

Development of North American Consensus Guidelines for Medical Laboratories That Perform and Interpret Platelet Function Testing Using Light Transmission Aggregometry

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Upon completion of this activity you will be able to:

- describe the overall purpose of the guidelines for platelet light transmittance aggregometry testing for bleeding disorder assessments and why the bleeding time is no longer recommended.
- describe the recommended approach to determine a reference range for light transmission platelet aggregometry.
- list the recommended agonists for light transmission platelet aggregometry assessments for common and rare platelet function disorders.
- describe the recommended approach to interpreting platelet light transmittance aggregometry results, using guidelines, and describe the recommended additional testing to consider when abnormalities are identified.

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Abstract

Platelet function testing is important for the diagnostic evaluation of common and rare bleeding disorders. Our study goals were to promote best practices and reduce unnecessary testing variances by developing North American guidelines on platelet function testing. Guidelines were developed by consensus for expert recommendations (minimum level for approval, 70%) that included recommendations on the evaluation and interpretation of light transmission platelet aggregometry (LTA). To assess consensus, medical opinions on recommendations were gathered from diagnostic laboratories that perform LTA, in collaboration with the Quality Management Program–Laboratory Services (QMP-LS) in Ontario, Canada (10 laboratories), and the North American Specialized Coagulation Laboratory Association (NASCOLA; 47 laboratories, 5 overlapping the QMP-LS group). Adequate consensus was achieved for all and 89% of recommendations for the QMP-LS and NASCOLA groups, respectively. The recommendations adopted provide North American laboratories with additional guidance on platelet function testing, including how to interpret LTA abnormalities.

Platelet function testing is important for the diagnostic evaluation of common and rare bleeding disorders.¹⁻⁵ The International Society on Thrombosis and Haemostasis (ISTH) has provided guidelines for some platelet function tests, such as closure times⁶ and platelet function testing for “drug resistance,” which is not recommended outside of clinical studies.⁷ In 2008, the Clinical and Laboratory Standards Institute (CLSI) published guidelines on platelet function testing that provide clinical laboratories with information on acceptable methods for platelet function testing by a variety of methods, including light transmission aggregometry (LTA).⁸ However, the CLSI guidelines do not address test interpretation. The British Society for Haematology published guidelines on platelet function testing in 1988⁹ and is presently updating its recommendations. It is unclear whether medical laboratories that perform LTA in North America agree with or are following available guidelines.

LTA has been the focus of preguideline practice surveys¹⁰⁻¹³ because it is recognized to be the most important and common test that medical laboratories perform to diagnose platelet function disorders.^{1,14} LTA provides more specific diagnostic information than the bleeding time, and it is also more sensitive to common bleeding problems, when performed by standardized methods, with validated reference intervals (RIs).¹⁵ However, recent surveys on LTA practices for bleeding disorder assessments, including the largest worldwide survey by the ISTH, have illustrated variability in testing and that many laboratories assess LTA without appropriate

RIIs.¹⁰⁻¹³ These surveys have identified wide variations in choice of agonists and agonist concentrations and in the use of healthy control samples for LTA quality monitoring.¹⁰⁻¹³

In an effort to improve and standardize the evaluation of platelet function disorders by medical laboratories, we pursued the development of consensus guidelines on platelet function testing. The guideline development was done by a process of collaboration and consultation, involving diagnostic laboratories in North America that assess platelet function and perform LTA. Recommendations were first developed in collaboration with the Quality Management Program–Laboratory Services (QMP-LS), a department of the Ontario Medical Association that acts on behalf of the Ontario Ministry of Health and Long-Term Care and is responsible for setting standards of practice guidelines for laboratory tests and services, in addition to providing external quality assessment and accreditation of licensed medical laboratories in Ontario. Subsequently, the collaboration was broadened to include the medical laboratories that belong to the North American Specialized Coagulation Laboratory Association (NASCOLA), a nonprofit organization that promotes the development of guidelines on the appropriate use, performance, and interpretation of coagulation tests and provides proficiency testing to North American laboratories that perform diagnostic testing for bleeding and thrombotic disorders. These collaborative consultations generated consensus guidelines that cover important aspects of platelet function testing, including how to interpret and follow up on LTA abnormalities.

Materials and Methods

To establish the level of agreement with expert recommendation statements on platelet function testing, opinions were sought from medical laboratories belonging to several North American external quality assessment organizations that had evaluated platelet function testing practices. First, on October 5, 2009, the medical laboratories that perform platelet function testing in the Canadian provinces of Ontario and Manitoba participated in a full-day review in Hamilton, Canada, cosponsored by QMP-LS and McMaster University, on platelet function testing for bleeding disorder assessments. The meeting ended with an open forum to develop, discuss, and assess consensus for recommendations on platelet function testing practices. Each site was invited to contribute by presenting, sharing evidence, or suggesting program topics. The final program for guideline development covered a broad range of topics (references indicate some of the cited literature reviewed) relevant to platelet function assessment including an overview of platelet function and platelet disorders in adults and children⁴; methods to evaluate platelet function

and establish valid RIIs for these assays¹⁶; surveys on practices for testing platelet function^{10-13,17}; an overview of important recommendations in the CLSI guidelines on platelet function testing⁸ (presented by one of the authors, Margaret Rand, PhD, Hospital for Sick Children, Toronto, Canada); the diagnostic usefulness of bleeding times, LTA, electron microscopy assessments for dense granule deficiency,¹⁵ and platelet adenosine triphosphate release assays⁵; and a presentation on LTA findings for platelet-rich plasma (PRP) samples, tested without (native) or with an adjustment of the sample platelet count that included a review of the literature¹⁸⁻²¹ and an interim analysis of a prospective cohort study, by the Hamilton Regional Laboratory Medicine Program, comparing findings for both types of samples for healthy controls and patients referred for bleeding disorder assessments.

Next, recommendation statements **Table 1**, **Table 2**, and **Table 3** were discussed and developed, with input from the participating medical laboratories that perform platelet function testing whose representation included experts on platelet disorders (Margaret Rand, PhD, S.I., and C.P.M.H.). Key aspects of the evidence reviewed and discussions were summarized in the QMP-LS broadsheet publication on platelet function testing.²² Next, an online survey (opened March 4 and closed April 2, 2010) was used to determine whether NASCOLA medical laboratories agreed with the recommendation statements that were developed and approved at the Hamilton meeting. The online survey was structured so that only the laboratories that performed LTA were able to rate the recommendation statements.

Each site that participated in the study was instructed to provide 1 response that represented its medical opinion on each proposed recommendation statement. At the QMP-LS meeting, a designee recorded the level of agreement and transcribed comments from the open discussions on recommendations, including some qualifying statements from experts (Table 2). Some recommendations were put forward to obtain consensus for CLSI recommendations⁸ that many clinical laboratories in North America and other regions do not follow^{10,11} (eg, regular testing of healthy control subjects and estimation of RIIs using nonparametric analysis). Others were put forward to refine recommendations on LTA agonists made in CLSI guidelines⁸ (eg, the need for a lower limit for ristocetin concentrations appropriate for LTA).

Data from the online survey of NASCOLA participants were analyzed anonymous to participant identity, after removing duplicate responses and vendor responses from the database. To accurately reflect NASCOLA opinions, information for the laboratories that had also participated in the QMP-LS group was not removed until the data were analyzed for all unique participants in the combined group. For each group, the percentage of participants that agreed with each statement were calculated (with correction for skipped responses among

Table 1
Level of Agreement for General Recommendation Statements on the Preevaluation, Examination, and Postevaluation Aspects of Platelet Function Testing*

Recommendation Statements	Agreement			Acceptance Grade
	QMP-LS (n = 10)	NASCOLA (n = 40)	Combined (n = 45)	
1 Platelet function testing should only be performed at the request of a hematologist.	10 (100)	20 (50)	25 (56)	Accepted by QMP-LS; rejected by NASCOLA
2 Peripheral blood platelet morphology should be assessed when patients are undergoing platelet function testing.	10 (100)	31 (78)	36 (80)	Accepted
3 a) i. Samples from a healthy control volunteer will be run on each day patient samples are analyzed. The healthy control volunteer must not be taking drugs known to inhibit platelet function (eg, nonsteroid anti-inflammatory drugs [NSAIDs] that inhibit cyclooxygenase-1, or other platelet inhibitory drugs, such as clopidogrel).	10 (100)	28 (70)	33 (73)	Accepted
a) ii. Individuals with bleeding problems or who have been referred to a hematologist for the investigation of a bleeding disorder should not be healthy controls for platelet function tests.	10 (100)	34 (85)	39 (87)	Accepted
b) The platelet-rich plasma (PRP) of the control sample will be adjusted by adding autologous platelet-poor plasma (PPP) to either the standard adjusted PRP platelet count for testing or adjusted to a lower value, to match the test patient's PRP platelet count, when the patient is thrombocytopenic and has a PRP platelet count below the standardized adjusted value.	10 (100)	31 (78)	36 (80)	Accepted
4 A precollection drug questionnaire will be issued to patients and healthy control volunteers, prior to collection of blood samples to limit interference from drugs and to record the subject's current medications to aid test interpretation.	10 (100)	34 (85)	39 (87)	Accepted
5 The precollection drug questionnaire will be administered by the laboratory staff on the day of collection prior to procuring the blood sample.	10 (100)	25 (63)	34 (76)	Accepted by QMP-LS; rejected by NASCOLA
6 Platelet function testing by LTA will be performed using PRP samples adjusted with autologous PPP to a final, standardized platelet count between 200 and 300 × 10 ⁹ platelets/L.	10 (100)	37 (93)	42 (93)	Accepted
7 a) Diagnostic laboratories are to determine reference intervals for the % maximal aggregation response, specific for each concentration of agonist tested.	10 (100)	38 (95)	43 (96)	Accepted
b) The % maximal aggregation will be determined for each concentration of agonist used in LTA on a minimum of 40 individual healthy control volunteers.	9 (90)	28 (70)	32 (71)	Accepted
c) Nonparametric statistical analysis will be used for the determination of the reference intervals for each agonist.	10 (100)	33 (83)	38 (84)	Accepted
d) LTA reference intervals established on healthy adult volunteers can be applied to children older than neonates.	10 (100)	30 (75)	35 (78)	Accepted
8 When a single agonist is abnormal, this finding should be considered as a potential false-positive or nondiagnostic finding.	10 (100)	16 (40)	21 (47)	Accepted by QMP-LS; rejected by NASCOLA
9 The % maximal aggregation and LTA tracings should be reviewed and the final interpretive comment shall be prepared by a laboratory physician.	10 (100)	37 (93)	42 (93)	Accepted
10 The bleeding time is no longer a recommended test for a bleeding disorder.	10 (100)	36 (90)	41 (91)	Accepted

LTA, light transmission platelet aggregometry; NASCOLA, North American Specialized Coagulation Laboratory Association; QMP-LS, Quality Management Program—Laboratory Services.

* Data are given as number (percentage) of responses. The data for the combined group were corrected for overlap of 5 participants. Grades indicate whether recommendations were accepted or rejected.

Table 2
Level of Agreement for Recommendation Statements on LTA Agonists and Agonist Concentrations*

Recommendation Statements	Agreement			Acceptance Grade	CLSI-Recommended Final Concentrations for the Agonist ⁸
	QMP-LS (n = 10)	NASCOLA (n = 40)	Combined (n = 45)		
Collagen should be run at a low concentration that is verified to detect impaired platelet function from aspirin and other cyclooxygenase 1 inhibitors. <i>QMP-LS: Testing with a higher concentration should be performed when there is reduced maximal aggregation with the low concentration of collagen.</i>	10 (100)	34 (85)	39 (87)	Accepted	1-5 µg/mL (type 1 fibrillary); typically 2 µg/mL to start
Epinephrine should be used at a final concentration of 5-10 µmol/L. <i>QMP-LS: A higher concentration of epinephrine does not have diagnostic utility and should not be included in the panel for LTA.</i>	10 (100)	32 (80)	37 (82)	Accepted	0.5-10 µmol/L; typically 5 µmol/L to start
Arachidonic acid should be used at a final concentration of 0.5-1.64 mmol/L.	10 (100)	38 (95)	43 (96)	Accepted	Single concentration between 0.5 and 1.6 mmol/L
Thromboxane analogue U46619 should be used at a final concentration of 1.0 µmol/L.	10 (100)	16 (40)	42 (47)	Accepted by QMP-LS; rejected by NASCOLA	1-2 µmol/L
Ristocetin should be used at final concentrations of Low: 0.5-0.6 mg/mL High: 1.2-1.5 mg/mL	10 (100)	37 (93)	42 (93)	Accepted	≤0.6 mg/mL 0.8-1.5 mg/mL
ADP should be used at a final concentration of 2.0-10 µmol/L. <i>QMP-LS: Higher concentrations should be tested if aggregation is impaired with 2.0-2.5 µmol/L ADP.</i>	10 (100)	36 (90)	41 (91)	Accepted	0.5-10 µmol/L; typically 5 µmol/L to start

ADP, adenosine diphosphate; CLSI, Clinical and Laboratory Standards Institute; LTA, light transmission platelet aggregometry; NASCOLA, North American Specialized Coagulation Laboratory Association; QMP-LS, Quality Management Program–Laboratory Services.

* Data are given as number (percentage) unless otherwise indicated. The agonist concentrations recommended in the CLSI guidelines⁸ are shown for comparison. The level of agreement for the combined group was corrected for overlap of 5 participants. Grades indicate whether recommendations were accepted or rejected. Items shown in italics are additional expert comments provided to the QMP-LS participant group that were not included in recommendations rated by NASCOLA participants.

Table 3
Level of Agreement With Recommendation Statements on the Interpretation of LTA and Follow-up Investigations to Consider for Different Types of Abnormalities*

LTA Finding	Recommended Interpretation	Follow-up Investigations to Consider
Aggregation is absent or markedly reduced with arachidonic acid, normal with thromboxane analogue, and reduced with low concentrations of collagen. There is absent secondary aggregation with epinephrine.	Aspirin-like defect (drug induced or inherited); drug history should be reviewed.	Repeat testing when subject is not taking aspirin or other nonsteroidal anti-inflammatory drugs
Aggregation is present only with ristocetin.	Possible Glanzmann thrombasthenia (inherited or acquired)	Glycoprotein analysis to assess the platelet fibrinogen receptor α Ib β 3
Aggregation is absent with high concentrations of ristocetin, and the patient has thrombocytopenia with very large platelets (can be normal if the defect is acquired).	Possible Bernard Soulier syndrome (inherited or acquired); von Willebrand factor deficiency should be excluded.	Glycoprotein analysis to assess glycoprotein I bIXV, the platelet von Willebrand factor receptor
Aggregation is reduced with high concentrations of ristocetin, and the patient does not have thrombocytopenia.	Interpretation should consider the possibility of von Willebrand disease.	von Willebrand factor levels
Aggregation is abnormally increased with low concentrations of ristocetin.	Possible type 2B or platelet-type von Willebrand disease	von Willebrand factor levels; consider genetic testing for type 2B or platelet-type von Willebrand disease
Aggregation is abnormal with a number of agonists but markedly impaired with ADP, with significant deaggregation.	The possibility of a platelet ADP receptor defect (P2Y12) should be considered. A drug-induced defect should be excluded as the cause.	Repeat aggregation testing
Other abnormalities with ≥ 2 agonists	The findings suggest that a platelet function disorder is present. The findings should be confirmed on another sample, if clinically indicated.	Platelet ATP release and/or electron microscopy for dense granule deficiency
Abnormalities seen with only 1 agonist (excluding collagen or ristocetin)	Aggregation responses indicate a single agonist abnormality that is nondiagnostic and could represent a false-positive.	Repeat aggregation testing, platelet ATP release, and/or electron microscopy for dense granule deficiency

ADP, adenosine diphosphate; ATP, adenosine triphosphate; LTA, light transmission platelet aggregometry; NASCOLA, North American Specialized Coagulation Laboratory Association; QMP-LS, Quality Management Program–Laboratory Services.

* Data are given as number (percentage) unless otherwise indicated. The level of agreement for the combined group was corrected for overlap of 5 participants. Grades indicate if recommendations were accepted or rejected.

NASCOLA participants), using the number responding to each question as the denominator (“nonapplicable” responses were considered nonagreement).

All recommendation statements were graded as accepted or rejected, based on whether the predetermined minimum 70% level of agreement was achieved to adopt the statement as a consensus recommendation.

Results

Ten diagnostic laboratories (9 from Ontario and 1 from Manitoba) participated in the QMP-LS group, and 47 diagnostic laboratories (7 from Canada and 40 from the United States) participated in the NASCOLA group. Five Canadian laboratories participated in both groups. Almost all QMP-LS (9 [90%]) and NASCOLA (43 [92%]) participants performed platelet function testing by LTA. While the QMP-LS participants rated all recommendation statements, 13 (28%) of NASCOLA participants did not complete the entire online survey (including 2 sites that did not perform LTA).

The level of agreement with the proposed recommendation statements (corrected for skipped responses), is shown in Tables 1 through 3. All recommendation statements were

approved by QMP-LS participants and most (13 [89%] of 15 statements) were approved by NASCOLA participants. Removal of the overlapping participants did not alter the approval or rejection of recommendation statements by the NASCOLA group (not shown).

There were differences between Canadian and US NASCOLA participants for some responses that could reflect differences in medical practices between these countries. For example, all Canadian participants agreed with the recommendation that platelet function testing should be performed only at the request of a hematologist (recommendation 1, Table 1), whereas only 13 (33%) of US laboratories agreed with this recommendation. Some US laboratories commented that they allowed hematologists, pathologists, oncologists, cardiologists, cardiovascular surgeons, and other physicians to order platelet function testing and that some requests were to evaluate drug effects and “drug resistance.”

There was general agreement with the recommendation that peripheral blood cell morphologic features should be assessed for patients undergoing platelet function testing (recommendation 2, Table 1). However, some NASCOLA participants thought it was acceptable to do this before an assessment of platelet function or to restrict this evaluation to people with thrombocytopenia, abnormal platelet size, or “flagged” abnormalities in CBCs.

All recommendations pertaining to the collection and processing of appropriate healthy control samples (recommendations 3a and 3b, Table 1) achieved acceptable consensus. Nevertheless, some QMP-LS and NASCOLA participants indicated that they had difficulties obtaining healthy control samples and/or that they tested control samples less frequently than recommended (eg, when the patient’s test result was abnormal or there was unusual behavior of a specimen, reagent, or equipment; when new reagents were being assessed; and when quality controls were being evaluated).

There was general agreement with the recommendation (recommendation 4, Table 1) that a precollection drug questionnaire be issued to patients and healthy control volunteers, before blood sample collection, to limit interference from drugs known to affect platelet function and to record the subject’s current medications to aid test interpretation. While most Canadian laboratories (10 [100%] in the QMP-LS group and 6 [86%] of 7 in the NASCOLA group) agreed that this should be done by laboratory staff (recommendation 5, Table 1), there was insufficient overall agreement (25 [63%] for all NASCOLA participants) to make this a NASCOLA recommendation.

The recommendation for laboratories to perform LTA on samples adjusted to a standard platelet count (recommendation 6, Table 1) was approved. QMP-LS and NASCOLA participants acknowledged that there were recent publications reporting that adding autologous platelet-poor plasma (PPP) to

QMP-LS (n = 10)	Agreement		Acceptance Grade
	NASCOLA (n = 34)	Combined (n = 39)	
10 (100)	34 (100)	39 (100)	Accepted
10 (100)	31 (91)	36 (92)	Accepted
10 (100)	32 (94)	37 (95)	Accepted
10 (100)	32 (94)	37 (95)	Accepted
10 (100)	32 (94)	37 (95)	Accepted
10 (100)	30 (88)	35 (90)	Accepted
10 (100)	32 (94)	37 (95)	Accepted
10 (100)	26 (76)	31 (79)	Accepted

adjust PRP samples to a standard platelet count is not always necessary for the assessment of some agonist responses and that it may introduce artifact.¹⁸⁻²¹ All QMP-LS participants agreed that laboratories should continue their current practice of adjusting PRP to a standardized platelet count with PPP until more data emerge from large prospective studies of people referred for bleeding disorder assessments. The recommendation was also made because none of the published studies reported on a full agonist panel used for bleeding disorder assessments and none had provided data for a full range of subjects with bleeding disorders,¹⁸⁻²¹ including people with type 2B or platelet-type von Willebrand disease. There was recognition that changing practices to use native samples would require establishment of new RIs and more data on an acceptable range of platelet counts for testing native samples. Among NASCOLA participants, a few disagreed with the recommendation to test a thrombocytopenic patient's sample in parallel with a control PRP sample adjusted to match the low platelet count (a strategy previously reported¹⁶), with 1 laboratory commenting that its site only tested adjusted PRP when samples had very high platelet counts. Another laboratory indicated it would not test PRP with platelet counts of less than $80 \times 10^3/\mu\text{L}$ ($80 \times 10^9/\text{L}$).

There was consensus among QMP-LS and NASCOLA participants for the recommendations that RIs be established for each agonist and agonist concentration used to test maximal aggregation, using a minimum of 40 healthy control samples to obtain a valid RI determination by nonparametric statistical analysis²³ (recommendations 7a-7d, Table 1). A minority thought that the 20 samples recommended in the CLSI guidelines⁸ was more reasonable and/or commented that it would be difficult to collect 40 control samples. The QMP-LS group, but not the NASCOLA group, was presented with the information that LTA findings for neonates and preterm infants, but not older children, differ from those for adults.⁴ However, both groups agreed that adult RIs are acceptable for interpreting data for children older than neonates (recommendation 7d, Table 1).

There was complete agreement among QMP-LS participants, but insufficient agreement (16 [40%]) among NASCOLA participants, with the recommendation (recommendation 8, Table 1) that an abnormality with a single agonist should be considered as a potential false-positive or nondiagnostic finding, as reported.¹⁵ However, there was consensus for the reworded recommendation (Table 3) to consider abnormalities with only 1 agonist (excluding collagen or ristocetin) as nondiagnostic findings that could represent a false-positive.

There was agreement with the recommendation (recommendation 9, Table 1) that a laboratory physician should review the percentage of maximal aggregation and LTA tracings and prepare the final interpretive comment for a report.

A few NASCOLA participants disagreed and stated that this could be delegated to a medical technologist.

Both groups agreed that the bleeding time is no longer a recommended test for a bleeding disorder (recommendation 10, Table 1). A few NASCOLA sites indicated that they were still performing the test.

No formal recommendations on instrumentation were presented. The laboratories that participated in the QMP-LS group discussed that there was no evidence to support endorsement of any specific manufacturer's aggregometer(s) and that there was a lack of direct comparison information on how different instruments perform in assessing LTA for bleeding disorder investigations. They acknowledged that standardization to a single type of instrument might facilitate result comparisons, but without government or other funding to standardize instrumentation, this would not be possible.

All recommendations on LTA agonists (adenosine diphosphate [ADP], epinephrine, arachidonic acid, collagen, thromboxane analogue U46619, and ristocetin) and agonist concentrations (detailed in Table 2) for a testing panel were approved with the exception that only QMP-LS participants approved the use of thromboxane analogue U46619. No participants in either group raised objections to the recommended concentration (1.0 $\mu\text{mol/L}$ final) of thromboxane analogue U46619. Although 15 NASCOLA laboratories agreed and 2 disagreed with the recommendation to use thromboxane analogue U46619, 22 sites thought the recommendation statement was not applicable because they did not use this agonist. A minority of NASCOLA laboratories expressed uncertainties about other agonists and/or commented that they tested a higher or lower concentration.

The QMP-LS group discussed the fact that there is heterogeneity among collagen preparations for LTA but no evidence to recommend purchasing this reagent from a specific vendor. There was consensus from both groups to test collagen at a low concentration, verified to detect impaired platelet function from aspirin and other cyclooxygenase 1 inhibitors (Table 2). QMP-LS participants were also provided with the qualifier that testing should be performed with higher collagen concentrations if maximal aggregation with the low concentration is reduced (Table 2). One QMP-LS participant indicated that it had published that a low concentration of collagen, verified to detect aspirin-induced abnormalities, was sensitive to common platelet function defects due to other causes.¹⁵ Some NASCOLA participants indicated that they relied on other agonists (including arachidonic acid) to detect an aspirin-like abnormality. QMP-LS participants discussed that an LTA panel needed to include arachidonic acid and thromboxane analogue to distinguish aspirin-like defects from other abnormalities. The QMP-LS participants were provided with the expert recommendation qualifiers that higher concentrations of ADP should be tested if aggregation is impaired with 2.0 to 2.5

$\mu\text{mol/L}$ ADP⁵ and that a high concentration of epinephrine (eg, 100 $\mu\text{mol/L}$) does not have diagnostic usefulness¹⁵ and should not be tested (Table 2). The QMP-LS group discussed differences in performance of some lots of ristocetin and whether this may explain why detection of the gain-of-function abnormalities in some subjects with type 2B von Willebrand disease had required more than 0.6 mg/mL ristocetin.²⁴

There was a high level of consensus on how to interpret LTA findings (summarized in Table 3; a lower proportion of NASCOLA participants assessed these recommendations). Some NASCOLA participants commented that they would add to the proposed LTA interpretive comment for some findings. For example, some would comment that drug-induced problems account for most aspirin-like abnormalities. Some laboratories that accepted aggregation requests from cardiologists and cardiovascular surgeons commented that tests showing aggregation only with ristocetin would be reported as indicative of congenital Glanzmann thrombasthenia, afibrinogenemia, or acquired abnormalities due to anti-integrin drugs or autoantibodies. Some NASCOLA laboratories commented that they would report that drug-induced abnormalities should be considered as potential explanations for multiple aggregation abnormalities suggestive of a platelet function defect (but not typical of aspirin-like defects). Some NASCOLA laboratories would assess von Willebrand factor multimers, in addition to von Willebrand factor levels, if there was increased aggregation with ristocetin suggestive of type 2B or platelet-type von Willebrand disease. Others commented that they test for ATP release abnormalities and/or dense granule deficiency at the same time as evaluating LTA.

Discussion

Guidelines are important for the proper application, performance, and interpretation of many laboratory assays. Guidelines for platelet function tests have been particularly challenging to develop because of the assay complexity, the need for fresh blood samples, variations in medical laboratory practices, and uncertainties about best practices.¹⁴ For other assays, participation in surveys on practices, with review of guideline items, has led to increased compliance with guideline recommendations.²⁵

NASCOLA and QMP-LS had participated in questionnaire surveys on platelet function testing,^{10,11,17} and both organizations support laboratory use of the CLSI guideline on platelet function testing⁸ for important guidance on LTA, based on expert opinion and published literature. Both organizations recognized that the CLSI guidelines do not address some important issues, such as LTA interpretation. Both organizations also postulated that it would be feasible to involve medical laboratories that perform LTA

in the development of additional recommendations, based on consensus.

Some differences in QMP-LS compared with NASCOLA responses seem to reflect regional differences in practices, such as whether physicians who are not hematologists (eg, oncologists) commonly order platelet function tests and whether laboratory staff have access to patients during sample collection and/or are willing to administer a precollection drug questionnaire. It is possible that the higher level of consensus achieved for QMP-LS compared with NASCOLA participants was influenced by the fact that unlike the QMP-LS participants, the NASCOLA members did not attend prevote lectures (which reviewed relevant evidence) or participate in group discussions to develop recommendations before determining the level of agreement. It is possible that the lectures led to a greater consideration of evidence when voting on recommendations. Accordingly, there may have been greater awareness among QMP-LS compared with NASCOLA participants that aggregation abnormalities with single agonists often represent false-positives¹⁵ and that thromboxane analogue U46619 is useful for aggregation testing because it is sensitive to common platelet function defects.¹⁵ Alternatively, the prevote lectures may have biased some opinions of QMP-LS participants.

While there were no abstentions among the QMP-LS group, some NASCOLA laboratories did not rate all recommendations, particularly those at the end of the online survey, which has been a problem with other Web-based questionnaire surveys.¹¹ Nevertheless, most recommendation statements in the consensus initiative were adopted as guidelines for North American laboratories.

Acceptance of some key items from the CLSI guidelines (eg, the use of control samples and LTA RIs, determined by nonparametric analysis of maximal aggregation data)⁸ will require updates to the practices of many clinical laboratories, including those that find it difficult to procure healthy control samples in the numbers required to establish valid RIs. The recommendation that the bleeding time (which is much less sensitive to common platelet function disorders than LTA)¹⁵ should no longer be performed to evaluate bleeding problems may stimulate the remaining laboratories that still perform bleeding times to discontinue it.

Most clinical laboratories that offer LTA use the test to evaluate bleeding problems, although some permit its use to evaluate drug responses or drug resistance.¹¹ In our study, some laboratories commented that they perform platelet function testing at the request of cardiologists and cardiovascular surgeons, and some commented that they permitted testing for purposes other than bleeding disorder assessments. The development of recommendations on LTA use for assessing drug effects or drug resistance was not pursued because this is not recommended in the ISTH guidelines, except for research studies.⁷

CLSI guidelines give laboratories the option of testing LTA using native or platelet count–adjusted PRP samples.⁸ The consensus in our study was to use adjusted PRP (prepared by dilution with autologous PPP) when performing LTA for bleeding disorder assessments until native samples are validated to be appropriate for all agonists, including ristocetin, to detect platelet-type and type 2B von Willebrand disease. These recommendations may require a future update if prospective comparison studies, using a full agonist panel, demonstrate that native or adjusted PRP perform better for evaluation of common platelet function disorders.

Our efforts to develop consensus guidelines refined some CLSI guideline⁸ recommendations, such as the recommended concentrations of collagen, given the heterogeneity in preparations, and the appropriate low and high concentrations of ristocetin for LTA to evaluate bleeding problems (Table 2). An aggregation panel that includes ADP, collagen, epinephrine, arachidonic acid, thromboxane analogue U46619, and ristocetin, to detect common and rare platelet function disorders by LTA, was approved by QMP-LS participants, and this panel was approved by NASCOLA participants, except for inclusion of thromboxane analogue U46619. The QMP-LS group approved inclusion of thromboxane analogue U46619 after reviewing and discussing evidence on its sensitivity to common platelet function disorders¹⁵ and its use in distinguishing aspirin-like defects from other function abnormalities.²⁶ It is likely that the NASCOLA laboratories were less certain about the usefulness of thromboxane analogue U46619 because many had never used this agonist and they did not have an organized opportunity to review recent evidence on the usefulness of this agonist. Nevertheless, the QMP-LS recommendation may help guide practices.

The consensus guidelines that our collaborative QMP-LS/NASCOLA study generated provide diagnostic laboratories with additional practical recommendations on how to assess and interpret platelet function by LTA for bleeding disorder assessments. It may prove useful to do a follow-up survey to determine if additional or modified recommendations are needed to optimize practices and to promote compliance with platelet function testing recommendations because surveys have been helpful for promoting compliance with guidelines for other complex diagnostic assays.²⁵ Our study also suggests that participation in educational activities is important to ensure that diagnostic laboratories understand and follow recommendations on how to perform and interpret platelet function testing for bleeding disorder assessments.

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