LAB DETECTION OF *NAEGLERIA FOWLERI:* CONTINUING EDUCATION VIDEO

An educational video for all clinical laboratory personnel.

Produced by The Jordan Smelski Foundation for Amoeba Awareness

http://www.jordansmelskifoundation.org/

Directed by Stanley Pomianowski

http://www.duckduckvideo.com/

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Introduction: What is the Purpose of this Video?

The purpose of this video is to provide education to clinical laboratory professionals regarding the importance of awareness of Primary Amebic Meningoencephalitis (PAM), and the critical role the laboratorian can play in the case outcome of this all-too-often-fatal, but also potentially treatable, infection. The laboratorian's awareness that a CSF specimen received for a bacterial (or viral) meningitis work-up may, in fact, be related to PAM, and the practice of communicating with the ordering physician to ascertain nasal freshwater exposure history, are vital pieces leading to the detection or exclusion of a PAM case.

Quite literally, there is an enormous potential to save lives with the PAM awareness and lab detection methods conveyed in this video.

This is a "peer-to-peer" training, with presentations from two laboratorians; **Shiela Black**, MHM, BSMT (ASCP) of Florida Hospital, Orlando, Florida, and **Franke Johns**, BSMT, SH (ASCP), recently retired from Arkansas Children's Hospital, Little Rock, Arkansas. Specimens from the latest two U. S. PAM cases with successful treatment outcomes were managed by Shiela and Franke.

Shiela and Franke provide information on how these cases were managed in the lab, as well as valuable recommendations and guidance to the viewer, all with the objective of ensuring rapid detection so that treatment can be initiated as quickly as possible, thus contributing to the best chances for a successful outcome.

In addition, **Dr. Jennifer Cope**, medical epidemiologist and infectious disease physician overseeing the free-living amoeba program in the Waterborne Disease Prevention Branch at the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, provides an overview on PAM, including the mode of infection with the causative agent, *Naegleria fowleri* (a very important aspect of the disease for the laboratorian), clinical signs, treatment, and another very important aspect for the laboratorian: the recently changing epidemiology of the disease.

The attendee will also view Jordan's Story, presented by Steve and Shelly Smelski, Jordan's parents. At 11 years of age, Jordan Cole Smelski passed away in Central Florida from Primary Amebic Meningoencephalitis, after vacationing in Costa Rica.

(Please see additional information on the presenters, and on Jordan's Story, below, under: "**II. Presenters**" and "**V. About The Jordan Smelski Foundation for Amoeba Awareness**")

Background:

Why is this video directed to laboratorians?

To date, there have been only 4 survivors of PAM in the United States; 3 of which experienced full recoveries with no significant neurologic deficits. In each of these 3 cases, the laboratory personnel were the first to suspect the infection and to identify the organism in the clinical specimen (CSF). The rapid detection of the causative organism, *Naegleria fowleri*, allowed for the rapid initiation of the very specific treatment needed for this often-fatal infection, which contributed to the successful outcomes. (1-4)

More about PAM

PAM is a devastating, rapidly-progressing infection of the brain and spinal cord which begins when fresh water containing the ameba, *Naegleria fowleri*, enters the nose and comes into contact with the nasal mucosa. The ameba penetrates the nasal mucosa, travels along the olfactory nerves, crosses the cribiform plate, and enters the brain. The infection causes a hemorrhagic, necrotizing meningoencephalitis with extensive destruction of cerebral hemispheres, arachnoid and pia mater, and other structures. The infection results in overwhelming cerebral edema which causes brain herniation and death. (5,6)

There have been 143 confirmed PAM cases with exposures in the US, from 1962 through 2016. (7).

The case fatality rate for PAM in the United States is over 97%. (5) This is the highest US case fatality rate of any infectious disease in the past 54 years.

What are the reasons for this extremely high mortality rate?

The worst answers to this question would be:

"There is no way to detect the organism" and… "There is no treatment"……..however, this is not the situation. *There are* ways to rapidly detect the organism in the clinical laboratory, and *there is also* a recommended treatment (https://www.cdc.gov/parasites/naegleria/treatment-hcp.html) which has resulted in the 3 cases in the U.S. in which there were full recoveries with no significant neurologic deficits; each occurring when the organism was detected in the lab rapidly. (1-4)

Background, continued

What, then, has been the issue contributing to this rate? There are actually 3 critical issues, which occur in a combined way to produce the devastating results we have seen:

1) The initial signs and symptoms are identical to those of bacterial meningitis. (5) PAM is also often mistaken for other, more common types of meningitis. (8) The presumptive diagnosis in Jordan's case was viral meningitis. (9)

2) This is not a commonly occurring infection: the awareness that a patient may be infected with *Naegleria fowleri* when they present with meningitis signs and symptoms is low.

Not only has the awareness been low at the point of care (the emergency department in most cases), but also in the clinical laboratory. This low awareness is not limited to the clinical arena; this is also seen among the general public, ie, the patient (or patient's parents) quite often are not aware of the ameba, how the infection occurs, and the fulminant nature of the infection.

3) This is an extremely fulminant infection- the median time from infection to signs and symptoms is 5 days, and the median time from symptom onset to death is 5 days. (5,6)

All of the fatal cases (and the one case of survival with profound persistent mental disability) have resulted from this combination of factors. (5,6,10)

Historically, almost all patients presenting to the ED with PAM are treated as outpatients for common conditions other than bacterial meningitis, once that is ruled out by the lab. By the time PAM may be suspected, it is too late for successful treatment, due to the fulminant nature of the infection. (5,6,10)

The awareness message to laboratorians is very urgent, now more than ever, and now for <u>laboratorians throughout the United States:</u>

The awareness message has always been urgent; recent changes in the epidemiology of the disease, however, bring an even greater urgency for both awareness and for understanding the simple techniques for rapid detection of *Naegleria fowleri* in the clinical lab, for laboratorians throughout the United States.

These recent changes have been in two areas: (1) exposures have occurred in states never reporting confirmed cases before; northern states whose freshwater bodies historically have not been ideal environments for the thermophilic organism, and (2) new exposure scenarios unlike the more common exposures (immersion in natural bodies of fresh water; for example: lakes, rivers, or streams).

Cases with exposures in Minnesota, Indiana, Maryland, and Kansas have been reported over the past few years. (8)

Background, continued

Recently, confirmed cases have occurred in the U.S. that are associated with neti pot usage, a lawn water slide, and ritual nasal ablution (U.S. Virgin Island). (11-13)

Additionally, there have been recent exposures in novel bodies of water which further underscores the urgent need for increased awareness in the laboratory (and, of course, at the point of care). (8,9)

A patient may present with symptoms far from the location where he or she was infected; this scenario has the potential to further reduce the awareness level at the point of care, which further underscores the need for awareness in the laboratory to support the overall goal of a rapid diagnosis. (9)

Therefore, laboratorians throughout the United States should be familiar with the awareness messages and simple, rapid techniques described in this video to be prepared to significantly contribute to successful outcomes

Despite the complex issue of multiple exposure scenarios and contributing factors, there is only one route of infection that is known to cause PAM in these cases in the United States; *Naegleria fowleri* enters the nose in fresh water that contains the ameba. The question concerning exposure for both the clinician at the point of care and the laboratorian to ensure a rapid diagnosis and detection, will center around any freshwater exposure in the past 2 weeks that could involve fresh water entering the nose, particularly untreated or under-treated fresh water.

Contact:

For questions or comments relating to this document or the video, and for the answer key to the evaluation questions, please contact:

Jack Tracy

Florida Department of Health in Orange County

Epidemiology Program

Jack.tracy@flhealth.gov

407-723-5050

Steve Smelski

Jordan Smelski Foundation for Amoeba Awareness

Ssmelski@jordanssmile.org

Presenters, Acknowledgements, and Links to the Video

SHIELA BLACK, MHM, BSMT (ASCP)
Laboratory Coordinator, Florida Hospital Orlando, Florida

Shiela Black, MHM, BSMT (ASCP), received her B.S. degree in Health Science in Medical Technology from the University of Florida and her Master's degree in Heath Management from St. Thomas University in Miami. She is nationally certified as a medical technologist by the American Society for Clinical Pathology and licensed in the State of Florida as a clinical laboratory supervisor. Her thirty-three years of experience in the clinical laboratory includes positions as assistant laboratory director at Pembroke Pines General Hospital in South Florida as well as Central Florida Regional Hospital in Sanford. She has been a member of the Florida Hospital laboratory team for the past twenty-seven years. Her awareness of the possibility of *Naegleria fowleri* infection in a meningitis case in 2016 led her to the rapid detection of the organism, which resulted in the rapid initiation of the specific treatment protocol for primary amebic meningoencephalitis (PAM), which resulted in only the third successful PAM outcome since 1978.

JENNIFER R. COPE, MD, MPH
Medical Epidemiologist, Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention

Jennifer Cope, MD, MPH is a medical epidemiologist and infectious disease physician with the Waterborne Disease Prevention Branch in the National Center for Emerging and Zoonotic Infectious Diseases. She oversees the free-living amoeba program which supports clinical, epidemiologic, laboratory, and communication activities related to the free-living amebae (Naegleria, Balamuthia, Acanthamoeba, and Sappinia). Dr. Cope began her career at the Centers for Disease Control and Prevention in 2009 as an Epidemic Intelligence Service (EIS) officer assigned to the North Dakota Department of Health. She joined the Waterborne Disease Prevention Branch as a Preventive Medicine Resident in 2011 and transitioned to her current role as medical epidemiologist in 2013. Dr. Cope obtained a BS in Microbiology from Penn State University and received her medical degree from the University of Pittsburgh School of Medicine. She completed residency training in internal medicine at the Emory University hospitals and Grady Memorial Hospital in Atlanta and infectious disease fellowship training at the University of Maryland Medical Center in Baltimore. She is board certified in infectious diseases.

FRANKE JOHNS, BSMT, SH (ASCP)
Evening Shift Supervisor, Arkansas Children's Hospital, Laboratory (retired)

Franke Johns, BSMT, SH (ASCP), received her B.S. degree in Medical Technology from the University of Central Arkansas in 1984. She completed her internship at Spark's Regional Medical Center in Fort Smith, Arkansas. Franke has 33 years of clinical laboratory experience, 32 of which were at Arkansas Children's Hospital. She has a specialty in hematology. Franke served for 28 years as the Evening Shift Supervisor. She preferred the evening shift as it allowed her to be involved in all facets of laboratory work, and because typically "the most interesting cases" were seen during that shift. Her awareness of the possibility of *Naegleria fowleri* infection in a meningitis case in 2013 led her to the rapid detection of the organism, which resulted in the rapid initiation of the specific treatment protocol for primary amebic meningoencephalitis (PAM), which resulted in the first successful PAM outcome since 1978.

Acknowledgements

We wish to gratefully acknowledge Maria Brock, BSMT, (S)ASCP, ART, Laboratory Manager, Coagulation and Urinalysis at Florida Hospital, Orlando, FL for proposing this video and for all of her work towards making it a reality. Many thanks to all the video participants, and to all those who contributed to the production of the video.

Links to the Video

The video is located under "Facts" on the Jordan Smelski Foundation for Amoeba Awareness website.

For presentations from this website, click on the "Full Screen" icon in the lower right of the video screen.

The Centers for Disease Control and Prevention provides a link to the video from the diagnosis page in the Public Health and Medical Professionals section of the *Naegleria fowleri* – Primary Amebic Meningoencephalitis (PAM) website.

About the Jordan Smelski Foundation for Amoeba Awareness

The Jordan Smelski Foundation

The Jordan Smelski Foundation for Amoeba Awareness was started in September of 2014, shortly after eleven-year old Jordan Cole passed away from Primary Amoebic Meningoencephalitis, after vacationing in Costa Rica. Jordan was infected by *Naegleria fowleri* after swimming in a resort pool that was filled with unchlorinated hot spring water. The Foundation's Mission is to: *Create global awareness about the risks associated with amoebas in warm fresh water and to educate families and medical communities about the risks from Naegleria fowleri and Primary Amoebic Meningoencephalitis (PAM).*

Shortly after Jordan's death, the Foundation created a list of items that we determined needed to change, in-order to have a successful outcome from a PAM infection (Previously 99% fatal). The Foundation has been committed to changing these items and in August of 2016, Sebastian DeLeon became just the 4th survivor of PAM in the US in the last 65 years! The educational information shared at the initial PAM Summit in 2015 helped a Lab Technician identify *Naegleria fowleri* early in Sebastian's case, allowing for a positive outcome!

The Foundation holds several fund-raising events every year (golf event and walks) to finance research, fund educational/training videos, fund awareness videos and to participate in conference events.

In 2017 the Foundation has:

- Provided a grant for research to Dr. Francine Marciano-Cabral, Virginia Commonwealth University on four unknown environmental conditions for *Naegleria fowleri*.
- The Foundation has committed funding for an educational video for emergency physicians at the Florida College of Emergency Physician (FCEP) and the American College of Emergency Physicians (ACEP).
- The Foundation produced a 12-minute video detailing "Jordan's Story" to help educate parents and children on the risks of *Naegleria fowleri* exposure. https://www.facebook.com/jordansmelskifoundation/videos/1158368060974840/?hc_ref=ARQaDbJH1WEJ8UzZesbZhkHbk41HcLA7JjJGccm2jhgYqETfSyKhIbrlt-FGtCSfLUM

About the Jordan Smelski Foundation, continued

- The Foundation just completed funding and production of a 60-minute training video called "*Lab Detection of Naegleria fowleri*" for help in early identification of PAM cases. http://www.jordansmelskifoundation.org/lab-detection-naegleria-fowleri
- The Foundation is currently producing a series of short videos, detailing answers to the 40 most common questions on *Naegleria fowleri* and PAM, featuring world experts with their answers.
- And this past September 29th the Foundation hosted the 3rd Annual PAM Summit, partnering with Florida Hospital for Children once again. http://hospitalchurch.org/sermon/amoeba-summit-2017/

Jordan Smelski Foundation
PO Box 470430
Lake Monroe, FL 32747

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The Jordan Smelski Foundation for Amoeba Awareness welcomes suggestions, ideas, and comments regarding awareness efforts to all audiences, including the general public, children and adults, and the clinical audience: both laboratory personnel and physicians who would see patients presenting with the signs and symptoms of PAM.

Please contact us at ssmelski@jordanssmile.org with any suggestions, ideas, and comments.

Objectives

(number may be shortened according to needs)
(these objectives are designed as guidelines and may be modified to more closely fit the exact terminology, audience, and needs of your specific training)
Below includes both a suggested set, and below this set is an additional bank if more, or different objectives are preferred

Suggested objectives set:

1) The laboratorian will understand the critical importance of considering the possibility of *Naegleria fowleri* infection when CSF specimens are received on suspect meningitis cases.
2) Learn that a history of nasal freshwater exposure within 14 days of presentation associated with any CSF specimen in suspect meningitis cases represents an urgent situation that should result in the immediate processing of the specimen using lab procedures described in the video to detect the ameba.
3) Learn that the two ways to detect *Naegleria fowleri* in the lab are: direct microscopy for visualization of motility, and staining after cytocentrifugation (eg. Cytospin™) with Wright-Giemsa staining, and both methods should be performed.
4) Understand the "do's and don'ts" in specimen processing for *Naegleria fowleri* detection, and tips for rapid detection.
5) Learn that Gram stain is not the stain of choice for detecting *Naegleria fowleri* in the lab, the Wright's stain is the preferred stain.
6) Know the different warming tips to increase motility of the organism for enhanced detection.
7) Understand that when a large number of neutrophils and no bacteria are seen on the Gram stain, *Naegleria fowleri* should be suspected.
8) The viewer will understand that part of the *Naegleria fowleri* detection protocol in the lab should be to call CDC, 24/7, immediately upon suspicion or detection for immediate support and consultation.
9) Learn that *Naegleria fowleri* is normally found in warm fresh water such as lakes, rivers, streams and hot springs
10) Learn that infection typically occurs when people go swimming or diving in warm fresh water.
11) Learn that the ameba, *Naegleria fowleri*, infects people when water containing the ameba enters the body through the nose, it then travels up the olfactory nerves to the brain, where it causes PAM

Suggested objectives set, continued:

12) Learn that in very rare instances, PAM may also occur when water from other sources, such as inadequately treated swimming pool water, or contaminated tap water enters the nose.
13) Understand that recently, cases of PAM have been identified in states as far north as Minnesota, Indiana, and Maryland, possibly due to rising average water temperatures.
14) Understand that clinically, a patient with PAM presents much like a patient with Bacterial meningitis
15) Understand that the most important clues pointing to a diagnosis of PAM in a patient with the symptoms of severe headache, fever, vomiting, neck stiffness, and seizures are found in the patient's freshwater exposure history within the past 2 weeks

Objectives bank:
Lab awareness and detection techniques: Shiela and Franke's presentations

1) Understand the importance of lab personnel asking about freshwater exposure history when a CSF specimen is received for meningitis work up.
2) Learn that the Wright's stain (Wright-Giemsa), the Trichrome, and Periodic Acid Schiff are the recommended stains for detecting *Naegleria fowleri*.
3) Understand that any clinical lab is equipped to perform the procedures for rapid detection of *Naegleria fowleri.*
4) Understand that the recommended spin-down speed for concentrating the CSF for the direct microscopy process (for visualization of motility) is 2,000 RPM and the recommended spin-down time is 5 minutes.
5) Learn that there are 3 recommended ways to look for the organism in a wet mount set up: (1) counting chamber, CSF placed directly on a slide, (3) CSF aliquot spin-down at 2,000 rpm for 5 minutes.
6) Understand that, even with an extremely high mortality rate, there can be successful treatment outcomes when early detection in the laboratory occurs.
7) Become familiar with the laboratory detection methods and procedures in the laboratory for detection of *Naegleria fowleri* in CSF.
8) Know that the organism has been detected successfully (with successful treatment outcomes) in a different way in 2 different hospitals: 1 was visualizing motility, one was with the stain technique, when no moving organisms were seen in the counting chamber.

Objectives bank, continued:

9) The viewer will learn how awareness and rapid detection in the laboratory resulted in successful treatment outcomes at both Florida Hospital, and Florida Hospital for Children and Arkansas Children's Hospital: Sebastian's Story and Kali's Story

Dr. Cope's presentation:

1) Understand that the trophozoite stage is the infective stage of the ameba
2) Learn when and where in the United States, *Naegleria fowleri* infection typically occurs.
3) Understand that the only certain way to prevent PAM due to swimming is to refrain from water-related activities in warm fresh water.
4) Know that recently, ritual nasal rinsing, the use of neti pots, and playing on a backyard water slide have all been identified as exposures that have caused PAM
5) Understand that when a diagnosis of PAM is considered, CDC should be consulted immediately.
6) Know that the recommended combination of drugs with antimicrobial activity is: Amphotericin B, rifampin, fluconazole, azithromycin, and miltefosine
7) Learn that management of elevated intracranial pressure and cerebral edema in PAM survivors has included hyperosmolor therapy, moderate hyperventilation, and induced hypothermia.

Objectives for Jordan's Story

1) Learn about the tragic loss of Jordan Smelski, Steve and Shelly Smelski's son, to Primary Amebic Meningoencephalitis.
2) Learn about the timeline of PAM in Jordan's story
3) Learn about the type of exposure that occurred in Jordan's story
4) Learn about the clinical response in Jordan's story

Suggested evaluation questions

As with the objectives, directly below is a suggested set of questions, if questions are used in the program, and additional questions are in the bank below the suggested set.

Please contact Steve Smelski at ssmelski@jordanssmile.org *or Jack Tracy at* Jack.tracy@flhealth.gov *for the answer key to all the questions below, and for any other comments or questions regarding this section.*

1.) Why is it critically important for the laboratory technician to ask the ordering physician about freshwater exposure history when a CSF specimen is received for a meningitis work up?

A. Test reagents can be ordered from specialty labs as soon as a *Naegleria fowleri* infection is suspected
B. Additional lab personnel can be summoned to the lab for a collaborative approach to detection of *Naegleria fowleri*
C. There can be successful treatment outcomes for PAM when early detection of *Naegleria fowleri* occurs in the laboratory
D. The electron microscope will be put into use as well as the software dedicated to microscopic movies

2.) "Time is of the Essence" in *Naegleria fowleri* detection, because:

A. As soon as the organism is detected, the strain tests can be initiated, and strain reports can be made, providing a clear picture for treatment.
B. As soon as the organism is detected, antimicrobial sensitivity testing can begin, and the results can be reported out for treatment and monitoring of antibiotic effectiveness.
C. The faster the identification of the ameba is made, the sooner a decision can be made on the proper stain to use for further speciation and work-up.
D. The faster the identification of the ameba is made, the faster the treatment can be initiated, and the better the chances will be for a positive treatment outcome

Suggested evaluation questions, continued:

3.) Which of the following is correct regarding the detection method(s) for *Naegleria fowleri* presented in the video?

A. Use of the dissecting microscope after the specimen has been frozen and prepared in paraffin with an acid fuchsin stain, followed by immunohistochemistry.
B. Looking for amoeboid movement in either the counting chamber, by direct wet mount, or by the enhanced method (centrifugation), and by using Wright-Giemsa staining after cytocentrifugation (eg Cytospin™). .
C. Immunohistochemistry (IHC) utilizing either immunoperoxidase staining, fluorescein, or rhodamine, and ultracentrifugation with a freezing and thawing cycle.
D. Monoclonal antibodies available on order from specialty labs, and either the Luxol fast blue stain or the Reticulin stain

4.) In looking for the ameba in the counting chamber or the direct wet prep, which of the following is important to remember?

A. A quick glance is all that is needed; the ameba is very distinctive from macrophages and artifacts- it can be seen immediately, and usually is present in large numbers.
B. It is important to add 5 % dextrose and phosphate-buffered saline to the aliquot, this will slow the ameba's movement to allow it to be seen after about 2 hours.
C. It can easily be mistaken for an artifact or a macrophage, especially if it is not moving or moving slowly; the entire chamber or slide should be studied carefully.
D. Both (B) and (C) are correct.

5.) If an automated system is routinely used for cell counts with CSF specimens for suspect meningitis cases, which of the following is correct?

A. It is important to also perform at least one of the wet prep procedures to look for the distinctive amoeboid movement of *Naegleria fowleri*, along with cytocentrifugation (eg Cytospin™) with wright's stain.
B. No further work-up is needed; the automated cell counting system will also detect, to species level, any protozoa present, and the instrument will also perform antibiotic sensitivity testing
C. An aliquot should be sent to the chemistry lab for processing in the blood chemistry analyzer to detect *Naeleria fowleri*–specific enzymes; this will also report strain type which will guide treatment.
D. Both (B) and (C) are correct.

Suggested evaluation questions, continued:

6.) Which of the following was discussed in the video regarding Cytocentrifugation (eg: Cytospin™) with Wright-Giemsa stain?

A. It should be performed, in addition to performing any of the 3 wet prep procedures to detect the ameba, and the slide should be scanned in its entirety.
B. It is never necessary to perform this step to detect the ameba, and the Wright-Giemsa stain is not the correct stain.
C. This procedure renders the ameba impossible to see. The centrifuging step kills the ameba.
D. None of these.

7.) Whenever the ameba is observed or suspected, which of the following should occur?

A. The pathologist should be called for confirmation of visual results.
B. The ordering physician should be contacted immediately, and infection control as well.
C. CDC should be contacted at the 24/7 support phone number provided in the video.
D. All of the above

8.) The absence of a specific request from the ordering physician for a *Naegleria fowleri* (or “ameba”) work-up with CSF on a suspect meningitis case means:

A. The tests do not need to be done; you should assume this has already been ruled out, as ameba infection is always thought of at the point of care
B. Ameba and PAM may not have been considered- communication with the physician about the freshwater exposure history is critical in this scenario
C. If no freshwater exposure history is available with the order or from the physician right away, testing for the ameba should begin along with the ordered tests.
D. Both (B) and (C)

Suggested evaluation questions, continued:

9.) To detect the ameba, warming is recommended, because it will usually increase the movement of the ameba and make it easier to see. The slide can be warmed before, or even after inoculation by:

A. Placing the slide in an incubator (35ºC to 37ºC)
B. Placing a warmed penny on the slide
C. Simply leaving the plated slide (or counting chamber) on the microscope with the light on (conventional bulb) for about 5-10 minutes
D. All of these

10.) Regarding "Do's and Don'ts" for CSF specimen storage and handling temperatures for detection of the ameba, which of the following is true?

A. Do freeze the specimen, don't store the specimen at room temp
B. Don't refrigerate or freeze the sample at any time, Do keep the sample at room temp
C. Don't keep the specimen below 45ºC
D. There were no "do's and don'ts" provided regarding storage and handling temperatures

11.) A "best practices" tip to optimize recovery of *Naegleria fowleri* is:

A. The ameba will become more motile the darker its environment is; be sure to completely darken the room for 30 minutes prior to looking at the slide
B. The ameba can stick to the sides of the CSF collection tube; be sure to thoroughly mix specimens, gently shake the tube, and flick the sides of the tube while mixing
C. The ameba can only be detected after it has been plated on trypticase soy agar (TSA) and stained with Luxol fast blue stain or the Reticulin stain
D. All of the above

Suggested evaluation questions, continued:

12.) Which of the following is true regarding the successful PAM treatment outcomes at Florida Hospital for Children in 2016 and Arkansas Children's Hospital in 2013 (the only 2 successful treatment outcomes in the United States since 1978)?

A. Both successful cases are the direct result of the physician at the point of care specifically asking for the lab to look for the ameba in the CSF specimen
B. In both cases, laboratory personnel awareness of the possibility of the ameba infection, and the detection of the organism in the CSF specimen contributed to the successful outcomes
C. Both hospitals had CSF transferred to their labs from community hospitals where *Naegleria fowleri* infection was suspected by the physicians there
D. Both cases are the direct result of the patient telling the physician that they believed they had primary amebic meningoencephalitis due to their recent freshwater exposure.

13.) Another scenario where processing CSF for ameba detection should also occur, especially where the freshwater exposure history is not known, or not available from the physician, is:

A. When you see a large number of neutrophils and no bacteria on the Gram stain.
B. When you see a large number of bacteria and very few neutrophils in the Gram stain.
C. When a large number of fungi and acid-fast bacteria are seen on the Gram stain
D. When a large amount of enzymatic activity is seen right after the Gram stain is done.

14.) In suspect meningitis cases where a history of freshwater exposure is known, or cannot be absolutely ruled out:

A. In addition to the usual CSF tests, an aliquot of the CSF should be frozen and left for the next shift to examine for fungi
B. In addition to the usual CSF tests, an aliquot of the CSF should be heated to 87°C and then tested for the Dengue fever virus
C. There is no connection between meningitis symptoms and a history of freshwater exposure
D. In addition to the usual CSF tests, the CSF should also be processed for the detection of *Naegleria fowleri*

Suggested evaluation questions, continued:

15.) Why is the Gram stain ***not*** the correct stain to use with a CSF specimen to detect *Naegleria fowleri*?

A. It washes all bacteria and white cells away, rendering it very difficult to obtain a clear overall picture of the condition of the CSF
B. The ameba's morphology is destroyed with the heat fixation process of Gram staining, rendering it very difficult to see and very easy to miss
C. It immediately converts the organism's appearance to mimic that of the macrophages, which necessitates special containing techniques
D. All of the above

16.) Clinically, a patient with PAM presents much like a patient with:

A. Hepatitis B infection
B. Lower respiratory tract
C. Bacterial meningitis
D. Norovirus infection

17.) The ameba, *Naegleria fowleri*, infects people when:

A. Water containing the ameba enters the body through an open cut or abrasion in the skin and travels to the brain via the bloodstream
B. Water containing the ameba is swallowed; the ameba then penetrates the intestinal mucosa and travels to the brain via the bloodstream
C. Water containing the ameba enters the body through the nose, it then travels up the olfactory nerves to the brain
D. None of the above

18.) Which of the following is true regarding the ways in which infection with *Naegleria fowleri* could occur?

A. Infection typically occurs when people go swimming or diving in warm freshwater
B. In very rare instances, PAM may also occur from exposure to water from inadequately treated swimming pools
C. In very rare instances, PAM may also occur from exposure to contaminated tap water
D. All of the above

Suggested evaluation questions, continued:

19.) Which of the following is true regarding the distribution of PAM cases in the United States?

A. No laboratory-confirmed PAM cases have occurred north of the state of Virginia, as water temperatures are always too cold in the northern states for the ameba
B. Recently, cases of PAM have been identified in states as far north as Minnesota, Indiana, and Maryland, possibly due to rising average water temperatures
C. The only states in the south with no confirmed PAM cases are Florida and Texas due to the dry climate seen in those states
D. There have been no laboratory-confirmed PAM cases in the southern states in the past 10 years, presumably due to low rainfall

20.) In the last few years, some additional types of water exposures have been identified which have caused PAM. These have been:

A. Ritual nasal rinsing (as part of the person's Muslim faith) with *Naegleria fowleri* contaminated home tap water
B. The use of Neti Pots for nasal saline irrigation apparently with *Naegleria fowleri* contaminated home tap water
C. Playing on a backyard water slide supplied with contaminated home tap water
D. All of the above

21.) When a diagnosis of PAM is considered, CDC should be consulted immediately, because:

A. 24/7 diagnostic and treatment consultation is available
B. Contact tracing can begin immediately with support from CDC
C. Testing reagents can be shipped to the laboratory
D. None of these

Evaluation question bank:

Shiela Black's Presentations:

1.) Quite often, primary amebic meningoencephalitis (PAM) may not be "on the radar" at the point of care for the initial diagnosis, therefore:

A. You, as the laboratory practitioner with PAM awareness, may be the first one to know that the patient's signs and symptoms are due to infection with *Naegleria fowleri*
B. As a laboratory practitioner, you should complete the 2 year course of specialized training in protozoology, and lab detection techniques
C. Additional equipment should be ordered which is designed specifically for the detection of *Naegleria fowleri*, followed by training on the equipment.
D. None of these.

2.) Why is it beneficial for the laboratory order system to have an automated reminder to the ordering physician to ask the patient about freshwater exposure history with all CSF specimen orders in suspect meningitis cases?

A. The lab will be alerted to the possibility of *Naegleria fowleri* infection and can query the physician if this is not answered in the order.
B. This serves as a reminder to the technologist and the physician that infection with the ameba could be a possibility, and procedures for detection can be undertaken.
C. Both (A) and (B)
D. None of these.

3.) In addition to looking for the ameba in the counting chamber and/or direct wet prep, an *enhanced* wet prep recovery process can be performed; this is:

A. Alternate freezing (-10ºC) and heating (73º C) an aliquot of CSF for 3 times, then placing the aliquot in the automated counting instrument
B. Spinning an aliquot of CSF at 8,000 RPM for 25 minutes in a standard centrifuge, then placing the pellet on a slide for direct microscopy.
C. Fix an aliquot of CSF with gluteraldehyde, washing 4 times with PBS, then looking for the ameba in the scanning electron microscope.
D. Spinning an aliquot of CSF at 2,000 RPM for 5 minutes in a standard centrifuge, then placing the pellet on a slide for direct microscopy.

Evaluation question bank, continued:

4.) In addition to the slide warming tips to increase the movement of any *Naegleria fowleri* amebae in the specimen, other ways discussed in the video to warm the specimen were:

A. No additional warming tips were provided beyond those for the slide
B. Placing an aliquot of CSF in a water bath or incubator (35ºC- 37ºC)), or combining a few drops of warm water with an aliquot of CSF before placing on slide
C. Placing an aliquot of the CSF in the thermal cycler (at highest temp setting) for 30 minutes before placing on slide
D. None of these

5.) Which of the following is correct regarding contrast stains and the detection methods for the ameba *Naegleria fowleri*?

A. Contrast stains and the Gram stain should not be used- Wright-Giemsa (also termed Wright's stain or Giemsa-Wright) should be used on the Cytocentrifugation (eg Cytospin™) slide.
B. No staining of any type should be done, and Cytocentrifugation (eg Cytospin™) is an unnecessary step
C. Only the gram stain should be used
D. Any contrast stain can be used with the Cytocentrifugation (eg Cytospin™) step to detect the ameba

6.) The ameba in a Wright stained (also called Wright-Giemsa, or Giemsa-Wright stain) Cytocentrifugation (eg Cytospin™) slide can be differentiated from white cells in which way?

A. The ameba's nucleus has a centrally-located nucleolus that is almost pin-point, and is lightly stained, while the cytoplasm is a dark purple.
B. There is no difference in the appearance of the ameba and the white cells.
C. Only the gram stain should be used
D. The ameba's nucleus is violet, and has a large, centrally-located nucleolus that is densely-staining; the cytoplasm is sky blue.

Evaluation question bank, continued:

Dr. Cope's presentation:

7.) Where is *Naegleria fowleri* commonly found?

A. Lakes and rivers
B. Hot Springs
C. Streams
D. All of the above

8.) Which of the following is the infective stage of *Naegleria fowleri*?

A. Trophozoite
B. Cyst
C. Flagellate
D. None of the above

9.) Regarding ways to reduce the risk of PAM due to swimming, which of the following is true?

A. Swimming only in freshwater with a temperature of 55 degrees Fahrenheit or lower will ensure a 100% reduction in risk
B. Refrain from swimming in freshwater when there are open cuts or skin abrasions, and if there is a viral infection present
C. The only certain way to prevent PAM due to swimming is to refrain from water-related activities in warm fresh water
D. Refrain from swallowing water while swimming in freshwater

10.) If freshwater swimming activities do occur, additional recommendations that may reduce risk of PAM are:

A. Holding your nose shut using nose clips, or keeping your head above water when taking part in activities in bodies of warm, fresh water
B. Avoid putting your head under the water in hot springs or other untreated thermal waters, and avoid stirring up the sediment in shallow, warm, freshwater areas
C. Avoid water related activities in freshwater water during periods of high water temperature and low water levels
D. All of the above

Evaluation question bank, continued:

11.) The most important clues pointing to a diagnosis of PAM in a patient with the symptoms of severe headache, fever, vomiting, neck stiffness, and seizures are found

A. In the patient's social history: have they had close, prolonged contact with anyone with similar symptoms in the past 2 to 5 weeks?
B. In the patient's vaccination and travel history: have they been vaccinated for PAM and have they had recent travel to a PAM-endemic country?
C. In the patient's freshwater exposure history within the past 2 weeks: swimming in a lake, river, stream, or nasal or sinus irrigation for medical or religious purposes, or any other nasal fresh water exposure?
D. None of these

12.) Initial testing in a patient with signs and symptoms of PAM and a history consistent with PAM should include:

A. A lumbar puncture to obtain CSF for laboratory examination
B. Brain imaging; lesions diagnostic for PAM are seen early in the disease
C. Arboviral disease panel: Dengue, chikungunya, zika virus
D. Both (B.) and (C.)

13.) The recommended combination of antimicrobial drugs for treatment of PAM is:

A. Posaconazole, amoxicillin, bacitracin, metronidazole, and pentostam
B. Butoconazole, omalizumab, tinidazole, erythromycin, and dicloxacillin
C. Amphotericin B, rifampin, fluconazole, azithromycin, and miltefosine
D. None of these

14.) Management of elevated intracranial pressure and cerebral edema in PAM survivors has included:

A. Administration of steroids
B. Drainage of CSF
C. Hyperosmolor therapy, moderate hyperventilation, and induced hypothermia
D. All of the above

Evaluation question bank, continued:

Franke Johns' presentation:

15.) Which of the following is true regarding the ways in which the ameba, *Naegleria fowleri*, was detected at the hospitals in the video?

A. One hospital detected it by looking for it on a wet prep (in a counting chamber), and seeing the ameboid movement; the other detected it by looking for it on a stained cytocentrifuged (eg Cytospin™) slide, using Wright-Giemsa (or Giemsa-Wright) stain.
B. Both laboratories detected it the same way: looking for it on a wet prep (in a counting chamber), and seeing the ameboid movement.
C. Both laboratories detected it the same way: by looking for it on a stained cytocentrifuged (eg Cytospin™) slide, using Gram Stain.
D. One hospital detected it by looking for it in a frozen, Gram stained section, and the other detected it by heating an aliquot of the CSF to 83ºC and then Gram staining it.

16.) If only a Gram stain is used on a CSF specimen, and the patient is infected with *Naegleria fowleri*, which of the following would most likely occur?

A. The ameba would be detected immediately, as this is the recommended stain to use on CSF for *Naegleria fowleri*
B. The ameba would be detected in the exam room by a rapid test with the patient's saliva, there is no need to test the CSF in the lab for *Naegleria fowleri*
C. The ameba would be missed, and therefore the specific treatment needed immediately for PAM would not be initiated, the patient would have virtually no chance of survival
D. None of these

17.) The laboratorian plays a critical role in the detection of the ameba *Neagleria fowleri* in CSF specimens submitted in suspect meningitis cases. Quite often, Primary Amebic meningoencephalitis is not considered by the ordering clinician. It is strongly recommended that all hospital labs provide training for all shifts on:

A. Awareness of the possibility of *Naegleria fowleri* infection in suspect meningitis cases
B. The importance of asking the ordering clinician about freshwater exposure history when a CSF specimen is received
C. The importance of saving CSF for the ameba detection steps.
D. All of these.

Evaluation question bank, continued:

18.) Since time is of the essence for ameba detection in suspect meningitis cases when a history of freshwater exposure is known, or cannot be ruled out, it is strongly recommended that all hospital labs have a pre-arranged standard operating procedure for detecting *Naegleria fowleri*, which includes a protocol for answering which of the following questions?

A. Which procedure will be done first, wet mount (wet prep) or stain?
B. Can both the wet mount and stain be done simultaneously? What is the time limit on results?
C. Which department will do the Wright-Giemsa stain? Which department will do the wet prep? Does this change according to the shift?
D. All of these.

19.) Which of the following are the stains which can be used to detect *Naegleria fowleri*?

A. Wright-Giemsa (or Giemsa-Wright), Gram stain, acid fuchsin stain
B. Gram stain, Luxol fast blue stain, Reticulin stain, acid-fast stain, iodine
C. Wright-Giemsa (Wright's or Giemsa-Wright), Hematoxylin and Eosin, Periodic Acid Schiff (PAS), and Trichrome
D. Endospore stain (Schaffer-Fulton), Ziehl-Nulsen, Acridine Orange, and Gram Stain

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