**Use**

The motile sperm are harvested and maximized for intrauterine insemination to enhance fertility

**Principle**

As semen is centrifuged through a two layer gradient system (Isolate), contaminants, including dead sperm, white cells, abnormal forms and other miscellaneous debris are reduced, therefore, separating the motile fraction of sperm from the seminal fluid. Sperm are then washed in a medium that utilizes the basic formulation and pH of human tubal fluid.

A. Scheduling Instructions for spin and wash with hospital lab:

* Schedule with as much notice as possible.
* Procedure will be scheduled following collection at 06:30.
* Information needed for scheduling is: donor and recipient, names, both birth dates, patients home phone number, doctor, and date.
* Hospital lab records information on calendar and have a bag of supplies ready when patient arrives, if needed.
* Make sure patient is within 30 minutes of hospital, if it is a home collection.

B. Lab instructions for preparing bags of supplies for collections:

* Use brown paper bags.
* Place the “Important instruction” label on outside of the bag
* The bag of supplies should contain:

- Sterile container with name label attached

- Verification form

- Copy of patient instructions and information

- New unused biohazard bag

C. Patient instructions for collection and handling of semen sample.

**Reagents, Supplies, & Equipment**

1. Sperm washing medium - 12 ml

* PH is 7.2 - 7.4
* Store at 2-8⁰C. Warm to 37+2⁰C just before use.
* Do not freeze or expose to temperatures greater than 39⁰C.
* Do not use beyond expiration date.
* Vial is good for 2 weeks after opening.
* Do not place in CO2 incubator.
* Do not use if media shows evidence of particulate matter, cloudiness or is not salmon to rose in color.
* Does not contain any antibiotic. Supplementation may be added just prior to use.

 2. Isolate

 Colloidal suspension of silica particles stabilized with covalently bound hydrophilic

 saline in a HEPES-buffered HTF and is sterile filtered.

* Store at 2 - 8⁰ C and warm to 37⁰ C just before use. Discard 30 days after opening.
* May appear slightly cloudy. This is normal.
* Do not use any vial of medium which shows evidence of particulate matter or contamination (extreme cloudiness).
* This product contains no preservatives or antibiotics. To preserve sterility, the product must be used with aseptic technique.
* Do not use beyond expiration date.
* Do not place in CO2 incubator.

3. Makler Counting Chamber

The Makler Counting Chamber is a simple to use device for rapid and accurate sperm count and motility evaluation.

The chamber is composed of two parts:

1. Metal base
2. Cover glass

At the center of the cover glass is a 1 mm squared grid, subdivided into 100 squares each 0.1 x 0.1 mm.

When the cover glass is placed on the four tips, the space bounded by the two surfaces and any row of 10 squares of the grid is exactly 0.001 mm cubed or one millionth of ml.

1. Sperm Quality Analyzer – SQA-V

 The SQA-V is an automated sperm quality analyzer. The system performs a highly

 reliable 70-second semen analysis that follows the WHO guidelines for analyzing Sperm

 Concentration, Motility and Normal Morphology and other parameters. The SQA-V can

 test FRESH, FROZEN, WASHED and POST VASECTOMY samples and it runs assayed

 QwikCheck-beads (latex) for QC purposes.

1. SQA-V capillary

 6. Qwik Chek Control Beads

 7. Sterile specimen containers.

8. Sterile centrifuge tubes with screw cap

1. Sterile pipettes

10. Sterile luer tip caps

11. Padded manila envelope

12. 10cc and 3cc syringes

13. 1 ½ inch blunt end 19 gauge needles

14. 37+2⁰ C. Incubator

15. 36+3⁰ C. Heating block

16. 56-70⁰ C. Heating block

**Specimen Requirements**

*Patient should be given specific instructions on the proper method of collecting semen specimens by their physician. \*Specimens collected outside of the laboratory area should be kept near body temperature and delivered to the laboratory within 30 min of collection.*

 Specimen type: **Semen**

 Specimen volume: **Entire ejaculate**

* The entire specimen must be collected into a sterile container (supplied by the physician’s office or the laboratory.
The specimen container must be clearly labeled with the patient name and identifying information
* Transport the specimen to the laboratory right after collection. Offsite collection is an option **if** the sample can be delivered **to the lab within 30 minutes of collection.**
* Keep the sample at ambient temperature during transportation within the hospital or body temperature if collected outside the hospital.

**Sample Rejection Criteria**

* The physician’s office will make an appointment for the procedure. Only appointments in which transportation of the IUI specimen to the physician’s office can occur within 30 minutes will be accepted (local)
* Collect the entire ejaculate by masturbating into the sterile container provided by physician or laboratory. Other containers will be rejected. Contact physician before rejecting.
* The person receiving the specimen must check to see if it is labeled. Unlabeled specimens may be rejected. Contact physician before rejecting.
* If the specimen is delivered greater than 30 minutes after ejaculation, note on patient report and notify physician.

**Safety**

* Handle all specimens as capable of transmitting disease.
* Gloves must be worn when working with semen specimens.

**SQA-V Auto-calibration & Self-Test**

The SQA-V automatically performs a five minute Auto-Calibration and Self-Test when the system is turned ON from both the back panel and the keypad. [See screen shots below.]

* During this time do not touch the system or insert a capillary for testing
* When the MAIN MENU appears, the SQA-V is ready for testing
* Turn on the V-Sperm computer (while SQA-V is calibrating)
* Enter the system from the log-on screen by typing in:
* User Name: **administrator**
* Password: **fertility**

**SQA-V VERSION 2.60**

**PLEASE WAIT**

**SYSTEM STABILIZATION AND**

**AUTOCALIBRATION**

**#TESTS REMAINING: 78**

**PRESS ENTER TO CONTINUE**

**PRINTING DATA**

**SYSTEM SELF-TEST:**

**Quality Control**

 Makler Chamber

 1. Test the cleanliness by placing the cover glass on the four tips and look for color

 fringes at the four points (Newton’s Phenomenon).

1. Each day of use one level of QC beads is counted.
2. Swirl the Qwik Chek quality control beads to promote even distribution of the beads.

 4. Immediately open the cap and pipette the required volume of bead solution onto the

 counting chamber.

 5. Wait at least 20 seconds to allow to settle

 6. Place the counting chamber on the microscope stage.

 7. Examine the sample for uniform bead distribution

 8. Count the beads.

 9. Record the value.

 If controls are out of range, repeat using fresh material. Clean the Makler Chamber if necessary

 and repeat.

**Procedure**

**A. IUI - Preanalytical**

* 1. When specimen is brought to lab, immediately place it in the 37+2⁰C incubator, and maintain

 the specimen at that temperature throughout the procedure. Sections 3 and 4 of consent

 form must be signed. Sections 1 and 2 should be signed prior to delivery.

2. Turn on the SQA-V and allow to calibrate

 3. Disinfect work area with disinfectant.

 4. Allow isolate and sperm washing media to come to 37+2⁰C temperature, and maintain it at

 37+2⁰C throughout the procedure; it takes about a half hour.

 5. Processing of semen specimen must begin within 30 minutes of ejaculation.

 6. Allow semen to liquefy at 37+2⁰C with occasional agitation. Place container at 45º angle if it

 creates more surface area. It takes about 15-20 minutes. If collected off site, semen may

 already be liquid.

 If specimen is not liquid at 45 minutes, help the liquefying process by mixing with a sterile

 pipette or blunt end needle and syringe.

 7. Mix the specimen well by rotating the container.

 8. Withdraw semen into syringe with a 1.5 inch, blunt needle attached to measure and record the

 volume.

 9. Perform motility analysis on the SQA-V. Perform manual sperm count using the Makler

 counting chamber.

**B. Semen Motility Analysis on the SQA-V**

1. Place a couple of drops of semen into a test tube cap using a sterile pipette.
2. From the **MAIN MENU** use arrows to select: **TEST NEW PATIENT** and press **ENTER**
3. **ENTER PATIENT/SAMPLE DATA** in the screen below.
* Patient ID – Enter the female patient’s Medical Record #
* Birth Date - enter female patient birth date
* Sample/Accession # - Enter the patient’s accession number
* Collected - Enter the date and time the sample was collected
* Received - Enter the date and time the sample was received.
1. Press **ENTER** to view the **SAMPLE TYPE** screen: use arrows to select.
* Select: **FRESH**
* Volume: Enter the volume of the ejaculate in milliliters
* WBC Conc.: Select </=1M/mL
* Enter through pH, Appearance, Liquefaction, and Viscosity (no responses required)
* After entering patient and sample data, the following screen will appear:

|  |
| --- |
| IS SAMPLE VOLUME SUFFICIENT FOR COMPLETE TESTING >=.5 Ml? YES/NO  |

Answer **NO** to perform motility.

* When **LOW VOLUME SPECIMEN** screen appears select:

 **LOW VOLUME – 20 MICROLITERS ONLY** follow the prompts on the screen.

1. Mix specimen by gently rotating the container and fill capillary with 20 microliters of semen.

 **Filling the capillary**

1. Push the syringe piston in fully. Place only the thin part of the capillary into the bottom of the sample.
2. Pull the piston back slowly while the capillary is in the sample. Fill only the (thin) capillary chamber with 20 microliters of semen. Aspirate the sample until it just appears in the cuvette part. Withdraw the capillary tip from the semen sample and visually inspect the capillary to ensure the sample has completely filled the thin section (no meniscus) and that no bubbles are present.
3. Quickly (to avoid wicking) and thoroughly wipe the outer surface of the capillary – both top and bottom with a Kimwipe. Visually confirm that the thin chamber of the capillary is still full of semen after completing the cleaning process.
4. Remove the separating valve from the capillary by pulling it out or pushing it out with a pen.
5. **Please note: test low volume samples as soon as the sample is aspirated into the capillary.**
6. Insert the capillary into the SQA-V chamber when prompted to do so by the instrument.

Do not touch the system during the testing cycle.

1. Test results will be displayed on the screen. Press **[ENTER]** to Print.
2. Remove sample capillary from the instrument.
3. The screen below will be displayed in order to transfer the test results to the V-Sperm computer.

|  |
| --- |
| TO TRANSFER TEST RESULTS TO V-SPERM: PRESS:”**IMPORT TEST**” BUTTON IN V-SPERM |

1. At the computer: **press IMPORT TEST** button on the left hand side of the screen.
2. Press **[ENTER]** on SQA-V to return to main menu.

**C. Manual Sperm Count on Makler Chamber**

 1. Put a small drop of the 0.5 cc suspension in the center of the lower disc.

* 1. Grasp the cover glass opposite the 2 dark points and place it on the four tips. Look for the

 appearance of color fringes (Newton’s phenomenon)and ensure that the drop has spread to

 the 10 micron thickness. It is recommended that the drop will spread on the entire area of the

 lower disc. Some surplus does not interfere with proper analysis as long as the tips are not

 over flooded.

1. Place the Makler chamber on the 56-70⁰C heating block for minutes to immobilize the sperm.
2. Lift the Chamber by the handles and place it on the stage of the microscope in the chamber

 holder.

1. Focus with the 4 or 10x objective and then switch to the 20x objective.

 **NEVER USE A 40 X OBJECTIVE AS THIS MAY DAMAGE THE COVERGLASS**

1. Bubbles or debris in the grid area will interfere with the count. The chamber should be

 reloaded.

1. Count the number of sperm in a strip of 10 squares. This represents their concentration in

 millions per ml. Record the results.

1. If the SQA-V is unavailable to use for the motility on the washed IUI specimen, perform a

 manual count and motility on the washed specimen and use these numbers to calculate the

 total motile count in the IUI specimen. See Manual Sperm Motility and Calulations below.

 **D. IUI Wash Procedure**

 1. Withdraw the prewarmed sperm wash media into a 10cc syringe. Withdraw the remaining

 wash media into a 3cc syringe (at least 0.5cc).

 2. Disinfect the top of the Isolate bottle with alcohol. Using a 3cc syringe with a 1 inch needle,

 pierce the top of the Isolate bottle and transfer 1.5 - 2.0 mL of the lower layer of Isolate into a

 sterile, disposable, conical centrifuge tube. Always use aseptic technique. With a black

 marker, mark the top of this layer on the outside of the tube.

 3. Using a 3cc syringe with a 1inch needle and sterile technique, transfer an equal volume of

 upper layer on top of the lower layer. This is done by contacting the surface of the lower layer

 at the side of the tube with the tip of the needle. Carefully dispense the upper layer by

 spiraling the needle tip around the circumference of the tube in an upward motion as the

 level for the upper layer rises.

 4. If the total volume is greater than 2 ml or the specimen remains viscous after (1) hour,

 proceed with the Prewash Procedure, otherwise, omit the Prewash Procedure.

 **Prewash Procedure (if needed)**

1. Add warmed sperm washing media to achieve a ratio of at least (2) parts sperm washing media to (1) part semen. Use additional centrifuge tubes if necessary. Cap and gently invert 3-5 times to mix. Centrifuge at 1600 rpms for 10 minutes.
2. Discard the supernatant down to 1 or 2 ml. If more than (1) centrifuge tube was used, combine the pellets here. Mix gently and proceed with the gradient procedure.

 5. Gently place 1.5 - 2.0ml of liquefied or prewashed semen onto the upper layer.

 6. Centrifuge for 20 minutes at 300 x g (1600 rpm). Most of the sample is left at the top.

 7. If no pellet is visible or if it is a small pellet, at the completion of the centrifugation procedure,

 only remove and discard the sperm layer. Place the lower layers in a separate centrifuge tube

 and wash them along with the pellet centrifuge tube.

 8. Remove supernatant from above the pellet with a pipet or syringe, moving from the top down

 being careful not to disturb the pellet. If supernatant sticks to side of centrifuge tube, remove it

 with a sterile swab. Add 5mL ( can be less) of sperm washing media and resuspend the pellet.

 9. Repeat washing procedure one more time. If you have done the prewash, you only have to

 wash once. Make sure you keep back at least 0.5 ml sperm washing media for the next step.

 10. Remove supernatant and resuspend the pellet in a volume of sperm washing medium, so that

 the total volume is approximately 0.5 cc. Aspirate solution with a blunt needle and a 3cc

 syringe. Record the volume of the IUI suspension.

 11. Place 2 to 3 drops of the IUI suspension in a test tube cap and perform a motility count on the

 SQA-V.

 12. Pull back media from needle into syringe, cap with luer tip cap. Record the IUI insemination

 volume. Label syringe with recipient’s name. Place syringe in biohazard bag in the 37º C

 incubator

13. Hold at 37º C temperature until patient arrives for pickup. When patient arrives, have

 patient sign consent form for pickup and give specimen to patient.

**E. IUI Washed Specimen Motility Analysis on the SQA-V**

1.From the **MAIN MENU** use arrows to select: **TEST NEW PATIENT** and press **ENTER**

2. **ENTER PATIENT/SAMPLE DATA** in the screen below.

* Patient ID – Enter the female patient’s Medical Record #
* Birth Date - enter female patient birth date
* Sample/Accession # - Enter the patient’s accession number
* Collected - Enter the date and time the sample was collected
* Received - Enter the date and time the sample was received.
1. Place three drops of washed IUI specimen from the blunt needle in a test tube cap.
2. Record the volume of the IUI specimen.
3. Press **ENTER** to view the **SAMPLE TYPE** screen: use arrows to select.
	* + Select: **WASHED**
		+ Volume: Enter the volume of the **IUI** insemination volume in milliliters (i.e., approximately 0.5 mL)
		+ WBC Conc.: Select </=1M/mL
		+ Enter through pH, Appearance, Liquefaction, and Viscosity (no responses required)
		+ After entering patient and sample data, the following screen will appear:

|  |
| --- |
| IS SAMPLE VOLUME SUFFICIENT FOR COMPLETE TESTING >=.5 Ml? YES/NO  |

* Answer **NO** to perform motility.
* When **LOW VOLUME SPECIMEN** screen appears select:

 **LOW VOLUME – 20 MICROLITERS ONLY** follow the prompts on the

 screen.

1. Mix specimen by gently rotating the container .
2. Fill the SQA-V capillary with 20 microliters of the IUI sample.
3. Wipe the outer surface of the capillary – both top and bottom.
4. Remove the separating valve from the capillary
5. Insert the capillary into the SQA-V chamber
6. Test results will be displayed on the screen. Press **[ENTER]** to Print

**Note: If the SQA-V result indicates that no progressively motile sperm are present**, perform a manual motility count on the IUI specimen. See Manual Motility Evaluation below.

1. Remove sample capillary from the instrument
2. At the computer: **press IMPORT TEST** button on the left hand side of the screen.
3. Press **[ENTER]** on SQA-V to return to main menu.
4. Record results on patient log sheet and in computer (See Reporting Results Section)

**F. Manual Sperm Motility Evaluation**

To be performed if Motility is unavailable from the SQA-V

 1. Prewarm the Chamber on the 37º + 2⁰C heating block:

 2. Put a small drop of the semen or 0.5 cc IUI suspension in the center of the lower disc.

 3. Grasp the cover glass opposite the 2 dark points and place it on the four tips. Look for the

 appearance of color fringes and ensure that the drop has spread to the 10 micron thickness

 . It is recommended that the drop will spread on the entire area of the lower disc. Some

 surplus does not interfere with proper analysis as long as the tips are not over flooded.

 4. Lift the Chamber by the handles and place it on the stage of the microscope in the chamber

 holder.

 5. Focus with the 4 or 10x objective and then switch to the 20x objective.

 6. Evaluate within 3-5 minutes after application of the sample. If you see the motility slowing,

 rewarm the chamber for a short time.

 7. Count all non-motile sperm and sperm in grade 1 and 2 within 9, 16, or 25 squares.

 Number of squares counted depends on the count.

 8. Count all progressively motile sperm (grade 3 & 4) within the same sqares.

|  |  |
| --- | --- |
| Grade | Type of sperm Motility |
| 0 | Sperm not moving |
| 1 | Sperm moving in place. Motion with no forward progression (shaking or immobilized motion) |
| 2 | Sperm moving with sluggish speed in a random direction |
| 3 | Sperm moving with reasonable speed in a linear direction |
| 4 | Sperm moving with fast speed in a linear direction |

Grading chart

 9. Or count non-motile and progressivly motile together up to 100 or 200.

 10. Calculate the percent progressively motile.

 (Motile/(Immotile + Motile) x 100). Record the results.

**G. Cleaning the Makler Chamber**

1. Clean the chamber with a wash bottle with DI water. The brush in the kit may be used with

 DI water.

 2. Use lens paper to blot and dry, being careful not to wipe the grid or the posts.

 3. Never use tap water.

**H. Calculations**

1. Calculation of Total Motility(%) on the initial semen analysis

 MSC(M/ml)

 Concentration(M/ml) = % Total Motile Sperm

 2. Calculation of Progressive Motility(%) on the initial semen analysis

 PMSC(M/ml)

 Concentration(M/ml) = % Progressive Motile Sperm

 3. Calculation of Total Motile Count (in millions) in the IUI insemination sample for **manually**

 **performed counts**

 Total Motile Count (in millions) = Insemination vol. X Tot. Washed Sperm Count X %Prog. Mot.

 Sperm

 (decimal form)

**I. Results**

 1. The SQA-V will automatically print and save the test results.

 2. Record SQA-V and manual results on the patient worksheet and perform manual

 calculations.

 3. Attach instrument printout to worksheet.

 4. Enter results from the worksheet into the LIS system.

 5. Release results from the LIS.

6. Print a patient report from the LIS and place it in with the IUI specimen.

7. Record Manual QC count on QC log sheet and store all log and worksheets in the Spin and

 Wash Notebook.

 **Results to be reported**

 Semen analysis

 Specimen Volume

 Concentration(M/ml)

 Total Motility (%)

 Progressive motiltity(%)

 MSC (M/ml)

 PMSC (M/ml)

 SMI

IUI analysis

 IUI Total Volume

 Total Motile Sperm

 Total Prog. Motile Sperm

 **Reference Range**

|  |  |
| --- | --- |
| Volume  | >/= 2 ml  |
| **Concentration** (Count)Manual sperm count  | **>/= 15 M/mL (WHO 5th Manual)** |
| Total Motility (PR+NP) | **>/= 40% (WHO 5th Manual)** |
| Progressive Motility (PR) | **>/= 32% (WHO 5th Manual)** |
| MSC (Motile Sperm Conc) | **>/= 6M/mL** |
| SMI (Sperm Motility Index) | **>/=80** |
| IUI Total Motile Count | 3-20 million ideally  |

**Notes and Limitations**

1. Specimen is stable for several hours once the processing is complete.

2. Ideally 3-20 million sperm should be present to inseminate. Pregnancy has resulted with a

 count as low as 1.5 million.

3. If infertility is a female cervix problem, there is a 60-70% chance of fertility with 3-4

 procedures. If the infertility is a male problem the success rate is very low.

4. Culture the specimen if it sits for 6 hours before it is signed out.

 **References**

Sperm washing medium, package insert 1/92 Irvine Scientific, Santa Ana, CA.

Gundersen Clinic Procedure, LaCrosse, WI.

Irvine Scientific Isolate Procedure, Santa Ana, CA.

Abbott Northwest Procedure, Minneapolis, MN.

 Makler Counting Chamber instructions, Haifa, Israel.

 World Health Organization, WHO Laboratory Manual for Examination of Human Semen, 5th

 edition, Cambridge University Press, Cambridge, 2010.

 Medical Electronic Systems LLC; SQA-V GOLD User Guide, Revision 15\_July, 2013

 Package insert; Medical Electronic Systems, Qwik-Check Beads

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| **Date:** | **Author:** | **Date:** | **Validated by:** | **Date:** | **Approved by:** |
| 8/93 | J. Paulsen |  | 2/3/2014 | Elaine Krueger |  |
|  |
| **Annual Review** |
| **Date:** |  |  |  |  |  |  |  |  |  |  |  |  |
| **Initials:** |  |  |  |  |  |  |  |  |  |  |  |  |
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| 2/3/2014 | EK | Reformatted and revised to meet new instrumentation and practice |
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