B-281

# Analytical Validation of the Mindray High Sensitivity Cardiac Troponin I Assay

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## ABSTRACT

**Background:** According to recent global guidelines for patients presenting with ischemia, high sensitivity cardiac troponin I and T (hs-cTn) are the preferred biomarkers for the diagnosis and risk assessment of acute myocardial infarction and myocardial injury. The purpose of our study was to perform an analytical validation study of the Mindray hs-cTnI assay.

**Methods**: Analytical studies were designed according to Clinical and Laboratory Standards Institute protocols (CLSI). We used one reagent lot and one CL1200i chemiluminescence Immunoassay Analyzer (Mindray Bio Medical Electronics Co., Shenzhen, China). Following the CLSI EP17 A2 document, the limit of blank (LoB) and limit of detection (LoD) were assessed following analysis of a) Mindray controls without cTnI concentrations added for the LoB and b) 4 lithium heparin (LiHep) fresh samples with low cTnI concentrations, for 3 days for LoB and LoD, with 5 replicates per day (n = 60). For the precision study, according to the CLSI EP15-A3 protocol, we used 12 LiHep fresh samples, measured for 10 days, with 2 runs per day (separated by a minimal time of 2 hours) and in replicates of 3 (n = 720). For the 12 samples for the precision study, 7 were chosen with hs-cTnI concentrations close to the manufacturer sex-specific 99th percentile upper reference limits (URL; 31 ng/L for males and 15 ng/L for females). The CLSI EP06 protocol was followed to study linearity, using 9 fresh LiHep samples analyzed the same day in replicates of 7 (n = 63). A blank sample between every sample was used to avoid carryover. Our analytical specification for linearity was an allowable deviation <10%. Analyses were performed with Analyze it for Excel.

**Results:** Using both parametrical and non-parametrical analyses, LoB was <0.1 ng/L, with a relative light unit (RLU) of 5,394. LoD was 0.1 ng/L according to the parametrical analysis. Regarding imprecision, for samples ranging from cTnI concentrations of 1 to 106 ng/L, repeatability had a coefficient of variation (%CV) from 1.15% to 3.83%, and within-laboratory imprecision ranging from 1.69% to 5.03%. Using samples with hs-cTnI concentrations close to the manufacture's sex-specific upper reference limits (9-36 ng/L), the repeatability and within-laboratory precision was 1.27-1.61% and 1.92-2.52%, respectively. The measuring interval for the linearity study ranged from 1.1 to 30,772 ng/L. All analytical parameters met CLSI limits of acceptable performance. No carry over was detected.

**Conclusion:** Our analytical observations of the Mindray hs-cTnI assay demonstrate excellent LoB, LoD, imprecision and linearity, that were in alignment of with the manufacturer's claims and published guidelines for hs-cTnI.

## CONTACT

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## BACKGROUND

- According to recent global guidelines for patients presenting with ischemia, high-sensitivity cardiac troponin I and T (hs-cTn) are the preferred biomarkers for the diagnosis and risk assessment of acute myocardial infarction and myocardial injury.
- The purpose of this study was to perform an analytical validation study of the Mindray hs-cTnI assay in terms of limit of blank (LoB), limit of detection (LoD), precision, linearity, analytical specificity and sex-specific 99th percentile upper reference limits (URL).

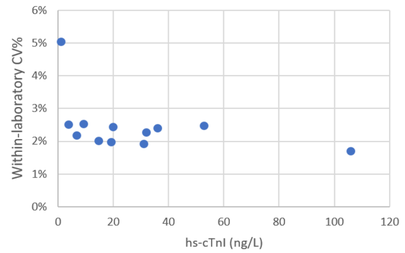
## METHODS

- Analytical studies were designed according to Clinical and Laboratory Standards Institute protocols (CLSI).
- We used one reagent lot and one CL1200i chemiluminescence immunoassay analyzer (Mindray Bio Medical Electronics Co., Shenzhen, China).
- **LoB and LoD study** (CLSI EP17 A2): 4 Mindray controls without cTnI concentrations for LoB and 4 lithium heparin (LiHep) fresh samples with low cTnI concentrations for LoD were analyzed for 3 days, with 5 replicates per day (n = 60). LoB was analyzed by using both parametrical and non-parametrical analyses, while LoD by parametrical analysis.
- **Precision study** (CLSI EP15-A3): 12 LiHep fresh samples, measured for 10 days, with 2 runs per day (separated by a minimal time of 2 hours) and in replicates of 3 (n = 720). For the 12 samples, 7 were chosen with hs-cTnI concentrations close to the manufacturer's sex-specific 99th percentile URLs (31 ng/L for males and 15 ng/L for females) . Repeatability and within-laboratory imprecision were calculated.
- **Linearity study** (CLSI EP06): 9 LiHep fresh samples were analyzed the same day in replicates of 7 (n = 63). A blank sample between every sample was used to avoid carryover. Our analytical specification for linearity was an allowable deviation <10%.
- **Analytical specificity study** (CLSI EP07-3): skeletal troponin I, skeletal troponin T, cardiac troponin T, troponin C, actin, tropomyosin, myosin light chain, myoglobin and CK-MB were studied for cross-reactivity. An interference screening test was carried out by spiking a Li-hep sample pool to obtain 50,000 ng/L of interferent (2,000 ng/mL in the case of biotin). Testing were performed in replicates of 5 for the control and spiked sample pool (n= 90). Interference from biotin was also examined. Our analytical specification for specificity was an allowable deviation <10%. If the interference screening test was positive, a dose-response experiment was conducted.
- **99th percentile URL study**: Healthy males and females (400 each) using Li-hep plasma from the AACC Universal Sample Bank were be measured in replicates of 1. Non-parametric statistic was used to calculate overall and sex-specific 99th percentile URLs. Analyses were performed before and after excluding those participants with any of the following criteria:
  - Abnormal health questionaire;
  - Increased NT-proBNP (>125 ng/L if <75 years of age or >450 ng/L if ≥75 years);
  - Hemoglobin A1c (HbA1c) ≥6.5%;
  - Estimated glomerular filtration rate (eGFR) <60 mL/min/1.73$m^2$.
- Analyses were performed with Analyze it for Excel (version 6.15).

## RESULTS

- **LoB and LoD study**: Using both parametrical and non-parametrical analyses, LoB was <0.1 ng/L, with a relative light unit (RLU) of 5,394. LoD was 0.1 ng/L according to the parametrical analysis.
- **Precision study:** for samples ranging from cTnI concentrations of 1 to 106 ng/L, repeatability had a coefficient of variation (%CV) from 1.15% to 3.83%, and within-laboratory imprecision ranging from 1.69% to 5.03% (Figure 1). Using samples with hs-cTnI concentrations close to the manufacturer's sex-specific URL (9-36 ng/L), the repeatability and within-laboratory imprecision was 1.27-1.61% and 1.92-2.52%, respectively (Figure 1).

**Figure 1: Within-laboratory imprecision of Mindray high-sensitivity cardiac troponin I**




- **Linearity study**: The measuring interval for the linearity study ranged from 1.1 to 30,772 ng/L. All analytical parameters met CLSI limits of acceptable performance. No carry over was detected.

**Table 1: Analytical specific for Mindray high-sensitivity cardiac troponin I**

| | hs-cTnI$_{control}$ (mean, ng/L) | hs-cTnI$_{spiked}$ (mean, ng/L) | Interferent concentration (ng/L) |
|---|---|---|---|
| Skeletal troponin I | 15.2 | 16.2 | 10,000 ng/L |
| Skeletal troponin T | 15.5 | 15.2 | 50,000 ng/L |
| Cardiac troponin T | 14.8 | 15.1 | 50,000 ng/L |
| Troponin C | 15.9 | 16.9 | 10,000 ng/L |
| Actin | 15.5 | 15.5 | 50,000 ng/L |
| Tropomyosin | 15.9 | 17.5 | 50,000 ng/L |
| Myosin light chain | 15.7 | 17.4 | 50,000 ng/L |
| Myoglobin | 15.8 | 15.6 | 50,000 ng/L |
| CK-MB | 6.3 | 6.1 | 10,000 ng/L |
| Biotin | 26.9 | 27.4 | 2,000 ng/mL |

**Table 2: Mindray AACC Universal Sample Bank upper reference limits (ng/L) by non-parametric statistic**

| | N (pre exclusion) | 99th (pre exclusion) | N (post exclusion) | 99th (post exclusion) |
|---|---|---|---|---|
| Overall | 822 | 15 | 702 | 11 |
| Female | 421 | 9 | 350 | 5 |
| Male | 401 | 15 | 352 | 14 |

## CONCLUSIONS

- Our analytical observations of the Mindray hs-cTnI assay demonstrate excellent LoB, LoD, precision and linearity, that were in alignment with the manufacturer's claims and guidelines for hs-cTnI.
- Mindray hs-cTnI has clinically aceptable analytical specificity for all of the interferents studied.
- We observed unique sex-specific 99th percentile URLs as expected between males and females.
- The Mindray hs-cTnI assay is aceptable for clinicial investigation for patient-oriented studies.

## ACKNOWLEDGEMENT

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