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# Technical Manual of Microbiological Media

# Foreword

The Clinical and Laboratory Standards Institute (CLSI) has developed a standard specifying the requirements for quality control testing of prepared culture media, *Quality Control for Commercially Prepared Microbiological Culture Media*, Approved Standard, M22. Commercially prepared media listed as EXEMPT, in Table 1B of M22, need not be retested, provided the user is assured that the criteria have been met by the manufacturer; however, as stated in CLSI M22, “categorization of media as exempt does not preclude a laboratory from performing complete quality control on any manufactured medium type when deemed necessary.” Media listed in Table 1B as NONEXEMPT, require user quality control. The table following this Foreword, though not all-inclusive, lists commonly used Remel prepared media and the applicable category into which it falls.<sup>1</sup>

Remel has developed the *Technical Manual of Microbiological Media* to ensure the customer that our quality control conforms with or exceeds CLSI guidelines. The General Information section includes information regarding packaging, precautions, product storage and deterioration, quality control performance, certificates of quality, general limitations, and references. Product-specific Instructions for Use (IFU) for Remel prepared media are also included in this manual. Each IFU contains information relating to specific media use and product quality control.

The recommendations in M22 apply to the following types of media: bacteriological, fungal, and mycobacterial, and include biplates, paddles, and other kits used for primary isolation. These recommendations do not apply to media used for isolation of parasites, viruses, mycoplasmas, and chlamydiae; nor to commercially prepared media packaged in kits consisting of two or more different substrates primarily used for microbial identification. In regard to media specifically used for antimicrobial susceptibility testing, CLSI states that such media “have different quality control recommendations that are detailed in separate CLSI documents.”<sup>1</sup>

The Remel packing slip, included with each delivery, contains the lot number and expiration date of each product received. By retaining the Remel packing slip, a laboratory meets the CLSI guidelines for documentation of lot specific quality control of commercially prepared media. Two packing slips are included with each shipment, one for the receiving department and one for the laboratory. It is the responsibility of the laboratory to ensure that the media is delivered to the laboratory by the receiving department in a timely manner. Upon receipt in the laboratory, the technologist should visually inspect all media for breakage, contamination, proper appearance, and evidence of freezing or overheating, following the protocol outlined in CLSI Standard M22. A space is provided on the Remel packing slip for documenting this inspection. Media should continue to be monitored by the laboratory technologist and any deficiency documented. Remel Technical Service should be notified of deficiencies so that appropriate action can be taken. In order to accurately document a deficiency, the lot number, batch number (if available), and expiration date of the product must be provided, as well as other information requested by Technical Service. Remel Regulatory Affairs is responsible for conducting investigations based on the information provided to the Technical Service Representative by a customer.

Providing the best quality control program possible is a primary objective for Remel. Quality control procedures are continually updated to reflect the most current CLSI guidelines, and as new products are added to the Remel catalog, IFUs will be prepared with usage and quality control information.

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**Prepared Media, Exempt and Nonexempt, as adapted from CLSI, M22, Table 1B.**

<b>CATEGORY</b>	<b>EXEMPT*</b>	<b>NONEXEMPT</b>
GENERAL BACTERIOLOGIC MEDIA	Blood agar Brain heart infusion (BHI) media Chocolate agar * Peptone broth Thioglycollate broth Tryptic soy broth (TSB) Urease agar	Nutrient broth
SELECTIVE AND DIFFERENTIAL MEDIA	BCYE agar (buffered charcoal yeast extract) * Charcoal selective agar w/ CVC (cycloheximide, vancomycin, cefoperazone) CIN agar (cefsulodin, irgasan, novobiocin) Columbia agar w/ CNA (colistin, nalidixic acid) Citrate agar CLED agar (cystine lactose electrolyte deficient) EMB agar (eosin methylene blue) SF broth ( <i>Enterococcus faecalis</i> ) GN broth (gram-negative) HE agar (hektoen enteric) LIM broth MacConkey agar Mannitol salt agar PC agar ( <i>Burkholderia cepacia</i> ) * PEA agar (phenylethyl alcohol) Selective agar for Group A Streptococcus Selective media for enterococci w/ or w/o azide Selenite broth Sheep blood agar w/ SXT (trimethoprim-sulfamethoxazole) SS agar ( <i>Salmonella-Shigella</i> ) TCBS agar (thiosulfate citrate bile salts sucrose) TSI agar (triple sugar iron) TSA w/ sheep blood w/ ampicillin XLD agar (xylose lysine desoxycholate) Thayer-Martin agar (modified) *	BCYE w/ DGVP Bordet Gengou Agar Campylobacter agar CVA (cefoperazone, vancomycin, amphotericin B) Campy blood agar (Blaser) Chocolate agar w/ bacitracin Chocolate agar w/ pyridoxal Desoxycholate broth Martin-Lewis agar New York City agar * OFFPBL agar (polymixin B, bacitracin, lactose) SMAC agar (MacConkey agar w/ sorbitol) Todd-Hewitt broth Trans-vaginal broth
ANAEROBIC MEDIA	Anaerobic blood agar Anaerobic phenylethyl alcohol (PEA) agar BBE agar ( <i>Bacteroides bile esculin</i> ) Brucella agar Brucella agar w/ hemin and Vitamin K Brucella laked blood agar w/ KV (kanamycin, vancomycin) CDC anaerobe laked blood agar w/ KV CDC anaerobic 5% sheep blood agar w/ KV Egg yolk (modified) agar Kanamycin laked blood agar	CDC anaerobe 5% sheep blood agar w/ PEA
MYCOBACTERIA (AFB) MEDIA	Lowenstein-Jensen media Middlebrook agar Middlebrook 7H9 broth	ATS agar (American Trudeau Society) Middlebrook 7H10 agar and 7H11 agar Mitchison agar
FUNGAL MEDIA	Brain Heart Infusion agar w/ 5% sheep blood w/ CG (chloramphenicol, gentamicin) Cornmeal agar Inhibitory mold agar Inhibitory mold agar w/ gentamicin Soy peptone agar w/ CC w/o pH indicators (cycloheximide, chloramphenicol) Potato dextrose agar Sabouraud dextrose agar Sabouraud dextrose agar w/ CG (chloramphenicol, gentamicin)	Cornmeal agar w/ Tween® BHI agar w/ 5% sheep blood w/ CC (cycloheximide, chloramphenicol) BIGGY agar Birdseed agar BHI agar w/ 5% sheep blood w/ PS (penicillin, streptomycin) Dermatophyte test medium Potato Flake agar w/ or w/o CC (cycloheximide, chloramphenicol)

\*CLSI strongly recommends quality control of exempt media used for fastidious organisms (in particular exempt media for recovery of *N. gonorrhoeae*, *H. influenzae*, *Campylobacter* spp., *Legionella* spp., and *B. cepacia* among others) to ensure optimum recovery of organisms. Refer to the current edition of M22 for the complete table.

# General Information

Quality, service, and technology have been our objectives at Remel since we began operations in 1973. We firmly believe every laboratory is entitled to these three basic commitments from a supplier. Therefore, Remel will endeavor to fulfill these commitments, to the best of our ability, for any laboratory we are privileged to serve.

Remel was founded in 1973 as a small, regional manufacturer of prepared culture media. Since then, we have grown to be a recognized global leader in the manufacture and distribution of a wide range of trusted laboratory products for clinical, industrial, and research applications.

Remel continues to expand its product portfolio, which now covers several laboratory disciplines including microbiology, parasitology, virology, serology, immunomicrobiology, and coagulation. Our line of products includes prepared and dehydrated media, stains, reagents, identification and susceptibility test disks, organism identification systems, quality control organisms, animal blood products, collection and transport systems, diagnostic test kits, and other related products.

The Remel administration office and main manufacturing facility are located in Lenexa, Kansas, a suburb of Kansas City. Our company-owned network of regional warehouses in the United States and other distribution partnerships permit rapid response to meet the needs of laboratories around the world and ensure products are delivered in a timely manner.

Remel adheres to the current Quality System Regulations for Medical Devices, CFR Title 21, part 820, and is routinely inspected by the United States Food and Drug Administration (FDA) for compliance.<sup>2</sup> Our products undergo stringent quality assurance testing, including pre-testing of raw materials, performance testing of finished goods, and microbial load analysis. Performance testing of the final product complies with or exceeds standards established by the Clinical and Laboratory Standards Institute (CLSI) or the United States Pharmacopeia (USP) as applicable. Remel also holds an ISO Certificate of Registration for a Quality Management System compliant with the requirements of BS EN ISO 9001:2000 Quality Systems Management—Requirements and ISO 13485:2003 Quality Systems—Medical Devices—Particular Requirements for the Application of ISO 9001.

## ABOUT THIS MANUAL




The Instructions for Use (IFU) contained in this manual provide information regarding intended use, principle, classical formulation, media preparation (if applicable), test procedures and interpretation, quality control, and references for each product.

Technical support is available by contacting our Technical Service Department at (800) 447-3641. Our experienced staff of microbiologists can provide you with prompt and reliable responses to your inquiries regarding appropriate product selection, test performance, expected results, and quality control.

## PACKAGING

Remel media products are available in a variety of sizes, volumes, and package configurations. Consult the Remel catalog for a list of available products. Refer to the legend below for an explanation of symbols used on product labels.

## Symbol Legend

<b>REF</b>	Catalog Number
<b>IVD</b>	In Vitro Diagnostic Medical Device
<b>LAB</b>	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
<b>LOT</b>	Batch Code (Lot Number)
	Use By (Expiration Date)

## REAGENTS (CLASSICAL FORMULA)

The formulae included in this manual are based on the classical formulations and may be adjusted as required to meet performance standards.

## PRECAUTIONS

The Instructions for Use in this manual are for Remel prepared media products that are labeled For *In Vitro* Diagnostic Use and For Laboratory Use. Each product should be used by properly trained individuals. Appropriate safety precautions should be taken for successful isolation of the causative agent of disease. This process may require special hazard labels and containers, protective clothing, and timely transport.

The identification of mycobacterial or fungal organisms may require the use of certain safety equipment, such as biological safety cabinets, splash-proof containers, and appropriate disinfectants.<sup>3</sup> Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media in approved biohazard bags after their use. Standards for disposal may vary per institution protocol and state, county, or city regulations.

## STORAGE

Store plated media products inside their original packaging (cellophane bag and box) at the appropriate temperature indicated on the product label. In order to prevent dehydration, product should not be stored in close proximity to a fan, or for prolonged periods under a laminar flow hood or in a Biological Safety Cabinet. Do not freeze or overheat the product unless specifically indicated on the package label or IFU. Media should be protected from light.

Tubed media products should be stored inside the product package at the temperature indicated on the package label.

Remel has an established Stability Study Program designed to ensure performance claims are supported through assigned expiration dates for our products. The results of stability studies indicate the media continues to meet the designated performance specifications when inoculated up to and including the labeled date of expiration and incubated for recommended incubation times as referenced in the individual product IFU.

Allow products to equilibrate to room temperature prior to use. Media products stored at room temperature, for daily use, must be stored inside their cellophane bag, away from a UV light, not under a laminar flow hood, and not for extended periods of time.

Products should be used prior to the expiration date indicated on the package label. Any reagent requiring reconstitution should be used by the expiration date indicated in the IFU or the expiration date on the package, whichever comes first. Expiration date applies to a product in an unopened container, stored as directed.

The majority of Remel products currently follow the expiration date format of Year-Month-Day expressed as YYYY-MM-DD (e.g., 2005-07-27). For those products that still carry Month- Year or Year-Month formats, the Expiration Dates should be interpreted as expiration on the last day of the designated month.

To ensure maximum performance and recovery after inoculation, optimal environmental conditions must be followed. Aerobic incubation of product must occur under conditions appropriate to the medium, away from fans, and at the proper temperature and humidity. Incubator humidity should be maintained at 70-80% and monitored on a regular basis.<sup>4</sup>

#### PRODUCT DETERIORATION

Do not use a product if (a) there is evidence of dehydration, (b) the product is contaminated, (c) the color has changed, (d) the expiration date has passed, or (e) there are other signs of deterioration.

#### SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected in suitable containers, transported to the laboratory without delay, and protected from excessive heat or cold. If there is any delay in processing a specimen, it should be maintained in a suitable transport medium at the appropriate temperature.

Consult appropriate references for recommended guidelines regarding proper specimen collection and transportation.<sup>5-7</sup> Certain specimens require special transport, processing, and safety precautions. Refer to the product IFU and current microbiology reference manuals for proper procedures to successfully culture specific pathogens.

#### MATERIALS REQUIRED BUT NOT SUPPLIED

1. Inoculating loops, swabs, collection containers
2. Loop sterilization device
3. Incubators, alternative environmental systems
4. Supplemental media
5. Quality control organisms
6. Centrifuge
7. Microscope, slides, cover slips, immersion oil
8. Biological safety cabinet, safety equipment
9. Gloves, personal protective equipment
10. Pipettes and pipetting device
11. Splash-proof containers, alcohol-sand flask
12. Culture transfer spade, teasing needle
13. Disinfectant(s)
14. Autoclave, biohazard bags, sharps disposal
15. Shrink seals, gas permeable and impermeable bags

#### QUALITY CONTROL PERFORMANCE

All lot numbers of Remel products have been tested using quality control organisms derived from ATCC® strains and have been found to be acceptable. These quality control organisms are listed on each IFU and strains specified in the current CLSI standard M22, are specifically noted.<sup>1</sup> Additional quality control organisms are often used by Remel to further validate the performance of a specific medium.

Control organisms should be tested in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported until the discrepancy is resolved. Microorganisms used in quality control procedures should be pure and well isolated. These organisms may be used to test for growth or selective performance of a medium. Refer to CLSI standard M22 for detailed instructions pertaining to source, handling and storage of quality control organisms.

#### Direct Inoculation of Media

As stated in CLSI Standard M22, care must be exercised if using direct inoculation. "An inoculum that is either too heavy or too light will mask both the growth and/or inhibitory properties of a medium. If a quality control failure occurs while using direct inoculation, repeat using a standardized suspension as part of corrective action."

#### Preparation of a Cell Suspension (Working Control):

1. Prepare a suspension in sterile, nonbacteriostatic saline (0.85% w/v NaCl) to match a 0.5 McFarland standard ( $1 \times 10^7$  to  $1 \times 10^8$  cfu/ml). Use an 18-24 hour culture of the organism.
2. Alternatively, prepare a suspension by inoculating 3 to 5 colonies from a 24-hour culture into sterile broth. Incubate for several hours to achieve a suspension equivalent to a 0.5 McFarland standard.

#### Nonselective Media Growth Performance:

##### Plated Media

1. Dilute the working control 1:100 in sterile broth or nonbacteriostatic saline.
2. Inoculate the test medium with 10  $\mu$ l of the diluted suspension using a calibrated loop or pipette. Streak plate for isolation.
3. Incubate under conditions appropriate to medium with applicable incubation duration.
4. If the 1:100 dilution inoculum proves too dense, repeat using a 1:1000 dilution to produce isolated colonies.

##### Tubed Media

1. Inoculate with 10  $\mu$ l of the undiluted 0.5 McFarland suspension.
2. Incubate under conditions appropriate to medium with applicable incubation duration.

#### Selective Media Growth Performance:

##### Plated Media

1. Dilute the working control 1:10 in sterile broth or nonbacteriostatic saline.
2. Inoculate the test medium with 10  $\mu$ l of the diluted suspension using a calibrated loop or pipette. Streak plate for isolation.
3. Incubate under conditions appropriate to medium with applicable incubation duration.
4. If the 1:10 dilution inoculum proves too dense, repeat using a 1:100 dilution to produce isolated colonies.

##### Tubed Media

1. Inoculate with 10  $\mu$ l of the undiluted 0.5 McFarland suspension.
2. Incubate under conditions appropriate to medium with applicable incubation duration.

#### INTERPRETATION OF RESULTS

Nonselective Media: Performance is satisfactory if the quality control organism(s) exhibits adequate growth, expected colony size, and typical colony morphology.

Selective Media: Performance is satisfactory if the quality control organism(s) exhibits little or no growth of organisms susceptible to inhibitory agents and growth of expected organisms with typical colony size and morphology.

#### Biochemical Performance:

1. Follow instructions listed on the product IFU.
2. Always use a fresh, pure subculture of the test organism.
3. Inoculate the surface of the medium by stabbing, streaking, and/or agitating the inoculum in or on the medium.
4. Incubate in the appropriate atmosphere with applicable incubation duration.
5. Observe medium for desired biochemical reaction.
6. Specific instructions for mycology and mycobacteriology biochemical performance may apply.

Quality control organisms used to validate growth, selectivity, and biochemical reactions for certain fungal and mycobacteriology media should be derived from an actively growing culture. These organisms either require direct inoculation to a medium being tested or a dilution equal to a 0.5 or 1.0 McFarland turbidity standard or equivalent, prepared in sterile water, saline, or appropriate medium with subsequent transfer to the medium being tested. Some exceptions may apply.

The user should consult the product IFU and/or appropriate fungal and mycobacteriology manuals for organisms that serve as the test quality control organisms.

#### **CERTIFICATES OF QUALITY**

Certificates of Quality certify that specific lot numbers of products have met all performance and quality control criteria for the product. For the purpose of quality assurance documentation, Certificates of Quality are available upon request and from My Remel Online.

#### **CHEMICAL HAZARDS AND MATERIAL SAFETY DATA SHEETS**

Products ordered from Remel are intended for use by qualified laboratory professionals who are trained in good laboratory procedures and aware of potential hazards. Material Safety Data Sheets (MSDS) are prepared in accordance with the OSHA Hazard Communications Standard and are available for specified products upon request and from My Remel Online.

#### **LIMITATIONS**

The Instructions for Use contained in this manual are designed to provide a general description of the products as commonly used. Appropriate references should be consulted for detailed information regarding testing methodologies.<sup>5-19</sup>

The use of prepared and dehydrated culture media is only part of the overall scheme for identification of microorganisms. Variations in results may occasionally be observed. These variations may be the result of improper specimen collection, storage or transportation procedures, improper procedural technique or use, possible interference from other chemicals and biological substrates, and strain variation or mutation.

Slight to moderate color variations of broth media may occur and do not affect performance. While every process is put in place to minimize the potential, nonviable organisms may be seen when Gram staining some broth culture media resulting from their presence in various media components.

A pure culture of the organism is recommended for biochemical, serological, and other confirmatory tests for identification of the organism. Organisms vary in their requirements for temperature, humidity, and atmospheric conditions. Optimal environmental conditions must be determined and followed for each organism being tested. Consult appropriate references for further information.<sup>5-19</sup>

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen due to the degree of selectivity or nonselectivity of the medium. The agents in a selective media may inhibit some strains of the desired species or permit growth of a species they were designed to inhibit, especially if the species are present in large numbers in the specimen, or resistant to the selective agent (i.e., antibiotic, dye, alcohol). Cultures of specimens grown on selective media should be compared with specimens cultured on nonselective media to obtain additional information about potential pathogens.

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