World-wide field study of FVIII activity assay variability of ADYNOVATE, the PEGylated form of rFVIII ADVATE, in clinical hemostasis laboratories

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INTRODUCTION

Most clinical laboratories, for analysis of post infusion human plasma samples, use one-stage clotting assays which may differ in instruments, method of clot detection, assay set-up, reference standard calibration, reagent source and reagent composition, all contributing to assay variability. In addition chromogenic assays are in use also having inherent variability resulting from different methods, instruments and assay kits. Baxalta has performed an international collaborative study among clinical laboratories to analyze plasma from patients with hemophilia A spiked in vitro with ADYNOVATE (PEGylated form of rFVIII ADVATE) or ADVATE (human recombinant full length FVIII; Baxalta) at high (0.80 IU/mL), medium (0.20 IU/mL) and low (0.05 IU/mL) FVIII concentrations. Thirty-five results from clinical laboratories world wide were received and evaluated for assay variability.

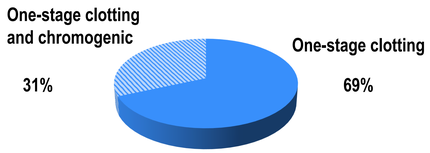
OBJECTIVE

The study was intended to generate data on assay variability in clinical routine laboratories when analyzing ADYNOVATE and ADVATE in hemophilic plasma.

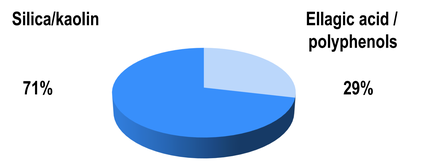
METHODS

Study participants were asked to use their routinely established methods for FVIII activity analysis in human plasma samples.
Twenty-seven laboratories reported data obtained with their one-stage clotting method (OSCA), two laboratories provided data from two different OSCA's and one laboratory performed four different OSCA's. Activator reagents for one-stage clotting assays were ellagic acid (8), polyphenols (2) or silica/kaolin (25), clot detection was either mechanical or optical. The majority of laboratories used immunodepleted FVIII-deficient plasma for the OSCA. All together, thirty-five data sets were available for evaluation Eleven participants in addition also provided results from a chromogenic assay. Statistics with a linear mixed effects model were performed by Quintiles (Bloemfontein, South Africa), comprehensive analysis is ongoing.

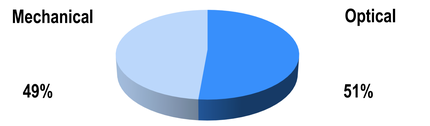
Assay methods



OSCA activator reagents

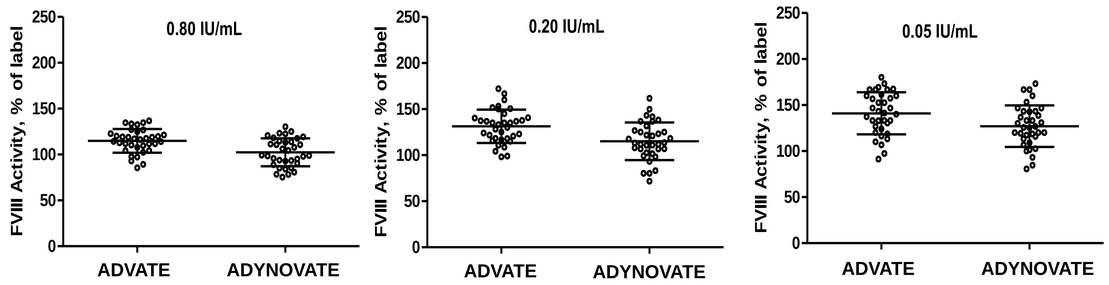


OSCA clot detection

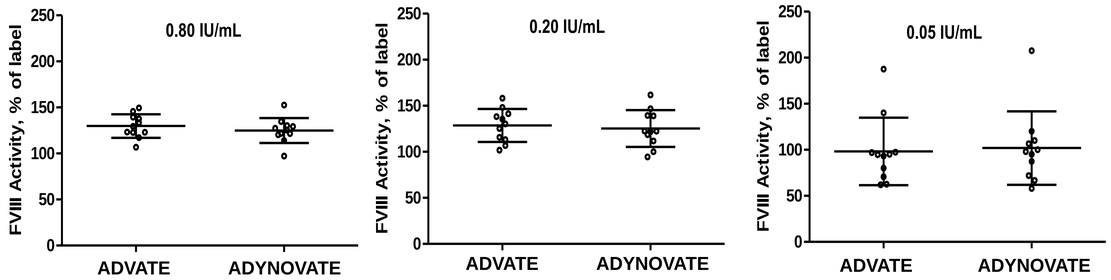


RESULTS

One-stage clotting assays (n=35)



Chromogenic assays (n=11)



	Label activity (IU FVIII/mL)	One-stage clotting assays (OSCA)			Chromogenic assays			Agreement of OSCA and chromogenic assay methods	
		Mean recovery, % of label (n = 35 per level)	Intra-laboratory % CV (n = 35)	Inter-laboratory % CV (n = 35)	Mean recovery, % of label (n = 11 per level)	Intra-laboratory % CV (n = 11)	Inter-laboratory % CV (n = 11)	Ratio OSCA/chrom (n = 11/11)	
								Mean	Range
ADVATE	0.80	114.0	6.8	10.9	129.0	7.7	9.0	0.89	0.67 – 1.19
	0.20	129.8	8.8	13.0	127.4	4.8	13.9	1.07	0.79 – 1.57
	0.05	138.3	12.9	16.1	92.4	15.1	32.5	1.60	0.71 – 2.58
	Mean	**127**	n.a.	n.a.	**116**	n.a.	n.a.	**1.19**	n.a.
ADYNOVATE	0.80	101.0	7.4	14.7	124.0	7.6	10.1	0.82	0.65 – 1.29
	0.20	112.9	9.5	17.8	123.7	5.4	15.9	0.96	0.65 – 1.45
	0.05	124.3	12.4	17.5	95.4	17.3	33.7	1.39	0.57 – 2.76
	Mean	**113**	n.a.	n.a.	**114**	n.a.	n.a.	**1.06**	n.a.

- The mean ratio between one-stage clotting and chromogenic assays was very similar for ADYNOVATE and ADVATE
- Ratios for individual laboratories ranged between 0.7 and 1.6 (for 0.8 and 0.2 IU/mL). At the lowest concentration, between laboratory variation was higher
- Within-laboratory and between-laboratory assay variability for one-stage clotting assays and chromogenic assays was similar for ADYNOVATE and ADVATE
- With both assays, variability increased with lower FVIII concentrations
- No obvious impact from assay reagents or assay set-up

STUDY PARTICIPANTS

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CONCLUSION

- **ADYNOVATE activity in human hemophilia A plasma samples can be reliably analyzed with one-stage clotting assays and chromogenic assays using routinely established methods in clinical laboratories**
- **All laboratories used one-stage clotting assays, one third used chromogenic methods in addition**
- **Assay variability for one-stage clotting assays and chromogenic assays for ADYNOVATE is similar to that of the parent FVIII molecule ADVATE**
- **Ratio between one-stage clotting and chromogenic assays for ADYNOVATE and ADVATE is very similar and close to 1.0**
- **There is no need for a product specific standard**

REFERENCE

P.L. Turecek, S. Romeder-Finger, C. Apostol, A. Bauer, A. Crocker-Buque, D.A. Burger, R. Schall, H. Gritsch. A world-wide survey and field study in clinical hemostasis laboratories to evaluate FVIII:C activity assay variability of ADYNOVATE and OBIZUR in comparison to ADVATE. Haemophilia. 2016 Jun 28. doi: 10.1111/hae.13001. [Epub ahead of print].

DISCLOSURES

Peter L. Turecek, Claudia Apostol, Stefan-Romeder-Finger, Alexander Bauer and Herbert Gritsch are full-time employees of Baxalta Innovations GmbH, now part of Shire. Divan Burger is employee of Quintiles.

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