DNA Fragmentation
sperm chromatin dispersion test

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REF SP/SFT/DNA-009

User Manual

IVD
Turnaround time for test: 90min

Store at: 2°C - 8°C after receiving

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Importance of DNA Fragmentation:

• For couples planning to undergo IVF or ICSI, these tests evaluate the impact of sperm DNA damage on reproductive outcomes (Fertilization, Embryo Development, Pregnancy, Miscarriage, Post-natal Development).

• Unexplained Infertility: Based on evidence, tests 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 levels 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 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.

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.

Thus the inclusion of an assessment of DNA damage is more comprehensive for semen quality.
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.

- 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 5 millions/mL) should be processed to obtain sperm concentration 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

- 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

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.
**Required BUT NOT PROVIDED IN KIT**

**Equipments**
- 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

**Disposable Materials**
- Hand Gloves
- semen Collection Container
- Non-spermicidal semen Collection Pouch (Sperm Collect™)
- Microtips
- Pasteur Pipettes
- Test Tubes
- Glass Slides
- Coverslips
- Filter Papers

**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°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)
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](image)

   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](image)

   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](image)

   Sperm are shrunken than normal size.

DNA Fragmentation - Sperm 360
Categorization of Sperm

A. Non-fragmented DNA
   Ratio \( R \) \( \geq 1.4 \) [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 50\( \mu \)L of \( \text{H}_2\text{O}_2 \) (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):

\[ \text{DFI} = \frac{Y + Z}{S} \times 100 \]

Normal reference value / range - DFI % \( \leq \) 15%
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.

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.
**Examination by Automated Software**

- Result interpretation is supported with - **CASA with Auto & innovative Expert Mode**

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

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**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.

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**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
- ⏳️ EXP. use by
- 🔧 manufacturer
- 🎈 IVD health surveillance device for in-vitro diagnostic
- Ⓟ Σ 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=7lBTM2y8ysQ](www.youtube.com/watch?v=7lBTM2y8ysQ)

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