EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Technozym ADAMTS13 Activity
DECISION SUMMARY

I Background Information:

A De Novo Number

DEN230024

B Applicant

Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH

C Proprietary and Established Names

Technozym ADAMTS13 Activity

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
SAC	Class II with special controls	21 CFR 864.7297	Hematology

II Submission/Device Overview:

A Purpose for Submission:

De Novo request for evaluation of automatic class III designation for Technozym ADAMTS13 Activity

B Measurand:

ADAMTS13 Activity

C Type of Test:

Manual enzyme linked immunosorbent assay (ELISA)

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

III Indications for Use:

A Intended Use(s):

See Indications for Use below

B Indication(s) for Use:

The Technozym ADAMTS13 Activity assay is an enzyme-linked immunosorbent assay (ELISA) intended for the qualitative determination of ADAMTS13 activity in platelet poor human citrated plasma. The assay is intended to be used in conjunction with other clinical and laboratory findings as an aid in the diagnosis of thrombotic thrombocytopenic purpura (TTP) in adult and pediatric patients being evaluated for thrombotic microangiopathy (TMA).

C Special Conditions for Use Statement(s):

For Prescription Use Only
For In Vitro Diagnostic Use Only

D Special Instrument Requirements:

Microplate reader

IV Device/System Characteristics:

A Device Description:

The Technozym ADAMTS13 Activity assay is an enzyme linked immunosorbent assay (ELISA) used for detection of ADAMTS13 activity in citrated human plasma.

The assay contains:

- ADAMTS13 Activity anti-GST coated test plate – microplate coated with anti-GST antibody
- ADAMTS13 Activity GST-VWF73 – reagent that contains GST tagged peptide of 73 amino acids from the A2 domain of VWF with specific cleavage site for ADAMTS13 and serves as the in vitro substrate for ADAMTS13
- ADAMTS13 Activity Calibrators – consists of six vials containing lyophilized plasma, each with a different level of ADAMTS13 activity
- ADAMTS13 Activity Controls – consists of two vials of lyophilized plasma, each with high or low levels of ADAMTS13 activity
- ADAMTS13 Activity Conjugate – reagent that contains horseradish peroxidase (HRP) conjugated monoclonal antibody directed against the neoepitope exposed due to cleavage of GST-VWF73 by ADAMTS13 present in plasma
- ADAMTS13 TMB substrate – reagent contains tetramethylbenzidine (TMB) substrate for HRP
- ADAMTS13 Activity Stop Solution – reagent contains 2.5% sulfuric acid for stopping the conversion of TMB substrate

B Test Principle

ADAMTS13, a disintegrin and metalloprotease with thrombospondin type 1 motif 13, is an enzyme (VWF-cleaving protease) that specifically cleaves von Willebrand factor (VWF) under high shear stress conditions. The Technozym ADAMTS13 Activity assay is an enzyme linked immunosorbent assay for the detection of ADAMTS13 activity in human citrated plasma. GST-VWF73, a substrate that can be specifically cleaved by ADAMTS13 in vitro, is immobilized on to wells of a microplate that is pre-coated with an antibody specific to glutathione S-transferase (GST). After washing away unbound GST-VWF73, samples (i.e., clinical specimens, controls, and calibrators) are pipetted into wells and incubated with immobilized GST-VWF73. ADAMTS13 present in the samples cleaves the VWF73 peptide of immobilized GST-VWF73 at specific sites, exposing the neoepitope on VWF73. After washing away the excess sample, a second mouse monoclonal antibody specific to the neoepitope on GST-VWF73 that has been conjugated to the enzyme horseradish peroxidase (HRP) is added to the well. After washing away unbound HRP-conjugated antibody, the chromogenic substrate is added to the well. The HRP enzyme catalyzes a specific reaction with the chromogenic substrate, which produces a colored product that is detected as absorbance measurement (optical density, OD) at 450 nm with a microplate reader. The amount of absorbance (OD) generated is proportional to ADAMTS13 activity in the well. The results for the wells containing calibrators are used to create a reference curve to quantify the ADAMTS13 activity in the sample.

In line with the recommendation of the International Society of Thrombosis and Haemostasis (ISTH) in the Journal of Thrombosis and Haemostasis (2020), the assay results should be interpreted at the ADAMTS13 Activity assay cut-off of 0.1 IU/mL for thrombotic thrombocytopenic purpura (TTP). Technozym ADAMTS13 Activity assay results > 0.1 IU/mL will be TTP negative and results ≤ 0.1 IU/mL will be TTP positive. The ADAMTS13 Activity assay results should be interpreted in conjunction with other clinical and laboratory findings.

V Standards/Guidance Documents Referenced:

CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition

CLSI EP06-A2: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline – Second Edition

CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition

CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

VI Performance Characteristics:

Analytical Performance:

1. Precision/Reproducibility:

Precision studies were conducted according to recommendations in CLSI EP05-A3 using quality controls and nine human plasma sample pools, which were prepared by mixing human plasma from normal donors with clinical samples from patients diagnosed with thrombotic thrombocytopenic purpura (TTP) and deficient in ADAMTS13 activity or heat inactivated plasma.

Within-laboratory precision
To evaluate the within-laboratory precision, each sample was tested for five days with two runs per day and two replicates per run at a single site, using three reagent lots for a total of 30 replicate measurements per sample. The samples tested included levels below, around and above the assay cut-off of 0.1 IU/mL. The quantitative and qualitative results are summarized in the tables below.

Sample	N	Mean (IU/mL)	Repeatability		Between-run		Between-day		Between-lot		Within-laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
S1	30	0.65	0.03	4.46	0.03	5.10	0.02	3.60	0.00	0.00	0.05	7.66
S2	30	0.45	0.01	2.26	0.04	8.50	0.00	0.00	0.01	3.00	0.04	9.26
S3	29	0.24	0.01	3.25	0.01	2.50	0.00	1.00	0.00	1.70	0.01	4.52
S4	29	0.19	0.00	1.84	0.01	4.20	0.00	0.00	0.00	2.10	0.01	4.97
S5	30	0.14	0.00	2.19	0.01	4.90	0.00	0.00	0.00	0.00	0.01	5.17
S6	30	0.08	0.00	2.05	0.01	8.10	0.00	0.00	0.00	0.00	0.01	7.87
S7	30	0.65	0.03	4.88	0.03	3.80	0.02	2.90	0.01	0.90	0.05	6.90
S8	30	0.23	0.01	3.04	0.01	5.40	0.00	0.00	0.00	0.80	0.01	6.24
S9	30	0.12	0.00	2.62	0.01	4.10	0.00	0.00	0.00	0.00	0.01	4.91

Sample	Mean (IU/mL)	Total results	Qualitative agreement	
			Number of correct results	% Correct call
S1 negative	0.65	30	30/30	100
S2 negative	0.45	30	30/30	100
S3 negative	0.24	29	29/29	100
S4 negative	0.19	29	29/29	100
S5 negative	0.14	30	30/30	100

Sample	Mean (IU/mL)	Total results	Qualitative agreement	
			Number of correct results	% Correct call
S6 positive	0.08	30	30/30	100
S7 negative	0.65	30	30/30	100
S8 negative	0.23	30	30/30	100
S9 negative	0.12	30	30/30	100

Operator-to-operator

The study was conducted over five days using one reagent lot with two runs per day and two replicates per run by three operators for a total of 30 mean results per sample level. The study design included six samples prepared by mixing plasma from normal human donors with native deficient plasma (TTP patient plasma) in different ratios. In addition, three sample levels were prepared by mixing plasma from normal human donors with heat inactivated plasma. The samples tested included levels below, around and above the assay cut-off of 0.1 IU/mL.

Sample	N	Mean (IU/mL)	Repeatability		Between-run		Between-day		Between-operator		Within-laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
S1	30	0.67	0.03	4.17	0.03	5.10	0.03	4.80	0.01	1.90	0.06	8.27
S2	30	0.45	0.01	2.26	0.04	9.70	0.00	0.00	0.01	1.30	0.05	10.08
S3	29	0.24	0.01	2.90	0.01	4.30	0.00	0.00	0.01	2.30	0.01	5.59
S4	29	0.19	0.00	2.19	0.01	5.40	0.00	0.00	0.00	2.20	0.01	6.10
S5	30	0.13	0.00	1.77	0.01	3.60	0.01	3.40	0.00	2.50	0.01	5.99
S6	30	0.07	0.00	2.69	0.00	6.00	0.00	0.00	0.00	4.10	0.01	8.12
S7	30	0.65	0.03	4.81	0.03	4.80	0.00	0.00	0.01	1.80	0.05	7.11
S8	30	0.23	0.01	2.26	0.01	4.90	0.00	1.70	0.00	1.60	0.01	5.84
S9	30	0.12	0.00	2.05	0.01	5.20	0.00	1.20	0.00	2.40	0.01	6.18

Sample	Mean (IU/mL)	Total results	Qualitative agreement	
			Number of correct results	% Correct call
S1 negative	0.67	30	30/30	100
S2 negative	0.45	30	30/30	100
S3 negative	0.24	29	29/29	100
S4 negative	0.19	29	29/29	100

Sample	Mean (IU/mL)	Total results	Qualitative agreement	
			Number of correct results	% Correct call
S5 negative	0.13	30	30/30	100
S6 positive	0.07	30	30/30	100
S7 negative	0.65	30	30/30	100
S8 negative	0.23	30	30/30	100
S9 negative	0.12	30	30/30	100

Site-to-site reproducibility

The study was performed at three study sites. At each site, the samples were assayed on each of five days, with two runs per day and two replicates per run, using one lot of reagents, resulting in a total of 30 mean results per sample level. The study design included six sample levels prepared by mixing plasma from normal human donors with native deficient plasma (TTP patient plasma) in different ratios. In addition, three sample levels were prepared by mixing plasma from normal human donors with heat inactivated plasma. To prepare heat inactivated plasma with no residual ADAMTS13 activity, citrated plasma samples from normal donors were heat-inactivated for 1 hour at 56°C. The samples tested included levels below, around and above the cut-off of 0.1 IU/mL.

Sample	N	Mean (IU/mL)	Repeatability		Between-run		Between-day		Between-site		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
S1	30	0.67	0.03	4.81	0.04	6.20	0.05	6.60	0.00	0.00	0.07	10.24
S2	30	0.46	0.02	4.67	0.04	8.40	0.00	0.00	0.01	2.20	0.05	9.86
S3	29	0.25	0.01	3.75	0.02	6.60	0.00	0.00	0.01	4.80	0.02	8.99
S4	29	0.19	0.01	4.46	0.02	7.40	0.00	0.00	0.00	0.00	0.02	8.64
S5	30	0.14	0.01	4.17	0.01	9.00	0.00	0.00	0.00	0.00	0.01	9.88
S6	30	0.07	0.00	4.53	0.01	6.30	0.00	0.00	0.01	7.00	0.01	10.45
S7	30	0.66	0.03	4.46	0.04	5.70	0.01	2.00	0.03	3.90	0.06	8.49
S8	30	0.23	0.01	4.31	0.01	5.30	0.00	0.00	0.00	0.00	0.02	6.85
S9	30	0.12	0.00	2.97	0.01	6.40	0.00	0.00	0.00	3.60	0.01	8.01

Sample	Mean (IU/mL)	Total results	Qualitative agreement	
			Number of correct results	% Correct call
S1 negative	0.67	30	30/30	100
S2 negative	0.46	30	30/30	100

Sample	Mean (IU/mL)	Total results	Qualitative agreement	
			Number of correct results	% Correct call
S3 negative	0.25	29	29/29	100
S4 negative	0.19	29	29/29	100
S5 negative	0.14	30	30/30	100
S6 positive	0.07	30	30/30	100
S7 negative	0.66	30	30/30	100
S8 negative	0.23	30	30/30	100
S9 negative	0.12	30	30/30	100

2. Analytical Specificity/Interference:

Interference studies were conducted based on the CLSI EP07 3rd Edition guideline. Three base pools mimicking high (1.0 IU/mL), medium (0.5 IU/mL) and low (0.1 IU/mL) levels of ADAMTS13 activity were prepared by mixing human citrated plasma (non-icteric, non-turbid and non-hemolyzed) with plasma rendered ADAMTS13 deficient by heat inactivation. Interference testing was conducted by paired-difference testing using one lot of reagents for both common endogenous and extrinsic interferents. Each sample was tested in five replicates. Samples with and without the interferent were measured, and the measurand concentration difference was determined.

None of the substances in the following table were found to lead to clinically significant interference.

Potential interfering substance	No interference up to the following evaluated clinically significant concentration:
Exogenous	
Acetaminophen	15.6 mg/dL
Acetylcysteine	15.0 mg/dL
Ampicillin Na	7.5 mg/dL
ASA	3.0 mg/dL
Biotin	0.351 mg/dL
Caplacizumab	0.15 mg/dL
Cefoxitin Na	660.0 mg/dL
Cyclosporine	0.18 mg/dL
Doxycycline	1.8 mg/dL
Heparin	330 units/dL
Ibuprofen	21.9 mg/dL
Levodopa	0.75 mg/dL
Methyldopa	2.25 mg/dL

Potential interfering substance	No interference up to the following evaluated clinically significant concentration:
Exogenous	
Metronidazole	12.3 mg/dL
Phenylbutazone	32.0 mg/dL
Prednisolone	0.12 mg/dL
Rifampicin	4.8 mg/dL
Rituximab	50.0 mg/dL
Theophylline	6.0 mg/dL
Endogenous	
Intralipid	500 mg/dL
Hemoglobin	220 mg/dL
Unconjugated Bilirubin	66.0 mg/dL
Conjugated Bilirubin	66.0 mg/dL
GST	0.02 mg/dL
VWF	2.0 IU/mL
Human anti mouse antibody	titer >12
Rheumatoid factor	156 IU/mL

3. Assay Reportable Range:

   Not applicable

4. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Traceability
   Target values for calibrators and controls are traceable to the first International Standard for ADAMTS13 Activity and Antigen in Plasma (NIBSC WHO 1st international Standard ADAMTS13 Plasma 12/252).

   Stability of calibrators and controls
   Stability of calibrators and controls were evaluated in accordance with CLSI EP25A. Three lots of calibrators and controls were used in the study and stored in their final packaging at 2–8°C. At time points 0, 12, 24 and 30 months, sets of calibrators and controls were placed into stable storage (-70°C). At the end of the study (t=30 months), all calibrators and controls were tested in triplicate in one single run on one instrument using one lot of reagents. The data supported a real-time stability of 24 months.

5. Assay Cut-Off:

   Not applicable

B Comparison Studies:

1. Clinical Performance Study:

The clinical performance study was conducted at two external sites, one located in U.S. and the other located outside of the U.S. Testing was performed double blinded. The clinician making the diagnosis decisions and selecting the samples was blinded to the Technozym ADAMTS13 activity results and the laboratory technician conducting the Technozym assay was blinded to the diagnosis. Samples were tested in duplicate using the Technozym ADAMTS13 Activity assay. One kit lot was used per study site. At each study site, tests were performed by one laboratory professional. The study samples used in testing were residual samples selected from a local repository of frozen human citrated plasma from patients diagnosed with thrombotic microangiopathies (TMA) (i.e., clinical suspicion of thrombotic thrombocytopenic purpura (TTP)) by board-certified clinician according to the local testing algorithm for TMAs. All patient samples were from donors > 6 months of age and patient population is representative of intended use population.

Combined agreement analysis for both sites with a total of 137 samples included in the clinical performance study.

		Clinical diagnosis of TTP		
		Positive	Negative	Total
Technozym ADAMTS13 Activity	Positive	28	3	31
	Negative	5	101	106
	Total	33	104	137
Sensitivity = 84.8% (28/33); 95% CI: (69.1% to 93.3%) Specificity = 97.1% (101/104); 95% CI: (91.9% to 99%) Positive Predictive Value (PPV) = 90.2% (28/31); 95% CI: (75.2% to 96.6%) Negative Predictive Value (NPV) = 95.3% (101/106); 95% CI: (90.0% to 97.8%)				

C Clinical Studies:

1. Clinical Sensitivity:

Refer to Clinical Performance Study

2. Clinical Specificity:

Refer to Clinical Performance Study

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable

D Clinical Cut-Off:

The clinical cut-off for TTP diagnosis is 10% or 0.1 IU/mL ADAMTS13 activity.

E Other Supportive Performance Characteristics Data:

1. Prozone Effect (Hook Effect)

   Information was provided to support that no significant hook effect was observed up to activity levels of 8 IU/mL.

2. Cross-contamination Studies

   A study was performed to evaluate if cross-contamination and/or carryover occurs between samples in the plate wells during the assay procedure. Low samples with a target concentration of 0.1 IU/mL and high samples with a target concentration of 1.0 IU/mL were used to perform the studies. In the first stage, the signal of only low samples was evaluated throughout the microplate. In the second stage, two test plates were run with an alternating pattern over all available patient sample locations. The pattern consisted of two wells containing only the low samples followed by two wells containing the high sample. The study was performed by three operators performing testing with three microplate readers and plate washer combinations with one lot of reagents. No cross-contamination was observed.

3. Reagent Stability Studies:

   Real-time Shelf-life Stability Studies
   The real-time stability study was conducted in accordance with CLSI guideline EP25-A. The study was conducted with three lots of Technozym ADAMTS13 Activity assay kits.
   Eight samples were prepared by mixing citrated human plasma in human ADAMTS13 activity deficient plasma (HIP) in different ratios. These samples were aliquoted and frozen at -20°C and a fresh aliquot was used for every test time point. Reagent kits were stored in their final packaging at 2–8°C. Time points used in the real time stability study included: 0, 6, 12, 24 and 30 months. Reagent kits were retrieved and tested with different ADAMTS13 activity sample levels at the end of each designated time point in the study. The testing was done in duplicates for each ADAMTS13 activity level. Based on the real-time stability results, the data supports a shelf-life of the Technozym ADAMTS13 Activity assay kit for up to 24 months at 2–8°C.

4. Sample Stability Studies

   Frozen sample stability
   Eight samples were prepared by mixing citrated human plasma with heat treated citrated human plasma in different ratios, and aliquots was stored frozen at < -20°C. At each test time point, a randomly selected set of aliquots was thawed at 37°C using a water bath and testing was performed. Samples were tested in duplicates in the Technozym ADAMTS13 Activity assay within one run. Testing was performed at the time points 0 (stored at -20°C for minimum of 5 days before testing), 6, 12, 18 and 24 months. One reagent lot was used

throughout the study and testing was performed on one instrument. The study supports frozen sample stability of 12 months.

Fresh Sample stability
Six samples were prepared by mixing freshly drawn citrated human plasma with native TTP plasma (no ADAMTS13 activity) and aliquoted for testing at room temperature (18–25°C) and under refrigerated conditions (2–8°C). For samples stored at room temperature, testing was performed at time points 0, 4, 8, 9, 24 and 25 hours. For samples stored under refrigerated conditions, testing was performed at time points 0, 24, 25, 48 and 49 hours. All samples were tested in duplicates using three different reagent lots. The study supports a sample stability for up to 8 hours at room temperature (18–25°C) and up to 24 hours under refrigerated conditions.

VII Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

VIII Identified Risks and Mitigations:

Risks to Health	Mitigation Measures
Clinical action based on false positive results may lead to inappropriate patient management, or unnecessary treatments.	Certain design verification and validation activities and documentation, including certain studies. Certain labeling information, including certain limiting statements and performance characteristics.
Clinical action based on false negative results may lead to delayed diagnosis, misdiagnosis, or discontinuation of treatment.	Certain design verification and validation activities and documentation, including certain studies. Certain labeling information, including certain limiting statements and performance characteristics.

IX Benefit/Risk Assessment:

A Summary of the Assessment of Benefit:

There is currently no FDA market-authorized device for determining ADAMTS13 activity. Patients with thrombotic thrombocytopenic purpura (TTP) typically present with thrombocytopenia, microangiopathic hemolytic anemia (e.g., low hemoglobin, low hematocrit, low haptoglobin, elevated LDH, presence of schistocytes in peripheral blood smear) and various degrees of organ damage. These changes, however, are non-specific for TTP and can also occur in many of the differential diagnoses. TTP is caused by ADAMTS13 deficiency. The availability of the test may aid in the differential diagnosis of thrombotic thrombocytopenic purpura (TTP) from other thrombotic microangiopathies (TMA) as described in the Journal of Thrombosis and

Haemostasis (2020) "ISTH guidelines for the diagnosis of thrombotic thrombocytopenic purpura" (e.g., "although diagnosis of TTP relies on a high index of suspicion, based on clinical presentation and laboratory results, the panel recognized that the importance of having an ADAMTS13 activity test in the diagnosis and initial management process").

B Summary of the Assessment of Risk:

When used as intended, the risks of the device are mainly related to false positive or false negative test results. For a false positive test result, the risk could include unnecessary further testing or inappropriate patient management, including cessation of investigation for other diseases, resulting in missed opportunities to properly treat the patient. Additionally, a false positive test may lead to unnecessary treatments with side effects such as bleeding, fatigue, pyrexia, headache, paresthesia, urticaria, fatal infusion reactions, tumor lysis syndrome, severe mucocutaneous reaction and progressive multifocal leukoencephalopathy. Risks of a false negative test include a missed or delayed diagnosis, improper patient management including continuation of investigating the etiology of a patient's symptoms, which usually consists of further history, physical examination, and testing. The additional risk associated with a false negative test is related to the inappropriate discontinuation of treatment which can lead to missed opportunities for the timely treatment of TTP positive patients. Such treatment has been associated with faster normalization of platelet count, lower incidence of TTP-related death, lower rate of recurrence of TTP and lower incidence of thromboembolic event than placebo in clinical trials.

C Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

D Summary of the Assessment of Benefit-Risk:

Device design verification and validation, including precision, method comparison, and interference studies will help ensure that the device functions as intended and mitigate the risk of false positive or false negative test results. A limitation statement conveying that results from the assay alone should not be used in making treatment decisions will be included in the labeling, as an additional mitigation against the risk of false positive and false negative results. Overall, while general controls are insufficient to mitigate the risks of the device, in light of the special controls, the probable benefits outweigh the probable risks of incorrect test results for the proposed indications for use.

X Conclusion:

The De Novo request is granted, and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code(s): SAC
Device Type: ADAMTS13 activity test system
Class: II
Regulation: 21 CFR 864.7297