



Slide preparations for the cytology  
of pleural fluid or ascitic fluid

In addition to mesothelial cells, pleural fluid and ascitic fluid also contain lymphocytes and eosinophilic and neutrophilic granulocytes. The protein content is frequently around 30 g/l. The cell content often varies considerably. Both fluids may contain blood and ascitic fluid may also contain lipids.

## Advantages of the Hettich Method

1. **Cyto chambers for different cell contents and volumes**
2. **High cell yields**
3. **Good quality of the cells**
4. **Optimum spreading of the cells**

## Preparation

### 1. Preparation of samples with a high erythrocyte content

Since a high erythrocyte content will have a negative effect on the quality of the preparation, it is recommended that the erythrocytes be removed using a lysis buffer before derivation of the cyto preparation.

#### a) Preparation of the lysis buffer

8.29 g ammonium chloride ( $\text{NH}_4\text{Cl}$ )  
1.0 g potassium hydrogen carbonate ( $\text{KHCO}_3$ ) and  
0.037 g ethylene-diamine-tetraacetic acid (EDTA) are made up to 1 litre with distilled water.

The lysis buffer should be autoclaved to extend its shelf-life.

#### b) Lysis of the erythrocytes

- Centrifuge the sample fluid for 10 minutes at 350 x g (corresponding to 1,700  $\text{min}^{-1}$  in the 6-place rotor and 1,900  $\text{min}^{-1}$  in the 4-place rotor).
- Pipette off the supernatant and set it aside.
- Resuspend the sediment in lysis buffer (1 part by volume sediment to 10 parts by volume buffer).
- Mix well and allow to stand for 10 minutes at room temperature. The haemoglobin liberated results in a transparent red coloration.
- Stop the reaction by adding 10 x the volume of physiological saline (0.9% NaCl) or phosphate-buffered saline.
- Centrifuge at 350 x g for 10 minutes.
- Decant the supernatant containing the lysed erythrocytes and discard it.
- Resuspend the sediment in 10 ml 0.9% NaCl (or PBS), mix it thoroughly and centrifuge it again for 10 minutes at 350 x g.
- Discard the supernatant and resuspend the sediment in the original sample supernatant that was set aside, and mix it well. A cyto preparation can now be derived from the sample.

**Important:** Do not allow the cells to stand in the lysis buffer for too long as they may also be lysed.

### 2. Selection of suitable accessories

The 2 ml chamber (Cat. No. 1664) with a sedimentation area of 60  $\text{mm}^2$  and the 4 ml chamber (Cat. No. 1665) with a sedimentation area of 120  $\text{mm}^2$  are recommended for the centrifugation of pleural fluid and ascitic fluid. The 2 ml chamber is suitable for samples containing up to 100,000 cells and the 4 ml chamber for samples containing up to 200,000 cells.

### 3. Assembly of the cyto insert

Information on the assembly of the cyto insert is provided in our leaflet "Perfect preparations – with the Hettich cyto system all it takes is a turn". For slide preparations from pleural fluid and ascitic fluid it is generally necessary to use dry fixation. The cyto insert should therefore be assembled with the filter card (see B1 in the illustration). If the samples are infectious then we recommend the use of our lid No. 1661 (see B2 in the illustration).

## 4. Centrifugation

### a) Sedimentation

Fill the samples into the centrifugation tubes and centrifuge them for **10 minutes** at **490 x g** (corresponding to 2,000 min<sup>-1</sup> with the 6-place rotor and 2,200 min<sup>-1</sup> with the 4-place rotor).

### b) Removal of the cell-free supernatant

The cell-free supernatant remains in the chamber after centrifugation and must be removed by careful aspiration.

It is important that the sediment is not disturbed whilst the supernatant is being removed, as this can affect the quality of the preparation and/or lead to cell loss. It is therefore recommended that the supernatant be removed using a Pasteur pipette with its tip just below the surface of the supernatant, moving downwards. The pipette should not come into contact with the slide - a small drop of liquid should be allowed to remain over the sediment!

### c) Drying of the sediment

The sediment must be dried if the cells are to be stained using Giemsa stain. This requires a second centrifugation step. After removal of the supernatant, loosen the slide ring and remove it together with the chamber (see B4). Place the slide carrier with the slide and the filter card together with the suspension back in the centrifuge and centrifuge them for **1 minute** at **1,100 x g** (corresponding to 3,000 min<sup>-1</sup> with the 6-place rotor and 3,400 min<sup>-1</sup> with the 4-place rotor). The remaining liquid will be removed through centrifugal force and absorbed by the filter card. The cells will remain as a sediment on the slide. Their morphology will be intact and they will be well distributed over the surface. Evaporation artefacts such as leukocytes that are reduced in volume and the development of crystals will be avoided through the dry centrifugation.

### d) Fixing and staining

The dry preparation can be fixed immediately and then stained.

### Good to know

If Giemsa stain or May-Grünwald-Giemsa stain is used then the slides must be rinsed with Weise or Sörensen buffer in a final step. They must then be dried.

**This can be achieved quickly and easily using the Labora System frame for 6 microscope slides (Cat. No. 1285):**

- Place the slides rinsed in Weise buffer in the frame and insert the frame in the centrifuge.
- Centrifuge for **1 minute** at **275 x g** (corresponding to 1,500 min<sup>-1</sup> in the 6-place rotor and 1,700 min<sup>-1</sup> in the 4-place rotor).
- Remove the frame holding the dry slides from the centrifuge.
- The preparations are now ready for microscopy and/or mounting of a cover slip.
- Wipe the suspension dry.

Up to **36 slides** can be dried at the same time in the 6-place rotor.

## Ordering information

Centrifuge	Cat. No.
ROTOFIX 32A	1206
UNIVERSAL 320 / UNIVERSAL 320 R	1401 / 1406

Selection of accessories <sup>1)</sup>	Cat. No.
4-place rotor	1624
6-place rotor	1626
cyto suspension	1660
lid fitting onto 1660	1661
slide carrier with fastening ring	1662
cyto chamber 1 x 2 ml (60 mm <sup>2</sup> )	1664
cyto chamber 1 x 4 ml (120 mm <sup>2</sup> )	1665
labora-system frame for 6 slides	1285

<sup>1)</sup> The complete range of Hettich cyto accessories is listed in our brochure on cyto centrifugation, which can be ordered free of charge.