Validation of an original ETP-based APC resistance assay for the evaluation of prothrombotic states

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VALIDATION OF AN ORIGINAL ETP-BASED APC RESISTANCE ASSAY FOR THE EVALUATION OF PROTHROMBOTIC STATES

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BACKGROUND

- The activated protein C resistance assay based on the endogenous thrombin potential (ETP-based APCr assay) is recommended in guidance from medicines regulatory authorities (e.g. EMA and FDA) for the investigation of steroid contraceptives.[1]
- The results are usually "normalized" with a reference plasma to provide the "normalized APC sensitivity ratio" (nAPCsr).[2]
- However, the methods described in the literature are home-made and mostly without standardization of the method, the reagents, the reference plasma and the quality controls.

AIM

To validate the analytical procedure of an ETP-based APCr assay according to the regulatory standard ICHQ2R1 and CLSI guidelines.[3]

METHOD

- Three quality controls (QCs) representing plasmas with different levels of coagulation and one reference plasma (Ref plasma) were used.
- The method targets a 90% inhibition of the ETP in a pool of plasma from healthy donors (10 men and 10 women not using hormonal contraception, with no coagulation abnormalities [i.e. FV Leiden nor G20210A mutation carrier)] in presence of APC compared to the same condition in absence of APC. [▶ Figure 1]
  - Inhibition % = 100% - $\frac{Sample\ ETP\ (+TM)}{Sample\ ETP\ (-TM)}$

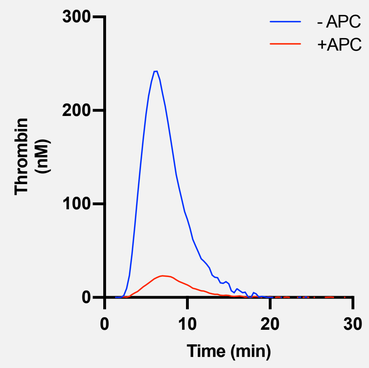

Figure 1 : Thrombin generation in absence of APC (blue curve) and in presence of APC (red curve).

- As the pool of healthy donors is not produced at large scale, specific algorithms are applied to the commercial reference plasma to correlate with the pool.

RESULTS

- Limits of acceptability of QCs and Ref plasma [▶ Table 1] were defined as
  - the mean of results obtained in the entire study (N=24) ± 2*SD
  - SD = the highest CV of the accuracy study * the mean of the entire accuracy study

QC low (hypocoagulable)	100 ± 0%
QC intermediate (intermediate coagulable)	45 ±15 %
QC high (hypercoagulable)	12 ± 10%
Ref plasma	89 ± 6%

Table 1 : Limits of acceptability (mean ± 2*SD) of QCs and Ref plasma.

- Intra-run (into a same plate) and inter-run (between plates) repeatability passed the acceptance criteria : <10% of standard deviation. [▶ Table 2]

	Intra-run variability [SD]	Inter-run variability [SD]
QC low (hypocoagulable)	0%	0%
QC intermediate (intermediate coagulable)	1%	7%
QC high (hypercoagulable)	3%	4%
Reference plasma	0%	3%

Table 2 : Intra- and inter-run repeatability (expressed in SD). Intra-run repeatability was based on 5 measurements of the Ref plasma and QCs and inter-run repeatability was based on 10 runs measuring the Ref plasma and QCs, performed by the same operator.

- Intermediate precision passed the acceptance criteria : standard deviation <10% and no significant difference between operators. [▶ Table 3]

	Operator 1 [SD]	Operator 2 [SD]	Operator 3 [SD]	p-value
QC low (hypocoagulable)	0%	0%	0%	0.8503
QC intermediate (intermediate coagulable)	4%	5%	2%	0.6969
QC high (hypercoagulable)	3%	4%	0%	0.8253
Reference plasma	2%	2%	1%	0.9459

Table 3 : Intermediate precision (expressed in SD and p-value) based on 3 runs measuring the ref plasma and QCs and performed by 3 different operators.

- The assay demonstrated a curvilinear dose-response to protein S and APC concentrations (R^2>0.99). [▶ Figure 2 and ▶ figure 3]

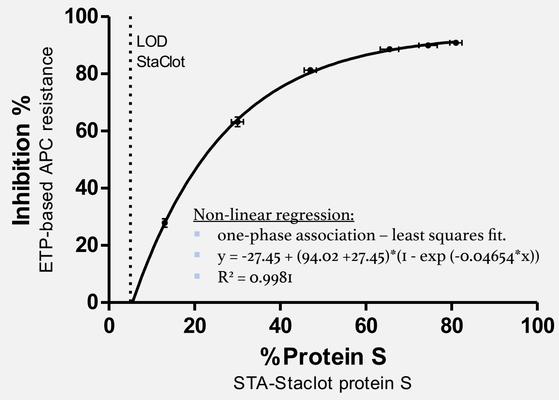

Figure 2: Inhibition percentage depending on a protein S deficiency. Vertical dotted line represents the limit of detection of the STA®-Staclot® protein S kit.

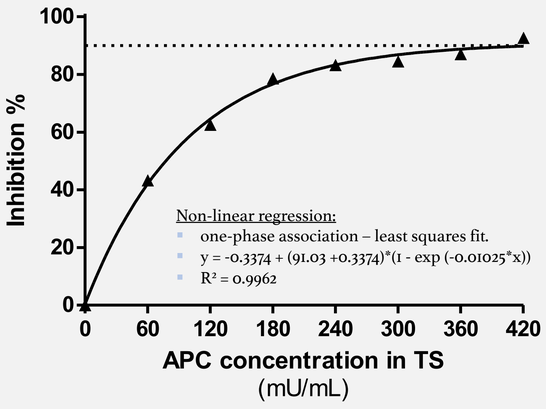

Figure 3: Inhibition percentage depending on concentration of spiked APC. Horizontal dotted line represents 90% inhibition.

- Analysis of plasma samples from 50 healthy individuals (22 women not taking combined oral contraceptive (COC) and 28 men, no FV Leiden carrier) confirmed the validity of the tests [acceptance criteria: mean = 90% (± 2,5%)] with a mean inhibition percentage of 89%.
- Investigations in women taking COC confirmed the good sensitivity of the assay. [▶ Figure 4]

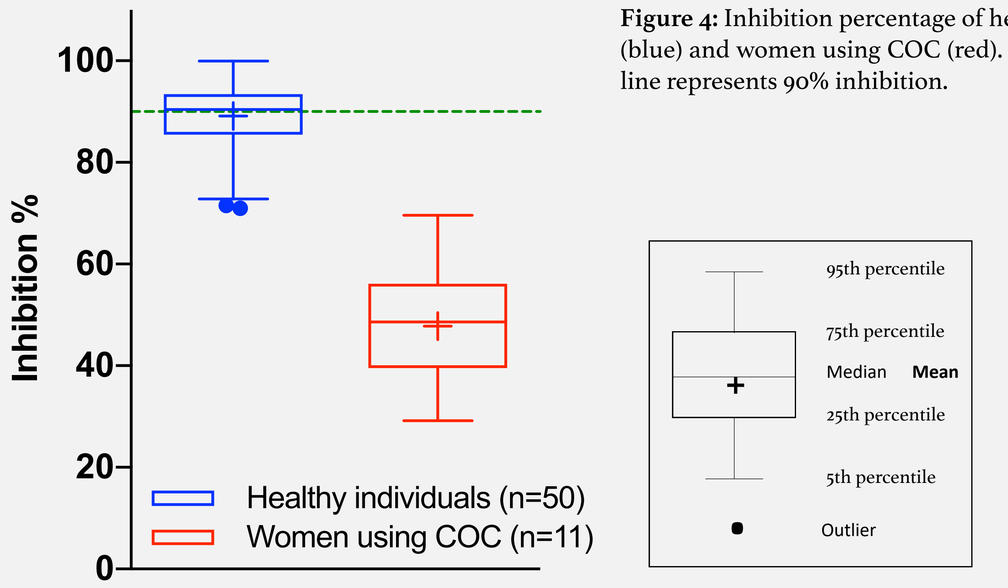

Figure 4: Inhibition percentage of healthy individuals (blue) and women using COC (red). The dotted green line represents 90% inhibition.

CONCLUSION

This study is the first describing the validation of ETP-based APCr assay according to regulatory standards.

It provides the stakeholders, the regulatory bodies and the physicians with a reproducible, sensitive and validated assay.

This will allow study-to-study comparison as well as perspectives for the establishment of specific thresholds to reflect the prothrombotic state in the individual patient.

Conflict of Interest :
Jonathan Douxfils reports personal fees from Daiichi Sankyo, Diagnostica Stago, Roche and Roche Diagnostics outside the submitted work. Jonathan Douxfils is the CEO and founder of QUALIblood s.a.

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