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현장검사용 SelexOn™ B형 나트륨이뇨펩티드 면역분석기의 성능 평가

Performance Evaluation of a Point of Care SelexOn™ B-Type Natriuretic Peptide Immunoassay

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Background: This study was conducted to evaluate the analytical performance of the SelexOn™ B-type natriuretic peptide (BNP) assay (Osang Healthcare Inc., Korea), a new rapid lateral flow immunoassay for point of care (POC) testing using whole blood.
Methods: The imprecision, linearity, and method comparison of SelexOn™ BNP assay were evaluated. Two commercial BNP assays, the ADVIA Centaur® BNP (Siemens Health Care diagnostics Inc., USA) and the Triage® BNP assays (Alere, USA), were included for method comparison using 100 whole blood samples from patients. The reference interval was verified using 120 residual samples from health examination participants.
Results: The SelexOn BNP had total CVs of 20.3%, 13.3%, and 10.3% in BNP concentrations of 89.44 pg/mL, 480.71 pg/mL, and 1,201.84 pg/mL of control materials, respectively. Linearity was observed from 56 pg/mL to 1544 pg/mL. The SelexOn BNP (y) regression equation was $y=0.9706x-21.68$ with Centaur BNP (x) ($r=0.930$) and $y=0.7600x+0.0506$ with Triage BNP (x) ($r=0.845$), respectively. The predicted mean difference (%) of the SelexOn BNP at the clinical decision levels (100 pg/mL) was up to 25% lower than the two comparative methods. The SelexOn BNP levels were below 50 pg/mL in 114 (95%) of the 120 samples.
Conclusions: The SelexOn BNP using EDTA was developed as a POC test for differential diagnosis or treatment monitoring for acute heart failure. However, clinical decision values must be improved to be compatible with other BNP methods.

Key Words: Point of care testing, B-type natriuretic peptide, Lateral flow immunoassay

INTRODUCTION

Heart failure is a major global cause of disease and death [1]. B-type natriuretic peptide (BNP) is currently regarded as a clinically useful biomarker for diagnosis of heart failure and monitoring treatment [2]. BNP is a biologically active hormone composed of 32 amino acids produced in the myocardium [2]. BNP increases in response to an increased myocardial wall stress [3]. Plasma BNP levels of 100 pg/mL and 400 pg/mL are helpful for exclusion and consideration of heart failure in untreated patients with symptoms of a heart failure, respectively [1, 3].

Various commercial BNP tests are performed using automated analyzers in many clinical laboratories [2]. The point of care (POC) test continues to be needed because it can reduce the clinical potential decision time in emergency situations by providing test results within approximately one hour [1]. However, the POC test should be verified to have a good correlation and precision compared to those in the clinical laboratory assays [1].

A new SelexOn™ B-type natriuretic peptide assay (SelexOn

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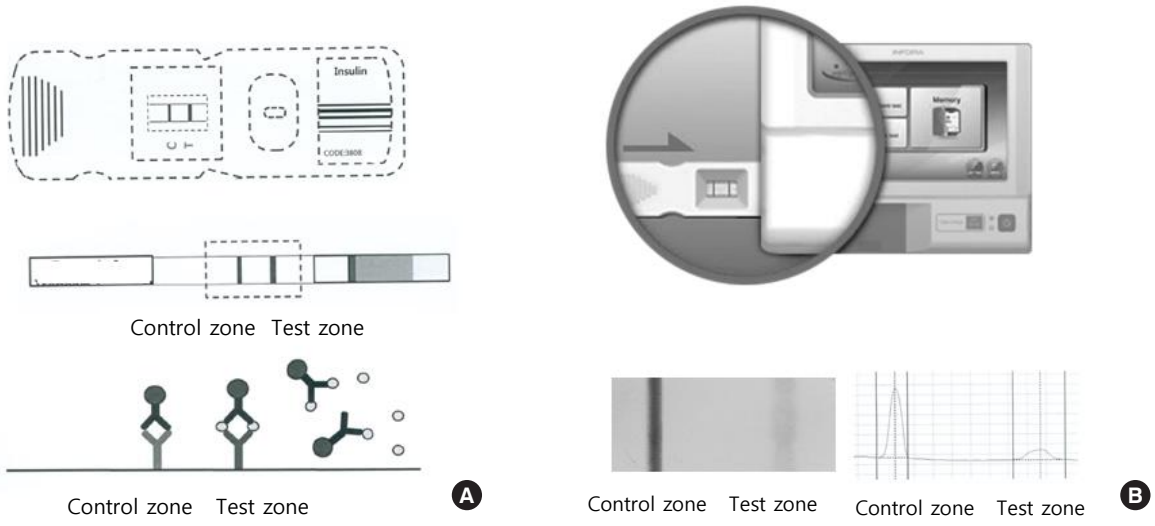

Fig. 1. The Selexon™ B-type natriuretic peptide (BNP) assay system. (A) The disposable cartridge uses a lateral flow immunoassay to detect BNP. Control and test lines are visible on the nitro-cellulose membrane. The control line indicates that the sample has migrated across the membrane as intended. (B) The camera inside the analyzer detects the signal line intensity. The integrated software converts the signal intensity to a quantitative result and shows it on the display.

BNP) (Osang Healthcare Inc., Anyang, Korea) was recently developed for POC testing. It consists of a single-use, disposable SelexOn™ cartridge and a portable automated analyzer system. The principle for quantifying BNP is a rapid sandwich immunoassay using gold particles conjugated with a specific antibody on a test cartridge using lateral flow with K_2EDTA-anticoagulated whole blood. The camera inside the analyzer detects the signal line intensity. The integrated software converts the signal intensity to a quantitative result by comparing with an internal calibration curve (Fig. 1).

MATERIALS AND METHODS

We evaluated the analytical performance of the SelexOn BNP compared to two BNP assays based on the Clinical and Laboratory Standards Institute (CLSI) guidelines. This study was approved by the Catholic Medical Center Institutional Review Board (OC16-DISI0012).

1. Imprecision

Imprecision was evaluated according to the CLSI guideline EP15-A3 [4]. Low, medium, and high levels of the quality control materials were provided by the manufacturer. The control materials were measured in five replicates a day for five days. Repeatability and within-laboratory imprecision at each level were assessed. Within-laboratory imprecision was compared to the desirable imprecision specification (15%) recommended by Apple et al. [5]. In addition, two K_2EDTA-anticoagulated whole blood samples near a clinical decision level of 100 pg/mL were measured in 20 replicates a day per sample to obtain repeatability since BNP should be measured within 4 h of collection at room temperature [5].

2. Linearity

Linearity was evaluated according to CLSI guideline EP6-A [6]. The claimed measuring interval ranged from 50 pg/mL to 2,000 pg/mL. Two patient samples with low (L) and high levels (H) were mixed as follows: 0.75*L+0.25*H, 0.5*L+0.5*H, 0.25*L+0.75*H. Each sample was measured three times using the SelexOn, and the mean values were compared to the expected values. Linear fit, second order, and third order polynomial regression analyses were performed. The regression equation was obtained (y, measured concentration; x, expected concentration) using a linear fit model.

3. Method comparison

Method comparison was performed according to CLSI guideline EP9-A3 [7]. Two BNP assays, the ADVIA Centaur® XPT BNP (Centaur BNP) (Siemens Health Care Diagnostics Inc., Tarrytown, NY, USA) and the Triage® BNP test (Triage BNP) (Alere, San Di-

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ego, CA, USA), were selected. The Centaur BNP is an automated sandwich immunoassay using EDTA plasma for central laboratory testing. The Triage BNP is a fluorescence immunoassay using EDTA plasma or whole blood for POC testing. K_2EDTA-anticoagulated whole blood in a plastic tube was used to perform the SelexOn BNP and the Triage BNP, and the corresponding EDTA plasma was used to perform the Centaur BNP. Regression equations between the compared assays were obtained using the Passing-Bablok fit.

4. Reference interval verification

A total of 120 EDTA whole blood residual samples obtained from individuals (aged from 22 years to 75 years; mean, 57 years), including 48 males (aged from 24 years to 75 years; mean, 60 years) and 72 females (aged from 22 years to 74 years; mean, 54 years) who participated in a health examination program without a medical history of cardiovascular disease or renal disease, were included to verify the reference interval (RI) of 100 pg/mL as a clinical decision point claimed by the manufacturer [1, 3, 8].

5. Statistics

Analyze-it version 5.20 (Analyze-it Software, Ltd., Leeds, UK) was used to analyze imprecision, linearity, and method comparison.

RESULTS

1. Imprecision

The repeatabilities were 15.8%, 9.0%, and 8.1% for three mean BNP control levels of 89 pg/mL, 481 pg/mL, and 1,202 pg/mL, respectively (Table 1). The total CV was 20.3% at 89.44 pg/mL, exceeding the desirable imprecision specification (15%), 9.0% at 480.71 pg/mL and 8.1% 1201.84 pg/mL, which met the desirable imprecision specification advised by Apple et al. [5]. However, mean BNP levels in two clinical samples were 91 pg/mL and 116 pg/mL, with repeatabilities of 4.6% and 6.9%, which were lower than 15.8% of the 89 pg/mL for the control material.

Table 1. Precision of the SelexOn™ B-type natriuretic peptide (SelexOn BNP) assay

Material	Mean BNP (pg/mL)	Repeatability		Within-laboratory	
		SD	CV	SD	CV
Control 1*	89	14	15.8%	18	20.3%
Control 2	481	43	9.0%	64	13.3%
Control 3	1,202	98	8.1%	124	10.3%
Sample 1†	91	4	4.6%	-	-
Sample 2	116	8	6.9%	-	-

*CLSI guideline EP15-A3 (five repeats a day for five days) for control materials.
†20 repeats a day for the K_2EDTA-whole blood samples.

2. Linearity

Linearity was observed from 56 pg/mL to 1544 pg/mL (Fig. 2). The regression equation between the measured (y) and the assigned values (x) was y = 16.38 + 0.9728x based on a linear fit (R^2 = 0.992, recovery rate: 95.8%-112.6%). The expected bias for non-linearity between the linear fit and the nonlinear (third order polynomial) fit was -20.6% (95% CI, -78.3% to 37.1%), 8.5% (0.1% to 16.8%), -1.1% (-4.6% to 2.4%), -3.9% (-6.9% to -0.8%), and 1.9% (-0.2% to 4.0%) at 56 pg/mL, 428 pg/mL, 800 pg/mL, 1,172 pg/mL, and 1,544 pg/mL, respectively.

3. Method comparison

A total of 100 patient specimens within the measuring range instructed by the SelexOn BNP manufacturer (50 pg/mL to 2,000 pg/mL) were available for method comparison (Table 2 and Fig. 3). The SelexOn BNP (y) showed correlation coefficients of 0.930 with the Centaur BNP (x) and 0.845 with the Triage BNP (x). The

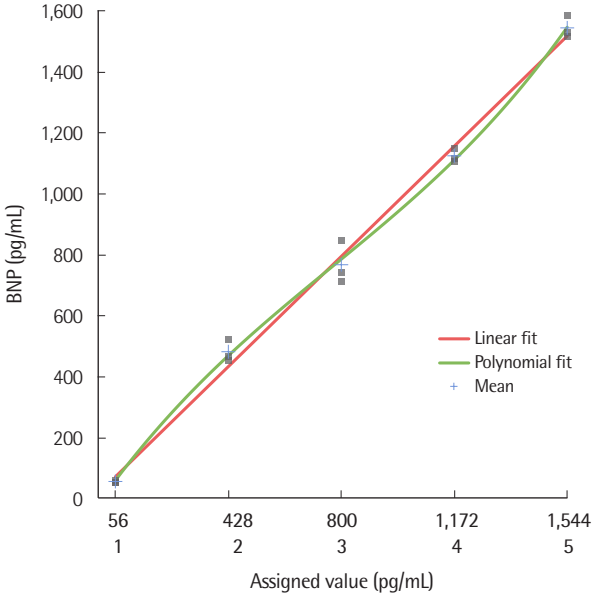

Fig. 2. Linearity of the SelexOn™ B-type natriuretic peptide assay. The red and green regression lines represent the linear and polynomial fits, respectively. The regression equation between the measured (y) and the assigned values (x) was y=16.38+0.9728x based on a linear fit (R^2=0.992).

Table 2. Method comparison of the SelexOn™ BNP with the Centaur® BNP and the Triage® BNP assays (N=100)

Methods compared (y vs. x)	Intervals measured (y, pg/mL)	*r*	Passing-Bablok fit equation	y-intercept (95% CI)	Slope (95% CI)
SelexOn vs. Centaur	52-1995	0.930	y=0.9706x-21.68	-49.27 to -5.654	0.8969 to 1.108
SelexOn vs. Triage	52-1995	0.845	y=0.7600x+0.0506	-22.60 to 30.62	0.6217 to 0.8576

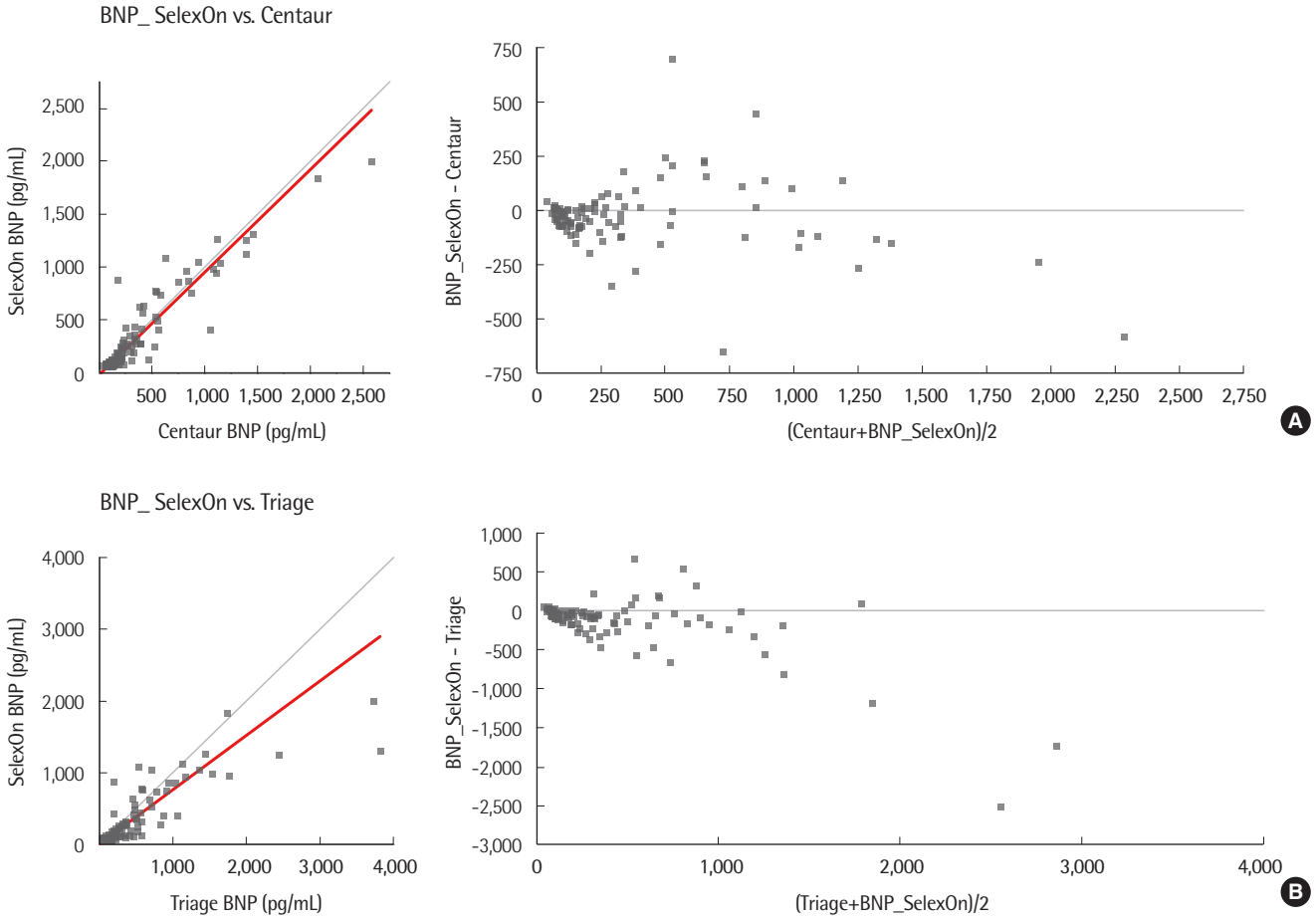

Fig. 3. Scatter plot of method comparison for the SelexOn BNP, the Centaur BNP, and the Triage BNP assays. The red line represents the linear regression equation by the Passing-Bablok fit (left) and difference plots (right). The SelexOn BNP (y) regression equation was (A) y=0.9706x-21.68 with Centaur BNP (x) (*r*=0.930), and (B) y=0.7600x+0.0506 with Triage BNP (x) (*r*=0.845).

Table 3. Distribution of BNP results from non-heart failure samples participating in the health examination program (N=120)

Age group	All (%)	All ages (N=120)		<45 years (N=22)		45-54 years (N=22)		55-64 years (N=37)		65-75 years (N=39)	
BNP (pg/mL)		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
<50	114 (95)	45	69	3	18	9	10	14	22	19	19
50-74	1 (0.8)	1	0	-	-	-	-	-		1	
75-100	2 (1.7)	1	1	1	-	-	1	-			
101-160	3 (2.5)	1	2	-	-	1	1	-	1		

regression equations were y=0.9706x-21.68 with the Centaur BNP and y=0.7600x+0.0506 with the Triage BNP, respectively. The predicted mean difference (%) ((y–x)/y×100) of the SelexOn BNP (y) was -24.63% at 100 pg/mL and -8.63% at 400 pg/mL, respectively, compared to the Centaur BNP (x). The predicted mean difference (%) of the SelexOn BNP (y) was -24.0% at 100 pg/mL and -24.0% at 400 pg/mL, respectively, compared to the Triage BNP (x).

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4. Verification of reference interval

Out of the 120 samples, 95.0% were less than 50 pg/mL, 96.3% were less than 75 pg/mL, and 97.5% were less than 100 pg/mL. The distribution of BNP results by age and gender have been summarized in Table 3. Of the 120 individuals across all ages, 45 males and 69 females showed BNP results below 50 pg/mL.

DISCUSSION

We evaluated the analytical performance of the SelexOn BNP for imprecision, linearity, method comparison, and verification of reference interval based on CLSI guidelines.

The total imprecision in the control material ranged from 10.3% to 20.3%, and the CV (20.3%) at 89 pg/mL exceeded 15% of a desirable specification for imprecision [5, 9]. Although repeatability was only evaluated in consideration of BNP stability in whole blood [5], similar low concentration clinical samples had lower repeatability (4.6%-6.9%) than control (15.8%). This may be because the substrate of the manufacturer-provided control material was not the same as that of the clinical sample. On the basis of the intra-individual biological variation (CV_I), the goal for imprecision should be less than or equal to half of the CV_I [5, 10]. BNP has a very wide intra-individual biological variation (CV_I, 30%-50%) like other hormones, and the total imprecision of the SelexOn BNP is considered less than the minimum requirement of analytical imprecision goal (25%) [9-12, 14].

BNP usually increases in heart failure, and patients with a higher BNP value (≥100 pg/mL) in the acute setting should be evaluated via further work-up to rule out heart failure [1, 3, 13]. If the BNP level rises above 35 pg/mL in patients with a suspected heart failure in the non-acute setting, additional echocardiography should be recommended for heart failure [13]. BNP can also increase in various cardiac and non-cardiac conditions like acute coronary syndrome, acute myocardial infarction, pulmonary embolism, hyperthyroidism, renal failure, or septic shock [11, 13]. Therefore, BNP is reportedly a useful biomarker for ruling out rather than diagnosing heart failure because BNP at clinical decision levels has high negative predictive values in both the acute and the non-acute settings [13]. In addition, High BNP during heart failure follow-up and monitoring is also reportedly associated with adverse outcomes [13].

Here, the correlation between the SelexOn BNP with the other two BNP assays was not high (r=0.930 for the Centaur BNP; r=0.845 for the Triage BNP), and the predicted mean difference (%) of the SelexOn BNP at clinical decision levels the 100 pg/mL and 400 pg/mL was up to 25% lower than that in the two comparable assays. The substantial differences between methods agree with the previous BNP immunoassay comparison studies [1, 2, 10, 14, 15]. Similar results have been reported in previous studies comparing the Triage and the Centaur BNPs [10, 14]. The main putative cause of the non-harmonized BNP assays is that there are no primary reference materials for BNP measurements; many manufacturers use different antibodies and calibrators for BNP immunoassays [1, 2, 16]. Therefore, it is important to identify and harmonize the systematic differences in BNP assays, and more appropriate clinical decision thresholds may be necessary for the individual BNP assay [2, 14]. If the BNP assay has a negative systematic bias, it may be interpreted as a false negative in disease differential diagnosis at low concentrations near 100 pg/mL. Our results suggest that calibration adjustments may be necessary to improve the negative systematic bias of the SelexOn BNP. Variations between lots can have a significant impact on the test results for cartridge-type POT tests, but the lot-to-lot variability was not evaluated in the present study.

In the reference interval verification, most (95% of the 120 individuals) with the SelexOn BNP exhibited values below 50 pg/mL. There was no difference observed between gender and age in the ratio outside the reference interval for the clinical decision point, but further study will be needed for stratified reference intervals. The BNP reference intervals for the healthy elderly are higher than the clinical decision level used for the heart failure guidelines [13, 17, 18]. Therefore, for the elderly, data should be interpreted in relation with the clinical symptoms [17, 18].

In summary, the SelexOn BNP using EDTA whole blood was developed as a POC test for differential diagnosis or treatment monitoring for acute heart failure. However, harmonization should be further strengthened to ensure that clinical decision values are compatible with other BNP methods.

요 약

배경: 이 연구는 전혈을 이용한 현장검사용 새로운 신속 측방유동 면역분석법인 SelexOn™ B형 나트륨이뇨펩티드(B-type natriuretic peptide, BNP) 검사(Osang Healthcare, Korea)의 분석적 성능을

평가하기 위해 수행되었다.

방법: SelexOn™ BNP 검사의 비정밀도, 직선성 및 검사법 비교를 평가하였다. 방법 비교를 위해서는 두 가지 상업용 BNP 분석법인 ADVIA Centaur® BNP (Siemens Healthcare diagnostics Inc., USA)와 Triage® BNP (Alere, USA) 검사를 100명의 환자 전혈을 이용하여 실시하였다. 참고구간은 건강검진 참가자 120명의 잔여 검체로 검정하였다.

결과: SelexOn BNP는 BNP 농도가 각각 89.44 pg/mL, 480.71 pg/mL 및 1,201.84 pg/mL인 경우 총 변이계수(CV)가 20.3%, 13.3% 및 10.3%였다. 직선성은 평가된 56 pg/mL-1,544 pg/mL범위에서 관찰되었다. SelexOn BNP (y)의 회귀 방정식은 각각 Centaur BNP (x)와 y=0.9706x-21.68 (r=0.930), Triage BNP (x)와 y=0.7600x+0.0506 (r=0.845)이었다. 임상적 의사결정농도(100 pg/mL)에서 SelexOn BNP의 예측 값은 두 개의 비교 가능한 분석법보다 최대 25% 낮았다. 120 검체 중 114 (95%) 검체에서 SelexOn BNP 수치가 50 pg/mL 미만이었다.

결론: SelexOn BNP가 EDTA 전혈을 사용하여 신속하게 급성 심부전의 감별 진단과 치료 모니터링을 위한 현장검사로 사용되기 위해서는 다른 BNP 분석법과 호환가능한 임상적 의사결정농도를 갖도록 더욱 향상되어야 할 것이다.

Conflicts of Interest

None declared.

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