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| PurposePrinciple | This procedure provides instructions forperforming Unfractionated and Low Molecular Weight Heparin assay.  The quantitative determination of the plasma levels of unfractionated (UFH) and  low molecular weight heparin (LMWH) is based on the measurement of their  anti-Xa activity on antithrombin in a competitive system using a synthetic  chromogenic substrate. The normal function of a molecule of factor Xa, as soon  as it appears in plasma, is to cleave its natural substrate, prothrombin, to  generate thrombin, the enzyme responsible for the fibrin clot formation. In the  presence of heparin, competition occurs between this mechanism and the  inhibitory mechanism exerted by the heparin-antithrombin III complex, this  inhibition being largely responsible for the action of heparin. The proposed  method is a one-step reaction based on a similar principle: as soon as factor Xa  is added to the plasma-substrate mixture, two reactions take place  simultaneously:   * hydrolysis of the substrate by factor Xa * inhibition of factor Xa by the heparin-antithrombin III complex\*   After the necessary period of time for the competitive reaction to reach equilibrium, the quantity of paranitroaniline (pNA) that is released is inversely proportional to the concentration of heparin present in the plasma. |

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| Scope | This procedure is intended for Clinical Laboratory Scientist (CLS) and Medical Laboratory Technicians (MLT) who are trained and competent in performing the Heparin (UFH and LMWH) assay.  This Procedure replaces LCoM 219 STA-R Evolution Quality Control and LCoM 221 STA-R Evolution Sample Processing. |

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| Specimen sources | Whole Blood collected in 9:1 ratio of blood in a 3.2% (0.109M) Sodium Citrate tube. Sample must be centrifuges within 1 hour of collection. |

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| Specimen collection | Refer to LPhM 200 Performing Venipuncture at KP LAMC |

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| Specimen transport | Refer to LSPM 203 Specimen Collection, Handling, Packaging and Transportation |

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| Specimen storage | Whole blood or plasma can be stored at 20 + 5◦ C for 4 hours.  Do not store whole blood or plasma at 2-8◦ C |

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| Specimen rejection | Reject specimen under any of the following conditions:   * Clotted * Samples collected in wrong collection tube * Short draws and over draws * Samples with visible hemolysis * Samples with unusually shortened results must be checked for clot |

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| Equipment | * Stago STA-R Evolution * Centrifuge |

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| Material and supplies | * Pipettes * Pipette tips * Cuvette Roll-1000 * Magnetic Stir Bars * STA® Reducer * Reagent Grade Water * STA – DESORB U * STA® - Cleaner Solution |

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| Reagent/s | **Description** | **Preparation** | **Stability** |
| STA® - Liquid Anti-Xa Assay Kit:  **Reagent 1:** Ready to use chromogenic substrate.  **Reagent 2:** Ready to use bovine factor Xa. | Allow the reagent to stand 30 minutes at room temperature (18-25 °C) before use. Swirl vial gently to ensure each vial is completely mixed; place an STA® **-** Reducer in the vial and replace the perforated plastic cap on the vial.  Write the date and initial on the vial before loading on the instrument.  Allow the reagent to sit at room temperature (18-25 °C) for 30 minutes. Swirl vial gently to ensure each vial is completely mixed; then place an STA® **-** Reducer in the vial and replace the perforated plastic cap on the top.  Write the date and initial on the vial before loading on the instrument. | 7 days on analyzer in its original vial with the STA® - Reducer and perforated cap in place.  7 days on analyzer in its original vial with the STA® - Reducer and perforated cap in place. |
|  | STA® - Owren-Koller Buffer | Ready to use buffer. Used by the STA-R Evolution® to perform dilutions of controls and patients’ plasmas.  Write the date and initial on the vial before loading on the instrument. | 72 hours on the STA-R Evolution® |

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| 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, and 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 LGM   8000, 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 LGM 8005, specifically, but not limited to, exposure to body fluids. * For proper handwashing, see LGM 8010, specifically, not limited to, proper handwqashing. * For proper infection control, see LGM 8004, specifically, but not limited to, proper use of gloves. * For proper handling of regular and infectious waste, see LGM 8006, specifically, but not limited to, proper disposal of regular and biohazardous waste. * For proper cleaning of work area, see LGM 8007 – 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 LGM 8012. |
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**Calibration**  Refer to LCOM 416 STA-R Evolution® Calibration/Verification

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| QualityControl |

Unfractionated Heparin (UFH or Anti-Xa) Controls must be ran at least once per shift using the STA® - Quality HNF/UFH.

Low Molecular Weight Heparin (LMWH) Controls must be ran when only when

there is patient testing using STA® - Quality HBPM/LMWH.

When Quality Control tolerance limits are exceeded based on the QC criteria

defined in LGM 2022 Qulaity Control (QC) Policy, corrective action must be

taken and documented before analyzing patient samples.

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|  | |  |  |  | | --- | --- | --- | | **Controls** | **Preparation** | **Stability** | | **STA® - Quality HNF/UFH: (used for UFH or Anti-Xa )**  (STA® - Quality HNF/UFH 2 and STA® - Quality HNF/UFH 7) | Reconstitute each vial with 1.0 ml distilled water. Let stand 30 minutes at room temperature. Swirl gently to ensure the vial is completely mixed.  Write the date and initial on the vial after reconstitution. | Reconstituted stability on the STA-R Evolution® is 4 hours. | | **STA® - Quality HBPM/LMWH: (used for LMWH)**  (STA®- Quality HBPM/LMWH Control 8 and STA®- Quality HBPM/LMWH Control 14) | Reconstitute each vial with 1.0 ml distilled water. Let stand 30 minutes at room temperature. Swirl gently to ensure the vial is completely mixed.  Write the date and initial on the vial after reconstitution. | Reconstituted stability on the STA-R Evolution® is 4 hours. | | | | |
|  | * Follow the steps below to run Unfractionated Heparin or Low Molecular Weight Heparin Quality Control | | | |
| Step | Action |
| 1 | Click the icon |
| 2 | |  |  | | --- | --- | | **If…** | **Then…** | | running quality control for Unfractionated Heparin (or Anti-Xa) | Click the **LIQ** **UFH** test abbreviation | | running quality control for Low Molecular Weight Heparin (LMWH) | Click the **LIQ** **LMWH** test | |
| 3 | Click the **Control Level #** tab (#corresponds to the number of the level) |
| 4 | Click the button |
| 5 | Confirm by clicking **OK** |
| 6 | Repeat for each level |
| 7 | All control ranges are monitored automatically by the STA–R Evolution®. Control results are automatically filed in the STA–R Evolution® QC file. All results for a 24-hour period will be converted to a “mean” value at midnight. This mean is used in the statistical data and is plotted on the Levy-Jennings chart as a daily mean. Each point can be viewed on the Levy-Jennings Daily control chart by clicking on the left arrow. |

* Follow the step below to Display Daily Controls

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| Step | Action |
| 1. | Click on the  The **Daily controls** screen is displayed: |
|  | * For Quality control result >2SD, the value will be flagged ‘To Validate’. ‘To Validate’ denotes that the result must be reviewed and accepted or appropriate action taken such as repeating the quality control run. Follow the step below to validate the quality control result. | | | |
| Step | Action |
| 1 | From the **Daily Control** **screen**, **double click** on the quality control to be validated. The following options will display:   * Validate * Rerun * Delete |
| 2 | |  |  | | --- | --- | | **If…** | **Then…** | | The quality control result is acceptable | Choose ‘Validate’ | | The quality control requires repeat or rerun | Choose ‘Rerun’ | | The quality control needs to be excluded from the daily statistical data. | Choose ‘Delete’ | |

* + - * Follow the step below to print daily Quality Control results.

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| Step | Action |
| 1. | From the **Daily Control screen**, **click** on  to print daily quality control results. |

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| Procedure | * + - * Follow the steps below to run Prothrombin Time on patient samples. | | | |
| Step | Action |
| 1 | Place tubes in rack |
| 2 | Place rack(s) on tray |
| 3 | Place tray on STA-R. Tubes are automatically loaded, their barcoded labels are read, work lists are downloaded from the host computer or the predefined profile is applied according to the chosen option. Tests are automatically carried out by the analyzer. |
| 5 | **Test Panel**  Click on |
| 6 | If the instrument is unable to read the barcode, the automatic unloading of not read tubes is selected  and all tubes which are not read are automatically unloaded.  The **Manual Input of Patient ID’s** window is displayed for re-identification. |
| 7 | Enter the sample ID |
| Clinical Significance | Unfractionated Heparin (UFH) and Low molecular weight heparins (LMWH) are used for the prevention and treatment of thromboembolic diseases. The quantitative determination of plasma UFH and LMWH levels are useful for monitoring treatment efficacy. | | | |
| Reference Range | |  |  | | --- | --- | | UFH or Anti-Xa Reference Range | 0.3-0.7 IU/ml | | LMWH Reference Range | 0.5-1.0 IU/ml | | UFH or Anti-Xa Clinical Reportable Range | 0.1-1.1 IU/ml | | LMWH Clinical Reportable Range | 0.1-4.0 IU/ml | | | | |

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| Interpretation Results / Critical Values | |  |  | | --- | --- | | UFH or Anti-Xa Critical Value | >0.79 IU/ml | | LMWH Critical Values | >1.3 IU/ml | |
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| Limitations | * + - * Any release of platelet factor 4 (PF4), which is a potent heparin inhibitor, will lead to an under-estimation of the heparin level in the plasma being tested. Careful and adequate centrifugation is essential: the higher the level of residual platelets, the greater the risk of PF4 release.       * The STA® - Liquid Anti-Xa assay relies on the Antithrombin level of the patient. Low levels [less than 60%] may reflect an underestimation of the heparin level result.       * The STA® - Liquid Anti-Xa assay UFH is insensitive to the following substances:   Hemoglobin: up to 1.5 g/l  Conjugated bilirubin: up to 288 mg/l – 342 umol/l  Unconjugated bilirubin: up to 138 mg/l – 236 umol/l  Triglycerides: up to 6.9 g/l – 8 mmol/l |

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| Controlled Documents | The following non-controlled documents support this procedure. | | | |
| Document No. | Name of Documents |
| LPhM 200 | Performing Venipuncture at KP LAMC |
| LSPM 203 | Specimen Collection, Handling, Packaging and Transportation |
| LGM 2022 | Quality Control (QC) Policy |
| LCoM 41 | STA-R Evolution® Calibration/Verification |
| LGM 8000 | Safety Practices |
| LGM 8004 | Infection Control |
| LGM 8005 | Universal Body Substance Precautions |
| LGM 8006 | Handling of Regular and Infectious Waste |
| LGM 8007 | Cleaning Work Areas |
| LGM 8010 | Hand washing Policy |
| LGM 8012 | Storage and disposal of Chemical Hazardous Waste |

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| Non-Controlled Documents | The following controlled documents support this procedure. |

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| Document No. | Name of Documents |
|  | STA-*R* Evolution Reference Manual |
|  | STA® Package Inserts |

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| Author(s) | Rosalie I. Fajardo, MS CLS(ASCP) |