UK NEQAS
International Quality Expertise

Histocompatibility & Immunogenetics

UK NEQAS for H&I Schemes to Support Platelet Investigations – An Analysis of Errors in HPA Genotyping and HPA Antibody Detection / Specification

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Introduction

UK NEQAS for Histocompatibility and Immunogenetics (H&I) offers 19 H&I-specific external quality assessment schemes to over 350 participants in more than 50 countries worldwide.

Human Platelet Antigen's (HPA) are polymorphic and can stimulate the production of HPA specific antibodies. HPA antibody detection and specification, along with HPA genotyping Is important for several clinical situations, including neonatal alloimmune thrombocytopenia (NAIT), post-transfusion purpura (PTP) and cases of platelet transfusion refractoriness.

UK NEQAS for H&I has two Schemes that support laboratories providing testing to support platelet investigations, Scheme 10 for HPA genotyping and Scheme 10 for the detection and specification of HPA antibodies.

Scheme 10 - HPA Genotyping

The purpose of this Scheme is to assess Participants' ability to correctly determine HPA polymorphisms.

- 10 samples are sent per year as 2 batches of 5 samples.
- Participants can register for assessment for any combination of: HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, HPA-6, HPA-15. They can report other HPA polymorphisms but these will not be assessed.
- Assessment of results is achieving consensus with >75% of laboratories agreeing the presence/absence of each allele.
- Participants that report a full HPA genotype in agreement with the consensus are assessed as acceptable.
- Those out of consensus or not submitting a result without a valid reason are assessed as unacceptable.
- Participants must achieve ≥9 acceptable HPA genotypes in order to achieve satisfactory performance each year.

Table 1 shows the number of Participants and levels of unsatisfactory performance (UP) in Scheme 10 since its introduction as a pilot in 2016.

Table 1: Summary of Scheme 10 Participant Numbers and UP

Scheme 10	2016	2017	2018	2019	2020
Number of Participants	12	15	37	38	40
Number with UP (%)	N/A	1 (6.7%)	1 (2.7%)	3 (7.9%)	0 (0%)

Table 2 shows a summary of false negative and false positive errors in HPA genotypes submitted since 2017. The most errors were found at HPA-15b (n=6, error rate 0.49%), -3b (n=5, error rate 0.41%), -15a (n=4, error rate 0.33%), -1b (n=4, error rate 0.32%), -3a (n=3, error rate 0.24%) and -5b (n=3, error rate 0.24%). Interestingly there was an even split of false positive (n=15) and false negative (n=14) errors. There was no correlation in errors made and the method of detection noted.

Table 2: Summary of Scheme 10 Errors 2017-2020

Errors 2017-20	HPA-1 a	HPA-1 b	HPA-2 a	HPA-2 b	HPA-3 a	HPA-3 b	HPA-4 a	HPA-4 b	HPA-5 a	HPA-5 b	HPA-6 a	HPA-6 b	HPA-15 a	HPA-15 b	Total
False Neg	2	3	1	0	2	4	0	0	0	0	0	0	1	2	15
False Pos	0	1	0	1	1	1	0	1	0	3	0	0	3	4	15
Total	2 (0.16%)	4 (0.32%)	1 (0.08%)	1 (0.08%)	3 (0.24%)	5 (0.41%)	0	1 (0.09%)	0	3 (0.24%)	0	0	4 (0.33%)	6 (0.49%)	30 (0.19%)
Total Tested	1258	1258	1228	1228	1228	1228	1048	1048	1248	1248	890	890	1228	1228	16256

9 labs made at least one error in an HPA genotype. Of these 9 labs, 2 labs submitted 4 wrong genotypes, 4 labs submitted 2 wrong genotypes and 3 labs submitted 1 wrong genotype.

The overall error rate in HPA genotyping is low (0.19%) but concerning, especially that many of the errors are at clinically relevant HPA-1, -3 and -15 polymorphisms.

Scheme 11 – HPA Antibody Detection/Specification

The purpose of this Scheme is to assess Participants' ability to correctly detect the presence of HPA antibodies and determine the specificity of the antibody present.

- 8 samples are sent per year as 2 batches of 4 samples.
- Participants must report the presence/absence of HPA antibodies and designate the antibody specificity. HLA antibody presence or absence may also be reported.
- Consensus is determined by >75% of laboratories agreeing the presence of the specificity or >95% of laboratories agreeing the absence of a specificity.
- Participants are assessed as acceptable if they assign a consensus specificity.
- An unacceptable result is assigned if a participant missed a consensus specificity or assigned a specificity where the consensus is negative or did not submit a result for assessment without a valid reason.
- Satisfactory performance is getting >75% of specificities in agreement with consensus in a year.

Table 3 shows the number of Participants and levels of UP in Scheme 11 since its introduction as a pilot scheme in 2017. There was a notable increase in the number of labs with UP in 2020 (7.1%).

Table 3: Summary of Scheme 11 Participant Numbers and UP

Scheme 11	2017	2018	2019	2020
Number of Participants	13	35	39	42
Number with UP (%)	N/A	1 (2.9%)	1 (2.6%)	3 (7.1%)

Table 4 shows a summary of false negative and false positive errors in HPA antibody specification since 2018. Most errors where found at HPA-5b (n=10, error rate 0.88%), -1b (n=9, error rate 0.79%), -3b (n=8, error rate 0.70%), -1a (n=6, error rate 0.53%) and -15b (n=5, error rate 0.44%), Overall there was a slightly greater tendency to report false negatives (n=26) than false positives (n=25).

Table 4: Summary of Scheme 11 Errors 2018-2020

Errors 2018-20	HPA-1 a	HPA-1 b	HPA-2 a	HPA-2 b	HPA-3 a	HPA-3 b	HPA-4 a	HPA-4 b	HPA-5 a	HPA-5 b	HPA-6 a	HPA-6 b	HPA-15 a	HPA-15 b	Total
False Neg	5	8	0	0	0	5	0	0	0	8	0	0	0	0	26
False Pos	1	1	1	0	2	3	1	1	7	2	0	0	1	5	25
Total (n=1135)	6 (0.53%)	9 (0.79%)	1 (0.09%)	0	2 (0.18%)	8 (0.70%)	1 (0.09%)	1 (0.09%)	7 (0.62%)	10 (0.88%)	0	0	1 (0.09%)	5 (0.44%)	51 (0.32%)

17 labs made one error, 10 labs made 2 errors, 2 labs made 3 errors, 1 lab made 4 errors, 2 labs made 5 errors and 1 lab made 6 errors. The overall error rate was low (0.32%).

We have noted a number of differences in the methodology used by participants and this might be affecting their potential to detect certain HPA specificities due to limitations of the methods employed.

Comment

►These Schemes aim to improve the overall quality of laboratory testing by identifying differences in clinical practice that could affect patient care through the comparison of laboratories across the world.

► Participants can view anonymised results tables and the methods each laboratory uses which allows for further analysis of kits and testing strategies.

► Participants with UP are asked to perform a root cause analysis to ascertain the cause of the issue. The aim of NEQAS is the improvement of lab performance through education. As such all our participants have access to experts in the field to provide assistance if required.

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