LeucoScreen

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

For in vitro diagnostic use - Reagent for professional use only

INTRODUCTION

Most human ejaculates contain white blood cells (WBC), the majority being peroxide-positive granulocytes1,2,3,4. 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. This includes reductions in the volume of the ejaculate, sperm concentration, and sperm motility, as well as loss of sperm function as a result of oxidative stress5,6 and/or secretion of cytotoxic cytokines7. There is currently no clear threshold concentration of leucocytes beyond which fertility will be impaired. The clinical impact depends upon the site at which the leucocytes enter the semen, the type leucocyte involved, and their state of activation. As a general rule, semen should not contain more than 1 x 104 peroxide-positive cells per ml8.

Note 1: When the threshold is exceeded, we recommend to perform microbiologic tests and assess for accessory gland markers (e.g. with Citric Acid test, EpiScreen Plus and Fructose Test) to investigate if there is an accessory gland infection.

Note 2: The absence of leucocytes does not exclude the possibility of an accessory gland infection.

Note 3: The number of tests that can be performed with the LeucoScreen kit is not specified, instead, the kit has been designed for 20 days of analysis.

MATERIAL INCLUDED WITH THE TEST

- Reagent 1 - 20ml of LeucoScreen stain (Contains: benzidine, cyanosine and methanol)
- Reagent 2 - 1ml of 3% Hydrogen peroxide

A certificate of analysis and the MSDS can be downloaded from our website (www.fertipro.com).

MATERIAL NOT INCLUDED WITH THE TEST

Object glasses, cover glasses, pipettes, microscope

PRINCIPLE OF THE TEST

Granules in the polymorphonuclear WBC contain peroxidase. Peroxidase catalyses hydrogen peroxide into water and free oxygen ions, which in turn, oxidize benzidine. Oxidized benzidine colours brown and consequently, peroxide-positive cells have a brown coloration. Reagent 1 also contains a red contrast fluid to differentiate peroxidase positive round cells from peroxidase negative round cells.

SPECIMEN TYPE

When round cell concentration in an ejaculate exceeds 1 x 10⁴ per ml, the nature of these cells should be assessed for peroxidase activity9. The LeucoScreen test is preferably done ± 1 hour after ejaculation.

METHOD

We recommend to view our demonstration video (download via link on our website, or scan barcode e.g. with the App "REA PharmaScan"): 1. Perform the test 1 hour after ejaculation.
2. We strongly recommend to work under a fume hood, as Reagent 1 is poisonous.
3. Preparation of work solution: Add 30µl of Reagent 2 to 1ml of Reagent 1. This work solution remains stable for 1 day.
4. Mix 1 drop (10µl) of sperm with 1 drop (10µl) of work solution, using the edge of the cover slip. Mix thoroughly for at least 1 minute.
5. Wait 1 minute. Place the cover slip on top of the mixture, avoid air bubbles. Formation of small air-bubbles is normal and due to peroxidase reaction. The higher the concentration of peroxidase positive cells, the more bubbles will form. Note: In case of excessive bubble formation, read slide immediately.
6. Read the result after 2 minutes at a magnification of 400x (read at least 20 separate microscope fields).

CALCULATION OF THE CONCENTRATION OF PEROXIDASE-POSITIVE WHITE BLOOD CELLS

1. KNOWN CONCENTRATION OF SPERMATOZOA

Count the number of peroxide-positive WBC and the number of spermatozoa. Calculate the concentration of peroxide-positive WBC based on this formula:

\[
\text{Number of peroxide-positive WBC} \times \text{sperm concentration (mill/ml)}
\]

This method will only work if the semen sample contains spermatozoa (preferably more than 10mill/ml).

2. UNKNOWN CONCENTRATION OF SPERMATOZOA

In this case the concentration of peroxide-positive WBC can be determined by multiplying the number of peroxide-positive WBC with a known factor based on the volume of the semen mixture in one microscopic field. To this end, take the following steps:

1. Determine the surface area of 1 microscopic field: Measure the diameter of 1 microscopic field with a micrometer. Calculate the radius in µm (≈ half of the diameter).
2. Surface area of one microscopic field (µm²) = radius² x 3.14
3. Depth of sample: distance between microscopic glasses (µm): Volume semen mixture (µl) = Length (mm) x width (mm) x cover glass x 1000
4. Calculation of FACTOR: 1000000 µm²/surface area (µm²) x distance between glasses (µm)
5. Examine at least 20 different microscopic fields and tally the number of peroxide-positive WBC.
6. Calculate the concentration of peroxide-positive WBC (mill/ml) based on this formula:

\[
\text{Total number of counted peroxide-positive WBC} \times \text{FACTOR}
\]

INTERPRETATION

- Yellow to brown stained cells are peroxide-positive cells: polymorphonuclear white blood cells.
- Pink stained cells: all other cells

STORAGE

Suitable for transport or short term store at elevated temperatures (up to 5 days at 37°C). Store reagents between 2°C-25°C.

REMARKS

Formation of a sediment in Reagent 1 is normal. Simply pour Reagent 1.

SENSITIVITY AND SPECIFICITY

Sensitivity and specificity for leucocytospermia is 90% when compared with the immunohistological test10, with a threshold for the peroxidase stain of 1 mill. WBC/ml and for the immunohistological test of 2 mill. WBC/ml.

WARNINGS AND PRECAUTIONS

All semen samples should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis.

Reagent 1 is very poisonous by inhalation, skin contact or swallowing. Risk of unrepairable damage. Wear protective clothing and take off contaminated clothing immediately. Work under a fume hood. In case of any accident, seek medical attention. Reagent 2 is corrosive and causes burns. After contact with skin wash immediately with water and soap. Wear eye / face protection.

BIBLIOGRAPHY


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