

Body Fluid Analysis for Cellular Composition; Proposed Guideline

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Objectives

To provide users with Recommendations for: •

- Collection and transport of body fluids, . ١
- Numeration and identification of cellular components, . ٢
and
- Guidance for qualitative and quantitative assessment . ٣
of body fluid.

Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity •

Body Fluids

Body fluids will be discussed today: •

Cerebrospinal,CSF . ١

Serous (pleural, peritoneal, pericardial), . ٢

Synovial fluids. . ٣

Standard Precautions •

Preanalytical Variables

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The test request procedure is important to address in the quality system model for laboratory testing.

An error in the order • entry procedure or In a test request being • incorrectly ordered, either as tests added to or deleted from the original test request.

Preanalytical Variables, anticoagulant

- The **type of anticoagulant** (additive) used for the collection of specific body fluids may also affect test results.
 - eg, using an additive when it is not required (cerebrospinal fluid, or **CSF**) may possibly affect the enumeration of white and red blood cells.
- Using the **wrong additive** (synovial) could possibly introduce artifacts and therefore interfere with the identification of cellular elements present on a slide.

Preanalytical Variables

In some body fluids, the proper order of draw is important (the incidence of cellular contamination from tube to tube is reduced).

It is also necessary so that a microbiology specimen is not contaminated.

In addition, hemolyzed and clotted specimens are not recommended as specimens of choice for analysis

However, circumstances may arise when it is not possible to acquire another specimen from a patient.

These exceptions to standard practice must be clearly defined in the site's Standard Operating Procedures.

Preanalytical Variables

Specimen transport may be affected, eg, the •
temperature at which a specimen is transported could
affect the integrity, degradation, or deterioration of the
constituents of the fluid.

The transport time must also be acceptable to maintain •
specimen integrity.

The method of transport may also affect the integrity of •
the specimen.

For example, the use of a *pneumatic tube system* must •
be approached with caution because excessive shaking
of body fluids may result in a breakdown of the cellular
constituents

Pneumatic System



Preamanalytical Variables

The date and **time** on the specimens should be **verified** against the **actual collection time** of the specimen. If a significant discrepancy between the times exists, the unit should be notified to rectify the discrepancy before test analyses.

If these specimen identification guidelines are not met either electronically or manually, the unit will be notified that a new specimen will be required.

If specimen identification guidelines are not met, the laboratory personnel must follow the administrative guidelines of the laboratory for **analysis or rejection** of the specimen.

In addition, a **Laboratory Incident Report** should be filed.

Specimen Collection

Cerebrospinal Fluid, CSF

Specimen Collection

Cerebrospinal Fluid, CSF

- CSF is usually collected by **lumbar puncture(LP)**, but may also be obtained by **lateral cervical** or **cisternal puncture**.
- **Sterile technique** is mandatory to avoid introducing bacteria.
- **Manometric measurements** may be done and are the responsibility of the clinical service rather than the laboratory.
- Usually, fluid is collected into **three or four tubes** for **chemical, microbiologic**, and **cellular analysis**.
- The tubes should be labeled according to the **sequence of collection**.

Specimen Collection

Cerebrospinal Fluid, CSF

- It is preferable to have the **first tube** analyzed for **chemical** and **serologic** studies.
- Subsequent tubes should be used for **microbial** and **cellular analysis** to obtain accurate cell counts and decrease the chance of bacterial contamination.
- ***No anticoagulant is necessary***, since spinal fluid does not clot except occasionally if the puncture is traumatic.

Specimen Collection

Cerebrospinal Fluid, CSF

Since the volume of CSF is relatively small, the total amount collected is limited and usually varies from **10 to 20 mL** in adults. •

Up to 8 mL may be safely removed from the smallest infant. •

Complications of lumbar puncture include headache, infection, and brain herniation. •

Rarer complications may also occur. •

Specimen Collection

Cerebrospinal Fluid, CSF

Table 1. Specimen Requirements for Cerebrospinal Fluid

<u>Test</u>	<u>Anticoagulant</u>	<u>Volume (mL)</u>	<u>Comments</u>
(e.g., protein, glucose, other special tests)	None	3-5	Tube #1 If traumatic tap is suspected, cell count should also be performed on Tube 1.
Gram stain and culture	None	3-5	Tube #2
Cell count and differential	None	3-5	Tube #3 or 4
Other tests as required (e.g., cytology)	None	3-5	Tube #4

Specimen Collection

Serous Fluid

Specimen Collection

Serous Fluid

- Serous fluids (e.g., pleural, peritoneal) from large volume collections may be **aliquoted** into smaller volumes before transport to the laboratory or in the laboratory.
- *Specimens should be **gently agitated** during collection, before aliquoting, and before testing for cell counts and differentials.*
- **EDTA** is the recommended anticoagulant for cell counts and differentials.
- *Refrigerated storage* is adequate for cell counts and differentials for **up to 24 hours**.
- Although testing can be done on small volumes of fluid, **5 to 8 mL** is recommended in the event follow up studies are needed (e.g., flow cytometry).
- A sterile collection tube must be used for microbial studies.

Specimen Collection

Serous Fluid

For **cytology specimens**, a wide range of volumes may be sent to the laboratory. •

As little as **15** to greater than **100 mL** may be sent for analysis. •

A **50-mL** specimen is recommended. •

Sterility is not required and no anticoagulant is necessary. •

However, heparin and EDTA are also used. •

If clumps of material are present, they can be processed as a **cell block**. •

Specimen Collection

Serous Fluid

Table 2. Specimen Requirements for Serous Fluids

<u>Tests</u>	<u>Anticoagulant</u>	<u>Volume (mL)</u>
RBC, WBC, differential	EDTA	5-8
Total protein, LD, glucose amylase	Heparin, none	8-10
Gram stain, bacterial culture	SPS*, none, or anticoagulant without bactericidal or bacteriostatic effect	8-10
AFB culture	SPS, none, or anticoagulant without bactericidal or bacteriostatic effect	15-50
PAP stain, cell block	None, heparin, EDTA	5-50

*SPS = Sodium polyanetholsulfonate

Specimen Collection

Synovial Fluid

Specimen Collection

Synovial Fluid

- The amount of fluid depends on the **size** of the joint and **effusion**.
- A **3- to 5-mL** sample is ideal for laboratory analysis. (However, since this may not be possible in smaller joints, the physician should prioritize the requested tests and clearly communicate with the laboratory.)
 - Specimens ***should not be rejected*** because ***of small volumes***, since ***even a drop may provide definitive diagnosis in crystalline joint disease*** and only small volumes are needed for cell count and differential.

Specimen Collection

Synovial Fluid

- The on the collection tray, previously used to remove fluid from a medicinal vial.
- Fluid should be **thoroughly mixed** after collection and before analysis in the laboratory to obtain accurate cell counts.
- Some texts indicate that **lithium heparin** and **EDTA** should not be used as anticoagulants because they produce crystalline material that can be confused with pathologic crystals.
- However, others have used lithium heparin and EDTA without difficulty.

Specimen Collection

Synovial Fluid

Table 3. Specimen Requirements for Synovial Fluid*

<u>Test</u>	<u>Anticoagulant</u>	<u>Volume (mL)</u>	<u>Comments</u>
Cell count, differential, crystals, inclusions	Heparin, EDTA	3-5	Can be done on a few drops of fluid. Mix thoroughly.
Glucose	Fluoride or none	3-5	8-hr. fast preferred
Protein	None		
CH50	None		Freeze if not tested immediately.
C3, C4	None or EDTA		Requires 1 mL
Culture	SPS, none, or anticoagulant without bactericidal or bacteriostatic effect	3-5	Sterile tube required

* Requirements may change with advances in technology.

Specimen Handling and Transport

Specimen Handling and Transport

- Specimens should be transported to the laboratory **promptly**.
- Cellular degeneration of CSF can begin **within one hour** of collection, so cell counts should be completed as soon as possible.

Specimen Handling and Transport

CSF specimens should be transported at •
ambient temperature to the testing site as
soon as possible following completion of the
collection procedures.

CSF for microbiology testing should **never be** •
refrigerated before or after transport; since
some organisms are **fastidious** and
temperature sensitive, they have the
capability of becoming nonviable.

Serous Fluids

It is also recommended that pleural, pericardial, and peritoneal fluids be transported to the testing site at *ambient temperature*. •

To preserve the integrity of these specimens, however, the testing site should be in receipt of these specimens *as soon as* possible after the completion of the collection procedures. •

Otherwise, *cell lysis, cellular degradation,* and *bacterial growth* could occur and possibly affect the test results. •

Serous Fluids

- Serous fluids for the **cytology** laboratory should be sent **as soon as** possible.
- If storage is necessary, the specimen should be *refrigerated at 4 °C without a fixative*.
- Serous fluids have a **high protein content**, cellular detail with Papanicolaou (PAP), H & E, or other stains will be adequately preserved with **refrigeration** for **several days**.

Synovial Fluids

Synovial fluid specimens may be transported •
and analyzed at **room temperature.**

Quantitative Assessment Manual Counting, Procedure

Quantitative Assessment

Manual Counting, Procedure

Mix the specimen well by rotation on an **automated mixer** for a maximum of **2 to 5 min.** (*excessive rocking may damage cells*) or **hand mix** by inverting the tube **10 to 15 times.** •

The exception is **synovial fluid**, which must be mixed for **5 to 10 min** due to the viscosity of the fluid. •

If the fluid is in a conical tube, **flick** the bottom of the conical tube several times to dislodge cells before mixing the specimen. •

The **more turbid the sample**, the greater the mixing process impacts cell count accuracy. •

Specimen Dilutions

- The sample should be **well mixed** before analysis.
- Both **erythrocytes** and **nucleated cells** are enumerated in the **same chamber**.
- Specimens are usually **counted undiluted**, unless they are **bloody** or **cloudy**.
- Typical dilutions for any fluid can range from **1:10 to 1:200** or higher, depending on the turbidity of the specimen.

Specimen Dilutions

- Different diluents can be used to dilute the fluids. *Isotonic saline* can be used for both white and red cell dilutions while *acetic acid* or *hypotonic saline* may be used to lyse red cells for white cell dilutions.
- Acetic acid should not be used as a diluent for synovial fluid manual nucleated cell counts, since *mucin will coagulate*.
- If manual nucleated cell counts are performed on synovial fluid samples, erythrocytes can be lysed, with preservation of nucleated cells, by using a *hypotonic saline solution (0.3%)*.

Hemocytometer Preparation and Charging

- Before charging the hemocytometer chamber, make sure it is clean and dry. Place a coverslip on the hemocytometer.
- Place the hemocytometer in a petri dish lined with moist paper. Elevate the hemocytometer on two sticks so it does not come in direct contact with the moist paper.
- Fill both sides of the hemocytometer, being careful not to overfill. After the hemocytometer is loaded, allow the cells to settle for **5 to 10 minutes** (the amount of time required for the cells to settle depends on the **cellularity** of the specimen).
- Label the petri dish using a crayon or by attaching a computer label. The label must include the patient's name or specimen number and the set-up time.
- Cells must be counted as soon as possible. If the fluid has drawn back from the sides of the hemocytometer, the sample has begun to dry out and the counts are invalid. Re-mix the sample and set the hemocytometer counts up again.

Recommendation for counting

The following guidelines are recommended for counting areas:

- a) If **less than** an estimated **200 cells** are present in all **nine** squares, count **all nine squares**. This area counted is 9 mm².
- b) If **more than** an estimated **200 cells** are present in **all nine squares**, then count the **four corner squares**. This area counted is 4 mm².
- c) If **more than** an estimated **200 cells** are present **in one square**, then count **five of the squares within the center square** for an area of 0.2 mm².

Cell Counting Procedures

- Place the hemacytometer under the microscope, using low power only (10X), and adjust to see the cells.
- Scan the large squares. For accuracy, there should be **even distribution of cells** (*approximately no more than ten cells variation in the large squares*).
- Cells should not overlap. *For diluted samples, a minimum of 200 cells should be counted.*
- Then, switch to high power magnification (**40X**). The count is performed under high power.
- Depending on the number of cells present, an appropriate number of squares should be counted.
- The more cells present, the smaller and fewer the numbers of squares that need to be counted.

White and Red Blood Cell Counts

- Nucleated cells may be counted in the **same chamber** as erythrocytes.
- Count and average the result.
- Count the appropriate areas on both sides of the hemacytometer for the dilution and number of cells present as follows:
 - *All **nine** squares if **no** dilution*
 - *All **nine** squares for **1:10** dilution*
 - ***Four** corner squares for **1:20** dilution*
 - ***Center** square for **1:100** dilution*
 - ***Red cell** area for **1:200** dilution*

Calculations

$$\text{Cells/mm}^3 = \frac{\text{\# of cells counted} \times 10^4 \times \text{dilution factor}}{\text{\# of squares counted}}$$

$$\text{Total cells} = \text{cells/mL} \times \text{volume of original cell suspension}$$

Automated Methods

The key issue in using automated counters is to • ensure that the instrument can provide **reliable counts** at the **low levels** of cells encountered in body fluids.

Thus, each laboratory must define the lower limits • for counting nucleated cells and erythrocytes, below which the use of automated or semiautomated counters is not reliable.

The lower limit for counting should not exceed the • limits recommended by manufacturers.

Carry-Over

The effect of one sample on the next sample immediately following it should obviously be minimized. •

This is especially **true for clear and colorless** CSF that may follow a *bloody CSF*. •

Any carry-over that may be present should be of no clinical significance whatsoever. •

There are two types of carry-over: •

positive carry-over; and ()

negative carry-over. ()

Positive carry-over is the effect of an elevated sample on a subsequent sample of lower concentration. •

Negative carry-over is the effect of a low concentration sample on a subsequent sample of higher concentration. •

This condition can be possibly observed in instruments where a dilution effect can occur by the diluent/rinsing agent during the rinse cycle that occurs between sample analyses. •

QC of automated BF cell count

The College of American Pathologists (CAP) • states that if the same instrument is used for cell counting of blood specimens, there is no need to have separate control runs for body fluid cell counting

Thank you, any question?

