DRG® CH50 ELISA (EIA-3989)

CE IVD

REVISED 31 DEC. 2008 (VERS. 6.0) **RUO IN THE USA**

1 INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of complement functionality.
For in vitro diagnostic use only. In the United States, this kit is intended for Research Use Only.

1.1 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.

2 PRINCIPLE

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

3 REAGENTS, MATERIAL AND INSTRUMENTATION

3.1 Reagents and materials supplied in the kit

1. **Reference Calibrator** (1 vial) 0.6 mL
   Ready to use
2. **Incubation Buffer** (1 vial) 12 mL
   Phosphate buffer 50 mM pH 7.35;
3. **Immune Complex** (1 bottle) 6 mL
   β-galactosidase/anti-β-galactosidase,
4. **Microplate,** 1x, breakable
5. **oNPG-Substrate** (1 vial) lyophilized
   Phosphate buffer 15 mM, pH 7.0; o-NPG 2.3 mM - (avoid any skin contact)
6. **Ethandiol** (1 bottle), 1.0 mL
7. **Stop Solution** (1 vial) (7 mL)
   Sodium Carbonate 16%, - (avoid any skin contact).
8. **Control** with different levels of solubilisation, (0.6 mL)
   Low Control (1 vial) liquid
   High Control (1 vial) liquid

3.2 Reagents necessary not supplied

Distilled water.

3.3 Auxiliary materials and instrumentation

Automatic dispenser
Microplates reader (filter at 420 or 405 nm)
Incubator 37°C
Centrifuge for Eppendorf Tube (12.000 rpm)
Reference Serum (1 vial) Lyophilized

3.4 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 °C – 8 °C in the dark.
Bring all reagents to room temperature 22 °C – 28 °C.
Use only serum samples (avoid using plasma samples)
Human serum is stable for one month at –20°C (six months at -80°C).

4 PROCEDURE

4.1 Preparation of the Immunocomplex

Use the reagent without any dilution.
Before use mix well the immune complex with vortex.
Stable for 3 months at 2 °C – 8 °C

4.2 Preparation of the ONPG-Substrate

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

4.3 PROCEDURE

Step 1 in Eppendorf tubes
Dispense each sample (serum), the reference calibrator and control and a not solubilizing control in an Eppendorf tube:

	Reference Calibrator, Control	Sample	Not-solubilising Control
Incubation Buffer	100 µl	100 µl	150 µl
Reference Calibrator, Control	50 µl	---	---
Sample	---	50 µl	---
Immune Complex	50 µl	50 µl	50 µl

Mix well.
Incubate at 37°C for 2 hours.
Centrifuge at 10,000 – 12,000 rpm for 15 minutes.
Transfer with care 50 µl of supernatant of each Eppendorf tube in the microwells.
Avoid touching the pellet with the pipette.

Step 2 in the Microplate

	Blank	Reference Calibrator, Control	Sample	Not-solubilising Control
Incubation Buffer	50 µl			
Supernatant		50 µl	50 µl	50 µl
oNPGSubstrate)	100 µl	100 µl	100 µl	100 µl
Shake; incubate at +37°C for 15 minutes.				
Stop Solution	50 µl	50 µl	50 µl	50 µl
Read the absorbance (O.D.) against the Blank at 420 nm (405/420).				

5 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 past 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.

6 LIMITATIONS

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.

6.2 Interpretation of results

The CH50 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 CH50 ELISA 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 or abnormalities in the alternative pathway can exist despite a normal CH50
The traditional method for the activity determination of complement is the method of total haemolysis.
The DRG CH 50 method is based on the capacity of complement to solubilize the immune complex.
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 immune complex disease.

7 RESULTS

7.1 Mean absorbance

Calculate the mean of the absorbances (OD) of reference calibrator, control and of each sample.

7.2 Calculation of results

The result can be expressed as
a. CH50 value or as
b. % of Reference Calibrator

Determinate the results using the following formula:
a. OD_{Sample} / $OD_{Reference}$ Calibrator x CH50 Value of Reference Calibrator = CH50 Value of sample

b. OD_{Sample} / $OD_{Reference}$ Calibrator x CH50 % of Reference Calibrator = % of Reference Calibrator

Example:
CH50 Value of Reference Calibrator Vial = 100
CH50 % of Reference Calibrator Vial = 50

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

Absorbance of Reference Calibratoe = 0.350
Absorbance of Sample = 1.108

a. CH50 Value of Sample = 1.108/0.350 × 100 = 316
b. % of Reference Calibrator = 1.108/0.350 × 50 = 158 %

8 INTERPRETATION OF RESULTS

% of Reference	CH 50 Value	Interpretation
0 – 50	0 – 100	Absence or low
51 – 150	101 – 300	Normal
> 151	> 301	High

9 PERFORMANCE AND CHARACTERISTICS

9.1 Expected Values

The CH50 ELISA kit was performed using serum samples from 60 randomly selected, apparently healthy, blood donors. These samples gave CH50 mean values of 305 ranging from 121 to 397 with a standard deviation of 55.

9.2 Correlation

The CH50 ELISA was compared to another commercially available assay. The linear regression curve was calculated
y = 1.0324 x -0.1164

10 WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

11 BIBLIOGRAPHY

1. Miller G.W, Nussenzweig V.: A new complement function: solubilization of antigen-antibody aggregates. PNAS, 72, 418 – 1975.
2. Takahaschi M., Takahaschi S., Brade U., Nussenzweig V.: Requirement for the solubilization of immuno-aggregates by complement. J. Clin. Invest. 62, 349 – 1978.
3. Migliorini P, et al. J. of Immunological Methods, 77 119-130 (1985)

DRG

DRG® CH50 ELISA (EIA-3989)

CE IVD

REVISED 31 DEC. 2008 (VERS. 6.0) **RUO IN THE USA**

SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..