INSTRUCTIONS FOR USE

Complement TCC

Semi-Quantitative test

Enzyme immunoassay for assessment of terminal complement complex (TCC)

Document No. LABEL-DOC-0333, 3.0

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

REF **COMPL TCC RUO**

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PURPOSE OF RESEARCH PRODUCT

The Complement TCC is an enzyme immunoassay for the semi-quantitative determination of soluble terminal complement complex (sTCC also known as TCC or sC5b-9) in human EDTA plasma.

The product is intended for professional use. The result shall not be used for clinical diagnosis or patient management. FOR RESEARCH USE ONLY.

SUMMARY AND EXPLANATION

In all cases where determination of complement function is wanted, TCC levels can be very informative as a supplement to functional assessment of the three complement pathways. It reflects the historical *in vivo* activity of complement in a given sample.

The complement system plays an essential role in chronic, autoimmune and infectious disease. There are three pathways of complement activation, namely the classical, the alternative and the lectin pathway. The soluble terminal complement complex is a product of the terminal pathway and can be a result from all three complement activation pathways. Since TCC reflects activation to the end of the final terminal pathway irrespective of the initial pathway involved, it is a particularly good candidate for general evaluation of complement activation. (Harboe 2011).

It is well-known that the complement system plays a key role in the development and amplification of the inflammatory process at the tissue level in various pathological conditions. Increased levels of TCC can be detected in for example hemolytic uremic syndrome (HUS) (Noris 2012), Systemic lupus erythematosus (SLE) (Porcel 1995, Mollnes 1999) and rheumatoid arthritis (RA) (Struglics 2016). The complement system can also be activated by artificial surfaces, for example during hemodialysis or cardiopulmonary bypass, resulting in increased levels of TCC. (Deppish 1990, Ovrum 1996). TCC is also well suited for studies of complement activation by biomaterials in medical devices (Stang 2014).

TCC can like other activation products of complement be measured in assays using neo-epitope specific monoclonal antibodies. Neoepitopes are hidden in the native complement component and exposed after complement activation. (Mollnes 1985, Mollnes 1993)

PRINCIPLE OF THE COMPLEMENT TCC ASSAY

The assay is a colorimetric sandwich ELISA. Samples are diluted in assay diluent and 100µL diluted sample is transferred to the microtiter wells and incubated at room temperature for 60 minutes. During this first incubation TCC in the sample is captured by the anti-TCC monoclonal antibody, pre-coated on the surface of the microtiter wells. After washing to remove unbound material, a second horseradish peroxidase (HRP) labelled monoclonal antibody is added to detect the TCC bound to the well. After incubation for 30 minutes the wells are washed again and a substrate is added and incubated. The color development is stopped after 30 minutes and the color is measured in a spectrophotometer. The color is directly proportional to the amount of TCC bound to the well. The amount of TCC is determined by comparison to the color development of the calibrator samples.

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WARNINGS AND PRECAUTIONS

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The human serum components used in the preparation of the calibrators and controls in the kit have been tested for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen by FDA approved methods and found negative. Because no test methods can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
- The Centers for Disease Control and Prevention and National Institutes of Health recommended that potentially infectious agents be handled at the Biosafety Level 2.
- Calibrators, controls, diluent, conjugate and wash solution contain ProClin 300 as a preservative. Never pipette by mouth or allow reagents or samples to come into contact with skin. Reagents containing ProClin may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water. Handle ProClin 300 containing reagents as hazardous waste.
- The stop solution contains 0.5 M sulphuric acid. Do not allow the reagent to get into contact with the skin.
- TMB (3, 3', 5, 5'-tetramethylbenzidin) is toxic by inhalation, in contact with skin and if swallowed. Observe care when handling the substrate.
- Safety data sheet for all hazardous components contained in this kit is available on request from Svar Life Science.

SPECIMEN COLLECTION

Blood samples are to be collected using aseptic venipuncture technique and EDTA plasma obtained using standard procedures. A minimum of 5 mL of whole blood is recommended. Centrifuge blood samples and transfer cell-free plasma to a clean tube. Plasma must be properly handled to prevent in vitro complement activation.

The centrifuged EDTA plasma may be kept at 4°C up to 8 hours and analysis should be performed within this timespan. For longer storage, plasma should be frozen at -70° C or lower. Samples should not be frozen and thawed more than once.

KIT COMPONENTS AND STORAGE OF REAGENTS

- One frame with microtiter wells (12x8) coated with anti-TCC monoclonal antibody, sealed in a foil pack with a dry pack.
- 1.5mL Low control (LC). Ready to use.
- 1.5mL High control (HC). Ready to use.
- 2 x 30 mL Diluent (Dil). Ready to use.
- 15 mL Conjugate containing HRP-labelled antibodies to TCC. Ready to use.
- 15 mL Substrate TMB. Ready to use.
- 15 mL Stop solution (0.5M H_2SO_4). Ready to use.
- 30 mL Wash solution, 30x concentrated.
- 6 x 1.5mL vials with calibrators containing human purified TCC, 0 ng/mL, 10ng/mL, 50 ng/mL, 100ng/mL, 200ng/mL and 400ng/mL. Ready to use.

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Please note:
All reagents in the kit are ready to use except the wash solution. The reagents should be stored at 2-8°C. Components from different lots shall not be mixed.

Materials or equipment required but not provided

- Microplate reader with filter 450nm and 620nm.
- Precision pipettes with disposable tips.
- Washer for strips, absorbent tissue, tubes and a timer.
- Dilution plate.

PROCEDURE

1. Equilibrate reagents (microtiter plate, calibrators, controls, diluent, wash solution, conjugate, substrate and stop solution) to room temperature (20°C - 25°C).
2. Dilute the wash solution 30x (ex. 30 ml concentrated wash solution + 870 ml dH_2O)
3. Thaw EDTA plasma samples at room temperature.
4. Store thawed plasma samples on ice.
5. Dilute samples 1/10 with diluent (ex. 30 µl plasma + 270 µl diluent). Using a pre-dilution plate is recommended.
6. Transfer 100µL diluted sample, calibrator and controls to the microtiter wells in duplicates and incubate at room temperature for 60 minutes.
   NOTE: A quick transfer of samples, calibrators and controls to the plate is recommended to avoid drift of signal.
7. Wash 3 times with 300 µl diluted wash solution by filling and emptying the wells. After last wash, empty the wells by tapping the strip on an absorbent tissue.
8. Add 100 µl conjugate to the wells. Incubate at room temperature for 30 minutes.
   Wash 3 times with 300 µl diluted wash solution by filling and emptying the wells. After last wash, empty the wells by tapping the strip on an absorbent tissue.
9. Add 100 µl substrate to the wells. Incubate at room temperature for 30 minutes.
10. Add 100 µl stop solution to the wells.
11. Read absorbance at 450 nm om the microplate reader. Read at 620 nm as reference wavelength.

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CALCULATION OF RESULT

Subtract the reference wavelength from the 450nm wavelength and calculate mean OD values for all samples. Construct a calibrator curve by plotting the OD of the calibrators against the concentrations. Values of the six calibrators are 0 ng/mL, 10 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL and 400 ng/mL. Read the concentrations of the un-known samples against the calibrator curve.

NOTE: All performance data in this IFU were calculated using a 4-parameter curve fit. Users may select a different curve fit. It is recommended that each laboratory establish a reference range with the selected curve fit.

NOTE: to calculate the concentration of TCC in the sample, compensation for dilution must be done i.e. 10x according to the protocol above. If the sample result exceeds the calibrator curve a higher dilution is recommended and should be taken into account when calculating the concentration in sample.

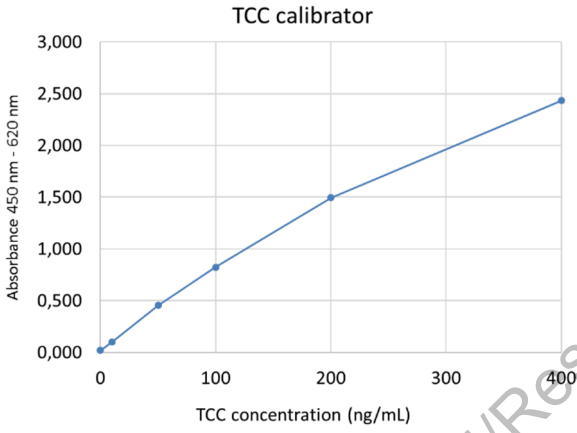

Figure 1.

Example of a calibrator curve

Please Note: The figure above shows an example of a semi-quantitative standard curve and should not be used for actual sample interpretation.

QUALITY CONTROL

The OD for Calibrator 1 shall be <0.15
The OD for Calibrator 6 shall be >1.0
The high and low controls are intended to monitor for substantial reagent failure. If any of the controls are not within their respective range, the test should be considered as invalid and repeated.

LIMITATIONS

This kit is for research use only and is not intended for diagnostic use.
This kit has been used to test EDTA plasma. Other matrices have not been tested.

EXPECTED RESULTS

EDTA plasma from 144 blood donors were tested and the normal 95% reference range was calculated (Table 1). It is recommended that each laboratory establish a reference range with samples commonly used since results may vary between different sample panels.

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Table 1. Plasma from 144 blood donors were tested and the normal 95% reference range was calculated

Reference limit	Concentration	90% CI
Lower limit 2.5%	3 ng / mL	0.0 to 5.6
Upper limit 97.5%	15 ng / mL	12.8 to 17.8

Note: Compensating for the 1/10 sample dilution the values correspond to neat sample titers of 30 ng/mL respectively and 150 ng/mL.

PRECISION

Between-run precision was determined by analyzing 10 EDTA plasma samples across the measuring range in 8 replicates at 4 occasions. One of the test occasions was used for the calculations of within-run precision (Table 2).

Table 2. Between-run and within-run precision. Note: results below are mean concentrations at 1/100 dilution according to assay procedure.

	Within run[1]		Between run[2]	
Sample	**Mean conc. (ng/mL)**	**CV (%)**	**Mean conc. (ng/mL)**	**CV (%)**
1	10	9	10	8
2	11	3	11	5
3	17	3	17	4
4	38	5	37	4
5	55	5	53	3
6	64	4	63	2
7	83	4	80	4
8	118	5	115	5
9	168	5	162	4
10	281	6	268	6

[1]n=8 replicas [2]n=4 runs

Batch-to-batch precision was determined by analyzing 10 EDTA plasma samples across the measuring range in three different kit batches (Table 3).

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Table 3. Batch to batch precision. Note: results below are mean concentrations at 1/100 dilution according to assay procedure.

Sample	Batch 1 (ng/mL)	Batch 2 (ng/mL)	Batch 3 (ng/mL)	Mean (ng/mL)	SD	CV (%)
1	10	9	10	10	0.47	5
2	15	14	15	15	0.68	5
3	35	35	38	36	1.91	5
4	53	51	52	52	1.13	2
5	65	63	69	66	3.12	5
6	90	73	75	79	8.89	11
7	110	108	118	112	5.20	5
8	152	144	167	154	11.2	7
9	261	231	288	260	28.8	11
10	252	223	248	241	15.7	7

LINEARITY/RECOVERY

A dilution series was prepared for three EDTA plasma samples. A 1/10 dilution has been used in all reported results in the IFU but dilutions between 1/10 and 1/40 will yield accurate TCC concentrations (Table 4). Note that using a different sample dilution than 1/10 may shift the reference range. It is recommended that each laboratory establish a reference range with the dilution of choice.

Table 4. Linearity and dilution recovery.

Sample	Dilution factor	Mean measured concentration	Expected concentration	Recovery
1	10	328	328	100%
	15	207	219	95%
	20	145	164	88%
	25	132	131	101%
	30	108	109	99%
	40	76	82	93%
2	10	298	298	100%
	15	180	199	90%
	20	138	149	93%
	25	136	119	114%
	30	105	99	106%
	40	88	76	116%
3	10	273	273	100%
	15	181	182	99%
	20	151	137	110%
	25	119	109	109%
	30	97	91	107%
	40	83	68	122%

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LIMIT OF DETECTION

Limit of Detection (LOD) for the Complement TCC ELISA RUO has been estimated to 3ng/mL, determined by dilution of TCC containing samples until signal to noise (diluent) was between 2 and 3.

INTERFERING SUBSTANCES

The substances in Table 5 were tested in the Complement TCC RUO assay and not found to interfere.

Table 5 Non-interfering substances

Substance	Concentration
Hemoglobin	484 mg/dL
Bilirubin F (free/unconjugated)	20.8 mg/dL
Bilirubin C (conjugated)	20.0 mg/dL
Chyle (Lipids)	140 FTU (Formazine Turbidity Units)
Reuma factor (RF)	10 mg/mL (ref to total IgG conc.)
HAMA	20 mg/mL
C9	60 mg/L

HOOK EFFECT

No hook effect has been observed in Complement TCC ELISA RUO up to 62000ng/mL. Three normal EDTA plasma samples were spiked with purified TCC to final concentrations of 62000 ng/mL (High 1), 39000 ng/mL (High 2) and 13000ng/mL (High 3). The samples were diluted in 2-step dilution from 1/5 to 1/2560 in diluent.

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TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSES	SOLUTION
Calibrator or control values out of range	Incorrect temperature, timing or pipetting, reagents are not mixed	Check that the time and temperature were correct. Repeat test.
	Cross contamination of controls	Pipette carefully.
	Optical pathway is not clean.	Check for the dirt or air-bubbles in the wells. Wipe plate and reread.
All test results negative	One or more reagents are not added or added in wrong sequence.	Recheck procedure. Check for unused reagents. Repeat test.
	Antigen coated plate is inactive	Check for obvious moisture in unused wells. Wipe plate bottom and reread.
All test results yellow.	Contaminated buffers or reagents.	Check all solutions for turbidity.
	Washing solution is contaminated.	Use clean container. Check the quality of water used for preparation of solution.
	Improper dilution of plasma.	Repeat test.
Poor precision.	Pipette delivery CV >5% or samples not mixed.	Check the calibration of pipette. Use reproducible technique. Avoid air bubbles in pipette tip.
	Plasma or reagents are not mixed sufficiently or not equilibrated to room temperature.	Mix all reagents gently but thoroughly and equilibrate to room temperature.
	Reagent addition is taking too long time, inconsistency in timing intervals.	Develop consistent uniform technique and use multi-tip device or auto-dispenser to decrease time.
	Optical pathway not clean.	Check for air bubbles in the wells. Wipe plate bottom and reread.
	Washing not consistent, trapped bubbles, washing solution left in the wells.	Check that all wells are filled and aspirated uniformly. Dispense liquid above level of reagent in the well. After last wash, empty the wells by tapping the strip on an absorbent tissue.
	The complement system has self-activated in the sample	Draw new sample and keep strict to the timelines and temperatures recommended in specimen collection section

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EXPLANATION OF SYMBOLS

	Batch number.
	Catalogue number.
	Use-by date.
	Temperature limit.
	Biological risk.
	Consult instructions for use.
	Warning.
	Corrosive substance.
 96	Content sufficient for 96 tests.

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Ab	Antibody.
DIL	Diluent.
CONJ	Conjugate
BUF WASH 30X	Wash solution 30x conc.
SUB TMB	Substrate TMB
CONTROL -	Low control
CONTROL +	High control
CAL 1-6	Calibrator
STOP	Stop solution

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