Comparison of Analytical Performance of Two Single-Step Measurement Devices of B-Type Natriuretic Peptide

Junichi Ishida, MD, Toru Suzuki, MD, Kenichi Aizawa, MD, Daigo Sawaki, MD, and Ryozo Nagai, MD

SUMMARY

Rapid measurement of B-type natriuretic peptide (BNP) plays a practical role in the diagnosis of congestive heart failure. Analytical evaluation of a new small-footprint immunochromatography reader of BNP (Rapidpia®) was performed and compared with the commercially available SHIONOSPOT® Reader as the index. The new BNP assay had a within-run coefficient of variation (CV) of 9.0% and a between-run CV of 2.1%. Correlations between whole blood and plasma samples and those with the index SHIONOSPOT® Reader were y = 0.93x + 0.88, R² = 0.98 and y = 1.08x - 6.67, R² = 0.93, respectively. Based on our findings, the two point-of-care (POC) assays for BNP, Rapidpia® and SHIONOSPOT® Reader, showed comparable results. (Int Heart J 2012; 53: 320-323)

Key words: Congestive heart failure, Point-of-care

B-type natriuretic peptide (BNP) is a diagnostic and prognostic biomarker in congestive heart failure. According to the AHA/ACC guidelines, measurement of BNP in patients presenting to the emergency room with dyspnea is a Class IIA procedure, and according to Japanese guidelines, it is classified as Class I to diagnose acute and chronic heart failure. Because rapid measurement of BNP is especially useful for ruling out heart failure, point-of-care (POC) assays have been recently developed.

In the present study, the performance of a new immunochromatographic reader, Rapidpia® (Sekisui Medical Co., Ltd., Tokyo), was examined.

METHODS

Principle of measurement: In the test cartridge, anti-human BNP monoclonal antibody was immobilized on a nitrocellulose membrane, and the paired BNP antibody labeled with colloidal gold was stored on a conjugate pad. Basic measurements of the sandwich immunoassay such as the calibration curve were recorded on a calibration card. BNP concentration was indicated by the density of colloidal gold captured at the test line using the antigen-antibody sandwich reaction. BNP conjugated to the antibody labeled with colloidal gold, and the complex moved onto the membrane. This then bound to the immobilized antibody on the membrane, and a purple-red test line, indicating colloidal gold, was observed. Density was determined by measuring the absorbance of green light-emitting diode, followed by calculation of BNP concentration.

Assay procedures: The assay procedure was as follows: (i) A Rapidpia® (Sekisui Medical Co., Ltd., Tokyo) read the calibration card; (ii) an unopened test cartridge was kept at room temperature, and then inserted into the reader; (iii) a 120 μL sample was applied to its well; and (iv) BNP concentration was automatically measured within 15 minutes.

Collecting whole blood and plasma samples: Samples containing various BNP concentrations were prepared by adding synthetic BNP to whole blood or plasma samples from healthy subjects to evaluate the performance of Rapidpia®; i.e., precision, recovery ratio, detection limit, linearity, analytical sensitivity, prozone reaction, and correlation between specimen types.

To assess the correlation between assays, samples were obtained from patients who had undergone cardiac catheterization at the University of Tokyo Hospital. All patients provided written informed consent. Patients with acute myocardial infarction, unstable angina, acute heart failure, and end-stage renal disease were excluded. Five mL of whole blood was collected in a tube containing disodium EDTA (1 mg/mL blood) and stored at 4°C. Samples were separated by centrifugation at 2400 g for 15 minutes at 4°C and plasma samples were prepared. Within 6 hours, BNP levels were measured with Rapidpia® and SHIONOSPOT® Reader (Shionogi & Co., Ltd., Osaka, Japan).

Method correlation: Measurements using Rapidpia® were compared to those using SHIONOSPOT® Reader. Clinical samples (n = 57) were analyzed by both assays.
Statistical analysis: Statistical analysis for method comparison and correlation between plasma and whole blood was performed by Passing-Bablok regression analysis.5)

RESULTS

The detection limit was determined as the concentration corresponding to the signal obtained at two standard deviations from the mean of the signal of samples with zero BNP concentration. Ten replicates of the zero concentration samples were assayed and used to determine the mean and standard deviation. The apparent concentration at two standard deviations from this mean was extrapolated. The minimum detectable concentration, defined as the concentration at two standard deviations from the mean zero standard signal, was determined for 3 lots (7.5, 8.6, and 9.8 pg/mL) (Figure 1). The minimum detectable concentration of the assay was less than 10 pg/mL.

![Figure 1](image1)

Figure 1. The limit of detection was determined by replicate measurements (n = 10) of several plasma samples with different BNP levels and blank solution. Error bar indicates ± 2SD.

Table I. Precision of the Rapidpia® Assay

<table>
<thead>
<tr>
<th>Within-run</th>
<th>Within-run</th>
<th>Between-run</th>
</tr>
</thead>
<tbody>
<tr>
<td>(whole blood)</td>
<td>(plasma)</td>
<td>(plasma)</td>
</tr>
<tr>
<td>Low</td>
<td>Middle</td>
<td>High</td>
</tr>
<tr>
<td>Mean</td>
<td>61.4</td>
<td>148.9</td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.5</td>
<td>3.2</td>
</tr>
</tbody>
</table>

CV indicates coefficient of variation.

Table II. Recovery of the Rapidpia® Assay

<table>
<thead>
<tr>
<th>Theoretical value</th>
<th>Measurement value</th>
<th>Recovery ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 (30.4) + P2 (76.2)</td>
<td>53.3</td>
<td>52.5</td>
</tr>
<tr>
<td>P3 (100.9) + P4 (189.3)</td>
<td>145.1</td>
<td>143.1</td>
</tr>
<tr>
<td>P5 (281.5) + P6 (612.6)</td>
<td>447.1</td>
<td>444.0</td>
</tr>
</tbody>
</table>

![Figure 2](image2)

Figure 2. Linearity at both (A) moderately-high and (B) extremely-high BNP levels, (C) analytical sensitivity, and (D) prozone reaction.

![Figure 3](image3)

Figure 3. Method comparison using the Passing and Bablok regression model. A: Whole blood BNP (pg/mL) versus plasma BNP, Whole blood BNP = 0.93 (plasma BNP) + 0.88, $R^2 = 0.98$. B: Rapidpia® versus SHIONOSPOT®, Rapidpia®, BNP = 1.08 (SHIONOSPOT®) - 6.67, $R^2 = 0.93$. 

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Both within-run and between-run precision were determined using 3 samples. Within-run precision was calculated by consecutively testing whole blood or plasma samples in triplicate 10 times. Between-run precision was tested on 5 different days. Samples were stored at -80°C and thawed once for measurement. Within-run precision ranged from 3.0%-9.0% coefficient of variation (CV) (Table I). Between-run precision ranged from 1.2%-2.1% CV (Table I).

For recovery evaluation, 6 plasma samples were prepared at concentrations of 30.4 (P1), 76.2 (P2), 100.9 (P3), 189.3 (P4), 281.5 (P5), and 612.6 (P6) pg/mL. All samples were mixed at a ratio of 1:1 and assayed 3 times. Recovery was achieved by mixing samples of known concentrations ranging from 98.6%-99.3% (Table II).

Dilution linearity was assessed by serial dilution of moderately-high (400 pg/mL) or extremely-high (800 pg/mL) concentration samples. Results were plotted and observed values were compared to expected values. The upper detection limit and prozone reaction were evaluated using a serial dilution of prepared samples. Dilution linearity was determined by serial dilution of naturally occurring high-concentration samples or spiked samples. Serial dilution of high-concentration samples in ratios from 4:5 to 1:5 resulted in a linear plot of observed values compared to expected values (Figures 2A, 2B, 2C). BNP values were recorded as > 800 pg/mL, whereas during examination of prozone reaction, samples went up to 7968 pg/mL exceeding the upper limit of the measurement range (Figure 2D).

Comparison between whole blood and plasma samples was performed. Thirty matched samples were analyzed by linear regression analysis, which resulted in the following equation: whole blood = plasma (0.93) + 0.88, with a correlation coefficient of 0.99 (n = 30) (Figure 3A).

Correlation between assays was assessed. Passing-Bablok regression analysis of the BNP values revealed the following equation: Rapidpia® = SHIONOSPOT® Reader (1.08) - 6.67, with a correlation coefficient of 0.93 (n = 57) (Figure 3B).

**DISCUSSION**

At the present time, there are 7 instruments, [Rapidpia®, SHIONOSPOT® Reader, MI02® (Shionogi & Co., Ltd., Osaka, Japan), AIA® (Tosoh Co., Ltd., Tokyo), PATHFAST® (Mitsubishi Chemical Medicine Co., Ltd., Tokyo), CL-JACK® (Kyowa Medex Co., Ltd., Tokyo) and ARCHITECT® (Abbott Japan Co., Ltd., Tokyo)], which are commercially available for the measurement of BNP in Japan. Two of them, Rapidpia® and SHIONOSPOT® Reader, are POC test devices. Rapidpia® exhibited analytical performance, including detection limit, within-run, between-run, recovery, dilution-linearity, comparison between whole blood and plasma samples, and at comparable levels with the commercially available SHIONOSPOT® Reader. The values with Rapidpia® were about 1.08 times as large as those with the SHIONOSPOT® Reader. The reason for this difference remains to be identified. However, at a cutoff value of 100 pg/mL, the diagnostic agreement was very high (93.0%; Table III). Thus this marginal difference likely has little effect on the interpretation of results to diagnose or treat heart failure in actual clinical practice, especially in the emergency department. Correlations among BNP assays commercially used in Japan showed similar values, whereas Triage-BNP®, widely-used in the United States, exhibited about 1.5 times higher results than SHIONORIA® (Shionogi & Co., Ltd., Osaka, Japan) with close correlation. Physicians should be aware of the difference between BNP assays.

The measurement range of BNP concentration with Rapidpia® is 10-800 pg/mL, which is narrower than the SHIONOSPOT® Reader, but sufficient to diagnose heart failure. Accurate BNP values can be measured by adding diluted solutions with samples, if necessary.

In Japan, the normal upper limit of BNP is 18.4 pg/mL, whereas BNP levels are known to be affected by age, gender, obesity, and renal dysfunction in the presence or absence of heart failure. It is likely that the use of cut-offs adjusted for various factors will improve the clinical sensitivity of all BNP assays while maintaining high specificity.

Several features make the system easy to use, including its use of cartridge-type reagents, freedom from key operations for measurement, and minimal preanalytical tasks. In addition, as suitable for POC use, Rapidpia® weighs only 1.4 kg, has a footprint of only 30 cm², and can determine the BNP concentration in whole blood or plasma samples. Importantly, it allows consecutive measurements for approximately 10 samples as needed in clinical laboratory use and stored plasma samples can also be measured making this advantageous for retrospective research use.

While rapid measurement of BNP has become increasingly important, especially in the emergency room, it is not yet widespread in Japan. Furthermore, BNP-guided therapy, which reduces the risk of CHF-related death or hospital stay in CHF patients, requires faster determination of the analyte. Rapid, sensitive, and qualitative POC assays for BNP might help diagnose and treat heart failure.

**Conclusion:** Two POC assays for BNP, Rapidpia® and SHIONOSPOT® Reader, were compared and showed comparable results.

**ACKNOWLEDGMENTS**

Sekisui Medical Co., Ltd. provided Rapidpia® and Rapidchip® reagents, and Shionogi & Co., Ltd. provided SHIONOSPOT® Reader and SHIONOSPOT® BNP reagents.

**REFERENCES**

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