SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

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

Device Trade Name: AAV5 DetectCDx

Device Procode: QWQ

Applicant's Name and Address: ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT, 84108

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P190033

Date of FDA Notice of Approval: June 29, 2023

II. INDICATIONS FOR 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.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

When drawing blood for the AAV5 DetectCDx assay, universal precautions for bloodborne pathogens should be observed.

Rheumatoid factor levels greater than 476 IU/mL will interfere with the ability of the AAV5 DetectCDx to accurately detect anti-AAV5 antibodies.

Triglyceride levels greater than 500 mg/dL will interfere with the ability of the AAV5 DetectCDx to accurately detect anti-AAV5 antibodies.

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 ("Detected") can occur due to the 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."

V. DEVICE DESCRIPTION

The AAV5 DetectCDx is a companion diagnostic (CDx) device intended for use with ROCTAVIAN (valoctocogene roxaparvovec-rvox), a gene therapy indicated for hemophilia A patients that is a recombinant, replication incompetent AAV5 vector containing a DNA genome. The AAV5 DetectCDx uses a bridging immunoassay and electrochemiluminescence (ECL) reaction 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 detect antibodies to the AAV5 capsid. A positive result in the screening step is confirmed in the confirmatory step prior to providing a test result of "Detected" to indicate the presence of anti-AAV5 antibodies. A "Not Detected" test result indicates that anti-AAV5 antibodies were not detected in the screening step or that the confirmatory step did not confirm the presence of anti-AAV5 antibodies. The AAV5 DetectCDx is performed only at ARUP Laboratories, a single laboratory site located at 500 Chipeta Way, Salt Lake City, UT 84108. The ARUP clinical laboratory responsible for testing and reporting results is ISO15189, CLIA, and CAP certified.

The AAV5 DetectCDx utilizes reagents manufactured exclusively for use with the AAV5 DetectCDx by ARUP Laboratories, as well as utilizing reagents and instrumentation which have been specifically validated for, and approved for use as part of, the AAV5 DetectCDx (Tables 1 – 3, below).

Table 1: Critical Reagents Manufactured for Use with AAV5 DetectCDx	
Reagents	Use in Assay
AAV5 Coating Reagent	Unlabeled capsid used as capture antigen to coat bottom of plates
AAV5 Confirmatory Reagent	Unlabeled capsid used in confirmatory step
AAV5 Detection Reagent	SULFO-TAG/ruthenylated capsid for ECL reaction
AAV5 Run Controls	Quality controls that include a Negative Control (NEG), Low Positive Control (LPC), High Positive Control (HPC), and Cut point Control (CC)

Table 2: Other Critical Reagents/Components Used in AAV5 DetectCDx	
Reagent/Component	Use in Assay
96-well ECL immunoassay plates	Assay plates
Read Buffer	Contains tripropylamine (TPA) substrate for ECL reaction
Tris-buffered saline with 1% Casein (TBS-C)	Blocking buffer

Table 3: Instrumentation and Software Used in AAV5 DetectCDx	
Instrument/Software	Use in Assay
ECL-based plate reader *	ECL-based plate reader
Plate reader software version 4.0 †	Off-the-shelf software that runs and supports the plate reader
Millennium Helix Unified Case Manager Software version 2018.13.02 (Cerner Corporation)†	Off-the-shelf data management software used to manage workflow of assay, calculate screening and confirmatory results from raw data, and determine assay output based on screening and confirmatory results
Microplate Washer	Plate washer

*AAV5 DetectCDx is intended to be performed on specific serial number-controlled instruments at ARUP Laboratories.

†Software and cybersecurity were reviewed for in-vitro diagnostic use with the AAV5 DetectCDx on serial number-controlled instruments at ARUP Laboratories.

Specimen preparation and transport to ARUP Laboratories

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). Collect the patient's whole blood in a 3.2% sodium citrate tube. Centrifuge the specimen and separate plasma within 72 hours of collection. Transfer 1mL (minimum of 0.5mL) of plasma into a pour-off polypropylene transport tube. Performance of the AAV5 DetectCDx has not been evaluated for samples stored/transported in tube types other than the ARUP Transport Tube (polypropylene). An optional specimen shipping kit from ARUP Laboratories specifically indicated for use with the AAV5 DetectCDx is available. Plasma specimens must be frozen (-10°C or below) before they are shipped to ARUP

Laboratories. Plasma specimens must be transported to ARUP Laboratories frozen on dry ice.

Assay Principle and Format
The AAV5 DetectCDx is a manually run ECL-based bridging immunoassay performed in 96-well plate format. The 96-well ECL immunoassay plates coated with AAV5 Coating Reagent (followed by washing and blocking steps) are incubated with diluted patient plasma specimens. If anti-AAV5 antibodies are present in the patient specimen, the antibodies bind to the unlabeled AAV5 capsid (AAV5 Coating Reagent) coating the wells. After washing the plate, AAV5 Detection Reagent is added to each well and wells with patient samples that contain anti-AAV5 antibodies will bind the SULFO-TAG capsid in the AAV5 Detection Reagent, which participates in the ECL reaction. After incubation and washing, Read Buffer (containing TPA substrate) is added to each well. The plate is then read on the ECL-based plate reader . Each well of the plate is electrically stimulated and the resultant ECL signal is measured.

Anti-AAV5 antibodies in the patient specimen form a bridge between the AAV5 capsid coating the plate and the ruthenylated (Ru-) AAV5 capsid in the AAV5 Detection Reagent. With addition of the TPA substrate in the Read Buffer , an electrochemiluminescent signal is generated in wells with patient specimen containing anti-AAV5 antibodies.

Patient specimens are run in the screening and confirmatory steps of the AAV5 DetectCDx in parallel, in separate wells of the 96-well plate. The confirmatory step methodology is identical to that of the screening step, except that patient specimens are pre-incubated with unlabeled capsid (in the AAV5 Confirmatory Reagent) to compete for any anti-AAV5 antibodies that are present, prior to addition to the 96-well plate. If AAV5-binding antibodies are present in the patient specimen, 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.

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/plate acceptance and for patient results to be reported, 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.

Interpretation of Results
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[1]. Results for the

[1] Disease-specific screening and confirmatory cut points for the assay were determined using statistical analysis of data collected in a study that utilized sodium citrated (3.2%) plasma samples from eighty (80) hemophilia A-affected male donors. The screening cut point (SCP) is defined as the signal to noise (S/N) value at which a specimen will be considered negative if the specimen S/N is less than the calculated cut point value. The screening cut point was empirically determined to obtain a 5% false positive rate. The confirmatory cut point (CCP) was empirically determined to obtain a 1% false negative rate. See Section IX.A.1, below.

confirmatory step are expressed as a Confirm Index (CI). The CI is obtained by calculating the ratio of mean signals obtained for the confirmatory and screening steps and dividing this by the confirmatory cut point[1]. 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 (Figure 1).

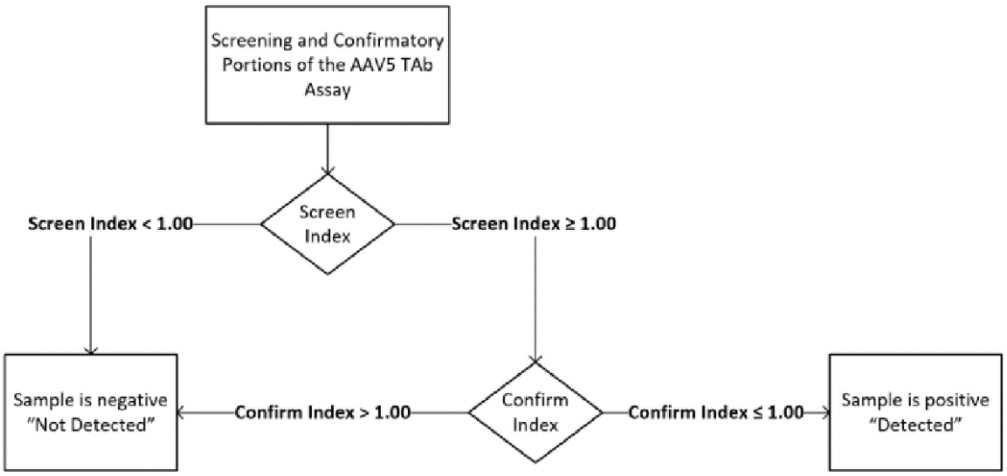

Figure 1: Summary of Resulting and Reporting for the two-step AAV5 DetectCDx

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.

Patients evaluated with the AAV5 DetectCDx who are anti-AAV5 antibody negative (result of Not Detected) are eligible for treatment with ROCTAVIAN (valoctocogene roxaparvovec-rvox) under the supervision of a physician.

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

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

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no FDA-cleared or -approved alternatives for detection of anti-AAV5 antibodies in human serum for the selection of hemophilia A patients who are eligible for treatment with ROCTAVIAN (valoctocogene roxaparvovec-rvox), an adeno-associated virus serotype 5 (AAV5)-based gene therapy.

VII. MARKETING HISTORY

The AAV5 DetectCDx has not been marketed in the United States. The AAV5 DetectCDx has been marketed in the European Union under DIRECTIVE 98/79/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 October 1998 on in vitro diagnostic medical devices.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Below is a list of the potential adverse effects (e.g., complications) associated with the use of the device.

Patients with false negative results for the AAV5 DetectCDx (patients with pre-existing anti-AAV5 antibodies who are indicated as Not Detected by the device) would receive the treatment and be exposed to the potential risks associated with the ROCTAVIAN treatment including the possibility of not experiencing the potential benefits of the treatment. Patients who receive treatment with ROCTAVIAN will have their FVIII activity monitored. Based on the patient's response, they may either be tapered off FVIII concentrates/hemostatic agents if the ROCTAVIAN treatment demonstrates efficacy, or will continue to receive FVIII treatment if response to the treatment is not achieved.

Patients with false positive results for the AAV5 DetectCDx would not be eligible to receive the ROCTAVIAN treatment. However, patients with a false positive result will continue to receive the current standard of care therapies that are known to be efficacious for hemophilia A.

Procedure-related complications for the assay itself are limited to obtaining the plasma specimen via a blood draw. These risks for the AAV5 DetectCDx are equivalent to risks of sample collection in other in vitro diagnostic tests and not unique to the AAV5 DetectCDx. The AAV5 DetectCDx is a non-invasive in-vitro companion diagnostic and as such, there is minimum impact on the patients from the test itself.

For the specific adverse events that occurred in the clinical studies, please see Section X below.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

1. Establishment of Screening and Confirmatory Cut Points

The screening and confirmatory cut points for the AAV5 DetectCDx were established prior to use of the investigational device in nonclinical studies and the 270-301 clinical study (and other clinical studies; see Section X below). Once established, the cut points for the device were locked and remain unchanged.

Disease-specific screening and confirmatory cut points were determined by analysis of plasma samples from eighty (80) previously unscreened hemophilia A patients. A

balanced experimental design was utilized to diminish the variability associated with different analysts, runs and plates (Shankar et al., 2008[2]). Two (2) analysts tested batches of five (5) plates, each plate containing a subgroup of 16 samples. For determination of both screening and confirmatory cut points, samples were run in duplicate in both the screening and confirmatory portions of the assay, for a total of four (4) wells on each plate. Each analyst tested each sample five (5) times, resulting in each sample being tested a total of 10 times on 10 separate runs.

AAV is a naturally occurring non-pathogenic virus endemic to the global population, and many individuals harbor pre-existing antibodies directed against the AAV capsid[3]. At the time of this study, no other method was currently available to detect infection or exposure to AAV5, therefore, it was not possible to know a priori which samples were negative or positive for anti-AAV5 antibodies. For this reason, a strategy was developed to identify samples containing pre-existing antibodies to AAV5, considered "true positives," so that these samples could be removed from further statistical analysis of the screening cut point. This strategy involved the removal of samples that generated signals greater than the Low Positive Control (LPC), a known anti-AAV5 positive sample, as well as the removal of statistical outliers that were identified as additional true positives. The assay-specific, fixed Screening Cut Point (SCP) was thus established based on the statistical analysis of the set of samples identified as negative for anti-AAV5 antibodies, to generate a 5% false positive rate. The resultant analysis produced a SCP value of 1.14. The SCP is used as a normalization factor to calculate the Screen Index (SI). The SI = (S/N)/SCP, where S/N is the signal to noise[4]. Thus when (S/N) = 1.14 for a sample, the SI = 1.0.

In order to calculate the Confirmatory Cut Point (CCP) for the assay, the Inhibition Ratio (IR) was calculated for each sample run in the screening and confirmatory steps of the assay (the IR = $\mu_{confirm}$ / μ_{screen}). Samples in which the mean IR was greater than or equal to the mean IR for the LPC were removed as true positives with pre-existing anti-AAV5 antibodies. The assay specific, fixed CCP was thus established based on the statistical analysis of a set of samples negative for anti-AAV5 antibodies, to generate a 1% false negative rate. The resultant analysis produced a CCP of 0.707. The CCP is used as a normalization factor to calculate the Confirmatory Index (CI). For samples with SI $\geq$ 1.00, a CI > 1.00 indicates the sample is negative for anti-AAV5 antibodies and samples with a CI $\leq$ 1.00 are deemed positive for anti-AAV5 antibodies.

2. **Anti-AAV5 Antibody Detection**
   The AAV5 DetectCDx, indicated as a device to detect anti-AAV5 antibodies, is a first-of-a-kind device, with no other previously cleared or approved devices of its type.

[2] Shankar et al. 2008. Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J Pharm Biomed Anal. 48:1267-1281.
[3] Klamroth et al. 2022. Global seroprevalence of pre-existing immunity against AAV5 and other AAV serotypes in people with hemophilia A. Human Gene Therapy 33(7-8): 432-441.
[4] The mean signal for samples (and controls) run in the screening step are normalized to a plate-specific normalization factor (which is the mean of replicate Cut Point Control, CC, wells run on each plate), to generate the S/N for the sample (or control), such that S/N = μ_{screen} / μ_{CC}.

No reference methods exist to detect anti-AAV5 antibodies, and human derived anti-AAV5 antibody reference material is not available. As such, it is important that there is empirical demonstration that the AAV5 DetectCDx detects anti-AAV5 antibodies, using multiple complementary approaches. The following information, studies, and approaches demonstrate that the AAV5 DetectCDx detect anti-AAV5 antibodies:

Design of the AAV5 DetectCDx:
The assay consists of both a screening portion, which is conducted in the absence of any unlabeled AAV5 capsid, and the confirmatory portion, in which unlabeled AAV5 capsid is added to the well to compete with the signal-generating Ru-labeled capsid. Reduction of assay signal in the presence of unlabeled capsid indicates that binding of AAV5 is required for generation of the signal.

Affinity-purification of human anti-AAV5 antibodies:
AAV5 capsid covalently bound to magnetic beads was used to affinity purify anti-AAV5 antibodies from a high titer human plasma sample. The resultant, small amount of affinity-purified material was determined to have a human IgG concentration of 200 ng/mL. A dilution series of the affinity-purified antibody was tested using the clinical trial assay (CTA), an earlier version of the AAV5 DetectCDx, to generate a dose-response of the assay results with decreasing levels of anti-AAV5 antibodies.

Assay response for clinical samples pre- and post-ROCTAVIAN dose:
Pre- and post-ROCTAVIAN dose samples from subjects enrolled in the 270-201 clinical trial were evaluated using the CTA. Patients who were Not Detected as measured by the assay at baseline (pre-dose) were shown to convert to Detected by the assay at 8 weeks post-dose with ROCTAVIAN due to the detection of the anti-AAV5 antibody response (see Table 4 below).

Table 4: Assay results pre- and post-ROCTAVIAN treatment

Subject	Pre-dose SI result	Post-dose SI result
1	0.87	438.08
2	0.97	286.86
3	0.88	455.94
4	0.90	208.54
5	0.81	358.62

AAV5 seroprevalence as determined by AAV5 DetectCDx:
Clinical study 270-901 was a non-interventional study conducted to assess the seroprevalence of antibodies against various serotypes of Adeno-Associated Viruses (including AAV5). The results of this study were published in 2022 (Klamroth et al., 2022[3]). The study tested patients with hemophilia A up to three (3) times over six (6) months, looking at both single timepoint incidence of seroprevalence and the consistency of antibody test results in a patient over time. Subjects in this study did not represent the intended use population of the device and were not used for the evaluation of efficacy and safety of ROCTAVIAN.

Overall, of the 540 participants tested at Day 1 in the study, 34.8% tested positive (Detected result) for anti-AAV5 antibodies. Factoring in the prevalence of hemophilia A in the countries being assayed, the global weighted average of AAV5 seroprevalence in hemophilia A participants was 29.7%. This AAV5 seroprevalence is consistent with previous reports of AAV5 seroprevalence (Boutin et al., 2010[5]; Kruzik et al., 2019[6]). Additionally, 62 of 72 participants with multiple assessments (86.1%) showed consistency in AAV5 antibody status across all assessed timepoints.

Table 5: AAV5 Seroprevalence as determined by AAV5 DetectCDx		
	Day 1	
Country	n	% AAV5 positive (Detected)
South Africa	56	51.8%
Russia	91	46.2%
Italy	20	40.0%
France	86	37.2%
Japan	84	29.8%
Germany	89	28.1%
Brazil	26	26.9%
USA	71	26.8%
United Kingdom	17	5.9%[c]
Overall	540[b]	34.8%
Global HA weighted average[a]	29.7%	

HA, hemophilia A

n = sample size tested at the Day 1 timepoint in the country/region indicated. Percentages represent percentage of participants testing AAV5-antibody positive relative to the sample size tested at the Day 1 timepoint (n).

[a]Global HA weighted average is calculated based on the percentage of 270-901 participants testing AAV5 seropositive on Day 1 in each country, multiplied by (the number of HA participants in that country per 2018 World Federation of Hemophilia (WFH) survey[7] / (the total number of HA participants in all the 270-901 countries per the WFH surveys)).

[b]Out of 546 participants enrolled in 270-901, 540 had at least one AAV5 antibody measurement.

[c]The UK rate in this study may be artificially low due to the small sample size.

3. Precision Studies

Description of samples in the precision studies.

Samples evaluated in the precision studies were made from pooling plasma samples from multiple non-hemophilia A donors to target the specified SI and CI values (see

[5] Boutin et al. 2010. Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors. Human Gene Therapy 21(6): 704-712.

[6] Kruzik et al. 2019. Prevalence of anti-adeno-associated virus immune responses in international cohorts of healthy donors. Methods & Clinical Development 14: 126-133.

[7] World Federation of Hemophilia (WFH). Report on the Annual Global Survey 2018. Available at https://www1.wfh.org/publications/files/pdf-1731.pdf as of 7 March 2023.

Table below). Aliquots of each sample type were stored in frozen storage (-70°C or colder) until use in the precision studies.

Table 6: Sample Types Evaluated in Precision Studies	
Sample Type	Target (Mean) Screen Index (SI) and Confirm Index (CI) Values
High Negative	SI < 1.00 and CI ~ 1.20
Cutoff*	SI > 1.00 and CI ~1.00
Low Positive	SI > 1.00 and CI ~0.80
Mid Positive**	SI ~1.80 and CI ~0.60
High Positive	SI > 10.0 and CI < 0.20

*The Cutoff Sample was not evaluated in the Lot-to-Lot Precision Study
**The Mid Positive Sample evaluated in the Lot-to-Lot Precision Study had SI ~ 4.00 and CI ~0.40

Precision Study #1: Within-laboratory precision (repeatability, between-run, and between-day components)

Design: The within-laboratory precision study was based on the single-site precision evaluation study as described in *Clinical and Laboratory Standards Institute* (*CLSI) EP05-A3 – Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition*. The study was performed over 20 days, with two runs (plates) per day, and two true replicate measurements per sample type (a true replicate measurement is 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. A total of 80 replicates were collected per sample (20 days x 2 runs/per day) x 2 replicates = 80 replicates per sample).

Table 7: 20-Day Precision Study – Qualitative Results					
Sample type	N	Mean		% Detected	% Not Detected
		SI	CI		
High Negative	80	0.88	1.181	0/80= 0%	80/80= 100%
Cutoff	79*	1.05	1.005	33/79 = 41.8%	46/79= 58.2%
Low Positive	80	1.64	0.673	80/80= 100%	0/80= 0%
Mid Positive	80	2.01	0.521	80/80= 100%	0/80= 0%
High Positive	79*	41.55	0.027	80/80= 100%	0/80= 0%

*One replicate was invalid

Table 8: 20-Day Precision Study – SI values										
Sample type	N	Mean	Repeatability		Between-Run		Between-Day		Total*	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Negative	80	0.88	0.029	3.3%	0.032	3.6%	0.010	1.2%	0.044	5.0%
Cutoff	79**	1.05	0.032	3.0%	0.045	4.3%	0.018	1.7%	0.058	5.5%
Low Positive	80	1.64	0.034	2.1%	0.069	4.2%	0.038	2.3%	0.086	5.2%
Mid Positive	80	2.01	0.048	2.4%	0.084	4.2%	0.149	7.4%	0.178	8.8%
High Positive	79**	41.55	1.266	3.0%	3.182	7.7%	3.521	8.5%	4.911	11.8%

*Total precision includes repeatability, between-run and between-day precision
**One replicate was invalid

Table 9: 20-Day Precision Study – CI values										
Sample type	N	Mean	Repeatability		Between-Run		Between-Day		Total*	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Negative ***	80	1.181	0.058	4.9%	0.039	3.3%	0.000	0.00%	0.070	5.9%
Cutoff	79**	1.005	0.031	3.1%	0.058	5.7%	0.033	3.2%	0.079	7.9%
Low Positive	80	0.673	0.030	4.5%	0.025	3.7%	0.021	3.1%	0.044	6.6%
Mid Positive	80	0.521	0.022	4.3%	0.015	7.0%	0.051	9.8%	0.058	11.0%
High Positive	79**	0.027	0.001	4.3%	0.002	7.6%	0.003	10.2%	0.004	13.4%

*Total precision includes repeatability, between-run and between-day precision
**One replicate was invalid
*** CI is not used to determine qualitative output for high negative samples, as high negative samples have SI < 1.0

Precision Study #2: Repeatability

Design: The repeatability study evaluated each of the five sample types in 16 true replicates on a single plate (run), using a single lot of reagents, and run on a single instrument system by a single operator. A true replicate is the mean of the measurements from two duplicate wells on the plate.

Table 10: Repeatability Study – Qualitative Results					
Sample type	N	Mean		% Detected	% Not Detected
		SI	CI		
High Negative	16	0.94	1.256	0/16= 0%	16/16= 100%
Cutoff	16	1.07	1.005	56.25% (9/16)	43.75% (7/16)
Low Positive	16	1.49	0.726	16/16= 100%	0/16= 0%
Mid Positive	16	1.80	0.638	16/16= 100%	0/16= 0%
High Positive	16	35.91	0.031	16/16= 100%	0/16= 0%

Table 11: Repeatability – SI values				
Sample type	N	Mean	Repeatability	
			SD	%CV
High Negative	16	0.94	0.052	5.6%
Cutoff	16	1.07	0.051	4.8%
Low Positive	16	1.49	0.035	2.4%
Mid Positive	16	1.80	0.070	3.9%
High Positive	16	35.91	1.71	4.8%

Table 12: Repeatability – CI values				
Sample type	N	Mean	Repeatability	
			SD	%CV
High Negative*	16	1.256	0.073	5.8%
Cutoff	16	1.005	0.050	5.0%
Low Positive	16	0.726	0.026	3.6%
Mid Positive	16	0.638	0.051	8.0%
High Positive	16	0.031	0.002	6.8%

* CI is not used to determine qualitative output for high negative samples, as high negative samples have SI < 1.0

Precision Study #3: Within-Laboratory Precision (Operator-to-Operator Variability)

Design: The study to evaluate operator-to-operator variability was based on CLSI EP05-A3. Each sample type was evaluated by each of three operators, over five (non-consecutive) days, with one run (plate) per day, and with five true replicates on each plate. A true replicate is the mean of the measurements from two duplicate wells on the plate. Each operator evaluated performance of the sample types on different plates (different runs), and as such, operator imprecision is confounded by run (plate). The

study was conducted using a single lot of critical reagents and was performed on a single instrument system. A total of 75 data points each were collected per sample analyzed (5 days x 3 Operator runs (1 per day) x 5 replicates = 75 data points per sample).

Table 13: Operator Precision – Qualitative Results							
Sample type	N	Mean		% Detected Overall	% Detected Operator 1	% Detected Operator 2	% Detected Operator 3
		SI	CI				
High Negative	75	0.86	1.191	0/75= 0%	0/25= 0%	0/25= 0%	0/25= 0%
Cutoff	73*	1.03	1.008	25/73= 34.2%	4/24= 17%	11/24= 46%	10/25= 40%
Low Positive	75	1.54	0.706	75/75= 100%	25/25= 100%	25/25= 100%	25/25= 100%
Mid Positive	75	1.90	0.537	75/75= 100%	25/25= 100%	25/25= 100%	25/25= 100%
High Positive	74**	38.48	0.028	75/75= 100%	25/25= 100%	25/25= 100%	24/24= 100%

*Two replicates were invalid
** One replicate was invalid

Table 14: Operator Precision – SI values									
Sample type	N	Mean	Repeatability		Between-Operator/Run		Between-Day		Total*	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Negative	75	0.86	0.025	2.9%	0.038	4.4%	0.020	2.4%	0.049	5.8%
Cutoff	73**	1.03	0.033	3.2%	0.037	3.6%	0.000	0.0%	0.050	4.8%
Low Positive	75	1.54	0.037	2.4%	0.087	5.6%	0.022	1.5%	0.097	6.3%
Mid Positive	75	1.90	0.048	2.5%	0.161	8.5%	0.000	0.0%	0.168	8.8%
High Positive	74***	38.48	1.864	4.8%	3.974	10.3%	0.000	0.0%	4.389	11.4%

*Total precision includes repeatability, between-operator/run and between-day precision
**Two replicates were invalid
*** One replicate was invalid

Table 15: Operator Precision – CI values										
Sample type	N	Mean	Repeatability		Between-Operator/Run		Between-Day		Total*	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Negative **	75	1.191	0.0443	3.7%	0.0153	1.3%	0.0274	2.3%	0.0543	4.6%
Cutoff	73***	1.008	0.428	4.3%	0.0311	3.1%	0.0225	2.2%	0.0575	5.7%
Low Positive	75	0.706	0.0311	4.4%	0.0121	1.7%	0.0086	1.25	0.0345	4.9%
Mid Positive	75	0.537	0.0214	4.0%	0.0219	4.1%	0.0215	4.0%	0.0374	7.0%
High Positive	74†	0.028	0.0020	7.0%	0.0016	5.6%	0.0007	2.4%	0.0027	9.3%

*Total precision includes repeatability, between-operator/run and between-day precision

** CI is not used to determine qualitative output for high negative samples, as high negative samples have SI < 1.0

*** Two replicates were invalid

† One replicate was invalid

Precision Study #4: Within-Laboratory Precision (Instrument-to-Instrument Variability)

Design: The study to evaluate instrument-to-instrument variability was based on CLSI EP05-A3. Each sample type was run on two instruments, over five (non-consecutive) days, with one run (plate) per day, and with five true replicates on each plate. A true replicate is the mean of the measurements from two duplicate wells on the plate. Samples were tested on each instrument on discrete plates, as independent runs. The study was conducted using a single lot of critical reagents and was performed on two instruments. A total of 50 replicates per sample were collected (5 days x 2 Instruments x 1 run/day x 5 replicates = 50 replicates per sample).

Table 16: Instrument Precision – Qualitative Results						
Sample type	N	Mean		% Detected Overall	% Detected Instrument 1	% Detected Instrument 2
		SI	CI			
High Negative	50	0.88	1.189	0/50= 0%	0/25= 0%	0/25= 0%
Cutoff	50	1.06	0.991	30/50= 60%	19/25= 76%	11/25= 44%
Low Positive	50	1.63	0.696	50/50= 100%	25/25= 100%	25/25= 100%
Mid Positive	50	2.06	0.512	50/50= 100%	25/25= 100%	25/25= 100%
High Positive	50	42.55	0.027	50/50= 100%	25/25= 100%	25/25= 100%

Table 17: Instrument Precision – SI values										
Sample type	N	Mean	Repeatability		Between-Instrument/Run		Between-Day		Total*	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Negative	50	0.88	0.030	3.4%	0.000	0.0%	0.012	1.4%	0.032	3.7%
Cutoff	50	1.06	0.041	3.8%	0.025	2.4%	0.000	0.0%	0.048	4.5%
Low Positive	50	1.63	0.051	3.1%	0.080	4.9%	0.026	1.6%	0.098	6.0%
Mid Positive	50	2.06	0.093	4.5%	0.080	3.9%	0.115	5.6%	0.168	8.2%
High Positive	50	42.55	3.149	7.4%	2.827	6.6%	2.310	23.0%	4.821	11.3%

*Total precision includes repeatability, between-instrument/run and between-day precision

Table 18: Instrument Precision – CI values										
Sample type	N	Mean	Repeatability		Between-Instrument/Run		Between-Day		Total*	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Negative **	50	1.189	0.0459	3.9%	0.0000	0.0%	0.0101	0.9%	0.0470	3.9%
Cutoff	50	0.991	0.0403	4.1%	0.0109	1.1%	0.286	2.9%	0.0506	5.1%
Low Positive	50	0.696	0.0289	4.1%	0.0092	1.3%	0.0186	2.7%	0.0356	5.1%
Mid Positive	50	0.512	0.0266	5.2%	0.0165	3.2%	0.231	4.5%	0.0390	7.6%
High Positive	50	0.027	0.0023	8.6%	0.0008	3.2%	0.0008	3.1%	0.0026	9.7%

*Total precision includes repeatability, between-instrument/run and between-day precision

** CI is not used to determine qualitative output for high negative samples, as high negative samples have SI < 1.0

Precision Study #5: Within-Laboratory Precision (Lot-to-Lot Variability)

Design: The study to evaluate critical reagent lot-to-lot variability was based on CLSI EP05-A3. Each sample type was run with three unique reagent lots, over six (non-consecutive) days, with one run (plate) per day, and with four true replicates on each plate. A true replicate is the mean of the measurements from two duplicate wells on the plate. Samples were tested with each reagent lot with one run per day on discrete plates, as independent runs. The study was run on a single instrument system by a single operator. A total of 72 replicates per sample were collected (6 days x 3 Lots x 1 run/day x 4 replicates = 72 replicates per sample).

Table 19: Critical Reagent Lot Precision – Qualitative Results							
Sample type	N	Mean		% Detected Overall	% Detected Lot 1	% Detected Lot 2	% Detected Lot 3
		SI	CI				
High Negative	72	0.85	1.195	0/72= 0%	0/24= 0%	0/24= 0%	0/24= 0%
Low Positive	71*	1.42	0.713	71/71= 100%	24/24= 100%	23/23= 100%	24/24= 100%
Mid Positive	72	6.21	0.162	72/72= 100%	24/24= 100%	24/24= 100%	24/24= 100%
High Positive	71*	42.04	0.026	71/71= 100%	24/24= 100%	24/24= 100%	23/23= 100%

*One replicate was invalid

Table 20: Critical Reagent Lot Precision – SI values									
Sample type	N	Mean	Repeatability		Between-Run/Day		Between-Lot		Total*	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Negative	72	0.85	0.022	2.6%	0.028	3.3%	0.000	0.0%	0.036	4.2%
Low Positive	71**	1.42	0.035	2.5%	0.034	2.4%	0.034	2.4%	0.060	4.2%
Mid Positive	72	6.21	0.192	3.1%	0.423	6.8%	0.409	6.6%	0.619	10.0%
High Positive	71**	42.04	1.087	2.6%	4.836	11.5%	3.074	7.3%	5.833	13.9%

* Total precision includes repeatability, between-run/day and between-lot precision

**One replicate was invalid

Table 21: Critical Reagent Lot Precision – CI values										
Sample type	N	Mean	Repeatability		Between-Run/Day		Between-Lot		Total*	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Negative**	72	1.195	0.042	3.5%	0.000	0.0%	0.044	3.7%	0.061	5.1%
Low Positive	71***	0.713	0.025	3.6%	0.010	1.3%	0.016	2.3%	0.032	4.5%
Mid Positive	72	0.162	0.009	5.5%	0.007	4.5%	0.012	7.4%	0.017	10.2%
High Positive	71***	0.026	0.001	4.9%	0.003	10.4%	0.002	7.9%	0.004	14.0%

* Total precision includes repeatability, between-run/day and between-lot precision

**CI is not used to determine qualitative output for high negative samples, as high negative samples have SI < 1.0

***One replicate was invalid

AAV5 DetectCDx Overall Precision

The tables below present estimates of the repeatability, between-run, between-day, between-operator, and between instrument components of precision using data from the studies described above (excluding between-lot study).

Table 22: Overall Precision AAV5 DetectCDx – SI values

Sample type	Mean	Repeatability†		Between-Run#		Between-Day*		Between-operator**		Between-instrument‡		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Negative	0.87	0.03	3.4%	0.032	3.7%	0.01	1.1%	0.038	4.4%	0.000	0.0%	0.06	6.8%
Cutoff	1.04	0.04	3.8%	0.045	4.3%	0.02	1.9%	0.037	3.6%	0.025	2.4%	0.08	7.5%
Low Positive	1.56	0.04	2.6%	0.069	4.4%	0.03	1.9%	0.087	5.6%	0.080	5.1%	0.15	9.3%
Mid Positive	1.96	0.06	3.1%	0.084	4.3%	0.13	6.6%	0.161	8.2%	0.080	4.1%	0.24	12.5%
High Positive	40.28	1.96	4.9%	3.182	7.9%	3.11	7.7%	3.974	9.9%	2.827	7.0%	6.89	17.1%

†Repeatability was estimated with pooling study1, study2, study3, study4, and between-production lot study (study details not shown).
#Between-run variation was estimated using study1.
*Between-day variation was estimated with pooling study1, study3, and study4.
**Between-operator variation was estimated using study3.
‡Between-instrument variation was estimated using study4.

Overall precision for SI for the AAV5 DetectCDx, including a between-lot component of precision, for samples near the SI cutoff is: 7.0% for High Negative and 13.5% for Low Positive.

Table 23: Overall Precision AAV5 DetectCDx – CI values

Sample type	Mean	Repeatability †		Between-Run#		Between-Day*		Between-operator**		Between-instrument‡		Total	
		SD	% CV	SD	%CV	SD	%CV	SD	%CV	SD	% CV	SD	%CV
High Negative ***	1.19	0.05	4.2%	0.039	3.3%	0.01	0.8%	0.0153	1.3%	0.0000	0.0%	0.07	5.5%
Cutoff	1.00	0.04	4.0%	0.058	5.8%	0.03	3.0%	0.0311	3.1%	0.0109	1.1%	0.08	8.3%
Low Positive	0.69	0.03	4.3%	0.025	3.6%	0.02	2.9%	0.0121	1.8%	0.0092	1.3%	0.05	6.7%
Mid Positive	0.53	0.03	5.7%	0.015	2.8%	0.05	9.4%	0.0219	4.1%	0.0165	3.1%	0.07	12.5%
High Positive	0.03	0.00	0.0%	0.002	7.4%	0.00	0.0%	0.0016	5.9%	0.0008	3.0%	0.00	9.9%

†Repeatability was estimated with pooling study1, study2, study3, study4, study5 between-production lot study (study details not shown).
#Between-run variation was estimated using study1.
*Between-day variation was estimated with pooling study1, study3, study4.
**Between-operator variation was estimated using study3.
‡Between-instrument variation was estimated using study4.
*** CI is not used to determine qualitative output for high negative samples, as high negative samples have SI < 1.0

Overall precision for CI for the AAV5 DetectCDx, including a between-lot component of precision, for samples near the CI cutoff is: 6.5% for High Negative and 12.9% for Low Positive.

4. Linearity study

Not applicable, qualitative assay.

5. Analytical Sensitivity/Detection Limit

Not applicable, as the AAV5 DetectCDx is a qualitative assay.
The detection capability of the AAV5 DetectCDx has been defined for internal quality control and qualification procedures.

6. Endogenous Interfering Substances (Analytical Specificity)

A study was performed based on guidance from *CLSI EP07 A3 – Interference Testing in Clinical Chemistry* and evaluated endogenous substances typically found in plasma samples and potential interferents to the assay. The study used a panel of three (3) plasma samples, designated as high negative, low positive, and high positive and generated from non-hemophilia A donors. The target assay output values are listed in the table below.

Table 24: Sample panel evaluated in analytical specificity studies		
Sample	SI Value	CI Value
	Target	Target
High Negative	< 1.00	~1.20
Low Positive	> 1.00	~0.80
High Positive	> 10.0	< 0.20

Five (5) replicates of each sample were tested at each concentration of each endogenous substance as per the recommended test concentration specified in Table 2 of *CLSI EP37 - Ed. 1 Supplemental Tables for Interference Testing in Clinical Chemistry* with the exception of triglycerides. An endogenous substance is not considered an interferent if addition of the test substance did not change the qualitative output of any of the treated samples compared to the control sample or the percent difference between treated samples and control sample was ≤ 10% for both SI and CI values. Of the endogenous substances evaluated, three were found to interfere at the top concentration(s) tested.

Table 25: Interfering endogenous substances	
Substance	Interfering test concentration(s)
Hemoglobin	1000 mg/dL
Rheumatoid Factor (RF)*	≥ 1285 IU/mL
Triglycerides	750 mg/dL

*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.

The following endogenous substances were not found to interfere with the AAV5 DetectCDx results at the indicated concentration(s).

Table 26: Non-interfering endogenous substances	
Substance	Non-interfering test concentration(s)
Albumin	6 mg/dL
Bilirubin, conjugated	40 mg/dL
Bilirubin, unconjugated	40 mg/dL
Hemoglobin	≤ 800 mg/dL
Rheumatoid Factor	≤ 476 IU/mL
Triglycerides	≤ 500 mg/dL

Cholesterol was not evaluated as a potential interferent to the AAV5 DetectCDx and the effect of this substance on the assay is unknown.

7. **Exogenous Interfering Substances (Analytical Specificity)**
A study was performed based on guidance from *CLSI EP07 A3 – Interference Testing in Clinical Chemistry* and evaluated exogenous substances to include anticoagulants, and concomitant medications commonly used by the patient population. The study used a panel of three (3) plasma samples, designated as high negative, low positive, and high positive and generated from non-hemophilia A donors. The target and mean assay output values are listed in Table 24 above.

Five (5) replicates of each sample were tested at each concentration of each exogenous substance as per the recommended test concentration specified in Table 1 of *CLSI EP37 - Ed. 1 Supplemental Tables for Interference Testing in Clinical Chemistry* or, for concomitant medications not listed in CLSI EP37, at levels based on the reported C_{max} values (3X C_{max} as highest concentration tested). An exogenous substance is not considered an interferent if addition of the test substance did not change the qualitative output of any of the treated samples compared to the control sample or the percent difference between treated samples and control samples was ≤ 10% for both SI and CI values. No exogenous substances were found to interfere with the assay at the concentration tested.

The following exogenous substances were not found to interfere with the AAV5 DetectCDx results at the indicated concentration(s).

Table 27: Non-interfering exogenous substances	
Substance	Test concentration
Oxycodone*	0.0324 mg/dL
Acetaminophen*	15.6 mg/dL
Naproxen*	36.0 mg/dL
Ibuprofen*	21.9 mg/dL
Omeprazole*	0.84 mg/dL
Atorvastatin*	0.075 mg/dL
Lisinopril*	0.0246 mg/dL
Bictegravir*	1.85 mg/dL
Tenofovir*	0.0978 mg/dL
Doravirine*	0.289 mg/dL

Table 27: Non-interfering exogenous substances	
Atazanavir*	1.95 mg/dL
Fexofenadine*	0.116 mg/dL
Vitamin C*	5.25 mg/dL
Biotin†	0.351 mg/dL
Heparin sodium*	330 IU/mL
Sodium citrate**	7.3%
Plasma concentrate (e.g. Hemofil-M)‡	150 IU/dL
Recombinant FVIII (e.g. Advate)‡	384 IU/dL
Extended half-life recombinant FVIII (e.g. Eloctate)‡	324 IU/dL
Emicizumab-kxwh (e.g. Hemlibra)‡	170 µg/mL

*Test concentration evaluated as recommended in Table 1 of CLSI EP37 Ed. 1.

** Anticoagulant sodium citrate at a concentration of 7.3%, to simulate a short draw 44% of the normal volume of blood, was not found to interfere with the assay. Higher concentrations of sodium citrate could not be evaluated with the AAV5 DetectCDx due to technical issues.

†Test concentration evaluated as recommended in *Testing for Biotin Interference in In Vitro Diagnostic Devices – Guidance for Industry.*

‡Test concentration evaluated is 3x Cmax.

Celecoxib (Celebrex) was not evaluated as a potential interferent to the AAV5 DetectCDx and the effect of this substance on the assay is unknown.

8. **Cross-reactivity study (Analytical Specificity)**

The AAV5 DetectCDx was not evaluated for potential interference from, or cross-reactivity to, AAV serotypes other than AAV5. The high degree of sequence similarity between capsid proteins of different AAV serotypes (Vandenberghe et al. 2009[8]) suggests that antibodies generated against one AAV serotype are likely to also bind other serotypes. Antibodies not specific to AAV5 may thus give a Detected result for the assay.

The AAV5 DetectCDx was not evaluated for potential interference from antibodies associated with other medical conditions. As such, it is not known if antibodies associated with other medical conditions (e.g. anti-HIV antibodies) can give a Detected result for the assay.

9. **Prozone/High-dose hook effect**

A high-dose hook study was performed to characterize the performance of the AAV5 DetectCDx assay when used to test a dilution series of specimens containing very high levels of AAV5 antibodies (SI ~ 90) that have the theoretical potential to cause a high-

[8] Vandenberghe, L. et al. 2009. Naturally occurring singleton residues in AAV capsid impact vector performance and illustrate structural constraints. Gene Therapy 16, 1416–1428.

dose hook effect. Samples with SI values > 90 were not evaluated for the potential of a high-dose hook effect. The study 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 an 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. Each dilution step was tested in three replicates using one lot of reagents. The results from this study indicated that there were no false negative (Not Detected) results observed for tested samples with high AAV5 titers, and that anti-AAV5 antibody at the elevated concentrations tested do not produce a prozone (hook) effect for the AAV5 DetectCDx.

It was noted that samples with higher SI values (SI > 90) are typically associated with CI values ≤ 1.00 to generate a "Detected" result. Since a potential prozone effect was not evaluated for samples with SI > 90, 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" to mitigate the risk of a possible false negative result from being reported.

10. Carryover study

A study was performed to evaluate the susceptibility of the AAV5 DetectCDx to within-assay sample carryover. The samples used in the study were composed of a negative sample (SI < 1.00 and CI > 1.00) and a high positive sample (SI between 50–85 and CI between 0.03–0.15). The sample set was used to create an alternating pattern of the negative and high positive samples in columns 3–10 of two (2) 96-well ECL immunoassay plates (coated). The two (2) 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. All negative sample replicates across both test plates had SI values of 0.8–0.9 (mean SI across replicates = 0.88) as expected (Not Detected qualitative results) with the exception of two replicates with SI values of 1.04. These two replicates generated CI values of 1.247 and 1.377, respectively, providing qualitative results that remained Not Detected. All (100%) negative and positive replicates on both plates provided the expected results (of Not Detected or Detected, respectively), demonstrating that the AAV5 DetectCDx meets the acceptance criteria and is not susceptible to within-assay plate carryover.

11. Sample Stability

A study was conducted to evaluate the effect of sample storage under various conditions and storage durations for whole blood and plasma samples. Samples evaluated in the study were plasma/whole blood samples from non-hemophilia donors handled under conditions intended/expected of patient samples.

Table 28: Sample Types Evaluated in Stability Studies				
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

All plasma samples evaluated in the study were stored in frozen storage (-70°C or colder) for a minimum of 24 hours prior to being subjected to test conditions, which mimics the condition in which plasma samples are expected to be handled prior to testing patient samples with the AAV5 DetectCDx.

Stability of the patient sample during collection and processing for use with the AAV5 DetectCDx was determined. The following plasma and whole blood collection stability claims are supported for use with the AAV5 DetectCDx:

Table 29: Sample Collection Stability	
Storage Condition	Duration of stability
Whole blood, room temperature (20° to 25°C)*	72 hours
Whole blood, refrigerated (2° to 8° C)*	72 hours
Plasma, room temperature (20° to 25°C)**	72 hours
Plasma, refrigerated (2° to 8° C)**	72 hours

*Stability prior to processing to plasma
**Stability post-processing to plasma and prior to freezing

Stability of the patient sample during transport to ARUP Laboratories for use with the AAV5 DetectCDx was determined. The following plasma sample transport stability claims are supported for use with the AAV5 DetectCDx:

Table 30: Plasma Sample Transport Stability*	
Transport Condition	Duration of stability
Room temperature/ambient	10 days
Refrigerated (with gel packs)	10 days
Frozen (on dry ice)	10 days
Frozen (ice pack)	7 days
Elevated temperature (37°C)	1 day

*Performance of the AAV5 DetectCDx has not been evaluated for plasma samples transported in tube types other than the ARUP Transport Tube (polypropylene).

The following plasma sample stability claims are supported for use with the AAV5 DetectCDx:

Table 31: Plasma Sample Stability*	
Storage Condition	Duration of stability
Room temperature (20° to 25°C)	72 hours
Refrigerated (2° to 8° C)	28 days
Frozen (-10°C)	12 months
Frozen (-70°C or colder)	12 months
Freeze/thaw cycles	7 events

*Performance of the AAV5 DetectCDx has not been evaluated for plasma samples stored in tube types other than the ARUP Transport Tube (polypropylene).

12. 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 AAV5 DetectCDx. Reagent stability studies were conducted as per *CLSI EP25-A – Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*, evaluating the performance of multiple vendor lots of each critical reagent using plasma samples from non-hemophilia donors (see Table 28 "Sample Types Evaluated in Stability Studies") with the AAV5 DetectCDx.

Table 32: Reagent Stability		
Reagent	Storage Condition	Duration of Stability
AAV5 Plate Components (AAV5 capsids*)	Frozen (-70°C)	12 months
AAV5 Run Control Set†	Frozen (-70°C)	12 months
Read Buffer (1X)	Room temperature (20° to 25°C)	12 months
AAV5 Coated Plate Set‡	Refrigerated (2° to 8° C)	7 days

*Used to make the AAV5 Coating Reagent, AAV5 Confirmatory Reagent, and the AAV5 Detection Reagent.
†The AAV5 Run Control Set is made up of the quality controls for use with the AAV5 DetectCDx, and includes a Negative Control (NEG), Low Positive Control (LPC), High Positive Control (HPC), and Cut point Control (CC).
‡Short-term/in-use stability of the 96-well plate coated with AAV5 Coating Reagent, the AAV5 Confirmatory Reagent, and the AAV5 Detection Reagent.

X. SUMMARY OF PRIMARY CLINICAL STUDY(IES)

The safety and effectiveness of the AAV5 DetectCDx 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). The results from this study were used to establish a reasonable assurance of safety and effectiveness of the AAV5 DetectCDx for the selection of adult hemophilia A patients for whom ROCTAVIAN treatment is being considered. Data from this clinical study were the basis for this PMA approval decision. A summary of the clinical study is presented below.

A. Study Design

A prospective, open-label, single-dose, single-arm, multicenter clinical study was initiated on December 19, 2017. A total of 134 subjects, aged 18 to 70 years, with severe hemophilia A were enrolled at multiple U.S. and Outside the United States (OUS) sites and received 6 x 10^{13} vg/kg body weight of ROCTAVIAN. The study was evaluated for success based on the correlation between negative AAV5 DetectCDx test results and responder status post-ROCTAVIAN treatment. The data analysis cutoffs are November 15, 2021 (2-year data) and November 15, 2022 (3-year data).

The Medical Monitor conducted ongoing reviews of individual subject safety, and an independent Data Monitoring Committee (DMC) conducted ongoing reviews of both safety and efficacy data. The DMC, consisting of independent experts in clinical trials, statistics, and hemophilia, convened regularly during the trial and had access to individual and aggregated FVIII activity levels, FVIII usage, and bleeding data as well as to all available safety data.

1. Clinical Inclusion and Exclusion Criteria

Inclusion criteria for patient enrollment in 270-301 study (abbreviated):

- Males ≥ 18 years of age with hemophilia A and residual FVIII levels ≤ 1 IU/dL as evidenced by medical history, at the time of signing the informed consent.
- Must have been on prophylactic FVIII replacement therapy for at least 12 months prior to study entry. High-quality, well-documented historical data concerning bleeding episodes and FVIII usage over the previous 12 months must have been available.
- Treated/exposed to FVIII concentrates or cryoprecipitate for a minimum of 150 Exposure Days (Eds).
- Must have been willing and able to provide written, signed informed consent after the nature of the study has been explained and prior to any study-related procedures.
- Must have had no previous documented history of a detectable FVIII inhibitor, and results from a Bethesda assay or Bethesda assay with Nijmegen modification of less than 0.6 Bethesda Units (BU) (or less than 1.0 BU for laboratories with a historical lower sensitivity cutoff for inhibitor detection of 1.0 BU) on two (2) consecutive occasions at least 1 week apart within the previous 12 months (at least one (1) of which should be tested at the central laboratory).
- Sexually active participants must have agreed to use an acceptable method of effective contraception.

Exclusion criteria for patient enrollment in 270-301 study (abbreviated):

- Detectable pre-existing antibodies to the AAV5 capsid.
- Any evidence of active infection or any immunosuppressive disorder, including HIV infection.
- Significant liver dysfunction with any of the following abnormal laboratory results:
  - -ALT (alanine aminotransferase) > 1.25x upper limit of normal (ULN);
  - -AST (aspartate aminotransferase) > 1.25x ULN;
  - -GGT (gamma-glutamyl transferase) > 1.25x ULN;
  - -Total bilirubin > 1.25x ULN;
  - -Alkaline phosphatase > 1.25x ULN; or

-INR (international normalized ratio) ≥ 1.4.

Subjects whose liver laboratory assessments fell outside of these ranges could have undergone repeat testing of the entire liver test panel within the same Screening window and, if eligibility criteria were met on retest, could be enrolled after confirmation by the Medical Monitor.

- Prior liver biopsy showing significant fibrosis of 3 or 4 as rated on a scale of 0-4 on the Batts-Ludwig (Batts 1995) or METAVIR (Bedossa 1996) scoring systems, or an equivalent grade of fibrosis if an alternative scale is used.
- Evidence of any bleeding disorder not related to hemophilia A.
- Platelet count of $< 100 \times 10^9$/L.
- Creatinine ≥ 1.5 mg/dL.
- Liver cirrhosis of any etiology as assessed by liver ultrasound.
- Chronic or active hepatitis B as evidenced by positive serology testing (HBsAg, HBsAb, and HBcAb) and confirmatory hepatitis B virus (HBV) DNA testing.
- Active hepatitis C as evidenced by detectable hepatitis C virus (HCV) RNA or currently on antiviral therapy.
- Active malignancy, except non-melanoma skin cancer.
- History of hepatic malignancy.
- History of arterial or venous thromboembolic events (e.g., deep vein thrombosis, non-hemorrhagic stroke, pulmonary embolism, myocardial infarction, arterial embolus), with the exception of catheter-associated thrombosis for which anti-thrombotic treatment is not currently ongoing.
- Known inherited or acquired thrombophilia, including conditions associated with increased thromboembolic risk, such as atrial fibrillation.
- Prior treatment with any vector or gene transfer agent.
- Use of systemic immunosuppressive agents, not including CS, or live vaccines within 30 days before the ROCTAVIAN infusion.

2. Follow-up Schedule

Disease assessment and other clinical assessments were conducted according to the protocol during the trial. Post-infusion and safety follow-up was conducted through 52-weeks post-infusion. Additional safety follow-up is conducted years two (2) through five (5) post-infusion. Patients receiving therapy should enroll in a 15-year registry to evaluate the long-term safety and efficacy of ROCTAVIAN.

3. Clinical Endpoints

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. All bleeding episodes, regardless of treatment, were counted towards the ABR. The pharmacodynamic effect of ROCTAVIAN was assessed by measuring circulating factor VIII activity levels.

B. Accountability of PMA Cohort

A total of 134 subjects were enrolled in the 270-301 study. All subjects were screened with the AAV5 DetectCDx assay and had a minimum follow-up post-infusion of 66 weeks with a median follow-up of 162 weeks (range: 66 to 255 weeks). Of the 134

subjects, 112 subjects previously participated in a non-interventional study (270-902) and had at least 6 months of prospectively collected baseline ABR data prior to enrollment (termed the rollover population). The remaining 22 patients had retrospectively collected baseline ABR data (termed the directly enrolled population). Both the rollover and the directly enrolled populations (all 134 subjects) are included in the safety population and in the analysis of FVIII activity, while only the rollover population (n=112) is included in the efficacy evaluable population.

C. Study Population Demographics and Baseline Parameters

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.

Table 33: 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)

D. Safety and Effectiveness Results

1. Safety Results

The AAV5 DetectCDx assay involves the testing of plasma processed from blood samples. Blood samples are routinely collected as part of the management of hemophilia A. Sample collection presents no additional safety hazard to the patient being tested.

Although the safety with respect to ROCTAVIAN treatment was addressed during the review of the BLA and is not addressed in detail in this SSED, safety data collected in study 270-301 have shown that ROCTAVIAN treatment was generally well-tolerated. Most common adverse reactions to ROCTAVIAN

(n=134) were nausea, fatigue, headache, abdominal pain, vomiting, and diarrhea and the most common laboratory abnormalities were elevations in ALT, AST, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), factor VIII activity levels, gamma glutamyl transferase (GGT), and bilirubin above upper limit of normal (ULN). The majority of adverse events (AEs) have been Grade 1 (mild) to Grade 2 (moderate) in intensity. Asymptomatic, transient ALT elevations (up to grade 3 in severity) were responsive to corticosteroid treatment. Infusion reactions (defined as adverse events occurring during and within 6 hours of ROCTAVIAN infusion) including anaphylaxis and other hypersensitivity reactions have occurred and were effectively mitigated by managing the infusion rate and treating with supportive medications. Although elevated factor VIII activity levels beyond the upper limit of normal have occurred, no thromboembolic events attributable to ROCTAVIAN have been reported, and no subjects have developed clinically meaningful anti-FVIII inhibitors. One subject with a history of hepatitis C and steatohepatitis was diagnosed with autoimmune hepatitis at the third year follow-up. No participants discontinued from studies as a result of a treatment emergent adverse event (TEAE). There are no long-term safety data. The long-term safety of ROCTAVIAN therapy is unknown. Please refer to ROCTAVIAN labeling for additional safety information on the treatment.

2. Effectiveness Results

The efficacy performance of the AAV5 DetectCDx as a companion diagnostic device for the detection of AAV5 antibodies in human plasma collected in 3.2% sodium citrate to aid in the selection of hemophilia A patients for treatment with ROCTAVIAN is based on data from 134 subjects in study 270-301 who had a "Not Detected" result.

The AAV5 DetectCDx test is used to help determine eligibility of hemophilia A patients for ROCTAVIAN treatment by identifying patients who are "Not Detected" for pre-existing anti-AAV5 antibodies, which may reduce transduction efficiency of the gene therapy. The efficacy data of ROCTAVIAN are used to evaluate the benefit of AAV5 DetectCDx. The effectiveness of AAV5 DetectCDx is determined based on the correlation between "Not Detected" AAV5 DetectCDx results and the responder status post-ROCTAVIAN treatment.

To evaluate the efficacy and safety of ROCTAVIAN, the sponsor conducted a prospective, open-label, single-dose, single-arm, multinational study (270-301 study) in adult male patients with severe hemophilia A. Adult hemophilia A patients in the study received a single intravenous dose of 6×10^{13} vg/kg body weight of ROCTAVIAN. The NI analysis met the pre-specified NI margin (set at 3.5 bleeds per year) in the efficacy evaluable population of 112 patients, indicating the effectiveness of ROCTAVIAN. Factor VIII activity levels post-ROCTAVIAN infusion showed inter-individual variability. Factor VIII activity, as measured by the chromogenic substrate assay (CSA), demonstrated mean (SD) and median (range) values of 25.0 (35.5) and 12.7 (5.1, 26.5), and 21.0 (34.0) and

10.0 (4.3, 19.8) at months 24 and 36 respectively (data from 98 and 96 of 112 patients at 24- and 36-month timepoints). Factor VIII activity analyzed using the one-stage assay clotting assay (OSA) showed higher values compared to the CSA. The results from this study support the clinical benefit of the AAV5 DetectCDx in the selection of hemophilia A patients for treatment with ROCTAVIAN.

3. Subgroup Analyses

Subgroup analysis was not performed for the 270-301 study due to limited sample sizes.

4. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included one investigator at the single test site ARUP Laboratories. The clinical investigator did not have disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Expected Values

The ROCTAVIAN clinical development program consists of six (6) interventional studies (including 270-301) and two (2) non-interventional studies. The AAV5 DetectCDx statistical analysis was designed to demonstrate that the device is appropriate for its intended use and purpose. For this analysis, a number of patient population demographic variables were analyzed for their potential association with assay results (Detected vs Not Detected).

Table 34: 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).

Table 35: 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%).

Table 36: 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.

XII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Hematology Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical effectiveness of the AAV5 DetectCDx assay was demonstrated in study 270-301, a prospective, open-label, single-dose, single-arm, multinational study consisting of 134 hemophilia A patients at multiple U.S. and OUS sites. A single intravenous dose of 6×10^{13} vg/kg body weight of ROCTAVIAN met the per-specified non-inferiority margin, 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.

B. Safety Conclusions

Patients with false positive results were not enrolled in the study to receive ROCTAVIAN treatment, and would continue with the current standard of care. The risk associated with a false positive result is minimal. Patients with false negative results would have been inappropriately determined eligible for the treatment. The benefit from gene therapy in the presence of pre-existing anti-AAV5 antibodies is unclear, however the patients may be exposed to potential short-term and long-term risks of therapy.

Although the safety with respect to ROCTAVIAN treatment was addressed during the review of the BLA and is not addressed in detail in this SSED, safety data collected in study 270-301 have shown that ROCTAVIAN treatment was generally well-tolerated. The long-term safety of ROCTAVIAN therapy is unknown.

C. Benefit-Risk Determination

The probable benefits of the device are based on data collected in study 270-301 as described above and the data from the study support the effectiveness of ROCTAVIAN.

The probable risks of the device are also based on data collected in study 270-301. The data have also shown a favorable safety and tolerability profile for ROCTAVIAN. The majority of AEs have been Grade 1 (mild) to Grade 2 (moderate) in intensity. No subjects have withdrawn from the study as a result of an AE.

Infusion-associated events (defined as adverse events occurring during and within 48 hours of ROCTAVIAN infusion) and ALT elevations were the most commonly reported treatment-related AEs. No thromboembolic events have been reported, and no subjects have developed clinically meaningful anti-FVIII inhibitors.

Additional factors, including the rarity of severe hemophilia A, the ability to manage false positive patients with standard of care and the lack of alternative testing method are considered in the assessment of benefit-risk. Additionally, the current device fills an unmet medical need for more effective gene therapy treatment of severe hemophilia A, an irreversibly debilitating disease.

When considering the above factors and additional mitigations provided by appropriate labeling, the probable benefit of this device outweighs the probable risk, and the data provide a reasonable assurance of safety and effectiveness for the proposed indications for use.

1. Patient Perspective

   This submission either did not include specific information on patient perspectives or the information did not serve as part of the basis of the decision to approve or deny the PMA for this device.

In conclusion, given the available information above, the data support that probable benefit of use of this device to identify adult hemophilia A patients without pre-existing anti-AAV5 antibodies for eligibility to receive ROCTAVIAN outweighs the probable risk associated with the device, when considering the mitigations provided by appropriate labeling.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use.

XIV. CDRH DECISION

CDRH issued an approval order on June 29, 2023.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

Boutin S, Monteilhet V, Veron P, Leborgne C, Benveniste O, Montus MF, Masurier C. Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors. Hum Gene Ther. 2010 Jun;21(6):704-12. doi: 10.1089/hum.2009.182. PMID: 20095819.

Klamroth R, Hayes G, Andreeva T, Gregg K, Suzuki T, Mitha IH, Hardesty B, Shima M, Pollock T, Slev P, Oldenburg J, Ozelo MC, Stieltjes N, Castet SM, Mahlangu J, Peyvandi F, Kazmi R, Schved JF, Leavitt AD, Callaghan M, Pan-Petesch B, Quon DV, Andrews J, Trinh A, Li M, Wong WY. Global Seroprevalence of Pre-existing Immunity Against AAV5 and Other AAV Serotypes in People with Hemophilia A. Hum Gene Ther. 2022 Apr;33(7-8):432-441. doi: 10.1089/hum.2021.287. Epub 2022 Mar 16. PMID: 35156839; PMCID: PMC9063149.

Kruzik A, Fetahagic D, Hartlieb B, Dorn S, Koppensteiner H, Horling FM, Scheiflinger F, Reipert BM, de la Rosa M. Prevalence of Anti-Adeno-Associated Virus Immune Responses in International Cohorts of Healthy Donors. Mol Ther Methods Clin Dev. 2019 Jun 7;14:126-133. doi: 10.1016/j.omtm.2019.05.014. PMID: 31338384; PMCID: PMC6629972.

Shankar G, Devanarayan V, Amaravadi L, Barrett YC, Bowsher R, Finco-Kent D, Fiscella M, Gorovits B, Kirschner S, Moxness M, Parish T, Quarmby V, Smith H, Smith W, Zuckerman LA, Koren E. Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J Pharm Biomed Anal. 2008 Dec 15;48(5):1267-81. doi: 10.1016/j.jpba.2008.09.020. Epub 2008 Sep 19. PMID: 18993008.

Vandenberghe LH, Breous E, Nam HJ, Gao G, Xiao R, Sandhu A, Johnston J, Debyser Z, Agbandje-McKenna M, Wilson JM. Naturally occurring singleton residues in AAV capsid impact vector performance and illustrate structural constraints. Gene Ther. 2009 Dec;16(12):1416-28. doi: 10.1038/gt.2009.101. PMID: 19727141; PMCID: PMC2795093.

World Federation of Hemophilia (WFH). Report on the Annual Global Survey 2018. Available at https://www1.wfh.org/publications/files/pdf-1731.pdf as of 7 March 2023.