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SEED Body fluids

Body fluid cell counts – how to perform and interpret the results

Fluids present in body cavities or hollow organs can be of physiological or pathological origin. The indication for an analysis of body fluids covers a wide range of diagnostic reasons and is therefore part of the diagnostic workup in most routine laboratories.
The requested analyses, alongside cell count and differentiation, include clinical chemistry parameters, serologic analyses, tumour marker identification, tumour cytology, bacteriological analyses and further specialised analyses. The cellular analysis of body fluids provides important diagnostic information needed for assessing various medical conditions, such as inflammatory diseases or malignancies.

Pre-analytical phase

Knowing the type of material is important for the laboratory to ensure the correct technical validation of the results. In case of cerebrospinal fluid (CSF) specimens, the material type is usually informed to the laboratory. However, with other body fluids the precise material classification is often missing.
CSF is a very special type of body fluid and, compared with other body fluids, has to be processed differently in some respect.
A main difference is the way in which the fluid is obtained, and, as a result, its often very small volume that is received by the laboratory as specimen.

Generally, all body fluids have to be processed immediately, possibly within one or two hours after collection [1, 2]. This is particularly true for cytospin preparations needed for differentiation purposes. Certain cell types, for example activated cells, do not survive as long as other types do [2].

General sampling recommendations for body fluids:

- *Native (tube without additives): for clinical chemistry and serologic analyses*
- *EDTA tube: to count and differentiate cells*
  Exceptions can be:
  - *CSF: normally used without EDTA additive (since CSF does not contain any clotting factors)*
  - *Synovial fluid: may also be anticoagulated with sodium heparin to avoid artefacts for the assessment of crystals*
- *Heparin tube: search for tumour cells*
- *Sodium fluoride tube: determination of lactate*
  Exceptions can be:
  - *CSF: normally used in native form*

The focus of this SEED article is to explain the findings in terms of cell count and differentiation for pleural, ascitic, cerebrospinal and synovial fluid as well as for continuous ambulatory peritoneal dialysate (CAPD). This article will neither deal with the clinical-chemical and serologic parameters, nor with the bacteriological analyses.

Body fluids in a compact overview

1. Pleural fluid

The pulmonary pleurae are two thin serous membranes that surround the lungs and line the inside of the thoracic cavity with a layer of mesothelium. The pleural cavity lies between these layers of mesothelium and contains physiologically a clear serous fluid of less than 15 mL. A pleural effusion results from excessive accumulation of fluid in the pleural cavity. The most common causes of a pleural effusion are congestive heart failure, tumours including carcinomas and inflammation [3].

When a pleural effusion is discovered, the primary question is whether it is a transudate or an exudate because the differential diagnosis has a crucial influence on the therapy. The classification as transudate or exudate depends on the results of various clinical-chemical, serologic and haematological parameters. Table 1 lists the main decision criteria [2]. The results are evaluated according to so-called 'cut-off limits' (decision limits). There are no reference values as such.

2. Ascitic fluid

Physiologically, the volume of peritoneal fluid is small. The term 'ascites' refers to an abnormal accumulation of fluid in the peritoneal cavity, and the fluid is commonly referred to as 'ascitic fluid' rather than 'peritoneal fluid'.
Since the underlying primary disease could be benign or malignant, the differentiation of ascites is of crucial importance for the further diagnostic and therapeutic approach. This also applies to the differentiation between non-infected and infected ascitic fluid [2].

The most common cause of ascites is liver cirrhosis (approx. 80 %), with the remaining 20 % being due to congestive heart failure, tuberculosis, cancer or other causes [3].

The results are evaluated based on the decision limits (cut-off values). Since there is no 'normal ascites', the most common case – liver cirrhosis – is used as an orientation for the cut-off values.

Composition of cells in ascitic fluid [4]

- WBC count < 500/µL
  - Commonly present: mononuclear cells (MN) such as lymphocytes and monocytes/macrophages, but also mesothelial cells (non-WBC)
  - Polymorphonuclear cells (PMN) are less frequently seen and should be below 10 %
- RBC count < 10,000/µL

The numbers of WBC and PMN attract particular attention when detecting spontaneous bacterial peritonitis (SBP). A PMN count of ≥ 250 cells/µL confirms the diagnosis of SBP in the absence of an evident intra-abdominal source of infection [5] and the treating physician should immediately initiate empirical antibiotic treatment.

3. Dialysate, CAPD

Some patients requiring dialysis can employ continuous ambulatory peritoneal dialysis (CAPD). The peritoneal cavity is filled with a sterile dialysis solution via a catheter. By way of osmosis, the fluid then removes those substances from the body that are usually eliminated via the kidneys. The dialysis fluid is exchanged every 4–6 hours via a bag. The patients are able to perform this themselves after a little training. This procedure gives dialysis patients a better quality of life because they do not have to attend a dialysis clinic constantly; however, it harbours the risk of contracting a peritonitis through the catheter access. Patients normally have to visit a physician once a month for a check-up.

Causes of CAPD peritonitis are germs introduced through e.g. contaminated dialysis tubing or extension of the catheter exit site, or tunnel infections. Other possible reasons include patients' allergic reactions to some components of the dialysis fluid or connection systems in the beginning of CAPD, which then lead to a so-called 'eosinophilic peritonitis'.

Table 1 *Main decision criteria for the characterisation of pleural effusions*

Transudate	Exudate
Total cell count < 1,000/µL = white blood cells (WBC) + non-WBC (e.g. mesothelial cells)	Total cell count > 1,000/µL = WBC + non-WBC (e.g. mesothelial cells)
Neutrophilic granulocytes < 250/µL	Neutrophilic granulocytes > 500/µL
Red blood cells (RBC) < 1,000/µL	RBC > 10,000/µL
Further clinical-chemical and serologic parameters taken into consideration: albumin gradient serum/pleural fluid, CEA, total protein, total protein serum/pleural fluid, α-amylase, cholesterol, LD, glucose and others	

Therefore, the most common question when examining CAPD fluids is:

- Inflammatory or non-inflammatory?
  - Number of neutrophilic granulocytes
  - Number of eosinophilic granulocytes

The cells found in CAPD fluid are comparable to those in ascitic fluid (i.e. mesothelial cells, macrophages, monocytes, lymphocytes = mononuclear cells, and the polymorphonuclear cells) because the dialysis solution is likewise in the peritoneal cavity. The number of cells in the dialysate will depend, in part, on the length of the dwell [6].

The International Society for Peritoneal Dialysis (ISPD) recommends peritonitis always to be diagnosed when at least two of the following criteria are met:

(1) clinical features that are consistent with peritonitis, i.e. abdominal pain and/or cloudy dialysis effluent;
(2) a dialysis effluent with WBC > 100/µL (after a dwell time of at least 2 hours), with > 50 % polymorphonuclear cells; and
(3) a positive dialysis effluent culture [6].

Eosinophilic peritonitis is defined by the presence of WBC > 100/µL, with eosinophils constituting > 10 % of the total white blood cell count.

4. Synovial fluid (SF)

The analysis of SF is a crucial tool for diagnosing patients with joint effusion, and classifying the fluid as non-inflammatory, inflammatory or septic. It is also often used to establish a diagnosis by exclusion. In any case, it is an important direct diagnostic criterion for gout, pseudogout, other crystal depositions and septic arthritis. Sodium heparin is recommended as anticoagulant since, besides cell count and differentiation, the assessment of crystals in the specimen is an important analytical step. The use of other anticoagulants such as lithium heparin, EDTA or oxalate can lead to artefacts when detecting crystals [1].

Characteristics of synovial fluid

Normally there is a small amount of SF present, e.g. around 3.5 mL in an adult's knee.

- It is pale to straw-coloured and clear.
- SF is normally very viscous.
- The biochemical composition is similar to that of plasma, containing fat, protein, water, and glucose. But it contains also hyaluronic acid and cells.
- Functions of SF are to reduce friction, enable shock absorption and ensure nutrient and waste transportation.

The viscosity of SF derives from the polymerisation of hyaluronic acid. If the viscosity is very high, this can lead to difficulties when determining cell counts. This can be resolved by preparing either a dilution with saline or adding hyaluronidase to the specimen (400 units to 1 mL synovial fluid, then incubate for 10 minutes at 37° C) [1].

Assessing counts of WBC and PMN in combination is diagnostically important for an immediate discrimination of non-inflammatory, inflammatory and infectious disorders.

- In a normal joint, the SF contains
  - WBC < 200/µL,
  - PMN < 25 %, and
  - no red blood cells.

The following traditional classification system was compiled by the American Rheumatism Association [3]:

- Non-inflammatory: WBC < 2,000 x 10^6/L, PMN < 25 %
- Inflammatory: WBC 2,000 – 50,000 x 10^6/L, PMN > 50 %
- Septic: WBC > 50,000 x 10^6/L, PMN > 75 %

The proportion of PMN to the total white blood cell count is of practical interest. With inflammatory types, it can rise to > 70 %, and with septic arthritides up to 95 %.

Further details on synovial fluid are discussed in two dedicated SEED articles. Part 1 explains the main characteristics and composition of SF, whereas part 2 focusses on laboratory examination and describes the different tests necessary for diagnosis. The two articles can be directly downloaded from the Sysmex Europe website: '*Synovial fluid - part 1: main characteristics*' *and* '*Synovial fluid - part 2: laboratory evaluation*'

5. Cerebrospinal fluid (CSF)

This material poses the biggest challenge for everybody involved, from sample collection through to processing in the laboratory. Statements made in the following text refer to CSF samples from adult patients.

The indication for an analysis of CSF covers a very broad spectrum. The following are possible issues:

- Inflammation
- Tumours
- Barrier dysfunctions
- Infection
- Bleeding

Spinal taps are performed with both a diagnostic and a therapeutic indication.

Reference range for cell counts

- CSF obtained by lumbar puncture: WBC < 5/µL [3]
- Lymphocytes or monocytes may normally occur
- No granulocytes
- No red blood cells

Depending on the medical condition, CSF may also contain non-haematopoietic cells (e.g. tumour cells, astrocytes, oligodendrocytes, etc.). A deliverable of an automated cell count using a haematology analyser, the preliminary differentiation between PMN and MN can provide a fast and helpful indication of the cell distribution.

Automated body fluid analysis

The combination of haemocytometry and stained cytocentrifugation is considered the gold standard for counting and differentiating cells in body fluid samples. However, a cell count can nowadays be carried out both manually and automatically [1, 3], and it is important to know where the limits and limitations of both methods lie.

Traditionally, body fluid counts are performed by manual counting of RBC and nucleated cells/WBC under a microscope using a haemocytometer (also referred to as 'counting chamber'). The accuracy of this device strongly depends on a number of variables including correct loading of sample volume, appropriate dilutions and the number of squares and cells counted. Additionally, it is a subjective and extremely labour-intensive process requiring a high level of expertise. Moreover, in many laboratories, the number of technical experts is decreasing, and with increasing workloads, laboratories now have the option of automating their cell counting using automated haematology or urinalysis analysers. These are designed to be faster, more precise and easier to use compared with the manual methods. In addition, they aspirate a larger sample volume than the counting chamber holds, leading to more cells being counted and in turn enhancing precision and accuracy. Table 2 summarises the advantages and disadvantages of haemocytometry and Sysmex analysers [3].

Table 2 *Summary of benefits and disadvantages of different methods, modified from Fleming C et al. [3]*

Method	Benefits	Disadvantages	Recommendations
Manual counting chamber	✔ low cost ✔ needs only a small sample volume ✔ TNC/WBC/RBC counts	■ high imprecision ■ high inter-observer variability ■ time-consuming	Use only a) if doubting results of the automated analysis and b) for samples with suspected malignancy.
Haematology analysers XN-Series and XN-L Series in XN-BF mode	✔ reduced TAT ✔ no sample preparation ✔ small sample volume ✔ low limit of detection ✔ 2-part WBC differential (PMN and MN) ✔ research 4-part WBC differential ✔ extended counting volume ✔ one flag to notify abnormalities ✔ QC material commercially available	■ lowest reportable RBC value: 1,000 cells/µL* ■ limitations in detecting malignant cells	Critically review scattergrams and histograms to detect abnormalities and follow up with manual method. Review each sample.
Urinalysis analysers UF-4000/5000 in body fluid mode	✔ BF mode always on board (WBC, RBC, EC, TNC, BACT) ✔ 2-part WBC differential (PMN and MN) ✔ low limits of detection for WBC, RBC and bacteria ✔ no sample preparation	■ requires a larger sample volume ■ currently no detection of atypical cells	TNC = WBC + EC

* This refers to the display range of RBC-BF (0.000 to 99.999 x 10^6/µL). For detailed information on performance specifications please always refer to the instructions for use of the respective analyser.

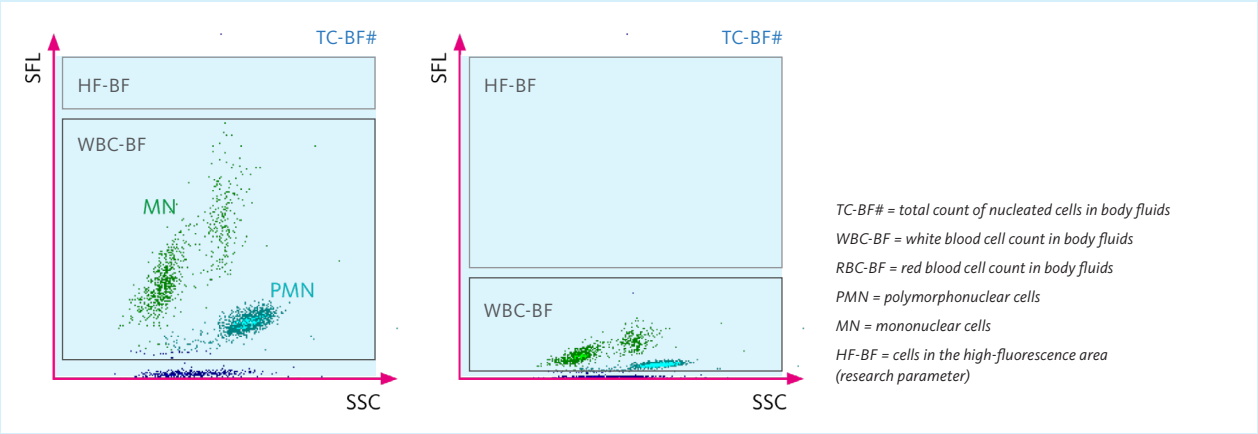

Fig. 1 *Body fluid parameters in the WDF scattergram (left) and WDF extended scattergram (right)*

The Sysmex XN-Series and XN-L Series offer a special measurement mode for the analysis of body fluids – the Body Fluid mode (XN-BF mode).

When the XN-BF mode is selected, a background check is carried out automatically. The measurement channels used are the

- WDF channel and
- RBC/PLT channel.

Without any special preparation, the sample is measured after manual aspiration. In the device, a differentiation is made between PMN and MN, and absolute (#) and relative (%) counts for these cells are provided on the basis of the WBC count (WBC-BF). Since various body fluids may also contain non-WBC, as explained in the previous text, the total cell count (TC-BF) data is also required. The scattergram parameters are explained in Fig. 1.

The TC-BF# parameter includes all the nucleated cells detected in the WDF channel. White blood cells are represented in the WBC-BF area, whereas other cells can be seen in the high-fluorescence area (HF-BF). Mesothelial cells, for example, which are much larger than white blood cells, have a higher RNA/DNA content and therefore may show up in the high-fluorescence range of the WDF scattergram. Tumour cells may equally appear in this area because of their RNA/DNA content and their size, as shown in the example in Fig. 2.

When interpreting the HF-BF area, the material type as well as the clinical context has to be taken into consideration. For example, mesothelial cells can be regularly found in pleural fluid samples, and when measured on the analyser they can be expected to show up in the HF-BF area. If, on the other hand, cells are discovered in the HF-BF area when measuring CSF, it is not a normal finding and has to be clarified by performing microscopy on a cytospin preparation. Publications support that the absence of highly fluorescent body fluid cells (HF-BF) could be used to exclude malignant conditions in samples of serous fluids [7-9]. These examples clearly show that the information from the HF-BF area has to be evaluated differently depending on the underlying clinical question and the type of material.

For interpretation support of the results generated by Sysmex haematology analysers, the special rule set for body fluids, which is part of the *Extended* IPU, may be used. Various criteria for the technical validation of the results, separated by type of material (i.e. two categories: (1) CSF and (2) all others), are taken into consideration.

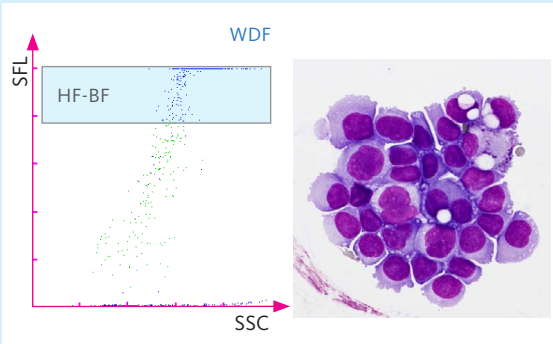

Fig. 2 *WDF scattergram (left) with increased cell counts in the HF-BF area and cytospin image (right, taken by a Sysmex DI-60) showing cell groups of tumour cells in CSF from a breast cancer patient*

These rules include the following:

- WBC Abn Scattergram judgement
- HF-BF indications for highly fluorescent cell groups
- EO-BF indications for eosinophilic granulocytes in CAPD
- Check for possible RBC interferences

For certain body fluids, laboratories prefer to report the total cell count, while for others the white blood cell count is preferred. The WBC differential of the analyser is always related to the WBC-BF count. In case the TC-BF# is selected for reporting in the *Extended* IPU, it also calculates PMN and MN, including the research parameters (e.g. HF-BF, EO-BF, NE-BF, LY-BF, MO-BF), on the basis of TC-BF#.

According to the recommendations in the guideline [1], the macroscopic examination (incl. colour, clarity, clot formation, viscosity) is an essential part of body fluid analysis. With the *Extended* IPU (from version 4.6 onwards) there are now four new parameters, which allow entering those sample characteristics during result validation.

The rules provide indications of interferences and recommendations for actions to be performed, such as chamber counts or cytospin preparations.

With an easy, standardised and automated measurement of body fluids in combination with a complementary set of rules dedicated to body fluids, Sysmex offers also less experienced laboratory staff an optimum level of safety and confidence when these critical specimens arrive in the lab and call for immediate attention.

Summary

- The reference ranges and cut-off values for cells in body fluids are very different, depending on the material in question.
- The key reference parameter – total cell or WBC count – depends on the type of material, which should be taken into account. Depending on the type of body fluid, non-WBC (e.g. mesothelial cells, tumour cells, etc.) may also be present alongside haematopoietic cells (WBC). This means that total cell count and WBC count are not always identical.
- Automated measurement of body fluids and appropriate technical validation of the counts help to improve result quality and their time to report.

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