150eP_ High robustness and reproducibility of the genomic signature EndoPredict through quality control programs in France since 2017.

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Background

Among breast cancers (BC), luminal ER+/HER2− is the most common subtype and, at early stage, the standard of care is adjuvant endocrine therapy post-surgery. However late recurrences occur and may require chemotherapy. In addition to classical risk factors such as age, tumor size(T) and nodal status (N), genomic signatures (GS) have been developed to evaluate patient's prognosis and guide treatment[1].
The GS EndoPredict, quantifies 8 genes (+4 controls) via qRT-PCR on RNA extracted from FFPE tissue and combines pT/pN to generate recurrence scores and risk of relapse.

Although inter-laboratory reproductibility has been reported in validations studies[2], no European nor national external quality assessment (EQA) exists for such tests. In France, EndoPredict is performed in decentralized labs under ISO 15189, requiring robust internal quality control (QC)[3].

Rationale : Create a **French national QC scheme**, under Gen&Tiss supervision, to fulfill ISO 15189 requirements.

Methods

Six to 12 french participating labs in a pilot program (2017) and therafter biennal programs (2018,2020,2022,2025).
Anonymized FFPE samples with pT/pN data shared and analyzed under routine conditions .

Test metrics collected:

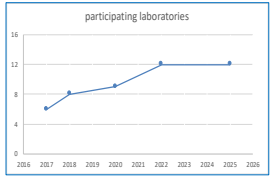
- **EP** Score: Molecular score derived from qRT-PCR analysis.
- **EPclin** Score: Molecular score combined with pathological parameters (pT/pN) provided with the sample.
- 10-Year **Recurrence Risk**: Calculated based on the Epclin
- **Risk Classification**
  Low Risk: EPclin < 3.33 (Risk < 10%)
  High Risk: EPclin > 3.33 (Risk ≥ 10%)

Technical Quality Control on Reference sample (qRef):
Each qRT-PCR plate includes a qRef provided in the kit and the 12-gene Ct values must fall within a range for valid test.
Long-term Monitoring: 15 qRef Ct values were collected per platform at each QC scheme.

1 Loibl S. et al. (2024) Early breast cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Annals of Oncol
2 Denkert C. et al, (2012) Decentral gene expression analysis for ER+/Her2- breast cancer: results of a proficiency testing program for the EndoPredict assay. Virchows Arch
3 Lehmann-Che J. et al (2018). First French Pilot Quality Assessment of the EndoPredict Test for Early Luminal Breast Carcinoma. Anticancer Res.

Results

Since 2017, 5 programs have been organized, enabling the Endopredict inter-laboratory reproducibility to be evaluated on a **regular basis**, for a growing number of laboratories (6 to 12) and a **large duration** (8 years).



Evaluation:
9 shared samples representing **88 tests** across participating labs

1. Scores and recurrence reproducibility:

88 tests (2017-2025)	max CV (%)
EP	4,5
Epclin	2,3
% recurrence risk	8,3*

* expressed as a integer, explaining the higher CV

2. EPclin Score Variability across labs and programs

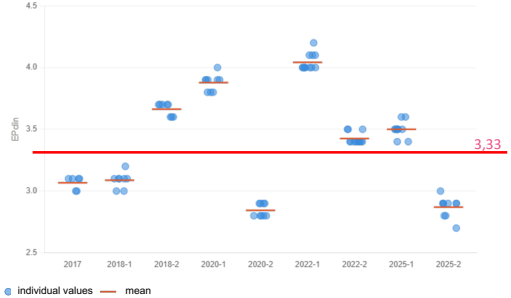


fig 1: Individual scores and means (small red bars) for the 5 QC schemes. The red line indicates the clinical threshold (3.33); values remain clearly separated from this threshold, guaranteeing consistent therapeutic decisions

100% concordance for low and high risk class including for case 2022-2 close to the threshold

3. qRef 11-gene Ct Values Stability (2018-2025)

542 qRef analyses across, at least 8 platforms on a period of 8-years with different RNA qRef batches, operators and instruments

The standard deviation (SD) of CT values is generally **close to 0.5** (8 out of 11 genes; mean = 0.41, range: 0.27–0.59). Only 3 genes exhibited greater dispersion, with an SD of 0.77 for OAZ1 and MGP (CV: 3.1%) and 1.32 for BIRC5 (CV: 5.4%). These genes correspond to lower expression levels.

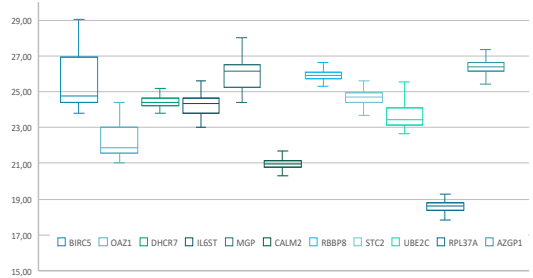


fig 2: Boxplot distribution of 542 Ct values for each of the 11 genes collected (BIRC5 to AZGP1)

qRef Ct values remain remarkably stable across 8+ labs over 8y, confirming **robustness of decentralized testing**

Conclusion

Over 8 years (2017–2025), the program demonstrated exceptional inter-laboratory reproducibility: the EndoPredict test achieved **100% concordance in risk class** and a coefficient of variation **(CV) of <10%** across all evaluations.
This ensure that **therapeutic decisions** (chemotherapy vs. no chemotherapy) are **consistent** regardless of the laboratory.
Moreover, the **decentralized test** proves **highly robust** over the 8-year period across 8+ French laboratories, with a CV of <5% for all reference RNA gene expression quantifications, except one.
These test reliability contributes to the clinical value of the prognostic tool, today largely recommended in early-stage ER+/HER2- breast cancer for safe therapeutic de-escalation.

This initiative serves as a critical model for real-time monitoring of all available GS, paving the way for an European EQA to ensure accurate medical decisions.

J Lehmann-Che declares no conflict of interests on the ePoster