



Answer to Photo Quiz: *Streptococcus pneumoniae*

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The figure in the photo quiz shows an uncommon Gram stain of a *Streptococcus pneumoniae* isolate. The subcultured isolate did not grow either on blood agar (BBL Columbia agar with 5% sheep blood; Becton Dickinson) or on chocolate agar (chocolate agar-PolyViteX; bioMérieux) even after 72 h of incubation under aerobic conditions (37°C; 5% CO₂). No other media were subcultured from the positive aerobic bottles.

Several factors may reduce the vitality of bacteria, thus modifying their appearance on the Gram stain and their ability to grow. In *Streptococcus pneumoniae*, one of these factors is certainly autolysis (1). Autolysis plays a role not only in reducing *in vitro* growth but also in pneumococcal pathogenesis, thereby shielding bacteria from the immune system and increasing toxin release (1). Autolysis is induced by specific enzymes (autolysins), which are activated in the stationary phase and degrade cell wall peptidoglycan, therefore modifying the cell appearance on the Gram stain (2).

After 30 h of incubation, a single anaerobic blood culture bottle turned positive, and bacteria with the same Gram stain features were evidenced. Only the use of anaerobically incubated Schaedler agar plates (BBL Schaedler agar with vitamin K1 and 5% sheep blood; Becton Dickinson) facilitated the growth of the isolate, and its definitive identification as *Streptococcus pneumoniae* was through matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Vitek MS; bioMérieux). To further elicit the growth, the cultured blood was inoculated in brain heart infusion broth (BBL brain heart infusion; Becton Dickinson). After a further 72 h, a significant growth was obtained on subcultured Schaedler agar plates, allowing antimicrobial susceptibility testing to be performed.

The isolate was susceptible to the most commonly used antipneumococcal antibiotics, including benzylpenicillin (MIC ≤ 0.06 μg/ml) (Vitek 2; bioMérieux), ceftriaxone (MIC ≤ 0.12 μg/ml), erythromycin (MIC ≤ 0.12 μg/ml), and levofloxacin (MIC, 0.5 μg/ml). Empirical therapy was therefore shifted to treatment with ceftriaxone only for a total of 14 days, leading to her making a complete recovery.

Importantly, during the complete investigation period, urinary pneumococcal antigen (UPA) (Alere BinaxNOW *Streptococcus pneumoniae*) test results were repeatedly and persistently negative. The sensitivity of this test varies from 59% to 75% in different studies (3, 4), and false-negative reports have been associated with some clinical factors, including low C-reactive protein (CRP) levels and warfarin therapy (5). A recent report described a low sensitivity to serotypes not included in the then-available seven-valent conjugated vaccine formulation (6). Interestingly, our isolate was later recognized as belonging to serotype 3, a serotype not included in the seven-valent vaccine.

This case can be seen as an example of the challenging laboratory presentation of pneumococcal pneumonia and sepsis. It is important to remember that in clinically suggestive cases, the suspicion of pneumococcal etiology should be borne in mind

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even when difficult-to-grow diplococci with an apparent negative Gram stain result are visualized and even where UPA test results are persistently negative.

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