

EDITORIAL

Validation of new technology for antibody detection by antiglobulin tests

Correctly performed spin-tube indirect anti-globulin tests (IAT) have provided the mainstay for antibody detection in compatibility testing for over 30 years. Significant improvements have been made in the production and standardization of anti-human globulin (AHG) reagents that avoid unwanted false-positives due to too much anti-C3d. However, NEQAS trials have consistently shown that 4-6% of workers miss 1+/2+ levels of reaction. Some of these failures may have been due to inefficient cell washing machines but the ISBT/ICSH working party on antiglobulin reagents demonstrated, by replicate tests with weak 1+/2+ sensitized cells, that most false-negative IAT are due to excessive agitation at the reading stage of the test (Voak *et al.*, 1986; 1988). The use of replicate tests is now recommended to evaluate the efficiency of cell washing machines and worker performance in reading IAT (BCSH, 1991).

It is recognized that spin-tube IATs require skilled expertise of hand and eye and that this is the major weakness of this technology. Nevertheless, a well performed spin-tube IAT is the standard against which any new system must be compared. Guidelines have been published to help in the validation of liquid phase microplate IAT (BCSH, 1990). This technology is essentially 96 small tubes per plate. Not only does it suffer from all the reading stage problems of tube tests, but it also has the added problem that many AHG reagents prozone with weakly sensitized cells. Liquid phase microplate technology, although as sensitive as conventional tube tests, cannot compete with new technologies that have a simple accurate reading stage. Several technology systems have emerged that have a much simpler reading phase than manually read spin-tube IAT; they are of three main types.

1 Solid phase IAT (Plapp *et al.*, 1984) or semi-solid phase IAT microplate (Ross & Gordon, 1986; Scott, 1991). Both provide a rapid sensitive IAT with the convenience of a 96-well plate and have a superior reading phase that is not subject to the errors of agitation tube or liquid phase microplate techniques. In all these procedures the test is read on a solid phase, either manually or photometrically and labelled anti-IgG can be used by an ELISA technique.

2 The Gel system IAT of Lappierre *et al.* (1990) is very easy to use with a simple reading stage. Agglutination is visible in the gel and the reactions can be graded for strength.

3 Automated reading of conventional spin tube tests (Szabo *et al.*, 1992). This system equals the sensitivity of a manual LISS spin tube IAT. The computer-controlled cell washing system incorporates the use of special bent glass tubes to facilitate photometric reading of the tests and provides grading of the strength of agglutination. Thus, it has the benefits of using established spin-tube techniques combined with a reliable automated reading procedure.

Validation studies of new systems have correctly compared the performance of new systems with that of conventional tests. However, some discrepancies have arisen that have highlighted the need to refine the validation procedure for the study of new systems. Bromilow *et al.* (1991), in an extensive study of antibody screening tests, found the Diamed Gel system IAT to be consistently more sensitive and with fewer false-positives than their conventional LISS spin-tube IAT. On the other hand, Voak *et al.* (1991) and Phillips *et al.* (1992) both found that the Gel IAT was less sensitive with some difficult antibodies (anti-Fy^a, Jk^aS).

In their studies, Voak *et al.* (1991) evaluated their semi-solid phase microplate IAT (LIS-SPAT) in comparison with the Diamed Gel IAT and LISS and polyethylene glycol (PEG) spin-tube manual tests. This revealed some important aspects of validation tests.

1 Titration studies with anti-D, anti-Kell and anti-Fy^a (spin tube IAT titres of not more than 32, as used in the standardization of antiglobulin reagents (Engelfriet & Voak, 1987)) revealed that the Gel Test IAT titres were equal to, but not greater than, those of the spin-tube tests. Whereas, the LIS-SPAT titres were three dilutions higher which suggested that this was a more sensitive system.

2 The detection of a panel of weak difficult antibodies (30) against red cells heterozygous for the appropriate antigen. This test confirmed that the Gel Test missed the four antibodies reported later by Phillips *et al.* (1992) and two other antibodies (anti-Jk^a and anti-K).

Furthermore, the sensitivity of the LIS-SPAT IAT was only as good as that of the spin tube test.

3. Replicate tests with weak 1+/2+ anti-D sensitized cells demonstrated the excellent reproducibility and sensitivity of all the systems.

4. Tests for false-positives with group AB inert sera and fresh ABO compatible sera, in simulated cross-match tests with red cells from CPD-A1 donor pack cross match lines, demonstrated that all the systems were relatively free from false reactions.

Each of the validation stages provides useful information. The titres with anti-D, anti-K and anti-Fy^a show the approximate sensitivity of a new system in parallel to standard tests. If dilutions of the antiglobulin reagent (AHG) are used, the problems of prozones can easily be resolved by selecting the correct dilution of AHG reagent for the weakest sensitized cells used in the chequerboard tests. However, although Voak *et al.* (1991) used the optimum dilution of the AHG reagent in the LIS-SPAT microplate tests, they did not find the predicted higher sensitivity in the tests with weak difficult antibodies.

The detection of weak difficult antibodies with red cells heterozygous for the corresponding antigens was the most stringent sensitivity test. It demonstrated that the LIS-SPAT and the Gel Test IATs were not more sensitive than well performed spin-tube tests. The weak antibodies missed by the Gel Test IAT were all detected when the tests were repeated with red cells homozygous for the corresponding antigens. Thus the Gel Test IAT is a reliable system for antibody screening provided that good quality screening panels are used.

The use of replicate tests with weak anti-D sensitized cells has many useful features. They can rapidly demonstrate a failure of reproducibility of reagents, between wells in a microplate, or between the different tubes of a Gel Test card. They can also check the efficiency of a new reading system using photometric or ELISA methods.

Claims for increased sensitivity of new systems should always be considered with caution, as many such claims have been marred by the unwanted occurrence of an increased number of false-positives. Tests for false-positives are therefore an essential part of the validation of a new system.

It is appropriate to proceed with what is called a clinical trial when a new system has successfully passed a validation procedure. At present there are no regulations to control the testing procedures for new systems being used in the U.K. However, the BCSH and SBT/ICSH Working Party on blood grouping reagents (Bruce *et al.*, 1990) are guided by the relevant FDA Regulations. These require a clinical trial to be performed at three or

more sites to assess a new system in parallel with the conventional system in use. A trial in the routine work situation of some 3,000 samples enables the reliability, ease of use and time-cost relationships of a new system to be accurately established.

It is also important to remember that validation and routine test trials must be performed by workers fully familiar with a new system and that it should be carried out strictly according to the manufacturer's instructions. Useful new systems that offer cost-effective sensitive antibody detection are now a fact of life, and several new technologies are finding their place in routine blood transfusion work.

Finally, weaknesses highlighted in assessment trials should be notified to the manufacturers, as it is quite reasonable to expect that fine tuning of reagents will be a feature of new technology systems.

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