Assay Improvement

Evaluation of Different Washing Buffers in Removing Non-Specific Reactivity in Complement-Binding HLA Antibody Detection Assays

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Introduction

Unexpected binding reactivity might arise from HLA-specific antibodies or non-HLA non-specific binding. A bead-based complement binding HLA antibody detection assay uses Phosphate-Buffered Saline (PBS) in its wash steps. While PBS provides a stable, isotonic environment, it may lack detergents that could reduce non-specific interactions. PBS with Tween-20 (PBST) contains Tween-20, a non-ionic detergent that helps reduce non-specific binding by breaking weak interactions.

Materials and methods

Sample Preparation

A total of 12 heat-inactivated sera were tested using PBS and PBST, including samples that previously demonstrated high negative control (NC) bead values.

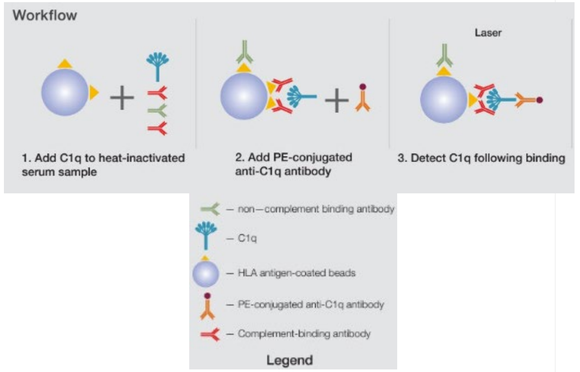
Test Method

One wash with PBS (200 µL) was compared to one PBST wash (200 µL) in a complement-binding HLA antibody assay after secondary antibody incubation. The trimmed mean fluorescence intensity (TMFI) values collected from bead-based antibody detection assays were compared between the two protocols.

Data Analysis

This feasibility study compared the use of PBS and PBST during the assay washing step to evaluate whether fluorescence signals were affected.

Figure 1. C1q assay overview



Results

Negative control bead reactivity results showed that for only 1 serum sample, the TMFI was < 500 with the PBS wash step method. In contrast, for 6 sera, the TMFI was < 500 with the PBST wash method (See Table 1). This suggests that the PBST washing method can be more effective in reducing the background signal on negative control beads. Positive controls and other immunoassay analytes did not show detrimental loss of reactivity between the two washing methods (See Figure 2).

Results

Figures

Figure 2. TMFI signals from representative sera tested with PBS and PBST washing on an HLA CI antibody detection product (top graph; 1 serum sample) and HLA CII antibody detection product (bottom 2 graphs; 2 sera). PBS results are in dark blue, and PBST results are in yellow.

Results

Figures & Tables

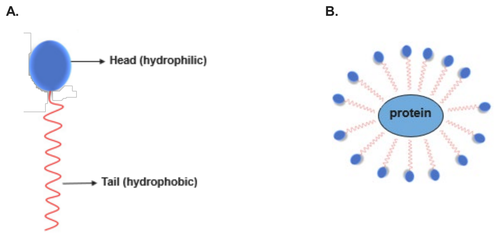


Figure 3. A: The general structure of Tween-20. B: Detergents solubilize hydrophobic proteins, which are often responsible for non-specific binding, by surrounding them with tween molecules, thereby disrupting weak hydrophobic interactions in aqueous solutions.

Table 1. Comparison of TMFI Values for PBS vs. PBST

Sample	TMFI of NC with PBS	TMFI of NC with PBST
1	23,197	22,549
2	21,505	21,788
3	30,268	30,299
4	26,921	17,980
5	27,581	87
6	20,480	23
7	1,154	10
8	27	3
9	3,898	17
10	6,940	14
11	22,424	21,987
12	19,909	953

Conclusions

In certain cases, immune complexes may bind to negative control beads, leading to elevated fluorescence signals. Additionally, electrostatic interactions between serum proteins and negative control beads might occur in some instances, leading to heightened TMFI signals. PBST can reduce non-specific binding to negative control beads without impacting other immunoassay interactions. As evidenced by the results from both washing methods, PBST washing may not reduce background in all cases; however, it was more effective than PBS for 50% of the tested samples (See Table 1).

References

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