Sperm 360°

Sperm 360° ™

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Nuclear Protein Assay

sperm nuclear protein assay
using "Aniline Blue" staining

SP/SFT/NP-008

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

Mammalian sperm & DNA is the most tightly compacted eukaryotic DNA which is in sharp contrast to DNA structure in somatic cells nuclei.

Compaction & organization help protect sperm chromatin during transport through the male & female reproductive tract. This also ensures delivery of the paternal genome in a form that allows developing embryo to accurately express genetic information.

Somatic cell nuclear DNA is wrapped around an octomer of histones & packaged in to solenoid structure. This type of packaging adds histones which increase the chromatin volume.

During spermiogenesis, sperm chromatin undergoes a series of modifications in which histones are lost & replaced by protamines.

Protamines are approximately half the size of histones. They are highly basic sperm-specific nuclear proteins that are characterized by an arginine-rich core & cysteine residues.

Protamines condense the DNA strands & form the basic packing unit of sperm chromatin called a toroid. Thereby, conferring a higher order of DNA packaging in sperm than that found in somatic cells.

Humans express equal quantities of two protamines, protamine 1 & protamine 2. The mean P1/P2 ratio is approximately 1.

In human chromatin, ~85% of the histones are replaced by protamines. Approximately 15% of the histones are retained subsequently making chromatin less tightly compacted.

During spermatogensis, First, somatic histones are replaced by testis-specific histone variants, which are replaced by transition proteins (Tp1a & Tp2) in a process involving extensive DNA rearrangement & remodeling. During the elongating spermatid stage, the transition proteins are replaced in the condensing chromatin by protamines.
Chromatin remodeling is facilitated by the coordinated loosening of the chromatin by histone hyperacetylation & by the DNA topoisomerase II (topo II), which produce temporary nicks in the sperm DNA to relieve torsional stress that results from super coiling. The same enzyme Topo II normally repairs these temporary nicks prior to completion of spermiogenesis & ejaculation. However, it these nicks are not repaired, DNA fragmented sperm may be present in the ejaculate.

Defects in the chromatin remodeling process result in the production of spermatozoa that are characterized by reduction in the efficiency of protamination, abnormal protamine 1 to protamine 2 ratio, & relatively high nucleohistone content. Thereby, creating a state of vulnerability where spermatozoa DNA become increasingly susceptibly to oxidative damage.

Abnormal Protamine P1/P2 ratio is associated with low sperm count, decreased sperm motility & morphology, diminished fertilization ability, & increased sperm chromatin damage.

Sperm nuclear protein assay (chromatin maturity) is done via aniline blue staining. Aniline blue stains persistent histones in the sperm nucleus.

- Mature chromatin - Histone <15% & Protamine (I & II) >85%.
- Immature chromatin - Histone >15%.

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** plasma must be thawed at 37°C (with Sperm Warmer™) before performing test.

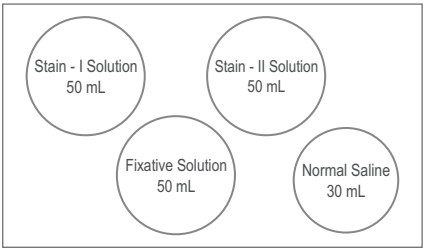
2 Kit Contents

- Fixative Solution : 50 mL
- Stain - I : 50 mL
- Stain - II : 50 mL
- Normal Saline : 30 mL

Other Reagents (Required But Not Provided In Kit) :

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

Content Box 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™)
- Stop-watch
- Slide-Warmer
- 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

Step 1 : Label plastic ware & disposable material with appropriate Patient ID & Sample ID.

Step 2 : Processed Semen Smear :

- Take 100µL of liquefied semen.
- Add 400µl of **Normal Saline (NS)**.
- Mix well & centrifuge at 2000rpm for 2 - 3 min.
- Discard the supernatant.
- Adjust the sperm concentration between **40 - 60** millions/mL with Normal Saline (NS) & use **5**µL to prepare the smear.
- If adjusted concentration is less than **40** millions/mL, use **10**µL to prepare the smear.

Step 3 : Allow the smear to air dry.

Step 4 :
Lay the air dried smear horizontally and cover theentire smear with **1** mL **fixative** solution. Keep it for **5** min.

Step 5 :
Drain off the **fixative** solution & rinse the smear in distilled water (DW).

Step 6 : Lay the smear horizontally and cover the entire smear with **1** mL of **Stain - I** solution. Keep it for **5** min.

Step 7 : Drain off the **Stain - I** solution & rinse the smear in distilled water (DW).

Step 8 : Lay the smear horizontally and cover the entire smear with **1** mL of **Stain - II** solution. Keep it for **2** min.

Step 9 : Drain off the **Stain - II** solution & rinse the smear in distilled water (DW).

Step 10 : Drain off the solution & allow the smear to air-dry (use Slide Warmer™).

Quick Glance

Processed or **neat** semen sample

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Take 5 µL / 10 µL semen sample, prepare smear & dry it

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1 mL Fixative solution for **5 min**

↓

Drain off Fixative Solution & **Rinse** in Distilled water

↓

1 mL Stain - I for **5 min**

↓

Drain off Stain - I & **Rinse** in Distilled Water

↓

1 mL Stain - II for **2 min**

↓

Drain off Stain - II & **Rinse** the in Distilled Water

↓

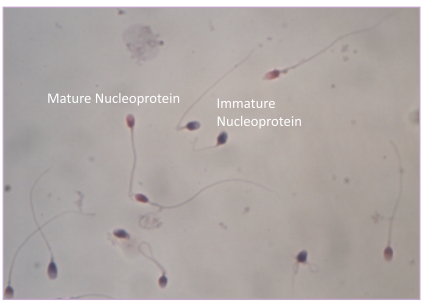
Dry the smear

Examine under **100x** (objective lens)

6 Examination

- Put a drop of immersion oil on the dry smear.
- Examine the smear under the microscope with the help of **100x** lens.
- Examine at least **200** sperms & count the following :
  - Sperm **head** with **blue stain** [Mostly in Post acrosomal region] (Indicates sperm with immature nuclear protein)
  - Sperm **head** with **red stain** [Mostly in Post acrosomal Region] (Indicates sperm with mature nuclear protein)

Reference Image :



7 Result

- No. Of Sperm Evaluated : _____
- Sperm With Mature Nuclear Protein : _____
- Sperm With Immature Nuclear Protein : _____

Normal reference value / range :

- **Normal:**
  Immature Nuclear Protein **< 15%**
- **Equivocal**:
  Immature Nuclear Protein **≥ 15%** & **≤ 25%**
- **Abnormal**:
  Immature Nuclear Protein **> 25%**

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

Limitations :

- This test provides presumptive quantitative information of 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 : Nuclear Protein** 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 of gloves & face mask.

11 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
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

www.spermprocessor.com/sft-nuclear-protein-assay.html

www.youtube.com/watch?v=XrD73zHyjoE