

## Using EDTA to Ameliorate the ‘Prozone Effect’ in UK NEQAS for H&I’s ‘HLA Antibody Specificity Analysis’ Scheme

Felicity May, Tracey Rees, Deborah Singleton & Christopher Darke

### Introduction

UK NEQAS for H&I’s ‘HLA Antibody Specificity Analysis’ scheme (Scheme 3) assesses participants’ ability to define HLA specificities in antisera using the same method(s) they employ for clinical samples.

81 laboratories participated in scheme 3 in 2015, and all used a Luminex-based method. The majority of participants used Luminex alone (n = 65), but some used Luminex in combination with other methods (Luminex + CDC n = 14; Luminex + ELISA n = 1; Luminex + ELISA + Flow Cytometry n = 1).

Human complement factors, notably C1, C3, C4, interfere with the Luminex assay often resulting in falsely low results (the so-called prozone effect). A number of test modifications can be used to overcome this problem, including EDTA treatment of sera, heat inactivation, and DTT treatment. These methods are increasingly widely used, particularly in highly sensitised patients.

### Participant Survey

A survey of serum treatment techniques was circulated to Scheme 3 participants. The survey comprised of 10 questions relating to serum treatment techniques with particular focus on EDTA treatment methodology.

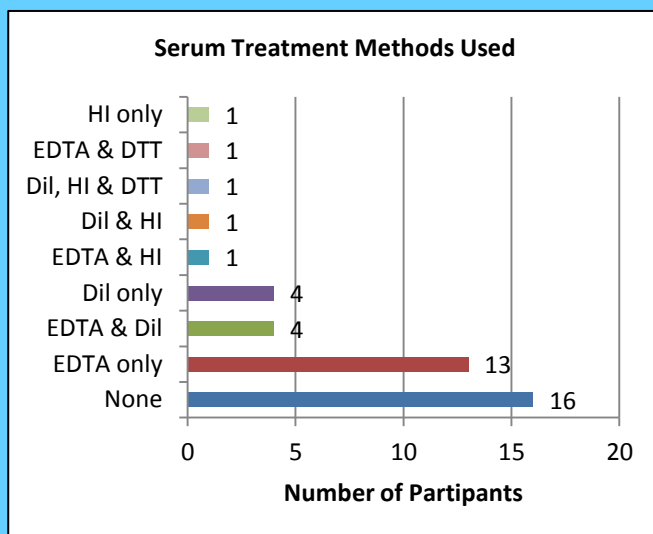
### Results

42 of the 81 participants (51.9%) returned responses to the survey.

- 61.9% of laboratories (n = 26) used some form of serum treatment
- 38.1% of laboratories (n = 16) currently have no routine treatment measures in place

The following methods were used by participants; some laboratories applied several methods depending on the clinical sample:

- EDTA addition (EDTA) - 73.1%, n=19/26
- Serum dilution (Dil) - 38.5%, n=10/26
- Heat inactivation (HI) - 15.4%, n=4/26
- DTT addition (DTT) - 11.5% n=3/26



Concentrations of EDTA stock solutions used included 6%, 0.1M, 0.2M and 0.5M. EDTA to serum ratios ranged between 1:10 and 1:20.

Of the 19 laboratories that used EDTA, 84.2% (n=16) added it directly to the sera while 3 laboratories added it to the wash buffer.

Of the 16 labs that added EDTA to serum, 11 (68.8%) tested samples immediately after treatment while 5 routinely stored sera prior to testing (3 at -20°C and 2 at 4°C). 5 laboratories commented that some samples were stored routinely whilst others were tested immediately depending on, e.g. sample urgency. Of the 11 laboratories that immediately tested samples after EDTA treatment, 8 (72.7%) applied a minimum EDTA/serum incubation time of 5 to 15 minutes. Three laboratories had no formal serum EDTA treatment time.

### Comment

Importantly, no laboratories that employed a treatment method (i.e. EDTA, dilution, heat inactivation or DTT) had unsatisfactory Scheme 3 results for 2015.

### Further Information

Full information on all UK NEQAS for H&I schemes is available at [www.neqashandi.org.uk](http://www.neqashandi.org.uk) or contact the Scheme Manager at [ukneqashandi@wales.nhs.uk](mailto:ukneqashandi@wales.nhs.uk)