New Developments in Blood Group Serology

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The last issue of INFUSIONSTHERAPIE UND TRANSFUSIONSMEDIZIN included four articles covering a wide range of developments in serology. These included a review of monoclonal antibodies used in routine and research [1], a study of antibodies to high-frequency red blood cell antigens [2], a contribution to isotypes of cold agglutinins with discussion on the rare occurrence of IgG antibodies to the Pr antigens and the rarity of Ig class switching in cold agglutinins [3] and a paper on a microplate solid-phase method for typing red blood cells [4].

In addition to the important impact of monoclonal antibodies in routine and research, the other most significant advances have been seen with the introduction of the new systems and technologies for blood typing, antibody screening and compatibility testing.

Laboratories have adopted new technologies to help improve aspects of performance such as to reduce work time or to handle increased work loads in conditions of financial restraints; especially with automated or semi-automated systems staff numbers can be reduced.

These new techniques must be shown by evaluation and clinical trial to be at least as good as existing techniques [5, 6] when in routine use. If new methods have greater sensitivity than standard tube tests, then this must be meaningful and cost effective, and not the detection of clinically non-significant antibodies that may not be cost effective or that may delay the issue of blood in urgent circumstances. Kretschmer et al. [7] considered that the sensitivity of the gel test versus their manual spin tube test reduced the frequency of delayed post-transfusion reactions. The move from serum to plasma samples for automated antibody screening has also raised consideration for the detection of difficult complement-requiring antibodies such as anti-Jk^a/Jk^b. Yates et al. [8] have shown that sub-agglutinating levels of IgM anti-Jk^a/Jk^b can far more easily be detected by anti-complement reaction than by

the much weaker anti-light-chain reactions of anti-IgG reagents seen with the use of plasma samples.

Different approaches have been used to evaluate new technology systems. Thus contradictory publications have appeared on the relative sensitivity of the DiaMed gel test for antibody detection (DiaMed, Cressier, Switzerland), e.g. Bromilow et al. [9] and Kretschmer et al. [7] found it more sensitive while Voak et al. [10], Phillips et al. [11] and Pinkerton et al. [12] found that the gel test had the same sensitivity as a well performed indirect spin tube antiglobulin test. The probable explanation for the gel test showing a higher performance than routine spin tube tests is due to false-negative results caused by excessive agitation at the reading phase of spin tube tests. This common fault explains most of the failures in external proficiency trials and is easily recognised and corrected by blind replicate tests [13, 14, 15] with 1+/-2+ IgG anti-D sensitised cells. The origin of excessive reading techniques was due to too much polyclonal anti-C3d complement in polyspecific antiglobulin reagents prior to 1987.

The ISBT/ICSH working party on reagents showed that the old FDA method for assessing freedom of reaction with red blood cells with antiglobulin reagents only used washed cells. Many AHG reagents specific by this test would nevertheless give a consistent 1+ unwanted positive result in cross-match tests when the cells are incubating with fresh serum. This is because red blood cells take up more C3d when incubating the fresh serum. The FDA in 1987 altered the specificity test for AHG reagents to incorporate simulated cross-match tests. Further progress at the same time came from the use of monoclonal anti-C3d which was much easier to standardise to give clean, yet potent polyspecific reagents [15].

The use of blind replicate tests to assess staff and quality assure cell washing machines [13, 14] was included in our UK guidelines for compatibility testing from 1987. Thus from 1990 to 1992 we had the very interesting situation of new technolo-

gy with simple endpoints that were easier to read than tube tests, e.g. micro-column and solid-phase systems, in wide use in our hospitals just after we had solved the major common problems of classical spin tube techniques. Certainly it is true that less skilled staff can use the new technology systems, especially those with automated reading devices.

However, serologists know that there is no perfect system for the detection of all antibodies. Thus, the micro-column systems (DiaMed and BioVue) for example may miss weak ABO incompatibility that is detectable by a rapid saline spin tube test [16]. Also micro-column techniques have been demonstrated to miss examples of other weak antibodies such as anti-Fy^a, anti-Jk^a, anti-S and anti-K [10, 11, 17]. These failures in detecting certain weak antibodies have been shown [17] to be due to shear forces breaking down weak agglutination as it is forced through the gel or bead matrix by centrifugation forces. For example, in one trial of 100 Gp B plasmas × A₂B red blood cells the DiaMed gel antiglobulin test missed 68 incompatibilities while the BioVue bead antiglobulin test missed 76, compared to a 5-min spin tube saline test that only missed 8 and a LISS spin tube antiglobulin test that only missed 7 [17].

However, these failures have to be put in perspective within a clinical setting. Even when performed properly, the rapid saline direct spin tube test does not always demonstrate ABO incompatibility. In addition, the frequency of an incompatibility occurring between a group B patient and an A₂B donor owing to limitations of the direct agglutination test in a laboratory where donor units are selected randomly regardless of recipients' ABO group is very rare. Also the missed incompatibilities are predominantly anti-A₁ cold antibodies i.e. titres less than 16 with A₁ cells and little reaction with A₂ cells, thus they are essentially not dangerous at 37 °C with A₂B cells that have even less reaction with low titre anti-A than A₂ cells.

A far more important issue is that new technology systems, just like classical tube systems, are only as good as the quality of the screening red blood cell panels used for antibody detection. The drive to achieve a cost-effective antibody screening of patients' specimens causes all laboratory practices to be reviewed, particularly when new technologies are introduced such as the DiaMed and Ortho micro-column tests. The standardisation of these tests and their reliable reading, together with the need for cost saving, led Lillevang et al. [18] to evaluate the use of pooled red blood cell samples for antibody screening using the DiaMed antiglobulin micro-column test. In a study of 5,446 antenatal and pretransfusion EDTA plasma samples, it was concluded that the use of two pools, each of two red cell samples, maintained transfusion safety and

compared favourably with their previous testing regime. There pools contained C, D, c, e, k, Fy^a, Fy^b, JK^a, Kp^b, M, N, S, s, Le^a, Le^b and Lu^b in the homozygous state and E, K, P₁, Lu^a and others present in the heterozygous state.

However, this was challenged by Eggington et al. [19] who reported on the use of pools of two and three red blood cell samples in the gel micro-column antiglobulin test using 2,000 antenatal serum samples respectively. This study used pools that contained at least one red blood cell sample with a homozygous expression of C, D, c, e, Fy^a, Fy^b, N, S, s, Le^a and Le^b. The K antigen was present in the heterozygous state. The authors concluded that the use of pooled red blood cells led to a decrease in sensitivity which caused difficulty in the interpretation of results, and that the detection of known antibodies was reduced. Similar conclusions were reached by Bombail-Girard et al. [20].

Microcolumn tests are able to separate unagglutinated from agglutinated red blood cells and, in theory, offer the ability to use pooled red blood cells to investigate the loading capacity of the gel or glass bead column matrix. Neither of the investigators used red blood cells with a homozygous expression of the K antigen, antibodies of which can be difficult to detect in low-ionic-strength antiglobulin tests.

The current guidelines for the UK Blood Transfusion Services [21] state that pooled red blood cells should not be used for the screening of patients specimens. This is based on long experience with tube test methods showing that the use of pooled red blood cells results in a down-grading of the reaction (by the presence of a mixture of red blood cells, some with and some lacking the corresponding antigen to antibody present in the serum), and presents reading difficulties in the interpretation of mixed-field reactions.

A far better move to cost-effective cell screening can be achieved by proper management of screening, selection and use of high-quality two-cell panels by a policy of having large-scale or national reagents laboratories. Methods are now available for the frozen storage and retrieval of matched pairs of reagent red blood cell units, containing antigens of probable clinical significance, without significant loss of these antigens over a usable period appropriate to screening cells. Such matched pairs of R₁R₁ and R₂R₂ donations with homozygous expression of all antigens of probable clinical significance but with heterozygous K occur with a frequency of some 10 matched pairs in 100,000 donors. Using them results in considerable resource and investment. However, their use, compared with a three red blood cell sample screen, could cause a saving of some 1.4 million GBP per year in consumable costs over the UK and a 30% saving in work load [22].

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