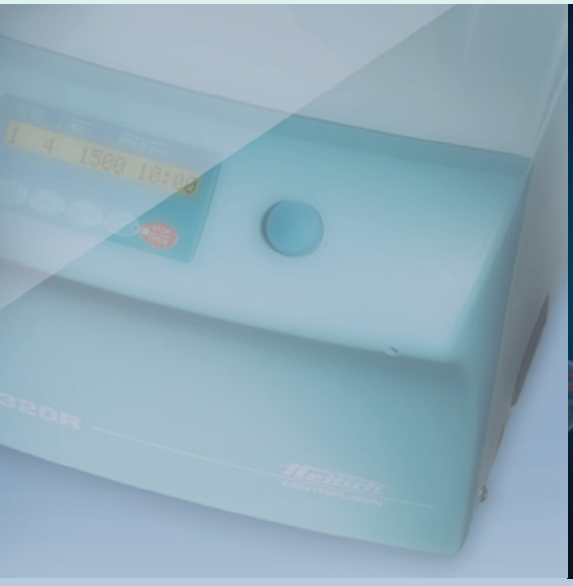
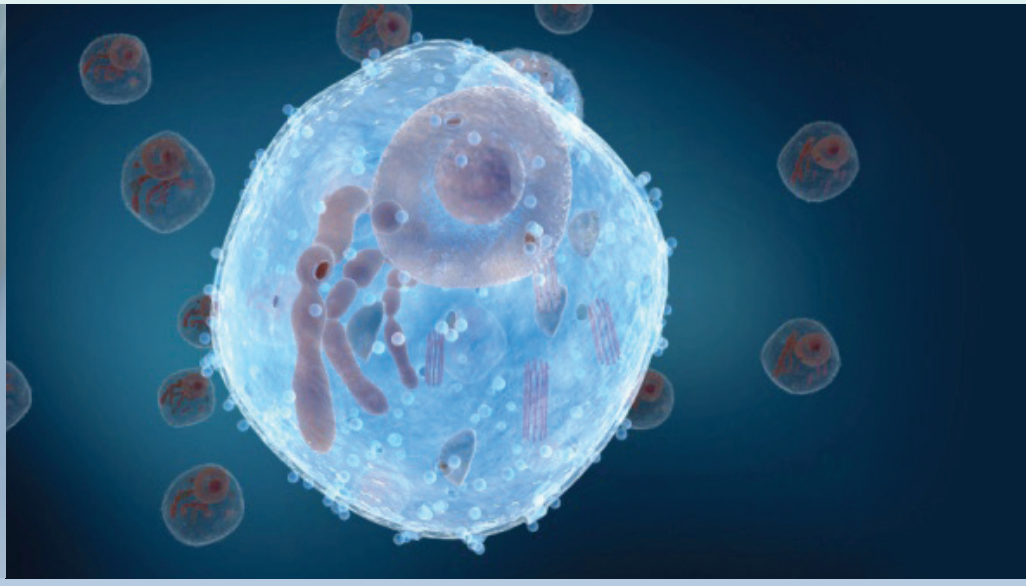
Hettich
Application Note





Slide preparations for urine cytology
Preparation of native urine

Hettich
ZENTRIFUGEN

To derive a good cytological preparation from a urine sample it is not sufficient to simply centrifuge native urine onto commercial microscope slides. Urine has a low cell concentration and those cells present adhere poorly to the slides. This would result in a preparation with an insufficient cell concentration. To increase the cell concentration it is necessary to coat the slide or use a suitable fixative. Poly-L-Lysine (PLL, Sigma, Cat. No. P8920) has been found to be suitable for slide coating and Saccomano's solution for fixing.

Advantages of the Hettich Method

1. High cell yield

- Through coating of the microscope slide
- Through incorporation of the cells in a fixing liquid which improves adhesion of the cells

2. Good quality of the preparation

- The use of Saccomano's solution yields sediments with a very clean background.
- The urothelium cells form a monolayer
- The PLL coating not only renders the cells visible, but also other smaller particles such as bacteria.

Preparation

A) Preparation of a cytological specimen with coated slides

1. Preparation of the slide

Two methods are available for coating of the slides with PLL – the immersion method and coating in the centrifuge. The immersion method is simple and easy to use, but the coating is occasionally not uniform. Coating in a centrifuge is a more time-consuming procedure, but will always deliver very good results.

a) *Immersion method*

- Dilute PLL 1:10 with distilled water and fill into a cuvette.
- Immerse commercial slides into this solution without any pre-treatment and leave them in the solution for 5 minutes. The PLL should be at room temperature.
- Remove the slides and dry them flat in a heated cabinet at approximately 60 °C. It will take longer to dry them at room temperature. The slides are ready to use as soon as the PLL layer is dry.

b) *Coating in the centrifuge*

- Dilute the PLL 1:10.
- Lay the uncleaned slides on the slide carrier (Cat. No. 1662) and secure a 8 ml chamber to it (Cat. No. 1666).
- Fill 100 µl diluted PLL into the cyto chamber.
- Centrifuge the cyto insert at **1100 x g** for **one hour** (corresponds to 3,000 min^{-1} with the 6-place rotor and 3,200 min^{-1} for the 4-place rotor. The sample can then be filled directly into the cyto chamber.

2. Suitable accessories

For centrifugation of urine we recommend the use of our largest cyto chamber with a volume of 8 ml and a sedimentation area of 240 mm^2. In many cases this will avoid the need for pre-centrifugation in tubes.

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 urine it is generally necessary to use wet fixation. The cyto insert should therefore be assembled without the filter card (see A1 in the illustration). If the specimens are infectious then we recommend the use of our lid No. 1661 (see A2 in the leaflet).

4. Centrifugation

a) *Sedimentation*

Centrifuge the cyto chambers for **5 minutes** at **1100 x g** (corresponds to 3,000 min^{-1} with the 6-place rotor and 3,200 min^{-1} with the 4-place rotor.

b) *Removal of the cell-free supernatant*

The cell-free supernatant remains in the chamber after centrifugation and is removed by careful decanting or pipetting. It is important that the sediment is not disturbed whilst the supernatant is being removed, as this can affect the quality of the procedure and/or lead to cell loss.

c) *Fixing and staining*

Once the supernatant has been removed the moist preparation can be fixed immediately (e.g., in 99% ethanol) and then stained.

B) Preparation of a cytological specimen using Saccomano's solution

1. Preparatory steps

a) *Preparation of the urine sample*

- Centrifuge the urine sample in a tube for **10 minutes** at 1700 x g (3,600 min^{-1} with the 6-place rotor and 4,000 min^{-1} with the 4-place rotor).
- Decant the supernatant.
  Important: The sediment in the centrifuge tube must be loosened before addition of the Saccomano's solution (best achieved through lightly tapping the base of the tube against a solid surface).
- Add the Saccomano's solution and allow it to stand for 30 minutes at room temperature.

b) *Preparation of the Saccomano's solution*

To prepare 100 ml Saccomano's solution, mix 43 ml distilled water, 53 ml 95 % ethanol and 4 ml polyethylene glycol (PEG) stock solution.

c) *Preparation of the polyethylene glycol stock solution:*

- Heat polyethylene glycol 1500 (Merck Darmstadt, Cat. No. 807489) and distilled water to 60 °C.
- The same volume of each (e.g., 50 ml PEG and 50 ml distilled water) are warmed and mixed.
- Allow the stock solution to cool to room temperature before the Saccomano's solution is made up.

2. Suitable accessories

See page 2.

3. Assembly of the cyto insert

See page 2.

4. Centrifugation

a) *Sedimentation*

The cyto chambers are centrifuged for **5 minutes** at **1100 x g** (corresponding to 3,000 min^{-1} with the 6-place rotor and 3,200 min^{-1} with the 4-place rotor).

b) *Removal of the cell-free supernatant*

The cell-free supernatant remains in the chamber after centrifugation and is removed by decanting.

c) *Fixing and staining*

After removal of the supernatant, remove the cyto chamber and allow the sediment to air-dry until a wax-like deposit has formed. The preparation may be mailed out in this state. Before staining, the wax-like deposit on the sediment must be removed through immersion in 50% ethanol for approx. 10 minutes.

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 4 ml (120 mm^2)	**1665**
cyto chamber 1 x 8 ml (240 mm^2)	**1666**

[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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