UK NEQAS
International Quality Expertise

Histocompatibility & Immunogenetics

Variability of Luminex Methods - Findings from a Survey of UK NEQAS for H&I Participants

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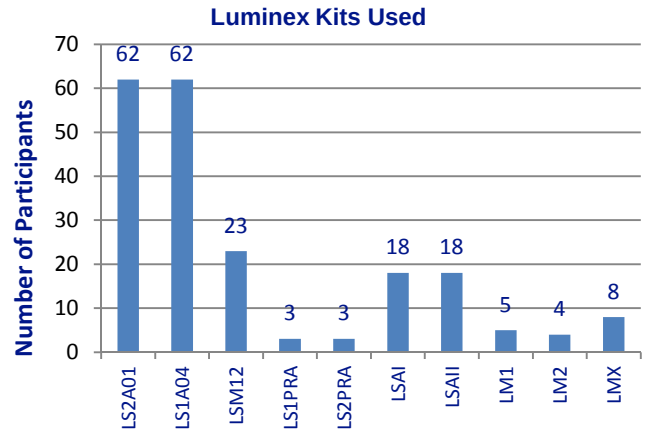
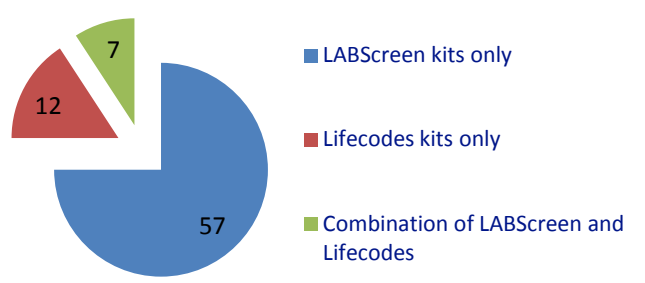
Introduction

In 2016, UK NEQAS for H&I requested detailed information on the Luminex methodology used by participants for its 'HLA Antibody Specificity Analysis' scheme (Scheme 3) .

76 out of the 80 (95.0%) participants responded.

Results





- Bead volumes ranged from 1.5µl to 5µl for LABScreen and from 2.5µl to 40µl for Lifecodes
- Serum volumes ranged from 8µl to 20µl for LABScreen and 5µl to 20µl for Lifecodes

Negative Control Sera

- 72.4% (n=55) of participants used the manufacturer's
- 9.2% (n=7) used 'in house'
- 2.6% (n=2) used no negative control sera
- 15.8% (n=12) did not specify

Positive Control Sera

81.6% (n=62) used a positive control of which:

- 69.4%(n=43) used patients' sera
- 85.5%(n=53) included this control in every run.

Sample Storage

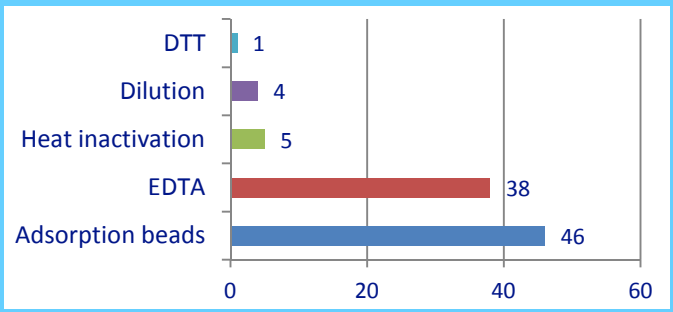
96.1%(n=73) stored samples before testing, including:

- Freezing 82.2% (n=60)
- Refrigeration 16.4% (n=12)
- Ambient 1.4% (n=1)

72.6% (n=53) routinely stored samples for >48 hours before testing.

Serum Treatment

79.0% (n=60) used serum treatment, 7.9% (n=6) did not, 13.2% (n=10) did not specify. Methods used are shown below (some labs applied several methods):



Wash Steps

All but 2 laboratories followed the manufacturer's incubation times and used the supplied wash buffer.

- 65.8% (n=50) removed buffer using a vacuum manifold
- 30.3% (n=23) used 'centrifuge, flick and blot'
- 1.3% (n=1) used LabXpress
- 2.6% (n=2) did not specify

Data Analysis

- 69.7% (n=53) used 'HLA Fusion',
- 17.1% (n=13) used MatchIT,
- 7.9% (n=6) used both HLA Fusion' and MatchIT
- 5.3% (n=4) used 'HLA Fusion' with other software.

Minimum positive control bead MFI varied from 400 – 10000 and the criteria for high background ranged from MFIs of 300 – 1500.

Comment

These findings indicate disturbing variations in Luminex practices.

Further Information

Full information on all UK NEQAS for H&I schemes is available at **www.neqashandi.org.uk** or contact the Schemes' Manager at **ukneqashandi@wales.nhs.uk**

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