HUMAN IgG SUBCLASSES
SINGLE DILUTION BINDARID™ KITS

For in-vitro diagnostic use
Product Code: RN106.3, RN107.3, RN108.3, RN109.3 & RK021

Product manufactured by:
The Binding Site Group Ltd., 8 Calthorpe Road, Edgbaston, Birmingham, B15 1QT, UK.
www.bindingsite.co.uk
Telephone: +44 (0)121 456 9500
Fax: +44 (0)121 456 9749
e-mail: info@bindingsite.co.uk

FDA USA Information:
Complexity Cat.: High
Analyte ID Code: 2807
Test System ID Code: 61071

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INTENDED USE

This kit is for use in quantitating human IgG subclasses 1, 2, 3 or 4 in serum and other biological fluids.

SUMMARY AND EXPLANATION

In normal adults, IgG constitutes approximately 75% of the total serum immunoglobulins. Within the IgG class, the order of concentration of the 4 subclasses is IgG1 > IgG2 > IgG3 > IgG4, but the actual concentration of each may vary markedly between individuals.

The four IgG subclasses show considerable difference in their properties, including ability to fix complement, to bind to macrophages and to pass through the placenta. Abnormally high levels of one or more subclass may be associated with certain conditions, including anaphylaxis, autoimmune and gut diseases as well as hypo- and hyper-gammaglobulinaemia (Ref. 1). In particular, reduced production of IgG2 in children may be associated with recurrent infections (Ref. 2). The subject has been reviewed (Refs 3 & 4).

Single radial immunodiffusion methods are derived principally from the work of Fahey & McKelvey (Ref. 5) and Mancini et al (Ref. 6 & 7). These methods allow for quantitative determination of immunoglobulins and other proteins in biological fluids. Fahey and McKelvey demonstrated a relationship between the antigen concentration and the square of the ring diameter during diffusion. This relationship becomes linear on complete diffusion (Mancini & McKelvey, 1957). This is based upon important findings.

PRINCIPLE OF THE ASSAY

The method involves antigen diffusing radially from a cylindrical well through an agarose gel containing an appropriate mono-specific antibody. Antigen-antibody complexes are formed which, under the right conditions, will form a precipitin ring. The ring size will increase until equilibrium is reached between the formation and breakdown of these complexes, this point being termed ‘completion’. At this stage, a linear relationship exists between the square of the ring diameter and the antigen concentration. By measuring the ring diameters produced by a number of samples of known concentration, a calibration curve may be constructed. The concentration of the antigen in an unknown sample may then be determined by measuring the ring diameter produced by that sample and reading off the calibration curve.

There are three different procedures that may be used with this kit (see Section 8.4). Procedures ONE and TWO require that the rings are measured at completion. A linear calibration curve is constructed for Procedure THREE, whereas for Procedure ONE a reference calibration curve is provided, which converts ring diameters directly to protein concentrations. Using Procedure THREE, ring diameters are measured before completion; the calibration curve produced will be non-linear.

REAGENTS

4.1 RID plates (supplied in foil pouches). These contain monospecific antibody to IgG1, 2, 3 or 4 in agarose gel. Up to fourteen samples can be run per plate (including calibrator(s)). Preservatives: 0.099% sodium azide, 0.1% E-amino-n-capric acid (EACA), 0.01% benzamidine.

4.2 Calibrators. These are supplied in stabilised liquid form, made from stabilised pooled normal human serum. The concentration of IgG Subclasses given on the vial labels may have been obtained by comparison with the DA470x international reference material (Ref. 10 & 11). Preservatives: 0.099% sodium azide, 0.1% EACA, 0.01% benzamidine.

4.3 7% Bovine Serum Albumin (BSA) solution. This is supplied in stabilised liquid form and is used for use as a diluent. Preservatives: 0.099% sodium azide, 0.1% EACA, 0.01% benzamidine.

4.4 Control Serum. This is supplied in stabilised liquid form and is prepared from normal human serum. The expected concentrations for each subclass are marked on the vial label. Preservatives: 0.099% sodium azide, 0.1% EACA, 0.01% benzamidine.

CAUTION

All donors of human materials supplied in this kit have been tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either approved by the FDA (USA) or cleared for in vitro diagnostic use in the EU (Directive 98/78/EC, Annex II); however, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infectious material including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical attention.
medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained personnel for the purposes stated in the Intended Use. Strict adherence to these instructions is essential at all times. Reagents from different batch numbers of kits are NOT interchangeable. If large numbers of tests are performed care should be taken to ensure that all the reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kits should be stored at 2-8°C and can be used until the expiry date given on the kit box label. DO NOT FREEZE. RID plates should be stored at 2-8°C and are damaged by temperature extremes. Freezing will destroy the gel, therefore RID plates should be kept away from cooling elements in refrigerators. High temperatures should also be avoided as this will result in moisture loss from the gel, affecting performance. Unopened plates should be stored flat and upside down (pouch label uppermost) to prevent condensation accumulating in the wells. Handle plates with care to prevent gel damage. Unopened calibrators and controls should be stored at 2-8°C. Once opened they are stable for at least one week at 2-8°C, but for longer storage they should be aliquoted and frozen (-20°C or below). All other reagents should be stored at 2-8°C.

7 SPECIMEN COLLECTION AND PREPARATION

Use fresh or deep frozen serum samples. Microbiologically contaminated, haemolysed and very lipaemic serum samples or those containing particulate matter should not be used. Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum may be stored at 2-8°C for up to 48 hrs prior to assay. For prolonged storage aliquot and store at -20°C or below. Repeated freezing and thawing should be avoided. The BSA included in the kit should be used as diluted when required, as this will maintain the viscosity of the sample. Results can therefore be accurately compared with the calibrators which have a similar viscosity as the serum. BSA should also be used when necessary for diluting other less viscous specimens (eg. urine).

8 METHODOLOGY

8.1 Materials provided

<table>
<thead>
<tr>
<th>Individual kits: Codes RN106.3, RN107.3, RN108.3, RN109.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 3 x Human IgG Subclass 1/2/3/4 SD BINDARID (Radial immunodiffusion plates in foil pouches)</td>
</tr>
<tr>
<td>2. 8 x Gel dividers</td>
</tr>
<tr>
<td>3. 1 x Human IgG Subclass 1/2/3/4 SC Calibrator (Liquid calibrator)</td>
</tr>
<tr>
<td>4. 3 x 5%L, 7% BSA Solution for RN106.3, RN107.3</td>
</tr>
<tr>
<td>5. 1 x Human IgG Subclass Control Serum</td>
</tr>
<tr>
<td>6. 1 x instruction leaflet, including RID Reference table.</td>
</tr>
</tbody>
</table>

Human IgG Subclass Combi kit RK021

| 1. 4 x Human IgG Subclass 1/2/3/4 SD BINDARID (Radial immunodiffusion plates in foil pouches) |
| (One plate for each IgG subclass) |
| 2. 8 x Gel dividers |
| 3. 4 x Human IgG Subclass 1/2/3/4 SC Calibrator (Liquid calibrator) |
| 4. 2 x 5%L BSA Solution |
| 5. 1 x Human IgG Subclass Control Serum |
| 6. 1 x instruction leaflet, including RID Reference table. |

8.2 Materials required but not provided

| 8.2.1 Equipment for collection and preparation of test samples, eg sample tubes, centrifuge etc. |
| 8.2.2 Pipettes for accurate dilution of samples, when required. |
| 8.2.3 Micropipettes for sample application. These should be capable of accurately delivering 5uL volumes. Binding Site Micropipettes (code AD041) or ‘Hamilton’ syringes are recommended. |
| 8.2.4 Jewellers’ Eyepiece (code AD040) or digital RID plate reader (AD400) for magnifying and accurately measuring the precipitin ring diameters to 0.1mm. |
| 8.2.5 Graph paper |

8.3 Reagent preparation

8.3.1 RID plates

To avoid contamination of the gel, plates should be used in a dust-free environment. Take the plate from the foil pouch and store in a dust free environment. Make sure the list, showing the antigen and the test samples, is kept up to date and any wells which are not used are closed with paraffin wax. For shelf life of the gel, the list has to be kept up to date and removed from plates any time they are not used.

Plate partitioning: The plates may be partitioned into up to four sections using the gel divider(s) provided prior to use. Each divider should be positioned carefully on the gel, cutting edge downward, with the stabilising arm resting on the central plate label. Press firmly on the arm to cut the gel and leave in position.

Plate partitioning is recommended if only part of the plate is to be used initially or when measuring high concentration samples which could (by diffusing over a wide area) result in antibody depletion occurring elsewhere on the plate. After initial use, partitioned plates should be resealed in their foil pouches and stored at 2-8°C with the gel divider(s) in place. Store partitioned plates right side up and use within four weeks.

8.3.2 Calibrator(s)

The calibrator is liquid and must be mixed gently before use. It should be applied to the plates neat. Dilutions of the calibrator must be made if a calibration curve is required (as for Procedures ONE and TWO). These dilutions should normally be a medium dilution (60%, ie 6 parts in 10) and a low dilution (10%, ie 1 part in 10). It is recommended that 60L of calibrator is mixed with 40L of the diluent provided (7% BSA), for 25L of dilution, and 25L of calibrator is mixed with 225L of the diluent for a 10% dilution.

8.3.3 Controls

The liquid control serum should be treated in the same way as a patient sample. For a 1/10 dilution recommended for IgG1 and IgG2 assays, it is suggested that 25L of control is mixed on the day of assay with 225L of the BSA provided. Dilution of the control is not required for IgG3 and IgG4 assays.

8.3.4 Samples

IgG1 and IgG2 samples should be diluted 1/10 (1 part in 10) on the day of assay. To obtain adequate accuracy it is recommended that 25L of test sample is mixed with 225L of 7% BSA. IgG1 and IgG4 samples do not normally require dilution. However, with myeloma paraproteins, greatly increased dilutions may be required. In such cases it is advisable to estimate the approximate concentration of the myeloma protein by archetypal methods before measuring the dilution (eg by determining the increase in the total protein concentration compared with normal serum). For samples having very low IgG subclass concentrations (eg paediatric serum samples), one of the following is recommended:

i. Apply the sample undiluted or at a reduced dilution (IgG1 and IgG2 only)
ii. Concentrate the sample
iii. Make a double fill of the well (see section 8.5).

8.4 Procedures

8.4.1 Procedure ONE: RID reference table

This method does not require the construction of a calibration curve – sample concentrations corresponding to each ring diameter are read directly from the RID Reference Table. Rings must be allowed to develop to completion which will require a minimum diffusion time of 72 hours. The neat calibrator should be run on each plate used to ensure all are performing correctly.

8.4.2 Procedure TWO: Calibration curve at completion

In this method, the neat calibrator plus two dilutions are used to produce a linear calibration curve. Rings must be allowed to develop to completion, which will require a minimum diffusion time of 72 hours. To complete the calibration, the neat calibrator must be run concurrently for several plates of the same batch. In such cases, the neat calibrator should be run on each plate to ensure all are performing correctly.

8.4.3 Procedure THREE: Calibration curve prior to completion

In this method, the neat calibrator plus the two dilutions are used to produce a calibration curve, instead of a non-linear one, for complete calibration. The recommended diffusion time is 6 hours but more accurate results will be obtained after 24 hours diffusion. It is advisable that a separate calibration curve be constructed for each used plate.

8.5 Application of calibrators and samples

The calibrator(s), control and test samples should be gently mixed immediately before use. Fill required number of wells with 5uL of the neat calibrator using a micropipette. If Procedure TWO or THREE is being followed, also fill the required number of wells with the medium and low dilution calibrators. The remaining wells should then be filled with 5uL of appropriate diluted test samples and controls. Plates should not be left open for long periods during calibrator/test sample application, as this will cause excessive drying of the gel.

Note: For those samples suspected of containing low concentrations of the specific proteins, ‘dilute’ samples may be used. For calibration, neat calibrator/test samples are run in parallel with the ‘dilute’ sample. The ‘dilute’ sample should be included in the calibration procedure.

8.6 Incubation

After sample application, the lid is tightly closed and the plate stored flat with the lid uppermost at room temperature 20-24°C. It is essential that the gel is not allowed to dry out during incubation. To minimise evaporation, it is suggested that plates should either be resealed in the foil pouch or stored in a moist box (a sealed plastic box containing damp tissue paper) during incubation. The minimum incubation time for Procedure THREE is 6 hours and for complete calibration (Procedures ONE and TWO) is 72 hours. Final ring diameters may be affected by temperature; the expected ring size for the neat calibrator is 9mm (± 0.3mm) when incubated at 20-24°C. Extremes of temperature should be avoided. On complete diffusion, rings may remain stable for several days if maintained at the diffusion temperature. Beyond this, drying of the gel may lead to ring distortion.

8.7 Quality control

The control serum should be treated exactly like a test sample. Values obtained for each control should be within ±15% of the concentration stated on the vial label.

9 RING MEASUREMENT AND RESULT PROCESSING

After the required diffusion time, ring diameters should be measured to the nearest 0.1mm, using jeweller’s eyepiece or a RId reader. When reading with an eyepiece, use bright side lighting and a dark background. If difficulties are experienced, view the plate macroscopically and mark the edges of the rings on the back of the plate using a needle. The distance between these marks may then be more easily measured.

If any patient sample shows an unexpected result, this procedure must be repeated to confirm initial findings.

Note: For Procedures ONE and TWO ring diameters must have developed to completion. If there is any doubt, rings should be remeasured after a further 24 hours incubation. If there is still any doubt, rings should be remeasured again after a further 24 hours incubation.

If any patient sample shows an unexpected result, this procedure must be repeated to confirm initial findings.

Note: For Procedures ONE and TWO ring diameters must have developed to completion. If there is any doubt, rings should be remeasured after a further 24 hours incubation. If there is still any doubt, rings should be remeasured again after a further 24 hours incubation.
Example:

<table>
<thead>
<tr>
<th>Test Sample</th>
<th>Dilution</th>
<th>Ring Diameter (mm)</th>
<th>Table Value (mg/L)</th>
<th>Original Sample Conc. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1 Serum A</td>
<td>1/10</td>
<td>6.4</td>
<td>6700</td>
<td>6100</td>
</tr>
<tr>
<td>IgG1 Serum B</td>
<td>1/10</td>
<td>&lt;4.0</td>
<td>&lt;1180</td>
<td>&lt;1180</td>
</tr>
<tr>
<td>IgG1 Serum B (repeat)</td>
<td>1/2</td>
<td>5.8</td>
<td>4660</td>
<td>930</td>
</tr>
</tbody>
</table>

*Calculated as follows: Table value x Recommended Diln. / Actual Diln. i.e. 4660mg/L x (110)/(1/2).

Note: The calibrators provided are prediluted and applied to the plates neat. Therefore IgG1 neat calibrator (1400mg/L) giving a 9.0mm ring is equivalent to an original sample concentration of 1400 x 10 = 14000mg/L, the table value.

Procedure TWO

Plot the square of the diameters of the precipitin rings formed by the neat calibrator and the two dilutions versus their IgG subclass concentration (given on the calibrator vial label). IgG subclass concentrations should be along the horizontal (x) axis, ring diameters squared along the vertical (y) axis. A line of best fit is drawn through the three points; the y-intercept should be in the range 10 - 15mm². The IgG subclass concentration is determined from the calibration curve; remember to adjust the sample concentration obtained by any dilution factor used.

Sample Calculation:

IgG2 calibrators (ie the neat calibrator plus 60% and 10% dilutions) gave the following diameters on an IgG2 test plate:

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Concentration (mg/L)</th>
<th>Diameter (D) (mm)</th>
<th>D squared (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat</td>
<td>800</td>
<td>9.0</td>
<td>81.0</td>
</tr>
<tr>
<td>60%</td>
<td>480</td>
<td>7.3</td>
<td>53.3</td>
</tr>
<tr>
<td>10%</td>
<td>80</td>
<td>4.1</td>
<td>16.8</td>
</tr>
</tbody>
</table>

A calibration curve was plotted using these results:

An unknown serum sample, diluted 1/10 with 7%BSA as recommended, gave a 6.3mm diameter ring on this plate. From the above curve, this corresponds to an IgG2 concentration of 3430mg/L. Therefore, the IgG2 concentration in the undiluted sample = 10 x 343 = 3430mg/L.

Procedure THREE

Plot the calibration curve as for Procedure TWO. The graph will not be a straight line but a curve, the gradient of which decreases with increasing protein concentration. The y-intercept should be as indicated for Procedure TWO. Test sample protein concentrations are read off the calibration curve; remember to adjust the sample concentration obtained by any dilution factor used.

Sample Calculation:

IgG2 calibrators (ie the neat calibrator plus 60% and 10% dilutions) gave the following diameters on an IgG2 test plate after 24 hours:

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Concentration (mg/L)</th>
<th>Diameter (D) of ring (mm)</th>
<th>D squared (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat</td>
<td>800</td>
<td>7.9</td>
<td>62.4</td>
</tr>
<tr>
<td>60%</td>
<td>480</td>
<td>6.8</td>
<td>46.24</td>
</tr>
<tr>
<td>10%</td>
<td>80</td>
<td>4.2</td>
<td>17.64</td>
</tr>
</tbody>
</table>

A calibration curve was plotted using these results:

An unknown serum sample, diluted 1/10 with 7%BSA as recommended, gave a 6.3mm diameter ring on this plate. From the above curve, this corresponds to an IgG2 concentration of 3430mg/L. Therefore, the IgG2 concentration in the undiluted sample = 10 x 343 = 3430mg/L.

10 LIMITATIONS OF PROCEDURE

10.1 For Procedure ONE, accurate results are limited to the range given for the individual IgG subclasses on the RID Reference Table. However, results generated from ring diameters greater than the neat calibrator ring diameter (i.e. 9mm) should be regarded as approximate (see Section 9). For Procedures TWO and THREE, accurate results are limited to the calibration line between the neat and low calibrator dilution values (extrapolation beyond these points is not valid). Sample giving results outside these ranges must be diluted or concentrated as appropriate and retested (see Section 8.3.4).

10.2 These plates have been shown to give measurable results with a range of samples containing the relevant IgG subclass. However, it is not possible to exclude the existence of an occasional atypical with unusual determinants (particularly from a myeloma patient) that may be undetected by this technique. Similarly, an unusual variant of one subclass may infrequently occur that gives a faint second ring on a different subclass plate (Ref 9).

10.3 FDA (USA) Information – see front page.

10.4 TROUBLESHOOTING

A calibration curve was plotted using these results:

![Diameter Squared (mm²) vs IgG2 Concentration (mg/L) graph]

**Problem**

1. Neat calibrator (less than 8.7mm) (Procedures 1 and 2 only)
   - i) Inaccurate ring measurement
   - ii) Incorrect volume applied
   - As for B1 above

2. Test samples (above acceptable range – see Section 10.1)
   - i) Concentration too high
   - ii) Inaccurate volume applied
   - Dilute the sample(s) responsible and repeat assay using new plate.

D. Double/multiple rings:

- Non-specific precipitation close to well (due to PEG in gel)
- Poor sample application
- Calibrator deterioration
- Incubation temperature too low
- Incorrect volume applied

E. Non-circular rings:

- Poor sample application
- Gel dried out before use
- Local antibody depletion
- Uneven gel (plate not stored flat)

F. Cloudy gel:

- Male has been frozen
- Gel dried out before use
- For E (iii) above.
Normal IgG and IgG subclass concentrations were obtained for these kits using sera obtained from 124 normal adult British Blood donors. These results are illustrated below as histograms.

Serum IgG subclass concentrations are age dependent. The data below was obtained by measuring the total IgG and subclass content of paediatric serum samples from a Birmingham UK hospital using these kits. The number of samples used in each category is shown as N. All concentrations are in mg/L.

### RID paediatric data (mg/L)

<table>
<thead>
<tr>
<th>Age</th>
<th>IgG1 Mean (N)</th>
<th>IgG2 Mean (N)</th>
<th>IgG3 Mean (N)</th>
<th>IgG4 Mean (N)</th>
<th>Total IgG Mean (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2 years</td>
<td>0.1004 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1004 (5)</td>
</tr>
<tr>
<td>2-4 years</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
</tr>
<tr>
<td>4-6 years</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
</tr>
<tr>
<td>6-8 years</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
</tr>
<tr>
<td>8-10 years</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
</tr>
<tr>
<td>10-12 years</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
</tr>
<tr>
<td>12-14 years</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
</tr>
<tr>
<td>14-18 years</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
<td>0.1240 (5)</td>
</tr>
</tbody>
</table>

### PERFORMANCE CHARACTERISTICS

**Precision**

The precision (repeatability) of this kit is expressed as the mean and the percentage coefficient of variation (CV) which had been determined using prediluted samples. All analyses were performed in our laboratory. Each value was calculated from 10 measurements (duplicate determinations on five separate plates from a typical batch) unless otherwise stated. Rings were measured after 72 hours for Methods 1 and 2, and after 6 hours and 24 hours for Method 3.

<table>
<thead>
<tr>
<th>Sample pool</th>
<th>Method 1 (Tabular) mean conc. (mg/L) CV</th>
<th>Method 2 (End point) mean conc. (mg/L) CV</th>
<th>Method 3 (timed diffusion) 6 hour mean conc. (mg/L) CV</th>
<th>Method 3 (timed diffusion) 24 hour mean conc. (mg/L) CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1 High</td>
<td>12120 (2.4%)</td>
<td>12100 (2.6%)</td>
<td>11200 (8.6%)</td>
<td>12600 (4.5%)</td>
</tr>
<tr>
<td>Medium</td>
<td>7560 (2.5%)</td>
<td>7560 (2.6%)</td>
<td>6670 (3.4%)</td>
<td>7620 (3.8%)</td>
</tr>
<tr>
<td>Low</td>
<td>2740 (5.2%)</td>
<td>2730 (2.4%)</td>
<td>3000 (5.6%)</td>
<td>2680 (5.2%)</td>
</tr>
<tr>
<td>IgG2 High</td>
<td>7235 (0.8%)</td>
<td>7140 (0.8%)</td>
<td>6330 (3.5%)</td>
<td>7360 (2.8%)</td>
</tr>
<tr>
<td>Medium</td>
<td>4410 (1.5%)</td>
<td>4370 (1.0%)</td>
<td>3800 (2.7%)</td>
<td>4360 (2.3%)</td>
</tr>
<tr>
<td>Low</td>
<td>1580 (4.6%)</td>
<td>1580 (4.0%)</td>
<td>1740 (5.5%)</td>
<td>1470 (4.9%)</td>
</tr>
<tr>
<td>IgG3 High</td>
<td>1020 (2.2%)</td>
<td>1040 (1.9%)</td>
<td>1010 (4.9%)</td>
<td>1070 (2.5%)</td>
</tr>
<tr>
<td>Medium</td>
<td>601 (3.3%)</td>
<td>620 (2.9%)</td>
<td>737 (7.1%)</td>
<td>698 (3.2%)</td>
</tr>
<tr>
<td>Low</td>
<td>210 (4.71)</td>
<td>235 (4.55)</td>
<td>273 (8.51)</td>
<td>235 (4.55)</td>
</tr>
<tr>
<td>IgG4 High</td>
<td>435 (1.66%)</td>
<td>442 (1.64%)</td>
<td>373 (6.69%)</td>
<td>438 (3.25%)</td>
</tr>
<tr>
<td>Medium</td>
<td>251 (3.74%)</td>
<td>261 (3.60%)</td>
<td>278 (8.79%)</td>
<td>268 (2.51%)</td>
</tr>
<tr>
<td>Low</td>
<td>86.2 (2.93%)</td>
<td>97.0 (3.26%)</td>
<td>102 (5.66%)</td>
<td>93.2 (4.07%)</td>
</tr>
</tbody>
</table>

**If a problem cannot be resolved, please refer to supplier.**
Within-plate and inter-batch variation

The within-plate variation is expressed as the mean ± standard deviation (SD) of determinations of CV made using 3 plates from separate batches. Six measurements of end-point ring diameters were made per plate, using a pre-diluted human serum pool as the sample.

The inter-batch variation is expressed as the CV of mean diameter values obtained from 3 typical batches of plates. The mean diameter for each batch was calculated by measuring the end-point ring diameters obtained using a prediluted human serum pool sample, applied to three plates from each batch (six ring measurements per plate).

### Table: Within-plate variation

<table>
<thead>
<tr>
<th>Subclass</th>
<th>Within-plate variation (%)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>0.97 ±/− 0.94</td>
<td>0.32</td>
</tr>
<tr>
<td>IgG2</td>
<td>0.58 ±/− 0.32</td>
<td>0.26</td>
</tr>
<tr>
<td>IgG4</td>
<td>0.85 ±/− 0.13</td>
<td>1.14</td>
</tr>
<tr>
<td>IgG4</td>
<td>0.89 ±/− 0.36</td>
<td>0.33</td>
</tr>
</tbody>
</table>

### BIBLIOGRAPHY


### SUMMARY OF PROEDURE

14.1 Select Procedure ONE, TWO or THREE. Procedure THREE must be used if results are required quickly.

14.2 Prepare sample and control dilutions (1/10) for IgG1 and IgG2 assays. Sample dilutions are not normally required for IgG3 and IgG4 assays (see 8.3.3 and 8.3.4).

14.3 Allow condensation to evaporate from RID plate(s).

14.4 Apply calibration(s), control and samples to RID plate(s) in 5 µL volumes.

14.5 Replace lid(s) and incubate at room temperature 20-24°C for fixed time period - minimum 6 hours (Procedure THREE) or until rings are complete (minimum 72 hours) (Procedures ONE and TWO).

14.6 Measure the ring diameters.

14.7 Read results off RID Reference Table (Procedure ONE) or plot calibration curve and read off results (Procedures TWO and THREE).