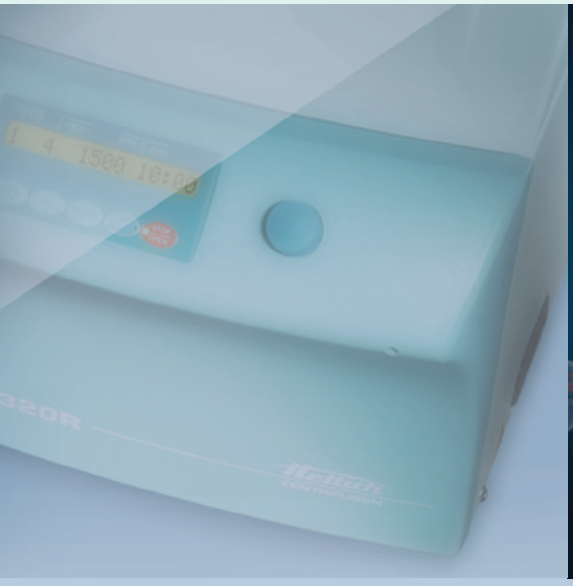
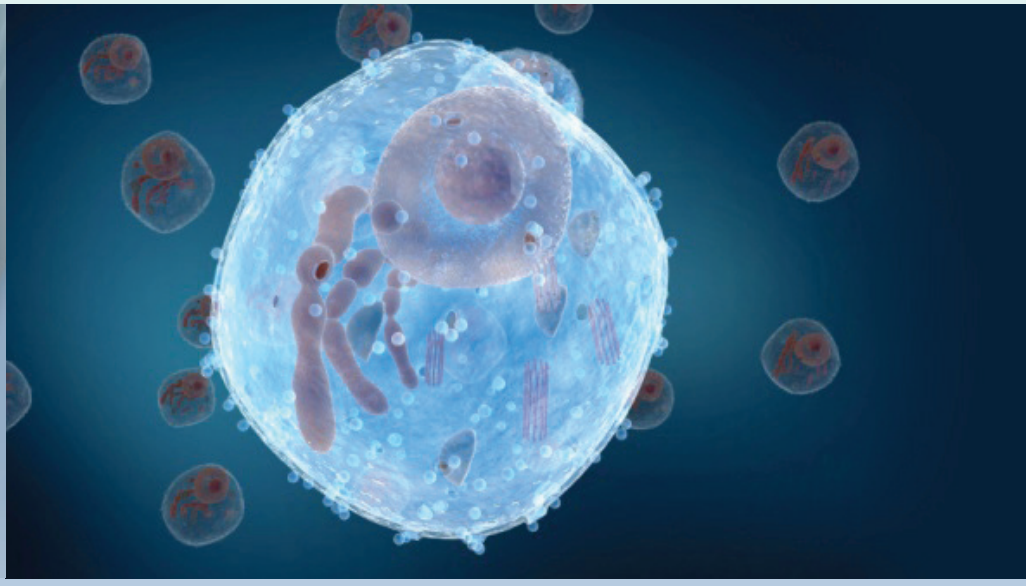
Hettich
Application Note





Single-step slide preparations with the angle chamber

Hettich
ZENTRIFUGEN

This method was developed in the CSF laboratory of the Institute of Clinical Chemistry and Laboratory Diagnostics (Prof. Kluge, Dr. Roskos) at the Friedrich Schiller University, Jena, Germany.

The aim was to develop a simple method for the preparation of cells on microscope slides for different cell types whilst maintaining the integrity of the cells. Comparative tests using original samples were extensively carried out to devise suitable sample preparation steps and determine the optimum centrifugation conditions.

The method described below allows differential cell counts to be performed for cerebrospinal fluid, pleural effusions, ascites and dialysates.

Advantages of the Hettich Method

It has become necessary to develop and implement simplified centrifugation procedures which nevertheless maintain cell integrity and deliver results that are reliable and reproducible for the body fluids submitted for cyto-logical analysis for a number of reasons – time pressure, the expectation of 24/7 availability of cytodiagnostics and a marked rise in the number of diagnostic parameters for the same or a reduced quantity of tissue available for analysis, coupled with financial/organisational constraints and thus personnel considerations (integration of special labs into central ones).

The method below fulfils these requirements. It delivers slide preparations of cell suspensions in a single step using angle chambers in Hettich centrifuges.

Preparation

A) Cerebrospinal fluid

The aim is for at least 200 cells to be detectable on the sedimentation area and to allow an adequate percentage differentiation. The cell count is firstly established before centrifugation using a counting chamber such as the Fuchs-Rosenthal. A recovery rate of at least 25% can be assumed under the centrifugation conditions given in Section 4 below. Consequently, if a CSF contains 2 cells / µl then at least 400 µl is to be used. If there is 1 cell / µl (1/3 to 3/3 in the Fuchs-Rosenthal counting chamber), then a CSF volume of at least 600 µl is required, and 800 µl is preferable. The volume used should not be less than 200 µl as this would reduce homogeneity to an undesirable level.
Pleural effusion and ascites samples are normally high in protein, so it is only necessary to add protein if the cells have to be diluted to a considerable degree because of high cell numbers (see Section 2.3.):

1. Preparation of the samples

Protein must be added to suspensions with less than 10 cells / µl and protein concentrations below 3000 mg / litre (this will be the case for most CSF samples) for buffering and to stabilise the cells. There is virtually no upper limit for the protein concentration. Normal serum that is available in the laboratory may be used (calf serum / albumin solutions may also be used).

50 µl serum is added to a *sample volume of 400 µl* to obtain a protein concentration above 3000 mg / litre for CSF that *does not contain blood and is undiluted*. This also applies for dialysates. If it is necessary to dilute a CSF which is rich in cells and either contains blood or is blood-free, then it may be diluted with physiological **NaCl solution** at a ratio of **9:1** (referred to below as "9:1" mixture). The NaCl solution must be prepared freshly on the day of use, be stored in a refrigerator and allowed to reach room temperature before use (see Section 2.1.), *or*
if there is sufficient homologous CSF then it may be diluted with its **cell-free supernatant** that can be derived through centrifugation. The centrifugation parameters are the same as for serum derivation from blood.

1.1. Blood-free CSF:

The following are approximate dilutions for a sample volume of 400 µl with cell numbers as follows:

< 50 cells / µl:	**400 µl** CSF plus **50 µl** serum
50 – 150 cells / µl:	**100 µl** CSF plus **300 µl** "9/1" mixture or homologous supernatant
150 – 500 cells / µl:	**50 µl** CSF plus **350 µl** "9/1" mixture or homologous supernatant
> 500 cells / µl:	**50 µl** CSF plus **1000 µl** "9/1" mixture. Of this, only 200 µl is to be filled into the angle chamber. Higher dilutions will be required if the cell numbers are extremely high.

1.2. **CSF containing blood:**

The dilutions given above will only apply for CSF containing blood to a limited degree since it will also contain erythrocytes in addition to the white cells. When selecting the dilution parameters and volume of diluted cell suspension to be used it is necessary not only to prevent inclusion of too many erythrocytes, but also to avoid diluting out the erythrophages and haemosiderophages that are required for diagnosis. It may be necessary to use a number of preparations with different dilutions to obtain ones that are suitable for evaluation.

2. Choice of suitable accessories

For 400 – 800 µl cell suspension the angle chamber No. 1672 is recommended. This has a sedimentation area of 60 mm² and a diameter of 8.7 mm. The corresponding filter card is No. 1697.

For conventional Pappenheim staining and routine preparations microscope slides coated with *Polysine*™[1)] are recommended. For the immunocytological demonstration of cellular surface markers, especially tumour markers, *Super Frost*®[1)] *Color* slides are recommended.

3. Assembly of the cyto insert

The microscope slide, cyto chambers and filter card are secured to the slide carrier (Cat. No. 1662) using the fastening ring. Turn the ring as far as possible and whilst maintaining tension turn it back to a small degree so that after centrifugation the entire supernatant is absorbed by the filter card and there is no residual liquid remaining on the sediment surface.

4. Centrifugation

a) ***Filling of sample***
To fill the sample, place the cyto insert upright on the bench or place it in the cyto suspension (Cat. No. 1680) and pipette the prepared sample into the funnel of the chamber.

b) ***Sedimentation***
One of two centrifuge programmes can be used:
Programme 1: 3 minutes at 100 x g
Programme 2: 4 minutes at 50 x g

Programme 1 should be used for routine use.

c) ***Disassembly of the cyto insert***
Ensure that all of the liquid has been absorbed by the filter card before disassembling the cyto insert. If this is the case, then remove the cyto insert from the centrifuge, loosen the fastening ring, remove the chamber and take out the microscope slide together with the filter card. Remove the filter card carefully, without disturbing the sediment. Allow the sediment to air dry.

Important: If the cells are dried for too long they may be damaged!

If, after centrifugation, there is still liquid in the angle chamber, then this must be removed whilst keeping the angle chamber vertical. Then loosen the fastening ring whilst keeping the angle chamber in a vertical position, so that liquid still on the sediment surface is slowly and uniformly distributed over the filter card and the homogeneity of the sediment is not affected.

d) ***Pappenheim staining of preparations***
Marking of the sediment area has been found to be useful before staining.
Allow the **May-Grünwald solution** (Merck) to act on the dry cell sediment for **3-5 minutes**, rinse in distilled water and then stain it for **10-15 minutes with diluted Giemsa solution** (1 ml stock solution from Merck plus 10 ml distilled water).

The intensity of staining with May-Grünwald solution is sufficient after 3 minutes, and can still be evaluated in most cases after 5 minutes. If left longer than 5 minutes, evaluation may not be possible if the cells have already been altered as a result of therapy or in the case of ventricular CSF.

Special staining and the detection of immunocytological cell surface markers (such as lymphocyte and tumour markers) can also be carried out with dried and non-fixed cell sediments in accordance with relevant operating procedures.

B) Dialysates

Dialysates do not generally need to be diluted. 50 µl serum is added per 400 µl sample volume.

C) Pleural effusions and ascites

With pleural effusions and ascites the cell count normally varies between 50 and 5,000 cells / µl, so if 400 µl is used as the sample volume then the

[1)] Registered trademark of the firm Gerhard Menzel Glasverarbeitungswerk GmbH & Co. KG

dilution conditions given for CSF that is blood-free can be adopted.

Since the protein content of both pleural effusions and ascites is very high – 20 to 40 g / litre – the samples can be diluted 1:10 with physiological saline without addition of protein. Since sufficient quantities of the effusions are generally available, sufficient supernatant that is free of cells can be derived for the dilutions.

Ordering information

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

Selected accessories [2]	Cat. No.
4-place rotor	**1624**
6-place rotor	**1626**
cyto suspension	**1680**
slide carrier with fastening ring	**1662**
angle chamber (30 mm^2)	**1671**
angle chamber (60 mm^2)	**1672**
angle chamber (120 mm^2)	**1673**
filter cards for 1671	**1696**
filter cards for 1672	**1697**
filter cards for 1673	**1698**

[2] Our complete range of accessories for cytology is listed in our cyto brochure, which is available free of charge.

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