Complement TCC Flexi

A Gyrolab® system application

For research and professional use only. Not for use in diagnostic procedures.

This application note contains a suggested protocol and performance data.
Each individual laboratory must set up their own method and perform relevant validations.

Background

In all cases where determination of complement function is wanted, soluble terminal complement complex (sTCC also known as TCC or sC5b-9) 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 (1).

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) (2), Systemic lupus erythematosus (SLE) (3, 4) and rheumatoid arthritis (RA) (5). The complement system can also be activated by artificial surfaces, for example during hemodialysis or cardiopulmonary bypass, resulting in increased levels of TCC (6, 7). TCC is also well suited for studies of complement activation by biomaterials in medical devices (8).

TCC can like other activation products of complement be measured in assays using neo-epitope specific monoclonal antibodies. Neo-epitopes are hidden in the native complement component and exposed after complement activation (9, 10).

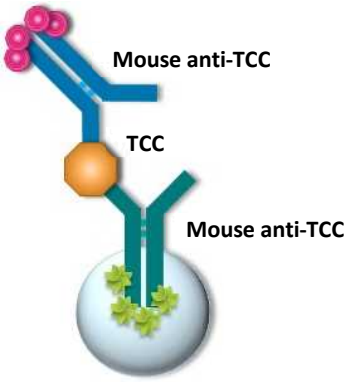
Purpose

The purpose of the Complement TCC assay and the Complement TCC Flexi reagents is to detect TCC in human EDTA plasma. The Complement TCC Flexi application note allows users to independently set up, customize and optimize a Complement TCC Flexi assay based on Svar Life Science's Complement TCC assay on the Gyrolab® automated platforms (Gyros Protein Technologies) or another platform of their choice using Svar Life Science's Flexi products.

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

Assay design

It is recommended to set up Complement TCC Flexi assays as 3-step sandwich assays with the capture and detection antibodies used in their designated functions to ensure optimal performance of the assay. This 3-step sandwich assay design is also used in Svar Life Science's Complement TCC ELISA (COMPL TCC RUO). A recommended Gyrolab® assay protocol based on the Complement TCC ELISA is described below, allowing for automated TCC quantification in EDTA plasma using Gyrolab® platforms.



Materials and methods

The following semi-quantitative Complement TCC Flexi assay was developed on a Gyrolab® xPlore using the Flexi reagents listed in Table 1 combined with additional materials and equipment listed in Table 2. The assay has not been validated on a multi-disc instrument. This protocol may not be directly transferrable to other platforms without further optimization and validation.

Table 1. Flexi reagents required.

Reagent	Volume	Concentration	Product code
Complement TCC Flexi Capture antibody (CAP Ab)	16 µL	1.25 mg/mL	FX1354
Complement TCC Flexi Detection antibody (DET Ab)	16 µL	1.25 mg/mL	FX1355
Complement Flexi Diluent (DIL)	32 mL	-	FX1344
Complement TCC Flexi Calibrator stock (CAL)	70 µL	400 ng/mL	FX1356
Complement TCC Flexi Low control (CONTROL L)	35 µL	CoA	FX1357
Complement TCC Flexi High control (CONTROL H)	35 µL	CoA	FX1358

Store all the Flexi reagents at 2–8°C. Once conjugated, follow the antibody conjugate storage instructions from the supplier of the respective conjugation kits. Reagents of different lots or different conjugation reactions shall not be mixed. Dispose of the reagents as hazardous waste.

Table 2. Materials or equipment required but not provided by Svar Life Science.

Item	Supplier	Product code
Gyrolab® platform (e.g. xPlore)	Gyros Protein Technologies	-
Biotinylation kit	See Gyrolab® User Guide	See Gyrolab® User Guide
Alexa Fluor® 647-labelling kit	See Gyrolab® User Guide	See Gyrolab® User Guide
Bioaffy™ 1000 CD	Gyros Protein Technologies	P0004253
PBS-T	See Gyrolab® User Guide	See Gyrolab® User Guide
Rexxip® F	Gyros Protein Technologies	P0004825
Gyrolab® wash buffer pH 11	Gyros Protein Technologies	P0020096
PCR Plate 96	Gyros Protein Technologies	P0004861
Microplate foil	Gyros Protein Technologies	P0003313
Microplate foil adapter	Gyros Protein Technologies	P0003697
Precision pipettes with disposable tips	-	-

Complement TCC Flexi Gyrolab® protocol

Before starting a Complement TCC Flexi Gyrolab® assay, equilibrate all materials to room temperature and perform the assay at room temperature. The assay protocol is summarized in Table 3.

Table 3. Complement TCC Flexi Gyrolab® protocol summary.

Capture	100 µg/mL biotinylated* capture antibody in PBS-T
Detection	10 µg/mL Alexa Fluor® 647-labeled* detection antibody in Rexxip® F
Analyte	TCC in EDTA plasma
Standard curve	Calibrator stock in diluent: 400, 200, 100, 50, 10, 0 ng/mL
CD-type	Bioaffy™ 1000 CD
Method	1000-3W-006-A
Wash buffer 1	PBS-T
Wash buffer 2	Gyrolab® wash buffer pH 11
PMT-setting	PMT 5%
Standard fit model	5-parameter logistic (5PL) curve fit excluding the blank
Expected dynamic range	10–400 ng/mL
Sample dilution	1:10 (MRD) to 1:80 in diluent.

*Conjugation of antibodies is required before use, see Gyrolab® User Guide.

Antibody conjugation

The anti-TCC capture antibody should be biotinylated according to the Gyrolab® standard protocols (Gyrolab® User Guide) prior to use in Gyrolab® assays. Centrifuge the antibody solution before conjugation to ensure sufficient antibody recovery and mix carefully with a pipette, avoid formation of foam, before aspirating the solution. In the Gyrolab® assay, the conjugated capture antibody is diluted to 100 µg/mL with PBS-T.

The anti-TCC detection antibody should be labeled with Alexa Fluor® 647 according to the Gyrolab® User Guide prior to use in Gyrolab® assays. Centrifuge the antibody solution before conjugation to ensure sufficient antibody recovery and mix carefully with a pipette, avoid formation of foam, before aspirating the solution. In the Gyrolab® assay, the conjugated capture antibody is diluted to 10 µg/mL with Rexxip® F.

Sample preparation

EDTA plasma samples should be equilibrated to room temperature and freshly diluted just before running the assay as the samples may have residual complement activity. Undiluted samples can be kept at 2–8°C for up to 8 h. All samples should be centrifuged prior to dilution (Gyrolab® User Guide). See **Specimen collection** for detailed information on collecting and storing specimen.

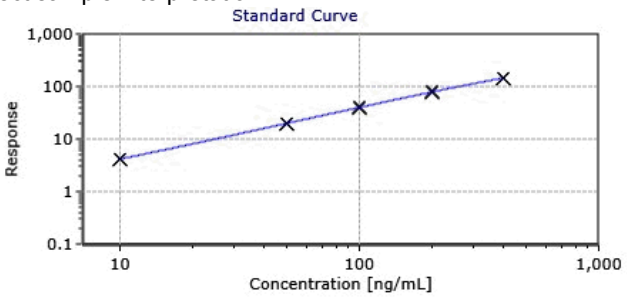
The centrifuged samples should be diluted with the Complement Flexi diluent to a minimum recommended dilution (MRD) of 1:10 up to 1:80. Samples containing high levels of TCC may need to be diluted further. When diluting samples, it is recommended to dilute at least 10 µL of sample (e.g. 10 µL sample + 90 µL diluent for 1:10 dilution). To calculate the concentration of TCC in the plasma sample, the measured analyte concentration should be compensated for the dilution factor, e.g. 10x if using the MRD above. It is recommended to measure all samples, calibrators and controls in duplicates to ensure that results are precise.

Standard curve

Purified human TCC is used as the assay standard. The standard curve should be diluted in a dilution series from the calibrator stock (400 ng/mL) using the Complement Flexi diluent, see recommended standard curve in Table 4. Analyte concentrations are interpolated from the recommended standard curve using the PMT 5% response and a 5-parameter logistic (5PL) curve fit excluding the blank. It is recommended to include the high and low control samples in each assay run to verify the performance of the assay. Using the recommended assay protocol, the controls should measure within the limits given on their respective certificate of analysis.

Table 4. Recommended standard curve preparation. Note: the graph shows an example of a semi-quantitative standard curve and should not be used for actual subject sample interpretation.

Calibrator	Conc. (ng/mL)	Calibrator solution	Diluent
A	400	10 µL	-
B	200	10 µL A	10 µL
C	100	10 µL B	10 µL
D	50	10 µL C	10 µL
E	10	10 µL D	40 µL
F	Blank	-	10 µL



Specimen collection

It is recommended to measure TCC levels in EDTA plasma. Blood samples are to be collected using aseptic venipuncture technique and EDTA plasma is 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.

Matrices other than EDTA plasma have not been tested, and the user should carefully validate the assay's performance in other matrices and pay special attention to matrix effects and false positives derived from potential in vitro complement activation.

Assay performance

All results reported below were generated using the recommended Complement TCC Flexi Gyrolab® assay protocol as described above.

Working range

Table 5. Assay working range. The lowest (LLOQ) and highest (ULOQ) concentrations of the standard curve that gave a Total Error (% absolute bias + % CV) < 30 % in three repeated assessments were assigned as LLOQ and ULOQ.

LOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)
10	10	400

Precision

Between-run precision was determined by analyzing 8 TCC-containing EDTA-plasma samples, some of which were spiked with calibrator material, at 1:5–1:10 dilution across the measuring range in 4 replicates at 3 occasions (Table 6). One of the test occasions was used to calculate within-run precision (Table 6).

Table 6. Within-run and between-run precision. Note: Results below are mean concentrations at the respective dilutions used. [1]n=4 replicates, [2]n=3 runs

Sample	Within-run[1]		Between-run[2]	
	Mean conc. (ng/mL)	CV (%)	Mean conc. (ng/mL)	CV (%)
1	24.0	1.5	24.4	4.4
2	26.7	9.4	29.1	11.7
3	49.6	5.3	48.1	3.9
4	86.6	4.2	83.6	3.0
5	117.5	3.0	119.1	1.9
6	195.8	5.8	206.6	4.8
7	194.5	24.4	226.9	12.4
8	261.8	11.3	267.0	2.1

Linearity

Dilution series were prepared with four TCC-containing EDTA-plasma samples and were used to assess linearity. As demonstrated here, dilutions between 1:10 and 1:80 yield accurate TCC results (Table 7).

Table 7. Linearity and recovery. Four TCC-containing EDTA-plasma samples were diluted so that they were in range of the standard curve and the linearity of measured concentrations was assessed. Theoretical true concentrations were calculated based on the 1:10 dilution. Dilutions outside the reported range may not be linear.

Sample	Dilution factor	Mean measured conc. (ng/mL)	Expected conc. (ng/mL)	Recovery (%)
1	1:10	280.0	280.0	100.0
	1:20	138.6	140.0	99.0
	1:40	77.2	70.0	110.3
	1:80	42.1	35.0	120.3
2	1:10	275.5	275.5	100.0
	1:20	151.8	137.8	110.2
	1:40	86.9	68.9	126.1
	1:80	45.7	34.4	132.8
3	1:10	280.8	280.8	100.0
	1:20	149.1	140.4	106.2
	1:40	81.5	70.2	116.1
	1:80	42.5	35.1	121.1
4	1:10	349.6	349.6	100.0
	1:20	178.9	174.8	102.3
	1:40	91.4	87.4	104.6
	1:80	53.6	43.7	122.7

Note: The use of different dilution factors may be required depending on assay setup, sample type as well as sample analyte concentration. Samples measuring above the ULOQ should be diluted further. Plasma samples without complement activation may be below the LOD at MRD.

Recommendations

It is recommended that users determine the concentrations of antibodies after conjugation and titrate their signal against the standard curve and controls to ensure that the assay is functional.

Usage on other platforms or with altered protocols should be carefully validated by the user.

If customizing this assay, it is important to screen matrices and assess backgrounds, in particular for disease-specific matrices. Assay parameters should be validated in-house. Data given in this document should only be considered as a guidance.

References

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