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Research Article

# Comparison of Population-Based Reference Intervals and Reference Change Values of Some Analytes Used to Diagnose Insulin Resistance

**Fatma Nesrin Turan,[1] Hakan Erbas,[2] Mustafa Sukru Senocak,[3] Suphi Vehid[4]**

[1]Department of Biostatistics and Informatics, Trakya University Faculty of Medicine, Edirne, Turkey
[2]Department of Biochemistry, Trakya University Faculty of Medicine, Edirne, Turkey
[3]Department of Biostatistics, Istanbul University Faculty of Medicine, Istanbul, Turkey
[4]Department of Public Health, Istanbul University, Faculty of Medicine, Istanbul, Turkey

## Abstract

**Objectives:** The aim of this study was to examine the reference change value defined as a normal difference in serial test results to investigate clinically significant changes in a given proportion of all healthy persons.

**Methods:** In all, 18 volunteers were included in a cross-sectional and methodological study. The participants provided 5-mL blood samples twice a day, before and after having breakfast, 3 times in 15-day periods. The levels of glycose and insulin were evaluated biochemically and with a hormone auto-analyzer. Biological variation, the reference change value, and the individual index were calculated.

**Results:** The mean participant age±SD was 40.1±7.81 years, and the median age was 41.0 years (min: 27, max: 55 years). Twelve (66.67%) were female and 6 (33.33%) were male. While fasting blood sugar (FBS) was influenced by both time and individual characteristics (p=0.030 and 0.006, respectively), fasting insulin resistance (FIR) changed over time, but was not influenced by individual characteristics, according to the LOG10 base (p=0.796 and p=0.015, respectively). The level 1 FBS intra-individual variation was 7.62 and the inter-individual variation was 7.69. For level 2, the intra-individual variation was 7.71 and the inter-individual variation was 7.65. The intra-individual variation < the inter-individual variation for FIR in both level 1 (15.50<18.44) and level 2 (15.69<18.37).

**Conclusion:** Measurements of glucose and insulin levels should be examined using the analytic variation values of the device. This study demonstrates the importance of analytical variation, intra- and inter-individual variation, the index of individuality, and reference change value.

**Keywords:** Analytical variation, index of individuality, intra- and inter-individual variation, reference change value

The reference intervals, which play an important role in diagnostics, first observed when the laboratory tests were improved in 1965.[1] They were first used by the clinicians at the end of 1990s. As a result of that the importance of the reference intervals increased after 2000, plenty of authors stressed the importance of the careful choice of reference individuals[1, 2] of authors stressed the importance of the careful choice of reference individuals.[5, 22] Beside this, other medical decisions are important and laboratory tests provide essential information for the medical decision process. But when several serial test results for one person are available, there is another interesting approach for the interpretation of laboratory results. These laboratory results vary person to person.[3] It is an important fact that the population-based reference intervals (PBRI) for the use in medical decision process. However, PBRI may reflect

**Address for correspondence:** Fatma Nesrin Turan, MD. Trakya Universitesi Tip Fakultesi, Biyoistatistik ve Enformatik Anabilim Dali, Edirne, Turkey
**Phone:** +90 212 414 30 00 **E-mail:** fnesrin@trakya.edu.tr
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different results for different studies for the same analyte. The reasons for these differences depend on the various factors; individuals are different in age, growth properties, hormones, nutritional habits, pregnancy, menopause, and seasonal, geographical settlement, genetic, ethic , etc conditions.[4-6] Each specially adequate-sized population based data of an analyte may represent a "mean" and a Gaussian distribution of values.[5-7]

The aim of this study is to describe "reference change value" defined as "normal" difference between serial test results to investigate clinically significant changes in a given proportion of all healthy persons.

## Methods

After obtaining approval from Trakya University Medical Faculty Scientific Research Ethics Committee, Edirne, Turkey, all the volunteers were interrogated whether they had a disease which could affect their analyte levels before their blood was taken and whether they had a recently developed health problem or were not on the day of blood taking. Blood of volunteers, who were suspected of being diseased, were not taken. Informed consent form was received from all volunteers.

35 volunteers, who would take part in this study, were tested as for their ASL, ALT, Creatinin, Sodium, Potassium, Hemoglobine, HgA1C, HOMA-IR (Insulin resistance) levels for once and their waist circumferences were measured.

After the evaluation of these analytes, 25 volunteers were randomly chosen out of 35 volunteers, who were detected to be healthy, were included in the blood taking process, in which they would give 5 ml blood samples twice a day before and after having breakfast. It was repeated 3 times in 15-day periods. However, 8 volunteers were disqualified from this blood-taking process due to the exclusion criteria after the measurements. During the blood-taking evaluation period, the volunteers were required to meet the criteria of leading a healthy life. The volunteers were given standart breakfast after their fasting blood samples were taken and before postprandial blood samples were taken. The blood-taking process was based on some rules such as the same blood-taking technician should take the samples at the same hour on condition that the tourniquet time was equal and after allowing the volunteers to have a rest for 10 minutes before taking their blood. The samples were examined as for the levels of glycose and insulin with biochemistry and hormone auto-analyzer.

### Statistical Analysis

The required sample size was calculated using the following formula: $n=(1.96^2 \times \text{Standart Deviation}^2)/\text{margin of error}^2$ and when post-hoc power analysis was performed, 9 cases were found sufficient. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 19.0 Armonk, NY: IBM Corp., MedCalc V14.12.0 and Microsoft

Excel version 2010. HOMA –IR analyte was calculated downloading HOMA2 Calculator software.

Shapiro Wilk test was used to assessed the suitability of the data within the normal distribution. Logarithmic transformation was used for data out of normal distribution. For within group comparisons and individual differences, repeated measures ANOVA and two way analysis of variance was performed. Data was presented as mean±SD and median (min-max). Two tailed probabilities less than 0.05 were considered as significant.

For Fasting Blood Sugar (FBS) and Postprandial Blood Glugose (PBG) analytes were normal distribution, Reference change value (RCV) and Index of individuality (II) were calculated using raw data. For Fasting insulin resistance (FIR) and Postprandial insulin resistance (PIR) analytes where results were not normally distributed, data were log-transformed (to base LOG10) and then assessed in the same way as for the raw data.[4, 5, 8, 9]

Biological variation consists of three components. These are the intra-individual ($CV_I$), inter- individual ($CV_G$) and analytical variation ($CV_A$ ).[3, 6, 10, 11]

Serum insulin levels were measured with a chemiluminescence method using ADVIA Centaur XP Immunoassay System (Siemens Healthcare GmbH, Erlangen, Germany). Serum glucose were determined spectrophotometrically using an ARCHITECT c16000 chemistry system (Abbott Laboratories, Abbott Park, Illinois, U.S.A.).

Analytical variation was calculated using the 20-day calibration results of both auto analyzers.

The calculation of biological variation of analytes required the following formula:

$CV_I = (CV_{TI}^2 - CV_A^2)^{1/2}$,

$CV_G = (CV_T^2 - CV_I^2 - CV_A^2)^{1/2}$ formulas are being used.[2, 12] In these formulas;

$CV_I$: intra-individual variation, $CV_G$: inter- individual variation, $CV_{TI}$: Total intra-individual variation, $CV_T$: Total variation , $CV_A$ : analytical variation are the symbols.

The RCV calculation was based on the following formula: $RCV=\sqrt{2} \times Z(CV_A^2+CV_I^2)$, where $\sqrt{2}$ denotes the probability of two-tailed change and Z denotes 1.96 for %95 confidence interval. And then the individual index (II) calculation was based on the following formula: $II={}^{CV_I}/_{CV_G}$.[6, 12]

When II ≤0.6 and II≥1.4, PBRI are more appropriate to use, and when between 0.6-1.4, PBRI should be used caution.[5, 10]

## Results

35 volunteers were included in the study in accordance with the pre-defined criteria. The Mean±SD and Median (Min-Max) of volunteers were 37.00±9:35 and the mean age was 38 (23-56). 19 (54.29%) volunteers were female and 16 (45.71%) volunteers were male. The absence of insulin resistance was our exclusion criterion. Two volunteers had insulin resistance. 25 volunteers were chosen randomly from the pool of volunteers. The study was completed with 18 volunteers after the exclusion of 7 volunteers as not meeting the inclusion criteria. Mean age±SD 40.1±7.81 and 41.0 (27-55) Median (Min-Max) and respectively 12 (66.67%) were female, and 6 (33.33%) were male.

Fasting blood sugar is influenced by both time and the individual characteristics (p=0.030 and 0.006, respectively). While there was a significant difference between measurement 2 with the measurements 1 and 3 (p=0.050), there was no statistically significant difference between the measurements 1 and 3 (p=0.531).

Fasting insulin resistance changes on the time, but is not influenced by individual characteristics according to the LOG10 base (p=0.796 and p=0.015, respectively) There was no statistically significant difference between measurements 1 and 2, 1 and 3, 2 and 3 (p=0.999, 0.842 and 0.999, respectively).

Postprandial blood glucose level does not change depending on the time but is influenced by individual characteristics (p=0.315 and 0.005). There was no statistically significant difference between measurements 1 and 2, 1 and 3, 2 and 3 (p=0.999, 0.842 and 0.815, respectively).

Postprandial insulin resistance does not change depending on time and also not influenced by individual characteristics according to LOG10 base (p=0.985 and 0.060). There was no statistically significant difference between measurements 1 and 2, 1 and 3, 2 and 3 (p = 0.998, 0.993 and 0.998, respectively).

Table 1 shows the conducted analysis, the mean±standart deviation and median (minimum-maximum) values of the studied sample.

Table 2 and Table 3 show the analytical variation, intra and inter individual variation, the index of individuality and one- and two-tailed RCV at 95% and 99% confidence intervals.

**Table 1.** The descriptive statistics (mean±SD, Median (Min-Max)) of study parameters

| Parameters | Mean±SD | Median (Min-Max) |
|---|---|---|
| Age | 40.1±7.8 | 41.0 (27.0-55.0) |
| BMI | 26.2±4.1 | 26.2 (20.6-35.5) |
| Waist circumference (cm) | 87.4±12.1 | 86.5 (65.5-112.0) |
| AST (U/L) | 18.4±3.4 | 18.0 (13.0-24.0) |
| ALT (U/L) | 20.4±10.6 | 17.5 (7.0-49.0) |
| Creatinine (mg/dL) | 0.8±0.2 | 0.8 (0.5-1.2) |
| Na (mmol/L) | 140.9±2.5 | 141 (136.0-145.0) |
| K (mmol/L) | 4.3±0.3 | 4.3 (3.8-5.0) |
| Hemoglobin (g/dL) | 13.7±1.6 | 13.8 (10.0-16.2) |
| Hemoglobin A1c (%) | 4.2±0.2 | 4.2 (3.9-4.7) |
| HOMA-IR‡ | 1.7±0.8 | 1.5 (0.4-3.5) |
| FBS (mg/dL)* 1 m | 89.4±7.7 | 90.5 (77.0-102) |
| FBS (mg/dL) 2 m | 82.9±9.1 | 83.5 (69.0-96.0) |
| FBS (mg/dL) 3 m | 88.1±10.6 | 88.5 (63.0-105.0) |
| FIR (mU/L)** 1 m | 13.0±5.9 | 11.6 (3.5-27.5) |
| FIR (mU/L) 2 m | 13.6±8.9 | 11.1 (5.7-41.3) |
| FIR (mU/L) 3 m | 18.1±21.5 | 12.3 (3.7-94.2) |
| PBG (mg/dL)*** 1 m | 85.2±7.3 | 86.0 (69.0-96.0) |
| PBG (mg/dL) 2 m | 81.1±12.5 | 79.5 (59.0-108.0) |
| PBG (mg/dL) 3 m | 85.5±16.1 | 84.0 (52.0-118.0) |
| PIR (mU/L)****1 m | 19.8±13.6 | 17.4 (3.8-53.6) |
| PIR (mU/L) 2 m | 19.9±14.2 | 15.6 (4.7-50.1) |
| PIR (mU/L) 3 m | 20.6±17.8 | 14.0 (5.2-70.1) |

‡: Homeostatic Model Assessment- Insulin Value; *: Fasting blood sugar; m: measurement; **: Fasting insulin value; ***: Postprandial blood glucose; ****: Postprandial insulin value.

**Table 2.** According to Level 1, Reference change values and Individual Index according to biological variation of study parameters

| Analyte | $CV_A$ | $CV_I$ | $CV_G$ | RCV %99 | | RCV %95 | | II |
|---|---|---|---|---|---|---|---|---|
| | | | | One tailed | Two tailed | One tailed | Two tailed | |
| FBS(mg/dL) | 1.53 | 7.62 | 7.69 | 25.60 | 28.34 | 18.12 | 21.53 | 0.99 |
| FIR* | 1.97 | 15.50 | 18.44 | 51.48 | 57.00 | 36.45 | 43.30 | 0.84 |
| PBS(mg/dL) | 1.53 | 10.01 | 10.83 | 33.37 | 36.95 | 23.63 | 28.07 | 0.93 |
| PIR* | 1.97 | 19.18 | 16.09 | 63.53 | 70.35 | 44.99 | 53.44 | 1.19 |

*: data were log-transformed (to base LOG10).

**Table 3.** According to Level 2, Reference change values and individual index according to biological variation of study parameters

| Analyte | $CV_A$ | $CV_I$ | $CV_G$ | RCV %99 | | RCV %95 | | II |
|---|---|---|---|---|---|---|---|---|
| | | | | One tailed | Two tailed | One tailed | Two tailed | |
| FBS(mg/dL) | 1.27 | 7.71 | 7.65 | 25.74 | 28.50 | 18.22 | 21.65 | 1.01 |
| FIR* | 0.49 | 15.69 | 18.37 | 51.73 | 57.28 | 36.63 | 43.52 | 0.85 |
| PBS(mg/dL) | 1.27 | 10.08 | 10.76 | 33.48 | 37.07 | 23.71 | 28.16 | 0.94 |
| PIR* | 0.49 | 19.42 | 15.91 | 64.02 | 70.89 | 45.34 | 53.86 | 1.22 |

*: data were log-transformed (to base LOG10)

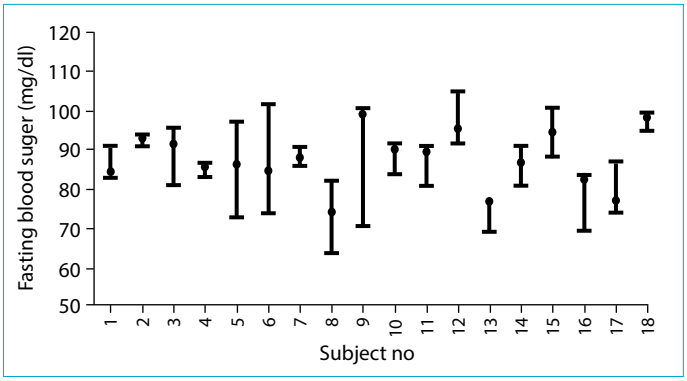


**Figure 1.** Fasting blood sugar (Median (Min-Max))

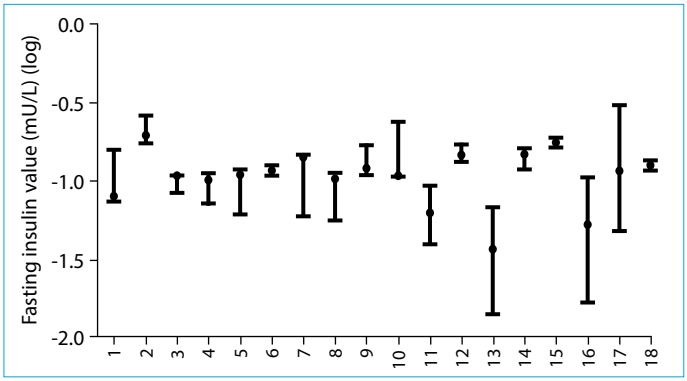


**Figure 3.** Fasting insulin value (Log) (Median (Min-Max))

Figures 1, 2, 3 and 4 show the median and absolute (minimum and maximum values) range of values FBS, PBS, FIV (log) and PIV (log).

## Conclusion

In this study, we demonstrated the calculation of RCV values for FBS, PBG, FIR and PIR parameters using the $CV_a$ value, which was obtained from the internal quality controls of the biochemistry and hormone auto analyzers, and the $CV_g$ value, which was obtained from our own database. Totally four different RCV values were calculated from the internal quality controls at two different levels of 0.95 and 0.99 confidence intervals and one- and two-tailed probability. The usage of RCV would be an answer to understand the changes in the value of an analyte at different times in the clinical laboratory and it will be useful in terms of deciding whether these decreases and increases show significant changes according to the laboratory results.

Many biological changes show that intra individual coefficient of variation is often lower than the variation between inter individual coefficient variation.[11, 13, 14] In our study, for FBS, CVI was 7.62 and CVg was 7.69 in level 1, CVI was 7.71 and CVg was 7.65 in level 2. Therefore, while CVI<CVg for level 1, CVI>CVg for Level 2. In this case, when the test results of individuals are compared with reference change interval, the results may not be useful in level 1. The situation is the totally reversed when we carry out an evaluation according to level 2. That the intra-individual biological variation of FBS was greater than the inter-individual variation in level 2 reflects that RCV is necessary to be attentive to the evaluation on the basis of population (Table 2) (5, 10). In other words, test results of the individuals might be

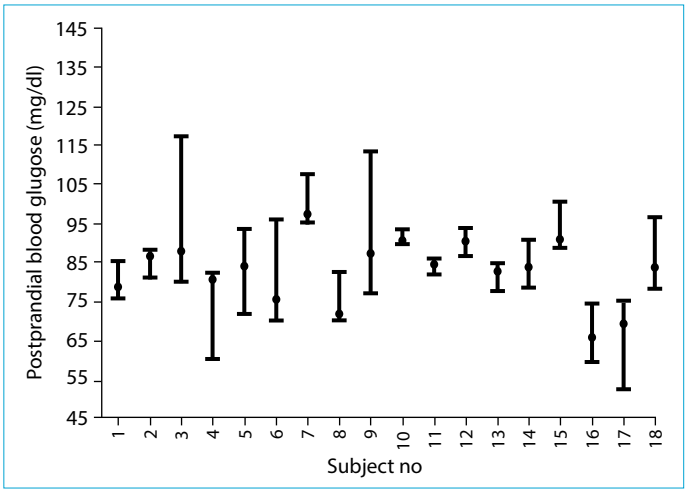


**Figure 2.** Postprandial blood glucose (Median (Min-Max))

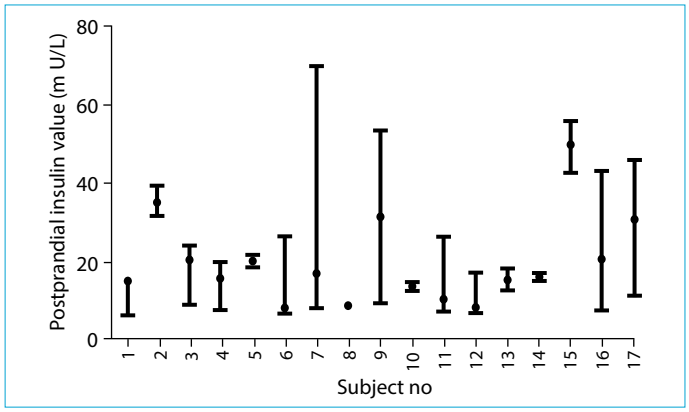


**Figure 4.** Postprandial insulin value (Log) (Median (Min-Max))

evaluated by population-based reference change interval in level 2, it is not possible for level 1.

CVI<CVg for FIR both in level 1 and level 2 (15.50<18.44 and 15.69<18.37, respectively) and it will be more useful to use intra individual variation based on RCV.[13]

Another criterion in the evaluation of laboratory results report is II. While the II is lower than 0.6, the usage of population-based reference intervals will be not convenient. Because, PBRI will cover only a small portion of the population. When the II is greater than 1.4, the usage of PBRI will be convenient. However, it should be treated very carefully if the II is in the range of 0.6 - 1.4.[13, 15] In our study, the II value was between 0.99 - 1.01 in accordance with the level 1 and level 2 for FBS, was between 0.84-0.85 for FIR, was between 0.93-0.94 for PPG and was located between 1:19 to 1:22 for PIR. In this case, the evaluation of the analytes should be carried out very carefully for both level 1 and 2.

In our study, when we carried out an evaluation for the lowest and the highest percentage change within the intervals of 2 weeks with two individuals concerning FBS, the decrease in the value (1.14%) of FBS was not significant in accordance with the confidence interval, which was between the reference values considering either level 1 with 95% (3.59) and 99% (5.07) or level 2 with 95% (2.97) and 99% (4.19).

The individual, who has the highest percentage change, is outside the reference values with 95% (59.82) and 99% (84.48) according to both level 1 level 2 and the decrease in the value of FBS is significant according to the confidence interval (30.69%) (Table 4).

The results of the evaluation carried out for FBS within the 2-week intervals showed that the individual with the lowest percentage change 95% (7.27) and 99% (20.29) in level 1 as well as in level 2 by 95% (5.90) and 99% (20.99) is within the reference value and the decrease in the value of FBS is not significant according to the confidence interval (1.04%).

Furthermore, if we consider the individual with the highest percentage change; in level 1 with 95% (53.76) and 99% (75.92) and in level 2 with 95% (53.04) and 99% (74.89), the increase in the value of FIR is within the reference value and is not significant according to the confidence interval (% 59.73) (Table 4).

When we carried out an evaluation for the lowest and the highest percentage change within the intervals of 4 weeks with two individuals concerning FBS, the individual with the lowest percentage change 95% (3.58) and 99% (5.06) in level 1 as well as in level 2 by 95% (2.96) and 99% (4.18) and the decrease in the value of FBS is not significant according to the confidence interval (0.00%).

The individual, with the highest percentage change, is outside the reference values with 95% (43.24) and 99% (61.06) according to both level 1 and level 2, and the decrease in the value of FBS is significant according to the confidence interval (23.17%) (Table 5).

As a result of the evaluation carried for FIR, it is seen that the individual, who has the lowest percentage change, is within the reference values with 95% (4.60) and 99% (6.49)

according to level 1 and 95% (2.60) and 99% (3.68) according to level 2 and the increase in the value of FIR is not significant in accordance with the confidence interval (1.20%).

When we carried out an evaluation for the individual, who has the highest percentage change, s/he is outside the reference values with 95% (97.60) and 99% (137.82) according to both level 1 and level 2, and the increase in the value of FIR is significant according to the confidence interval (59.73%) (Table 5).

As a result, it is important to remember that some individuals are within the boundaries of population-based reference ranges while some others may be outside.

In this study, the glucose and the insulin levels of the in-

**Table 4.** Evaluation of two individuals with the lowest and the highest percentage change (1st and 2nd measurements)

| | Analyte | 1. Result | 2. Result | Change (%) | Level 1 | | Level 2 | | Reference Interval |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | One-tailed RCV (%99) | Two tailed RCV (%95) | One-tailed RCV (%99) | Two tailed RCV (%95) | |
| 1. Individual | FBS(mg/dL) | 87 | 86 | 1.14 | 5.07 | 3.59 | 4.19 | 2.97 | 70-105 |
| (minimum) | FBS(mg/dL) | 85 | 84 | 1.18 | 5.06 | 3.58 | 4.18 | 2.96 | 74-140 |
| | FIR (mU/l) | 11.24 | 10.96 | 1.04 | 20.29 | 7.27 | 20.29 | 5.90 | 3-25 |
| | PIR(mU/l) | 17.53 | 16.65 | 1.80 | 12.01 | 6.15 | 12.00 | 8.50 | 3-25 |
| 2. Individual | FBS(mg/dL) | 101 | 70 | 30.69 | 84.48 | 59.82 | 84.48 | 59.82 | 70-105 |
| (maximum) | FBS(mg/dL) | 83 | 60 | 27.71 | 74.95 | 53.08 | 74.89 | 53.04 | 74-140 |
| | FIR (mU/l) | 3.48 | 7.33 | 59.73 | 75.92 | 53.76 | 75.92 | 53.76 | 3-25 |
| | PIR(mU/l) | 3.79 | 8.86 | 63.73 | 186.77 | 132.26 | 186.77 | 132.26 | 3-25 |

**Table 5.** 1st and 3rd measurement evaluation for two individuals with the lowest and the highest percentage change

| | Analyte | 1. Result | 3. Result | Change (%) | Level 1 | | Level 2 | | Reference Interval |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | One-tailed RCV (%99) | Two tailed RCV (%95) | One-tailed RCV (%99) | Two tailed RCV (%95) | |
| 1. individual | FBS | 77 | 77 | 0.00 | 5.06 | 3.58 | 4.18 | 2.96 | 70-105 |
| (minimum) | FBS | 91 | 90 | 1.09 | 5.06 | 3.58 | 4.18 | 2.96 | 74-140 |
| | FIR (mU/l) | 15.27 | 15.78 | 1.20 | 6.49 | 4.60 | 3.68 | 2.60 | 3-25 |
| | PIR(mU/l) | 14.15 | 12.84 | 3.81 | 8.73 | 6.18 | 8.70 | 3.93 | 3-25 |
| 2. individual | FBS | 82 | 63 | 23.17 | 61.06 | 43.24 | 61.06 | 43.24 | 70-105 |
| (maximum) | FBS | 80 | 118 | 47.50 | 89.48 | 63.37 | 89.43 | 63.33 | 74-140 |
| | FIR (mU/l) | 11.83 | 94.23 | 83.99 | 137.82 | 97.60 | 137.82 | 97.60 | 3-25 |
| | PIR(mU/l) | 12.82 | 5.23 | 54.19 | 99.35 | 70.36 | 99.35 | 70.36 | 3-25 |

dividuals were measured using the biochemistry and hormone auto analyzers. After using the analytical variation values of the devices, and examining the biological variation of both the sample and the individuals, it has become obvious that the interpretation of the laboratory results requires care and sensitivity. Furthermore, it is recommended that a software should be uploaded on the devices to facilitate giving PBRI for the devices as well as giving the biological variations while evaluating the analytes.

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**Ethics Committee Approval:** The study was approved by Trakya University Medical Faculty Scientific Research Ethics Committee.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** None declared.

**Authorship contributions:** Concept – M.S.S., F.N.T.; Design – M.S.S., F.N.T.; Supervision – M.S.S., F.N.T., SV.; Materials – H.E., S.V.; Data collection &/or processing – H.E., F.N.T.; Analysis and/or interpretation – M.S.S., F.N.T.; Literature search – M.S.S., F.N.T.; Writing – M.S.S., F.N.T., H.E., S.V.; Critical review – M.S.S., F.N.T.

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