Q&A column, 10/17

Editor: Frederick L. Kiechle, MD, PhD

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Submit a Question

Q. Our doctors request strep group A culture on throat specimens that are negative for rapid strep group A. On culture workup, if we have beta-hemolytic strep, we perform latex grouping only for group A strep; we report negative for GAS if latex is negative and positive if latex is positive. I think we should confirm all GAS with pyrrolidonyl arylamidase (PYR), and group and report other non-GAS. What do you think?

A. Definitive identification of group A strep requires both a presumptive identification and demonstration of the group A antigen. Testing should be performed on pure cultures isolated on five percent sheep blood agar with trypticase soy base. Presumptive identification of GAS may be established by testing for susceptibility to bacitracin or by demonstration of PYR activity.[1] *S. pyogenes* is not the only beta-hemolytic *Streptococcus* that may exhibit the group A antigen; this property has also been reported in *S. dysgalactiae* subsp. *equisimilis* and *S. anginosus*. Therefore, confirmation of *S. pyogenes* based on latex grouping alone may be inaccurate.[2]

Although less prevalent than GAS, streptococci groups C and G (*S. dysgalactiae* subsp. *equisimilis*) may result in acute and epidemic pharyngitis and invasive infections clinically indistinguishable from GAS.[3,4] Disease due to non-GAS is likely under-recognized. Although definitive benefits of antibiotic administration for non-GAS pharyngitis have been debated,[4,5] identification and reporting of non-GAS in symptomatic patients may justify treatment to shorten symptoms, prevent the chance of severe sequelae, and reduce the possibility of transmission of non-GAS to susceptible contacts.[6] Detection of non-GAS may be achieved using culture on five percent sheep blood agar. Isolation of large-colony b-hemolytic streptococci will require additional biochemical testing in addition to Lancefield grouping to confirm groups C or G.[1]

An alternative to the culture-based approach is through the use of molecular detection of GAS-specific target genes via isothermal helicase-dependent amplification (HDA). A newer HDA method (Quidel Solana, Quidel Inc., San Diego) demonstrated 98.2 percent sensitivity and 97.2 percent specificity as compared with standard culture methods. This method is FDA approved, and the sensitivity is such that culture confirmation of negative results is not required. A molecular approach to detection of group A strep would allow for more rapid laboratory turnaround time to result, with the potential to specifically target therapy. The disadvantage to many molecular assays is that they are specific for GAS only and will not detect other streptococcal pathogens such as groups C or G.[7]

To address this issue, the FDA approved, in late October 2016, the Quidel Solana Strep Complete assay, which allows for detection and differentiation of GAS (*S. pyogenes*) in addition to streptococcal groups C and G (*S. dysgalactiae*) by helicase-dependent amplification. This moderate-complexity test is performed on throat swabs from symptomatic individuals and does not require confirmation by culture methods. Based on clinical trial data, the average sensitivity and specificity for GAS was 98.8 percent and 98.9 percent. Average sensitivity for groups C and G streptococci was 100 percent and 99.5 percent.[8]

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Q. How can a laboratory concentrate joint fluid samples to have a better yield of crystals?

A. Although the components included in the examination of synovial fluids vary somewhat between laboratories, virtually all examinations include a white cell count, differential count, culture, Gram stain, and a polarized light exam for crystals. The pathologic crystals are monosodium urate (MSU), the cause of gout, and calcium pyrophosphate dehydrate (CPPD), the cause of pseudo gout.

Several studies have concluded that the detection threshold for crystals is 10–100 µg/mL. It has also been demonstrated that sensitivity and specificity of crystal detection are variable. Detection of MSU crystals by polarized light exam has a sensitivity of 63–78 percent and a specificity of 93–100 percent. CPPD detection has a sensitivity of 12–83 percent and a specificity of 78–96 percent. It would seem logical that concentration of the crystals in synovial fluid prior to examination would be common. However, that is not the case. Standard laboratory procedure texts do not routinely address concentration of synovial fluids. Rather, procedures use unconcentrated

fluid to prepare a slide for polarized light examination.

A study by Yuan, et al., described increased yield if fluids that are initially negative are held in the refrigerator for 24 hours and reexamined. Overall crystal yield increased six percent. Additional brief mentions are made of examining cytospin preparations with polarized light and centrifuging an aliquot of synovial fluid and preparing a slide from the pellet. The centrifuge speed and the size of the aliquot are not specified. None of these methods for increasing yields are commonly used, although multiple laboratories reexamine initially negative fluids after refrigeration. The concentration of synovial fluid is not contraindicated and may improve the efficacy of the polarized light examination. If a method of concentration is chosen, it would be necessary to appropriately validate the method.

As noted above, there is no widely accepted and utilized method for concentration of joint fluid samples to improve crystal detection. Thorough education of staff members is the method favored by most laboratories to improve sensitivity and specificity of crystal detection.

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