For Individual Laboratory to Complete:

Laboratory Name		
Adopted		
Reviewed		
Reviewed		
Revised		
Supercedes		

EZ Complement CH50 Test

(Hemolytic Method)
Prod No. 789-001
-24 tests

Method: **Diamedix Corp., Immunosimplicity®**
For *In Vitro* Diagnostic Use.

Clinical Significance

The complement system participates in the immunological defense of the human body. The complement system consists of a group of several proteins, which normally exist in serum in an inactive form. The classic pathway is initiated by the complexing of antigen to its specific antibody, either IgG or IgM, and is the primary amplifier of the biological effects of humoral immunity.[1,2] Activation of the complement sequence leads to the consumption of complement components which, in turn can lead to a decrease in their concentration. Thus, the determination of complement activity can indicate whether the complement system has been activated by an immunologic and/or pathogenic mechanism.

Complement levels may be abnormal in certain disease states such as rheumatoid arthritis or systemic lupus erythematosus (SLE) and in some genetic disorders. Increased complement levels are often associated with inflammatory conditions, trauma, or acute illness such as myocardial infarction. Since separate complement components are acute-phase proteins, the elevations however are common and non-specific. Deficiencies of complement account for a small percentage of primary immunodeficiencies but depression of complement frequently co-exists with SLE and other disorders associated with an immunopathologic process. Thus, low levels of complement can be found in rheumatic diseases, glomerulonephritis, infectious diseases and in deficiency disorders. [1,2]

The traditional method for determination of functional complement activity is total hemolytic (CH50) assay. This assay measures the ability of the test sample to lyse 50% of a standardized suspension of sheep erythrocytes coated with anti-erythrocyte antibody. Both the classic activation and the terminal complement components are measured in this reaction. Total complement activity is usually abnormal if any component is defective.[3]

Assessment of CH50 is useful in screening for genetic deficiencies in the complement system and in monitoring the progress of patients with immune complex disease.

Principle of the Procedure

Total complement consists of a number of distinct components. When sheep erythrocytes are sensitized with anti-sheep erythrocyte antibody, an antigen-antibody complex is formed. This complex, when exposed to the complement in human serum, will activate the components resulting in lysis of the erythrocytes and the release of hemoglobin. The degree of lysis is proportional to the concentration of total complement in the serum.

The Diamedix EZ Complement CH50 Test consists of test tubes that contain sheep erythrocytes sensitized with antibodies against sheep erythrocytes in 3 ml of a standardized buffer solution.

The cell suspension has been adjusted so that approximately 5 µl of sample from an individual with normal complement levels will lyse approximately 50% of the cells. Cell lysis can be read in a standard spectrophotometer at 415 nm. Patient values are then compared to a Reference Serum with a known CH50 value.

Specimen Collection

Whole blood should be collected by accepted medical techniques. A minimum of 5 ml of whole blood is recommended. Allow blood to clot for approximately 60 min at room temperature (18-30ºC) Centrifuge the sample and transfer the serum to a clean tube at 2-8ºC. Samples must be handled and stored correctly to avoid erroneous results. If the serum is not tested on the day it is separated, store at -70ºC, preferably in aliquots. If storage does not exceed 24 hours, serum can be kept frozen at -20ºC. Prior to testing, bring frozen sera to room temperature and mix gently avoiding foam formation. All patient sera should then be kept cold (2-8ºC) until used. Multiple freeze-thaw cycles should be avoided. Hemolysed specimens should not be used.

CAUTION: Serum samples must not be heat-inactivated prior to use.

Reagents

Sensitized Cells 24 tubes, 3 ml per tube. Standardized suspension of buffered sheep erythrocytes sensitized with antibodies to sheep erythrocytes. Preserved with sodium azide.

Store these reagents UPRIGHT at 2 to 8° C.
DO NOT FREEZE.

Other Materials Required

Pipettors capable of dispensing appropriate volumes.
Tube rack.
Vortex mixer.
Timer.
Centrifuge.
Reader capable of reading absorbance at 415nm with a 1-cm light path (EZ Reader recommended)
Reference and control material.

The following materials may be obtained from Diamedix.

EZ Complement Reference Serum **Cat. No.789-006**

Lyophilized Human Serum containing normal complement levels as determined by the EZ CH50 Test. 8 X 0.3 ml vials. Store at -20ºC.
This is a reference preparation suitable for standardizing the EZ CH50 Test. This Reference is prepared from human serum and is stabilized by lyophilization. This material is assigned both a CH50 value and a % Value. Reconstitute immediately before use with 0.3 ml of distilled water in accordance with the instructions that accompany the product.

EZ Complement Low Control **Cat. No.789-008**

Lyophilized Human Serum containing low complement levels as determined by the EZ CH50 Test. 8 X 0.3 ml vials. Store at -20ºC.
The Low Control is prepared from human serum and is stabilized by lyophilization. This material is assigned both a CH50 value and a % Value. Reconstitute immediately before use with 0.3 ml of distilled water in accordance with the instructions that accompany the product.

EZ Complement High Control **Cat. No.789-009**

Lyophilized Human Serum containing high complement levels as determined by the EZ CH50 Test. 8 X 0.3 ml vials. Store at -20ºC.
The High Control is prepared from human serum and is stabilized by lyophilization. This material is assigned both a CH50 value and a % Value. Reconstitute immediately before use with 0.3 ml of distilled water in accordance with the instructions that accompany the product.

Warnings:

1. Handle samples, Reference Serum, controls and the materials that contact them as potential biohazards. Each donor unit in the Reference Serum and controls has been found negative for Hepatitis B surface antigen and HIV-I antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1, Hepatitis B virus, or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories", 1993.
2. Never pipette by mouth.
3. Avoid contact with open skin and mucous membranes.
4. The Sensitized Cells contain sodium azide as preservative. Azides are reported to react with lead and copper in plumbing to form compounds that may become explosive. When disposing of solutions containing sodium azide, flush with copious amounts of water to minimize buildup of metal azide compounds.
5. Do not use Sensitized Cells beyond the expiration date imprinted on each tube (day-month-year).
6. Procedural steps should be strictly adhered to in order to obtain consistent and reliable results.
7. Diamedix makes every effort to ensure that the Sensitized Cells are packaged and shipped in a manner that will render them useful throughout their shelf-life. However, due to factors that are difficult for Diamedix to control regarding the shipping and receiving of this product, it is recommended that the end user check each box of tubes randomly selecting tubes, centrifuging the tubes at 1800 RPM for 10 minutes, then reading the “spontaneous lysis” absorbances. If the “spontaneous lysis” absorbance exceeds 0.150, please contact Diamedix Technical Services Dept. at 1-800-327-4565.

Calibration

This test uses an EZ Complement Reference Serum that has been calibrated against an in-house Gold Standard. All lots of Reference Serum are traceable to the Gold Standard. Each has been prepared from a pool of sera containing normal complement levels. The Reference Serum functions as an internal reference preparation and is assigned a CH50 and % value. The Reference Serum should be included in every test and is run in the same way as a test sample. Users can also utilize internally obtained material. Patient values are then calculated against this material.

Quality Control

a) The absorbance of the "spontaneous lysis" control must be no greater than 0.150 when read at 415 nm.

b) The Low and High controls, if utilized, should be within their assigned ranges.

The test is considered valid if these criteria are met.

Procedure

1. Set up as many tubes as there are samples for testing plus one tube each for the Reference Serum, Low and High Controls, and one tube for the "spontaneous lysis" control.
2. Place the required number of tubes containing the Sensitized Cells in a suitable rack and allow them to warm to room temperature (18 - 30ºC) for at least 60 minutes.
   NOTE: In order to avoid incubation and mixing differences, Diamedix recommends that runs be limited to 12 – 15 tubes.
3. VIGOROUSLY vortex or shake the tubes for 10 seconds to resuspend the cells.
4. Remove the caps from all tubes. Add 5 µl of patient sample, Reference and controls to the appropriately labeled tube. After each sample addition, replace the cap and mix IMMEDIATELY by shaking the tube vigorously.
   The "spontaneous lysis" tube should also be mixed.
5. Allow the tubes to stand at room temperature (18 - 30ºC) for 60 ± 5 minutes.
6. Mix the contents of all tubes again by inverting 3-4 times.
7. Centrifuge the tubes at approximately 1800 RPM for 10 minutes.
8. Read the absorbances of the supernatants at 415 nm within 15 minutes after centrifugation. Diamedix recommends the use of the EZ Reader for absorbance determinations.
9. Read the absorbance of the "spontaneous lysis" control.
   If the absorbance is greater than 0.150, the results of the assay are not considered valid. Repeat the test.
10. Zero the reader using the "spontaneous lysis" control as a blank. This will correct for the degree of "spontaneous lysis" in the cells.
11. Read and record the absorbance values of the Reference Serum and each control and patient serum.

Calculation of Results

Results can be expressed either as % of the EZ Complement Reference Serum or as CH50 Values. Determine the results using the formula below:

Absorbance of Sample / Absorbance of Reference x % Reference or CH50 Value of Reference (from vial label) = % Reference or CH50 Value of Sample

Examples: CH50 Value of Reference Serum Vial = 210
% Value of Reference Serum = 105

Absorbance of Reference = 0.726 Absorbance of Sample = 1.023

a. $\frac{1.023}{0.726} \times 210 = 296$ *CH50 Value*

b. $\frac{1.023}{0.726} \times 105 = 148\%$ *of Reference*

Interpretation of Results

% of Reference	*CH50 Value*	*Interpretation*
0 to 50	0 – 100	Absent or low
51 to 150	101 – 300	Normal
> 151	> 301	High

Limitations

1. EZ Complement CH50 Test results are not diagnostic in themselves. Test results should be interpreted in conjunction with other laboratory tests as well as the clinical presentation of the patient.
2. The EZ Complement CH50 Test will provide an assessment of the functional activity of total complement. This test can determine abnormal complement levels but cannot identify the abnormal component or components.
3. Individual component abnormalities or abnormalities in the alternative pathway can exist despite a normal CH50 value.

References

1. Turgeon, M.L. 1996. Soluble Mediators of the Immune System. In: Immunology and Serology in Laboratory Medicine. 2nd Edition. Mosby, St Louis, MO. p. 89-107.
2. deShazo, R.D., Lopez, M. L. and Salvaggio, J.E. 1987. Use and Interpretation of Diagnostic Immunologic Laboratory Tests, JAMA. Vol. 258, No. 20, p 3019-3023.
3. Kabat, E. A. and Mayer, M.M. 1961. Complement and Complement Fixations. In: Experimental Immunochemistry, 2nd Edition, Charles C. Thomas, Springfield, IL. p.133-240.