

URIGNOST S REAGENT

IVD *In vitro* diagnostic medical device



Modification acc. to Sternheimer for staining and microscopic analysis of urine sediment

INSTRUCTIONS FOR USE

REF Product code: UGS-500 (50 mL) UGS-1000 (100 mL)

Introduction

Microscopic examination of urine sediment is an extremely important test in detecting various disorders in kidney functions and urogenital tract. By conducting microscopic examination it is possible to view and differentiate between leukocytes, erythrocytes, epithelial cells, microorganisms and cylinders. UriGnost S reagent is stabilized and modified acc. to Sternheimer and is specially prepared according to the European Confederation of Laboratory Medicine (ECLM) instructions. UriGnost S reagent is used for qualitative and quantitative analysis of urine sediment. Contains Alcian Blue and Pyronin B dyes. UriGnost S reagent can also be acquired as a part of UriGnost S kit that contains all the necessary equipment for sampling, concentrating, counting cells and kidney cylinders and urine sediment analysis.

Product description

- **URIGNOST S REAGENT** – reagent for staining and analysis of urine sediment.

Other equipment necessary for staining procedure:

- Tube with retentive bottom, such as BioGnost's UriGnost tube vol. 15 mL with screw cap, retentive bottom vol. 0.2 mL, graduated (product code 4020-2501, 500 pcs in packaging)
- Pipette tips 200 µL, Eppendorf/Universal type, yellow (product code 3601-500, 500 pcs in packaging)
- Urine analysis plates, such as BioGnost's UriGnost 10 plate, 1 pc for 10 urine sediment analyses (product code UG10P-50, 50 pcs in packaging)

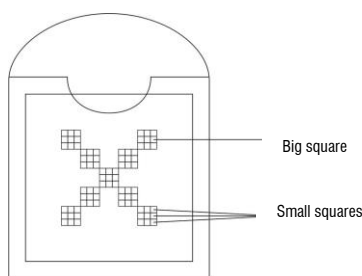
Preparing the sample for staining

- Analyze the urine sample immediately after sampling. If the analysis cannot be performed within 2 hours, store the sample at 4°C for up to 4 hours. Let the sample reach room temperature before use.
- Pour 12 mL of freshly sampled and stirred urine into the tube with retentive bottom and close it with appropriate cap. Urine sample volume may be smaller in certain cases (pediatric samples or special clinical conditions). The volume must be accurately measured and recorded because of calculation and expression of results.
- Centrifuge for 5 min at 1500 rcf.
- Remove the supernatant formed above the urine sediment after the centrifuge. Turn the tube over for 3-5 seconds in order to remove the supernatant. The tube must not be shaken during removal of the supernatant. Turn the tube back into vertical position. Retentive bottom retains 0.2 mL of urine with sediment after decanting.

Staining samples using UriGnost 10 plate

- Add 1 drop of UriGnost S reagent for staining into the retentive bottom of UriGnost tube that contains urine with sediment.
- Mix the urine sample with sediment and UriGnost S reagent with a pipette or gently agitate the tube by hand.
- This causes the sample to get stained immediately.
- By using the pipette and the same pipette tip, add 1 drop of stained sample into the chamber located on the plate for microscopical analysis and counting urine sediment elements. The sample is then spread around the chamber by capillary action.
- UriGnost 10 plate has 10 separated and numbered chambers that enable testing 10 different samples on the same plate.
- Each chamber contains 9 big squares, and each square is made of 9 small squares.
- The sample is viewed under low magnification (10x) in order to notice the cell and cylinder distribution, and high magnification (40x) is used for identification of cylinders and counting cells. Small cells and cylinders are counted in at least 9 small squares, big cells in at least 5 small squares, and the result is expressed as average value of number of cells. Average value of number of cells and cylinders in a small square is the total number of cells and cylinders in small squares divided by the number of counted small squares.
- One field of view under high magnification (40x) is equivalent to small square within a chamber.

UriGnost 10 plate



- Chamber volume: 14 µL
- Chamber depth: 0.1397 mm
- Big square dimensions: 0.846x0.846 mm
- Big square volume: 0.1 µL
- Small square volume: 0.01111 µL

Formulas for calculating cells and cylinders per µL of urine sample

$$\text{conversion factor} = \frac{9 \times 10 \times \text{sediment volume (mL)}}{\text{sample volume (mL)}}$$

- 9- calculates the volume of a small square (0.01111 µL) to the volume of a big square (0.1 µL).
- 10- calculates the volume of a big square (0.1 µL) to 1 µL.

$$\text{average value of number of cells in a small square} = \frac{\text{total amount of cells and cylinders in small squares}}{\text{number of counted squares}}$$

cells and cylinders in 1 μL of urine sample = average value of cells and cylinders in a small square x conversion factor

If a 12 mL urine sample produces 0.2 mL of urine with sediment, the amount of cells and cylinders per μL of the sample is obtained by multiplying the average value of the cells in small squares with factor 1.5. Conversion factor 1.5 is obtained by the following calculation:

$$\frac{9 \times 10 \times 0.2 \text{ mL}}{12 \text{ mL}} = 1.5$$

Example of calculating the amount of white blood cells per μL of urine sample from the data stated in the table for calculating the amount of cells and cylinders per μL of urine sample.

$$\text{average value of white blood cells in small squares} = \frac{36}{9} = 4 \quad \text{white blood cells in } 1 \mu\text{L of urine sample} = 4 \times 1.5 = 6$$

Calculation of cells and cylinders per μL of urine sample:

Cells and cylinders	Number of counted small squares	Total number of cells and cylinders in small squares	Average value of cells and cylinders in small square	Conversion factor	Cells and cylinders in 1 μL of urine sample
White blood cells	9	36	4	1.5	6
Red blood cells	5	120	24	1.5	36
cylinders	9	6	0.66	1.5	1

Conversion factors: In case another sample or urine sediment volume is used, a different conversion factor must be used. Examples are stated in the table.

Sediment volume / Sample volume	0.2 mL	0.4 mL	0.5 mL	1.0 mL
2.5 mL	7.2	14.4	18	36
5 mL	3.6	7.2	9	18
10 mL	1.8	3.6	4.5	9
12 mL	1.5	3	3.75	7.5

If a non-centrifuged urine sample is used, the conversion factor is 90.

Results: color of cells and cylinders

- Leukocytes: cytoplasm - red to purple, nucleus - red, granules - dark red
- Neutrophils: cells - light blue, granules - grey
- Erythrocytes: light blue
- Epithelial cells: light blue (vaginal epithelial cells - light red or purple)
- Bacteria: light red
- Cylinders: light red to blue

Note

Staining procedures are not standardized and they depend on standard operating procedures of individual laboratories and the experience of the personnel conducting the staining procedure. Depending on personal requests and standard laboratory operating procedures, sample processing and staining can be carried out according to other protocols.

Preparing the sample and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples with modern technology and mark them clearly. Follow the manufacturer's instructions for use. In order to avoid mistakes, the staining procedure and diagnostics should only be conducted by authorized and qualified personnel. Use only microscope according to standards of the medical diagnostic laboratory. In order to avoid an erroneous result, a positive and negative check is advised before application.

Safety at work and environmental protection

Handle the product in accordance with safety at work and environmental protection guidelines. Used solutions and out of date solutions should be disposed of as special waste in accordance with national guidelines. Chemicals used in this procedure could pose danger to human health. Tested tissue specimens are potentially infectious. Necessary safety measures for protecting human health should be taken in accordance with good laboratory practice. Act in accordance with signs and warnings notices printed on the product's label, as well as in BioGnost's material safety data sheet which is available on demand.














Storing, stability and expiry date

Keep UriGnost S reagent in a tightly sealed original packaging at temperature of 15°C to 25°C. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Sterheimer, R.; Malbin, B., (1951): Clinical Recognition of Pyelonephritis with a New Stain for Urinary Sediments, Am. J. Med., 11, 312
2. Topić, E.; Primorac, D.; Janković, S., (2004): Medicinskobiochemijska dijagnostika u kliničkoj praksi, Medicinska naklada

UGS-X, V2-EN2, 10 November 2016, IŠP/VR

 Refer to the supplied documentation	 Storage temperature range	 Number of tests in package	 Product code	 European Conformity
 Refer to supplied instructions	 Keep away from heat and sunlight	 Valid until	 Lot number	 Manufacturer
 For <i>in vitro</i> diagnostic use only	 Keep in dry place	 Caution - fragile		

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