TITLE: MICRO – 736 Cerebrospinal Fluid Culture

PRINCIPLE / PURPOSE:

Cerebrospinal fluid (CSF) circulates around the brain, ventricles, and spinal cord and carries essential metabolites into neural tissues and cleanses the organs of waste. Infection within the leptomeninges is called meningitis and is caused by bacterial or viral pathogens, which cross the blood brain barrier. Patients with impaired host defenses are at increased risk for the development of meningitis. Patients experience fever, headache, stiff neck, nausea, vomiting, lethargy, confusion and mental disorientation. CSF is collected aseptically by inserting a needle into the subarachnoid space.

ORGANISMS OF INTEREST:

Any isolate from CSF is considered significant.

All organisms which infect the meninges are very fastidious.

AGENTS OF CHRONIC MENINGITIS

*Mycobaerium tuberculosis*

*Cryptococcus neoformans*

*Coccidioides immitis*

*Histoplasma capsulatum*

*Blastomyces deratitidis*

*Candida* species

Other fungi

*Actinomyces*

*Treponema pallidum*

*Brucella*

*Salmonella*

Rare Parasites

AGENTS OF ACUTE BACTERIAL MENINGITIS

*Streptococcus pneumoniae*

*Haemophilus influenzae*

*Neisseria meningitidis*

*Streptococcus agalactiae* (Group B strep)

Gram negative bacilli

*Listeria monocytogenes*

Staphylococci

*Leptospira*

*Treponema Pallidum*

*Mycobacterium tuberculosis*

SCOPE:

This procedure applies to the processing and culture of cerebral spinal fluid.

POLICIES:

* Use a biological safety cabinet when opening a culture container or potentially creating aerosols.
* Always wear gloves when handling a specimen.
* CSF specimens must be processed immediately. A delay in processing my result in the death of a patient.

SPECIMEN:

Type:

Minimum of 500 µL (0.5 mL) of CSF.

Note: If ≤500 µL is received, notify the physician of modified processing; only a gram stain and chocolate plate will be set up. “Interpret results with caution due to limited specimen volume” must be documented in LIS.

Handling Conditions:

1. Aseptically cleanse puncture site with alcohol.
2. Insert needle at L3, L4-L5, or L5-S1 interspace.
3. Spinal fluid will appear in the needle hub.
4. Collect CSF into sterile leakproof tubes (included in LP kit). Generally, tube #2 is used for culture.

EQUIPMENT AND MATERIALS:

Equipment:

Loop

Biological safety cabinet

35ºc, CO2 incubator

Vortex

Sterile disposable pipet

Centrifuge

Cytospin

Media:

# TSA blood agar – CO2

Modified thayer Martin- CO2

Chocolate Agar- CO2

MacConkey agar – CO2

* add sabourands if yeast or fungus is suspected.

 All media are BBL and stored at 2-8ºC. After plating, media are incubated at 35-37ºC.

QUALITY CONTROL:

Selected types of media are checked for sterility and performance using ATCC strains.

PROCEDURE:

1. Centrifuge the CSF tube for 10 minutes if the quantity of fluid is greater than 1 mL.

2. Aspirate the supernatant using a sterile pipet, leaving approximately 0.5 to 1 mL of sediment

1. Save the supernatant for additional studies.
2. Cytospin all CSF gram stains. Use Cytospin in Micro department; perform under hood.

 Add 2-3 drops of CSF to a slide-holder.

 Add 1 drop of Albumin, cap the tube.

 Load the cytospin.

 Press load 1 and start.

 Spin at 1000 RPM for 5 minutes.

3. Make and stain a gram stain. Record stain results on the worksheet and in the LIS.

 Any positive finding on the gram stain must be called immediately (critical value).

 Note: The gram stain is considered a STAT test and must be processed and

 reported ASAP (within 1 hour of receipt to the lab).

4. Vortex the sediment 30 seconds to resuspend the pellet.

5. Inoculate the media using 1 to 2 drops of the sediment.

6. Incubate media according to media section of this procedure.

DAY 1

Examine aerobic plates at 12-24 hours for potential pathogens. Reincubate media.

Day 2

Examine aerobic plates for potential pathogen. Reincubate media.

Day 3

Examine aerobic plates for potential pathogens.

Perform ID and AST (where appropriate for organism) on all isolates. Call all positive results (critical value) ASAP.

Finalize report.

All plates are saved for 7 additional days at room temperature in the biohazard waste room.

INTERPRETATION & REPORTING RESULTS:

Procedures for Abnormal Results:

Positive gram stains are to be called and reported immediately.

Reporting Format:

Semi-quantitative growth using the terms:

 Rare: 1-2 colonies

 LIGHT GROWTH: 1nd quadrant

 MODERATE GROWTH: 2nd quadrant

 HEAVY GROWTH: Final quadrant

For cultures that are negative, use the following LIS codes.

NG24-NO GROWTH IN 24 HOURS

NG48- NO GROWTH IN 48 HOURS

NGA3 – NO GROWTH AEROBICALLY IN 3 DAYS

PROCEDURE NOTES:

CSF may contain very few organisms per milliliter of fluid, so concentration of the specimen is important.

Age specific microorganisms are as follows.

1. Premature or neonatal infants less than 2 months of age usually develop E.Coli and Lancefield’s group B beta hemolytic streptococci.
2. In children more than 2 months of age, up to 5 years Haemophilus Influenza (typeB) Streptococcus pneumoniae and Neisseria meningitidis. (Since the Haemophilus influenzae vaccine, fewer cases of meningitidis caused by this organism are occurring).
3. The significant microorganisms in adults which may cause meningitidis are Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae in-patients of age 65 years or older.
4. CSF gram stain results: When a gram stain is called positive for any microorganism check to see if a CSF bacterial antigen study has been ordered. Ask the physician if the bacterial antigen study should be cancelled.
5. If a CSF bacterial antigen study is ordered, it must be ordered and sent STAT to the reference lab for testing.
6. Extra CSF is stored in double-door refrigerator after set-up completion.
7. Any delay in processing CSF should be placed in 35° CO2 incubator and processed ASAP.
8. Turnaround times for CSF gram stains should be within a reasonable amount of time and will be monitored by Microbiology.

The specificity and sensitivity of CSF bacterial antigen studies are not adequate. Meningitis may be caused by an organism which is not covered by the antigen tests.

RELATED PROCEDURES:

Gram Stain

CytoPro

Critical Result Reporting

REFERENCES:

ASM: Clinical Microbiology Procedures Handbook, ASM, SEC 1.9, 2008

Fingold, S.M. and W.J. Martin, “ Diagnostic Microbiology”, 6th ED. C.V. Mosby Co., St. Louis, pp213-222, 1982.

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HISTORY PAGE

SOP Number: MICRO - 736

SOP Title: CSF Culture

Written By: Jacee Farmer

Manual in which Hard Copy of this SOP is located: Microbiology Manual IV

Distribution: Sharepoint

Supersedes Procedure:

SOP CHANGE CONTROL

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