Microbiology

Requirements for good laboratory practice and COLA Laboratory Accreditation programs are underlined.

SUBSPECIALTY - BACTERIOLOGY

Infectious diseases are caused by a wide variety of microorganisms. The selection of the proper specimens and test procedure are important in the detection and identification of the microorganisms responsible for the disease. Various methods such as microscopic examination, cultures, biochemical, and immunological techniques are used to analyze patient specimens.

SPECIMEN COLLECTION AND HANDLING

Proper collection and storage is critical for quality testing and reliable results. All laboratories must have written policies, procedures, and instructions for the collection, labelling, reporting, and referral of patient specimens. Be sure to include in the procedure manual the following: specific specimen collection requirements (also see manufacturer's instructions), specimen stability, special patient preparation (such as for clean catch urine specimens), verification procedures for patient identification, and proper labelling and handling techniques for each microbiological test procedure performed.

CULTURE MEDIA

1. Keep the plates wrapped in their plastic sleeve until used. Mark the date received on the plastic sleeve wrapper.

2. Note the date of expiration and do not use after that date.

3. Store the plates in the refrigerator using recommended manufacturer's instructions.

4. Check each plate before using it, looking for:
- cracked petri dish
- uneven filling of agar
- very pale color in the sheepblood plates
- dry or cracked media
- too many bubbles
- organisms growing on unused plates

NOTE: If any of the above conditions are identified, notify the manufacturer of the problem. Document this.

5. All culture plates should be brought to room temperature before use.

Media plates must be checked for hydration and sterility, and reference cultures should be used to check the media for the ability to grow the appropriate microorganisms.

Make sure the media is not dried up. **Expiration dates** should also be checked since using expired media can prevent growth of organisms or result in improper growth. Call the manufacturer or supplier for replacement of expired or damaged media. If the proper media to set up a culture is not available, then refer the specimen to the reference lab after consulting the reference laboratory manual for specimen transport requirements.

A **daily sterility check** can be performed by visually checking the media for growth. Dispose of media with growth and record this action in the quality control logs. Sterility checks for all media should also be performed when new batches arrive from the manufacturer. Simply incubate a plate from each batch of different type of media and observe at 24 hours for growth. If there is no growth, the plates can be used for control specimens. Record the results in the quality control logs.

Reference organisms are used as controls for cultures and should be maintained and sub-cultured weekly to substantiate the growth of a particular organism on the different types of media used in the laboratory. Incubate the plates overnight and store in the refrigerator between subculture. The GC reference organism will need to be subcultured more frequently since it is difficult to keep alive. The following are suitable reference organisms:

Group A Beta Streptococcus
Non-Group A Beta Streptococcus
Coagulase negative Staphylococcus species
Staphylococcus aureus - ATCC # 29213
Escherichia coli - ATCC # 25922
Pseudomonas aeruginosa - ATCC # 27853
Neisseria gonorrhoeae - ATCC # 43070

Reference organisms can be obtained from the supplier of your culture plates and reagents. You may also consider asking a local hospital or reference laboratory to supply you with the reference organisms you need. Proven microorganisms from patient specimens may also be used. The exception to this is in the case of antimicrobial susceptibility testing

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and microbe identification systems when the organism must be a certain ATCC (American Type Culture Collection) strain as listed above, since these organisms exhibit specific reactivity patterns which are necessary to verify the expected reactivity of the system being used.

A plate from every new batch of culture plates should be tested with the appropriate reference organism to show that the media supports, selects, or inhibits bacterial growth, or has the biochemical reactivity that is expected. These quality control steps are not necessary if there is documentation available from the manufacturer (such as package inserts) to show that these specifications have been checked according to the National Committee on Clinical Laboratory Standards (NCCLS).

NOTE: This exception does not include chocolate agar, Campylobacter media, and selective media for the isolation of Neisseria species. These must be controlled upon receipt in the laboratory due to a high percentage of failure during shipment.

DISCS

With each batch or shipment of discs, note the expiration date. Discs must be stored with a desiccant and at the temperature recommended by the manufacturer. Be sure to check the desiccants that are supplied with multi-disc dispensers. Replace the desiccant in multi-disc dispensers according to the manufacturer's instructions. The appropriate reference organisms (positive and negative) should be used with the corresponding culture media to check for the expected activity of the discs.

If bacitracin (A) discs are used for throat cultures, you must use a Group A beta-hemolytic Streptococcus reference organism as your positive control and a non-Group A beta-hemolytic Streptococcus reference organism (e.g. Group C Streptococcus) as a negative control each week of use and when a new batch or lot number is being introduced.

If optochin (P) discs are used, then you must use a Streptococcus pneumoniae reference organism as a positive control and an alpha-hemolytic Streptococcus (non-Streptococcus pneumoniae) reference organism as a negative control. When using sensitivity discs, the appropriate reference organisms and culture media must be used to check for the expected zone of inhibition of each type of disc.

If novobiocin discs are used, then you must use a coagulase negative Staphylococcus species reference organism for the negative control and the Staphylococcus saprophyticus reference organism as a positive control. The recommended reference organisms to be used with the corresponding antimicrobial discs are: Staphylococcus aureus for Gram positive susceptibility, Escherichia coli for Gram negative susceptibility, and Pseudomonas aeroginosa for Pseudomonas aeroginosa susceptibility. Refer to manufacturer package inserts of the ATCC reference organisms for the expected zone sizes. Refer to manufacturer package inserts for additional information on antimicrobial disc quality control. Record all results in the quality control logs.

GRAM STAIN

Control slides should be prepared using the appropriate reference organism and stained with the Gram stain weekly to verify the proper staining characteristics of the stain. Staphylococcus or Streptococcus reference organisms can be used to demonstrate the Gram positive reaction, and the Escherichia coli reference organism can be used to demonstrate the Gram negative reaction. Freshly-prepared Gram stain reagents should be labelled with the date prepared and the expiration date. Commercially-prepared or freshly-prepared Gram stain reagents should not be used after the expiration date. Record all results in the quality control logs.

CATALASE TEST

The catalase test is used to differentiate the Staphylococcus species from the Streptococcus species which may be present on a culture plate. Positive and negative controls must be performed each day of use. This involves using an inoculum from one of the Staphylococcus reference organism plates as a positive control and an inoculum from one of the Streptococcus reference organism plates as a negative control. Record results in the quality control logs.

COAGULASE TEST

The coagulase slide, tube test, or coagulase latex agglutination test is used to differentiate Staphylococcus aureus from other Staphylococcus species which may be present on a culture plate. Positive and negative controls must be performed each day of use. This involves using an inoculum from the Staphylococcus aureus reference organism plate as a positive control and an inoculum from a coagulase negative Staphylococcus species reference organism plate as a negative control. If controls are provided with the coagulase latex agglutination test, then they may be used in lieu of the control organisms mentioned above. Record all results in the quality control logs.

OXIDASE TEST

The oxidase test is frequently used to verify the presence of Gram negative diplococci which may be present on a modified Thayer-Martin or Martin-Lewis culture plate. It is also used to differentiate Pseudomonas species and similar organisms from the Enterobacteriaceae group. Positive and negative controls must be performed each day of use. This involves using an inoculum from the Neisseria gonorrhoeae or Pseudomonas aeruginosa reference organism plates as a positive control and an inoculum from one of the Staphylococcus species or Escherichia coli reference organism plates as a negative control. Record all results in the quality control logs.

"X" AND "V" FACTORS

X and V factors are used to differentiate between the various species of Haemophilus. The most common Haemophilus species have been isolated from specimens such as eye discharges, sputum, vaginal discharges, or blood. X and V factors are supplied as individual filter strips or discs, or as a combination of the two factors on filter strips or discs. When X and V factors are being used, quality control must be performed each week of use. The appropriate Haemophilus species reference organisms should be used to verify the expected activity for each factor or combination of the two factors. Refer to manufacturer package inserts for additional information. Record all results in the quality control logs.

BETA-LACTAMASE

The Beta-Lactamase test is used to detect penicillin-resistant strains of Neisseria gonorrhoeae. Check with your microbiology manufacturer, local hospital laboratory, or reference laboratory for positive and negative Beta-Lactamase GC reference organisms to use for quality control. Quality control must be performed each day of use with the reference organisms or controls supplied by the Beta-Lactamase test kit manufacturer. Record all results in the quality control logs.

ANTIMICROBIAL SUSCEPTIBILITY TESTING (Kirby-Bauer)

This method of testing is used to determine the susceptibility or resistance of a particular microorganism to a selection of appropriate antimicrobial agents (antibiotics). When using this method of susceptibility, the inoculum (saline or a brain heart infusion [BHI] broth suspension of bacteria) must be of a specific turbidity (cloudiness). The turbidity of the inoculum is compared to a 0.5 McFarland Standard (0.5 Barium sulfate solution). The McFarland standard can be purchased from the supplier of your microbiology culture media.

Please note the expiration date of the standard and replace as recommended by the manufacturer. When the inoculum suspension matches the turbidity of the McFarland standard, it is considered the right strength for susceptibility testing. If your suspension is too "weak" you may obtain zones larger than what would represent the susceptibility of the organism and you may report out that an organism is susceptible to an antibiotic to which it is really resistant. The reverse is also true, too strong a suspension may result in reporting that an organism is resistant to an antibiotic to which it is really susceptible.

In order to check the proper performance of your antibiotic discs, you should set up susceptibility tests using reference organisms which are indicated in the package inserts of the antibiotic discs you use. These reference organisms will produce zones of inhibition as specified in the package inserts. This quality control procedure should be performed each day of use for infrequent users of susceptibility testing. For

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frequent users of susceptibility testing, controls may be run for 30 consecutive days of testing using the appropriate reference organisms and antimicrobial discs. The zones of inhibition are recorded and observed during the 30 day test period. If the results are within the expected ranges, then the quality control can be run once a week. Refer to COLA *LabFacts 31--Antimicrobial Susceptibility (Sensitivity) Testing*.

These microbiology controls and procedures must be DOCUMENTED. You should develop a form which includes a place to chart procedures (such as media checks) and a place to record the results of each of the controls you have performed. Include a place to put the date and the initials of the person who performed the controls.

For a more in-depth discussion of biochemical tests used for organism identification and helpful charts on tracking daily QC acitivities, please request COLA FastFacts #29--General Microbiology by calling the COLA Information Resource Center at (800) 981-9883. You may also access this information on line at http://www.cola.org/fastfacts/ffintro.htm.