CE

DiaMetra
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DCM040-11
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CH50

for routine analysis

- FUNCTIONALITY TEST OF COMPLEMENT (CH 50) -

RUO

LOT
See external label

2°C 8°C

Σ = 96 test

REF DKO040

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

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 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-convertasi by classic way.
The quantity of complex β-galactosidase/Anti β-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-nitrophenilgalactopiranoside (o-NPG) is used as substrate and the measure of reagent product (o-nitrophenol) is read at 420nm (or 405 nm).

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Reference Calibrator (1 vial, 0.6 mL) **REF DCE030/4030-0**
2. Incubation Buffer (1 vial, 12 mL)
   Phosphate buffer 50 mM pH 7.35 **REF DCE024-0**
3. Immunocomplex (2 vials, 3 mL each)
   β-galactosidase/anti-β-galactosidase **REF DCE025-0**
4. Microplate (1 breakable microplate)
   Empty microplate **REF DMZ003**
5. ONPG Substrate (1 vial, lyophilized)
   Phosphate buffer 15 mM pH 7.0 o-NPG 2.3 mM
   (avoid any skin contact) **REF DCE026-0**
6. Ethanediol (1 vial, 1 mL)
   (Harmful if swallowed) **REF DCE027-0**
7. Stop Solution (1 vial, 7 mL)
   Tris buffer **REF DCE028-0**
8. Controls with different levels of solubilisation (2 vials, 0.6 mL each)
   Low Control **REF DCE045/4001-0**
   High Control **REF DCE045/4002-0**

3.2. Reagents necessary not supplied
Distilled water.

3.3. Auxiliary materials and instrumentation
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 to use plasma samples). Human serum are stable one month at -20°C (six months if stored at -80°C).

4. 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 mucosa.
- 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.

5. 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 expiry 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 expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry 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 lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. 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.

6.2. Preparation of the ONPG-Substrate

Add 10 mL of distilled water to the reagent. Once the reagent is dissolved, add 0.5 mL of Ethanediol. Stable for 2 months at 2-8°C.
Important: for a better repeatability (inter-assay), we suggest to bring the substrate at room temperature (22-28°C) before using (avoid the dispension of reagent just removed from the fridge).

6.3. Procedure

Step 1 in Eppendorf tubes

Dispense each serum sample, the reference calibrator and a not solubilising control in a eppendorf tube:

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

Vortex and invert few times the tube to be sure that the solution is well mixed. Incubate 2 hours at 37°C
Centrifuge at 10000-13500 xg "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 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 to touch the bottom of the tube with the tip).
- do not shake the centrifugate
- take slowly the supernatant in order to avoid turbulences that cause the suspension of pellet

(the pellet is composed of not solubilised immunocomplex with high enzymatic activity (β-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 solubiliz. control
Incubation Buffer	50 L	/	/	/
Supernatant	/	50 L	50 L	50 L
ONPG-Substrate	100 L	100 L	100 L	100 L
Incubate for 15 minutes at 37°C in the dark.				
Stop solution	50 L	50 L	50 L	50 L
Shake the microplate gently. Read the absorbance (O.D.) at 420 nm (or 405) against Blank.				

7. 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.

8. LIMITATIONS OF PROCEDURE

8.1. Interpretation of results

Diametra 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.

The Diametra 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 or abnormalities in the alternative pathway can exist despite a normal CH50.

The traditional method for the activity determination of complement is the method total haemolysis. The Diametra CH 50 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.

9. RESULTS

9.1. Mean Absorbance

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

9.2. Calculation of Results

The result can be expressed as:

a. CH50 value or
b. % 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:

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
Absorbance of Reference Calibrator = 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%*

10. 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 Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

11. PERFORMANCE AND CHARACTERISTICS

11.1. Correlation

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

Sensitivity	100.0%
Specificity	94.4%
Overall agreement	95.5%

12. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

ERRORE CAUSE POSSIBILI/ SUGGERIMENTI

Nessuna reazione colorimetrica del saggio

- mancata dispensazione del coniugato
- contaminazione del coniugato e/o del Substrato
- errori nell'esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

Reazione troppo blanda (OD troppo basse)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

Reazione troppo intensa (OD troppo alte)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta
- qualità scadente dell'acqua usata per la soluzione di lavaggio (basso grado di deionizzazione,)
- lavaggi insufficienti (coniugato non completamente rimosso)

Valori inspiegabilmente fuori scala

- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)

CV% intrasaggio elevato

- reagenti e/o strip non portate a temperatura ambiente prima dell'uso
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)

CV% intersaggio elevato

- condizioni di incubazione non costanti (tempo o temperatura)
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)
- variabilità intrinseca degli operatori

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

ERROR / POSIBLES CAUSAS / SUGERENCIAS

No se produce ninguna reacción colorimétrica del ensayo

- no se ha dispensado el conjugado
- contaminación del conjugado y/o del substrato
- errores en la ejecución del ensayo (p. ej., dispensación accidental de los reactivos en orden incorrecto o procedentes de frascos equivocados, etc.)

Reacción escasa (DO demasiado bajas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado corto, temperatura de incubación demasiado baja

Reacción demasiado intensa (DO demasiado altas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado largo, temperatura de incubación demasiado alta
- calidad escasa del agua usada para la solución de lavado (bajo grado de desionización)
- lavados insuficientes (el conjugado no se ha retirado completamente)

Valores inexplicablemente fuera de escala

- contaminación de pipetas, puntas o contenedores- lavados insuficientes (el conjugado no se ha retirado completamente)

CV% intraensayo elevado

- los reactivos y/o tiras no se encontraban a temperatura ambiente antes del uso
- el lavador de microplacas no funciona correctamente (sugerencia: limpiar el cabezal del lavador)

CV% interensayo elevado

- condiciones de incubación no constantes (tiempo o temperatura)
- controles y muestras no dispensados al mismo tiempo (con los mismos intervalos) (controlar la secuencia de dispensación)
- variación en función de los operadores

ERREUR CAUSES POSSIBLES / SUGGESTIONS

Aucune réaction colorimétrique de l'essai

- non distribution du conjugué
- contamination du conjugué et/ou du substrat
- erreurs dans l'exécution du dosage (par ex., distribution accidentelle des réactifs dans le mauvais ordre ou en provenance des mauvais flacons, etc.)

Réaction trop faible (DO trop basse)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop court, température d'incubation trop basse

Réaction trop intense (DO trop élevée)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop long, température d'incubation trop élevée
- mauvaise qualité de l'eau utilisée pour la solution de lavage (bas degré de déionisation)
- lavages insuffisants (conjugué non entièrement éliminé)

Valeurs inexplicablement hors plage

- contamination des pipettes, embouts ou récipients - lavages insuffisants (conjugué non entièrement éliminé)

CV% intra-essai élevé

- les réactifs et/ou les bandes n'ont pas atteint la température ambiante avant usage
- le laveur de microplaques ne lave pas correctement (suggestion : nettoyer la tête du laveur)

CV% inter-essai élevé

- conditions d'incubation non constantes (temps ou température)
- contrôles et échantillons non distribués en même temps (avec les mêmes intervalles) (contrôler l'ordre de distribution)
- variabilité intrinsèque des opérateurs