Effects of Reagent and Instrument on Prothrombin Times, Activated Partial Thromboplastin Times and Patient/Control Ratios

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Key words

Prothrombin time – Activated partial thromboplastin time – Proficiency testing – Statistic, robust estimation – Analysis of variance

Summary

Activated partial thromboplastin times accumulated from two proficiency testing surveys were analyzed to determine simultaneously the effects of the method and reagent used. Prothrombin time results were reevaluated concomitantly for comparison.

A robust two-way analysis of variance was applied to determine the effect of method and reagent on APTT results. The effect of the reagent and method on the ratio of abnormal to normal plasma clotting times was determined. We found a substantial difference in ratios for the PT using different reagents on the same instrument. There was an even larger effect of reagents on APTT ratios.

Our finding of substantial reagent effects for the PT and APTT clearly support the need for standardization. We found standardization to be feasible only for the PT, and only if applied in a form consistent with the inherent error structure of the data. For the APTT, the present methodology and plasma samples did not achieve consistent standardization.

Introduction

The activated partial thromboplastin time (APTT), used as an overall measure of the intrinsic clotting system, is – apart from the prothrombin time (PT) – the coagulation test most routinely performed by clinical laboratories. The main functions of the APTT include screening for intrinsic coagulation defects, monitoring of patients on heparin therapy, and identifying of patients with circulating anticoagulants. The optimal APTT system is sensitive to factor deficiencies of <30% activity, such as those of factors VIII and IX, and the assay is prolonged with defects of the contact factors, XI and XII. Patients with a lupus-type circulating anticoagulant often have a prolonged APTT but normal PT.

In APTT testing a platelet substitute or partial thromboplastin is provided, usually with some type of phospholipid. A surface activator such as ellagic acid, celite, micronized silica or kaolin is added to the thromboplastin to initiate activation of the contact phase of coagulation. A pool of lyophilized normal human plasma and the individual plasmas contributing to it are frequently employed to establish a control value and a normal range respectively. Therapeutic ranges for heparin and oral anticoagulant drugs are derived from this normal baseline value or range.

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The PT and APTT results may be reported in several ways, including the actual clotting times and/or the ratio of the patient's times to those of the control. However, such ratios do not normalize the results (1). The therapeutic ratio for PT has been calculated by many authors (1–3), and its validity has been disputed (2). Intralaboratory control times are generally used for the PT and APTT ratios to reduce the effects of interlaboratory variation. In the present analysis we used results from our normal control plasmas to calculate the ratios.

Many different phospholipids of human, animal and vegetable sources and various types of activators are used as APTT reagents. The need to standardize reagents and techniques has been emphasized in many reports (4–6) and motivated our study. The international normalized ratio (INR) was recommended by Loeliger et al. (3) for the normalization of the prothrombin times in oral anticoagulant control. The INR is defined as the PT ratio result that would be obtained if the WHO primary international reference thromboplastin was used to test the patient sample. Central to using an adjustment such as the INR is the requirement that a constant set of adjustments for the PT ratio apply to all orally anticoagulated patients' plasmas. We studied the applicability of a single set of adjustments for both PT and APTT ratios.

Materials and Methods

The data were the results of proficiency tests administered by the New York State Department of Health in July and October 1985. Approximately 340 laboratories participated in each test. In each mailout three plasma samples were tested, one normal and two abnormal. The participant laboratories were to report the average of duplicate measurements on each specimen.

Plasma Samples

Samples for the July 1985 test were citrated human plasmas, supplied in lyophilized aliquots, prepared by the New York Blood Center laboratory in Melville, NY. Three distinct levels of PT and APTT activity were prepared from two batches of pooled plasma; each approximately 3–4 l. The abnormal plasmas were prepared by absorption of clotting factors in normal plasma by barium sulfate.

The samples for October 1985 were obtained from Ortho Diagnostic Systems, Inc., Raritan, NJ. These samples were batches of Ortho "off-the-shelf" control specimens, one normal and two artificially depleted plasmas, with different levels of prothrombin-complex activity. The samples were to be reconstituted with distilled water and allowed to stand at room temperature for 15 min before testing.

Assay Methods and Reagents in Participating Laboratories

For the PT roughly 11% of the participating laboratories used manual, 35% mechanical, and the rest photooptical methods (Table 1). Twelve commercial thromboplastins were used for the October 1985 samples, but most participating laboratories used Dade C (42%), Ortho OBT (24%), or Simplastin (12%).

For the APTT about 6% of the participating laboratories used manual, 35% mechanical and the rest photooptical methods. There were combina-

Table 1 PT: number of laboratories using each combination of method and reagent, October 1985

Method	Reagent TA	TB	TC	TD	TF	TG	TH	TM	TN	TP	TR
Manual											
MA									1		
MB		1	1		1		3		2		
MC		3	7			1	7	2	13		
Mechanical											
MG					1	5	3				
MH							1		1		
MJ		3	44	3	2	3	47	10	14		
MK							1				
Photooptical											
MM		1	12			3	5	2	7	1	10
MN			9			1	2		4	1	11
MP			1						1		1
MR			5		1	1	4		2		
MT			84	2	1		11	5	3	1	1
MU						5					
MX	2		1								
MY			1				8				
MZ							1			1	

Method codes: MA, Applicator Stick; MB, Loop; MC, Tilt-tube; MG, Clotek (Hyland); MH, Data Clot 2 (Helena); MJ, Fibrometer (Bio Quest); MK, other (specify); MM, Coag-a-mate-150 or Dual Beam (General Diagnostic); MN, Coag-a-mate 2001 or X2 (General Diagnostic); MP, Coagulation Profiler (BioData); MR, Coagulyzer (Sherwood); MT, Electra (MLA); MU, Fibrintimer II (Hyland); MX Unimeter (Biodynamics); MY, Koagulab (Ortho); MZ, other.
Reagent codes: TA, Biodynamics; TB, Dade Activated-liquid; TC, Dade C; TD, Dade Reagent; TF, Hyland Dried; TG, Hyland Liquid; TH, Ortho OBT; TM, PH/CMS Thromboscreen; TN, Simplastin; TP, Simplastin A; TR, Simplastin Automated.

tions of 3 manual methods with 5 reagents, 3 mechanical methods with 8 reagents, and 9 photooptical methods with 8 reagents. In October 1985, for example, 119 laboratories used 12 combinations of mechanical methods and reagents, while 198 laboratories used 34 combinations of photooptical methods and reagents (Table 2). Nine commercial reagents were in use, but more than 82% of the participating laboratories used three reagents. Roughly 83% of laboratories with photooptical methods used Dade Actin (50%), GD automated APTT (20%), or Ortho Thrombofax (13%). Most of the laboratories with mechanical or manual (tilt-tube) methods used either Ortho Thrombofax (40 and 25% respectively) or Dade Actin (33 and 35%).

For the PT ratio analysis, data from the following combinations were used: photooptical methods, the 91% of laboratories using 5 methods and 7 reagents; mechanical methods, the 93% using the Fibrometer and 8 reagents; manual methods, the 79% using tilt-tube and 6 reagents. For the APTT ratio analysis, data from the following combinations were used:

Table 2 APTT: number of laboratories using each combination of method and reagent, October 1985

Method	Reagent AA	AB	AE	AF	AH	AK	AP	AR	AX
Manual									
MA							1		
MB	1				1				
MC	7				1	3	5	1	
Mechanical									
MG			1	3			3		
MH					1		1		
MJ	39	4	4		7	2	47	7	
Photooptical									
MM	12	2	1		13	1	5	1	
MN	7	2	1		17		1		
MP	1				2				
MR	6		1		3		2		
MT	69	13	1		4	1	10	5	1
MU	1		4				1		
MX	1								
MY	1						6		
MZ							1		1

Method codes: MA, Applicator Stick; MB, Loop; MC, Tilt-tube; MG, Clotek (Hyland); MH, Data Clot 2 (Helena); MJ, Fibrometer (BioQuest); MM, Coag-a-mate 150 or Dual Beam (General Diagnostic); MN, Coag-a-mate 2001 or X2 (General Diagnostics); MP, Coagulation Profiler (BioData); MR, Coagulyzer (Sherwood); MT, Electra (MLA); MU, Fibrintimer II (Hyland); MX Unimeter (Biodynamics); MY, Koagulab (Ortho); MZ, other.
Reagent codes: AA, Dade Actin; AB, Dade Actin FS; AE, Hyland A-Plus APTT; AF, Hyland Kaolin APTT; AH, GD Automated APTT; AK, GD Platelin Thrombofax; AP, Ortho Thrombofax; AR, PH/CMS Thrombofax; AX, Helena APTT Reagent.

Table 3 Logarithmic grand mean and CV for PT and APTT at three plasma activity levels assayed by three methods

Test period and sample	Photooptical Mean ($\log_e$)	CV (%)	n	Mechanical Mean ($\log_e$)	CV (%)	n	Manual Mean ($\log_e$)	CV (%)	n
July 1985: PT									
Normal	2.59	3.4	177	2.57	5.9	122	2.63	8.2	35
Abnormal I	2.81	3.4	177	2.84	4.6	122	2.90	7.3	35
Abnormal II	3.09	5.4	177	3.16	5.5	122	3.22	7.6	35
October 1985									
Normal	2.49	3.6	193	2.52	4.9	137	2.58	7.8	33
Abnormal I	2.85	3.5	193	2.89	4.9	137	3.00	8.9	33
Abnormal II	3.12	5.1	193	3.20	6.0	137	3.28	10.7	33
July 1985: APTT									
Normal	3.57	6.0	171	3.50	9.7	110	3.70	17.9	19
Abnormal I	3.83	6.4	171	3.75	8.8	110	3.95	13.1	19
Abnormal II	4.17	14.3	171	3.98	14.9	110	4.11	15.4	19
October 1985									
Normal	3.49	5.7	181	3.40	9.9	110	3.65	15.0	17
Abnormal I	3.94	7.5	181	3.84	12.8	110	4.01	14.1	17
Abnormal II	4.27	9.5	181	4.11	17.0	110	4.29	18.81	17

photooptical methods, the 93% of laboratories using 5 methods and 6 reagents; mechanical methods, the 92% using the Fibrometer and 7 reagents; manual methods, the 85% using tilt-tube and 5 reagents.

Rare combinations were eliminated from the analysis, because estimation of their overall variance was impossible (7).

Statistical Analysis

The data were analyzed by a self-critical robust procedure for two-way analysis of variance, similar to that previously reported (8). Logarithms of both PT and APTT data were used to ensure homoscedasticity and normal error structures. As in our previous study, analysis of reference laboratory data showed no significant interaction between methods and reagents. The interaction terms will therefore be omitted in this report.

To more nearly parallel clinical practice, we also determined the clotting time ratios. For each mailout we calculated the ratio of the clotting time for each abnormal plasma (I and II) to the time for the normal plasma. Each ratio is reported as the antilogarithm of the difference between the logarithms of the abnormal and normal clotting times. The self-critical robust procedure was applied to the difference of the logarithms of the abnormal and normal clotting times. The model is

$$Y_{ijk} = \log_e(X_{ijkm}) - \log_e(X_{ijkl}) = \mu + \alpha_i + \beta_j + e_{ijk}$$

where X_{ijkm} = the observed clotting time of an abnormal plasma level l from laboratory k using instrument i and thromboplastin j; X_{ijkl} = the observed clotting time of the normal plasma from laboratory k using instrument i and thromboplastin j; μ = the overall mean of the logarithm of ratio (abnormal PT/normal PT); α_i = the ith instrument effect; and β_j = the jth thromboplastin effect; e_{ijk} = the random error term. Since we are assuming $\log_e X_{ijkm}$ and $\log_e X_{ijkl}$ to be normally distributed (8), Y_{ijk} is also normally distributed with mean μ and the variance σ^2. Also the model assumes that e_{ijk} is a normally distributed error with a mean = 0 and with constant variance σ^2 (homoscedasticity). The σ^2 is estimated by the robust variance. The CV is the standard deviation of the random error term e_{ijk} in the above stated model.

The self-critical procedure simultaneously estimates the value for each parameter and checks the consistency of the assumed model with the data. The level of scrutiny of the data is controlled by the user, who specifies a coefficient of self-criticism c (9). This method is a generalization of widely accepted maximum-likelihood estimates when c = 0. Qualitatively, if the underlying data structure is normal, the parameter estimates are stable with increasing c values of 0 to 0.3. Weights are automatically assigned to each observation, and the weight of an outlying observation decreases rapidly, while that of an observation within the normal distribution stabilizes close to 1 as c moves from 0.1 to 0.3. In this way, the influence of any observations inconsistent with the model and the bulk of data was removed on the estimation of parameters. Thus, the estimated parameters

are robust against outliers. In practice, even clerical errors could be included in the analysis without influencing the estimated parameters.

Since normality and homoscedasticity tests for self-critical ANOVA are not available, three goodness-of-fit tests were used as alternatives: Anderson-Darling, Kolmogorov-Smirnov, and Cramer-von Mises (10) for the normality of residuals and Levene's test for equal variances (11) for homoscedasticity. These tests were applied after outlying observations were removed. Observations beyond 3 robust SD were treated as outliers using c = 0.25. The automatic weighting technique allows inclusion of all observations in the estimation of parameters, and these outliers were not eliminated from the self-critical analyses but were for the normality tests.

Results

Clotting Times

Results of the PT analysis were in good agreement with our previous findings (8). The coefficients of variation (CV) for all six samples ranged from 3.4 to 5.4% for photooptical, 4.6 to 6.0% for mechanical, and 7.3 to 10.7% for manual methods (Table 3). The assumptions of normality and homoscedasticity of the errors were satisfied for all three types of methods.

For the APTT the CV for all six samples ranged from 5.7 to 14.3% for photooptical, 8.8 to 17.0% for mechanical, and 13.1 to 18.8% for manual methods (Table 3).

The APTT estimates were less consistent (i. e., had larger CV) than those for PT (Table 3). A test of assumption of a constant residual variability across all reagents was not satisfied by the mechanical methods. In proficiency testing the efficiency and uniformity of grading can be improved if large subsets of the data can be described by a model with constant error variance (8). No such model could be achieved for mechanical methods. However, for the photooptical and manual methods the assumption of normality and homoscedasticity was not rejected. Hence improved efficiency and uniformity of grading could be achieved for these methods.

Effects of Method and Thromboplastin Effects on PT Ratios

For each combination of method and reagent, ratios of abnormal clotting times to normal clotting times, Ratios I and II, and the grand means and CV of those ratios (Table 4 A–D) were

Table 4A   PT: cell mean ratios for various method-reagent combinations with c = 0.25, October 1985*

Method	Reagent								Grand mean	CV (%)	n
Ratio I											
Mechanical	TB	TC	TD	TF	TG	TH	TM	TN			
MJ	1.47	1.42	1.36	1.30	1.50	1.49	1.39	1.53			
									1.45	4.1	126
Photooptical	TC	TG	TH	TM	TN	TP	TR				
MM	1.36	1.41	1.42	1.39	1.50	1.50	1.54				
MN	1.39	1.43	1.45		1.53	1.54	1.57				
MR	1.41	1.46			1.56						
MT	1.39	1.43	1.45	1.41		1.53	1.57				
									1.45	3.8	185
Manual	TB	TC	TH	TM	TN						
MB	1.47	1.48	1.45		1.56						
MC	1.47	1.48	1.45	1.53	1.56						
									1.51	7.6	39
Ratio II											
Mechanical	TB	TC	TD	TF	TG	TH	TM	TN			
MJ	1.96	1.88	1.80	1.75	2.10	2.10	1.91	2.06			
									1.98	6.7	126
Photooptical	TC	TG	TH	TM	TN	TP	TR				
MM	1.75	1.89	1.83	1.82	2.02	1.96	2.04				
MN	1.83	1.98	1.92		2.12	2.06	2.14				
MR	1.86	2.01			2.15						
MT	1.81	1.95	1.90	1.88		2.03	2.12				
									1.88	5.5	185
Manual	TB	TC	TH	TM	TN						
MB	1.78	1.78	1.86		1.96						
MC	1.93	1.93	2.03	2.01	2.14						
									2.00	8.4	39

* c = coefficient of self-criticism (9).

For the method and reagent codes and the number of laboratories using each combination, see Table 1.
n = the number of observations for which the CV was calculated.

calculated by the self-critical robust technique (9). The assumptions of homoscedasticity and normality of error structures were satisfied. As clotting times for the PT were lognormally distributed, Ratios I and II were also lognormally distributed.

The use of ratios had little effect on residual variability of PT results for photooptical and mechanical methods. The CVs for photooptical and mechanical were only slightly different from those for direct analysis of PT (Table 4A–C). For manual methods the use of ratios resulted in a moderate narrowing of the CV range.

For photooptical methods greater variability was associated with the reagent than with the instrument. Roughly 75% of the total variability explained by this analysis was associated with thromboplastin effects and 25% with instrument effects (Table 5A and B). These factors resulted in a substantial difference in ratios for different reagents on the same instrument.

Effects of Method and Thromboplastin on APTT Ratios

For the APTT, the use of ratios substantially reduced the residual variation. For example, the CVs for mechanical methods with the October 1985 results were reduced from 12.8% (Abnormal I) to 9.9% (Ratio I) and from 17.0% (Abnormal II) to 13.6% (Ratio II) (Table 3 vs Table 4B). The CV for manual methods were reduced from 14.1 to 6.8% and from 18.8 to 7.9% respectively. Thus for these methods the reduction of interlaboratory variation substantially increased the precision of the estimates. For photooptical methods the reduction in variability

was smaller, from 7.5 to 6.5% and from 9.5 to 8.9% respectively. Nevertheless, the precision of ratio estimates remains better for the PT than for the APTT ratios, as evidenced by their differences in CV.

For the APTT ratios the assumptions of homoscedasticity and normality of error structures was satisfied. We reduced the between laboratory variations (interlaboratory variation) by removing the individual laboratory bias in the normal PTs. Thus a model with a proportional error structure was appropriate for these data once interlaboratory variation was reduced, and the advantage of a single model for all observations was attained (8). As stated earlier, this was not the case for absolute clotting times when APTT was measured by mechanical methods. For the photooptical methods 94% of the instruments or reagent-induced variation in ratios stemmed from reagent effects (Table 5), and the differences among ratios for various reagents on the same instrument were substantial and significant (p <0.0001).

Reagent effects for mechanical methods were also substantial and significant (p <0.0001). For example, the range of ratios associated with Dade Actin was similar to the range for Ortho Thrombofax; thus the two are said to be homoscedastic. However, the means of the two groups differed by about 1 standard deviation (1.405 and 1.600 respectively) (Fig. 1). Thus a significant reagent effect was demonstrated for these two groups. Similarly the cell mean ratios showed generally larger absolute reagent effects for APTT than for PT (Table 4A–C). For example, the Ratio II range was 1.75–2.10 for PT and 1.60–2.20 for APTT on the same instrument (Fibrometer).

Table 4B   APTT: cell mean ratios for various method-reagent combinations with c = 0.25, October 1985

Method	Reagent AA	AB	AE	AH	AK	AP	AR	Grand mean	CV (%)	n
Ratio I										
Mechanical										
MJ	1.40	1.59	1.54	1.36	1.33	1.60	1.48			
								1.50	9.9	110
Photooptical										
MM	1.48	1.77	1.58	1.46		1.66	1.58			
MN	1.52	1.83	1.64	1.51		1.71				
MP	1.43			1.42						
MR	1.57		1.69	1.54		1.76				
MT	1.53	1.84	1.65	1.52		1.72	1.65			
								1.57	6.5	179
Manual										
MC	1.51			1.40	1.40	1.51	1.72			
								1.49	6.8	17
Ratio II										
Mechanical										
MJ	1.80	2.20	1.97	1.87	1.60	2.11	1.90			
								2.11	13.6	110
Photooptical										
MM	2.05	2.64	1.95	2.10		2.35	2.10			
MN	2.09	2.69	1.99	2.15		2.40				
MP	2.00			2.06						
MR	2.19		2.09	2.25		2.52				
MT	2.10	2.71	2.01	2.16		2.42	2.16			
								2.18	8.9	179
Manual										
MC	1.95		1.54	1.93	2.07	2.12				
								1.95	7.9	17

* c = coefficient of self-criticism (9).

For the method and reagent codes and the number of laboratories using each combination, see Table 1.
n = the number of observations for which the CV was calculated.

Table 4C   PT: cell mean ratios for various method reagent combinations with c = 0.25, July 1985

Method code	Thromboplastin code TB	TC	TD	TF	TG	TH	TM	TN	TR	Mean	CV (%)	n
Ratio I												
Mechanical												
MJ	1.30	1.29	2.21	1.28	1.28	1.31	1.29	1.39	1.23			
										1.31	4.8	122
Photooptical												
MM		1.22				1.26	1.27	1.29	1.32			
MN		1.22				1.26		1.29	1.22			
MP		1.21						1.28	1.31			
MR		1.24				1.28		1.32				
MT		1.26				1.26	1.28	1.30	1.33			
										1.25	3.2	173
Manual												
MC	1.23	1.27	1.29		1.17	1.33	1.27	1.33				
										1.30	4.9	35
Ratio II												
Mechanical												
MJ	1.81	1.75	1.63	1.73	1.74	1.81	1.83	1.89	1.58			
										1.79	5.3	122
Photooptical												
MM		1.55				1.69	1.64	1.75	1.65			
MN		1.61				1.75		1.81	1.71			
MP		1.63						1.83	1.73			
MR		1.69				1.84		1.89				
MT		1.63				1.76	1.72	1.83	1.72			
										1.66	5.8	173
Manual												
MC	1.73	1.73	1.73		1.61	1.87	1.75	1.81				
										1.79	6.6	35

* c = coefficient of self-criticism (9).

For the method and reagent codes and the number of laboratories using each combination, see Table 1.
n = the number of observations for which the CV was calculated.

Normalization of Reagent Effects on PT and APTT Ratios

The World Health Organization (WHO) has recommended the use of international normalized ratios (INR) in an effort to standardize PT ratios (3). Central to using an adjustment such as INR's is the requirement that a constant set of adjustments apply to all orally anticoagulated patients' plasmas. The ANOVA technique employed here allows for a feasibility study of the application of a similar standardization to APTT ratios. Specifically, a test for the significance of the interaction between ratios (plasmas) and reagents directly tests the hypothesis that a single set of reagent adjustments is applicable to the various ratios. These tests were applied to PT and APTT ratios from both mailouts for the photooptical instruments. Although there was a significant interaction between reagent and plasmas in the original scale (time/sec) for both PT and APTT ratios, there was no

Table 4D APTT: cell mean ratios for various method-reagent combinations with c = 0.25, July 1985

Method code	Reagent code						Grand mean	CV (%)	n
Ratio I									
Mechanical	AA	AB	AH	AK	AP	AR			
MJ	1.27	1.25	1.45	1.25	1.26	1.23			
							1.27	7.2	106
Photooptical	AA	AB	AE	AH	AP				
MN	1.25	1.37	1.51	1.31	1.28				
MN	1.27	1.40	1.54	1.33	1.31				
MR	1.30	1.43		1.36	1.34				
MT	1.27	1.39	1.53	1.32	1.30				
							1.30	6.2	168
Manual	AA	AF	AH	AK	AP	AR			
MC	1.25	1.64	1.45	1.40	1.19	1.49			
							1.31	10.3	20
Ratio II									
Mechanical	AA	AB	AH	AK	AP	AR			
MJ	1.79	1.32	1.89	1.22	1.49	1.49			
							1.62	13.0	106
Photooptical	AA	AB	AE	AH	AP				
MM	1.75	1.65	2.76	1.71	1.44				
MN	1.84	1.73	2.91	1.80	1.52				
MR	2.04	1.92		1.90	1.68				
MT	1.92	1.81	3.03	1.88	1.58				
							1.83	14.0	168
Manual	AA	AF	AH	AK	AP	AR			
MC	1.75	2.89	1.63	1.51	1.09	1.55			
							1.56	19.6	20

* c = coefficient of self-criticism (9).

For the method and reagent codes and the number of laboratories using each combination, see Table 1.
n = the number of observations for which the CV was calculated.

Table 5A PT ratios: nonorthogonal two-way ANOVA for photooptical laboratories, October 1985

Sample	Source of variation	Sum of squares	d.f.	Mean squares	F test	p value
Ratio I	Method	0.015	3	0.005	3.4	0.019
	Reagent	0.236	4	0.059	40.7	0.000
	Residual	0.236	163	0.001		
	Total	0.236	170	0.003		
	Multiple R = 0.775					
	Multiple R squared = 0.589					
	Eta squared* for method = 0.17					
	Eta squared for reagent = 0.56					
	Method-induced variation = 23%					
	Reagent-induced variation = 77%					
Ratio II	Method	0.051	3	0.017	5.6	0.001
	Reagent	0.447	4	0.112	37.2	0.000
	Residual	0.491	163	0.003		
	Total	1.159	170	0.007		
	Multiple R = 0.76					
	Multiple R squared = 0.58					
	Eta squared for method = 0.19					
	Eta squared for reagent = 0.53					
	Method-induced variation = 26%					
	Reagent-induced variation = 74%					

* Eta squared: The proportion of total sum of squares explainable by the predictor (Method or Reagent) (25).

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Table 5 B APTT ratios: nonorthogonal two-way ANOVA for photooptical laboratories, October 1985

Sample	Source of variation	Sum of squares	d.f.	Mean squares	F test	p value
Ratio I	Method	0.067	3	0.022	6.0	0.001
	Reagent	0.546	4	0.137	36.6	0.000
	Residual	0.546	152	0.004		
	Total	1.244	159			
	Multiple R = 0.69					
	Multiple R squared = 0.48					
	Eta squared* for method = 0.03					
	Eta squared for reagent = 0.46					
	Method-induced variation = 6%					
	Reagent-induced variation = 94%					
Ratio II	Method	0.045	3	0.015	2.2	0.09
	Reagent	0.911	4	0.2228	32.9	0.000
	Residual	1.051	152	0.007		
	Total	2.022	159	0.013		
	Multiple R = 0.69					
	Multiple R squared = 0.48					
	Eta squared for method = 0.03					
	Eta squared for reagent = 0.46					
	Method-induced variation = 6%					
	Reagent-induced variation = 94%					
	Four methods are MM, MN, MR, and MT.					
	Five reagents for PT are TC, TG, TH, TN, and TR.					
	Five reagents for APTT are AA, AB, AE, AH, and AP.					

* Eta squared: The proportion of total sum of squares explainable by the predictor (Method or Reagent) (25).

significant interaction for either mailout in the logarithm transformed scale for PT ratios, meaning that no evidence was found to negate applying a single set of adjustment factors to Ratios I and II for either mailout. On the other hand, there were highly significant interactions for the APTT and PT ratios (p <0.0001) both in the original scale (time/sec) and the logarithm transformed scale. Thus, we rejected the hypothesis that a single set of reagent adjustments was applicable to APTT Ratios I and II. For example, from Table 4B we find for reagent AE (Hyland A-Plus APTT) using instrument MN in the October mailout, that AE-specific Ratio I was 104% ([1.64/1.57] ∗ 100) of the grand mean of Ratio I, but that the AE-specific Ratio II was 91% ([1.99/2.18] ∗ 100) of the grand mean of Ratio II.

Discussion

The results of the present analysis of PT are in good agreement with our previous report (8). Furthermore, the use of ratios of abnormal to normal PT resulted in only a small decrease in residual variation. The fact that in both forms of analysis the reagent effects are substantial support the consensus for standardization of PT reagents (12–18).

The residual variability of APTT results was substantially greater than for PT, and assumption of constant variability across all reagents was not satisfied for mechanical measurements of APTT. The use of APTT ratios did satisfy the assumption of constant variance across the reagents and did reduce the residual variations. These improvements indicate that the use of ratios provides a valuable means for reducing interlaboratory variation for APTT. These improvements will also lead to improvements in the efficiency and uniformity of the grading of proficiency testing programs in a manner analogous to that previously reported for the grading of PT proficiency testing (8). On the other hand the fact that even with these improvements reagent effects and residual variation remains large indicate the need for further improvement in the measurement of APTT. The large reagent effects for APTT support the assertion of many other investigators of the even larger need to standardize APTT reagents (4–6, 18).

The larger effect of reagent on the APTT may reflect the greater variety of APTT reagents (phospholipids and activators) currently being marketed. Also these results are consistent with previous reports that found different APTT reagents to have varied sensitivities to plasma levels of the intrinsic coagulation factors. Many reports have studied the sensitivities of the
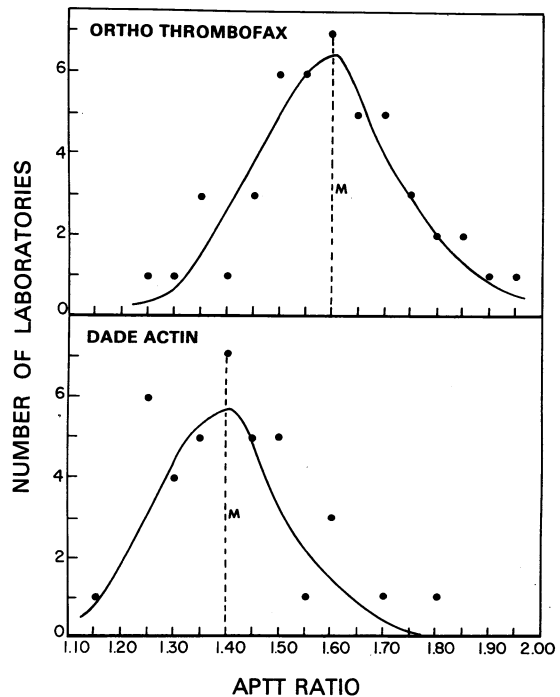

Fig. 1 Histogram of observed and expected APTT Ratio 1 for laboratories using mechanical methods (Fibrometer) with two reagents (Dade Actin and Ortho Thrombofax), where M's denotes group means, ● denotes the observed number, and curves are the expected number of laboratories. Coefficients of variation for Dade Actin and Ortho Thrombofax are 9.7 and 9.9%, respectively (this difference is not statistically significant, p >0.49 from Levene's test) (11)

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different APTT reagents and their lipid composition to a variety of coagulation disorders (5, 19, 20).

While the clinical impact of false-positive or false-negative APTT ratio cannot be assessed on the basis of proficiency data alone, our results indicate a substantial potential for the misclassification of APTT ratios when reagent effects are not properly accounted for. Even when APTT ratios are used only as a screening tool, an incorrect ratio could needlessly delay implementation of treatment and entail an added expense.

The data presented in this paper are based on assays of human plasmas artificially depleted of prothrombhin-complex clotting factors. There is a published report which addresses the effect of plasma origin and composition on sample mean (21). Plasmas derived from patients treated with coumarin were compared with artificially depleted plasmas. The study found a large differences between the artificially depleted plasmas prepared by a commercial source and that prepared by a non-commercial provider. As indicated by the authors, these differences may have stemmed from different conditions of preparation, such as use of aluminum hydroxide, incubation time, centrifugation, filtration and freeze-drying.

In an earlier report in which we analyzed both artificially depleted plasmas and lyophilized pooled patient plasmas, we found no remarkable differences between these plasmas (8) in the residual variances. Reexamination of other unpublished results from this previous study in light of the findings reported here, again indicated no substantial differences in PT values between the sources of plasma. In particular, the use of PT ratios had only a small effect on reducing interlaboratory variation and substantial reagent effects also were observed for lyophilized patient plasmas. However, there were slightly smaller reagent induced effect with the lyophilized patient plasmas than with the artificially depleted plasmas. In a clinical setting, the quality of clot formation in patient plasmas in comparison to that of normal plasmas is also an important consideration, and further studies of these effects are clearly desirable. However, in poroficiency testing where a laboratory's performance is evaluated on its ability to measure accurately the clotting times of uniform plasma samples using the same instrument and reagent combinations, the use of patient plasma is unnecessary. Since comparisons of this type are not currently available for the APTT either in our data or in the published literature, further study of these effects using naturally depleted plasmas is warranted. In addition, studies of the APTT with plasmas artificially or naturally deficient in factors VIII and IX, the contact factors, or other intrinsic pathway factors should provide novel and relevant information.

The lack of standardization of therapeutic ratios for the PT precludes definition of the optimal therapeutic range. The lower limit of anticoagulation recommended at one center can actually be the upper limit of safety at another (12). In 1983, WHO published recommendations for normalization of the PT test in oral anticoagulant control. This proposal has been supported by the International Committee for Standardization in Haematology (ICSH) and the International Committee on Thrombosis and Haemostasis (ICTH). Successful implementation of the WHO recommendation in clinical practice has been reported (22, 23). To achieve conformity an international normalized ratio (INR) has been proposed (3, 24). The INR is defined as the PT ratio result that would be obtained if the WHO primary international reference thromboplastin was used to test the patient sample.

Our analysis showed that PT standardization was feasible only if the form of standardization to be applied was consistent with the inherent error structure of the data. In particular, unless proportional adjustment (i.e. analysis conducted in the logarithmic scale) was applied to PT ratios, adjustments of PT results were not consistent across plasmas. When proportional adjustments were applied to PT ratios, our analysis showed no significant variation in the required adjustment across plasmas. In contrast, no adjustment scheme was identified for the APTT that could be consistently applied across plasmas.

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Holter Monitoring Technique

Technical Aspects and Clinical Applications

In this book a survey is given on the technical development and reliability of the Holter monitoring technique as well as the clinical relevance of the results obtained. Following the introductory presentations on general technical problems of the Holter monitoring technique, the analysis of the ST segment of the surface ECG were discussed. Thereafter the recovery and clinical significance of cardiac arrhythmias, particularly with respect to their relevance to sudden cardiac death, were debated, based on both experimental and clinical experiences.

In addition to the Holter ECG technique the final session focussed on long-term ambulatory recording of different biological parameters like arterial blood pressure, pulmonary artery pressure, arterial pulse wave contours, and of the electrical activity of the brain.