PROFICIENCY TESTING SERVICE
AMERICAN ASSOCIATION OF BIOANALYSTS
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PARTICIPANT STATISTICS **FIRST QUADRIMESTER 2018**

GRAM STAIN

	Specimen 1		Specimen 2		Specimen 3		Specimen 4		Specimen 5		
Gram Stain	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**No. of Labs**
	69	6	2	74	1	75	4	72	69	7	76
Flagging		***	***		***		***			***	

	Specimen 1		Specimen 2		Specimen 3		Specimen 4		Specimen 5	
Morphology	**No.**	**Flagging**	**No.**	**Flagging**	**No.**	**Flagging**	**No.**	**Flagging**	**No.**	**Flagging**
Cocci	2	***	4	***	63		58		19	
Diplococci	0	***	5	***	3	***	5	***	45	
No diplococci present	2		1		1		1		0	***
Coccobacilli	4	***	9	***	1	***	1	***	2	***
Rods (Bacilli)	60		49		0	***	0	***	2	***
Streptococci	0	***	0	***	0	***	0	***	0	***
Yeast	0	***	0	***	0	***	0	***	0	***
Total Population	**68**		**68**		**68**		**65**		**68**	

*Specimen 2 was evaluated based on referees. The organism present was Listeria monocytogenes.

Educational

	Specimen 1				Specimen 2			
Gram Stain	**Neg**	**Pos**	**Yeast**	**No pathogen found**	**Neg**	**Pos**	**Yeast**	**No pathogen found**
	6	22	6	0	0	35	0	0

	Specimen 1		Specimen 2	
Morphology	**No.**		**No.**	
Cocci	2		10	
Diplococci	0		10	
No diplococci present	0		1	
Coccobacilli	1		4	
Rods (Bacilli)	15		2	
Streptococci	7		8	
Yeast	8			
Total Population	**33**		**35**	

Gram Stain #1 -

History:
Clinical Presentation:
A 42-year-old male presented to the ED with progressive weakness in his legs and a productive cough (with dyspnea) that developed several weeks before. On the physical exam, the patient was gaunt, displayed white plaques on the tongue and oral cavity, and rales were heard throughout the left lung. Neurological deficits were noted (e.g., cognitive, diminished strength and tendon reflexes in the lower extremities), as well. Initial testing revealed that the patient was positive for HIV (by ELISA) with an HIV-RNA burden of 30,018 copies/mL. In addition, a brain and spinal cord MRI demonstrated many ring-enhancing lesions; however, CSF studies (i.e., Gram stain, fungal stain, cryptococcal antigen, cultures, and VDRL testing) were negative. Subsequently, CT of the chest showed multiple cavitary and nodular pulmonary infiltrates. Transbronchial tissue specimens (obtained by biopsy) were sent for microbiological and pathological examination. The attached image is a Gram stain of the culture growth.

Discussion narrative:
The differential diagnosis of ring-enhancing CNS lesions in an individual with AIDS is broad, but typically consist of infectious or neoplastic disorders. In this particular case, the work-up focused on the causative differential diagnoses of pyogenic CNS lesions (e.g., *Listeria* spp., *Nocardia* spp. , other pyogenic bacteria, *Cryptococcus* spp. , *M. tuberculosis*, etc.) as it is unusual for primary CNS lymphoma to present with multiple ring-enhancing lesions located in the brain and spinal cord. As it turned out, the transbronchial tissue biopsy grew Nocardia, consistent with the Gram stain morphology (see below).

Laboratory evaluation for nocardia begins with macroscopic examination (i.e., if granules are observed, they should be carefully washed in saline, crushed, and examined microscopically), followed by microscopy using modified Kinyoun acid-fast) and Gram stains. While not always visualized on smears of clinical material, microscopic detection is especially important, when possible, for such slow-growing organisms in order to a provide rapid presumptive diagnosis while awaiting the results of the culture. As can be visualized from the enclosed image, nocardiae are seen microscopically as beaded gram-positive, thin, branching, filamentous organisms, usually in a background of purulence with many polymorphonuclear leukocytes. Gram staining is the most sensitive method by which to visualize and recognize nocardiae in clinical samples. Nocardiae will grow on most nonselective media, but require a minimum of 48-72 hours before colonies are evident. Therefore, consideration to extended routine culture incubation (i.e., up to 14 days) may need to be considered for optimal detection. Colonial morphology should also be observed macroscopically (for the “cotton-candy-like” presence of aerial hyphae) and microscopically. Nocardiae colonies are highly variable, but typically display a chalky and often wrinkled appearance with a wide-range of color pigmentation. Traditionally, nocardiae were identified to species biochemically (e.g., hydrolysis of adenine, casein, tyrosine, xanthine, etc.), but due to their nonreactive nature and the increasing number of described species, this approach no longer provides reliable identification for all of the currently recognized species. Consequently, for laboratories with molecular capabilities, gene sequencing (e.g., 16S rRNA) can provide rapid and reliable identification for most Nocardia isolates, as can the proteomic approach (e.g., MALDI-TOF mass spectrometry) with many Nocardia species now represented in commercially available databases.
While nocardiosis is not a common opportunistic infection in this patient population, it should be suspected in a patient presenting with simultaneous pulmonary and intracranial disease and a prolonged time course, as in this case. Pulmonary manifestations are the most common and include nodule formation, cavitation, as well as pneumonia and empyema. In the case patient, trimethoprim-sulfamethoxazole was administered along with anti-HIV therapy, to which he had an excellent response with resolution of the pulmonary and brain pathologies, an undetectable viral load, 30-lb weight gain, and nearly full recovery of his gait.

Gram Stain #2 -

History:
. Seropurulent fluid was submitted to the microbiology laboratory for routine culture and Gram stain to confirm the suspected diagnosis.
Clinical Presentation:
A 56-year-old woman, with a medical history of splenectomy following an automobile accident (10 years prior) and a 25-year history of smoking, presented to the ED with fever, shortness of breath, persistent cough, and purulent sputum of three days in duration. On exam, she had a temperature of 38.3°C, respiratory rate of 19/min, pulse of 104 beats/min, blood pressure of 149/109 mm Hg, and pO_2 of 92 mm Hg. Auscultation of the chest revealed coarse breath sounds at the right lower base with bibasilar fine crackles. The patient was admitted to the hospital and started on ceftriaxone, intravenously. Blood and sputum cultures (obtained at admission) were both positive for the same organism. The Gram stain from the sputum specimen can be seen in the attached photomicrograph.

Discussion narrative:
Based on the initial physical exam findings (e.g., fever, productive cough, shortness of breath, adventitious breath sounds on chest auscultation of the right lower lung) coupled with subsequently confirmed right lower lobe infiltrates upon radiographic imaging (i.e., not revealed in case history), this patient had a lower respiratory tract infection most consistent with community-acquired bacterial pneumonia (CABP). In patients suspected of CABP, attempts are typically made to determine the causative agent so that the patient can be properly managed through specific antimicrobial therapy. Such confirmation is typically obtained through one (or more) of three common laboratory testing alternatives: 1) sputum examination; 2) blood culture; and 3) urinary antigen detection.

In lobar pneumonia, as was seen upon physical and radiologic examination of this patient, the most common etiologic agent is *Streptococcus pneumoniae*. In fact, as typical in cases with pneumococcal pneumonia, this case was diagnosed by its characteristic Gram stain (as seen in the appended photomicrograph) in which the stained sputum demonstrated numerous polymorphonuclear leukocytes in the presence of spherical-to-ovoid ("lancet-shaped"), gram-positive diplococci. While the Gram stain sensitivity is fairly high (≈80%) with quality sputum specimens (i.e., ≥25 neutrophils and <10 squamous epithelial cells/low-power field), it is not uncommon to receive poor-quality specimens in the clinical setting. Therefore, the laboratory must have appropriate acceptance/rejection criteria in place to ensure that the clinical material is predominately mucopurulent sputum and not oropharyngeal secretions, as pneumococci can be a part of the resident microbiota of the oropharynx. Consequently, the presence of gram-positive diplococci in "sputum" specimens cannot be reliably associated with the diagnosis of pneumococcal pneumonia, and such reporting could lead to false positives and subsequently, the overuse of antibiotics and promotion of antibacterial resistance.
The predominant phenotypic characteristics associated with *S. pneumoniae* (*Sp*) as observed in sputum (and/or blood) culture are: 1) a-hemolysis on blood agar plates; 2) *Sp* colonies are moist, can occasionally appear mucoid and typically demonstrate a central navel-like depression (viridans group streptococci (VGS) lack this feature and have a dome-like appearance). In addition, biochemical testing such as the catalase test (negative f or *Sp*), bile solubility (positive for *Sp*) and optochin sensitivity (*Sp* is sensitive to optochin) can further differentiate this organism from the closely-related VGS. In addition, automated phenotypic testing platforms are also used to identify this organism and, in general, perform very well. Also (as mentioned above), another common test modality for invasive pneumococcal disease is the urinary antigen test. This test performs well, and is extremely useful in circumstances where antimicrobials have already been administered, making it much less likely that organisms with be detected through culture. Lastly, molecular methods such as DNA probe-hybridization, MALDI-TOF mass spectrometry, and nucleic acid amplification tests have also been used with success, either as a stand-alone identification method, or as a means to confirm manual or automated phenotypic testing with ambiguous results.