EQA FOR RARELY PERFORMED SPECIALISED HAEMOSTASIS ASSAYS – THE VIAPATH CHALLENGE

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INTRODUCTION

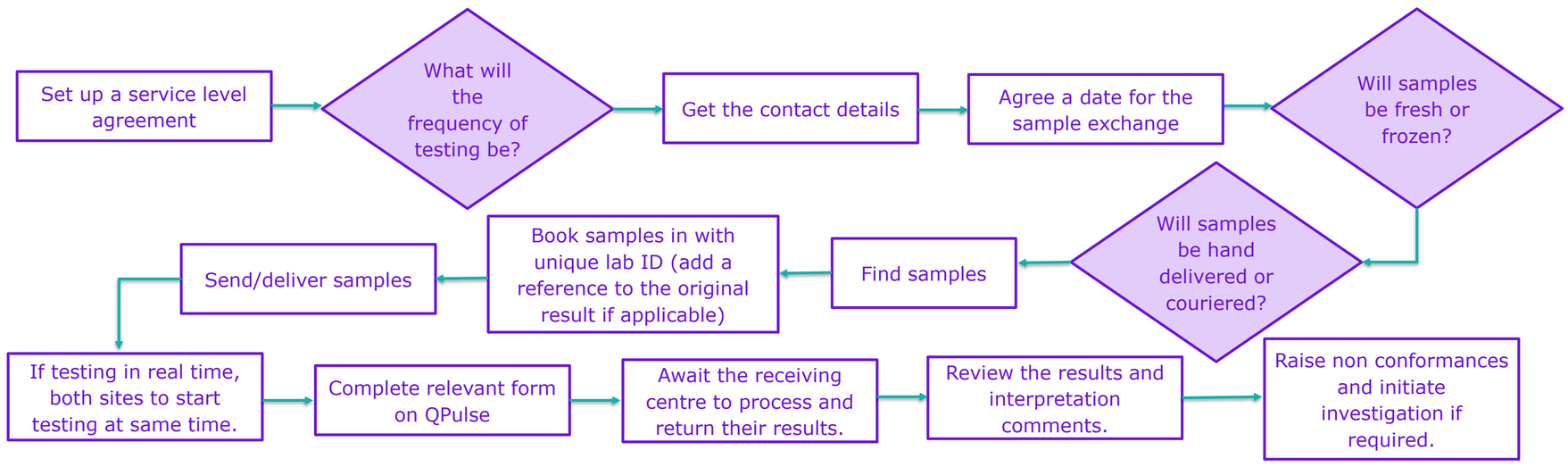
There are no External Quality Assurance (EQA) schemes for some of the rare, highly specialised assays performed in specialist centres linked to a Haemophilia Centres and / or trauma centres. In order to comply with the International ISO standard 15189:2012 for maintaining UKAS accreditation the Haemostasis laboratory at Viapath routinely coordinates a Laboratory Quality Assurance For Rare Specialist Assays (LQARSA) EQA scheme. The scheme involves the exchange of both fresh and previously tested samples , between Viapath and other specialist Centres across London and the South East.

THE CHALLENGES are many, but main considerations are; 1. Identifying suitable partner Centres, 2. Finding appropriate samples (abnormal and normal) , 3. The limitations and restrictions on sample transport for assays which require processing within a certain time after blood draw, (such as Platelet Aggregation LTA , chronolog and PFA analysis) , 4.Ideally comparing like with like for analysers, reagents and methodology and 5. Using a robust process of reporting to assess consensus agreement.

Table 1: LQARSA test & participant list

Test	Participant	Sent/rec'd Frequency	Sample type
Platelet agg	Basingstoke Royal free	2/year	Whole blood
Plt chronolog	Basingstoke Hammersmith Royal Free	2/year	
Platelet Glycoprotein	GOSH	2/year	
Fibrinogen Ag	Basingstoke Royal free	2/year	Frozen plasma aliquots
Reptilase time	Basingstoke	2/year	
AF8 antibodies (ELISA)	Basingstoke Royal free	2/year	
VWF:FVIII binding	Basingstoke Royal free	2/year	
Platelet Nucleotides	Royal free	2/year	Frozen platelet lysate
PK and HMWK	Royal Free	2/year	Frozen plasma aliquots
FVIII, FIX and FXI Ag	Royal Free	2/year	
Non FVIII/IX inhibitors Porcine & Bovine Bethesda	Royal Free	2/year	
Thrombin Antithrombin antibodies	Royal Free	2/year	
Prothrombin fragment F1 & F2	Royal Free	2/year	
TSVT/ET	HSL (TDL)	4/year	

Figure 1: LQARSA process flow chart



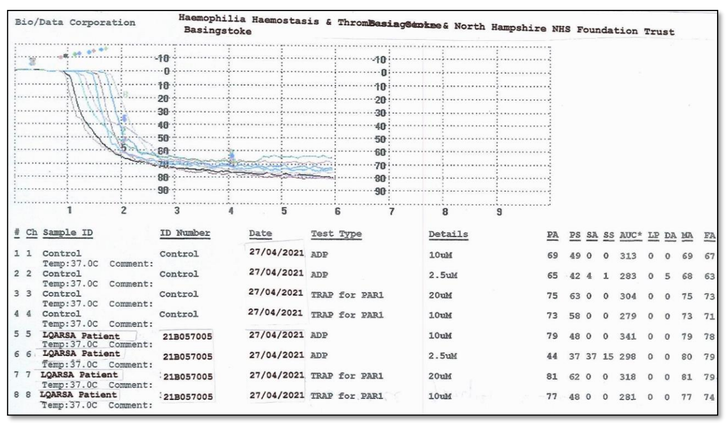
HOW REPORTS ARE GENERATED AND ASSESSED.

Reports are generated by issuing an interpretation comment, compared, and performance rated as Good, Acceptable (taking in to account the reference ranges and methodology), or poor. As there are only two results for comparison no application of statistical analysis is performed. Examples of LTA traces shown in fig 2 and 3, showed poor agreement and initiated an investigation lead under the guidance of the Principal BMS, see actions taken in figure 4. See example of disagreement in figure 5 and an example of good agreement in figure 6, with actions taken.

A disclaimer comment " Not clinically significant " may be appropriate even if >10% deviation of numerical results.

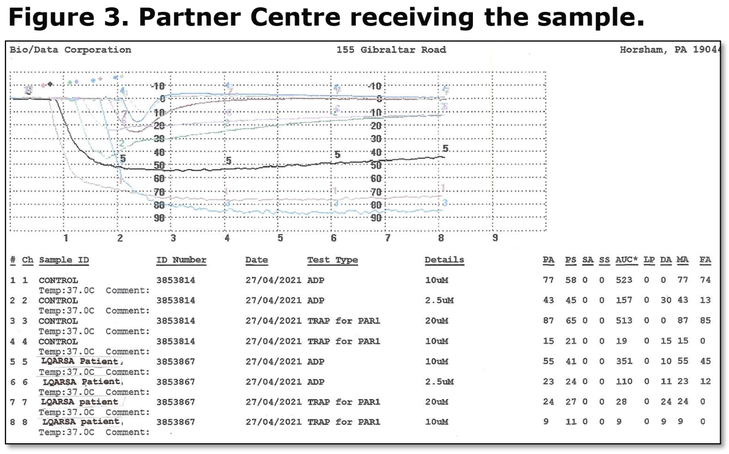
Examples of LTA traces from a sample exchange – survey 0221.

Figure 2. Partner centre sending the sample.



#	Ch	Sample ID	ID Number	Date	Test Type	Details	PA	PS	SA	SS	AUC*	LP	DA	MA	FA
1	1	Control Temp:37.0C Comment:	Control	27/04/2021	ADP	10uM	69	49	0	0	313	0	0	69	67
2	2	Control Temp:37.0C Comment:	Control	27/04/2021	ADP	2.5uM	65	42	4	1	283	0	5	68	63
3	3	Control Temp:37.0C Comment:	Control	27/04/2021	TRAP for PAR1	20uM	75	63	0	0	304	0	0	75	73
4	4	Control Temp:37.0C Comment:	Control	27/04/2021	TRAP for PAR1	10uM	73	58	0	0	279	0	0	73	71
5	5	LQARSA Patient Temp:37.0C Comment:	21B057005	27/04/2021	ADP	10uM	79	48	0	0	341	0	0	79	78
6	6	LQARSA Patient Temp:37.0C Comment:	21B057005	27/04/2021	ADP	2.5uM	44	37	37	15	298	0	0	80	79
7	7	LQARSA Patient Temp:37.0C Comment:	21B057005	27/04/2021	TRAP for PAR1	20uM	81	62	0	0	318	0	0	81	79
8	8	LQARSA Patient Temp:37.0C Comment:	21B057005	27/04/2021	TRAP for PAR1	10uM	77	48	0	0	281	0	0	77	74

Figure 3. Partner Centre receiving the sample.



#	Ch	Sample ID	ID Number	Date	Test Type	Details	PA	PS	SA	SS	AUC*	LP	DA	MA	FA
1	1	CONTROL Temp:37.0C Comment:	3853814	27/04/2021	ADP	10uM	77	58	0	0	523	0	0	77	74
2	2	CONTROL Temp:37.0C Comment:	3853814	27/04/2021	ADP	2.5uM	43	45	0	0	157	0	30	43	13
3	3	CONTROL Temp:37.0C Comment:	3853814	27/04/2021	TRAP for PAR1	20uM	87	65	0	0	513	0	0	87	85
4	4	CONTROL Temp:37.0C Comment:	3853814	27/04/2021	TRAP for PAR1	10uM	15	21	0	0	19	0	15	15	0
5	5	LQARSA Patient, Temp:37.0C Comment:	3853867	27/04/2021	ADP	10uM	55	41	0	0	351	0	10	55	45
6	6	LQARSA Patient, Temp:37.0C Comment:	3853867	27/04/2021	ADP	2.5uM	23	24	0	0	110	0	11	23	12
7	7	LQARSA patient Temp:37.0C Comment:	3853867	27/04/2021	TRAP for PAR1	20uM	24	27	0	0	28	0	24	24	0
8	8	LQARSA patient, Temp:37.0C Comment:	3853867	27/04/2021	TRAP for PAR1	10uM	9	11	0	0	9	0	9	9	0

Figure 5: Example with disagreement – LAQRSA for TSVT/ET analysis sample 23.10.20

Six samples were transported from HSL to Viapath Laboratory on 23.10.20 for TSVT/ET analysis. Samples were frozen and transported on dry ice by courier.

HSL/Viapath lab no.	TSVT(R) Viapath	ET (R) Viapath	TSVT(R) HSL	ET (R) HSL	Viapath interpretation	HSL interpretation
1.3745865	1.76	1.05	1.64	1.18	POS	POS
2.3745867	1.04		0.91		NEG	NEG
3.3745868	1.37	1.17(14% correction)	1.21	1.10	POS	Equivocal
4.3745871	1.22	1.16 (4.9% correction)	1.10		Insignificant correction. LA not detected	NEG
5.3745872	1.41	1.06	1.49	1.13	POS	POS
6.3745873	1.77	1.03	1.85	1.54	POS	POS

Action: Conclusion is a adequate agreement (taking into account method and interpretation). Difference in interpretation is due to different reference range. Viapath are " in house" ranges and HSL are clinician based ranges. Difference in ET ratios are noted, and the protocols will be streamlined on the CS analysers used at each site.

Figures 4: LAQRSA survey report 0221 - Detailing the differences observed for the exchange sample at the partner Centres and actions needed

Date: 27.04.21	Viapath lab no. 3853815			BNHH lab no. 21B057005			
	Delivered to Viapath by courier. Time of arrival at Viapath: 11:30am. Spun at 11:45am			Samples taken at Basingstoke at 09:00am, spun at 09:30 am			
	PRP count Viapath = 183			PRP count Basingstoke = 251			
Analyst Viapath: KMH				Analyst Basingstoke : MS			
ADP	slope	F aggregation		slope	F aggregation		comment
ADP 2.5uM	24	12	Reduced response	37	79	Normal	None
10.0uM	41	45	Reduced response	48	78	Normal	None
TRAP 20 uM	14	0	Reduced response	62	79	Normal	None
Interpretation	Platelets noted to be varied in size with a large MPV. Patient platelets showed reduced response to response to both concentrations of ADP and TRAP, epinephrine and low dose collagen tested. The results are suggestive of a global platelet defect, potentially of the weak agonist pathway.			Results normal with all agonists tested.			Variation likely to reflect transport of sample, and time difference in preparation time most effect on ADP and TRAP. For future exchanges the samples will be delivered by hand and processing will be started in parallel at both sites.
Overall Interpretation		**Abnormal**		**Normal**			
Score	POOR – Out of consensus agonists + interpretation						

Action: The decision was made to deliver exchange samples by hand to minimum activation of platelets and also to start processing samples in parallel as soon as samples have reached their destination**.** Sample exchange to be repeated within 6 months.

Figure 6: Example with good agreement – LAQRSA for Anti FVIII antibodies (ELISA) sample 27.04.21

Site	BNHH			Viapath		
Performed by	NH			WM		
	Result	**Lab no.**	**Interpretation**	**Result**	**Lab No.**	**Interpretation**
	2.52	21B057007	POSITIVE	2.265	3853853	POSITIVE
Reference Range g/L (Negative cut off)	**OD <0.80**			**OD <0.775**		
Survey 0121 Score	**GOOD**					

Action: No action needed as results and interpretation in good agreement

CONCLUSION AND FUTURE IMPROVEMENTS

Communication is key between the Partner Centres , EQA lead and principal BMSs to resolve any poor performances. In 2020 the EQA lead generated a questionnaire requesting the Partner Centres to share experiences on how to resolve and close non agreements. Several useful feedback comments included 1. Developing a streamlined process for recording error logs and corrective actions,2. using the "Anytest" NEQAS EQA samples to perform the specialised tests, so that a statistical analysis is generated and 3. Exchange complicated and interesting samples which require multiple assays to generate an interpretation authorisation comment which can be compared. The LQARSA EQA scheme is still evolving and all improvements will aid future planning and challenges experienced.

Acknowledgements
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References

United Kingdom Accreditation service (UKAS) website www.ukas.com