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# Variability among commercial batches of normal pooled plasma in lupus anticoagulant testing

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## Abstract

**Introduction:** Lupus anticoagulant (LA) testing requires normal pooled plasma (NPP) in performing mixing studies and can be used for normalized ratios of clotting times (CTs). The aims were to demonstrate whether significant differences in clotting times between two batches of a same commercial NPP (CRYOcheck™) directly affect NPP-based cut-off values.

**Methods:** Diluted Russell Viper venom time (DRVVT) and activated partial thromboplastin time (aPTT) were used for LA testing. Screening, mixing and confirm tests were performed with Stago® instruments and reagents. Two batches of commercial NPP (A1291 and A1301 from CRYOcheck™; frozen) were compared in the determination of cut-off values. Cut-off values were defined as 99th percentile values of 60 healthy donors and compared with Mann–Whitney *U* test.

**Results:** Cut-off values obtained with the two NPP batches were significantly different for DRVVT (screen normalized ratio: 1.09 vs. 1.24, screen mix: 41.9 s vs. 38.9 s; index of circulating anticoagulant: 5.0 vs. 8.4; all had *p*-value <.001). On the contrary, no significant differences were observed for aPTT (screen normalized ratio: 1.32 vs. 1.34; *p*-value = .4068, screen mix: 37.8 s vs. 38.1 s; *p*-value = .1153) except for index of circulating anticoagulant: 9.6 versus 10.4 (*p*-value <.05).

**Conclusion:** This study demonstrates that differences between two commercial NPP batches produced by a same manufacturer influenced LA cut-off values used for mixing studies and normalized ratios. Adequate cut-off setting, taking into account NPP CTs, is important to provide accurate conclusion about the presence or absence of a LA and avoid potential clinical impact.

## KEYWORDS

cut-off values, laboratory diagnosis, lupus anticoagulant, mixing study, normal pooled plasma

## 1 | INTRODUCTION

Laboratory detection of lupus anticoagulant (LA), which has important clinical implications,<sup>1,2</sup> is based on two clotting test systems: diluted Russell Viper venom time (DRVVT) based tests and sensitive activated

partial thromboplastin time (aPTT) based tests. LA testing is conducted through three different steps: screening, mixing and confirmation. LA is deemed positive when both screening and mixing steps yield a result above the cut-off values while the confirmation step evidences a phospholipid dependence.<sup>3</sup> According to the last International Society on

Thrombosis and Haemostasis (ISTH) guidelines, these cut-off values should be derived from 99th percentile obtained from 120 healthy donors.<sup>3</sup> Alternatively, cut-off values of LA testing reagents manufacturers can be adopted locally via a validation exercise, if they fit with the internal laboratory cut-off values derived from at least local 20 healthy donors, with no more than 2/20 outside the manufacturer's range.<sup>4</sup> The second step, which is the mixing test, consists of mixing a normal pooled plasma (NPP) with patient's plasma in a 1:1 ratio. This aids discrimination between coagulation inhibitors and coagulation factor deficiencies.<sup>3,5-9</sup> Furthermore, to reduce interlaboratory and intralaboratory variability, the guidelines recommend to compute normalized ratio with CTs of NPP.<sup>3,9,10</sup> As presented in an international survey conducted in 2019 by the ISTH, most laboratories apply normalized ratios in LA testing by dividing patient's plasma CT by NPP CT.<sup>11</sup> In short, the implementation of normalized CT ratios reduces laboratory variability and mixing studies with NPP increases LA diagnosis specificity.<sup>12-16</sup> However, the problems surrounding NPPs are substantial. Most of laboratories use commercial lyophilized or frozen plasmas while in-house production of NPPs is recommended as stated in the latest updated ISTH guidelines.<sup>3</sup> Indeed, the use of commercial plasmas is not encouraged because of the lack of specifications in manufacturer's product inserts and alterations due to the preparation process and unavoidable use of additives, which are undisclosed. The use of commercial lyophilized or frozen plasmas is nevertheless allowed provided they meet the specifications for clotting factors levels and number of residual platelets.<sup>3,9</sup> It is not described whether one or several NPP batches should be used for the determination of cut-off values; the only mention is that NPPs should be produced from at least 40 normal donors.<sup>3</sup> It is assumed that NPPs prepared from 40 healthy donors would result in a homogeneous plasma mixture with consistently close to 100% clotting factor contents.<sup>3,9</sup> Nevertheless, Moore et al. demonstrated that NPPs prepared by diverse manufacturers gave different CTs and were not interchangeable.<sup>17</sup> They however did not study the impact of different batches of a same commercial NPP source on cut-off values. Our hypothesis is thus that different batches of a same commercial NPP (we used CRYOcheck™ Pooled Normal Plasma) may show significant variability in CTs and therefore influence mixing study cut-offs and normalized ratios of CTs with potential clinical impact.<sup>17,18</sup>

The objectives of this study are to investigate whether batches of commercial frozen NPP produced by the same manufacturer (CRYOcheck™) may show significant differences in the CTs of the two test systems used for LA testing. Another objective is to investigate whether these differences may directly affect the mixing study as well as normalized ratio cut-off values. In addition, we compared CTs obtained from two sources of NPP (commercial and in-house NPPs).

## 2 | MATERIALS AND METHODS

### 2.1 | Methods

The study was conducted at the hematology laboratory of the CHU UCL Namur. First, CTs of commercial NPP batches used during the

last five years (2017–2021) were extracted from the laboratory information system (LIS) for screen step in aPTT and DRVVT system and for screen and confirm steps in DRVVT system, to study the variation of CTs. Second, CTs and characteristics of two commercial NPP batches from the same source were compared to assess the potential differences. The third part of this study was thus to determine NPP-based cut-off values with 60 healthy donors, by calculating the 99th percentile value with both NPP batches. The following part of the study was the comparison of, NPP CTs from two sources, including four batches of commercial frozen NPP and two batches of in-house NPP. Last, mixing studies were performed with patient's plasmas with two commercial NPPs. Depending on the plasma volume collected from routine samples, DRVVT screen, DRVVT confirm and/or aPTT screen mixing tests were performed.

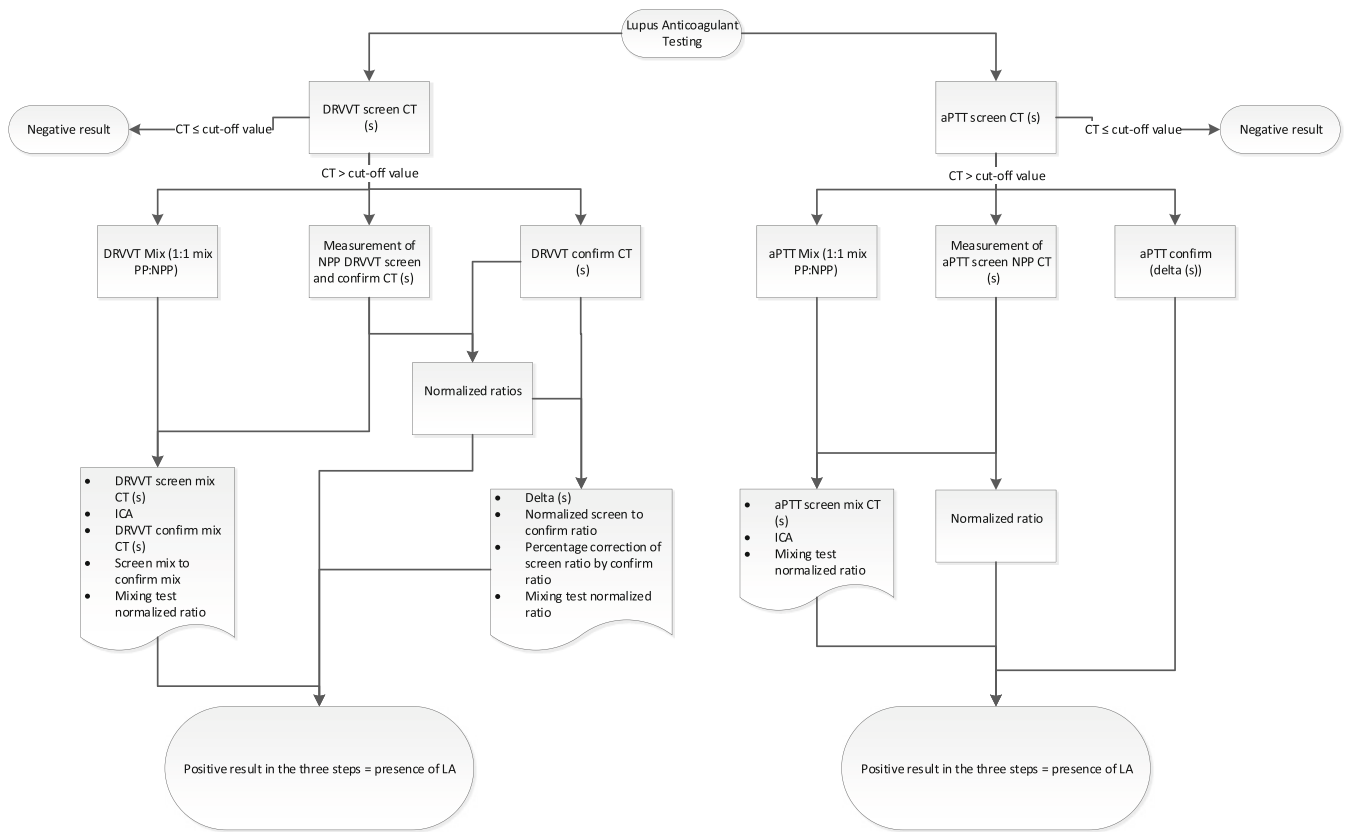
#### 2.1.1 | Retrospective analysis and data extraction of commercial NPP CTs

CTs of NPPs have been extracted for the last five years at Mont-Godinne hospital, CHU UCL Namur. The extraction was carried out from the LIS Glims version 8 (MIPS, Gent, Belgium).

#### 2.1.2 | Lupus anticoagulant testing

LA testing was carried out according to a three-step procedure used in medical laboratory at Mont-Godinne hospital, CHU UCL Namur (Figure 1): screening, mixing and confirm with two test systems. Assays used for screening step were: diluted Russell viper venom time (DRVVT–STA<sup>®</sup>–Staclot<sup>®</sup> DRVV Screen kit, Stago Diagnostica, Asnières-sur-Seine, France) and activated partial thromboplastin time (aPTT–PTT–LA<sup>®</sup> reagent from Stago). For confirm step, Staclot<sup>®</sup> LA (Stago) was used for aPTT system and STA–Staclot<sup>®</sup> DRVV confirm (Stago) for DRVVT system. According to our routine diagnostic testing protocol, only patients with at least one screen CT above the cut-off value were investigated further, with the calculation of normalized ratio (by dividing CT of patient's plasma by CT of NPP) and performance of mixing and confirmatory studies (Figure 1). Mixing studies were carried out with a patient's plasma diluted 1:1 with commercial frozen NPP (CRYOcheck™ Pooled Normal Plasma, Precision BioLogic). Mixing studies in DRVVT pathway were performed with the same assays (DRVVT screen and confirm) using patient's plasma diluted 1:1 with NPP (CRYOcheck™). In our laboratory, mixing test with CRYOcheck™ NPP is not performed for aPTT confirm but only for aPTT screen (PTT–LA). Performing mixing test with Staclot<sup>®</sup> confirm reagent would be redundant. Indeed, in Staclot<sup>®</sup> confirm test, patient's plasma is already mixed with normal plasma containing hexagonal phase phosphatidylethanolamine reagent. Shortening of prolonged CT with high phospholipids in confirmation step is indicative of a LA.

DRVVT screen, DRVVT confirm and aPTT screen tests were all performed with STA R Max2 instrument (Stago). aPTT confirm was performed with STart Max instrument (Stago). aPTT confirm was



**FIGURE 1** Lupus anticoagulant testing in medical laboratory. aPTT, activated partial thromboplastin time; CT, clotting time; DRVVT, diluted Russell Viper venom time; LA, lupus anticoagulant; ICA, index of circulating anticoagulant; NPP, normal pooled plasma; PP, patient's plasma

performed with another instrument because dead volume on STA R Max2 is substantial (200  $\mu$ l) and consumes too much reagent volume compared to STart Max instrument (25  $\mu$ l with no dead volume). The operators who carried out the tests were proficient for test methods and use of instruments (>10-year experience in hemostasis laboratory).

### 2.1.3 | Determination of cut-off values involving NPPs

The determination of cut-off values in this study was fully aligned with the recommendations of last ISTH guidelines except for the number of healthy donors.<sup>3,19</sup> The cut-off values were defined as the 99th percentile of normal CTs, obtained from 60 healthy donors. A non-parametric approach was used as CTs or ratios were not all normally distributed and because it improves specificity arising from the potential reduction in false positives, tempered by the statistically inevitable reduction in sensitivity. The deviation from the normal distribution was assessed by Kolmogorov–Smirnov test. Outliers were analyzed with Reed method.<sup>3,19</sup> Cut-off values were computed by taking the 99th percentile value using the “robust method,” after exclusion of outliers.<sup>4</sup>

The patient's plasma refers to healthy donor in the equations below. Screening cut-off values were expressed as CTs in seconds and as normalized ratios. Normalized ratios were computed by dividing the CT of the patient's plasma (PP) by the CT of the NPP for screening steps. CT and normalized ratios were also determined for confirm step in DRVVT system:

$$\text{Normalized ratio} = \frac{CT_{PP}}{CT_{NPP}}$$

Mixing study cut-off values were expressed as CTs on 1:1 mix PP: NPP and as normalized ratios. For mixing study, index of circulating anticoagulant (ICA), also referred as Rosner index was obtained by the following calculation:

$$ICA = \frac{(CT_{mix} - CT_{NPP})}{CT_{PP}} * 100,$$

$$\text{Mixing test normalized ratio} = \frac{CT_{mix}}{CT_{NPP}}$$

In DRVVT system, overall interpretation was possible by computing interrelated step cut-off values:

Percentage correction of normalized ratios

$$= \frac{\frac{CT_{PP} \text{ screen}}{CT_{NPP}} - \frac{CT_{PP} \text{ confirm}}{CT_{NPP}}}{\frac{CT_{PP} \text{ screen}}{CT_{NPP}}} * 100,$$

$$\text{Normalized} \frac{\text{screen}}{\text{confirm}} \text{ ratio} = \frac{\frac{CT_{\text{screen PP}}}{CT_{\text{screen NPP}}}}{\frac{CT_{\text{confirm PP}}}{CT_{\text{confirm NPP}}}},$$

$$\text{Mixing ratio} = \frac{CT_{\text{screen mix}}}{CT_{\text{confirm mix}}}.$$

Mann-Whitney *U* nonparametric statistic test was used to assess the differences between cut-off values derived from the two commercial NPP batches. *p*-Value <.05 was considered to be statistically significant.

### 2.1.4 | Statistics software

Statistics, including 99th percentile cut-off determination and graphic elements were obtained using MedCalc® software.

## 2.2 | Materials

### 2.2.1 | Normal pooled plasmas

The commercial NPPs compared in this study were frozen platelet-poor plasmas constituted from a minimum of 20 healthy donors mixed in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (CRYOcheck™ Pooled Normal Plasma, Precision Biologic, Dartmouth, Canada). The batches were released if acceptance criteria were met: they must comply for the levels of factors II, V, VII, VIII, IX, X, XI and XII (>85%) and the donors must be negative for LA screening. The CRYOcheck™ NPPs were stored at -80°C and used before the expiration date indicated on the packaging. Each vial of NPP was thawed at 37°C in a water-bath for 5 min and was gently inverted for homogenization before LA testing. After thawing, NPP vials were used within 1 h and were not allowed to be refrozen. CTs of NPPs were measured under the same conditions as CTs of healthy donor's plasmas, by the same operator, with the same reagents and instruments and on the same day, to reduce operational variation.

CTs of four commercial frozen NPP batches (A1278, A1291, A1301 and A1313) were measured for DRVVT screen, confirm and aPTT screen and were included in the comparison of NPP characteristics. Two of these NPP batches were used for the determination of NPP-based cut-off values (A1291 and A1301). Results of certificates of analysis for these two batches are available in supporting information, Table A.1. Comparison between the CTs of the two NPP batches was performed using Mann-Whitney *U* test (nonparametric), to assess if they were statistically significantly different.

Two in-house NPPs were also included in the comparison of NPP characteristics. They were provided by Namur Biobank-eXchange (NAB-X). The first one was constituted of 48 healthy individuals

(17 men and 31 women) and the second one was constituted of 53 healthy individuals (17 men and 36 women). Details regarding the characteristics of healthy volunteers and the collection method of plasmas are available in supporting information.

### 2.2.2 | Individual plasmas of healthy donors

Sixty samples from healthy individuals (13 men and 47 women) were analyzed for the determination of cut-off values. All samples were stored and managed by the NAB-X. Details regarding the characteristics of healthy individuals and the collection method of plasmas are available in supporting information.

## 3 | RESULTS

### 3.1 | Changes over time of DRVVT and aPTT CTs obtained with commercial NPPs

From 2017 to 2021, a total of 395 CT measurements were recorded in the LIS for DRVVT screening step with an average number of tests of 79 per year. For aPTT system, 294 CT measurements were recorded and the average number of tests per year was 59. DRVVT confirm test with NPPs was started in our laboratory from September 2018 with a total of 284 CT measurements until end of December 2021.

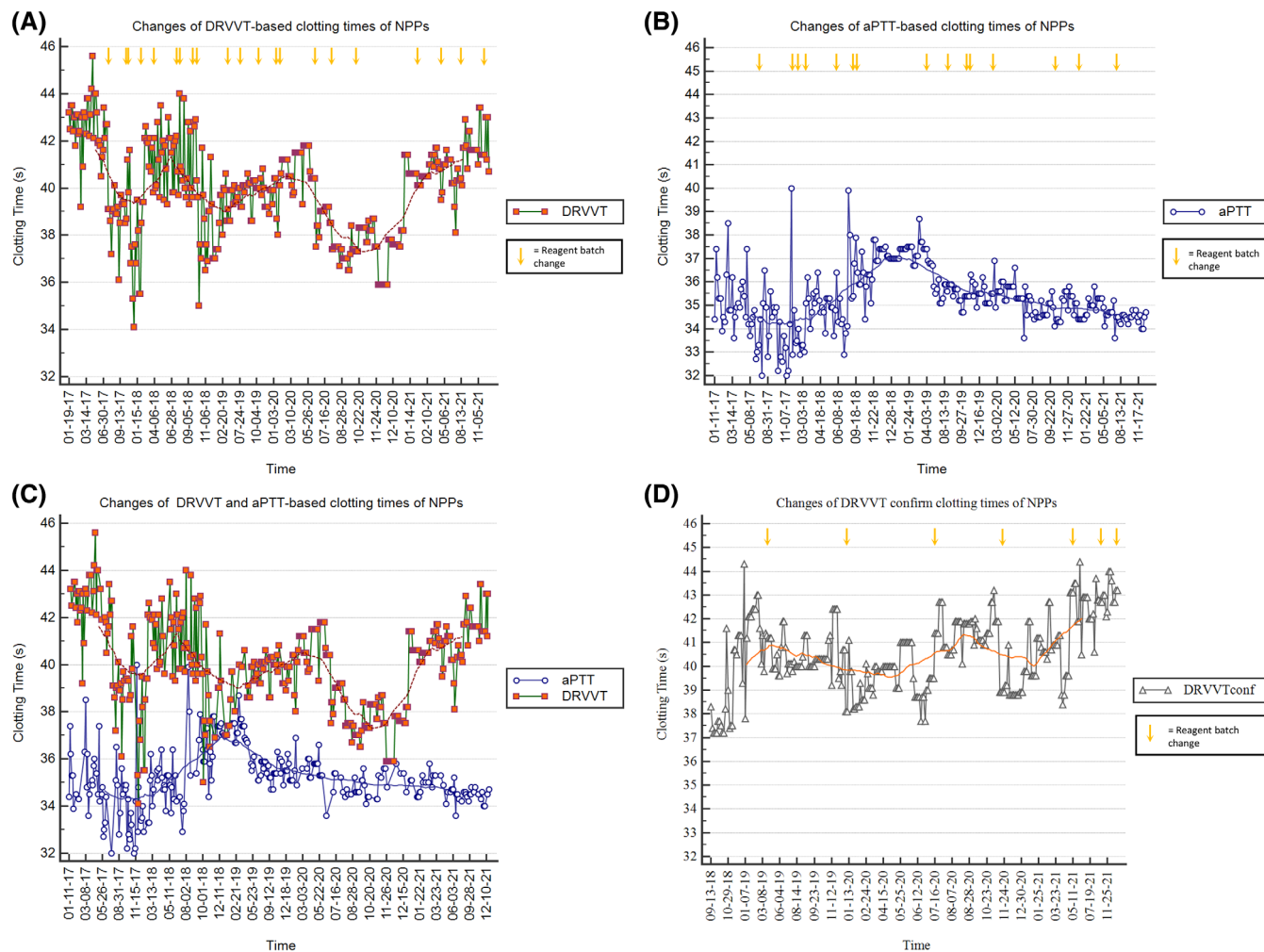
Changes over the five last years were discernible (Figure 2). The maintenance of instruments was carried out and no significant event was recorded in the logbooks. Changes of reagent batches were recorded in logbooks and are indicated by a yellow arrow on Figure 2. We did not find consistent associations with those changes. DRVVT screen CT varied at a greater degree compared to aPTT screen CT with clear-cut CT shifts at specific time points. However, aPTT system was also subject to variability even if it has been very stable since 2019. DRVVT confirm CTs also showed some fluctuations, in a lesser extent.

Concomitant variation of CTs for DRVVT screen and aPTT screen were also retrospectively analyzed (Figure 2C). DRVVT-based screening CTs were more subject to variations while aPTT-derived tests were more stable at equivalent time points.

Following this observation, it was assumed that commercial NPP CTs might fluctuate based on batch changes. Unfortunately, we were not able to prove that the changes of commercial NPP CTs were significantly attributed to NPP variability alone, as the change of NPP batches was not tracked and recorded in the quality system.

### 3.2 | Differences between two commercial batches of NPP

The two commercial frozen NPPs batches A1291 and A1301 (CRYOcheck™) have been compared with our testing instruments to evaluate the batch-to-batch variability of CTs (Table 1).



**FIGURE 2** Changes over time of clotting times of NPPs. aPTT, activated partial thromboplastin time; DRVVT, diluted Russell Viper venom time; NPP, normal pooled plasma

Characteristics of both batches are presented in Table A.1, supporting information. Fourteen separate runs were performed for DRVVT screen, DRVVT confirm and aPTT screen for each batch to assess between-run precision. All tests were performed by one trained operator on the same instrument and with the same reagents to limit the influence of other variability factors. Precision between replicates was good with a maximum coefficient of variation of 1.6% for batch A1291, on DRVVT confirm analysis. Batch A1301 gave different values in DRVVT CTs compared to batches A1291. The main difference was observed for DRVVT screen CT with a mean calculated at 35.8 s for batch A1301 while the mean DRVVT screen CT for the batch A1291 was 40.7 s. The difference between both batches is nearly 5 s for DRVVT screen CT. In aPTT system, the difference in the CTs was less marked but significant ( $p = .0015$  for aPTT system against  $p < .0001$  for DRVVT system).

Based on the low levels of imprecision indicating minimal between-run variation, reagent change logs and analyzer logs indicating no equipment failures, we considered that the CT fluctuations shown in Figure 2 could be at least partly due to variability of NPP batches. To study the impact of NPP CTs, it was decided to compare

cut-off values determined from these two NPP batches (A1291 and A1301) with samples obtained from 60 healthy donors.

### 3.3 | Determination of cut-off values with two different commercial NPPs batches

The complete testing panel for LA was carried out for all 60 individual plasmas of healthy donors, with both commercial NPP batches (A1291 and A1301). Kolmogorov–Smirnov values are available in supporting information. No outlier was detected. Comparison between NPP-dependent cut-off values is presented in Table 1.

Every cut-off value of DRVVT system was statistically significantly different when derived from one NPP batch compared to the other. This reflects the large difference observed in the CTs between NPPs presented in Table 1. Cut-off values derived from NPPs in the aPTT system were in contrast more consistent, again demonstrating the influence of NPP CT which is more stable for aPTT on the studied batches. aPTT screen normalized ratios and screen mixing CT cut-offs did not show statistically significant difference while ICA did.

**TABLE 1** Comparison of CTs (DRVVT screen, DRVVT confirm, and aPTT screen) and NPP-based cut-off values of two NPP batches (A1291 and A1301)

Comparison of CTs (DRVVT screen, DRVVT confirm and aPTT screen)						
	NPP A1291 aPTT screen CT (s)	NPP A1301 aPTT screen CT (s)	NPP A1291 DRVVT screen CT (s)	NPP A1301 DRVVT screen CT (s)	NPP A1291 DRVVT confirm CT (s)	NPP A1301 DRVVT confirm CT (s)
Run 1	34.4	34.3	40.5	35.9	41.7	40.8
Run 2	34.6	34.2	40.3	36.0	41.7	41.0
Run 3	34.9	34.4	40.7	36.3	41.7	40.6
Run 4	34.4	34.2	40.4	35.8	42.6	40.7
Run 5	34.8	34.2	40.5	35.8	43.1	40.7
Run 6	34.9	34.1	40.7	35.4	42.5	40.6
Run 7	34.6	34.1	41.1	35.9	42.3	40.2
Run 8	34.6	34.1	41.1	35.5	42.4	40.7
Run 9	34.0	34.1	40.4	35.7	42.7	40.9
Run 10	34.8	33.3	39.6	35.0	43.4	40.8
Run 11	34.7	34.4	40.7	35.9	42.6	39.9
Run 12	34.3	34.0	41.0	35.8	43.4	40.1
Run 13	34.5	34.0	41.1	35.8	41.9	41.1
Run 14	33.7	34.0	41.0	36.5	41.1	40.4
Mean	34.5	34.1	40.7	35.8	42.4	40.6
SD	0.3	0.3	0.4	0.4	0.7	0.3
CV (%)	1.0	0.8	1.0	1.0	1.6	0.9
p-Value	.0015		<.0001		<.0001	
NPP-based cut-off values			NPP A1291 (NPP1)	NPP A1301 (NPP2)	p-Value	
DRVVT screen normalized ratio			1.09	1.24	<.0001	
DRVVT screen mix CT (s)			41.9	38.9	<.0001	
Index of circulating anticoagulant (DRVVT)			5.0	8.4	<.0001	
DRVVT screen mix normalized ratio			1.03	1.09	<.0001	
DRVVT confirm normalized ratio			0.98	1.02	<.0001	
Normalized screen/confirm ratio (DRVVT)			1.21	1.32	<.0001	
Percentage correction of normalized ratios (%)			20.0	26.5	<.0001	
DRVVT confirm mix CT (s)			42.7	41.1	<.0001	
DRVVT confirm mix normalized ratio			1.01	1.02	.0446	
DRVVT screen/confirm mix			1.04	1.01	.0021	
aPTT screen normalized ratio			1.32	1.34	.4068	
aPTT screen mix CT (s)			37.8	38.1	.1153	
Index of circulating anticoagulant (aPTT)			9.6	10.4	.0026	
aPTT screen mix normalized ratio			1.10	1.12	.0033	

Note: Screen normalized ratio:  $\text{screen CT}_{\text{PP}}/\text{screen CT}_{\text{NPP}}$ ; screen mix:  $\text{screen CT on 1:1 mix PP:NPP}$ ; index of circulating anticoagulant:  $(\text{CT}_{\text{screen mix}} - \text{screen CT}_{\text{NPP}})/\text{screen CT}_{\text{PP}} \times 100$ ; screen mix normalized ratio:  $\text{CT}_{\text{screen mix}}/\text{screen CT}_{\text{NPP}}$ ; confirm normalized ratio:  $\text{confirm CT}_{\text{PP}}/\text{confirm CT}_{\text{NPP}}$ ; normalized screen/confirm ratio:  $\text{screen normalized ratio}/\text{confirm normalized ratio}$ ; percentage correction of normalized ratios:  $(\text{screen normalized ratio} - \text{confirm normalized ratio})/\text{screen normalized ratio} \times 100$ . Confirm mix:  $\text{confirm CT on 1:1 mix PP:NPP}$ ; confirm mix normalized ratio:  $\text{CT}_{\text{confirm mix}}/\text{confirm CT}_{\text{NPP}}$ ; screen/confirm mix:  $\text{screen CT}/\text{confirm CT on 1:1 mix PP:NPP}$ ; PP, patient plasma; NPP, normal pooled plasma. Abbreviations: aPTT, activated partial thromboplastin time; CT, clotting time; CV, coefficient of variation; DRVVT, diluted Russell Viper venom time; NPP, normal pooled plasma; SD, standard deviation.

### 3.4 | Extended comparison of two sources of NPPs

DRVVT screen, confirm and aPTT screen CTs of NPPs produced from two sources were compared. Four commercial frozen NPP batches

(CRYOcheck™: A1278, A1291, A1301 and A1313) and two in-house NPPs produced locally were included in the comparison. It was decided to analyze each batch once in the same conditions as these tests have good repeatability with small coefficients of variation (Table 1). The results are summarized in Table 2.

**TABLE 2** Comparison of CTs of two sources of NPPs

	APTT screen CT (s)	DRVVT screen CT (s)	DRVVT confirm CT (s)
NPP A1278	34.4	40.2	42.2
NPP A1291	34.5	40.7	42.4
NPP A1301	34.1	35.8	40.6
NPP A1313	37.8	40.1	43.6
In-house NPP 1	34.9	36.0	37.6
In-house NPP 2	37.5	38.7	36.6

Abbreviations: aPTT, activated partial thromboplastin time; DRVVT, diluted Russell Viper venom time; CT, clotting time; NPP, normal pooled plasma.

In either sources of NPPs, we observed variability from one batch to another. The aPTT screen CT seemed less prone to variability (Figure 2). Nevertheless, aPTT screen CTs may also be subject to variability as demonstrated with the commercial frozen batch NPP A1313. Variability is also observed for in-house NPPs collected during two different campaigns with local healthy donors. DRVVT confirm CT was always longer than DRVVT screen CT for commercial frozen NPPs while it was not always verified for in-house NPPs.

### 3.5 | Patient's LA testing with two commercial NPP batches

Mixing studies were performed with five nonanticoagulated patient's plasmas that had aPTT or DRVVT screen CT above the cut-off value, using two commercial NPP batches (A1301 and A1313). Of note, three of the five patients were found LA-positive. Each result has been compared to the cut-off values obtained with NPP A1291 and NPP A1301 (namely NPP1 and NPP2 in Table 1). For DRVVT screen mix CT of patient 2, although no discordance was obtained when the patient's plasma was mixed with NPP A1301, a discrepancy was observed for the results of the mix with NPP A1313 (Table 3). In comparison with the cut-off value obtained with NPP1 (41.9 s), the result was negative while with cut-off value obtained with NPP2 (38.9 s), the result was positive. The same conclusion was reached in DRVVT screen mix test with plasmas of patients 3 and 5. When aPTT screen mix test was performed with both different batches, no discordance was observed. This has to be correlated to the closeness of cut-off values that directly depend on NPP batch characteristics (Table 1).

## 4 | DISCUSSION

In this study, we found substantial variability of CTs among commercial (same provider) and in-house NPP batches (Tables 1 and 2). Such variability might well have a clinical impact. To the best of our knowledge, statistically significant differences between reference cut-off values derived from different NPP batches produced by a same manufacturer (Table 1) had not been reported yet. Figure 2 shows the variability of NPP CTs over 5 years. DRVVT screen CT of NPPs fluctuated to a greater extent than aPTT screen CT (Figure 2C). This lower

between-batch NPP variation in aPTT may be due to compensation by the additional steps of the intrinsic pathway. It is however not excluded that more variability could appear in the future, justifying a continuous monitoring, as illustrated with aPTT CT of NPP batch A1313 in Table 2. The variation in DRVVT confirm appeared slightly less marked than for DRVVT screen (Figure 2A, D). Phospholipid concentration and composition may be a contributing factor by reducing NPP CT variability in the confirm test. The phospholipid concentration does have an impact in some batches, for example NPP A1301 where DRVVT confirm CT is 4.8 s longer than DRVVT screen CT. We observed that the phospholipids had the properties to lengthen DRVVT CT for all commercial frozen NPP batches (see Table 2).

The analysis about the impact on cut-off values has been conducted with two NPP batches. We observed a more significant difference in DRVVT system compared to aPTT system, as logically inferred from changes in Figure 2. The smaller difference in the cut-off values was observed for the DRVVT screen/confirm mix ratio (1.01 vs. 1.04). It is worth noting this is a small difference relative to the 5 s difference in the NPP CTs. The use of this ratio as a main cut-off value might minimize the influence of NPP CTs. The sole parameter that showed significant difference in aPTT system was ICA, which is not surprising as the NPP operates in two different parameters of the equation (i.e., CT screen mix and CT NPP).

Results of DRVVT screen mix test of three patient's plasmas with two different commercial NPP batches (A1301 and A1313) were discordant when compared with both generated cut-off values (Table 3). This illustrates again the variability inherent to the characteristics of NPP batches used in this comparison (Tables 1 and 2). On the contrary, no discrepancies were observed for aPTT screen mix test with patient's plasmas emphasizing all involved NPPs had close characteristics in this coagulation pathway and therefore near cut-off values. Discrepancies regarding LA diagnostic efficiency with mixing studies may result from the use of NPPs with extreme CTs.

The use of NPP in LA assessment has always been of importance as recommended in several guidelines<sup>3,6-9</sup>: required for mixing studies and possible option for normalized ratios of CTs. Mixing study is interpreted either from mixing test specific cut-offs or ICA. Based on recent publications, the current ISTH guideline recommends the use of a cut-off specific for mixing test ratio and against the use of ICA, because the latter has been shown to be less sensitive to LA inhibition.<sup>3,15,16,20,21</sup> Although ICA tends to be abandoned in favor of

**TABLE 3** Patient's LA testing with two commercial NPP batches

	aPTT screen CT (s)	DRVVT screen CT (s)	DRVVT confirm CT (s)	Interpretation			
<b>Patient 1</b>							
NPP A1301	35.0	35.7	38.7	aPTT screen mix CT			
NPP A1313	38.5	40.4	42.4	Cut-off value NPP1 (37.8 s)		Cut-off value NPP2 (38.1 s)	
Patient's plasma undiluted	53.0	41.6	NA				
Patient's plasma 1 mix 1:1 with NPP A1301	39.2	INS	INS	Pos	Pos		
Patient's plasma 1 mix 1:1 with NPP A1313	41.8	INS	INS	Pos	Pos		
<b>Patient 2</b>							
NPP A1301	35.0	35.7	38.7	aPTT screen mix CT		DRVVT screen mix CT	
NPP A1313	38.5	40.4	42.4	Cut-off value NPP1 (37.8 s)	Cut-off value NPP2 (38.1 s)	Cut-off value NPP1 (41.9 s)	Cut-off value NPP2 (38.9 s)
Patient's plasma undiluted	65.2	53.5	71.7				
Patient's plasma 2 mix 1:1- with NPP A1301	41.0	37.0	INS	Pos	Pos	Neg	Neg
Patient's plasma 2 mix 1:1 with NPP A1313	42.8	40.9	INS	Pos	Pos	Neg	Pos
<b>Patient 3</b>							
NPP A1301	35.6	35.7	40.5	DRVVT screen mix CT			
NPP A1313	37.8	40.1	43.6	Cut-off value NPP1 (41.9 s)		Cut-off value NPP2 (38.9 s)	
Patient's plasma undiluted	45.6	49.3	44.4				
Patient's plasma 3 mix 1:1- with NPP A1301	INS	40.7	INS	Neg	Pos		
Patient's plasma 3 mix 1:1 with NPP A1313	INS	43.5	INS	Pos	Pos		
<b>Patient 4</b>							
NPP A1301	36.0	35.7	39.0	aPTT screen mix CT			
NPP A1313	37.5	39.9	42.4	Cut-off value NPP1 (37.8 s)		Cut-off value NPP2 (38.1 s)	
Patient's plasma undiluted	63.2	40.1	NA				
Patient's plasma 4 mix 1:1- with NPP A1301	54.5	INS	INS	Pos	Pos		
Patient's plasma 4 mix 1:1 with NPP A1313	56.4	INS	INS	Pos	Pos		
<b>Patient 5</b>							
NPP A1301	35.8	34.7	39	aPTT screen mix CT		DRVVT screen mix CT	
NPP A1313	38.2	39.1	42.3	Cut-off value NPP1 (37.8 s)	Cut-off value NPP2 (38.1 s)	Cut-off value NPP1 (41.9 s)	Cut-off value NPP2 (38.9 s)
Patient's plasma undiluted	55.6	46.1	46.7				
Patient's plasma 5 mix 1:1- with NPP A1301	45.7	36.6	INS	Pos	Pos	Neg	Neg
Patient's plasma 5 mix 1:1 with NPP A1313	47.5	40.9	INS	Pos	Pos	Neg	Pos

Note: The significance of color values in red indicates potential disagreement of final conclusion when the result is compared to the different cut-off values.

Abbreviations: aPTT, activated partial thromboplastin time; CT, clotting time; DRVVT, diluted Russell Viper venom time; INS, insufficient volume; NA, not applicable; Neg, negative result; NPP, normal pooled plasma; NPP1, NPP batch A1291; NPP2, NPP batch A1301; Pos, positive result.

mixing test ratio, ICA cut-off value was calculated in this study, as it is particularly sensitive to NPP variability. Despite international recommendations made by different groups of experts, the use of mixing studies is a source of substantive disagreement. The use of NPP for mixing studies is sometimes criticized because dilution effect may weaken LAs and generate false negative results.<sup>6,8,12,13,16,22-30</sup> For example, the mix of a weak-positive patient's plasma with a NPP showing an extremely short CT might result in a complete correction and therefore in a false-negative result. It was illustrated in our study with the plasma of patients 2 and 5 when mixed with NPP A1301 (Table 3). Other authors, in contrast argue that mixing studies are a powerful tool to differentiate factor deficiencies from coagulation inhibitors and increase the specificity of LA testing.<sup>12,31,32</sup> Mixing studies also allow the detection of paradoxical LA cofactor effect.<sup>33</sup>

Table 2 shows the comparison of CTs of two sources of NPPs. Although limited data is available in this comparison, variability is less important for in-house NPPs than for commercial frozen NPPs. Whereas commercial NPPs are qualified according to strict acceptance criteria (clotting factor levels close to 100%, normal aPTT and PT, low number of residual platelets [ $<10\ 000/\mu\text{l}$ ], absence of LA), they are not in all aspects similar to in-house NPPs, due to the manufacturing and recruitment process. First, CRYOcheck™ commercial frozen NPP batches are collected by plasmapheresis. Citrate levels are “equivalent” to 109 mM, but there is a margin of tolerance regarding this value, because numerous bags issued from different donors are mixed together. Second, manufacturing process introduces additives/stabilizing agents in the NPP composition, with potential to alter the correction properties during mixing tests. The freeze-drying process might influence the properties of lyophilized NPPs. Thrombin generation was studied with different sources of NPPs by Foulon-Pinto et al. and CRYOcheck™ frozen plasmas had natural coagulation inhibitor levels 15%–30% higher than lyophilized plasmas.<sup>34</sup> Finally, commercial plasmas are manufactured from remunerated donors recruited in North America. In Belgium, remunerated donation is not allowed, and ethics committees' clearance is mandatory to recruit healthy donors. Remunerated donors may alter or dissimulate information about their medical history, their recent travels and most importantly their ongoing treatment (such as anti-coagulant or antiplatelet treatment) for getting the money compensation of a donation. Donors in a volunteer-based system are less susceptible to alter their medical history or the treatment they have. All these elements could contribute to variability. For those reasons, in-house NPPs are preferred in LA testing as recently suggested by last ISTH guidelines.<sup>3</sup> It is likely that in-house NPPs use the same individual donors more often than commercial NPPs, potentially reducing between batch variations for in-house NPPs. Nevertheless, the in-house NPPs we have studied were not perfectly identical (Table 2), which warrants some consideration, that is, degree of intraindividual variability, collection tube batch changes and other less evident variability factors. The access to in-house NPPs is more difficult and it has to be expected that NPP status will evolve with the new regulation on in vitro diagnostic medical devices (IVDR)-Regulation EU 2017/746.<sup>35</sup>

To normalize screen and confirm CTs with ratios, CLSI guidelines advocate the use of reference interval (RI) mean CT in the denominator while ISTH guidelines and BCSH guidelines recommend using NPP CT measured in the same run of patient's plasma.<sup>3,6-8</sup> Normalized CT ratios have been incorporated in LA testing to reduce interlaboratory and intralaboratory variability.<sup>10</sup> Indeed, concomitant use of NPP and patient's plasma CTs reduces influence of variability factors such as operators, instruments or change of reagent batches by the normalization of results.<sup>5,8-10</sup> Moore et al. compared the LA testing of 1000 clinical samples by deriving normalized ratios from the RI mean or from different NPPs. They first demonstrated that NPPs prepared from different manufacturers gave different CTs and were not interchangeable, even if all were prepared from healthy donors. NPP-derived ratios had greater conclusion agreement with RI-mean derived ratios when CTs of NPPs matched closely the RI-mean.<sup>17</sup> As changes in NPP commercial batches may result in significant changes in NPP-based cut-off values (Table 1), solutions should be implemented to mitigate impact on LA diagnosis. NPPs could be prepared from more than 40 healthy donors to obtain a pool of plasma representative of healthy donor's characteristics by reducing the effects of donors with results at RI extremes. However, generating representative NPPs is more problematic than we give it credit for and it might be not just a function of donor numbers. For instance, ethnic differences have been described for DRVVT,<sup>36</sup> so a NPP batch of 100 donors from different ethnic backgrounds may be less representative than a 20 donor NPP from local, more representative donors. For the time being, solutions have to come from end-user laboratories. When a new NPP batch is used in daily clinical practice, it seems appropriate to release it with an in-house qualification based on an overlap testing with the in-use batch. In case of relevant difference demonstrated between two NPP batches, recalculation of cut-off values should be performed, with updating in the LIS. The impact of a new and different NPP batch on mixing cut-off values can only be overcome with testing of healthy donors, again igniting the debates about the relevance and feasibility of mixing studies. Clinical laboratories could also order several batches of NPP to choose the most suitable compared to their current batch, avoiding the need of new reference ranges. It is also possible to forecast the future amounts of NPP to last as long as possible with the same NPP batch while still matching stability requirements.

The arrows in Figure 2 showing the changes of reagent batches were not consistently associated with significant NPP CT shifts. Characteristics of NPPs may be more variable than those of reagents as they contain all the actors of the coagulation (intrinsic pathway for aPTT and common pathway for DRVVT). NPP characteristics are sensitive to biological interindividual variability while manufacturing of chemical reagents is highly standardized with narrow specifications and little space for variability. Analyzers are qualified for use and make part of a global maintenance plan. Logbooks are recorded for any undesirable event. In conclusion, for the reasons mentioned above and in the light of all the results of this study, the variability of NPP CTs was more likely due to the variability of NPP characteristics and not to other factors.

## 5 | STUDY LIMITATIONS

First, the change of NPP batches was not tracked and recorded in our quality system. We were not able to prove that the commercial NPP CT changes were significantly attributed to NPP variability. It is essential to record and assess any NPP batch change as this may result in significant CT and cut-off values changes. We were nevertheless able to demonstrate the variability of commercial NPPs with the four batches tested and that most of NPP CT shifts did not occur at the times of the changes of reagent batches (see Figure 2). Second, our LA testing protocol was not entirely ISTH 2020 guideline compliant but data were retrospectively analyzed from 2017 onwards meaning that last ISTH guidelines (2020) had not been released yet. Besides, DRVVT and aPTT screening tests were initially interpreted from clotting times in seconds not ratios and only being converted to ratios if the clotting time was elevated. Third, some of the individual plasmas used in the determination of cut-off values were sometimes collected more than two years ago. To our knowledge, the stability of frozen individual plasmas at  $-80^{\circ}\text{C}$  for LA testing has not been validated with such a long storage time. However, each individual plasma, whatever its storage time, has been compared with both different NPP batches. One other limitation was the number of healthy donors used for the comparison between NPP-based cut-offs (60). Last guidelines recommend at least 120 healthy donors, or alternatively 40 healthy donors with demonstrated matching with manufacturer's cut-off values of LA testing reagents.<sup>3</sup> Our study laid in an in-between area as we had more than 40 healthy donors but importantly our cut-off values did not match manufacturer's. It is not entirely unexpected that the local cut-offs were different to the manufacturer's because different reagents and analytical equipment were used, and the local donor population could be different. Additionally, manufacturer's cut-off values were only determined with a limited number of tests and with a limited number of normal plasmas (e.g., 27 for DRVVT screen CT).<sup>37</sup> Although the robust method was used to calculate cut-offs because there were <120 donors, it should be acknowledged that the CLSI guideline recommends that no less than 80 donors are used with this method (since 60 were used in the study), and that confidence intervals tend to be wide with this method.<sup>4</sup> The impact of NPP batch changes on cut-off values should be investigated with a larger sample of healthy donors to comply with the requirements of the last ISTH guidelines, with more NPP batches and with a wider range of commercial NPPs to corroborate our conclusion. The impact of NPP variability on LA testing and clinical outcome should be investigated with more patients and with more NPPs too.

## 6 | CONCLUSION

Changes in NPP CTs can bias mixing CTs and normalized ratios and therefore affect cut-off values. Given the importance of the NPP in the work-up of LA testing, properties of any new commercial NPP batch should be carefully assessed and scrutinized.

## AUTHOR CONTRIBUTIONS

Julien Cabo and François Mullier designed the study. Julien Cabo, Justine Baudar, Maité Guldenpfennig and François Mullier involved in the testing. Julien Cabo, Thomas Lecompte, Jonathan Douxfils and François Mullier involved in the analytical interpretation of data. Julien Cabo drafted the manuscript. Laure Morimont, Justine Baudar, Maité Guldenpfennig, Hugues Jacqmin, Reza Soleimani, Thomas Lecompte, Jonathan Douxfils and François Mullier involved in reviewing and editing the manuscript. All authors approved the final version of the manuscript.

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## CONFLICT OF INTEREST

Jonathan Douxfils is CEO and founder of QUALblood s.a. and reports personal fees from Roche, Roche Diagnostics, Stago, Gedeon Richter, Mithra, DOASense, YHLO and Daiichi-Sankyo, outside the submitted work. Thomas Lecompte reports fees for educational brochures of Stago company, outside the topic of LA testing. François Mullier reports institutional fees from Stago, Werfen, Nodia, Roche Sysmex and Bayer. He also reports speaker fees from Boehringer-Ingelheim, Bayer Healthcare, Bristol-Myers Squibb-Pfizer, Stago, Sysmex and Aspen all outside the submitted work.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## REFERENCES

1. Tektonidou MG, Andreoli L, Limper M, et al. EULAR recommendations for the management of antiphospholipid syndrome in adults. *Ann Rheum Dis*. 2019;78(10):1296-1304. doi:10.1136/annrheumdis-2019-215213
2. Chaturvedi S, McCrae KR. Diagnosis and management of the antiphospholipid syndrome. *Blood Rev*. 2017;31(6):406-417. doi:10.1016/j.blre.2017.07.006
3. Devreese KMJ, de Groot PG, de Laat B, et al. Guidance from the Scientific and Standardization Committee for lupus anticoagulant/antiphospholipid antibodies of the International Society on Thrombosis and Haemostasis: update of the guidelines for lupus anticoagulant detection and interpretation. *J Thromb Haemost*. 2020;18(11):2828-2839. doi:10.1111/jth.15047
4. CLSI. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, 3rd ed*. CLSI document EP28-A3c. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
5. Moore G. Current controversies in lupus anticoagulant detection. *Antibodies*. 2016;5(4):22. doi:10.3390/antib5040022
6. CLSI. *Laboratory Testing for the Lupus Anticoagulant; Approved Guideline*. CLSI Document H60-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

7. Moore GW. Recent guidelines and recommendations for laboratory detection of lupus anticoagulants. *Semin Thromb Hemost.* 2014;40(2): 163-171. doi:10.1055/s-0033-1364185
8. Keeling D, Mackie I, Moore GW, Greer IA, Greaves M, British Committee for Standards in H. Guidelines on the investigation and management of antiphospholipid syndrome. *Br J Haematol.* 2012;157(1): 47-58. doi:10.1111/j.1365-2141.2012.09037.x
9. Pengo V, Tripodi A, Reber G, et al. Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. *J Thromb Haemost.* 2009;7(10):1737-1740. doi:10.1111/j.1538-7836.2009.03555.x
10. Pradella P, Azzarini G, Santarossa L, et al. Cooperation experience in a multicentre study to define the upper limits in a normal population for the diagnostic assessment of the functional lupus anticoagulant assays. *Clin Chem Lab Med.* 2013;51(2):379-385. doi:10.1515/cclm-2012-0382
11. Cohen H, Mackie IJ, Devreese KMJ, International Society for Thrombosis and Haemostasis Scientific and Standardization Committee for Lupus Anticoagulant/Antiphospholipid Antibodies. Clinical and laboratory practice for lupus anticoagulant testing: an International Society of Thrombosis and Haemostasis Scientific and Standardization Committee survey. *J Thromb Haemost.* 2019;17(10):1715-1732. doi:10.1111/jth.14560
12. Devreese KM, de Laat B. Mixing studies in lupus anticoagulant testing are required at least in some type of samples. *J Thromb Haemost.* 2015;13(8):1475-1478. doi:10.1111/jth.13020
13. Devreese KM. No more mixing tests required for integrated assay systems in the laboratory diagnosis of lupus anticoagulants? *J Thromb Haemost.* 2010;8(5):1120-1122. doi:10.1111/j.1538-7836.2010.03807.x
14. Favalaro EJ, Bonar R, Zebelman D, Kershaw G, Marsden K. Laboratory investigation of lupus anticoagulants: mixing studies are sometimes required. *J Thromb Haemost.* 2010;8(12):2828-2831. doi:10.1111/j.1538-7836.2010.04090.x
15. Moore GW, Culhane AP, Daw CR, Noronha CP, Kumano O. Mixing test specific cut-off is more sensitive at detecting lupus anticoagulants than index of circulating anticoagulant. *Thromb Res.* 2016;139: 98-101. doi:10.1016/j.thromres.2016.01.019
16. Depreter B, Devreese KM. Differences in lupus anticoagulant final conclusion through clotting time or Rosner index for mixing test interpretation. *Clin Chem Lab Med.* 2016;54(9):1511-1516. doi:10.1515/cclm-2015-0978
17. Moore GW, Brown KL, Bromidge ES, Drew AJ, Ledford-Kraemer MR. Lupus anticoagulant detection: out of control? *Int J Lab Hematol.* 2013;35(2):128-136. doi:10.1111/ijlh.12006
18. Coppens A, Devreese KM. Evaluation of commercial normal pooled plasma used in the laboratory diagnosis of lupus anticoagulants. *Thromb Res.* 2010;126(3):246-249. doi:10.1016/j.thromres.2010.01.004
19. Vanoverschelde L, Kelchtermans H, Musial J, de Laat B, Devreese KMJ. Influence of anticardiolipin and anti-beta2 glycoprotein I antibody cut-off values on antiphospholipid syndrome classification. *Res Pract Thromb Haemost.* 2019;3(3):515-527. doi:10.1002/rth2.12207
20. Kumano O, Moore GW. Lupus anticoagulant mixing tests for multiple reagents are more sensitive if interpreted with a mixing test-specific cut-off than index of circulating anticoagulant. *Res Pract Thromb Haemost.* 2018;2(1):105-113. doi:10.1002/rth2.12069
21. Kumano O, Moore GW. Ruling out lupus anticoagulants with mixing test-specific cutoff assessment and the index of circulating anticoagulant. *Res Pract Thromb Haemost.* 2019;3(4):695-703. doi:10.1002/rth2.12245
22. Thom J, Ivey L, Eikelboom J. Normal plasma mixing studies in the laboratory diagnosis of lupus anticoagulant. *J Thromb Haemost.* 2003; 1(12):2689-2691. doi:10.1111/j.1538-7836.2003.0543h.x
23. Rosner E, Pazner R, Lusky A, Modan M, Many A. Detection and quantitative evaluation of lupus circulating anticoagulant activity. *Thromb Haemost.* 1987;57(2):144-147. <https://www.ncbi.nlm.nih.gov/pubmed/3110995>
24. Moore GW, Savidge GF. The dilution effect of equal volume mixing studies compromises confirmation of inhibition by lupus anticoagulants even when mixture specific reference ranges are applied. *Thromb Res.* 2006;118(4):523-528. doi:10.1016/j.thromres.2005.09.010
25. Devreese KM. Interpretation of normal plasma mixing studies in the laboratory diagnosis of lupus anticoagulants. *Thromb Res.* 2007; 119(3):369-376. doi:10.1016/j.thromres.2006.03.012
26. Hong SK, Hwang SM, Kim JE, Kim HK. Clinical significance of the mixing test in laboratory diagnoses of lupus anticoagulant: the fate of the mixing test in integrated lupus anticoagulant test systems. *Blood Coagul Fibrinolysis.* 2012;23(8):739-744. doi:10.1097/MBC.0b013e328358e899
27. Chandrashekar V. Dilute Russell's viper venom and activated partial thromboplastin time in lupus anticoagulant diagnosis. *Blood Coagul Fibrinolysis.* 2016;27(4):408-411. doi:10.1097/mbc.0000000000000463
28. Moore GW. Mixing studies for lupus anticoagulant: mostly no, sometimes yes. *Clin Chem Lab Med.* 2020;58(4):492-495. doi:10.1515/cclm-2019-1248
29. Pennings MT, De Groot PG, Meijers JC, Huisman A, Derksen RH, Urbanus RT. Optimisation of lupus anticoagulant tests: should test samples always be mixed with normal plasma? *Thromb Haemost.* 2014;112(4):736-742. doi:10.1160/TH14-02-0122
30. Reber G, Meijer P. In ECAT veritas? *Lupus.* 2012;21(7):722-724. doi:10.1177/0961203312446389
31. Chandler JB, Torres R, Rinder HM, Tormey CA. Lupus anticoagulant testing and anticoagulation do not mix: quantitation of discrepant results and potential approaches to reduce false positives. *Br J Haematol.* 2014;167(5):704-707. doi:10.1111/bjh.13030
32. De Kesel PMM, Devreese KMJ. The effect of unfractionated heparin, enoxaparin, and danaparoid on lupus anticoagulant testing: can activated carbon eliminate false-positive results? *Res Pract Thromb Haemost.* 2020;4(1):161-168. doi:10.1002/rth2.12264
33. Tripodi A. To mix or not to mix in lupus anticoagulant testing? That is the question. *Semin Thromb Hemost.* 2012;38(4):385-389. doi:10.1055/s-0032-1304717
34. Foulon-Pinto G, Jourdi G, Perrin J, et al. Study of thrombin generation with St Genesia to evaluate xaban pharmacodynamics: analytical performances over 18 months. *Int J Lab Hematol.* 2021;43(4):821-830. doi:10.1111/ijlh.13443
35. Testa S, Meijer P, Lasne D, Mullier F. Implementation of the new EUR IVD regulation and relation with ISO15189 accreditation: guidance is urgently required for haemostasis testing. *Int J Lab Hematol.* 2022;44-(suppl 1):71-78. doi:10.1111/ijlh.13936
36. Jerrard-Dunne P, Evans A, McGovern R, et al. Ethnic differences in markers of thrombophilia: implications for the investigation of ischemic stroke in multiethnic populations: the South London Ethnicity and Stroke Study. *Stroke.* 2003;34(8):1821-1826. doi:10.1161/01.STR.0000083049.65008.5F
37. STACLOT® DRVV insert kit—Detection of lupus anticoagulants.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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