LEIDEN UNIVERSITY MEDICAL CENTER

Yin and yang of current troponin assays

- analytical issues

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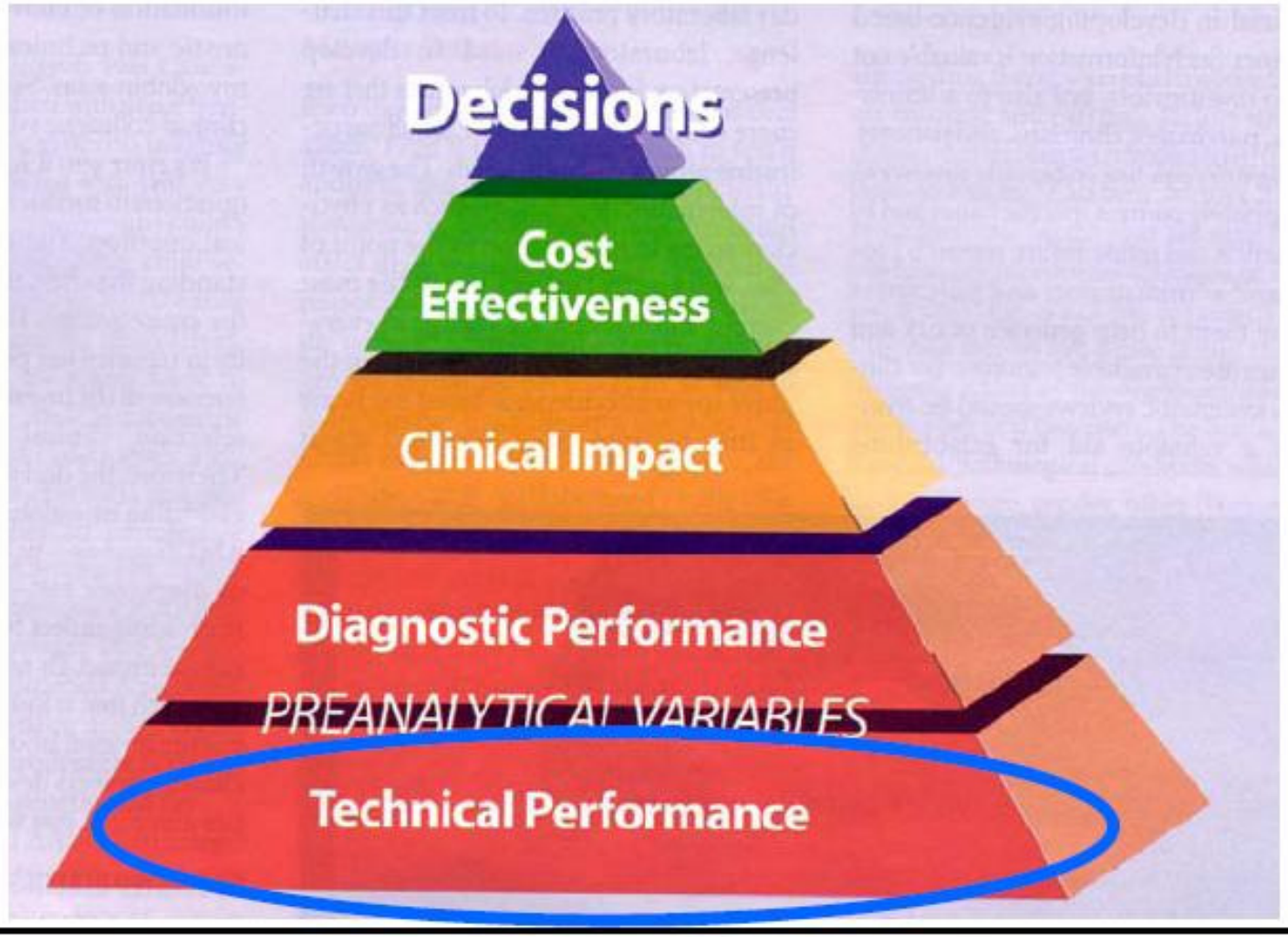
SKML sectie AC gebruikersdag
3 juni 2010

Hierarchy of Evidence-Based Medicine



Inhoud

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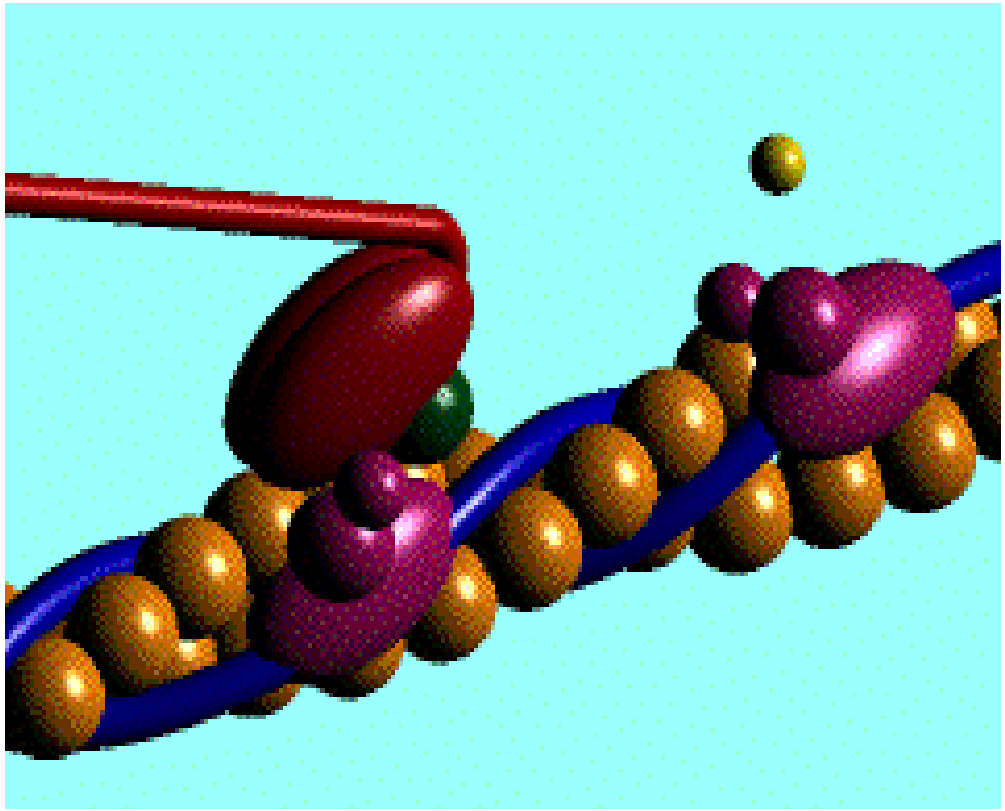
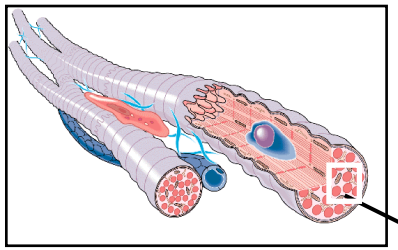
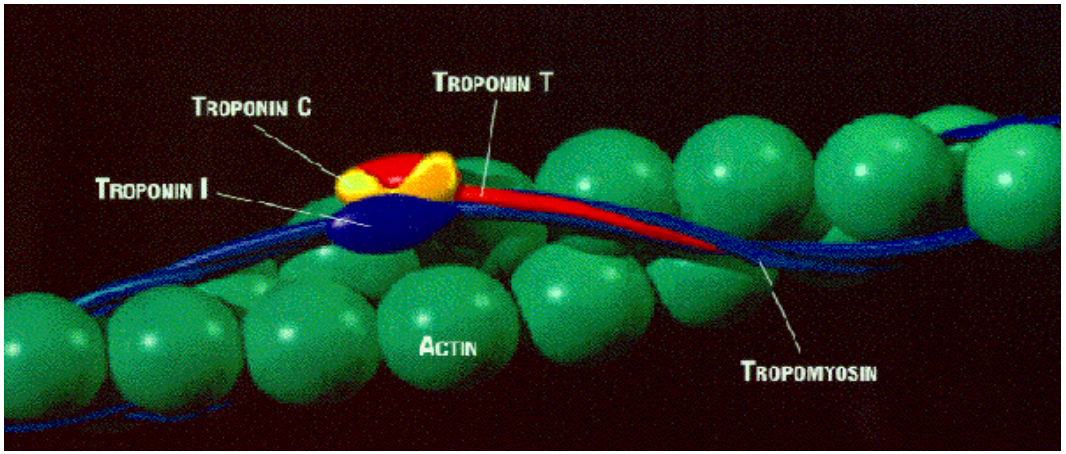


I. Introduction - cTroponin complex (Tn)



Myocardium Fiber



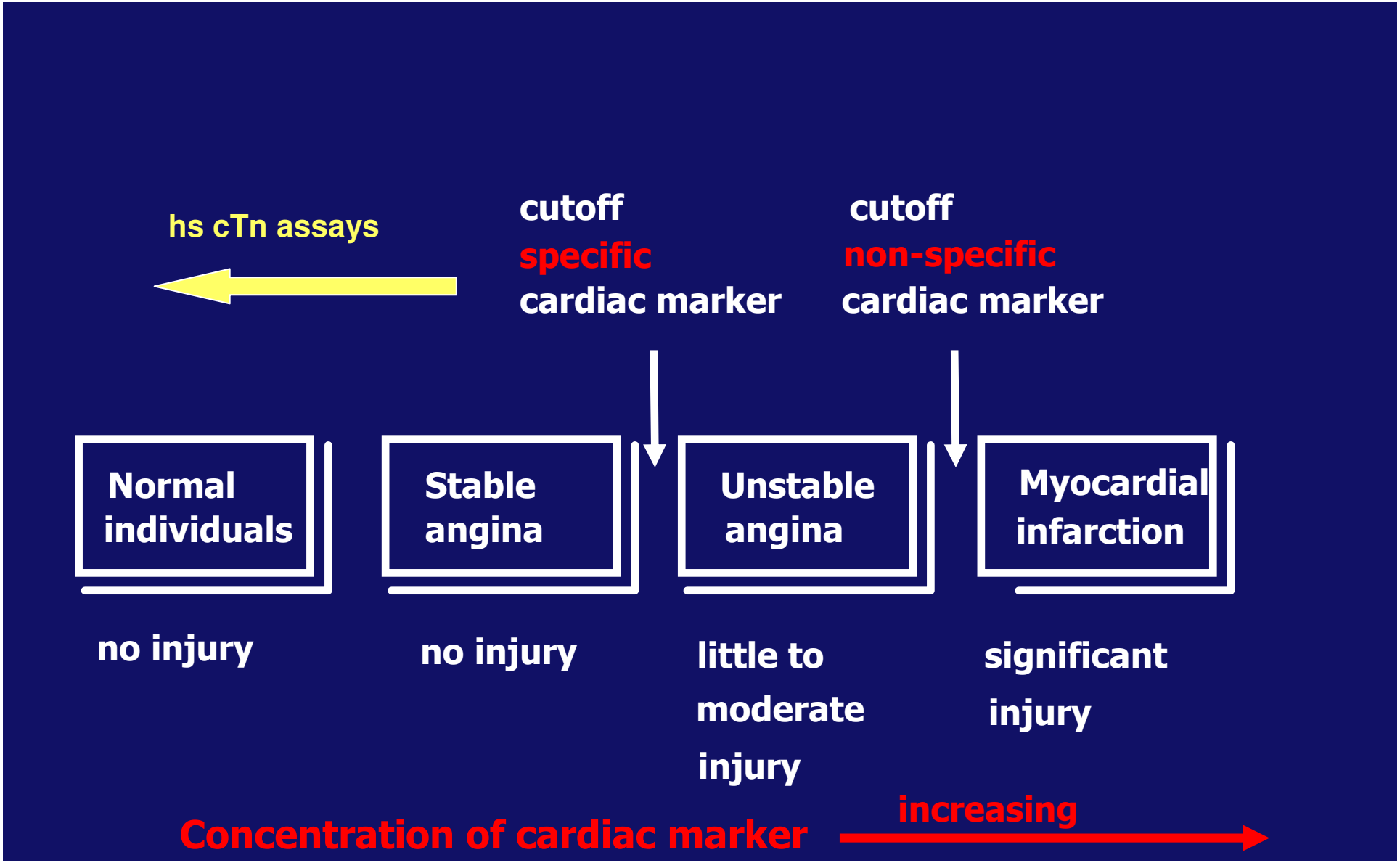
- Globular protein complex present in the thin myofilaments, involved in regulation of muscle contraction
- Different isotypes present in skeletal and cardiac muscle:

➢ Troponin C binds Calcium - *Identical in heart and skeletal muscle*

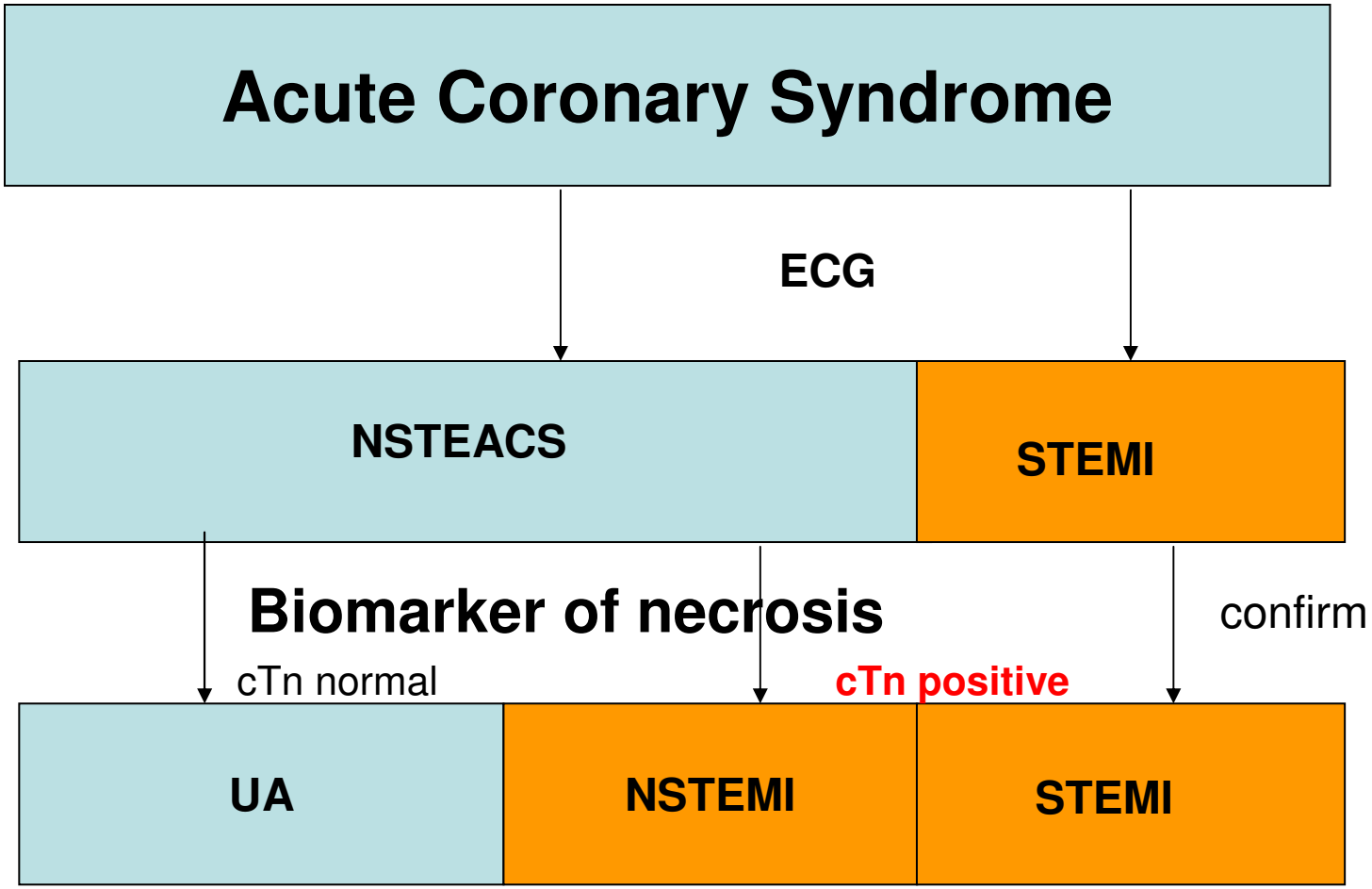
➢ Troponin I in absence of Ca++ binds to actin, inhibits actin-myosin ATPase induced contraction - *cardiac specific isoforms*

➢ Troponin T links troponin complex to tropomyosin, facilitates contraction - *cardiac specific isoforms*

Lower cutoffs with cardiac troponin



cTn allows better categorization and Tx of ACS pts



cTn & interpretation

- Increases of cTns are **INDICATIVE** of myocardial injury but do not identify the mechanism of injury. If an ischemic mechanism of injury is unlikely, other etiologies of myocardial injury should be pursued.

- The degree of the increase of cTns in ischemia-induced injury patients is related to the patient's prognosis.

Causes of Elevated Troponin in clinical settings other than ACS or PCI

Ischaemic causes other than plaque fissuring or rupture

- Coronary embolism (red cell or platelet thrombi, vegetation, atrial myxoma, calcification)
- Coronary spasm
- Coronary dissection
- Aortic dissection
- Transplant vasculopathy

Cardiac surgery

- Left ventricular venting
- Inadequate cardioplegia
- Traumatic atrial cannulation
- Manipulation of the heart
- Ischaemia related causes such as conduit or native vessel occlusion

Miscellaneous

- Tachyarrhythmia
- Hypertension
- Congestive heart failure
- Renal failure
- Drug toxicity (e.g. adriamycin, 5-fluorouracil, etc)
- Hypothyroidism
- Pulmonary embolism with right ventricular infarction
- Sepsis (including sepsis occurring with shock)
- Transient ischaemic attack, stroke or subarachnoid haemorrhage
- Pheochromocytoma
- Rhabdomyolysis with myocyte necrosis

Myopericarditis

- Rheumatic fever
- Rheumatoid arthritis
- Systemic vasculitis
- Post-viral

Infiltrative diseases of the myocardium

- Amyloidosis
- Sarcoidosis

Traumatic

- Atrioventricular ablation
- Defibrillation
- Chest wall trauma

Improved clinical performance with hs cTn assays

- **↑ clinical sensitivity for AMI diagnosis**
  - Rise and fall pattern should be typical
  - Timing of blood specimens: from 6-9 hrs after presentation → 3 hr
  - Early clinical sensitivity: > 90% at 3 hr!
  - Lowered specificity

- **↑ clinical sensitivity for risk stratification of adverse cardiac events**

- Higher % of non-ACS pts with abnormal cTn results!
- Higher % of chronic elevations (e.g. CRF)!

II. Guidelines for Acute Coronary Syndromes and re-definition of Myocardial Infarction

Guidelines with analytical focus

- NACB/IFCC working group
  1. Wu et al. Clin Chem. 45:1104-21, 1999;
  2. Morrow et al Clin Chem 53:552-74, 2007

Guidelines with clinical focus

- Joint ESC/ACC/AHA/WHF Task Force
  1. Alpert et al. JACC 36:959-69, 2000;
  2. Thygesen et al JACC 50:2173-95, 2007

European Heart Journal (2007) 28, 2525–2538
doi:10.1093/eurheartj/ehm355

EUROPEAN SOCIETY OF CARDIOLOGY®

Expert consensus document

Redefinition of Myocardial Infarction

Criteria for acute MI

The term MI should be used when there is evidence of myocardial necrosis in a clinical setting consistent with myocardial ischemia. Under these conditions any one of the following criteria meets the diagnosis for myocardial infarction:

- Detection of **a rise and/or fall of cardiac biomarkers** (preferably cTn) with at least **one value above the 99th percentile** together **with evidence of myocardial ischemia** with at least one of the following:
  - ✓ Symptoms of ischemia
  - ✓ ECG changes indicative of new ischemia (new ST-T changes or new left bundle branch block (LBBB))
  - ✓ Development of pathologic Q waves in the ECG
  - ✓ Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality

- Timing is essential: sampling at admission, 6-9h, 12-24h

Thygessen K et al *EHJ* 2007; 28, 2525-33. *JACC,* 502173-95.
Alpert J et al. The Joint ESC/ACC committee, *EHJ* 2000; 21:1502-1513. *JACC* 2000; 36: 959-969.

NACB Clinical Guidelines for ACS

2007 Clin Chem and Circulation

Class I Recommendation

In the presence of a clinical history suggestive of ACS, the following are considered indicative of myocardial necrosis consistent with MI (Level of Evidence: C):

- **Maximal concentration of cardiac troponin exceeding the 99th percentile of values (with optimal precision defined by total CV≤10%) for a reference control group on at least one occasion during the first 24 hours after the clinical event (Observation of a rise and fall in values is useful in discriminating the timing of injury).**

NACB Analytical Guidelines for ACS

2007 Clin Chem and Circulation

Class I (Level of Evidence C)

Identification of antibody/epitope recognition sites for each biomarker.

Assays for cardiac biomarkers should strive for a total imprecision (%CV) of ≤10% at the 99th percentile reference limit.

Cardiac biomarker assays must be characterized with respect to potential interferences, including rheumatoid factors, human anti-mouse antibodies, and heterophile antibodies.

Stability (over time and across temperature ranges) for each acceptable specimen type

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Assay specificity (interferences)

- Heterophile antibodies
  - Natural autoimmune rheumatoid factors
  - Incidence: 0.02%
  - False positive if binding to Fc constant domain of Ag-Ab complexes
  - False low if binding to variable regions of the capture antibody
- HAAA
  - Most commonly HAMA
  - Compete with cTn by cross-reacting with reagent antibodies of the same species → false high
- Autoantibodies
  - Incidence of falsely negative cTn: 3.5%
  - Major effect when cTn concentration is low
  - Incidence of interference will increase in high sensitive cTn assays

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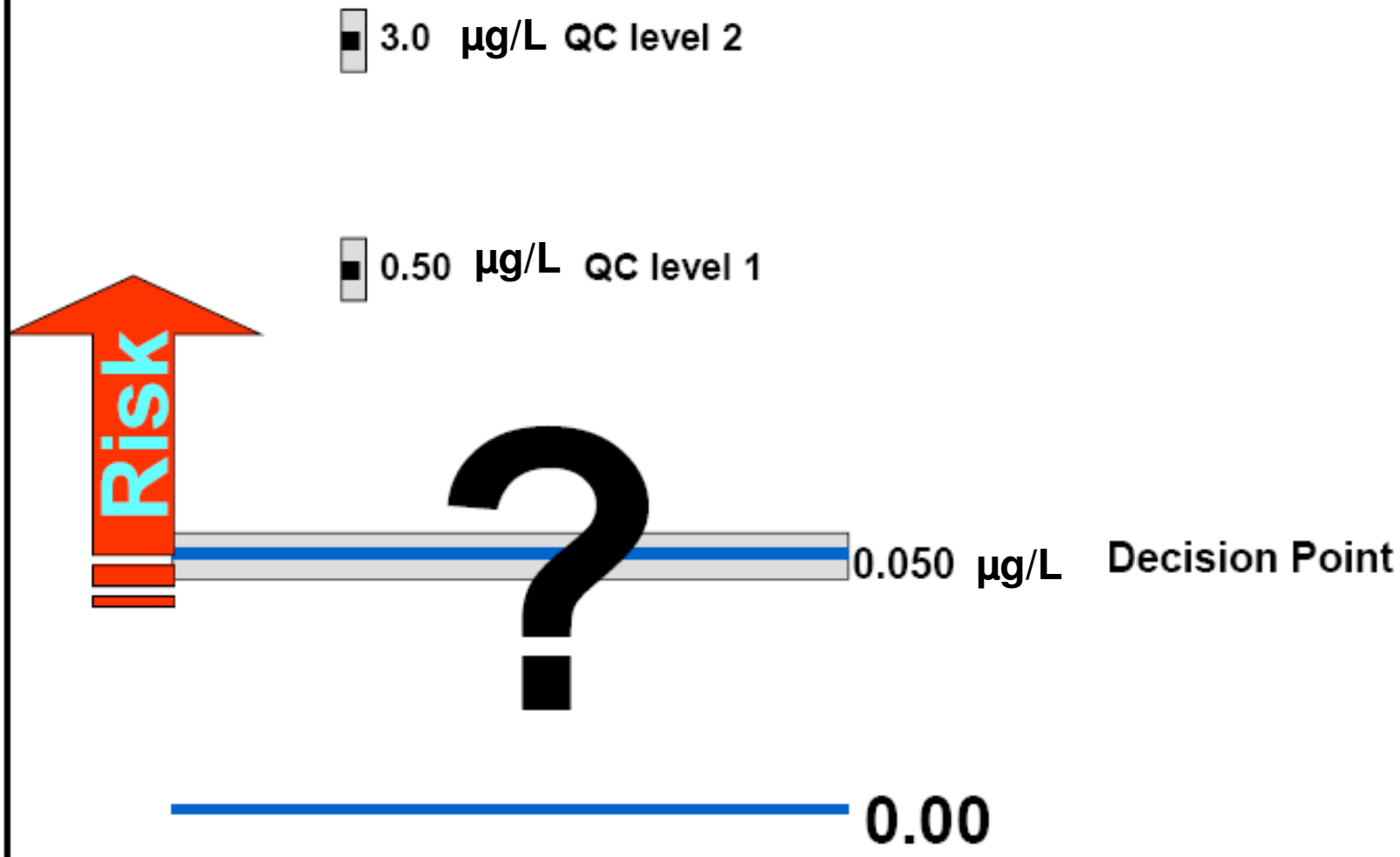
Where Should Quality Control Be?



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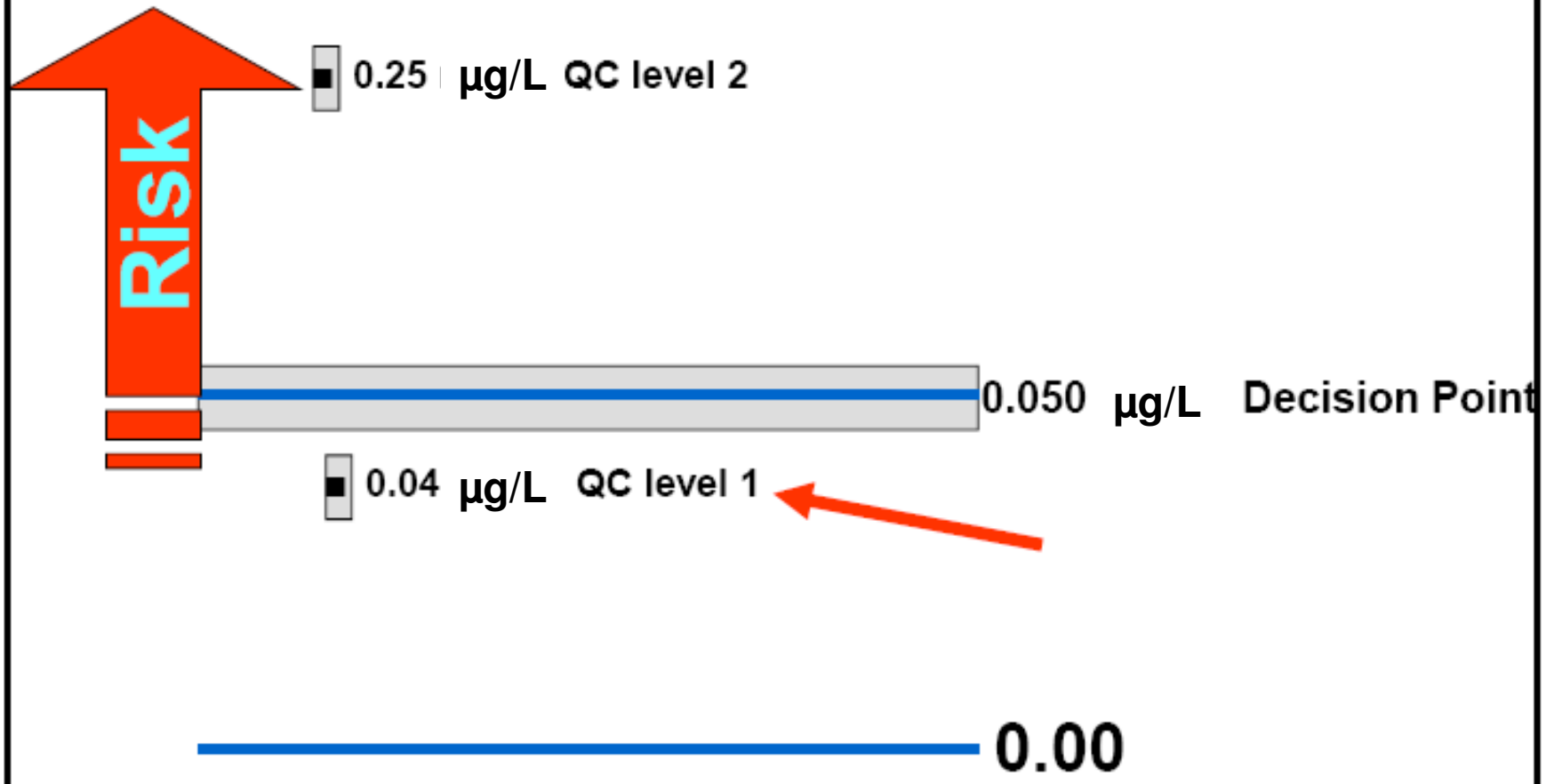
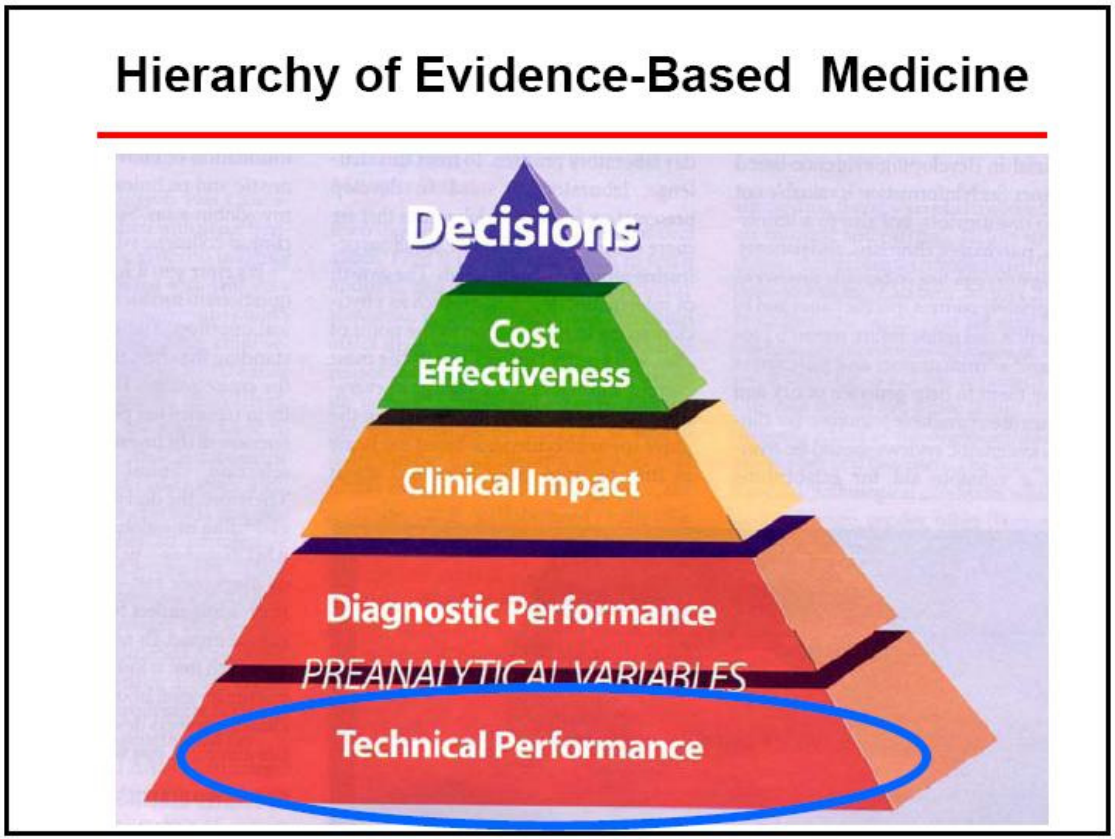


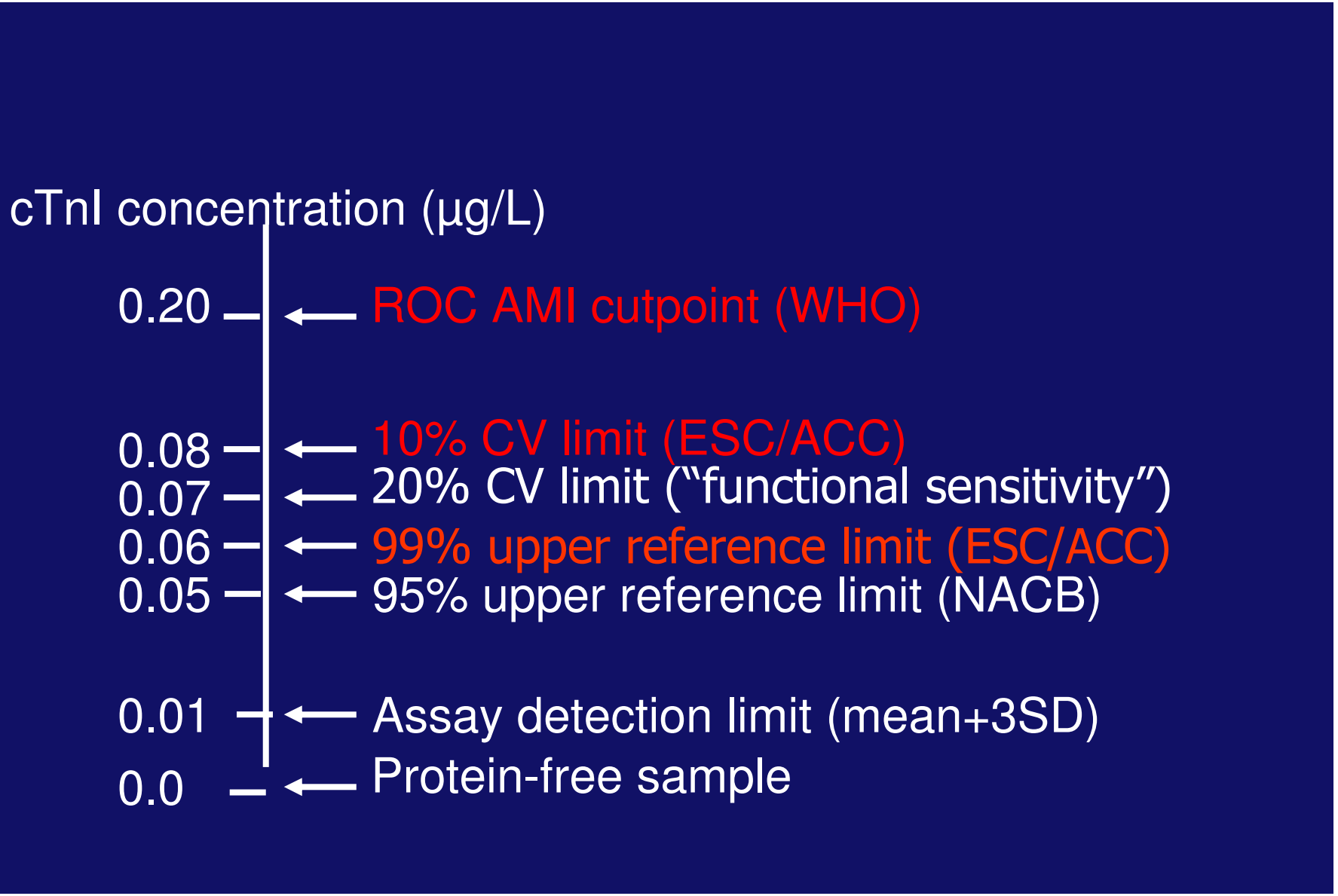
III. Methodological consequences of the redefinition of MI?

- cTn assays need to be **more sensitive at the low end** → lower cutoff points

- cTn assays need to be **more precise**



History of cTn Cutoffs



Cutoffs for cardiac markers

- ROC cutpoint: separating data from patients with unstable angina vs. confirmed AMI.
- 99th percentile with acceptable precision (≤10%): established by ESC/ACC in 2000/2007 redefinition of AMI.
- 10% CV surrogate cutoff in absence of assays with acceptable sensitivity to determine 99th percentile.

IFCC website for troponin assay characteristics

Analytical characteristics of commercial and research high sensitivity cardiac troponin I and T assays per manufacturer.

Compan y/platform/assay	LoD µg/L	99th % µg/L	%CV 99th %	10% CV µg/L	Risk Stratification*	Epitopes recognized by antibodies	Detection Antibody Tag
Abbott AxSYM ADV	0.02	0.04	15.0	0.16	Yes	C 87-91, 41-49; D 24-40	ALP
Abbott ARCHITECT	<0.01	0.028	15.0	0.032	No	C 87-91, 24-40: D: 41-49	Acridinium
Abbott i-STAT	0.02	0.08*	16.5	0.10	Yes	C: 41-49, 88-91; D: 28-39,62-78	ALP
Beckman Coulter Access Accu	0.01	0.04	14.0	0.06	Yes	C; 41-49; D: 24-40.	ALP
bioMerieux Vidas Ultra	0.01	0.01	27.7	0.11	No	C: 41-49, 22-29; D: 87-91, 7B9	ALP
Inverness Biosite Triage	0.05	<0.05	NA	NA	No	C: NA; D: 27-40	Fluorophor
Inverness Biosite Triage (r)	0.01	0.056	17.0	NA	No	NA	Fluorophor
Mitsubishi Chemical PATHFAST	0.008	0.029	5.0	0014	No	C: 41-49; D:71-116, 163-209	ALP
Ortho Vitros ECi ES	0.012	0.034	10.0	0.034	Yes	C 24-40, 41-49; D 87-91	HRP
Radiometer AQT90	0.0095	0.023	17.7	0.039	NA	C; 41-49, 190-196; D: 137-149	Europium
Response Biomedical RAMP	0.03	<0.1	18.5	0.21	No	C: 85-92; D: 26-38	Flourophor
Roche E170	0.01	<0.01	18.0	0.03	Yes	C: 125-131; D: 136-147	Ruthenium
Roche Elecsys 2010	0.01	<0.01	18.0	0.030	Yes	C: 125-131; D: 136-147	Ruthenium
Roche Cardiac Reader	<0.05	<0.05	NA	NA	NO	C: 125 – 131; D:136-147	Gold particles
Siemens Centaur Ultra	0.006	0.04	10.0	0.03	Yes	C; 41-49, 87-91; D: 27-40	Acridinium
Siemens Dimension RxL	0.04	0.07	20.0	0.14	Yes	C: 27-32; D: 41-56	ALP
Siemens Immulite 2500 STAT	0.1	0.2	NA	0.42	No	C: 87-91:D: 27-40	ALP
Siemens Immulite 1000 Turbo	0.15	NA	NA	0.64	No	C: 87-91:D: 27-40	ALP
Siemens Stratus CS	0.03	0.07	10.0	0.06	Yes	C: 27-32; D: 41-56	ALP
Siemens VISTA	0.015	0.045	10.0	0.04	Yes	C: 27-32; D: 41-56	Chemiluminescent
Tosoh AIA II	0.06	<0.06	8.5	0.09	No	C: 41-49; D: 87-91	ALP
Research High Sensitive Assays							
Beckman Coulter Access hs-cTnI	0.0020	0.0086	10.0	0.0086	NA	C; 41-49; D: 24-40	ALP
Roche Elecsys hs-cTnT	0.001	0.013	8.0	0.012	NA	C: 125-131; D: 136-147	Ruthenium
Nanosphere hs-cTnI	0.0002	0.0028	9.5	0.0005	NA	C: 136-147; D: 49-52,70-73,88,169	Gold-nanoparticles
Singulex hs-cTnI	0.00009	0.0101	9.0	0.00088	NA	C: 41-49 ; D: 27-41	Capillary flow fluorescence

Version updated September 12, 2009; LoD = limit of detection; 99th % = 99th percentile concentration; 10% CV = lowest concentration that has been shown to have a 10% CV (total imprecision); risk stratification claim per FDA; Epitopes were supplied by manufacturers; (r) = revised assay submitted to FDA per Inverness; hs = high sensitivity designation per manufacturers.

Guideline acceptable troponin assays

Method	99th P µg/L	10% CV µg/L	10%CV/ 99th P ratio
Troponin I			
Mitsubishi PATHFAST	0.029	0.014	0.48
ORTHO Vitros	0.034	0.034	1.00
Siemens Centaur	0.04	0.03	0.75
Siemens stratus	0.07	0.06	0.86
Siemens Vista	0.045	0.04	0.89
Beckman Access	0.0086	0.0086	1.00
Nanosphere	0.0028	0.0005	0.170
Singulex	0.0101	0.00088	0.087
Troponin T			
Roche hs TnT	0.013	0.012	0.92

Apple Clin Chem 55:7 1303-6, 2009

IV. Know your assay!

Praktijkvoorbeeld

- **Validatie van de hs cTnT assay in het LUMC**

Troponin T Test Evolution

1st generation (1989)	ELISA Troponin T	1 cardio-specific monocl. Ab
2nd generation (1993)	Enzymun Troponin T Elecsys Troponin T	**2 cardio-specific monocl. Ab** **cal: bovine cTnT**
3rd generation (1996)	**Elecsys Troponin T**	**2 cardio-specific monocl. Ab** **cal: human rec. cTnT**
4th generation (2005)	**Elecsys Troponin T**	**No interferences with heparin**
5th generation (2009)	**High sensitive Elecsys cardiac Troponin T**	**Min. detectable conc: 0.003 µg/L *** **99th percentile: 0.014 µg/L *** **10% CV: 0.014 µg/L ***

*** ng/mL = µg/L**

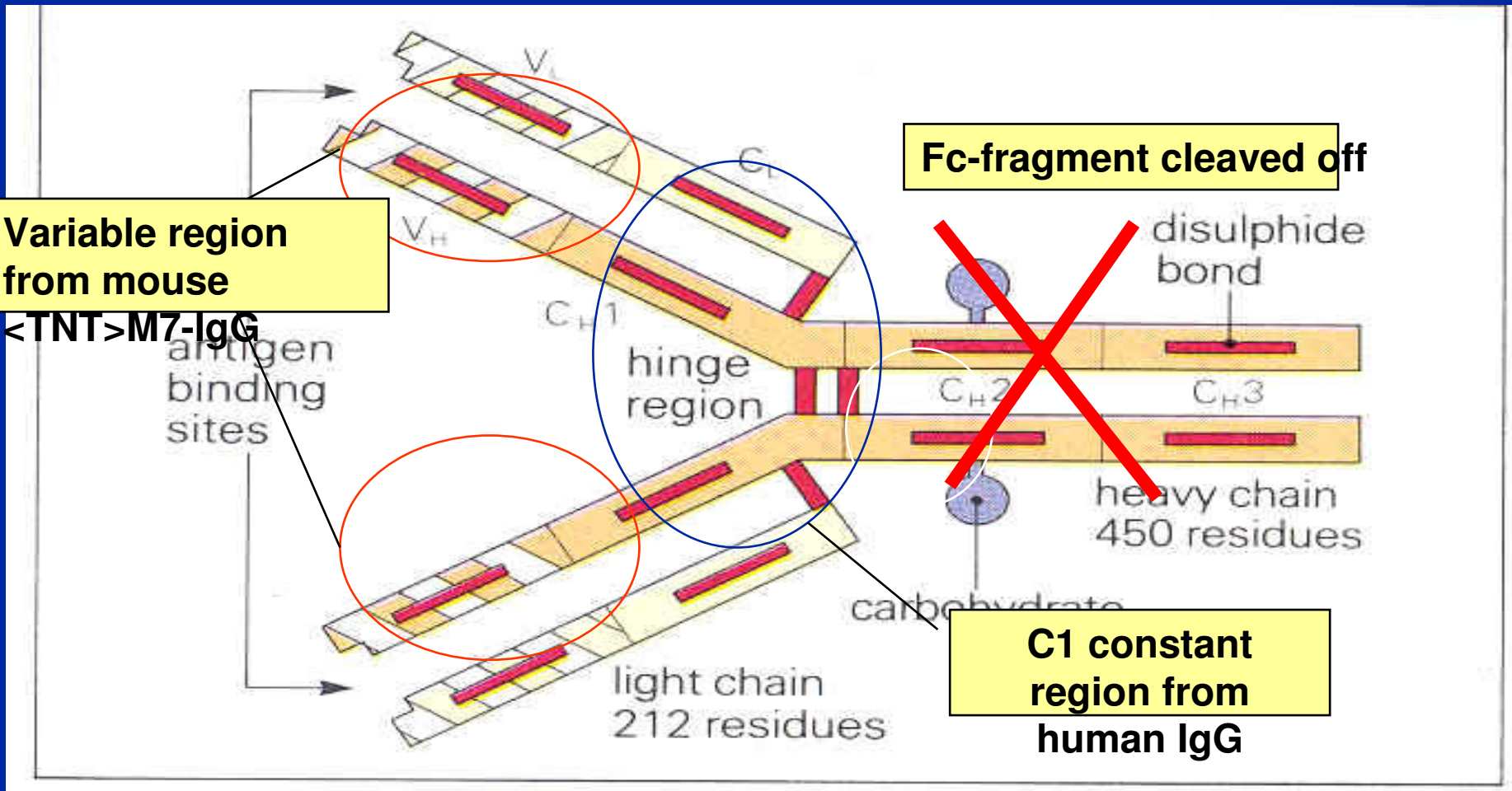
Development of a high sensitive TnT assay

Strategy:

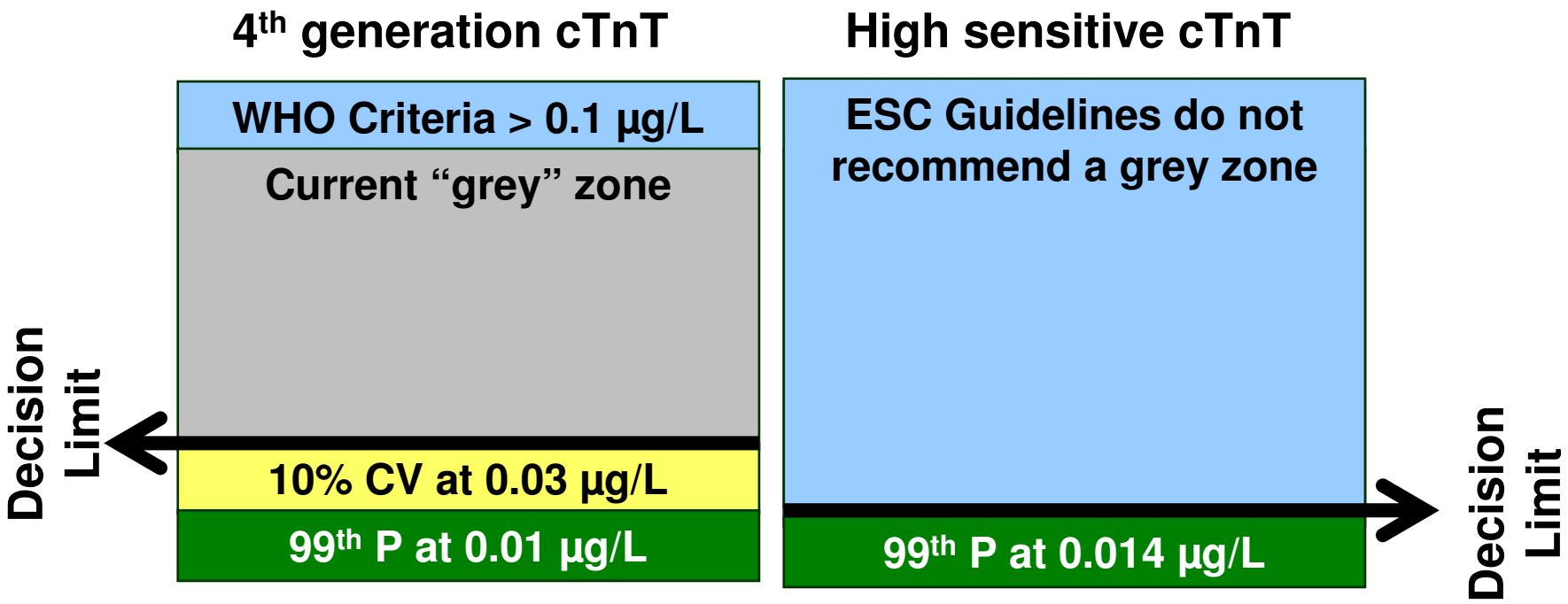
1. Increase of sample volume from 15 µl to 50 µl
2. Signal amplification by use of highly optimized antibody-Ru conjugates
3. Increase of signal-to-noise ratio by lowering of background signal
4. Use of same antibodies as in 4th gen Troponin T assay

Chimeric anti Troponin T Antibodies

constructed from IgG from 2 different species (mouse / human)



cTnT & the challenge about thresholds



Higher sensitivity, new guidelines means many more "false positives" !?

View from an Expert on clinical implications

Clinical implications of 99th P value as a cut-off for AMI

Dr Lefevre:

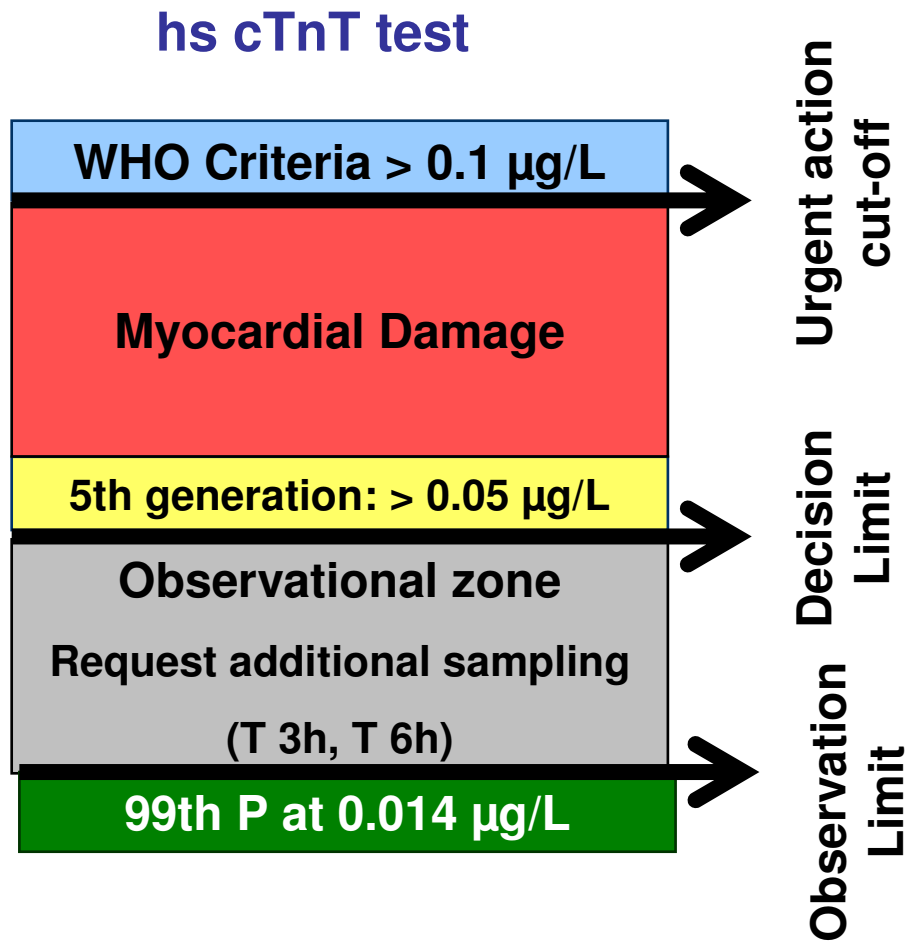
"*With the 99th P cut-off value, cTn tests are not used anymore for their diagnostic value but rather for their prognostic value. This is why the 99th P cut-off value doesn't meet the needs of ER & interventional cardiologists.*

The initial IFCC objective was to force the manufacturers to standardize test accuracy and sensitivity, and not to define an ACS cut-off.

The side effect of an increased sensitivity has always been a specificity decrease. Clinicians should know this".

LUMC nieuwsbrief en beslisgrenzen

Voorstel: rekening houden met pos. bias in lage gebied; cutpoint evenredig verhogen!



V. SKML data hartmerkers anno 2009/2010

I. cTnI/T Combi data; cTnI histogram en interlab CVs

II. Klinische effectiviteit vigerende cTn assays: is de klinische interpretatie identiek?

Hartmerker rondzending vlg Combi Nieuwe Stijl

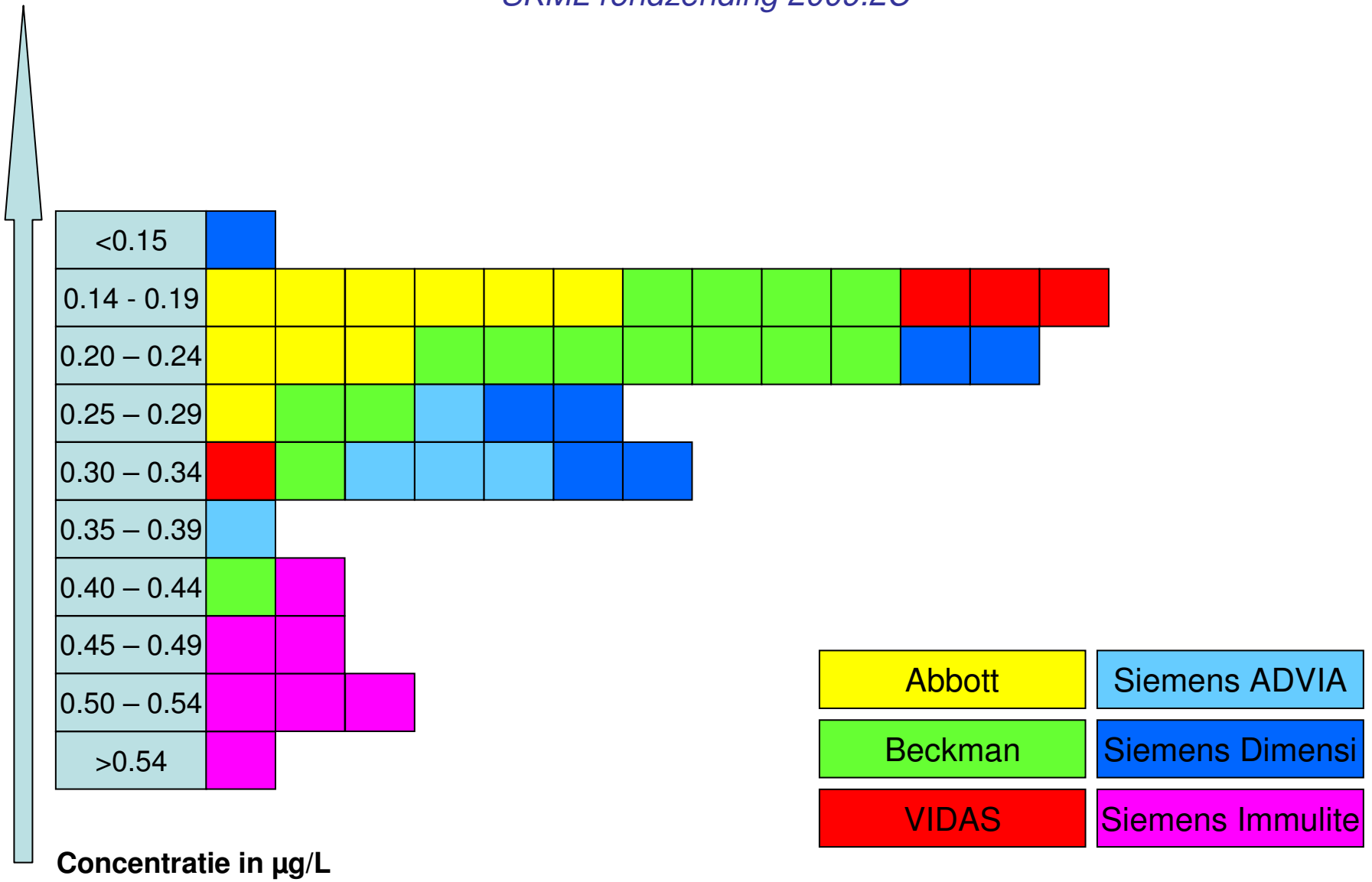
cTroponine Combi 2010.1:
gemiddelde van de meetbare resultaten (in ug/L)

Methode	n	A	B*	E	D	F	C
Abbott Architect	8	0.017	0.051	0.211	0.312	0.608	1.234
Beckman Access DxI	11	0.029	0.070	0.250	0.360	0.755	1.388
Beckman UniCel DxC	8	0.017	0.059	0.254	0.362	0.770	1.430
Siemens ADVIA	4	0.027	0.095	0.382	0.555	1.192	2.403
Siemens Dimension RxL	4	<0.020	0.072	0.250	0.357	0.755	1.260
Siemens Dimension Vista	7	0.010	0.077	0.423	0.539	1.142	2.034
Siemens DPC Immulite	5	<0.20	0.210	0.606	0.830	1.837	3.030
BioMerieux VIDAS	5	0.030	0.017	0.264	0.344	0.680	0.825
Totaal cTnI	52						
Roche Conventioneel	75	0.010	0.012	0.046	0.067	0.138	0.231
Hs-cTnT	4	0.008	0.019	0.065	0.086	0.160	0.250
Totaal cTnT	79						
Doelstelling cTnT		0.010	0.015	0.050	0.070	0.140	0.240

* Monster met borderline cTroponine dat doorloopt in 2011 en 2012

Landelijke cTroponine I data

SKML rondzending 2009.2C

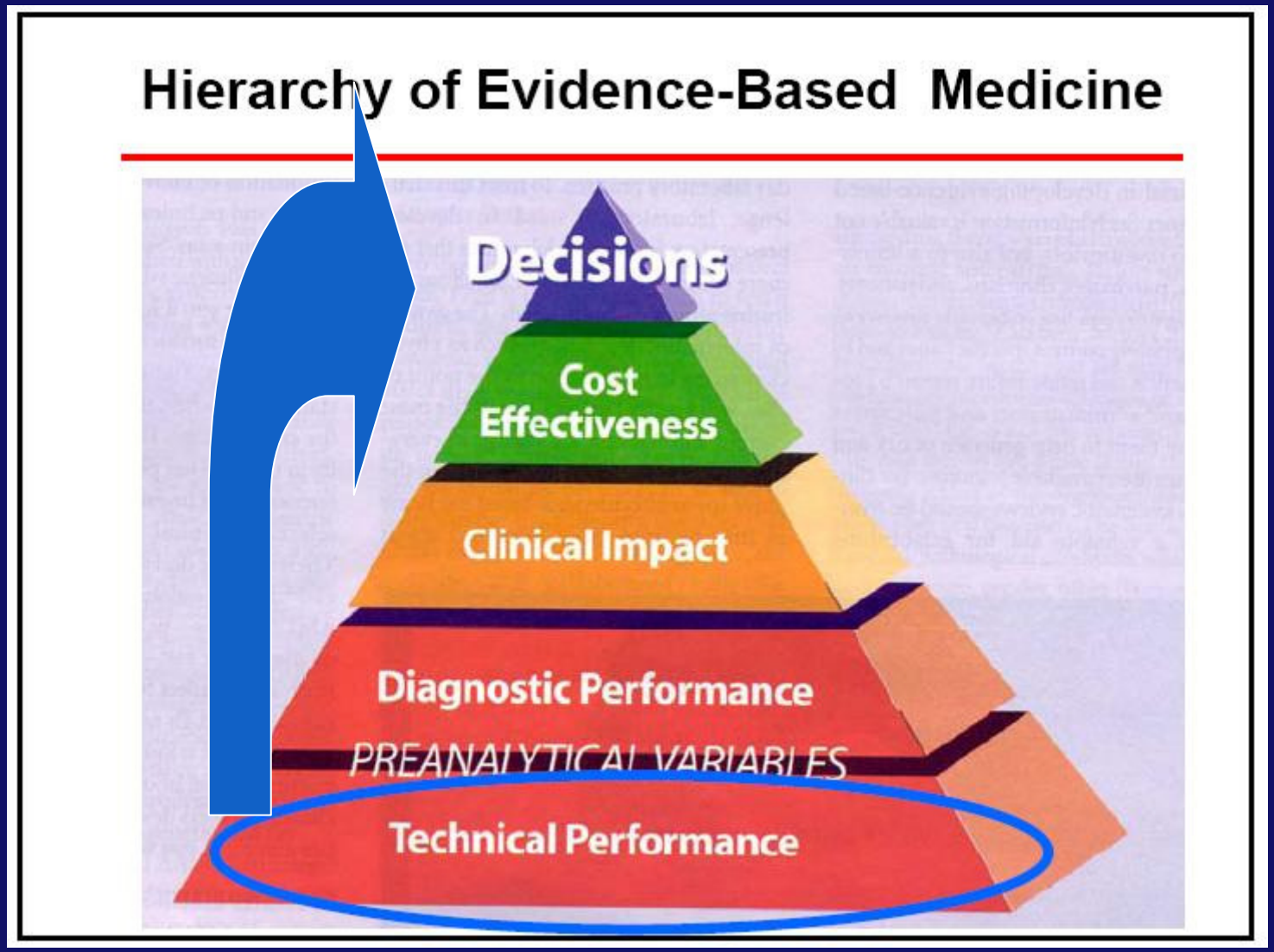


cTroponine I methodegroepen in Nederland

- interlaboratorium VC in monster 2009.3C

Methode	N	Gemiddelde (µg/L)	Interlab CV (%)
• Abbott Architect	10	0.19	17%
• Beckman	15	0.23	26%
• VIDAS	4	0.22	28%
• Siemens ADVIA	5	0.32	10%
• Siemens Dimension	8	0.25	26%
• Siemens Immulite	7	0.49	9%

Comparative Clinical Effectiveness of current cTn assays in the Netherlands



Comparative Clinical Effectiveness of current cTn assays in the Netherlands

DOEL:

- Onderzoek naar de impact van de cTn analysekwaliteit op de klinische interpretatie: diagnose MI [ja/nee]

- Gebruik makend
  - van landelijke SKML rondzenddata (2010)
  - IFCC tabel met cTn beslisgrenzen (99^{e} P, 10% CV) EN CV_a

- Kleurcoderen: aan een getal hangt een klinische interpretatie vast die is aangegeven met een kleurcode

Klinische interpretatie cTn methoden anno 2010

- Verschilt ngl. methode, generatie en fabrikant

- Meest gevoelige assays momenteel, zowel in termen van betrouwbaar positief en betrouwbaar negatief meten zijn:

  - Siemens Advia cTnI
  - Siemens Dimenstion Vista cTnI
  - Roche hs cTnT

Conclusies bij (hs) cTn bepalingen: 1/2

Keys to Analytical Quality

- Know your cardiac troponin assay
- Harmony with other troponin assays ?
- Imprecision (95% CI) at low levels ≤10%?
- Quality control monitoring at low (~99th percentile) troponin levels

Conclusies: 2/2

- **<u>Troponin-omics:</u> cardiac troponin is niet meer weg te denken…**
  - **CK-MB and Mb geen rol meer**
  - **Seriele testing c.q. delta criterium vergt meer onderzoek**
  - **Reviseer tijdsinterval tussen opeenvolgende bloedafnames.**
- **Langzame beweging naar hs cTn I/T assays in Nederland**
- **Educatieve worsteling teneinde hs cTn begrijpelijk en hanteerbaar weg te zetten voor zowel lab als kliniek.**
- **Partnerships tussen kliniek en lab zijn essentieel.**
- **SKML rondzendmonsters: 12 paren per 2010, CNS concept, alsook levels in het referentiegebied en rond de 99^{e} P!**