Light transmission aggregometry for platelet function testing: position paper on current recommendations and French proposals for accreditation

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Keywords:

Accreditation; laboratory practice; light transmission aggregometry; platelet.

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Abstract

Light transmission aggregometry (LTA) is a method used to investigate platelet functions in platelet-rich plasma (PRP), notably when screening for platelet disorders. Various national guidelines and recommendations help in setting up the LTA test in specialized laboratories. However, due to the nature of the sample matrix and its subsequent specificities, more accurate positions are needed to achieve LTA accreditation according to the standard NF EN ISO 15 189. We reviewed guidelines and recommendations as they can be useful in the accreditation process, and we conducted a survey on LTA practice among members of the *Société Française de Thrombose et d'Hémostase (SFTH*) in 2021. We formulated 28 proposals, which have been approved by vote within the SFTH. All aspects to take into consideration for the proper conduct of LTA assays and their accreditation have been covered. Notably, preanalytical, analytical and postanalytical aspects are depicted, including blood sampling, PRP preparation, instruments, agonists, performance assessment, personnel training and data interpretation. This document, essentially representing a French position paper on the current recommendations and subsequent proposals for LTA accreditation, might prove useful also outside France for relevant laboratories and auditors involved in LTA accreditation.

Introduction

Light transmission aggregometry (LTA) is a method used to investigate platelet functions and a functional test to explore heparin-induced thrombocytopenia (HIT). The specificities of the sample matrix must be taken into account in view of LTA accreditation according to the International Organization for Standardization (ISO) standard NF EN ISO 15 189.1,2 To date, several guidelines have been published to improve LTA standardization.3–9 Our proposals aim helping laboratories to obtain accreditation for the preanalytical, analytical and post-analytical aspects of LTA performed on platelet-rich plasma (PRP). A survey was conducted on LTA practice among the members of the SFTH (Société Française de Thrombose et d'Hémostase, in 2021) (Supplemental data S1), and current guidelines were reviewed. This resulted in 28 proposals (Table I) that have been approved by the French Centre de Référence des Pathologies Plaquettaires Constitutionnelles (CRPP) and SFHT members (Figure 1). Their main applications are bleeding syndromes suggesting a platelet disorder, and suspicion of type 2B or platelet-type von Willebrand dis- ease (VWD) where ristocetin-induced platelet agglutination (RIPA) is used. However, most of these proposals can be applied to other LTA-based assays.

Of note, this document does not cover whole blood impedance aggregometry nor instrument-related LTA specificities.

Overview and medical justification

LTA can notably be useful in the investigation of hemorrhagic syndromes, in addition to platelet count and coagulation factor assessments. LTA using PRP allows to screen for acquired or congenital platelet function disorders.10,11 LTA can also be used to diagnose HIT12,13 and, in a few situations, to evaluate the efficacy of antiplatelet therapy or document treatment compliance.14–16

Regardless of the medical context, preanalytical steps are crucial, with a precise evaluation of the indication, notably in a pediatric setting.

LTA characteristics

LTA principle

LTA records the transmission of light through a sample of stirred PRP at 37°C, which increases upon platelet aggregation after the addition of an agonist. The result is compared to that obtained with autologous platelet-poor plasma (PPP).17

It is recommended to perform LTA at 37°C, letting the sample stabilize for at least 1 minute or up to 5 minutes before adding the agonist,7 respectively, as per guidelines of the International Society on Thrombosis and Haemostasis (ISTH) or British guidelines.6 To initiate aggregation, a maximum of 1/10 final volume of agonist should be dispensed directly in the PRP,6,7 not down the wall of the cuvette.4 The change in light transmission should then be followed for 3−10 minutes.6,7

Instrument characteristics

The total volume should be sufficient to let the aggregometer record light transmission over time as a curve. Some suppliers propose mechanical devices for smaller sample volumes by elevating the cuvettes. LTA6,7 requires continuous stirring at 1000 rpm5 or between 1000 and 1200 rpm.6

Substantial differences pertain to light sources or stirring speed, without available comparative studies (Supplemental Table). The latter are difficult to perform, due to the large amounts of specimen required (180−500 μL) and numerous reagents available. Hence, LTA still lacks standardization. There does not seem to be any clinical trial regarding the sensitivity/ specificity of LTA for the diagnosis of platelet disorders, which will constitute a difficulty for the application of the In Vitro Diagnostic Regulation (IVDR) 2017/746, issued by the European Union.18

Connection with laboratory information systems

The absence of a connection between LTA instruments and laboratory information systems (LIS), with subsequent manual entry of results, requires specific procedures for result-recording control.1

Agonists for LTA

Platelet disorders can be suspected when a single agonist yields abnormal agglutination, but guidelines recommend the use of several agonists, because of shared platelet signalling and amplification pathways.11 There are, however, some discrepancies in their choice or concentration (Table II).4–7,19 First-line agonists are adenosine diphosphate (ADP), epinephrine/adrenaline, collagen, arachidonic acid and ristocetin, while second-line agonists comprise thrombin, thrombin-receptor activating peptides (TRAPs), collagen-related peptides (CRP), thromboxane A2 mimetic U46619, calcium ionophore A23187 and convulxin.6 UK guidelines suggest to use a full panel of 7 agonists and to choose concentrations sufficient to cause maximum aggregation of more than 50% and no complete disaggregation in 95% of normal controls (except for low-dose ristocetin and normal saline).9

Specificities of LTA-based tests and proposals

At least 70% agreement allowed 60 voters to validate these proposals if less than 20% disagreed. Rejected proposals (number 12) or with relevant comments (number 11, 15 and 16) were revised and resubmitted to vote. The main steps involved in LTA accreditation are summarized in Table III. Our proposals for LTA assessment and accreditation are summarized in Table I and were based on the following arguments.

Overall approach, risk management and personnel qualification

Overall approach

LTA provides both quantitative (maximal aggregation amplitude, velocity or slope of aggregation, length of the lag phase, amplitude at the end of the monitoring period) and qualitative data (presence/absence of aggregation, curve shape, presence of shape change, deaggregation, presence of a secondary wave). LTA tests can thus be considered either as a qualitative and/or quantitative method. LTA interpretation must consider results obtained with all agonists. RIPA (i.e. ristocetin) should be distinguished, because it determines the presence or absence of platelet agglutination at low-dose and high-dose ristocetin.

Table I. SFTH proposals for light transmission aggregometry (LTA) assessment and accreditation.

Proposal 1	Examinations based on LTA can be accredited, based on quantitative (reference intervals) or qualitative (curve shape) data.
Proposal 2	Procedures can be verified rather than validated if the instrument and all provided reagents are used exactly as validated by the manufacturer.
Proposal 3	LTA requires specifically trained laboratory operators. Their qualification should aim at mastering LTA preanalytical and analytical specificities.
Proposal 4	Medical personnel should be trained to review requests and interpretations, during a supervised period with qualified personnel. This competency can be evaluated through simultaneous interpretation of a series of results with all authorized personnel and/or by regular participation to postanalytical EQA.
Proposal 5	LTA tests should be performed in a defined clinical context and with prior review of the request.
Proposal 6	LTA should not be performed in patients who take medication or exogenous substances with potential interference on platelet functions. This must be taken into account during interpretation if the test has been done anyway.
Proposal 7	Venous blood should be drawn without or with minimum tourniquet pressure, in plastic (polypropylene) or siliconized glass tubes, containing buffered sodium citrate 0.109 M or 0.129 M, and with needles of at least 21 gauge.
Proposal 8	Sampling time should be recorded and centrifugation carried out after 15 minutes of whole blood stabilization. LTA studies should be completed within 4 hours after blood sampling. Specific arrangements may be adopted if they are validated by the laboratory.
Proposal 9	Hemolyzed samples should be discarded. Icterus and lipemia should be notified and considered when interpreting the results.
Proposal 10	Pneumatic systems should not be used for specimen transportation. Because these systems have very various specifications, if the laboratory intends to use it, this should be specifically validated for LTA-based platelet function exploration.
Proposal 11*	PRP should be prepared after centrifugation at 170–200 *g*, for 10–15 minutes, at ambient temperature, without brake, unless the laboratory has validated the use of minimal brake.
Proposal 12*	PRP platelet counts should not be adjusted, unless >600 G/L. Above 600 G/L, PRP dilution with autologous PPP can interfere with platelet functions. Considering the absence of recommendations about PRP dilution, it is suggested not to perform LTA in such conditions. If LTA is performed anyway, the precise conditions should be notified and taken into account during interpretation.
Proposal 13	For PRP platelet counts <150 G/L, LTA results should be interpreted with caution and consider the analytical thresholds provided by the instrument manufacturer and/or agonists used whenever appropriate.
Proposal 14	Calibration should systematically be set at 0% (PRP) and 100% (PPP) transmission for each sample.
Proposal 15*	The use of "control" subjects (defined as subjects from whom blood is specifically collected for this purpose, regardless of any medical indication for hemostasis investigation) is not mandatory to master LTA. If this is needed, the laboratory should comply with legal regulation
Proposal 16*	Each PRP should be controlled for the absence of spontaneous platelet aggregation in the absence of any agonist. The platelet maximal amplitude aggregation threshold should be defined by the laboratory. A positive reaction (platelet aggregation) should also be observed with at least one agonist.
Proposal 17	The laboratory should have a procedure for reagent validation, based on risk assessments, in the absence of IQC for LTA. The use of "control" samples from healthy subjects will be left to the appreciation of the laboratory (see Proposal 15)
Proposal 18	Due to the lack of EQA programs or proficiency testing for LTA: (i) the laboratory should regularly perform risk assessments and/or participate in a postanalytical EQA program for qualitative aspects (ii) for quantitative aspects, the evaluation of precision, bias and measurement uncertainty is not feasible
Proposal 19	Repeatability assays should be performed initially, upon acquisition of an LTA instrument, for each channel. At later stages, in case of doubt on LTA results repeatability, consecutive measures (i.e. 5), with at least one agonist should be performed on the channel involved. This can be performed with the remaining PRP of the patient, or with PRP from "control" subjects in compliance with legal regulations. It is proposed to set the acceptance limit of the CV at 15%, for usual agonist concentrations. Reproducibility assays are not recommended.
Proposal 20	Between channels concordance should be tested upon acquisition of a new LTA instrument. Later, whenever there is a doubt on concordance, it must be tested using at least one agonist with the remaining PRP of the patient, or with PRP from "control" subjects in compliance with legal regulations. Each channel must be tested. It is proposed to set the acceptance limit of the CV at 15%, when using usual agonist concentrations or good agreement for interpretation.
Proposal 21	If there is no other qualified instrument in the laboratory, the laboratory should use samples from "control" subjects in compliance with legal regulations to test performance for the initial qualification of an LTA instrument.
Proposal 22	Concordance between two instruments should be checked before use, if possible, in case of preventive maintenance, instrument failure or when the manufacturer proposes to loan an aggregometer. This should be performed with at least one agonist at the usual concentration with the same PRP issued from a patient test or with PRP from "control" subjects in compliance with legal regulations. It is proposed to set the acceptance limit of the CV at 15%.
Proposal 23	When platelet aggregation fails with only one agonist, the operator should check the presence of the stirring magnet in the cuvette and repeat the test.
Proposal 24	Result concordance should be checked at least upon acquisition of a new instrument, if the laboratory has several LTA instruments equally used for the same tests. This could be performed using patient samples, with agonists and concentrations inducing a high amplitude of platelet aggregation. It is proposed to set the acceptance limit for bias between instruments at 15% and/or consider concordance during interpretation.
Proposal 25	The performance of new batches of agonists or new shipments should be checked by comparison with a previous batch/vial, using residual PRP from patient tests or PRP from "controls," in compliance with legal regulations. It is proposed to set the acceptance limit for bias between agonist batches at 15% and/or consider concordance during interpretation.
Proposal 26	It is proposed to define reference intervals, based on published data, manufacturers of LTA instruments and reagent data, and, if possible, with local verification with ≥ 20 "control" subjects, well selected after a medical examination to avoid any drug interference, and in accordance with legal regulations.
Proposal 27	The interpretation of LTA results must be contextualized. The final report should mention agonists and their concentrations, and provide quantitative and qualitative interpretation and/or aggregation tracings. In case of abnormal responses, a control should be performed on another sample from the patient, using LTA and/or another method allowing for platelet function exploration.
Proposal 28	LTA for platelet disorder screening should not be used in children less than one-year-old. For the pediatric population above the age of one, it is proposed to consider any occurring difficulty during blood sampling, when interpreting LTA results.

EQA: External quality assessment, IQC: Internal quality control, PRP: platelet-rich plasma, PPP: platelet-poor plasma, CV: coefficients of variation
*Revised proposals resubmitted to vote

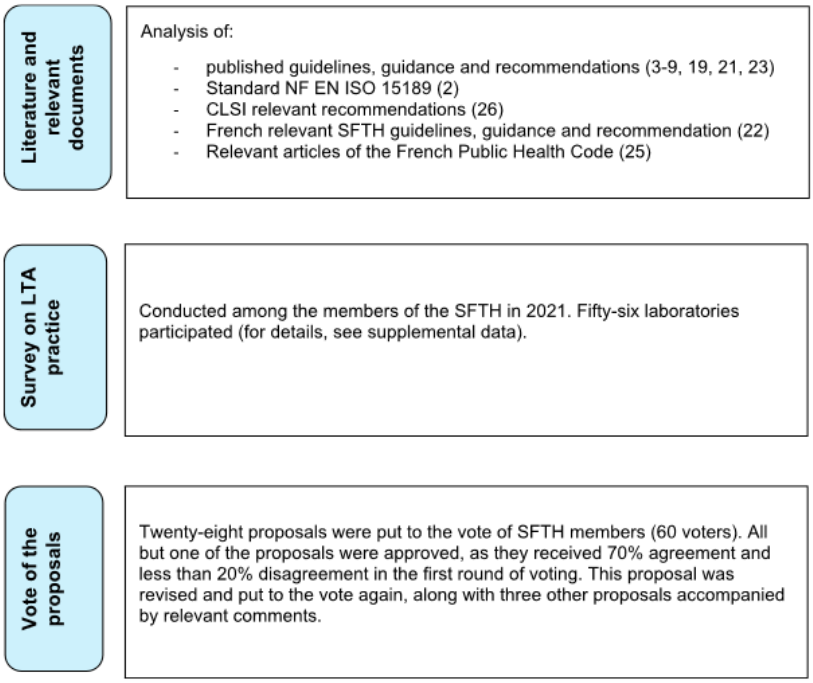

Figure 1. Flow chart for the proposal development.
SFTH: Société Française de Thrombose et d'Hémostase; CLSI: Clinical & Laboratory Standards Institute; LTA: light transmission aggregometry.

Risk management

Main specific critical risk points are i) knowledge of the clinical and therapeutic context that led to the order of the test, which impacts the choice of agonists and proper interpretation of LTA curves; ii) specimen transportation; iii) agonists and PRP preparation; iv) time delay between blood collection, PRP preparation and LTA; v) manual LIS entry of results.

Training and qualification of operators

All guidelines agree that LTA-based tests should be addressed to specialized laboratories,5,7 performed and interpreted by qualified and experienced personnel5,6 able to demonstrate competency in aggregation curves interpretation. External quality assessment (EQA) can be helpful.8 Laboratory management shall define and document personnel qualifications, training, and competency records, as for other tests. For technicians, the qualification should demonstrate the correct preparation of PRP samples and reagents. The remaining PRP samples can be used to test inter-operator variability.

Questionnaires are helpful to standardize knowledge assessment.

The qualification of medical personnel involves a period of supervised reviewing of prescriptions, results and interpretation. To maintain competency or evaluate interindividual variability, qualified personnel can regularly perform collegial reviews of data interpretation and/or participate in EQA.

Table II. Main agonists used to explore platelet functions in light transmission aggregometry. Final concentrations are indicated according to different guidelines: CLSI (Clinical & Laboratory Standards Institute),[4] North American Consensus Guidelines,[5] British Society for Haematology Committee,[6,9] ISTH (International Society on Thrombosis and Haemostasis)[7] and French *Centre de Référence des Pathologies Plaquettaires Constitutionnelles* (CRPP).[11]

Agonists	Targets	CLSI – 2008	USA – 2010	UK – 2011	ISTH – 2013	CRPP – 2017	UK – 2021
ADP	$P2Y_1$, $P2Y_{12}$	0.5 - 10 µM often 5 µM	2 – 10 µM	0.5 – 20 µM 2.5 µM initial	2 µM initial	2 µM – 10 µM – 100 µM	5 and 10 µM 10 µM if one
Collagen	GPVI, GPIaIIa	type I: 1 to 5 µg/mL Often 2 µg/mL initial	type I: 1 to 5 µg/mL Often 2 µg/mL	type I: 1 to 5 µg/mL/ 1.25 µg/mL initial	Horm collagen (equine tendon) 2 µg/mL initial	2 µg/mL – 10 µg/mL	1/1.25 and 2/2.5 µg/mL 2/2.5 µg/mL if one
Epinephrine (adrenaline)	α2A	0.5 to 10 µM Often 5 µM initial	0.5 to 10 µM > 10 µM not useful	0.5 to 10 µM 5 µM initial	5 µM initial	25 µM	5 and 10 µM 10 µM if one
Gamma thrombin	PAR-1 and PAR-4,	Concentrations not specified*		50 to 200 ng/mL			
TRAP	PAR-1 and PAR-4	Concentrations not specified		TRAP-6 = SFLLRN (PAR-1 agonist) 10 to 100 µM /AYPGKF (PAR-4) 100 to 500 µM	10 µM	TRAP-6 10 µM to 50 µM	5 and 10 µM (PAR-1)
Arachidonic acid	TXA_2 generation, agonist of TP_α receptor	0.5 – 1.6 mM	0.5 – 1.64 mM	0.5 – 1.0 mm/1 mM (only one concentration proposed)	1 mM initial	1 mM	
U46619	TPα	1 to 2 µM	1 µM	1 µM/single point	1 µM		1 µM
Ristocetin	VWF-GPIb interaction	2 concentrations: - Low: ≤ 0.6 mg/mL - High: 0.8 to 1.5 mg/mL	2 concentrations: - Low: 0.5 or 0.6 mg/mL - High: 1.2 to 1.5 mg/mL	2 concentrations: - Low: 0.5 to 0.7 mg/mL - High: 1.2 to 1.5 mg/mL	1.2 mg/mL initial If normal: 0.5 to 0.7 mg/mL If absent: 2 mg/mL		0.5 and 1.25 g/L
CRP	GPVI			10 to 1000 ng/mL			
Convulxine	GPVI			1 to 1000 ng/mL			
Ionophore A23187	Intracellular calcium signalling, procoagulant function			1.25 to 10 µM			
PMA	Protein kinase C			30 mM			

ADP, adenosine diphosphate; α_{2A}, adrenoreceptor; CRP, collagen-related peptide; EPHB2, ephrine transmembrane receptor B2 tyrosine kinases; PAR, protease activating receptor; PMA, phorbol 12-myristate 13-acetate; TPα, thromboxane receptor; TRAP, thrombin-receptor activating peptide, peptides activating PAR-1 (SFLLRN or TFLLRN) or PAR-4 (AYPGKF); TXA_2, thromboxane A_2; VWF, von Willebrand factor. *Depending on the preparation

Preanalytical requ*irements*

Clinical data

Access of medical personnel to patient clinical data is important. Platelet disorders are associated with mucocutaneous hemorrhagic manifestations, and VWD should be ruled out first. Clinical examination can be standardized using Bleeding Assessment Tools (i.e. ISTH-BAT –20). Clinical data should include personal and familial bleeding history, with a family tree when relevant.21 Physical examination should provide information about syndromic presentation of thrombocytopenia and/or platelet dysfunction, such as deafness, renal or neurological impairment. Exogenous substances potentially interfering with platelet functions should be listed before blood collection (see III.2.2). Sampling for LTA should not occur before clinical review, in order to optimize timing (treatment-dependent), and agonist choice, concentration and ranking, especially for small samples.

Patient preparation for specimen collection

Guidelines suggest to perform LTA tests in resting subjects to avoid platelet activation by adrenaline release after physical effort.6,7 CLSI and UK guidelines also recommend fasting before sampling4,6 although light meals are unlikely to affect LTA.

Smoking (30 minutes), ingestion of caffeine (2 hours), drug or any substance affecting platelet functions (CLSI and UK lists) 4, 6, 7, 10 should be avoided. For the latter, a 3−14 days delay after the last dose is recommended.6,7 All guidelines encourage to collect this information and take it into account in LTA interpretation.4–7,11

Specimen collection, transportation and conservation

LTA, sensitive to preanalytical steps, requires that venous blood is drawn in polypropylene or siliconized glass tubes, with 0.109 M/ 0.129 M buffered sodium citrate.22 CLSI guidelines recommend the dihydrate form of trisodium citrate at 0.105/0.109 M (3.2%), or anticoagulant citrate-dextrose formula A (ACD-A), excluding other anticoagulants including ACD.4 Blood sampling should use at least 21 gauge needles.22 Depending on patient age and sampling volume, tourniquets should be avoided, or released as soon as blood collection begins, discarding the first 3–4 mL. LTA interpretation should consider these conditions.

Samples should be kept at room temperature. ISTH guidelines recommend centrifugation after a "rest" time of 15 minutes.7 All guidelines suggest that LTA should be performed between 15 minutes and 4 hours after blood sampling,4,6,7 although this is not properly supported. LTA performed too early could be impacted by endogenous adrenaline or centrifugation-related ADP release.4 CLSI recommendations indicate that LTA results are significantly modified when performed on PRP older than 4 hours.4 ISTH suggests that such a delay should be mentioned in the report.7

Hemolysis induces both an overestimation of light absorbance and ADP release that could activate or desensitize platelets. PRP can also be contaminated with residual red blood cells (RBC) in case of suboptimal centrifugation or during PRP decantation. RBC absorb the transmitted light and may induce falsely decreased aggregation. Leukocytes can also induce overestimated light absorbance. Hyperlipidemia interferes with basal turbidimetry and light transmission.4 Icteric samples with bilirubin concentrations >34.2 µM (2 mg/dL) can decrease the maximal amplitude aggregation of about 12.5%.4 UK and CLSI guidelines suggest that LTA should not be performed in such circumstances.4,6 ISTH guidelines suggest to discard grossly hemolyzed samples and indicate lipemia in the final report.7

French guidelines (SFHT) suggest courier transportation or specific validation of pneumatic tube systems.22 CLSI recommends to transport samples in the upright position at room temperature (20° −25°C) 4 but not to use pneumatic systems. UK guidelines suggest that it is preferable for samples to be taken at the same site as the testing laboratory to prevent transport artefacts.9

Considering the importance of specimen collection quality, procedures should facilitate the traceability of time delays between collection and LTA, specimen transportation and total duration of examination. Laboratory proximity to the collection centre and delays between LTA test and prescription help to better control preanalytical processes.

New devices are being developed, such as devices for storing PRP at room temperature for several days. To date, there are no data on their evaluation in clinical diagnosis.

Preparation and stability of PRP and PPP

PRP is prepared after centrifugation between 170 and 200 g for 10 −15 min at ambient temperature without braking.4,6,7 However, due to various braking systems, the use of minimum brakes can be adapted in some laboratories. PRP should be collected with a plastic pipette and placed in an identified then capped plastic tube. Of note, this method is not suitable in case of thrombocytopenia with very large platelets.4,7 ISTH guidelines thus suggest to collect PRP after blood sedimentation in such cases. It is however uncertain whether tubes should be kept at a 45° angle, when preparing PRP by sedimentation of blood samples with very large platelets.7 PRP could also be prepared after first centrifugation at 65 g for 10 minutes, then at 200 g to collect normal-sized platelets (unpublished data).

The optimal platelet count for PRP should be between 150 and 600 G/L. ISTH guidelines suggest that LTA results can be impaired if the PRP platelet count is <150 G/L. They also recommend not to adjust to a standardized value with autologous PPP before LTA tests, below 600 G/L. Above this value, uncertainty remains over the correct interpretation of LTA results. Moreover, dilution with autologous PRP may interfere with platelet function.7 UK guide- lines indicate that unless it is >600 G/L, the platelet count should not be adjusted using PPP, as this may cause artefactual inhibition of platelet aggregation.6 Although this is not documented in UK recommendations, such artefacts are addressed elsewhere.23,24 Both

ISTH and UK guidelines consider that even with low PRP platelet counts LTA should be performed to exclude the major platelet disorders (Glanzmann thrombasthenia, Bernard-Soulier syndrome, type 2B and platelet-type VWD).6,7,9

Table III. Main steps involved in light transmission aggregometry accreditation.

	Validation procedure
Risk management	Procedures to: - gather information on the clinical and therapeutic context - master pre-analytical, analytical and post-analytical stages - train and qualify laboratory technicians and medical staff
Repeatability assay	- Initially for newly acquired instruments on each channel, later if doubt about the results - Consecutive measures (at least 5), with at least 1 agonist at usual concentration with residual PRP from a patient or PRP from a control subject in compliance with legal regulation - Acceptable limit of 15% for CV when interpretation is based on quantitative data
Concordance between the channels	- Initially for newly acquired instruments on each channel, later if doubt about concordance -With at least 1 agonist at usual concentration with residual PRP from a patient or PRP from a control subject in compliance with legal regulation - Acceptable limit of 15% for CV when interpretation is based on quantitative data
Comparison of instruments	Check the concordance between the instruments before use if: - several instruments are used for the same test, - in case of preventive maintenance or instrument failure, when the manufacturer proposes a loaned aggregometer Use of at least 1 agonist at usual concentration with residual PRP from a patient or PRP from a control subject in compliance with legal regulation - acceptable limit of 15% for CV when interpretation is based on quantitative data
Inter-assay variability	No possible studies
Interferences	No possible studies - Hemolysis: The release of purinergic nucleotides, including ADP, can activate and desensitize platelets and may interfere with optical reading. Samples with hemolysis should be rejected - Lactescence: Avoid fatty meals as sample lactescence may interfere with optical reading. Lactescent samples are to be rejected or interpreted with caution. - Icterus: There is no literature on the impact of bilirubin on optical aggregometry. Adjustment of 100% aggregation using PPP from the patient should make it possible to avoid the matrix effect specific to the patient. If turbidity or bilirubin levels are too high, calibration is impossible: the test cannot be performed. - Drugs: The patient questioned the patient to check whether anti-platelet drugs are being taken prior to sampling - Physical exertion: The adrenaline produced during physical exertion may cause platelet activation. It is recommended that the patient rests before blood sampling.
Intra-operator variability	Collegial interpretation of a series of results together with all authorized personnel and/or the regular participation in postanalytical EQA
Interpretation criteria	Based on - Qualitative data (curve shape) - Quantitative data (referred to reference intervals for maximal aggregation (%) and/or latency (sec), and/or slope (%) and or area under the curve (arbitrary units) Interpretation according to the clinical and therapeutic context
Accuracy	Not applicable in the absence of internal and external quality control. In case of participation in a post-analytical external quality assessment, the internal organization for the analysis of this external quality assessment must be described: participants (number and quality: medical staff, laboratory technicians, engineers, medical residents, etc.)
Sensitivity and specificity	Given the low prevalence of the pathologies concerned and the lack of control samples, an experimental study to check sensitivity and specificity in the laboratory is not feasible. Prescription for this test should always be accompanied by clinical and therapeutic information. Interpretation of results must consider hemorrhagic symptoms and other clinical data, and other tests carried out to investigate primary hemostasis, such as measurement of von Willebrand factor, platelet occlusion times measured by Platelet Function Analyzer, platelet glycoprotein studies by flow cytometry, dense granule studies, etc. In the event of an abnormal response to one or more agonists, a check must be carried out on a new sample, possibly combined with additional tests.
Measurement range/limit of detection	The measurement range should be predefined for each patient between 0% and 100%, established with, respectively, PRP and PPP from the patient. When possible, this step should be applied to each channel. It is not possible to define a detection limit.
Contamination	The use of single-use glass reaction cuvettes and disposable pipette tips for each patient and for each reagent avoids inter-sample contamination. It is mandatory to change the pipette tip between each reagent to avoid contamination.
Reagent robustness and stability	Package inserts and stress data sheets for each reagent provide this information. If applicable, details of tests carried out locally should be recorded.
Reference range	Supplier information, published data and/or local verification with ≥ 20 "control" subjects, well selected after a medical examination to avoid any drug interference, and in accordance with legal regulation.

PRP: platelet-rich plasma, PPP: platelet-poor plasma, CV: coefficient of variation

PPP is prepared from whole blood or from the same tube after PRP preparation, by centrifugation at 1500−2000 g for 15 min, or 2000−2500 g for at least 10 min at ambient temperature, according to SFTH recommendations.22

Quality criteria in LTA

No appropriate internal quality controls (IQC) or EQA are currently provided by manufacturers for LTA. There also is no efficient method to stabilize PRP i.g. freezing, or lyophilization. Inter- laboratory comparison programs are also very difficult to set-up, due to the narrow time window after blood sample collection before platelet functions are impaired. Moreover, large volumes would be required, even with a limited number of participants.8

In such cases, ISO 15 189 states that "the laboratory shall develop other approaches and provide objective evidence for determining the acceptability of examination results." All guide- lines propose various options.4–8 The qualitative approach of LTA, however, may work without IQC or EQA, if risk management is correctly assessed and regularly reviewed, and if comparison assays are performed.

Calibration of aggregometer channels

It is crucial for an optical method to calibrate 0% (PRP) and 100% (PPP) transmission for each sample.4,6,7 This step should be applied for each channel, except for instruments that apply the 0% set of the first channel to all others. The CLSI suggests that each laboratory should:

measure its own precision, by testing a minimum of 10 samples in duplicate and calculating the coefficient of variation of the duplicate pair (the nature of samples and acceptance limits are not specified) validate between-instruments reproducibility if more than one aggregometer is available. The medical personnel should establish performance criteria for between-analyzers variance.

test transmission linearity, using for instance a 50% point (1:1 PRP/PPP dilution), however, the relevance of such a test is questionable.

validate the temperature and mechanical stirrers RPM (acceptance limits not defined), which is challenging for a laboratory, but feasible by the manufacturer.

Internal quality controls

Use of « control » platelets.

Current guidelines suggest using normal platelets from healthy donors as controls. This is appropriate in the following cases:

- reagent or batch validation.4–6

- if an unexpectedly abnormal LTA test is observed in a patient,4–6 or always in parallel with each patient.7 This is controversial because of platelet response variability with some agonists.

- when testing a low platelet count PRP (<150 G/L), after adjusting the control PRP sample with its PPP to match the PRP count of the patient.5,6 This is controversial, as previously discussed.23,24

- to establish reference ranges or normal cutoffs4–6 (see below).

It is, however, recognized that these approaches are unrealistic for most clinical laboratories, due to the inherent variability of the test even in healthy subjects5 and mostly to the large number of donors that would be required at matching times. This emphasizes that LTA results should be interpreted by experienced staff.6

In France, blood collection from healthy "control" subjects is regulated by article L.1221–4 of the French Public Health Code. It specifies that the subject must provide consent and that blood collection must be supervised by a physician and tested for a list of diseases.25

Therefore, the use of such "control" samples should be limited to situations with no other option, such as the definition of reference ranges. Moreover, "control" samples used in LTA should be qualified for that purpose.

Sample matrix quality control.

The sample matrix for quality should be controlled with appropriate negative and positive results. A negative control is the absence of platelet aggregation without any agonist (or with isotonic NaCl solution) for 5 minutes at least. Conversely, platelet aggregation with any agonist can be considered a positive control.

In spite of the absence of IQC, reagents should be validated with a frequency adapted to each laboratory activity. Normal, abnormal and absent reactions should be observed during the initial validation and use of each reagent. Curve shapes should also be compatibility with expected typical curves. Medical personnel should be able to validate reagent conformity, with results obtained in one or several series and/or testing the same reagents with another method and/or by confirming an abnormal response with another vial of the same batch or another reagent preparation (dilution). Notably, if the

laboratory performs LTA for several patients on the same day, both normal and abnormal aggregations can be observed and recorded.

Reagent validation. See III.4.6

Interlaboratory comparisons

Interlaboratory comparisons, as defined by ISO 15 189, are not feasible for LTA, for the reasons detailed above. Without EQA or a stable sample matrix, the evaluation of precision, bias and uncertainty is not feasible. Postanalytical EQA programs will mainly help standardize the interpretation of LTA results.

Performance assessment

Instrument qualification

Repeatability assays can be performed using residual PRP after the test has been performed. In case of insufficient residual volumes, several PRPs can be mixed (unpublished data from the group). Reproducibility is not feasible, because of the absence of an appropriate quality control matrix (see below).

Scarce data have been published, still without any precise and systematic documentation on instruments or reagents. Intra-assay coefficients of variation (CV) for maximal amplitude of platelet aggregation in healthy donors ranged from 6.7% to 11.4%, using ISTH-suggested agonist concentrations (collagen 2 µg/mL, arachidonic acid 1 mm, ristocetin 1.2 mg/mL and adrenaline 5 µM). CVs were greater (17.4%) with lower ADP concentrations (2 µM).7 Inter-assay LTA CVs were available for arachidonic acid-induced platelet aggregation when exploring the effect of aspirin (0.6 and 21.1%) and for ADP-induced platelet aggregation when exploring clopidogrel effect (4.3 and 13.4%) (32). Moreover, VWF ristocetin cofactor activity (VWF:RCo) performed using LTA with lyophilized platelets displayed inter-assay CVs of 20−30% (33). Acceptable CVs for repeatability assays were then set at 15%, based on these data and results from a survey in French laboratories (Supplemental data).

During the initial qualification of LTA instruments, or after instrument maintenance, the conformity assessment produced by the manufacturer should be analyzed, notably for comparable light transmission in each channel. If the laboratory has no other qualified LTA instrument, the use of "control" subjects in compliance with legal regulations is necessary for repeatability assays during initial qualification.

Analytical sensitivity and specificity

These parameters are difficult to calculate for each agonist, because of the low prevalence of platelet disorders and the lack of proper control materials. However, three items can help ensuring conformity:

- calibration based on patient PRP and PPP for each tested sample;

- IQC sample matrix: negative and controls (see above)

- absence of platelet aggregation with only one agonist. In such cases, it is necessary to check the stirring magnet in the cuvette.

Detection limit

This parameter is not relevant for LTA.

Comparison of two instruments

Comparison assays should be performed between LTA instruments, if used for the same tests, or while using a loaned instrument. Comparisons should be performed with the same PRP, the same agonist at the same concentration, using residual PRP after initial LTA, or samples from "control" subjects in compliance with legal regulations. The laboratory must also choose specific agonist(s) and concentration(s) for comparisons. In the absence of specific recommendations, bias acceptance can be set at 15% for quantitative data. For qualitative data, results obtained with different instruments should be concordant.

Interferences

Assaying for the effect of substances possibly interfering with LTA results is not feasible. As mentioned above, icteric or lipemic samples are not critical nonconformities when testing PRP in LTA, but this should be taken into account in interpretation. Hemolyzed samples constitute a preanalytical nonconformity. These characteristics should be known and mentioned when interpreting LTA results (see Table I, Proposal 9).

Reagent batch assessment

The laboratory should organize traceability for all agonists because LTA instruments usually do not integrate these data. A process should also be established for the evaluation and validation of any new reagent batch.

Both CLSI and UK guidelines suggest to assess the performance of new agonist batches by comparison with the previous batch,4,6 using "normal platelets."4 The CLSI suggests, for instance, that the laboratory could plot cumulative duplicate values of LTA results obtained with "normal platelets" when testing in duplicate new and previous batches on a quality control chart, thus assessing for maintained reproducibility.4 ISTH guidelines suggest that each laboratory should validate test performance with each new reagent batch yet do not provide any modality.7 North American guidelines remind of the difficulties for some laboratories to obtain healthy control samples when new reagents are assessed.5

In the absence of proper IQC, it is possible to assess new agonist batches performance by comparison with previous batches, using control samples (i.e. residual post-LTA PRP samples or PRP from "control" subjects)

Postanalytical process

Interpretation and report of LTA results

LTA results can be quantitative (maximal aggregation amplitude expressed as percentage or area under the curve, velocity, initial slope, lag time before collagen-induced aggregation) and/or qualitative (presence/absence of aggregation, initial curve shape change, mono- or

biphasic curve shape, reversible or irreversible aggregation) (see Proposal 1). It is also possible to provide LTA curves in the result report.

The CLSI describes all possibly reported data: slope (initial slope of aggregation curve), minimal agonist concentration required to induce secondary wave aggregation (ADP or adrenaline), percent of final aggregation, percent of maximum aggregation.4 The ISTH also proposes items for evaluation (see above) and reporting of LTA results, together with comments for lipemic samples or studies performed more than 4 hours after blood collection.7 UK guidelines suggest to establish reference ranges if quantitative measures of aggregation are reported versus a purely subjective visual assessment of aggregation curves.8

Interpretation and postanalytical steps

Reference ranges.

If the laboratory has a quantitative approach, reference ranges should also be noted in the report; these ranges could also determine the percentage of aggregation below which a test is considered abnormal. The "establishment of reference intervals," as per the CLSI requires 120 specimens, which is not achievable.26 When reference intervals are already available, the CLSI suggests that each laboratory should establish its own reference intervals in correlation with published reference intervals, using a minimum of 20 normal subjects in strict conditions.4 North American and UK guidelines recommend that each laboratory should determine reference intervals for the percentage of maximal aggregation at each agonist concentration, based on 40 individual "healthy control volunteers" at minimum, using nonparametric statistical analyses.5,9 These intervals can be applied to children but not to neonates.5

Interpretation of LTA.

All guidelines agree on the importance of interpreting LTA results in the context of clinical and therapeutic data as well as all other investigations. Such an integrated interpretation should also take into account complementary methods such as flow cytometry, platelet secretion assays, electron microscopy4–6 or even genetic analyses in some cases.11 Abnormal results should be controlled on another specimen before establishing a diagnosis of platelet disorder.

ISTH guidelines recommend to consider a whole array of parameters when interpreting LTA results: platelet shape changes, lag-time length, aggregation slope, aggregation amplitude/percentage maximal and final values, deaggregation, aggregation tracings (visual examination) and the presence of a "secondary wave" with epinephrine.7

Other guidelines provide details to help interpreting LTA curves for the diagnosis of the most common platelet disorders. North American guidelines offer general recommendations.5 UK guidelines display detailed examples of LTA curves in a series of platelet disorders.6 The French CRPP proposes a practical guide for the interpretation of LTA results. It covers not only fairly common platelet disorders with well-known mechanisms and complementary diagnosis methods (flow cytometry, electron microscopy, granular composition analysis, genetic exploration, etc.) but also platelet disorders with uncertain mechanisms. This guide provides a description of pathophysiological mechanisms, tables and examples of aggregation curves.3,11 Moreover, UK guidelines suggest to repeat all unexpected, abnormal LTA tests with a fresh sample, in parallel with a normal control sample,6,8 and North American guidelines suggest to consider as a potential false-positive or non-diagnostic finding any abnormality with a single agonist.5

Finally, UK guidelines provide some data for the pediatric population.6 The total blood volume (20 mL) required for LTA, which has to be less in children, the absence of reference intervals for newborns and the use of finer needles (23 gauge), UK guidelines suggest to perform LTA after the age of one, if possible, in parallel with a normal control sample, using adult reference intervals. It is also recommended to test other family members whenever possible.

Conclusions

Laboratories performing platelet function analysis with LTA should have regular practice of this test and implement specific procedures as proposed in this document. This is necessary due to the absence of analytical quality controls and to the characteristics of the sample matrix. With current technological developments, platelets produced in vitro could help provide quality control material close enough to human platelets, if production costs are kept reasonable.27

Acknowledgements

Medical writing for this manuscript was assisted by MPIYP (MC Béné), Paris, France.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported that there is no funding associated with the work featured in this article.

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