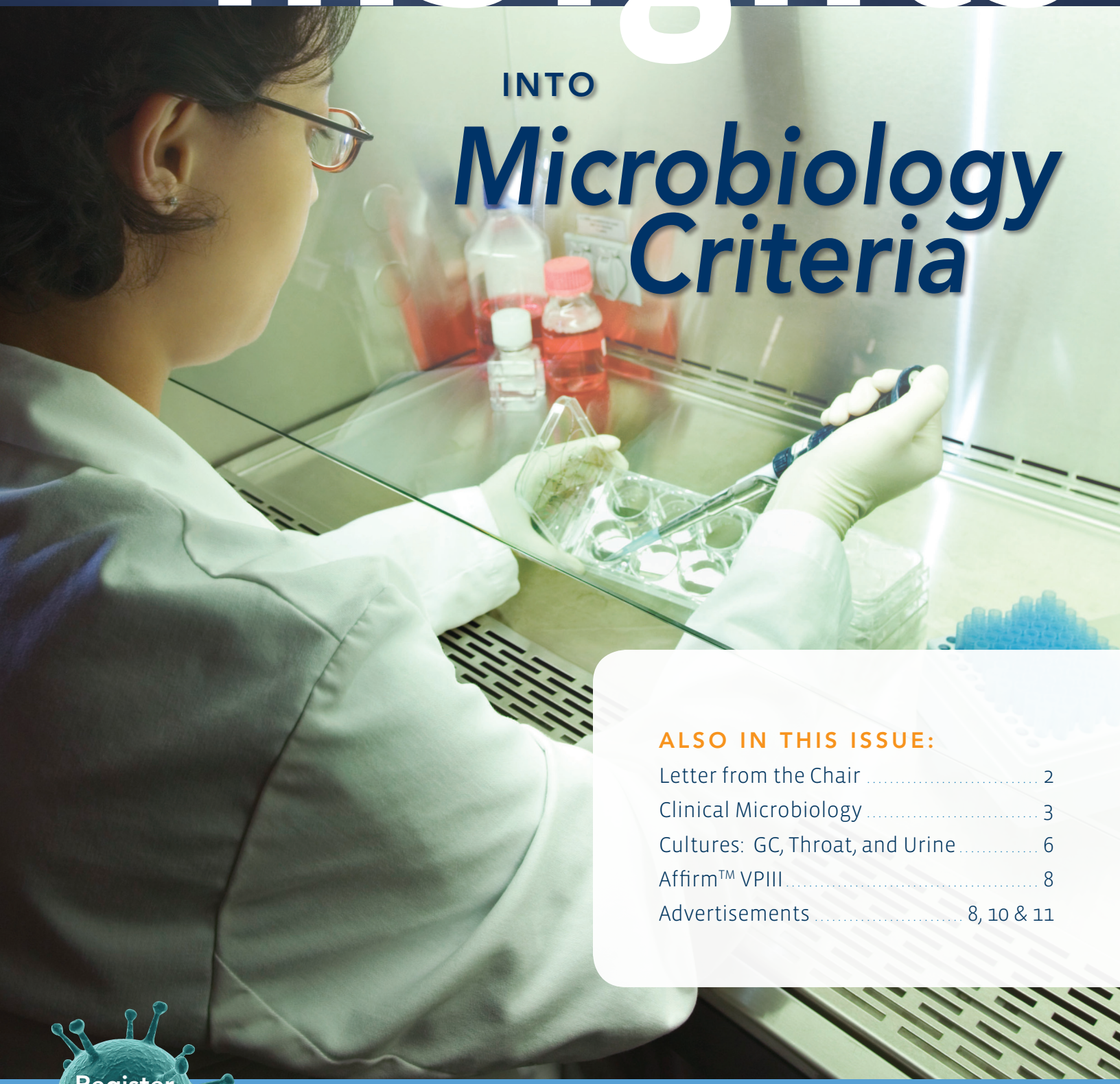
SEPTEMBER / OCTOBER '12

COLA'S

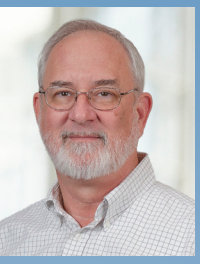
inSights



INTO Microbiology Criteria

ALSO IN THIS ISSUE:

"FLU: SEASONAL OR PANDEMIC, WHAT SHOULD WE DO?" *Webinar Info on Page 12*



FROM THE CHAIR

In past issues of *Insights*, we have highlighted Complete Blood Counts and the flags that you may see with automated analyzers (Hematology). We have also focused on the importance of PT/INR testing and the hand held devices that can be used for point-of-care-testing (Coagulation).

With this issue, we again concentrate on one particular specialty. This time we delve deeper into Microbiology. Specifically, we look at the COLA criteria that we use to assess this specialty. The general "M" category criteria are discussed in our cover article, "Clinical Microbiology."

Criteria that are often cited with three commonly performed cultures (throat cultures to isolate group A Strep, urine cultures, and GC cultures) are explained in another article. The final article in this series clarifies confusion seen by our surveyors in laboratories using a particular test kit (the Affirm VPIII kit from BD).

The common thread throughout the issue is that we want to help you understand what you are doing and why you're doing it. This will lead to better test performance, more accurate test results, and higher quality patient care – our ultimate goal.

W. James Stackhouse, MD, MACP
Chair, COLA Board of Directors

COLA

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Clinical Microbiology

Definition: "Clinical microbiology is concerned with the investigation, diagnosis and, in an advisory capacity, management of infection caused by microorganisms – viruses, bacteria, parasites and fungi. There are also clinical scientists in this area working in immunology and public health." [1]

Traditionally, clinical microbiology has been synonymous with "culture & sensitivity." To determine the cause of infection, a specimen (such as urine, stool, swab of the infected area, etc.) was sent to the lab to be cultured. The specimen was inoculated onto selective agar plates containing nutrient rich media that supported the growth of the suspected causative agent. The agar plates were then incubated at specific environmental conditions for 24 to 48 hours. After microbial colonies were isolated, the agent's sensitivity to antibiotics could be determined. After 3 – 5 days, antimicrobial treatment was identified, and patient treatment could finally be established. This timing was not ideal, since patients often suffered through symptoms for 7 – 10 days before seeking help.

Research advances have dramatically changed the face of clinical microbiology. From early immunological studies detailing how the body develops antibodies to fight foreign agents to the most recent molecular / genetic discoveries, the knowledge has been used to create ever evolving techniques to manage patient diagnosis and treatment. The quick, waived test methods in use today are a direct result of this research.

In Microbiology, waived test methods exist for a wide variety of organisms and the number continues to increase. The widely used Monospot® and rapid strep kits have been joined by waived tests for *H. pylori*, Influenza, Lyme disease, RSV (Respiratory Syncytial Virus) and HIV (Human Immunodeficiency Virus), to name a few. However, as with all clinical diagnostic testing, it is important to confirm the complexity of your test method[2] as this will determine Proficiency Testing requirements and the qualifications that laboratory personnel must meet.

Regardless of whether the test method is waived or non-waived, the quality of the result obtained depends on how the test is performed. Since test results directly impact patient care, testing personnel should ensure the test is performed correctly by following ***all*** instructions, including those addressing regulatory guidelines, Quality Control, and when applicable, instrument maintenance.

COLA CRITERIA

For the remainder of this article, we will offer compliance tips for the Microbiology category of COLA criteria.

M 1

M 1: *Are specimens for microbiology cultures collected using the appropriate type of swab or collection device?*

"Using the appropriate type of swab or collection device" is important for test kits as well as cultures. There are several factors involved in understanding what is appropriate. Among others, some of these factors are:

- The principle of the test;
- The nature of the collection device; and
- The suspected causative agent.

For example, when culturing bacteria, the bacteria must remain viable during transport to the laboratory, and inoculation of the nutrient agar. The swab (collection device) must be placed in specific transport medium (preservative) to maintain the viability of the organism.

>> CONTINUED ON PAGE 4

When using rapid strep kits for the identification of Group A Streptococcus, viable bacteria are not required. Therefore, it is not necessary to place the collection device in transport medium / preservative. Placing the collection device in transport medium may dilute the specimen which can lead to false negative results.

Neisseria gonorrhea provides another example of the importance of using the appropriate type of swab or collection device. Fatty-acids contained in cotton are toxic to *Neisseria gonorrhea,* a very highly fastidious organism; therefore, cotton swabs should not be used for collection and transport when *N. gonorrhea* infection is suspected.

M 2, M 3, and M 4

M 2: Are specimens plated on appropriate media to support the growth of potential pathogens?

M 3: Is the culture medium at room temperature prior to plating the specimen?

M 4: Are cultures incubated using the appropriate incubation conditions for atmosphere and temperature?

Different microorganisms grow under different conditions, including varying nutrient requirements and assorted environmental needs. Similar to M 1, knowing the suspected causative agent will help in understanding what conditions are appropriate for growth.

In general, microorganisms can be divided into three categories based on atmospheric needs:

- Aerobic – organisms require the presence of oxygen to grow;
- Microaerophilic – organisms require less oxygen and a higher degree of CO_2; and
- Anaerobic – organisms require the removal of all oxygen.

Special incubators are available to ensure that the required atmospheric conditions are achieved. Other procedures, such as the use of candle jars or gas packs, can be implemented to create an increased CO_2 or anaerobic environment.

Since microorganisms can be very sensitive to cold, it is important that agar plates are at room temp when inoculated. The likelihood of obtaining bacterial growth of any significance is diminished when inoculated onto a cold culture plate. For optimal growth, most microorganisms require an incubation temperature range of 35 – 39°C; however, several organisms grow best at alternate temperatures – such as 42°C or room temp (20 – 25°C).

It is important to know the suspected causative agent to ensure that the appropriate environmental conditions are met.

M 5, M 6, and M 7

M 5: Is each batch or shipment of media checked to show:

- *It is sterile; AND*
- *Supports, selects, or inhibits bacterial growth, (as appropriate based on type of media); OR*
- *Has the biochemical reactivity that is expected?*

Documentation available to show that the manufacturer has checked these specifications according to the standards of the Clinical and Laboratory Standards Institute (CLSI) can satisfy this requirement.

M 6: *Are media visually inspected before use?*

M 7: Does the laboratory report any deteriorated or substandard media to the manufacturer?

Contaminated plates are not uncommon, even with commercially-prepared media; therefore, it is important to ensure that the media is in good condition prior to inoculation. False positives will occur if specimens are inoculated on contaminated plates. The general condition of media should be checked upon receipt and this visual inspection should be documented. This can be easily accomplished by including comments on inventory or receipt logs (e.g., "Lot received mm/dd/yy; visually inspected – OK – mm/dd/yy by initials"). Media should also be checked prior to inoculation, but this inspection may or may not be documented.

Deteriorated or substandard media should be reported to the manufacturer. Most manufacturers will provide free replacements for substandard media reported to them, since this helps identify a potential quality issue in their manufacturing process. Documenting this notification and replacement will also help you monitor your inventory. Documentation of an increase in contaminated or damaged agar plates sent by a particular manufacturer may help you consider alternative suppliers.

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CLINICAL MICROBIOLOGY

Many manufacturers routinely check their media in accordance with standards established by the Clinical and Laboratory Standards Institute (CLSI) and provide documentation ensuring their media conforms to these standards. For most media, this documentation provides evidence to meet criterion M 5; however, there are selective media that are exceptions. For example, the CLSI documentation is not applicable to chocolate agar, Campylobacter media, and selective media for the isolation of Neisseria species. Due to a high failure percentage that may occur during shipment, identification systems with multiple sections of selective or indicator producing media must also be controlled upon receipt in your laboratory.

M 8, M 9, and M 10

M 8: *Where applicable, are positive, negative, and graded reactivity checked with each batch, lot number, and shipment of microbiology reagents, discs, stains, and anti-sera when prepared or opened?*

M 9: *Are positive and negative or graded reactivity checked with each batch, lot number, and shipment of identification systems when prepared, received, or when first opened?*

M 10: *Does the microbiology record, for each sample, include documentation of the reactivity noted for each step of the identification process?*

Whether reagents are prepared in house or purchased commercially, you must perform and document quality control to ensure accurate reagent performance; either prior to, or concurrent with, patient testing. If performed concurrently with patient testing, and QC results are out of acceptable range, patient test results must not be reported. The tests must be repeated after the problem has been identified and resolved, and acceptable performance has been verified.

M 9 refers to all biochemical identification systems (either single or combinations of tests) for an organism, and systems, such as API® strips or MicroScan® panels. It also includes pre-packaged media combinations, such as Uricult® or Bullseye® plates, when they are used for presumptive identification of microorganisms. (The listed systems are merely examples and do not constitute an all-inclusive list.)

If the systems are used to report presumptive identification of organisms, verification of key system components is required to ensure reagents give the appropriate reactions in the presence of known organisms. Maintaining a complete cadre of organisms the systems are able to identify may not be feasible – nor necessary. The organisms used to test the systems are organisms which are

- Suggested by the manufacturer;
- Commonly available;
- Easily maintained; and
- Represent those organisms most commonly identified among your patient population.

You must document that you have verified reagents, anti-sera, systems, etc. are performing to specification. When surveyed, your surveyor will review these records, which may include log sheets, worksheets, or electronic files. Records cannot include documentation on bottles, containers, or kit boxes that may eventually be discarded. The documented information will prove valuable during:

- Result interpretation;
- Investigation of unexpected results; or
- When assessing competency of personnel to interpret test results.

The face of clinical microbiology is changing with emerging, innovative test methodologies; however, the goal of providing accurate, reliable patient test results leading to quality patient care remains the same. ■

RESOURCES:

[1] American Society for Microbiology; *Clinical Microbiology Portal*; http://clinmicro.asm.org/index.php/about-clinical-microbiology/what-is-clinical-microbiology

[2] The FDA provides a searchable database of approved test systems to determine if your methodology is waived, moderate complexity or high complexity. You can search by test system, manufacturer and/or analyte. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCLIA/search.cfm

Cultures: GC, Throat, and Urine

Sources of confusion and citations for commonly performed cultures.

In our cover article, Clinical Microbiology, we discussed the general microbiology category of COLA criteria. This article delves deeper into three commonly performed cultures.

THROAT CULTURE FOR STREP

This section addresses throat cultures for Strep only; throat cultures to isolate organisms other than Strep are beyond the scope of this article.

While some rapid strep screening kits may be waived, throat cultures are non-waived. They may be either moderate or high complexity based on several different factors. Determining whether you perform moderate or high complexity throat cultures is important since it affects laboratory personnel requirements.

For throat cultures to be moderate complexity, you must

- Use selective agar that inhibits growth of bacteria other than Strep, such as SSA (strep selective agar) or BAP with SxT (blood agar plate with sulfamethoxazole and trimethoprim);
- Using media other than a selective agar, such as TSA (trypticase soy agar) or BAP (blood agar plate – without additives), makes the throat culture a high complexity test;
- Apply bacitracin discs that contain 0.04 units of bacitracin;
- Read zones of inhibition for only beta-hemolytic colonies; and
- Report only presumptive (initial) ID;
- Report as "Presumptive positive for group A Strep" or "No group A Strep recovered;"
- Any identification or interpretation makes the culture high complexity; and
- Reporting results of "negative" or "positive" should be avoided because it does not give the clinician complete information.

Some laboratories have protocols that allow the use of rapid strep kits to test colonies isolated from a throat culture plate. Be aware that this is a high complexity procedure!

The Food & Drug Administration (FDA) is responsible for categorizing test methodologies as waived, moderate complexity, or high complexity. Any test, kit, or method (or any use or modification of these) which is not evaluated and approved by the FDA is automatically considered high complexity. The kit you use as a screening method may not be FDA approved to test plated colonies, making this practice high complexity. Testing plated colonies is considered high complexity even when the kit is FDA approved for this practice since the kit is now being used to identify the colony. Additionally, testing plated colonies is still considered high complexity when the manufacturer's instructions recommend or require further testing of the colonies.

URINE CULTURE

Note: *To decrease the possibility of contamination, urine cultures should be performed on clean-catch, midstream specimens, collected in sterile containers (or other appropriately collected specimens). Random samples can be contaminated and contamination leads to falsely-elevated colony counts. This gives the impression of an infection when, in reality, none exists.*

Like throat cultures, urine cultures may be moderate or high complexity. If the organisms are identified, cultures are considered high complexity, regardless of whether it is a presumptive (initial) ID or a definitive (final) ID. Results reported as "Gram Positive," "Gram Negative," and/or "Lactose-fermenting" are considered identification, and are therefore high complexity. The use of multi-chambered plates (such as the Bullseye urine plate) or the use of dipslide culture tests (such as the Uricult system) is also considered high complexity.

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For urine cultures to be considered moderate complexity, results must be reported as colony counts; the unit of measurement is CFU/ml (colony forming units/milliliter). Results reported as "Growth" or "No Growth" are also considered moderate complexity; however, Proficiency Testing (PT) is required if this method of reporting results is utilized. PT is *not* required if you report only colony counts and "No growth" is reported as zero(0) colonies or 0 CFU/ml. However, it is considered good laboratory practice to perform PT even if it is not required.

It is also permissible to describe the colonies when reporting colony counts without offering an interpretation. This means of reporting maintains the moderate complexity nature of the culture since the result states only what is seen (objective knowledge); subjective knowledge is not being used to offer an interpretation or identification. For example, reporting "31,000 CFU/ml, clear colonies on MacConkey agar" is moderate complexity and "31,000 CFU/ml, presumptive *Pseudomonas* species" is high complexity.

Urine cultures are still moderate complexity if a biplate (such as Blood/MacConkey or MacConkey/EMB) is used, as long as the results are reported as colony counts without an identification or interpretation.

GC CULTURE FOR *NEISSERIA GONORRHOEAE*

GC cultures are high complexity procedures; therefore, you must adhere to all requirements for high complexity testing if you perform these cultures. Proficiency Testing is required for both moderate and high complexity procedures; however, personnel requirements differ depending on the complexity of the procedure you utilize.

GC cultures for presumptive identification of *Neisseria gonorrhoeae* can be moderate complexity ONLY if all of the following conditions are met

- Specimens must be urogenital or rectal samples;
- Specimens must be plated on selective media (Thayer-Martin, Martin-Lewis, or NYC);
- Incubate the plates at 35°C in a CO_2 rich environment for optimal growth of *N. gonorrhoeae*;
- Further testing must be performed on suspected colonies: oxidase test and Gram stain;
- To be reported as presumptive positive, colonies must be oxidase positive and display characteristic Gram stain morphology (Gram negative diplococci); and
- Results must be reported as "Presumptive positive for *Neisseria gonorrhoeae*" or "No N*eisseria gonorrhoeae* recovered."

Any other testing and any other result is high complexity.

It is good laboratory practice to send out any presumptive positive result for confirmation and this does not change the complexity of the culture performed in your laboratory. Note that cotton swabs are toxic to *Neisseria gonorrhoeae*, so Dacron or rayon swabs must be used for specimen collection when this is the suspected causative agent. Additionally, the swab must be placed in specific transport medium (preservative) to maintain the viability of the organism.

Special notes regarding Gram stains:

Do not report "Gram positive" or "Gram negative" based on plate morphology alone (without actually performing a Gram stain). It is technically incorrect; it constitutes a preliminary identification based on sight and smell; and it is high complexity since an identification / interpretation has been made.

Gram stains are considered moderate complexity ONLY if

- The specimen is an endocervical or urethral sample; and
- The slide is made directly from the clinical specimen.

All other specimen sites are high complexity.

Gram stains are also considered moderate complexity during the procedure for presumptive identification of *Neisseria gonorrhoeae* if

- The slide is made from a picked colony from the selective media; and
- The result for the entire procedure is reported as "Presumptive positive for *Neisseria gonorrhoeae*" or "No *Neisseria gonorrhoeae* recovered."

If there are any additional questions regarding subject matter reviewed in this article, please contact us at info@cola.org or 800-981-9883. ■

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Affirm™ VPIII

The Affirm™ VPIII test[1] is a DNA probe test intended for use in the detection and identification of three common causes of bacterial vaginosis.

This article is not meant to endorse or discourage the use of the Affirm™ VPIII system. It is only meant to assist COLA labs that already use the Affirm™ VPIII system.

Vaginitis, one of the most common problems encountered in the field of women's health, can be divided into three main categories based on causative agent:

- Bacterial vaginitis (BV, the majority of which are caused by *Gardnerella vaginalis*);
- Yeast vaginitis (candidiasis, commonly called "yeast infections"); and
- *Trichomonas vaginalis* vaginitis (trichomoniasis).

The Affirm™ VPIII identification test is designed to detect and identify any of these three causative agents when found in vaginal fluid specimens from patients with symptoms of vaginitis. Test performance with other specimens or other patient populations has not been established. Other laboratory methods for the identification of these organisms include other rapid identification kits, microscopic evaluation, amine test, Gram stain, pH, and culture.

The principle of this test and the proper procedure to utilize during performance of the test are beyond the scope of this article. For this information, as well as in depth information on intended use and result interpretation, please refer to the Affirm™ VPIII package insert on the BD website.

QUALITY CONTROL

The Affirm™ VPIII test includes positive and negative internal controls that are tested simultaneously with each patient specimen. In addition to ensuring all components of the test system work properly, the positive control ensures there is no specimen interference, and the negative control ensures there is no non-specific specimen binding.

Patient results cannot be reported if the internal controls do not produce the expected results. Please refer to the Affirm™ VPIII package insert, which details specific scenarios of how to interpret the control results and what patient results can be reported in each instance.

Note: Internal control results, including failures and subsequent corrective actions, must be documented each day of patient testing.

COLA CRITERIA

Quality control requirements must be performed in accordance with applicable local, state, and/or federal regulations. Accreditation requirements and your organization's protocols must also be followed. The most stringent rules must be observed.

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AFFIRM™ VPIII

M 9:
Are positive and negative or graded reactivity checked with each batch, lot number, and shipment of identification systems when prepared, received, or when first opened?

QC 10:
Are manufacturer's instructions for the use of reagents, controls, and kits followed?

Applicable COLA criteria referenced when using the Affirm system include M 9 and QC 10.

It is important to know that when the manufacturer's instructions "recommend" or "suggest" you perform a procedure, or if the instructions state that a procedure "may be performed," COLA interprets this as the procedure MUST be followed. Failure to follow the manufacturer's suggestions, recommendations, and the like will lead to citations for failing to comply with criterion QC 10. Failure to document your compliance will also lead to this citation.

To ensure that you are using the most current version of the instructions, periodically compare package inserts in use in your laboratory to what is posted on the manufacturer's website. The manufacturer's technical service representatives can also provide the most current package insert. Then, ensure that the new insert is available for reference and that all staff are aware of any changes to be implemented.

According to criterion M 9 and the current manufacturer's instructions,[1] each lot number and shipment of the Affirm test system must be controlled with a positive and negative external control for each organism that the system detects (in addition to the internal controls run simultaneously with patient specimens). QC may be performed using fresh stock cultures or commercially prepared swabs of specific ATCC (American Type Culture Collection) cultures. This can be accomplished in two ways.

1. Use of three separate swabs; one that will test positive for each of the three organisms.

   With this option, each individual positive swab serves as the negative control for the other two organisms. An additional negative swab is NOT needed.

2. Use of a trivalent swab that will test positive for all three organisms AND an additional negative swab.

   A separate negative swab is needed with this option since the trivalent swab is formulated to produce positive results for all three organisms. A negative control does not exist if the trivalent swab is the only control tested.

Proficiency Testing (PT) criteria:

PT must be performed and applicable PT criteria must be met since *Candida* and *Gardnerella* are regulated analytes. Refer any PT questions to your PT provider as they are best equipped to answer questions and offer recommendations.

Additional criterion:

COLA criterion QC 18 warrants an explanation even though it does not apply to the Affirm system.

Many common test methods involve some form of extraction of a particular antigen or DNA sequence from a specimen to determine the presence or absence of a given organism. There are a number of different extraction methods, including chemical extraction and heat extraction to break down the cell wall (cell lysis) to release the desired antigens. QC 18 is applicable to those methods that perform a chemical based extraction.

Methods that rely on heat extraction can be assessed by monitoring the temperature during the lysing process. Since the Affirm system utilizes heat extraction, QC 18 does not apply.

QC 18:
If you perform any direct antigen tests with an extraction phase included, do you check the test system with two control materials (including one capable of detecting errors in the extraction process) each day of patient testing?

However, rather than monitoring the temperature, the Affirm package insert states that each reagent lot and shipment must be tested for adequate sample lysis by testing a fresh stock culture or commercially prepared swab of *Candida albicans*. The Quality Control section of the Affirm™ VPIII package insert provides detailed information on sample lysis, why *Candida* species must be used, and what constitutes acceptable control results.

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AFFIRM™ VPIII

Since QC 18 does not apply, the *Candida* controls do not have to be run each day of patient testing; however, as per the package insert, they do have to be run with each lot number and shipment. Using the methods stated above to control each lot and shipment (three separate swabs or a trivalent swab-negative swab combination) also satisfies the requirement to confirm adequate sample lyses. *Candida* controls do not have to be performed in duplicate.

Failure to control each lot and shipment, and/or failure to document the controls, will result in citations due to failure to comply with COLA criterion M 9.

If there are any additional questions regarding the subject matter reviewed in this article please contact us at info@cola.org or 800-981-9883. ■

RESOURCES:

[1]The product insert for the BD AffirmTM VPIII Microbial Identification Test was referenced extensively to create this article. It can be found as a pdf on the BD website: http://www.bd.com/ds/technicalCenter/inserts/670160JAA(201008).pdf

[2]Refer to the package insert for the specific ATCC cultures for each organism.

DON'T FORGET!

REMINDER FROM THE CEO

In order to manage survey requirements for all laboratories, it is necessary to coordinate travel and scheduling of surveyors across the entire country. COLA makes every effort to schedule a surveyor in your area on a timely basis, within the 18 – 24 month time period following your last onsite survey. Efficient scheduling of our surveyors may mean that your survey will be scheduled closer to the 18 month end of this time period, rather than the 24 month end. Please inform us of possible scheduling difficulties for the entire six month period.

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