Minor Product Modification Improves Specificity of PGD® Test

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Background

Following introduction of the Platelet PGD Test to the market as a quality control test, Verax sponsored a multi-center study of the PGD Test at 18 hospital transfusion services. The study population comprised more than 27,000 leukocyte reduced apheresis platelet units suspended in plasma (LRAP) that had been distributed to the hospitals as culture negative following testing with a growth-based system cleared by FDA for quality control testing of platelets for transfusion. The purpose of the study was to determine the performance of the PGD Test in actual use conditions in order to estimate:

- The bacterial detection or True Positive (TP) rate: 1:3,069 (95% Confidence Interval [CI] 1:6,711 – 1:1,617)
- Specificity (for 10,424 doses that had additional concurrent culture performed at the time of PGD testing: 99.3% (CI 99.1% -- 99.4%)
- The False Positive (FP) rate for 27,620 doses: 0.51% (CI 0.43 – 0.61)

The results of this large study[1,2] supported FDA's designation of the Platelet PGD Test as a "safety measure" when LRAP are tested within 24 hours prior to platelet transfusion following testing with a growth-based culture system FDA-cleared for testing platelet components.

FDA Guidance, finalized in September, 2019[3] provided a pathway to extending expiration dating of LRAP to 7 days when a safety measure is used as did prior (draft) versions of the Guidance.

Investigation

Verax initiated a project to improve the specificity of the PGD Test. Verax investigated FP PGD results to first determine their cause(s) and then develop an assay modification that would reduce FP results below 0.5%.

Immunoassays are known to be susceptible to interference from human endogenous antibodies that may interact nonspecifically with antibodies used in the immunoassay.

Two types of endogenous antibodies are anti-animal antibodies and heterophile antibodies:

- Human anti-animal antibodies are species specific, e.g. human anti-mouse (HAMA). Human anti-mouse antibodies may develop after patient exposure to monoclonal antibody based medicine. In the case of veterinarians or pet owners, human antibodies against domestic animals, such as rabbits, guinea pigs, etc., may develop over time.[4]
- Heterophile antibodies develop in the absence of exposure to a specific immunogen and may react with varying avidity to multiple species.[5]

Assay interference by human endogenous antibodies may cause either falsely reactive or falsely negative test results, depending on the architecture of the assay.[6] In antibody sandwich immunoassays, such as the Platelet PGD Test, the antigen of interest is sandwiched between capture and detector antibodies, forming an antibody/antigen sandwich. When human endogenous antibody bridges the assay's capture and detector antibodies, false reactivity occurs.

Typical antibodies may be depicted as Y shapes, with the two identical arms (regions) of the Y referred to as Fab (fragment antigen binding) regions. When subjected to cleavage with certain enzymes, the Fab regions, which contain antigen binding capacity, are separated from the tail of the Y or the Fc (fragment crystallizable) region. The Fc region contains no antigen-binding activity.[7]

Figure 1 is an illustration of an antibody sandwich assay format such as that of the PGD Test. In this example, the capture antibody (Ab) and the detector antibody are whole antibodies. Each whole antibody comprises both crystallizable (Fc) region and specific antigen binding region or F(ab). The bacterial antigen (Ag) of interest is shown "sandwiched" between two antibodies. Note that antibody/antigen binding occurs in the F(ab) region. Immediately below the illustration is a sample photo of how the PGD Test would demonstrate such a test result – a true positive (TP) result.

Figure 1

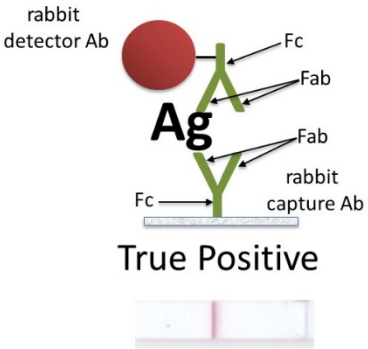
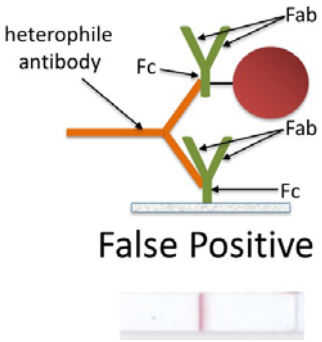


Figure 2 shows how a human endogenous antibody can bridge a capture Ab and a detector Ab and generate a false positive (FP) signal. As shown in the illustration, false reactivity due to anti-animal antibodies or heterophilic antibodies typically occurs in the Fc region of the antibody rather than the specific antigen-binding region F(ab). Immediately below the illustration is a sample photo of how the PGD Test would demonstrate such a FP result.

Figure 2



Verax reviewed reports of FP results and determined that one of the four regions of the PGD Test strip was the region most often associated with FP results. This region (line 1 or L1, on the Gram-positive (GP) side of the Test device) used the same rabbit IgG antibodies as both capture and detector antibodies. These antibodies were whole antibodies and, therefore, included both Fc and F(ab) regions.

Assay modification

For decades, pepsin has been one method used to cleave whole antibodies to separate the Fc portion of whole antibodies from the Fab fragments. In this process the antibody is cut such that the two antigen binding fragments remain linked together, thus creating a single $F(ab')_2$, which retains the same antigen binding characteristics as the whole antibody.[8]

Verax initiated a project to evaluate the use of pepsin to cleave the PGD Line 1 (L1) capture antibodies, which are currently whole antibodies, into Fc and $F(ab')_2$ fragments. The $F(ab')_2$

fragments were proposed for use as L1 capture antibodies. Line 1 detector antibodies would remain whole antibodies.

Figure 3

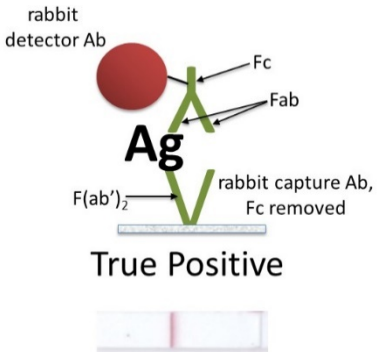
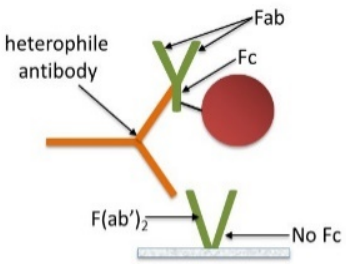


Figure 3 depicts the newly modified PGD assay format. Note the F(ab')$_2$ as the capture antibody. A sample photo shows how the modified PGD Test would demonstrate a TP result.

Figure 4 shows how implementation of this modification to antibody processing was expected to address FP. Note that the heterophile antibody is unable to bridge the capture and detector antibodies, since the capture antibody has no Fc region to which it can bind. As shown in the sample photo immediate underneath the illustration, the modified PGD Test would not show a Reactive or FP result.

Assay Validation

Figure 4



Verax performed studies to evaluate post-modification performance vs. performance of the original PGD Test. These studies would determine whether or not specificity of the PGD Test was improved without negatively affecting other performance attributes. All studies involved testing the modified PGD Test side by side with the original PGD Test.

Confirmation Studies

These studies included confirmation of the Limit of Detection established with the original PGD Test, prozone or hook effect, and interfering substances (both donor related and sample related conditions). Results of these studies showed that that the minor processing modification did not have a negative impact.

Specificity

Specificity was assessed by testing 5,410 samples from culture-negative platelets with both the current, unmodified PGD Test version and the modified version. Seven sites participated in the study. Platelet types tested were: LRAP in 100% plasma (LRAP), LRAP suspended in PAS-C/plasma (PAS), prestorage pools of leukocyte reduced whole blood derived platelets suspended in plasma (PSP), and post-storage pools of non-leukocyte reduce whole blood derived platelets (nLRWBDP).

Thirty of the 5,410 samples were FP in the original version of the PGD Test. Only six of those 30 samples were FP in the modified PGD Test and none of these six were FP due reactivity in the modified region (L1) of the test device. Overall observed specificity of the modified version of the PGD Test was 99.9%, compared to overall specificity of 99.4.%. Table 1 shows results of this testing.

Table 1: Summary of Specificity Testing Results

Platelet Type	Total Tested	Observed Specificity (LCL*)	
		Original PGD	Modified PGD
LRAP	3303	99.4% (99.1%)	99.9% (99.8%)
PAS	416	99.8% (98.9%)	99.8% (98.9%)
PSP	1193	99.7% (99.4%)	99.9% (99.6%)
nLRWBDP	498	98.8% (97.7%)	99.4% (98.5%)

*LCL (lower one-sided 95% confidence limit)

Product Identification

The modified product can be identified by its refreshed design. Figure 5 shows the pouch of the modified PGD Test on the right and the pouch design of the original PGD product on the left.

Figure 5 PGD Test Device Pouch Designs: Modified product on right

Figure 6 shows the design of the original PGD Test device on the left and the modified PGD Test device on the right.

Figure 6 PGD Test Device Designs: Modified product on right

Summary

Validation testing of the minor modification to the PGD product demonstrated improved specificity performance and no loss in ability of the assay to detect bacteria. Overall observed specificity of the modified PGD Test was approximately 99.9% compared to overall specificity of 99.4% for the original PGD Test. The change to the L1 region had no impact on the assay procedure and no new training is required prior to implementation.

Improved specificity can be expected to positively impact platelet availability, laboratory workflow and costs.

[1] https://www.veraxbiomedical.com/wp-content/uploads/2019/06/Platelet-PGD-Test-US-Rev-J-June-2019-Version.pdf

[2] Jacobs, MR, Smith, D, Heaton, WA, Zantek, ND, Good, CE. Detection of bacterial contamination in prestorage culture-negative apheresis platelets on day of issue with the Pan Genera Detection test. Transfusion 2011; 51: 2573-82

[3] US Food and Drug Administration. Bacterial Risk Control Strategies for Blood Collection Establishments and Transfusion Services to Enhance the Safety and Availability of Platelets for Transfusion. September 2019.

[4] Emerson J, Keane KY.Endogenous Antibody Interferences in Immunoassays. Lab Medicine 2013; 44(1): 69-73

[5] *Id*

[6] *Id*

[7] Janeway CA Jr, Travers P, Walport M, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. The structure of a typical antibody molecule. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27144/

[8] *Id.*