Semen Analysis **Technical Procedure #1310.t**

PRINCIPLE

A semen evaluation is usually performed as part of a comprehensive study to determine the cause of infertility. The evaluation consists of measurement of volume, assessment of viscosity, motility count, sperm count, and morphological assessment of spermatozoa.

SPECIMEN

The physician will instruct the patient how to collect the specimen for examination. The specimen should be collected in a sterile 4oz screw cap container. After collection, the specimen should be kept as close to body temperature as possible and must be received in the Laboratory within 1 hour of collection. Analysis must be started within one hour of collection. Analysis will be performed up to two hours post collection.

Unacceptable specimens include specimens with volume less than 50 ul; specimens exposed to temperatures outside of normal 20^0-37^0C range; days of abstinence less than one day; any contamination (soap, gel, etc.); condom collection; or semen evaluation, post vasectomy, or urine retrograde specimen greater than two hours old.

Appointments are made through Client Services by calling 734-7373. Semen Analysis post vasectomy, or urine retrograde specimen are performed Tuesday, Wednesday, and Thursday from 1000 to 1300 by appointment only.
To access appointment schedule: EMR-Schedule (menu bar)-LAB-choose Lab Cancer Center. Go to Resources and click on Appt CCRLab to display appointments.

When the specimen is delivered to the Laboratory by the patient a CPT will speak with the patient and inquire as to:

- Method of collection
- Complete collection
- Time of collection
- Days of abstinence

REAGENTS, EQUIPMENT and SUPPLIES

- Microscope with 20X ocular, phase capability and warmed stage set to 35^0C
- Centrifuge: Baxter Scientific or Centrifuge 5702
- Makler counting chambers
- Disposable 5 ml or 10ml serological pipettes
- Pipette capable of delivering 5 ul
- Pipette tips
- Water bath set for 55^0C
- Microscope slides and cover slips
- 15ml polypropylene, screw cap conical tube, BD Falcon 352096
- Quick III Stain Kit, Three Step Procedure; Astral Diagnostics Incorporated Hem 5316
- Coplin Jars
- Transfer pipettes
- Kova tubes

QUALITY CONTROL

- Slides are labeled with minimum two specimen identifiers.
- Slides prepared for each seminal fluid are examined by the technologist for:
  - Proper staining
  - An adequate number of undistorted cells within the reading area

- If cells appear distorted beyond recognition, there are too many cells (packed field), or cells appear imperfectly stained, the slide should be remade with attention to correction of the problem noted.
- Select an area of the slide where sperm are evenly distributed, staining is uniform, and there is sufficient space between cells to adequately assess characteristics.
- The semen counts and differential are correlated with each other, making sure that cell ratios appear consistent between them.

☐ Stains are checked for contamination and reactivity each day of use.

PROCEDURE

SEMEN EVALUATION

Upon receipt of the specimen, the container should be allowed to liquefy for 15-30 minutes at room temperature. Liquefaction typically occurs 15 minutes post collection. Specimens may sit up to one hour from collection to allow for liquefaction. Note long liquefaction time on worksheet and in results. Specimen should be received in sterile specimen container. Note on worksheet and in results if received in other container. All motility and progression evaluation testing will be done immediately on receipt of specimen.

☐ Volume

- Measure and record volume of specimen using a disposable 10ml serological pipette. Normal semen volume is $\geq$1.5 mL.

☐ Appearance

- Normal appearance is homogenous, grey in color, and opaque.
- Report as “Normal” or “Abnormal”
    - For Abnormal specimen describe color (usually red-brown or yellow) and opalescence of specimen.

☐ Viscosity

- After liquefaction, the viscosity of the sample can be estimated by gently aspirating it into a plastic disposable pipette, allowing the semen to drop by gravity and observing the length of any thread. A normal sample leaves the pipette in small discrete drops. If viscosity is abnormal the drop will form a thread more than 2 cm long (High viscosity).

- Report viscosity as "Normal" or "High"

- Report gelatinous clumps it any are present.

 Evaluation for Presence of Sperm

- Evaluation for presence of sperm, motility, and sperm counts are all performed on Makler counting chambers. Mix the sample by inversion then by gently mixing using a transfer pipette. Plate the Makler counting chamber using a well mixed 5 ul sample and place in the center of the lower disc. Place the cover glass, holding it with fingers opposite the black dots, on the four pins of the chamber. Place chamber on microscope stage and examine using a 20x objective. Never use a 40x objective; it will break the cover glass.

- If no sperm are present report as “No sperm present”. If sperm are present proceed to Agglutination and Progression.

 Agglutination and Progression

- Evaluate for Agglutination. Agglutination is defined as any motile sperm that stick to each other. Immotile sperm clumps, motile sperm stuck to other cells or debris are not agglutination. Report as None, Isolated (<10 sperm per agglutinate), Moderate (10-50 sperm per agglutinate), Large (>50 sperm agglutinate), or Gross (all sperm agglutinated).

- Evaluate Progression as normal or abnormal. Normal progression is fast, forward movement. Abnormal progression is twitching, sluggish motion, or no movement.

 Motility and Sperm Count

Motility is a two-fold procedure. A well-mixed undiluted drop of 5 ul semen is placed on the Makler counting chamber and the cover is placed on the chamber. Only the non-motile sperm are counted in 10 Makler squares. Then an aliquot of semen is immobilized by heating to 55^0C in a water bath. This is then counted on the Makler chamber using 5 ul semen and placing the cover on the chamber. Count all sperm present in 10 squares. This is the total sperm count. The % motility is calculated as (total sperm counted in 55^0C sample minus the non-

motile sperm counted in the original sample) divided by the total sperm count in 55^0C sample; multiplied by 100.

- Using a Makler counting chamber charged with 5ul undiluted semen and cover and place on microscope.
  - ◊ Using a 20X ocular and phase settings count the number of immotile sperm in a strip of 10 squares. Count heads only. Do not count motile sperm.
- Place approximately 250 ul aliquot of the well mixed undiluted specimen in a tube and place in a 55^0C water bath for 5 minutes. This will immobilize all sperm.
  - Plate 5 ul of well mixed, heated specimen in the center of Makler counting chamber and cover placed on chamber.
  - Using a 20X ocular count the number of sperm in a strip of 10 squares.
  - This is the total sperm count. Report as # million/mL.
  - Use the following formula to calculate % motility:

$$\frac{\text{(\# sperm in heated specimen - \# immotile sperm in unheated specimen)}}{\text{\# sperm in heated specimen}} \times 100 = \%$$

- Total Sperm Number
  - The Total Sperm Number is the sperm count in 10 squares per ml times the volume of specimen (ml). The reporting unit is “million”.

$$\frac{\text{(\#sperm in heated specimen) million}}{\text{ml}} \times \text{(semen volume) ml} = \text{(sperm) million}$$

- Leukocyte Count
  - Count leukocytes on Makler Counting Chamber. Round cells are non-sperm cells in semen and include epithelial cells, leukocytes, germ cells, and isolated sperm heads. Count same 10 squares used for total sperm count. Count only leukocytes, verify count on stained slide.

- Report as "none seen" or "# million/ml". Normal value is less than 1 million/ml

- Other cell present

  - Report contaminants, erythrocytes, bacteria, etc if present. Phone the physician if any bacteria is present..

- Strict Kruger Spermatozoa Morphology:

  - Prepare at least two thin wedge smears of semen sample. Dilute the semen with saline approximately 1:1.

    - ◊ Allow wet smears to air dry.

    - ◊ Stain slides using Quick III Stain Kit three step procedure

      - Dip slide into Fixative three to seven times. Each dip should be one second. Touch slide to inside of Fixative chamber to allow excess solution to drain, then blot edge of slide on absorbent towel.

      - Repeat for Solution I and Solution II

      - Rinse with DIH_2O and air dry.

    - ○ Wright stain may be used to enhance review of leukocytes or other round cells present.

  - Place the stained slide under 100X bright field oil immersion to assess morphology.

  - Count 200 spermatozoa using a key counter noting normal and abnormal forms.

    - ○ Normal is defined as follows:

- Head must have a smooth oval configuration, with a well-defined acrosome comprising 40-70% of the anterior sperm head.
- A second, slightly differing type of normal head form is also recognized, i.e. and oval form still having a smooth contour, but slightly tapered at the post-acrosomal end.
- Normal head length is between 3 and 5micron, width between 2 and 3micron.
- The head width must be between three-fifths and two-thirds the head length.
- No neck/midpiece and/or tail defects may be present.
- The midpiece must be slender, axially attached, less than or equal to 1 micron in width, and approximately one and one-half times the head length.
- Cyotplasmic droplets may be present, but those which compromise more than half the size of a normal sperm head consign to abnormality.
- Tails must be straight, uniform, and slightly thinner at midpiece, uncoiled and approximately 45 micron long.
- The heads of stained spermatozoa are slightly smaller than the heads of living spermatozoa, although the shapes are not appreciably different. The length to width ratio should be 1.5 to 1.75

◊ All borderline forms should be counted as abnormal.

◊ It is unnecessary to distinguish between all variations in head size and shape, or between the various tail defects. However, if a region of the sperm cell is abnormal in a majority of the cells, an additional comment should be made regarding the predominant defect(s).

○ Report as % Normal Spermatozoa present. Strict Kruger Morphology Normal $\geq$4%.

RETROGRADE EJACULATION URINE

In some men, semen passes into the bladder at ejaculation, resulting in aspermia, or no apparent ejaculate. Conformation of the situation is obtained by examining a sample of post-ejaculatory urine for the presence of spermatozoa. Analysis will consist of Semen Evaluation, and post-ejaculated urine specimen. Small numbers of sperm may remain in the urethra after normal antigrade ejaculation and these usually number only a few thousand. The presence of more than 5 million sperm in a post-ejaculate sample makes the presence of retrograde ejaculation very likely.

- Semen analysis is performed as stated above.
- Total volume of urine is recorded
- Two 10 ml aliquots of well mixed urine put into centrifuge tubes. The sperm are killed by urine and dead sperm will sink.
- Centrifuge at 1600 rpm's for 10 minutes.
- Remove supernate, leaving 1 ml of urine on packed sediment.
- Plate a well mixed sample on Makler counting chamber. If no sperm are present, report as "no sperm present". If sperm are present, count sperm and calculate the estimated total sperm count in urine.
  - Record urine volume
  - Count ten squares in Makler chamber. This is sperm million per 10 ml urine
  - Calculate the estimated total sperm count in urine as "million sperm in total urine sample".

    $$\frac{\text{(sperm \# from Makler)}}{\text{10 ml}} \times \text{volume urine ml} = \text{sperm (M) in total sample}$$
  - Calculate the number of sperm per ml as follows:

    $$\frac{\text{Sperm \# from Makler}}{\text{10 ml}} = \text{number of sperm (M) per ml}$$

POST VASECTOMY ANALYSIS

Post vasectomy success is not restricted to azoospermia but should include those men whose post vasectomy semen analysis show rare non-motile sperm ($\leq$100,000 non-

motile sperm/ml). Post vasectomy semen analysis must include evaluation for count and motility.

- Mix semen thoroughly by pipeting up and down 5 times with a disposable pipette. Avoid creating bubbles.
- Immediately after mixing place 5 ul of undiluted semen on a Makler counting chamber, apply cover and place the chamber on the microscope stage.
- Evaluate for the presence of sperm.
    - If sperm are present, count and motility must be noted.
        - If less than 1 sperm is seen in 10 Makler squares report as "Rare (motile or non-motile) sperm seen".
        - If 1 or more sperm is seen in 10 Makler squares proceed with count.
        - If sperm are motile proceed with motility count, unless only rare sperm seen.
- Report as "No sperm seen", "Rare (motile or non-motile) sperm seen", or sperm count (million/mm^3) and motility.

REFERENCE RANGES

- Semen Properties:
    - Semen volume $\geq$1.5 ml
    - Semen viscosity Normal
    - Sperm agglutination none or isolated
- Sperm Count:
    - Sperm concentration $\geq$ 15 million
    - Total sperm number $\geq$ 39 million
- Sperm Motility:
    - Percent motility $\geq$ 40 %

- Progression Normal

- Sperm Morphology
  - Normal (Kruger Strict Criteria) $\geq$ 4%
- Semen Leukocyte Concentration < 1 million/ mL
- Post-vasectomy specimen
  - No sperm present, or $\leq$100,000 non-motile sperm/ml.

NOTES

- Semen analysis evaluates certain characteristics of a male's semen and the sperm contained in the semen. It is usually done as part of a comprehensive study to determine the cause of infertility or may be used for the purposes of sperm donation.
- Low semen volume is characteristic of obstruction of the ejaculatory duct of congenital bilateral absence of the vas deferens, a condition in which the seminal vesicles are also poorly developed. Low semen volume can also be the result of collection problems (loss of a fraction of the ejaculate), partial retrograde ejaculation or androgen deficiency.
- High semen volume may reflect active exudation in cases of active inflammation of the accessory organs.
- Sperm count or concentration measures the concentration of sperm in an ejaculate. The total sperm count is the sperm count multiplied by the volume.
- Excessive viscosity may interfere with the ability of sperm to travel from the site of deposition to the cervix or uterus.
- The presence of agglutination is not sufficient evidence to deduce an immunological cause of infertility but is suggestive of anti-sperm antibodies. Further testing (anti-sperm antibodies) is necessary.

- The presence of a large proportion of viable but immotile sperm may be indicative of structural defects in the flagellum; a high percentage of immotile and non-viable cells may indicate epididymal pathology.

- The presence of non-sperm cells in semen may be indicative of testicular damage (immature germ cells), pathology of the efferent ducts (ciliary tufts) or inflammation of the accessory glands (leukocytes).

- Human semen samples contain spermatozoa with different kinds of malformations. Defective spermatogenesis and some epididymal pathologies are commonly associated with an increased percentage of spermatozoa with abnormal shapes. The morphological defects are usually mixed. Abnormal spermatozoa generally have a lower fertilizing potential, depending on the types of anomalies, and may also have abnormal DNA. Morphological defects have been associated with increased DNA fragmentation, an increased incidence of structural chromosomal aberrations, immature chromatin and aneuploidy.

- Fructose is produced in the male reproductive tract by the seminal vesicles and is released into the semen during ejaculation. Fructose is the energy source for sperm motility

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PROCEDURE HISTORY

Date	Written by	Revision	Approve date	Approved by
2/88		Adopted	2/4/88	N Levy MD
7/9/94	B. Gosselin	New format	7/11/94	E Larkin MD
		Annual Review	10/10/95	E Larkin MD
11/4/96	J Cannon	Minor Revisions	11/96	E Larkin MD
		Annual Review	12/30/97	C Miller, PhD
1/9/98	J Cannon	Retired		
6/2011	R.B.Duplantier L Gandy	Updated/Revised	06/24/11	D Dwyre, MD
8/24/12	L Gandy	Minor	8/24/12	D Dwyre MD
2/2013	L Gandy	PVSA motility and no centrifugation	2/19/13	D Dwyre MD
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