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REF **Art.-Nr. ZR10302020**

20 Applikations

IVD ***in vitro* Diagnostik**

Manual

(PLEASE READ CAREFULLY)

SemenLeu

(Semen leucocyte test)

Distribution:

GYNEMED

For professionel use only

Application

The determination of leukocytes in seminal fluid serves as a marker for the functioning of the accessory sex glands. Leukocytes, especially polymorphic polynuclear leucocytes (PML), are present in most human ejaculates. By normal microscopy these cells can be morphologically easily mixed up with multi-nuclear spermatids. It is known that peroxidases are histochemically exclusively characteristic for the PM granulocytes.

Principle of the Methode

By using hydrogen peroxide (H2O2) peroxidase-positive leukocytes (neutrophils polymorphic granulocytes) can be stained yellow to brown. Other cells (sperm, lymphocytes, monocytes, macrophages and multinucleated spermatids) remain unstained (peroxidase-negative). With this kit the seminal fluid is treated with the reagents 1 and 2 in which only peroxidase stainpositive cells remain brown. These cells can be identified with a phase contrast microscope.

Storage and Stability

2-8°C

24 months from date of manufacture. The work solution AB is usable after storage in the fridge until the next day.

Content

- Reagent 1 20 ml

- Reagent 2 1 ml

Necessary Utensils

- Coverslips (18 x 18 mm)
- Wet-chamber
- Gloves
- Contrasting phase microscope
- Native ejaculate or washed sperm (100 µl)
- Slides
- Paper towels
- Pipettes and tips (10-100 and 100-1000 µl)
- Test tube (2 ml)
- Test tube holder

Cell Counting chamber

Preparation of work solution AB:

Mix 1 ml of reagent 1 with 20 µl of reagent 2. In the case of studying more samples you have to calculate the appropriate amount of solution AB.

Procedure

1. Pipette 100 µl ejaculate in a test tube
2. Add 900 µl of solution AB
3. Mix gently solution AB and ejaculate (avoid foaming)
4. Incubating the mixture at room temperature 20-30 min
5. Repeat step 3
6. Pipette the mixture to a counting chamber. Put the counting chamber four minutes in a wet chamber to let sink all large cells.

Evaluation objective: number of leukocytes in ejaculate
By microscopic view leukocytes are colored yellow to brown by peroxidases. The total number of peroxidasepositive cells per ejaculate can be calculated in one of the following options:

Known concentration of spermatozoa:

Count the peroxidase-positive cells and spermatozoa in at least 20 fields of view at 400x magnification. The concentration of the white blood cell is calculated using the following formula: (Number of white blood cells / number of spermatozoa) x sperm concentration (million / ml)

(number of white blood cells / number of spermatozoa) x sperm concentration (Mio/ml)

This method is only suitable for samples which contain more than 10 million sperm cells/ml.

Unknown concentration of spermatozoa:

In this case, the concentration of white blood cells is determined by multiplication by a factor which results from the size of a field of view and the height of the distance between the counting chamber and the coverslip (or the depth of the semen sample). The diameter of a field of view can be measured by a micrometer. The surface area (s) corresponds to the square of the radius (r) multiplied by pi ($s = \pi r^2$).

Example: view field diameter = 250 µm, radius = 125 µm → area (s) = 49086 µm2 .

The height between the slide and the coverslip can be calculated with the following formula: height [µm] = volume [µl] / (length [µl] x width [mm] of the coverslip. Example: sample volume = 20 µl. Coverslip = 24 x 40 mm → height = 20/(24x40) = 0.0208 mm = 20.8 µm.

The factor by which the concentration of white blood cells has to be multiplied is calculated from these values: Factor = 1,000,000 µm3 (area x height).

Example: Factor = 1,000,000 µm³ / (49086 µm2 x 20.8 µm) = 0.98.

For example, if five white blood cells in a field of view are counted it results by this factor a concentration of 4.9 million white blood cells per ml of ejaculate. In fertile men the value of peroxidase-positive leukocytes is between 0.5x106 and 106 at a total leukocyte number (peroxidase-positive and peroxidasenegative cells) from 106 and 2x106 per ml of ejaculate [6].

Excessive presence of these cells (sperm-induced leukocytosis) can display a seed head infection. The sperm-induced leukocytosis can also be associated with a disturbance of the seed profile, including the reduction of semen volume, sperm concentration and sperm motility and a loss of sperm function as a result of oxidative stress [1, 2] or the secretion of cytotoxic cytokines.. It is therefore difficult to give an exact limit of the leukocyte concentration at which fertility is impaired. The influence of these cells depends on the place in the reproduction channel from where the leukocytes enter the sperm, the type of leukocytes and the degree of activation. If the seminal fluid contains more than 1x106 white cells per ml, the samples to be tested microbiologically for gland infection.

Note: The absence of leukocytes does not exclude the possibility of glandular infection.

Safety information / precautions

(Please read also the safety data sheets)

- All semen samples should be considered potentially infectious. Handle with all samples like HIV or hepatitis infected material.
- When working with samples and reagents wear always protective clothing (gloves, gowns, eye / face protection).
- Reagent 1 contains ortho-toluidine, which is classified as carcinogenic. Skin contact or ingestion should be avoided.
  Reagent 2 contains hydrogen peroxide (H_2O_2). It is corrosive and toxic by inhalation. Skin contact or ingestion should be avoided.

  In case of an accident with reagent 1 and/or 2 contaminated clothing should take off immediately and consult a doctor.

References

1. **Aitken RJ**, West KM (1990) Analysis of the relationship between reactive oxygen species production and leucocyte infiltration in fractions of human semen separated on Percoll gradients. International, Journal of Andrology, 13:433-451
2. **Aitken RJ et al. (1989)** Generation of reactive oxygen species, lipid peroxidation and human sperm function. Biology of Reproduction, 41:183- 187
3. **Barratt CLR et al. (1990)** Functional significance of white blood cells in the male and female reproductive tract. Human Reproduction, 5:639-644
4. **Hill JA et al. (1987)** Effects of soluble products of activated lymphocytes and macrophages (lymphokines and monokines) on human sperm motion parameters. Fertility and Sterility, 47:460- 465
5. **Politch JA et al (1993)** Comparison of methods to enumerate white blood cells in semen. Fertility and Sterility, 60: 372-375
6. **WHO Press (2010)** laboratory manual for the examination and processing of human semen.
7. **Wolff, H, Anderson, DJ (1988)** Immunohistological characterization and quantification of leukocyte subpopulation in human semen. Fertility and Sterility, 53:528-536

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consult instructions for use
IVD *in vitro* diagnostics
Temperature limitation

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