Il ruolo del laboratorio nella diagnosi e nel monitoraggio della PTT

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HIGHLIGHTS IN EMATOLOGIA
TREVISO, 1-2 DICEMBRE 2023

Disclosures of Francesca Tosato

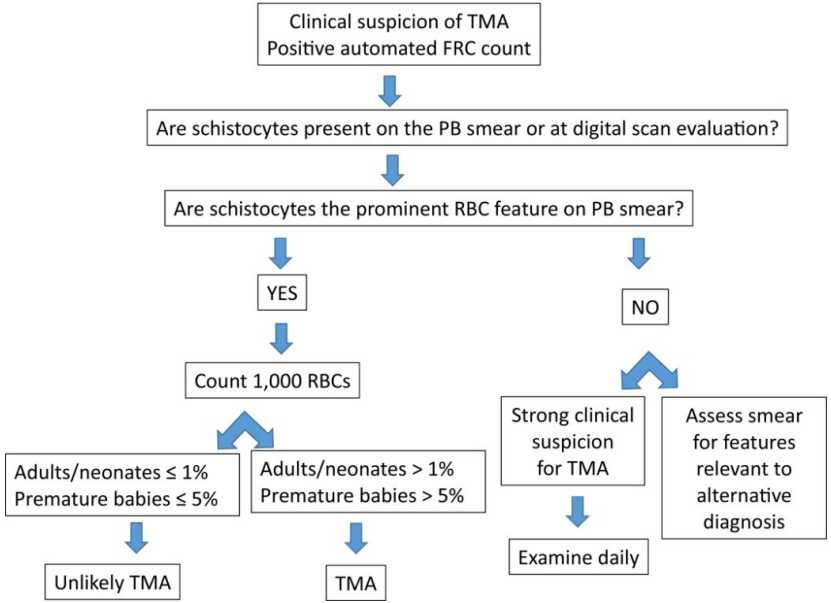
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
		NOTHING TO DECLARE					

LABORATORY FINDINGS

- Thrombocytopenia (< 30 x 10 /L)
- MAHA with Schistocytes
- Lactate dehydrogenase (LDH) is raised due to tissue ischemia and cell lysis
- Low plasma haptoglobin marker of haemolysis: it binds to free haemoglobin and the complex is cleared by macrophages
- Coombs test is generally negative
- Coagulation screen is normal (DD with CID)
- ADAMTS13 deficiency (<10%)
- N.B. During acute presentation it is important to check troponin levels, even in the absence of chest pain, due to high levels of reported ischaemia

Int J Lab Hematol. 2022;44(Suppl. 1):101–113

2021 update of the 2012 ICSH Recommendations for identification, diagnostic value, and quantitation of schistocytes: Impact and revisions



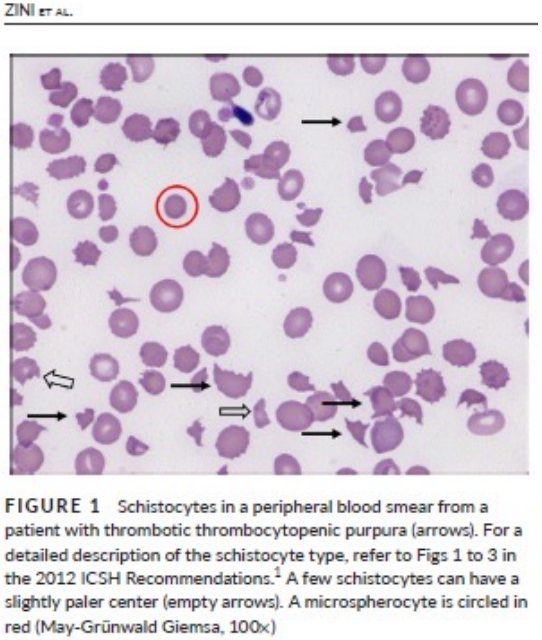


FIGURE 1 Schistocytes in a peripheral blood smear from a patient with thrombotic thrombocytopenic purpura (arrows). For a detailed description of the schistocyte type, refer to Figs 1 to 3 in the 2012 ICSH Recommendations.[1] A few schistocytes can have a slightly paler center (empty arrows). A microspherocyte is circled in red (May-Grünwald Giemsa, 100×)

Int J Lab Hematology, Volume: 43, Issue: 6, Pages: 1264-1271, First published: 24 August 2021, DOI: (10.1111/ijlh.13682)

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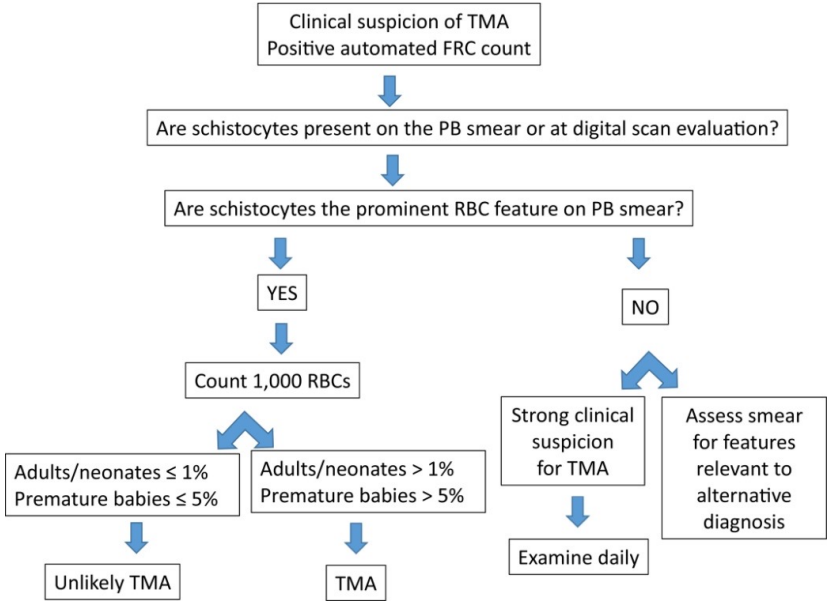

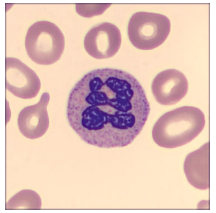

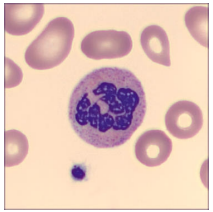

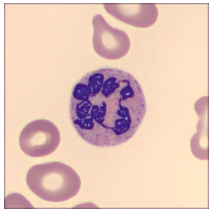

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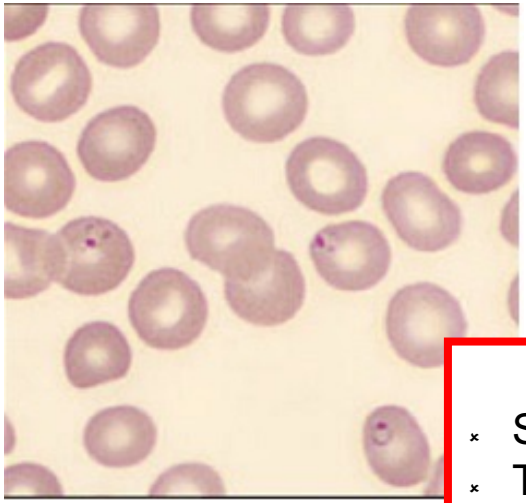
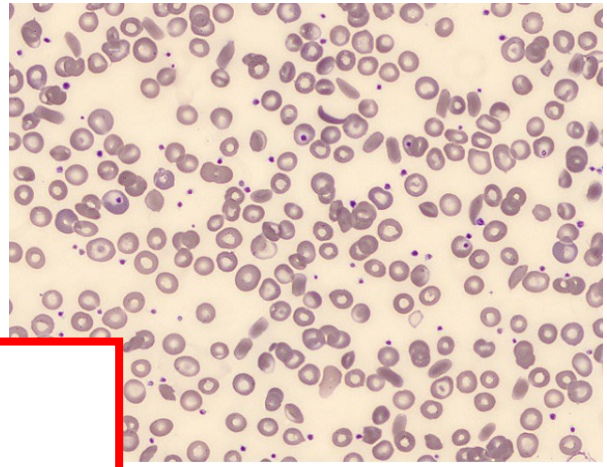
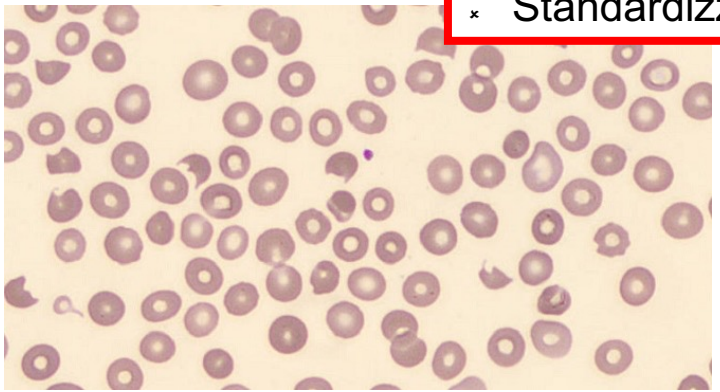
- ✓ L'importanza dell'**interpretazione morfologica applicata al contesto clinico** rimane essenziale: gli schistociti, anche associati a grave trombocitopenia, possono essere osservati in una serie di numerose altre condizioni oltre alla TMA. L'importanza di una accurata diagnosi differenziale è fondamentale soprattutto in contesti clinici potenzialmente confondenti (es. anemia da carenza di B12 e/o folati).
- ✓ La **morfologia digitalizzata** degli eritrociti aiuta i professionisti nell'**armonizzazione** dei criteri specifici per l'identificazione degli schistociti e nel calcolo della percentuale con un conteggio di RBC adeguato e standardizzato.

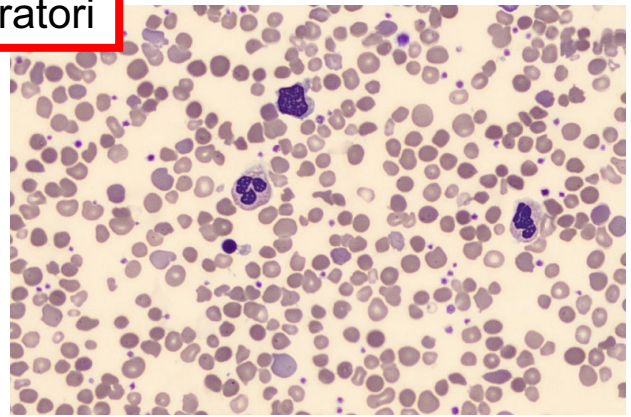


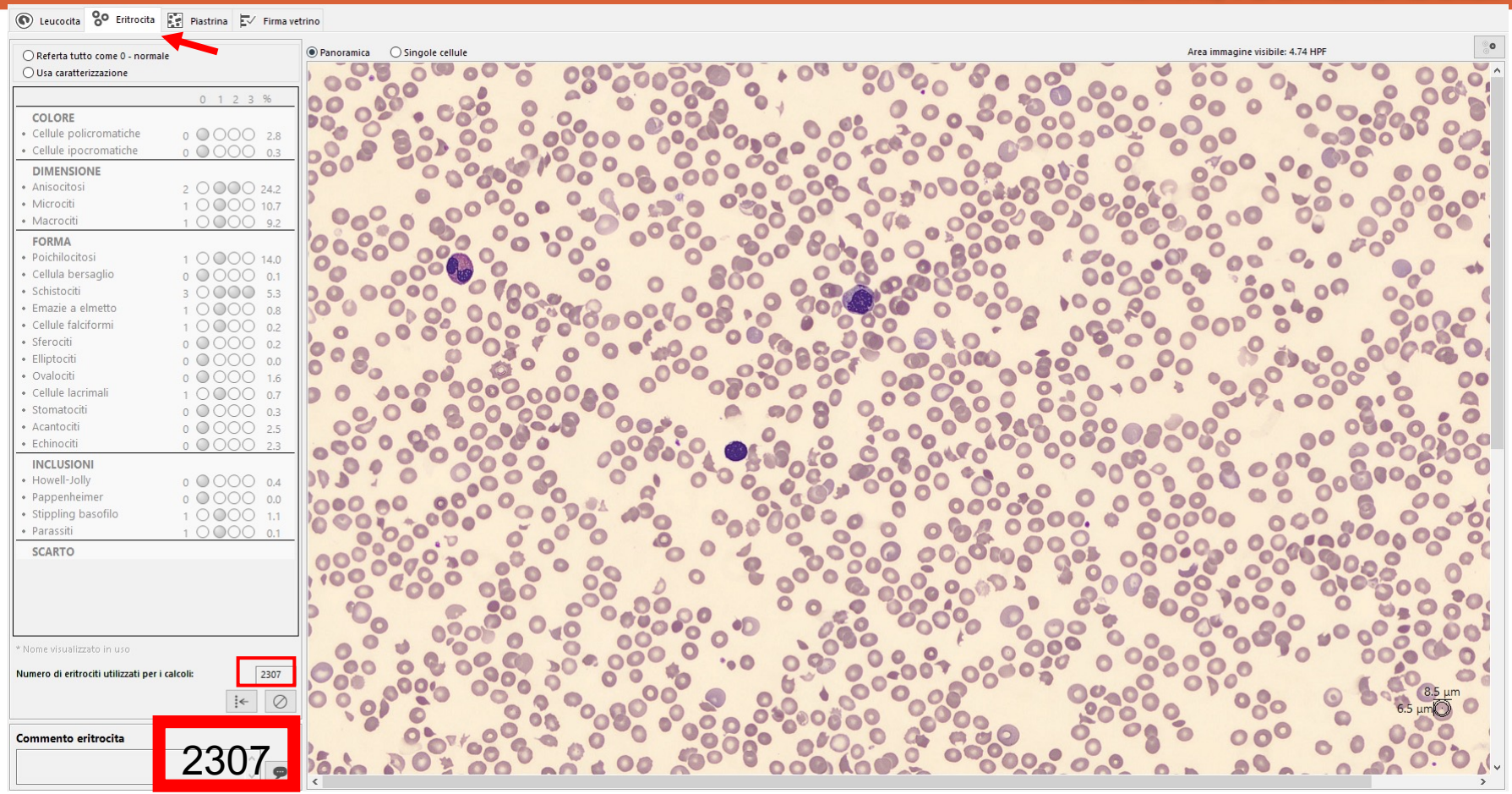


Reperibilità - Urgenza

- Semplicità
- TAT
- Standardizzazione tra operatori



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Pappenheimer 0 0.0
Stippling basofilo 1 1.1
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8.5 µm
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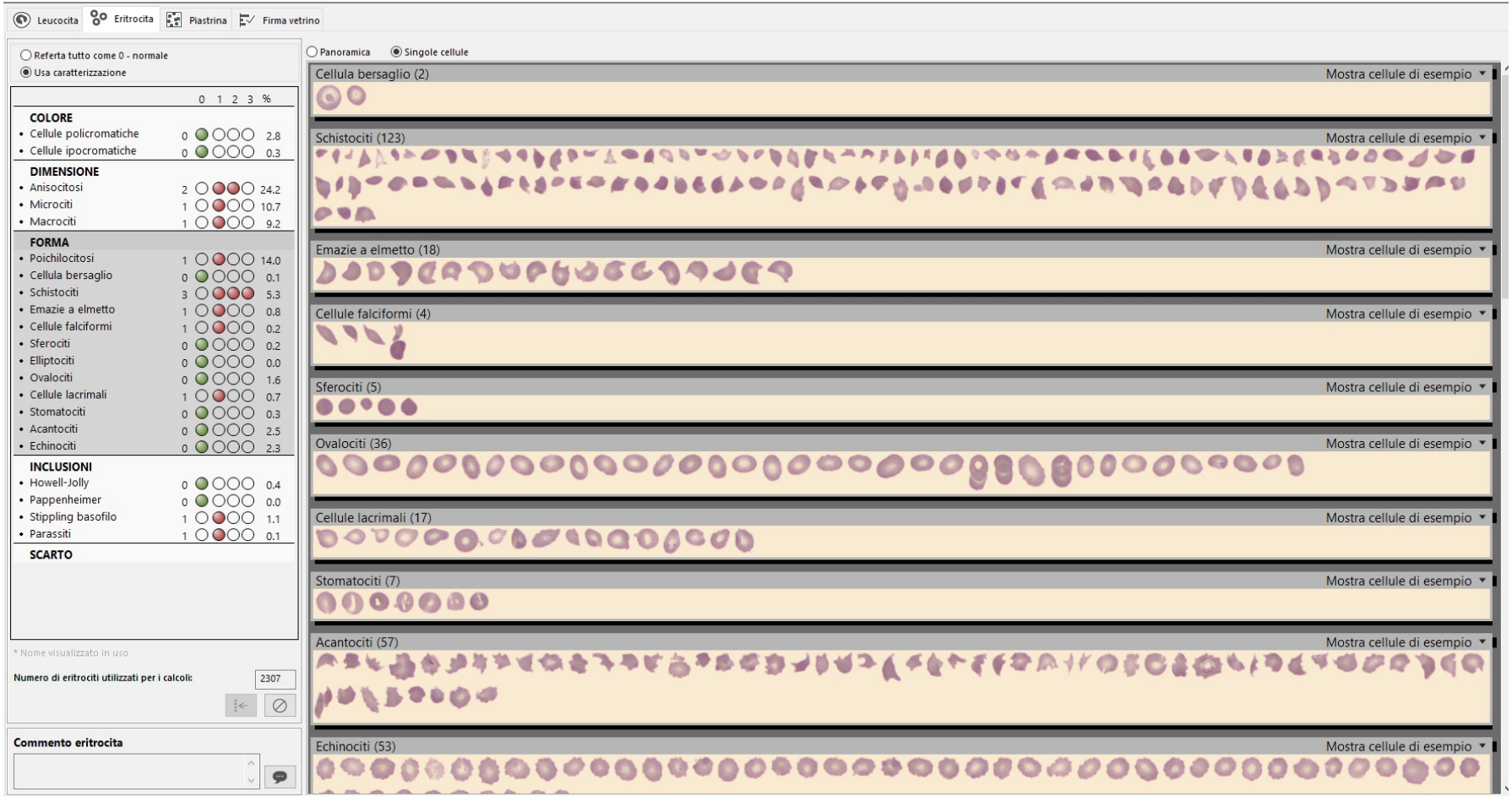
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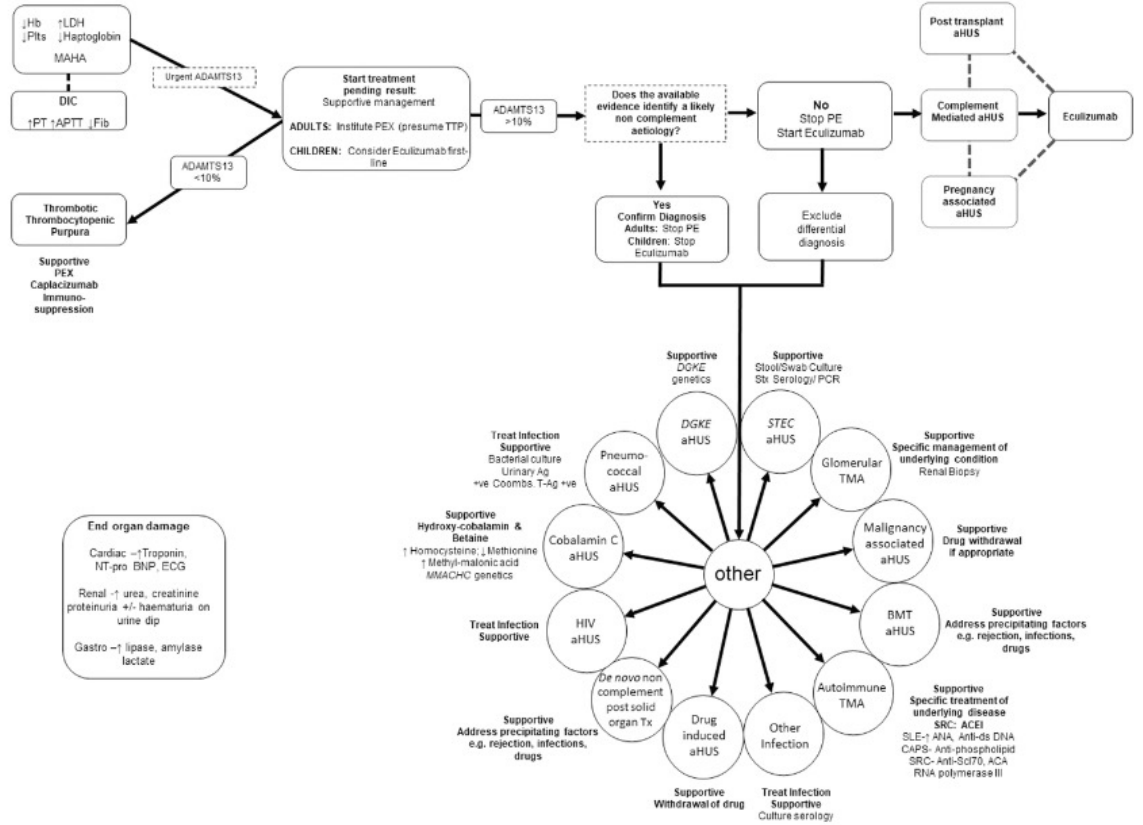
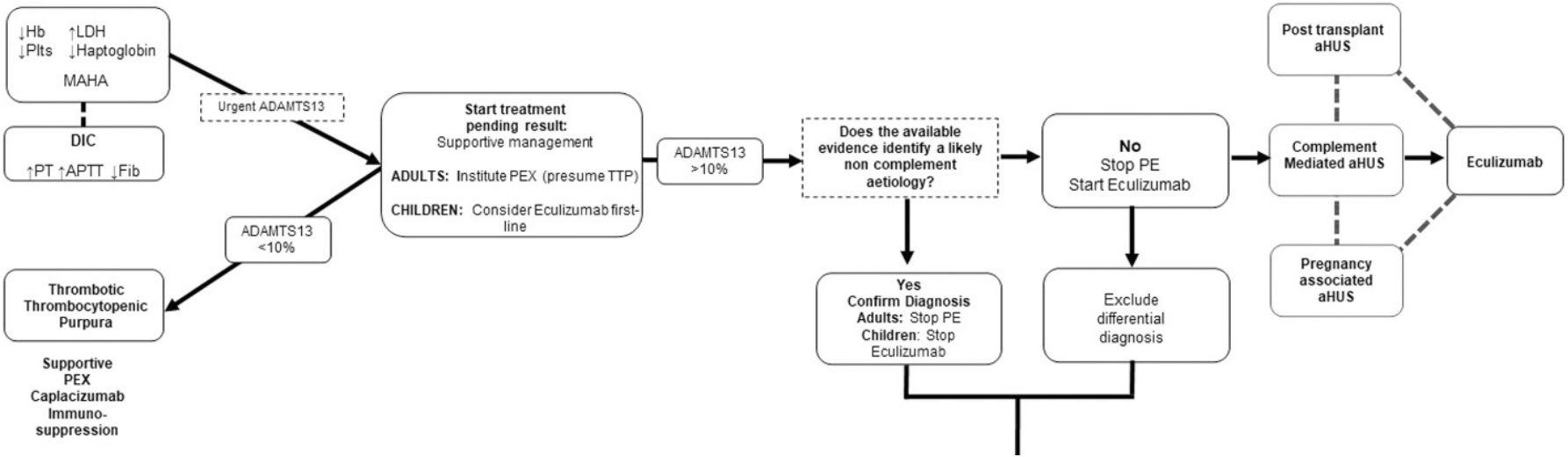
Singole cellule
Cellula bersaglio (2) Mostra cellule di esempio
Schistociti (123) Mostra cellule di esempio
Emazie a elmetto (18) Mostra cellule di esempio
Cellule falciformi (4) Mostra cellule di esempio
Sferociti (5) Mostra cellule di esempio
Ovalociti (36) Mostra cellule di esempio
Cellule lacrimali (17) Mostra cellule di esempio
Stomatociti (7) Mostra cellule di esempio
Acantociti (57) Mostra cellule di esempio
Echinociti (53) Mostra cellule di esempio



Int J Lab Hematol. 2022;44(Suppl. 1):101–113



Int J Lab Hematol. 2022;44(Suppl. 1):101–113

The evidence-based guideline of the International Society on Thrombosis and Haemostasis (ISTH) for the diagnosis of thrombotic thrombocytopenic purpura

The panel agreed on three recommendations covering the initial diagnosis with emphasis on the importance of **ADAMTS13 testing** (eg, activity, anti-ADAMTS13 IgG or inhibitor) and assessment of the **pretest probability of TTP** by clinical assessment and/or the risk assessment models like the PLASMIC or French score.

The panel noted how **availability and turnaround time of ADAMTS13 test results might affect early diagnosis and management**, in particular the use of caplacizumab.

J Thromb Haemost. 2020;18:2486–2495.

Pretest probability of TTP

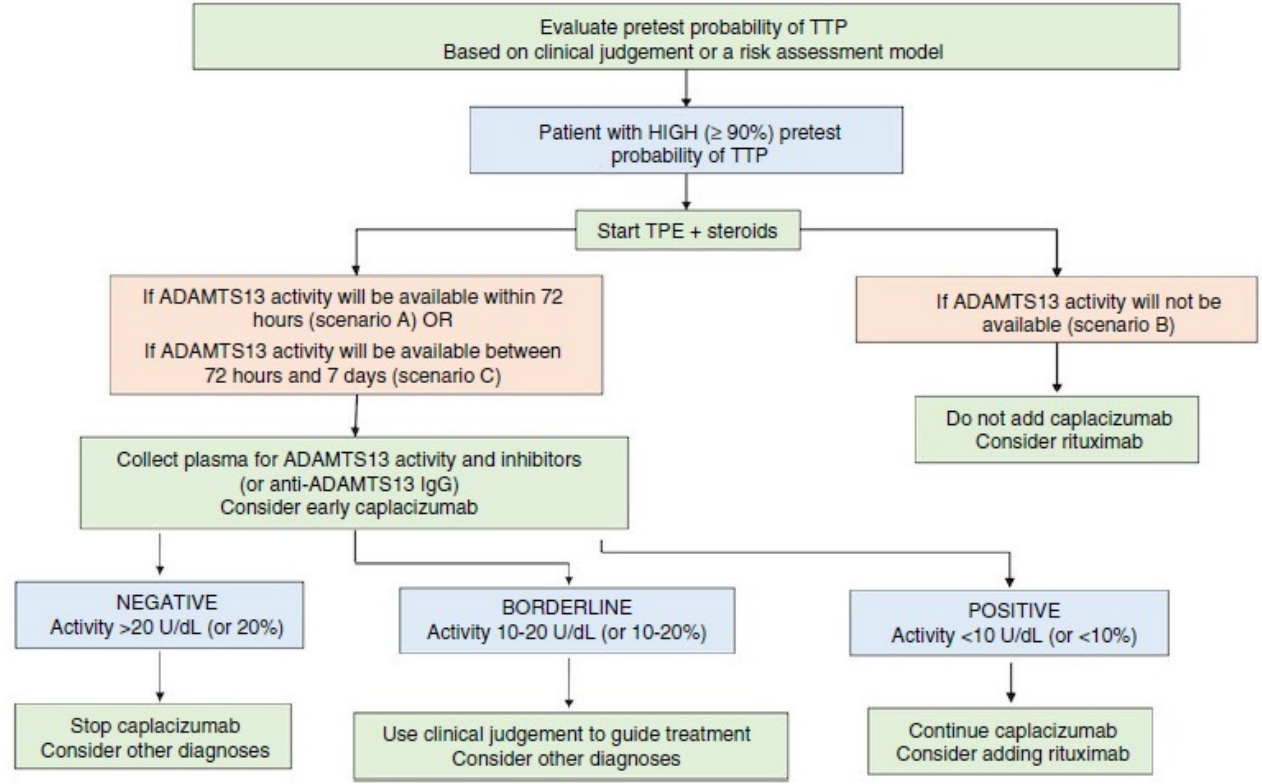
TABLE 1 PLASMIC score or French score predicts the likelihood of severe ADAMTS13 deficiency in a suspected TTP

Parameters	French Score	PLASMIC Score
Platelet count	<30 × 10^9/L (+1)	<30 × 10^9/L (+1)
Serum creatinine level	<2.26 mg/dL (+1)	<2.0 mg/dL (+1)
Hemolysis		
Indirect bilirubin >2 mg/dL or reticulocyte count >2.5% or undetectable haptoglobin	[a]	+1
No active cancer in previous year	[a]	+1
No history of solid organ or SCT	[a]	+1
INR < 1.5	[a]	+1
MCV < 90 fL	NA	+1
Likelihood of severe deficiency of ADAMTS13 activity (<10%)	0: 2%	0-4: 0%-4%
	1: 70%	6: 5%-24%
	2: 94%	6-7: 62%-82%

French score considered patients with thrombotic microangiopathy that included hemolysis and schistocytes in their definition and assumed that there was no history or clinical evidence for associated cancer, transplantation, or disseminated intravascular coagulation. Therefore, these items were intrinsic to the scoring system

Both the PLASMIC and the French scores were designed for adult populations with no comorbid conditions (eg, pregnancy, cancer, sepsis, organ/tissue transplantation, etc.) which may not be reliable in assessing children and patients with other comoridities

PLoS One. 2010;5:e10208.
Lancet Haematol. 2017 Apr;4(4):e157-e164
J Thromb Haemost. 2020;18:2486–2495.



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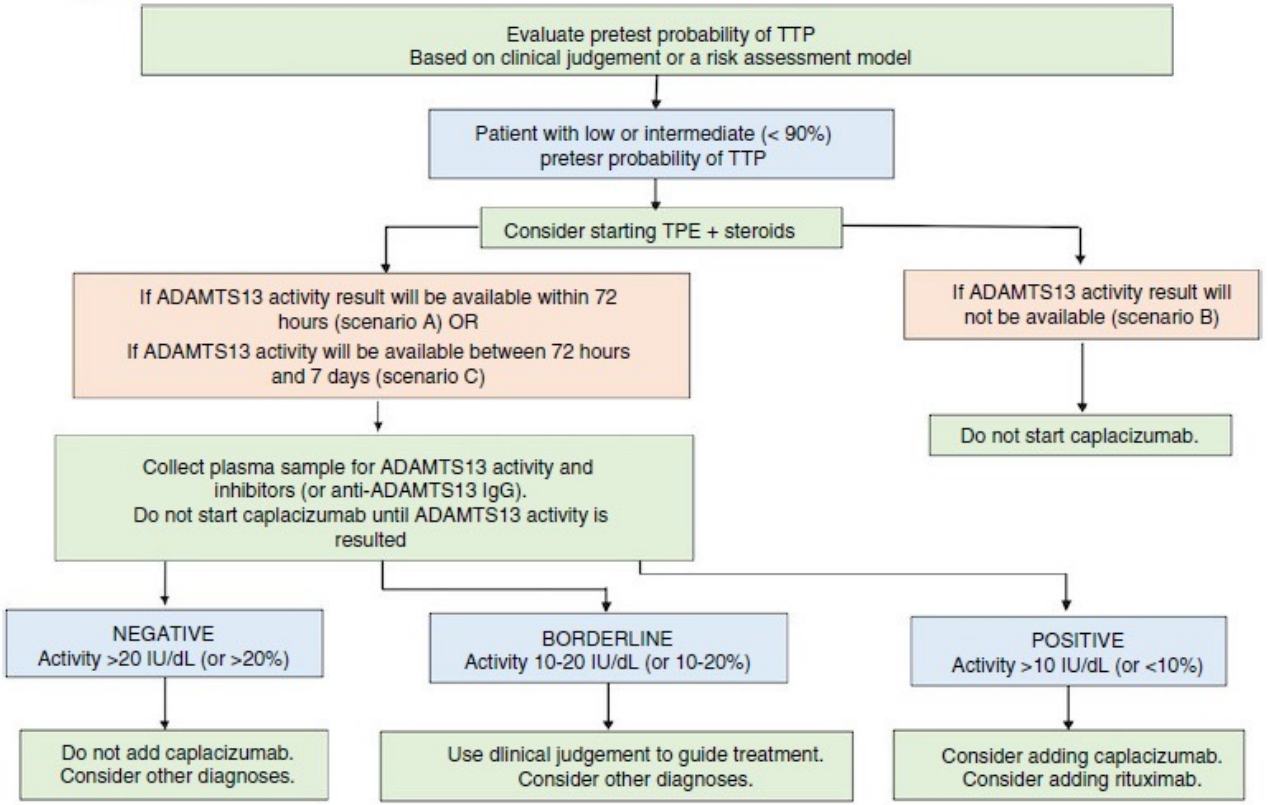


J Thromb Haemost. 2020;18:2486–2495.

International Council for Standardization in Haematology (ICSH) recommendations for laboratory measurement of ADAMTS13

I. PRE-ANALYTICAL VARIABLES

II. ADAMTS13 ASSAYS: three types

- ACTIVITY
- ANTIGEN
- ANTIBODY/INHIBITOR

I. VALIDATION OR VERIFICATION OF ASSAY PERFORMANCE

II. CLINICAL UTILITY OF ASSAYS

Int J Lab Hematol. 2020;42:685–696.

Consensus recommendations on sample collection and handling for ADAMTS13 assays

- Citrated plasma (centrifuged to ensure platelet depletion) should normally be used
- Heparin plasma or serum samples may be used depending on the assay type and if it is validated for these sample types
- EDTA plasma is not suitable for ADAMTS13 assays
- Samples should be centrifuged and plasma separated from the cells as rapidly as possible after blood collection to avoid in vitro changes
- Unless assays are performed immediately, plasma samples should be stored and shipped below -40°C to avoid potential proteolysis

Int J Lab Hematol. 2020;42:685–696.

ADAMTS13 ACTIVITY ASSAYS

TABLE 1 Some popular clinical laboratory methods for ADAMTS13 activity

Method	Performance	Calibrant	Diluent; dilution factor	VWF substrate type	Detection; assay type
[a]CBA[29,48,60,76]	LLOQ = 5%-6% Intra-assay CV = 9% Inter-assay CV < 15%	PNP diluted in assay buffer	5 mmol/L Tris/BSA, pH 8 1 mmol/L Pefabloc SC 3 mmol/L $BaCl_2$ 1.5 mol/L Urea DF: 12	Full-length plasma-derived VWF	Residual VWF binding to collagen, chromogenic ELISA End-point
[a]FRET[30,31,52,77]	LLOQ: 1%-3% Intra-assay CV: NR Inter-assay CV: ≤6%	PNP diluted in ADAMTS13-inactivated plasma	5 mmol/L Bis-Tris, pH 6 1 mmol/L Pefabloc SC 25 mmol/L $CaCl_2$ 0.005% Tween-20 DF: 50	VWF73 substrate	Fluorescence change (ex 355 nm, em 450/460 nm) Kinetic
[a]FRET[30,46,48,49,50,78]	LLOQ: 3%-5% Intra-assay CV: 6% Inter-assay CV: <10%	PNP diluted in assay buffer	5 mmol/L Bis-Tris, pH 6 1 mmol/L Pefabloc SC 25 mmol/L $CaCl_2$ 0.005% Tween-20 DF: 30	VWF-73 substrate	Fluorescence change (ex 340 nm, em 450/465 nm) Kinetic
FRET[3]	LLOQ = 0.3% Intra-assay CV = 1.8% Inter-assay CV = 1.7%	Heparinized PNP (minimally diluted in assay buffer)	50 mmol/L HEPES, pH 7.4 150 mmol/L NaCl 10 mmol/L $CaCl_2$ 1 mg/L BSA 0.05% Tween-20 protease inhibitor cocktail and 1 mmol/L PMSF DF: 2	rVWF71 substrate	Fluorescence change (ex 638 nm, em 658 nm) Kinetic
FRET[48]	LLOQ = 5% Intra-assay CV = NR Inter-assay CV = 5%-18%	Commercial calibrant diluted in ADAMTS13-inactivated plasma	Undisclosed components DF:20	VWF86-ALEXA substrate	Fluorescence change (ex 485 nm, em 530 nm) Kinetic
Chromogenic activity ELISA[22,35,48,50]	LLOQ: 0.3%-3% Intra-assay CV: ≤5.4% Inter-assay CV: ≤8%	Commercial calibrant diluted in assay buffer	Undisclosed components DF:31	VWF73-GST substrate	Chromogenic ELISA format (anti-GST capture; HRP conjugated anti-N10 monoclonal Ab to detect substrate cleavage) End-point

1st generation: full-length plasma-derived VWF multimers as substrate

2nd generation: recombinant peptides or fragments derived from VWF (73 amino acid peptide based on the sequence around the cleavage site in the VWF A2 domain)

CHROMOGENIC ELISA

Substrate cleavage may be detected by loss of signal (eg removal of GST) or the increased binding of monoclonal antibodies against a neo-epitope

Multiple steps (incubation, washing, reagent addition, etc.) and many laboratories have microplate readers

FRET

Increased fluorescence signal due to the loss of quenching when the peptide is cleaved and the quencher is no longer in close proximity to the fluorophore

Automatic reagent addition, incubation and measurement, minimizing hands-on time, although a fluorescence microplate reader is required

Int J Lab Hematol. 2020;42:685–696.

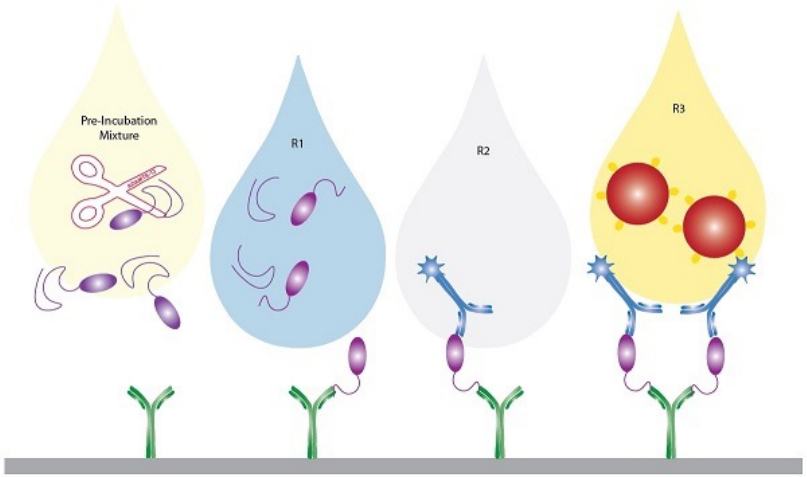
Currently, most of the commonly utilized assays (in-house or commercial) for measuring ADAMTS13 activity are based on enzyme linked immunosorbent assay (**ELISA**) or fluorescence resonance energy transfer (**FRET**)-based technologies utilizing **recombinant VWF substrates.**

While all the above test approaches provide valid results, most **do not easily accommodate urgent testing** for many reasons, including assay time (typically 4–5 h) and technical skill required.
Also, for cost/labour efficiency, most current assays are best performed in batches and not for individual patients.
These factors compromise timely testing for ADAMTS13 activity despite clinical urgency.

It is widely accepted that timely testing (ie, test results within the same day as patient management decisions are required) would assist rapid identification or exclusion of TTP, with patients thereby better managed, with potentially reduced risk and cost burden by avoiding unnecessary plasma exchange.
Recently, several **rapid ADAMTS13 activity assays** have become available to potentially fill this void.

Am J Hematol. 2021;96:1049–1055.

TECHNOSCREEN ADAMTS13 ACTIVITY ASSAY



- Rapid (30 minutes), semiquantitative screening assay which utilizes a flow through cartridge and an activity ELISA principle, without the need for specialized instrumentation or personnel
- Four indicator points (zero, 10, 40 and 80 IU/dL)
- Quality specifications: 88.7% sensitivity, 90.4% specificity, 74.6% positive predictive value, and 96.2% negative predictive value
- The method is limited by its subjective visual interpretation and potential for interference by lipids (which might block the device membrane), haemolysis and icterus
- The test is suitable for use in a POC environment as a **screening** tool and for the **negative exclusion of TTP**
- When decreased activity is detected, it should be confirmed by bioassay in an accredited laboratory

Int J Lab Hematol. 2020;42:685–696.
J Thromb Haemost. 2020;18:1686-1694.

HEMOSIL ACUSTAR ADAMTS13 ACTIVITY ASSAY

Figure 1. Assay scheme of HemosIL® AcuStar ADAMTS13 Activity

Fully automated, quantitative chemiluminescence-based two-step immunoassay (**CLIA**)

magnetic particles coated with GST-VWF73 peptide substrate

chemiluminescent detection based on an isoluminol labelled monoclonal antibody that reacts with the cleaved peptide

Strenghts

Rapid assay (33 minutes), with good sensitivity and precision

Good correlation between the assays and high agreement in classifying samples with ADAMTS13 levels below 10 IU/dL

Not affected by icterus, lipaemia or plasma turbidity

Weaknesses

Only three point calibration curve

Some discrepancies compared to FRET-VWF73 and a chromogenic activity ELISA, with some samples showing inter-assay disparity at normal and high activity

Thromb Haemost. 2018;118:942-944. Thromb Haemost. 2019;119:1767-1772.
J Thromb Thrombolysis. 2020. https://doi.org/10.1007/s1123 9-020-02086-8

The CLIA is accurate and very rapid compared to standard commercial or other in-house based testing, taking only 33 m to derive a test result as opposed to 3–5 h for ELISA, and may therefore be regarded as a valuable option for supporting **urgent/emergency** testing.

Am J Hematol. 2021;96:1049–1055.

A multicenter laboratory assessment of a new automated chemiluminescent assay for ADAMTS13 activity

Essentials

- TTP is a potentially fatal disorder due to ADAMTS13 deficiency
- Rapid ADAMTS13 testing permits timely/appropriate TTP diagnosis/therapy but is lacking
- A large multicenter evaluation of a new rapid assay for ADAMTS13 activity
- The AcuStar method provides fast and accurate ADAMTS13 activity and inhibitor levels

J Thromb Haemost. 2021;19:417–428.

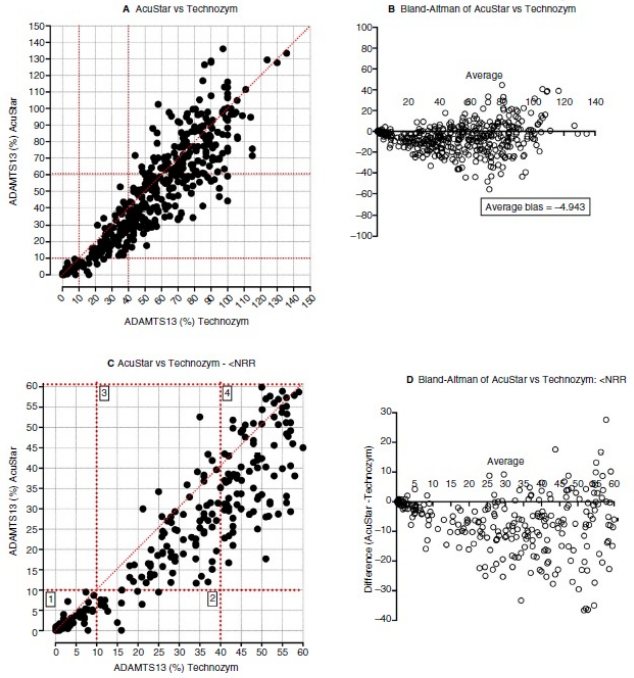


FIGURE 1 Comparative assessment of ADAMTS13 Activity testing by automated HemosIL AcuStar ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) Activity chemiluminescence versus Technozym ADAMTS13 Activity enzyme-linked immunosorbent assay. A, All data (retrospective and prospective) combined compared by linear regression analysis ($r = .956$; $P < .001$). B, Same data as (A) shown as Bland-Altman difference plot. The average negative bias was 4.94. C, Same data as (A), but shown only for "low" ADAMTS13 test values, characterized as being lower than the respective assay normal reference ranges (NRRs: 60.6%-130.6% for HemosIL; 40%-130% for Technozym). The dotted red lines identify the respective low limit NRR values, and also the important decision point of 10%, which reflects the standard for identification of severe ADAMTS13 deficiency, as consistent with a diagnosis of thrombotic thrombocytopenic purpura. The noted quadrants thus reflect: "1": Values of ADAMTS13 identified as severe (≤10%) using both test systems. "2": Values of ADAMTS13 identified as severe (≤10%) using HemosIL and "abnormal" (but not severe) using Technozym. Refer to Table 1 for further elucidation of these samples. "3": Values of ADAMTS13 identified as low but not severe using both test systems. "4": Values of ADAMTS13 identified as low (but not severe) using HemosIL and normal by Technozym. D, Same data as (B), but shown only for "low" ADAMTS13 test values

The HemosIL AcuStar ADAMTS13 Activity assay provided results rapidly, which were largely comparable with the Technozym ADAMTS13 Activity ELISA assay, albeit **lower** on average.
Conversely, inhibitor levels tended to be identified at a **higher** level on average.

J Thromb Haemost. 2021;19:417–428.

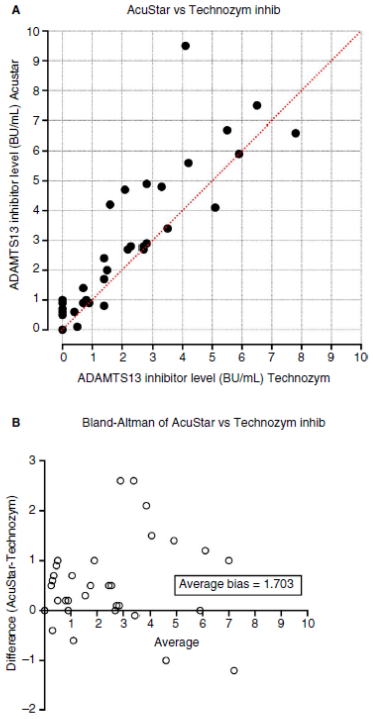


FIGURE 4 Comparative assessment of inhibitor testing using ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) Activity assays, being automated HemosIL chemiluminescence (AcuStar) versus Technozym. A, Data compared by linear regression analysis ($r = .987$; $P < .001$). B, Same data as (A) shown as Bland-Altman difference plot. The average positive bias was 1.70

ADAMTS13 activity testing: evaluation of commercial platforms for diagnosis and monitoring of TTP

Essentials

- Accurate ADAMTS13 activity testing is essential to diagnosing and monitoring thrombotic thrombocytopenic purpura
- We saw good concordance of several methods
- However, the AcuStar assay can **underestimate** ADAMTS13 activity in a small number of cases
- Therefore, it is important to consider the clinical context when utilizing the AcuStar assay

Res Pract Thromb Haemost. 2023;7:e100108

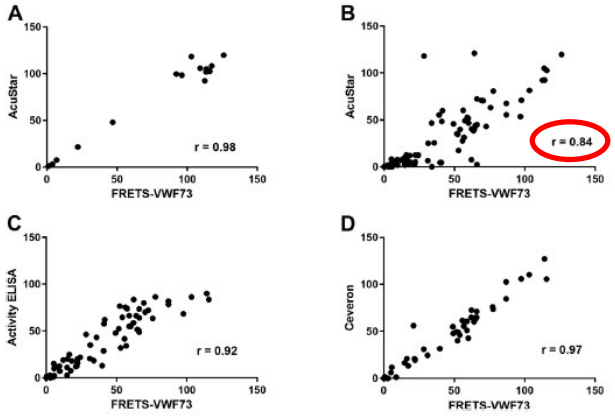


FIGURE 1 Pearson correlation between ADAMTS13 activity as measured by FRETS-VWF73 and commercial assays. (A) Eight healthy control samples, 1 pooled normal plasma sample and a range of dilutions of this on the AcuStar (Hemosil) platform. (B) Patient samples on the AcuStar. (C) Patient samples on Technozym activity ELISA. (D) Patient samples on the Ceveron Technofluor (FRETS) platform.

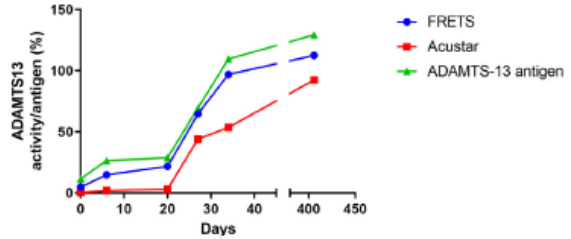


FIGURE 3 Longitudinal ADAMTS13 activity as measured by FRETS-VWF73 (blue circles) or AcuStar (red squares) and ADAMTS13 antigen levels (green triangles) from initial presentation to follow up during remission in a patient.

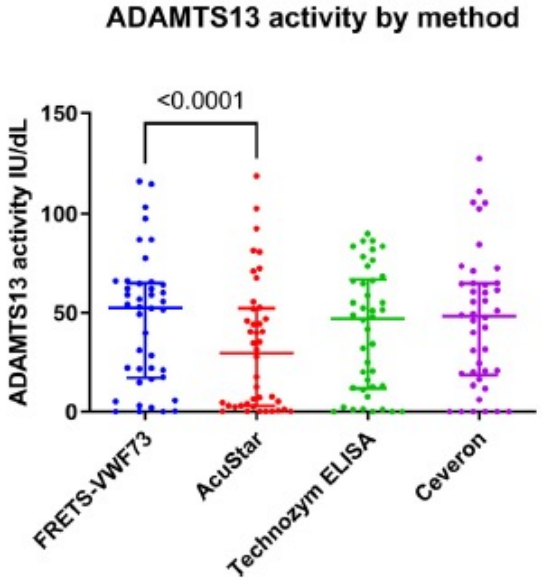


FIGURE 2 ADAMTS13 activity results tested on all four platforms. For all 42 samples that were tested across the four different platforms, the individual results are plotted with the median and interquartile range shown as long and short lines respectively.

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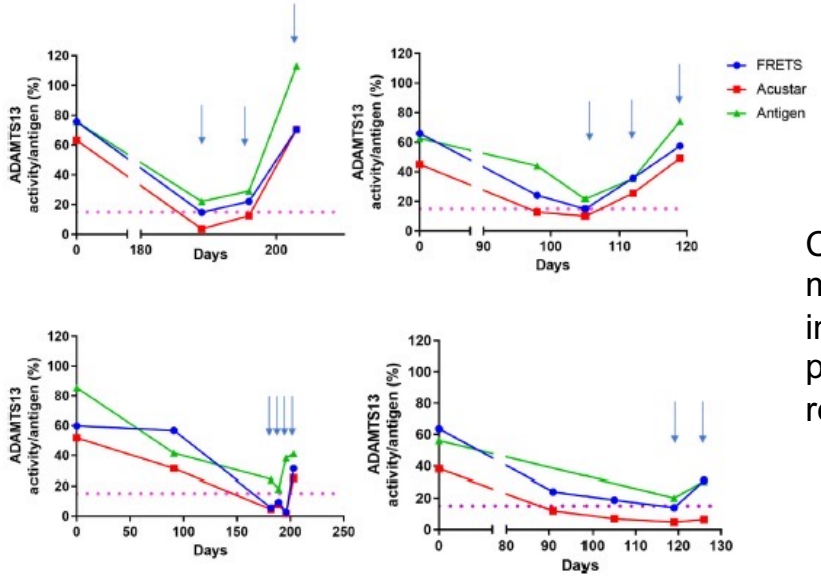

FIGURE 4 Longitudinal ADAMTS13 activity measurements prior to elective Rituximab treatment as measured by FRETS-VWF73 (blue circles) or AcuStar (red squares). Light blue arrows indicate when a dose of Rituximab was given. Rituximab is usually offered when the ADAMTS13 activity as measured with FRETS-VWF73, is approaching or lower than 15IU/dL (horizontal pink dotted line). ADAMTS13 Antigen levels are also indicated (green triangles).

Conclusion: when AcuStar is used upfront to guide management, a second testing method should be used in patients with an atypical thrombotic thrombocytopenic purpura presentation or unexpectedly slow ADAMTS13 recovery.

Discrepant Results

TABLE 3 Discrepant results on the AcuStar platform.

Sample	FRETS-VWF73 (IU/dL)	AcuStar (IU/dL)	Technozym ELISA (IU/dL)	Ceveron (IU/dL)	Anti-ADAMTS13 IgG RR (<6.1%)	ADAMTS13 antigen (RR 74%–134%)
Neonate with anemia & schistocytes on film	66	2	49	65	2	94
Sepsis	40	5	13	32	1	51
HUS	41	4	29	N.A.	1	35
Sepsis	62	8	59	65	5	62
Sepsis	16	10	N.A.	N.A.	1	N.A.
TTP 6 week follow-up	17	3	8	13	107	47
TTP 6 week follow-up	53	18	32	49	10	54
TTP 8 week follow-up	34	0	18	N.A.	N.A.	N.A.
TTP 4 week follow-up	22	5	16	19	25	14
TTP 1 week follow-up	15	2	3	N.A.	N.A.	26
TTP 2 week follow-up	22	3	13	21	18	29
TTP 3 week follow-up	21	3	12	56	11	38

- In total, 32 TMA presentation samples were tested, of which TTP was confirmed in 13 cases
- All 4 platforms showed results <10 IU/dL for the TTP samples tested, suggesting comparable **sensitivity**
- However, 5 of the 19 (26%, **15%** of tot) remaining TMA samples had **discrepant results** between AcuStar and FRETS-VWF73, suggesting TTP in the AcuStar assay
- As these cases were not confirmed as TTP, it suggests a somewhat lower **specificity** of the AcuStar assay
- We also tested 51 samples from patients with TTP undergoing **monitoring** during remission, and in 7 cases (**14%**), there was a **discrepancy** between AcuStar and FRET-VWF73 that would impact treatment decisions (e.g., further immunosuppression or continuation of caplacizumab)

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Discrepant Results

- As AcuStar manufacturer's instructions suggested that **interference by VWF** was tested only up to 200 IU/dL, we spiked normal plasma with Voncento, up to 600 IU/dL, which showed **no interference**

- We established whether **inhibitory antibodies** could account for the lower results by spiking normal control plasma with the mouse monoclonal inhibitory antibody 3H9, which showed that this had a **much bigger impact** on AcuStar and Technozym ELISA assays than FRETS-based assays

  We tested whether this could be because of the lower **pH** in the FRETS assay (pH 6) potentially minimizing the inhibitory effect in FRETSVWF73, but increasing the pH to 7.6 did not reduce the ADAMTS13 activity result

  The **concentration of VWF73** (which competes with inhibitory antibody) in the AcuStar assay is not disclosed and could explain this discrepancy in theory

- Although these different effects of inhibitory antibodies on AcuStar vs FRETS-VWF73 could potentially explain the discrepancies in **TTP follow-up samples**, they do not explain the aberrantly low results in **non-TTP samples** with the AcuStar

- For these, further investigation of possible causes is necessary to clarify the limitations of the assay

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ADAMTS13 testing update: Focus on laboratory aspects of difficult thrombotic thrombocytopenic purpura diagnoses and effects of new therapies

- It is important to note that **mild to moderate** (or rarely severe) **ADAMTS13 decreases** can be seen in a variety of **non-TTP** medical illnesses including liver disease (decreased synthesis), DIC (consumption), acute inflammatory states (cytokine-mediated suppression of hepatic synthesis), and in pregnant women and newborns (hepatic function or maturity)
- Mild or moderate deficiency has been reported in **rare cases of TTP** and could occur if the specimen is collected after treatment initiation
- Activity assays are variably affected by **interfering substances** such as free hemoglobin due to hemolysis, or icteria
- While ADAMTS13 activity results are an incredibly helpful tool in the diagnosis of TTP, definitive diagnosis always requires close **clinico-pathologic correlation**

Interferences	Hemoglobin	None up to 500 mg/dL
	Bilirubin	None up to 18 mg/dL
	Triglycerides	None up to 1,250 mg/dL
	Rheumatoid Factor (RF)	Presence of RF can cause underestimation
	Human Anti-Mouse Antibody (HAMA)	None up to 1 µg/mL
	von Willebrand Factor (VWF)	None up to 200 IU/dL (200%)
	Heparin (LMWH, UFH)	None up to 2 IU/mL

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ADAMTS13 ANTIGEN ASSAYS

- Immunoblotting procedures and **ELISA** assays have been used for ADAMTS13 antigen measurement and the latter is the method of choice in the clinical diagnostic laboratory when an assay is indicated
- There are a variety of commercial kits and numerous polyclonal and monoclonal antibodies available for assays
- The capture and detection antibody selection is critical as monoclonal antibodies directed against the central domains of the protein may detect truncated forms and full-length ADAMTS13, while antibodies directed against the C-terminus may underestimate functional ADAMTS13 molecules
- ADAMTS13 antigen tests are **not commonly used** in clinical practice as these identify only quantitative, but not functional, deficiency

Int J Lab Hematol. 2020;42:685–696.

ANTIBODY AND INHIBITOR ASSAYS

- ADAMTS13 autoantibody assays demonstrate the presence of specific immunoglobulins and are detected in **iTTP**
- Depending on the ADAMTS13 domain specificity of the immunoglobulins, they may or may not inhibit the proteolytic activity
- **Inhibitory antibodies** can be demonstrated in mixing tests and Bethesda type assays
- **Non-neutralizing antibodies** can influence ADAMTS13 survival in the circulation and are clinically relevant
- **Autoantibody assays**, where available, are therefore preferred

Int J Lab Hematol. 2020;42:685–696.

AUTOANTIBODY ASSAYS

- Western blotting, immunocapture and ELISA assays for determination of ADAMTS13 autoantibody levels have been described
- **ELISA** methods for immunoglobulin (Ig) G class autoantibodies are most widely used in clinical laboratories
- The calibration of these assays, their reporting units and cut-off values vary
- The results may not be interchangeable

Int J Lab Hematol. 2020;42:685–696.

INHIBITOR TESTS

- Patient plasma is heated at 56°C for at least 30 minutes to inactivate ADAMTS13 and other haemostasis proteins, while leaving IgG unaffected and
- Centrifuged to remove any precipitated protein
- Doubling dilutions are incubated with untreated pooled normal plasma as a source of ADAMTS13 for 1 hour at 37°C
- A control comprising heat-treated normal control plasma or buffer and untreated PNP is incubated in parallel
- After incubation, the residual ADAMTS13 activity is measured in the patient and control samples
- If an IgG inhibitor is present in the patient sample, the ADAMTS13 activity in the test sample will be lower than the control
- In this case, the incubated dilutions of heat-treated patient plasma are assayed to determine inhibitor potency in a Bethesda type assay

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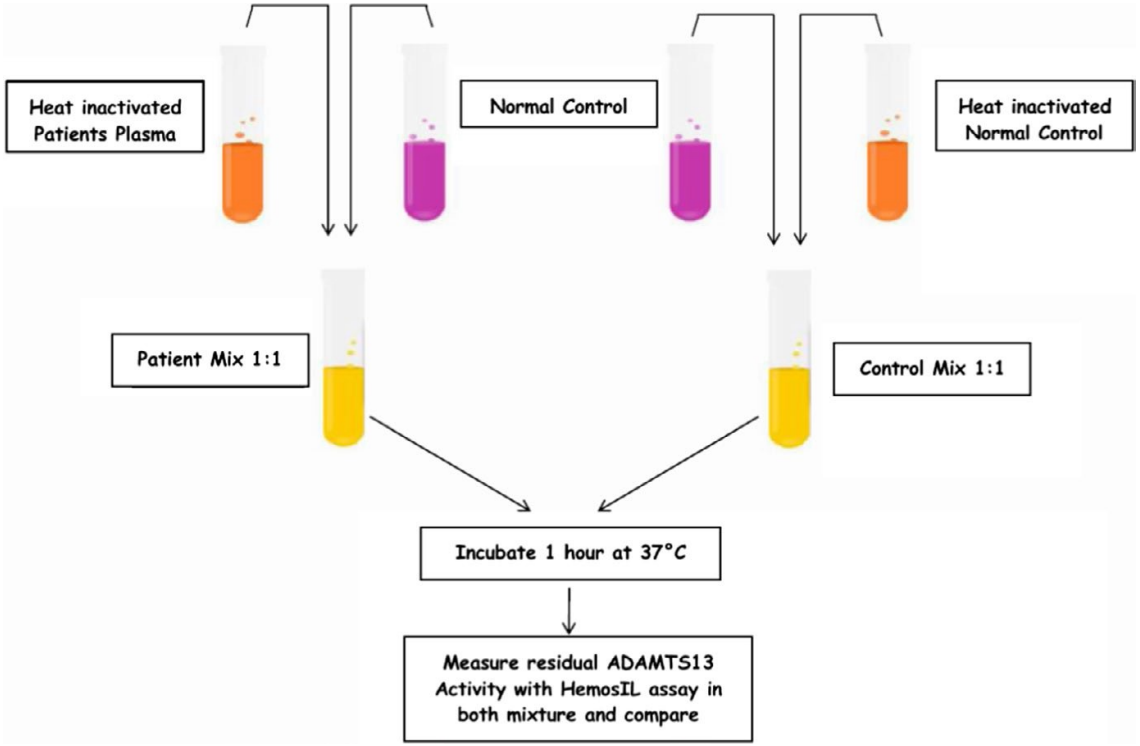
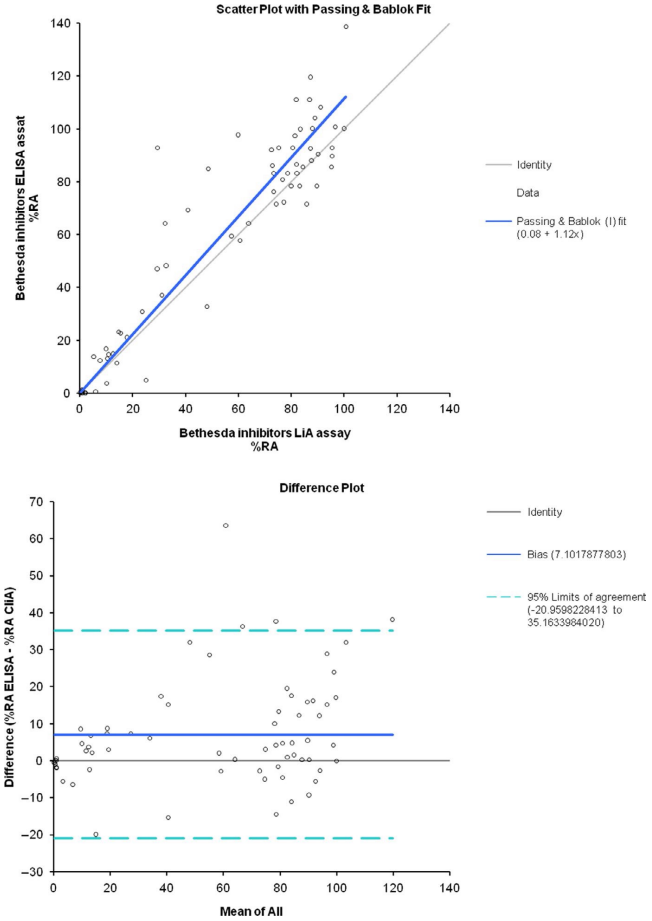


Int J Lab Hematol. 2021;43:298–304.

CliA and ELISA Bethesda assays showed **very good agreement** with samples run at the same time using ELISA ADAMTS13-autoantibody assay.

Albeit more expensive, the CliA Bethesda assay identified inhibitory anti-ADAMTS13 within almost the same TAT as ELISA, but with better automation and limited operator involvement.



Int J Lab Hematol. 2021;43:298–304.

Consensus recommendations on assays for ADAMTS13, autoantibodies and inhibitors

- Functional **FRET**-based assays or chromogenic activity **ELISA** methods are recommended as front line assays as they are sensitive, show good precision and are simpler to use, being completed in a few hours
- Rapid point of care assays may have utility as screening methods or “out of hours” **emergency** tests
- Every **calibrator** should be traceable to the International Standard Plasma for assaying ADAMTS13 activity in citrated plasma samples
- When **reporting** results: indicate the type of assay performed, use the correct units (eg IU/dL) for activity and antigen assays, and state the reference range for the method
- High and low activity **controls** should be included in each assay run
- Protocols should be **validated** after any modification
- If gross **icterus** interferes in some FRET assay methods, the problem can be resolved by assaying at a higher dilution, treatment with bilirubin oxidase, or using a chromogenic activity ELISA (A comment regarding potential assay interference should be added to the laboratory report)
- If artefactual in vitro **haemolysis** is likely (eg secondary to difficult venipuncture or use of a small gauge needle) fresh blood samples should be obtained (If this is not possible, a comment regarding potential assay interference should be added to the laboratory report)
- Where assay results do not match the clinical picture, or congenital TTP is suspected and ADAMTS13 activity results are normal or only show a mild reduction, collagen-binding assays should be considered
- If decreased ADAMTS13 activity (<20 IU/dL) is detected in a new patient, an ADAMTS13 antibody assay or inhibitor test should be performed
- Wherever possible, use the same ADAMTS13 assay when studying a patient **longitudinally** to manage treatment

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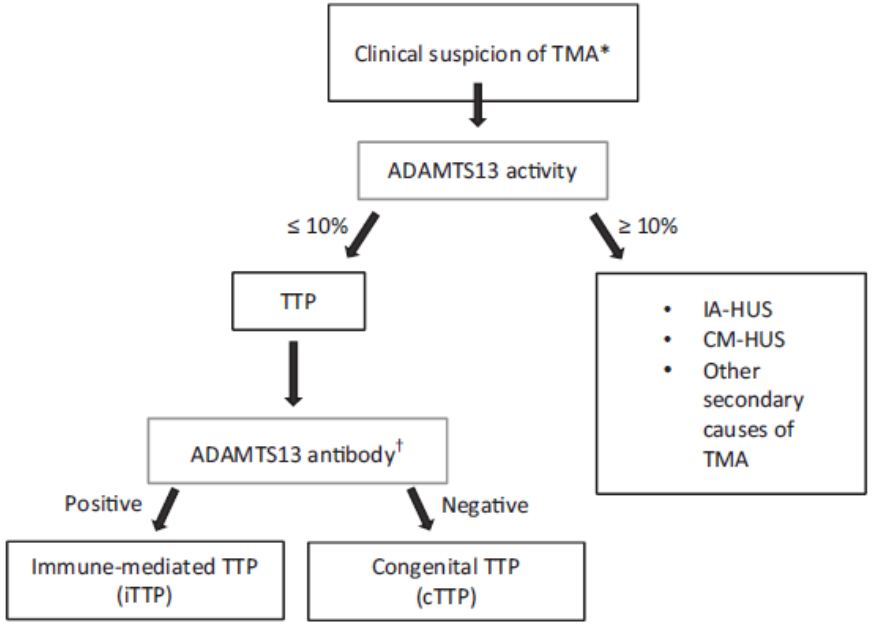
CLINICAL UTILITY OF ASSAYS



Int J Lab Hematol. 2020;42:685–696.

TABLE 2 Combined impression of ADAMTS13 testing results

Activity	Inhibitor (Bethesda)	Antibody (ELISA)	Interpretation
>20%	N/A	N/A	Negative for severe deficiency, seek alternative diagnosis; rare cases of TTP or post-treatment sample collection could display this pattern
10%-20%	See below if performed	See below if performed	Equivocal for severe deficiency, close clinical correlation required, consider alternative diagnosis; some cases of TTP or post-treatment sample collection could display this pattern
<10%	Positive	Not done, positive, or negative	Acquired TTP with neutralizing antibodies
<10%	Negative	Positive	Acquired TTP with non-neutralizing antibodies
<10%	Negative	Negative	Consider inherited TTP, acquired immune TTP without detectable antibodies due to titer or technical limitations, or possibly acquired TTP due to nonimmune mechanisms; consider genetic testing, close clinical correlation required, ADAMTS13 levels during clinical remission may help to differentiate inherited from acquired

Int J Lab Hematol. 2021;43(Suppl. 1):103–108.

Consensus recommendations on clinical utility of assays

- ADAMTS13 activity <10 IU/dL is diagnostic for TTP in patients presenting with a clinical scenario and blood film consistent with TMA
- An alternative diagnosis and repeat ADAMTS13 assay should be considered in TMA patients presenting with an indeterminate range of 10-20 IU/dL ADAMTS13 activity
- Alternative diagnosis/therapy should be considered in patients with an ADAMTS13 activity >10 IU/dL, especially when >20 IU/dL, as they rarely respond to PEX
- **The overall clinical picture/treatment of the patient should always be reviewed with close interaction with clinicians. This is important to avoid unnecessary tests and to ensure that appropriate tests meet clinical needs**, especially when non-TTP expert clinicians request assays. Clinical scoring systems such as the PLASMIC score may assist in providing guidance for the necessity of ADAMTS13 testing
- ADAMTS13 antibody assays should be used to decide whether the patient has cTTP or iTTP; definite cTTP diagnosis requires ADAMTS13 genetic testing
- Nomenclature, normal range, reporting units and methodology (eg inhibitory and noninhibitory antibodies) need further standardization
- Follow-up ADAMTS13 activity testing is important as persistently low levels increase the risk of exacerbation and TTP relapse
- ADAMTS13 antigen levels are not clinically useful in the absence of activity assays, but may prove helpful in predicting mortality risk in iTTP patients with high IgG ADAMTS13 antibody level, although further study is needed

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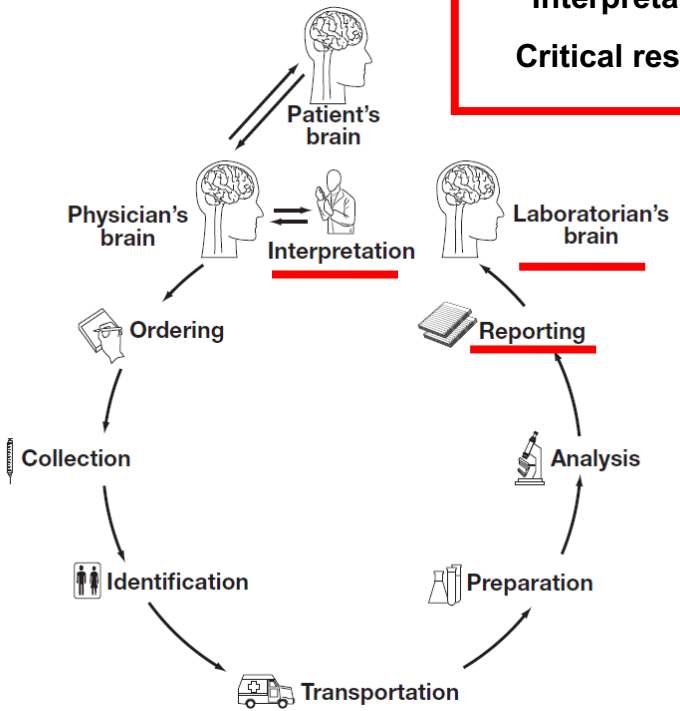
Brain - to - Brain loop and TTP

Interpretative comments

Critical results notification



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