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Leukocytes

detection of leukocytes
by using peroxidase test

REF SP/SFT/L-006

IVD



User Manual



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Turnaround time for test: 30min



Store at: 2°C - 8°C after receiving

Concept

All semen samples contain **round cells** & it is important to know whether these are **leukocytes** (white blood cells) or **germinal cells**.

They are **normally present** in seminal fluid but their numbers should be limited. In a normal ejaculate, there should be **no more than 5 million germinal cells/mL** & there should be no more than **1 million white blood cells/mL**. It is almost **impossible** to tell the **difference** between these two types of cells **without staining** the preparation.

Leukocytes in semen **produce** oxidative stress (the generation of reactive oxygen species - **ROS**) & cytotoxic cytokines secretions (lymphokines & monokines). These products may interfere with sperm progression by affecting motility, morphology & DNA integrity.

Peroxidase positive granulocytes are **predominant** form of leukocytes (WBC) in semen. **Elevated** concentration of WBC & detection of peroxidase positive WBC is an important **indicator of acute infection & inflammation** which produces ROS.

- Semen sample is collected with :
 - **Abstinence period** of **2-7days**.
 - **Ideal collection** through **masturbation** in sterile container.
 - **Non-spermicidal polyurethane semen collection pouch (Sperm Collect™)** can be used when required.
- Semen sample is allowed to liquefy and then well mixed for performing test.

Special Instructions :

- **Hyperviscous** semen sample should be processed to bring towards normal viscosity. (**Viscosity-CH™** or **Viscosity-BR™** kit can be used)

- Reagent - I : 50 mL
- Reagent - II : 04 mL

Content Box Diagram :



Storage Conditions :

- The kit should be stored in dark at 2°C - 8°C after receiving.
- Bring all the reagents to room temperature before use.
- Once opened, store reagents in the fridge protected from light.
- Expiry date is printed on the out side of the box.

REQUIRED BUT NOT PROVIDED IN KIT

- Microscope
- Controlled Temperature 37°C Dry bath (Sperm Warmer™ / Water bath)
- Centrifuge Machine (Androspin™)
- Slide Warmer™
- Semen Analysis Chamber (Sperm Meter™)
- Pipettes Set
- Stopwatch
- Microtip Box

REQUIRED BUT NOT PROVIDED IN KIT

- Hand gloves
- Semen Collection Container
- Non-spermicidal Semen Collection Pouch (Sperm Collect™)
- Microtips
- Pasteur Pipettes
- Test Tubes
- Micro Tubes / Storage Vials
- Glass Slides
- Coverslips
- Filter Papers

Step 1 : Label plastic ware & disposable material with appropriate **Patient ID & Sample ID**.

Step 2 :
Measure the **volume** of semen (**approx**) in **mL**.

Step 3 :
Take 0.9 mL Reagent - I in micro tube.



Step 4 :
Add 0.1 mL of well mixed & liquefied semen to Step 2 micro tube.

Step 5 :
Mix well the micro tube contents.

Step 6 :
Add a drop of **Reagent - II** to **step 3** micro tube.
Shake the micro tube for **1-2 min**.

Step 7 :
Place the **micro tube** at room temperature for **20 - 30 min**.

Quick Glance

* **Measure** the **volume** of semen (approx) in **mL** (upto one decimal).

0.9 mL Reagent - I

Add 100 μ L of liquefied semen & mix well

Add drop of Reagent - II & mix well

Put micro tube at room temperature for **20 - 30 min.**

Take 10 μ L from this tube to **10 micron** depth chamber (Sperm Meter™)



Examine
using **20x** objective lens
(Preferably Phase Contrast)

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Examination

- Take **10 μ L** sample from **step 5** on Semen Analysis Chamber (Sperm Meter™) or 10 micron depth chamber & put cover glass.
- **Examine** under **20x** objective lens preferably phase contrast microscope by adjusting around **10 squares** in the given field of focus.
- **Count** : - Number of WBC in **10 squares**.
- Number of **brown colored WBC (Peroxidase Positive)** in **10 squares**.

NOTE : One may use Slide Coverslip method with 40x objective lens (preferably phase contrast microscope).

- Semen **Volume** : __ **mL**
- White Blood Cells : __ **millions/mL**
- White Blood Cells : __ **millions/ejaculate**
- **Peroxidase Positive**
White Blood Cells : __ **millions/mL**
- **Peroxidase Positive**
White Blood Cells : __ **millions/ejaculate**

• **Normal Reference Range :**

- Normal** : < 1 million/mL
Equivocal : ≥ 1 million/mL & ≤ 2 million/mL
Abnormal : > 2 million/mL

(As per Fifth edition of WHO laboratory manual for examination and processing of Human Semen)

Limitations :

- This test provides presumptive quantitative information of sperm.
- This parameter should be analyzed by a specialist.
- The result should be evaluated taking into account all clinical & laboratory findings related to the same sample.

- All patient samples & reagents should be treated as potentially infectious & the user must wear protective gloves, eye protection & laboratory coats when performing the test.
- The kit should be discarded in a proper biohazard container after testing.
- Do not eat, drink or smoke in the area where specimens & kit reagents are handled.
- Do not use beyond the expiration date which appears on the package label.
- It is recommended to use of gloves & face mask.

- Do not release the products used into the environment. Follow centre guidelines for the storage & disposal of toxic substances.
- Biological samples must be handled as potentially infectious.

Description of Symbols



consult instructions of use



product reference



lot number



use by



manufacturer



health surveillance device
for in-vitro diagnostic



contains sufficient for 'n' tests



temperature limitation



keep dry



CE mark (Conformité Européene)

Accreditations & Registered Certificates

- **ISO 13485 : 2003** Certified
- **CE** Certified
- **GMDN** Registered
- **US FDA** Registered

For more information & procedure videos

 www.spermprocessor.com/sft-leukocytes.html



www.youtube.com/watch?v=xvSiDMAUq9I

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