Nidacon Product Manual

Nidacon International AB

Nidation

1. The building of a nidus, a nest, as with birds

2. Implantation of the fertilized ovum (zygote) and the building of a nest, the placenta, in the endometrium.

Conception

1. The union of male and female gametes, the sperm and egg, to form a conceptus (also known as a zygote, or a pre-implantation embryo).

2. An impression or idea.

Contents

Nidacon
International AB

Introduction

Nidacon International AB (hereafter called Nidacon) manufactures and sells Medical Devices mainly for Assisted Reproduction Technologies (ART), with IVF, ICSI and insemination (IUI) solutions. The company was founded in 1996 by Assoc. Prof. Paul V. Holmes MSc, PhD, DrMedSc, an embryologist and endocrinologist from the Dept.of Obstetrics and Gynaecology at Sahlgrenska University Hospital in Gothenburg, Sweden.

Nidacon considers many different factors when designing its products. We hope that the attention to detail has helped to create products which will lead to better results. We aim to work in close relation with our customers; they are the cornerstones of our research department.

One of the first products to result from the company's research and development, PureSperm®, was introduced onto the market in November 1996. It has gained rapid acceptance and is now the global market leader for isolation and preparation of sperm used in human assisted reproduction. It was the first product of its kind to achieve both 510(k) clearance from the US FDA and CE marking with the European authorities.

Quality

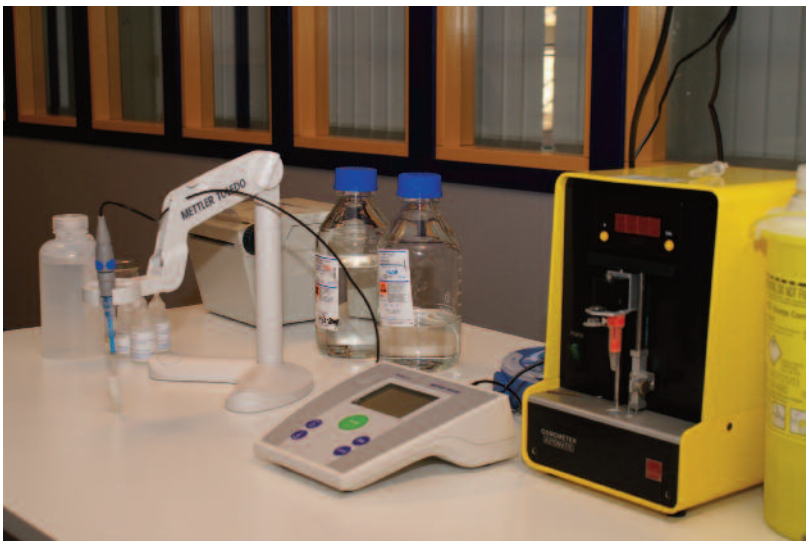
Nidacon is certified according SS-EN ISO 9001 (implemented 2000-12-15) and SS-EN ISO 13485 (implemented 2003-08-15). The management system secures continued development of the organisation.

We register our products according to the valid directives and requirements in different countries of the world. This also ensures our high quality on the market and it shall continue to be our beacon.

CE 0413

Nidacon intends to always maintain the high quality of its products and, in order to achieve this, all batches are tested at Nidacon before they are cleared for the market. Sterility controls are performed on each batch manufactured, the endotoxin level is measured and biological efficacy tests are carried out. A batch is only released for sale if it meets specific criteria.

Each batch is accompanied by a quality control certificate which records the results of the tests. Using this rigorous quality control system, we ensure that each batch meets the correct standards. Consequently the customers are secure in the knowledge that our products are reliable and will provide good results when used correctly.



Shelf life

Nidacon is conscious of customer requirements and always tries to provide products which are convenient. This convenience includes ease of transportation and long shelf life. Therefore, the products have a shelf life of one to two years at room temperature.

All ingredients are chosen for their temperature tolerance and their stability in aqueous solution. Rigorous shelf life testing has been carried out in Nidacon's laboratory to ensure that the theoretical stability of the salt formulations is matched by their actual stability when combined in the product.

Packaging

The packaging for Nidacon's products has received the same care and attention to detail as the design of the products.

Bottles; For most of our products we have chosen borosilicate glass instead of sodium silicate glass to avoid the leaching of sodium from the bottles into the contents during the long shelf life. Research in our laboratory has shown that sufficient sodium ions can leach from a sodium silicate bottle to have a negative effect on the development of two-cell mouse embryos. Therefore, we avoid exposing all cells to raised sodium-ion levels in the products by packaging in borosilicate glass.

Stoppers; Based on embryo-toxicity testing of three types of commercially available rubber stoppers approved for pharmaceutical use today, Nidacon chose silicone rubber as the material for the stoppers. We found that both natural latex rubber and butyl rubber are toxic to embryos, preventing development and maybe causing embryonic death. Silicone rubber did not have any detrimental effect, allowing embryonic development and hatching to proceed as usual. Therefore, stoppers made from pharmaceutial silicone rubber were chosen for our products.

"This convenience includes ease of transportation and long shelf life."

Background • Product composition • Buffer Glucose • Antibiotics • Additives

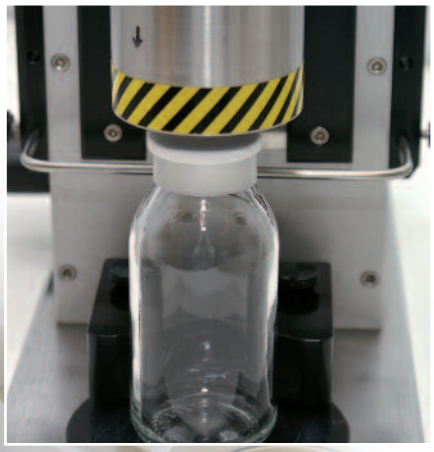
Background

Under normal physiological circumstances, sperm undergo a series of maturation changes after ejaculation which enables them to negotiate the different sections of the female reproductive tract, and eventually locate and fertilise the egg. If sperm are to be used for ART, it is essential that any product which is used for sperm preparation must match the sperm's physiological requirements as closely as possible. If sperm are stimulated excessively, particularly ionically, they become "hyperactive", a process which results in the sperm using up its energy resources and dying before fertilisation is achieved.

Therefore, the pH and osmolality of the sperm solutions must be adjusted very specifically to avoid ionic chock and subsequent hyperactivation.

Product composition

The component salts of Nidacon's products are balanced with specific regard to the ion composition of both the ejaculate and the female reproductive tract. This balance ensures a smooth transition from ejaculate to fertilisation medium via the gradient and wash.



Buffer

The zwitterion buffer, HEPES, is included to maintain the pH of the PureSperm® gradient and PureSperm® Wash while working with the sperm on the bench. Fluids designed to maintain pH in a CO_2 environment, i.e. in the incubator, are unsuitable for use outside the incubator as they do not possess sufficient buffering capacity to maintain the pH.

Fluctuations in pH and temperature are detrimental to sperm survival on the bench. In addition, HEPES has an anti-oxidant effect, reducing reactive oxygen species (ROS) which can be damaging in the sperm preparation.

Glucose

Glucose is a component of PureSperm® products. Glucose is the primary energy substrate available to sperm in the human female reproductive tract.

Antibiotics

Antibiotics are not included in our products for several reasons. Penicillin G, a commonly used antibiotic in cell culture medium only lasts for approximately 10 days in aqueous solution, being inactivated after this time and the degradation products are cell-toxic. Furthermore, this antibiotic is ineffective against some of the bacteria most commonly found in semen. Streptomycin and gentamycin are cytotoxic. Gentamycin, in particular, has been shown to be toxic to embryos.

Therefore, it seems prudent to avoid including potentially spermicidal components in sperm preparation fluids. Moreover, bacterial contamination in the ejaculate is removed by density gradient preparation. Therefore, the absence of antibiotics in the gradient will not be detrimental to the sperm preparation, and avoids exposing the sperm to potentially toxic compounds.

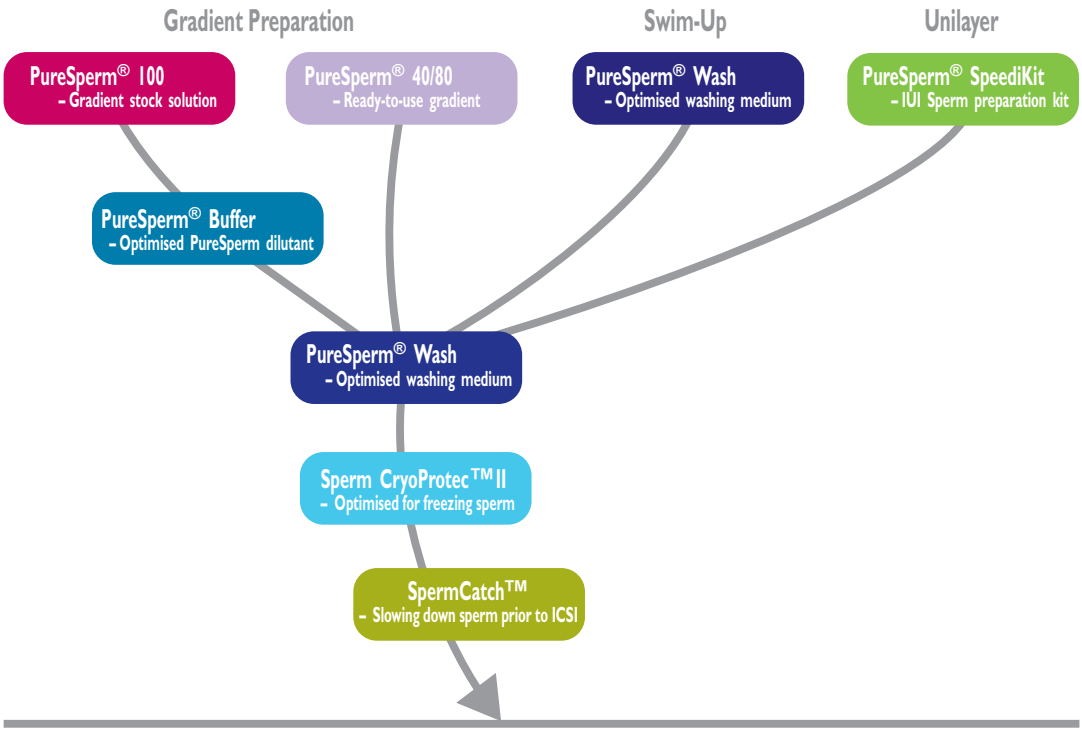
Additives and Phenol Red

No preservatives or unstable ingredients are added to Nidacon products. In addition, we have decided not to use phenol red in our media, since it has been proven to have estrogenic effects. Gametes have receptors for estrogen and they can be affected by its presence. For instance, it has been shown that estrogen inhibits sperm motility and the acrosome reaction.

Diagnostic Products

- **Sperm VitalStain™** – One-step vitality stain
- **Sperm MorfoStain™** – One-step morphology stain

Sperm Preparation



Culture Products

- **VitriBlast™** – Vitrification of blastocysts
- **ThermoBlast™** – Warming of vitrified blastocysts
- **NidOil™** – Overlay during embryo culture

Ordering information

Cat. No.	Description	Size
PSK-020	***PureSperm® 40/80***	2 × 20 mL
PS40-100	***PureSperm® 40***	100 mL
PS80-100	***PureSperm® 80***	100 mL
PS100-100	***PureSperm® 100***	100 mL
PS100-250	***PureSperm® 100***	250 mL
PS100-1000	***PureSperm® 100***	1000 mL
PSB-100	***PureSperm® Buffer***	100 mL
PSW-100	***PureSperm® Wash***	100 mL
PSW-020	***PureSperm® Wash***	2 × 20 mL

Cat. No.	Description	Size
PSSK-070	***PureSperm® SpeediKit***	5 patient preps
SC-100	***SpermCatch™***	6 × 100 µL
SCP-020	***Sperm CryoProtec™ II***	2 × 20 mL
NO-100	***NidOil™***	100 mL
NO-300	***NidOil™***	300 mL
SVS-010	***Sperm VitalStain™***	2 × 10 mL
SMS-250	***Sperm MorfoStain™***	250 mL
VBK-010	***VitriBlast™ Kit***	3 × 10 mL
TBK-010	***ThermoBlast™ Kit***	4 × 10 mL

10 items or more on one purchase order gives 5% discount.
We have distributors in most countries, for a complete list take a look at our web page www.nidacon.com

Products

PureSperm® 100

is a sterile (autoclaved SAL-10^{-3}), silane-coated, silica colloid in an isotonic salt solution. It is optimised for the preparation of discontinuous density gradients for the separation and purification of human sperm.

Shelf life 2 years. CE 0413

Components	
Silane-Coated Silica	*H_2O*
NaCl	*CaCl*
Glucose	*KCl*
EDTA	
HEPES	

PureSperm® 40 PureSperm® 80

Ready-to-use density gradient solutions, 40 and 80%. Makes lab-work easier and minimizes the risk for mistakes.

Shelf life 2 years. CE 0413

Components	
Silane-Coated Silica	*KCl*
NaCl	*Citrate*
Glucose	*Lactate*
Na Pyruvate	*HEPES*
EDTA	*H_2O*

PureSperm® Buffer

a balanced salt solution designed specifically for diluting PureSperm® 100 to make up the layers of different densities for a discontinuous gradient. The optimised formulation of PureSperm® Buffer is designed to maximise sperm survival during gradient centrifugation.

Shelf life 2 years. CE 0413

Components	
NaCl	*EDTA*
KCl	*Citrate*
HEPES	*Glucose*
Lactate	*H_2O*
Pyruvate	

PureSperm® Wash

a sterile isotonic salt solution. It is optimised for washing the sperm recovered from density gradient preparations, for use in swim-up procedures, for extension of sperm prior to IUI or as a medium for maintaining sperm.

Shelf life 1 year.

Components	
NaCl	*Pyruvate*
$MgSO_4$	*hSA*
	(human serum albumin)
KCl	*EDTA*
KH_2PO_4	*H_2O*
Glucose	*HEPES*
$NaHCO_3$	

PureSperm® SpeediKit

is a kit that provides you with all the components required to prepare 5 human sperm samples for IUI. It contains semen sample tubes, ready-to-use tubes for a single layer colloid centrifugation and ready-to-use tubes with PureSperm® Wash for the washing of the pellet after centrifugation. A perfect product for the small clinic, 5 patients /kit.

Shelf life 1,5 years.

Components	
Silane-Coated Silica	*KCl*
NaCl	*Citrate*
Glucose	*Lactate*
Na Pyruvate	*HEPES*
EDTA	*H_2O*
$MgSO_4$	*$NaHCO_3$*
hSA (human serum albumin)	

Sperm CryoProtec™II

a sterile salt solution containing glycerol, optimised for freezing both gradient-prepared sperm and for unprocessed ejaculates. Nidacon recommends the nitrogen-vapour freezing technique, since this technique seems to provide the best results after thawing

Shelf life 1 year. CE 0413

Components	
NaCl	*EDTA*
KCl	*$NaHCO_3$*
HEPES	*Lactate*
Glucose	*Glycerol*
$MgSO_4$	*Pyruvate*
KH_2PO_4	

Sperm VitalStain™

a one step staining technique for assessment of sperm vitality, one of the basic tools in semen analysis.

Shelf life 2 years. CE 0413

Components	
NaCl	*Nigrosine*
Eosin	*Formalin*
H_2O	

Sperm MorfoStain™

a classical Romanovsky stain. It is a one-step stain optimised for assessment of sperm morphology.

Shelf life 2 years. CE 0413

Components	
Methanol	*May Grunwald*
Eosin Y	*Giemsa*
Methylene B	*Azur B*

Products

SpermCatch™

for slowing down sperm prior to ICSI without using polyvinylpyrrolidone (PVP). To avoid ICSI injection of PVP, it contains only natural products for increasing the viscosity.

Shelf life 1 year.

Components	
NaCl	*Pyruvate*
$MgSO_4$	*hSA*
	(human serum albumin)
KCl	*EDTA*
KH_2PO_4	*H_2O*
Glucose	*HEPES*
$NaHCO_3$	*Hyaluronic acid*

NidOil™

a sterile, light paraffin oil for use as an overlay during gamete, zygote and pre-embryo culture in the incubator, or during manipulations outside the incubator. No additives, UV-protective packaging.

Shelf life 2 years. CE 0413

Components
Light paraffin oil

VitriBlast™

A kit for vitrification of blastocysts. based on well tested formulations. Numerous publications demonstrates their effectiveness regarding both survival rates and pregnancy rates.

Shelf life 6 months.

Components	
NaCl	*KCl*
$MgSO_4$	*KH_2PO_4*
Glucose	*$NaHCO_3$*
Pyruvate	*hSA*
	(human serum albumin)
EDTA	*H_2O*
HEPES	*Lactate*
Sucrose	*Ethyleneglycol*
DMSO	*Ficoll*

ThermoBlast™

A kit optimised for warming blastocysts vitrified with VitriBlast™ Kit. Ready-to-use solutions.

Shelf life 6 months.

Components	
NaCl	*KCl*
$MgSO_4$	*KH_2PO_4*
Glucose	*$NaHCO_3$*
Pyruvate	*hSA*
	(human serum albumin)
EDTA	*H_2O*
HEPES	*Lactate*
Sucrose	

General information

Background

A normal semen sample (ejaculate) is made up of seminal fluid which contains a number of different cells, cell debris, microbiological and biological substances.

The different cell types contained in semen are normal motile sperm, juvenile sperm and senescent sperm (no fertilisation function) and sperm with DNA breaks. Epithelial cells from the male reproductive tract, male immune cells and cell debris (detritus) are also present in the semen, as are bacteria and possibly viruses.

Moreover, the seminal fluid contains biologicals such as sperm decapacitating factors and reactive oxygen species (ROS), both of which negatively affect fertilisation.

After ejaculation in vivo, normal sperm quickly migrate from the liquefied semen into the uterine cervix of the female, thereby separating themselves from adverse affects of the factors mentioned above.

In the andrology laboratory of an IVF-clinic, separation of the normal motile sperm from seminal fluid and its contents can be achieved by using either a "discontinuous density gradient" or a "swim-up".

Positive features of a discontinuous density gradient according to Nidacon.

Feature	Density Gradients	Swim-Up
Separates motile sperm from other cell types	✔	✔
Separates out immature, aged and dying sperm	✔	—
Separates out morphologically abnormal sperm	✔	—
Separates out sperm with damaged chromatin	✔	—
Removes bacteria and viruses	✔	—

If the density gradient has been made correctly, the sperm pellet should contain only functional, fertile sperm.

General care and use

- All solutions should be brought to room temperature before use to avoid the temperature fluctuations which are detrimental to sperm survival.
- Open and reseal bottles in a laminar air-flow bench using sterile techniques to avoid contamination.
- Store all opened bottles at 2-8°C after re-sealing.

Density Gradient Preparation

PureSperm® 100 **PureSperm® 40** **PureSperm® 80**

PureSperm® Buffer **PureSperm® Wash**

Recommendations

If you have a sample with a high volume (>3mL), you can prepare two PureSperm gradients for each semen sample. This reduces the risk of overloading a single gradient, provides security when handling tubes or recovering sperm pellets and provides two tubes to balance the centrifuge rotor.

Reagents and Equipment

PureSperm® 100 plus PureSperm Buffer or
PureSperm® 40 and 80
Sterile Pasteur pipettes
PureSperm® Wash
Sterile 2 mL and 10 mL pipettes
Bench top centrifuge with swing out rotor

Procedure

1. If you use PureSperm® 100, dilute with PureSperm® Buffer to make your gradient solutions, for example add 2 mL PureSperm® Buffer to 8 mL PureSperm® 100 to obtain 10 mL 80% PureSperm. Add 6 mL PureSperm® Buffer to 4 mL PureSperm® 100 to obtain 10 mL 40% PureSperm. Instead you can use the ready-to-use PureSperm® 40 and PureSperm® 80 solutions.

2. Use a sterile pipette to add 2 mL of 80% PureSperm to a conical tube.

3. Use a new pipette to carefully layer 2 mL 40% PureSperm on top of the 80% layer. It is important not to disrupt the two layers and to maintain a sharp interface.

4. Layer the liquefied semen onto the gradient. We recommend that you don't take more than 1,5 mL /gradient or you risk overloading the gradient and not getting a good result.

5. Centrifuge at 300 x g for 20 minutes. Make sure that your centrifuge uses the correct g-force (use equation). Do not use the brake.

6. Aspirate in a circular movement from the surface everything except the pellet and 4-6 mm of the 80% PureSperm layer. If no pellet is seen after centrifugation, remove all fluid except the lowest 0.5 mL.

7. Use a new pipette to aspirate the pellet (or the lowest 0.5 mL). Transfer sperm pellet to a new tube and re-suspend pellet in 5 mL PureSperm® Wash. Always use a new tube with PureSperm® Wash to avoid contamination from the ejaculate. Combine sperm pellets if double procedure has been used.

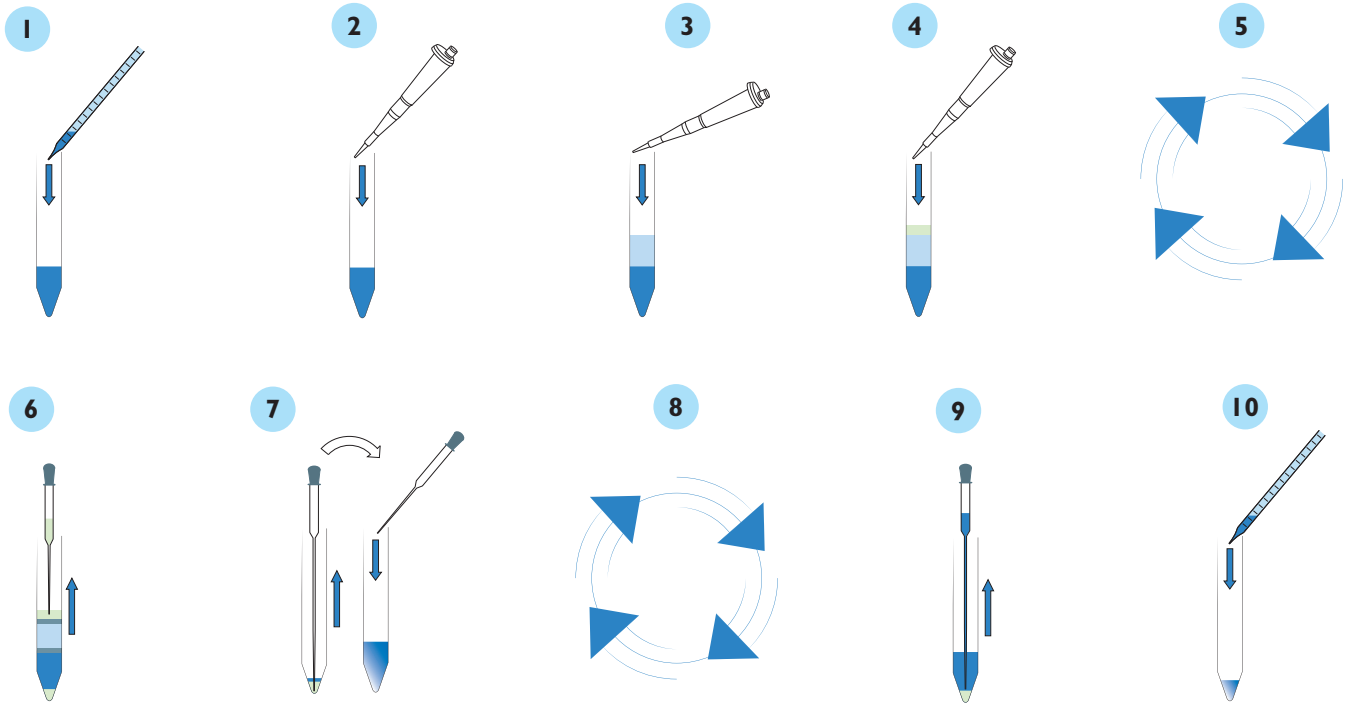
8. Centrifuge at 500 x g for 10 minutes. Do not use the brake.

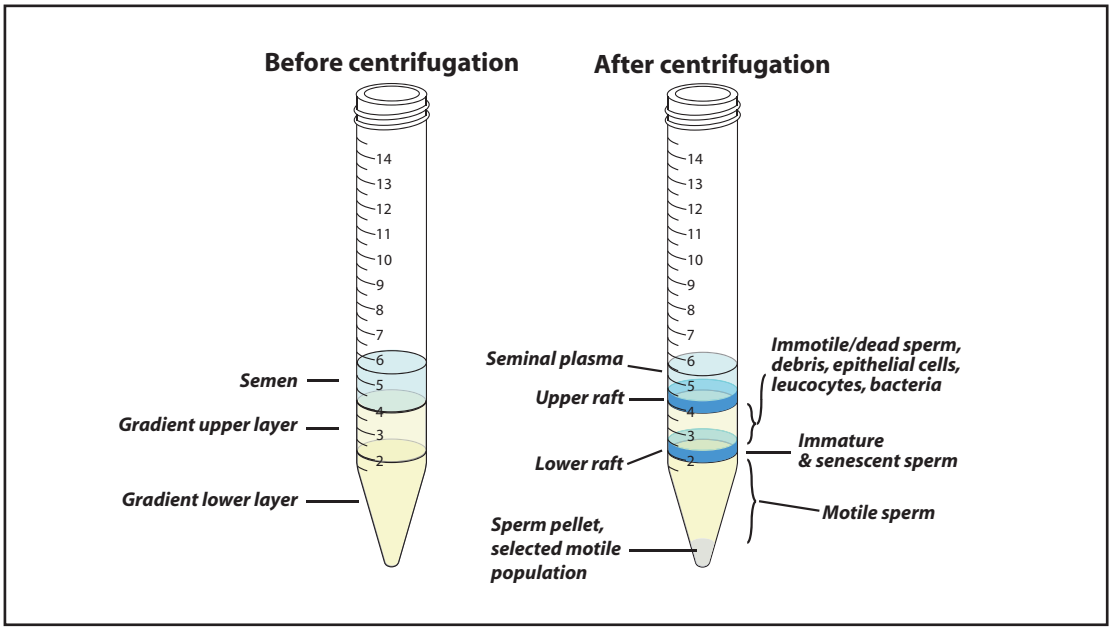
9. Aspirate PureSperm® Wash supernatant leaving as little liquid as possible above the pellet. If no pellet is seen, leave the bottom 0.25 mL fluid.

10. Resuspend the sperm pellet in a suitable volume of media. The sample is now ready for use.

Tips

- Gradients should be layered immediately prior to use but the different density solutions of PureSperm can be prepared in advance, provided that they are stored at 4°C and brought to room temperature before use.
- When retrieving the pellet after the gradient centrifugation, care must be taken to avoid contaminating the pellet with components of the ejaculate or upper gradient layer. Therefore we recommend that you use a new pipette after removing most of the gradient to avoid contamination, for example, by bacteria.





Calibrate the centrifuge; to achieve the correct g force, use the equation:

Rpm = $\sqrt{[\ (g/(1.118 \times r)]} \times 10^3$

g = the centrifugal force

r = rotational radius, the distance (mm) from the centre of the rotor to the bottom of a centrifuge tube in the bucket when raised to horizontal position

For example; to achieve 300 x g when radius = 165mm the centrifuge speed must be:

Rpm = $\sqrt{[\ (300/(1.118 \times 165)]} \times 10^3 = 1275$

PureSperm® SpeediKit

Background

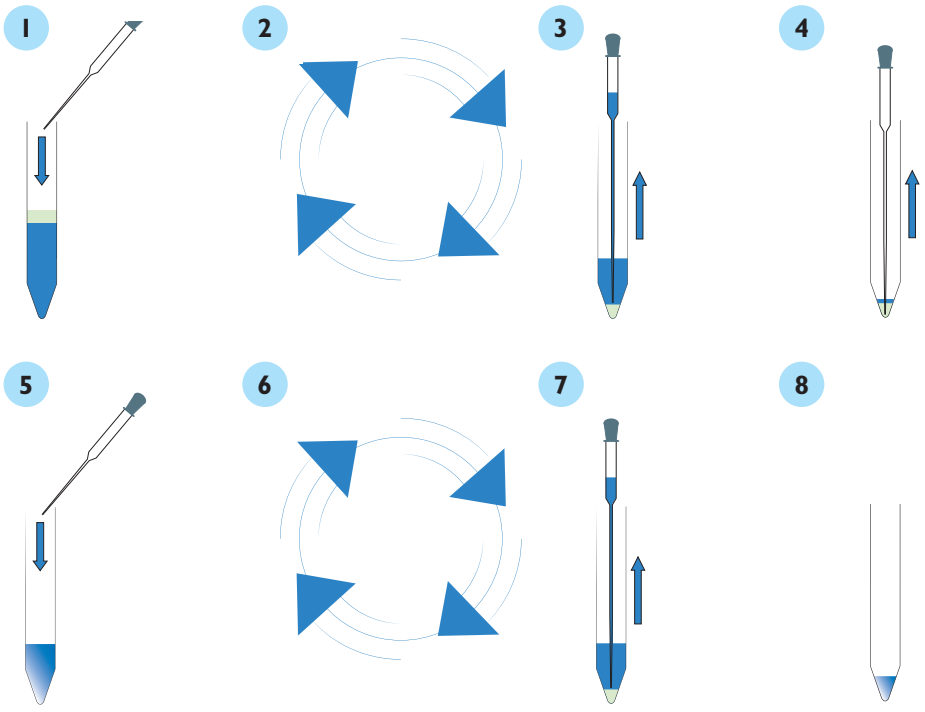
We especially recommend PureSperm® SpeediKit for the smaller clinics or for IUI clinics. This SpeediKit is a rapid and efficient alternative to sperm-preps using two-layer, discontinuous density 'gradient' centrifugation. Everything is included in a convenient kit form for rapid sperm preparation, all based on the effective centrifugation through a single layer of PureSperm colloid, instead of a two-layer gradient, followed by rinsing the sperm with PureSperm® Wash. The kit contains both the PureSperm colloid and the PureSperm® Wash for 5 patients, already dispensed in centrifuge tubes, plus semen collection tubes. You do not need an incubator.

Reagents and Equipment

Semen collection tubes (included in the kit)
Ready-to-use tubes of PureSperm® Unilayer and PureSperm® Wash (included in the kit)
Balance tubes (included in the kit)
Bench top centrifuge with swing-out rotor
Sterile Pasteur pipettes

Procedure

1. Use a sterile pipette to carefully layer liquified semen (up to 1.5 mL) on top of the PureSperm® Unilayer. If you have a sample volume greater than 1.5 mL, use two tubes.
2. Centrifuge at 300 x g for 30 minutes. Do not use the brake.
3. Use a new sterile pipette to aspirate the supernatant, leaving about 5 mm of liquid above the pellet. If no pellet is seen after centrifugation, remove all fluid except the lowest 0.5 mL.
4. Use a new pipette to aspirate the pellet (or the lowest 0.5 mL).
5. Transfer sperm pellet to the tube containing PureSperm® Wash. Resuspend the sperm.
6. Centrifuge at 500 x g for 10 minutes. Do not use the brake.
7. Use a new pipette to aspirate the supernatant, leaving as little liquid as possible above the pellet. If no pellet is seen, leave the bottom 0.25 mL fluid.
8. Resuspend the pellet in the remaining PureSperm® Wash. The sperm preparation is now ready for IUI.



Tips

- All you need are the semen samples, a centrifuge and sterile pipettes; the rest you will find in the SpeediKit.

PureSperm® Wash

Background

For most situations Nidacon recommends using a discontinuous density gradient for preparing human sperm from semen. However, many customers at some time need to use the Swim-up technique and the most ideal product for this purpose is PureSperm® Wash.

PureSperm® Wash is a salt solution balanced and adjusted for the nutrition and long survival of human sperm. It functions exceeding well in this role

Recommendations

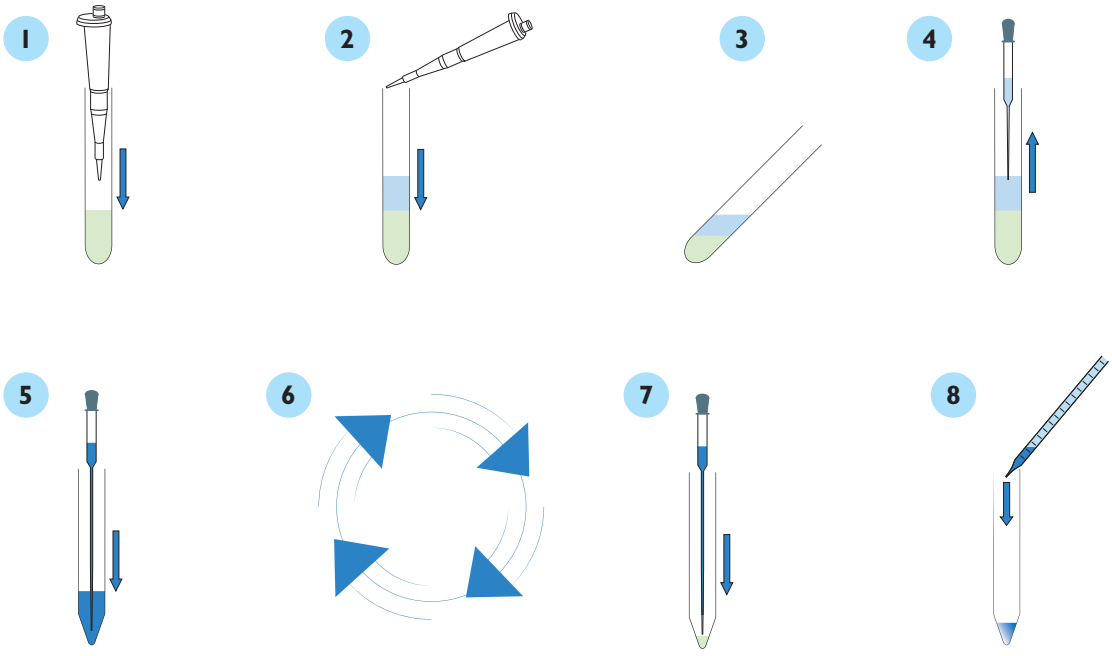
Since PureSperm® Wash does not contain any antibiotics and since Swim-Up cannot guarantee removal of bacterial contamination, it is recommended to add antibiotics when using Swim-Up to prepare sperm for ART. We recommend that you add Penicillin at a concentration of 100 U/mL.

Reagents and Equipment

PureSperm® Wash
Round bottomed centrifuge tubes
Disposable sterile conical centrifuge tubes
Sterile pipettes
CO_2 incubator
Bench top centrifuge with swing out rotor

Procedure

1. Transfer 1 mL of liquefied semen to a sterile round bottomed centrifuge tube. If the sample is too viscous, try diluting it with PureSperm® Wash before.
2. Use a new pipette to carefully layer 1,5 mL PureSperm® Wash over the semen.
3. Without disturbing the layers, place the centrifuge tube at a 45° angle into a CO_2 incubator, at 37°C for 60 minutes.
4. Carefully remove the uppermost (0,5-1,0 mL) of medium containing motile sperm using a sterile pipette.
5. Place this fluid in a sterile conical centrifuge tube containing 5 mL PureSperm® Wash.
6. Centrifuge at 500 x g for 10 minutes. Do not use the brake.
7. Aspirate the supernatant, leaving no more than 2 mm depth of liquid above pellet.
8. Resuspend the sperm pellet in a suitable volume of medium to obtain the required sperm concentration. The sample is now ready for analysis or use.



Tips

- If you have a viscous sample, be careful when you remove the upper layer after incubation. It is easy to get hold of the semen sample and disrupt the layers.

Freezing of spermatozoa

Sperm CryoProtec™II

Background

Sperm CryoProtec™II does not contain material of animal origin, such as egg yolk. The cryoprotectant in Sperm CryoProtec™II is glycerol, the proportion being reduced as far as possible to minimize toxicity to sperm, while still providing cryoprotection. Moreover, a high concentration of glucose is present as an osmotic agent to reduce intracellular water, thus diminishing damage due to ice-crystal formation.

Recommendations

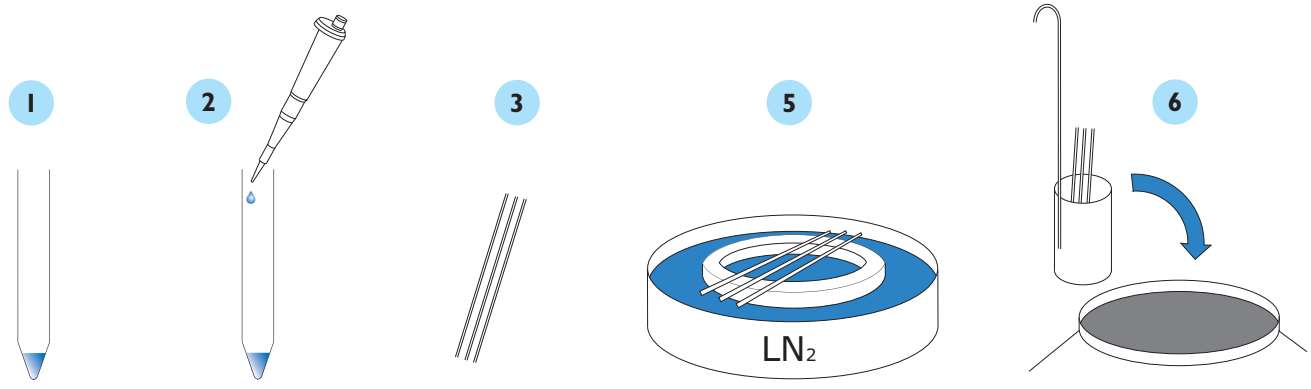
Although it is possible to freeze unprocessed semen, we recommend that you prepare the ejaculate using a PureSperm density gradient. This method removes seminal plasma as well as ROS and their sources, thereby ensuring optimal recovery of motile sperm on thawing.

Reagents and Equipment

Sperm CryoProtec™II and PureSperm® Wash
Sterile pipettes
Disposable sterile centrifuge tubes (e.g. Falcon 2075)
Disposable sterile cryopreservation vials or plastic straws
Scissors

Processed ejaculate

1. When using prepared sperm, resuspend sperm pellet in a small volume of PureSperm® Wash to obtain the desired concentration of sperm.
2. Add 1 part of Sperm CryoProtec™II to 3 parts of sample (see dilution table) ensuring thorough mixing after adding each drop.
3. Fill straws with sperm suspension or aliquot into vials.
4. Equilibrate straws or vials in refrigerator for 30-60 minutes.
5. Place the straws horizontally in nitrogen vapour, 1 cm above the liquid nitrogen surface on a piece of styrofoam (cryo floater). Leave for 30 minutes.
6. Transfer the straws quickly into the liquid nitrogen and, thereafter, store in liquid nitrogen.



Unprocessed ejaculate

1. Add 1/3 of Sperm CryoProtec™II (see dilution table) drop wise, ensuring thorough mixing after adding each drop.
2. Continue as for gradient-prepared sperm.

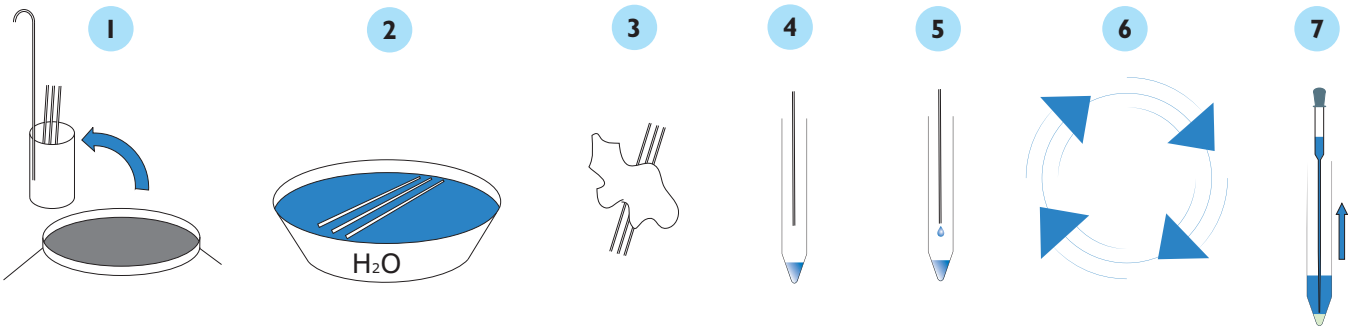
Dilution table

Sperm Sample (µL)	SCPII™ (µL)	Sperm Sample (µL)	SCPII™ (µL)	Sperm Sample (µL)	SCPII™ (µL)
100	33	1100	367	2100	700
200	67	1200	400	2200	733
300	100	1300	433	2300	767
400	133	1400	467	2400	800
500	167	1500	500	2500	833
600	200	1600	533	2600	867
700	233	1700	567	2700	900
800	267	1800	600	2800	933
900	300	1900	633	2900	967
1000	333	2000	667	3000	1000

For other volumes than those listed; calculate:	
Volume Sperm Sample / 3 = Volume SCPII	Example: 300 µL Sperm Sample / 3 = 100 µL SCPII

Thawing procedure

1. Remove the straws from the liquid nitrogen tank.
2. Place straws in water at 37°C for 30 secs.
3. Dry surface of straw.
4. Cut one end of straw.
5. Hold the straw over a tube with 5 ml PureSperm® Wash and cut the other end of the straw. Any sperm suspension remaining in the straw can be expelled using a pipette.
6. Centrifuge at 500 x g for 10 minutes. Do not use the brake.
7. Aspirate PureSperm® Wash supernatant leaving as much liquid as required for desired concentration. If no pellet is seen, leave the bottom 0.10 mL fluid.
8. For the thawed unprocessed ejaculate we recommend that you perform a density gradient preparation (see density gradient preparation).



Tips

- To avoid osmotic chock for the sperm, it is important to slowly mix Sperm CryoProtec™II with your sperm sample but don't mix for longer that 5 minutes.
- The incubation time before freezing can be reduced to 15 minutes but we recommend 60 minutes.
- When thawing unprocessed sperm, expel the straw contents into 0.5 – 1 mL PureSperm® Wash prior to gradient preparation.

SpermCatch™

Background

SpermCatch™ is an alternative to PVP (polyvinyl-pyrrolidone) which today is the most common substance product used for slowing down sperm prior to ICSI. However, PVP has been reported to cause problems, like damaging the sperm plasma membrane and it may also interfere with sperm nucleus decondensation.

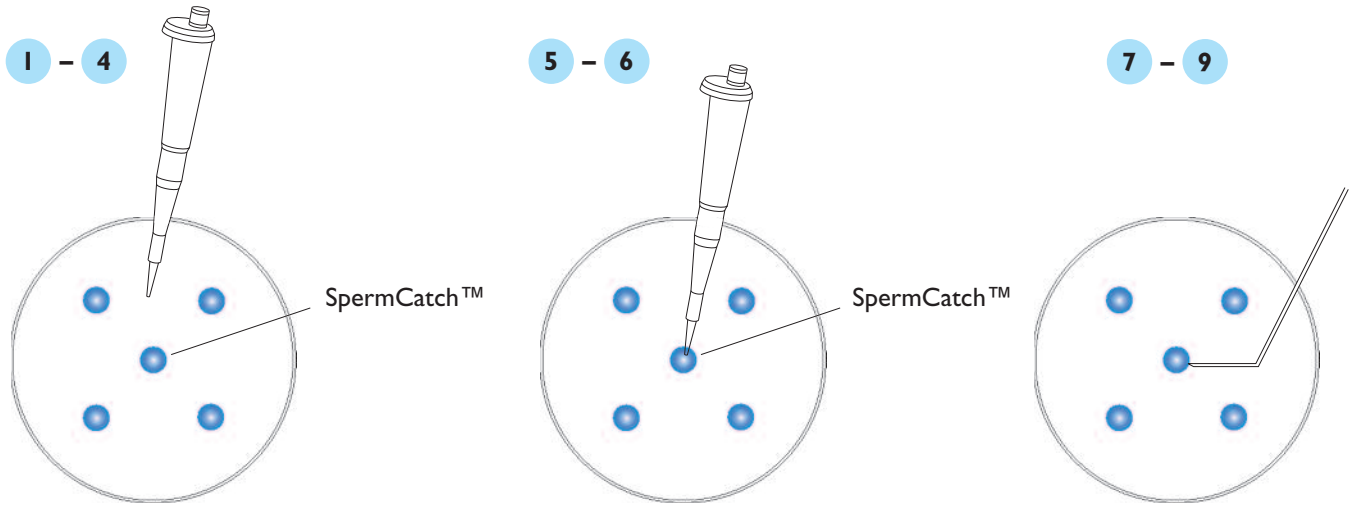
SpermCatch™ is a solution without PVP and contains instead hyaluronic acid which is a natural component. Several studies have shown that SpermCatch™ gives the same or even better results than PVP. Since Sperm-Catch™ is a solution containing hyaluronic acid, see the following reference for the advantages. (ref 21)

Reagents and Equipment

SpermCatch™
NidOil™
Injection media
Sterile pipettes
ICSI equipment
Petri dish

Procedure

1. Place a 10 µL drop of SpermCatch™ in the middle of a petri dish.
2. Place 4 drops of 10 µL injection media around the SpermCatch™ drop in the petri dish.
3. Immediately cover the drops with NidOil™.
4. Incubate for 30 minutes in CO_2 environment at 37°C.
5. Add 1 µL of prepared sperm suspension to the middle of the SpermCatch™ drop.
6. Incubate for 10 minutes in CO_2 environment at 37°C.
7. Fill your injection pipette with SpermCatch™ to avoid the sperm sticking to the inside of your pipette. It will also help you to make a controlled injection.
8. Immobilise the individual sperm by using the injection pipette to ”knick” the sperm tail.
9. Aspirate the immobilised sperm.
10. Move to one of the oocyte droplets. Focus on the oocyte and position the oocyte with the holding pipette. Bring down your injection pipette and inject the sperm. Make sure that the oolemma is broken before you expel the sperm.



Tips

- ICSI dishes must be prepared quickly to avoid osmolarity changes in the media. Only make two at a time.
- It is convenient to have two dishes per patient.

NidOil™

Background

Mineral oil to overlay the embryo culture is used extensively in IVF laboratories. NidOil™ contains a paraffin oil which has been specifically chosen and then treated in our production laboratories to ensure that its purity and handling characteristics be suitable for using as an overlay when culturing gametes and embryos.

NidOil™ does not require washing before use, and it is neither too sticky nor too viscous, to facilitate pipetting.

Stringent quality assurance controls are carried out on each batch to ensure freedom from microbiological contamination and low endotoxin levels.

There have been several reports of paraffin oils becoming embryo-toxic after exposure to light on the laboratory bench. As a precaution against any possible light-induced changes, NidOil™ is packaged in amber, screw-top bottles.

Recommendations before use

NidOil™ should be equilibrated in the same way as the culture medium before use to avoid differences in temperature and gaseous content between the components of the culture system.

Vitrification and warming of blastocysts

VitriBlast™ Kit ThermoBlast™Kit

Background

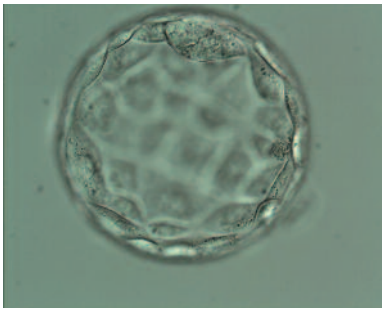
The main problem when freezing cells is the formation of intracellular ice crystal both during cooling and warming, since these ice crystals have a detrimental effect on cell survival. Vitrification, the extremely rapid freezing of cellular material, makes it possible to freeze cells without forming ice crystals within the cells. The result of vitrification is a very homogenous structure, an amorphous crystalline structure.

Recommendations

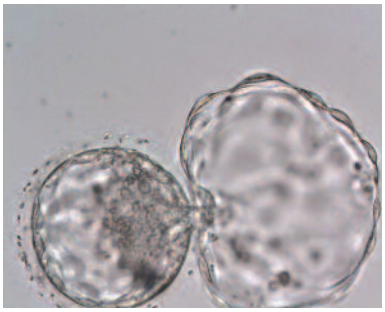
VitriBlast™ can be used with different types of vitrification-devices, like cryotop, cryoloop or the HSV (high security straw). The described method below is with the cryoloop but the same protocol can be used for all devices. Work on a heated stage at all times when manipulating the blastocyst. Do not let the blastocyst-remain exposed to the microscope light during incubation.

Reagents and Equipment

VitriBlast™ and ThermoBlast™ kit
Sterile pipettes
Device for vitrification
CO_2 incubator
Stop watch or timer
Liquid nitrogen reservoir
Liquid nitrogen
Culture dishes (NUNC 4-well)
Heated stage
Inverted microscope



Vitrified and warmed blastocyst with excellent morphology.



Hatching blastocyst.

Vitrification procedure using the cryoloop

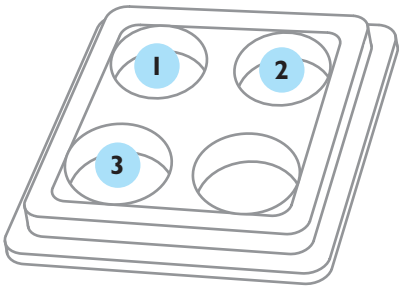
Additional equipment:
Cryocane for storage of cryo tubes
Crystalwand
Vial Clamp for holding the cryo tube
Cryoloop
(Hampton Research)

Note: If the additives are stored in the refrigerator, remove them in good time prior to use. DMSO turns solid below +18°C. The additives can be stored outside the refrigerator in their little box, even after opening. The DMSO can be warmed in the hand if urgent.

1. Label the NUNC-dish with the patient ID and each well with each solution number, i.e. 1, 2 and 3.

2. Pipette the vitrification media as described below. When adding the DMSO and Ethylene glycol (EG), which are included in the kit, to solution 2 and 3, pipette the two up and down a few times to obtain optimal mixing of the media.

Well 1	
VitriBlast™ 1	1000 µL
Well 2	
VitriBlast™ 2	850 µL
DMSO:	75 µL
EG:	75 µL
Well 3	
VitriBlast™ 3	700 µL
DMSO:	150 µL
EG:	150 µL



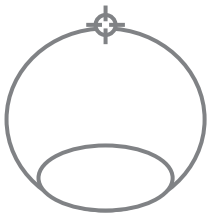
Vitrification procedure using the cryoloop

3. Incubate at 37°C in CO_2 for 30 minutes (maximum 1 hour, since longer time makes it difficult to create a film on the loop).

4. During the 30 minutes incubation of the dish, collapse the blastocyst. This can be done either by laser (Fertilase, red, 5, see pictures below) or by using an ICSI-pipette.

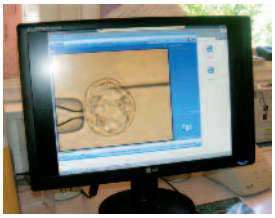
Laser

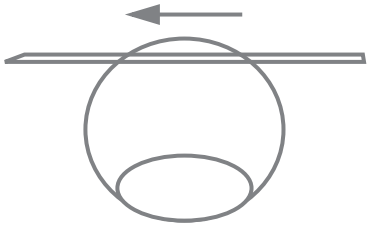
- If laser is used, shoot as far from inner cell mass (ICM) as possible. The laser beam shoots vertically, aim as illustrated below.
- Be sure that you create a hole through the zona and the trophectoderm.



ICSI-pipette

- If an ICSI-pipette or other sharp instrument is used, puncture right trough the trophoblast cell layer into the blastocoele, and be sure to puncture as as possible from the ICM.
- The pipette should be inserted at the one o´clock position and through the blastocyst at 11 o´clock.



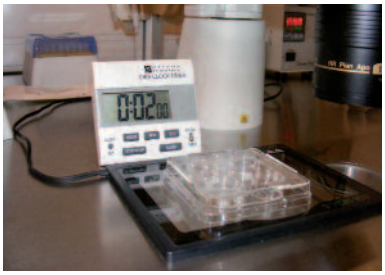


5. Remove the NUNC-dish from the incubator and place it on a heating stage (make sure the heat controller is set high enough to obtain 37°C in the media).

6. Place the punctured and collapsed blastocyst in solution nr 1. Start the stop watch.

7. After 1.5-2 minutes, move the blastocyst by aspirating solution no 2 into the pipette tip, then by collecting the blastocyst from solution 1, and thereafter by transferring it to solution no 2 (well 2).

8. Incubate on the heating stage for EXACTLY 2 minutes. Start the stopwatch and observe when 2 minutes is approaching (it is easier to start the stopwatch and let it run towards 2 minutes, than to set it on 2 minutes countdown, this removes the stress of the beeping noise). While incubating; proceed to step 9 below.



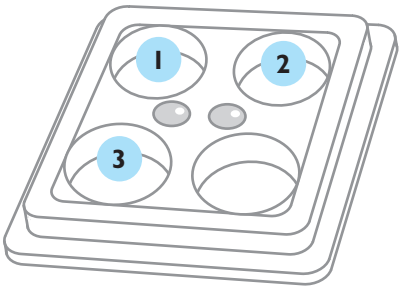
Do not let the blastocyst remain exposed to the microscope light during the incubation.

Vitrification procedure using the cryoloop

9. During the 2 minutes incubation; prepare 2 x 10 µL drops of solution 3 in the middle of the dish (see diagram below). The droplets evaporate quickly; prepare them as late as possible.

10. Attach the loop to the Crystalwand.

11. At the correct time, move the blastocyst by aspirating solution no 3 from the well into the pipette tip, collect the blastocyst from solution 2 in the second well, and then transfer it to solution no 3 in the 10 µL droplet. The blastocyst must remain in solution 3 for exactly **30 seconds**, including the time in the loop. No shorter or longer time.



12. Coat the loop with the solution in the other 10 µL droplet, and place the blastocyst in the loop.

Note: Using drops reduces the risk of loosing the blastocyst. The blastocyst tends to float in the viscous medium nr 3. It is also important to incubate nr 3 in the same condition as the other two solutions, hence the use of 1 mL.

13. Immerse in liquid nitrogen.

14. Attach the cryo tube to the Vial Clamp and immerse the tube in the liquid nitrogen allowing it to fill. Insert the loop into the tube, very carefully. Keep the loop in liquid nitrogen during the whole procedure. Use the Crystalwand to close the tube.

15. Attach the tube to the Cryocane for storage in liquid nitrogen.

Warming procedure

1. Label the NUNC-dish with the patient ID and each well with each solution number, i.e. 4, 5, 6 and 6 respectively.

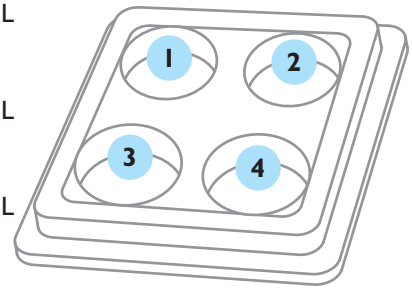
2. Pipette the warming media 4, 5 and 6 as described below.

Well 1
ThermoBlast™4: 1000 µL

Well 2
ThermoBlast™5: 1000 µL

Well 3
ThermoBlast™6: 1000 µL

Well 4
ThermoBlast™6: 1000 µL



3. Incubate at 37°C in CO_2 for 30 minutes.

4. Carefully detach the loop from the cryo tube, making sure not to touch the inside of the tube with the loop (the blastocyst may be lost). Unscrewing the top and moving the loop from the tube are the most risk-filled moments in the procedure.

5. Immerse the loop in the surface of solution 4. Let the blastocyst fall off. Identify its presence in the well and incubate for 2 minutes on the heating stage. (2 minutes includes time for "finding" the blastocyst).

6. Move the blastocyst by aspirating solution no 5 into the pipette tip, collect the blastocyst from solution 4, and transfer to solution 5. Incubate for 3 minutes in solution 5.

7. Move the blastocyst by aspirating solution no 6 into the pipette tip, collect the blastocyst from solution 5, and transfer to solution 6. Move around the blastocyst to rinse it, and then transfer to the second well containing solution 6.

8. Incubate for 5 minutes in solution 6 on the heating stage.

9. Thereafter, transfer the blastocyst to culture medium and allow the blastocyst time to reexpand. Wait for 1 to 4 hours before final judgement. If the blastocyst has not reexpanded after 4 hours, the chance of reexpansion is negligible.

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Vitality Staining

Sperm VitalStain™

Background

Sperm vitality should be determined in semen samples with 50% or more immotile spermatozoa according to the WHO laboratory manual for the examination of human sperm.

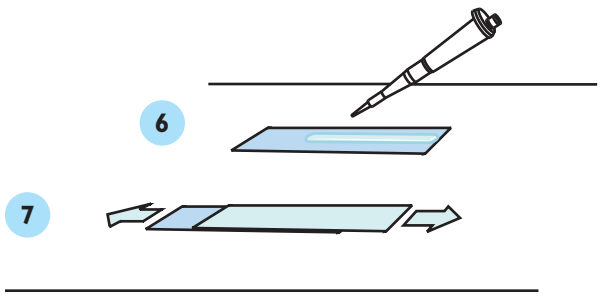
SpermVitalStain uses the eosin-nigrosine technique in a one-step method to establish the percentage of live spermatozoa. It is based on the principle that dead cells (i.e. those with damaged plasma membranes) will take up the eosin and stain red. Nigrosine provides the background to facilitate visualisation of the unstained (white) live cells.

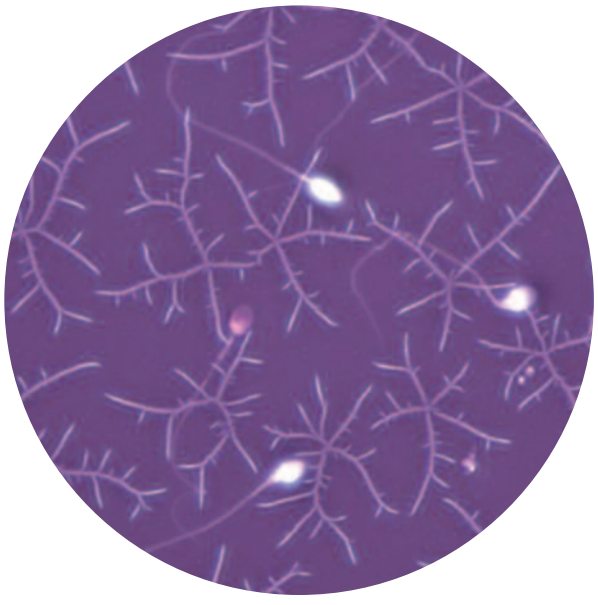
Reagents and Equipment

Light microscope (40 – 100 x magnification)
Microscope slides
Pipette
Test tube

Procedure

1. Shake the bottle of Sperm VitalStain™ before use.
2. Take an equal amount of Sperm VitalStain™ and the sperm sample (eg. 50 µL SVS + 50 µL sample). Use for example an eppendorf tube.
3. Mix well.
4. Leave for 30 secunds at room temperature.
5. Prepare a slide using your conventional method or use the method recommended by Nidacon.
6. Transfer a 20 µL drop onto a labelled microscope slide with a pipette, making a string/line of fluid in the middle of the slide.
7. Cover this slide with a second microscope slide and, when the drop is evenly spread between the two slides, pull them apart from each other horizontally. You then have two good slides.
8. Air dry the two slides and examine. If you want to store for later use, mount the slides with DPX or equivalent mountant, and a cover slip.
9. Examine using a bright field 40 x objective or a 100 x objective under oil immersion.
10. Count 200 sperm, the white (unstained) are classified as alive and the red or pink are classified as dead. Sperm coloured only at the neck region are classified as alive.





Tips

- The 100x objective with immersion oil will give you a very clear picture of stained versus unstained sperm.

Sperm MorfoStain™

Background

The technique is based on the principle that sperm with different characteristics will stain so that one can differentiate them. The numerical estimation of abnormal sperm in an ejaculate can aid in the judgement of whether, and which kind, of infertility treatment will be necessary.

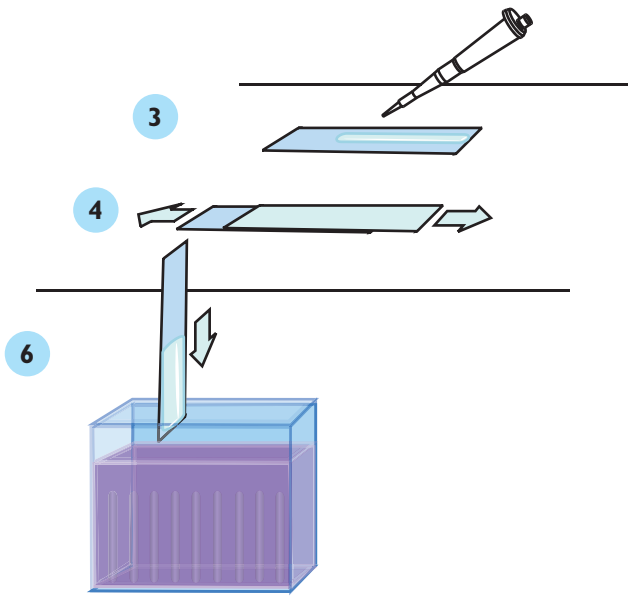
The sperm will be stained in a darker colour (blue) and the background will be lighter. Consequently, the shape, size and integrity of the sperm can easily be determined using 100x objective, oil immersion microscopy.

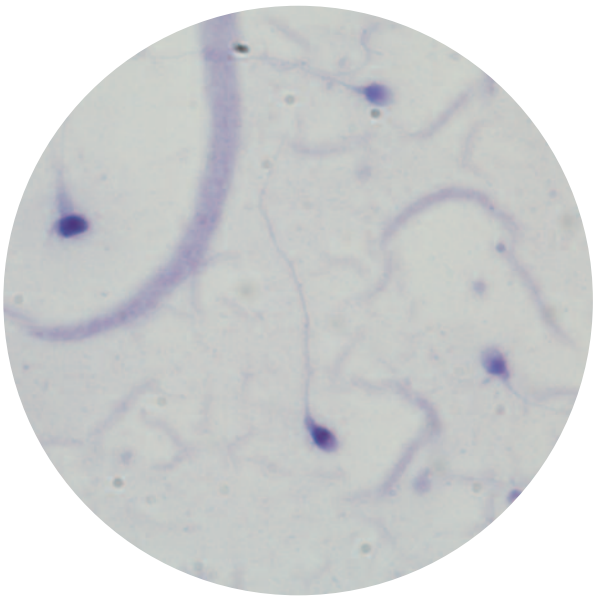
Reagents and Equipment

Coplin jar or similar
Microscope slides
Light microscope (40-100 x objectives)
Pipette

Procedure

1. Let sample liquefy 30 minutes before preparation.
2. Make a semen smear on a microscope slide using your conventional method or the method recommended by Nidacon.
3. Transfer a 20 µL drop onto a labelled microscope slide with a pipette, making a string /line of fluid in the middle of the slide.
4. Cover this slide with a second microscope slide and, when the drop is evenly spread between the two slides, pull them apart from each other horizontally. You then have two good slides.
5. Air dry the smears.
6. Dip the dry smears into the staining solution for 8 seconds.
7. Rinse in double distilled water, changing the water 3 times. Let slides air dry lying flat.
8. Mount the slides with coverslips and DPX, or equivalent mounting fluid, and let them dry completely before examination.
9. Examine using a bright-field 100 x objective under oil immersion.
10. Classify at least 200 sperm, classification according to the 2002 NAFA and ESHRE-SIGA manual on Basic Semen Analysis.





Tips

- Use a pencil to mark your sample slides since the stain will remove permanent markers

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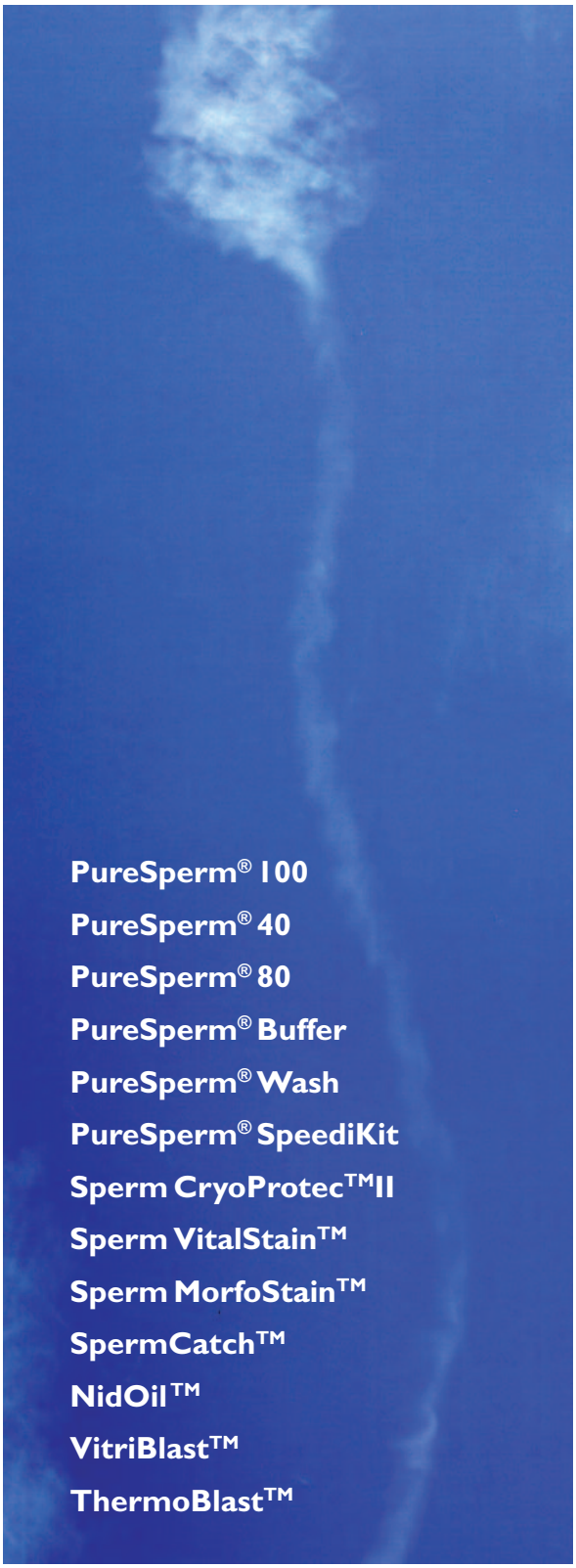

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