SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Enzyme-linked immunosorbent in vitro diagnostic assay for the semi-quantitative detection of antibodies (IgG) to AAVrh74 capsid in human serum
Device Trade Name:	Quest Diagnostics AAVrh74 Antibody ELISA CDx
Device Product Code:	QWQ
Applicant's Name and Address:	Quest Diagnostics Nichols Institute 33608 Ortega Highway San Juan Capistrano, CA 92675
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P250002
Date of FDA Notice of Approval:	July 24, 2025

Breakthrough Device: Granted breakthrough device status on July 27, 2023, because (1) the device is intended to diagnose a life threatening or irreversibly debilitating disease or condition, (2) no approved or cleared alternatives exist, and (3) the availability of the device is in the best interest of patients.

II. INDICATIONS FOR USE

The Quest Diagnostics AAVrh74 Antibody ELISA CDx is an enzyme-linked immunosorbent in vitro diagnostic assay intended for the semi-quantitative detection of antibodies (IgG) to AAVrh74 capsid in human serum. The test reports an antibody titer, and a semi-quantitative interpretation of the test results derived from the antibody titer. Patients with an AAVrh74 antibody titer <1:400 are reported as not elevated for AAVrh74 antibody titers and may be eligible for treatment with the gene therapy. Patients with an AAVrh74 antibody titer ≥1:400 are reported as elevated for AAVrh74 antibody titers and are ineligible for treatment with the gene therapy. The test is for prescription use only. The test is intended to be used in conjunction with other available clinical information as an aid to identify patients eligible for treatment with indicated gene therapies.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

Patient specimens used with the Quest Diagnostics AAVrh74 Antibody ELISA CDx may be potentially infectious. Proper handling (e.g., universal precautions for bloodborne pathogens) and disposal methods in compliance with national, state, or local regulations and guidelines of the agencies holding jurisdiction over the laboratory.

Ensure conditions are met as stated in the Specimen Collection and Handling Section and Shipping Instructions Section of the labeling.

Samples received that visually appear to be hemolyzed or turbid, or with any bacterial contamination present, will not be tested and another blood draw from the patient will be required.

Test is to be performed only on the instruments (i.e., plate, washer, and reader) which are designated to perform the Quest Diagnostics AAVrh74 Antibody ELISA CDx and are labelled as "For Quest Diagnostics AAVrh74 Antibody ELISA CDx only".

V. DEVICE DESCRIPTION

The Quest Diagnostics AAVrh74 Antibody ELISA CDx is a companion diagnostic (CDx) device intended for use with Sarepta Therapeutics, Inc.'s ELEVIDYS (delandistrogene moxeparvovec-rokl), a gene therapy using AAVrh74 capsids. The Quest Diagnostics AAVrh74 Antibody ELISA CDx provides semi-quantitative immunodetection of antibodies (IgG) to AAVrh74 capsid using the indirect-ELISA method based upon a colorimetric reaction and is intended to be used as an aid in identifying patients eligible for treatment with ELEVIDYS, a gene therapy for Duchenne Muscular Dystrophy (DMD). Patients whose blood samples are analyzed using Quest Diagnostics AAVrh74 Antibody ELISA CDx and test negative for antibodies to the AAVrh74 capsid and reported as "Not Elevated" may be eligible to receive Sarepta Therapeutics' gene therapy.

The Quest Diagnostics AAVrh74 Antibody ELISA CDx utilizes reagents manufactured exclusively for use with the Quest Diagnostics AAVrh74 Antibody ELISA CDx by Quest Diagnostics, as well as utilizing reagents and instrumentation which have been specifically validated for, and approved for use as part of, the Quest Diagnostics AAVrh74 Antibody ELISA CDx (Tables 1–3, below). The reagents are supplied as a kit and consist of refrigerated and frozen components. All these components combined contain all the reagents needed for the generation of four test results.

Table 1. Refrigerated Critical Reagents Manufactured for Use with Quest Diagnostics AAVrh74 Antibody ELISA CDx

Reagent	Use in Assay
AAVrh74 Antibody ELISA AAVrh74 Coated Microtiter Plate, 96 wells	Assay plate
AAVrh74 Antibody ELISA Anti-human IgG [HRP] Conjugate	Off-the-shelf antibody to bind to the target antigen
AAVrh74 Antibody ELISA Sample Diluent	Sample diluent

Table 1. Refrigerated Critical Reagents Manufactured for Use with Quest Diagnostics AAVrh74 Antibody ELISA CDx	
AAVrh74 Antibody ELISA Conjugate Diluent	Conjugate diluent
AAVrh74 Antibody ELISA TMB Substrate	Off-the-shelf substrate to create a detectable signal
AAVrh74 Antibody ELISA Stop Solution	Off-the-shelf solution to terminate the enzyme-catalyzed reaction
AAVrh74 Antibody ELISA 20X Wash Buffer	Assay wash buffer

Table 2. Frozen Critical Reagents Manufactured for Use with Quest Diagnostics AAVrh74 Antibody ELISA CDx	
Reagent	**Use in Assay**
AAVrh74 Antibody ELISA Calibrator	Assay calibrator
AAVrh74 Antibody ELISA Negative Control (NC)	Assay negative control
AAVrh74 Antibody ELISA Low Positive Control (LPC)	Assay low positive control to target cut-off
AAVrh74 Antibody ELISA Positive Control (PC)	Assay positive control

Table 3. Instrumentation and Software Used in Quest Diagnostics AAVrh74 Antibody ELISA CDx	
Instrument/Software	**Use in Assay**
Agilent BioTek Epoch2 Microplate Spectrophotometer *†	Plate reader
Gen5 Microplate Reader and Imager (Gen5 IVD) Software - Version: 3.11.19 †	Off-the-shelf software that runs and supports the plate reader
Bio-Tek 50TS Microplate Washer: supported by the embedded onboard software "PN 1550200-FW Instrument Base Code" *†	Off-the-shelf analysis software
Polypropylene deep well plate (2 mL) or tubes	Sample and control dilutions
Plate sealers	Seals the microtiter plate

* Quest Diagnostics AAVrh74 Antibody ELISA CDx is intended to be performed on specific serial number-controlled instruments at Quest Diagnostics Nichols Institute.
† Software and cybersecurity were reviewed for in-vitro diagnostic use of the Quest Diagnostics AAVrh74 Antibody ELISA CDx on serial number-controlled instruments at Quest Diagnostics Nichols Institute.

Specimen Collection and Shipping

Draw blood in either a Serum Separator Tube (SST, i.e., gold-top serum separator tube*) or a non-gel serum separating collection tube (i.e., red top tube**). For proper additive performance, mix the blood by inverting the tube five times. Allow the blood to clot in an upright position for at least 30 minutes, but no longer than 1 hour before centrifugation. Separate serum from whole blood by centrifugation according to the collection tube type. Refer to the manufacturer's manual for recommended centrifuge speed and duration. Patient specimens must be stored according to stability information described below and shipped in a manner that maintains the integrity of the specimen during transport and until arrival at the laboratory. The serum sample must be received within 72 hours of shipping. Overnight

delivery in an insulated container at refrigerated temperature with pre-frozen cold packs is the preferred shipping method as described in the Shipping Instructions, below, and in the instructions in a specimen shipping kit provided to the ordering laboratory or physician. All specimens to be tested with Quest Diagnostics AAVrh74 Antibody ELISA CDx are to be shipped overnight in an insulated container with pre-frozen cold packs.

*If using the gold-top SST, after centrifugation place the labeled gold-top tube in the shipping container. Do not freeze the gold-top SST collection tubes. Do not ship the gold-top SST collection tubes frozen.

**If using a red-top tube or a non-gel serum separating collection tube, after centrifugation transfer the serum into a labeled plastic transport tube without any additives and place in the shipping container.

Assay Principles and Format

The assay is performed in 96-well microtiter plates. Microtiter wells coated with recombinant rAAVrh74 (Ag+ wells) are tested alongside wells which do not contain rAAVrh74 (Ag- wells). The test procedure involves diluting patient serum samples and the Positive Control (PC) 1:400, 1:800, 1:1600 and 1:3200 and the Calibrator (CAL), Negative Control (NC) and Low Positive Control (LPC) 1:400, using Sample Diluent. Patient samples, Controls, Calibrator and Blank (Sample Diluent only) are tested in duplicate in both Ag+ and Ag- wells on the same plate. Antibodies (IgG) to AAVrh74 capsid present in the sample bind to the immobilized rAAVrh74 on the plate. After a wash step, the bound antibodies are detected using goat anti-human IgG labeled with HRP diluted with Conjugate Diluent. Addition of TMB Substrate leads to the production of a blue product, which turns yellow after the reaction is stopped by acidification using Stop Solution. Signal intensity is quantitated by reading the absorbance at 450 nm using an off-the-shelf microplate spectrophotometer, BioTek Epoch2 microtiter plate absorbance reader. The intensity of the color is proportional to the titer of antibodies (IgG) to AAVrh74 capsid present in the serum sample, the higher the signal intensity (optical density, "OD") the more antibody present in the sample.

A microtiter plate absorbance reader is operated using off-the-shelf GEN5 IVD software (v 3.11.19). The average signal in the wells without antigen (Ag-) is subtracted from the average signal of the wells with antigen to calculate sample specific absorbance (Spec OD). Then the sample normalized absorbance (Normalized Optical Density or Norm OD) is calculated using the lot-dependent Calibrator Correction Coefficient (Norm OD) value. The microtiter plate absorbance reader calculates the assay results.

Interpretation of Results

Samples that do not yield a positive result at any of the dilutions will be reported as <1:400 and are considered negative and reported as "Not Elevated." A patient with a Not Elevated result may be eligible for gene therapy. The highest dilution that yields a Norm OD greater than the cutoff (Norm OD=1.10) is reported as the titer. Samples with titer ≥1:400 are considered positive and reported as "Elevated." A patient with an Elevated result is ineligible for gene therapy. Samples positive at all dilutions (e.g., positive at 1:400 dilution, 1:800 dilution, 1:1600 dilution and 1:3200 dilution) will be reported as ≥1:3200. If the results are deemed "inconclusive", the

ordering provider is notified of "Test Not Performed (TNP)".

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no alternative FDA-cleared or -approved alternatives for the detection of anti-AAVrh74 antibodies in human serum for the selection of patients with DMD who are eligible for treatment with ELEVIDYS (delandistrogene moxeparvovec-rokl), an adeno-associated virus serotype rh74 (AAVrh74)-based gene therapy.

VII. MARKETING HISTORY

The Quest Diagnostics AAVrh74 Antibody ELISA CDx has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Patients with false negative results (patients with pre-existing anti-AAVrh74 antibodies but are indicated as Not Elevated by the device) would potentially receive the treatment and be exposed to the potential risks associated with ELEVIDYS treatment including the possibility of not experiencing the potential benefits of the treatment. For the specific adverse events related to the approved gene therapy, please see the approved gene therapy product label.

Patients with false positive results (patients without pre-existing anti-AAVrh74 antibodies but are indicated as Elevated by the device) would potentially not receive treatment with ELEVIDYS. Such patient will continue with the current standard of care, such as corticosteroids and physical therapy. The risk associated with a false positive result is minimal.

Procedure-related complications for the assay itself are limited to obtaining the serum specimen via a blood draw. These risks for the Quest Diagnostics AAVrh74 ELISA CDx are equivalent to risks of sample collection in other in vitro diagnostic tests and not unique to the Quest Diagnostic AAVrh74 ELISA CDx. The Quest Diagnostic AAVrh74 ELISA CDx is a non-invasive in-vitro companion diagnostic and as such, there is minimum impact on the patients from the test itself.

IX. SUMMARY OF NON-CLINICAL STUDIES

A. Laboratory Studies

1. Assay Cut-off Determination

During the clinical development of ELEVIDYS, clinical trial assays (CTAs) other than the Quest Diagnostics AAVrh74 Antibody ELISA CDx were utilized to test the baseline AAVrh74 antibody titer of the patient to determine the eligibility for therapy administration. The Quest Diagnostics AAVrh74 Antibody ELISA CDx assay was developed as a colorimetric immunoassay based upon CTA1. The developed CTA1 assays were used to establish a clinical cut-off titer for the AAVrh74 assay before clinical trials. In SRP-9001-102 and SRP-9001-103 clinical trials, CTA1 adopted the pre-existing antibody titer of ≤1:400 as eligibility criteria based on the safety and efficacy data from

non-human primate studies performed on CTA1.

The Norm OD cutoff of ≥ 1.1 was established using colorimetric immunoassay methods by testing serum specimens from subjects presumed to be negative for AAVrh74 antibodies (i.e., apparently healthy adult donors; n = 120; 60 females and 60 males) at a titer of 1:400 using CTA2. The cut off was defined as Norm OD ≥1.10. In order to utilize a fixed cutoff (Norm OD ≥ 1.1), CTA2 developed an assay calibrator to reduce run to run variability (i.e., assay drift) over time. The calibrator is identical to the calibrator used for the Quest Diagnostics AAVrh74 Antibody ELISA CDx.

Quest Diagnostics AAVrh74 Antibody ELISA CDx was transferred from CTA2 to the Quest Diagnostics laboratory in San Juan Capistrano CA. Successful transfer of the assay from CTA2 to the Quest Diagnostics laboratory in San Juan Capistrano was shown with a panel of 100 specimens from a healthy adult population of adults that included 50 positives and 50 negatives. There was 100% agreement between the specimens.

To determine the assay cut-off of the Quest Diagnostics AAVrh74 Antibody ELISA CDx, serum specimens from subjects presumed to be negative for AAVrh74 antibodies (i.e., apparently healthy adult donors; n = 120; 60 females and 60 males) were tested at a titer of 1:400. No significant difference in the distribution of values by gender was observed (p-value = 0.85). The cut off was defined as ≥1.10 based on the upper limit (97.5th percentile) of the assay cut-off.

2. Accuracy

An accuracy study was performed to verify the signal obtained from the Quest Diagnostics AAVrh74 Antibody ELISA CDx was from antibodies in serum samples over a range of samples with titers above Norm OD 2.00 and low positive samples with titer 1:400 but not exceeding 1:800.

The study was performed using two reagent kit lots by two operators with 10 serum samples. Seven samples were prepared from adult male healthy donors and positive for AAVrh74 antibodies with Norm OD values greater than 2.0 and three from clinical trial patients collected at timepoint week two (2) after ELEVIDYS dosing was provided by Sarepta Therapeutics Inc. Two 1:400 dilutions for each of the samples positive for AAVrh74 antibodies were prepared to create dilution 1 (untreated) and dilution 2 (AAVRH74 spike). The data shows that all untreated positive samples yielded positive results. All positive samples pre-incubated with AAVrh74 yielded negative results. 100% of samples passed acceptance criteria and yielded the expected results. The results from the accuracy study demonstrate that the Quest Diagnostics AAVrh74 Antibody ELISA CDx detects and measures antibodies (IgG) against AAVrh74.

3. **Precision**

Description of samples in the precision studies.

All samples were collected from adult healthy donors. All native, non-pooled samples from potential donors or commercial pooled samples were pre-screened at the 1:400 dilution using the Quest Diagnostics AAVrh74 Antibody ELISA CDx.

Table 4: Sample Description

Sample IDs	Description	Targeted Norm OD Range	Target Norm OD
NC	Low Negative	<50% below cutoff	0.02
S2-2	Moderate Negative	~30% below cutoff	0.66
S4-3	Moderate Negative	~30% below cutoff	0.66
PC-4	High Negative	~20% below cutoff	0.88
S2-1	Low Positive	~20% above cutoff	1.32
S4-2	Low Positive	~20% above cutoff	1.32
PC-3	Low Positive	~20% above cutoff	1.50
LPC	Low Positive	~30% above cutoff	1.60
S4-1	Positive	~100% above cutoff	2.20
PC-2	Positive	~100% above cutoff	2.50
PC-1	High Positive	~200% above cutoff	3.60

Precision Study #1: Repeatability:

The first study (Study 1) characterized repeatability using six replicates per sample dilution within the same day, using one reagent lot, one operator and one instrument pair (one plate washer and one plate reader) for a total of six measurements. The second study (Study 2) characterized repeatability using 16 replicates per sample dilution within the same day, using the same reagent lot, one operator and one instrument pair for a total of 16 measurements per sample.

Table 5: Repeatability Study Result Summary

Samples (Target Norm OD)	N	Norm OD			Qualitative Agreement (%QA)
		Mean	SD	%CV	
Study 1					
NC (0.02)	6	0.02	0.00	0.00	100% (6/6)
PC-4 (0.80)	6	0.80	0.06	7.06	100% (6/6)
PC-3 (1.50)	6	1.45	0.14	9.88	100% (6/6)
LPC (1.60)	6	1.60	0.09	5.85	100% (6/6)
PC-2 (2.50)	6	2.46	0.24	9.72	100% (6/6)
PC-1 (3.60)	6	3.58	0.18	5.15	100% (6/6)
Study 2					
NC (0.02)	5*	0.02	0.01	55.90	100% (5/5)
LPC (1.60)	5*	1.36	0.08	5.59	100% (5/5)
S2-1 (1.32)	16	1.28	1.11	8.35	100% (16/16)
S2-2 (0.66)	16	0.70	0.05	6.67	100% (16/16)
S4-1 (2.20)	16	2.25	0.09	3.94	100% (16/16)
S4-2 (1.32)	16	1.25	0.06	4.89	100% (16/16)
S4-3 (0.66)	16	0.68	0.04	5.28	100% (16/16)
* NC and LPC are are tested per plate, i.e. per run in Study 2					

Precision Study #2: Within-laboratory precision (repeatability, between-run, and between-day components)

The second set of studies characterized between-run and between-day precision in accordance with CLSI EP05-A3. The studies measured each sample across two replicates per sample, two runs per day, over 20 days for a total of 80 measurements per sample. A single lot of reagents, one operator and one instrument pair were used in both studies. The Norm OD values were utilized to estimate between-run, and between-day precision.

Table 6: Between-Day and Between-Run Precision Study Result Summary

Samples (Target Norm OD)	N	Norm OD	Between-day		Between-run		Repeatability		Total*	
		Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Study 1										
NC (0.02)	80	0.02	0.00	0.00	0.01	44.57	0.01	45.18	0.02	63.46
PC-4 (0.80)	80	0.86	0.03	3.35	0.02	2.01	0.07	8.23	0.08	9.11
PC-3 (1.50)	80	1.60	0.01	0.86	0.05	3.19	0.11	6.68	0.12	7.46
LPC (1.60)	80	1.61	0.00	0.00	0.06	3.79	0.7	4.66	0.10	6.01
PC-2 (2.50)	80	2.64	0.06	2.28	0.07	2.82	0.19	7.10	0.21	7.97
PC-1 (3.60)	80	3.62	0.10	2.85	0.13	3.63	0.27	7.56	0.32	8.85
Study 2										
NC (0.02)	41	0.01	0.00	0.00	0.01	70.59	**	**	0.01	70.56
LPC (1.60)	41	1.40	0.00	0.00	0.07	5.33	**	**	0.07	5.33
S2-1 (1.32)	80	1.33	0.01	0.74	0.05	4.06	0.05	3.69	0.07	5.53
S2-2 (0.66)	80	0.73	0.01	1.84	0.02	2.83	0.04	4.84	0.04	5.90
S4-1 (2.20)	82	2.18	0.00	0.00	0.12	5.44	0.09	4.14	0.15	6.84
S4-2 (1.32)	82	1.26	0.02	1.37	0.06	4.62	0.05	4.04	0.08	6.29
S4-3 (0.66)	80	0.69	0.00	0.16	0.04	5.48	0.02	3.55	0.05	6.53

* Total precision includes repeatability, between-day and between-run.
** Data for Within-run (Repeatability) cannot be estimated for NC or LPC as only one of each were tested per plate, i.e. per run in Study 2.

Table 7: Qualitative Agreement Result Summary

Samples (Target Norm OD)	Expected Status (Positive/ Negative)	%Positive (#positive/Total)	%Negative (#negative/Total)
Study 1			
NC (0.02)	Negative	0	100% (80/80)
PC-4 (0.80)	Negative	0	100% (80/80)
PC-3 (1.50)	Positive	100% (80/80)	0
LPC (1.60)	Positive	100% (80/80)	0
PC-2 (2.50)	Positive	100% (80/80)	0
PC-1 (3.60)	Positive	100% (80/80)	0
Study 2			
NC (0.02)	Negative	0	100% (41/41)
LPC (1.60)	Positive	100% (41/41)	0

Samples (Target Norm OD)	Expected Status (Positive/ Negative)	%Positive (#positive/Total)	%Negative (#negative/Total)
S2-1 (1.32)	Positive	100% (80/80)	0
S2-2 (0.66)	Negative	0	100% (80/80)
S4-1 (2.20)	Positive	100% (82/82)	0
S4-2 (1.32)	Positive	98.8 (81/82)	1.2% (1/82)
S4-3 (0.66)	Negative	0	100% (80/80)

Precision Study #3: Within-laboratory precision (Operator-to-Operator Variability)
The third set of studies characterized between-operator precision. The study design included testing three replicates per sample, two runs per day over three days, by three operators. This study used a single lot of reagents and one instrument pair (1 washer and 1 reader). A total of 54 measurements were obtained per sample.

Table 8: Between-operator Precision Study Result Summary

Samples (Target Norm OD)	N	Mean Norm OD	Between-Day		Between-Run		Repeatability		Between-Operator		Total*	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Study 1												
NC (0.02)	54	0.02	0.00	13.52	0.00	0.00	0.01	50.29	0.00	0.00	0.01	52.07
PC-4 (0.80)	54	0.82	0.04	5.22	0.00	0.00	0.09	11.13	0.05	5.52	0.11	13.48
PC-3 (1.50)	54	1.49	0.06	3.80	0.04	2.98	0.14	9.23	0.06	3.78	0.17	11.08
LPC (1.60)	54	1.58	0.04	2.76	0.00	0.00	0.09	5.50	0.02	1.12	0.10	6.25
PC-2 (2.50)	54	2.43	0.10	4.07	0.03	1.26	0.20	8.26	0.00	0.00	0.23	9.29
PC-1 (3.60)	54	3.36	0.15	4.43	0.00	0.00	0.31	9.14	0.00	0.00	0.34	10.16
Study 2												
NC (0.02)	19	0.02	0.01	32.35	0.00	0.00	**	**	0.00	0.00	0.01	32.35
LPC (1.60)	19	1.38	0.11	7.86	0.00	0.00	**	**	0.00	0.00	0.11	7.86
S2-1 (1.32)	56	1.34	0.12	9.26	0.00	0.00	0.10	7.41	0.00	0.00	0.13	9.32
S2-2 (0.66)	54	0.72	0.07	9.09	0.00	0.00	0.06	8.52	0.00	0.00	0.07	9.12
S4-1 (2.20)	54	2.27	0.22	9.69	0.06	2.57	0.13	5.75	0.07	3.08	0.23	9.97
S4-2 (1.32)	54	1.29	0.08	6.27	0.00	0.00	0.05	3.88	0.1	0.87	0.08	6.36
S4-3 (0.66)	54	0.70	0.06	7.94	0.00	0.00	0.03	4.58	0.02	3.34	0.06	8.25

* Total precision includes repeatability, between-day, between-run and between-operator.
** Data for Repeatability cannot be estimated for NC or LPC as only one of each were tested per plate, i.e. per run in Study 2.

Table 9: Qualitative Agreement Results summary

Samples (Target Norm OD)	Expected Status (Positive/ Negative)	%Positive (#positive/Total)	%Negative (#negative/Total)
Study 1			
NC (0.02)	Negative	0	100% (54/54)
PC-4 (0.80)	Negative	2% (1/54)	98% (53/54)

Samples (Target Norm OD)	Expected Status (Positive/ Negative)	%Positive (#positive/Total)	%Negative (#negative/Total)
PC-3 (1.50)	Positive	98% (53/54)	2% (1/54)
LPC (1.60)	Positive	100% (54/54)	0
PC-2 (2.50)	Positive	100% (54/54)	0
PC-1 (3.60)	Positive	100% (54/54)	0
Study 2			
NC (0.02)	Negative	0	100% (19/19)
LPC (1.60)	Positive	100% (19/19)	0
S2-1 (1.32)	Positive	96.4% (54/56)	3.6% (2/56)
S2-2 (0.66)	Negative	0	100% (54/54)
S4-1 (2.20)	Positive	100% (54/54)	0
S4-2 (1.32)	Positive	100% (54/54)	0
S4-3 (0.66)	Negative	0	100% (54/54)

Precision Study #4: Within-laboratory precision (Lot-to-Lot Variability)

The fourth set of studies characterized between-lot precision for the assay and controls. The first study (Study 1) included testing three replicates per sample, two runs per day over five days and three reagent lot combinations. The second study (Study 2) included testing four replicates per sample, two runs per day over five days and three reagent lot combinations. One instrument pair and one operator were used in both studies. A total of 90 measurements per sample in Study 1 and a total of 120 measurements per sample in Study 2 were obtained to estimate between-lot precision.

Table 10: Between-lot Precision Study Result Summary

Sample (Target nOD)	N	Mean Norm OD	Between-Day		Between-Run		Repeatability		Between-Lot		Total Precision*	
			SD	%CV	SD	%CV	SD	%CV	SD	% CV	SD	% CV
Study 1												
NC (0.02)	90	0.03	0.0	0.00	0.00	13.11	0.02	25.59	0.02	70.84	0.02	76.46
PC-4 (0.80)	90	0.84	0.02	2.35	0.00	0.00	0.01	7.94	0.01	1.40	0.07	8.39
PC-3 (1.50)	90	1.60	0.05	3.04	0.03	1.75	0.04	7.43	0.04	2.32	0.13	8.54
LPC(1.60)	90	1.59	0.00	0.00	0.03	1.98	0.04	4.65	0.04	2.52	0.09	5.65
PC-2 (2.50)	90	2.48	0.04	1.67	0.07	2.97	0.08	6.27	0.08	3.19	0.20	7.82
PC-1 (3.60)	90	3.64	0.00	0.00	0.17	4.80	0.16	7.25	0.16	4.57	0.35	9.82
Study 2												
NC (0.02)	60**	0.02	0.00	0.00	0.00	10.86	0.01	31.62	0.01	43.70	0.01	55.02
LPC (1.60)	60**	1.45	0.00	0.00	0.04	2.56	0.07	4.68	0.11	7.77	0.14	9.43
S2-1 (1.32)	120	1.31	0.03	2.05	0.05	3.67	0.06	4.64	0.02	1.24	0.08	6.38
S2-2 (0.66)	120	0.70	0.02	2.95	0.02	2.49	0.04	5.35	0.01	1.36	0.05	6.73
S4-1 (2.20)	120	2.28	0.06	2.46	0.14	6.27	0.09	4.13	0.00	0.00	0.18	7.90
S4-2 (1.32)	120	2.48	0.00	0.00	0.08	6.29	0.06	4.36	0.00	0.00	0.10	7.66
S4-3 (0.66)	120	0.71	0.01	1.80	0.04	5.69	0.04	5.56	0.00	0.00	0.06	8.16

*Total Precision includes repeatability, between-day, between-run and between-lot.
**NC and LPC are tested per plate, i.e. per run in Study 2

Table 11: Between-Lot Qualitative Agreement (QA) Results Summary

Samples (Target Norm OD)	Expected Status (Positive/ Negative)	%Positive (#positive/Total)	%Negative (#negative/ Total)
Study 1			
NC (0.02)	Negative	0	100% (90/90)
PC-4 (0.80)	Negative	0	100% (90/90)
PC-3 (1.50)	Positive	100% (90/90)	0
LPC (1.60)	Positive	100% (90/90)	0
PC-2 (2.50)	Positive	100% (90/90)	0
PC-1 (3.60)	Positive	100% (90/90)	0
Study 2			
NC (0.02)	Negative	0	100% (60/60)
LPC (1.60)	Positive	100% (60/60)	0
S2-1 (1.32)	Positive	100% (120/120)	0
S2-2 (0.66)	Negative	0	100% (120/120)
S4-1 (2.20)	Positive	100% (120/120)	0
S4-2 (1.32)	Positive	97.5% (117/120)	2.5% (3/120)
S4-3 (0.66)	Negative	0	100% (120/120)

Precision Study #5: Between-Instrument precision

The fifth set of studies characterized between-instrument (plate washers and plate readers) precision for the instruments that are used for the Quest Diagnostic AAVrh74 Antibody ELISA assay. A total of four instruments: two washers and two readers were tested with three replicates per sample, two runs per day over five days. In Study 1, three washer/reader combinations were tested as washer 1/reader 1, washer 1/reader 2, and washer 2/reader 1. In Study 2, two washer/reader combinations were used as washer 1/reader 2 and washer 2/reader 1. Both studies used a single lot of reagents by a single operator. A total of 90 measurements per sample in Study 1 and 60 measurements per sample in Study 2 were obtained to estimate between-instrument (between reader and between washer) precision.

Table 12: Between-Instrument Precision Summary

Samples (Target Norm OD)	N	Mean Norm OD	Between-Day		Between-Run		Repeatability		Between-Instrument		Total Precision*	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Study 1												
NC (0.02)	90	0.02	0.00	9.58	0.00	18.32	0.00	21.10	0.00	5.48	0.01	30.05
PC-4 (0.80)	90	0.78	0.00	0.00	0.06	7.31	0.08	10.19	0.00	0.00	0.10	12.50
PC-3 (1.50)	90	1.49	0.05	3.18	0.06	4.12	0.13	9.03	0.00	0.00	0.16	10.42
LPC (1.60)	90	1.60	0.02	1.46	0.03	2.19	0.10	6.18	0.00	0.00	0.11	6.72
PC-2 (2.50)	90	2.59	0.09	3.35	0.11	4.32	0.20	7.61	0.00	0.00	0.24	9.37
PC-1 (3.60)	90	3.75	0.19	4.93	0.14	3.72	0.23	6.14	0.00	0.00	0.33	8.71
Study 2												
NC (0.02)	20	0.02	0.00	0.00	0.01	29.50	**	**	0.00	0.00	0.01	29.50

Samples (Target Norm OD)	N	Mean Norm OD	Between-Day		Between-Run		Repeatability		Between-Instrument		Total Precision*	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
LPC (1.60)	20	1.61	0.04	2.25	0.08	5.26	**	**	0.00	0.00	0.09	5.72
S2-1 (1.32)	60	1.31	0.03	2.34	0.05	3.54	0.09	7.15	0.00	0.00	0.11	8.31
S2-2 (0.66)	60	0.67	0.03	4.59	0.00	0.00	0.04	6.37	0.00	0.00	0.05	7.85
S4-1 (2.20)	60	2.27	0.09	3.96	0.09	4.22	0.10	4.56	0.00	0.00	0.17	7.37
S4-2 (1.32)	60	1.27	0.07	5.62	0.00	0.00	0.05	4.18	0.00	0.00	0.19	7.00
S4-3 (0.66)	60	0.67	0.03	3.91	0.01	1.16	0.04	5.31	0.00	0.00	0.05	6.70

* Total Precision includes Between-instrument, Between-run, Between-day and repeatability.
** **Study 2**: Data from washer 1/reader 2 (Set 1) or washer 2/reader 1 (Set 2) for Within-run (Repeatability) cannot be estimated for NC or LPC as only one of each were tested per plate.

Table 13: Between-Instrument Qualitative Agreement Results Summary

Samples (Target Norm OD)	Expected Status (Positive/ Negative)	%Positive (#positive/Total)	%Negative (#negative/Total)
Study 1			
NC (0.02)	Negative	0	100% (90/90)
PC-4 (0.80)	Negative	2% (2/90)	98% (88/90)
PC-3 (1.50)	Negative	100% (90/90)	0
LPC (1.60)	Positive	100% (90/90)	0
PC-2 (2.50)	Negative	100% (90/90)	0
PC-1 (3.60)	Positive	100% (90/90)	0
Study 2			
NC (0.02)	Negative		100% (20/20)
LPC (1.60)	Positive	100% (20/20)	0
S2-1 (1.32)	Positive	95% (57/60)	0
S2-2 (0.66)	Negative	0	100% (60/60)
S4-1 (2.20)	Positive	100% (60/60)	0
S4-2 (1.32)	Positive	100% (60/60)	0
S4-3 (0.66)	Negative	0	100% (60/60)

Quest Diagnostics AAVrh74 Antibody ELISA Overall Precision

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

Table 14: Total Precision Summary

Samples (Target Norm OD)	Within-run		Between-Day		Between-Run		Between-operator		Between-lot		Between-instrument		Total Precision	
	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
S2-2 (0.66)	0.05	6.67	0.03	4.59	0.02	2.83	0.07	9.12	0.05	6.73	0.05	7.85	0.05	7.19
S4-3 (0.66)	0.04	5.28	0.03	3.91	0.04	5.48	0.06	8.25	0.06	8.16	0.05	6.70	0.05	7.47
PC-4 (0.80)	0.06	7.06	0.00	0.00	0.02	2.01	0.11	13.48	0.07	8.39	0.10	12.50	0.07	21.76
S2-1 (1.32)	0.11	8.35	0.03	2.34	0.05	4.06	0.13	9.32	0.08	6.38	0.11	8.31	0.10	7.25
S4-2 (1.32)	0.06	4.89	0.07	5.62	0.06	4.62	0.08	6.36	0.10	7.66	0.09	7.00	0.09	6.92
PC-3 (1.50)	0.14	9.88	0.05	3.18	0.05	3.19	0.17	11.08	0.13	8.54	0.16	10.42	0.12	20.32
S4-1 (2.20)	0.09	3.94	0.09	3.96	0.12	5.44	0.23	9.97	0.18	7.90	0.17	7.37	0.18	7.83
PC-2 (2.50)	0.24	9.72	0.09	3.35	0.07	2.82	0.23	9.29	0.20	7.82	0.24	9.37	0.18	18.52
PC-1 (3.60)	0.18	5.15	0.19	4.93	0.13	3.63	0.34	10.16	0.35	9.82	0.33	8.71	0.27	17.98

Table 15. Overall Precision Study Qualitative Result

Samples (Target Norm OD)	Expected Status (Positive/ Negative)	%Positive (#positive/Total)	%Negative (#negative/Total)
Study 1			
NC (0.02)	Negative	0	100% (320/320)
PC-4 (0.80)	Negative	99.1% (317/320)	0
PC-3 (1.50)	Negative	99.7% (319/320)	0
LPC (1.60)	Positive	100% (320/320)	0
PC-2 (2.50)	Negative	100% (320/320)	0
PC-1 (3.60)	Positive	100% (320/320)	0
Study 2			
LPC (1.60)	Positive	100% (145/145)	0
S2-1 (1.32)	Positive	98.5% (327/332)	0
S2-2 (0.66)	Negative	100% (330/330)	0
S4-1 (2.20)	Positive	100% (332/332)	0
S4-2 (1.32)	Positive	98.8% (328/332)	0
S4-3 (0.66)	Negative	0	100% (332/332)
NC(0.02)	Negative	0	100% (145/145)

4. Linearity

A linearity study was designed based on CLSI EP06-A *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline* to characterize the linearity of the Quest Diagnostics AAVrh74 Antibody ELISA CDx. The study used one reagent kit lot. Samples were collected from individual, adult healthy

donors, and were prescreened at the 1:400 dilution using the Quest Diagnostics AAVrh74 Antibody ELISA. Samples with known Norm OD values (at the 1:400 dilution) were pooled and tested prior to use. The study utilized six individual high antibody positive samples tested at the serial dilution range of 1:400 to 1:12,800 (assay titer reportable range of 1:400–≥1:3200). The specific OD values were graphed to a best fit linear equation using five points of the dilution series with one or more point below the cutoff value. The first order polynomial regression analysis was performed to evaluate the linearity of the assay. The regression coefficients, standard error of the slope, and a t-test to test whether the linear coefficients are statistically significant were considered. R-Squared, the goodness of fit measure for linear regression models, was also reported per sample. Results demonstrate that the Quest Diagnostics AAVrh74 Antibody ELISA CDx is linear across the reportable range of the assay titer between 1:400 and 1:3200, as well as below the cutoff (Norm OD <1.10), and the maximum OD of the spectrophotometer.

5. **Prozone/High-dose Hook Effect**
   A high-dose hook study was performed to characterize the performance of the Quest Diagnostic AAVrh74 Antibody ELISA CDx when used to test a dilution series of specimens containing very high levels of AAVrh74 antibodies. Very high AAVrh74 antibody tittered sera was diluted from 1:400 and tittered to end-point. Each sample was run in identical plate runs per day by one operator using one instrument setup (1 washer/reader) and the same reagent kit lot. Each dilution was tested in singlicate. For data analysis, the numerical quantitative equivalent of dilutions 400 to 13107200 (1/dilution) was plotted against the Norm OD values for the samples to demonstrate absence of a Hook Effect (no dilution prior to the end-point titer will have an NOD <1.10). Results demonstrates the absence of a high-dose hook effect for the Quest Diagnostics AAVrh74 Antibody ELISA CDx.

6. **Analytical Sensitivity (Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantitation (LoQ))**
   The studies were designed based on CLSI EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition* to establish the analytical sensitivity (LoB, LoD and LoQ) of the Quest Diagnostics AAVrh74 Antibody ELISA CDx.

   The LoB was assessed by one operator with two reagent lots and one instrument pair (one washer and one plate reader combination). Serum specimens were collected from adult male healthy donors. Four native negative serum samples (Norm OD <1.1 at 1:400 dilution) were further immunodepleted to prepare four blank samples in the LoB study. The four low measurand level samples were prepared by spiking one native positive serum sample to the four immunodepleted blank samples. LoB was calculated as the 95th percentile of blank replicates using non-parametric data analysis for each reagent lot. The LoD of the device was calculated for each reagent lot using parametric data analysis. The LoB, as the maximal value of the LoB for the two reagent lots tested, is Norm OD = 0.03. The LoD, as the maximal value of the LoD for the two reagent lots tested, is Norm OD = 0.05.

   A study was performed to establish the Lower Limit of Quantitation (LLoQ) and Upper Limit of Quantitation (ULoQ) following the variant approach in CLSI EP17-A2. Eight

multilevel serum samples were prepared from adult healthy male donors and prescreened at the 1:400 dilution using the Quest Diagnostics AAVrh74 Antibody ELISA CDx. Samples with known Norm OD values (at the 1:400 dilution) were pooled and tested prior to use. Aliquots of each sample were tested at the 1:400, 1:800, 1:1600, 1:3200 and up to 1:25600 for high positives in order to determine the titer and confirm the targeted Norm OD values below or above the cutoff (Norm OD 1.10) at the 1:400 dilution. Using the subject assay, each of the eight samples were tested in eight replicates for five days using each of the two reagent lots replicates for 80 total measurements per sample, or 40 results per sample per lot.

The LLoQ was determined as the Norm OD value with the highest %CV at the low end and ULoQ was determined as the Norm OD value with the highest %CV at the high end of the measurement range across two reagent lots. LLoQ was determined as Norm OD = 0.46, Titer <1:400 and ULoQ was determined as Norm OD = 1.64, Titer 1:12800.

7. Analytical Specificity (Interfering Substances)

An analytical specificity study was performed to characterize the effect of endogenous substances commonly present in serum samples and exogenous substances (e.g., medications utilized to treat the target population (individuals with DMD)) on the performance of the Quest Diagnostics AAVrh74 Antibody ELISA CDx. The study design was based on the recommendations in CLSI EP07 *Interference Testing in Clinical Chemistry 3rd Edition*. Samples were collected from adult healthy donors and were prescreened at the 1:400 dilution using the Quest Diagnostics AAVrh74 Antibody ELISA CDx. Donor specimens and serum pools were selected and used to create pooled samples S1–S3 targeting different Norm OD ranges. Each sample was tested in 15 replicates with interferent (Test) and in 15 replicates with Control (diluent only) at a single dilution 1:400 on the same plate. Testing was performed by one operator using one plate reader and one plate washer. The results from the interference study demonstrate that none of the 29 potentially endogenous and exogenous substances at the tested concentration interfere with the performance of the Quest Diagnostics AAVrh74 Antibody ELISA CDx.

Table 16: Serum Specimen Requirements

Sample	Expected average Norm OD after spike addition	Description
S1	~20% below cutoff	High negative
S2	~20% above cutoff	Low positive
S3	2–3 times cutoff	Moderate positive

Table 17: Summary Results of Analytical Specificity

Endogenous Interferents and Test Concentrations	
Albumin	6 g/dL
Bilirubin, conjugated	40 mg/dL
Bilirubin, unconjugated	40 mg/dL
Cholesterol	400 mg/dL
Glucose	1,000 mg/dL
Hemoglobin	1,000 mg/dL
Rheumatoid factor	600 IU/mL

Endogenous Interferents and Test Concentrations	
Triglycerides, total	1,500 mg/dL
Alkaline phosphatase (ALP)	1.29 U/mL
Lactate dehydrogenase (LDH)	4.0 U/mL
Creatine Kinase (CK-MM)	50,000 U/L
Aspartate aminotransferase (AST)	3,500 U/L
Alanine aminotransferase (ALT)	3,500 U/L
Exogenous Interferents and Test Concentrations	
Acetaminophen	15.6 mg/dL
Biotin	0.351 mg/dL
Deflazacort	1.2 mg/dL
Human growth hormone	30.3 ng/mL
Ibuprofen	21.9 mg/dL
Prednisone	9.9E-03 mg/dL
Prednisolone	1.20E-01 mg/dL
Ciprofloxacin	1.2 mg/dL
Amikacin	14.4 mg/dL
Losartan	2.36E-01 mg/mL
Perindopril	2.36E-03 mg/mL
Lisinopril	2.46E-04 mg/mL
Hydrochlorothiazide	1.13E-03 mg/mL
Furosemide	1.59E-02 mg/mL
Spironolactone	5.55E-04 mg/mL
Omeprazole	8.4E-03 mg/mL

8. Carryover

A study was performed to assess well-to-well carry over due to liquid transfer steps and well-to-well cross-talk during absorbance detection. This study utilized one lot of reagents, two instruments and two operators for a total of three days across Stage 1 (replicates of negative sample in one plate setup) and Stage 2 (replicates of alternating negative and positive samples across the plate). The study used serum specimens from adult male, healthy donors. All samples collected from donors were prescreened at the 1:400 dilution using this AAVrh74 Antibody ELISA CDx.

All negative and positive samples yielded 100% negative and positive results, respectively. The results from the carry-over and cross-talk analyses demonstrated there was no carry-over or cross-talk.

9. Specimen Stability

A specimen stability study was performed to characterize serum specimen stability for testing with the Quest Diagnostics AAVrh74 Antibody ELISA CDx. The isochronous study design was based on the recommendation in CLSI EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*. A single lot of reagents and one single instrument pair for each temperature tested, with the exception of the frozen (-60°C to -90°C) condition which used three reagent kit lots.

Based on the sample collection and processing conditions used during Sarepta's clinical

studies, as well as Quest's expected commercial specimen shipping conditions, serum stability was evaluated after exposure to the following conditions: ambient storage (18–26°C), refrigerated storage (2–8°C), frozen storage (-10°C to -30°C and -60°C to -90°C), and repeated freeze/thaw cycles. Native serum samples targeting different Norm OD value ranges were collected from adult healthy male donors. Six samples were tested in five replicates at each timepoint.

The specimen stability supports the following specimen stability claims:

- Freeze/Thaw cycles: 9 cycles
- Ambient (18°C to 26°C): 5 days
- Refrigerated (2°C to 8°C): 10 days
- Frozen (-10°C to -30°C): 21 days
- Frozen (-60°C to -90°C): 12 months

A transport stability study was performed to determine the stability of serum specimens during transport for testing with Quest Diagnostics AAVrh74 Antibody ELISA CDx, where specimens were subjected to simulated worst-case summer and winter transport conditions. This study used two lots of reagents and six native serum samples targeting different Norm OD value ranges. Small and medium insulated shipment containers were used for temperature cycling testing by FedEx in three shipping configurations: ambient shipment (18°C to 26°C), shipment on cold-packs (2°C to 8°C) and frozen shipping condition on dry ice (-60 ºC to -90ºC) that mimic commercial shipping conditions of specimens. After return of the shipping containers with specimens to Quest Diagnostic's testing site at CA, specimens were frozen (-60 to -90ºC) and subsequent testing was performed. The Norm OD values for samples S1-S6 in each shipment condition were compared to their Norm OD values at baseline. The results of this study support a specimen shipping/thermocycling stability claim of up to 72 hours for use in the Quest Diagnostics AAVrh74 Antibody ELISA CDx.

10. Reagent Stability

The reagent stability study was performed based on the recommendation in CLSI EP25-A to characterize the stability (shelf-life and in-use) of the Quest assay kit components. Kit components are stored at two different temperatures: the controls and calibrator are stored frozen at -60ºC to -90ºC and the remainder of the reagents (i.e., the prepared plates and additional kit components) are stored refrigerated at 2–8ºC. All reagents are single use and are stored as single use, ready-to-use aliquots except the Anti-human IgG (Fc) [HRP] Conjugate Stock and the 20X Concentrated Wash Buffer. These two reagents are stored as single use but require dilution before use. Stability was characterized by testing five serum specimens collected from adult healthy donors, targeting different Norm OD value ranges.

Three reagents lots were tested. Each of five samples was assessed in five replicates per plate, two plates per timepoint (n = 7 timepoints) after 0, 1, 3, 6, 9, 12 and 15 months of storage at the indicated temperatures. T=0 was defined as the timepoint at which the first testing occurred and was within 7 days of kit release. The results from the reagent shelf-life demonstrated that reagents are stable when stored as indicated for up to at least 12 months and the in-use stability study demonstrated that reagents are stable when removed from their indicated storage temperatures and placed at ambient temperature for 28 hours.

X. SUMMARY OF CLINICAL STUDIES

The efficacy of ELEVIDYS was evaluated in two double-blind, placebo-controlled studies (Study 1 [NCT 03769116] and Study 3 [NCT 05096221]) and one open-label study (Study 2 [NCT 04626674]) in which a total of 214 male patients with a confirmed disease-causing mutation in the DMD gene were dosed. Please refer to ELEVIDYS therapeutic approval page for key demographics and baseline characteristics of the patient population in the ELEVIDYS clinical trials.

A. SRP-9001-102 (NCT03769116) Study Design: A randomized, double-blind, placebo-controlled study in male patients with a confirmed disease-causing mutation in the *DMD* gene.

In SRP-9001-102, the study population consisted of male ambulatory DMD patients (N=41) aged 4 through 7 years with either a confirmed frameshift mutation, or a premature stop codon mutation between exons 18 to 58 in the *DMD* gene.

Patients were randomized 1:1 to receive either ELEVIDYS (N=20) or placebo (N=21), as a single intravenous infusion via a peripheral limb. Randomization was stratified by age (i.e., aged 4 to 5 years vs. aged 6 to 7 years). In the 4 through 5-year-old subgroup, the mean age, mean weight and mean NSAA total score (range) for the ELEVIDYS-treated patients (n=8) were 4.98 years, 20.1 kg and 20.1 (17-23), and for the placebo patients (n=8) were 5.15 years, 19.8 kg and 20.4 (15-24). In the ELEVIDYS group, eight patients received 1.33×10^{14} vg/kg of ELEVIDYS, and 12 patients received lower doses. All patients were on a stable dose of corticosteroids for DMD for at least 12 weeks prior to ELEVIDYS infusion. All randomized patients had baseline anti-AAVrh74 antibody titers <1:400 as determined by an investigational total binding antibody ELISA (CTA 1).

B. SRP-9001-103 (NCT04626674) Study Design: An open-label, ongoing study in male patients with a confirmed disease-causing mutation in the *DMD* gene.

Study SRP-9001-103 included 5 cohorts of 48 male DMD patients. Patients in Cohorts 1, 2 and 3 have a confirmed frameshift, splice site or premature stop codon mutation anywhere in the DMD gene, while patients in cohort 4 included patients with mutations in the DMD gene starting at or after exon 18. All patients in cohort 5 had mutations that partially or fully overlap with exons 1–17 in the DMD gene. All patients had baseline anti-AAVrh74 antibodies titers ≤1:400 as determined by the investigational total binding antibody ELISA (CTA 1). Patients received a single intravenous infusion of 1.33×10^{14} vg/kg ELEVIDYS if they weighed less than 70 kg or 9.31×10^{15} vg/kg total fixed dose if they weighed 70 kg or greater.

Cohorts 1, 2, 4 and 5a enroll 40 ambulatory patients 3 to 12 years of age, with weights ranging from 12.5 to 50.5 kg, baseline mean NSAA total score of 20.3 (11 to 30), and mean time to rise from floor of 4.7 seconds (2.4 to 9.7). Cohorts 3 and 5b include 8 non-ambulatory patients 10 to 20 years of age, with weights ranging from 36.1 to 80.1 kg. The primary objection of Study 103 drug efficacy was to compare micro-dystrophin expression from baseline to the week 12 group.

C. SRP-9001-301 (NCT05096221): A multi-center, randomized, double-blind, placebo-controlled study in male patients with a confirmed disease-causing mutation in the *DMD*

gene.

Study SRP-9001-301 included 125 ambulatory male patients aged 4 through 7 years, with a confirmed frameshift, splice site, premature stop codon, or other disease-causing mutation in the DMD gene starting at or after exon 18, were dosed. Patients with exon 45 (inclusive), or in-frame deletions, in-frame duplications, and variants of uncertain significance ("VUS"), were excluded. All patients had baseline anti-AAVrh74 antibodies titers <1:400 as determined by the investigational total binding antibody ELISA (CTA 2) and received a single intravenous infusion of 1.33×10^{14} vg/kg ELEVIDYS. The efficacy outcome measure of the study was to evaluate the effect of ELEVIDYS on physical function as assessed by the NSAA total score. Key secondary outcome measures were to evaluate expression of micro dystrophin in skeletal muscle, time to rise from floor, and time of 10-meter walk/run. Additional efficacy outcome measures included time of 100-meter walk/run, and time to ascend 4 steps.

D. Clinical Bridging Studies

During the clinical development of ELEVIDYS, CTAs were established and utilized to test the baseline AAVrh74 antibody of patients to assess the immunogenicity to the capsid and determine the subject eligibility for gene therapy administration. Drug efficacy is not correlated to the immunogenicity to the capsid. Two different CTAs were utilized during the Sarepta Therapeutics' clinical trials. CTA 1 was utilized in clinical trials SRP-9001-102 and SRP-9001-103, and CTA 2 was utilized in clinical trial SRP-9001-301 part 1. The Quest Diagnostics AAVrh74 Antibody ELISA CDx was not utilized in any of the clinical trials. Therefore, Quest Diagnostics conducted two retrospective clinical validation studies, i.e., bridging or concordance studies – Bridging Study 1 and Bridging Study 2, to compare the performance of the Quest Diagnostics AAVrh74 Antibody ELISA CDx to that of the CTAs. Samples from the following clinical studies submitted in the Biologics License Application (BLA) for ELEVIDYS were utilized in the Bridging Studies.

a. Bridging Study 1

Bridging Study 1 was conducted to assess the concordance between the Quest Diagnostics AAVrh74 Antibody ELISA CDx and CTA 1 used in Sarepta Therapeutic's SRP-9001-102 and SRP-9001-103 clinical trials.

A total of 118 samples were included consisting of 98 native screening specimens as well as 20 contrived samples near the 20% above and below CTA's cutoff. Quest obtained all available (evaluable) specimens from subjects at different timepoints including screening and post-dose samples from SRP-9001-102 and SRP-9001-103. Although the candidate device is intended for use only at screening, positive specimens were needed to meet the recommendations found in CLSI EP12-A2 *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition*.

The Quest Diagnostics AAVrh74 Antibody ELISA CDx was defined as the test method and the CTA 1 as the comparative method. Positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) were reported. The calculations of PPA and NPA exclude inconclusive and unevaluable results. A 95% two-sided Wilson score confidence interval was calculated for each reported measure of agreement.

Table 18. Concordance Analysis Results

	CTA + (Positive)	CTA – (Negative)	Total	Concordance (95% CI)
CDx + Positive	62	5	67	PPA=92.5% (82.7, 97.2)
CDx- Negative	5	46	51	NPA=90.2% (77.8, 96.3)
Total	67	51	118	OPA=91.5% (84.6, 95.6)

b. Bridging Study 2

Bridging Study 2 was conducted to assess the concordance between the Quest Diagnostics AAVrh74 Antibody ELISA CDx and CTA 2 used in Sarepta Therapeutic's SRP-9001-301 Part 1 study. A total of n=142 screening specimens from Sarepta's SRP-9001-301 Part 1 study were included for concordance analysis.

The Quest Diagnostics AAVrh74 Antibody ELISA CDx was defined as the test method and CTA 2 as the comparative method. PPA, NPA and OPA were reported together with their 95% two-sided Wilson score confidence interval. The calculations of PPA and NPA excluded inconclusive and unevaluable results.

Table 19. Concordance Analysis Results

	CTA + (Positive)	CTA – (Negative)	Total	Concordance (95% CI)
CDx + Positive	16	0	16	PPA=94.1% (73.0, 99.0)
CDx- Negative	1	125	126	NPA=100% (97.0, 100)
Total	17	125	142	OPA=99.3% (96.1, 99.9)

The safety and efficacy of ELEVIDYS for the treatment of DMD has been established in patients at least 4 years of age with a confirmed mutation in the DMD gene, as per ELEVIDYS drug label (Clinical Pharmacology (12.2), Clinical Studies (14).

XI. FINANCIAL DISCLOSURE

The Financial Disclosure by Clinical Investigators regulation (21 CFR. *Part* 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any non-employee clinical investigator conducting clinical studies covered by the regulation. The clinical validation study included one (1) investigator that Quest considers an employee. None of the non-employee clinical investigators had 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.

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

For the intended use of semi-quantitative detection of antibodies (IgG) to AAVrh74 capsid in patients with DMD for treatment with ELEVIDYS, the effectiveness of the Quest Diagnostics AAVrh74 Antibody ELISA CDx was demonstrated through analytical studies using patient samples from the clinical studies. The data from the analytical validation and clinical bridging studies support the reasonable assurance of safety and effectiveness of the Quest Diagnostics AAVrh74 Antibody ELISA CDx when used in accordance with the indications for use.

Data regarding the efficacy of ELEVIDYS are part of the gene therapy approval and are summarized in the approved gene therapy label. Refer to FDA Label for the most recent ELEVIDYS product labeling.

B. Safety Conclusions

The risks of the device include the potential for a false positive, false negative, or delayed result. A false positive result would exclude the potential patient from ELEVIDYS treatment. A false negative result would lead to incorrect consideration for the gene therapy. Patients with a false negative result may be exposed to potential short-term and long-term risks of the gene therapy. A delayed result could potentially lead to delayed access to the gene therapy. However, the rate of occurrence of a false positive, a false negative, or a delayed result has been reduced to its lowest possible level with controls and mitigations as demonstrated by the Quest Diagnostics AAVrh74 Antibody ELISA CDx non-clinical and clinical robust performance characteristics.

Data regarding the safety of ELEVIDYS are summarized in the approved gene therapy label. Long-term safety of ELEVIDYS continues to be monitored as outlined in the risk management plan in ongoing clinical trials and proposed post-approval studies.

C. Benefit-Risk Determination

The benefit of the Quest Diagnostics AAVrh74 Antibody ELISA CDx, as a companion diagnostic device, is to detect pre-existing AAVrh74 antibodies in human serum and aid in the selection of DMD patients for ELEVIDYS treatment. The clinical assessment of Sarepta Therapeutics' ELEVIDYS was conducted under two double-blind, placebo-controlled studies (SRP-9001-102 and SRP-9001-301) and one open-label study (SRP9001-103). For the ELEVIDYS studies, clinical trial assays were used to select DMD patients for ELEVIDYS treatment by identifying patients with pre-existing anti-AAVrh74 antibody titers <1:400. All patients in the three clinical studies had baseline anti-AAVrh74 antibody titers <1:400 as determined by the CTASs and received ELEVIDYS. In the current PMA submission, the effectiveness of the Quest Diagnostics AAVrh74 Antibody ELISA CDx is

determined based on the concordance between its test results and those generated by the CTAs in two bridging studies. The results of the bridging studies met the pre-defined acceptance criteria.

The efficacy of Sarepta Therapeutics' ELEVIDYS is used to indirectly assess the clinical benefit of the Quest Diagnostics AAVrh74 Antibody ELISA CDx. DMD patients with false positive results (negative for antibodies but tested positive by the assay) will be ineligible for treatment. Such patients will continue with the current standard of care, such as corticosteroids and physical therapy. The risk associated with a false positive result is minimal.

Patients with false negative results (positive for antibodies but tested negative by the assay) will be inappropriately deemed eligible for treatment with ELEVIDYS. In the current PMA, patients with pre-existing AAVrh74 antibody titer exceeding 1:400 were excluded. The clinical safety of ELEVIDYS in the presence of high titers of anti-AAVrh74 total binding antibodies (false negative result) has not been evaluated. Refer to FDA for the most recent ELEVIDYS product labeling.

The benefits of the Quest Diagnostics AAVrh74 Antibody ELISA as an aid in determining eligibility for therapy with ELEVIDYS in DMD patients outweigh the risks. As noted above, any occurrence of a false positive, false negative, or delayed results with Quest Diagnostics AAVrh74 Antibody ELISA CDx have been reduced to the lowest possible level as shown by the fact that the assay met all acceptance criteria in both analytical and clinical studies.

The Quest Diagnostics AAVrh74 Antibody ELISA CDx has met all accuracy, specificity, sensitivity, precision, stability, linearity, high-dose hook effect, carry over and cross-contamination performance acceptance criteria. The assay correlates with the clinical trial assays in accuracy and clinical performance. The use of this assay would not change the benefit and risk profile of the therapeutic product, which has been approved by CBER. Considering the natural history of the disease and unmet medical needs, approval of the current PMA is recommended.

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 available data supports the probable benefit of use of the Quest Diagnostics AAVrh74 Antibody ELISA CDx to identify individuals for the treatment of DMD that may be eligible to receive ELEVIDYS 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. PREDETERMINED CHANGE CONTROL PLAN

The Quest Diagnostics AAVrh74 Antibody ELISA CDx includes a predetermined change control plan (PCCP) approved by the US Food and Drug Administration (FDA). The PCCP describes the specific test methods for analytical validation for the expansion of the patient population for whom treatment with a specific Sarepta Therapeutics' gene therapy is being considered without further regulatory review.

The Quest Diagnostics AAVrh74 Antibody ELISA CDx is intended to be used as an aid to identify patients with DMD for whom treatment with Sarepta Therapeutics' ELEVIDYS (SRP-9001) is being considered. The intent of this PCCP is to expand that intended use population to patients for whom treatment with an additional gene therapy manufactured by Sarepta Therapeutics' is being considered should the therapy receive future approval.

Sarepta Therapeutics' ELEVIDYS and the additional gene therapy included in this PCCP share the same Capsid Antigen which is a critical reagent utilized to prepare the AAVrh74 Antibody ELISA AAVrh74 Coated Microtiter Plate. Since the capsid utilized for the gene therapy programs remains unchanged, pre-existing antibodies the Quest Diagnostics AAVrh74 Antibody ELISA detects would be the same, regardless of the therapy. The Quest CDx Assay procedure, all reagents and components, specimen type (human serum), assay cut-off (1:400), and result interpretation will remain the same for the expanded indication. Once all changes to the Quest Diagnostics AAVrh74 Antibody ELISA CDx are made in accordance with this PCCP, the commercial Quest CDx Assay will use the same AAVrh74 Capsid Antigen for plate coating to support the eligibility determination for the commercially available gene therapies.

Due to this generalizability, a more limited set of validation data is scientifically justified to support future inclusion of the new patient population and gene therapy indication as compared to the data in support of the DMD indication. Therefore, Quest will leverage the analytical performance data submitted in this PMA to support the expansion of use in determining eligibility of treatment with the future gene therapy once the PMA is approved. The additional confirmatory studies to be conducted are endogenous and exogenous interference substances study utilizing the same methodology and protocols as the interference studies submitted in support of this PMA and the DMD indication.

Quest Diagnostics will perform an analytical specificity study to test additional interference substances, specifically supplements taken by and medications prescribed to patients that could potentially interfere with the assay. If the validation data meets the specific acceptance criteria, the Quest Diagnostics AAVrh74 Antibody ELISA CDx will become a companion diagnostic for Sarepta Therapeutics' approved gene therapy without additional premarket review.

The plan describes the specific analytical protocols which are designed in accordance with recommendations found in CLSI EP07, *Interference Testing in Clinical Chemistry 3rd Edition*. Each substance will be tested at the levels defined in CLSI EP37, 1st Edition, when guidance is available.

The acceptance criteria were approved to ensure that the device maintains the following

performance characteristics:

- Samples must meet pre-established sample and run acceptability criteria.
- Per CLSI EP07, 3rd Edition, section 5.3.1 (analyzing the results for interference) and section 6.2 (dose-response study), interference will be assessed by calculating the percent difference. If the percent difference is >10% for at least one sample, a dose-response study is to be performed at 1/2, 1/4, 1/8 and 1/16 of the initial interferent concentration to determine the lowest concentration at which interference occurs.
- For each interferent and interferent concentration, the qualitative agreement will be calculated accordingly: Qualitative agreement is the number of replicates with expected negative or positive results divided by total number of replicates.
- If the percent difference between the control and test spike sample is ≤10%, the effect of the interferent is considered non-significant.
- S1 (High Negative, 20% below cutoff) must score (%QA) negative ≥80% of the time; S2 (Low Positive 20% above cutoff) must score (%QA) positive ≥80% of the time; S3 must score (%QA) as positive ≥95% of the time.

Labeling for the Quest Diagnostics AAVrh74 Antibody ELISA CDx will be updated to reflect the new indication, and all labeling changes will be reported to the FDA in a PMA Annual Report.

XV. CDRH DECISION

CDRH issued an approval order on July 24, 2025.

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

XVI. 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.