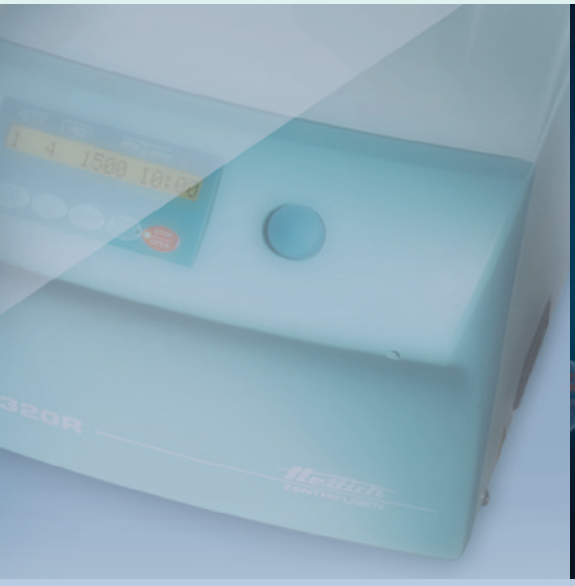
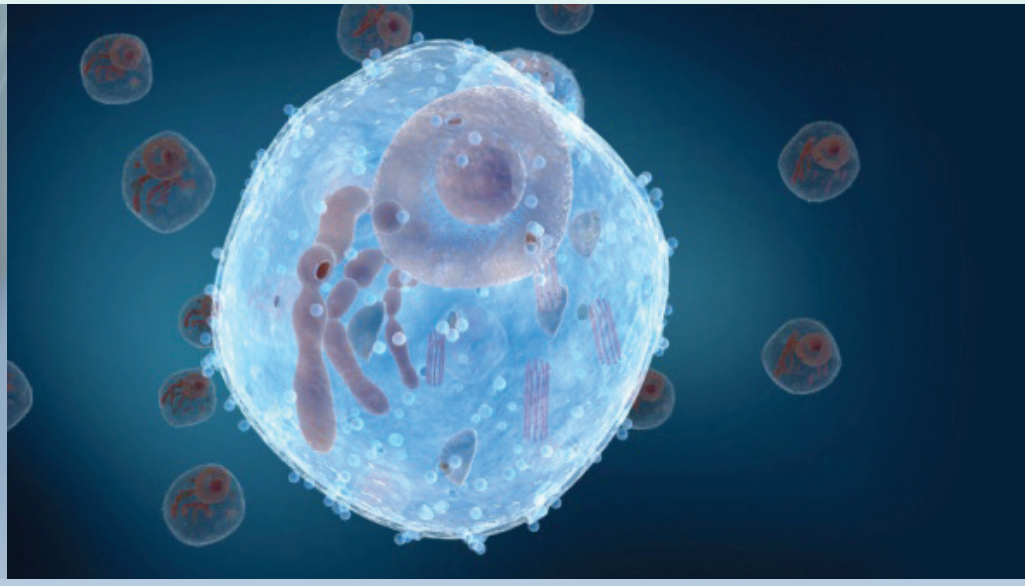
Hettich
Application Note





Slide preparation of bronchial washings for cytological examination

Hettich
ZENTRIFUGEN

Bronchial washing is the flushing of the respiratory tract with physiological saline solution. This procedure is used to derive cellular material and/or any invaded foreign bodies, for examination under a microscope.

Bronchial washings from healthy non-smokers comprise approximately 90% macrophages and a maximum of 15% lymphocytes, up to 3% granulocytes and 0.5% eosinophils. A washing is rich in cells and in most cases also contains mucus. In patients with certain diseases this cellular constitution changes and provides valuable information to aid diagnosis.

The presence of inhaled foreign bodies such as asbestos particles and pathogens (e.g., tubercle bacteria and pneumocystis carinii in patients with AIDS) can be detected directly.

Therefore, cytological preparations are derived from bronchial washings for the diagnosis of a large number of pulmonary and respiratory diseases. The preparations are generally stained immunocytochemically or by using Pappenheim's staining method.

Advantages of the Hettich method

1. Easy preparation

2. Excellent preparation quality

- Uniform cell distribution in the sediment
- Optimal spreading of the cells
- No selective cell loss, all cell types are represented
- Evaluation is simple since cells are concentrated over a defined area

Preparation

1. Sample preparation

Since bronchial washings often contain mucus which would affect the quality of the preparation, the mucus should be removed in advance.
The washing is therefore filtered through 2 layers of gauze.

2. Suitable accessories

As the washing is rich in cells, 2 ml and 4 ml chambers with sediment areas of 60 mm^2 or 120 mm^2 should be used.

The sample volume to be filled into the chambers depends on the cell content. Every mm^2 of sediment area in the chamber should contain between 1,000 and 2,000 cells – so approx. 60,000 to 120,000 cells should be filled into the 60 mm^2 chamber and 120,000 to 240,000 into the 120 mm^2 chamber. (The number of cells can be determined with a Fuchs-Rosenthal counter).

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". Microscope slide preparations derived from a bronchial washing are centrifuged, then air dried. For this reason, the insert should be assembled **without** a filter card (see illustration A1 in above mentioned leaflet). When working with infectious samples, the inserts should be closed with lid no. 1661 (see illustration A2 in the leaflet) to prevent the escape of aerosols.

4. Centrifugation

a) *Sedimentation*
Centrifuge the cyto chambers for **20 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).

b) *Removal of the cell-free supernatant*
After centrifugation, the cell-free supernatant is still in the chamber and should be removed as **completely as possible** through careful pipetting. This step is not necessary, if Dr. Barkhofen's method is used (see *c, 2.*).

c) *Drying of the sediment*
There are different approaches to drying the sediment:

1. After pipetting-off the supernatant, remove the cyto chamber (see illustration A4 in the leaflet) and allow the sediment to air dry.

2. Method of Dr. Barkhofen (Schillerhöhe Stuttgart/Gerlingen)
After centrifugation, take the cyto insert out of the centrifuge and tilt it to one side to allow the liquid to flow out. Leave the cyto insert in this position for at least 1 hour to dry or for longer if possible (e.g., overnight). Then remove the cyto chamber and allow the remaining ring of liquid residue to air dry.

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

Good to know:
With the Giemsa and the May-Grünwald-Giemsa staining methods, the microscope slides are rinsed with a buffer (e.g. Weise or Sörensen) after staining. 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 in buffer in the frames and centrifuge them for **1 minute** at **275 x g** (this corresponds to 1,500 min^{-1} in the 6-place rotor and 1,700 min^{-1} in the 4-place rotor).

Ordering information

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

Selection of accessories [1]	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^2)	**1664**
cyto chamber 1 x 4 ml (120 mm^2)	**1665**
labora-system frame for 6 slides	**1285**

[1] 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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