KAISER PERMANENTE®
COLORADO LABORATORY

SEMEN ANALYSIS (COMPLETE)

PRINCIPLE:

Examination of freshly ejaculated semen yields an index of male fertility.

SPECIMEN:

Freshly obtained, masturbated sample (complete) in a clean container, (glass or hard plastic). A specimen obtained during interrupted coitus is NOT recommended.

Patient should abstain for 48-96 hours but not more than 7 days. Obtaining the specimen at intervals approximating normal sexual activity leads to inconsistent results. Intervals greater than 7 days may lead to decreased motility and increased non-viable forms. A complete fresh sample is mandatory. Time of collection should be noted. DO NOT use a condom for collection of the sample if at all possible. If masturbation is contraindicated (e.g. for religious reasons), it may be necessary to use a condom for collection. A condom without added spermicide (Milex Sheath) should be used and the sample transferred immediately into an appropriate container. The report should include a comment that the specimen was collected in a condom.

Specimens are accepted M-F, 9:00 AM to 3:00 PM. at specific Kaiser Permanente laboratories. See the patient instruction sheet for the list of laboratories that perform testing. All specimens will be tested. Enter the temperature of the specimen during transport as a "Result Comment" in the LIS. If the specimen is not maintained at body temperature during transport, enter a "Result Comment" in the computer – "Interpret results with caution - specimen not maintained at body temperature during transport."

Laboratory personnel need to follow the OSHA regulations for blood or body fluids when handling semen specimens. Ideally, the specimen should be received within 60 minutes of collection. The specimen must be brought to the laboratory within 2 hours of collection. If the specimen is received more than 2 hours past the collection time, enter a comment on the report.

REAGENTS-SPECIAL SUPPLIES AND EQUIPMENT:

1. Micro Cell slides (get from Franklin lab)
2. 15cc graduated plastic disposable centrifuge tube
3. Glass slides
4. Color pHast Indicator Strips pH 5-10 (Pharmacy)
5. Reticle for microscope
6. Sperm Diluting Fluid (#S1042) Rocky Mt Reagents, Inc

PROCEDURE:

- Evaluate the motility, pH, gross description, liquefaction and viscosity as soon as possible after the specimen is received in the laboratory.
- The count, volume and morphology can be performed at a later time.
- All testing is performed at room temperature.

1. **Collection Time, Container, Days of Abstinence**
   a. Record the time the specimen is received in the laboratory.
   b. Record the type of container. If the container is not a sterile plastic urine cup, enter a comment on the report.
   c. Record the time the specimen was obtained and the number of days of abstinence.
   d. Mix the sample well.

2. **Temperature of Specimen During Transport -** Record temperature of the specimen during transport

   NOTE: Enter the temperature of the specimen during transport as a "Result Comment" in the LIS. If the specimen is not maintained at body temperature during transport, enter a "Result Comment" in the computer – "Interpret results with caution – specimen not maintained at body temperature during transport."

3. **Appearance**
   a. Record the appearance: Parchment (off white color), translucent or opaque.
   b. Record the presence of blood or gel pieces (gel pieces have no significance)

4. **Volume -** Record the volume using a graduated plastic disposable centrifuge tube or a 3 ml syringe

5. **Liquefaction**
   a. If the sample is not already liquefied, return the specimen to the original container and allow to sit at room temperature for 30 minutes or until self-liquefaction is complete.
   b. If liquefaction does not occur after 30 minutes, report "no liquefaction after 30 minutes".
   c. Pulling the sample through an 18-gauge needle on a syringe can help with liquefaction.

NOTE: Normal semen coagulates immediately upon ejaculation and liquefies within 5-25 minutes.

6. **Viscosity**
   a. Determine the viscosity after complete liquefaction.
   b. Report as normal, increased or decreased.

NOTE: Normal viscosity – semen can be poured drop by drop and has slight to moderate stringing.
Decreased viscosity – semen has no stringing when dropped from a dropper.
Increased viscosity – semen is difficult to draw into the pipette and no distinct drops are seen.

7. **pH -** Test with color pHast Indicator Strips pH 5-10. Compare color to the chart on the container and record.

8. Morphology

a. Mix a few drops of Sperm Diluting Fluid with the semen sample before making slides.
b. Make 2 thin smears and allow to air dry thoroughly.
c. Send slides and left over specimen to the Franklin lab for morphology evaluation by the Strict Kruger method.

9. Sperm Count

a. Using a small pipette, carefully load the Micro Cell slide and wipe away any excess fluid.
b. Use the microscope fitted with the reticle to do the count. The chamber is a uniform depth all over so you can move the field around to do your count.
c. It is best to count 100 to 200 sperm.
d. Record the number of boxes that you count. **NOTE: THIS IS THE MOST IMPORTANT PARAMETER.**

10. Motility

a. Using the same sample from #9 above count the number of motile sperm in the boxes you counted to get the total sperm count. (For example if you needed 20 boxes to get the sperm count, use 20 boxes to get the motility.)
b. Motility is expressed as a percentage of total sperm counted. (In this case the motile sperm divided by motile + non-motile sperm X 100.)
c. Record the direction of movement (e.g. progressive or non-progressive).
    * Sperm progression may be graded on a 0-4 scale:
        0- non-motile
        1- movement but no forward progression
        2- sluggish movement with random direction
        3- good sperm motility with approximately straight line movement
        4- high speed motility with forward progression

NOTE: 0-2 refers to non-progressive motility and 3-4 progressive motility

d. Report if over 10% of the sperm are in clumps (agglutinates) - True agglutinates are composed only of sperm and do not contain cellular material.

11. Azoospermatic Specimens: (Absence of spermatozoa in the semen).

a. Perform a microscopic analysis on a centrifuged specimen.
b. Confirm azoospermatic specimens with a second tech.
c. When the sperm count is <0.2 mil/ml, the LIS reflexes a Fructose test, prepare the specimen and send to the appropriate reference laboratory.

CALCULATIONS:

1. The formula for calculating the sperm concentration is: $C=N \times F$

where **C**=sperm concentration
N=average number of sperm per box
F=Factor (this is determined using a micrometer and is microscope and

magnification dependent:

Ex. the F is 7.8 at 10X and 31.25 at 20X for the microscope KP #0197655 at FMC. See the manufacturer's directions for detailed explanation of determining F.)

N is determined by dividing the number of sperm by the number of boxes counted

CRITERIA FOR SPECIMENS WHICH HAVE NOT BEEN PROPERLY COLLECTED

1. Specimens more than 120 minutes old, perform testing and enter a "Result Comment" in the LIS "Interpret results with caution – specimen received in Laboratory more than 2 hours post collection"
2. Specimens not maintained at body temperature during transport to the laboratory: perform testing and enter a "Result Comment" in the LIS – "Interpret results with caution – temperature of specimen not maintained at body temperature during transport to the laboratory"
3. Specimen received in condom, perform testing and enter a "Result Comment" in the LIS – "Interpret with caution, specimen received in a condom.
4. Specimen is an incomplete ejaculate , perform testing and enter a "Result Comment" in the LIS – "Interpret with caution - specimen is an incomplete ejaculate"

NORMALS

Volume.....1.5 - 5.0 ml
pH.....7.0 - 8.5
Count.....>20 million/ml
Motility.....50%, progressive
Normal Forms.....>14%
Liquification.....Complete in 30 minutes
Viscosity.....Normal
Appearance.....Parchment color, translucent or opaque

PROCEDURAL NOTES

Motility, pH, evaluation of gross description, liquefaction, viscosity need to be evaluated as soon as possible after the specimen is received in the laboratory. The count, volume and morphology can be evaluated at a later time. All testing is performed at room temperature.

REFERENCES

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7. Articles submitted by Dr Donald Kreger to include: "An AUA Best Practice Policy and ASRM Practice Committee Report"; "Handbook of Andrology" page 50 and Fertility and Sterility Vol 73 Issue 3 2000.