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Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard—Third Edition

This document contains quality assurance procedures for manufacturers and users of prepared, ready-to-use microbiological culture media.

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

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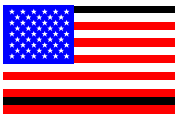

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Abstract

The M22 standard provides information on quality control of commercially prepared microbiological culture media to users and manufacturers. M22-A3 is a revision of the approved standard, M22-A2, published in December 1996. The standard applies to all commercial media listed in Table 2 regardless of packaging, plate, or tube design. The media included in M22-A3 are from three surveys conducted by the College of American Pathologists. The third survey, conducted in the fall of 2001, was performed in response to the many requests for further expansion of the exempt media list. M22-A3 lists an additional 27 exempt media.

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Foreword

Quality control of commercially prepared media imposes a substantial financial burden on licensed microbiology laboratories. In response, the College of American Pathologists (CAP) conducted three laboratory surveys to determine the failure rates of commonly used media.[1,2]

The first two surveys provided data that allowed exemption of 24 of 35 assessed media from quality control. The third survey, conducted in 2001, allows the addition of 27 media to the exempt list. The data, however, cause concern. Manufacturers perform quality control on all media sold to customers. Why, then, do certain media repeatedly exhibit failure rates ≥0.3 or 0.5%? Less than optimum storage conditions may contribute to medium failure. Media are shipped, stored, and delivered nonrefrigerated by the manufacturer or distributor. Specialty media that require more fastidious quality control organisms also often exhibit higher failure rates. Separate, limited surveys of different U.S. and Canadian[3,4] clinical microbiology laboratories revealed a lack of standardization in the quality control of media, including processing, storage, and inoculation of quality control organisms. Until resolution of these issues, clinical laboratories must continue to verify the performance of certain medium types.

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Key Words

Commercially prepared, ready-to-use culture media; culture media; quality assurance; quality control

Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard—Third Edition

1 Scope

The M22 standard provides information on quality control of commercially prepared microbiological culture media to users and manufacturers. M22-A3 is a revision of the approved standard, M22-A2, published in December 1996.

The basic premise of this standard is that the retesting of commercially prepared microbiological culture media is unnecessary for those media that are of proven reliability. The categorization of media that do not require retesting by the user is based on quality control data collected from surveys of clinical laboratories enrolled in the bacteriology proficiency-testing program conducted by the College of American Pathologists (CAP). The media types listed in the M22 standard are well established for recovery of clinically significant microorganisms. Exemption of certain media from routine quality control by the clinical laboratories assumes that media performance is monitored by an overall quality program that correlates test methods with clinical information, and monitors test procedures and specimen quality. **Media used for antimicrobial susceptibility testing have different quality control recommendations that are detailed in separate NCCLS documents.**

Changes or additions to this newest revision are the following: 1) Designation of the responsibilities of the manufacturer, distributor, and user; 2) clarification of the media included in various categories; 3) simplification of the basic protocols for the maintenance of quality control organisms; 4) incubation conditions for media quality control; 5) recommendations for the quality control of media used for certain fastidious organisms; and 6) expansion of the cutoff for acceptable failure rate from 0.3% to 0.5% and the categorization of an additional 27 media as exempt from user testing.

2 Introduction

The NCCLS Subcommittee on Media Quality Control was formed in 1984 to develop a standard that would specify the requirements for quality control of culture media. The work of this subcommittee resulted in the publication of M22 as a proposed standard in 1985 and an approved standard in 1990. A revision of M22 was published in 1996. In 2001, the document was scheduled for a second revision and the responsibility was assigned to a working group within the original subcommittee. From the inception of M22, the subcommittee has utilized the recommendations of the College of American Pathologists for the categorization of media that require quality control by the user.

CAP evaluated the failure rates of commercially prepared media in three surveys mailed to participants of the CAP Microbiology Proficiency Testing Surveys (see Table 1).[1,2] Failure rates are calculated as a raw percentage score of "total number of lots failing QC/total number of lots tested." An extrapolated failure rate is then determined by calculating what proportion of the raw rate is attributed to some type of failure detected by a QC organism. Only those media with a significant QC experience as defined by >1000 lots or >100 000 items which exhibit QC strain-related failures meet the criteria for calculation of the extrapolated failure rate.

The most recent survey (2001) evaluated 262 968 lots, among which were 32 702 833 plates, tubes, or bottles.[2] Failure rates were calculated for the 38 most commonly used media (97% of the reported lots). Reasons for media failures for all three surveys are listed in Table 1A. The extrapolated failure rate limit was raised from 0.3% to 0.5% based on analysis of the distribution of failures rates from the three surveys. Users are exempt from performing quality control of media with failure rates ≤0.5% (see Table 1B). Media with failure rates >0.5% continue to require user quality control.

In the United States, the Clinical Laboratory Improvement Amendments of 1988 (CLIA) issues standards for laboratory control procedures that are separate from the recommendations of NCCLS. Each laboratory must confirm the acceptance of the recommendations of NCCLS document M22 by any inspection or licensing agency used by the laboratory. See the References section for CLIA citation.[5]

3 Standard Precautions

Standard precautions should be followed when performing quality control procedures using viable microorganisms. Standard 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 infection from laboratory instruments and materials and for recommendations for the management of exposure, refer to the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections.*

4 General Responsibilities of the Manufacturer, Distributor, and User

4.1 The Manufacturer

In the United States, commercially prepared microbiological media are categorized as medical devices and are regulated by the Department of Health and Human Services (HHS), Food and Drug Administration (FDA). The Code of Federal Regulations describes Current Good Manufacturing Practice (CGMP) requirements that apply to manufacturers of commercial media.[6] This regulation includes requirements for production and process controls, establishing and maintaining activities that ensure each lot meets acceptance criteria, corrective and preventive action, labeling, device packaging, and maintaining certain records including complaint files.

Some specific elements of the Quality System Regulation (QSR) are:

- Each manufacturer shall establish and maintain procedures for finished device acceptance to ensure that each production run, lot, or batch of finished devices meets acceptance criteria (CFR Part 820, Subpart H).[6]

- Each manufacturer shall ensure that device package and shipping containers are designed and constructed to protect the device from alteration or damage during the customary conditions of processing, storage, handling, and distribution (CFR Sec. 820.130).[6]

- Each manufacturer shall establish and maintain procedures to ensure that mix-ups, damage, deterioration, contamination, or other adverse effects to product(s) do not occur during handling (CFR Sec. 820.140).[6]

- Each manufacturer shall establish and maintain procedures for the control of storage areas and stock rooms for a product to prevent damage, deterioration, contamination, or other adverse effects pending use or distribution and to ensure that no obsolete, rejected, or deteriorated product is used or distributed. When the quality of a product deteriorates over time, it shall be stored in a manner to facilitate proper stock rotation, and its condition shall be assessed as appropriate. Each manufacturer shall establish and maintain procedures that describe the methods for authorizing receipt from and dispatch to storage areas and stock rooms (CFR Sec. 820.150).[6]

- Each manufacturer shall establish and maintain procedures for control and distribution of finished devices to ensure that only those devices approved for release are distributed, and that purchase orders are reviewed to ensure that ambiguities and errors are resolved before devices are released for

distribution. Where a device's fitness for use or quality deteriorates over time, the procedures shall ensure that expired devices or devices deteriorated beyond acceptable fitness for use are not distributed. Each manufacturer shall maintain distribution records which include or refer to the location of: 1) the name and address of the initial consignee; 2) the identification and quantity of devices shipped; 3) the date shipped; and 4) any control number(s) used (CFR Sec. 820.160).[6]

4.1.1 User Information

The following minimum information should be available to the user from the manufacturer:

- Documentation of successful growth performance for all media using well characterized bacterial strains as recommended in the M22-A3 standard. Table 2 lists the manufacturer's minimum quality control requirements (exempt media only).

- Identification of the medium type, formulation, lot number, expiration date, appropriate handling and storage conditions, to include both the shipping box and each individual box or sleeve of medium within the shipping box (if applicable).

4.1.2 Documentation of Quality Control

Availability of technical information for the user that indicates the manufacturer's basic specifications and controls for each medium type, including the following:

- General information and recommended uses for each medium type;

- Media formulations, excluding proprietary ingredients;

- Media specifications (contamination level, pH, pour depth, volume dispensed, presence/absence of nonviable contaminants, or other requirements);

- Performance criteria, including testing to detect gross contamination;

- Organisms used for growth checks and the expected reactions to include any growth variables observed; and

- End process controls with acceptance criteria (lot release criteria) for contamination, pH, pour depth, volume dispensed, presence/absence of nonviable contaminants, or other specifications. Labeling should include a means by which the user may be assured that the product meets the net quantity of contents, expressed terms of weight or volume, numerical count, or any combination of these or other terms which accurately reflect the contents of the package.

4.2 The Distributor

The distributor should perform the following:

- Comply with manufacturer's labeled specifications for medium storage and handling before and during delivery to the user, including conditions to prevent physical and temperature-related damage.

- Maintain documentation, which verifies compliance with such specifications and is available to users and manufacturers if requested.

4.3 The User

The user should perform the following:

- Develop an agreement with the distributor to establish expectations for storage and handling from the time the media leave the distribution warehouse until they are delivered to the end user's facility.

- Develop procedures within the user's institution to ensure adherence to the manufacturer's recommendations for media storage and handling from the time it is received by the institution's central receiving area (loading dock), until delivery to the laboratory.

- Comply with manufacturer's recommendations for media storage and handling from the time it is delivered to the laboratory storage area until it is used for patient testing.

- Retain the most current version of technical information provided by the manufacturer.

- Adhere to procedures outlined in Section 5. These include satellite laboratories that obtain media from an institutional central supply source, unless the satellite laboratory receives media already tested by the system's core laboratory. In this case, the core laboratory is responsible for ensuring appropriate shipping conditions to the satellite laboratory.

- Document all media quality control activities and corrective action as stipulated in NCCLS document M22-A3.

- Document that media used for purposes other than those stipulated by the manufacturer and/or approved by the FDA provide adequate recovery of organisms.

4.3.1 Reporting Deficiencies Identified by the User

The user should perform the following:

- Identify and/or correct the cause of any media failure. Document all activities.

- Notify the manufacturer and/or distributor. Document the manufacturer's/distributor's response in corrective action.

Media replacement depends on the nature of the problem and negotiation with the manufacturer.

5 Categories of Microbiological Media

Media are divided into two main descriptive categories: selective and nonselective (nutritive). These categories are based on the purposes for which the media were designed. Selective media contain antimicrobial or chemical agents that inhibit the growth of certain microorganisms. Nonselective media promote the growth of organisms. Some nonselective and selective media also contain components that allow for organism differentiation. The organisms selected for quality control are determined by these categories.

Commercial media are divided into two additional categories—exempt and nonexempt—which are determined by quality control performance data collected by CAP.

5.1 Exempt Media Category

This category includes commercial media documented to maintain consistent user performance with minimum variation and requires minimum quality control (see Table 1B).

Laboratories are advised to carefully monitor the performance of media used for fastidious organisms or specialty testing, regardless of categorization as exempt, in order to ensure that recovery of isolates from clinical specimens is satisfactory. Categorization of media as exempt does not preclude a laboratory from performing complete quality control on any manufactured medium type when deemed necessary.

5.2 Nonexempt Media Category

This category includes commercial media documented by user quality control performance data collected by CAP to vary in performance from lot to lot, or any media prepared by the user. Nonexempt media require complete user quality control including confirmation of satisfactory performance with recommended organisms (see Table 1B).

5.3 General Quality Control Requirements for Both Exempt and Nonexempt Media

5.3.1 Receiving Media from the Manufacturer

Perform the following procedures when a media shipment arrives in the laboratory:

(1) Verify delivery of the ordered amount. Check each medium type for multiple lot numbers and/or impending expiration dates. Report recurring problems to the manufacturer or distributor.

(2) Record the lot number, expiration date, and the received date (optional) for each medium type.

(3) Store the media as specified by the manufacturer (generally at 2 to 8 °C) pending quality control. Separate exempt media from nonexempt media. Refer to Sections 5.3.2 and 5.3.3 for further handling instructions.

5.3.2 Visual Inspection

Record the numbers of plates per lot that exhibit any of the characteristics listed below. If excessive, notify the manufacturer or distributor.

Visually inspect each medium lot for obvious problems such as:

- cracked or damaged plates;
- agar detached from the petri plates;
- frozen or melted agar;
- unequal filling of the plates;
- insufficient agar in the plates (<3 mm);
- hemolysis of blood containing media;
- change in the expected color of the media (possible pH problem);

- excessive bubbles or rough surfaces;
- excessive moisture or dehydration;
- obvious contamination; and
- presence of precipitates.

5.3.3 Checking for Contamination

Because contamination testing is routinely performed by manufacturers, commercially prepared media need not be retested for sterility by the end user. Instead, careful inspection for contamination should take place immediately before inoculation with patient specimens.

5.3.4 Quality Assurance

Observations have confirmed that both exempt and nonexempt media have the potential to perform less than optimally or may fail quality control after receipt from the manufacturer. In order to monitor for these potential failures, users are encouraged to adopt the following procedures:

(1) Laboratories are strongly encouraged, regardless of exempt status, to confirm the ability to support growth for all media used for the recovery of fastidious organisms (see Table 3) such as:

- anaerobes;
- *Bordetella pertussis;*
- *Burkholderia cepacia;*
- *Campylobacter/Helicobacter;*
- *Legionella;*
- *Neisseria gonorrhoeae;* and
- any isolate with fastidious or unique growth requirements.

(2) Laboratories must use media for primary culture which will support the growth of a wide variety of organisms, for example, blood agar and chocolate agar.

5.4 User Quality Control Requirements for Nonexempt Media Only

Nonexempt media require performance of complete quality control by the user. Perform visual and contamination checks. In addition, verify acceptable growth and/or inhibitory properties with appropriate bacterial or fungal control organisms as described in Tables 2 and 3.

5.5 Quality Control Organisms

5.5.1 Source of Quality Control Organisms

Quality control organisms are available from the American Type Culture Collection (ATCC®). ATCC-derived cultures are also available from commercial sources. Previously characterized organisms isolated from patients and shown to be phenotypically stable are also appropriate for media quality control.

Documentation records of biochemical characterizations and identification of these isolates should be maintained.

5.5.2 Storage of Quality Control Organisms

See below for instructions.

Quality Control Organisms	Storage Conditions	Type of Stock Culture	Length of Storage
All	Per manufacturer	Lyophilized	Until expiration date
Rapidly growing bacteria and yeast	2-8 °C	Working QC Cultures	4 weeks
	2-8 °C	Stock QC Cultures	≤12 months
	≤-20 °C	Suspension in Cryopreservative	≤12 months
	≤-50 °C	Suspension in Cryopreservative	Indefinitely
Anaerobes, mycobacteria, bacteria w/special growth requirements	≤-20 °C	Suspension in Cryopreservative	≤12 months
	≤-50 °C	Suspension in Cryopreservative	Indefinitely
Moulds	25-30 °C	Oil Overlay or Water Cultures	Indefinitely
	≤-20 °C	Spore Suspension	Indefinitely

5.5.3 Processing of Quality Control Organisms

5.5.3.1 Organisms Required for Daily or Weekly Quality Control

NOTE: Processing of quality control organisms for antimicrobial susceptibility testing is detailed in separate NCCLS documents.

(1) At least once a year, prepare a culture of each quality control organism routinely used by the laboratory. Use lyophilized or frozen organisms.

(2) Inoculate one to two slants of an appropriate nonselective medium. Incubate under conditions favorable for growth. Label as stock control, then the name of the organism and the date of inoculation. Tightly seal and store the slants(s) for ≤12 months at 2 to 8 °C. Moulds and certain fastidious bacteria such as *N. gonorrhoeae* may require 25 to 35 °C storage. In addition, more fastidious organisms such as *N. gonorrhoeae* may have limited survival on stored slants and may require more frequent subculture.

(3) From the stock control, prepare a second subculture. Label as working control, then the name of the organism and date of inoculation. Incubate under conditions favorable for growth. Store the working control at 2 to 8 °C for less than four weeks. Seal tightly if plates are used. Prepare a fresh working culture at least once per month from the refrigerated stock control. Avoid multiple serial subcultures of quality control organisms over extended periods of time.

5.5.3.2 Organisms Required for Intermittent Quality Control

For growth checks of less frequently used nonexempt media, inoculate frozen or lyophilized control organisms on appropriate nonselective media. Incubate under conditions favorable for the organism's growth. Subculture before use to ensure full growth potential and characteristics for quality control.

6 Inoculation of Quality Control Media

6.1 Direct Inoculation

Exercise care if using direct inoculation. An inoculum that is either too heavy or too light will mask both the growth and/or inhibitory properties of a medium. If a quality control failure occurs while using direct inoculation, repeat using a standardized suspension as part of corrective action (see Section 6.2).

6.2 Standardized Suspension

Using a standardized suspension of the quality control organism is a better challenge of media performance than direct inoculation. A standardized suspension also allows comparison of quality control results between users. A more concentrated suspension tests the ability of selective media to successfully inhibit certain organisms. A lighter suspension challenges the ability of nonselective media to adequately support growth.

6.3 Preparation of Suspension (See Figure 1.)

(1) Prepare a suspension in sterile, nonbacteriostatic saline (0.85% w/v NaCl) to match a 0.5 McFarland standard (0.08 to 0.1 absorbance units at 625 nm) ($1x10^7$ to $1x10^8$ cfu/mL). Use an 18- to 24-hour culture of the quality control organism.

(2) Alternatively, prepare a suspension by inoculating three to five colonies from a 24-hour culture into sterile broth. Incubate for several hours to achieve a suspension equivalent to a 0.5 McFarland standard.

6.3.1 Dilution for Nonselective Media

Dilute the suspension 1:100 in sterile broth or nonbacteriostatic saline. Inoculate each test plate with 10 µL (0.01 mL) of the suspension. Streak for isolation. If the 1:100 dilution inoculum proves too dense, use a 1:1000 dilution to produce isolated colonies.

6.3.2 Dilution of Suspension for Selective Media

Dilute the suspension 1:10 using sterile broth or nonbacteriostatic saline. Inoculate each test plate with 10 µL (0.01 mL). Streak for isolation. Use a 1:100 dilution if isolated colonies are not produced.

6.3.3 Tubed Media

Inoculate with 10 µL (0.01 mL) of the undiluted 0.5 McFarland suspension.

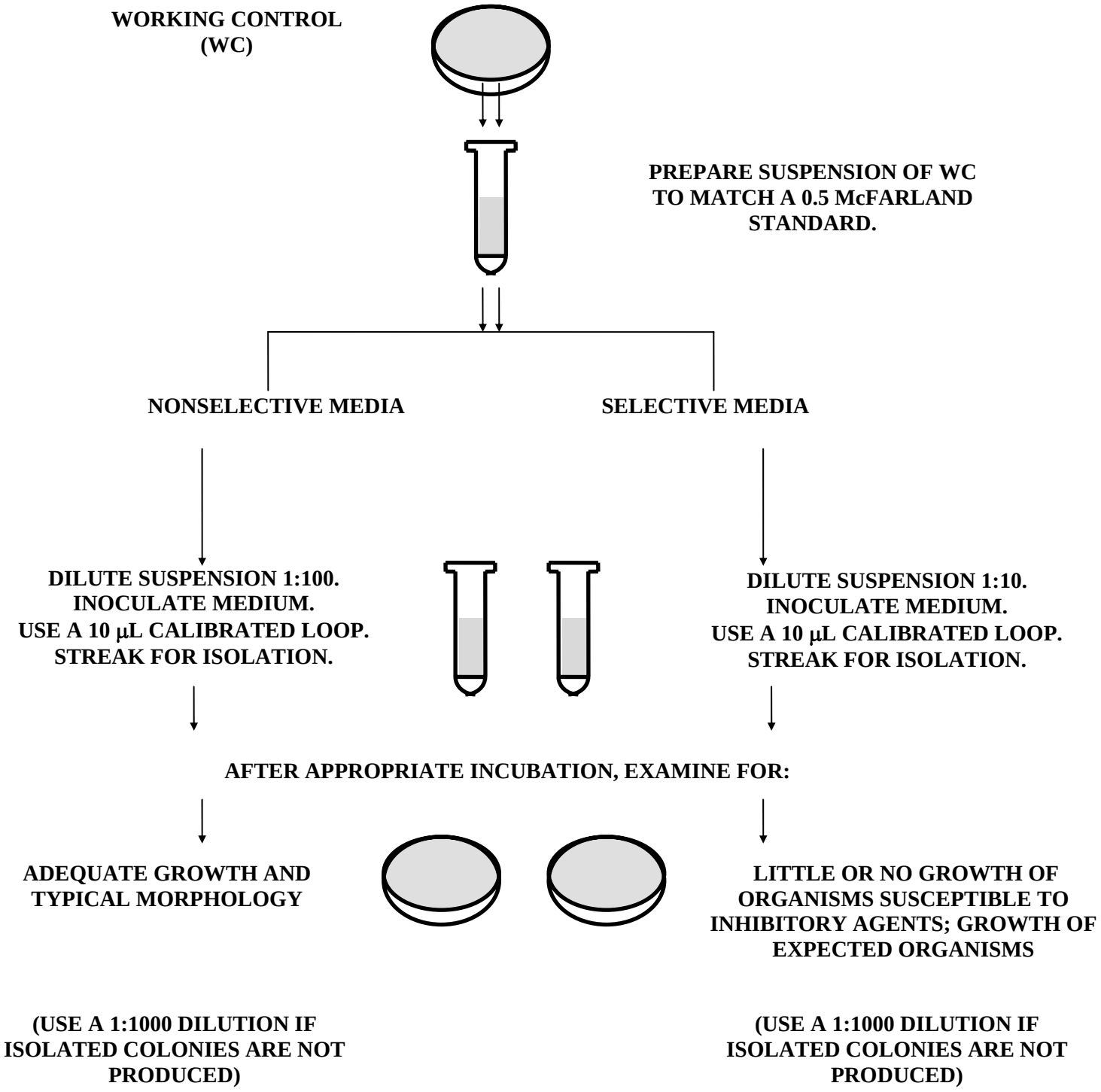

Figure 1. Preparation of Suspensions for Quality Control of Nonexempt Media

7 Incubation Conditions

Quality Control Organisms	Incubation Temperature	Incubation Atmosphere	Length of Incubation
Rapidly growing bacteria	35-37 °C	Ambient Air* or CO_2 Enriched	18-24 hours
Bacteria with special growth requirements	35-37 °C	CO_2 Enriched	24-72 hours
Anaerobes	35-37 °C	Anaerobic Gas	24-72 hours
Campylobacter	42 °C	Campy Gas	24-48 hours
Mycobacteria	35-37 °C	CO_2 Enriched	7-21 days
Yeast	35-37 °C	Ambient Air	≤72 hours
Moulds	25-30 °C	Ambient Air	≤72 hours

* Atmosphere depends on medium type. Check manufacturer's recommendations.

8 Interpretation of Results

- Selective media perform satisfactorily if the quality control organism(s) exhibits adequate growth, expected colony size, typical colony morphology, and inhibition of growth of certain organisms.

- Nonselective (growth) media perform satisfactorily if the quality control organism(s) exhibits adequate growth, expected colony size, and typical colony morphology.

Table 1. Extrapolated Failure Rates (EFR) of Media Included in the Three College of American Pathologists Surveys (1984, 1988, 2001)[a] (From Jones RN, Krisher K, Bird DS, and the College of American Pathologists Microbiology Resource Committee. Results of the survey of the quality assurance for commercially prepared microbiology media. *Arch Pathol Lab Med.* 2003;127(6):661-665. Reprinted with permission from the College of American Pathologists.)

1984 Survey		1988 Survey		2001 Survey	
Media	**EFR**[a]	**Media**	**FR**	**Media**	**EFR**
Anaerobic blood agar	0.071	Middlebrook 7H10	0.9	Automated blood culture bottle[d]	0.05
BHI blood culture broth	0.000	Middlebrook 7H11	0.7	Automated blood culture bottle[d]	0.26
Blood agar	0.015	Anaerobic PEA[b] agar	0.4	Bacteroides bile esculin agar	0.14
Campylobacter agar	0.338	Blood culture bottle[d]	0.0	BHI agar w/sheep blood w/CG[b]	0.17
Chocolate agar	0.18	Bacteroides bile esculin agar	0.7	BHI agar w/sheep blood w/CC[b]	0.74
Columbia nalidixic acid agar	0.11	Biphasic blood culture bottle	0.3	Brucella agar w/hemin/vitamin K	0.11
Eosin methylene blue agar	0.11	Blood culture broth	0.3	Brucella agar	0.13
Enterococcosel agar w/wo[b] azide	0.040	*Campylobacter* blood agar	0.9	Brucella laked blood agar w/KV[b]	0.33
Fungal agar w/CC[b,c]	0.046	Charcoal yeast extract agar	0.6	Campy blood agar (Blaser)	0.54
GN[b] broth	0.018	Chocolate agar	0.4	CDC laked blood agar w/KV[b]	0.20
Hektoen agar	0.056	CIN[b] agar	0.1	CDC sheep blood agar w/KV[b]	0.18
Lowenstein-Jensen medium	0.037	Citrate agar	0.0	CDC sheep blood agar w/PEA[b]	0.78
MacConkey agar	0.019	CLED[b] agar	0.0	Charcoal selective agar	0.39
Mannitol salt agar	0.078	Centrifugation/isolation tube	0.4	Chocolate agar	0.17
Mueller-Hinton agar	0.043	Inhibitory mould agar	0.4	Cornmeal agar	0.33
Mueller-Hinton blood agar	0.031	Kanamycin vancomycin agar	0.6	Cornmeal agar w/Tween	1.34
Mueller-Hinton chocolate agar	0.022	Martin-Lewis agar	0.9	*Campylobacter* (CVA[b]) agar	0.60
Middlebrook agar	0.000	Radiometric blood culture bottle[d]	0.3	Egg yolk (modified) agar	0.25
PEA[b] agar	0.078	*Streptococcus faecalis* broth	0.0	GC[b] (CA[b]/IsoVitaleX®) agar	0.68
Sabouraud dextrose agar	0.111	Thayer-Martin agar	0.5	GC[b]-Lect™ agar	0.30
Selenite broth	0.014	TCBS[b] agar	1.4	Inhibitory mould agar	0.40
Salmonella-Shigella agar	0.132	Triple sugar iron agar	0.2	Inhibitory mould agar w/G[b]	0.12
Thayer-Martin agar	0.31	Urease agar	0.3	Kanamycin laked blood agar	0.15
Thioglycolate broth	0.016			*Legionella* selective agar	0.38
Thiol blood culture broth	0.000			LIM broth	0.24
TSB blood culture broth	0.000			MacConkey w/sorbitol agar	0.61
XLD[b] agar	0.062			Martin-Lewis agar	0.52
				Nutrient broth	1.32
				PC[b] (*Burkholderia*) agar	0.48
				Potato dextrose agar	0.50
				Reagan-Lowe agar	1.29
				Sabouraud's dextrose agar w/CG[b]	0.08
				Selective agar for Group A Strep	0.04
				Sheep blood agar w/SXT[b]	0.10
				TCBS[b] agar	0.27
				Thayer-Martin agar	0.49
				Todd-Hewitt broth	0.63
				Sheep blood agar w/AM[b]	0.31

[a] Media categorization is based on a failure rate (FR) calculated from the total number of medium lots failing QC divided by the number of lots tested. The proportion of the FR caused by QC organism failure (extrapolated failure rate or EFR) is then determined for total media with ≥1000 lots and/or ≥ 100 000 items. See References 1 and 2 for details. Only FR data available from 1988 survey.

[b] Abbreviations: AM (Ampicillin); BC (Blood Culture); BHI (Brain Heart Infusion); CA (Chocolate Agar); CC (Cycloheximide/Chloramphenicol); CIN (Cefsulodin Irgasan-Novobiocin); CG (Chloramphenicol/Gentamicin); CLED (Cystine-Lactose-Electrolyte-Deficient); CVA (Cefoperazone/Vancomycin/Amphotericin B); GC (Gonococcal); G or GM (Gentamicin); GN (Gram Negative); PC (*Pseudomonas cepacia*); PEA (Phenylethyl Alcohol); KV (Kanamycin/Vancomycin); PS (Penicillin/Streptomycin); SXT (Sulfamethoxazole/Trimethoprim); TCBS (Thiosulfate Citrate Bile Sucrose); XLD (Xylose Lysine Deoxycholate); w/wo (with/without).

[c] Represents specific commercial soy peptone formulations such as Mycosel or Mycobiotic media.

[d] Represents bottle broth formulations from BD Diagnostic Systems (Sparks, MD) or BioMérieux (Raleigh/Durham, NC). Refer to manufacturer's package insert for specific QC requirements of different blood culture media.

Table 1A. Reasons Given for Lot Failures (From Jones RN, Krisher K, Bird DS, and the College of American Pathologists Microbiology Resource Committee. Results of the survey of the quality assurance for commercially prepared microbiology media. *Arch Pathol Lab Med.* 2003;127(6):661-665. Reprinted with permission from the College of American Pathologists.)

	Survey Year					
Reason	**1984**		**1988**		**2001**	
Contaminated	217	(35)[a]	98	(34)	149	(18)
Hemolyzed	124	(20)	49	(17)	60	(7)
No Growth of QC Organisms	83	(14)	63	(22)	332	(40)
Media Too Soft	46	(8)	—		—	
Frozen Media	37	(6)	9	(3)	—	
Media Not Inhibitory	24	(4)	34	(12)	155	(19)
Poor Growth of QC Organisms	23	(4)	13	(5)	—	
Wrong pH	17	(3)	8	(3)	—	
Antibiotic Zone Discrepancy	15	(2)	—		—	
Broken Plates	14	(2)	4	(1)	—	
Wrong Agar Depth	10	(2)	6	(2)	—	
Media Dried Out	3		3	(1)	—	
Defect, Surface or Other	—		—		136	(16)
Total No. of Responses	**613 (100)**		**287 (100)**		**832 (100)**	

[a] Number and (%) of responses

Table 1B. Exempt and Nonexempt Categories for Media Included in CAP Surveys (1984, 1988, 2001)[a] (From Jones RN, Krisher K, Bird DS, and the College of American Pathologists Microbiology Resource Committee. Results of the survey of the quality assurance for commercially prepared microbiology media. *Arch Path Lab Med.* 2003;127(6):661-665. Reprinted with permission from the College of American Pathologists.)

CATEGORY	EXEMPT [c]	NONEXEMPT[d,e]
General bacteriologic media	Blood agar Chocolate agar[c] Thioglycolate broth Urease agar	Nutrient broth
Blood culture media[b]	Brain heart infusion (BHI) blood culture broth Biphasic blood culture bottle medium Centrifugation/isolation tubes (adult) Thiol blood culture broth Trypticase soy blood culture broth Peptone broth	
Media for gram-positive bacteria	Columbia (CNA) agar Selective media for enterococci with or without azide LIM broth Mannitol salt agar Phenylethyl alcohol (PEA) agar Selective agar for Group A Streptococcus Sheep blood agar with sulfamethoxazole/trimethoprim (SXT) *Enterococcus* (*Streptococcus*) *faecalis* broth	Todd-Hewitt broth Desoxycholate broth[e] Trans-vaginal broth[e] Chocolate agar with pyridoxal[e]
Media for gram-negative bacteria	Cefsulodin irgasan novobiocin (CIN) agar Citrate agar Cystine lactose electrolyte deficient (CLED) agar Eosin methylene blue (EMB) agar Gram-negative (GN) broth Hektoen (HEK) agar MacConkey agar *Salmonella-Shigella* (SS) agar Selenite broth Thiosulfate citrate bile salts sucrose (TCBS) agar Triple sugar iron (TSI) agar Trypticase soy agar with sheep blood with ampicillin Xylose lysine desoxycholate (XLD) agar	MacConkey sorbitol agar Chocolate agar with bacitracin[e]
***Neisseria gonorrhoeae* (GC) media**	Thayer-Martin agar (modified)[c] GC-Lect[c,d] ™	Martin-Lewis agar Chocolate agar with IsoVitaleX® New York City agar[c]

Table 1B. (Continued)

CATEGORY	EXEMPT [c]	NONEXEMPT[d,e]
***Bordetella pertussis* media**		Reagan-Lowe agar Bordet Gengou agar[e]
***Legionella* media**	*Legionella* selective (CYE/BCYE) agar [c,d]	Selective *Legionella* agar with DGVP[d,e]
***Burkholderia cepacia* (PC) media**	*Pseudomonas cepacia* (PC) agar[c]	OFPBL agar[d,e]
***Campylobacter* media**	Charcoal selective agar with CVC[d]	*Campylobacter* blood agar (Blaser) *Campylobacter* agar with CVA[d]
Anaerobic media	Anaerobic blood agar Anaerobic phenylethyl alcohol (PEA) agar Bacteroides bile esculin (BBE) agar Brucella agar Brucella agar w/hemin/Vitamin K Brucella laked blood agar with KV[d] CDC anaerobe laked blood agar with KV[d] CDC anaerobic 5% sheep blood with KV[d] Egg yolk (modified) agar Kanamycin laked blood agar	CDC anaerobe 5% sheep blood agar with PEA
Mycobacteria (AFB) media[d]	AFB biphasic bottle medium[d] Middlebrook 7H9 broth Lowenstein-Jensen media Middlebrook agar Automated AFB bottle broths[b,d]	Middlebrook 7H10 agar Middlebrook 7H11 agar American Trudeau Society (ATS) agar[e] Mitchison's agar[e] Petragnani medium[e]
Fungal media	Cornmeal agar Inhibitory mould agar Inhibitory mould agar with gentamicin Soy peptone agar with CC without pH indicators[d] Potato dextrose agar Brain heart infusion agar with 5% sheep blood/CG[d] Sabouraud's dextrose agar Sabouraud's dextrose agar with CG[d]	Cornmeal agar with Tween Brain heart infusion agar with 5% sheep blood/CC[d] BIGGY agar[d,e] Birdseed agar[e] Brain heart infusion agar with 5% sheep blood/PS[d,e] Dermatophyte test medium[e] Potato flakes agar with or without CC[d,e]

[a] Exempt: Extrapolated Failure Rate of ≤ 0.5%; Nonexempt: Extrapolated Failure Rate of > 0.5%; media with insufficient data for categorization is considered nonexempt and QC is required.

[b] Represents formulations from BD Diagnostic Systems (Sparks, MD) or BioMérieux (Raleigh/Durham, NC). Refer to manufacturer's package insert for specific QC information.

[c] Quality control of exempt media used for fastidious organisms (in particular exempt media for recovery of *N. gonorrhoeae*, *H. influenzae*, *Campylobacter* sp., *Legionella* sp., and *B. cepacia* among others) strongly recommended to ensure optimum recovery of organisms. Refer to Table 3.

[d] Abbreviations: AFB (Acid Fast Bacilli); BIGGY (Bismuth sulfite Glucose Glycine Yeast); CC (Cycloheximide/Chloramphenicol); CG (Chloramphenicol/Gentamicin); CVA (Cefoperazone/Vancomycin/Amphotericin B); CVC (Cefoperazone/Vancomycin/Cycloheximide); CYE/BCYE (Buffered Charcoal Yeast Extract); DVGP (Dye,Vancomycin, Glycine, Polymyxin B); GC (Gonococcal); KV (Kanamycin/Vancomycin); OFPBL (Oxidative Fermatative Polymyxin B, Bacitracin and Lactose); PS (Penicillin/Streptomycin).

[e] Media deemed nonexempt because of insufficient data for calculation of extrapolated failure rate. See footnote a.

Table 2. Manufacturers' Minimum Quality Control Requirements for Commercially Prepared Media

Medium	Incubation Atmosphere, Length, and Temperature	Control Organisms (ATCC® No.)[a]	Expected Results
Anaerobic sheep blood and laked blood agar	Anaerobic, 24-48 h, 35 °C	*B. fragilis* (25285) *C. perfringens* (13124) *F. nucleatum* (25586) *P. anaerobius* (27337) *P. melaninogenica* (25845)	Growth Growth, beta hemolysis Growth Growth Growth
Anaerobic broths—see thioglycolate medium			
Blood agar—nonselective sheep blood agar media	Aerobic or CO_2, 18-24 h, 35 °C	*S. pyogenes* (19615) *S. pneumoniae* (6305) *S. aureus* (25923) *E. coli* (25922)	Growth, beta hemolysis Growth, alpha hemolysis Growth Growth
Blood agar-CAMP test (trypticase soy agar [TSA] with sheep blood only)	Aerobic, 18-24 h, 35 °C	*S. aureus* (33862) or (25923) *S. agalactiae* (12386) *S. pyogenes* (19615)	Growth Positive reaction (Arrowhead area of clearing) Negative reaction (No arrowhead formation)
Blood agar—Selective sheep blood agar media (Columbia [CNA] agar, phenylethyl alcohol [PEA] agar)	CNA, CO_2, 24-48 h, 35 °C PEA, CO_2, 24-48 h, 35 °C	*S. pyogenes* (19615) *S. pneumoniae* (6305) *S. aureus* (25923) *P. mirabilis* (12453) *S. pyogenes* (19615) *S. aureus* (25923) *P. mirabilis* (12453)	Growth, beta hemolysis Growth, alpha hemolysis Growth Inhibition (partial) Growth Growth Inhibition (partial)
Blood culture media. This applies to brain heart infusion, trypticase soy broth, and thiol-based media. Other media for blood culture are exempt from user performance testing provided that manufacturers certify that additional organisms appropriate for their intended use are tested.	Anaerobic, 5 days, 35 °C Aerobic, 5 days, 35 °C	*B. fragilis* (25285) *S. pneumoniae* (6305)	Growth Growth
Campylobacter agar (user quality control required)	Reduced O_2 with CO_2, 48 h, 42 °C	*C. jejuni* (33291) *E. coli* (25922)	Growth Inhibition (partial)

Table 2. (Continued)

Medium	Incubation Atmosphere, Length, and Temperature	Control Organisms (ATCC® No.)[a]	Expected Results
Chocolate agar	CO_2, 24 and 48 h, 35 °C	*N. gonorrhoeae* (43069) *H. influenzae* (10211)	Growth Growth
Cefsulodin irgasan novobiocin (CIN) agar	Aerobic, 24-48 h, 25 °C	*Y. enterocolitica* (9610) *E. coli* (25922) *P. aeruginosa* (27853) *E. faecalis* (29212)	Growth; deep red center transparent border (bull's-eye) Inhibition (partial to complete) Inhibition (partial to complete) Inhibition (partial to complete)
Cystine lactose electrolyte deficient (CLED) agar	Aerobic, 24-48 h, 35 °C	*E. coli* (25922) *P. vulgaris* (8427) *S. aureus* (25923)	Growth; yellow centers Growth; bluish, spreading Inhibited (partial) growth; uniform deep yellow
Buffered charcoal yeast extract (BCYE) (CYE/BCYE) agar	Aerobic, 48-72 h, 35 °C	*L. pneumophila* (33152) *L. bozemanii* (33217) *L. micdadei* (33204)	Growth; yellow-green fluorescence under long-wave UV light Growth; blue-white fluorescence Growth
Enrichment broths for enterics (gram-negative [GN] broth, selenite broths)	Aerobic, 18-24 h, 35 °C	*S. typhimurium* (14028) *S. sonnei* (9290) *E. coli* (25922)	Growth on subculture Growth on subculture (may be inhibited by selenite media) Inhibition (partial to complete) on subculture Growth on subculture from GN broth
Eosin methylene blue media (Levine EMB agar; EMB agar, modified)	Aerobic, 18-24 h, 35 °C	*S. typhimurium* (14028) *E. coli* (25922) *E. faecalis* (29212)	Growth, colorless to amber colonies Growth, blue-black colonies with green metallic sheen Inhibition (partial)
Hektoen enteric (HEK) agar	Aerobic, 18-24 h, 35 °C	*S. typhimurium* (14028) *S. flexneri* (12022) *E. faecalis* (29212) *E. coli* (25922)	Growth, colonies blue to green-blue with black centers Growth, colonies green to blue-green Inhibition (partial); colonies yellow Inhibition (partial to complete); colonies yellow to salmon colored
MacConkey agar	Aerobic, 18-24 h, 35°C	*E. coli* (25922) *P. mirabilis* (12453) *S. typhimurium* (14028) *E. faecalis* (29212)	Growth, pink colonies Growth, colorless colonies, partial inhibition of swarming Growth, colorless colonies Inhibition (partial)

Table 2. (Continued)

Medium	Incubation Atmosphere, Length, and Temperature	Control Organisms (ATCC® No.) [a]	Expected Results
Mannitol salt agar	Aerobic, 24 and 48 h, 35 °C	*S. aureus* (25923) *S. epidermidis* (12228) *P. mirabilis* (12453)	Growth, colonies have yellow zones at 48 h Growth, colonies have red zones at 48 h Inhibition (partial)
Mycobacteria media (Lowenstein-Jensen agar and Middlebrook) broth medium used for recovery of AFB are exempt from user quality control provided that manufacturers certify acceptable performance using the QC isolates listed.	CO_2, <21 days, 35 °C	*M. tuberculosis H37Ra* (25177) *M. kansasii* Group I (12478) *M. scrofulaceum* Group II (19981) *M. intracellulare* Group III (13950) *M. fortuitum* Group IV (6841) *E. coli* (25922)	Growth Growth Growth - May be inhibited on selective media Growth - May be inhibited on selective media Growth Inhibition (partial to complete on selective media)
PC (*Burkholderia cepacia)* agar	Aerobic, 48-72 h, 30 °C	*B. cepacia* (25416) *E. coli* (25922) *P. aeruginosa* (27853) *S. aureus* (25923)	Growth with red zone Inhibition (partial to complete) Inhibition (partial to complete) Inhibition (partial to complete)
Nonselective mycology media	Aerobic, ≤72 h, 25-35 °C	*C. albicans* (60193 or 10231) *T. mentagrophytes* (9533)	Growth Growth
Salmonella-Shigella (SS) agar	Aerobic, 24 h, 35 °C	*S. typhimurium* (14028) *S. flexneri* (12022) *E. faecalis* (29212) *E. coli* (25922)	Growth, colonies colorless with or without black centers Growth, colorless colonies Inhibition (complete) Inhibition (partial to complete; colonies pink to rose-red with precipitate)
Selective mycology media	Aerobic, ≤7 days, 25 °C	*A. niger* (16404) *C. albicans* (10231) *T. mentagrophytes* (9533) *E. coli* (25922)	Inhibition (partial to complete) on media containing cycloheximide Growth Growth Inhibition (partial to complete) on media containing chloramphenicol

Table 2. (Continued)

Medium	Incubation Atmosphere, Length, and Temperature	Control Organisms (ATCC® No.)[a]	Expected Results
Selective media for pathogenic *Neisseria* spp.	CO_2, 24-48 h, 35 °C	*N. gonorrhoeae* (43069) *N. meningitidis* (13090)[b] *P. mirabilis* (43071) *E. coli* (25922)[b] *N. sicca* (9913)[b] *C. albicans* (60193)[b] *S. epidermidis* (12228)	Growth Growth Inhibition (partial) use only for media containing trimethoprim Inhibition (partial) Inhibition (partial) Inhibition (partial) Inhibition (partial)
Selective media for enterococci, with azide	Aerobic, 24-48 h, 35 °C	*E. faecalis* (29212) *S. pyogenes* (19615) *E. coli* (25922)	Growth, blackening around colonies Inhibition (partial to complete) Inhibition (partial) - Colorless colonies on bile esculin agar
Selective media for enterococci, without azide	Aerobic, 24-48 h, 35 °C	*E. faecalis* (29212) *S. pyogenes* (19615)	Growth, blackening around colonies Inhibition (partial to complete)
Thioglycolate broth, with or without indicator	Aerobic, 48 h (tightened cap), 35 °C	*B. fragilis* (25285) *S. aureus* (25923)	Growth Growth
Thioglycolate broth, enriched with vitamin K and hemin	Aerobic, 48 h (tightened cap), 35 °C	*P. anaerobius* (27337) *B. vulgatus* (8482) *C. perfringens* (13124)	Growth Growth Growth
Tubed media (brain heart infusion and tryptic soy broth)	Aerobic, 18-24 h, 35 °C	*E. coli* (25922) *S. aureus* (25923)	Growth Growth
Xylose lysine desoxycholate (XLD) agar	Aerobic, 24 h, 35 °C	*S. typhimurium* (14028) *S. flexneri* (12022) *E. faecalis* (29212) *E. coli* (25922)	Growth - colonies red with black centers Growth - colonies red Inhibition partial Inhibition (partial to complete; colonies yellow to yellow-red)

[a] ATCC is a registered trademark of the American Type Culture Collection.
[b] Required for commercial manufacturers; not necessary for testing by users.

Table 3. Minimum User Quality Control Recommendations for Certain Categories of Commercially Prepared Media[a,b]

Medium	Incubation Conditions	Control Organisms (ATCC® No.)[c,d]	Expected Results
Chocolate agar	CO_2, 24-48 h, 35 °C	*N. gonorrhoeae* (43069) *H. influenzae* (10211)	Growth Growth
Campylobacter agar	Reduced O_2, enriched with CO_2, 24-48 h, 42 °C	*C. jejuni* (33291) *E. coli* (25922)	Growth Inhibition (partial)
Selective media for *Neisseria gonorrhoeae*[b]	CO_2, 24-48 h, 35 °C	*N. gonorrhoeae* (43069 or 43070) *P. mirabilis* (43071) *S. epidermidis* (12228) *C. albicans* (10231)	Growth Inhibition (partial) - (media with trimethoprim) Inhibition (partial) Inhibition (partial)
Media specifically for isolation of *B. cepacia*	Aerobic, 48-72 h, 35 °C	*B. cepacia* (25416) *P. aeruginosa* (27853)	Growth with red zone Growth
Media specifically for isolation of *Legionella*	Aerobic, 48-72 h, 35 °C	A well characterized clinical isolate in addition to organisms listed in Table 2	Growth
Media specifically for isolation of *B. pertussis*	CO_2, 48-72 h, 35 °C	A well characterized clinical isolate plus *B. pertussis* (12742)	Growth
Nonexempt media for primary isolation of fungi Moulds Yeast	 Aerobic, <72 h, 25 °C Aerobic, <72 h, 35 °C	 *T. mentagrophytes* (9533) *A. niger* (16404) *C. neoformans* (34877) *C. albicans* (10231)	 Growth Growth Growth (dependent on medium type) Growth
Nonexempt media for primary isolation of anaerobes	Anaerobic atmosphere, 24-72 h, 35 °C	As indicated in Table 2	As indicated in Table 2
Any media listed in Table 1B that have a performance deficiency	As indicated in Table 2 or manufacturer's recommendations	As indicated in Table 2 or manufacturer's recommendations	As indicated in Table 2 or manufacturer's recommendations
All other media for primary isolation of bacteria and fungi not listed in Table 1B	As indicated in Table 2 or manufacturer's recommendations	As indicated in Table 2 or manufacturer's recommendations	As indicated in Table 2 or manufacturer's recommendations

[a] Quality control of exempt media is strongly encouraged when using specialty media for the recovery of anaerobes or fastidious organisms. Media components such as antimicrobial agents or other special additives may deteriorate if exposed to adverse conditions during delivery to the laboratory. Quality control is strongly encouraged on any exempt media used for the isolation of *N. gonorrhoeae*.

[b] Minimum requirements for media quality control include at least one organism to document support of growth. Testing additional organisms is recommended to confirm the performance of inhibitory or selective media. Refer to Table 2 and/or manufacturer's recommendations for a listing of appropriate quality control organisms.

[c] The identification number used by the American Type Culture Collection.

[d] Although not formally documented, some quality control isolates ("lab-adapted" strains) are suspected of producing growth on media even under substandard conditions. The use of clinical isolates is recommended to ensure adequate quality control of certain specialty media.

Table 4. Performance Check of Nonexempt Media

Performance Check	Instructions[a]	Satisfactory[b]	Unsatisfactory
Nonselective media	Prepare a 1:100 dilution of the PS in sterile saline. Inoculate medium with 10 µL to achieve 10^3-10^4 cfu/plate.	Colonies of sufficient size and characteristic colony morphology to allow initiation of routine testing within 24-48 hours incubation.	No growth within 48 hours or insufficient colony size for observation of typical characteristics or initiation of additional testing procedures.
Selective media	Prepare a 1:10 dilution of the PS in sterile saline. Inoculate medium with 10 µL to achieve 10^4-10^5 cfu/plate.	Colonies of sufficient size and characteristic colony morphology to allow initiation of routine testing within 24-48 hours incubation. Total to partial inhibition of QC organisms used to test selective properties.	No growth within 48 hours or insufficient colony size for observation of typical characteristics or initiation of additional testing procedures. Inadequate inhibition of QC organisms used to test selective properties.
Tube media	Inoculate each tube with 10 µL of the primary suspension.	Colonies of sufficient size and characteristic colony morphology to allow initiation of routine testing within 24-48 hours.	No growth within 48 hours or insufficient colony size for observation of typical characteristics or initiation of additional testing procedures.
Mycobacterial media	Emulsify the growth in sterile diluent containing bovine serum albumin (0.2%) and Tween 80 (0.002%). Add 8-12 sterile glass beads and mix vigorously for 10 min. Let stand for 3 hours to allow large particles to settle. Transfer the supernatant to a sterile vial. Adjust the cell suspension to match a 0.5 McFarland standard. Proceed as described for preparation of dilutions and inoculation of nonselective and selective media.	Colonies of sufficient size and characteristic colony morphology to allow initiation of routine testing after appropriate incubation. Total to partial inhibition of QC organisms used to test selective properties.	No growth or insufficient colony size for observation of typical characteristics or initiation of additional testing procedures. Inadequate inhibition of QC organisms used to test selective properties.
Fungal media	Yeast: Procedure is the same as for bacteria. Moulds: Transfer a portion of the colony to the plate.	Yeast: Colonies of sufficient size and characteristic colony morphology within 72 h. Moulds: Colonies of sufficient size and characteristic colony morphology within 3-5 days. Total to partial inhibition of QC organisms used to test selective properties.	No growth or insufficient colony size after recommended incubation period. Inadequate inhibition of QC organisms used to test selective properties.

[a] Inoculum preparation for aerobic/anaerobic bacteria: Prepare a suspension of a 24-hour culture in sterile saline to match a 0.5 McFarland standard. (Primary Suspension) (PS).

[b] Acceptable performance should generally allow initiation of routine testing within 24-48 h for nonfastidious, more rapidly growing bacteria. Refer to Table 2 for specific recommendations.

Table 5. Processing of Exempt Media

Process	Instructions	Satisfactory	Unsatisfactory
Media Arrival	Record the amount of media received, the arrival date (optional), lot numbers, and expiration dates in the QC records. Sequester the lot(s) pending QC testing. Label boxes with the received date.	Boxes properly labeled. Dates, lot numbers, and amount received are recorded in QC records. Media are stored at 2-8 °C.	Boxes unlabeled. Required information not recorded in QC records. Failure to store media at recommended temperatures.
Visual Inspection	Remove media packages from outer boxes. Examine the packages for obvious damage. For every 10-20 plates in the lot, remove 1 plate. Perform visual inspection. Record results.	1. Intact petri dish 2. Agar attached to bottom of dish 3. Blood containing medium is opaque 4. Medium is appropriate color 5. Minimal moisture in package 6. Not frozen or desiccated 7. No visible contamination 8. Agar has even depth of at least 3 mm 9. Smooth agar surface 10. No precipitates	1. Cracked/damaged petri dish 2. Agar dislodged or loose 3. Hemolysis of blood in medium 4. Color differs from characteristic appearance 5. Excessive accumulation of moisture 6. Evidence of freezing or medium desiccated 7. Gross contamination 8. Uneven or underfilled plates/agar desiccation 9. Excessive bubbles/rough surface 10. Presence of precipitates
Sterility Check (optional)	Incubate the plates removed for examination during the Visual Inspection (see above) and incubate at 35 °C for ≤72 hours. Record results.	No bacterial or fungal growth	Bacterial or fungal growth detected

References

[1] Mac Lowry JD, Edson DC, Dreskin R. CAP Microbiology Resource Committee Survey of Commercially Prepared Media. In: *The Role of Clinical Microbiology in Cost Effective Health Care.* Smith JW, ed. Skokie, IL: College of American Pathologists; 1985:555-559.

[2] Jones RN, Krisher K, Bird D. Results of the survey of the quality assurance for commercially prepared microbiology media. *Arch Pathol Lab Med*. 2003;127(6):661-665.

[3] Zoutman D, Fleming C, Richardson H. Compliance with NCCLS approved standard M22-A2 for bacteriologic media quality assurance: a survey of 124 Ontario microbiology laboratories. *Diagn Microbiol Infect Dis*. 2002;42:29-34.

[4] Krisher K. Survey results: The use of the NCCLS standard, M22-A2, by clinical laboratories. *Clinical Microbiology Newsletter*. April 2000.

[5] 42 CFR Part 493. *Medicare, Medicaid, and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; Final Rule;* 2003.

[6] 21 CFR 820. *Food and Drugs. Food and Drug Administration Department of Health and Human Services. Quality System Regulation. Labeling and Packaging Control;* 2003.

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 on M22-A2

M22-A2: *Quality Assurance for Commercially Prepared Microbiological Culture Media—Second Edition; Approved Standard*

General Comments

1. I have heard that there are going to be some major changes with regard to quality control of anaerobic media in that the requirement for growth of some of the more fastidious strains (*B. levii)* will no longer be required. I think this sets a bad precedent in that anaerobic media should be able to support the growth of a wide variety of potentially pathogenic anaerobic isolates, not just *Bacteroides fragilis* and *Clostridium perfringens*. This seems like the equivalent of requiring that chocolate agar grow *E. coli* and *S. aureus* because *N. gonorrhoeae* is too difficult to maintain as a stock culture. This does not provide the end user with any assurance that the medium will do what it is supposed to do.

- **The subcommittee agrees that quality control using more fastidious organisms would measure the performance of anaerobic media more accurately. Only a limited list of commercially prepared anaerobic quality control isolates is available. Clinical laboratories would not have ready access to more fastidious organisms. A compromise is the addition of *P. melaninogenica* (ATCC 25845) to the list of required quality organisms for anaerobic media.**

2. I question the recommendation in M22-A2 (Dec. 1996) that manufacturer QC testing of CYE/BCYE testing is sufficient for adequate QC of this medium. As I understand it, this recommendation is based on unpublished CAP data on QC failure rates for this medium tested with *L. pneumophila* ATCC 33152. The problem with this recommendation, and the data on which it is based, is that stock strains of *L. pneumophila*, such as ATCC 33152, are media-adapted. Media-adapted *L. pneumophila* strains do not reliably detect bad BCYE medium. The last three editions of the ASM *Manual of Clinical Microbiology* warn against this practice. The fact that participants in the CAP survey detected few problems with BCYE medium, when tested with media-adapted strains, does not provide much assurance to me. In fact, proper QC testing of BCYE medium requires the use of nonadapted strains. I do not know whether growth of *L. bozemanii*, ATCC 33217, serves as any better index of BCYE medium quality. My guess would be that it would not, especially given your qualitative testing standards and allowance for serial passage of working controls. To my knowledge, parallel testing with nonadapted strains of *L. bozemanii* has not been performed. In addition to my concern about the lack of good QC data on commercially prepared BCYE medium, I have serious questions about the adequacy of your suggested testing method. The recommendation that serially passed working control cultures of *L. pneumophila* be used for the QC testing may simply foster media adaptation. In addition, the absence of quantitative standards for the QC testing makes interpretation unclear. Does this mean that a plate inoculated with 10^4 cfu of *L. pneumophila* would pass your standards if any growth were detected? I do agree that the general quality of commercial BCYE medium is quite high. In fact, using a much more rigorous standard, the failure rate of BCYE medium in our laboratory is 0%(95%CI=0 to 5%, n=72). Regardless, the use of bad medium can be avoided by using more rigorous standards than those in M22-A2. As an index of this, the failure rate in our laboratory of selective BCYE medium with PAC is 4%(95%CI=0 to 11%, n=72). This is despite apparently "appropriate" QC testing by the manufacturer. My concern about BCYE medium is that I have not tested several hundred lots of commercial medium, and that the true failure rate may be closer to 5% than it is to 0%. I would like to see more data on this point using rigorous standards. I request that the committee reconsider its recommendation for QC testing of BCYE medium, until a more rigorous standard has been established and studied with commercial media.

- **The subcommittee recommends the use of a well characterized clinical isolate in addition to the listed ATCC strains for quality control of media used for isolation of more fastidious isolates.**

3. I believe the NCCLS committee should reconsider the current recommendation on the choice of QC organisms for anaerobic blood agar. The current list of organisms is not adequate to identify performance problems related to oxygen toxicity of media and the subsequent lack of growth of many anaerobic clinical isolates. What is needed is for the manufacturer to test anaerobic blood agar with an organism that is a compromise.

- **See the response to Comment 1.**

4. I would like to suggest an alternative organism to the committee: *Porphyromonas asaccharolytica* can be easily maintained and truly tests the anaerobic blood agar medium for oxygen toxicity—and, in addition, tests for medium for pigment production, which the current recommendations do not allow. This change will help ensure user quality assurance of anaerobic media.

This change is essential, because pigmented, anaerobic, gram-negative rods represent about 7 to 8% of clinical isolates and have different nutritional and oxygen requirements than other clinically isolated anaerobes. Furthermore, *Porphyromonas asaccharolytica* will detect performance problems, as has been previously documented to occur with anaerobic media. See *J Clin Microbiol*. 1989;27:2268-2271.

- **See the response to Comment 1.**

5. My question involves the addition of more media to the standard. In the abstract, it states that there have been many requests for additions. It is also stated that there is a lack of independent data for these new media. Are these media presently in testing and what is the time frame for inclusion in the document? If they are not presently in testing, what will it take to start this process?

   For instance, LIM Broth is widely used in Group B Strep testing. Also in the state of Utah, our Health Board has requested we use MacConkey/Sorbitol to screen for pathogenic *E. coli.* Others that we use here include V agar for *Gardnerella* and CCFA for *Clostridium difficile*. Some common media such as TSI and motility medium are also not covered.

- **The proposed new revision exempts a larger number of media types from required complete quality control.**

6. For testing the nutritive capacity of Regan Lowe plate media, we have used 10 microliters of a 1:100 dilution of a 0.5 McFarland suspension as stated in the NCCLS guidelines, but our organisms have failed to grow. We have tried two different ATCC strains of *B. pertussis*. Talking to other labs (including reference labs), the story seems to be the same that the 1:100 dilution does not grow, but if you use a 1:10 dilution the organisms grow.

   Are there any provisions for testing Regan Lowe plate media? Have other labs brought this concern to your attention? And are there any suggestions of what we can do? As I said we have tried different lot numbers, different suppliers, and different ATCC strains.

- **A direct inoculation is appropriate for quality control. If problems occur, inoculation of dilutions of the bacterial suspension is recommended for corrective action.**

7. I enclose the Dermatophyte Test Medium (DTM) clarification from the HCFA website that states, "Under CLIA, a laboratory that uses DTM must perform end-user quality control by performing media checks for sterility, selectivity and/or inhibition, the ability to support growth and the appropriate biochemical response for each batch or shipment or media." (Communication dated December 15, 1997 in which Dr. George Evans, chairholder of NCCLS Subcommittee on Culture Media, is attributed with stating that media listed in Table 2 of M22-A2 as "selective mycology media" (media containing cycloheximide and chloramphine excluding inhibitory mold agar) is only for Mycosel and Mycobiotic. I enclose the package inserts DTM from two suppliers. These formulae contain cycloheximide and chloramphenicol, and the control organisms include all those listed in the NCCLS Table 2 for selective mycology media.

   I am aware that some manufacturers of DTM have changed the formula or do not use the specified control organisms. Nevertheless, I question the rationale for requiring end-user quality control by those laboratories using a medium that is consistent with the requirements of Table 2A. A possible solution to this added burden on our members who use a correctly formulated and tested medium is to have HCFA issue a further clarification that end-user QC is only required for media that do not meet the specifications of Table 2. Correct formulation and QC testing could be verified via the package insert.

- **DTM was not included in the media surveys of 1984 or 1988. Data from the 2001 survey indicate that DTM does not meet the qualifications for the currently accepted method for calculation of failure rates (>1000 lots or >100 000 items used by participants). The formulation for DTM also contains a pH indicator and gentamicin. These items are not included in similar soy peptone-based fungal media containing cycloheximide and chloramphenicol.**

Summary of Delegate Comments and Subcommittee Responses on M22-P2

M22-P2: *Quality Control for Commercially Prepared Microbiological Culture Media; Proposed Standard—Second Edition*

General

1. Why did you change the title from Quality Assurance (last edition) to Quality Control?

- **Although the two processes are closely related, quality control, which involves the analysis and inspection for defects in manufactured products, more closely represents the intent of the document.**

2. Since NCCLS M22-P2 is based primarily on the results published by the CAP, concerns with this publication also pertain to M22-A2 as follows:

a) My first concern is that survey response rates are not provided and the potential for bias in the estimated lot failure rates cannot be checked. In addition, the confidence intervals for the estimated failure rates are not provided.

b) My second concern is the calculation of the extrapolated lot failure rates. Since M22 proposes using these rates to classify media as exempt or non-exempt from routine quality control, the potential for bias and imprecision in the estimated failure rates needs to be considered more formally.

- **The data are based on survey evaluations performed by the CAP and provided to NCCLS. The statistical calculation methods are the sole purview of the CAP and accepted in good faith by NCCLS in the publication of M22 since its inception 15 years ago. The primary quality control of manufactured media is the responsibility of the media manufacturer.**

Section 4, General Responsibilities of the Manufacturer, Distributor, and User (Formerly Section 3)

3. Please define "QSR" as referenced in Section 3.1.

- **The abbreviation "QSR" represents the term Quality System Regulation. This text has been added to Section 4.1.**

4. Section 3.3: the first bullet point is also a responsibility of the distributor, and so should be listed in Section 3.2 as well.

- **The first bullet point is the responsibility of the user.**

5. Section 3.2: there should be an introductory sentence before these bullets.

- **The title of the section serves as the introductory sentence for each separate subsection. However, text has been added to Section 4.2 to avoid any confusion.**

6. Section 3.3: there should be an introductory sentence before these bullets.

- **The title of the section serves as the introductory sentence for each separate subsection. However, text has been added to Section 4.3 to avoid any confusion.**

Section 5.5.2, Storage of Quality Control Organisms (Formerly Section 4.5.2)

7. Storage of QC organisms requires a lot of extra work than current recommended practice. We are wondering if this will be mandatory or optional. Also, many organisms are available from commercial sources more easily than the method outlined.

- **Users have the option of opening a new package of a lyophilized organism whenever quality control is performed. The procedure outlined is an option for those sites that want to prepare QC organisms once yearly for laboratory use. Standardization of the protocol is recommended in order to reduce passage and possible subsequent mutations.**

Section 6, Inoculation of Quality Control Media (Formerly Section 5)

8. The recommendations in M22-P2 for media requiring QC require a lot more work than current methods (i.e., dilution protocols are cumbersome and sometimes impossible for routine labs).

- **The document gives the user the option of using direct inoculation. Since direct inoculation produces a less consistent inoculum, an alternative more standardized procedure is also presented for users.**

9. Page 8, Section 5.3 (1) – the concentration for saline is not exact: saline is 0.85% or 0.9%.

- **Percentages in Section 6.3 were corrected to 0.85%.**

Section 7, Incubation Conditions (Formerly Section 6)

10. Page 10, Section 6, Incubation Conditions: for *Campylobacter*, the incubation should be at 35-37 °C or 42 °C depending on the strain.

- **The incubation condition of 42 °C represents the more common use of the medium.**

Tables

11. For tube media, what is the acceptable performance for broth?

- **Acceptable performance of broth is the same as for tube media.**

12. In Table 1, are these percentages? Footnote (a) calls them proportions. Please explain (e.g., proportion failing *100).

- **Proportion and percentage convey the same meaning: a part of the whole/total.**

13. In the 1988 survey column in Table 1, are these failure rates or extrapolated failure rates (FR or EFR)? If they are really FR (column heading), then please explain in the text.

- **Only the failure rates were available from the 1988 CAP data. As listed in the text, additional information is available from the two references cited.**

14. In Table 1A, it is understandable why percentages do not sum to 100% in tables like this. However, they sum to 100% for 1984, 95% for 1988, and only 84% for 2001. The table should be extended, or some other explanation offered for the inconsistency.

- **The table was corrected and all values equal 100%.**

15. In Table 1A, it would be useful to note which of the listed reasons were included as "detected by QC strains" in the calculation of EFR in the published manuscript.

- **The reasons that could be detected by QC strains are evident.**

16. In Table 1B, please explain whether the exempt classification comes from any of the three surveys, or the latest one or what was done when a medium was "exempt" based on one survey but not on another, as given in Table 1.

- **The exempt category was expanded based on the data received from each of the three CAP surveys.**

NOTES

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 document 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

M22-A3 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
GP2					X M2 M6 M7 M11						

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: preanalytic, analytic, and postanalytic. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

M22-A3 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 7following page.

Preanalytic					**Analytic**		**Postanalytic**	
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpretation	Results Report	Post-test Specimen Management
					M2 M6 M7 M11	M2 M6 M7 M11	M2 M6 M7 M11	M6

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

Related NCCLS Publications*

GP2-A4 **Clinical Laboratory Technical Procedure Manuals; Approved Guideline—Fourth Edition (2002).** GP2-A4 contains guidelines that address the design, preparation, maintenance, and use of technical procedure manuals in the clinical laboratory.

M2-A8 **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eighth Edition (2003).** *American National Standard.* This newly revised standard contains updated recommended techniques, interpretive criteria, and quality control parameters for disk susceptibility testing. This document is complete with disk susceptibility testing tables updated for 2003 (M100-S13/M2).

M6-A **Evaluating Production Lots of Dehydrated Mueller-Hinton Agar; Approved Standard (1996).** M6-A addresses procedures for evaluating production lots of Mueller–Hinton agar and the development and application of reference media.

M7-A6 **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Sixth Edition (2003).** *American National Standard.* 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. This document contains MIC interpretive criteria and quality control parameters tables updated for 2003 (M100-S13/M7).

M11-A6 **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Sixth Edition (2004).** This standard provides reference methods for the determination of minimal inhibitory concentrations (MICs) of anaerobic bacteria by agar dilution and broth microdilution.

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

NOTES

NOTES

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St. Francis Medical Center (CA)
St. John Hospital and Medical Center (MI)
St. John's Hospital & Health Center (CA)
St. Joseph Mercy Hospital (MI)
St. Joseph's Hospital-Marshfield Clinic (WI)
St. Joseph's Hospital & Medical Center (AZ)
St. Jude Children's Research Hospital (TN)
St. Mary of the Plains Hospital (TX)
St. Michael's Hospital (Toronto, ON, Canada)
Ste. Justine Hospital (Montreal, PQ, Canada)
Salem Clinic (OR)
San Francisco General Hospital (CA)
Santa Clara Valley Medical Center (CA)
Seoul Nat'l University Hospital (Korea)
Shands at the University of Florida
So. California Permanente Medical Group
South Bend Medical Foundation (IN)
South Western Area Pathology Service (Australia)
Southern Maine Medical Center
Southwest Texas Methodist Hospital
Spartanburg Regional Medical Center (SC)
Specialty Laboratories, Inc. (CA)
State of Connecticut Dept. of Public Health
State of Washington Department of Health
Stony Brook University Hospital (NY)
Stormont-Vail Regional Medical Center (KS)
Sun Health-Boswell Hospital (AZ)
Sunnybrook Health Science Center (ON, Canada)
Swedish Medical Center - Providence Campus (WA)
Temple University Hospital (PA)
Tenet Odessa Regional Hospital (TX)
The Toledo Hospital (OH)
Touro Infirmary (LA)
Tripler Army Medical Center (HI)
Truman Medical Center (MO)
UCLA Medical Center (CA)
UCSF Medical Center (CA)
UNC Hospitals (NC)
Unidad de Patologia Clinica (Mexico)
Union Clinical Laboratory (Taiwan)
Universita Campus Bio-Medico (Italy)
University Hospitals of Cleveland (OH)
University of Alabama-Birmingham Hospital
University of Chicago Hospitals (IL)
University of Colorado Hospital
University of Debrecen Medical Health and Science Center (Hungary)
University of Illinois Medical Center
University of the Ryukyus (Japan)
The University of Texas Medical Branch
The University of the West Indies
University of Virginia Medical Center
University of Washington
UZ-KUL Medical Center (Belgium)
VA (Hampton) Medical Center (VA)
VA (Hines) Medical Center (IL)
VA (Tuskegee) Medical Center (AL)
Valley Children's Hospital (CA)
Vejle Hospital (Denmark)
Virginia Beach General Hospital (VA)
Virginia Regional Medical Center (MN)
ViroMed Laboratories (MN)
Washington Adventist Hospital (MD)
Washoe Medical Center Laboratory (NV)
Waterford Regional Hospital (Ireland)
Wellstar Health System (GA)
West Jefferson Medical Center (LA)
Wilford Hall Medical Center (TX)
William Beaumont Army Medical Center (TX)
William Beaumont Hospital (MI)
William Osler Health Centre (Brampton, ON, Canada)
Winn Army Community Hospital (GA)
Winnipeg Regional Health Authority (Winnipeg, Canada)
Wishard Memorial Hospital (IN)
Yonsei University College of Medicine (Korea)
York Hospital (PA)

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