Sperm 360°

Sperm 360° ™

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Morphology-D

evaluation of sperm morphology
by Diff-Quik Staining method

REF SP/SFT/MP-005-A

IVD

User Manual

a

Sperm 360

product

Turnaround time for test: 1min

Store at: 2°C - 8°C after receiving

Index

Morphology-D

CONCEPT

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.

1 Specimen Preparation

- 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)

- Severe **oligospermic** semen sample (i.e. sample with Sperm Concentration less than 5millions/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.

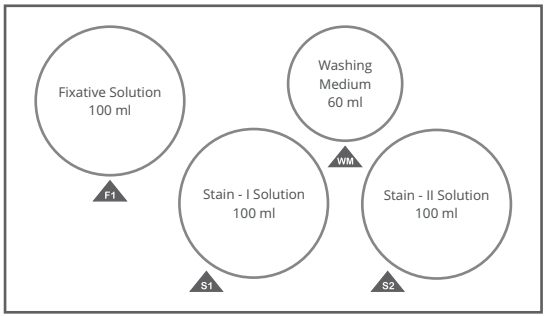
2 Kit Contents

- 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 :



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

5 Procedure

A. Labeling :

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

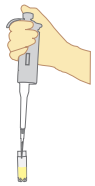
B. Semen Smear Preparation :

- **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.

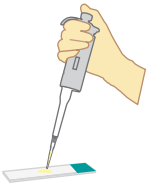
  - If Sperm Concentration is **less than 40 millions/mL**, place **10 µL** of liquefied semen sample on glass slide. Form the smear.

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).

Quick Glance

Liquefied Semen Sample Or Process Semen Smear

↓

5µL / 10µL – Prepare Smear & dry

↓

1 mL Fixative for 15 sec

↓

Drain off Fixative & air dry

↓

1 mL Stain - I for 12 sec

↓

Drain off Stain - I

↓

1 mL Stain - II for 8 sec

↓

Drain off Stain - II

↓

Rinse smear in Distilled Water

↓

Drain off Distilled Water & Dry the smear

Examine

under **100x** (objective lens)

6 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)**.

STRICT CRITERIA

Abnormal Sperm : Defect Details

• **Head :**		
	Size	Micro
		Macro
	Shape	Tapered
		Pyriform
		Round
		Amorphous
	Vacuole	Present
	Acrosome	Absence of acrosome
		Small sized
		Large sized
• **Neck :**		
	Insertion	Bent insertions
		Asymmetrical
	Size	Thin neck
		Thick neck
• **Tail :**		
	Insertion	Bent
	Length size	Short
		Coiled
	No. of tails	More than one

7 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%)

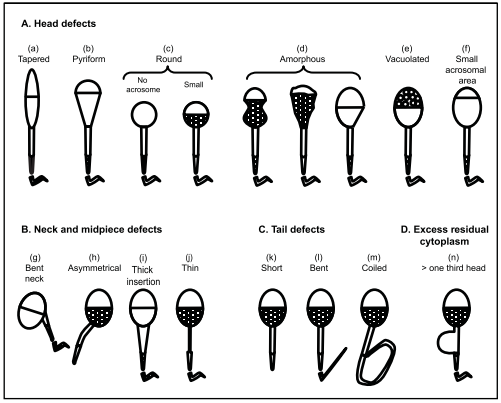
- Calculate sperm deformity index and teratozoospermic index.

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).

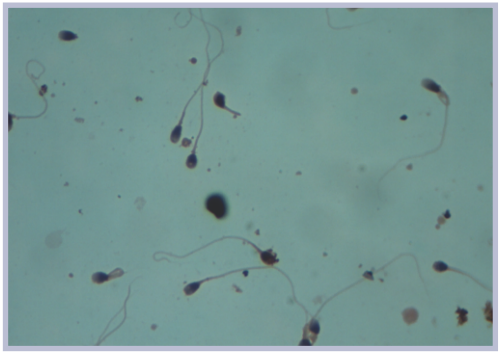
Schematic Representation :



Adapted from Kruger et al., 1993 and reproduced by permission of MQ Medical.

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

Reference Image :



- Result interpretation is supported with -

  Sperm Soft CASA with **Auto** & innovative **Expert Mode**

- Individual test module - **Sperm Soft : Morph / Vitality** is also available.

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.

Head Defects

- **Head Size**
  - Large Heads (LH) : _____
  - Small Head (SH) : _____
  - Normal Head (NH) : _____
- **Head Shape**
  - Tapered Heads (TP) : _____
  - Elongated Heads : _____
  - Amorphous Heads (AH) : _____
  - Round Head : _____
  - Normal Head (NH) : _____
- **Acrosomal Area**
  - Small Acrosomal Area : _____
  - Large Acrosomal Area : _____
  - Acrosomal Area Absent : _____
  - Normal Acrosomal Area : _____
- **Vacuoles**
  - Vacuolated Head (VH) : _____
- **Duplicate Head (DH)**
  - Duplicate Head (DH) : _____

Neck & Mid-piece Defects

- **Insertion**
  - Bent : _____
  - Asymmetrical : _____
  - Normal : _____
- **Size**
  - Thickened : _____
  - Thin : _____
  - Normal : _____

Principal Piece (Tail) Defects

- **Insertion**
  - Bent : _____
  - Normal : _____
- **Size**
  - Bent : _____
  - Short : _____
  - Coiled : _____
  - Irregular Tails : _____
  - Normal : _____
- **Principal Piece (Tail Defects)**
  - Loose Heads (Only Tails) : _____

(As per **fifth edition** of **WHO** laboratory manual for examination and processing of **human semen**).

10 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.

11 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.

12 Safety & Environment

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

Description of Symbols

	consult instructions of use
REF	product reference
LOT	lot number
	use by
	manufacturer
IVD	health surveillance device for in-vitro diagnostic
	contains sufficient for 'n' tests
	temperature limitation
	keep dry
CE	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

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