nAbCyte™
Anti-AAVRh74var HB-FE Assay

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012-099-003

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IVD For in vitro diagnostic use

Rx only (For prescription use only)

NAME

Device Trade Name: nAbCyte™ Anti-AAVRh74var HB-FE Assay

Device Generic Name: nAbCyte Anti-AAVRh74var HB-FE Assay for BEQVEZ™ (fidanacogene elaparvovec) Eligibility in moderate to severe Hemophilia B

INDICATIONS FOR USE

The nAbCyte Anti-AAVRh74var HB-FE Assay for BEQVEZ (fidanacogene elaparvovec) Eligibility in moderate to severe Hemophilia B, or nAbCyte Anti-AAVRh74var HB-FE Assay, is a cell-based qualitative *in vitro* companion diagnostic device intended for the detection of neutralizing antibodies to the AAVRh74var capsid in serum specimens from adult patients previously diagnosed with moderate to severe hemophilia B to determine eligibility for treatment with the hemophilia B gene therapy, BEQVEZ. Patients who test positive for neutralizing antibodies to the AAVRh74var capsid are not eligible for treatment with BEQVEZ. Patients who test negative for neutralizing antibodies to the AAVRh74var capsid are eligible for treatment with BEQVEZ.

This diagnostic device is a single-site assay for professional use only and is to be performed only at Labcorp-Monogram Biosciences by trained and qualified laboratory personnel.

HUMANITARIAN DEVICE

Authorized by federal law for use as a companion diagnostic in the selection of patients to receive the Pfizer hemophilia B gene therapy, BEQVEZ (fidanacogene elaparvovec). The effectiveness of this device for this use has not been demonstrated.

MANUFACTURER'S ADDRESS

Laboratory Corporation of America, Holdings
Labcorp-Monogram Biosciences
347 Oyster Point Blvd.
South San Francisco, CA 94080

CUSTOMER SERVICE

Client Services email:
Monogram@labcorp.com

Client Services phone numbers:
Toll: 1-650-635-1100
Toll Free: 1-800-777-0177 (U.S. and Canada only)

CONTRAINDICATIONS

There are no known contraindications.

WARNINGS AND PRECAUTIONS

- Follow specimen collection, sample preparation, and shipping instructions to ensure that adequate samples are sent to Labcorp-Monogram Biosciences for testing. Samples that are received hemolyzed or thawed will not be tested and another blood draw from the patient will be required.
- The time interval between serum sample acquisition and subsequent testing with the nAbCyte Anti-AAVRh74var HB-FE Assay and BEQVEZ (fidanacogene elaparvovec) infusion should be as short as possible (e.g., approximately 8 weeks).

LIMITATIONS

- For *In Vitro* Diagnostic (IVD) use.
- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- The test results are intended to be used in conjunction with the full prescribing information for BEQVEZ (fidanacogene elaparvovec) and should not be used as the sole factor when determining the appropriateness of prescribing BEQVEZ (fidanacogene elaparvovec).
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care.
- Given the lack of a reference method, or an orthogonal method for reference, the false negative rate of the test has not been determined in the clinical setting.
- The performance of the assay in the presence of ibuprofen and naproxen has not been established. Patients should refrain from taking ibuprofen or naproxen within 12 hours or 4 days, respectively, of a blood draw for screening with nAbCyte Anti-AAVRh74var HB-FE Assay.

- The test is only intended to be performed by trained laboratory personnel at Labcorp-Monogram Biosciences.
- The test is intended to be performed using specific serial number-controlled instruments by Labcorp-Monogram Biosciences.

SUMMARY AND EXPLANATION OF THE TEST

The nAbCyte Anti-AAVRh74var HB-FE Assay is a qualitative, cell-based, antibody-mediated neutralization or transduction inhibition assay that is intended to be a companion diagnostic for BEQVEZ (fidanacogene elaparvovec). Patients may exhibit a pre-existing humoral response through exposure to naturally occurring AAVs that could limit gene transduction after the administration of BEQVEZ (fidanacogene elaparvovec). For this reason, testing to determine that a patient is Negative for neutralizing antibodies (nAbs) to the AAVRh74var vector is important to facilitate the safe administration of BEQVEZ (fidanacogene elaparvovec). Results are reported as “Positive” or “Negative” for nAbs to anti-AAVRh74var vector-based on a clinically validated assay cutoff.

TEST PRINCIPLE

nAbCyte Anti-AAVRh74var HB-FE Assay is performed exclusively as a laboratory service in a single CAP/CLIA laboratory at Labcorp-Monogram Biosciences.

The assay uses a recombinant AAV transduction inhibition assay to evaluate human serum for anti-AAVRh74var nAb activity. Briefly, a recombinant AAV vector (AAVRh74var CAG lucP) comprised of a luciferase gene-expression cassette and AAVRh74var capsid is incubated with serial dilutions of human serum and subsequently used to transduce cells. If anti-AAVRh74var nAb activity is present in the serum specimens, target cell transduction and subsequent luciferase signal are inhibited. Positive and Negative Controls are run on each assay plate.

A luminometer is used to measure light levels resulting from luciferase activity. The luminescence signals are analyzed using an Analysis Workbook, which has been validated to determine if the assay plate is valid through established quality control measures that include acceptance criteria associated with the Positive Control, Negative Control, and the patient sample. Once the assay plate is determined to be valid, results for the assay are reported qualitatively as either Positive or Negative. Results are not provided if the assay plate is invalid due to a failure of the quality control measures. A Positive result is defined as > 50% inhibition at the cut-off dilution (1:1). A Negative result is defined as ≤ 50% inhibition at the cut-off dilution (1:1).

ORDERING INSTUCTIONS

For ordering patient testing, contact Client Services:

e-mail: nAbCyteHemB@labcorp.com

Phone: 844-294-7361

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Physicians who are considering prescribing BEQVEZ (fidanacogene elaparvovec) for their patients with moderate to severe hemophilia B should order a patient blood draw to prepare serum for testing with nAbCyte Anti-AAVRh74var HB-FE Assay. A minimum of 2 mL of blood will be collected using standard serum collection and handling techniques using either a glass silicone coated red top tube or a plastic clot activator, silicone coated red top tube. The collection tubes should be filled and thoroughly mixed by manual inversion to mix the blood with the clotting activation agent. After mixing, allow the blood to clot for 30 minutes with the tube standing upright. Centrifuge the tube at 1500-2000 g for 15 minutes to separate the clot from serum.

Transfer the serum into appropriately labeled 1.5-2.5 mL screw-top plastic vials. A minimum of 0.2 mL of serum is required for

testing. With the vials standing upright, freeze the serum immediately at -20 °C or colder in a non-defrosting freezer until shipment. Do not thaw the sample after freezing.

Note: Samples that are received hemolyzed or thawed will not be tested and another blood draw from the patient will be required.

SHIPPING INSTRUCTIONS

Ship the frozen serum on dry ice to Labcorp-Monogram Biosciences:

Labcorp-Monogram Biosciences
345 Oyster Point Blvd.
South San Francisco, CA 94080

Questions about specimen collection, preparation for analysis, and shipping can be sent to Labcorp-Monogram Biosciences Client Services at Monogram@labcorp.com, 650-635-1100, or 1-800-777-0177.

INSTRUMENTS

nAbCyte AAVRh74var HB-FE Assay is intended to be performed using the PerkinElmer EnSight Multimode Plate Reader Luminometers Model HH3400, which are identified by specific serial numbers.

INTERPRETATION OF RESULTS

Results for the assay are reported qualitatively as either Positive or Negative based on the transduction of cells by an AAV vector in the presence of the serum sample relative to the transduction signal in the absence of the serum sample. Inhibition > 50% at the cut-off dilution (1:1) is defined as Positive. Inhibition ≤ 50% at th cut-off dilution (1:1) is defined as Negative.

A negative result for anti-AAVRh74var nAbs is one of the eligibility criteria for BEQVEZ (fidanacogene elaparvovec) therapy for patients with moderate to severe hemophilia B. See Limitations related to treating physician decisions on patient care and treatment.

EXPECTED VALUES AND POPULATION CHARACTERISTICS

The C0371004 study (NCT03587116) was a non-investigational product, multi-center, lead-in study designed to evaluate current Factor IX (FIX) prophylaxis replacement therapy, in the usual care setting of moderately severe to severe adult hemophilia B participants (FIX:C≤2%) who are negativ for neutralizing antibodi s (nAb) to adeno associated virus vector AAVRh74var. The study consisted of two phases: the screening phase, which included a laboratory blood draw to determine nAb status to AAVRh74var for the group of hemophilia B participants, and the second phase consisted of the data collection phase for those hemophilia B participants who were below the established threshold for nAb to AAVRh74var. The study was designed to provide prospective efficacy and selected safety data of FIX prophylaxis replacement therapy in the usual setting of those hemophilia B participants. Upon successfully completing the C0371004 study, participants were consented and screened for entry into the therapeutic Phase 3 hemophilia B gene therapy study described in the Clinical Performance section below.

All patients were tested for presence of anti-AAVRh74var nAbs in C0371004 study that included 314 hemophilia B participants. Of the 314 hemophilia B patients, 193 tested positive for anti-AAVRh74var nAbs while 121 tested negative for anti-AAVRh74var nAbs with the nAbCyte Anti-AAVRh74var HB-FE Assay. Based on this data, anti-AAVRh74var nAb negativity using nAbCyte Anti-AAVRh74var HB-FE Assay is expected in approximately 39% of patients.

SPECIFIC PERFORMANCE CHARACTERISTICS

Unless otherwise noted, a contrived set of five serum samples were used for the analytical studies. These samples are called the samples throughout this Technical Information. Human sera samples were generated using single or pooled human sera to span the potential % inhibition range of the assay. The samples used are as follows:

1. Sample 1: Low titer negative sample generated to exhibit approximately 30% inhibition.
2. Sample 2: Low titer negative sample generated to exhibit approximately 40% inhibition.
3. Sample 3: Low titer positive sample generated to exhibit approximately 60% inhibition.
4. Sample 4: Low titer positive sample generated to exhibit approximately 70% inhibition.
5. Sample 5: High titer positive sample generated to exhibit approximately >90% inhibition.

ANALYTICAL SENSITIVITY

The limit of blank, limit of detection, and C5-C95 interval were calculated based on CLSI EP12-A2. The limit of blank, which is the highest measurement result that is likely observed for a blank sample, is estimated to be 10% inhibition at the 1:1 dilution. The limit of detection, which is the lowest amount of analyte in a sample that can be detected, is 25% inhibition at the 1:1 dilution and is well below the assay cutoff.

The C5-C95 interval, which is the range of analyte concentrations around the cutoff such that observed results at concentrations outside this interval are consistently negative (concentrations <C5) or consistently positive (concentrations >C95), is 43-57% inhibition.

CONCORDANCE BETWEEN SEMI-AUTOMATED AND FULLY MANUAL OPERATION

Unless otherwise noted, a semi-automated process using three different liquid handlers was used to perform the analytical validation studies. Liquid handlers were used solely for the purpose of the analytical validation to reduce the potential for injury to operators due to the repetitive nature of validation activities. The commercial assay will be conducted manually due to expected low testing volume.

The performance of three liquid handlers was validated by demonstrating concordance between the manual and semi-automated methods based on CLSI EP12-A2 guidelines for estimating agreement between a candidate method and comparative method. The acceptance criteria specified that the semi-automated assay method shall d monstrate ≥ 95% OPA, positive percent agreement (PPA), and negative percent agreement (NPA) with the manual assay method using a minimum of 40 Factor IX-deficient clinical samples that were collected from a lead-in study (C0371004, NCT03587116).

All three liquid handlers showed 100% positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA), meeting the acceptance criteria, indicating that the use of the liquid handlers did not impact the performance of the assay.

PRECISION AND REPRODUCIBILITY

Precision studies were conducted as outlined in CLSI EP12-A2 by assessing positive/negative agreement for assay determinations. Five samples (defined above) spanning low-to-high % inhibition and the Negative and Positive Controls were evaluated in each precision study.

Percent agreement was 100% for all samples in both the intra-assay and the inter-assay precision studies.

INTRA-ASSAY PRECISION

Intra-assay precision was established by assessing repeatability of assay determinations (Positive or Negative) generated for the samples within a single day (20 replicates of the samples and 40 replicates of the Negative and Positive Controls). The intra-assay precision study design and results are shown in **Table 1**. To evaluate the intra-assay precision around the assay cutoff, the number of determinations using Samples 2 and 3 were double that of the other samples. Overall agreement with the expected result was 100% for every sample and control.

Table 1. Intra-assay precision study design and results

Sample	Mean % Inhibition	Operator	Lots	Determinations/ Run	Runs/ Day	Days	Total Determinations	Expected Result	Actual Result/Total
1	21.7%	1	1	20	1	1	20	Negative	20/20
2	32.7%	1	1	40	1	1	40	Negative	40/40
3	67.6%	1	1	40	1	1	40	Positive	40/40
4	63.6%	1	1	20	1	1	20	Positive	20/20
5	100%	1	1	20	1	1	20	Positive	20/20
Positive Control	75.7%	1	1	140	1	5	140	Positive	140/140
Negative Control	-15.2%	1	1	140	1	5	140	Negative	140/140

INTER-ASSAY PRECISION

Inter-assay precision included day-to-day and run-to-run variation by running each panel sample twice per day with two determinations per run for 20 days (80 total determinations for each panel sample and 400 determinations of each control). The inter-assay precision study design and results are shown in **Table 2**. To evaluate the inter-assay precision around the assay cutoff, the number of determinations using Samples 2 and 3 were double that of the other samples. Overall agreement with the expected result was 100% for every sample and control.

Table 2. Inter-assay precision study design and results

Sample	Mean % Inhibition	Operator	Lots	Determinations/ Run	Runs/ Day	Days	Total Determinations	Expected Result	Actual Result/ Total
1	23.6%	1	1	2	2	20	80	Negative	80/80
2	33.2%	1	1	2	2	20	80	Negative	80/80
3	66.5%	1	1	2	2	20	80	Positive	80/80
4	66.1%	1	1	2	2	20	80	Positive	80/80
5	100%	1	1	2	2	20	80	Positive	80/80
Positive Control	75.9%	1	1	2	10	20	400	Positive	400/400
Negative Control	-16.2%	1	1	2	10	20	400	Negative	400/400

ACCURACY

The accuracy of nAbCyte Anti-AAVRh74var HB-FE Assay was established by detecting anti-AAVRh74var nAb reactivity using clinical samples from participants in a clinical study (Study C0371002, NCT03861273). All participants whose data supported the accuracy endpoint tested Negative for anti-AAVRh74var nAbs. These participants were then administered BEQVEZ (fidanacogene elaparvovec). Samples were collected at a scheduled visit from participants approximately 52 weeks after the administration of BEQVEZ (fidanacogene elaparvovec). The post-administration test result using nAbCyte Anti-AAVRh74var HB-FE resulted Positive (100% inhibition at the 1:1 sample dilution) for all 42 participants.

ASSAY VARIATION

Sources of variation associated with multiple operators, lots, and luminometers were evaluated using the Sera Sample panel and Positive and Negative Controls. Results of Assay Variation testing were tabulated as the percent agreement with the expected result for each sample and control. Overall agreement with the expected results for each sample and control were calculated as a measure of assay precision.

OPERATOR-TO-OPERATOR VARIATION

1. Semi-automated: Operator-to-Operator variation was evaluated using three operators. Each operator ran the five (5) samples across ten runs with two determinations per run using a single lot of components and a single luminometer. A total of 30 plates were tested each day over a period of 20 days (10 days x 2 days/run) The total assay plate count was 300. A total of 60 determinations per serum sample and 300 determinations per assay control were generated. Overall agreement with the anticipated result was 100% for Sample 1, Positive Control, and Negative Control, 98% for Sample 2, Sample 4, and Sample 5, and 97% for Sample 3.

2. Manual: A manual operator-to-operator study was performed to compare the result of using semi-automated assay conduct with the manual assay conduct that will be used commercially due to expected low testing volume. For Operator-to-Operator variation, three operators tested Sample 2 and Sample 3 over ten days with two determinations per day. The acceptance criteria for each sample and for each operator was defined as ≤10 percentage points. Differences in mean percent inhibition for each pairwise comparison of operators was defined as ≤ 10 percentage points.

   As shown in **Table 3**, the standard deviation for each sample for each operator was <5 percentage points, satisfying the acceptance criteria of <10 percentage points. Absolute differences in mean percent inhibition for each pairwise comparison of operators were <4 percentage points, satisfying the acceptance criteria of ≤10 p rcentage points. Variation using the fully manual method was less than or equivalent to the variation observed with semi-automated method.

Table 3. Operator-to-Operator variation using manual assay

Test Serum	Mean Inhibition (%)			Standard Deviation (%)			Difference in Mean Inhibition (%)*		
	Op 1	Op 2	Op 3	Op 1	Op 2	Op 3	Op 2 vs 1	Op 3 vs 2	Op 3 vs 1
Sample 2	47.1	50.7	50.1	4.3	4.3	3.1	3.6	-0.7	2.9
Sample 3	67.2	70.0	69.0	2.9	3.8	3.6	2.9	-1.1	1.8

*Some calculations were affected by rounding in the decimal place.

LOT-TO-LOT VARIATION

Lot-to-lot variation was evaluated using the sample in ten assay runs consisting of two determinations per run performed by a single operator using a single luminometer and three device lots. A total of 30 plates were tested each day over a period of 20 days (10 days x 2 days/run). The total assay plate count was 300. A total of 60 determinations per serum sample and 300 determinations per assay control were generated over the three lots.

Overall agreement with the anticipated result was 100% for Sample 1, Sample 3, Sample 4, Positive Control, and Negative Control, and 98% for Sample 2 and Sample 5.

INSTRUMENT-TO-INSTRUMENT VARIATION

Instrument-to-instrument variation was evaluated by reading all plates from both Operator-to-Operator and Lot-to-Lot studies using three luminometers. Each plate was barcoded within a batch and the batches were read on the luminometers in sequence. A total of 120 determinations per sample and 600 values per assay control per instrument were generated and assessed. Overall agreement with the anticipated result was 100% for Sample 1, Sample 2, Sample 4, Sample 5, Positive Control, and Negative Control, and 99% for the Sample 3 for all pairwise comparisons.

SPECIFICITY

Assay specificity was performed to evaluate the ability of nAbCyte Anti-AAVRh74var HB-FE Assay to detect anti-AAVRh74var nAb activity in the presence of potentially interfering anti-viral antibodies, endogenous, and exogenous components in the sample matrix.

CROSS REACTIVITY

Cross-reactivity with heterologous anti-viral antibodies at 200 µg/mL in serum was evaluated using the nAbCyte Anti-AAVRh74var HB-FE Assay on samples ranging from low to high % inhibition at the 1:1 sample dilution. The nAbCyte Anti-AAVRh74var HB-FE Assay showed no cross-reactivity to any of the anti-viral antibodies tested at 200 µg/mL: polio, type 1,2,3; adenovirus (all 41 types); hepatitis B virus (HBV); chicken pox; mumps; measles; hepatitis C virus (HCV); or human immunodeficiency virus (HIV).

REACTIVITY (TRUE POSITIVITY)

1. Three different approaches were used to demonstrate the specific detection of anti-AAVRh74var nAb:

    a) Empty AAVRh74var capsid was spiked into the Positive Control and three of the samples that were manufactured to exhibit high % inhibition at the 1:1 sample dilution.

    b) The Positive Control and three of the samples that were manufactured to exhibit high % inhibition at the 1:1 sample dilution were depleted of immunoglobulins using Protein G beads.

    c) The reactivity of antibodies to other AAV capsids, AAV6 and AAV9, were evaluated against the AAVRh74var CAG LucP vector for recognition in the nAbCyte Anti-AAVRh74var HB-FE Assay. Anti-AAVRh74var monoclonal antibody (mAb) reactivity against AAV6-CMVIVS-fLuc and AAV9-CMVIVS-fLuc transduction and anti-AAV6 and anti-AAV9 mAb control sera to neutralize AAVRh74var CAG lucP vector transduction were evaluated.

2. The results for each approach to assess reactivity (true positivity) were as follows:

    a) Capsid inhibition results are shown in **Table 4**. For each sample tested, samples exhibited reduced % inhibition after treatment with empty capsid, demonstrating expected reactivity to the AAVRh74var capsid.

    b) Immunoglobulin G (IgG) depletion results are shown in **Table 4**. For each sample tested, samples exhibited reduced % inhibition after treatment with Protein G beads, demonstrating expected reactivity to IgG depletion.

Table 4. Specificity of nAbCyte Anti-AAVRh74var HB-FE Assay: AVRh74var capsid and IgG inhibition

Sample	Mean % Inhibition				Samples with reduction in % Inhibition	
	Empty capsid		Protein G		Empty Capsid	Protein G
	Untreated	Treated	Untreated	Treated		
Positive Control	74.7	-37.9	72.3	-0.4	5/5	5/5
Sample 3	53.5	-51.2	59.5	23.3	5/5	5/5
Sample 4	68.2	-23.6	59.3	-3.8	5/5	5/5
Sample 5	100.0	97.7*	100.0	100.0**	5/5	5/5

* Titers (1/Dilution at 50% inhibition) were reduced from 143 to 11.
** Titers (1/Dilution at 50% inhibition) were reduced from 225 to 82.

c) Reactivity results with AAV vectors and mAbs are shown in **Table 5**. Neither anti-AAV6 mAb nor anti-AAV9 mAb caused interference with the nAbCyte Anti-AAVRh74var HB-FE Assay. Specificity of anti-AAVRh74var mAb was demonstrated by the lack of inhibition of AAV6 and AAV9 transduction.

Table 5. Anti-AAVRh47var mAb recognition of AAV vectors

Capsid (or Vector)	anti-AAV6 Vector mAb (Mean % inhibition, Result/Expected)	anti-AAV9 Vector mAb (Mean % inhibition, Result/Expected)	anti-AAVRh74var Vector mAb (Mean % inhibition, Result/Expected)
AAV6 (AAV6-CMVIVS-fLuc)	72.1%, POS, 5/5	40.0%, NEG 5/5	-75.5%, NEG, 5/5
AAV9 (AAV9-CMVIVS-fLuc)	-13.2%, NEG, 5/5	74.1%, POS, 5/5	-20.2%, NEG, 5/5
AAVRh74var (AAVRh74var CAG LucP)	2.0%, NEG, 5/5	2.8%, NEG, 5/5	72.6%, POS, 5/5

ENDOGENOUS INTERFERENCE

Interference studies were conducted with common endogenous substances using CLSI EP07-A2 and EP37 as guidelines. Five samples exhibiting anti-AAVRh74var nAb throughout the assay range (0 to 100% inhibition) and Negative Control were used unless otherwise noted (n=5). Absolute allowable differences of 10% inhibition were defined to be acceptable for Samples 2-5 and 15% for Sample 1 because expected variability of the assay is higher at lower % inhibitions. The concentrations listed in **Table 6** represent the supplemental amount of respective interfering substance added to the serum samples.

Table 6. Interference Testing – Endogenous Components

Endogenous Components	Concentration
Hemoglobin	1000 mg/dL
Bilirubin (conjugated)	40 mg/dL
Bilirubin (unconjugated)	40 mg/dL

Endogenous Components	Concentration
Triglyceride	1500 mg/dL
Albumin	6 g/dL
Rheumatoid Factor	20 IU/mL
Cholesterol*	400 mg/dL

*Cholesterol was only tested with two samples (Samples 3 and 4, n=5) that were made to be approximately 10% above and below the assay cutoff, respectively.

The results did not show a significant effect for the assay to detect anti-AAVRh74var nAb in the presence of the interfering substances identified in **Table 6** with the following exceptions:

1. Rheumatoid Factor at 60 IU/mL. Rheumatoid factor was re-evaluated at a 3-fold lower concentration, 20 IU/mL, which also corresponds to the upper end of the normal reference range in circulation, 0-20 IU/mL. Rheumatoid Factor satisfied the acceptance criteria for all test sera at 20 IU/mL.

2. Sunflower-derived triglycerides were initially tested with an equal mixture of five triglycerides provided in a commercially available kit. Upon a failure, further analysis determined that tributyrin interfered with the assay at the concentration initially tested. Butyric acid, a hydrolysis product of tributyrin, is a histone deacetylase inhibitor and will thereby increase the transcriptional activity of the luciferase reporter enzyme. Upon dilution, tributyrin did not interfere at a level that was 100-fold higher than normal levels of butyric acid. A human-derived triglyceride mixture was later tested at 1500 mg/dL with two samples (Samples 3 and 4, n=5) that were made to be approximately 10% above and below the assay cutoff, respectively. Triglyceride interference using this mixture was not observed at the conditions tested.

DRUG INTERFERENCE

Interference studies were conducted using drugs routinely administered in hemophilia B patients. Five samples exhibiting anti-AAVRh74var nAb throughout the assay range (0 to 100% inhibition) and Negative Control were used unless otherwise noted (n=5). Absolute allowable differences of 10% inhibition were defined to be acceptable for Samples 2-5 and 15% for Sample 1 because expected variability of the assay is higher at lower % inhibitions. The concentrations listed in **Table 7** represent the supplemental amount of the respective interfering substance added to the samples.

Table 7. Interference Testing – Exogenous Components (medications)

Exogenous Components (Medications)	Type of Medication	Concentration
Factor IX	Replacement therapy: BeneFIX	210 IU/dL
Human-derived Factor IX	Replacement therapy: AlphaNine SD	300 IU/dL
Celecoxib	Pain	0.879 mg/dL
Acetaminophen	Pain	30 µg/mL
Darunavir	HIV/AIDS; Protease inhibitor	1.59 mg/dL
Efavirenz	HIV/AIDS; NBNRTI inhibitor	1.2 mg/dL
Dolutegravir	HIV/AIDS; Integrase inhibitor	0.0064 mg/dL
Tenofovir	HIV/AIDS; NRTI inhibitor	0.0978 mg/dL

Exogenous Components (Medications)	Type of Medication	Concentration
Tranexamic Acid	Bypass agent: clotting	49.23 µg/m
Amino CaproicAcid	Bypass agent: clotting	0.900 mg/dL
Oxycodone	Pain	0.0342 mg/dL
Harvoni (ledipasvir, sofosbuvir)	HCV; NS5A Inhibitor, NS5B Inhibitor	969 ng/mL, 1854 ng/mL
Zepatier (elbasvir, grazoprevir)	HCV; NS5A Inhibitor, NS3/4A Inhibitor	363 ng/mL, 495 ng/mL

The results did not show a significant effect for the assay to detect anti-AAVRh74var nAbs in the presence of the interfering substances identified in Table 7 with the following exceptions:

1. Acetaminophen at 15.6 mg/dL increased inhibition relative to the solvent-only reference for negative human sera, Samples 1 and 2. Acetaminophen was retested at the recommended high concentration in CLSI EP07-A2 of 30 µg/mL and satisfied the acceptance criteria.

2. Dolutegravir at 0.0192 mg/dL reduced inhibition relative to the solvent-only reference for Sample 2. Dolutegravir was retested at 0.0064 mg/dL, the highest drug concentration under therapeutic treatment, according to CLIS EP37. Dolutegravir at 0.0064 mg/dL satisfied the acceptance criteria for all test sera and therefore shows no evidence of interference at therapeutically relevant concentrations.

WELL-TO-WELL CROSS-TALK AND CROSS-CONTAMINATION

This study measured the effect of placing sample wells adjacent to either low or high relative luminescence units (RLUs). A different plate layout compared to the commercial assay was used to exaggerate the conditions to assess cross-talk and cross-contamination. To assess crosstalk between the Negative Control and Sample 2, duplicate wells of Sample 2 were placed below the Negative Control (a high RLU well). Additionally, Sample 2 was placed above the Sample 5. A similar plate layout was used to evaluate cross-talking and cross-contamination using Sample 3 and Sample 5. A total of eighteen plates were used, and each plate layout was evaluated six times. Vertical and horizontal adjacent determinations were compared. This study design yielded 60 vertical comparisons per sample and 30 horizontal comparisons per sample. All samples were in 100% agreement with expected results for Sample 2, Sample 4, and Sample 5. There were no statistical differences between samples adjacent to wells with high RLU and low RLU.

HOOK EFFECT

nAbCyte Anti-AAVRh74var HB-FE Assay was evaluated for the possibility of a hook effect, whereby a sample with high anti-AAVRh74var nAb levels would diminish luciferase inhibition, potentially leading to an erroneous negative result. This study was conducted by two different methods:

1. Mouse anti-AAVRh74var monoclonal antibody was spiked into anti-AAVRh74var negative human serum and then serially diluted.

2. Clinical samples exhibiting high anti-AAVRh74var titer following administration with BEQVEZ (fidanacogene elaparvovec) were serially diluted.

Both sets of samples exhibited monotonically increasing inhibition as expected with increased antibody concentration until inhibition reached a plateau at approximately 100%, with no evidence of a hook effect.

SAMPLE STABILITY

Sample Conditions	Stability
Freeze/Thaw Cycles	Samples can undergo three freeze/thaw cycles.
Transport	Samples are stable at 37 °C for three days in a shipping container containing dry ice.
Short-Term Stability	Samples are stable for 20 hours at ambient temperatures. Samples are stable for three days at 4 °C.
Long-Term Stability	Samples are stable at –20 °C for one month. Samples are stable at –70 °C for twelve months.

CLINICAL PERFORMANCE

A total of 51 participants who completed a lead-in study (C0371004, NCT03587116) were screened for eligibility to participate in the phase 3 evaluation of BEQVEZ (fidanacogene elaparvovec) (C0371002, NCT03861273). Of those 51 participants, 45 received a single 5×10^{11} vg/kg administration of BEQVEZ (fidanacogene elaparvovec). One of six subjects screened out of the C0371002 study due to the presence of anti-AAVRh74var nAbs above the established threshold.

No adverse events were associated with the use of nAbCyte Anti-AAVRh74var HB-FE Assay.

The major efficacy outcome of the C0371002 study was to demonstrate non-inferiority on annualized bleeding rate (ABR) for total bleeds (treated and untreated) from Week 12 to Month 15 versus standard-of-care Factor IX prophylaxis replacement regimen. Efficacy results for study C0371002 using the patient population selected in part by nAbCyte Anti-AAVRh74var HB-FE Assay are summarized in **Table 8**.

Table 8. Efficacy results in study C0371002

	Factor IX Prophylaxis (N=45)	BEQVEZ (fidanacogene elaparvovec) (N=45)
Number (%) of participants without any bleeds	13 (28.9)	29 (64.4)
Number of bleeds		
n	45	45
Mean (SD)	5.0 (9.19)	1.2 (2.34)
Median (Min, Max)	2.0 (0, 41)	0.0 (0, 11)
(Q1, Q3)	(0.0, 5.0)	(0.0, 1.0)
Annualized bleeding rate (ABR)		
n	45	45
Mean (SD)	4.55 (9.380)	1.39 (2.651)
Median (Min, Max)	1.27 (0.0, 53.9)	0.00 (0.0, 10.4)
(Q1, Q3)	(0.00, 3.29)	(0.00, 1.25)

	Factor IX Prophylaxis (N=45)	BEQVEZ (fidanacogene elaparvovec) (N=45)
Model derived ABR		
Estimate (95% CI)	4.43 (1.81, 7.05)	1.30 (0.59, 2.02)
Treatment difference		
Negative binomial estimates*	Estimate (95% CI)	-3.13 (-5.44, -0.81)
Negative binomial p-value*	P-value	0.0081
Percentage reduction		
Negative binomial estimates**	Estimate (95% CI)	70.63 (49.17, 83.03)
Negative binomial p-value**	P-value	<.0001

* The treatment difference and P-value are obtained from a repeated measures generalized linear model (GLM) with negative binomial distribution and identity link function.

** The treatment difference and P-value are obtained from a repeated measures generalized linear model (GLM) with negative binomial distribution and log link function.

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2. CLSI Reference: Interference Testing in Clinical Chemistry 3rd ed. CLSI guideline EP07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

3. CLSI Supplemental Tables for Interference Testing in Clinical Chemistry, 1st Edition. CLSI guideline EP37. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

SYMBOLS GLOSSARY

Symbol	Meaning	Symbol	Meaning
REF	Catalog number		Use-by date
IVD	In vitro diagnostic medical device	LOT	Batch code
Rx Only	For prescription use only		Caution

Symbol	Meaning	Symbol	Meaning
	Contains sufficient for <n> tests		Temperature limit
	Manufacturer		Keep away from sunlight
	Consult instructions for use		Contains biological material of animal origin
	Contains biological material of human origin		Contains human blood or plasma derivatives
	Do not use if package is damaged and consult instructions for use		

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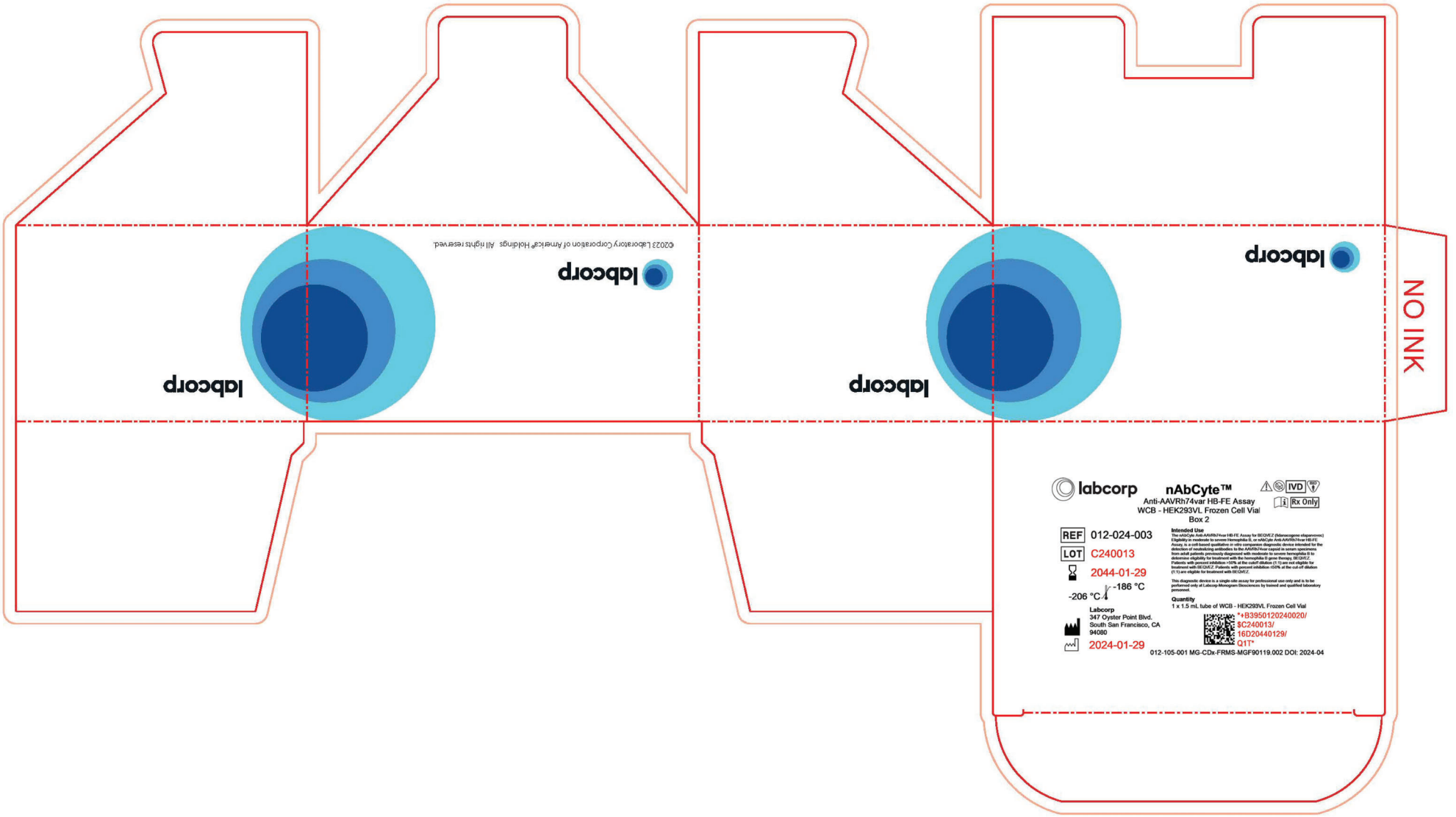
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NO INK
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nAbCyte™
Anti-AAVRh74var HB-FE Assay
WCB - HEK293VL Frozen Cell Vial
Box 2
IVD
Rx Only
Intended Use
The nAbCyte Anti-AAVRh74var HB-FE Assay for BEQVEZ (fidanacogene elaparvovec) Eligibility in moderate to severe Hemophilia B, or nAbCyte Anti-AAVRh74var HB-FE Assay, is a cell-based qualitative in vitro companion diagnostic device intended for the detection of neutralizing antibodies to the AAVRh74var capsid in serum specimens from adult patients previously diagnosed with moderate to severe hemophilia B to determine eligibility for treatment with the hemophilia B gene therapy, BEQVEZ. Patients with percent inhibition >50% at the cutoff dilution (1:1) are not eligible for treatment with BEQVEZ. Patients with percent inhibition ≤50% at the cut-off dilution (1:1) are eligible for treatment with BEQVEZ.
This diagnostic device is a single-site assay for professional use only and is to be performed only at Labcorp-Monogram Biosciences by trained and qualified laboratory personnel.
Quantity
1 x 1.5 mL tube of WCB - HEK293VL Frozen Cell Vial
REF 012-024-003
LOT C240013
2044-01-29
-206 °C -186 °C
Labcorp
347 Oyster Point Blvd.
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