DRG® IgM CIC ELISA (EIA-3987)

Revised 30 Aug. 2010 rm (Vers. 6.1) **RUO in the USA**

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

1 INTENDED USE

Immunoenzymatic colorimetric method for determination of IgM-CIC concentration in serum and plasma.

1 PRINCIPLE

C3-fixing circulating immune complexes (CIC) are first blocked by an anti-C3 antibody immobilized on a microplate. To quantify IgM-CIC complexes, a specific peroxidase anti-IgG antibody and H_2O_2-TMB as the enzyme substrate are then employed.
The amount of the enzyme reaction products formed, which are measured by reading absorbance at wavelength 450 nm, are proportional to the levels of solid-phase IgM-CIC complexes.

2 REAGENT, MATERIAL AND INSTRUMENTATION

2.1 Reagent and material supplied in the kit

1. **Incubation Buffer** (1 bottle) 27 ml
   100 mM Borate buffer, pH 7.4, 10 g/L BSA
2. **Conjugate** (1 bottle) 0.75 mL
   peroxidase-conjugate anti-IgM
3. **Conjugate Buffer** (1 bottle) 25 ml
   100 mM Borate buffer, pH 7.4, 10 g/L BSA
4. **Coated Microplate**, (1 microplate breakable)
   strips coated with Anti-C3 $(Fab')_2$
5. **TMB-substrate** (1 bottle) 12 mL
   H_2O_2-TMB 0.25gr/L (avoid any skin contact)
6. **Stop solution** (1 bottle) 12 mL
   Sulfuric acid 0.15 mol/L (avoid any skin contact)

2.2 Reagents necessary not supplied

0.9% NaCl solution (Saline solution)

2.3 Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader
37°C incubator

Note
All reagents and the microplate should be stored at 2÷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.

3 PRECAUTION

- Sera of control individuals may be stored at -20°C for several months.
- Samples should be dispensed in small aliquots (50 µL) and thawed only once.
- Do not use either lyophilised serum or pool of sera as controls.
- Handle control and sample sera in the same way.

4 PROCEDURE

4.1 Preparation of Conjugate

Dilute concentrated Conjugate (reagent 2) 1/20 with Conjugate buffer (reagent 3).
E.g. 100 µl of concentrated conjugate can be diluted to 2 mL with conjugate buffer.
Mix well and avoid foaming.
Reagents are stable until three hours at room temperature

4.2 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	25 µL
Incubation Buffer	500 µL

Mix gently with mixer.

4.3 Procedure

As it is necessary to perform in duplicate, each determination should also include two wells for each Sample, two wells for any Control and two wells for Blank.
Dispense:

	Sample	Control	Blank
Diluted Serum/plasma	100 µL	100 µL	---
Incubation buffer	---	---	100 µL

Incubate at 37°C for *1/2 hour*.
Remove the contents from each well. Wash three times each well with 300 µL of Saline solution. Leave the excess liquid to drain away by inverting the plate on absorbent paper.
Dispense into each well:

	Sample	Control	Blank
Diluted conjugate	100 µL	100 µL	100 µL

Incubate at 37°C for *1/2 hour*.

Remove the contents from each well. Wash three times each well with 300 µL of Saline solution. Leave the excess liquid to drain away by inverting the plate on absorbent paper.
Dispense into each well:

	Sample	Control	Blank
TMB Substrate	100 µL	100 µL	100 µL

Incubate at room temperature 22÷28°C for *15 min.*
Dispense:

	Sample	Control	Blank
Stop solution	100 µL	100 µl	100 µL

Read absorbance at 450 nm against Blank within 30 minutes after the addition of the Stop solution.

5 QUALITY CONTROL

It should be mentioned that a suitable parameter for comparing concentration of CIC is not currently available.
Generally, for comparison purposes, it is considered the concentration of IgG complexes, which, as CIC, can combine with the complement.
However, there are several limitations in using IgG complexes to estimate levels of CIC:

- Only CIC containing IgG can be measured (the procedure cannot be adapted to IgA, IgM and IgE-containing CIC);
- IgG complexes are not stable;
- Products of temperature aggregation may not be reproducible.

Because of these observations, it has been suggested that a population of healthy blood donors represents a more suitable control.
A screen of 15 - 20 individuals is sufficient to establish a *"normal range"* of values.
Performing each time duplicate determination of 5 - 6 control samples may eliminate assay-to-assay variations.

6 LIMITATION OF PROCEDURE

6.1 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.

7 RESULTS

7.1 Calculation

Results are expressed as *standard deviation units* (*sd*)
(whereas sd is not the statistically defined standard deviation!)
For the calculation of *sd* you need to determine

- the absorbance (OD) to calculate Standard Deviation (SD) (SD is the statistically defined standard deviation) and
- the Mean OD of controls (see chapter 7, healthy specimens)

In run 1, you have to measure samples of 5 – 6 healthy specimens, you use as controls.

You get $SD1_{Controls}$, $Mean1_{Controls}$

Now you calculate the *standard deviation units* of values you got from specimens, if you measured them in run 1.
Absorbance of the specimens = $OD_{specimen}$

$$sd = \frac{OD_{specimen} - Mean1_{Controls}}{SD1_{Controls}}$$

These results should be corrected because of assay-to-assay variations.

If you measure specimens in another run (for example run 2), you have to correct "*sd* " with the assay-to-assay variation factor.
Now in run 2, you take these controls and measure them together with samples of specimens.

You get $SD2_{Controls}$, $Mean2_{Controls}$, Absorbance of the specimens ($OD_{specimen}$)

These basics are necessary to calculate the *corrected standard deviation units (corrected sd)* to interpret absorbance values of the specimens:

$$corrected\ sd = \frac{Mean1_{Controls}}{Mean2_{Controls}} \times \frac{OD_{specimen} - Mean2_{Controls}}{SD2_{Controls}}$$

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Example:

RUN 1

healthy controls	$OD_{control}$
C1	0,45
C2	0,679
C3	0,521
$Mean1_{Controls\ 1-3}$	0,55
$SD1_{Controls\ 1-3}$	0,1172

specimens	$OD_{specimen}$	sd	Interpretation: negative results < 2 neg	Interpretation: positive results ≥ 2 pos
P1	0,765	1,834	<2 neg	
P2	0,875	2,7725		≥ 2 pos
P3	0,489	-0,5204	<2 neg	
P4	1,543	8,4711		≥ 2 pos

RUN 2

healthy controls	$OD_{control}$
C1	0,512
C2	0,732
C3	0,601
$Mean2_{Controls\ 1-3}$	0,615
$SD2_{Controls\ 1-3}$	0,111

specimens	$OD_{specimen}$	corrected sd	Interpretation: negative results < 2 neg	Interpretation: positive results ≥ 2 pos
P1	0,821	2,081		≥ 2 pos
P2	1,314	7,063		≥ 2 pos
P3	0,45	-1,667	<2 neg	
P4	1,893	12,913		≥ 2 pos

8 WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

9 BIBLIOGRAPHY

Pereira,A.B., et al J. Immunol. 125: 763-770 (1980).
E. Sagnelli, et al J. Clin. Lab. Immunol. 12: 11-15 (1983)
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10 TROUBLESHOOTING

ERROR POSSIBLE 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.)

Unexplainable outliers

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