

**A COMPARATIVE EVALUATION OF THREE POINT-OF-CARE  
PROTHROMBIN TIME TESTS, PROTINE, PROTINE3 AND  
INRATIO, TO A LABORATORY REFERENCE STANDARD**

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## SUMMARY

**Purpose:** An in-house study was conducted using clinical specimens obtained from the ITC outpatient clinic to compare three point-of-care (POC) Prothrombin Time (PT) systems, ProTime (“ProTime5,” full volume 5-channel test), ProTime3 and INRatio, to the laboratory reference.

**Methods:** Whole blood samples (fingerstick and venous) were obtained from patients on oral anticoagulant therapy (Coumadin, Warfarin) and tested on the POC systems. Venous samples were tested on two instruments to evaluate precision. Venous samples (plasma) also underwent testing on a laboratory reference system using an MLA Electra 900 (Medical Laboratory Automation, Pleasantville, NY) and Dade Innovin reagent (ISI=0.98, Dade Behring, Newark, DE) to assess accuracy.

**Results:** *Accuracy using Venous Samples:* All POC tests were highly correlated to the lab reference system with  $r=0.96$ ,  $0.93$ , and  $0.96$  for ProTime5, ProTime3, and INRatio, respectively. Relative differences between each POC test and the reference system were calculated with respect to INR range,  $<2.5$  and  $\geq 2.5$ . INRatio was statistically different ( $p<0.05$ ) from the reference system in both ranges while ProTime5 and ProTime3 were equivalent to the reference system, regardless of INR range.

*Accuracy with Fingerstick Samples, ProTime3 vs. INRatio:* Both POC tests were highly correlated to the reference system with  $r=0.92$  and  $0.97$  for ProTime3 and INRatio, respectively. Relative differences were calculated between each POC test and the reference with respect to INR range,  $<2.5$  and  $\geq 2.5$ . INRatio was statistically different from the reference system for INR values  $\geq 2.5$  ( $p<0.05$ ). ProTime3 was equivalent to the reference system, regardless of INR range.

*Accuracy with Fingerstick Samples, ProTime5 vs. INRatio:* Both POC tests were highly correlated to the lab reference system with  $r=0.97$  and  $0.98$  for ProTime5 and INRatio, respectively. Relative differences were calculated between each POC test and the reference with respect to INR range,  $<2.5$  and  $\geq 2.5$ . INRatio was statistically different from the reference system ( $p<0.05$ ) for INR values  $\geq 2.5$ . ProTime5 was equivalent to the reference system, regardless of INR range.

*Precision:* Pooled SD, which provides a measure of reproducibility, was calculated with duplicate results collected with ProTime3 and INRatio. Pooled SD= $0.24$  and  $0.37$  for ProTime3 and INRatio, respectively.

*“No Result” Rate:* During the performance of this study, no result was periodically obtained due to various sampling, internal control or technical test errors. The “no result” rate was lower for both ProTime assays compared to INRatio with rates equal to  $5.4\%$ ,  $4.2\%$ , and  $11.2\%$  for ProTime3, ProTime5, and INRatio, respectively.

**Conclusion:** Results demonstrate all POC systems were highly correlated to a standard laboratory reference method. INRatio demonstrated a positive bias that was exaggerated in the higher INR range. The ProTime Microcoagulation System (ProTime5 and ProTime3) demonstrated a smaller bias and accurate INR results across the entire INR range. Results confirm both ProTime assays are appropriate for monitoring the coagulation status of patients on oral anticoagulant therapy. It is important that all sites establish their own correlation with the local laboratory to identify institutional variation between POC systems and their unique reference systems.

## INTRODUCTION

The ProTime Microcoagulation System (ITC, Edison, NJ) has been used to manage patients on Coumadin therapy in professional and patient self-testing settings since 1995 and 1996, respectively. This system employs a 5-channel assay (ProTime5) which is unique in triplicate PT testing and on-board controls. A second assay, ProTime3, was added to the system in 2001 to meet the need for a PT test requiring less blood. This assay is a single PT test with on-board controls. Other test systems have entered the POC PT marketplace, including the INRatio PT Monitoring System (HemoSense, Milpitas, CA). To date, no comparison of these systems has been published.

POC system accuracy is typically validated by comparing INR results between the POC system and a common laboratory reference. POC precision is evaluated by obtaining repeat test measurements. This study compares accuracy and precision of these POC systems.

## METHOD

Fresh whole blood samples (n=37), collected from patients on long-term anticoagulant therapy via venipuncture, were tested simultaneously using a split-sample experimental design. Multiple lots (n=3) of each POC assay were tested in this study. Two lots were used for each POC assay in the venous phase of the study. Different lots were evaluated in the fingerstick phase for each POC assay. Venous samples were tested on two instruments simultaneously for ProTime3 and INRatio, creating the duplicate testing for precision evaluation of these systems. Venous blood was collected (3.2% sodium citrate) from patients at the time of POC testing in both the venous and fingerstick phases for comparative laboratory plasma results. Plasma was tested using the laboratory reference system, MLA Electra 900 (Medical Laboratory Automation, Pleasantville, NY) and Dade Innovin reagent (ISI=0.98, Dade Behring, Newark, DE). The MLA-Innovin system used in this study has been verified against a national reference laboratory (Midwest Hemostasis, Muncie, IN). The acceptability of this system is substantiated as it has been part of a proficiency test program (College of American Pathologists or CAP) since 1999 in which it consistently performed within  $\pm 1$  SD of the proficiency program's nominal result (internal ITC data). Furthermore, MLA instrumentation is cited in the package insert of all POC systems under investigation as the reference instrument.

Fingerstick testing was performed by obtaining two separate fingerstick samples from each patient (n=34); one fingerstick sample was tested on ProTime5, the other on INRatio. The same procedure was used to compare fingerstick results on ProTime3 and INRatio (n=56 patients). The reference INR from venous blood was obtained for all participants in the fingerstick evaluation. Fingerstick samples were independent and unique for each POC assay tested. No randomization was employed.

Linear regression, bias and outlier analyses were used to assess agreement between each POC system and the lab reference. Bias (relative differences between each POC system and the reference) and outlier analyses were performed with data separated into two INR classes,  $< 2.5$  and  $\geq 2.5$ . This arbitrary INR separation was established to provide study balance for meaningful evaluation. Classification was determined by lab reference system (MLA-Innovin) where INR values ranged from 0.91 to 4.76 for the study. Paired differences [POC-reference] were categorized by agreement within 0.4 INR, and as discrepant by 0.7 or 1.0 INR. INR differences of 0.4 and 0.7 have been used as benchmarks in comparative evaluations of the prothrombin time test<sup>1</sup>. Clinically significant inter-laboratory variations in INR have been documented in several studies<sup>2,3,4,5</sup>; 0.4 INR is considered acceptable variation<sup>1</sup>, based on the negative impact on clinical decision.

Pooled SD, which provides a measure of precision, was calculated using duplicate results collected with ProTime3 and INRatio. ProTime5 was not included in the precision evaluation as the ProTime5 cuvette performs triplicate PT tests and reports the median value. ProTime3 and INRatio are more analogous in that each cuvette or test strip performs a singlet PT test.

### RESULTS & DISCUSSION – ACCURACY WITH VENOUS SAMPLES

Regression analyses were performed to compare each POC system with the lab reference (Table 1, Figure 1). All three systems were highly correlated to the lab reference system with correlation coefficient ( $r$ ) = 0.96, 0.93, and 0.96 for ProTime5, ProTime3 and INRatio, respectively. However, slope values varied among POC systems. INRatio vs. Reference produced a slope=1.40, indicating a strong positive bias to the laboratory system, while ProTime5 and ProTime3 both generated slopes closer to 1.0, suggesting small or no bias.

Table 1: Regression Analysis, POC vs. Reference System, Venous Samples

POC System	n	slope	intercept	r
ProTime5	36	0.96	0.31	0.96
ProTime3	37	0.90	0.27	0.93
INRatio	36	1.40	-0.36	0.96

Figure 1 – Accuracy using Venous Samples, All POC Methods vs. Reference

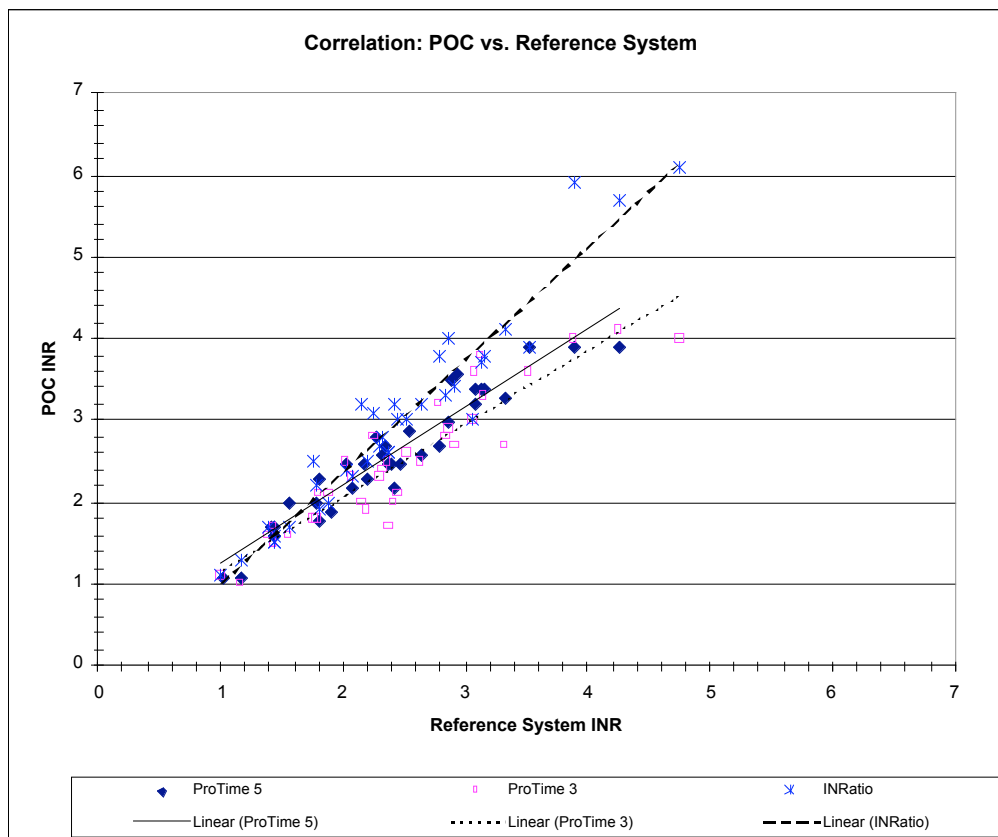


Table 2 summarizes relative differences for each system. Differences calculated for the population were lower for both ProTime assays as compared to INRatio. Analysis of variance (ANOVA) was performed to statistically compare the POC methods to the lab reference system. Both ProTime assays demonstrated statistical equivalence. INRatio results were significantly different ( $p < 0.05$ ). ANOVA findings were consistent for both INR ranges. Table 2 also shows how frequently each POC system was in agreement with the lab reference within 0.4 INR. For values  $< 2.5$  INR, both ProTime assays were within 0.4 INR of the lab reference 82% of the time while INRatio was in agreement less frequently (68%). Frequency of agreement was somewhat diminished with ProTime3 for values  $\geq 2.5$ . INRatio frequency of agreement dramatically decreased for values  $\geq 2.5$  such that agreement with the lab reference was within 0.4 only 14% of the time.

Table 2: Bias Analysis [POC-Reference System], Venous Samples

		ProTime5	ProTime3	INRatio
<2.5 INR*	n	22	22	22
	population avg. (INR)	2.1	1.9	2.2
	avg. relative difference vs. Reference	<b>0.20</b>	<b>0.03</b>	<b>0.35**</b>
	SD of difference	0.19	0.29	0.28
	within 0.4 INR (n, %)	18 (82%)	18 (82%)	15 (68%)
$\geq 2.5$ INR*	n	14	15	14
	population avg. (INR)	3.3	3.3	4.1
	avg. relative difference vs. Reference	<b>0.20</b>	<b>0.01</b>	<b>0.80**</b>
	SD of difference	0.28	0.38	0.53
	within 0.4 INR (n, %)	12 (86%)	10 (67%)	2 (14%)

\*INR classification determined by Reference System result

\*\* Statistically significant difference ( $p < 0.05$ ) when compared to the Reference System

Table 3 identifies values for each POC system that disagreed with the reference system by greater than 0.7 and 1.0 INR. Almost no discrepant results occurred with the ProTime assays while INRatio yielded numerous outliers.

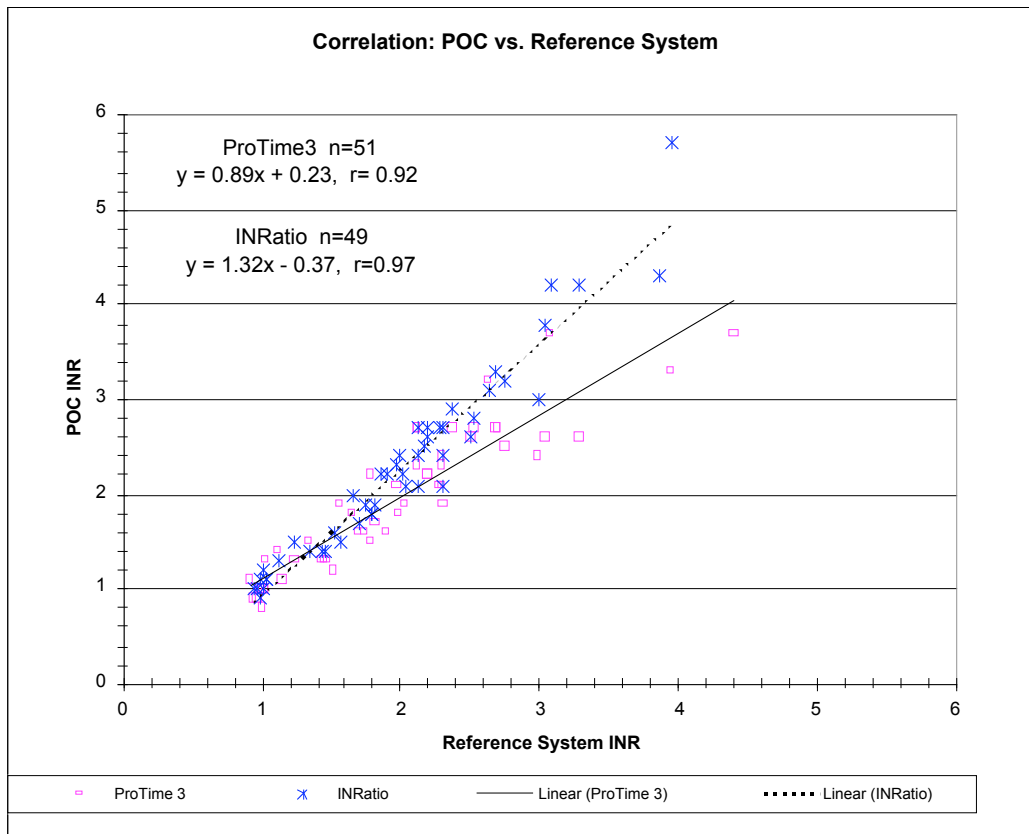
Table 3: Discrepant INR Results [Comparison of POC and Reference Lab System], Venous Samples

		ProTime5	ProTime3	INRatio
<2.5 INR	n	22	22	22
	values discrepant by $> 0.7$ INR (n, %)	0 (0%)	0 (0%)	4 (18%)
	values discrepant by $> 1.0$ INR (n, %)	0 (0%)	0 (0%)	1 (5%)
$\geq 2.5$ INR	n	14	15	14
	values discrepant by $> 0.7$ INR (n, %)	0 (0%)	1 (7%)	6 (43%)
	values discrepant by $> 1.0$ INR (n, %)	0 (0%)	0 (0%)	5 (36%)

**RESULTS & DISCUSSION – ACCURACY WITH FINGERSTICK SAMPLES**  
**ProTime3 vs. INRatio**

Regression analyses were performed to compare both POC systems with the lab reference (Figure 2). Both were highly correlated to the laboratory reference with correlation coefficient (r) = 0.92 and 0.97 for ProTime3 and INRatio, respectively. Slope values were different between the two systems. Slope=1.32 for INRatio, whereas slope=0.89 for ProTime3. These slopes were directionally consistent with venous sample testing on each system.

Figure 2 – Accuracy using Fingerstick Samples, ProTime3 and INRatio vs. Reference Lab System



Relative differences are summarized in Table 4. Differences calculated for the population were smaller with ProTime3 in both INR classifications. Table 4 also shows the frequency of agreement for each POC system relative to the lab reference result within 0.4 INR. Agreement with the lab reference system was less frequent in the higher INR range for both POC systems. ProTime3 demonstrated a greater percent agreement with the lab reference system in both INR classifications. An ANOVA was performed to compare the POC methods with the lab reference system. ProTime3 demonstrated statistical equivalence in both INR ranges. INRatio differences were statistically significant ( $p < 0.05$ ) for INR values  $\geq 2.5$ .

Table 4: Bias Analysis [POC-Reference System], Fingerstick Samples, ProTime3 and INRatio

		ProTime3	INRatio
<2.5 INR*	n	39	38
	population avg. (INR)	1.7	1.9
	avg. relative difference vs. Reference	<b>0.02</b>	<b>0.17</b>
	SD of difference	0.23	0.19
	within 0.4 INR (n, %)	35 (90%)	33 (87%)
≥2.5 INR*	n	12	11
	population avg. (INR)	2.9	3.7
	avg. relative difference vs. Reference	<b>-0.16</b>	<b>0.62**</b>
	SD of difference	0.47	0.50
	within 0.4 INR (n, %)	5 (42%)	3 (27%)

\*INR classification determined by Reference System result

\*\* Statistically significant difference (p<0.05) when compared to the Reference System

Table 5 identifies values for each POC system that disagreed with the lab reference system by greater than 0.7 and 1.0 INR. No such discrepant results occurred with the ProTime3 while INRatio yielded several discrepant results in the higher INR range.

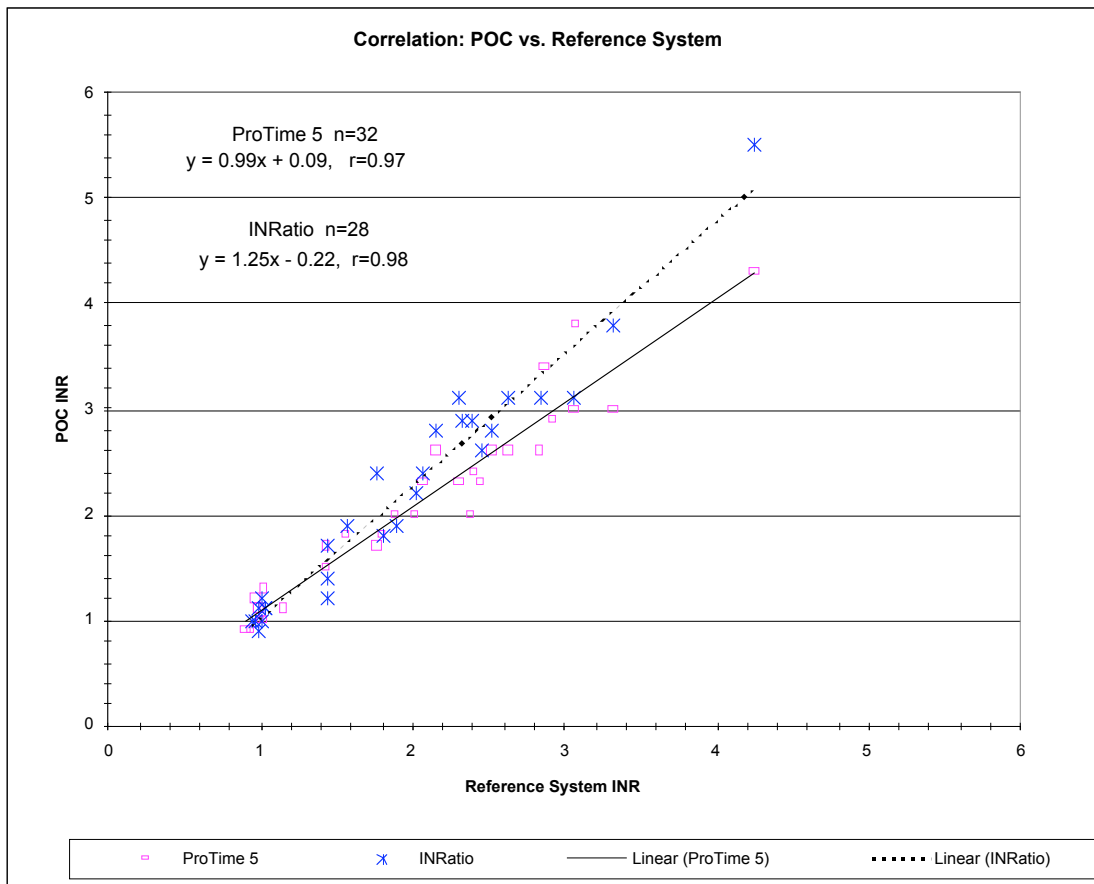
Table 5: Discrepant INR Results [Comparison of POC and Reference System], Fingerstick Samples, ProTime3 and INRatio

		ProTime3	INRatio
<2.5 INR	n	39	38
	values discrepant by > 0.7 INR (n, %)	0 (0%)	0 (0%)
	values discrepant by > 1.0 INR (n, %)	0 (0%)	0 (0%)
≥2.5 INR	n	12	11
	values discrepant by > 0.7 INR (n, %)	0 (0%)	4 (36%)
	values discrepant by > 1.0 INR (n, %)	0 (0%)	2 (18%)

## RESULTS & DISCUSSION – ACCURACY WITH FINGERSTICK SAMPLES ProTime5 vs. INRatio

Regression analyses were performed to compare both POC systems with the reference lab system (Figure 3). Both were highly correlated to the reference system with correlation coefficient ( $r$ ) = 0.97 and 0.98 for ProTime5 and INRatio, respectively. Slope values were different between the two systems. INRatio produced slope=1.25 whereas ProTime5 generated slope=0.99. As expected, these slopes are directionally consistent with those observed with venous samples on each system.

Figure 3 – Accuracy using Fingerstick Samples, ProTime5 and INRatio vs. Reference Lab System



Relative differences are summarized in Table 6. Differences were smaller with ProTime5 in both INR classifications. Table 6 also shows frequency of agreement for each POC system compared to the lab reference result within 0.4 INR. Agreement with the lab reference system was less frequent in the higher INR range for both POC systems. ProTime5 demonstrated significantly closer agreement with the lab reference system, overall, in both INR classifications. An ANOVA comparing the POC methods to the lab reference system demonstrated ProTime5 was statistically equivalent in each INR range. INRatio differences were statistically significant ( $p < 0.05$ ) for INR values  $\geq 2.5$ .

Table 6: Bias Analysis [POC-Reference System], Fingerstick Samples, ProTime5 and INRatio

		ProTime5	INRatio
<2.5 INR*	n	23	22
	population avg. (INR)	1.55	1.70
	avg. relative difference vs. Reference	<b>0.06</b>	<b>0.21</b>
	SD of difference	0.17	0.27
	within 0.4 INR (n, %)	22 (96%)	17 (77%)
≥2.5 INR*	n	9	6
	population avg. (INR)	3.13	3.57
	avg. relative difference vs. Reference	<b>0.08</b>	<b>0.46**</b>
	SD of Difference	0.34	0.42
	within 0.4 INR (n, %)	7 (78%)	3 (50%)

\*INR classification determined by Reference System result

\*\* Statistically significant difference (p<0.05) when compared to the Reference System

Table 7 identifies values for each POC system that disagreed with the lab reference system by greater than 0.7 and 1.0 INR. ProTime5 generated fewer discrepant than INRatio.

Table 7: Discrepant INR Results [Comparison of POC and Reference Lab System], Fingerstick Samples, ProTime5 and INRatio

		ProTime5	INRatio
<2.5 INR	n	23	22
	values discrepant by > 0.7 INR (n, %)	0 (0%)	1 (5%)
	values discrepant by > 1.0 INR (n, %)	0 (0%)	0 (0%)
≥2.5 INR	n	9	6
	values discrepant by > 0.7 INR (n, %)	1 (11%)	1 (17%)
	values discrepant by > 1.0 INR (n, %)	0 (0%)	1 (17%)

The percentage of discrepant values noted for the INRatio assay varied with the multiple fingerstick evaluations (36% and 17%). This may be explained by the smaller population tested in the second study (n=56 and n=34) or lot-to-lot variation of the INRatio assay.

## RESULTS & DISCUSSION – PRECISION

Duplicate results were obtained by testing venous blood samples with two ProTime3 and two INRatio instruments. Pooled standard deviation (pooled SD) was calculated. Results are summarized in Table 8. ProTime3 demonstrated a lower pooled SD than INRatio.

Table 8: Summary of Precision

	<b>ProTime 3</b>	<b>INRatio</b>
n (number of patients )	36	35
population avg. (INR)	2.4	3.0
pooled SD	<b>0.24</b>	<b>0.37</b>

## RESULTS & DISCUSSION – “NO RESULT” RATE

During the conduct of this study, no result was obtained periodically due to various test errors. Table 9 summarizes for each POC system the rate at which no result was reported during the accuracy evaluations. ProTime errors were a combination of sampling (not enough blood), internal control and technical system errors. Almost all INRatio errors were related to sampling (not enough blood). “No result” rate was lower for both ProTime assays compared to INRatio.

Table 9: Summary of “No Result” Rate.

	<b>ProTime3</b>		<b>ProTime5</b>		<b>INRatio</b>	
	<b>tests (n)</b>	<b>no result (n)</b>	<b>tests (n)</b>	<b>no result (n)</b>	<b>tests (n)</b>	<b>no result (n)</b>
Venous study	37	0	37	1	37	1
Fingerstick study (1)	56	5	(n/a)		56	7
Fingerstick study (2)	(n/a)		34	2	34	6
TOTALS	93	5	71	3	127	14
<b>% Incidence, No Result</b>	<b>5.4%</b>		<b>4.2%</b>		<b>11.0%</b>	

## CONCLUSION

Results demonstrate all POC systems were highly correlated to a standard laboratory reference method. The slopes generated from the regression analysis show directional differences of the ProTime and INRatio systems relative to the MLA. INRatio demonstrated a positive bias that was exaggerated in the higher INR range resulting in significantly different results compared to the MLA for INR values  $\geq 2.5$ . The ProTime Microcoagulation System (ProTime5 and ProTime3) demonstrated a smaller bias and accurate INR results across the entire INR range, with few discrepant values, consistent with published reports<sup>6,7</sup>. Results confirm both ProTime assays are appropriate for monitoring the coagulation status of patients on oral anticoagulant therapy. It is important that all sites establish their own correlation with the local laboratory to identify institutional variation between POC systems and their unique reference systems.

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