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Commutability of Calibration and Control Materials for Serum Lipase

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Background: **To effectively assess and correct for intermethod variability, calibration and control materials (CCMs) must show the same intermethod behavior as patient sera, i.e., they must be commutable. We describe the commutability of selected CCMs for lipase assays, the impact of noncommutability of CCMs in normalizing patient results, and characteristics of reagents that affect assay specificity and commutability.**

Methods: **Lipase was measured in 98 patient sera and in 29 commercial CCMs, with 2 commercial methods using different substrates and with 4 experimental methods using 1,2-*o*-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester as substrate and colipase as cofactor, but differing in the stabilizing proteins used and in the size of the substrate micelles.**

Results: **The noncommutability rate, i.e., the frequency of aberrant intermethod behavior of CCMs in comparison with patient sera, was 27% for liquid CCMs and 47% for lyophilized CCMs. The normalized residuals, measuring the degree of noncommutability, were −2.3 to 2.4 for CCMs with "normal" lipase activity, and −3.5 to 21.7 for CCMs with higher lipase activity. Recalculation of patient results with CCMs as calibrators decreased or increased the original bias according to whether the CCMs were commutable.**

Conclusions: **For the lipase methods in this study, the frequency of noncommutability of CCMs is affected by assay-specific characteristics, including size of substrate micelles and the presence or absence of added proteins.**

The measurement of serum lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) activity is a well-established diagnostic test for acute pancreatitis *(1, 2)*. Large discrepancies among different methods have been described *(1, 3-5)*, possibly leading to incorrect interpretation of test results, notwithstanding the moderately high biologic variation of serum lipase (intraindividual, 23%; interindividual, 33%) *(6)*. Discrepancies among the results of different methods may reflect, in part, the lack of a reference method for the titration of calibrators and the use of calibrators exhibiting differences in catalytic properties *(7)*.

In the standardization process, the intermethod behavior of calibration and control materials (CCMs)[4] must be the same as that of fresh patient sera to effectively measure intermethod variability and to equalize catalytic activity measurements. The ability of CCMs to show intermethod behavior comparable to that observed when measuring the same quantity in patient sera is referred to as "commutability" *(8-10)*. Many commercially available CCMs lack such commutability for many analytes *(10-14)*, including lipase *(3)*. The lack of commutability may not be attributable to the declared characteristics of the CCMs, but rather to the interaction between the characteristics of the CCMs and the specificity of the analytical methods. Accordingly, during the process of setting up a new method, evaluation of the frequency of noncommutability of CCMs would be useful when the new method is compared with either a reference method or any other method.

The aims of this work were the following: (*a*) to assess the commutability of several commercially available CCMs in two commercial lipase methods that use different substrates; (*b*) to assess the impact of commutable and noncommutable CCMs on normalizing patient sera results with different methods; and (*c*) to evaluate some

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[4] Nonstandard abbreviations: CCM, calibration and control material; DG, method using 1,2-diglyceride as substrate; CH, method using 1,2-*o*-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester as substrate; P250 and P400, methods using stabilizing proteins 1,2-*o*-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester as substrate, and micelles with 250-nm and 400-nm diameter, respectively; and NP250 and NP400, methods using 1,2-*o*-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester as substrate and micelles with 250-nm and 400-nm diameter, respectively, but without stabilizing proteins.

characteristics of reagents that affect assay specificity and commutability.

Materials and Methods

We assayed 98 fresh patient serum samples (singleton), covering a wide interval of concentration values, and 29 commercially available CCMs (duplicate analyses) from Beckman Analytical, Bio-Rad Laboratories, Merck, Ortho-Clinical Diagnostics, Roche Diagnostics, and Sentinel. All the CCMs had a human matrix.

Lipase measurements were performed on a Hitachi 912 automatic analyzer (Roche Diagnostics) at 37 °C with two commercial methods and with four additional experimental methods. One commercial method, designated as "DG" (Sentinel), used 1,2-diglyceride as substrate *(3, 15, 16)*. The other commercial method, designated as "CH" (Roche Diagnostics), and the four experimental methods used 1,2-*o*-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester as substrate *(17)*. All four experimental methods (experimental reagents from Sentinel) contained colipase, but differed from one another in stabilizing proteins and in the size of substrate micelles: two methods contained protein (0.3 g/L) and micelles with either a 250-nm (P250) or 400-nm (P400 method) diameter, respectively; the two methods without added protein used micelles with either a 250-nm (NP250) or 400-nm (NP400) diameter.

In all our experiments, the CH method was arbitrarily kept as the comparison method. The intermethod differences observed in the assay of CCMs were compared graphically with those of patient sera *(18)*. The intermethod relationship in the assay of patient sera was also assessed by means of the Passing and Bablok *(19)* nonparametric linear regression; the dispersion around such a line is estimated as residual SD ($S_{y|x}$). The nonparametric correlation coefficient (Spearman *r*) was also calculated. For each CCM, the residual was computed and then divided by the residual SD of patient sera to yield the normalized residual. The normalized residual of each CCM was taken as the measure of its degree of commutability *(20, 21)*; normalized residuals outside the ±3 interval were considered to indicate lack of commutability.

To assess the effect of CCM noncommutability on recalibration, the results for the entire set of patient sera by the *y*-axis method (i. e., DG) were recalculated, taking CCMs as calibrators, with values assigned by the *x*-axis method (i. e., CH). Both single-point and two-point mathematical recalibrations were used because intermethod comparison with patient sera revealed constant and proportional components of intermethod differences. The differences [(original *y*-axis value) − (*x*-axis value)] and [(recalculated *y*-axis value) − (*x*-axis value)] were then computed, and the distributions of such differences were compared to assess the effect of recalibration *(12-14)*.

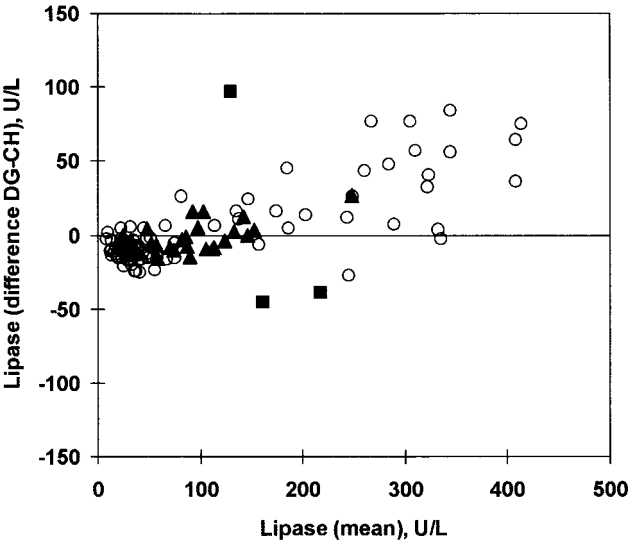

Fig. 1. Distribution of intermethod differences (DG − CH) vs concentration. ○, patient sera; ▲, commutable CCMs; ■, noncommutable CCMs.

Results

The intermethod differences (DG − CH) observed in the assay of patient sera and CCMs are plotted against mean enzyme activity in Fig. 1; the aberrant intermethod behaviors of some noncommutable CCMs are clearly shown. Table 1 reports the statistical assessment (nonparametric linear regression and correlation) of method-comparison results. The normalized residuals of the 29 CCMs are listed in Table 2; the noncommutability rate was 3/29 (10%), 18/29 (63%), 15/29 (52%), 5/29 (17%), and 13/29 (45%) when the DG, P250, P400, NP250, and NP400 methods, respectively, were compared with the CH method. CCMs 1, 2, 3, 4, 5, 6, 7, 8, 14, and 18 were always commutable, whereas CCM 11 was noncommutable in all cases. CCMs 24 and 25 were noncommutable when the four experimental methods (P250, P400, NP250, and NP400, respectively) were compared with the CH method. In our experiments, the overall noncommutability rate was 19/70 (27%) for liquid CCMs and 35/75 (47%)

Table 1. Statistical assessment (nonparametric linear regression and correlation) of method-comparison results in the assay of patient sera.[a]

Statistical parameters	Compared method				
	DG	P250	P400	NP250	NP400
n	98	98	98	98	98
Intercept, U/L	−15.3	14.8	4.0	26.4	14.7
Slope	1.14	0.36	0.50	0.34	0.36
Residual SD, U/L	16.7	2.4	3.7	3.0	3.4
Correlation coefficient, Spearman *r*	0.943	0.977	0.984	0.967	0.970

[a] Results from each compared method (dependent variable) are compared with those from the CH method (independent variable).

Table 2. Main characteristics and normalized residuals of the commercially available CCMs included in this study.

No.	Product name	Physical state	Lipase,[a] U/L	Normalized residual				
				DG	P250	P400	NP250	NP400
1	Synchron level 3[b]	Liquid	26	0.7	0.9	0.5	0.3	0.3
2	Multiqual assayed 1[c]	Liquid	34	0.4	−1.0	0.4	−2.3	−0.3
3	Multiqual unassayed 1[c]	Liquid	36	0.2	−1.5	−0.3	−2.2	−1.1
4	Ortho liquid control 1[d]	Liquid	47	0.8	0.9	1.6	−1.9	1.4
5	Multiqual unassayed 2[c]	Liquid	56	0.1	1.1	0.9	−0.6	−0.2
6	Lyphochek unassayed 1[c]	Lyophilized	61	0.0	2.4	1.8	0.2	0.8
7	Lyphochek assayed 1[c]	Lyophilized	64	−0.5	1.0	0.5	−0.6	0.1
8	Liquichek unassayed 1[c]	Liquid	67	−0.6	1.2	0.7	−0.3	0.2
9	Roche control serum N[e]	Lyophilized	76	−0.3	3.5[f]	2.4	0.6	2.6
10	Synchron level 2[b]	Liquid	79	−0.4	3.8[f]	1.4	−0.5	2.2
11	Multiqual assayed 3[c]	Liquid	82	6.0[f]	21.7[f]	18.2[f]	13.4[f]	11.1[f]
12	Roche control serum P[e]	Lyophilized	84	0.0	3.5[f]	2.8	−0.2	2.7
13	Calibrator lot F0915[g]	Lyophilized	85	1.1	4.6[f]	3.0	0.2	3.5[f]
14	Ortho liquid control II[d]	Liquid	87	0.1	2.3	2.6	−1.5	2.4
15	Precinorm U[e]	Lyophilized	92	−0.4	4.0[f]	3.1[f]	−0.9	4.0[f]
16	Normal control serum[g]	Lyophilized	96	1.1	4.9[f]	3.4[f]	0.0	5.1[f]
17	Lyphochek unassayed 2[c]	Lyophilized	96	0.4	8.6[f]	6.8[f]	2.1	7.0[f]
18	Multiqual assayed 2[c]	Liquid	98	−0.8	0.6	0.5	−2.3	0.5
19	Lyphochek assayed 2[c]	Lyophilized	110	−0.6	6.7[f]	4.9[f]	0.0	5.5[f]
20	Qualitrol HS N[h]	Lyophilized	117	−0.7	8.7[f]	6.3[f]	2.0	6.9[f]
21	Liquichek unassayed 2[c]	Liquid	118	−0.6	8.1[f]	5.9[f]	0.8	6.6[f]
22	Synchron level 1[b]	Liquid	126	−0.4	9.4[f]	4.9[f]	0.0	7.2[f]
23	Clinical chemistry calibrator[g]	Lyophilized	133	−0.1	9.9[f]	7.2[f]	3.0	9.9[f]
24	Qualitrol HS P[h]	Lyophilized	137	0.5	12.0[f]	7.5[f]	4.0[f]	9.9[f]
25	c.f.a.s. calibrator[e]	Lyophilized	146	−0.4	12.2[f]	7.8[f]	3.8[f]	9.4[f]
26	Precipath U[e]	Lyophilized	151	−0.2	10.3[f]	6.5[f]	1.9	8.8[f]
27	Multiqual unassayed 3[c]	Liquid	185	−3.4[f]	10.9[f]	7.6[f]	3.7[f]	1.9
28	Calibrator lot H0622[g]	Lyophilized	234	0.5	0.3	3.8[f]	−3.2[f]	1.7
29	Ortho liquid control III[d]	Liquid	237	−3.5[f]	7.1[f]	3.9[f]	0.1	2.6

[a] CH method.
[b] Beckman Analytical.
[c] Bio-Rad Laboratories.
[d] Ortho-Clinical Diagnostics.
[e] Roche Diagnostics.
[f] Noncommutable CCM.
[g] Sentinel.
[h] Merck.

for lyophilized CCMs. The degree of noncommutability of each CCM, measured by the value of its normalized residual, was somewhat related to its lipase activity when the P250, P400, and NP400 methods were compared with the CH method (Fig. 2).

To show the effect of recalibration with commutable and noncommutable CCMs, the results generated by the DG method for patient sera were recalculated, using either commutable or noncommutable CCMs as calibrators: CCMs 28 and 11 (normalized residuals, 0.5 and 6.0, respectively) were chosen for single-point calibration; the pairs of CCMs 3/28 and 27/29 (normalized residuals, 0.2/0.5 and −3.4/−3.5, respectively) were chosen for two-point calibration. Table 3 and Fig. 3 show the changes in intermethod differences (method DG vs method CH) after recalibration with commutable and noncommutable CCMs. When two commutable CCMs were used, the distribution of the intermethod differences improved: the median intermethod difference tended to approach 0, the slope of the nonparametric regression line tended to approach 1, and the intercept value decreased. When one commutable CCM was used, only the slope of the regression line improved because the single-point calibration could not correct the constant component of intermethod differences. When noncommutable CCMs were used instead, the distributions of the differences deteriorated: the median tended to move away from 0, the slope of the regression line tended to move away from 1 (single-point calibration), and the intercept of the regression line increased (two-point calibration).

When a noncommutable CCM was used for recalibration, 5 pathologic results changed to nonpathologic val-

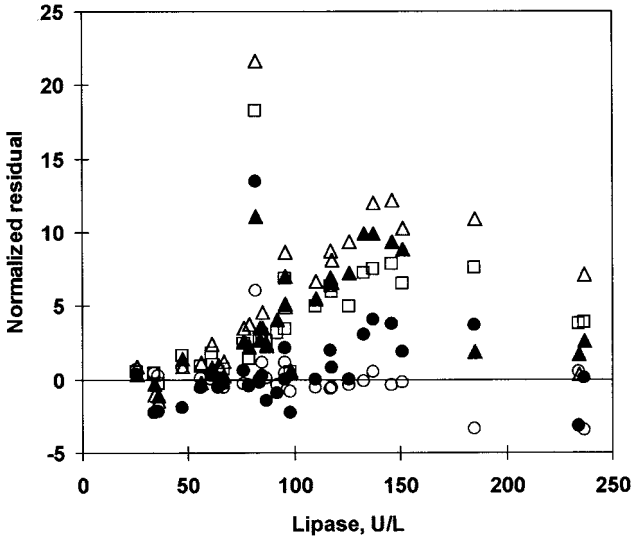

Fig. 2. Normalized residuals vs lipase activity concentration of the commercial CCMs included in this study.

Comparison method (*abscissa*), CH. Compared methods (*ordinate*): ○, DG; △, P250; □, P400; ●, NP250; ▲, NP400.

ues; when two noncommutable CCMs were used for recalibration, 67 nonpathologic results turned to pathologic.

Discussion

When dealing with an enzyme acting at a lipid–water interface, different assay methods, with different configurations of the substrate and in the presence of different matrices, can be expected to give different results. Nonetheless, one approach to standardizing enzyme activity assays is to calibrate routine methods with CCMs that have values assigned by a "comparison" method *(22)*. For such a procedure to be effective, the CCMs must be commutable *(23)*. Consistent with its definition *(9)*, commutability of CCMs with patient sera, in a stated pair of methods, is considered here as a "relative" property, comparing the intermethod behavior of each CCM with the population of patient sera, whichever dispersion the latter may show. The "standardized residual" is taken as the measure of the intermethod behavior of a single material (either patient serum or CCM), and to stay on the safe side, the interval from −3 to +3 is taken as the "reference interval" of the standardized residuals of patient sera. A CCM showing a standardized residual outside the ± 3 interval has <1% probability for its intermethod behavior to belong to the patient sera population and is, therefore, classified as noncommutable. As already discussed *(11)*, the wider the distribution of patient sera standardized residuals, the higher the probability for a CCM to be commutable with the patient sera in the specific pair of methods.

The examples given here show that the recalibration with commutable CCMs may permit a correction of the original intermethod differences (Table 3), as already shown for other components *(13, 14, 23, 24)*. Two-point recalibration more effectively corrected the constant component of intermethod differences. The perverse effect of recalibrating with noncommutable CCMs and the consequent misinterpretation of patient results were also shown. Many CCMs assayed for lipase in our experiments were noncommutable in this work.

If noncommutable CCMs similar to those included in the present study are used in external quality-assessment schemes, the interlaboratory (intermethod) variability measured may not be representative of the variability observed in the assay of patient sera *(25)*.

The reasons for the lack of commutability of the CCMs are not always evident, but they are often ascribed to the

Table 3. Statistical analysis of the intermethod differences (DG method vs CH method) before and after recalibration with commutable and noncommutable CCMs.

	Recalibration				
		Commutable CCMs		Non-commutable CCMs	
Statistical parameter	None	Single-point	Two-point	Single-point	Two-point
Intermethod differences					
Lowest value, U/L	−28.0	−52.0	−51.1	−194.3	7.2
2.5 centile, U/L	−25.0	−30.5	−29.9	−176.2	29.0
25 centile, U/L	−14.0	−17.4	−11.3	−62.3	43.4
Median, U/L	−9.0	−12.4	−5.2	−29.5	48.4
75 centile, U/L	4.8	−5.3	−0.4	−21.8	55.4
97.5 centile, U/L	76.2	35.9	33.0	−10.5	93.9
Highest value, U/L	84.0	45.3	43.9	−4.9	104.0
Linear regression/correlation					
Intercept, U/L	−15.3	−13.8	−4.8	−6.5	47.7
Slope	1.14	1.03	0.99	0.52	1.01
Residual SD, U/L	16.7	14.9	14.3	7.9	15.1
Correlation coefficient, Spearman *r*	0.943	0.942	0.942	0.946	0.952

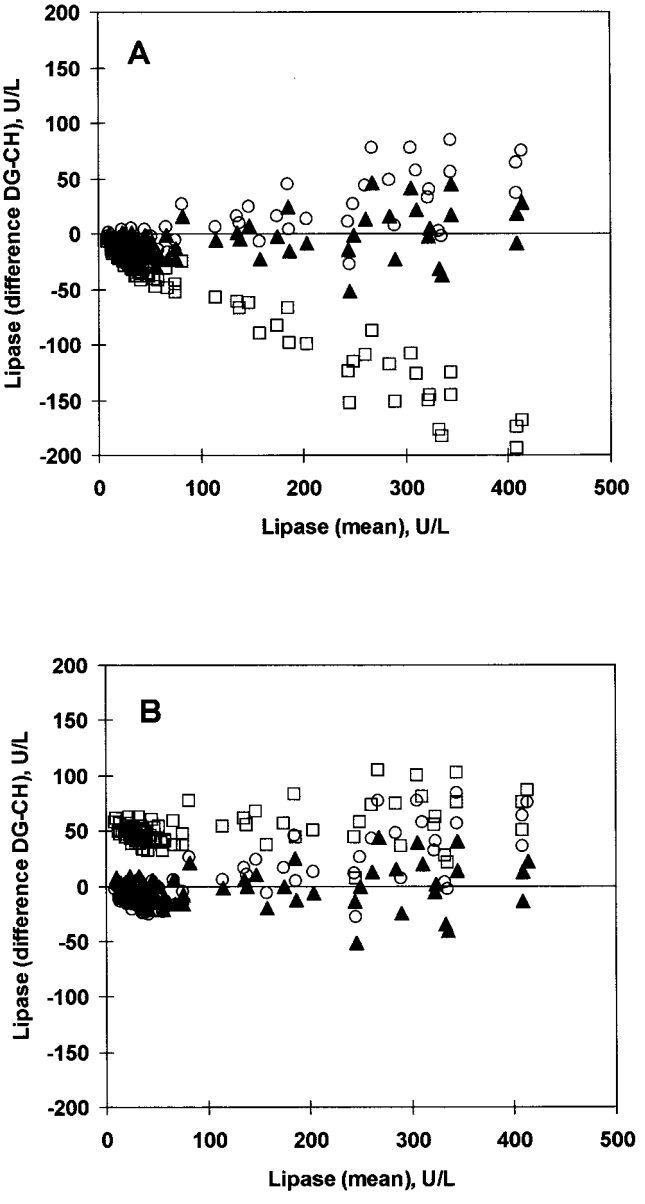

Fig. 3. Distribution of intermethod differences (method DG − method CH) vs concentration.

(*A*), single-point recalibration. (*B*), two-point recalibration. ○, original differences for patient sera; ▲, differences after recalibration with commutable CCMs; □, differences after recalibration with noncommutable CCMs.

matrix *(25-27)*. Reportedly, all the CCMs assayed in our experiments had a human matrix, but it was not declared in their labels whether human, animal, or plant enzyme had been added. Our data (Table 2) show that noncommutability seems to be a more frequent event for lyophilized CCMs: the overall noncommutability rate was 27% for liquid CCMs and 47% for lyophilized CCMs. Also, analyte concentration and degree of noncommutability appeared related (Fig. 2). The degree of noncommutability may be either related or not related to the analyte concentration: whereas the latter pattern may be caused mainly by matrix effects, it has been argued that differences in the analyte characteristics between patient samples and CCMs are mainly responsible for the former, as may be the case with enzymes *(24)*.

Because the occurrence of noncommutability may be attributable to the interaction of the characteristics of the CCMs with the specificity of the analytical methods, we evaluated the frequency of noncommutability by analyzing our set of patient sera and CCMs with four experimental methods and with an established method. From a general point of view, in clinical enzymology, one should pay attention not to change the clinical reliability of an established assay by changing the measuring method. However, if new or modified assay methods are suggested, these should be tested for their compatibility with fresh human sera and with CCMs. Our data show that, for the lipase assays that use 1,2-*o*-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester as substrate, the frequency of noncommutability may be affected by some characteristics of the analytical method, such as size of substrate micelles and the presence of stabilizing proteins different from colipase. It seems interesting to note that with substrate micelles of 250 nm, the simple addition of the stabilizing proteins caused the frequency of noncommutability to shift from 5/29 to 18/29.

We conclude that, as the lack of commutability seems to be an unpredictable event attributable to the interaction of the characteristics of the CCMs with the properties of the analytical methods, it is necessary to check the commutability of CCMs if they are to be used for assessing the performance of different methods by external quality-assessment schemes or to normalize patient results by different methods. To minimize the frequency of noncommutability, efforts should be made to improve both the quality of the CCMs and the robustness of the methods, thus allowing harmonization of results produced by different methods. The production of analytical results that are true and comparable worldwide represents an important contribution to the practice of evidence-based medicine *(28)*.

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