Morphology-D
evaluation of sperm morphology
by Diff-Quik Staining method

REF SP/SFT/MP-005-A

www.spermprocessor.com

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Turnaround time for test: 1min

Store at: 2°C - 8°C after receiving
Sperms in human ejaculate exhibit considerable morphological variations in size & shape of Head & Acrosome. Also changes are seen in degree of nuclear vacuolations, size of persisting cytoplasmic droplets, mid-piece disturbances & tail abnormalities.

Sperm is considered normal when Head, Mid-piece, Neck & principal piece (Tail) are normal. Normal head has smooth oval configuration with well defined acrosome comprising of 40 - 70% of head. The cytoplasmic droplet, if present, should not be more than one half the size of sperm head. The mid-piece should be slender, less than 1µm in width, about one & a half times the length of the head, & attached axially to the head. The principle piece (tail) should be straight, uniform, thinner than mid-piece, uncoiled & approximately 45 µm long.

In order to achieve accurate sperm morphological evaluation, following principles & steps must be followed:

• Correct preparation of slides & smears.
• Correct fixation
• Correct staining of the smear
• Appropriate magnification used
• Number of fields used to evaluate the sperm & number of sperm evaluated.
Specimen Preparation

- Semen sample is collected with:
  - **Abostinence 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)*

- 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 Sperm Warmer™) before performing test.

Kit Contents

- **Fixative Solution (F1)** : 100 mL
- **Stain - I Solution (S1)** : 100 mL
- **Stain - II Solution (S2)** : 100 mL
- **Washing Medium (WM)** : 60 mL

**Other Reagents :** Required But Not Provided In Kit:

- Distilled Water
- Xylene (Neoclear)
- Mounting Solution
- Immersion Oil

**Kit Content Layout Diagram :**

```
  Fixative Solution 100 ml
     F1
  Washing Medium 60 ml
     WM
  Stain - I Solution 100 ml
     S1
  Stain - II Solution 100 ml
     S2
```
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.

3 Equipments

REQUIRED BUT NOT PROVIDED IN KIT

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

4 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
- Glass Slides
- Coverslips
- Filter Papers
A. Labeling:

Label plastic ware & disposable materials with appropriate Patient ID & Sample ID.

B. Semen Smear Preparation:

• Evaluate the sperm concentration of given semen sample and note it down.

• Neat Semen Smear
  - If Sperm Concentration is greater than 40 - 60 millions/mL, place 5 µL of liquefied semen sample on glass slide. Form the smear.
  - If Sperm Concentration is less than 40 millions/mL, place 10 µL of liquefied semen sample on glass slide. Form the smear.

C. Processed Semen Smear:

• Take 100 µL of liquefied semen.

• Add 400 µL of washing medium.

• Mix well & centrifuge at 2000 rpm for 2 - 3 minutes.

• Discard supernatant.

• Adjust the sperm concentration between 40 - 60 millions/mL with washing medium & use 5 µL to prepare the smear.

• If adjusted concentration is less than 40 millions/mL, use 10 µL to prepare the smear.
D. Test Procedure:

**Step 1:** Allow smear to air dry (use Slide Warmer™)

**Step 2:** Lay smear horizontally & cover the entire smear with **1 mL** of **Fixative** solution. Keep it for **15** seconds.

**Step 3:** Drain off the **fixative** solution & allow smear to air-dry.

**Step 4:** Lay smear horizontally & cover the entire smear with **1 mL** of **Stain - I** solution. Keep it for **12** seconds.

**Step 5:** Drain off **Stain - I** solution

**Step 6:** Lay smear horizontally & cover the entire smear with **1 mL** of **Stain - II** solution. Keep it for **8** seconds.

**Step 7:** Drain off **Stain - II** solution

**Step 8:** Rinse smear in **DW**

**Step 9:** Drain off **DW** & clean the back of the slide with a filter paper.

**Step 10:** Allow smear to air-dry (Use Slide-warmer).

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**Quick Glance**

Liquefied Semen Sample Or Process Semen Smear

1. 5µL / 10µL - Prepare Smear & dry
2. 1 mL Fixative for 15 sec
3. Drain off Fixative & air dry
4. 1 mL Stain - I for 12 sec
5. Drain off Stain - I
6. 1 mL Stain - II for 8 sec
7. Drain off Stain - II
8. Rinse smear in Distilled Water
9. Drain off Distilled Water & Dry the smear

Examine under **100x** (objective lens)
Morphology of Normal Sperm

• **Head**
  - Smooth oval configuration

• **Dimensions**
  - Length: 5.0µm - 6.0µm
  - Width: 2.5µm - 3.5µm

• **Acrosome**
  - Acrosome should comprise of 40 - 70% of the anterior sperm head.

• **Borderline Forms**
  - Consider all borderline forms as abnormal.

• **Neck / mid-piece**
  - No abaxial implantations - Slender approximately 1 µm in width & 6 - 7 µm in length.
  - Cytoplasmic droplets >30% of head size are considered abnormal.

• **Tail**
  - Uniform, slightly thinner than the mid-piece, uncoiled with principal piece 45-50µm & terminal segment of 4-6µm.

Normal Sperm:

- If sperm dimensions for Head, Neck & Tail are within normal limits, then it is called as **Normal Sperm**.

Abnormal Sperm:

- When any one of the parameters of sperm for Head, Neck or Tail is not within normal limits, then it is called as **Abnormal Sperm** (Strict Criteria).
Examination

• Put a drop of immersion oil on dry smear.
• Examine the smear under microscope with the help of 100x lens.
• Examine at least 200 sperm and evaluate 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.

Calculations

• Calculate the percentage of normal & abnormal sperm.
• Calculate total number of head, mid-piece & tail defects of the abnormal sperm.

Normal reference value / range:
For Normal Sperm 4% (3% - 4%)

• 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
(As per fifth edition of WHO laboratory manual for examination and processing of Human Semen).

Schematic Representation:

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

Reference Image:
Sperm Evaluation

- No. of Sperm Evaluated : ________
- Normal Sperm : ________
- Abnormal Sperm : ________

Total Sperm Defects

- Head Defects : ________
- Neck & Mid-piece Defects : ________
- Principal Piece (Tail) Defects : ________

Sperm Deformity Index : ________
Teratozoospermic Index : ________

Reference Value For Normal Sperm :
  - Normal : > 4%
  - Equivocal : > 3% & ≤ 4%
  - Abnormal : < 3%

Limitations :

- This test provides presumptive quantitative information of sperm Morphology.
- 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 interpretation is supported with -

CASA with Auto & innovative Expert Mode

Individual test module - Sperm Soft : Morph / Vitality is also available.
### Head Defects

<table>
<thead>
<tr>
<th>Head Size</th>
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<tbody>
<tr>
<td>Large Heads (LH)</td>
<td></td>
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<tr>
<td>Small Head (SH)</td>
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<tr>
<td>Normal Head (NH)</td>
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<table>
<thead>
<tr>
<th>Head Shape</th>
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<tbody>
<tr>
<td>Tapered Heads (TP)</td>
<td></td>
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<tr>
<td>Elongated Heads</td>
<td></td>
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<tr>
<td>Amorphous Heads (AH)</td>
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<tr>
<td>Round Head</td>
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<tr>
<td>Normal Head (NH)</td>
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<table>
<thead>
<tr>
<th>Acrosomal Area</th>
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<tbody>
<tr>
<td>Small Acrosomal Area</td>
<td></td>
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<tr>
<td>Large Acrosomal Area</td>
<td></td>
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<tr>
<td>Acrosomal Area Absent</td>
<td></td>
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<tr>
<td>Normal Acrosomal Area</td>
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<table>
<thead>
<tr>
<th>Vacuoles</th>
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<tbody>
<tr>
<td>Vacuolated Head (VH)</td>
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<table>
<thead>
<tr>
<th>Duplicate Head (DH)</th>
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<td>Duplicate Head (DH)</td>
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### Neck & Mid-piece Defects

<table>
<thead>
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<th>Insertion</th>
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<tbody>
<tr>
<td>Bent</td>
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<tr>
<td>Asymmetrical</td>
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<table>
<thead>
<tr>
<th>Size</th>
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<tbody>
<tr>
<td>Thickened</td>
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<tr>
<td>Thin</td>
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<tr>
<td>Normal</td>
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### Principal Piece (Tail) Defects

<table>
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<th>Insertion</th>
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<td>Bent</td>
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<td>Normal</td>
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<table>
<thead>
<tr>
<th>Size</th>
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<tr>
<td>Bent</td>
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<tr>
<td>Short</td>
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<tr>
<td>Coiled</td>
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<tr>
<td>Irregular Tails</td>
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<tr>
<td>Normal</td>
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<table>
<thead>
<tr>
<th>Principal Piece (Tail Defects)</th>
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<tbody>
<tr>
<td>Loose Heads (Only Tails)</td>
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*As per fifth edition of WHO laboratory manual for examination and processing of human semen.*
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.

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

🔗 http://www.spermprocessor.com/sft-morphology.html

🔗 www.youtube.com/watch?v=YFLSHZE31N8