ADAMTS-13 is the enzyme responsible for cleaving ultra large von Willebrand factor multimers. Deficiency of ADAMTS-13 is associated with thrombotic thrombocytopenic purpura (TTP), a serious disorder usually diagnosed with clinical criteria.

Historic test methods used to measure ADAMTS-13 activity were technically challenging and time consuming. Commercial kits first appeared which utilized fluorescence resonance energy transfer (FRET) methodology to measure ADAMTS-13 activity. Recently a kit using an ELISA methodology has been released.

In this study, we compared results from three commercial kits using samples from patients referred for a possible diagnosis of TTP. Two of the kits used a FRET methodology and the other employed an ELISA method. Our evaluation also included linearity and imprecision studies.

### Results

A total of 38 samples were tested, which included normal and abnormal values. Refer to Graphs.

Technocline ELISA identified 13 samples with less than 5% activity while Technocline FRET identified 12 of these samples as less than 5% and 1 sample with 6% activity.

All 13 of these markedly low samples were less than 33% by the GTI method (range 10-32%), suggesting the cutoff for TTP is higher with the GTI kit compared to the Technocline kits.

Imprecision results were acceptable for all three kits. The ELISA kit demonstrated the best precision with less than 10% CVs across a range of values. Refer to Table 1.

All three kits demonstrated acceptable linearity. Refer to Table 2.

### Materials and Methods

Results from three commercially available kits were compared: ADAMTS-13 Activity Assay (GTI, Waukesha, Wisconsin, USA), Technozym ADAMTS-13 Activity/Antigen ELISA assay (FRET method) and Technozym ADAMTS-13 Activity ELISA assay (Technoclone kits, Vienna, Austria). All methods were performed according to the manufacturer’s package insert protocols.

In the GTI method 50 µL of the diluted patient sample (18 µL patient sample and 132 µL specimen diluent) was pipetted into each well. 50 µL of substrate (a synthetic fragment of von Willebrand protein with fluorescent tags on either side of the ADAMTS-13 cleavage site) was then added and a time zero reading was taken. The plate was incubated at room temperature for 30 minutes and a second reading was obtained.

In the Technoclone FRET method, the kit plate is coated with anti-ADAMTS-13 antibody. 50 µL of sample was added to each well and allowed to incubate at room temperature for 2 hours. The plate was washed and 50 µL of fluorogenic substrate was added. Kinetic measurements were taken for 15 minutes (one measurement per minute) at 30 °C. The TechnoLine test can also be continued to measure the ADAMTS-13 antigen level. For the purposes of this study, only the activity was measured.

In the Technoclone ELISA method, the kit plate is coated with Anti-GST. 100 µL of GST–WF73 Substrate was added to each well and incubated for 1 hour. The plate was washed and 100 µL of diluted patient sample (20 µL patient sample and 600 µL reaction buffer) was added and incubated for 30 minutes to allow the ADAMTS-13 in the sample to cleave the WF73 Substrate. The plate was washed and 100 µL of HRP conjugated Ab directed against cleavage site of the WF73 Substrate was added and allowed to incubate for 60 minutes. The plate was washed and TMB substrate added and the color reaction was stopped by sulphuric acid after 30 minutes. All incubations were at room temperature.

All plates were read on a SpectraMax M5 microplate reader (Molecular Devices Corp., Sunnyvale, California, USA). Assay results were obtained using evaluation software provided by each company. Results were reported as a percentage of normal.

Plasma samples from 38 patients sent to ARUP Laboratories were included in the study. All patient samples were de-identified according a University of Utah Institutional Review Board approved protocol to meet the Health Information Portability and Accountability Act (HIPAA) patient confidentiality guidelines.

Imprecision was studied using normal and abnormal patient samples (three levels of activity). At least 10 replicates were performed for the within run studies. Between run studies included at least 6 replicates on 3 separate runs.

Linearity was assessed using patient samples diluted with the appropriate diluent provided in each kit.

### Conclusion

We observed a bias between the Technoclone (both FRET and ELISA methods) and GTI kits in samples with markedly low ADAMTS-13 activity.

Cutoffs for severe deficiency need to be kit specific.

Availability of ELISA methodology for measurement of ADAMTS-13 may improve accessibility for testing, supporting more rapid and accurate diagnosis of TTP.

### Tables

#### Table 1. Summary of Linearity Results

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<tr>
<th>Sample</th>
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<th>Diluted 1:4</th>
<th>Diluted 1:8</th>
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<td>Patient A</td>
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<td>Patient B</td>
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#### Table 2. Summary of Linearity Results

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