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| 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. |

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| Policy  | Semen Analysis Manual Method is a backup method when Semen Analysis Automation is not available. |

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| Workplace Safety | All laboratory employees are expected to maintain a safe working environment and an injury-free workplace. Laboratory employees are responsible for their own safety, the safety of others and adhering to all departmental and medical center safety policies and procedures.* For standard precautions and safety practices in the laboratory; see **Safety Practices**, specifically, but not limited to, equipment safety, proper body mechanics, sharps exposure and proper use of personal protective equipment (PPE).
* For Universal Body Substance precautions, see **Universal Body Substance Precautions**, specifically, but not limited to, exposure to body fluids.
* For proper hand-washing, see **Hand washing Policy**, specifically, not limited to, proper hand-washing.
* For proper infection control, see **Infection Control**, specifically, but not limited to, proper use of gloves.
* For proper handling of regular and infectious waste, see **Handling of Regular and Infectious Waste**, specifically, but not limited to, proper disposal of regular and biohazardous waste.
* For proper cleaning of work area, see **Cleaning Work Areas**.
* For proper handling of chemicals and reagents, see the Chemical Hygiene Plan.

For proper storage and disposal of chemical hazardous waste, see **Storage & Disposal of Chemical Hazardous Waste**. All laboratory employees are expected to maintain a safe working environment and an injury-free workplace. Laboratory employees are responsible for their own safety, the safety of others and adhering to all departmental and medical center safety policies and procedures. |

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| Specimen  | * Specimen Type: Fresh Semen
* Specimen Volume: Entire ejaculate is required for determining sample volume
* Minimum Volume:
* 0.1 mL(SQA-Vision Manual)
* Maximum Ejaculation to Test Time: 1 hour
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| Specimen Collection | Provide the patient with local instructions for semen collection, and verify that they have followed these instructions summarized below:* 2-7 days abstinence from ejaculation prior to specimen collection
* Collect sample by masturbation or by special direction from physician
* Lubricants, spermicides and other contaminants are not to be used.
* The entire specimen must be collected into a clean container supplied only by the provider’s office or laboratory.
* The specimen container should be clearly labeled with the patient’s first and last name, medical record number, and date and time of collection.
* Keep specimen at room temperature. DO NOT refrigerate or expose to heat.
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| Specimen Transport and Temperature | * Transport the specimen to the laboratory right after collection (within 60 minutes after collection) for an accurate evaluation of sperm motility.
* During transport to the laboratory, the sample should be kept between 20 °C and 37 °C.
* Do not heat or cool the sample nor the container

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| Specimen Stability | * The semen sample must be tested within one hour of collection because motility will decline.
* Semen samples must be tested by the laboratory on a priority basis upon delivery, and expedited to the testing area.
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| Specimen Handling Prior to Testing | * When a patient arrives at the laboratory with his specimen, he is given the Patient Questionnaire Form by the receiving laboratory personnel to fill out. See **Procedure for Managing the Semen Analysis – Patient Questionnaire Form** and **Semen Analysis – Patient Questionnaire Form**.

***Important Note:*** Use the information in the completed Patient Questionnaire Form to result in Cerner. * The collection container should remain at room temperature until liquefaction is complete or 45 minutes, whichever is shorter.
* Some samples will not liquefy within 45 minutes (most will liquefy within 15 minutes).
* If a specimen is not liquefied, the accuracy of the analysis will be compromised.
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| Specimen Rejection | * The following rejection criteria are recommended by the vendor/manufacturer.
* If testing is greater than 60 minutes but less than 2 hours after sample collection, results are questionable due to age of specimen.
* If testing is greater than 2 hours after collection, reject the specimen.
* See procedure block ***Cerner Resulting*** to report the required **Analysis Time** and **Analysis Time Comment** in Cerner.
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| Equipment, Reagents, Materials and Supplies | * SQA-Vision Fixed cover-slip Slide
* QwikCheck Liquefaction Kit (Catalog #0900)
* QwikCheck Beads (Catalog #0200)
* QwikCheck Test Strips for Semen Analysis (Catalog #0700) using BioRad Urinalysis Controls
* QwikCheck Dilution Kit (Catalog #0800)
* Semen Dilution Fluid(immobilizes the spermatozoa)
* pH Indicator Paper
* Vortex Mixer
* Dilution Container
* Timer
* Thermometer with Humidity Sensor
* Incyto Disposable Hemocytometer
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| Quality Control | * QC Qwik Check Test Strips using Biorad Urine Controls:

Level 1 = <1 M/ml Leukocytes; pH = 5.0 – 6.5Level 2 = ≥1 M/ml Leukocytes; pH = 7.0 – 8.0* QC is only performed when a sample needs to be performed under manual method.
* Acceptable QC is good for 24 hours.
* Perform QC sperm concentration by performing count on QwikCheck Beads following the sperm concentration section below.
* Document QC performed on **Semen Analysis Manual Test QC and Patient Log** found in the Semen Analysis Manual Test QC and Patient Log binder.
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| Macroscopic Examination | Follow the steps below to perform macroscopic examination |
| Macroscopic Examination – Semen Analysis |
| **Step** | **Action** |
|  | 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 manipulations must be recorded in the report***.
 |
|  | Mix liquefied specimen well. |
|  | Pour specimen into a graduated plastic centrifuge tube and determine the volume to the nearest 0.1 mL. Record volume. |
|  | Determine the viscosity of the liquefied sample.* Draw the specimen into a disposable pipette and allow to drop back into container by gravity.

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| 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.
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| Microscopic Examination | Follow the steps below to perform microscopic examination |
| Microscopic Examination – Wet preparation using SQA-Vision Fixed Cover-slip Slide(20 µm deep) |
| **Step** | **Action** |
|  | Mix semen sample gently and thoroughly. Use of SQA-Vision Fixed Cover-slip Slide **does not require** dilution. |
|  | Using a Biopette (0.5-10uL) pipette deliver ̴ 3.5 μL of well mixed liquefied semen sample onto a SQA-Vision Fixed Cover-slip Slide on both chambers. Load the sample where instructed by the arrow(s).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).
 |
|  | Perform initial evaluation at 100X total magnification. 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 mentioned in the report.
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|  | Process for sperm motility, sperm concentration, and normal forms/morphology |
|  | Use the **Semen Analysis Manual Test QC and Patient log** provided to document manual sperm motility and sperm concentration reports. |

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| Assessment of Sperm motility  | Follow the steps below to perform assessment of sperm motility |
| Assessment of Sperm motility – Semen Analysis using SQA-Vision Fixed Cover-slip Slide |
| **Step** | **Action** |
|  | Examine wet preparation slide by using a microscope at 400X total magnification or using SQA-Vision Visualization mode at zoomed out (1188X) magnification. ***See procedure LAMC-PPP-0764 Automated Semen Analysis Using SQA-Vision***. |
|  | Examination is performed within one hour of specimen collection, once complete liquefaction has occurred. |
|  | Differentiate motility by following criteria below. Classify at least 200 spermatozoa or 20 microscopic fields.

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| IM (Immotile) | no movement |
| NP (Non-Progressive) | All other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed. |
| PR (Progressive) | Spermatozoa moving actively, either linearly or in a large circle, regardless of speed. |

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|  | Motility differentiation will be reported in percentages (IM%+NP%+PR%=100%) |

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| Assessment of Sperm Concentration  | Follow the steps below to perform assessment of sperm concentration

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| Determining the required dilution using a 20 µm deep wet preparation |
| **Step** | **Action** |
|  | Examine the 20 um deep wet preparation made using the SQA-Vision Fixed cover slip or similar. |
|  | If *spermatozoa are observed*, count them, determine the necessary dilution from table below. If spermatozoa are not observed go to *step 5 of Assessment of Sperm Concentration – using InCytochip.*

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| Microscope at 400X magnification spermatozoa/field | SQA-Vision visualization zoomed out(1188X)spermatozoa/field | Dilution required |
| >101 | >33 | 1:20(1+19) |
| 16-100 | 5-33 | 1:5(1+4) |
| <15 | <5 | 1:2(1+1) |

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|  | Perform required dilution using QwikCheck Dilution Kit or Semen Diluting fluid and proceed to ***Assessment of Sperm Concentration – using InCytochip***  |

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| Assessment of Sperm Concentration –using InCytochip |
| **Step** | **Action** |
|  | InCytochip consists of 9 large squares, each measuring 1x1mm, and the depth of the chamber is 0.1mm. Each large square has a total volume of **0.1mm3**. |
|  | Mix the diluted semen sample well and load 10 µl of sample into the injection area so that it fills the chamber by capillary action.(Please be careful not to make air bubbles.) Repeat step on the 2nd chamber and store InCytochip for at least 4 minutes at room temperature in a humid chamber (e.g. on water-saturated filter paper in a covered Petri dish) to prevent drying out. |
|  | Examine inCyto chip slide, at 400X total magnification count number of spermatozoa on 1 large square using grid 5 or 1 row with in grid 5(multiply count by 5 to obtain a large square count). Count only spermatozoa with a complete head and tail composition. Perform count on both chambers to produce 2 replicate counts.Check for acceptable differences between two replicate counts for a given sum. Acceptable difference is the maximum allowable difference between replicates.

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| **Sum between replicates** | **Acceptable difference** |  | **Sum between replicates** | **Acceptable difference** |
| <144 | acceptable |  | 329-249 | 36 |
| 144-156 | 24 |  | 347-366 | 37 |
| 157-169 | 25 |  | 367-385 | 38 |
| 170-182 | 26 |  | 386-406 | 39 |
| 183-196 | 27 |  | 407-426 | 40 |
| 197-211 | 28 |  | 427-448 | 41 |
| 212-226 | 29 |  | 449-470 | 42 |
| 227-242 | 30 |  | 471-492 | 43 |
| 243-258 | 31 |  | 493-515 | 44 |
| 259-274 | 32 |  | 516-538 | 45 |
| 275-292 | 33 |  | 539-562 | 46 |
| 293-309 | 34 |  | >563 | 47 |
| 310-328 | 35 |  |  |  |

*Continued…*Example1: Chamber 1= 120, Chamber 2= 155, Sum= 120+155=275, Difference= 155-120=35; Acceptable difference for **275 is 33** which makes the count *Unacceptable*. Perform a recount or recharge on a new Fixed cover slip slide.Example2: Chamber 1= 130, Chamber 2=145,Sum= 130+145=275, Difference= 145-130=15; Acceptable difference for **275 is 33** which makes the count ***Acceptable***. Proceed to next step.*Note: Sum between replicates that falls below 144 is considered acceptable regardless the difference.* |
|  | Average replicate count between 2 chambers, this count will be your **cellscounted** / 0.1 mm3, follow formula below to convert to M/ml where in: * C = sperm concentration
* D = dilution factor
* 0.1 mm3 = volume per large square.

C= (**Cellscounted** / 0.1 mm3) X (1,000 mm3 / 1 ml) X ( 1 M / 1,000,000) X DC= [(**Cellscounted** X 10,000) / ml ] X (1 M / 1,000,000) X DC= (**Cellscounted** X 0.01)M/ml X DC= M/mlExample: 1:5 Dilution sample: Average replicate count for 130 and 145 on 2 chambers = 138 cells counted per 0.1 mm3 (*counted on 1 large square per chamber on grid 5*).C= (**Cellscounted** X 0.01) X DC= 138 X 0.01 X 5**C= 6.9 M/ml** |
|  | For **Sperm Concentration of <2M/ml**(lowest reportable range which means anything below 2 M/ml will be reported in Cerner as <2M/ml) – Choose under sperm concentration comment if **No sperm/hpf or Rare sperm/hpf** was observed. |

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| **Processing of Normal Morph/Forms%** | Follow steps below to process send out of Sperm Cell Morphology. |

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| Process of Normal Morph%/Sperm Morphology – Semen Analysis |
| **Step** | **Action** |
|  | If Normal Forms% is not resulted by instrument and sperm concentration is ≥2M/ml then Manual Normal Morph/Forms% will be sent out for analysis as test order **Sperm Cell Morphology**(KPHC Order code: 87205ZS/ CPT Code: 87205/ Collection label code: Sperm Morp). |
|  | Mix the semen well. Slide must be prepared within 2 hours from time of collection |
|  | Place a drop of semen on a slide and with a second slide drag the sample along the surface. Make 2 feathery edge smears |
|  | Label smear and air dry smear |
|  | Call provider to order Sperm Cell Morphology |
|  | Once order is up, send slides in slide holders to Regional Laboratory Specimen Processing |
|  | Report in Cerner for Normal Morph/Forms% as “==” and enter result comment “Specimen send out for Sperm Cell Morphology”  |

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| Resulting in Cerner | Follow the steps below to result in Cerner |
| Resulting in Cerner – Semen Analysis |
| **Step** | **Action** |
|  | Access Cerner ARE. |
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| **Parameter** | **Reportable Range** |
| Semen CollTime | Enter Military Time |
| Days Abstained | Enter days from questionnaire |
| Method of Collection | Enter method from questionnaire |
| Semen coll contr | Enter container from questionaire |
| Coll Issues | Enter any collection issues |
| Trnsp Issues | Enter any transportation issues |
| Spec Rec Time | Enter Receipt time |
| Analysis Time | Enter analysis time |
| Analysis Time Comm | Enter any analysis delay comments |
| Semen Appear | Enter semen appearance |
| Semen Appear Comm | Enter any appearance comments |
| Semen Liq & Visc | Enter Normal or Abn |
| Semen pH | Enter pH |
| Semen WBC | Enter <1 or >=1 |
| Semen Vol | Enter volume |
| Sperm Conc | Enter Sperm Concentration |
| Sperm Conc Comm | Enter comment if <2 M/ml |
| Immotility % (IM) | Enter Immotile % **If sperm concentration is <2M/ml report as N/A(free text)** |
| NonProgMot % (NP) | Enter NP % **If sperm concentration is <2M/ml report as N/A(free text)** |
| Prog Mot % (PR) | Enter PR% **If sperm concentration is <2M/ml report as N/A(free text)** |
| Tot PR Mot Cnt | Cerner will calculate **If sperm concentration is <2M/ml report as N/A(free text)** |
| Normal Morphs % | Enter Normal Forms % Auto if available, **if not and sperm conc. is >2M/ml then see section Processing of Normal Morph% above,**  **if sperm concentration is <2M/ml report as N/A(free text)** |
| Total Sperm/Ejac | Calculation: Conc X Volume **If sperm concentration is <2M/ml report as N/A(free text)** |
| Tot Mot % (PR + NP) | Calculation: PR + NP **If sperm concentration is <2M/ml report as N/A(free text)** |
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|  Motility

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| **Immotile** | IM | No movement |
| **Non Prog. Motility** | NP | sperm moving, but with no to very slight forward motion orsperm moving aimlessly, but with more direct slow forward motion |
| **Progressive Motility** | PR | sperm moving at moderate speed in a straight forward motion orsperm moving at high speed in a straight forward motion |
| **Total Progressive Motility** | Tot PR Mot | Cerner will automatically calculate and autopopulate.(Semen Vol)\*(Sperm Conc)\*(Prog Mot Pct PR/100) |

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|  | Verify Results in Cerner. |

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| Reference Range | * The ranges below are based on WHO 5th reference.
* The table below shows the reference ranges for Kaiser Permanente.
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| Semen 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 | >= 4% |
| Non-Controlled Documents | The following non-controlled documents support this procedure.* CAP Laboratory Accreditation Standards Checklist
* Product Insert; Medical Electronic Systems, QwikCheck Beads
* Product Insert; Medical Electronic Systems, QwikCheck Test Strips
* Product Insert; Medical Electronic Systems, QwikCheck Liquefaction
* Product Insert; Medical Electronic Systems, QwikCheck Dilution
* WHO laboratory manual for the Examination and processing of human semen, 5th Edition
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| Controlled Documents | The following controlled documents support this procedure.

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| **Document Number** | **Document Name** |
| LAMC-PPP-0123 | Safety Practices |
| LAMC-PPP-0127 | Infection Control |
| LAMC-PPP-0128 | Universal Body Substance Precaution |
| LAMC-PPP-0129 | Handling of Regular and Infectious Waste |
| LAMC-PPP-0130 | Cleaning Work Areas |
| LAMC-PPP-0132 | Hand-washing Policy |
| LAMC-PPP-0134 | Storage and Disposal of Chemical Hazardous Waste |
| LAMC-PPP-0277 | Beckman Coulter Unicel DxH 800 Quality Control |
| Attachment MS | Semen Analysis Manual Test QC and Patient Log |

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| Procedure |
| Semen Analysis Collection from local laboratory or LabNet |
| Procedure for Managing the Semen Analysis – Patient QuestionnaireForm |
| LAMC-PPP-0319 | Semen Analysis: Automated- Spermalite SQA-V |
| LAMC-PPP-0323 | Semen Analysis: Specimen Collection Information |
| Form |
| Semen Analysis – Patient Questionnaire Form |
| Author(s) | Alvin Castillo, CLS |

Attachment MS

