AAV5 DetectCDx™
For In Vitro Diagnostic Use | Rx Only

AAV5 Total Antibody Assay for ROCTAVIAN (valoctocogene roxaparvovec-rvox) Eligibility in Hemophilia A

Proprietary and established product name:

- **Device Trade Name:** AAV5 DetectCDx™
- **Device Generic Name:** AAV5 Total Antibody (TAb) Assay for ROCTAVIAN (valoctocogene roxaparvovec-rvox) Eligibility in Hemophilia A

Intended use:

The AAV5 Total Antibody Assay for ROCTAVIAN (valoctocogene roxaparvovec-rvox) Eligibility in Hemophilia A ("AAV5 TAb Assay") or AAV5 DetectCDx is a qualitative in vitro diagnostic test by electrochemiluminescence intended for detection of antibodies in human plasma collected in 3.2% sodium citrate that bind to the adeno-associated virus serotype 5 (AAV5). The AAV5 TAb Assay is indicated as an aid in the selection of adult hemophilia A patients for whom ROCTAVIAN treatment is being considered. Patients that are anti-AAV5 antibody positive (result of Detected) are not eligible for treatment with ROCTAVIAN; patients that are anti-AAV5 antibody negative (result of Not Detected) are eligible for treatment with ROCTAVIAN. This assay is for professional use and is a single-site assay performed at ARUP Laboratories.

Contraindications:

None

Warnings and precautions:

- When drawing blood for the AAV5 DetectCDx™ assay, universal precautions for bloodborne pathogens should be observed.
- Patients with rheumatoid factor levels greater than 476 IU/mL will interfere with the ability for the AAV5 DetectCDx™ to accurately detect anti-AAV5 antibodies.
- Patient samples with triglyceride levels greater than 500 mg/dL will interfere with the ability of the AAV5 DetectCDx™ to accurately detect anti-AAV5 antibodies.

- Patient samples with Hemoglobin levels greater than 800 mg/dL will interfere with the ability of the AAV5 DetectCDx™ to accurately detect anti-AAV5 antibodies.
- Patient samples collected for the AAV5 DetectCDx™ must not exceed 7.3% sodium citrate as higher concentrations could not be evaluated.
- Cross-reactivity in the AAV5 DetectCDx™ assay to antibodies other than anti-AAV5 antibodies is unknown. A positive assay result can occur due to detection of antibodies other than anti-AAV5 antibodies.
- Since a potential prozone/hook effect was not evaluated for samples with SI > 90 with the AAV5 DetectCDx, it is recommended that if a sample with an SI value > 90 generates a CI value > 1.00 (typically indicative of a "Not Detected" result), that the sample still be considered "Detected."

Summary and explanation of the test:

The AAV5 DetectCDx™ uses a bridging immunoassay to detect antibodies to AAV5 in human sodium citrated (3.2%) plasma specimens. The AAV5 DetectCDx™ uses a combination of concurrently conducted screening and confirmatory steps to reliably detect antibodies specific for AAV5 capsid. Patients evaluated with the AAV5 DetectCDx™ who are anti-AAV5 antibody negative (result of Not Detected) are eligible for treatment with valoctocogene roxaparvovec-rvox (ROCTAVIAN) under the supervision of a physician. Patients evaluated with the AAV5 DetectCDx™ who are anti-AAV5 antibody positive (result of Detected) are not eligible for treatment with ROCTAVIAN.

Valoctocogene roxaparvovec-rvox (ROCTAVIAN), or AAV5-hFVIII-SQ drug product, is a gene therapy treatment for severe hemophilia A, an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. Hemophilia A is caused by deficiency in the activity of coagulation factor VIII (FVIII), an essential cofactor in the intrinsic coagulation cascade. This disorder can be inherited or acquired, leading to insufficient quantities of FVIII or a dysfunctional FVIII.

ROCTAVIAN is an adeno-associated virus serotype 5 (AAV5)-based gene therapy vector that expresses the SQ form of human FVIII (hFVIII-SQ) under the control of a liver-specific promoter. The AAV5 viral capsid mediates binding and uptake into cells, as well as trafficking to the cell nucleus. The vector genome contains a transgene expression cassette inserted between the AAV DNA terminal sequences (referred to as ITRs). After unpackaging of the vector genome in the cell nucleus, recombination between the ITRs generates double-stranded, circular vector genomes that persist mainly as un-integrated episomes. The transgene codes for an active form of FVIII that is used in the coagulation process. ROCTAVIAN is delivered by single intravenous dose and was designed to achieve stable expression of active FVIII in the plasma, synthesized from vector-transduced liver tissue.

Since pre-existing anti-AAV5 antibodies may neutralize ROCTAVIAN, only patients who demonstrate no detectable anti-AAV5 antibodies as determined by the AAV5 DetectCDx will be eligible for treatment with ROCTAVIAN. The presence of neutralizing activity against AAV capsids in non-human primates (NHPs) can inhibit liver transduction and expression of the transgene product (Jiang, 2006, Blood); (Wang, 2011, Hum Gene Ther), while immune-deficient mice reconstituted with purified human immunoglobulins demonstrated a titer-dependent reduction in transgene expression when dosed with AAV vectors (Scallan,

2006, Blood). Diminished efficacy, correlating with the presence of pre-existing immunity, has also been suggested in the clinical setting by treatment of small numbers of hemophilia B patients with an AAV2-vectored Factor IX (FIX) transgene (Manno, 2006, Nature Med).

The AAV5 DetectCDx™ uses a bridging immunoassay to detect antibodies to AAV5 in human sodium citrated (3.2%) plasma specimens. The AAV5 DetectCDx™ uses a combination of concurrently conducted screening and confirmatory steps to reliably detect antibodies specific for AAV5 capsid. The screening step assesses for the presence of anti-AAV5 antibodies, while the confirmatory step determines if the electrochemiluminescence (ECL) signal is specific. In the confirmatory step, samples are pre-incubated with unlabeled capsid (referred to as AAV5 Confirmatory Reagent) to compete for any anti-AAV5 antibodies that are present. If AAV5-binding antibodies are present, they will be bound by the unlabeled AAV5 capsid, resulting in a reduced ECL signal for the confirmatory step as compared to the screening step. The cut points for the screening and confirmatory assays were determined based on the statistical analysis of a set of samples negative for anti-AAV5 antibodies yielding a 5% false positive rate for the screening step and 1% false positive rate for the confirmatory step.

Principles of the test procedure:

The AAV5 DetectCDx™ is to be performed only at ARUP Laboratories, a single laboratory site located at 500 Chipeta Way, Salt Lake City, UT 84108.

A MULTI-ARRAY® 96-well plate is coated with unlabeled AAV5-CMV-GFP capsid, washed, blocked with assay diluent containing casein, and washed again. The patient plasma specimen is diluted and then added in duplicate to specific wells of the plate. If anti-AAV5 antibodies are present in the specimen, they will bind to the unlabeled AAV5-CMV-GFP capsid coating the wells. After incubation with patient specimen, the plate is washed, and SULFO-TAG AAV5-CMV-GFP capsid is added to each well. Anti-AAV5 antibodies bind to SULFO-TAG capsid (also referred to as ruthenylated capsid), which participates in an electrochemiluminescence (ECL) reaction. After incubation and washing, tripropylamine (TPA) substrate is added to each well. The plate is read on a research use only (RUO) ECL-based plate reader. Each well of the plate is electrically stimulated and the resultant ECL signal is measured.

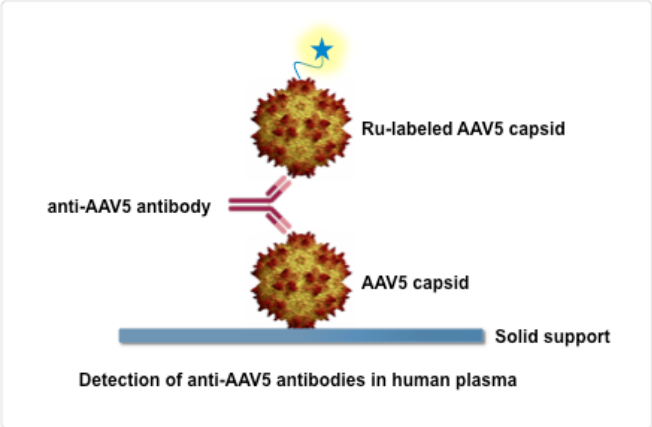

Figure 1. Anti-AAV5 antibody forms a bridge between AAV5 capsid coating the immunoassay plate and ruthenylated AAV5 capsid. The Ru-label participates in the generation of an electrochemiluminescent signal that indicates the presence of anti-AAV5 antibodies.

Each 96-well plate includes a cut point control (CC), negative control (NEG), a low antibody positive control (LPC), and a high antibody positive control (HPC). For run acceptance, the NEG, CC, HPC, and LPC must meet the pre-established criteria for the between-well coefficient of variation (CV) for replicate wells. The HPC and LPC must screen and confirm positive, and the HPC, LPC, and NEG signals must fall within the established acceptance range.

Results for the screening step are expressed as a Screen Index (SI). The SI is calculated by dividing the normalized screening result by the screening cut point. Results for the confirmatory step are expressed as a Confirm Index (CI). The confirm index (CI) is obtained by calculating the ratio of mean signals obtained for the confirmatory and screening assays and dividing this by the confirmatory cut point. The CI is not considered if anti-AAV5 antibodies are not detected in the screening step. Results are based on the values obtained for the SI and CI (see Figure 2):

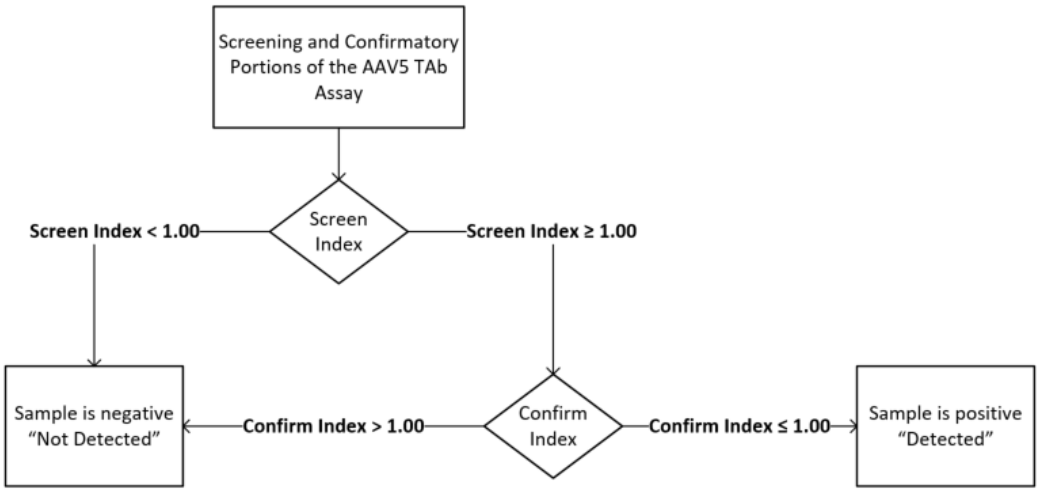

Figure 2. Summary of resulting and reporting for the two-step AAV5 DetectCDx™

Specimens with SI < 1.00, or SI ≥ 1.00 with a CI > 1.00, are reported as Not Detected for anti-AAV5 antibodies.

Specimens with SI ≥ 1.00 and CI ≤ 1.00 are reported as Detected for anti-AAV5 antibodies.

Reported Results:

Patients evaluated with the AAV5 DetectCDx™ who are anti-AAV5 antibody negative (result of Not Detected) are eligible for treatment with ROCTAVIAN under the supervision of a physician.
Upon completion of testing at ARUP Laboratories, a test report with the results of the AAV5 DetectCDx™ will be sent to the designated physician. The following are the standard report results:

- **Detected:** patient **is not** eligible for treatment with ROCTAVIAN (valoctocogene roxaparvovec - rvox)
- **Not Detected:** patient **is** eligible for treatment with ROCTAVIAN (valoctocogene roxaparvovec - rvox)

Reagents:

The primary reagents for the AAV5 DetectCDx™ include:

AAV5 DetectCDx™ Reagents and Storage Conditions		
Reagent	**Storage Conditions**	**Component(s)**
AAV5 Coated Plate Set	Refrigerated (2 to 8 ºC)	• MULTI-ARRAY® 96-well plate • AAV5 Confirmatory Reagent • AAV5 Detection Reagent
AAV5 Control Set	Frozen (-70 ºC)	• Low positive control • High positive control • Cut point control • Negative control
Read Buffer T (1X)	Room temperature (20 to 25 ºC)	Read Buffer T (1X)

Additional reagents used in the AAV5 DetectCDx:

- TBS Buffer (1X) with 1% casein
- 1X DPBS
- Tween 20 (proteomics grade), 1.0% (v/v)
- ProClin 300, 0.05% (v/v)

Instruments: The MULTI-ARRAY® 96-well plate used in the AAV5 DetectCDx™ is read on a MESO QuickPlex SQ 120 instrument, as identified by a specific serial number.

General laboratory instruments and materials that are also used in the AAV5 DetectCDx™ include:

- Refrigerator capable of 2 to 8 ºC
- Freezers capable for -10 °C or colder and -70 °C or colder
- Single-channel pipette set
- Multi-channel pipettes
- Vortex mixer
- Minicentrifuge capable of 400 RPM
- Microplate shaker
- Microplate washer
- Microplate adhesive film
- PCR aluminum sealing film
- 0.2 mL and 1.2 mL 8-well strip tubes with cap

Software: The software used with the AAV5 DetectCDx™ is comprised of the MSD Discovery Workbench® version 4.0 and Cerner's Millennium Helix Unified Case Manager® version 2018.13.02.

Specimen shipping kit contents:

A specimen shipping kit provided by ARUP Laboratories may be used by the ordering laboratory or physician. Use of the shipping kit is optional; the kit will be provided to customers upon request. The specimen shipping kit includes the following components:

- Tube, Round False Bottom, Std Transport, 4mL screw cap
- Collection and Shipping Instructions
- Bag, ARUP Sample, Frozen, Ziploc w/ Absorbent pad
- Box, Shipping (cardboard with foam cooler insert; comes with a UN3373 and dry ice label)
- FedEx Priority Pre-Paid Shipping Label

Specimen collection and shipping:

To order the AAV5 DetectCDx™ assay, use the ARUP test requisition form (TRF) or ARUP's web-based ordering interface (available only to existing ARUP clients). The TRF must be fully completed. All samples must be transported to ARUP Laboratories frozen on dry ice. Any specimens not received frozen will result in specimen rejection and will not be tested.

Collection Instructions

- Collect the patient's whole blood in a 3.2% sodium citrate tube.
- Samples that exceed 7.3% sodium citrate cannot be evaluated and may require patient redraw.
- NOTE: When drawing blood for the AAV5 DetectCDx test, universal precautions for bloodborne pathogens should be observed.
- Centrifuge the specimen and separate the plasma within 72 hours of collection. Refer to your manufacturer's manual for recommended centrifuge speed and duration.
- Transfer 1 mL (minimum: 0.5 mL) of plasma into a polypropylene pour-off (transport) tube. Sample stability for the AAV5 DetectCDx™ has not been evaluated in tube types other than the ARUP Transport Tube (polypropylene).
- Failure to provide sufficient volume may result in the need for patient redraw.
- Label the transport tube with the patient's first and last name, date of birth, and sex.
- Freeze plasma specimen at -10°C or below.
- Ship frozen plasma specimens to ARUP as soon as possible on dry ice and use overnight delivery to ensure next day arrival at ARUP. NOTE: Plasma specimens must be frozen before they are shipped to ARUP Laboratories.
- Plasma samples can be stored frozen (-10 to -70°C) for up to 12 months. Minimize number of freeze/thaw events, not to exceed 6 events.

Please refer to the AAV5 DetectCDx™ Collection and Shipping Instructions in the optional specimen shipping kit or found online at http://www.aruplab/aav5 for further details about test ordering, specimen collection, and shipping samples to ARUP Laboratories.

Limitations:

This assay is intended for professional use only and is to be performed only at ARUP Laboratories, a single laboratory site located at 500 Chipeta Way, Salt Lake City, UT, 84108.

- For *in vitro* diagnostic use
- For professional use only
- For prescription use only
- This test is intended to be performed on specific serial number-controlled instruments at ARUP Laboratories

Performance characteristics:

Detection capabilities:
The detection capability of the AAV5 DetectCDx™ has been defined for internal quality control and qualification procedures.

Precision:
The precision of the AAV5 DetectCDx™ assay was evaluated across days, operators, instruments and reagents. The precision studies were based on CLSI EP05-A3 - Evaluation of Precision of Quantitative Measurement Procedures and CLSI EP12-A2 - User Protocol for Evaluation of Qualitative Test Performance. AAV5 DetectCDx precision was assessed using five sample types, as indicated in the table below.

Sample Types Used in DetectCDx™ Precision Evaluation				
Sample Type	**SI Value**		**CI Value**	
	Target	**Measured (mean)**	**Target**	**Measured (mean)**
High negative	< 1.00	0.87	~1.20	1.193
Cutoff	> 1.00	1.04	~1.00	1.005
Low positive	> 1.00	1.56	~0.80	0.695
Mid positive	~1.80	1.95	~0.60	0.538
High positive	> 10.0	40.01	< 0.20	0.0360

Results (summarized in the table below) indicate that inter- and intra-assay precision in the AAV5 DetectCDx™ is acceptable and that operator-to-operator, instrument-to-instrument, and reagent lot-to-lot variations do not impact assay results.

Results of AAV5 DetectCDx™ Precision Evaluation				
Study	**Experimental Conditions**	**Runs**	**Qualitative Agreement**	**% Coefficient of Variance**
Within-laboratory	Single operator, single instrument, single raw material reagent lot	2 runs per day 20 test days 2 replicates per sample	100% QA for high negative, low positive, mid positive and high positive samples	%CV ≤ 15% for all sample types tested
Repeatability	Single operator, single instrument, single raw material reagent lot	16 replicates per sample	100% QA for high negative, low positive, mid positive and high positive samples	%CV ≤ 15% for all sample types tested
Operator-to-operator	3 operators, 1 instrument, 1 production reagent lot	1 run per day per operator 5 test days 5 replicates per sample	100% QA for high negative, low positive, mid positive and high positive samples	%CV ≤ 15% for all sample types tested
Instrument-to-instrument	1 operator, 2 instruments, 1 production reagent lot	1 run/day 5 test days 5 replicates per sample	100% QA for high negative, low positive, mid positive and high positive samples	%CV ≤ 15% for all sample types tested
Reagent Lot-to-Lot	1 operator, 1 instrument, 3 vendor reagent lots	1 run/day 6 test days 4 replicates per sample	100% QA for high negative, low positive, mid positive and high positive samples	Between-lot %CV < 15%

The data for the sample near cutoff (SI > 1.00, CI ~1.00) was collected and evaluated to determine whether the performance of the assay at sample near cutoff was as expected. A total of 308 results were generated for the cutoff sample from the precision studies. The observed results (n=308) were determined as Detected 132 times and determined as Not Detected 176 times. The percent Detected was 43% (132/308) with 95% CI: (37%; 48%). The sample near cutoff performed as expected. Details of AAV5 DetectCDx sample analysis is presented in the table below.

Results of Sample Near AAV5 DetectCDx™		
	SI	**CI**
n	308	308
Mean	1.04	1.01
Median	1.04	1.00
SD	0.054	0.060
%CV	5.2%	6.0%

Interference:

The AAV5 DetectCDx™ was evaluated for interference by endogenous (naturally present in human plasma) and exogenous substances (e.g. common over-the-counter medicines, prescription drugs). Interference testing was based on CLSI EP07-A3 - Interference Testing in Clinical Chemistry, 3rd Edition; CLSI EP37-ED1 - Supplemental Tables for Interference Testing in Clinical Chemistry, 1st Edition. The interference study evaluated the impact of substances on the assay results using three sample types that

corresponded to a high negative sample, a low positive sample, and a high positive sample, as indicated in the tables below.

Sample Types Used in Endogenous & Exogenous Substances Evaluation				
Sample Type	**SI Value**		**CI Value**	
	Target	**Measured (mean)**	**Target**	**Measured (mean)**
High negative	< 1.00	0.850	~ 1.20	1.278
Low positive	> 1.00	1.260	~ 0.80	0.862
High positive	> 10.0	23.670	< 0.20	0.048

Rheumatoid factor (RF) interference was tested by evaluating the change in AAV5 DetectCDx assay results when a low positive sample was added to a high negative sample in the presence of different concentrations of rheumatoid factor.

A substance was considered an interferent to the AAV5 DetectCDx™ if addition of the test substance changed the qualitative output of the sample compared to control. A substance was also considered an interferent if the change in the SI/CI values of the high negative or low positive sample, samples above and below the critical assay cutoff, compared to control were > 10% with a high degree of confidence.

Interfering Substances to AAV5 DetectCDx		
Substance	**Test concentration**	**Impact on Qualitative Test Result**
Hemoglobin	1000 mg/dL	Could convert sample to Not Detected result
Triglycerides	750 mg/dL	Could convert sample to Not Detected result
Rheumatoid Factor†	1285 IU/mL, 1750 IU/mL, 3695 IU/mL	No expected impact

† RF interfered with the AAV5 DetectCDx in a dose-dependent manner with > 10% difference in assay values compared to control

Non-interfering Endogenous and Exogenous Substances*	
Substance	**Test concentration**
Albumin	6 mg/dL
Bilirubin, conjugated	40 mg/dL
Bilirubin, unconjugated	40 mg/dL
Triglycerides	500 mg/dL
Triglycerides	200 mg/dL
Hemoglobin	800 mg/dL
Hemoglobin	400 mg/dL
Rheumatoid Factor	476 IU/mL
Acetaminophen	15.6 mg/dL
Advate	384 IU/dL
Atazanavir	1.95 mg/dL
Atorvastatin	0.075 mg/dL
Bictegravir	1.85 mg/dL
Biotin	0.351 mg/dL
Doravirine	0.289 mg/dL

Non-interfering Endogenous and Exogenous Substances*	
Substance	**Test concentration**
Eloctate	324 IU/dL
Fexofenadine	0.116 mg/dL
Hemlibra	170 µg/mL
Hemofil-M	150 IU/dL
Heparin	330 IU/dL
Ibuprofen	21.9 mg/dL
Lisinopril	0.0246 mg/dL
Naproxen	36.0 mg/dL
Omeprazole	0.84 mg/dL
Oxycodone	0.0324 mg/dL
Sodium citrate†	7.3% (short draw)
Tenofovir	0.0978 mg/dL
Vitamin C	5.25 mg/dL

† Higher concentrations of sodium citrate could not be evaluated with the AAV5 DetectCDx™

* Cholesterol and Celebrex have not been evaluated as potential interferents to the AAV5 DetectCDx™ assay, so the effect of these substances on the assay is unknown

Cross-reactivity with other antibodies:

Cross-reactivity in the AAV5 DetectCDx™ assay to antibodies other than anti-AAV5 antibodies is unknown. A positive assay result can occur due to detection of antibodies other than anti-AAV5 antibodies.

Prozone effect:

The AAV5 DetectCDx™ was evaluated to determine whether elevated concentrations of anti-AAV5 antibody produce a prozone (hook) effect. The study samples utilized distinct plasma samples from three (3) non-hemophilia A donors that represent the highest AAV5 titer positive samples that were previously identified in historical studies conducted at ARUP Laboratories. Individual two-fold dilution series were created by diluting the high titer positive AAV5 plasma samples into the anti-AAV5 negative plasma sample for eight (8) dilution steps to cover the range from high positive to negative Screen Index and Confirm Index values.

The results from this test indicate that a prozone effect was not observed for samples with starting SI values of ~90. Human specimens with SI values greater than 90 were not evaluated in this study and may exhibit a prozone effect.

Carryover:

The possibility of carryover and well-to-well cross-talk was evaluated for the AAV5 DetectCDx™ assay. The study sample set indicated in the table below was used to create an alternating pattern of negative and high positive samples.

Sample Types Evaluated in Carryover and Cross-Talk Evaluation				
Sample Type	**SI Value**		**CI Value**	
	Target	**Measured (mean)**	**Target**	**Measured (mean)**
Negative	< 1.00	0.88	>1.00	1.427
High positive	50-85	49.40	0.03-0.15	0.026

The two (2) AAV Coated Plates were arranged so that the locations of the screening and confirmatory assay modes and the negative and high positive samples were swapped between plates to address all sections of the plate.

Summary of AAV5 DetectCDx™ Carryover and Cross-Talk Evaluation	
Sample Type	**Reported values**
Negative	100% Not Detected
High positive	100% Detected

Based on these results, it was concluded that carryover or well-to-well cross-talk were not observed in the study.

Stability:
Stability of the reagents, collections, and samples for the AAV5 DetectCDx™ assay were evaluated based on CLSI EP25-A - Evaluation of Stability of In Vitro Diagnostic Reagents. Stability studies evaluated the impact of various storage and transport conditions of reagents and human whole blood and plasma using three sample types that corresponded to a high negative sample, a low positive sample, and a high positive sample, as indicated in the table below.

Sample Types Evaluated in Stability Tests				
Sample Type	**SI Value**		**CI Value**	
	Target	**Measured (mean)**	**Target**	**Measured (mean)**
High negative	< 1.00	0.89	~1.20	1.245
Low positive	> 1.00	1.46	~0.80	0.768
High positive	> 10.0	31.08	< 0.20	0.038

A condition and/or timepoint was considered to impact the AAV5 DetectCDx™ if the qualitative output of the sample compared to control was changed. A condition and/or timepoint was also considered to impact the results of the AAV5 DetectCDx™ if the change in the SI/CI values of the high negative or low positive sample, samples above and below the critical assay cutoff, compared to control were > 10% with a high degree of confidence.

AAV5 DetectCDx™ Plasma Sample Stability	
Storage Conditions	**Stability**
Room temperature (20 to 25 ºC)	72 hours
Refrigerated (2 to 8 ºC)	28 days
Frozen (-10 ºC or colder)	12 months
Frozen (-70 ºC or colder)	12 months
Freeze-thaw cycles	7 events

AAV5 DetectCDx™ Sample Collection Stability	
Conditions	**Stability**
Plasma, room temperature (20 to 25 ºC)	72 hours
Plasma, refrigerated (2 to 8 ºC; for storage post-processing prior to freezing)	72 hours
Whole blood, room temperature (20 to 25 ºC)	72 hours
Whole blood, refrigerated (2 to 8 ºC; for storage prior to processing to plasma)	72 hours

AAV5 DetectCDx™ Plasma Sample Transport Stability	
Transport Conditions	**Stability**
Refrigerated (with gel packs)	10 days
Ambient temperature	10 days
Frozen (on dry ice)	10 days
Elevated temperature (37 ºC ± 2 ºC)	1 day
Frozen (on ice pack)	7 days

AAV5 DetectCDx™ Established Reagent Stability		
Reagent(s)	**Conditions**	**Stability**
AAV5 Plate Components	Frozen (-70 ºC)	12 months
AAV5 Run Control Set	Frozen (-20 ºC)	12 months
AAV5 Coated Plate Set	Refrigerated (2 to 8 ºC)	7 days
Read Buffer T (1X)	20 to 25 ºC	12 months

Expected Values

Patient Population Demographics:

A number of patient population demographic variables were analyzed for their potential association with AAV5 DetectCDx assay results (Detected vs Not Detected).

Percent of Detected AAV5 DetectCDx Results Stratified by Race and Ethnicity		
Race	***N***	***Percent Detected***
White	618	27.8% (172/618)
Asian	159	28.3% (45/159)
Black or African American	110	34.5% (38/110)
Native Hawaiian or other Pacific Islander	2	0.0% (0/2)
Not Provided or Multiple	138	40.6% (56/138)
Combined	1,027	30.3% (311/1,027)
Ethnicity		
Hispanic or Latino	27	29.6% (8/27)
Not Hispanic or Latino	965	29.8% (288/965)
Not provided	35	42.9% (15/35)
Combined	1,027	30.3% (311/1,027)

Higher seropositivity (percent of results Detected) was observed for the “Black or African American” group (34.5% Detected).

Percent of Detected AAV5 DetectCDx Results Stratified by Country of Origin		
Country of Origin	***N***	***Percent Detected***
Australia	45	15.6% (7/45)
Belgium	19	21.1% (4/19)
Brazil	102	32.4% (33/102)
France	116	37.1% (43/116)
Germany	101	25.7% (26/101)
Israel	12	8.3% (1/12)
Italy	24	33.3% (8/24)
South Africa	112	35.7% (40/112)
Spain	14	21.4% (3/14)
South Korea	6	33.3% (2/6)
Taiwan	40	35.0% (14/40)
United Kingdom	94	18.1% (17/94)
United States	168	28.0% (47/168)
Russia	91	46.2% (42/91)
Japan	84	29.8% (25/84)
Combined	1,028	30.4% (312/1,028)

A high level of seropositivity (percent results Detected) was observed in Russia (46%) and a low level was observed in Israel (8%) and United Kingdom (18.1%).

Percent of Detected AAV5 DetectCDx Results Stratified by Type of FVIII Replacement		
	N	***Percent "Detected"***
On demand	108	45.4% (49/108)
Prophylaxis	891	26.4% (235/891)
Combined	999	28.4% (284/999)

The "on-demand" group experienced a higher seropositivity rate (percent results Detected) than the prophylaxis group.

Summary of clinical study:

Study Design:
The ROCTAVIAN clinical development program consists of six (6) interventional studies and two (2) non-interventional studies. The AAV5 DetectCDx was utilized in five (5) of these clinical studies. The safety and effectiveness of the AAV5 DetectCDx for its intended use was demonstrated through testing of specimens from hemophilia A patients enrolled in the clinical study 270-301 (study objective to evaluate the safety and efficacy of ROCTAVIAN; ClinicalTrials.gov Identifier NCT03370913). Samples were all evaluated at ARUP Laboratories in Salt Lake City, Utah using the AAV5 DetectCDx™ assay.

Study Population Demographics
The effectiveness of the AAV5 DetectCDx as a companion diagnostic device for the testing of human plasma collected in 3.2% sodium citrate samples for the presence of AAV5 antibodies to aid in the selection of hemophilia A patients for treatment with ROCTAVIAN is based on the 134 patients from

study 270-301, who had a "Not Detected" AAV5 DetectCDx result and were enrolled in the clinical study. In study 270-301, 134 subjects, aged 18 to 70 years (median: 30 years), received ROCTAVIAN. The population was 72% White (96 patients), 14% Asian (19 patients), and 11% Black (15 patients). All except two (2) subjects were HIV negative. Subjects were previously treated only with prophylactic FVIII replacement therapy. There were no subjects on Emicizumab prophylaxis.

Demographics of 270-301 study population	
Age at enrollment, years	
Mean (SD)	31.7 (10.3)
Median (Range)	30.0 (18, 70)
Sex, n (%)	
Male	134 (100)
Race, n (%)	
Asian	19 (14.2)
Black or African American	15 (11.2)
Native Hawaiian or other Pacific Islander	1 (0.7)
White	96 (71.6)
Not provided due to patient privacy	3 (2.2)
Ethnicity, n (%)	
Hispanic or Latino	7 (5.2)
Not Hispanic or Latino	127 (94.8)
Type of FVIII treatment for hemophilia A, n (%)	
Prophylaxis	134 (100)

Study Results:

The results from study 270-301 support the clinical benefit of the AAV5 DetectCDx in the detection of anti-AAV5 antibodies as an aid in the selection of hemophilia A patients for treatment with ROCTAVIAN. Adult hemophilia A patients in the study received a single administration of 6E13 vg/kg dose of ROCTAVIAN. The primary efficacy outcome was a non-inferiority (NI) test of the difference in annualized bleeding rate (ABR) in the efficacy evaluation period following ROCTAVIAN administration compared with ABR during the baseline period with the NI margin set at 3.5 bleeds per year. The NI analysis met the pre-specified NI margin in the efficacy evaluable study subjects, indicating the effectiveness of ROCTAVIAN. The results from this study support the clinical benefit of the AAV5 DetectCDx in the selection of hemophilia A patients for treatment with ROCTAVIAN.

The safety evaluation of AAV5 DetectCDx as a companion diagnostic device for the testing of human plasma collected in 3.2% sodium citrate samples for the presence of AAV5 antibodies to aid in the selection of hemophilia A patients for treatment with ROCTAVIAN is based on the data generated in one (1) clinical study (270-301) on 134 subjects with severe hemophilia A exposed to ROCTAVIAN. All subjects received a single dose of 6×10^{13} vg/kg of body weight of ROCTAVIAN with a minimum follow-up of 66 weeks and a median follow-up of 162 weeks (range 66 to 255 weeks).

ROCTAVIAN was found to have an acceptable safety and tolerability profile that supports a positive benefit-risk assessment. All subjects successfully completed their full-dose infusion of ROCTAVIAN, with no participants discontinued from the study as a result of a treatment emergent adverse event (TEAE). The

most common adverse reactions to ROCTAVIAN were rash, headache, nausea, fatigue, diarrhea, and lab abnormalities. The most common laboratory abnormalities to ROCTAVIAN were alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), factor VIII activity levels, gamma glutamyl transferase (GGT), and bilirubin above upper limit of normal (ULN). Refer to the ROCTAVIAN Full Prescribing Information for more information.

Long-term safety of ROCTAVIAN continues to be monitored as outlined in the risk management plan in ongoing clinical trials and proposed post-approval studies.

Refer to the most recent ROCTAVIAN product labeling.

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