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Centre for Metrological Traceability in Laboratory Medicine (CIRME)

Director: Prof. Mauro Panteghini

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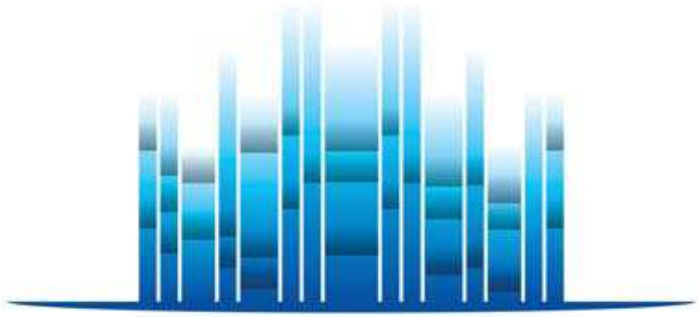

5th International CIRME Meeting

STANDARDIZATION OF CARDIAC TROPONIN I: THE ONGOING IINTERNATIONAL EFFORTS

NOVEMBER 30th, 2011

Performance requirements for cTnI measurement in clinical setting: need to standardize as well

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Centre for Metrological Traceability in Laboratory Medicine (CIRME)

Milano, Italy

Clin Chem Lab Med 2001; 39(2):174–178

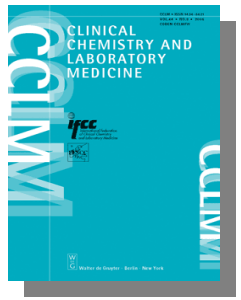
Quality Specifications for Cardiac Troponin Assays

International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)[1) 2)]

IFCC Scientific Division
Committee on Standardization of Markers of Cardiac Damage[3)]

Prepared for publication[4)] by

Mauro Panteghini[1, 5)], Willie Gerhardt[2], Fred S. Apple[3], Francesco Dati[4], Jan Ravkilde[5] and Alan H. Wu[6]



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Editorial

Performance of Today's Cardiac Troponin Assays and Tomorrow's

[Panteghini M, Clin Chem 2002;48:809]

- ✓ *Powerful tests, such as cardiac troponins, on which critical decisions will rest, need highly reliable measurements.*
- ✓ *A number of assay-related issues can markedly affect the performance of cardiac troponin measurement in everyday practice, and different assays are not equal in their ability to measure this biomarker.*
- ✓ *It is vital that all information on the troponin assays is given and that performance characteristics of the assays are objectively assessed and adequately described.*

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Quality requirements for cardiac troponin assays
Main issues to be addressed

- *Analytical factors:*
  - *Calibration characterization & traceability*
  - *Assay specificity (including identification of epitopes recognized by the employed antibodies and crossreactivity to structurally related molecules present in blood)*
  - *Assay detection limit & limit of quantitation*
  - *Interferences*
- *Pre-analytical factors:*
  - *Sample type & stability*

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FINAL DOCUMENT

Global Harmonization Task Force

Title: Summary Technical Documentation (STED) for Demonstrating Conformity to the Essential Principles of Safety and Performance of In Vitro Diagnostic Medical Devices

Authoring Group: Study Group 1 of the Global Harmonization Task Force

Date: March 17th, 2011

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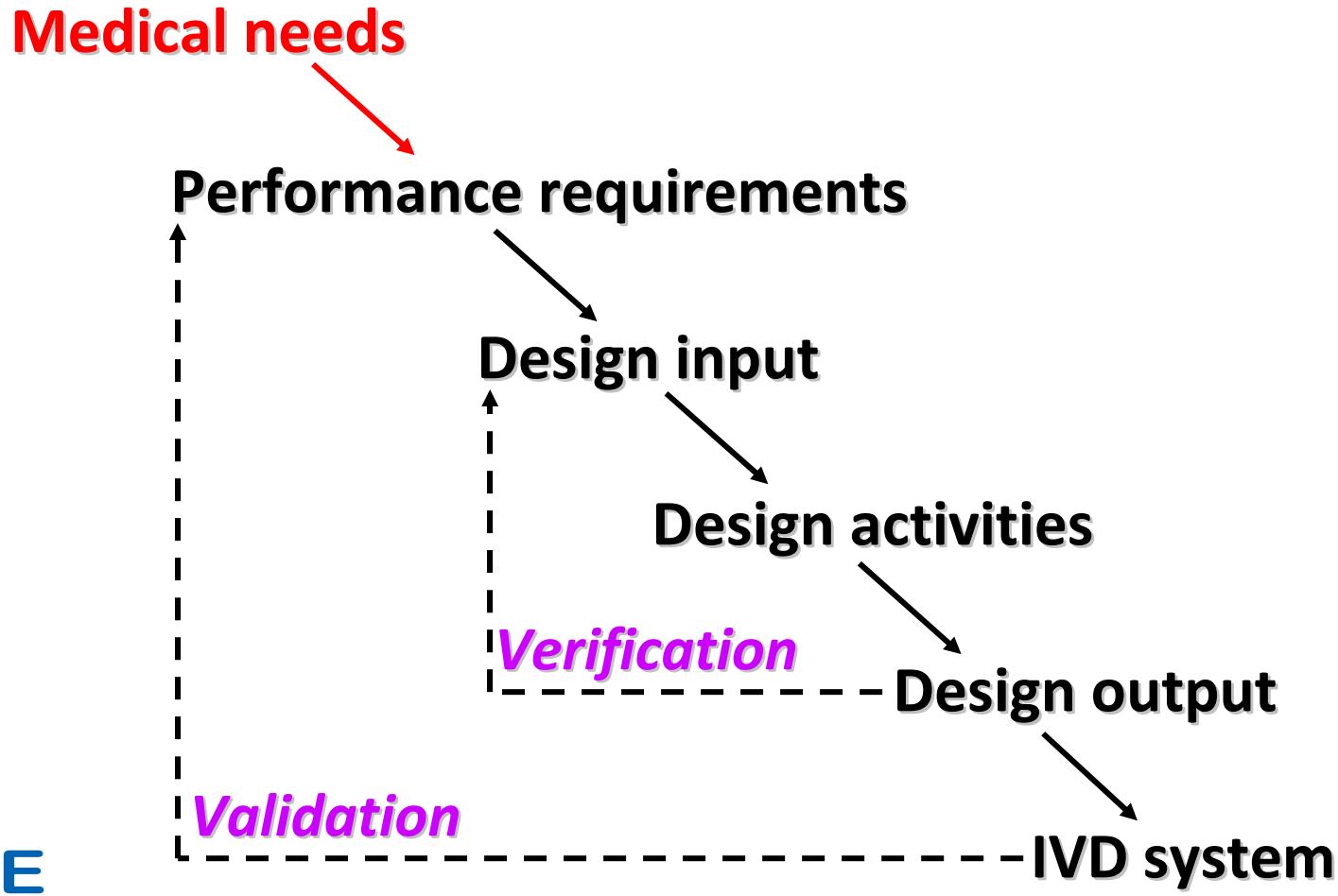
The design control process of an in vitro diagnostic (IVD) system



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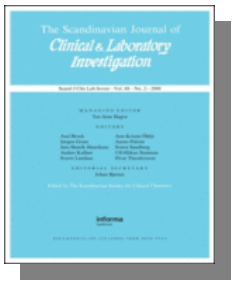
[Adapted from Powers DM & Greenberg N, Scand J Clin Lab Invest 1999;49:539-44]



Performance requirements for troponin assays

Confounding factors

- Terminology correctness
- Experimental protocols
- Statistical approaches
- Source of available information

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CLSI EP17-A Definitions [2004]

Limit of detection (LoD)

"Lowest amount of troponin in a biological sample that can reliably be detect by the method."

Limit of blank (LoB)

"Highest measurement result that is likely to be observed (with a stated probability) for a blank sample."

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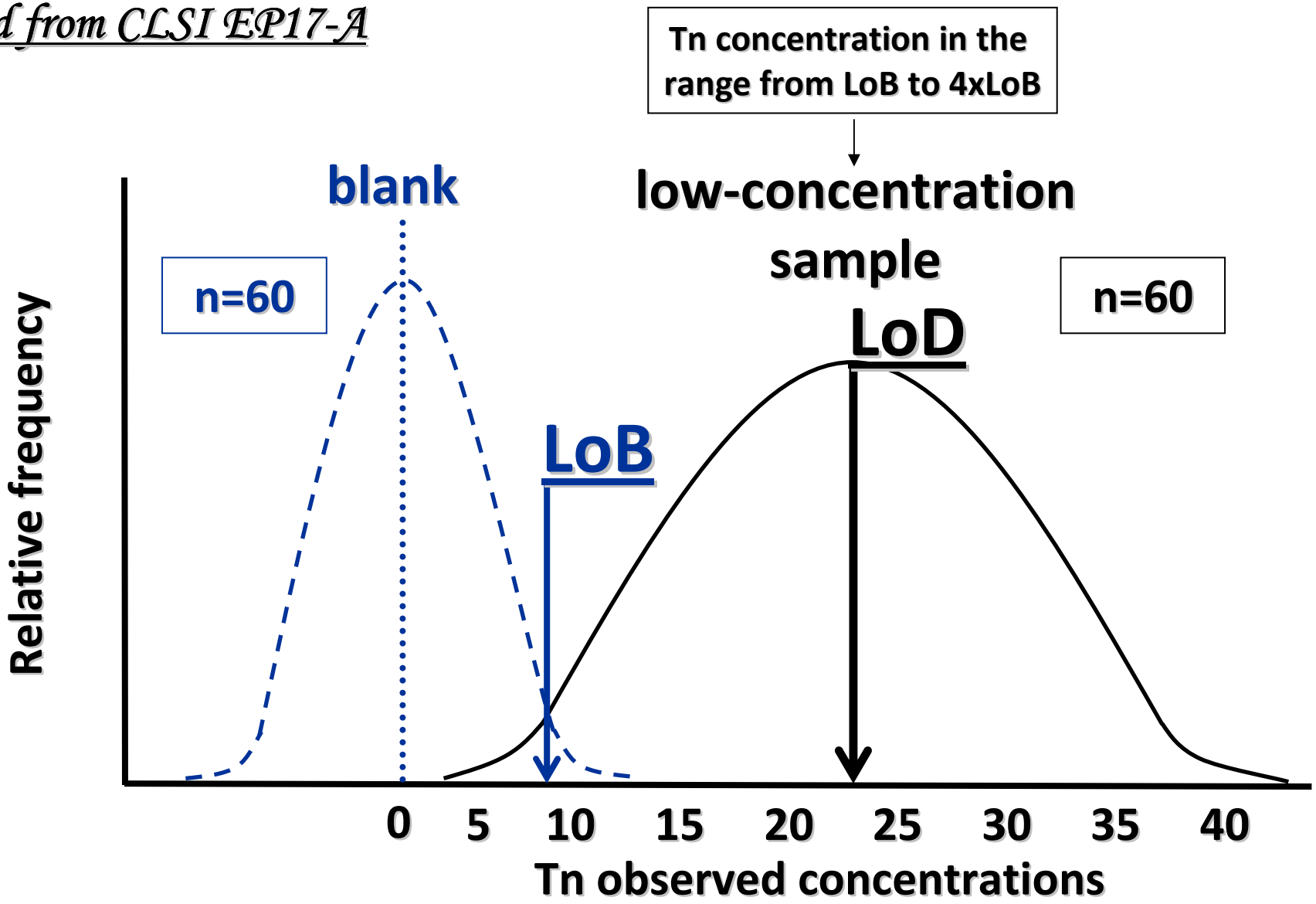
Adapted from CLSI EP17-A



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$$LoB = mean_B + 1.645\ SD_B$$

$$LoD = mean_B + 1.645\ SD_B + 1.645\ SD_S$$

In the majority of cases, LoD is practically estimated simply as the troponin concentration corresponding to a signal 2 SD above the mean of 20 replicates for a sample in which troponin is absent, e.g. the zero calibrator, which is similar to the illustrated procedure for estimating LoB, it is not surprising that many commonly reported 'LoD' values are lower than they would be if more correct experimental and statistical procedures are used.

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Table 1. Scorecard designations of cTn assays.

Acceptance designation	Total imprecision at the 99th percentile, CV%
Guideline acceptable	≤10
Clinically usable	>10 to ≤20
Not acceptable	>20
Assay designation	Measurable normal values below the 99th percentile, %
Level 4 (third generation, hs)	≥95
Level 3 (second generation, hs)	75 to <95
Level 2 (first generation, hs)	50 to <75
Level 1 (contemporary)	<50

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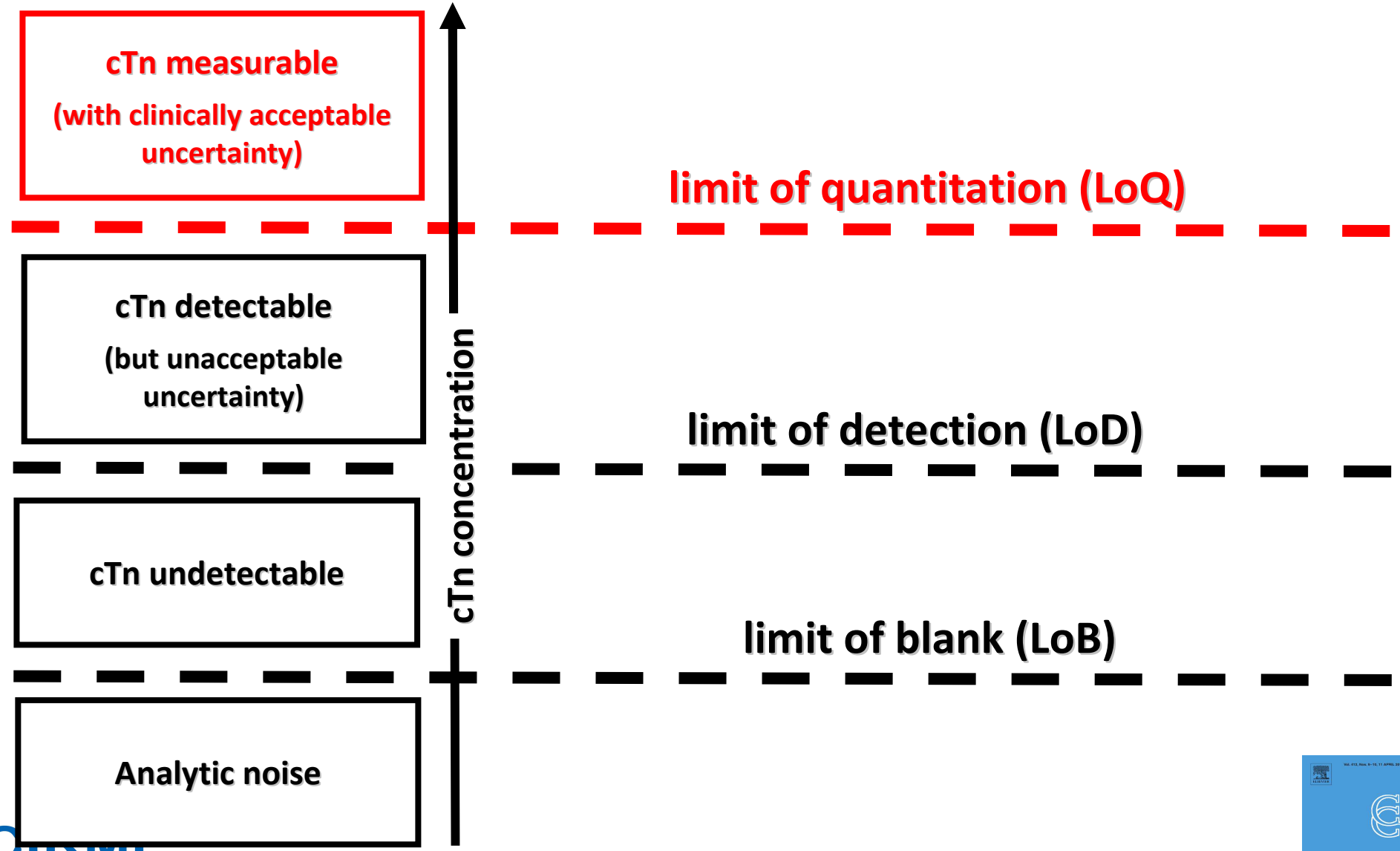
[Apple FS, Clin Chem 2009;55:1303]



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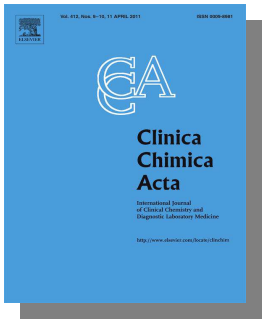
[Panteghini M, Clin Chim Acta 2009;402:88]



Limit of Quantitation (LoQ): definition

[Adapted from WHO-ECBS 1995]

"Lowest amount of troponin that can be quantitatively determined with stated acceptable (read "clinically meaningful") imprecision and bias."

→ Thus, to be clinically usable the 99th upper reference limit cannot be lower than the limit of quantitation of the given troponin assay.

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Analytical performance of troponin assays: central issues

- Definition of the limit of quantitation
- Derivation of information on assay performance

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Allowable Limits

IFCC-IUPAC Stockholm Conference 1999
for setting quality specifications in Laboratory Medicine

1. Evaluation of the effect of analytical performance on clinical outcomes in specific clinical settings (e.g. misclassification in diagnosis)
2. Evaluation of the effect of analytical performance on clinical decisions in general
    a. Data based on components of biological variation
    b. Data based on analysis of clinicians opinions
3. Published professional recommendations from national and international expert bodies
4. Performance goals set by
    a. Regulatory bodies
    b. EQAS organizers
5. Goals based on the current state of the art (e.g. as demonstr data from EQAS)

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[Scand J Clin Lab Invest 1999;49:475-585]

Effect of analytical performance of troponin measurement on diagnostic misclassification

[Sheehan P et al., Ann Clin Biochem 2002;39:213]

Limit of quantitation CV* (assuming unbiased results)	% misclassification
36.2%	7.7-15.2
24.6%	3.8-7.7
16.3%	1.8-3.8
13.0%	1.4-1.8
11.2%	1.2-1.4
9.4%	0.9-1.2
6.7%	0.5-0.9

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*Note that this was probably a conservative estimate given that the impact of imprecision was evaluated by duplicate measurements in a single assay run.

Allowable analytical goals based on components of biological variation

→ We need to know biological variability of troponin (I and T) and this information should be produced using well designed protocols

Standard for production

Fraser & Harris
Crit Rev in Clin Lab Sci
1989;27:409-37

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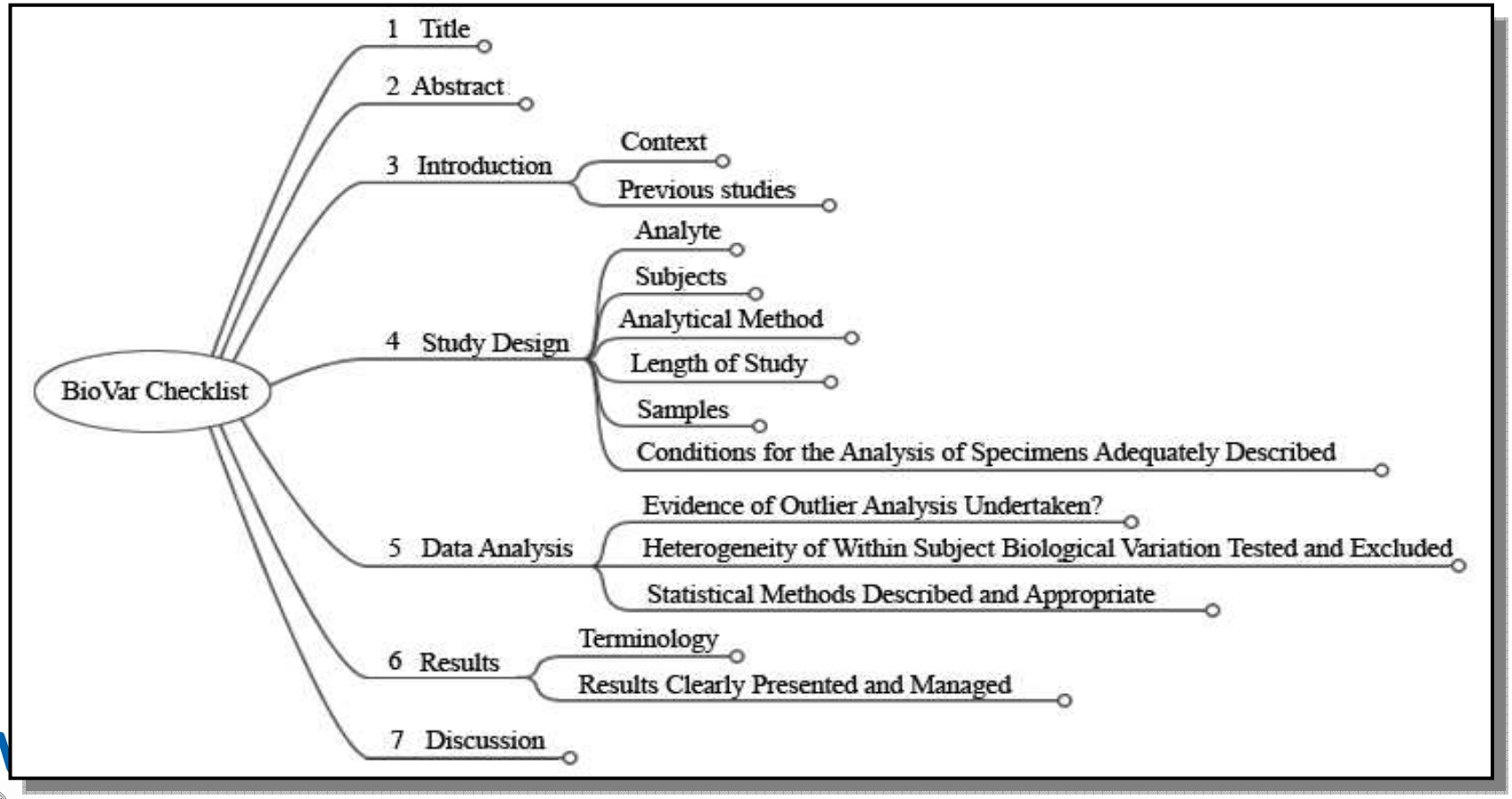
The checklist for critical appraisal of biological variation studies proposed by the Working Group on Biological Variation of the European Federation of Clinical Chemistry and Laboratory Medicine (EFCC) (http://biologicalvariation.com/ESW/Files/BERLIN_final_2011PD.pdf)



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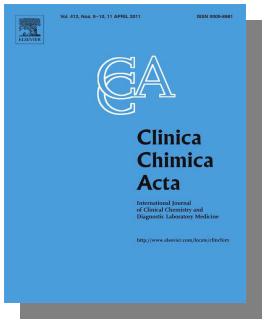
Is available information on biological variability reliable?

Table 4
Summary of the characteristics of studies on biological variability of HbA_{1c} evaluated in this systematic review.

Study no.	Method specificity as per HbA_{1c} measurand definition	Recruitment of healthy subjects	Optimal study duration	Optimal protocol of sample analysis	Statistical analysis described
1	No	Yes	±	No	No
2	No	No	Yes	No	Yes
3	No	No	No	No	Yes
4	±	No	No	No	No
5	±	Yes	Yes	No	Yes
6	±	Yes (F only)	Yes	±	Yes
7	Yes	No	No	No	No
8	No	Yes (M only)	Yes	No	Yes
9	±	No	No	No	Yes



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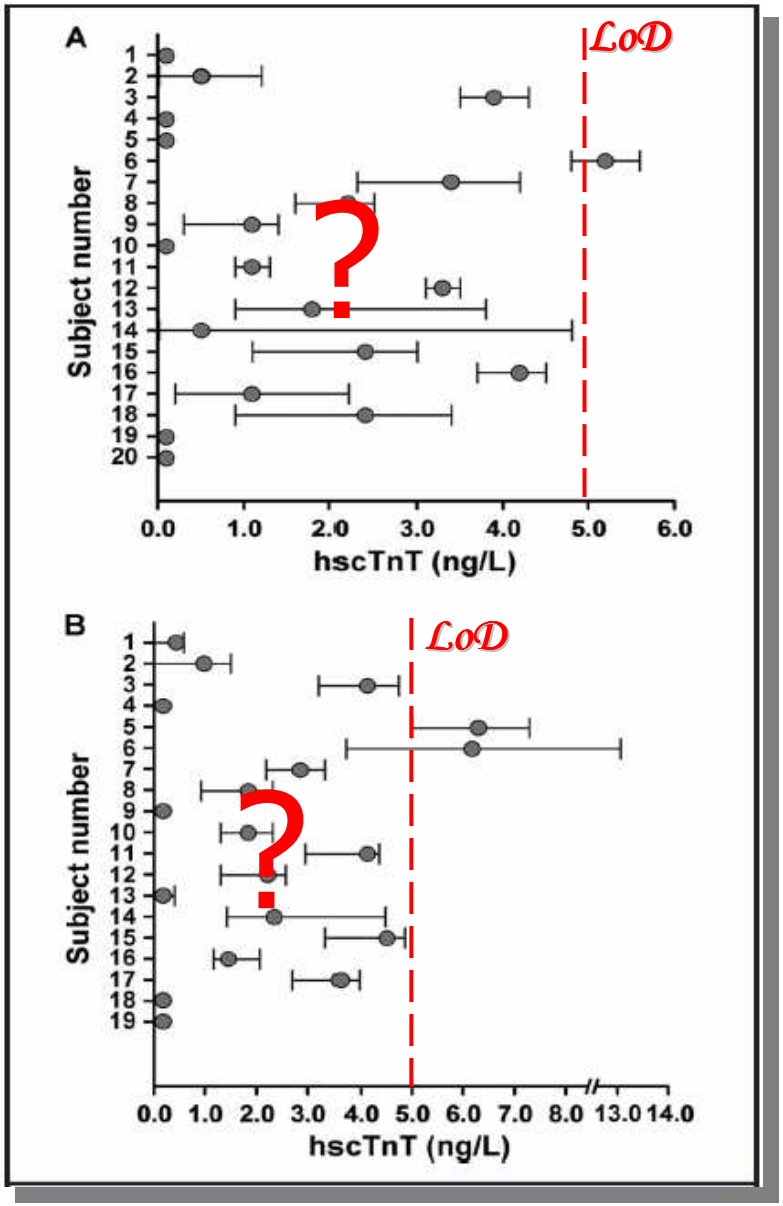
[Braga F et al, Chim Clin Acta 2010;411:1606]

Is available information on biological variability of troponin T reliable?



Short-term

Long-term

[Vasile VC et al., Clin Chem 2010;56:1086]

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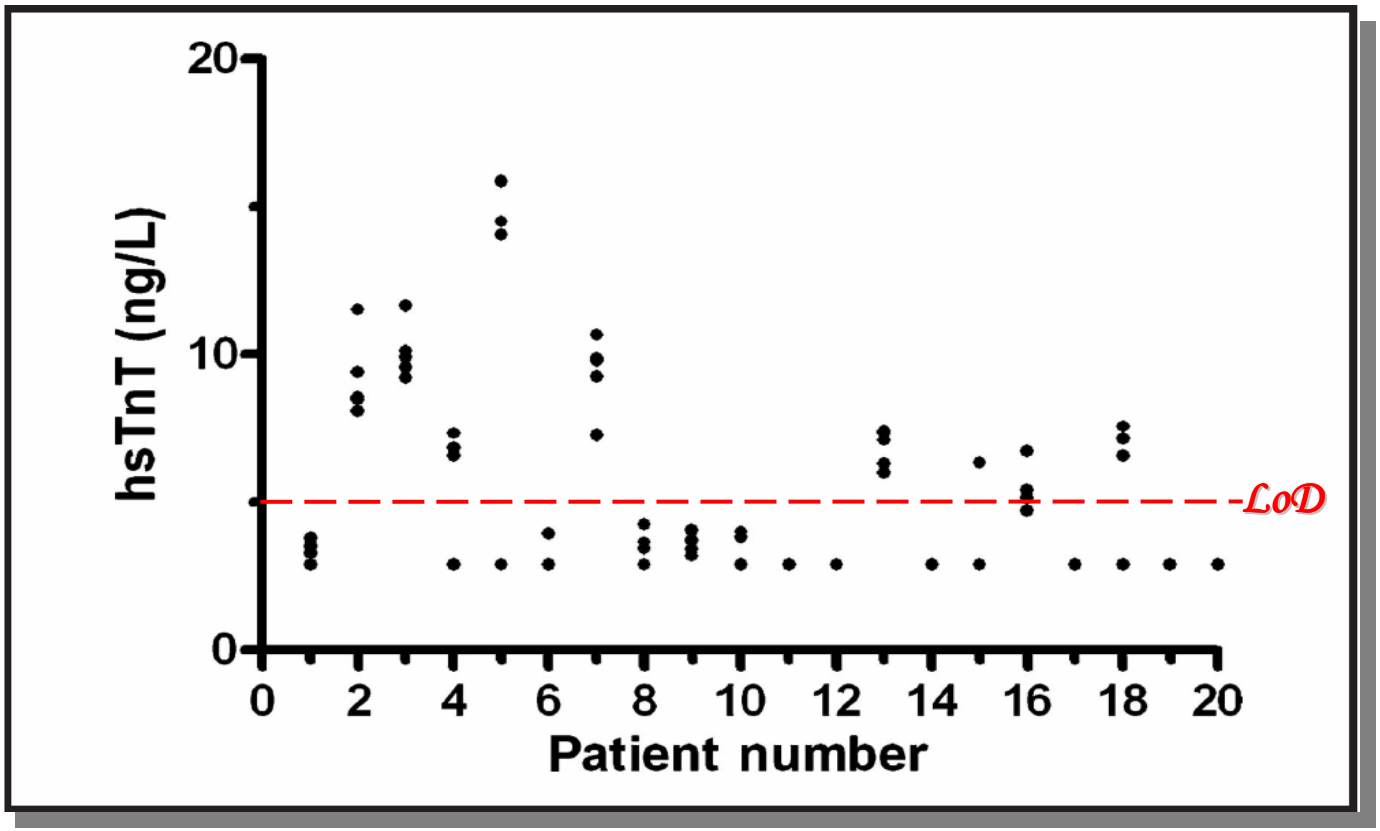
Is available information on biological variability of troponin T reliable?



48% of results were lower than the LoB of the assay!

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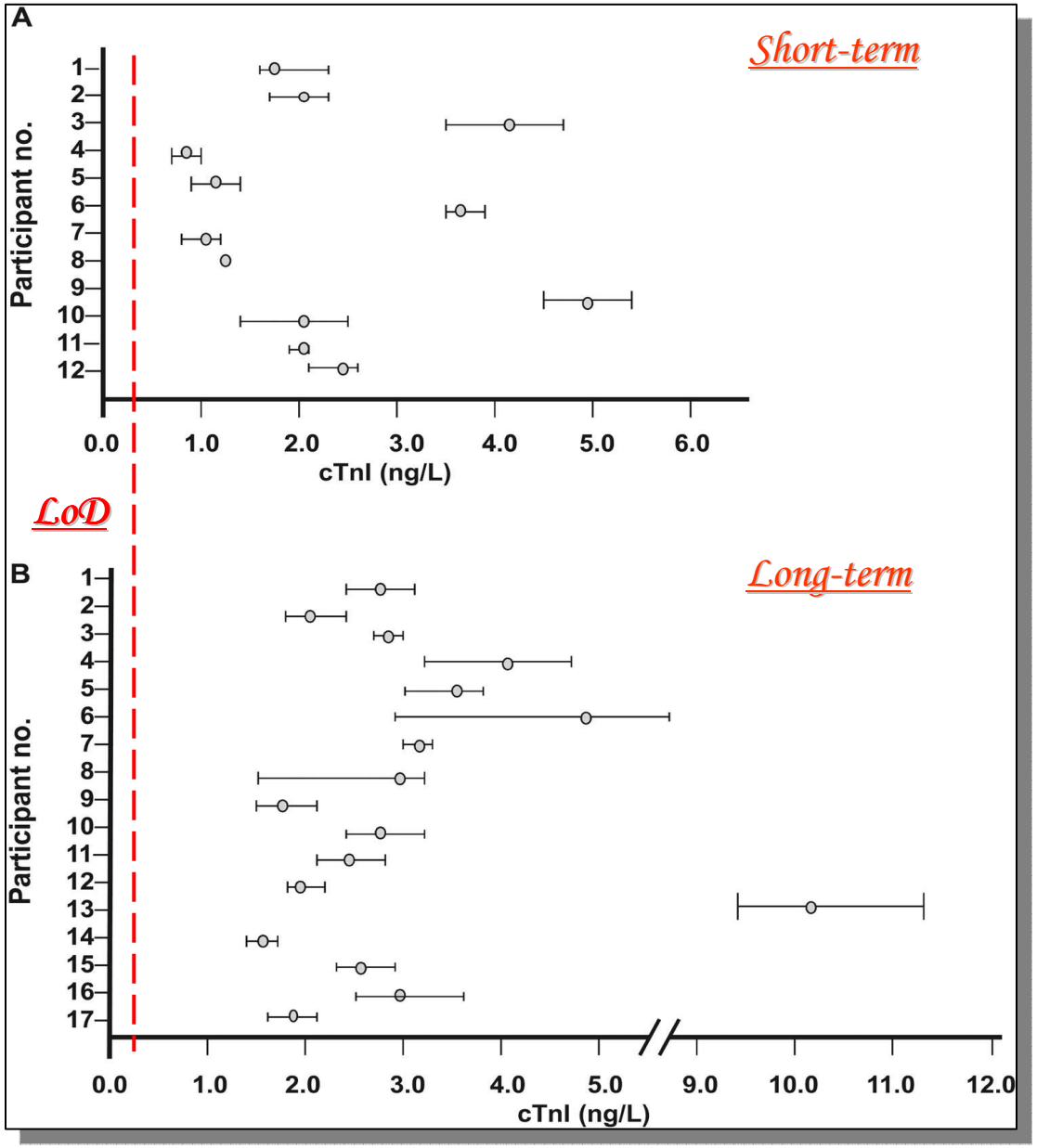
[Frankenstein L et al., Clin Chem 2011;57:1068]

Is available information on biological variability of cTnI reliable?

Singulex Erenna single-photon assay
[under development & not available for commercial use]



[Wu A.H.B. et al. Clin Chem 2009;55:52-58]

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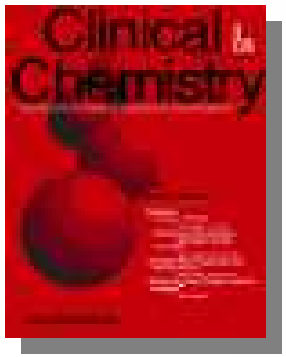
Is available information on biological variability of cTnI reliable?



[Vasile VC et al. Clin Chem 2011;57:1080]

Biologic Variation of a Novel Cardiac Troponin I Assay

- The assay LoD is 2.1 ng/L.
- The median value in the study population was 2.2 ng/L, so that approx. 50% of results were <LoD!

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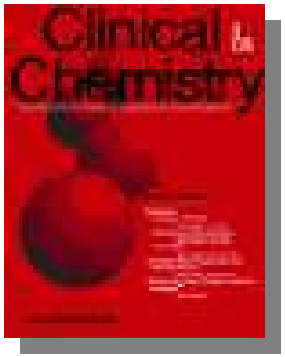
Is available information on biological variability of cTnI reliable?

Biological variability of cTnI determined using the Abbott Architect highly sensitive assay

[declared LoD 1.2 ng/L]



AACC 2011 Annual Meeting

Variable	Goldberg J et al.	Apple FS et al.
No. of subjects	24	12
Mean cTnI	1.9 ng/L	3.5 ng/L
CV_A	16.8%	13.8%
CV_I	24.4%	15.2%
CV_G	124.0%	70.5%

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Analytical performance goals based on data of biological variability of the analyte

[Fraser CG et al. Ann Clin Biochem 1997;34:8]

- ***Minimum***

  $CV_A < 0.75 \times CV_I$

  $B < 0.375 \times (CV_I^2 + CV_G^2)^{0.5}$

  $TE < [1.65 \times 0.75 \times CV_I + 0.375 \times (CV_I^2 + CV_G^2)^{0.5}]$

- ***Desirable***

  $CV_A < 0.50 \times CV_I$

  $B < 0.250 \times (CV_I^2 + CV_G^2)^{0.5}$

  $TE < [1.65 \times 0.50 \times CV_I + 0.250 \times (CV_I^2 + CV_G^2)^{0.5}]$

- ***Optimum***

  $CV_A < 0.25 \times CV_I$

  $B < 0.125 \times (CV_I^2 + CV_G^2)^{0.5}$

  $TE < [1.65 \times 0.25 \times CV_I + 0.125 \times (CV_I^2 + CV_G^2)^{0.5}]$

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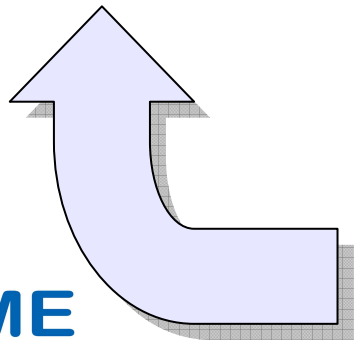
Analytical performance goals for cardiac troponin I measurements using routine methods based on data of biological variability of the analyte obtained by Wu et al.

Quality level	Imprecision goal as CV	Bias goal	Total error goal
Minimum	≤7.3%	±21.6%	<±33.6%
Desirable	≤4.9%	±14.4%	<±22.5%
Optimum	≤2.4%	±7.2%	<±11.2%



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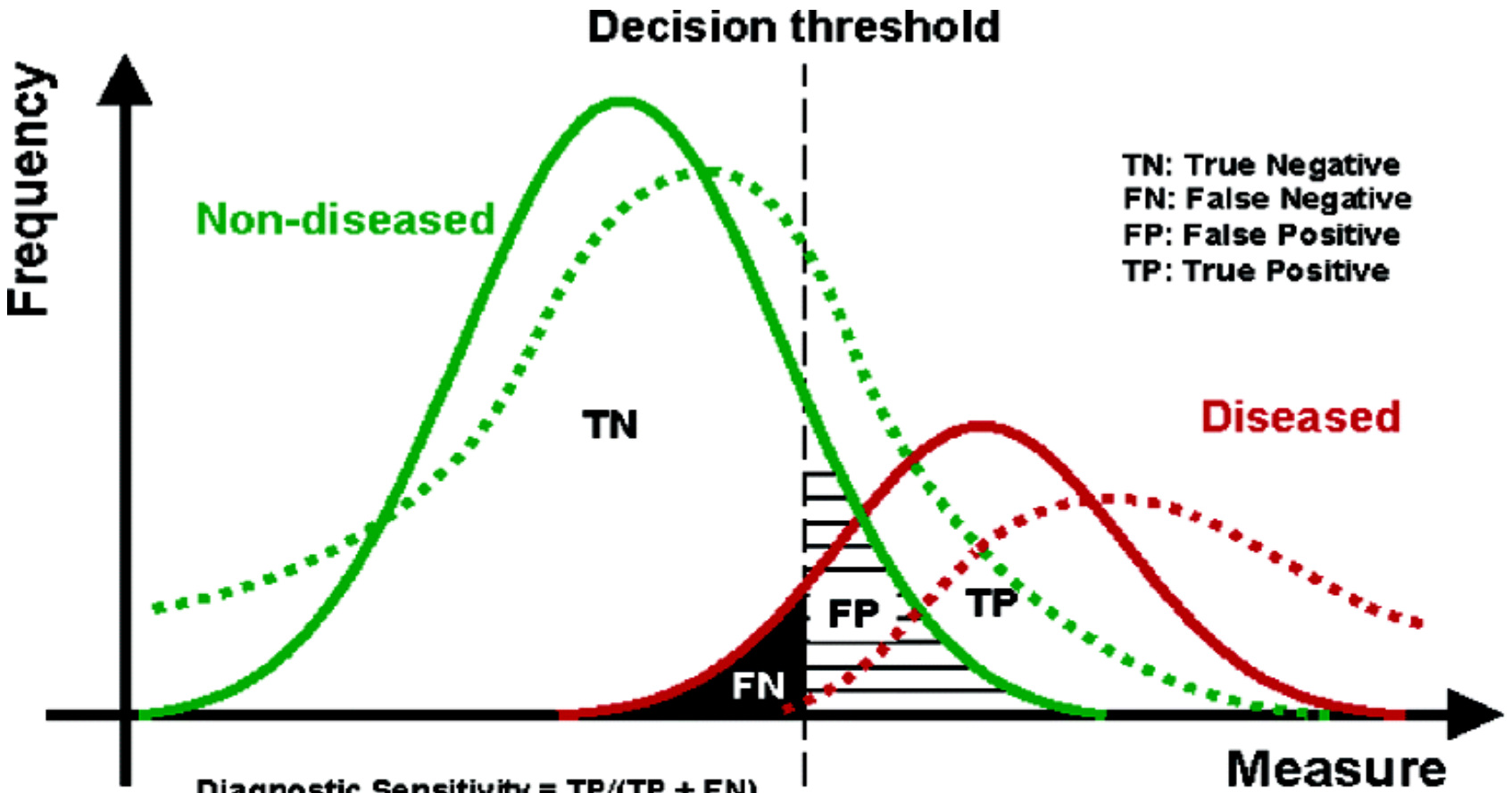
Variable	Short term (0–4 h)	Long term (0–8 weeks)
Analytical variation		
CV_A, %[a]	8.3	15
Biological variation		
CV_I, %	9.7	14
CV_G, %	57	63
Index of individuality	0.21	0.39
RCV: log-normal increase, %	+46	+81
RCV: log-normal decrease, %	−32	−45

Setting quality specifications: III: Data based on experts' opinion and published recommendations

- Expert opinion* has suggested that cTn assays with imprecision of up to a 20% CV at the 99th percentile limit may reasonably be used in clinical practice, even if a CV <10% is desirable.
- No limits for evaluation of bias were given; however, the level of analytic bias producing a systemic shift of cTn results that can produce changes in the clinical decisions has to be defined.

* Jaffe AS et al. for the Biochemistry Subcommittee of the Joint European Society of Cardiology/American College of Cardiology Foundation/American Heart Association/World Heart Federation Task Force for the definition of myocardial infarction (2010).
Thygesen K et al. for the Study Group on Biomarkers in Cardiology of the European Society of Cardiology Working Group on Acute Cardiac Care (2010).

How Bias in Measurements May Affect Medical Decision-Making



Diagnostic Sensitivity = TP/(TP + FN)
Diagnostic Specificity = TN/(TN + FP)

Positive Predictive Value (PPV) = TP/(TP + FP)
Negative Predictive Value (NPV) = TN/(TN + FN)

Disease frequency ($AUC_{diseased}/AUC_{non\text{-}diseased}$) affects PPV and NPV

Impact of test accuracy (bias shifts and imprecision skews and broadens curves)

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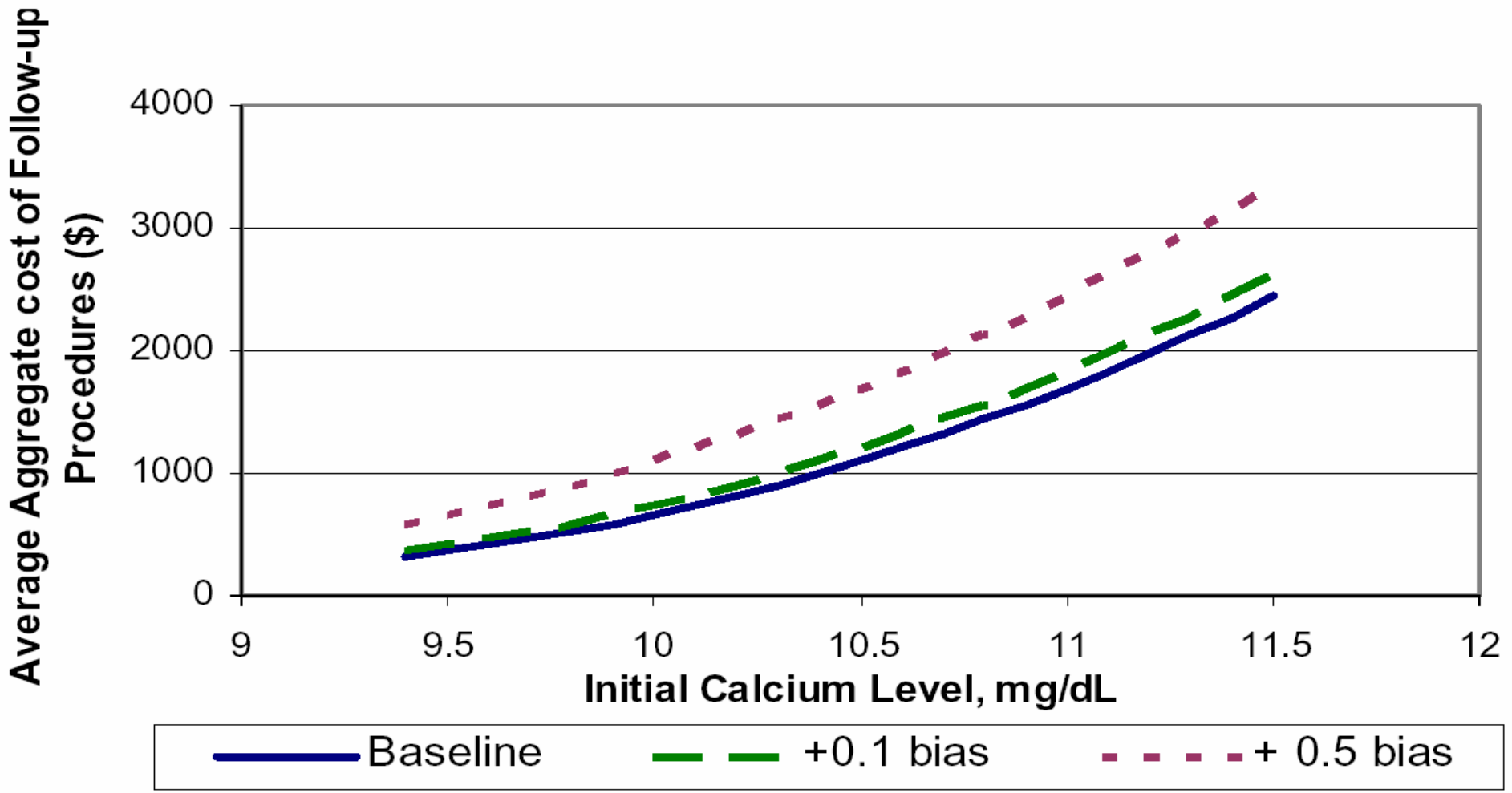
How Bias in Measurements May Affect Health Costs



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[Gallaher MP et al., NIST Planning Report 04-1 - The impact of calibration error in medical decision making, 2004]

Synopsis of proposed analytical performance goals for cardiac troponin measurement

Quality level	Imprecision goal (as CV)			Bias goal
	Outcome-based	Biological variability	Expert opinion	Biological variability
Minimum	<13%	<7.3%	<20%	±21.6 %
Desirable	<10%	<4.9%	<10%	±14.4 %
Optimum	<6%	<2.4%	-	±7.2 %

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[Panteghini M, Troponin Monograph 2011]

Linit of quantitation for troponin measurement: recommendations

1. The cTn measurement performance goals must be targeted at the concentration corresponding to the 99th percentile upper reference limit.
2. Although the definition of analytical performance goals for cTnI and cTnT measurements is still under discussion, a total CV <10% together with an assay bias within ±15% may reasonably represent a good compromise for minimum requirements. This is consistent with the minimum total error goal for serum cTn measurement estimated at ~33%.
3. If a bias greater than ±15% is detected, a process of reassigning the manufacturer's calibrators must be undertaken to decrease this bias. The manufacturer should provide information on any known bias, and the uncertainty of that bias, compared with selected internal higher order references (e.g. the manufacturer's working calibrator) as determined using the commercial method under ideal conditions.

[Panteghini M, Troponin Monograph 2011]

How to derive information on the performance of troponin assays ?

- From industry data (assay package inserts)
- From peer-reviewed literature
- From *ad hoc* performed experimental protocols
- From quality control programmes

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How to derive information on the performance of troponin assays ?

→ **Results from appropriately structured internal & external quality programmes give optimal estimation of troponin assay perfomance and may significantly differ from those obtained from other listed sources**

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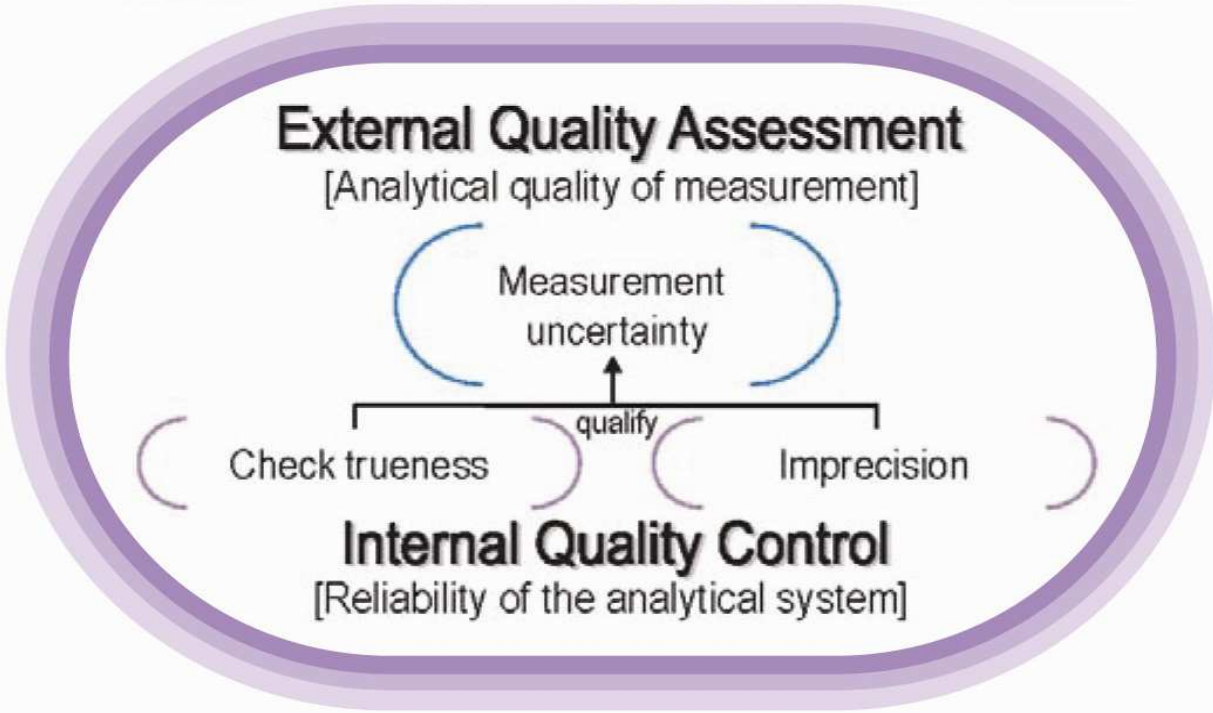

MILANO - *NOVEMBER 30th, 2010*

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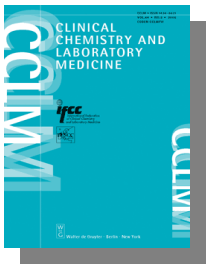
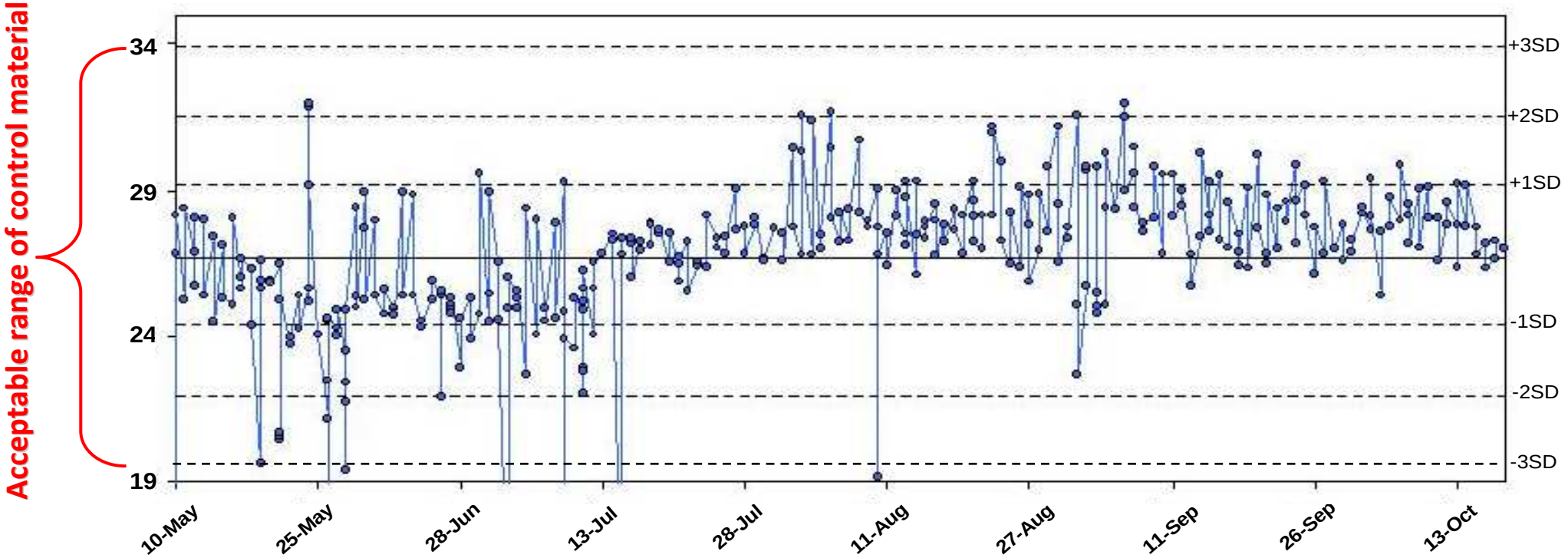
[Adapted from Panteghini M, Clin Chem Lab Med 2010;48:7]



Monitoring the reliability of the analytical system through IQC: I. Check alignment

Clinical laboratories must verify the consistency of the performance declared by the manufacturer of their troponin assays during daily routine operations performed in accordance with the manufacturer's instructions, by analyzing the system control materials and confirm that current measurements are acceptable ('unbiased') according to the manufacturer's established range.



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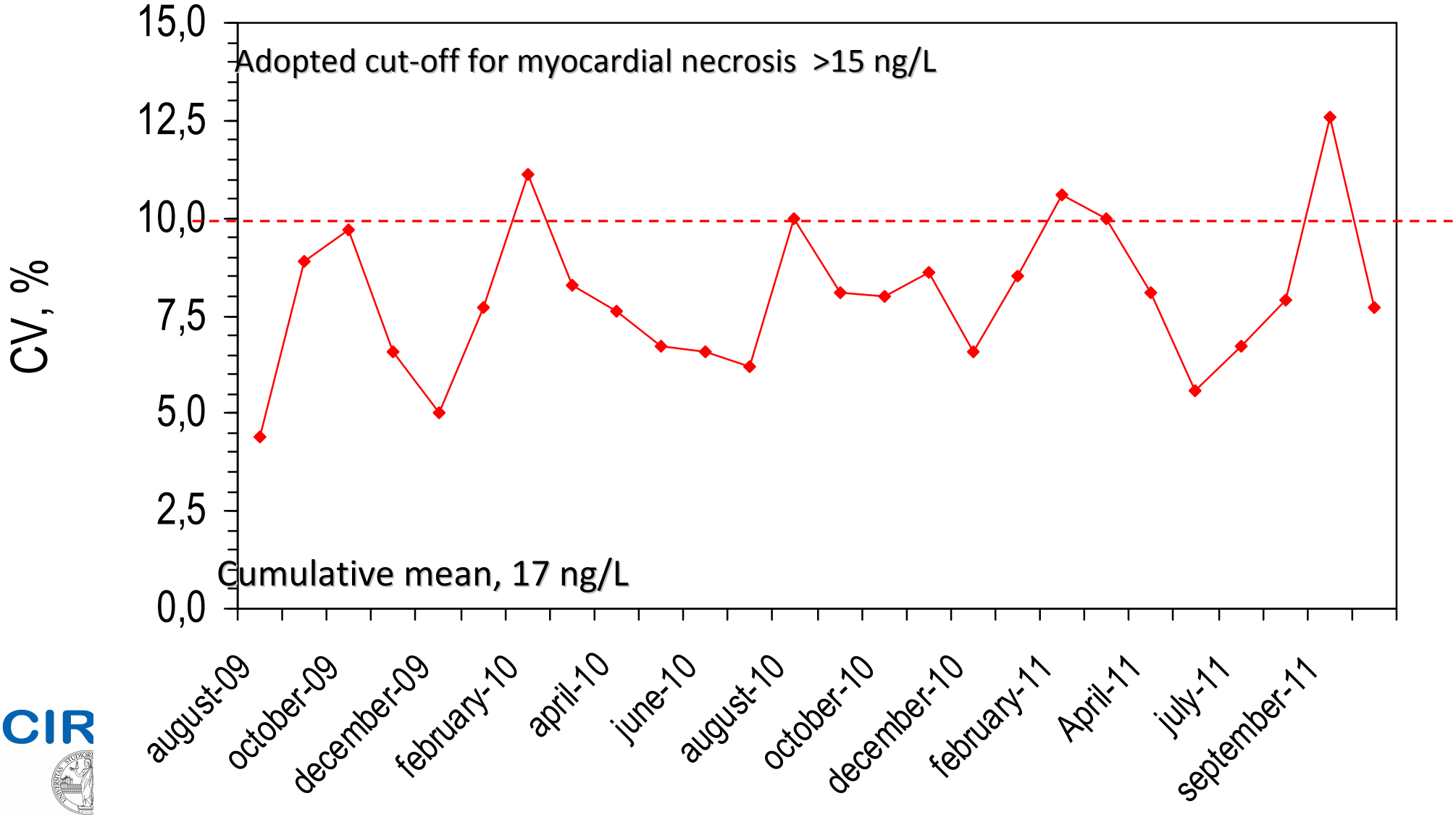
Monitoring the reliability of the analytical system through IQC: II. Evaluate the system imprecision



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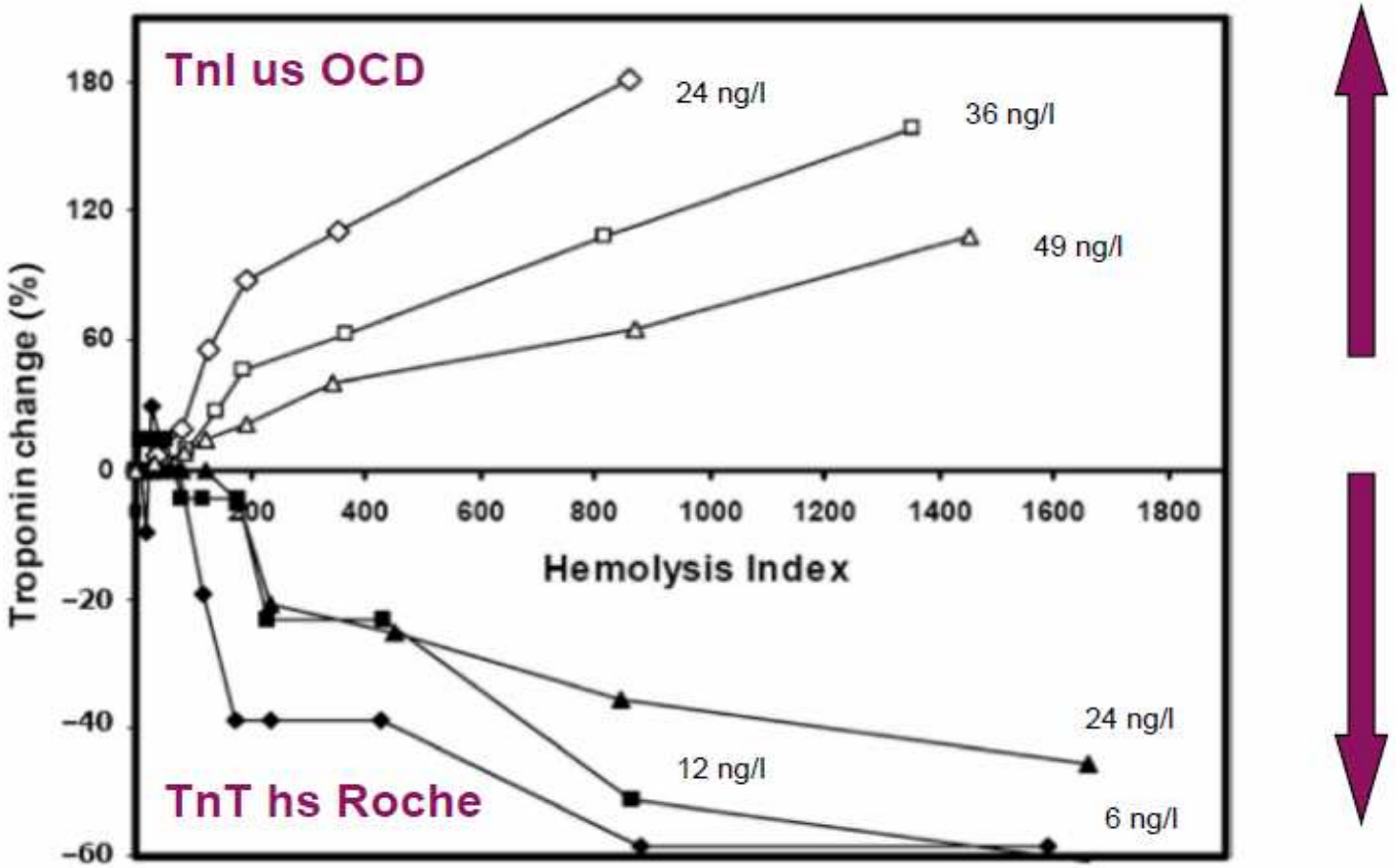
Interfering factors: hemolysis



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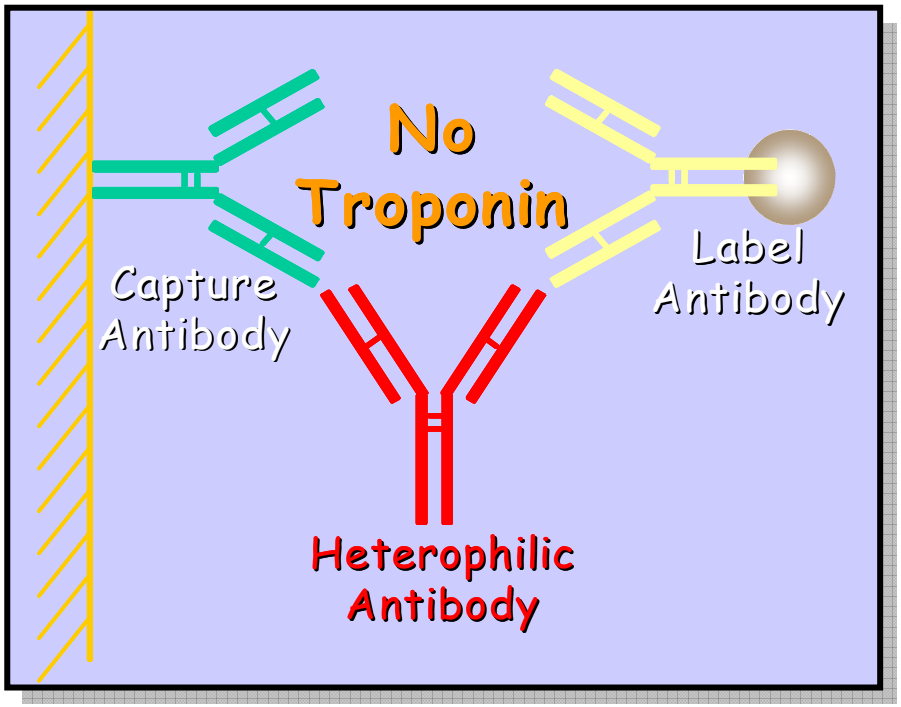
[Bais R et al. Clin Chem 2010;56:1357]

Interfering factors: heterophile antibodies (HA)



The susceptibility of different troponin assays to HA may be related to:

1. the type of immunoglobulin class,
2. concentrations of the circulating HA,
3. effectiveness of the type of blocking agents incorporated into the reagent formulation, and
4. the immunoassay format.

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3-site troponin I immunoassays: an approach to improve the analytical sensitivity

cTnI Assay System	Antibody specificity: a.a. residues	
Abbott AxSYM/Architect	MAb1 (capture) MAb2 (capture) MAb3 (detection)	41-49 87-91 24-40
BioRad BioPlex 2200	MAb1 (capture) MAb2 (capture) MAb3 (detection)	31-34 41-47 88-94
Radiometer AQT90	MAb1 (capture) MAb2 (capture) MAb3 (detection)	41-49 190-196 137-148
Mitsubishi Pathfast	MAb1 (capture) MAb2 (detection) MAb3 (detection)	41-49 71-116 163-210
Ortho Clinical Diagn. ECi	MAb1 (capture) MAb2 (capture) MAb3 (detection)	24-40 41-49 87-91
Siemens ADVIA Centaur	MAb1 (capture) MAb2 (capture) PAb3 (detection)	41-49 87-91 27-40

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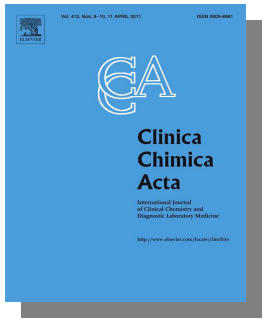
[mod. from Panteghini M, Clin Chim Acta 2009;402:88]



BUT: 3-site assays may be more prone to HA interference with an estimated frequency that is at least twice of that in 2-site immunoassays

Clinica Chimica Acta 395 (2008) 181–182

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Letter to the editor

Heterophilic antibody interference in an ultra-sensitive 3-site sandwich troponin I immunoassay

Siemens ADVIA Centaur Ultra™ assay

Analyte	Native	HBT treated	Recovery
cTnI	1999 ng/L	30 ng/L	0.15%
Ferritin	1001 µg/L	951 µg/L	95%
TSH	5.1 mU/L	4.1 mU/L	80%
FSH	11.2 IU/L	11.5 IU/L	100%

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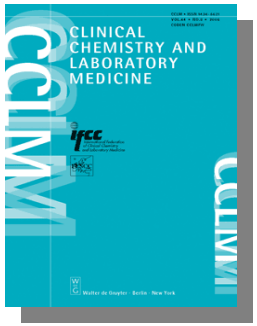
AND: this may be also true in 3-site assays with 1 capture antibody and 2 detection antibodies

Clin Chem Lab Med 2009;47(7):829–833 © 2009 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2009.182

Evaluation of analytical performance of the Pathfast® cardiac troponin I

Mitsubishi Pathfast® assay



Results: No significant differences were found when cTnI concentrations of 40 lithium-heparin plasma samples were compared with the matched values of K_2-EDTA plasma, whole blood and serum samples. The LoB and the LoD of the cTnI method were 0.0048 and 0.0066 µg/L, respectively. cTnI mean values from 0.66 to 6.0 µg/L showed a total CV% from 6.0 to 6.4. cTnI at a concentration of 0.02 µg/L was associated with a total CV of 9.6%. The method gave a linear response for cTnI concentrations within the measurement range. In six of 12 samples containing HA, a positive interference was demonstrated. The 99th percentile limit of the cTnI distribution in the reference population was 0.013 µg/L.

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Recommendation

The lack of interference of HA in a troponin assay system should be carefully documented by measuring samples containing high HA concentrations in conjunction with treatment of the sample with HA blocking tubes.

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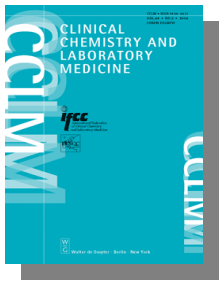
[Panteghini M et al. Clin Chem Lab Med 2001]



HAMA interference: sample examination protocol

HA sample

The sample is assayed in all of the evaluated immunoassays (in duplicate)

Result (untreated)

HA sample

Heterophilic Blocking Tube (HBT)

The sample is assayed in all of the evaluated immunoassays (in duplicate)

Result (treated)

Interfering factors: anticoagulants

- The sample type issue cannot be considered closed using current generation assays as recently two out 5 commercial assays for troponin I have shown significant differences between serum and heparin plasma with more heparin samples below assay limit of blank (Clin Chim Acta 2010;411:1095).
- The use of anticoagulants for sample collection should, therefore, be studied and validated thoroughly by using appropriate experimental and statistical approaches before it can be recommended for practical use in troponin measurement.

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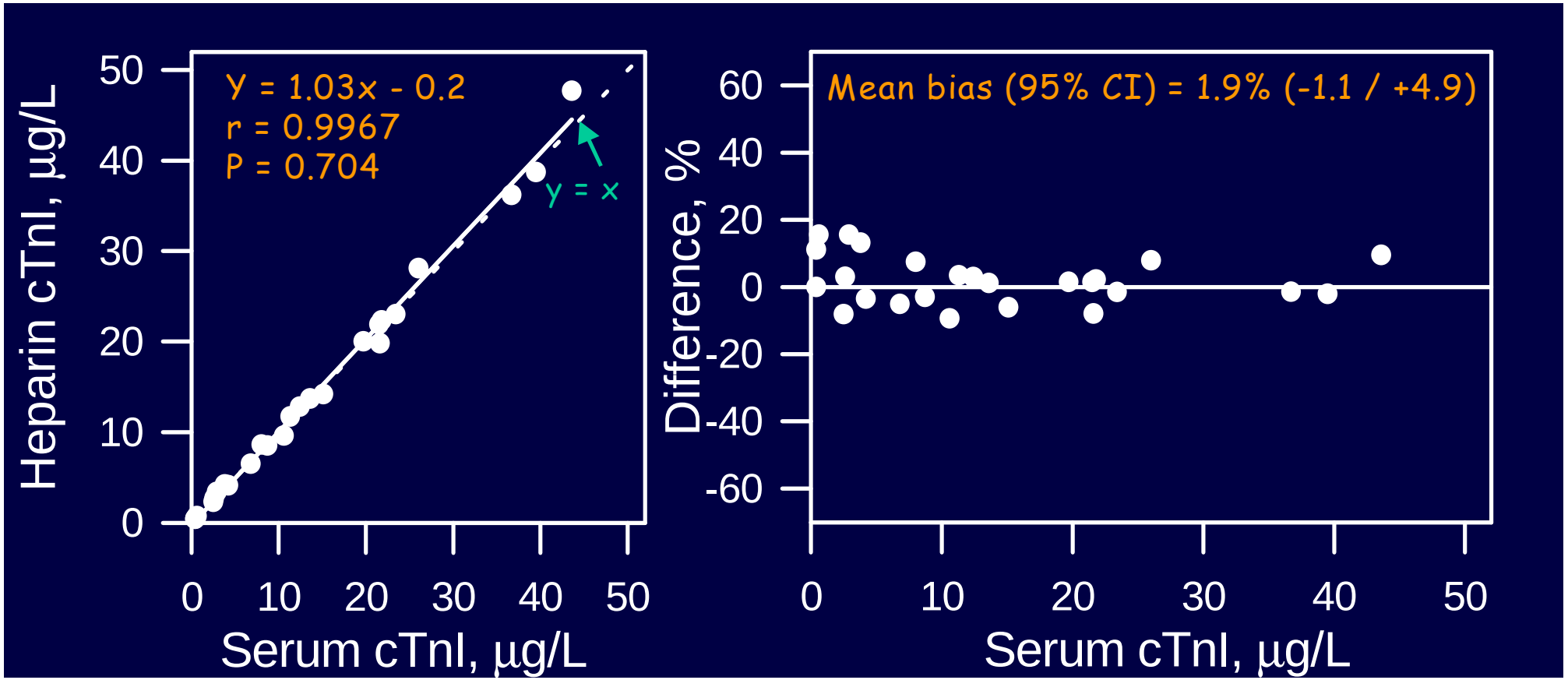

The essential preconditions for interchangeability between serum and plasma values are the obtainment of a slope equal to 1 with no significant intercept in the regression equation, or zero percentage difference in the bias plot.

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Interfering factors: *in vitro* stability

- Depending on the assay antibody configuration, sample stability is method dependent, creating a need for specific data for each commercially available troponin assay.
- Published and manufacturers' data on the *in vitro* stability of troponin assays are, however, limited.
- For clinical research, information on the long-term stability of troponin in stored samples is important prior to the use of archived samples.

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The ages of troponin performance requirements