Automated Assessment of the Complement System

Haemostasis Research Unit

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Conflict of interests disclosure

Research Support/P.I.	**UCL has received unrestricted research grants from Sysmex**
Employee	**Hiroshi Kurono is an employee of Sysmex**

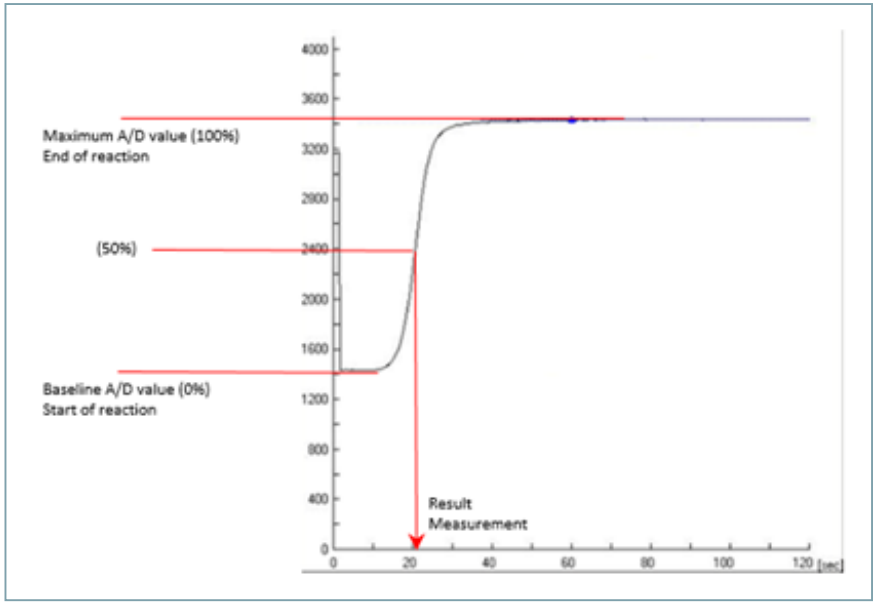
Background

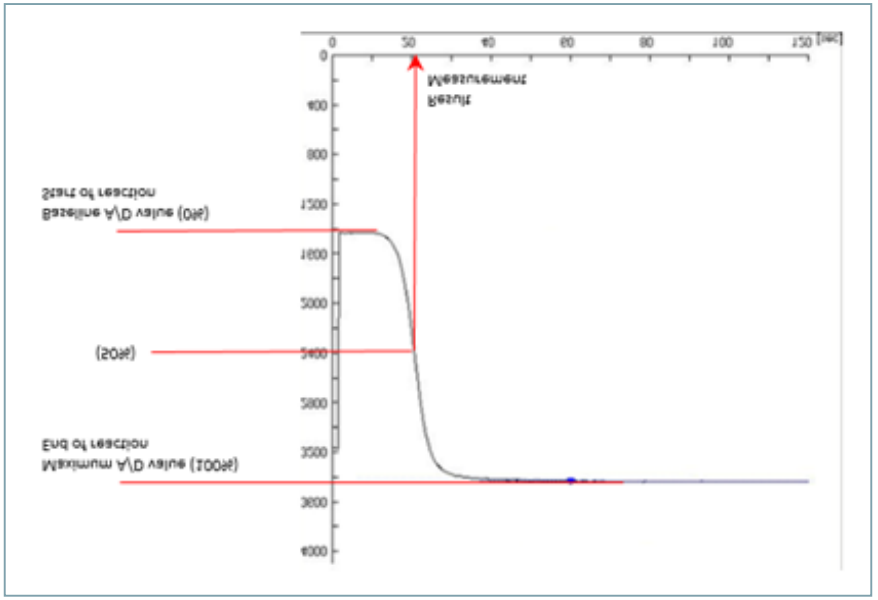
- Abnormalities of complement function plays an important role in a wide range of diseases including: rheumatoid arthritis, Crohn's disease, ischaemia reperfusion injury, sepsis, myasthenia gravis, PNH, age related macular degeneration, thrombotic microangiopathies, SLE and the antiphospholipid syndrome.
- Eculizumab (Soliris) is FDA/NICE-approved for the treatment of aHUS and PNH. Cost: approximately $500,000 (€430,000) per patient per year.
- A standard dosing regime is used and complement activity is not monitored.
- A variety of pharmaceutical agents that inhibit complement are in development.

Complement inhibitors in development

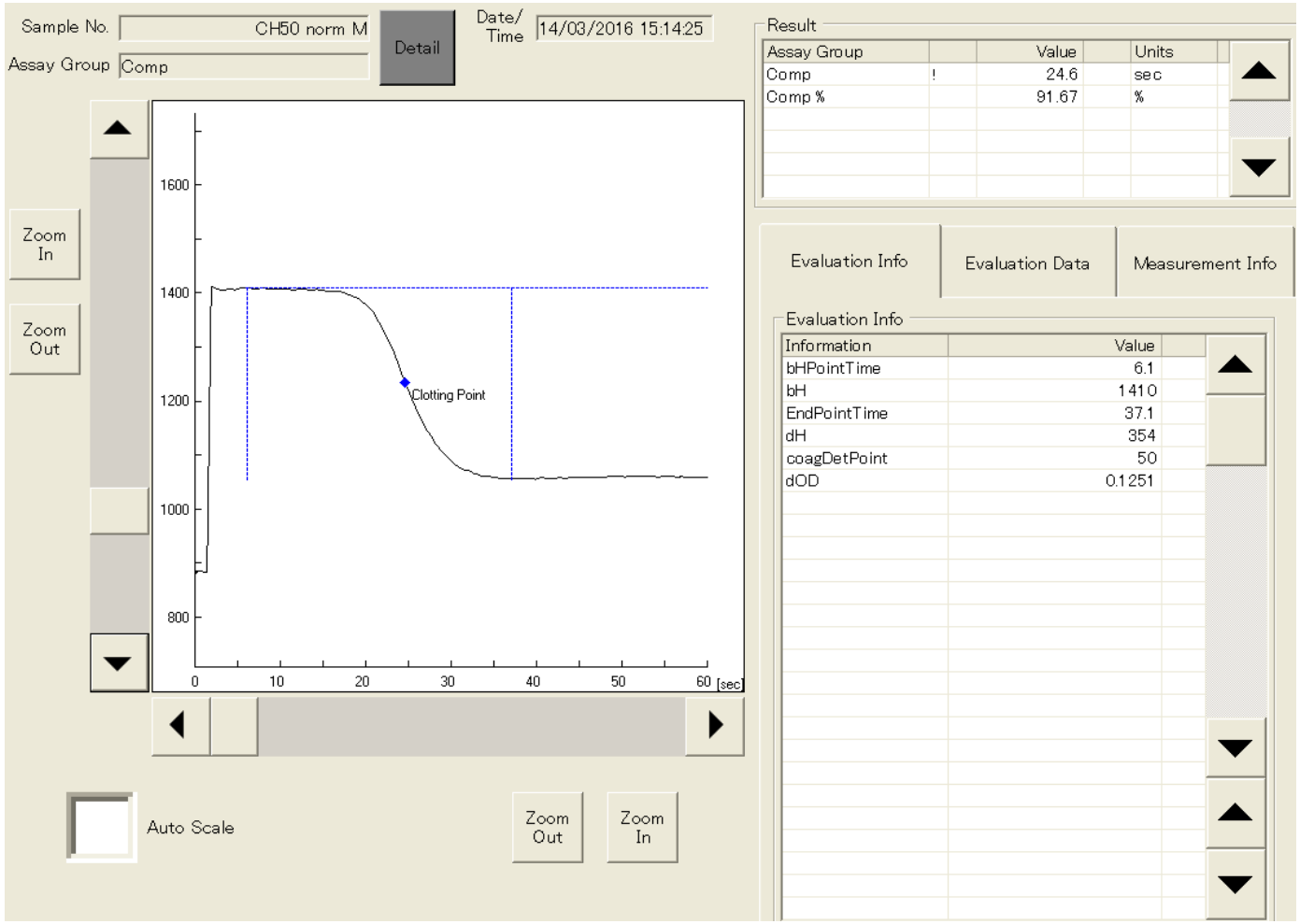
Company	Place	Product	Target	Molecule	Route	Indication	Phase
Amyndas	USA	Compstatin Mini FH	C3 FH	Peptide Peptide	Intraocular Intraocular	AMD AMD	Preclin Preclin
Alnylam	USA	ALN225	C5	RNAi peptide	s.c.	PNH	Preclin
Omeros	USA	OMS721	MASP2	Mini antibody	s.c.	aHUS, TTP	PII
Akari Therapeutics	UK	Coversin	C5	Protein	s.c.	aHUS, PNH	PI
Adienne	Italy	Mubodina Ergidina	C5 C5	Mini antibody Mini antibody	s.c. s.c.	aHUS, DDD Transplant	Preclin Preclin
Alexion	USA	ALX1103	C3	Protein	s.c.	PNH	PI
Apellis	USA	APL-1	C3	Peptide	Inhaled	Asthma/COPD	PI/II
InflaRx	Ger.	IFX-1 IFX-2	C5a	Antibody Antibody	i.v.	Sepsis	PI Preclin
Alligator	Swe.	ADC1104	C5aR	Antibody	i.v.	IRI	Preclin
NovoNordisk	Den.	NN8209	C5aR	Antibody	i.v.	Rh arthritis	PI
Noxxon	Ger.	NOX-D20	C5a	Spiegelmer	? i.v. or s.c.	Sepsis	Preclin
Ophthotech	USA	Zimura	C5	Antibody	Intraocular	Wet AMD	PII

CS-2100i method development





CS-2100i Method (research software)



Siemens Complement Reagents

Erythrocyte Reagent

- Sheep erythrocytes in stabiliser solution (NaCl, HEPES, Methylisothiazolinone)

Amboreceptor Reagent

- Rabbit antibodies to sheep erythrocytes, NaCl, $CaCl_2$, $MgCl_2$, HEPES, NaN_3

Reagent Preparation

- Add 5 mL amboreceptor reagent to 1 vial of erythrocyte reagent and incubate at 37°C for 45 minutes

Analysis protocol

- The analyser mixed 20 μL serum with 200 μL combined reagent (sensitized cells)
- The time taken to achieve 50% red cell lysis was measured, using absorbance at 660nm
- Assay standardised with pooled normal serum

Study design

A commercial CH50 ELISA (Quidel Corp) and an in-house lytic CH50 assay (using sheep erythrocytes (TCS Biosciences) and hemolysin (Sigma) were used as reference methods.

Although citrate plasma can be used in the automated assay, serum was preferred to avoid any irreversible effects of chelation on complement activity.

Calibration curve stability was measured over 5 days

The detection limit was determined by testing serial dilutions of normal serum.

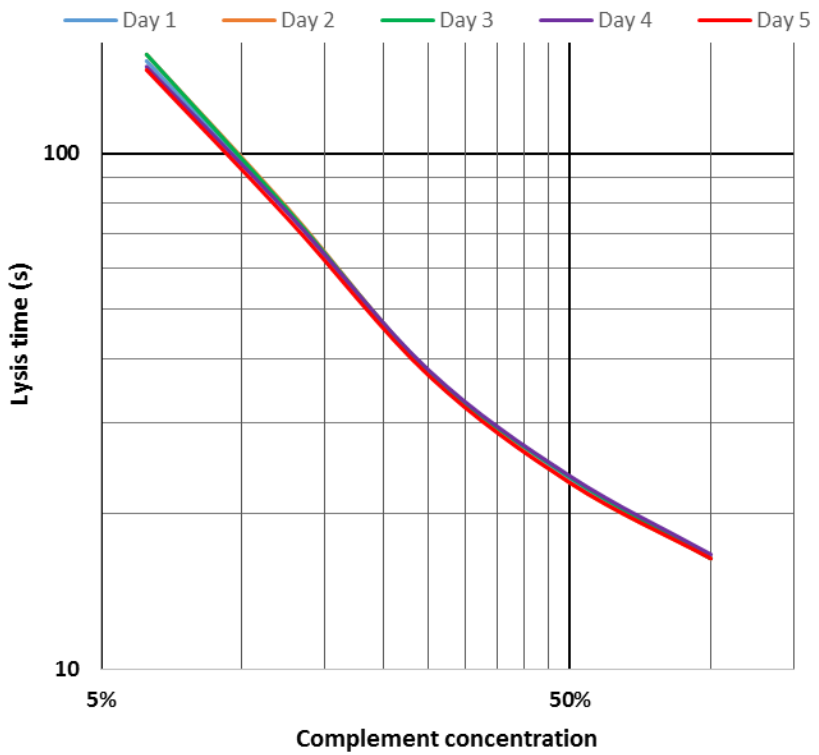
Within day and between day imprecision was assessed by testing freshly thawed aliquots of serum

Comparability was assessed in 75 sera from normal controls, patients with liver disease, thrombotic APS and non-APS thrombosis controls.

Results

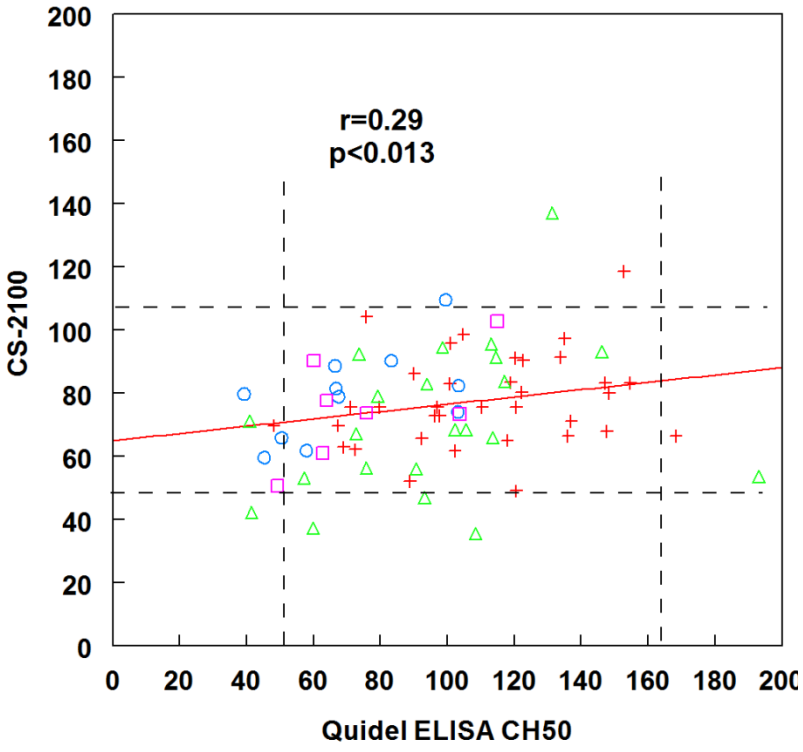
- Detection limit 1.6%
- Intra- & Inter-assay precision 2.5 & 7.0% (normal serum)
- Reagents stable for >24hr on analyser (but need manual mixing prior to each run)
- Serum prepared within 1 hr stable for 4hr at ambient temperature.
- Serum prepared after 48 hours suffered a 10% loss of activity

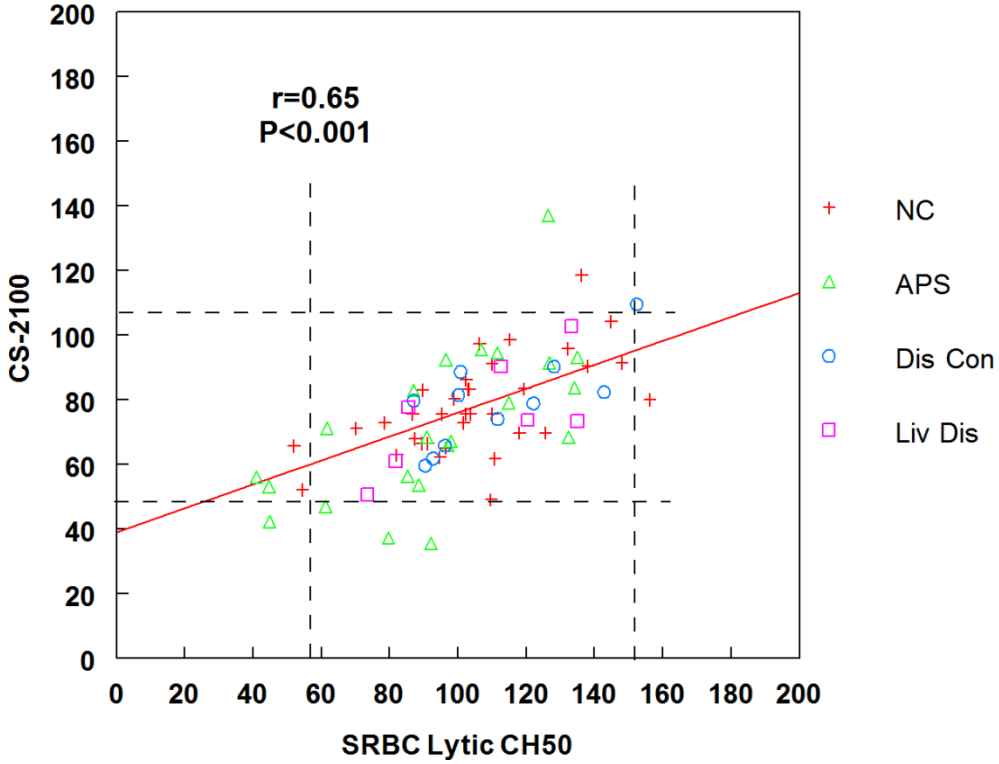
Calibration curve stability



	Lysis time (s)					
Concentration	Day 1	Day 2	Day 3	Day 4	Day 5	%CV
6.25%	151.2	155.6	155.6	147.4	145.2	3.12
12.50%	77.1	78.4	77.8	76.8	74.8	1.78
25%	37.3	37.6	37.8	38	37.1	0.97
50%	23.4	23	23.6	23.7	23	1.41
100%	16.4	16.4	16.4	16.7	16.4	0.82

Comparability (75 Samples)





35 Normal subjects, 22 APS, 11 Thrombosis Controls, 7 Liver Disease

Conclusions

- The automated method showed good linearity, precision and comparability.
- It allows the routine assessment of complement activity in a highly standardized manner. This may be particularly useful for monitoring the efficacy of new complement inhibitory drugs.