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Quality Control of Microbiological Transport Systems; Approved Standard

This document provides criteria to assist manufacturers and end users of transport devices in providing and selecting dependable products for the transport of microbiological clinical specimens.

A standard for global application developed through the NCCLS consensus process.

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Quality Control of Microbiological Transport Systems; Approved Standard

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Abstract

NCCLS document M40-A—*Quality Control of Microbiological Transport Systems; Approved Standard* presents the criteria that shall be considered when choosing a microbiological transport device, to facilitate sample preservation. Quality control considerations for the manufacturer and testing laboratory are presented, as well as techniques, control organisms, and acceptability criteria. This document provides a consistent protocol for initial testing of microbiological transport devices by manufacturers and a method by which laboratories can validate manufacturer claims and compare devices.

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Foreword

In 1893, Councilman first described making transport swabs by wrapping cotton pledgets around the end of wires, enclosing these wires in test tubes, and sterilizing them in a hot air sterilizer.[1] After sterilization, the wires, "still enclosed in the test tubes," were carried to the wards where the wire could be removed and used to rub the pharyngeal membrane of patients suspected to have diphtheria. After collection, the test tubes were labeled and sent to the laboratory where specimens could be plated.

The development of transport devices was a result of public health concerns.[2] Maintaining organism viability during transport to the public health laboratory was imperative for isolation and identification of the agents responsible for relevant infectious diseases. During the 1930s, 1940s, and 1950s, infections of public health concern, particularly gonorrhea and bacterial diarrhea, were the driving force behind development of transport media and devices.[3] Most studies focused on evaluation of performance rather than establishment of an acceptable standard of expected performance.[4] It is difficult to determine when systematic quality control began to be applied to transport systems. However, it was Rubbo and Benjamin who noted that certain batches of cotton wool used on swabs were associated with faster microbial death rates than others and that this phenomenon (toxicity) could be countered by the addition of serum onto the transport swabs.[5]

Within the hospital setting, use of transport devices for various "routine cultures" began as investigators determined variability in recovery from specimens plated at the bedside compared to those routed to the laboratory via established mechanisms.[6] Today, a number of factors contribute to the increasing emphasis on the use of transport devices to maintain specimens for microbiological testing. These include increased use of outpatient treatment that has accompanied shortened hospital stays, and centralization of laboratory services due to both managed care and shortage of individuals with expertise in clinical microbiology. Standardization of the quality control of transport devices is long overdue.

As new technologies provide the opportunity to redefine the method of recovery or detection of organisms of interest, standardizing the quality control testing and acceptance criteria will become important to assure the highest level of care to patients. This document on quality control of transport devices will assist in standardization of the performance of these devices.

In the United States, basic manufacturing requirements for medical devices, including *in vitro* diagnostic (IVD) devices, were established via the Medical Device Amendments of 1976. This legislation gave the Food and Drug Administration (FDA) authority to regulate medical devices (premarket notification, [510(k)] and premarket approval [PMA]), and develop consistent manufacturing requirements (Good Manufacturing Practices). Good Manufacturing Practices include the requirement to perform product quality control testing prior to distribution. Each manufacturer is required to establish the type of testing to be performed, as well as acceptance criteria based on the product and its intended use. Additionally, the European Union (EU) has recently adopted the Medical Device Directive 93/42/EEC and the *In Vitro* Diagnostic Device Directive 98/79/EC that have requirements very similar to those in the U.S. The Directives include provisions to utilize harmonized standards as a method of demonstrating conformity to the Directive requirements. Likewise, the FDA has recently formalized the use of these types of standards by manufacturers to demonstrate performance in premarket submissions. Further discussion of regulatory considerations for these markets can be found in Appendix B.

Acknowledgment

This standard was developed through the cooperation of the NCCLS Area Committee on Microbiology and its Subcommittee on Quality Control of Microbiological Transport Systems, and Committee E13, Culture Media of the Department for Medical Standards (Normenausschuss Medizin) at the German Standards Institution (Deutsches Institut für Normung [DIN]). Representatives of both NCCLS and DIN participated in the development of each organization's respective standard. It is expected that this effort will advance the international harmonization of this important microbiology standard, thereby improving healthcare delivery worldwide.

Key Words

Acceptable performance, acceptance criteria, biological properties, control strains, microbiological, microbiological testing, molecular transport, performance criteria, quality control, regulatory considerations, specimen transport, standards, storage conditions, transport devices, transport medium, viral transport

Quality Control of Microbiological Transport Systems; Approved Standard

1 Scope

It is clear that the transport of clinical specimens is a critical component for accurate diagnosis. The preservation of inherent, interpretive attributes of microorganisms and/or nucleic acids can be quickly compromised when the transport conditions or transport devices are suboptimal. The advent of antigen detection methods, methods for amplification and detection of genetic elements, and the requirement for local or distant transport of these specimens to a testing facility has imposed further considerations on manufacturers to provide products that will not compromise the ability of the laboratory to provide clinically relevant data to physicians. Clinicians should be able to collect and submit specimens to the laboratory with a reasonable assurance that the transport device will maintain the viability of microorganisms and/or preserve nucleic acids present in the specimen. Laboratorians should be able to retrieve specimens from containers, devices, and transport media with a reasonable assurance that representative components of the specimen were maintained during transport.

This standard provides criteria to the manufacturers and end users of transport devices to assist in provision of dependable products for the transport of microbiological clinical specimens. Manufacturers will be able to state whether or not the performance characteristics of a particular product satisfy the performance standards as specified in this document. Furthermore, manufacturers shall state whether or not any additional testing is required prior to the use of a particular product.

In this document, except as specifically noted, quality control consists of an assessment of the performance characteristics of a complete device and not the individual components. There are multiple variables involved in the manufacture of a transport device, including, but not limited to, the container, transport medium, collection device, packaging, and atmosphere. It is fundamental that the assessment of the device be directed at measurable performance characteristics for the particular device.

This document is not intended to provide proprietary information on product development, but rather to provide to the device user assurance that manufacturer claims are met following standardized testing and acceptance criteria. It provides guidance to the manufacturer in addressing critical issues related to specimen integrity specific to the type of testing to be performed, e.g., bacterial and viral culture, or nucleic acid detection. This document does not address the technique of transport device manufacture, but focuses on the methods for quality control testing and acceptance criteria to provide a product suitable for analysis of clinical specimens for agents of disease.

Transport devices are essential components of the preanalytical process of microbiology laboratory testing. It is recognized that these early steps in the total testing process are critical to production of clinically relevant information. Patients, physicians, healthcare providers, and laboratorians expect products that meet the highest standards of laboratory practice. This document will facilitate this goal.[a] And while it is beyond the scope of this document to address the design of devices, it is imperative that device design promotes correct use, and that laboratorians select devices that best serve the user needs of the physician and the patient.

Although a discussion of specimen transport conditions is beyond the scope of this document, it is recognized that temperature has a significant effect on preservation of microorganisms in various transport devices. There are a number of recent studies that have compared performance of transport devices inoculated with various organisms at room temperature (20 ºC to 25 ºC) and cold temperature (4 ºC to 8 ºC). These studies have found simulated transport performance at cold temperatures to be

[a] For example, in the U.S., the Clinical Laboratory Improvement Amendments (CLIA) guidelines place the responsibility for acceptance of quality specimens on the laboratorian.

superior to room temperature. These data support the suggestion that the current recommendations of room temperature transport do not represent the optimal holding temperature for maximum preservation of microbiological samples.[7-14] If transport conditions of the end user differ from those validated by the manufacturer, actual transport conditions should also be tested in order to determine viability and overgrowth of organisms (i.e., in insulated coolers with cold packs).

We encourage manufacturers to perform quality control of microbial transport devices at both 20 ºC to 25 ºC and 4 ºC to 8 ºC, and furthermore to specify this in their package inserts and regulatory submissions. This will permit users who wish to transport specimens for testing by their laboratories at 4 ºC to 8 ºC assurance that manufacturers performed testing at the temperature used by their laboratories. If further investigations lead to changes in the current recommendations for room temperature specimen transport, manufacturers performing quality control at both temperatures would not have to make any subsequent changes in their quality control procedures or package inserts. Finally, the members of the subcommittee wish to emphasize that few standards for quality control have been suggested for many microbiology transport devices. For this reason, some of the protocols provided are general outlines designed, in part, to promote discussion among the manufacturers, laboratories, and users regarding what would constitute an appropriate standard, as well as promote research and publication that may serve as the foundation of new standards as activity in this area moves forward.

Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996;Vol 17;1:53-80), (MMWR 1987;36[suppl 2S]2S-18S), and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials and for recommendations for the management of blood-borne exposure, refer to the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections.*

2 Introduction

Prior to production of this document, there had not been a recognized standard procedure for determining the effectiveness of microbiological transport devices. As a result, manufacturers have not had the benefit of external guidelines and standards to measure the performance characteristics of their products, and have had to rely on internally developed protocols for testing product performance. For this reason, it has been difficult to independently validate manufacturers' performance claims.

A variety of microbiological transport systems, formulations, and devices exist. It is the goal of this document to provide a standard that will enable both manufacturers and end users to systematically evaluate systems for performance effectiveness, ensure standards of performance, and allow for internal validation of product effectiveness.

3 Definitions

Active Ingredient – A component that is included in a reagent or medium to provide pharmacological effect to organisms present, or undergo a chemical change in the reagent media which provides a specified activity or effect (e.g., acts as an inhibitor, a nutrient, preservative, or stabilizer).

Breakage – Loss of vessel integrity due to the production of unintentional cracks or openings in the vessel's walls.[15]

Container failure – Any situation that leads to the loss of the specimen or its alteration so as to render it unsatisfactory for analysis.

Leakage – The loss of a seal between the container and its closure resulting in materials moving into or out of the container.[15]

Overgrowth studies – The process to determine increases in organism concentrations during simulated transport.

Room temperature – The temperature prevailing in a working area[16]; **Controlled room temperature –** A temperature maintained thermostatically that encompasses the usual and customary working environment of 20 degrees to 25 degrees Celsius; **NOTE:** Labeling can indicate storage at 'controlled room temperature' or 'up to 25 °C.'

Sampling device – An object (often made of plastic material) with a carrier, used for taking the specimen containing the microorganism; **NOTES:** a) This carrier can be fitted with absorbent material (e.g., cotton wool, synthetic polyester, rayon, polyurethane foam); b) The holder of the sampling device can be integrated into the design of the cap of the transport system ***(DIN 58 942, Part 4).***[15]

Transport – The act of moving a sample from one point to another; **NOTES:** a) Usually from collection to testing site; b) Transport may include intramural (e.g., pneumatic tube system) and extramural (car, plane, bus) components; c) Any container that is used for transporting a microbiology sample shall fall under the standards of this section.[15]

Transport container – The container of the transport system is a transparent container accommodating the transport medium and the specimen containing microorganism and, in some cases, the sampling device ***(DIN 58 942, Part 4).***[15]

Transport medium – Liquid or semisolid medium designed to preserve and maintain the integrity of the specimen for the time period between specimen collection and laboratory processing of the sample; **NOTE:** Transport media usually contain substances that do not promote multiplication of microorganisms, but ensure their preservation (e.g., Stuart's or Amies transport medium ***[DIN EN 1659]***. For some fastidious organisms, culture media have been employed as part of a transport system.[15]

Transport swab – A device design for collecting and transporting a sample taken from a patient and transported to a testing laboratory; **NOTES:** a) The device is normally supplied as a kit comprising: 1) Swab applicator stick(s), which at one end is covered with an absorbent fiber material such as rayon or synthetic polyester or with a foam material such as polyurethane. The swab applicator stick is used for collecting a sample from the patient; 2) A container or tube, into which the swab applicator is placed after the sample is collected. This container or tube is intended to secure the sample during transport; 3) A liquid or semisolid transport medium, which may or may not be present in the container or tube; b) A transport swab may or may not have a stated claim for the maintenance of viable organisms, or for the preservation of antigenic or molecular components of an organism(s).

Transport system – For the purposes of this standard, the transport system is a device that ensures the integrity of the specimen and allows for safe handling during the interval between specimen collection and processing the specimen in the laboratory; **NOTES:** a) It consists of the liquid or semiliquid (gel) transport medium, the container of the transport system and in some cases, also a sampling device ***(DIN 58 942, Part 4);*** b) It does not include packaging materials that conform to various regulations pertaining to the transport of etiologic agents.[15]

4 General Microbiological Transport System Considerations

This document deals only with the specimen collection transport systems used with clinical specimens for microbiological testing, and does not provide recommendations for specimen collection transport systems for general laboratory testing or for testing reagents and devices used in actual analyte testing. Regulatory considerations are summarized in Appendix B.

4.1 Container Structural Requirements

The design and construction of the container and sampling device shall satisfy the physical and chemical requirements given in this standard up to and including the manufacturer's "use-by" date.

4.1.1 Internal Stresses

The container and the sampling device shall be free from internal stresses in order to prevent the occurrence of cracks that might result in the contamination or dehydration of the transport medium.

4.1.2 Impact Strength

Transport systems shall be capable of withstanding the range of mechanical forces to which they can reasonably be expected to be subjected without breakage. In addition, systems consisting of an agar column or plug into which a sampling device is placed must also be capable of withstanding forces encountered in transport without a significant separation or splitting of the agar column or plug.

4.1.3 Tightness

Design of the transport closure shall be capable of withstanding the range of mechanical forces and pressures to which it can reasonably be expected to be subjected without leakage.

4.2 Assessment of Structural Requirements

It is beyond the scope of this standard to provide detailed procedures for assessment of the factors listed in Section 4.1. Manufacturers should consult regional regulations for protocols to assess structural requirements for containers and sampling devices.[b] Other organizations have established procedures that may be used to perform such assessments. (See Appendix A). The appropriateness of a procedure may vary between the various transport systems. Appendix A is not meant to be exhaustive. Comparable standardized procedures from other organizations may be used.

4.3 Biological Requirements of the Transport System

The transport system shall preserve the specimen during transport when the device is utilized as specified in the package insert, up to and including the manufacturer's use-by date.

[b] In the U.S., the Food and Drug Administration has not established protocols for manufacturers to follow, but does interpret 21CFR subpart G 820.70 and 820.80 as well as subpart H 820.80 (d) to specify that manufacturers must establish processes and conduct testing to assure structural integrity.

4.3.1 Container and Additive Interferences

Some transport systems contain different types of transport media and stabilizers to preserve viability of fastidious microorganisms for which transport is intended. Any known incompatibilities related to the intended use(s) of the transport system shall be indicated plainly by appropriate warnings in the package insert. End users shall verify incompatibility by including controls against inhibition in their internal protocols and may consider whether each lot number and shipment of a transport device type should be tested for interference before use. Manufacturers are encouraged to list all ingredients so that the user can determine if certain ingredients (e.g., glycol, charcoal, antibiotics) are known inhibitors for microorganism or nucleic acid assays. Each transport device type shall be tested for interference before using.

4.3.2 Swabs

Some components of collection swabs are known to be problematic to isolation of certain microorganisms or amplification and/or detection of various nucleic acids.[17-31] For example, calcium alginate minitipped swabs commonly used to collect nasopharyngeal specimens for *Bordetella pertussis* may decrease recovery of lipid-enveloped viruses.[17,18,22,24,28,30] Similarly, some lots of cotton used in swab tips have been shown to be inhibitory to the growth of some microorganisms; hence, cotton-tipped swabs shall not routinely be used for specimen collection for culture.[19,32] Reduced recovery has also been reported when using calcium alginate-tipped swabs for *Neisseria gonorroheae*.[27] In addition, certain swab components have been found to interfere with molecular detection methods.[29,31] The glue used by some swab manufacturers may be inhibitory to certain organisms, and may also be extracted during specimen preparation (such as enzymatic digestion or chemical extraction processes) and interfere with molecular detection methods.[27] Wire/metal shafts may also contribute to interference problems if the shaft comes in contact with extracting reagents in molecular testing.[29]

4.3.3 Assessment of Biological Requirements

Testing shall be carried out as specified in this document. It is difficult to simulate the submission of clinical specimens by challenging a transport system with a pure suspension of microorganism. It is recognized that the absence of other biomaterial such as protein, mucous, exudate, and other competing microorganisms may influence recovery. Nonetheless, although it is recognized that testing pure suspensions of organisms is not without problems, it is necessary in order to reproducibly standardize the testing and make it possible for the user to evaluate and compare products.

4.4 Bio-burden

Microbiological transport systems may contain liquid or semisolid gel formulas. Semisolid gels contain agar, a polysaccharide extract from seaweed with an intrinsic bio-burden. Although manufacturers attempt to minimize or eliminate all sources of bio-burden originating from product components or from the process of manufacture, agar raw material still represents an important potential source of bio-burden.

Transport systems are normally sterilized by lethal ionizing irradiation; therefore, bio-burden generally refers to nonviable organisms observed on microscopic examination of Gram-stained smears prepared from swabs transported inside the medium. Because nonviable organisms are present in the transport media, the possibility exists that bacterium may be interpreted as originating from the patient, regardless of whether or not they are recovered on culture. A clearly defined standard is necessary to limit bio-burden to acceptable levels.

A Gram-stained smear must be prepared from the final product of each lot of transport swabs or transport system containing liquid or semisolid gels. Using a diamond-tipped or similar glass marker, inscribe an area 20 x 50 mm on a microscope slide. Using the collection swab or device supplied with the transport

system, apply liquid or semisolid gel medium evenly across the area marked on the slide. In the case of semisolid gels, apply between 0.1 and 0.2 grams of the medium, taking special care to distribute the medium smoothly and evenly, avoiding agar lumps. Microscopic examination must identify no more than two bacteria in ten microscopic fields with 10 x 100 magnification in order to be considered acceptable. If more than two bacteria are observed in ten microscopic fields, a minimum of five transport products from that lot should be further examined, extending the microscopic assessment to 50 adjacent microscopic fields. If the total number of bacteria observed in 50 fields is less than ten, the lot is acceptable. If the total number of bacteria observed exceeds ten, the lot is unacceptable. When conducting this investigation, care must be taken to avoid all other extraneous sources of bacterial cells. The use of washed and alcohol-treated glass slides, sterile filtered Gram-stains, and a fresh bottle of oil immersion is recommended.

4.5 Product Information

Packaging shall include descriptive product identification, product identification number, lot number, expiration date, appropriate usage instructions, and storage conditions. The package insert and ideally the packaging of the transport system itself should clearly state the intended and validated uses and any known limitations (e.g., an aerobic swab that cannot be used for anaerobic cultures).

Standardized procedures used to assess the structural parameters of the transport container shall also be listed.

4.6 DOT/IATA Regulations Governing Shipping

On August 12, 2002, the U.S. Department of Transportation (DOT) issued a Final Rule under U.S. transportation regulations. The effective date for this new final rule was extended from the initial effective date of October 1, 2002, to February 14, 2003. This regulation affects all shipments, by air or ground transportation within and between the United States, and harmonizes U.S. DOT regulations (Title 49 CFR) with the international rules for air shipment of Infectious Substances and Diagnostic Specimens: the ICAO (International Civil Air Organization) Technical Instructions.

The rule affects the way in which the broad categories of infectious substances, diagnostic specimens, biological products, and regulated medical waste are defined, classified, packaged, and transported. The most significant changes are found in the rules for air shipment of liquid specimens. The emphasis is clearly on sturdy, leakproof specimen collection containers. Under the new rule, triple packaging is required for all diagnostic specimens (primary container, leakproof secondary container, sturdy outer shipping container), and either the primary or secondary container must meet new leakage requirements (95kPa pressure test). The Final Rule can be viewed at http://hazmat.dot.gov/67fr-53118.pdf.

5 Quality Control

5.1 Sampling

5.1.1 Number of Sampling Devices/Transport Systems

Testing of microbiological transport media shall be performed by the manufacturer in triplicate (three devices for each product, organism, and time point).

5.1.2 Number of Lots

Each lot of packaged product shall be tested by the manufacturer as specified in this standard.

Manufacturers may have their own in-house standards for “product release,” which they are required to maintain. However, it is the responsibility of the manufacturer to establish whether their product meets

the performance criteria set forth in this NCCLS standard. It is also the responsibility of the manufacturer to ensure that the product performance continues to meet these performance criteria, up to and including the expiration date.

6 Quality Control of the Transport Container

6.1 Manufacturer

It is the responsibility of the container manufacturer to ensure that the various factors listed in Section 4.1 are addressed during product design and manufacturing. Standardized methods from recognized sources in the manufacturing industry (see Appendix A) shall be used to assess acceptable performance.

The manufacturer shall satisfy physical and chemical requirements of transport devices required by Good Manufacturing Practices (see Appendix B).

The manufacturer shall also subject a sample from each lot number to at least one standardized test for mechanical strength and one standardized test for the tightness of the closure. In addition, the manufacturer is responsible for performing testing and meeting acceptable performance criteria for which the device is specifically intended, as specified in this standard.

6.2 Laboratory End User

Laboratorians shall be familiar with those factors that affect the performance of specimen containers. In particular, the various conditions under which the containers are to be used must be addressed during the selection of a transport system. However, due to the nature of the tests and the expertise required to perform them efficiently, end users are not responsible for routine container quality control.

Laboratories evaluating new transport systems shall subject them to the extremes of conditions that may be encountered. Testing shall include the extremes of temperature, pressure, and mechanical forces (including travel through pneumatic tube systems, airplanes, and in vehicles routinely employed in the transport of specimens). The transport system shall perform to acceptable levels under each of these conditions. Once such evaluation has occurred, routine quality control testing directed to the specimen container need not be performed. Procedures used by the laboratory in making this evaluation need not be as standardized as those utilized by manufacturers.

7 Bacteriological Swab Transport Devices

Apart from the quality control currently performed by manufacturers, there is little published information to use as the basis for the standards proposed in this section. Thus, the proposed quality control standards for bacteriological swab transport devices were developed based upon review of the available literature.[33-37] Limited validation studies were performed by three committee members in their laboratories.

7.1 Storage Conditions

Sampling devices in the appropriate transport system shall be stored prior to use according to the manufacturer's recommendations.

7.2 Shelf-Life Studies

At least three lots of transport devices shall be tested at established intervals by the manufacturer to validate expiration dates. Transportation and storage conditions are taken into account when expiration dates are established (see the current edition of NCCLS document M22—*Quality Assurance for Commercially Prepared Microbiological Culture Media*). Expiration shall be provided to the user on the device labeling on the basis of this information. If a manufacturer wishes to modify an expiration date claim for a product, performing the process of testing at established intervals with at least three lots would be necessary to validate the new claim.

7.3 Organism-Specific Standards

It is not anticipated that all transport devices/systems will be able to satisfy the standards outlined in this document for all microorganisms. Hence, bacterial strains are divided into three main groups for quality control testing: aerobic and facultative anaerobic bacteria; anaerobic bacteria; and fastidious bacteria. Manufacturers can make or publish claims for any or all of the three groups based upon satisfactory quality control results for that specific group. The required quality control strains are listed in Tables 1 through 3 (Section 7.11.1) and Tables 5 through 7 (Section 7.12.1).

7.4 Source of Control Strains

All of the control strains of bacteria in Tables 1 through 3 (Section 7.11.1) and in Tables 5 through 7 (Section 7.12.1) are available from the American Type Culture Collection[c] (ATCC®). ATCC®-derived cultures from commercial sources may also be used. The strains indicated in Tables 1 through 3 and 5 through 7 are the minimum that must be included in the test battery used to evaluate each of the media listed. Additional strains may be employed by the manufacturer as needed for intended use. Refer to control strains listed in Tables 1 through 3 and Tables 5 through 7.

7.5 Maintenance of Control Strains

Maintenance of stock cultures to be used for quality control purposes must be standardized in a manner that will minimize the opportunity for contamination or alteration of growth characteristics. This can be ensured by the use of either frozen cultures or standardized cultures obtained from commercial sources.

The following procedures may be used to prepare control cultures of all strains of bacteria utilized for quality control testing. If cultures from a commercial source are used, the manufacturer's directions are to be followed.

7.6 Preparation of Frozen Stock Cultures of Bacteria

- Stock cultures are prepared from lyophilized cultures obtained from ATCC.® Reconstitute these cultures according to the directions obtained from ATCC.®

- Using a sterile loop, inoculate by streaking two or three TSA with 5% sheep blood agar, chocolate agar, or anaerobic blood agar plates with the suspension from the reconstituted vial. Incubate the inoculated plates for 18 to 24 hours (some fastidious and anaerobic bacteria may require 48 hours of incubation) in the appropriate atmosphere at 35 to 37 ºC.

- After incubation, check for purity and verify identity by colony morphology. Perform biochemical tests, if necessary. Remove sufficient growth from the confluent area of the plates and suspend in about 50 to 100 mL of a cryoprotective medium. The cryoprotective medium may be sterile

[c] ATCC is a registered trademark of the American Type Culture Collection.

defibrinated sheep blood, rabbit blood, skim milk, or soybean-casein digest broth (tryptic soy broth, TSB) containing glycerol in a final concentration of 10 to 15% (v/v). If these frozen cultures are used to inoculate test media, the suspension shall be adjusted to the turbidity of a 0.5 McFarland standard (approximately 1 to 2 x 10^8 colony-forming units [CFU] per mL for *Escherichia coli* ATCC® 25922) before freezing. Use a No. 1 McFarland standard to adjust the suspension for *N. gonorrhoeae.* The cell suspension shall contain a sufficient number of viable cells to propagate the culture after one freeze-thaw cycle.

- Dispense a small volume (0.5 to 1.0 mL) of the bacterial suspension into small, sterile glass or plastic vials. Prepare enough stock vials to provide a source of inoculum for up to one year. Check the purity before the vials are made available for quality control testing.

- Place the vials in a freezer maintained at less than or equal to -50 °C and store until needed. The vials may be stored in an ultra-low-temperature freezer or in the vapor phase of a liquid nitrogen storage tank. Strains may be kept indefinitely at temperatures less than -70 °C. Strains shall be kept for one year only at temperatures between -50 °C and -70 °C; they shall not be stored at temperatures above -50 °C.

- When needed, remove a single vial from the freezer or liquid nitrogen storage tank and rapidly thaw the contents. The contents of the vial are used to initiate the test or, they may be used to prepare working control cultures. Alternatively, a subculture may be used for testing. Inoculate solid medium to obtain isolated colonies. After appropriate incubation, isolated colonies are used to initiate the test or to inoculate agar slants or plates to serve as primary working control cultures. Unused cell suspensions shall be discarded; never refreeze them for later use.

7.7 Working Control Cultures

- A fresh working culture should be prepared at least once per month from the refrigerated stock control. Avoid multiple serial subcultures of quality control organisms over extended periods of time.

- To produce a working control culture, inoculate an agar slant or plate with inoculum from the frozen stock culture, and incubate overnight or until adequate growth is obtained. These working agar slant or plate cultures may then be stored at 4 °C to 8 °C, or at room temperature, for up to four weeks. Always check for purity and typical colony morphology when preparing each subculture.

- A working frozen culture may also be prepared from the frozen stock culture using the procedure described in Section 7.6. Either stock vials or working control cultures may be used to inoculate the media to be tested.

- Working control cultures of anaerobic organisms shall be maintained in chopped meat without carbohydrates stored at room temperature[38] or other suitable anaerobic broth media, rather than agar slants or plates, unless frozen cultures are used.

7.8 Turbidity Standard for Inoculum Preparation

To standardize the inoculum density, a $BaS0_4$ turbidity standard equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension) shall be used. These may be prepared in-house or purchased from a commercial source. The use of a spectrophotometer to prepare organism suspensions is recommended. Use a spectrophotometer with a 1-cm light path and matched cuvettes to determine the absorbance. The absorbance at 625 nm shall be 0.08 to 0.10 for the 0.5 McFarland standard as referenced in the current edition of NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.*

7.9 Direct Colony Suspension Inoculum Preparation Method

The inoculum is prepared by making a direct suspension in 0.85% physiological saline (pH 6.8 to 7.2) of isolated colonies selected from an 18- to 24-hour agar plate (some fastidious and anaerobic bacteria may require 48 hours of incubation). A nonselective medium shall be used. Refer to Tables 1 through 3 (Section 7.11.1) and Tables 5 through 7 (Section 7.12.1) for the appropriate medium.

7.10 Quality Control Protocols

In the routine clinical laboratory, the roll-plate method is the primary means of inoculating swab transport devices to plated media. A limitation of the roll-plate method for quality control purposes is that it is not a quantitative method; it is, at best, a semiquantitative approximation. When counting colonies on a plate, statistical validity is only ascribed to colony counts of 30 to 300 on a 100-mm plate, equivalent to a one-log change in the colony count.[39] This narrow range is unacceptable for the precise type of quality control required for manufacturer performance validation of swab transport devices. On the other hand, quantitative quality control methods such as the swab elution method described below do not reflect the standard protocol used in most clinical laboratories. Whereas the swab elution method allows a quantitative measurement of the ability of a transport system to maintain viable organisms, the roll-plate technique takes into consideration some mechanical variables of the direct swabbing action that exist in the clinical laboratory, and which can influence the release of the sample onto culture plates. Because of this, two methods of testing are included.

It is expected that manufacturers will perform both methods of testing to ensure that quality control methods are sensitive enough to detect small changes related to performance characteristics of individual device components (swab elution method), as well as an approximation of whether the device performs acceptably under usage in the clinical laboratory (roll-plate method). End users may wish to perform both methods of testing to validate performance characteristics of the transport device or choose the method most appropriate for validating performance based on specimen inoculation methodologies in use.

Determination of the survivability or overgrowth of microorganisms on swab transport devices may be performed at two different ranges of temperature, 4 °C to 8 °C and 20 °C to 25 °C, corresponding to refrigerator and room temperatures, respectively. For the purposes of this document, these are considered potential transport and/or holding temperature ranges, i.e., the temperature range at which the swab transport devices are maintained after inoculation with microorganisms as described in the following sections.

7.11 Swab Elution Method (Quantitative)

7.11.1 Inoculum Density

Inoculum preparation shall be performed just prior to transferring organism suspension to microdilution plates or tubes, where swab absorption will be performed. The entire quality control procedure shall not exceed 20 minutes in order to reduce loss of organism viability in inoculum prior to incubation of inoculated swabs at appropriate holding temperature(s). Prepare inocula in 0.85% physiological saline (pH 6.8 to 7.2) to a concentration of approximately 1.5×10^8 CFU/mL (equivalent to 0.5 McFarland standard) from 18- to 24-hour growth of organism on an agar plate (some fastidious and anaerobic bacteria may require 48 hours of incubation). Dilute inoculum tenfold in 0.85% physiological saline (pH 6.8 to 7.2) solution to provide a concentration of approximately 1.5×10^7 CFU/mL (see Figure 1).

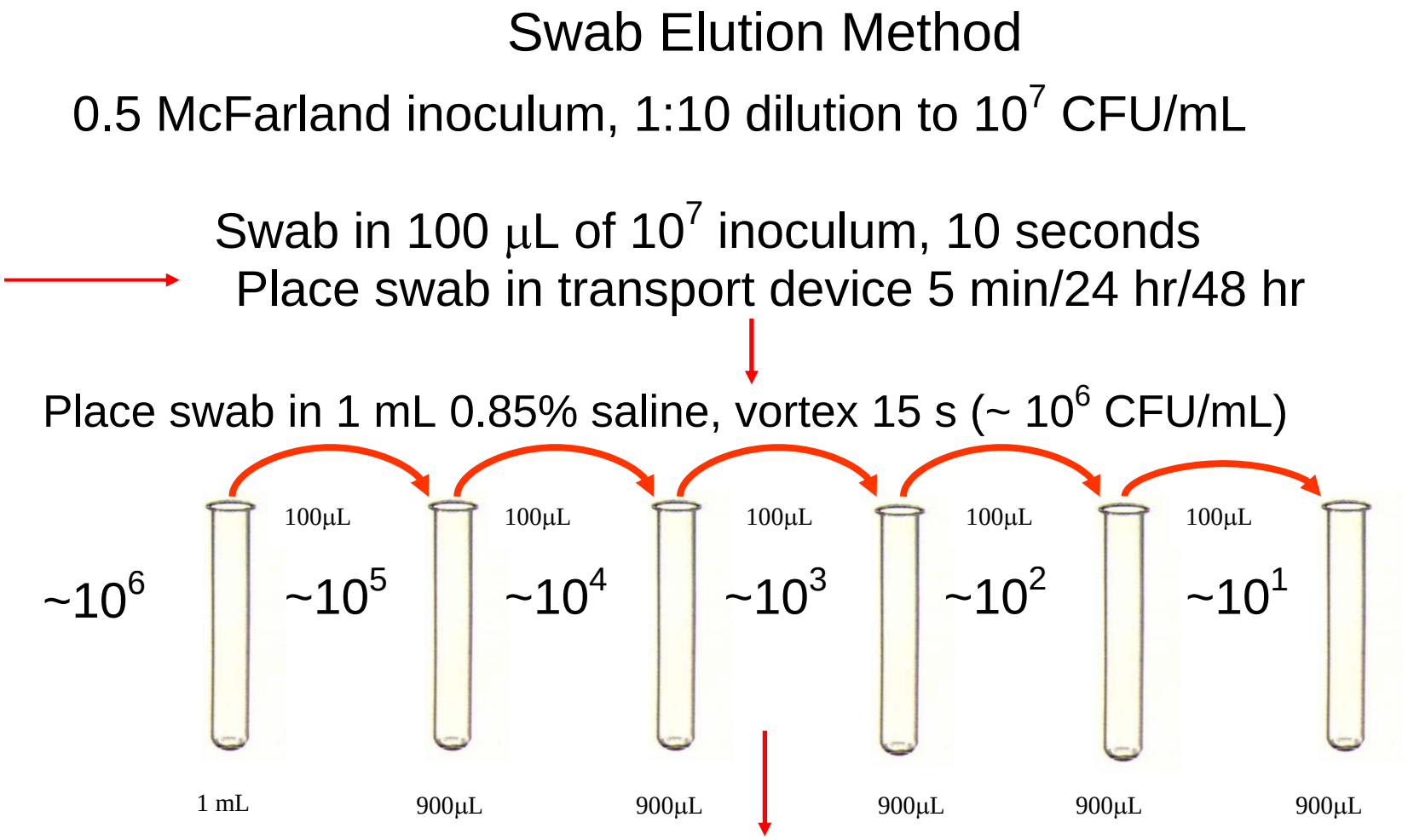

Figure 1. Schematic Diagram of Swab Elution Method

The initial inocula (1.5 x 10^8 CFU/mL) shall be verified by serial dilutions and plating to nonselective plated media. See Tables 1 through 3 below. Acceptable inoculum limits include 1.5 x 10^7 to 1.5 x 10^9 CFU/mL.

This inoculum concentration is not intended to necessarily reflect the concentration of microorganisms present in clinical specimens. Rather, this concentration has been chosen to permit accurate assessment of the performance characteristics of the transport devices.

Table 1. Aerobic and Facultative Anaerobic Bacteria

Species	Strain	Inoculum (CFU)	Plate Media	Incubation T ºC	Incubation Atmosphere	Testing Time (hours)
Pseudomonas aeruginosa	ATCC® BAA-427	1 x 10^6	5% sheep blood agar	35-37	Aerobic	48
Streptococcus pyogenes	ATCC® 19615	1 x 10^6	5% sheep blood agar	35-37	5% CO_2	48
Streptococcus pneumoniae	ATCC® 6305	1 x 10^6	5% sheep blood agar	35-37	5% CO_2	48
Haemophilus influenzae	ATCC® 10211	1 x 10^6	Chocolate blood agar	35-37	5% CO_2	48

Table 2. Anaerobic Bacteria

Species	Strain	Inoculum (CFU)	Plate Media	Incubation T ºC	Incubation Atmosphere	Testing Time (hours)
Bacteroides fragilis	ATCC® 25285	1 x 10^6	Brain-heart infusion agar	35-37	Anaerobic	48
Peptostreptococcus anaerobius	ATCC® 27337	1 x 10^6	Brain-heart infusion agar	35-37	Anaerobic	48
Fusobacterium nucleatum	ATCC® 25586	1 x 10^6	Brain-heart infusion agar	35-37	Anaerobic	48
Propionibacterium acnes	ATCC® 6919	1 x 10^6	Brain-heart infusion agar	35-37	Anaerobic	48
Prevotella melaninogenica	ATCC® 25845	1 x 10^6	Brain-heart infusion agar	35-37	Anaerobic	48

Table 3. Fastidious Bacteria

Species	Strain	Inoculum (CFU)	Plate Media	Incubation T ºC	Incubation Atmosphere	Testing Time (hours)
Neisseria gonorrhoeae	ATCC® 43069	1 x 10^6	Chocolate agar	35-37	5% CO_2	24

7.11.2 Inoculation Procedure

Different types of swab devices can routinely absorb different volumes of inoculum fluid. For example, a standard rayon or synthetic polyester fiber-tip swab can consistently absorb 100 µL while a rayon or synthetic polyester fiber-tip minitip device can only absorb approximately 20 µL of inoculum. A polyurethane foam device can absorb approximately 50 µL of inoculum (See Table 4).

Each microorganism/device combination requires six samples (three for zero-time inoculum determination and three for poststorage inoculum determination). Therefore, based upon the swab type, the following volume of inocula is pipetted into each of six tubes or wells of a microtiter plate for each microorganism to be tested (use of a multichannel pipettor can expedite transfer to microtiter plate):

- Standard applicator swab 100 µL
- Polyurethane swabs 50 µL
- Minitip swabs 20 µL

Table 4. Inoculum Adjustment

Standard applicator swabs	Dilute the 0.5 McFarland suspension 1:10	Absorb 100 µL onto each swab tip
Polyurethane swabs	Dilute the 0.5 McFarland suspension 1:5	Absorb 50 µL onto each swab tip
Minitip swabs	Dilute the 0.5 McFarland suspension 1:2	Absorb 20 µL onto each swab tip

Place each of the six swabs for a particular microorganism/device combination into a tube or microtiter well until it touches the bottom and allow it to absorb for a minimum of ten seconds. Next, place sampling devices directly into transport containers/holders. Crush ampules as appropriate for the particular device type. No manipulation of the devices, beyond what is indicated in the package insert, shall be performed. It is important to process all devices in the same manner.

No longer than 20 minutes shall be allowed from preparation of organism suspension to placement of sampling devices in the appropriate transport system and placement at appropriate holding temperature. Extending the processing time may result in significant reductions in viable organisms, especially when working with *N. gonorrhoeae* and fastidious anaerobes. The significance of set-up time for QC of anaerobes on swab transport devices is yet to be established. Hold all transport devices for the length of time indicated in Tables 1 through 3 (Section 7.11.1).

7.11.3 Plating

Colony counts are determined using the following procedure (see Figure 1) [33,40]:

(1) Remove a swab from the transport device, and place it into a tube containing 1 mL, of 0.85% physiological saline (pH 6.8 to 7.2).

(2) While holding the swab, mix vigorously using a vortex mixer for a minimum of 15 seconds.

(3) Express as much liquid as possible from the swab by rotating it on the inside of the tube.

(4) Perform five tenfold serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) in 1 mL of 0.85% physiological saline pH 6.8 to 7.2 (100 µL added to 900 µL), resulting in dilution concentrations of approximately 10^5, 10^4, 10^3, 10^2, and 10^1CFU/mL.

(5) Thoroughly mix the primary tube on a vortex mixer. Pipette 100 µL to appropriate plated media. Repeat pipetting step to second plate for duplicate determination.

(6) Spread inoculum evenly across the surface of the media with a sterile spreader.

(7) Repeat with the remainder of the dilutions.

(8) Place in incubator at 35 °C to 37 ºC in the appropriate atmosphere of incubation.

Three of the test swabs for each microorganism/device type are utilized as zero-time controls. Remove the three devices from the transport container/holder within 5 to 15 minutes after they are inoculated. These

three swabs serve as zero-time growth controls against which the remainder of test swabs shall be compared. Store the remaining three test swabs for each organism and temperature/combination for the time specified in Tables 1 through 3.

7.11.4 Quantitation—Acceptance Criteria

Following appropriate incubation, count and average CFU. For testing to be valid, zero-time plates must have counts between 30 and 300 CFU. The final count is an average of the CFU of the six plates (three swabs with duplicate subcultures) from the dilution providing 30 to 300 CFU.

7.11.5 Recovery

Specimens held at 20 °C to 25 °C

To be considered acceptable, there shall be no more than a 3 $\log_{10}$ ($1 \times 10^3 \pm 10\%$) decline in CFU between the zero-time CFU count and the CFU of the swabs that were stored. For example, if 1×10^6 CFU are present in the zero-time plating, then a value of $1 \times 10^3 \pm 10^2$ CFU to $1 \times 10^6 \pm 10^5$ CFU count after incubation for a specified period is acceptable for that particular microorganism/device combination.[34-37] Overgrowth of microorganisms, particularly gram-negative bacilli, in swab systems transported or held at 20 °C to 25 °C continues to be a significant problem (unpublished data). At present, none of the commercially available swab transport devices are able to adequately inhibit microbial overgrowth at room temperatures. Therefore, quality control testing, for purposes of assessing significant microbial overgrowth, is not performed when the holding temperature is 20 °C to 25 °C.

Specimens held at 4 °C to 8 °C

Quality control for specimens held at 4 °C to 8 °C includes assessment of bacterial overgrowth (defined as no more than a 1-log increase in CFU between the zero-time CFU count and the CFU count after incubation for a specified period). Therefore, to be considered acceptable, for specimens held at 4 °C to 8 °C, there should be no more than a 1-log increase in CFU and no more than a 3 $\log_{10}$ ($1 \times 10^3 \pm 10\%$) decline in CFU between the zero-time CFU and the CFU after the specified holding period. For example, if 1×10^6 CFU are present in the zero-time plating, than a value of $1 \times 10^3 \pm 10^2$ CFU to $1 \times 10^7 \pm 10^6$ CFU following storage is acceptable for that particular microorganism/device combination.[34-37]

For a lot number to be released, each test organism must meet acceptance criteria. In the event that one organism fails quality control testing while the other organisms tested are acceptable, that one organism may be retested. If the repeat results are acceptable, then the lot is considered acceptable. No lots shall be released until acceptable quality control results are achieved for all organisms in Tables 1 through 3 (Section 7.11.1) for which survival in the transport device is claimed.

7.12 Roll-Plate Method (Qualitative)

7.12.1 Inoculum Density

Inoculum preparation shall be performed just prior to transferring organism suspension to microtiter plates or tubes where swab absorption will be performed. The entire quality control procedure shall not exceed 20 minutes in order to reduce loss of organism viability in inoculum prior to incubation of inoculated swabs at appropriate holding temperature(s). Inocula are prepared in 0.85% physiological saline (pH 6.8 to 7.2) to a concentration of approximately 1.5×10^8 CFU (equivalent to 0.5 McFarland standard) from 18- to 24-hour growth of organism (some fastidious bacteria and anaerobes may require 48 hours of incubation). See Figure 2.

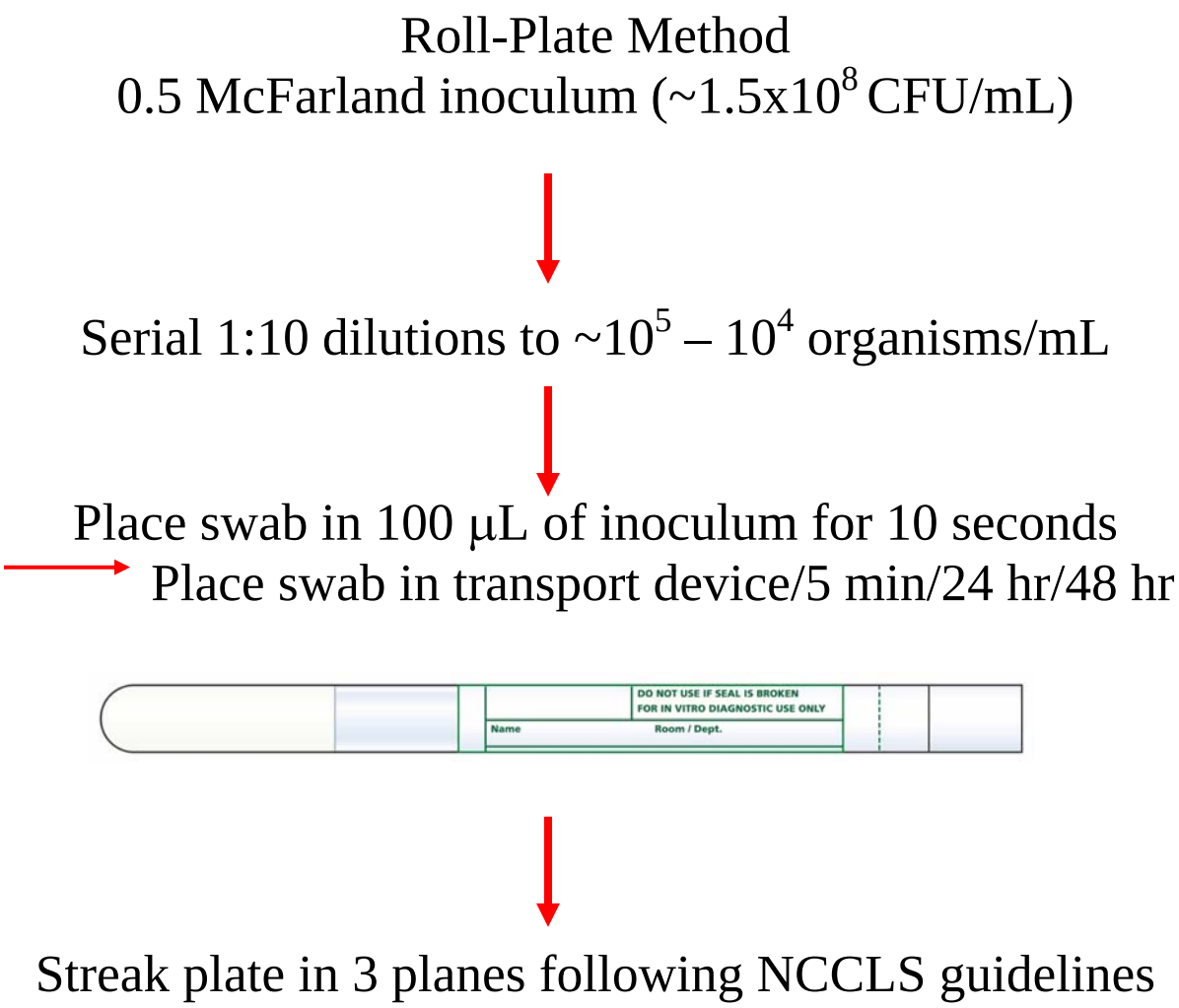

Figure 2. Schematic Diagram of Roll-Plate Method

For viability studies, **on standard applicator swabs**, four tenfold dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) are performed (2.5 mL added to 22.5 mL or 1 mL added to 9 mL saline solution) to provide approximate final working concentrations ranging from approximately 1.5 x 10^7 CFU/mL to 1.5 x 10^4 CFU/mL. The 10^4, 10^5, and 10^6 dilutions are used to inoculate standard applicator swabs with 100 µL using tubes or wells of a microdilution plate as described in Section 7.11.2. This results in final swab inocula of approximately 10^3, 10^4, and 10^5 as listed in Tables 5 through 7. For polyurethane foam and minitip swabs, the dilutions are modified to take into consideration the smaller volume that can be absorbed by these particular swabs (see Table 4).

Suspensions are prepared in greater volumes than necessary in order to reduce effects of oxidation on organism viability and shall be mixed frequently and immediately using a vortex mixer for a minimum of 15 seconds prior to transfer, to ensure consistent distribution of organisms in suspension.

This inoculum concentration is not intended to necessarily reflect the concentration of microorganisms present in clinical specimens. Rather, this concentration has been chosen to permit accurate assessment of the performance characteristics of the transport devices (approximately 300 countable colonies on zero-time plates).

Overgrowth studies are performed only on swab transport devices that are held at 4 °C to 8 °C. For overgrowth studies, five tenfold serial dilutions (2.5 mL added to 22.5 mL saline solution) are performed from the initial inoculum (approximately 1.5 x 10^8 CFU/mL) to provide a final inoculum concentration of approximately 1.5 x 10^3 CFU/mL, resulting in a swab inoculum of 10^2, as listed in Table 5. The initial inoculum shall be verified by serial dilutions and plating to nonselective media.

This inoculum concentration is not intended to necessarily reflect the concentration of microorganisms present in clinical specimens. Rather, this concentration has been chosen to permit accurate assessment of the performance characteristics of the transport devices (≤1 log increase in growth).

Table 5. Aerobic and Facultative Anaerobic Bacteria

Species	Strain	Roll-Plate Method Inoculum (CFU)		Plate Media	Incubation Temp (ºC)	Incubation Atmosphere	Testing Time (hours)
		Viability	Overgrowth				
Pseudomonas aeruginosa	ATCC® BAA-427	N/A	1 x 10^2	5% sheep blood agar	35-37	Aerobic	48
Streptococcus pyogenes	ATCC® 19615	1 x 10^3 - 1 x 10^5		5% sheep blood agar	35-37	5% CO_2	48
Streptococcus pneumoniae	ATCC® 6305	1 x 10^3 - 1 x 10^5		5% sheep blood agar	35-37	5% CO_2	48
Haemophilus influenzae	ATCC® 10211	1 x 10^3 - 1 x 10^5		Chocolate blood agar	35-37	5% CO_2	48

Table 6. Anaerobic Bacteria

Species	Strain	Roll-Plate Method Inoculum (CFU)	Plate Media	Incubation Temp (ºC)	Incubation Atmosphere	Testing Time (hours)
Bacteroides fragilis	ATCC® 25285	1 x 10^3 - 1 x 10^5	Brain-heart infusion agar	35-37	Anaerobic	48
Peptostreptococcus anaerobius	ATCC® 27337	1 x 10^3 - 1 x 10^5	Brain-heart infusion agar	35-37	Anaerobic	48
Fusobacterium nucleatum	ATCC® 25586	1 x 10^3 - 1 x 10^5	Brain-heart infusion agar	35-37	Anaerobic	48
Propionibacterium acnes	ATCC® 6919	1 x 10^3 - 1 x 10^5	Brain-heart infusion agar	35-37	Anaerobic	48
Prevotella melaninogenica	ATCC® 25845	1 x 10^3 - 1 x 10^5	Brain-heart infusion agar	35-37	Anaerobic	48

Table 7. Fastidious Bacteria

Species	Strain	Roll-Plate Method Inoculum (CFU)	Plate Media	Incubation Temp (ºC)	Incubation Atmosphere	Testing Time (hours)
Neisseria gonorrhoeae	ATCC® 43069	1 x 10^3 - 1 x 10^5	Chocolate agar	35-37	5% CO_2	24

7.12.2 Inoculation Procedure

Follow the inoculation procedure in Section 7.11.2.

7.12.3 Plating

Determine colony counts using the following procedure:

7.12.3.1 Zero-Time (Initial Counts) Samples

(1) Hold swabs in transport tubes for 5 to 15 minutes before initial (zero-time) plating.

(2) Remove the swab from the transport device.

(3) Inoculate swab to the dried surface of appropriate culture media by streaking the swab over the entire sterile agar surface, rotating the swab between the thumb and the index finger to ensure that all surfaces of the swab equally contact the surface of the culture media. Repeat by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum, as outlined in NCCLS document M2—*Performance Standards for Antimicrobial Disk Susceptibility Tests*. Avoid the rim of the agar to allow countable CFU.

(4) Within 15 minutes, place the sample in an incubator at 35 to 37 ºC in an appropriate atmosphere as described in Tables 5 through 7.

7.12.3.2 Samples Held for 24 and 48 Hours

The following day, count CFU on the zero-time plates with inoculum density most closely approaching 300 colonies, to complete viability studies.

7.12.4 Quantitation-Acceptance

Following appropriate incubation, CFU are counted and averaged. The same dilution must be counted for the zero-time and 24- or 48-hour plating as indicated in Tables 5 through 7. To ensure accurate counts, the zero-time subculture plates shall not exceed 300 CFU.[39] The final count is an average of the CFU of the three plates (three swabs per set of organism per holding condition).

For viability studies, the inoculum dilution yielding zero-time plates most closely approaching 300 CFU shall be utilized to complete viability studies.

For overgrowth studies, zero-time plates shall yield average counts of 5 to 50 CFU for testing to be valid. Note that for overgrowth studies, acceptable recovery from zero-time plates falls below those described for statistical validity (30 to 300).[39] The range of 5 to 50 CFU was chosen based on the lowest number of colonies expected to be recovered reproducibly, in order to more accurately detect overgrowth up to a 1-log increase in CFU.

7.12.5 Recovery

For viability studies to be considered acceptable, there shall be ≥5 CFU following the specified holding time from the specific dilution that yielded zero-time plate counts closest to 300 CFU (see Section 7.12.4).

For overgrowth studies to be considered acceptable, there shall be no more than 1 log increase in CFU between the zero-time count and the counts of the swabs that were held. For example, if 15 CFU are present in the zero-time plating, then a value of no more than 150 CFU following storage is acceptable for that particular microorganism/device combination.

For a lot number to be released, each test organism must meet acceptance criteria. In the event that one organism fails quality control testing while the other organisms tested are acceptable, that one organism

may be retested. If the repeat results are acceptable, then the lot is considered acceptable. No lots shall be released until acceptable quality control results are achieved for all organisms in Tables 5 through 7 (Section 7.12.1) for which survival in the transport device is claimed.

7.12.6 Reporting Quality Assurance Data to the User

The manufacturer shall indicate that performance testing has established that the product conforms to this standard through a label, package insert, technical manual, or other document. The manufacturer may also indicate any additional performance testing, sterility testing, and pH measurement that is performed.

8 Specialized Transport Devices

8.1 General Considerations

Many specialized devices are vessels without transport media and/or preservative. These devices that include tubes and cups for fluid, semisolid, and solid specimens will be governed by quality control testing that determines the ability of the device to seal and not leak under conditions of transport (extremes and variation of temperature and pressure). This type of testing of physical parameters is addressed in Section 4. In general, however, package inserts for such devices shall state the physical parameters that have been tested and the suitability for use without breakage or leakage in pneumatic tube systems for aircraft transport (governed by 49 CFR §173.127). Devices not suitable for use in pneumatic tube systems shall be labeled to advise against inadvertent use in such systems; however, users of pneumatic tube systems should perform device testing in their own systems, as system speeds may vary.

8.2 Urine Culture Containers with Preservative

Urine transport containers are addressed by the most recent version of NCCLS document GP16—*Routine Urinalysis and Collection, Transportation, and Preservation of Urine Specimens,* but transport devices for urine culture are not addressed in this standard. Quality control testing for urine culture containers with preservative shall include seeding devices with sterile physiological saline suspensions of relevant uropathogens including representative isolates of organisms commonly recovered from patients with urinary tract infections. These species should be used with the following strains recommended: *Escherichia* coli (ATCC® 25922), *Staphylococcus saprophyticus* (ATCC® 15305), *Proteus mirabilis* (ATCC® 7002), *Enterococcus faecalis* (ATCC® 29212), *Pseudomonas aeruginosa* (ATCC® BAA-427), and *Candida albicans* (ATCC® 24433). Testing shall be conducted at intervals that mimic actual use, which could be any time between 2 and 24, 48, or even 72 hours, depending on the claim made by the manufacturer and the needs of the customer. Plate counts shall remain within 1 $\log_{10}$ of the initial organism concentration when tested with calibrated loops (or quantitative plating of dilutions). Toxicity is an issue when some devices containing urine preservative are underfilled. For this reason, manufacturers shall establish a “fill volume range” for minimum and maximum filling, and laboratories shall monitor this volume to assure that the viability of transported pathogens is maintained.

8.3 Growth Devices That Are Used as Transport Devices

Quality control combined culture media and transport devices are addressed by NCCLS document M22—*Quality Assurance for Commercially Prepared Microbiological Culture Media.*

8.3.1 Blood Cultures

Recent automated blood culture systems have been evaluated as transport devices and have ‘delayed vial entry’ claims with the FDA. In contrast, those devices in use prior to the enactment of the Medical Device Amendments have not been evaluated when there is a delay in incubation because there was no requirement for such testing. For this reason, if manual blood culture media are to be used as a transport

device when blood cultures are collected in a site remote from the laboratory (physician office, patient service center, hospital satellite facility, etc), in the absence of clinical parallel validation studies demonstrating acceptable recovery with delayed transport, refer to manufacturers' recommendations. Validation could be demonstrated by use of seeded cultures with clinically relevant colony-forming units per milliliter. Quality control shall establish the length of time that bottles can be held prior to being placed into an incubator without compromising recovery; whether the bottles should be held at room temperature; or whether they should be placed in an incubator awaiting transport and then transported at room temperature without adversely affecting recovery.

9 Molecular Transport and Specimen Types

See also NCCLS document MM3—*Molecular Diagnostic Methods for Infectious Diseases.*

Sterile transport containers; blood collection tubes; rayon and synthetic polyester fiber swabs alone, or in various transport media; viral transport media; and chaotrope/surfactant/divalent metal ion chelator, etc., have all been used for transporting specimens requiring molecular testing.

Generally, transport systems used for specimens collected for culture ensure viability and recovery of target microorganisms from the specimen. For molecular transport, specimens must be transported to minimize reaction with any inert ingredients or denaturing of nucleic acids if the organisms are to be lysed during the transport phase. If organism viability is maintained, nucleic acids will be protected.

Molecular transport/collection systems are assay specific and must be assessed for utility with the assay to be used and the specimens to be tested. For example, urine specimens, because of high urea content and low pH, would require different transport conditions than sputum samples.

9.1 General Considerations

Regional regulatory agencies may specify elements for specimen collection/transport devices.[41] For example, in the U.S., the FDA has specified criteria for kit-based assays, which serve as an excellent starting point for "home-brew" assays. In particular, the FDA states:

(1) Detail the method(s) that must be used to collect optimal specimens for testing.

(2) For specific collection or transport of devices recommended or included in test kit, specify ways the user shall assure that specimens were collected and transported appropriately.

   a. Specify the types and volume (if applicable) of all specimens acceptable for testing with the device. Discuss the effects of testing inadequate or inappropriate specimens.

   b. List the appropriate specimen transport conditions (e.g., time, temperature, etc.) for each type of specimen. List and discuss the effect(s) of inappropriate transport.

   c. Describe recommended storage time and temperature.[41]

Details are further outlined in NCCLS document MM3—*Molecular Diagnostic Methods for Infectious Diseases.*

It is important that specimen collection devices do not add substances that degrade or alter the target nucleic acids (e.g., nucleases), or that interfere with target amplification and/or detection. Plastics and other collection devices utilized for transport of specimens for nucleic acid detection shall be subjected to testing for known contaminants that may interfere with reactions, as well as for any substances that may affect the testing for which the device is intended. Specific assays may have substrates that behave

differently with various types of transport systems, and it is extremely important that testing facilities are aware that validation is required before using transport devices for purposes other than the intended use specified by the manufacturer.

Collection devices intended for transport of samples for nucleic acid detection shall be nuclease-free if organisms are lysed during the transport time. Generally, collection devices used to transfer specimen material for culture will be suitable if organism viability is maintained; however, the end user must confirm the appropriateness of the transport device through use of inhibition controls in the specific nucleic acid amplification/detection protocol in use, especially when usage is outside the specific intended use. Details for testing are further outlined in NCCLS document MM3—*Molecular Diagnostic Methods for Infectious Diseases*.

Swabs may also contain material that may inhibit detection of the target nucleic acid. For example, some swabs contain adhesives to secure the rayon or synthetic polyester fiber to the shaft, and some shafts may be constructed from synthetic materials.

Functional testing must be performed by the manufacturer to ensure proper performance of the finished product.[41,42]

This document discusses the specimen collection transport systems used for transport only of clinical specimens for microbiological molecular testing, and does not provide recommendations for qualifying reagents and devices used in actual analyte testing. Analyte-specific reagents (FDA 21 CFR 864.4020[a])—the active ingredients used by laboratories in the development and performance of "home-brew" clinical diagnostic tests (analyte-specific oligonucleotide primers and probes)—and nonanalyte-specific components—general-purpose reagents (sample-preparation kits, *Taq* DNA polymerase, reverse transcriptase, RNA polymerase, dNTPs, and label-detection reagents)—are not covered by this document. Readers are referred to NCCLS document MM3—*Molecular Diagnostic Methods for Infectious Diseases*.

9.2 Quality Control

9.2.1 Manufacturer

Manufacturers are required to comply with regional quality systems requirements.[d,41,42]

9.2.2 End User

Clinical specimens have inherent factors that may inhibit amplification of target RNA and DNA. In addition to the testing performed by the manufacturer, the end user is responsible for testing all parameters of molecular testing through the use of appropriate positive, negative, and inhibition controls incorporated into molecular test protocols. See NCCLS document MM3—*Molecular Diagnostic Methods for Infectious Diseases* for further information. Internal controls help ensure that inhibitory factors of transport devices, the specimens contained, reagents, and test conditions are not responsible for failure to detect the target analyte in question.

The stability of the clinical specimen, i.e., target analyte in the transport system, shall also be determined based on data accumulated in-house or reported in the scientific literature. RNA targets are less stable than DNA targets and would require more stringent transport conditions.[42]

[d] In the U.S. manufacturers must also comply with requirements outlined in NCCLS document MM3—*Molecular Diagnostic Methods for Infectious Diseases*.

10 Viral Culture Transport Devices

Unlike bacteriology, for which there are numerous well-defined isolates and procedures used for quality control characteristics of growth media that can be extrapolated for use in the quality control of bacterial transport devices, there are few standardized procedures for assessment of viral recovery. There are only a few studies that have compared recovery from different transport media/systems from clinical specimens and a few using seeded specimens. Each approach has advantages. Direct comparison of recovery from multiple specimens—each in a different transport—can give a reliable comparison of performance for unadapted viral isolates. Alternately, use of standardized inocula prepared from laboratory isolates yields a method that can be standardized for intra- and interlaboratory evaluation of performance. For this reason, this document proposes use of well-characterized laboratory strains in a specified culture protocol. The protocol may not be considered optimal for recovery of all viruses, but it does provide a method to assess performance.

Stock suspensions of virus can be prepared according to the directions of the supplier (e.g., ATCC® strains). This usually involves adding the contents of an ampule (frozen or lyophilized) from the supplier to a specified tissue culture cell line monolayer of specified confluence, and monitoring for the development of cytopathic effects. If foci of CPE fail to develop and spread within a specified time limit, the infected cells are split 1:2 and observed for CPE as monolayer reforms. Once CPE is observed, the virus is harvested from the infected cells by scraping the monolayer or disrupting it with glass beads and thoroughly mixing it, using a vortex mixer. This suspension can then be titered by using tenfold serial dilutions and performing either plaque assays or fluorescent foci assays. However, because many virus titers will decrease with even one freeze-thaw cycle, aliquots shall be frozen with cryoprotectant medium (i.e., skim milk, glycerol, bovine serum albumin), and titers for use in quality control shall be determined after a single freeze-thaw cycle.

Ideally, stock preparations used to assess recovery would mimic the upper range of clinically relevant viral titers. For example, Herpes simplex virus is often present in vesicles at titers 10^3 to 10^7 tissue culture infectious dose (TCID); viral titers of stock suspensions would best contain about 5 x 10^4 TCID/mL, so that a 0.2 mL inoculum contained about 10^3 TCID.

Specimen	Primary agents	Commonly cultured secondary agents
Respiratory	Adenovirus, Influenza A or B, Parainfluenza viruses 1-3, Respiratory syncytial virus, Rhinovirus	Cytomegalovirus, Enteroviruses, measles, Varicella-zoster, Herpes simplex
Skin lesions	Herpes simplex virus, Varicella-zoster virus	Adenovirus, Enteroviruses
Ocular specimen	Adenovirus, Coxsackie viruses, Echoviruses, Herpes simplex virus	Cytomegalovirus, Varicella
Genital specimens	Herpes simplex virus	Adenovirus, Cytomegalovirus, mumps
Blood, bone marrow	Cytomegalovirus	Herpes simplex virus, mumps, measles
Urine	Cytomegalovirus	Adenovirus, measles, mumps

10.1 Transport and Specimen Types

Transport devices for virus cultures include sterile, empty containers; swab systems; and tubes or vials containing liquid transport medium. As with other transport, empty vials, cups, and tubes shall be subjected to testing by the manufacturer for the physical parameters outlined in Sections 4.1 through 4.3. During device selection, the end user shall evaluate devices under conditions of simulated use for their specific applications.

Specimens for virus culture include the array of specimens cultured for bacteria, but the conditions of transport are not similar.

10.2 Quality Control

Herpes simplex virus Type 2 ATCC® VR-734, respiratory syncytial virus ATCC® VR-1032, and cytomegalovirus ATCC® VR-977 are recommended as viral strains to be used for quality control testing. To assess performance of a transport medium/device, the devices would be inoculated to contain 5×10^4 TCID/mL of the virus. The device would be held at the temperature(s) for which a claim is desired for the desired simulated time of transport (24 to 96 hours). After that time, serial tenfold dilutions would be prepared, and 0.2-mL portions would be inoculated to a suitable tissue culture monolayer. Any recovery of virus would be acceptable performance.

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Appendix A. Sources of Specimen Container Failure

Type of Failure	Factor	Where Possibly Encountered	QC/ Evaluation Point	Potentially Useful ASTM Protocol*
Breakage	Brittleness	Low temperature	Design	D746
		Sterilization (radiation)	Design	
		Changes in storage	Post-manufacture	
	Mechanical impact	Dropping from bench; abrupt stops as in pneumatic tube systems	Post-manufacture	D5276
	Stress cracks	Formed during production	Design	D2561
	Flexural stress		Design	D6395
Leakage	Pressure variation (inside vs. outside container)	During air transport (up to 5 atmospheres); gas buildup by microorganisms (as in stool samples)	Design	D4991
	Vibration (resulting in the loosening of the closure)	Ground transportation, pneumatic tube systems	Design	D746 & D5094
	Gasket material (material fails to maintain a proper seal)	Low temperatures, excessive pressure variation	Design	
Specimen alteration	Excessive CO_2	CO_2 from dry ice permeating plastic or through closure/container seal	Design	
	Residual extractable substances	PCR inhibitors	Post-manufacture	
	Sample swab becoming separated from surrounding transport media	Mechanical impact causes an agar column of medium to break apart and separate from the swab	Post-manufacture	

*Procedures listed are given as examples. The appropriateness of a procedure to a particular situation must be assessed by the individual. Standardized procedures from similar organizations are equally suitable.

Additional ASTM Documents*

D746-98	Standard Test Method for Brittleness Temperature of Plastics and Elastomers by Impact
D4991-94	Standard Test Method for Leakage of Empty Rigid Containers by Vacuum Method
D4812-99	Standard Test Method for Unnotched Cantilever Beam Impact Strength of Plastics
D5094-90	Leakage of Liquids from Containers with Threaded or Lug-Style Closures
E2097-00	Standard Guide for Determining the Impact of Extractables from Non-Metallic Materials on the Safety of Biotechnology Products
D5276-98	Standard Test Method for Drop Test of Loaded Containers by Free Fall
D6395-99	Standard Test Method for Flatwise Flexural Impact Resistance of Rigid Plastics
D2561-95	Standard Test Method for Environmental Stress-Crack Resistance of Blow-Molded Polyethylene Containers

* Available from ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

Appendix B. Regulatory Considerations

United States Market

Medical devices, including *in vitro* diagnostic devices, are subject to the requirements of the Federal Food, Drug & Cosmetic (FD&C) Act, which are contained in the final procedural regulations Title 21 Code of Federal Regulations Parts 800-1200 (21 CFR Part 800–1299). These baseline requirements apply to all medical devices and are necessary for marketing, proper labeling, and monitoring of performance once the device is on the market. Good Manufacturing Practices (GMP) set forth in the Quality System (QS) regulation require that United States and foreign manufacturers establish a quality system for the design, manufacture, packaging, labeling, storage, installation, and servicing of finished medical devices intended for human use in commercial distribution in the United States. The QS regulation also requires that specifications and controls be established for devices and that the finished devices meet these specifications prior to being released for sale. Manufacturers are also required to collect and analyze quality data (i.e., trend analysis, customer feedback) to identify and correct quality problems. Manufacturer's compliance with the QS regulation help, assure that medical devices continue to be safe and effective for their intended uses. Further information concerning GMP and QS regulation can be found at www.fda.gov/cdrh/devadvice.

Prior to a manufacturer placing a medical device on the market, the device must be classified according to its intended use. The Food and Drug Administration (FDA) places medical devices within three classes based on the risk associated with the use of the device and the level of control needed to assure that the product is safe and effective.

FDA divides medical devices into three classes:

- Class I – Devices subject to general controls (GMP, QS regulation, labeling, premarket notification [510(k)] if indicated), low-risk device
- Class II – Devices subject to general controls and special controls (FDA guidance documents, premarket notification [510(k)]), moderate-risk device
- Class III – Devices subject to general controls, special controls, and premarket approval, high-risk device

More detailed information describing regulatory classes for medical devices in the United States can be found on the FDA web page at www.fda.gov/cdrh/devadvice/3132.html.

Microbiological specimen collection and transport devices are identified as Class I devices. There are three generic device categories that the FDA uses to group specimen collection and transport devices.

Transport Culture Medium (21CFR§866.2390)

Transport culture medium is a device that consists of a semisolid, usually nonnutrient medium that maintains the viability of suspect pathogens contained in patient specimens while in transit from the specimen collection area to the laboratory. The device aids in the diagnosis of disease caused by pathogenic microorganisms and also provides epidemiological information on these diseases.

Transport systems in this category include anaerobic transport culture media; nonpropagating transport culture media; and propagating transport culture media. These Class I devices require premarket notification [510(k)] to the FDA.

Microbiological Specimen Collection and Transport Device (21CFR§866.2900)

A microbiological specimen collection and transport device is a specimen-collecting chamber intended for medical purposes to preserve the viability or integrity of microorganisms in specimens after their collection and during their transport from the collecting area to the laboratory. The device may be labeled or otherwise represented as sterile. The device aids in the diagnosis of disease caused by pathogenic microorganisms.

Transport systems in this category include parasite concentration devices; specimen collection devices; aerobic transport systems; and anaerobic transport systems. These Class I devices require premarket notification [510(k)] to the FDA.

Specimen Transport and Storage Container (21CFR§866.3250)

A specimen transport and storage container, which may be empty or prefilled, is a device intended to contain biological specimens, body waste, or body exudates during storage and transport so that the matter contained therein can be destroyed or used effectively for diagnostic examination. If prefilled, the device contains a fixative solution or other general purpose reagent to preserve the condition of a biological specimen added to the container.

Transport systems in this category include specimen containers such as urine collection containers and specimen mailer and storage containers. These Class I devices do not require premarket notification [510(k)] to the FDA unless labeled as sterile devices.

European Market – CE Mark (CE)

In June 1998, a mandatory European Medical Devices Directive (93/42/EEC) regulating the safety and marketing of medical devices came into effect. The Directive set a new quality standard and harmonized the controls within a single system across the European Union (EU), instead of having different standards and rules in each country. A *Competent Authority* in each EU member state (usually a department within the health administration) is responsible for enforcing and policing the Directive. Each *Competent Authority* designates one or more *Notified Body(ies)* within the country to carry out conformity assessment of all medical device manufacturers.

Directive 93/42/EEC states that devices must not compromise the health and safety of patients, users, and where applicable, any other persons. Devices must also achieve their intended and claimed performance. Manufacturers undergo one or more conformity assessment procedures to confirm that the design and production of the device ensures conformity. Under the Directive, the degree of control and conformity of the manufacturer has to be matched, as far as possible, to the degree of risk inherent in the device. Medical devices have been grouped into four classes based on a series of classification rules:

Class I	Low-risk
Class IIa	Medium-risk
Class IIb	Medium-risk
Class III	High-risk

The classification rules (Rules 1-18) are based on the intended use of the device and the terms related to *duration of contact* with the patient, *degree of invasiveness*, and the *anatomy affected* by the use of the device. If more than one rule applies to a device, the highest classification stands. With respect to this NCCLS standard, the European Medical Devices Directive is only pertinent to microbiology specimen transport systems that incorporate a sample collection swab that is required to obtain a sample from the patient by direct contact with that patient.

Surgically Invasive Devices. All surgically invasive devices intended for transient use are in Class IIa (Rule 6).

The Directive provides the following definitions for the terms used in these device classification rules: *Body orifice* is any natural opening in the body, as well as the external surface of the eyeball, or any permanent artificial opening, such as a stoma. *Transient use* is defined as normally intended for continuous use for less than 60 minutes. A *surgically invasive device* is an invasive device that penetrates inside the body through the surface of the body, with the aid or in the context of a surgical operation.

Invasive Devices. All invasive devices with respect to body orifices (other than surgically invasive devices, which are not intended for connection to an active medical device) are in Class I if they are intended for transient use. These devices are in Class IIa if they are intended for short-term use, except if they are used in the oral cavity as far as the pharynx; in an ear canal up the ear drum or in a nasal cavity; and are not liable to be absorbed by the mucous membrane, in which case they are in Class I (Rule 5).

The level of conformity required by manufacturers depends on the classification of the device. For Class I sterile devices, Class II and Class III manufacturers are required to have a quality management system in place, which is independently audited and accredited by a *Notified Body*. For Class IIa devices, the Directive clearly requires the imposition of a full quality management system by the manufacturer, for example, ISO 9000 and EN 46000 with conformity to the appropriate Annex of the European Directive 93/42/EEC.

The Directive also dictates a mandatory vigilance system in which manufacturers are required by law to report any adverse incidents involving devices they produce to the *Competent Authority* in that country. Information about serious incidents is collected centrally by each EU country and is made available to other EU Members. The aim is to prevent the same type of incident recurring anywhere in the EU.

Only those manufacturers' products that meet the requirements of the Medical Device Directive will be legally allowed to be distributed and sold throughout the EU. Manufacturers conforming to the essential requirements of the Directive will be entitled to display the CE mark. More information on the European Medical Devices Directive can be found at the website provided by the European Commission www.newapproach.org.

Additionally, any transport device that incorporates a transport medium as a component of the device will also need to comply with the *In Vitro* Diagnostic Device Directive (IVDD) 98/79/EC as of December 7, 2003. The IVDD requires that devices must not compromise the health and safety of patients, users, and where applicable, any other persons. Devices must also achieve their intended and claimed performance.

Under this Directive, technical documentation supporting the safety and effectiveness of the device must be compiled and made available upon request by a *Competent Authority*. For transport devices that are covered under this Directive, self-certification to the Directive requirements is acceptable for placement into the EU market.

Other International Markets

Regulatory requirements of each country will need to be assessed on an individual basis to determine what is needed prior to placing a collection and transport system on the market for sale.

NCCLS consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Subcommittee Responses

M40-P: *Quality Control of Microbiological Transport Systems; Proposed Standard*

General

1. The microbiologist working in the parasitology unit was concerned by the absence of any specific quality control standard for transport devices in this area of microbiology (e.g., vessels with preservative.)

- **The subcommittee agrees, but in the interest in getting a document published, some devices were omitted. Future revisions of the document could address devices for parasitology as well as other devices not addressed in detail.**

Section 5.1.2, Number of Lots

2. The subcommittee proposed that the manufacturer retest each lot of the product every six months to ensure a level of compliance demonstrating performance. It is felt that this six-month monitoring is unrealistic unless a change occurs that may warrant testing. Since product is made and released to established specifications, compliance is met and guidelines are followed. Therefore, the six-month interim testing is not warranted.

- **The subcommittee agrees. The reference to retest each lot of product every six months has been removed as suggested.**

Section 8.2, Urine Culture Containers

3. The committee proposed that control testing be conducted to establish the performance claim of the product by seeding devices with relevant uropathogens and site flora, including *Escherichia* coli (ATCC® 25922), *Staphylococcus saprophyticus* (ATCC® 15305), *Proteus mirabilis* (ATCC® 7002), *Enterococcus* (ATCC® 29212), *Pseudomonas aeruginosa* (ATCC® BAA-427), and *Candida* (ATCC® 24433).

   As stated, the text seems to require use of the above ATCC strains. Devices on the market may have been developed and obtained FDA clearance with similar organisms, but not necessarily the specific strains mentioned. We request that the ATCC strains be listed as recommendations rather than requirements.

   "Control testing should include seeding devices with liquid suspensions of relevant uropathogens including representative isolates of organisms commonly recovered from patients with urinary tract infections. The following strains are recommended: *Escherichia coli* (ATCC 25922), *Staphylococcus saprophyticus* (ATCC 15305), *Proteus mirabilis* (ATCC 7002), *Enterococcus* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC BAA-427), and *Candida* (ATCC 24433)."

- **The subcommittee agrees to the modified language that was proposed with the exception that both genus and species names were used.**

4. The committee proposed that plate counts (colony counts) remain within +/-1 $\log_{10}$ of the initial organism concentration. We recommend that the comparison be made to the refrigerated, nonchemically preserved organism concentration, since this is the result that would be rendered had no preservative been used. For example: If a manufacturer has a claim that supports bacterial maintenance (organism concentration) of a product due to the chemical preservation, comparisons should be made per time interval between the chemically preserved urine and the unpreserved urine. This is a direct demonstration of product performance.

- **The subcommittee disagrees with this suggestion/comment. Urine preservative should be validated to be comparable to urine at the time of specimen collection, so the comparison should be to the original colony**

count or perhaps the colony count within some reasonable time frame of collection (one hour is the standard from early studies). Comparison to unpreserved urine held for the same time interval, as simulated transport, will not ensure maintenance of the quality of the original specimen during transit. Refrigeration was deemed an acceptable alternative, because it met this one-hour comparison standard (Mou TW and Feldman, HW. The enumeration and presentation of bacteria in urine. *Am J Clin Pathol*. 1961;35:572-575). A three-way comparison of the initial organism concentration at the time of collection to the concentration after refrigeration to chemically preserved urine would increase the likelihood that there could be 2 $\log_{10}$ differences between the original specimen and the preserved specimen. The concentration of organisms in the preserved specimen should be directly compared to the original (within one hour of collection) concentration.

5. The committee proposed that due to toxicity issues existing from underfilled urine preservative containers, manufacturers should establish a "fill volume range." It is also proposed that laboratories should be monitoring this volume to assure pathogen viability.

   It is recommended that manufacturers establish minimum fill-line indicators on the specimen collection containers for additives with critical ratios. Underfilling may result in toxicity due to preservative not being fully utilized. However, in an evacuated system, overfilling is not a concern because of draw control. For nonevacuated systems, the maximum fill line should be indicated on the specimen collection device or labeling.

- **The subcommittee would welcome the presence of fill lines on containers, but the recommendation in the standard is only that the manufacturer establish a "fill volume range," which could be listed in the package insert or available from a technical services department. The standard is not stipulating that the labeling include the fill volume indicators. The subcommittee believes this should be monitored, because devices (including those that are evacuated and designed to control filling) may be used incorrectly by those responsible for specimen collection. Monitoring the fill volume may alert the laboratory to incorrect use of the device.**

Summary of Delegate Comments and Subcommittee Responses

M40-A: *Quality Control of Microbiological Transport Systems; Approved Standard*

General

1. I have a concern about packaging of the Culturette® swab. In recent years, especially with the advent of gel and charcoal based transport media, the swab can no longer be packaged inside the shaft. The swab is unwrapped inside the package and can easily become contaminated once the package is opened.

- **This issue is beyond the scope of this document. Any concerns with specific brands of swabs should be communicated to the manufacturer directly.**

Section 4.3.1, Container and Additive Interferences

2. The last sentence needs clarification/expansion regarding responsibility and frequency. Who is responsible? The end user? The manufacturer? Or both? How often? Each lot? Each shipment? Each whatever?

- **Text has been added to this section to clarify end-user responsibility for verifying incompatibility and frequency of testing.**

Section 4.5, Product Information (Formerly Section 4.4)

3. The package insert and ideally, the packaging of the transport system itself should clearly state the intended and validated uses, and any known limitations (e.g., an aerobic swab that can/cannot also be used for anaerobes).

- **Text has been added to clarify the necessary product information.**

Section 7.10, Quality Control Protocols

4. The last sentence is unclear. Is 4 to 8 degrees the transport temperature range and 20 to 25 degrees the holding range which is implied by the ordering, or vice versa?

- **The text in this section has been clarified to reflect that these temperatures can be considered either the transport or holding temperature.**

Summary of Comments and Subcommittee Responses

5. For Comment 1, I agree with the issue that other transport systems, such as that for ova and parasites, needs to be addressed.

- **The subcommittee acknowledges this concern and hopes to address other transport devices in future editions of this document.**

The Quality System Approach

NCCLS subscribes to a quality system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents through a gap analysis. The approach is based on the model presented in the most current edition of NCCLS HS1—*A Quality System Model for Health Care.* The quality system approach applies a core set of "quality system essentials (QSEs)," basic to any organization, to all operations in any healthcare service's path of workflow. The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The quality system essentials (QSEs) are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Service & Satisfaction
Personnel	Process Control	Assessment	Facilities & Safety

M40-A addresses the quality system essentials (QSEs) indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
					X GP16 M7 M22 MM3						M29

Adapted from NCCLS document HS1— *A Quality System Model for Health Care.*

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, GP26-A2 defines a clinical laboratory path of workflow which consists of three sequential processes: preanalytical, analytical, and postanalytical. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

M40-A addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the following page.

Preanalytic					**Analytic**		**Postanalytic**	
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpretation	Results Report	Post-test Specimen Management
			X GP16	GP16	GP16 M7	GP16 M7	GP16 M7	

Adapted from NCCLS document HS1— *A Quality System Model for Health Care.*

Related NCCLS Publications*

GP16-A2 **Routine Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline—Second Edition (2001).** This guideline describes routine urinalysis test procedures that address materials and equipment, macroscopic examinations, clinical analyses, and microscopic evaluations.

M7-A6 **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Sixth Edition (2003).** This newly revised standard provides updated reference methods for the determination of minimal inhibitory concentrations (MICs) for aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

M22-A2 **Quality Assurance for Commercially Prepared Microbiological Culture Media; Approved Standard—Second Edition (1996).** This standard contains quality assurance procedures for manufacturers and users of prepared, ready-to-use microbiological culture media.

M29-A2 **Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Second Edition (2001).** Based on U.S. regulations, this document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

MM3-A **Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline (1995).** This document contains guidelines for use of nucleic acid probes and nucleic acid amplification techniques for detection of target sequences specific to particular microorganisms. Limitations, quality assurance, proficiency testing, and interpretation of results are also described.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

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