



Specimen Preparation :

- Ideally semen sample, having abstinence period of 2-7 days, must be collected by masturbation in a sterile container.
- Always use liquefied, normal viscous, well mixed semen sample to perform the test.

Special Instructions :

- ☛ One can use a non-spermicidal polyurethane semen collection pouch (Sperm Collect) if needed.
- ☛ **Hyper-viscous** semen sample should be processed to obtain normal viscous semen sample (Use 'Viscosity' kit) before proceeding for the test.
- ☛ Severe **oligospermic** semen sample (i.e. sample with Sperm Concentration less than 5 millions / ml) should be processed to concentrate the sperm concentration to around 8-10 millions / ml before performing the test.
- ☛ Frozen semen must be thawed at 37°C with Controlled Temperature 37°C Dry bath (Sperm Warmer / Water bath) before test procedure.

Equipments, Disposable Materials & Reagents not provided with the kit :

➤ Equipments :

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| • Microscope | • Semen Analysis Chamber (Sperm Meter) |
| • Controlled Temperature 37°C Dry bath (Sperm Warmer / Water bath) | • Microtip Box |
| • Set Of Pipettes | • Staining Tray |
| • Centrifuge Machine (Androspin) | • Glass Slide Stand |
| • Slide-warmer | • Glass Slide Tray |
| • Stop-watch (Andro Watch) | • Coupling Jar |

➤ Disposable Materials :

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| • Hand-gloves | • Test-tubes |
| • Semen Collection Container | • Glass-slides |
| • Non-spermicidal Semen Collection Pouch (Sperm Collect) | • Coverslips |
| • Microtips | • Filter Papers |
| • Pasture Pipettes | |

➤ Reagents :

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| • Oil Immersion | • Xylene (Neoclear) | • Mounting Solution |
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Kit Reagents :

- Harris's Haematoxylin : Spray x 1
- Ethanol (95%) : Spray x 1
- Ethanol (70%) : Spray x 1
- Ethanol (50%) : Spray x 1
- Acid Ethanol : Spray x 1
- OG-6 Stain : Spray x 1
- EA-50 Stain : Spray x 1

Procedure :

- ☞ **Step 1 :** Label Plastic ware & Disposable material with appropriate Patient ID & Sample ID.
- ☞ **Step 2 :** Neat Semen Smear :
 - If Sperm Concentration is greater than 20 millions / ml, place 5 µls of liquefied semen sample on glass slide. Form the smear.
 - If Sperm Concentration is less than 20 millions / ml, place 10 µls of liquefied semen sample on glass slide. Form the smear.
- Processed Semen Smear :
 - Take a 100µl of liquefied semen.
 - Add 200µl of semen washing medium.
 - Mix well, centrifuge at 2000rpm for 2 – 3 minutes.
 - Discard the supernatant.
 - Use the pellet (5-10µl) for preparation of the smear.
- ☞ **Step 3 :** Allow the smear to air dry
- ☞ **Step 4 :** Lay the air dried smear in **Ethanol (95%) fixative solution**.
Keep it for minimum of **15 minutes**.
- ☞ **Step 5 :** Drain off the fixative solution allow the smear to air-dry .
- ☞ **Step 6 :** Lay the smear horizontally and cover the entire smear with **Ethanol (70%) spray**.
Keep it for **30 seconds**.
- ☞ **Step 7 :** Cover the entire smear with **Ethanol (50%) spray**. Keep it for **30 seconds**.
- ☞ **Step 8 :** Keep the smear in distilled water for **30 seconds**. Drain off the water.
- ☞ **Step 9 :** Lay the smear horizontally and cover the entire smear with 1 ml of **Harris's Haematoxyline**. Keep it for **4 minutes**. Drain off the stain.
- ☞ **Step 10 :** Keep the smear in distilled water for **30 seconds**. Drain off the water.
- ☞ **Step 11 :** Lay the smear horizontally and cover the entire smear with **Acid Ethanol Spray**.
Keep it for **10 seconds**.
- ☞ **Step 12 :** Hold the smear in the running tap water for 5 minutes. Drain off the water.
- ☞ **Step 13 :** Lay the smear horizontally and cover the entire smear with **Ethanol (50%)**.
Keep it for **30 seconds**.
- ☞ **Step 14 :** Lay the smear horizontally and cover the entire smear with **Ethanol (70%)**.
Keep it for **30 seconds**.
- ☞ **Step 15 :** Lay the smear horizontally and cover the entire smear with **Ethanol (95%)**.
Keep it for **30 seconds**.
- ☞ **Step 16 :** Lay the smear horizontally and cover the entire smear with **OG-6 Spray**.
Keep it for **1 minute**.



- ☞ **Step 17 :** Lay the smear horizontally and cover the entire smear with **Ethanol (95%)**. Keep it for **90 seconds**.
- ☞ **Step 18 :** Lay the smear horizontally and cover the entire smear with **EA-50 Spray**. Keep it for **1 minute**.
- ☞ **Step 19 :** Lay the smear horizontally and cover the entire smear with **Ethanol (95%)**. Keep it for **90 seconds**.
- ☞ **Step 20 :** Drain off the solution & allow the smear to air-dry (use slide warmer).

Examination :

- Put a drop of oil immersion on the dry smear
- Examine the smear under the microscope with the help of 100X lens.
- Examine at least 200 sperm and count the followings as per Kruger's strict criteria / WHO criteria :
 - Normal Sperm
 - Abnormal sperm
- In abnormal sperm, detailed abnormality (defects) pertaining to head, mid piece and tail should be noted down.

Results' Interpretation:

- Calculate the percentage of normal and abnormal sperm
- Calculate total number of head, mid-piece and tail defects of the abnormal sperm.
- Calculate Sperm deformity index and teratozoospermic index.
 - ☞ Sperm Deformity Index = Total No. Of Defects / No. Of Sperm Evaluated
 - ☞ Teratozoospermic Index = Total No. Of Defects / Total No. Of Abnormal Sperms
- Normal reference value / range : For Normal Sperm **4%** (3% - 4%)
(As per fifth edition of WHO laboratory manual for examination and processing of human semen).

Coverslipping Stained Slides (Permanent Stained Slide) :

- Dip dried stained - slide into Xylene (Neoclear) solution just prior to coverslipping.
- Place the mounting media on the slide.
- Place the coverslip on to the slide as quickly as possible to avoid air-drying & air bubbles.

Safety & Environment Precautions :

- Store the kit in appropriate conditions as per the instructions given on the package.
- Do not use the kits beyond expiry date given on the package.
- All the reagents are potentially hazardous and it is strictly advised to follow the MSDS instructions.
- All the patients' samples should be treated as potentially infectious. Laboratory personnel are advised to wear protective gloves, face masks, laboratory coats etc while performing the tests.
- Dispose off all the waste generated, during and after the tests, in an appropriate manner as per the governing compliance laws of the land.

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