DRG® CIC C3d (EIA-3170)

RUO in the USA

Revised 15 June 2010 rm (Vers. 6.0)

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

INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of CIC C3D concentration in serum and plasma.

CLINICAL SIGNIFICANCE

The importance of the immunocomplex (CIC) and their relation with several diseases have been object of investigations for many years.
The enstablishment of immunocomplex is a normal protecting process of the immune system. The circulating immunocomplex are removed from the circulation by means of various cellular, biochemical and enzymatic processes. Key of elimination of many CIC is the activation of the classic way of the complement.
In some diseases, of difficult understanding, the immunocomplex can begin the damaging of tissue and organs. In this case the activation of the complement can lead to the anafilotoxine production, stimulation of leukocyte and activation of macrophage and other cells.
In some cases of glomerulonephritises, in which the immunocomplex fix to the cellular membranes, it has the destruction of the tissue.
Circulating immuno-complexes (CIC) are present in many individuals affections from systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), especially in those affections from vasculitides complications.
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 some test to determinate fragments of the complex are available (Es. C1q and C3d), that have an important diagnostic mean.

PRINCIPLE

C3d-fixing circulating immune complexes (CIC) are first blocked by the anti-C3d immobilized on the microplate. During this phase, the immune complex bind the anti-C3d 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 of 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 per ml (µg Eq/ml) is the unit of measure of the results.

Reagent, material and instrumentation

Reagent and material supplied in the kit

1. CIC C3d **Standards** STD0 – STD2 (3 vials, 1.5 mL each)
2. **Negative Control** (1 vial = 1.5 mL)
   Positive Control (1 vial = 1.5 mL)
3. **Incubation Buffer** (1 bottle) 50 ml
   Phosphate buffer 74 mM pH 7.4; BSA 1 gr/L
4. **Conjugate** (1 bottle) min. 0.4 mL
   Anti-Human IgG-HRP conjugate
5. **Conjugate Buffer** (1 bottle) 20 ml
   Phosphate buffer 74 mM pH 7.4; BSA 1 gr/L
6. **Coated Microplate**, (1 microplate breakable)
   CIC C3D coated on microplate
7. Conc. **Wash solution** 10X, 2x (1 bottle = 50 ml)
   NaCl 16 g/L; tween 20 1 g/L, 20 mM Phosphate buffer, pH 7.4
8. **TMB Substrate** (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 to 8 °C in the dark and used within the expiration 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

Preparation of Conjugate

Dilute the concentrated Conjugate (reagent 4) **1/100** with Conjugate buffer (reagent 5).
The quantity of diluted Conjugate is proportional at the number of tests. Mix well and avoid foaming.

Stable for 3 hours at 18 °C to 25 °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 taking care also to transfer crystals with washing of the bottle, then mix until crystals are completely dissolved.

Preparation of the Sample

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. Avoid vortexing.

Assay Procedure

As it is necessary to perform in duplicate, each determination should include two wells for each *Sample*, two wells for each point of standard curve (S0-S2) and two for each Control..
Dispense:

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

QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of CIC C3d 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 lipemeic 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) for each point of the standard curve and of each sample.

Standard Curve

The Std. has the following concentration:
S0 0 µg Eq/mL
S1 16 µg Eq./mL
S2 64 µg Eq/mL
Plot the values of absorbance of the standards 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 the concentrations expressed in µg Eq/mL.

Reference Value

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

Performance and 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%.

Accuracy

The recovery of 12.5 – 25 – 50 – 100 µg Eq/mL IgG of CIC C3d added to "plasma-free" sample gave an average value (±SD) of 99.5% ± 4.4% with reference to the original concentrations.

Sensitivity

The lowest detectable concentration of CIC C3d that can be distinguished from the zero standard is 1,0 µg Eq/ml at the 95 % confidence limit.

Specificity

92 serum and plasma specimens collected from normal and asymptomatic subjects were tested with CIC C3d 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 was tested with CIC C3d. The overall clinical sensitivity was 92 %

Comparatives data

Circulating Immune complex (CIC) collected from 160 patient with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), or other disorders subjects and 95 from normal, asymptomatic subject were measured CIC C3d ELISA (EIA-3170) and with another kit commercially available.
The overall agreement between the two test methods was 87 %.
The average CIC concentration was 2.1 µgEq/mL (S.D. = 1.6)

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

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

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

RUO in the USA

Revised 15 June 2010 rm (Vers. 6.0)

Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità