

Cardiac Troponin Assays
Public Workshop
November 28, 2017

Goals of the workshop

- Share FDA's experience with these devices
- Open lines of communication between FDA and stakeholders
- Get input from stakeholders
- Our shared goal is to have reliable devices available for patient use

General information

- Meeting room is equipped with WIFI
 - Password is “publicaccess”
- Participants will only have access to Bldg 31
 - Breakfast, lunch (pre-made sandwiches) and snacks will be available for purchase
 - Pre-order box lunches will also be sold in the morning at the kiosk in Bldg. 31

Agenda

Cardiac Troponin Assays Public Workshop Tuesday November 28, 2017 8:30 a.m. to 4:30 p.m.		
Time	Topics	Panel
8:35-8:45 a.m.	Introduction/Welcome Remarks	
8:45-9:30 a.m.	Cut-Off Determination/Reference Interval Studies	Fred Apple, Ph.D., Jeff Bishop, Ph.D., Rob Christenson, Ph.D., Amy Saenger, Ph.D., Dina Greene, Ph.D., Norberto Pantoja-Galicia, Ph.D., Jackie Wieneke, M.D., and Stayce Beck, Ph.D. (moderator)
9:30-9:45 a.m.	Break	
9:45-10:55 a.m.	Clinical Trial Design	Christopher deFilipi, Ph.D., Allan Jaffe, M.D., James McCord, M.D., Richard Mowak, M.D., Zivjema Vucetic, M.D., Ph.D., Paula Caposino, Ph.D., Jackie Wieneke, M.D., and Courtney Lias, Ph.D. (moderator)
10:55-12:00 p.m.	Pre-Analytical And Analytical Considerations For Clinical Trials	Fred Apple, Ph.D., Rob Christenson, Ph.D., Dina Greene, Ph.D., Jane Phillips, Ph.D., Amy Saenger, Ph.D., Paula Caposino, Ph.D., Kerry Welsh, M.D., Ph.D, and Brittany Schuck, Ph.D. (moderator)
12:00-1:00 p.m.	Lunch	
1:00-2:10 p.m.	Clinical Trials For Point Of Care Devices	Anna Marie Chang, M.D., Allan Jaffe, M.D., James McCord, M.D., Frank Peacock, M.D., Rick San George, Ph.D., Juliane Lessard, Ph.D., Paula Caposino, Ph.D., and Kellie Kelm, Ph.D. (moderator)
2:10-2:25 p.m.	Break	
2:25-3:15 p.m.	Use Of Existing Clinical Data To Support Claims	Anna Marie Chang, M.D., Rakesh Engineer, M.D., Alberto Gutierrez, Ph.D., Karen Richards, Yader Sandoval, M.D., Kellie Kelm, Ph.D., Courtney Lias, Ph.D., and Ian Pilcher (moderator)
3:15-4:15 p.m.	Public Comments	
	Closing Remarks	

Panelists

- Invited experts that we identified from the workshop registration list
- Reached out to associations representing device manufacturers for a list of participants from industry interested in participating
- All workshop participants are welcome speak during the public comment period



Cut-off Determination Reference Interval Studies

Paula Caposino, Ph.D.

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Cut-off determination/reference interval studies for troponin assays

- Why are these needed?
 - Troponin assays are not standardized/harmonized and test results are not interchangeable
- How are these used?
 - Some sponsors choose the 99th percentile URL as the clinical cut-off
 - Some sponsors use cut-off determination studies to define clinical cut-offs
- Why are these important?

Study design

- Different Approaches
 - Differences in inclusion/exclusion criteria
 - Differences in statistical methods
 - Differences in claims (e.g., sex-specific URLs)
- What is the right approach?

Discussion topics for panel

- Discuss best practices for trial design including:
 - The subjects to enroll
 - How to analyze the data
 - What to do with results that are outliers
- Why do many sponsors choose to perform large reference interval studies?
- What information about these studies would be helpful to clinicians and laboratorians?



Clinical Trial Design

Paula Caposino, Ph.D.

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Clinical trials for troponin assays

- Why do we need clinical data for troponin devices?
- How can we stimulate innovation of cardiac troponin devices?
- What do troponin clinical trials look like?
- What are some challenges with these trials?

Why do we need clinical data?

- Troponin assays are not standardized
- Test results are not interchangeable
- Each assay has a unique cut-off(s)
- Each assay has different performance characteristics (e.g., precision around the cut-off)
- Clinical trials provide reasonable estimates of clinical performance

Why do we need clinical data?

- Some manufactures are interested in different claims:
 - Prognosis claims
 - Rapid rule out of MI
- Clinical trials provide estimates of clinical performance for different claims

Why are some assays not available in the United States?

- Clinical validity may not be needed OUS
- Assay has not been submitted for FDA review
- Some cases, assay has performance issues

Why are some assays not available in the United States?

- Poor clinical performance
 - In a study more than 20% of MI patients did not have a single investigational test result above the cut-off
- Imprecision around the clinical cut-off
- Differences between sites
- Trial design contributes to these issues

What do troponin clinical trials look like?

- Clinical trials for troponin assays
 - Cut-off determination study
 - “all comer” clinical validation study
- Central adjudication of subjects
 - Different approaches to adjudication
- Clinical performance of new device compared to adjudicated diagnosis

What are some challenges with these trials?

- Informed consent
 - Delay in enrollment/data collection
- Clinical cut-off(s)
- Bias related to study design
 - Preanalytical issues
 - Excluded subjects
 - Adjudication

What are some challenges with these trials?

- Bias related to study execution
- Pre-analytical issues
- Missing samples
 - Matrix type not collected
 - Timepoints not collected

Discussion topics for panel

- Discuss best practices for trial design to minimize challenges
- Discuss best practices for adjudication
- What information from a clinical trial do clinicians and laboratorians need to understand the clinical performance of a troponin device?



Pre-analytical and analytical considerations for clinical trials

Kerry J. Welsh, MD, PhD

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Overview of topics

- Specimen stability
- Tube type
- Detection limits
- Trial considerations
- Biotin interference

Specimen Stability

- Stability studies are used to support routine specimen handling conditions in clinical use and to support the use of banked clinical specimens in clinical studies.
- Specimen stability study conditions:
 - Room temperature
 - Refrigerated (2 - 8°C)
 - Freeze thaw
 - Frozen storage (-20 versus -80°C)
- Specimens used in clinical studies may not be analyzed immediately, and thus may be subjected to a variety storage conditions.
- We have observed different troponin stability results depending on the assay used.

Specimen Stability

- Some troponin assays show instability at room temperature by two hours, but not at refrigerated, freeze/thaw, or frozen conditions.
- The following table shows an example troponin assay with a male cut-off of 25 ng/L, an overall cut-off of 17 ng/L, and a female cut-off of 13 ng/L. Specimens were measured at baseline, and stored at room temperature for up to eight hours.

	Baseline, ng/L	2 Hours, ng/L	4 Hours, ng/L	8 Hours, ng/L
1	27.2	22.3	21.8	19.8
2	20.2	15.1	15.4	15.8
3	17.3	13.8	11.4	11.2
4	23.5	18.8	18.3	17.6

Specimen Stability

- Some troponin assays are stable at room temperature, but not after freezing.
- The following table shows another example troponin assay. Specimens were measured at baseline, and subjected to multiple freeze/thaw cycles.

	Baseline, ng/L	Freeze/Thaw 1, ng/L	Freeze/Thaw 2, ng/L	Freeze/Thaw 3, ng/L
1	17.8	9.6	9.7	10.0
2	9.8	15.2	15.0	15.4
3	36.7	29.6	28.1	28.2
4	52.4	42.2	39.8	39.1

Tube type

- Sponsors often choose to perform clinical and analytical validation studies in one tube type, and transfer the performance claims to other tube types by a matrix comparison study.
- The following table is an example of a matrix comparison study in which the sponsor showed nearly identical performance between serum and lithium heparin tube types. Clinical concordance was also shown between the two sample types at the assay cut-off.

Passing Bablok Regression by Concentration Ranges

Range (ng/mL)	n	Slope (95% CI)	Intercept (95% CI)	Correlation
0.03 to 20	86	1 (1.00 to 1.00)	0.00 (0 to 0)	r = 1.00
0.03 to 5	83	1 (1.00 to 1.00)	0.00 (0 to 0)	r = 0.99
0.03 to 0.4	68	1 (1.00 to 1.00)	0.00 (0 to 0)	r = 0.99

Tube type

- We have observed different troponin results depending on the matrix used for some troponin assay systems. The following is an example of different results observed in different matrices:

	Lithium heparin, ng/L	EDTA plasma, ng/L	Serum, ng/L	Serum with thrombin-based clot activator, ng/L
1	17.9	22.3	24.6	27.5
2	28.7	22.9	21.9	19.6
3	60.2	48.6	59.2	63.7
4	230.2	199.4	175.5	207.2

Tube type

- We also sometimes see unusual troponin results with certain tube types.

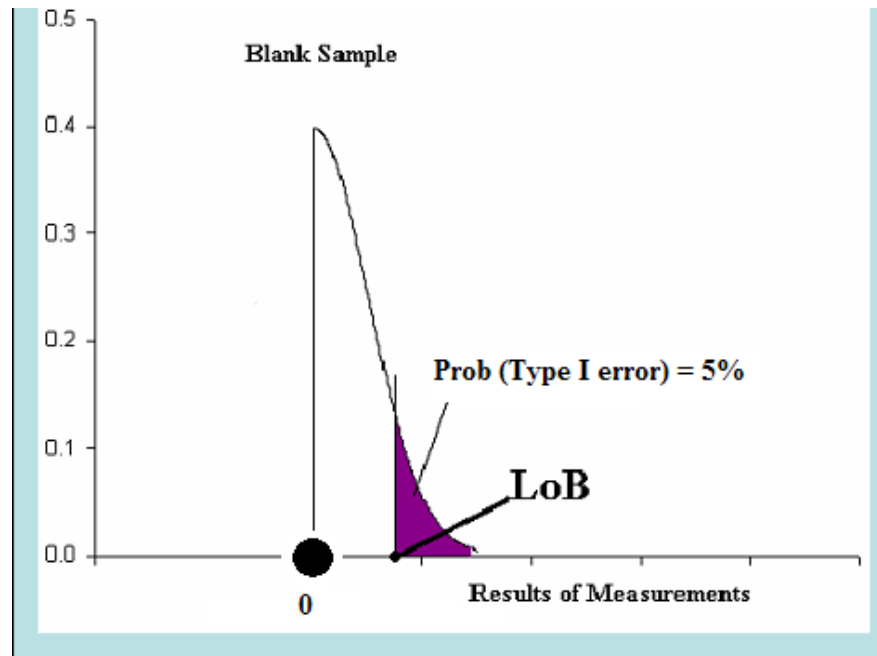
Specimen	EDTA, ng/L			Serum, ng/L			Lithium Heparin, ng/L		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
001	8.8	9.8	9.6	11.6	10.4	12.0	8.8	295.5	554.0
002	19.0	17.4	--	20.0	22.2	--	17.5	81.9	---



Detection limits

Limit of Blank (LoB)

LoB is typically defined as the limit only exceeded 5% of the time by a Blank sample measurement.

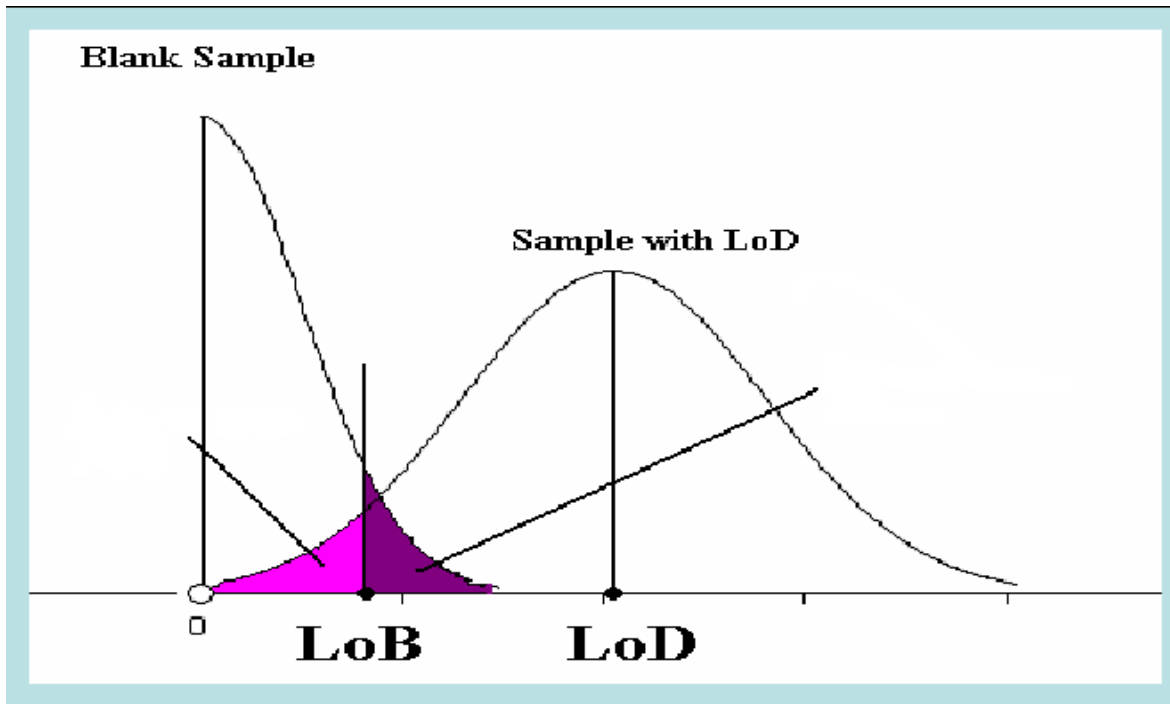


$\alpha = 5\%$
LoB = 95th percentile of
all measurements of
blank samples

Measurement result \leq LoB \rightarrow "Not Detected"
Measurement result $>$ LoB \rightarrow "Detected"

Limit of Detection (LoD)

The LoD is typically defined as the lowest analyte concentration likely to be reliably distinguished from a blank sample with 95% confidence.



Results > LoD = detected

When a samples have a concentration at the LoD, only 5% of the measurements are erroneously declared as not detected.

Limit of Quantitation (LoQ)

LoQ is usually defined as the lowest amount of an analyte in a sample that can be reliably detected *and* at which a stated performance goal is met.

Depending on the defined performance goal, the LoQ could be equal to the LoD or it could be much higher. It should not be lower than LoD.

$$\text{LoB} < \text{LoD} \leq \text{LoQ}$$

Detection Limits

	LoD, ng/L	LoQ % CV ≤ 20%, ng/L	% CV ≤ 10%, ng/L
Lot 1	0.8	1.2	4.3
Lot 2	1.7	2.1	5.9
Lot 3	1.4	2.5	8.9

Detection limits

Target Value, ng/L	Lot 1, % Total Error	Lot 2, % Total Error
0.8	80	140
1	68	110
2	30	66
4	37	37
6	19.3	23
8	15.4	14.7
10	15.4	13.2
12	16	16

Detection limits

- Manufacturing of these reagents is not able to ensure consistent performance of the device near the detection limits.
- Calibrator and control target values are often not near the LoQ, and thus performance at the LoQ is not able to be sufficiently monitored.
- FDA reviews clinical performance of the assay at the assay cut-offs.

Trial Issues: Lab-to-Lab and Analyzer Differences



- Sponsors sometimes choose to perform testing of clinical study specimens at multiple laboratory sites.
- We have sometimes observed different assay performance depending on the laboratory site. Questions may arise regarding if such data are poolable.
- We have also observed different analytical and clinical performance on different analyzer models from the same manufacturer.

	Laboratory 1		Laboratory 2		Laboratory 3	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Sensitivity	92.3%	79.5 - 97.0	74.2 %	56.5 - 87.0	80.1%	63.2 - 90.9
Specificity	88.2%	84.5 - 91.1	88.4%	84.5 - 91.2	85.5%	81.7 - 88.8
PPV	49.9%	37.4 - 62.3	44.1%	30.7 - 58.0	37.7%	26.2 - 50.1
NPV	97.0%	95.4 - 97.6	95.0%	92.4 - 96.5	95.8%	93.5 - 97.0

Trial Issues: Repeat Testing



- Respective review of data by sponsors sometimes identifies quality control issues or instrument issues.
- Repeat testing of all or a subset of clinical specimens is sometimes performed by sponsors.
- In general, intended users test a specimen once and report the results.
- The goal of the analytical and clinical validation studies is to generate representative performance estimates of what intended users will see.



Biotin Interference

- Biotin is being increasingly marketed as a beauty supplement.
- Many tablets contain over 10 mg, suggesting consumers may be taking much higher amounts of biotin than the recommended Dietary Reference Intake (30 µg per day).
- Biotin-streptavidin is a common component of assay architecture due to the high affinity of streptavidin for biotin and binding under many chemical conditions.
- High levels of biotin in a patient's specimen can interfere with many laboratory tests.
- Assay susceptibility to biotin interference is variable in magnitude, and can cause positive or negative interference depending on the assay design and conditions.

Biotin Interference

- A proposed strategy for avoiding biotin interference for some tests is to have the patient stop biotin supplementation and wait several hours to several days prior to laboratory testing.
- Patients do not always tell healthcare providers they are taking biotin or may not know they are taking biotin, and healthcare providers may not inquire about biotin intake.
- Laboratories do not have a way to easily identify specimens with high levels of biotin that may cause interference with troponin assays.
- FDA has received a death report due to a patient taking a high dose of biotin that was associated with a false negative troponin result.



Safety Communication on Biotin Interference

- <https://www.fda.gov/MedicalDevices/Safety/AlertsandNotices/ucm586505.htm>



Discussion Topics for the Panel

- How can specimen stability be incorporated into the trial design?
- What differences in troponin results have users observed with different tube types?
- How can manufacturers better control test performance at the limit of quantitation?
- How can the reliability of troponin testing at different sites be maintained?
- How can we prevent unsafe use of troponin devices because of biotin interference?
What steps are necessary to address this issue?



Clinical Trials for Point-of-Care (POC) Devices

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Goals of Today's Session

- Benefits/Challenges of POC Troponin devices
- Considerations for designing clinical trials for POC Troponin devices
 - Examples of challenges observed by FDA
- Panel Discussion

Troponin Devices in POC environments

Benefits

- Convenience, e.g. less sample processing
- Immediate availability of results = more timely intervention
- May be the only device available/feasible in certain settings

Challenges

- Less controlled/predictable environmental conditions
- Users with less IVD testing training
- May not be interchangeable with other methods



When reviewing POC Troponin devices, FDA considers the full scope and unique characteristics of each device to assess the benefit/risk profile, including access to testing, turnaround time for results, etc.

POC Clinical Trial Design Considerations

- POC clinical trials for Troponin are difficult!
- Sample matrix performance differences
 - Lower or higher sensitivity in whole blood vs. plasma, even when the assay cut-off is matrix specific
- Stability to support clinical study samples for POC Troponin devices
 - Use of retrospective samples, lack of or insufficient bridging to fresh samples
 - Testing outside a validated sample stability window

POC Clinical Trial Design Considerations

- POC environmental challenges, e.g. variability in temperature
 - Exclusion of 35% of clinical study samples because of temperature-related instrument errors for device with narrow operating range (20-24°C)
- Site-to-site performance differences
 - Sample handling procedures
 - Patient populations
 - Demographic differences
 - Cut-off establishment
 - Collection of different sample types

Discussion Topics for the Panel

- What expectations do you have for the performance of POC Troponin devices?
- What should manufacturers include in their labeling to aid users at moderately complex POC Troponin sites?
- What are some of the challenges encountered while planning/executing a clinical trial for a POC Troponin device and how could they be addressed?
- How limited is too limited for POC Troponin devices that are intended for use in a narrow range of operating conditions?



Use of Existing Data to Support Claims

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Center initiative to promote use of RWE

Use of Real-World Evidence to Support Regulatory Decision-Making for Medical Devices

Guidance for Industry and Food and Drug Administration Staff

- <https://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm513027.pdf>

What is valid scientific evidence?

- Well-controlled investigations
 - Partially controlled studies
 - Trials without matched controls,
 - Well-documented case histories conducted by qualified experts,
 - Reports of significant human experience with a marketed device, from which it can fairly and responsibly be concluded by qualified experts that there is reasonable assurance of the safety and effectiveness of a device under its conditions of use.
- Sec. 860.7 Determination of safety and effectiveness.

Discussion topics for panel

- Discuss best practices for using existing clinical data
- Discuss additional useful clinical uses for troponin
- What sources of data may be useful to support troponin assays?

