Introducing automated body fluid analysis into the haematology laboratory

Automated Body Fluid Analysis
The purpose of this newsletter is to introduce the concept of shifting body fluid analysis from the current manual method, which is traditionally the domain of the microbiology laboratory, to being incorporated into the routine workflow of automated haematology analysers.

Key words:
Body fluids, CSF, automation, XT-4000i, XE-5000

What are “body fluids”?
Body fluids can be defined as any liquid substance produced by the human body. Body fluids can be sub-divided into three main categories, namely normal, pathological and iatrogenic (table 1). Normal body fluids are always present and play an integral role in normal human biological function. Pathological body fluids are produced in response to an underlying disease process such as infection, inflammation, trauma or cancer. They usually accumulate in body cavities that are generally “dry” or have minimal quantities of fluid that are not ordinarily amenable to sampling or are found in abnormal tissues such as cysts and abscesses. Iatrogenically induced body fluids are generated when fluid is deliberately introduced into a body cavity by a clinician either for diagnostic or therapeutic purposes. The commonest iatrogenic body fluid is peritoneal dialysis fluid. Patients with renal failure need a “kidney substitute” to remove waste products ordinarily cleared by the kidney. If haemodialysis is not available, the abdominal cavity is filled with dialysis fluid, left for several hours and then drained out again.

This procedure is called continuous ambulatory peritoneal dialysis (CAPD). In bronchoalveolar lavage (BAL) fluid is introduced into lungs via bronchoscope and then sucked out. BAL is primarily used to try and look for tumour cells.

From a medical diagnostics perspective, the concept of “body fluids” is generally restricted to those fluids that are likely to be referred to a laboratory for analysis in order to facilitate a medical diagnosis.

a) Blood
Blood is very easy to sample hence it is very commonly used as a window to the functioning of the body, namely to confirm normal functioning or to identify pathology. Blood is the most important and most widely tested ‘body fluid’ used to aid diagnosis and well as to monitor response to treatment. Because of the frequency of investigation, numerous dedicated analysers exist, hence blood is not considered to be part of conventional body fluid analysis.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Pathological</th>
<th>Iatrogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Pleural fluid (chest cavity)</td>
<td>Peritoneal dialysis fluid</td>
</tr>
<tr>
<td>Urine</td>
<td>Ascitic fluid (abdominal cavity)</td>
<td>Bronchoalveolar lavage</td>
</tr>
<tr>
<td>Semen</td>
<td>Pericardial fluid (around the heart)</td>
<td></td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synovial fluid (joint space)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
b) Urine
Urine is the second most frequently tested ‘body fluid’ used primarily to confirm infection and to identify renal pathology. Because of the frequency of investigation and unique requirement for the identification of casts, automated analysers specific for urine have been developed. Urine is therefore also not considered to be part of conventional body fluid analysis.

c) Semen
The main reason for analysis is for infertility assessment. The focus here is on sperm motility, and morphology and chemistry. It requires highly skilled evaluation and functional assessment which is beyond the scope of routine body fluid analysis.

The body fluid analysis, in the context of laboratory diagnostics is therefore restricted to those fluids shown in table 2.

Table 2  Body fluids considered under the umbrella term body fluid analysis

<table>
<thead>
<tr>
<th>Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>Synovial fluid</td>
</tr>
<tr>
<td>Pleural fluid</td>
</tr>
<tr>
<td>Ascitic fluid</td>
</tr>
<tr>
<td>Pericardial fluid</td>
</tr>
<tr>
<td>CAPD fluid</td>
</tr>
</tbody>
</table>

How are body fluids sampled?
Sampling of all body fluids is an invasive procedure. There is always the risk of traumatic puncture which can cause bleeding and may need surgery to control. Sampling must always be performed under sterile conditions as there is a risk of introducing infection into what is normally a sterile cavity. Body fluid sampling can be equated to a minor surgical procedure. All specimens must therefore be treated as precious as repeat sample collection required due to careless handling is usually not an option.

Why are body fluids analysed?
Laboratory investigations serve the purpose of answering one of the following basic questions

- Is the patient getting better? – i.e. to monitor response to treatment

The primary purpose of body fluid analysis is to distinguish between infection, inflammation (reactive conditions) and malignancy. The baseline investigations performed on all body fluids are shown in table 3.

Table 3  Baseline body fluid investigations

<table>
<thead>
<tr>
<th>Cell Counts</th>
<th>White cell count (WCC) plus differential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Microscopy</td>
</tr>
<tr>
<td>Microbiology</td>
<td>Gram stain Culture</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>Glucose Proteins</td>
</tr>
</tbody>
</table>

Automated body fluid analysis, the subject of this newsletter, concerns itself with cell counting and morphology which are the labour intensive components in traditional analysis.

The questions that doctors seek to answer are fairly predictable for each type of body fluid.

a) Cerebrospinal fluid (CSF)
CSF is fluid that bathes the brain and spinal cord acting as a shock absorber as well as to provide nutrients and remove waste. The membranes covering the brain and spinal cord are called meninges. Infection of meninges gives rise to a very serious life threatening illness referred to as meningitis. The presence of infection can be detected by virtue of the fact that CSF composition changes. A patient presenting with sudden onset of headache, fever, changed mental state and photophobia having been previously well is suspected of having meningitis. The answer to the question “does this patient have meningitis?” lies in finding an elevated WCC (adults > 5-10 cells/µL; children > 10-30 cells/µL). Accurate cell counting at very low levels is therefore vital, especially if one considers that a patient with severe leucopenia (WCC ~0.5 x 10^3/µL in whole blood) has a WCC that is 50 times higher than the cut-off in CSF that is used to diagnose meningitis. Other causes of an elevated WCC include encephalitis, leukaemia/lymphoma with CNS involvement and other neurological disorders. The latter group of conditions are usually readily differentiated based on a longer duration of signs and symptoms. Meningitis
may be bacterial, viral, fungal or tuberculous in nature. As acute bacterial meningitis can be rapidly fatal it constitutes a medical emergency. The answer to the question “is this an acute bacterial meningitis?” lies in performing a white cell differential count and finding a predominance of neutrophils (polymorphonuclear cells). All other conditions with an elevated WCC will have predominance of lymphocytes (mononuclear cells).

The RCC is used primarily to see if the elevated WCC is due to contamination with peripheral blood. A “bloody tap” may occur when the lumbar puncture is difficult. This is readily identified as the CSF clears in sequential tubes during the collection process. In the case of an intracranial bleed (arachnoid haemorrhage), the CSF does not clear. Review of morphology is primarily required for suspected leukaemias and lymphomas in order to confirm central nervous system involvement. If present, CSF analysis is used to monitor response to treatment. Gram staining is used to confirm the presence and nature of bacteria.

b) Pleural and ascitic fluid
Accumulation of fluid in the chest and abdominal cavities is always abnormal. Fluid in the chest is referred to as pleural fluid because the membranes lining the chest cavity are called “pleura”. Fluid in the abdomen is generally referred to as ascitic fluid but sometimes peritoneal fluid (derived from the word peritoneum which is the membrane lining the abdominal cavity) is used as well. Common causes of pleural effusions and ascites include congestive heart failure, liver failure with portal hypertension (ascites mainly). As the reasons for this, as well as for trauma induced haemothorax (blood in the chest) and haemoperitoneum (blood in the abdomen), are usually obvious no analysis is generally undertaken. Other causes include malignancy ascitic as well as infection, primarily tuberculosis (TB). In the event of a suspected malignancy ascitic and/or pleural fluid specimens are not commonly referred for conventional ‘body fluid analysis’ (cell counting and microscopy) but are usually referred directly to cytology to look for malignant cells. In the event of suspected TB, the specimens are usually sent directly for TB culture or acid fast bacilli staining. Only in the event of suspected acute infection, which is usually superimposed on another cause of chronic ascites or pleural effusion, are samples generally submitted for conventional analysis. The main questions that doctors want answers for when submitting pleural and or ascitic fluid to a laboratory are “is the fluid due to a malignancy?” or “does the patient have tuberculosis?”.

c) Synovial fluid
Synovial fluid refers to fluid in the joint space. Its primary role is lubrication and ordinarily is only present in very small quantities that are not accessible to aspiration. Synovial fluid will however accumulate in the event of sepsis, inflammation and trauma. Joint swelling post trauma is usually due to a collection of blood which becomes obvious upon aspiration and generally requires no further laboratory intervention. The primary question that doctors have in mind when submitting synovial fluid for analysis is “what is the cause of arthritis?” with the differential diagnosis including acute sepsis (increased polymorphonuclear cells) or inflammation (increased mononuclear cells).

d) Continuous ambulatory peritoneal dialysis fluid
Patients undergoing peritoneal dialysis are at high risk of infection because the natural barrier of the abdominal cavity is constantly being breached. The peritoneal dialysate is regularly checked for signs of infection by performing WCC and differential counts. The development of acute peritonitis in a chronic renal failure patient is very serious as these patients are dependent on dialysis for survival. In most cases they are receiving peritoneal dialysis because for whatever reason haemodialysis is not an available treatment option in the first place. So here the primary question is “is there any sign of an evolving bacterial infection?”.

e) Pericardial fluid
The heart is enclosed in a sac of very tough membrane called the pericardium. Much the same as for all other body cavities, a fluid accumulation here is abnormal. Pericardial fluid is only rarely sampled because the cause is often obvious and it is a dangerous procedure. Pericardial fluid is therefore only rarely sent to the laboratory for analysis.

How are body fluids measured?
This section is restricted to cell counting and morphology as culture and biochemistry are specialised investigations in their own right.

a) Manual method
Body fluid cell counting has traditionally always been performed manually using a microscope. It requires pre-analytical processing which is dependent on sample type. Preanalytical preparation is cumbersome, time consuming and error prone, especially as it is influenced by the accuracy of pipetting. The actual counting process requires a special counting chamber and a skilled microscopist who knows exactly how to perform cell counting using the counting grid.
The approach depends on the estimated cell count and extent of red cell presence. The reported cell count is a calculated value which is dependent on the number and type of squares counted as well as the dilution factor if appropriate. The procedure requires highly skilled microscopists who need to be available day and night, especially for those urgent CSF evaluations. The manual differential count should be performed on a stained cytospin slide as this concentrates cells. The traditional wedge smear used for peripheral blood analysis is unsuitable for body fluid analysis. In practice most laboratories try to perform a differential count on the unstained sample at the same time as performing the cell count. Those that do make and stain smears tend to perform only a 2 part differential; namely mononuclear cells and polymorphonuclear cells. Very few laboratories actually perform a complete differential count and report on overall morphology using the recommended cytospin approach as most have neither the special cytospin centrifuge nor the skilled staff required to do so effectively.

Limitations of manual body fluid cell counting are numerous but can be summarised as follows:
- Labour intensive
- Accuracy of result depends on skill of technologist
- Wide variability of results resulting in a poor CV%
- Requires preanalytical preparation which is time consuming and error prone
- Requires very accurate pipetting for dilution steps
- Requires good working knowledge of counting chamber
  - User must know which one they are using in order to make adjustments for chamber depth to get accurate counts
- Must be properly filled
- Any delays in assessment will increase inaccuracy
- Must understand counting areas and calculations required

Limitations of manual body fluid differential counting and morphological review in practice can be summarised as follows:
- Cytospins are rarely performed
- Wedge smears are rarely representative
- Cells appear different in body fluids compared to blood – requires skilled person to recognise
- Malignant cells usually missed by inexperienced technologists
- Usually only produce a 2 part differential anyway
- In practice not performing appropriate morphological review

b) Automated body fluid analysis
Automated cell counting in peripheral blood has been standard practice in laboratory medicine for several decades now. Not only is automated cell counting much faster but is also performed with much better precision as many more cells can be counted than would ever be possible using a counting chamber and microscope. The speed and precision of analysis is what makes automated body fluid analysis a far wise choice, especially for laboratories that are under-resourced. Table 4 highlights the advantages of automated body fluid analysis over the manual method which is still widely believed to be the gold standard method. The only possible limitation of automated analysis is that direct morphological review is not possible but there are clues or flags that abnormal cells are present.

### Table 4: Advantages of automated body fluid analysis over the manual method

<table>
<thead>
<tr>
<th>Automated Analysis</th>
<th>Manual Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>No preanalytical sample preparation required</td>
<td>Not standardised</td>
</tr>
<tr>
<td>Quality control available</td>
<td>No quality control available</td>
</tr>
<tr>
<td>Operator independent</td>
<td>Very operator dependent and therefore prone to error</td>
</tr>
<tr>
<td>Much more accurate at very low and very high counts</td>
<td>Cumbersome</td>
</tr>
<tr>
<td>Fast turnaround time</td>
<td>Relatively long turnaround time</td>
</tr>
</tbody>
</table>
**CLSI guidelines on body fluid analysis**
The Clinical Laboratory Standards Institute has issued a guideline on “Body Fluid Analysis for Cellular Composition” (H56-A (2006)). The document is not a statutory regulation but rather a consensus statement derived from inputs from the wider healthcare community providing guidance on best laboratory practice. It makes no reference to the manual method being the preferred or “gold standard method, but rather outlines how body fluids should be analysed and reported depending on the chosen method, i.e. manual or automated. It is of interest to note that very few if any laboratories actual adhere to the manual method procedure as described in the CLSI guideline document. In contrast, automated body fluid cell counting available on the Sysmex haematology analysers is fully compliant with the guideline.

**Sysmex automated body fluid analysis**
In recognition of the major clinical benefit that would be derived by providing accurate cell counts within the same fast time as is the norm for full blood counts (FBC), dedicated body fluid analysis is now available on the Sysmex XE-5000 and XT-4000i haematology analysers. The principles of measurement are based on the same state of the art technology used for FBC testing including fluorescence flow cytometry for the differential. The major advantage that Sysmex analysers have over other competitor haematology analysers is the fact that the body fluid analysis has has a dedicated mode which permits the analytical range to be adjusted to accurately accommodate those very low cell counts that are so critical in the accurate diagnosis of meningitis. The lower limit of detection of WCC# on the Sysmex analyser is zero, whereas it is as high as 50 cells/µL which is of no use when the cut-off for adults is 10 cells/µL on other analysers. Other advantages over competitor analysers is the small sample volume required (e.g. 85 µL on the XT-4000i compared with 300 µL elsewhere). Sysmex body fluid analysis is approved for a wide range of body fluids (CSF, pleural fluid, ascetic fluid, synovial fluid, CAPD fluid) whereas competitor analysers are only permitted to process CSF samples.

The advantages of Sysmex automated body fluid analysis are:
- Inclusion of a highly fluorescent cell count which represents non-haemopoietic tumour cells or macrophages. Their presence would trigger a manual microscopic review.
- Operator independent - no special training or skills required
- Fast and fully automated (no manual preparation steps)
- Very rapid – 38 samples per hour
- Available 24 h a day
- Body fluid specific quality control material is available
- Very good precision at low levels – WBC 10 cell/µL which is essential for CSF analysis.

![Figure 1](the Sysmex XT-4000i haematology analyser with dedicated body fluid mode.)
What are the challenges of switching from manual to automated body fluid analysis?

The greatest challenge in introducing automated body fluid analysis is the fact that the traditional domain of body fluid analysis currently resides within the microbiology laboratory whereas automated cell counting for body fluids is a feature available on analysers that will be primarily used for FBC analysis under the custodianship of the haematology laboratory. It is always best to approach such situations with the patient in mind. The greatest advantage to be gained is for the laboratory to be able to provide an accurate WBC and differential count within minutes on CSF specimens and therefore rapidly identify those patients that have acute bacterial meningitis so that their care is prioritised and life-saving treatment commenced. The approach will always have to be one of co-operation between two traditional separate disciplines of laboratory medicine. Automated body fluid analysis has many advantages over manual counting but will never fully replace the manual method as special cases will always require microscopic review either by microbiology to look for crystals or by cytology to look for malignant cells. It is best to view the automated body fluid solution as a first pass screen for rapid counting, followed by manual review of selective cases. This is however no different for haematology samples – there will always be always be a select few that will still require manual microscopic review. Automated analysis will take away the bulk of the manual labour requirement and therefore free up staff to focus their attention on those cases that really need to be carefully reviewed.

Take home message

Sysmex automated body fluid analysis will provide both the busy laboratory as well as the smaller laboratory where highly skilled staff may not be available day and night with an excellent solution to eliminate the labour intensiveness and slowness of the current widely used manual microscopic cell counting on body fluids. It is widely acknowledged that the manual method has a significantly higher imprecision that automated cell counting, a fact which has been derived from many clinical studies where the manual methods were performed strictly according to the recommended guidelines. It is therefore highly likely that the precision of the manual method in routine daily practice is probably significantly worse and of questionable clinical value. In contrast the automated method has excellent precision, is operator independent and offers very rapid turnaround times which have huge potential to dramatically impact patient care.

Compiled by
Dr Marion Münster