IVF Media & Consumables Recommended Protocols

PLANER

PRESERVE / PROTECT / NURTURE


SemenLeu
GYNEMED


GM501 CULT
GYNEMED


SepaSperm® Solution

IVF Media & Consumables Recommended Protocols

About Planer

Planer Ltd is now part of the Hamilton Thorne Group, a leading provider of precision instruments, consumables, software and services to the Assisted Reproductive Technologies (ART) and developmental biology research markets.

In 1973, Planer started out by developing and producing pioneering one off machines for cryogenic researchers. Now, 45 years on, we are proud to have become the gold-standard supplier of equipment for cell preservation. Over this time, we have also helped scientists around the world achieve many notable breakthroughs, including the first baby born from a frozen embryo in 1984 and the first successful frozen ovary transplant 2014. In August 2019, we were acquired by the Hamilton Thorne Group, a move intended to significantly accelerate the growth of Planer operations around the world.

Our customers include laboratories, hospitals, pharmaceutical companies and the assisted reproduction fields, which we supply with the hardware, software and systems for the safe preservation, storage and monitoring of biological specimens such as embryos, blood products, tissue and biologicals.

In our factories near London's Heathrow airport, we design and build our controlled rate freezers, incubators, sensors and systems. Around 90% of these products are exported with the help of our 80 or so sales and service distributors around the world. Planer equipment can now be found in most countries and our watchwords are robust design, compliant operation, Just-in-Time manufacture and long-standing relationships with our customers and distributors.

Assisted conception consumables and media

Now, as UK distributor for Gynemed and Kitazato reproductive media and consumables, our offering to the UK IVF market has been considerably strengthened. These carefully selected ranges, which meet our demanding quality standards, aim to help ensure that IVF practitioners achieve the maximum level of success.

Take a look at our Assisted Conception catalogue to see the full range of consumables and media products we have available. If you have any questions, please get in touch with the Planer sales team by emailing enquiries@planer.com.

Our product ranges

Cryopreservation

Cryopreservation using controlled rate freezers is used in a variety of applications including IVF, ART and research as well as stem cell, blood and large scale vaccine storage. Our range of freezers comes in a variety of sizes and caters for a broad range of different needs, whether a single straw or 8,000 vials need to be slow frozen.

Incubation

Our space saving, precise benchtop incubators are increasingly used in human and veterinary assisted reproduction applications. They offer the best possible in-vitro environment with rapid gas and temperature recovery times – and all with a battery back-up.

Monitoring

To keep laboratories safe, both for samples and for operators, parameters such as temperature, humidity, carbon dioxide, liquid nitrogen level, oxygen, door status etc. need to be monitored around the clock. Our DATAssure™ wireless monitoring system meets the most stringent standards to help our customers to comply with HACCP, BRC, FDA and MRHA legislative requirements.

Cryo Storage

Ultra low cryo storage offers security for biological samples at -190 °C with long holding times and can be used in areas such as assisted reproduction, immunology, gene therapy, tissue banking, stem cells, cord blood, algae, fungi and viruses. The range we offer extends from small dewars to large capacity electronically controlled vessels.

UK Distributor for Hamilton Thorne and Gynemed products

Now, as part of the Hamilton Thorne Group, we are also the UK distributor for Hamilton Thorne clinical lasers and the Gynemed range of media and consumables for the assisted conception market.

Kitazato human IVF products now distributed in UK by Planer

Planer is now the UK distributor for Kitazato human IVF products, including the Cryotop vitrification system, catheters, needles and micropipettes.

Get in touch to find out more

Email enquiries@planer.com
Tel 01932 755000
Website www.planer.com

Kitazato IVF Products

Gynemed IVF Products

Kitazato
IVF products

Micro Tools

ICSI INJECTION PIPETTES

Micro Tools for ICSI Injection (MT-INJ): 10 pcs/box

Indication for use

The Intracytoplasmatic Sperm injection (MT-ICSI) Injection Pipettes are used to inject a single sperm into oocyte.

Instructions for use

Preparation

- Make the drop of culture medium and PVP, cover with Hypure™ Oil. Equilibrate it in the incubator for 15 minutes.
- Set the pipette to suitable manipulation holder.
  Note: Recommend choosing the product whose inner diameter matches diameter of sperm in order to prevent a large amount injection of PVP.
- Place oocyte in culture medium, place motile sperm in PVP.
  Instructions for use

Instructions for use

1. Aspirate sperm tail-first into MT-INJ.
2. Move the polar body at the 12 or 6 o'clock position, and hold oocyte with MT-HD.
3. Move sperm to tip of MT-INJ.
4. Penetrate oocyte with MT-INJ.
   Note: Make sure that the cell membrane is ruptured.
5. Inject single sperm into oocyte with minute amounts of PVP.
6. Gently withdraw MT-INJ.

HOLDING PIPETTE

Micro Tools for ICSI Holding (MT-HD): 10 pcs/box

Indication for use

The Holding pipettes (MT-HD) are used to hold the oocyte or embryo in position with the application of vacuum during the injection of a single sperm with the ICSI injection pipette, and other micromanipulation procedures.

Instructions for use

Preparation

Set the pipette to suitable manipulation holder.

Instructions for use

1. Hold oocyte or embryo with MT-HD.
2. Keep holding it until the manipulation is finished

BIOPSY PIPETTES

Micro Tools for Polar Body Biopsy (MT-BPPD): 10 pcs/box.
Micro Tools for Blastomere Biopsy (MT-BPBM): 10 pcs/box.

Indication for use

The Polar Body Biopsy pipettes (MT-BPPD) are tools used in IVF/Assisted Reproduction Technology (ART) laboratories for removal of polar bodies from oocytes, which may be done in order to perform pre-implantation genetic diagnosis on the genetic material in the biopsied cell(s).

The Blastomere Biopsy Pipettes (MT-BPBM) are tools used in IVF/Assisted Reproduction Technology (ART) laboratories for removal of blastomere(s) from embryos, which may be done in order to perform pre-implantation genetic diagnosis on the genetic material in biopsied cell(s).

Instructions for use

Preparation

- Make the drop of culture medium, cover with Hypure™ Oil. Equilibrate it in the incubator for 15 minutes.
- Set the pipette to suitable manipulation holder.
- Place oocyte or embryo in culture medium.

Instructions for use

1. Open the zona pellucida, and then extract polar body from oocyte, blastomere from embryo or trophectoderm from blastocyst.
2. Use the extracted cell and perform genetic diagnosis.

PARTIAL ZONE DISSECTION PIPETTE

Micro Tools for PZD (MT-PZD): 10 pcs/box

Indication for use

The Partial Zone Dissection pipettes (MT-PDZ) are used to cut an opening on the zone of an embryo mechanically in assisting embryo hatching prior to implantation.

Instructions for use

Preparation

- Make the drop of culture medium, cover with Hypure™ Oil. Equilibrate it in the incubator for 15 minutes.
- Set the pipette to suitable manipulation holder.
- Place embryo in culture medium.

Instructions for use

(Cross dissection process of zona pellucida):

1. Cut a part of zona pellucida in the cross shape with MT-PZD. Note: Do not allow pipette to touch blastomere at this time. This could damage embryo.

GENERAL INFORMATION

Raw Material Borosilicate glass

Quality Control Specification

Each lot of Micro Tools receives the following tests

- Endotoxin (≤ 0.5 EU/device (LAL)
- Mouse Embryo Assay (2-cell MEA test: ≥80%)

Storage instructions and stability

- Keep away from sunlight.
- Keep dry.

Contraindications

Note: Confirm specification of the product in the labeling prior use

- To avoid deterioration, do not re-sterilize.
- Do not re-use. Reuse may cause contamination.
- Do not use if the package is opened or damaged.
- Do not use the product after expiration date or infected prod-uct and discard it (see the warning).

Warnings

- Aseptic technique should be used.

Ki174/V2

Hypure™ Oil Light

Intended use

Hypure™ Oil is intended to be used as an overlay for culture of embryos, oocytes and sperm in ART and micromanipulation procedures.

Composition

Pharmaceutical grade quality paraffin oil.

General information

Hypure™ Oil is pre-washed, ready to use mineral oil for use in IVF, ICSI and related artificial reproductive techniques.

The oil allows small volumes of medium to be used as it prevents the evaporation of media and maintain stable osmolality and pH in the culture medium.

Instructions for use

CAUTION: The detailed usage of Hypure™ Oil is determined based on each laboratory`s procedure and protocols that are optimised for individual medical purposes.

Preparation :

- For culture, pre-equilibrate Hypure™ Oil as necessary, and the culture medium at 37 °C in a CO_2 incubator for several hours (or overnight),
- For ICSI, warm Hypure™ Oil and HEPES buffered medium at 37 °C.

Instructions for use :

1. Put culture medium on a dish or place micro drop of culture medium on a dish.
2. Gently pipette equilibrated Hypure™ Oil over the medium until they are completely covered.

Note: Washing of oil for embryo culture is not required.

Quality control specifications

The following tests were performed for each lot of this product:

- Sterility: SAL 10^{-3}
- Endotoxin: < 0.1 EU/ml
- Mouse embryo assay: ≥ 80% blastocysts after 120 hours in culture
- Density: 0.83-0.86 g/ml
- Viscosity: < 30 cP at 30 °C
- Peroxide Value (POV): <0.1mEq/kg

Storage instructions and stability

- Store between 15-25 °C.
- Keep away from sunlight.
- Once opened, store the product in the original packaging.
- Once opened, the product is stable for at least 28 days under aseptic conditions and at room temperature. After this period, please discard the remaining media.
- The product is stable after transport (max 5 days) at elevated temperatures (< 37 °C).
- This product is stable until the expiration date labelled on the bottle if the packaging is un-opened or undamaged.

Contraindications

- Do not re-sterilize.
- Do not freeze the product.
- Do not use the product after expiration date.
- Do not use if packaging is damaged or broken.
- Do not use if product becomes discoloured, cloudy or shows any evidence of microbial con-tamination.

Warnings

Note: “Lifetime of the device is determined by the applied AR technique and is maximum to the period of embryo culture.”

- Always works under strict hygienic conditions (e.g. LAF-bench ISO Class 5) to avoid contamination. Aseptic technique should be used.
- In case of eye or skin contact with Hypure™ Oil, immediately flush eye/skin with water.
- Observe all federal, state and local environmental regulations when discarding the product.
- In case of infection, dispose of the product appropriately in a prescribed manner.
- The user shall be responsible for any problems caused by non-conformity to the present IFU.
- The oil may appear cloudy if removed from the incubator and allowed to stand at room temperature for extended periods of time. Such cloudiness usually disappears if the oil is re-equilibrated by replacing it in the incubator for several hours; if it does not disappear, discard the product.
- This product is intended to be used by medical specialist trained in fertility treatment.

Hypure™ Oil Heavy

Intended use

Hypure™ Oil is intended to be used as an overlay for culture of embryos, oocytes and sperm in ART and micromanipulation procedures.

Composition

Pharmaceutical grade quality paraffin oil.

General information

Hypure™ Oil is pre-washed, ready to use mineral oil for use in IVF, ICSI and related artificial reproductive techniques.

The oil allows small volumes of medium to be used as it prevents the evaporation of media and maintain stable osmolality and pH in the culture medium.

Instructions for use

CAUTION: The detailed usage of Hypure™ Oil is determined based on each laboratory`s procedure and protocols that are optimised for individual medical purposes.

Preparation :

- For culture, pre-equilibrate Hypure™ Oil as necessary, and the culture medium at 37 °C in a CO_2 incubator for several hours (or overnight),
- For ICSI, warm Hypure™ Oil and HEPES buffered medium at 37 °C.

Instructions for use :

1. Put culture medium on a dish or place micro drop of culture medium on a dish.
2. Gently pipette equilibrated Hypure™ Oil over the medium until they are completely covered.

Note: Washing of oil for embryo culture is not required.

Quality control specifications

The following tests were performed for each lot of this product:

- Sterility: SAL 10^{-3}
- Endotoxin: < 0.1 EU/ml
- Mouse embryo assay: ≥ 80 % blastocysts after 120 hours in culture
- Density: 0.86-0.88 g/ml
- Viscosity: 95-140 cP at 30 °C
- Peroxide Value (POV): <0.1 mEq/kg

Storage instructions and stability

- Store between 15-25 °C.
- Keep away from sunlight.
- Once opened, store the product in the original packaging.
- Once opened, the product is stable for at least 28 days under aseptic conditions and at room temperature. After this period, please discard the remaining media.
- The product is stable after transport (max 5 days) at elevated temperatures (< 37 °C).
- This product is stable until the expiration date labelled on the bottle if the packaging is un-opened or undamaged.

Contraindications

- Do not re-sterilise.
- Do not freeze the product.
- Do not use the product after expiration date.
- Do not use if packaging is damaged or broken.
- Do not use if product becomes discoloured, cloudy or shows any evidence of microbial contamination.

Warnings

Note: “Lifetime of the device is determined by the applied AR technique and is maximum to the period of embryo culture.”

- Always works under strict hygienic conditions (e.g. LAF-bench ISO Class 5) to avoid contamination. Aseptic technique should be used.
- In case of eye or skin contact with Hypure™ Oil, immediately flush eye/skin with water.
- Observe all federal, state and local environmental regulations when discarding the product.
- In case of infection, dispose of the product appropriately in a prescribed manner.
- The user shall be responsible for any problems caused by non-conformity to the present IFU.
- The oil may appear cloudy if removed from the incubator and allowed to stand at room temperature for extended periods of time. Such cloudiness usually disappears if the oil is re-equilibrated by replacing it in the incubator for several hours; if it does not disappear, discard the product.
- This product is intended to be used by medical specialist trained in fertility treatment.

Gamete Buffer

Intended use

Gamete Buffer is used for washing and handling of human gametes and embryos outside the incubator, including ICSI, IUI and embryo transfer procedure.

Composition

Gamete Buffer contains:

- HEPES buffered
- Bicarbonate
- Physiologic salts
- Glucose
- Lactate

General information

This medium is suitable to use in ambient atmosphere. Gamete Buffer does not contain human albumin (Supplement medium with human albumin solution before use is recom-mended).

Instructions for use

The medium must be prepared under sterile conditions (LAF bench ISO class 5 and sterile recipient).

Depending on the number of procedures that will be performed on one day, remove the required volume of medium under aseptic conditions in an appropriate sterile recipient. This is in order to avoid repeated openings / warming cycles of the medium. Discard excess (un-used) media.

Preparation:

1. Addition of human albumin before use:
   a. Add albumin with a protein end con-centration of 4g/L to Gamete Buffer.
   b. Close the bottle and turn at least 10 ti-mes upside down (do not shake), to make sure that the albumin is homo-genously mixed through the medium.
   c. Once the bottle is opened and added albumin, store dark at 2°C - 8 °C and do not use the product after 7 days.
2. Warm Gamete Buffer over night at 37 °C with closed lid.

Note: The cap of the bottle should be kept tightly closed during pre-warming.

Instructions for use:

This medium is used for washing and short-term handling of human gametes and embryos outside the incubator, including washing after denuding, sperm washing, ICSI, embryo transfer, IUI proce-dure. The detailed usage of Gamete Buffer is determined based on each laboratory`s procedure and protocols that are opti-mized for individual medical purposes.

Quality control specifications

The following tests were performed for each lot of this product:

- pH: between 7.30-7.60
- Osmolality: 270-290 mOsm/kg Sterility: sterile (SAL 10^{-3})
- Endotoxins: < 0.25 EU/ml
- MEA (blastocysts after 96h): ≥ 80 % after 1 hour of exposure (zygote stage)

All results are reported on Certificate of Analysis which is available upon request.

Storage instructions and stability

- Store between 2-25 °C before first use.
- Keep away from sunlight.
- Once opened, store the product in the original packaging.
- Once opened, the product is stable for at least 7 days in aseptic conditions and stored between 2-8 °C. After this period, please discard the remaining media.
- The product is stable after transport (max 5 days) at elevated temperatures (< 37 °C).
- This product is stable until the expiration date labelled on the bottle if the packaging is unopened or undamaged.

Contraindications

- Do not re-sterilize.
- Do not freeze the product.
- Do not use the product after expiration date.
- Do not use if packaging is damaged or broken.
- Do not use if product becomes discoloured, cloudy or shows any evidence of microbial contamination.

Warnings

- Always work under strict hygienic conditions (e.g. LAF-bench ISO Class 5) to avoid contamination. Aseptic technique should be used.
- In case of eye or skin contact with Gamete Buffer, immediately flush eye/skin with water.
- Observe all federal, state and local environmental regulations when discarding the product.
- In case of infection, dispose of the product appropriately in a prescribed manner.
- The user shall be responsible for any problems caused by non-conformity to the present IFU.
- This product is intended to be used by medical specialist trained in fertility treatment.

Ki153/V2

SepaSperm® Solution

Intended use

SepaSperm® Solution is a ready to use medium used for separation of motile sperm from seminal fluid with density gradient method.

Composition

SepaSperm® Solution medium contains:

- Silane-coated silica particles
- HEPES-buffered
- EBSS (Earle's balanced salt solution)

General information

SepaSperm® Solution are available in bottles of 20 ml, 50 ml and 100 ml.

Instructions for use:

CAUTION: The detailed usage of SepaSperm® Solution is determined based on each laboratory`s procedure and protocols that are optimised for individual medical purposes.

Preparation

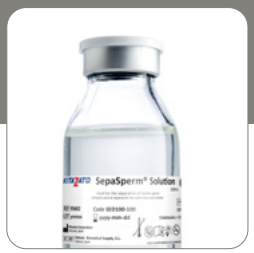
- Allow the semen to liquefy at 37 °C and initiate the procedure.
- When gelatinous pellet is detected in the semen, leave it at room temperature for 30 more minutes or remove those using filters or other appropriate equipment.
- Warm SepaSperm® Solution to 37 °C prior to use. The products should be tightly capped when used in CO_2 incubator to prevent pH alteration.
- Properly mix the prepared density gradients by inverting the bottles several times.

Instructions for use

1. Prepare a dual gradient system (45% – 90% or 40% – 80%) for each semen sample with a rinsing medium.

- 90% gradient: Dilute SepaSperm® Solution with 10% of a rinsing medium.
- 45% gradient: Dilute SepaSperm® Solution with 55% of a rinsing medium. Note: Gradients should be prepared and repacked under sterile conditions (e.g. LAF bench ISO class 5 and sterile recipient). For optimal results:
- Prepare the gradients maximum 24 hours prior to use and store at 2-8 °C and warm the gradients to room temperature or 37 °C one hour before use.
- Prepare the gradients needed for one day in one time and mix well. Discard excess unused prepared gradient.

2. Carefully place 2.5 ml of 90% density gradient on the bottom of the centrifuge tube.
3. Add 2.5 ml of 45% density gradient on top of the 90% density gradient.
4. Gently place the liquefied semen on the top layer (45% density gradient).
5. Centrifuge the tubes at 350 to 400 g for 15 to 18 minutes. If the pellets are not visible, centrifuge for a further 3 minutes.
6. Remove the supernatant.



Quality control specifications

The following tests were performed for each lot of this product:

- Sterility: SAL 10^{-3}
- Endotoxin: < 0.5 EU/ml
- Sperm Survival test: ≥ 80% survival after 4 hours exposure; ≥ 75% survival after 24 hours exposure.
- pH: 7.20 – 7.90 (Lot release criteria:7.20-7.60)
- Osmolality: 300-330 mOsm/kg

All results are reported on Certificate of Analysis which is available upon request.

Storage instructions and stability

- Store between 2-8 °C.
- Keep away from sunlight.
- Once opened, store the product in the original packaging.
- Once opened, the product is stable for at least 7 days under aseptic conditions and store between 2-8 °C. Discard the remaining media if this period has passed.
- The product is stable after transport (max. 5 days) at elevated temperatures (< 37 °C).
- This product is stable until the expiration date labelled on the bottle if the packaging is unopened or undamaged

Contraindications

- Do not re-sterilize.
- Do not freeze the product.
- Do not use the product after expiration date.
- Do not use if packaging is damaged or broken.
- Do not use if product becomes discoloured, cloudy or shows any evidence of microbial contamination.

Warnings

- Always works under strict hygienic conditions (e.g. LAF-bench ISO Class 5) to avoid contamination. Aseptic technique should be used
- In case of eye or skin contact with SepaSperm® Solution, immediately flush eye/skin with water.
- Observe all federal, state and local environmental regulations when discarding the product.
- In case of infection, dispose of the product appropriately in a prescribed manner.
- The user shall be responsible for any problems caused by non-conformity to the present IFU.
- This product is intended to be used by medical specialist trained in fertility treatment.

Ki154/V2

Cryotop®

Intended Use

Cryotop® is used for storage of vitrified human oocytes and embryos.

Quality Control Testing

Each lot of Cryotop® receives the following tests:

- Sterility by the current USP Sterility Test
- Endotoxin by LAL methodology
- Mouse Embryo Assay (one cell)

All results of each lot are reported on a Certificate of Analysis which is available upon request.

Storage Instructions and Stability

Store in original sterile pack at 15-30 degree Celsius.

Cryotop® is stable until the expiration date shown on package label when stored as directed.

Precautions and Warnings

Sterile if the package is unopened or undamaged. Do not use if package is broken.

Each Cryotop® is intended for single use.

Perform all vitrification and thawing operations for oocytes or embryos at room temperature (23-27 degree Celsius).

Liquid nitrogen may cause freeze burns if in contact with skin or eyes. Use appropriate precautions when working with liquid nitrogen.

Do not re-use. Re-use may cause change in product quality and raise the risk of a poor embryo survival rate.

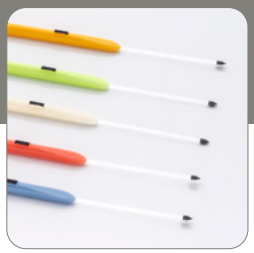
This product is intended to be used by medical specialists trained in fertility treatment.

Aseptic technique should be used.

Instructions for Use

Vitrification

1. Open the sterile pack of Cryotop® and write necessary information about a patient on the handle of Cryotop®.
2. Fill 90% of a container with fresh liquid nitrogen.
3. Prepare embryos or oocytes for vitrification according to laboratory protocol.
4. Take the straw cap of Cryotop® and plunge it into the fresh liquid nitrogen.
5. Place the Cryotop® under the microscope and adjust the focus on the black mark of the Cryotop®. Logo should be up.
6. Gently place the oocytes or embryos by the black part of Cryotop® sheet with minimal volume of vitrification solution using a suitable pipette.
   Note: The recommended load of the Cryotop® is up to 4 oocytes or embryos.
7. Quickly plunge the Cryotop® into fresh liquid nitrogen.
8. Hold the straw cap using tweezers and fit the can onto the Cryotop®, securing by tightening securely by hand.
9. Put the Cryotop® in a cane and store it in a tank for long term storage.
   Caution: Take care that the Cryotop® remains immersed in liquid nitrogen at all times until thawing.



Thawing

1. Prepare the thawing media according to laboratory protocol.
2. Retrieve the cane which has the specific Cryotop® and quickly immerse the cane in a container filled with fresh liquid nitrogen. Collect the specific Cryotop® from the cane in the liquid nitrogen. Check the information about the patient on the handle of the Cryotop®.

Caution: The Cryotop® except the handle should remain immersed in liquid nitrogen at all time. Place a container for liquid nitrogen by the stereo microscope.

3. Carefully twist and remove the straw cap from the Cryotop® in liquid nitrogen.
4. Quickly immerse the Cryotop® sheet into the thawing solution. It should be one second.
5. One minute after immersing into the thawing solution, gently aspirate the oocyte/embryo using a suitable pipette.
6. Perform the thawing procedure according to laboratory protocol.

Cryotop® SC

Intended Use

Cryotop®SC is used for storage of vitrified human oocytes and embryos.

Quality control testing

Each lot of Cryotop®SC receives the following tests: ■

• Sterility by the current USP Sterility Test ■

• Endotoxin by LAL methodology

• Mouse Embryo Assay (one cell)

All results of each lot are reported on a Certificate of Analysis which is available upon request.

Storage instructions and stability

Store in original sterile pack at 15-30 degree Celsius. Cryotop®SC is stable until the expiration date shown on package label when stored as directed.

Precautions and warnings

Sterile if the package is unopened or undamaged. Do not use if package is broken. Each Cryotop®SC is intended for single use.

Perform all vitrification and thawing operations for oocytes or embryos at room temperature (23-27 degree Celsius).

Liquid nitrogen may cause freeze burns if in contact with skin or eyes. Use appropriate precautions when working with liquid nitrogen.

Do not re-use. Re-use may cause change in product quality and raise the risk of poor embryo survival rate.

This product is intended to be used by medical specialists trained in fertility treatment. Que Aseptic technique should be used.

Instructions for use■

Vitrification

1. Open the sterile pack of Cryotop®SC and write necessary information about a patient on the handle of Cryotop®SC.
2. Fill 90% of a container with fresh liquid nitrogen.
3. Prepare oocytes or embryos for vitrification according to laboratory protocol.
4. Place the Cryotop®SC under the microscope and adjust the focus on the black mark of the Cryotop®SC. Logo should be up.
5. Gently place the oocytes or embryos by the black mark of Cryotop®SC sheet with minimal volume of vitrification solution using a suitable pipette.
   Note: The recommended load of the Cryotop®SC is up to 4 oocytes or embryos.
6. Quickly plunge the Cryotop®SC tip to the middle of handle into liquid nitrogen. Then prop it against the edge of container.
7. Plunge the straw cap into liquid nitrogen and cut the above marking point.
8. Hold up the Cryotop®SC sheet within the 2.5cm height from the surface of liquid nitrogen. Insert the CryotopSC into the cap straw and seal it with a heat sealer.
   Note: Do not let liquid nitrogen into the cap straw.
9. Immerse the capped Cryotop®SC into liquid nitrogen.
10. Put the Cryotop®SC in a cane and store it in a tank for long term storage.
    Caution: Take care that the Cryotop®SC remains immersed in liquid nitrogen at all times until thawing.

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Thawing

1. Prepare the thawing media according to laboratory protocol.
2. Retrieve the cane which has the specific Cryotop®SC and quickly immerse the cane in a container filled with fresh liquid nitrogen. Collect the specific Cryotop®SC from the cane in the liquid nitrogen. Check the information about the patient on the handle of the Cryotop®SC.

Caution: The Cryotop®SC except the handle must remain immersed in liquid nitrogen at all time. Place a container for liquid nitrogen by the stereo microscope.

3. Cut the sealed part of the straw cap with scissors remaining the tip of Cryotop®SC in liquid nitrogen.
4. Remove the Cryotop®SC from the straw cap, and quickly immerse the Cryotop®SC sheet into the thawing solution. It should be one second.
5. One minute after immersing into the thawing solution, gently aspirate the oocytes or embryos using a suitable pipette.
6. Perform the thawing procedure according to laboratory protocol.

Vitrification Media

Intended use

This product is to be used for vitrification of oocytes (MII) and embryos.

Vitrification media

- No. 0 (colour code: white) Basic Solution (BS): 1×1.5 ml vial (only for oocyte (MII) vitrification)
  ! Caution: Before use, check the specifications of the container and labeling (number marked on top of the cap, cap colour, vial labeling colour, solution name, and volume). If you notice anything unusual regarding the items mentioned above, do not use the product and please contact the distributor

Recommended equipment

- Cryotop: 1 Cryotop stores up to 4 oocytes (MII) or 4 Embryos as a recommendation.
- ReproPlate: with 6 wells
- Cooling Rack
  ! Caution: This product is sterilized. Please handle the product in a sterile field with a clean bench.

Instructions for use

Preparation

- Fill 90% of the Cooling Rack with fresh liquid nitrogen.
- Compare the width of perivitelline space with the thickness of zona pellucida and record it.
- Bring BS, ES and VS to room temperature (23-27 °C as recommendation).
- Use a sterilised pipette as a handling tool, with a suitable internal diameter for oocytes or embryos.
- The recommended internal diameters are as follows: 120 µm for oocytes (MII), 120 µm for pronuclear stage embryos, 150 to 180 µm for cleavage stage embryos, and 180 to 250 µm for blastocyst stage embryos.

Equilibration

! Caution: Equilibration procedures for Oocyte (MII) and Embryo are different.

Equilibration of oocytes (MII)

1. Using a pipette place a 20 µl of BS into the first well and 300 µl of VS in both the second and third wells of the Reproplate.
2. Step 0: Transfer the Oocyte (MII) from the culture dish to the BOTTOM of BS.
3. Step 1: Immediately add ES 20 µl gently on the TOP of BS of the first well and leave it for 3 minutes.
   Add another ES 20 µl gently on the TOP of the first well and leave it for 3 minutes.
   Add another ES 240 µl gently on the TOP of the first well and leave it for 9 minutes.

Equilibration of embryos

1. Drop each 300 µl of ES into first well, VS into second and third well on the Repro plate using pipette.
2. Step 1: Transfer the Embryo to the TOP center of ES from the culture dish. It will spontaneously begin to shrink and then gradually return to its original size by absorbing the ES solution (within 15 minutes).

Vitrification

! Caution: The following steps from 1 to 9 should be completed between 60 and 90 seconds.

1. Aspirate the oocyte (MII)/embryo from ES with the tip of a pipette.
2. Step 2: Transfer the oocyte (MII)/embryo to the TOP centre of the VS of second well.
3. Aspirate the oocyte (MII) / embryo with a pipette and blow it out. Repeat this process 3 times, changing the position in the VS of second well.
4. Transfer the oocyte (MII)/embryo to the VS of third well.
5. Change the position of the oocyte (MII)/embryo in the VS of third well with a pipette.
6. Place the oocyte (MII)/embryo by the black line on the Cryotop.
7. Make a planar droplet.
8. Make sure if the oocyte (MII)/embryo is on the Cryotop with a minimal volume of the VS of third well (less than 0.1µl) under a microscope.
9. Plunge the Cryotop immediately in liquid nitrogen.
10. Put the Cryotop in a cane and store it in a storage tank.

Quality control specification

The following tests were performed for each lot of this product:

- Sterility by the Sterility Test (EP)
- Endotoxin by LAL methodology
- Mouse Embryo Assay (One Cell)
- pH (EP)
- Osmolality (EP)

Storage instructions and stability

Store the vials at 2 to 8 °C.
This product is stable until the expiry date labeled on the vial.

Composition

- HEPES within Basic Culture Medium
- Ethylene Glycol
- Dimethyl Sulfoxide
- Trehalose
- Hydroxypropyl Cellulose
- Gentamicin

Ki170/V2

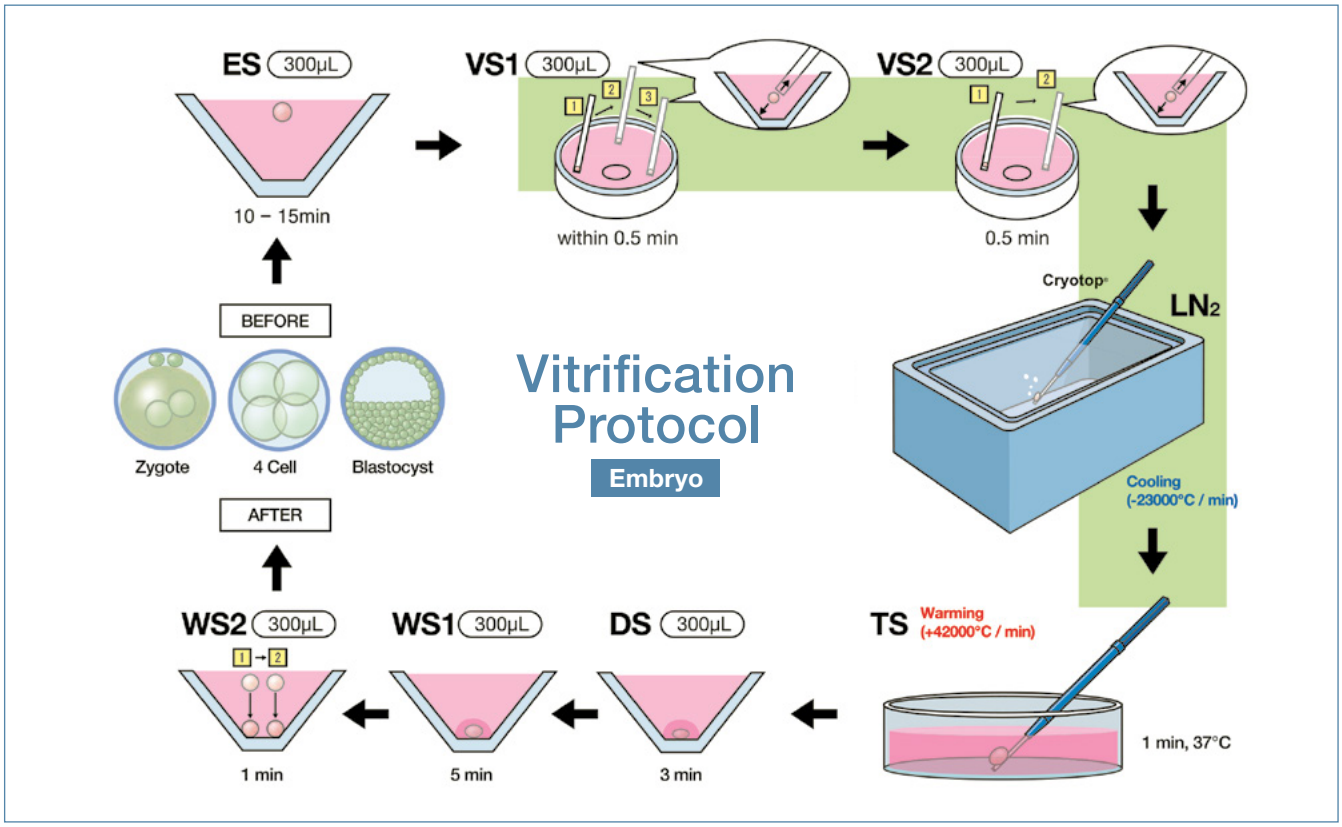
Contraindications

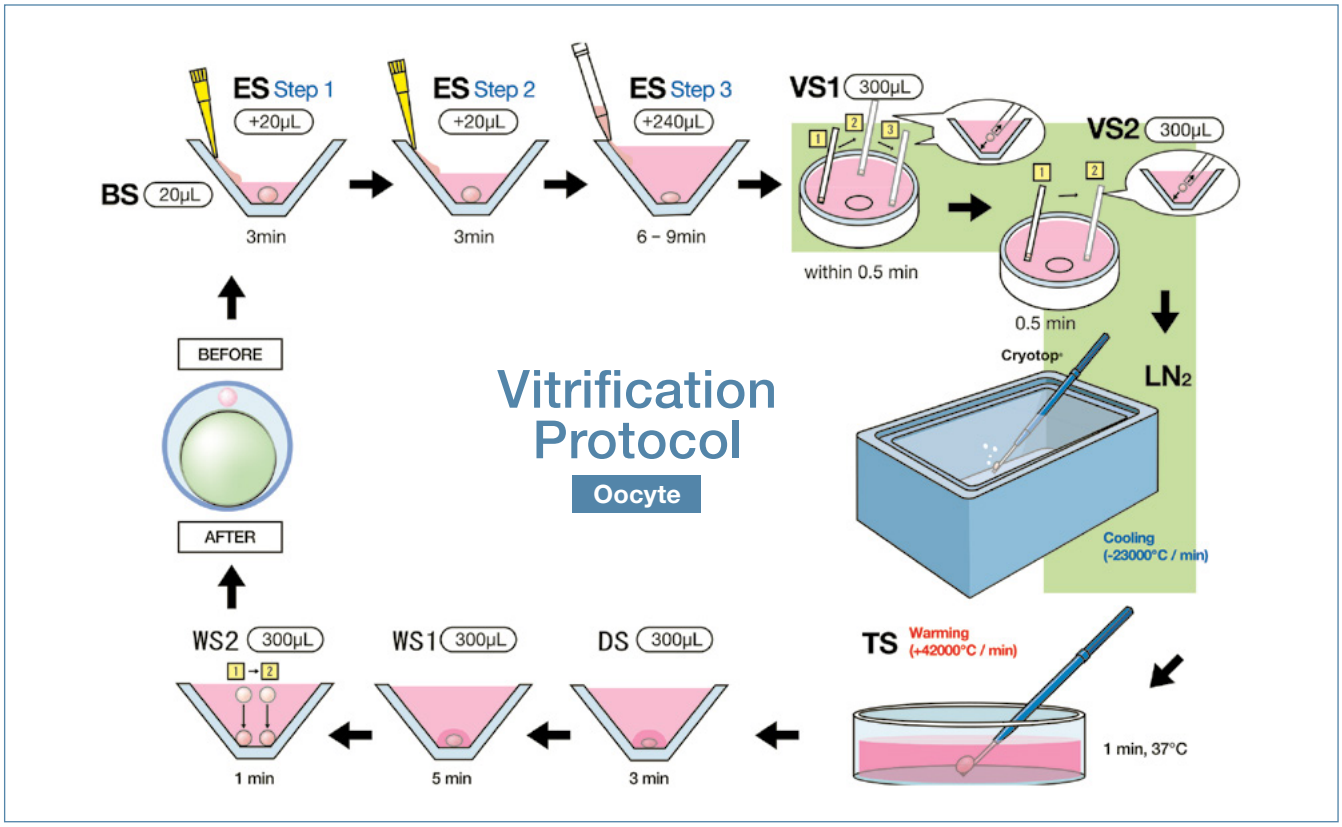
- Do not re-sterilise.
- Do not re-use. Re-use may cause contamination.
- Do not use solution that shows cloudiness or becomes discoloured.
- Do not use the product if you notice anything unusual regarding the specifications on the label (number, colour, name, volume).
- Device is sterilised if the package or container is unopened or undamaged. Do not use if the package or container are opened or damaged.
- Upon delivery media must be stored in original unopened container and refrigerated at 2-8 °C.
- Do not use and please discard if the media is not stored under refrigeration (2 to 8 °C).
- Do not use the product if past the expiration date.

Warning

- Read the instructions for use prior to use.
- Use the KITAZATO thawing media to thaw the oocytes (MII) or embryos vitrified with KITAZATO vitrification media
- This product is intended to be used by medical specialists trained in fertility treatment.
- Aseptic technique should be used.
- Use sterilised equipment and materials only.
- In case of eye or skin contact with Vitrification/Thawing media,immediately flush eye/skin with water.
- Morphologically abnormal oocytes, embryos, or significantly poor grade oocytes or embryos are unsuitable for cryopreservation.
- Observe all federal, state and local environmental regulations when discarding the product.
- In case of infection, dispose of the product appropriately in a prescribed manner.
- The user shall be responsible for any problems caused by non-conformity to the present IFU.
- Vitrification media contain the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitised to this antibiotic.

Note: The long-term safety of the vitrification technique and maximum storage in liquid nitrogen has not been established and is unknown.


ES 300µL
10 – 15min
VS1 300µL
within 0.5 min
VS2 300µL
0.5 min
Cryotop
LN2
Cooling
(-23000°C / min)
BEFORE
Zygote
4 Cell
Blastocyst
AFTER
Vitrification
Protocol
Embryo
WS2 300µL
1 min
WS1 300µL
5 min
DS 300µL
3 min
TS Warming
(+42000°C / min)
1 min, 37°C


ES Step 1
+20µL
BS 20µL
3min
ES Step 2
+20µL
3min
ES Step 3
+240µL
6 – 9min
VS1 300µL
within 0.5 min
VS2 300µL
0.5 min
Cryotop
LN2
Cooling
(-23000°C / min)
BEFORE
AFTER
Vitrification
Protocol
Oocyte
WS2 300µL
1 min
WS1 300µL
5 min
DS 300µL
3 min
TS Warming
(+42000°C / min)
1 min, 37°C

Ki170/V2

Thawing Media

Intended use

This product is to be used for thawing of Oocytes (MII) and embryos.

Thawing media

- No. 1 (Colour Code: Red) Thawing Solution (TS): 2×4.0 ml vials
- No. 2 (Colour Code: Yellow) Diluent Solution (DS): 1×4.0 ml vial
- No. 3 (Colour Code: White) Washing Solution (WS): 1×4.0 ml vial

! Caution: Before use, check the specifications of the container and labeling (number marked on top of the cap, cap colour, vial labeling colour, solution name, and volume). If you notice anything unusual regarding the items mentioned above, do not use the product and please contact the distributor.

Recommended equipment

- Petri Dish: 35 mm for TS
- ReproPlate: with 6 wells

! Caution: This product is sterilised. Please handle the product under sterilisation on a clean bench.

Instructions for use (IFU)

Preparation

- Warm TS vial (sealed) with a Petri Dish in an incubator to 37 °C (1.5 hours). Pour the full contents of TS into the Petri Dish.
- Bring DS and WS to room temperature (23-27 °C as recommendation) ■
- Using a pipette place 300 µl of DS into the first well and 300 µl of WS in both the second and third wells of the Reproplate.

! Caution: Use a sterilised pipette as a handling tool, with a suitable internal diameter for oocytes or embryos. The recommended internal diameters are as follows: 180 µm for oocytes (MII), 180 µm for pronuclear stage embryos, and 250 µm for cleavage or blastocyst stage embryos.

Thawing

Step 1: Quickly immerse the Cryotop strip completely into the TS. Leave it for 1 minute.

Step 2: Aspirate the oocyte (MII)/embryo with the pipette and gently place it on the BOTTOM of the DS. Leave it for 3 minutes.

Step 3:

1. Aspirate the oocyte (MII)/embryo with the pipette and gently place it on the BOTTOM of the WS of second well. Leave it for 5 minutes.
2. Aspirate the oocyte (MII)/embryo with the pipette, gently place it on the TOP of the WS of third well. After the oocyte (MII)/embryo drops to the bottom of the WS of third well, repeat this process one more time.
3. Transfer the oocyte (MII)/embryo to a culture dish containing the appropriate culture medium. Incubate the oocyte (MII)/embryo in a 37 °C incubator to complete recovery.

Caution 1: It is recommended to incubate oocytes (MII) and embryos for 2 hours.

Caution 2: Wash the embryo and incubate embryo for recovery in appropriate media to avoid the WS of the third well to be transferred into the patient's body.

Quality control specification

The following tests were performed for each lot of this product:

- Sterility by the Sterility Test (EP)
- Endotoxin by LAL methodology
- Mouse Embryo Assay (One Cell)
- pH (EP)
- Osmolality (EP)

Storage instructions and stability

Store the vials at 2 to 8 °C.
This product is stable until the expiry date shown in the labeling on the vial.

Composition

- HEPES within Basic Culture Medium
- Trehalose
- Hydroxypropyl Cellulose
- Gentamicin

Contraindication

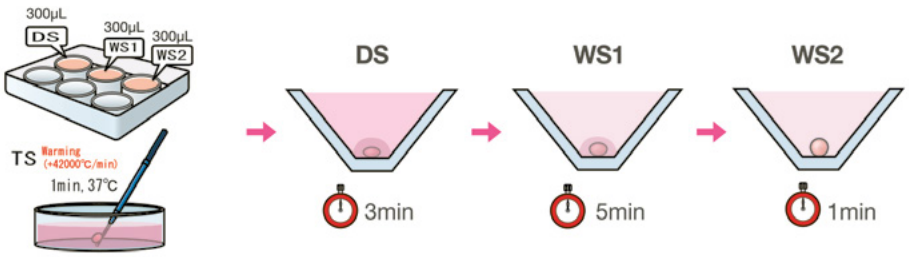
- Do not re-sterilise
- Do not re-use. Reuse may cause in contamination.
- Do not use solution that shows cloudiness or becomes discoloured.
- Do not use the product if you notice anything unusual regarding the specifications on the label (number, colour, name, volume).
- Device is sterile if the package or container is unopened or undamaged. Do not use if the package or container are opened or damaged.
- Upon delivery media must be stored in original unopened container and refrigerated at 2-8 °C.
- Do not use and please discard if the media were not stored under refrigeration (2 to 8 °C).
- Do not use the product if past the expiration date.

Warning

- Read the instructions for use prior to use.
- This product is intended to be used by medical specialists trained in fertility treatment.
- Aseptic technique should be used.
- Use sterilised equipment and materials only.
- Observe all federal, state and local environmental regulations when discarding the product.
- In case of eye or skin contact with Vitrification/Thawing media, immediately flush eyes/skin with water.
- The user shall be responsible for any problems caused by non-conformity to the present IFU.
- Thawing media contain the antibiotic gentamicin sulfate. Appropriate precautions should be taken to ensure that the patient is not sensitive to this antibiotic.

Note: The long-term safety of the vitrification technique and maximum storage in liquid nitrogen has not been established and is unknown.

Thawing - Cryotop Open System



Ki171/V2

Plastic Ware for ART

Vitrification / Thawing plate

Indication for use

Repro Plate is the plate used for holding the solution/media that performs vitrification/thawing of oocytes or embryos during Assisted Reproductive Technology (ART).

Instructions for use

Preparation

- Prepare the device and instruments needed for laboratory's procedures and protocols.
- Write necessary information about the patient on the lid/base (main body) of Plate/Dish.

Instructions for use

1. Put vitrification/thawing medium on Plate/Dish based on laboratory's procedures and protocols.
2. Transfer oocytes or embryos to the vitrification/thawing medium, and then manipulate them according to the laboratory's procedures and protocols.

Note: For vitrification and thawing process, follow the protocol of the KITAZATO product.

Caution: The detailed usage of Repro Plate is determined based on each laboratory's procedures and protocols that are optimised for individual medical purposes.

Culture dish

Indication for use

Culture Dish is the dish used for holding the solution/media that cultures oocytes or embryos during ART.

Instructions for use

Preparation

- Prepare the device and instruments needed for laboratory's procedures and protocols.
- Write necessary information about the patient on the lid/base of Dish.

Instructions for use

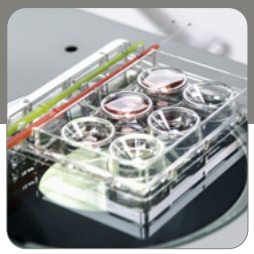
1. Put culture medium on Dish based on laboratory's procedures and protocols.
2. Transfer oocytes or embryos to the culture medium, and then manipulate them according to the laboratory's procedures and protocols.

Caution: The detailed usage of Dish is determined based on each laboratory's procedures and protocols that are optimised for individual medical purposes.

Quality control specification

Each lot ensures the following.

- Endotoxin by Photometric Techniques (LAL): ≤0.5EU/device
- Mouse Embryo Assay (one cell) : ≥80%
- Sterilised by gamma irradiation and validated to meet sterility assurance level (SAL): 10^{-6}



Storage instructions, warnings and stability

- Store at 15 to 30 °C.
- This product is sterile until the expiry date shown on the label if the package or container is unopened or undamaged.
- Keep away from sunlight.

Contraindications

- To avoid deterioration, do not re-sterilise.
- To avoid contamination, do not re-use.
- Do not use if the package or container are opened or damaged.
- Do not use the product after expiration date or infected product or used product, and discard it (see the warning).
- This product is intended to be used by medical specialists trained in fertility treatment.

Warnings

- Read the instructions for use prior to use.
- Aseptic technique should be used.
- Use sterilised equipment and materials only.
- In case of infection or used product, dispose of the product appropriately in a prescribed manner.
- The user shall be responsible for any problems caused by non-conformity to the present IFU.
- Observe all federal, state and local environmental regulations when discarding the product.

Note: Single use device. Lifetime of the device is determined by applied Assisted Reproductive Techniques and is maximum to the period of embryo culture.

Gynemed
IVF products

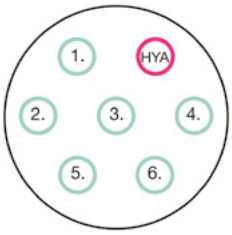
GM501 Hyaluronidase

Preparation for denudation of fresh oocytes

Preparation of the 4-well dish

- One 4-well dish (“Hya-dish”) needs to be prepared for 10 oocytes.
- First fill well 2 to 4 with 400 µl GM501 Wash medium and cover the filled wells with GM501 Mineral Oil. Equilibrate the dish overnight in a humidified CO_2 incubator.
- Warm the GM501 Hyaluronidase and fill the first well with 400 µl warmed GM501 Hyaluronidase.

Preparation of microdrop dish



- One 60 mm Petri dish (“Hya-dish”) needs to be prepared for 10 oocytes.
- Each dish contains a total of 7 drops, 80 µl each (6x GM501 Wash medium, 1x GM501 Hyaluronidase). In the diagram the wash drops are shown in green and the hyaluronidase drop is shown in pink.
- To facilitate identification mark the spot for the Hyaluronidase drop on the bottom of the Petri dish. First add the 6 drops with GM501 Wash medium to the dish and cover with GM501 Mineral Oil. Equilibrate the dish overnight in a humidified CO_2 incubator.
- Warm the GM501 Hyaluronidase and add one drop of 80 GM501 Hyaluronidase to the dish at the marked position.

Denudation procedure using the microdrop dish

1. Pipette up to 10 oocytes into the first drop (“1”) of the dish.
2. Transfer 5 oocytes to the “Hya” drop.
3. Pipette the oocytes immediately up and down (5 to 10 times) using for example a pipette with 100 µl tip (MEA-tested) adjusted to 50 µl. The cumulus cells will detach and the oocytes still surrounded by corona cells will be visible. **ATTENTION: The oocytes should not be in the Hyaluronidase for more than 30 seconds!**
4. Pick up the oocytes using the denudation pipette (inner diameter 125-155 µm) and transfer them to the next GM501 Wash containing drop (“2”). Aspirate and blow back the oocytes repeatedly to remove residual hyaluronidase.
5. Transfer the oocytes to the next GM501 Wash containing drop (“3”). Aspirate and blow back the oocytes repeatedly until nearly all corona cells are removed.
6. Transfer the oocytes to the next GM501 Wash containing drop (“4”). Leave the denuded oocytes in this drop.
7. Repeat steps 2 to 6 with the remaining oocytes.
8. When all oocytes are collected in GM501 Wash drop “4” and are free of cumulus and corona cells, wash them in the last two drops (“5” and “6”).
9. The denuded oocytes can then be transferred to a dish containing GM501 Cult for further incubation until ICSI is performed or directly to an ICSI dish.

Gi128/V3

Embryo Culture

4-well dish

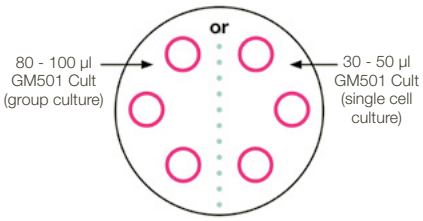
Day “-1”:
Prepare a 4-well dish by filling each well with 400 µl of GM501 Cult. Cover the wells completely with GM501 Mineral Oil and equilibrate overnight in a CO_2 atmosphere at 37 °C.

Day “0”:
ICSI - Directly after microinjection add up to 6 oocytes per well for culture.
IVF - Add up to 6 oocytes per well for fertilisation and add the appropriate amount of prepared spermatozoa. Prepare an additional 4-well dish in the same fashion for the next day (“culture” dish).

Day “1”:
ICSI - No further intervention necessary. If desired, assess pronuclear status.
IVF - Carefully remove remaining cumulus and corona cells by pipetting and transfer the fertilised oocytes to the prepared “culture” dish.

Microdrop dish

Day “-1”:
Prepare a microdrop dish with sufficient 80 - 100 µl drops (group culture) or 30 - 50 µl drops (single embryo culture) of GM501 Cult and cover completely with GM501 Mineral Oil. Equilibrate overnight in a CO_2 atmosphere at 37 °C.



Day “0”:
ICSI - Directly after microinjection add the desired amount of oocytes to the respective drops (e.g. 3 oocytes per drop for group culture).
IVF - Add 2-3 oocytes per drop for fertilisation and add the appropriate amount of prepared spermatozoa. Prepare an additional dish in the same fashion for the next day (“culture” dish).

Day “1”:
ICSI - No further intervention necessary.
If desired, assess pronuclear status.
IVF - Carefully remove remaining cumulus and corona cells by pipetting and transfer the fertilised oocytes to the prepared “culture” dish.

Simple “one-step” culture

Day -1	Day 0	Day 1	Day 2 or 3
Preparation of dishes for IVF or ICSI using GM501 Cult Media	Fertilisation by IVF or ICSI and culture in GM501 Cult Media	Ongoing culture in GM501 Cult Media	Embryo transfer

Extended “one-step” culture

Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Preparation of dishes for IVF or ICSI using GM501 Cult Media	Fertilization by IVF or ICSI and culture in GM501 Cult Media	Ongoing culture in GM501 Cult Media	Ongoing culture in GM501 Cult Media. Preparation of new dishes for day 3 if wanted	Ongoing culture in new dishes with GM501 Cult Media	Ongoing culture in GM501 Cult Media	Embryo transfer

Gi129/V3

GYNEMED

Swim-Up

Preparation

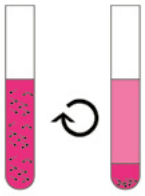
We recommend one of the products from the GM501 MediaLine:

- GM501 SpermAir or
- GM501 SpermActive

Washing 1

To prepare the swim-up tubes transfer 4.5 ml medium into a new conical centrifuge tube. Mix well with 1.0-3.0 ml liquefied semen.

Centrifuge the tube at 300-400x g for 10 minutes



Swim - Up

Aspirate the supernatant without dispersing the pellet and discard it.

Carefully overlay the pellet with equilibrated GM501 SpermActive or warmed GM501 Sperm Air medium.

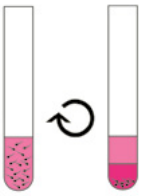
Put the tube in the CO_2-incubator (GM501 SpermActive). Or if using GM501 SpermAir, put in a heating cabinet for 1 hour with top not firmly closed.

Aspirate the supernatant containing the motile sperm and pour into a new conical centrifuge tube.

Washing 2

Resuspend the pellet with 1 ml equilibrated GM501 SpermActive or warmed GM501 SpermAir medium.

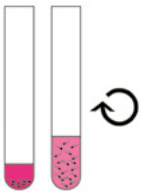
Centrifuge the tube at 300-400x g for 6 minutes.



Aspirate the supernatant without dispersing the pellet and discard it.

Resuspend the pellet with 1 ml equilibrated GM501 SpermActive or warmed GM501 SpermAir medium. Centrifuge the tube at 300-400x g for 6 minutes.

Repeat the step.



Aspirate the supernatant and discard it.

For IVF resuspend the pellet in 0.1–1.0 ml equilibrated culture medium (e.g. GM501 Cult) to the desired sperm concentration, for ICSI or Insemination resuspend it in GM501 SpermActive or GM501 SpermAir.

Gi130/V3

Swim-Up

Preparation

We recommend one of the products from the GM501 MediaLine:

- GM501 SpermAir or
- GM501 SpermActive

Swim-Up

To prepare the swim-up tubes, transfer 2 ml medium into a new conical tube.

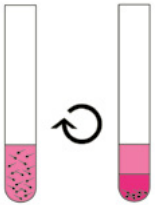
Underlay gently 1 ml of liquefied semen.

Place the tube in the CO_2-incubator (GM501 SpermActive) or heating cabinet (GM501 Sperm-Air) for 1 hour with top not firmly closed.

Washing

Aspirate the upper media layer containing the motile sperms without dispersing the native ejaculate and fill it into a new conical centrifuge tube.

Centrifuge the tube at 300-400 g for 6 minutes.

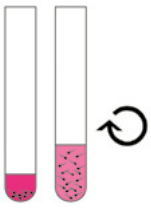


Aspirate the supernatant without dispersing the pellet and discard it.

Resuspend the pellet with 1 ml equilibrated GM501 SpermActive or warmed GM501 SpermAir medium.

Centrifuge the tube at 300-400x g for 6 minutes.

Repeat the step.



Aspirate the supernatant and discard it.

For IVF resuspend the pellet in 0.1–1 ml equilibrated culture medium (e.g. GM501 Cult) to the desired sperm concentration. For ICSI or Insemination resuspend it in GM501 SpermActive or GM501 SpermAir.

Gi131/V3

GYNEMED

Density centrifugation

Preparation

Before use warm all components of the system and the samples to 37 °C or to room temperature.

Mix the density gradient bottles by a minimum of 5 bottles inversions before use.

Pipette 2.5 ml of the lower density gradient (e.g. 45%) into a sterile disposable centrifuge tube.

Using a 3 ml syringe with a 21 G needle, layer 2.5 ml of the higher density gradient (e.g. 90%) under the lower density gradient (e.g. 45%) free of air bubbles.

Take care that the two layers are distinctly separated. This is done by placing the tip of the needle at the bottom of the centrifuge tube and slowly dispensing the higher density gradient.

These two layers of density are stable for about two hours.

Gently place 2.5 ml of liquefied semen onto the upper layer using a transfer pipette or syringe.

Centrifuge at 350-400x g for 15-18 minutes.

In case, no pellet is visible after this step, centrifuge for another 3 minutes.

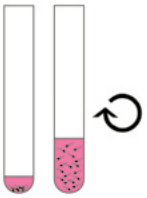
Centrifugation force should not be increased over 500x g

Aspirate the supernatant.

Using a syringe, resuspend the pellet with 2-3 ml of fresh washing medium.

Centrifuge at 300x g for 8-10 minutes.

In case you want to gain higher sperm concentrations it is advisable to centrifuge for the whole 10 minutes.



Aspirate the supernatant and repeat the last two steps.

Finally remove the remaining liquid to leave the pellet resuspended in the required amount for use with the subsequent procedure of assisted reproductive medicine (e.g. IVF, ICSI, IUI).

Gi132/V3

Insemination Kit

1. Warm up the GM501 SpermAir vial to 37 °C.
2. Remove metal cap from the stopper and desinfect the stopper´s surface with isopropylalcohol (70%).
3. Insert the enclosed short cannula through the stopper. (It serves as a pressure balance valve).
4. Aspirate liquefied, analysed ejaculate into an enclosed 2 ml syringe and attach a long cannula.
5. Hold the syringe with its tip upwards to collect air in the upper part of the syringe and press it out.
6. Insert the syringe's cannula (tip downwards) through the vial´s stopper until the tip touches the bottom of the vial.
7. Now release the ejaculate slowly and carefully by depressing the syringe and let it suspend under the preparation medium without mixing the two liquids.
8. Remove the syringe with the cannula carefully while leading the tip along the inner wall of the vial. Discard the syringe and the cannula.
9. Now carefully place the vial´s neck into the rack´s fork and store the vial at 37 °C in an incubator (no CO_2) or in a warming cabinet for least 45 minute and not longer than 3 hours.
10. At the appropriate time carefully take the vial out and turn it upright. Attach a fresh long cannula on the tip of a fresh 2 ml syringe, aspirate 1ml of air and insert it again through the disinfected stopper.
11. Aspirate 0.5 to 1.0 ml of the upper media layer and remove the syringe with the cannula. The syringe now contains the SpermAir fraction with the isolated motile sperms. Until the insemination procedure place the syringe with the cannula with the attached protection cap of the cannula in a incubator (no CO_2)/ warming cabinet at 37 °C.
12. To inseminate remove the cannula from the syringe and attach the enclosed IUI-catheter to the tip of the syringe.
13. The position assistance is adjusted corresponding to the anatomical proportions determined before.
14. The catheter is inserted until the assistance is positioned on the outer uterine orifice.
15. As soon as the requested position has been reached, the catheter will be turned, so that the marks on the grip are lying visible on top. In this way both of the lateral openings at the very end of the catheter are lined up towards the applicators orifice.
16. The suspension with the spermatozoa is injected slowly into the cave uteri.
17. Finally the catheter is slowly extracted out of the uterus.

Advice

If the sperm is not liquefied sufficiently 30 minutes after ejaculation, liquefy it by aspirating it into a sterile disposable syringe (2 or 5 ml) and flushing it out serveral times.

Before doing this, let disturbing rude particles sediment and do not aspirate them into the syringe.

It is recommended that the sperm concentration is analysed prior to insemination. At least 2 million grade A spermatozoa should be present. An insemination with lower than 0.5 million/ml motil spermatozoa is not recommended.

If performed optimally, the sperm suspension should contains no or very few immotile spermatozoa.

Gi133/V3

GYNEMED

Vitrification

Preparation

Ensure all media are warmed up to 37 °C and mixed well before use.

We would strongly advise you to read through all the steps of the vitrification/thawing procedure before starting the procedure.

Preliminary steps

1. In a 4-well dish fill the first well with 300 µl of VitriStore Pre-vitrification Medium, the second with VitriStore Freeze 1 and the third with Vitri-Store Freeze 2 solution.
2. Make sure that the liquid nitrogen is available to ensure fast work flow.
3. Next open as many packs of vitrification devices as will be required for the vitrification step. Place the separate parts of the vitrification device on the workbench for easy access later in the procedure.

Freezing preparation

1. Transfer the embryos from the blastocyst cell culture medium into the first well (PVM).
2. Process the embryos according to the schemes by transfering them from one well to the next.

Vitrification

1. Using an attenuated pipette or an equally suitable device, place a maximum of 2 blastocysts in a volume of approximately 0.3 µl of VitriStore Freeze 2 on the tip of your vitrification straw.
2. Place the vitrification straw in the outer sheath and seal it as indicated in the instructions for use of the vitrification device.
3. Plunge the sealed device into the liquid nitrogen.

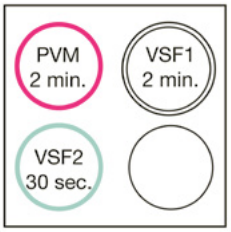
Morulae

Early blastocysts

Blastocyst after artificial shrinking

Solutions and application at room temperature

Well 1	Well 2	Well 3
PVM	VSF 1	VSF 2
2 min.	2 min.	30 sec.



Blastocysts

Expanded blastocysts

Solutions and application at 37 °C

Well 1	Well 2	Well 3
PVM	VSF 1	VSF 2
2 min.	3 min.	30 sec.

** Before starting the vitrification procedure, in order to reduce the negative effect of the blastocoel, expanded blastocysts should be collapsed by reducing the volume of the blastocoel artificially with a glass pipette. (Vanderzwalmen et. al., 2002; Sonet al., 2003; Hiraoka, 2004)*

Gi135/V3

Vitrification

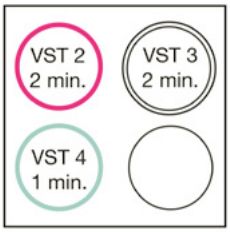
Preparation

Ensure all media are warmed up to 37 °C and mixed well before use.

We would strongly advise you to read through all the steps of the vitrification/thawing procedure before starting the procedure.

Thawing

1. In a 4-well dish fill the first well with 300 µl of VitriStore ThawMedium 1, the second with VitriStore ThawMedium 2, the third with VitriStore ThawMedium 3 and the 4th with VitriStore ThawMedium 4.



2. Remove the vitrifcation straw from the outer sheath as indicated in the instructions for use of the vitrification device.
3. Immediately plunge the vitrification straw into pre-heated VitriStore Thaw Medium 1 (37 °C) and leave in Thawing 1 medium for 3 minutes.

4. Transfer into VitriStore Thaw Medium 2 (37 °C) and leave in this medium for 2 minutes.
5. Transfer into VitriStore Thaw Medium 3 (37 °C) and leave in this medium for 2 minutes.
6. Finally transfer into VitriStore Thaw Medium 4 (37 °C) and wash for at least 1 minute.
7. Transfer into blastocyst culture medium for continued cell culture (e.g. GM501 Cult media).

35 mm Petri dish	Well 1	Well 2	Well 3
VST 1	VST 2	VST 3	VST 4
3 min.	2 min.	2 min.	1 min.

Gi136/V3

Slow freezing

Preparation

Ensure all media are warmed up to room temperature and mixed well before use.

Freezing

1. Using a sterile pipette place 1 ml of Embryo Store Freeze medium in a centre well dish (at room temperature).
2. Add the embryos to the freezing medium and allow them to settle for about 30 seconds.
   Caution: Due to density differences, the embyros tend to float upwards and shrink.
3. Load the embryos in straws leaving about 1/5 air in the straw.
4. Seal the straws and label with name, date and number of embryos.
5. Start freezing program within 5-10 minutes.
   Below is an example of a freezing protocol.

	Temperature range	Freezing rate	Time
Phase 1	RT to +4 °C	-10 °C/min	2 min
Phase 2	+4 °C to -6 °C	-2 °C/min	5 min
Phase 3	-6 °C (autoseeding)	0 °C/min	10 min
Phase 4	-6 °C to -30 °C	-0.3 °C/min	80 min
Phase 5	-30 °C to-196 °C	-199 °C/min	1 min

Thawing

1. In a 4-well culture dish place 1 ml of each EmbryoStore Thaw thawing solution (1, 2 & 3). This leaves 1 well empty to retrieve the frozen/thawed embryos.
2. Prepare a water bath at 37 °C to thaw the straws. Prepare a 1 ml tuberculin-syringe by filling it with 0.8 ml of air first followed by 0.2 ml of EmbryoStore Thaw 1 medium.
3. Remove the straws from liquid nitrogen and leave at room temperature for about 5 seconds.
4. Submerge the straw in the water bath at 37 °C for another 5 seconds (ensure no frozen part remains in the straw).
5. Empty the straw by opening both ends of the straw (above the empty well) and blowing the contents of the syringe through the straw.
6. Retrieve the embryos and place them in EmbryoStore Thaw 1 thawing solution.
7. Transfer the embryos to EmbryoStore Thaw 2 thawing solution after 3-5 minutes.
8. After another 3-5 minutes the embryos are transferred to EmbryoStore Thaw 3 thawing solution. Leave for a further 3-5 minutes before proceeding.
9. At the final stage the embryos are transferred in IVF culture medium (e.g. GM501 Cult media) for washing and further culture.

Gi134/V3

Sperm freezing

Preparation

Ensure all media are well mixed before use.

Before freezing

In case of very low sperm concentrations it is advisable to concentrate the sperm before freezing. GM501 Gradient can be applied before freezing to remove debris and to enrich the concentration of motile cells in a sample. This may increase sperm quality after thawing and will reduce the number of straws to be frozen. GM501 SpermStore needs to be warmed to room temperature before use to avoid cold-shock.

Freezing

1. Allow the semen to liquefy at room temperature for 30 minutes.
2. Mix 1.00 ml of sperm with 0.70 ml GM501 SpermStore. Add the medium in drops while gently swirling.
3. Leave the mixture for 10 minutes at room temperature for equilibration.
4. Aspirate the sample/medium mixture into the freezing straws, leaving approximately 1.5 cm of air at the end of the straws.
5. Seal the straws.
6. Dry off individually with a lint-free wipe.
7. Shake to move the air-bubble to the centre of the straw.
8. Place the straw horizontally on a styrofoam board in a liquid nitrogen bath to allow for freezing in vapour phase. Leave for (at least) 15 minutes.
9. Transfer straws quickly into liquid nitrogen and store at -196 °C.

Thawing

1. Remove as many straws as required from the liquid nitrogen.
2. Place the samples in tap water for 5 minutes.
3. Cut off the end of the straw, place the open end inside a container (e.g. a conical centrifugation tube) and tap the straw lightly against the side of the container to allow complete evacuation of the mixture.
4. Dilute the thawed sperm in a suitable insemination medium (at least 3.0 ml per 0.5 ml semen) and mix thoroughly.
5. Centrifuge for 15 minutes at 300-350x g.
6. Resuspend pellet in a suitable insemination medium.

After thawing

If necessary, use sperm preparation techniques after thawing the semen to eliminate dead sperm cells and debris.

Gi137/V3

Liquefaction

1. Warm the Bromelain in Dulbecco´s PBS to 37 °C.
2. Dilute the semen sample with the same volume of Bromelain (for highly viscous, tenacious ejaculates we recommend to grind the ejaculate roughly beforehand.
3. Swirl the semen solution carefully.
4. Incubate the ejaculate for approx. 10 minutes at 37 °C.
5. Use the liquefied semen sample for evaluation.
6. Continue IVF treatment according to internal standard procedures.

   Attention: To calculate the sperm cells concentration the dilution with factor of semen with Bromelain must be accounted.

   WHO laboratory manual-quote: These treatments may affect seminal plasma biochemistry, sperm motility and sperm morphology, and their use must be recorded

6. Continue IVF treatment according to internal standard procedures.

Gi138/V3

Collagenase treatment

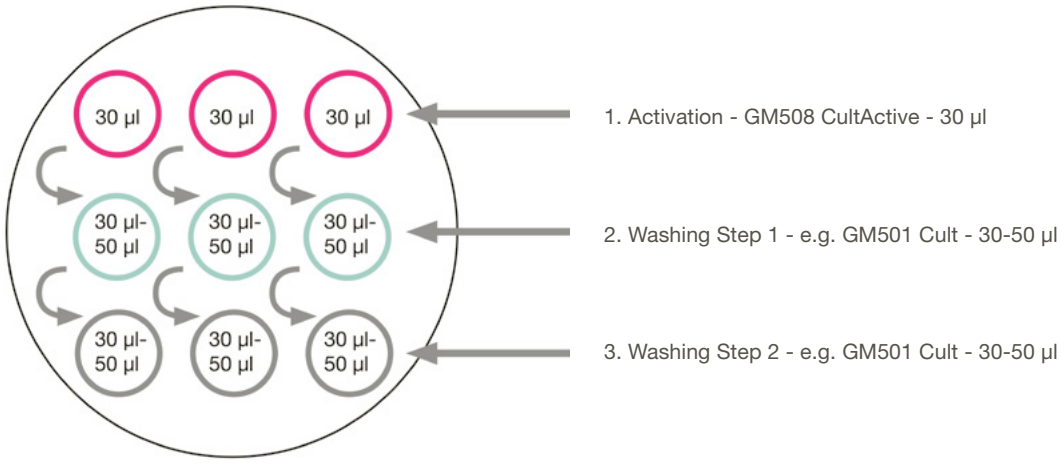
1. Transfer 1.5 ml GM501 Collagenase into a 5 ml round-bottom centrifugation tube.
2. Warm the GM501 Collagenase at 37 °C. GM501 Collagenase is HEPES-buffered. Incubation in a CO_2-Incubator will lower the pH.
3. For digestion of testicular tissue carefully pick up the chosen tissue pieces with a fine syringe cannula. For easier handling, if necessary, fill the tissue suspension into a 60 mm petri dish. Let adhesive transport or cryo medium drop off occur as much as possible and transfer into Collagenase tubes.
4. Close the tube completely and place in the incubator (or ideally in a heat cabinet for digestion of the tissue) for 60 minutes. Slight agitation every 20-30 min will facilitate the formation of a single cell suspension.
5. Suspend the digested tissue by carefully pipetting up and down. Under ideal conditions a suspension of single testicular tissue cells and free semen cells has been formed. If coarse tissue pieces are still visible, repeat step 4 for a further 20 to 30 minutes.
6. Now centrifuge the tissue cell suspension and wash twice with 1–2 ml HEPES-buffered sperm processing medium (e. g. GM501 SpermAir). Discard the obtained supernatant. Alternatively, the cell suspension can be processed using a density gradient system (e. g. GM501 Gradient).
7. Resuspend the pellet in a small volume of 30–80 µl HEPES-buffered sperm processing medium. Add a few µl of this suspersion into a dish.
8. Continue IVF treatment according to internal standard procedures.
9. If no motile sperms can be found Gynemed recommends the application of GM501 Sperm-Mobil.

GM501 SpermMobil

1. Do not equilibrate GM501 SpermMobil in a CO_2- incubator, just warm up to 37 °C.
2. To facilitate sperm activation add 1.50 - 2.00 µl GM501 Sperm Mobil to the sperm cells containing drop (approx. 30.00 - 40.00 µl/dilution 1:20) of processing media inside the Petri dish.
3. GM501 SpermMobil should be added to the opposing side from where the sperm cells are to be aspirated.
4. Wait for 10 minutes. The activating effect initiates after a few minutes and lasts approximately for one hour.
5. The dish should be placed on a heating plate at 37 °C during the diagnostic evaluation.

GM508 CultActive

1. GM508 CultActive must be shaken directly before use for approximately 30 sec.
2. GM508 CultActive must be equilibrated 4 hours in a vial not firmly closed at 5 - 7% CO_2 and 37 °C prior to use.
3. Equilibrate culture medium for washing (e.g. GM501 Cult) for 4 hours in a vial not firmly closed at 5 - 7 % CO_2 and 37 °C prior to use.
4. Prepare for each oocyte 1 drop (30.00 µl) GM508 CultActive and 2 drops (30.00 - 50.00 µl) culture medium MOPS and HEPES free, (e.g. GM501 Cult). An oil overlay of the drops using suitable oil (e.g. GM501 Mineral Oil) is recommended. Please be aware that protein-free media drops (e.g. GM508 CultActive) can exhibit slightly different dynamic properties compared to other media.
5. Immediately after the ICSI procedure incubate the oocytes for 15 minutes in the pre-equilibrated Ca^{2+}-Ionophore GM508 CultActive drops. (See picture below - Step 1 - Activation)
6. Remove the oocytes from the GM508 CultActive drop and wash twice in culture media. This has to be done in a HEPES or MOPS free media, e.g. GM501 Cult media. (See picture below - step 1 and 2 - washing)
7. Put the oocytes in your culture medium for further culture.
8. Assess the development on select time points.



Gi141/V3

SemenHos

Application

This SemenHos test is used to test the vitality of sperm cells. The hypo-osmotic swelling is based on the semi-permeability of the intact cell membrane and their ability of active water transport, in order not to burst. In sperm with intact membranes the flagellum swells up within 5 min. This change remains stable for up to 30 min.

Principle

In this hypo-osmotic swelling test swelling of cells only occurs in vital cells with an intact membrane by using hypotonic solution.

Storage and stability

2-8 °C. Sterile sampling. Contains no antibiotic.

24 months from date of manufacture.
After opening use within 7 days.

Content

SemenHos solution, 20 x 900 µl

Necessary utensils

- Coverslips (18 x 18 mm)
- Gloves
- Contrasting phase microscope
- Native ejaculate or washed sperm (105-110 µl)
- Slides
- Paper towels
- Pipettes and tips (10-100 µl)
- Water bath or heating cabinet (37°C)

Preparation of SemenHos solution

Preheat the SemenHos solution to 37 °C

Procedure

1. Occasionally native ejaculate without additions includes hypo-osmotic sperm forms. Transfer 5-10 µl liquefied semen without air bubbles to a slide and cover it with cover slips. Microscope at 400x magnification. This is the zero-value.
2. Examine the percentage of sperm with swollen flagellum by observing 100 sperm, calculated in duplicate.
   Note this value note* (a_0 %).
3. Add 100 µl ejaculate without air bubbles to 900 µl preheated SemenHos solution and mix
4. Incubate this mixture for 10 min at 37 °C.
5. Transfer 10 µl of the mixture to a slide and place and cover it with a cover slip.
6. Microscope at 200x or 400x magnification.
7. Repeat twice step 2 to 6.

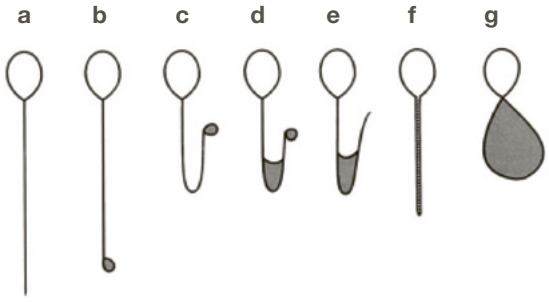


Fig.1: Schematic representation of typical morphological changes of human spermatozoa after exposure with hypo-osmotic solution (extract WHO 2010).

No change (a). Different tail changes (b-g).
The swelling in the tail region is indicated in gray.

Evaluation

Percentage of vitality of spermatozoa

Calculate the difference between the percentage of sperm with swollen flagellum before and after incubation with the SemenHos solution.

Example:

Before incubation:

1. Count 2/100
2. Count 3/100

Mean value: 2.5 /100= 2.5%

After incubation:

1. Count 88/100
2. Count 97/100

Mean value: 92.5/100= 92.5%

Result: 92.5% - 2.5%= 90%

90 % of the sperm are vital

The SemenHos test is regarded as normal, if after incubation more than 60% of the sperm show a swollen flagellum.
The sample is not normal when the result is less than 50% (WHO 2010).

Safety information / Precautions

(Please read also safety data sheets)

- All semen samples should be considered potentially infectious. Handle all samples as though they are HIV or hepatitis infected material.
- When working with samples and reagents always wear protective clothing (gloves, gowns, eye /face protection).
- All ingredients of reagents are classified as non-toxic

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SemenIgA

Application

“Immunological Infertility” describes the absence of a concept as a result of an impaired immune system of the reproductive organs. The vast majority of these disorders are based on the presence of anti-sperm antibodies (ASA) that can occur in both genders.

Immunoglobulins (antibodies) are considered as defense mechanisms of the body against foreign objects or substances. When the body detects a “foreign” substance, it tries to build an immunity using antibodies against the substance. However, in the case of anti-sperm antibodies, the body recognizes the sperm as foreign, it then builds anti-bodies against it and affects the reproductive process. The antibodies built by the defense system of a woman inhibit the sperm to enter through the cervical mucus and to reach the egg. In men, antibodies which bind to the spermatozoa impede the passage of sperm through the cervical mucus. Causes for the development of these antibodies may be infections and / or injury to the genital tract or an autoimmune disease.

Sperm antibodies in the ejaculate, almost all belong to the IgA and / or IgG classes. Data suggest that IgA antibodies are more clinically significant than IgG antibodies, but rarely occur alone. The antibody in ASA of serum is predominantly IgG, but in ASA of the ejaculate or cervical mucus it is IgA.

Principle

For screening tests combined anti-Ig beads can be used which are able to detect all Ig types. By using the immune beads tests (IBT) different types of antibodies against sperm can be detected in different biological samples, like blood, cervical mucus and sperm. The test may indicate the presence of antibodies and the severity of antibody formation, as well as which part of the sperm cells specifically is affected.

The type of ASA has an influence on the function of spermatozoa only when complement fixing antibodies are present. A survey of reproductive medical centers in the UK showed (Krapež et al., 1998) that the MAR test ((“mixed antiglobulin reaction test”) or the IBT (immuno bead test) was used. Both tests are easy to perform. They work with beads which are coated with anti-immunoglobulins and bound on the surface of the spermatozoa attached by ASA.

The WHO referred the MAR test as compulsory screening test. The in vitro detection of antigens or antibodies is only possible when the antigen-antibody reaction is made visible or measurable. The selection of the detection technique is dependent on the properties of the antigen (size, number and structure of the antigenic determinants), the properties of the corresponding anti-body (avidity and specificity) and the concentration of the analyte to be determined. Antigen or anti-bodies are principally determined by the following techniques:

- Direct readout
- Indirect readout
- Proof due to the marking of a reactant

For the determination of IgA antibodies against spermatozoa in this test direct evidence can be used with blue microspheres.

Storage and stability

Store at 2-8 °C. Do not freeze

Time: 18 months from date of manufacture

Content

1 bottle containing 300 µl red latex particles (3 µm diameter) coated with spec. Anti-IgA.

Note: preserved with sodium azide (final concentration 0.09%).

Necessary utensils (not included)

- Cover slips (18 x 18 mm)
- Moist chamber
- Gloves
- Light microscope, 400x-600x magnification
- Ejaculate (10 µl)
- Slides
- Paper towels
- Pipettes and tips (0.5-10 µl)

Procedure (see also diagram below)

Performing the direct testing of the IgA class in human spermatozoa.

1. Warm up semen sample and reagents to room temperature. Use only native ejaculate.
2. Pipette 3.5 µl fresh ejaculate 3.5 µl latex particles on a glass slide (mix latex particles before).
3. Mix with the tips of the pipette 5x the semen sample with the latex particles.
4. Place a cover slip to the mixture and incubate in a moist chamber.
5. Evaluate the mixture after 3 minutes. Examine the sample under a light microscope at 400x-600x magnification. It may be beneficial to evaluate the slide under phase contrast or dark field. If positive, the latex particles are deposited on the motile sperm. This reaction between the sperm and the latex particles indicates that anti-sperm antibodies are present. Count 100 sperm to determine the percentage of IgA-positive sperm. Count again after 10 minutes.

Keep the slides while waiting in a humid environment (e.g. in a culture dish containing moist filter paper).

Evaluation:
A suspected immunological infertility is when 10-39% of motile sperm have adhering latex particles. If 40% or more of the sperm have adhesive latex particles, an immunological infertility is very likely.

The direct IgA test can be carried out only with motile sperm. Semen samples with very low sperm concentration or a small number of motile sperm can lead to erroneous results. It is therefore recommended to count a larger number.

Important note:
The decision to an ART therapy should not be primarily the result of the IgA-dependent test, but could value as an additional factor beside the number of sperm, sperm motility, sperm morphology and other traits are valued.

Safety information / precautions

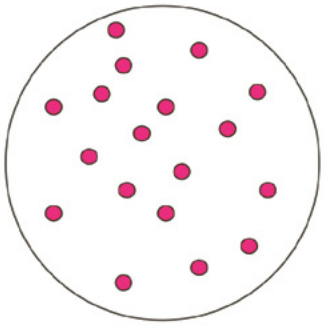
(Please read also the safety data sheets)

- All semen samples should be considered potentially infectious. Handle all samples as if they are HIV or hepatitis infected material.
- When working with samples and reagents always wear protective clothing (gloves, gowns, eye / face protection).
- SemenIgA contains 0.1% bovine serum albumin.
- Sample slides with samples and remaining sample should be inactivated after evaluation and then discarded.

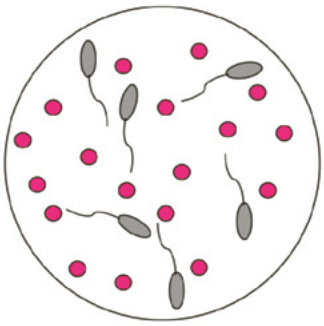
Gi181/V1

Diagram of the procedure of the seminal IgA test

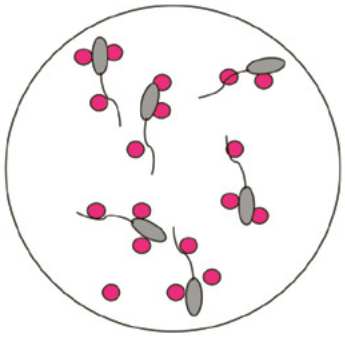
3.5 µl Anti-IgA- latex particles



3.5 µl fresh, native sperm



Mix 5x latex particles with 3.5 µl fresh native sperm



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SemenIgG

Application

“Immunological Infertility” describes the absence of a concept as a result of an impaired immune sys-tem of the reproductive organs. The vast majority of these disorders are based on the presence of anti-sperm antibodies (ASA) that can occur in both genders.

Immunoglobulins (antibodies) are considered as defense mechanisms of the body against foreign objects or substances. When the body detects a “foreign” substance, it tries to build an immunity using antibodies against the substance. However, in the case of anti-sperm antibodies, the body rec-ognizes the sperm as foreign, it then builds anti-bodies against it and affects the reproductive process. The antibodies build by the defense system of a woman inhibit the sperm to enter through the cervical mucus and to reach the egg. In men, antibodies which bind to the spermatozoa impede the passage of sperm through the cervical mucus. Causes for the development of these antibodies may be infections and / or injury to the genital tract or an autoimmune disease.

Sperm antibodies in the ejaculate almost all belong to the IgA and / or IgG classes. Data suggest that IgA antibodies are more clinically significant than IgG antibodies, but rarely occur alone. The antibody in ASA of serum is predominantly IgG, but in ASA of the ejaculate or cervical mucus it is IgA.

Principle

For screening tests combined anti-Ig beads can be used which are able to detect all Ig types. By using the immune beads tests (IBT) different types of antibodies against sperm can be detected in differ-ent biological samples, like blood, cervical mucus and sperm. The test may indicate the presence of antibodies and the severity of antibody formation, as well as which part of the sperm cells specifically is affected.

The type of ASA has an influence on the function of spermatozoa only when complement fixing antibodies are present. A survey of reproductive medical centers in the UK showed (Krapež et al., 1998) that the MAR test ((“mixed antiglobulin reaction test”) or the IBT (immuno bead test) was used. Both tests are easy to perform. They work with beads which are coated with anti-immunoglobulins and bound on the surface of the spermatozoa attached by ASA.

The WHO referred the MAR test as compulsory screening test. The in vitro detection of antigens or antibodies is only possible when the antigen-antibody reaction is made visible or measurable. The selection of the detection technique is de-pendent on the properties of the antigen (size, number and structure of the antigenic determi-nants), the properties of the corresponding anti-body (avidity and specificity) and the concentration of the analyte to be determined. Antigen or anti-bodies are principally determined by the following techniques:

- Direct readout
- Indirect readout
- Proof due to the marking of a reactant

For the determination of IgG antibodies against spermatozoa in this test direct evidence can be used with blue microspheres.

Storage and stability

Store at 2-8 °C. Do not freeze

Time: 18 months from date of manufacture

Content

1 bottle containing 300 µl latex particles (3 µm diameter) coated with spec. Anti-IgG.

Note: preserved with sodium azide (final concentration 0.09%).

Necessary utensils (not included)

- Cover slips (18 x 18 mm)
- Moist chamber
- Gloves
- Light microscope, 400x-600x magnification
- Ejaculate (10 µl)
- Slides
- Paper towels
- Pipettes and tips (0.5-10 µl)

Procedure (see also diagram below)

Performing the direct testing of the IgA class in human spermatozoa.

1. Warm up semen sample and reagents to room temperature. Use only native ejaculate.
2. Pipette 3.5 µl fresh ejaculate 3.5 µl latex particles on a glass slide (mix latex particles before).
3. Mix with the tips of the pipette 5x the semen sample with the latex particles.
4. Place a cover slip to the mixture and incubate in a moist chamber.
5. Evaluate the mixture after 3 minutes. Examine the sample under a light microscope at 400x-600x magnification. It may be beneficial to evaluate the slide under phase contrast or dark field. If positive, the latex particles are deposited on the motile sperm. This reaction between the sperm and the latex particles in-dicates that anti-sperm antibodies are present. Count 100 sperm to determine the percentage of IgG-positive sperm. Count again after 10 minutes.

Keep the slides while waiting in a humid environment (e.g. in a culture dish containing moist filter paper).

Evaluation:
A suspected immunological infertility is when 10-39% of motile sperm have adhering latex particles. If 40% or more of the sperm have adhesive latex particles, an immunological infertility is very likely.

The direct IgG test can be carried out only with motile sperm. Semen samples with very low sperm concentration or a small number of motile sperm can lead to erroneous results. It is therefore recommended to count a larger number.

Important note:
The decision to an ART therapy should not be primarily the result of the IgG-dependent test, but could value as an additional factor beside the number of sperm, sperm motility, sperm morphology and other traits are valued.

Safety information / precautions

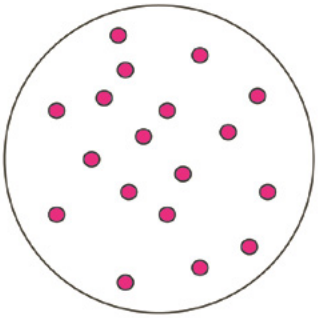
(Please also read the safety data sheets)

- All semen samples should be considered potentially infectious. Handle all samples as though they are HIV or hepatitis infected material.
- When working with samples and reagents always wear protective clothing (gloves, gowns, eye / face protection).
- SemenIgG contains 0.1% bovine serum albu-min.
- Sample slides with samples and remaining sample should be inactivated after evaluation and then discarded.

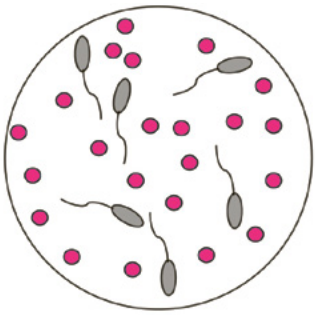
Gi184/V2

Diagram of the procedure of the seminal IgG test

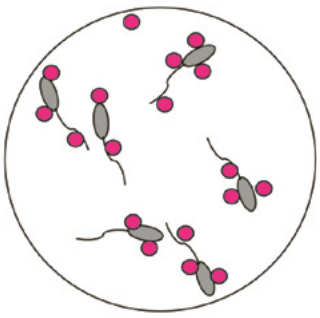
3.5 µl Anti-IgG- latex particles



3.5 µl fresh, native sperm



Mix 5x latex particles with 3.5 µl fresh native sperm



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SemenLeu

Application

The determination of leukocytes in seminal fluid serves as a marker for the functioning of the accessory sex glands. Leukocytes, especially polymorphic polynuclear leucocytes (PML), are present in most human ejaculates. By normal microscopy these cells can be morphologically easily mixed up with multi-nuclear spermatids. It is known that peroxidases are histochemically exclusively characteristic for the PM granulocytes.

Principle of method

By using hydrogen peroxide (H2O2) peroxidase-positive leukocytes (neutrophils polymorphic granulocytes) can be stained yellow to brown. Other cells (sperm, lymphocytes, monocytes, macrophages and multinucleated spermatids) remain unstained (peroxidase-negative). With this kit the seminal fluid is treated with the reagents 1 and 2 in which only peroxidase stainpositive cells remain brown. These cells can be identified with a phase contrast microscope.

Storage and stability

Temperature: 2-8 °C

Time: 24 months from date of manufacture. The work solution AB is usable after storage in the fridge until the next day.

Content

- Reagent 1 20 ml
- Reagent 2 1 ml

Necessary utensils

- Coverslips (18 x 18 mm)
- Wet-chamber
- Gloves
- Contrasting phase microscope
- Native ejaculate or washed sperm (100 µl)
- Slides
- Paper towels
- Pipettes and tips (10-100 and 100-1000 µl)
- Test tube (2 ml)
- Test tube holder
- Cell counting chamber

Preparation of work solution AB

Mix 1 ml of reagent 1 with 20 µl of reagent 2. In the case of studying more samples you have to calculate the appropriate amount of solution AB.

Procedure

1. Pipette 100 µl ejaculate into a test tube
2. Add 900 µl of solution AB
3. Mix gently solution AB and ejaculate (avoid foaming)
4. Incubate the mixture at room temperature 20-30 min
5. Repeat step 3
6. Pipette the mixture into a counting chamber. Put the counting chamber for four minutes in a wet chamber to let all large cells sink.

Evaluation objective: number of leukocytes in ejaculate

By microscopic view leukocytes are colored yellow to brown by peroxidases. The total number of peroxidasepositive cells per ejaculate can be calculated in one of the following options:

Unknown concentration of spermatozoa:
Count the peroxidase-positive cells and spermatozoa in at least 20 fields of view at 400x magnification. The concentration of the white blood cell is calculated using the following formula: (Number of white blood cells / number of spermatozoa) x sperm concentration (million / ml)

This method is only suitable for samples which contain more than 10 million sperm cells/ml.

Unknown concentration of spermatozoa:
In this case, the concentration of white blood cells is determined by multiplication by a factor which results from the size of a field of view and the height of the distance between the counting chamber and the coverslip (or the depth of the semen sample). The diameter of a field of view can be measured by a micrometer. The surface area (s) corresponds to the square of the radius (r) multiplied by pi ($s = \pi r^2$).

Example: view field diameter = 250 µm, radius = 125 µm area (s) = 49086 μm^2 .

The height between the slide and the coverslip can be calculated with the following formula: height [µm] = volume µl / (length [µl] x width mm of the coverslip. Example: sample volume = 20 µl. Coverslip = 24 x 40 mm height = 20/(24x40) = 0.0208 mm = 20.8 µm.

The factor by which the concentration of white blood cells has to be multiplied is calculated from these values: Factor = 1,000,000 μm^3 (area x height).

Example: Factor = 1,000,000 μm^3 / (49086 µm2 x 20.8 µm) = 0.98.

For example, if five white blood cells in a field of view are counted it results by this factor a concentration of 4.9 million white blood cells per ml of ejaculate. In fertile men the value of peroxidase-positive leukocytes is between $0.5x10^6$ and 10^6 at a total leukocyte number (peroxidase-positive and peroxidasenegative cells) from 10^6 and $2x10^6$ per ml of ejaculate [6].

Excessive presence of these cells (sperm-induced leukocytosis) can display a seed head infection. The sperm-induced leukocytosis can also be associated with a disturbance of the seed profile, including the reduction of semen volume, sperm concentration and sperm motility and a loss of sperm function as a result of oxidative stress [1, 2] or the secretion of cytotoxic cytokines. It is therefore difficult to give an exact limit of the leukocyte concentration at which fertility is impaired. The influence of these cells depends on the place in the reproduction channel from where the leukocytes enter the sperm, the type of leukocytes and the degree of activation. If the seminal fluid contains more than $1x10^6$ white cells per ml, the samples should be tested microbiologically for gland infection.

Note: The absence of leukocytes does not exclude the possibility of glandular infection.

Safety information / precautions

- Please also read all safety data sheets
- All semen samples should be considered potentially infectious. Handle all samples as possible HIV or hepatitis infected material.
- When working with samples and reagents always wear protective clothing (gloves, gowns, eye /face protection).
- Reagent 1 contains ortho-toluidine, which is classified as carcinogenic. Skin contact or ingestion should be avoided.
- Reagent 2 contains hydrogen peroxide (H_2O_2). It is corrosive and toxic by inhalation. Skin contact or ingestion should be avoided.
- In case of an accident with reagent 1 and/or 2 contaminated clothing should take off immediately and consult a doctor.

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Gi149/V3

SemenMar

Application

“Immunological Infertility” describes the absence of a concept as a result of an impaired immune system of the reproductive organs. The vast majority of these disorders are based on the presence of anti-sperm antibodies (ASA) that can occur in both genders.

Immunoglobulins (antibodies) are considered as defense mechanisms of the body against foreign objects or substances. When the body detects a “foreign” substance, it tries to build an immunity using antibodies against the substance. However, in the case of anti-sperm antibodies, the body recognizes the sperm as foreign, it then builds antibodies against it and affects the reproductive process. The antibodies built by the defense system of a woman inhibit the sperm to enter through the cervical mucus and to reach the egg. In men, antibodies which bind to the spermatozoa impede the passage of sperm through the cervical mucus. Causes for the development of these antibodies may be infections and / or injury to the genital tract or an autoimmune disease.

Sperm antibodies in the ejaculate belong almost to the IgA and / or IgG classes. Data suggest that IgA antibodies are more clinically significant than IgG antibodies, but rarely occur alone. The antibody in ASA of serum is predominantly IgG, but in ASA of the ejaculate or cervical mucus it is IgA.

Principle of method

For screening tests combined anti-Ig beads can be used which are able to detect all Ig types. By using the immune beads tests (IBT) different types of antibodies against sperm can be detected in different biological samples, like blood, cervical mucus and sperm. The test may indicate the presence of antibodies and the severity of antibody formation, as well as which part of the sperm cells specifically is affected.

The type of ASA has an influence on the function of spermatozoa only when complement fixing antibodies are present. A survey of reproductive medical centers in the UK showed (Krapež et al., 1998) that the MAR test (“mixed antiglobulin reaction test”) or the IBT (immuno bead test) was used. Both tests are easy to perform. They work with beads which are coated with anti-immunoglobulins and bound on the surface of the spermatozoa attached by ASA.

The WHO referred the MAR test as a compulsory screening test. The in vitro detection of antigens or antibodies is only possible when the antigen-antibody reaction is made visible or measurable. The selection of the detection technique is dependent on the properties of the antigen (size, number and structure of the antigenic determinants), the properties of the corresponding antibody (avidity and specificity) and the concentration of the analyte to be determined. Antigen or antibodies are principally determined by the following techniques:

- Direct readout
- Indirect readout
- Proof due to the marking of a reactant.

For the determination of IgA and IgG antibodies against spermatozoa in this test direct evidence can be used with yellow microspheres.

Storage and stability

Store at 2-8 °C. Do not freeze

18 months from date of manufacture

Content

1 bottle containing 300 µl latex particles (3 µm diameter) coated with spec. Anti-IgA and Anti-IgG.

Note: preserved with sodium azide (final concentration 0.09%).

Necessary utensils (not included)

- Cover slips (18 x 18 mm)
- Moist chamber
- Gloves
- Light microscope, 400x-600x magnification
- Ejaculate (10 µl)
- Slides
- Paper towels
- Pipettes and tips (0.5-10 µl)

Procedure (see also diagram below)

Performing the direct testing of the IgA and IgG class in human spermatozoa.

1. Warm up semen sample and reagents to room temperature. Use only native ejaculate.
2. Pipette 3.5 µl fresh ejaculate and 3.5 µl latex particles on a glass slide (mix latex particles before).
3. Mix with the tips of the pipette 5x the semen sample with the latex particles.
4. Place a cover slip on the mixture and incubate in moist chamber.
5. Evaluate after 3 minutes the mixture. Examine the sample under at light microscope at 400x-600x magnification. It may be beneficial to evaluate the slide under phase contrast or dark field. If positive, the latex particles are adhered on the motile sperm. This reaction between the sperm and the latex particles indicates that antibodies are present. Count 100 sperm to determine the percentage of IgA and IgG-positive sperm. Count again after 10 minutes.

Keep the slides while waiting in a humid environment (e.g. in a culture dish containing moist filter paper).

Evaluation

A suspected immunological infertility is when 10-39% of motile sperm have adhering latex particles. If 40% or more of the sperm have adhesive latex particles, an immunological infertility is very likely.

The direct IgA and IgG test can be carried out only with motile sperm. Semen samples with very low sperm concentration or a small number of motile sperm can lead to erroneous results. It is therefore recommended to count a larger number.

Important note:
The decision to an ART therapy should not be primarily the result of the IgA-dependent test, but could value as an additional factor beside the number of sperm, sperm motility, sperm morphology and other traits are valued.

Safety information / precautions

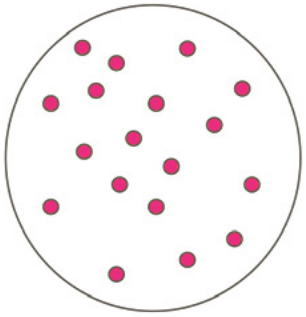
(Please read also the safety data sheets)

- All semen samples should be considered potentially infectious. Handle all samples as though they are HIV or hepatitis infected material.
- When working with samples and reagents always wear protective clothing (gloves, gowns, eye /face protection).
- SemenIgA contains 0.1% bovine serum albumin.
- Sample slides with samples and remaining sample should be inactivated after evaluation and then discarded.

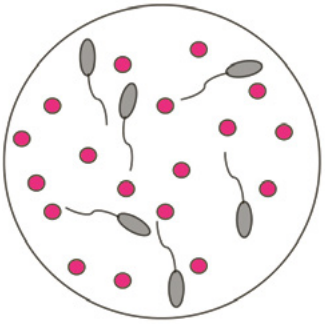
Gi185/V1

Diagram of the procedure of the seminal IgA and IgG test

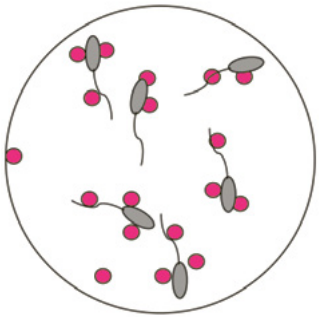
3,5 µl (IgG, IgA-Latex particles)



3,5 µl fresh, native sperm



Mix 5x latex particles with 3,5µl fresh native sperm



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SemenStain

Application

SemenStain is a quick-staining-method to assess the morphology of sperm (spermiogram). This method is composed of a staining kit which allows differential staining of the sperm parts due to their different basophilic, eosinophilic and neutrophilic properties.

Principle

The sperm are fixed. Here, the succedan-staining is used, which means, three dyes are used one after the other and it results to differentiate staining of different tissues with individual dyes.

Storage and stability

15-25 °C

36 months from date of manufacture

Content

- Reagent 1, 1x 50 or 250 ml
- Reagent 2, 1x 50 or 250 ml
- Reagent 3, 1x 50 or 250 ml
- Reagent 4, 1x 50 or 250 ml

Necessary utensils (not included)

- Native ejaculate or washed sperm (5-10 µl)
- Staining cuvettes (8x) or tubes (50 ml, 8x)
- Gloves
- Tweezers
- Paper towels
- Slides
- Slides rack (if more than five slides are to dye)
- Immersion oil
- Microscope

Procedure (see also diagram)

1. Apply 5-10 µl sperm per slide, smear the sperm with a coverslip and let dry. We recommend preparing 2 slides per patient.
2. Fill the staining cuvettes with reagent 1, reagent 2, reagent 3 and reagent 4, respectively. Fill 4 other empty cuvettes with water. Place the cuvettes side by side. Label them from 1 to 8.
3. Immerse slides 3 minutes by repeated immersion in cuvette 1 (reagent 1) to fix the preparation. Wash slides 3 minutes in cuvette 2 (water). Then place the slides vertically on paper towels to remove excess water.
4. Colour the slides 1 minute by repeated immersion in cuvette 3 (reagent 2). Wash the slides in cuvette 4 (water). Change the water several times until water stays clear; remove with paper towel the excess water of the slides.
5. Colour the slides 1 minute by repeated immersion in cuvette 5 (reagent 3), wash the slides according to step 4 in cuvette 6 (water) and remove from the slides with paper towel the excess water.
6. Colour the slides 1 minute by repeated immersion in cuvette 7 (reagent 4), wash the slides according to step 4 in cuvette 8 (water) and remove from the slides with paper towel the excess water.
7. Dry the slides in the open air.

Evaluation

Evaluate the sperm with immersion oil at 1000x magnification on the side of the slide with lower sperm density. Here the sperm are better to assess individually.

The criteria for classification of sperm by their morphology can be found in the WHO laboratory manual (2010).

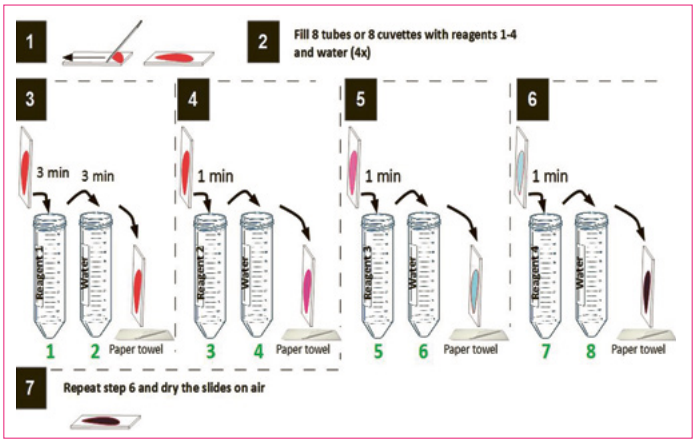
Sperm cell parts	Staining/colour
Head - Nucleus	red
Head - Acrosome	dark green
Middle part	pale green
Tail	green

After the evaluation the immersion oil can be gently removed from the slide with a paper towel. Then the slide can be immersed in reagent 1 for 5 min, dried and stored. It is also possible to produce preparations with a coverslip and glue for long term storage.

Safety information / precautions

- All semen samples should be considered potentially infectious.
- Handle all samples as though they are HIV or hepatitis infected material.
- When working with samples and reagents always wear protective clothing (gloves, gowns, eye / face protection).
- Reagent 1 is containing methanol: toxic by inhalation, skin contact or ingestion. May cause organ damage. There is a risk of irreversible damage.
- All other ingredients are not classified as toxic

Diagram (see also procedures outlined above)



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Gi186/V1

SemenVit

Application

This SemenVit kit is used to examine the motility and vitality of sperm. It is particularly important in semen samples with less than 40% of the forward moving (motile) sperm.

Principle

The determination of the vitality of sperm cells is judged by the integrity of the sperm membrane. The dye exclusion method is based on the fact that dead sperm with damaged plasma membrane absorb certain dyes.

Storage and stability

Store at 2-8 °C.

Time: 24 months from date of manufacture

Content

- Reagent 1 20 ml
- Reagent 2 230 ml

Required utensils

- Gloves
- Immersion oil
- Microscope
- Native ejaculate or washed sperm (20-50 µl)
- Slides
- Paper towels
- Pipettes and tips (10-100 µl)
- Test tubes (1.5 or 2 ml)
- Test tube holder

Procedure

This test should begin immediately after liquefaction of the semen sample, preferably after 30 minutes and not later than 60 minutes to avoid negative influences:

1. Pipette 20-50 µl sperm ejaculate in a test tube.
2. Add 2 drops of reagent 1, mix (avoid foaming) and incubate at room temperature for 30 seconds.
3. Add 3 drops of reagent 2 and mix again.
4. Transfer 10 µl of the mixture to a slide, smear the mixture with a cover slip and let air dry.
5. Evaluate the sperm with immersion oil at 1000x magnification.

Evaluation

Vital sperm appear colourless, transparent or light pink; dead or not viable cells are stained red. Sometimes in sperm within the coloured neck region and not coloured head and flagellum are observable- This caused by damage to the membrane and these sperm are classified as vital.

Count 200 cells and distinguish between vital sperm from dead sperm. The total number of membrane-intact sperm is of biological significance. The value is determined by multiplying the total number of sperm in the ejaculate by the percentage of membrane-intact cells.

Total number of vital sperm =
Total number of sperm x percentage vital sperm

Example

Total number of sperm in ejaculate: 20 Million Percentage of vital sperm 55% or 0.55, respectively

Total number of vital sperm = 20 Million x 0.55 = 11 Million

Vital sperm are not necessarily motile. Therefore, it is of clinical importance, whether immotile sperm are living or dead cells. Test results should be made in connection with the evaluation of the motility of the same semen sample. Vital but immotile sperm may have structural defects in the flagellum.
A high number of immotile and dead sperm (necrozoospermia) may indicate a dysfunction of the epididymis. The lowest reference value for the vitality of the sperm is 58% (WHO 2010).

Safety information / precautions

- All semen samples should be considered potentially infectious.
- Handle all samples as if they are HIV or hepatitis infected material.
- When working with samples and reagents always wear protective clothing (gloves, gowns, eye /face protection).
- Reagent 1 is containing eosin Y and reagent 2 is containing nigrosine. Both substances are not classified as toxic

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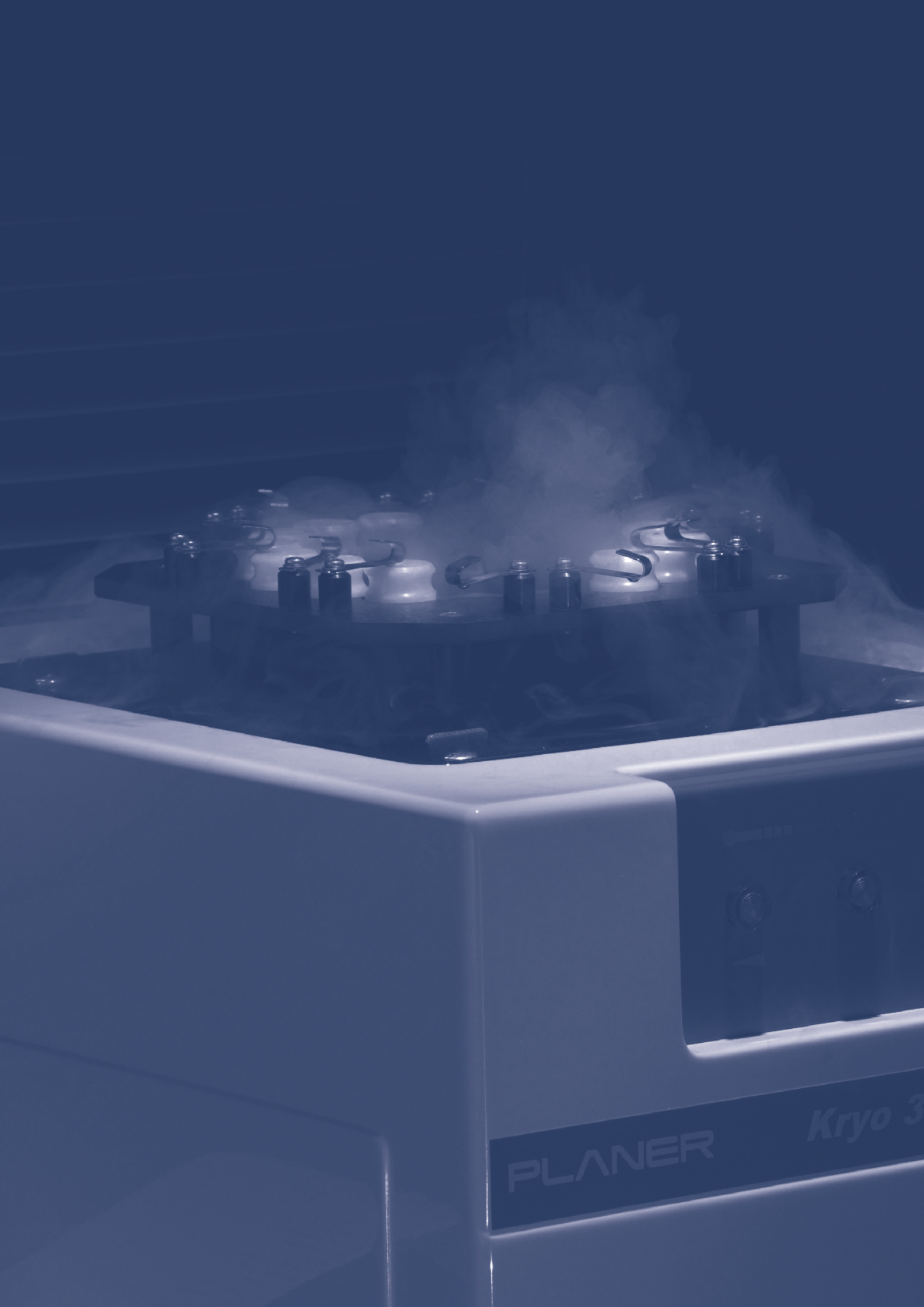
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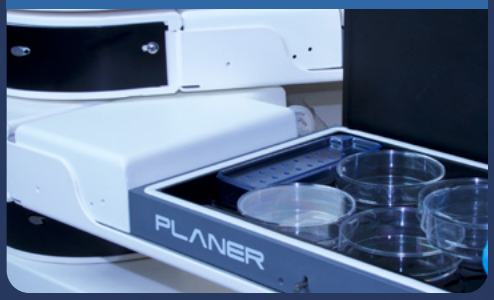

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