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Abbreviated Identification of Bacteria and Yeast; Approved Guideline

This document provides the minimum identification criteria that can be used to rapidly identify organisms commonly isolated from clinical specimens.

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

NCCLS

NCCLS...

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Abbreviated Identification of Bacteria and Yeast; Approved Guideline

Ellen Jo Baron, Ph.D., Chairholder
Mike Cox
Marian Heyliger, M.S., SM (ASCP)
J. Michael Miller, Ph.D.
Kathryn L. Ruoff, Ph.D.
Melvin P. Weinstein, M.D.
Mary K. York, Ph.D.

Abstract

Many microorganisms commonly isolated in human diagnostic microbiology laboratories exhibit specific morphologic or biochemical traits that can be determined rapidly upon obtaining a pure colony. When such rapidly obtained parameters allow reliable identification of the organism with a high degree of certainty, the necessity of performing more time-consuming tests is decreased, and timely patient care is enhanced.

NCCLS document M35-A—*Abbreviated Identification of Bacteria and Yeast; Approved Guideline* includes the minimum identification criteria that can be used to rapidly identify a limited number of organisms commonly isolated from patient specimens. Although these tests do not rule out an occasional misidentification, those errors may not have important consequences with regard to patient outcome. Those situations in which rapid test results may have limitations are described. With those exceptions, confirmatory identification is not clinically useful and need not be done.

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Committee Membership

Area Committee on Microbiology

James H. Jorgensen, Ph.D.
Chairholder
University of Texas Health Science Center
San Antonio, Texas

Mary Jane Ferraro, Ph.D., M.P.H.
Vice-Chairholder
Massachusetts General Hospital
Boston, Massachusetts

Subcommittee on Abbreviated Identification of Bacteria and Yeast

Ellen Jo Baron, Ph.D.
Chairholder
Stanford University Medical School
Stanford, California

Mike E. Cox
Anaerobe Systems
Morgan Hill, California

Marian Heyliger, M.S., SM (ASCP)
Food and Drug Administration
Rockville, Maryland

J. Michael Miller, Ph.D.
Centers for Disease Control and Prevention
Atlanta, Georgia

Kathryn L. Ruoff, Ph.D.
Massachusetts General Hospital
Boston, Massachusetts

Mary K. York, Ph.D.
MKY Consulting
Walnut Creek, California

Advisors

Kay Baitz
Key Scientific Products
Round Rock, Texas

Karen Carroll, M.D.
ARUP
Salt Lake City, Utah
Dr. Jose B. Casals
Rosco Diagnostics
Denmark

Hans O. Hallander
Swedish Institute for Infectious Disease Control
Stockholm, Sweden

Lisa R. Henderson, MPA, M.T. (ASCP), NCA (CLS)
AAFP
Kansas City, Missouri

James McLaughlin, Ph.D.
University Hospital – Albuquerque
Albuquerque, New Mexico

Mary Motyl, Ph.D.
Beth Israel Medical Center
New York, New York

Roxanne G. Shively, M.S.
Food and Drug Administration
Rockville, Maryland

Lisa L. Steed, Ph.D.
Medical University of South Carolina
Charleston, South Carolina

Ronald J. Zabransky, Ph.D.
Shaker Heights, Ohio

Staff

Lois M. Schmidt, D.A.
Staff Liaison
NCCLS
Wayne, Pennsylvania

Tracy A. Dooley, M.L.T. (ASCP)
Project Manager
NCCLS
Wayne, Pennsylvania

Patrice E. Polgar
Editor
NCCLS
Wayne, Pennsylvania

Donna M. Wilhelm
Assistant Editor
NCCLS
Wayne, Pennsylvania

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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

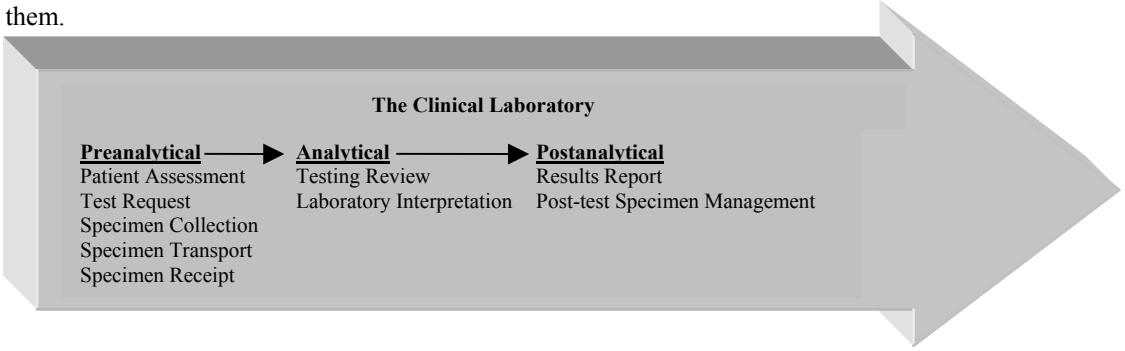
M35-A addresses the following Quality System Essentials (QSEs)

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

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 post analytical. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information. The arrow depicts the sequence, from left to right, that any clinical laboratory follows. In addition, the necessary steps or subprocesses are listed below them.



Most of NCCLS's documents relate to the clinical laboratory, so the most common path of workflow will be that depicted above. The path of workflow for other healthcare activities, e.g., respiratory services, imaging services, etc., or for other types of organizations, e.g., medical device manufacturers, will differ from that of the clinical laboratory. All such paths of workflow describe the sequence of activities necessary to produce the organization's or an entity's specific product or services. For those documents that relate to other paths of workflow, the icon will reflect different process steps.

M35-A addresses the following steps within the Clinical Laboratory Path of Workflow

Preanalytical					Analytical		Postanalytical	
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpretation	Results Report	Post-test Specimen Management
					X	X	X	

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

Contents

Foreword

Microorganisms isolated in the clinical microbiology laboratory demonstrate unique biochemical and morphologic characteristics. These characteristics provide a mechanism for determination of microbial identity to the genus (and sometimes, species) level. Identification of pathogenic bacteria and yeast provides the basis for guidance of treatment with antimicrobial and antifungal agents. In the clinical microbiology laboratory, the need for accurate reporting to the clinician of microbial identification is coupled with a demand for rapid turnaround time to allow for initiation of therapy. These requirements often demand the expenditure of resources in a healthcare environment where laboratory personnel are faced with ever increasing financial and manpower constraints. The recognized need for finding cost-effective approaches to diagnostic microbiology obviates the use of time- and resource-consuming, comprehensive microbial identification techniques. M35 was created to supplement this effort.

This document contains instructions and flowcharts outlining the minimal characteristics required to identify the listed microorganisms to genus, and in some cases, to species, with enough reliability (≥ 95% accuracy) for clinical laboratory reports. It is expected that additional organisms will be added to successive versions of the document, and NCCLS would appreciate being informed of any problems that occur as a result of using the guidelines in this document for determining patient results.

Protocols for noncommercial tests used for abbreviated identifications are listed in separate appendixes at the end of this document.

Standard Precautions

Because it is often impossible to know what might be infectious, all human specimens are to be treated as infectious and handled according to "standard precautions." Standard precautions are new 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 recommendations for the management of blood-borne exposure, refer to NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections*.

Key Words

Biochemical characteristics, microbiological identification

Abbreviated Identification of Bacteria and Yeast; Approved Guideline

1 Introduction

A variety of methods can be used to identify microorganisms of clinical importance. The most well characterized employ a battery of biochemical and enzymatic tests that are used after characterization based on initial gram stain and colony morphological characteristics. Often these methods require time and materials that add to the cost of the final identification. Even simple tests such as the bacitracin and optochin disk tests require overnight incubation. Waiting for results of these test methods may unnecessarily delay reporting of clinically important isolates and may slow the laboratory workflow.

Although many laboratorians use rapidly determined characteristics, such as odor, immediate enzymatic reactions (spot tests), and other criteria for "presumptive" or initial identification, many of these rapid methods have not been standardized or validated. The methods described in this guideline are those believed to yield a result reliable enough for clinical decision-making but are cost-effective, take less time, and are easier to perform than conventional methods. Several authors have examined the cost savings of using rapid methods or the overall patient care benefits (economic and general) that rapid reporting of results yield.[1,2,3] Tests that may be included in this category are single-tube, slide, spot, agglutination, disk, chromogenic media, fluorogenic, enzymatic, microscopic, morphologic, or plate methods that can be performed within a few hours. Proprietary multitest and molecular-based systems are not included. A critical factor in the performance of these tests is the competency and experience of the microbiologist. Initial correct interpretation of colonial and gram stain characteristics is essential to achieving the desired results.

Laboratory directors, managers, and supervisors are responsible for assuring that these methods are only used in situations in which the competency of the tester is adequate. Isolates to be tested should have been determined by the experienced tester to have a high likelihood of verifying the suspected identification. Inexperienced laboratorians should be under the direct supervision of an experienced technologist or use alternate methods until proficiency has been achieved.

Isolates conforming to the reactions described in the appendix will identify the named organism with >95% accuracy, and their identification can thus be reported without qualification, with the caveats listed below. Confirmation by additional procedures is unnecessary. It should also be emphasized that lack of a positive result in the rapid tests included here does not rule out the identification of any isolate. It simply indicates the need for further testing.

2 Scope

Many laboratories use FDA-approved commercial systems for identification of microorganisms, because they lack the confidence in or resources for performing in-house validations of alternative methods. Use of such panels has resulted in greater standardization and more accurate taxonomic identifications, albeit at relatively higher cost. M35-A—*Abbreviated Identification of Bacteria and Yeast; Approved Guideline* provides well-documented, published studies to guide laboratories in choosing rapid, reliable, and often less expensive alternatives for laboratories that otherwise could not adopt such methods. This guideline shares the experience and expertise of other microbiologists for reporting bacterial and yeast identifications more rapidly than by traditional methods.

3 Definitions[a]

Algorithm, *n* – A set of rules for solving a problem in a finite number of steps, as for finding the greatest common divisor.

Quality control, *n* - The operational techniques and activities that are used to fulfill requirements for quality

4 Indications for Performing Rapid Microbial Identification Tests

Accurate, significant, and clinically relevant information from the laboratory need not always require complex test methods. Some level of identification is indicated for any organism associated with an infectious process and isolated from a properly collected clinical specimen taken from the infected site. The determination of whether the isolated organism is actually contributing to the infectious process is based on its association with polymorphonuclear leukocytes in the gram stain; the numbers and variety of organisms present in the culture; the types of organisms historically causing infection in that site; and the knowledge and experience of the microbiologist and the clinician evaluating the results.

Whenever a microorganism is determined to require identification, rapid methods, if available, can be employed for initial characterization. Whether conventional identification must follow to verify the initial results is based on the potential use of the information. Certain organisms from normally sterile body sites, organisms of importance to infection control practitioners or involved in nosocomial outbreaks, and other important organisms, as determined by individual laboratory deliberations, should be considered for further testing to validate the rapid results. In most cases, however, confirmatory identification need not be done. The identifications derived from the procedures outlined in this guideline are intended to be reported directly, not as "presumptive" identifications, since the likelihood of correct identification (>95%) is comparable to that of other conventional and automated methods.

Quality control procedures for each rapid test should follow the minimum criteria required by regulatory or licensure agencies. At a minimum, a positive and negative control should be provided for each test procedure. The frequency of testing may vary.

5 Organisms Included in the Document

5.1 Aerobes and Facultative Anaerobes

Gram-negative

Escherichia coli
Haemophilus influenzae
Moraxella catarrhalis
Proteus species
Pseudomonas aeruginosa

Gram-positive

Enterococcus species
Staphylococcus aureus
Streptococcus agalactiae (Group B)
Streptococcus pneumoniae
Streptococcus pyogenes

[a] Some of these definitions are found in NCCLS document NRSCL8—*Terminology and Definitions for Use in NCCLS Documents.* For complete definitions and detailed source information, please refer to the most current edition of that document.

Yeast species

Candida albicans
Candida glabrata
Cryptococcus neoformans

5.2 Anaerobes

Gram-negative

Bacteroides fragilis group
Bacteroides ureolyticus
Prevotella species
Prevotella intermedia
Porphyromonas species
Bilophila wadsworthia
Fusobacterium nucleatum
Veillonella spp.

Gram-positive

Peptostreptococcus
Clostridium difficile
Clostridium perfringens
Clostridium septicum
Clostridium sordellii
Clostridium tetani
Propionibacterium acnes

6 Organisms Specifically Excluded from the Document[4-9]

Due to the often sensitive or legal nature of the laboratory identification of *N. gonorrhoeae*, rapid methods for neither *N. gonorrhoeae* nor *N. meningitidis* have been included in this document. Organisms for which no single set of simple tests can be recommended, including nonfermentative gram-negative rods other than *P. aeruginosa*, Enterobacteriaceae such as *Salmonella* and *Shigella*, and others not listed above, do not lend themselves to abbreviated identification schemes.

7 *Escherichia coli*[10-21]

Colonies must be gram-negative, nonswarming, spot indole positive, and oxidase negative (Figure 1) (see Appendix A1 and A2). Hemolytic colonies on sheep blood agar (BAP) are *E. coli*. Colonies that are nonhemolytic on blood agar and lactose-positive on either MacConkey or eosin methylene blue agar are identified as *E. coli* by a negative rapid pyrrolidonyl arylamidase (PYR) test. Colonies that are nonhemolytic on BAP and lactose negative, are identified as *E. coli* by a positive rapid methylumbelliferyl-beta-D-glucuronidase (MUG) test (see Appendix A3). Limitations: (1) Occasional *Shigella* can be indole-positive and MUG-positive, so lactose-negative isolates from blood, feces, or other gastrointestinal sites should be tested by alternative methods; (2) *E. coli* O157:H7 is MUG-negative; conventional tests should be performed to identify this species. A positive MUG test, however, may be used as a preliminary screening test to rule out *E. coli* O157:H7; (3) Rare *E. coli* O157:H7 may be MUG-positive, such as those seen in a 1998 food-borne outbreak involving a rare strain of *E. coli* O157:H7 that was urease- and MUG-positive; however 99% of O157:H7 isolates are MUG-negative; and (4) In a few geographic areas, *M. morganii* and *Proteus* spp. can be beta-hemolytic on BAP. However, since they do not ferment lactose, a negative MUG test can be used to separate them from MUG-positive *E. coli*. When

mixed cultures are observed on MacConkey or EMB agar, care must be taken that the colony picked from the blood agar plate for rapid testing corresponds to the colony with the appropriate lactose reaction on the differential medium.

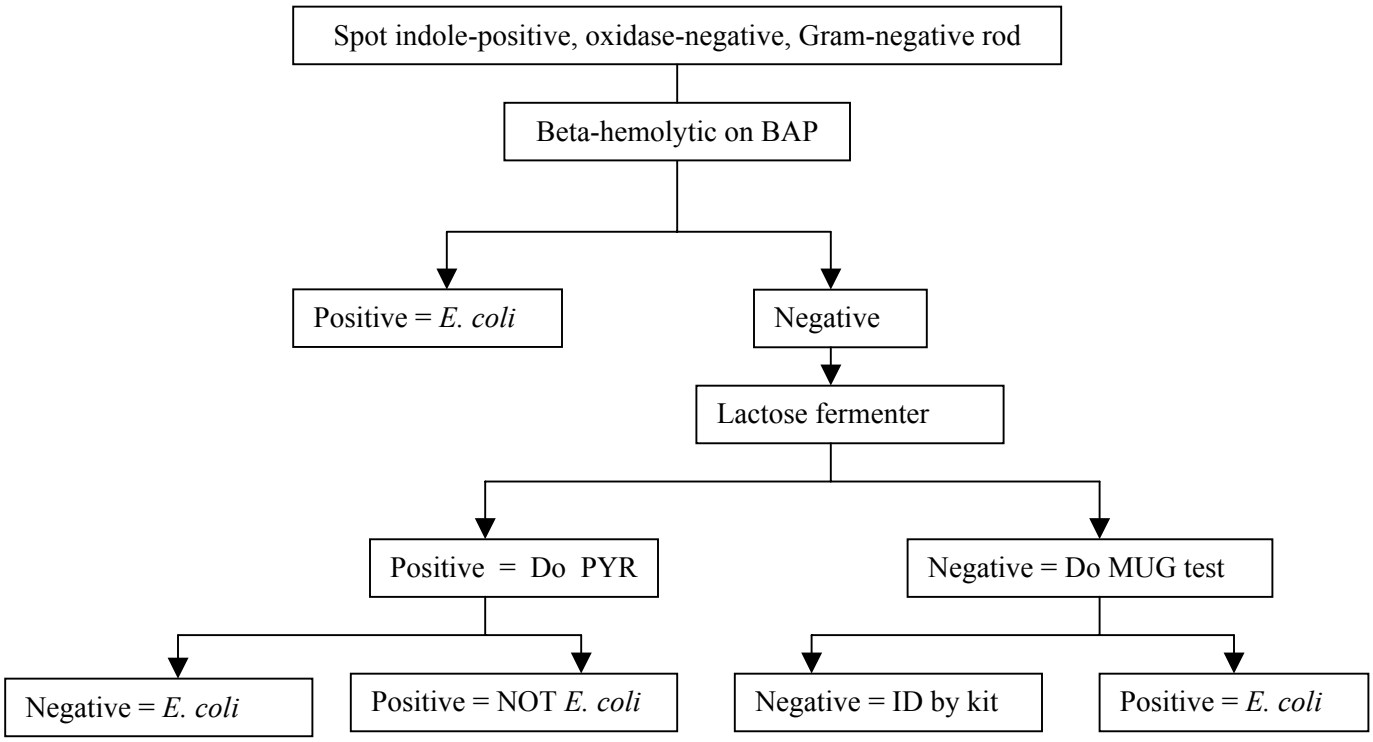


Figure 1. Algorithm for Identification of *E. coli*

8 *Haemophilus influenzae*[22]

For isolates from respiratory or cerebral spinal fluid (CSF) specimens; gram-negative small rods or coccobacilli; colonies >1 mm diameter on chocolate agar in 5% CO_2, but not on sheep blood agar, in 24 hours; or satelliting growth around other colonies on sheep blood agar and a negative rapid test for porphyrin synthesis, the delta-aminolevulinic acid test identifies *H. influenzae* (see Appendix A5). Limitation: *H. haemolyticus* cannot be differentiated from *H. influenzae* except by the beta-hemolysis of *H. haemolyticus* on rabbit or horse blood agar. Since there are no reports of *H. haemolyticus* causing disease, misidentifying this species as *H. influenzae* may result in over-treatment of some patients. To avoid misidentification of *Francisella tularensis*, do not use this algorithm if growth is poor in 24 hours. *F. tularensis* is negative in the porphyrin test but does not satellite around staphylococci and grows poorly on chocolate agar in 24 hours.

9 *Moraxella catarrhalis*[23,24]

For gram-negative diplococci that are oxidase positive (see Appendix A2) and grow on both chocolate and sheep blood agars, a positive rapid butyrate esterase or tributyrin test identifies *M. catarrhalis*. Limitation: Most other Moraxella species are positive for butyrate esterase, but are coccobacilli, not diplococci.

10 *Proteus* species [25-29]

Colonies must display swarming growth. *P. mirabilis* is indole-negative and *P. vulgaris* is indole-positive (see Appendix A1). Limitation: Ampicillin-resistant, indole-negative isolates should be identified using maltose or ornithine, as all *P. penneri* and some *P. mirabilis* are ampicillin-resistant (Table 1). Alternatively, one could report *P. mirabilis/penneri* or "indole-negative *Proteus"* as an alternative to further identification of indole-negative *Proteus* spp.

Table 1. Differentiation of *Proteus penneri* and *Proteus mirabilis*

Species	Maltose	Ornithine
P. penneri	+	-
P. mirabilis	-	+

11 *Pseudomonas aeruginosa* [30-37]

Oxidase-positive colonies (see Appendix A2) of a gram-negative rod displaying typical smell (Concord grapes or corn tortilla), recognizable colony morphology (metallic or pearlescent, rough, pigmented, or extremely mucoid) are *P. aeruginosa*. Limitations: Rare *Aeromonas* isolates may resemble *P. aeruginosa* (lacking the typical smell) but can be distinguished by spot indole (see Appendix A1), which is positive for *Aeromonas* and negative for *P. aeruginosa*. Isolates from cystic fibrosis patients should be evaluated carefully, because atypical morphotypes of *P. aeruginosa* are common and some *Burkholderia cepacia* isolates from such patients can resemble *P. aeruginosa.*

12 *Staphylococcus aureus* [38-58]

For white to yellow, creamy, opaque colonies on sheep blood agar plate (BAP) that are gram-positive cocci in clusters, catalase-positive (see Appendix A4), the conventional slide coagulase (clumping factor) and rapid four-hour tube coagulase tests will differentiate *S. aureus* (coagulase-positive) from non-*S. aureus* isolates. Limitations: *S. schleiferi* and *S. lugdunensis* may also be coagulase-positive by the slide test. Such strains are expected to comprise much less than 5% of all coagulase-positive isolates, conforming to the >95% accuracy of the methods presented. The tube test should be read at least hourly for up to four hours due to clot lyses. Some strains of *S. aureus* produce a staphylokinase that dissolves the clot, giving a false-negative result. The final reading of the tube test at 35 °C should be made at 4 hours, since some animal strains, rarely isolated from humans (*S. intermedius* and *S. hyicus)* may yield positive tube coagulase tests in 12 to 24 hours. Rare *S. aureus* strains require >4 hours to clot the tube coagulase reagent; therefore, this rapid algorithm cannot be used. If a suspicious isolate is coagulase-negative after the first 4 hours, incubating the coagulase tube for 20 additional hours at room temperature is less likely to yield false-negative results. Only sterile EDTA rabbit plasma is recommended for coagulase testing.

Positive results from commercial latex agglutination kits are accurate for identification of *S. aureus*. However, *S. saprophyticus* may yield a positive latex agglutination test in some cases, although the isolate will be negative in the conventional clumping factor slide test and tube coagulase test; thus, commercial agglutination tests should not be used to identify non-hemolytic staphylococci from urine. Some kits performed less well with methicillin-resistant *S. aureus* (MRSA) than with methicillin-sensitive *S. aureus* (MSSA). A positive result in the rapid (four-hour) thermonuclease test accurately identifies *S. aureus*.

13 *Enterococcus* species [59-73]

For colonies >1 mm that are non-beta hemolytic on sheep blood agar, catalase-negative (see Appendix A4), with gram stain morphology of positive cocci or coccobacilli in pairs and chains, a positive

pyrrolidonyl arylamidase (PYR) test identifies *Enterococcus* spp. Limitations: Occasional *Lactococcus garvieae* may be misidentified as *Enterococcus* spp.

14 *Streptococcus agalactiae* (Group B)[69,74-92]

S. agalactiae are gram-positive cocci in pairs and chains, catalase-negative (see Appendix A4), usually displaying a narrow zone of beta hemolysis with a soft periphery on sheep BAP. Rapid (two to four hours) hippurate hydrolysis tests or a rapid (30 minutes) spot test for CAMP factor (see Appendix A7) are both accurate for identification of this organism. Commercial particle agglutination tests approach 100% accuracy. Limitation: Beta-hemolytic enterococci may be hippurate-positive (see Appendix A6); enterococci can be differentiated by a positive PYR test. Non-hemolytic *S. agalactiae* exist, but they could not be identified using rapid hippurate hydrolysis, since many viridans streptococci are also hippurate positive.

15 *Streptococcus pneumoniae*[93-104]

S. pneumoniae are gram-positive cocci in pairs and chains, catalase-negative (see Appendix A4), and alpha hemolytic on sheep BAP. Colonies are usually transparent, slightly mucoid, or flattened (resemble a checkers playing piece), not peaked. Positive bile (either 2% or 10%) solubility (see Appendix A8) on the plate is accurate for identification of this organism. Limitation: Some *S. pneumoniae* may not be bile-soluble.

16 *Streptococcus pyogenes*[62,65,69,70,75,79,91,105-111]

S. pyogenes are gram-positive cocci in pairs and chains, catalase-negative (see Appendix A4), displaying beta-hemolytic colonies >0.5 mm in diameter on sheep BAP after 24 hours incubation. Colonies are usually dry, peaked, or convex with a sharp periphery to the zone of hemolysis. A positive PYR test identifies *S. pyogenes*. Serogrouping by particle agglutination approaches 100% accuracy. Limitation: Beta-hemolytic enterococci are also PYR-positive; colony morphology must be carefully evaluated. Large (>1 mm), moist colonies with a softer periphery of the zone of beta hemolysis should be identified using another method.

17 *Candida albicans*[112-133]

For a colony suspected of being a yeast, microscopic confirmation is necessary. *C. albicans* are seen as oval-shaped, budding yeast cells (the microscopic step—either gram stain or wet preparation—is very important and must not be skipped). The germ tube test (see Appendix A9), properly interpreted, identifies *C. albicans* within three hours. Human serum, because of its inherent risks, is not recommended for this test. Germ tubes detected after >3 hours incubation should not be considered, since yeast other than *C. albicans* share this trait. Colonies less than 48 hours old on blood-containing medium that exhibit mycelial projections into the agar, called "star-like" or "feet," are identified as *C. albicans*. Rapid enzymatic tests (N-acetyl-beta-D-galactosamide, methylumbelliferyl-N-acetyl-beta-D-galactosaminide, or L-proline-beta-naphthylamide) will also identify *C. albicans*, although some commercial products may require that colonies to be tested be grown on antibiotic-free medium (see product inserts). Limitations: *C. dubliniensis*, a recently recognized species often isolated from the oral cavities of AIDS patients and thought to develop antifungal resistance readily, may not be distinguished easily from *C. albicans*. Most *C. dubliniensis* fail to grow at 45 °C, whereas most *C. albicans* will grow at that temperature, a test that can help distinguish between them. *C. tropicalis* may rarely display a fringed type colony after 24 hours that may be mistaken for "feet."

18 *Candida (Torulopsis) glabrata*[112-133]

C. (Torulopsis) glabrata are seen as small yeast colonies on 5% sheep blood agar. Small (smaller than *C. albicans*), circular yeast cells are observed microscopically. Colonies are larger and growth is more luxuriant on eosin methylene-blue agar (EMB) than on blood agar at 24 and 48 hours. It is the only yeast that ferments trehalose rapidly at 42 °C. Growth at 42 °C is not used as an identifying characteristic, but ability to ferment trehalose at 42 °C is unique. This characteristic is determined by using a rapid three-hour tube test (commercial reagents only). A rapid (one-hour) assimilation test (see Appendix A10) performed at 35 °C using a cycloheximide-containing medium is also useful for identifying this species. Limitation: Occasional strains of *C. tropicalis* and other species may also yield positive results, so colony and cellular morphology should be evaluated carefully.

19 *Cryptococcus neoformans*[112-133]

C. neoformans display spherical, budding yeast cells. Colonies are nonpigmented on Sabouraud or blood agar and may be mucoid but do not necessarily exhibit a mucoid phenotype. A rapid (30 minutes to 4 hours) phenol oxidase test by commercial disk (caffeic acid disk) identifies *C. neoformans*. The disk must not be over-moistened, as this can cause false-positive results. Additionally, the test is less accurate with colonies taken directly from glucose-containing media such as Sabouraud dextrose agar. This species is also urease-positive, often turning positive within 30 minutes, although that characteristic alone is not sufficient for accurate identification.

20 Anaerobes[134-143]

Using gram-stained cellular morphology, growth characteristic on primary agar (supplemented anaerobic blood agar [BAP], laked sheep blood agar with kanamycin and vancomycin [LKV], Bacteroides bile esculin agar [BBE]), and spot tests only, several species, groups, or genera of anaerobes can be presumptively identified with enough accuracy for routine purposes. Colonies from anaerobically incubated original plates must be subcultured to anaerobic blood agar for anaerobic incubation, to yield a pure culture and isolated colonies, and to chocolate agar for 5 to 7% CO_2 incubation. Chocolate agar is essential to rule out *Haemophilus* spp., which grow on anaerobic blood agar incubated anaerobically but not on sheep BAP incubated in CO_2. Those isolates that fail to grow in CO_2 are considered to be anaerobes, for which the following criteria can be used. Of several choices, only the 1% paradimethylaminocinnamaldehyde spot indole reagent has been validated for anaerobic bacteria.

Gram-negative anaerobic bacteria (Table 2): Large (>1 mm diameter), convex colonies on anaerobic BAP exhibiting similar-sized dark (gray or black) colonies on BBE and seen as regular gram-negative rods on gram stain, are identified as *B. fragilis* group. Breadcrumb or opalescent colonies on anaerobic BAP that fail to grow on BBE, are spot indole-positive, and exhibit thin, pointed, fusiform cells on gram stain are identified as *F. nucleatum*. Small (<1.0 mm diameter), translucent colonies on anaerobic BAP with corresponding tiny colonies on BBE after at least 48 hours incubation, often displaying a black dot in the center of the colony due to H_2S production, that are strongly catalase-positive (see Appendix A11), are identified as *Bilophila wadsworthia*.

On anaerobic BAPs, black-pigmented colonies (or those that fluoresce brick-red under long-wave ultraviolet [UV] light) on LKV with corresponding small, translucent or opaque colonies that consist of small coccobacilli are identified as *Prevotella* spp. If they are spot indole-positive, they can be identified as *P. intermedia*. Organisms with similar colony morphology on anaerobic BAP, brick-red fluorescence under UV light, and similar cellular morphology on gram stain but no growth on LKV, also indole-positive, are *Porphyromonas* spp. Flat, transparent colonies that pit the agar, are catalase-negative, urease-positive, and fail to grow on BBE are *B. ureolyticus*. Small (0.5- to 1.0-mm diameter) transparent-to-opaque colonies on anaerobic BAP that fluoresce red under UV light, fail to grow on BBE, and exhibit tiny, gram-negative diplococci on gram stain are *Veillonella* spp.

Gram-positive anaerobic bacteria (Table 3): Organisms with large (>2 mm diameter), irregular colonies exhibiting a double zone of beta hemolysis on anaerobic BAP, no growth on BBE, catalase-negative, and large, boxcar-shaped, blunt-ended, gram-positive or gram-variable cells without spores on gram stain are *C. perfringens*. An anaerobic organism that shows smoothly swarming growth over the surface of the BAP, which is spot indole- and catalase-negative, and displays thin gram-positive rods with swollen, subterminal spores on gram stain is *C. septicum*. An anaerobic organism exhibiting similar, but more slowly moving swarming growth that is spot indole-positive, catalase-negative, and whose gram-positive cells exhibit swollen terminal spores (tennis-racquet shaped) is *C. tetani* (a laboratory may never recover this species, particularly since the diagnosis is a clinical one). Another swarming *Clostridium* species with serpentine-edged colonies and slowly spreading growth that is spot indole- and rapid urease-positive, catalase-negative, with subterminal spores is *C. sordellii*. Organisms with small (approximately 1 to 2 mm diameter), enamel-white, opaque colonies on anaerobic BAP that fail to grow on BBE, are spot indole-positive, catalase-positive, and reveal pleomorphic, coryneform rods on gram stain are *Propionibacterium acnes*. *C. difficile*, a thin, spore-forming, gram-positive rod with rarely visible subterminal spores, can be identified when a selective agar (cycloserine-cefoxitin fructose agar [CCFA]), is used. The colonies on CCFA are large and flattened. Corresponding colonies on anaerobic BAP fluoresce chartreuse under UV light and exhibit a strong, cow manure, barnyard-like smell. Anaerobic gram-positive cocci are usually either *Peptostreptococcus* species or a member of one of the newly named genera (*Peptoniphilus, Schleiferella, Anaerococcus, Finegoldia, Micromonas*). Until the new genera are better recognized, it is still prudent to report them as "*Peptostreptococcus* species" or "anaerobic gram positive cocci."

Table 2. Abbreviated Identification of Anaerobic Gram-Negative Bacteria

Identification	Blood/LKV Colony Morphology	Cell Morphology	Bacteroides Bile Esculin Colony Morphology	Indole Reaction
***Bacteroides fragilis* group**	Large, convex	Regular	Large, convex, gray-black	Not Done
Bacteroides ureolyticus	Translucent, pitting the agar (catalase-negative with 15% H_2O_2)	Tiny rods, coccobacilli	No growth	Negative
Bilophila wadsworthia	Tiny, translucent (catalase +++ with 15% H_2O_2)	Regular to filaments	Translucent with black center at 72 hours	Negative
Fusobacterium nucleatum	Opalescent, breadcrumb	Fusiform, thin pointed	No growth	Positive
***Porphyromonas* spp.**	Small, translucent or opaque, fluoresce brick-red on Brucella agar	Tiny coccobacilli	No growth	Positive
Prevotella intermedia	Small, translucent or opaque, fluoresce brick-red on LKV or Brucella agar	Tiny coccobacilli	No growth	Positive
***Prevotella* spp.**				Negative
***Veillonella* spp.**	Small, transparent or opaque, fluoresce brick-red on Brucella agar	Tiny diplococci	No growth	Negative

Table 3. Abbreviated Identification of Anaerobic Gram-Positive Bacteria

Identification	Blood Agar Colony Morphology	Cell Morphology	Indole Reaction
C. difficile	Large, flat colonies; barnyard (cow manure) smell; chartreuse fluorescence	Thin rods, rare spores	Negative
C. perfringens	Large, irregular-shaped, double zone beta-hemolysis	Boxcar, large, square rods	Not Done
C. septicum	Smoothly swarming	Thin rods, subterminal spores	Negative
C. sordellii	Very large, lobate, irregular, flat	Thin rods, subterminal spores	Positive
C. tetani	Smoothly swarming but slow growing	Swollen terminal spores	Positive
***Peptostreptococcus* spp.**	Small, peaked, circular	Cocci, pairs and chains	Not Done
Propionibacterium acnes	Small, opaque, enamel-white, circular (catalase+ with 15% H_2O_2)	Coryneform rods	Positive

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Candida albicans, Candida (Torulopsis) glabrata, Cryptococcus neoformans

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Appendix. Test Procedures

Protocols for Some of the Noncommercial Tests Used for Abbreviated Identifications

(Protocols included here do not contain all of the required contents of an NCCLS-formatted procedure or a 16-point CLIA procedure outline, but key information is presented. Formal procedures should be prepared by each laboratory using its preferred format, which would include appropriate reporting guidelines. Protocols for many of these tests can be found in the *Clinical Microbiology Procedures Handbook,* Vols. 1 and 2 (Isenberg HD, ed. 1992 and Supplements. ASM Press, Washington, DC). Reagents can be made easily within the laboratory. Quality control strains must be tested with each lot of reagents and thereafter in accordance (in the U.S.) with national CLIA regulations or the package inserts for commercial reagents. Not all QC protocols are included here.)

A1. Spot Indole Test

A1.1 Principle

The conversion of tryptophan to indole by tryptophanase is indicated by a color change following addition of an aldehyde indicator of the presence of indole.

A1.2 Reagents

Use either 5% (w/v) *p*-dimethylaminobenzaldehyde (this is not the same reagent as Kovac's, which uses an alcohol-acid base) or 1% paradimethylaminocinnamaldehyde in 10% (vol/vol) concentrated HCl. Store at 4 °C in a dark bottle. The reagent is stable for one year from the date of preparation.

NOTE: Only the paradimethylaminocinnamaldehyde reagent has been validated for use with anaerobic bacteria.

A1.3 Procedure

(1) Moisten a piece of Whatman No. 1 filter paper with the reagent.

(2) Using a wooden stick or bacteriological loop, rub a portion of a colony from sheep blood agar onto a small area of the moistened filter paper. The growth medium must contain an adequate amount of tryptophan for optimal production of trytophanase.

> **NOTE:** Do not use colonies from media containing dyes, Mueller-Hinton agar, or high glucose-content agar for the spot indole test. Since indole can diffuse through the media, colonies <5 mm from morphologically distinct adjacent colonies should not be chosen for testing.

A1.4 Interpretation

A1.4.1 Observe for the development of a brown-red to purple-red color (benzaldehyde reagent) or blue color (cinnamaldehyde reagent) within 20 seconds, indicating the presence of indole.

A1.4.2 The formation of white-yellow color indicates an absence of indole within the bacterial colony.

A1.5 Limitation

Detectable indole will diffuse to adjacent colonies that are within 5 mm of a 2- to 3-mm diameter colony, giving false-positive results.

A2. Spot Oxidase Test

A2.1 Principle

Cytochrome oxidase, an enzyme in the oxidation-reduction pathway, oxidizes the substrate tetramethyl-*p*-phenylenediamine dihydrochloride to indophenol, a purple-colored end product.

A2.2 Reagents

Tetramethyl-*p*-phenylenediamine dihydrochloride (1% w/v) in sterile distilled water, prepared fresh each day of use. Once the pale purple color begins to darken, the reagent should be discarded.

A2.3 Procedure

(1) Moisten a piece of Whatman No. 1 filter paper with a drop of the reagent.

(2) Using a wooden stick or plastic or platinum bacteriological loop, rub a portion of a nonpigmented colony from growth on any suitable agar medium (not MacConkey or other purple agar) onto a small area of the moistened filter paper.

A2.4 Interpretation

A2.4.1 Observe for the development of a dark blue to purple color within ten seconds, indicating the presence of cytochrome oxidase enzyme. Color that develops after ten seconds should not be considered.

A2.4.2 Formation of white-yellow color or absence of color indicates failure to detect the enzyme.

A2.5 Limitation

Some fastidious organisms may require several subcultures to develop sufficient enzyme for detection by this method, but that should not be a consideration for the organisms included in this guideline. Nickel-base alloy wires containing chromium and iron used to rub the colony paste onto the filter paper may cause false-positive results.

A2.6 Quality Control

Test a fresh colony of known *Neisseria* species or *P. aeruginosa* (positive) and *E. coli* (negative).

A3. Rapid Methylumbelliferyl-beta-D-glucuronidase (MUG) Test

A3.1 Principle

E. coli and a number of other bacteria produce the enzyme beta-D-glucuronidase, which hydrolyzes beta-D-glucopyranosiduronic derivatives to aglycons and D-glucuronic acid. The substrate 4-methylumbelliferyl-beta-D-glucuronide is hydrolyzed by the enzyme to yield the 4-methylumbelliferyl moiety, which fluoresces blue under long-wave UV light.

A3.2 Reagent

(1) M/15 Sorensen's phosphate buffer, pH 7.5

- M/15 Na_2PO_4 (Solution A)
  a) 4.730 g Na_2HPO_4
  b) qs to 500 mL with sterile distilled water

- M/15 KH_2PO_4 (Solution B)
  a) 4.535 g KH_2PO_4
  b) qs to 500 mL with sterile distilled water

- Combine 85 mL of Solution A and 15 mL of Solution B.

  NOTE: Check final pH; adjust to pH 7.5 with either Solution if necessary (Solution A to make more alkaline; Solution B to acidify).

(2) Dissolve 50 mg 4-methylumbelliferyl-ß-D-glucuronide in 10 mL 0.05 M Sorensen's phosphate buffer, pH 7.5.

(3) Prepare a 1:16 dilution of the stock MUG and add 1.25 mL to a vial containing 50 sterile, 6 mm-diameter paper disks. Allow to thoroughly saturate the disks so no fluid remains in the vial (approximately 0.78 µg/disk).

(4) Spread the saturated discs onto a metal screen and place in a dry environment at ambient temperature until the disks are completely dry.

(5) Store disks at –20 °C in a sealed container protected from light for up to one year or at 4 °C for no more than one month from date of preparation.

A3.3 Procedure

(1) Wet a disk with one drop of water.

(2) Using a wooden stick or bacteriological loop, roll a portion of a colony from a suitable agar medium onto the disk.

(3) Incubate at 35 °C in a closed container for up to two hours.

A3.4 Interpretation

A3.4.1 Observe disks using a long-wave (366 nanometers) ultraviolet light. A positive reaction is indicated by an electric blue fluorescence.

A3.4.2 Lack of fluorescence indicates a negative reaction.

A4. Catalase Test for Aerobic Bacteria

A4.1 Principle

Some aerobic bacteria produce a catalase enzyme that hydrolyzes hydrogen peroxide into water and oxygen (bubbles).

A4.2 Reagent

Hydrogen peroxide (3%, aqueous). Dilute 30% (v/v) hydrogen peroxide 1:10 in sterile distilled water.

A4.3 Procedure

(1) Touch the center of an isolated colony with a wooden stick and transfer some of the cell paste onto a clean glass slide so that it is visible. Be careful to avoid picking up any of the agar medium. (Blood may produce false-positive reaction due to peroxidase.)

(2) Place a drop of 3% hydrogen peroxide directly onto the cell paste and observe closely for the immediate formation of bubbles. Holding the slide over a dark background is helpful.

A4.4 Interpretation

A4.4.1 The immediate appearance of bubbles indicates a positive reaction.

A4.4.2 No bubbles, or the slow elaboration of a few bubbles after 20 seconds, indicates a negative reaction.

A4.5 Limitations

Selecting colonies with some metal bacteriological loop materials will yield false-positive results. In fact, platinum loops do not yield false-positive results.

A4.6 Precautions

Rapid evolution of oxygen may generate droplet or aerosol formation. The catalase reaction does not affect organism viability.

A4.7 Quality Control

Perform the procedure on a fresh 18-hour subculture of *Staphylococcus aureus* (positive reaction) and *Streptococcus pyogenes* (negative reaction).

A5. Rapid Porphyrin Synthesis (Delta-aminolevulinic Acid; ALA) Test

A5.1 Principle

The enzymes necessary to synthesize porphyrins (X-factor or hemin) from aminolevulinic acid are lacking in organisms that require hemin for growth, such as *H. influenzae*. Bacteria that can utilize the ALA as a substrate produce porphyrins, which fluoresce orange-red under UV light.

A5.2 Reagent

Add 33.5 mg delta-aminolevulinic acid and 20 mg $MgSO_4 \bullet 7H_2O$ to 100 mL of 0.1 M Sorensen's buffer, pH 6.9. Dispense in 0.5-mL amounts and store at –20 °C in a dark bottle. Alternatively, commercial reagent-impregnated disks are available.

100 mL 0.1 M Sorensen's phosphate buffer, pH 6.9

1. 7.1 g Na_2HPO_4 in 500 mL deionized water.
2. 6.8 g KH_2PO_4 in 500 mL deionized water.

3. Combine 55.4 mL of Na_2HPO_4 solution and 44.6 mL of KH_2PO_4. Check pH; adjust with either solution if necessary (Na_2HPO_4 to make more alkaline; KH_2PO_4 to acidify).

A5.3 Procedure

(1) Place the filter paper on a clean, dry surface.

(2) Using a wooden stick or a bacteriological loop, transfer growth from a suitable agar medium onto the filter paper.

(3) Slowly pour reagent drop wise over each spot.

(4) Incubate the filter paper in a closed environment at 35 °C for two hours.

(5) Observe reactions for red fluorescence under long-wave (366 nanometers) ultraviolet light.

A5.4 Interpretation

A5.4.1 The presence of a red fluorescence indicates a positive test (organism is hemin-independent, such as *H. parainfluenzae*).

A5.4.2 Lack of red fluorescence indicates a negative result (organism cannot convert ALA to hemin, such as *H. influenzae)*.

A5.5 Quality Control

Test colonies from overnight subcultures on chocolate agar of *H. influenzae* (negative fluorescence) and *H. parainfluenzae* (positive fluorescence).

A6. Rapid Hippurate Hydrolysis Test

A6.1 Principle

Hydrolysis of sodium hippurate by Group B streptococci produces benzoic acid and glycine. When ninhydrin (a protein detector) is added to hydrolyzed sodium hippurate, it reacts with the amino acid glycine and produces a deep blue color. Ninety-nine percent (99%) of Group B streptococci hydrolyze hippurate while other groups of beta streptococci do not.

A6.2 Reagents

A6.2.1 Sodium Hippurate (1% w/v)

Add 1 gram sodium hippurate to 100 mL distilled water. Mix well to dissolve completely. Dispense in capped tubes in 0.4-mL amounts. Freeze at –20 ^{o}C until needed. Shelf life: until quality control no longer performs appropriately.

A6.2.2 Ninhydrin

Mix 50 mL acetone and 50 mL 1-butanol thoroughly in a dark glass bottle. Add 3.5 g ninhydrin, mix, and store at room temperature. **Caution: Flammable.**

A6.2.3 Quality Control

Test a fresh subculture of *Streptococcus agalactiae* and *S. sanguis* growing on soybean casein digest agar with 5% sheep blood or equivalent monthly. The *S. agalactiae* should yield a positive result and the *S. sanguis* should yield a negative result. If the quality control reagents fail to show correct reactions, repeat the test with fresh isolates. If QC is still incorrect, discard all reagents, prepare fresh reagents, and retest.

A6.3 Procedure

(1) Defrost one tube containing 0.4 mL sodium hippurate reagent for each organism to be tested.

(2) Use a wooden stick or bacteriological loop to inoculate the sodium hippurate with a heavy inoculum of the suspected organism from a fresh subculture on blood agar. (Take care not to pick up pieces of agar, as the protein present will cause a weak positive reaction).

(3) Emulsify the organism in the substrate.

(4) Incubate tubes for two hours in a 37 ^{o}C heating block or water bath.

(5) Add 0.2 mL of the ninhydrin solution and mix gently; do not shake or vigorously agitate the tubes.

(6) Return tubes to the heating block or water bath for ten minutes. Incubation for longer than 30 minutes may result in false-positive readings.

(7) Observe color.

A6.4 Interpretation

A6.4.1 Organisms such as *S. agalactiae* that are able to hydrolyze sodium hippurate produce a deep-blue color (about the color of crystal violet), indicating a positive result.

A6.4.2 A negative reaction results in a colorless broth or faint tinge of purple in the broth.

A7. Rapid Spot CAMP Test

A7.1 Principle

S. agalactiae produces a diffusible protein (CAMP factor) that acts synergistically with the beta-lysin elaborated by *S. aureus* to produce a zone of enhanced hemolysis. The overnight CAMP test relies on elaboration of the two toxins during growth. The rapid spot test utilizes an extract containing staphylococcal beta-lysin that acts directly with the CAMP factor that is diffused into the medium around a young colony (less than 24 hours of incubation) of *S. agalactiae* to show enhanced hemolysis within 30 minutes after addition of the staphylococcal reagent to the colony area.

A7.2 Reagents

Spot test reagent – Staphylococcal Beta lysin

(1) Inoculate two 5-mL tubes of brain-heart infusion broth with a small amount of growth from a fresh subculture of *S. aureus* ATCC 25923.

(2) Incubate overnight (shaking is optional) at 35 °C in air.

(3) Working in a laminar flow biological safety cabinet, mix the two broth suspensions. Sterilize by filtration using a 0.45-µm cellulose-acetate filter.

(4) Aliquot 1-mL portions of the filter-sterilized broth (now the spot CAMP test reagent) into small tubes. Label each tube with the name of reagent (spot CAMP reagent), date of preparation, preparer's initials. Label with a six-month expiration date. Store at -20 °C or lower temperature.

(5) Quality control

(a) Defrost one tube of the new reagent before the previous tube has expired or been depleted.

(b) Parallel test both the new reagent and the old reagent on a fresh colony of Group B streptococcus, a beta-hemolytic *Enterococcus* species (or a nonhemolytic *Enterococcus* spp. if a beta-hemolytic one is unavailable), and fresh colony of *S. aureus*. Place another drop in an uninoculated area of the plate to test for autohemolysis.

(c) Observe for enhancement of hemolysis in the area immediately adjacent to the colony, indicating a positive result, as in the procedure (below). Expected results are:

Table A1. Expected Reaction

Organism	Reaction
Group B streptococci	Positive
Enterococcus spp.	Negative
Staphylococcus aureus	Negative
No colony	No hemolysis

(d) If the reagent does not yield expected results, discard all frozen tubes and make a fresh batch using a different subculture of the *S. aureus*.

(6) Store defrosted reagent in the refrigerator at 4 to 6 °C for no longer than two weeks. Do not refreeze.

A7.3 Procedure

(1) Place one drop or 10 µL loopful of reagent next to a suspected group B streptococcal colony growing on casein soy digest with 5% sheep blood (after overnight incubation, in 5% CO_2). The liquid may touch or even engulf the colony.

(2) Incubate the plate in air at 35 °C (right side up to prevent the spot CAMP reagent from running over the plate's surface) for 20 minutes.

(3) Examine using transmitted light for a zone of enhanced hemolysis next to the colony. Reincubate for up to 30 minutes if reaction is initially negative. Use a hand lens if necessary to examine the plate.

Refrigeration after the initial incubation period may enhance the reaction.

A7.4 Interpretation

A7.4.1 The presence of a clear zone of enhanced hemolysis only where the diffused, slight hemolysis overlaps is considered a positive reaction.

A7.4.2 Lack of hemolysis near the colony in the presence of the spot CAMP reagent (staphylococcal hemolysin) is a negative reaction.

A7.5 Quality Control

See A7.2 (5) above.

A8. Rapid Bile Solubility Test

A8.1 Principle

The active autocatalytic enzyme of *Streptococcus pneumoniae* is enhanced by bile or sodium deoxycholate. The 10% bile w/v (oxgall) reagent may yield more rapid reactions, but both 2% and 10% have been used successfully.

A8.2 Reagents

Use either 2% (w/v) or 10% bile or oxgall. Add 1 g sodium deoxycholate to 9.0 mL of sterile, distilled water (10% solution), or dilute this solution 1:5 in water to make a 2% solution. Store at 4 °C in a dark bottle. The reagent is good for one year from the date of preparation, but a new lot should be prepared if it becomes cloudy or contaminated.

A8.3 Procedure

A8.3.1 Plate Method

(1) Place a drop of the bile reagent directly onto an isolated colony to be tested.

(2) Without tipping the plate, incubate at room temperature or at 35 °C for 15 minutes or until the liquid has evaporated or adsorbed into the medium. Placing the plate on top of a 35 °C-heating block is an alternative to carrying it to an incubator.

(3) Observe carefully for a flattening of the colony. Be certain that the colony did not simply float away.

A8.3.1.1 Interpretation

(1) A flattened colony is a positive reaction, indicative of *S. pneumoniae.*

(2) An intact colony is a negative reaction, indicative of a non-pneumococcal organism.

A8.3.2 Tube Method

(1) Place 0.5 mL of bile reagent into a small, sterile tube. Place 0.5 mL sterile saline into another tube for a control.

(2) Inoculate enough test organism into each tube to create a slightly turbid suspension.

(3) Incubate at room temperature or at 35 °C for 5 to 15 minutes. Watch for a decrease in turbidity in the tube containing the bile salt suspension relative to the control tube.

A8.4 Interpretation

(1) Decreased turbidity or clearing of the suspension in the bile-containing tube is considered a positive bile solubility reaction and indicates the presence of an active catalytic enzyme such as that which *S. pneumoniae* produce.

(2) No decrease in turbidity of the suspension in the bile-containing tube is considered a negative bile solubility reaction and suggests that the organism is not *S. pneumoniae*.

A8.5 Quality Control

Test growth from an overnight subculture of *S. pneumoniae* (positive for autocatalysis) and a non-pneumoniae viridans streptococcus (does not flatten on agar or show clearing in broth) with each new lot number of bile reagent prepared.

A9. Germ Tube Test

A9.1 Principle

The blastoconidia of *Candida albicans* begin to produce mycelial elements in the presence of serum more rapidly than do most other yeast species. Unlike those of some other yeast species, the pseudohyphae of *C. albicans* possess a characteristic structure without a constriction between the mother cell and the hyphal element.

A9.2 Reagent

Purchase fetal or newborn calf serum or, if no other alternative exists, use outdated blood bank serum that has been tested and found free of HIV and hepatitis. If human serum is used, the entire lot must be validated for reactivity with known positive and negative controls before placing into use. Aliquot 0.5 mL each into small, sterile tubes with caps. The reagent is good for several years if frozen at –20 °C or colder. Bring serum to room temperature for testing.

A9.3 Procedure

(1) Inoculate a small amount of the yeast colony into a tube of serum. Incubate at 35 °C for up to three hours.

(2) After a minimum of 30 minutes of incubation, make a wet preparation of the suspension and examine under high dry (400x) magnification for the characteristic germ tube structures.

(3) Alternatively place a drop of the serum on a glass slide, emulsify a small amount of the organism in the serum, and apply a cover slip. Incubate the slide at 35 °C in a moist slide container for a minimum of two to a maximum of three hours.

(4) The slide can be removed for microscopic examination periodically and returned to the incubation chamber if germ tubes are not yet visible.

A9.4 Interpretation

A9.4.1 The presence of germ tubes (no constriction at site of origin) within two to three hours is a positive test, consistent with *C. albicans.*

A9.4.2 The absence of germ tubes does not rule out the identification of *C. albicans*, but additional methods must be used.

A9.5 Quality Control

Test a known *C. albicans* (produces germ tubes) and another yeast, such as *C. glabrata* (does not produce germ tubes) with each new lot number of serum.

A10. Rapid Trehalose Assimilation at 35 °C

A10.1 Principle

The rapid trehalose assimilation test is used as a rapid screen for *C. glabrata*, a small yeast that is germ tube-negative. The yeast is emulsified in trehalose broth with brom cresol green and incubated at 35 °C for one hour. *C. glabrata* utilizes trehalose rapidly and changes the indicator from blue to yellow. Cycloheximide inhibits new enzyme formation by the organism. If the expected result is not obtained, other assimilation tests or conventional methods should be used.

A10.2 Reagents

A10.2.1 Yeast Nitrogen Base (Double Strength)

Add 6.7 g yeast nitrogen base to 100 mL distilled water. Heat gently in a 56 °C-water bath to dissolve and sterilize by filtration. Add 20 mL yeast nitrogen base to 80 mL distilled water.

A10.2.2 Trehalose (40% w/v Solution)

Add 4 g trehalose to 10 mL distilled water. Heat gently in a 56 °C-water bath to dissolve and filter sterilize.

A10.2.3 Brom Cresol Green (0.08%)

Add 0.1 g brom cresol green to 7.1 mL 0.01N NaOH. Add this slowly to distilled water to bring total volume to 125 mL and sterilize by filtration.

A10.2.4 Cycloheximide (10,000 µg/mL)

Add 0.1 g cycloheximide (Actidione) to 10 mL distilled water. Heat gently in a 56 ºC-water bath to dissolve and filter sterilize. Dispense in 0.5-mL aliquots and store at –30 °C or below for up to six months.

A10.2.5 Working Solution of Trehalose Rapid Assimilation Broth

Mix together 8 mL of the 2x yeast nitrogen base, 1 mL of the 40% trehalose, 1 mL of the 0.08% brom cresol green, and 0.4 mL of the cycloheximide solution. Adjust the pH to 5.4 to 5.5. This solution can be refrigerated for up to one week.

For each organism to be tested, dispense 0.1 mL into a single well of a microtiter plate.

A10.3 Procedure

(1) Emulsify one or two colonies of the yeast from any primary medium into the broth. Cover the plate to prevent drying.

(2) Incubate the microtiter plate at 35 °C in a non-CO_2 atmosphere for one hour.

(3) Observe the color reaction.

A10.4 Interpretation

A10.4.1 **Positive**–A change in the broth color from blue to yellow.

A10.4.2 **Negative**–No change in the blue broth color in the allotted time.

A10.4.3 If the test is positive on a tiny yeast (pinpoint colonies on sheep blood agar after overnight incubation), report as *C. glabrata*. For negative reactions, other tests must be performed.

A10.5 Quality Control

Using the following organisms, verify the performance of the broth with each test performed.

Candida glabrata ATCC® 2001: positive-control; *Candida albicans* ATCC® 14053: negative-control.

A11. Catalase Test for Anaerobic Bacteria

A11.1 Principle

Some anaerobic bacteria produce a weak catalase enzyme that hydrolyzes hydrogen peroxide into water and oxygen (bubbles). The substrate must be more concentrated than the hydrogen peroxide used to detect the catalase of aerobic bacteria.

A11.2 Reagent

Hydrogen peroxide (15 % aqueous). Dilute 30% hydrogen peroxide 1:1 in sterile, type I reagent grade water.

A11.3 Procedure

(1) Touch the center of an isolated colony with a wooden stick and transfer some cell paste onto a clean glass slide. Be careful to avoid picking up any blood from the agar.

(2) Place a drop of 15% hydrogen peroxide directly onto the cell paste and observe closely for the immediate formation of bubbles. Holding the slide over a dark background is helpful.

A11.4 Interpretation

A11.4.1 The immediate appearance of bubbles indicates a positive reaction.

A11.4.2 No bubbles, or the slow elaboration of a few bubbles after 20 seconds indicates a negative reaction.

A11.5 Quality Control

Perform the test on a fresh 48-hour subculture of *B. fragilis* (positive reaction) and *C. perfringens* (negative reaction).

Summary of Comments and Committee Responses

M35-P: *Abbreviated Identification of Bacteria and Yeast; Proposed Guideline*

NCCLS consensus procedures include an appeals process that is described in detail in Section 9 of the Administrative Procedures. For further information contact the Executive Offices or visit our website at www.nccls.org.

Section	Comment	Response
1. General	Is there any data that details the loss of accuracy if all screening tests are not utilized for gram-positive organisms (i.e., performing catalase or gram stain, instead of both tests)?	The subcommittee is not aware of any current data regarding loss of accuracy of identification of gram-positive organisms if fewer than the recommended screening tests are performed.
2. General	The document states that the spot indole test (for *E. coli* and *Proteus sp.*) uses 5% p-dimethylaminobenzaldehyde or 1% paradimethylaminocinnamaldehyde (nonalcohol based). Is there a problem with using Kovac's reagent with an alcohol base?	Kovac's reagent is not recommended. Please refer to Comment 41.
3. General	On page one, the document states, "organisms from sterile body sites should be considered for complete identification to validate the rapid results." On page three of the document it states that the negative porphyrin synthesis is sufficient to identify *Haemophilus influenzae* from CSF specimens. This appears to be a contradiction.	The caveat against using these methods for sterile body fluids has been removed. Laboratories always have the option to perform more tests if they choose.
4. General	There is concern that isolates obtained from sterile body sites/fluids be completely identified for medico-legal reasons.	For the organisms included, the identifications derived from following these protocols and algorithms precisely are at least as accurate as those derived from multiple reagent kits or biochemical tests. Laboratories must document the competency of their scientists as the proper basis of preparing for potential medico-legal challenges.
5. General	Due to a shortage of experienced technologists/microbiologists and/or the pressure being placed on laboratories to replace technologists with less skilled personnel, there is concern that these tests only be used by experienced personnel. These are skills that cannot be quickly taught. Inexperienced laboratorians should be directly supervised or use alternate methods until proficiency is documented.	A sentence regarding the importance of direct supervision for inexperienced technologists has been added to the Introduction.
6. General	The issue of experience of the personnel involved in performing the abbreviated identifications, of course, is key to the success of this whole approach. Unfortunately, the trend seems to be in the opposite direction. It is becoming more and more difficult to replace the experienced retirees with personnel with the	Expertise and confidence in technologists is seen as a key factor in implementing these protocols. Even with multitest kits, erroneous identifications will be made unless the correct assumptions are made about the identity of the isolate tested. Laboratories with relatively inexperienced personnel or those with less skilled personnel and rotators

Section	Comment	Response
	background training necessary to achieve the 95% confidence level desired. Specific questions that were asked: How many misidentifications will be made because the worker does not recognize that results may be inaccurate because of a mixed culture? Also, how might empiric therapy based on published in-house antibiotic profiles be affected if a higher than desirable percentage of incorrect organism identifications are made by less experienced personnel?	may wish to parallel the rapid methods and conventional methods for some period to gain confidence in the laboratorians' abilities. See Comment 5.
7. General	For busy laboratories, particularly those serving an outpatient clientele, implementing a same-day identification would require setting up a parallel system of additional set of tests, thereby perhaps adding to rather than decreasing the work load. On the other hand, one can argue that it might not be necessary to put most *E. coli* or *P. aeruginosa* isolates, for example, into an automated identification system, thereby saving kit and reagent costs.	This document does not recommend parallel identification testing, but use of the rapid method alone where appropriate, thus eliminating the need for additional tests.
8. General	Information in this document would be useful to laboratories if used carefully and by those with experience in microbiology. It could have negative results if used by smaller laboratories with our experienced technologists.	The requirements for experience and validation, in addition to competency assessment and monitoring, have been addressed.
9. General	The inclusion of a list of sites for which these tests would be appropriate would be good, or a list of sites with the required extent of work-up of organisms (e.g., isolates from MSU work-up) by abbreviated tests acceptable.	The subcommittee removed the limitations on the types of specimens from which rapid testing of isolates should be performed. The tests described here, performed properly, yield results at least as reliable as those from commercial multitest products. Laboratories have the option of performing more tests if they choose.
10. General	For *Enterobacteriaceae* it would be useful to include a description of their colonial morphology on MacConkey agar.	The only *Enterobacteriaceae* included were *E. coli*, which does not have a distinctly characteristic colony, and *Proteus*, which must be swarming to fit the algorithm. Since either lactose-positive or -negative *E. coli* can be identified, morphology on MacConkey agar is probably not particularly helpful.
11. Introduction	M35-P would be good if used in the appropriate situation. Unfortunately, some very important points are buried in the Introduction, a section many people skip over when reading a document. It is stated in this area, "A critical factor in the performance of these tests is the skill and experience of the microbiologists," and "Laboratory directors are responsible for assuring that these methods are only used in situations in which the competency of the users is adequate." This is of particular	A comment regarding the expertise of microbiologists and the director's responsibility for competency of laboratory workers has been placed in the Introduction and bolded to highlight its importance.

Section	Comment	Response
	importance in smaller laboratories in which microbiology staff are not often very experienced for quick consultation. Also, these laboratories may have restricted budgets and may be more likely to lean towards abbreviated methods. These points should be more highlighted in the document and not "lost" in the Introduction	
12. Introduction	What is being attempted by advocating a same-day definitive species identification of these select organisms whenever possible? Some of the experienced microbiologists seem a bit reticent to report a definitive species identification based on a few observations or "spot" characteristics. However, in most instances when quizzed "what else could it be," alternative "look-alikes," other than those mentioned in the NCCLS documents, were rarely forthcoming. The issues of cost-savings, reduction in personnel time, and ease of performance, as indicated in the NCCLS document, were generally accepted. Whether or not the decision-making processes by physicians and alterations of empiric courses of therapy based on rapid laboratory identifications benefits overall patient care were not as clear, particularly in the private hospital practice sector. The overall cost/benefit ratio of implementing rapid automated methods, for example, still needs to be elucidated.	Three references examining the economic benefits of faster reporting of microbiological results are cited in the Introduction (York et al., Doern et al., and Barenfanger et al.)
13. Section 4	In the community hospital laboratory that may also serve a large outpatient population, how can one know beforehand when a case may be of "legal importance"? Situations where complete identifications for organisms of importance to infection control practitioners or involved in nosocomial outbreaks can be defined; however, the statement "other important organisms as determined by individual laboratory deliberations" seems a bit vague and arbitrary, and obviously difficult to standardize. For example, it may be decided in a given laboratory that "quick identifications" will not be made on any pure culture isolates from peripheral blood or sterile body fluids.	The concept of appropriate isolates to test using this method should be left to the discretion of the laboratory, based on the level of expertise available and the supervisor's confidence in the workers' abilities. We removed language that sterile body site isolates should not be tested using the algorithm.
14. Section 5.2 Anaerobes	I realized that not all the anaerobic bacteria included are given on the list in the introduction section. Included are the gram-negative anaerobes *Porphyromonas*, *Bacteriodes ureolyticus*, *Prevotella* species and *Prevotella intermedia* and the gram-positive anaerobes *Peptostreptococcus* (now called *Finegoldia*) and *Clostridium difficile* in the text section but not on the list (p. 10).	Additional anaerobic bacteria included in the algorithms have been listed in the beginning of the document.

Section	Comment	Response
15. Section 7	*Escherichia coli*: The only question in the NCCLS algorithm is the observation of lactose fermentation, which assumes that a MacConkey agar plate was set up for primary isolation. Will a less experienced person be able to match the lactose fermenter with the blood agar growth and distinguish pure isolate from a mixed culture?	A sentence about problems associated with mixed cultures has been added to the text (see Section 7).
16. Section 7	*Escherichia coli:* Many laboratories do not perform an oxidase on lactose fermenters, while a few labs voiced concern at possibly missing *Aeromonas species* if an oxidase test is not performed. Is there any data available defining the accuracy of the *E. coli* algorithm if some of the tests are not performed (i.e., oxidase, PYR, or MUG)?	Refer to Reference 1 for the publication validating the importance of oxidase for identifying lactose fermenting gram-negative rods.
17. Section 7	*Escherichia coli:* Can EMB be substituted for MacConkey in the *E. coli* algorithm?	EMB can be substituted for MacConkey. A statement regarding determination of lactose fermentation on either MacConkey or EMB has been added to Section 7.
18. Section 7	*Escherichia coli:* We would suggest that odor be included in the algorithm for *E. coli* on page three. Some organisms, especially *Citrobacter sp*., have distinctive odors and can be used to rule out *E. coli*.	The subcommittee agreed that there is not sufficient information available to add odor to the *E. coli* algorithm.
19. Section 7	*Escherichia coli:* I would like to share with you that some strains of *E. coli* O157:H7 produce B-glucuronidase (MUG positive). A single case of MUG+ *E. coli* O157:H7 was reported in *JCM* Vol. 33, No. 12, Dec. 1995, p333 by Peggy Hayes, CDC. In 1998, we investigated a food-borne outbreak involving a rare strain of *E. coli* O157:H7 which was MUG- and urea-positive recovered from food (cole slaw) and humans. I have attached our outbreak findings. However, in the last 11 years, >99% of the *E. coli* O157:H7 isolates we identified were MUG-negative, which is a very quick, useful, and helpful test for screening isolates.	A statement was added to the text regarding the MUG+ *E. coli* strain reported in 1995 and the outbreak detected in 1998.
20. Section 7	*Escherichia coli:* Section 7 says *E. coli* O157 is MUG-negative. If a fecal specimen screened on Mac-Sorbitol is positive, spot indole-positive, and MUG-positive, is it safe to say it is not *E. coli* O157. We are a small hospital lab and rarely encounter O157 but screen for it with Mac-Sorb plates. Suspicious isolates are sent to a reference lab for confirmation. This is an effort to reduce the number of isolates sent out.	The indole and MUG disk tests must be performed from a colony on a blood agar plate, not on isolates on MacConkey Sorbitol agar. If there are no sufficiently isolated colonies on the initial blood agar plate, a subculture must be performed before the rapid tests can be done. Assuming accurate rapid test results, less than 1% of isolates that are sorbitol- and MUG-negative and indole-positive will be *E. coli* O157:H7.
21. Section 8	*Haemophilus influenzae*: Some nervousness expressed here, particularly for spinal fluid isolates. Many laboratories set up all spinal fluids on blood agar using a staph streak and look for	The method using a staphylococcal streak on blood agar is an overnight method, whereas the ALA test can be performed on the first day. Multitest systems are always an option.

Section	Comment	Response
	satelliting. It may be worth advocating this in the protocol. The identification of an organism satelliting around the staph streak with the gram stain morphology as described, giving a negative spot ALA test, caused less apprehension. Most still would prefer to use one of the rapid *Neisseria/Haemophilus* ID systems.	
22. Section 8	*Haemophilus influenzae:* In reading the M35-P document I had trouble understanding what came after the limitation statement of *Haemophilus influenzae*. "Limitation: *H. haemolyticus* cannot be differentiated from *H. influenzae* except by the beta-hemolysis of *H. haemolyticus* on rabbit or horse blood agar. The clinical importance of separating these two species is unknown." I am of the opinion that *H. haemolyticus* is "nonpathogenic" and should not be confused with *H. influenzae*, and that statement is to me stating otherwise.	*H. haemolyticus* is a commensal inhabitant of the gingival crevice, and there is no published association with infectious disease. The text has been modified to clarify this limitation of the rapid test (see Section 8).
23. Section 9	*Moraxella catarrhalis:* Most labs feel more comfortable with the use of the QuadFerm tube—which includes both beta lactamase and DNAse, and gives a three- to four-hour reading.	The QuadFerm product is no longer available.
24. Section 10	*Proteus* species*:* Some labs may report *Proteus mirabilis/penneri* or "indole-negative Proteus" in lieu of further speciating indole-negative *Proteus* spp.	Reporting *Proteus mirabilis/penneri* or "indole-negative *Proteus"* is an acceptable alternative to further identification of indole-negative *Proteus* spp.; additional text has been added in Section 10.
25. Section 10	*Proteus* species*:* Regarding Section 10 on *Proteus* species, I understood you to say that an oxidase and indole should be performed on swarming lactose-negative, gram-negative rods (you didn't substitute oxidase for indole). The NCCLS document doesn't mention performing an oxidase test on these organisms. Was this just an oversight, or does the committee think that an oxidase test is not necessary?	The subcommittee does not know of any oxidase-positive, truly swarming gram-negative rods; therefore, oxidase is not mentioned in the *Proteus* algorithm.
26. Section 11	*Pseudomonas aeruginosa*: Most laboratories rely on some means to detect pyocyanin production (green pigment/odor) as a guide to rapid identification. In an outpatient setting, whether a specimen comes from a cystic fibrosis patient may not be known. An atypical *P. aeruginosa* isolate (nonpigmented, for example) or a *B. cepacia* may be missed. An experienced person probably will not fall into the trap, but there may be a problem with the less experienced worker. Working through this falls into the "local laboratory deliberation" category.	As stated in the document, laboratories that receive any specimens from cystic fibrosis patients need to become aware of the sources of such specimens and to evaluate cultures very carefully. Physicians need to be educated to inform the laboratory if the patient has cystic fibrosis, otherwise necessary selective media may not be used and clinically important isolates may not be detected or identified properly.

Section	Comment	Response
27. Section 12	*Staphylococcus aureus:* Many labs incubate the tube coagulase at room temperature overnight for staphylococci that are tube coagulase-negative after four hours. The document recommends that the tube coagulase not be incubated beyond four hours. Could this be clarified?	This has been clarified as suggested in Section 12.
28. Section 12	*Staphylococcus aureus:* The document states that *Staphylococcus saprophyticus* may give a false-positive latex agglutination result. These colonies have a distinctive bright white appearance. Experienced microbiologists would recognize these colonies and test the organism with an alternate method (i.e., tube coagulase).	Colony morphology of *S. saprophyticus*, although usually distinctive, can vary, and cream-colored colonies, albeit non-beta hemolytic, have been observed.
29. Section 14	*Streptococcus agalactiae* (Group B): No mention was made of nonhemolytic *Streptococcus agalactiae* as a potential limitation.	Text regarding nonhemolytic *S. agalactiae* has been incorporated in Section 14. However, readers should remember that the document states that colonies not fulfilling all criteria must be identified using other means.
30. Section 15	*Streptococcus pneumoniae*: Experienced personnel will not miss the identification by following the protocol suggested. Perhaps the strains producing "checker-piece" morphology should also be mentioned. I personally have more difficulty in interpreting the direct bile solubility test on the agar surface, but this is less of a problem with those more experienced than I.	A comment regarding checker piece morphology has been added to the text (see Section 15), as well as additional text for performing bile solubility (see Appendix A8).
31. Section 16	*Streptococcus pyogenes*: The experienced workers will be able to distinguish between group A strep and beta-hemolytic enterococci as described in the protocol. Do you want to advocate the use of a direct "A" disk on SXT agar? I know that many POLs use this approach. I'm not sure, however, this will achieve the 95% sensitivity you want. Also, should the antigen extraction approach be mentioned? The comfort level is also higher in most laboratories that use a particle agglutination system, which is briefly mentioned in the protocol.	The use of a bacitracin disk on SXT agar is an acceptable method for early detection of *S. pyogenes* on throat swab cultures, but the subcommittee chose to deal with colonies detected on routine standard laboratory media in this case. Antigen extraction is not considered to be as rapid as the PYR test, which is equally accurate and less difficult to perform.
32. Section 16	*Streptococcus pyogenes:* Is it appropriate to report "beta hemolytic streptococci-not group A" based on colony morphology and a negative PYR or based on colony morphology alone (i.e., beta hemolytic colonies not consistent with typical group A streptococci)?	The subcommittee did not comment on the appropriateness of reporting "beta hemolytic streptococci, not group A," as this sort of report is left to the discretion of the individual institution/laboratory. However, reliance on colony morphology alone is not recommended.
33. Section 17	*Candida albicans:* The document states that a microscopic exam is necessary on suspected yeast colonies. Later in that same paragraph, it states that colonies with "feet" or "star-like" projections on blood-containing media <48 hours old are	The subcommittee has agreed that microscopic morphology is important in addition to colony morphology for identification of *C. albicans*. Retaining the gram stain for identification of yeast colonies displaying "feet" is important, because some technologists may have trouble

Section	Comment	Response
	identified as *Candida albicans*. Is it necessary to perform a microscopic exam on these distinctive colonies? Please clarify.	differentiating "feet" from "fringe," as well as observing the appropriate oval-shaped cells.
34. Section 17	*Candida albicans:* The document states that "germ tubes detected after >3 hours should not be considered…"; however, in appendix A9, the procedure states "incubate at 35 °C up to four hours." Please clarify.	This has been clarified in Appendix A9.
35. Section 18	*Candida (Torulopsis) glabrata*: The small colonies and small yeast cells are key initial observations that may be missed by the less experienced. The rapid trehalose assimilation test is important for laboratories to know about. Growth at 42 °C does not seem to be a "rapid" test. Can one expect same-day growth?	The text has been modified to clarify that fermentation of trehalose is the identifying characteristic evaluated at 42 °C, not growth (see Section 18).
36. Section 18	*Candida (Torulopsis) glabrata:* The document states that *Candida glabrata* is the only yeast that ferments trehalose and grows at 42 °C. The procedure in the appendix describes the test being incubated at 35 °C.	For identification of *C. glabrata*, trehalose fermentation is performed using a commercial product at 42 °C. The test described in the appendix is the assimilation test, which is performed at 35 °C. This has been clarified in Section 18.
37. Section 19	*Cryptococcus neoformans*: Perhaps it should be suggested that the India ink or nigrosin mount can be used to study the cell morphology, which is obviously most helpful in identifying encapsulated strains. I also put much stock in irregular size of the spherical yeast cells. I am not up to date on the direct caffeic acid disk test. The last time I studied this issue, the strips were insufficiently sensitive to recommend, and we always advocated using "bird seed" agar. The latter, of course, is overnight. I would assume that if the caffeic acid test is positive, the ID of *C. neoformans* can be made (high predictive value positive?). If it is negative, additional tests will need to be done anyway; therefore, an identification will not be missed. The rapid urease test also is mentioned, which is used in many laboratories.	As for all test systems in this document, when all criteria are met, the organism is correctly identified >95% accurately. This is true for the caffeic acid disk test. False-negative results may be the result of adding too much liquid to moisten the disk before inoculating the colony paste, or the result of using colonies from Sabouraud dextrose or other glucose-containing medium. Additional text has been added (see Section 19).
38. Appendix, Section A9	We used to identify *C. albicans* by "star-like" or "feet" or "fingering" appearance on blood agar but some were misidentified as such. We discontinued the practice, since it could be subjective to some.	For *C. albicans* the differentiation between "feet" and "fringe" has been mentioned in the text (see Section 17).
39. Appendix, Section A3	The rapid MUG test lists Sigma as a source for the MUG, but no source is given for the diluent (0.05 M Sorensen's phosphate buffer - pH 7.5). Please add this to the final guideline. I was not able to find it in Sigma's catalog and was unable to find any other source either.	The formula for Sorensen's buffer has been added to the protocol for the MUG test (see Appendix A3).
40. Appendix	The following are tests mentioned in the various protocols that are not included in the "Appendix of Test Procedures":	• DNase rapid test is no longer available, and it has been removed from the algorithm.

Section	Comment	Response
	DNase Butyrate Esterase Test Caffeic acid disk test.	• Butyrate esterase is available from several commercial manufacturers, each of whom has a slightly different protocol. The subcommittee suggests following the manufacturer's recommendations. • Caffeic acid disk is also available commercially, and the subcommittee suggests following the manufacturer's recommendations. A few suggestions have been added to the text.
41. Appendix, Section A1	Regarding the spot indole test, why isn't Kovac's reagent recommended? Are the other reagents more sensitive? We've been using Kovac's reagent and aren't aware of any problems. I know that some labs do not do oxidase testing on lactose fermenters (especially when the organisms are not hemolytic on sheep blood agar). I'd be interested in hearing some feedback on this topic.	There were two reasons that the subcommittee did not recommend Kovac's reagent for the indole test: 1. None of the articles used as references for the rapid indole test, which compare different reagents, recommend Kovac's reagent. 2. The chemical substrate powder is the same for both Kovac's and the aldehyde reagent; however, the subcommittee agrees that for a reagent to be available at the workbench at all times during the day, an aqueous solution is safer than the amyl-alcohol solution required for the Kovac's formulation. The odor of amyl alcohol can cause headaches in a portion of the normal population, and breathing this odor over time as it evaporates from a filter paper is not desirable.
42. Appendix, Section A1	I would be interested in hearing about the possibility of not gram staining organisms and doing spot testing (i.e., catalase tests on colonies that are consistent with streptococci or staphylococci) and identifying the organisms based on colonial morphology and biochemical reactions.	The subcommittee concurred that the gram stain be retained. Staphylococci colonies can resemble streptococci (particularly those from patients with cystic fibrosis), and some enterococci may show a weak catalase test. In addition, yeast and staphylococci may produce colonies that are indistinguishable.

Related NCCLS Publications*

GP6-A **Inventory Control Systems for Laboratory Supplies; Approved Guideline (1994).** This document provides recommendations for inventory control systems to ensure the availability of reagents and supplies in the laboratory.

M2-A7 **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Seventh Edition (2000).** This standard contains updated recommended techniques, interpretive criteria and quality control parameters for disk susceptibility testing.

M7-A5 **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Fifth Edition (2000).** This 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—Second Edition; Approved Standard (1996).** This document contains quality assurance procedures for manufacturers and users of prepared, ready-to-use microbiological culture media.

M27-A2 **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Second Edition (2002).** This document addresses the selection and preparation of antifungal agents; implementation and interpretation of test procedures; and quality control requirements for susceptibility testing of yeasts that cause invasive fungal infections.

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.

NRSCL8-A **Terminology and Definitions for Use in NCCLS Documents; Approved Standard (1998).** This document provides standard definitions for use in NCCLS standards and guidelines, and for submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

* 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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YD Diagnostics (Seoul, Korea)

Trade Associations

AdvaMed
Association of Medical Diagnostic Manufacturers
Japan Association Clinical Reagents Ind. (Tokyo, Japan)
Medical Industry Association of Australia

Associate Active Members

20th Medical Group (SC)
31st Medical Group/SGSL (APO, AE)
67th CSH Wuerzburg, GE (NY)
121st General Hospital (CA)
Academisch Ziekenhuis-VUB (Belgium)
Acadiana Medical Laboratories, LTD (LA)
Adena Regional Medical Center (OH)
Advocate Healthcare Lutheran General (IL)
Akershus Central Hospital and AFA (Norway)
Albemarle Hospital (NC)
Allegheny General Hospital (PA)
Allegheny University of the Health Sciences (PA)
Allina Health System (MN)
Alton Ochsner Medical Foundation (LA)
American Medical Laboratories (VA)
Antwerp University Hospital (Belgium)
Arkansas Department of Health
ARUP at University Hospital (UT)
Armed Forces Research Institute of Medical Science (APO, AP)
Associated Regional & University Pathologists (UT)
Aurora Consolidated Laboratories (WI)
Azienda Ospedale Di Lecco (Italy)
Bay Medical Center (MI)
Baystate Medical Center (MA)
Bbaguas Duzen Laboratories (Turkey)
Bermuda Hospitals Board
Bo Ali Hospital (Iran)
British Columbia Cancer Agency (Vancouver, BC, Canada)
Brooks Air Force Base (TX)
Broward General Medical Center (FL)
Calgary Laboratory Services
Carilion Consolidated Laboratory (VA)
Cathay General Hospital (Taiwan)
CB Healthcare Complex (Sydney, NS, Canada)
Central Peninsula General Hospital (AK)
Central Texas Veterans Health Care System
Centre Hospitalier Regional del la Citadelle (Belgium)
Centro Diagnostico Italiano (Milano, Italy)
Champlain Valley Physicians Hospital (NY)
Chang Gung Memorial Hospital (Taiwan)
Changi General Hospital (Singapore)
Children's Hospital (NE)
Children's Hospital & Clinics (MN)
Children's Hospital Medical Center (Akron, OH)
Children's Hospital of Philadelphia (PA)
Children's Medical Center of Dallas (TX)
Clarian Health–Methodist Hospital (IN)
Clendo Lab (Puerto Rico)
Clinical Laboratory Partners, LLC (CT)
CLSI Laboratories (PA)
Columbia Regional Hospital (MO)
Commonwealth of Kentucky
Community Hospital of Lancaster (PA)
CompuNet Clinical Laboratories (OH)
Cook County Hospital (IL)
Cook Children's Medical Center (TX)
Covance Central Laboratory Services (IN)
Danish Veterinary Laboratory (Denmark)
Danville Regional Medical Center (VA)
Delaware Public Health Laboratory
Department of Health & Community Services (New Brunswick, Canada)
DesPeres Hospital (MO)
DeTar Hospital (TX)
Detroit Health Department (MI)
Diagnosticos da América S/A(Brazil)
Dr. Everett Chalmers Hospital (New Brunswick, Canada)
Doctors Hospital (Bahamas)
Duke University Medical Center (NC)
E.A. Conway Medical Center (LA)
Eastern Maine Medical Center
East Side Clinical Laboratory (RI)
Eastern Health (Vic., Australia)
Elyria Memorial Hospital (OH)
Emory University Hospital (GA)
Esoterix Center for Infectious Disease (TX)
Fairview-University Medical Center (MN)
Federal Medical Center (MN)
Florida Hospital East Orlando

Foothills Hospital (Calgary, AB, Canada)
Fort St. John General Hospital (Fort St. John, BC, Canada)
Fox Chase Cancer Center (PA)
Fresenius Medical Care/Spectra East (NJ)
Fresno Community Hospital and Medical Center
Frye Regional Medical Center (NC)
Gambro Healthcare Laboratory Services (FL)
Gateway Medical Center (TN)
Geisinger Medical Center (PA)
Grady Memorial Hospital (GA)
Guthrie Clinic Laboratories (PA)
Hahnemann University Hospital (PA)
Harris Methodist Erath County (TX)
Harris Methodist Fort Worth (TX)
Hartford Hospital (CT)
Headwaters Health Authority (Alberta, Canada)
Health Network Lab (PA)
Health Partners Laboratories (VA)
Heartland Regional Medical Center (MO)
Highlands Regional Medical Center (FL)
Hoag Memorial Hospital Presbyterian (CA)
Holmes Regional Medical Center (FL)
Holzer Medical Center (OH)
Hopital du Sacre-Coeur de Montreal (Montreal, Quebec, Canada)
Hôpital Maisonneuve – Rosemont (Montreal, Canada)
Hospital for Sick Children (Toronto, ON, Canada)
Hospital Sousa Martins (Portugal)
Hotel Dieu Hospital (Windsor, ON, Canada)
Houston Medical Center (GA)
Huddinge University Hospital (Sweden)
Hurley Medical Center (MI)
Indiana State Board of Health
Indiana University
Institute of Medical and Veterinary Science (Australia)
International Health Management Associates, Inc. (IL)
Jackson Memorial Hospital (FL)
Jersey Shore Medical Center (NJ)
John C. Lincoln Hospital (AZ)
John F. Kennedy Medical Center (NJ)
John Peter Smith Hospital (TX)
Kadlec Medical Center (WA)
Kaiser Permanente Medical Care (CA)
Kaiser Permanente (MD)
Kantonsspital (Switzerland)
Keller Army Community Hospital (NY)
Kenora-Rainy River Regional Laboratory Program (Ontario, Canada)
Kern Medical Center (CA)
Kimball Medical Center (NJ)
King Faisal Specialist Hospital (Saudi Arabia)
King Khalid National Guard Hospital (Saudi Arabia)
King's Daughter Medical Center (KY)
Klinični Center (Slovenia)
Laboratories at Bonfils (CO)
Laboratoire de Santé Publique du Quebec (Canada)
Laboratório Fleury S/C Ltda. (Brazil)
Laboratory Corporation of America (NJ)
Laboratory Corporation of America (MO)
LAC and USC Healthcare Network (CA)
Lakeland Regional Medical Center (FL)
Lancaster General Hospital (PA)
Langley Air Force Base (VA)
LeBonheur Children's Medical Center (TN)
L'Hotel-Dieu de Quebec (Canada)
Libero Instituto Univ. Campus BioMedico (Italy)
Salina Regional Health Center (KS)
San Francisco General Hospital (CA)
Santa Clara Valley Medical Center (CA)
Seoul Nat'l University Hospital (Korea)
Shanghai Center for the Clinical Laboratory (China)
South Bend Medical Foundation (IN)
Southwest Texas Methodist Hospital (TX)
South Western Area Pathology Service (Australia)
Louisiana State University Medical Center
Maccabi Medical Care and Health Fund (Israel)
Magee Womens Hospital (PA)
Malcolm Grow USAF Medical Center (MD)
Manitoba Health (Winnipeg, Canada)
Martin Luther King/Drew Medical Center (CA)
Massachusetts General Hospital (Microbiology Laboratory)
MDS Metro Laboratory Services (Burnaby, BC, Canada)
Medical College of Virginia Hospital
Medicare/Medicaid Certification, State of North Carolina
Memorial Medical Center (IL)
Memorial Medical Center (LA) Jefferson Davis Hwy
Memorial Medical Center (LA) Napoleon Avenue
Methodist Hospital (TX)
Methodist Hospitals of Memphis (TN)
MetroHealth Medical Center (OH)
Michigan Department of Community Health
Mississippi Baptist Medical Center
Monte Tabor – Centro Italo - Brazileiro de Promocao (Brazil)
Montreal Children's Hospital (Canada)
Montreal General Hospital (Canada)
MRL Pharmaceutical Services, Inc. (VA)
MRL Reference Laboratory (CA)
Nassau County Medical Center (NY)
National Institutes of Health (MD)
Naval Hospital – Corpus Christi (TX)
Naval Surface Warfare Center (IN)
Nebraska Health System
New Britain General Hospital (CT)
New England Fertility Institute (CT)
New Mexico VA Health Care System
North Carolina State Laboratory of Public Health
North Shore – Long Island Jewish Health System Laboratories (NY)
Northwestern Memorial Hospital (IL)
O.L. Vrouwziekenhuis (Belgium)
Ordre professionnel des technologists médicaux du Québec Ospedali Riuniti (Italy)
The Ottawa Hospital (Ottawa, ON, Canada)
Our Lady of Lourdes Hospital (NJ)
Our Lady of the Resurrection Medical Center (IL)
Pathology and Cytology Laboratories, Inc. (KY)
The Permanente Medical Group (CA)
Piedmont Hospital (GA)
Pikeville Methodist Hospital (KY)
Pocono Hospital (PA)
Presbyterian Hospital of Dallas (TX)
Queen Elizabeth Hospital (Prince Edward Island, Canada)
Queensland Health Pathology Services (Australia)
Quest Diagnostics Incorporated (CA)
Quintiles Laboratories, Ltd. (GA)
Regions Hospital
Reid Hospital & Health Care Services (IN)
Research Medical Center (MO)
Rex Healthcare (NC)
Rhode Island Department of Health Laboratories
Riyadh Armed Forces Hospital (Saudi Arabia)
Royal Columbian Hospital (New Westminster, BC, Canada)
Sacred Heart Hospital (MD)
Saint Mary's Regional Medical Center (NV)
St. Alexius Medical Center (ND)
St. Anthony Hospital (CO)
St. Anthony's Hospital (FL)
St. Barnabas Medical Center (NJ)
St-Eustache Hospital (Quebec, Canada)
St. Francis Medical Ctr. (CA)
St. John Hospital and Medical Center (MI)
St. John Regional Hospital (St. John, NB, Canada)
St. Joseph Hospital (NE)
St. Joseph's Hospital – Marshfield Clinic (WI)
St. Joseph Mercy Hospital (MI)
St. Jude Children's Research Hospital (TN)
St. Luke's Regional Medical Center (IA)
St. Mary of the Plains Hospital (TX)
St. Mary's Hospital & Medical Center (CO)
St. Paul's Hospital (Vancouver, BC, Montreal)
St. Vincent Medical Center (CA)
Ste. Justine Hospital (Montreal, PQ, Canada)
Southern Maine Medical Center
Specialty Laboratories, Inc. (CA)
Stanford Hospital and Clinics (CA)
State of Washington Department of Health
Stony Brook University Hospital (NY)
Stormont-Vail Regional Medical Center (KS)
Sun Health-Boswell Hospital (AZ)
Sunrise Hospital and Medical Center (NV)
Swedish Medical Center – Providence Campus (WA)
Tampa General Hospital (FL)
Temple University Hospital (PA)
Tenet Odessa Regional Hospital (TX)
The Toledo Hospital (OH)
Touro Infirmary (LA)
Trident Regional Medical Center (SC)
Tripler Army Medical Center (HI)
Truman Medical Center (MO)
UCSF Medical Center (CA)
UNC Hospitals (NC)
University College Hospital (Galway, Ireland)
University Hospital (Gent) (Belgium)
University Hospitals of Cleveland (OH)
The University Hospitals (OK)
University of Alabama-Birmingham Hospital
University of Alberta Hospitals (Canada)
University of Colorado Health Science Center
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University of the Ryukyus (Japan)
University of Texas M.D. Anderson Cancer Center
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University of Washington
UZ-KUL Medical Center (Belgium)
VA (Denver) Medical Center (CO)
Virginia Department of Health
VA (Kansas City) Medical Center (MO)
VA (Western NY) Healthcare System
VA (San Diego) Medical Center (CA)
VA (Tuskegee) Medical Center (AL)
VA Outpatient Clinic (OH)
Vejle Hospital (Denmark)
Washington Adventist Hospital (MD)
Washoe Medical Center Laboratory (NV)
West Jefferson Medical Center (LA)
West Shore Medical Center (MI)
Wilford Hall Medical Center (TX)
William Beaumont Army Medical Center (TX)
William Beaumont Hospital (MI)
Williamsburg Community Hospital (VA)
Winn Army Community Hospital (GA)
Winnipeg Regional Health Authority (Winnipeg, Canada)
Wishard Memorial Hospital (IN)
Yonsei University College of Medicine (Korea)
York Hospital (PA)

NCCLS ▼ 940 West Valley Road ▼ Suite 1400 ▼ Wayne, PA 19087 ▼ USA ▼ PHONE 610.688.0100
FAX 610.688.0700 ▼ E-MAIL: exoffice@nccls.org ▼ WEBSITE: www.nccls.org ▼ ISBN 1-56238-459-7