

Autokit CH50

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.

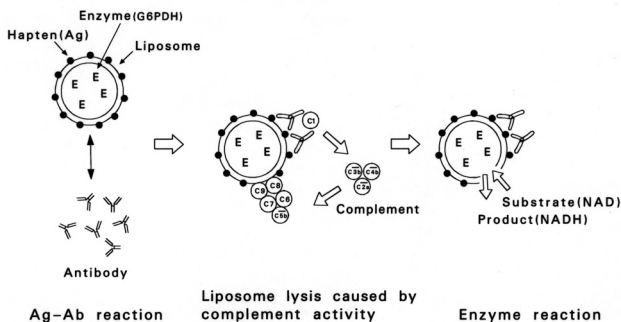
Summary and explanation of the test

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.

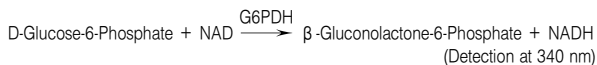
Liposomes, consisting of concentric shells of lipid bilayers separated by aqueous interspaces, have been used extensively to study complement-mediated immune damage to cell membranes.^{4,5} A homogeneous assay for total complement activity based on immune lysis of liposomes has been reported previously.⁶ The degree of liposome lysis is determined from entrapped alkaline phosphatase activity, and the procedure, which is performed manually, can not be applied to automated laboratory analyzers. This method requires adding many reagents to reaction tubes, a long reaction time, and the use of antibodies binding liposomes, which might induce aggregation and sedimentation of the liposomes in the prepared reagent. 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.



Enzyme reaction



Reagents

Contents and storage conditions

- (1) Liposome
4 units/mL liposome containing G6PDH
Store at 2-10°C. (Do not freeze.)
- (2) Substrate
Anti-DNP antibody (goat)
24 mmol/L G6P
9 mmol/L NAD
Store at 2-10°C.
- (2a) Diluent
10 mmol/L maleate buffer (pH 5.0)
Store at 2-10°C.

Code No.	995-40801
(1) Liposome	2 bottles × 20 mL
(2) Substrate	1 bottle × for 20 mL
(2a) Diluent	1 bottle × 20 mL

Warnings and precautions

- (1) For in vitro diagnostic use.
- (2) Not to be used internally in humans and animals.
- (3) Do not mix the reagents from one test unit with those of another test unit, which has a different lot number.
- (4) Do not use reagents past the expiration date stated on each reagent container label.
- (5) Do not use the reagents described above for any purpose other than described herein.
- (6) 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.
- (7) UDI (Unique Device Identification) is on the box. Keep box until reagent is finished.

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. (See Performance Characteristics.)

Warning/Biohazard

Since all specimens are potentially infectious, they should be handled at the Biosafety Level 2 as recommended for any potentially infectious body fluid in the USA Centers for Disease Control/USA National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", and in accordance with any other local or national regulations relating to the safe handling of such materials.

Procedure for WAKO-30R

Materials supplied

Refer to the section entitled "Reagents".

Materials required but not supplied

WAKO-30R analyzer.
CH50 Calibrator (Code No. 997-43801).
Complement Control (Code No. 991-43701).

Reagent preparation

Reagent 1: (1)

Use Liposome (1) as supplied. This solution is stable until expiration date.

Reagent 2: (2) + (2a)

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

Calibrator :

Remove vials from freezer. Remove the cap and stopper and gently tap down all lyophilized material to the bottom of the vial. Accurately add 0.5 mL of distilled or deionized water to dissolve the contents of each of the Calibrators. Replace the stopper securely. Make sure that all the lyophilized material is covered with fluid and allow the vials to stand at room temperature for 5 minutes with occasional, gentle shaking. When all the material has been reconstituted at the bottom of the vial, turn the vial on its side and allow the reconstituted material to "roll around" the walls of the vial by rotating the vial slowly. Invert the vial and allow the fluid to cover the stopper for 20-30 seconds with gentle shaking. Return the vial to the upright position and remove the stopper. Allow any fluid which may have been trapped in the stopper to drain down.

Once the Calibrator is reconstituted, keep the solution on ice for at least 30 minutes. Store at 2-10°C thereafter and use it within 8 hours.

Control :

Accurately add 0.5 mL of distilled or deionized water to dissolve the contents of each of the Control. Once the Control is reconstituted, keep the solution on ice for at least 30 minutes. Store at 2-10°C thereafter and use it within 8 hours.

Test procedure

Parameter setting (WAKO-30R)

Test : CH50

Temperature : 37°C

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REACTION MODE	1	RATE
REFERENCE TEST ID	0	
TEST WAVELENGTH	1	340
BLANK WAVELENGTH	14	700
MULTI-ASSAY TEST ID	0	
TEST READ TIMING	58-62	
BLANK READ TIMING	0-0	
SAMPLE VOLUME	10 µL	
DILUTED SAMPLE VOLUME	0 µL	
SAMPLE REPEAT #	0	

REAGENT D	VOLUME	0 µL
	POSITION	0
	BOTTLE	0 mL
REAGENT 1	VOLUME	250 µL
	POSITION	*1
	BOTTLE	50 mL
REAGENT 2	VOLUME	125 µL
	POSITION	*2
	BOTTLE	50 mL
STIRRER		1 ON

* 1:Any number from 8-36
* 2:Any number from 28-47

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CALIBRATION MODE	4	LOGIT-4
CALIBRATION INTERVAL	0	0
FACTOR	0	
SLOPE FACTOR	100%	
BASE FACTOR	0	
BLANK #	3	
STANDARD #	3	

	CUP NO.	CONC.
BLANK	0	0
STANDARD 1	*3	*4
STANDARD 2	*3	*4
STANDARD 3	*3	*4
STANDARD 4	*3	*4
STANDARD 5	*3	*4

* 3 Defined by operator.
* 4 Input the assigned value of the calibrator.

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SAMPLE RANGE	0	
REACTION MODE	0	UP
MAX RATE LIMIT	0%	
RANGE (mABS)	0	
CALIBRATOR'S ABSORBANCE WINDOW		
SAMPLE	LOW	0
BLANK	LOW	0
SLOPE	0-0	0

RATIOS	REACTION MODE	1	RATE
	TEST READ TIMING	[] []	
	REF. READ TIMING	[] []	
	CHECK VALUES	[] []	

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

- (1) The measurable range of Wako Autokit CH50 is 10 - 60 U/mL.
- (2) Assay results may not be accurate if samples are diluted.

Expected values

Serum : 31.6 - 57.6 U/mL⁸

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.

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)

Preliminary Precision Test - Within-Run Precision

Run #	Sample #	Replicates	Mean (U/mL)	SD	CV (%)
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

The data was collected according to NCCLS Guidelines.

Concentration Level	# of assay Days	Mean (U/mL)	SD	CV (%)	S _{wr}	S _T
High	21	48.3	1.57	3.2	18.9	22.1
Low	21	26.9	1.54	5.7	16.6	16.7

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

Specificity (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 Pathol 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 O, Kanno T. Clin Chem 1995 ; 41 : 586-90.
8. Fujio K, Nagao K, Shiraiishi N. Jpn J. Med Pharm Sci 2012 ; 67 (2) : 291-5.

Ordering information

Code No.	Products	Package
995-40801	Autokit CH50	Liposome 2×20 mL Substrate 1×for 20 mL Diluent 1×20 mL
997-43801	CH50 Calibrator	5 conc. × for 0.5 mL
991-43701	Complement Control	2 conc. × 10 bottles × for 0.5 mL

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Manufactured by
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