Proctor Health Care, Inc
Department of Pathology
& UnityPoint Health Proctor
Peoria, IL 61614

Effective Date: April 7, 2000
Date Reviewed:

Semen For Fertility

Manual Methods

No Instrumentation

SEMEFE

I. CLINICAL SIGNIFICANCE

This procedure is used in a comprehensive investigation of infertility

II. SPECIMEN

The best method of collection is masturbation directly into a sterile wide mouthed plastic container provided by the lab. If the specimen cannot be collected in the lab, the specimen should remain at body temperature and be brought to the lab within 30 minutes.
Preferably, the lab will provide the sterile container. Condoms are unacceptable.
If there are significant problems in specimen collection, consult the pathologist for alternatives (i.e. silicon condoms from an urologist).
Semen Culture has a special cleansing instruction. See end of this procedure for the directions provided to our patients by the Customer Service Desk.
For a valid exam the patient should not have ejaculated during the previous 48 hours

III. HOURS OF THE TEST

Monday-Friday 7:30 a.m. to 12:00 pm and 8:00 am to Noon on Saturday excluding hospital designated Holidays.

IV. REAGENTS AND EQUIPMENT

A. MLA pipet & tip
B. Hand held counter
C. Hemocytometer
D. Large test tubes
E. Slide and Cover slip
F. Serological Pipettes
G. Plastic pipet
H. Microscope
I. Reagent Grade Water
J. Eosin/Nigrosin Stain Kit
K. QwikCheck™ Test Strips for semen WBC and pH
L. QwikCheck™ Liquefaction Kit

V. QUALITY CONTROL

We use procedural Quality Control for manual Semen Analysis. The specimen is diluted and counted in duplicate and must match within 20%. When no sperm are visible, the specimen is centrifuged, and the pellet microscopically analyzed for the presence of any sperm. Motility is counted in duplicate and must match within 10. The vitality stain is evaluated for contamination and reactivity.

VI. PROCEDURE:

A. The laboratory receptionist will provide the patient with the instructional form for collecting a Semen Analysis Work Form and labeled sterile plastic container. The patient should fill out the information through previous semen analysis. The patient will be directed to the outpatient collecting room for specimen collection. If the sample is collected by means other than masturbation, in a non-sterile or non-plastic container, document this information on the report.

B. Our receptionist will deliver the specimen and the completed work form to hematology immediately after collection. The specimen should kept at room temperature.

C. Use the work form (a copy can be found in the procedure manual) to record all results before entering them into Sunquest.

D. Make sure that the collect time has been documented on the form.

E. Liquefaction**:** Liquefaction time is the time it takes for the semen to turn to liquid. Allow the specimen to sit at room temperature for 30 minutes, so that the sample can liquefy and be mixed thoroughly. After 30 minutes has elapsed from the time of collection, the sample is checked for liquefaction. If the specimen is not liquefied, allow another 30 minutes for liquefaction to complete. If the specimen is not liquefied in 60 minutes, report liquefaction as abnormal.

Before any further testing is begun, the sample should be thoroughly mixed in the container.

F. Appearance: Record the appearance of the sample. Normal semen is opaque white to gray white. Note the presence of blood, other pigmentation, gelatinous clumps, mucous clumps, or trichomonas, odor or any abnormal characteristic or contaminant. Microscopic characteristics such as erythrocytes and white blood cells should also be reported during the morphology analysis.

Viscosity: Determine the viscosity of the sample by gently aspirating the sample into a pipette and allow the semen to drop by gravity. Observe the length of the thread formed. The longer the thread the more viscous the sample. A normal semen specimen will be expelled from the pipette in distinct drops. Grade as normal or abnormal. If the sample is hyper viscous, chymotrypsin may be added to the sample to aid in liquefaction. See the Liquefaction Kit package insert for instructions.

G. Volume: Measure the volume of the sample using a disposable serological pipette.

H. WBC concentration and pH: remove a QwikCheck test strip from the bottle and tightly cap the bottle. Mix the semen sample thoroughly. Using a pipette, place one drop of the specimen on each test pad (pH and Leukocytes). Do not touch the test pad areas and leave the strip in a horizontal position. Wait 1 minute to read leukocytes and pH. Compare the color of the test pad to the appropriate color scale for pH and WBC on the bottle label. Note: test pads will grow darker if read after 1 minute. WBC should only be reported as >1 M/ml if the test pad is a dark purple color.

I. Motility: Motility should be observed 30 to 60 minutes after specimen collection. Mix the sample well. Place a 10ul drop of semen on two glass slides and cover slip them with a 22 X 22 cm glass coverslip. Do the motility in duplicate when the sperm stop drifting on the slide. The duplicate motility should match within 10.

J. Quality of Motility: Use Sunquest Function Differential Result Entry
Keyboard: YPEODF and click Ok
Click on Options and select Offline Counter

1. Click on Set Count and select 200.
2. Assess the motility of the sperm in each field by scoring each sperm.
   a. Progressively Motile: Sperm moving actively in a line or circle. Press the "K" key
   b. Non-Progressively Motile: All other patterns of motility with the absence of progression. Press the "L" Key.
   c. Immotile: No movement. Press the ";" key.
3. The counter will stop when 200 sperm have been evaluated. Enter the result on the worksheet.
4. Determine the motility in the second slide by following steps 1-6. The Motility determinations should match within 10.
5. Average the counts and record each category to the nearest whole number.
6. Report % Motility as the percent of PR + NP.

K. When no sperm are visible use a Concentrated Sample

1. Centrifuge an aliquot of sample in the Coag centrifuge.
2. Decant the supernatant.
3. Examine the pellet microscopically for motility as above.

L. Vitality/Viability: If the % motility is less than 40%, a sperm viability stain should be performed.

1. Place a drop of the eosin and nigrosin stain on a glass slide with coverslip to check the stain for debris. Record on QC sheet.
2. Mix one drop of semen with two drops of Eosin Y stain in a labeled test tube. Wait 30 seconds and mix again.
3. Add 3 drops of Nigrosin stain and mix.
4. Make 4 smears within 30 seconds and allow to air dry.

5. Verify the spermatozoa are visible against a dark background on the stained slide and record on the QC log.
6. Count the number of live and dead sperm by using one counter for the number of (live) unstained (white) sperm and a second counter for the number of (dead) dark pink sperm.
7. Count 200 sperm on 100 oil of a light or phase microscope. Repeat on another slide and average the counts. Counts should match within 20%.
8. Report the % of live sperm as Percent Viability

% Vitality = # unstained sperm X 100
 # unstained + # stained

M. Semen Concentration (Count): After complete liquefaction, prepare two separate dilutions of the semen sample by using distilled water as the diluent. You have an idea of the sperm count while viewing the motility.

1. Dilution of Semen Sample:
   a. If the sperm count is approx.< 3 million, make a 1:10 dilution. (0.1ml semen and 0.9ml water)
   b. If the sperm count is approx. 3-20 million make a 1:20 dilution. (0.1ml semen and 1.9ml water)
   c. If the sperm count is approx. 20-40 million make a 1:100 dilution (0.1ml semen and 9.9ml water)
   d. If the sperm count is approx. >40 million make a 1:200 dilution (make a 1:2 of a 1:100 or 0.05 semen in 9.95 water)
   e. If no sperm were seen when observing for Motility in a concentrated specimen, report no sperm seen.
   f. If sperm were seen on the concentrated sample and the sperm count is 0 when plated straight, report "rare sperm seen on concentrated sample".

2. Charge the hemocytometer and allow the specimen to settle for 1 to 5 minutes.
3. Do the sperm count on 40X. Count the two different dilutions. The results should check within 20%, then average the counts.
4. Calculations:
   Plastic Hemocytometer: The more squares you count the more accurate the results will be. Count no less than 27 squares and if possible count all 81 squares.

   $$\frac{\text{\# sperm counted X 90 X dilution X 1000}}{\text{\# small squares counted}} = \text{sperm/ml}$$

   Glass Hemocytometer: Count all 9 large squares, if possible

   $$\frac{\text{\# sperm counted X dilution X 1000}}{} = \text{sperm/ml}$$

squares counted X 0.1 (volume)

N. When all results are complete and recorded in Sunquest, give the specimen, cumulative report and completed work form to Histology. A concentrating technique is used and slides are prepared from the sediment for the pathologist to do morphology. A PAP stain is used to distinguish leukocytes from immature sperm or other round cells. If Histology is gone when you deliver the specimen, store the sample in the refrigerator and put the paperwork on the counter.

VII. INTERPRETATION OF RESULTS:

After the Pathologist does the sperm morphology, an interpretive comment is added to the Semen for Fertility results.

VIII. RESULT REPORTING

A. Click on Result Entry
B. Manual Mode
C. Configuration Mode: YSEMEN PRP SEMEN ANALYSIS
D. Click Result
E. The Accession number is ready to result. For any parameter not assessed manually, type HIDE for "do not report".
   1. ABSTIN: Number of days since last ejaculation
   2. SPAPP: Semen appearance: Normal or abnormal
   3. VISCO: Enter semen viscosity: Normal (within one hour) or abnormal (>1 hour)
   4. LIQUE: Normal or abnormal.
   5. SPPH: Semen pH
   6. SQCONT: Container type- enter "Sterile"
   7. SQCONC: Sperm concentration. Enter the count using a ; and placing commas where appropriate ;75,666,666
   8. SQMOT5: Enter the total % sperm motility
   9. SQPMOT: Enter the % progressively motile sperm
   10. NPMOT5: Enter the % non-progressively motile sperm
   11. IMMOT5: Enter the % immotile sperm
   12. SPVOL: Volume of the sample
   13. SQWBCC: WBC concentration (M/ml). See package insert for instructions.
   14. SQVITD: Type "HIDE". We only report the % live for viability testing.
   15. SQVITL: Enter % of viable sperm. Hide if motility is >40%.
   16. SPCOMM: Enter any additional comments, or HIDE if no comments are necessary.

17. ROUDEB: Enter debris/round cells (few, moderate, many, gross)

IX. REFERENCE INTERVALS

Semen Parameter	Reference Value	Source
Concentration (Count)	≥15 M/ml	WHO 5th manual
Total Motile (PR+NP)	≥40 %	WHO 5th manual
Progressive (PR)	≥32 %	WHO 5th manual
Non-progressive (NP)	-	-
Immotile (IM)	-	-

X. REFERENCES

A. Urinalysis and Body Fluids, Susan King Stasinger, pp. 158-162.
B. Urinalysis and Body Fluids A ColorText and Atlas, Ringsrud and Linne, pp. 206-213.
C. Body Fluids, 3rd Ed., Kjeldsbert and Knight, 1993.
D. Atlas of Sperm Morphology, M. Adelman, E. Cahill, 1989, pp. 2-13
E. Package insert; Medical Electronic Systems, QwikCheck Test Strips
F. Package insert; Medical Electronic Systems, QwikCheck Liquefaction Kit
G. WHO Laboratory manual for the examination of human semen, 5th Edition 2010

REVISION HISTORY (began tracking 2016)			
Rev	**Description of Change**	**Author**	**Effective Date**
0	Initial Release	Cindy Schroeder	1/22/16
1	Added Viability QC and Interpretation	Sheanea LaCock	4/23/17
2	Changed specimen transport to room temperature; updated result reporting to reflect changes in Sunquest resulting.	Sheanea LaCock	7/17/19

Reviewed by

Lead	Date	Coordinator/Manager	Date	Medical Director	Date
Sheanea LaCock	4/23/17	Cindy Schroeder MT(ASCP)	4/23/17		5/15/17
Sheanea LaCock	7/17/19				