Angioedema diagnostics

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November 16th 2017

Disclosures

(potential) conflicts of interest	
Relation	Company
• Appointed	Sanquin

Sanquin Diagnostiek

Non-for-profit organisatie

- bloodbank
- plasmaproducts*
- tissues and cells
- reagents*
- research
- diagnostics*

- Bulk diagnostic tests are mostly performed in hospitals
- Low volume specialties are not profitable: Sanquin Diagnostics performs those tests for hospitals throughout the Netherlands
- Sanquin courier collects samples in all hospitals
- SERVICE

Sanquin Plasma products

Sanquin diagnostics: follow-up of production process and end product

Cinryze
C1-inhibitor from different plasma
Nationalities for their own market

Angioedema diagnostics

- Clinical observation / family history / therapeutic response
- Laboratory testing

Types of angioedema

Acquired

IH-AAE
InH-AAE
ACE1-AAE
C1-INH-AAE type I
C1-INH-AAE type II

Hereditary

C1-INH-HAE Type I
C1-INH-HAE Type II
FXII-HAE
U-HAE

Farkas et al, Clin Rev Allerg Immunol 2016

Types of acquired angioedema

IH-AAE	idiopathic histaminergic acquired AE (response to standard allergy therapy, no identifyible allergen)
InH-AAE	Idiopathic non-histaminergic AAE, unknown (FXII mutation excluded)
ACEI-AAE	acquired AE related to angiotensin-converting enzyme inhibitors
C1-INH-AAE Type I	acquired C1-INH deficiency, secondary to lymphoproliferative disease, no autoantibodies
C1-INH-AAE Type II	acquired C1-INH deficiency with autoantibodies against C1-INH

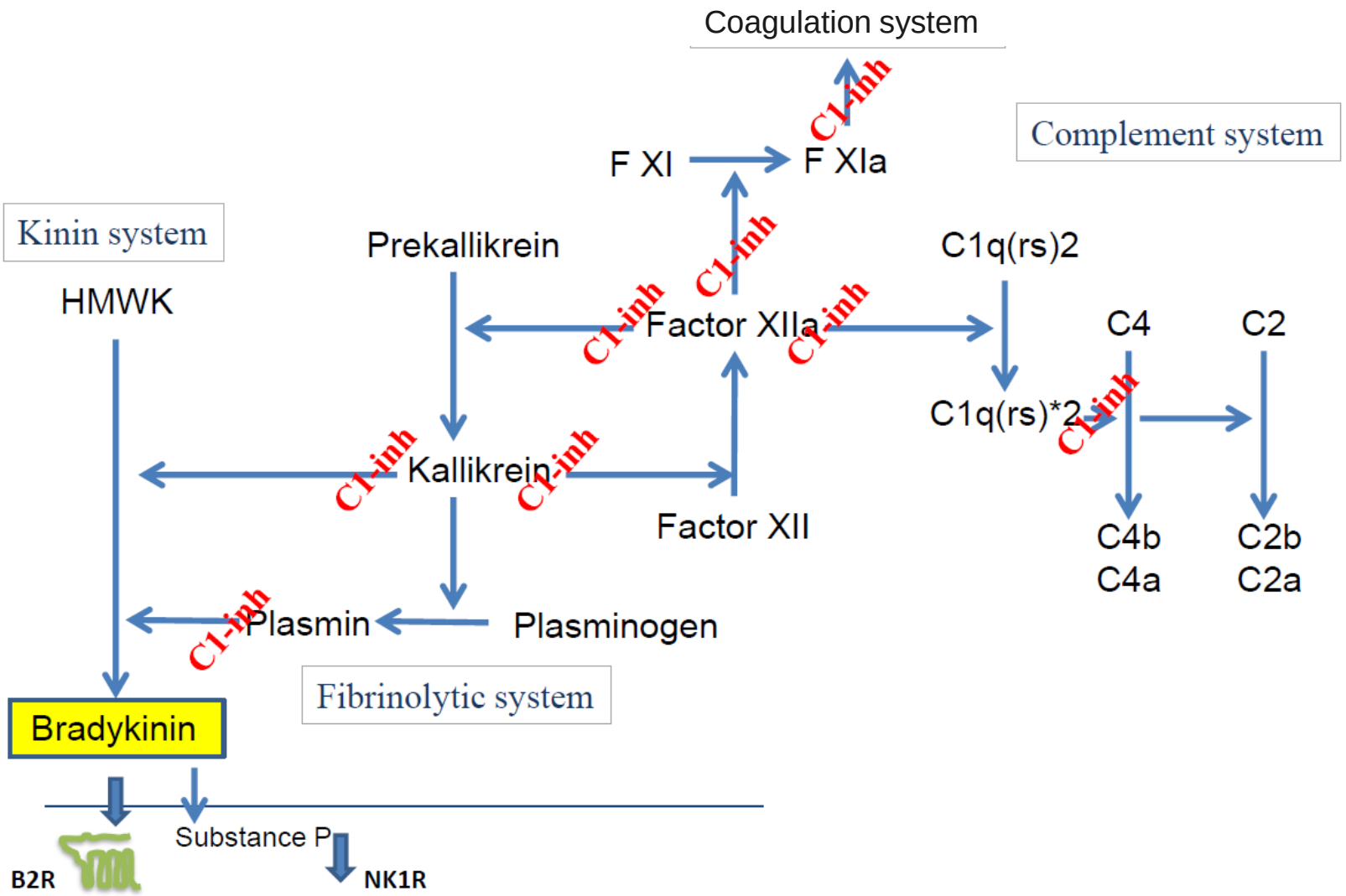
Types of hereditary angioedema

C1-INH-HAE Type I	C1-INH deficiency (~85%), mutation in *SERPING1*
C1-INH-HAE Type II	C1-INH functional deficiency, mutation in *SERPING1*
FXII-HAE	mutation in *F12* (mostly females)
U-HAE	unknown origin (type III)

Available lab tests

- C1-inhibitor function
- C1-inhibitor antigen concentration
- C4 concentration
- C1q concentration
- C1 inhibitor autoantibody (levels, function)
- *F12* mutation analysis
- *SERPING1* mutation analysis

C4 / C1q are used as readout



C1-inhibitor and C1

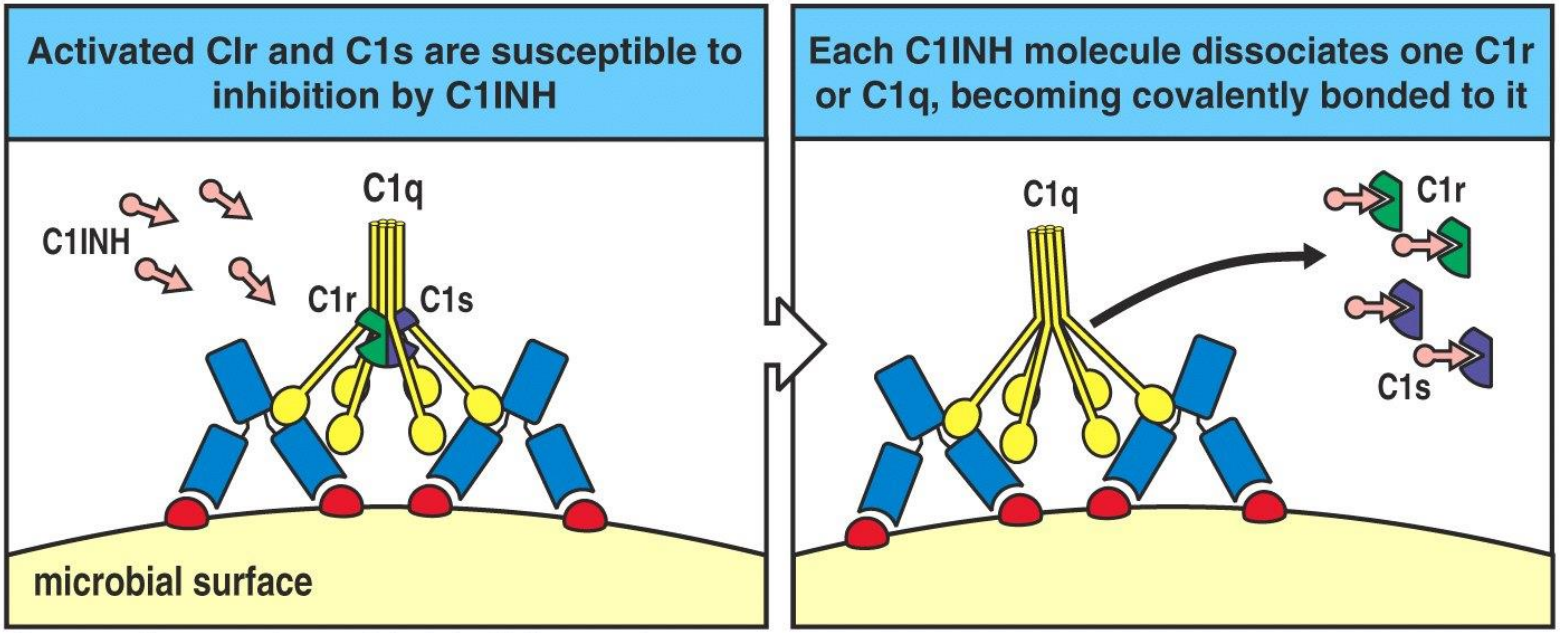


Figure 7-47 The Immune System, 2/e (© Garland Science 2005)

The complement classical pathway

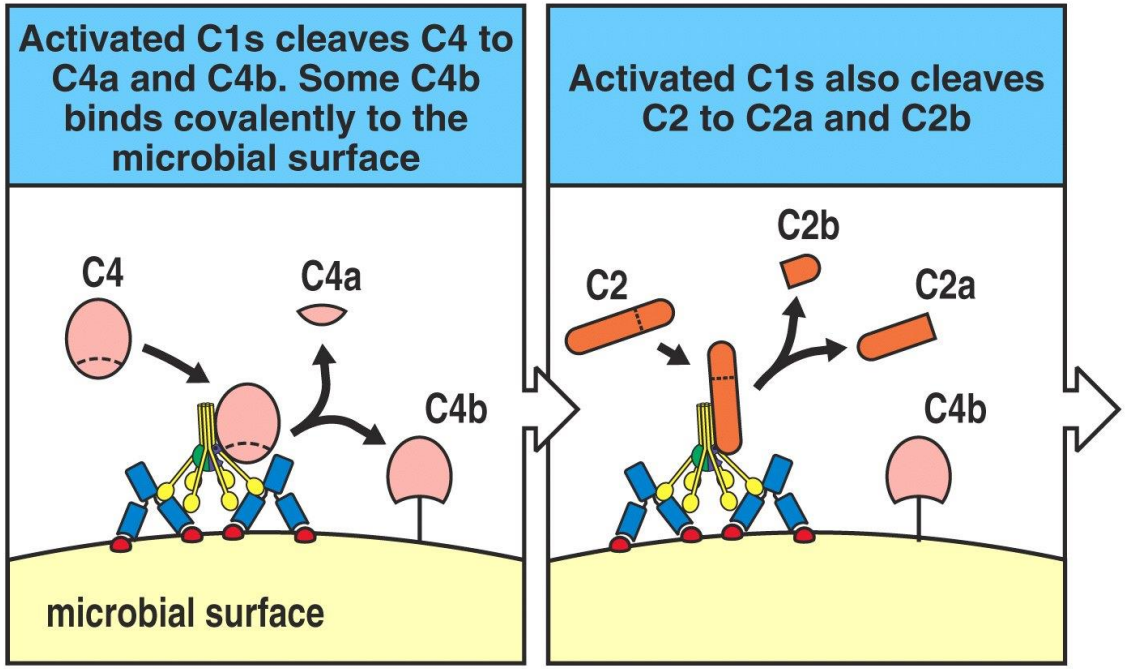
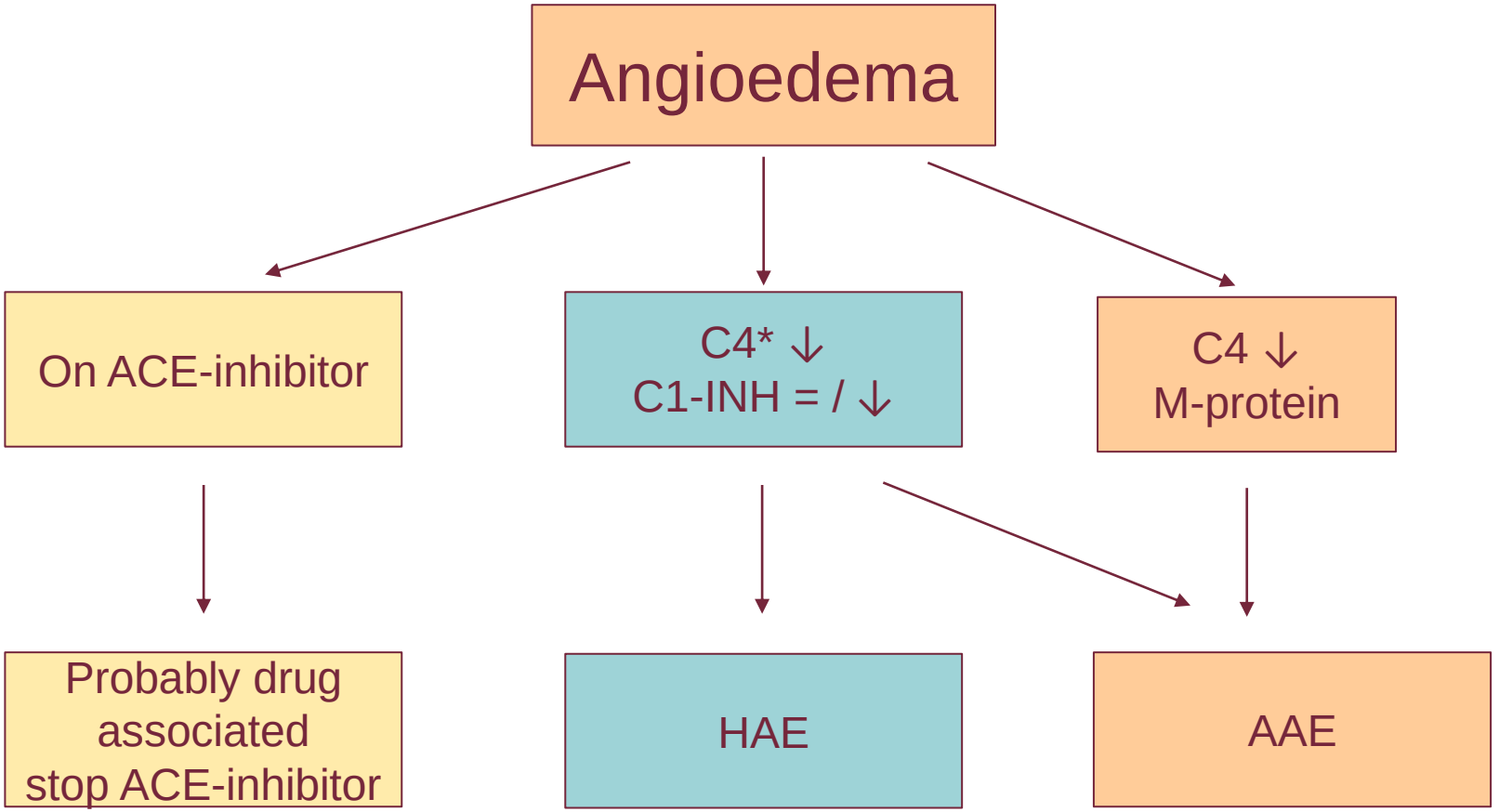


Figure 7-34 part 1 of 2 The Immune System, 2/e (© Garland Science 2005)

Simple diagnostic algorithm



* C4 is not very specific or sensitive, but OK for screening

Sanquin Blood Supply

Risk of screening with C4

Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2007.03438.x

An evaluation of tests used for the diagnosis and monitoring of C1 inhibitor deficiency: normal serum C4 does not exclude hereditary angio-oedema

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**Department of Immunopathology, St Barts and the London NHS Trust, London, UK, and †Institute for Human Genetics, University of Würzburg, Germany*

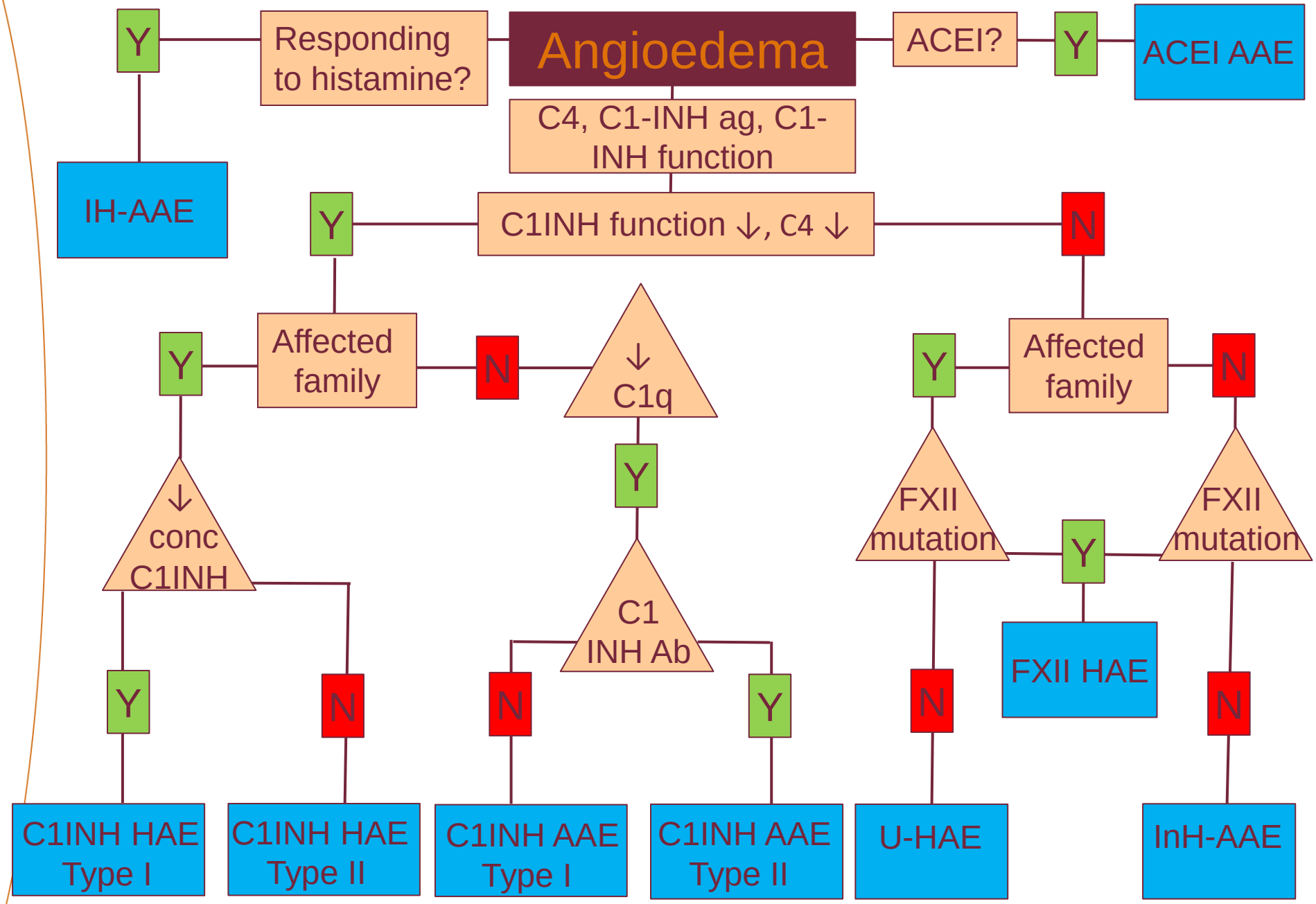
Summary

Reduced levels of serum C4 have been considered a ubiquitous finding in hereditary angio-oedema (HAE), and consequently low C4 is often used to 'request manage' access to C1 inhibitor assays in the United Kingdom. However, in our experience normal C4 may occasionally be compatible with HAE. We audited the results of serum C4, C1 inhibitor antigen (C1inhA) and C1 inhibitor function (C1inhF) in 49 HAE patients, compared to a control

Table 3. Diagnostic performance of low C4, low C1inhA/C1inhF for hereditary angio-oedema (HAE) in untreated patients.

	Low C4 (< 0·14 g/l)	C1inhA (< 150 mg/l)	C1inhF (m = manufacturers' range, < 68%)	C1inhF (optimized range, < 84%)	Low C4 and low C1inhF (optimized range)
Sensitivity	81%	97%*	57%	78%	78%*
Specificity	85%	100%*	100%	100%	100%*

*Refers to HAE type I.



Farkas et al, Clin Rev Allerg Immunol 2016

Diagnostic outcomes

Type	Disorder	C1-INH		C4	C1q	Ab	Mutation	
		function	antigen		(low in 70%)		*SERPING1*	*F12*
acquired	IH	=	=	=	=	No	No	No
(AAE)	InH	=	=	=	=	No	No	No
	ACEI	=	=	=	=	No	No	No
	Type I	↓	↓	↓	↓	No	No	No
	Type II	↓	↓/=	↓	↓	Yes	No	No
hereditary	Type I	↓	↓	↓	=	No	Yes	No
(HAE)	Type II	↓	N/↑	↓	=	No	Yes	No
	FXII	=	=	=	=	No	No	Yes
	U (type III)	=	=	=	=	No	No	No

Farkas et al, Clin Rev Allerg Immunol 2016

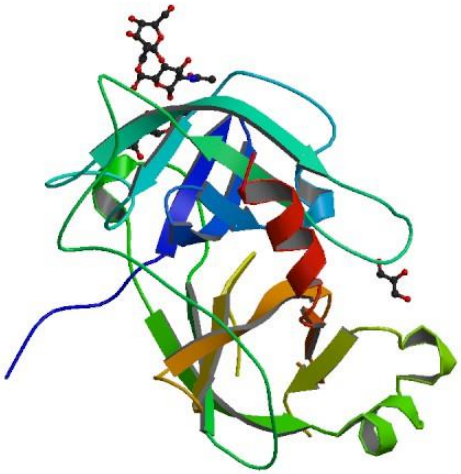
FXII HAE

- Deletion of exon 9
- c.983 C>A (p.Thr328Lys)
- c.983 C>G (p.Thr328Arg)

Overactive protein

Oestrogen ↑ FXII

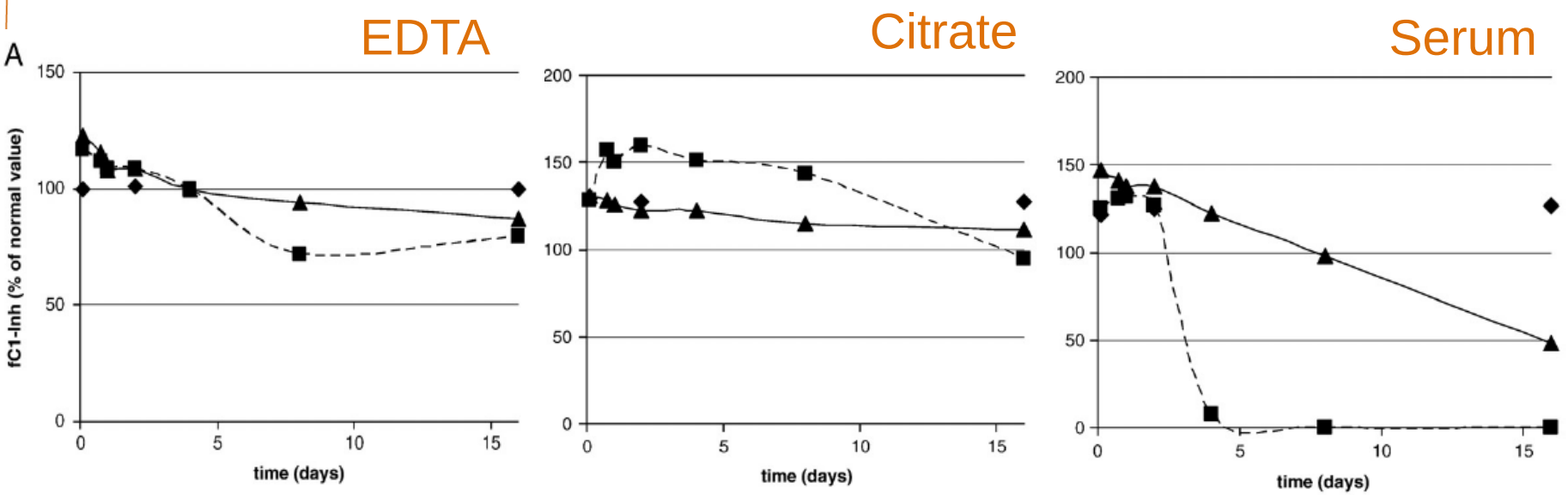
>> bradykinin



www.rcsb.com

Cold inactivation

- Plasma for C1 inhibitor activity should be stored at -20ºC.
- Storage at 4ºC leads to cold inactivation (lower functional levels)
- This is coincided by normal C4 levels



Wagenaar et al 2008

Plasma vs serum

- Complement activation is Ca^{2+} and Mg^{2+} dependent
- Complement activation is inefficient in EDTA-plasma
- Complement activation is less efficient in citrate plasma than in serum
- Complement activation continues at RT/37^{o}C, especially in absence of C1-INH

→ C1-INH function and C4 and C1q levels can best be measured in plasma that has been stored at -20^{o}C. Storage at RT or serum may results in activation and falsely lower results

C1-INH function: assay types

- Chromogenic assay
- Complex formation ELISA

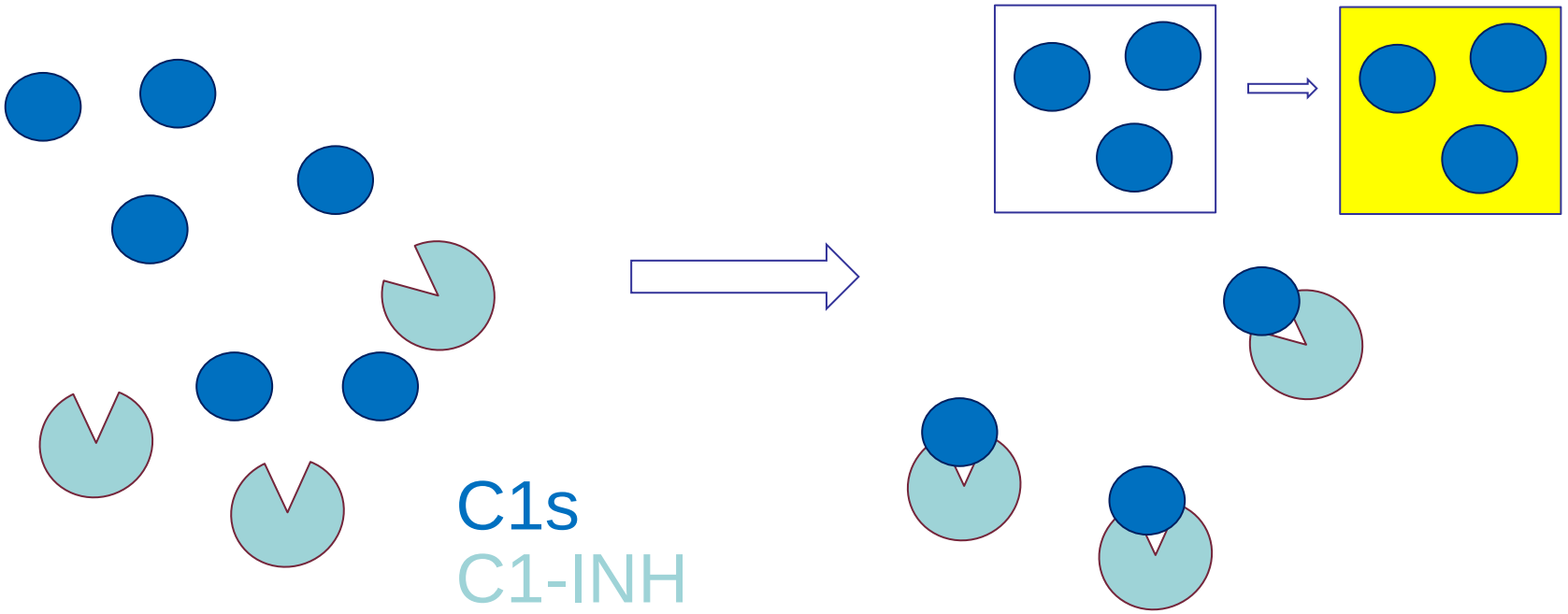
www.wikipedia.org

Chromogenic assay

$C1\text{-}INH_{sample} + C1s_{excess} \rightarrow C1INH/C1s\ complex + C1s_{remainder}$

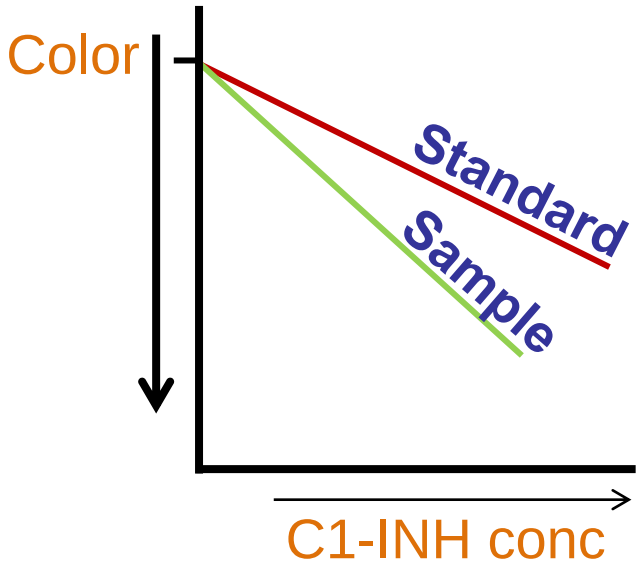
$C1s_{remainder}$

Colorless substrate → Colored substrate



C1-INH activity at Sanquin

Ratio between slopes is a measure for activity compared to standard



Tecan Freedom Evo (CV% < 8%)

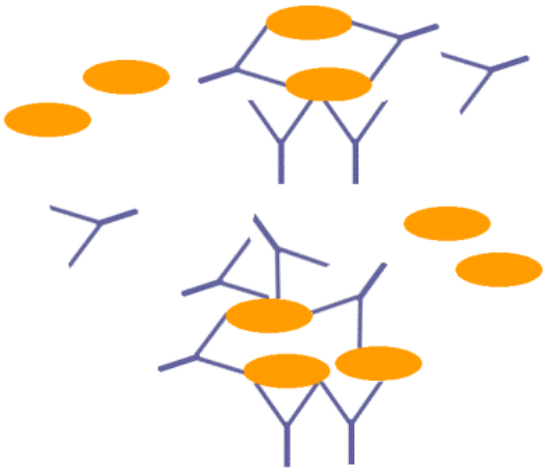
Alternative C1-INH function tests

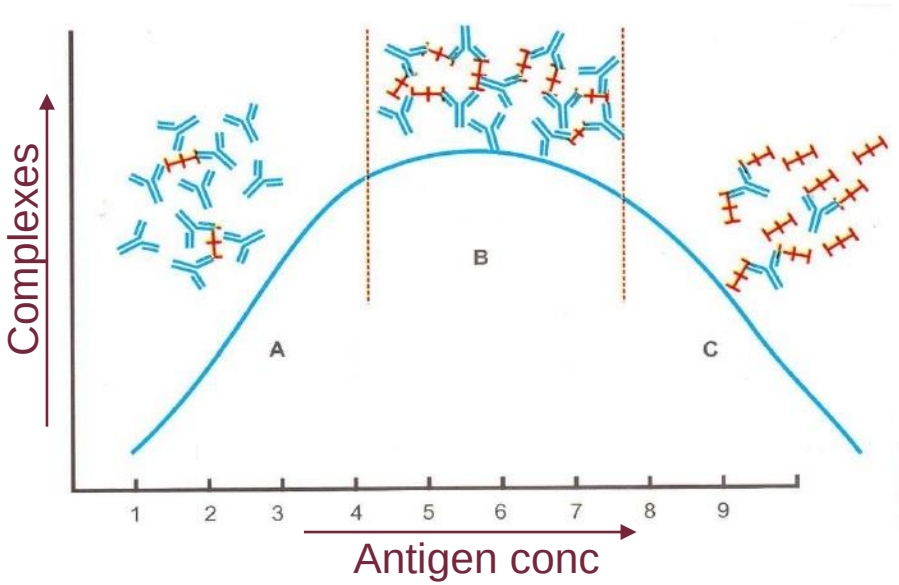
Tests focussing on the contact/kinin system

New ELISA: detection of FXIIa-C1INH or kallikrein-C1INH complexes but other inhibitors exist that may interfere (Joseph et al 2015)

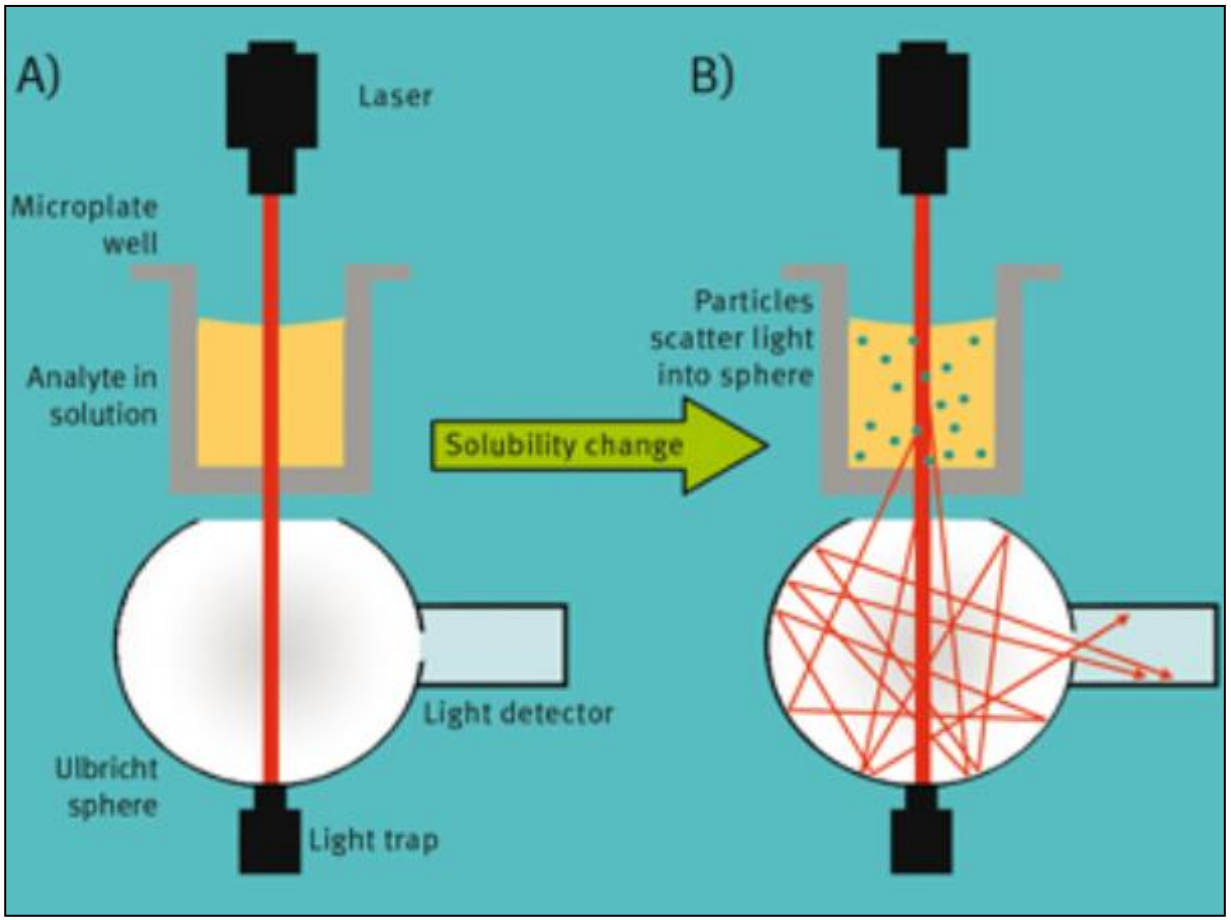
BK assays: poor reproducibility (Hofman et al 2016)

Nephelometric tests
(C4, C1-INH, C1q)

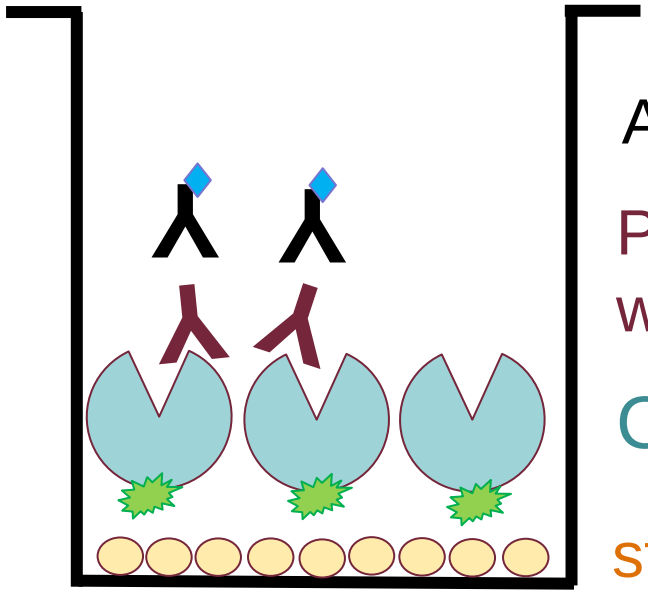




Nephelometry principle



Regular C1-INH antibody ELISA



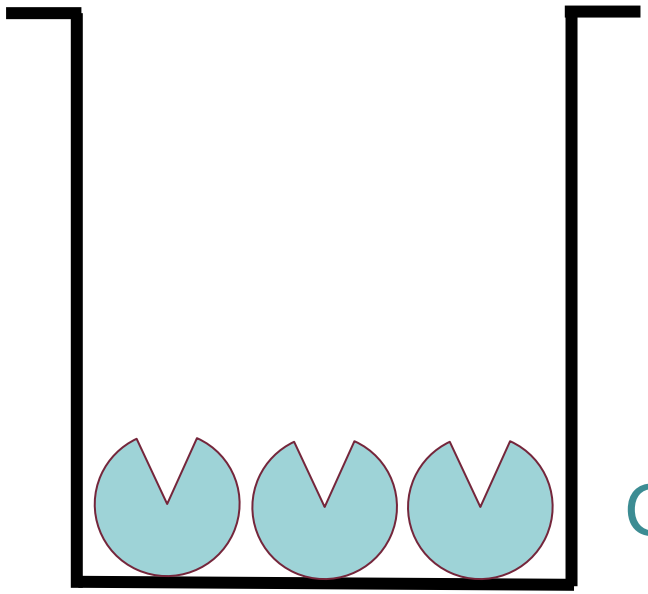
Anti-human IgG/A/M-HRP

Pt serum/plasma
with/without antibodies

C1-INH-biotin

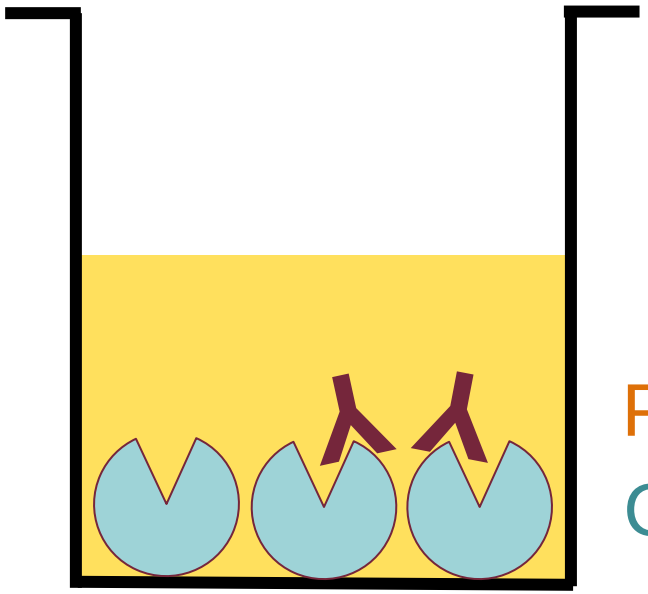
streptavidin

Function neutralising antibodies



C1-INH

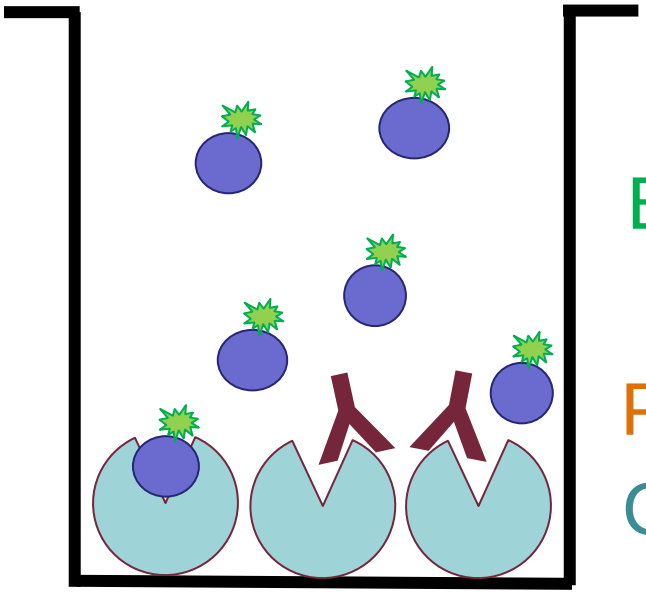
Functional neutralising antibodies



Pt serum with/without antibodies

C1-INH

Functional neutralising antibodies

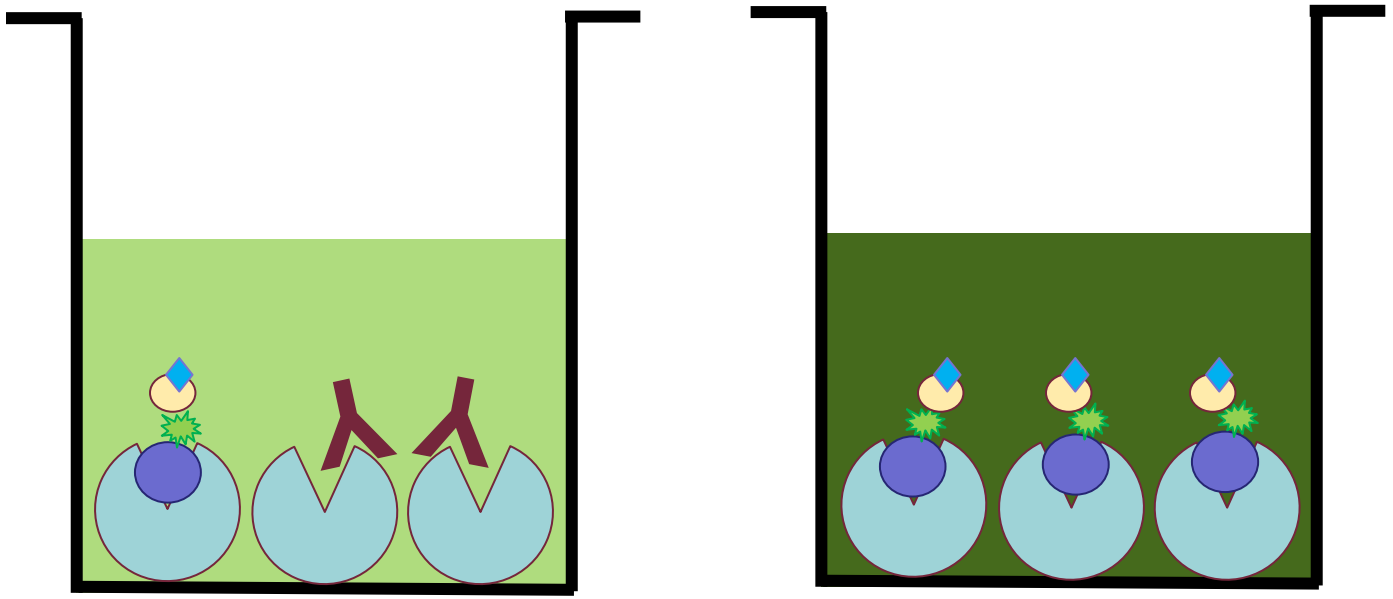


Biotinylated C1s

Pt serum with/without antibodies

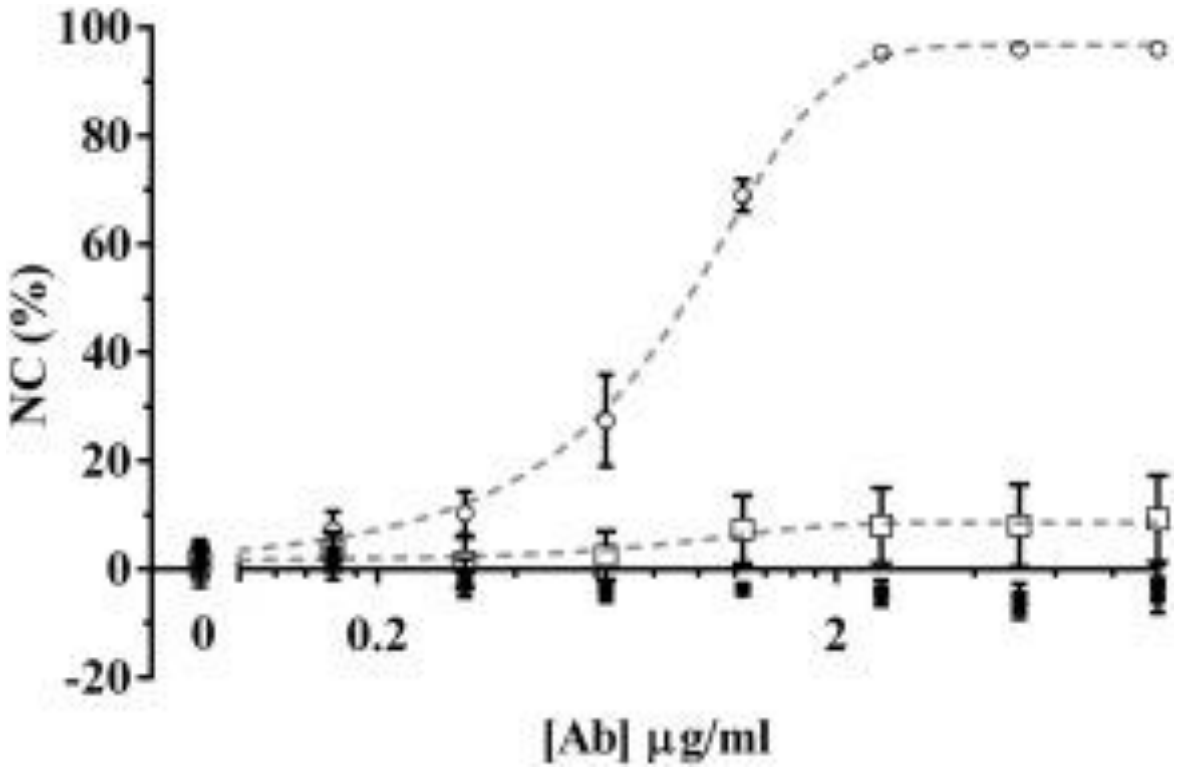
C1-INH

Functional neutralising antibodies



Development of ELISA

Specificity of neutralising Ab ELISA



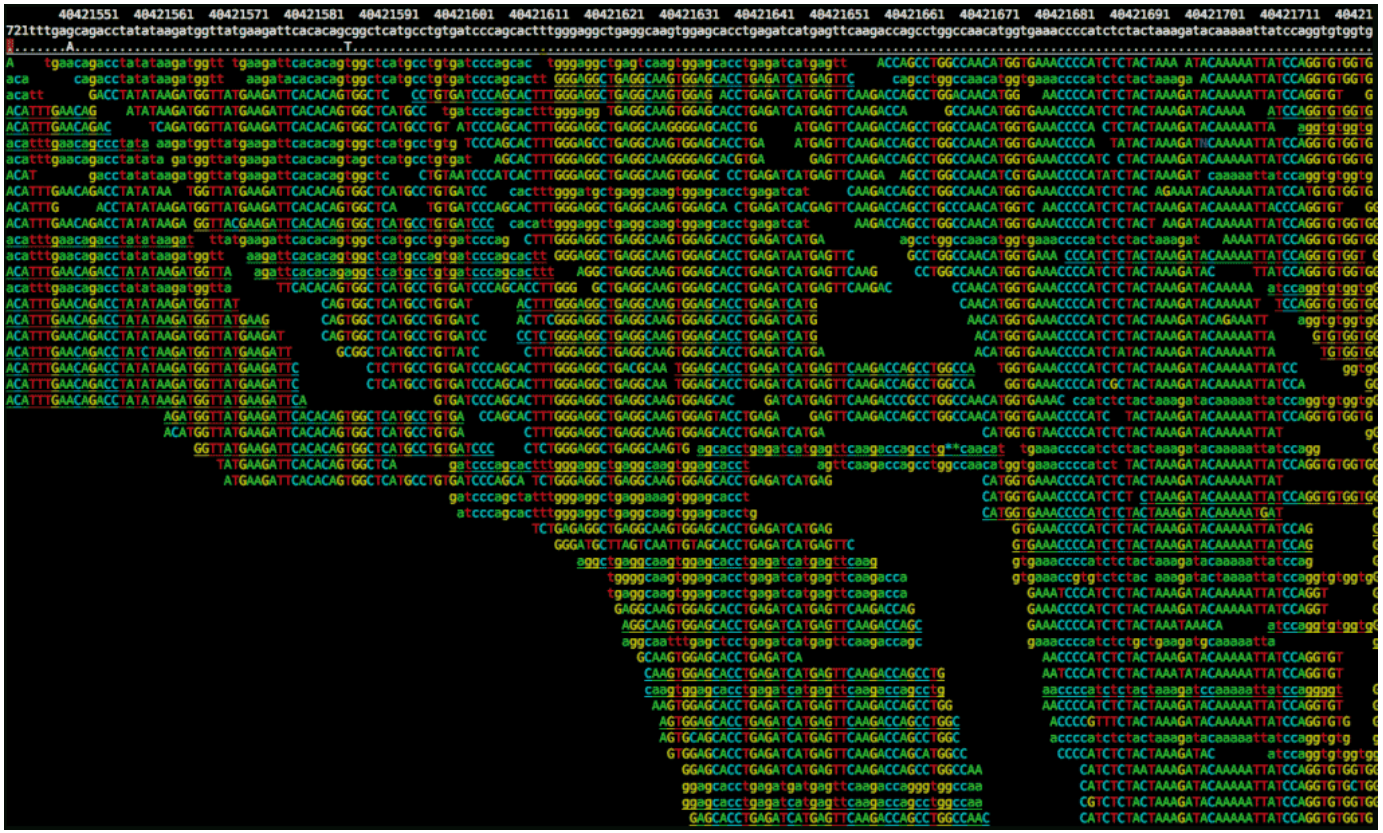
○ Neutralising Ab

□ Non-neutralising Ab

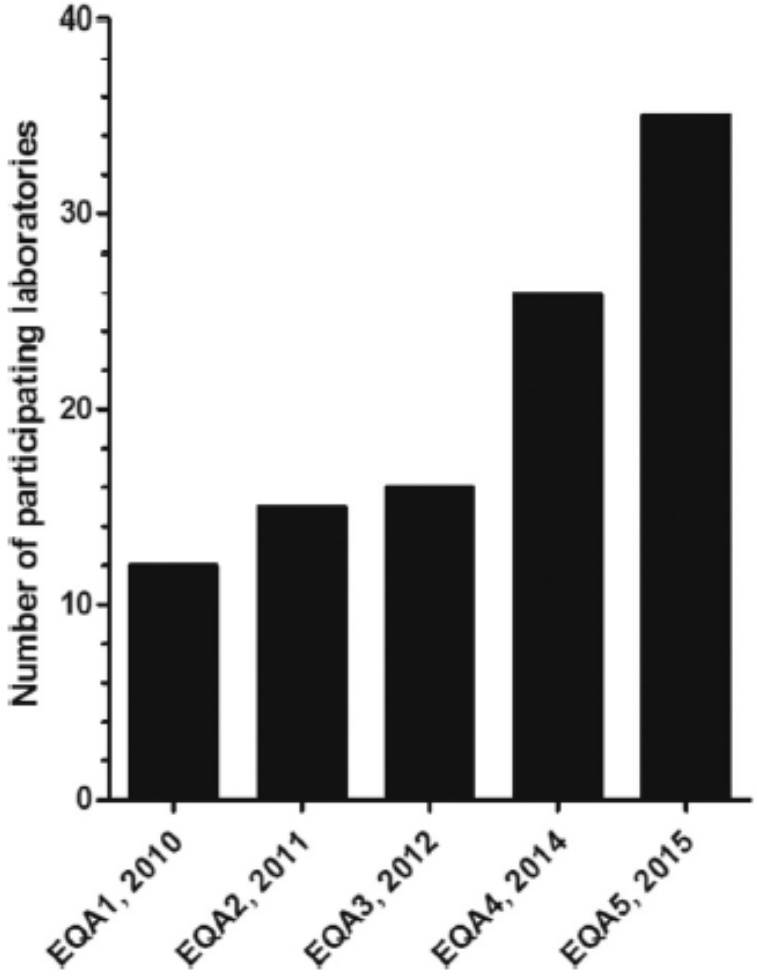
Engel at al Journal of Immunological Methods 426 (2015) 114–119

SERPING1 and *F12* mutation analysis

- Searching for known mutations by PCR / Sanger sequencing
- Next generation sequencing (targeted, exome, whole genome)



Standardisation of complement diagnostics (EQA)



- To improve the quality of complement testing
- To formulate recommen-dations/guidelines on the best tests to use

Standardisation of complement diagnostics (EQA)

Results of the 5th External Quality Assessment quantitative results.

Assay	No. of responses	Median of normalized results	25th Percentile of normalized results	25th Percentile of normalized results	Between-laboratory coefficient of variation***
C4	26	1.25	1.17	1.32	0.10
		0.98	0.92	1.02	0.10
C1-inhibitor antigen	20	0.78	0.73	0.89	0.19
		0.61	0.59	0.65	0.16
C1-inhibitor function	18	0.64	0.45	0.82	0.43
		0.62	0.49	0.80	0.28
C1q	18	0.60	0.52	0.73	0.40
		0.66	0.58	0.71	0.16

Prohaszka et al 2016

International standards

ERM-DA470k: C4
NIBSC 08/262: C1INH potency

EQA future initiatives

Set up recommendations on:

- Assay preference
- Assay calibration
- Assay presentation
- Result interpretation

Small working groups:

No 2: C1 inhibitor function and autoantibodies
Facilitators: Marco Cicardi, Lilian Varga

Summary

- Different complement-based assays to diagnose angioedema
- Algorithm depends on lab possibilities
- Storage conditions are important
- Standardisation initiatives in progress