Hemolyzed Specimens: A Reason for Rejection or a Clinical Challenge?

To the Editor:

Clinical laboratories must improve the preanalytical phase, a phase highly susceptible to mistakes *(1)*. In some reports, hemolyzed specimens, the most common reason for rejection, account for ~60% of rejected specimens, fivefold more than the second most common cause *(2)*. Cellular contents can falsely increase values for some plasma constituents, such as potassium, lactate dehydrogenase, and aspartate aminotransferase *(3)*. Moreover, hemolysis produces spectrophotometric interference with other laboratory methods.

In vitro hemolysis depends mainly on the way in which the blood samples are drawn and treated, and it may in particular depend on the blood being forced through too fine a needle *(4)* or through the large-bore needle of a syringe into a tube; it may also be caused by shaking the tube too vigorously and/or centrifuging blood specimens before clotting is complete. In vivo hemolysis, on the other hand, may have at least 50 causes. We evaluated the causes of hemolysis in samples received by our STAT section in the Department of Laboratory Medicine of the University Hospital of Padova, which performs all clinical chemistry tests on specimens collected using lithium heparin as anticoagulant (Becton Dickinson).

Specimens, collected by physicians or nurses from hospitalized patients, were mainly from the departments of internal medicine (28%) and surgery (21%), intensive care units (23%), the emergency department (16%), and the department of organ transplantation (9%). Over a 30-day observation period, we evaluated 27 540 blood specimens from 15 323 sample requests for clinical chemistry, coagulation, and toxicological tests. According to the study protocol, each time hemolysis was visually identified, even if it was only slight, the laboratory contacted the phlebotomists to find out the procedure utilized for vascular access, the technique used for drawing blood, and to obtain information on the transportation, preservation, and storage of the specimens. If no errors in these procedures were identified and in vivo hemolysis was not suspected clinically, serum haptoglobin was measured immediately and at 24 h with a view to confirming the presence of any acute hemolysis, which was clinically evaluated and then confirmed in a further phase.

We identified 505 hemolyzed specimens (3.3%); of these, 64% were affected by a small degree (<50 mg/L of hemoglobin) of hemolysis, 31% by an intermediate degree, and 5% by a high degree (>300 mg/L of hemoglobin). The concentration of hemoglobin in plasma was measured by a colorimetric assay (Plasma hemoglobin; Sigma-Aldrich). The percentages of hemolyzed specimens were similar in the internal medicine and surgery departments (3.1%), intensive care units (3.5%), the emergency department (3.3%), and in the department of organ transplantation (3.4%).

In most cases it was possible to relate the presence of hemolysis to a specific cause because of the cooperation of phlebotomists and nurses, and only 26 (5.1%) cases remained unresolved (Table 1). Importantly, hemolysis from excessive aspiration force was relatively frequent, mainly in the case of small or superficial veins. Another frequent cause was the presence of a partial obstruction of an arterial catheter, leading to an increase in the aspiration force when a syringe was used to collect the sample. Yet another cause was hemolysis caused by forcing blood from a syringe into a tube, which was confirmed by observing a difference in the degree of hemolysis in the different tubes filled with blood from the same syringe. Collection of samples by syringe was associated with a higher rate of hemolysis as 83.8% of hemolyzed specimens were collected with syringes, vs 70% of the total number of specimens. In vivo hemolysis accounted for 16 of 505 cases (3.2%); 7 of these cases were associated with prolonged extracorporeal circulation during cardiac surgery; 2 with of acute ethanol toxicosis, 3 with transfusional reactions, 1 with necrotic-hemorrhagic pancreatitis, 1 with rhabdomyolysis from drug overdose, and 2 were of unknown etiology. Importantly, in 5 of the 16 cases, the presence of hemolysis was not suspected by clinicians, and the laboratory finding was essential in identifying the presence of a critical situation, thus potentially improving the medical outcome.

We conclude that:

(*a*) Hemolyzed specimens are a critical preanalytical problem calling for well-designed and implemented laboratory guidelines and recommendations.
(*b*) The frequency of in vitro interferences can be reduced by introducing and correctly utilizing evacuated tube systems, instead of syringes.
(*c*) If a specimen is found to be

Table 1. Causes of specimen hemolysis.

	n	%
Blood drawn too vigorously through		
Needle into syringe	155	30.7
Butterfly needle into syringe	101	20
Intravenous catheter into syringe	83	16.5
Infusion access into syringe	58	11.5
Catheter partially obstructed	35	6.9
Blood forced into the tube	26	5.1
In vivo hemolysis	9	1.8
Extracorporeal circulation	7	1.4
Specimen frozen	4	0.8
Errors in handling	1	0.2
Cause unknown	26	5.1
Total	505	100

hemolyzed, it cannot simply be rejected, but the laboratory should alert the clinician so that any in vivo hemolysis can be ruled out. The laboratory guidelines for in vivo hemolysis should include the measurement and immediate transmission of results of some laboratory tests, at least potassium in emergencies, which can provide the clinician with essential information, thus allowing identification of clinical situations requiring immediate intervention.

(*d*) A consensus between the medical laboratory and clinicians should be reached to assure the correct use of and improvement in these guidelines.

References

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Effect of Rheumatoid Factor on Cardiac Troponin I Measurement Using Two Commercial Measurement Systems

To the Editor:

The Abbott AxSYM cardiac troponin I (cTnI) immunoassay may generate false-positive results in the presence of specific human anti-animal antibodies (HAAAs) *(1)*. We use the term HAAAs rather than heterophile antibodies, a term used for weak, multispecific antibodies against diverse antigens *(2)*, either human or

Table 1. Comparison of cTnI results obtained with Beckman Access and Abbott AxSYM.

A. Effect of RF and HBT treatment on cTnI measured on two instruments

	cTnI, μg/L			
	Beckman Access		Original Abbott AxSYM	
RF, kIU/L	No HBT	HBT-treated	No HBT	HBT-treated
89	0.0	0.0	0.0	0.0
90	0.1	0.1	0.5	0.5
103	0.0	0.0	0.1	0.1
109	0.0	0.0	0.4	0.4
185	0.0	0.0	0.5	2.1
201	0.0	0.0	0.1	0.1
209	0.0	0.0	0.1	0.0
387	0.0	0.0	5.1	0.7
503	0.0	0.0	1.0	ND[a]
503	0.0	0.0	1.2	0.4
573	0.0	0.0	1.1	1.9
748	0.0	0.0	2.0	2.2
764	0.0	0.0	3.1	1.3
800	0.0	0.0	6.3	0.5
832	0.0	0.0	1.1	1.2
841	0.0	0.0	11.4	9.5
935	0.0	0.0	0.8	1.9
1129	0.0	0.0	6.9	ND
1270	0.0	0.0	10.4	0.6
1300	0.0	0.0	0.7	ND
1346	0.0	0.0	13.1	2.8
1568	0.0	0.0	11.8	24.7
2938	0.0	0.0	3.4	6.3

B. Effect of RF on two versions of the Abbott AxSYM cTnI immunosassay

	cTnI, μg/L	
RF, kIU/L	Original	Modified
83	2.0	0.0
84	0.0	0.0
98	0.0	0.0
101	0.1	0.0
114	3.1	0.0
125	0.0	0.0
131	0.2	0.4
145	0.1	0.0
146	1.3	0.0
177	0.1	0.0
181	1.3	0.0
219	1.0	0.0
444	0.2	0.0
486	0.2	0.0
500	10.0	0.0
584	3.2	0.0
832	4.3	0.5
899	0.3	0.0
935	0.2	0.0

[a] ND, not determined.