EVALUATION OF A CHROMOGENIC FVIII ACTIVITY ASSAY FOR THE MEASUREMENT OF THREE LONG-ACTING RECOMBINANT FVIII CONCENTRATES POTENCY

Anne Lochu[1], Alexandra Chenier[1], Fanny Lemaire[1],
Marie-Eglantine Dujaric[1], Pierre-Olivier Sevenet[2]

[1] Diagnostica Stago, Recherche et Développement, Gennevilliers 92230 ;
[2] Diagnostica Stago, Développement Clinique, Asnières-sur-Seine, 92600.

INTRODUCTION

Haemophilia A is the most common severe inherited bleeding disorder in human. Treatment and prevention of bleeding consist in injections of plasma-derived or recombinant FVIII protein replacement. Recent improvement in bioengineering led to the production of FVIII molecules with a prolonged half-life of approximately 18 hours, compared to 12h for conventional products, resulting in a reduction of injections and a more convenient treatment schedule.
Factor VIII (FVIII) activity testing can be performed using several techniques, including one-stage clotting and chromogenic assays. However, FVIII chromogenic assays have recently been reemphasised by the European Medicines Agency for new long-acting recombinant FVIII therapy monitoring[1].

The aim of this study is to assess the performances of a chromogenic assay TriniCHROM™ FVIII:C for measuring FVIII activity (FVIII:C) in plasma samples containing long-acting recombinant FVIII concentrates.

MATERIAL AND METHODS

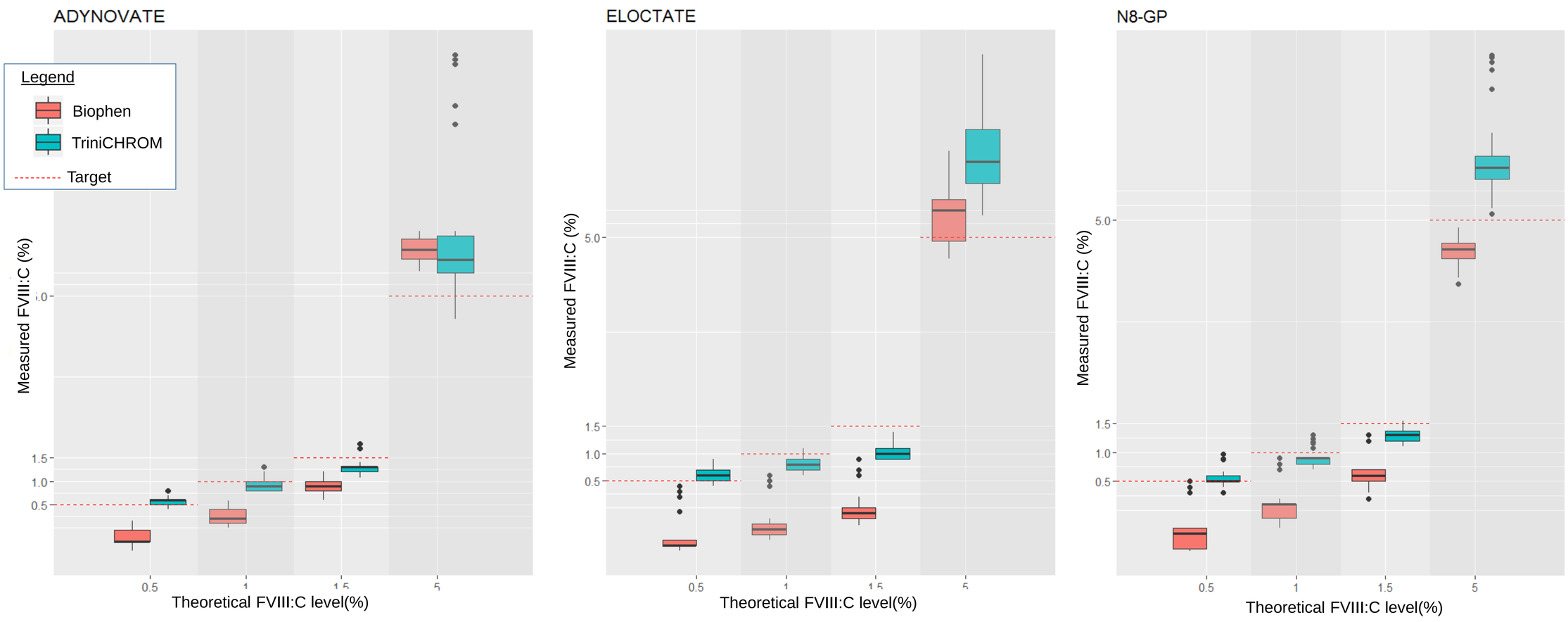
FVIII-immunodepleted plasma samples were spiked with increasing concentrations (0.5-100%) of three different long-acting recombinant FVIII concentrates: **Adynovate**® (Shire/Baxalta), **Eloctate**® (Bioverativ/Biogen) and **N8-GP** (Novo Nordisk).
FVIII:C was measured (n=25 for each sample) with TriniCHROM™ FVIII:C (Tcoag) on STA R Max® analyser (Stago). Results were compared to theoretical FVIII:C values.
Furthermore, low range values (0.5-5 %) were compared to a secondary chromogenic assay: Biophen® FVIII:C (Hyphen Biomed).

RESULTS

Mean and standard deviation (SD) of TriniCHROM FVIII:C results of FVIII-deficient plasma samples spiked with long-acting FVIII concentrates.

Theoretical FVIII:C of spiked samples (%)	TriniCHROM™ FVIII:C					
	Adynovate		Eloctate		N8-GP	
	Mean (%)	SD (%)	Mean (%)	SD (%)	Mean (%)	SD (%)
0,5	0,6	0,10	0,6	0,12	0,5	0,16
1.0	0,9	0,14	0,8	0,13	0,9	0,15
1,5	1,3	0,22	1.0	0,14	1,3	0,11
5	6	1,70	7	0,71	6	0,83
10	12	2,62	14	1,54	13	2,30
20	23	3,88	26	2,70	25	3,20
50	53	8,74	62	9,46	58	10,05
100	134	9,72	122	13,12	131	19,23
Coefficient of correlation (95 % confidence interval)	**r = 0,995** (0,9715-0,9991)		**r = 0,999** (0,9986-1,000)		**r = 0,998** (0,9916-0,997)	

Distribution of FVIII:C level with TriniCHROM FVIII and Biophen FVIII:C according to theoretical values



In the low range, TriniCHROM shows to be very accurate, while Biophen underestimate FVIII:C of the three concentrates.

CONCLUSION

- **TriniCHROM™ FVIII:C shows very good correlations with spiked concentrations of three long-acting FVIII concentrates. We observed overestimation for FVIII:C > 50%. However, a very good precision is obtained in the values of interest, i.e. < 5%, for a safe patient management.**
- **TriniCHROM™ FVIII:C seems promising for the monitoring of these new long-acting FVIII concentrates. Further studies are however necessary to confirm the effectiveness of the assay in plasmas samples from treated patients.**

[1] European Medicines Agency, EMA/135928/2014. Workshop report: ttp://www.ema.europa.eu/docs/en_GB/document_library/Report/2014/07/WC500169760.pdf. June 2014. Characterization of new clotting factor concentrates (FVIII, FIX) with respect to potency assays used for labelling and testing of post infusion samples.