Sperm 360°

Sperm 360° ™

www.spermprocessor.com

Sperm Processor Pvt. Ltd.
6, Welcome Nagar, Garkheda,
Aurangabad (MS) - 431005, India.
Ph.: +91 240 6603800
Fax: +91 240 2341694
Email: info@spermprocessor.com

DNA Fragmentation

sperm chromatin
dispersion test

REF SP/SFT/DNA-009

IVD

User Manual

a

Sperm 360

product

Turnaround time for test: 90min

Store at: 2°C - 8°C after receiving

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CONCEPT

The single goal of the spermatozoon is to deliver the paternal counterpart of genetic material to the oocyte.

When one refers to the sperm as a whole functional cell, it is not just for its role as a carrier but for its contents as well.

DNA damage in human sperm is associated with impaired conception, disrupted embryonic development, increased rate of miscarriage & morbidity in the offspring.
Damage may involve single stranded breaks of nicks, double stranded breaks or fragments, deletion/addition & base modification.
The cause of DNA damage seen in human sperm is not yet precisely understood.
Three main theories have been proposed as :
Defective sperm chromatin packaging (Immature Sperm Chromatin), **Apoptosis** (Cell Death Physiological / Programmed) & **Oxidative Stress**.
High levels of Sperm DNA Fragmentation are positively correlated with lower fertilization rate, impaired implantation rate & an increased incidence of abortion.

It is expected that the tests that access sperm quality should identify not only the ability of sperm to reach the oocyte with an intact DNA molecule but also their ability to fertilize the oocyte & activate embryo growth.
Thus the inclusion of an assessment of DNA damage is more comprehensive for semen quality.

Importance of DNA Fragmentation :

- For couples planning to undergo IVF or ICSI, these tests evaluate the impact of sperm DNA damage on reproductive out comes (Fertilization, Embryo Development , Pregnancy, Miscarriage, Post-natal Development).
- Unexplained Infertility : Based on evidence, test of sperm DNA damage may be predictors of failed natural pregnancy.
- Recurrent pregnancy loss: Based on evidence, high levels of DNA damage are associated with recurrent pregnancy loss.
- IUI Failures: Based on evidence, high level of DNA damage are associated with Repeated IUI Failures. These couples have to be advise for ICSI.
- Couples with a history of spontaneous miscarriages
- Selection of the best donor
- Selection of the best seminal sample prior to vasectomy or oncology treatment or varicose repair
- Men over 40 years old; smokers; those exposed to toxics & pollutants
- Men treated for cancer; on certain prescription medications
- Men who have had repeated urogenital infections
- Men with infectious disease, fever & varicocele indicators
- Poor embryo quality on second egg donation cycles
- Idiopatic male factor

DNA Test Procedure

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.

- Ideally test is to be performed within 30 to 60 min of collection.

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 obtain sperm concentration around 8-10 millions/mL before performing the test.

- **Frozen semen** must be thawed at 37^{0}C (with Sperm Warmer™) before performing test.

2 Kit Contents

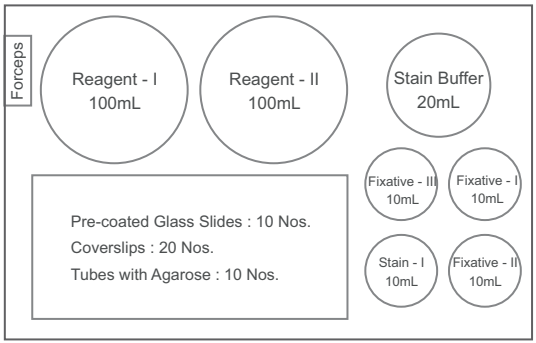
•Pre-coated Glass Slides : 10 Nos.

- Coverslips : 20 Nos.
- Tubes with Agarose : 10 Nos.
- Reagent - I : 100 ml
- Reagent - II : 100 ml
- Fixative - I : 10 ml
- Fixative - II : 10 ml
- Fixative - III : 10 ml
- Stain - I : 10 ml
- Stain Buffer : 20 ml

Other Reagents (Required But Not Provided In Kit) :

- Distilled Water
- Xylene (Neoclear)
- Mounting Solution

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)
- DNA Warmer™ (70°C & 37°C)
- DNA Slide Immersion Tray
- Black Tray
- Staining Tray
- Set Of Pipettes
- Centrifuge Machine (Androspin™)
- Slide Warmer™
- Stopwatch
- Semen Analysis Chamber (Sperm Meter™)
- Microtip Box
- Glass Slide Stand
- Glass Slide Tray

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

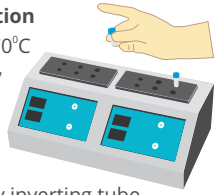
Step 1: Label Plastic ware & Disposable materials with appropriate Patient ID & Sample ID.

Step 2: Bring all the reagents at room temperature.

Step 3: Semen Sample Preparation

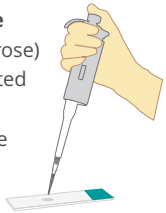
- Calculate sperm concentration (millions / mL) of given semen sample.
- Dilute / concentrate the semen sample to achieve sperm concentration as **10-15** millions / mL, with the help of normal saline or sperm washing medium.

Step 4: Agarose Tubes Preparation



- Keep agarose tube at 70°C for **15-20** min to liquify agarose completely. (Use **DNA Warmer™** / Water Bath of 70°C).
- Confirm liquification by inverting tube.
- Transfer agarose tube from 70°C to 37°C.
- Keep for **5** min.
- Add **60 µL** of prepared semen from **step 3**.
- Mix well & maintain at 37°C.

Step 5 : Preparation of precoated slide



- Put slide (precoated with agarose) horizontally with agarose coated side facing upwards.
- Put **15 - 20 µL drop** of agarose semen sample from **step 4** on slide.

> **Note 1 :** The technician has to decide whether to put 1 drop or 2 separate drops of agarose semen.

- Gently put coverslip over drop to spread it completely (Avoid formation of air-bubbles).

> **Note 2 :** Coverslip is placed in manner that both of its outer edges protrude outside the width edge of the glass slide.

> **Note 3 :**
> - Thus we recommend the size of the coverslip as 24 X 32 mm.
> - Ideally the width of the glass slide to be used is 25 mm.

- Keep slide on precooled (5 mins) Tray at 2 - 8^{0}C for 5 mins.
- Take slide out and remove coverslip gently without disturbing smear.

Step 6 : Treatment with Reagent – I

- Put slide horizontally from **step 5** in staining tray. (**Use DNA Slide Immersion Tray**).
- Pour **8 - 10** mL of Reagent-I such that slide is completely dipped in reagent.
- Allow reagent for **7** mins.

- Lift slide from immersion tray.

- Clean back side of slide with filter paper.

Step 7 : Treatment with Reagent – II

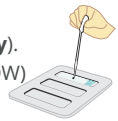
- Put slide horizontally from **step 6** in staining tray (**Use DNA Slide Immersion Tray**).
- Pour **8 - 10** mL of Reagent-II such that, slide is completely dipped in reagent.
- Allow reagent for **20** mins.

- Lift slide from immersion tray.

- Clean back side slide with filter paper.

Step 8 : Treatment with Distilled Water

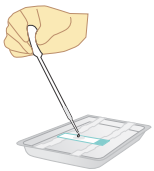
- Put slide horizontally from **step 7** in staining tray. (**Use DNA Slide Immersion Tray**).
- Pour **8 - 10** mL Distilled Water (DW) such that slide is completely dipped.
- Allow DW for **5** min.



- Lift slide from immersion tray.
- Clean the back side of slide with filter paper.

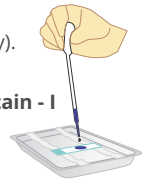
Step 9 : Fixation of smear

- Put slide horizontally from **step 8** on staining tray (Use Glass Slide Staining Tray).
- Pour **1** mL of **Fixative - I** covering entire smear.
  - Allow for **2** min.
  - Drain off by tilting slide.
- Pour **1** mL of **Fixative - II** covering entire smear.
  - Allow for **2** min.
  - Drain off by tilting slide.
- Pour **1** mL of **Fixative - III** covering entire smear.
  - Allow for **2** min.
  - Drain off by tilting slide.



Step 10 : Wright Staining of Fixed smear

- Put fixed smear of **step 9** horizontally on staining tray (Use Glass Slide Staining Tray).
- Pour **0.5** mL (6-7 drops) of **Stain - I** covering entire smear. Wait for **1 - 2** minutes.



- Pour **1** mL (14-15 drops) of **Stain Buffer** covering entire smear. Wait for **10 - 15** minutes.
- Drain off solution by tilting.

- Clean back side of slide with filter paper.

- Rinse slide with distilled water.

- Allow slide to air-dry (Use the Slide Warmer™).

Quick Glance

1 Semen Sample Preparation

Sperm concentration
15 – 20 million / µL

2 Agarose Tube Preparation

Incubate at 70˚c for 15 min

↓

Incubate at 37˚c for 5 min

↓

Add 60µL to Agarose tube & incubate at 37°C for 5 min

3 Agarose Slide Preparation

Put 10 - 15µL of Incubated Semen with Agarose from Step 2

↓

Put a Cover Slip on slide

↓

Keep slide with cover slip on pre-incubated tray (2 – 8°C) for 5 min

↓

Remove Coverslip

4 Prepared Slide (Agarose + Semen + Agarose) from Step 3

Reagent – I for 7min

↓

Reagent - II for 20 min

↓

Distilled Water for 5 min

↓

Fixative – I for 2 min

↓

Fixative – II for 2 min

↓

Fixative – III for 2 min

↓

0.5mL of Stain - I for 1 - 2 min

↓

Stain Buffer 1mL for 10 - 15 min

↓

Rinse in Distilled Water

↓

Dry the smear

Examine

under **40x** (objective lens)

6 Examination

- Examine 200-500 sperms in air-dried smear under microscope using **40x** (objective lens).
- Identify sperm with tail & observe their head as follows :

A. SPERM WITH HALO

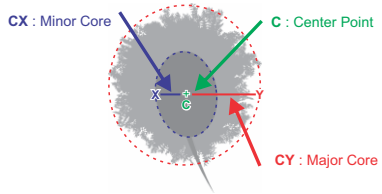


Fig. 1

a. Identify & mark center of head (**C**).

b. **MINOR CORE :**
- Mark periphery of central dark area
- This area is known as **Minor core**.
- Note down radius **CX** (approx) [as in **Fig. 1**]

c. **MAJOR CORE :**
- Mark periphery of **Chromatin Dispersion** (called **'HALO'**)
- This area is known as **Major core**.
- Note down radius **CY** (approx) using center of head. [as shown in **Fig. 1**]

d. **RATIO (R)**
- Calculate ratio **(R)** of major core with minor core.

$$R = \frac{CY}{CX}$$ (**CX** & **CY** from **Fig. 1**)

B. SPERM WITHOUT HALO :

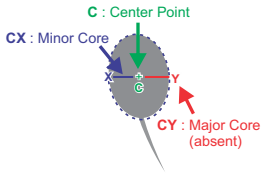


Fig. 2

a. Identify & mark center of head (**C**).

b. **MINOR CORE :**
- Central dark area is known as **Minor core**.
- Note down radius (**CX**) (approx).

c. **MAJOR CORE :**
- Absent
- Radius (**CY**) same as **CX.**

d. **RATIO (R) :**

$$R = \frac{CY}{CX} = 1$$ (**CX** & **CY** from **Fig. 2**)

C. DEGRADED SPERM :

Fig. 3

Sperm are shrunken than normal size.

Categorization of Sperm

A. **Non-fragmented DNA**

Ratio (**R**) ≥ 1.4 [Sperm with Halo] {**Fig. 1**}

B. **Fragmented DNA**

Ratio (**R**) < 1.4 [Sperm with Halo] {**Fig. 1**}

Ratio (**R**) = 1 [Sperm without Halo] {**Fig. 2**}

C. **Degraded Sperm**

Sperm with Shrunken size (from **Fig. 3**)

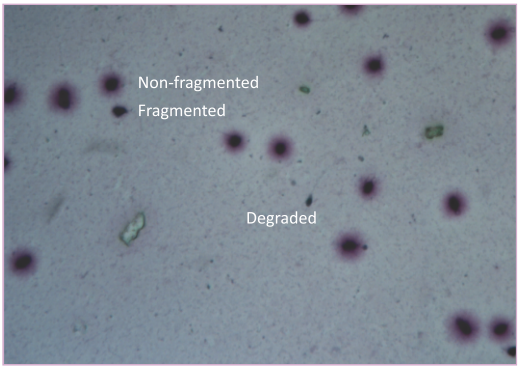
Positive & Negative Control

Positive Control : Sperm without halo

- Follow procedure **up to Step 5.**
- **Add 50µL** of H_2O_2 (300 mM) covering entire slide.
- **Incubate** at **2-8°C** for **5 min**.
- **Continue** from **Step 6**.

Negative Control : Sperm with halo

- **Follow** procedure up to **Step 5**.
- **Skip Step 6**
- **Continue** from **Step 7**.



Calculation :

- Calculate :
  - No. of sperm evaluated (**S**)
  - No. of sperm with Non-fragmented DNA (**X**)
  - No. of sperm with fragmented DNA (**Y**)
  - Degraded sperm (**Z**)

S = X + Y + Z

- **DFI (DNA Fragmentation Index)** :

$$DFI = \frac{\text{No. of sperm with fragmented DNA (Y)} + \text{No. of Degraded Sperm (Z)}}{\text{No. of sperm evaluated (S)}} \times 100$$

= [(Y + Z) / S] x 100

Normal reference value / range - DFI % ≤ 15%

7 Result

Sperm Evaluation

- No. of Sperm Evaluated : ____
- Sperms with Non-fragmented DNA : ____
- Sperms with Fragmented DNA : ____
- Degraded Sperms : ____

DNA Fragmentation Index (DFI) : ____

Normal reference value / range for DFI Index :

- Normal : DFI < 15%
- Equivocal : DFI ≥ 15% & DFI ≤ 25%
- Abnormal : DFI > 25%

Limitations :

- This test provides presumptive quantitative information of DNA Fragmentation in 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.

8 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.

9 Examination by Automated Software

- Result interpretation is supported with -

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

- Individual test module - **Sperm Soft : DNA** is also available.

10 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 gloves & face mask.

11 Safety & Environment

- **Attention ! Slide processing must be performed under fume hood.**
- Avoid inhalation & contact with the solutions supplied. The acid solution (DA) contains Hydrochloric acid, & lysing solutions (LS) contains Dithiothreitol & Triton X-100. Consult specifications supplied by manufacturers.
- 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
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-dna-fragmentation.html

www.youtube.com/watch?v=7IBTM2y8ysQ