# ANALYTICAL CHARACTERIZATION OF A NOVEL NT-PROBNP ASSAY ON A CENTRAL LABORATORY PLATFORM

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A-009

## BACKGROUND

This study aims to define the analytical characteristics, perform Method Comparisons and establish valid Reference Limits for the novel Access NT-proBNP assay that will be widely available for clinical management of patients. Measurements were conducted with the Access NT-proBNP assay on the DxI 9000 Immunoassay Analyzer* with LiHep plasma samples.

## STUDY DESIGN

| Analytical Characteristic | Result(s) | Guidance Followed | Comment |
|---|---|---|---|
| Limit of Blank (LoB) | 1.1 ng/L | CLSI EP17-A2 | 4 reagent lots tested; 240 NT-proBNP-free data points |
| Limit of Detection (LoD) | 4.8 ng/L | CLSI EP17-A2 | 4 Li-hep samples between LoB and 4.5-fold higher. |
| Limit of Quantitation (LoQ) | 4.8 ng/L | NTproBNP level @ 20% CV | NT-proBNP concentration at 20% CV of assay |
| Imprecision | 3.0 to 7.9% Reproducibility; Table 1 | CLSI EP05-A3 | 3 reagent lots@ 3 labs; 7 concentrations 38 ng/L to 23,847 ng/L |
| Linearity | 38 ng/L to 25,000 ng/L | CLSI EP06-Ed2 | 3 reagent lots; 5 replicates of 15 samples across assay range |
| Interferences / Cross reactivity | No interferences | CLSI EP07-A3 | 74 drugs and substances & 15 biologicals tested. |
| Method Comparison | Figure 1 & 2; Table 2 | CLSI EP09C-ED3:2018 | 210 Residual Li-Hep samples; Passing Bablok linear regress; Bland Altman bias plots |
| Reference Interval Study | Table 3 & 4; Figure 3 | CLSI EP28-A3c & IFCC 2019 | 306 males and 369 Females |

Table 1. Imprecision of Access NT-proBNP across the Analytical Measuring Range

| LiHep Sample | N | Mean \ng/L | Between Site /Lot , CV (SD) | Between Day CV (SD) | Between Run \CV (SD) | Repeatability CV (SD) | Reproducibility CV (SD) |
|---|---|---|---|---|---|---|---|
| Sample1 | 60 | 38.24 | 6.9% (2.6) | 0.0% (0.0) | 1.7% (0.7) | 3.5% (1.3) | 7.9% (3.0) |
| Sample2 | 59 | 127.31 | 5.6% (7.1) | 0.6% (0.8) | 0.0% (0.0) | 2.9% (3.6) | 6.3% (8.0) |
| Sample3 | 59 | 288.19 | 4.9% (14.0) | 1.6% (4.7) | 0.7% (2.0) | 2.7% (7.8) | 5.8% (16.8) |
| Sample4 | 60 | 428.00 | 4.1% (17.4) | 0.0% (0.0) | 1.7% (7.3) | 1.5% (6.5) | 4.7% (20.0) |
| Sample5 | 60 | 1708.42 | 0.3% (5.2) | 1.4% (23.2) | 1.7% (29.5) | 2.0% (33.7) | 3.0% (50.7) |
| Sample6 | 60 | 11096.6 | 1.4% (159.7) | 0.8% (91.7) | 1.2% (135.2) | 1.7% (191.6) | 2.7% (298.2) |
| Sample7 | 60 | 23848.6 | 1.4% (334.0) | 0.0% (0.0) | 1.5% (367.5) | 2.2% (527.5) | 3.0% (724.4) |

## METHOD COMPARISON

Figure 1. Passing-Bablok Regression- Method Comparison between Access NT-proBNP and Elecsys NT-proBNP assays

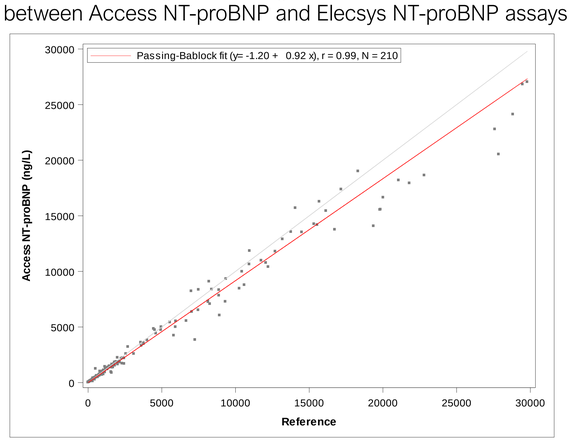



Figure 2. Bland-Altman Bias Plot comparing the Access and Elecsys NT-proBNP assays

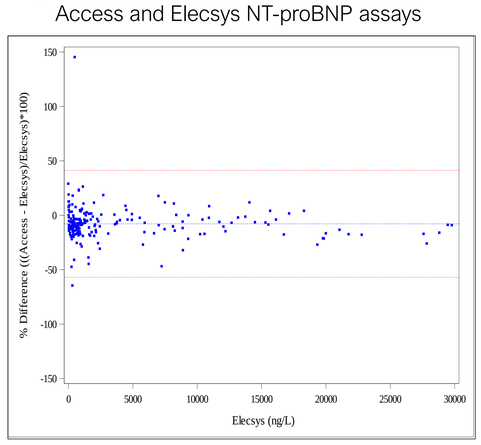



Table 2. Positive (PPA), Negative (NPA), & Overall (OPA) Method Agreement at Critical Decision Points

| Cutoff, ng/L | PPA | | NPA | | OPA | |
|---|---|---|---|---|---|---|
| | % | 95% CI | % | 95% CI | % | 95% CI |
| 125 | 97.9 | 94.8–99.2 | 100.0 | 82.4–100.0 | 98.1 | 95.2–99.3 |
| 300 | 97.7 | 94.3–99.1 | 100.0 | 89.6–100.0 | 98.1 | 95.2–99.3 |
| 450 | 96.6 | 92.4–98.6 | 100.0 | 94.1–100.0 | 97.6 | 94.5–99.0 |
| 900 | 91.4 | 84.9–95.3 | 96.8 | 91.0–98.9 | 93.8 | 89.7–96.3 |
| 1800 | 94.9 | 87.7–98.0 | 100.0 | 97.2–100.0 | 98.1 | 95.2–99.3 |

## REFERENCE INTERVAL STUDY

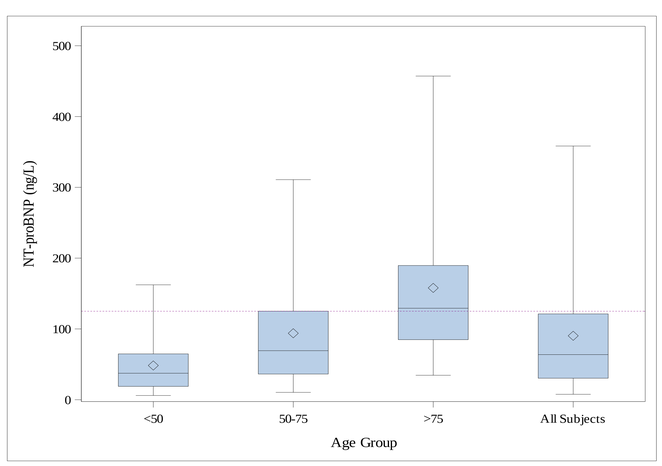
Table 3. Characteristics of Enrolled Reference Population

| | Female (N=369) | Male (N=306) | Total (N=675) |
|---|---|---|---|
| Age (years) | 62 (39, 75) | 54 (37, 72) | 57 (38, 74) |
| hsTnI (ng/L) | 2 (2, 3) | 3 (2, 5) | 3 (2, 4) |
| Overweight (25+) | 27.85% | 29.04% | 56.89% |
| eGFR (60+) | 51.56% | 43.26% | 94.81% |
| White | 47.56% | 31.56% | 79.11% |
| Non-Hispanic or Latino | 44.59% | 37.33% | 81.93% |

Table 4. Age and Sex Stratified Access NT-proBNP Values in a Healthy Population.. NHANES data in right-most column

| Age category (years) | Sex | N | Access NT-proBNP 2.5th Percentile (95% CI) | Access NT-proBNP 97.5th Percentile (95% CI) | Access NT-proBNP 50th Percentile (95% CI) | NHANES 50th Percentile (95% CI) |
|---|---|---|---|---|---|---|
| 21–29 | Female | 46 | 11 (7.9, 12.7) | 152 (148.1, 162.3) | 51 (39.9, 66.6) | 44 (41, 47) |
| | Male | 38 | 3 (3.1, 6.6) | 108 (75.4, 107.5) | 22 (15.6, 28.2) | 16 (15, 18) |
| 30–39 | Female | 51 | 12 (11.3, 14.9) | 152 (140.6, 178.2) | 54 (44.0, 64.9) | 50 (47, 54) |
| | Male | 52 | 6 (4.6, 6.3) | 103 (84.1, 118.1) | 23 (14.6, 30.8) | 21 (19, 23) |
| 40–49 | Female | 38 | 16 (16.4, 22.3) | 215 (205.8, 215.1) | 53 (44.6, 68.6) | 59 (54, 55) |
| | Male | 42 | 5 (4.6, 6.0) | 103 (92.1, 364.1) | 23 (18.8, 31.0) | 24 (21, 27) |
| 50–59 | Female | 42 | 17 (4.9, 19.1) | 273 (199.3, 369.8) | 60 (39.8, 76.2) | 71 (61, 81) |
| | Male | 55 | 8 (3.9, 10.1) | 188 (185.0, 266.8) | 31 (27.7, 43.8) | 41 (35, 46) |
| 60–69 | Female | 19 | 25 (3.9, 10.1) | 247 (185.0, 266.8) | 82 (54.4, 123.4) | 90 (75, 105) |
| | Male | 19 | 9 (3.9, 10.1) | 169 (185.0, 266.8) | 51 (36.1, 67.0) | 61 (49, 75) |
| 70–93 | Female | 173 | 31 (20.2, 40.5) | 443 (376.5, 521.0) | 136 (121.2, 152.1) | 177 (141, 222) |
| | Male | 100 | 19 (9.5, 28.0) | 411 (283.6, 461.8) | 100 (77.8, 117.6) | 155 (123, 193) |

Figure 3. Box and Whisker Plot for Reference Interval by Age Group and All-ages




Upper bound of the whisker indicates 97.5th Percentile Upper Reference Limit

## CONCLUSIONS

- The novel Access NT-proBNP assay offers robust analytical performance as an aid to acute HF management
- Imprecision of assay is acceptable (Table 1)
- Agreement with a reference assay is ≥94% (Table 2)
- Reference interval study shows that median results for all age and sex strata very similar to the NHANES national study. (Table 4)
- NT-proBNP Upper Reference Limit varies substantially with age, precluding use of a single URL for all patients (Figure 3)

*Full name: DxI 9000 Access Immunoassay Analyzer