ORIGINAL ARTICLE

Multi-centre evaluation of pre-transfusional routine tests using 8-column format gel cards (DG Gel®)

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SUMMARY

Background: Advances in immunohaematology laboratory practice to improve performance, cost-effectiveness and patient safety are desirable.

Objectives: To perform a multi-centre evaluation of the 8-column Grifols DG Gel® cards and reagent system to assess its performance, suitability and adaptability to the daily blood transfusion laboratory routine in the United Kingdom.

Methods/Materials: A total of 4281 immunohematological analyses {1825 ABO/D grouping, 1921 antibody screening, 75 Rh phenotyping and K antigen determination, 361 antibody identification and 99 neonates [ABO/D and DAT (direct anti-globulin test)]} were performed on 2255 specimens. All cases were run in parallel with the reference method of each laboratory (DiaMed-ID® cards or conventional tube technique in some cases).

Results: Concordant results between Grifols DG Gel® system and the reference method were obtained in 97·7% of tests. For ABO grouping by the Grifols DG Gel® system, sensitivity was 99·95%, specificity was 99·96%, predictive positive value (PPV) was 99·89% and predictive negative value (PNV) was 99·98%. For D grouping, sensitivity was 99·78%, specificity was 100%, PPV was 100% and PNV was 99·78%. For antibody screening, sensitivity was 90·63%, specificity was 99·94%, PPV was 99·32% and PNV was 99·15%. Of the Rh subgroups and K types, results were 100% concordant. For antibody specificity detection, accuracy was 96·95% for Grifols DG Gel® system and 95·29% for DiaMed-ID® system. For the newborn tests, concordant results were obtained in 100% of ABO/D grouping and in 89·9% of DAT.

Conclusion: The Grifols DG Gel® 8-column system is reliable and safe for routine tests performed in the immunohaematology laboratory.

Key words: immunohaematology, microtube gel column agglutination, transfusion laboratory routine.

Since the invention of the anti-globulin test in 1945 (Coombs, 1998), the classical tube test has been refined to improve speed and sensitivity with the introduction of solid-phase methods and column agglutination technology (Knight & Poole, 1995). Solid-phase immunoassays were first described in the mid-1980s (Plapp et al., 1984; Sinor et al., 1985). In solid-phase immunoassay, one of the immunoreactive components, either antigen or antibody, is immobilised on a solid carrier prior to testing.

Column agglutination technology comprises a group of methods in which agglutination does not take place in a liquid phase, but red cells are centrifuged through a gel or bead suspension contained in special microtubes to detect red cell agglutination. Column agglutination offers advantages: reagents are already dispensed, the washing step in the anti-globulin phase is omitted and the reaction in the column is stable. Commercial kits of column tests for pre-transfusion purposes have been available for over 20 years and have progressively replaced conventional tube tests in most laboratories (Lapierre et al., 1990).

Increasingly, a major focus of laboratories has been to look for new techniques to improve the aspects of performance, including turnaround times, or to deal with increased workloads. New techniques must be shown...
to be better, or at least as good as, existing techniques when they are in routine use. This manifests as an ongoing trend to rationalise pre-transfusion testing protocols. In particular, antibody screens to detect only clinically significant antibodies have been developed to maximise efficiency without adverse effect on patient safety (Casina, 2006). Further efficiencies have been realised through the use of methods that have a decreased incubation period, such as antibody screening using low-ionic-strength solution (LISS). Finally, there is also a move towards automation or semi-automation, to facilitate streamlining and batch testing, provide traceability and decrease sources of error from staff manual manipulations.

Grifols DG Gel® (Diagnostic Grifols S.A., Barcelona, Spain) is a microtube gel column agglutination-based system, which is new to some European markets for pre-transfusion testing. The system’s unique feature is an 8-column gel card which is intended to provide in some cases a more complete test profile in comparison to the traditional 6-column format, to simplify pre-transfusion testing and, as a consequence, optimise cost-effectiveness. The 8-well format is accommodated in cards that are similar in size to 6-column format systems. The Grifols DG Gel® system can be used in manual techniques or through full automation instrumentation [WADiana® Compact analyser (Diagnostic Grifols S.A., Barcelona, Spain)]. The aim of this study was to assess the performance of DG Gel® cards reagent system and automation in a large multi-centre evaluation.

MATERIALS AND METHODS

Aims and study design

A multi-centre evaluation of Grifols DG Gel® cards and reagents system was performed in the United Kingdom. The primary objective was to confirm its sensitivity and specificity in daily blood transfusion laboratory routine. Secondary objectives were related to qualitative evaluation assessments.

From September to November 2009, 4281 immunohaematological analyses [1825 for ABO/D grouping, 1921 for antibody screening, 75 for Rh phenotyping and K antigen determination, 361 for antibody identification and 99 for neonates’ ABO/D grouping and direct anti-globulin test (DAT)] were performed on a total of 2255 specimens of whole blood samples collected in EDTA (ethylenediaminetetraacitic acid). Before testing, samples were centrifuged to separate cells and plasma, the latter of which was used for reverse ABO typing and antibody screening and identification. In addition, antibody identification panels were performed on plasma samples stored below −30 °C.

Samples were collected from five different centres: Birmingham Heartlands Hospital, University Hospital in Leicester, Manchester Royal Infirmary, NHS Blood and Transplant (NHSBT) Laboratories in Leeds and Newcastle. All tests were run in parallel with Grifols DG Gel® system and the reference method of each laboratory [DiaMed-ID® (DiaMed GMBH, Cressier, Switzerland) cards in all cases except in a small number of newborn tests in which reference method was the conventional tube technique].

Reagents

The following cards containing monoclonal antisera were used for the 8-column format card test procedures:

(i) Grifols DG Gel® ABO/Rh (2D) + K, which includes complete forward and reverse ABO group determination (anti-A, -B and -A,B), Rh D determination (two anti-DVI− clones), K antigen determination and diluent/inert negative control; (ii) Grifols DG Gel® Rh Pheno, which includes Rh phenotype determination for two patients per card (anti-C, -E, -c and -e); (iii) Grifols DG Gel® Coombs, which includes eight columns with polyclonal anti-human globulin reagent (AHG), for the direct and indirect anti-globulin test (DAT/IAT); (iv) Grifols DG Gel® anti-IgG, which includes eight columns with monospecific anti-IgG for the DAT/IAT; (v) Grifols DG Gel® Neutral, which includes eight columns with neutral gel suspension to perform saline and enzyme techniques and (vi) Grifols DG Gel® Neonatal, which includes complete ABO forward typing (anti-A, -B and -A,B), Rh type determination (double anti-D determination: DVI+ & DVI− clones), negative control and DAT (IgG and AHG) for haemolytic disease of the foetus and newborn test (HDFN).

The following 6-column format gel cards were used for the DiaMed-ID® card test procedures: (i) ID-Card ‘DiaClon ABO/D + reverse grouping’, which includes forward and reverse ABO group determination (anti-A and -B), Rh D determination (two anti-DVI− clones); (ii) ID-Card ‘DiaClon ABO/D + reverse grouping’, which includes forward and reverse ABO group determination (anti-A and -B), Rh D determination and negative control well; (iii) ID-Card ‘DiaClon Rh-Subgroups + K’, which includes complete Rh phenotype and K antigen determination; (iv) ID-Card ‘LISS/Coombs’ which includes six columns with polyclonal AHG, for DAT/IAT; (v) ID-Card ‘Coombs Anti-IgG’, which includes six columns with monospecific anti-IgG for DAT/IAT; (vi) ID-Card ‘NaCl, enzyme test and cold agglutinins’, which includes neutral gel suspension to perform saline and enzyme techniques and (vii) ID-Card ‘DiaClon ABO/Rh for newborns’, which includes complete ABO forward typing (anti-A, -B and -A,B), Rh type determination (anti-DVI−...
The following reagents were obtained from Grifols: reagent red blood cells (RBC) Serigrup Diana A1/B for reverse grouping, SeraScan Diana 4 (four cells) for antibody screening, Identisera Diana and Identisera Diana P (1–11 cells) and Identisera Diana Extend and Identisera Diana Extend P (12–15 cells), for antibody identification; Grifols DG Gel® Control (for internal quality control); Grifols DG Gel® Sol (to prepare RBC suspensions); DianaFluid A and DianaFluid B (solutions for internal washing of the automatic instrument, WADiana®).

For DiaMed-ID® card test ‘ID-Diluent 2’ (modified LISS for red cell suspensions) and ‘ID-Cell Stab’ (stabilisation solution for red cells) were used and each participating laboratory used its routine set of reagent RBC: In laboratories using automation DiaMed wash solution concentrates A and B for DiaMed-ID®-automates and RBC for DiaMed-ID® tests were obtained from DiaMed (‘DiaCell ABO human’ for the reverse grouping; ‘DiaCell I + II + III’ for antibody screening, and ‘DiaPanel’ for the antibody identification); in centres which used manual processing of the samples, RBC for DiaMed-ID® tests were obtained from NHSBT (‘3 Cell screen’ and ‘ID Panel 1’).

**Procedures**

All cases were run in parallel with Grifols DG Gel® cards and the reference method. Most samples (85-8%) were processed through full automation instrumentation (WADiana® Compact analyser in the case of Grifols DG Gel® system; ID-Gel Classic station with DiaMed cards), including testing for ABO/D grouping, antibody screening and some samples for antibody identification. A small number of samples (14-2%) including those for Rh phenotyping and K antigen determination, most of the antibody identifications and all those for neonatal testing were processed through manual instrumentation (Grifols DG Therm and Grifols DG Spin in case of DG Gel® system; DiaMed-ID® cards were processed through manual instrumentation DiaMed Gel Test ID-Micro Typing System). The study was performed by trained and experienced biomedical scientists (BMS).

Standard procedures for ABO/D grouping (1825 samples), antibody screening (1921 samples) and Rh phenotype and K antigen determination (75 samples) were carried out following the manufacturer’s instructions. An RBC suspension for use in the microtube system was made in modified LISS provided by the manufacturers according to their instructions (final concentration 0.8% RBC re-suspended in Grifols DG Gel® Sol diluted in case of DG Gel® system and re-suspended in ID-CellStab in case of DiaMed-ID®).

Additionally, Group and Screen analyser batch times (eight samples per batch) for DG Gel® and DiaMed-ID® were measured.

The antibody identification was carried out in 212 samples with known antibodies in indirect anti-globulin test (IAT) and enzyme (papain) techniques in adherence to the manufacturer’s instructions. The antibody detection test included an LISS IAT and a two-stage enzyme test. For Grifols DG Gel® system, the test comprised cells selected from a 15-cell identification panel and for DiaMed-ID® system cells selected from 11-cell identification panel or a 10-cell identification panel when reagent RBCs were from NHSBT. The technique consisted of mixing 50 μL of reagent RBC with 25 μL of plasma sample followed by 15 min incubation at 37 °C. Centrifugation using the preset cycle (9 min for the Grifols DG Gel® system and 10 min for the DiaMed-ID® system) was performed.

Forward ABO/D typing and DAT were determined in 99 samples from newborns in adherence to the manufacturers’ instructions. The reference method was DiaMed-ID® in 58 samples and the conventional tube technique in 41 samples. In case of Grifols DG Gel® system, two different anti-D (D^{VI+} and D^{VI−}) were tested, and DAT was performed with monospecific (anti-IgG) and polyspecific reagents media.

For qualitative evaluation, the BMS using the system reported differential operational features of the Grifols DG Gel® system, particularly those regarding the ease of use, clarity and stability of reactions, and advantages of 8-column over 6-column system.

**Interpretation of results**

All manual tests were read macroscopically after centrifugation. The strength of reaction was given on a scale as −, +, 1+, 2+, 3+ and 4+. All discrepancies were investigated. The criteria to identify discrepancies were as follows: if reactions gave results of opposite sign in each system (i.e. negative in one method and positive in the other), the discrepancy was classified as major or qualitative; if the sign of the reactions were the same in both systems but with a difference of two or more grades (i.e. 1+ in one method and 3+ in another), the discrepancy was classified as minor or quantitative. Differences of one grade (i.e. 1+ in one method and 2+ in another) were not considered discrepancies.

Antibody identification was performed as per British Committee for Standards in Haematology (BCSH) Blood Transfusion Task Force guidelines (Chapman et al., 2004). The result was considered false-positive when a positive result in the antibody identification panel did not match with the known antibody of the sample.
Statistical analysis

Statistical analysis was descriptive. Absolute and relative frequencies of variables in percentage were presented. In qualitative discrepancies, estimated sensitivity, specificity, predictive positive value (PPV), predictive negative value (PNV) and the efficiency of the tested microtube systems were used to compare the results between the Grifols DG Gel® system and the DiaMed-ID® reference method. Calculations were performed according to standard formulae (Vamvakas, 2001) including the 95% confidence intervals (CI).

RESULTS

In the global study, concordant results between Grifols DG Gel® microtube gel column agglutination system and the reference method were obtained in a total of 4182 of the 4281 performed tests (97.7%).

ABO/D grouping

Of the 1825 analyses performed for the ABO/D grouping, 1808 (99.1%) were concordant.

Discrepant results were obtained in 13 (0.71%) of the ABO grouping tests (12 qualitative and 1 quantitative, all of them due to reaction in the reverse group strength, mainly negative in one system and weak-positive in the other) and in 4 (0.22%) of the D grouping tests.

Of the 12 qualitative discrepancies for the ABO reverse grouping, 4 Group A samples (false-negative anti-B) and 5 Group O samples (4 false-negative anti-B and 1 false-negative anti-A) were correctly detected by the Grifols DG Gel® system but not by the DiaMed-ID®, whereas 2 Group A samples (1 false-positive with A cells and 1 false-negative with B cells) and 1 AB sample (false-positive anti-A) were correctly identified by the DiaMed-ID® but not by the Grifols DG Gel® system.

Of the four discrepancies for the D grouping, four weak-positive D (three of them from the same patient taken at different times during the study) were detected by the DiaMed-ID® system but not by the Grifols DG Gel® system.

These results are summarised in Table 1.

For ABO grouping by the Grifols DG Gel® system (in relation to DiaMed-ID® reference method), the sensitivity was 99.95% (99.69–99.99 CI), the specificity was 99.96% (99.87–99.99 CI), the PPV was 99.89% (99.60–99.97 CI) and the PNV was 99.98% (99.90–99.99 CI).

For D grouping, the sensitivity was 99.78% (99.44–99.91 CI), the specificity was 100% (99.79–100 CI), the PPV was 100% (99.79–100 CI) and the PNV was 99.78% (99.44–99.91 CI).

Antibody screening

Of the 1921 antibody screens performed, discordant results were observed in 19 samples (1.0%). Detailed concordances and discrepancies are summarised in Table 2.

Of the 17 results that were negative in Grifols DG Gel® system and positive in DiaMed-ID®, 15 were false-negative and 2 were false-positive. The 15 false-negative corresponded to one sample containing allo-anti-e and auto-anti-c, 1 LISS dependent pan-agglutination, 2 anti-K and 11 anti-D. Of the 11 anti-D results, 3 were weak alloimmune anti-D and 8 were residual prophylactic anti-D, all of them showing a + reaction strength with DiaMed-ID®.

Of the two results that were positive in Grifols DG Gel® and negative with DiaMed-ID®, one was a false-positive of Grifols DG Gel® and one was a false-negative of DiaMed-ID® (both for DAT).

The sensitivity by the Grifols DG Gel® system for antibody screening was 90.63% (85.11–94.24 CI), the specificity was 99.94% (99.68–99.99 CI), the PPV was

<table>
<thead>
<tr>
<th>Table 1. ABO/D grouping tests (n = 1825) comparing the Grifols DG Gel® system and the reference method (DiaMed-ID®)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>True</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>AB</td>
</tr>
<tr>
<td>O</td>
</tr>
<tr>
<td>D−</td>
</tr>
<tr>
<td>D+</td>
</tr>
</tbody>
</table>

Number of correctly (true) and incorrectly (false) identified group tests, are shown.
and 18% through full automation technique).

(82% of samples were processed by manual technique

A total of 361 tests for antibody identification, including

Antibody identification

·

E=2 6

DiaMed-ID

were 100% concordant between Grifols DG Gel

Of the 75 Rh subgroups and K types performed, results

Rh phenotyping and K antigen determination

·

Antibody screening tests (Table 2.

Positive Negative 17

Negative Positive 2

Negative Negative 1758

Positive Positive 144

99.32% (96.22–99.88 CI) and the PNV was 99.15% (98.61–99.49 CI).

A batch of eight Group and Screen samples took

Rh phenotype

n

·

CDe/cDE 26 26 34

·

cDE/cde 6 6 8

·

cDE/cDE 14 14 18.7

·

CDe/cDE 11 11 14.7

·

CDe/cde 26 26 34.7

·

CDe/CDc 0 0 0

·

Cde/cde 1 1 1.3

·

Cde/cde 8 8 10.7

·

cDE/cde 7 7 9.3

·

cDE/cDE 2 2 2.7

·

K– 23 23 92

·

K+ 2 2 8

Table 2. Antibody screening tests (n = 1921) comparing the Grifols DG Gel® system and the reference method (DiaMed-ID®)

<table>
<thead>
<tr>
<th>Grifols DG Gel®</th>
<th>DiaMed-ID®</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>1758</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>17</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>144</td>
</tr>
</tbody>
</table>

Number of concordant and discrepant results in antibody detection are shown.

99.32% (96.22–99.88 CI) and the PNV was 99.15% (98.61–99.49 CI).

A batch of eight Group and Screen samples took 46.5 min for Grifols DG Gel® and 45 min for DiaMed-ID®. Grifols DG Gel® used a 4-cell panel for antibody screening whereas a 3-cell panel was used by DiaMed-ID®.

Rh phenotyping and K antigen determination

Of the 75 Rh subgroups and K types performed, results were 100% concordant between Grifols DG Gel® and DiaMed-ID® systems. Table 3 shows the detailed blood group determinations.

Percentages of antigens were D = 88.0%, C = 69.3%, E = 26.7%, c = 81.3% and e = 97.3%.

Antibody identification

A total of 361 tests for antibody identification, including IAT and enzyme tests, were carried out on 212 samples (82% of samples were processed by manual technique and 18% through full automation technique).

Table 3. Rh phenotyping (n = 75) and K antigen
determination tests (n = 25) comparing the Grifols DG Gel®
system and the reference method (DiaMed-ID®)

<table>
<thead>
<tr>
<th>Rh phenotype and K antigen</th>
<th>Grifols DG Gel®</th>
<th>DiaMed-ID®</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDe/cDE</td>
<td>11</td>
<td>11</td>
<td>14.7</td>
</tr>
<tr>
<td>CDe/cde</td>
<td>26</td>
<td>26</td>
<td>34.7</td>
</tr>
<tr>
<td>CDe/CDe</td>
<td>14</td>
<td>14</td>
<td>18.7</td>
</tr>
<tr>
<td>CDe/CDe</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cde/cde</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>cde/cde</td>
<td>8</td>
<td>8</td>
<td>10.7</td>
</tr>
<tr>
<td>cDE/cde</td>
<td>6</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>cDE/cde</td>
<td>7</td>
<td>7</td>
<td>9.3</td>
</tr>
<tr>
<td>cDE/cDE</td>
<td>2</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>K–</td>
<td>23</td>
<td>23</td>
<td>92</td>
</tr>
<tr>
<td>K+</td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

Number and relative percentages of the blood groups found are shown.

Discordant results were observed in 53 (14.7%) tests, 20 of them were quantitative and 33 were qualitative (the antibody was detected only by one system).

Of the 33 qualitative discrepancies, 5 were nonspecific positive results (2 from Grifols DG Gel® and 3 from DiaMed-ID®) and 28 were related to determined antibody specificity. Table 4 shows the list of identified antibodies as well as those in which discrepancy of identification between Grifols DG Gel® and DiaMed-ID® systems were observed.

Of the 28 antibody specificity discrepancies (7.8% of the 361 total analyses), the Grifols DG Gel® system detected the antibody in 17 occasions (350 identified out of 361; 96.95% of accuracy), whereas the DiaMed-ID® system detected the antibody in 11 occasions (344 identified out of 361; 95.29% of accuracy).

There were 17 discrepancies between Grifols DG Gel® and the reference method in IAT. These included three antibodies identified using DG Gel® only (single examples of anti-E, -Jk b and -K). Using enzyme-treated cells, 11 discrepancies were identified with 4 antibodies being detected in DG Gel® but not DiaMed-ID® (single examples of anti-D, and -E and two examples of anti-C) and 2 antibodies detected with DiaMed-ID® only (single examples of anti-Jk b, and -Kp a). All these results are summarised in Table 4.

Newborn tests

Concordant results were obtained in 89 of the 99 newborn ABO/D and DAT tests performed (89.9%). Discrepant results were observed only in DAT, seven corresponded to weak-positive DAT detected by Grifols DG Gel® and not detected by the reference method (one of them was in the tube technique) and three corresponded to positive DAT detection only by DiaMed-ID® system.

Qualitative evaluation assessments

The BMS using the system reported that the well apertures of the 8-column system of the Grifols DG Gel® cards were slightly larger than those of the reference system. In addition, Grifols DG Gel® cards reactions were considered clear, stable and easy to read. Some reports highlighted that the plastic of the Grifols DG Gel® cards was clearer than that of the reference method. General performance was considered at least as good as DiaMed-ID®.

DISCUSSION

Advances in transfusion laboratory practice, which lead to improved performance, costs and patient safety are clearly desirable. This study was carried out to evaluate the sensitivity, specificity, suitability and adaptability
Table 4. Antibody identification tests (n = 361 in 212 samples)

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Total detected</th>
<th>Identification discrepancies (cases of antibody detected by one system only)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IAT Grifols DG Gel®</td>
<td>DiaMed-ID®</td>
<td>Enzyme Grifols DG Gel®</td>
</tr>
<tr>
<td>Anti-C</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Anti-c</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-C + D</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-C + e</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-c + E + K</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-C*</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-D</td>
<td>17</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-D + C + K</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-e</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-E</td>
<td>24</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Anti-E + K</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-f</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Fy*</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Jka</td>
<td>20</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Anti-Jkb</td>
<td>5</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Anti-K</td>
<td>26</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Anti-K + Jka</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Kpa</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Lea</td>
<td>3</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Anti-Lea + Leb</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Leb</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-M</td>
<td>21</td>
<td>6</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Anti-P1</td>
<td>2</td>
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<tr>
<td>Anti-S</td>
<td>14</td>
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<tr>
<td>Auto anti-Wk</td>
<td>2</td>
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<td></td>
<td></td>
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<tr>
<td>Pan/auto-agglutination</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LISS dependant pan/auto</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown specificity</td>
<td>1</td>
<td></td>
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</tbody>
</table>

Total antibodies as well as the number of discrepant results in identification (antibodies detected only by Grifols DG Gel® system and antibodies detected only by DiaMed-ID® method) for IAT and enzyme tests are shown.

1Two prophylactic and 1 weak.
2One pan-agglutination.
3One weak.

of Grifols DG Gel® cards (an 8-column microtube agglutination-based system new to the UK) to the daily blood transfusion laboratory routine. Grifols DG Gel® cards were compared with the reference method of five laboratories in the United Kingdom (DiaMed-ID® cards, a system with a well known performance reliability) (Bromilow et al., 1991; Weisbach et al., 1999; Grey et al., 2002; Novaretti et al., 2003, 2004; Paz et al., 2004; Cid et al., 2006; Schoenfeld et al., 2009) in almost all cases, and with the conventional tube technique in some newborn tests.

In this study, procedures and methodologies were set up according to two premises: first, to follow the gel card manufacturers’ instructions (including the use of different RBC suspension media and different centrifugation settings for Grifols DG Gel® and DiaMed-ID®) and secondly, application to the regular transfusion practice of the centres participating in the study, which raised procedural differences such as the use of manual techniques or automation instrumentation, that we have taken into account.

Regarding ABO/D grouping, Rh phenotyping/K antigen determination and antibody screening, two centres used fully automated instrumentation and RBC from DiaMed for DiaMed-ID® tests; whereas the other three centres used manual processing of the samples and RBC from NHSBT for DiaMed-ID® tests. For, newborn testing, one centre used DiaMed-ID® as the reference...
method, whereas the other centre used the conventional tube technique.

Since the objectives of our study were focused on the daily blood transfusion laboratory routine, results were evaluated collectively, acknowledging technical diversity. In fact, this is a common issue found when comparing the results reported by different laboratories, as different approaches and techniques are used for evaluation (Voak, 1992). In general, the Grifols DG Gel® system compared favourably with the reference system, (mostly DiaMed-ID®) as 97.7% of the results on both systems, in a sample size that was considered adequate (Voak, 1999), were fully concordant. Results obtained with Grifols DG Gel® met the BCSH validation guideline criteria (British Committee for Standards in Haematology, 1995).

For ABO/D grouping, sensitivity and specificity was between 99.78% and 100%. The observed discrepancies (0.9%) were all in the reverse group and were similarly distributed between both systems. Gel tests are known to show a high sensitivity in reverse grouping (Lapiere et al., 1990).

Four patient samples known to have weak D expression were detected by the reference technique but not by DG Gel®. Three of the four samples, however, were of the same patient. The second patient with a weak Rh D had very weak reactions in only one of the Diamed anti-D wells and negative for both Grifols D wells. It should be noted that differing reactions would be expected because Diamed and Grifols use different anti-D clones.

BCSH guidelines for pre-transfusion testing (United Kingdom Haemophilia Centre Doctors’ Organisation, 2003) and for blood grouping and antibody testing in pregnancy (Gooch et al., 2007) state that the evidence that weak D foetal red cells is minimal and indicate that the detection of most weak D types is adequate with high affinity anti-D reagents. Similarly there is no specific requirement to detect weak D in transfusion recipients, as non-detection results in the selection of D-red cells without risk of D alloimmunisation.

Antibody screening studies yielded interesting results as most of the 1% of discrepancies (11 of 17) was related to anti-D antibodies: three weak anti-D and eight residual prophylactic anti-D that were only detected by DiaMed-ID®. It is acknowledged in BCSH guidelines that no technique can detect all red cell antibodies (Chapman et al., 2004). Furthermore, it should be taken into account that residual prophylactic antibodies are of limited clinical significance (Castella et al., 2001), and the detection of such antibodies can potentially lead to further cost-increasing and time-consuming laboratory investigations. It has been suggested that individual cost-benefit analysis should be performed in every institution to decide whether a high-sensitive screening system should be applied (Weisbach et al., 2006). Sensitivity and specificity for Grifols DG Gel® were 90.63% and 99.94%, respectively. When comparing a new routine immunohaematology system with DiaMed-ID®, Schoenfeld et al. (2009) found suitable sensitivity of 83.3% and specificity of 92.8%. In a study by Cid et al. (2006) comparing three microtube column systems, including Grifols DG Gel® and DiaMed-ID®, 100% of sensitivity and specificity was observed for Grifols DG Gel®.

The number of samples tested for Rh phenotyping and K antigen determination was small. Nevertheless, 100% of concordance was observed between the reference method and Grifols DG Gel®. The frequency of Rh and K antigens strongly coincided with that found in the Caucasian population (Reid & Lomas-Francis, 2004).

Regarding antibody-specificity identification, both systems worked well with similar diagnostic accuracy. The rate of detection was similar, although slightly higher for Grifols DG Gel® (96-95%). However, the rate observed for DiaMed-ID® (95.29%) was higher than that reported by Weisbach and co-workers (91.6%) in a study comparing the performance of microtube systems (Weisbach et al., 1999). There were only three cases of antibodies detected by one system alone (anti-D, anti-E and anti-Kp²). In the case of anti-M in IAT and anti-K in enzyme test, there was a difference of three or more positives missed by one or another system (in benefit of Grifols DG Gel® for anti-M and in benefit of the reference method DiaMed-ID® for anti-K). It should be pointed out, however, that due to the proteolytic effect of the enzyme, the clinical significance of antibodies detected only with enzyme-treated cells is controversial (Mollison, 2005).

Altogether 17 examples of antibodies in IAT were identified in one test system only, of which 12 were detected only in DG Gel® and 5 only in DiaMed-ID®. Of these, eight were anti-M, and in BCSH guidelines M-units are not required for patients in whom anti-M is not detectable at 37°C (Chapman et al., 2004). Anti-M is a very rare cause of transfusion reactions. A further two were anti-Lea which is a clinically benign antibody. Of the remainder, three examples were in the Kidd blood group system in which weak antibodies are notoriously difficult to detect consistently. Overall, the performance of the systems was similar in antibody identification.

Grifols DG Gel® cards for newborns appeared to be more sensitive than the reference method. Six weak-positive DAT were missed by DiaMed-ID® and one was missed by using the conventional tube technique whereas only three were missed by DG Gel®. It is important to point out that although there is a trend to replace conventional tube test by microtube gel tests (Lapiere et al., 1990), the former is still a widely used reference method (Tissot et al., 1999).
Performance time measurements for both Grifols DG Gel® and DiaMed-ID® systems showed almost identical batch times for ABO/D grouping and antibody screen analysis. Taking into account that Grifols DG Gel® procedures were carried out on 8-column and 4-cell panel card format versus 6-column and 3-cell panel card format in the reference methods, the Grifols DG Gel® system appeared to perform more efficiently. For instance, the Grifols DG Gel® 8-column format allowed performing the complete ABO/D grouping (forward and reverse), K antigen determination and full Rh phenotype by using one and a half cards, whereas two complete 6-column DiaMed-ID® cards were needed for obtaining the same information. Additionally, Grifols DG Gel® used two cards with a 15-cell panel for antibody determination, whereas two cards with a 10- or 11-cell panel was used by DiaMed-ID® cards. This was abbreviated in most of the antibody identification cases. Moreover, Grifols DG Gel® cards for newborns allowed testing both forward ABO/D typing and DAT in one card, which was considered by the technical staff to reduce potential mistakes due to fewer steps having to be performed. Finally, the staff also reported some other helpful features of the Grifols DG Gel® 8-column card system, such as the large well apertures, making an easier target for pipetting and also reducing the problem of bubbles, and the easy reading of reactions due to the clear plastic of the cards.

As a general conclusion, the Grifols DG Gel® system (equipment and reagents) is reliable and safe for routine tests performed in the immunohaematology laboratory (ABO/D grouping, Rh phenotyping and K antigen determination, antibody screening and identification of the most clinically significant alloantibodies and newborn testing). The 8-column format of the Grifols DG Gel® cards allows performing a higher number of tests per cycle when 2- or 3-cell panel screenings are used, and is a suitable alternative for those laboratories using a microtube agglutination system.

DECLARATIONS OF INTEREST

The authors declare and acknowledge the support of Grifols to the present study. The authors state that opinions in this paper are those of themselves, and that they are fully responsible for what is written in it and were not influenced by the sponsor company. The authors are grateful to Dr. Jordi Bozzo for his help in the compilation of data for the manuscript preparation. All authors contributed equally to the study.

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