BIOGNOST®

URIGNOST S KIT

IVD In vitro diagnostic medical device CE

UriGnost S kit for sampling, staining and microscopic analysis of 500 urine sediments

INSTRUCTIONS FOR USE

REF Catalog number: USK-500

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 kit is used for qualitative and quantitative analysis of urine sediment. UriGnost S kit contains UriGnost S reagent modified according to instructions of European Confederation of Laboratory Medicine (ECLM) and all the necessary equipment for sampling, concentrating, counting cells and kidney cylinders and urine sediment analysis.

Product description

- **URIGNOST S KIT**– the kit contains 1 reagent and disposables sufficient for 500 tests

The kit contains:	Packaging for 500 tests
UriGnost S reagent for 500 tests	50 mL (UGS-500)
UriGnost tube vol. 15 mL with screw cap, retentive bottom vol. 0.2 mL, graduated	500 pcs (4020-2501)
Pipette tips 200 µL, Eppendorf/Universal type, yellow	500 pcs (3601-500)
UriGnost 10 plate, 50 pcs for 500 urine sediment analyses	50 pcs (UG10P-50)

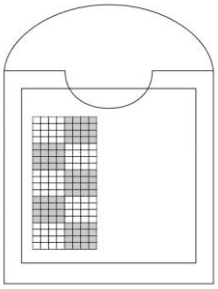
Preparing the sample for staining

- 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 UriGnost 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.

Sample staining procedure

- 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 UriGnost 10 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 10 big squares, and each square is made of 16 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. 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: 7 µL
- Size of the grid: 2x5mm
- Depth of the grid: 0.1 mm
- Big square dimensions: 1x1 mm
- Big square volume: 0.1 µL
- Small square volume: 0.00625 µL

Formulas for calculating cells and cylinders per µL and mL of urine sample

$$\text{Cells/µL} = \frac{(\sum \text{total number of cells in small squares n}) \times \text{concentration factor} \times 10}{N}$$

$$\text{Cells/mL} = \frac{(\sum \text{total number of cells in small squares n}) \times \text{concentration factor} \times 10^4}{N}$$

- Concentration factor = Sediment volume / Centrifuged urine volume
- **10:** calculates 0.1µL into 1µL
- **N**: number of counted large squares

10^4=calculates 0.1µL into 1mL

In case that less than 1 entire large square is counted (less than 16 little small squares), the following formulas are used:

$$\text{Cells/µL} = \frac{(\sum \text{total number of cells in small squares n}) \times 16 \times \text{concentration factor} \times 10}{n}$$

$$\text{Cells/mL} = \frac{(\sum \text{total number of cells in small squares n}) \times 16 \times \text{concentration factor} \times 10^4}{n}$$

n: number of counted small squares

For example, if only 5 individual squares are counted, 16/5 is used for the calculation in order to achieve equivalent for the entire grid

Method of counting cells for diluted samples (number of cells/mL)

After placing the sample in the chamber, cells distributed in N squares are counted.

Because the grid contains 10 squares, and each square has dimensions 1x1 mm, depth 0.1 mm and volume 0.1μL, the formula for detecting cell concentration (number of cells/mL) is as follows:

$$\text{Cells/mL} = \frac{(\sum \text{total number of cells in small squares n}) \times \text{concentration factor} \times 10^4}{N}$$

Pay attention to the cells found on the edges: it is necessary to count the cells located in the upper right hand (or lower left hand) side of the chamber in order to avoid the risk of multiple counting of the same cells

The example shows counting of cells of a sample that has been diluted 100 times

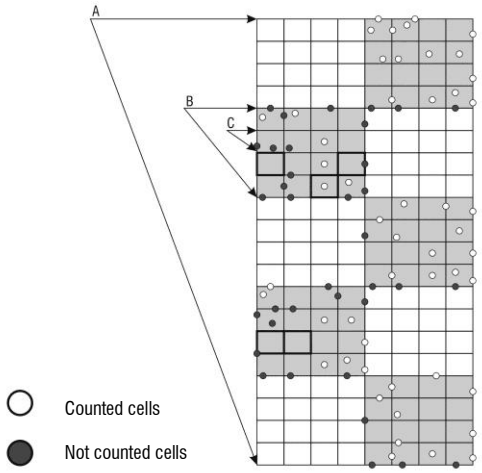
N= 5 (number of counted large squares)

$\sum$ number of cells counted in 5 large squares = 67

Dilution factor = 10^2

[number of cells/mL] = $(67/5) \times 10^2 \times 10^4 = 13.4 \times 10^6$

Example



A: GRID
B: LARGE SQUARE
C: SMALL SQUARE

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 kit 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): Medicinskobiokemijska dijagnostika u kliničkoj praksi, Medicinska naklada

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Refer to the supplied documentation	Storage temperature range	Number of tests in package	REF	Product code	CE	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	CE
Refer to supplied instructions	Keep away from heat and sunlight	Valid until	LOT	Lot number		Manufacturer		
IVD For *in vitro* diagnostic use only	Keep in dry place	Caution - fragile						