**Intended Use**

This assay is designed for the *in-vitro* measurement of circulating immune complexes (CIC) that bind C1q, present in human serum. It is intended for the determination of CIC in serum of patients with various autoimmune and other CIC-related diseases, and is used in conjunction with other clinical findings.

Sufficient materials are supplied to allow a maximum of 41 samples to be tested in duplicate or 89 in single, with a calibration curve and a positive and negative control.

**Summary and Explanation of the test**

Antigen-antibody interactions can result in the formation of immune complexes. This is part of the body's normal and continuous immunological function. Antigens may be fixed in the tissue or free in plasma or other body fluids forming circulating immune complexes. Normally CIC are removed harmlessly by the reticuloendothelial system. However, large deposits of immune complexes in vascular structures with subsequent activation of inflammatory pathways such as the complement cascade, can result in an immune complex disease state with accompanied tissue damage. The fate of these complexes depends upon many variables, including the nature of antigen and antibody class, the size of the complex, their rate of production and the functional integrity of the phagocytic system.

Because of the pathogenic role of CIC in many diseases, specific methods have been developed to detect them. These take advantage of the specific physicochemical properties of CIC's and/or of their ability to bind to cell surfaces eg Raji cells and platelets, or molecules such as C1q\(^1, 2, 3\). Indeed, a World Health Organisation study recommended that CIC measurement be performed by at least two distinct assay methods\(^4\).

Measurement of the serum concentrations of C1q binding CIC by ELISA is prognostically important\(^5\). It is particularly suitable for monitoring CIC levels in patients with systemic lupus erythematosus, where the levels vary with disease activity and depressed complement responses\(^6\).

**Principles of the Procedure**

Microwells are pre-coated with purified human C1q. The calibrators, controls and diluted patient samples are added to the wells and C1q binding immune complexes are bound by the C1q during the first incubation. After washing the wells to remove all unbound proteins, purified peroxidase labelled goat anti-human IgG conjugate is added. The conjugate binds to the captured immune complex and the excess unbound conjugate is removed by a further wash step.

The bound conjugate is visualised with 3,3',5,5' tetramethylbenzidine (TMB) substrate which gives a blue reaction product, the intensity of which is proportional to the concentration of CIC in the sample. Sulfuric acid is added to each well to stop the reaction. This produces a yellow end point color, which is measured at 450nm. The concentration of CIC in the sample is read from the calibration curve and results are expressed as heat aggregated human IgG equivalents per mL (μg Eq/mL).

**Reagents**

1. Polystyrene microwell ELISA plate coated with human C1q antigen (12-1 x 8 wells), with holder in foil package containing desiccants
2. ELISA Negative Control, 1 vial of buffer containing preservative and human serum with no human antibodies to C1q, prediluted, 1.2mL
3. C1q CIC ELISA Calibrator A, 1 vial of buffer containing preservative and aggregated human IgG to C1q, prediluted, 1.2mL
4. C1q CIC ELISA Calibrator B, 1 vial of buffer containing preservative and aggregated human IgG to C1q, prediluted, 1.2mL
5. C1q CIC ELISA Calibrator C, 1 vial of buffer containing preservative and aggregated human IgG to C1q, prediluted, 1.2mL
6. C1q CIC ELISA Calibrator D, 1 vial of buffer containing preservative and aggregated human IgG to C1q, prediluted, 1.2mL
7. C1q CIC ELISA Calibrator E, 1 vial of buffer containing preservative and aggregated human IgG to C1q, prediluted, 1.2mL
8. C1q CIC Positive Control, 1 vial of buffer containing preservative and aggregated human IgG to C1q, prediluted, 1.2mL
9. Type III Sample Diluent, 2 vials – colored yellow containing Tween 20, protein stabilizers and preservative, 50mL
10. HRP Wash Concentrate, 1 vial of 40x concentrate – colored red containing Tris-buffered saline, Tween 20 and a preservative, 25mL. Refer to the Methods Section for dilution instructions.
11. HRP C1q CIC IgG Conjugate, 1 vial – colored blue containing buffer, protein stabilizers and preservative, 10mL
12. TMB Chromogen, 1 vial containing stabilizers, 10mL
13. HRP Stop Solution, 0.344M Sulfuric Acid, 1 vial – colorless, 10mL
**Warnings**

1. **WARNING:** This product contains a chemical (0.02% chloramphenicol) in the Sample Diluent, controls and conjugate known to the State of California to cause cancer. The sample diluent also contains Proclin 150. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
2. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the C1q CIC Positive Control, C1q CIC Calibrators A through E and ELISA Negative Control should be handled in the same manner as potentially infectious material.
3. Sodium Azide is used as a preservative. Sodium Azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
4. The HRP C1q CIC IgG conjugate contains a dilute poisonous/corrosive chemical, which may be toxic if ingested in large amounts. To prevent possible chemical burns, avoid contact with skin and eyes.
5. TMB Chromogen contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
6. The HRP Stop Solution consists of a dilute sulfuric acid solution. Avoid exposure to bases, metals, or other compounds, which may react with acids. Sulfuric acid is a poison and corrosive, which may be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
7. Use appropriate personal protective equipment while working with the reagents provided.
8. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

**Precautions**

1. This product is for **In Vitro** Diagnostic Use.
2. Appropriately trained personnel should only use this product.
3. Strict adherence to the protocol is recommended. Any deviation may affect assay performance, and the results obtained. Pay attention to specific ‘Notes’ and warnings throughout these Instructions for Use.
4. 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 reagents are from the **SAME** batch. All strips used must be taken from the same foil pouch. Substitution of any component may lead to incorrect results.
5. To avoid reagent contamination, only use new or clean plastic/glassware. **Never** return unused reagents to the bottles.
6. Do **not** leave reagent bottles uncapped; any resulting evaporation or contamination will lead to inconsistent results.
7. TMB substrate must not be exposed to light or water.
8. Microbiolally contaminated, haemolysed or lipaemic serum and specimens containing particulate matter should not be used.
9. Inaccurate sample dilution cannot be checked, as kit controls are ready to use. The use of calibrated pipettes and appropriate internal QC samples is recommended.
10. The use of automated assay systems, sample dilutors and other automated equipment may lead to differences in results when compared to the manual procedure. It is the responsibility of any laboratory to fully validate the system, and ensure the results fall within the limits as defined in this insert and associated QC certificate.
11. All equipment used must be calibrated and maintained according to the manufacturer’s instruction.

**Storage Conditions**

1. The kit should be stored at 2-8°C and should **not** be frozen. Inappropriate storage temperatures will affect the results.
2. Diluted wash buffer is stable for 1 week at 2-8°C.
3. The expiry date of the kit is shown on the outer label.

**Specimen Collection**

1. Blood samples should be collected by venepuncture allowed to clot naturally and the serum separated.
2. The serum may be stored at 2-8°C for up to 7 days prior to assay, or for prolonged storage, aliquoted and stored at -20°C or below.
3. Repeated thawing and freezing should be avoided.
4. Serum samples should **not** be heat-inactivated, as this may give false positive results.
Procedure
Materials provided

- **Instruction leaflet:** Giving full assay details.
- **QC Certificate:** Indicating the expected performance of the batch.
- **C1q Coated Wells:** 12 breakapart 8 well strips coated with human C1q. Each plate is packaged in a re-sealable foil bag containing two desiccant pouches.
- **ELISA Negative Control:** 1 bottle containing 1.2mL of diluted human serum. The expected value is given on the QC certificate. Ready to use.
- **C1q CIC Calibrators:** 5 bottles, each containing 1.2mL of diluted aggregated human IgG, with the following concentrations of immune complex: 100, 33.3, 11.1, 3.7, 1.23 µg Eq/mL. Ready to use.
- **C1q CIC Positive Control:** 1 bottle containing 1.2mL of diluted aggregated human IgG. The expected value is given on the QC certificate. Ready to use.
- **Type III Sample Diluent:** 2 bottles containing 50mL of buffer for sample dilution. Colored yellow, ready to use.
- **HRP Wash Concentrate:** 1 bottle containing 25mL of a 40-fold concentrated buffer for washing the wells.
- **HRP C1q CIC IgG Conjugate:** 1 bottle containing 10mL of purified peroxidase labelled antibody to human IgG. Colored blue. Ready to use.
- **TMB Chromogen:** 1 bottle containing 10mL of 0.344M Sulfuric Acid. Ready to use.
- **HRP Stop Solution:** 1 bottle containing 10mL of 0.344M Sulfuric Acid. Ready to use.

Additional materials required but not provided

- **Automatic microplate plate washer:** This is recommended, however, plate washing can be performed manually.
- **Plate reader:** Capable of measuring optical densities at 450nm referenced on air.
- **Distilled or deionised water:** This should be of the highest quality available
- **Calibrated micropipettes:** For dispensing 1000, 100 & 10µL
- **Multichannel pipette:** Recommended for dispensing 100µL volumes of conjugate, substrate and stop solution.
- **Glass/plastic tubes:** For sample dilution

Method
Before you start

1. **Bring the kit to room temperature**
   The kit is designed for room temperature operation (20-24°C).
   Remove the kit from storage and stand at room temperature for approximately 60 minutes. Wells must not be removed from the foil bag until they have reached room temperature.

2. **Kit components**
   Gently mix each kit component before use.

3. **Wash buffer (40x concentrate) dilution**
   Dilute the HRP Wash Concentrate 1:40 by adding the contents of the HRP Wash Concentrate bottle to 975mL of distilled or deionized water. If the entire plate will not be run within this period, a smaller quantity can be prepared by adding 2.0mL of the concentrate to 78mL of distilled or deionized water for every 16 wells that will be used. The diluted buffer is stable for 1 week at 2-8°C.

4. **Sample dilution**
   Dilute 10mL of each sample with 1000mL of sample diluent (1:101) and mix well. **Note:** Diluted sample must be used within 8 hours.

5. **Strip and frame handling**
   Place the required number of wells in the strip holder. Position from well A1, filling columns from left to right across the plate. When handling the plate, squeeze the long edges of the frame to prevent the wells falling out. **Note:** Return unused wells to the foil bag immediately with the two desiccant pouches and re-seal tightly to minimise exposure to moisture. Take care not to puncture or tear the foil bag, see below. **WARNING:** Exposure of wells to moisture or contamination by dust or other particulate matter will result in antigen degradation, leading to poor assay precision and potentially false results.

Assay procedure

1. **Sample addition**
   Dispense 100µL of calibrator, assay controls and diluted (1:101) sample into the appropriate wells of the plate provided. **Note:** Samples must be added to the plate as quickly as possible to minimise assay drift, and the timer started after the addition of the last sample. **Incubate at room temperature for 30 minutes.**
2. **Washing**

The washing procedure is critical and requires special attention. An improperly washed plate will give inaccurate results, with poor precision and high backgrounds. After incubation remove the plate and wash wells 3 times with 200-300µL wash buffer per well. Wash the plate either by using an automatic plate washer or manually as indicated below. After the final automated wash, invert the plate and tap the wells dry on absorbent paper.

Plates can be washed manually as follows:

a. Flick out the contents of the plate into a sink.

b. Tap the wells dry on absorbent paper.

c. Fill each well with 200-300µL of wash buffer using a multichannel pipette.

d. Gently shake the plate on a flat surface.

e. Repeat a-d twice.

f. Repeat a and b.

3. **Conjugate addition**

Dispense 100µL of conjugate into each well, blot the top of the wells with a tissue to remove any splashes. **Note:** To avoid contamination, never return excess conjugate to the reagent bottle.

**Incubate at room temperature for 30 minutes.**

4. **Washing**

Repeat step 2.

5. **Substrate (TMB) addition**

Dispense 100µL of TMB substrate into each well, blot the top of the wells with a tissue to remove any splashes. **Note:** To avoid contamination never return excess TMB to the reagent bottle.

**Incubate at room temperature in the dark for 30 minutes.**

6. **Stopping**

Dispense 100 µL of stop solution into each well. This causes a change in color from blue to yellow.

7. **Optical density measurement**

Read the optical density (OD) of each well at 450nm on a microplate reader, within 30 minutes of stopping the reaction.

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**Quality Control**

1. **Quality Control**

In order for an assay to be valid, all the following criteria must be met:

- Calibrators and the positive and negative controls must be included in each run.
- The values obtained for all the controls should be in the ranges specified on the QC Certificate.
- The curve shape should be similar to the calibration curve, shown on the QC Certificate.

**If the above criteria are not met, the assay is invalid and the test should be repeated.**

2. **Calculate mean optical densities**

For each calibrator, control and sample calculate the mean OD of the duplicate readings. The percentage coefficient of variation (%C.V.) for each duplicate OD should be less than 15%.

3. **Plot calibration curve**

The calibration curve can be plotted either automatically or manually as follows by plotting the immune complex concentration in µg Eq/mL on the log scale against the OD value on the linear scale for each calibrator:

- Automatic - use appropriately validated software, and the curve fit that best fits the data.
- Manual - using log/linear graph paper, draw a smooth curve through the points (not a straight line or point to point).

4. **Treatment of anomalous points**

If any one point does not lie on the curve, it can be removed. If the absence of this point means that the curve has a shape dissimilar to that of the sample calibration curve, or more than one point appears to be anomalous, then the assay should be repeated.

5. **Calculation of CIC levels in controls and samples**

Read the level of the immune complex binding in the controls and diluted samples directly from the calibration curve. The control values should fall within the range given on the QC Certificate. **Note:** The calibrator values have been adjusted by a factor of 100 to account for a 1:101 sample dilution. No further correction is required.

6. **Assay calibration**

The assay is calibrated in µg Eq/mL using internal reference calibrators whose values were assigned in an assay calibrated using the WHO reference aggregated Human IgG preparation.

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**Limitations of the Procedure**

1. This kit is used to aid diagnosis only. A positive result suggests certain diseases that must be confirmed by clinical findings and other serological tests.

2. The results obtained from this assay are not diagnostic proof of the presence or absence of disease.

3. Please note sera may contain interfering substances such as chelating agents, DNA, anti-C1q autoantibodies, rheumatoid factor or monomeric immunoglobulin.
Expected Values

The normal range was determined on serum from 200 normal adult blood donors and the following values expressed in μg Equivalent per mL (μg Eq/mL), were obtained.

The ranges below are provided as a guide only. ELISA assays are very sensitive and capable of detecting small differences in sample populations. It is recommended that each laboratory determine its own normal range, based on the population techniques and equipment employed.

<table>
<thead>
<tr>
<th>Value Range</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 4.4μg Eq/mL</td>
<td>Negative Result</td>
</tr>
<tr>
<td>4.4 -&lt;10.8μg Eq/mL</td>
<td>Equivocal</td>
</tr>
<tr>
<td>≥10.8μg Eq/mL</td>
<td>Positive Result</td>
</tr>
</tbody>
</table>

Normal Range

C1q-CIC levels were measured in serum from 200 normal blood donors, the results are displayed in the graph below.

C1q-CIC are found sporadically in the normal population as a result of infection, and can also be elevated after eating. To arrive at a logical cut-off for the 'true normals', the elevated samples have been eliminated by three iterative cycles. This results in a cut-off of 4.4μg Eq/mL.

An equivocal range has been set between 4.4 and 10.8μg Eq/mL, with positives being ≥10.8μg Eq/mL.

On this basis 9/200 normal samples have been shown to be positive with values between 11.6-13.5μg Eq/mL.

This normal range is provided as a guide only. It is recommended that each laboratory determine its own normal range.

Performance Characteristics

Precision

The intra- and inter-assay precision was measured using three samples within the range of the assay. The mean concentration and % C.V. for each sample are given below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (μg Eq/mL)</th>
<th>% C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>5.4</td>
<td>13.0</td>
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<tr>
<td>Sample 2</td>
<td>28.7</td>
<td>7.1</td>
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<tr>
<td>Sample 3</td>
<td>50.7</td>
<td>3.9</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (μg Eq/mL)</th>
<th>% C.V.</th>
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<tbody>
<tr>
<td>Sample 4</td>
<td>5.3</td>
<td>7.2</td>
</tr>
<tr>
<td>Sample 5</td>
<td>24.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Sample 6</td>
<td>67.4</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Analytical Sensitivity

Sensitivity was determined as the mean concentration + 2 SD given by 20 determinations of the sample diluent, a value of 0.1μg Eq/mL was obtained.

Relative Specificity, Sensitivity, Agreement

The relative specificity, sensitivity and agreement were determined against an alternative CIC-C1q EIA kit using 48 test samples, including 29 samples from adult blood donors and 12 from confirmed SLE patients.
Alternative EIA

<table>
<thead>
<tr>
<th>BINDAZYMEN C1q-CIC EIA</th>
<th>+</th>
<th>Eqvocal</th>
<th>-</th>
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<tbody>
<tr>
<td>+</td>
<td>14</td>
<td>4</td>
<td>0</td>
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<tr>
<td>Eqvocal</td>
<td>1</td>
<td>0</td>
<td>7</td>
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<tr>
<td>-</td>
<td>0</td>
<td>1</td>
<td>21</td>
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</table>

Relative sensitivity: 95.0%
Relative specificity: 75.0%
Relative agreement: 83.3%

For calculation of the relative sensitivity, specificity and agreement, equivocal values are regarded as positive.

A correlation of the values from the two kits gave an R² of 0.80.

**Interfering Substances**

C1q binding CIC negative and positive samples were assayed to test the possible effect of interfering substances. The method used to check these substances was based on the Interference Check A plus™ kit - Kokusai Shiyaku, Japan.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Bilirubin F (Free)</td>
<td>18.3mg/dL</td>
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<tr>
<td>Bilirubin C (Conjugate)</td>
<td>19.0mg/dL</td>
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<tr>
<td>Haemolysed Haemoglobin</td>
<td>490mg/dL</td>
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<tr>
<td>Chyle</td>
<td>1930 Units</td>
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<tr>
<td>Rheumatoid factor</td>
<td>500 IU/mL</td>
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</tbody>
</table>

No interference was observed at the above concentrations of bilirubin, haemoglobin or chyle. However rheumatoid factor (RF) does interfere and samples suspected of containing rheumatoid factor should be screened by an appropriate RF assay before C1q CIC determination.

**Measuring Range**

The measuring range of this kit is 1.23 - 100µg Eq/mL.

**Plate template**

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**Summary of procedure**

1. Add 100µL of each calibrator, control and 1:101 diluted sample to the appropriate wells.
   **Incubate for 30 minutes.**
   Wash.
2. Add 100µL of conjugate to each well.
   **Incubate for 30 minutes.**
   Wash.
3. Add 100µL of substrate to each well.
   **Incubate for 30 minutes.**
4. Add 100µL of stop solution to each well.
   **Measure the absorbance at 450nm**

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References


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