Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Table of Contents **See Page**

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Abbreviations

Term	Description
%CV	Percent Coefficient of Variation
AAV5	adeno-associated virus serotype 5
AAV5 TAb Assay	adeno-associated virus serotype 5 Total Antibody Assay
BMN 270	Biomarin 270 (valoctocogene roxaparvovec)
CC5	Confirmatory Cut Point
CI	Confirm Index
DPBS	Dulbecco's Phosphate Buffered Saline
ECL	Electrochemiluminescence
HPC5	High Antibody Positive Control
ITRS	Inverted Terminal Repeats
LoB	Limit of Blank
LoD	Limit of Detection
LPC5	Low Antibody Positive Control
MSD	Meso Scale Diagnostics
NEG5	Negative Control
QA	Qualitative Agreement
RF	rheumatoid factor
RPM	Rotations Per Minute
RUO	Research Use Only
SD	Standard Deviation
SI	Screen Index
TAb	Total Antibody
TPA	tripropylamine
TBS-C	Tris-Buffered Saline with 1% casein

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

1. Proprietary Name

- **Device Trade Name:** AAV5 DetectCDx™ Kit
- **Device Generic Name:** AAV5 Total Antibody (TAb) Assay for Valoctocogene Roxaparvovec Eligibility in Hemophilia A
- **INN (International Non-Proprietary Name):** Valoctocogene Roxaparvovec

2. Intended Purpose

The AAV5 DetectCDx™ Kit for Valoctocogene Roxaparvovec Eligibility in Hemophilia A is a qualitative *in vitro* diagnostic test by electrochemiluminescence intended for detection of antibodies in human plasma collected in 3.2% sodium citrate that binds to AAV5. The AAV5 TAb Assay is indicated as an aid in the selection of adult Hemophilia A patients for whom Valoctocogene Roxaparvovec treatment is being considered. Patients that are anti-AAV5 antibody positive (result of "Detected") are not eligible for treatment with Valoctocogene Roxaparvovec; patients that are anti-AAV5 antibody negative (result of "Not Detected") are eligible for treatment with Valoctocogene Roxaparvovec.

3. Intended Use

The AAV5 DetectCDx™ Kit is a distributable *in vitro* diagnostic test used for screening adult Hemophilia A patients for the presence of anti-AAV5 antibodies as an aid in determining eligibility for Valoctocogene Roxaparvovec.

4. Intended User

The AAV5 DetectCDx™ Kit is intended for use by laboratory healthcare professionals only.

5. Contraindications

None

6. Warnings and Precautions

Please read the Instructions for Use carefully prior to starting the assay procedure and follow each step closely.

- IVD This product is for *In Vitro* Diagnostic Use.
- When drawing blood for the AAV5 DetectCDx™ Kit, universal precautions for bloodborne pathogens should be observed.
- Patient samples with triglyceride levels greater than 500 mg/dL will interfere with the ability for the AAV5 DetectCDx™ Kit 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™ Kit to accurately detect anti-AAV5 antibodies.
- Patient samples with levels of rheumatoid factor (RF) greater than 500 mg/dL will interfere with the ability of the AAV5 DetectCDx™ Kit to accurately detect anti-AAV5 antibodies.
- Patient samples with levels of Vitamin C greater than 5.25 mg/dL will interfere with the ability of the AAV5 DetectCDx™ Kit to accurately detect anti-AAV5 antibodies.

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

- Collected patient samples must not exceed 7.3% sodium citrate as higher concentrations could not be evaluated.
- Follow standard precautions. All patient specimens and antigen sets and run controls should be considered potentially infectious and handled accordingly.
- Proper personal protective equipment including lab coats, gowns, gloves, eye protection is recommended.
- Use of non-recommended reagent volumes and concentrations may result in a loss of performance and may also decrease the reliability of the test results.
- Use of non-recommended consumables may decrease the reliability of the test results.
- Use of non-recommended equilibration and incubation times as well as temperatures may result in a loss of performance and may also decrease the reliability of the test results.
- Do not mix reagents from different lots or kits.
- Do not use run control acceptance ranges other than those lot-specific ranges provided in the statement of release document included with each kit.
- Do not modify any reagents. Use reagents as received from manufacturers.

7. Limitations

- IVD For *in vitro* diagnostic use
- For professional use only
- For prescription use only

8. Summary and Explanation of the Test

Valoctocogene Roxaparvovec, or AAV5-hFVIII-SQ drug product, is a gene therapy treatment for severe Hemophilia A, an X-linked recessive bleeding disorder that affects approximately one (1) in 5,000 males. Hemophilia A is caused by a 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.

Valoctocogene Roxaparvovec 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. Valoctocogene Roxaparvovec is delivered by a single intravenous dose and is designed to achieve stable, potentially life-long, expression of active FVIII in the plasma, synthesized from vector-transduced liver tissue.

Since pre-existing anti-AAV5 antibodies may neutralize Valoctocogene Roxaparvovec, only patients who demonstrate no detectable anti-AAV5 antibodies will be eligible for treatment with Valoctocogene Roxaparvovec. This approach is based on prior studies in other systems (capsids,

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

cassettes, levels of purity, etc.). 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). In this study, patients with various levels of pre-existing neutralizing antibodies (NAb) against the AAV2 capsid were treated with up to 2E10+12 vg/kg. A single patient with elevated NAb (1:17) against the AAV2 vector exhibited reduced expression of the FIX transgene compared to another subject with a lower NAb titer (1:2).

To evaluate whether or not pre-existing AAV5 antibodies preclude the possibility of achieving efficacy with Valoctocogene Roxaparvovec, a pre-clinical non-human primate investigation was undertaken to test this hypothesis and clarify the predictive value of the pre-existing AAV5 TAb screening assay. Data indicated that animals could be pre-screened and enrolled based upon the AAV5 TAb assay results to predict those most likely to respond to Valoctocogene Roxaparvovec. Therefore, to maximize the likelihood of establishing proof-of-concept in the Valoctocogene Roxaparvovec clinical trials, only Hemophilia A patients with no detectable pre-existing antibodies against the AAV5 vector were enrolled.

The AAV5 DetectCDx™ Kit uses a bridging immunoassay to detect antibodies to AAV5 in human sodium citrated (3.2%) plasma specimens. The AAV5 DetectCDx™ Kit uses a combination of concurrently conducted screening and confirmatory assay modes to reliably detect antibodies specific for AAV5 capsid. The screening mode assesses for the presence of anti-AAV5 antibodies, while the confirmatory mode determines if the electrochemiluminescence (ECL) signal is specific. In the confirmatory mode of the assay, samples are incubated with an excess amount of unlabeled capsid (referred to as AAV5 Confirmatory Reagent) prior to addition to the AAV5 Coated Plate. If anti-AAV5 antibodies are present, they will bind to the unlabeled AAV5 capsid greatly reducing the amount of anti-AAV5 antibody available for binding to the Coated Plate, resulting in a reduced ECL signal for the confirmatory mode of the assay as compared to the screening mode.

9. Principles of The Procedure

A MULTI-ARRAY® 96-well plate is coated with unlabeled AAV5-CMV-GFP capsid, washed, blocked with assay buffer 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. The SULFO-TAG capsid binds to the anti-AAV5 antibodies already bound to the unlabeled capsid coating the plate thus forming a bridge (Figure 1). The SULFO-TAG capsid contains a ruthenylated moiety that allows it to participate in an ECL reaction. After incubation and washing, tripropylamine (TPA) substrate is added to each well, which when electrically stimulated an ECL signal is produced and is measured on a Research Use Only (RUO) plate reader.

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

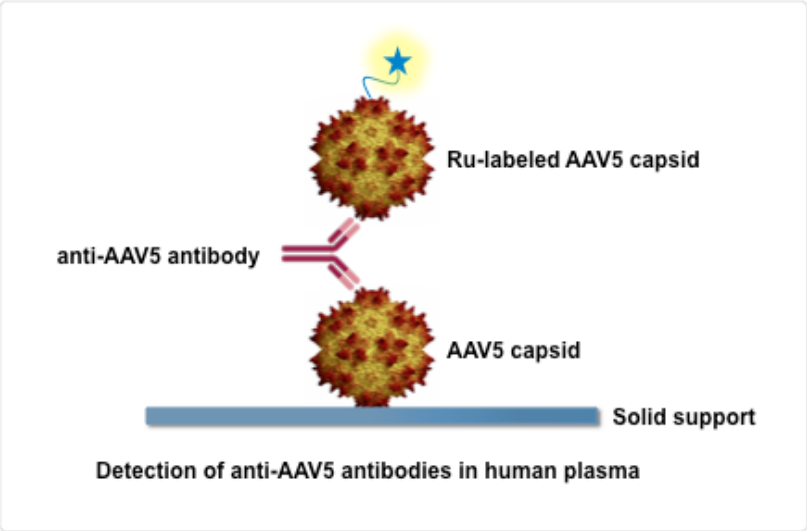


Figure 1. Anti-AAV5 antibody forms a bridge between AAV5 capsid coating the immunoassay plate and ruthenylated AAV5 capsid. The Ru-label participates in the generation of an electrochemiluminescent signal indicating the presence of anti-AAV5 antibodies

Each 96-well plate includes a cut point control (CC5), negative control (NEG5), a low antibody positive control (LPC5), and a high antibody positive control (HPC5). For run acceptance, the NEG5, CC5, HPC5, and LPC5 must meet the pre-established criteria for between-well coefficient of variation (CV) for replicate wells. The HPC5 and LPC5 must screen and confirm positive, and the HPC5, LPC5, and NEG5 signals must fall within the established acceptance range.

Results for the screening mode are expressed as a Screen Index (SI). If the SI < 1.00 the sample is deemed negative for anti-AAV5 antibodies and is reported as “Not Detected”. The results of the confirmatory mode are not considered when SI < 1.00. If the SI ≥ 1.00, the results of the confirmatory mode are considered. Results for the confirmatory mode are expressed as a Confirm Index (CI). If the CI > 1.00 the sample is considered negative for anti-AAV5 antibodies and is reported as “Not Detected”. If the CI ≤ 1.00, the sample is deemed positive for anti-AAV5 antibodies and is reported as “Detected” (see **Figure 2**).

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

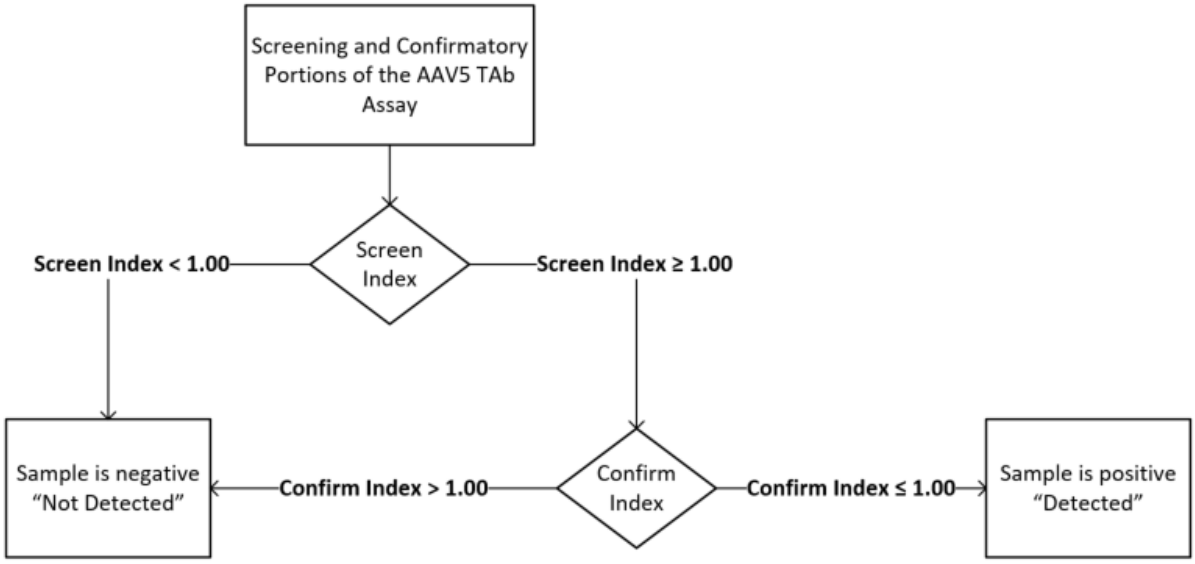


Figure 2. Summary of Resulting and Reporting for the Two-Step AAV5 DetectCDx™ Assay

10. Reagents and Materials

10.1. Materials Provided in the AAV5 DetectCDx™ Kit

The primary reagents for the AAV5 DetectCDx™ Kit (shown in **Table 1**) are usable until the labeled expiration date.

Table 1. Primary Reagents for AAV5 DetectCDx™ Kit

Reagent Name	Storage Temperature	Expiration
AAV5 Antigen Reagent Set	-70 ºC	TBD
AAV5 Run Control Set	-70 ºC	TBD

10.2. Materials Required But Not Provided

Note: These reagents are not to be modified in any way and are to be used as received from manufacturer(s)

- Tris-Buffered Saline with 1% casein (TBS-C, 1X)
- Dulbecco's Phosphate Buffered Saline (DPBS,10X)
- ProClin 300
- Meso Scale Diagnostics (MSD) Read Buffer 4X
- Refrigerator (2 to 8 °C)
- Freezer (-10 °C or colder)
- Freezer (-70 °C or colder)
- Vortex mixer
- Minifuge

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

- Microplate shaker capable of reaching 400 Rotations Per Minute (RPM)
- Single channel and multi-channel pipette sets
- Pipette tips, filtered pre-sterilized
- Reagent reservoirs, sterile disposable
- Microplate adhesive film
- PCR aluminum sealing film
- 0.2 mL and 1.2 mL 8-well strip tubes with cap
- 5 mL and 15 mL polypropylene tubes with screw caps, amber and clear, preferably low protein binding type

11. Equipment Required but not Provided

Note: Prior to use, ensure that instrument and equipment have been maintained and calibrated according to the manufacturer's recommendations.

- Meso Scale Diagnostics MESO QuickPlex SQ 120 system w/ Discovery Workbench v4.0 Software
- Bio-Tek 405 LS Microplate washer

12. Specimen Collection and Preparation

The AAV5 DetectCDx™ Kit is used with human blood samples collected in 3.2% sodium citrate. The blood collection tube must be filled per the manufacturer's guidelines to prevent an elevated concentration of sodium citrate in the collected sample. Samples that exceed 7.3% sodium citrate cannot be evaluated and may require a patient redraw. Plasma must be separated from whole blood within 72 hours of collection. A delay in this step could potentially convert samples to "Not Detected". Refrigerate plasma at 2 to 8 ºC within 48 hours of processing. A delay in this step could potentially convert samples to "Not Detected". Specimens may be refrigerated for up to 72 hours before freezing.

12.1. Transport

Specimens are shipped in an insulated box containing dry ice. Although specimens may be stable under other conditions during transport, frozen specimens are preferred to maximize stability. Specimens should be received within 11 days of shipping on dry ice.

13. Ordering the AAV5 DetectCDx™ Kit

To order the AAV5 DetectCDx™ Kit contact Associated Regional and University Pathologists (ARUP) Laboratories.

14. Assay Procedure

14.1. Day One (1): Plate Coating Procedure

1. Obtain the required amount of AAV5 Coating Reagent from AAV5 Antigen set and DPBS Buffer (1X).
2. Allow AAV5 Coating Reagent to equilibrate to room temperature (20 to 25 °C) for a minimum of 30 minutes and no longer than 2 hours.

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

3. Prepare AAV5 Plate Coating Reagent:
   - Aliquot 3100 µL DPBS Buffer (1X) into an appropriately sized tube.
   - Add 100 µL AAV5 Coating Reagent.
   - Gently vortex mix for 5-10 seconds.

   Note: Each tube of AAV5 Coating Reagent makes one (1) plate. Making more than three (3) plates at a time is not recommended.
4. Add 30 µL of the prepared AAV5 Coating Reagent to each well of the MULTI-ARRAY® 96-well plate(s). Gently tap the individual sides of the plate moving clockwise and counterclockwise on a solid surface to ensure each well is completely covered with AAV5 Plate Coating Reagent. Visually confirm that all wells are completely covered.

   Note: An electronic 8-channel pipette is recommended for dispensing the AAV5 Coating Reagent.
5. Label plates with preparation date.

   Note: Coated plates expire 7 days after preparation and storage at (2 to 8 °C).
6. Seal MULTI-ARRAY® plate(s) with microplate adhesive film and incubate on a microplate shaker (setting: 400 RPM at room temperature (20 to 25 °C) for 60 ± 5 minutes).
7. Wash AAV5 coated plate(s) with ECL Wash Buffer using a plate washer programmed according to the steps shown in **Appendix A**.

   Note: The plate washer program defined in **Appendix A** will be used for all Coated Plate preparation and assay procedure wash steps.
8. Invert MULTI-ARRAY® plate(s) and tap firmly on absorbent towels to remove excess liquid from wells.
9. Add 200 µL of TBS-C (1X) to all columns on the MULTI-ARRAY® 96-well plate(s) using an 8-channel pipette.
10. Seal MULTI-ARRAY® plate(s) with microplate adhesive film and incubate on a microplate shaker (setting: 400 RPM at room temperature (20 to 25 °C) for 60 ± 10 minutes).
11. Wash AAV5 coated plate(s) with ECL Wash Buffer using the appropriate wash program.
12. Invert MULTI-ARRAY® plate(s) and tap firmly on absorbent towels to remove excess liquid from wells.
13. Seal MULTI-ARRAY® plate(s) with aluminum adhesive film
14. Store AAV5 MULTI-ARRAY® 96-well Coated Plate(s) in a refrigerator (2 to 8 °C) for a minimum of 12 hours up to a maximum of 7 days before use.

14.2. Day Two (2): Specimen Preparation Procedure

15. Obtain the required amount of AAV5 Coated Plates from Day One (1), AAV5 Confirmatory and Detection Reagents, AAV5 Total Antibody Assay Run Controls, TBS-C (1X), and the patient plasma sample(s) to be analyzed.

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

16. Allow reagents to equilibrate to room temperature (20 to 25 °C) for a minimum of 30 minutes and no longer than 2 hours.
17. Prepare AAV5 Confirmatory Reagent:
    - Aliquot 3700 µL TBS-C (1X) into appropriately sized tube.
    - Add 100 µL AAV5 Confirmatory Reagent.
    - Gently Vortex 5-10 seconds
18. Prepare AAV5 Detection Reagent:
    - Aliquot 3100 µL TBS-C (1X) into appropriately sized tube
    - Add 100 µL AAV5 Detection Reagent
    - Gently Vortex 5-10 seconds
19. Pulse-vortex the patient samples for a minimum of 10 seconds and centrifuge briefly prior to setting up the required stock 0.2 mL 8-well strip tube.
20. Transfer 25 µL of the patient samples to labeled 0.2 mL 8-well strip tubes in the order they will be placed into the plate as per plate layout (see suggested plate layout in **Figure 3**).

    Note: It is recommended to use 0.2 mL strip tubes to avoid sample mix-ups in subsequent assay steps. Up to 16 specimens can be assessed per AAV5 Coated Plate Set for a maximum of two (2) 8-well sample strip tubes as shown in **Figure 3**.

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Figure 3. Suggested plate map

21. Transfer 15 µL of each AAV5 Run Control to the designated tubes (as per plate layout) of a labeled 0.2 mL strip tube. It is recommended the CC5 and NEG5 are dispensed prior to the HPC5 and LPC5, capping the tubes after each run control addition.
22. Create two (2) sets of 1.2 mL dilution tubes for each patient sample strip tube. Create one (1) set of 1.2 mL dilution tubes for the run controls.
    - One (1) set of sample dilution tubes is to be used for patient sample Screening mode and one (1) set is to be used for the patient sample Confirmatory mode
    - Both Screening and Confirmatory dilutions are performed using one (1) dilution strip tube for the run controls
23. Aliquot 190 µL of TBS-C (1X) into the patient sample and control screening dilution tubes and aliquot 190 µL of AAV5 Confirmatory Reagent into the patient sample and control confirmatory dilution tubes.
24. Aliquot 10 µL of the samples and run controls into the appropriate Screening and Confirmatory dilution strip tubes using an 8-tip multi-channel pipette. Rinse any residual sample or control volume from the pipette tips by aspirating and dispensing three (3) times in the sample diluent tubes being careful to avoid cross-contamination.

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

25. Gently vortex-mix the sample and control dilution strip tubes for a minimum of 10 seconds being careful to avoid cross-contamination. Cover the strip tubes and incubate them at room temperature (20 to 25 °C) for 55 to 60 minutes.

    Note: Allow a minimum of 5 minutes between sample incubation start times if more than one (1) plate is being run at a time. Creating a 5 minute gap between plates allows for adequate time for pipetting in subsequent assay steps.

14.3. Coated Plate Preparation

Note: Coated Plate preparation should take place during the incubation step of the sample preparation procedure (step 25), to ensure that the samples and controls are incubated for no longer than 60 minutes.

26. Remove the AAV5 Coated Plate from the appropriate storage location (2 to 8 ºC).
27. Wash the AAV5 Coated Plate with ECL Wash Buffer using the appropriate wash program.
28. Invert the AAV5 Coated Plate on to an absorbent towel(s) and tap firmly to remove excess liquid from the plate wells.

14.4. Sample Analysis Procedure

Note: It is recommended to use an 8-channel pipette to avoid sample mix-ups and to maintain consistent incubation times between the wells of the plate for all steps of the assay procedure.

29. Using reverse pipetting technique, aliquot 30 µL of the prepared sample and control dilutions into the washed AAV5 Coated Plate based on self-generated or suggested plate map (**Figure 3**).
30. Gently tap the individual sides of the plate on a solid surface to ensure that the bottom of each well is completely covered with the added sample volume.
31. Seal the AAV5 Coated Plate with microplate adhesive film and incubate at room temperature (20 to 25 °C) on a microplate shaker at 400 RPM for 60±2 minutes.
32. Wash the AAV5 Coated Plate with ECL Wash Buffer using the appropriate wash program.
33. Invert the AAV5 Coated Plate on to an absorbent towel(s) and tap firmly to remove excess liquid from the plate wells.
34. Using reverse pipetting technique, aliquot 30 µL of AAV5 Detection Reagent to all wells of the AAV5 Coated Plate.
35. Gently tap the individual sides of the plate on a solid surface to ensure that the bottom of each well is completely covered with the added sample volume.
36. Seal the AAV5 Coated Plate with adhesive aluminum film and incubate at room temperature (20 to 25 °C) on a microplate shaker at 400 RPM for 60±2 minutes.
37. Wash the AAV5 Coated Plate with ECL Wash Buffer using the appropriate wash program.

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

38. Invert the AAV5 Coated Plate on to an absorbent towel(s) and tap firmly to remove excess liquid from the plate wells.
39. Aliquot 150 µL of Read Buffer T (1X) into each well of the AAV5 Coated Plate.

    Note: The AAV5 Coated Plate must be analyzed within 5 minutes of Read Buffer T (1X) addition.

14.5. Coated Plate Analysis Procedure

Note: Use of software other than the vendor installed operating software for the MESO QuickPlex SQ 120 to generate the data for the controls or samples will violate the safety and effectiveness of this assay.

40. Read the AAV5 Coated Plate using the MESO™ QuickPlex SQ 120 instrument.
41. The MESO™ QuickPlex SQ 120 result file will need to be saved as a read-only file to a computer or network location.
42. Refer to the MESO™ QuickPlex SQ 120 instruction manual provided by the instrument manufacturer for the operation of the MESO™ QuickPlex SQ 120.

14.6. Calculations

43. All rounding to set decimal places must use Banker's Rounding rules.
44. Calculate mean and Coefficient of Variation (%CV) for samples and controls
    - Calculate the average electrochemiluminescence (ECL) signal and %CV to one (1) decimal place for each duplicate set of controls (HPC5, LPC5, NEG5), each duplicate set of samples, and the six cut point controls (CC5) in the screening mode wells.
    - Calculate the average ECL signal and %CV to one (1) decimal place for each duplicate set of positive controls (HPC5, LPC5) and each duplicate set of samples in the confirmatory mode wells.
45. Screen Index Calculation: The SI for each control (HPC5, LPC5, NEG5) and each sample is calculated by dividing the control or sample specific ECL mean for the Screening Mode wells by the Cut Point Control ECL mean. The result is divided by 1.14 to equal the Screen Index (calculated to two (2) decimal places) for the given control or sample. See equation below:

$$\frac{\left(\frac{mean\ sample\ specific\ Screening\ mode\ ECL\ value}{mean\ CC\ ECL\ value}\right)}{1.14} = Screen\ Index$$

46. Confirm Index Calculation: The CI for the positive controls (HPC5, LPC5) and each sample is calculated by dividing the control or sample specific ECL mean for the Confirmatory Mode wells by the mean from the corresponding Screening Mode wells multiplied by 0.707. The formula for CI is shown below (calculated to three (3) decimal places):

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

$$\frac{mean\ Confirmatory\ mode\ ECL}{(mean\ Screening\ mode\ ECL\ x\ 0.707)} = Confirm\ Index$$

47. Optional CC5 Outlier Removal: If the %CV for the CC5 controls is > 25% the Dixon Q test can be used to identify a single outlier to remove from the %CV calculation for CC5. Calculate a Q-test for each suspected outlier in the cut point control using the following formula (calculated to three (3) decimal places):

$$\text{Q test} = \left| \frac{(suspect\ value - closest\ value)}{(highest\ value - lowest\ value)} \right|$$

48. If the result from the Q test outlier calculation for a set of six (6) CC well signals is greater than 0.625 (Q test α = 0.05 for six (6) values), then the identified outlier can be excluded. A maximum of one outlier may be removed per run. The %CV of the mean ECL values for the remaining CC5 results must be ≤ 25% after outlier removal. If the %CV of the CC5 wells is >25% after removal of a single outlier, then the entire run will fail.

15. Interpretation of Results

15.1. AAV5 Run Control Interpretation

Controls must meet the criteria in **Table 2**. If any one of these criteria are not met, the run must be rejected and repeated.

Table 2. Criteria for Run Controls

Control	Criteria
HPC5	%CV for duplicate wells must be ≤ 25%
	Must have SI ≥ 1.00
	Must have CI ≤ 1.00
	Must be within lot-specific defined range[1]
LPC5	%CV for duplicate wells must be ≤ 25%
	Must have SI ≥ 1.00
	Must have CI ≤ 1.00
	Must be within lot-specific defined range[2]
NEG5	%CV for duplicate wells must be ≤ 25%
	Must have SI < 1.00
CC5	%CV for all cut point control wells must be ≤ 25%[2]

[1] Use the statement of release lot-specific defined acceptance ranges provided in each kit.

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

[2] The Dixon Q test may be used to deactivate an outlier CC5 sample if %CV > 25%.
The Dixon Q test to determine outliers uses the following formula**:**

$$Q = \frac{|suspect\ value - closest\ value|}{highest\ value - lowest\ value}$$

If the result from the Dixon Q test is greater than 0.625 (Q test α = 0.05 for six (6) values), then the identified outlier will be excluded**.**

15.2. Patient Sample Result Interpretation

Patient sample(s) must meet the criteria in **Table 2**. If the patient sample criteria are not met, the patient sample must be rejected and repeated.

Table 3. Criteria for patient samples

Measurement	Criteria
Individual Sample Measurements	%CV for duplicate wells must be ≤ 25%
'Detected' Output	Screen Index ≥ 1.00 **AND** Confirm Index ≤ 1.00
'Not Detected' Output	Screen Index < 1.00

Patients evaluated with the AAV5 DetectCDx™ Kit who are anti-AAV5 antibody negative (result of "Not Detected") are eligible for treatment with Valoctocogene Roxaparvovec under the supervision of a physician.

Upon completion of testing, a test report with the results of the AAV5 DetectCDx™ Assay will be sent to the designated physician. The following are the standard report results:

- **Detected:** patient **is not** eligible for treatment with Valoctocogene Roxaparvovec
- **Not Detected:** patient **is** eligible for treatment with Valoctocogene Roxaparvovec

16. Retesting

16.1. Failed Runs

Table 4. AAV5 DetectCDx™ Run Control Output Interpretation

IF…	THEN…
All Run Control indicators pass	Proceed with sample assessment
Any Run Control indicator fails	• Identify the cause of failure and remediate (as applicable) • Repeat AAV5 DetectCDx™ in its entirety **Note:** Procedure may be repeated a total of two (2) additional times
Any Run Control indicator fails after second repeat	Contact PharmaDx (CDxtechnicalsupport@aruplab.com)

Table 5. AAV5 DetectCDx™ Specimen Output Interpretation

IF…	THEN…

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Sample indicators pass (e.g., %CV values > 25% Screening and Confirmatory Modes)	Proceed with sample resulting and reporting
Sample indicator fails (e.g., %CV values > 25% for Screening AND/OR Confirmatory Modes)	Identify the cause of failure and remediate (as applicable) Repeat sample AAV5 DetectCDx™ Assay in its entirety one (1) additional time **Note:** Sample may be repeated only once
After first repeat Sample indicator fails (e.g., %CV values > 25% for Screening AND/OR Confirmatory Modes)	The operator will change the indicator from 'FAIL' to 'Inconclusive' to report sample results as 'Inconclusive'

17. Non-Clinical Performance Evaluation

17.1. Detection Capabilities

The detection capabilities of AAV5 TAb Assay (AAV5 DetectCDx™) were evaluated based on "CLSI EP17 A2 - Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures". Detection capabilities were defined using the Limit of Blank (LoB) and Limit of Detection (LoD) thresholds. The evaluation was conducted using the following conditions: single operator, single instrument, two (2) assay reagent lots, four (4) non-consecutive test days, four (4) blank samples, four (4) low measured samples, and four (4) replicated measurements per sample per reagent/day combinations. The results of this experiment shown in **Table 6** demonstrate that AAV5 DetectCDx™ is capable of resolving anti-AAV5 antibody levels at the assay cutoff.

Table 6. Summary of AAV5 DetectCDx™ detection capabilities evaluation

Summary of AAV5 DetectCDx™ Detection Capabilities Evaluation		
	Screening Mode	**Confirmatory Mode**
Estimated LoD	SI = 1.00	CI = 1.115
Results	Accepted	Accepted

17.2. Prozone Effect

The AAV5 DetectCDx™ was evaluated to determine whether elevated concentrations of anti-AAV5 antibodies produce a prozone (hook) effect. Individual two-fold dilution series were created by diluting a high titer positive anti-AAV5 plasma sample (10 µg/mL total IgG rabbit anti-AAV5 polyclonal serum) into the anti-AAV5 negative plasma sample for an eight-fold dilution to cover the range from high positive to negative SI and CI values. Each dilution series was analyzed on a single plate for a total of four (4) individual analysis runs.

The results from this test indicate that there were no false negative ("Not Detected") results observed for tested samples with high anti-AAV5 titers and that elevated concentrations of anti-AAV5 antibodies do not produce a prozone (hook) effect in the AAV5 DetectCDx™ assay (**Table 7**).

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Table 7. Summary of AAV5 DetectCDx™ Prozone Effect Evaluation

Summary of AAV5 DetectCDx™ Prozone Effect Evaluation		
Sample	**Reported Values Based on Dilution**	**Results**
High positive (high anti-AAV5 titer)	Detected at all dilutions	Accepted
High positive (high anti-AAV5 titer)	Detected up to 1:16 dilution; Not Detected at 1:32 or greater dilution	Accepted
High positive (high anti-AAV5 titer)	Detected up to 1:8 dilution; Not Detected at 1:16 dilution (series 1) and 1:32 dilution (series 2)	Accepted
Artificially high positive	Detected at all dilutions	Accepted

17.3. Contamination and Cross Talk

The possibility of cross-contamination and cross-talk was evaluated for the AAV5 DetectCDx™ assay. The study sample set indicated in **Table 8** was used to create an alternating pattern of negative and high positive samples.

Table 8. Sample Types Evaluated in Contamination and Cross-Talk Evaluation

Sample Types Evaluated in Contamination 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. Based on the results of this Cross-Talk Evaluation, it was concluded that no contamination or cross-talk is introduced into the design of the assay (**Table 9**).

Table 9. Result summary of AAV5 Cross-Talk Evaluation

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

17.4. Stability

17.4.1. AAV5 Detect CDx™ 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

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

conditions of reagents and human whole blood and plasma using three (3) sample types that corresponded to a high negative sample, a low positive sample, and a high positive sample, as indicated in **Table 10**.

Table 10. Sample Types Evaluated in Stability Tests

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 results of the AAV5 DetectCDx™ assay if addition of 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.

Table 11. Conditions and/or timepoints that may interfere with AAV5 DetectCDx™ performance

Conditions and/or Timepoints That May Interfere					
Item	**Test Conditions**	**Timepoint**	**Sample type(s) which did not maintain stability**	**Impact on SI/CI values**	**Impact on Qualitative Test Result**
Specimen	Refrigerated (2 to 8 ºC)	28 days	Low positive	Increase in SI value; decrease in CI value	No expected impact
Specimen	Frozen (-70º C)	2 months	Low positive	Increase in CI value	Could convert sample to “Not Detected”
Specimen transport	Ambient (room) temperature	8 days	Low positive	Decrease in SI value; increase in CI value	Could convert sample to “Not Detected” result
Specimen transport	Elevated (37 ± 2 ºC)	2 days	Low positive	Increase in CI value	Could convert sample to Not Detected
Plasma collection	Room temperature (20 to 25 ºC)	48.9 hours	Low positive	Decrease in SI value	Could convert sample to “Not Detected” result

Table 12. AAV5 DetectCDx™ Established Reagent Stability

AAV5 DetectCDx™ Established Reagent Stability

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Reagent(s)	Conditions	Stability
AAV5 Coated Plate Set	2 to 8 ºC	7 days
Read Buffer T (1X)	20 to 25 ºC	12 months

Table 13. AAV5 DetectCDx™ Sample Stability

AAV5 DetectCDx™ Sample Stability	
Storage Conditions	**Stability**
Room temperature (20 to 25 ºC)	72 hours
Refrigerated (2 to 8 ºC)	9.6 days
Frozen (-10 ºC or colder)	24 months
Frozen (-70 ºC or colder)	24 months
Freeze-thaw cycles	7 events

Table 14. AAV5 DetectCDx™ Collection Stability

AAV5 DetectCDx™ Collection Stability	
Conditions	**Stability**
Whole blood, room temperature (20 to 25 ºC)	72 hours
Whole blood, refrigerated (2 to 8 ºC)	72 hours
Plasma, room temperature (20 to 25 ºC)	48.9 hours
Plasma, refrigerated (2 to 8 ºC)	72 hours

AAV5 DetectCDx™ Sample Transport Stability	
Transport Conditions	Stability
Frozen (on dry ice)	11 days
Refrigerated (with gel packs)	11 days
Ambient temperature	8 days
Elevated temperature (37 ºC ± 2 ºC)	2 days

17.4.2. AAV5 DetectCDx™ Kit Stability

Stability of the reagents for the AAV5 DetectCDx™ Kit 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 using three (3) sample types that corresponded to a high negative sample, a low positive sample, and a high positive sample, as indicated in **Table 15**.

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Table 15. Sample Types Evaluated in Stability Tests

Sample Types Evaluated in Stability Tests				
Sample Type	**SI Value**		**CI Value**	
	Target	**Measured (mean)**	**Target**	**Measured (mean)**
High negative	< 1.00	0.83	~1.20	1.340
Low positive	> 1.00	1.41	~0.80	0.814
High positive	> 10.0	55.21	< 0.20	0.197

A condition and/or timepoint for the AAV5 DetectCDx™ Kit was considered acceptable if the following criteria were met:

- All runs must meet the run acceptance criteria outlined in the assay technical procedure. Qualitative Agreement percentage must be = 100%.
- If the regression slope for SI and CI is not statistically significant ($p \geq 0.05$), then the stability duration for that sample is taken as the maximum time point tested.

If the regression slope for either SI or CI is statistically significant ($p < 0.05$), then the stability duration by measurand drift for that sample is taken as the time at which the two-sided 95% confidence interval of the regression line intersects with ±10% of the mean value of t=0. This may be determined visually from the linear regression plot. If the 95% confidence interval does not intersect with ±10% of the mean value of t=0, then the time point/condition is accepted.

Table 16. Conditions and/or timepoints that may interfere with AAV5 DetectCDx™ Kit performance

Item	Test Conditions	Timepoint	Sample type(s) which did not maintain stability	Impact on SI/CI values	Impact on Qualitative Test Result
AAV5 DetectCDx™ Kit Reagent Stability	Refrigerated (2 to 8 ºC)	0.2 d	Low positive	Increase in SI value, Decrease in CI value	No Impact
AAV5 DetectCDx™ Kit Open Container Stability	Ambient (room) temperature	18 h	High negative High positive	Increase in SI value Increase in CI value	No Impact
AAV5 DetectCDx™ Kit Open Container Stability	Ice (4 ºC)	3.4 h	High negative	Increase in SI value	No Impact

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Table 17. AAV5 DetectCDx™ Kit established reagent stability

Reagent(s)	Conditions	Stability
AAV5 DetectCDx™ Kit	-70 ºC	TBD, Study on-going
	2 to 8 ºC	0.2 d
	20 to 25 ºC	18 h
	4 ºC (Ice)	3.4 h
	-70 ºC (dry ice)	9 d

17.5. 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 - Interference Testing in Clinical Chemistry, 3rd Edition" and "CLSI EP37 - Supplemental Tables for Interference Testing in Clinical Chemistry, 1st Edition". The interference study evaluated the impact of substances on the assay results using three (3) sample types that corresponded to a high negative sample, a low positive sample, and a high positive sample, as indicated in **Table 15**.

Table 18. Sample types used in endogenous and 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 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™ Assay 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 and non-interfering substances are shown in **Table 16** and **Table 17** respectively.

Table 19. Interfering endogenous and exogenous substances

Substance	Test concentration	Sample type for which interference was seen	Impact on SI/CI values	Impact on Qualitative Test Result
Hemoglobin	1000 mg/dL	High negative	Decrease in SI value	No expected impact

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Substance	Test concentration	Sample type for which interference was seen	Impact on SI/CI values	Impact on Qualitative Test Result
Hemoglobin	1000 mg/dL	Low positive	Decrease in SI value	Could convert sample to "Not Detected" result
Triglycerides	750 mg/dL	Low positive	Increase in CI value	Could convert sample to "Not Detected" result
Rheumatoid Factor†	476 mg/dL, 1285 mg/dL, 1750 mg/dL, 3695 mg/dL	Low positive	Increase in SI value, decrease in CI value	No expected impact

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

Table 20. Non-interfering endogenous and exogenous substances

Substance	Test concentration	No interference for listed sample type(s)
Albumin	6 mg/dL	High negative, low positive, high positive
Bilirubin, conjugated	40 mg/dL	High negative, low positive, high positive
Bilirubin, unconjugated	40 mg/dL	High negative, low positive, high positive
Triglycerides	750 mg/dL	High negative, high positive
Triglycerides	500 mg/dL	High negative, low positive, high positive
Triglycerides	200 mg/dL	High negative, low positive, high positive
Hemoglobin	1000 mg/dL	High positive
Hemoglobin	800 mg/dL	High negative, low positive, high positive
Hemoglobin	400 mg/dL	High negative, low positive, high positive
Acetaminophen	15.6 mg/dL	High negative, low positive, high positive
Advate	384 IU/dL	High negative, low positive, high positive
Atazanavir	1.95 mg/dL	High negative, low positive, high positive
Atorvastatin	0.075 mg/dL	High negative, low positive, high positive
Bictegravir	1.85 mg/dL	High negative, low positive, high positive
Biotin	0.351 mg/dL	High negative, low positive, high positive
Doravirine	0.289 mg/dL	High negative, low positive, high positive
Eloctate	324 IU/dL	High negative, low positive, high positive
Fexofenadine	0.116 mg/dL	High negative, low positive, high positive
Hemlibra	170 µg/mL	High negative, low positive, high positive
Hemofil-M	150 IU/dL	High negative, low positive, high positive
Heparin	330 IU/dL	High negative, low positive, high positive
Ibuprofen	21.9 mg/dL	High negative, low positive, high positive

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Substance	Test concentration	No interference for listed sample type(s)
Lisinopril	0.0246 mg/dL	High negative, low positive, high positive
Naproxen	36.0 mg/dL	High negative, low positive, high positive
Omeprazole	0.84 mg/dL	High negative, low positive, high positive
Oxycodone	0.0324 mg/dL	High negative, low positive, high positive
Sodium citrate†	7.3% (short draw)	High negative, low positive, high positive
Tenofovir	0.0978 mg/dL	High negative, low positive, high positive
Vitamin C	5.25 mg/dL	High negative, low positive

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

17.6. Precision

17.6.1. AAV5 DetectCDx™

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 (5) sample types, as indicated in **Table 21**.

Table 21. 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 **Table 19**) 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.

Table 22. Results of AAV5 DetectCDx™ precision evaluation

Study	Experimental Conditions	Runs	Qualitative Agreement (QA)
Inter-assay	Single operator, single instrument, single raw material reagent lot	Two (2) runs per day 20 test days Two (2) replicates per sample	100% QA for high negative, low positive, mid positive, and high positive samples
Intra-assay	Single operator, single instrument, single raw material reagent lot	Five (5) runs Multiple days	100% QA for high negative, low positive, mid positive, and high positive samples
Operator-to-operator	Three (3) operators, one (1) instrument, 1 production reagent lot	One (1) run per day per operator Five (5) test days	100% QA for high negative, low positive, mid positive, and high positive samples

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Study	Experimental Conditions	Runs	Qualitative Agreement (QA)
		5 replicates per sample	
Instrument-to-instrument	One (1) operator, two (2) instruments, 1 production reagent lot	One (1) run/day Five (5) test days 5 replicates per sample	100% QA for high negative, low positive, mid positive, and high positive samples
Reagent Lot-to-Lot	One (1) operator, one (1) instrument, three (3) production reagent lots	One (1) run/day Six (6) test days Five (5) replicates per sample	100% QA for high negative, low positive, mid positive, and high positive samples

The data for the cutoff sample was collected and evaluated to determine whether the performance of the assay at the 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 called "Detected" 132 times and called "Not Detected" 176 times. Analysis by Fisher's exact test with an expected ratio of 1:1 for "Detected"/"Not Detected" resulted in a p-value of 0.0897 (not significant), indicating that the observed result is not different than the expected result of a 1:1 ratio of "Detected"/"Not Detected". The cutoff sample performed as expected, with a balance between "Detected" and "Not Detected" results obtained as expected for a sample at the cutoff (**Table 20**).

Table 23. Results of AAV5 DetectCDx™ cutoff sample analysis

	SI	CI
n	308	308
Mean	1.04	1.01
Median	1.04	1.00
SD (Standard Deviation)	0.054	0.060
%CV	5.2%	6.0%

17.6.2. AAV5 DetectCDx™ Kit

The inter-laboratory precision of the AAV5 DetectCDx™ Kit was assessed using three (3) sample types, as indicated in **Table 24**. These precision studies were based on "CLSI EP05 A3 - Evaluation of Precision of Quantitative Measurement Procedures".

Table 24. Sample types used in AAV5 DetectCDx™ Kit inter-laboratory precision evaluation

Sample Type	SI Value		CI Value	
	Target	Measured (mean)	Target	Measured (mean)
High negative	< 1.00	0.86	~1.20	1.307
Low positive	> 1.00	1.38	~0.80	0.810
High positive	> 10.0	24.17	< 0.20	0.056

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

The multi-site reproducibility study design was a 3 x 5 x 2 x3 format conducted across Site 1 and Site 2 with three (3) operators, of whom two (2) are located at Site 2. A total of 90 measurements for each sample (3 operators x 5 days; 2 runs per day x 3 replicates per run). Plate coating and assay analysis were performed by the same operator.

The high negative, low positive, and high positive samples had 100% Qualitative Agreement, for this study and Inter-operator precision had %CV ≤ 25% for all samples tested.

Site to site precision was assessed using a variance components analysis and mean difference. No statistically significant difference was observed between sites for all samples tested.

18. Clinical Performance Evaluation

The AAV5 DetectCDx™ assay was used in five (5) clinical studies which were used to establish the clinical utility of the test. Four (4) of the studies were prospective studies in which the AAV5 DetectCDx™ assay was used. One (1) study was a retrospective study where ARUP analyzed the specimens after conclusion of the investigation. Samples were all evaluated at ARUP Laboratories in Salt Lake City, Utah using the AAV5 DetectCDx™ assay.

18.1. Patient Population Demographics

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 variables were analyzed for their potential association with assay results ("Detected" vs "Not Detected"). Results of the analysis by patient demographic are shown in **Table 21**.

Table 25. AAV5 DetectCDx™ results by patient demographic

	Detected	Not Detected	P-value: t.test (Wilcoxon)
n	**212**	**428**	
RACE (%)			0.4018
Asian	25 (14.9)	70 (19.1)	
Black or African American	18 (10.7)	26 (7.1)	
Multiple	0 (0.0)	1 (0.3)	
Native Hawaiian or other pacific islander	0 (0.0)	1 (0.3)	
White	125 (74.4)	268 (73.2)	
ETHNIC (%)			1
Hispanic or Latino	4 (2.0)	8 (2.0)	
Not Hispanic or Latino	194 (98.0)	402 (98.0)	
SEX (%)			1
Female	0 (0.0)	2 (0.5)	
Male	212 (100.0)	426 (99.5)	
COUNTRY (%)			0.003
Australia	3 (1.4)	12 (2.8)	
Belgium	2 (0.9)	11 (2.6)	

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

	Detected	Not Detected	P-value: t.test (Wilcoxon)
Brazil	13 (6.1)	12 (2.8)	
France	39 (18.4)	56 (13.1)	
Germany	23 (10.8)	49 (11.4)	
Great Britain	17 (8.0)	65 (15.2)	
Israel	4 (1.9)	7 (1.6)	
Italy	8 (3.8)	13 (3.0)	
Japan	22 (10.4)	55 (12.9)	
Russia	35 (16.5)	40 (9.3)	
Spain	0 (0.0)	2 (0.5)	
South Africa	16 (7.5)	21 (4.9)	
USA	30 (14.2)	85 (19.9)	
Type of FVIII replacement (%)			0.043
On-demand	29 (17.0)	41 (10.0)	
Prophylaxis	142 (83.0)	366 (89.7)	
hFVIII total antibody screen (%)			1
Negative	9 (100.0)	46 (95.8)	
Positive	0 (0.0)	2 (4.2)	
hFVIII total antibody confirm (%)			1
Negative	7 (100.0)	37 (94.9)	
Positive	0 (0.0)	2 (5.1)	
AGE (mean (SD))	39.74 (14.40)	36.96 (12.70)	(0.0389)
Baseline annualized bleeding rate (all bleeds) (bleeds/year) (mean (SD))	6.00 (9.38)	8.16 (17.01)	0.519 (0.716)
Baseline annualized bleeding rate (treated bleeds) (bleeds/year) (mean (SD))	6.00 (9.38)	9.09 (15.00)	0.86 (0.53)
Baseline annualized FVIII usage (IU/kg/year) (mean (SD))	5222.65 (1127.73)	4693.80 (1796.21)	0.824 (0.349)
Baseline annualized number of FVIII infusions (infusions/year) (mean (SD))	137.00 (46.80)	131.51 (50.09)	0.704 (0.73)
Baseline FVIII activity, IU/dL (mean (SD))	11.32 (15.16)	13.45 (26.06)	0.818 (0.131)
hFVIII inhibitor level, BU = <0.6 (%)	7 (100.0)	52 (100.0)	
hFVIII protein level (mean (SD))	5.61 (5.61)	6.46 (8.81)	0.761

Age: A logistic regression was performed and showed that Age is lower for the "Not Detected" group and is linearly correlated with the AAV5 DetectCDx™ result. This observation is in line with an increased chance of AAV5 exposure over one's lifetime.

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Country: The Country of Origin appeared to be associated with AAV5 DetectCDx™ result. For example, a high level of seropositivity was observed in Russia (47%) and a low level was observed in Great Britain (21%). This is in line with literature findings that AAV seropositivity varies based on geographic area.

Type of FVIII replacement: The type of FVIII replacement (on-demand vs. prophylaxis) appeared to have an association with the AAV5 DetectCDx™ result, with the "on-demand" group experiencing a higher seropositivity rate than the prophylaxis group. However, it is possible that Age and Country of Origin may be responsible for the observed trend. Indeed, the average age of the "on-demand" group is significantly higher than the average age of the prophylaxis group, at 43.6 years and 37.7 years respectively. Also, the Country of Origin of the subjects in each group is widely different.

18.2. Efficacy Assessment

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 Valoctocogene Roxaparvovec is based on 47 patients who had a "Not Detected" result and were enrolled in clinical studies. The results from two studies 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 Valoctocogene Roxaparvovec. The clinical outcomes from both studies demonstrated a statistically significant and highly therapeutically impactful increase in FVIII activity following administration of either 6E13 vg/kg and 4E13 vg/kg doses of Valoctocogene Roxaparvovec in a durable and disease-transforming manner. The data show that the effect of the FVIII protein produced by transduced hepatocytes, as measured by FVIII activity, is strongly correlated with reducing the risk of bleeding and use of FVIII concentrates. Investigations have shown clear and consistent correlations between chromogenic substrate and one-stage clotting assay FVIII activity levels, as well as FVIII activity levels and the risk of bleeds using either assay. Importantly, in a population representative of the broader, real-world population of Hemophilia A patients, the magnitude of the clinical benefit, in terms of reductions in both annualized bleed and FVIII utilization rates, are ≥85% and 95%, respectively, post-Valoctocogene Roxaparvovec in the absence of any further treatment represents a unique therapeutic profile in Hemophilia A. This transformative treatment outcome is further supported by improvements in health-related quality of life.

18.3. Safety Conclusion

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 Valoctocogene Roxaparvovec is based on the data generated in four (4) clinical studies on 49 subjects with severe Hemophilia A who were exposed to Valoctocogene Roxaparvovec for up to 3.5 years.

The results from four clinical trials support that AAV5 DetectCDx™ in the detection of anti-AAV5 antibodies as an aid in the selection of Hemophilia A patients for treatment with Valoctocogene Roxaparvovec was demonstrated to have a favorable safety and tolerability profile in the course of this study. The majority of adverse events (98%) have been Grade One (1) (mild) to Grade Two (2) (moderate) in intensity; six (6) adverse events (2%) were Grade

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

Three (3) (severe). No Grade Four (4) or Five (5) adverse events have been reported. Only one (1) adverse event was immune related that emerged within 24 hours of receiving treatment with BMN 270 (Biomarin 270 (valoctocogene roxaparvovec)). It was resolved without clinical sequela within 48 hours following medical management. Infusion-related reactions were effectively mitigated by managing infusion rate and medications.

19. References

1. Jiang, H, Couto L, Patarroyo-White, S, Liu, T et al. Effects of transient immunosuppression on adenoassociated, virus-mediated, liver-directed gene transfer in rhesus macaques and implications for human gene therapy. *Blood* **2006**; 108(10): 3321–3328.
2. Wang L, Calcedo R, Bell P, Lin J, et al. Impact of pre-existing immunity of gene transfer to nonhuman primate liver with AAV8. *Hum Gen Ther* **2011**; 22(11):1389–1401.
3. Scallan C, Jiang H, Liu T, Patarroyo-White S, et al. Human immunoglobulin inhibits liver transduction by AAV vectors at low neutralizing titers in SCID mice. *Blood* **2006**; 107: 1810-1817.
4. Manno CS, Pierce GF, Arruda V, Glader B et al. Successful transduction of liver in Hemophilia by AAV-factor IX and limitations imposed by the host immune response. *Nat Med* **2006**; 12: 342-347.

20. Technical and Customer Service

Contact Information

ARUP Laboratories
500 Chipeta Way
Salt Lake City, Utah 84108
USA

FPhone: +1 800 522 2787

Fax: +1 800 522 2706

Website: www.aruplab.com

Authorized Representative

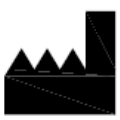
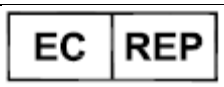
EC REP Qarad EC-REP BV
Pas 2572440 Geel
Belgium

Phone:

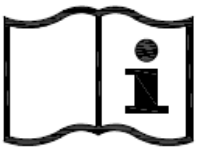
Website:

21. Symbols

The following symbols are used in ARUP Laboratories product labeling:

	Manufacturer
	Authorized Representative in the European Community

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

	CE Mark
	For *In Vitro* Diagnostic Use
	Non-sterile
	Storage Conditions
	Consult Instructions for Use

22. Legal Notice

TBD.

This is an *in vitro* diagnostic product.

AAV5 DetectCDx™ is a registered trademark of ARUP Laboratories.

23. Appendix A

Table A1. Plate washing program

Cycle #	3	
Aspirate	Aspirate Type	**TOP**
	Travel Rate	**5 (9.4 & 2.0 millimeters/second)**
	Aspirate Delay	**2000 milliseconds**
	Aspirate Position	**X: 00 Y:-10**
	Aspirate Height	**026 (3.303 millimeters)**
	Secondary Aspirate?	**NO**
Dispense	Dispense Rate	**5**
	Dispense Volume	**320 microliters/well**
	Vacuum Delay Volume	**0 microliters/well**
	Dispense Position	**X: 00 Y: 00**

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

	Dispense Height	**111 (14.101 millimeters)**
Options – Pre-wash	Wash Pre-Dispense?	**NO**
	Bottom Wash?	**NO**
Options – Mid Cycle Wash	Wash Shake?	**NO**
	Wash Soak?	**NO**
	Home Carrier?	**NO**
	Between Cycle Pre-Dispense?	**NO**
Options – Post-Wash	Final Aspirate?	**YES**
	Aspirate Type	**TOP**
	Travel Rate	**5 (9.4 & 2.0 millimeters/second)**
	Aspirate Delay	**2000 milliseconds**
	Final Aspirate Position	**X: 00 Y:00**
	Aspirate Height	**026 (3.303 millimeters)**
	Secondary Aspirate?	**YES**
	Final Aspirate Secondary Position	**X: 00 Y:00**
	Final Aspirate Secondary Height	**026 (3.303 millimeters)**

24. Appendix B

24.1. ECL Wash Buffer Recipe (20 liters)

1. Combine in the following order:
   a. Clinical laboratory reagent water (Class 1, 18 MΩ), 17 liters
   b. Dulbecco's Phosphate Buffered Saline (10X), 2 liters
   c. Tween 20 (proteomics grade), 200 milliliters
   d. ProClin 300, 10 milliliters
2. Stir for 10 minutes
3. Add enough clinical laboratory reagent water to make a final volume of 20 liters
4. Transfer to an appropriate container for use with plate washer
5. ECL Wash Buffer expires 1 month after preparation

24.2. Read Buffer T (1X)

1. Measure 250 milliliters of Read Buffer T (4X) and transfer to a 1 liter disposable plastic bottle
2. Measure 750 milliliters of clinical laboratory reagent water (Class 1, 18 MΩ) and combine with Read Buffer T in the plastic bottle
3. Mix thoroughly

Instructions for Use: AAV5 DetectCDx™ Kit IVDD

4. Read Buffer T (1X) expires one year after preparation