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On the cover: **Non-encapsulated Pulmonary Cryptococcosis in a patient infected with Cryptococcus sp. fungal organisms**

Cryptococcosis is an infection caused by fungi that belong to the genus Cryptococcus. There are over 30 different species of Cryptococcus, but two species – Cryptococcus neoformans and Cryptococcus gattii – cause nearly all cryptococcal infections in humans and animals. Although many people who develop cryptococcosis have weakened immune systems, some are previously healthy.

Photo courtesy of the CDC

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Article 445
1 Clock Hour

Understanding the Causative Issues of Pre-Analytic Hemolysis in the Health Care Setting

By Michael Shymko and Tina Shymko

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Our blood has four primary components: red blood cells erythrocytes, white blood cells leukocytes, platelets and serum or plasma.

Pre-analytical hemolysis affects only the red blood cells, or erythrocytes, prematurely destroying them. The typical RBC lives approximately 120 days and then the cell wall ruptures, releasing hemoglobin and cell fragments which are poured out into the blood plasma, and is a normal occurrence within the body to be recycled back to the bone marrow maintaining homeostasis. During a blood draw, pre-analytic hemolysis can be created and is detrimental to laboratory results. Erythrocyte cell wall membranes rupture very easily, not only spilling hemoglobin but elevating serum potassium, bilirubin, acid phosphate, zinc, magnesium, albumin, creatine kinase CK, and also cause an elevation in D-dimer results.[3] On average, it has been proven that pre-analytic hemolysis can interfere with 39 different blood results. Phlebotomists have a great appreciation and even a greater understanding of this concept as opposed to healthcare personnel who routinely draw blood but are not certified phlebotomists. Certified laboratory phlebotomists utilize specific collection practices, protocols and procedures in order to help prevent pre-analytic hemolysis.[3, 4] The breakage of red blood cells or "lysis" is easily detected in the lab, whereas the spilled hemoglobin turns blood plasma pinkish to red dependent on the concentration of RBCs lysed or hemolized in the sample.[4]

Because it is poorly understood, reducing the occurrence of pre-analytic hemolysis of specimens drawn in the healthcare environment is a consistent nemesis that is experienced by healthcare personnel who routinely draw blood, but are not professional laboratory phlebotomists. The misconception may stem from the fact that healthcare personnel may not be properly trained in the art and science of correct and consistent blood draw protocols and procedures as laboratory phlebotomists are. Healthcare personnel either do not understand, or simply underestimate the conditions and the complexities of proper procedure, proper protocol, and proper training to draw blood accurately and correctly.[5]

The concept of phlebotomy best practices as established by the World Health Organization (WHO) is now being introduced and becoming a growing focus for hospitals across the nation. The best practices concept is rapidly bringing to light an awareness of what can happen when collectors of blood severely underestimate and misunderstand blood specimen collecting practices and procedures.[6] There is a comprehensive training program given to phlebotomists to promote and provide a best practice standard of care.[6]

The use of a standard protocol for blood draws can reduce the rate of pre-analytic hemolysis by more than 7-fold.[2] The rate of hemolysis from collectors of blood other than phlebotomists was shown to be on average 12.4 percent as compared to 1.6 percent drawn by phlebotomists.[2]

The American Society for Clinical Pathology has established a benchmark of 2 percent or lower for hemolysis rates in laboratory blood samples, and is also the standard of care for laboratory phlebotomists.[3]

According to Ana K. Stankovic, MD, PhD,

Case Thirty-Two: An Unusual Gram-Negative Bacillus Recovered from a Pediatric Patient with Chronic Otitis Media

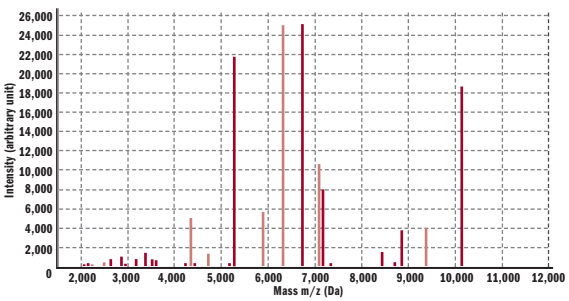
By Dr. Morgan McCoy; Jenny Pfeffer; and Dr. Joel Mortensen

EDITOR'S NOTE: BEFORE reading the Case Follow-up and Discussion below, study the Case Description on page 2 of this issue, and formulate your own answers to the questions posed.

Case Discussion

The Vitek 2, a semi-automated biochemical identification system, was unable to identify the organism. Rather than relying on biochemical reactions, Matrix-assisted laser desorption ionization time of flight (MALDI –TOF) mass spectrometry relies on the generation of a mass spectrum of microbial proteins through their ionization and time of flight measurement. The resultant spectrum is then compared to a curated library of known spectra. MALDI-TOF mass spectrometry identified the organism as *Bordetella trematum* utilizing a non-FDA approved library; which was confirmed by 16S ribosomal RNA gene sequencing.

Figure 3 *MALDI-ToF Spectra*



Though labor intensive, ribosomal RNA gene sequencing has proven an excellent technique for the identification of microbial species. This is due to the highly conserved ribosomal RNA genes of microbial organisms. Sequencing of these genes has proven effective in discriminating related species to a level far superior to conventional or semi-automated biochemical analysis and is currently rivaled only by MALDI-TOF.

Antimicrobial susceptibility testing (AST) was performed by E-test (bioMerieux) and are shown in table 1. Results were not released to the patient's chart due to insufficient *in vitro* and *in vivo* susceptibility data and a lack of accepted breakpoints.

Table 1. AST results

Antimicrobial agent	MIC in µg/ml
Levofloxacin	1.5
Ticarcillin/Clavulanic acid	1.0
Cefepime	6
Meropenem	0.047
Aztreonam	128
Tobramycin	2
Amikacin	16
Colistin	0.19

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Article 447
1 Clock Hour

Three Rapid Screening Tests for *Cryptococcus Neoformans* in the Laboratory

By Marjorie D. Palmer-Newball

Abstract

The timing seems right to reintroduce clinicians and medical laboratorians to the important use of three rapid screening laboratory tests: cell count, India ink, and gram stain that presumptively identify *Cryptococcus neoformans* in cerebrospinal fluid. The tests are time sensitive and studies have shown the India ink to be 86%, gram stain 89%, and cell count 90% positive in patients co-infected with HIV and *Cryptococcus neoformans*, the leading cause of mortality and disability, in areas of Sub-Saharan African. The major environmental sources of *Cryptococcus neoformans* are soil contaminated with pigeon feces, and in fruits, vegetables, and trees.

A 29-years-old male presented at the emergency room complaining of a headache, sensitivity to light, seizures, and stiff-neck. Upon admission, he was treated with Dilantin and Phenobarbital for the seizure, concurrently, with Amphotericin B and flucytosine every six hours for two weeks for cryptococcal meningitis. The concurrent use of the three rapid screening tests provided ease in detecting the pathogen, but their limitations can produce erroneous results and misdiagnosis. Serological replacement test may be considered, the urease and cryptococcal antigen test, but these tests are neither cost-effective nor routine. While no data was found on the statistical benefit in using these three tests concurrently, their individual use has yielded high sensitivity. It is assumed their benefits increase significantly when performed simultaneously; therefore, they are still preferred in the preliminary assessment of *Cryptococcus neoformans*, because they are routine, cost-effective, fast, and highly accurate, ideal especially for limited resource countries.

Marjorie D. Palmer-Newball, MT (AMT), Public Hospital Authority, Department of Pathology and Laboratory Medicine, Medical Microbiology Section, Princess Margaret Hospital, Nassau, Bahamas

Three Rapid Screening Tests for *Cryptococcus Neoformans* in the Laboratory

It is imperative that clinicians and medical laboratorians, primarily medical technologists, are reintroduced to three rapid screening tests used in the detection of *Cryptococcus neoformans*. The severity of individuals co-infected with *Cryptococcus neoformans* and HIV often result in a fatal outcome; therefore, these three presumptive laboratory tests, cell count, India ink, and gram stain, are of clinical importance. The purpose of this case report is to recount the clinical experience of a patient; identify causative agent of his medical condition, highlight treatment management, outcome, and recognize the diagnostic laboratory tests used in the assessment process. A brief literature review is provided that includes the lack of research evidence identifying *Cryptococcus neoformans* within the Caribbean region. The discussion will focus on clinical implications of the report, why it is important to disseminate these findings to clinicians and medical laboratorians, and options of advanced technological methods to identify the opportunistic pathogen.

A patient suspiciously infected with *Cryptococcus neoformans* will need rapid laboratory testing and aggressive medical intervention for the preservation of life**.** Awareness of the concurrent use of the three tests is expected to increase their application in medical laboratories. According to World Health Organization (WHO), "rapid tests are better for emergency testing…. designed for use where a preliminary screening test result is required and are especially useful in resource-limited countries…. Quick and easy to perform – 10 minutes to 2 hours" (2017, p. 1). Cerebrospinal fluid (CSF) is the specimen of choice for laboratory investiga-

Article 448
1 Clock Hour

Meningococcal Disease: A Trail of Incapacitations and Fatalities

By Dr. Jude Okoyeh

Jude N. Okoyeh, PhD, Department of Clinical Laboratory Science, School of Health Sciences, Winston-Salem State University, Winston-Salem, NC

Introduction

Meningitis can be caused by bacterial and nonbacterial organisms. Meningitis from bacterial infection is more severe and poses a greater danger to life than viral meningitis. Essentially, most cases of meningitis are as a result of infections from three bacteria, namely, *Neisseria meningitidis*, *Streptococcus pneumonia* and *Haemophilus influenza* type b, that cause meningococcal meningitis, pneumococcal meningitis and influenza, respectively. Most of the bacterial meningitis in newborns, such as those caused by Group B Streptococcus spp, are from their mothers. The occurrence of meningitis is highest in infants less than one month. Children that are older are more often infected by *H. influenza*, *N. meningitidis*, and *S. pneumoniae*. Either *N. meningitidis* or *Streptococcus pneumonia* can cause meningitis in adults, but they are mainly due to *Strep pneumoniae* infections. These bacterial spread mostly through the blood, some use the nerve as a pathway to enter the brain (such as viral meningitis from rabies and herpes simplex virus). Factors that predispose people to meningitis include inadequate treatment of infections of the sinus or ear, diabetes, low innate immunity, cancer, treatment with steroids, HIV, advanced age, traveling to endemic areas for an extended period of time, active and passive smoking, kissing, over-crowded places, patronizing pub and bars and low economic status.[1,2,3] *Neisseria meningitidis*, which belongs to the kingdom Bacteria, phylum Proteobacteria and genus *Neisseria*, is responsible for causing most of the illnesses that are collectively referred to as meningococcal disease and it is the only microorganism that is capable of causing epidemic outbreaks of meningitis.

Infections from various forms of *N. meningitidis* occur world-wide with highly fatal outcome[1]. The occurrence of *N. meningitidis* in different populations of the world is highly unpredictable because of the differences in distribution (Fig. 1), frequency of the disease and serological groupings.[1] Globally, about 1.2 million cases of meningococcal infection occur annually, with about 135,000 deaths.[1] Most of the morbidity effects and mortality of meningococcal disease occur in children less than1 year old, with lesser risks in adolescent and young adults of 15-25 years. The carriage pattern of *N. meningitidis* tends to be higher in adolescents and younger adults than in children and new-born.[1] Epidemics of the disease occur seasonally, with the tendency to start in the dry season and end at the commencement of the rainy season.[2] Out of the 13 meningococcal strains identified so far: A, B, C, E-29, H, I, K, L, W-135, X, Y, Z, AND Z' (29E), six serogroups - A, B, C, W (formerly W-135), X and Y - cause almost all invasive disease cases.[2] Sero-grouping is the usual method of classifying meningococcus spp using biochemical properties that are targeted against their polysaccharide capsule. The capsule is one of the determinants of virulence and it is a focus of interest in the development of meningococcal vaccines.[2] Other classification methods include the use of monoclonal antibodies, PorinA, PorinB, lipo-oligosaccharide LOS structures and multilocus sequence typing (MLST) of their DNA genes or a combination of these methods.[1] If these DNA genes are closely related, they are called clonal

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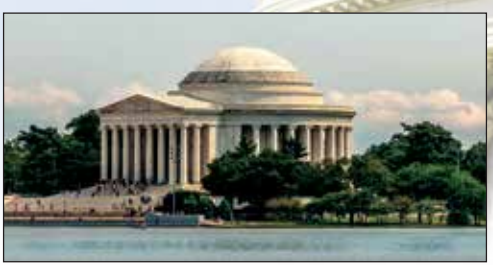

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