Interpretive Educational Scheme (iED)
Clinical Scenario 3/2024 – Transfusion/Platelet Immunology Case

Dispatched on 29th October 2024

Summary of Results

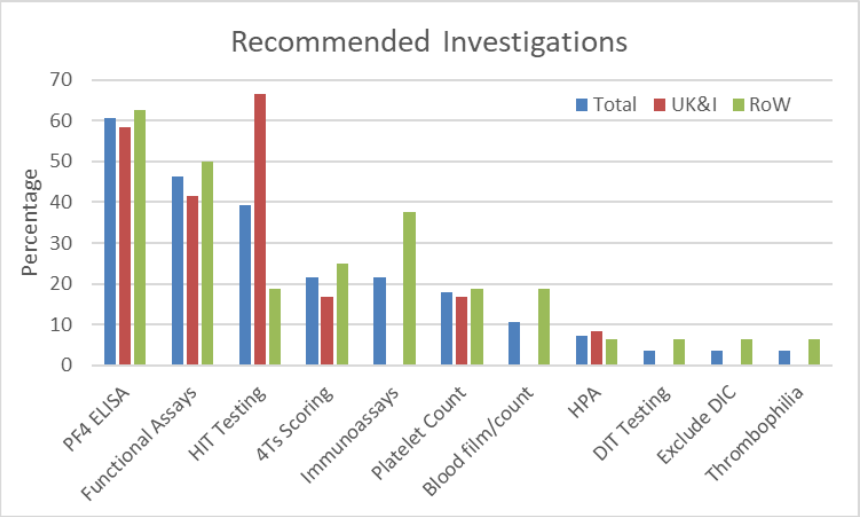
There were 28 responses received. 12 from laboratories based in the UK and Ireland (UK&I) and 16 from laboratories based in the rest of the World (RoW).

A 54-year-old male is referred to your laboratory on the 22nd August 2024. The patient's platelet count had fallen from 149 x 10^9/L on the 9th August 2024 to 98 x 10^9/L on the 22nd August 2024.

The patient has early onset peripheral vascular disease which was diagnosed at 31 years old. The patient had an angioplasty at 34 years of age and started on regular aspirin. At 45 years old the patient had Aortobifemoral bypass surgery and started on Rivaroxaban. This treatment was changed to Warfarin after a pulmonary embolism. On the 9th August 2024 the patient had an ilio-popliteal bypass and was prescribed Dalteparin, Teicoplanin and Co-amoxiclav.

1. Given the patient's thrombosis and thrombocytopenia Heparin Induced Thrombocytopenia (HIT) is being considered. What investigations would you perform?

Recommended Investigations	Total (n=28)		UK&I (n=12)		RoW (n=16)	
	Count	%	Count	%	Count	%
PF4 ELISA	17	**61**	7	**58**	10	**63**
Functional Assays	13	**46**	5	**42**	8	**50**
HIT Testing	11	**39**	8	**67**	3	**19**
4Ts Scoring	6	**21**	2	**17**	4	**25**
Immunoassays	6	**21**	0	**0**	6	**38**
Monitor Platelet Count	5	**18**	2	**17**	3	**19**
Blood film / count	3	**11**	0	**0**	3	**19**
HPA Antibody/Genotyping	2	**7**	1	**8**	1	**6**
DIT Testing	1	**4**	0	**0**	1	**6**
Exclude DIC	1	**4**	0	**0**	1	**6**
Test for Thrombophilia	1	**4**	0	**0**	1	**6**



The patient tested negative for HIT. After HIT was ruled out the patient was referred for autoimmune thrombocytopenia investigation.

The patient's serum was screened for platelet reactive antibodies by indirect Platelet Immunofluorescence Test (PIFT), see Table 1.

Table 1: Results from the Indirect PIFT (Patient Serum 22/08/24)

	Platelet Donor ID	PD1	PD2	PD3
	Platelet Donor HPA Type	1a1a 2b2b 3a3a 5a5a	1a1a 2a2a 3b3b 5b5b	1b1b 2a2a 3a3a 5a5a
Assay	PIFT IgG	1046	1053	1083
	PIFT IgM	729	908	1103
Cut-off*	PIFT IgG Mean + 4SD	5021	4873	4850
	PIFT IgM Mean + 4SD	1110	1269	1679

**The Indirect PIFT threshold for a positive result is > Mean + 4 Standard Deviations (SD) of the negative sera.*

The patient's platelets were isolated and a Direct PIFT was carried out to ascertain if immunoglobulins were bound to the patient's platelet glycoproteins, see Table 2.

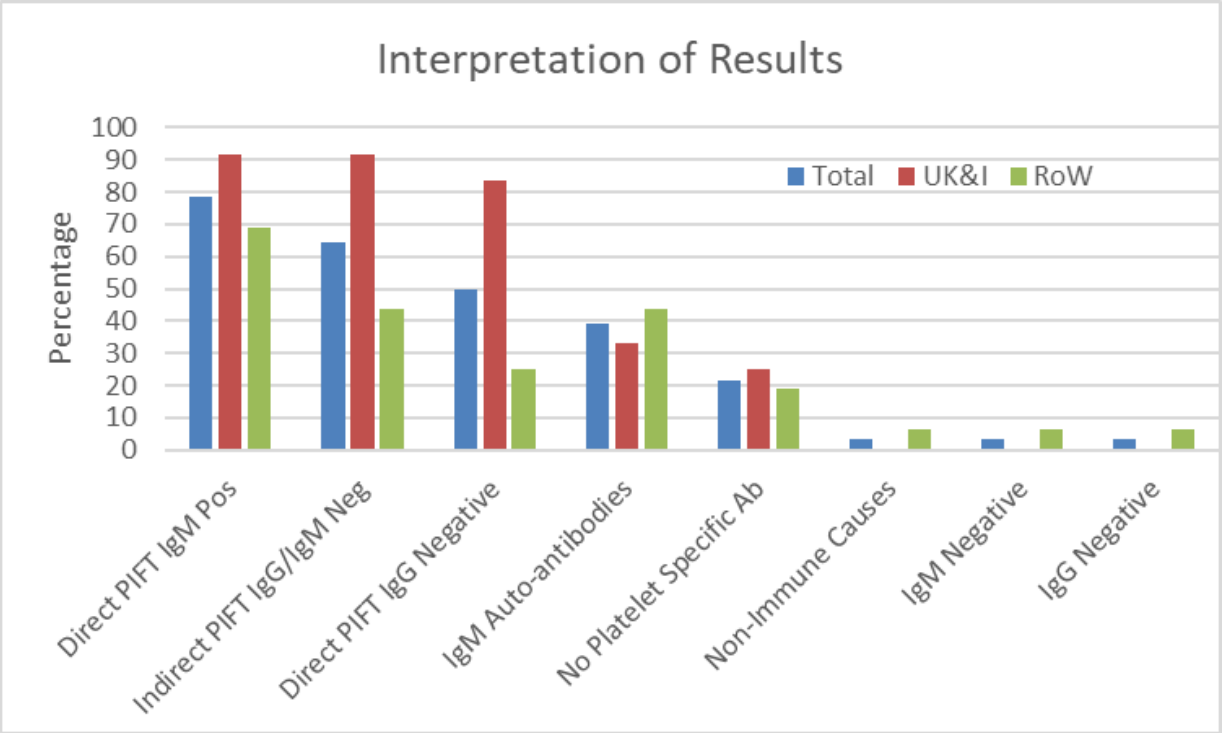
Table 2: Results from the Direct PIFT (Patient Serum 22/08/24)

Assay	PIFT IgG	1644
	PIFT IgM	9859
Cut-off*	PIFT IgG Mean + 4SD	3753
	PIFT IgM Mean + 4SD	2276

**The Direct PIFT cut-off value is derived from platelets isolated from 6 ABO O donors, a positive result is > Mean + 4SD.*

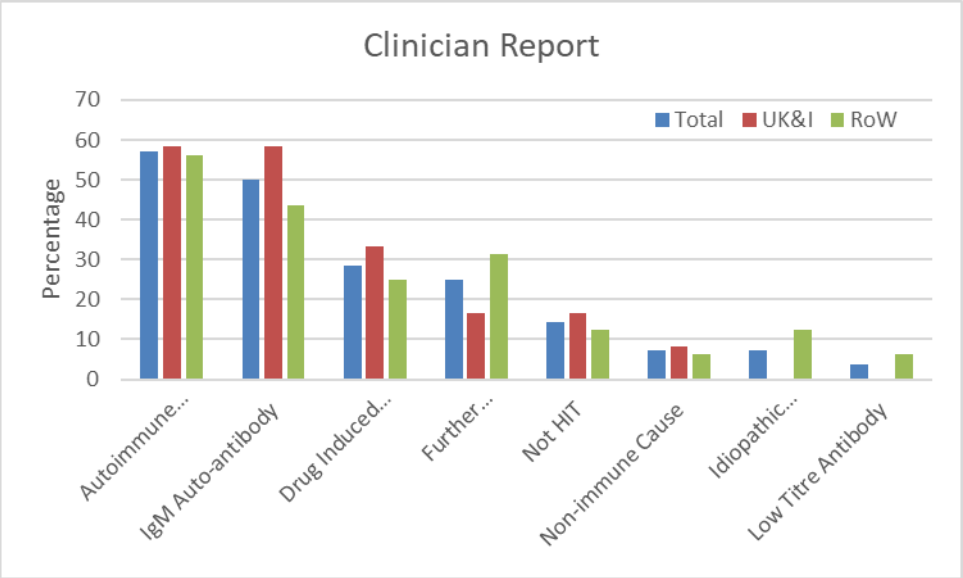
2. How would you interpret these results?

Interpretation of Results	Total (n=28)		UK&I (n=12)		RoW (n=16)	
	Count	%	Count	%	Count	%
Direct PIFT IgM Positive	22	**79**	11	**92**	11	**69**
Indirect PIFT IgG and IgM Negative	18	**64**	11	**92**	7	**44**
Direct PIFT IgG Negative	14	**50**	10	**83**	4	**25**
IgM Auto-antibodies	11	**39**	4	**33**	7	**44**
No Platelet Specific Antibodies	6	**21**	3	**25**	3	**19**
Exclude Non-Immune Causes	1	**4**	0	**0**	1	**6**
IgM Negative	1	**4**	0	**0**	1	**6**
IgG Negative	1	**4**	0	**0**	1	**6**



3. What would you report to the referring clinician?

Clinician Report	Total (n=28)		UK&I (n=12)		RoW (n=16)	
	Count	%	Count	%	Count	%
Autoimmune thrombocytopenia	16	**57**	7	**58**	9	**56**
IgM Auto-antibody	14	**50**	7	**58**	7	**44**
Possible Drug Induced Thrombocytopenia	8	**29**	4	**33**	4	**25**
Further Investigations Required	7	**25**	2	**17**	5	**31**
HIT Diagnosis Not Supported	4	**14**	2	**17**	2	**13**
Possible Non-immune Cause	2	**7**	1	**8**	1	**6**
Acute Idiopathic Thrombocytopenia	2	**7**	0	**0**	2	**13**
Low Titre Antibody	1	**4**	0	**0**	1	**6**



The referring clinician also wanted Drug Induced Thrombocytopenia (DIT) to be investigated as the patient's platelet count had fallen from 98 x 10^9/L on the 22nd August 2024 to 20 x 10^9/L at testing on the 28th August 2024.

Investigation for DIT involves the addition of drug at a suggested concentration into an indirect PIFT. The assay is run with and without the drug of interest.

Dalteparin is a low molecular weight heparin, as HIT had been ruled out this was not investigated.

Co-amoxiclav is an antibiotic costing of amoxicillin and clavulanic acid at various concentrations. The patient's serum was screened for platelet reactive antibodies by Indirect PIFT in the presence of Co-amoxiclav, see Table 3.

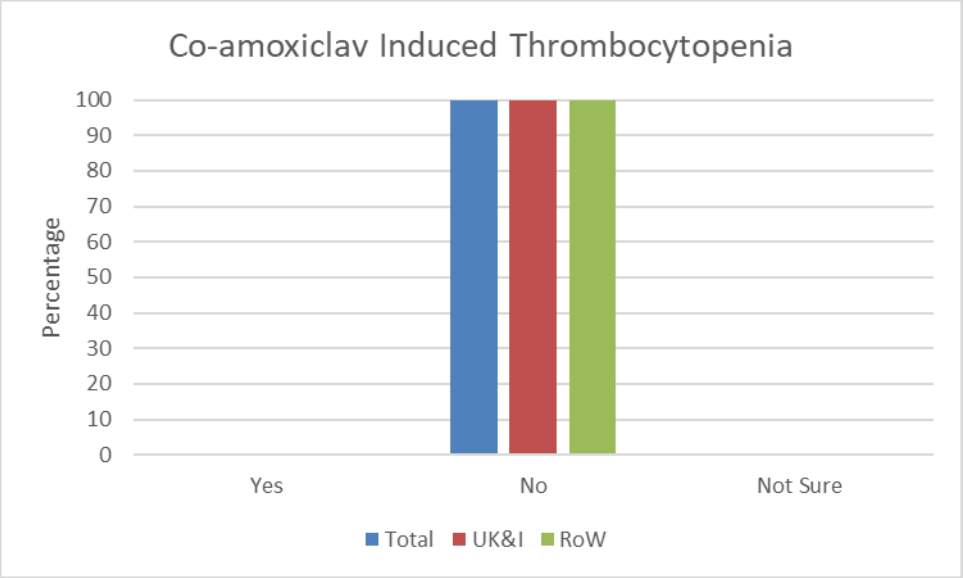
Table 3: Results from the Indirect PIFT in the Presence of Co-Amoxiclav (Patient Serum 22/08/24)

	Platelet Donor ID and Drug of Interest	Control EDTA/BSA		Co-Amoxiclav 10mg/mL amoxicillin & 3mg/mL clavulanic acid		Co-Amoxiclav 2.5mg/mL amoxicillin & 0.75mg/mL clavulanic acid	
		PD2	PD3	PD2	PD3	PD2	PD3
	Platelet Donor HPA Type	1a1a 2a2a 3b3b 5b5b	1b1b 2a2a 3a3a 5a5a	1a1a 2a2a 3b3b 5b5b	1b1b 2a2a 3a3a 5a5a	1a1a 2a2a 3b3b 5b5b	1b1b 2a2a 3a3a 5a5a
Assay	PIFT IgG	5394	5025	4615	4525	2429	2789
	PIFT IgM	2729	2275	2854	2359	3113	2485
Cut-off*	PIFT IgG Mean + 4SD	5457	5224	6504	4990	2474	5694
	PIFT IgM Mean + 4SD	3208	2822	3031	2509	3380	2614

**The Indirect PIFT threshold for a positive result is > Mean + 4SD of the negative sera.*

4. Do these results suggest that Co-amoxiclav is implicated?

Response	Total (n=28)		UK&I (n=12)		RoW (n=16)	
	Count	%	Count	%	Count	%
Yes	0	**0**	0	**0**	0	**0**
No	28	**100**	12	**100**	16	**100**
Not Sure	0	**0**	0	**0**	0	**0**



Teicoplanin is also an antibiotic. The patient's serum was screened for platelet reactive antibodies by Indirect PIFT in the presence of Teicoplanin, see Table 4.

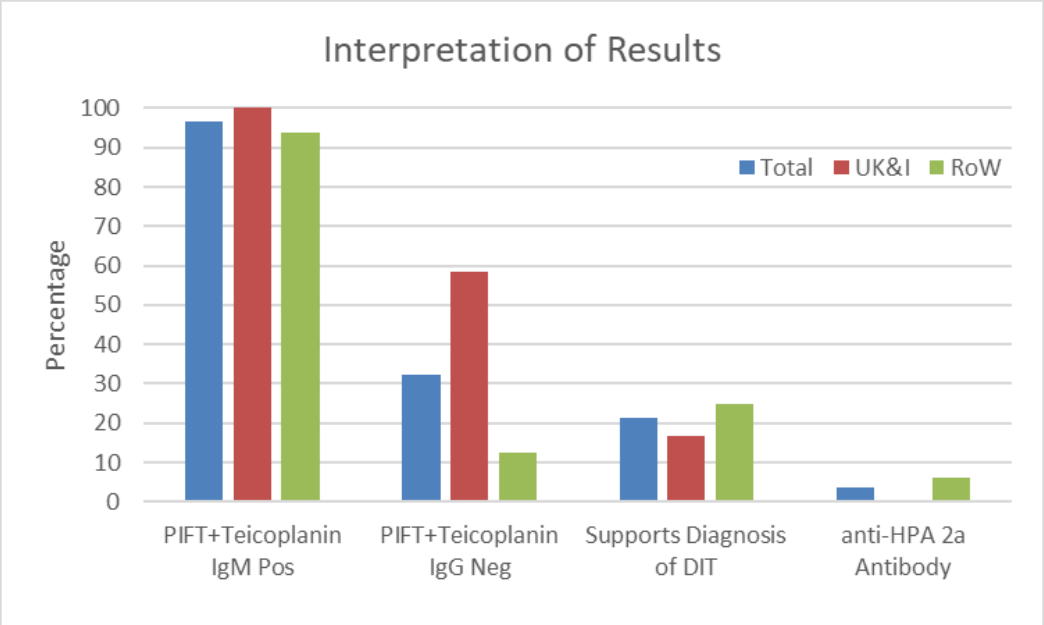
Table 4: Results from the Indirect PIFT in the Presence of Teicoplanin (Patient Serum 22/08/24)

	Platelet Donor ID	EDTA/BSA control		Teicoplanin 1mg/mL	
		PD2	PD3	PD2	PD3
	Platelet Donor HPA Type	1a1a 2a2a 3b3b 5b5b	1b1b 2a2a 3a3a 5a5a	1a1a 2a2a 3b3b 5b5b	1b1b 2a2a 3a3a 5a5a
Assay	**PIFT IgG**	7612	6881	4206	2963
	PIFT IgM	2594	2483	20164	14177
Cut-off*	**PIFT IgG Mean + 4SD**	8375	8539	4330	3856
	PIFT IgM Mean + 4SD	3372	2953	3609	3187

**The Indirect PIFT threshold for a positive result is > Mean + 4SD of the negative sera.*

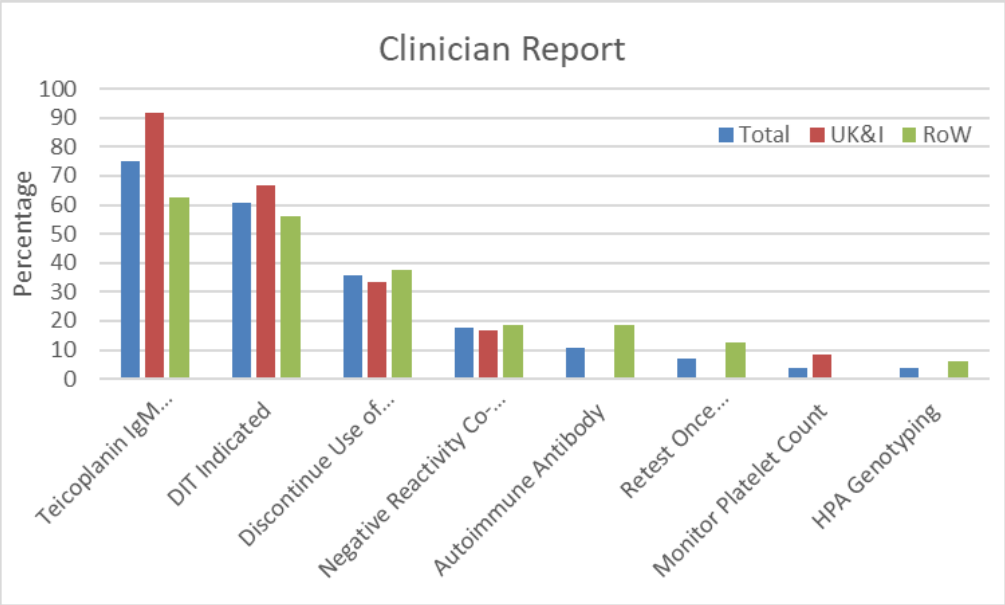
5. a) How would you interpret the results in Table 4?

Interpretation of Results	Total (n=28)		UK&I (n=12)		RoW (n=16)	
	Count	%	Count	%	Count	%
PIFT+Teicoplanin IgM Pos	27	**96**	12	**100**	15	**94**
PIFT+Teicoplanin IgG Neg	9	**32**	7	**58**	2	**13**
Supports Diagnosis of DIT	6	**21**	2	**17**	4	**25**
anti-HPA 2a Antibody	1	**4**	0	**0**	1	**6**



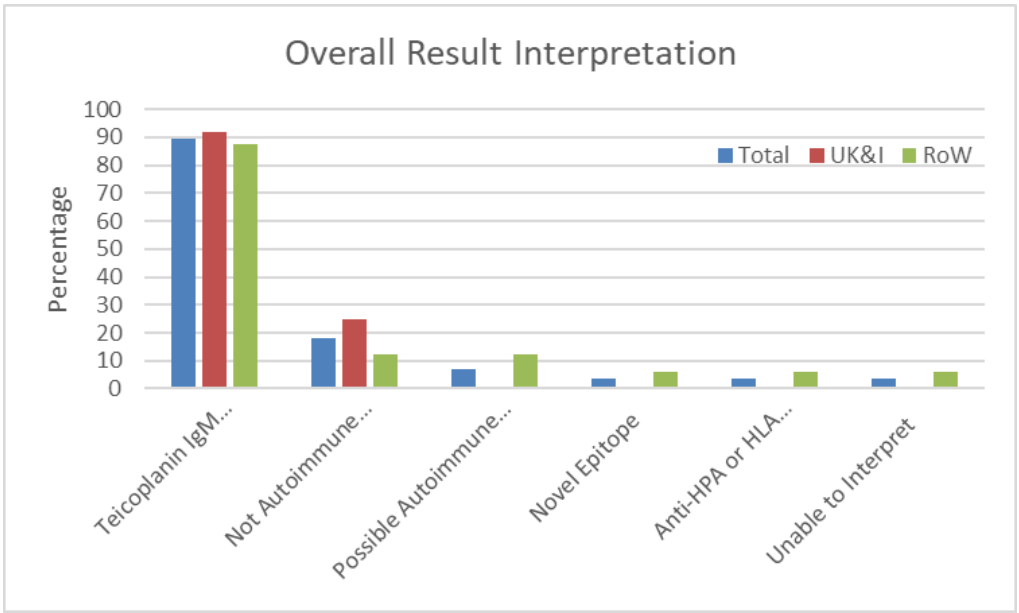
b) What would you report to the referring clinician in respect of all results?

Clinician Report	Total (n=28)		UK&I (n=12)		RoW (n=16)	
	Count	%	Count	%	Count	%
Teicoplanin-dependent IgM Antibody	21	**75**	11	**92**	10	**63**
DIT Indicated	17	**61**	8	**67**	9	**56**
Discontinue Use of Teicoplanin	10	**36**	4	**33**	6	**38**
Negative Reactivity in Presence of Co-Amoxiclav	5	**18**	2	**17**	3	**19**
Autoimmune Antibody	3	**11**	0	**0**	3	**19**
Retest Once Teicoplanin Ceased	2	**7**	0	**0**	2	**13**
Monitor Platelet Count	1	**4**	1	**8**	0	**0**
HPA Genotyping	1	**4**	0	**0**	1	**6**



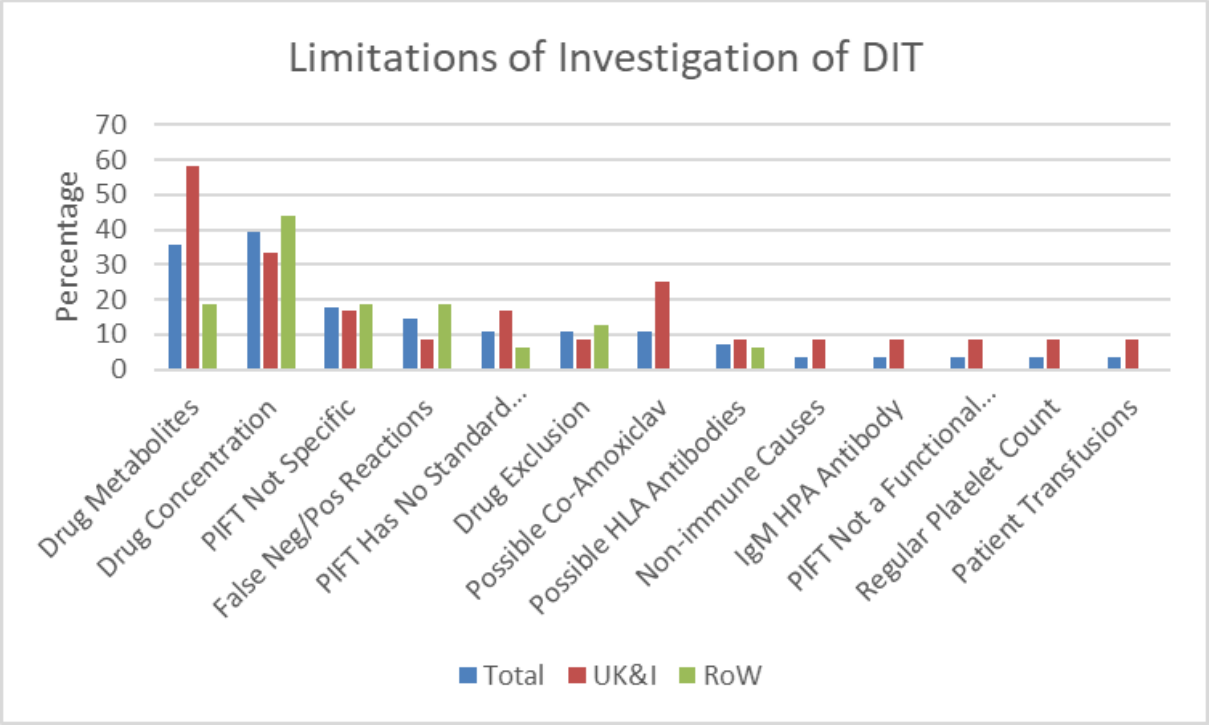
6. How would you interpret the results from the Direct PIFT assay (see Table 2) in light of the results of the DIT testing?

Result Interpretation	Total (n=28)		UK&I (n=12)		RoW (n=16)	
	Count	%	Count	%	Count	%
Direct PIFT IgM Positivity Due to Teicoplanin-dependent IgM Antibodies	25	**89**	11	**92**	14	**88**
Autoimmune Thrombocytopenia Ruled Out	5	**18**	3	**25**	2	**13**
Possible Autoimmune Reactivity	2	**7**	0	**0**	2	**13**
Novel Epitope	1	**4**	0	**0**	1	**6**
Anti-HPA or HLA Antibodies	1	**4**	0	**0**	1	**6**
Unable to Interpret	1	**4**	0	**0**	1	**6**



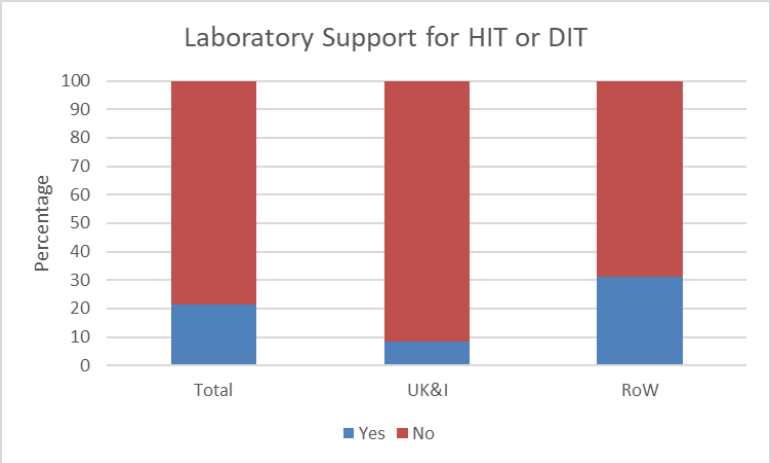
7. Are there any limitations that you would highlight to the clinical team on the investigation of DIT?

Limitations	Total (n=28)		UK&I (n=12)		RoW (n=16)	
	Count	%	Count	%	Count	%
Cannot Detect Drug Metabolites/Interactions	10	**36**	7	**58**	3	**19**
Unknown Drug Concentration In Vivo	11	**39**	4	**33**	7	**44**
PIFT Not Specific	5	**18**	2	**17**	3	**19**
Possible False Neg / Pos Reactions	4	**14**	1	**8**	3	**19**
PIFT Has No Standard Controls	3	**11**	2	**17**	1	**6**
Diagnosis of DIT Based on Clinical Symptoms and Drug Exclusion	3	**11**	1	**8**	2	**13**
Cannot Rule Out Co-Amoxiclav	3	**11**	3	**25**	0	**0**
Cannot Rule Out HLA Antibodies	2	**7**	1	**8**	1	**6**
Non-immune Causes Not Excluded	1	**4**	1	**8**	0	**0**
Possible IgM HPA Antibody	1	**4**	1	**8**	0	**0**
PIFT Not a Functional Assay	1	**4**	1	**8**	0	**0**
Need Regular Platelet Count	1	**4**	1	**8**	0	**0**
Knowledge of Patient Transfusions	1	**4**	1	**8**	0	**0**



8. Does your laboratory provide testing to support testing for HIT or DIT?

Response	Total (n=28)		UK&I (n=12)		RoW (n=16)	
	Count	%	Count	%	Count	%
Yes	6	**21**	1	**8**	5	**31**
No	22	**79**	11	**92**	11	**69**



9. Do you have any further comments on the case?

- Clinical management is complex in these scenarios.
- It was educational, although outside our scope of practice.
- Patient HPA type not known.
- Interesting teaching question focussing on the differential diagnosis thrombocytopenia and transfusion. The patient could require platelets to counteract his anticoagulant medication.
- The concentration used for Teicoplanin is approx. 100x higher than expected from regular usage, which might affect test results. Also, without proper screening on induced drug-metabolites, sufficient clinical advice on DIT is limited.
- If Thrombocytopenia persists after stopping the drug and is cleared from the patient body, it is recommended to perform another autologous test to determine if the direct IgM is drug-induced or autoimmune thrombocytopenia.
- Concentration of drug in drug induced thrombocytopenia testing should be data from treatment or reference table.
- Do we need to screen for autoimmune disease in this patient?
- Monitor Clinical Response After Drug Discontinuation. Consider Repeat Testing if Clinical Suspicion Remains High. Interpret Results Within the Clinical Context.
- It is the first time ever that I am involved in interpretation of this kind of case.

Comments and suggested responses from the UK H&I experts providing this scenario*

Question 1

The patient had a heparin induced thrombocytopenia (HIT) diagnostic 4T score of 5 (intermediate). The 4T score considers thrombocytopenia, timing of platelet fall, thrombosis and other potential causes for thrombocytopenia. As such, testing for HIT would be appropriate.

Question 2

The indirect PIFT for IgG and IgM antibodies was negative as was the direct PIFT for IgG antibodies. However, the direct PIFT for IgM antibodies was positive.

Question 3

The results indicate a raised level of IgM antibodies bound to the patient's platelets.
These results support a diagnosis of autoimmune thrombocytopenia (AITP). Serum platelet autoantibodies are only found in approximately 20-40% of AITP patients. Determination of platelet associated immunoglobulin (PAIg) is a more reliable indicator of platelet autoimmunity.

Question 4

No, the results do not support DIT in the presence of Co-amoxiclav. The addition of the drug into the assay does not indicate that an antibody is binding to the donor platelets.

Question 5

The indirect PIFT in the presence of Teicoplanin was negative IgG antibodies but positive for IgM antibodies.

The results suggest that Teicoplanin is implicated in the thrombocytopenia. There is a positive IgM reaction when Teicoplanin is added to the assay. This supports a diagnosis of Teicoplanin induced thrombocytopenia.

Question 6

The positive IgM result in the direct PIFT assay (Table 2) may have been caused by the assay detecting a IgM immune complex of Teicoplanin bound to the patient's platelets when the sample was taken.

Question 7

The limitations of testing for DIT include establishing the correct drug concentration to use in the assay that mimics *in vivo* concentrations. The DIT may be due to metabolite of the drug or interactions with other blood components which cannot be tested for in the assay. There may also be interactions between multiple drugs adding further complexity to testing.

Please note:
These comments have been compiled by subject matter experts from the UK NEQAS for H&I Steering Committee in accordance with current guidelines. We accept that guidelines are not always explicit for every situation and therefore the responses may be aligned with the clinical practices of an individual transplant centre and may not be directly applicable across all settings. UK NEQAS are not necessarily endorsing these responses as the only correct action, just one possible view which, we acknowledge, may be biased towards UK practice.