Version 21-08/24

User's Manual

CIC C1q ELISA Kit

REF **DEIA1661**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The CIC-C1q EIA is designed for detection of C1q binding circulating immune complexes in human serum and plasma.

General Description

The importance of circulating immune complexes (CIC) and their relationship to various diseases has been the subject of investigation for a number of years. Formation of immune complexes is a protective, ongoing, and usually benign, process of a normally functioning immune system. CIC are removed from the circulation in the normal host by a number of complex biochemical, enzymatic, and cellular processes. Key to the elimination of many CIC is the activation of the classical complement pathway. However, under certain disease conditions that are still poorly understood, immune complexes may initiate complement-dependent injury of various organs and tissues. This activation of complement may begin a series of potentially destructive events in the host including anaphylatoxin production, cell lysis, leukocyte stimulation, and activation of macrophages and other cells. When immune complexes become fixed to vessel walls or cell membranes, destruction of normal tissue can occur, as in some cases of glomerulonephritis. Certain properties of CIC influence their potential pathogenicity. Of particular importance are: (1) nature, size and concentration of the antigen; (2) nature, size and concentration of the antibody; and, (3) rate of formation and clearance of the immune complexes. Circulating immune complexes have been measured in a variety of conditions: infections, autoimmune disorders, trauma, and neoplastic proliferative diseases. Current studies suggest that CIC determination can be important in the evaluation of certain diseases and, sometimes, in monitoring efficiency of therapy. This is especially true in systemic lupus erythematosus (SLE) and some forms of rheumatoid arthritis (RA). Classically, the first disease state linked to the formation of immune complexes was serum sickness, described in the early 1900's by von Pirquet. Since that time elevated levels of CIC have been described in autoimmune diseases (SLE, SLE-related syndrome, RA), glomerulonephritis, neoplastic disease (Hodgkin's, leukemia), bacterial infections (subacute bacterial endocarditis [SBE], leprosy), parasitic infections (malaria, schistosomiasis) and viral infections (hepatitis, mononucleosis). Over 40 assay techniques have been described to detect or quantitate CIC. Such tests as the Raji Cell assay, C1q deviation test, conglutinin test, fluid phase C1q binding procedures, rheumatoid factor assay, PEG precipitin test, and solid phase C1q assays have been described. Since the size and physiochemical properties of CIC vary markedly, none of these assays has been accepted as a standard. A collaborative study sponsored by the World Health Organization in 1978 determined that no single method was appropriate in all suspected disease states and recommended that at least two different assay techniques be performed to detect and measure CIC adequately.

Principles of Testing

The CIC-C1q EIA is based on the principle that complement fixing CIC will bind to immobilized human C1q purified protein.

In the first stage, standards and serum or plasma specimens diluted in Complement Specimen Diluent are added to the C1q-coated microtiter wells and incubated. During this incubation period, immune complexes that bind to C1q will complex with the C1q-coated microassay wells. To confirm a positive CIC result, the

specimen may be diluted in Confirmation Diluent, which contains a high salt concentration known to inhibit the binding of CIC to C1q, and then added to the microtiter wells and incubated. A wash cycle removes unbound serum proteins.

In the second stage, horseradish peroxidase (HRP)-conjugated goat anti-human IgG is added to each test well. During this incubation, the conjugate will bind to the immune complexes that are now bound to the C1qcoated microassay wells. A wash cycle removes unbound conjugate.

In the third stage, an enzyme substrate is added to each test well. The bound HRP-conjugated antibody reacts with the chromogenic substrate forming a green color. After incubation, a reagent is added to stop color development.

The standard and test specimen absorbances (A405 values) are measured spectrophotometrically. The intensity of the green color is proportional to the amount of CIC IgG antibodies binding the solid-phase C1q. A standard curve is generated by plotting the A405 values obtained with each standard versus its concentration. The concentration of immune complexes present in the test specimen is determined by reference to the standard curve. Results are expressed as heat aggregated human gamma globulin equivalents per mL (µg Eq/mL).

Reagents And Materials Provided

1. CIC-C1q Standards A-C. 2 each, 2 mL.(lyophilized) When reconstituted, each contains a known quantity of heat aggregated human gamma globulin (HAGG), in PBS, 2.5% stabilizers
2. Microassay Plate. 12 each. 96-well with retainer and holder consisting of eight-well strips coated with purified human C1q protein in a resealable foil pouch.
3. Stop Solution. 6 mL. Contains 250 mM oxalic acid
4. 20× Wash Solution Concentrate. 2 each, 50 mL. Each contains phosphate buffered saline (PBS), 1.0% Tween-20®, and 0.035% ProClin® 300
5. Complement Specimen Diluent. 50 mL. Contains PBS, 0.05% Tween-20, 2.5% stabilizers, 0.035% ProClin 300
6. Substrate Diluent. 25 mL. Contains 0.1M citrate buffer and 0.05% H_2O_2
7. Substrate Concentrate. 1.5 mL. Contains 0.7% 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), diammonium salt
8. CIC-C1q Conjugate. 2 each, 3 mL. Peroxidase-conjugated (goat) anti-human IgG suspended in HRP stabilizing buffer
9. Hydrating Reagent. 25 mL. Contains 0.035% ProClin 300
10. Confirmation Diluent. 2 each, 10 mL. Contains PBS, 2.5% stabilizers, 1.2 M NaCl, 0.035% ProClin 300

Materials Required But Not Supplied

1. Timer (60 minute range)
2. Calculator or other computational method to validate the assay
3. Clean, unused microassay plates and/or test tubes and racks
4. Container for wash buffer dilution
5. Wash bottle or other immunoassay washing system

6. Adjustable multichannel pipette (8 or 12 channels) or repeating micropipettes (optional)
7. Clean pipettes, 1 mL, 5 mL, and 10 mL
8. Micropipettes and pipette tips
9. Plate reader capable of optical density A405 readings between 0.0 and 2.0
10. Deionized or distilled water

Storage

Store unopened kit at 2°C to 8°C. After the kit is opened, the 20X Wash Solution Concentrate and Hydrating Reagent may be store at 2°C to 30°C.

After selecting the reagents or materials to be used in the assay, return the unused reagents immediately to their appropriate storage temperatures. Bring reagents and materials to room temperature (15°C to 30°C) before use.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

The Substrate Concentrate may range in color from colorless to pale or dark green. This condition will not influence performance. However, the freshly prepared Substrate Solution should be colorless to pale green. A dark green color indicates that the prepared Substrate Solution has deteriorated, must be discarded, and new Substrate Solution prepared in clean glassware.

Cloudiness or discoloration of the diluted Wash Solution indicates a deterioration of this reagent. If this occurs, the solution should be discarded.

Specimen Collection And Preparation

Handle and dispose of all specimens using Universal Precautions.

The assay requires at least 10 µL of serum or EDTA plasma. All specimens should be collected aseptically and prepared using standard techniques for clinical laboratory testing.

Do not heat-inactivate the specimens. Any particulate matter should be cleared from the specimens by low speed centrifugation before testing.

Samples may be stored at 2°C to 8°C for up to 7 days. If specimens are stored for longer periods, they should be frozen at–20°C or below, in a freezer which is not self-defrosting.

Reagent Preparation

Refer to Table 1 for the amounts of Substrate Solution and microassay strips required per number of tests. After removing the needed reagents and materials, return the unused items to their appropriate storage temperatures (see STORAGE). Bring all reagents and materials for the assay to room temperature (15°C to 30°C) before using.

1. Wash Solution

Mix the 20× Wash Solution Concentrate by inverting the bottle several times. If the 20× Wash Solution Concentrate has been stored at 2°C to 8°C, crystals may have formed. To dissolve the crystals, warm the bottle in a 37°C to 50°C water bath until all crystals have dissolved. Mix thoroughly. Prepare the Wash Solution for washing the microassay wells by diluting the entire contents of one of the bottles of 20× Wash

Solution Concentrate up to one liter with distilled or deionized water. Mix thoroughly. The Wash Solution is stable for 30 days when stored in a clean container at 2°C to 8°C. If discoloration or cloudiness occurs, discard the reagent.

2. Selecting the Microassay Strips

Remove the strip retainer from the assembled plate. Determine the number of strips required for the assay by referring to Table 1. Remove the unneeded strips and place them in the storage bag, reseal the bag, and return to storage at 2°C to 8°C. Secure the strips to be used in the assay by replacing the strip retainer securely onto the microassay plate.

3. CIC-C1q Standard Reconstitution

Add 2.0 mL Hydrating Reagent to standard vials, A-C. Allow lyophilized standards to rehydrate for at least 15 minutes, followed by thorough mixing. Avoid formation of foam or bubbles during mixing. Reconstituted standards are stable for 30 days when stored at 2°C to 8°C.

4. Specimen Dilution

Caution: Treat all specimens as if potentially infectious. Do not use heat-inactivated or contaminated specimens. Determine the number (N) of specimens to be tested. Label test tubes #1 through #N and record which specimen will correspond to each tube on the data sheet provided. Prepare a 1-to-50 dilution of each specimen using the Complement Specimen Diluent (e.g., 10 µL test specimen mixed with 490 µL Complement Specimen Diluent). Mix thoroughly, but avoid formation of foam and bubbles. Do not store or re-use diluted specimens. If the measured concentration of immune complexes in a sample is greater than the concentration of Standard C and a more accurate end point result is desired, repeat testing of the specimen at a 1:200 dilution (fourfold dilution of the 1:50 dilution) is recommended. **Note: Specimens that have measured concentrations of CIC less than Standard C should not be diluted further and retested.**

5. Adding diluted specimens to the microassay wells

Either of two methods can be used to add your diluted specimens, standards, controls and buffer to the wells. See Step 3 of Assay Procedure. For small assay runs where only a few specimens are being tested, the diluted specimens and other reagents can be added directly to their assigned well with a micropipette (100 µL/well). For small or large runs, but especially larger runs, we recommend the use of a multi-channel pipettor for adding specimens as follows. (This latter procedure may be used to conveniently add the conjugate, substrate and stop solution as well.)

In order to load the standards, controls and diluted specimens into the microassay wells as rapidly as possible, a "replica plating" procedure can be employed. Instead of adding 100 µL of each standard, control or diluted specimen to the C1q coated wells individually, 120-130 µL of each solution can be added to individual wells in a blank plate (not provided) corresponding to the final EIA pattern desired. After all the solutions to be tested have been added to the microassay wells in the blank plate, rapidly transfer 100 µL from each blank well to the C1q coated wells using a multi-channel micropipettor. To avoid the possibility of cross-contamination, pipette tips must be changed each time there is a change in the composition of the samples to be transferred.

6. Confirmation Dilution (optional)

If confirmation is desired, determine the number (N) of specimens to be confirmed. Label test tubes #1c through #Nc and record which specimens will correspond to each tube on the provided data sheet. Prepare the appropriate dilution (1:50 or 1:200) using the Confirmation Diluent. A specimen diluted in Confirmation Diluent must be run concurrently with the same dilution of specimen in Complement Specimen Diluent.

7. Preparation of Substrate Solution

Prepare just prior to use. Determine the required volume of Substrate Solution from Table 1. Prepare the Substrate Solution by adding 50 µL of Substrate Concentrate per mL of Substrate Diluent. Mix thoroughly.

Table 1
Assay requirements

Wells[1]	8-Well Strips	Substrate Solution Required (mL)	Substrate Diluent (mL)	Substrate Concentrate (µL)
16	2	1.6	2.0	100
24	3	2.4	3.0	150
32	4	3.2	4.0	200
40	5	4.0	5.0	250
48	6	4.8	5.0	250
56	7	5.6	6.0	300
64	8	6.4	7.0	350
72	9	7.2	8.0	400
80	10	8.0	9.0	450
88	11	8.8	9.0	450
96	12	9.6	10.0	500

[1]Determine the number of specimens to be tested and add seven (7) wells for the three standards (to be tested in duplicate) and one blank well. It is recommended that duplicate standards be tested in separate microassay strips when possible.

Assay Procedure

Read entire product insert before beginning the assay.

See REAGENT PREPARATION before proceeding. Grip the plate firmly during all handling to prevent accidental removal of the strip retainer.

1. Record the well positions corresponding to all test samples and standards, as well as the indicated lot number from the vial labels. Label one corner of the Microassay Plate for orientation.
2. Prepare the microassay strips as follows:

   a. Rehydrate microassay wells by adding approximately 300 µL of Wash Solution to each well using a wash bottle or automated plate-washing device.

   b. Incubate at room temperature (15°C to 30°C) for 15 to 20 minutes.

   c. Remove the fluid from the wells.

   d. Invert the plate and tap firmly on absorbent paper twice to remove any remaining fluid. Do not allow the wells to dry.
3. Add 100 µL of Complement Specimen Diluent to the well(s) that will be used to blank the plate reader.
4. Add 100 µL of each reconstituted CIC-C1q Standard (A, B, C,) to duplicate wells.
5. Add 100 µL of each diluted specimen to its assigned microassay well. See REAGENT PREPARATION, item 5.
6. Incubate at room temperature (15°C to 30°C) for 60 ± 1 minutes.
7. Wash the microassay wells as follows: **Note: The microassay well-washing procedure can be done manually or with an automated plate washer.**

   a. After the incubation in step 6 (and in step 9 below), remove the contents from each well.

b. Add approximately 300 µL Wash Solution to each well using a wash bottle or automated plate-washing device.

c. Incubate the wells for 1 minute at room temperature (15°C to 30°C).

d. Remove the contents from each well.

e. Add approximately 300 µL Wash Solution to each well.

f. Remove the contents from each well.

g. Repeat steps e-f three additional times.

h. After this fifth wash cycle, invert the plate and tap firmly on absorbent paper twice to remove any remaining liquid.

8. Using a multi-channel or repeating pipette, dispense 50 µL of the Conjugate into each washed test well, including the blank well(s).
9. Incubate the microassay strips at room temperature (15°C to 30°C) for 30 ± 1 minutes. Prepare the Substrate Solution during this incubation (see REAGENT PREPARATION, item 8).
10. Wash the microassay wells after the 30-minute incubation (step 9), as described under ASSAY PROCEDURE, step 7.
11. Immediately following the wash procedure, dispense 100 µL of the freshly prepared Substrate Solution into each well, including the blanks.
12. Incubate the microassay strips at room temperature (15°C to 30°C) for 30 ± 1 minutes.
13. Add 50 µL of Stop Solution to each well to stop the enzymatic reaction. The Stop Solution should be added to the wells in the same order and at the same rate as was the Substrate Solution. Gently tap the plate to evenly disperse the color.
14. Determine the absorbance reading at 405 nm for each test well within one hour after the addition of the Stop Solution (step 13), making a blank correction in accordance with the spectrophotometric system in use.
15. Dispose of the remaining diluted specimens, substrate, and the used microassay strips (see Precautions).

Quality Control

RECOMMENDED CONFIRMATION METHOD

If independent confirmation of a positive result is required, or if a positive result is inconsistent with the clinical interpretation, the positive specimen may be assayed using a confirmation test. A negative result cannot be confirmed. The confirmation method utilizes a specimen diluent (the Confirmation Diluent), which contains a high concentration of sodium chloride.6,7 To confirm a positive result, an aliquot of the specimen must be diluted (1:50 or 1:200) in the Confirmation Diluent and a second aliquot diluted similarly in the

Complement Specimen Diluent. Both samples are then assayed according to the usual CIC-C1q assay procedures.

See REAGENT PREPARATION, and INTERPRETATION OF RESULTS sections for details.

QUALITY CONTROL

Good laboratory practice recommends that positive and negative controls be included in each assay.

Controls for the CIC-C1q assay are available from CD for this purpose, and should be used in accordance with their Package Insert.

In addition to controls, this assay provides a RECOMMENDED CONFIRMATION METHOD and VALIDATION.

Interpretation Of Results

Calculations

The standard curve is generated using the blank-subtracted A405 values for each standard (on the y axis) and the assigned concentration for each standard (along the x axis). The standard curve must meet the validation requirements. Most computers and calculators are capable of performing this calculation. An example of a typical standard curve is shown in Figure 1.

The µg Eq/mL concentration for each sample is calculated from the standard curve using linear regression analysis. Calculated values determined against the standard curve should be evaluated against the cut-off for positive reporting. See Interpretation below.

Dilution Factor Adjustment. The assigned CIC concentration has been determined assuming a 1:50 dilution of the specimen. If a higher dilution of the specimen was assayed, the user must multiply the calculated result by the appropriate dilution factor. For example, if the specimen dilution assayed was 1:200, the calculated result must be multiplied by 4.

Confirmation Test Calculation. To confirm a positive result, the immune complex concentration [CIC] determined in the sample diluted in Confirmation Diluent is divided by the immune complex concentration measured in the sample diluted in the Complement Specimen Diluent to generate a ratio:

Ratio = [CIC] in Confirmation Diluent / [CIC] in Complement Specimen Diluent

Validation

Determine the slope, y-intercept, and correlation coefficient of the derived best fit line. The values must be within the following ranges to qualify the assay.

Correlation coefficient (r): Greater than 0.95

Slope (m): 0.022 to 0.056

y-intercept (b): (–) 0.108 to (+) 0.238

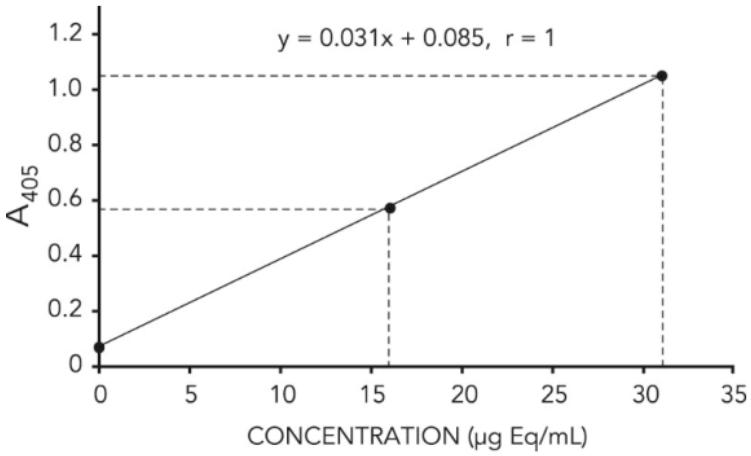
Interpretation

1. Negative Results: Values less than 4.0 µg Eq/mL are considered negative for significant levels of CIC.
2. Positive Results: Values greater than or equal to 4.0 µg Eq/mL are considered positive for significant levels of CIC.
3. Confirmation Results: If the ratio is less than 0.7, the positive CIC result is confirmed. In other words, greater than 30% reduction confirms a positive result.

Occasionally, positive specimens may not confirm. Nonconfirming specimens may be due to, among other reasons: (1) mishandled specimens (e.g. contaminated or heat-inactivated) or (2) specimens which contain autoantibodies to C1q. These specimens are not necessarily negative for CIC. The material causing the apparent false positive result may mask concomitantly occurring CIC which, if they were present alone, would

otherwise give a confirmable positive CIC result.

Figure 1
Example of Standard Curve



Sample	(A_{405})	µg Eq/mL
Standard A	0.09	0.2
Standard B	0.57	15.6
Standard C	1.05	31.1
Specimen 1	0.19	3.4
Specimen 2	0.82	23.7
Specimen 3	0.40	10.2
r = 1.00	m = 0.031	b = 0.085

Reference Values

Circulating immune complexes (CIC) were measured in sera from 312 subjects using the CIC-C1q Enzyme Immunoassay. One hundred six (106) sera were collected from normal, asymptomatic subjects. The average CIC concentration was 2.1 µg Eq/mL (S.D. = 1.9).

Specimens obtained from 206 patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), or other disorders were tested using the CIC-C1q Enzyme Immunoassay and another commercially available EIA kit. The overall agreement between the two test methods was 87%.

Within the above population, eighty-one (81) SLE patients and thirty-three (33) RA patients were tested. The two kits agreed for 82% of these specimens. These results are shown in Table 2.

Table 2
Comparative Data for Specific Patient Groups

CD Test Result	–	+	–	+
Alternative Test Result	–	+	+	–
RA Patients	18	9	3	3
SLE Patients	40	23	15	3
Other	0	90	2	0

Performance Characteristics

Accuracy

An immune complex standard prepared by the World Health Organization (WHO) using heat aggregated IgG was used to standardize the assay. To test the accuracy of the assay, five dilutions of the WHO standard were tested in triplicate in nine runs in the kit. The assayed values were determined to be predictive of the expected standard concentrations (coefficient of determination = 0.97).

Forty-one (41) paired serum and plasma (EDTA anticoagulant) samples from SLE and RA patients were compared to demonstrate the suitability of plasma samples for the assay. No significant difference between the serum and plasma results was observed (a = 0.05).

Precision

Three serum samples and three standards were tested in nine assay runs in three different kit lots. Each standard was tested in triplicate within each assay run. Each serum specimen was run in a single well in each run; the average variation between each run for the specimens and standards is shown in Table 3 as interassay variation. The average variation within each run for the standards is shown in Table 3 as intraassay variation.

Table 3
Assay Reproducibility

	Mean (Eq/mL)	Intraassay S.D. (% CV)	Interassay S.D. (% CV)
Specimen 1	30	NT	3.1 (10)
Specimen 2	7	NT	2.6 (37)
Specimen 3	0	NT	0.3 (N/A)
Standard 1	37	3.2 (9)	3.9 (11)
Standard 2	20	2.1 (10)	1.8 (9)
Standard 3	0	0.1 (N/A)	0.0 (N/A)

NT = not tested N/A = not applicable

Sensitivity

The analyte sensitivity of the CIC-C1q Enzyme Immunoassay is 1.0 µg Eq/mL.

Specificity

One hundred six (106) serum and plasma specimens collected from normal, asymptomatic subjects were tested for CIC. The overall specificity of the assay was 94%.

Precautions

1. Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
2. Dispose of containers and unused contents in accordance with Federal, State and Local regulations.
3. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
4. Wear suitable protective clothing, gloves, and eye/face protection when handling contents of this kit.

5. Store assay reagents as indicated.
6. Do not use Coated Strips if pouch is punctured.
7. Test each sample in duplicate.
8. ProClin 300 is used as a preservative. Incidental contact with or ingestion of buffers or reagents containing ProClin can cause irritation to the skin, eyes or mouth. Use good laboratory practices to reduce exposure. Seek medical attention if symptoms are experienced.
9. Use of multichannel pipettes or repeat pipettors is recommended to ensure timely delivery of reagents.
10. For accurate measurement of samples, add samples and standards precisely. Pipette carefully using only calibrated equipment.
11. Proper collection and storage of test specimens are essential for accurate results.
12. Avoid microbial or cross-contamination of specimens, reagents, or materials. Incorrect results may be obtained if contaminated.
13. Do not use a microassay well for more than one test.
14. Decontaminate all specimens, reagents, and materials by soaking for a minimum of 30 minutes in a 1:10 solution of household bleach (sodium hypochlorite) or autoclave at 121°C for 30 minutes at 15 psi.
15. Using incubation times and temperatures other than those indicated in the Procedure section may give erroneous results.
16. The Substrate Concentrate is light sensitive. Avoid prolonged exposure to bright or direct light. Store reagents in the dark when not in use.
17. Do not allow microassay wells to dry once the assay has begun.
18. When adding or aspirating liquids from the microassay wells, do not scrape or touch the bottom of the wells.
19. Heat-inactivated, hyperlipemic or contaminated specimens may give erroneous results.
20. To avoid aerosol formation during washing, use an apparatus to aspirate the wash fluid into a bottle containing household bleach.
21. This assay may be performed with any validated washing method.
22. Wash hands thoroughly after handling.

Limitations

1. Heat inactivations of specimens may cause false positive results. Because this test measures aggregates of human IgG non-selectively, sample collection and processing must avoid conditions that promote aggregation of IgG.
2. The CIC-C1q Enzyme Immunoassay has been used to test specimens collected as serum or as plasma in EDTA anticoagulant. Other anticoagulants have not been tested.