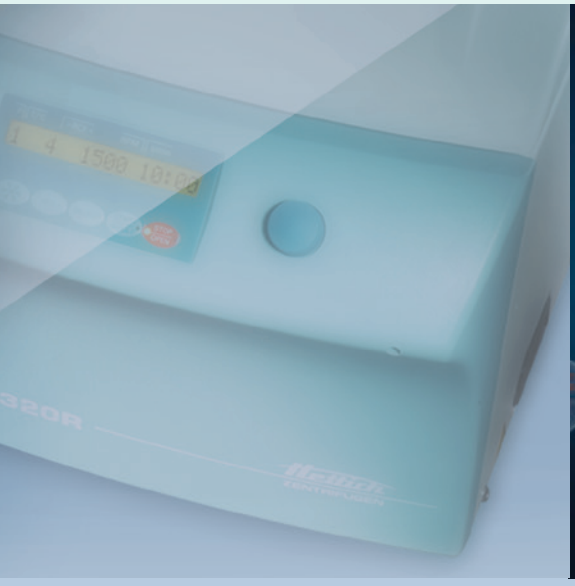
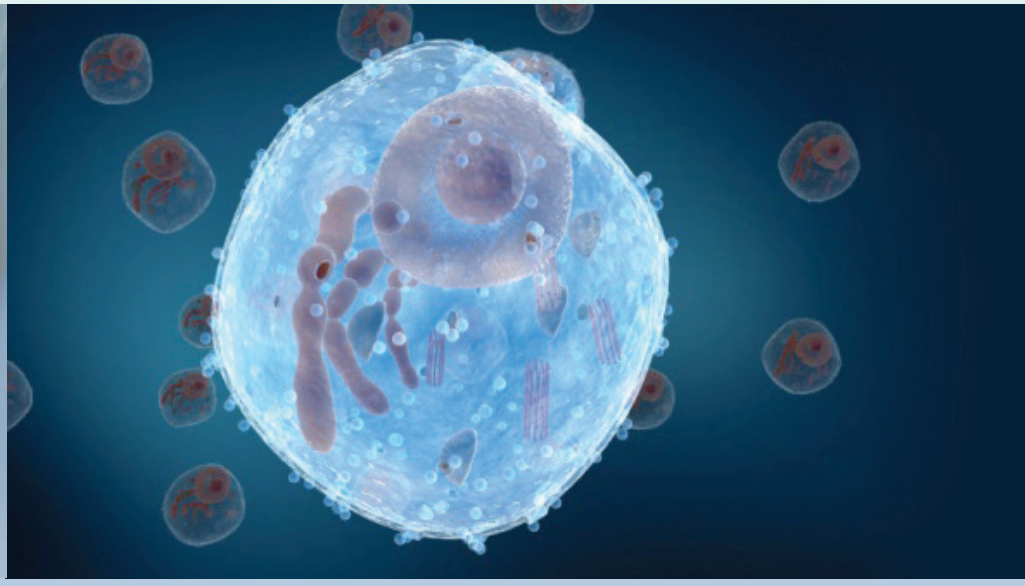
Hettich
Application Note





Slide preparation of cerebrospinal fluid for cytological examination

Hettich
ZENTRIFUGEN

In liquor diagnostics, conventional liquor cytology plays an important role in the detection and classification of infectious inflammatory diseases of the CNS, in the detection of bleedings in subarachnoid spaces and in the discovery of neoplastic cells. In addition, cytological preparations are used for follow-up tests and therapy control.[1)]

As cerebrospinal fluid usually contains few cells and little albumin, cells start suffering damage a few hours after punction. Therefore, liquor samples taken to prepare slides for cytological examination **should not be older than 2 hours**.[2)]

Not only is the age, but also the processing of the samples of vital importance for the diagnostic value of the preparations. Good preparations of cerebrospinal fluid require optimal cell recovery and the preclusion of selective cell loss. The morphology of the cells needs to be maintained to enable a correct identification of the different cell populations.

Advantages of the Hettich method

1. Immediate processing of the liquor samples

- Suitable for samples of various cell content and different volumes up to 8 ml
- Use of the cell-free supernatant for further analyses

2. Excellent preparation quality

- Optimal preservation of the morphology
- Optimal spreading of the cells
- Optimal yield

Preparation

1. Sample preparation

Often the quality of preparations of cell- and albumin-poor liquor samples is not satisfactory. We, therefore, recommend adding human serum or albumin solution.

The addition of albumin to samples with cell counts below 10/µl and albumin rates below 3000 mg/l is considered vital for the stabilisation of cells. To guarantee an albumin concentration above 3000 mg/l, 50 µl normal serum with a total protein content of approx. 70 g/l is added to a liquor sample of 400 µl. Autogenic serum is not required.[3)]

The use of coated slides can also increase cell yield. For routine and conventional staining it is recommended to use slides coated with *Polysine*™, for the immunocytological detection of cellular surface marker, particularly tumour markers it is recommended to use *Super Frost*® *Color* slides.[4)] (*Polysine*™ and *Super Frost*® are registered trademarks of the Gerhard Menzel Glasbearbeitungswerk GmbH & Co. KG).

2. Suitable accessories

The available quantity and the cell content of the samples determine the adequate chamber. With liquor samples, we generally recommend the use of 1 ml and 2 ml chambers with sedimentation areas of 30 mm² or 60 mm².

cells/sample	up to 2,000	2,000 – 20,000	more than 20,000
sedimentation area	30 mm²	30 mm²	60 mm²
volume	100 µl – 1 ml	100 µl – 1 ml	400 µl – 2 ml
addition of albumin	yes	no	no

3. Assembly of the cyto insert

How to assemble a cyto insert can be learnt from our leaflet „Perfect preparations – with the HETTICH cyto-system all it takes is a turn“. Slide preparations of liquor cerebrospinalis usually require dry fixation. For this reason, the insert should be assembled **with** a filter card (see illustration B1 in above mentioned leaflet). When working with infectious samples, the inserts should be closed with hygienically sealed lid no. 1661 (see illustration B2 in above mentioned leaflet).

1) Kluge et al., Stellenwert der praktischen (klassischen) Liquorzytologie im Gesamtspektrum der Liquordiagnostik, in Kluge et al. (Hrsg.) Atlas der praktischen Liquorzytologie, Stuttgart 2005, p. 2-3.
2) Ibidem, p. 7.
3) Ibidem, p. 8.
4) Recommended by the Liquor Laboratory of the Institute for Clinical Chemistry and Laboratory Diagnostics (Prof. Dr. Kluge, Dr. Roskos) of the Clinical Centre of the University of Jena.

4. Centrifugation

a) *Sedimentation*
Centrifuge the cyto chambers for **3 minutes** at **275 x g** (this corresponds to 1,500 min^{-1} with the 6-place rotor and 1,700 min^{-1} with the 4-place rotor).
During centrifugation, the cells sediment onto the slide.

b) *Removal of the cell-free supernatant*
After centrifugation, the cell-free supernatant is still within the chamber and needs to be removed except for a small remainder. When draining the chamber, take care not to stir the sediment. Otherwise, the quality of the preparation will suffer and cells may get lost. We advise to use a Pasteur pipette for removing the supernatant. Do not position the tip of the pipette on the slide, but follow the liquid level with it carefully downwards. Do not drain the chamber completely, but leave a drop of supernatant on the sediment!
If no albumin has been added to the sample, the recovered supernatant may be used for biochemical analyses.

c) *Drying the sediment*
With the most frequently performed Giesma method of staining, the sediment needs to be dry. Dry the sediment by centrifuging it a second time.
After the supernatant has been removed, twist off the fastening ring and lift it off with the chamber (see illustration B4). Put the cyto suspension with slide carrier, slide and filter card back into the centrifuge and spin for **1 minute** at **1,100 x g** (this corresponds to 3,000 min^{-1} with the 6-place rotor and 3,400 min^{-1} with the 4-place rotor).
The residual supernatant is spun off by centrifugal force and absorbed by the filter card. The cells which constitute the sediment stay on the slide. They are well preserved and ideally spread. By drying the preparation by centrifugation, evaporation artefacts like shrunk leucocytes or crystallisations can be avoided.

d) *Fixing and staining*
The dry preparation can be fixed and stained immediately.

Good to know:
In the final stage of the Giemsa and May-Grünwald-Giemsa methods of staining, the slides are rinsed with a buffer (Weise, Sörensen). Thereafter, they need to be dried.
This can be done quick and easily with the labora-system frame for 6 slides (Cat. No. 1285).

- Put the slides rinsed with Weise buffer into the frames, and put the frames into the centrifuge.
- Centrifuge for **1 minute** at **275 x g** (this corresponds to 1,500 min^{-1} in the 6-place rotor, 1,700 min^{-1} in the 4-place rotor).
- Take the frames containing the dry slides out of the centrifuge.
- The preparations are ready for examination under the microscope and/or mounting now.
- Wipe the cyto suspensions dry

With the 6-place rotor up to **36 slides** can be dried at a time.

Ordering information

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

Selected accessories [5]	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 1 ml (30 mm^2)	**1663**
cyto chamber 1 x 2 ml (60 mm^2)	**1664**
filter cards for 1663 and 1664, pack of 200	**1675**
labora-system frame for 6 slides	**1285**

[5] The complete range of Hettich cyto accessories is listed in our brochure on cyto centrifugation, which can be ordered free of charge.

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