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Mechanism of Platelet Interference with Measurement of Lactate Dehydrogenase Activity in Plasma

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Platelets reportedly inhibit lactate dehydrogenase activity in plasma under reaction conditions of low osmolality. We describe observations inconsistent with these reports, and we attribute this "inhibition" to optical interference by platelets during the course of a reaction. We conclude that when platelet lysis is prevented and the optical interference of platelets corrected, platelet-rich plasma, platelet-poor plasma, and serum show essentially the same lactate dehydrogenase activity. Furthermore, platelet contamination can cause unexpected problems when lactate dehydrogenase is assayed with centrifugal analyzers. Results can be high or low, depending on the volume of diluent pipetted with the sample, and extreme within-run variations in activity are possible. When plasma is used instead of serum for routine analyses, regular checks for platelet contamination should be performed as a quality-control procedure, especially by laboratories separating plasma with bench-top centrifuges. Platelets can also interfere optically with assay of other enzymes and metabolites.

Additional Keyphrases: *variation, source of · quality control · centrifugal analyzer · plasma/serum comparisons*

For many years, the relationship between the lactate dehydrogenase (LD, EC 1.1.1.27) activity of serum and plasma has been the subject of controversy.[3] Rothwell et al. (*1*), in an attempt to resolve conflicting data in the literature (*2–8*), proposed that serum and platelet-poor plasma have essentially the same LD activity. They also suggested that intact platelets can inhibit the LD activity of plasma at low electrolyte concentration, a concept supported in a later study (*9*). However, our observations, reported here, suggest that intact platelets may not "inhibit" LD activity as previously suggested, but rather may interfere by a mechanism that has important consequences for other assays.

In measuring enzyme activities in our own laboratory with commercial substrates, we observed that although platelets appeared to "inhibit" LD activity, the α-hydroxybutyrate dehydrogenase (HBD, no EC no. assigned) activity of the same samples was increased. It seemed to us unlikely that platelets would "inhibit" LD activity yet activate HBD activity for the same samples measured on the same instrument under similar osmotic conditions (osmolality >2000 mmol/kg). We also observed that platelets interfere with the assay of other enzymes and metabolites, especially for reactions generating small changes in absorbance. In addition, we found that platelet-contaminated samples have either falsely high or falsely low LD activity, as determined with a centrifugal analyzer, depending on the volume of diluent pipetted with the sample, and that extreme within-run variations in activity accompany the use of excess diluent. We therefore further investigated the mechanism of platelet interference with the measurement of plasma LD activity.

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[3] Nonstandard abbreviations: LD, lactate dehydrogenase; HBD, α-hydroxybutyrate dehydrogenase; PRP, platelet-rich plasma; PPP, platelet-poor plasma.

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Materials and Methods

Venous blood samples from 10 laboratory staff members were either allowed to clot or added to lithium heparin (15 int. units/mL). Platelet-rich plasma (PRP) was prepared by centrifugation at 500 × *g* for 10 min. Serum and platelet-poor plasma (PPP) were obtained by centrifugation at 3000 × *g* for 15 min.

We measured LD activity (lactate-to-pyruvate reaction) by the Beckman single-vial modification (cat. no. 682304) of the method of Amador et al. (*10*), using both an enzyme rate analyzer (Beckman Enzyme Activity Analyzer System TR; Beckman Instruments, Inc., Fullerton, CA 92634) and a centrifugal analyzer (Cobas Bio; Roche Analytical Instruments, Nutley, NJ 07110). We also measured LD activity in the pyruvate-to-lactate direction with the System TR by a modification of the method of Rothwell et al. (modification C, Table 3, of ref. *1*). In the latter method, intact platelets have been reported to inhibit LD activity at low osmolality. To measure HBD activity with the System TR, we used the Beckman single-vial modification (cat. no. 682305) of the method of Rosalki and Wilkinson (*11*).

The System TR was used in both a single-probe and double-probe mode. In the single-probe mode, 35 μL of sample was *directly* mixed with 535 μL of substrate and the reaction monitored. In the double-probe mode, an additional 35 μL of sample was added to a blank reagent so that the double-beam spectrophotometric arrangement could automatically correct for nonspecific changes in absorbance. In preparing blank reagents, we omitted either NAD^+ or NADH from complete reagents of known composition, omission of substrate being an unacceptable alternative (*12*). We monitored all reactions with a recorder. The centrifugal analyzer was also used with two variations: 10 μL of sample was pipetted with either 20 or 80 μL of water diluent (Table 1).

We determined the enzyme activities of PRP, PPP, and serum in duplicate for each subject at 37 °C, and the standard error of the mean activity for each set of results was calculated from the standard deviation of the difference between duplicate results.

We used a Coulter S Plus IV (Coulter Electronics, Luton, Bedfordshire, England) for cell counts in the PRP and PPP samples. Where clumping was suspected, or platelet counts were very low, we counted the cells visually (*13*).

Results and Discussion

The LD and HBD activities of PRP, PPP, and serum from 10 normal subjects, summarized in Table 2, indicate that serum and plasma have essentially the same enzyme activity unless plasma is contaminated with platelets. Two mechanisms of platelet interference with the measurement of plasma LD activity have been previously proposed (*1*). Our

Table 1. Principal Instrument Settings for Determination of LD Activity with the Cobas Bio

2	Calculation factor	4020
6	Limit	0.088
7	Temp, °C	37.0
8	Type of analysis	2
9	Wavelength, nm	340
10	Sample vol, μL	10
11	Diluent vol, μL	20 or 80
12	Reagent vol, μL	255
13	Incubation time, s	120
14	Start reagent vol, μL	0
15	Time of first reading, s	0.5
16	Time interval, s	10
17	Number of readings	10
18	Blank mode	1
19	Printout mode	1

Table 2. Effect of Platelets on LD and HBD Activity in 10 Subjects

Sample	Technique[a]	Enzyme Activity, U/L (Mean ± SEM)
LD: System TR, modification of Rothwell et al. (1)[b]		
Serum	SP	302.6 ± 0.39
Serum	DP	299.1 ± 0.56
PRP[c]	DP	292.9 ± 0.68
PPP	DP	285.0 ± 0.47
PPP	SP	283.2 ± 0.47
PRP	SP	216.7 ± 0.59
LD: System TR, Beckman reagent		
PRP	DP	151.4 ± 0.86
Serum	DP	119.8 ± 0.53
Serum	SP	119.1 ± 0.27
PPP	DP	114.5 ± 0.34
PPP	SP	111.1 ± 0.21
PRP	SP	77.1 ± 0.60
LD: Cobas Bio, Beckman reagent		
PRP	80 μL	469.0 ± 8.32
Serum	20 μL	111.1 ± 0.35
Serum	80 μL	110.5 ± 0.19
PPP	80 μL	108.3 ± 0.24
PPP	20 μL	104.2 ± 0.23
PRP	20 μL	66.5 ± 1.04
HBD: System TR, Beckman reagent		
PRP	SP	214.8 ± 0.67
PRP	DP	177.6 ± 0.67
Serum	SP	176.5 ± 0.45
Serum	DP	171.9 ± 0.31
PPP	SP	170.6 ± 0.66
PPP	DP	163.7 ± 0.52

[a] Diluent volume (Cobas Bio) on blanking mode (System TR) SP, single probe mode; DP, double probe mode. [b] Method in which platelets reportedly inhibit LD activity at low osmolality. [c] Mean platelet count in PRP (n = 10): 340.2 × 10^9/L (range: 120–688 × 10^9/L).

results support the mechanism of increased LD activity from platelet lysis, but contradict the mechanism of inhibition of LD activity by intact platelets at low electrolyte concentration. We propose that the second mechanism is a misinterpretation of optical interference caused by intact platelets. Our reasons are as follows:

When we repeated the experiment that led to the concept of platelet-inhibited LD activity (Rothwell et al., modification C) we found, like previous authors (*1, 9*), that PRP appears to have less activity than PPP. In the single-probe mode on the System TR, the mean LD activity of PRP was approximately 65 U/L less than that of PPP. In the double-probe mode, however, PRP and PPP had essentially the same LD activity. In this system, the absorbance decrease in the LD reaction, related to the consumption of NADH, is masked by an increase in the absorbance of PRP over the reaction period. Thus, at an osmolality of approximately 125 mmol/kg, platelets do not "inhibit" LD activity as previously suggested, but instead optically interfere with the assay without lysing.

With the high-osmolality (>2000 mmol/kg) Beckman LD reagent on the System TR, we also observed that PRP appears to have less LD activity than PPP—about 35 U/L less in the single-probe mode. In the double-probe mode, however, PRP had about 35 U/L more LD activity than did PPP. These results can be explained as follows: At high osmolality, the absorbance of PRP does not increase as in the previous example, but actually *decreases* over the 1-min time interval during which the System TR normally computes LD activity, as indicated by the PRP (blank) trace in Figure 1. In this system, the absorbance increase in the LD reaction related to the formation of NADH is masked by a decrease in the absorbance of PRP over the reaction period, with the net result that platelets once again appear to "inhibit" LD activity in the single-probe mode. In the double-probe mode, the greater activity of PRP was due to an initial very slow lysis of platelets and a correction for the optical interference discussed above. Thus platelets, rather than inhibiting LD activity as one might interpret without blank correction, are actually responsible for higher activity than PPP with this reagent.

Although the absorbance of PRP decreases with both Beckman LD and HBD blank reagents on the System TR, in the LD reaction (lactate-to-pyruvate) NADH is generated, while in the HBD reaction NADH is consumed. This explains our observation that platelets appear both to inhibit LD activity and to activate HBD activity. The osmolality of the HBD reagent is not as high as the LD reagent and is insufficient to lyse platelets over the time interval where HBD activity is measured.

The LD activity determined with the Cobas Bio was essentially the same for serum or PPP for 10 μL of sample pipetted with 20 or 80 μL of water diluent. With PRP, on the other hand, there were extremely large differences in activi-

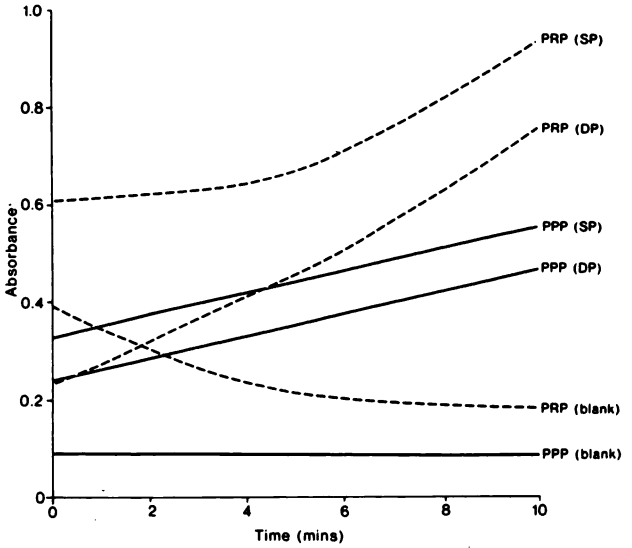

Fig. 1. Reaction rates for either PRP (broken lines) or PPP (solid lines) measured with Beckman LD reagent and a System TR

PRP (blank) and PPP (blank): reaction rates of PRP and PPP, respectively, with LD reagent without NAD^+. PPP (SP) and PPP (DP): the "true" reaction rate for PPP in either a single- or double-probe mode. PRP (DP): reaction rate of PRP corrected for the optical interference of platelets. PRP (SP): the reaction rate of PRP measured with only a single probe. The initial slow reaction rate can easily be interpreted as platelet "inhibition" of LD activity. The LD activity in serum and PPP from this sample was 125 U/L and the PRP contained 270 × 10^9 platelets per litre

ty when different volumes of diluent were used. With 20 μL of diluent, LD activity in PRP was nearly half that of serum and PPP; with 80 μL of diluent, it was about fourfold that of serum or PPP. By simply altering the volume of water pipetted with PRP, LD activity in plasma from two subjects with approximately 600 × 10^9 platelets per liter varied by more than 12-fold.

When 10 μL of PRP is pipetted into the sample well of the cuvette rotor with 80 μL of water, the low osmolality produces a time-dependent lysis of platelets while the remaining samples are pipetted. Because the extent of lysis varies with the sample's position within a series of samples, extreme within-run variations in LD activity are possible. For example, the LD activity of PRP from one subject ranged from 700 U/L for the first samples pipetted to approximately 200 U/L for the last sample (Figure 2). LD activity of the serum and PPP from this subject was only 125 U/L. In contrast, when 20 μL of water diluent is used, the LD results for PRP are low because the LD reaction is masked by a decrease in the absorbance of PRP as on the System TR.

Thus, the absorbance of PRP at 340 nm is substantially more than for PPP. The presence of 100 × 10^9 platelets per liter can increase the absorbance of undiluted plasma by almost 2.000 *A* at 340 nm. Moreover, such a number of platelets can readily be found in routine plasma samples because few bench-top centrifuges can completely remove platelets from plasma in less than 15 min. When we examined 103 unselected plasma samples, prepared with a commonly used Sorvall GLC series bench-top centrifuge (DuPont Instruments, Newton, CT 06470) at maximum speed (2600 rpm; 1240 × *g*), the mean platelet count was 88 × 10^9/L; 31% of samples had platelet counts in excess of 100 × 10^9/L, and a patient with thrombocytosis had 346 × 10^9 platelets per liter. The contamination of this last sample with platelets is sufficient to quadruple the LD activity of normal subjects under certain conditions and halve LD activity under others.

The most extreme increases of LD activity occur when platelets lyse, and the time-dependent lysis of platelets can be a special problem with centrifugal analyzers. However, non-lysed platelets can also alter the true measurement of LD and HBD activity due to absorbance changes in the sample itself. For some subjects with sufficiently high platelet counts, we observed that the LD reaction (lactate-to-pyruvate) was masked by optical interference to such an extent that the reaction appeared to go in the wrong direction. We have found that measurement of other metabolites (e.g., uric acid at 290 nm) and enzymes (e.g., alkaline phosphatase at 405 nm) can be similarly affected by changes in the optical properties of platelets during the course of a reaction. Whether the absorbance changes are due to clumping or unclumping or to swelling or shrinking of platelets is uncertain, but the effect can be overcome by dialysis or appropriate blanking. The preferred solution, however, is to ensure that plasma is essentially free of platelets.

We therefore recommend that:

- documents on sample preparation (e.g., *14*) include centrifugation conditions necessary to remove platelets from plasma (*1*),
- regular checks of platelet contamination be performed as a quality-control procedure,
- opalescent plasma samples be regarded with suspicion, and,
- the effects of platelet contamination be assessed during method development.

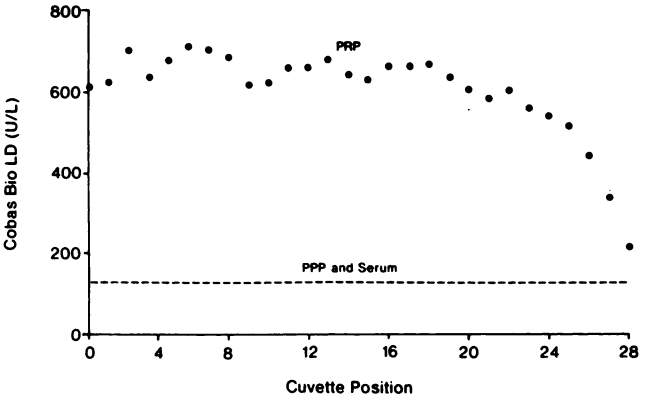

Fig. 2. Falsely increased LD activity, as measured in the Cobas Bio, from platelet contamination of plasma

The LD activity of serum and PPP was approximately 125 U/L. In this example, 10 μL of a sample containing approximately 550 × 10^9 platelets per liter was pipetted with 80 μL of water diluent, and LD activity was measured with Beckman LD reagent

A careful assessment of the extent of platelet contamination in plasma samples and its effect on various assays may lead to some reappraisal of the relative merits of serum and plasma for certain laboratory tests.

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