# Luminex method variability in HLA antibody specificity testing for UK NEQAS for H&I samples

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## Introduction

The UK National External Quality Assessment Service for Histocompatibility and Immunogenetics (UK NEQAS for H&I) have operated an HLA-specificity testing scheme since 1988.

In 2013 98% of participants tested samples using Luminex technology. We have noted considerable variation in reported HLA specificities despite using Luminex kits from the same manufacturer

Here we report findings from Scheme 3 – 'HLA-Antibody Specificity Analysis', for the last 5 samples distributed in 2013.

## Scheme material

10 serum samples known to contain HLA antibodies are provided in 2 batches of 5 samples each year. Specificities were assessed if >74% of participants reported their presence and were considered absent if assigned by <6% of labs. Specificities reported by 6-74% participants were not assessed.

## Results

All 66 participants (bar 1) tested the last 5 samples in 2013 using Luminex technology. 47 (71%) laboratories reported results based on Luminex testing only.

From the two available Luminex kit manufactures 45 participants used LABScreen (One Lambda), 13 LIFECODES (Immucor) and 7 used both.

### *LABScreen only users*

- 6 different combinations of Mixed, PRA and Single Antigen bead (SAB) kits were used. 25 (56%) participants used SAB kits only, 13 (29%) used Mixed and SAB, 3 (7%) used Mixed, PRA and SAB, 3 (7%) used PRA and SA and 3 (7%) participants did not use SAB kits (PRA ± Mixed)
- The volume of beads used varied from 1.8-5µl (table 1)
- All participants used HLA Fusion Software but the version varied from 2.0, 3.0 and 3.2
- Of the 42 participants that completed lot information 36 used Class I SAB kit lot 008 and 6 used lot 007. All used lot 009 for Class II SAB.

**Table 1: LABScreen SAB volume and MFI cut off values**

| Bead Volume (µl) | Number of participants | MFI Cut Off | Number of participants |
|---|---|---|---|
| 1.8 | 1 | Variable (60-2685) | 6 |
| 2.0 | 6 | 500 | 6 |
| 2.5 | 11 | 1000 | 19 |
| 3.0 | 5 | 1500 | 2 |
| 5.0 | 13 | 2000 | 3 |

- SAB cut off values ranged from 60-2000 (table 1). 6 participants varied their cut off value for each sample.
- There was considerable variation in the SAB positive and negative control values reported for each sample (table 2).

**Table 2: LABScreen SAB Control Bead Values**

| Sample | Positive Control Range (mean) | Negative Control Range (mean) | Pos/Neg Ratio Range (mean) |
|---|---|---|---|
| 306 | 3118-17950 (12243) | 23-617 (80) | 29.1 – 695.9 (282.2) |
| 307 | 3228-18695 (12283) | 15-362 (42) | 51.6 – 928.7 (378.8) |
| 308 | 913-19200 (11418) | 19-941 (195) | 8.5 – 837.9 (176.8) |
| 309 | 1917-17272 (11661) | 20-589 (58) | 20.4 – 660.9 (333.5) |
| 310 | 3086-17420 (12280) | 30-363 (71) | 47.1 – 513.3 (222.8) |

38 labs had reported full information on the bead volume, kit lot and cut-off value used for LABScreen SAB testing. 8 used the same volume of beads (2.5 µl), kit lots (008, 009) and cut-off value (1000 MFI). These were compared to the remaining 30 labs with differing test variables.

The number of non-assessed Class I specificities decreased from 55 to 38, suggesting more consistent results in the group using identical test variables (table 3).

**Table 3: Non-assessed Class I Specificities**

| Sample | Number of non-assessed Class I Specificities (specificities reported by 6-74% of participants) | | |
|---|---|---|---|
| | Differing SAB test variables (n=30) | Consistent SAB test variables (n=8) | All participants (n=65) |
| 306 | 14 | 8 | 50 |
| 307 | 3 | 6 | 4 |
| 308 | 6 | 3 | 6 |
| 309 | 8 | 5 | 15 |
| 310 | 24 | 16 | 26 |
| Total | 55 | 38 | 101 |

## Comment

These recent UK NEQAS for H&I Scheme 3's findings highlights Luminex testing variability between different laboratories using the same manufacture's kit. These differences are probably contributing to the inter-laboratory variation seen with Luminex specificity assignments.

## Further information

Full information on all UK NEQAS for H&I schemes is available at **www.neqashandi.org** or contact the Scheme Manager - Deborah Singleton

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