HealthSupport Queensland

Paediatric Mixed Bag

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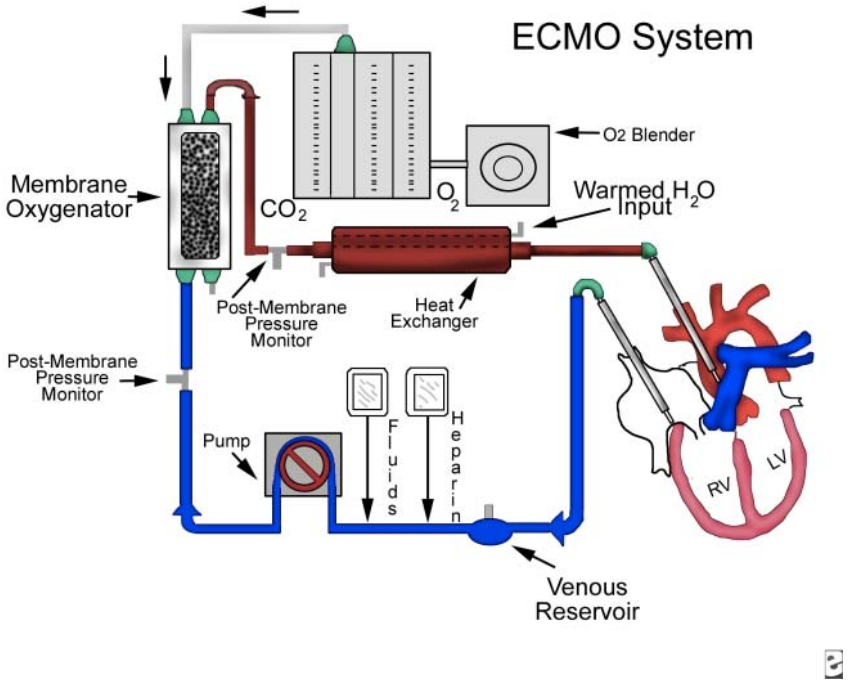
Presentation Overview

- Paediatric ECMO from a laboratory perspective
- Challenges faced in a paediatric laboratory
- Various case studies

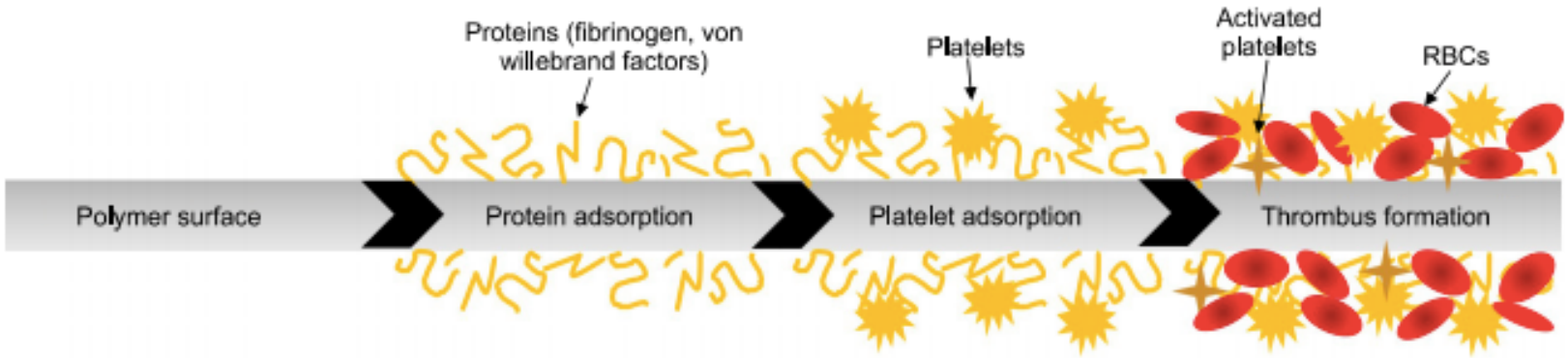
What is ECMO?

- Principle
  - oDesaturated blood drained via a venous cannula
  - oCO2 is removed, O2 added through an extracorporeal device
  - oBlood returned to systemic circulation via another:
    - – Vein: VV ECMO
    - – Artery: VA ECMO



Components

- **Centrifugal pump** (replaced roller pumps – reduces haemolysis due to less negative pressure)
- **Membrane oxygenator & heat exchanger** (controls temperature and gas exchange)
- **Controller** (control panel for monitoring ECMO system and blood gas monitoring)
- **Cannulas**
- **Tubing** (non-endothelial surfaces induces clotting) need for anti-coagulation



ECMO Coagulation Monitoring

Requested Coagulation Panel (6 hourly or 4hours if heparin adjustments required)

- PT
- aPTT
- aPTT (heparin neutralisation)
- FibC
- Anti-Xa assay
- Antithrombin assay
- TEG testing (performed at bedside)

+

- FBC per 6 hour blood draw
- Once daily TRIG/Plasma free HgB/Chem20/CRP/PCT
- Other as required tests

ECLS Blood Product Support

- Maintain 2 Fresh < 7days red cells
  - o +/- Freshly <24hours irradiated (?Di-George or Oncology)
- Maintain 1 bag of platelets
- Ready supply of Thrombotrol-VF (Antithrombin Concentrate)
- Ready supply of Cryoprecipitate / FFP
- Blood product management largely driven by close coagulation monitoring and clinical oversight

Antithrombin (AT)

- AT previously known as Antithrombin III
- Physiological inhibitor of blood coagulation
  - Predominantly Thrombin and Xa
- Produced by the liver
- Effect markedly enhanced by heparin

Heparin + AT

- Rate of antithrombin-thrombin inactivation
  - Accelerated 2000-4000 fold
- Rate of Factor Xa inhibition:
  - Accelerated 500-1000 fold
- Enhances Factor IXa inhibition
  - In presence of physiological Calcium

Antithrombin Deficiency

- Physiological:
  - *Neonates (normal AT Ag but different isoforms i.e. activity)*
- Acquired types:
  - *Increased secretion* - Renal Failure associated with nephrotic syndrome
  - *Decreased production* – cirrhosis, liver failure
  - *Accelerated consumption* – **ECMO**, CPB, severe trauma, DIC
- Inherited:
  - Autosomal dominant. Mutations in SERPINC1 gene.
  - Homozygous - most are presumed lethal (excluding heparin binding defects)

Case Study 1

- 8 day old male
  - Complex congenital heart disease - surgery
  - multiple venous thromboses
  - ECMO?
  - Request for COAG, UFH-anti-Xa and AT“3”

Case Study 1

- COAG Results:

GENERAL COAGULATION		Specimen: Blood		
INR	1.4 H			
Prothrombin Time	17 H	THROMBOPHILIA SCREEN		
APTT	103 D	Antithrombin 3	*	U/mL
Fib (derived)	1.5 L			
Unfrac. Hep. Assay	0.44			

- AT result transmitted from analyser as 0.01XL
- Note: aPTT result and UFH-Anti-Xa result

Case Study1: Review of results on ACL TOP 500

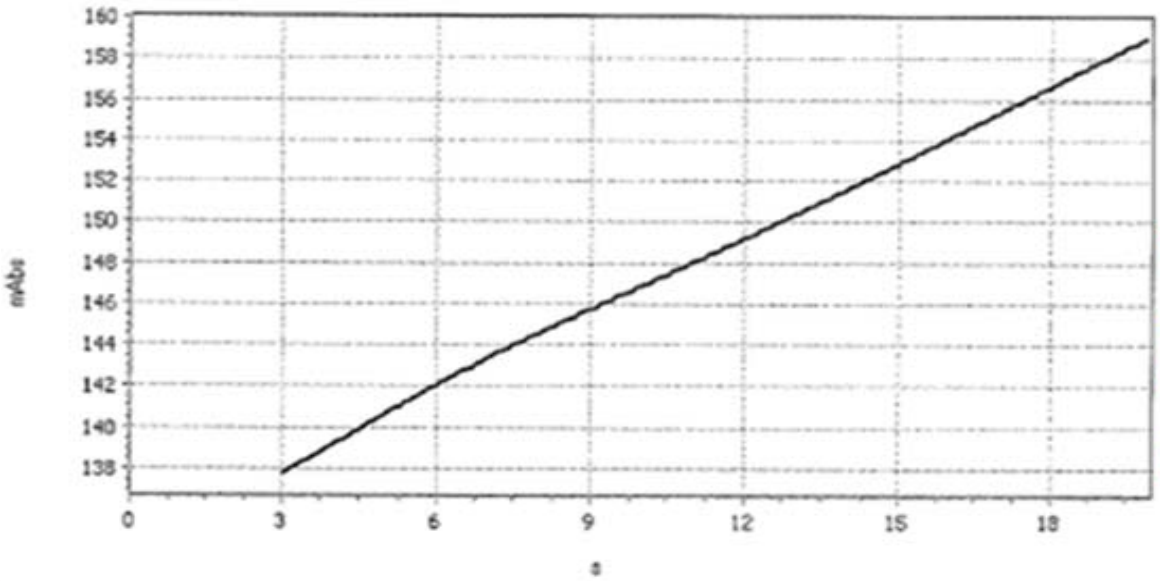
Test Code:	AT LIQ	**Test Type:**	Patient	**Rack ID:**	59
Sample ID:		**Sample Type:**	Patient	**Position ID:**	1

Completed Date & Time:

		Replicate 1	Replicate 2	Mean
Measured	**mAbs/min**	FAILED		FAILED
	%	FAILED		FAILED
	U/mL	FAILED		FAILED

Replicate 1

Display derivative curves



Errors and warnings

		Replicate 1
		Measured (mAbs/min)
CE	5060	(Data) Normalized curve delta too low
		Unit 1 (%)
RE	5100	Measured result failed
		Unit 2 (U/mL)
RE	5100	Measured result failed

Test Code: AT LIQ

Error Code: (Data) Normalized curve delta too low

Result: FAILED

Case Study 1

- Interpretation of result reported initially as 0.01U/mL based on:
  - Transmitted result to LISS of 0.01XL (L = low)
  - Error code “normalised curve delta too low”
  - ?Makes sense to report a low result

Case Study 1

- **Understanding Reagents and the assay:**
- AT Reagent – Intermediate reagent
  - Factor Xa + heparin
- AT Chromogenic Substrate – Start reagent
  - S-2765

Case Study

1. AT + Heparin → [AT-Heparin]
2. [AT-Heparin] + FXa (excess) → [AT-Heparin-FXa] + FXa (residual)

FXa (residual)

3. S-2765 → Peptide + pNA

- AT binds to heparin.
- AT-Heparin complex binds to excess Fxa (Xa reagent)
- Residual FXa determined by rate of hydrolysis of S-2765. (Xa Substrate)
- pNA measured at 405nm (?Interferences at this wavelength)
  - **Inversely proportional** to AT level (residual Xa activity)
  - Neonatal isoforms have less affinity for Xa (perceived reduced activity)

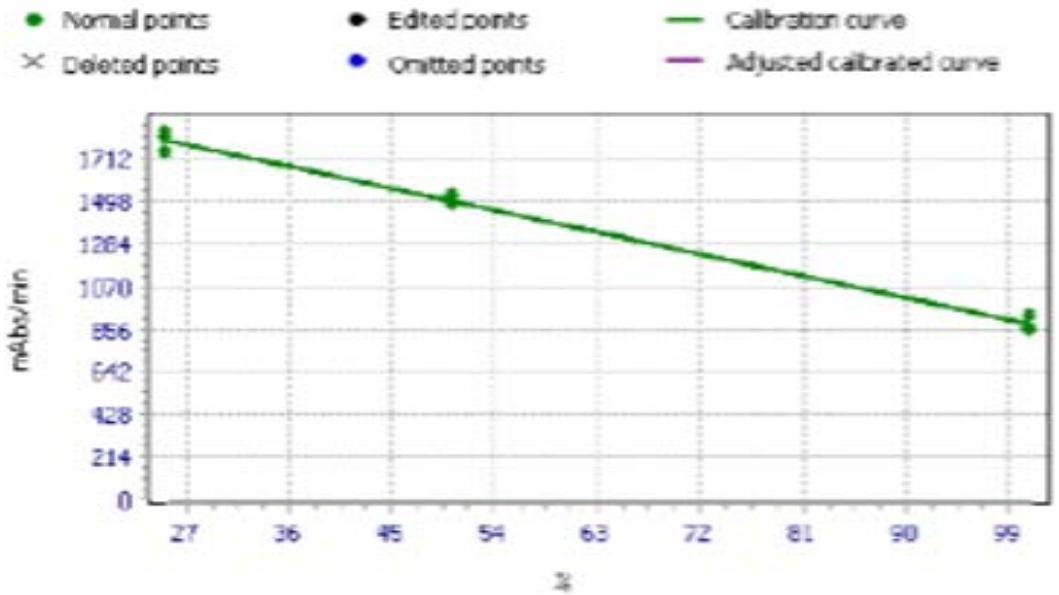
Case Study 1

Calibration Curve

Regression Type:	Linear regression
X - Transformation:	No transformation
Y - Transformation:	No transformation
Y-Intercept:	2.108E+03
Slope:	-1.221E+01
r2:	0.992
Adjusted Y:	
NPP:	
NPP Value:	

Errors and warnings

		General
MT	5600	Maintenance overdue or failed



Note:

- High change in mAbs noted at low levels AT

- Low change in mAbs noted at high levels AT

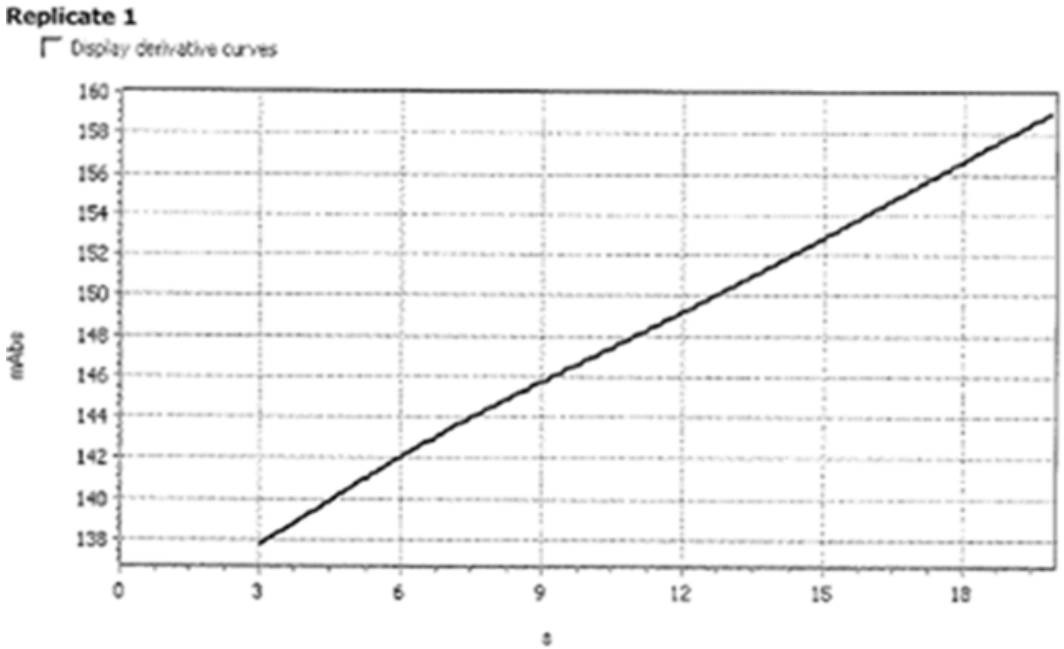
Concentration	Target	%CV	Mean Value	Replicates		
100.00 %	101	4.747	871.34	918.18	855.95	839.87
60.00 %	51	1.698	1501.89	1508.81	1523.22	1473.64
25.00 %	25	2.911	1792.90	1828.64	1817.05	1733.02

Case Study 1: Review of initial results

GENERAL COAGULATION		Specimen: Blood		
INR	1.4 H			
Prothrombin Time	17 H	THROMBOPHILIA SCREEN		
APTT	103 D	Antithrombin 3	*	U/mL
Fib (derived)	1.5 L			
Unfrac. Hep. Assay	0.44			

- UFH assay and APTT indicate presence of heparin
- Xa Stago assay is dependant on AT (from patient plasma)
  - Xa IL assay now in-use also requires AT level >0.40U/mL
  - UFH 0.44 theoretically not possible with AT 0.01U/mL

Caste Study 1: Review of initial results



- Change in mAbs = 22
- This indicates a very **high** AT3 level (Out of range high!)
  - Rate of change is inversely proportional to AT level

Case Study 1: Patient Cumulative History

- Previous AT = 0.27U/mL 7 hours prior
- On ECMO
  - Known process for AT consumption
- Considering therapeutic UFH level and extended APTT result with low mAbs result?
- Causes for AT result to be “out of range high” when low 7 hours prior?

Case Study 1: Transfusion History

- Patient transfused **Thrombotrol**
  - Thrombotrol (Concentrated AT blood product)
  - Issued prior to sample collection.
  - ?Was sample collected whilst Thrombotrol being administered
  - Large dose AT to such a small baby – possibly real result

Case Study 1: Reporting

- Result should have been reported as >1.50U/mL
  - Linear range for AT assay 0.10 – 1.50U/mL

- Given the therapeutic UFH, low mAbs result from analyser and transfusion history a “low” result was not possible or reasonable.

- Error code for AT Liq Test with high AT levels misleading.

Case Study 1: AT3 LCCH Test Code

- ‘AT LIQ’ test profile no longer used

- ‘AT3 LCCH’ test generated and now in use
  - Performs routine AT test (i.e. 1 in 40dilution)
  - If fail performs automatic ‘Alternate pre-dilution’
    - 1in80 dilution
  - If alternate pre-dilution fails new error code:
    - “(Range) Above measured result test range”

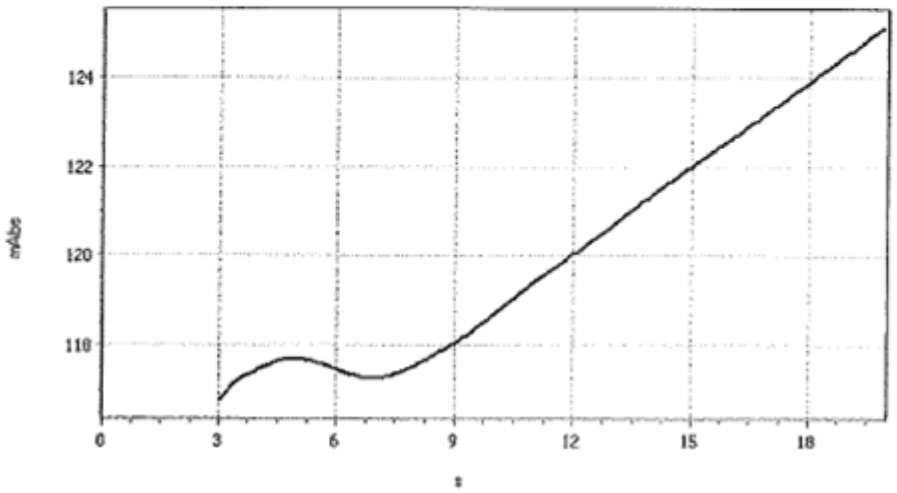
Case Study1: AT LIQ

Completed Date & Time:

		Replicate 1	Replicate 2	Mean
Measured	mAbs/min	FAILED		FAILED
	%	FAILED		FAILED
	U/mL	FAILED		FAILED

Replicate 1

Display derivative curves



Errors and warnings

		Replicate 1
		~~Measured (mAbs/min)~~
CE	5060	(Data) Normalized curve delta too low
		Unit 1 (%)
RE	5100	Measured result failed
		Unit 2 (U/mL)
RE	5100	Measured result failed

- Note: on initial run original error code

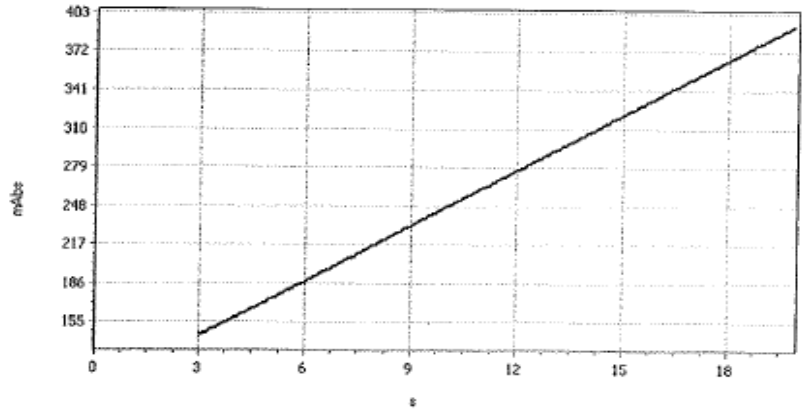
Case Study 1: AT3 LCCH

Completed Date & Time:

Measured		Replicate 1	Replicate 2	Mean
	mAbs/min	FAILED		FAILED
	%	FAILED		FAILED
	U/mL	FAILED		FAILED

Replicate 1

Display derivative curves



Errors and warnings

		Replicate 1
		Measured ~~(mAbs/min)~~
CE	5068	(Range) Above measured result test range
		Unit 1 (%)
RE	5100	Measured result failed
		Unit 2 (U/mL)
RE	5100	Measured result failed

- Note: Automatic reflex with alternate pre-dilution
  - New ‘clearer’ error code

Case Study 1: 'AT3 LCCH' on Patient 3 weeks later

Test Code:	AT3 LCCH	**Test Type:**	Patient	**Rack ID:**	59
Sample ID:		**Sample Type:**	Patient	**Position ID:**	5

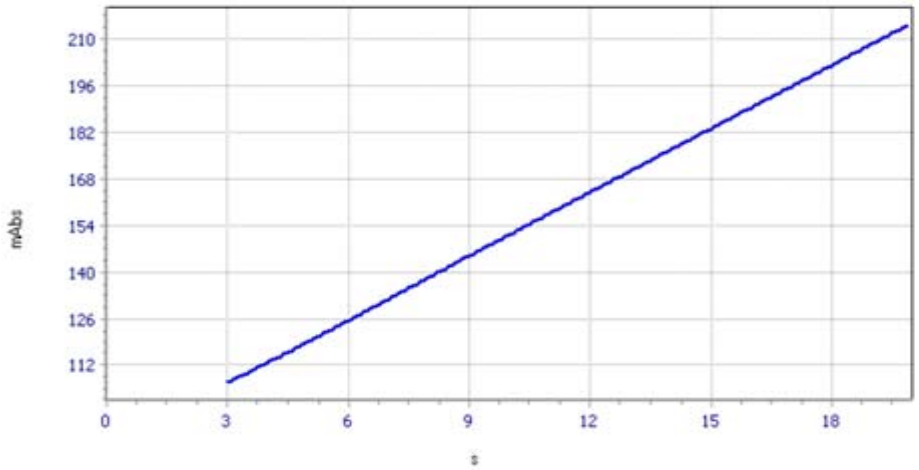
Completed Date & Time:

		Replicate 1	Replicate 2	Mean
Measured	**mAbs/min**	360.31		360.31
	%	144		144
	U/mL	1.44		1.44

Replicate 1

Display derivative curves

Errors and warnings



- Note: mAbs = 98; AT = 1.44U/mL

Case Study 2

- 6 week old Female
  - Post Cardiac Surgery
  - Placed on ECMO
  - Request for COAG, UFH-anti-Xa and AT“3”

Case Study 2: Patient Results

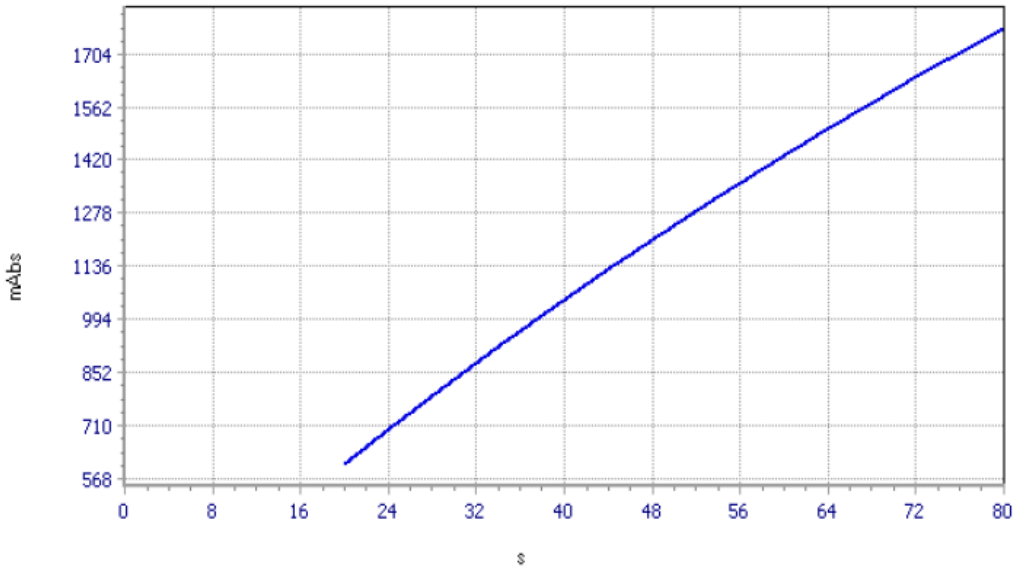
- **Results**
- APTT: >200s
- APTT-P: 48s
- PT: 33s
- Fib-C: <0.4g/L
- UFH: Failed
- AT3: Failed

Case Study 2: UFH Anti-Xa

		Replicate 1	Replicate 2	Mean
Measured	**mAbs/min**	1149.96		1149.96
	mAbs/min	1149.96		1149.96
	U/mL	FAILED		FAILED
	IU/mL	*0.00*		*0.00*

Replicate 1

Display derivative curves



- Markedly high change in mAbs

- Result: 0.00U/mL

- Anti-Xa UFH assay principal similar to AT assay. Heparin:AT neutralise Xa. Change in mAbs inversely proportional to AT result.

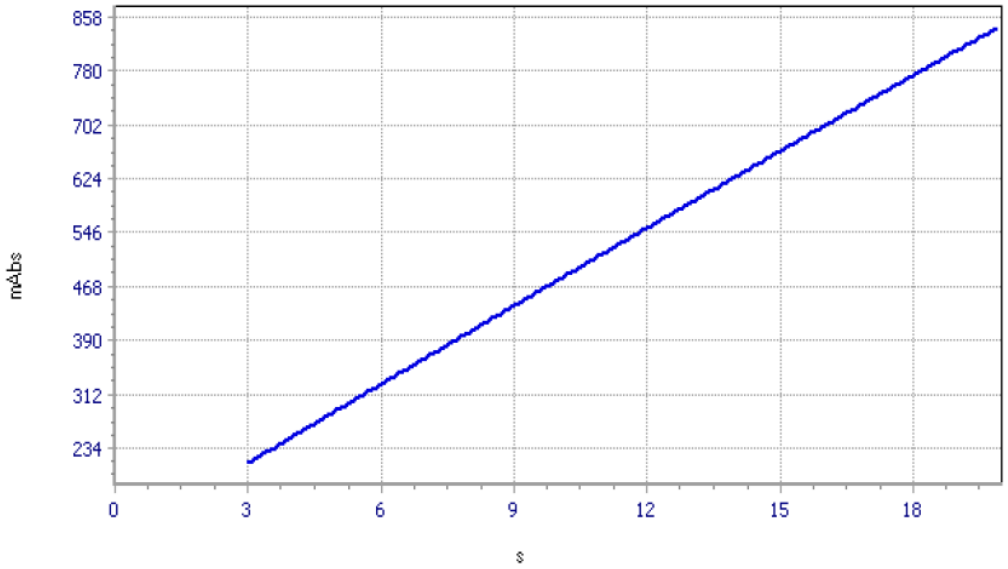
- Consistent with low result

Case Study 2: AT

		Replicate 1	Replicate 2	Mean
Measured	**mAbs/min**	2107.82		2107.82
	%	FAILED		FAILED
	U/mL	FAILED		FAILED

Replicate 1

Display derivative curves



- High change in mAbs
- Failed result
- Consistent with low result
- Report as: <0.01U/mL

Case Study 2: Summary

- APTT and APTT-P results indicate presence of heparin

  - APTT: >200s
  - APTT-P: 48s

- Abnormal Coag results cause relates to possible mixture of:
  - Consumption, dilution, synthetic & age related

- Knowing our assays:
  - **UFH Anti-Xa:**
    - Dependant on patient endogenous AT (IL reagent >0.4U/mL)
    - Patient AT <0.01U/mL
    - Cannot report UFH Anti-Xa result due to assay limitations

- Patient was immediately given 2 units of Cryoprecipitate to resolve fibrinogen deficiency and vial of Thrombotrol-VF for AT deficiency

- Repeat UFH Anti-Xa: 0.12U/mL and AT=1.04U/mL (heparin infusion reduced due to bleeding)

- Supplementation of AT has been associated with increased mortality

Case Study 3:

- 12 yr old male
- PICU patient post-op for routine cardiac surgery

GENERAL COAGULATION		Specimen: Blood Arce	
INR	>10.0 C	Platelets	52 D
Prothrombin Time	>100 D	Reptilase Time	17
APTT	>200 C	Fib (derived)	*
aPTT (Hep.neut)	>200 H	Fib (clottable)	0.9 C
		Unfrac. Hep. Assay	0.87 H
		Antithrombin 3	0.09 L U/mL

- Results indicate Gross Heparin
  - Normal Reptilase (not DIC)
- UFH result does not fit with gross heparin?!
- Doctors stated heparin given many hours ago and don't believe it is heparin

Case Study 3:

- Antithrombin result = 0.09U/mL = very low
- UFH Anti-Xa requires 0.40U/mL AT for true assessment of result
- Normal Pool AT run with AT of ~1.00U/mL (normal)
- A 50:50 NP with patient mix performed and AT run offline
  - o 50:50 mix AT = 0.6U/mL (above assay requirement)
- A 50:50 mix run for UFH Anti-Xa = >4.00U/mL
- Disccused with haematologist and treating doctor notified. Protamine given.
- ~120 blood and blood products over 2 days. Massive haemorrhage.

Hyperbilirubinaemia

- Chromogenic IL assays for AT and UFH Anti-Xa are read at 405nm
- This leads to potential interferences by Triglycerides, Plasma Hb and Bilirubin
- Routine clot based testing on ACL TOP analysers are read at 671nm (less spectral interferences at this range)
- Very ill children on ECLS with **Bilirubin** levels reported at LCCH of up to **1765 umol/L**. (This child was on 2 x ECLS circuits)

- Packet insert states no interference Bilirubin:
  - o up to 342umol/L (UFH Anti-Xa)
  - o up to 684 umol/L (AT)

Bilirubin Interference AT Assay:

When Bilirubin >1765umol/L* add comment: "Due to increased levels of Bilirubin the AT3 result may be questionable – please interpret with due caution." Continue to add comment on subsequent AT3 requests until Bilirubin level drops to appropriate level.

- * The interference of bilirubin was extended from 684 to 1765umol/L using the following data.

	Passing Bablok Regression	Passing Bablok 95% CI	Acceptable Y/N (or Comment)
Samples with bilirubin <684 µmol/L (n=15)	Y= 0.98x + 0.04	Slope: 0.8551 to 1.056 Intercept: -0.0394 to 0.1020	Yes
Samples with bilirubin >684 µmol/L (n=18)	Y= 1.00x - 0.05	Slope: 0.7857 to 1.167 Intercept: -0.2147-0.1632	See comment[1]

	Difference Plot Mean (U/mL)	95% CI for mean difference (U/mL)	Acceptable Y/N (or Comment)
Samples with bilirubin <684 µmol/L (n=15)	0.003	-0.0206 to 0.0273	Yes
Samples with bilirubin >684 µmol/L (n=18)	-0.051	-0.0790 to -0.0221	See comment[2]

1: The data shows that we are 95% confident that the slope of the Passing Bablok regression falls between 0.7857 to 1.167 and note the 95% CI for the intercept does not include zero.
2: The mean difference for samples with bilirubin <684umol/L is 0.003U/mL with 95% CI -0.0206 to 0.0273 while the samples with high bilirubin samples (>684umol/L) have a mean difference of -0.051U/mL with 95% CI of -0.0790 to -0.0221. Although the CI does not include zero the mean difference is not thought to be clinically significant.
Dominic Burns 19/09/2017 on behalf HDWP.

- Utilised 'AT3 LCCH' alternate pre-dilution test profile
- Assay utilises alternate pre-dilution of 1in100 (normal AT assay 1 in 40).
- Allowed for a reduction in bilirubin interference of 2.5x i.e. up to levels of 1710umol/L
- 34 samples tested
  - 16 with bilirubin normal range
  - 18 with bilirubin >684umol/L

Summary

- Have an understanding of:
  - Reagents used
  - Test profiles
  - What test results mean
  - Pre-analytical factors
- Tie together all results, transfusion history & clinical features
- Liaise with other laboratories, analyser experts and clinical staff

Quotes:

Internal auditor from another laboratory:

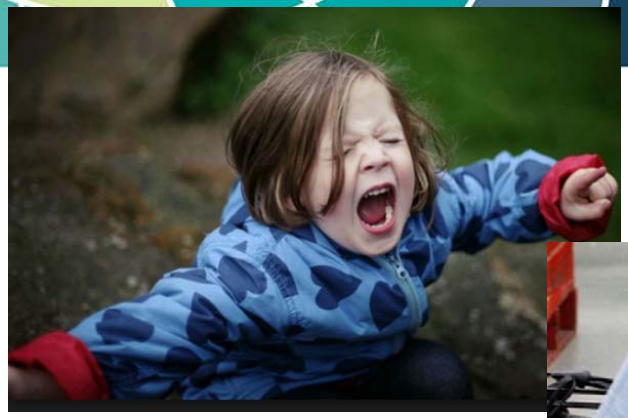
“They were short in phlebotomy outpatients so I had to assist with a hold. I was traumatised by the experience and don’t want to come back! Paediatric collectors have my complete respect – I could not do it”.

Phlebotomist:

“There was one little girl that used to smile all day until ward rounds. She would then have a meltdown and throw some of her breakfast she had hidden at me as I walked in”.

Phlebotomist:

“One boy needed both parents one on each leg, two additional phlebotomists one on each arm. Phlebotomists begins taking blood and child realises he can’t hit or kick so proceeds to headbutt the phlebotomist. Successful venepuncture + headache!



Kids Just Don't Want Their Blood Taken!