TRAINING UPDATE

Lab Location:	SGAH and WAH	**Date Distributed:**	5/28/2012
Department:	Microbiology	**Due Date:**	6/28/2012

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:
Specimen Processing for Microbiology, SGAH.M04.005, WAH.M04.005
Description of change(s):
1. Specimens for STOOL CULTURE are inoculated into a Para-Pak C&S Transport (Modifed Cary Blair Medium) and plated media for stool cultures will no longer be inoculated at the hospital lab. 2. The proper storage and transport temperature is room temperature. 3. Hektoen Enteric agar (HE), CIN agar, TCBS agar, blood agar with ampicillin, and Campy agar are no longer needed at the hospitals. 4. The plating chart has been updated.

Name of procedure:
Media Quality Control, SGAH.M11.003, WAH. M11.003
Description of change(s):
1. Hektoen Enteric agar (HE), CIN agar, TCBS agar, blood agar with ampicillin, and Campy agar are no longer needed at the hospitals. 2. Quality control organisms for Campy agar and blood agar with ampicillin are no longer needed.

EMPLOYEE SIGNATURES

I have read and understand the procedure described above:

Name	**Signature**	**Date**

Employee signatures are not necessary. Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training all sites (version 005)

Non-Technical SOP

Title	**Specimen Processing for Microbiology**	
Prepared by	Ronald Master	Date: 4/14/2009
Owner	Ronald Master	Date: 4/14/2009

Laboratory Approval		
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		
Local Effective Date:		

12 month (or new) management review and approval: Signature acknowledges SOP version remains in effect with NO revisions.		
Print Name	**Signature**	**Date**

TABLE OF CONTENTS

1. PURPOSE

To describe the process for microbiology specimen setup, plating and management.

2. SCOPE

The scope of this SOP is to ensure the pre analytic processes for microbiology specimens are outlined. These procedures are imperative in determining what pathogenic organisms are present in specimens obtained from patients.

3. RESPONSIBILITY

It is the responsibility of all personnel assigned to Microbiology to read, understand and to perform all procedures as described in this SOP.

4. DEFINITIONS

Plating – inoculation of plated/tubes media with clinical specimen for microbiology culture.

Inoculation – to implant microorganisms or infectious material onto a culture medium.

Streaking – The use of a loop or other plating tool to inoculate a specimen in order to differentiate microorganisms by color or texture from its surroundings on a culture medium.

5. PROCEDURE

5.1 Routine Procedure for Plating Cultures:

All specimens are to be plated in a biosafety cabinet.

1. **Media and its location:**

All routine media will be stored in the refrigerator. Microbiology media should be kept in the refrigerator until needed. Media should be allowed to warm to room temperature before use. A working supply (minimum amount) is left at room temperature for use.

2. **Loops, Swabs, and Pipettes:**

   a. Loops - A wire loop is used for streaking specimens, with the exception of urines. A 0.001 mL calibrated loop must be used to inoculate urine. For sterile urines (cystoscopy, suprapubic aspirate, etc.) use both 0.001 and 0.01 mL calibrated loops.

   b. Swabs - Swabs are used in making the initial inoculation of plates, for preparing smears, and for inoculating specimens into broth media. If a specimen is submitted on a swab it must be submitted in a culturette containing holding medium to prevent drying out. A swab is convenient for inoculating certain specimens onto media, e.g., stool, sputum. Sterile swabs are available at the plating bench.

   c. Pipettes - A sterile pipette may be used to inoculate liquid specimens into broth media, such as thioglycolate, and any plated media. A sterile pipette should be used to inoculate CSF and other body fluids and environmental cultures of liquids. To inoculate thioglycolate with a pipette, insert pipette to bottom of tube and slowly evacuate sample as you with draw the pipette.

3. **Preparation of Smears and Gram Stains:**

   a. Smears - Write the accession number, specimen source, date and the patient's last name on the slide. Using a sterile loop or swab, make a smear about the size of a nickel near the center of the slide. Let the slide air dry, then heat fix. ALWAYS MAKE SMEAR AFTER INOCULATING MEDIA TO AVOID CONTAMINATING THE SPECIMEN.

4. **Inoculation and Streaking of Media:**

   a. The first process in the cultural examination of clinical specimens is the selection of appropriate isolation media. Addenda A, Figure 3 lists the media suitable for the isolation of microorganisms most commonly recovered from various clinical specimens. It is desirable to inoculate more than one kind of isolation medium.

   b. The purpose of isolation is to obtain bacterial colonies representing progeny of a single cell and thus provide the source of a pure culture. The streaking of materials onto the surface of the medium provides such results. The streaking method must be such that, (a) part of the medium is inoculated with a large amount of material, and (b) subsequent streaking will allow for growth of isolated colonies. When done properly, the completed streaking should cover essentially the entire surface of the medium. When streaking plates, flame the loop between the first and second streak area to avoid overly heavy growth. An illustration and

explanation of an acceptable method is provided in Addendum A, Figure 1. Addendum A, Figure 2 illustrates the proper streaking procedure for a urine colony count.

5.2 Incubation of Plates:

Plates and broth media are incubated in a CO_2 incubator at 35 ±2°C.

5.3 Specimens and Special Requirements:

5.3.1 Stool Cultures

1. If *E. coli* O157 is ordered, use test code XECOL.
2. Stool in transport media is to be sent to Chantilly. Stool specimens will be plated in Chantilly.

5.3.2 IV Catheter Tips

1. Perform all steps in a biological safety cabinet.
2. Using sterile forceps, remove catheter tip from transport tube.
3. Lay the catheter tip on a blood agar plate, and using sterile forceps, roll tip 4-5 times over entire plate. If the catheter tip is longer than 2 inches (5 cm), use sterile scissors or scalpel to cut the end closest to the top of the tube (proximal end) prior to rolling the distal end on the plate. The proximal end may be rolled on a second plate, if desired.
4. Leave the catheter tip on the plate, do not press it into the agar.

6. RELATED DOCUMENTS

N/A

7. REFERENCES

N/A

8. REVISION HISTORY

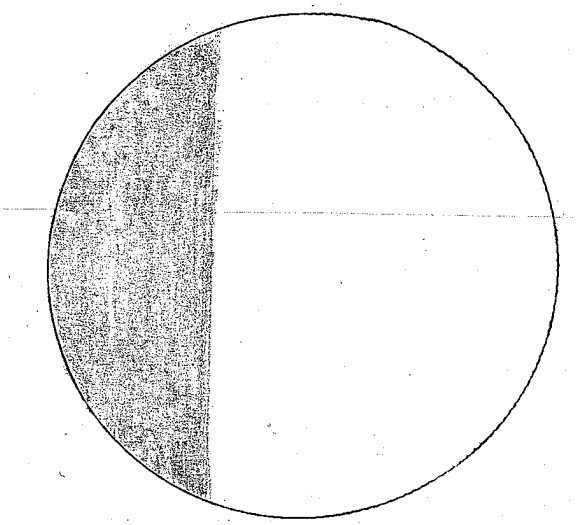
Version	Date	Reason for Revision	Revised By	Approved By
		Supersedes SOP M006.007		
000	5/26/09	Addenda D: media change for MRSA screen	L. Barrett	R. Master
001	3/10/10	Section 5: Change stool culture, add IV cath tip	R. Master	R. Master
002	5/17/10	Section 5.1: Delete requirement for date on opened media 5.2: Change temperature to 35 ± 2°C	R. Master	R. Master
003	7/12/11	5.3.2 Specified catheter length	R. Master	R. Master
004	5/21/12	5.3.1 Deleted plated media for stool cultures	R. Master	R. Master
004	5/21/12	Figure 4: Updated stool cultures, add location	R. Master	R. Master

9. ADDENDA

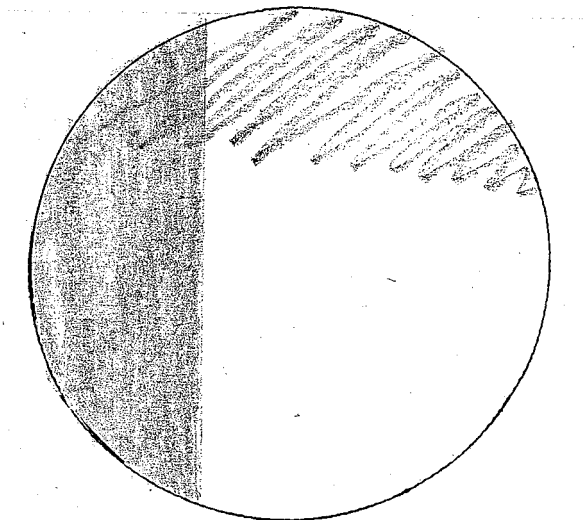
A. Figure 1 – One Acceptable Method of Plate Streaking

B. Figure 2 – Proper Streaking for a Urine Culture and Colony Count
C. Figure 3 – Proper Streaking for a Biplate
D. Figure 4 – Plating Chart for Media by Source and Test Code (see Attachment tab of Infocard)

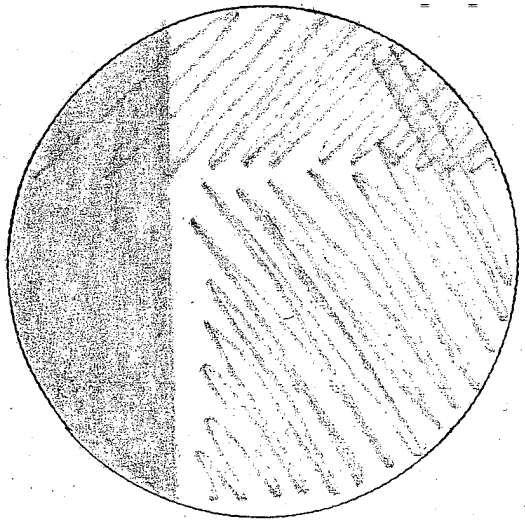
Figure1 **<u>One Acceptable Method of Plate Streaking</u>**



Step 1: Using a loop or a swab inoculate the specimen onto one edge of the plate, covering about one third of the plate.

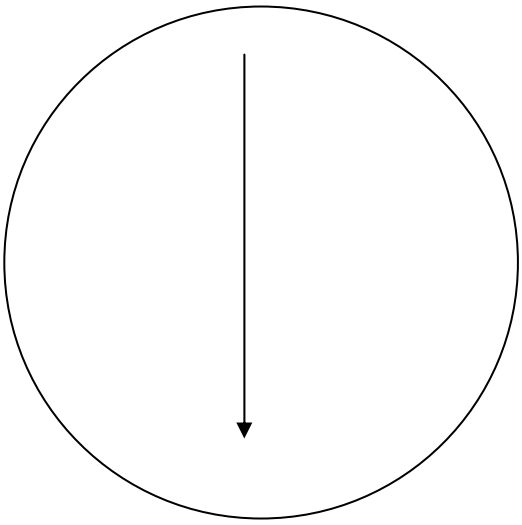


Step 2: Flame loop arid cool it by stabbing into the sterile agar. With cooled loop, streak at a right angle to the initial inoculum going back and forth many times.

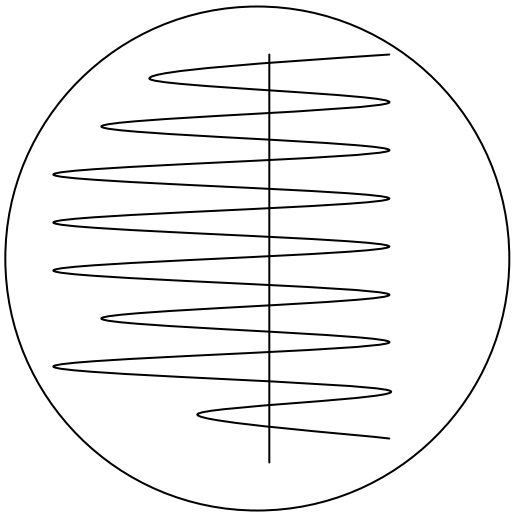


Step 3: Flame loop if specimen is likely to contain a lot of normal flora. Cool the loop. Rotate plate again, and entering only the isolation area, draw loop over the-previously uninoculated portion of the plate. (Be careful to not streak over the initial inoculum.)

Figure 2 **Proper Streaking for a Urine Culture and Colony Count**

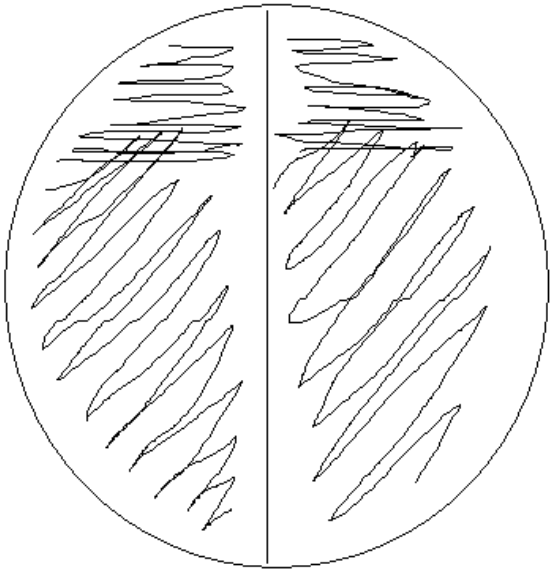


Step 1: Swirl urine to mix. Select sterile calibrated loop. Dip into the bottom of the urine sample and streak down the middle of the plate.



Step 2: Starting at the top, go back and forth numerous times over the initial streak line to facilitate the isolation of bacterial colonies.

Figure 3 **Proper Streaking of Bi-plates (non-urine specimens)**



Biplates are inoculated by initially streaking about 15-20% of the plate, then flaming before performing the downward streak.

Quest Diagnostics

☐ Germantown Emergency Center
☐ Shady Grove Adventist Hospital
☐ Washington Adventist Hospital

PLATING CHART FOR MEDIA BY SOURCE AND TEST CODE

Culture	Test Code	Blood	MAC	CHOC	Martin/ Lewis	ParaPak C&S vial	HE	CNA	TCBS	CIN	BAP w/ ampicillin,	Ana Blood	Thio	Gram Stain	V agar	LIM broth	Misc
Stool Culture, Enteric Profile X *Salmonella, Shigella, Campylobacter, Aeromonas, Plesiomonas, Yersinia* & *Vibrio*	15292					X											
Stool, *E. coli* O157	4221					X											
Respiratory Culture	769	X	X	X				X									
Eye Culture	76950	X	X	X				X									
Ear Culture	76951	X	X	X				X									
Throat Culture	5870	X		X													
Group A Strep Only Culture	6470	X															
Genital Culture	778		X	X				X							X		
GBS Screen	14537															X	
GC Screen	657				X												
Urine Culture	775	X	X														0.001 mL loop
Urine, sterile source	2983	X (0.01 + 0.001)	X (0.01 + 0.001)														0.001 mL and 0.01 mL loops
CSF Culture	127350	X	X	X				X					X	X			
Sterile body Fluid Culture	1273	X	X	X				X					X	X			
Wound Culture	783 / 78351	X	X	X				X									
Tissue Culture	78350	X	X	X				X					X	X			
Surgical Culture	78353	X	X	X				X					X				
Anaerobic Culture	15871	X										X					ana BBE/LKV biplate
VRE Screen	8557							X									
MDRO Screen	78354		X														KPC Chromagar
IV Catheter tip	78352	X															
Type 1 Lab water	4120																1 mL SMA

Quest Diagnostics

☐ Germantown Emergency Center
☐ Shady Grove Adventist Hospital
☐ Washington Adventist Hospital

Culture	Test Code	Blood	MAC	CHOC	Martin/ Lewis	ParaPak C&S vial	HE	CNA	TCBS	CIN	BAP w/ ampicillin,	Ana Blood	Thio	Gram Stain	V agar	LIM broth	Misc
Environmental Culture	6320																
Dialysis water /Dialysate																	1 mL T-soy agar
TAC soln				1 mL													
Breast milk		0.01 mL															
Physical / Resp Ther																	Swab in T-soy broth
Surgical Instruments																	Rinse in T-soy broth
Pharmacy sterility check																	TSB
Mold settle plate																	SAB
Bacterial settle plate		X															
Positive Blood Culture		X	X	X				X				X					SAB if yeast seen on Gram stain

Non-Technical SOP

Title	**Media Quality Control**	
Prepared by	Ron Master	Date: 7/30/2009
Owner	Ron Master	Date: 7/30/2009

Laboratory Approval		
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		
Local Effective Date:		

12 month (or new) management review and approval: Signature acknowledges SOP version remains in effect with NO revisions.		
Print Name	**Signature**	**Date**

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1. PURPOSE

All media should be subjected to quality control testing to determine the adequacy of the media regarding sterility and performance. The quality control procedures performed by commercial suppliers with test organisms can be accepted for some types of media. The laboratory must maintain the media manufacturer's documentation that QC practices conform to NCCLS specifications. All media should be stored according to the manufacturer's recommendations and disposed of when outdated. All media is required to be tested prior to use. Results of controls must be verified for acceptability before patient results are released. Media is removed from use on the expiration date. All media that passes QC should be labeled with a departmental green sticker and signed with the date. All QC results should be documented on the appropriate QC sheet.

2. SCOPE

This SOP covers most aspects of Quality Control procedures and documentation for commercially prepared media.

3. RESPONSIBILITY

It is the responsibility of personnel assigned to Microbiology personnel to learn understand and perform quality control procedures for all media as described in this SOP.

4. DEFINITIONS

Exempt media - includes commercial media documented to maintain consistent user performance with minimum variation and required minimum quality control. However, categorization of media as exempt does not preclude a laboratory from performing quality control on any manufactured medium type when deemed necessary.

Nonexempt media - Nonexempt media require complete user quality control including confirmation of satisfactory performance with recommended organisms.

5. PROCEDURE

I. Receiving Media from the Manufacturer:

A. Verify delivery of the ordered amount. Check medium type for multiple lot numbers and/or impending expiration dates. Report recurring problems to a Supervisor so that the manufacturer or distributor may be contacted.

B. Record the lot number, expiration date, and the received date for each medium type, including blood culture media.

C. Store the medium as specified by the manufacturer (usually 2-8ºC) pending quality control. Separate exempt media from nonexempt media.

II. Initial Examination of Media:

A. Media from a commercial source should be submitted to the following:

1. Examine individual packages for breakage, contamination, excessive moisture, and dehydration and for adequate appearance, evidence of overheating or freezing.

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

3. In addition, each shipment of purchased media must be inspected for the following:

cracked/damaged Petri dishes
unequal filling of plates
cracked medium in plates
hemolysis (if blood containing)
excessive number of bubbles
freezing
agar detached from plates
insufficient agar in the plates (<3 mm)
change in the expected color of the media (possible pH problem)
expiration date
adequate hydration
appropriate color and thickness
tube media not dried or loose from sides
broth media not cloudy
contamination
frozen/melted agar
plates smooth

4. If acceptable, record the date of receipt on each package. Place a label from the inspected lot of media on the Media Labels and Quality Assurance Form. For blood culture bottles, obtain the copy of the Quality Control Certificate and place a Blood Culture Media Visual Inspection label on the Certificate above the lot # and expiration date. Record the date received, initials, and check the OK box if the visual check was acceptable. If unacceptable, record the problem and inform a Supervisor of the lot number of unacceptable media. The Supervisor will contact the manufacturer regarding the failed media. Microbiology staff must document and notify the media manufacturer of deficiencies found during the use of the media. If the shipment contains more than one (1) lot, each individual lot should be tested prior to use.

5. If the QC organism is not viable or a medium does not perform acceptably, do not use that lot of medium until the problem is resolved. Record the corrective action. If media that has acceptable QC results is not available, media may be borrowed from Washington Adventist Hospital or Quest Diagnostics in Chantilly. If media that has passed QC cannot be obtained from WAH or Chantilly, specimens can be sent to Chantilly to be plated. This option should only be used in a critical situation as it will delay turn-around-time.

III. Sterility Testing:

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

IV. Processing of Quality Control Organisms

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

B. Inoculate one nonselective medium. Incubate under conditions favorable for growth. Label as a stock control with the name of the organism and the date of inoculation. Tightly seal and store at 2-8º C for up to 12 months. Certain fastidious bacteria such as *Neisseria gonorrhoeae* may require 25 to 35ºC storage.

C. From the stock control, prepare a second subculture. Label as working control with the name of the organism and date of inoculation. Incubate under conditions favorable for growth. Store the working control at room temperature or 35± 2°C CO_2 for one to two weeks. Prepare a fresh working culture at least once per month from the refrigerated stock control. Avoid multiple serial subcultures of quality control organisms over extended periods of times. For *Neisseria gonorrhoeae* and *Neisseria meningitidis*, maintain the

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stock culture at room temperature. It may be required to replenish the stock on a monthly basis.

V. Performance Testing:

Evaluation of each new lot or shipment of nonexempt media should be made for growth supporting, selective and differential properties prior to use. An adequate variety of stock organisms will be maintained to perform this testing. One positive and one negative control organism should be used to test each property of a medium.

VI. Satisfactory Performance of Media:

A medium performs satisfactorily if all test strains provide satisfactory growth with typical colony morphology and size or in the case of selective media, if growth of the appropriate organisms is inhibited. Tubed media are generally evaluated by testing strains that will give negative and positive results with each reaction what can be observed. The appropriate reactions should be obtained before the medium can be considered to be satisfactory.

VII. Limitations:

Quality control is intended to detect defects among a small sample of media. It may not detect every defect from every agar plate or tubed medium.

6. RELATED DOCUMENTS

None

7. REFERENCES

1. Krisher et. al, Bartlett RC et al: *Quality Control for Commercially Prepared Microbiological Culture Media: Approved Standard—3rd Edition* NCCLS Publication M22-A3 (ISBN 1-56238-536-4), NCCLS, 940 West Valley Road, Suite 1400, Wayne Pennsylvania 19087-1898 USA, 2004.

8. REVISION HISTORY

Version	Date	Reason for Revision	Revised By	Approved By
		Supersedes SOP M016.004		
000	8/20/2010	Addendum C: Delete MacConkey Sorbitol	R. Master	R. Master
001	8/23/2011	Addendum B: Added Hektoen agar	R. Master	R. Master
002	5/21/2012	Addendum A: Deleted Campylobacter	R. Master	R. Master
002	5/21/2012	Addendum B: Deleted CIN, TCBS, Campy agar, HE, XLD, and BAP w/ ampicillin	R. Master	R. Master
002	5/21/2012	Addendum C: Deleted BAP w/ ampicillin and Campy agar	R. Master	R. Master
002	5/21/2012	Addenda D & E: Location added	L. Barrett	R. Master

9. ADDENDA AND APPENDICES

Addendum A - Incubation Conditions
Addendum B - Exempt and Nonexempt Categories for Media
Addendum C - Quality Control Organisms for Nonexempt Media
Addendum D - Media Labels and Quality Assurance (see Attachment tab of Infocard)
Addendum E - Blood Culture Media Visual Inspection Labels (see Attachment tab of Infocard)

Addendum A

Incubation Conditions

Quality Control Organisms	Incubation Temperature	Incubation Atmosphere	Length of Incubation
Rapidly growing bacteria	35-37^{o}C	Ambient Air* or CO_2 Enriched	18-24 hours
Bacteria with special growth requirements	35-37^{o}C	CO_2 Enriched	24-72 hours

Addendum B

Exempt and Nonexempt Categories for Media

CATEGORY	EXEMPT	NONEXEMPT
General bacteriologic media	Blood agar Chocolate agar Thioglycolate broth	Nutrient broth
Blood culture media	Automated blood culture bottle represents broth formulations from BD Diagnostics Systems.	
Media for gram-positive bacteria	Columbia (CNA) agar LIM broth	
Media for gram-negative bacteria	MacConkey agar	
Neisseria gonorrhoeae (GC) media		Martin-Lewis agar Thayer-Martin agar (modified)
Anaerobic media	Anaerobic blood agar	

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Addendum C

Quality Control Organisms for Nonexempt Media

Medium	Atmosphere, length of incubation	Control organisms	Expected results
Chocolate agar	CO_2, 24-48 hrs	*N. gonorrhoeae* (ATCC 43069 or 43070) *H. influenzae* (ATCC 10211)	Growth Growth
Martin-Lewis agar	CO_2, 24–48 hrs	*N. gonorrhoeae* (ATCC 43069)	Growth
Thayer Martin	CO_2, 24-48hrs	*N. gonorrhoeae* (ATCC 43069)	Growth
V agar	CO_2, 24–48 hrs	*G. vaginalis* (ATCC 14018)	Growth