

## RESEARCH OUTPUTS / RÉSULTATS DE RECHERCHE

### **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

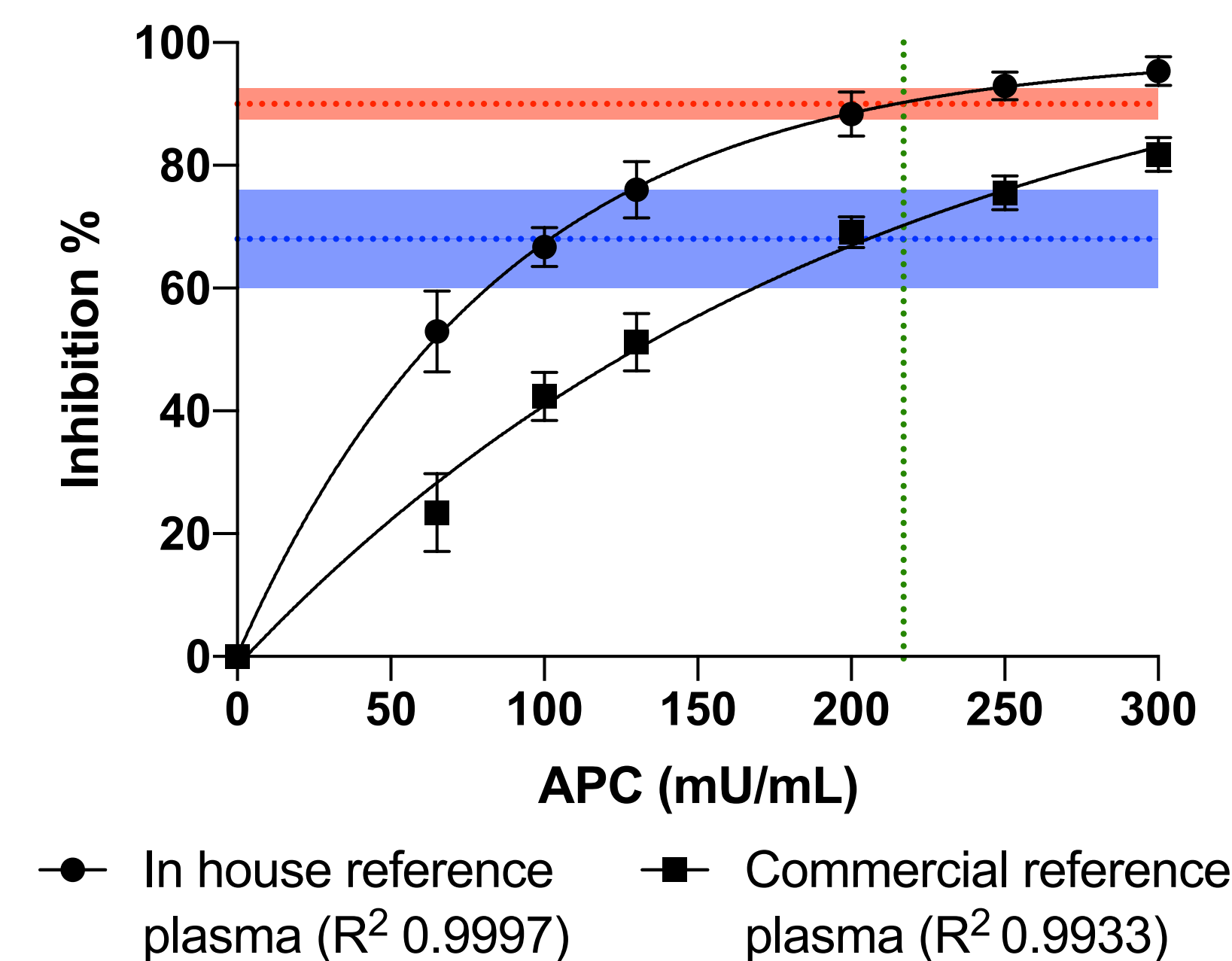
- Analyses were performed on 3 Calibrated Automated Thrombogram (CAT) with Thrombinoscope software (version 5.0 for CAT<sub>1&2</sub>; version 2.0 for CAT<sub>3</sub>) and by 3 different operators.
- Dose-response curves (n=3) were performed on each device to defined the amount of APC, leading to
  - (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<sub>1</sub> and CAT<sub>2</sub> (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.**

**FIGURE 1:** Total variability between CAT<sub>1</sub> and CAT<sub>2</sub>. 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<sub>1</sub> and CAT<sub>2</sub>. Inhibition % in blue\* are outliers



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

**TABLE 2:** Inhibition percentage and absolute difference between CAT<sub>1</sub> and CAT<sub>2</sub>.

	Inhibition %		Difference
	CAT 1	CAT 2	
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%

- 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<sub>3</sub> 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<sub>3</sub> at low ETP-values.

## 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.

## REFERENCES

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- <sup>3</sup> Douxfils, J., et al. (2020). "Validation and standardization of the ETP-based activated protein C resistance test for the clinical investigation of steroid contraceptives in women: an unmet clinical and regulatory need." *Clin Chem Lab Med* **58**(2): 294-305.

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