EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
ConcizuTrace ELISA
DECISION SUMMARY

I Background Information:

A De Novo Number

DEN240035

B Applicant

Randox Laboratories Ltd.

C Proprietary and Established Names

ConcizuTrace ELISA

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
SES	Class II	21 CFR 864.7298 Non-factor replacement product test system	Hematology

II Submission/Device Overview:

A Purpose for Submission:

De Novo request for evaluation of automatic class III designation for ConcizuTrace ELISA

B Measurand:

Concizumab-mtci (ng/mL)

C Type of Test:

Enzyme-linked immunosorbent assay (ELISA)

III Indications for Use:

A Indication(s) for Use:

For in vitro diagnostic use.

The ConcizuTrace ELISA (enzyme linked immunosorbent assay) is intended for the quantitative measurement of concizumab concentration in human 3.2% citrated plasma samples from Hemophilia A and B patients after 4 weeks from the initiation of treatment with concizumab. The measurement of concizumab concentration is used for dose adjustment decision in accordance with the drug label.

The ConcizuTrace ELISA is a manual assay performed by qualified healthcare professionals on the microplate reader qualified by Randox.

For prescription use only.

B Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

C Special Instrument Requirements:

Molecular Devices SpectraMax ABS Microplate Reader and SoftMax Pro GxP version 7.2 (qualified by Randox)

IV Device/System Characteristics:

A Device Description:

The ConcizuTrace ELISA is based on a standard sandwich enzyme-linked immunosorbent assay (ELISA) method in a classic 96-well plate format used for detection of concizumab in human plasma.

The assay contains the following components:

- Microtiter Plate – a 96-well plate coated with Tissue Factor Pathway Inhibitor (TFPI)
- ELISA Diluent/Wash Buffer Concentrate – buffer contains detergent and preservatives
- Assay Buffer – buffer containing detergent, blockers, and preservatives
- Acidification/ Neutralization Plate – blank microtiter plate
- Acetic Acid – 12% acetic acid
- Neutralizing Solution – buffer containing detergent, blockers, and preservatives
- Conjugate – labeled with horse radish peroxide (HRP) in stabilizing solution
- TMB Substrate – tetramethylbenzidine substrate supplied ready to use
- ELISA Stop Solution – acidic solution
- ConcizuTrace Calibrators – eight vials containing lyophilized plasma each with a different concizumab concentration
- ConcizuTrace QC – three vials containing lyophilized plasma (low, medium or high concizumab concentration)

Materials required but not provided
The following are required to run the ConcizuTrace ELISA assay but not provided in the kit:

- Microplate Reader: SpectraMax ABS from Molecular Devices, to measure absorbance at 450 nm with subtraction of 620 nm
- Microplate Reader Software: SoftMax Pro GxP version 7.2 to control the Microplate Reader and to perform non-linear regression using 5 parameter curve fitting with 1/Y2 weighting.
- Automated Plate Washer
- Plate Shaker
- Pipettes
- Plate Seals, and
- Centrifuge

B Principle of Operation

Plasma samples are pre-treated with acetic acid followed by addition of a TRIS buffered solution to the acidified samples. After sample pre-treatment, concizumab present in sample is captured on a human Tissue Factor Pathway Inhibitor (hTFPI) coated microtiter plate. Mouse anti-human IgG4 HRP conjugate is used for detection. A substrate solution is added, and color develops in proportion to the amount of concizumab bound in the initial step. The absorbance is measured at 450 nm (reference wavelength at 620 nm is subtracted) using a plate reader. The color intensity is proportional to the concizumab concentration in the sample.

V Standards/Guidance Documents Referenced:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI EP06, Evaluation of the Linearity of Quantitative Measurement Procedures; Approved Guideline – Second Edition
- CLSI EP07, Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition
- CLSI EP25-A2, Evaluations of Stability of In Vitro Diagnostic Reagents; Approved Guideline – Second Edition
- CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition
- IEC 60601-1-2:2014; 4th Edition: Medical electrical equipment – Part 1-2: General requirements for basic safety and essential performance – Collateral Standard: Electromagnetic disturbances – Requirements and tests
- IEC 61010-1 Edition 3.0, Standard for Safety for Electrical Equipment For Measurement, Control and Laboratory Use; Part 1: General Requirements
- IEC 61010-2-101: 2015, EN 61010-2-101:2017, Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment

- IEC 61010-1:2010/AMD1:2016, EN 61010-1:2010/A1:2019, Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements

VI Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

Repeatability
The repeatability study was conducted in accordance with CLSI EP05-A3 using six surrogate samples, which were prepared from pooled plasma spiked with concizumab. Each sample was assessed in 20 replicates using a single lot of reagents on a single instrument system by a single operator. A replicate is the mean of the measurements from two duplicate wells on the plate. Analysis was conducted in accordance with the CLSI document EP05-A3. The results are summarized below.

Sample Description	Mean Value (ng/mL)	N	Repeatability	
			SD	%CV
PM1	223.60	20	18.54	8.29
PM2	338.91	20	17.98	5.31
PM3	2144.26	20	122.03	5.69
PM4	3060.36	20	190.86	6.24
PM5	4808.61	20	274.98	5.72
PM6	5091.00	20	69.30	7.25

Within-Site Precision
The within-site precision study was conducted according to recommendations in CLSI EP05-A3 using six surrogate samples, which were prepared from pooled plasma spiked with concizumab. Each sample was assessed in replicates of five per run, two runs per day, for 20 days. A replicate measurement was defined as an average of two replicates of the same sample on the same plate. A single lot of critical reagents was used in the study, and the study was run on a single instrument system by a single operator. The results are summarized below.

Sample Description	N	Mean (ng/mL)	Repeatability		Between-Run		Between-Day		Within-laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
PM1	200	193.63	11.49	5.9%	14.36	7.4%	8.93	4.6%	20.45	10.6%
PM2	200	307.49	30.68	10.0%	13.03	4.2%	10.78	3.5%	35.04	11.4%
PM3	200	2091.76	102.27	4.9%	78.34	3.7%	132.67	6.3%	184.93	8.8%
PM4	200	2850.08	119.41	4.2%	157.15	5.5%	164.21	5.8%	256.75	9.0%
PM5	200	4477.38	297.79	6.7%	261.83	5.8%	355.85	7.9%	532.79	11.9%
PM6	200	5050.73	465.70	9.2%	271.27	5.4%	601.92	11.9%	807.94	16.0%

Lot-to-Lot Precision

The lot-to-lot precision study was conducted according to recommendations in CLSI EP05-A3 using six surrogate samples, which were prepared from pooled plasma spiked with concizumab, and clinical samples from patients taking concizumab. Each sample was assessed in replicates of four per run, one run per day, for five non-consecutive days, and using three reagent lots. A replicate was defined as the mean of the measurements from two duplicate wells on the plate. The study was run on a single instrument by a single operator. The results are summarized below.

Sample	N	Mean (ng/mL)	Repeatability		Between-Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
PM1	60	181.29	10.09	5.57	1.18	0.65	19.77	10.91	22.23	12.26
A2 Pool	60	210.96	15.31	7.26	0.00	0.00	19.87	9.42	25.09	11.89
PM2	60	270.98	23.04	8.50	10.97	4.05	23.45	8.65	34.65	12.79
PM3	60	2112.89	134.91	6.39	0.00	0.00	137.62	6.51	192.72	9.12
PM4	60	3194.58	123.74	3.87	0.00	0.00	386.15	12.09	405.49	12.69
PM5	60	4173.76	397.93	9.53	0.00	0.00	526.63	12.62	660.07	15.81
PM6	60	4407.67	207.46	4.71	0.00	0.00	614.67	13.95	648.74	14.72

Operator-to-Operator Precision

The operator-to-operator precision study was conducted according to recommendations in CLSI EP05-A3 using six surrogate samples, which were prepared from pooled plasma spiked with concizumab, and clinical samples from patients taking concizumab. Each sample was assessed in replicates of five per run, one run per day, for five non-consecutive days using one reagent lots. The study was run on two instruments by two operators. The results are summarized below.

Sample Description	N	Mean Value (ng/mL)	Between-Operator/Instrument	
			SD	%CV
PM1	50	153.21	7.52	4.91
PM2	50	223.40	15.17	6.79
PM3	50	1983.91	175.28	8.84
PM4	50	3229.88	435.53	13.48
A2 Pool	50	3500.96	438.95	12.54
PM5	50	4444.87	505.36	11.37

2. Linearity:

A study was conducted to determine the linear range for the ConcizuTrace ELISA according to the recommendations in the CLSI EP06 (Second Edition) guideline. Pooled clinical samples with 13 different concizumab concentrations were evaluated. Each sample was tested in five replicates using two lots of reagents and one instrument. Imprecision at each sample concentration was examined through determination of SD, %CV, and variances.

Linearity analysis was based on expected values at each sample level. The linear range for the ConcizuTrace ELISA was determined to be 122.63 – 4224.93 ng/mL.

3. Analytical Specificity/Interference:

A study was conducted to determine the impact of endogenous and exogenous interferents on the ConcizuTrace ELISA according to the recommendations in the CLSI EP07-A3 guideline. Six surrogate samples, which were prepared from pooled plasma spiked with concizumab, and clinical samples from patients taking concizumab were included in this study. Five (5) replicates of each sample were tested at each concentration of each substance as recommended in Table 1 of *CLSI EP37-Ed. 1 Supplemental Tables for Interference Testing in Clinical Chemistry*. For concomitant medications not listed in CLSI EP37, levels based on the reported Cmax values (3X Cmax as highest concentration) were tested. The following substances were not found to interfere with the ConcizuTrace ELISA results at the indicated concentrations.

Table 4: Endogenous Interferents

Endogenous Interferents	**No interference up to the following concentration**
Free Bilirubin (Unconjugated)	40 mg/dL
Conjugated Bilirubin	40 mg/dL
Hemoglobin	1000 mg/dL
Biotin	19.7 mg/dL
HAMA IgG Type 1	1000 ng/mL
HAMA IgG Type II	1000 ng/mL
RF IgM	1350 IU/mL
Ascorbic Acid	5.25 mg/dL
Triglycerides (Intralipid 20% Emulsion)	1500 mg/dL
Cholesterol	400 mg/dL
Recombinant TFPI	500 ng/mL
Uric Acid	23.5 mg/dL

Table 5: Exogenous Interferents

Exogenous Interferents	**No interference up to the following concentration**
Acetaminophen (paracetamol)	15.6 mg/dL
Ibuprofen	21.9 mg/dL
Naproxen	36.0 mg/dL
Angiotensin-converting enzyme (ACE) inhibitor (Lisinopril)	0.0246 mg/dL
Proton-pump inhibitor (omeprazole)	0.84 mg/dL
Statin/HMG-CoA Reductase inhibitor (atorvastatin)	0.075 mg/dL
Antihistamine (fexofenadine)	0.116 mg/dL
Integrase inhibitor (raltegravir)	1.5 mg/dL
Protease inhibitor (atazanavir)	1.95 mg/dL
Non-nucleoside reverse transcriptase inhibitors (doravirine)	2.886 µg/mL
Reverse transcriptase inhibitor (tenofovir)	1.14 mcg/mL
Excess sodium citrate	0.64%

Exogenous Interferents	No interference up to the following concentration
Ribavirin	11.046 µg/mL
Sofosbuvir	0.1854 mg/dL
Ledipasvir	0.0969 mg/dL
Dasabuvir	2 µg/mL
Velpatasvir	777 ng/mL
Elbasvir	362 ng/mL
Grazoprevir	495 ng/mL
N pegylated Factor IX (N9-GP SRM	561 IU/dL
Recombinant Factor VII (rFVII SRM)	504 IU/mL
Recombinant Factor VIII (N8 SRM)	609 IU/dL
N pegylated Factor VIII (Turoctocog Alfa Pegol, Esperoct)	594 IU/dL
Recombinant Factor IX (rFIX SRM)	480 IU/dL
Entecavir	24.6 ng/mL
Human Plasma Derived Factor VIII (Haemoctin 1000)	3 IU/mL
Human Plasma Derived Factor IX	1.5 IU/mL

4. Assay Reportable Range:

   122.63 – 4224.93 ng/mL

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

   Traceability

   This study is a lot-specific traceability study. Calibrator standardization/assignment was carried out across three calibrator lots. The master calibrator lot was prepared from pooled plasma spiked with concizumab and standardized via against the concizumab reference material. Subsequent batches of calibrators were assigned using the master calibrator set as reference. Each lot was assigned by two operators in one experimental run on one instrument (plate reader and plate washer). The result demonstrated that the acceptance criteria and design inputs were met as all assigned calibrator lots encompassed the target assay range.

   Reagent Stability

   Reagent stability studies were performed to establish real-time shelf-life stability and in-use stability for critical reagents when used with the ConcizuTrace ELISA kit. Reagent stability studies were conducted as recommended in *CLSI EP25-A Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*, evaluating the performance of multiple lots of each critical reagent using surrogate samples prepared from pooled plasma spiked with concizumab, and clinical samples from patients taking concizumab. The result demonstrates that the ConcizuTrace ELISA kit is stable for 12 months when stored under refrigeration (2–8ºC); the in-use critical reagents (except plate, calibrators, and QCs) in the ConcizuTrace ELISA kit is stable for 28 days when stored at 2–8ºC; the in-use TFPI Coated Microtiter Strippable Plates (MTP) in the ConcizuTrace ELISA kit is stable for 28 days when stored at 2ºC to 8ºC; the in-use calibrators and quality controls in the ConcizuTrace ELISA kit is stable for 21 days under frozen (stored at -18 to -24°C) or refrigerated (2–8°C); and the

transportation stability for ConcizuTrace ELISA kit (packaged with ice packs) is 72 hours under shipping conditions.

Table 6: Reagent Stability

Reagent	Storage Condition	Duration of Stability
ConcizuTrace ELISA kit (Shelf life)	Refrigeration (2–8ºC)	12 months
ConcizuTrace ELISA kit (In-Use)	Refrigeration (2–8ºC)	28 days
TFPI microplate (In-Use)	Refrigeration (2–8ºC)	28 days
Calibrators and Quality Control (In-Use)	Frozen (-18 to -24°C) or refrigerated (2–8°C)	21 days
ConcizuTrace ELISA kit (Transportation)	Shipping (packaged with ice packs)	72 hours

Sample Stability

A study was conducted to evaluate sample stability under various storage conditions. Surrogate samples, which were prepared from pooled plasma spiked with concizumab, and clinical samples from patients taking concizumab were included in this study. Each sample was tested in five replicates at each condition using a single reagent lot on one instrument by one operator at each storage condition. Results of the study are summarized below.

Storage Condition	Duration of stability
Ambient temperature (15° to 30°C)	24 hours
Frozen (-18 to -24°C)	60 days
Frozen (-75 to -90°C)	60 days
Freeze/Thaw	Up to 6 cycles

Sample Transportation Stability

The transportation stability study was conducted to assess the impact of environmental variability throughout shipment of sample specimen on the performance of ConcizuTrace ELISA. This study assessed both real-time (i.e. air freight) and simulated transportation conditions (sequence of changes in temperature: 3 days at 35 – 39°C to -18°C – -24°C, and 3 days at 2 to 8°C, repeated to duration of 12 days). Surrogate samples, which were prepared from pooled plasma spiked with concizumab, and clinical samples from patients taking concizumab were included in this study. At each condition, samples were tested in five replicates using one reagent lot on one instrument by one operator. The result demonstrates that the specimen is stable up to 18 days during -80°C frozen shipment on dry ice.

6. Detection Limit:

Detection limits were evaluated in accordance with the CLSI EP17-A2 guideline.

The Limit of Blank (LoB) was evaluated using four plasma samples containing no detectable levels of concizumab. Samples were tested in five replicates, using three reagent lots and one instrument, for three days (n=60 replicates per reagent lot). The LoB for the ConcizuTrace ELISA was determined to be 26.71 ng/mL.

The Limit of Detection (LoD) was evaluated using four plasma samples with concizumab concentration between the established LoB and 5 x times the established LoB. Samples were tested in five replicates, using three reagent lots and one instrument, for three days (n=60 replicates per reagent lot). The LoD for the ConcizuTrace ELISA was determined to be 62.44 ng/mL.

The Limit of Quantitation (LoQ) was evaluated using four plasma samples with a concizumab concentration above the established LoD. Samples were tested in three replicates, using three reagent lots and one instrument, for three days (n=36 replicates per reagent lot). The LoQ for the ConcizuTrace ELISA was determined to be 99.72 ng/mL.

7. Assay Cut-Off:

Not applicable

8. Accuracy:

Dilution Recovery

This study was conducted in accordance with CLSI EP34. Recovery was assessed by analyzing ten (10) samples spanning the measuring range of the assay. Surrogate B2 samples (3.2% sodium citrate plasma pool spiked with concizumab reference material) were included in the dilution recovery study. Recovery samples were prepared by spiking 3.2% sodium citrate plasma pool with concizumab reference material and diluted 20-fold. These samples were analyzed in 5 replicates across one analytical run per operator across two operators and two reagent lots.

ConcizuTrace™ ELISA meets design input requirements for recovery of 85–115%.

9. Carry-Over:

The study was conducted to evaluate the susceptibility of the ConcizuTrace ELISA to within-assay sample carryover. This study was assessed using two samples with different concizumab concentrations (low-level sample at 160 ng/mL and high-level sample at 50,000 ng/mL). These two samples were arranged in an alternating pattern to assess for potential well-to-well cross-contamination from high measurand samples to low measurand samples. All samples met the acceptance criteria, demonstrating that the ConcizuTrace ELISA is not susceptible to within-assay plate carryover.

B Comparison Studies:

1. Bridging Study:

A bridging study was conducted to assess the concordance between the clinical trial assay used in the explorer7 (phase 3) clinical trial (hereafter referred to as the comparator assay), and the ConcizuTrace ELISA. A total of 146 samples were included in the study. Samples

were analyzed in one replicate (two wells) on the ConcizuTrace ELISA and two replicates (two wells per replicate, mean measurement was taken) for the comparator assay. Passing-Bablok regression, regression coefficients and their 95% confidence intervals, in addition to an agreement analysis with an evaluation of assay performance at the relevant medical decision levels, are summarized below.

N	Result Range (ng/mL)	Correlation Coefficient (r)	Intercept (95% CI)	Slope (95% CI)
86*	125–4065.8	0.973	-4.76 (-23.1, 13.59)	1.04 (0.98, 1.09)

** 86 samples are within the AMR which can be included in the regression analysis.*

		Comparator assay			Total
		>4000 ng/mL	200–4000 ng/mL	<200 ng/mL	
ConcizuTrace ELISA	>4000 ng/ml	32	1	0	33
	200-4000 ng/ml	2	56	6	64
	<200 ng/ml	0	2	47	49
	Total	34	59	53	146
Cutoff at 200 ng/mL	PPA (95% CI)	97.8% (92.5%, 99.4%)			
	NPA (95% CI)	88.7% (77.4%, 94.7%)			
Cutoff at 4000 ng/mL	PPA (95% CI)	94.1% (80.9%, 98.4%)			
	NPA (95% CI)	94.6% (88.8%, 97.5%)			

2. Matrix Comparison:

Not applicable.

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable.

2. Clinical Specificity:

Not applicable.

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

The efficacy of Alhemo in patients with hemophilia A and B with inhibitors (HAwI and HBwI) was evaluated in the explorer7 trial (NCT04083781), a multi-national, multi-center, open-label, phase 3 trial that investigated the safety and efficacy of Alhemo for routine prophylaxis in 91adults (58 HAwI and 33 HBwI) and 42 adolescents (22 HAwI and 20 HBwI) male patients with hemophilia A or B with inhibitors who have been prescribed, or

are in need of, treatment with bypassing agents. Eptacog alfa was the rFVIIa used in explorer7. Patients were excluded if they had a history, current signs or symptoms, or at high risk of thromboembolic events, ongoing or planned immune tolerance induction treatment, in addition to patients with planned major surgery.

Among the 133 patients treated with Alhemo in the trial the mean age was 29 years (range: 12 to 79); 42 patients were 12 to <18 years of age, 89 patients were 18 to 64 years of age, and 2 patients were ≥65 years of age, and all were male. Seventy-eight (78) patients were White, 37 patients were Asian, 9 patients were Black or African American, 6 patients had race information unreported, and 3 patients were American Indian or Alaska Native; 6 patients identified as Hispanic or Latino, 122 patients identified as not Hispanic or Latino and 5 patients had ethnicity information unreported.

No analyses were performed for sex-, gender-, age-, race-, ethnicity-, or any other relevant characteristic- specific subgroups.

The trial was comprised of 4 arms, two randomized arms and two non-randomized arms:

- Arms 1 and 2: 52 patients (27 HAwI, and 25 HBwI), previously treated on-demand, were randomized 1:2 to no prophylaxis (arm 1: on demand treatment with bypassing agents) or Alhemo prophylaxis (arm 2), with stratification by hemophilia type (HAwI, HBwI) and prior 24-week bleeding rate (< 9 or ≥9)
- Arms 3 and 4: 81 additional patients (53 HAwI and 28 HBwI) treated with Alhemo prophylaxis

Treatment with Alhemo included a loading dose of 1 mg/kg on the first day and a once-daily dose of 0.20 mg/kg starting on the second day. The dose was individualized to 0.25 mg/kg or 0.15 mg/kg if Alhemo plasma concentration measured once after 4 weeks of treatment was <200 ng/mL or >4000 ng/mL, respectively. Measurement of concizumab-mtci plasma concentration after 4 weeks was used to optimize the daily maintenance dose. In the trial, a total of 108 patients received their individualized dose, 1 patient on 0.15 mg/kg, 79 patients on 0.20 mg/kg and 28 patients on 0.25 mg/kg.

Efficacy was evaluated in hemophilia A and B patients with inhibitors when all patients in arms 1 and 2 had completed at least 24 or at least 32 weeks, respectively), by comparing the number of treated bleeding episodes between Alhemo prophylaxis (arm 2) and no prophylaxis (arm 1). Using a negative binomial model, a ratio of the annualized bleeding rates (ABR) was estimated to 0.14 ($p<0.001$), corresponding to a reduction in ABR of 86% for subjects on Alhemo prophylaxis compared to no prophylaxis. The estimated mean ABR was 1.7 [95%CI: 1.01; 2.87] for patients on Alhemo prophylaxis (arm 2) and 11.8 [95%CI: 7.03; 19.86] for patients on no prophylaxis (arm 1).

Recommended Dosing Regimen:

- Day 1: Loading dose of 1 mg/kg
- Day 2: Once-daily dose of 0.2 mg/kg until individualization of maintenance dose (see below)
    - 4 weeks after initiation of treatment: For dose optimization measure concizumab-mtci plasma concentration by Concizumab Enzyme-Linked Immunosorbent Assay (ELISA) prior to administration of next scheduled dose.

- Once the concizumab-mtci concentration result is available, individualize the maintenance dose of Alhemo. no later than 8 weeks after initiation of treatment, based on the following concizumab-mtci- plasma concentrations:
  - Less than 200 ng/mL: adjust to a once-daily dose of 0.25 mg/kg
  - 200 to 4,000 ng/mL: continue once-daily dose of 0.2 mg/kg
  - Greater than 4,000 ng/mL: adjust to a once-daily dose of 0.15 mg/kg

  Additional measurements of concizumab-mtci plasma concentration should be taken at routine clinical follow-ups provided the patient has been on the same maintenance dose for 8 weeks of treatment to ensure steady-state plasma concentration. Maintenance of concizumab plasma concentration above 200 ng/mL is important to decrease the risk of bleeding episodes. If concizumab-mtci plasma concentration remains below 200 ng/mL at two consecutive measurements, the benefits of continued Alhemo treatment should be evaluated versus the potential risk of bleeding events, and alternative therapies if available should be considered.

  As Alhemo is dosed by body weight (mg/kg), it is important to recalculate the dose when patients experience body weight changes.

D Clinical Cut-Off:

The cut-off values were evaluated based on phase 2 and initial phase 3 clinical trial data generated using the comparator assay.

Concizumab Concentration (X) at Clinical Cut-Off	**Adjusted Concizumab Dose**
$X < 200$ ng/ml	Dose adjustment to 0.25 mg/kg
$200 \text{ ng/ml} \leq X \leq 4000$ ng/ml	No dose adjustment (dose kept at 0.20 mg/kg)
$X > 4000$ ng/ml	Dose adjustment to 0.15 mg/kg

X = Concentration level in patient sample

E Expected Values/Reference Range:

Not applicable

F Other Supportive Performance Characteristics Data:

<u>High Dose Hook Effect</u>

A high-dose hook effect study was performed to characterize the performance of the ConcizuTrace ELISA when used to test a dilution series of specimens containing very high levels of concizumab that have the potential of a high-dose hook effect to generate false negative results. The study utilized Surrogate B2 samples (3.2% sodium citrate plasma pool from healthy subjects spiked with concizumab reference material). The samples were diluted in six-fold series by using the negative serum as a diluent. Each dilution step was tested in

three replicates using two lots of reagents. The results from this study indicated that there is no-high dose hook effect (up to 400,000 ng/mL) for the ConcizuTrace ELISA.

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
False test results may lead to inappropriate or delayed treatment decisions.	Certain design verification and validation activities and documentation, including certain studies. Certain labeling information, including certain limiting statements and performance characteristics.
Failure of the test system to perform as intended or indicated	Certain design verification and validation activities and documentation, including certain studies. Certain labeling information, including certain limiting statements and performance characteristics.
Failure to correctly interpret test results	Certain design verification and validation activities and documentation, including certain studies. Certain labeling information, including certain limiting statements and performance characteristics.

Pediatric Extrapolation

In this De Novo request, existing clinical data were not extrapolated to support the use of the device in a pediatric patient population. Alhemo is intended to be used in patients 12 years and up and patients 12-21 years of age were studied in the clinical study.

IX Benefit/Risk Assessment:

A Summary of the Assessment of Benefit:

The drug concizumab will be used for routine prophylaxis to prevent or reduce the frequency of bleeding episodes in hemophilia A and B patients. Maintenance dose setting, based on concizumab exposure as measured by ConcizuTrace ELISA, was introduced in the concizumab clinical development program. Concizumab exposure was found to be the most tangible measure for maintenance dose setting to support safety and efficacy of concizumab. The performance of the device has been demonstrated through design verification and validation studies, which include clinical studies.

The studies demonstrate that the ConcizuTrace ELISA can appropriately and reproducibly measure the concentration of concizumab in 3.2% citrated human plasma samples. The

ConcizuTrace ELISA informs dose decision for patients after 4 weeks from the initiation of treatment with concizumab thus reducing the risk for uncontrolled bleeding episodes or thrombotic events. Based on the performance data from the precision and bridging studies, the extent of uncertainty for the benefits is low.

B Summary of the Assessment of Risk:

The risk of ConcizuTrace ELISA assay is associated with discordant results, as a result of false test results, failure of the system to perform as intended, or failure to correctly interpret results. Such incorrect results may lead to inappropriate or delayed treatment decisions. Patients with discordantly high-test results may fail to receive dose escalation, have concizumab dose inappropriately reduced, or fail to switch to alternative therapies. As a result, such patients may have inadequate prophylaxis and are at increased risk of bleeding, which can be serious and life threatening. Patient with discordantly low results may receive incorrect dose escalation or fail to have appropriate dose reduction. With increased exposure to concizumab, there is an increased risk for concizumab associated adverse events (AEs), including thrombosis, hypersensitivity reaction and increase in markers of coagulation activation (D-dimer and prothrombin fragment). The discordant results are sources of uncertainty for the risks. As the ConcizuTrace ELISA assay shows acceptable PPA and NPA comparing to the clinical trial assay, the extent of uncertainty for risks is low.

C Patient Perspectives:

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

D Summary of the Assessment of Benefit-Risk:

The clinical, verification and validation studies have shown evidence of clinical benefits for the device performance. The studies demonstrate that the ConcizuTrace ELISA can appropriately and reproducibly measure the concentration of concizumab in 3.2% citrated human plasma samples. There is probable risk associated with false results, including due to failure of the system to perform as intended or failure to correctly interpret results. The risks of false results are mitigated by the requirement of certain design verification and validation, including certain studies to ensure high analytical accuracy, precision, and specificity performance. Certain labeling information, including limiting statements in the test reports and labeling, serve to reduce the chances of false positives or negatives as a result of incorrect performance of the test. While general controls are not sufficient to mitigate the risks, in light of the special controls, the benefits of the device outweigh the risks.

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): SES
Device Type: Non-factor replacement product test system
Class: II
Regulation: 21 CFR 864.7298