DiaMetra

CIC C1q

for routine analysis

Direct immunoenzymatic determination of CIC C1q in human serum or plasma

IVD | LOT See external label | 2°C 8°C | Σ = 96 tests | REF DKO016

INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of CIC C1q concentration in human serum or plasma.
CIC C1q kit is intended for laboratory use only.

1. 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.

2. PRINCIPLE

CIC C1q kit is based on the binding of C1q-linked immunocomplexes to C1q adsorbed on microplate.
In the first step, the samples are added to the microplate adsorbed with C1q; during the following incubation, C1q-fixing circulating immune complexes (CIC) bind to the C1q immobilized on the microplate. The microplate is washed for remove the unbound serum proteins.
In the second step, the anti-human IgG conjugated with horseradish peroxidase (HRP) is added; it binds to the immunocomplex fixed on the microplate. The washing step removes the unbound conjugate.
In the third step, the TMB Substrate is added, and this reacts with the conjugate fixed on the microplate, developing a colorimetric reaction.
The quantity or CIC IgG complex is proportional to the colour intensity read at 450 nm wavelengths.
The immunocomplex concentration in the sample is calculated based on a serie of standards.
Heat aggregate human gamma globulin pro mL (µgEq/mL) is the unit of measure of the results.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. CIC C1q Standards (3 vials, 1.5 mL each)
   - STD0 — REF DCE002/1606-0
   - STD1 — REF DCE002/1607-0
   - STD2 — REF DCE002/1608-0
2. Controls (2 vials, 1.5 mL each, ready to use)
   74 mM Phosphate buffer, pH 7.4, 1 g/L BSA
   - Negative Control — REF DCE045/1601-0
   - Positive Control — REF DCE045/1602-0
3. Incubation Buffer (1 vial, 50 mL)
   74 mM Phosphate buffer, pH 7.4 — REF DCE008-0
4. Conjugate (1 vial, 0.5 mL)
   Anti human IgG conjugated with horseradish peroxidase (HRP) — REF DCE002/1602-0
5. Conjugate Buffer (1 vial, 20 mL)
   74 mM Phosphate buffer, pH 7.4 — REF DCE009-0
6. Coated Microplate (1 breakable microplate)
   Microplate coated with C1q — REF DCE002/1603-0
7. 10x Conc. Wash Solution (2 vials, 50 mL each)
   NaCl 160 g/L; tween-20 10 g/L - 0.2M Phosphate buffer, pH 7.4 — REF DCE054-0
8. TMB Substrate (1 vial, 15 mL)
   H_2O_2-TMB 0.26 g/L *(avoid any skin contact)* — REF DCE004-0
9. Stop Solution (1 vial, 15 mL)
   Sulphuric acid 0.15 mol/L *(avoid any skin contact)* — REF DCE005-0

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm)

Note

All reagents and the microplate should be stored at 2÷8°C in the dark and used within the expiration date printed on the package.

Leave the microplate at room temperature for few minutes prior to removing the number of wells necessary for the assay.

Place the unused strips into the storage bag and reseal the bag with tape.

4. WARNINGS

- This kit is intended for in vitro use by professional persons only.
- Use appropriate personal protective equipment while working with the reagents provided.
- All human source material used in the preparation of standards and controls for this product has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Standard and the Controls should be handled in the same manner as potentially infectious material.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or Proclin 300^R as preservatives. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
- The TMB Substrate 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.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry dates printed on the labels of the box and of the vials must be observed. Do not use any kit component beyond their expiry date.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposable tips. For this purpose, Diametra supplies a separate decontamination reagent for cleaning needles.
- If you use automated equipment is your responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- 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 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated should not be used in the assay. Highly lipemeic or haemolysed specimens should similarly not be used
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Standards preparation

Standards, as Controls, are ready to use. The Standards have the following concentration:

	S_0	S_1	S_2
µgEq/mL	0	16	64

Allow the standards to reach room temperature (22-28°C) before use. Mix gently.

6.2. Preparation of Diluted Conjugate

Dilute the Conjugate (reagent 4) 1:100 with Conjugate buffer (reagent 5). The exact quantity is proportional to the number of the assays.

Mix well and avoid foaming. Stable for 3 hours at room temperature (22÷28°C).

6.3. Preparation of Wash Solution

Dilute the content of the vial "10X Conc. Wash Solution" 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 taking care also to transfer crystals, then mix until crystals are completely dissolved.

6.4. Preparation of the Sample

The CIC assay can be performed in human serum or plasma. Samples that are not immediately processed should be stored at -20°C. Samples should not be thawed more than once.

Prepare the samples by pipetting in a test tube:

Sample	10 µL
Incubation Buffer (reagent 3)	500 µL

Mix gently. Avoid using vortex.
The Controls are ready to use.

6.5. Procedure

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

Reagents	Standard	Sample or Controls	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 and 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**
Shake the microplate gently. Read Absorbance (E) at 450 nm against Blank within 15 minutes after the addition of stop solution.			

7. 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.

8. LIMITATIONS OF PROCEDURE

8.1. Assay Performance

Samples microbiologically contaminated should not be used in the assay. Highly lipemic or haemolysed specimens 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 it lasts more than ten minutes, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve. Addition of the TMB Substrate initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop 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 steps may result in poor replication and spurious results.

8.2. 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.

9. RESULTS

9.1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample.

9.2. Standard Curve

Plot the mean value of absorbance of each standard (Em) against concentration. Draw the best-fit curve through the plotted points.

9.3. Calculation of Results

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

10. REFERENCE VALUES

	µgEq/mL of aggregates IgG
Negative Sample	<16
Uncertain Sample	between 16 and 18
Positive Sample	>18

11. PERFORMANCE AND CHARACTERISTICS

11.1. Precision

11.1.1. Intra Assay Variation

Within run variation was determined by replicate the measurement (16x) of two different control sera in one assay. The within assay variability is ≤ 5.3%.

11.1.2. Inter Assay Variation

Between run variations was determined by replicate the measurements of two different control sera in 2 different lots. The between assay variability is ≤ 6.0%.

11.2. Recovery

The recovery of 12.5 – 25 – 50 – 100 µgEq/mL IgG aggregates added to a sample gave values between 94.3% and 105.7% with reference to the original concentrations.

11.3. Detection limit

The lowest detectable concentration of CIC C1q that can be distinguished from the zero standard is 1.0 µgEq/mL at the 99% confidence limit.

11.4. Diagnostic Specificity and sensitivity

11.4.1. Clinical Specificity and sensitivity

92 serum specimens collected from normal, asynptomatic subjects were tested with CIC C1q ELISA DiaMetra. The clinical specificity of the assay was 96%.

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

11.4.2. Specificity and sensitivity vs commercial reference method

Specimen obtained from 209 patients with SLE, RA, or other disorders were tested using the Diametra CIC C1q kit and QUIDEL EIA kit. The obtained results are shown in the table below:

DIAMETRA kit	**-**	**+**	**-**	**+**
QUIDEL kit	**-**	**+**	**+**	**-**
RA Patients	20	12	4	4
SLE Patients	38	25	12	6
Others	0	85	2	1

From the 209 tested samples the following diagnostic sensitivity and specificity are obtained:

	RA	**SLE**	**Others**	**RA+ SLE**	**RA+ SLE+ Others**
Sensitivity	75,0%	67,6%	97,7%	69,8%	87,1%
Specificity	83,3%	86,4%	--	85,3%	84,1%
Agreement	-	-	-	78,5%	86,1%

11.4.3. Comparative data

Circulating Immunocomplexes (CIC) collected from 160 patient with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), or other disorders subjects, and 95 form normal, asymptomatic subjects were measured.
The overall agreement between the two test methods was 87%.
The average CIC concentration of healthy donors was 2.1 µgEq/mL (S.D. = 1.6).

12. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

- Triolo G., et al J.Clin.Lab.Immunol 13, 35-39 (1984)
- Rong-jia Xu et al J.Immunol.Met. 135, 225-231(1990)
- Menzel J.E.,et al J.Immunol. Met. 138, 16 (1991)
- Muso E, et al Nippon Jinzo Gakkai Shi.36(4):345-54 (1994)
- Yoshinoya S,et al J Clin Lab Immunol. 38(4):161-73 (1992)

Ed 03/2011 **DCM016-10**

DiaMetra S.r.l. Headquarter: Via Garibaldi, 18
20090 SEGRATE (MI) Italy
Tel. 0039-02-2139184 – 02-26921595
Fax 0039–02–2133354.
Manufactory: Via Giustozzi, 35 – Z.I Paciana
06034 FOLIGNO (PG) Italy
Tel. 0039-0742–24851
Fax 0039–0742–316197
E-mail: info@diametra.com

	DIA.METRA SRL	
Mod. PIS	**PACKAGING INFORMATION SHEET**	

IT *Spiegazione dei simboli* **GB** *Explanation of symbols* **FR** *Explication des symboles* **ES** *Significado de los simbolos*
DE *Verwendete Symbole* **PT** *Explicaçao dos simbolos*

IVD	DE ES FR GB IT PT	In vitro Diagnostikum Producto sanitario para diagnóstico In vitro Dispositif medical de diagnostic in vitro In vitro Diagnostic Medical Device Dispositivo medico-diagnostico in vitro Dispositivos medicos de diagnostico in vitro		DE ES FR GB IT PT	Hergestellt von Elaborado por Fabriqué par Manufacturer Produttore Produzido por
REF	DE ES FR GB IT PT	Bestellnummer Nûmero de catálogo Réferéncès du catalogue Catalogue number Numero di Catalogo Número do catálogo	yyyy-mm	DE ES FR GB IT PT	Herstellungs datum Fecha de fabricacion Date de fabrication Date of manufacture Data di produzione Data de produção
yyyy-mm-dd	DE ES FR GB IT PT	Verwendbar bis Establa hasta (usar antes de último día del mes) Utiliser avant (dernier jour du mois indiqué) Use by (last day of the month) Utilizzare prima del (ultimo giorno del mese) Utilizar (antes ultimo dia do mês)		DE ES FR GB IT PT	Biogefährdung Riesco biológico Risque biologique Biological risk Rischio biologico Risco biológico
	DE ES FR GB IT PT	Gebrauchsanweisung beachten Consultar las instrucciones Consulter le mode d'emploi Consult instructions for use Consultare le istruzioni per l'uso Consultar instruções para uso	LOT	DE ES FR GB IT PT	Chargenbezeichnung Codigo de lote Numero de lot Batch code Codice del lotto Codigo do lote
Σ = xx	DE ES FR GB IT PT	Ausreichend für "n" Tests Contenido suficiente para "n" tests Contenu suffisant pour "n" tests Contains sufficient for "n" tests Contenuto sufficiente per "n" saggi Contém o suficiente para "n" testes	**Cont.**	DE ES FR GB IT PT	Inhalt Contenido del estuche Contenu du coffret Contents of kit Contenuto del kit Conteúdo do kit
Max Min	DE ES FR GB IT PT	Temperaturbereich Límitaciôn de temperatura Limites de température de conservation Temperature limitation Limiti di temperatura Temperaturas limites de conservação			