Semen Analysis: Complete (Manual)

Safety Message	• Semen is considered a biological hazardous material and is subject to laboratory protocol for handling such material. • Dispose all contaminated supplies in the appropriate biohazard containers.

Purpose	• This document will define the procedure for the use of performing a manual complete semen analysis which will indicate the presence of sperm from a collected fresh sample or a post-vasectomy sample.

Policy	• Patients are instructed to bring specimens to the laboratory within 60 minutes of collection. The specimen must be maintained at room temperature, 20C – 37C and not exposed to heat or cold. Laboratory personnel are not to refuse already collected specimens submitted outside of the times stated above. • Semen analysis is performed at room temperature between 68^0-75.2^0 F (20^0 – 24^0C). Temperature deviations are noted in report.

Specimen	• Fresh – samples collected within an hour of testing and not enriched, diluted or treated. • Post-vasectomy – fresh samples collected within an hour of testing and designated as post-vasectomy specimens. • The patient's physician or the laboratory provides the collection instructions (see attachment "A") and the sterile plastic container. • Specimen must be completely labeled with patient's name and medical record number. • Specimen must be protected from extremes of temperature (less than 20C or more than 37C) during transport to the laboratory. • Specimen must reach the testing laboratory within 60 minutes

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	for an accurate evaluation of sperm motility. • When patient arrives at the lab with his specimen, he is given a short questionnaire (see attachment "B") to fill out asking him whether the specimen was maintained at room temperature. If the patient indicates that there was a temperature problem, the clerk notifies the CLS who is then responsible to enter that information in the final report. Questionnaire is attached to slip and submitted to laboratory testing section with specimen.

Equipment and Reagents	Assemble the following supplies before proceeding: • Incyto Disposable Hemocytometer • Semen Diluting Fluid (formaldehyde, sodium bicarbonate, water) • 10 μL,1000 μL,& 50 μL MLA pipettes • 12 x 75 mm glass tube • microscope slides • glass coverslip 22 X 22 mm • Biorad Urine Controls & Qwik Check Test Strips

Quality control	• QC Qwik Check Test Strips using Biorad Urine Controls: Level 1 = <1 M/ml Leukocytes; pH = 5.0 – 6.5 Level 2 = ≥1 M/ml Leukocytes; pH = 7.0 – 8.0 • QC is run once every 24 hours when you receive a sample

Macroscopic Examination:

Step	Action
1	Allow the specimen to liquefy. Swirl semen in container to determine if coagulum has liquefied. A liquefied specimen will take the shape of the container. **Note:** • Normal semen sample liquefies within 60 minutes at room temperature. Continuous gentle mixing or rotation of specimen during liquefaction may reduce errors in determining sperm concentration. Failure of specimen to liquefy within 60 minutes must be recorded in the report. When samples do not liquefy within 60 minutes, extend incubation time and pipette the specimen repeatedly with a sterile pipette. If this method fails, use of an enzymatic treatment may be necessary. Use of these

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	manipulations must be recorded in the report.
2	Mix liquefied specimen well.
3	Pour specimen into a graduated plastic centrifuge tube and determine the volume to the nearest 0.1 mL. Record volume.
4	Determine the viscosity of the liquefied sample. • Draw the specimen into a disposable pipette and allow to drop back into container by gravity. Normal: Specimen slightly mucoid, but will fall in separate drops. Decreased: Specimen watery, not viscous or mucoid. Increased: Specimen very mucoid, will not fall in separate drops. The drop will form a thread more than 2 cm long. • Results are directly entered in the LIS. **Note:** • High viscosity can interfere with determination of sperm motility and concentration. The methods to reduce viscosity are the same as those for delayed liquefaction.

Microscopic Examination

Step	Action
1	Using an MLA pipette deliver 10 μL of well mixed liquefied semen sample onto a clean glass slide and cover sample with a 22 mm X 22 mm glass cover slip. The freshly made wet preparation is left to stabilize for approximately one minute. **Note:** • Examination is performed at room temperature between 20C-25C (68F–77F) .
2	Perform initial evaluation at 10X. Scan slide for mucus strand formation, sperm aggregation, and evenness of spread of sperm on slide. If the number of sperm per visual field varies considerably, sample is not homogeneous. Remix the sample thoroughly and repeat exam. **Note:** • Lack of homogeneity may also be due to abnormal consistency, abnormal liquefaction, aggregation of sperm in mucus threads, or from sperm agglutination. These abnormalities must be

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	mentioned in the report. • If no spermatozoa are seen during the initial examination at 10X and at 40X, centrifuge entire sample **2000 RPM for 3 minutes**. A complete and systematic search of all the re-suspended precipitate must be done.
3	At 40X scan slide for motility, sperm concentration, debris, epithelial cells, erythrocytes, round cells, other cellular elements (e.g. bacteria).
5	If round cells are present, perform enumeration during sperm concentration determination or stained smear morphological evaluation. **Note:** • The number of round cells per 100 spermatozoa is calculated as round cells per milliliter of semen (round cells/100 sperm X sperm concentration in millions/mL divided by 100). • If >5 X 10^6 round cells/mL are present staining is indicated to determine if the cells are leukocytes or sperm precursors. • WBCs indicate infection or inflammation, while sperm precursors may point to physiologic or reproductive problems.
6	Assess sperm motility: At least five microscopic fields are assessed in a systematic way to classify 200 spermatozoa. Examination is performed within one hour of specimen collection, once complete liquefaction has occurred. IM (Immotile) — no movement NP (Non-Progressive) — sperm moving, but with none to very slight forward motion or sperm moving aimlessly, but with more direct slow forward motion PR (Progressive) — sperm moving at moderate or high speed in a straight forward motion

Assessment of Sperm Concentration:

Step	Action
1	Mix semen sample gently and thoroughly.
2	Using a 1000 µl pipette, pipette 1000 µl of semen diluting fluid into a clean glass tube.

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3	Using a 50 µl pipette, remove 50 µl semen diluting fluid from the tube and discard.
4	With a new pipette tip, deposit 50µl of semen sample into the 950 µl of semen diluting fluid.
5	Mix well
6	Charge both sides of the hemocytometer with the dilution.
7	Allow the chamber to set for about 1 - 2 minutes to allow the sperm to settle.
8	Using the high dry objective, count the sperm in the 5 small squares in the center square of the chamber (RBC counting area) in each chamber and average the count. **Note:** • Both sides of the chamber are counted and counts must be within 10%. Rejected specimens must be mixed and recounted. If still not acceptable, new dilutions should be set-up and recounted.
9	Calculate the sperm count according to the type of counting chamber using the following formula: <u>Hemocytometer:</u> Ave count x depth factor x area factor x dilution = Count/mm^3 Count/mm^3 x 1000 = sperm/mL or Ave count x 10^6 = sperm/mL **Note:** • Semen diluting fluid contains formalin. At conclusion of sperm count procedure, the diluted sample is discarded into special formalin waste receptacle located on Body Fluid bench. Empty full receptacle into pathology formalin waste container in path cutting room.

Assessment of Sperm Morphology:

Step	Action
1	Evaluate sample for <u>Normal</u> forms present. WHO 5th strict criteria are applied in assessing the morphological normality of the spermatozoon. For spermatozoon to be considered normal, both its head and tail must be normal. All borderline forms should be considered abnormal. • Mix the semen well. • Place a drop of semen on a slide and with a second slide drag the sample along the surface. Make 2 smears. • Air dry smear, label, and stain on the Hematek slide stainer.

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- Using oil immersion objective, evaluate 200 spermatozoa in slide area where sperm are evenly distributed. Spermatozoa are recorded as either normal or abnormal; number of round cells per 100 spermatozoa is also recorded. The number of round cells per 100 spermatozoa is calculated and reported as round cells per milliliter of semen (round cells/100 sperm X sperm concentration in millions/mL divided by 100).
- Report % normal forms.

Head Defects	Neck & Midpiece Defects	Principal piece Defects	Excess residual Cytoplasm (ERC)
• Large • Small • Tapered • Pyriform • Round • Amorphous head • Vacuolated heads (more than 2 vacuoles or >20% head area occupied by unstained vacuolar areas) • Vacuoles in the post-acrosomal region • Small or large acrosomal areas (<40% or >70% of the head area) • Double heads • Or any combination of these.	• Asymmetrical insertion of the midpiece into the head • Thick or irregular midpiece • Abnormally thin midpiece • Or any combination of the above.	• Short • Multiple • Broken • Smooth hairpin bends • Sharply angulated bends • Irregular width • Coiled tails • Or any combination of the above.	• This is associated with abnormal spermatozoa produced from a defective spermatogenic process. • Large amount of irregular stained cytoplasm • 1/3 or more of sperm head size

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Resulting in Cerner:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Access Cerner ARE.</td></tr>
<tr><td>2</td><td>
<table>
<tr><th>Parameter</th><th>Reportable Range</th></tr>
<tr><td>Semen CollTime</td><td>Enter Military Time</td></tr>
<tr><td>Days Abstained</td><td>Enter days from questionnaire</td></tr>
<tr><td>Method of Collection</td><td>Enter method from questionnaire</td></tr>
<tr><td>Semen coll contr</td><td>Enter container from questionaire</td></tr>
<tr><td>Coll Issues</td><td>Enter any collection issues</td></tr>
<tr><td>Trnsp Issues</td><td>Enter any transportation issues</td></tr>
<tr><td>Spec Rec Time</td><td>Enter Receipt time</td></tr>
<tr><td>Analysis Time</td><td>Enter analysis time</td></tr>
<tr><td>Analysis Time Comm</td><td>Enter any analysis delay comments</td></tr>
<tr><td>Semen Appear</td><td>Enter semen appearance</td></tr>
<tr><td>Semen Appear Comm</td><td>Enter any appearance comments</td></tr>
<tr><td>Semen Liq & Visc</td><td>Enter Normal or Abn</td></tr>
<tr><td>Semen pH</td><td>Enter pH</td></tr>
<tr><td>Semen WBC</td><td>Enter <1 or >=1</td></tr>
<tr><td>Semen Vol</td><td>Enter volume</td></tr>
<tr><td>Sperm Conc</td><td>Enter Sperm Concentration</td></tr>
<tr><td>Sperm Conc Comm</td><td>Enter comment if <2 M/ml</td></tr>
<tr><td>Immotility % (IM)</td><td>Enter Immotile %</td></tr>
<tr><td>NonProgMot % (NP)</td><td>Enter NP %</td></tr>
<tr><td>Prog Mot % (PR)</td><td>Enter PR%</td></tr>
<tr><td>Tot PR Mot Cnt</td><td>Cerner will calculate</td></tr>
<tr><td>Normal Forms %</td><td>Enter Normal Forms %</td></tr>
<tr><td>Total Sperm/Ejac</td><td>Calculation: Conc X Volume</td></tr>
<tr><td>Tot Mot % (PR + NP)</td><td>Calculation: PR + NP</td></tr>
<tr><td></td><td></td></tr>
</table>
Motility
<table>
<tr><td>Immotile</td><td>IM</td><td>No movement</td></tr>
<tr><td>Non Prog. Motility</td><td>NP</td><td>sperm moving, but with no to very slight forward motion or
sperm moving aimlessly, but with more direct slow forward motion</td></tr>
<tr><td>Progressive Motility</td><td>PR</td><td>sperm moving at moderate speed in a straight forward motion or
sperm moving at high speed in a straight forward motion</td></tr>
<tr><td>Total Progressive Motility</td><td>Tot PR Mot</td><td>Cerner will automatically calculate and autopopulate.

(Semen Vol)*(Sperm Conc)*(Prog Mot Pct PR/100)</td></tr>
</table>
</td></tr>
</table>

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3	Verify Results in Cerner.

Reference Ranges

Parameter	Reference Range
Volume	>= 1.5 mL
Semen pH	>= 7.2
Semen WBC	<1 Million/ml
Total Sperm/Ejaculation	>= 39 Million
Sperm Concentration	>= 15 Million/ml
Total Motility (PR + NP)	>= 40%
Progressive Motility (PR)	>= 32%
Morphology Normal Forms (WHO 5th)	>= 4%

Procedural Notes

- When the semen viscosity is greatly increased, counting will be facilitated by adding mucolytic agent such as Qwik Check Liquefaction. If a significant amount is added, a dilution correction factor must be used to calculate the count.
- Another option for liquefying very viscous semen is by drawing up the sample up and down through a needle with a syringe to break up the coagulum.
- Sperm are susceptible to sudden temperature changes that can affect motility. The specimen must be maintained at room temperature 20C – 25C (68^0F-77^0F).
- All glass and plastic ware must be clean to avoid contaminants that can affect sperm motility.
- In normal semen, the majority of cells are mature sperm. Other typical cells are epithelial cells, immature germ cells, and WBCs.

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	High percentages of these cells, however, should be reported, as should gross bacterial, RBCs, trichomonas or yeast, as a comment in Cerner at the Morphology DTA. • If patient indicated that there was a temperature problem during transportation of specimen to laboratory, the CLS must enter the information in Cerner as a comment at the Collection Issues DTA . ▪ Manual Methodology is performed on the following: 1. Sperm Concentration < 2 M/mL 2. Motility of 0% 3. Viscous samples 4. Too many particles to give an accurate count

Limitations	▪ Some specimens may be too viscous, even after addition of a mucolytic agent, or contain too many particles to give an accurate count. In this case, attach a comment, "Unable to perform accurate sperm count due to incomplete liquefaction of the seminal fluid." On the Sperm Count DTA.

Downtime	• Use pre-accessioned barcodes or downtime barcodes • Test semen according to the sample testing procedure • Print the results and fax according to the System Downtime protocol

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References	• WHO Laboratory Manual for the examination and processing of human semen , 5TH ed.. World Health Organization 2010 • Baker, DJ.. Performing A Quality Semen Analysis in the Clinical Laboratory, MLO. December 2000. • SQA-Vision User Guide. Version 44.3.148

Attachments	A. Directions for Collection of Semen Analysis Specimen B. Semen Analysis Questionnaire and Worksheet

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Document History Page

Effective Date: **02-18-08**

Change type: New, Major, Minor etc.	Changes Made to SOP – describe	Signature responsible person/date	LabManager Reviewed/ Date	Med. Director reviewed/ date	Date change implemented
New Format		Mina Acosta,CLS 08-14-04	Nancy Muneno,CLS 08-18-04	R. Doshi, MD 08-19-04	08-19-04
Major	Added Safety Message and Purpose. Updated the policy according to the automated semen analysis protocol.	C.Edora 02-13-08	N.Muneno 02-14-08	R.Doshi,MD 02-14-08	02-14-08
Existing	New Lab Med. Director			S.Wirio,MD 09/12/12	09/12/12
Minor	Updated for Cerner	N.Taft 09/26/14	J. Wolf 09/29/14	S.Wirio,MD 09/29/14	09/29/14
Major	Updated to WHO 5th				

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Attachment A

KAISER PERMANENTE
Southern California Permanente Medical Group

SOUTHERN CALIFORNIA PERMANENTE MEDICAL GROUP
25825 S. Vermont Ave., Harbor City, CA 90710

DIRECTIONS FOR COLLECTION OF SEMEN ANALYSIS SPECIMEN

- Abstain from sexual intercourse for a minimum of 72 hours (3 days) but no longer than 7 days prior to collection of specimen.
- Use only the container supplied by the Laboratory. DO NOT USE A CONDOM to collect specimen unless directed to do so by your doctor.
- Obtain specimen by masturbation or by special direction from the physician. DO NOT USE LUBRICANTS of any kind when collecting specimen.
- Collect the entire specimen.
- Keep specimen at room temperature. DO NOT REFRIGERATE specimen. DO NOT EXPOSE SPECIMEN TO HEAT.
- Label specimen container with last name, first name, date and time of collection.
- Bring specimen to the Laboratory as soon as possible after collection. (30 minutes or less is preferable)
- Please do NOT submit semen specimens to the Gardena or Long Beach Office Laboratories.

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Attachment B

Semen Analysis – Patient Questionnaire

Purpose This form provides patient collection information for semen specimens, and the maintenance of such records per regulatory requirement.

PATIENT NAME: _____

MEDICAL RECORD NUMBER: _____

TIME OF COLLECTION: _____

DAYS OF ABSTINENCE (Circle the day(s) of abstinence):

1 2 3 4 5 6 7

METHOD OF COLLECTION (Check box):

☐ Masturbation

☐ Special directions from physician

SPECIMEN COLLECTION CONTAINER (Check box):

☐ Plastic container provided by the laboratory or clinic

☐ Other (describe) _____

DESCRIBE ANY COLLECTION ISSUES (i.e., spilled specimen, incomplete specimen):

_____

DESCRIBE ANY TRANSPORT ISSUES (i.e., in hot car for a long time):

_____

TIME SPECIMEN RECEIVED AT CHECK-IN: _____