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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