DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Leuco test technical's information's
Technical's card 13-106
Product code 13-106
Pack 2x50 ml or on request
Stability of product properly conserved at 4°C 12 months
CND code W01030799

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For emergency contact your nearest anti-poison unit.

Principle
Most human ejaculates contain leucocytes, the predominant cell type being the neutrophil. Excessive presence of these cells (leucocytospermia) may indicate the existence of reproductive tract infection.
Furthermore, leucocytospermia may be associated with defects in the semen profile, including 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 (Aitken et al, 1989; Aitken and West, 1990) and - or secretion of cytotoxic cytokines (Hill et al, 1987).
It is difficult to define a threshold concentration of leucocytes beyond which fertility will be impaired. The impact of these cells depends upon the site at which the leucocytes enter the semen, the type leukocyte involved, and their state of activation. As a general rule, a normal ejaculate should not contain more than 5x 106 round cells/ml, while the number of leucocytes should not exceed 1x 106/ml (WHO, 1992). When the semen contains more than 1x 106/ml white blood cells, microbiological tests should be performed to investigate if there is an accessory gland infection.

The granules in the neutrophilic polymorphous leucocytes contain peroxidase, which together with the hydrogen peroxide form water and free oxygen ions, these oxygen ions oxidize the benzidine which colours brown, thus staining the cells brown. Eosin 10b 1 contrast fluid to differentiate peroxidase positive round cells from peroxidase negative round cells.

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

Method
1) Preparation of work solution:
Add 30 µl of Hydrogen peroxide (reagent B) to 1 ml of benzidine (reagent A). This working solution remains stable for 1 day.
2) Mix 1 drop (10µl) of sperm with 1 drop (10 µl) of working solution, using the edge of the cover slip.
Mix thoroughly for at least 1 minute.
3) Cover with the cover slip approximately two minutes after initial mixing, avoiding 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.
4) Read the result after 2 minutes at a magnification of 400x (read at least 20 separate microscope fields).

Calculation of the concentration of white blood cells
Known concentration of spermatozoa. Count the number of WBC and the number of spermatozoa. Calculate the concentration of WBC based on this formula. (Number of WBC - number of sperm cells) x sperm concentration (mill/ml). This method will only work if the semen sample contains sperm cells (preferably more than 10 mill/ml).

Unknown concentration of spermatozoa
In this case the concentration of WBC can be determined by multiplying the number of WBC with a known factor based on the size of a microscope field and the height between the object glass and the cover glass (or the depth of the semen sample).
The diameter of the microscope field can be measured using a micrometer. The surface area in one field is equal to the square of the radius multiplied with pi ($s = \pi r^2$).
e.g. Diameter = 250 µm ⇨ radius = 125 µm ⇨ surface area = 49086µm^2

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The distance between the object glass and the cover glass can be calculated using the formula:
height (in µm) = volume (in µl) / (length x width of cover glass in mm) e.g. volume of the sample = 20 µl, coverglass = 24 x 40 mm
Height = 20 / (24 x 40) = 0.0208mm or 20.8µm
Knowing these figures a factor can be determined using this formula:
Factor = 1 000 000 / (surface area x height)
e.g. Factor = 1 000 000 µm3 / (49086 µm2 x 20.8 µm) = 0.98
This means that if 5 WBC's are counted in one microscope field, the corresponding concentration would be 4.9 mill/ml.

Result
Yellow to brown stained cells are peroxidase positive cells: neutrophil polymorphous leucocytes
Pink stained cells: all other cells

Limitations of the method
LeucoScreen only stains peroxidase positive white blood cells, other types of white blood cells (e.g. lymphocytes and monocytes) cannot be detected.

Reagent

Benzidine (WHO modified) solution A	50 ml
Hydrogen peroxide solution B	50 ml

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

References
WHO laboratory manual for the examination and processing of human semen, 5th edition, WHO, 2010