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ORIGINAL ARTICLE

A multi-center evaluation of TECHNOSCREEN® ADAMTS-13 activity assay as a screening tool for detecting deficiency of ADAMTS-13

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Abstract

Background: Quantifying A disintegrin-like and metalloprotease with thrombospondin type 1 motif, member 13 (ADAMTS-13) activity enhances thrombotic thrombocytopenic purpura (TTP) diagnosis but most assays are time consuming, technically demanding, and mainly available in reference centers.

Objective: Evaluate a simple, semiquantitative ADAMTS-13 activity screening test for early identification/exclusion of TTP.

Patients/Methods: Plasma from 220 patients with suspected thrombotic microangiopathy at three reference centers were tested with TECHNOSCREEN® ADAMTS13 activity screening test in comparison with TECHNOZYM® ADAMTS-13 activity ELISA at two centers, and in-house fluorescence resonance energy transfer assay at the third center. The screening test indicates if ADAMTS-13 activity is at one of four level-indicator points: 0, 0.1, 0.4, or 0.8 IU/mL.

Results: Screen results were interpreted as binary data in that ADAMTS-13 activity was above or below the 0.1 IU/mL TTP clinical threshold. Combining all sites' data, the screen exhibited 88.7% sensitivity, 90.4% specificity, 74.6% positive predictive value, and 96.2% negative predictive value, comparable to published data for quantitative assays. Five samples with quantitative results below the threshold gave screen readings of 0.1 IU/mL and seven marginally above the threshold gave screen readings of zero. All would warrant plasma exchange while the level is quantified. Nine samples with normal/near normal results gave screens of zero and confirmatory quantifications would prompt early treatment withdrawal, as is current practice. One sample generated screen/quantitative results of 0.4/0.00 IU/mL respectively and was the only clear false-negative.

Conclusions: The screening test provides more rapid ADAMTS-13 level evaluation than most currently available assays. Its simple operation renders it suitable for adoption in routine or specialist laboratory environments.

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KEYWORDS
ADAMTS-13, ADAMTS-13 activity, screening test, thrombotic microangiopathy, thrombotic thrombocytopenic purpura

1 | INTRODUCTION

Thrombotic thrombocytopenic purpura (TTP) is a rare, life-threatening thrombotic microangiopathy (TMA) associated with a severe functional deficiency of the enzyme ADAMTS-13 (A disintegrin-like and metalloprotease with thrombospondin type 1 motif, member 13).[1] ADAMTS-13 is physiologically responsible for cleaving ultra-large von Willebrand factor (VWF) multimers upon their release from endothelial cells into the circulation, at the Tyr 1605-Met 1606 bond in the A2 domain of VWF.[2,3] Severe ADAMTS-13 deficiency can be congenital, also known as Upshaw-Schulman syndrome, but is more commonly acquired because of the presence of polyclonal autoantibodies against ADAMTS-13 that either inhibit function or promote increased clearance.[4,5] The deficiency leads to accumulation of hyperfunctional ultra-large VWF multimers in the microcirculation, resulting in indiscriminate attachment to platelets and spontaneous formation of VWF-rich thrombi in small arterioles that cause end-organ ischaemia.[6] In turn, these thrombi precipitate consumptive thrombocytopenia and a mechanical hemolytic anemia arising from red blood cell shearing as they travel past and through the microthrombi.

Clinical diagnosis of TTP was originally based on the classical pentad of thrombocytopenia, hemolytic anemia, neurological symptoms, renal dysfunction, and fever.[5] However, clinical presentation is now recognized as variable and other TMAs, such as "typical" and atypical hemolytic uremic syndromes, have similar features and differentiating between them clinically can be challenging.[1,5] A finding of an ADAMTS-13 activity level of <0.1 IU/mL distinguishes TTP from other TMAs in most cases and supports implementation of disorder-appropriate treatment, which for TTP is prompt plasma exchange (PEX) to replenish the missing enzyme and partly to remove antibodies if present.[1,5,7]

Several methods have been described for the determination of plasma ADAMTS-13 activity in clinical diagnostic laboratories such as fluorescence resonance energy transfer (FRET) technique or immunoassays based on measuring the extent of cleavage of a synthetic, labelled VWF fragment.[8] Although they can improve outcomes, most of these assays have a turnaround time of several hours, and in any case, are generally only available in specialized laboratories.[7,9,10] Timely diagnosis of TTP is critical as the mortality rate in untreated patients is as high as 90% and around one-half of the deaths occur within 24 hours of presentation.[5,11] A simple to perform, semiquantitative screening tool for assessing ADAMTS-13 activity levels has recently entered the diagnostic armory suitable for adoption by routine or specialist laboratories. The present study sought to evaluate its performance in determining whether ADAMTS-13 activity is above or below the clinical threshold of <0.1 IU/mL against the two commonly used types of specialist ADAMTS-13 activity assays, ELISA and FRET assay, in clinically relevant patient populations.

Essentials

- ADAMTS-13 activity assays are valuable in thrombotic thrombocytopenic purpura (TTP) diagnosis.
- A simple, rapid ADAMTS-13 activity screening test was evaluated with samples of known levels.
- Diagnostic performance data were comparable to more time consuming fully quantitative assays.
- The rapid screen can be used in routine or specialist laboratories to enhance TTP diagnosis.

2 | MATERIALS AND METHODS

The participating sites were the Viapath Diagnostic Haemostasis & Thrombosis Laboratories, St. Thomas' Hospital, London, UK (site 1); Department of Hemostaseology, MEDILYS Laborgesellschaft mbH, Hamburg, Germany (site 2); and Immune Pathology and Haemostasis Laboratories, Sanquin Diagnostics BV, Amsterdam, Netherlands (site 3). All sites are reference centers and routinely run diagnostic assays for ADAMTS-13 activity and inhibitors.

2.1 | Blood samples

A total of 253 double-centrifuged, citrated plasma samples from typical ADAMTS-13 activity assay requests received at each study site laboratory were tested with the TECHNOSCREEN® ADAMTS-13 activity screening test (Technoclone, Vienna, Austria). Double centrifugation techniques at all three sites conformed to recommended practice[12,13] and referring centers were all required to send double centrifuged plasma. Because TTP is a rare disorder with a reported incidence of ~10 cases/million people,[14] the majority of the samples tested were sourced from repository samples which had been stored at −70°C for up to 6 months. In any case, the aim was to test samples with a range of activities and not restrict validation testing to samples with levels below the clinical threshold. The remainder of the samples were sourced from routine samples received on the day of testing and were not subjected to freeze/thaw processing. Site 1 tested 99 samples, all of which were from the repository, as were the 50 samples tested at site 3. At site 2, 43/104 (41%) of the samples tested were repository samples. The study period was from August

2018 to September 2019. Patient data were obtained from laboratory databases, but because these each of these sites act as reference centers, clinical data were not available for all samples tested as the information was omitted on referral. The sample populations from sites 1 and 2 were similar with regards to age, sex, and percentage of TTP patients tested. Site 3 had not tested pediatric samples and had less clinical data. The populations are summarized in Table 1.

2.2 | Reference ADAMTS-13 activity assays

Sites 1 and 2 used TECHNOZYM® ADAMTS-13 activity ELISA (Technoclone) as their routine ADAMTS-13 activity assay. Briefly, glutathione S-transferase (GST) tagged VWF fragment (VWF73), which contains the ADAMTS-13 cleavage site, is immobilized via anti-GST antibodies bound to an ELISA plate. Diluted plasma is added to the well and ADAMTS-13 present cleaves the VWF73, which is detected with a horse radish peroxidase (HRP) conjugated monoclonal antibody specific for this cleavage site. A chromogenic substrate for HRP is used to visualize the amount of cleaved VWF73, the color intensity being directly proportional to the ADAMTS-13 activity in the sample. Activity levels are determined by reading from a calibration curve generated with calibrators standardized to international standard 12/252.[15] The assay was automated on a Dynex DS2 analyser (Werfen UK, Warrington, UK) at site 1 and at site 2 a manual locally developed ELISA technique was used with a Tecan HydroFlex wash automate (Tecan Trading AG, Männedorf, Switzerland) and a Tecan Sunrise ELISA reader with Magellan software (Tecan Trading AG). Both sites used TECHNOZYM® ADAMTS-13 inhibitor ELISA assay (Technoclone) to detect antibodies to ADAMTS-13, performed according to the manufacturer's instructions. The ELISA plates are precoated with recombinant ADAMTS-13 to capture any antibodies in test plasma and the antibodies detected with an HRP-conjugated anti-human IgG, which is reacted with a chromogenic substrate. The assay was automated on the Dynex DS2 at site 1 and performed manually at site 2 with the same wash automate and reader as the activity assay.

Site 3 used an in-house FRET assay for ADAMTS-13 activity. Briefly, citrated patient plasma was incubated with bilirubin oxidase (Sigma-Aldrich) for 30 minutes at 37°C. Thereafter, ADAMTS-13 activity was measured in three independent dilutions using the substrate FRETS-VWF73 (Peptides International) according to the manufacturer's instructions. Cleavage of the FRETS-VWF73 substrate by ADAMTS-13 results in a fluorescent signal which increases in direct proportion with ADAMTS-13 activity. Fluorescence was measured every minute for 2 hours. A normal pool plasma calibrated against the World Health Organization 1st International Standard ADAMTS-13 plasma NIBSC code 12/252 (NIBSC) was used for the calibration curve. An in-house ELISA was used to detect ADAMTS-13 antibodies. The ELISA plates were coated with recombinant ADAMTS-13 (NIBSC) to capture any antibodies in test plasma and the antibodies detected with HRP-conjugated IgG1, IgG3, and IgG4 (Bio-Connect Services BV), and HRP-conjugated IgG2 (Thermo Fisher Scientific). Color development was terminated by adding 1 mol/L H_2SO_4. Extinctions were measured at 450 and 620 nm. Results are expressed as a ratio between test and normal pooled plasma, with a ratio >1.88 regarded as positive.

TABLE 1 Summary of patient data whose samples were tested at each site

	Site 1	Site 2	Site 3
Sex			
Male (n/%)	21/25.3	33/35.5	12/27.3
Female (n/%)	62/74.7	59/63.4	32/72.7
No data available	–	1	–
Age			
Median (y)	50	46	52
Range (y)	0-85	1-81	16-89
Clinical data			
TTP[a]	54	52	7
Hemolytic uremic syndrome	–	2	–
Upshaw Schulmann syndrome	–	2	1
Others (eg, renal disease)	18	4	–
No data available	11	33	36

[a]Patients at various stages of thrombotic thrombocytopenic purpura (TTP) from newly diagnosed, before/after treatment and relapsed.

2.3 | ADAMTS-13 activity screening test

TECHNOSCREEN® ADAMTS-13 activity (Technoclone) is based on flow through technology and uses GST tagged VWF73 substrate, similar to the ELISA assay. Plasma samples are preincubated with VWF73 for 20 minutes at room temperature to permit cleavage by ADAMTS-13 present in the plasma. Pretreated sample is then applied to the test unit that contains a membrane with an immobilized IgG capture antibody specific for the cleaved VWF73, similar to that used in the TECHNOZYM® ADAMTS-13 activity ELISA, whereas a corresponding control well contains polyclonal anti-GST antibodies to capture both noncleaved and cleaved VWF73. As the sample flows through the membrane into an absorbent pad, the cleaved VWF73 binds to the immobilized antibody in the test well. Cleaved and noncleaved VWF73 bind to the control membrane. A secondary biotinylated anti-GST antibody is used to detect the membrane-bound complex and is revealed by reactivity with a streptavidin-gold conjugate, producing a red color. The color intensity of the test well is proportional to the level of ADAMTS-13 activity in the plasma sample and is compared with a color card, allowing semiquantitative analysis of the patient sample which is classed as 0, 0.1, 0.4, or 0.8 IU/mL. The control well color intensity must match or exceed that given on the color card for the assay results to be valid. Results for a normal plasma and an acute TTP plasma are shown in Figure 1.

Plasma samples with ADAMTS-13 activity levels ranging from 0.00 to 1.82 IU/mL were tested with the TECHNOSCREEN®

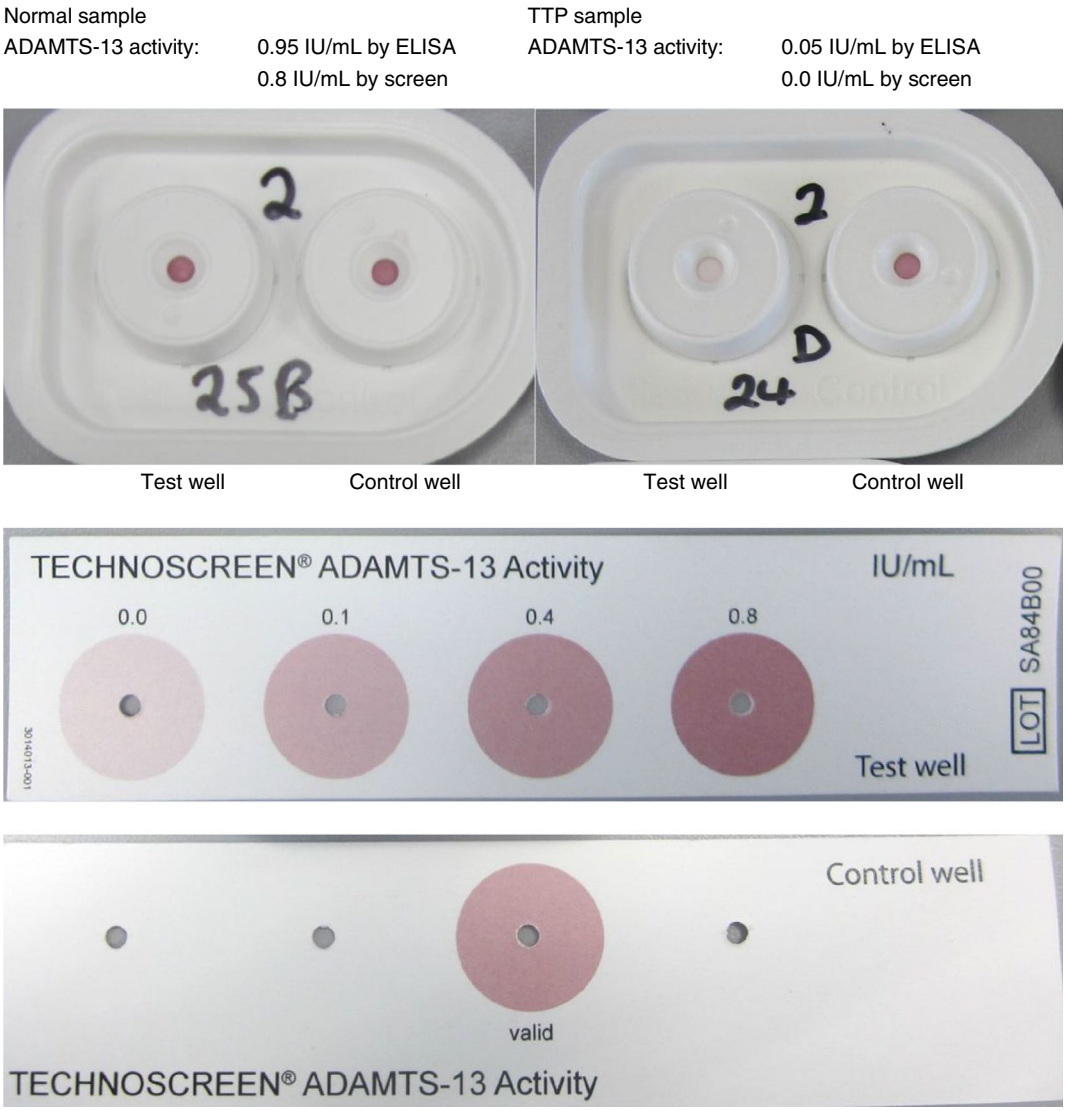

FIGURE 1 Examples of TECHNOSCREEN® ADAMTS-13 activity results from a normal plasma and a plasma from a patient with acute thrombotic thrombocytopenic purpura (TTP), and comparative interpretation cards

ADAMTS-13 activity test. Site 1 ran up to five samples at a time and the end-point was read/interpreted by between three and six observers. Site 2 ran the samples individually which were read/interpreted by the operator who performed the test, which is the anticipated way in which the assay would normally be used in the acute setting. Site 3 ran up to four samples at a time and the end-point was read/interpreted by three observers. All interpretations were undertaken within 10 minutes of the end-point color generation, as recommended by the manufacturer. Site 3 additionally took readings at 20 minutes to assess for any effects of overincubation. Individual readings were noted and the mean value recorded as the reported result. The manufacturer recommends no more than 10 should be run simultaneously to permit timely end-point readings. In the case of flow through issues, where the sample/reagent step takes >50 seconds to flow through the membrane, or unacceptable color development in the control well, the instance was documented and described. All readers of the screening test results were blinded to the quantitative results, which were obtained from the routine analysis of each laboratory for the data analysis.

2.4 | Interferences

Interferences were assessed by spiking plasma with hemoglobin in a hemolysate in the range of 0 to 1000 mg/dL, unconjugated bilirubin in the range of 0 to 4000 mg/dL, and intralipid in the range of 0 to 120 mg/dL.

2.5 | Statistical analysis

Because full clinical data were not available on all patients, statistical evaluation of TECHNOSCREEN® ADAMTS-13 Activity was established by considering the assay as a qualitative test and grading

TABLE 2 Sub-categorization of quantitative vs screening results

Category	A	B	C	D
Quantitative ADAMTS-13 activity (IU/mL)	<0.1	≥0.1	<0.1	≥0.1
TECHNOSCREEN® ADAMTS-13 activity (IU/mL)	<0.1	<0.1	≥0.1	≥0.1
Site 1 (n = 83)	8	5	1	69
Site 2 (all) (n = 93)	30	5	5	53
Site 2 (fresh samples only) (n = 50)	8	3	0	39
Sites 1 and 2 (all) (n = 176)	38	10	6	122
Site 3 (n = 44)	9	6	0	29
All sites (n = 220)	47	16	6	151

results as either positive or negative.[16] Thus, an ADAMTS-13 activity of ≥0.1 IU/mL was taken as the negative response because, in the context of how the screening test will be applied in clinical practice, it represented values above the clinical threshold for TTP. Conversely, an ADAMTS-13 activity of <0.1 IU/mL corresponds to presence of TTP (when married with clinical data and other laboratory data) and was taken as the positive response. Samples were classified as being positive in both screen and quantitative assay (class A), positive in screen only (class B), positive in quantitative assay only (class C), or negative in both assays (class D), corresponding to disease present/test positive, disease absent/test positive, disease present/test negative, and disease absent/test negative in the statistical software. The numbers of samples in each class for each site and combined sites are shown in Table 2.

Eighty-one samples at site 1 were rated by four or more readers so interreader agreement was assessed by calculating the intraclass correlation coefficient for the first four readings because they were not always the same readers for each sample. Intraclass correlation coefficient was separately calculated for 14 samples that had been rated by six readers. Statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

3 | RESULTS

The quantitative ELISA ADAMTS-13 activity levels in the samples from site 1 ranged from 0 to 1.82 IU/mL, median 0.48 IU/mL, with 10 having levels <0.1 IU/mL. The locally derived reference range is 0.66 to 1.08 IU/mL. Eight of the 10 samples with activity levels <0.1 IU/mL had results from TECHNOZYM® ADAMTS-13 inhibitor (Technoclone), an ELISA assay, which are interpreted as negative if ≤15 U/mL. Two samples from known TTP patients were negative for the presence of an inhibitor at the time of sampling, and the other six were positive, with results ranging between 18 and 93 U/mL, median 26.4 U/mL. The quantitative ELISA ADAMTS-13 activity levels in the samples from site 2 ranged from 0 to 1.10 IU/mL, median 0.34 IU/mL, with 35 having levels <0.1 IU/mL. The locally derived reference range is 0.50 to 1.20 IU/mL. All samples with activity levels <0.1 IU/mL had results from the TECHNOZYM® ADAMTS-13 inhibitor ELISA assay, 24 of which were positive, with results ranging from 16 to 100 U/mL, median 72 U/mL. Four of the 11 inhibitor-negative samples were from patients with known TTP, two had Upshaw-Schulman syndrome, and the others did not have clinical data available. The quantitative FRET ADAMTS-13 activity levels in the samples from site 3 ranged from 0 to 1.20 IU/mL, median 0.50 IU/mL, with 10 having levels <0.1 IU/mL. The locally derived reference range is 0.50 to 1.40 IU/mL. Five of the 10 samples with activity levels <0.1 IU/mL had results from the in-house ELISA, all of which were positive for the presence of an inhibitor, with ratios between 4.4 and 55.2, median 25.1.

There was high between-reader agreement for TECHNOSCREEN® ADAMTS-13 activity at sites 1 and 3. A complete consensus of all readers or only one of the readers recording a different level to the others was recorded in 80% of the samples at site 1, and in all samples for site 3. When differing interpretations of the end-point color occurred, only two different levels were reported, which were always sequential. Intraclass coefficient for the 81 samples at site 1 with four readings from separate random raters was 0.83 (95% confidence interval [CI], 0.77-0.88) for single ratings and 0.95 (95% CI, 0.93-0.97) for averaged ratings, indicating good (>0.75) to excellent (>0.90) reliability.[17] For the 14 samples that had been rated by six separate random raters, intraclass correlation coefficient for single ratings was 0.87 (95% CI, 0.75-0.95), and 0.98 (95% CI, 0.95-0.99) for averaged ratings. Extended flow-through in TECHNOSCREEN® ADAMTS-13 activity was encountered in 16/99 (16.1%) samples at site 1, 11/104 (10.6%) at site 2, and 6/50 (12.0%) at site 3, leaving 86, 93, and 44 samples from sites 1, 2, and 3, respectively, with paired screen and quantitative assay results for statistical analysis. All samples with extended flow-through were repository samples that had been frozen and thawed before analysis. To evidence whether other results from frozen/thawed samples without extended flow-through were reliable, a set of 10 additional samples were analyzed fresh and then after subjection to one freeze/thaw cycle with storage at −70°C for 1 week. The results between these two sets were not significantly different and within the previously described assay performance, and no samples exhibited extended flow through. Thus, borderline samples around the 0.1 IU/mL cutoff can vary from 0 to 0.1 IU/mL, resulting in the respective classification.

Three of the 56 samples (5.4%) at site 3 that were read after 10- and 20-minute incubation periods changed from readings of zero to readings of 0.1 IU/mL, which was closer to the quantitative values. All others were unchanged.

Of the 220 samples that gave results with the screening test, quantitative results for 55 were <0.10 IU/mL (mean 0.02 IU/mL, median 0.00 IU/mL), 14 were between 0.10 and 0.20 IU/mL (mean 0.14 IU/mL, median 0.15 IU/mL), 24 were between 0.20 and 0.40 IU/mL (mean 0.33 IU/mL, median 0.35 IU/mL), 84 were between 0.40 and 0.80 IU/mL (mean 0.59 IU/mL, median 0.57 IU/mL), and 43 were >0.80 IU/mL (mean 1.02 IU/mL, median 0.98 IU/mL). Quantitative results mapped to screening test readings are shown in Figure 2. Diagnostic performance data for each site, sites 1 and 2 combined, and all three sites combined are shown in Table 3.

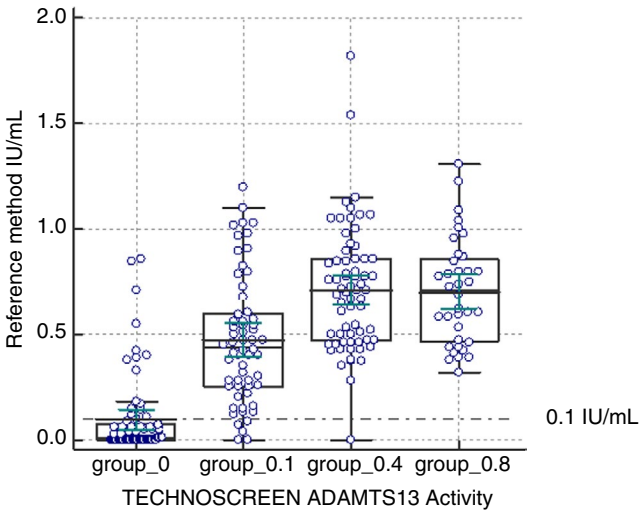

FIGURE 2 Quantitative ADAMTS-13 activity results grouped into readings from TECHNOSCREEN® ADAMTS-13 activity assay

Applying the TTP clinical threshold ADAMTS-13 activity level of 0.1 IU/mL as the diagnostic discriminator, 22/220 (10.0%) samples were misclassified by TECHNOSCREEN® ADAMTS-13 activity. The results were categorized into three groups, as shown in Table 4. The 12 samples in group 1 were all ADAMTS-13 deficient based on results from quantitative assays, five of which were below 0.1 IU/mL, but with screening test readings of 0.1 IU/mL, whereas seven samples had quantitative results just above the cutoff but with screening test readings of zero. The nine samples in group 2 had normal or near-normal quantitative results but had screening test readings of zero. The single sample in group 3 had a quantitative result below 0.1 IU/mL but a screening test reading of 0.4 IU/mL. Nineteen of these samples (86.4%) were from repositories and had been frozen and thawed.

No differences were recorded in result interpretations between spiked and unspiked plasma for hemoglobin ≤200 mg/dL, unconjugated bilirubin ≤15 mg/dL, and intralipid ≤500 mg/dL. Above those values, extended flow-through prevented end-point generation within the 50-second time window. All samples used in the study were subjected to visual inspection for hemolysis, icterus, and lipemia. Only one sample, from site 3, was reported as visually icteric but it did not experience delayed flow-through.

4 | DISCUSSION

Clinical differentiation of TTP from other TMAs is important because treatments for these disorders differ and not all benefit from PEX.[1] Early instigation of PEX is a lifesaving intervention for acute TTP yet it is expensive, invasive, and not without clinical risk.[7,10] Although currently available quantitative ADAMTS-13 assays have been shown to enhance TTP diagnosis and clinical outcomes most are time consuming, technically demanding, generally only available in specialist departments, and commonly only during core working hours.[7,9,10] The present study evaluated a simple to perform, rapid screening test against the two commonly used quantitative ADAMTS-13 activity assay types to assess its ability to distinguish ADAMTS-13 activity levels above and below the clinical threshold for diagnosis of acute TTP and initiation of PEX. Although FRET assays tend to be considered the gold standard for ADAMTS-13 activity measurement the TECHNOZYM® ADAMTS-13 activity ELISA is reported by the ECAT international external quality assurance scheme to be in more widespread use,[18] so it was valuable to include both assay types in the study. A recent study comparing results from the TECHNOZYM® ADAMTS-13 activity ELISA assay generated at multiple centers against a FRET assay at a reference center, concluded that the ELISA assay is comparable to the FRET assay and suitable for use in the clinical diagnosis of TTP.[19]

The samples used encompassed a full spread of ADAMTS-13 levels, including levels below and close to the 0.1 IU/mL threshold, and levels between that value and the lower limits of local reference ranges. All of the samples tested were from clinically relevant patients in whom TTP formed part of the differential diagnosis, or were from patients with established TTP at different stages of the disease and treatment, including acute presentation, thereby reducing spectrum bias.[20] As the screening test is read qualitatively to generate semiquantitative interpretations, it was not considered necessary or entirely feasible to calculate a reference range from a set of normal donors. Furthermore, the screening test is designed to indicate whether ADAMTS-13 levels are below a specific cutoff and not the lower limit of a reference range. Classifying TECHNOSCREEN® ADAMTS-13 activity as ostensibly a qualitative assay permitted generation of diagnostic performance data from its operation as a binary discriminator of ADAMTS-13 activity above or below the TTP clinical threshold. Sensitivity, specificity, positive predictive value, and negative predictive value (NPV) for each site and combined data were comparable with those reported for quantitative assays.[10,21-23] Importantly for a screening test, NPV values were all high (>91%) with narrow CIs, indicating a low likelihood of missing genuine TTP cases while awaiting quantitative confirmation.

The goal of rapid tests is to expedite a clinical decision, so erroneous binary results can have a directly adverse effect. According to current good laboratory practice, rapid diagnostic test results should not be final results[20] and the TECHNOSCREEN® ADAMTS-13 activity manufacturer recommends in its literature that deficiency of ADAMTS-13 activity identified in the screening tool should be confirmed with a quantitative assay. In terms of potential effects on immediate clinical decision making, the results on the 22 misclassified samples are revealing. In view of between-assay type variability[19,21-23] and the deficiency in some TTP patients being above the 0.1 IU/mL threshold,[5,11,24-26] rigid adherence to a generically defined cut-off is inadvisable. Thus, the five samples in group 1 from Table 4 with quantitative results below the threshold but a screening test value of 0.1 IU/mL would warrant initiation of PEX based on the screen result while immediate confirmatory quantitative testing is undertaken. All of these patients had acute TTP and the screening test result would not have delayed treatment. Similarly, the seven samples with screen

TABLE 3 Diagnostic performance data for individual sites and combined sites

Site	n	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Accuracy % (95% CI)
1	83	88.9 (56.5-99.4)	93.2 (85.1-97.1)	61.5 (35.5-82.3)	98.6 (92.3-99.9)	92.3 (84.9-97.3)
2 (all)	93	85.7 (70.6-93.7)	91.4 (81.4-96.3)	85.7 (70.6-93.7)	91.4 (81.4-96.3)	89.3 (81.1-94.7)
2 (fresh)	50	100.0 (67.6-100.0)	92.9 (81.0-97.5)	72.7 (43.4-90.3)	100.0 (91.0-100.0)	94.0 (83.5-98.8)
1 + 2[a]	126	83.3 (68.1-92.1)	92.2 (84.8-96.2)	81.1 (65.8-90.5)	93.3 (86.1-96.9)	89.7 (83.0-94.4)
1 + 2 (All)	176	86.4 (73.3-93.6)	92.4 (86.6-95.8)	79.2 (65.7-88.3)	95.3 (90.2-97.8)	90.9 (85.7-94.7)
3	44	100.0 (70.1-100.0)	82.9 (67.3-91.9)	60.0 (35.8-80.2)	100.0 (88.3-100.0)	86.4 (72.7-94.8)
All	220	88.7 (77.4-94.7)	90.4 (85.0-94.0)	74.6 (62.7-83.7)	96.2 (91.9-98.2)	90.0 (85.3-93.6)

Abbreviations: CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
[a]Repository samples only.

TABLE 4 Misclassified results at ADAMTS-13 activity cutoff of 0.1 IU/mL

Group	n	Subgroup (n)	Quantitative ADAMTS-13 activity (IU/mL)	TECHNOSCREEN® (IU/mL)
1	12	5	0.00/0.00/0.04/0.07/0.09	0.1
		7	0.10/0.11/0.12/0.14/0.15/0.17/0.18	0
2	9	–	0.33/0.38/0.39/0.40/0.42/0.55/0.71/0.85/0.86	0
3	1	–	0.00	0.4

results of zero but quantitative results marginally above the clinical threshold would initiate the same response, and clinical considerations and ADAMTS-13 antibody results would guide the decision to continue or withdraw PEX. The nine samples in group 2 with screening results of zero but normal or near-normal quantitative results would prompt initiation of PEX in the acute setting but early withdrawal once quantitative results were available, which maps to current practice in institutions where ADAMTS-13 assays are available.[7,9,10] The single group 3 sample was the only clear potential misclassification that could have precluded PEX treatment because the quantitative result was below the 0.1 IU/mL threshold. Interestingly, although the patient had concordantly reduced ADAMTS-13 antigen, the ADAMTS-13 antibody assay was negative, which in tandem with an only mildly reduced platelet count of 117×10^9/L, and hemoglobin of 126 g/L, would have triggered an alternative diagnosis to TTP. Figure 2 shows that apart from the single group 3 sample, all others with screen readings of 0.4 IU/mL had quantitative results well above the 0.1 IU/mL clinical threshold. In the context of detecting ADAMTS-13 activity levels above or below the clinical threshold, all samples that gave a reading of 0.1 IU/mL apart from the five in group 1 were accurate in that respect. However, it does reveal a wide spread of quantitative results so readings of 0 or 0.1 IU/mL will require immediate quantitative confirmation in the acute diagnostic setting. Particularly where screening tests are read by only one observer, such as lone workers in an out-of-core-hours situation, results that are ambiguous between two readings results should be reported as the lower value. This permits initiation of PEX for readings of 0 or 0.1 IU/mL in clinically appropriate patients while immediate quantitative analysis is undertaken, and treatment modified if the quantitative result indicates it is appropriate to do so. Confirmatory quantitative assays for readings of 0.4 and 0.8 IU/mL can be undertaken at the next batched analysis, which in the participating centers would normally be within 1 week.

Technical limitations of the screening tool were minimal because it is designed for use as a nonspecialized technique. The main difficulty encountered was prolonged flow-through that prevented color generation. This was only encountered in frozen-thawed samples and was most likely from protein aggregation during thawing potentially interfering with flow of plasma through the test device membrane. Most of the misclassified samples were also frozen-thawed. The separate experiment with additional samples subjected to one freeze/thaw sample suggested that the effects were minimal. Although sensitivity and NPV were 100% for the population of fresh samples, it should be noted that this was a smaller population than the combined numbers of frozen samples. The assay is intended for use on fresh, nonfrozen plasma in an emergency situation, so the issue would not impact significantly, if at all, in the clinical diagnostic setting. Subjective interpretation of the coloured end point against a batch-specific color chart is a potential limitation and interpretations at site 1 were undertaken by multiple readers to account for variability in visual acuity. Intraclass correlation coefficient values indicated good to excellent interreader agreement. Figure 1 shows that there is clear distinction between color intensity for each reading threshold, and although there were interpretive discrepancies, only two different levels were reported on each occasion, which were always sequential. It is conceivable that introducing further color readings on the chart indicating additional ADAMTS-13 levels would introduce greater ambiguity because color intensities would be less distinguishable from adjacent

levels. Using quantitative ADAMTS-13 assays with 0.1 IU/mL as the decision point for definite TTP has been reported to have similar sensitivity and specificity to that we report here for the screening test,[10,11,22,23] emphasizing that laboratory data can enhance and inform diagnosis and treatment of TTP but the diagnosis cannot be made on laboratory data alone.[1,11,24,25,27] A potential limitation of reading the color chart is that of red-green color blindness, although no such individuals were available to assess whether this affected ability to distinguish differences in intensity. Only three of the 56 samples at site 3 that were read after 10- and 20-minute incubation periods were rated differently. All three changed from readings of 0 to 0.1 IU/mL, suggesting that overincubation may occasionally affect low readings.

The manufacturer indicates that EDTA samples, and samples with excessive hemolysis, icterus or lipemia, should not be used with TECHNOSCREEN® ADAMTS-13 activity. ADAMTS-13 is inhibited by EDTA, and hemolysis, icterus, and lipemia can cause extended flow-through. Hemoglobin, conjugated bilirubin, and triglycerides are reported to be visually apparent in plasma or serum at 30, 2.0, and 300 mg/dL, respectively,[28,29] which are all markedly below the thresholds at which they were shown to interfere with TECHNOSCREEN® ADAMTS-13 activity. Only one sample, which was icteric, had a visually apparent potential interference but no flow-through issues, so there were no significant interferences in the samples studied.

Diagnosis of TTP is challenging because of diverse clinical manifestations and difficulties in clinically distinguishing it from other TMAs. Although clinical scoring systems can aid identification of patients most likely to have acute TTP and benefit from PEX, it is documentation of severe ADAMTS-13 deficiency and, subsequently, presence of an inhibitor, that secure the diagnosis.[30-32] Quantitative ADAMTS-13 activity assays are rarely available in the emergency situation, and even in reference centers that do have them, the results are commonly not available for several hours and may not be performed out of core hours. Although more rapid quantitative assays are entering the market, they are limited to performance on dedicated analytical platforms.[33,34] Availability of a simple and rapid screening test with comparable sensitivity and specificity to lengthy and more expensive quantitative assays has potential to inform whether the quantitative assay, and initiation of PEX, are necessary. ADAMTS-13 is stable in plasma at room temperature for up to 48 hours[35] so nonfrozen samples can be transported to remote testing sites where clinicians experienced in diagnosing and treating TTP/TMAs, and experienced laboratory staff, are based. Additionally, the screening test can be used in nonspecialist departments in order that normal results obviate the need to refer either samples or critically ill patients to specialist centers.

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DISCLOSURE OF CONFLICT OF INTEREST

M. Griffiths, S. Geiter, and N.B. Binder are employees of Technoclone. G.W. Moore reports consultancy fees from Technoclone. All other authors state they do not have any conflict of interest.

ADDENDUM

G.W. Moore, U. Budde, N.B. Binder, D. Meijer, and A. Leyte designed the study. M. Griffiths, G.W. Moore, and R. Dittmer interpreted the data. M. Griffiths, G.W. Moore, and D. Meijer wrote the manuscript. L. Rushen, A. Brown, B. Schocke, and A. Moes performed the screening tests and collected data. S. Geiter performed statistical analyses. J.A. Cutler and D. Meijer supervised testing and data collection. All authors critically reviewed the manuscript and approved the final version.

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