AUTOSTAT™II C1q-CIC

C1q-containing Circulating Immune Complex Product Code - DIO002

CE IVD

PROPRIETARY AND COMMON NAMES

Hycor Biomedical Ltd Autostat™II C1q-CIC enzyme linked immunosorbent assay (ELISA) specific for C1q-containing circulating immune complexes.

INTENDED USE

Enzyme-linked immunosorbent assay method for the semi-quantitative determination of specific C1q-containing circulating immune complexes in human serum. The results of the C1q-CIC assay can be used as an aid in the diagnosis and monitoring of immune dysfunction. Levels of these complexes are one indicator in a multi-factorial diagnostic regime.

For *in vitro* diagnostic use only.

INTRODUCTION

Circulating immune complexes are present in many individuals with Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA), especially with any of the vasculitides complications. Levels of CICs have been reported to show correlation with disease activity in that higher levels are reported during active phases of the disease.

Many tests have been developed for the detection of CICs, including PEG precipitation and radial immunodiffusion and cellular based assays such as the Raji cell assay. No single procedure appears to detect all types of CIC's however, those procedures which detect CIC's containing fragments of complement (e.g.C1q and C3d) appear to detect clinically relevant events. C1q binds with greatest avidity to immune complexes ranging in size from 19 to 27 S. In serum sickness, which is the prototype immune complex disease, this size of complex has typically been found to be depoited in tissue leading to damage.

The Autostat™II test system for C1q circulating immune complexes detects immune complexes containing both C1q and IgG. The concentration is expressed as µg/ml.

The Autostat™II test range also includes a kit for C3d circulating immune complexes which detects immune complexes containing both C3d and IgG.

PRINCIPLE OF THE PROCEDURE

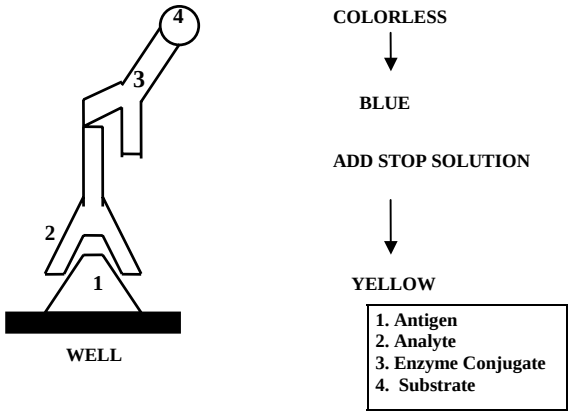
The Autostat™II assay is a solid phase immunosorbent assay (ELISA) in which the analyte is indicated by a colour reaction of an enzyme and substrate. The Autostat™II wells are coated with anti-C1q monoclonal antibody (1).

On adding diluted serum to the wells the C1q-containing CICs (2) present bind to the antibody. After incubating at room temperature and washing away unbound material, horseradish peroxidase conjugated anti-IgG monoclonal antibody (3) is added, which binds to the immobilised complexes.

Following further incubation and washing, tetra-methyl benzidine substrate (TMB) (4) is added to each well. The presence of the complex turns the substrate to a dark blue colour. Addition of the stop solution turns the colour to yellow.

The colour intensity is proportional to the amount of immune complexes present in the original serum sample.

FIGURE 1



MATERIALS PROVIDED

- Mouse anti-human IgG conjugate: One vial containing 15ml of ready-to-use HRP conjugate. Conjugate diluent contains 0.05% Proclin 300. Conjugates are color coded pink.

- Microplate: One microplate is supplied which contains 12 strips of breakapart wells. The wells are coated with mouse-anti-C1q antibody.

- Standard: One vial containing 1 ml of standard. The standard contains human antisera and 0.09% sodium azide as a preservative. The concentration of the standard is 100µg/ml.

- Positive control: One vial containing 0.45 ml of concentrated positive control which contains human antisera and 0.09% sodium azide as a preservative.

- Negative control: One vial containing 0.45 ml of concentrated negative control which contains normal human serum and 0.09% sodium azide as a preservative.

- TMB substrate solution: One vial containing 15ml of ready-to-use tetra-methylbenzidine (TMB) substrate.

- Sample diluent: One bottle containing 100ml of ready-to-use sample diluent buffer. The buffer includes 0.09% Sodium azide. Sample diluent buffer is color coded blue.

- Wash buffer: One bottle containing 50ml of wash buffer concentrate. Wash buffer concentrate contains 0.06% Proclin 300.

- Stop solution: One bottle containing 20ml of 0.25M H_2SO_4 stop solution.

WARNINGS OR PRECAUTIONS

Warning - Potentially Hazardous and Biohazardous Materials

Sera used in the preparation of the standards and controls have been tested for the presence of antibodies to Human Immunodeficiency Virus (HIV 1 and 2), as well as for Hepatitis B Surface Antigen (HBsAg) and HCV and found to be negative. All material is tested with FDA approved assays. Because no test method can offer complete assurance that HIV, HBsAg or other infectious agents are absent, it is recommended that human serum based products be handled with the same precautions used for patient specimens.

Dispose of reagent solutions containing sodium azide and thimerosal as preservatives according to all local, state and national regulations. To dispose of reagents containing azide, flush away using copious amounts of water. Dispose with caution as sodium azide can form explosive compounds on prolonged contact with lead or copper piping.

The performance data represented here were obtained using specific reagents listed in the package insert. Do not use reagents from other manufacturers in the kits. Do not use reagents from other Autostat™II kits with this kit. Do not dilute or adulterate the kit reagents, unless directed by the kit protocol. Do not use the substrate solution if it has begun to turn blue. Do not use heat-inactivated serum.

Reagents contain preservatives which may be toxic if ingested. Do not pipette by mouth. Avoid contact of reagents or patient samples with skin or mucous membranes. If contact occurs, immediately flush with large quantities of water. Avoid splashing or creation of aerosols. Reusable glassware must be thoroughly washed and rinsed so that it is free of all detergents.

Microplate washing is important. Improperly washed wells will give erroneous results. Do not allow the wells to dry between incubations. Do not vary reagents and incubation temperatures above or below room temperature (18 - 25°C).

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Distilled or deionized water.
2. Wash bottle, automated or semi-automated microwell plate washing system.
3. Rack for sample dilution.
4. Micropipettes including multichannels capable of accurately delivering 5-1000µl (less than 3% cv).
5. Reagent reservoirs for multichannel pipettes.
6. One-liter graduated cylinder.
7. Disposal basins and 0.5% sodium hypochlorite (50 ml bleach in 950ml water).
8. Single or dual wavelength microplate reader with 450nm filter. If dual wavelength is used, set the reference filter to 600 - 650 nm.
9. Paper towels, pipette tips and timer

STORAGE AND SHELF LIFE

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

INTERFERENCES AND HOOK EFFECT

Grossly haemolysed, lipaemic or microbiologically contaminated samples should not be used. Samples with abnormally elevated levels of haemoglobin, bilirubin and especially EDTA may interfere with assay performance and accuracy.

A 'hook effect' may only be seen with very high samples which are above the assay range. No hook effect is observable or of significance in this assay.

SPECIMEN COLLECTION AND PREPARATION

Use serum in this procedure. It is most important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Obtain patient samples by non-traumatic venipuncture, using a vacuum tube or sterile syringe. If a syringe is used, transfer the blood immediately to a vacuum tube (plain red-top or serum separator).

Allow samples to clot at room temperature (18-25°C) for at least 20-30 minutes, until the clot just begins to retract. Spin the sample in a centrifuge. Immediately following the centrifugation, transfer the cell-free serum to a tightly stoppered storage bottle.

Do not use sera samples showing signs of haemolysis. If it is necessary to store a sample prior to analysis, it is recommended that, for a period of up to 72 hours, store the sample in a sealed container at 2-8°C. Freeze samples at -20°C if longer storage is required. Avoid repeat freeze-thawing.

LIMITATIONS OF USE

A negative result should not be used as a sole criterion to rule out immune dysfunction but must be taken in relation to other clinical observations and diagnostic tests. While the precision of the Autostat™II kit is sufficient to allow samples to be measured in single determinations, this is done at the clinical laboratory's discretion. It is advised that duplicate determinations should be used to enable identification of potential pipetting error or to allow for confirmation in the equivocal range.

It should be noted that C1q-containing CICs occur at low levels in other autoimmune and non-autoimmune conditions. Therefore all other clinical observations and diagnostic tests should be taken into account for clinical diagnosis.

REAGENT PREPARATION

- Wash buffer: Measure and dilute 50 ml of the 20 X wash buffer to one liter with distilled or deionized water. Mix well before use. Store this solution at 2-8°C if it is not to be used immediately. The diluted wash buffer is stable at 2-8°C for one week.

- Positive and negative controls: These are provided in a concentrated form and should be diluted 1/5 (50µl + 200µl) with sample diluent buffer before use. Prepare fresh control dilutions before each assay run. Vortex all samples and controls before testing.

The conjugate, substrate and stop solution in Autostat™II kits are supplied in a ready-to-use format.

MANUAL ASSAY PROTOCOL

1. Bring all reagents to room temperature (18-25°C).
2. Remove protective covering and select sufficient wells to accommodate the patient samples, standards and assay controls. Each sample is to be tested in duplicate. **Wash plate three times with diluted wash buffer immediately prior to commencing the assay (remove excess wash buffer by tapping on absorbent paper but do not allow the plate to dry out).**
3. Dilute all serum samples and assay controls 1/5 in sample diluent
4. Pipette 100µl of the standards, diluted control or diluted patient sample into the wells. To achieve blanking on the plate reader add a 'no serum' control of 100µl of sample diluent to the first two wells. This will act as the zero point for the curve fit.
5. Incubate the wells at room temperature (18-25°C) for 30 minutes.
6. Wash the wells three times with diluted wash buffer. This can be done manually with a multichannel pipette or on an automatic plate washer. Empty the wells, invert and tap dry on paper towel.
7. Add 100µl of ready-to-use conjugate to each well.
8. Incubate the wells at room temperature (18-25°C) for 15 minutes.
9. Repeat washing as in section 6. above.
10. Add 100µl of ready-to-use TMB substrate to each well.
11. Incubate the wells at room temperature (18-25°C) for 15 minutes.
12. Add 50µl of stop solution to each well. Tap gently to ensure uniform color distribution and read within 15 minutes.
13. To read the plate, ensure the base is free from moisture and no air bubbles are in the wells. Read the absorbance of the well contents at 450nm on a suitable plate reader. On readers equipped with a dual wavelength facility set the reference filter to 600 - 650nm.
14. Subtract the blank (or mean of blanks) from the optical densities of the standard, controls and patient samples. If the assay was performed in duplicate, the mean of the wells should be taken.

CALCULATION OF RESULTS

Calculate the mean, blank corrected, absorbance value (OD) for duplicates of the kit standard. Calculate the mean, blank corrected, absorbance value (OD) for duplicates of the kit controls and patient samples. Using the following algorithm, calculate the concentration of each of the samples:

$$\frac{\text{Concentration of standard}}{\text{OD of standard}} \times \text{OD of sample or control}$$

The concentration of the standard is 100 µg/ml.

MANUAL TYPICAL RESULTS

The data below is for example only and should not be used for calculation of results. The results were calculated using the formula described in the 'calculation of results' section:

	OD	OD	Mean OD	Result
Reagent Blank	0.090	0.103	0.097	
Standard	2.059	2.111	2.085	
Sample 1	2.283	1.963	2.123	101.9 µg/ml
Sample 2	0.222	0.191	0.206	5.5 µg/ml

INTERPRETATION OF RESULTS

The assay cut-off was determined by combining data from a panel of 44 asymptomatic normal samples and a panel of other autoimmune positives. These are as follows:

µg/ml	Negative	Equivocal	Positive
C1q CIC	<40	40 - 50	>50

PERFORMANCE CHAREACTERISTICS

Analytical Specificity and Sensitivity.
A panel of 51 'normal' asymptomatic individuals was run on the kit. 88% of the normals gave negative results.

The sensitivity of the assay was established by calculation of the mean plus three standard deviations of a minimum of 20 replicates of the zero standard which gave a value of 1.23 µg/ml

Reproducibility
Intra- and inter- assay variation were checked using a number of samples.

Intra-assay variation	A	B	C
x	6.4	86.5	116.7
%cv	4.3	6.6	3.6

Inter-assay variation	A	B	C
x	6.4	86.5	116.7
%cv	13.5	8.3	7.7

QUALITY CONTROL
Good laboratory practice indicates that with each assay run, one or more quality control samples of known antibody level should be analyzed as though they were clinical samples. Positive and negative control samples are supplied with each kit which may be assayed with each run. The results of these quality control samples should fall within the limits indicated on the Certificate of Analysis.

Should the results fall outwith this range repeat the assay using freshly prepared controls. Should the results continue to fall outside the specific range, and after equipment, adherence to the protocol and laboratory procedure have been verified, seek assistance from the supplier. Do not report patient results if the control results fall outwith the acceptable ranges.

Relative Sensitivity and Specificity.
The assay was compared to another commercially available test. The results are shown below:

		Autostat II		
		Positive	Negative	Equivocal
Predicate Device	Positive	10	1	5
	Negative	2	40	2

Relative sensitivity	90.9 %
Relative specificity	95.2%
Overall agreement	94.3 %

Equivocal results were omitted from the above calculation.

HY•TEC INSTRUMENT ASSAY PROTOCOL

Please refer to the HY•TEC automated EIA system procedure manual for specific instructions on using this assay with the HY•TEC automated instrument. Dilutions of controls and samples are performed automatically by the HY•TEC instrument.

Important: Wash plate three times with diluted wash buffer immediately prior to commencing the assay (remove excess wash buffer by tapping on absorbent paper but do not allow the plate to dry out).

Assay results are calculated by the HY•TEC system automatically.

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