LeucoScreen

IVD

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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 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, 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]. 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 1x 10^6 peroxidase-positive cells per ml[4].

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, peroxidase-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.

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[7]

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.
2. When round cell concentration exceeds 1x10^6 per ml, perform the LeucoScreen test.
3. 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.
4. 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.
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. 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, or scan barcode):

CALCULATION OF THE CONCENTRATION OF PEROXIDASE-POSITIVE WHITE BLOOD CELLS

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

PROPORTION POSITIVE ROUND CELLS =

$$\frac{\text{Number of POSITIVE round cells}}{(\text{Number of POSITIVE round cells} + \text{Number of NEGATIVE round cells})}$$

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

CONCENTRATION (mill/mL)=
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

Suitable for transport or short term storage 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 over filter-paper to eliminate sediment.

LIMITATIONS OF THE METHOD

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

SENSITIVITY AND SPECIFICITY

Sensitivity and specificity for leukocytospermia is 90% when compared with the immunohistological test[8], 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.

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