Yumizen BIO

HIL (Hemolysis, Icterus, Lipemia) and the Importance of Auto-Check Systems in the Hemostasis Laboratory

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Introduction:

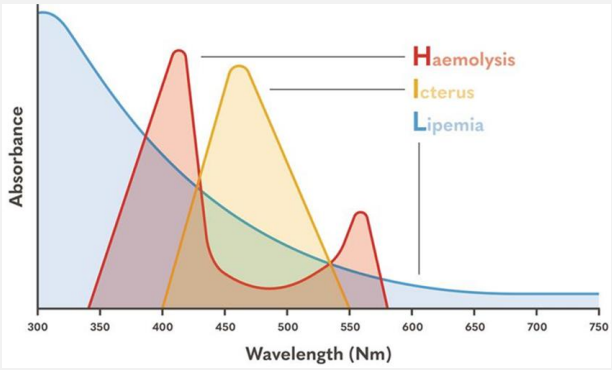
HIL interference is a common problem in routine coagulation laboratory practice.

In accordance with studies[7,8], hemolyzed samples represent between 3% and 6% of tubes in laboratories. Interference due to hemolysis, icterus or lipemia (HIL) may lead to inaccurate results. The aim of this study was to assess HIL interference on prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen, using mechanical and optical detection methods.

Checking tube filling is a crucial step in Hemostasis pre-analysis. It is imperative to respect the hemostasis tube filling procedure.

Interference in Hemostasis testing due to spurious hemolysis is attributed to both analytical and biologic elements, namely high absorbance of cell-free hemoglobin at wavelengths used by optical instrumentation, and release of cytoplasmatic and plasma membrane molecules (e.g., tissue factor, proteases, phospholipids, and ADP) which may activate blood coagulation and platelets.

The interference attributable to hyperbilirubinemia is mostly due to spectral overlap, whereas that of hypertriglyceridemia mainly reflects elements of light scatter and volume displacement, as well as direct interference of lipid particles with hemostasis.



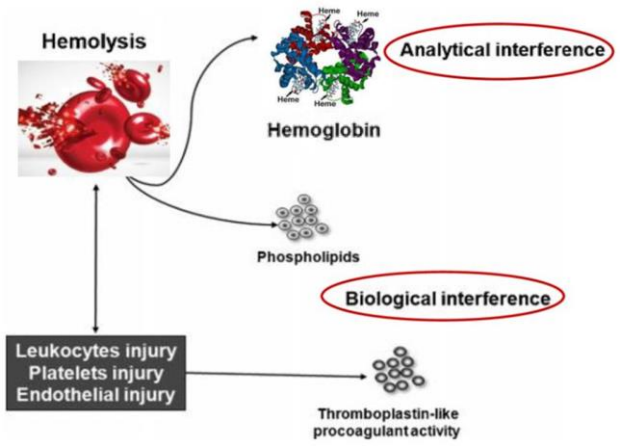
A graph displaying wavelengths at which interference caused by Hemolysis, Icterus & Lipemia is likely to be evident.

Effects of Interfering Substances Including Hemolysis, Icterus and Lipemia (HIL)

International Council for Standardization in Hematology recommendations (1)

Hemolysis:

Hemolysis may affect hemostasis tests due to release of hemoglobin, intra cellular components and thromboplastic substances from damaged cells causing misleading results. For example, the release of tissue factor and subsequent thrombin activation, along with the release of red cell ADP and membrane phospholipids, platelet activation, and the release of PF4 can all contribute to sample activation and clotting factor depletion.



Lippi et. Al. Seminars in Thrombosis & Hemostasis 2013

Recommendation 1: Plasma for coagulation testing, which has been prepared from citrated blood samples, should be checked for the presence of in vitro hemolysis, preferably using an automated system for consistency.

Recommendation 2: APTT should not be performed on samples with hemolysis that has occurred in vitro during sample collection, transport and processing.

Remark: The impact of moderate in vitro hemolysis (i.e., <10 g/L) on PT/INR is usually clinically irrelevant.

Recommendation 3: The possibility that hemolysis has occurred in vivo should be considered. Samples from patients with in vivo hemolysis for determining coagulation tests can be accepted and tested.

Icterus:

The impact of icterus on PT, APTT or fibrinogen with a mechanical system was clinically irrelevant in one study, while another study reported some shortening of PT and APTT (all <10%), increase in Fibrinogen (up to 20%) and elevation of D-dimer (<10%) in the presence of increasing bilirubin concentrations, depending upon reagent/instrument combination. Bilirubin levels >100 µmol/L can reduce ADAMTS 13 activity in some fluorescence assays by quenching fluorescence, which can sometimes be avoided by sample dilution.

Sample dilution may be an option in other tests, such as chromogenic assays, if bilirubin interference is suspected. Where a manufacturer identifies a level of bilirubin that causes interference in a particular test, this should be considered when interpreting and reporting test results.

Lipemia:

Lipemia in citrated plasma samples can lead to inaccurate results when samples are analyzed for coagulation tests due to increased light scatter or absorbance using photo-optical and mechanical systems.

Recommendation: If the lipid level is so high that the analyzer cannot accurately detect clotting, the sample can be analyzed after removal of the lipid from the plasma by centrifugation at 10 000 g for 10 minutes at ambient temperature.

Remark: If high speed centrifugation is used, a reference range should be established for tests on samples processed using this procedure.

Vacutainer /Tube Filling issues in Hemostasis testing:

Insufficient mixing may have a greater effect on specialized hemostasis assays. Citrate tubes must be adequately filled (to the mark noted on the tube if provided) or to not less than 90 % of this total volume. Inadequate filling of the collection tube will lead to inaccurate results.

Recommendation		Potential Risk
	Anticoagulant Recommended Sodium citrate 3,2 % (109 mmol/l); Acceptable Sodium Citrate 3,8% (0,129 M); CTAD acceptable in some circumstances.	Serum or any other anticoagulant can lead to an incorrect result.
	Sufficient volume. Respect the required ratio of sodium citrate to whole blood (1:9). **Fill volume: ≥90 %.** Do not transfer from 1 tube to another. Check expiry date of the tube.	Sample dilution (excess CaCl2-Citratre binding)[9] False results.

As the anticoagulant used (sodium citrate) is liquid, the anticoagulant/blood ratio must be 1 volume of anticoagulant to 9 volumes of blood. In practice, a 90% filling ratio is recommended, 80% being the acceptable limit (6). If this is not the case, the sample is over-diluted and therefore the result may be underestimated.

Conversely, an overfilled tube does not respect the Anticoagulant/Sample ratio either. In this case, the anticoagulant may be in insufficient quantity and its action partially ineffective.

How can HIL auto-check help?

Building on past experience and the available literature, HORIBA carried out a study on the effect of hemolysis, common pre-analytical and analytical issue, on the Yumizen G1550 automated coagulation analyzer, with integrated HIL auto-check at the Sheffield Teaching Hospitals NHS Foundation Trust, UK. (2,3,4,5)

Aim of the study:

To assess acceptable values for hemolysis using the HIL auto-check with regards to in vitro hemolysis on hemostasis assays on the HORIBA Yumizen G1550.

Method:

Plasma was prepared from samples collected into 0.109M trisodium citrate (Vacutainer Plus, Becton Dickinson) by single centrifugation at 2000g for 10 mins.

Hemolyzed plasmas that failed automated detection (Sysmex, CS5100) and their matched repeat from the same patient free of hemolysis and collected within 4 hours of the initial hemolyzed sample were stored at -80oC.

Plasmas were analyzed for prothrombin time (Yumizen G PT5, Yumizen G PT5 Liq, Yumizen G PT5 Reco), activated partial thromboplastin time (Yumizen G APTT, Yumizen G APTT Liq), fibrinogen assay (Yumizen G Fibrinogen) and DDimer assay (Yumizen G Ddi).

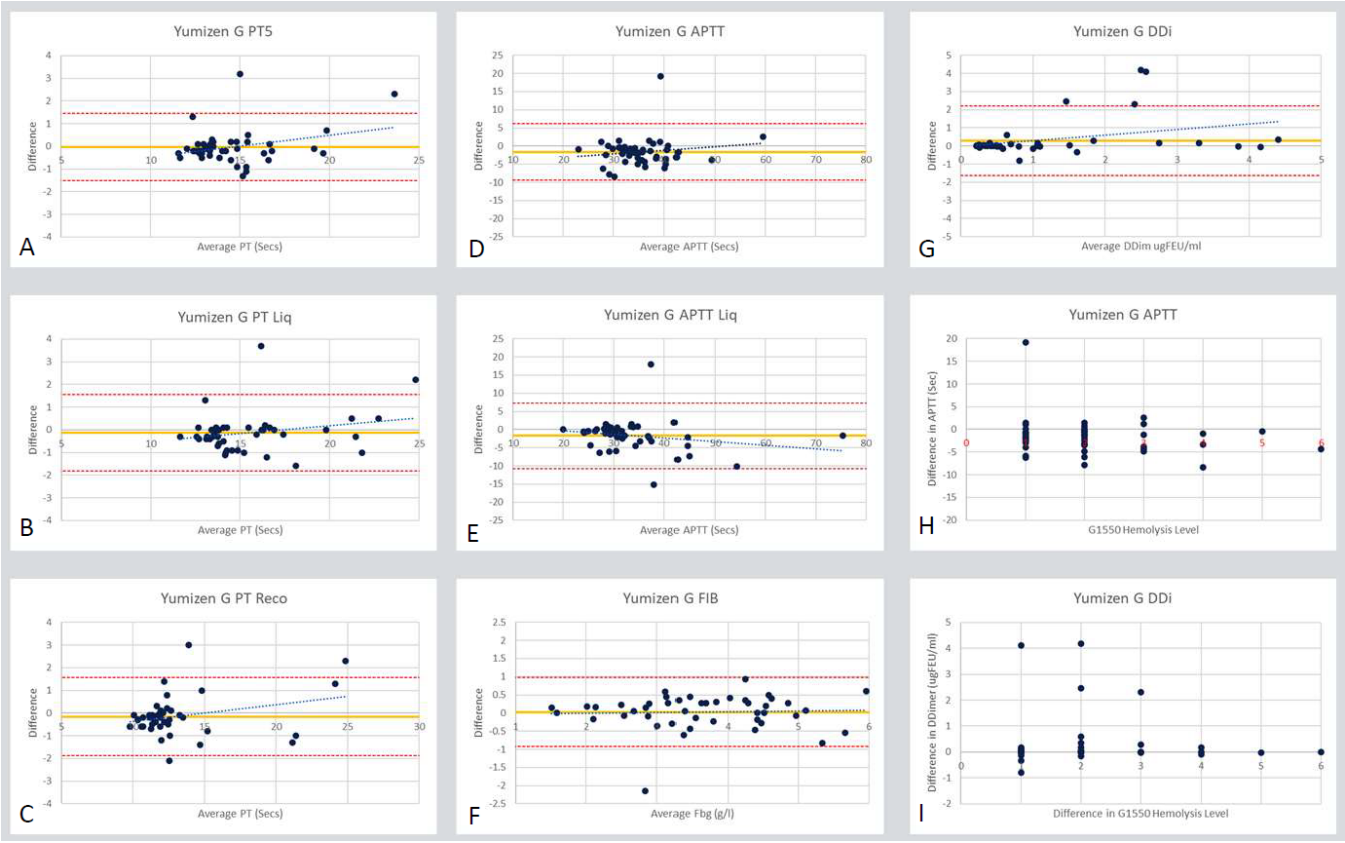
Results:

Spontaneous hemolysis that occurred during sample collection/processing had no clinically relevant effect on PT for any tested thromboplastin or fibrinogen determination (Table 1.) However, APTT showed clinically relevant, reagent-specific changes in some samples. Yumizen G APTT 16/46 >3 second decrease, 1/46 >3 second prolongation. Yumizen G APTT Liquid 6/46 >3 second decrease, 1/43 >3 second prolongation. For DDimer there was an overall increase of 0.3 ugFEU/ml, but 4/46 increased by 2.0 ugFEU/ml or more. There was no correlation between the degree of hemolysis and any changes that occurred.

Tables & Figures:

Mean results of initial rejected Hemolyzed sample and non-Hemolyzed replacement.

			Rejected Heamolysed Sample			Clear Repeat			Difference			
			Mean	Range		Mean	Range		Mean	Range		
Test		Units		Lower	Upper		Lower	Upper		Lower	Upper	N=
Haemolysis	Sysmex XN Hb	g/l	**1.7**	*0.5*	*9.0*	**0.0**	*0*	*0.5*	**1.63**	*0.5*	*9*	46
Haemolysis	G1550 Heam Level		**2.1**	*1.0*	*6.0*	**0.2**	*0*	*1*	**1.89**	*0*	*6*	46
	Reagent											
PT	Yumizen G PT5	Sec.	**14.4**	*11.4*	*24.8*	**14.5**	*11.7*	*22.5*	**0.0**	*-1.3*	*3.2*	45
PT	Yumizen G PT5 Liq	Sec.	**15.4**	*11.5*	*25.9*	**15.5**	*11.8*	*23.7*	**-0.1**	*-1.6*	*3.7*	45
PT	Yumizen G PT5 Reco	Sec.	**12.8**	*9.5*	*26.0*	**12.9**	*10.1*	*23.7*	**-0.2**	*-2.1*	*3*	46
APTT	Yumizen G APTT	Sec.	**34.6**	*22.6*	*60.8*	**36.2**	*23.5*	*58.2*	**-1.6**	*-8.4*	*19.2*	46
APTT	Yumizen G APTT Liq	Sec.	**32.9**	*20.0*	*74.5*	**34.6**	*20*	*76.2*	**-1.7**	*-15.2*	*18*	43
Fib	Yumizen G Fibrinogen	g/l	**3.7**	*1.6*	*6.3*	**3.6**	*1.44*	*5.94*	**0.0**	*-2.15*	*0.94*	45
DDIM	Yumizen G Ddi	ugFEU/ml	**1.199**	*0.220*	*4.620*	**0.900**	*0.220*	*4.230*	**0.298**	*-0.794*	*4.188*	46



Conclusion:

Results from the study confirm that,

- PT and fibrinogen were not clinically significantly affected by hemolysis.
- The APTT of some hemolyzed samples was falsely reduced.
- The D-Dimer of some hemolyzed samples was significantly higher.
- Hemolyzed samples for APTT determination and for accurate D-Dimer determination should be rejected.

However, as hemolyzed D-Dimer results were raised falsely, they may still be safely used for VTE exclusion.

Check points:

- The auto-check, tube filling, integrated hemostasis system eliminates the need for the lab technician to manually check each tube before loading it into the automated system, increasing productivity and guaranteeing quality results.
- The above study and its observations highlight the importance of both pre-analytical HIL auto-check in the coagulation laboratory and the use of automation for quality and lean laboratory operations.

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Authors:

Dr. Prakash Suvasia, Scientific & Medical Officer, HORIBA

Bruno Pougault, International Marketing Hemostasis Product Manager, HORIBA

Alice Tenti, International Marketing Hemostasis Product Manager, HORIBA