

**Definition of cTnI measurand:  
What is clinically relevant?**

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Turku, Finland**

# Cardiac Troponin I

## Some biochemical features

**Molecular mass:** 23876 Da; 209 aar

**Isoelectric point:** ~10.0

**Structure:** 4 regions with helix structure (42-78, 89-134, 150-157, 162-187);

other parts of the molecule do not have a regular structure

**TnI- TnC comp:** Regions 32-79 and 129-149;

high affinity;

pI:~5.0

**TnI- cTnT comp:** Site 80 and 97, lower affinity

**Phosphorylation sites:** Ser 22, Ser 23, Thr 30, Thr 50, Thr 128, Thr 142

**To be precise in cTnI measurements we need to know the biochemical properties of the analyte.**

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# Characterization of a New Certified Reference Material for Human Cardiac Troponin I

DAVID M. BUNK\* and MICHAEL J. WELCH

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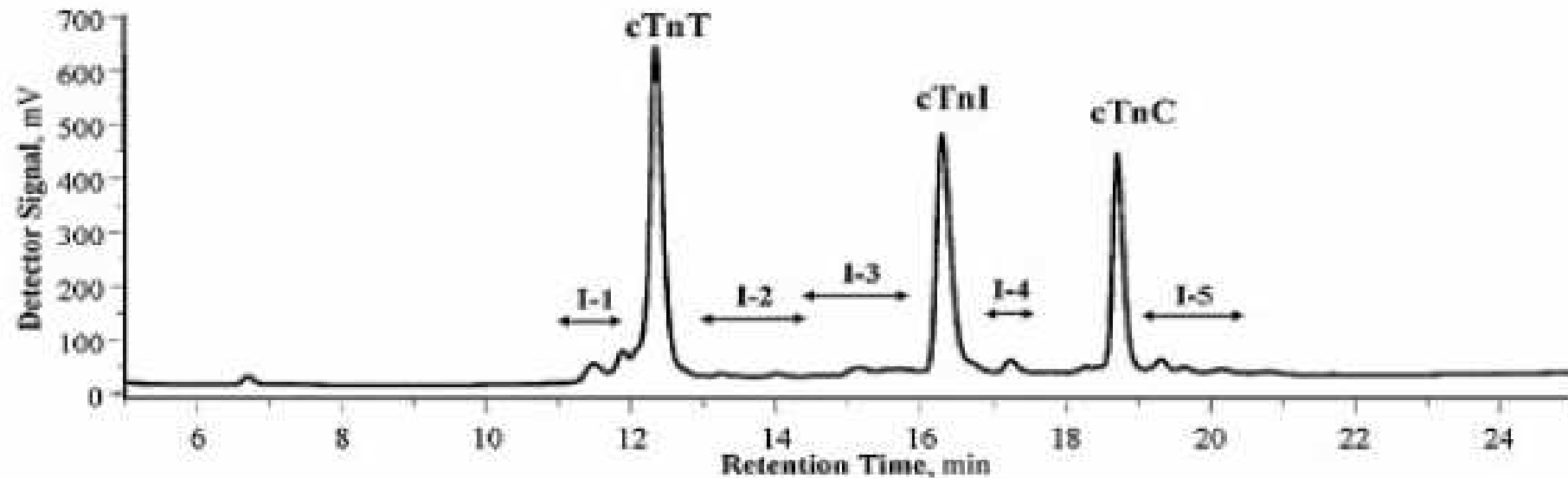
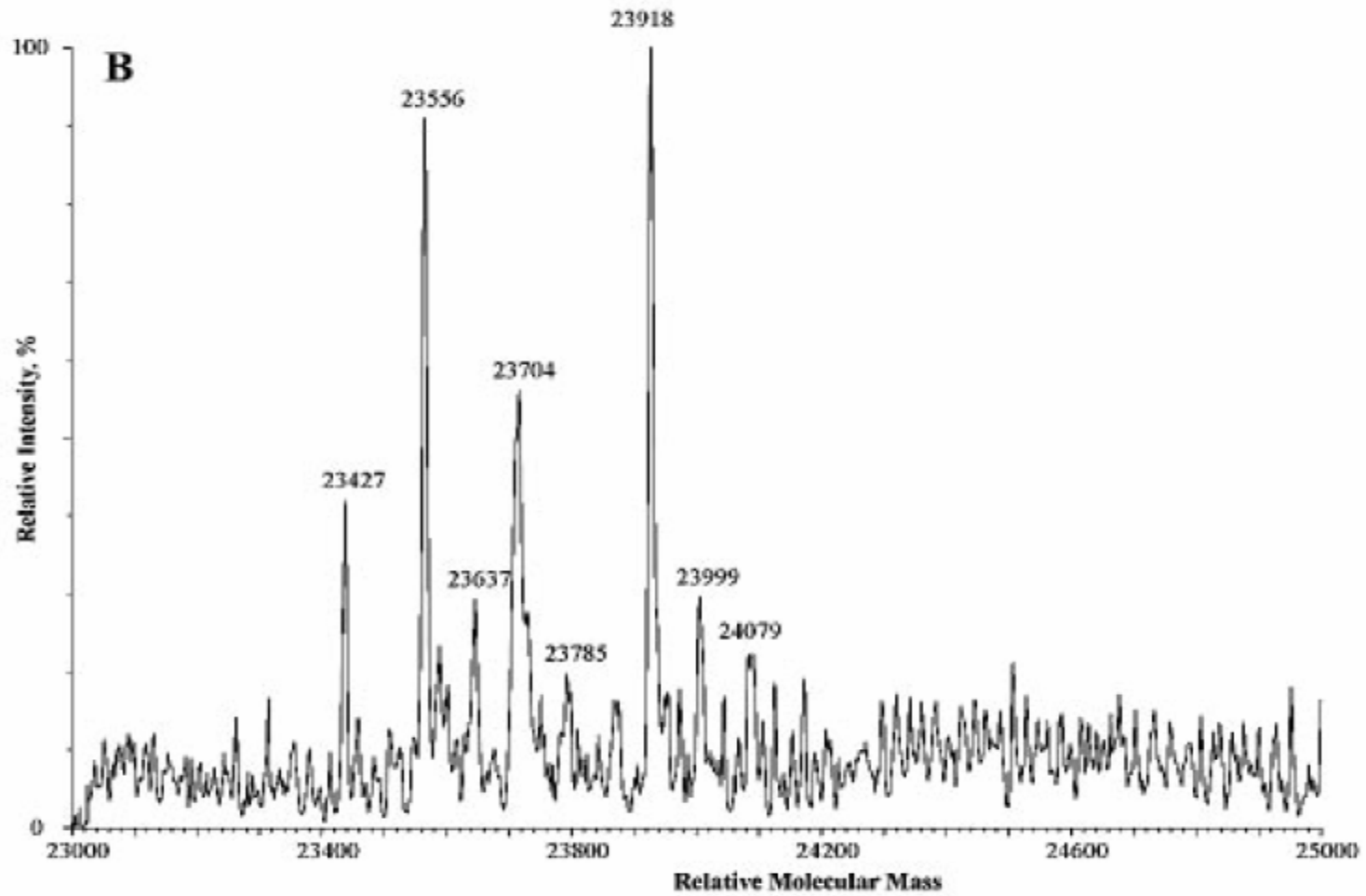


Fig. 1. RPLC chromatogram of SRM 2921 with UV absorbance detection (220 nm).

**Stoichiometric analysis ccTnT/TnI/TnC –0.84/1/1.04**

# cTnI forms in SRM 2921



# cTnI forms in SRM 2921

**Table 2. Relative molecular masses observed in the LC-MS and MALDI-MS analyses of SRM 2921.**

Component	Calculated $M_r^a$	Observed $M_r^b$		Tentative identification
		LC-MS	MALDI-MS	
cTnT	34 459.0	33 775 (8)	33 771 (20)	cTnT(Ac1-281) <sup>c</sup>
		34 374 (8)		cTnT(Ac1-286)
		<b>34 503 (6)</b>	<b>34 501 (15)</b>	N-Acetylated cTnT [cTnT(Ac1-287)]
		34 524 (8)		
cTnI	23 918.4	23 427 (8)	23 424 (20)	cTnI(Ac1-205)
		23 556 (6)	23 552 (15)	cTnI(Ac1-206)
		23 637 (8)	23 636 (20)	Monophosphorylated cTnI(Ac1-206)
		23 704 (8)	23 700 (15)	cTnI(Ac1-207)
		23 785 (8)	23 789 (20)	Monophosphorylated cTnI(Ac1-207)
		<b>23 918 (5)</b>	<b>23 920 (15)</b>	N-Acetylated cTnI [cTnI(Ac1-209)]
		23 999 (6)		Monophosphorylated cTnI(Ac1-209)
		24 079 (8)	24 079 (20)	Bisphosphorylated cTnI(Ac1-209)
cTnC	18 444.6	18 426 (6)		N-Acetylated cTnC [cTnC(Ac1-161)]
		<b>18 445 (4)</b>	<b>18 440 (10)</b>	
		18 463 (8)		
		18 477 (8)		

<sup>a</sup> Calculated from the amino acid sequence obtained from the Swiss-Prot protein sequence database (<http://us.expasy.org/sprot/sprot-top.html>) using entry names TNNT2\_HUMAN for cTnT, TNNT3\_HUMAN for cTnI, and TNNT1\_HUMAN for cTnC.

<sup>b</sup> The component with the highest observed signal intensity is indicated in bold. The values in parentheses are the uncertainties calculated at the 95% confidence level.

<sup>c</sup> Ac, acetylated.

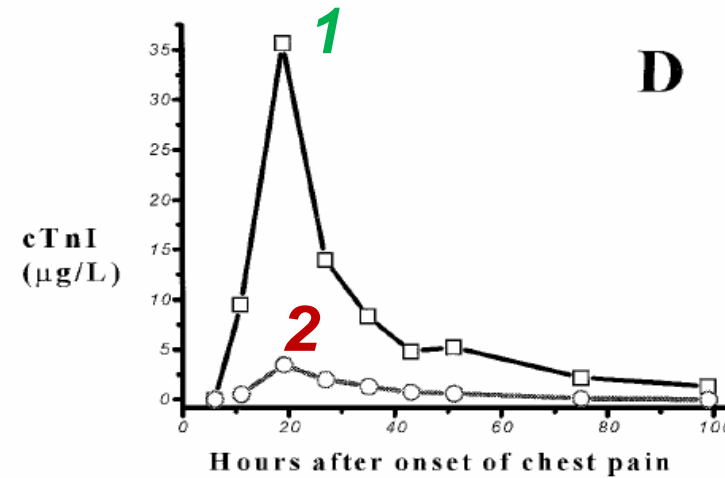
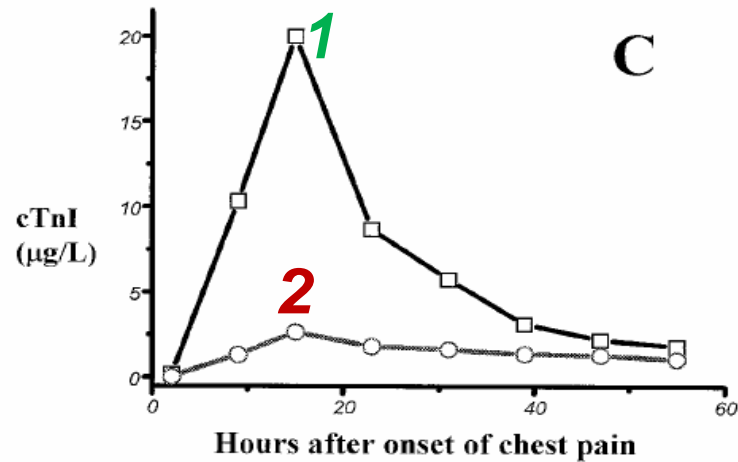
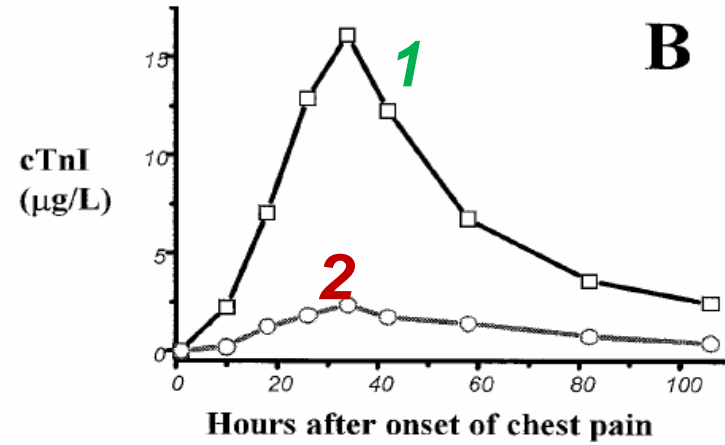
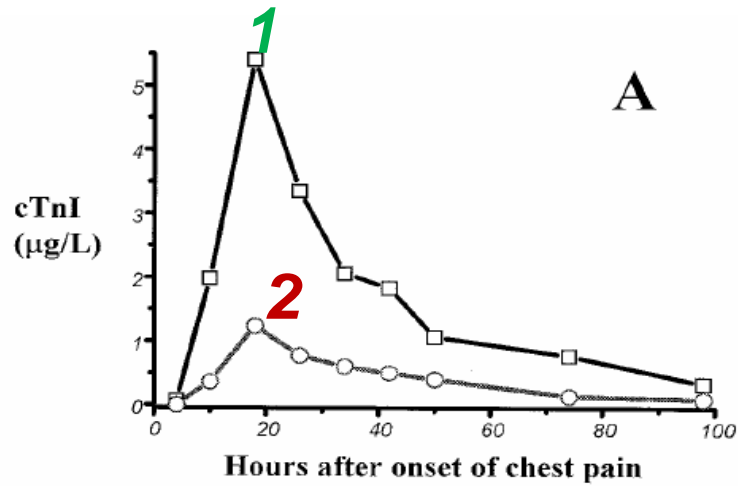
**cTnl in patient's blood**

***more questions than answers...***

*Free or complex?*

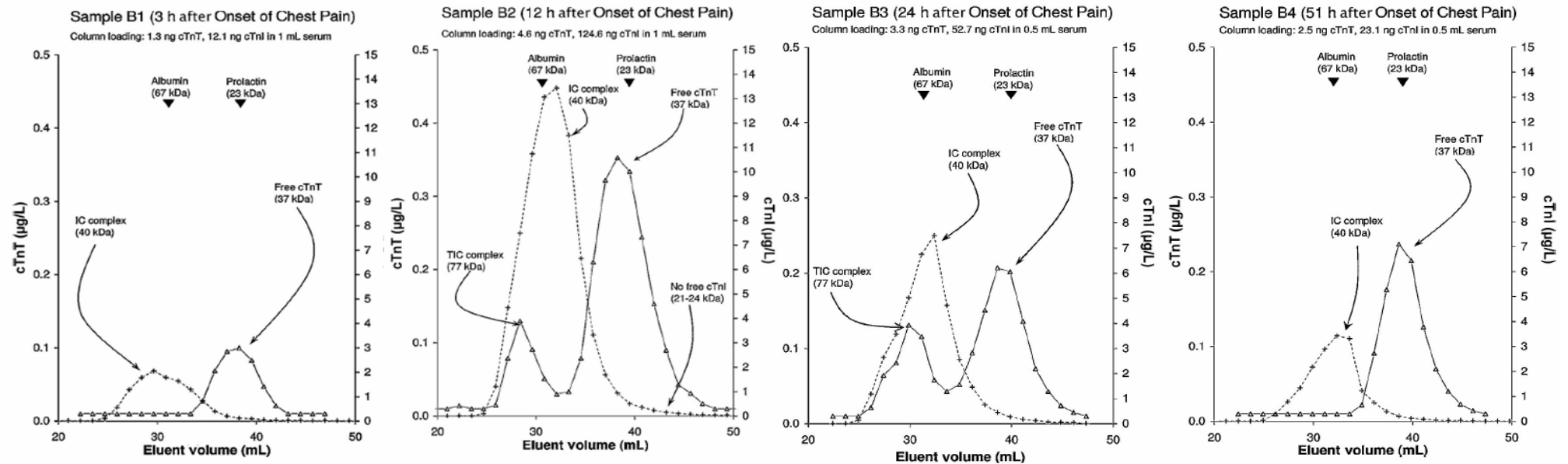


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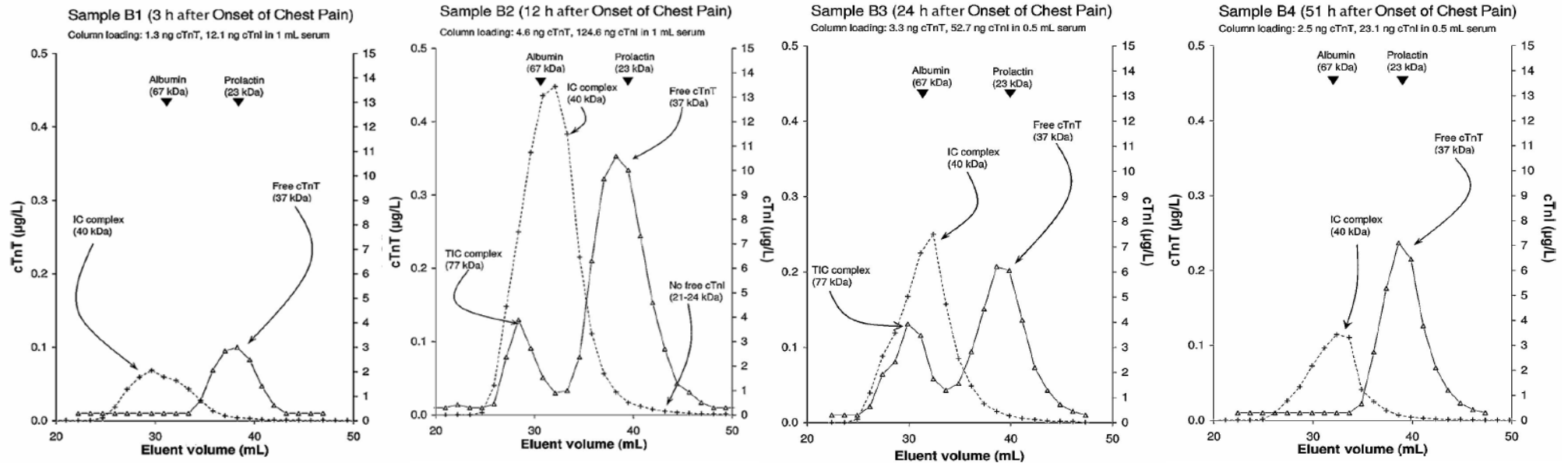


# Free or complex?

## GF studies of blood samples



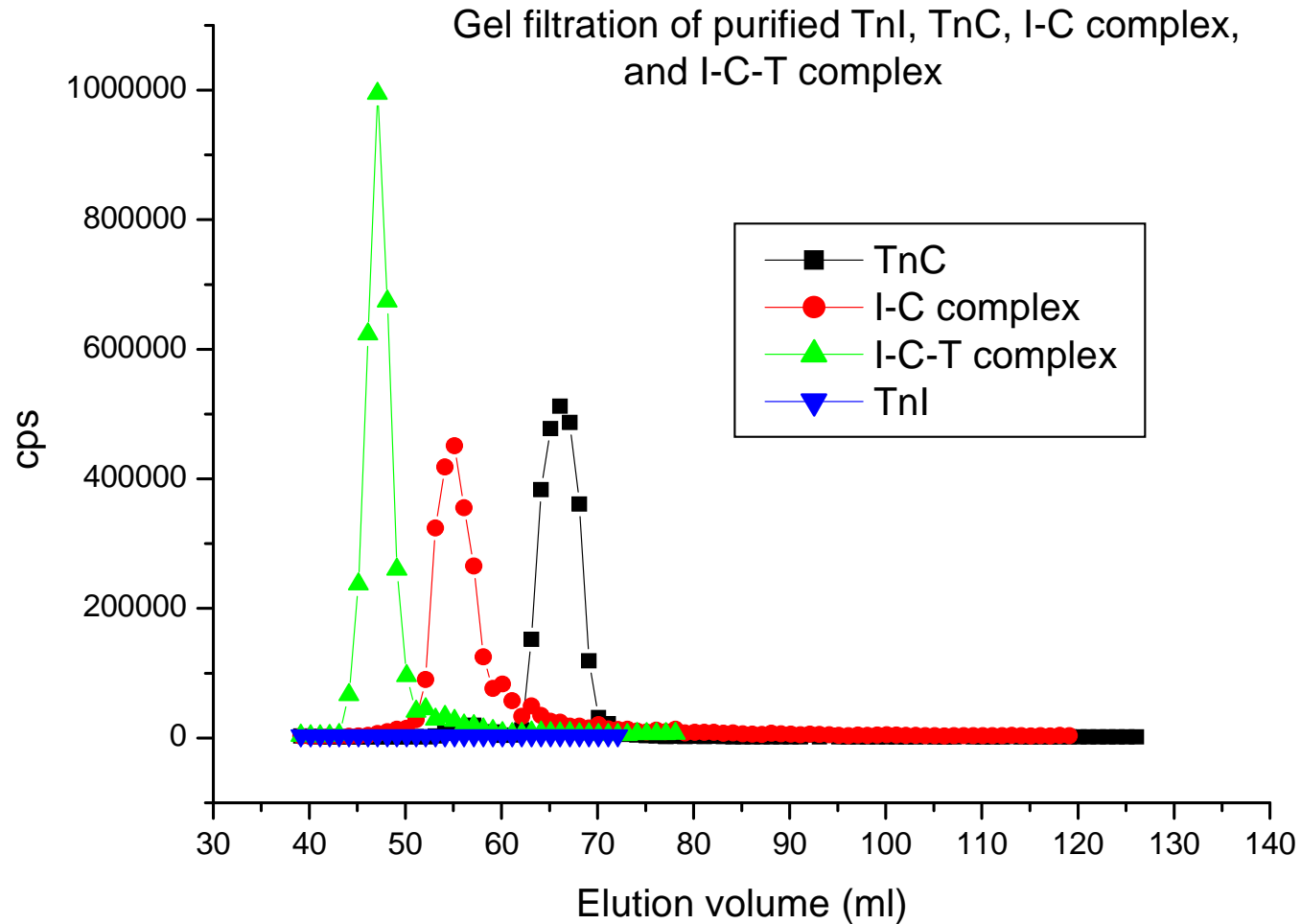
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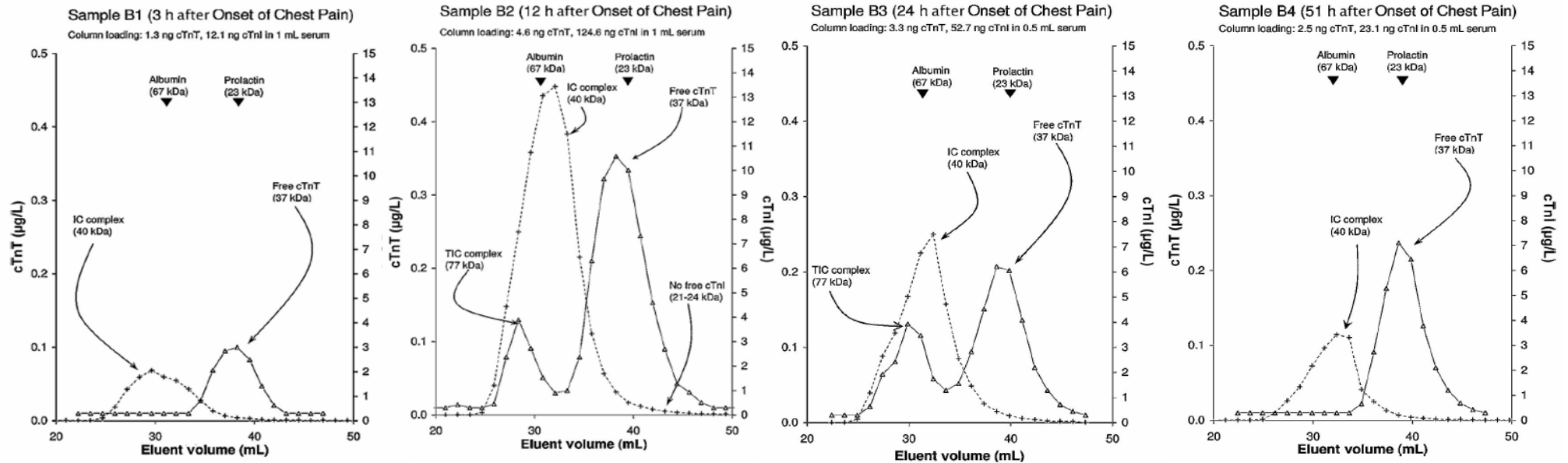
**Recovery: cTnT assay 86% (41-110)**

**cTnI assay 49% (23-134)**

# *GF studies of troponins and troponin complexes*



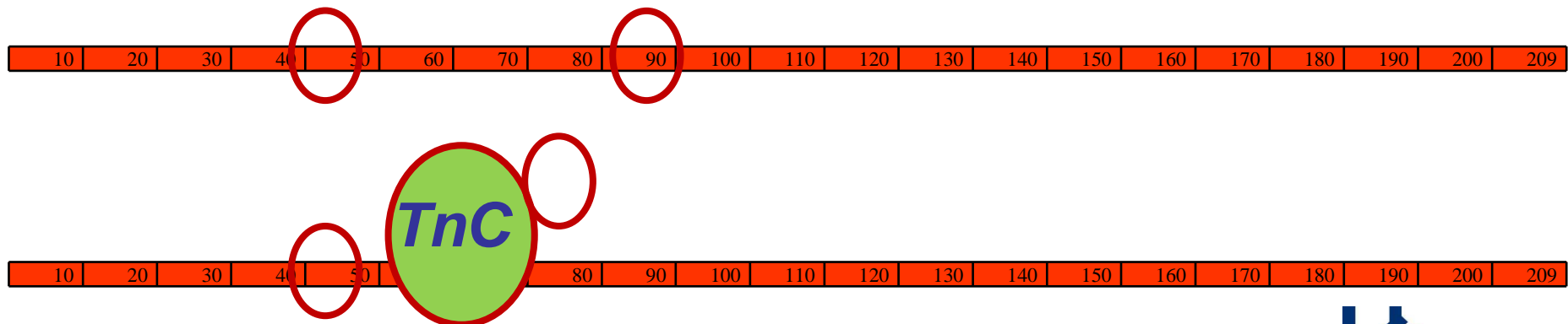
# GF studies of blood samples



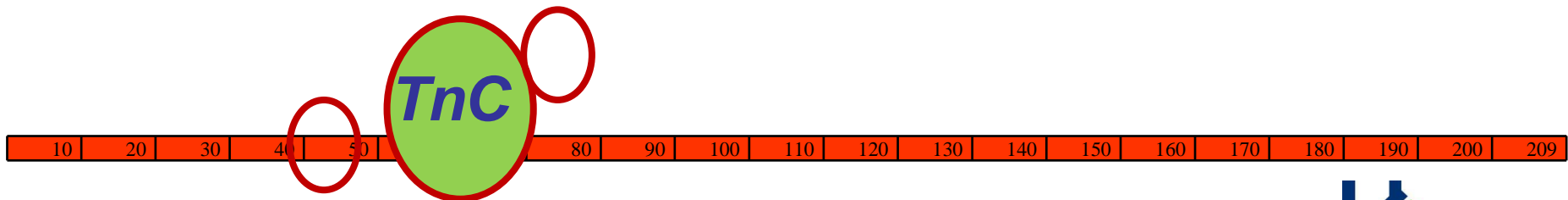
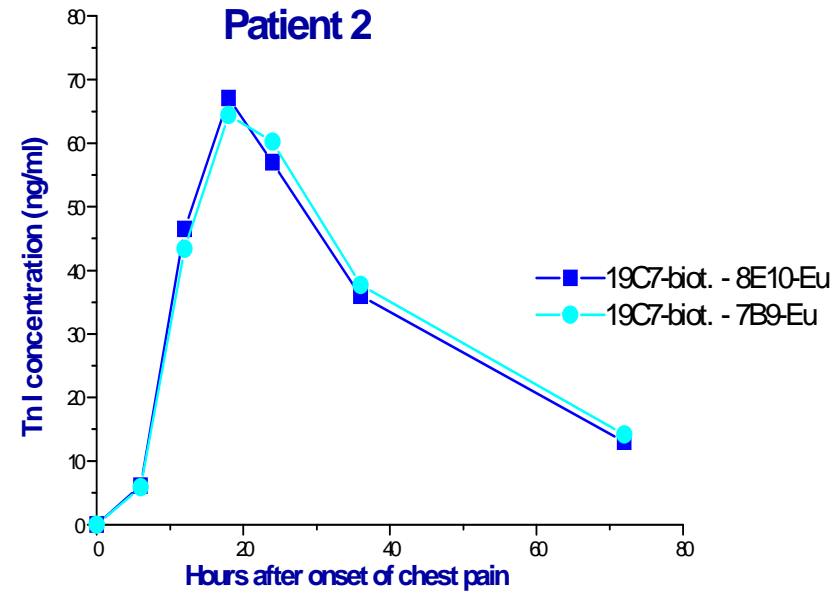
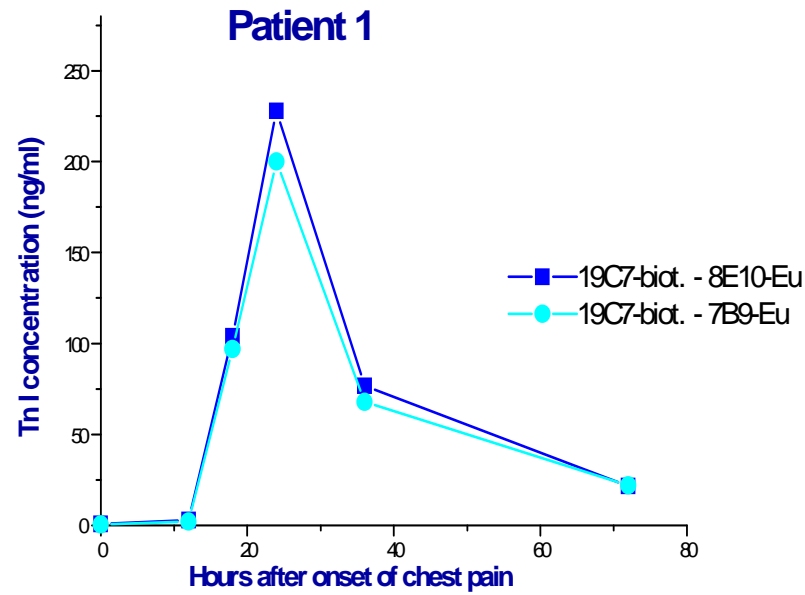
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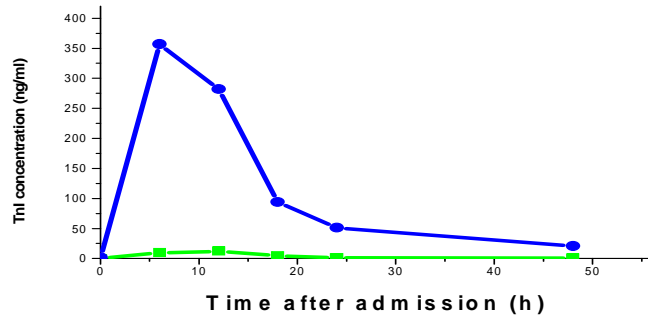
# Free or complex with troponin C?



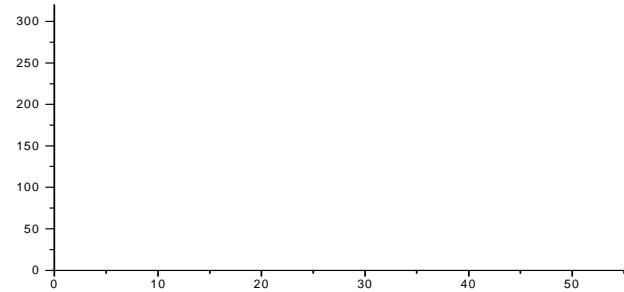
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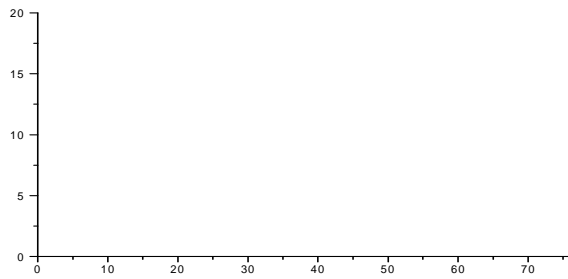
# Complex with troponin T?



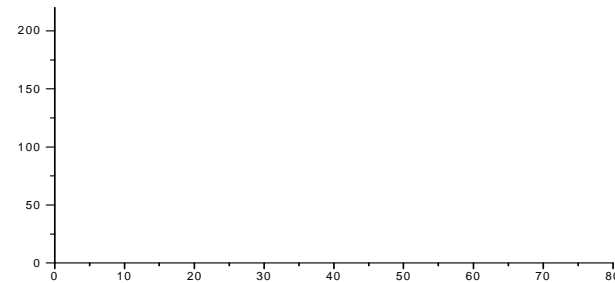
A



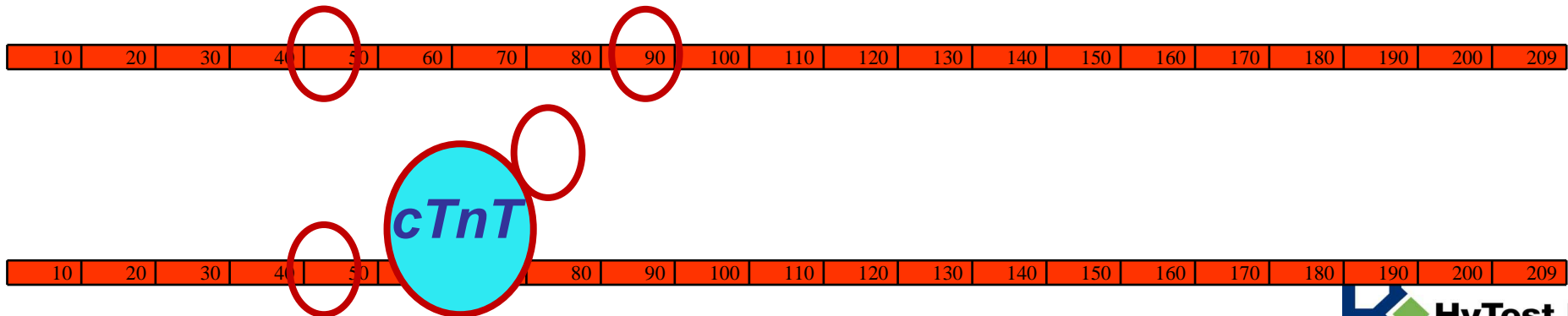
B



C



D



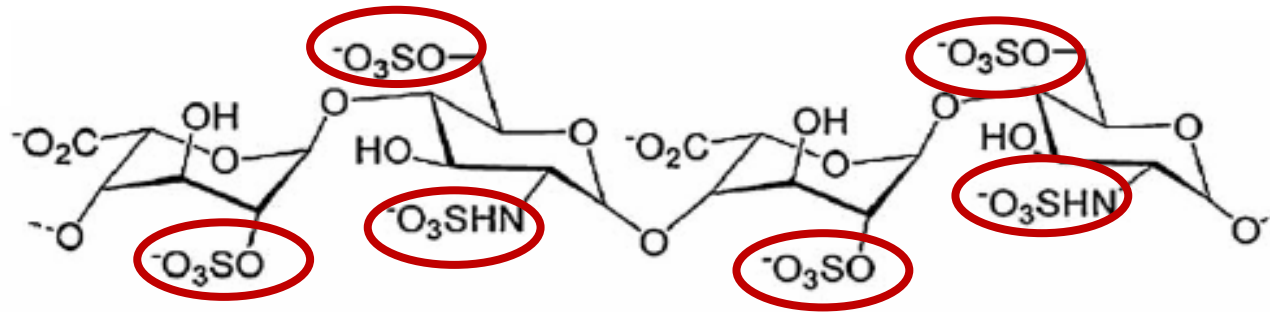


## **Questions:**

- Does “free” (not complexed) cTnl exist in patients blood?***
- What epitopes are affected by complex formation with TnC***

# *Heparin and cTnl measurements*

## Heparin and cTnI measurements



cTnI pI~10

cTnI – TnC complex pI ~ 5

# Heparin and cTnI measurements

**Table 2. Results for cTnI and cTnT in heparin-plasma compared with serum (n = 100).**

Method	Results for cTnI and cTnT in heparin-plasma compared with initial serum result, %			Percentage of results lower in heparin-plasma than in serum		
	Mean $\pm$ 95% CI <sup>a</sup>	Range	-/+ of mean	Difference >10%	Difference >15%	Difference >20%
Abbott AxSYM cTnI	101 $\pm$ 2	76–123	-25/+22	8	5	2
ACS:Centaur cTnI	94 $\pm$ 3	50–120	-44/+26	35	28	11
Roche Elecsys cTnT	99 $\pm$ 3	34–129	-65/+30	17	11	9

<sup>a</sup> CI, confidence interval.

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## ***Questions:***

- Why effect of heparin is different in different blood samples?***
- The mapping of the epitopes sensitive to heparin was not done yet***

# Phosphorylation



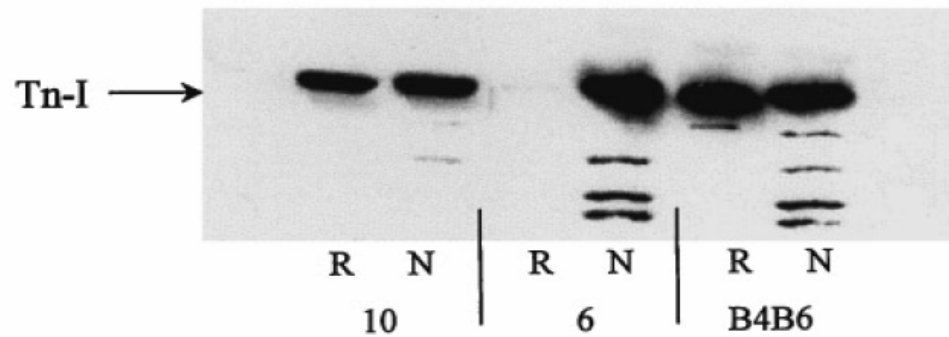
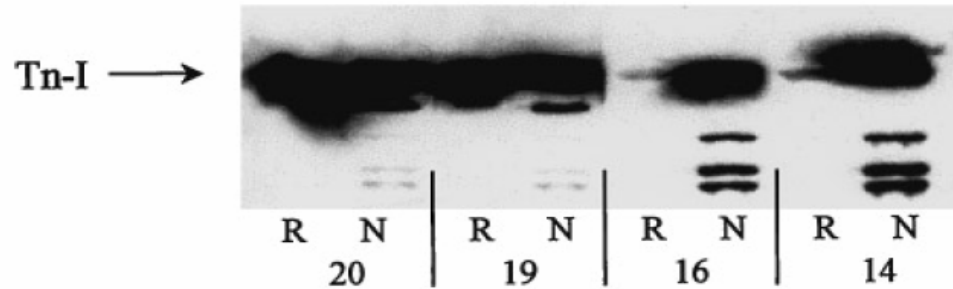
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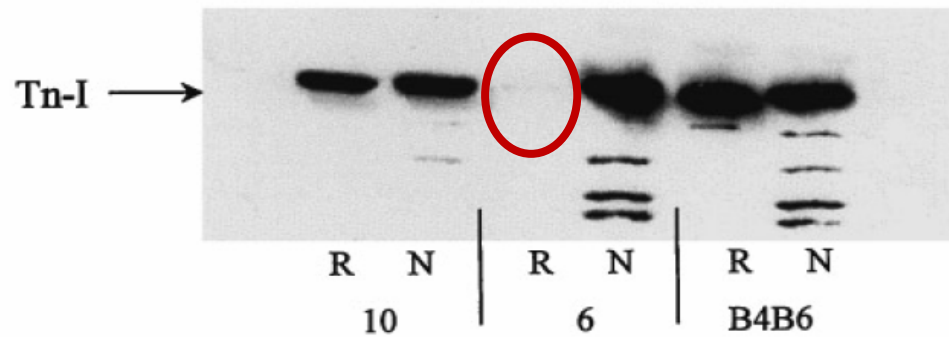
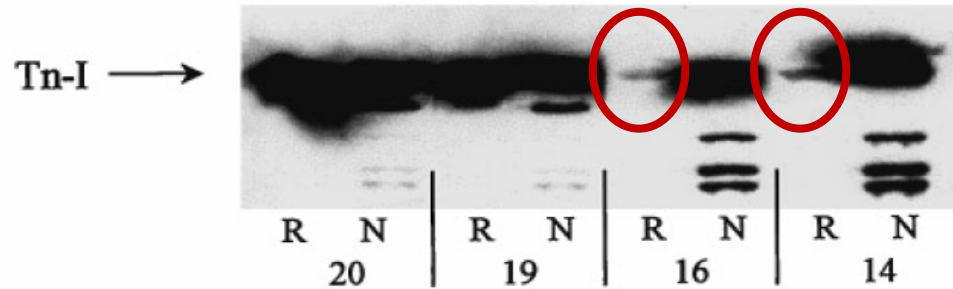
# Phosphorylation specific antibodies for cTnI



Pi Pi

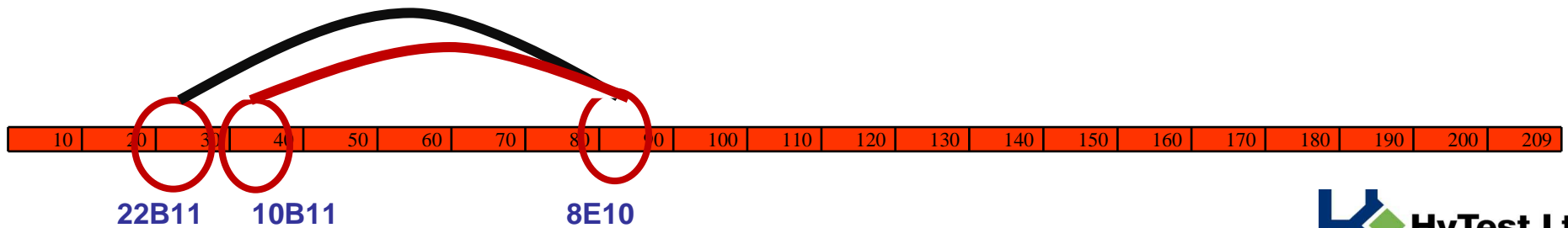
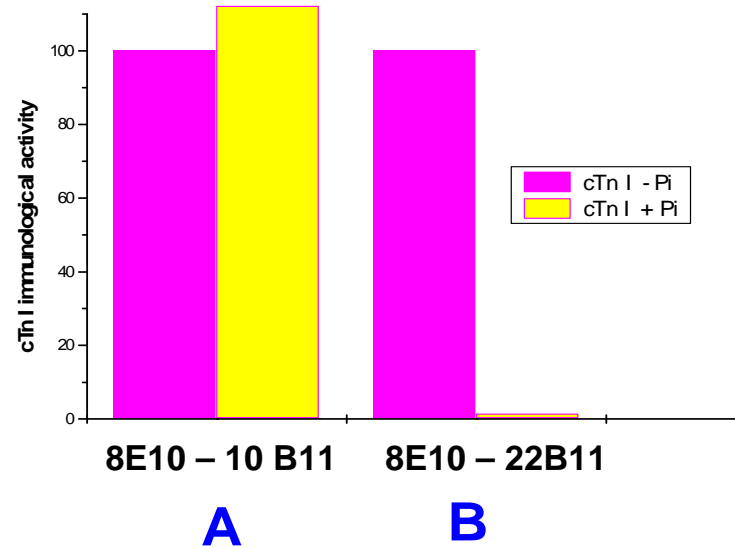
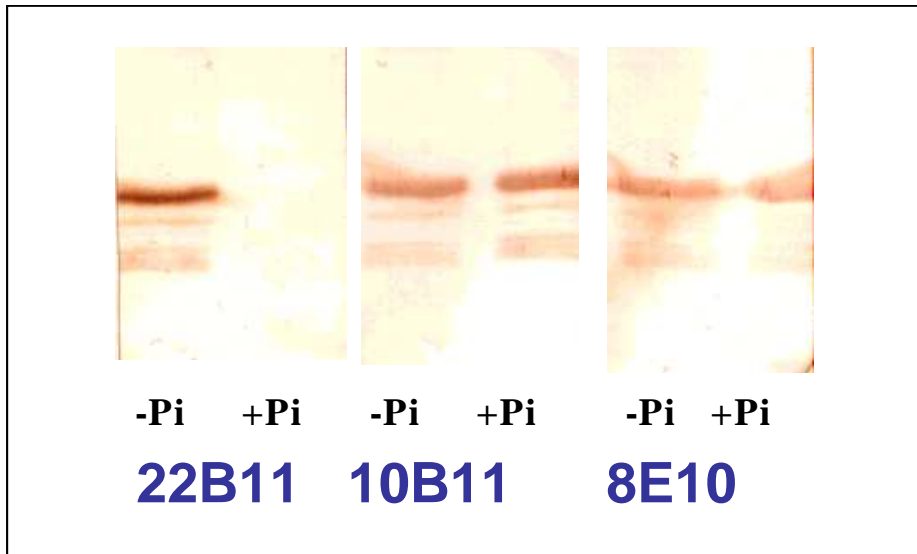


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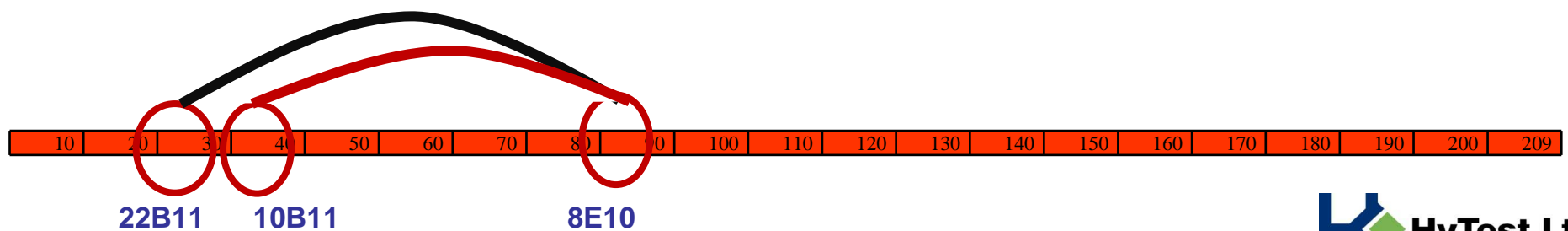
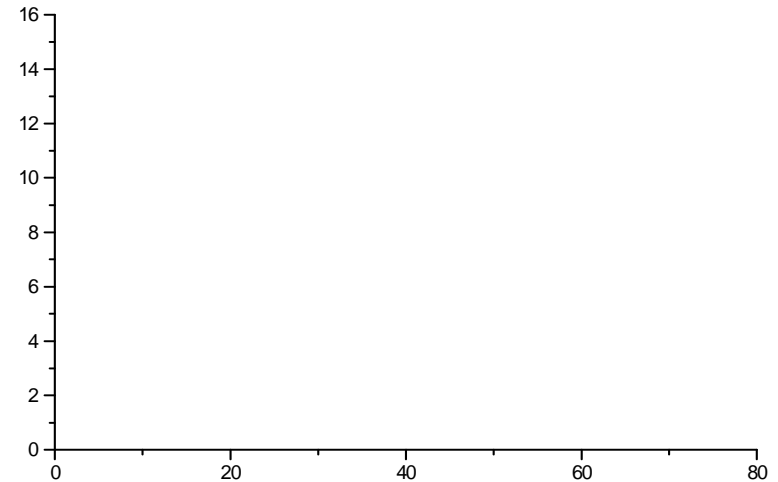
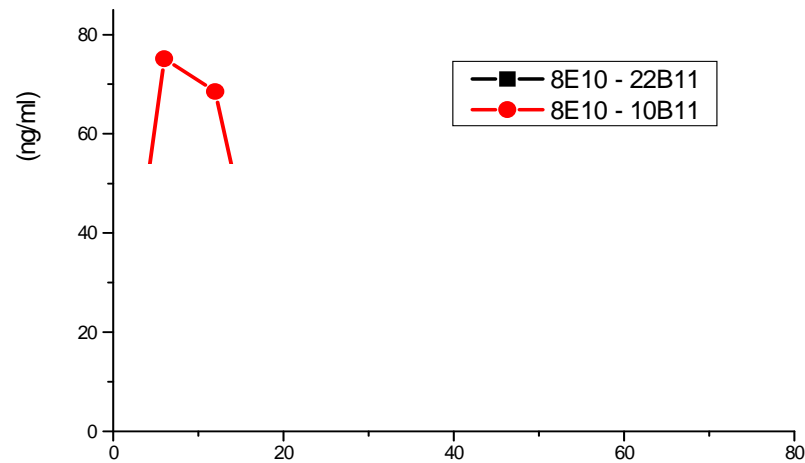


Pi Pi





# Measurements of dephosphorylated cTnI



# Questions

- We do not know what is the ratio of phospho/dephospho forms (Ser 22 and Ser 23) in patients' blood; is it of clinical value?**
- We have no information about phosphorylation of other known sites at all**

# **Autoantibodies**



# Autoantibodies

[Bohner J](#), [von Pape KW](#), [Hannes W](#), [Stegmann T](#).  
False-negative immunoassay results for cardiac troponin I probably due to circulating troponin I autoantibodies.  
[Clin Chem](#). 1996 Dec;42(12):2046.

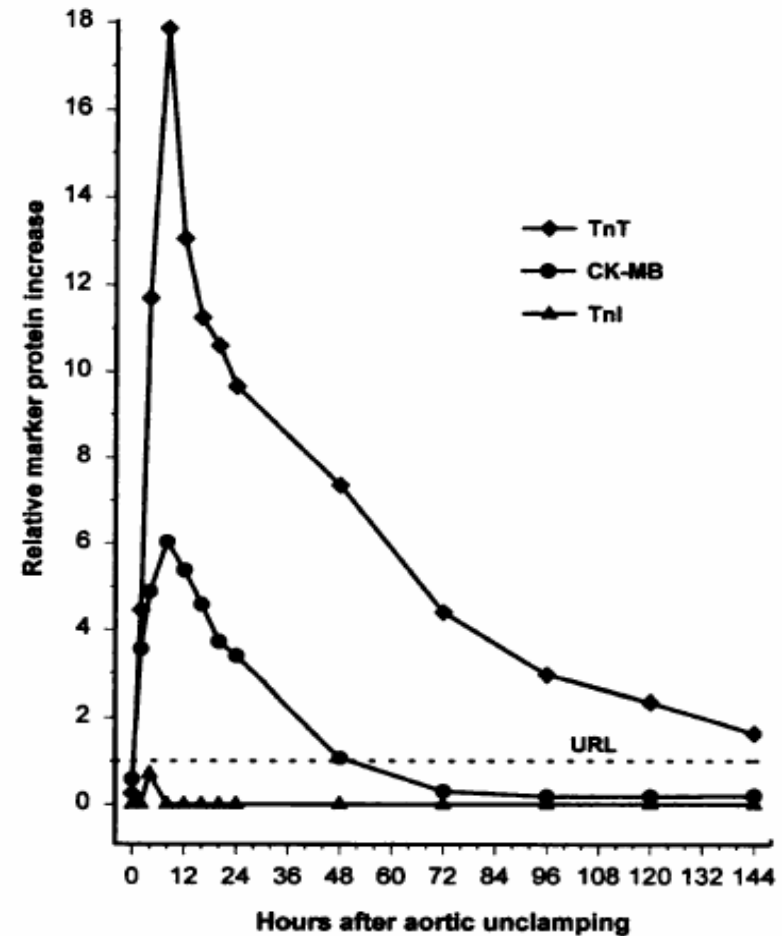


Fig. 1. Changes in serum concentrations of cardiac marker proteins in a patient after coronary artery bypass grafting.

*Ordinate:* multiples of URL. Note the missing increase of cTnl above the URL (*dotted line*).

# Autoantibodies

**TABLE 1.** Prevalence of Cardiac Troponin-I Autoantibodies in Normal Donor and Biomarker Positive Sample Cohorts

	(n)	Age (range)	Median Response S/LC (95% CI)	% Prevalence <sup>a</sup> (n)
Healthy Controls				
Normal Blood Donors				
All	750	37.8 (18–77)	1.400 (1.271–1.500)	12.7 (95)
M	413	37.1 (18–77)	1.600 (1.300–1.800) <sup>b</sup>	15.7 (65) <sup>c</sup>
F	282	38.9 (18–74)	1.250 (1.100–1.500) <sup>b</sup>	9.9 (28) <sup>c</sup>

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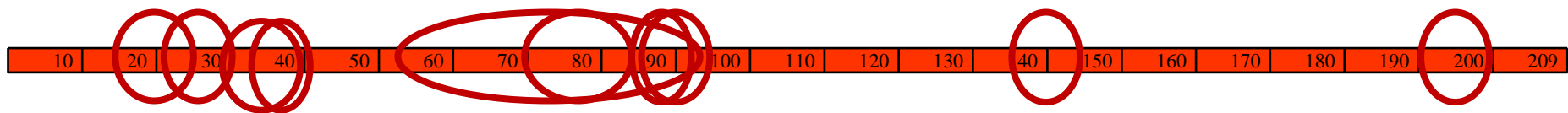
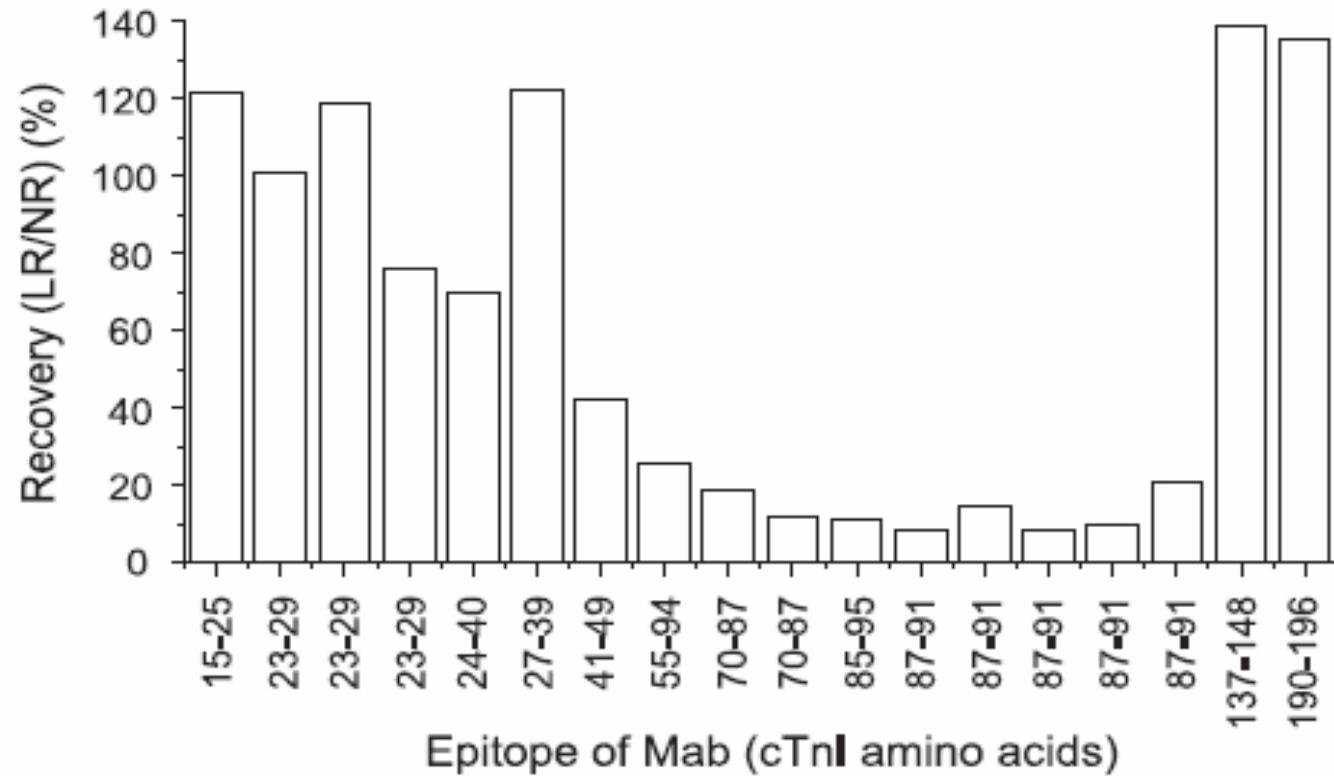
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Epitope mapping using peptide (15 aar) library:

***“ The most ubiquitous strong response was to epitopes close to amino and carboxy terminal regions of the protein.”***

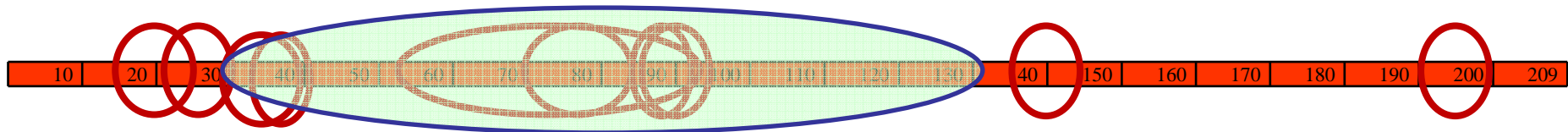
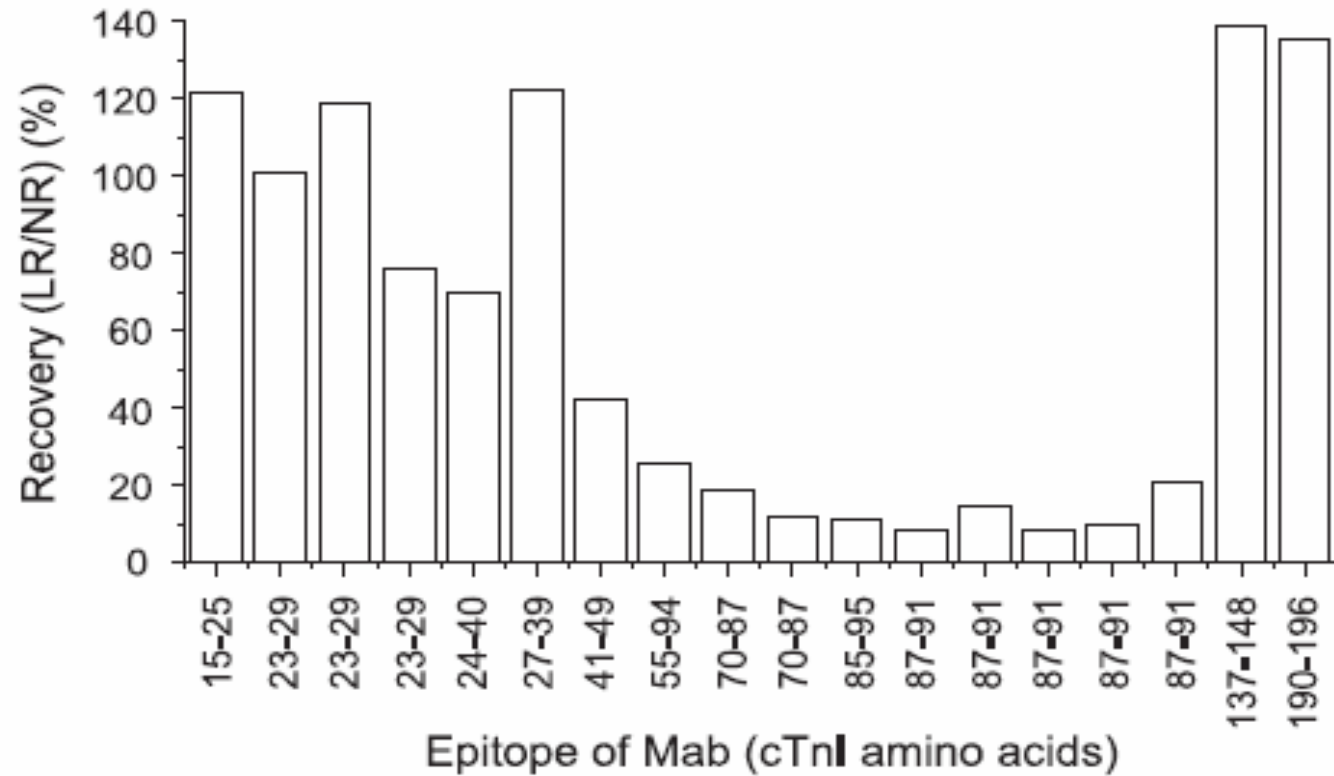
# Autoantibodies

Serum sample with high level of autoantibodies



# Autoantibodies

Serum sample with high level of autoantibodies



# Autoantibodies

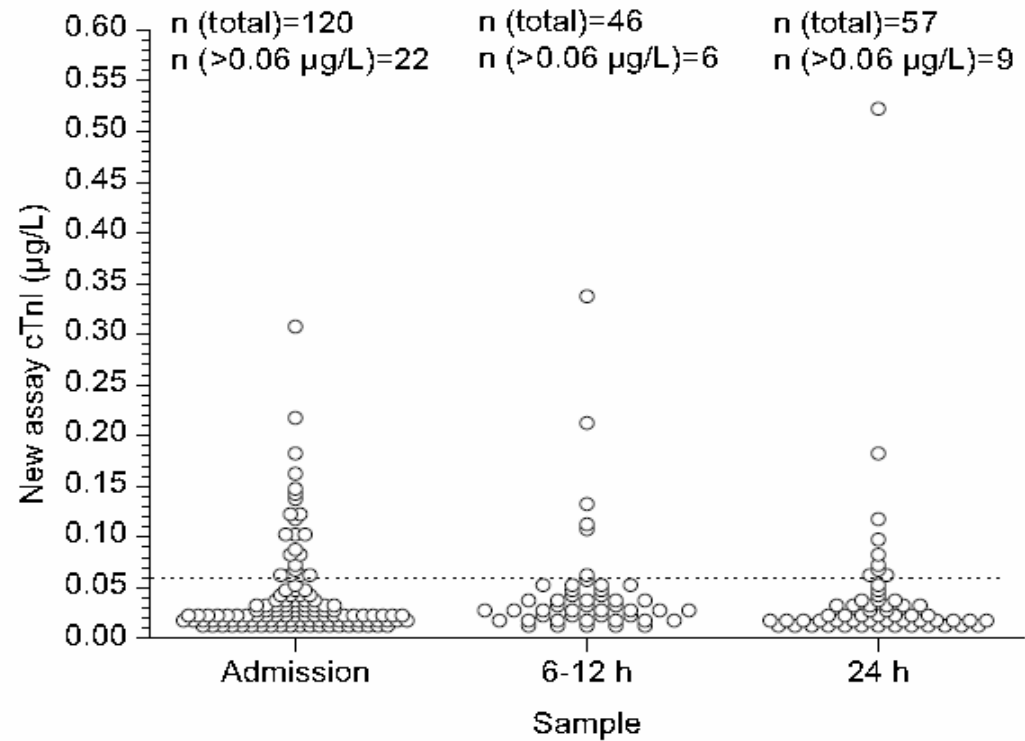
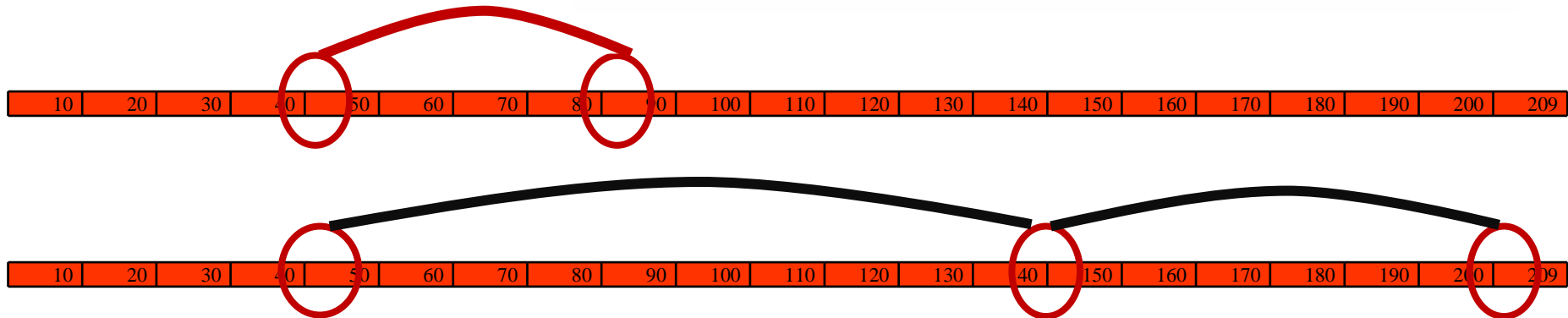


Fig. 2. cTnI concentrations measured by the new assay in samples that were below the detection limit of the Aio cTnI assay (0.05 µg/L). The *horizontal line* indicates the concentration giving a total CV of 10%.



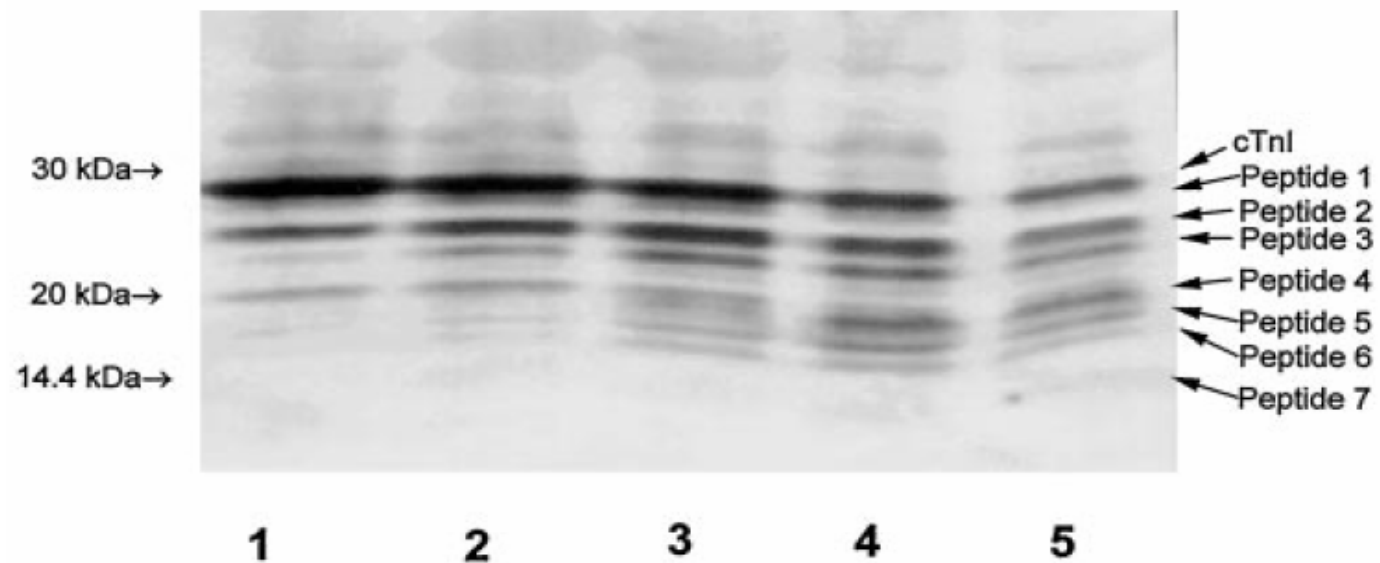
## **Questions:**

- What factors are responsible for the appearance of anti-cTnI autoantibodies?**
- What is the epitope specificity of the autoantibodies?**
- How can we avoid the negative interference of autoantibodies?**

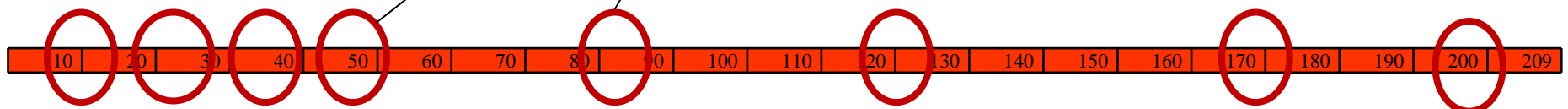
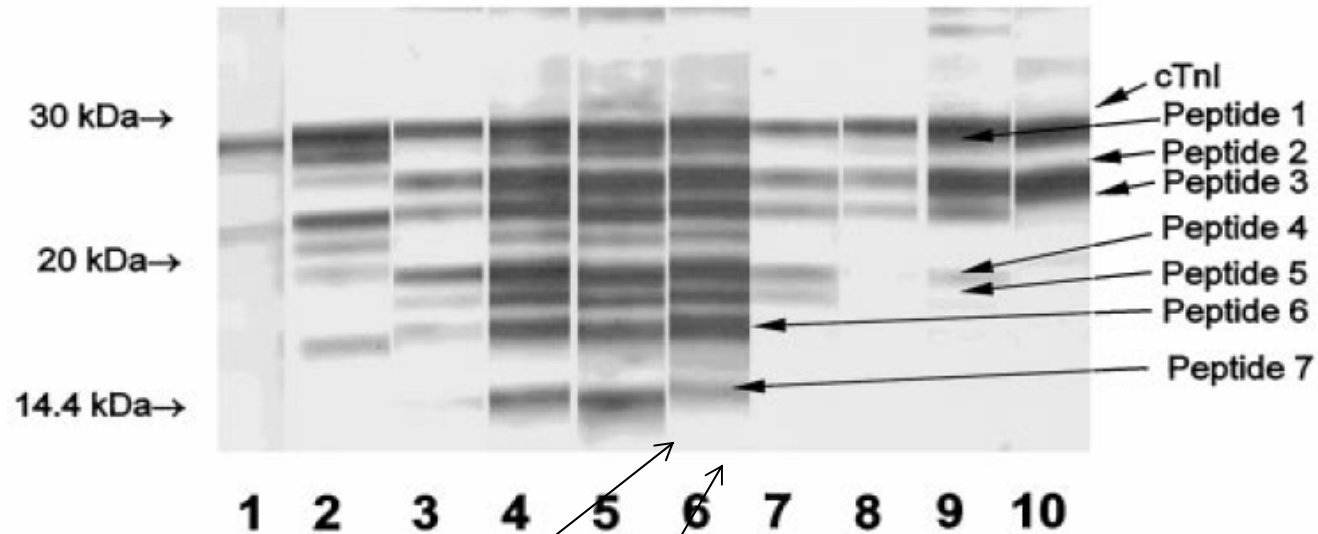


# **Troponin I degradation**

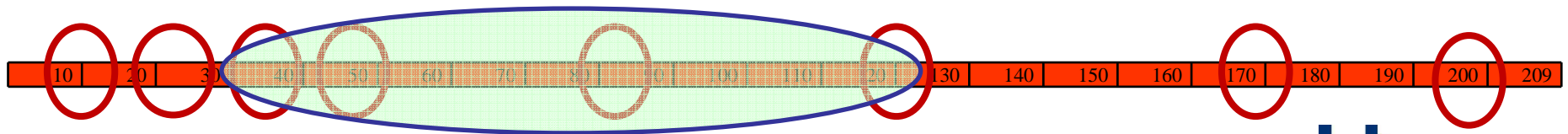
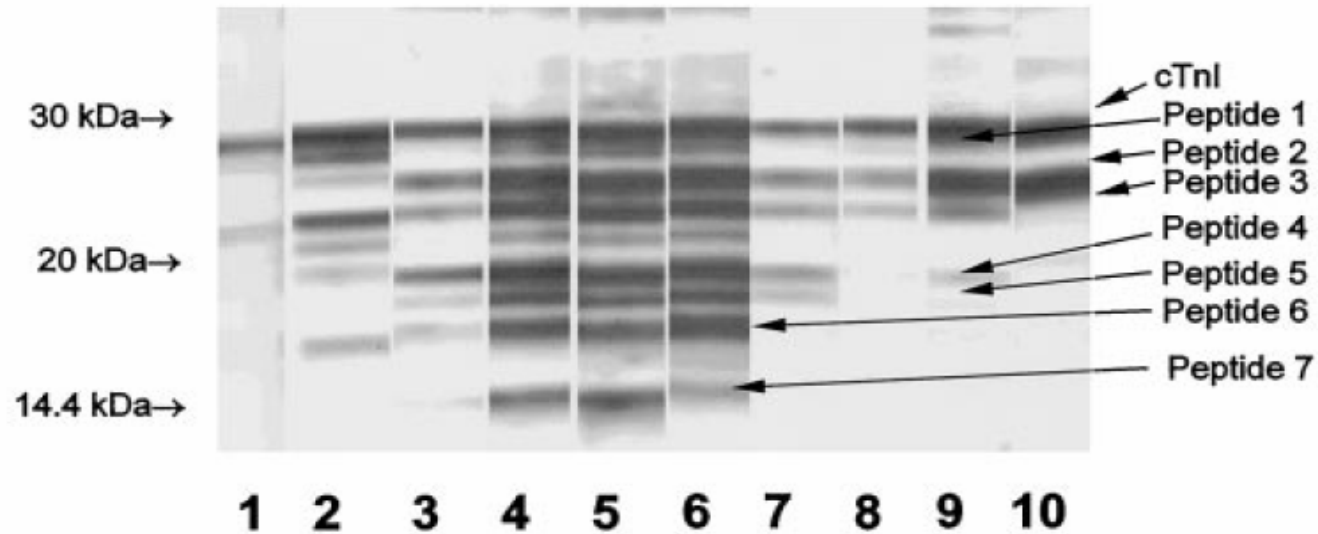
# Troponin I degradation *in vitro*



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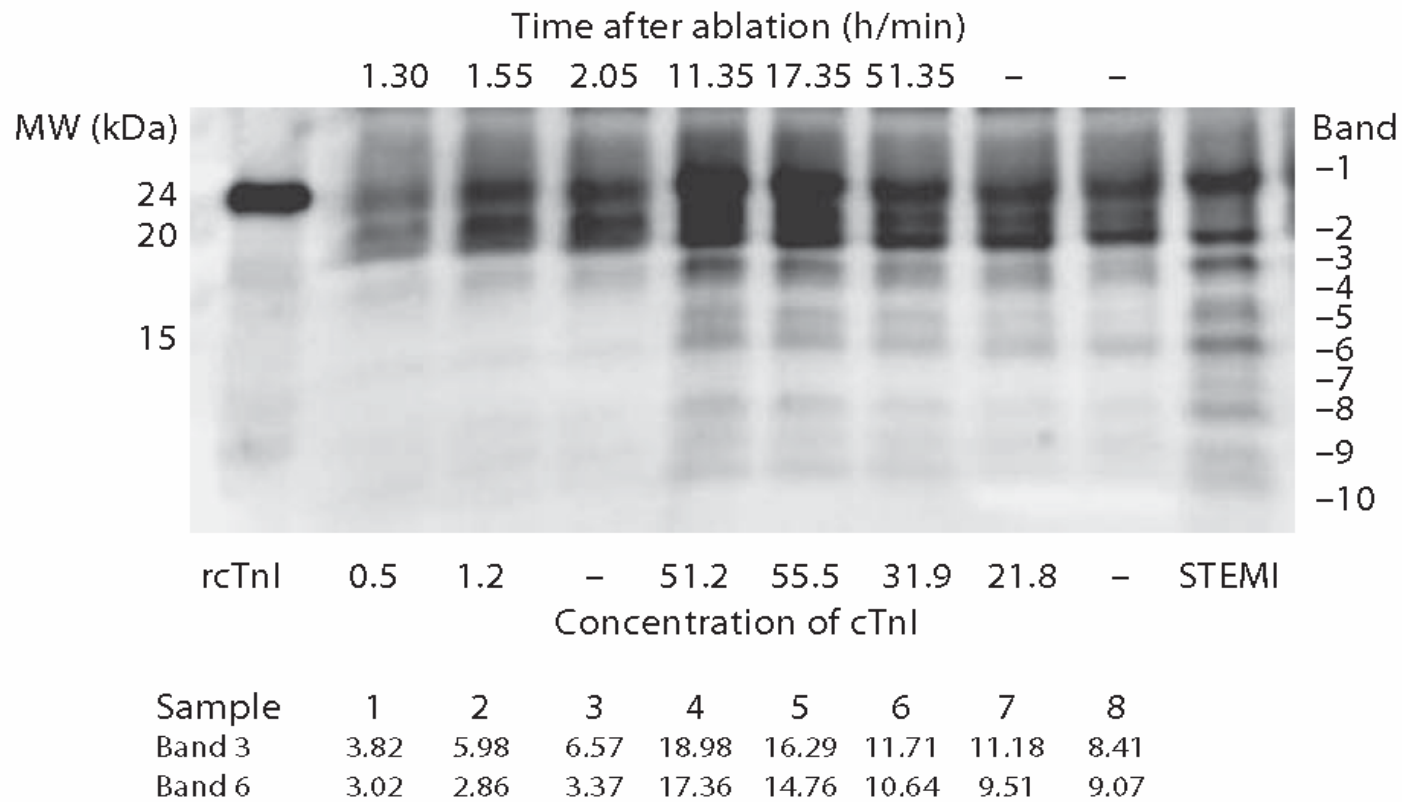


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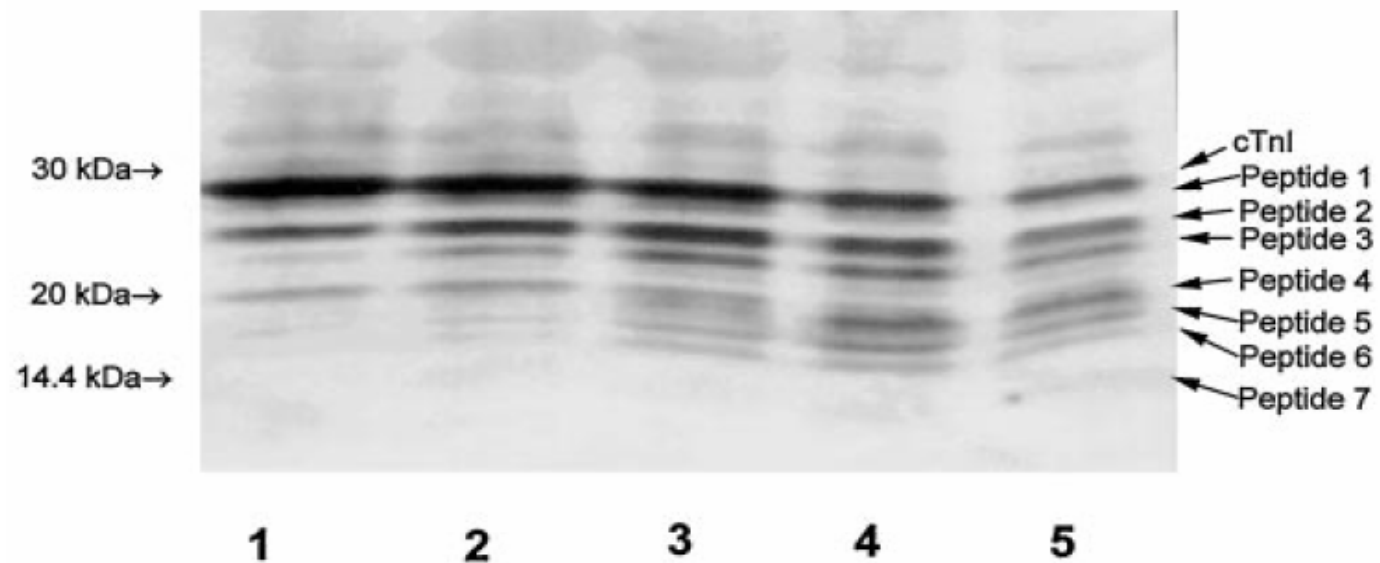


# Troponin I degradation

## Blood studies

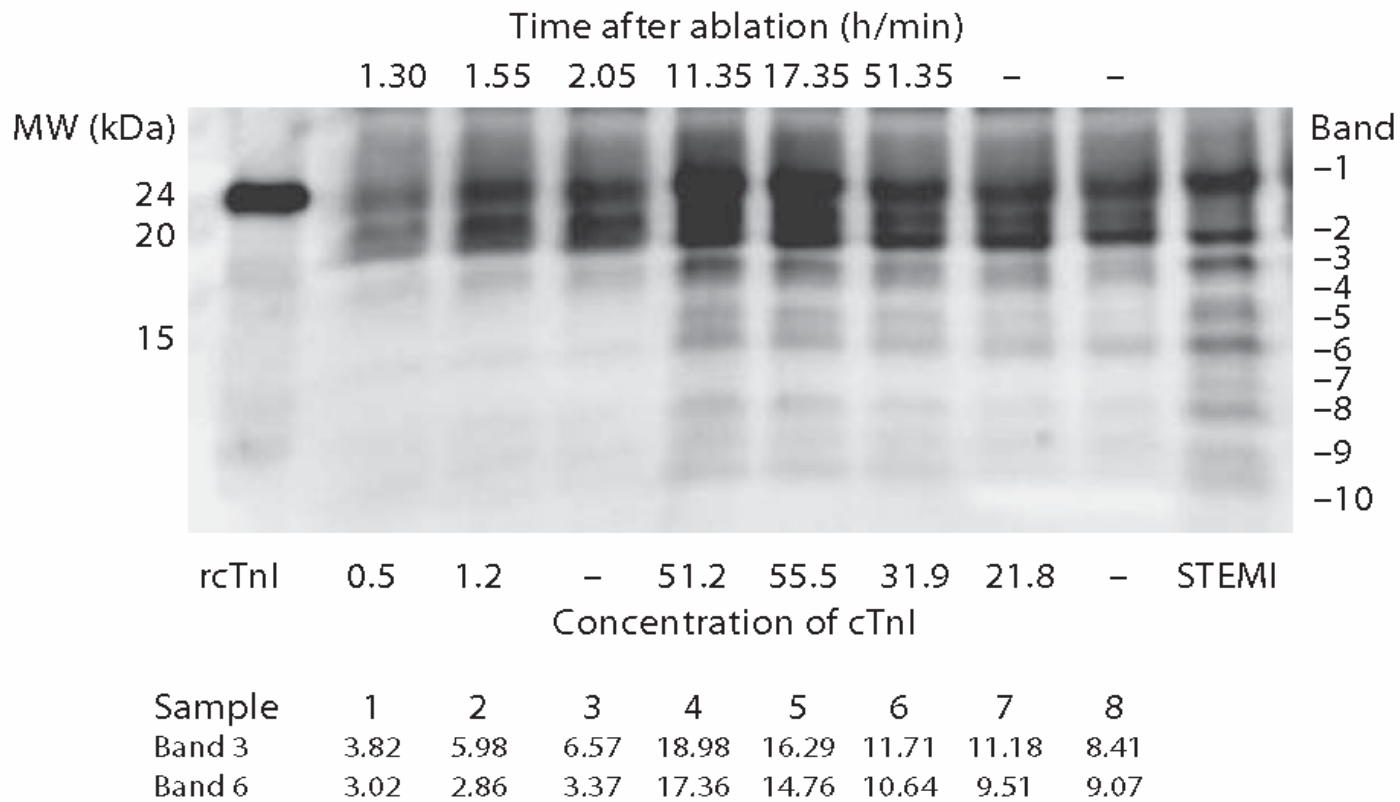


# Troponin I degradation *in vitro*



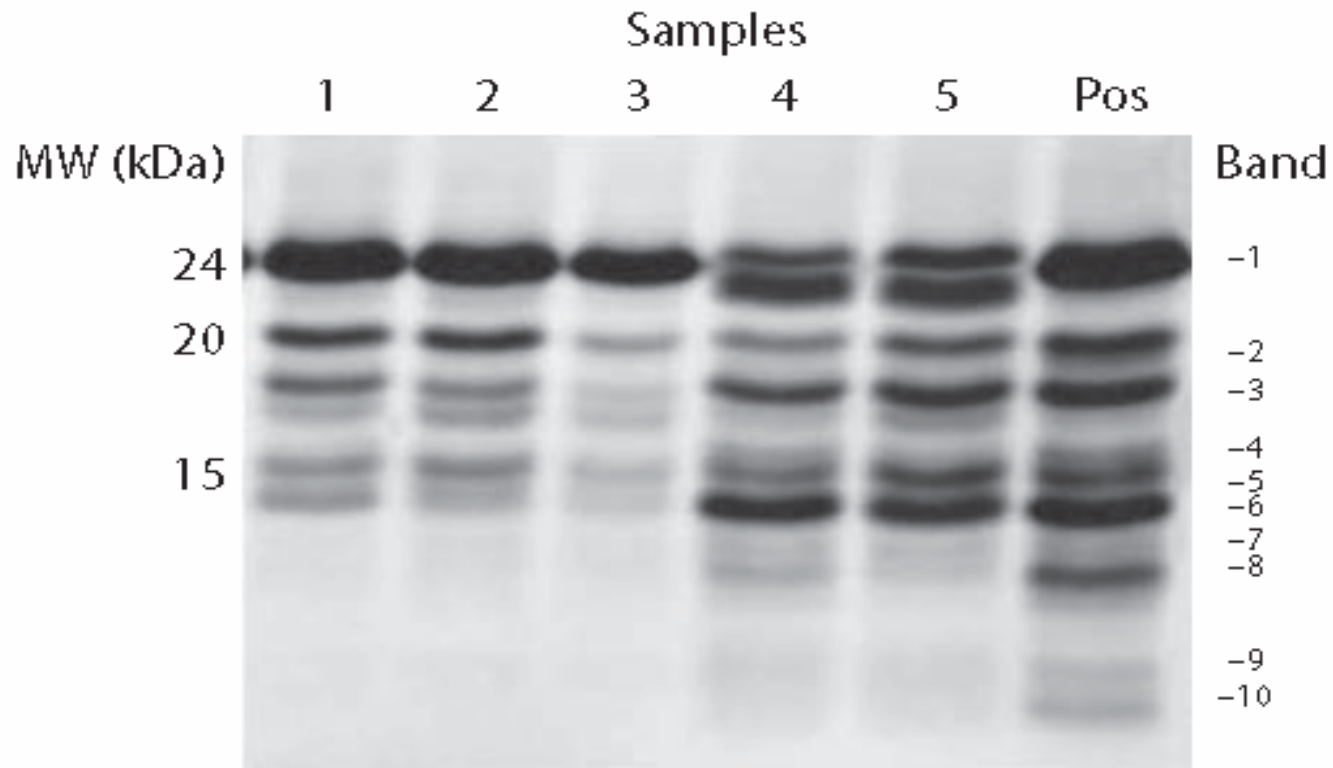
# Troponin I degradation

## Blood studies



# Troponin I degradation

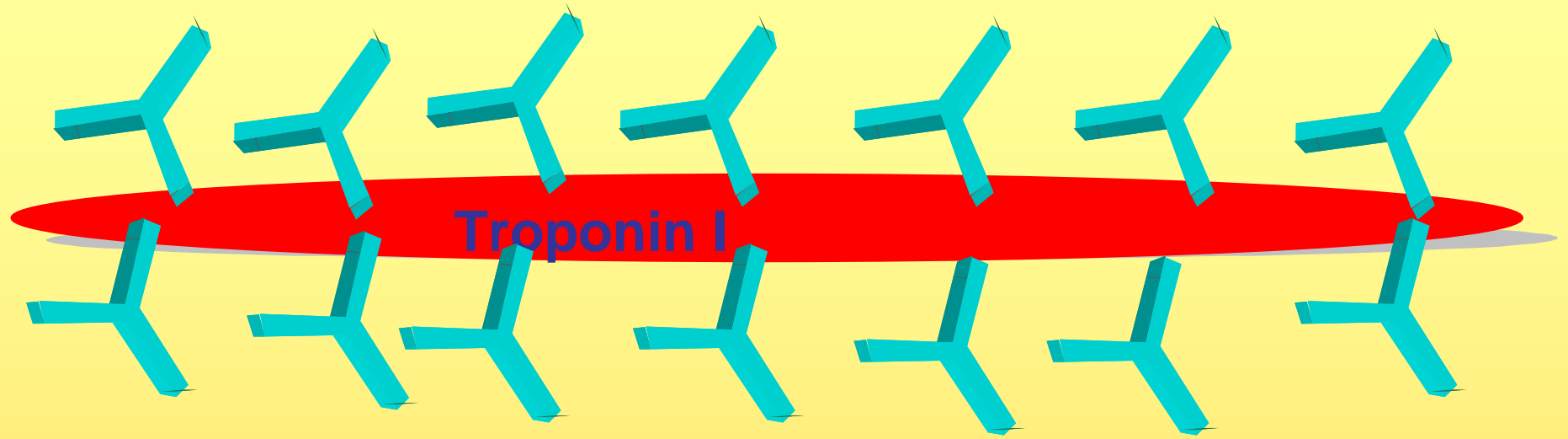
*Blood studies. Immunoprecipitation.*

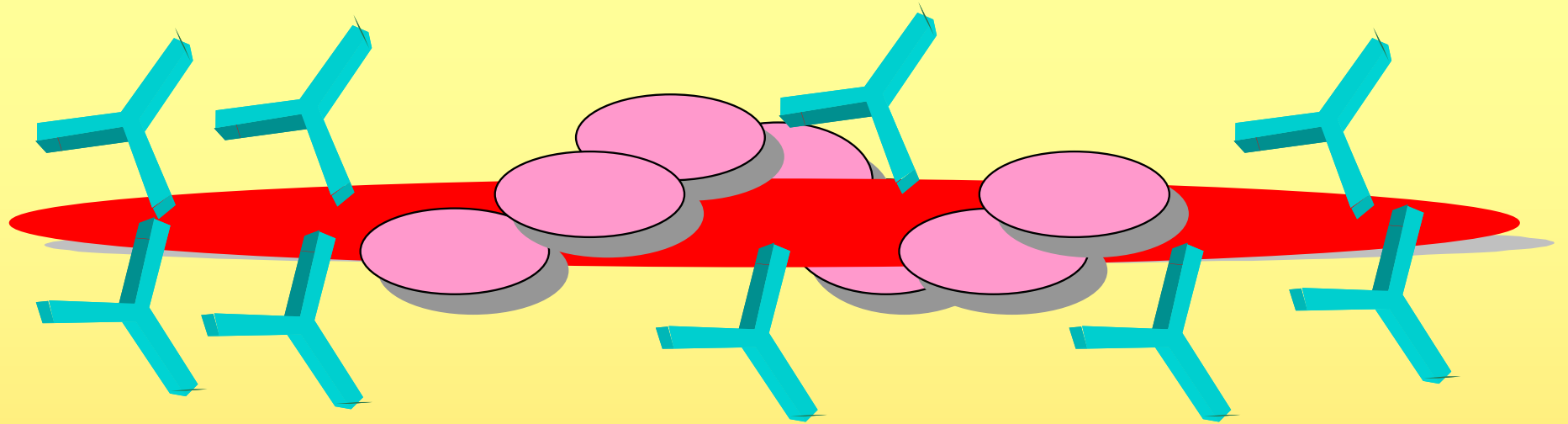




## **Questions:**

- In what form cTnI is released in the blood stream – intact or truncated?**
- What is the ratio of intact and truncated forms in the:**
  - early samples**
  - peak samples**
  - late samples?**
- Can we use antibodies specific to the terminal epitopes for the assay development?**

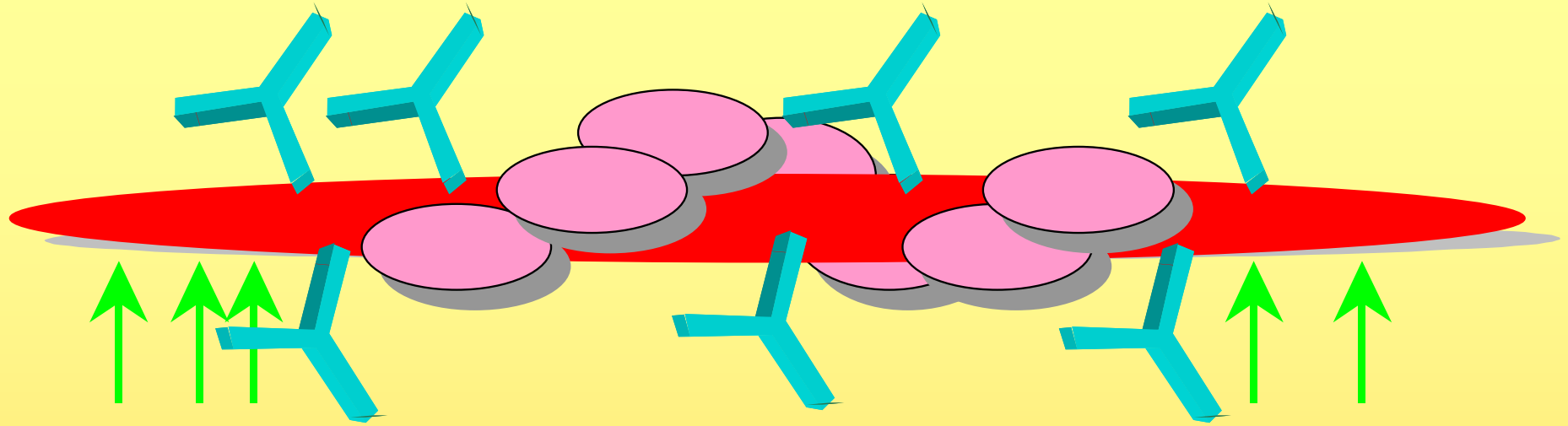




**Troponin I**

**Troponin C**

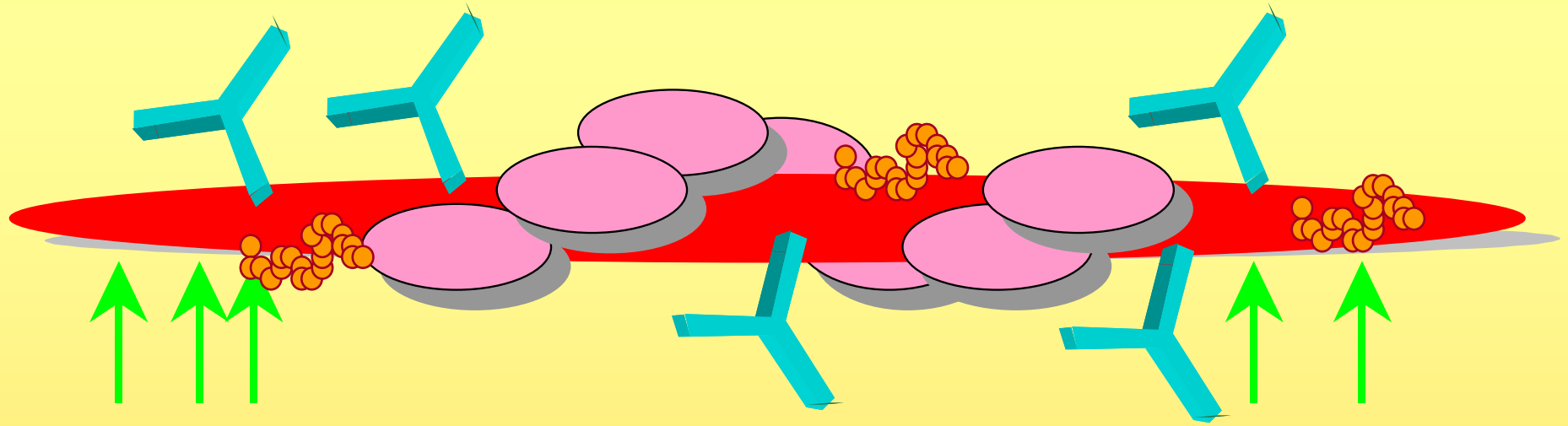




**Troponin I**

**Troponin C**

**Sites of proteolytic degradation**

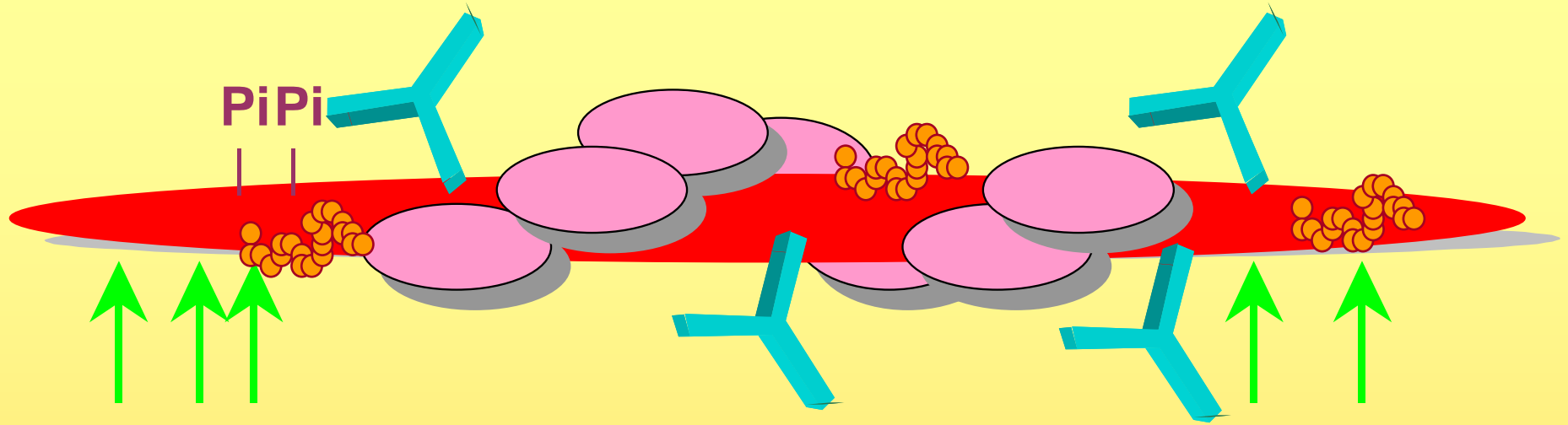


**Troponin I**

**Troponin C**

**Sites of proteolytic degradation**

**Heparin**



**Troponin I**

**Troponin C**

**Sites of proteolytic degradation**

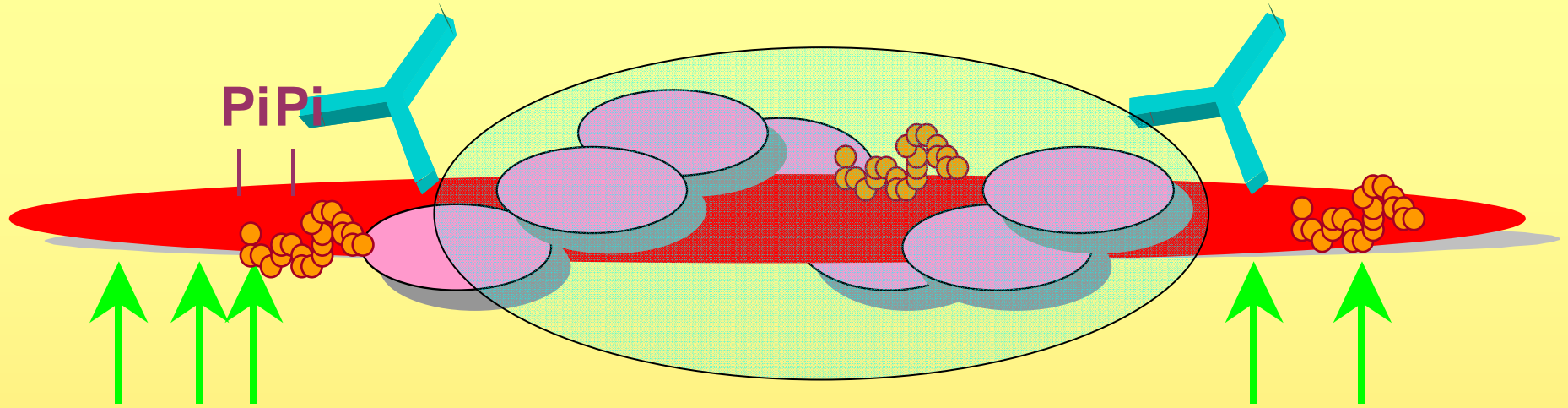
**Heparin**

**Phosphorylation**



**PiPi**





**Troponin I**



**Troponin C**



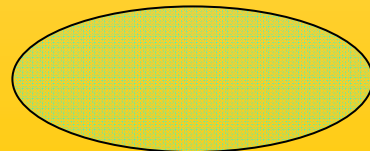
**Sites of proteolytic degradation**



**Heparin**

**PiPi**

**Phosphorylation**



**Autoantibodies**

# IFCC site

[http://www.ifcc.org/PDF/ScientificActivities/IFCC\\_Troponin\\_Table\\_vDec\\_2010\\_FINAL\\_ng\\_L\\_28Jan11.pdf](http://www.ifcc.org/PDF/ScientificActivities/IFCC_Troponin_Table_vDec_2010_FINAL_ng_L_28Jan11.pdf)

## Epitopes of antibodies utilized in cTnI assays

Table 1. Analytical characteristics of commercial and research cardiac troponin I and T assays declared by the manufacturer.

Commercially available assays - Company/ platform(s)/ assay	LoB* (ng/L)	LoD* (ng/L)	99 <sup>th</sup> % (ng/L)	%CV at 99 <sup>th</sup> %	10% CV (ng/L)	Risk Stratification	Epitopes recognised by Antibodies	Detection Antibody Tag
Abbott AxSYM ADV	20		40	14.0	160	Yes	C: 87-91, 41-49; D: 24-40	ALP
Abbott ARCHITECT	<10		28	14.0	32	Yes (No in US)	C: 87-91, 24-40; D: 41-49	Acridinium
Abbott i-STAT	20		80	16.5	100	Yes	C: 41-49, 88-91; D: 28-39, 62-78	ALP
Alere Triage SOB	50		NAD	NA	NA	No	C: NA; D: 27-40	Fluorophor
Alere Triage Cardio 3 (r)	10		NAD	17.0 (at 20)	NA	No	NA	Fluorophor
Beckman Coulter Access Accu	10		40	14.0	60	Yes	C: 41-49; D: 24-40	ALP
bioMerieux Vidas Ultra	10		10**	27.7	110	No	C: 41-49, 22-29; D: 87-91, 7B9	ALP
Mitsubishi Chemical PATHFAST	8		29	5.0	14	No	C: 41-49; D: 71-116, 163-209	ALP
Ortho VITROS Troponin I ES	7	12	34	10.0	34	Yes	C: 24-40, 41-49; D: 87-91	HRP
Radiometer AQT90 FLEX TnI		9.5	23	17.7	39	NA	C: 41-49, 190-196; D: 137-149	Europium
Radiometer AQT90 FLEX TnT		10	17	15.2	25	NA	C: 125-131; D: 136-147	Europium
Response Biomedical RAMP	30		NAD	18.5 (at 50)	210	No	C: 85-92; D: 26-38	Fluorophor
Roche Cardiac Reader cTnT	<50		NAD	NA	NA	No	C: 125-131; D: 136-147	Gold particles
Roche E 2010 /cobas e 411 / E 170 / cobas e 601 / 602 TnT (4 <sup>th</sup> gen)	10		NAD	NA	30	Yes	C: 125-131; D: 136-147	Ruthenium
Roche E 2010/cobas e 411 / E 170 / cobas e 601 / 602 hs-TnT		5	14	10.0	13	NA	C: 125-131; D: 136-147	Ruthenium
Roche E 2010/cobas e 411 / Roche E 170/cobas e 601 / 602 cTnI		160	160**	NA	300	No	C: 87-91, 190-196; D: 23-29, 27-43	Ruthenium
Siemens Centaur Ultra	6		40	8.8	30	Yes	C: 41-49, 87-91; D: 27-40	Acridinium
Siemens Dimension RxL	40		70	20.0	140	Yes	C: 27-32; D: 41-56	ALP
Siemens Dimension EXL	17		56	10.0	50	Yes	C: 27-32; D: 41-56	Chemiluminescence
Siemens Immulite 2500 STAT	100		200	NA	420	No	C: 87-91; D: 27-40	ALP
Siemens Immulite 1000 Turbo	150		NA	NA	640	No	C: 87-91; D: 27-40	ALP
Siemens Stratus CS	30		70	10.0	60	Yes	C: 27-32; D: 41-56	ALP
Siemens VISTA	15		45	10.0	40	Yes	C: 27-32; D: 41-56	Chemiluminescence
Tosoh ST AIA-PACK	60		60**	8.5	NA	No	C: 41-49; D: 87-91	ALP



# Epitopes of antibodies utilized in cTnI assays

Table 1. Analytical characteristics of commercial and research cardiac troponin I and T assays declared by the manufacturer.

Commercially available assays - Company/ platform(s)/ assay	LoE <sup>a</sup> (ng/L)	LoD <sup>a</sup> (ng/L)	99 <sup>th</sup> % (ng/L)	%CV at 99 <sup>th</sup> %	10% CV (ng/L)	Risk Stratification	Epitopes recognised by Antibodies	Detection Antibody Tag
Abbott AxSYM ADV	20		40	14.0	160	Yes	C: 87-91, 41-49; D: 24-40	ALP
Abbott ARCHITECT	<10		28	14.0	32	Yes (No in US)	C: 87-91, 24-40; D: 41-49	Acridinium
Abbott i-STAT	20		80	16.5	100	Yes	C: 41-49, 88-91; D: 28-39, 62-78	ALP
Alere Triage SOB	50		NAD	NA	NA	No	C: NA, D: 27-40	Fluorophor
Alere Triage Cardio 3 (r)	10		NAD	17.0 (at 20)	NA	No	NA	Fluorophor
Beckman Coulter Access Accu	10		40	14.0	60	Yes	C: 41-49; D: 24-40	ALP
bioMérieux VIDAS Ultra	10		10**	27.7	110	No	C: 41-49, 22-29; D: 87-91, 7B9	ALP
Mitsubishi Chemical PATHFAST	8		29	5.0	14	No	C: 41-49; D: 71-116, 163-209	ALP
Ortho VITROS Troponin I ES	7	12	34	10.0	34	Yes	C: 24-40, 41-49; D: 87-91	HRP
Radometer AQT90 FLEX TnI		9.5	23	17.7	39	NA	C: 41-49, 190-196; D: 137-149	Europium
Radometer AQT90 FLEX TnT		10	17	15.2	25	NA	C: 125-131; D: 136-147	Europium
Response Biomedical RAMP	30		NAD	18.5 (at 50)	210	No	C: 85-92; D: 26-38	Fluorophor
Roche Cardiac Reader cTnT	<50		NAD	NA	NA	No	C: 125-131; D: 136-147	Gold particles
Roche E 2010/cobas e 411 / E 170 / cobas e 601 / 602 TnT (4 <sup>th</sup> gen)	10		NAD	NA	30	Yes	C: 125-131; D: 136-147	Ruthenium
Roche E 2010/cobas e 411 / E 170 / cobas e 601 / 602 hs-TnT		5	14	10.0	13	NA	C: 125-131; D: 136-147	Ruthenium
Roche E 2010/cobas e 411 / Roche E 170/cobas e 601 / 602 cTnI		160	160**	NA	300	No	C: 87-91, 190-196; D: 23-29, 27-43	Ruthenium
Siemens Centaur Ultra	6		40	8.8	30	Yes	C: 41-49, 87-91; D: 27-40	Acridinium
Siemens Dimension RxL	40		70	20.0	140	Yes	C: 27-32; D: 41-56	ALP
Siemens Dimension EXL	17		56	10.0	50	Yes	C: 27-32; D: 41-56	Chemiluminescence
Siemens Immulite 2500 STAT	100		200	NA	420	No	C: 87-91; D: 27-40	ALP
Siemens Immulite 1000 Turbo	150		NA	NA	640	No	C: 87-91; D: 27-40	ALP
Siemens Stratus CS	30		70	10.0	60	Yes	C: 27-32; D: 41-56	ALP
Siemens VISTA	15		45	10.0	40	Yes	C: 27-32; D: 41-56	Chemiluminescence
Tecoh ST AIA-PACE	60		60**	8.5	NA	No	C: 41-49; D: 87-91	ALP

