HI-TECH SOLUTIONS

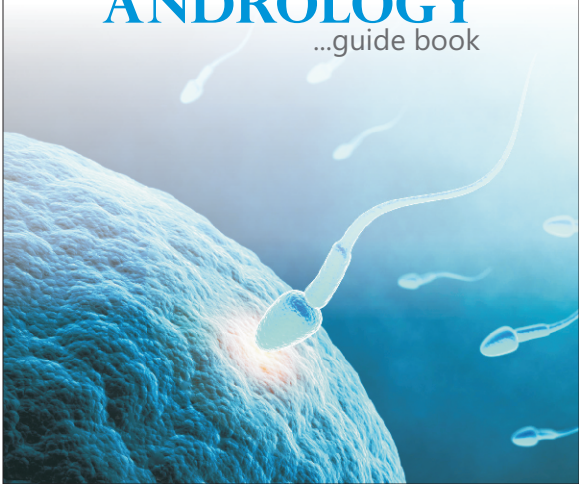
ASSISTED REPRODUCTIVE TECHNOLOGY

HI-TECH CRYOBANK

The Sperm's world

ANDROLOGY

...guide book



Manufacturers & Service Providers :

IUI
IVF
ICSI

MEDIA,
DISPOSABLES
& EQUIPMENTS

SEMEN BANKING

FROZEN DONOR SEMEN SAMPLES

HI-TECH CRYOBANK
The Sperm's world

HI-TECH SOLUTIONS
ASSISTED REPRODUCTIVE TECHNOLOGY

An ISO 9001:2008 Registered Company

Contents Page

PROCEDURAL SCHEDULE

SR. NO.	TIME AFTER EJACULATION	ACTION
1.	0-5 Minutes	Register sample in the Laboratory. Place the sample in Semen Warmer at 37°C
2.	20-25 Minutes	Examine sample for completion of liquefication, appearance and odour.
3.	30 Minutes	(A) Measure the pH.
		(B) Measure ejaculated volume and assess viscosity
		(C) prepare specimen for microbiological evaluation if requested
		(D) Make eosin-nigrosin smears.
		(E) Examine a wet preparation; asess: a. sperm motility and progression b. Sperm agglutination c. Presence of other cells and debris.
		(F) Make video recordings for subsequent sperm movement and analysis, if required.
		(G) Determine the Sperm Concentration by using 10 μ depth Sperm Counting Chamber.
		(H) Prepare smears to be stained for Sperm Morphology/Function
		(I) Take any aliquots for antibody titrations & biochemistry assays. Including the spot test for fructose, if performed.
		(J) Prepare a washed sperm population for the direct Immunobead test.
		(K) It indicated from the wet preparation use one of the specific methods for detecting leukocytes.
4.	As soon as possible	Complete the preparation of aliquots
	(1)	(2)

NORMAL VALUES OF SEMEN VARIABLES
(As per WHO Standards)

STANDARD TESTS	REFERENCE VALUES
Volume	2.0 ml. or more
pH	7.2 - 7.8
Sperm Concentration	20 millions spermatozoa/ml. or more
Total Sperm Count	40 millions spermatozoa/ejaculate or more
Motility	50% or more with forward progression or 25% or more with rapid progression within 60 min. of ejaculation.
Morphology	30% or more with normal form
Sperm Function	>50% or more with normal form
Vitality	75% or more live (i.e. excluding dye)
White Blood Cells	< 1 million/ml
Immunobead Test	<20% spermatozoa with adherent particles
MAR Test	<10% spermatozoa with adherent particles
Optional Tests	
Glucosidase (Neutral)	20mU or more/ejaculate
Zinc (Total)	2.4 µmol or more/ejaculate
Citric Acid (total)	52 µmol or more/ejaculate
Fructose	13 µmol or more/ejaculate

BACKGROUND:

Many reports have shown problems with the high variability in results of semen analysis. The special interest group in Andrology (SIGA) of the European Society of Human Reproduction and Embryology (ESHRE) implemented a standardized training course, which has been run in different regions of the world on more than 20 occasions since 1994. The aim of the present analysis was to investigate to what extent training resulted in any immediate effects on the variability of assessments made by different observers.

METHODS:

The variability in participant's results from the beginning to the end of each course was analyzed in eight courses given between 1995 and 1999.

RESULTS:

For assessments of sperm concentration, motility, vitality and morphology, substantial improvement was seen over the duration of the course.

CONCLUSION:

A comprehensive, structured training course does lead to substantial reductions inter-observer variability in semen analysis. This supports our contention that providing a thorough theoretical background and repeated practical training, combined with daily feedback of results, is highly effective in raising the technical skills of laboratory personnel performing semen analysis.

INTRODUCTION:

The problem of high variability in basic semen analysis results and thereby doubts as to their clinical value has been described in several reports that showed large variations in results both among different investigators and for individual investigators on different occasions. Semen analysis remains the cornerstone of the male infertility workup. For most men, however, a semen specimen in a cup is all that is required for their initial evaluation. If it is "normal" the male fertility evaluation is usually not pursued. However, who is to say, "what is normal"? Do the current standards for Semen Analysis predict who has a "normal" fertility potential and who does not? Male fertility is dependent upon sperm quality. Ideally, the fertilizing capacity may be directly appraised by sperm incubation with oocytes under natural reproductive or controlled laboratory conditions. Unfortunately, such a procedure is difficult, if not impossible, for routine semen analysis evaluations. As a practical and viable substitute, several sperm assays have been developed to put indirectly infer sperm fertilizing capacity; Standard attributes routinely reported during such diagnostic evaluation include sperm concentration, motility %, and percent normal sperm morphology. Since large variations may occur even between consecutive ejaculates from the same individual.

BASIC SEMEN ANALYSIS: In 1866 Sims stressed the importance of the presence of spermatozoa for fertilization, which performed

postcoital examinations on fluids of the vagina and the endocervix and stated that spermatozoa had to be present in the endocervical mucus for conception to occur. It is only since the turn of this century that a more scientific approach for semen analysis was adopted and, according to Ross, it was only in the 1940s that Wiesman stated that a **semen analysis was not complete unless the volume, motility, concentration and morphology were determined.** This was further supported by the work of Hotchkiss in 1945. The American Fertility Association established minimum requirements for semen analysis and semen parameter standards in 1951, in 1966 by Freund, and in 1971 by Ellisen. The minimum requirements for a complete semen analysis are still being extended with time, and today must include so called screening tests for the presence of Antispermatozoa Antibodies such as the mixed antiglobulin reaction (MAR) test.

NEED OF STANDARDIZATION:

There have been several efforts made to improve the quality of semen analysis by recommending suitable methods and organizing training courses or programmers but the effect of at least the earliest activities have in the long run, apparently been limited mainly to the centers involved in the initial projects. Several studies have emphasized the importance of standardization of laboratory methods and training of staff to achieve reliable results in routine semen analysis.

BASIC DIFFICULTY IN STANDARDIZATION:

As semen analysis are used mainly to investigate male fertility potential of an infertile population, the datum point for establishing standards for fertility evaluation should not be based on what is average in normal population, but rather on what minimum semen parameters values are needed to give a reasonable chance for conception. These values will be much lower than the mean values for normality of a population with proven fertility. For this the WHO manual does not provide guidelines. Semen parameters should be classified for diagnostic prognostic purposes, for example fertile, sub fertile, infertile and sterile, when azoospermia is present.

Process of Standardization: A complete semen analysis can be divided into the following five categories (a) Background Data [b] Physical Parameters (c) Quantitative & Qualitative Analysis (d) Bio- Chemical Analysis (e) Screening Tests. These components cannot be isolated aspects, it is therefore important to regard all these aspects with equal importance, as all these factors are interrelated.

(a) Background Data: A semen analysis cannot be interpreted unless certain basic facts are known, Abstinence period, method of collection, type of container used, place of collection. Each of the parameter play major role in interpretation of semen analysis result hence each laboratory must have their own SOP (standard operated procedure).

(b) Physical Parameter: As physical parameters include colour, liquefaction time, viscosity, PH and volume. Out of these the crucial parameters are liquefaction & viscosity. As per WHO criteria liquefaction time should be monitored as: within 30 minutes, within 30-60 minutes and more than 60 minutes. Viscosity within normal limit, equivocal and increased. For measured of pH both colorimetric and glass electrode methods are use to determine the pH of semen. Although the electronic method is more accurate preference should be given to pH measurement by colorimetric method using a special indicator paper.

(C) Analysis of Quantitative & Qualitative Parameter: Parameters that are classified under this heading are those that are regarded by many laboratories to comprise a complete or standard semen analysis and this include sperm concentration, estimation of quantitative (% motile) & qualitative motility, agglutination, supravital staining & morphology of the spermatozoa . The MAR test should also be included as a routine procedure. A semen is a body fluid, viscous In nature, as far as possible it should be examined without dilution, during examination avoid overlapping of sperm (sperm should be in single layer) In the era of Assisted Reproductive Technology every semen examination parameter count in terms of fertility. There is importance of calculation of total motile sperm in a given ejaculate. This can be achieved only by utilizing 10 micron depth chamber.

Hence the results of quantitative & qualitative parameter are mainly influenced by Depth of smear (chamber), Area to be counted & choice of objective lens.

SEMEN ANALYSIS

- Semen is biological fluid, viscous in nature
- Semen contains Sperms & Seminal Plasma.
- During semen analysis two basic parameters are most important

SPERM CONCENTRATION

1st of all note down the exact volume of semen,

Then calculate sperm concentration

(1) Formula for Sperm Concentration

Sperm Concentration :

$$\frac{\text{No. of Sperms x Dilution factor x Depth factor}}{\text{Total Area Counted}} \times 10^4 \text{ millions ml.}$$

(2) Sperm Motility Assessment

Assessment of Motility is done by :

Qualitative Motility Assessment _____Actively Motile
Quantitative Motility_____Motility %

(3) Motility % =

REMARK : Motility % is always calculated with reference to Sperm concentration.

(4) Total Sperm count =

Volume x sperm concentration millions/ejaculate

5) Total motile sperm count=

$$\frac{\text{Sperm Count x motility \%}}{100}$$

SPERM MORPHOLOGY & FUNCTION

TEST- I : SPERM pH

Procedure to determination of Semen pH :

Place a drop of liquefied semen on pH strip wait for minute, compare the color change with chart given on strip **(NORMAL pH of Semen 7.1 to 7.4)** Significance-pH Test is less than 7.0 in a sample with azoospermia, there may be obstruction of ejaculatory ducts of bilateral congenital absence of the vasa.

Vitality Test

TEST-II : Membrane integrity of Sperms Physical Intactness of Sperm : Procedure

- Take one drop of liquefied semen
- Add two drops of Eosin and add three drops of Nigrosin MIX WELL
- TAKE one drop of mixture on slide make a smear, air dry it, evaluate under microscope with 100x objective lense with oil immersion

SIGNIFICANCE:

1. Evaluation of physical intactness of sperm membrane by supra vital staining.
2. This test provides estimation of motility with good internal control.
3. The sum of dead spermatozoa (i.e. Stained) and motile spermatozoa (I.e. Stained) and motile spermatozoa (i.e. unstained) should not be more than 100%

Conclusion:

Stained Spermatozoa (Dead Sperms) Unstained Spermatozoa (Motile Sperms)

TEST-III : Morphology Stain : Procedure

- Take One drop of liquefied semen on as slide make a smear dry it on warmer.
- For fixing the smear put 0.5mi of FIXATIVE again dry it,
- Put 8-10 drops of morphology stain. Hold it for 45 seconds. wash with distilled water, Air dry it and see under microscope with oil immersion

SIGNIFICANCE : Evaluate defects of Head, neck and tail of sperm.

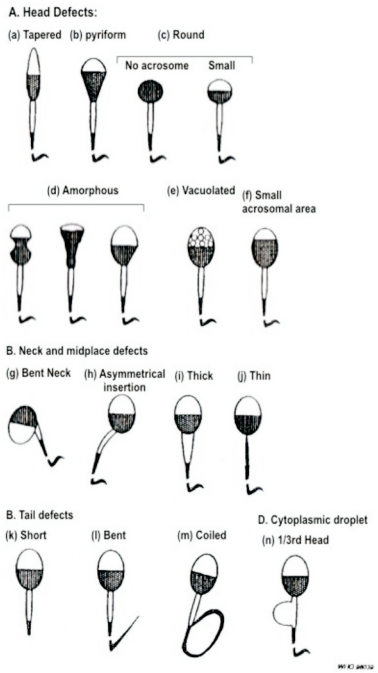


Fig.: Schematic Drawings of some abnormal forms of human spermatozoa (Adapted from Kruger ct al., 1993 (A) Head defects (a) Tapered (b) Pyriform (c) Round, small and acrosome either absent or present (d) Amorphous. (e) Vacuolated. (I) Acrosomal area small. (B) Neck and midplace defects. (g) Bent neck. (h)Asymmetrical insertion of mid place. (i) Thick midplace (i) Thin midplace (c) Tail defects (k) Short tail (l) Bent tail (m) Coiled tail (D) Cytoplasmic droplet defect. (n) Droplet greater than one third the area of the normal sperm head.

Fructose Test

TEST-IV : Chemical Analysis of Seminal Plasma. Fructose Determination (qualitative Analysis)

Principle:

Seminal Plasma is produced by the seminal vesicles & the prostate gland. The components of the seminal plasma are specific for these glands. Therefore, the determination of these components effects the activity of the concerned gland, e.g fructose specifically represents functionality of the seminal vesicles.

Reagents : Selivanoff's solution - 25ml.

Specimen : Lique?ed semen OR Seminal plasma OR Thawed cryopreserved seminal plasma.

Procedure :

- Take 5 ml. of Selivanoff's reagent in a glass test tube.
- Add 0.5 ml. of the specimen.
- Hold the test tube on the burner with the holder.
- Boil it for 30 to 60 seconds.
- Observe the color of the mixture after 5 to 10 minutes for the change in color.

Interpretation :

- Change in color from colorless to red tone indicates the presence of fructose in the given specimen.
- No change in color indicates absence of fructose.

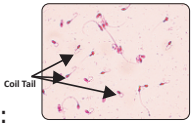
Note : Absence of fructose must be verified by performing the same test, but with the another sample.

SPERM FUNCTION

hTS-HOST(Hypo-Osmotic Swelling Test) :

This test evaluates response of spermatozoa to hypo-osmotic stress. This test is a good index of healthy spermatozoa.

Normal : >60% spermatozoa with tail coiling.



Procedure :

Take 500 micro litre of HTS-H.O.S. Solution in a small test tube. Add 100 micro litre of Liquified semen sample to the hos solution and mix well . Keep at 37^0 Centigrade for 30 minutes.

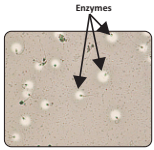
Add 50 micro litre of stop solution and take just one drop on the plain glass slide. Put The cover slip and examine under microscope in 40x objective. Calculate percentage of Coiled tail.

Normal percentage > 60%

hTS-AIST(Acrosome Intactness Slide Test) :

This test evaluates the functional status of sperm acrosome and is a goos indicator of sperm's ability to penetrate the oocyte investments.

Normal : >50% Spermatozoa with Halos.



Procedure :

Take 500 micro litre of hTS-acrosome solution in a test tube. Add 100 micro litre Liquified semen sample to the centrifuge tube and mix well. Incubate

at 37^{0}C for 30 minutes. Put a drop of mixture solution on the hTS-gelatin coated slide & make smear very smoothly, air dry for 2 minutes to remove excess moisture.

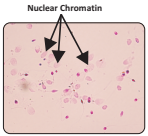
Incubate for 2 hours at 37^{0}C. Air dry and examine under 10x-40x objective. Count the spermatozoa with halos.

Normal percentage > 50%

hTs-NCDT (Nuclear Chromatin Decondensation Test) :

This test cab be used to find out whether decrease in fertilizing potential has relation to sperm head chromatin.

Normal : >70% decondensed head.



Procedure :

Take 100 micro litre liquified semen in a test tube, add 1ml. Wash solution And mix well and cetrifuge for 5 minutes at 1500 rpm. After centrifugation discard All the supernatant without disturbing pellete. Dissolve the NCD powder in 10ml distilled water properly. Add 200 micro litre hTs-N.C.D. solution to the pellete and mix well. Keep at room temperature for 30 minutes. Add 50-100 micro litre of stop solution and transfer a drop of mixture solution to The glass slide and cover with a coverslip. Examine under 40x objective. Count the number of condensed and decondensed heads. Normal percentage > 70% decondensed spermatozoa.

SEMEN ANALYSIS REPORT

Donor ID No.:

Doctor	Date & Time	Abstinence (Days)	Produced at
			[ ]Centre [ ]Home

Purpose: [ ] IUI [ ] ANALYSIS **[ ] FREEZING [ ] TRIAL WASH**

Basic Semen Analysis			
	Pre wash	Post wash	Acceptable (WHO) Values
Volume (ml)			2.0-5.0
Sperm count (mill/ml)			>20
Motility (%)			>50
Activity (%)	(Rapid)	(Moderate)	(Slow) (Sluggish)
Viscosity	[ ]0	[ ]1+	[ ]2+ [ ]3+
Agglutination	[ ]0	[ ]1+	[ ]2+ [ ]3+
Debris	[ ]0	[ ]1+	[ ]2+ [ ]3+

Normal Morphology (%)	Normal Function (%)	Acceptable (WHO) Values (%)
Morphological Abnormalities (%)		

Head		**Mid-Piece**		Tail		HOS		
Amorphous		Cytoplasmic Droplet		Coiled tail		AIS		
Elongated		Others		Short tail		NCD		
Pyriform				Hairpin tail				
Macrocephalic				Double tail				
Microcephalic				OTHERS				
Broken Neck								
Double Head								
OTHERS								

Hypo-osmotic Swelling Test : %		
Semen Frozen : [ ] Yes [ ] No	No. of Vials frozen :	Location :
Remark : Acceptable / Not Acceptable	Biologist : Name : Signature	

SEMEN COMPONENT

POINT OF ORIGIN

Semen	Testis	Epididymis	Seminal Vesicle	Prostate
Physical Characteristics				
Coagulation	...	...	✔	✔
Liquefaction	...	...	✔	✔
Volume	✔	✔	✔	✔
spermatozoa				
Count	✔	...	...	...
Motility	✔	✔	✔	✔
Morphology	✔	✔	...	...
Seminal Plasma				
pH	...	...	✔	✔
Leukocytes	...	✔	✔	✔
Fructose	...	...	✔	...
Zinc	...		...	✔
a-glucosidase	...	✔	...	...

Semen Preparing and Processing

Assisted conception (ART-Assisted Reproductive Technology) techniques have fast evolved into major treatment modalities in management of infertile couples. Semen preparation and processing is the cornerstone of all the assisted conception techniques. Out of many methods of semen preparation density gradient solution is universal technique, which gives maximum yield and clean sample.

Disposables Required

- Semen Collection Container
- Two Conical Centrifuge Test Tubes
- Two Pasture Pipette
- One IUI Catheter / Cannula for Insemination concentrated sperms

All above disposables must be sterilized by Gamma/ETO

Equipment Required

- Digital Semen Warmer (37^0 C.)
- Binocular Microscope
- Digital Centrifuge Machine
- Laminar Flow
- Sperm Counting Chambe
- Hi-Tech Sperm Viewer Camera

Important : Examine the packing of media (Ampoules/Vials). Showing any cracks or change in color of the solution to either dark pink or yellow, or appear cloudy or hazy should be discarded.

Occasionally samples do not liquefy properly and remain too viscous. Increasing the speed (RPM) of

centrifuge and time will did in collection of good number of motile sperm.

If sample showing heavy amount of debris and cellular contaminants, may leave behind some debris in the prepared sample. To reduce this contamination due to sticking of interface to the tube after removing seminal plasma layer, the interface can be gently scraped and stirred by the pipette without disturbing the pellet, so that all the interface sticking to tube is loosened and freed from the wall and can be aspirated.

Warranty

This product is based on research methods. Its clinical utility has to be established by the method using the product. This kit is ampoulised at state of the art facilities with GMP & WHO standards. We accept limited liability to replace defective material. Other liability including injuries arising out of misuse of this product will not be accepted.

Important : Swim up technique is not suitable for highly viscous sample. Oligospermic semen sample can be processed with using multiple tubes technique to increase the surface area for swim up. All the supernatants can be pooled after swim up.

Single Layer Density Gradient Technique (CRYO-DENS 1)

Single layer high density gradient technique, effectively removes contaminates left behind by other sperm processing methods, yielding a sample that contains mostly motile sperm almost free from dead and abnormal sperms, debris and round cells. The procedure is effective for both high and low count semen specimen. It is also effective in highly viscous sample and sample need not be treated with enzymes for liquefaction.

CD 1 single layer high density gradient technique is highly efficient, fast & cost effective method of processing semen sample for procedure like IUI/IVF. It is coated colloidal suspension of sterile silica particles which effectively removes all contaminates and concentrates motile sperms. This solution effectively separates normal and healthy sperms from lymphocytes, epithelital cells, abnormal or immature sperms, debris, bacteria and seminal fluid. This solution helps to avoid premature hyperactivation and improves fertilization potential.

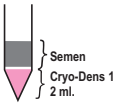
Material Needed :

1. CD1 (CRYO-DENS 1) 2 ml. for high density gradient preparation
2. Modified HTF/HAM-F10 with HEPES buffered 5 ml. for washing

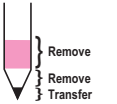
Procedure :

1. Bring all the required solution and semen sample to semen warmer at 37^{0}C before use. This will avoid cold shock to the spermatozoa and eliminate condensation on the ampoules.

2. Preferably clean the external surface of the ampoules with spirit and allow drying. Open the ampoules by holding the colored ring on the ampoules neck and firmly flicking the tip backwards. Ampoule will break clean at the neck.
3. Transfer entire contents of CRYO-DENS 1 solution (2ml) to the conical centrifuge test tube.



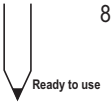
4. Examine a small drop of well mixed semen sample for pre-processing evaluation. Gently layer entire semen sample over the CRYO-DENS 1 solution using the same pipette. Care should be taken, so as mixing of two layers avoided.
5. Centrifuge at 1500 RPM for 15 minutes. If the sample is highly viscous centrifugation can be done even at 2000 RPM. Following centrifugation pellet will be visible.



6. Remove seminal plasma layer and interface, rapidly aspirating the debris at the interface. Then carefully remove CRYO-DENS 1 solution layer without disturbing the sperm pellet. Add 2-4 drops of modified HTF/HAM-F10 solution to the sperm pellet using second pipette. Resuspend carefully and transfer to second tube. Add 3-4 ml of Modified HTF/HAM-F10 solution

and mix thoroughly.

7. Centrifuge at 1500 RPM for 5 minutes. Remove supernatant (without disturbing the pellet) and re-suspend in 0.5 ml Modified HTF/HAM-F10 solution.



8. Examine a drop of suspended prepared sample for post processing parameters and calculate the yield.

Double Layer Density Gradient Technique (CRYO-DENS 2)

Double layer density gradient technique, effectively removes contaminates left behind by other sperm processing methods, yielding a sample that contains mostly motile sperm almost free from dead and abnormal sperms, debris and round cells. The procedure is effective for both high and low count semen specimen. It is also effective in highly viscous sample and sample need not be treated with enzymes for liquefaction.

It effectively removes contaminates left behind by other sperm processing methods, yielding a sample that contains mostly motile sperm almost free from dead and abnormal sperms, debris and round cells. The procedure is effective for both high and low count semen specimen. It is also effective in highly viscous sample and sample need not be treated with enzymes for liquefaction.

CD 2 double layer density gradient technique is highly efficient, fast & cost effective method of processing semen sample for procedure like IVF/ICSI/IUI. It is coated colloidal suspension of sterile silica particles which effectively removes all

contaminates and concentrates motile sperms. This solution effectively separates highly strong and healthy sperms from lymphocytes, epithelital cells, abnormal or immature sperms, debris, bacteria and seminal fluid. This solution helps to avoid premature hyperactivation and improves fertilization potential.

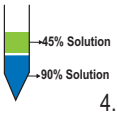
Material Needed :

1. CD2 (CRYO-DENS 2) 90% & 45% 1ml. each for double layer density gradient preparation
2. Modified HTF/HAM-F10 with HEPES buffered 5 ml. for washing

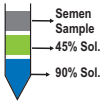
Procedure :

1. Bring all the required solution and semen sample to semen warmer at 37^{0}C before use. This will avoid cold shock to the spermatozoa and eliminate condensation on the ampoules.

2. Preferably clean the external surface of the ampoules with spirit and allow drying. Open the ampoules by holding the colored ring on the ampoules neck and firmly flicking the tip backwards. Ampoule will break clean at the neck.

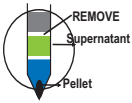


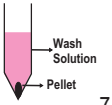
3. Transfer entire contents of CRYO-DENS 2(90%) solution (1ml) to the conical centrifuge test tube. Carefully layer 1ml of CRYO-DENS 2 (45%) solution over the 90% layer so that the layers do not mix.
4. Examine a small drop of well mixed



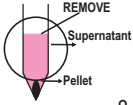
semen sample for pre-processing evaluation. Gently layer entire semen sample over the CRYO-DENS 2 (45%) solution using the same pipette. Care should be taken, so as mixing of two layers and semen is avoided.

5. Centrifuge at 1500 RPM for 15 minutes. If the sample is highly viscous centrifugation can be done even at 2000 RPM. Following centrifugation pellet will be visible.
6. Remove seminal plasma layer and interface, rapidly aspirating the debris at the interface. Then carefully remove CRYO-DENS 2 (45% & 90%) solution layers without disturbing the sperm pellet. Add 2-4 drop of modified HTF/HAM-F10 solution to the sperm pellet using second pipette. Re-suspend carefully and transfer to second tube. Add 3-4 ml. of Modified HTF/HAM-F10 solution and mix thoroughly.

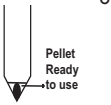




7. Centrifuge at 1500 RPM for 5 minutes. Remove supernatant (without disturbing the sperm pellet) and re-suspend in 0.5 ml Modified HTF/HAM-F10 solution.



8. Examine a drop of suspended prepared sample for post processing parameters and calculate the yield.



Swim up Technique
(Modified HTF/HAM-F10)

Modified HAM-F10/HTF solution contains all necessary ingredients like protein, glucose, EDTA, Glutamine, HEPES buffer, HSA etc. The optimized formulation of this solution is designed and based on maximum survival time of sperm to improve fertilization potential.

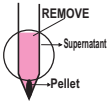
Swim up technique is a preferred method for normospermic semen specimen, in oligospermic semen sample, it may not be possible to collect enough sperms for insemination. Different method of processing based on density gradient separation method like CD1 or CD2 needed.

Procedure :

1. Put the modified HAM-F10 or HTF solution ampoules in semen warmer at 37°C for 10 minutes along with the semen samples & plasticware accessories.

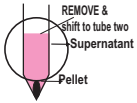
2. Preferably clean the external surface of the ampoules with sprit and allow to dry.
3. Examine a small drop for pre wash evaluation
4. Transfer liquefied and well mixed semen samples up to 3ml to the conical bottom tube.

5. Add equal quantity HAM-F10 or HTF solution to the semen sample and mix slightly.
6. Centrifuge for 10 minutes at 1500 RPM.



7. Following centrifugation remove all the supernatant without disturbing the pellet.
8. Layer 1.5ml of HAM-F10 or HTF solution over the pellet. (without disturbing the pellet.) If the pellet gets accidently disturbed, re-centrifuge at 1000 RPM for 3-5 minutes.

9. Keep the tube inclined in the semen warmer at 37°C for 45-60 minutes.
10. Aspirate the supernatant (which contain active motile sperms) without disturbing the pellet and transfer to second tube. Centrifuge the tube at 1000 RPM for 5 minutes.



11. Remove & discard all the supernatant without disturbing the sperm pellet and re-suspend in 0.5 ml HAM-F10 or HTF solution.

12. Examine a drop of suspended prepared sample for post processing parameters and calculate the yield.

Sperm Freezing, Thawing and Preparation

Before freezing:

In case the sperm count is very low, it is advisable to concentrate the sperms before freezing. This may increase sperm quality after thawing and will reduce the number of freeing vials to be frozen.

After thawing:

Use sperm preparation/concentration techniques after thawing the semen to eliminate debris and dead sperm cells. Dilute the concentration sperm pellet with a suitable semen washing solution (e.g. Hi-Tech HTF/Ham-f10 solutions)

Post wash freezing procedure

Allow the semen to liquefy at 37^0 C temperatures in Semen Warmer for 30 minutes after collection.

Separates spermatozoa can be prepared with density gradient solutions (single layer/double layer density gradient technique) and washed concentrated sperm pellet with washing solutions (Modified HTF/Modified HAM-F10). Resuspend the final pellet with washing solutions (Modified HTF/Modified HAM-F10) and dilute with equal volume (1:1) of SpermPlus+freeze and washing solutions.

Caution : make sure freezing solution is at room temperature to avoid cold shock.

(To avoid osmotic shock) add SpermPlus+freeze slowly drop by drop and mix carefully tilted after each drop added. Close the cap tightly and turn the tube upside down 10 times, being careful not to create bubbles.

Leave the mixture for 10 minutes at room temperature for equilibration.

Load the mixture sample into the freezing vials.

Close the vials cap, coding identity and Place in refrigerator at 4^0 C for 10 minutes.

Hang and Freeze the vials just above the Liquid Nitrogen label (without contacting with LNO_2) for 20 minutes.

Completely dip & store in liquid nitrogen.

Thawing procedure

Remove as required vials from liquid nitrogen container.

Place the vials in semen warmer at 37^0 C for 20 minutes.

Gently mix the semen and take a drop on the sperm counting chamber and again put the slide back to the semen warmer at 37^0 C for 10 minutes. Close the vials cap and place in semen warmer.

Now take the sperm counting chamber, examine under 20 x objective for post thaw parameters and calculate the yield.

Pre wash freezing procedure

Allow the semen to liquefy at room temperature for 30 minutes.

Add 0.7 ml SpermPlus+freeze with 1 ml liquefied mix semen

(To avoid osmotic shock) add SpermPlus+freeze slowly drop by drop and mixture carefully tilted after each drop added. Close the cap tightly and turn the tube upside down 10 times, being careful not to create bubbles.

Caution: make sure freezing solution is at room temperature to avoiding cold shock.

Leave the mixture for 10 minutes at room

temperature for equilibration.

Load the mixture sample into the freezing vials.

Close the vials cap, coding identity and Place in refrigerator at 4^0 C for 10 minutes.

Hang and Freeze the vials just above the Liquid Nitrogen label (without contacting with LNO_2) for 20 minutes.

Completely dip & store in liquid nitrogen.

Thawing procedure

Remove as required vials from liquid nitrogen container.

Place the vials and other required material in semen warmer at 37^0 C for 15 minutes.

Layer the semen over a suitable density gradient processing solution in a conical centrifuge tube (at least 2 ml per 1 ml semen) without mixing.

Centrifuge for 15 minutes at 1500 RPM. (After following centrifugation pellet will be visible)

Remove seminal fluid, dead cells and debris (without disturbing the concentrated sperm pellet).

Dilute the concentrated sperm pellet in a suitable sperm washing solution 0.5 ml (e.g. Hi-Tech modified HTF/Ham-f10) Mix slightly and transfer to new centrifuge tube. Add 3-4 ml same washing solution mix well.

Centrifuge at 1000 RPM for 5 minutes. Remove supernatant (without disturbing the pellet) and re-suspend the sperm pellet in 0.5 ml suitable washing solutions (e.g. Hi-Tech modified HTF/Ham-f10).

Examine a drop of suspended prepared semen sample for post thaw processing parameters and

Sperm Processing Media

CRYO-DENS 1

Single Layer Density Gradient Solution

10 x 2 ml high density gradient
10 x 5 ml wash solution (Modified HTF / HAM-F10)

Advantages:

- **MAXIMUM SURVIVAL TIME OF 30+ HOURS**
- Concentrate More Healthy Sperms by High Density Gradient Solution
- Increase Yield Percentage
- Increase Fertility Rate
- Remove Bacteria
- Remove ROS level
- Remove Abnormal and Immature Sperms
- Remove White Cells, Pus Cells and Cell debris

Tested for:

- pH
- Osmolarity
- Endo toxin level
- Sterility
- Sperm survival

Sperm Processing Media

CRYO-DENS 2

Double Layer Density Gradient Solution

10 x 1 ml 45% density gradient (upper layer)
10 x 1 ml 90% density gradient (lower layer)
10 x 5 ml wash solution (Modified HTF/HAM-F10)

Advantages:

- **MAXIMUM SURVIVAL TIME OF 30+ HOURS**
- Concentrate more highly healthy and strong sperms by double density gradient solution
- Increase yield percentage
- Increase fertility rate
- Remove bacteria
- Remove ROS level
- Remove abnormal and immature sperms
- Remove white cells, pus cells and cell debris

Tested for:

- pH
- Osmolarity
- Endo toxin level
- Sterility
- Sperm survival

Sperm Processing Media

MODIFIED HTF / MODIFIED HAM-F10

Swim up / Wash solutions

10 x 5 ml wash solution
(Modified HTF/Modified HAM-F10)

This solution is used for the separation & purification of human sperm cell by swim up method or washing the sperm pellet recovered from density gradient preparation.

Advantages:

- **MAXIMUM SURVIVAL TIME OF 30+ HOURS**
- Concentrate motile sperms by swim up method
- Increase yield percentage
- Increase fertility rate
- Remove abnormal and immature sperms

Tested for:

- pH
- Osmolarity
- Endo toxin level
- Sterility
- Sperm survival

Store this solution at 2^0-8^0C.

HI-TECH CRYOBANK
The Sperm's world

Donor's Frozen Semen Sample

Advantages:

- Ready to use
- Minimum 20-30 million motile sperm with 50-70% progressive rate in each vial
- Highly qualified donor
- Infection Tested: HIV, VDRL, HCV, HBsAg, Thalassemia, Chlamydia, Hemoglobin & urine
- Q. C. Tested: Sperm survival, Sperm Function, Sperm count & motility, Motility percentage and pH
- Quarantined period of minimum for six month
- Donor selection based on educational qualification, medical and qualification history of family, genetic and basic semen parameters.

HI-TECH
MICROSCOPE

(Monocular / Binocular / Trinocular Microscope)
For use in clinical & biological laboratories ideally suited for semen analysis, morphology, medical colleges, hospitals & higher education.

REMI
CETRIFUGE MACHINE

(R4C/ R8C)
Compact model for routine work in medical clinical Laboratories. Digital Time, Display with Speedometer Safety Lid Lock, Swing out head with 8x15 ml. tubes.

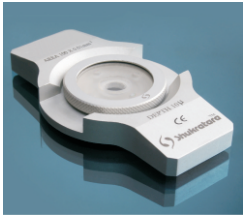
HI-TECH
SEMEN WARMER

Digital Display with Micro processor based temperature controller. Straight & inclined angle (30°C) Block for swim down and swim-up Technique Middle space for Semen Collection container and other disposable

HI-TECH
LAMINAR FLOW

0.3 microns HEPA Filers, 99.997% filteration efficiency Work table covered Stainless Steel Fitted with Static pressure Indicator (Manometer). Sterilization through UV - Germiciadal Tubes Size 2"x2"x2" or 4"x2"x2"

HI-TECH
SPERM COUNTING CHAMBER-SHUKRATARA



Specially designed for Semen Analysis Easy & quick Result

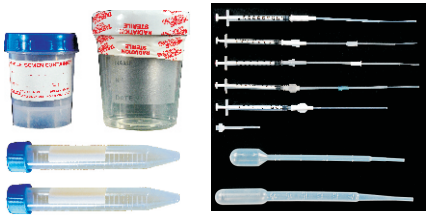
Count Spermatozoa in any 10 squares & concentrate in million / ml. directly. 10 micron depth.

HI-TECH
SPERM VIEWER CAMERA

Digital Eyepiece for video capturing

Just connect with microscope to Computer and capture Morphology, motility counts of semen & other pathological use.

A.R.T. Disposables
Centrifuge Tube, Pasture Pipette, Semen Container, IUI Catheter



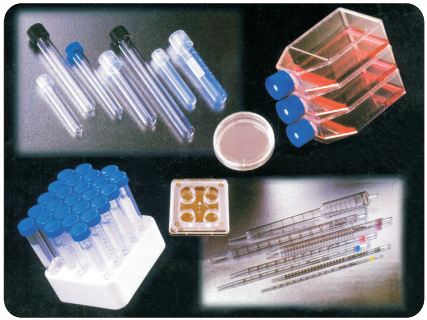
Vaginal Probe Cover

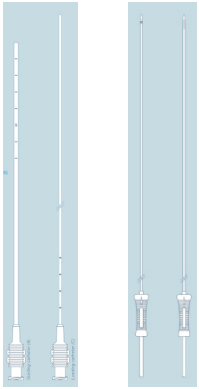
Powder Free Gloves

Endometrial Biopsy Curette
(Rampipella)

- All disposables are sterilized
- Individually or pair packing in sterilization indicator pouch
- Medical grade plastic.
- Batch, lot, manufacturing and expiry date indicated on product sticker

Falcon IVF Disposables





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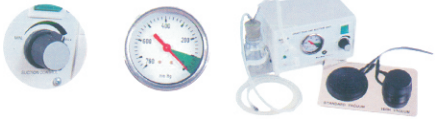
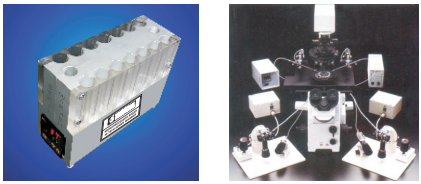
Practise better Medicine

Vitrolife IVF media

IVF/ICSI Equipments





InVitroCare IVF media

HTF HEPES

retrieval, follicle flushing, ICSI procedures and cryopreservation
Formulated with EDTA and a stabilized form of glutamine
12 month shelf life at 2-8°C
Cat # 2002 - 100ml or Cat # 2002-5 - 500ml

HTF

use as a **fertilization medium** and for early embryo culture through 2PN stage
Formulated with EDTA and a stabilized form of glutamine
120 day shelf life at 2-8°C
Cat # 2001 - 100ml

IVC-ONE™

For **early embryo development** from Day O through compaction
Glucose and Phosphate Free
Formulated with EDTA, Glutathione,
and a stabilized form of glutamine
120 day shelf life at 2-8°C
Cat # 2006 - 100ml

IVC-TWO™

For use in **Day 3 embryo transfers** and ICSI procedures
Low Glucose and Phosphate Free
Formulated with EDTA, Glutathione,
and a stabilized form of glutamine
120 day shelf life at 2-8°C
Cat # 2008 - 100ml

IVC-THREE™

For **extended embryo culture** and transfer through expanded **Blastocyst (Day 5)**
Phosphate Free and elevated levels of Glucose
Supplemented with amino acids and vitamins
Formulated with Glutathione
120 day shelf life at 2-8°C
Cat # 2007 -100ml

SpermPlus⁺Freeze

Cat. no. SPF1006

*SpermPlus⁺**Freeze*** is a chemically balanced based (without egg yolk) freezing medium for cryopreservation of human semen specimen used in assisted reproductive procedure. It contains sucrose & glycerol as cryoprotective agents and gentamicin as an antibiotic.

It is clinically tried and proven to give excellent sperm motility and other post thaw parameters.

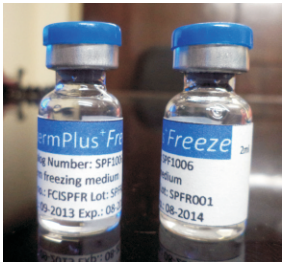
Contains: HSA, sucrose, Glycerol, potassium chloride, sodium chloride, magnesium chloride, sodium phosphate, HPLC water, EDTA, sodium bicarbonate, D-glucose and Gentamicin. All ingredients are cell culture grade.

Advantages:

- Small volume pack size to avoid cross contamination
- Give maximum motility percentage post thaw
- Increase survival time and success rate
- More protection during freezing step
- Long period shelf life of one year

Packaging:

1x 5 ml
10x2 ml



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