

CH50 ELISA TEST SYSTEM

INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of complement functionality in human serum. CH50 kit is intended for laboratory use only.

CLINICAL SIGNIFICANCE

The primary utility of the CH50 in the practice of an allergist-immunologist is to screen for complement-deficiency associated immunodeficiency (primarily classic or terminal complement component deficiencies). Absent or significantly reduced individual complement components may result in infections, Neisserial meningitis, or sepsis. A reduced CH50 in this situation warrants quantification and functional assays of individual complement components. Reduction of the CH50 occurs when individual complement component(s) are deficient or consumed.

PRINCIPLE OF THE TEST

The complex galactosidase/anti-galactosidase, is solubilized by serum through the deposition of C3b molecules. The formation of C3b quantity necessary for the solubilization is mediated by alternative pathway, but it is accelerated from activity of C3-convertase by classic way. The quantity of complex-galactosidase/Anti-galactosidase dissociated from the antibody, detectable by enzymatic activity in the supernatant at the end of the reaction, is a measure of serum capacity to form C3b molecules. The o-nitrophenylgalactopyranoside (o-NPG) is used as substrate and the measure of reagent product (o-nitrophenol) is read at 420nm (or 405 nm).

MATERIALS AND COMPONENTS

- Reference Calibrator (1 vial, 0.6 ml)
- Incubation Buffer (1 vial, 12ml)
Phosphate Buffer 50m M pH 7.35
- Immunocomplex (2 vials, 3ml each)
Galactosidase/anti-galactosidase
- Microplate (1 breakable micTplate)
Empty Microplate
- ONPG Substrate (1 vial, lyophilized)
Phosphate buffer 15 mM pH 7.0 o-NPG 2.3 mM
(Avoid any skin contact)
- Ethanediol (1 vial, 1ml)
(Harmful if swallowed)
- Stop Solution (1 vial, 7ml)
ris buffer
- Controls with different levels of solubilization
(2 vials, 0.6ml each)
Low Control
High Control

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled water.

AUXILIARY MATERIALS

- Automatic dispenser
- Microplates reader (filter at 420 nm or 405 nm) Incubator 37°C
- Centrifuge (10000 - 13500 xg "RCF")

Note

The Reference calibrator and Controls are synthetic; they guarantee higher reproducibility and stability compared with the reference of human origin.

Store all reagents at 2-8°C in the dark and use them before the expiry date of the kit. Bring all reagents to room temperature (22-28°C) before using.

Maintain the same order in reagents dispensation.

Use only serum sample (avoid using plasma samples). Human serum is stable one month at -20°C (six months if stored at -80°C).

WARNINGS

This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.

Use appropriate personal protective equipment while working with the reagents provided.

Follow Good Laboratory Practice (GLP) for handling blood products.

Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy, and the bovine proteins have been obtained from countries not infected by BSE; however, these materials should be handled as potentially infectious.

Some reagents contain small amounts of Sodium Azide as preservative. Avoid the contact with skin or mucus. Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover, it may react with lead or copper plumbing' to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build up. The ONPG Substrate contains an irritant, which may be harmful if inhaled, ingested, or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes. Ethanediol is harmful if swallowed; In case of ingestion consult a physician immediately. Avoid the exposure of reagent ONPG Substrate to directed sunlight, metals, or oxidants. Do not freeze the solution.

PRECAUTIONS

Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction for Use. All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable

until the expiration date when stored and handled as indicated. Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to

use. Do not interchange kit components from different lots. The expiration date printed on box and vials labels must be observed. Do not use any kit component beyond their expiration date.

If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested. 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 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.

Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. Maximum precision is required for reconstitution and dispensation of reagents.

Samples microbiologically contaminated, highly lipemic or hemolyzed should not be used in the assay.

Plate readers measure vertically. Do not touch the bottom of the wells.

PREPARATION OF THE IMMUNOCOMPLEX

Use the reagent without any dilution.

Before using mix well, the immunocomplex with vortex. Stable 3 months at 2-8°C.

PREPARATION OF THE ONPG SUBSTRATE

Add 10 ml of distilled water to the reagent. Once the reagent is dissolved, add 0.5 ml of Ethanediol. Stable for 2 months at 2-8°C.

Important: for a better repeatability (inter-assay), we suggest bringing the substrate at room temperature (22-28°C) before using (avoid the dispersion of reagent just removed from the fridge).

PROCEDURE

Step 1

Dispense each serum sample, the reference calibrator and a solubilizing control in an Eppendorf tube:

	Reference Calibrator	Sample or Controls	Not solubilizing control
Incubation Buffer	100µL	100 µL	150 µL
Reference calibrator	50µL	/	/
Sample or Controls	/	50 µL	/
Immunocomplex	50 µL	50 µL	50 µL

Vortex and invert a few times to be sure that the solution is well mixed.

Incubate 2 hours at 37 °C. Centrifuge at 10,000-13,500xg "RCF" for 15 minutes. Transfer with care, avoid touching the pellet with the pipette, 50µl of supernatant of each Eppendorf tube in the well of the microplate.

Important:

- Avoid the suspension of the pellets (NB: the pellet is often not very visible, but it is at the bottom of the tube; thus, avoid touching the bottom of the tube with the tip).
- Do not shake the centrifugate.
- Shake slowly the supernatant to avoid turbulences that cause the suspension of the pellet.

The pellet is Composed of non-solubilized immunocomplex with high enzymatic activity (p-Galactosidase); the presence of a small quantity of pellet in the supernatant can cause false positives and erroneous values for controls).

Step 2
In the Microplate

	Blank	Reference Calibrator	Sample or Controls	Not solubilize control
Incubation Buffer	50 µL	1	1	1
Supernatant	1	50 µL	50 µL	50 µL
ONPG-Substrate	100 µL	100 µL	100 µL	100 µL
Incubate for 15 minutes at 37°C in the dark.				
Stop solution	50 µL	50 µL	50 µL	50 µL
Shake the microplate gently, Read the absorbance (O.D.) at 420 nm (or 405) against Blank.				

QUALITY CONTROL

Each laboratory should assay controls at normal, high, and low levels range of CH50 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 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.

LIMITATIONS OF PROCEDURE

Interpretation of results CH50 results are not diagnostic themselves. Test results should

be interpreted in conjunction with other laboratory tests as well as the clinical presentation of the patient. CH50 kit 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 in the alternative pathway can exist despite a normal CH50. The traditional method for the activity determination of complement is the method total hemolysis. CH50 method is based on the capacity of complement to solubilize the immunocomplex. 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. Assessment of CH50 is useful in screening for genetic deficiencies in the complement system and in monitoring the progress of patients with immunocomplex disease.

RESULTS

Mean Absorbance

Calculate the mean absorbencies (EM) of reference calibrator, controls and of each sample.

Calculation of Results

The results can be expressed as:

- CH50 value or
- % of Reference Calibrator

The exact CH50 Value of Reference Calibrator is lot dependent and is reported on the label.

Determine the results using the following formula:

$$OD(\text{sample}) / OD(\text{Reference Calibrator}) \times CH50(\text{Value of Reference Calibrator}) = CH50 \text{ Value of Sample}$$

$$OD(\text{sample}) / OD(\text{Reference Calibrator}) = \% \text{ of reference Calibrator}$$

Example:

CH50 Value of Reference Calibrator Vial = 100
CH50 % of Reference Calibrator Vial = 50
Absorbance of Reference Calibrator = 0.350
Absorbance of Sample = 1.108

a. CH50 Value of Sample = $1.108 / 0.350 \cdot 100 = 316$

b. % of Reference Calibrator = $1.108 / 0.350 \cdot 50 = 158\%$

REFERENCE VALUES

% Reference	CH50 Value	Interpretation
0-50	0-100	Absence or low
51-150	101 - 300	Normal
> 151	> 301	High

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore, each laboratory should consider the range given by the manufacturer as a general indication and produce their own range

of expected values based on the indigenous population where the laboratory works.

PERFORMANCE AND CHARACTERISTICS

Correlation

22 samples were tested from a healthy blood donor with CH50 Test System and with a similar commercially available kit. The results were processed by ROC curves analysis on two levels (low and normal) showing:

Sensitivity	100%
Specificity	94.4%
Overall agreement	95.5%

WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

REFERENCES

Miller G.W. Nussenzweig V., PNAS , 72,418-1975
 Takahaschi M., Takahaschi S., Brade U., Nussenzweig V. J. Clin. Invest. 62, 349-1978
 Migliorini p, et al. J. of immunological Methods, 77,119-130 (1985)

PRESENTACIÓN:

CONT. 96 TEST CODIGO: RSET074-2