Leukocytes

detection of leukocytes by using peroxidase test

REF SP/SFT/L-006
a product

Sperm 360

Turnaround time for test: 30min
Store at: 2°C - 8°C after receiving

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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.
Specimen Preparation

- Semen sample is collected with:
  - Abstinence period of 2-7 days.
  - 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)

Kit Contents

- 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 outside of the box.
**Equipments**

**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

**Disposable Materials**

**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

**Procedure**

**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 µL of liquefied semen & mix well

Add drop of Regent - II & mix well

Put micro tube at room temperature for 20 – 30 min.

Take 10 µL from this tube to 10 micron depth chamber (Sperm Meter™)

Examine using 20x objective lens (Preferably Phase Contrast)

Examination

- Take 10 µL 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).
### 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.

### Result

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Semen Volume</strong></td>
<td>___ mL</td>
</tr>
<tr>
<td>White Blood Cells</td>
<td>___ millions/mL</td>
</tr>
<tr>
<td>White Blood Cells</td>
<td>___ millions/ejaculate</td>
</tr>
<tr>
<td><strong>Peroxidase Positive</strong></td>
<td></td>
</tr>
<tr>
<td>White Blood Cells</td>
<td>___ millions/mL</td>
</tr>
<tr>
<td><strong>Peroxidase Positive</strong></td>
<td></td>
</tr>
<tr>
<td>White Blood Cells</td>
<td>___ millions/ejaculate</td>
</tr>
</tbody>
</table>

**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)

### Precautions

- 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.

### Safety & Environment

- Do not release the products used into the environment. Follow centre guidelines for the storage & disposable 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.youtube.com/watch?v=xvSiDMaUq9I](http://www.youtube.com/watch?v=xvSiDMaUq9I)

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