FVIII activity of OBIZUR can be measured in hemophilic plasma with standard FVIII one-stage clotting assays

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INTRODUCTION

OBIZUR (antihaemophilic factor recombinant porcine sequence; rpFVIII) is a purified glycoprotein produced in Baby hamster Kidney (BHK) cells by recombinant DNA technology. Each vial of OBIZUR is labeled with the actual rpFVIII activity expressed in units determined by a one-stage clotting assay (OSCA), using a reference rpFVIII material calibrated against the World Health Organization (WHO) 8th International Standard for human FVIII concentrates. 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 also chromogenic assays are in use also having some 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 OBIZUR or ADVATE at high (0.80 U/mL), medium (0.20 U/mL) and low (0.05 U/mL) FVIII concentrations. Thirty-five results from clinical laboratories world wide were received and evaluated or assay variability.

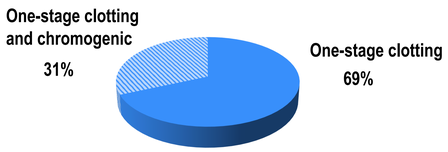
OBJECTIVE

A global field study performed to determine how OBIZUR is measured in comparison with ADVATE using locally established FVIII assays.

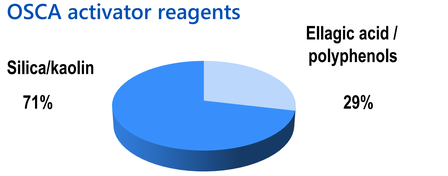
METHODS

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 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 are performed by Quintiles (Bloemfontein, South Africa), comprehensive analysis is ongoing.

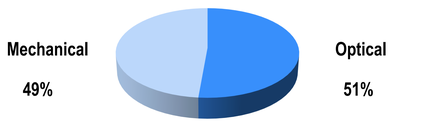
Assay methods



OSCA activator reagents



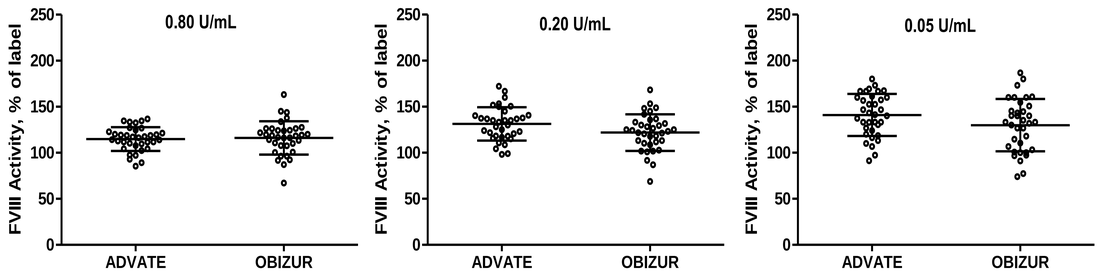
OSCA clot detection



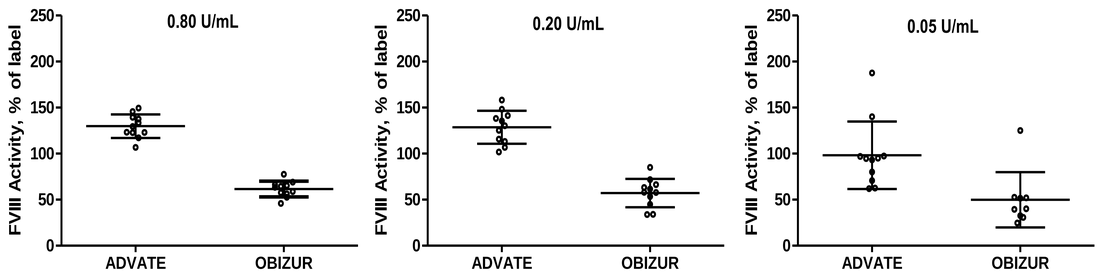
WFH World Congress; July 24–28, 2016; Orlando, USA

RESULTS

One-stage clotting assays (n=35)



Chromogenic assays (n=11)



	Label activity (U 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	8.9	0.89	0.67 – 1.19
	0.20	129.8	8.8	12.9	127.4	4.8	13.8	1.07	0.79 – 1.57
	0.05	138.3	12.9	16.0	92.4	15.1	31.7	1.60	0.71 – 2.58
	Mean	**127**	n.a.	n.a.	**116**	n.a.	n.a.	—	—
OBIZUR	0.80	114.4	8.9	15.8	60.9	9.3	13.2	1.85	1.04 – 3.00
	0.20	119.6	12.3	16.1	54.9	12.0	28.8	2.25	1.19 – 4.29
	0.05	125.9	15.4	21.6	43.5	29.5	46.8	3.00	0.76 – 4.41
	Mean	**120**	n.a.	n.a.	**53**	n.a.	n.a.	—	—

One-stage clotting assay (OSCA)

- FVIII recovery at 0.8 IU/U/mL was 114% for ADVATE and OBIZUR
- At lower FVIII concentrations, recoveries were between 120% and 138%
- Intra-laboratory variation was low (7% to 15%)
- Inter-laboratory variation was similar for both products (11% to 22%)

Chromogenic assay

- 11 clinical laboratories reported in vitro FVIII activity recoveries showing similar assay variability for ADVATE and OBIZUR
- On average, recoveries were 116% (ADVATE) and 53% (OBIZUR)
- Inter-lab variation was high at low FVIII concentrations
- In absolute levels, chromogenic assays reported lower data for OBIZUR as compared to ADVATE
- Results are as expected
  - OBIZUR Product Information: The potency values of OBIZUR determined by the chromogenic assay vary and are approximately 20-50% lower than those of the one-stage clotting assay.

STUDY PARTICIPANTS

Jovan Antovic, Peter Baker, Valdas Banys, Jerome Beltran, Sarah Berberich, Maria Berndtsson, Sarah Bruty, Roger Buchanan, Meera B. Chitlur, Marina Dzhelali, Marion Echenagucia, Audrone Eidukaite, Mary Elaine Eyster, Fabienne Floc'h, Kenneth Friedman, Sara Gay, Laurine Genesta, Paul Giangrande, Lucy Goff, Marc Grimaux, Ralph Gruppo, Sandra Haberichter, Catherine P. M. Hayward, Lourdes Herrera, Margareta Holmström, Shawn Jobe, Rashid Kazmi, Richard Ko, Barbara Konkle, John Lazarchick, Petra Linden, Steven Lobel, Anne Lochu, Maria Fernanda López Fernandez, Jonathan Lowe, Paige A. Macy, Janine Martin, Karen Moffat, Jens Müller, Sukesh C. Nair, Johannes Oldenburg, Amparo Santamaria Ortiz, Rafael Parra, Julia Phillips, Steven Pipe, Lina Rageliene, Heesun Rogers, Arlette Ruiz de Sàez, Wade Sandau, Jeff Sanders, Bernard Silver, Alok Srivastava, Andrew Sutherland, Fikre Tadesse, Desiree Tan-Castillo, Michael Tarantino, Gayle Teramura, Stefan Tiefenbacher, Laila Vengal, Gilbert C. White, Anne M. Winkler, Guy Young

CONCLUSION

- **One-stage clotting assay is most widely used FVIII assay**
- **OBIZUR can be measured as human FVIII with one-stage clotting assays with similar assay variability as ADVATE**
- **OBIZUR is underestimated by the chromogenic FVIII assay**
  - **Confirms Baxalta's statement in the Product Information**
- **Measurement of FVIII activity levels provides the ability to objectively monitor safety and efficacy**

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; Aaron R. Novack is employee of Baxalta US Inc, now part of Shire. Divan Burger is employee of Quintiles.