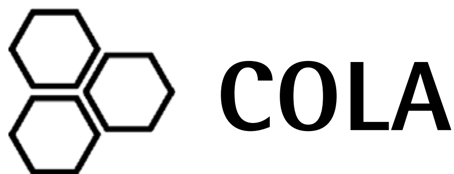

FAST FACTS 29

GENERAL MICROBIOLOGY

Requirements for COLA's Laboratory Accreditation and Community Hospital Laboratory Accreditation programs are underlined for your information. The reader is assumed to have a working knowledge of clinical microbiology.

Microbiology is a demanding specialty. The purpose of this COLA Fast Facts is to outline requirements for performing cultures and identifying organisms from miscellaneous sources.

Complexity

How a laboratory isolates and identifies organisms will determine the complexity level of the laboratory.

1. **Moderate Complexity**
   - Moderate complexity procedures for performing throat cultures, GC cultures, and urine colony counts have been described in COLA LabGuides available to COLA Laboratory Accreditation program participants. For cultures other than these three types, moderate complexity laboratories have three choices of how to report the growth of organisms. These are:
     - Growth
     - Growth - Referred for identification or interpretation
     - No Growth of bacterial colonies on solid media.

Helpful Tip for Reporting Growth or Lack of Growth of Organisms

Do not use the "No Growth" report if any of the following are present:

- Normal flora
- Contaminants
- Any organism not considered a pathogen

2. **High Complexity**
   - All procedures involving identification are considered to be high complexity.
   - All cultures inoculated into a broth medium, or to broth blood cultures, are considered to be high complexity.

Personnel

Call COLA's Information Resource Center at (800) 298-8044 to determine if your personnel are qualified by education and training to perform moderate or high complexity microbiology procedures.

Specimen Collection and Transport

In addition to ensuring the accurate and complete reporting of microbial growth, the laboratorian also should <u>follow the written instructions for proper specimen collection and transport, and monitor and enforce compliance to these instructions</u>. The proper specimen collection and transport instructions will vary depending on the site of specimen collection. The written instructions should reflect the different criteria necessary for the individual specimen type.

For collection of specimens from the lower respiratory tract, urinary tract, and genital tract the written instructions should include methods:

- For removing normal flora.
- To prevent contamination of the specimen.

Instructions for specimen collection from body sites normally considered sterile, such as a blood culture or joint aspirate, should include:

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- The procedure for decontaminating the skin prior to collection.
- The appropriate anticoagulant required to prevent the clotting of body or joint fluids.
- The adequate volume of specimen necessary to ensure proper specimen/anticoagulant ratio.

It is equally important to have written criteria established for specimen rejection. Always confer with the physician prior to rejecting any specimen. For example, sputum specimens are commonly collected incorrectly, and frequently consist of saliva heavily contaminated with oral cavity flora. To ensure an adequate sputum specimen, the laboratory may perform a microscopic examination for the relative numbers of leukocytes, macrophages, and squamous epithelial cells. Specimens containing greater than 25 squamous epithelial cells per low power field (100x) may indicate a saliva specimen and should not be cultured.

Document the time of specimen collection as part of the laboratory record. Process all specimens within three hours of collection to ensure the recovery of pathogens.

Helpful Tips for Specimen Collection and Transport

- Do not use heparin preparations that contain the preservative benzyl alcohol, as this preservative may inhibit organism recovery.
- If using swabs, use the less toxic Dacron swabs and place them in a non-nutrient transport medium such as Cary-Blair or Stuart transport medium.
- In general, swabs are not suitable for culturing Mycobacteriae or fungi.

Quality Control for Media

1. Prepared on-site

A laboratory may choose to prepare media for its own use and should follow the manufacturer's guidelines for preparing media from dehydrated stock. With each batch prepared, test the prepared media for sterility, final pH, and performance with stock cultures of organisms.

The stock cultures are usually described by the manufacturer of the dehydrated product and should:

- Represent the most delicate strains that are to be isolated.
- Represent the most hardy strains that are to be inhibited.

Confirm appropriate physical and biochemical reactions of the stock cultures by testing the prepared media with:

- The most weakly positive reactive strains.
- The strains most likely to give a false positive reading.

Helpful Tips for Media Prepared On-Site

- Store prepared media at 4°C in sealed bags to retard dehydration or consult the manufacturer's directions.
- Refer to the National Committee for Clinical Laboratory Standards (NCCLS) Document M22-A for more detailed guidelines on quality control for prepared media.

2. Commercially prepared media

When commercially prepared media are used, the manufacturer should provide the user with all of the information outlined above for each lot and type of media received.

If this information is not present, then perform the quality control (QC) testing for prepared on-site media. In addition to maintaining a record of this QC performance, complete the following:

- Record the date the media is received.
- Inspect the product for:
- Cracked petri dishes or tubes.
- Unequal filling of plates or tubes.
- Media that is cracked in the petri dish.
- Undesired hemolysis.
- Evidence of freezing.
- Excessive numbers of bubbles in the medium.
- Sterility.
- Evidence of leakage from tubes or bottles.

Whenever any of the above are observed, discard the media, notify the manufacturer, and document the notification in the laboratory's quality assurance records.

Additional Media Quality Control

Certain commercially-prepared media require a performance check upon receipt in the laboratory. In general, these media are fragile or contain components that are easily degraded during transport. Media include those used to isolate *Neisseria gonorhoeae* (e.g., Thayer-Martin), chocolate agar, and Campylobacter agar.

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Document once for each new lot or shipment of these specific media the demonstration of the inhibition of normal flora, and adequate growth of the target organisms.

Environment

Many organisms have specific environmental requirements in addition to their nutritional needs. Therefore, perform the following to closely monitor the environmental parameters:

- Maintain the laboratory at a room temperature range of 23-29°C with the relative humidity at 30-50%, unless otherwise specified by the manufacturers of your instrumentation. Values outside of this range may have undesired effects on culture media and may introduce error into serological testing.
- Record daily the actual temperatures of temperature-controlled equipment.
- Maintain the humidity in incubators at 40-50% and CO_2 levels at 5-10%.
- Place an oxygen-sensitive indicator in all anaerobic jars to ensure that anaerobic conditions are achieved during incubation.
- Semi-annually, monitor biological safety cabinets for the proper velocity of the air flow, and for the integrity of the high-efficiency air filters they contain.
- Periodically check the condition of inoculation loops and wires and calibrate quantitative loops.
- Decontaminate countertops daily and after any spills.
- Maintain documentation of all of these important monitors.

Media Selection

The choice of media for primary specimen inoculation will vary depending on the specimen type. **Appendix 1** contains a suggested protocol for routine cultures.

Examination and Retention of Cultures

Examine bacterial cultures after 18-24 hours of incubation. Many cultures are readable at this time and a preliminary report may be issued providing valuable information to the clinician. However, many bacteria require additional incubation for visible growth.

Helpful Tips for Examination and Retention of Cultures

- Retain for one week cultures of normally sterile body fluids, wounds, abscesses, and anaerobic cultures.
- Discard plates after 48 hours and reincubate only the broth cultures for the longer period of time.
- Examine stool cultures and subcultures for the presence of lactose negative colonies. If none are observed, discard cultures and subcultures.
- Hold cervical and vaginal cultures for 48 hours of incubation before issuing a negative report.
- Hold sputum cultures for one day.

Identification of Organisms

Once microbial growth has occurred, several methods can be used to identify the organisms. Since most clinical specimens are inoculated onto or into several media, including selective or differential agars, the first clue to identification of an isolated colony is the nature of the medium on which the organism is growing.

For example, with the exception of enterococci, only gram-negative bacteria grow well on MacConkey or other gram-negative selective agars. Media containing substances inhibitory to gram-negative bacteria, such as Columbia CNA agar, support the growth of gram-positive organisms. Most bacteria and fungi will flourish on nutrient or supplemented media, such as 5% sheep blood agar, chocolate agar, or brain heart infusion agar.

Although many experienced laboratorians may be tempted to identify organisms based on their characteristic morphological appearance, it is unwise to place total confidence on morphology for preliminary identification. For example, yeast colonies often resemble staphylococci.

Helpful Tips for Identification of Organisms

- Wet preps or gram stains of material from isolated colonies can provide valuable information on cellular morphology.
- Rapid spot biochemical tests can reliably be applied to many organisms.
- Coagglutination and latex particle agglutination tests are available commercially and provide rapid and accurate results.
- The most cost-effective measure available to the microbiologist is to presumptively identify bacteria based on a combination of these techniques.

The remainder of this COLA Fast Facts will focus on some of the available microbiology procedures and tests that are within the scope of the small laboratory.

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Gram-Positive Cocci

There are a few Gram-positive cocci that are frequently isolated in a small laboratory. These are: *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, Enterococcus species, and *Staphylococcus aureus*.

Helpful Tips for Identification of Gram-Positive Cocci

- On a Gram stain, staphylococci appear as clusters or groups of cocci and streptococci appear as chains of cocci.
- Streptococci and staphylococci can be differentiated with the use of a catalase reaction. Staphylococci test positive for the catalase reaction, while streptococci test negative.
- The coagulase test can be used to quickly screen isolates for *Staphylococcus aureus*, which are almost always coagulase-positive.
- *Streptococcus pyogenes* (group A strep) and *Enterococcus* species are able to hydrolyze the substrate PYR (L-pyrrolidonyl b-naphthylamide). The released free b-naphthylamide reacts with a second substrate, N,N-dimethyl-aminocinnam-aldehyde to produce a red color. This test is as specific as the bile esculin agar and salt broth tests used to identify enterococci and more specific than the overnight bacitracin test used to presumptively identify group A streptococci. Perform this test on isolated colonies with characteristic Streptococcus culture morphology.
- A simple and reliable presumptive test for the identification of *Streptococcus agalactiae* (group B Strep) is the CAMP reaction. An arrowhead shaped area of hemolysis occurs when group B streptococci grow in the presence of β-toxin-producing staphylococci on sheep blood agar.
- Group B β-hemolytic streptococci, *Gardnerella vaginalis*, and *Listeria* species all hydrolyze sodium hippurate.
- *Streptococcus pneumoniae* may readily be identified by an optochin susceptibility test, where an appropriate-sized zone of inhibition indicates susceptibility and that the organism is a pneumococcus. Refer to the package literature supplied with the disks used for zone interpretations. A bile solubility test may be utilized, as well, by directly applying 10% sodium desoxycholate solution to suspect colonies. Those alpha hemolytic colonies that are *S. pneumoniae* will lyse.

Appendix 2 contains additional information on presumptive groupings for streptococci and enterococci.

Gram-Negative Coccobacilli

Gram-negative coccobacilli that grow well on chocolate agar but not on sheep blood agar are typical of *Haemophilus* species. These organisms require the presence of hemin (X factor) and/or nicotinamide adenine nucleotides (V factor) for growth. V factor may be supplied by a streak of staphylococci across the agar surface about which satellite colonies of V-dependent strains of *Haemophilus* can grow. The requirements for X and V factors may be determined by placing filter paper disks impregnated with the individual factors onto a soybean-casein digest agar surface that has been inoculated with the test organism.

Gardnerella vaginalis is a pleomorphic gram-negative bacillus that is usually isolated from the genital/urinary tract. It requires neither X or V factor for growth, but is best isolated on HBT agar where it produces small β-hemolytic colonies. The organism can also be isolated on chocolate agar where it produces alpha hemolytic colonies. *G. vaginalis* is catalase-negative, oxidase-negative, but will hydrolyze sodium hippurate.

Gram-Negative Bacilli

Colonies morphologically resembling *Pseudomonas aeruginosa* may be presumptively identified based on observation of β-hemolysis, blue-green pigment, characteristic grape-like odor, and positive oxidase test results.The oxidase test may be performed by flooding the agar surface with the oxidase reagent and observing for the characteristic blue-purple color development of positive colonies. The Kovac's method may be more useful as viable colonies remain for other testing. Moisten filter paper with the oxidase reagent, and, using a wooden applicator stick or platinum loop, apply material from the bacterial colonies to the filter paper. Blue to purple color development within 10 seconds is interpreted as a positive reaction. Oxidase reagent-impregnated paper is commercially available and only requires application of bacterial cultures.

The spot indole test can be used to differentiate swarming *Proteus* species from one another and in the presumptive identification of *Escherichia coli*. Clear colonies of bacteria that swarm on sheep blood agar and spot test positive with the indole reagent may presumptively be reported as *P. vulgaris* while those that are indole-negative are *P. mirabilis*. Be aware that bacteria other than *Proteus* species may swarm. Non-mucoid, flat, pink lactose-positive colonies on MacConkey agar with a positive spot indole test and a negative oxidase test may presumptively be reported as *E. coli*.

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Multi-test systems

Many classical biochemical tests have been modified to allow more rapid results with a smaller volume of reagent and a heavier inoculum. Many of these have been packaged so that multiple tests can easily be inoculated at the same time. The biochemical reaction patterns may be interpreted with the aid of a computer-generated database in which reaction patterns translate into an organism identification with a calculated predictive probability. Some manufactured products can produce identification results in as few as four hours, while others require overnight incubation. Some test systems include susceptibility testing as well as identification components on the same microtiter tray. Innovative formats for microbial testing continue to be developed in one of the fastest changing laboratory arenas.

Quality Control

Performance monitoring of every procedure used in microbial identification is as important as choosing the proper testing protocol. Even rapid spot tests should have quality control performed on a regular schedule with organisms that will demonstrate positive and negative reactions. Appendix 3 is a guideline of a QC protocol with the required frequency of testing.

Quality control of X, V, and XV disks or strips for the identification of Haemophilus species may be performed in the following fashion:

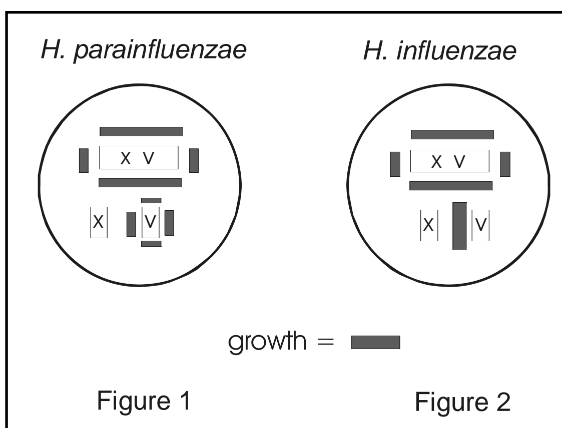

Figure 1 Figure 2

1. Inoculate a suspension of the test organism as you would inoculate a plate for disk susceptibility testing.
2. Place the individual X and V disks about 1 cm apart. Incubate overnight at 35°C and 5-10% CO_2
   - For *H. parainfluenzae*, growth should occur around the XV strip, and around the V strip, but not around the X strip (Figure 1).
   - For *H. influenzae*, growth should occur around the XV strip and between the X and V strips, but around neither X nor V, indicating the nutritional requirement that both be present for growth. (Figure 2).
3. Perform quality control with each new lot or shipment of factor disks or strips, and weekly thereafter.

For multi-test identification systems, the best guideline for quality control requirements will be found in the manufacturer's package literature. These brochures will usually identify the organisms necessary for adequate quality control of their product. In general, expect to use several (three to five) organisms to verify the performance of the identification portion of the product.

Perform quality control testing once upon receipt of a new lot or shipment of product to verify that the manufacturer's stated performance characteristics have not been compromised during handling and transport. The susceptibility portion of the test system must be tested with quality control organisms each day of use, or once weekly if your laboratory has satisfied the requirements for weekly quality control testing.

Appendices 4 and 5 contain daily and weekly QC forms for you to use as a guide.

Proficiency Testing

Proficiency testing (PT) requirements, for the specialty of microbiology, are handled differently from other laboratory specialties. In microbiology, a laboratory needs to perform a minimum of five samples per testing event in each subspecialty of microbiology. These samples should represent all of the types of microbiology testing the laboratory performs.

Proficiency testing must be performed for organisms that are regulated within each subspecialty. The five PT specimens per subspecialty can be spread out over the testing performed within each subspecialty as long as each type of testing is assessed. For example, if a laboratory performs three different direct antigen tests and three different types of cultures/ID methods, then the lab has the option of choosing which antigen detection test(s) and which type(s) of cultures/ID methods to include within the five total challenges.

The proficiency testing samples must be treated in the

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same manner as patient samples and identified to the same level of identification and susceptibility as patient samples.

Culture Considerations for Anaerobic Organisms

The basic procedures used for anaerobes differ from those used for aerobic organisms mainly in that incubation is carried out in the absence of air. Plastic anaerobic incubation jars and commercially prepared anaerobic media have provided a convenient and reliable method for isolation of these organisms.

Isolation of pure colonies remains the critical procedure in anaerobic microbiology. Anaerobes are usually present in mixed culture along with other aerobic or anaerobic organisms. Extra care taken during subculturing these organisms will allow you to obain a pure colony more quickly. Anaerobe identification can be a very slow and time-consuming activity since a longer incubation of two days is generally required.

Helpful Tips for Culturing Anaerobic Organisms

- As with aerobic organisms, collection and transport of specimens is crucial to successful diagnosis of infection. Many commercial manufacturers have anaerobic transport products.
- Take care to avoid contamination with normal flora, to maintain the specimen under anaerobic conditions, and to promptly process the specimen in the laboratory.
- When possible, collect aspirates with a needle and syringe or collect tissue samples for culture.
- Do not use swabs as they may expose anaerobes to air, tend to dry out, and limit sample volume.
- Perform a Gram stain, as the Gram stain report of a direct smear helps the physician choose a course of therapy during the period of several days between specimen collection and the culture report.
- Observations on the Gram stain will also provide a guide for culture media selection and the procedures that follow. If organisms observed in the smear are not isolated on culture, suspect defective procedures in specimen collection and transport, environment, handling, etc.
- Store solid media for primary isolation in an anaerobic atmosphere for at least 24 hours before use to allow “dissolved” oxygen in the medium to diffuse.
- Include an enriched broth medium as a “backup” to solid media during culture set-up.
- Include an oxygen-sensitive indicator device in the anaerobic incubation jar or chamber to monitor that anaerobic conditions have been met during incubation.
- Anaerobic jars should be incubated at 35°C for at least 48 hours prior to opening, as the organisms are most sensitive to the effects of oxygen in the first 24 hours of incubation.
- Identification of anaerobic organisms is carried out much as it is for aerobes. Most commonly encountered anaerobes can be presumptively identified by a relatively small number of tests, including colony and growth characteristics on solid and in liquid media, gram stain morphology, inhibition by certain antibiotics, and biochemical characteristics.
- The analysis of metabolic products by means of gas-liquid chromatography is a classic method used in sophisticated laboratories for organism identification.
- Several commercially available packaged identification systems are available to the laboratory staff. Some rapid methods may provide results in four hours, relying on preformed enzymes to break down test substrates.
- Methods that rely on organism growth will have a longer turnaround time.
- All of the pathogenic anaerobic bacteria are part of the normal flora of the body, and all of them are pathogens of opportunity.
- Do not wait until the organism species is identified to communicate with the physician. The laboratory staff may be of considerable assistance to the physician in assessing the significance of an isolate.
- A Working Group of the National Committee for Clinical Laboratory Standards (1989) has determined that susceptibility testing of anaerobic bacteria is generally unnecessary.
- When the patient’s clinical situation indicates the need for susceptibility information, it is recommended that these procedures be performed at a reliable referral laboratory.

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References

1. Baron, E., Peterson, L., Finegold, S. *Bailey and Scott's Diagnostic Microbiology*, 9th ed. St. Louis, C.V. Mosby. 1994.

2. Henry, J.B. *Clinical Diagnosis & Management By Laboratory Methods*, 18th ed. Philadelphia, PA. W.B. Saunders Company. 1991.

3. National Committee for Clinical Laboratory Standards. *Physician's Office Laboratory Guidelines; Tentative Standard*. NCCLS Document POL1-T2, Vol.12, No. 5. Villanova, PA, 1992.

Additional Resources

1. National Committee for Clinical Laboratory Standards, 940 West Valley Road, Suite 1400, Wayne, PA 19087. Phone: (610) 688-0100, Fax: (610) 688-0700.

2. National Committee for Clinical Laboratory Standards. *Quality Assurance for Commercially Prepared Microbiological Culture Media; Approved Standard*. NCCLS Document M22-A, 1990.

3. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Disk Susceptibility Tests, ed. 5; Approved Standard*. NCCLS Document M2-A5, 1993.

4. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, ed. 3; Approved Standard*. NCCLS Document M7-A3, 1993.

5. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Susceptibility Testing; Fifth Informational Supplement*. NCCLS Document M100-S5, 1994.

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APPENDIX 1

MEDIA FOR PRIMARY SPECIMEN INOCULATION

SPECIMEN TYPE	MEDIA	ADDITIONAL MEDIA
Abcess	Blood agar, MacConkey, Anaerobic blood agar, Thioglycolate broth	Columbia agar with colistin and nalidixic acid (CNA) as Gram stain indicates.
Blood	Chocolate agar, Anaerobic blood agar	as Gram stain indicates
Joint Fluid	Blood agar, Chocolate agar, Thioglycolate broth	
Ear, external	Blood agar, MacConkey agar, Chocolate agar	CNA
Ear, internal	Blood agar, MacConkey agar, Chocolate agar, Thioglycolate broth	CNA
Conjunctiva	Blood agar, MacConkey agar, Chocolate agar, Thioglycolate broth	CNA, Thayer-Martin agar
Nasopharynx	Blood agar, Chocolate agar	CNA
Nose	Blood agar, CNA	Mannitol Salt
Sputum	Blood agar, MacConkey agar, Chocolate agar	CNA
Deep wounds	Blood agar, MacConkey agar	Chocolate agar, CNA
Traumatized areas	Blood agar, MacConkey agar, Chocolate agar, Thioglycolate broth	CNA
Foley catheter	Do not culture	
Urethra, female	Thayer-Martin agar	Chocolate agar with 1% IsoVitaleX
Urethra, male	Thayer-Martin agar	Chocolate agar with 1% IsoVitaleX
Cervix	Thayer-Martin agar	Chocolate agar with 1% IsoVitaleX
Vagina	Thayer-Martin agar	Human Blood-Tween Bilayer agar (HBT)
Prostate Fluid	Blood agar, MacConkey agar, Thayer-Martin agar,	CNA
Feces	Blood agar, MacConkey agar, Hektoen Enteric agar, Xylose Lysine Deoxycholate (XLD) agar, Gram-negative broth, Campylobact*er* agar (42°C)	

APPENDIX 2

PRESUMPTIVE GROUPINGS FOR STREPTOCOCCI AND ENTEROCOCCI

Test	Beta Hemolytic Strep Groups		Non-Beta Hemolytic Strep Group D	
	A	B	Enterococcus	Non-enterococcus
Bacitracin*	+	—	n/a	n/a
Hippurate	—	+	n/a	n/a
Camp reaction	—	+	n/a	n/a
PYR	+	—	+	—
Bile esculin agar	n/a	n/a	+	+
NaCl broth	n/a	n/a	+	—

*** Almost 10% of non-group A Beta-hemolytic streptococci will be inhibited by Bacitracin.**

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APPENDIX 3

GUIDELINE FOR QUALITY CONTROL PROTOCOL

Test	Frequency	Positive Control		Negative Control	
		Organism	Result	Organism	Result
Bacitracin	**lot, weekly**	***S. pyogenes***	**A zone of inhibition around the disk.**	***S.agalactiae***	**Uniform growth up to the edge of the disk.**
Bile esculin	**lot**	***E. faecalis***	**Blackening of agar slant.**	***S. bovis***	**No blackening of agar slant.**
Bile solubility	**lot**	***S. pneumoniae***	**Lysing of colony.**	***S. mitis***	**No lysing of colony.**
Camp test	**daily**	***S. agalactiae***	**Enhanced hemolysis where the streak of the control organism approaches the streak of *S. aureus*.**	***S. pyogenes***	**No enhanced hemolysis.**
Catalase	**lot, daily**	***S. aureus***	**Production of bubbles.**	***S. pyogenes***	**No change.**
Coagulase	**lot, daily**	***S. aureus***	**Clumping or coagulation of rabbit, human, or pig plasma.**	***S. epidermidis***	**No clumping or coagulation.**
Gram stain	**lot, weekly**	***S. aureus***	**Organism stains blue.**	***E. coli***	**Organism stains red.**
Hippurate	**lot**	***S. agalactiae***	**Deep purple color produced.**	***E. faecalis***	**No color change.**
Indole	**lot**	***E. coli***	**Red color produced.**	***Enterobacter cloacae***	**No color change.**
NaCl broth	**lot**	***E. faecalis***	**Turbidity occurs.**	***S. bovis***	**No turbidity.**
Optochin	**lot, weekly**	***S. pneumoniae***	**A zone of inhibition around the disk.**	***S. pyogenes***	**Uniform growth up to the edge of the disk.**
Oxidase	**lot, daily**	***P. aeruginosa***	**Pink or purple color develops depending on methodology**	***E. coli***	**No color change.**
PYR	**lot**	***S. pyogenes***	**Red color produced.**	***S. agalactiae***	**No color change.**

Daily = QC testing each day of use for patient testing.
Lot = QC testing with each new lot or batch of reagent or product upon receipt.
Weekly = QC testing at least once a week.

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Appendix 4

Microbiology Daily Quality Control

Month/Year__________________________ **Supervisor Review**____________________

Indicate the observed reaction

Day	**Tech initials**	**Oxidase**		**Catalase**		**Coagulase**		**PYR**		**Camp**		**Indole**	
		P. aeruginosa +	*E. coli* -	*Staph.* species +	*Strep.* species -	*S. aureus* +	*S. epidermidis* -	*S. pyrogenes* +	*S. agalactiae* -	*S. agalactiae* +	*S. pyogenes* -	*E. coli* +	*E. cloacae* -
1													
2													
3													
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Document Corrective Action on the Next Page, marked Appendix 5.

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Appendix 5

Microbiology Quality Control
WEEKLY

Month/Year___________________________ **Supervisor Review**____________________

Indicate the observed reaction

Date	Tech	Gram Stain		Bacitracin		Optochin		XV Disks	X & V Disks	
		Staph. sp. +	*E. coli* -	*Strep. pyogenes* +	*Strep. agalactiae* -	*Strep. pneumoniae* +	*Strep. mitis* -	*Haemophilus sp.* Growth	*H. parainfluenza* Growth around V disk only	*H. influenza* Growth between disks

PER LOT OR SHIPMENT

Month/Year___________________________ **Supervisor Review**_____________________

Indicate the observed reaction

Date	Tech	Bile Esculin		NaCl Broth		Hippurate		Bile Solubility		PYR		Indole	
		Lot #		Lot #		Lot #		Lot #		Lot #		Lot #	
		Exp. Date:		Exp. Date:		Exp. Date:		Exp. Date:		Exp. Date:		Exp. Date:	
		E. faecalis +	*Strep. pyogenes* -	*E. faecalis* +	*Strep. bovis* -	*Strep. agalactiae* +	*E. faecalis* -	*Strep. pneumonaie* +	*Strep. mitis* -	*Strep. pyogenes* +	*Strep. agalactiae* -	*E. coli* +	*E. cloacae* -

Corrective Action:

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