BIOGNOST®

CYTOGNOST KIT

IVD *In vitro* diagnostic medical device CE

Three-reagent kit for manual blood cell counting

INSTRUCTIONS FOR USE

REF Product code: HCG-K-100 (3 x 100 mL) HCG-K-500 (3 x 500 mL) HCG-K-1L (3 x 1000 mL)

Introduction

CytoGnost kit contains three reagents that are standardly used in clinical and hematology laboratories for manual blood cells counting: Hayem's solution is used for manual erythrocyte counting, Tuerk's solution is used for manual leukocyte counting, and ThromboGnost solution is used for manual thrombocyte counting. Each reagent is used separately. It is important to correctly prepare and dilute the sample of blood in the specified volume during every counting method.

Product description

- **CYTOGNOST KIT** – three-reagent kit for manual erythrocyte, leukocyte and thrombocyte counting

Testing sample

- Uncoagulated venous blood or capillary blood

Other necessary preparations:

- Staining chamber (Neubauer or Bürker-Türk net)
- Erythrocyte or leukocyte mixer
- Cover glass
- Sterile lancet
- Microscope

Preparing the sample for staining

Use Hayem's solution for counting **erythrocytes:** a) filling the mixer: draw blood into erythrocyte mixer by the 0.5 mark, then draw in Hayem's solution by the 101 mark. Dilution is 1/200. Carefully stir the blood sample and Hayem's solution. Use the preparation within a few hours.

b) filling the counting chamber: discard the first two drops and then fill the counting chamber.

Use Tuerk's solution for counting **leukocytes:** a) filling the mixer: Draw blood into the vortex mixer to the 1.0 mark, then draw Tuerk's solution to the 11 mark. The dilution ratio is 1-10. It is possible to make a 1-20 dilution ratio by drawing blood to the 0.5 mark, and Tuerk's solution to the 11 mark. Carefully stir the blood sample and Tuerk's solution. Use the preparation within 1 hour.

b) filling the counting chamber: discard the first three drops and then fill the counting chamber.

Use ThromboGnost solution for counting **thrombocytes:** a) filling the mixer: draw ThromboGnost solution into the mixer by the 0.5 mark, then draw blood from fingertip (previously disinfected and pricked using sterile lancet and with first blood drop wiped using cotton wool) by the 0.5 mark (previously drawn ThromboGnost solution that way gets pushed by the 1 mark). Top part of the mixer should be wiped clean of blood using cotton wool; draw more diluting solution (ThromboGnost solution) by the 101 mark. Dilution is 1/200.

b) filling the counting chamber: the mixer is stirred for 2-3 minutes, and then the counting chamber gets filled with contents*. The chamber filled with the mixer content should be left in humid chamber for 15 min.

*Note - should the thrombocytes not be counted right away after placing the content in the cell counting chamber, the mixer should be stirred again for 6 minutes.

Staining procedure

During using Hayem's solution: counting is carried out under the microscope with a 10x magnifying factor lens. It is necessary to lower the condenser and move the front lens outwards. Count the erythrocytes in the center part of the grid. Four diagonal fields are most commonly counted (64 squares). For more precision, count one peripheral field (total of 80 squares).

During using Tuerk's solution: Counting is carried out under the microscope with a 10x magnifying factor lens. It is necessary to lower the condenser and move the front lens outwards. Count the leukocytes in 4 big angular squares with sides 1 mm in length. Recounting is recommended. The results must not differentiate more than 15%.

During using ThromboGnost solution: counting is conducted under medium microscope magnification (40x), on a $1mm^2$ net (central big square in the net). All thrombocytes within the square are counted, as well as those touching two adjacent edges of the square (for instance, left and lower edge, and erythrocytes touching the other two squares are not being counted). All blood cells are visible in microscope field of view, and thrombocytes are dimly flickering, so they must be carefully watched as to net get confused with dust particles.

Result

Counting **erythrocytes:**

One side of a square is 1/20 mm in length; depth is 1/10 mm (after positioning the cover glass). Calculate the mean value of erythrocytes per square and then number of erythrocytes in 1 mm^3 of blood. Do not omit the dilution factor; multiply the result with 200.
The results are expressed as a mean value of double counting.

Normal erythrocyte values: Females: $3.86 - 5.08 \times 10^{12}/L$
Males: $4.34 - 5.72 \times 10^{12}/L$

Counting **leukocytes:**

Number of leukocytes = $(x \cdot 10 \cdot 10)/4$ (dilution 1-10)

Number of leukocytes = x·25 (No. cells/µl)

X = total amount of counted cells in 4 angular squares
The results are expressed as a mean value of double counting.

Normal leukocyte count range/µL:

Grownups	4,000 – 9,000
School children	5,000 – 12,000
Toddlers	6,000 – 15,000
Infants	7,000 – 17,000
Newborns	10,000 – 30,000

Counting **thrombocytes:**

The attained number of thrombocytes on 1mm^2 surface should be multiplied with dilution and chamber depth, This provides the number of thrombocytes in 1 µL.

Thrombocytes/µL = A x 200 x 10

A = number of thrombocytes counted in 1mm^2

200 = dilution

10 = chamber depth

Normal thrombocytes values: Grownups: 150 - 400 x 10^9/L

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 handling. 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.

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. Reagents 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.

Storing, stability and expiry date

Keep CytoGnost kit in a tightly sealed original packaging at temperature of +15°C to +25°C. Do not keep in cold 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. Nagahashi, H. et al. (2000): Improved Sensitivity in the Measurement of Residual Leukocytes in Platelet Products Using an Automated Leukocyte Counter, *Labile Blood Components and Blood Donation,* 79; p 34-39.
2. Pal, G.K. et Parvati, Pal. (2006): Textbook Of Practical Physiology, 2nd ed., Orient Blackswan
3. Softić, N. (1988): *Hematološke laboratorijske pretrage*, Tisak Sveučilišna naklada Liber, Zagreb.
4. Teijlingen van, M. E. et al. (2000): In vivo visualization of hemodialysis-induced alterations in leukocyte-endothelial interactions. *Kidney Internalional,* 57; pp 2608-2617.

HCG-K-X, V1-EN1, 23 May 2022, IŠP/VR

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						