INTENDED USE
Confirmation of the blood groups of the ABO and Rh (D) system, in gel technique.

INTRODUCTION
The ABO system was the first human blood group system discovered, by Landsteiner in 1900, and is still the most important in transfusion practice. The ABO system is defined by the presence or absence of the A (ABO1) and/or B (ABO2) antigens in the red blood cells and by the presence of antibodies in the serum corresponding to the antigen or antigens missing in the red blood cells. The determination of Rh (D) is defined by the presence or absence of the D antigen (RH1) in the red blood cells.

The anti-A, anti-B and anti-D<sup>+</sup> reagents are used to perform the ABO and Rh blood group typing.

PRINCIPLE OF THE TEST
The principle of the test is based on the gel technique described by Y. Lapierre<sup>2</sup> for detecting red blood cell agglutination reactions. The agglutination occurs when the red blood cell antigens contact the corresponding antibodies, present in the reagent or in the serum or plasma sample. The DG Gel card is a plastic support consisting of 8 microtubes. Each microtube consists of a column and a dispensing/ incubation chamber. Each column contains polymerised dextran microspheres in buffered medium which act as a filter. The dextrans are mixed with a reagent that contains specific antibodies or a buffer. The microtubes that contain the specific antibodies into the gel solution act as a reaction medium and the red blood cells agglutinate in contact with the antibodies.

The microtubes without antibodies are used in techniques in which the antibodies react directly with the red blood cells in the incubation chamber and for controls. During centrifugation and depending on their size, the red blood cell agglutinations are trapped in the surface or along the gel column. The non-agglutinated red blood cells descend to the bottom of the microtube.

REAGENTS
Each microtube of the DG Gel Confirm P card contains polymerised dextrans in buffered medium, with preservatives and mixed with different reagents. The different microtubes are identified by the front label of the card:
- microtubes A: monoclonal anti-A (IgM antibodies of murine origin, clone Bima-1).
- microtubes B: monoclonal anti-B (IgM antibodies of murine origin, LB-2).
- microtubes D<sup>+</sup>: monoclonal anti-D (IgM antibodies of human origin, clone MS-201).
- microtubes Ctl. buffered solution without antibodies (control microtubes).

Reagent ready to use. Use the microtubes immediately once the seal has been opened.

STABILITY
DG Gel Confirm P is stable up to the expiry date stated on the label, with the seal intact and stored at 2–25 °C in the position indicated on the outer packaging. Do not freeze.

MATERIAL REQUIRED BUT NOT PROVIDED
- 10 μl, 50 μl and 2 ml automatic pipettes.
- Disposable pipette tips.
- Glass tubes.
- DG Gel Sol.
- Centrifuge for DG Gel cards.

SAMPLES
Blood samples recently collected with the routinely used anticoagulants (EDTA, citrate and heparin). Do not use haemolysed, cloudy or contaminated samples or with clot presence.

The procedure of extracting, collecting and handling the blood must be performed by qualified technician personnel according to current standards and directives<sup>1-4</sup> and following the instructions of the manufacturer of the material used for collecting the sample.

- Determination of the antigens of the ABO/Rh system: use the red blood cells collected with anticoagulants. If necessary, samples stored at 2–8 °C can be used up to 48 hours after their extraction.

Red blood cells from bags collected in CPD, CPDA or SAG-Mannitol can also be used until the expiry date indicated on the label, of the bag, if stored at 2–8 °C.

If red blood cells from the bag segment are used, it is recommended to wash them with physiological saline solution before preparing the suspension. Do not use if clots or haemolysis are observed.

TEST METHOD
DG Gel Confirm P can be used both in a manual method and with semi-automatic or automatic instruments. For the automatic system, see the instrument user manual.

Allow the samples and reagents to reach room temperature (18–25 °C). Inspect the state of the cards before use (see section LIMITATIONS "in relation to the product"). Identify the cards and samples to be used.

Each card is individually identified by a bar code. Unless a bar code reader is used, identify them manually.

If manual method is used: carefully peel off the metal film that covers the microtubes to prevent cross-contaminations among them and carefully dispense the red blood cell suspension, avoiding the pipette tip to come into contact with the wall or the content of the microtubes.

Manual method:
1. Preparation of the ABO/Rh groups (microtubes A/B/D<sup>+</sup>/Ctl.):
   - Centrifuge for DG Gel cards.
   - DG Gel Sol.
   - Disposable pipette tips.
   - Microtube A
   - Microtube B
   - Microtube D<sup>+</sup>

   Interpretation
   - Microtube A:
     - ++: A positive
     - +: A negative
   - Microtube B:
     - ++: B positive
     - +: B negative
   - Microtube D<sup>+</sup>:
     - ++: D positive
     - +: D negative

RESULT
Result Reading:

<table>
<thead>
<tr>
<th>Negative</th>
<th>+/–</th>
<th>+</th>
<th>+/+</th>
<th>+/+</th>
<th>DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/–</td>
<td>Scarce small-sized agglutinations in the lower half of the column.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td>Some small-sized agglutinations in the column.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2+</td>
<td>Small or medium-sized agglutinations throughout the column.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3+</td>
<td>Upper band of medium-sized agglutinations in the upper half of the column.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4+</td>
<td>Band of agglutinated red blood cells in the upper part of the column.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP</td>
<td>Double Population (double band of red blood cells, at the bottom and in the upper part of the column).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Stability of the results: it is recommended an immediate reading of the results after centrifuging the cards. Do not leave processed cards in horizontal position.

If necessary, a delayed reading can be made up until 24 hours after processing the cards if kept in vertical position, refrigerated (2–8 °C) and sealed with paraffin or similar material to avoid evaporation of the supernatant.

Interpretation of the results:

**ABO system**

<table>
<thead>
<tr>
<th>Microtube A</th>
<th>Microtube B</th>
<th>Microtube Ctl.</th>
<th>ABO Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>A</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>0</td>
<td>B</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>0</td>
<td>A/B</td>
</tr>
</tbody>
</table>

**Rh system (D antigen)**

<table>
<thead>
<tr>
<th>Microtube D&lt;sup&gt;+&lt;/sup&gt;</th>
<th>Microtube Ctl.</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>0</td>
<td>D positive</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>D negative</td>
</tr>
</tbody>
</table>

English
Notes:
1. The results by themselves alone are not a diagnosis. They must be evaluated together with the patient's clinical information and other data.
2. The microtube Ctl. must be negative. If it is positive, invalidate the test. Repeat the determination after washing the red blood cells with physiological saline solution and preparing a new suspension of the washed red blood cells. If the microtube Ctl. of the repetition is negative, the results of the test can be interpreted; if it is positive, invalidate the test.
3. ABO/Rh system: in the event of positive reactions, presence of a weak antigen must be investigated. In the event of +/- to 3+ reactions, presence of a weak antigen must be investigated.
4. Negative reactions with the anti-D\textsuperscript{\textcircled{R}} reagent must be verified, using other reagents and techniques which may detect different variants of the D antigen.
5. The observation of complete or partial haemolysis (pinkish supernatant and/or gel column) in microtubes must be interpreted as a positive result, after verifying that it is not due to a problem of extraction and/or handling of the sample.
6. Occasionally, a red blood cell retention in the incubation chamber may occur with positive 4+ samples, without interfering in result reading.

QUALITY CONTROL
1. It is recommendable to include known positive and negative controls in each series of tests.
2. If an unexpected control value is obtained, a complete verification of the instrument, reagents and material used must be made.

PERFORMANCE CHARACTERISTICS
Diagnostic sensitivity and specificity: ABO/Rh system
The diagnostic sensitivity and specificity of the antibodies present in the DG Gel Confirm P card\textsuperscript{5,6}, for the determination of the antigens of the ABO and Rh systems, have been studied in a representative number of positive and negative samples.

\[
\begin{align*}
\text{Antibody} & & \text{No. of Samples} & & \text{Sensitivity}\% & & \text{Specificity}\% \\
\text{Anti-A} & & 3041\textsuperscript{7} & & 100 & & 100 \\
\text{Anti-B} & & 3046\textsuperscript{7} & & 100 & & 100 \\
\text{Anti-D}\textsuperscript{\textcircled{R}} & & 1329\textsuperscript{8,9} & & 99.7 & & 100 \\
\end{align*}
\]
(a) Sensitivity: (number of true positive results / (number of true positive results + number of false negative results)) x 100
(b) Specificity: (number of true negative results / (number of true negative results + number of false positive results)) x 100
(c) These samples include 49 samples with weak expression of the D antigen. If only these samples are considered, the sensitivity of the anti-D\textsuperscript{\textcircled{R}} reagent is 93.9%.

Precision:
The precision\textsuperscript{10,11,12} of the reagents present in the DG Gel Confirm P card has been determined, including repeatability, inter-lot reproducibility and intra-laboratory reproducibility tests. No false positive or false negative results were obtained, and differences between agglutination intensities in positive samples were 1 agglutination grade or less in all assays.

LIMITATIONS
In relation to the sample:
Do not use haemolysed, cloudy or contaminated samples or with clot presence.
Sample: red blood cells
1. Red blood cells from individuals with A or B variants may present a weak expression of the antigens. Antigen expression may be weakened in red blood cells of people with leukaemia or other malignant diseases\textsuperscript{12}.
2. Abnormal concentrations of serum proteins, the presence of macromolecular solutions in the serum/plasma or the presence of Wharton’s jelly in cord blood samples may cause the non-specific agglutination of the red blood cells. It is recommended to wash the red blood cells before performing the test\textsuperscript{12}.
3. Transfused patients or those subjected to bone marrow transplant may present images of double population\textsuperscript{13}.
4. Patients with high-potency autoantibodies may coat the red blood cells completely, causing spontaneous agglutination\textsuperscript{13}.

In relation to the product:
Before use, inspect the microtubes of the cards:
1. Do not use the card if microbiological contamination or change in microtube colour is observed.
2. If due to improper transport or storage, dispersed drops at the top of the microtube are observed, it is recommended to centrifuge the card’s before use. If drops do not descend, do not use the card.
3. If trapped bubbles in the gel, any microtube without supernatant, a decrease in the gel volume or cracked gel are observed, do not use the card.
4. The anti-A reagent may not react with weak variants of Ax phenotype.

WARNINGS AND PRECAUTIONS
The use of “in vitro” diagnostic reagents for professional use requires taking into account the following indications:
- The reagents of the DG Gel Confirm P card of human monoclonal origin are manufactured using a non-reactive material for the HB, antigen nor for anti-HIV and anti–HCV antibodies, when tested with authorised reagents. Nevertheless, there is no known procedure to ensure that products of human origin will not transmit hepatitis or AIDS. Human blood products and samples must be handled as if they were potentially capable of transmitting infectious diseases.
- The product must only be used by qualified personnel.
- A red blood suspension of a concentration other than that indicated may cause false positive or negative results.
- The use of diluents other than DG Gel Sol for the red cell suspension may modify the reaction.
- The addition of volumes other than those indicated in the method may modify the reaction.
- Do not use the card beyond the expiry date.
- Once used, the product must be disposed of in special containers for biological waste.
- If you have any doubts or need further information on the use of this product, consult the authorised distributor in your country.

BIBLIOGRAPHY
6. External evaluation and internal study Diagnostic Grifols, S.A. (REGD-000124659, January 2013

PRESENTATION
210351 DG Gel Confirm P 50 cards Profile: 2x(A/B/D\textsuperscript{\textcircled{R}}/Ctl.)
Revision date: January 2013

This document is available in several languages. The translations have been made from the master document in Spanish. In the event of doubts or discrepancies, the wording in the master document in Spanish shall take precedence.

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