Autokit CH50

Read this instruction sheet thoroughly before use.

CE

***In vitro* Liposome Immunoassay**

For the quantitative determination of Total Complement Activity in human Serum

995-40801
0413 D4

Intended use

The Autokit CH50 is an *in vitro* Liposome Immunoassay (LIA) for the quantitative determination of total complement activity (CH50) in human serum, using an automated procedure.

Summary and explanation of the test

The complement cascade, consisting of ~ 20 serum proteins, plays an important role in the body's immunological defense system. Complement activity in human sera can provide important information in the diagnosis of many diseases. Clinically, complement activity is a direct indicator of abnormalities of the complement system, and is different from immunoreactive components of the system. Complement activity has been correlated with the active stage of systemic lupus erythematosus, rheumatoid arthritis, cryoglobulinemia-vasculitis, some forms of nephritis, and inherited deficiencies of the complement system.[1] Previously, the most commonly used assay for total complement activity was based on complement-mediated hemolysis of antibody-sensitized erythrocytes.[2] In this method, appropriate serum dilutions are necessary to measure lysis of the indicator cells. A simpler method, which does not require serum dilution, has been developed.[3] However, both methods are complicated and time-consuming, and the reagents are not stable because of the use of erythrocytes. In addition, it is difficult to automate a hemolytic complement assay because of the unstable nature of the erythrocyte dispersion.

Liposomes, consisting of concentric shells of lipid bilayers separated by aqueous interspaces, have been used extensively to study complement-mediated immune damage to cell membranes.[4,5] A homogeneous assay for total complement activity based on immune lysis of liposomes has been reported previously.[6] The degree of liposome lysis is determined from entrapped alkaline phosphatase activity, and the procedure, which is performed manually, cannot be applied to automated laboratory analyzers.

This method requires adding many reagents to reaction lubes, a long reaction lime, and the use of antibodies binding liposomes, which might induce aggregation and sedimentation of the liposomes in the prepared reagent.

Recently, we developed an automated homogeneous liposome-based assay for total complement activity in human serum. We used a homogeneous population of small-size liposomes (200 nm), which gave a stable dispersion, and glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) as the entrapped enzyme (the optimum pH of G6PDH is neutral in comparison with that of alkaline phosphatase). Using these liposomes we developed a fully automated assay system for total complement activity.[7]

Principle of the method

When a sample is mixed with the liposome and the substrate, the antibodies in the reagent combine with dinitrophenyl (DNP) on the liposomes and then complements in the sample are activated by the antigen-antibody complex. The activated complements break the membrane of the liposome. The enzyme, G6PDH, contained in the liposome, reacts with NAD and glucose 6-phosphate (G6P) in the reagent. During this enzyme reaction the NAD is reduced to NADH. As a result of this reduction, absorbance at 340 nm increases. The absorbance increase is proportional to the complement activity in the sample.

Reactions

Enzyme reaction

D-Glucose-6-Phosphate + NAD $\xrightarrow{\text{G6PDH}}$ ß-Gluconolactone-6-Phosphate + NADH (Detection at 340 nm)

Reagent preparation

Reagent 1: (R1)

Use Liposome (R1) as supplied. This solution is stable until expiration date.

Reagent 2: (R2) + (R2a)

Reconstitute one bottle (for 20 mL) of Substrate (R2) with one bottle (20 mL) of Diluent (R2a) to prepare the Substrate Solution. The Substrate Solution is stable for 40 days at 2 - 10°C.

Reagents

Contents and storage conditions

R1: Liposome	2 bottles x 20 mL	Store at 2 - 10°C (Do not freeze)
R2: Substrate	1 bottle x for 20 mL	Store at 2 - 10°C
R2a: Diluent	1 bottle x 20 mL	Store at 2 - 10°C
R1: Liposome	Contains liposome G6PDH	4 U/mL
R2: Substrate	Contains anti-DNP antibody, goat	24 mmol/L G6P 9 mmol/L NAD
R2a: Diluent	Contains maleate buffer, pH5.0 **Mixture containing:** 5-chloro-2-methyl-2H-isothiazol-3-one [EC No 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC No 220-239-6] (3:1)	5 mmol/L

This kit (R2a) contains components classified as following:

Classification according to Regulation (EC) No 1272/2008

• Signal word Warning

• Hazard-determining components of labelling:

Mixture containing: 5-Chloro-2-methyl-2H-isothiazol-3-one [EC No 247-500-7] and 2-Methyl-2H-isothiazol-3-one [EC No 220-239-6] (3:1).

• Hazard statements

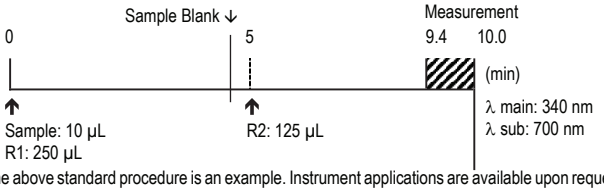
May cause an allergic skin reaction.

• Precautionary statements

Avoid breathing dust/fume/gas/mist/vapours/spray.
Wear protective gloves/protective clothing/eye protection/face protection.
Contaminated work clothing should not be allowed out of the workplace.
If skin irritation or rash occurs: Get medical advice/attention.
Wash contaminated clothing before reuse.
IF ON SKIN: Wash with plenty of soap and water.
Specific treatment (see on this label).
Dispose of contents/container in accordance with local/regional/national/international regulations.

Standard procedure

Temperature: 37°C (Hitachi®717)



The above standard procedure is an example. Instrument applications are available upon request.

Warnings and precautions

- For *in vitro* diagnostic use only.
- The usage and application of this test is reserved for professional use only. Please refer to respective national and local regulations and legislation.
- Not to be used internally in humans and animals.
- Operate the instruments according to operator's manuals under appropriate conditions.
- Do not mix the reagents from one test unit with those of another test unit which has a different lot number.
- Do not use the containers and other materials in the package for any purposes other than those described herein.
- Clinical diagnosis must be determined with clinical symptoms and other test results by a physician.
- Store the reagents under the specified conditions. Do not use reagents past the expiration date stated on each reagent container label.
- After opening the reagents, it is recommended to use them immediately. When the opened reagents are stored, cap the bottles and keep them under the specified conditions.
- Since all specimens are potentially infectious, they should be handled with appropriate precaution. Refer to respective good laboratory practice protocols for preventing transmission of infection and handle samples in accordance with any other local or national regulations relating to the safe handling of such materials.
- If the reagents come in contact with the mouth, eyes or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
- When discarding the reagents, dispose of them according to local or national regulations.

Physical or chemical Indications of instability

The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent instability.

Instruments

The reagent is designed to be used on commercially available automated analyzers. Refer to the operating manual for a description of instrument operation and specifications. Performance standards on alternative instrumentation must be established by the end user.

Specimen collection and preservation

Use serum as a specimen.

It is recommended to measure the complement activity in the specimen immediately after separation of serum. If needed, store specimens at -70°C or lower. Ascorbic acid, bilirubin, hemoglobin, and lipemic turbidity do not have a significant effect on the measurement.

Manufacturer:

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Materials supplied
Refer to the section entitled „Reagents".

Materials required but not supplied
Automated analyzer
CH50 Calibrator (Code No. 997-43801)
Complement Control (Code No. 991-43701)

Results
The final results are automatically calculated and printed in concentration.

Calibration
The CH50 assay produces a calibration curve by plotting absorbance vs. concentration. It is recommended to perform calibration at least once a week.

Quality control
A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. The values obtained for controls should fall within the manufacturer's acceptable ranges. If values are to be established for unassayed control material, the laboratory should assay each level of control material a sufficient number of times to generate a valid mean and acceptable range.

Limitations of the procedure
The measurable range of Wako Autokit CH50 is 10 - 60 U/mL.

Expected values
Serum : 31,6 - 57,6 U/mL.
The expected values are calculated by a normal value distribution. 243 persons, showing assay results within the expected values in 12 biochemicals parameters, were selected from 880 persons. These data were performed in Japan. (Internal data).
Since expected values are affected by age, sex, diet, geographical location and other factors, each laboratory should establish its own expected values for this procedure.

Interfering substances
Ascorbic acid concentrations up to 50 mg/dL, hemoglobin concentrations up to 500 mg/dL and bilirubin concentrations up to 40 mg/dL do not have a significant effect on the Autokit CH50 assay.

Performance characteristics

Accuracy (WAKO-30R)

No.	Expected (U/mL)	Observed (U/mL)	Recovery (%)
1	27.1	31.0	114.4
2	36.5	40.0	109.6
3	47.3	47.0	99.4
4	54.6	53.5	98.0

Precision (WAKO-30R)

Within-Run Precision

Run #	Sample #	Replicates	Mean (U/mL)	SD	SV (%)
1	1	21	49.5	0.5	1.10
1	2	21	25.9	0.3	1.35
2	1	21	46.2	0.5	1.14
2	2	21	27.9	0.3	1.05

Total Precision

Concentration level	# of assay days	Mean (U/mL)	SD	CV (%)	S_{wr}	S_T
Low	21	26.9	1.54	5.7	16.6	16.7
High	21	48.3	1.57	3.2	18.9	22.1

The data was collected according to NCCLS Guidelines.

Sensitivity: The minimum detectable level of CH50 is estimated to be 10 U/mL.

Specifity (WAKO-30R)
Additive Study

Ascorbic acid (mg/dL)	none	10	20	30	40	50
CH50 (U/mL)	36.0	36.0	36.0	35.0	35.5	35.5

Bilirubin (mg/dL)	none	8	16	24	32	40
CH50 (U/mL)	35.0	36.0	36.0	36.0	37.0	37.0

Hemoglobin (mg/dL)	none	100	200	300	400	500
CH50 (U/mL)	40.0	40.0	40.0	40.0	40.0	39.5

References

1. Schur PH : Complement studies of sera and other biologic fluids. Hum Palhol 1983 ; **14** : 338-42.
2. Mayer MM : Complement and complement fixation. In : Kabat EA, Mayer MM , eds. Experimental immunochemistry, 2nd ed. Springfield, IL: Charles C Thomas. 1967 ; 133-240.
3. Kitamura H , Inai S , Nagaki K : A simple procedure for the titration of total hemolytic complement activity. Jpn J Clin Chem 1983 ; **12** : 143-7.
4. Kinsky SC : Antibody-complement interaction with lipid model membranes. Biochim Biophys Acta 1972 ; **265** : 1-23.
5. Akots G , Braman JC , Broeze RJ , Bowden DW : Rapid, homogeneous phase, liposome-based assays for total complement activity. Complement 1984 ; **1** : 125-33.
6. Bowden DW , Rising M , Akots G , Myles A , Broeze RJ : Homogeneous liposome-based assay for total complement activity in serum. Clin Chem 1986 ; **32** : 275-8.
7. Yamamoto S , Kubotsu K , Kida M , Kondo K , Matsuura S , Uchiyama S , Yonekawa 0 , Kanno T : Clin Chem 1995 ; **41** : 586-90.

Ordering information

Code No.	Product	Package
995-40801	Autokit CH50	R1: 2 x 20 mL R2: 1 x for 20 mL R2a: 1 x 20 mL
997-43801	CH50 Calibrator	CAL: 5 conc. x for 0,5 mL
991-43701	Complement Control	H: 10 x for 0,5 mL (high) L: 10 x for 0,5 mL (low)

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