

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MI\SFLD\v05	Page 1 of 1
Section: Sterile Fluids Manual	Subject Title: Table of Contents	
Issued by: LABORATORY MANAGER	Original Date: March 20, 2000	
Approved by: Laboratory Director	Revision Date: April 6, 2005	
	Review Date: April 6, 2005	

STERILE FLUIDS CULTURE MANUAL

TABLE OF CONTENTS

Cerebrospinal Fluids	2
Other Sterile Fluids	4
Peritoneal Dialysis Effluent.....	7
Predialysis Fluid	9
Bone Marrow (Aspirates or Biopsies).....	11
Blood, Platelets, & Other Transfusion Products	13
Cryptococcal Antigen	
Yeast Identification	
Record of Edited Revisions	15

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MI\SFLD\01\v04	Page 1 of 3
Section: Sterile Fluids Manual	Subject Title: Cerebrospinal Fluids	
Issued by: LABORATORY MANAGER	Original Date: March 20, 2000	
Approved by: Laboratory Director	Revision Date: April 6, 2005	
	Review Date: April 6, 2005	

CEREBROSPINAL FLUIDS

I. Introduction

Bacterial meningitis is the result of infection of the meninges (lining around the brain). This section includes central nervous system shunt fluid, fluid from Omayya reservoirs, external ventricular drainage fluid as well as routine CSF. The examination of CSF from patients suspected of having meningitis is always considered to be a STAT procedure.

II. Specimen Collection and Transport

See [Pre-analytical Procedure - Specimen Collection QPCMI02001](#)

III. Reagents / Materials / Media

See [Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001](#)

IV. Procedure

A. Processing of Specimens:

See [Specimen Processing Procedure QPCMI06003](#)

a) Direct Examination:

Gram stain: Note the presence or absence of organisms and WBCs. DO NOT quantitate.
Make 2 smears if the specimen is grossly bloody.

b) Culture:

Media	Incubation
Blood Agar	CO ₂ , 35°C x 4 days
Chocolate Agar	CO ₂ , 35°C x 4 days
Fastidious Anaerobic Broth	O ₂ , 35°C x 4 days

If fungus or Cryptococcus is requested, add :	
Inhibitory Mould Agar ²	O ₂ , 28°C x 3 weeks
Esculin Base Medium ²	O ₂ , 28°C x 3 weeks

¹Forward inoculated fungal media to Mycology section for incubation and work-up.

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # M1\SFLD\01\v04	Page 3 of 3
Sterile Fluids Manual		

B. Interpretation of Cultures:

Examine the Blood Agar and Chocolate Agar plates and the Fastidious Anaerobic Broth daily for 4 days incubation. If no growth on the culture plates but evidence of growth in Fastidious Anaerobic Broth, perform Gram stain and sub-culture Fastidious Anaerobic Broth onto Chocolate Agar, Brucella Agar and other media as appropriate and incubate and process as above. All isolates are to be identified.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. **Reporting**

a) Gram stain: Report the presence or absence of organisms and WBCs. Do not quantitate.

b) Culture:

Negative Report: “No growth”.

Positive Report: Report all isolates with appropriate sensitivities. Do not quantitate except if it is from the fluid medium only – add ISOLATE Comment “From broth culture only, indicative of small numbers and/or contamination.”

Refer to Technical Manual for [Cryptococcal Antigen](#) reporting.

Report results ASAP by telephone to the ward/ordering physician for the following:

- All positive or STAT Gram stain
- All Gram stain results for TRI, CAMH, Bridgepoint and Toronto Grace patients
- Cryptococcal antigen test
- All positive culture.

VI. **References**

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Tenover, R.M. Tenover. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Blood Cultures-General Detection and Interpretation, p.3.4.1.1-3.4.1.19 In Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

QMP-LS Practice Guidelines – Cerebral Spinal Fluid

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # M1SFLD\07\v04	Page 1 of 3
Section: Sterile Fluids Manual	Subject Title: Other Sterile Fluids	
Issued by: LABORATORY MANAGER	Original Date: March 20, 2000	
Approved by: Laboratory Director	Revision Date: April 6, 2005	
	Review Date: April 6, 2005	

OTHER STERILE FLUIDS

I. Introduction

Pleural (Thoracentesis/ Empyema) Fluids:

Infection of the pleural space may result in severe morbidity and mortality. Therefore rapid and accurate microbiological assessment is required. Any organism found in pleural fluid must be considered significant (although specimen contamination may occur during collection).

Peritoneal (Ascites) Fluids:

Peritonitis may be classified as primary (spontaneous), secondary or tertiary. Primary peritonitis usually occurs in someone with pre-existing ascites (e.g. patients with chronic liver disease) in which there has been no entry into the abdominal cavity. Secondary and tertiary peritonitis occur after surgery or trauma to the abdomen. Although enteric Gram negative organisms are the most common isolates associated with these types of infections, polymicrobial infection is common with a mixture of both Gram positives and negatives including anaerobes.

Synovial (Joint) & Pericardial Fluids:

These are normally sterile fluids. Infection of these fluids may be due to a variety of different organisms as a result of direct infection, contamination at the time of surgery/trauma or hematogenous spread.

Amniotic Fluids:

Amniotic fluid is that fluid which surrounds the developing fetus in utero. As with other normally sterile fluids, infection of the amniotic fluid may result in severe morbidity and mortality to the mother and fetus. Any organism isolated must be considered significant (although contamination may occur during collection).

Other Fluids:

Infection of normally sterile body fluids may result in severe morbidity and mortality. Any organism isolated must be considered significant (although specimen contamination may occur during collection). Specimens include tympanocentesis fluid, intraocular fluid, hydrocele fluid, cyst fluid, etc.

II. Specimen Collection and Transport

See [Pre-analytical Procedure - Specimen Collection QPCMI02001](#)

III. Reagents / Materials / Media

See [Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001](#)

IV. Procedure

A. Processing of Specimens:

See [Specimen Processing Procedure QPCMI06003](#)

- a) Direct Examination: Gram stain: Note the presence of organisms and WBCs.
Do not quantitate.
Calcofluor white stain (If fungus is requested).
- b) Culture:

Media	Incubation
Blood Agar (BA)	CO ₂ , 35°C x 4 days
Chocolate Agar (CHOC)	CO ₂ , 35°C x 4 days
Fastidious Anaerobic Agar (BRUC)	AnO ₂ , 35°C x 48 hours
Kanamycin/Vancomycin Agar (KV)	AnO ₂ , 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O ₂ , 35°C x 4 days

If fungus is requested, add :	
Inhibitory Mould Agar (IMA) ¹	O ₂ , 28°C x 3 weeks
Esculin Base Medium (EBM) ¹	O ₂ , 28°C x 3 weeks

¹Forward inoculated fungal media to the Mycology section for incubation and work-up.

B. Interpretation of Cultures:

Examine the BA, CHOC plates and THIO daily for up to 4 days. Examine the BRUC after 48 hours incubation. If no growth on the culture plates but evidence of growth in THIO, perform a Gram stain and sub-culture the THIO onto CHOC, BRUC and other media as appropriate and incubate and process as above. All isolates are to be identified.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MISFLD\07\v04	Page 3 of 3
Sterile Fluids Manual		

V. Reporting

a) Gram stain: Report the presence or absence of organisms and WBCs. Do not quantitate.

b) Culture:

Negative Report: "No growth".

Positive Report: Report all isolates with appropriate sensitivities. Do not quantitate except if it is from the fluid medium only – add ISOLATE Comment “From broth culture only, indicative of small numbers and/or contamination.”

Telephone results of a positive Gram stain and all positive cultures to the ward / ordering physician.

VI. References

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Tenover, R.M. Tenover. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Blood Cultures-General Detection and Interpretation, p.3.4.1.1-3.4.1.19 In Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # M1SFLD\04\v03	Page 1 of 2
Section: Sterile Fluids Manual	Subject Title: Peritoneal Dialysis Effluent	
Issued by: LABORATORY MANAGER	Original Date: March 20, 2000	
Approved by: Laboratory Director	Revision Date: April 6, 2005	
	Review Date: April 6, 2005	

PERITONEAL DIALYSIS EFFLUENT

I. Introduction

Dialysis solution is infused into the patient's abdominal cavity through a permanently implanted tube. The solution remains there for several hours, picking up waste from the blood stream. The dialysis solution may become infected while in the patient's abdomen or from external contamination due to the tubing which enters the patient's abdominal cavity. A variety of both Gram negative and positive organisms may infect the dialysis solution.

II. Specimen Collection and Transport

See [Pre-analytical Procedure - Specimen Collection QPCMI02001](#)

III. Reagents/Materials/Media

See [Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001](#)

IV. Procedure

A. Processing of Specimens

NB: No more than one dialysis fluid per patient should be processed every other day. If a bag of cloudy fluid is received after a clean one is processed, culture and sensitivity is always done. A portion of the fluid should be sent to Haematology for cell count if requested.

See [Specimen Processing Procedure QPCMI06003](#)

a) Direct Examination:

- i) If specimen is cloudy: Gram stain
- ii) If specimen is clear: No Gram stain is needed.

b) Culture:

Media	Incubation
--------------	-------------------

If specimen is clear:

Bact/Alert bottles* Processed as per blood culture protocol

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MI\SFLD\04\v03	Page 2 of 2
Sterile Fluids Manual		

Media	Incubation
If specimen is cloudy:	
Blood Agar (BA)	CO ₂ , 35°C x 4 days
Chocolate Agar (CHOC)	CO ₂ , 35°C x 4 days
MacConkey Agar (MAC)	CO ₂ , 35°C x 4 days
BacT/Alert bottles*	Processed as per blood culture protocol

*Inoculate both an aerobic and anaerobic bottle with 8 to 9 mL of fluid each.

B. Interpretation of Cultures:

Examine the BA, MAC and CHOC daily for up to 4 days incubation. Identify all isolates.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. **Reporting**

a) Gram stain: Report the presence or absence of organisms and WBCs.
Do not quantitate.

b) Culture:

Negative Report: "No growth".

Positive Report: Report all isolates with appropriate sensitivities. Do not quantitate except if it is from the fluid medium only – add ISOLATE Comment "From broth culture only, indicative of small numbers and/or contamination."

Telephone all positive Gram stain and culture results to ward/ordering physician. When out-patient units are closed, page the Nephrology Fellow/Resident with the results of the Gram stain and/or culture results.

VI. **References**

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Tenover, R.M. Tenover. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Blood Cultures-General Detection and Interpretation, p.3.4.1.1-3.4.1.19 In Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MI\SFLD\05\v03	Page 1 of 2
Section: Sterile Fluids Manual	Subject Title: Predialysis Fluid	
Issued by: LABORATORY MANAGER	Original Date: March 20, 2000	
Approved by: Laboratory Director	Revision Date: April 6, 2005	
	Review Date: April 6, 2005	

PREDIALYSIS FLUID

I. Introduction

This is fluid collected prior to dialysis and should normally be sterile.

II. Specimen Collection and Transport

See [Pre-analytical Procedure - Specimen Collection QPCMI02001](#)

III. Reagents / Materials / Media

See [Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001](#)

IV. Procedure

A. Processing of Specimens:

See [Specimen Processing Procedure QPCMI06003](#)

1. Examine the fluid noting the colour and turbidity. Record the observations in the LIS under COMMENT in the SOURCE screen.
2. Centrifuge specimens (3500 rpm x 20 minutes).
3. Decant supernatant leaving approximately 2-3 mL. Vortex well to resuspend the sediment.
 - a) Direct Examination:
 - i) If specimen is cloudy: Gram stain
 - ii) If specimen is clear: No Gram stain is needed.

b) Culture:

Media	Incubation
If specimen is clear:	
Fastidious Anaerobic Broth (THIO)	O ₂ , 35°C x 4 days
If specimen is cloudy:	
Blood Agar (BA)	CO ₂ , 35°C x 4 days
MacConkey Agar (MAC)	O ₂ , 35°C x 4 days
Chocolate Agar (CHOC)	CO ₂ , 35°C x 4 days
Fastidious Anaerobic Broth (THIO)	O ₂ , 35°C x 4 days

B. Interpretation of Cultures:

Examine the BA, MAC and THIO daily for up to 4 days incubation. Examine BRUC and KV after 48 hours. If no growth on the culture plates, but evidence of growth in the THIO perform Gram stain and subculture the THIO onto CHOC, BRUC and other media as appropriate and incubate and process as above. All isolates are to be identified.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. **Reporting**

a) Gram stain: Report the presence or absence of organisms. Do not quantitate.

b) Culture:

Negative report: "No growth"

Positive report: Report all isolates with corresponding sensitivities. Do not quantitate except if it is from the fluid medium only – add ISOLATE Comment "From broth culture only, indicative of small numbers and/or contamination."

Telephone all positive Gram stain and culture results to ward/ordering physician.

When outpatient units are closed, page the Nephrology Fellow/Resident with the results of the Gram stain and/or culture results.

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MI\SFLD\09\v03	Page 1 of 2
Section: Sterile Fluids Manual	Subject Title: Bone Marrow (Aspirates or Biopsies)	
Issued by: LABORATORY MANAGER	Original Date: March 20, 2000	
Approved by: Laboratory Director	Revision Date: April 6, 2005	
	Review Date: April 6, 2005	

BONE MARROW (ASPIRATES OR BIOPSIES)

I. Introduction

Infection of bone marrow is uncommon. However, it may be a site of infection with fungus or tuberculosis in patients with disseminated disease.

II. Specimen Collection and Transport

See [Pre-analytical Procedure - Specimen Collection QPCMI02001](#)

III. Reagents / Materials / Media

See [Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001](#)

IV. Procedure

A. Processing of Specimens:

See [Specimen Processing Procedure QPCMI06003](#)

- a) Direct Examination: Gram stain.
Calcofluor white stain.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO ₂ , 35°C x 4 days
Chocolate Agar (CHOC)	CO ₂ , 35°C x 4 days
Fastidious Anaerobic Agar (BRUC)	AnO ₂ , 35°C x 48 hours
Kanamycin / Vancomycin Agar (KV)	AnO ₂ , 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O ₂ , 35°C x 4 days
Inhibitory Mould Agar (IMA) ¹	O ₂ , 28°C x 4 weeks
Esculin Base Medium (EBM) ¹	O ₂ , 28°C x 4 weeks
Blood Egg Albumin Agar (BEAA) ¹	O ₂ , 28°C x 4 weeks

¹Forward inoculated fungal media to the Mycology Section for incubation and work-up.

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MI\SFLD\09\v03	Page 2 of 2
Sterile Fluids Manual		

B. Processing of Cultures:

Examine the BA, CHOC plates and THIO daily for up to 4 days incubation and the BRUC and KV plates after 48 hours incubation. If no growth on the culture plates but evidence of growth in THIO, perform Gram stain and sub-culture the THIO onto BA, CHOC and other media as appropriate.

If bone marrow received in BacT/Alert bottle(s), process as a routine blood culture. Do **NOT** inoculate BacT/Alert bottle(s) in the lab. (Refer to the Blood Culture Manual).

All isolates are to be identified.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

IV. Reporting

a) Gram stain: Report the presence or absence of organisms.

b) Culture:

Negative Report: "No growth"

Positive Report: Report all isolates with appropriate sensitivities. Do not quantitate except if it is from the fluid medium only – add ISOLATE Comment “From broth culture only, indicative of small numbers and/or contamination.”

Telephone all positive Gram stains and cultures to ward/ordering physician.

VI. References

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Tenover, R.M. Tenover. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Blood Cultures-General Detection and Interpretation, p.3.4.1.1-3.4.1.19 In Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MI\SFLD\10\v02	Page 1 of 2
Section: Sterile Fluids Manual	Subject Title: Blood, Platelets, and Other Transfusion Products	
Issued by: LABORATORY MANAGER	Original Date: March 20, 2000	
Approved by: Laboratory Director	Revision Date: April 6, 2005	
	Review Date: April 6, 2005	

BLOOD, PLATELETS, & OTHER TRANSFUSION PRODUCTS

I. Introduction

Occasionally blood, platelets and other transfusion products may become infected at the time of collection from donors, during processing or at the time of infusion into patients. Any organism isolated must be considered significant.

II. Specimen Collection and Transport

See [Pre-analytical Procedure - Specimen Collection QPCMI02001](#)

III. Reagents / Materials / Media

See [Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001](#)

IV. Procedure

A. Processing of Specimens:

See [Specimen Processing Procedure QPCMI06003](#)

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO ₂ , 35°C x 4 days
Chocolate Agar (CHOC)	CO ₂ , 35°C x 4 days
Fastidious Anaerobic Broth (THIO)	O ₂ , 35°C x 4 days

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MI\SFLD\10\v02	Page 2 of 2
Sterile Fluids Manual		

B. Processing of Cultures:

Examine the BA and CHOC plates after 24 and 48 hours incubation. Examine the THIO daily for up to 5 days. If no growth on culture plates but evidence of growth on THIO, perform Gram stain and sub-culture THIO onto CHOC, BRUC and other media as appropriate and incubate and process as above. All isolates are to be identified.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting

a) Gram stain: Report the presence or absence of organisms.

b) Culture:

Negative Report: No growth

Positive Report: Report all isolates with appropriate sensitivities. Do not quantitate except if it is from the fluid medium only – add ISOLATE Comment “From broth culture only, indicative of small numbers and/or contamination.”

Telephone results of all positive Gram stains and cultures to ward / ordering physician.

VI. References

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Tenover, R.M. Tenover. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Blood Cultures-General Detection and Interpretation, p.3.4.1.1-3.4.1.19 In Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.