



Leuko-TIC®

Supplement Information for Counting Low Values of Leukocytes (WBCs). (Supplement, e.g. for Transfusion Medicine)

This is a supplemental product information for visual microscopic counting of low values of leukocytes (white blood cells; WBCs) with Leuko-TIC®, e.g. in the Transfusion Medicine (residual WBC, rWBC). Please also refer to the basic product information.

Principle

This product information describes the counting of extremely low leukocyte numbers (mostly < 1000/µL). This protocol must only be used if the regular method yielded a leukocyte (WBC) count below 2000/µL, as well as for counting residual leukocytes in leucocyte depleted infusion products.

These instructions supplement those for regular counting. For further details refer to the Leuko-TIC® product information.

Attention!

This additional information is a supplement to the product information. It is also important to observe the information in the product information!

Sample Material

Process freshly collected capillary blood immediately. Sterile and sealed K₂- or K₃- EDTA blood can be processed within max. 24 hrs when stored at +4 ... +8 °C. Do not freeze!

Also suitable for other sample material such as CSF (Liquor cerebrospinalis)*¹, erythrocyte concentrates, thrombocyte concentrates, blood plasma (transfusion medicine: determination of residual WBCs), synovial/joint fluids (limited)^{3, 4}, thoracentesis/pleural fluids and others².

For leukocytes counts from blood lower than 1000/µl, use of EDTA blood is recommended.

Reference Ranges

EDTA-blood	[10 ⁹ /L = 10 ⁹ /µL]	
Neonates:	10.0 ...	30.0
Nurselings:	7.0 ...	17.0
Infants:	6.0 ...	15.0
Scholars:	5.0 ...	12.0
Adults:	4.0 ...	9.0

rWBC of Transfusion concentrates	[10 ⁶ /280 ml]	[/µl] = [10 ⁶ /L]
Erythrocyte concentrate.....	< 1	< 3,57
Thrombocyte concentrate.....	< 1	< 3,57

Please refer to the relevant literature or regulations for reference ranges.

Procedure

Dilution A = 1 : 20 (20 µL sample/Leuko-TIC) (Dilution for Erythrocyte (RBC) Concentrates):

This is the recommended method and regular dilution for Leuko-TIC®. However, cells are counted in the Fuchs-Rosenthal counting chamber (see section "counting/calculation").

Discard the first drop of capillary blood before filling the 20 µL end-to-end volume capillary.

Fill a 20 µL end-to-end volume capillary bubble-free with blood from end to end. We urgently recommend using a capillary holder for this. Remove blood on the outside with a lint-free tissue without sucking blood from the capillary. Place filled volume capillary into the opened TIC-tube, close and shake thoroughly until all blood is flushed from the capillary. Leave capillary in the vial.

Alternative:

As an alternative to the 20 µL capillary, you may use a 20 µL automatic micropipette. Wipe off sample stuck to the outside of the pipette tip with a lint-free tissue. Flush the pipette tip repeatedly with the Leuko-TIC® solution.

Dilution B = 1 : 4.8 (100 µL sample/Leuko-TIC®) (Dilution for Thrombozytes (PLT) Concentrates):

Special dilution with 5 times the sample volume for counting in Neubauer counting chamber (see section "counting/calculation").

Add 100 µL sample to the content of a Leuko-TIC® vial. Use either a capillary pipette with ring mark at 100 µL or, preferably, an automatic pipette which is easier to handle.

Wipe off sample adhering to the outside of the pipette tip or capillary with a lint free tissue. Flush the pipette tip or the capillary repeatedly with the Leuko-TIC® solution.

General procedure (continued)

Close vial and shake sufficiently between thumb and index finger until all blood is flushed from the capillary or mixed with solution. Wait at least 30 seconds for complete lysis of RBCs. Shake the tube once more before loading the counting chamber.

Fill the chamber filling capillary about a quarter to half its length by capillary action and seal the upper end with your finger. Touch the tilted capillary (narrow angle) against the edge of the cover slip and load the counting chamber.

Whereas the use of fresh whole blood leukocytes (WBC) can be counted up to 48 hours, we recommend for blood products to count within 1 hr.

Counting/calculation

Shake the vial once more before loading the counting chamber. Fill the chamber filling capillary about halfway by capillary action and seal the upper end with your finger (wear gloves). Touch the tilted capillary (narrow angle) against the edge of the cover slip and load the counting chamber.

Counting in a Fuchs-Rosenthal chamber results in statistically more exact values due to the larger area assessed (only valid for leukocyte counts below 2000/ μ l).

For microscopic counting, use phase-contrast optics or bright field (lowered condenser) at 100 \times magnification.

Nageotte counting chamber:

Use dilution A (20 μ l sample).

Count the WBCs in all 40 lines each 10 \times 0.25 mm in meandering form. This is a volume of 100 mm² \times 0.5 mm = 50 μ L.

$$\begin{aligned} \text{Gezählte Leukozyten} \times 0.4 &= \text{Leukozyten}/\mu\text{l} \\ \text{Gezählte Leukozyten} \times 0.4 &= \text{Leukozyten} \times 10^6/\text{l} \\ \text{Gezählte Leukozyten} \times 0.112 &= \text{Leukozyten} \times 10^6/\text{Spendereinheit 280 ml} \end{aligned}$$

Fuchs-Rosenthal counting chamber:

Use dilution A (20 μ L sample).

Count the WBCs in all 16 large squares (1 mm² each). This is a volume of 16 mm² \times 0,2 mm = 3,2 μ L.

$$\begin{aligned} \text{Total count from the 16 squares} \times 6.25 &= \text{WBCs}/\mu\text{l} \\ \text{Total count from the 16 squares} \times 6.25 &= \text{WBCs} \times 10^6/\text{L} \\ \text{Total count from the 16 squares} \times 1.75 &= \text{WBCs} \times 10^6/\text{Unit of 280 ml} \end{aligned}$$

Neubauer or Neubauer "improved" counting chamber

Use dilution B (100 μ L sample).

Count the WBCs in the 4 large corner squares (1 mm² each). This is a volume of 4 mm² \times 0,1 mm = 0,4 μ L.

$$\begin{aligned} \text{Total count from the 4 large corner squares} \times 12 &= \text{WBCs}/\mu\text{l} \\ \text{Total count from the 4 large corner squares} \times 12 &= \text{WBCs} \times 10^6/\text{L} \\ \text{Total count from the 4 large corner squares} \times 3.36 &= \text{WBCs} \times 10^6/\text{Unit of 280 ml} \end{aligned}$$

Information

Also refer to the basic Leuko-TIC[®] product information

Instruction for Use

For professional use only.

To avoid errors, the use of qualified personnel is carried out. National guidelines for work safety and quality assurance must be followed.

The used equipment must comply with the state of technology and the laboratory requirements.

Protection against infection

It is important to ensure effective protection against infection according to laboratory guidelines.

Laboratory personnel working with human samples should at a minimum be immunized against Hepatitis B (HBV).

Support/Information service

For methodological and technical support, please contact us by E-Mail at support@bioanalytic.de.

Periodically check for updates of this product information on our website.

Feedback

Information from users can be reported to support@bioanalytic.de.

Suggestions will be considered for further development.

If a serious incident has occurred during or as a result of use, please report it to the manufacturer and/or its authorized representative and to your national authority.

Waste Management

Please observe your national laws and regulations.

Used and expired solutions must be disposed of in accordance with your local regulations.

Inside the EU, national regulations apply that are based on the current, amended version of Council directive 67/548/EEG on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

Decontaminated packaging can be disposed of as household waste or recycled, unless otherwise specified.

Footnotes

- *1) Samson's Concentrate REF 006688 can also be used to count WBCs in small volumes of CSF (cerebrospinal fluid). Instructions on request.
- *2) Non-blood specimens should ideally be free of blood contamination and insoluble components like fibrin clots.
- *3) Samples containing substances that form precipitates with acetate or acetic acid cannot be used. Further information on request.
- *4) For synovial fluids (SF) we recommend strictly use Leuko-TIC SF. This special fluid is not biding precipitates with SF.