DRG® CIC C1q (EIA-3169)

Revised 23 June 2009 (Vers. 8.0) **RUO in the USA**

Please use only the valid version of the package insert provided with the kit.

INTENDED USE

Immunoenzymatic colorimetric method for the quantitative determination of CIC C1q concentration in serum and plasma.

CLINICAL SIGNIFICANCE

The complement system is a biochemical cascade of the immune system that helps clear pathogens from an organism. It is derived from many small plasma proteins that work together to form the primary end result of cytolysis by disrupting the target cell's plasma membrane.
Activation of this system leads to cytolysis, chemotaxis, opsonization, immune clearance, and inflammation, as well as the marking of pathogens for phagocytosis. The complement system consists of more than 35 soluble and cell-bound proteins, 12 of which are directly involved in the complement pathways. The proteins account for 5% of the serum globulin fraction. The complement proteins are synthesized mainly by hepatocytes; however, significant amounts are also produced by monocytes, macrophages, and epithelial cells in the gastrointestinal and genitourinary tracts.
C1q is involved in the classical complement pathway. The classical pathway is triggered by activation of the C1-complex (which consists of one molecule C1q and two molecules C1r and C1s), either by C1q's binding to antibodies from classes M and G, complexed with antigens, or by its binding C1q to the surface of the pathogen.
The complement system might play a role in many diseases with an immune component, such as Barraquer-Simons Syndrome Alzheimer's disease, asthma, lupus erythematosus, various forms of arthritis, autoimmune heart disease and multiple sclerosis.Deficiencies of the terminal pathway predispose to both autoimmune disease and infections (particularly meningitis).
There are many tests for the determination of CIC, included the test of precipitation with PEG, radial immunodiffusion, and cellular tests like the test of Ray cell. Does not exist one procedure to determinate all types of immunocomplex; in commerce exist some test to determinate fragments of the complex (Es. C1q and C3d) that have an important diagnostic mean.

PRINCIPLE

C1q-fixing circulating immune complexes (CIC) are first blocked by anti-C1q immobilized on the microplate.
During this phase, the immune complex bind the anti-C1q coated on the microplate. The microplate is washed to remove the unbound serum protein.
In the second phase anti-human IgG-peroxidase conjugate is added which will bound to the immune complex fixed on the microplate. The washing removes the unbound conjugate. In the third phase, TMB substrate is added, and this reacts with the conjugate fixed on the microplate.
The quantity or CIC IgG complex is proportional to the colour intensity read at 450 nm wavelengths.
The immune complex concentration in the sample is calculated based on a series of standard.
Heat aggregate human gamma globulin pro mL (µg equiv./mL) is the unit of measure of the results.

Reagents, materials and instrumentation

REAGENTS AND MATERIALS SUPPLIED WITH KIT

1. CIC C1q **Standards**, S0 – S2, (3 vials) 1.5 mL each, (ready to use)
2. **Controls** (Negative + Positive), 2x (1 bottle= 1.5 mL), (ready to use)
   50 mM Phosphate buffer, pH 7.4, 1 g/L BSA
3. **Incubation Buffer** (1 bottle) 50 mL
   100 mM Phosphate buffer, pH 7.4
4. **Enzyme Conjugate** (1 bottle) 0.5 mL
   Peroxidase-coupled anti-IgG antibody
5. **Conjugate Buffer** (1 bottle) 20 mL
   100 mM Phosphate buffer, pH 7.4
6. **Coated Microplate** (1 microplate breakable), 96 wells coated with C1q
7. Conc. **Wash Solution** 10X (2 x1 bottle, 50 mL each)
   NaCl 16 g/L; Tween 20 1 g/L, 20 mM Phosphate buffer, pH 7.4
8. **TMB Substrate Solution** (1 bottle) 15 mL
   H_2O_2-TMB 0.25g/L (avoid any skin contact)
9. **Stop Solution** (1 bottle) 15 mL
   Sulphuric acid 0.15 mol/L (avoid any skin contact)

REAGENTS NECESSARY NOT SUPPLIED

Distilled water.

AUXILIARY MATERIALS AND INSTRUMENTATION

Automatic dispenser.
Microplates reader

Note:
All reagents and the microplate should be stored at 2 °C – 8 °C in the dark and used within the date written on the package.
Leave the microplate at room temperature for few minutes prior to removing a number of wells necessary for the assay. Place the unused microwell strips into the storage bag and reseal the bag with tape.

Procedure

STANDARD PREPARATION

Standards and Controls are ready to use. The Standards have the following concentrations:

Revised 23 June 2009 (Vers. 8.0) **RUO in the USA**

	S_0	S_1	S_2
µgEq/mL	0	16	64

Allow standards to reach room temperature before use. Mix gently.

PREPARATION OF CONJUGATE

For each series of 16 wells, dilute 20 µl of Conjugate in 2.0 mL of Conjugate buffer.
Mix well and avoid foaming. Stable for 3 hours at 22 °C to 28 °C.

PREPARATION OF WASH SOLUTION

Dilute the content of the vial "Conc. Wash Solution 10X" with distilled water to a final volume of 500 mL prior to use.
For smaller volumes respect the 1:10 dilution ratio.
The diluted wash solution is stable for 30 days at 2-8°C.
In concentrated wash solution is possible to observe the presence of crystals, in this case mix at room temperature until complete dissolution of crystals, for greater accuracy dilute the whole bottle of concentrated wash solution to 500 mL on taking care also transfer crystals with washing of the bottle, then mix until crystals are completely dissolved.

SAMPLE PREPARATION

The CIC assay can be performed in both serum and plasma.
Samples which are not immediately processed should be stored at -20°C. Samples should not be thawed more than once.
Pipette in a test tube:

Serum/Plasma	10 µl
Incubation Buffer	500 µl

Mix gently.

ASSAY PROCEDURE.

As it is necessary to perform in duplicate, each run should also include two wells for each *Sample*, two wells for each control and for each point of standard curve.

Revised 23 June 2009 (Vers. 8.0) **RUO in the USA**

Reagents	Standard	Sample	Blank
Standard S_0-S_2	**100 µL**		
Controls	**100 µL**		
Diluted Sample		**100 µL**	
Incubate 30 minutes at 37°C. Remove the contents from each well; wash the wells three times with 300 µL diluted wash solution.			
Diluted Conjugate	**100 µL**	**100 µL**	
Incubate 30 minutes at 37°C. Remove the contents from each well; wash the wells three times with 300 µL diluted wash solution.			
TMB substrate	**100 µL**	**100 µL**	**100 µL**
Incubate 15 minutes in the dark at room temperature (22-28°C).			
Stop solution	**100 µL**	**100 µL**	**100 µL**
Read Absorbance (E) at 450 nm against Blank within 15 minutes after the addition of stop solution.			

QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of CIC C1q for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

LIMITATION OF PROCEDURE

ASSAY PERFORMANCE

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic or haemolysed specimen(s) should similarly not be used. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

INTERPRETATION OF RESULTS

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

RESULTS

MEAN ABSORBANCE

Calculate the mean of the absorbance (Em) corresponding to the single point to the standard curve and to each sample.

STANDARD CURVE

Plot the mean values of absorbance of the standards (Em) against concentration. Draw the best-fit curve through the plotted points.

CALCULATION OF RESULTS

Interpolate the values of the samples on the standard curve to obtain the corresponding values of IgG concentrations expressed in µg Equiv./mL.

Revised 23 June 2009 (Vers. 8.0) **RUO in the USA**

REFERENCE VALUES

	µg Equiv./mL of aggregates IgG
Negative Sample	< 16
Uncertain Sample	between 16 –18
Positive Sample	> 18

PERFORMANCE CHARACTERISTICS

PRECISION

Intra Assay Variation

Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 6.6%.

Inter Assay Variation

Between run variations was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 13.4%.

SENSITIVITY

The lowest detectable concentration of CIC C1q that can be distinguished from the zero standard is 1.0 mg Equiv./mL at the 95 % confidence limit.

ACCURACY

The recovery of 12.5 – 25 – 50 – 100 µg equiv/mL IgG aggregates added to a sample gave an average value (±SD) of 99.5% ± 4.4% with reference to the original concentrations.

SPECIFICITY

92 serum and plasma specimens collected from normal and asymptomatic subjects were tested with CIC C1q ELISA. The overall specificity of the assay was 96 %.

125 serum and plasma specimen collected from patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) or other disorders were tested with CIC C1q. The overall clinical sensitivity was 92 %

COMPARATIVE DATA

Circulating Immune complexes (CIC) collected from 160 patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), or other disorders subjects and 95 form normal, asymptomatic subject were measured.

The overall agreement between the two test methods was 87 %.

The average CIC concentration was 2.1 µg Equiv/mL (S.D. = 1.6)

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

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3. Menzel J.E.,et al J.Immunol. Met. 138, 16 (1991)
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TROUBLESHOOTING

POSSIBLE ERROR CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed)

too high within run (CV%)

- reagents and/or strips not pre-warmed to room temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run (CV%)

- incubation conditions not constant (time, temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

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