Antithrombin Activity Measurement via the INNOVANCE Antithrombin Assay to Manage Fitusiran Dosing

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Introduction

- Hemophilia A and hemophilia B are rare bleeding disorders caused by Factor VIII (FVIII) or Factor IX (FIX) deficiencies, respectively, leading to insufficient thrombin generation.[1]
- The FDA-approved Qfitlia (fitusiran) is a subcutaneous prophylactic antithrombin (AT)-lowering therapeutic that increases thrombin generation to restore hemostasis in people with hemophilia A or B, with or without inhibitors.[2-4] It is approved in the U.S. and UAE for routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adults and children ≥ 12 years.
- To optimize the benefits and reduce the risks associated with Qfitlia, an antithrombin-based dosing regimen (AT-DR) targeting AT activity between 15% and 35% of normal was implemented in clinical studies.[5]
- Qfitlia dosing is individually adjusted based on AT measurement and thus requires an AT activity assay with high analytical sensitivity and high precision, especially at the low end of the measuring range.[6]

Objective

- To evaluate the analytical performance of the INNOVANCE Antithrombin assay (Siemens Healthineers, Marburg, Germany) at the low end of the measuring range, as required for optimizing Qfitlia dosing.

Methods

Performance data were generated using the BCS XP System (Siemens Healthineers, Marburg, Germany) according to the CLSI (Clinical and Laboratory Standards Institute) guidelines comprising the following studies:

Limit of quantitation (LoQ)

- Design: CLSI EP17-A2[a]
- Sample types: Native citrated plasma samples from Qfitlia-treated patients diluted with assay buffer to AT activities at the low end of the measuring range (≤ 10% of normal).

Potential interference by other hemophilia therapeutics

- Design: CLSI EP07-ED3[a] (paired-difference model)
- Sample types: Native citrated plasma from Qfitlia-treated patients with AT activities at the low end of the measuring range (around 15% of normal) as well as Control Plasma N (90-100% of normal).
- The tested doses of recombinant FVIIA (rFVIIa), Factor VIII (FVIII), FIX, and aPCC were based on the Bleed Management Guidelines (BMG) provided in the United States Prescribing Information (USPI). The tested doses for desmopressin and tranexamic acid are the doses recommended for hemophilia patients per each drug's labeling

Precision (reproducibility)

- Design: Precision studies were conducted to determine repeatability/within-device precision, impact of combined reagent lots, and combined system precision by analysis of variance (ANOVA) according to CLSI guideline EP05-A3.[a]
- Repeatability/within-device precision was evaluated using one reagent lot over 20 days in two runs/day of two replicates/sample on a single system (20x2x2)
- The impact of combined reagent lots was evaluated using three reagent lots over 20 days in two runs/day of two replicates/sample on a single system (3x20x2x2)
- Combined (multiple) system precision was evaluated using one reagent lot tested on three systems over five days in two runs/day of three replicates/sample (3x5x2x3)
- Samples comprised native citrated plasma from Qfitlia-treated patients with AT activities at the low end of the measuring range (around 10% and 15% of normal AT activity). In addition, Control Plasma P (CPP) was diluted 1:3 with INNOVANCE Antithrombin Buffer and used to show comparability in performance to native samples.

[a] All CLSI Guidelines can be found on the CLSI website: https://clsi.org.

Results

Precision (repeatability/within device)

Precise AT activity quantification around the Qfitlia target range is crucial to guarantee proper dosing. This study was performed to supplement the precision characteristics of the assay as described by Merz et al. using samples from patients treated with Qfitlia at the lower end of the target range.[7] Coefficients of variation (CV) and standard deviations (SD) presented in Tables 1–3 were obtained using the INNOVANCE Antithrombin assay on the BCS XP System and indicated low variability at the lowest end of the AT activity range (~15% of normal) recommended for determining the Qfitlia target dose for both the native samples and the diluted plasma-based control (CPP; 1:3 in assay buffer). For native sample 1—which represents this minimum—both repeatability and intra-assay precision were consistently <10% CV, even when additional factors representative of real-world routine settings were considered, such as using more than one reagent lot or multiple systems across serial measurements.

Table 1. Repeatability/within device using design 20x2x2 on the BCP XP System (one reagent lot).

Sample	Mean (% of norm)	n	Repeatability CV (%)	Repeatability SD (% of norm)	Within-device/ lab CV (%)	Within-device/ lab SD (% of norm)
Native sample 1	15.82	80	7.46	1.18	8.13	1.29
Native sample 2	9.94	80	12.67	1.26	15.10	1.50
CPP 1:3	11.80	80	9.41	1.11	12.16	1.43

Table 2. Total precision using combined reagent lots using three reagent lots on the BCS XP System (design 3x20x2x).

Sample	Mean (% of norm)	n	Repeatability CV (%)	Repeatability SD (% of norm)	Combined assay lots CV (%)	Combined assay lots SD (% of norm)
Native sample 1	15.73	240	8.77	1.38	9.95	1.57
Native sample 2	9.75	240	13.94	1.36	16.31	1.59
CPP 1:3	12.07	240	10.31	1.24	18.32	2.21

Table 3. Total precision across combined systems (three BCS XP Systems, one reagent lot, design 3x5x2x3).

Sample	Mean (% of norm)	n	Repeatability CV (%)	Repeatability SD (% of norm)	Combined sites CV (%)	Combined sites SD (% of norm)
Native sample 1	15.54	90	5.93	0.92	8.85	1.38
Native sample 2	11.05	90	7.31	0.81	19.82	2.19

Interference

Interference by other hemophilia therapeutics was evaluated to demonstrate the analytical specificity of the INNOVANCE AT assay for the hemophilia patient population (Table 4). This is particularly important to ensure that AT quantification is not affected by co-administered therapeutics.

Based on these studies, no relevant interference was detected.

Table 4. Interference study conducted on the BCS XP System using the paired-difference method.

Therapeutics*	Tested concentration
Desmopressin	0.0144 µg/mL
Tranexamic acid	0.48 mg/mL
Recombinant factor VIIa	2.16 µg/mL
Coagulation factor VIII	0.96 IU/mL
Coagulation factor IX	1.44 IU/mL
activated Prothrombin complex concentrate (aPCC)	2.4 IU/mL

*Concentration tested based on recommended dose as per each drug's United States Prescribing Information (USPI).

Limit of quantitation (LoQ)

An LoQ study was performed to reflect increasing quantitative certainty at the lower end of the measuring interval relevant for Qfitlia dosing (15-35% of normal AT activity). The minimal measurand amount that can reliably be quantitated by the INNOVANCE Antithrombin assay with respect to defined accuracy goals was determined to be 7.32% of normal (Table 5), which is well below the Qfitlia target range ensuring reliable performance for Qfitlia dosing.

Table 5. LoQ study on the BCS XP System.

Test	Result
Limit of Quantitation (LoQ)	7.32% of norm

Conclusion

Analytical performance evaluation of the INNOVANCE Antithrombin assay demonstrated high precision and reproducibility across reagent lots and systems. The assay provided high quantitative certainty at the low-end region of the measuring range (LoQ). In addition, therapeutics which may be used in patients treated with Qfitlia were not found to interfere with the assay.

In conclusion, the assay can precisely and reliably quantify antithrombin activity at clinical decision points (15–35% of normal AT activity) even in the presence of other hemophilia-specific therapeutics, thus fulfilling requirements needed to support Qfitlia dose establishment and monitoring in individuals with hemophilia A or B.

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