Technical Report No. 33 (Revised 2013)

Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods

2013

Parenteral Drug Association

PDA Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods <u>Technical Report Team</u>

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ISBN: 978-0-939459-63-6

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1.0 Introduction 概述

Microbiological testing plays an ever-increasing role in the pharmaceutical laboratory .In response to this, a variety of alternative and rapid methodologies that automate existing methods, make use of surrogate markers, or are based on wholly new technologies have emerged in recent years. These alternative methodologies offer significant improvements in terms of speed, accuracy, precision, and specificity over traditional, or classical, microbiology test methodologies.
微生物检测在制药实验室中的作用日趋重要。对此，在最近几年已经出现了通过自动化现有的方法，利用的替代标记物，或是基于全新的技术的各种非传统或快速检测方法。这些非传统方法在检测速度，精度，准确性和专属性方面比传统的或经典的微生物检验方法显着改善。

The majority of testing performed today relies on century-old, conventional methods based on the Recovery and growth of microorganisms using solid or liquid microbiological growth media .This is true in part because these methods can be appropriate for their intended use and have a long history of application in both industrial and clinical settings .They often are limited, however, by slow microbial growth rates ,the unintended selectivity of microbiological culture, and the inherent variability of microorganisms in their response to culture methods. In spite of the limitations of classical culture methods, acceptance of alternative and potentially superior methods has only started to gain momentum with in the pharmaceutical, biotechnology, and medical device industries. The Technical Report Team believes that the lack of clear guidance both on how to demonstrate the equivalence of alternative/rapid methods to existing methods in a manner acceptable to regulatory agencies and on how to validate the equipment associated with alternative/rapid methods is one impediment to the widespread adoption of these methods.
现在使用的绝大多数测试还依赖于百年前的，基于使用固态或液态微生物生长介质的微生物复原和生长的方法。在某种程度上这是真实的，因为这些方法非常有效并且在工业和临床的应用上有很长历史。但这常常受到缓慢的微生物增长速度，微生物培养的无选择性以及不同培养方法下的微生物固有可变性等因素的限制。尽管存在当前培养方法的局限性，仅仅在制药、生物和医疗器械行业开始接受新的具有潜在优势的方法。技术报告小组认为，缺少证明非传统/快速方法与监管机构接受的当前方法等效以及如何验证新方法的设备的清晰指南，阻碍了这些方法的广泛采用。

Considerable guidance can be found regarding the validation of chemical methods. Examples include USP General Informational Chapter<1225>Validation of Compendia Methods and the International Conference on Harmonization (ICH) guideline Validation of Analytical Methods (1,2).These publications provide very specific instruction regarding the demonstration of alternative analytical chemistry methods and their equivalence to existing methods. Chapters introduced by the compendia, including USP General Information Chapter <1223> Validation of Alternative Microbiological Methods, and Ph. Eur. Informational Chapter5.1.6 Alternative Methods for Control of Microbiological Quality, provide guidance on the steps needed to validate an alternative microbiological method (3,4). However, additional guidance is needed ,as an understandable and holistic approach to the qualification and implementation of novel alternate microbiological methods, including rapid microbiological methods, still does not exist that would satisfy all regulatory agencies.
可以找到关于化学方法验证的重要指南，例如USP一般信息章节<1225>中药典方法验证和ICH公布的分析方法验证（1，2）。这些出版文献为证明新化学分析方法与现行方法的等效性验证提供了具体说明。在美国药典通用信息章＜1223＞微生物替代法的验证，和欧洲药典的第5.1.6章节-控制微

生物质量等章节对非传统微生物方法的验证步骤提供指导（3,4）。因为目前还不存在能够满足政府监管部门要求的可理解并能全面实施的用于确认和执行新的可选择的微生物学方法，包括快速微生物方法，因此需要有更多的指南。

The original PDA Technical Report No.33 was published in 2000 to fill this void. Industry, compendia, and regulatory developments since then, however, have necessitated this update to the guidance .The team believes that this revision is timely and will provide additional guidance to assist with the evaluation, validation, and implementation of the alternative microbiological methods.
自2000年的PDA第33号技术报告发表填补了空白。然而从那时起，工业、出版物、监管的发展上要求必须更新指南。本小组认为，本次修订是及时的，这将有助于非传统微生物学方法的评估，验证和实施提供额外的指导。

This Technical Report was developed as a collaborative effort amongst representatives from alternative method suppliers and vendors, the pharmaceutical, biopharmaceutical and medical device industries, and regulatory agencies. It is intended to provide a comprehensive approach to the introduction of alternative microbiology methods in a government-regulated environment. It is anticipated that by providing agreed upon performance standards, the development, qualification and implementation of alternative microbiological methods will be greatly accelerated.
本技术文件是由新方法供应商，药品和医疗设备行业，标准组织和监管机构的代表共同努力下编写的。其目的是，在政府监管环境下，提供一个引入新的微生物学方法的通用的方法。期望通过提供统一的性能标准，能大大加速新的微生物方法的开发、验证和实施。

1.1 Scope and Purpose of the Technical Report技术报告的范围和目的

This Technical Report is intended to provide guidance for the successful evaluation, validation, and implementation of alternative and rapid microbiological methods needed by the pharmaceutical, biotechnology and medical device industries to assure product quality. Applications for these methods include, but are not limited to, the testing of microbial limits, sterility, and antimicrobial effectiveness; microbiological monitoring of clean rooms and other controlled environments and water for pharmaceutical purposes; microbial characterization and identification; and microbiological in-process control testing.
本技术报告的目的是为那些被制药、生物技术、医疗器械保证产品质量所需要的新的或快速微生物检测方法的成功评估、验证和实施提供指南。这些方法的应用包括但不限于微生物限度测试、无菌测试、抗菌效力测试，洁净室和其他受控环境的微生物监测、制药用水监测，以及微生物的识别、鉴定和过程控制检测。

The Technical Report Team authored this document for microbiologists responsible for the validation of the microbiological test methods used in the routine microbiology testing laboratory; the document also should be of interest to suppliers of testing equipment, microbiology managers and supervisors, validation specialists, quality control personnel responsible for the approval of validation protocols and the release of product and regulatory agencies.
这份报告的目标对象是常规微生物测试实验室中负责对使用的微生物测试方法进行验证的微生物学人员。本文件对测试设备供应商、微生物经理和主管、验证专家、负责批准验证方案和产品放行的质量控制人员以及监管机构应该会有所帮助。

1.2 Overview of Technical Report Structure技术报告的结构概述

This Technical Report was written to establish industry-wide criteria on what constitutes an acceptable alternative/rapid microbiology test to the compendial or classical method and how to prove it to the satisfaction of quality organizations and regulatory agencies.

此文件是为了建立行业范围内的标准,为药典和和经典的微生物测试提供一个可接受的可选择的货或快速检测的方法，并指导如何证明其达到质量管理组织和机构要求。

The Technical Report Team arranged the guidance in such a way as to describe the technical, quality, regulatory, and business attributes of alternative and rapid microbiological methods, the scientific basis for available technologies, and an efficient process for the validation and implementation of such methods.

技术报告的团队对可选择的或快速的微生物检测方法的技术、质量、管理以及商业属性的描述是以可用技术的科学性和这些方法验证、实施过程的有效性为依据的。

2.0 Glossary of Terms 术语表

Accuracy准确性

The closeness of the actual test results obtained by the new method to the actual test results obtained by the existing method.

用新方法和传统方法实际测得值之间的接近程度。

Alternative or Rapid Microbiological Method(RMM)可选择的或快速微生物检测方法

A novel, modern and/or fast microbiological testing method that is different from a classical or traditional growth-based method, such as agar-plate counting or recovery in liquid broth media. The alternative or rapid method may utilize instrumentation and software to manage the testing and resulting data, and may provide quantitative, qualitative and/or microbial identification test results. Automated technologies that utilize classical growth-based methods may also be designated as being novel, modern or rapid, based on their scientific principle and approach to microbial detection. The terms alternative, rapid microbiological method, rapid method and the acronym RMM are used interchangeably within this technical report.

一个新的，现代的和/或快速的微生物检测的方法，不同于经典的或传统的培养基培养生长的方法，如琼脂平板计数或在液体介质恢复。可选择的或快速的方法，可以利用仪器和软件管理测试所产生的数据，并可提供定量，定性和/或微生物鉴定试验结果。利用经典的培养基为基础的自动化技术方法也可以被指定为可选择的、现代的和快速的、基于他们的科学原理和微生物检测方法。将术语新方法，快速微生物的方法，快速法和缩写RMM在本技术报告中交替使用。

Equivalence/Comparative Testing对等/对比试验

A measure of how similar the test results are when compared with the existing method.

与现有的方法相比，以确定测量的结果与现有方法的相似程度。

Exclusivity排外性

The capacity of an assay not to detect microorganisms closely related to a target microorganism.

检测不到与目标微生物相近（相似）的微生物的能力。

False Negative假阴性
A test result that is erroneously classified in a negative category (e.g. the absence of a viable Microbial detection result when viable microorganisms are present).
错误的阴性检测结果（例如，当存在活的微生物的时候，没有检测到存在的存活微生物）。

False Positive假阳性
A test result that is erroneously classified in a positive category (e.g. a viable microbial detection result when viable microorganisms are not present).
错误的阳性检测结果（例如，在一个没有活的微生物条件下检测到活的微生物的检测结果）。

Inclusivity包容性
The ability of an assay to detect a target microorganism.
评价一个分析方法对目标微生物的检测能力。

Intermediate Precision中间精密度
The precision within the same laboratory using different analysts, equipment, reagents and/or on different days.
是指在相同实验室中用、不同的化验员、不同的仪器、不同批的反应剂和/或不同的时间内，获取的测试结果的精度。

Limit of Detection(LOD)检测限
The lowest concentration of microorganisms in a test sample that can be detected, but not necessarily quantified, under the stated experimental conditions.
在规定的试验条件下，可以检测到的测试样品中的最低微生物浓度，但这个值不一定是量化的。

Limit of Quantification(LOQ)定量限
The lowest number of microorganisms in a test sample that can be enumerated with acceptable accuracy and precision under the stated experimental conditions.
在规定的条件下，样品中能被检测到最小的符合精密度和准确度的微生物数量。

Linearity线性
The ability to elicit results that are proportional to the concentration of microorganisms present in the sample within a given range, where accuracy and precision are demonstrated.
指检测结果与样品中微生物浓度的比例是否在给定范围内的能力，这里的精度和准确性应该经过证明。

Precision精度
The degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of the same suspension of microorganisms and using different suspensions across the range of the test .Also known as repeatability.
指在同一个微生物样品的悬浮液并使用检测范围内的的不同的悬浮液来重复取样检测的结果的一致程度。也称为重复性。

Range范围

The interval between the upper and lower levels of microorganisms that have been demonstrated to be determined with accuracy, precision and linearity.

用于描述在已被证明的精度、准确性和线性的条件下，检测上限和下限之间的一个数量范围。

Reproducibility重现性

The precision between laboratories, for example, through collaborative studies.

是指在不同实验室，例如通过合作研究，得到的检测精度。

Robustness鲁棒性

A method's capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

是指方法参数出现了小的但是考虑到的变化时，测量能力不受影响。可以为检测方法的日常检测的可靠性提供依据。

Ruggedness耐用性

The degree of intermediate precision or reproducibility of test results obtained by assessing the same samples under a variety of normal test conditions.

在各种常规试验条件下，对同一样品的检测结果的中间精密度和重现性的程度。

Specificity专属性

The ability to detect a range of microorganisms, which demonstrate that the method is fit for its intended use.

表明该方法适用于预期用途，可以检测一系列微生物的能力。

3.0Classical Microbiology and the Move Toward Alternative and Rapid Methods

经典的微生物学及可选择的快速方法的发展

3.1 Classical Microbiological Methods

经典的微生物方法

Classical pharmaceutical microbiology methods and strategies are comparatively simple techniques encompassing little technology and limited in their scope of detection. Methods for the detection and enumeration of bacteria , yeast , and mold have been appropriate for a variety of applications, including sterility testing, microbial limits, antimicrobial or preservative effectiveness, environmental monitoring, bioburden and microbial identification, to name a few. Biopharmaceutical production processes based on biotechnology and the use of cell cultures also require the industry to test for the presence of other adventitious microbiological agents, such as viruses and mycoplasma, in finished product, in-process samples and cell line/fermentation processes. Many of these microbiological testing methods have served the pharmaceutical industry for almost four decades, a period of growth in complexity and quantity of manufactured drug products.

经典的药品微生物方法和原理是相对简单的技术，所包含的技术含量低及检测范围有限。细菌、酵母菌和霉菌的检测和计数方法已有多种应用，包括无菌检测、微生物限度、抑菌剂或防腐剂效能、环境监控、生物负荷和微生物鉴别等。基于生物技术和细胞培养的生物制品生产工艺同样要求检测成品、中间样品和细胞线/发酵工艺中外来微生物的存在，如病毒和支原体。其中许多微生物检测

方法已经被制药行业采用了近四十年，期间生产的药品复杂性和数量都有明显增长。

Each of the above mentioned microbial tests are all considered as "growth-based" techniques that have primarily relied on three basic nutrient media for detection of significant microorganism contamination—Trypticase Soy Broth/Agar (developed by NASA in the 1960's) for aerobic bacteria , Sabouraud's Dextrose Broth/Agar for fungal growth (developed in the 1940's), and Fluid Thioglycolate Medium (also developed in the 1940's) for anaerobic microorganisms . These three media combined with the "aerobic plate count" method, published in the 1965 United States Pharmacopeia (USP) XXI-IV, have provided the basis for quantitative microbial challenge assays for microbial safety and quality product release, validation of in-process microbial tests, and sterilization validation for pharmaceuticals and medical devices in the twentieth century. The precision , accuracy, and standard error of these classical microbiological methods have been affected by the distribution of microorganisms in the test samples, cellular arrangement , sample dilution , and the plate count method itself such that the antimicrobial or preservative effectiveness test error is ±0.5 logarithm (5) and standard plate count errors can range from 18% t o 100% (error as % of the mean counts) when the mean number of colony form in g units per plate is 30 to 1, respectively (6).
以上各微生物检测都被认为是以基于微生物培养生长为基础的技术，主要依赖三种基本营养介质以检测微生物污染-用于需氧菌的胰蛋白大豆肉汤培养基/琼脂（1960’由NASA研制），用于真菌生长的沙保罗氏葡萄糖培养基/琼脂（1940’研制）以及用于厌氧菌的液体巯基乙酸盐培养基/琼脂（1940’研制）。这三种培养基与“需氧平皿计数”方法结合，发表于1965年的USP XXI- IV，为20世纪的微生物安全和产品放行、中间体微生物检测的验证、药品和医疗器械的灭菌验证提供了定量微生物挑战检测的基础。这些经典的微生物方法的精密度、准确度和标准偏差受到样品微生物分布、细胞组成排列、样品稀释和平皿计数方法本身的影响，因此当每平皿菌落数平均值为30至1时，抑菌剂或防腐剂效能检测的误差达±0.5对数单位并且标准平皿计数的误差可达18%至100%（与平均数量的误差百分比）。

Although the growth of microbial cells on agar surfaces or in liquid media provides the laboratory with critical information about the amount and the type of organisms that may be present in a sample under evaluation, the time-to-result is usually longer than what is desired. Days and even weeks may elapse before microbial colonies are visually detected, and in many cases, confluent growth prevents individual organisms from being isolated, necessitating subculture onto additional agar media, delaying the time-to-result even further . This delay may hamper the industry in making forward processing decisions and confirming that manufacturing processes are in a microbiological state of control.
尽管微生物细胞在琼脂表面或液体培养基的生长为实验室提供了被评估的样品中可能存在的菌种的数量和类别的关键信息，但得到结果的时间通常长于期望值。在目视观察到微生物菌落前可能浪费几天甚至几个星期，并且很多情况下，混合生长阻碍了单个微生物的分离，使在额外的琼脂上的次培养成为必需，从而更延迟了得到结果的时间。这种延误可能妨碍行业做出进一步的决定以及确认生产工艺处于微生物可控状态。

Additionally, microorganisms that are stressed due to nutrient deprivation following exposure to sublethal concentrations of antimicrobial agents, such as preservatives, disinfectants, heat, or decontaminating gases, or as a result of certain pharmaceutical manufacturing processes, may be stressed or physiologically injured; nor will they replicate when cultured on artificial media, because the media and/or incubation parameters may not be optimal for the resuscitation and subsequent proliferation of

organisms that may be present. The inability of stressed microorganisms, or even nonstressed microorganisms, to grow in artificial media has also been referred to as Viable But Non Culturable (VBNC). Additionally, when microorganisms experience unfavorable conditions a t the beginning of the stationary phase, environmental challenges can induce some cells into dormancy, and these cells will not grow. VBNC and dormancy states are further discussed in the public literature (7).
另外，那些由于暴露于尚不至于致死浓度的抑菌溶液而导致营养缺失的微生物，如防腐剂、消毒剂、热或气体灭菌，或某种药品生产工艺的结果，可能被影响或生理受损；当在人造培养基培养时他们也不会复制，因为该培养基或培养参数对于可能存在的微生物复苏及其后的繁殖不是最佳条件。无法在人造培养基中生长的受压制或未受压制的微生物，被视为活的但不可繁殖的微生物VBNC。另外，当微生物在稳定期开始时遭受了不利条件，环境挑战可导致某些细胞休眠，并且这些细胞不会生长。VBNC和休眠状态在文献资料中做了进一步讨论。

For these reasons, the modern microbiological laboratory should look toward developing innovative approaches for the detection, quantification and identification of microorganisms. From a quality risk management perspective, the industry can benefit from implementing alternative microbiological testing strategies to:
基于这些原因，现代微生物实验室应该期望发展微生物检测、定量和鉴别的新方法。从质量风险管理的角度来说，行业可从实施可选择的微生物检测原理受益：
• design robust processes that prevent contamination
设计可靠的工艺以防止污染
• ensure that a state of microbial control is maintained
确保维持微生物受控状态
• develop more effective strategies to correct a contamination problem
发展更有效的策略以解决污染问题
• continually improve processes and products
持续改进工艺和产品
• assess the potential impact of failing results on the patient
分析失败对于患者可能造成的潜在影响

The motivation for using risk management principles is supported by a number of quality and regulatory initiatives, and these have a direct impact on microbiological monitoring and control. For example, FDA's Pharmaceutical cGMPs for the 21st Century: A Risk Based Approach and Guidance for Industry: Process Analytical Technology, A Framework for Innovative Pharmaceutical Development, Manufacture and Quality Assurance indicate that using a scientific framework to find ways of mitigating risk while facilitating continuous improvement and innovation in pharmaceutical manufacturing is a key public health objective (8,9). These initiatives further promote the use of the latest scientific advances in manufacturing and technology, and this can apply to the implementation of alternative and rapid microbiological methods (RMMs) and testing strategies.
使用风险管理原理的动机受到了大量质量和法规倡议的支持，并且其对于微生物监控和控制有直接影响。例如，FDA的21世纪制药cGMP：基于风险的方法和工业指南：工艺分析技术，创新药物研发、生产和质量保证的结构中指出，在有利于药品生产的持续改进和创新过程的同时，采用科学的框架以找到减少药品生产的风险，是一项关键公共健康目标。这些倡议进一步促进采用最先进的科学生产技术，同时也适用于实施可选择的和快速的微生物方法（RMMs）和检测策略。

3.2 **Alternative and Rapid Microbiological Methods**可选择的和快速微生物方法

For more than 20 years, the field of alternative and RMMs has been gaining momentum as an area of research and application across a number of technology sectors. In fact, much of the development of new systems for the detection and identification of microorganisms has been driven by the food and beverage, environmental, municipal water, clinical, personal care and military/homeland security sectors. It is only since the introduction of the first version of this Technical Report that the pharmaceutical and biopharmaceutical industry took notice that these methods and technologies were available and could be validated as alternatives to existing microbiology testing.

在过去超过20年中，可选择的和快速微生物方法领域作为一个跨多技术分支的研发范围和应用领域已经受到很大重视。实际上，大多数关于微生物检测和鉴别新系统的发展受食品、饮料、环境、市政用水、临床、个人保健和军方/国家安全等领域的驱使。直到该技术报告第一版的介绍，药品和生物制品行业才开始关注到这些方法和技术已经可以应用并能被验证用作现有微生物检测的供选方案。

Alternative and RMMs are based on a wide variety of scientific principles and can be used for a number of testing applications. Technologies can detect the presence of diverse types of microorganisms or a specific microbial species, enumerate the number of microorganisms present in a sample, and can identify microbial cultures to the genus, species and subspecies levels. The manner in which microorganisms are detected, quantified or identified will be dependent on the specific technology, procedures and/or instrumentation employed. Additionally, a number of these methods are considered to be more sensitive, accurate, precise, and reproducible when compared with classical, growth-based methods. Some methods are fully or semi-automated, offer increased sample throughput, provide significantly reduced time-to-result (e.g., from days or weeks to hours or minutes), and for a few technologies, afford results instantaneously and in real-time. A more thorough review of technology platforms and the science behind these methods is provided in Section 4.0 of this Technical Report.

可选择的和快速微生物方法是基于多种科学原理并可被用于多种检测应用。这些技术可检测不同种类的微生物的存在或某一特定的微生物种类，计算样品中微生物数量，并可鉴别微生物到属，种和亚种。检测、定量和鉴别微生物的方法将依赖于具体的技术、规程和/或采用的仪器。另外，与经典的培养生长的方法相比，许多方法都被认为更灵敏、准确、精确和重现性更高。有些方法是全自动或半自动，提供了更大的样品处理能力，大大减少了等待结果的时间（如从几天或几个星期到几小时或几分钟），并有少数技术能提供即时结果。本技术报告第4.0节对这些方法背后的技术平台和科学研究进行了深入的分析。

3.3 Regulatory Perspectives法规观点

Alternative and RMMs have been understood, accepted and encouraged by regulatory authorities in numerous regions including the United States, Europe, Japan and Australia. Regulators will generally accept a change in a manufacturing or testing process if the change has been proven to be equivalent to, superior to, and/or non-inferior than the system currently in place. While this Technical Report contains recommendations for the validation studies to demonstrate such a concept, the final decision on acceptance may be through the affected regulatory agencies. This may be especially true if the method is incorporated in a previously approved regulatory dossier, such as a New Drug Application (NDA), Abbreviated New Drug Application (ANDA) or Marketing Authorisation. However, there are also instances where a formal regulatory submission or post-approval change may not be necessary, and in order to develop an appropriate strategy for the validation and implementation of these methods, it is

important to fully understand the current regulatory expectations. Therefore, it is highly recommended that an open dialogue between the interested parties (i.e., the firm intending to implement the method and the relevant regulatory authority) be initiated early in the planning process. This dialogue can include discussions about the proposed method, impacted products, the validation approach and acceptance criteria, as well as regulatory submission requirements. These types of meetings have been very helpful and have enabled the potential alternative method users to move forward with greater assurance that they will be successful in gaining regulatory approval, when required.
可选择的和快速微生物方法已被多个药政机构广泛理解、接受并受到鼓励，包括美国、欧洲、日本和澳大利亚。法规通常可以接受生产工艺和检测方法的变更，如果该变更被证明能够等同、更优和/或不低于现有系统。该技术报告中包括对于证明该理念的验证活动的建议，最终决定可能取决于受影响的相关药政机构。如果该方法已包含在已批准的药政文件中，则更是如此，如新药申报（NDA），仿制药申报（ANDA）或注册批准。但是，也有不需要正式的药政文件递交或批准后变更的例子，为了开发适宜的策略以验证和实施这些方法，全面理解现在的法规要求非常重要。因此，强烈建议在计划初期利益相关各方之间（如欲采用该方法的工厂和相关药政机构）发起公开对话。该对话可包括提议的方法的讨论，受影响的产品，验证方法和接受标准以及法规注册要求。这些对话对于潜在的新方法使用者非常有用，并使其能够有更高的保证获得药政机构的批准。

There exists a variety of different perspectives on alternative and RMM validation and submission strategies, depending on with which regulatory body a firm’s products are registered and/or which local inspectorate is responsible for conducting GMP audits at a firm's manufacturing facilities. The following sections will summarize the most current regulatory expectations for validation, submission and implementation; it is always recommended, however, to monitor any regulatory updates or changes in this area, as appropriate.
对于新和快速微生物方法的验证和药政注册策略有多种不同观点，依赖于该工厂的产品在哪个药政当局注册和/或哪个当地检察机构负责对该工厂执行GMP审计。以下章节将总结对于验证、药政注册和实施最近的法规要求。但是总是建议关注该领域任何法规更新或变更。

3.3.1 Unite States美国

The FDA has been accepting of alternative and RMMs for a number of years, and this position is echoed in a variety of guidance documents and quality initiatives. For example, FDA's 2004 Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing-cGMP recommends the use of rapid genotypic methods for microbial identification, as these methods have been shown to be more accurate and precise than biochemical and phenotypic techniques (10). The guidance also states that these methods are especially valuable for investigations into significant microbiological adverse events, such as sterility test failures and contaminated media fills. The guidance additionally confirms that other suitable microbiological tests (e.g., RMMs) can be considered for environmental monitoring, in-process control testing, and finished product release testing after it has been demonstrated that these new methods are equivalent or better than conventional methods.
FDA已于多年前便已接受可选择的和快速微生物方法，并可在多个指南文件和质量倡议中得以体现。如：FDA于2004发布的工业指南：无菌工艺生产的无菌制剂就推荐采用快速基因学方法进行微生物鉴别，因为这些方法已被证明比生物化学和表型技术更准确和精确。该指南也指出这些方法对于重大的微生物不良事件的调查也有重要作用，如无菌检测失败和受污染的模拟灌装。该指南还认为其他适宜的微生物检测（如快速法）在经证实其等同或优于传统方法时可用于环境监控、过程控制检测和成品放行检测。

In 2008, FDA's Center for Biologies Evaluation and Research (CBER) published draft Guidance for Industry Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products, and in 2011 published a proposed rule in the U.S. Federal Register amending the sterility test requirements for biologic product s (11). The latter states that advances in technology (in recent years) have allowed the development of new sterility test methods that yield accurate and reliable test results in less time and with less operator intervention than the currently prescribed methods. The proposed rule also included a discussion on expanding the potentially acceptable sterility test methods to non-culture-based methods in addition to culture-based methods, as well as guidance on validation principles, such as limit of detection, specificity, ruggedness, and robustness. The proposed rule was finalized in 2012 as "Amendments to Sterility Test Requirements for Biological Products" (12).
2008年，FDA生物制品评价和研究中心发布了草稿版工业指南：用于细胞和基因疗法药品的无菌检测的培养生长的快速微生物检测方法的验证，并于2011年发布了对于US联邦注册修正生物制品无菌检测要求的建议规则。后者指明近年来的先进技术已允许新的无菌检测方法的发展，与现有法定方法相比其在更短时间内能够得出准确可靠的结果，并且操作者的干预更少。提议的规则还包括将可能接受的无菌检测方法扩大基于培养生长的方法乃至到非培养为基础的方法的讨论，以及验证原理的指南，如检测限，专属性，偏离度和可靠性。提议的规则已于2012年定稿为“生物制品的无菌检测要求的增补案”。

A separate initiative, known as FDA's Strategic Plan for Regulatory Science, calls for the development of sensitive, rapid, and high-throughput methods to detect, identify, and enumerate microbial contaminants and validate their utility in assessing product sterility (13).
一个单独的倡议，FDA的法规科学战略计划，号召发展灵敏、快速和高流量的方法以检测、鉴别和计数微生物污染并验证其分析产品无菌性的作用。

The FDA suggests that USP <1223>, Ph. Eur. Chapter 5.1.6, or PDA Technical Report No. 33, be used as the basis for developing an appropriate validation plan (3,4). Additionally, many firms have successfully utilized the FDA comparability protocol (CP) as a means to manage the validation plan (14). Briefly, the CP is a regulatory submission (typically a prior approval supplement) that contains a validation protocol for the alternative or rapid microbiological method. The CP describes the proposed validation studies and the acceptance criteria to be met to demonstrate that the alternate method is acceptable. Once the CP has been approved, the applicant can use the protocol in the CP to validate the alternative method for its intended use. The CP can be particularly useful for changes of a repetitive nature, such as the use of a RMM for multiple products or processes. Moreover, because the CP is reviewed by the FDA, deficiencies in the validation plan can be corrected prior to performing the studies, eliminating the need to repeat some or all of the testing.
FDA建议采用USP<1223>，EP第5.1.6节，或PDA技术报告33作为开发验证计划的基础。另外，许多工厂已成功利用 FDA相似性方案（CP）作为管理验证计划的方式。总的来说，CP是一个药政注册文件（典型的批准前变更）其包含可选择或快速微生物检测方法的验证方案。CP描述计划的验证研究及需要达到的可接受标准以证明该可选择方法可接受。一旦CP批准，申请人可采用CP中的方案来验证可选择方法。CP对于重复性的变更尤为有用，如在多个产品或工艺中采用RMM方法。同时，由于CP已经过FDA审核，验证计划中的缺陷项可在实施研究之前得以更正，以避免重复部分或全部测试的必要。

When the applicant has completed the studies and met the acceptance criteria as outlined in the CP, they need to notify FDA when they are ready to implement the alternative method. This notification can be accomplished by the submission of a Special Report (21 CFR 314.81(b) (3) (ii)) (IS). The Special Report is a simple letter to FDA stating that they have completed the validation, the acceptance criteria have been met and that the alternative or rapid method is being implemented. In addition, a reduced reporting category can be used when notifying the FDA, such as a Changes Being Effected (CBE)-30 or CBE-0, the latter allowing the firm to immediately implement the method for routine use.
当申请人完成研究并符合CP中列出的接受标准，其需要通知FDA何时可执行可选择方法。该通知可通过递交特殊报告(21CFR 314.81(b)(3)(ii))来完成。该特殊报告是一封致FDA的简信以说明申请人已完成验证，满足接受标准并且可选择或快速方法待执行。另外，简化的报告形式可用于通知FDA，如CBE-30或CBE-0，后者允许工厂立即实施方法用于日常使用。

Finally, if the alternative or RMM will impact in-process microbiology assays that are not included in a formal product submission, such as a n NDA or ANDA, the implementation of the method may be better managed through a firm's internal change control program instead of going through a formal regulatory process. For this reason, it is always recommended that a firm discuss their validation and implementation plan with the FDA early in the planning phase.
最后，如果可选择或RMM方法会影响在线微生物检测，且其不包括在正式的产品注册文件中，如NDA或ANDA，则方法的实施应通过内部变更程序进行管理而不是通过正式的法规途径。由于这个原因，建议工厂在计划阶段与FDA就其验证和实施计划进行讨论。

3.3.2 Europe欧洲

Like their U.S. counterparts, European regulators have supported the validation and implementation of alternative and RMM technologies. However, there are subtle differences with respect to validation expectations and submission requirements. Furthermore, although individual member states have approved RMMs for routine use and for product marketed in the European Union (EU), many of the validation and implementation tools provided by the FDA did not exist until 2010.
与US一样，欧洲药政机构也支持可选择和RMM技术的验证和实施。但是，关于验证和药政注册要求有一些微小的差异。此外，尽管各成员国已经批准了RMM作为日常使用和在欧盟上市的药品，直到2010年由FDA提供的验证和实施工具才出现。

Commission Regulation (EC) 1234/2008 went into effect in 2010 and applies to variations to a Marketing Authorisation granted in a Mutual Recognition/Decentralized Procedure and to Community or Centralized Authorisations (16). The new variations regulation introduces a number of features aimed at reducing the workload for both competent authorities and applicants. One of the most important changes relating to alternative and RMMs is that it is now possible to group variations under the same Marketing Authorisation such that they can all be assessed at the same time. Furthermore, it is possible to combine the same variations or group of variations from different Marketing Authorisations and have all of these assessed at the same time under what is called a "Work Sharing Process" or "Common Assessment." This could be the case for a single alternative or RMM technology being used for multiple products.
委员会法规（EC）1234/2008于2010年生效并适用于在共有酬劳/分散程序中的市场授权和社团或集中授权。新法规引入了大量针对减少药政当局和申请人的工作量的特点。关于可选择和RMM最重要的变化之一就是现在已可在相同的市场授权下对变化进行分类因此其可同时进行评估。此外，可

以结合不同市场授权的相同的变化或变化的分组并使其通过“工作共享程序”或“通用评估”同时进行评估。这可能成为单个的可选择或RMM技术用于不同产品的实例。

Next, there is an opportunity for scientific dialog with regulators through the EMA Scientific Advice (SA) procedure (17). Here, a firm may ask for advice on their validation and implementation strategies. The SA working party includes representatives from all EU member states and a written report is provided with the results of the SA process.
接下来，有通过EMA科学建议（SA）与药政机构展开科学对话的机会。在此，工厂可针对其验证和实施策略寻求建议。SA工作组包括来自于欧盟所有成员国的代表，并可提供书面报告作为SA程序的结果。

On the basis of the Regulation (EC) 1234/2008, the Commission published a "guideline on the details of the various categories of variations to the terms of marketing authorisations for medicinal products for human use and veterinary medicinal products" which implements the 'post approval change management protocol' (PACMP) (18). This voluntary process, which is very similar to FDA's CP, provides a strategy for managing the review of proposed validation plans prior to the start of testing (19).
基于EC1234/2008，委员会发布了“关于人用药和兽用药市场授权变化的不同分类细节的指南”使“批准后变更管理方案”（PACMP）生效。该自愿程序与FDA的CP非常相似，提供了在开始测试前提议的验证计划的审核管理策略。

In this two-step process, a change management testing protocol is first submitted as a Type 2 Variation. The protocol should include the overall testing strategy, such as the planned studies, acceptance criteria and methods. Prior to submitting the PACMP, a firm may also discuss their testing strategies with the EMA under the SA procedure. Once the protocol is approved, the submitting company will perform the testing as specified in the protocol.
在这个两步的程序中，变更管理测试方案首先作为2类变更进行递交。该方案应包含总的测试策略，如计划的研究，接受标准和方法。递交PACMP之前，工厂可基于SA规程与EMA就其测试策略进行讨论。一旦方案批准，递交的公司可以按照方案进行测试。

The second step of the PACMP process involves submitting the resulting data (assuming they have met the protocol's acceptance criteria) as either a Type 1A or 1B Variation. The decision as to whether the data is submitted as a Type 1A versus a Type 1B variation is determined at the time of protocol review and approval. If the data is submitted under a Type 1A Variation, the company can immediately implement the alternative or rapid method, while a Type 1B Variation requires a 30-day waiting period while the data is reviewed. These strategies are very similar to the FDA's CBE-0 and CBE-30 reduced reporting policies.
PACMP的第二步涉及递交最终结果（如果满足方案中规定的接受标准）作为1A或1B类变更。结果作为1A还是1B类变更递交在方案审核和批准时确定。如果作为1A类变更递交，该公司可以立即实施可选择或快速方法，而1B类变更需要30天等待数据的审核。这些原则与FDA的CBE-0和CBE-30简化报告方针很类似。

The European Pharmacopeia (Ph. Eur.) contains a chapter that addresses the validation of alternative microbiology methods (4). Along with this document, Ph. Eur. 5.1.6 provides a framework for the development of an appropriate validation plan. The validation plan can then be included in the PACMP as

the basis for the studies that will be performed.
欧洲药典包含一个章节用来阐述可选择的微生物方法的验证。除此以外，欧洲药典5.1.6节提供了建立适宜的验证计划的框架。验证方案可包含在PACMP中作为研究的基础。

The EMA has recently extended the competencies of the PAT team (which is currently responsible for QbD matters) to also cover all matters related to alternative microbiological methods.
EMA最近扩大了PAT小组的职能（其现已负责QbD事宜）以也涵盖与可选择的微生物方法相关的所有事务。

Finally, it is recommended to have discussions with the EMA, the relevant competent authorities and/or the local inspectorate early in the implementation planning phase, especially if it is determined that a formal Type Variation change may not be required (this will depend upon the alternative or RMM's intended application, such as an in-process microbiology test that is not in a Marketing Authorisation).
最后，建议在实施计划的早期与EMA、相关药政机构和/或本地检查机构进行讨论，特别是如果当认为可能不需要正式的变更（这将取决于可选择的或RMM预期的申请，如没有包含在注册批准中的过过程中的微生物检测。）

3.3.3 Japan and Australia日本和澳洲

Both the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) and the Australian Therapeutic Goods Administration (TGA) are also accepting of alternative and RMM technologies.
PMDA和TGA都接受可选择的和RMM技术。

The TGA utilizes relevant sections in the Ph. Eur., British Pharmacopoeia, USP Chapter<1223>, Ph. Eur. Chapter 5.1.6, ISO 17025, as well as this technical report, when working with companies wishing to validate and implement alternative and RMM technologies (3,4,20).
在处理意欲验证和实施可选择的和RMM技术的问题的公司时，TGA使用欧洲药典5.1.6节，英国药典，USP <1223>和ISO 17025以及本技术报告。

The Japanese PMDA requires evaluation and approval for alternative or rapid methods that will be used for product release, such as a rapid sterility test. As of the publication date of this TR, at least one rapid sterility test has been approved by the PMDA. However, in the case of in-process control tests, the validation and implementation of alternative or RMM technologies would be managed under the responsibility of the drug manufacturer. Furthermore, the Japanese Pharmacopoeia (JP) is preparing a chapter on RMMs and will refer to USP chapter <1223> and Ph. Eur. Chapter 5.1.6.
日本PMDA要求对用于产品放行的可选择的或快速方法时进行评估和批准，如快速无菌测试。从该TR发布之日起，至少一种快速无菌测试已经过PMDA的批准。但是，作为过程控制检测，可选择的或RMM技术的验证和实施可由药品生产商进行管理。此外，日本药典正在起草章节针对RMM并将引用USP <1223>和欧洲药典 5.1.6节。

3.3.4 Rest of World (ROW)世界其他国家

The implementation of alternative and RMMs has also been successful in non-U.S. and non-European regions, including the Asia-Pacific and Central/South America. As with U.S. and European agencies, it is recommended that an open dialogue with the affected regulators is initiated early in the implementation process. And as many of these agencies may be unfamiliar with the proposed technologies, it may also be recommended to have the vendor involved as well. Where some ROW countries may follow USP Chapter <1223> and/or Ph. Eur. Chapter 5.1.6, other countries may only follow their own, local Pharmacopeias (3,4). In either case, it is recommended that this technical report serve as a framework for discussion of the planned validation and implementation plans.

可选择的和RMM已在非美国和非欧盟国成功实施，包括亚太区和中/南美地区。与美国和欧洲药政机构一样，建议在实施初期与相关药政机构进行沟通。由于许多这些地区的药政机构可能对于提议的技术不胜熟悉，因此也建议将供应商涵盖其中。部分ROW国家可能遵照USP《1223》和/或欧洲药典5.1.6节，其他国家可能只遵照其自己的药典。两种情况下，都建议将本技术报告作为验证和实施计划讨论的框架。

3.3.5 When an RMM is Approved by One Regulatory Authority but not Another 当RMM被一个药政机构批准而未被其他药政机构批准

Companies that want to utilize a RMM for a product that is marketed in multiple countries may require approval from each country's regulatory authority. However, it is possible that one regulatory authority may not approve the RMM, even though another regulatory authority has approved it. In this case, the company may need to establish an alternative product testing strategy for the country in which the RMM cannot be utilized, and this may include using the original microbiology method for that particular product.

那些才用RMM方法用于一个在多个国家上市的产品的公司可能会被要求获得各个国家药政机构的批准。但是，也有可能即使得到其他药政机构的批准，而某个药政机构不批准该RMM。在这种情况下，该公司可能需要建立一个可选择的的产品检测策略针对不能采用RMM的国家，并且可能包含该产品原来使用的微生物方法。

3.4 Business/Economic, Quality and Technical Considerations商业/经济，质量和技术考虑

Significant opportunities exist for improving the efficiency of manufacturing and quality assurance through the application of modern process analytical tools including alternative and RMMs. For many companies, the implementation of these technologies has provided a more in-depth understanding of their manufacturing processes in terms of microbial quality control. As such, some companies have been able to reduce variability and wasteful activities, and increase manufacturing capacity and efficiencies.

通过现代工艺分析工具的应用包括可选择的和RMM对于生产效能和质量保证的改进存在重要机会。对许多公司来说，采用这些技术对其微生物质量控制方面的生产工艺提供了更为深入的理解。因此，这些公司可减少可变性和浪费型活动，并增加生产能力和效能。

To aid in the evaluation of which particular alternative or RMMs are appropriate for specific applications, firms should consider the business and economic, quality and technical considerations of the proposed method(s).

为了帮助评估哪一种特定的可选择的或RMM适用于特定的应用，工厂应考虑所提议方法的商业和经济性，质量和技术条件。

3.4.1 Business and Economic Considerations商业和经济性考虑

The business and economic or cost requirements when implementing alternative or RMM should be well understood. These considerations may have a significant impact on the decision to validate and implement new technologies for routine use. From a business perspective, a number of factors should be considered, including, but not limited to, the following:

当实施可选择的或RMM时应充分考虑商业和经济性或成本要求。这些考虑可能对日常应用的新技术的验证和实施有巨大影响。从商业角度来说，有许多应当考虑，包括但不限于：

• Potential for reduced in-process microbiology testing and finished product release cycle times
减少中间控制微生物检测和成品放行周期的可能
• Reduction in risks associated with forward processing (e.g., bacterial contamination of mammalian cell cultures)
减少未来工艺的风险（如：哺乳动物细胞的细菌污染）
• Elimination or reduction of off-line assays
消除或减少离线检测
• Increases in laboratory automation and reductions in manual testing, sample handling and/or data management
增加实验室自动操作减少人工检测、样品处理和/或数据管理
• Reduced overhead and/or headcount for sampling and/or testing
减少取样和/或检测的经费或人员
• Ability to make immediate microbiology decisions on the state of microbial control
在微生物受控状态做出快速的微生物学决策的能力
• Faster response to contamination events or microbial data deviations, and the initiation of investigations
对污染事件或微生物数据偏差的快速反应，并发起调查
• Reduced repeat testing, lot rejection, reprocessing and rework
减少重复检测，不合格批次，返工和重新加工
• Reduction in plant downtime and investigations
减少工厂停工和调查
• Increased product yields
产品收率提高
• Reduced raw material, in-process and finished goods inventory holdings
减少原料、在制品和成品的库存
• Reduced warehousing space/cost and work-in-process (WIP).
减少库房空间/成本和中间过程

Many of these business considerations, in terms of potential cost savings and/or cost avoidances, may be analyzed using an appropriate financial or economic model, and the resulting information used to support, from an economic standpoint, the implementation of a new technology. However, it should be noted that the financial results of conducting such an exercise should not solely be used to make a final decision on whether or not to implement the technology; but rather, should be considered in addition to all of the other relevant factors, including the quality and technical benefits, when implementing the technology.
这些与可能的成品节约和或避免花费有关的商业考虑，可采用适宜的财务或经济模型进行分析，结论信息可从经济性为出发点用于支持新技术的实施。但是，应注意的是这些财务结论不应成为判断是否采用这些技术的唯一标准，应用这些技术时还应考虑除此之外的所有相关因素，如质量和技术优势。

A variety of financial models can be used to assess whether an alternative or RMM will provide a cost savings or cost avoidance when implemented. These include Return-On-Investment (ROI), Net Present Value, and Payback Period, to name a few. For example, ROI is the ratio of money gained or lost on an investment relative to the amount of money invested. In this case, the investment is the implementation of

an alternative or RMM. Payback Period is the time required for the return on an investment to "repay" the sum of the original investment. A company's financial organization can assist in determining the most appropriate model to use, depending on the technology, its applications, and other financial factors. In all cases, the operating costs associated with the existing method and the alternative or RMM are identified, as well as the potential cost savings/cost avoidances and the investment costs for validation and implementation. These values are then used in the relevant financial model to calculate either a potential financial benefit or loss.
Examples of operating costs may include, but are not limited to, the following:
可采用多种财务模型评估一个可选择的或RMM是否能够带来成本降低或花费避免。包括投资回报，目前净价值，投资回报周期等。例如，ROI是回报或损失的资金与投入的资金的比例。此时，投入一个可选择的或RMM的实施。投资回报周期是指收回原来投入所需要的时间。根据技术本身，应用和其他财务因素，一个公司的财务部门可协助确定最适宜采用的模型。总之，应确认现有方法与可选择的或RMM方法的操作成本，以及可能的成品降低以及验证和实施的投入成本。这些数据随后被用于相关的财务模型以计算可能的财务收益或损失。操作成本包括但不限于以下示例：

• Cost per test (e.g., consumables)
每次测试的成本（如耗材）
• Labor time and labor costs
人工时间和人工成本
• Equipment depreciation, calibration, qualification and maintenance
设备折旧，校验，确认和维护
• Laboratory overhead
实验室经费
• Data management and storage
数据管理和储存
• Additional testing (e.g., if the RMM is not approved in all countries).
额外测试（如，如果该RMM没有被所有国家批准）
Examples of investment costs may include, but are not limited to, the following:
投入成本包括但不限于以下示例：
• The capital costs for the new technology
新技术的资产成本
• Software updates
软件更新
• Training
培训
• Validation
验证
• Regulatory filings and associated costs, when applicable.
如适用，药政注册和相关费用

Finally, the values associated with potential cost savings and/or cost avoidances may be similar to what was previously discussed in the beginning of this section. Additional information on how to develop a financial assessment may be found in the public literature (21, 22).
总之，与潜在的成本降低和/或花费避免相关的价值观点与本节开始时讨论的一致。关于如何开展

财务分析的更多信息可参考公共文献。

3.4.2 Quality Considerations质量考虑

There are also potential quality benefits that a firm should consider. For example, the implementation of alternative or RMMs may provide the means to better monitor and control the overall microbial quality of a manufacturing process and its associated product, thereby reducing risk to the patient. Also, the use of technologies that provide a greater understanding of manufacturing variability, enhance process knowledge, and contribute to continuous process and product improvement should be considered. Furthermore, some technologies allow for more accurate, sensitive and reproducible monitoring, as well as enhanced trending of microbiological data, and automation, all which can contribute to increases in compliance, process knowledge and the improved detection of microorganisms.

公司同样应考虑可能的质量收益。例如，实施新或RMM可能更好的监管和控制一个生产工艺及其相关产品的整体微生物质量，因而减少病人的风险。同时，应考虑采用那些能够更好的理解生产变量，加强工艺理解并能提供工艺和产品持续改进的技术。另外，某些技术能够实现更准确、灵敏和重现性更高的监控，并能加强微生物数据的趋势分析和自动化操作，这些特点都能增加法规符合性、工艺理解并改进微生物的检测。

3.4.3 Technical Considerations技术考虑

Many alternative or RMMs offer increased accuracy, precision, reproducibility, sensitivity and specificity, as compared with classical microbiological methods. These technologies may also be fully automated, offer increased sample throughput, operate in a continuous data-collecting mode, provide significantly reduced time-to-result (e.g., from days or weeks to hours or minutes), and for some technology platforms, obtain results in real-time. Therefore, the potential technical benefits should also be understood when considering an alternative or RMM for implementation.

与经典微生物方法相比，许多可选择或RMM提供了更高的准确度、精密度、重现性、灵敏度和专属性。这些技术可以全自动化，增加样品量，在持续数据采集模式下进行操作，获得结果的时间大大减少（如，从几天或几星期到几小时或几分钟），并且对于一些技术平台，可时时获得结果。因此，在考虑实施可选择或RMM时应充分了解可能的技术优势。

3.5 Risk Analysis风险分析

Generally, when a change to a manufacturing or testing process is proposed, the potential risks associated with these changes should be identified. This is also the case when implementing an alternative or rapid microbiological method; therefore, a risk assessment should be performed prior to the start of any validation and implementation activities.

一般来说，当提起生产或检测工艺相关的变更时，应识别与这些变更相关的潜在风险。当实施可选择或快速微生物方法时也是如此。因此，在开始任何验证和实施活动前应完成风险分析。

The risk level in adopting an alternative or rapid method may vary depending on the technology considered and the methodology it replaces, the technology supplier, the nature of the measurements taken (qualitative, quantitative or identification) and the unit of measure (e.g., as compared with classical microbiology measurements, such as the colony forming unit), the particular product or process attribute being evaluated, method variability, the potential for false positive or false negative results, test sample requirements, computer system security, alarm notifications, the location of the measurement in the manufacturing process chain, worldwide regulatory acceptance, and various other factors.

采用可选择或快速方法的风险级别可能因以下方面而不同：所考虑采用的技术、欲替代的方法、技术供应商、采用的测量方法的性质（定性的、定量的或鉴别）、测量单位（如：与经典微生物测量方法相比，如cfu），所评估的具体产品或工艺的特性、方法变量、假阳性或假阴性结果的可能性、检测样品要求、计算机系统安全性、报警通知、生产过程中的监测点位置、世界范围内法规可接受性、以及其他因素。

The evaluation of risk to using an alternative or rapid method, as well as the risk to product quality, should be based on scientific knowledge and a link to patient safety. As such, the level of effort, formality and documentation should be relative to the level of risk. Briefly, the steps in conducting a risk analysis will include identifying the risks or hazards, and determining the likelihood of occurrence and severity of harm for each of the risks identified. The ability to detect the risks may also be considered. Then, each risk is analyzed against predefined criteria, and the output is a quantitative risk score or a qualitative risk ranking (e.g., low/medium/high). Based on the output, it should be determined whether the risks are acceptable or not. If they are not acceptable, the process may be changed and/or the risk may be reduced to an acceptable level. Additional activities may include routine risk reviews to ensure that no new risks have been introduced, and that risk controls are effective, especially during the routine use of the alternative or rapid method.
对于采用可选择或快速方法的风险评估以及对产品质量的风险评估应基于科学的理解并联系到患者安全。因此，投入的程度、正式程度和文件应与风险的级别相关。简单来说，风险分析的步骤应包括识别风险或危险源，确定各个风险的发生率和危害性。应考虑发现风险的能力。随后，对各个风险与预定标准进行分析，输出应是定量的分数或定性的风险分级（如：低/中/高）。根据输出结果，决定改风险被接受或拒绝。如果不被接受，则工艺可能进行变更和/或风险可能被降低直至可接受水平。其他活动可能包括常规的风险回顾以确认没有引入可选择的风险，并且风险控制措施是有效的，特别是在可选择或快速方法的日常使用过程中。

Risk Analysis Model tools, such as Failure Modes and Effects Analysis (FMEA) or Hazard Analysis and Critical Control Points (HACCP), may be utilized in order to effectively decide on which alternative or rapid method to implement, to assist in the justification of technology implementation, or to better understand the impact of implementation upon the product and the business. For additional information on these two methods of risk analysis or other risk analysis methods the reader is referred to the literature, which includes PDA Technical Report Number 44, Quality Risk Management/or Aseptic Processes (23-25).
可采用风险分析模型工具，如FMEA或HACCP，以更有效地确定采用哪种可选择或快速方法，帮助解释技术实施，或更好的理解对于产品和商业的影响。关于这两种风险分析方法或其他方法可参考文献，包括PDA TR44，质量风险管理/或无菌生产工艺。

3.6 Vendors, Suppliers and Audits供应商和审计

Vendors of alternative or rapid method technologies should be assessed for their ability to provide high quality instrumentation, consumables, software, and/or technical support. For example, vendors should have in place an appropriate quality system for designing, manufacturing, testing and release of equipment, software, reagents and consumables throughout the technology life cycle. Additionally, vendors may provide technical documentation, training, troubleshooting, calibration services, preventive maintenance programs and/or field service support. To ensure that the vendors meet a firm’s internal quality requirements, as well as GMPs, vendors should be evaluated for their ability to meet these

expectations. Vendor assessments or audits may be conducted through a review of relevant documentation provided by the vendor and/or a physical audit at the vendor's manufacturing, design/development and testing facilities. Some of the assessment areas that a company may focus on include, but are not limited to:
应当对可选择或快速方法技术的供应商进行评估以确定其提供高质量仪器、耗材、软件和/或技术支持的能力。例如，供应商应有适宜的质量体系以在整个技术生命周期中设计、生产、检测和放行设备、软件、溶剂和耗材。另外，供应商应提供技术文件、培训、问题处理、校验服务、预防性维护程序和/或现场服务支持。为了确保供应商满足工厂内部质量要求以及GMP要求，供应商应进行评估。供应商评估或审计可通过回顾供应商提供的相关文件进行和/或进行现场审计。审计范围可包括但不限于：

• Quality assurance procedures and standards, including change control and ISO certification
质量保证规程和标准，包括变更控制和ISO证书
• Results from other audits (regulatory or other end-users)
其他审计结果（官方或其他使用者）
• Financial stability
财务稳定性
• Availability in providing consumables and instrumentation
提供耗材和仪器的能力
• Ability to respond to field issues of a technical nature
技术本身现场问题的反馈能力
• Training and validation support, and associated documentation
培训和验证支持及相关文件
• The vendor's internal validation procedures and data
供应商内部验证程序和数据
• How software updates are managed, including the impact on validation activities
如何管理软件更新，包括对验证活动的影响
• Additional information that may support the end-user's justification to implement the vendor's technology, such as peer-reviewed publications, user manuals and other relevant documentation.
其他可能支持采用该技术的信息，如经同行评议的刊物，使用手册和其他相关文件

Some vendors may also have submitted additional technology performance information to FDA in the form of a Drug Master File (DMF; 21 CFR 314.420). The contents of the DMF may include method development information or data from validation studies performed by the vendor. For example, certain types of validation studies, such as ruggedness and robustness, may be performed by the vendor and included in a DMF. End-users may utilize the data supplied in the DMF as supporting information for their own validation studies and submissions to the FDA, when appropriate.
有些供应商可能向FDA递交了DMF文件。DMF的内容可包括供应商验证研究中获得的方法开发信息或数据。如：某种类型的验证研究，如偏离度和稳定性，可能包含在DMF中。如适用，使用者可利用DMF中的这些数据作为其自己的验证研究和FDA药政递交资料的支持信息。

3.7 Automated Methods自动化方法
Some alternative or rapid technologies may be considered as automated traditional or compendial microbiological test methods, especially when the results are in colony forming units (CFU). These

technologies may be qualified for their intended use without the need for demonstrating certain method validation requirements as specified in Section 5.0 of this Technical Report. For these technologies, at least accuracy and precision assessments should be performed, in addition to method suitability and equivalence/comparability studies. A risk assessment should be performed to determine the required testing that would support the validation of the alternative or rapid technology.
某些可选择或快速技术被视为自动化的传统或药典微生物检测方法，尤其是当结果为cfu时。这些技术不需要按照本技术报告第5.0节所描述的要求进行方法验证。对于这些技术，除了方法适应性和等效性/可比性研究外，至少应进行准确度和精密度的评估。应进行风险评估以确定可能支持可选择或快速技术验证所要求的检测。

4.0 Technology Review 技术审核

There exists a wide variety of alternative and rapid technologies, and they can be grouped in one or more of the following categories: qualitative, quantitative and/or identification.
微生物中有很多选择性的快速的技术，可归为以下几类：定性技术、定量技术和/鉴别。

Qualitative technologies will detect the presence of diverse types of microorganisms (e.g., total aerobic bacteria, yeast and mold), or a specific type of microorganism (e.g., E. coli or Mycoplasma). For comparison, the presence/absence sterility test is an example of a classical microbiological qualitative method.
定性技术用于检测微生物的种类（如总好氧性细菌，酵母菌和霉菌），或者检测某种特定的微生物种类（如：大肠埃希菌或支原体）。无菌检测法是微生物定性检测的一种经典方法。

Quantitative technologies will enumerate the number of microorganisms present in a test sample. For comparison, the standard agar plate count method is an example of a classical microbiological quantitative method.
定量技术用于计算供试品中出现的微生物的数量。为了进行对比，标准琼脂平板计数法是是微生物定量的经典方法。

Identification technologies can identify microbial cultures, and for some systems, single cells, to the Genus, species, subspecies and even strain levels. Some of these technologies represent fully automated strategies of classical, growth-based biochemical and carbohydrate methods, while other rely on a completely novel scientific principles that are similar to chemical or analytical methods.
鉴定技术可以鉴定群落或者单细胞微生物培养物到属，种，亚种甚至变种的水平。一些鉴定技术代表了在基于培养生长的生化和碳水化合物方法的全自动的经典方法，还有一些技术是完全依靠类似于化学或者分析方法的完全可选择的科学方法。

The manner in which microorganisms are detected, quantified and/or identified will be dependent on the specific technology, their scientific principles and the instrumentation employed. For example，alternative and rapid technologies may be further classified as those that rely on microbial growth, the use of viability stains, the detection of cellular markers or targets, optical spectroscopy, nucleic acid amplification, and Micro-Electro-Mechanical Systems, or MEMS.
微生物检测、定量和/或鉴别方法需依据特定的技术（特定的技术包括它们的科学原则和使用的仪器）。例如选择性的快速鉴定技术可以进一步分为微生物生长的方法，细胞标记或靶细胞的检测方

法、光谱法、核酸扩增法和微机电系统法或微电子机械系统法，这些检验方法都是以微生物的生长，有生存能力菌株的利用为基础的。

This chapter provides an overview of the different types of alternative and rapid technologies that are commercially available or are known to be in development. For ease of understanding, these technologies are discussed under their primary scientific principle; however, it should be noted that some technologies might provide one or more detection capabilities (i.e., qualitative, quantitative and/or identification). When applicable, this Technical Report provides additional clarity for technologies that fall under each of these detection categories. For example, certain nucleic acid amplification technologies that are primarily used to identify microorganisms may also provide an estimation of cell count based on the number of amplification cycles required to elicit a positive response (i.e., this is related to the number of genetic copies of the target starting material in the original sample). Additionally, certain spectroscopic technologies are able to provide both a microbial identification as well as a cell count.
本章概述了可供选择性的和快速的不同技术，这些技术在商业中用到或在发展完善中。为了便于理解，这些技术在它们主要的科学原则下进行讨论，然而，应注意到一些技术可能有一种或多种检测能力（如定性、定量和/或鉴别）。此项技术报告在每种检测分类项下都会更清晰的说明此技术的方法。例如，核酸扩增技术主要用于鉴别微生物，也可用于细胞计数的评估，此细胞计数是基于扩增周期的数量需要引出的阳性回应(例如,这与原始样品中目标原始材料的基因复制品的数量相关)。此外，光谱技术也可以用于微生物的鉴别和细胞计数。

The reader should also be aware that because alternative and rapid technologies are constantly evolving, specific vendor names and their respective technologies are not specified in this Technical Report; rather, the scientific principles of known technologies and developing methods are discussed. Additional information regarding specific technologies may be found in the public literature and in relevant online resources (26-28).
读者应注意到由于可选择性的和快速的方法在不断的改进，一些供应商的名字和他们的技术也没有在此技术报告中出现；更准确的说，应讨论已知技术的的科学原则和发展方法。关于特定技术的更多的信息可在公开的文献和相关在线资源中找到。

4.1 Growth-based基于培养生长的技术

Alternative and R MM that employ the use of growth-based platforms have been shown to reduce the time at which actively growing microorganisms can be detected, although the actual time-toresult may be impacted by the physiological state of microorganisms and/or the lag period in which microorganisms adapt to growth conditions. Many growth-based systems continue to use conventional liquid or agar media. As a result, the same types of applications that traditional methods are used for can also be applied to growth-based alternative and rapid methods. Examples include bioburden testing, Microbial Limits, environmental monitoring, sterility testing, and the identification or presence/absence of microorganisms. A summary of growth-based technologies is presented below.
利用基于培养生长技术平台的可供选择性的技术和 RMM 技术在微生物生长中检测可缩短检测时间，尽管实际的即时出的结果受受微生物的生理状态和微生物适应生长条件而产生的生长停滞期的影响。许多以微生物生长为基础的系统继续使用传统的液体或琼脂培养基。结果，使用传统方法的同种类型的用途可用于基于生长的可选择性的和快速的方法。例子包括生物负载测试，微生物限度，环境监测，无菌检测和微生物的检出及其鉴别。基于生长技术的概括如下。

4.1.1 Electrochemical Measurement 电化学测量法

Electrochemical methods measure changes in the electrical properties of microbiological media as a result of microbial metabolism. Liquid growth media comprise relatively large uncharged or weakly charged molecules, and microorganisms growing in this media will break down the large molecules into smaller more highly charged components (e.g., proteins into amino acids, fats into fatty acids and polysaccharides or sugars into lactic acid). These technologies can rapidly detect changes in measurable electrical threshold during microbial growth by monitoring the movement of ions between electrodes (conductance), or the storage of charge at the electrode surface (capacitance). By measuring the changes in electrical impedance, capacitance and/or conductance, growing microorganisms can be detected much faster than observing turbidity in the media.

电化学法测量微生物的新陈代谢产物在微生物培养基中电性能的变化。液体生长培养基包括不带电荷的大分子的或有微电荷的分子，生长于此培养基中的微生物将大分子分解为小的有高度电荷的成分（例如，蛋白质分解为氨基酸，脂肪分解为脂肪酸，多聚糖或糖分解为乳酸）。通过检测电极之间离子运动产生的电子临界值变化或电极表面的电荷储存能力这些技术可以快速的检测出变化。对微生物生长的测量，测量电阻抗的变化，电容或者电导的变化比观察培养基的浊度快得多。

4.1.2 Detection of Carbon Dioxide (CO_2) CO_2 的检测

Microorganisms, when grown in liquid culture, produce carbon dioxide (CO_2) and other metabolites. In a closed container, the amount of CO_2 produced may be monitored and used as a measure of organism viability (i.e., the presence of growing microorganisms). Test samples are added to media bottles that contain a liquid emulsion or silicone sensor. During microbial growth, CO_2 in the medium diffuses into the sensor. Hydrogen ions will then interact with the sensor resulting in a decrease in pH, and the sensor will turn color (e.g., from gray to yellow). The rate at which CO_2 is detected depends on the initial concentration of microorganisms; for example, a higher initial concentration will provide a faster detection response.

生长于液体培养基中的微生物产生 CO_2 和其他的代谢物。在密闭容器中，可检测产生的 CO_2 的量，被用于微生物活性的测量（例如生长微生物的存在）。将供试品加于包含液体乳剂或硅胶传感器的培养瓶中。在微生物生长过程中，培养基中的 CO_2 扩散到传感器上。氢离子将与传感器相互作用导致 PH 降低，传感器便会改变颜色（由灰变黄）。检测 CO_2 的速率取决于微生物的原始浓度；例如，原始浓度越高，检测速度越快。

4.1.3 Utilization of Biochemical and Carbohydrate Substrates 生化和碳水化合物的利用

There are a variety of technologies that employ a microorganism's ability to utilize biochemical and carbohydrate substrates as sole carbon or energy sources for the rapid and automated identification of microorganisms. A suspension of a pure culture (usually from an isolated colony on an agar plate) is inoculated onto test cards or strips. Each card or strip is composed of incubation wells, and individual wells contain a single substrate in dehydrated form. The inoculated cards or strips are incubated, and if the organism under test utilizes any of the substrates for cellular metabolism and growth, the turbidity, color and/or fluorescence in the well will change. The resulting data (normally in the form of positive and negative responses in each well) are compared with an internal database or reference library and a microbial identification (e.g., Genus and/or species) is provided.

有很多技术都是根据微生物以何种生化物质或者碳水化合物为唯一碳源或者能源物质。将纯培养物悬液（通常是从一个琼脂平板上分离的单菌落）接种至测试卡或测试条上。每个测试卡或测试条都有培养孔培养孔中有单个的脱水基质。培养接种卡或接种条，如果微生物在测试条件下利用孔中

的物质进行细胞生长或新陈代谢，孔的浊度、颜色和/或荧光将会发生改变。结果数据（通常是每个孔的阳性或阴性结果的形式）与内部数据库或相关信息库的数据对比，便提供了微生物的鉴别（例如属和/或种）

4.1.4 Digital Imaging and Auto-fluorescence of Micro-Colonies

数位成像和微生物菌落的自发荧光

During microbial growth, cells will fluoresce in the yellow-green spectral region when illuminated with blue light. Cellular auto-fluorescence in this spectral region is a property of all microbial cells due to the presence of ubiquitous fluorescent biomolecules including flavins, riboflavins, and flavoproteins. Test samples are filtered and the membrane is placed onto an agar surface and incubated. During incubation, a Light Emitting Diode (LED) excites micro-colonies to autofluoresce, which are quantitated by a Charge Coupled Device (CCD) imaging system in approximately one-half the time an operator would normally be able to observe colonies on the agar surface. Incubation of the agar can continue to allow for the recovery of larger colonies for subsequent analysis, such as microbial identification.

在微生物生长过程中，在黄绿光谱区用蓝光照射时，细胞发荧光。在此光谱区细胞自发荧光是所有微生物细胞的特性，这是因为无所不在的荧光生物分子的存在，荧光分子包括黄素、核黄素和黄素蛋白。过滤供试品，将薄膜置于琼脂表面，培养。在培养期间，发光二极管刺激微生物菌落自发荧光，微生物由电荷耦合原件成像系统定量，培养到规定时间的一半时，操作者可观察到琼脂表面的菌落。继续培养琼脂平板，回收大量的菌落用于以后的分析，例如微生物鉴定。

4.1.5 Fluorescent Staining and Laser Excitation of Micro-Colonies

荧光染色法和微生物菌落激光刺激法

Viability staining and laser excitation can also be used to detect and quantify micro-colonies. A test sample is filtered and the membrane is placed onto an agar surface. Following an appropriate incubation period, the membrane is stained with a nonfluorescent substrate. Microorganisms on the filter will take up the substrate, which is then enzymatically cleaved, liberating free fluorochrome in the microorganism cytoplasm. As the fluorochrome accumulates inside the cells, the signal is amplified. T he membrane is subsequently placed into a reader and exposed to the excitation wavelength of the fluorochrome. Fluorescent micro-colonies are then enumerated. Incubation of the agar can continue to allow for the recovery of larger colonies for subsequent analysis.

活性染色法和激光刺激法可以用于检测和定量微生物菌落。过滤供试品，将薄膜置于琼脂表面。培养适当的周期后，将薄膜用非荧光基质染色。微生物与附着酶的基质互换位置，并在微生物细胞质内释放荧光物质。随着荧光物在细胞中聚集，信号便扩大。随后将薄膜置于读数器中，暴露在荧光物的激发波长中。然后计算荧光微菌落的数量。继续培养琼脂平板，回收大量的菌落用于后续分析。

4.1.6 Use of Selective Media for the Detection of Specific Microorganisms

特定微生物选择性培养基的使用

Selective media is used to selectively promote the growth of specific types of microorganisms while preventing the growth of other types of microorganisms. This is also known as inclusivity and exclusivity. A test sample is added to a liquid-based selective medium that also contains unique dyes. During incubation, optical sensors detect changes in the medium's color or fluorescence, thereby indicating the presence of growing microorganisms.

选择性培养基用于选择性的促进某种限制菌的生长，同时阻止其他种菌的生长。这也叫做包含性和排他性。将样品加于包含特殊染料的液体培养基上。在培养期间，光学传感器检测到培养基颜色或

荧光物的变化，预示着生长菌的存在。

4.1.7 Measurement of Change in Head Space Pressure 顶部空间压力变更的测量

These technologies are based on noninvasive, continuous, automated monitoring of growing microbial cultures. The test sample is added to a liquid medium and electronic transducers will detect positive or negative pressure changes in the headspace of the container as a result of microbial growth (i.e., the production and/or consumption of gases).

这些技术基于微生物生长的非侵害性、持续、自动检测。将供试品加于液体培养基中，电子传感器将会检测到容器顶部空间压力的阳性或阴性的变更，作为微生物生长的结果。例如气体的产生和消耗。

4.1.8 Microcalorimetry 微量热法

Microbial catabolic activity produces heat, which can be measured on a sensitive microcalorimeter. This heat production can be measured by flowing the test sample continuously through a microcalorimeter, or by placing the test sample suspended in a growth medium inside a sealed metal ampoule within the microcalorimeter. Themeasured heat associated with the test sample is compared with the heat evolved from a sterile medium standard or baseline value.

微生物分解代谢活动产生的热量可以由灵敏的微热量计测量到。这种热量是供试品不断的流入微热量计来测量的，或将供试品悬浮于密闭的有微热量计的金属安瓿生长培养基中。供试品的热量与无菌标准培养基或空白值对比。

4.2 Viability-based 基于活性的技术

Viability-based technologies use viability stains and laser excitation for the detection and quantification of microorganisms without the need for cellular growth. For this reason, organisms that are stressed, injured, fastidious, in a dormant state or are considered viable, but nonculturable, may now be detected when these same organisms will not grow in or on classical microbiological media. These types of technologies can be used for a variety of applications that require the detection and enumeration of microorganisms, such as bioburden and Microbial Limits testing, environmental monitoring, process water analysis and sterility testing.

基于活性的技术利用活性菌株和激光刺激检测和定量微生物，不需要细胞生长。由于这个原因，受影响的、受损害的、需要复杂营养的微生物，在休眠状态或是被认为是有活性的，但未经培养的微生物，当上述微生物没有在传统的微生物培养基中生长，同样可以被检测到。这些技术类型适用于多种用途的微生物检测和计数，例如生物负载和微生物限度检测，环境监测，工艺用水分析和无菌检测。

4.2.1 Flow Cytometry 流式细胞技术

Flow cytometry involves labeling microorganisms with a viability marker and injecting the labeled sample into the instrument's flow cell. As individual, labeled cells pass through a focused laser beam, they will fluoresce and are enumerated. Because the flow cell is very narrow, usually small volumes of test sample, such as 1 mL or less, are usually evaluated. The process of labeling and evaluating viable cells in these types of technologies can be accomplished in as little as a few minutes, and for some systems, operate with minimal operator manipulation. Flow cytometry provides a relatively large range of detection operation (e.g., lO'-lO6 cells/mL) and has been demonstrated to enumerate a wide variety of microorganisms.

流式细胞技术包括用活性标示物标注微生物和将标注的样本注入仪器的流动池中。标注的细胞通过聚焦激光束后发荧光，同时被计数。由于流动池很窄，通常供试品体积都很小，例如 1ml 甚至更少，通常体积的多少都经过验证。在这些技术类型中标记和评估活性细胞的过程可在几分钟内完成，对于一些系统，用最低的操作量来操作。流式细胞计数法提供了一个相对大范围的检测操作（如 IO-IO6cell/ml）,且已经被证明可以为多种微生物计数。

Test samples that are applicable to the use of flow cytometry include liquid matrices and material that cannot be filtered, such as creams and lotions.
适用于流体细胞计数的供试品包括液体基体间质和不可过滤的物料，如膏体和洗剂。

4.2.2 Laser Scanning Solid Phase Cytometry 激光扫描固相细胞计数

Solid-phase cytometry uses a similar staining and laser excitation method as does flow cytometry; however, the microorganisms are first captured onto a solid phase (e.g., a 0.4 |j,m membrane filter) and he filter is subsequently labeled with a nonfluorescent, viability staining substrate. Microorganisms that are present on the surface of the filter will take up this substrate, and within the cytoplasm of metabolically active cells, the substrate is enzymatically cleaved by an esterase. This process results in the release of a fluorochrome, which can be excited when exposed to a laser of an appropriate wavelength. If the retained organisms have an intact cell membrane, the fluorescent label is concentrated within the cell, and this signal is detected when the laser scans the membrane surface. Following laser scanning, the technology provides a viable organism count, with a limit of quantitation of a single cell. The time from filtration to staining to enumeration is approximately 90 minutes.
固相细胞计数法与流式细胞计数法用的染色和激光刺激法相同；然而，微生物首先被采集到固相中，随后将过滤器标注为非荧光物、活性染色基质。在过滤器表面出现的微生物将占据基质的空间，在新陈代谢有活性的细胞的细胞质中，基质被酯酶酶解。此过程导致荧光物的释放，将荧光物置于合适的激光波长中将受到刺激。如果保留的微生物有完整的细胞膜，荧光标签集中于细胞中，当激光扫描薄膜表面时，会检测到此信号。激光扫描后，会得出活的微生物的数量。从过滤到染色到计数的时间大约 90 分钟。

Test samples for use in solid phase cytometry must be filterable, as the microorganisms must be retained on the membrane for viability staining and laser excitation.
用于固相细胞计数法的供试品必须是可过滤的，因为必须将微生物保留在薄膜上进行染色和激光刺激。

4.2.3 Direct Epifluorescence Filter Microscopy 直接荧光过滤显微镜术

Direct epifluorescence filter microscopy (DEFT) is essentially a precursor to laser scanning solid phase cytometry. DEFT technology is based on membrane filtration followed by microorganism staining using viability dyes and enumeration. Following sample filtration, the membrane is treated with fluorescent dyes, such as acridine orange, or 4',6-diamidino-2-phenylindole (DAPI), and viewed under an epifluorescence microscope. Viable microorganisms accumulate acridine orange and stain orange, while nonviable microorganisms stain green. A number of fluorescent redox dyes can also be used with DEFT, such as 5-cyano-2,3-ditolyl-tetrazolium chloride (CTC) for respiring cells.
直接荧光过滤显微镜术本质上是激光扫描固相细胞计数法的前身。DEFT 技术基于使用活性染料和计数法的微生物染色法的薄膜过滤法。供试品过滤后，用荧光染料处理薄膜，如吖啶橙，或 4,6-二脒基-2-苯基吲哚（DAPI），然后再荧光显微镜下观察。有活性的微生物沉积吖啶橙，染成了橙色，

而非活性微生物则染成了绿色。一些荧光氧化还原染料也可以与 DEFT 一起使用，例如 5-氰基-2,3-二甲苯基-四唑氯化物（CTC）

4.3Cell Component-based 基于细胞成分的技术

Cellular component-based technologies rely on the detection and analysis of specific portion of the microbial cell, including ATP, endotoxin, proteins and surface macromolecules. The types of applications these methods can be used for are wide spread and include sterility testing, bioburden and Microbial Limit testing, environmental and process water monitoring, microbial identification and endotoxin analysis.

基于细胞成分的技术是对微生物特殊部分的检测和分析，包括 ATP，内毒素，蛋白质和表面高分子。这些技术用途广泛，包括无菌检测、生物负载和微生物限度检测，环境和工艺用水监测，微生物鉴别和内毒素分析。

4.3.1 ATP Bioluminescence 三磷酸腺苷生物发光反应

ATP bioluminescence is the generation of light by a biological process. In the presence of the enzyme luciferase and the substrate luciferin, adenosine triphosphate (ATP) is enzymatically broken down to produce photons of light. An instrument equipped with a photomultiplier tube can detect these photons. Because ATP is a key intracellular energy source in all cells, measuring ATP can be a marker for viable microorganisms. Depending on the technology, some systems will detect the general presence of microorganisms by measuring the total relative light units from the test sample, while other systems can detect ATP bioluminescence from individual micro-colonies, thereby providing a quantitative assessment of the number of microorganisms from the original sample under evaluation.

三磷酸腺苷生物发光是在生化过程中产生光的反应。在荧光素酶和基质荧光素出现时，ATP 被酶解产生光子。装有光电倍增管的仪器可以检测到这些光子。因为 ATP 是所有细胞关键能量来源，测量 ATP 可以作为活性微生物的标志。依靠技术，一些系统通过测量供试品的总的相对光子检测一般微生物，其他的系统可以检测来自个别的微生物菌落的 ATP 生物发光。因此，可以提供原样微生物数量的定量评价。

The sensitivity and time-to-result of the ATP bioluminescence assay may also be improved with a two-phase reaction that begins by using an enzyme-catalyzed reaction to generate ATP to levels significantly higher than what is naturally contained in the microorganism. In the presence of microorganisms, specific microbial enzymes can be used to convert adenosine diphosphate (ADP) provided to the reaction into ATP and adenosine monophosphate (AMP). The enzymes are not consumed by the reaction; therefore, ATP is continuously generated as long as ADP is present. The amplified ATP levels are then detected using the typical ATP bioluminescence reaction previously described.

ATP 生物发光分析的灵敏性、即时性可以用双相反应来改进，即用酶催化反应催化产生大量 ATP 使之高于微生物原本包含的 ATP. 对于一些微生物,特定的微生物酶可以转变 ADP 为 ATP 和 AMP。反应过程中没有消耗酶，因此，只要存在 ADP 就会持续产生 ATP。产生的 ATP 就可以用之前描述过的特有的 ATP 生物发光反应来检测。

4.3.2 Fatty Acid Profiling 脂肪酸剖析

The cellular membrane contains lipid biopolymers, and one component of this cellular layer is chains of fatty acids. Fatty acids can be extracted from a pure culture of microorganisms, and following a series of chemical conversion steps; the purified fatty acids are analyzed via automated gas chromatography (GC).

The resulting gas chromatogram is compared with a previously established library of known microorganisms, and if a match is found, the identification is provided.
细胞膜有脂质生物聚合物，此细胞膜的成分之一是脂肪酸。脂肪酸可以从微生物的纯培养物中提取，随后经过一系列的化学转换步骤；最后通过自动气相色谱法分析纯脂肪酸。将气相色谱法的结果与已建立的已知的微生物信息库比较，如果结果匹配，此法可以作为鉴别方法。

4.3.3 Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) Mass pectrometry 基质辅助激光解析电离飞行时间质谱法

MALDI-TOF mass spectrometry provides an accurate molecular weight measurement and characterization of biomolecules, including proteins, peptides, polysaccharides and nucleic acids. Whole cells from an isolated colony are smeared onto a stainless steel plate and mixed with a UV-absorbing matrix. A laser ionizes the cells' biomolecules, which are then accelerated in an electric field. Within this field, the ionized molecules are separated according to their mass to charge ratio and the resulting mass spectrum is compared with an internal library of previously identified microorganisms.
MALDI-TOF 质谱提供了准确的分子重量测量法和对生化分子的特性描述，包括蛋白质、多肽、多聚糖和核酸。单菌落的整个细胞都被涂抹到不锈钢平板上并混合成具有紫外吸收矩阵的样品。激光电离细胞的生化分子，在电场中此过程会加速。在电场中，根据电离分子的质荷比将其分开，将最终的质谱与之前的微生物鉴别的内部信息库进行比较。

4.3.4 Surface Enhanced Laser Desorption Ionization Time of Flight (SELDI-TOF) Mass pectrometry 表面增强激光解析离子化飞行时间质谱法

SELDI-TOF mass spectrometry utilizes a chip array that contains one of a variety of chemical or biochemical surface receptors that will bind very specific protein molecules. The captured proteins are then analyzed by MALDI-TOF mass spectrometry, and when compared with other mass spectrum from known microorganisms, a microbial identification maybe obtained. This technology also allows for the direct profiling of proteins from complex biological samples, thereby bypassing the complicated steps of purification.
SELDI-TOF 质谱利法是利用一种包含有化学或生化表面受体的芯片排列，该表面受体可以结合特定的蛋白质分子，从而识别蛋白质分子。MALDI-TOF 质谱法分析采集到的蛋白质，与其他已知的微生物质谱对比时，就可获得微生物的鉴别。此项技术允许从复杂的生物样品中直接剖析蛋白质，因此忽略了提纯的复杂步骤。

4.3.5 Fourier Transform-Infrared (FT-IR) Spectrometry 傅里叶变换红外光谱

Molecular functional groups can absorb infrared radiation to generate a transmission spectrum specific for a material under evaluation. The material can also include microorganisms, and FT-IR can generate organism-specific spectra without the need for staining, labeling or amplification. For example, cellular material from a pure culture is spread onto a micro-plate and dried at 40-45°Cunder vacuum to create a biofilm. The dried biofilm is then analyzed in the FT-IR spectrometer. Each cell's FT-IR spectra reflects its biochemical composition, including proteins, lipids, DNA and RNA, and carbohydrates. This spectral fingerprint is then compared with other spectra from a previously established library of known microorganisms, and if a match if found, an identification is provided.
供试品分子官能团可以吸收红外线产生特定的透射光谱，微生物也可以。FT-IR 不需经过染色、标记和放大便可以产生生物体专一的光谱。例如，纯培养的细胞物散布到显微感光板上，在 40-45℃

下的真空干燥以得到生物膜，然后再用 FT-IR 分光仪进行分析。每个细胞的 FT-IR 光谱都反应了他的生化成分，包括蛋白质，脂质，DNA 和 RNA 和碳水化合物。然后将此图谱与之前建立的已知的微生物信息库的其他图谱对比，如果匹配的话，则进行了鉴定。

4.3.6 Endotoxin Detection 内毒素检测

The detection of endotoxin, or lipopolysaccharide (LPS) may now be performed using a quantitative, kinetic chromogenic method via a hand-held, point-of-use instrument within 15-20 minutes. The system uses LAL (Limulus Amoebocyte Lysate) reagents and has a sensitivity level similar to larger laboratory-based instrumentation (e.g., 0.05 - 5.0 EU/mL). Another technology utilizes an ELISA-based procedure where phage-derived protein binds LPS to the bottom of test wells in a microtiter plate. The plate can then be washed to remove components or conditions which may lead to interference during the endotoxin assay (e.g., salt or extremes in pH). Following the washing step, the bound endotoxin is detected using recombinant factor C and a fluorescent substrate. The sensitivity of this technology is between 0.05 - 500 EU/ML.

内毒素或脂多糖的检测可以使用定量的、动态发色法在 15-20 分钟内完成。系统运用鲎试剂内毒素检测试剂，灵敏度与大的实验室仪器监测结果相同（如 0.05-5.0EU/ml）。另一种技术是使用酶联免疫吸附试验，噬菌体衍生蛋白质滴定脂多糖在微孔板测试孔的底部。然后清洗微孔滴定板以防止检测内毒素造成交叉污染（盐，或 pH 的极端条件）。清洗步骤结束后，用重组因子 C 和荧光基质检测内毒素。这项方法的灵敏度在 0.05-500EU/ML 之间。

4.4 Optical Spectroscopy 光谱法

Optical spectroscopy methods utilize light scattering and other optical techniques to detect, enumerate and identify microorganisms without the need for microbial growth, labeling or amplification, and in many cases, obtain results in real-time.

光谱法利用光散射和其他的光学技术进行微生物检测、计数和鉴别，而微生物不必要进行培养、标记或放大，并且在很多情况下，会即时得出结果。

4.4.1 Light Scattering/Intrinsic Fluorescence 光散射/内源荧光

Light scattering is a phenomenon in which the propagation of light is disturbed by its interaction with particles. Instrumentation that utilizes Mie scattering (i.e., where the scattered light intensity is dependent upon the particle size in a certain size range) and fluorescence detection techniques can provide information about the size and number of viable microorganisms in air. As microorganisms pass through a laser of a specific wavelength, certain metabolites, such as NADH, riboflavin and dipicolinic acid, are excited and provide an intrinsic fluorescent signal that distinguishes the microorganisms from other airborne particulates. Therefore, these types of systems offer the simultaneous and instantaneous detection, sizing and counting of both viable and total particulates per cubic volume of air. Other instruments utilize Mie scattering to detect the presence of certain classes of microorganisms in water, such as coliforms, pseudomonads and protozoa.

光散射是光在传播过程中被与其有交互作用的粒子阻挡的现象。利用米氏散射（散射光强度取决于一定范围内的粒子大小）和荧光检测技术，可以提供空气中活性微生物的大小和数量。当微生物通过特定波长的激光时、某些代谢物，例如 NADH(还原型烟酰胺腺嘌呤二核苷酸)，核黄素和吡啶二羟酸，会被激活并发出固有的荧光信号，这就可以与空气中的其他悬浮粒子区分开。因此，这种技术提供了每立方空气中活性及所有粒子的大小和数目的及时的检测方法。运用其他仪器根据米氏散射原理可以检测水中的微生物，例如大肠菌，假单胞菌和原生动物。

4.4.2 Raman Spectroscopy 拉曼光谱法

Raman spectroscopy is an established analytical method based on the Raman scattering properties of a material under evaluation. When a laser interacts with the material, molecules are excited, resulting in a photon energy shift, which is related to the vibrations and rotations of the molecule. Because each molecule has its own unique Raman spectrum, this technique can be used for the identification of microorganisms. For example, a sample can be filtered, and each of the captured particulates is evaluated. The resulting Raman spectra are then compared with spectra from a previously established library of known microorganisms, and if a match if found, an identification is provided. Because Raman can target individual particulates, there exists the potential for obtaining a simultaneous identification as well as a microbial count.

拉曼光谱法是依据供试品的拉曼散射性能而建立的分析方法。当激光与供试品相互作用时，激发分子，引起光子能量的转变，这与分子的振动与旋转有关。由于每个分子有自己独特的拉曼光谱，因此拉曼光谱法可以鉴别微生物。例如，样品过滤后，可以对采集到的微粒进行分析。将供试品的拉曼光谱结果与已建立的已知的微生物信息库对比，如果匹配成功，则进行了鉴定。因为拉曼可以将个别的粒子作为目标，因此鉴别的同时也可以得出微生物的计数值。

4.5 Nucleic Acid Amplification 核酸扩增法

Nucleic acid and gene amplification-based technologies employ a variety of scientific principles, including, but not limited to, DNA-based Polymerase Chain Reaction (PCR), RNA-based reverse-transcriptase amplification, 16S rRNA typing, gene sequencing and other novel techniques. Many of these methods will detect the presence of a specific microorganism, such as an "objectionable" or pharmacopoeia "specified" organism, or can provide a microbial identification, in some instances, to the strain or subspecies level. Additional methods can be used to estimate the number of viable microorganisms in a sample, based on the number of amplification cycles required to reach a baseline or threshold level. Because there are many methods and systems based on nucleic amplification techniques, it is not possible to cover all of the scientific principles in this technical report; therefore, a brief example of key methods is provided, and additional information may be found in the literature and in online resources (26,27,29,30).

核酸和基因扩增技术利用多种科学技术，包括但不限于以 DNA 为模板的聚合酶链反应，以 RNA 为模板的反向转录酶扩增，16S rRNA 序列，基因序列测定和其他的新颖技术。这些技术中的一些方法能检测出特定微生物，例如“讨厌的”或药典中的“有规定的”的微生物，或者是鉴别微生物，在某些情况下甚至能测出菌株或亚种水平。其他的方法可用来判断样品中微生物的数量，但要基于扩增周期需要达到的基准线或临界线的数量。因为有很多方法和系统是基于核酸扩增技术，在此技术中不可能涵盖所有的科学技术；因此，在信息库和在线资源中只能找到关键方法的主要的例子和附加的信息。

4.5.1 Polymerase Chain Reaction (PCR)聚合酶链反应

In a classical PCR reaction, DNA is extracted from microorganisms (e.g., from an isolated colony on an agar plate) and heated to separate the double strands. DNA primers are then added which will bind to unique target sequences on the template DNA. The primer is elongated when a heat-stable DNA polymerase and nucleotide bases are added. T he result is two new copies of the template DNA. This PCR process is then repeated, resulting in millions of copies of the target DNA.

经典的 PCR 反应，从微生物中（琼脂平板中的单菌落）提取 DNA 然后加热使其分为双链。然后

加入 DNA 引物，DNA 引物在 DNA 模板上与特有的目标序列结合在一起。加入热稳定的 DNA 聚合酶和核苷酸碱基，引物被延长。结果得到两个新的 DNA 模板的复制品。然后重复此 PCR 程序，得到数以百万计的目标 DNA 复制品。

One of a variety of probes that also contain a fluorescent dye is included in the process, which allows for the rapid and real-time detection and quantitation of the number of amplification cycles needed to reach a threshold level, which in turn can be related to the number of copies of the target sequence in the original sample. There are many different types of probes that bind to double-stranded DNA or to specific sequences as they amplify and accumulate in the test system. The increase in fluorescent signals is then detected. As an example, if a sample contains a DNA target sequence associated with a particular microorganism, following PCR amplification of that target, the fluorescence signal from the probe will be detected, and the system will provide a positive response for that particular microorganism. If the target is not present in the original sample, then no amplification will occur and no fluorescence will be detected (above the threshold or background level). If the method utilizes real-time, quantitative PCR, the DNA amplification reaction is measured as it occurs.
有荧光染料的探针也包含在此程序中，此方法可以快速、实时的检测和定量需达到临界值的扩增周期。有很多不同类型的探针可以与双链 DNA 或特定序列联结，因为他们可以检测到系统中扩增和积聚的荧光信号。例如，如果样品包含有特殊的微生物相关的靶序列 DNA,在 PCR 扩增后，将会检测探针的荧光信号，系统将会提供那个特殊微生物的阳性反应结果。如果靶序列没有出现在原始样品中，那么将没有扩增现象，也检测不到荧光（在临界线与背景值上）。如果此方法采用即时的定量 PCR 方法，那么可以即时测量出 DNA 的扩增反应。

4.5.2 Reverse Transcriptase (RT) PCR 反向转录酶 PCR

RT-PCR uses RNA, instead of DNA, as a starting template for the PCR reaction. In this process, RNA is extracted from the cell, and the enzyme reverse transcriptase will create a complimentary strand of DNA. RNAse H will then remove the original single-strand of RNA. A second primer and DNA polymerase is then used to create double stranded DNA, which will be used in the classical PCR reaction as described above. RT-PCR has some advantages over classical PCR, such as a lower risk of contamination from nonviable cell DNA or residual DNA from the sample and/or work environment. RT-PCR is now being used for the detection of specific types of microorganisms as well as the estimation of viable cell count.
RT-PCR 使用 RNA 而不是 DNA 作为 PCR 反应的开始模板。在此过程中，从细胞中提取到 RNA，反向转录酶将会合成一条 DNA 链。核糖核酸酶 H 会降解原先的单链 RNA。然后在第二个引物和 DNA 聚合酶的作用下合成双链 DNA，此法用于传统的以上描述的 PCR 反应中。RT-PCR 优于传统的 PCR，例如来自非活性细胞 DNA 的低污染风险，或样品残留的 DNA 和/或工作环境。RT-PCR 现在被用于特定类型的微生物检测和活性细胞计数的评估。

4.5.3 Ribotyping 核糖体分型

To maintain correct RNA structure and ribosome function in bacteria, the 16S sequence of rRNA is highly conserved at the Genus and species level, but there are also nonconserved fragments within the rRNA operon that can be used for microbial identification and for strain differentiation. This method, known as ribotyping, uses restriction enzymes to cut DNA into fragments, which are then separated according to size by gel electrophoresis. The double-stranded DNA is then denatured to single-stranded DNA, which is subsequently hybridized with an rRNA operon probe and chemiluminescent agent. The resulting bands emitted by the fragments are compared with previously developed patterns from known

microorganisms, and a bacterial identification is provided. Additionally, the differences observed within the same patterns can be used to provide information related to strain differentiation between bacteria belonging to the same Genus and species.
为了维持细胞中正确的 RNA 结构和核糖体功能，16S rRNA 序列在种属水平中是高度保守的，但是 rRNA 操纵子中也有一些未保存的可用于微生物鉴别和菌株辨别的片段。此方法叫做核糖体分型，用限制性内切酶将 DNA 切成片段，然后根据凝胶电泳后片段的大小将其分离。双链 DNA 变性为单链 DNA 后，与一个 rRNA 操纵子探针和化学发光剂杂交。然后将所得到的 DNA 片段图谱与已知微生物的片段模式进行比对，就可以鉴别细菌。另外，通过观察片段模式的不同方面也可以为同种属的细菌提供菌株差异性信息。

4.5.4 Gene Sequencing 基因序列测定

Gene sequencing is used for the identification of a wide variety of microorganisms, including bacteria, yeast, mold and Mycoplasma. The scientific principle involves sequencing each nucleotide base of a specific DNA target after PCR amplification. Typically, the first 500 base pairs of the 16S rRNA gene are used, although the entire 16S rRNA gene has also been employed for greater accuracy.
基因序列测定用于细菌、酵母菌、霉菌和支原体多种微生物的鉴别。其科学原理是当 PCR 扩增后，为特定的 DNA 的核苷酸碱基排序。通常，使用 16S rRNA 的前 500 对碱基，但是为了得到更精确的结果也使用全部的 16S rRNA。

DNA is first extracted from a pure culture of cells and then amplified via PCR in four separate reactions; one reaction for each of the four deoxynucleotide bases: adenine (A) thymine (T) guanine (G) and cytosine (C). However, a mixture of these standard nucleotides and dideoxyribonucleotides are used, where the latter nucleotides lack a 3'-hydroxyl (-OH) group on their deoxyribose sugar. When a dideoxyribonucleotide is randomly incorporated during the amplification reaction, elongation of the PCR primer is terminated. This provides DNA fragments of varying lengths. Because each dideoxyribonucleotide is labeled with a different fluorescent dye, a series of fluorescently labeled copies of the amplified sequence, each terminating at a different base, is formed. These copies differ in molecular weight and can be separated and detected (based on their fluorescence) using capillary electrophoresis of the reaction mixes. By simultaneously analyzing each of the four reaction mixes representing the four deoxynucleotide bases, software within the gene sequencer will reconstruct the linear arrangement of these bases in the sequence being analyzed. The resulting sequence is then compared with a library of known microorganism sequences, and if a sequence match is found, a Genus and species identification is provided.
首先从细胞的纯培养物中将 DNA 提取出，然后通过 PCR 扩增；扩增需四种脱氧核苷酸碱基：腺嘌呤（A）胸腺嘧啶（T）鸟嘌呤（G）和胞嘧啶（C）。然而，使用这些标准的核苷酸和双脱氧核糖核苷酸的混合物，脱氧核糖核苷酸是氧核糖上缺少 3-羟基。因此，在扩增反应中，当有双脱氧核糖核苷酸被结合时，PCR 引物的扩增将终止。因此 DNA 片段的长度会有不同。由于每个双脱氧核糖核苷酸标有不同的荧光染料，就形成了标有荧光扩增序列的副本。这些副本的分子重量不同，因此可以使用毛细管电泳法对其进行分离和检测。通过同时分析代表四个脱氧核苷酸碱基的四个反应混合物，基因序列软件将会对所要分析的序列进行线性重组。把最终的序列与信息库中已知的微生物序列进行对比，如果序列匹配，即可作为种属的鉴别。

4.5.5 PCR and MALDI-TOF Mass Spectrometry PCR 和 MALDI-TOF 质谱

Combination gene amplification and mass spectrometry systems are also available for the detection of

specific microorganisms and for microbial identification. PCR is first performed using primers and probes specific for one or more target sequences. In one system, the resulting PCR amplified sequences, or amplicons are transferred onto a silicon chip and MALDI-TOF mass spectrometry is performed. In a separate system, the amplicons are introduced into an electrospray ionization-TOF mass spectrometer. The ionized amplicons willreach the detectors based on their nucleotide composition and length, and the resulting spectra are compared with an internal library of known microorganisms. These technologies can detect a wide variety of microorganisms, including viruses, and can detect multiple microbial species in a single sample (i.e., multiplexing).
将基因扩增法和质谱法结合使用也可以用来检测特定微生物和进行微生物鉴定。PCR首先在一个或多个目标序列中使用引物和探针。在一个系统中，PCR扩增的序列或扩增子被转移到硅胶条或MALDI-TOF质谱上。在分离的系统中，扩增子被引入到电喷射离子-TOF质谱中。基于的核苷酸成分和长度不同，扩增子被电离后，它们会进入检测器中，将最终的图谱与信息库中已知的微生物的图谱进行对比。此项技术可以用来检测包括病毒的更广范围内的微生物，在单个样品中可以检测多种微生物。

4.6 Micro-Electro-Mechanical Systems (MEMS) 微电子机械系统

MEMS utilize microarrays, biosensors, Lab-On-A-Chip or micro-fluidic systems, andnanottechno- logy, all which provide miniaturized technology platforms as compared with conventional, bench-top instrumentation.
微电子机械系统使用微阵列、生物传感器、芯片实验室或微流控分析系统和纳米技术，所有的这些技术与传统的、试验台上用仪器相比都提供微型技术平台。

4.6.1 Lab-On-A-Chip and Microfluidic Systems芯片实验室和微流控分析系统

Lab-On-A-Chip technologies employ sample preparation, fluid handling, analysis and detection steps in a microchip format. Samples are processed through the use of microfluidics, where pressure or voltage gradients move pico-or nanoliter volumes through miniaturized channels. Protein, DNA, RNA and whole cells are able to be analyzed in fluid samples.
芯片实验室技术在微芯片中进行样品制备、流体的处理、分析和检测。样品通过微流控系统，压力或电压通过微型管道以纳升的体积进行梯度移动。在样品流动中可以分析样品的蛋白质、DNA、RNA和所有的细胞。

One example of this type of technology utilizes a microchip to separate PCR amplicons for the purpose of identifying and strain typing microorganisms. The system targets short, repeating sequences of unknown function that occur randomly throughout the DNA of an organism. Primers bind to these sequences, resulting in multiple fragments of various lengths, which are subsequently added to the microchip and separated by size and charge. The amplicons then pass through a laser, causing fluorescence of an intercalating dye, and the resulting profile is compared with an internal database of similar profiles. If a match is found, a microbial identification or strain type is provided.
使用此项技术的一个例子：为了进行微生物的鉴定和菌株分型，使用微型板分离PCR扩增子。当微生物DNA中偶然进行未知功能的重复时，系统目标将缩短。引物与这些序列结合，导致不同长度的多种碎片，这些碎片随后被加入到微型条中，根据形状和电荷进行分离。然后扩增子通过激光，将嵌入染料的荧光和最终的剖析结果与相似的数据库比较。如果结果匹配，就进行了微生物鉴别或菌株分型。

4.6.2 Microarrays微阵列法

Microarrays are composed of an orderly arrangement of proteins, DNA, RNA or other biological fragments on a solid substrate, and can rapidly detect microorganisms of clinical importance, such as influenza A and Avian H5N1 (bird flu) strains.
微阵列法是蛋白质、DNA、RNA或其他微生物碎片在固体基质上的有序安排，可以快速检测临床中重要的微生物，例如流行性感冒A病毒和禽流感病毒。

One technology can identify up to 40 different species of Mycoplasma. DNA is first extracted from the Mycoplasma culture and PCR is performed using primers specific for conserved and speciesspecific regions of the 16S-23S rRNA intergenic transcribed spacer (ITS) of the Mycoplasma DNA. The fluorescently labeled fragments are then hybridized to the microarray chip. The chip contains probes for both species-specific targets and a universal probe for all Mycoplasmas, and if one of the targets is present, the system will provide a positive response.
一项技术可以鉴别到多达40种不同的支原体。首先从支原体培养物中提取DNA，然后对支原体DNA的16S-23S rRNA 基因间隔区利用引物进行PCR. 然后有荧光标记的碎片在微阵列条上杂交。微阵列条既包括特有目标物种的探针也包括可用于所有支原体的探针，如果其中任何一个目标出现，系统都会做出阳性反应。

4.6.3 Other Technologies其他技术

MEMS technologies continue to be developed, and include biosensors, nanoarrays, and micro and nanocantilever platforms for the rapid detection of microorganisms, viruses and other biological material. As microbiology detection systems evolve and continue to miniaturize, there is the potential for incorporating these novel methods directly into pharmaceutical manufacturing process streams and to reduce the footprint for lab-based methods that are still in use today.
微电子机械系统技术仍然在发展中，包括用于微生物、病毒和其他生物的快速检测的生物传感器、纳米阵列和微悬臂或纳米悬臂。随着微生物检测系统的发展并进一步微型化，有可能将这些新颖的技术合并直接用于制药工艺并减少至今仍在使用的基于实验室方法的印记。

5.0 The Validation Process

There are many definitions of validation. For example, ICH Q2(R1) states that (2):"The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended urpose."
有许多关于验证的定义。如，ICH Q2（R1）描述：分析方法规程的验证目的是证明其适用于预期用途。
Another example of a representative one of these definitions used by the EMA is from CPMP/QWP/848/96; EMEA/CVMP/598/99, September 2001 (31):"Validation is the act of demonstrating and documenting that a procedure operates effectively. Process validation is the means of ensuring and providing documentary evidence that processes (within their specified design parameters) are capable of consistently producing a finished product of the required quality."
另一个有代表性的定义被EMA所采用，出自CPMP/QWP/848/96; EMEA/CVMP/598/99, 2001年9月：验证是证明和记录一个规程有效运行的行为。工艺验证是为了保证和提供书面证据以证明工艺（在具体的设计参数内）能够持续的生产满足质量要求的产品。

Therefore, two components of validation are the appropriateness or suitability of the process or procedure

(meaning it does what it is intended to do) and reproducibility (meaning it continues to perform as expected). In the context of a new microbiological method, it is, therefore, important to be able to demonstrate the appropriateness of the method for the intended analytical application and ensure that there are procedures in place to show it continues to perform to the same standard of quality over time.
因此，验证的两个要素是工艺或规程的适宜性或适用性以及重现性（表示其按预期持续运行）。对于一个可选择的微生物方法来说，重要的是能够证明该方法对于预期的分析应用的适宜性并确保有规程证明其能够持续的按照同样的质量标准运行。

Validation should be more than a study conducted on a new method or sample matrix. Instead it should encompass the entire process that commences with the decision to change some aspect of the microbiological testing program and continues through ongoing routine use of the method. It follows, therefore, that validation starts from the outset, and the validation plan is designed to include each stage of the process that is required to implement a new test method. Adoption of this approach is intended to streamline and expedite the introduction of the new method by ensuring that each step in the process is considered in depth and documented before moving onto a subsequent stage.
验证应比新方法或样品组合研究的要求更高。验证应围绕从开始决定变更微生物检测程序的某些方面到持续的使用该方法的整个过程。因此它遵循验证从最开端开始并且验证计划应包含要求实施新测试方法的工艺的每一个步骤。采用这种方法是为了通过确保工艺的每一个步骤在进入下一步之前都经过深思熟虑并有记录而简化和加速新方法的引入。

As such, and in relation to alternative and rapid methods, a validated system may actually consist of equipment or instrumentation, associated software and an analytical test method. There are many industry guidance documents on the need for validation, and descriptions of a validation process. This Technical Report focuses on the unique aspects associated with validation of alternative and rapid methods (and their associated systems). Should you wish a more detailed review on the general requirements for validation, appropriate references are provided to discuss these topics.
因此，与可选择的和快速方法有关的验证系统可能由设备或仪器，相关软件和分析测试方法组成。关于验证需求和验证过程描述有许多工业指南文件。该技术报告关注与可选择的和快速方法（及其相关系统）有关的特有方面。如果你想对验证的一般要求有更为详细的回顾，本文也提供了适宜的参考资料。

Note: Some alternative and rapid methods may not utilize any equipment or software, and depending on the simplicity of the method, there may not be a need to validate anything other than the method itself. However, for the majority of commercially available alternative and rapid methods/systems that utilize a variety of substantially more complex hardware, software, incubators, specialized consumables, or other instrumentation, an analysis should be conducted to assess the extent of the validation strategy.
注意：某些可选择的和快速方法可能没有采用任何设备或软件，并且由于方法简单，因此除了方法本身没有必要验证其他部分。但是，对于大部分商业可得的采用了多种更为复杂的硬件、软件、培养箱、特殊耗材或其他仪器的可选择的和快速方法/系统，应进行分析以评估验证策略的范围。

There are many different approaches that have been successfully used for the validation of alternative and rapid methods. Furthermore, these approaches have been accepted by regulatory authorities. While examples of specific validation strategies are provided in this section of the Technical Report, it may be necessary to modify or customize these strategies to most appropriately address a specific technology,

method and/or application.
有许多不同方法成功的对可选择和快速方法进行了验证。另外，这些方法已被药政机构所接受。尽管在本节中介绍了具体的验证策略的实例，可能有必要对这些策略进行修改以更适于解决具体的技术、方法和/或应用问题。

The original issuance of PDA Technical Report No. 33 was published in May of 2000 followed by the United States and European Pharmacopeias each subsequently publishing a new chapter, USP<1223> and Ph. Eur. 5.1.6 (3,4), respectively, regarding the validation of alternative microbiological methods. At the time these documents were written, very few companies had implemented and validated alternative or rapid microbiological technologies and methods. The validation approach used generally followed the validation criteria for chemical methods in the various compendia, but redefining these criteria in microbiological terms. Within these documents there are provisions for some of the validation testing to be conducted by the supplier/vendor of the system (equipment, software and method) as well as validation testing that should be conducted by the end-user.
PDA TR33上一版发布于2000年5月，随后美国和欧洲药典分别发布了新的章节USP<1223> and Ph. Eur. 5.1.6 (3,4)，专门针对可选择微生物方法的验证。在这些文件起草的阶段，很少有公司实施和验证可选择的或快速微生物技术和方法。所采用的验证方法通常遵循不同药典中的化学方法的验证标准，但是在微生物条件下重新定义了这些标准。在这些文件中有针对供应商执行的验证测试（设备、软件和方法）和最终用户执行的验证测试的规定。

Broadly speaking, the validation criteria which need to be satisfied for microbiological testing methods can be divided, with the exception of microbial identification, into two categories: quantitative (a test that results in an enumeration of recovered microorganisms) and qualitative (a test with two outcomes; either positive or negative). However, minor differences existed between the three guidance documents, including terminologies, procedures employed during the validation process, data interpretation, acceptance criteria and the use of statistics. Therefore, it is the purpose of this current version of PDA Technical Report No. 33 to help potential users of alternative and RMM to achieve a harmonization of performance parameters requiring validation for any new method such that one strategy and/or regulatory submission can be acceptable in all geographic regions and by all regulatory authorities, and most importantly, be scientifically defendable and justified. It is also the recommendation of this Technical Report that the appropriate regulatory agencies be contacted and involved in the decision making process for both the validation activities and the implementation/use of an alternative or rapid microbiological detection, enumeration or identification method that will be utilized during the development and/or manufacture of pharmaceutical products.
大致来说，满足微生物检测方法的验证标准除了微生物鉴别以外，可以分为两类：定量（回收微生物计数）和定性（两种结果：阳性或阴性）。但是，这三种指南文件的差异很小，包括专业术语，验证过程中采用的程序，数据解释，接受标准和统计学应有。因此，这一版的TR33旨在帮助潜在的可选择和RMM用户获得需要对新方法进行验证的一致的性能参数使得一种策略和/或药政文件递交能够被所有地区和药政机构接受，更重要的是，能够进行科学的解释。本技术报告也建议在药品研发和/或生产过程中确定验证活动和实施/使用一个可选择的或快速微生物检测、计数或鉴别方法时联系适当的药政机构。

5.1 Pre-Validation Activities验证前活动

There are a variety of activities that may be successfully completed prior to initiating validation of the

system, as some of these tasks are critical to the subsequent validation activities required. Completion of these tasks can aid in completing a successful validation and, hopefully, reducing the number of deviations and retesting that may be necessary during the course of the validation activities.
在验证开始前有各种活动需要成功完成，因为其中一些工作对于接下来的验证活动十分关键。这些工作的完成可帮助完成成功的验证并有可能减少偏差和验证活动中必要的复测。

5.1.1 Proof of Concept (POC)概念论证

The alternative or rapid microbiological method selected for use should be initially evaluated to ensure confirmation of proof of concept (POC), feasibility or principle (i.e., assessing whether the method and accompanying system is actually suitable for its intended purpose and that it is compatible with the intended product or sample matrix). This proof of concept phase is most appropriate if the method supplier has no supporting data on similar products or sample matrices that the end-user will routinely test; therefore, POC testing can be conducted prior to making the final decision to purchase the equipment or instrumentation. This activity can be conducted either by the end-user or the supplier of the system/method. For example, the products or other sample matrices for evaluation during POC testing and, when appropriate, the number and types of microorganisms chosen to challenge the new method, should be carefully selected in order to ensure that the resulting data provides a compelling indication that the validation of the intended method and accompanying system will have a high probability of success.
所选的可选择的或快速微生物方法应进行初始评估以确保证实其概念论证，可行性或原理（即：评估该方法及其系统适用于其预期用途并与预期产品或样品组合具有相容性。）。概念论证阶段尤其适用于当方法供应商没有用于最终用户进行日常测试的相似产品或样品组合的支持数据；因此，POC测试可在最终确定购买设备或仪器之前执行。该活动可由最终用户或系统/方法供应商执行。例如：在POC测试中用于评估的产品或其他样品组合以及用于挑战新方法的微生物的数量和类型应进行仔细选择以确保结果数据能够提供有力证据说明预期方法及其系统的验证成功可能性很高。

5.1.2 Assessment of Supplier Capabilities/Supplier Audit供应商能力评估/供应商审计

It is important to assess the ability of a potential supplier to meet the specified requirements (e.g., as outlined in a User Requirements Specification, which is described in more detail in Section 5.2.2). This can include the supplier's ability to provide validation support, when required, as well as field service, software maintenance, and an uninterrupted supply of consumables and reagents. An assessment of the supplier's financial viability, size of the company, and understanding of applicable regulatory requirements may also be required. Some end-users generate an assessment or audit checklist for the supplier to complete, while others may choose to audit the supplier's development and manufacturing sites (i.e., capital equipment and consumables production facilities). These activities may also include an assessment of the supplier's primary design (hardware and software) and validation of the technology.
评估潜在供应商能否满足规定要求的能力十分重要（如：按URS的要求，在5.2.2节中有详细描述）。该评估可包括供应商提供验证支持的能力，必要时可包括现场服务、软件维护和持续供应耗材和试剂。还要求评估应商财务情况，公司规模，对法规要求的理解等。有些最终用户建立了评估或审计清单由供应商来完成，而有些可能选择对供应商的研发和生产产地进行现场审计（即：固定设备和耗材生产场地）。这些活动也可能包括对供应商初始设计（硬件和软件）和技术验证的评估.

5.1.3 Business Benefits or Return on Investment Considerations商业效益或投资回报考虑

Based on the POC evaluation and other pre-validation activities as described in the previous sections, a thorough analysis should be conducted to determine the technical appropriateness of the alternative or

rapid method for its intended use. Additionally, a Return on Investment (ROI) analysis may also be helpful to support or justify the business case for purchasing the equipment and its subsequent validation and implementation for routine use (refer to Section 3.4.1 for further discussion on this topic). The final instrument selection and purchase should, of course, take place before initiating validation.
基于前一章节描述的POC评估和其他验证前活动，应进行彻底的分析以确定可选择的或快速方法对于其预期用途的技术适宜性。另外，投资回报分析也可以帮助支持或解释采购设备的商业问题以及随后的日常使用的验证和实施（详见3.4.1节）。最终的仪器选择和采购应在发起验证前进行。

5.2 Validation of the Equipment, Software and Method设备、软件和方法的验证
The validation of an alternative or rapid method involves the entire system. Therefore, prior to validating the actual microbiological test method, the testing equipment/instrumentation and the associated software/computer system should be qualified. For this process, an adaptation to the Analytical Equipment Qualification Model maybe used (32).
一个可选择的或快速方法的验证涉及到整个系统。因此，在验证前，测试设备/仪器以及相关软件/计算机系统应经过确认。这一过程可采用分析设备确认模型。

The validation steps described in the following sections provide a useful framework that can be applied to the validation of a complete system (i.e., all the components of the new test method including any instrumentation, software, firmware and reagents), and guides the end-user through the process steps involved in the decision-making and practical work required for implementing a new alternative or rapid microbiological analytical instrument and associated method(s). It is of note that the framework provided here reflects a highly detailed approach, and some companies may not consider some of the validation deliverables a requirement for commercial off-the-shelf equipment (e.g., conducting a Design Qualification). As such, the overall validation strategy for particular equipment should be evaluated in a Risk Assessment and documented in the Validation Plan (refer to Section 5.2.1 for further detail).
下述章节描述的验证步骤提供了一个有用的框架可用于整个系统的验证（即：新测试方法的所有部件，包括仪器、软件、计算机固件和试剂），并可指导最终用户在实施一个可选择的可选择或快速微生物分析仪器和相关方法的整个过程中所涉及的决策和实际工作。应注意的是这里提供的框架描述了非常详细的方法，有些公司可能不会考虑某些现货设备的验证要求（如：设计确认）。因此，某设备的全面验证策略应进行风险评估并记录在验证计划中（详见5.2.1节）。

Not all the activities in each section need to be carried out in serial order, and as such, parallel path activities can occur (e.g., combining the IQ and OQ, if appropriate). However, it should be noted that some activities should not begin until previous activities have been completed (e.g., the PQ should be initiated only after it has been demonstrated that the OQ acceptance criteria have been met, and this phase of the validation plan has been reviewed and approved). The deliverables to be considered for validation, in addition to the recommended responsibilities for each task, are provided in Table 5.2-1.
不是各章节描述的所有活动都需要按顺序执行，因此，可以同时进行（如适应可合并IQ和OQ）。但是，应注意某些活动应在前期活动完成时再进行（如PQ只能在证明OQ满足接受标准时开始，并且这一阶段的验证计划已经过审核和批准）。除了各项工作建议的职责之外，应交付的验证项目见表5.2-1

Table 5.2-1 Validation Deliverables and Responsibilities
应交付验证项目和职责

Validation Deliverable 应交付验证项目	Responsibility职责	
	User用户	Supplier供应商
Risk Assessment and Validation Plan风险分析和验证计划	√	
User Requirements Specification (URS)用户需求规格	√	
Design Qualification (DQ)设计确认	√	√
Functional Design Specification (FDS)功能设计细节	√	
Requirements Traceability Matrix (RTM)需求追踪组合	√	
SOPs and Technology Training SOP和技术培训	√	√
System Integration系统整合	√	√
Installation Qualification(1)安装确认	√	√
Operational Qualification(1)运行确认	√	√
Performance Qualification (Method Validation)性能确认（方法验证）	√	
Suitability Testing适应性测试	√	
On-going Maintenance and Periodic Reviews 持续维护和定期回顾	√	√

Suppliers and end-users may perform their own IQ, OQ and on-going maintenance. The end-user should determine the extent of each party's roles in meeting the deliverables described above.
供应商和最终用户可执行各自的IQ、OQ和持续维护。最终用户应确定各方职责范围以满足上述要求。

5.2.1 Risk Assessment and Validation Planning风险分析和验证计划

In keeping with the principles introduced in Section 3.5, the first deliverable of any validation exercise should be a documented Risk Assessment. This is essentially a high level review of the potential risks associated with implementing a new microbiological method, as well as the factors that are likely to influence the overall approach to the validation of the new method (e.g., criticality of the generated data, system complexity/maturity, use of electronic records/signatures). Critical method steps and parameters, technical and scientific risks on method performance and the resulting data should be considered in this assessment. The output from this activity will be an appropriately scaled and focused validation effort that supports the pre-defined use of the new method.
与3.5节描述的原理一致，任何验证活动的第一步都应该是书面的风险分析。应深入评估与实施可选择的微生物方法相关的潜在风险，以及可能影响总体的验证方法的因素（如：产生的数据的关键性，系统的复杂程度/成熟度，使用的电子记录/签名）。关键方法步骤和参数，方法性能的技术和科学风险以及最终数据应在该评估中考虑到。这项活动的结果将作为支持新方法既定用途的量化的和关键的验证成果。

The next phase is the development of an overall validation strategy, or Validation Plan. It is the responsibility of the end-user to ensure that the Validation Plan is appropriate and correctly documented. Depending upon a company's requirements, the Validation Plan may or may not include those activities as described under the pre-validation activities (Section 5.1).

下一个步骤是开发总体验证策略或验证计划。最终用户应负责确保验证计划进行了适宜的准确的记录。按照一个公司的要求，验证计划可能包括或不包括如5.1节描述的验证前活动。

When it is determined that the new method requires validation then an approved Validation Plan should be followed which will govern the process from beginning to end and will detail precisely what activities are necessary to produce an appropriately validated system. Another key component of the Validation Plan is the definition of system validation responsibilities, such as the identification of the individuals or organizations/departments responsible for performing, reviewing and approving the work. The Validation Plan should also specify how deviations from the approved testing strategy are handled, documented, reviewed and approved. There are also situations where validation responsibilities may fall on both the end-user and the supplier of the new method (see Table 5.2-1).
当确定一个新方法需要验证，应遵循批准的验证计划以控制从始至终的整个过程并将详细描述必要的活动以生成适宜的验证系统。验证计划的另一个关键部分是系统验证职责的定义，如确定负责执行、审核和批准工作的各人员或组织/部门。验证计划也应明确如何处理、记录、审核和批准偏离已批准测试策略的偏差。也有最终用户和新方法供应商共同承担验证职责的情况（见表5.2-1）。

Finally, it may not be possible to verify every feature associated with every piece of equipment. Therefore, a decision should be made regarding the relevance of testing those features of the system (e.g., during the IQ and OQ) that will not be used during the routine use of the new test method. One way to address this type of concern is to obtain a certificate of conformance for those particular features within the instrument received from the supplier of the system. Alternately, the Validation Plan should specifically exclude those features and the reason why they will not be tested. For example, a system may offer the option to document assay parameters via a thermal printer or a regular printer. Depending on which option a company decides to pursue, the other may not be applicable and therefore not in scope for testing. Similarly, if a computer function will not be used routinely, then that computer function may not have to be tested during validation.
最后，不可能确认与每一个设备相关的所有特征。因此，应确定如何测试（如：在IQ和OQ中）该系统不会被日常使用到的特征。解决这个问题的一个方法是从供应商处获得这些特有特征的符合性证明。验证计划应明确排除那些特征以及明确为什么这些特征不被测试的原因。例如，一个系统可提供通过热敏纸或常规打印以记录检测参数的选择。

5.2.2 User Requirements Specification (URS)用户需求规格

The User Requirements Specification (URS) is a key document that explicitly describes the characteristics of the method that will be required for routine use. As such, the content of the URS may well determine the success or failure of the method selection process. The specification is typically prepared by the end-user; however, it is important to seek input from other internal stakeholders, such as Regulatory Affairs, Quality, Information Technology (IT), and other relevant validation groups, as well as potential suppliers of the method. Many alternative and rapid method suppliers possess their own system specification, often referred to as an External Specification or Supplier URS. Careful review and comparison of the supplier's URS and the end-user's URS may help identify criteria not included in one or the other which may be critical performance characteristics to be considered during the system selection process. This may include not only the microbiological aspects of the method and accompanying system, but also throughput, automation and environmental requirements, supplier expectations and/or communication and computer system capabilities (e.g., Laboratory Information Management Systems or

LIMS interface, data management). In cases where the end-user requires a very specific workflow (e.g., based on sample matrices and/or applications), the URS may serve as a starting point for additional performance characteristics that the end-user and the supplier may need to consider and/or develop.
URS是明确描述将用于日常使用的方法特征的关键文件。因此，URS的内容可能决定方法选择的成功或失败。该要求一般由最终用户起草；但是，寻求内部相关者的意见非常重要，如药政部门、质量部、IT及其他相关的验证小组以及潜在的方法供应商。许多可选择和快速方法供应商都有其自己的系统要求，通常被引用为外来要求或供应商URS。仔细审核和对比供应商URS以及最终用户的URS可能帮助识别彼此文件中可能未包含的标准，这些标准可能在系统选择过程中被认为是关键的性能特征。这一过程可能不仅包含该方法及其相关系统的微生物方面，也应包含生产能力，自动化程度和环境要求，供应商期望和/或沟通以及计算机系统能力（如：实验室信息管理系统或LIMS界面，数据管理）。如果最终用户要求非常具体的工作流程（如：基于样品组合和/或应用），URS可作为最终用户和供应商需要考虑和/或开发的其他性能特征的起始点。

The URS will also be the starting point for establishing the validation criteria that will be tested by the end-user. Therefore, the URS should describe the functions that the method and accompanying system must be capable of meeting that will be very specific for the end-user s needs and the materials to be assessed. Essentially, the requirements specified in the URS will directly influence the entire validation strategy and acceptance criteria.
URS也是建立最终用户验证标准的起始点。因此，URS应描述方法和相关系统必须满足的功能，这些功能是专门针对最终用户的需求和需要评估的物料。本质上来说，URS中的要求将直接影响整个验证策略和接受标准。

The functions that the method and accompanying system must be capable of performing will most likely be specific for each technology and the manner in which they are to be used. Examples may include, but are not limited to, the application, the level of sensitivity (limit of detection or quantification), microorganisms to be detected or identified (specificity), sample handling, automation and data management.
方法和相关系统必须具有的功能应具体针对其将使用的各技术和使用的方式。示例包括但不限于，应用、灵敏度（检测限或定量限），需要检测或鉴别的微生物种类，样品处理，自动化程度和数据管理。

5.2.3 Design Qualification (DQ)设计确认

Design Qualification (DQ) is documented review and verification that the proposed design of the equipment or system is suitable for its intended purpose. Therefore, the DQ is most critical if no commercial off-the-shelf equipment is available for the intended application, and must be specifically designed for that purpose. However, since most alternative and rapid microbiology methods/systems are commercial off-the-shelf equipment, the DQ serves to verify that the equipment specifications will meet the requirements as provided in the URS. The DQ may also take data from proof of concept testing into consideration, when appropriate. While this is termed "qualification" and is part of the qualification process, the DQ activity typically occurs prior to purchasing and validation of the method and accompanying system.
设计确认是书面审核和核实设备或系统的设计能够满足预期用途的过程。因此，如果没有现货设备用于预期应用并必须专门设计，DQ就非常关键。但是，因为大多数可选择和快速微生物方法/系统都是现货设备，DQ一般用于确认设备规格是否满足URS中的要求。如适用DQ也可考虑采用概念论

证测试中产生的数据。由于将其定义为“确认”并作为确认过程的一部分，DQ活动一般在方法和相关系统的采购和验证之前进行。

The DQ can be performed by the end-user and/or the supplier; however, the end-user is responsible for verifying that the method and accompanying system meets the requirements as specified in the URS. The methods for accepting the DQ and the instruments' suitability for use will be determined by the nature of the instrument, the complexity of the proposed application, the complexity of the software used for instrument operation and data analysis and the prior history with the supplier. Vendor audits, vendor-supplied documentation reviews and/or direct examination of the system can satisfy the DQ requirement.
DQ可由最终用户和/或供应商执行。但是，最终用户有职责确认方法和相关系统满足URS中的要求。接受DQ和仪器使用的适宜性的方法应由仪器的性质、应用的复杂性、仪器操作和数据分析软件的复杂性以及供应商历史数据决定。供应商审计，供应商文件审核和/或系统直接检查可满足DQ要求。

5.2.4 Functional Design Specification (FDS)功能设计确认

Prior to initiating the validation phase, a separate Functional Design Specification (FDS) document that describes all of the performance functions and requirements for the method and accompanying system, and what will be tested to ensure that the method and accompanying system performs as specified in the URS, should be written. The FDS can include microbiological and performance characteristics such as system and method functionality, configuration, input/outputs, environmental conditions, utilities, computer and communication architecture, interfaces, data management and security. The FDS can also point directly to the specific test scripts where each performance function or requirement will be evaluated and verified against pre-established acceptance criteria. For example, test scripts can be placed within the Installation Qualification (IQ), Operational Qualification (OQ) or Performance Qualification (PQ) sections of the validation plan. The FDS may be written by the end-user, the supplier or both parties, and may also be incorporated into other relevant documents, including the URS, depending on a company's validation requirements or preferences.
验证阶段开始前，应起草单独的FDA文件以描述方法和相关系统的所有性能功能和要求，需要的测试以确保方法和相关系统如URS要求的运行。FDS可包含微生物学和性能特征如系统和方法的功能性，构造，输入/输出，环境条件，公用设施，计算机和交流，界面，数据管理和安全性。FDS也可直接指出具体的测试方案以描述何时性能功能或要求将与既定接受标准对比进行评估或确认。例如，测试方案可被放在IQ，OQ或PQ中。FDS可由最终用户、供应商或双方共同起草，也可与其他相关文件合并，包括URS，这依赖于公司的验证要求或选择。

5.2.5 Requirements Traceability Matrix (RTM)需求追踪矩阵

The Requirements Traceability Matrix (RTM) provides traceability that all the requirements listed in the FDS and/or URS have been verified and/or tested. This may be considered a validation checklist and is, for all intent and purposes, a living document during the execution of the validation test scripts or protocols.
RTM用于追踪FDS和/或URS的要求是否已进行确认和/或测试。其可被视为验证清单以及在执行验证测试方案中用于所有预期用途的现有文件。

5.2.6 Standard Operating Procedures (SOPs) and Technology Training　SOP和技术培训

Standard Operating Procedures (SOPs) that facilitate the proper execution of the method and system

instrumentation should be written and approved prior to the execution of the validation plan. The need for effective instructions is important in order that personnel can understand exactly how to perform the new test, operate and maintain the associated instrumentation. SOPs should be appropriate, clear, accurate and approved by the proper individual/organization. Often the supplier of the new method will provide instructions for the system in order that they can be incorporated into an end-user's internal SOPs with minimum effort. However, it is the responsibility of the end-user to ensure that all SOPs are appropriate for their intended use.
在实施验证计划前应起草和批准用于方便执行方法和系统仪器的SOP。应按步骤进行指导以便于操作人员能够准确的理解如何执行新方法，操作和维护相关仪器。SOP应适宜、清楚、准确并有适宜人员/部门批准。一般来说新方法供应商会提供系统操作步骤指导以便于可写入最终用户的内部SOP并减少差错。但是，最终用户有职责确保所有SOP适于其内部使用。

Additionally, those analysts that will conduct the validation and/or operate the system should be appropriately qualified for these purposes. Therefore, training with the system supplier should be completed prior to the start of the validation activities, and this training may be conducted in-house (usually during the initial commissioning of the equipment) and/or at the supplier's own training facility, when available.
另外，实施验证和/或操作系统的分析人员应经过确认。因此，应在开始验证活动之前完成供应商培训，并且该培训应在公司现场（通常在设备初始调试阶段）和/或在供应商的培训场地进行。

5.2.7 System Integration系统整合

System integration usually refers to information technology (IT), or computer systems, and involves bringing together all of the component subsystems into a single, operating system and ensuring that all of the components of the system function appropriately. If the alternative or rapid technology requires to be connected to a separate data management storage and retrieval system, such as a Laboratory Information Management System (LIMS) or similar platform, system integration between these various components may be required. The supplier may need to work directly with the end-user's IT organization in developing an acceptable integration plan.
系统整合一般指IT或计算机系统，并涉及结合所有部件支系统，操作系统和确保所有系统部件功能适宜。如果可选择或快速技术要求连接单独的数据管理储存和追踪系统，如LIMS或类似平台，可能要求不同部件间的系统整合。供应商需要与最终用户直接配合以开发一个可接受的整合计划。

5.2.8 Installation Qualification (IQ)安装确认

Installation Qualification (IQ) studies should establish that the equipment is properly and safely installed with the correct utilities in an appropriate laboratory or, in some cases, manufacturing environment. A significant part of installation qualification is a verification that all new equipment was received and meets the design specifications for the equipment ordered. Any exceptions to the original specifications should be documented, showing the corrected specification and approvals. It should also be noted that an IQ is instrument specific and portions of an IQ may need to be repeated if the equipment is moved within the laboratory or to another user site. An exception to this rule may be for instrumentation designed to be portable.
IQ研究需要确定设备在适宜的实验室或生产环境中正确和安全的安装并连接正确的公用系统。IQ的重要部分是确认所有新设备已接收并满足设备采购的设计规格。应记录所有违背初始规格的异常，显示更正的规格和批准情况。应注意IQ是仪器专属的并且如果设备在实验室内搬动或转移至其他

使用地点IQ的一部分需要重复。移动式仪器可免于此原则。

IQ studies should be performed in accordance with an approved protocol. Examples of the fundamental types of information to be included in an IQ document include system descriptions, utility requirements, operating environmental conditions, safety features, calibration requirements, software to be installed, and supporting documentation (e.g., technical manuals, blueprints, drawings). Computerized or microprocessor controlled systems should also document important features such as dip switch settings, cabling connections, microprocessor chips utilized, the computer configuration, any special features of the equipment required, printer connections, buffers, files, and memory requirements. It is also important to document the software required and appropriate version numbers. This includes any operating systems used by the computer.
IQ研究应按批准的方案进行。IQ文件中包含的基本信息类型应涵盖系统描述、公用系统要求、操作环境条件、安全特点、校验要求、软件安装和支持文件（如技术手册、蓝图、图纸）。计算机或微处理机控制系统应记录重要特征如变光开关设置、卷缆柱连接、微处理机系统连接、缓冲、文件夹和记忆要求。记录需要的软件和适宜的版本号也很重要。包括计算机使用的所有操作系统。

The IQ may be carried out by the supplier during the initial installation or system integration of the equipment and witnessed by the end-user. However, depending on the extent of the supplier's IQ as compared with receiving company's validation requirements, a separate and more extensive IQ may also be performed by the end-user.
IQ可在初始安装或设备系统整合阶段由供应商执行并由最终用户见证。但是，根据供应商IQ的范围与接收公司的验证要求对比，可能需要最终用户进行单独的范围更广的IQ。

5.2.9 Operational Qualification (OQ)运行确认
The Operational Qualification (OQ) verifies and documents that relevant system parts or functionalities (and if applicable, associated software) work within pre-determined limits when operated in accordance with their operational procedures. Typical OQ parameters may for instance be verification of specified heating or cooling rates, adequate performance of optical systems or proper functioning of the user interface.
OQ确认和记录当按照操作规程进行操作时相关系统部分或功能性（及相关软件）在既定限度内工作。典型的OQ参数比如确认具体的加热或冷却速度，光学系统适宜性能或使用者界面的适当功能。

Furthermore, there is an expectation that any system, which will be used to generate regulated electronic records (and/or electronic signatures), is appropriately validated for this use (e.g., to generate records which are accurate and reliable and which can be appropriately maintained and accessed, or to comply with the expectations in 21 CFR Part 11). Other examples of computer testing that will be performed during the OQ include, but are not limited to, administrator control and operator access, user ID and password set up, user and system lockout, data, audit trails, report generation, data transfer and server communication, data backup and recovery, database management and integrity, and interference (radio frequency, electromagnetic or wireless). Additional guidance and requirements regarding the validation of computer systems may be found in EU Annex 11, 21 CFR Part 11, and Good Automated Manufacturing Practice (GAMP) (33-35).
另外，希望任何将用于产生受控电子记录（和/或电子签名）的系统对其使用进行验证（如：用于产生准确可靠的记录以及能被适宜的维护和权限设置，或符合21CFR第11章的要求）。其他OQ中

的计算机测试的实例包括但不限于管理权控制和操作权限，数据转移和终端交流，数据备份和恢复，数据库管理和完整性，界面（电台频率，电磁或无线电）。其他与计算机验证相关的指南和要求可参见欧盟GMP附件11,21CFR第11章和GAMP。

5.2.10Performance Qualification (PQ)性能确认

Performance Qualification (PQ) provides documented confirmation that the equipment, as installed and operated in accordance with operational procedures, consistently performs in accordance with predetermined criteria and thereby yields correct and appropriate results. This may, for instance, be demonstrated through the successful validation of a microbiological test method using that particular equipment or through application of another experimental setup, which may also encompass an appropriate selection of test microorganisms and/or other material (e.g., nucleic acid standards, fluorescent particles, etc., depending upon the technology).

PQ为设备按照操作规程进行安装和操作，按既定标准持续运行并由此产生正确和适宜的结果提供书面证明。PQ可通过微生物测试方法的成功验证得以证明，验证过程使用特定设备或通过应用其他实验性设置，其可能围绕测试微生物和/或其他物料（如核酸标准品、荧光微粒等，基于技术而定）的成功选择。

Method Validation方法验证

Method validation is the process by which it is experimentally demonstrated that the alternative or rapid method is adequate for the intended application and performs in a reliable manner. Furthermore, where applicable, it has to be demonstrated that the alternative or rapid method performs at least equivalently (i.e., it is non-inferior) to the existing or reference/compendial method intended to be replaced. This is also referred to as equivalence or comparative testing. Section 5.3 provides detailed guidance on the parameters that should be addressed during method validation.

方法验证是用试验证明可选择或快速方法适用于预期应用并且运行可靠的过程。另外，如适用，必须证明可选择或快速方法的性能至少与现有或欲替代的参考/药典方法等同。它也被称为等效性或对比测试。5.3节提供了应在方法验证中阐述的参数的具体指南。

Suitability Testing适应性测试

Since the validated method will be used with actual product or test samples, suitability testing should also be performed to demonstrate that the presence of a particular product, material or sample matrix does not significantly impact the performance of the alternative or rapid method. Section 5.4provides detailed guidance on the parameters that should be addressed during suitability testing, including false-positive and false-negative assessments.

由于被验证的方法将用于实际产品或测试样品，应进行适应性测试以证明特定产品、物料或样品组合的存在不会显著影响可选择或快速方法的性能。5.4节提供了应在适应性验证中阐述的参数的具体指南，包括假阳性和假阴性评估。

5.2.11 On-going Maintenance and Periodic Reviews持续维护和定期审核

Following validation of the new method and accompanying system, appropriate procedures should be established to maintain the system in a validated state, for example, SOPs should be implemented, the method and accompanying system should be included in a change control program, and all instrumentation or equipment should be maintained in good working condition.

新方法和相关系统验证之后，应建立适宜的规程以保持系统在验证状态，如，执行SOP，方法和相

关系统应包含在变更控制程序内，所有仪器或设备应保持良好的工作状态。

Once the new method and accompanying system has completed validation, subsequent change controls should assess the impact of the change on the validation status of the system. Additionally, a formal mechanism should be put in place to periodically review the method and accompanying system's performance, as well as the overall validation program in relation to current GMPs.
一旦新方法和相关系统完成验证，随后的变更控制应评估变更对于系统验证状态的影响。另外，应建立正式的机制以定期审核方法和相关系统的性能以及与cGMP相关的总体验证规程。

A special area of concern is the preventive maintenance program, frequently handled by the equipment manufacturer of specialized equipment. Many programs include updating system software with the most up-to-date software versions, extension of databases and periodic calibration checks. It is important to ensure that appropriate re-qualification testing is performed before placing the system back into use and that the end-user is cognizant of GMP requirements and performs the maintenance accurately in addition to documenting the activities that were performed. The Supplier should also provide information on the potential impact of repairs or spare part changes, in order to define re-qualification or verification requirements.
有一个特殊的关注就是预防性维护规程，由具体设备的生产商定期执行。许多规程包括更新系统软件至最新软件版本，数据库扩展和定期校验检查。重要的是要确保适宜的再确认测试在重新使用系统之前的执行，并且最终用户知道GMP要求并且除了记录行动之外还要准确的维护。供应商应提供关于维修或备件更换可能造成的影响的相关信息，以明确再确认或确证要求。

Finally, the results or outputs of this phase should feed back into the first phase of the validation process, namely, risk assessment, to ensure that the overall operation of the new method and accompanying system does not introduce additional quality risks associated with the lifecycle of the products and sample matrices that will be tested routinely.
最后，该阶段的结果应反馈至验证过程的第一阶段，也就是风险评估，以确保新方法和相关系统的总体运行不会引入其他的与产品欲测试的产品生命周期和产品组合相关的质量风险。

5.3 Establishment of Method Validation Criteria建立方法验证的标准

The task force for this revision of this Technical Report has considered the USP, Ph. Eur. and the original PDA Technical Report No. 33 recommendations, in addition to current industry practice and regulatory expectations for the validation of alternative and rapid methods, in developing a selection of acceptable strategies for satisfying the validation criteria for quantitative and qualitative alternative and RMM (microbial identification methods are addressed later in this Technical Report). The recommendations are found in Table 5.3-1.
对于定量和定性的可选择和RMM方法（微生物鉴别方法在本TR随后的章节中进行阐述），在开发选择满足验证标准的可接受策略时，除了考虑现在的行业规范和法规期望，改版技术报告工作组已考虑了USP，欧洲药典和TR33的上一版的建议。这些建议可见表5.3-1

Method Validation方法验证

Method validation is the process by which it is experimentally demonstrated that the alternative or rapid method is adequate for the intended application and performs in a reliable manner. Further-more, where applicable, it has to be demonstrated that the alternative or rapid method performs at least equivalently

(i.e., it is non-inferior) to the existing or reference/compendial method intended to be replaced. This is also referred to as equivalence or comparative testing. Section 5.3 provides detailed guidance on the parameters that should be addressed during method validation.
方法验证的过程是通过实验证明了可选择或快速的方法是适合预期应用并以可靠的方式执行。进而，在适用情况下,它打算替代已存在的方法/药典方法时必须证明可选择方法或快速方法执行至少等同于(例如，它不是劣等的方法。)现有的或对照/ 药典方法。这也称为等价或比较测试。第5.3节提供了详细的指导,应该解决方法验证的参数。

Suitability Testing适用性测试
Since the validated method will be used with actual product or test samples, suitability testing should also be performed to demonstrate that the presence of a particular product, material or sample matrix does not significantly impact the performance of the alternative or rapid method. Section 5.4 provides detailed guidance on the parameters that should be addressed during suit-ability testing, including false-positive and false-negative assessments.
当验证的方法用于实际生产或测试样品或组合，适用性测试应该被执行以证明存在的特殊的产品、材料或者样品组合中不会显著的影响可选择或快速方法的性能。第5.4部分参数方面提供了在详细的指导，这些参数在适用性测试期间要进行测试，包括假阳性、假阴性的评价。

Table5.3-1 method validation criteria
表5.3-1 方法验证参数

Validation Criteria 验证参数	Quantitative Method 定量方法	Qualitative Method 定性方法
Accuracy准确度	Yes是	No否
Precision精密度	Yes 是	No否
Specificity专属性	Yes 是	Yes是
Limit of Detection检测限	Yes 是	Yes是
Limit of Quantification定量限	Yes 是	No否
Linearity线性	Yes 是	No否
Range范围	Yes 是	No否
Ruggedness耐用性	Yes 是	Yes是
Robustness鲁棒性	Yes 是	Yes是
Equivalence/Comparative Testing等价性/比较测试	Yes 是	Yes是

All of the validation criteria, with the exception of Equivalence/Comparative Testing, are usually proven with standardized microbial suspensions (e.g., different cultures of bacteria, yeast and mold) in suitable diluents. During validation criteria testing, these microorganisms are used to demonstrate that the acceptance criteria (specific to each validation criterion) are met, and, when applicable, are at least equivalent with the results of the existing method (i.e., when using these same standardized microbial suspensions or an equivalent preparation).
所有的验证标准除了等效性/比较测试外，通常要用不同稀释剂中的标准微生物混悬剂来验证（例如，不同种类细菌，酵母菌和霉菌）。验证标准测试期间，这些菌悬液用来验证这些可接受标准是合适的（针对每个验证标准），并且如果适合，至少与现有的方法（例如，当用这些标准菌悬

液或等同的贮备液）是等同。

During Equivalence/Comparative Testing, actual product and/or sample matrices (i.e., test samples from the working environment as opposed to standardized laboratory cultures) are utilized. However, when the product or sample matrices are not expected to contain viable microorganisms (e.g., Water for Injection [WFI], air samples from ISO 5 environments, or test samples that typically provide a negative result during sterility testing or the microbiological examination of nonsterile products), challenging these test samples with known levels of microorganisms, and comparing the response in both the alternative or rapid method and the existing microbiological method, may be required.
等效性评价测试会用真实样品或者样品组合被利用（例如，测试样本来自与工作环境中而不是标准实验室菌种中）。但是当样品或样本模型可能不含有活的微生物（例如，注射用水，ISO5环境下的空气样本，无菌试验典型的阴性结果的测试样品或有菌的样品微生物限度检查结果阴性的样品），挑战这些已知微生物水平的测试样并比较可选择方法和快速检测法与已经存在的微生物检测方法的响应是需要的。

Actual product and sample matrices are also utilized during the assessment of background noise, interference and the potential for false positive or false negative results (i.e., this is covered in the section on Suitability testing).
真实的样本和样品组合也被用来评价背景噪音、干扰和潜在的假阳性、假阴性结果。（例如，这个被包括在适用性检查中）

The following sections provide definitions and protocol recommendations for each of the validation criteria, including testing procedures, acceptance criteria and statistical analyses, for quantitative and qualitative alternative and RMM.
下面这个部分为定性和定量的可选择方法和快速方法提供了每个验证标准的定义和推荐方案，包括测试程序、可接受标准、数据统计分析。

Validation testing may also be designed and executed where the data from one validation criteria study may be used for several other validation criteria requirements. For example, the data derived during the test for Linearity may also be used for the test for Accuracy.
验证测试也可能通过设计和执行来实现一个验证标准的数据可以用去几个其它验证标准要求。例如，线性测试的数据参数可能适合准确度测试。

It should also be noted that these recommendations are not all inclusive, and end-users may find alternative strategies (e.g., revised, additional or reduced testing, based on risk assessments) that are also satisfactory for use and accepted by regulators. In this instance, end-users are strongly encouraged to discuss their validation strategies with the relevant regulatory authorities prior to initiating their proposed validation plan.
值得注意的是这些推荐不是包括所有的，最终用户要找好可选择方法（修订、增加、减少测试都要根据风险评估来确定）使用户满意和符合法规。在此情况下，鼓励终端用户在实施他们的验证计划之前与相关法规当局讨论他们的验证策略。

5.3.1 Accuracy 准确度

Definition定义

The accuracy of a quantitative alternative or rapid method is the closeness of the actual test results obtained by the new method to the actual test results obtained by the existing method (e.g., standard plate count). Accuracy should be demonstrated across the practical range of the test, and the range may be dependent upon the new method's quantitative recovery capabilities.
可选择或快速测定法定量的准确性是指新方法测试的结果与已经存在的方法测试结果比较的接近程度（如标准评板计数法）。准确性必须通过在实际测试范围内测试来证明，测试范围将视新方法定量回收率而定。

When using the standard plate count method, the range will be limited by the countable numbers of colonies on a plate for a particular dilution of a microbial suspension (e.g., 25 to 250 cfu). Accuracy is usually expressed as the percentage recovery of microorganisms by the new method as compared with the actual recoveries from the existing or traditional method. Accuracy in terms of microbial identification systems is separately addressed in Section 5.7.
当用标准平板计数法时，技术范围受到特殊稀释级别的菌悬液在平板上形成的菌落可数数目限制（如25到250cfu）。准确度通常用新方法实际测试数量与已经存在的或传统的方法测得的数量比较而得的百分回收率来表示。微生物鉴别系统准确度将在5.7中进行阐述。

Procedure程序
Prepare a suspension of microorganisms in a suitable diluent at the upper end of the range of the test and serially dilute down to the lower end of the range of the test. At least five (5) suspensions across the range of the test should be analyzed. Additionally, accuracy should be measured at each of the suspensions using an appropriate number of replicates (e.g., at least triplicate), especially at the lower concentrations, where the variability on recoveries may be more pronounced. Actual recovery counts are obtained for each suspension using the new and the existing methods. The percent recovery for the new method is determined by comparing the recovered counts obtained by the new method with the recovered counts obtained by the existing method.
准备一份在适合的稀释剂中制备的菌悬液，浓度为测试范围的上限，连续稀释至低限级别。至少准备五个级别的菌悬液来测试。另外，每个级别至少平行制备多个平皿（例如，至少三个）来确定准确度，尤其是低浓度，回收率的可变性很明显。实际回收率来自于每个级别新方法和老方法计数。新方法的百分回收率是新方法得到的菌落数比上老方法得到的菌落数。

Acceptance Criteria and Statistical Analysis接受标准和统计分析
The new method should provide equivalent or better results than the existing method. The new method should provide a recovery of viable microorganisms not less than 70% of the actual recovery provided by the existing method for each suspension.
新方法测试的结果应该等效或优于老方法。每个稀释级别的菌液新方法测试得到的活的微生物的回收率比老方法得到的微生物回收率不少于70%。

Alternatively, a statistical comparison between the new method and the actual recoveries using the existing method may be performed. If the methods generate data that are normally distributed and have equal variances, a simple approach is to apply a Student t-test. If variances are not equal, a Student's t-test with Welch's correction may be used. If CFU counts do not follow a normal distribution, these should be transformed in order to have an almost Gaussian distribution by using, for example, the log of the counts or the square root +1 (which allows inclusion of "0" values). If CFU counts do not follow a normal

distribution despite transformation, an adequate nonparametric test should be applied (e.g., Mann-Whitney-Wilcoxon test).
另外,可能会执行统计比较使用新方法和使用老方法的实际回收率。如果方法产生的数据是正态分布,方差相等,一个简单的方法是应用t检验。如果方差不相等，t检验可以使用韦尔奇的修正。如果CFU计数不遵循正态分布,这些应该转变，例如,用log值或平方根+ 1(它允许包含“0”值)，来实现一个近高斯分布。如果CFU计数不遵循正态分布无法转换，那么要采用成一个适当的非参数检验（例如，Mann-Whitney-Wilcoxon测试)。

Analysis of variance (ANOVA) can also be utilized. The use of ANOVA is primarily indicated if more than two groups of data should be compared (cases with only 2 groups can be covered with Student's t-test). Also ANOVA requires normally distributed data and equal variances. If CFU counts do not follow a normal distribution, these should be transformed in order to have an almost Gaussian distribution by using the log of the counts or the square root +1 (which allows inclusion of "0" values). If CFU counts do not follow a normal distribution despite transformation, an adequate nonparametric test should be applied (e.g., the Kruskal-Wallis one -way analysis of variance test). If a significant difference between the data sets exists, Post-tests (e.g., Tukey's test) indicate the data sets, which are significantly different. Some Post-tests rely on comparison of confidence intervals. If the confidence interval for the differences between the true means of the new and the existing methods contains zero (i.e., the upper limit is a positive number and the lower limit is a negative number), then there is no statistically significant difference between the two methods. Alternatively, because microbiological counts tend to follow a Poisson distribution, the ratio of the means maybe used; if the confidence limit of the ratio contains one, no statistically significant difference exists (36,37).
方差分析也可以被使用。有多组数据用来比较可以使用方差分析(例，t检验只能分析两组数据)。还有方差分析需要正态分布数据和方差相等。如果CFU计数不遵循正态分布,这些应该转变为了有一个几乎高斯分布使用在计数日志或平方根+ 1(它允许包含“0”值)。如果CFU计数不遵循正态分布无法转换,应该用一个适当的非参数检验(如适用,Kruskal-Wallis单向方差分析测试方法)。如果试验后数据集之间的显著差异存在,Post-tests(如,tukey’s的测试)可用于分析这些明显不同的数据集。一些Post-tests测试依靠置信区间的比较。如果新方法和已有方法差别的置信区间包含零值，(即上限是一个正数,下限是负数),那么这两种方法之间没有统计上的显著差异。另外,因为微生物计数往往遵循泊松分布,也许会使用比率方法,如果比率的置信区间包含一,那么就不存在统计上的显著差异(36、37)。

However, it should be noted that the two methods may be statistically significantly different but this difference is of no practical consequence. In other words, the results should be reviewed against the requirements of the test. The reason is that even smaller differences become significant with increasing sample size, leading to situations where the difference is significant but the actual recovery of the new method is clearly higher than 70%.
然而，值得注意的是两个方法可能在统计学上存在差异，但是实际结果可能没有差异，换言之，根据需要所做的测试结果应该进行回顾。原因是当批量增大时，原本微小的差异可能变得严重,导致两种方法的差别变得严重，但实际新方法回收率明显高于70%。

On the other hand, insufficient sample size may lead to situations where no statistical difference is detectable, although mean recovery may be below 70% (this may be the case with high standard deviations). Therefore, it may be advisable to use test power calculations in order to verify appropriateness of the used number of replicates. If no systematic power calculations are performed, used

sample size should not be lower than six (6) replicates per data group and statistical test (36, 38).
另一方面，样本量不足可能会导致可检测的情况下无统计差异,虽然平均回收率可能低于 70%(这可能是高标准差)。因此较聪明的做法是使用测试功率计算,以验证适当的使用平行样品的数量。如果没有执行系统的功率计算, 每个数据组和统计检验使用样本大小不应低于 6 次平行，(36,38)。

As opposed to classical hypothesis testing which focuses on whether the application of different experimental methods leads to different outcomes, statistical equivalence or non-inferiority tests may also be used.
不同于经典假设检验专注于不同的实验方法是否导致不同的结果,统计等价或非劣性测试也可以使用。

Due to potential differences in microbial detection methods, there exists the possibility that the new method will recover a higher number of microorganisms than the existing method (e.g., when the new method does not rely on microbial growth). Because it is not possible to predict the outcome of microbial recovery of all alternative or RMM and for all sample types, this TR does not recommend an acceptable upper level for recovery during Accuracy studies. The end-user may want to consider establishing relevant upper levels, as applicable for the technology being evaluated, to ensure that recovery counts are acceptable and not due to sample, method or instrumentation issues. Here, in-put from the supplier of the alternative or rapid method may be appropriate.
由于潜在的微生物检测方法的差异, 存在新方法微生物的回收率比现有的方法微生物数量高（如当新方法不依赖于微生物增长）。因为它是不可能预测用于各种样品类型的所有可选择方法或快速方法的微生物回收率的结果, 所有在进行回收率准确度研究时，本TR不建议设置回收率的可接受的上限标准。如果对于评估某一技术合适，最终用户可能想要考虑建立相关上限，以确保回收率方面是可以接受的, 不受样本、方法或仪器的问题的影响。在这里，可选择方法或快速方法的供应商的数据可能是合适的。

5.3.2 Precision 精密度

Definition定义

The precision of a quantitative alternative or rapid method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of the same suspension of microorganisms and using different suspensions across the range of the test. In the context of this section, precision may also be referred to as repeatability, within-run variability or intra-assay precision, and makes use of the method within the same laboratory over a short period of time using the same analyst with the same equipment. The precision of a microbiological method is usually expressed as the standard deviation or, for the purposes of this technical report, the relative standard deviation, which is also referred to as the coefficient of variation.
当使用相同的微生物混悬液反复测试多个的微生物的样品和在测试范围内使用不同的菌悬液时，单个结果之间的一致性就是一种定量的可选择方法或快速方法的精密度。在本节中, 精度也可以被称为重复性，批内精密度，利用该方法在同一个实验室在短时间内使用相同的分析师相同的设备。微生物方法的精度通常表示为标准偏差或本技术报告所用的相对标准偏差，也称为变异系数。

Precision may also include a measure of either the degree of intermediate precision or reproducibility of the microbiological method under normal operating conditions. For example, intermediate precision (also referred to as run-to-run variability) refers to the use of the method within the same laboratory using

different analysts, equipment and on different days. Reproducibility expresses the precision between laboratories, for example, through collaborative studies. Intermediate precision and reproducibility are addressed in Section 5.3.8, Ruggedness. Therefore, when testing for precision, firms may include the use of single or multiple analysts, as desired.
精度也可能包括在正常操作条件下的微生物方法测量的中间精密度的程度或重现性。例如，中间精密度(也称为控制可变性)指的是在同一个实验室，使用相同的方法、不同的分析师、不同的设备和不同的日子做出的结果的精密度。重现性表示实验室之间的精密度。例如，通过协作研究。精密度和重现性将在5.3.8节中进行详细的阐述。因此，当测试精密度时，公司可能会需要单个或多个分析师。

Procedure程序

Prepare a suspension of microorganisms in a suitable diluent at the upper end of the range of the test and serially dilute down to the lower end of the range of the test. As counts approach the lower end of the range of the test (e.g., a single cell), variability in precision will increase. Therefore, suspensions should be used that will be appropriate for assessing precision. At least two (2) to five (5) suspensions across the range of the test should be analyzed . For each suspension, at least five (5) to ten (10) replicates should be assayed for recovered counts in order to calculate the coefficient of varia-tion. Precision calculations are to be performed on both the new method and the existing method.
准备合适浓度的菌悬液（范围上限），再用无菌的操作逐渐稀释至范围的下限备用。当测试计数接近范围下限时（例如，单个菌落），精密度的可变性增加。因此，菌悬液的浓度要适合用作评估精密度。至少要在范围内分析2到5个浓度的菌悬液。每个浓度至少重复做5到10个含量，计数回收率RSD。新方法和现有方法都要进行精密度计算。

Acceptance Criteria and Statistical Analysis可接受标准和统计分析

Generally, the precision of the assay should be appropriate for its intended purpose. The alternative method should not have a variability that is significantly larger than that of the existing method, except when a clear rationale or justification exists why such higher variability can be tolerated. Whether a comparison to the existing method is adequate has to be evaluated by the end-user. For a traditional plate count method, a coefficient of variation of less than 35% for a microbial number higher than 10 cfu is generally expected. If the new method meets the 35% value, there is no need to compare the new method's coefficient of variation with the existing method's coefficient of variation. However, if the new method does not meet a 35% coefficient of variation, then the new method should have a coefficient of variation that is not significantly greater than the coefficient of variation of the existing method.
通常情况，含量的精密度计数要适合它的预期目的。可选择的方法跟现有的方法相比不应该有一个显著的可变系数，除了当有一个清晰的论据或者理由说明为什么一个高的可变系数是可接受的。终端用户应评估与现有的方法的比较是否充分。传统的平板计数法，微生物数目超过10CFU时可变系数低于35%是可以接受的。假如新方法的可变系数满足35%，那么就没有必要同现有的方法进行可变系数的比较。但是当可变系数没有达到35%，那么新方法的可变系数跟现有方法比不能有显著的增大。

To demonstrate that the new method's coefficient of variation is not greater than the existing method's coefficient of variation, the McKay approximation may be used (39). For example, the coefficient of variation of the new method will be declared not statistically greater than the coefficient of variation of the existing method if the confidence intervals intersect, and the upper limit of the 95% confidence

interval for the new method coefficient of variation does not exceed the upper limit of the 95% confidence interval of the existing method by more than 10% (e.g., if the upper limit of the 95% confidence interval of the existing method would be 35%, the upper limit of the 95% confidence interval of the new method must not be higher than 45%).
McKay近似值将被用来证明一个新方法的可变系数没有比现有方法显著的变大。例如，如果新方法的可变系数的95%的置信区间的上限与已有方法的可变系数的95%的置信区间上限相比，没有超过10%，那么可以说明新方法的可变系数没有比已有方法的可变系数显著增高。（例如假如已经存在方法的95%的置信区间上限值是35%，那么新方法95%的的置信区间的上限是值不会超过45%。）

Another approach may be to use a statistical test for equal variance (e.g., Bartlett's test for normal distributed data or Levenes test for not normal distributed data). A third approach may be to statistically compare the coefficient of variation values of the new method with the coefficient of variation values of the existing method using a paired t-test (i.e., coefficient of variation values obtained for the same microbial suspension and from the same test run should be paired).
另外一种方法可以采用统计学方法计算同等变异值（例如，Bartlett's测试用在正态分布数据或Levenes测试用于非正态分布数据上。）。第三种方法就是统计比较新方法和现有方法的可变系数，然后用配对T检验（例如，从同一个菌悬液和相同的测试中获得的可变系数值应该配成对。）

5.3.3 Specificity专属性

Definition定义
The specificity of an alternative or rapid method is its ability to detect a range of microorganisms, which demonstrate that the method is fit for its intended use. For alternative or rapid methods that qualitatively detect a target panel of specific microorganisms, such as those that employ nucleic acid amplification techniques (e.g., as described in Ph. Eur. 2.6.21 and USP <1125>), inclusivity and exclusivity should be demonstrated (40,41).
可选择的或者是快速检验法的专属性是他们检测一系列微生物的能力，就是证明检测方法适用预期的检测目的。对于能够定性检测出某一特别微生物的可选择的方法或者快速方法，比如核酸扩增技术（例如，ep 2.6.21和USP《1125》所描述），包容性和排外性一定要证明。

Specificity should be conducted for both quantitative and qualitative alternative or RMM.
定性的和定量的可选择方法或者RMM方法都应有专属性。

Specificity testing has also been used to demonstrate that an alternative or rapid method is compatible with specific product or sample matrices (e.g., the absence of false positive or false negative results); however, these types of assessments should be conducted under Suitability Testing (refer to Section 5.4).
专属性测试是用来证明可选择方法或快速方法与特殊的产品或者样品组合相适应（例如，无假阳性或者假阴性结果）；但是，这一类评估的应该在稳定性测试项下进行（参照5.4部分）。

Procedure程序
To demonstrate that the new method is able to detect a range of microorganisms (if relevant to the new method and its application), a representative selection of microorganisms, such as Gram-positive and Gram-negative bacteria, yeast, mold and/or bacterial and fungal spores, should be utilized. The end-user should determine what types of microorganisms are to be used (and the number of replicates) during the assessment of each of the validation criteria as described in this Technical Report. This includes the use

of stressed microorganisms, and when appropriate, mixed cultures. It is the responsibility of the end- user to determine the appropriate panel of microorganisms to use for this purpose, which may include standard laboratory or culture collection strains (e.g., ATCC),environmental or facility isolates, in-process or sterility failure isolates, slow-growing, fastidious or anaerobic strains, and/or clinically relevant cultures.
证明新方法可能检测的微生物（如果与新方法和其应用有关联），需要挑选有代表性的微生物，比如革兰氏阳性或者革兰氏阴性的细菌、酵母菌、霉菌或者是细菌和真菌孢子将被使用。在评估验证标准的时候，终端用户必须决定使用那种类型的微生物（和平行测定次数）。
这里包括受影响的微生物，如果合适，混合菌种。终端用户需要决定验证用的合适的微生物种类，可能包括标准实验室或者培养基保藏菌（例如ATCC），环境或者厂房设施分离出的菌种，过程控制或者无菌失败分离出的菌种，生长缓慢菌种，需要复杂培养的或者厌氧生长，或者/和临床相关菌种

Inclusivity and exclusivity testing should be performed for those new methods/systems that rely on the detection of a specific target microorganism(s) but will not report a positive result if another non-target microorganism is present in the test sample.
For example, if a selective, growth-based method or a PCR technique is specific for the detection of E. coli, then inclusivity testing should demonstrate that the presence of E. coli in the test sample would render a positive result; however, when S aureus is present in the test sample, a negative result should be provided.
新方法/系统应该进行兼容性和排外性测试，要能检测出某一特定微生物但当有别的非目标微生物存在样品中时不会报告阳性结果。例如，假如一个选择性的、基于培养生长的方法或者PCR技术专门用来检测大肠埃希菌，这时兼容性测试应该证明大肠埃希菌在检测样品中存在报告阳性的结果；但是金黄色葡萄球菌在检测样品中存在时，结果应该是阴性的。

The end-user should determine the extent of inclusivity and exclusivity testing based on the number of microbial targets the new method is intended to detect, and to select microorganisms that will be detected by the new method (i.e., show inclusivity) in addition to selecting unrelated and closely related microorganisms that should not be detected by the new method (i.e., show exclusivity).
An appropriate number of replicates (e.g., at least 3) should be considered for each target and nontarget microorganism. Additionally, a relevant concentration of microorganisms may also be considered (e.g., lower concentrations for inclusivity and higher concentrations for exclusivity testing).
最终用户应该确定进行包容性和排外性测试的程度，基于新检测方法打算检测的目标微生物的数目，并选择新方法将要检测的微生物（例如，证明兼容性）除此之外还要选择性不应被新方法检测的无关的和密切相关的微生物(例如，证明专属性)。每个目标或非目标微生物都应考虑一个合适数量的平行测试（例如，至少3次）。另外，相关微生物的浓度同样需要考虑（例如，低浓度用来测试兼容性测试和高浓度用来专属性测试）。

When applicable, controls relevant to the new method may also need to be evaluated (e.g., nucleic acid standards). However, these may not be sufficient to evaluate inclusivity in all cases, as they may not cover several aspects of method sample preparation, such as microorganism lysis, nucleic acid capture and purification.
如果合适，新方法有关控制需要进行相关的评价（例如，核酸标准）。但是，这些用来评价所有情况的兼容性是不充足的，因为他们可能不包括方法中样品准备的一些方面，例如微生物分解、核酸捕获和净化。

Mixed Culture Testing混合培养物测试

Mixes may be used to demonstrate that the new method will detect or enumerate more than one type of microorganism during the evaluation of test samples against the validation criteria specified in this technical report, such as accuracy, precision and limit of detection .

在针对本报告中列出的验证标准如准确性、精密度和检测限进行测试样品评价时，混合菌种可能用于证明新方法将检测或者计数多余一种微生物。

It is the responsibility of the end-user to determine the appropriate panel of microorganisms to use for this purpose, if it is determined that this type of testing is required.

最终用户有责任决定用于这个目的的合适的微生物种类，假如这个类型的测试需要进行。

Mixed cultures may be evaluated in the presence of the test sample during Equivalence/- Com-parability Testing or in a suitable diluent during the assessment of other validation criteria, such as accuracy and precision testing.

在进行等效/相容性测试时，存在测试样品时应评价混合菌种，在评价其它验证标准如准确性和精密度测试时，在适当的稀释剂中应评价混合菌种。

Stressed Microorganism Testing受影响的微生物测试

When validating alternative or rapid methods for certain applications, such as sterility testing, process water or environmental monitoring, there may be a requirement to include stressed microorganisms, for example, when evaluating the limit of detection or during equivalence testing.

当验证可替代方法或快速方法的用于特定用途时，比如无菌测试，工艺用水或者环境监控，那么可能会需要包括受影响的微生物，例如，当评价检测限时或者评价等效性测试时。

Exposing to environmental (e.g., UV, heat, cold, pH, extremes in tonicity), antimicrobial (disinfectants, drug product) or sublethal sterilization conditions are examples of methods an end- user may use to obtain stressed microorganisms.

将菌种暴露在环境条件（例如UV，热，冷，ph，极端张力），抗菌的（消毒剂、药品生产）或者半灭菌条件是最终用户会用来获得受影响的微生物的例子。

The stressing method should provide a reliable and reproducible challenge, and therefore, may need to be qualified before use.

施压方法应该提供一个可靠的和可重复挑战，因此在用之前要进行确认。

The goal of the application of stress protocols may either be to force microorganisms into an injured, though still viable state prior to the experiment, from which the microorganisms may recover upon start of incubation if conditions are favorable, or challenge the organisms with a continuous presence of an adverse agent.

施压方案的目标可以是迫使微生物变成受伤的，在实验前是活的状态，在合适条件下还能够开始复制，也可以是在有不利试剂持续存在的情况下挑战微生物。

Whilst the former typically leads to a prolonged lag phase followed by normal growth, the latter may lead to a prolonged generation time.

前者会导致在正常生产前会有延长的停滞期，后者可能会导致长期的处于一代。

A relevant panel of microorganisms that will undergo stress protocols should be chosen as described above.
要按照前面描述的方法来选择经历影响的方案的相关微生物种类。

Additional information on how to stress microorganisms for use in Specificity studies may be found in the public literature (42).
另外专属性实验中如何影响微生物将在公共专著里阐述（42）

Acceptance Criteria and Statistical Analysis接受标准和统计分析
All microorganisms (including stressed microorganisms or mixed cultures, where appropriate) utilized during the testing should be successfully detected and/or enumerated, and meet the specific acceptance criteria.
在测试中使用的所有的微生物（包括受影响微生物或者混合菌种，如果使用）应应该被成功的检测和计算，并且符合特定的接受标准。

Acceptance criteria similar to those that are recommended for other validation parameters may be utilized.
可以使用的接受标准类似于其它验证参数推荐的。
For qualitative methods it should be noted that the number of replicates required may be significantly lower than suggested for assessment of the limit of detection, since higher microbial inoculate may be applied.
对于定性法，应该注意重复测试的需求显著低于用于评估检测限中建议的次数，因为可能使用更高级的微生物接种。

Inclusivity testing should demonstrate that the new method detects the target microorganism(s) it is intended to, and does not produce a positive detection result for unrelated or closely related microorganisms (i.e., exclusivity for nontarget microorganisms). For example, if a known nontarget organism is tested three (3) times, the new method should not detect the organism.
兼容性测试应该证明新方法能够检测到目标微生物，而不相关或者是非常相近的微生物不会产生一个阳性结果（即，非目标微生物的排外性）。例如，假如一个已知的非预期目标的微生物被检测出三次，新方法就不应该用来检测该微生物。

5.3.4 Limit of Detection检测限

Definition定义
The limit of detection of an alternative or rapid method is the lowest concentration of microorganisms in a test sample that can be detected, but not necessarily quantified, under the stated experimental conditions.
可替代方法或快速方法的检测限就是在相应的实验室条件下，样品中能被检测出的最低的微生物数量，不需要定量。

Limit of detection applies to a microbiological limit test, which determines the presence or absence of microorganisms.
检测限适用于微生物限度测试，可以确定微生物是否存在。

The limit of detection refers to the concentration of organisms present in the original sample, before any incubation or enrichment step, and not the concentration of organisms present at the time of the assay.
检出限指的是原始样品中微生物的浓度，没有经过培养或富集步骤，不是在微生物鉴定时存在的微生物浓度。

Also, the amount of sample tested, and the dilution of that sample, may determine the limit of detection.
同时抽样测试时的数量和样品稀释可能检测限。

For example, when 10 grams of test material is diluted in 90 mL of diluent, and 1 mL of the resulting preparation is plated on conventional agar medium, the absence of colonies (CFU) on the plate would be reported as <10 cfu per gram, because the limit of detection is 10 cfu.
例如10g样品用90ml来稀释，移取1ml到琼脂培养基平板中，没有菌落存在，将报告<10 cfu/g，因为检测限是10 cfu。

For alternative or rapid methods that qualitatively detect a target panel of specific microorganisms, such as those that employ nucleic acid amplification techniques, inclusivity should be demonstrated at the intended limit of detection (i.e., the lowest number of target microorganisms that will be detected). This may relate to the limit of detection for specific microorganisms or genomic copy equivalents that are present in the test sample prior to, or after, amplification steps, depending on the intended sensitivity and workflow of the new method.
可替代方法或者快速方法用来定性检测目标的特定微生物，比如使用核酸扩增技术，应该在预期的检测限上证明（即，能被检测出的最低数量的目标微生物）。这可能与特定微生物的检测限或者放大前后的测试样品的基因复制当量有关,取决于新方法的灵敏度和工作流程。

Procedure程序
As it is not possible to consistently obtain a reliable sample containing a very low level of microorganisms (e.g., a single viable cell), it is essential that the limit of detection of an assay is determined from an appropriate number of replicates.
事实上，不可能连续的获得含有很低水平微生物的可靠的样本（例如，单个活细胞），因此在含量检测限时选择一个合适的重复次数是必要的。

Since experiments aiming towards demonstration of the detection limit of an assay typically apply very low microbial concentrations, the number of replicates used may be higher as compared with other validation parameters.
因为实验通常应用微生物浓度非常低来证明的检出限，与其他验证参数比较，重复检测次数可能更多。

Furthermore, the appropriate number of replicates also depends on the statistical method(s) used (see below).
此外，合适的重复次数取决于使用的统计方法（如下）

As the intent of demonstrating that a new method is capable of detecting very low levels of microorganisms, then challenging a suitable diluent with a concentration of microorganisms that is

appropriate for the new method's application range is an acceptable strategy.
可接受的策略是：目的是为了证明新方法能够检测很低水平的微生物，那么用一个具有合适的微生物浓度范围的稀释液来挑战新方法。

For example, if a new method purports to have a limit of detection of less than 5 cfu, then challenging the system with 1-5 cfu is an adequate approach.
例如，一个新方法的检测限是小于5cfu，那么就用1-5cfu系列浓度来挑战是合适的。

Alternatively, the level of inoculation can be adjusted until at least 50% of the samples show growth in the existing test, or the inoculation levels can be diluted into the fractional range (e.g., dilutions to be assayed may contain 50, 5, 0.5, and 0.05 cfu).
或者，浓度可以调整到至少现有测试方法的50%或能够分次稀释到合适的范围（稀释的范围必须包括50,5,0.5,0.05cfu）

Regardless of the procedure used, the rate of recovery between the new method and the existing method are then compared for each dilution or microbial concentration that is being evaluated.
不管用什么程序，应比较每个稀释级别或每个微生物浓度的新方法和现有方法的回收率应。

Another approach may be to use a microbial suspension with a low mean microbial concentration (e.g., on average 1 cfu per inoculation volume)， and repeatedly challenge the new method with this suspension. The fraction of samples in which the microorganisms are successfully detected should be noted and compared to the expected value based on an appropriate statistical model. A statistical model that is often applied for this purpose is the Poisson distribution. With a mean microbial inocu- lum of 1 cfu，the Poisson distribution would predict that microbial growth occurs in 63% of samples
If the LOD is 1 cfu , but only in 26% of the samples if the LOD is 2 cfu. Thus, if a sufficient number of replicates is tested， the detection limit which best explains the obtained results can be evaluated.
另一种途径是使用较低平均浓度的菌悬液(如平均接种量为1CFU)并反复用此菌液挑战新检测方法。应关注那些微生物被成功检出的样品，并和基于相应的统计学模型得出的预期数值进行比较。泊松分布是一种经常用于这方面用途的统计学模型。当平均接种量为1cfu，检测限为1CFU时，泊松分布可预测的微生物生长概率为63%，而当检测限为2CFU时概率仅为26%。因此，只有当检测了足够数量的样本后，才能得到对检测结果最为适用的检测限。

As previous stated under the specificity section, the end-user should determine the most appropriate types of microorganisms to utilize during limit of detection testing. These may include standard cultures, environmental or facility isolates, in-process or sterility failure isolates, slow growing, fastidious or anaerobic, clinically relevant, stressed microorganisms and/or difficult to detect strains.
就如之前在专属性章节中提到的，最终用户在实验时应确定最为合适的微生物种类，可包括标准菌株，环境或设施中分离的菌株，中间体或失败的无菌检查样品中分离出的菌株，生长缓慢的微生物，需要复杂培养的菌或是厌氧菌，临床相关菌种，受影响的微生物和/或难检测到的菌株。

Acceptance Criteria and Statistical Analysis接受标准和统计学分析
The detection limit should be adequate for the intended application and may be assessed in comparison to a reference method if appropriate. In general, the limit of detection of the alternative or rapid method should not be significantly worse than that of the existing method, except when a clear rationale or

justification exists why a higher detection limit can be to lerated. For nucleic acid amplification detection methods, the limit of detection may be demonstrated for aspecific concentration of microorganisms, or for an approximate number of genomic copy equivalents before the amplification process.
检测限应与设计的用途相适应，若合适应与参考方法进行比较。一般来说，除非存在明确或合理的理由能解释为何可接受更高的检测限，可替代方法或快速方法的检测限不应显著差于现有方法。对于核酸扩增检测方法，其检测限应能证明适用于特定浓度的微生物，或是扩增前大致数量的基因复制当量。

The Fisher's exact test or Chi-Square test can be used for statistical evaluation of results. Alternatively, a statistical equivalence test may be used. However, depending on the statistical test utilized, the number of replicates should also be carefully considered. For example, the Chi-Square test should be used with relatively high sample sizes because it is based on an approximation to a certain distribution, and such approximation becomes less precise with a lower sample size. When smaller sample sizes are used, the Fisher's exact test may be the better choice, because it does not rely on approximations. Furthermore, when using a challenge level of less than5cfu, the statistical power of these evaluations may also be reduced. If no systematic test power calculations are performed, not less than 50 replicates per test method should be included in the statistical evaluation (this value is derived from a test power simulation with the Fisher's exact test, assuming a mean microbial inoculums of 2cfu). Therefore, it may be advisable to pool the results obtained for different test runs and microbial strains for the statistical evaluation in order to achieve adequate test power.
确切概率法或卡方检验可用于检验结果的统计学评价，此外，也可使用统计等价性检验。然而，当使用统计学方法时对试验次数应进行仔细考虑。例如，卡方检验需与较大样本数配合使用。因为卡方检验是基于对特定分布的近似，而随着样本数量的减少近似度会变差。当使用较小的样本数量时，确切概率法是一个更好的选项，因为其不依赖近似值。此外，当试验中的挑战水平低于5cfu时，这些评价方法的统计学意义都会被削弱。如果没有进行系统的实验效力计算，为进行统计学评价，每种试验方法应重复至少50次（该数值来源于假设平均接种量为2cfu的前提下，使用确切概率法对实验效力模拟得到的）。因此，建议大量积累不同试验和菌株的结果进行统计学评价，以获得合适的检验功效。

When using multiple microbial concentrations (similar to the fractional dilutions described above), a Most Probable Number (MPN) technique may be employed, where MPN tables are used to calculate the MPN value and upper and lower confidence intervals. In this case, no significant difference exists between the methods if the confidence levels overlap. In such fractional approaches, it is recommended to use not less than 10 replicates per dilution, in order that the MPN can be adequately estimated .Also, if the MPN values permit, a paired t-test or non-inferiority test may be conducted using these numbers in order to further strengthen the argument of a comparable limit of detection.
当使用多种浓度的菌液时（与前面所述的分次稀释类似），可应用最大可能数（MPN）方法，即使用MPN表计算 MPN值及其上下置信区间。在这种情况下，如果置信水平重叠则说明各检测方法之间没有明显的区别。在出现这种差异微小的情况时，推荐每个浓度稀释液的试验重复次数不少于10次，以得到合适的MPN值。同样的，如果MPN值允许，组合使用t检验和非劣效性假设检验可用于处理这些数值以进一步强化类似的参数检测限。

5.3.5 Limit of Quantification定量限

Definition定义

The limit of quantification alternative or rapid method is the lowest number of microorganisms in a test sample that can be enumerated with acceptable accuracy and precision under the stated experimental conditions.
可替代方法或快速方法的定量限是指在测定条件下，满足可接受的精密度和准确度的可计数的最低微生物数量。

Procedure测定

As it is not possible to consistently obtain a reliable sample containing a very low level of microorganisms (e.g., a single viable cell), it is essential that the limit of quantification of an assay is determined from an appropriate number of replicates. Therefore, at least five (5) to ten (10) replicates are recommended. In order to demonstrate the limit of quantification, it may be advisable to use different concentration of microorganisms in a suitable diluent. Because limit of quantification is intended to determine the lowest number of microorganisms that can be enumerated, the range of concentrations should be at or near the desired level of quantification for this purpose. For example, if the intended limit of quantification of a new method is expected to be that of a plate count method, then the theoretical range of concentrations tested may, for instance, be between 0.5 and 10 cells.
由于持续稳定获得极低数量水平的微生物（比如单个细胞）是不可能的，故而为获得方法的定量限，制定合适的重复实验次数就显得尤为重要。推荐的重复次数是至少5至10次。为了确定定量限，建议使用在合适稀释液中不同浓度的菌液。因为定量限的目的是决定可计数的最低微生物数量，所以菌液浓度的范围应在能达到此目的的预期定量限或是定量限的附近。例如，若一种新方法的期望定量限与平板计数法相当，则理论上被检微生物含量范围应在0.5到10个细胞。

As previously stated under the Specificity section, the end-user should determine the most appropriate types of microorganisms to utilize during limit of quantification testing.
就如之前在专属性章节中提到的那样，最终用户应决定最为合适的微生物种类以在定量试验中发挥最佳效用。

Acceptance Criteria and Statistical Analysis接受标准和统计学分析

The limit of quantification must be appropriate for the intended application. In general, the limit of quantification for the alternative or rapid method should be at least as sensitive as the existing method is to similar levels of microorganisms, except when a clear rationale or justification exists why a higher quantification limit can be tolerated. To demonstrate this, confidence intervals of count results assuming a Poisson distribution may be used. To that end, Poisson confidence intervals for the count results of the existing method may be calculated. If the values obtained with the alternative or rapid method are within that range, a similar limit of quantification may be assumed. However, caution should be taken when using this approach for low microbial concentrations that result in frequent sterile samples. In this case，mean count results of several replicates should be obtained for the comparison.
定量限必须与用途相适应。一般来说，在相似的微生物水平上，除非存在明确或合理的理由能解释为何可接受更高的定量限，可替代方法可替代方法或快速方法的定量限的灵敏程度至少应与现有方法相当。为了说明这一点，可假设计数结果的置信区间是泊松分布的。为此应计算现有方法计数结果的泊松置信区间。如果使用可替代方法和快速方法得到的数值在此范围内，则可假设一个近似的定量限。应注意的是，将此方法用于低微生物含量的情形时，会经常导致无法检出。在这种情况下，应使用数次重复试验计数结果的平均值进行对比。

Alternatively, the methods outlined for the validation parameters accuracy and/or precision may be used,

if extended to concentrations of microorganisms near the desired or expected detection level.
此外，如果微生物浓度延伸到接近期望的检测水平，可使用那些用于验证参数准确度和/或精密度的方法。

5.3.6 Linearity线性

Definition定义

The linearity of aquantitative alternative or rapid method is its ability to elicit results that are proportional to the concentration of microorganisms present in the sample within a given range, where accuracy and precision are demonstrated.
定量可替代方法或快速方法的线性是指在指定范围内，计数结果与样品中微生物浓度成比例的能力。

Procedure测定

At least five (5) replicates from at least five(5) different concentrations of microorganisms in a suit-able diluents and across the range of the assay are recommended. The mean of the replicates from each concentration may be used when calculating linearity. The end-user should determine the most appropriate types of microorganisms to utilize during linearity testing.
建议使用来自跨检测范围的至少5个不同浓度微生物悬液，每个浓度至少重复测试5次。计算线性时可能需要得到每个浓度的计数平均值。最终用户在测试线性时需决定最为适用的微生物种类。

Acceptance Criteria and Statistical Analysis接受标准和统计学分析

Linearity is demonstrated through linear regression analysis. Results can be considered satisfactory if the correlation coefficient, R2 ,Is 0.9 or better and the slope of the line is not diverging more than 20% from 1.0. An exception from the acceptance criterion for the slope may be appropriate if the alternative or rapid method consistently recovers higher numbers than the existing method (this may particularly be the case for nongrowth-based methods in comparison to a growth-based reference).
线性情况可通过线性回归分析得到。若相关系数R2=0.9或更优，且其斜率离散度不超过1.0的20%，则可被认为是满意的结果。当可替代方法或快速方法的微生物复苏水平高于现有方法（这尤其适用于非培养基培养生长的检测方法与基于培养生长的检测方法的比较）时，斜率超出接受标准仍是可接受的。

5.3.7 Range范围

Definition定义

The range of aquantitative alternative or rapid method is the interval between the upper and lower levels of microorganisms that have been demonstrated to be determined with accuracy, precision and linearity.
根据定量的可替代方法或快速方法，精密度，准确度和线性得到证明的微生物上下限之间的区间。

Procedure测定

The range is determined from studies of accuracy, precision and linearity.
通过对精密度，准确性和线性的研究确定范围。

Acceptance Criteria接受标准

The range is validated by verifying that the new method provides acceptable accuracy, precision and linearity when applied to samples containing microorganisms in a suitable diluents at the upper and lower

concentrations of the range, as well as within the range.
通过在范围上下限和范围内测试样品以确认新方法能提供可接受的精密度，准确性和线性，从而得到验证。

5.3.8 Ruggedness耐受性

Definition定义

Ruggedness is the degree of intermediate precision or reproducibility of test results obtained by assessing the same samples under a variety of normal test conditions, such as different analysts, different instruments, different lots of reagents or on different days. Intermediate precision is performed within the same laboratory, and reproducibility is performed between laboratories. Ruggedness can also be considered the intrinsic resistance to the influences exerted by operational and environmental variables on the results of the method.
耐受性是指相同样品在不同的常规检测条件下，测试结果的中间精密度或重现程度，如不同的分析人员，设备，不同批号的试剂以及不同的检测日期。中间精密度是在同一个实验室内获得的，而重现性则需在不同的实验室中获得。耐受性也可被看作是操作和环境变量作用在特定方法获得检测结果上的固有影响程度。

Ruggedness is a validation parameter that is best determined by the supplier of the alternative or rapid method, who has easier access to multiple instruments and batches of components. The data provided by the supplier are admissible to prove validation of ruggedness. However, it is the responsibility of the end-user to review the supplier's data and identify gaps with respect to any modifications of the method for in-house use. An evaluation should be performed whether these modifications are critical in nature and should be covered in a systematic study.
耐受性最好由可替代方法或快速方法的生产商方面作为已验证的参数提供，因为生产商更容易在不同设备和不同批号的试剂中对其进行测试。由生产商提供的耐受性验证数据的是可以被接受的。不过，最终用户有责任查看生产商的数据，并发现与用户根据自身实际使用所做任何修改后的差异。应对这些修改进行评估，以确认其是否是关键的，并进行系统研究。

Some end-users may also want to perform their own ruggedness testing; therefore, the following recommendations are provided.
部分最终用户也可能想自己进行耐受性测试，为此提供如下的建议。

Procedure测定

Prepare a suspension of microorganisms and evaluate at least five(5) to ten(10) replicates against each relevant test condition. Depending on the method, either changes in Precision(e.g., coefficients of variation) or the general ability to detect microorganisms could be evaluated.
制备菌悬液，对每一种相关测试条件进行至少5到10次的重复测试。根据具体方法，无论是精密度（如变异系数）还是检测微生物的能力都要进行评估。

Acceptance Criteria and Statistical Analysis接受标准和统计学分析

It should be demonstrated that the different test variables do not significantly impact outcome of the analysis. Acceptance criteria, statistical analyses and data evaluation approaches previously described in the sections covering accuracy, precision, specificity or limit of detection may be applied, depending on whether the assay is qualitative or quantitative.

要说明的是，不同的测试变量不会显著影响分析结果。可根据检测方法是定性还是定量分别应用之前在准确度，精密度，专属性章节中所述的接受标准，统计分析和数据评估。

5.3.9 Robustness鲁棒性

Definition定义

Robustness is a measure of a method's capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

鲁棒性是指有意使检测方法的参数发生微小变化，而该方法的检测能力不受影响的特性，以提供其在日常使用中的可靠性。

Robustness is a validation parameter that is best determined by the supplier of the alternative or rapid method. The data provided by the supplier are admissible to prove validation of robustness. However, it is the responsibility of the end-user to review the supplier's data and identify gaps with respect to any modifications of the method for in-house use. An evaluation should be performed whether these modifications are critical in nature and should b e covered in a systematic study.

鲁棒性最好由可替代方法或快速方法的生产商方面作为已验证的参数提供。由生产商提供的鲁棒性验证数据的是可以被接受的。不过，最终用户有责任查看生产商的数据，并发现与用户根据自身实际使用所做任何修改后的差异。应对这些修改进行评估，以确认其是否是关键的，并进行系统研究。

Some end-users may also want to perform their own ruggedness testing; therefore, the following recommendations are provided.

部分最终用户也可能想自己进行鲁棒性测试，为此提供如下的建议。

Procedure测定

The same procedure recommended for ruggedness testing may be used while changes on identified critical method and system parameters are introduced. Such critical method or system parameters may be evaluated through a risk assessment. These may include, but are not limited to, reagent concentrations, instrument operational limits, and incubation parameters (e.g., time and temperature for methods requiring microbial growth).

当确认有方法和测试系统参数有关键变化时，建议使用与鲁棒性性相同的测定方式。可通过风险分析确认什么是关键方法或系统参数，可包括但不限于试剂浓度，设备操作限以及培养参数（如检测方法所需的微生物生长时间和培养温度）。

Acceptance Criteria and Statistical Analysis接受标准和统计学分析

For each test condition a range should be demonstrated, within which the alternative or rapid method operates in a robust manner. If the method is shown to fail at providing robust results for particular test conditions, this should be accepted as the limitation(s) of the method. If a method is shown to be particularly sensitive towards a certain type of procedure, system parameter or manipulation, the results should be used to define adequate precautions or limitations when the method is used routinely.

应说明在每种测试条件下可替代方法或快速方法能稳定应用的范围。如果某方法不能在特定的测试条件下提供稳定的结果，这可被认为是该方法的局限。如果一个方法对特定检测过程，系统参数或操作表现得很敏感，其对在日常使用中的结果应有纠正预防措施或是限制。

5.3.10 Equivalence/Comparative Testing等效性/对比测试

Definition定义

The equivalence of an alternative or rapid method is a measure of how similar the test results are when compared with the existing method.

可替代方法或快速方法与现有方法的检测结果的相似性评价。

Variability may be a limiting factor with microbiological samples; therefore, the first phase of validation criteria testing requires that the two methods initially be run in parallel using standardized cultures(e.g., pure or mixed cultures in a suitable diluent) to demonstrate that the validation criteria previously discussed in this section of the technical report are being met.

微生物样品的易变性可能是一个限制因素，因此在验证标准的第一阶段要求两种方法使用标准菌株（如在合适溶液中的纯菌种或混合菌种）平行测试，以确认是否符合之前在本报告各章节中的验证标准。

Equivalence or comparative testing involves the use of actual product and other sample matrices that will be routinely tested using the alternative or rapid method once it is validated and implemented. The new method is run in parallel with the existing method for a specified period of time or number of product batches or test samples. The end-user should determine the most appropriate strategy for the duration and extent of these comparative studies, which may be influenced by the critical nature of the test method, the material being analyzed, the statistical methods used when interpreting the resulting data, regulatory expectations and/or other quality requirements.

一旦可替代方法或快速方法完成验证并实施，等效性或对比测将使用日常检验的实际产品或其它样品组合进行。新方法与现有方法在指定时期或产品批数或检验样品数内平行使用。最终用户应确认对比研究的时长和程度的最佳策略，其影响因素可能来自检验方法的关键性质，被检验物质的特性，解释数据时所使用的统计方法，监管方面的预期和/或其它质量方面的要求。

Actual product and sample matrices should also have previously been assessed for their potential to cause interference; false positive or false negative results (refer to Section 5.4 for further detail). When a supplier tests the end-user's product or sample matrices against the validation criteria, it is the end-user's responsibility to determine if additional, in-house testing is required, or if the testing performed by the supplier is adequate.

由于实际产品和样品组合潜在可能造成干扰，假阳性或假阴性结果（详见5.4节），所以应事先得到评估。当由生产商根据验证标准测试最终用户的产品或样品样品组合时，最终用户的责任是决定是否需要额外的内控测试，或是生产商的测试已经足够。

Finally, advances in technology can offer greater precision and sensitivity in comparison to conventional or compendial-referenced methodologies. As a result, an increase in organism recovery (i.e., detection or enumeration) may be observed. To address this, statistical treatment of the data generated from both methods should be used to demonstrate that the alternative or rapid method results are equivalent (i.e., non-inferior) or better than (i.e., superior) that of the existing method.

最后，技术的进步能提供比药典参考方法或传统方法更高的精密度和灵敏度。因此，可能会观察到微生物复苏性（即检测或计数）的增加。为解决该问题，对于由两种方法所获数据应进行统计学处理以表明可替代方法或快速方法等效（即不差于）或优于现有方法。

Procedure测定

Test samples should be identified that are expected to contain microorganisms and, when appropriate, test samples that do not contain microorganisms, in order to test the suitability of the alternative or rapid method. The latter is most important when determining that the test sample will not provide a false positive result.
应确定含有预期微生物的测试样品，并且在合适时应使用不含微生物的样品，以测试可替代方法或快速方法是否适用。当确认测试样品是否会出现假阳性时，后者是极其重要的。

However, it should also be noted that if the test sample does not contain microorganisms, then it is not possible to determine whether the new method will detect or enumerate microorganisms, nor will it be possible to perform a statistical comparison for equivalency between the new method and the existing method, as both methods will report the absence of microorganisms or no recovered counts (i.e., cannot compare zeros to zeros). Therefore, it may be necessary to challenge product or sample matrices with microorganisms in order to demonstrate equivalence with the existing method. The strategies for inoculating actual product or sample matrices should be similar to the methods described for the initial phase of validation criteria testing, namely, the use of microorganisms in a suitable diluent.
然而，应该注意的是，如果样品中不含微生物，则不可能确认新方法能检测或计数微生物，也不能对新方法和现有方法的等效性进行统计学比较，因为两种方法都会报告检不出微生物或是没有复苏计数（即不能用零计数和零计数进行比较）。因此，使用含有微生物的产品或样品组合进行挑战，以证明新方法和现有方法的等效性。接种实际产品或样品组合的方式应与验证初始阶段相同，也就是使用适合的菌液。

Additionally, the end-user must determine the numbers, types and physiological state of the challenge microorganisms in order to provide for meaningful data to support the successful validation of the alternative or rapid method. For example, regulatory authorities expect that very low levels (e.g., at the level of detection or quantification) of a wide variety of stressed microorganisms, including standard compendial cultures, anaerobes, slow growing strains and environmental or facility isolates, be utilized when validating a new method for the sterility testing of pharmaceuticals. For qualitative nucleic acid amplification detection methods/systems, product or sample matrices may need to be inoculated with actual target microorganisms, or with nucleic acid standards that will be relevant to the method under evaluation.
此外最终用户必须确定挑战微生物的数量，种类和生理状态，以为可替代方法或快速方法验证的成功提供有意义的数据。例如，监管机构期望包括各种受关注的微生物，如药典的标准菌株，生长缓慢的菌株和环境及设施中检出的微生物能被用于制药行业无菌检查新方法的验证。对于定性核酸扩增检测方法/系统，产品或样品组合需接种实际的目标微生物或是与方法相关的标准核酸以进行评估。

When conducting equivalence/comparative testing using actual product or sample matrices in the new method and the existing method, similar procedures and data analyses may be employed that were previously utilized for the validation criteria with standardized cultures in a suitable diluent. For example, when demonstrating equivalency for a new sterility test, the new method should be compared with the existing, compendia sterility test method using the limit of detection assay, where very low levels of microorganisms are inoculated into sterile product, and the resulting rates of positive to negative results can be statistically compared to demonstrate the new method is at least equivalent or superior in terms of detecting the challenge inoculum. Similarly, a new quantitative bioburden method can be shown to be

equivalent or superior to an existing compendia bioburden assay by demonstrating that the recovered counts are not statistically different, or are higher, than the existing method.
当使用实际产品或样品组合对新方法和现有方法进行等效性/对比测试时，可使用与之前用标准菌株进行验证类似的过程和数据分析。例如，在说明一种新的无菌检查方法的等效性时，新方法应与现有方法进行比较，药典的无菌检查法使用的检测限是接种很少量微生物到无菌产品中，阳性对阴性结果的比率可用于在统计学上比较说明新方法在检测挑战微生物时至少等效或优于参考方法。类似的，对于一种新的定量微生物限度检查方法，可通过在复苏计数方面与已有方法比较，在与统计学上没有差异或更好，来说明其是否等效或优于现有方法。

In all cases, the comparison between the alternative or rapid method and the existing method should be specific for the new method's intended application(s).
在所有的例子中，可替代方法或快速方法与现有方法的比较应针对新方法的预期应用进行。

Acceptance Criteria and Statistical Analysis接受标准和统计学分析
The alternative or rapid method must be shown to be at least statistically equivalent (i.e., it is the same), or statistically non-inferior (i.e., it is not worse), to the existing method. The new method may also be shown to be statistically superior (i.e., better results, higher recovery, a greater amount of microbial detection or a lower limit of detection) to the existing method, although the need to show superiority is not required, as the test for equivalency is to demonstrate that the new method is at least as good as the existing method.
可替代方法或快速方法必须至少表现出与现有方法在统计学上等效（即相同）或非劣（即不差于）。尽管未必需要表现出对现有方法的优越性，因为等效性测试是为了说明新方法至少与现有方法至少一样好，新方法也可以在统计学上优于（即更好的结果，更高的复苏水平，更高的检出水平或更低的检测限）现有方法。

Statistical methods and data analysis for equivalence using actual product or sample matrices may be the same as what was used when testing standardized cultures(in a suitable diluents) against other validation criteria.
使用实际产品或样品组合进行等效性测试所用的统计学方法和数据分析方式与使用标准菌株（在适合的稀释度）进行其它验证时相同。

5.4 Suitability Testing适用性测试
To demonstrate that the new method is compatible with specific product or sample matrices that will be routinely assayed, each material should be evaluated for the potential to produce interfering or abnormal results, such as false positives (e.g., a positive result when no viable microorganisms are present in the test sample) or false negatives (e.g., a negative result when microorganisms are present in the test sample).This may also include evaluating whether cellular debris, dead microorganisms or mammalian cell cultures have any impact on the ability of the new method and accompanying system to operate as it is intended to.
为了说明新方法适用于日常检验的特定产品或样品组合，对每种物质潜在可能产生的干扰或异常结果，诸如假阳性（比如样品中并无活性微生物，但得到了阳性结果）或是假阴性（比如样品中存在微生物，但得到了未检出的结果）均应进行评估。这同样包括评估细胞残片，死亡微生物或哺乳动物细胞对新方法及配套系统预期检测能力的影响。

Although it is possible that certain classes of materials will produce similar results, it is the end-user's responsibility to ensure that all test samples will be compatible with the new method.
尽管特定类别的材料产生类似结果的可能性是存在的，最终用户仍有责任确保所有的样品都适用于新方法。

The following guidance on false positive and false negative may be considered or modified to meet the specific needs of the end-user performing these analyses, when appropriate.
以下关于假阳性和假阴性的指南在最终用户进行分析时可参考或根据需要进行修改以适应实际需要。

5.4.1 False Positive Testing假阳性检测

This evaluation determines if the product or sample matrix contains any material that may produce background noise or interfering signals, resulting in a false positive outcome. This evaluation may also be conducted with the diluents used to prepare standardized suspensions that are utilized in the testing of other validation criteria (e.g., accuracy, precision), if it is thought that the diluents may produce a false positive result.
该评估确认产品或样品组合样品组合是否产生可能导致假阳性的背景噪声或干扰信号的物质。如果认为制备标准菌液的稀释液可能会产生假阳性，该评估也适用于制备用于验证其它标准（如精确度，精密度）所用的配制标准化菌悬液的稀释液。

Whenever possible, an appropriate test sample should contain no viable microorganisms (i.e., is sterile). In the event that the test sample is not supplied as sterile, then the sample should be treated such that it will not contain any viable microorganisms and it will not alter the sample properties that may have an adverse effect on this study.
只要有可能，合适的样品中应不含活性微生物（即无菌）。在提供的样品不是无菌的情形下，样品应进行处理以使其不含有活性微生物，且不改变可能对研究有不利影响的样品性质。

Evaluate the sterile test sample using the new method. The size of the test sample (e.g., individual sample volume) evaluated should be the same as what will be used during routine analysis. This evaluation should be performed using an appropriate number of replicates and test sample batches.
使用新方法评估无菌样品。用于评估的样品量（比如单个样品体积）应与日常分析中的用量相同。该评估应使用合适数量的样品批和检测重复次数进行。

Acceptance Criteria and Statistical Analysis接受标准和统计学分析

Product or sample matrices analyzed for their potential to cause a false positive result should not produce a positive result (i.e., the system detects microorganisms or provides a viable cell count, when it is known that no microorganisms are present in the test material). If the new method provides a positive result, but a positive result is not expected (e.g., the test sample is sterile), then the new method should be reviewed to determine if the result is due to a true false positive or interference condition, and not due to contamination of the supposedly sterile test sample. The end-user should be aware; however, that some contaminants may not be detectable by classical methods, but may be detected by the new method (e.g., viable but nonculturable, or VBNC organisms). Therefore, sterile test samples should be utilized to the end-user's best ability, and that acceptance criteria should be defined accordingly (e.g., a comparison to the results obtained with classical methodologies may not always be appropriate in such cases).

被分析潜在导致假阳性的产品或样品组合不应产生阳性结果（即当已知样品中不含有微生物时系统检测到微生物或给出了一个活性细胞计数）。如果新方法给出了一个阳性结果，但这个结果与预期不符（比如样品是无菌的），应对新方法进行回顾分析，确认该结果是一个真正的假阳性或是干扰情形，而不是由于推测为无菌的样品受到了污染。最终用户应该清楚的是，一些污染无法被传统方法检出，但是能被新方法检出（比如，有活性但无法被培养的微生物，即所谓VBNC微生物）。因此，无菌样品是最终用户的测试最佳选择，接受标准的定义则应有针对性（比如，与传统方法获得结果的比较在这些例子中就不一定适用）。

True false positives should be resolved before the new method is used to routinely to test that particular product or sample matrix. This may be achieved through measures that potentially reduce impact of the product or sample matrix, like for instance, increasing the dilution factor or adjustment of the rinsing protocols. If false positives cannot be resolved, then the specific test sample may be incompatible with the new method. However, some end-users may find that a low false positive rate is still acceptable if a follow-up confirmatory test is utilized.
真正的假阳性应在新方法被用于日常检测特定产品或样品组合之前得到解决。这可以通过一些可以潜在减少产品或样品组合样品组合带来影响的措施实现，如增加稀释倍数或增加淋洗量。如果假阳性情况得不到解决，则新方法不适用于检测特定样品。不过，部分最终用户发现，在使用后续确认检测的情况下，较低的假阳性率仍是可接受的。

Additionally, the presence of normal background noise should be fully understood, and in the event this interference is unavoidable, the end-user should determine if the background noise can be incorporated into the use of the new method (e.g., background noise may be an acceptable baseline for detecting microorganisms in the test sample under routine use).
此外，应被充分了解正常背景噪声的存在，在这种干扰无法避免时，最终用户应确认背景噪声是否可在新方法的使用中被接受（比如，背景噪声在日常使用中被接受为检测样品中微生物的检测基线）。

5.4.2 False Negative Testing假阴性检测

This evaluation determines if the product or sample matrix contains any material that may quench, mask or otherwise prevent the detection or enumeration of microorganisms when they are present, thereby producing a false negative result. Furthermore, it can be used to determine if a particular product or sample matrix does not exhibit pronounced antimicrobial properties. This evaluation may also be conducted with the diluents used to prepare standardized suspensions that are utilized in the testing of other validation criteria, if it is thought that the diluents may produce a false negative result.
该评估确认产品或样品组合中是否含有能抑制，掩盖或其它在微生物实际存在时阻止其被检出或计数的物质。此外，其也能被用于确认特定产品或样品组合中是否不存在显著的抑菌性。如果认为制备标准菌液的稀释液可能会产生假阴性，该评估也适用于制备用于验证其它标准（如精确度，精密度）所用得配制标准化菌悬液的稀释液。

The test sample should be inoculated with a known level and type of microorganism. The end-user should determine the appropriate levels and types of microorganisms specific for the method being validated and/or the test sample. Furthermore, an appropriate positive control should be prepared which is inoculated the same way but does not contain the product or sample matrix of interest. The microorganism-containing test sample and positive control should be evaluated using the new method.

The size of the test sample (e.g., individual sample volume) evaluated should be the same as what will be used during routine analysis. This evaluation should be performed using an appropriate number of replicates and test sample batches.
测试样品中应接种已知含量水平和类型的微生物。最终用户应决定针对待验证方法和/或样品的适用微生物含量水平和种类。此外，应准备同样接种但不含特定产品或样品组合成分的阳性对照样。应使用新方法对接种了微生物的样品和阳性对照样进行评估。用于评估的样品量（比如单个样品体积）应与日常分析中的用量相同。该评估应使用合适数量的样品批和检测重复次数进行。

When applicable, markers specific for the technology being evaluated may be added to the test sample to demonstrate that the marker will be detected under the conditions of the test. For example, ATP may be added to a test sample to demonstrate that the test sample will not interfere with the detection of ATP and result in a false negative result.
如果可行，可在样品中添加针对待评估新方法所用技术的特异性标记物，以用于说明在测试条件下标记物会被检出。比如，可在样品中加入ATP以说明样品不会影响ATP的检出而导致假阴性。

Acceptance Criteria and Statistical Analysis接受标准和统计学分析
Product or sample matrices analyzed for their potential to cause a false negative result should not produce a negative result (i.e., the system does not detect microorganisms or provide a viable cell count when it is known that microorganisms are present in the test material).
被分析的潜在导致假阴性的产品或样品组合不产生阴性结果（即在已知样品中存在微生物的情况下，系统没有检测到微生物或是给出一个活性细胞计数）。
If the new method provides a negative result, but the positive control provides a positive result, then the detection of microorganisms in the new method may be quenched, masked or prevented. Alternatively, the product or sample matrix may exhibit pronounced antimicrobial properties. For a quantitative method, the recovered counts from the product or sample matrix should not be significantly lower than from the positive control (e.g., not less than 70% recovery).
如果新方法给出了一个阴性结果，但是阳性对照给出了一个阳性结果，则使用新方法进行的微生物检测可能是被抑制，掩盖或是屏蔽了。或者产品或样品组合产生了显著的抑菌作用。对于定量方法，产品或样品组合的微生物复苏计数不应显著低于阳性对照样（比如，不低于70%）。

True false negatives should be resolved before the new method is used to routinely to test that particular product or sample matrix. This may be achieved through measures that potentially reduce impact of the product or sample matrix, like for instance, increasing the dilution factor or adjustment of the rinsing protocols. If false negatives cannot be resolved, then the specific test sample may be incompatible with the new method or accompanying system.
真正的假阴性应在新方法被用于日常检测特定产品或样品组合之前得到解决。这可以通过一些可以潜在减少产品或样品组合带来影响的措施实现，如增加稀释倍数或增加淋洗量。如果假阴性情况得不到解决，则新方法或附属系统不适用于检测特定样品。

5.5 Variability of Microbiological Methods: Additional Considerations
微生物方法的变异性：其他注意事项
An important point to consider during the validation of a new microbiological test method is the inherent variability in microbiology. There are three sources of variation: sample distribution error, cellular arrangement (chains or clumping) and metabolic activity.

在一个新的微生物测试方法的验证中，需要重点考虑的一点是微生物的固有变异性。固有变异有三种来：样本分布误差、细胞排列（链式或聚集型）和代谢活动。

For any given test procedure, the relative importance and contribution from these sources will depend on the principle of the test method and must be carefully considered.
对于任何指定的测试程序，这些变动来源的相对重要性和贡献大小将取决于测试方法的原理，需要仔细加以考虑。

5.5.1 Preparation of Test Samples测试样品的制备
Unlike chemical analytes where one can accurately weigh out a quantity of a chemical of known purity and dissolve it in a solvent such as water to obtain a standard solution, it is more difficult to consistently prepare a bacterial inoculum with a uniform cell count per unit volume of water especially with low microbial counts. Therefore, care should be taken when preparing in-house cultures, especially at low concentrations, or to utilize commercially prepared cultures that have been specifically designed to deliver low levels of microorganisms in a test system.
在化学分析中，可以准确称量一定量已知纯度的化学物质，溶解于溶剂（如水）中制备成一个标准溶液，而微生物测试则不同，你很难一致地制备出单位体积水中均一细胞计数的细菌接种物，特别是当微生物计数低的时候。因此，在自制菌液尤其是低浓度时，或者使用标明低浓度微生物含量的商业化制作的菌液是要特别注意。

Next, some level of error is normally expected to be associated with sampling, dilution, plating, incubation, counting, and calculation of microorganisms.
其次，取样、稀释、接种、培养、计数和微生物的计算通常都存在一定程度的误差。

Additionally, if you have a well-mixed homogeneous suspension of a pure culture, the counts in aliquots taken from that suspension should approximately follow a Poisson distribution. Additional variability can occur, however, when the distribution of microorganisms in the suspension (or the inoculated test sample) is not uniform (see 5.5.2 Sample Distribution Error) and can be introduced through handling.
而且，如果你有一个混合均匀的纯种培养物悬浮液，则从中所取的一部分悬浮液的菌落计数应大致遵守泊松分布。但当悬浮液中微生物的分布不均匀（见5.5.2样本分布误差）时，会出现其他变异，这可能在处理过程中引入。

Next, the precision between replicates of an assayed suspension of microorganism increases as number of microorganisms increase; conversely, the precision decreases as the number of microorganisms decrease. This should be understood especially when working with microorganism concentrations at very low levels.
随着微生物数量的增加，一个菌悬液重复检测结果之间的精密度也增加；相反精密度随着微生物数量降低而减小。认识到这一点比较重要，特别是在操作极低浓度微生物的时候。

An important issue is the determination of the number of replicates that are used in a validation protocol. The number of replicates required declaring a statistically significant difference between two microbial counting methods differs with a stated level of confidence. The number of replicates depends on the true percent difference (e.g., 0, 20, 25, 50 or 100%) that you want to detect, the probability (e.g., 50, 70 or 90%) of being able to detect the difference and the target concentration (e.g., 1, 10, 50 or 100 cfu) of the sample. Therefore, the appropriate statistical model and analysis parameters should be carefully selected for use and test power calculations may help in definition of an appropriate sample size.

一个重要的问题是确定验证方案中重复样品数量。表明两种微生物计数方法之间差异具有统计学意义所需的重复样品数量不同于一个已知的置信水平，它取决于你想检测的真实差异值（例如，0、20、25、50或100%）、能够检测出差异的概率（例如，50、70或90%）以及样品的目标浓度（例如，1、10、50或100CFU）。因此应仔细选择所用的适当统计模型和分析参数，测试的效能计算也有助于确定适当的样本量。

5.5.2 Sample Distribution Error样本分布误差

Distribution error is the largest source of error contributing to the variability in microbiological testing. The natural distribution of microorganisms is heterogeneous and rarely follows normal Gaussian distribution even after log transformation. The distribution tends towards a negative binomial or Poisson distribution and is extremely difficult to assess and predict particularly at the low contamination levels that are routinely found in relatively 'clean' test samples (e.g., from ISO 5 environmental monitoring samples and purified water systems). For this reason, appropriate statistical approaches should be carefully considered when comparing the results from an alternative or rapid method and an existing microbiological method.

在微生物测试中分布误差是变异的最大来源。微生物的自然分布是不均匀的，及时经过对数转换也很少服从正态分布。它的分布更趋向于一个负二项分布或泊松分布，而且极难评估和预测，尤其在测试样品相对"洁净"微生物污染水平低的时候（例如ISO5 环境监控样品和纯化水系统样品）。因此在比较一个可替代或快速检测方法与现有微生物测试方法结果时，应仔细考虑采用适当统计方法。

5.5.3 Cellular Arrangement细胞排列

Traditional culture methods detect and/or enumerate microorganisms by monitoring changes in turbidity or by counting CFU visible to the naked eye. For enumeration, the assumption is that one colony forming unit is derived from a single microorganism that was uniformly distributed within the test sample. However, microorganisms have a variety of arrangements and can occur singly and in pairs, chains, tetrads or irregular clusters. Microorganisms also have a tendency to colonize surfaces and form biofilms, which may also affect the manner in which individual cells are structurally arranged and are distributed in a test sample. Therefore, the number of CFU (or cell density) in a plated sample and therefore the accuracy of the viable count estimate are directly affected by colonial arrangement. Consequently, the coefficient of variance for microbiological methods may be large, especially for growth-based assays.

传统培养方法通过监视浊度变化或肉眼菌落计数来进行微生物检测和/或计数。对于计数，假设是一个菌落形成单元来自于测试样品中均匀分布的一个单一微生物。但是，微生物有不同的排列方式，可以是单个和成对的、链式的、四分体的或不规则的聚集体。微生物也倾向于在表面上繁殖，并形成生物膜，这可能也会影响单个细胞在测试样品中的排列和分布方式。因此，菌落排列将直接影响一个接种样品中CFU数（或细胞密度）和活菌计数准确性。结果是微生物方法的变异系数可能较大，尤其对于微生物培养生长的检测方法。

5.5.4 Metabolic Activity代谢活动

Successful detection and/or enumeration of microorganisms are influenced by their metabolic activity, genotype and readiness for growth. In any microbiological test method the presence of interfering factors must also be considered. Microorganisms may be stressed due to exposure to processing, environmental and experimental conditions or inhibitory components in the product or test sample itself. Stressed cells

may require a period of resuscitation and repair before they can be detected by cultural methods. Therefore, stressed organisms should be utilized in determining the time-to-result for growth-based alternative or rapid methods. Additionally, inactivating agents added to media that neutralize some product components, may also stress cells and inhibit their growth. Conversely, some products may contain nutrients sufficient to support microbial survival or even growth. Each of these points should be considered when developing the validation plan and testing strategy for an alternative or rapid method.
成功进行微生物检测和/或计数是受到微生物的代谢活动、基因型和是否处于待生长状态影响的。在任何微生物测试方法中，还必须考虑干扰因素的存在。微生物可能要经受工艺、环境和试验条件或产品/样品中抑菌成分的影响。在能够通过培养方法检测出之前，受影响的细胞可能需要一个复苏修复期。因此对于基于微生物生产的可替代或快速测试方法，应采用受影响的微生物确定获得检测结果所需时间。另外，加入培养基中和一些产品组分的的灭活剂也会影响细胞的活性，并阻止其生长。相反，部分产品可能含有足够微生物存活或生长的营养物质。在开发一个可替代或快速微生物方法的验证计划和测试策略时，应考虑上述每点内容。

5.6 Validation of Microbiological Methods: Additional Considerations
微生物方法的验证：其他注意事项
5.6.1 Alternative and Rapid Endotoxin Detection Methods
可替代和快速内毒素检测方法
The introduction of alternative and rapid methods for endotoxin detection requires that these new systems be qualified for their intended use. Therefore, it is expected that these types of methods/ systems meet the appropriate compendial requirements for the validation of endotoxin methods. This includes the qualification of the testing laboratory and the analysts. Validation of the instrumentation usually involves verification that standard or reference solutions of endotoxin yield the specified standard curves, analytical responses and/or limit of detection or quantification. Endotoxin detection methods that do not meet existing compendial requirements should be validated as an alternative method.
采用可替代和快速方法进行内毒素检测时，需要确认新系统满足预期用途。因此，这些类型的方法/系统应满足药典对内毒素方法的验证要求。这包括测试实验室和检验员的确认。仪器的验证通常包括确认内毒素标准或参比溶液能够产生指定的标准曲线、检验响应和/或检测限或定量限。不满足现有药典要求的内毒素检测方法应作为替代方法进行验证。

5.6.2 Unique Methods Requiring Additional or Modified Validation Strategies
需要额外验证策略或修订验证策略的独特方法
The method validation testing strategies contained within this TR primarily use standardized microbial cultures in liquid suspension. However, there exists the possibility that some alternative or rapid detection systems may need to consider different approaches, especially if a liquid suspension cannot physically be introduced into the detection system for analysis. Technologies that utilize airborne, aerosol, or other non liquid-based samples may fall into this category. Therefore, expanding or adapting the validation strategies within this TR may be appropriate, as long as the testing methods are scientifically justified. Because the end-user may not posses the expertise or specialized equipment to conduct such studies, the vendor may need to play a greater role in supporting the end-user during the validation of these types of technologies.
本技术报告中方法学验证的测试策略基本上采用液体悬浮液中的标准微生物菌株。但是部分替代或快速检测系统可能需要采用不同的方法，尤其是当无法用检测系统进行液体悬浮液分析时。采用空气、气溶胶或其他非液体样品可能就属于这一类。因此可适当扩展或调整本技术报告中的验证策略，

只要该测试方法有科学依据。因为用户可能不具备相应技能或特殊设备进行这些研究，供应商应为这些技术的验证提供更多支持。

5.6.3 Guidance on Changing Acceptance Criteria可接受标准变更的指南

Microbiological methods are subject to varying degrees of variability. For example, most of the methods used for environmental monitoring programs are performed in the absence of standard methods for the conduct of these tests. The results can be variable for a variety of reasons including: media selection, incubation time, incubation temperature, incubation conditions (e.g., humidity, oxygen tension and the like), intrusiveness of the sampling method, presence or absence of disinfectant residues, and the actual method being used. For traditional microbiological methods that measure in terms of CFUs, one does not actually know whether it is a single free-living cell, a clump of cells floating freely, or a cell(s) associated with a particulate. If one is performing surface monitoring with a swab or contact plate, recovery methods can vary yielding typically 20-60% of the number of organisms that are actually on the surface. In addition to these sources of variability, the actual method of enumerating the cells can be difficult and can have an impact on the results obtained. Even automated systems for enumeration can show variability because the method is dependent upon the technique used by the operator in preparing the sample. These are just a few examples of the ways that variability is manifested in conventional microbiological methods.

微生物方法受变异性的程度的影响。例如，环境监测所使用的大多数方法都没有标准的方法。结果会影响不同原因而不同，包括培养基的选择、培养持续时间、培养温度、培养条件（如湿度、氧含量以及其他类似条件）、取样方法的影响、消毒剂残留存在与否和实际使用的测试方法。对于测量菌落形成单元的传统方法，一个人实际上并不知道它是单个活细胞、一簇自由漂浮的细胞或附在颗粒上的细胞。当用擦拭法或接触碟进行表面监控时，回收结果通常为表面上实际微生物数量的20-60%。除了这些变异来源，细胞计数的实际操作难度大，也影响所获得的结果。即使自动计数系统也存在变异性，因为检测方法还取决于操作者制备样品的技术。这些只是表明传统微生物方法中变异性的几个例子。

In addition to the variability show within the conventional methods, it is also possible with alternative or rapid methods to see differences due to the actual measurements being reported. For example, a conventional quantitative method will provide results in terms of a CFU, while the alternative or rapid counterpart may provide results as the number of viable cells, a spectral analysis, relative light units, fluorescent units, and so forth. This may present a challenge. It is a frequent regulatory expectation to require data be evaluated or trended to create a history of the microbiological attributes present in an environment or in a test sample. If data are to be compared over time, then test methods must remain the same, which is fundamental to trend analysis. However, some of the alternative or rapid technologies provide a greater level or improved level of detection sensitivity over its conventional method counterpart, either through design or the ability to detect stressed or VBNC microorganisms. Therefore, the implementation of these new methods/systems may also require the establishment of new acceptance criteria. And in some instances, the trending of data may be lost at some point in time in order to bridge the gap between "old" and "new" data analysis. Regulators are aware of these issues, and these concepts were discussed in a 2006 publication by FDA microbiologists, Hussong and Mello (43).

除了传统方法所显示的变异性，因为实际测量结果报告方法不同，替代或快速方法结果也可能不同。例如，传统定量方法结果表示为 CFU，而替代或快速方法结果表示为活细胞数、光谱分析结果、相对光强度、荧光单位等。这可能带来一个挑战。监管部门要求对数据进行评估和趋势分析，建立

环境或测试样品中存在的微生物特性的历史数据。如果将不同时间的结果进行比较，则测试方法必须保持不变，这是进行趋势分析的基本要求。但是，通过设计或者拥有检测受影响或活的但不可培养的微生物的能力，部分替代或快速检测技术比传统方法具有更高检测灵敏度，因此，实施这些新方法/系统时，需要建立新的可接受标准。在部分情况下，不对一些时间点的数据进行趋势分析，以消除“老”数据分析和“新”数据分析差异。监管部门都清楚这些问题，这些概念也在 FDA 微生物专家 Hussong and Mello2006 年出版物中进行了讨论（43）。

In the event that an alternative or rapid method is qualified to provide greater sensitivity than the method intended to be replaced, an understanding of the impact to existing acceptance levels, in process or product specifications, and compendia and regulatory expectations is required. A statistical analysis and comparison between the data observed between the two methods should be performed, when appropriate, and this information may be used to justify any recommendations for changes to currently employed in-process or product acceptance levels and/or specifications. Discussions with relevant regulatory agencies and/or Pharmacopeias may also be required or regulatory submissions made prior to any changes being finalized.
当可替代或快速检测方法经确认具有比传统方法更高的灵敏度时，需要理解其对现有可接受水平、中间控制或产品标准以及药典和法规要求的影响。适当时，应对两种方法所观察到的数据进行统计分析和比较，并可作为中间控制或产品可接受水平和/或标准变更的依据。也可能需要与相关监管部门和/或药典进行讨论，或者在变更最终实施前提交注册申报资料。

Ultimately, any changes to established specifications must be related to fitness for use and be suitable for the application of interest. Additionally, final specifications should be scientifically sound, justified and based upon appropriate risk analysis.
最后对已有标准的任何变更必须适用于预期用途并获得利益。另外，最终的标准应科学合理，并基于适当的风险分析。

5.7 Alternative and Rapid Microbial Identification Methods
可替代和快速微生物鉴别方法

A variety of new alternative and rapid methods exist for the characterization and identification of microorganisms. According to the recently published USP Chapter <1113>, the verification of an identification test system may include one of the following three options (44):
有许多新的可替代和快速方法用于微生物的表征和鉴别。根据最新发布的USP通则<1113>，一个鉴别系统的确认可包括以下三种方法之一（44）：
"1) Using an existing system for parallel testing of microbial isolates obtained from routine testing (the number of isolates tested may be as high as 50, and any discrepancies in identification can be arbitrated using a referee method);
采用现有系统进行日常测试中获得微生物分离物的平行测试（测试的分离菌数量可高达50，并用一个参比方法对鉴别中任何偏差进行仲裁）；
2) testing 12-15 known representative stock cultures of different commonly isolated species for a total of 50 tests; or
取12-15种不同的分离菌的代表性储备培养物进行测试，总共50次测试；或者
3) confirming that 20-50 organism identifications, including 15-20 different species, agree with the results of a reference laboratory testing of split sample."
确认20-50次的微生物鉴别结果与对照试验室检测结果一致，应使用15-20个不同菌种。

The USP monograph further indicates that it is appropriate to use the quality control microorganisms specified by the supplier of the system and organisms identified in the applicable compendia.
USP专论进一步指出应采用该系统供应商指定的质量控制微生物和药典指明的微生物进行确认。

The monograph also states that verification of the identity of the species should be evaluated and the level of agreement should be considered. Typically greater than 90% agreement can be achieved with samples of microorganisms that are appropriate for the identification system.
USP专论还指出应对菌种鉴别确认的结果进行评估，并考虑一致性水平。通常对于适用于该系统的微生物可达到大于90%的一致性。

According to USP <1113>, the most important verification tests are accuracy and reproducibility (44). These parameters have been defined as:
根据USP<1113>，最重要的确认试验是准确度和重现性（44）。这些参数定义如下：
Accuracy % = (Number of correct results/Total number of results) x 100
准确度%=（正确结果的数量/结果总数）x100
Reproducibility % = (Number of correct results in agreement/Total number of results) x100
重现性%=(具有一致性的正确结果数量/结果总数) x100

The user should establish suitable acceptance criteria for accuracy and reproducibility/precision, taking into account method capability (44). However, these criteria should be applied critically, as the results will depend on the organisms selected in the verification. For example, the microbial identification system may not be able to identify an isolate because the organism is not included in the database, the system parameters are not sufficiently comprehensive to identify the organism, the isolate may be nonreactive in the system, or the species may not have been taxonomically described.
用户应建立适当的准确度和重现性/精密度可接受标准，并考虑方法的能力（44）。然而，应严格使用这些标准，因为结果将取决于确认中使用的微生物。例如，微生物鉴别系统可能无法鉴别一个分离菌，因为该微生物不在数据库中，系统参数不足以全面鉴别该微生物，分离菌在系统中是不起反应的，或者可能没有对该菌种进行分类描述。

Additional instrumentation and method validation activities may also be necessary, as required by the end-user, and as appropriate for the technology platform representative of the alternative or rapid system. For example, additional precision studies, similar to what has been described under Ruggedness (Section 5.3.8) may be employed, where testing is conducted on different days and/or by different analysts. Robustness testing (see Section 5.3.9) may also be warranted in the event modifications to the original method or instrumentation have been made. Additional suitability testing (see Section 5.4) may also be required if the sample being analyzed has the potential to cause false identification results.
根据最终用户要求，对于替代或快速检测系统的代表性技术平台，也许还要进行额外的仪器和方法验证活动。例如，可进行额外的精密度研究，这类似于鲁棒性（5.3.8节）相关内容，即在不同日期和/或由不同检验人员进行测试。在对原方法或仪器进行变更时，可能需要进行鲁棒性试验（见5.3.9节）。如果待测样品可能导致错误的鉴别结果，可能还需进行额外的适用性试验（见5.4节）。

5.8 Alternative and Rapid Methods for Mycoplasma Detection
可替代和快速支原体检测

PDA's Technical Report Number 50, Alternative Methods for Mycoplasma Testing, defines mycoplasma as follows (45):
PDA技术报告50，支原体测试的替代方法将支原体定义为（45）：
"Mycoplasma (trivial names for members of the class of Mollicutes) are an unusual group of bacteria distinguished by the absence of a cell wall, a small genome and low G+C content. The class includes pathogenic, saprophytic, and commensal species."
“支原体（软体纲中成员的俗称）是一组不常见细菌，特征为没有细胞壁、基因组简单、低G+C碱基含量。分类包括致病性、腐生性和共生性物种。”

This same technical report provides a comprehensive review of the various methods available for mycoplasma testing as well as describing the methods to use in evaluating the appropriateness of these methods.
PDA TR50对支原体测试不同方法进行了全面回顾，并描述了评估这些测试方法适宜性的方法。

Many of the recommendations provided in this (the PDA Technical Report No. 33) may be used in support of validating an alternative or rapid mycoplasma method; however, the reader is encouraged to review the recommendations provided in TR50 for a thorough understanding of what is expected. Furthermore, Ph. Eur. Chapter 2.6.7 may provide additional guidance (46).
本技术报告（PDA TR33）中许多建议也可用于可替代或快速支原体测试方法的验证；但是，建议读者浏览TR50中的建议，以全面理解应达到什么样的要求。另外，Ph. Eur.通则2.6.7也提供相关指南（46）。

6.0 Implementation: Guidance on Site Commissioning versus Initial Validation

实施：现场安装和初步验证指南

6.1Guidance for the Transfer of an Alternative or Rapid Method from an Originating Qualification Lab to a Separate Site/ Manufacturing Facility
一种可替代的或快速检测方法从合格的开发实验室转移到独立的场所或者生产工厂的指导

The first time an alternative or RMM technology platform is qualified, it is expected that a comprehensive qualification test plan, such as the one recommended in this technical report, is appropriately performed. However, it is usually not necessary to repeat the same qualification test plan for identical technologies that will be installed in the same location or at secondary facilities at a different geographic location (e.g., manufacturing sites)for routine use. In this case, a copy of the original qualification package can be provided to the secondary location, and a reduced test plan developed for the installation of the identical technology at that site. Each new installation, or technology transfer, must be separately evaluated to determine the extent of additional IQ, OQ, or PQ testing to be performed. This section outlines what this reduced testing plan may cover.
首先，如果一个可替代的或RMM技术平台是合格的，那它应该符合预期，应该按照一个完整的确认测试方案，如本技术报告中推荐的方案进行。然而通常情况下，对于安装在相同地方，或是安装在处于不同的地理位置（比如生产场地）的第二场地并用于常规使用的同一技术的相同测试方案没必要重复进行。这种情况下，原有的授权材料可以用于第二场地，并减少针对该场所的相同安装技术的测试方案。每一次可选择的安装或者技术转让都必须经过评估，以确定附加的IQ、OQ或者PQ测试的程度。本章节概述的精简的测试方案应该包含哪些内容。

6.2 Reduced Installation and Operational Qualification at the Site
简要的现场IQ和OQ

When an identical technology is installed at the secondary location, it is expected that a standard equipment IQ and OQ be performed following the original qualification package (refer to Section 5.0). Furthermore, the original hardware/software security configuration testing may not need to be repeated unless there will be systems in use at a secondary site that were not evaluated in the initial test plan. An example might be the use of a different data handling or archiving platform (e.g., LIMS, LA N server, external drive) than what was originally qualified. There may also exist environmental or physical conditions related to the secondary location that could warrant additional qualification considerations, such as extremes in altitude, relative humidity or temperature. For example, additional studies may need to be performed for methods that depend on the generation of gases as an indicator of microbial growth, as differences in performance may be observed at higher elevations due to decreased atmospheric pressure.

当一项相同的技术被安装在第二场所时，按照预期，将按照原有的确认包要求进行标准的设备IQ、OQ（请参见第5.0章节内容）。此外，原来的硬件/软件安全配置可以不用测试，除非第二场所使用的程序在原有的资质包中没有进行过评估，比如说使用一个不同于原来确认合格的数据处理或存储平台（例如，LIMS，局域网服务器，外部驱动器）。或者可能由于环境或者物理条件对第二场所的影响，如极端的海拔，相对湿度或温度，可以考虑额外的确认，例如：额外的研究需要一些方法通过检测气体的生成来监控微生物的生长，但是由于高海拔导致大气压降低可能造成性能上的差异，需要额外的进行确认。

6.3 Performance Qualification at the Site现场PQ

Because an exhaustive microbiological testing plan will be completed during method validation at the initial qualification facility, it may not be necessary for the secondary facility to repeat this testing in its entirety when a like-for-like instrument (e.g., the exact same methods and components with the exact same version numbers for all software, microprocessors, computers) is installed. However, it is recommended that a reduced microbiological challenge from the original qualification, in addition to representative isolates recovered at the secondary site (if applicable; see below), be performed to demonstrate that the system is operating as intended. For example, a few reference organisms, identical to what was used during the original qualification, may be used to confirm basic functionality and demonstrate that key qualification requirements are met(e.g., accuracy and precision). Additionally, it may be appropriate to determine if the original qualification will meet current GMPs and internal requirements, taking into consideration the date the original qualification was completed.

因为一个详细的微生物测试方案在最初确认场地的方法验证过程中已经完成，所以当安装仪器时（如，相同的方法和完全相同的版本号的组件，微处理器，计算机），可能没有必要在第二场地重复全部的测试方案。不过建议在按预期进行设备运行时，减少在原有的确认的微生物挑战，而采用第二场所分离的代表菌株，来证明设备能够满足预期的用途。例如，一些参照微生物，与原来确认中使用的一样，可以用来确认基本功能和证明符合关键质量要求（准确性和精度）。此外，可以根据原有确认的完成日期，应该确定一下原有的确认是否符合现行GMP和国内要求。

A review of locally recovered microbial isolates should indicate if the reference strains used during the original method validation are representative of the isolates recovered at that site. If it is determined that they are not, then the local qualification plan should include a list of microbial isolates to be evaluated

and what qualification requirements they will be tested against.
应该回顾分离回收到的微生物菌株，并且明示原始确认中使用的参照菌株能否代表现场分离出来的菌株。如果确定不是，那么此处的确认方案应该评估这些分离出来的微生物，还要包括要对这菌株做哪些确认。

Following the limited method validation using reference cultures and/or local isolates, the local equivalency testing will be performed. Even though the original equivalency testing may assess the same type of test sample as what the secondary site will be evaluating routinely, the microbial load (number and type of microorganisms) may not be the same. Therefore, equivalency testing should be conducted using the actual test material at the secondary site. Additionally, if the originating qualification did not include the actual product and/or process material that the site will be evaluating routinely, then these materials must be evaluated during the local method suitability testing, where the potential impact of sample material on the test method is assessed, such as system interference, false positives and/or false negatives. Section 5.0 of this Technical Report provides the guidance for conducting these types of studies.
在接下来用对照菌株或/和本土分离株的进行有限方法学验证时，应该进行现场的等效性测试。即便第二场地常规评估的测试样品的种类与最初等效测试中使用的种类相同是，微生物负载（微生物的数量和类型）可能不一样。因而，第二场所的等效性测试应该用实际使用的试验材料进行。此外，如果原来的确认中不包含现场需要常规评价的产品和/或者工艺材料时，那么应将这些材料在当地的方法适用性试验过程中进行评价，以评估样品材料对测试方法的潜在影响，如系统干扰，假阴性或假阳性。本技术报告的第5.0章节为进行这类研究提供了指导。

During the development of the local equivalency test, the site will determine the nature of the test plan to provide meaningful data about the ability of the system to operate as it is intended. This may include the required number of batches, number of replicates, number and location of sampling points, length of study, etc. Most importantly, sufficient data will be required to evaluate whether a shift in the data from the alternative or rapid method is statistically significant than the method being replaced, and whether the data warrants a modification to the base line acceptance levels or specifications.
在当地进行等效性测试的过程中，当地应该明确测试方案的性质，以确保提供能够证明系统按照预期运行的可靠数据。这可能包括所需批次的数量，重复次数，采样点的数量和位置，研究时间的长短等等。最重要的是，从数据变化来评估一个非传统或快速的检测方法比被替代的方法更具有统计学意义，以及这些数据是否确保在接受水平或标准的及格线上有所改进，都需要大量的数据来评估上述内容。

6.4 Implementation of the Alternative or Rapid Method at the Site
可替代的或快速检测方法的实施

As part of the installation, qualification and technology transfer activities at the secondary site, all necessary training will be conducted, relevant procedures will be written and approved, and maintenance and calibration programs will be developed prior to performing the analyst qualification and subsequent routine use.
作为在第二场地的安装、确认和技术转移的一部分内容，在进行分析和日常使用之前，要进行必要的培训、起草并批准相关规程，应建立维护保养和校准程序。

Once the alternative or rapid method has been successfully installed and qualified for use at a secondary

site and regulatory approval as required is sought, the system may be used routinely.
一旦该可替代的或快速检测方法被成功的安装并且确认适用于第二场所以及符合监管部门的要求，该系统就可以常规使用。

In the event the site intends to qualify the same instrument for use with a new product or process material, a robust new qualification plan is required to be performed, and the test plan and acceptance criteria are previously discussed in this document.
当该场地打算使用新产品或者工艺材料来确认同一个仪器适用，需要执行一个可替代的有效确认方案，而且测试方案和可接受标准必须符合本报告上文的要求。