

SUMMARY OF PROCEDURE

1. Allow the EZ Complement Cells to equilibrate at room temperature (18-30° C) for at least 1 hour. Resuspend them with a vortex or by shaking vigorously.
2. Add 5 μ l of patient samples, reference or control sera to the tubes containing the Sensitized Cells and thoroughly mix each tube immediately after sample is added.
Reserve one tube for the "spontaneous lysis" control (no sample).
3. Incubate at room temperature (18-30° C) for 60 \pm 5 min. Then mix all tubes by inverting 3-4 times.
4. Centrifuge at 1800 RPM for 10 minutes.
5. Read the absorbances of the supernatants at 415 nm against the "spontaneous lysis" control.
6. Calculate results.

INTENDED USE

For the determination of total complement activity (CH50) in human serum. This assay is used to determine the functional integrity of the entire classic complement pathway.

SUMMARY AND EXPLANATION

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 biologic 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 the 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 by antibody against sheep erythrocytes 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 Cells consist of test tubes that contain sheep erythrocytes sensitized with antibodies against sheep erythrocytes in 3 ml of standardized buffer solution.

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

REAGENTS

EZ Complement Cells 24 tubes, 3 ml per tube. Standardized suspension of sheep erythrocytes in buffer, 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 415 nm with a 1-cm light path. (EZ Reader recommended).
Reference and control sera.

The following materials may be obtained from Diamedix.

EZ Complement Reference Serum : **Catalog # 789-006**

Lyophilized Human Serum containing normal complement levels as determined by the EZ CH50 Test. 8 x 0.3 ml vials. Store at -20° C.

This is a reference preparation suitable for standardizing the EZ CH50 Test. The Reference is prepared from human sera 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 this product.

EZ Complement Low Control: **Catalog # 789-008**

Lyophilized Human Serum containing low levels of complement as determined by the EZ CH50 Test. 8 x 0.3 ml vials. Store at -20° C.

The Low Control is prepared from human sera 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 this product.

EZ Complement High Control: **Catalog # 789-009**

Lyophilized Human Serum containing high levels of complement as determined by the EZ CH50 Test. 8 x 0.3 ml vials. Store at -20° C.

The High Control is prepared from human sera 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 this product.

WARNINGS AND PRECAUTIONS

REAGENTS: For *in vitro* Diagnostic Use.

1. Handle samples, reference and controls and the materials that contact them as potential biohazards. Each donor unit in the reference and controls has been found negative for Hepatitis B surface antigen and HIV-1 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. Sodium Azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts", in the Manual Guide – Safety Management No. CDC-22, issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

European Communities Hazardous Substance Risk Phrases (Regulation (EC) No 1272/2008)

H300 –Fatal if swallowed.
H310 – Fatal if contact with skin.
EUH032 – Contact with acids liberates very toxic gas.
H410 – Very toxic to aquatic life with long lasting effect.
P264 – Wash all exposed external body areas thoroughly after handling.
P302+P352 – IF ON SKIN: Wash with plenty of water and soap.
P301+P310/P330 – IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Rinse mouth.

P270 – Do not eat, drink or smoke when using this product.
 P501 – Dispose of contents/container as hazardous waste.
 P391 – Collect spillage.

P273 – Avoid release to the environment. Refer to special instructions/
 Safety Data Sheet.

Sodium Azide inhibits horseradish peroxidase activity. Care must be taken to ensure that azide is not carried over from other reagents into conjugate and substrate steps.

- Do not use EZ Complement Cells beyond the expiration date imprinted on each tube (day-month-year).
- Procedural steps should be strictly adhered to in order to obtain consistent and reliable results.
- Diamedix makes every effort possible to ensure that the EZ Complement Cells are packaged and shipped in a manner that will render them usable 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 by randomly selecting tubes, centrifuging the tubes at 1800 RPM for 10 minutes and 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.

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 minutes at room temperature (18-30° C). Centrifuge the sample and transfer the serum to a clean tube at 2-8° C. 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 -70° C, preferably in aliquots. If storage does not exceed 24 hours, serum can be kept frozen at -20° C. Prior to testing, bring frozen sera to room temperature and mix gently avoiding foam formation. All patient sera should then be kept cold (2-8° C) until used. Multiple freeze-thaw cycles should be avoided. Hemolyzed specimens should not be used.

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

DO NOT USE PLASMA.

PROCEDURE

- Set up as many tubes as there are samples for testing plus one tube each for the Reference and Low and High Controls and one tube for the "spontaneous lysis" control. The "spontaneous lysis" control is a tube of EZ Complement Cells in buffer to which no sample is added.
- Place the required number of tubes containing the EZ Complement Cells in a suitable rack and allow them to warm to room temperature (18 - 30° C) for at least 60 minutes.
 NOTE: In order to avoid incubation and mixing differences, Diamedix recommends that runs be limited to 12-15 tubes.
- VIGOROUSLY vortex or shake the tubes for 10 seconds to resuspend the cells.
- Remove the caps from all tubes. Add 5 µl of patient samples, 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 thoroughly mixed.
- Allow the tubes to stand at room temperature (18 - 30° C) for 60 ± 5 minutes.
- Mix the contents of all tubes again by inverting 3-4 times.
- Centrifuge the tubes at approximately 1800 RPM for 10 minutes.
- 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.
- Read the absorbance of the "spontaneous lysis" control.
 If the absorbance value is greater than 0.150, the results of the assay are not considered valid. Repeat the test.
- Zero the reader using the "spontaneous lysis" control as a blank. This will correct for the degree of "spontaneous lysis" in the test samples.
- Read and record the absorbance values of the Reference Serum and each control and patient sample.

QUALITY CONTROL

- The absorbance of the "spontaneous lysis" control must be no greater than 0.150 when read at 415 nm.
- The Low and High Controls should be within their assigned ranges.

The test is considered valid if these criteria are met.

RESULTS

The concentration of EZ Complement Cells has been adjusted to yield approximately 50% hemolysis in the presence of 5 µl of normal human serum. Studies conducted by Diamedix have shown that the test is linear ($R^2 \geq 0.99$) for CH50 values of at least 400. Since the assay is linear over a broad range, a single point can be used for calibration of this method.

1. 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:

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of Reference}} \times \frac{\% \text{ Reference or CH50 Value of Reference (from vial label)}}{\% \text{ Reference or CH50 Value of Sample}} = \text{Value of Sample}$$

Examples: CH50 Value on Reference Serum vial = 210
 % Value on Reference Serum Vial = 105

Absorbance of Reference = 0.726 Absorbance of Sample = 1.023

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

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

2. 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

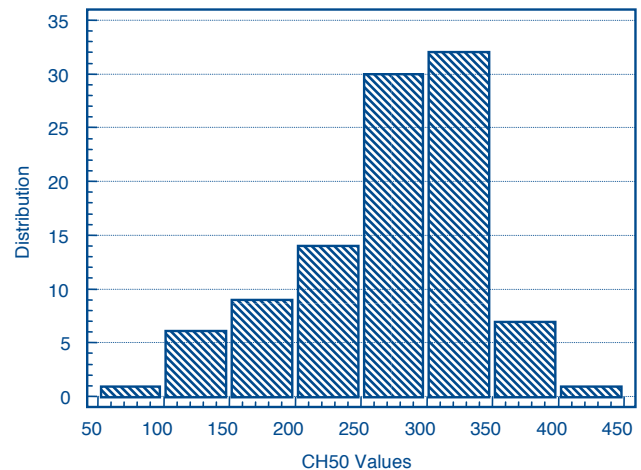
- EZ Complement CH50 Test results are not diagnostic in themselves. Test results should be interpreted in conjunction with other laboratory tests as well as the clinical presentation of the patient.
- The EZ Complement CH50 Test will provide an assessment of the functional activity of total complement.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

EXPECTED VALUES

Normal values in populations can vary widely. Complement proteins are acute-phase reactants and levels tend to increase with intercurrent illnesses. There is also a tendency for levels to be higher in females who are either pregnant or using oral contraceptives. In addition, it has been noted that levels tend to rise slightly in aged individuals (4).

The EZ CH50 Test was performed using serum samples from one hundred randomly selected, apparently healthy, blood donors from the S. Florida area. These samples gave CH50 values ranging from 91 to 404. The mean CH50 value obtained was 274 with a Standard Deviation of 68. The distribution of these values is shown in Figure 1.

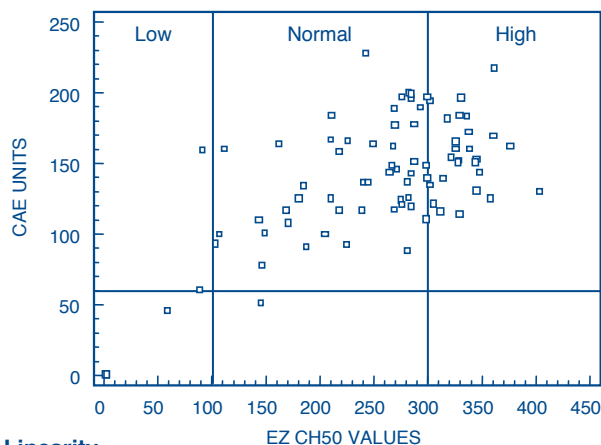
FIGURE 1
Distribution of CH50 Values from 100 Normal Sera



A. Correlation Studies

The EZ CH50 Test was compared to another commercially available test for the determination of total complement activity in human serum. Seventy-four serum samples from normal blood donors were assayed by both methods. In addition, a representative lot of EZ CH50 Reference, low and high controls were tested by both methods. Also tested were several commercially purchased deficient sera, Standard sera, as well as the WHO International Reference Preparation (5) for a total of eighty-two samples. The correlation between the methods is shown in Figure 2.

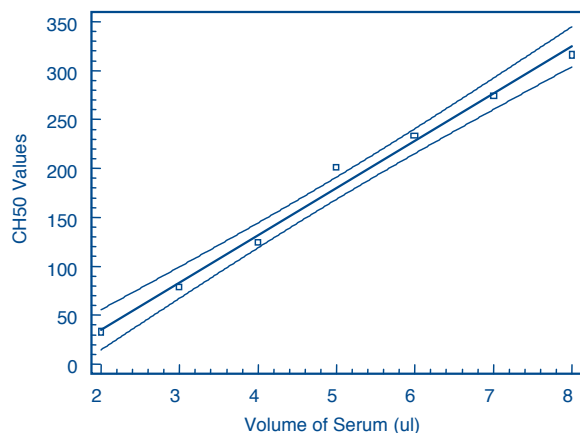
FIGURE 2
EZ CH50 Test vs. another commercially available test



B. Linearity

An assessment was made of the linearity of the assay procedure. Different amounts of sera were added to the Sensitized cell tubes and tested. Several different serum samples were tested in this manner. These data indicated that the assay is linear ($R^2 \geq 0.99$) to at least 400 CH50 units. Figure 3 shows the linearity of the in-house 'Gold' Standard material.

FIGURE 3
Linearity of EZ CH50



C. Accuracy

The accuracy of the EZ CH50 Test was assessed by testing several commercially available reference materials. The ability of the EZ CH50 test to detect deficiencies in complement components was assessed by testing two commercially available materials: one deficient for Factor B and the other deficient for C3. Results are shown in Table 1.

TABLE 1
EZ CH50 Results with Purchased Reference Materials

Sample	Expected CH50 Value	EZ CH50 Value
Factor B deficient	N/A	3.0
WHO Ref. Prep. (Functional Whole Complement)	100	94
Standard Complement Serum	122	112
C3 deficient Human Complement Serum	N/A	221

N/A - Not available

D. Precision Testing

The precision of the test method was assessed by testing eight samples in triplicate in two runs per day for three days. Samples included the in-house 'Gold' Standard, a representative EZ Reference Serum, EZ High Control serum, EZ Low Control serum and four patient samples. Mean values are expressed in CH50 units.

SERUM	INTRA-ASSAY DAY1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY3			INTERASSAY		
	MEAN VALUE	SD	CV%	MEAN VALUE	SD	CV%	MEAN VALUE	SD	CV%	MEAN VALUE	SD	CV%
A	214	4.2	2.0	214	7.9	3.7	214	1.6	0.8	214	5.4	2.5
B	200	7.2	3.6	212	6.6	3.1	204	11.8	5.8	205	10.4	5.1
C	338	47.6	14.1	387	44.6	11.5	315	16.3	5.2	347	50.3	14.5
D	67	4.8	7.2	75	7.6	10.1	76	4.5	6.0	73	7.2	9.9
E	107	7.3	6.9	109	10.3	9.5	106	3.9	3.7	107	7.9	7.4
F	287	9.6	3.3	283	5.6	2.0	275	5.6	2.0	282	9.2	3.3
G	283	7.8	2.8	287	18.8	6.6	270	11.8	4.4	280	15.8	5.6
H	262	8.4	3.2	260	18.5	7.1	237	18.2	7.7	253	19.8	7.8

REFERENCES

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Diamedix Corporation • A Subsidiary of ERBA Diagnostics, Inc.
14100 NW 57th Court – Miami Lakes, Florida 33014 - USA
(305) 324-2300 / (800) 327-4565
www.erbadiagnostics.com

EC REP Delta Biologicals S.r.l., Via Nicaragua 12/14, 00071 - Pomezia, Rome Italy
Telephone #: +39-06-91190.1 Fax #: +39-069105244



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