

LeucoScreen Plus



Semi-quantitative histochemical kit for the determination of peroxidase-positive white blood cells in human semen

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For *in vitro* diagnostic use only - Reagent for professional use only

INTRODUCTION

Most human ejaculates contain leucocytes and the predominant form of leucocytes in human semen are peroxidase-positive granulocytes^{1,2,3,4}. Excessive presence of these cells (leucocytospermia) may indicate the existence of reproductive tract infection. Leucocytospermia may also be associated with defects in the semen profile (reduction in sperm motility and DNA integrity, raise of sperm viscosity as well as loss of sperm function as a result of oxidative stress, and/or secretion of cytotoxic cytokines by these white blood cells). Although leucocytospermia is not an absolute indication of infertility, this condition is observed on average in 10 to 20% of all infertile men⁵.

When a typical semen analysis is performed, it is very difficult to differentiate white blood cells from other types of round cells in the semen sample (for example spermatogenic precursor cells⁷). A relatively rapid and inexpensive method of differentiating peroxidase positive white blood cells from other round cells in a semen sample makes use of the intrinsic peroxidase activity of these cells⁷. LeucoScreen Plus is, just like LeucoScreen, based on this technique and can therefore be used to stain the peroxidase positive white blood cells in a human semen sample.

According to the World Health Organization, the presence of more than one million peroxidase positive white blood cells (WBC) per ml ejaculate is considered abnormal and is labelled as "leucocytospermia"⁴. However, this threshold is under debate, as some have found this value too low and others too high. Indeed, threshold levels from 0.2×10^6 – 2×10^6 have been reported⁸⁻¹⁰.

When the threshold of one million peroxidase positive WBC per ml ejaculate is exceeded, microbiologic tests should be performed to investigate if there is an accessory gland infection. Assessment of accessory gland markers can provide additional useful information about the proper functioning of the epididymis (EpiScreen Plus, FertiPro nv), seminal vesicles (Fructose Test, FertiPro nv) or prostate. Importantly, the absence of leucocytes does not exclude the possibility of an accessory gland infection.

The number of tests that can be performed with the LeucoScreen Plus kit is not specified, instead, the kit has been designed for 40 days of analysis during the lifetime of the kit (20 working solutions can be made, which are stable for 2 consecutive days).

MATERIAL INCLUDED WITH THE TEST

- Reagent 1 – 6 ml of Substrate solution (4-CN in methanol)
- Reagent 2 – 300 µl of 30% Hydrogen peroxide
- Reagent 3 – 22 ml of Buffer solution
- Reagent 4 – 1.2 ml of Counter stain solution

A certificate of analysis and the MSDS are available on our website (www.fertipro.com).

MATERIAL NOT INCLUDED WITH THE TEST

Microscope slides, cover glasses, test tubes (Eppendorf), pipettes, bright field microscope.

Note: It is recommended to use a light microscope and not to use a phase-contrast microscope as the latter could lead to interpretation difficulties.

SPECIMEN TYPE

The test should be performed on fresh human semen samples containing more than 1×10^6 round cells per ml. The test should be performed within the same day of semen collection.

PRINCIPLE OF THE TEST

Myeloperoxidase, present in granules of certain leucocytes, oxidizes 4-CN to a bluish-purple 4-chloro-1-naphthol precipitate, utilizing H_2O_2 as the oxidant. Reagent 4 contains a counter stain solution to facilitate the differentiation between peroxidase positive round cells and peroxidase negative round cells.

INTERPRETATION

- **Peroxidase positive round cells:** contain (bluish-)black granules and have an anthracite-grey cytoplasm. This anthracite area can cover the complete cell or only part of it.
- **Peroxidase negative round cells:** remain unstained or can be pink-colored. Granules seen in unstained cells are not surrounded by the typical anthracite color seen in positive cells.

PERFORMANCE

In an independent validation study, 44 samples were analyzed by different operators with LeucoScreen and LeucoScreen Plus. Performance characteristics:

- **Accuracy:** Both kits provided comparable results based on Passing Bablok analysis.

- **Precision:** Both kits can distinguish between peroxidase-positive and -negative round cells with comparable precision (LeucoScreen Plus: CV_{intra} : 12%; CV_{inter} : 10%)

PRE-USE CHECK

Reagent 1 should contain a clear fluid, do not use the kit if the fluid has turned yellow.

METHOD

Before first use of the LeucoScreen Plus kit, we strongly recommend to follow the training program and to view our demonstration video, which are both available on our website. The video can also be downloaded by scanning this QR-code:



1. Count the number of round cells whilst determining the sperm concentration during routine semen analysis. Calculate and write down the total concentration of round cells in mill/mL, as this will be needed for the calculation of the concentration peroxidase-positive white blood cells. When round cells concentration exceeds 1×10^6 per ml, the LeucoScreen Plus test is indicated.
2. Prepare working solution: Add the following volumes to an Eppendorf tube and mix thoroughly.
 - 200 µl Reagent 1
 - 5 µl Reagent 2
 - 1 ml Reagent 3.This working solution is stable for 48 hours when stored between 2-8°C, protected from (sun)light.
3. Take 10 µl of the semen sample and add 20 µl of working solution. Mix thoroughly.
4. Incubate for two minutes at room temperature **in the dark**.
5. Transfer 10 µl of the mix to the middle of a microscope glass and add 10 µl of Reagent 4. Mix thoroughly using the edge of the cover slip.
6. Cover with the cover slip
7. Count and classify a total of 200 round cells using a magnification of 400x. Scan different microscopic fields (preferably 20).

Note: In some semen samples, you might prefer to enrich the round cell concentration to facilitate cell counting. Therefore, centrifuge the sample for 15 minutes at 350g, remove some volume of the semen sample and resuspend the pellet.

In case of very high concentration of round cells (i.e. above 20×10^6 per ml), it is strongly advised to dilute the sample in PBS or in FertiCult™ Flushing medium.

CALCULATION OF THE CONCENTRATION OF PEROXIDASE-POSITIVE WHITE BLOOD CELLS

- Calculate the proportion of peroxidase-positive cells as follows:

$$\text{PROPORTION POSITIVE ROUND CELLS} = \frac{\text{Number of POSITIVE round cells}}{\text{(Number of POSITIVE round cells + Number of NEGATIVE round cells)}}$$

- Now, calculate the concentration of peroxidase-positive white blood cells in the semen sample as follows:

$$\text{CONCENTRATION (mill/mL)} = \text{Proportion positive round cells} \times \text{total concentration of round cells}$$

Example:

- Total concentration of round cells is 2 mill/mL (determined during sperm concentration analysis)
- With the LeucoScreen Plus test, 120 round cells are found positive and 80 round cells are found negative
- Proportion positive round cells = $\frac{120}{(120+80)} = 0.6$
- Concentration of peroxidase-positive white blood cells = $0.6 \times 2 \text{ mill/mL} = 1.2 \text{ mill/mL}$

STORAGE AND STABILITY

Store reagents between 2°C-25°C. Suitable for transport or short-term storage at elevated temperatures (up to 5 days at 37°C). Do not freeze. Protect from (sun)light. The kit is stable for at least 12 months after production date (even after opening), do not use after expiry date mentioned on the product label. The bottles must be kept tightly closed at all times. The working solution can be stored up to 48 hours at 2-8°C, protected from (sun)light.

LIMITATIONS OF THE METHOD

This test is an aid in the diagnosis of male infertility and, as for other biological tests, interpretation of the results must be performed within the framework of clinical findings and data of history taking. The LeucoScreen Plus test only stains peroxidase-positive WBC, other types of WBC (e.g. lymphocytes and monocytes) are not detected.

WARNINGS AND PRECAUTIONS

Use of gloves is advised. Reagent 1 contains methanol: avoid inhalation of vapors. Reagent 2 contains H₂O₂: corrosive, causes burns. After contact with skin wash immediately with water and soap. Wear eye / face protection. All semen samples should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Always use fresh pipette tips for each step to avoid cross-contamination. Do not discard product into the environment.

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