



Objective

To compare normal pooled plasma (NPP) with Ci-Trol (Dade® Citrol 1® Coagulation Control Level 1) when used in the Nijmegen Assay (NA) for inhibitor measurement

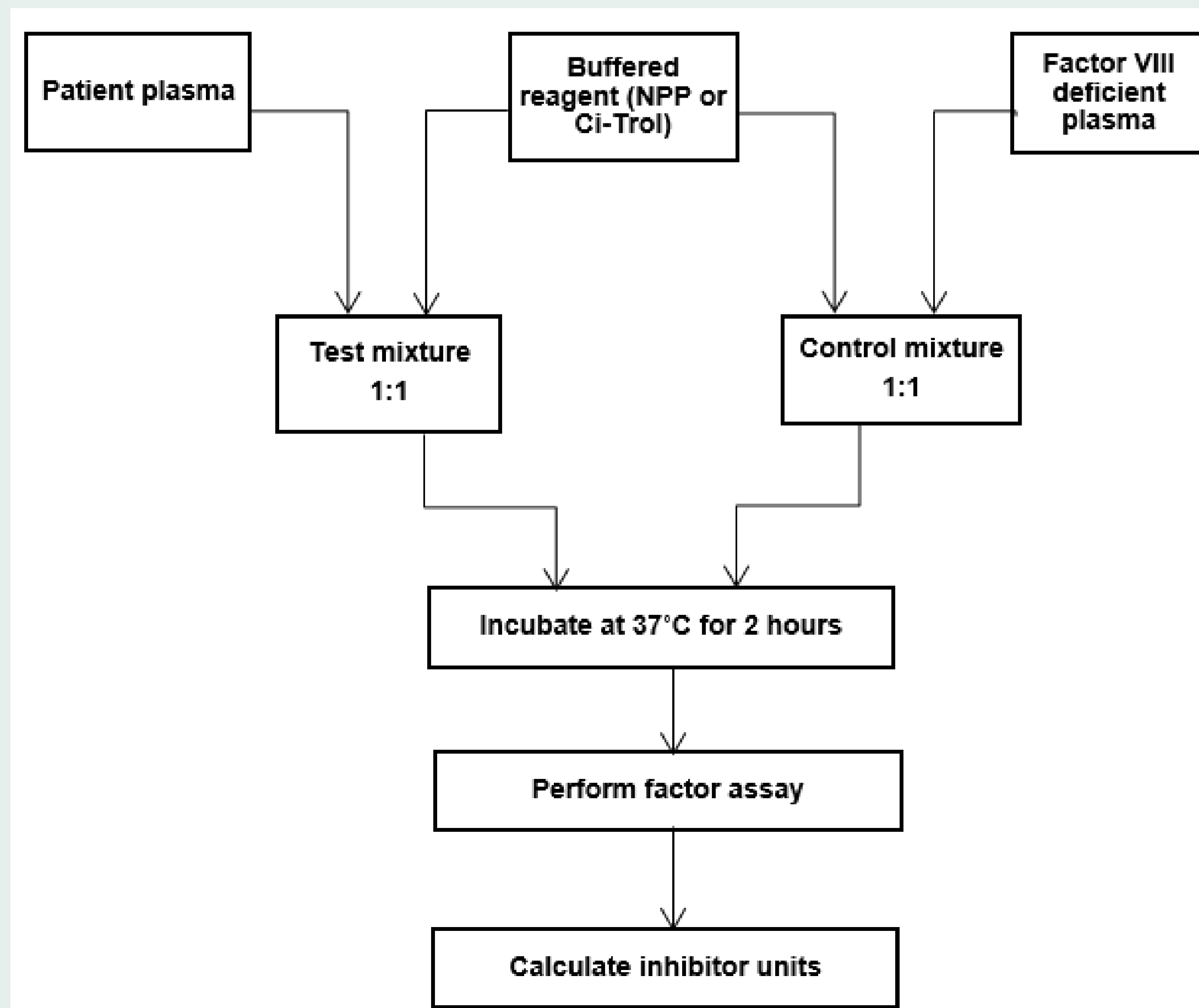
Introduction

Inhibitor measurement is important in the diagnosis, therapeutic monitoring and surveillance of inhibitor in haemophilia patients. At low inhibitor values, the Nijmegen assay (NA) has better reliability compared with the Bethesda assay (BA). Reliability of the BA and NA is partially dependent on effectively buffered reagents such as normal pooled plasma (NPP) and Ci-Trol.

Material and Methods

Set-up and optimization of the NA was as described by Verbruggen *et al* in 2014^{1,2}. The NPP and Ci-Trol were buffered with imidazole. Using either the NPP or Ci-Trol, the NA was performed on control plasmas with assigned values (from ECAT foundation) and known inhibitor patient plasmas from our centre [Figure 1]. Precision results done on control samples were expressed as standard deviation (SD) and coefficient of variation (CV). Accuracy results were expressed as bias and 95% confidence intervals (CI). A bias of <5% and precision within 2SD of mean were considered acceptable. In addition, correlation studies between NA and BA were performed and presented as linear regression graphs.

Figure 1. Flow diagram of Nijmegen Assay.



Results

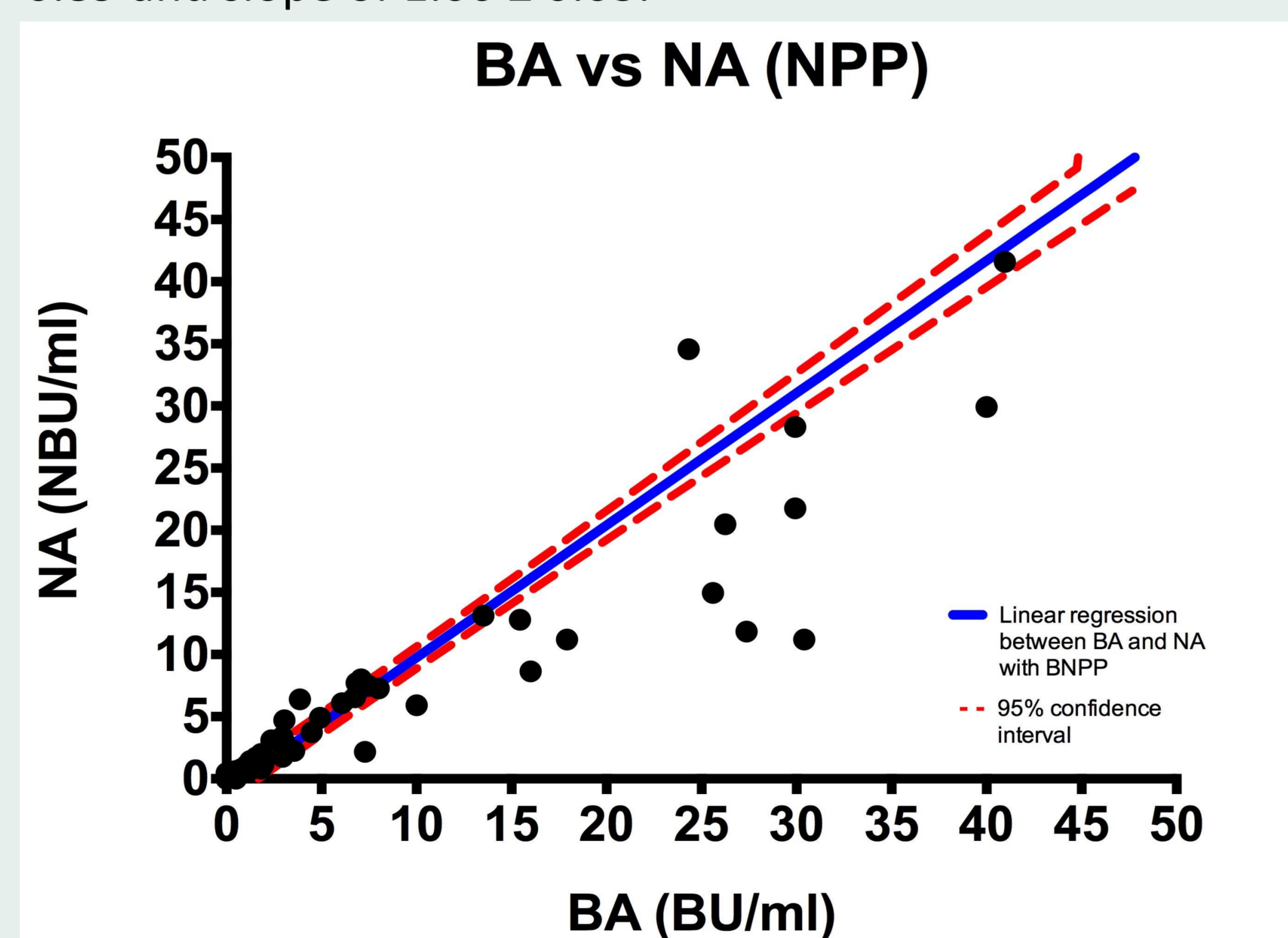
Of the 100 patient plasmas, 100 were analyzed with NPP and 43 with Ci-Trol. In the NA using NPP, the control plasma CV was 8.44% (95%CI of 0.77 ± 0.05) with a SD of 0.06. In the NA using Ci-Trol, the control plasma CV was 10% (95%CI of 0.93 ± 0.02) and SD was 0.09. In the Bland-Altman plot of NA vs BA using the NPP, the bias was 0.49 (95%CI of -8.1 to 9.1) [Figure 4]. The bias between NA and BA using Ci-Trol was 0.8 (95%CI of -6.8 to 8.5) [Figure 5]. The correlation coefficient of NA vs BA using the NPP was 0.93 and that of the NA vs BA analysis using the Ci-Trol was 0.76 [Figure 2 & 3]. All control plasma analyses using NPP were within reference values whilst only 2 of 3 values were within the assigned values using Ci-Trol [Table 1].

Table 1. Analysis of NA with different buffered reagents using ECAT control samples

	NA with NPP (NBU/mL)	NA with Ci-Trol (NBU/mL)	Expected value (NBU/mL)	ECAT mean range
INH-24	0,65	1,03	0,50	0.00 - 1.20
INH-25	3,64	4,00	2,80	1.00 - 4.60
INH-26	8,24	10,24	6,10	2.10 - 10.10

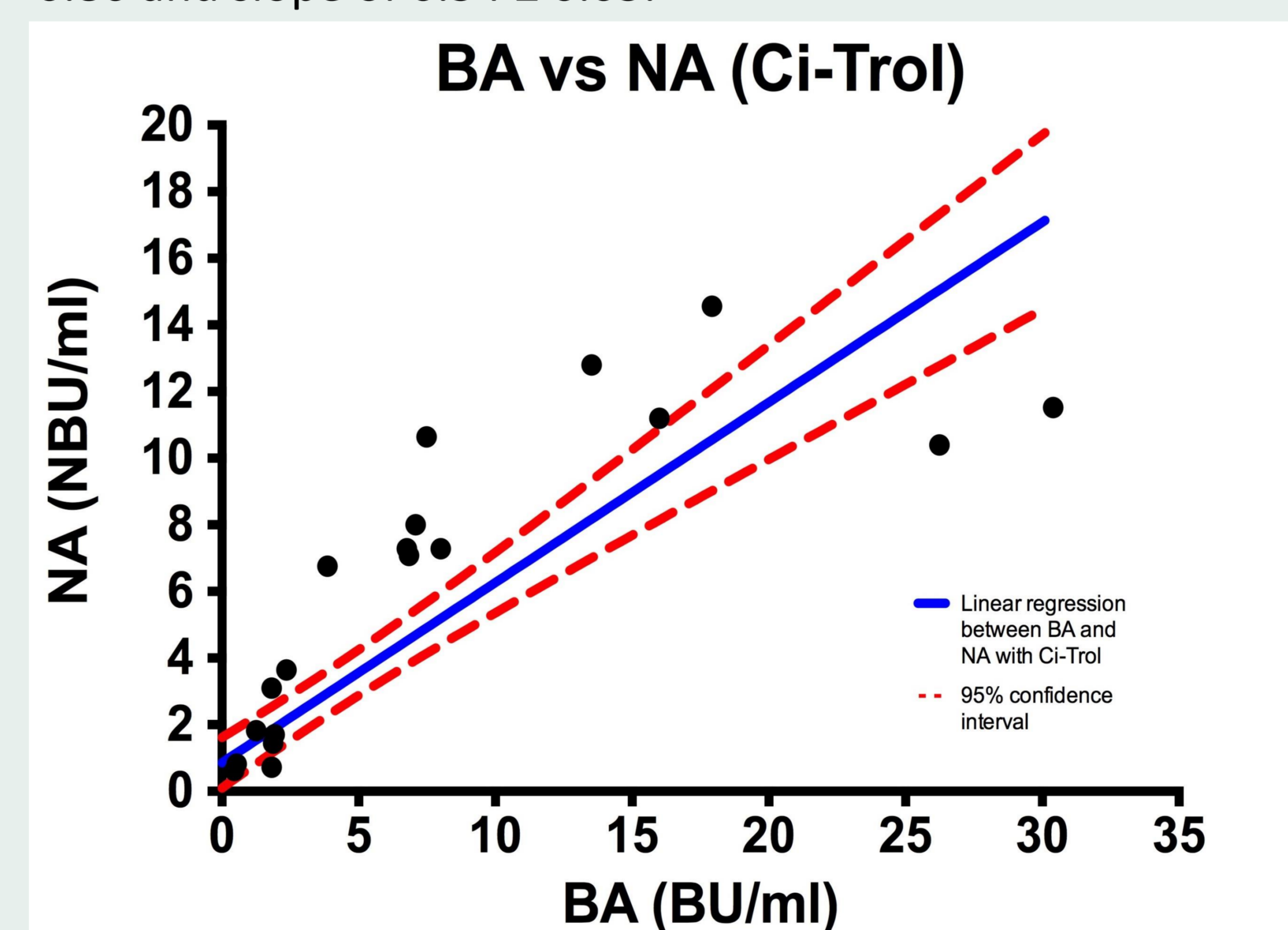
Abbreviations: ECAT, ECAT foundation; SD, standard deviation; INH-24, -25, -26, ECAT control samples; NA, Nijmegen Assay; NPP, normal pooled plasma; Ci-Trol, Dade® Citrol 1® Coagulation Control Level 1; NBU/mL, Nijmegen-Bethesda unit per millilitre.

Figure 2. Linear regression graph demonstrating NA using NPP against BA. Correlation studies between NA (NPP) and BA showed a $R^2 = 0.93$ (P-value <0.0001), Y-intercept = -0.89 and slope of 1.06 ± 0.03 .



Abbreviations: BA, Bethesda Assay; NA, Nijmegen Assay; BU/mL, Bethesda unit per millilitre; NBU/mL, Nijmegen-Bethesda unit per millilitre; NPP, buffered normal pooled plasma.

Figure 3. Linear regression graph demonstrating NA using Ci-Trol against BA. Correlation studies between NA (Ci-Trol) and BA shows $R^2 = 0.76$ (P-value <0.0001), Y-intercept = 0.86 and slope of 0.54 ± 0.05 .

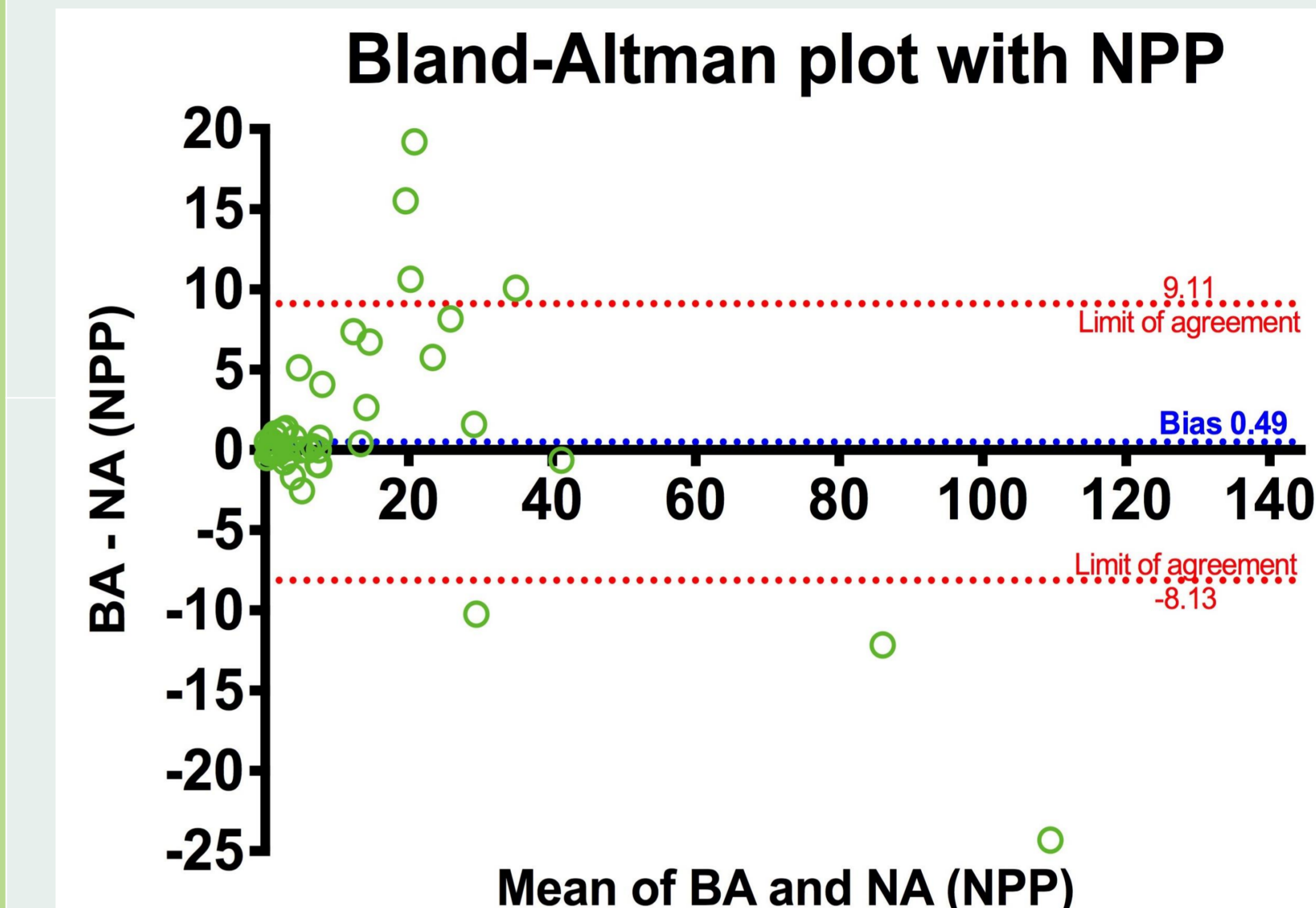


Abbreviations: BA, Bethesda Assay; NA, Nijmegen Assay; BU/mL, Bethesda unit per millilitre; NBU/mL, Nijmegen-Bethesda unit per millilitre; Ci-Trol, buffered Dade® Citrol 1® Coagulation Control Level 1.

References

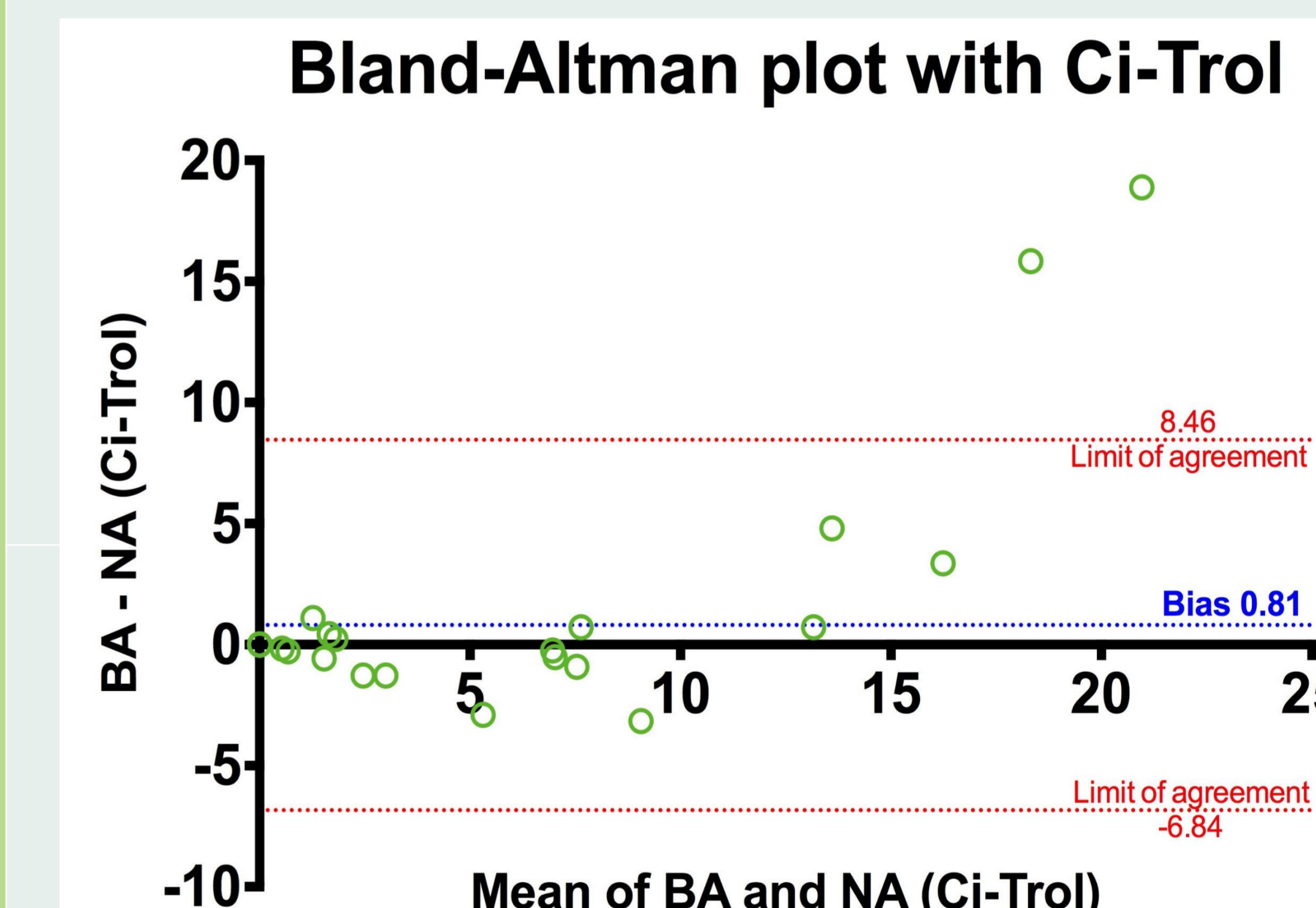
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- Favaloro EJ, et al. Haemophilia. 2014;20 Suppl 4:94-8.

Figure 4. Bland-Altman plot demonstrating accuracy between BA and NA with NPP.



Abbreviations: BA, Bethesda Assay; NA, Nijmegen Assay; NPP, buffered normal pooled plasma.

Figure 5. Bland-Altman plot demonstrating accuracy between BA and NA with Ci-Trol.



Abbreviations: BA, Bethesda Assay; NA, Nijmegen Assay; Ci-Trol, buffered Dade® Citrol 1® Coagulation Control Level 1.

Conclusion

In this limited analysis of patient and control plasmas, the performance of the Nijmegen assay with buffered normal pool plasma was better than that of the same assay using buffered Ci-Trol.

Disclosure

Authors report no conflict of interest in this study.