

Evaluation of the Fully Automated HemosIL Acustar ADAMTS13 Activity Assay

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ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 repeats, member 13) is responsible for the proteolysis of ultra-large von Willebrand factor (VWF) multimers into smaller multimers.¹ Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy (TMA) caused by severe ADAMTS13 deficiency (activity < 10%) leading to the formation of platelet-rich microthrombi within small arterioles responsible for the destruction of platelets and erythrocytes.^{2,3} TTP is a life-threatening condition with a mortality rate of 10 to 20% despite correct management.¹ The assessment of ADAMTS13 is of clinical importance as it is the only marker to be specific and sensitive toward TTP.³ The rapid differential diagnosis between TTP and other TMA (e.g., haemolytic uraemia syndrome [HUS, no ADAMTS13 deficiency]) is essential to initiate the appropriate treatment as it can be difficult to differentiate TMA based on clinical presentation.^{1,4} Furthermore, monitoring of ADAMTS13 activity is useful to ensure biological remission (ADAMTS13 > 10%) as well as predicting relapse (usually preceding clinical signs).^{5,6} It has effectively been demonstrated that 40% of patients diagnosed with autoimmune TTP will experience at least one relapse.¹

It has been shown that any delay in ADAMTS13 results will have a negative impact on the diagnosis, treatment and prognosis of the patient.^{3,7,8}

Today, in-house methods using full-length VWF and the fluorescence resonance energy transfer (FRET)-VWF73 are considered reference methods.^{5,9,10} However, these methods require considerable skill, are time-consuming and not widely available.¹

In our laboratory (Université catholique de Louvain, CHU UCL Namur, Hematology Laboratory, Yvoir, Belgium), the activity of ADAMTS13 is determined using the Technozym chromogenic enzyme-linked immunosorbent assay (ELISA) assay (Technozym, Technoclone, Vienna, Austria) based on the direct detection of cleavage products from a VWF peptide

substrate (recombinant GST-VWF73 peptide [glutathione S-transferase-von Willebrand factor of 73 amino acids]) containing the cleavage site of the ADAMTS13 (Tyr1605-Met1606) (Technozym insert kit). This assay is calibrated against the first World Health Organization international standard ADAMTS13 plasma.¹¹ Unfortunately, the Technozym chromogenic ELISA assay is time-consuming, highly manual (risk of error) and requires a new calibration at each run. Therefore, we aimed to compare this method with the new and first fully automated HemosIL AcuStar ADAMTS13 activity assay (Instrumentation Laboratory, Bedford, Massachusetts, United States). This latter assay is a two-step immunoassay also using the GST-VWF73 substrate but with magnetic particles and chemiluminescence technology with a release of first results within 33 minutes (HemosIL AcuStar preliminary insert kit).

For this method comparison, citrated plasma from 38 patients were selected from our sample collection preserved at -20°C for 1 to 11 months. These samples included 8 TTP patients, 2 patients with moderate ADAMTS13 activity (> 10–40%) and 28 patients with normal ADAMTS13 activity level (> 40%) based on our routine assay (Technozym). Assays were performed according to the manufacturers' instructions during October 2017. MedCalc software was used for statistical analysis (version 12.7.0). The mean bias between methods was determined with a Bland-Altman plot. Inherent characteristics and the pros and cons of each method were also compared (►Table 1).

The results of our external controls for the Technozym assay obtained from the ECAT Foundation (External quality Control for Assays and Tests with a focus on Thrombosis and Haemostasis) between July 2015 and August 2017 were also analysed ($n = 16$).

The absolute mean bias on Bland-Altman plot was 4.8%, but was not constant (95% confidence interval [CI], -0.27 to 9.83%) and the limits of agreement (95% CI) were -25.3% and

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Table 1 Inherent characteristics and pros and cons of each method

Assay	Technozym Chr ELISA assay	HemosIL AcuStar chemiluminescent assay
LOQ	Good (2%) ¹²	Excellent (0.3%) ¹³
Linearity	Good (0.3–105%)	Good (0.2–160%) ¹³
Intra-assay CV	2.6–5.4% ^{5,12}	2.7–4.4% ¹³
Inter-assay CV	6.0–6.7% ^{4,5,12}	3.7–5.1% ¹³
Time to first results	Long (> 3.5 h)	Rapid (33 min) ¹³
Substrate	Chr GST-VWF73	GST-VWF73
Calibration curve	6 points	3 points
No. of tests per kit	10	10
Pros	Low imprecision WHO calibrated ¹¹	Fully automated 24 h/24 availability Rapid Low imprecision WHO calibrated
Cons	Highly manual Require a new calibration at each run Technical skills Time-consuming	Need further validation

Abbreviations: Chr, chromogenic; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; GST-VWF73, glutathione S-transferase-von Willebrand factor of 73 amino acids; LOQ, limit of quantification; WHO, World Health Organization.

+34.9%, reflecting a high variability between both assays (►Fig. 1). This variability has already been reported for several commercial ADAMTS13 assays.¹² Based on these results, the same method should be used for the monitoring of ADAMTS13 and each laboratory must determine their own reference values. We should note that for long-term monitoring purposes, biological and analytical variation should be further investigated.

More important to notice is that based on the well acceptable TTP diagnosis cut-off (< 10%), no misclassification was pointed out. If considering only values < 10%, the bias was near to zero. Consequently, both methods appeared to be effective to identify acute TTP.

Based on our external quality control program, the calculated Z-score always fell into the –2 to +2 interval except in one sample (August 2016, sample 16.58) (data not shown)

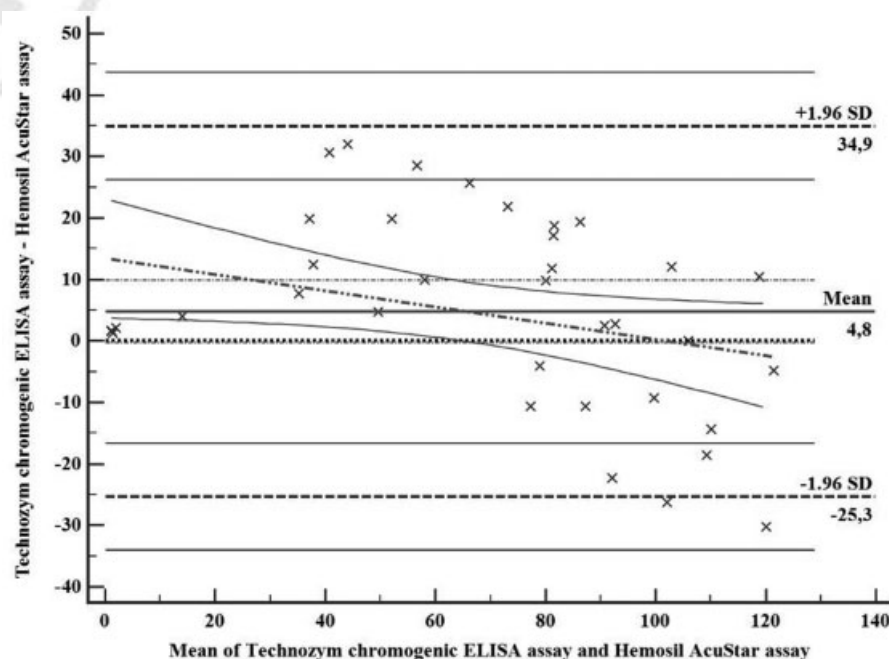


Fig. 1 Bland–Altman plot of ADAMTS13 activity (%) according to the Technozym chromogenic ELISA assay (Technozym, Technoclone, Vienna, Austria) and the HemosIL AcuStar ADAMTS13 activity assay (Instrumentation Laboratory, Bedford, Massachusetts, United States). Very low ADAMTS13 activity levels were rounded to the limit of quantification (LOQ) of each assay.

but no misclassification was observed based on the < 10% cut-off value.

In this method comparison, we used the Technozym assay as reference which showed a non-significant mean bias of – 0.02% (95% CI, –1.75 to 1.71%) with 95% CI limits of agreement of –16.83 to 16.80% in 97 patients compared with the well-established FRET reference method developed by Kokame et al in 2005.^{9,10} Another study evaluating the Technozym assay showed that 88% (83/94) of TTP patients were correctly correlated to an in-house FRET-VWF73 assay considered being the reference.⁵ In this same study, 2.6% (4/154) of non-TTP patients were classified as falsely positive with the Technozym assay.⁵ Taken together, this study calculated the sensitivity, specificity, predictive positive value and predictive negative value of the Technozym assay for the diagnosis of TTP at 88, 97, 95 and 93%, respectively, which supported its use in our daily routine practice. Recently, Langley et al also claimed that the Technozym assay was suitable for use in clinical settings.⁸

In case of discordant results between ADAMTS13 activity and clinical information (e.g., fever, thrombocytopenia, microangiopathic haemolytic, anaemia, neurological symptoms, renal insufficiency), a control with a reference method available in specialized laboratories may be suggested, in particular for borderline activity, which can be defined as < 30% with the Technozym assay based on the study of Joly et al.^{4,5}

Besides our evaluation, the new AcuStar assay was compared with the FRET Actifluor assay in 101 patients and presented at the International Society on Thrombosis and Haemostasis (ISTH) in July 2017.¹³ They showed that the AcuStar method reported values comparable to the FRET method except in the low activity range, where the FRET assay reported higher values. In agreement with these results, Nakashima et al recently showed that the Actifluor assay was the less performant assay to detect ADAMTS13 deficiency compared with the Technozym and Lifecodes assays as well as a reference laboratory method.¹²

In conclusion, our preliminary data suggest that the new AcuStar ADAMTS13 activity assay is a valuable tool to correctly diagnose acute TTP. Furthermore, the inherent advantages of automation (►Table 1) as well as the widespread availability 24 hours/24, the low imprecision and the fact that it does not require highly qualified personnel, make the AcuStar a platform of choice for measuring ADAMTS13

activity in haemostasis laboratories. Further studies comparing this new assay with reference methods on a larger number of patients are needed to confirm our data.

Conflict of Interest

None.

References

- 1 Joly BS, Coppo P, Veyradier A. Thrombotic thrombocytopenic purpura. *Blood* 2017;129(21):2836–2846
- 2 Levy GG, Nichols WC, Lian EC, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature* 2001;413(6855):488–494
- 3 Sadler JE. What's new in the diagnosis and pathophysiology of thrombotic thrombocytopenic purpura. *Hematology (Am Soc Hematol Educ Program)* 2015;2015:631–636
- 4 Mackie I, Langley K, Chitolie A, et al. Discrepancies between ADAMTS13 activity assays in patients with thrombotic microangiopathies. *Thromb Haemost* 2013;109(03):488–496
- 5 Joly B, Stepanian A, Hajage D, et al. Evaluation of a chromogenic commercial assay using VWF-73 peptide for ADAMTS13 activity measurement. *Thromb Res* 2014;134(05):1074–1080
- 6 Hie M, Gay J, Galicier L, et al; French Thrombotic Microangiopathies Reference Centre. Preemptive rituximab infusions after remission efficiently prevent relapses in acquired thrombotic thrombocytopenic purpura. *Blood* 2014;124(02):204–210
- 7 Sadler JE. Pathophysiology of thrombotic thrombocytopenic purpura. *Blood* 2017;130(10):1181–1188
- 8 Langley K, Fretwell R, Kitchen S, et al. Multiple centre evaluation study of ADAMTS13 activity and inhibitor assays. *Int J Lab Hematol* 2018;40(01):21–25
- 9 Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. FRET-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br J Haematol* 2005;129(01):93–100
- 10 Palla R, Valsecchi C, Bajetta M, Spreafico M, De Cristofaro R, Peyvandi F. Evaluation of assay methods to measure plasma ADAMTS13 activity in thrombotic microangiopathies. *Thromb Haemost* 2011;105(02):381–385
- 11 Hubbard AR, Heath AB, Kremer Hovinga JA; Subcommittee on von Willebrand Factor. Establishment of the WHO 1st International Standard ADAMTS13, plasma (12/252): communication from the SSC of the ISTH. *J Thromb Haemost* 2015;13(06):1151–1153
- 12 Nakashima MO, Zhang X, Rogers HJ, et al. Validation of a panel of ADAMTS13 assays for diagnosis of thrombotic thrombocytopenic purpura: activity, functional inhibitor, and autoantibody test. *Int J Lab Hematol* 2016;38(05):550–559
- 13 Mirabet M, Blanch S, Puig J, Serra J. Highly sensitive fully automated chemiluminescent immunoassay for rapid quantification of ADAMTS13 activity. *Res Pract Thrombosis Haemostasis* 2017;1 (Suppl 1):1297