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Inter-laboratory variability of the standardized ETP-based APC resistance assay (PB0386)

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INTRODUCTION

- Risk minimization measures need to be implemented to reduce the thrombotic risk associated with the use of combined oral contraceptives (COC). However, there is controversy on how to assess the risk at an acceptable cost for the society.
- Despite regulatory bodies recommend the assessment of the ETP-based activated protein C (APC) resistance during the development of steroid contraceptives; results are not comparable between studies due to lack of validation and standardization.
- To overcome this issue, our group recently validated and standardized this assay.
- However, further investigations are needed before a wide implementation of this method.

AIM

To assess the inter-laboratory variability of the validated ETP-based APC resistance assay.

METHOD

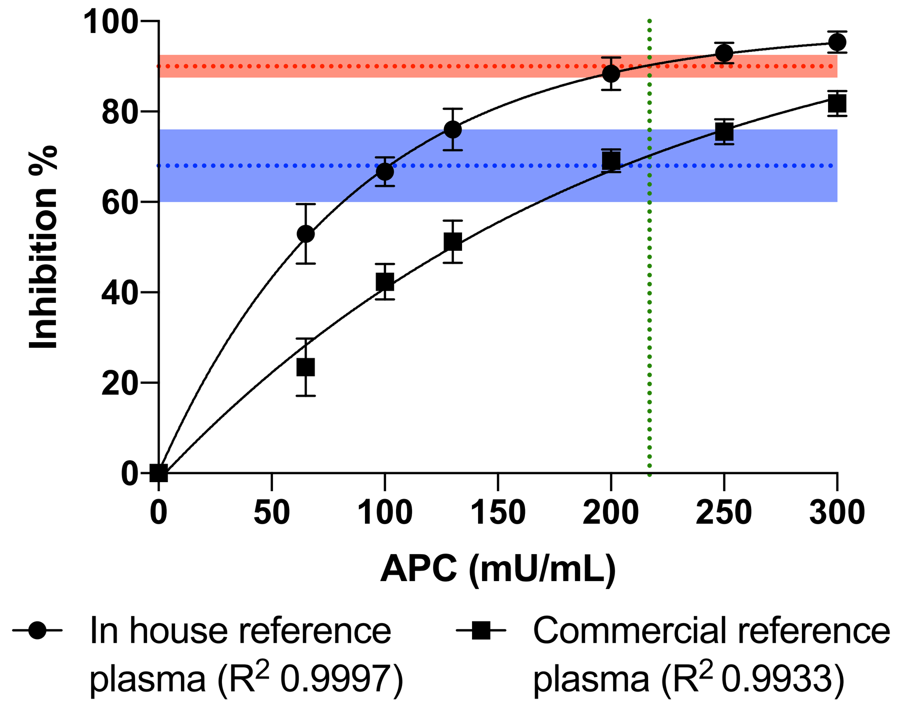
- Analyses were performed on 3 Calibrated Automated Thrombogram (CAT) with Thrombinoscope software (version 5.0 for $CAT_{1\&2}$; version 2.0 for CAT_3) and by 3 different operators.
- Dose-response curves (n=3) were performed on each device to defined the amount of APC, leading to
 - (a) 90% of inhibition of the ETP on an in-house reference plasma,
 - (b) the corresponding inhibition % on a commercial reference plasma.
- Quality controls (n=3) and a subset of real-life samples (n=11) were tested on each equipment.
- All measurements were performed in duplicate.

RESULTS

- Dose-response curves did not show any significant differences between CAT_1 and CAT_2 (Dunn's multiple comparison test; p-value > 0.9999) on both, commercial and in-house reference plasma ▶**Table/Figure 1.**
- An APC concentration of 217 mU/mL led to 90% of inhibition of the ETP on the homemade reference plasma and 68% of inhibition on the commercial reference plasma ▶**Table/Figure 1**.
- A mean deviation of 7.83% was observed when comparing samples tested on both CAT device at the defined APC concentration of 217 mU/mL (▶**Table 2**).
- As the software version of the CAT_3 was older, some modifications must be performed to ensure the transferability of the methodology. This is due to important background noise which limits the performance of this older version of the CAT_3 at low ETP-values.

FIGURE 1: Total variability between CAT_1 and CAT_2. The red dotted line represents the reference range of in-house reference plasma (90±2.5%) and the blue dotted line represents the reference range of commercial reference plasma (68±8%). The green dotted line represents the defined concentration of APC (217 mU/mL) to fit the reference ranges.

TABLE 1: Inhibition percentage at 6 concentration levels of APC with in-house reference plasma and commercial reference plasma on CAT_1 and CAT_2. Inhibition % in blue are outliers*


Inhibition %
100
80
60
40
20
0
0
50
100
150
200
250
300
APC (mU/mL)
In house reference plasma (R^2 0.9997)
Commercial reference plasma (R^2 0.9933)

[APC]	In-house reference plasma: Inhibition % CAT 1	In-house: Inhibition % CAT 2	In-house: p-value	Commercial reference plasma: Inhibition % CAT1	Commercial: Inhibition % CAT2	Commercial: p-value
	26,69*	52,05		14,65	27,48	
65 mU/mL	28,71*	56,13	>0.9999	17,18	31,35	>0.9999
	44,24	59,57		25,53	24,7	
	64,77	70,39		38,94	40,71	
100 mU/mL	62,63	64,34	>0.9999	37,51	47,3	>0.9999
	69,72	68,21		44,32	45,7	
	73,9	80,1		46,84	52,14	
130 mU/mL	68,15	76,11	>0.9999	44,37	56,42	>0.9999
	80,23	77,84		52,77	54,82	
	90,27	89,45		65,05	67,54	
200 mU/mL	87,92	81,62	>0.9999	68,86	71,88	>0.9999
	92,09	88,72		71,27	69,99	
	95,25	93,42		74,02	74,4	
250 mU/mL	91,58	89,48	>0.9999	72,16	78,83	>0.9999
	95,47	92,47		79,06	74,66	
	97,19	95,87		78,86	79,58	
300 mU/mL	96,13	82,89*	>0.9999	83,06	85,8	>0.9999
	96,55	95,08		83,69	79,92	
Defined concentration of APC (95%CI): **217 mU/mL** [197 mU/mL-249 mU/mL]						

TABLE 2: Inhibition percentage and absolute difference between CAT1 and CAT2.

	Inhibition % CAT 1	Inhibition % CAT 2	Difference
In-house ref plasma	88,66%	94,56%	5,90%
Commercial ref plasma	64,81%	74,36%	9,55%
QC Low	98,19%	100,16%	1,96%
QC Norm	31,22%	44,17%	12,95%
QC High	7,18%	9,70%	2,52%
S1	91,99%	96,34%	4,36%
S2	77,01%	81,54%	4,53%
S3	78,53%	86,07%	7,54%
S4	64,74%	74,16%	9,42%
S5	56,94%	64,92%	7,97%
S6	68,84%	79,33%	10,49%
S7	69,03%	76,53%	7,49%
S8	69,02%	76,42%	7,40%
S9	46,88%	60,55%	13,67%
S10	61,99%	71,36%	9,37%
S11	40,69%	50,90%	10,21%
	Mean deviation		7,83%

CONCLUSIONS

- These results demonstrate the transferability of the validated ETP-based APC resistance test between CATs using the same software version.
- In order to confirm the reliability of the results obtained with this validated method, its implementation in other centre is essential.
- The conduct of a larger multi-centre study should therefore be performed before implementing this technique in the routine setting.

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