Validation of a Laboratory Developed Test for the Detection of T-Activated Polyagglutination

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Background

The most common form of polyagglutination (PA) is T-activation, in which the bacterial enzyme neuraminidase alters sialoglycoproteins, MN, and Ss antigens by cleaving neuraminic acid residues on the red blood cell (RBC) surface, exposing the T cryptantigen, Gal-β(1-3)-GalNAc. IgM anti-T antibodies, normally present in human plasma, agglutinate T-activated RBCs in vitro and are also capable of causing in vivo hemolysis. AABB Standard 5.3.2.9 requires immunohematology laboratories to recognize and have a process to investigate PA. However, survey results indicate that current testing for PA in the United States is not standardized, and laboratories either use expired reagents, develop their own in-house testing methods, or no longer test for PA.

Introduction

Previous researchers at Weber State University Department of Medical Laboratory Sciences produced a Laboratory Developed Test (LDT) kit that can be used to detect T-activated PA. The LDT includes the T activation of red blood cells (RBCs) using the bacterial enzyme neuraminidase and the isolation of a lectin from Arachis hypogaea (peanut) seeds that detects the exposed T cryptantigen via agglutination. These T-activated RBCs are a positive control that confirms the lectin isolate accurately detects the exposed T cryptantigen while non-activated RBCs are used as a negative control. While creating the LDT, the previous researchers used Group O Rh Negative RBCs from Ethylenediaminetetraacetic acid (EDTA) whole blood to create the positive control RBCs. This study validates the LDT with the testing of 60 samples, provides additional information as to what additional ABO Rh blood types can be used to create the positive control cells, and investigates if specific anticoagulants or preservatives interfere with T-activation. Seven of the eight main ABO Rh blood types were evaluated and were sourced from whole blood anticoagulated with EDTA and packed RBC donor segments preserved in AS-1 and AS-3.

Acknowledgements

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Results

Initial testing and each subsequent interval of testing as weeks four, eight, and twelve resulted in all 60 T-activated samples producing strong 4+ agglutination and negative control reactions of 0 with the lectin. Further, plasma from an adult AB Rh negative sample containing the naturally occurring anti-T antibodies agglutinated the T-activated RBCs and did not react with a negative control of non-activated RBCs.

Table 1. Sample Blood Type and Anticoagulant/Preservative

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<th>ABORh</th>
<th>EDTA WB</th>
<th>AS-1</th>
<th>AS-3</th>
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<tr>
<td>Total</td>
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<td>27</td>
<td>3</td>
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Abbreviations: AS-1, Adsol; AS-3, Nutricel; EDTA, Ethylenediaminetetraacetic acid; WB, whole blood

Figure 1. Negative control and 4+ reaction

Figure 2. Hemagglutination results on a 0-4+ hemagglutination scale of the various T-activated RBC types of different ABORh blood types. Quantity of samples for each blood type is denoted by (#) and are individually tested each month.

Figure 3. Hemagglutination results on a 0-4+ hemagglutination scale of the various T-activated RBCs exposed to the anticoagulant/preservatives. Quantity of samples for each anticoagulant or preservative is denoted by (#) and are individually tested each month. Abbreviations: EDTA, Ethylenediaminetetraacetic acid; AS-1, Adsol; AS-3, Nutricel.

Study Design/Methods

Lectin Isolation

Arachis hypogaea lectin and T-activated control cells were prepared using a modification of Judd’s methods. Arachis hypogaea seeds were processed to allow for isolation of the lectin, which was stored with 0.1% sodium azide.

Enzyme Collection

Neuraminidase was obtained from Streptococcus pneumoniae strain ATCC 49619. After 24 hour growth in a 5% CO2 incubator at 37 degrees Celsius, 40 isolated colonies were mixed with a solution of 160 mL of tryptic soy broth (TSB) and 40 mL of a prepared 0.5% dextrose solution and incubated again at 5% CO2 for 24 hours to allow for neuraminidase production. The solution was centrifuged at 1400 rpm for 5 minutes, and the supernatant was filtered through a 0.45-micron filter and stored at 4 degrees Celsius.

Sample Preparation and T-Activation

The T-activated control cells were prepared from donated and deidentified samples including 30 EDTA whole blood samples and 30 segments from AS-1 and AS-3 packed RBC donor units. The RBCs were then washed three times in normal saline and treated with neuraminidase enzyme filtrate in a 1:1 ratio of packed RBCs to enzyme filtrate. The samples were incubated at 37 degrees Celsius for 2 hours to allow for T-activation.

Testing and Storage

Two drops of lectin isolate were combined with one drop of the T-activated cell suspension and gently mixed. The sample was centrifuged for 15 seconds and the agglutination was measured. Samples were then washed and stored in Alsever’s solution to preserve cellular integrity for long-term storage and testing.

Limitations

- T-activation only required two hours of incubation, where the original LDT stated 24 hours was required.
- Blood type B Rh Negative was not available for testing.
- The three anticoagulants/preservatives evaluated were EDTA, AS-1, and AS-3.

Conclusion

After successful T-activation of 60 samples demonstrating agglutination with Arachis hypogaea lectin, it was determined that this is a viable method to produce lectin and quality control cells for the detection of the T-activated form of PA. Further, seven of the eight major blood types in EDTA, AS-1, or AS-3 donor segments can be used for the creation of the T-activated control cells and demonstrates that the various anticoagulants or preservatives the cells have been collected and stored in does not inhibit the T-activation process.