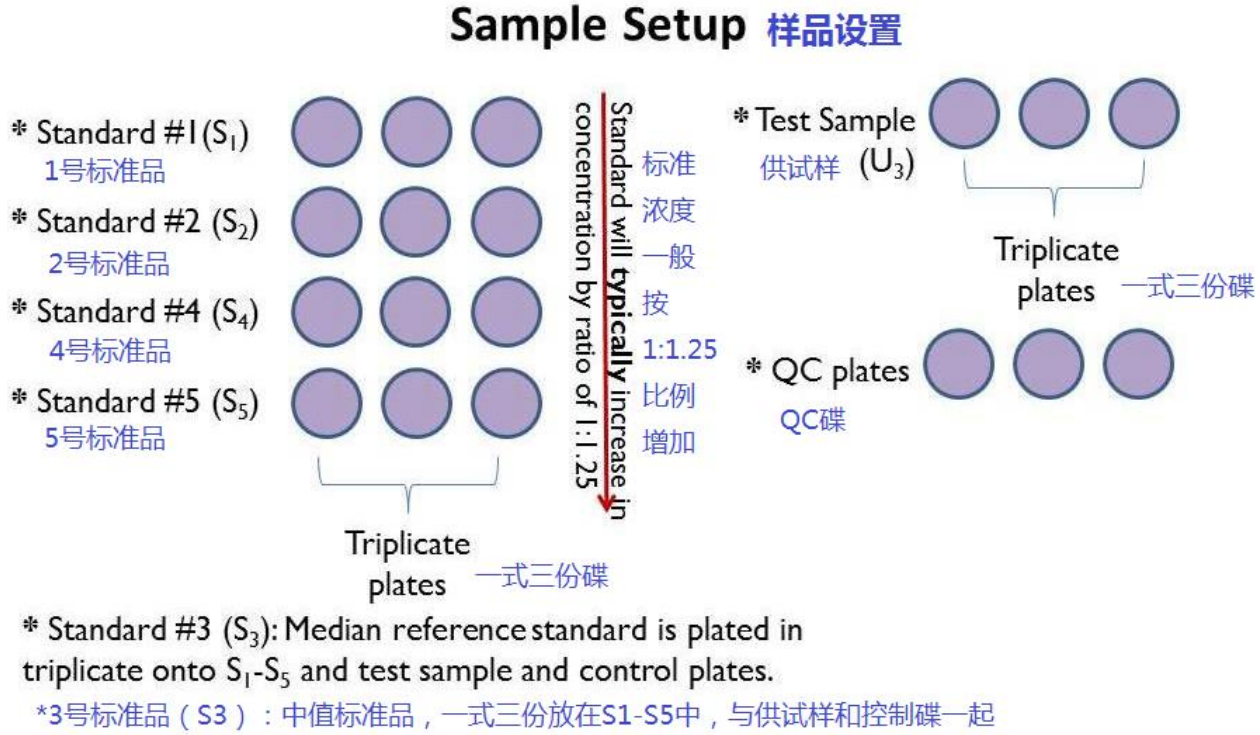


图 1：管碟法举例，标准曲线以及未知样品在琼脂碟上放置 6/19/17 MBB

Prior to testing, the unknown sample [U3] must be diluted to a known concentration. See USP <81> for the list of recommended concentrations of median reference standards used in test. The

target concentration is typically equivalent to the median reference standard [S3] of the standard curve.

在测试之前，未知样品【U3】必须稀释到已知浓度。测试中使用的中值标准品的推荐浓度列表参见 USP <81>。 目标浓度通常相当于标准曲线的中值标准品【S3】。

Diluting the unknown sample [U3] in this manner ensures a detection limit within the linear portion of the standard curve. For example, if the median reference standard [S3] has a concentration of 10.0 ug/mL, the unknown sample [U3] will also be diluted to a concentration of 10.0 ug/mL. See Diagram 1 for an example of one independent test run for the Plate Method and placement of the standard curve and unknown sample onto triplicate agar plates.

未知样品【U3】的稀释方式应确保检测限在标准曲线线性范围内。例如，如果中值标准品【S3】的浓度为 10.0 µg/mL，则未知样品 【U3】 也会稀释到 10.0 µg/mL 的浓度。碟法一次独立测试中将一式三份标准曲线和未知样品放置到琼脂平板上的示例参见图 1。

Quality control (QC) plates may include a test of respective diluents used for the reference, standard curve ([S1]-[S5]) and/or test/unknown sample [U3]. One of three QC plates should be dedicated for the evaluation of the inoculum used in test; specifically, this plate will only contain the test inoculum, will be free of penicylinders and should exhibit uniform growth (i.e. lawn) on top and within the agar. Microbial growth other than the lawn indicates the inoculum used in test may be contaminated and/or the technique used in preparing the single and agar plates may have been compromised the agar plates. The remaining two of three QC plates should be dedicated to each specific diluent used in test; for example, Water for Injection (WFI) and Buffer No. 4 are used in Vancomycin testing and therefore two QC plates, each containing WFI or Buffer No. 4 are prepared. Diluents are specific to each antibiotic and are listed in USP <81>.

质量控制 (QC) 板可包括对用于参考、标准曲线 (【S1】-【S5】) 和/或测试/未知样品【U3】的相应稀释剂的测试。三块 QC 板中的一块应专用于评估试验中使用的接种物；具体而言，该平板仅含测试接种物，没有小管，应在琼脂的顶部和内部表现出均匀的生长（即菌苔）。菌苔以外的微生物生长表明测试中使用的接种物可能受到污染和/或用于制备单板和琼脂板的技术可能已经损害了琼脂板。三块 QC 平板中的其余两块应专用于测试中使用的每种特定稀释剂；例如，注射用水(WFI) 和 4 号缓冲液用于万古霉素测试，因此制备两个 QC 板，每个板都包含 WFI 或 4 号缓冲液。USP <81> 中列出了每种抗生素专用的稀释剂。

E. Growth Media and Additional Test Solutions 生长培养基和其它检测溶液

In order to culture a pure and robust test organism, proper use and preparation of growth media (agar or broth), buffers and diluents is required. Components or final preparations of growth media, reagents or diluents may be prepared on-site and/or purchased from outside sources. Regardless of origin, any final product made from growth media components and/or preparations must be verified for identification, expiry, sterility and growth promotion, prior to use in test. Refer to USP <81> for preparation, use and storage of all growth media, buffers and diluents.

为了培养纯净且耐用的测试生物，需要正确使用和制备生长培养基（琼脂或肉汤）、缓冲液和稀释剂。生长培养基、试剂或稀释剂的成分或成品可以现场制备和/或从外部来源购买。无论来源如何，任何由生长培养基成分和/或成品制备的最终产品在用于测试之前都必须进行鉴定，并确认其有效期、无菌性和促生长能力。所有生长培养基、缓冲液和

稀释剂的制备、使用和储存参见 USP <81>。

F. Antibiotic Potency Testing: Plate Method 抗生素检测：管碟法

The Plate Method is the most commonly used USP <81> test. The Plate Method uses a solid medium (agar) to demonstrate antibiotic activity (i.e. zones of inhibitions (ZOIs)). This method requires the use of a five-point standard curve [S1] – [S5], median reference standard [S3], test antibiotic/ unknown sample [U3], verified inoculum, stainless steel penicylinders and petri-plates containing growth agar. See Diagram 1 for an example of one independent test run for the Plate Method and placement of the standard curve and unknown sample onto triplicate agar plates.

管碟法是最常使用的 USP<81>测试方法。管碟法使用固体培养基（琼脂）证明抗生素活性（即抑菌圈（ZOI））。该方法需要使用 5 点标准曲线【S1】-【S5】、中值标准品【S3】、供试抗生素/未知样品【U3】、经过确认的接种物、不锈钢小管和含有生长琼脂的培养碟。参见图 1 中一次独立碟法测试及一式三份标准曲线和未知样品琼脂平板的放置示例。

Depending upon the antibiotic, either a single layer or bi-layer agar plates are used in test. See USP <81> for preparation for single and bi-layer agar plates.

根据抗生素种类使用单层或双层琼脂进行测试。参见 USP<81>中单层或双层琼脂平板的制备。

1. Single Layer Plates: Prior to solidification, the growth agar is inoculated with a known volume of the verified test microorganism. The inoculated agar is thoroughly mixed, poured into a petri-plate and allowed to solidify.

   单层碟：将未知体积经过确认的测试菌接种到尚未固化的生长琼脂。充分混合接种后的琼脂，倾注到培养碟中，让其固化。

2. Bi-layer Plates: Prior to solidification, a portion of the growth agar is poured into the base of a petri-plate and allowed to solidify; this is the base layer of the test plate. The remaining portion of growth agar is further cooled and inoculated with a known volume of verified test organism. This inoculated agar is thoroughly mixed, poured onto the cooled base layer and allowed to solidify.

   双层碟：在固化之前，将一部分生长琼脂倾注到培养碟底部使其固化，这是测试碟的底层。将剩余部分生长培养基进一步冷却，将未知体积经过确认的测试菌接种至其中。充分混合接种后的琼脂，倾倒至冷却后的底层，让其固化。

Once the agar solidifies (either single layer or bi-layer agar plate), stainless steel penicylinders are applied to the agar surface using a penicylinder dispenser. Penicylinders are applied in an equidistant and upright fashion. Each penicylinder should be immediately dosed with two concentrations of antibiotic, the diluted unknown sample [U3] or the median reference standard (S3). Further, both antibiotics are dosed in equal volume into alternating penicylinders. See Diagram 2 for further details.

在琼脂固化之后（单层或双层琼脂碟），使用小管分配器将不锈钢小管以等距直立方式放置到琼脂表面。每支小管应立即注入 2 种浓度的抗生素、稀释后的未知样品【U3】或中值标准品（S3）。另取相同体积的 2 种浓度抗生素注入另外小管中。详情参见图 2。

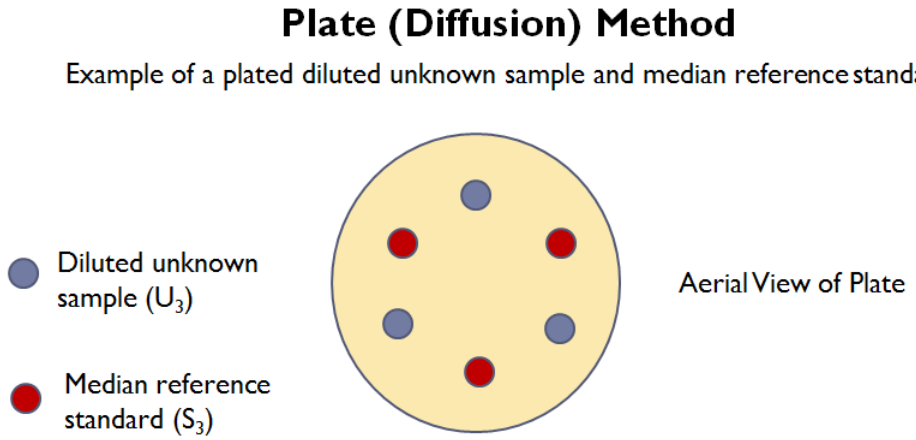


Diagram 2. Example of plated and dosed penicylinders containing the diluted unknown sample and median reference standard. 6/19/17 MBB.

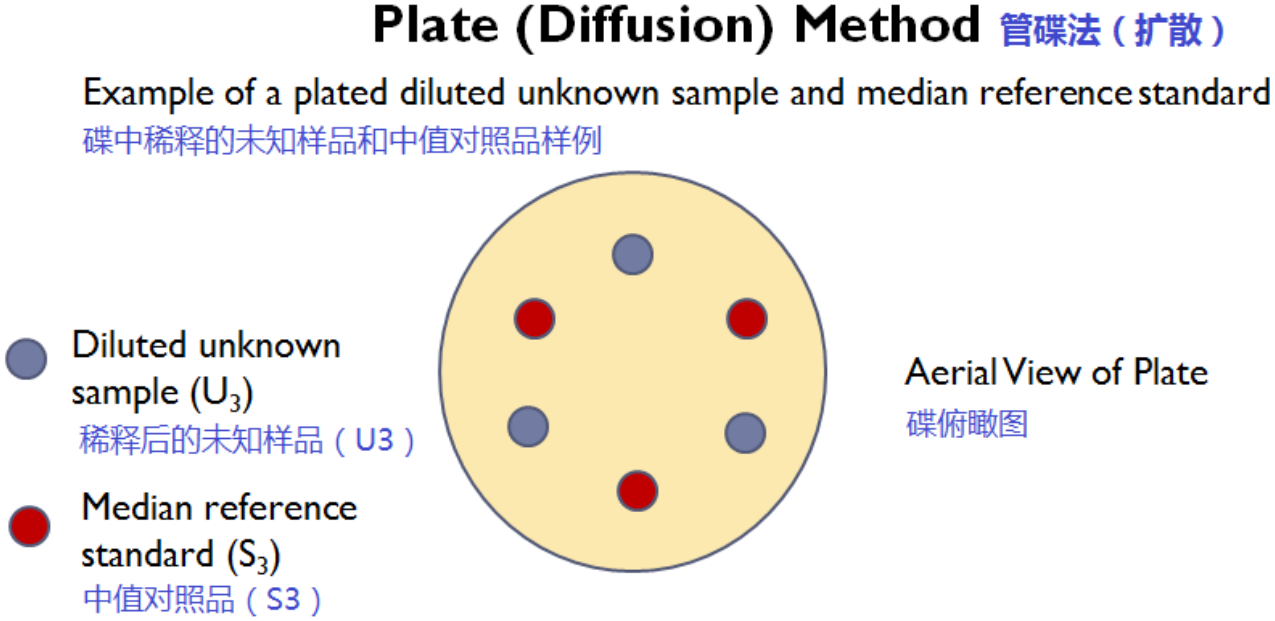


图 2 含已稀释未知样品和中值标准品的培养碟和定量小管示例 6/19/17MBB。

After dosing, all plates prepared in one independent test run are incubated at the same time. As the plates incubate, the antibiotics diffuse through the agar, creating a zone of clearing below and around penicylinder perimeter; this clearing is referred to as the zone of inhibition (ZOI). The ZOI demonstrates the antibiotic activity of the diluted unknown sample [U3] and the median reference standard [S3]. See Diagram 3 for further details.

在加入抗生素之后，同时培养一次检测中制备的所有培养碟。在平板培养期间，抗生素会在琼脂中扩散，在小管周边底下形成清晰的圆圈，该圈称为抑菌圈（ZOI）。ZOI 显示的是稀释后的未知样品【U3】和中值标准品【S3】的抗生素活性。详情参见图 3。

Plate (Diffusion) Method

Example of a plated diluted unknown sample and median reference standard with ZOIs surrounding dosed penicylinders

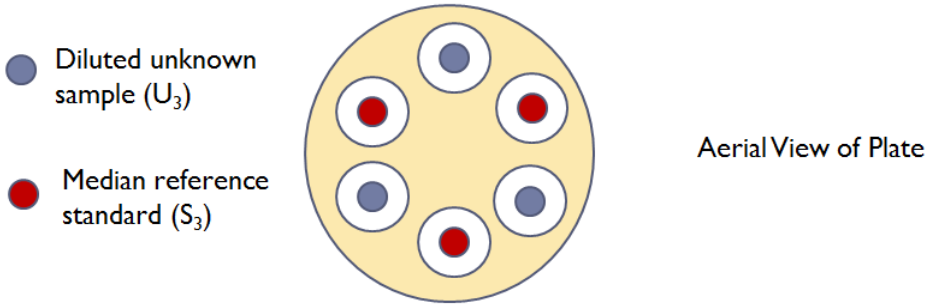


Diagram 3. Example of plated and dosed penicylinders on incubated plates showing zones of inhibition. 6/19/17 MBB.

Plate (Diffusion) Method
管碟（扩散）法

Example of a plated diluted unknown sample and median reference standard with ZOIs surrounding dosed penicylinders

稀释未知样和中值标准品碟示例，有ZOI围绕的定量小管

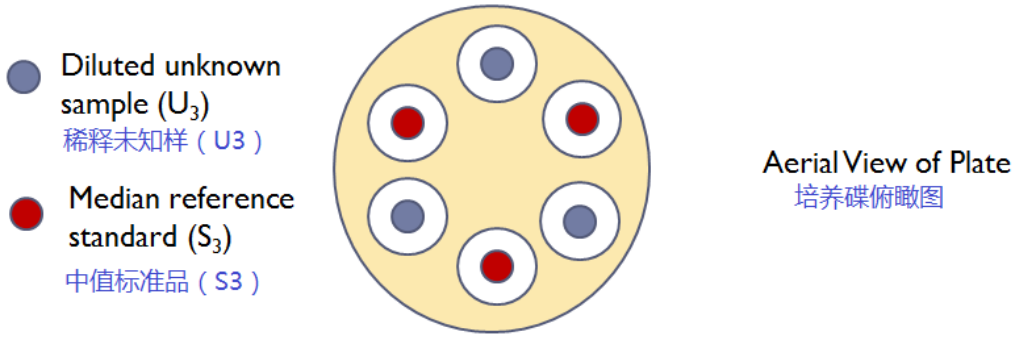


图 3 显示抑菌圈的带小管接种碟举例 6/19/17 MBB

After the specified incubation time, the penicylinders are removed, decontaminated (i.e. autoclaved) and washed with soap and water. The penicylinders are then heat sterilized prior to re-use in test. The diameter of each ZOI is measured with a manual/automatic plate reader. ZOIs should only be measured by qualified laboratory personnel. ZOI measurements are taken with the use of a manual or automatic plate reader. Examples of a manual reader include a Fisher-Lily zone reader or manual/electronic calipers; manual reads require data entry (e.g. handwritten and/or electronic) onto hardcopy and/or into electronic logbooks and/or spreadsheets. Examples of automated plate readers include OMNICON or Trinity V3. Unlike manual reads, automated plate readers allow a computerized system to measure the plates and do not require data entry of raw data.

经过指定的培养时间后，取掉小管，灭活（即灭菌）并用肥皂和水清洗。小管再次用于检测之前要加热灭菌。使用手动/自动读碟器测量每个 ZOI 的直径。ZOI 应由经过资格确认的实验室员工测量。使用手动/自动读碟器测量 ZOI。手动读碟器的示例包括 Fisher-Lily 读碟器或手动/电子圆规；手动读取需要将数据录入（例如手写和/或电子）纸质记录

和/或电子日志和/或数据表。自动读碟器的例子包括 OMNICON 或 Trinity V3。与手动读取不同，自动读碟器允许计算机化系统对培养碟进行测量，不需要录入原始数据。

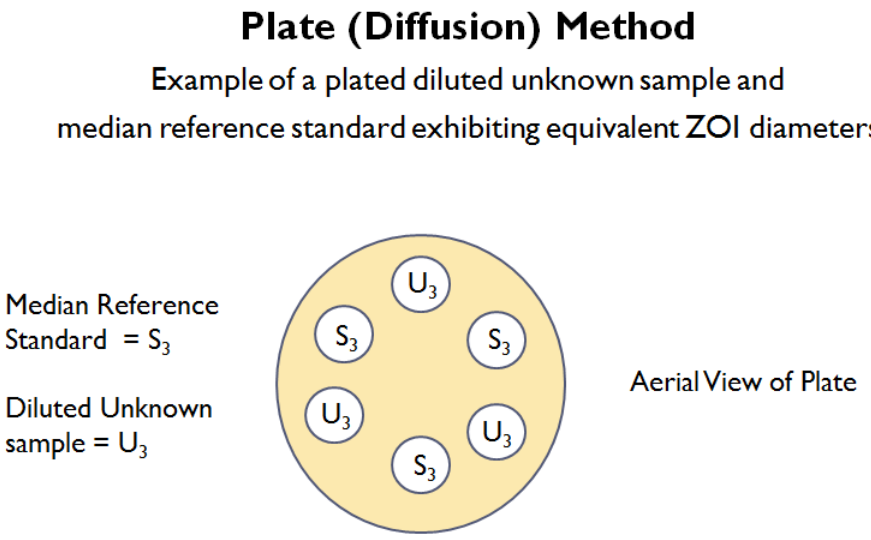


Diagram 4. Example of a plated diluted unknown sample and median reference standard exhibiting equivalent ZOI diameters. 6/19/17 MBB.

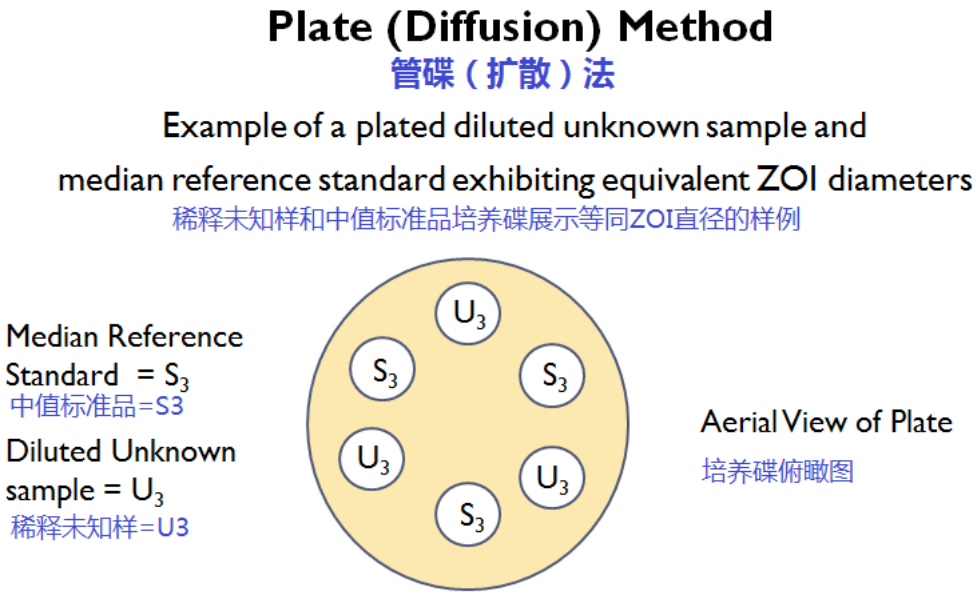


图 4：稀释未知样和中值标准品显示等同 ZOI 直径培养碟示例，6/19/17 MBB

The zones of inhibition exhibited by the unknown sample [U3] and the median reference standard [S3] should be approximately equivalent. See Diagram 4 for further details. The ZOI comparison between the diluted unknown [U3] and the median reference standard [S3] can be described mathematically as the percentage of reference concentration. The calculated potency of the unknown sample [U3] must have a percentage of reference concentration of 80% - 125%. If the concentration of the diluted unknown sample [U3] falls outside this range of 80%-125%, the same unknown sample must be retested using an estimated dilution to obtain an equivalent concentration of the median reference standard [S3]. Diluting the unknown sample [U3] in this manner ensures a final detection limit within the linear portion of the standard curve. Test sample preparation, storage and expiration require the dose and administration information (as per label claim) and procedures prescribed in USP <81> and the appropriate USP antibiotic

monograph.

未知样品【U3】和中值标准品【S3】显示的抑菌圈应该基本相同。详情参见图 4。稀释后的未知样品【U3】和中值标准品【S3】ZOI 之间的比较可以用算术方法描述为参比浓度的百分比。未知样品【U3】的计算效价必须为参比浓度的 80-125%。如果稀释后未知样品【U3】的浓度超出 80-125%范围，则必须使用估算稀释度复测相同未知样品，使其浓度相当于中值标准品【S3】浓度。未知样品【U3】的稀释方法应确保最终检出限度在标准曲线的线性范围内。供试样的制备、存贮和有效期需要 USP<81>和适当 USP 抗生素各论中所述的剂量和给药信息（按标签声明）和程序。

USP <81> states the following criteria for both the Plate and Tube Methods: 1) The calculated potency of the test antibiotic [U3] must be 80% to 125% of the median reference standard [S3] 2) Relative standard deviation for all measured and calculated data is NMT 10% and 3) Testing is performed in triplicate over a period of three independent test runs. The Plate Method further states: 1) The percentage coefficient of determinations (%R2) for each standard curve will be NLT 95% (i.e. correlation coefficient of NLT 0.9750) and 2) ZOIs for all media reference standard (S3 will measure between 14-16 mm). Refer to USP <81> for testing parameters and acceptable data requirements.

USP<81>要求管碟法和浊度法符合以下标准：（1）测试抗生素【U3】的计算效价必须为中值标准品【S3】的 80-125%，（2）所有测量数据和计算数据的相对标准偏差 NMT 10%，且（3）进行三次独立检测，每次测试一式三份样品。管碟法还要求：（1）每条标准曲线的测试百分比系数（%R2）NLT 95%（即相关系数 NLT 0.9750），且（2）所有中值标准品（S3）的 ZOI 应在 14-16mm。参见 USP<81>中检测参数和可接受数据要求。

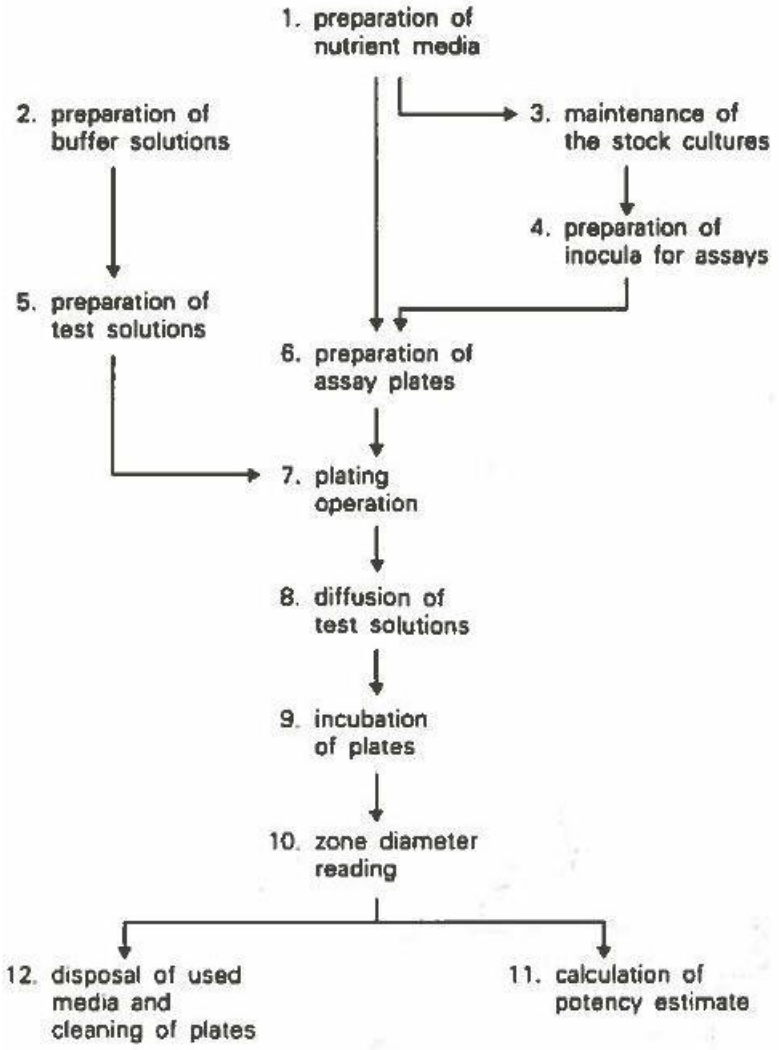


Diagram 5. Example of operations conducted prior, during and after testing using the Pate (Diffusion) Method (Hewitt and Vencent 1989).

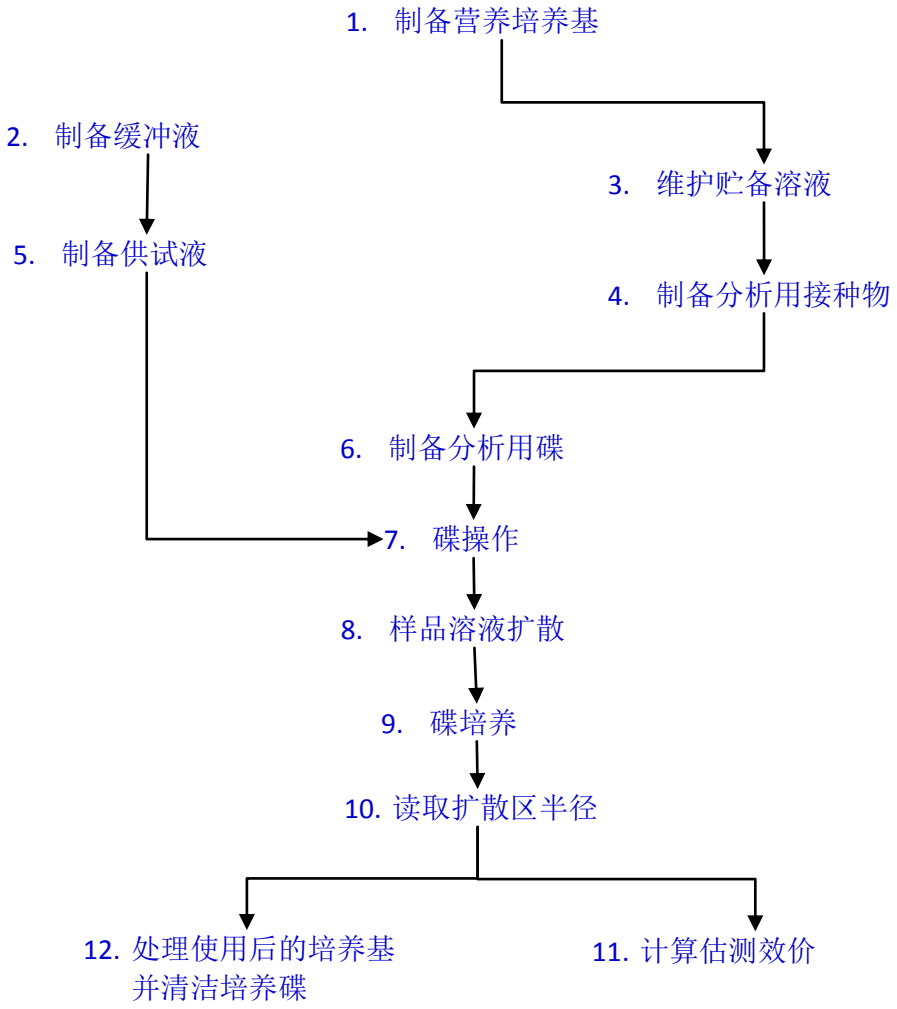


图 5：使用碟（扩散）法前、中、后所执行的操作示例（Hewitt and Vencent 1989）

G. Antibiotic Potency Testing: Tube Method 抗生素效价测试：浊度法

Of the two USP <81> methods, the Tube Method is less commonly used. The tube method uses a liquid medium (growth broth) to demonstrate antibiotic activity. This method requires the use of a test antibiotic/unknown [U3], five- point (minimum) standard curve ([S1]-[S5]) and verified inoculum. Growth inhibition is measured by qualified laboratory personnel with the use of a UV- VIS spectrophotometer. Test antibiotic will be analyzed in triplicate over a period of three independent test runs. Refer to USP <81> for Tube Method procedures.

在两种 USP <81> 方法中，浊度法不太常用。浊度法使用液体培养基（生长肉汤）来证明抗生素活性。该方法需要使用受测抗生素/未知物【U3】、（最少）5 点标准曲线(【S1】-【S5】) 和经过验证的接种物。由合格的实验室人员使用 UV-VIS 分光光度计测量生长抑制。抗生素检查应独立检测 3 次，每次一式三份。有关浊度法检测程序参见 USP<81>。

Test antibiotic/unknown [U3] tubes will contain the growth broth, inoculum and the test antibiotic/unknown [U3] in triplicate. The standard curve ([S1]-[S5]) tubes will contain the growth broth, inoculum and each of the respective standard curve concentrations [S1], [S2], [S3], [S4], and [S5] in triplicate. The QC tubes will contain additional median reference

standard [S3], will be used to perform preliminary absorbance checks and serve as a quality check for diluents and inoculum used in test. See Diagram 6 for an example of one independent test run for the Tube Method and placement of the standard curve and unknown sample into triplicate tubes.

一式三份抗生素/未知样品【U3】试管中含有生长肉汤、接种物和受检抗生素/未知样品【U3】。一式三份标准曲线（【S1】-【S5】）管含有生长肉汤、接种物和各代表性标准曲线浓度【S1】、【S2】、【S3】、【S4】和【S5】。QC 管含有另一份中值标准品【S3】，该管是用于进行初始吸光度检查，作为检测中所用稀释倍数和接种物的质量检查。参见图 6 浊度法一次独立检测，及标准曲线和未知样品一式三份样品管设置示例。

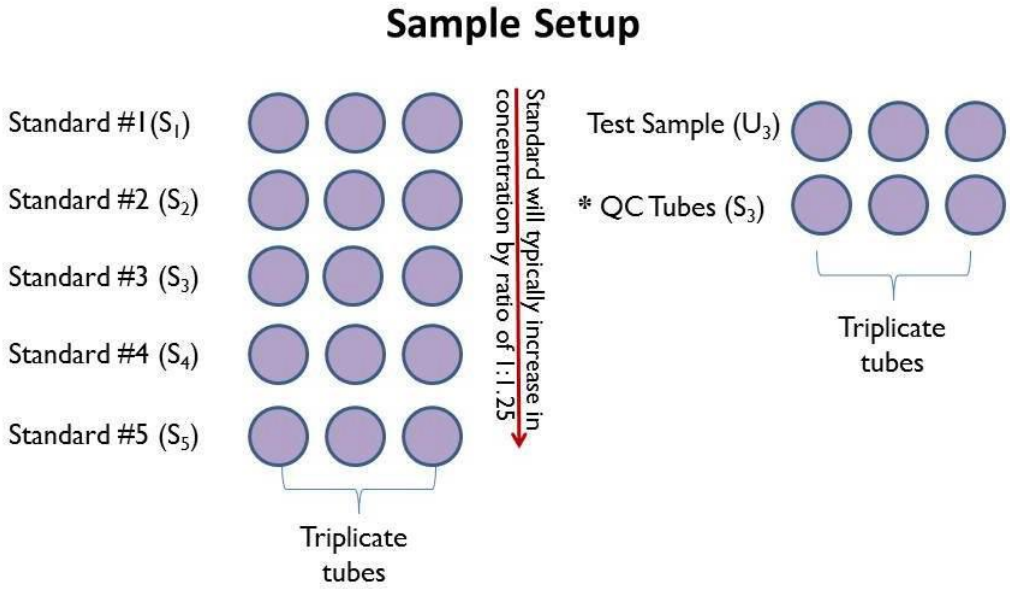


Diagram 6. An example of one independent test run for the Tube Method and placement of the standard curve and unknown sample into triplicate tubes. 6/22/17 MBB.

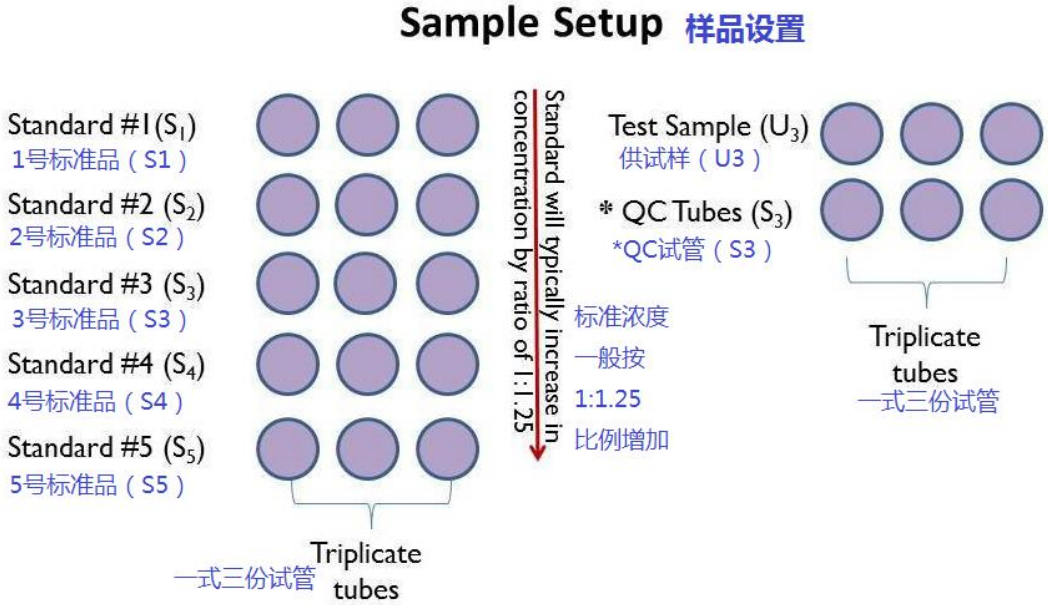


图 6：一次独立浊度法检测样例，标准曲线和未知样品一式三份放入试管，6/22/17 MBB

When a microorganism is placed into a broth containing the appropriate nutrients to support

growth, the microorganism flourishes and the broth becomes turbid. Turbidity is typically a simple visual indicator of microbial growth; this growth can be quantified by a UV-VIS spectrophotometer by measuring values absorbance or transmittance exhibited by the broth.

如果将一种微生物放进含有支持其生产的适当营养物质的肉汤中，该微生物会大量繁殖，肉汤会变混浊。浊度通常是微生物生长的简单视觉指标；通过紫外可见分光光度计通过测量肉汤显示的吸光度或透射率值可以量化该生长情况。

Test tubes containing growth broth, inoculum, test/unknown sample and standard curve ([S1] – [S5]), will be prepared and analyzed the same day. The test tubes are then placed into a circulating water bath for NMT 5 hours to reach a specified turbidity (i.e. absorbance). It is recommended, one or more QC tubes be used to perform an absorbance check at timed interval to assure the required absorbance is not exceeded; this activity may require the preparation of more than three QC tubes. After the specified absorbance is achieved, formaldehyde or a heat treatment is immediately added/applied to each test tube to inhibit additional microbial growth prior to absorbance reading and use of the UV-VIS. The absorbance or transmittance is read ad 580 nm or 530 nm.

在同一天制备含有生长肉汤、接种物、测试/未知样品和标准曲线 (【S1】-【S5】) 的试管并分析。然后将试管放入循环水浴中恒温不大于 5 小时，以达到指定的浊度（即吸光度）。建议使用一根或多根 QC 管定期进行吸光度检查，以确保不超过所需的吸光度；此操作可能需要准备三个以上的 QC 管。在达到指定的吸光度后，立即向各试管中加入甲醛，或对其进行热处理，在读取吸光度和使用 UV-VIS 之前抑制更多的微生物生长。在 580 nm 或 530 nm 处读取吸光度或透射率。

USP <81> states the following criteria for both the Plate and Tube Methods:

USP <81>中管碟法和浊度法标准如下：

1) The calculated potency of the test antibiotic [U3] must be 80% to 125% of the median reference standard [S3]

抗生素样品【U3】的计算效价必须为中值对照品【S3】的 80-125%。

2) Relative standard deviation for all measured and calculated data is NMT 10% and

所有测量和计算所得数据的相对标准偏差应 NMT 10%，且

3) Testing is performed in triplicate over a period of three independent test runs.

独立进行 3 次检测，每次检测使用一式三份样品。

The Tube Method further states: The percentage coefficient of determinations (%R2) for each standard curve will be NLT 90% (i.e. correlation coefficient of NLT 0.950) and
2) Absorbance values of the media reference standard [S3] are predetermined per antibiotic. Refer USP <81> for testing parameters and acceptable data requirements.

浊度法还要求，每条标准曲线的测定百分比系数（%R2）应 NLT90%（即相关系数 NLT0.950），且中值对照品【S3】的吸光度值要根据不同抗生素分别预先确定。参见 USP<81>中检测参数和可接受数据要求。

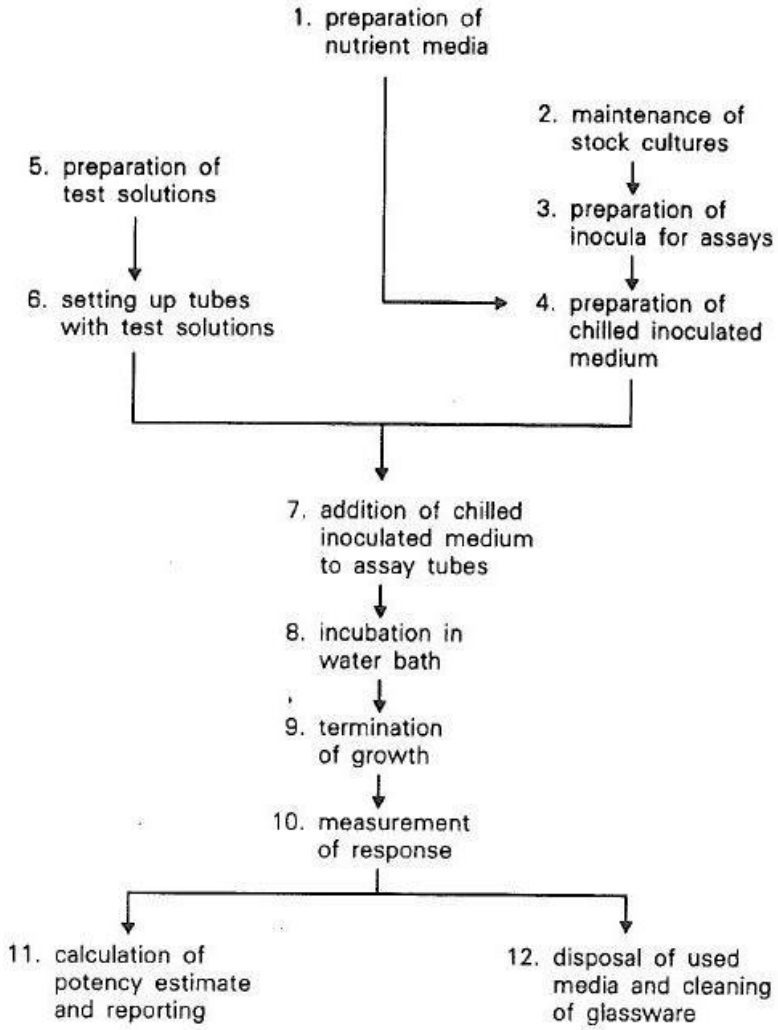


Diagram 7. Example of operations conducted prior, during and after testing using the Tube (Turbidimetric) Method (Hewitt and Vencent 1989).

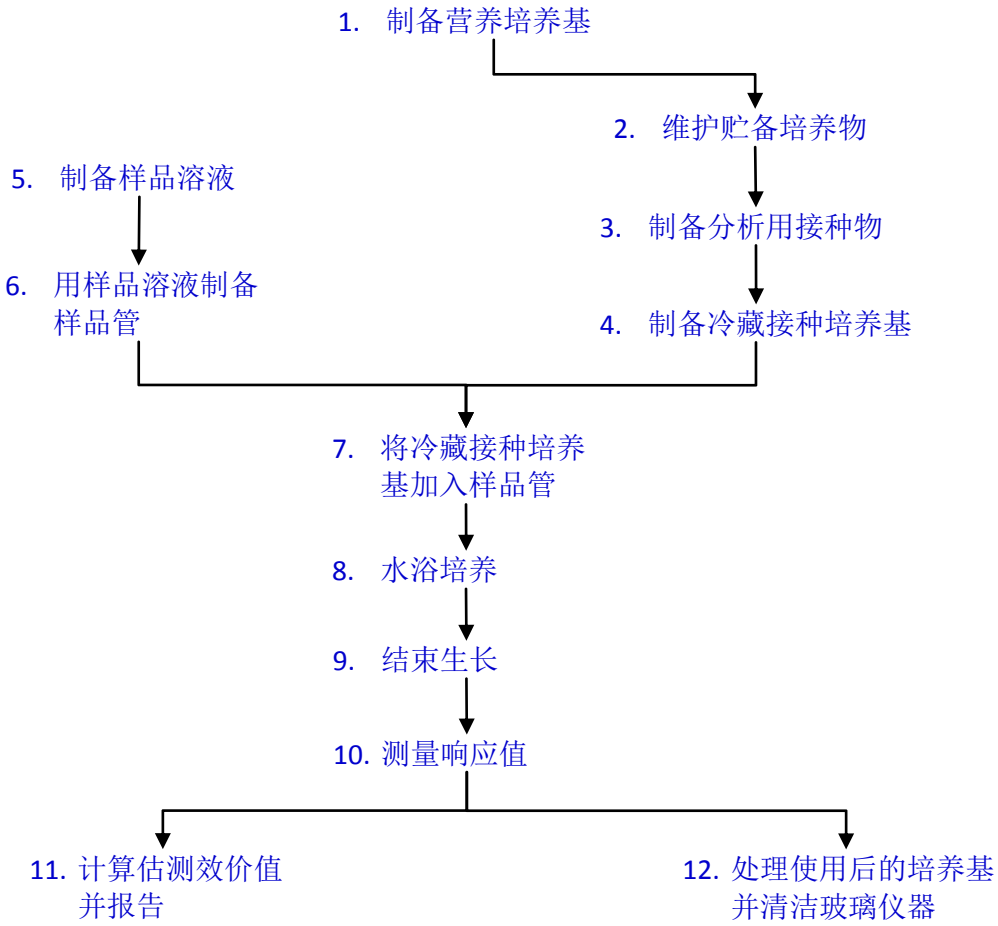


图 7：使用浊度法测量前、中、后所执行操作示例（Hewitt and Vencent 1989）

H. Calculations 计算

Potency calculations are listed in USP <81> and are specific to either the Plate or Tube Method. Calculations may be performed by traditional hand math or by electronic spreadsheet such as Microsoft Excel. In order to achieve reliable and reproducible results, electronic spreadsheets should be validated.

效价计算列在 USP<81>中，管碟法和浊度法有分开说明。可采用传统手工算术进行计算，亦可采用电子表格如微软 EXCEL 进行计算。为了得到可靠和可重复的结果，应对电子表格进行验证。

Likewise, automated readers performing calculations should also be validated prior to use in testing.

同样，进行计算的自动读数仪亦要在用于检测之前进行验证。

I. Inspectional Objectives 检查目标

When conducting an inspection of a human antibiotic manufacturer , contract manufacturer or test laboratory, inspectional objectives should include coverage of high risk product/s (unless stated otherwise through special assignment or investigation) and a comparison of both USP <81> and USP monograph requirements and acceptance criteria to three types of in-house

practice and procedures related to: 1) General laboratory and analytical operations 2) Initial training and requalification and 3) Data generation, review and archival. Employee practices should be documented in established written procedures. And, the original test data should match the final and/or released test results. All laboratory practices, procedures and test data should be based in sound science and meet the requirements and acceptance criteria of both USP <81> and related USP monograph.

在对人用抗生素生产商、合同生产商或检测实验室进行检查时，检查目标应该包括高风险产品（通过特殊指派或调查另行指明者除外），并将 USP<81>与 USP 各论要求和可接受标准与三种内部规范和程序进行比较：（1）通用实验室和分析操作，（2）初始培训和资质重新确认，以及（3）数据生成、审核和归档。员工规范应该制订有书面程序，并且原始检测数据应该与最终和/或放行检测结果相一致。所有实验室规范、程序和检测数据均应科学合理，并符合 USP<81>和相关 USP 各论中的要求和可接受标准。

Specifically, a CSO and/or Analyst should conduct observations and make a procedural comparison of media, reference standard, standard curve, culture control, unknown sample and equipment preparation and use; also, a review or practice and procedure of equipment calibration, maintenance (preventative and major) and use should be conducted. Practice and procedure comparisons should be made for laboratory personnel involved with data entry, transcription and/or review should be contemporaneously observed, followed by an immediate review of related documents for thoroughness and accuracy; this includes data transcription from handwritten and/or electronic data into logbooks, batch records and/or certificates of analyses. Additionally, it is necessary to understand which document serves as the original record; request for the most responsible person employed at the firm to identify the original record/s containing the raw data.

具体来说，CSO 和/或化验员应该对培养基、对照品、标准曲线、培养控制、未知样品和设备准备和使用进行观察，并与程序规定进行比较，并执行审核或规范，执行设备校正、维护（预防性维护和主要维护）和使用程序。应将参与数据录入、抄写和/或审核员工的操作情况与程序要求进行同步观察和比较，然后立即审核相关文件的完整性和准确性，其中包括数据从手写和/或电子数据转抄至日志、批记录和/或 COA 中。另外，有必要了解哪一种文件是作为原始记录，要求公司最终负责人员识别含有原始数据的原始记录。

If the firm utilizes contract manufacturing services, is a contract manufacturer and/or a contract test laboratory, an active Quality Agreement, as established by the firm and customer, may provide insight to the roles and responsibilities of each party for testing and data generation, review and archival.

如果公司使用合同生产服务，检查合同生产商和/或合同检测实验室是否签订有有效质量协议（就像公司与客户签订的协议一样），这样可以知悉各方在检测和数据生成、审核和归档方面的角色与职责。

J. References: 参考文献

1. Hewitt, W. & Vincent, S. (1989) Theory and Application of Microbiological Assay, Academic Press, Inc.

   Hewitt, W. & Vincent, S. (1989)，微生物检测理论与应用，Academic Press, Inc.

12. Chapter 8: Bioburden Estimation for Medical Devices 第八章：医疗器械的生物负载估测

According to FDA Compliance program 7382.845, *Inspections of Medical Device Manufacturers, Part IV*, - "Bioburden testing is to be performed in accordance with the guidance provided in *ISO 11737-1, Sterilization of medical devices – Microbiological methods – Part I: Estimation of population of microorganisms on products.* The methodology used for estimating the bioburden is to be validated. Twenty units are to be tested."[1]

根据 FDA 合规计划 7382.845《医疗器械生产商的检查》第四部分—"根据 ISO 11737-1 中的【医疗器械的灭菌—微生物学方法—第一部分：产品微生物群的估测】进行生物负载测试。用于评估行生物负载的方法学应进行验证。测试 20 个产品单位"。

The term "bioburden" is commonly used to describe the population of microorganisms present on unsterilized material or products. The bioburden quantity and types of bioburden organisms present can impact the sterilization process of the material or product. It is important to develop procedures which provide accurate, precise, and reproducible measurement of the bioburden population associated with the material or product. There are several approaches to remove microorganisms from a medical device. Some examples of these recovery methods include: filtration followed by plating; ultrasonic/shaking followed by filtration then placing on an agar medium; stomaching/rinsing/flushing followed by filtration and plating on an agar medium; if all else fails perform a direct swabbing or contact plate.

"生物负载"一词通常用于描述存在于非无菌物料或产品的微生物群。存在生物负载微生物的生物负载量和类型可以影响物料或产品的灭菌工艺。开发能够提供准确、精确和可重复测量的与物料或产品相关的生物负载量的程序非常重要。有几种方法可以从医疗器械中去除微生物。这些回收方法包括：过滤后电镀；超声/振荡后过滤，然后放在琼脂培养基上；擦洗/冲洗/冲刷后过滤并涂布在琼脂培养基上；如果所有都不行，那么就直接擦拭或涂抹。

The bioburden estimation of a medical device generally consists of four distinct stages:

医疗器械的生物负载评估通常由四个不同的阶段组成：

1. Collection of microorganisms from the medical device. (See Annex A and B)

   从医疗器械中收集微生物（见附录 A 和 B）

2. Enumeration of the collection sample containing recovered microorganisms.

   对含有已恢复的微生物的所采集样本计数

3. Bioburden characterization.

   生物负载表征

4. Application of the correction factor(s) determined during bioburden recovery studies in order to calculate the bioburden estimate from the raw presterilization count. The correction factor is derived from the determination of the recovery efficiency of a method. The calculation of a correction factor is illustrated in Appendix C, Section C.2.

   使用生物负载回收研究中确定的校正因子，从原始的预灭菌计数中计算生物负载估测值。校正因子是在方法回收率计算中得到的。校正因子的计算见附录 C 第 C.2 节。

It is not possible to define a single microbial collection technique because of the wide variety of materials used in health care products. Furthermore, the selection of conditions for enumeration will

be influenced by the types of microbial contamination which may be anticipated.

由于医疗产品所用材料各类繁多，因此不可能定义单一的微生物采集技术。此外，计数条件的选择亦会受到预期微生物污染各类的影响。

The current method *"ANSI/AAMI/ISO 11737-1:2018/(R) 2011 sterilization of health care products – Microbiological methods – Part 1: Determination of the population of microorganisms on product"* has the latest revisions and provides a great deal of information that will guide an analyst to the method needed for a particular type of product.

现行方法"ANSI/AAMI/ISO 11737-1:2018/(R) 2011 医疗产品的灭菌—微生物学方法—第一部分：产品微生物群的确定"有最新版本，其中提供了大量的信息，可指导分析人员了解特定类别产品所需的方法。

Annex A contains a decision tree "that addresses designing a bioburden method based on the nature of the product being tested and includes guidance for choosing such things as agitation techniques or filtration versus direct plating."[2] Annex A also addresses the procedures (repetitive recovery method, product inoculation method) available for the validation of the method for determining bioburden.

附录 A 包括一个决策树"阐述基于被测产品性质设计生物负载方法，并且包括用于选择诸如搅拌技术或过滤与直接电镀之类的指南"。附录 A 还阐述了可用于确定生物负载方法验证的程序（重复回收方法、产品接种方法等）。

Annex B has a comprehensive list of the different removal techniques that can be employed and alternatives for samples where removal of microorganisms by elution is not used.

附录 B 详细列出了可以使用的不同清除技术，以及不使用洗脱液清除样品中微生物的替代方法。

Annex C has a more in-depth explanation of the validation of the repetitive recovery and product inoculation methods.

附录 C 对于重复回收和产品接种方法的验证作了更深入的解释。

A. References:参考文献

1. FDA Compliance Program 7382.845 Inspections of Medical Device Manufacturers, February 2, 2011.

   FDA 合规程序 7382.845 医疗器械生产商检查，2011 年 2 月 2 日

2. ANSI/AAMI/ISO 11737-1:2018/(R) 2011, Sterilization of health care products – Microbiological methods – Part 1: Determination of the population of microorganisms on product.

   ANSI/AAMI/ISO 11737-1:2018/(R) 2011，医疗产品的灭菌—微生物学方法—第一部分：产品微生物群的确定

3. PDA Technical Report No. 21, Bioburden Recovery Validation. 1990

   PDA 第 21 号技术报告，生物负载回收验证，1990

13. Chapter 9: Environmental Monitoring 第九章：环境监测

ORS microbiologists may be required to assist CSOs during on-site inspections of pharmaceutical facilities to perform environmental monitoring (EM) sampling of those facilities to assess the microbiological bioburden of critical surfaces. This chapter describes a suggested procedure for conducting this activity. This should only serve as a guide with some modifications depending on the specific facility or special instructions from ORS Headquarters. Be sure to confirm with ORS Headquarters which ORS laboratory was designated to receive the EM samples. These procedures allow for a qualitative and quantitative assessment of environmental monitoring samples for the microbial presence in critical processing or laboratory area(s) being monitored.

在对制药设施进行现场检查期间，可能会要求 ORS 微生物学家协助 CSO 对这些设施进行环境监测（EMA）取样，用以评估重要表面的微生物负载。本章介绍了执行此活动的建议程序。这只能作为指导，需要根据特定的设施或 ORS 总部的特殊 指示进行。务必向 ORS 总部确认指定由哪个 ORS 实验室接收 EM 样品。通过这些程序，可定性定量地评估环境监测样品，确定受监测的关键工艺或实验室区域中的微生物存在情况。

A. Materials/Equipment 物料/设备

1. Sampling Materials (Disinfect all materials being brought into the ante-room, cleanroom, or area designated for compounding/processing by the firm.)

   取样物料（将所有准备带入公司配制/加工前厅、洁净间或区域的物料进行消毒）

   a. Sterile polyester or cotton swab with a sterile transport media solution.

      用无菌转运培养基溶液蘸湿的无菌涤纶或棉签

   b. Sterile sponge with detachable handle

      带有可拆卸手柄的无菌海绵

   c. *Hycheck or equivalent surface samplers

      Hycheck 或等同的表面取样器

   d. *RODAC/contact plates (Replicate Organism Detection And Counting)

      **Use media containing lecithin and tween neutralizers*

      *RODAC 碟/接触碟（两次生物检测和计数）

      *使用含有卵磷脂和吐温中和剂的培养基

   e. Sterile Whirl-pak® bags.

      无菌 Whirl-pak®袋

   f. Sterile water for irrigation or sterile saline held in screw cap containers.

      用于冲洗的无菌水或装在螺帽容器中的无菌盐水

   g. Dey/Engley (D/E) neutralizing broth

      Dey/Engley (D/E)中和肉汤

   h. Letheen Broth

      Letheen 肉汤

i. Sterile Disinfectant in a spray bottle

装在喷瓶中的无菌消毒剂

j. Sterile 70% alcohol spray bottle or wipes

无菌 70%乙醇喷瓶或抹布

k. Black marker (permanent, fine point).

黑色记号笔（永久，细头）

l. Digital Camera

数码照相机

2. Testing Equipment and Materials 检测设备和物料

a. Biological Safety Cabinet (BSC) with HEPA filtration

带 HEPA 过滤的生物安全柜（BSC）

b. Laminar Flow Hood (LFH) with HEPA filtration

带 HEPA 过滤的层流罩（LFH）

c. 10% Bleach or appropriate disinfectant/sporicidal

10%漂白剂或适当的消毒剂/杀孢子剂

d. Sterile 70% ethanol (ETOH) or Isopropyl Alcohol (IPA)

无菌 70%乙醇（ETOH）或异丙醇（IPA）

e. Sterile Sleeves

无菌护袖

f. Sterile Gloves

无菌手套

g. Hair Net

发网

h. Lab Coat/Sterile Gown

实验室工作服/无菌服

i. Beard Cover and/or Mask

胡须罩和/或面罩

j. Incubator set at 32.5℃ ±2.5℃. Incubator set at 22.5℃±2.5℃.

设置在 32.5℃ ±2.5℃和 22.5℃±2.5℃的培养箱

k. Modified Letheen Broth (MLB)

改良 Letheen 肉汤（MLB）

l. Modified Letheen Agar (MLA)

改良 Letheen 琼脂（MLA）

m. Sabouraud Dextrose Broth

沙氏葡萄糖琼脂培养肉汤

n. Sabouraud Dextrose Agar

沙氏葡萄糖琼脂培养基

o. Malt Extract Agar w/chlorotetracycline

麦芽提取琼脂培养基/金霉素培养基

p. TSA w/5% Sheep Blood Agar

TSA 5%绵羊血琼脂培养基

q. MacConkey Agar

麦康凯琼脂培养基

r. RODAC plates

RODAC 碟

s. Hycheck slides

Hycheck 培养条

t. Soybean Casein Digest Agar (TSA)

大豆胰蛋白肉汤培养基（TSA）

u. Soybean Casein Digest Broth (with neutralizers)

大豆胰蛋白肉汤培养基（带中和剂）

v. Neutralizers (i.e. lecithin, tween, etc...)

中和剂（即卵磷脂，吐温等）

B. Sampling Preparation 取样准备

1. Don appropriate personal protective equipment (PPE) as follows:穿戴适当的个人防护装备 (PPE)，如下所示：

Note: *All ORS full gowning and procedures should be performed prior to conducting the collection. The firm's procedures/guidelines should be adhered to when entering classified area; however, it is the expectation that the microbiologists entering the classified area, in order to perform sampling, will don sterile garments. If there is a dispute regarding the PPE to be donned for aseptic sampling, ORS Headquarters should be contacted prior to commencing gowning.*

注：在取样之前，要执行所有 ORS 完整更衣和程序。在进入洁净区时，要遵守公司的程序/指导，要求进入洁净区采集样品的微生物学家穿戴无菌洁净服。如果在无菌取样穿戴的 PPE 方面有争议，应在开始更衣前联系 ORS 总部。

a. Nonsterile Hair net and beard cover (if needed)

非无菌发网和胡须罩（如需要）

b. Sterile mask

无菌面罩

c. Shoe covers (non-sterile)

鞋套（非无菌）

d. Sterile Gloves

无菌手套

e. Sterile Disposable Coverall

一次性无菌衣

f. Sterile Hood

无菌罩

g. Sterile Goggles

无菌护目镜

h. Sterile Boot Covers

无菌鞋套

2. Sampling Equipment Controls 取样设备控制

a. Aseptic Technique Control: Place one (1) sterile Dacron or cotton swab into sterile water and place back into its sterile transport media solution. Place this negative control into a sterile Whirl- pak® bag.

Note: *This control may not be needed if the sponges and/or swabs are received pre-moistened. There is no surface contact for this control.*

无菌技术控制：将一个无菌涤纶或棉签放入无菌水中，并放回其无菌输送培养基溶液中，将此阴性对照放入无菌 Whirl- pak®袋中。

注：如果海绵和/或棉签在收到之前就已蘸湿则不需要该控制。该控制没有表面接触。

b. Swab/Sponge Sterility Control: Place an intact unus ed swab (or sponge) unit into a sterile Whirl-pak® bag.

棉签/海绵无菌控制：将完整的未使用的棉签（或海绵）单元放入无菌 Whirl-pak®袋中。

c. RODAC/Hycheck Sterility Control: Place an unused RODAC plate and/or Hycheck plate within a sterile Whirl-pak® bag.

RODAC/Hycheck 无菌控制：将未使用的 RODAC 碟和/或 Hycheck 碟放入无菌 Whirl- pak®袋中。

d. Whirl-pak® bag Sterility Control: Include one unopened Whirl- pak® bag as a closed control.

Whirl- pak®袋的无菌控制：放入一个未开封的 Whirl- pak®袋作为密封控制。

e. Glove Sterility Control: If the sampler uses ORS sterile gloves then have an intact unit

containing gloves placed into a sterile plastic bag and sent as a control.

手套无菌性控制：如果取样器使用 ORS 无菌手套，则将带有手套的完整装置放入无菌塑料袋中，并作为对照。

f. Sterile Equipment Control: Include any other sterile equipment used during EM sampling (i.e. sterile specimen cup, sterile media, etc.). (Coveralls, masks, and boot covers do not need to be included).

无菌设备控制：在环境监测取样过程中，加入所用的其它所有无菌设备（即无菌样品杯、无菌培养基等）。（无菌衣、面罩和鞋套不需要做对照样）

C. EM Sampling Procedure 环境监测取样程序

It is recommended that the investigative team bring equipment for both qualitative and quantitative EM methods. Qualitative methods utilizing sponges/ swabs are used for hard to reach areas. RODACs or Hychecks are employed for the quantitative method to enumerate microbes on open flat work surfaces. Each analyst is to perform the same role throughout the collection. (Ex. One analyst collects swabs and second analyst serves as an assistant.) See suggested sampling locations listed in section E of this procedure.

建议检查组携带定性和定量 EM 方法用取样设备。海绵/棉签的定性方法用于难取样区域。RODACs 或 Hychecks 用于定量方法，对开放式平坦工作表面的微生物计数。每个化验员在采样期间工作相同。（除了一个化验员采集擦拭样，第二个化验员作为助手外）。参见本程序第 E 部分所列的取样位置建议。

1. Disinfect gloved hands with a suitable sanitizing agent (i.e. sporicidal agent or sterile 70% alcohol).

用适合的消毒剂对套好手套的手消毒（即，杀孢子剂或 70%无菌乙醇）

a. Repeat this step between each EM sample.

每次 EM 取样之间重复该步骤

b. Allow gloves to air dry so no disinfectant is dripping from gloves.

让手套晾干，以免手套消毒剂滴下

c. Some swab/sponge sampling packages include a secondary set of sterile gloves. In these instances, the secondary glove can be aseptically used on top of the primary gloves to expedite the sampling process. The secondary gloves should not be removed, but additional gloves should be added and disinfected as needed.

有些棉签/海绵取样包装还有第二套无菌手套。这种情况下，第二副手套可以套在第一副外面直接用于无菌操作，以加快取样过程。不要摘下第二副手套，而应该增加手套并在必要时消毒。

d. When sampling an ISO 5 location.在 ISO 5 级区域取样时

i. Verify that the LFH/BSC certification is current.

核查 LFH/BSC 证书是否有效

ii. Allow LFH/BSC to run approximately 10 minutes before initiating sampling

让 LFH/BSC 运行约 10 分钟，然后开始取样

iii. Wipe down all outer sampling containers with a suitable sanitizing agent before placement in the LFH/BSC

用合适的消毒剂擦拭所有外部取样容器，然后放入 LFH/BSC

iv. Do not open sampling materials outside the LFH/BSC

不要在 LFH/BSC 外面打开取样材料

2. Qualitative Swabbing 定性擦拭取样

a. Open a sterile swab (or sponge). Dampen with wetting agent (sterile water, saline, or D/E neutralizing broth) and squeeze off excess by pressing against the inside of the container holding the wetting agent.

打开一个无菌棉签（或海棉），用润湿剂（无菌水、盐水或 D/E 中和肉汤）浸湿，按压装有湿润剂的容器挤出多余水分

b. Apply swab (or sponge with handle) to surface (or equipment) being monitored with **firm** application pressure.

将棉签（或带有手柄的海绵）用固定压力擦拭被监测表面（或设备）

c. When sampling (monitoring) flat surfaces allow the swab (or sponge with handle) to firmly rub an area of approximately 24 to 30 cm_2.

如果从平坦表面取样（监测）时，可将棉签（或带有手柄的海绵）固定摩擦约 24-30cm^2 的区域

d. Apply the swab (or sponge) within this contact area in both a horizontal and vertical direction for approximately 10 seconds.

在此接触区域内，用棉签（或海绵）沿水平和垂直方向擦拭约 10 秒钟

e. Place the swab (or sponge) back into the carrier container (if it came with one) and place into the additional sterile Whirl-pak® bag. Be sure to break off the handle portion of the sponge applicator stick.

将棉签（或海绵）重新放回装载容器（如果有）并放置在另一个 Whirl-pak®无菌袋中。确认已将海绵手柄折断去除。

3. Quantitative RODAC/Hycheck Sampling RODAC/Hycheck 碟定量取样

a. Carefully remove the lid of RODAC plate or loosen the cap on the Hycheck slide tube. Take care not to touch the agar surface.

Note: Examine *agar for contamination and or dehydration*

小心取下 RODAC 碟的盖子或松开 Hycheck 滑管的盖子。小心不要接触琼脂培养基表面

注：检查琼脂是否被污染或脱水

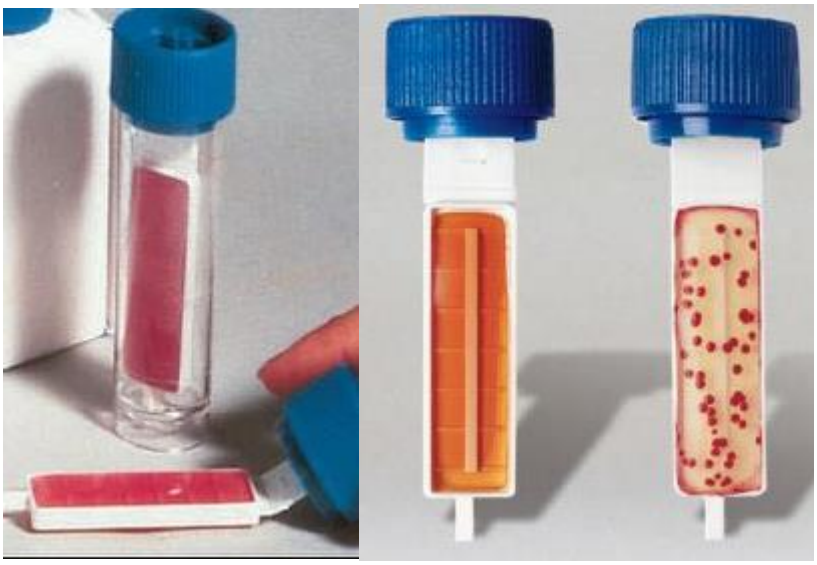
b. Gently but firmly touch the RODAC agar surface against the area being sampled, exert moderate, even, vertical pressure and then carefully replace lid. Avoid using rubbing motions of the plate at the sample site as this may break the agar.

将 RODAC 碟用于取样的琼脂表面轻轻但牢固地接触被取样的表面，垂直均匀按压，取下，小心地重新盖上盖子。避免碟表面与取样面摩擦，因为可能会使琼脂

裂开。

c. When using Hycheck press down on the spike to bend the paddle at the hinge line gently lowering the slide and press the agar to the surface with firm and even pressure. Repeat this step using the 2nd agar surface on an area adjacent to the initial test site. Replace slide in container and close tightly.

使用 Hycheck 取样条时，以尖头为着力点，手持盖按压，让取样条在折线处向下弯曲，垂直均匀地将培养基表面压在被取样表面。翻转，将反面培养基同样操作，从第一个取样点的邻近表面取样。将培养基条放回瓶中，盖紧。



（译注：原文未配图 Hycheck 取样条，因较少见，故译时配图，具体操作请自行脑补）

4. Sampling of Irregular Surfaces 不规则表面取样

A classified area may have an exposed irregular surface (i.e. particle board, wood, broken laminate, etc.) that requires sampling. In these situations, a colorless transport media such as letheen broth, saline or sterile water should be used to wet the sponge or swab prior to sampling. The microbiologist should avoid the use of D/E neutralizing broth which is a dark purple color and may discolor the irregular surface.

洁净区域可能会有暴露的不规则表面（即颗粒板，木头，断开的层流板等）需要取样。在此情形下，应在取样前使用无色转运培养基如 letheen 肉汤、沙林或无菌水湿润海绵或棉签。微生物学家应该避免使用 D/E 营养肉汤，该肉汤为深紫色，可能会使得不规则表面变色。

At the microbiologist's discretion, it may be determined that a RODAC plate should be used to sample the irregular surface. In this situation, a RODAC plate comprised of colorless agar such as tryptic soy agar would be appropriate. A colored agar such as D/E agar should be avoided.

根据微生物学家的判断，可能需要使用 RODAC 碟对不规则表面取样。在此情形下，适合使用无色琼脂 RODAC 碟如 TSA，应避免使用有颜色的琼脂如 D/E 琼脂。

5. Place the EM sample into a sterile Whirl-pak® bag and identify the bag immediately after.

将 EM 样品放入 Whirl-pak®无菌袋，然后立即对袋子进行标示。

6. Assign a consecutive number to the sample (i.e.1, 2, 3, etc.), in addition include the date, location of sample site (be specific) and your initials. Record in your inspectional record book the swab number and the location of the swab site.

   给样品连续编号（即 1、2、3 等），还要加上日期，取样位置（具体）和取样人首写字母。在检查记录本上记录拭子编号和取样位置。

7. After the sampling, the sampled area should be disinfected with sterile 70% alcohol. A sterile lint free wipe may be used to help remove sampling residue and expedite drying of the area.

   取完样品后，被取样的位置要用 70%无菌乙醇消毒。可使用无绒无菌抹布帮助除去取样残留物并让该区域快速干燥。

8. As soon as possible, place the double-bagged subs inside an insulated cooler, with pre-frozen gel packs to keep the samples cold, but not frozen, and transport/ship the sample to the servicing lab for analysis, within 24 hours of collection.

   尽快将装在双重袋中的样品放进装有预冷凝胶冰袋的隔热低温箱中保持样品低温，但不要冷冻。将样品在采集之后 24 小时内送至分析实验室。

9. Place the sample into a suitable mailing container to prevent crushing or physical damage to the swabs. The container should have some insulation capacity to prevent extreme temperature (freezing or excessive heat).

   将样品放在适当的邮递容器中，防止拭子受到撞击或物理损坏。容器应该有一定隔热能力，可防止极端温度（冷冻或高热）。

10. Contact with the receiving laboratory in advance regarding pending samples. This will ensure they have appropriate personnel and materials for sample set up within 48 hours of collection.

    应提前就准备运输的样品事宜联系接收实验室。这样可确保实验室在样品采集之后 48 小时内有样品检测所需的适当的人员和材料。

D. Recommended Environmental Monitoring Sites 建议环境监测点

When on an inspection, do not allow the firm to disinfect the work area prior to sampling. The facility and the equipment should be sampled during an in- process state as determined by the firm. The presence of disinfectant on the swab may reduce the microbial bioburden or increase inhibition during broth incubation. When collecting EM samples start in locations that are under the greatest control (ISO 5- HEPA filtered LFH/BSC or Isolator) and move to lesser controlled areas (areas outside the work station but still within the room).

在检查期间，不要让公司在取样前对工作区域消毒。应该在公司确定的工作状态下对设施和设备取样。拭子上有消毒剂可能会降低微生物负载，或增加肉汤培养期间的抑菌性。采集 EM 样品时，应从最高控制级别位置（ISO 5-HEPA 过滤 LFH/BSC 或隔离器）开始取样，然后取较低受控区域的样品（工作台以外区域但仍在房间内）。

1. Swab the frequently utilized surfaces within the controlled work station such as:

   擦拭受控工作站内频繁使用的表面，如

   a. Hooks for intravenous bags in the LFH or isolator

      LFH 或隔离器中的注射剂袋钩

b. Center of work surface

工作表面的中心

c. Fingertips & sleeves of Isolator gloves

隔离器手套的指尖和护袖

d. Peristaltic pumps

蠕动泵

e. Storage bins inside work station

工作站内的存贮箱

f. Shelving inside work station or any other stationary items

工作站内的棚架或任何其它固定装置

g. Equipment control panels including on/off switches of LFH/BSC

设备控制面板，包括 LFH/BSC 开关

h. Flexible plastic curtains used to separate multiple workstations

用于分隔多个工作台的易弯曲的塑料窗帘

2. Swab corner crevices inside the HEPA Filtered work station.

擦拭取样有 HEPA 过滤的工作站内的角落缝隙

3. Swab the handle, squeeze-trigger and nozzle of any bottle kept in the clean room or work station used for spraying (i.e., 70% alcohol, disinfectant solutions, etc.).

擦拭取样洁净写到或用于喷雾的（如 70%乙醇，消毒液等）工作站中保存的所有瓶子的手柄、挤 压扳机或喷嘴

4. Swab the underside of the chair in front of the work station. Specifically, on the front bottom rim where personnel would hold to pull up the chair.

擦拭取样工作台前面椅子下面，尤其是人员抓住拉动椅子的前底部边缘

5. Swab tables or benches within the controlled room where product container(s) or post sterilized product may be held outside of the HEPA filtered workstation.

擦拭取样控制室内但不在 HEPA 过滤工作台内，可能会放置产品容器或消毒后的产品的桌子或长凳

6. Swab the air in-take grid on each of the HEPA filtered work stations. Usually located on top of the unit holding the course filters.

擦拭取样每个 HEPA 过滤工作台上的进气格栅。通常在粗滤单元顶部。

7. Swab the exhaust (return) grid for the room air handling system that is connected to the facility air supply where the product manufacturing or compounding occurs.

擦拭取样连接到进行产品生产或配料的供气设施的房间空气处理系统的排气（回风）格栅。

8. Swab the light switch and door knob or handles leading into and out of the clean room and

the phone or intercom base.

擦拭取样灯开关和通往洁净间的门把手或手柄，以及电话或对讲机座

9. Swab any cardboard boxes, handles of plastic containers, tools (crimpers) or scissors, key pads on weighing scales, calculators or computers kept in the cleanrooms, keyboard, mouse and touch screen monitors.

   擦拭取样所有纸箱纸盒、塑料容器手柄、工具（卷曲器）或剪刀，键盘，氤和触屏监视器

10. Swab the exterior cuffs of the used lab coats worn by personnel during manufacturing or compounding. They may be hanging in the entry (ante) gowning room.

    擦拭取样取样生产或配料期间人员已用过的实验室外套的外袖口。它们可能挂在更衣间的入口（前厅）处。

11. Swab the bottom horizontal window sill within the clean room.

    擦拭取样洁净间内部的水平窗台

12. Swab any area under open or dislodged ceiling panels.

    擦拭取样开口或脱落的天花板下的区域

13. Sample areas of discoloration, stains or water and oil droplets.

    在变色、污迹或有水滴油滴处取样

14. Use your discretion to sample any other high-risk surface locations.

    自由判定在任何其它高风险表面位置取样

15. Photograph surfaces or equipment that display gross signs of contamination (i.e., particulate matter, fungi, discoloration, etc.). Try to include a distant picture of the targeted area along with a focused close-up. Be sure to sample this location, as well.

    对表面或显示污染迹象的设备（即颗粒物、真菌、变色等）拍照。尝试包括目标区域的远处图像以及聚集的近距离照片，确保在这个位置也有进行环境监测取样。

E. Analysis Preparation conducted by ORS laboratory ORS 实验室到要做的分析准备工作

1. All processing of swabs must be aseptically performed within a HEPA Filtered II Biological Safety Cabinet (BSC) or HEPA Filtered Laminar Flow Hood (LFH) with an air classification at the same rating or better than that from which the swab was collected.

   拭子处理必须在 HEPA 过滤 II 级生物安全柜（BSC）或 HEPA 过滤层流罩（LFH）内无菌操作，其中的空气级别应该等于或优于擦拭取样点。

2. All surfaces within the BSC or LFH must be thoroughly disinfected with a sporicidal disinfectant followed by filter sterile 70% ethanol or IPA prior to placing swabs under the hood and beginning analysis.

   将拭子放进层流罩开始分析之前，BSC 或 LFH 的所有表面必须用杀孢子剂和过滤器无菌 70%乙醇或 IPA 彻底消毒

3. In order to assure that the BSC/LFH and media are free of microbial contamination, standard open and closed controls used for sterility testing should be performed

concurrently with analysis.

为了确保 BSC/LFH 和培养基没有受微生物污染，无菌检测所用的标准打开和密闭控制样品要与测试样同步分析。

4. Don as appropriate the following PPE: 穿戴以下适当的 PPE
   a. Hair net 发网
   b. Disposable lab coat 一次性实验室外套
   c. Sterile disposable sleeves 一次性无菌袖套
   d. Sterile gloves 无菌手套
   e. Mask/beard covers 口罩/胡须罩
   f. Lab Safety Glasses 实验室安全眼镜
5. Sterile gloves must be decontaminated between the processing of each individual swab. Sterile gloves and sleeves should be discarded and replaced as needed.

   每个拭子处理中间必须对无菌手套除污染。无菌手套和袖套应该丢弃和更换（需要时）。
6. Sample Preparation 样品制备
   a. Examine swab containers for closure integrity to ensure tampering, leakage, or potential cross contamination has not occurred.

      检查拭子容器的密闭完整性，确保其未被损坏、无泄漏，亦没有潜在交叉污染。
   b. Carefully disinfect exterior of each swab container and place into the sanitized BSC/LFH and allow to air dry.

      仔细对每个拭子容器外部消毒，将其放入灭菌后的 BSC/LFH，晾干。
7. Media Selection 培养基选择
   a. Neutralizing additives (i.e. Tween/Polysorbate, Lecithin, etc.) are utilized to neutralize inhibitory disinfectant residues transferred to the swab during sampling that might inhibit microbial growth.

      利用中和添加剂（如吐温/聚山梨酯、卵磷脂等）中和在取样期间转移到拭子上的具有抑制性的残留消毒剂（这些消毒剂会抑制细菌生长）。
   b. For a broad-spectrum recovery of microorganisms, utilize a nutrient rich general-purpose media containing neutralizers (i.e. MLB, MLA, etc.).

      如要回收广谱微生物，应该使用含有中和剂（如 MLB、MLA）等的富含营养的通用培养基。
   c. When targeting fungal populations only, it is necessary to use an appropriate fungal media such as Sabouraud Dextrose or Malt Extract media. It is beneficial to use an antibiotic (i.e. Chlortetracycline) which will help to selectively inhibit bacterial growth and restrict the size and height of colonies of more rapidly growing molds.

      如果仅针对真菌种群，需要使用适当的真菌培养基，如沙氏葡萄糖琼脂培养基或

麦芽提取物培养基。使用抗生素（如金霉素）是有益有，这将有助于选择性找抽细菌生长并限制更快速生长的霉菌菌落的大小和高度。

d. TSA w/5% Sheep Blood Agar is beneficial for cultivating fastidious microorganisms.

TSA 5%绵羊血琼脂培养基有益于需要复杂营养的微生物的培养。

e. MacConkey Agar is used for the isolation and differentiation of Gram negative and enteric organisms.

麦康凯琼脂培养基用于革兰氏阴性菌和肠道微生物的分离和分化。

f. RODAC plates and Hycheck slides are used for the detection and quantification of microbiological contamination.

RODAC 碟和 Hycheck 条常用于微生物污染的检测和定量。

F. Analytical Procedure 分析方法

1. Approximately 100 ml of sterile media, MLB or other suitable media, should be aseptically added to each plastic bag containing a square sponge swab. Mix or swirl thoroughly.

取约 100ml 无菌培养基，MLB 或其它适当培养基，加至每个装有方形海绵拭子的塑料袋中，充分混合或旋转搅拌。

2. Approximately 10 ml of sterile media, MLB or other suitable media, should be aseptically added to a sterile container to which the swab and its transport media are added. Mix or swirl thoroughly.

取约 10ml 无菌培养基，MLB 或其它适当培养基，加入已放有拭子及其转运培养基的无菌容器中。充分混合或旋转搅拌。

3. All swabs are incubated at 25℃- 30℃ for at least 14 days to allow for the resuscitation of potentially stressed microbes.

所有拭子在 25℃- 30℃ 培养约 14 天，使其潜在被抑制的微生物得以恢复。

4. Hycheck slides and RODAC plates should promptly be incubated at 30-35℃ for 2 days and then 20 ℃ -25 ℃ for 5 days. Longer incubation times may be required when contaminants are suspected to be slow growing. Check plates daily for colony formation to minimize obscuring visualization of smaller colonies by over growth.

立即将 Hycheck 条和 RODAC 碟在 30-35℃ 充分培养 2 天，然后在 20 ℃ -25 ℃ 培养 5 天。如果容器可能导致生长缓慢，则可能需要更长时间。每天检查培养碟中菌群污染物情况，以减少较小菌落过分生长难以看清。

a. Count and record the number of colony forming units for RODAC plates. A representative of each colony type should be picked and re-streaked for purity and subsequently identified.

计算并记录 RODAC 碟上菌落数量，应挑选所有菌落类型重新纯化并鉴定。

b. Count the number of colonies on both sides of the paddle for the Hycheck slide. Report the colony counts for each side of the paddle and a representative of each all colony type should be picked and re-streaked for purity and subsequently identified.

计算 Hycheck 条两侧的菌落数，分别报告测试条两侧菌落计数，并挑选所有菌落

类型重新纯化和鉴定。

5. Check all swabs/sponges daily for turbidity and subculture for isolation as turbidity is observed. All subculturing must be performed under LFH or BSC. If container does not allow for turbidity observation to be made, then subculture all environmental samples between Day 5 and 7. All environmental samples will be subcultured following day 14 incubation regardless of previous subculturing.

   每天检查所有拭子/海绵的浊度，如发现混浊则分离并转种培养。所有转种培养必须在 LFH 或 BSC 中进行。如果容器无法观察到混浊，则将所有环境监测样本培养 5-7 天。无论之前是否转种培养，所有环境监测样本在 14 天培养之后均要进行转种培养。

6. Subculture all EM samples onto a combination of non-selective media (i.e. MLA, etc.) and selective/differential media (i.e. MacConkey agar, MEA, etc.). It is recommended to include TSA w/5% Sheep Blood Agar as one of the differential medias for subculturing. A minimum of two agars should be utilized, 1 must be a nonselective agar.

   转种所有 EM 样本至一个无选择性培养基（即 MLA 等）和选择性/差异性培养基（即麦康凯琼脂，MEA 等）的组合中。建议将含有 TSA 5%绵羊血的琼脂作为再培养用的差异性培养基的成分之一。最少要使用 2 种琼脂，其中一种必须是无选择性琼脂。

   a. Fungal media should be incubated at 20 ºto 25 ºfor 5 to 7 days. In some cases, extended incubation times may be appropriate, but generally not beyond 14 days unless there is a specific scientific justification.

      真菌培养基应该在 20 º- 25 ℃ 培养 5-7 天。有时需适当延长培养时间，但通常需要超过 14 天，有专门的科学依据者除外。

   b. All other culture media should be incubated at 30 ºto 35 ºfor 2 to 3 days.

      所有其它培养基应该在 30 º-35 ℃ 培养 2-3 天。

7. Re-incubate all cultured swabs until the full incubation (14 days) timeframe are met.

   再培养所有已培养的拭子直到满足全培养时间（14 天）。

8. Incubate all negative controls, such as system controls, media controls, under the same conditions as the sample.

   采用与样本相同的条件培养所有阴性控制样本，如系统控制样、培养基控制样。

9. Process and incubate submitted collector's controls under the same conditions as the sample.

   采用与样本相同的条件处理和培养所提交的采集者的控制样本。

10. Perform microbial characterization and identification following USP <1113> Microbial characterization, Identification and Strain Typing, as guidance. Typically, rapid identification systems (i.e. VITEK) are employed after primary screening and characterization are performed. Other identification platforms such as DNA sequencing may be beneficial if acceptable identification is not obtained through biochemical testing.

    按 USP<1113>微生物表征、鉴别和菌种分型要求对微生物进行表征和鉴别。一般来说，在初步筛查和表征之后会使用快速鉴别系统（即 VITEK）。如果通过生物化学测试无法得到可接受的鉴别结果，其它鉴别平台如 DNA 测序可能会有用处。

14. Chapter 10: Inspectional Guidance 第十章：检查指导

A. Microbiological Issues for Inspection of Pharmaceutical Laboratories 药物实验室检查的微生物问题

The following topics should be reviewed and evaluated during an inspection of a pharmaceutical microbiology laboratory.在对药物微生物实验室进行检查期间应查看和评估以下方面：

1. Finished product testing using USP or Non-compendia method

   使用 USP 或非药典方法检测成品

2. Review the original results for the following: sterility, bacterial endotoxin, microbiological examination of nonsterile products: specified microorganisms and enumeration, antimicrobial- effectiveness test, bioburden determination, water quality control testing.

   查看以下原始结果：无菌性、细菌内毒素、非无菌产品的微生物限度：控制菌和计数、抑菌效力测试、生物负载测定、水质控制检测。

3. Method Suitability (sterility), preparatory test (bacterial endotoxin), validation of method used for bioburden and water analysis

   方法适用性（无菌）：准备测试（细菌内毒素）、生物负载和水分析方法验证

4. Reagents and media- proper storage, expiration date, and growth promotion

   试剂和培养基适当存贮、有效期和促生长试验

5. Equipment and Instrumentation- (Steritest, manifold, automated/molecular identification system, Vitek, isolator and bio- decontamination system) review calibration, maintenance, validation (IQ, OQ, PQ)

   设备和仪器（Steritest 无菌检验系统、歧管试验、自动/分子识别系统、Vitek 系统等、隔离器和生物除污染系统），查看校验、维护、验证（IQ、OQ、PQ）

6. Sterility testing area design, operational procedures, monitoring, aseptic technique, gowning procedures, proper sample container disinfection, surface/air monitoring, HEPA filter certification, etc.

   无菌测试区设计、操作规程、监测、无菌技术、更衣程序、适当的样品容器消毒、表面监测、HEPA 过滤器证书等

7. Method description, modifications, and verification along with recording of sample results and appropriate review and evaluation by management

   方法描述、修改和确认，以及样品结果的记录和管理人员的适当审核与评价

8. Qualifications, training and identification of the personnel conducting each step of the analysis

   执行每一步分析操作人员的资格、培训和身份识别

9. Qualification and training of management to critically review data and interpret its significance

   管理人员批判性审核数据和解释其重要性的资格认证和培训

10. Microbial specifications set for raw material, finished product, water bioburden, and EM for analytical areas. Note: During pre-approval inspections questions about the appropriateness of finished product, raw material and in-process specifications should be discussed with the Center reviewer of the application.

    原料、成品、水生物负载和分析区域 EM 的微生物质量标准。注：在 PAI 检查期间，关于成品、原料和中控质量标准的适当性问题将与审评中心（CDER）的申报资料审评员进行讨论

11. Integrity and accuracy of the laboratory information management system (LIMS) for microbiology data entry, review and approval

    用于微生物数据输入、审核和批准选择、处理和生物指示剂储存的实验室有信息管理系统（LIMS）的完整性和准确性

12. Selection, handling, and storage of Biological Indicators (BIs)

    生物指示剂（BI）的选择、处理和存贮

13. Private (contract) testing laboratory quality agreements, data review, and associated problems; Have there been any changes in contract labs and why?

    私人（合同）检测实验室质量协议、数据审核和相关问题，合同实验室是否有变化，为什么？

14. Proper use and control of In-vitro diagnostic test kits, positive and negative controls, interpretation and reliability of results

    正确使用和控制体外诊断试剂盒，阳性和阴性对照，结果的解释和可靠性

15. Risk assessment of microbiological results for non-sterile products

    非无菌药品微生物结果的风险评估

16. A list of the entire laboratory's microbiological data deviations (Out of Specification (OOS)/ (Out of Limits (OOL) results) and Corrective Action Preventative Actions (CAPA) since last FDA on-site inspection

    自上次 FDA 现场检查以来整个实验室的微生物数据偏差清单（超标（OOS）/超限（OOL）结果）和纠正与预防措施（CAPA）清单

17. Stability Testing – sample storage conditions, missed sampling dates, etc.

    稳定性试验—样品存贮条件、错过取样日期等

B. Microbiological Issues for Inspection of Pharmaceutical Manufacturing Facilities 药品生产设施检查中的微生物问题

The following topics should be reviewed and evaluated by a microbiologist although some aspects will also be covered by the Consumer Safety Officer during the inspection.

虽然消费品安全官员亦要检查有些问题，但在检查期间，微生物专家应查看并评价以下问题

1. Product sterilization or bioburden reduction stage and validation- aseptic/filtration, steam, ETO, radiation, and other chemical processes

产品灭菌或生物负载降低阶段和验证—无菌/过渡、蒸汽、ETO、辐射和其它化学工艺

2. Depyrogenation- dry heat ovens for glass containers, wash/rinse for stoppers, adequacy of validation using spiked endotoxin, recovery studies before depryrogenation, filtration and column applications

   去热源—用于玻璃容器的烘箱、用于塞子的清洗/冲洗机、使用加标内毒素的充分性验证、去热原前的回收研究、过滤和色谱柱应用

3. Environmental monitoring- Types of equipment, calibration, operation and maintenance; surface, air, personnel and water; critical work areas for aseptically filled products (ISO 5, isolators, etc.); surface contact, surface sanitizer neutralizing media (e.g., TSA w/ Lecithin & Polysorbate 80), observe sampling technique, sample must represent dynamic/operational conditions, and trending/CAPA.

   环境监测—设备类型、校验、操作和维护；表面、空气、人员和水；无菌灌装产品的关键工作区域（ISO 5 级，隔离器等）；表面接触，表面灭菌剂中和培养基（例如，TSA w/卵磷脂和吐温 80）、观察取样技术、样品必须代表动态/运行条件，以及趋势分析/CAPA

4. Process simulation (media fills) studies - growth promotion testing to include when is it performed and which microorganisms are included, who is responsible for reading turbidity, volume adequacy in the product containers and accountability of product containers during and after incubation periods.

   工艺模拟（培养基灌装）研究—促生长试验，包括何时执行，有哪些微生物，培养基后谁负责读取浊度、产品容器中体积是否足够，产品容器的可靠性

5. Disinfection and sanitization- agents used (sporicidal?), preparation problems (over dilution); applicator (i.e., mop, spray. aerosol), time of exposure, areas of contact, supervision; residues, UV lights, water systems, filling equipment, work surfaces, process columns, verification and validation

   灭菌和消毒—所用试剂（杀孢子剂？），准备问题（过度稀释）；所用装置（即拖把、喷雾剂、气溶胶）、暴露时长、接触面积、监督、残留物、紫外线灯、水系统、灌装设备、工作表面、工艺用柱、确认与验证

6. Room design and Equipment- accessibility for disinfection and cleaning; aseptic filling critical area; HEPA filter certification and maintenance, air flow patterns/smoke studies, change evaluation/re- certification (rearranging cleanroom, adding equipment, HVAC, etc.), test during dynamic/operational conditions with maximum number of personnel in place, personnel and equipment flow, room differential pressure and temperature; adequacy of primary and secondary barriers

   房间设计和设备—消毒和清洁的可及性；无菌灌装关键区域；HEPA 过滤器认证和维护，气体流型/发烟试验、变更评估/重新认证（洁净区重排、增加设备、HVAC 等），动态/运行条件下最大人员数量情况下的测试，人流和设备流，房间压差和温度；初级和次级隔离装置的充分性

7. Water purification and delivery system: vulnerability of distillation process, RO, deionizers, cartridge filters, etc.; UV lights, dead legs, biofilm; corrosion (heat exchangers); waterborne microorganisms (nanobacteria) and endotoxin production; cold system problems,

disinfection problems

水的纯化和输送系统：蒸馏工艺、RO、去离子器、筒式过滤器等的易损性；紫外灯、死管、生物膜；腐蚀（热交换器）；水生微生物（纳米细菌）和内毒素的生长；冷却系统问题，消毒问题

8. Personnel- training procedures for aseptic technique, gowning procedures, cleaning and maintenance personnel training for ISO 5 room entry; glove and garment monitoring procedures

   人员—无菌技术的培训程序、更衣程序、清洁和维护的人员进入 ISO 5 级区域的培训；手套和洁净服监测程序

9. Product sampling: a representative sample is selected based on lot size as per USP <71> guidelines; quantity per container & units per batch, sample storage (time and temperature), sampling port sanitization or sterilization problems; be aware of skip lot testing on raw material.

   产品取样：按 USP<71>指南中的批量选择具有代表性的样品；每个包装&每批产品件数所取数量，样品存贮（时间和温度）、取样端口灭菌或消毒问题；了解原料的跳批检测

10. Maintenance records- determine dates, and location of equipment failures or out-of- service equipment that may have an impact on microbial contamination of product; looks for signs of roof leaks and water stains on ceiling panels, the degree of dirt and dust accumulation on supply and exhaust vents. Ask about new construction, plumbing or air handling system and the reason for change.

    维护记录—确定日期和可能影响微生物污染产品的设备故障和停止运行的位置；在天花板上寻找屋顶泄漏和水渍的迹象，供气和排气口上的污垢和灰尘积聚程度，询问新的建筑、管道或空气处理系统及其变化的原因

11. Compressed air systems—sterile process air, microbial particulate filtration (0.2 µm, hydrophobic), condensate causing blockage and microbial growth, routine point-of-use sampling, maintenance, filter integrity test

    压缩空气系统—无菌工艺空气，微生物颗粒过滤（0.2 µm，疏水），冷凝物引起堵塞和微生物生长，常规使用点取样、维护、过滤器完整性测试

C. Sample Data Review – When all results are negative 样品数据审核—所有结果为阴性时

The following should be reviewed when all the firm's sterility, nonsterile product and/or environmental monitoring test results indicate no microbial growth for validity and/or accuracy:

如果公司的所有无菌检查、非无菌产品/或环境监测结果显示无微生物生长，检查以下内容，确认其有效性和/或准确性：

1. Media- growth promotion; adequacy of the documented pH; low agar or broth volume in container, incubator temperature not set correctly; improper medium storage after QC (crystals from freezing, inadequate mixing prior to dispensing, agar plates dried out during incubation, etc.)

   培养基—促生长试验；所记录 pH 值的充分性；容器中琼脂或肉汤体积少，培养箱温度设置不正确；QC 之后培养基存贮不当（冷冻析晶，分装前混合不充分，培养期间琼脂平板干燥等）

2. Method suitability testing- validation for sterility; preparatory testing (BET). Appropriateness of neutralizers, product dilution, filtration; presence of toxic chemical contaminants in the water used to prepare the buffers. Not following method (excess of product added to broth during test but not during suitability testing, etc.)

   方法适用性试验—无菌性验证；准备测试（BET）。中和剂、产品稀释、过滤的适当性；在缓冲液制备用水含有毒化学污染物，不遵守方法（在检测中在肉汤中加入过量产品，但在方法适用性测试中则没有，等）

3. Improper tube or agar plate examination- check filter surface on submerged filter membrane (mold budding); surface film, light hazy growth in Thio broth, microbes settle to bottom of tube, pinpoint colonies (microaerophilic); medium not inoculated. Review disinfection process to assess the potential of antimicrobial residue being introduced during sample preparation; Review gas used for Isolator sterilization with medium inside chamber to assess the potential penetration into liquid broth and/or test product packaging.

   试管或琼脂平板检查不当—检查浸没滤膜的过滤器表面（霉菌生长）；表面膜，在硫代肉汤中轻微浑浊生长，沉降至管底的微生物，精确定位菌落（微好氧菌）；未接种培养基。检查消毒工艺，评估在样品制备过程中添加的潜在抑菌残留物；用放在隔离器内的培养基检查隔离器灭菌用气体，评估其是否可能穿透进入液体肉汤和/或测试产品包装中

4. Bioburden - Review the products bioburden for the presence of fastidious microorganisms which may require inclusion of special additives in the medium, such as halophilic contaminants in bicarbonate or high salt products need medium supplements with essential salts for survival. Where appropriate, the laboratory extend incubation time to improve the detection and recovery of fastidious microorganisms as part of their environmental monitoring program or part of investigation.

   生物负载—检查产品生物负载，看其中是否有可能需要在培养基中加入特殊添加剂的难养菌，例如碳酸氢盐或高盐产品中的嗜盐污染物需要含有必需盐的中等补充剂才能生存。适当时，实验室应该延长培养时间以提高难养菌的检出率和回收率，将其作为其环境监测项目的一部分，或调查的一部分。

5. Water test method- Evaluate the length of storage and storage temperature for collected samples prior to testing. Evaluate the type of collection bottles and their compatibility with the testing, for example endotoxin testing.

   水测试方法—评估所采集的样品在检测之前存贮的时长和存贮温度。用于内毒素检测时，要评估取样瓶的类型，及其与测试的相容性

6. Adherence to methods - Review data for adequacy of incubation time (for example 14 days for USP <71>), temperature (USP required temperatures) or appropriate media usage.

   是否遵守方法—审核数据，查看培养时间（例如 USP <71>要求的 14 天）、温度（USP 要求的温度）是否足够或培养其用途是否适当

7. Worksheets - Review possible falsification or incorrect entry onto worksheets or Laboratory LIM system.

   工作表—审核是否存在伪造或不正确录入记录或实验室 LIMS 系统的可能性

   Compare LIMs database entries to the analyst's laboratory notebook; Phrase the question

"When you get a positive test result…." not "If you get a positive test result..." Inspect the laboratory refrigerator or freezer for evidence of stored sample isolates. If they lyophilize sample isolates, ask to review the spread sheet data storage directly from the computer screen; hard copies could be obtained later. Review the Vitek or Micro Id isolate log book for all microorganisms identified and work backwards to the product lot number, filling rooms, equipment used, components or raw material used for that lot. This may allow you to find other lots associated with the contaminated lot.

将 LIMS 数据库录入的数据与化验员的实验室记录本进行比较，询问"你何时得到阳性检测结果……"，不要问"如果你得到一个阳性检测结果……"。检查实验室冰箱或冷藏柜，查找所存贮的样品分离物的证据。如果他们将样品分离物冻干，则要求直接在电脑屏幕上检查电子存贮数据表，可以随后要求查看纸质记录。检查 Vitek 或 Micro Id 分离物日志中所有鉴别出来的微生物，并追溯回产品批号、灌装间、所用设备、该批所用配料或原料。这可能会让你找到与受污染批有关的其它批次。

8. Personnel- review training records, personnel qualifications and experience. Observe analysts during sample collection, preparation, etc., and look for errors which may inhibit microbial recovery.

   人员—检查培训记录、人员资格和经验，在样品收集、制备等过程观察分析人员，寻找可能会抑制微生物回收的错误。

9. Laboratory - Visit the microbiology laboratory and look in the refrigerators, incubators, look at discarded plates from that day's work or request speciation log book and determine if microbial recovery has occurred, but not recorded on official worksheets or entered into LIMs. Determine where plates or other growth media are stored pending microbial identification. Review available plates or growth media to determine if the findings match those documented in the laboratory records.

   实验室—查看微生物实验室，查看当天工作的冰箱、培养箱、废弃的平板，或要求查看菌种鉴别登记本，确定是否曾发现微生物，却没有记录在正式的工作记录中或录入 LIMS。确定微生物鉴别时平板或其它培养基存放位置。检查现有平板或培养基，确定看到的东西与实验室记录中记录的内容是一致的。

D. Sample Data Review – When Microbial Growth Is Indicated 样品数据审核—显示有微生物生长时

When you encounter inspectional evidence that the firm has manufactured a microbiologically contaminated product, the few suggestions listed below should help you evaluate and proceed with this information. It is recommended that this evidence be communicated with the lead Consumer Safety Officer on the inspection so that appropriate communications with the responsible compliance office and the Center can occur. In addition, the Consumer Safety Officer can assist with the inspection of the manufacturing operations.

如果你查获证据证明公司生了的产品受到微生物污染，以下几点建议将有助于你对此类信息进行评估和处理。建议与负责检查的首席消费品安全官进行沟通，这样才能与负责合规办公室和中心进行合适的沟通。此外，消费品安全官可以在生产操作方面协助检查。

1. Documentation- Review and obtain copies of all records for lots indicating contamination; determine if there are other lots manufactured either before or after the "bad" lot(s). Review all associated activity and equipment related to the contaminated lot. There may be

common water sources, mixing tanks, piping, raw material, sterilizers, filters, etc. that may have been cross contaminated and transferred microbes to subsequent lots of products.

文件记录—审核并取得显示被污染的批次的所有记录副本；确定在“不良”批次前后是否生产了其它批次。审核与受污染批次相关的所有活动和设备。可能有普通水源、混合罐、管着、原料、灭菌器、过滤器等被交叉污染了，将微生物转给后续产品批次。

2. Validation - Review current established validation studies for product/component sterilization (disinfection for non-sterile products); has there been any equipment changed or modified; has there been a change of personnel or training; any new source material for equipment (vent filters, gaskets, filter manufacturer, etc.); any processing changes or room modifications, construction elsewhere in the facilities, etc.

   验证—审核当前已完成的产品/组份灭菌验证（非无菌产品是消毒）；是否有设备变更或改造；是否有人员或培训变更；设备是否有新来源材料（呼吸器，垫圈，过滤器生产商等）；是否有工艺变更或房间改造、设施内其它地方有建造工作等。

3. Environmental Monitoring (EM) - Review of environmental monitoring (EM) procedures and results for manufacturing and laboratory area- would the product contaminant grow on the EM medium; was the product contamination found in the manufacturing area; growth promotion potential of contaminant in other medium (i.e., TSB and Thio)

   环境监测（EM）--检查环境监测（EM）规程和生产与实验室区域的结果—产品污染物在 EM 培养基上是否生长；是否在生产区域发现产品污染；污染物在其它培养基上是否可能会促进生长（即 TSB 和硫基）。

4. Speciation- Record and copy the method of identification (i.e. API, Vitek, etc.). Determine if there were possible secondary contaminants that were not identified or recorded (check original plates, or isolates); verify accuracy of entry into the LIM system.

   物种形成—记录和复制鉴别方法（即 API，Vitek 等）。确定是否有可能未发现或记录二次污染（检查原始平板或分离物）；核查录入 LIMS 系统的数据准确性。

5. Source of potential contamination – Determine the potential source of contamination, which may include *Staphylococcus* (skin, insect, etc.); *Pseudomonas* (water, plants, etc.); yeast and mold (spores) (environmental)

   潜在污染来源—确定潜在污染来源，其中可能包括葡萄球菌（皮肤、昆虫等）；假单胞菌（水、植物等）；酵母和霉菌（孢子）（环境中）

6. Investigation Report – Review firm’s investigation report of microbiological out of specification (OOS) test results; source of contamination; where there corrective action(s) taken; if any repeat testing performed; batch disposition; does it include related lots and ancillary systems? Was the product rejected or released? If released ask why? Evaluate justification.

   调查报告—审核公司的微生物超标（OOS）结果调查报告；污染来源；是否采取了纠正措施；是否有重复检测；批处置；是否包括相关批次和辅助系统？产品拒收还是放行了？如果已放行要问为什么？评估其理由。

7. Product Test Failure - When a firm has a final product or in-process test result that indicates a failure (USP test failure, OOS, etc.) ask– Were the results due to laboratory error or a true

process contamination?

产品检测失败—如果一个公司有一个成品或中控检测结果显示失败（USP 检测失败，OOS 等），询问—结果是因为实验室错误还是真正的工艺污染？

8. Focus Areas - During the investigation, there are two areas for the review to focus: the manufacturing site and the laboratory that determined the OOS result. The investigations may run concurrently between manufacturing and the laboratory. For ease of review the questions listed in #9 (below) deal with laboratory data and those in Section E concern the manufacturing review. The laboratory section covers those questions that should be asked for a critical review of the microbiological data accumulated for sterility failures, non-sterile medical product failures, etc. Section E covers the manufacturing area and is divided into aseptic manufacturing (high risk) and terminally sterilized products (low risk).

   关注领域—在检查期间，有两个领域需要关注：生产现场和检出 OOS 结果的实验室。可以同步检查生产和实验室。为了便于审核，可按（以下）第 9 条中所列问题处理实验室数据，按 E 节所列审核生产模块。实验室部分包括了要询问对无菌检测失败、非无菌药品失败等累积微生物数据的批判性审核。E 节是针对生产领域，分为无菌生产（高风险）和终端灭菌（低风险）产品。

9. Laboratory Facility and Analytical Review 实验写到设施和分析审核

   a. Review QC records for proper/validated sterilization of all equipment and media used during the sterility test method: manifold/ Steritest; rinse fluid, culture media, canister kits, etc.

      审核 QC 记录，查看无菌检测中所用的所有设备和培养基是否均有经过恰当/经验证的灭菌：歧管/ Steritest 检验系统；冲洗液、培养基、灌装配套工具等

   b. Review the EM data acquired during sterility testing (i.e., settling plates, RODAC), simulation system controls, etc. What are the microbial species and their determined normal habitat (i.e., water, plants, people, etc.?)

      检查无菌试验（如沉降碟、RODAC）、模拟系统控制等过程中获得的 EM 数据。微生物是什么种属及其已知常规寄生地（如水、植物、人等？）

   c. Review training records and qualification of analysts performing the test; interview and/or observe analysts

      检查执行测试的化验员的培训记录和资格认证；对化验员进行询问和/或观察

   d. Review the qualification of the bio-clean room facilities or isolator chamber used during testing. Are there any leaks in the gloves, improper sanitization of product container before placement into work station or isolator? Has the isolator been evaluated for leaks?

      检查测试所用生物洁净室设施或隔离器的确认。手套是否有泄漏，产品容器在放入工作间或隔离器之前是否灭菌不当？隔离器是否经过泄漏评估？

   e. Review cleaning and sterilization requirements for reusable glassware and equipment. Poorly cleaned glassware will make sterilization of equipment more difficult and possibly shelter trapped microbes from the killing effect of the sterilant.

      检查重复使用的玻璃仪器和设备的清洁和灭菌要求。玻璃仪器清洁不良会导致设

备灭菌更加困难，并可能保护微生物使得灭菌剂无法有效杀灭

f. Review laboratory areas used for sub-culturing the sterility test medium onto enrichment plates. Cluttered work space or un- sanitized surfaces may cause plate contamination.

检查无菌测试培养基转移至增菌碟上培养时所用的实验室区域。工作空间杂乱或表面未灭菌可能会导致培养碟的污染。

g. Check the original plates used for isolation for possible pre- existing contamination (i.e. growth in non-streaked locations on the agar surface, subsurface growth)

检查分离时所用的原始碟，查看是否可能在使用前就被污染（即，在琼脂表面未接种位置生长，表面下生长）

h. Check to see if the medium had been recalled or has had past problems with contamination during manufacturing.

查看是否有培养基被召回或在生产期间曾发生过污染问题

i. It may be necessary to perform a genotype identification on the two isolates (product source and manufacturing area isolate) if they are the same species.

可能有必要在 2 份分离物上进行基因型鉴定（产品来源和生产区域分离物），查看其是否为同一种属

E. Manufacturing Facility Review 生产设施检查

1. Aseptically filled pharmaceuticals 无菌灌装药物

a. Check environmental monitoring (EM) data taken from production areas and the testing environment (i.e., S-T-A, settling plates, RODAC, etc.) for microbial contamination that matches the microbe isolated from the finished product sterility test

检查从生产区域和测试环境（即 STA，沉降碟，RODAC 等）采集的环境监测（EM）数据中微生物污染情况与从成品无菌检测中分离出来的微生物是否相符

If no microorganisms are detected, check the adequacy of the EM method used during manufacturing for proper sensitivity and applicability, for example

如果检出微生物，检查生产期间所用 EM 方法的充分性是否具备足够的灵敏度和适用性，例如

i. Are they using proper medium (i.e. non-selective medium)?

是否使用了合适的培养基（即，非选择性培养基）？

ii. Have they performed growth promotion?

是否进行了促生长试验？

iii. Did they use appropriate incubation time and temperatures?

是否使用了合适的培养时间和温度？

iv. Are they sampling in the appropriate room locations, during dynamic conditions, longest time between cleanings/sanitation and at frequency to assure reliability of the results?

是否在适当的房间位置取样，在动态条件下，清洁/消毒之间的最长时间和频

次，确保结果的可靠性？

v. If they recovered an anaerobic bacterium from the sterility test, (Thioglycollate broth) do they perform EM for anaerobic bacteria?

如果从无菌试验中回收到厌氧菌，是否对厌氧菌进行环境监测？

b. Have they performed a filter integrity test on the membrane used for the product sterilization? Review the products pre-filtration bioburden levels to assure that the concentration of bacteria in the bulk has not exceeded the membrane filtration capacity that was determined in their validation studies. Have they changed the source or model for the membrane filter cartridge used in the process?

是否对产品灭菌的膜进行了过滤完整性测试？检查产品过滤前的生物负载水平，确保细菌浓度没有超过在其验证研究中确定的膜过滤能力。是否工艺中使用的膜滤芯的来源或者型号？

c. Has the firm manipulated or excluded some of the data used in the final QC report? Perhaps raw data was averaged to bring the bioburden count below the alert or action levels. It can be helpful to request electronic Excel sheet version of data, to allow sorting (by frequency of organism, location, etc.) and trend analysis; hard copies can be requested later, if necessary.

公司是否操控或删除了最终 QC 报告中的一些数据？也许原始数据的平均值使得生物负载计数低于警戒或行动限。要求提供电子 EXCEL 表格版本的数据，允许进行分类（按微生物出现频次，位置等）和趋势分析，必要时可以稍后要求提供纸质副本

d. Review the media simulation studies. Did the microbial species recovered in past simulation studies match the microbe(s) recovered from the current product test failure?

检查培养基模拟研究。在以前的模拟研究中回收的微生物是否与目前产品测试失败中回收的微物相匹配？

e. Has there been a change or breach in the personnel barrier system to protect the product? Were there any interventions by maintenance or other staff personal during the manufacturing of the contaminated lots? Review glove/uniform monitoring results.

用于保护产品的人员屏障系统是否存在变化或破坏？在污染批次的生产过程中是否有维护人员或其它工作人员的干预？检查手套/工作服的监测结果。

f. Review the antimicrobial effectiveness challenge studies for the product if it multiple dose. Where there any changes to the container/closure component source or requirements?

检查产品抑菌效力挑战研究。容器/密闭组合的来源或要求有何变化？

g. Were there any changes to the disinfection procedure, reagents use, new personnel, application, equipment (mops, aerosols, etc.) etc.?

消毒程序、试剂使用、新人员、应用措施、设备（拖把、气雾剂等）是否有改变？

2. Terminally sterilized drug product 终端灭菌药品

a. Check autoclave validation studies for sterilization process- cold spot, heat penetration (challenged inside dry tubing, connectors/caps/stoppers, largest liquid volume, etc.),

changes in chamber load configuration, etc.

检查灭菌工艺中的高压灭菌器的验证研究—冷点、热穿透（在干燥试管内、连接器/盖子/塞子、最大液体体积等方面的挑战）、腔内装载参数变化等

b. Check maintenance records for house steam, records for autoclave repair, new plumbing

检查房间蒸汽维修记录，高压灭菌器维修记录，新的管道

c. Check Biological Indicator (BI) information- improper storage of BIs; changes in the culture (inoculum level and/or BI organism species) and incubation parameters

检查生物指示剂（BI）信息—生物指示剂储存不当；培养物（接种水平和/或 BI 微生物种属）和培养参数的变化

d. Evaluate the heat resistance characteristics of the microbial isolate found in the product during sterility testing and determine if it can survive during the process conditions, review product container/closure integrity data and possible recent supply source changes to vials or rubber stoppers; check possible post sterilization package integrity problems- mostly medical device issue.

评估在无菌测试过程中，在产品上发现的微生物分离物的耐热性，并确定其在生产过程中能否存活，检查产品容器/密闭器完整性数据以及西林瓶或胶塞最近可能的供应商变化；检查消毒后的包装完整性问题—医疗器械的主要问题

F. Inspectional Elements listed in the six (6) Inspectional Systems covered by the CP 7356.002 that cover ONLY Microbiological Issues 在 CP7356.002 的 6 个检查系统中所列的检查要素仅包括有微生物问题者

Some of the key coverage elements in five of the six Inspectional Systems listed in FDA Compliance Program Guidance Manual Program 7356.002 that relate to **microbiological** issues. Some examples are included for clarification. (Labeling system not included).

在 FDA 合规项目指导手册 7356.002 中列出的 6 个检查系统中有 5 个的关键要素与微生物问题有关。有些例子是为澄清问题（不包括标签系统）

1. Quality System 质量体系

a. Discrepancy and failure investigations related to manufacturing and testing: are they documented, evaluated and investigated in a timely manner; including corrective actions where appropriate.

与生产和测试有关的不符合和失败调查：是否及时记录、评估和调查；适当时包括纠正措施

b. Validation: status of required validation/revalidation (e.g., computer, manufacturing process, laboratory methods).

验证：验证/再验证所需的状态（例如，计算机，生产工艺，实验室方法）

c. Training/qualification of employees in quality control unit functions.

质量部门员工的培训/资格认定

2. Facilities and Equipment System 设施和设备系统

a. Facilities 设施

i. Cleaning and maintenance

清洁和维护

ii. Facility layout and air handling systems for prevention of cross-contamination (e.g. penicillin, beta-lactams, steroids, hormones, cytotoxics, etc.)

防止交叉的设施布局和空气处理系统（例如青霉素、β 内酰胺类、类固醇、性激素、细胞毒类等）

iii. Specifically, designed areas for the manufacturing operations performed by the firm to prevent contamination or mix-ups

为公司所执行的生产操作特意设计以防止污染或混淆的区域

iv. General air handling systems

一般空气处理系统

v. Lighting, potable water, washing and toilet facilities, sewage and refuse disposal

照明、饮用水、清洗和厕所设施，污水和废物处置

vi. Sanitation of the building, use of rodenticides, fungicides, insecticides, cleaning and sanitizing agents

厂房卫生，灭菌剂、杀菌剂、杀虫剂、清洁和消毒剂的使用

b. Equipment 设备

i. Adequacy of equipment design, size, and location

足够的设备设计、尺寸和位置

ii. Equipment surfaces should not be reactive, additive, or absorptive

设备表面不应该具有反应性、析出或吸收性

iii. Appropriate use of equipment operations substances, (lubricants, coolants, refrigerants, etc.) contacting products/containers/etc.

适当使用与产品/容器等接触的设备操作物质（润滑油、冷却剂、制剂剂等）

iv. Cleaning procedures and cleaning validation

清洁程序和清洁验证

v. Controls to prevent contamination, particularly with any pesticides or any other toxic materials, or other drug or non- drug chemicals

防止污染，尤其是被任何杀虫剂或任何其它毒性物质污染，或其它药品与非药品化学物质的污染的控制

vi. Qualification, calibration and maintenance of storage equipment, such as refrigerators and freezers for ensuring that standards, raw materials, reagents, etc. are stored at the proper temperatures

存贮设备确认、校正和维护，如确保对照品、物料、试剂等存贮在适当温度的冰箱和冷柜

3. Materials System 物料系统

a. Representative samples collected, tested or examined using appropriate means

使用恰当的方法采集、检测或检查代表性样品

b. Testing or validation of supplier's test results for components, containers and closures

组份、容器与密闭器供应商检测结果的测试或验证

c. Rejection of any component, container, closure not meeting acceptance requirements. Investigate the firm's procedures for verification of the source of components.

拒收所有不符合可接受标准的组份、容器和密闭器。检查公司用于核查组份来源的程序

d. Appropriate retesting/reexamination of components, containers, closures

对组份、容器、密闭器进行适当的复测/重新检查

e. Water and process gas supply, design, maintenance, validation and operation

水和工艺气体的供应、设计、维护、验证和运行

f. Containers and closures should not be additive, reactive, or absorptive to the drug product

容器和密闭器不应对药品有析出性、反应性或吸收性

g. Documented investigation into any unexpected discrepancy

记录对任何意外差异的调查

4. Production System 生产系统

a. Training/qualification of personnel

人员的培训/资格认证

b. Validation and verification of cleaning/sterilization/ depryrogenation of containers and closures

容器和密闭器的清洁/灭菌/除热原验证和确认

c. Established time limits for completion of phases of production (i.e. microbial growth potential of product)

建立生产步骤完成时限（即产品的微生物生长潜力）

d. Implementation and documentation of in-process controls, tests, and examinations (e.g., bioburden determination pH, adequacy of mixing)

实施并记录中控、检测和检查（例如生物负载检查 pH，混合充分性）

e. Justification and consistency of in-process specifications and drug product final specifications

中控标准和药品最终质量标准的论证和一致性

f. Prevention of objectionable microorganisms in non-sterile drug products

防止非无菌药品中的致病菌

g. Equipment cleaning and use logs

设备清洁和使用日志

h. Process validation, including validation and security of computerized or automated

processes (i.e. simulation studies)

工艺验证，包括计算机化或自动化工艺的验证和安全保护（即模拟研究）

i. documented investigation into any unexpected discrepancy

记录所有意外差异的调查

5. Laboratory Control System 实验室控制系统

a. Training/qualification of personnel

人员的培训/资格认证

b. Adequacy of staffing for laboratory operations

实验室操作人员数量是否足够

c. Adequacy of equipment and facility for intended use

设备和设施是否满足既定用途

d. Calibration and maintenance programs for analytical instruments and equipment

分析仪器设备的校正和维护程序

e. Validation and security of computerized or automated processes

计算机化或自动化流程的验证和安全保护

f. Reference standards; source, purity and assay, and tests to establish equivalency to current official reference standards as appropriate

对照品，来源，纯度和含量，建立其与现行官方对照品等同性的测试（适当时）

g. System suitability checks on chromatographic systems (e.g., GC or HPLC)

色谱系统的系统适用性检查（例如，GC 或 HPLC）

h. Specifications, standards, and representative sampling plans

质量标准，标准和代表性取样计划

i. Adherence to the written methods of analysis

遵守书面分析方法

j. Validation/verification of analytical methods

分析方法的验证/确认

k. Control system for implementing changes in laboratory operations

实验室变更实施控制系统

l. Required testing is performed on the correct samples

使用正确的样品执行所需检测

m. Documented investigation into any unexpected discrepancy

记录所有意外差异的调查

n. Complete analytical records from all tests and summaries of results

所有测试的完整分析记录和结果总结

o. Quality and retention of raw data (e.g., chromatograms and spectra)

原始数据的质量和保存（例如色谱图和光谱图）

p. Correlation of result summaries to raw data; presence of unused data

结果总结与原始数据的对应性，是否有未使用的数据

q. Adherence to an adequate Out of Specification (OOS) procedure which includes timely completion of the investigation

遵守充分的超标（OOS）程序，其中应包括有及时完成调查

r. Adequate reserve samples; documentation of reserve sample examination

留样充分，留样检查记录

s. Stability testing program, including demonstration of stability indicating capability of the test methods (i.e. container/closure, AET)

稳定性测试计划，包括检测方法的稳定性指示性能力的证明（即容器/密闭器，AET）

G. Sample Collection During an Establishment Inspection 现场检查期间样品采集

Samples of defective product constitute persuasive evidence that significant CGMP problems exist. Physical samples may be an integral part of a CGMP inspection where control deficiencies are observed. Physical samples should be correlated with observed control deficiencies. The investigator should consult Center and/or ORS Headquarters for guidance on quantity and type of samples (in-process or finished) to be collected. Documentary samples may be submitted when the documentation illustrates the deficiencies better than a physical sample. Divisions may elect to collect, but not analyze, physical samples, or to collect documentary samples to document CGMP deficiencies. Physical sample analysis is not necessary to document CGMP deficiencies.

缺陷产品的样品构成了有说服力的证据，表明存在显著的 CGMP 合规问题。实物样品可能是观察到的控制缺陷的 CGMP 检查组成部分。应该将实物样品与观察到的控制缺陷关联起来。检查员应该询问中心和/或 ORS 总部，获取所需采集的样品数量和类型（中间体或成品）的指导。当文档比实物样品更能说明缺陷时，可以提交文档样品。各地区可选择收集但不分析实物样品，或收集文档样品来记录 CGMP 缺陷。实物样品分析不需要记录 CGMP 缺陷。

When a large number of products have been produced under deficient controls, collect physical and/or documentary samples of products which have the greatest therapeutic significance, narrow range of toxicity, or low dosage strength. Include samples of products of minimal therapeutic significance only when they illustrate highly significant deficiencies.

如果在控制存在缺陷的情况下生产了大量产品，则应采集最有最大治疗意义、较小范围毒性，或最低剂量的产品的实物和/或文件样品。只有当最小治疗剂量的产品证明特别重大缺陷时，才需要采集其样品。

H. Appendix A: Literature and Resources 附录 A：文件和资源

A Comprehensive List of Only Microbiological Regulatory and Scientific Literature Resources

微生物法规和科学文献资源综合清单

The scope of this reading material will ONLY include microbiological scientific and regulatory publications or websites for conventional drugs, biologics and combinatorial products. Some references to medical device regulations will be included if relevant during an FDA investigation that covers microbiology.

本读物范围仅包括传统药物、生物制品和组合产品的微生物科学和法规出版物或网页。如果 FDA 检查期间会涉及一些医疗器械法规关于微生物的参考文献。

1. Legal requirements and regulations 法律法规要求
   a. CFR 210 & 211 "cGMPs for finished Pharmaceuticals"

      CFR 210 & 211，制剂 CGMP

   b. CFR 210 & 211 amended effective Dec 2008 (several changes that include microbiological requirements of aseptically filled products)

      CFR 210 & 211，2008 年 12 月修正（包括了无菌灌装产品的微生物要求的几个变更）

   c. CFR 610 General Biological Product standards" (Not covered during this review)

      CFR 610，一般生物制品标准（在本次审核中未包括）

   d. CFR 820 "Quality Systems Regulation (Devices, not covered)

      CFR 820，质量体系规定（器械，不包括）

   e. CFR 314.81(b)(3)(ii)- Applications for FDA approval to market a new drug (revised April 1, 2008) For submission of an alternate microbiological method with a comparability study

      FDA 批准上市新药申报要求（2008 年 4 月 1 日修订），采用可比性研究替代微生物方法的申报资料

   f. CFR 1271 Human cells, tissues, and cellular and tissue-based products

      人体细胞、组织和基于细胞与组织的产品

2. FDA Compliance Program Guidance Manuals FDA 合规程序指导手册
   a. FDA Compliance program Guidance Manual for FDA Staff: Drug Manufacturing Inspections program 7356.002

      FDA 员工合规程序指导手册：药品生产检查计划 7356.002

      During an inspection this program designated six (6) critical systems for review. They include: Quality System (always covered during an FDA inspection); Facilities &Equipment; Material; Production; Packaging and labeling; and Laboratory control systems.

      在检查期间，该计划指定要求检查 6 个关键系统。其中包括：质量系统（在 FDA 检查中都要包括）、设施&设备、物料、生产、包装和标签，以及实验室控制系统

   b. FDA Compliance program Guidance Manual for FDA Staff: Sterile Drug Process Inspections 7356.002A

FDA 员工合规程序指导手册：无菌工艺检查 7356.002A

The sections entitled "Inspectional" and "Analytical" and "Attachment A" are pertinent to an FDA microbiologist.

标题为"检查"和"分析"的章节与"附件 A"与 FDA 微生物学家有关。

3. Compliance Policy Guides 合规政策指导

   a. Sec. 100.550- Status and Responsibilities of Contract Sterilizers Engaged in the Sterilization of Drugs and Devices (CPG 7150.16) (Oct 2006)

      节 100.550 从事药品和器械灭菌的合同灭菌商的地位和职责(CPG 7150.16) (2006 年 10 月)

   b. Sec. 490.100 Process Validation Requirements for Drug Products and Active Pharmaceutical Ingredients Subject to Pre-Market Approval (CPG 7132c.08) (3/2004)

      节 490.100 需要上市批准的制剂和原料药的工艺验证要求(CPG 7132c.08) (3/2004)

   c. Manual of Policies and Procedures, CDER, MAPP 5040.1

      政策和程序手册，CDER，MAPP 5040.1

      Product Quality Microbiology Information in the Common Technical Document - Quality (CTD- Q)

      CTD 文件中的产品质量微生物信息—质量（CTD-Q）

   d. Compliance Policy Guidance for FDA Staff- Sec. 280.110 Microbiological Control Requirements in Licensed Anti-Human Globulin and Blood Grouping Reagents

      FDA 员工合规政策指导—节 280.110 已批准抗人体球蛋白和血型试剂的微生物控制要求

4. US Pharmacopeia (USP) Compendium 美国药典

   Review all relevant product monographs (not all have microbiological requirements); the following are regulatory chapters that contain enforceable microbiology requirements

   检查所有相关产品的各论（并不是所有产品都有微生物要求）；以下是含有强制微生物要求的法规章节

   General Notices and Requirements (page 1-13), Chart 10- Microbiology

   凡例和要求（页 1-13），第 10 节—微生物

   a. <1> Injections

      <1>注射剂

   b. <51> Antimicrobial Effectiveness test

      <51>抗菌效果测试

   c. <55> Biological Indicators-Resistance Performance tests

      <55>生物指示剂—抗性测试

   d. <60> Microbiological Examination of Nonsterile Products: Tests for Burkholderia cepacia complex

<60>非无菌药品的微生物检查：洋葱伯克霍尔德菌复合物检测

e. <61> Microbiological Examination of Nonsterile products: Microbial enumeration tests

<61>非无菌药品的微生物检查：微生物计数

f. <62> Microbiological Examination of Nonsterile products: Tests for Specified Microorganisms

<62>非无菌药品的微生物检查：

g. <63> Mycoplasma

<63>支原体

h. <71> Sterility Tests

<71>无菌检测

i. <81> Antibiotics-Microbial Assays

<81>抗生素微生物检测

j. <85> Bacterial Endotoxins Test

<85>细菌内毒素检测

k. <151> Pyrogen Test

<151>热原检测

l. <161> Transfusion and Infusion Assemblies and Similar Medical Devices

<161>输血和输液组件和类似医疗器械

m. <171> Vitamin B12 Activities Assay

<171>维生素 B12 活性测试

n. <797> Pharmaceutical Compounding-Sterile Preparations

<797>药品复方—无菌制剂

Dietary Supplements General Chapters Information

膳食补充剂通则信息

a. <2021> Microbial Enumeration Test-Nutritional and Dietary Supplements
<2021>微生物计数检测—营养和膳食补充剂

b. <2022> Microbiological Procedures for Absence of Specified Microorganisms-Nutritional and Dietary Supplements

<2022>检出控制菌的微生物方法—营养和膳食补充剂

c. <2023> Microbiological Attributes of Non-sterile Nutritional and Dietary Supplements

<2023>非无菌营养和膳食补充剂的微生物性质

USP Informational chapters<1000>through <1999> are informational chapters and provide guidance to the industry. These Informational chapters will help explain or expand on

scientific principles established in the regulatory chapters.

USP 的<1000>到<1999>章为非强制章节，为企业提供指导。这些参考章节帮助解释或扩展强制章节中所确立的科学原理。

a. <1035> Biological Indicators for Sterilization

<1035>灭菌生物指示剂

b. <1072> Disinfectants and antiseptics

<1072>消毒剂和灭菌剂

c. <1111> Microbiological Examination of Nonsterile Products: Acceptable Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use

<1111>非无菌产品的微生物检查：药物制剂和药用物质的可接受标准

d. <1112> Application of water activity Determination to Non-sterile pharmaceutical products

<1112>水活性检测在非无菌药品中的应用

e. <1113> Microbial Characterization, Identification, and Strain Typing

<1113>微生物表征、鉴别和菌分型

f. <1116> Microbiological evaluation of clean rooms and other controlled environments

<1116>洁净室和其它受控环境的微生物评估

g. <1117> Microbiological Best Laboratory Practices

<1117>微生物最佳实验室规范

h. <1207> Sterile Product Packaging—Integrity Evaluation

<1207>无菌产品包装—完整性评估

i. <1208> Sterility Testing –Validation of Isolator Systems

<1208>无菌性测试—隔离器系统的验证

j. <1209> Sterilization—Chemical and Physicochemical Indicators and Integrators

<1209>灭菌—化学和理化指示剂和积分器

k. <1211> Sterilization and Sterility Assurance of Compendial Articles

<1211>药典品种的灭菌和无菌保证

l. <1223> Validation of alternative microbiological methods

<1223>替代微生物方法的验证

m. <1227>Validation of Microbial Recovery from Pharmacopeial Articles

<1227>药典品种中回收微生物的验证

n. <1237> Virology Test Methods

<1237> 病毒学检测方法

5. AOAC international Includes chapters on disinfectants evaluation (i.e., Phenol coefficient

Methods; Hard surface carrier test methods; Use-Dilution Method)

AOAC 国际包括有消毒剂评估章节（即，苯酚系数法，硬表面载体测试法，应用稀释度法）

6. Association for the Advancement of Medical Instrumentation (AAMI)/ International Organization for Standardization (ISO).

   医疗器械促进协会（AAMI）/国际标准组织 （ISO）

   There are over fifty (50+) documents available through AAMI/ISO on the topic of "Sterilization Processes and Validation". These are internationally recognized standards and procedures recognized by FDA and Industry.

   AAMI/ISO 提供了超过 50 份以"灭菌工艺和验证"为主题的文件。这些都是国际和 FDA 与行业公认的标准和程序。

   AAMI/ISO Guidance documents- are available at the FDA intranet.

   AAMI/ISO 指南文件可在 FDA 内联网上找到。

7. FDA Inspection Guidance documents-FDA 检查指导文件

   Listed below are all the FDA guidance documents that contain only microbiological information relevant to inspection. In most cases these will be listed in the general website for CBER or CBER guidelines.

   以下均为只含有与检查有关的微生物信息的 FDA 指南文件。大多情况下，放在 CBER 通用信息或 CBER 指南网页中。

   a. Submission of Documentation in Applications for Parametric Release of Human and Veterinary Drug Products Terminally Sterilized by Moist Heat Processes (draft 8/2008)

      湿热工艺终端灭菌的人药和兽药参数放行申报资料中文件提交（2008 年 8 月，草案）

   b. Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products (draft guidance, 2/2008)

      基于生长快速微生物方法用于细胞和基因治疗产品无菌检测的验证（2008 年 2 月，草案）

   c. Guidance for Industry- Container and Closure system Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products (2/2008)

      行业指南—容器和密闭器系统完整性测试替代无菌检测，作为无菌药品的稳定性试验方案的一部分（2008 年 2 月）

   d. Guidance for Industry- Quality Systems Approach to Pharmaceutical cGMP Regulations (9/2006)

      行业指南—药物 CGMP 法规的质量体系方法（2006 年 9 月）

   e. Draft Guidance for Industry and FDA Staff- Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens (12/2005) Docket number 2005D-0434

      行业和 FDA 员工指南草案—基于核酸的体外诊断仪器用于微生物病原体检测（2005 年 12 月）文档号 2005D-0434

f. Guidance for Industry- Manufacturing Biological Drug Substances, Intermediates, or Products using Spore-forming Microorganisms (2/2005)

行业指南—采用形成孢子的微生物生产生物原液中间体，或产品（2005 年 2 月）

g. Sterile Drug Products Produced by Aseptic Processing —Current Good Manufacturing Practice, 9/2004

无菌工艺生产的无菌药品—CGMP，9/2004

h. Comparability Protocols - Chemistry, Manufacturing, and Controls Information, Required for industry interested in substituting an automated/Rapid Microbiological method in place of the USP compendial method cited in their original application (2/2003)

比较方案—研发、生产和检测信息，对有兴趣采用自动化/快速微生物方法取代在其初始申报资料中所引用的 USP 药典方法的企业的要求(2/2003)

i. Guidance for Industry- Sterility Requirement for Aqueous- Based Drug Products for Oral Inhalation—Small Entity Compliance Guide (11/2001)

行业指南—水性口服吸入药品的无菌要求—小微企业合规指南(11/2001)

j. Guide to Inspections of Quality Systems-Medical Device (8/1999)

质量体系检查指南—医疗器械(8/1999)

k. Guidance for Industry-Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product (1/1999)

行业指南—疫苗或相关产品的研发、生产和检测信息和设施描述信息内容与格式(1/1999)

l. Guide to Inspections of Lyophilization of Parenterals (10/18/97)

注射剂冻干检查指南(10/18/97)

m. Guide to Inspections of Cosmetic Product manufacturers (2/1995)

化妆品生产商检查指南(2/1995)

n. Guide to Inspections of Sterile Drug Substance Manufacturers, (7/1994)

无菌原料药生产商检查指南(7/1994)

o. Guide to Inspections of Topical Drug Products (7/1994)

外药品检查指南(7/1994)

p. Guidance for Industry- Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products (11/1994)

行业指南—人药和兽药申报资料中的灭菌工艺验证资料(11/1994)

q. Guideline for the manufacture of In Vitro Diagnostic Products (1/1994)

体外诊断产品生产指南(1/1994)

r. Guide to Inspections of Microbiological Pharmaceutical Quality Control Laboratories (7/1993)

微生物药学质量控制实验室检查指南(7/1993)

s. Guide to Inspections of High Purity Water Systems, (7/1993)

高纯水系统检查指南(7/1993)

t. Guide to Inspections of Validation of Cleaning Processes (7/1993)

清洁工艺验证检查指南(7/1993)

u. FDA Biotechnology Inspection Guide, Reference materials and training aids (11/1991)

FDA 生物技术检查指南，参考资料和培训辅助工具(11/1991)

v. Guidance for Industry, Pyrogen and Endotoxins Testing: Questions and Answers (June 2012)

行业指南—热原和内毒素测试问答（2012 年 6 月）

8. Inspectors technical guidance (ITG)-检查员技术指导（ITG）

The following are the ITGs that were written regarding microbiological issues.

以下为关于微生物问题的 ITG。

a. PYROGENS, STILL A DANGER (1/12/79 Number: 32)

热原，仍然很危险(1/12/79 编号: 32)

b. HEAT EXCHANGERS TO AVOID CONTAMINATION (7/31/79 Number: 34)

热交换器避免污染(7/31/79 编号: 34)

c. REVERSE OSMOSIS (10-21-80 Number: 36)

反渗透(10-21-80 编号: 36)

d. BACTERIAL ENDOTOXINS/PYROGENS (3/20/85 Number: 40)

细菌内毒素/热原(3/20/85 编号: 40)

e. LYOPHILIZATION OF PARENTERALS (4/18/86 Number: 43)

注射剂的冻干(4/18/86 编号: 43)

f. WATER FOR PHARMACEUTICAL USE (12/31/86 Number: 46)

制药用水(12/31/86 编号: 46)

g. MICROBIOLOGICAL CONTAMINATION OF EQUIPMENT GASKETS WITH PRODUCT CONTACT (12/31/86 Number: 48)

接触产品的设备垫片微生物污染(12/31/86 编号: 48)

9. Miscellaneous FDA Documents and References 其它 FDA 文件和参考文献

This list of references may not be entirely microbiology but very important none the less.
本参考文献清单并不全部都是微生物内容，但仍然非常重要。

a. FDA Inspectional Operational Manual

FDA 检查手册

b. FDA Warning Letters and Responses

FDA 警告信和回复

c. FDA Bacteriological Analytical Manual (BAM) (1/2001)

FDA 细菌学分析手册（BAM）（2001 年 1 月）

10. Important Government and International organizations: 重要政府机构和国际组织

a. National Institute of Health (www.nih.gov) 国立卫生研究院

b. Center for Disease Control and Prevention (www.cdc.gov) 疾控中心

c. CDC report on environmental monitoring CDC 环境监测报告

(http://www.cdc.gov/ncidod/dhqp/gl_environinfection.html)

d. World Health Organization (www.who.org) International pharmaceutical regulations along with monitoring of disease outbreaks around the globe may be important if assigned to work in a high-risk area. 世界卫生组织，如果是指派到高风险地区工作，则国际药品法规与全球疾病爆发监测可能很重要

11. Industry Technical references-行业技术参考文献

Parenteral Drug Association (PDA) Technical Reports- Although the scientific recommendations in these technical reports are not enforceable by FDA, they do contain industry current manufacturing practices and scientifically sound principles that support regulatory concerns. The following is a selection of PDA technical reports that may be useful:

注射剂协会（PDA）技术报告—虽然这些技术报告中的科学建议并非 FDA 强制要求，但其中确实有很多现行的行业生产实践，和支持监管关切的科学合理的原则。以下是可能有用的一些 PDA 技术报告。

Report No. 报告编号	Title	标题	Date
1	Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control	湿热灭菌工艺验证：灭菌周期设计、开发、确认和持续控制	July 2007
3	Validation of Dry Heat Processes Used for Sterilization and Depyrogenation	干热灭菌和除热原工艺的验证	1981
4	Design Concepts for the Validation of Water-for-Injection Systems	注射用水系统的验证设计理念	1983
5	Sterile Pharmaceutical Packaging: Compatibility and Stability	无菌药品包装：相容性和稳定性	1984
7	Depyrogenation	除热原	1985
11	Sterilization of Parenterals by Gamma Radiation	注射剂伽玛辐射灭菌	1988
13	Fundamentals of an Environmental Monitoring Program	环境监测计划基础	1990(Revised 2001)
15	Industrial Perspective on Validation of Tangential Flow Filtration in Bio-pharmaceutical Application	生物制药应用切向中流过滤验证的工业前景	1992
20	Report on Survey of Current Industry Gowning Practices	当前行业更衣实践调查报告	1990
21	Bioburden Recovery Validation	生物负载回收率验证	1990

Report No. 报告编号	Title	标题	Date
22	Process Simulation Testing for Aseptically Filled Products	无菌灌装产品的工艺模拟测试	2011
23	Industry Survey on Current Sterile Filtration Practices	当前无菌过滤实践的行业调查	1996
26	Sterilizing Filtration of Liquids	液体除菌过滤	2008
28	Process Simulation Testing for Sterile Bulk Pharmaceutical Chemicals	无菌散装药用化学品的工艺模拟测试	2006 (revised)
29	Points to Consider for Cleaning Validation	清洁验证考量要点	2012
30	Parametric Release of Pharmaceuticals Terminally Sterilized by Moist Heat	湿热灭菌的终端灭菌药品的参数放行	1999
33	Evaluation, Validation and Implementation of New Microbiological Testing Methods	新微生物检测方法的评估、验证和实施	2000
34	Design and Validation of Isolate Systems for the Manufacturing and Testing of Health Care Products	医疗产品生产和检测用隔离系统的设计和验证	2001
35	A Proposed Training Model for the Microbiological Function in the Pharmaceutical Industry	制药行业中微生物人员培训模式建议	2001
36	Current Practices in the Validation of Aseptic Processing -- 2001	无菌工艺验证的现行做法-2001	2002
40	Sterilization Filtration of Gases	气体除菌过滤	2005
41	Virus Filtration	病毒过滤	2005
45	Filtration of Liquids using Cellulose-based depth filter	使用纤维基深层过滤的液体过滤	2008
57	Analytical Method Validation and Transfer for Biotechnology products	生物技术产品的分析方法验证和转移	2012
61	Steam in place	在线蒸汽灭菌	2013

12. Books and Commercial Trade reports: 书籍和商贸报告

a. ASM, Manual of Clinical Microbiology;

ASM，临床微生物学手册

b. Disinfection, Sterilization, and Preservation, by S Block; Bergey's manual systematic Bacteriology

消毒、灭菌和防腐，S Block；伯吉氏系统细菌学手册

c. Remington's Pharmaceutical Sciences

雷明登氏药学大全

d. F-D-C Monthly Reports

F-D-C 月报

Excellent summary of conferences, FDA regulation changes, Key Industry personnel and often a list of the most recent Product Recalls and regulatory actions by FDA. Need to sign up for email membership. Instructions for membership enrollment are available at FDA website below.

优秀的会议总结，FDA 法规变化，关键行业人物和最新药品召回及 FDA 采取的强制措施清单。需要注册电子邮件成为会员。会员注册说明在以下 FDA 网页可

以找到。

(http://inside.fda.gov/Library/ElectronicResourcesWebLERN/Alphabeticallist/index.htm)

The "Gold Sheet"- Pharmaceutical & Biotechnology Quality Control The "Pink Sheet"- Prescription Pharmaceuticals and Biotechnology The "Gray Sheet"- Medical Devices Diagnostics & Instrumentation The "Silver Sheet"- Medical Device Quality Control reports

"金色表格"—药学和生物技术质量控制，"粉色表格"—处方药和生物技术，"灰色表格"—医疗器械诊断&仪器使用，"银色表格"—医疗器械质量控制报告

13. Free Trade publications available on line- 在线免费贸易出版物

Pharmaceutical Technology (www.pharmtech.com)

药物技术

Controlled Environments (www.cemag.us)

受控环境

American Pharmaceutical Review (www.americanpharmaceuticalreview.com)

美国药物评论

International BioPharm (www.biopharminternational.com/)

国际生物药品

14. Professional memberships 专业会员

These organizations have available searchable references.

以下组织有参考文献可供搜索

International Society of Pharmaceutical Engineers (www.ispe.org)

国际制药工程师协会

American Society for Microbiology (www.asm.org)

美国微生物协会

Parenteral Drug Association (www.pda.org)

美国注射剂协会

15. Glossary/Definitions 术语/定义

None 无

16. Records 记录

None 无

17. Supporting Documents 支持性文件

None 无

18. Document History 文件历史

Revision 版本号#	Status* 状态 (D, I, R)	Date 日期	Author Name and Title 作者姓名与职务	Approving Official Name and Title 批准官员姓名与职务
1.1	I	4/25/2014	Angele C. Smith, Microbiologist	
1.2	R	3/30/2015	Angele C. Smith, Microbiologist	
1.3	R	6/7/2016	Angele C. Smith, Microbiologist	Selen Stromgren, Deputy Director MPTSS
1.4	R	1/31/2018	Angele C. Smith, Microbiologist	George Salem, Staff Director OMPSLO
02	R	7/27/2020	Angele C. Smith, Microbiologist	Bryan Gamble, Acting Deputy Associate Director OMPSLO

* - D: Draft 草案, I: Initial 初版, R: Revision 修订本

19. Change History 修订历史

Revision 版本号#	Change 修订
1.1	ii.- updated Chapter 3. A. 2. a. iv. – revised Chapter 8. D. 1st paragraph – revised Chapter 10. H. 5. – author information updated
	ii.- 更新 第 3 章. A. 2. a. iv. – 修订 第 8 章. D. 第一段 – 修订 第 10 章. H. 5. – 作者信息更新
1.2	Chapter 3, D. 1. a. - revised Chapter 7 – Antibiotic Potency Assay (inserted)
	第 3 章, D. 1. a. – 修订 第 7 章– 抗生素效价含量（插入）
1.3	Chapter 9, A. 1. i – revised Chapter 9, B, B.1.a, C.4, C.7, C.9, C.10, E.1, E.2 – revised Chapter 10, A.6 – removed Appendix A, removed links
	第 9 章, A. 1. i – 修订 第 9 章, B, B.1.a, C.4, C.7, C.9, C.10, E.1, E.2 – 修订 第 10 章, A.6 – 删除附录 A，删除链接
1.4	Updated highlighted (grey) in document
	在文件中更新突出显示（灰色）
1.5	Updated to new template Updated author information Updated to 508 Compliance Updated Chapter 2 – revised to match new USP method Updated Chapter 3 – revised for clarification Updated Chapter 4 – revised for clarification Updated Chapter 5 – revised for clarification; added two USP references Updated Chapter 9 – revised for clarification Updated Chapter 10 – revised for clarification
	更新至新模板 更新作者信息 更新至 508 合规 更新第 2 章—修订使其符合新的 USP 方法 更新第 3 章—修订使其清晰 更新第 4 章—修订使其清晰

Revision 版本号#	Change 修订
	更新第 5 章—修订使其清晰，增加 2 个 USP 参考文献 更新第 9 章—修订使其清晰 更新第 10 章—修订使其清晰

（译注：按 18.文件历史，1.4 版之后貌似应该是 02 版，而不是 1.5 版）

20. Attachments 附件

None 无