



e-Network Forum

CALIFORNIA BLOOD BANK SOCIETY

"We help save lives of people who need blood"

Search CBBS Website

Experience using PEG versus LISS for antibody detection

A colleague wrote that his hospital discontinued using PEG in their pre-transfusion antibody screen due to a **large number of false positive test results** (which he referred to a "junk"). The inquiring blood banker stated that his laboratory would read their PEG antibody screen after addition of antihuman globulin reagent. His lab discontinued the use of PEG over concerns that frequent false positive results might cause delay in providing blood for transfusion. His lab is **now using LISS** for their RBC antibody screening. However, since switching to LISS, the inquiring blood banker's lab has encountered patients whose serum contains anti-Jka, but whose anti-Jka antibodies failed or nearly failed to react with the LISS antibody screening test (false negative results). For example, according to the inquiring blood banker, one patient showed a +/- to rough reaction with the LISS antibody screen, but a reference lab reported that the serum showed 2+ agglutination when PEG enhancement was employed. Another patient had a known anti-E, but a co-existing anti-Jka was not discovered until a reference laboratory tested the serum with a PEG enhanced method. The inquiring colleague would like to know **what other facilities have experienced when comparing PEG versus LISS**, and what e-network forum members have **done to minimize false positive and false negative results** when using these reagents.

The following input was received in response to the above:

1. For a related discussion that addresses **false-NEGATIVE** PEG antibody screen results see the earlier discussion entitled [False-negative Results with PEG Red Cell AntibodyTest - Due to Elevated Serum Globulins](#)
2. A blood banker wrote that she has been screening for unexpected antibodies using PEG for over 10 years. When she compared a PEG (Gamma PEG) tube technique to a LISS tube technique (Ortho Antibody Enhancement) she found **no increased occurrence of false positive reactions at the AHG phase**. In fact, she states that she read all of her reactions microscopically, and she never had a problem with a false positive reaction. Currently her laboratory is using **plasma** for all serological testing, although serum with anti-IgG provides the same "protection" from false positive reactions.
3. A **blood banker in California** commented that when her Transfusion Service was introduced to the use of PEG, they were warned of the exact problem experienced by the inquiring blood banker, namely, false positive results. Although the responding blood banker no longer works at a Transfusion Service, she feels confident to comment on this issue. She reports having worked with PEG, LISS, & albumin additives, and she **strongly supports the use of PEG**. The last procedure she wrote for using PEG included the use of a **prewarmed** test using PEG, if the regular screen with PEG showed a 1+ or weaker result. Most commonly, this prewarmed screen would show negative results, unless a clinically important antibody was present, such as weak Jka and E antibodies. In the responding blood banker's opinion, PEG is definitely worth using if you can incorporate a couple of 'next steps' to perform for complete evaluation of antibody screening.

EDITOR'S NOTE regarding reply #3: In my opinion, it is **NOT good practice to do reflex prewarmed testing** to determine if a 1+ or weaker PEG-AHG result is due to a clinically significant or insignificant alloantibody **WITHOUT FIRST KNOWING** which antibody you have detected. I base this on my own experience that prewarmed testing misses some clinically significant alloantibodies. That is why I suggest that the e-Network Forum review the following two articles on this subject:

- **Mallory, D:** Controversies in transfusion medicine. Prewarmed tests: pro - why, when, and how - not if. *Transfusion* 1995 35: 268-270.
- **Judd, WJ:** Controversies in transfusion medicine. Prewarmed tests: con. *Transfusion* 1995 35: 271-275.

In addition, so as not to impose my singular opinion on the network, I **queried two colleagues**, both of whom are well known experts in immunohematology. Here is what they had to say:

Expert #1 commented that his laboratory group will be presenting data (see the article by Leger RM, Garratty G. Weakening or loss of antibody reactivity following prewarm technique.

Transfusion 2001;41:30S) at the upcoming AABB meeting. These data confirm John Judd's observation several years ago, that some weak antibodies can be missed when using the prewarm technique. According to expert #1 (who works in Los Angeles), the prewarmed procedure still has a place when applied correctly (e.g., to determine if DEFINED antibodies such as anti-M, -N, -P1, etc. are reacting at 37C), but should NEVER be used to make unidentified "problem reactions" go away.

Expert #2 (who works in Michigan) commented that there was an abstract on pre-warmed PEG tests at the 1995 AABB Annual Meeting. [Champagne K, Spruell P, Chen J, Moulds M. Prewarming technique with a polyethylene glycol additive solution. Transfusion 1995;35(S):68.] The authors were able to detect small amounts of IgG alloantibodies (strength of reactivity not stated) using a prewarmed PEG method. Despite this, the authors' final statement was: "Before prewarming, it is important to evaluate autocontrol results and determine specificity of the antibody(ies)." Expert #2 said that he concurs whole-heartedly with this statement. Expert #2 wanted to know if the colleague who submitted reply #3 had determined what the unwanted PEG reactions were actually due to and how frequently they occurred? Were they due to cold-reactive autoantibodies? Were they due to nonspecific precipitation of IgG onto RBCs, or due to warm reactive antibodies? Expert #2 offered the opinion that substitution of saline for anti-IgG in the antiglobulin test should be informative. On a more critical note, the expert points out that the e-network member who submitted reply #3 chose PEG for its ability to detect weakly reactive antibodies, but then wants to render the screening tests nonreactive in order to conclude that the weak reactivity was due to a clinically insignificant antibody. This is false logic (for reasons given in the article by WJ Judd Controversies in transfusion medicine. Prewarmed tests: con Transfusion 1995 35: 271-275). Further, Expert #2 said that many of the blood group specific alloantibodies that are only found by "super-sensitive methods" are not clinically important. Evidence for this is found in a study conducted by Lown and Willis. [Lown J, Willis J. Monocyte monolayer assay (MMA) reactivity of alloantibodies reacting by the manual polybrene technique but not by an antiglobulin test. Transfusion Medicine 1995;5:281-4.] Finally the e-Network may be interested in a recent article by Combs and Bredehoft from Duke University. They switched from PEG to gel and noted a reduction in the incidence of unwanted positive tests but no increase in the number of delayed serological reactions following implementation of gel. This paper cites a number of publications that compare sensitivity of antibody detection methods. Relatively less sensitive but more specific antibody detection methods are certainly adequate for pretransfusion testing. After all, those who are not using PEG are not observing a plethora of patients with delayed hemolytic transfusion reactions! Further, no single method (including PEG) will detect all significant antibodies.

4. **A blood banker who works in New York State** reports that in his experience (in 26 years of fooling around with this subject) all techniques are tradeoffs between sensitivity and specificity. That said, repeated experience amongst an experienced technical staff over the years is the best recipe for consistent and clinically effective pre-transfusion testing. He does not think there is a material difference between gel, PEG, LISS etc. in experienced hands. He says this despite claims for the superiority of one over the other. In his opinion, it is a simple fact that the once or twice a year he has seen a detectable delayed hemolytic transfusion reaction in which the causative antibody was not only detectable by PEG (we use LISS), but also by a different technologist using their standard LISS technique. That is, operator to operator variability is MUCH greater than differences in sensitivity. Furthermore, the number of delayed hemolytic transfusion reactions (DHTRs in which an antibody is "missed" occurs about once in every 20,000 to 30,000 red cell transfusions, (they transfuse about 25,000 red cells a year, and use a standard manual LISS technique). The responding blood banker thinks that his institution's experience with keeping DHTRs at a minimum is about as good as one can do according to the literature. His conclusion is that **one should pick a technique that is simple, cheap and favored by the technical staff and stick with it**, learning its foibles and nuances, and stop worrying about missing antibodies because some other technique out there is more sensitive. Anything demonstrably more sensitive than LISS, such as using enzyme-treated cells, is going to detect lots of "garbage" and a few antibodies that will not usually cause clinically significant reactions, even though they are in the class of "usually clinically significant" antibodies. The responding member can remember perhaps one anti-Jka that was missed by LISS in about 20 years, or about half a million red cell transfusions. If one doesn't have an experienced technical staff, he can see the attraction of gel, which is a less operator dependent technique. Automated techniques also have this advantage of removing some of the human variability. Otherwise, the technology we have is probably as good as it is going to get in terms of sensitivity.
5. **A Scientific Director of a large immunohematology reference laboratory in Los Angeles** commented that there are many papers in the literature comparing PEG with other procedures (e.g., Slater JL et al. Evaluation of the polyethylene glycol-indirect antiglobulin test for routine compatibility testing. Transfusion 1989;29:686-689; Wenz B et al. Evaluation of the polyethylene glycol-potentiated indirect antiglobulin test. Transfusion 1990;30:318-321; deMan AJM et al. Evaluation of the polyethylene glycol antiglobulin test for detection of red blood cell antibodies. Vox Sang 1990;58:207-210; Shirey RS et al. Polyethylene glycol versus low-ionic-strength solution in pretransfusion testing: a blinded comparison study. Transfusion 1994;34:368-370; Barrett VJ et al.

Analysis of the routine use of polyethylene glycol (PEG) as an enhancement medium. Immunohematology 1995;11:11-13; Low KS et al. Improved detection of weak, clinically significant antibodies by supplementation of polyethylene glycol with a low-ionic solution. Immunohematology 1998;14:68-71; Combs MR et al. Selecting an acceptable and safe antibody detection test can present a dilemma. Immunohematology 2001;17:86-89). As always, it is a personal decision with pros and cons on both sides. **PEG is a good procedure and has been used routinely for many years in the responding scientific director's reference lab**, but as with all other procedures, is open to abuse (e.g., one cannot centrifuge tests before washing and AHG reagents containing anti-complement should not be used).

ADDENDA Sept. 21, 2001

6. A **blood banker in Sacramento** commented that in her opinion the most common cause of a false positive result with PEG is due to the **incomplete resuspension of the red cells during washing**. If a heavy precipitate is noted when the tubes are removed from the incubator, washing by hand or manual resuspension of the cells after each fill cycle of the washer will insure a thorough wash and prevent the cells sticking together rather than agglutinating after the addition of anti-IgG. Samples that do not have a heavy precipitate after incubation may also still stick together rather than be resuspended. After the 4th wash, she sets the cell washer for one cycle and pushes the check button. She checks to see if all the cells are fully resuspended immediately after the tubes are filled with saline, manually resuspending those that are not and continuing the cycle. If only a small amount was not resuspended she adds anti-IgG. If the cells are not easily resuspended she adds an additional wash and checks for resuspension again. **Another cause of positive reactions not matching any known antibody is reading the reactions microscopically**, Many of the hospitals in her area do this although the instructions say NOT to. PEG will detect many cold agglutinins and warm auto antibodies that would not normally be detected in LISS.

Please submit comments to the [e-Network Forum](#).



Printable PDF of this page

[Ira A. Shulman, MD](#)
CBBS e-Network Forum Editor & Moderator

The e-Network Forum is supported by the California Blood Bank Society (CBBS) and endorses collegial discussion among blood banking and transfusion medicine professionals. However, the CBBS does not necessarily endorse the specific views and opinions expressed in the forum. The forum is not intended as a substitute for medical or legal advice and the content should not be relied upon for any medical or legal purposes. Readers should make their own determinations as to: (i) what constitutes appropriate medical, technical, and administrative practices, and (ii) how best to comply with laws and regulations relevant to their questions. For the latter, they should consider consulting, as to any medical matters, a qualified physician, and, as to any legal matters, an attorney familiar with related state and federal laws. The user of the forum, by accessing same, assumes all risks arising out of such use and releases CBBS and their respective members, directors, officers and agents from and against any loss, damage, claim or liability arising out of such use of the forum.

Posted: September 20, 2001

Addenda: Sept. 21, 2001