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Journal of Immunological Methods

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Letter to the editor

Dear Editor,

I read, with great interest the report by [Ravindranath et al. \(2017, 450:73–80\)](#). This article purports that the Immucor Class I single antigen beads do not carry, on their surface, denatured HLA antigens in comparison to One Lambda single antigen beads(SAB) which does carry denatured HLA antigens (Abstract section, line 11–14).

In an effort to support/refute this claim we tested the Immucor single antigen kit (Lot 3005613, expiration date 2018-06-15) with two of three monoclonal antibodies used in their study. As demonstrated in the attached figure ([Fig. 1](#)), our testing produced contradictory results with the HC-10 monoclonal antibody. We were unable to test the TFL-006 antibody.

In contrast to the published article, we saw significant staining of the Immucor beads with the HC-10 monoclonal antibody. The authors state that testing was performed at a saturation concentration of 10 µg/ml (Section 2.2. SAB Luminex-based immunoassay, last paragraph) which, with the exception of a few specificities, produced minimal staining of the Immucor beads. However, in our testing, using the same concentration of HC-10, we observed a significantly higher staining than was reported in the article. When a saturation concentration of 1 mg/ml of monoclonal antibody was used, it clearly showed strong positive staining of all reactive Class I beads contained in the Immucor product. As reported and performed in the article, we tested the Immucor beads using the One lambda method. Given these data, it is obvious that the concentration of the monoclonal antibody purportedly used in this study was not at saturation as claimed. Clearly, sub-satur-

ating concentrations will provide lower MFI values. Hence, our HC-10 data clearly shows that the Immucor beads do have denatured antigen on their surface. Another monoclonal antibody, W6/32, produced results similar to the sub-saturation antibody concentration used in this article ([Fig. 2](#)). While we were unable to test the reactivity of TFL-006, it may be that this antibody was also not used at a saturating concentration.

A second important issue related to this publication is the fact that the particular lot of Immucor Class I beads used (03203F), had an expiration date of 3/31/2014. Therefore, this study might have been compromised unless the experiments were carried out before April 2014. This article was initially submitted on May 15, 2017. Manufacturers provide expiration dates based on their internal studies of product stability. It is completely unacceptable, under any circumstance, to use expired reagents in clinical testing. It would stand to reason that any study of clinical product, particularly one that may question product integrity/stability, be conducted under the same constraints as a clinical laboratory. Our studies were carried out using a current, unexpired, bead lot. Thirdly, even though the authors indicated that a One Lambda protocol was used in their experiments, the description in the Section 2.2 stated they used 2 µl of SABs rather than of 5 µl of SABs which is the quantity listed in the published One Lambda protocol. Experiments in [Fig. 1](#) used both variations of SAB quantities showing that neither achieved the saturation level of reactivity.

In summary, given the three critical points listed above, we are concerned that this article provides inaccurate information about a current clinical product which may lead to false interpretation of the

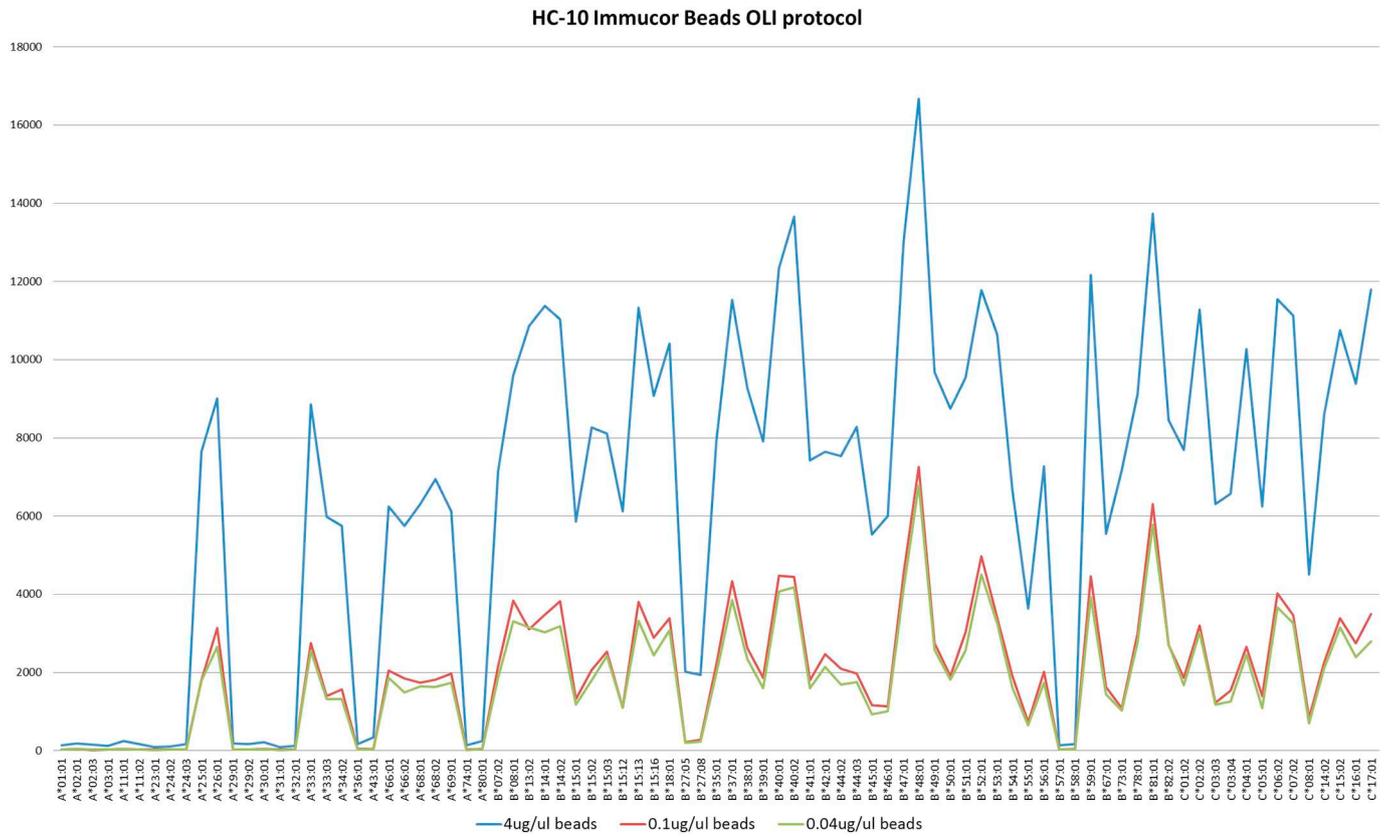


Fig 1. HC-10 monoclonal antibody reactivity with Immucor single antigen beads. 4 $\mu\text{g}/\mu\text{l}$ beads is equivalent to 1 mg/ml antibody and 5 μl beads; 0.1 $\mu\text{g}/\mu\text{l}$ beads is equivalent to 10 $\mu\text{g}/\text{ml}$ antibody and 2 μl beads; and 0.04 $\mu\text{g}/\mu\text{l}$ beads is equivalent to 10 $\mu\text{g}/\text{ml}$ antibody and 5 μl beads used in the experiments.

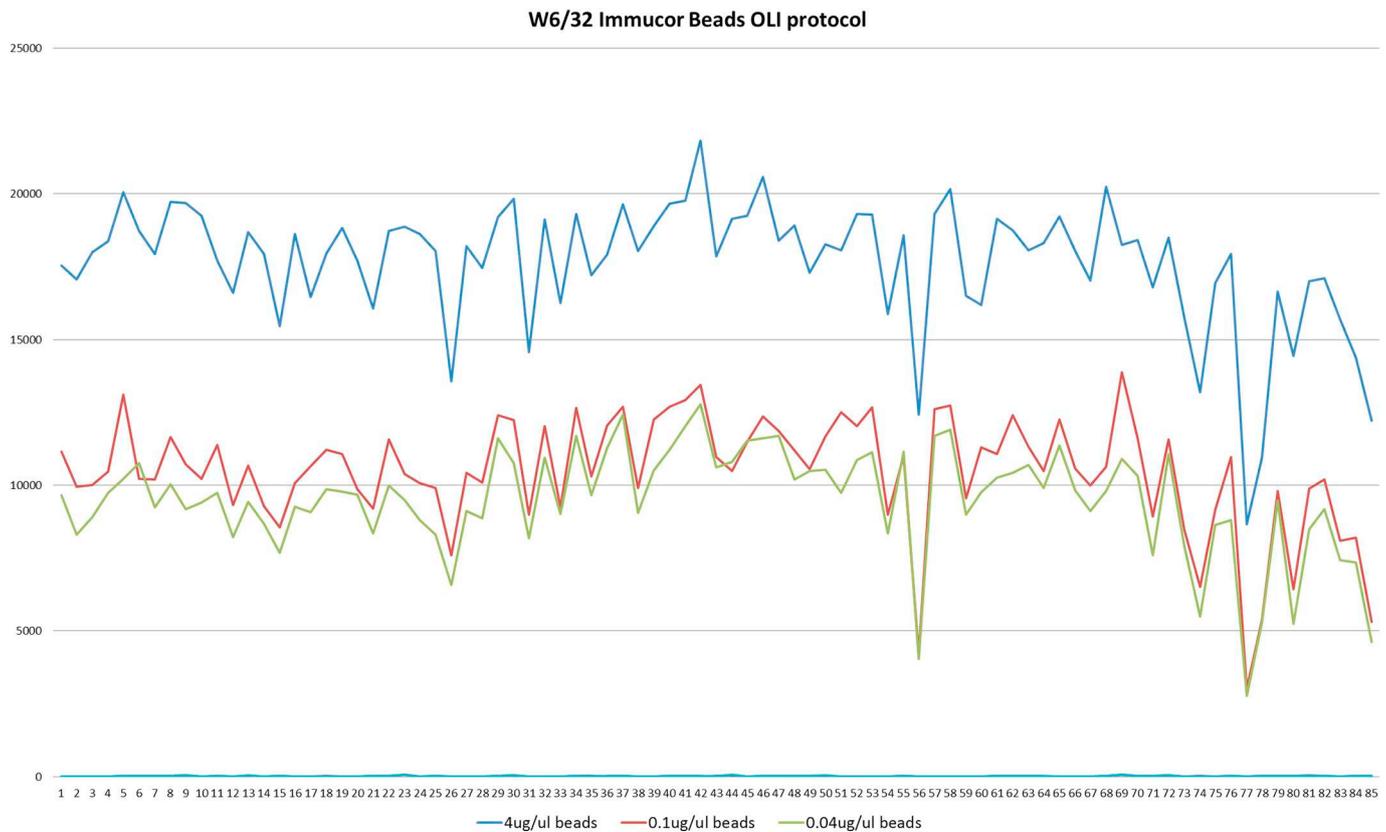


Fig. 2. W6/32 monoclonal antibody reactivity with Immucor single antigen beads. 4 $\mu\text{g}/\mu\text{l}$ beads is equivalent to 1 mg/ml antibody and 5 μl beads; 0.1 $\mu\text{g}/\mu\text{l}$ beads is equivalent to 10 $\mu\text{g}/\text{ml}$ antibody and 2 μl beads; and 0.04 $\mu\text{g}/\mu\text{l}$ beads is equivalent to 10 $\mu\text{g}/\text{ml}$ antibody and 5 μl beads used in the experiments.

clinical results and endanger patient safety. To be clear, we are not contending that the One Lambda product does not carry denatured Class I antigens just that the data related to the Immucor product were incorrect.

Sincerely,
Jar-How lee.

Reference

Ravindranath, M.H., Jucaud, V., Ferrone, S., 2017. Monitoring native HLA-I trimer

specific antibodies in Luminex multiplex single antigen bead assay: evaluation of beadsets from different manufacturers. *J. Immunol. Methods* 2017 (450), 73.

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