# Evaluation of 2-column agglutination versus conventional tube technique for antibody screening

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## تقييم نظام التراص ذي العمودين لتحرّي الأضداد ومقارنته مع أسلوب الأنبوب التقليدي

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**الخلاصة:** تهدف الدراسة إلى تحديد نوعية وحساسية نظام التراص ذي العمودين لكشف التركيزات المنخفضة للأضداد ذات الأهمية السريرية، في المصل. وقد تمت مقارنة نظام التراص ذي العمودين مع أسلوب الأنبوب التقليدي (اختبار كومس غير المباشر لتحري أضداد الغلوبولين). وقد تم استخدام اختبار البابزيم ذي المرحلتين لتمييز الفروق بين الطريقتين. وقد قمنا باختبار 3000 عينة مصلية من مرضى تم اختيارهم عشوائياً في مركز الملك حسين الطبي. وقد أسفر التحري عن نتائج سلبية في 2952 مريضاً، ونتائج إيجابية في 48 مريضاً. وقد توصلنا إلى أن نظام التراص ذي العمودين يُعدُّ أسلوباً أكثر حساسية، إلا أنه إذا ما تم تضمين الخلايا المعالجة بانزيم البابايين في أساليب الاختبار التقليدية عند تطبيقها على تحري الأضداد وكشفها، فقد تظهر الحساسية متقاربة في الأسلوبين.

ABSTRACT The study aimed to determine the specificity and sensitivity of the Ortho BioVue® two-column agglutination system for the detection of low concentrations of clinically significant antibodies in serum. The BioVue® system was compared with the conventional tube technique (LISS-Coombs indirect antiglobulin test), and the two-stage Papenzyme test was used to resolve discrepancies between the two methods. We tested 3000 serum samples from randomly selected patients at King Hussein Medical Centre. Both the antibody screening and identification gave negative results in 2952 patients and positive results in 48 patients. We found the BioVue® system to be the more sensitive technique. However, if papain enzyme-treated cells were included in the conventional tube technique when applied to antibody screening and identification, both methods would be of comparable sensitivity.

***Evaluation d'un système d'agglutination à deux colonnes pour le dépistage des anticorps par rapport à la technique classique en tube***

RESUME Le but de cette étude était de déterminer la spécificité et la sensibilité du système d'agglutination à deux colonnes Ortho BioVue® pour le dépistage de faibles concentrations d'anticorps cliniquement significatifs dans le sérum. Le système BioVue® a été comparé avec la technique classique en tube (test à l'antiglobuline ou LISS/Coombs indirect), et le test à papaïne à deux phases a été utilisé pour résoudre les écarts entre les deux méthodes. Nous avons testé 3000 échantillons de sérums prélevés chez des patients choisis au hasard au Centre médical King Hussein. Le dépistage et l'identification des anticorps ont donné des résultats négatifs chez 2952 patients et des résultats positifs chez 48 patients. Le système BioVue® s'est révélé être la technique la plus sensible. Cependant, si des cellules papaïnées étaient incluses dans la technique classique en tube appliquée pour le dépistage et l'identification des anticorps, les deux méthodes seraient d'une sensibilité comparable.

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## Introduction

Since 1985, new approaches to routine blood group determination and antibody detection in antenatal care and pretransfusion testing have been developed using solid-phase methods on microtitre plates [*1–3*]. A more recent development in transfusion medicine laboratory investigations has been the production of two-column agglutination technologies: the DiaMed-ID micro typing system which uses Sephadex gel [*4*]; and the Ortho BioVue® system, described in 1993 by Reis [*5*].

The BioVue® test is based on the sieving effect of glass bead microparticles in density gradient diluent contained within a microcolumn. On each card or cassette, six microcolumns are mounted. Each microcolumn contains micro glass beads, a diluent, and the specific reagent. Testing can be undertaken over a range of temperatures. After centrifugation in specifically designed centrifuges, agglutinated red cells are trapped by the micro glass bead matrix contained in the microcolumns, and unagglutinated cells are forced through the micro glass spheres to form a pellet at the bottom of the column.

Columns containing Ortho BioClone antihuman globulin (AHG) serum also contain a macromolecule density barrier. During centrifugation, this holds back the serum but allows the red cells to enter into the column and make contact with the antihuman serum. This density gradient principle diminishes the need to wash the red blood cells in the conventional tube test. Natural distribution of agglutinates occurs relative to the strength of the reaction. This allows for easy grading of the reaction strength.

At the same time, the OrthoScan system provides the benefits of automation for the manual BioVue® system in blood transfusion laboratories. The system software using the Microsoft Windows platform allows patients' tests to be requested, advising the operator which cassettes are required, and then prints off a label for each cassette using a unique identification system to ensure correct cassette identification. After the test has been performed, the cassettes are loaded onto a rack and the images scanned into the software. Image analysis is necessary—the software informs the operator if the analysis generates an invalid group or result. All images are stored for recall at any time. A bi-directional interface of results and requests is available and the instrument can easily be networked.

In order to establish which is the more sensitive technique, the study compared the Ortho BioVue® column agglutination system with the conventional tube technique for the detection of low concentrations of clinically significant antibodies in patients' serum.

## Methods

A total of 3000 serum samples from randomly selected patients at King Hussein Medical Centre, Amman, Jordan were screened for the presence of irregular red blood cell antibodies. The sera were separated from fresh clotted samples and tested immediately.

The BioVue® column agglutination system (Ortho Clinical Diagnostics Incorporated, Raritan, New Jersey, United States of America) was used in parallel with the conventional tube technique for antibody screening using low ionic strength solution (LISS) in the Coombs indirect antiglobulin test. Two-stage Papenzyme testing (Lorne Laboratories, Twyford, England), which uses papain enzyme-treated cells in the

conventional tube technique, was used to resolve discrepancies in the results obtained from the two methods.

The antibody screening cells used were the same in all tests performed, covering homozygosity for the following antigens: D, C, E, c, e, $Fy^a$, $Fy^b$, $Jk^a$, $Jk^b$, k, M, N, S, s, and at least one heterozygote ($K^+k^+$). The antibody identification cells (Resolve Panel-A) and the antibody screening cells (two-cell sets) were both obtained from Ortho Clinical Diagnostics. Since the technical details of the methods used for comparison of the conventional tube technique and the column agglutination technology are important, the methods used are reported in detail.

## Conventional tube method

The international standard technique was used for testing by the conventional tube method [6]. The technique employs one drop (50 µL) of a 3%–5% suspension of the antibody screening cells (Ortho Clinical Diagnostics) with four drops of the polyspecific AHG test serum, (DiaMed, Cressier-sur-Morat, Switzerland), and two drops of LISS-ADD (Lorne Laboratories) in the appropriate test tube. The mixture was incubated at 37 °C for 15 minutes.

Following incubation, the cells were washed three times with isotonic saline and the supernatant was carefully removed. Antiglobulin testing was then carried out using DiaClone polyspecific IgG (DiaMed) and complement AHG reagents.

After centrifugation for 15 seconds at 3400 rpm, all tubes were observed for either haemolysis or agglutination, using a gentle 'tip and roll' technique with macroscopic observation. Negative results were confirmed microscopically.

An antiglobulin control using Coombs control cells (Ortho Clinical Diagnostics) was included for all tubes with negative results.

## Two-stage Papenzyme test

Use of papain enzyme enhances the antibody–antigen reaction by the removal of the sialic acid (N-acetyl neuraminic acid) residues. A negative charge is generated that results in red blood cells coming into closer proximity for agglutination to occur. Removal of the sialic acid residues substantially reduces the negative charge on the red cell membrane, thus enhancing the second stage of red cell agglutination by IgG antibodies [7–9].

We used this technique in our study for the detection of anti-E and anti-$Le^a$, which were undetectable by the conventional tube LISS–Coombs technique. The antibody screening cells were washed with isotonic buffered saline (pH 6.9). Equal volumes of washed packed cells and Papenzyme-plus (sterile filtered stabilized papain solution, Lorne Laboratories) were mixed, incubated at 37 °C for 15 minutes, washed once in isotonic buffered saline (pH 6.9) and resuspended in LISS.

Following treatment of the antibody screening cells with Papenzyme-plus, a 2%–3% suspension of pre-papainized cells was mixed with an equal volume of the test serum, incubated at 37 °C for 5 minutes and centrifuged at 900–1000 rpm for 15 seconds. The pellet was then gently resuspended and examined macroscopically for agglutination and/or haemolysis.

A set of controls were used which included a negative control, using AB serum, a positive control using a weak IgG anti-D and appropriate cells, and an auto control using patients' own cells and serum.

## BioVue® column agglutination test

Cassettes of 6 columns containing polyspecific BioVue® AHG serum (rabbit and murine monoclonal antibodies, Green Mountain Antibodies, Burlington, Virginia,

USA) were used. For the assessment of this technology in the antibody screening, 50 µL of LISS solution (Ortho Clinical Diagnostics), 10 µL of 3%–5% suspended antibody screening cells and 40 µL of serum to be tested were added to each well in the microtyping cassettes. The cassettes were incubated for 10 minutes at 37 °C, spun in a centrifuge adapted for this purpose (supplied by the manufacturer), and observed for haemolysis and/or agglutination. Agglutination reaction grading was defined clearly in the test protocol described by the manufacturers (Ortho Clinical Diagnostics).

## Results

Table 1 summarizes the results of the 3000 serum samples, comparing the BioVue® column agglutination technique and conventional tube technique using the LISS-Coombs method in antibody detection and identification. Antibody screening in both methods gave negative results in 2952 patients (98.4%). Positive results were found in 48 samples (1.6%); positive antibody screening with definite specificity was found in 15 samples (0.5%) and positive antibody screening with unspecific reactions in 33 samples (1.1%). One sample of anti-E, and one sample of anti-$Le^a$ were undetectable by the tube technique, but were reactive in the BioVue® column technique and the two-stage Papenzyme testing by conventional tube technique.

## Discussion

The use of the BioVue® column agglutination technique showed several advantages. It is an easy and simple procedure, provides easily readable agglutination reactions and saves time.

Prior to the introduction of a new methodology, assessment of its sensitivity

Table 1 **Comparison of the BioVue® two-column agglutination technique and conventional tube techniques using the LISS-Coombs method for detection of antibodies in serum samples**

| No. of samples tested | Antibody specificity | BioVue® | Conventional tube enzyme | Conventional tube (papain treated cells) |
|---|---|---|---|---|
| 2952 | No antibody | Negative | Negative | Not included |
| 33 | Unspecified | Positive | Positive | Not included |
| 5 | Anti-D | Positive | Positive | Not included |
| 1 | Anti-C | Positive | Positive | Not included |
| 1 | Anti-c | Positive | Positive | Not included |
| 1 | Anti-e | Positive | Positive | Not included |
| 1 | Anti-E | Positive | Negative | Positive |
| 1 | Ant-$Le^a$ | Positive | Negative | Positive |
| 2 | Anti-M | Positive | Positive | Not included |
| 2 | Anti-S | Positive | Positive | Not included |
| 1 | Anti-K | Positive | Positive | Not included |

is required, particularly in a laboratory that relies on the AHG phase for antibody detection and compatibility testing. We compared the sensitivity of the BioVue® column agglutination technology with our standard conventional tube technique, which is regularly monitored within the laboratory to ensure its capacity for the detection of low concentrations of clinically significant antibodies.

Comparisons of the BioVue® column agglutination technology and conventional tube technique in the detection of clinically significant antibodies have previously been reported. Weisbach et al. compared four microtube column agglutination systems, including the conventional tube technique [*10*]. The rates of detection of clinically-significant antibodies were 91.6% by DiaMed gel technology, 92.2% by the BioVue® column agglutination technology, and 76.0% by conventional tube technique. The authors concluded that the sensitivity of all four microtube column systems in the detection of clinically significant antibodies was markedly superior to that of the LISS-Coombs tube technique. In another comparison study, Lamy et al. reported that the column agglutination system was more efficient than the manual tube technique [*11*]. They found that the number of positive samples containing specific antibodies was higher in the BioVue® column agglutination technology than in the conventional tube technique. Reis et al. reported that results on antibody screening obtained using the BioVue® column agglutination technology were more objective than those in the conventional tube technique [*5*]. South et al. reported that the BioVue® technology compared favourably with the standard tube testing concerning specificity and sensitivity [*12*].

Results obtained from the present study are consistent with these studies. We found a satisfactory correlation between the BioVue® column agglutination technology and the conventional tube technique. The sensitivity and specificity of the BioVue® technology in the present study did not exceed 0.01%. It was more sensitive in detecting antibodies of potential clinical significance in one run, which was not the case in the conventional tube technique. The results from the BioVue® system were not statistically significant. A larger number of samples would need to be studied to obtain a statistically significant result.

The washing procedure at the AHG phase in the conventional tube technique could be the reason for the elution of weakly bound antibodies from red blood cells. Furthermore, the washing phase in the conventional tube technique increases the possibility for false-weak or negative reactions due to neutralization of AHG by remaining traces of serum.

## Conclusion

We conclude that the Ortho BioVue® column agglutination technology has many advantages over the conventional tube technique for the detection of low concentrations of clinically significant antibodies. It is simple, sensitive, rapid and cost-effective, and results are more objective than those obtained by the conventional tube technique. However, if papain enzyme-treated cells were included in the conventional tube technique when applied to antibody screening and identification, both methods would be of comparable sensitivity.

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