1. **PRINCIPLE**
	1. Hemoclot thrombin inhibitor is an in-vitro test intended to be used for the quantitative measurement of direct thrombin inhibitor or DTI (e.g. hirudin, argatroban and dabigatran in human citrated plasma. The test uses a clotting method based on the inhibition of constant concentration of thrombin. Diluted patient plasma is mixed with pooled human plasma Reagent 1 (R1). Clotting is then initiated by adding a constant amount of highly purified human thrombin, in the form Reagent 2 (R2).
	2. The clotting time measured is directly related to the concentration of the DTI in the tested plasma. Since the calibrators for this test are spiked with argatroban, the results are specific for argatroban levels.
2. **SPECIMEN COLLECTION AND HANDLING**
	1. Refer to Coagulation Tests: Specimen Collection and Handling (Non-Platelet Function Tests Only) procedure.
3. **SUPPLIES/EQUIPMENT**
	1. IL Coagulation Analyzer
	2. Cuvette
	3. IL Reagent racks and sample racks
	4. Cleaning and Rinse solutions
	5. Serological and automatic pipettes
4. **REAGENTS**
	1. **Hyphen BioMed Hemoclot Thrombin Inhibitors R1 Normal pooled plasma:** Reconstitute vial with exactly 2.5 mL of distilled water. Shake until complete dissolution of the content, then vortex. Allow to homogenize for 15 minutes at room temperature while shaking the vial from time to time. . Reconstituted reagents are stable for 8h at room temperature, 24h at 2-8oC, 2 months frozen in the original vial.
	2. **Hyphen BioMed Hemoclot Thrombin Inhibitors R2 Human calcium thrombin:** Reconstitute each vial with exactly 2.5 mL of distilled water. Shake until complete dissolution of the content, then vortex. Allow to homogenize for 15 minutes at room temperature while shaking the vial from time to time. Reconstituted reagents are stable for 8h at room temperature, 24h at 2-8oC, 2 months frozen in the original vial.
	3. **HemosIL Cleaning Agent (Clean B)** Dilute 1 part Cleaning Agent + 7 parts DI water or equivalent.
5. **CONTROLS**
	1. **Hyphen BioMed Argatroban Control Plasma** **C1 (CONTROL 1)**: Human plasma, control plasma used for the quality control of argatroban (also called Argatra, Arganova,or Novastan) measurements. The human plasma is freeze-dried and supplemented with argatroban (level 1 at about 0.65 mcg/mL). Reconstitute each vial with exactly 1mL of distilled water. Shake thoroughly until completely dissolved, then vortex. Allow the reconstituted material to stand at room temperature (8-25˚C) for 30 min, while shaking the vial from time to time for 30 minutes. Mix thoroughly, then vortex until a completely homogenous solution. Reconstituted controls are stable for 7 days at 2-8˚C and 48 hours at room temperature (18-25˚C). **Do not freeze**.
	2. **Hyphen BioMed Argatroban Control C2 (CONTROL 2):** Human plasma, control plasma used for the quality control of argatroban measurement. The human plasma is freeze-dried, supplemented with argatroban (level 2 at about 1.25 mcg/mL). Reconstitute each vial with exactly 1 mL of distilled water. Shake thoroughly until completely dissolved, then vortex. Allow the reconstituted material to stand at room temperature (18-25˚C) for 30 minutes while shaking the vial from time to time. Mix thoroughly, then vortex until a completely homogenous solution**.** Reconstituted controls are stable for 7 days at 2-8˚C and 48 hours at room temperature (18-25˚C). **Do not freeze.**
6. **STANDARD**
	1. **Hyphen BioMed Argatroban Plasma Calibrator**
		1. **CAL1 – Calibrator 1** human plasma, freeze-dried, without any addition of argatroban (level 1 at 0 mcg/mL).
		2. **CAL2 – Calibrator 2** human plasma, freeze-dried, supplemented with argatroban (level 2 at about 0.5 mcg/mL)
		3. **CAL3 – Calibrator 3** human plasma, freeze-dried, supplemented with argatroban (level 3 at about 1.0 mcg/mL)
		4. **CAL4 – Calibrator 4** human plasma, freeze-dried, supplemented with argatroban (level 4 at about 1.5 mcg/mL)
		5. **CAL5 – Calibrator 5** human plasma, freeze-dried, supplemented with argatroban (level 5 at about 2.0 mcg/mL).
	2. Reconstitute CAL 1,2,3,4 and 5 with 1 mL of distilled water. Shake thoroughly until completely dissolved, then vortex. Allow the reconstituted material to stand at room temperature (18-25˚C) for 30 minutes while shaking the vial from time to time. Mix thoroughly, then vortex until a completely homogenous solution. Store unopened vials at 2-8˚C and use by the expiration date printed on the box. Reconstituted material is stable for 48h at room temperature and 7-8 days at 2-8˚C. **Do not freeze.**
7. **QUALITY CONTROL**
	1. Quality control consists of Hyphen BioMed C1 and C 2
	2. Frequency of Control Use:
		1. Controls should be run at least once every 8 hours shift and with a new calibration curve.
8. **PROCEDURE**
	1. Refer to IL Operations Procedure.
9. **ERROR CODES:**
	1. Refer to IL Operations Procedure Attachment D: Errors.
10. **EXPECTED VALUES**
	1. Any unreasonable result is to be repeated.
	2. Resulting is performed in LIS.
11. **NORMAL RANGE:**
	1. Refer to Coagulation Tests: Reportable Limits and Normal / Therapeutic Values procedure.
12. **REPORTABLE RANGE:**
	1. Refer to Coagulation Tests: Reportable Limits and Normal / Therapeutic Values procedure.
13. **TAT**
	1. Completion of result should be available within 24 hours of specimen collection date.
14. **LIMITATIONS:**
	1. Specimens should be centrifuged within 1h of collection.
	2. Bubbles interfere with IL ACL TOP liquid level sensor and therefore sampling. Ensure there are not any bubbles in any sample or reagent.
	3. The Hemoclot Thrombin Inhibitors reagents do not contain heparin inhibitors. Presence of heparin or of other anti-thrombin substances different from the one to be tested may interfere in the assay and prolong the clotting time.
	4. Blood activation,during specimen collection and plasma preparation, may interfere in the assay. Lipemic, hemolyzed and clotted specimen must be recollected.
15. **REFERENCES**
	1. ACL TOP Family On-Line Help Manual, Instrumentation Laboratory
	2. Hemoclot Thrombin Inhibitor package insert, Aniara Hyphen BioMed September 17, 2013

##### Document Control

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##### Document History

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| --- | --- | --- | --- | --- |
| Signature | Date | **Revision #** |  | **Related Documents****Reviewed/****Updated** |
| Prepared by: Carrie Canlas MT(ASCP)  | 07/02/2014 | **00** |  |  |
| Approved by: Mark Kolins, MD | 07/17/2014 |  |  |  |
|  |  |  |  |  |
| **Reviewed by: (Signature)** | **Date** | **Revision #** | **Modification** | **Related Documents****Reviewed/****Updated** |
| Marc Smith, MD | 07/16/2014 | 00 | New procedure. | OK |
| Marc Smith, MD | 11/10/2015 | 01 | Pg2 added stability for C1 and C2 controls. | OK |
| Marc Smith, MD | 04/01/2016 | 02 | Changed the title direct thrombin inhibitor to dilute thrombin time, added BCSXP operations in document | OK |
| Elizabeth Sykes, MD | 02/22/2018 |  |  |  |
| Marc Smith, MD | 04/16/2018 |  | Updated logo only | OK |
| Marc Smith, MD | 03/11/2019 |  | New instrumentation |  |
| Peter Millward, MD  | 03/27/2019 |  |  |  |
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