LeucoScreen

IVD

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

For in vitro diagnostic use - 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. This includes reductions in the volume of the ejaculate, decreased sperm concentration and sperm motility, as well as loss of sperm function as a result of oxidative stress[2,5] and/or secretion of cytotoxic cytokines[6]. Although leucocytospermia is not an absolute indication of infertility, this condition is observed on average in 10 to 20% of all infertile men[8].

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 cells[7]). 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 cells[7]. LeucoScreen is 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 x 10^6 – 2 x 10^6 have been reported[8-10].

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 (Citric Acid Test, FertiPro NV). 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 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 polymorphonuclear WBC contain peroxidase. The peroxidase catalyses hydrogen peroxide into water and free oxygen ions, which in turn, oxidize benzidine. Oxidized benzidine colours brown and consequently, peroxidase-positive cells have a brown coloration. Reagent 1 contains a red contrast fluid which facilitates the differentiation between peroxidase positive round cells and peroxidase negative round cells.

INTERPRETATION

- **Peroxidase-positive** round cells are stained yellow to brown/brown-reddish. These are polymorphonuclear white blood cells.
  Note: positive cells are completely or partially stained, sometimes only visible as brown spots.
- **Peroxidase-negative** round cells are stained pink. These are other round cells (e.g. spermatids, peroxidase-negative white blood cells)

SPECIMEN TYPE

Native liquified semen containing more than 1x10^6 round cells per ml.

METHOD[11]

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 can be used for the calculation of the concentration peroxidase-positive white blood cells. When round cell concentration exceeds 1x10^6 per ml, the LeucoScreen test is indicated.
2. Prepare work solution **in a fume hood** (Reagent 1 is poisonous): Add 30µl of Reagent 2 to 1ml of Reagent 1 and mix thoroughly. This work solution remains stable for 1 day.
3. Mix 1 drop (10µl) of semen with 1 drop (10µl) of work solution, using the edge of the cover slip. Mix thoroughly for at least 1 minute.
4. 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.
5. After 2 minutes, read at least 20 separate microscope fields and count the number of "peroxidase-positive" round cells and the number of "negative" round cells (see Chapter on INTERPRETATION). Use a magnification of 400x.

We recommend to view our demonstration video (download via link on our website: www.fertipro.com, or scan barcode):

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:

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

Example:

- Total concentration of round cells is 2 mill/mL (determined during sperm concentration analysis)
- With the LeucoScreen 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 x 2 mill/mL = 1.2 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. The kit is stable for at least 12 months after production date (even after opening), do not use after expiry date mentioned on the label. The working solution can be stored up to 24 hours in the dark at room temperature.

REMARKS

Formation of a sediment in Reagent 1 is normal. Simply pour Reagent 1 over filter-paper to eliminate sediment.

In case total round cell concentration has not been determined on the sample (deletion of step 1 in method section, which is not recommended!), it is possible to calculate the concentration of peroxidase-positive white blood cells based on the number of such cells counted per microscopic field. To this end, it is important that the exact volume of semen mixture examined in one microscopic field has been taken into account. This volume, expressed in µl, is calculated as follows:

- Measure the diameter of 1 microscopic field with a micrometer and calculate the radius:
  r = radius (mm) = ($\frac{diameter\ (\mu m)}{2}$)/1000
- Calculate depth of the sample (= distance between glasses):
  D = Depth (mm) = $\frac{Volume\ semen\ mixture\ (20\mu l)}{Length\ (mm)\ x\ width\ (mm)\ of\ cover\ glass}$
- V = Volume in one microscopic field (µl) = Depth x Radius^2 x 3.14

Examine at least 20 different microscopic fields and tally the number of peroxidase-positive round cells. Do the following calculations:

- A = average number of positive cells per microscopic field
- N = number of positive cells per semen mixture (cells/ml) = $\frac{A}{V}$ x 10^3
- Concentration peroxidase-positive WBC in native semen sample (cells/ml): 2 x N

Example:

- Diameter of microscopic field = 250 µm ➔ r = 0.125 mm
- Cover glass = 24x40 mm ➔ D = [20/(24x40)] = 0.0208 mm
- V = 0.0208 x 0.125^2 x 3.14 = 0.00102 µl
- 100 peroxidase-positive WBC counted in 20 fields ➔ A = 5
- N = 5/0.00102 x 10^3 = 4.900.000 cells/ml
- Concentration peroxidase-positive WBC in native semen sample (cells/ml) = 2 x 4.900.000 = 9.800.000 cells/ml.

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.

LeucoScreen only stains peroxidase-positive WBC, other types of WBC (e.g. lymphocytes and monocytes) cannot be detected.

PERFORMANCES

Sensitivity and specificity for leucocytospermia is 90% when compared with the immunohistological test[12], with a threshold for the peroxidase stain of 1 mill. WBC/ml and for the immunohistological test of 2 mill. WBC/ml.

The LeucoScreen kit can distinguish between peroxidase-positive and -negative round cells with an inter- and intra-assay CV below 10%.

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.

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