Reviewer’s report

Title: Decentral gene expression analysis: analytical validation of the Endopredict genomic multianalyte breast cancer prognosis test

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Reviewer: Nadarajen Vydelingum

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Report
Title: 'Decentral gene expression analysis: analytical validation of the Endopredict genomic multianalyte breast cancer prognosis test'

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Major Compulsory Revisions: None

Minor Essential Revisions: None

Discretionary Revisions: None

The manuscript is well written and the title correctly reflects the intent of the study. The manuscript describes in clear detail the validation of the EndoPredict (EP) multianalyte gene expression assay according to the adapted guideline MM17-A of the Clinical and Laboratory Standards Institute (CLSI). The authors have a demonstrated track record in this area of research and the study reported here is a continuation of this effort.

Based on their statistically sound analyses, the authors are justified in concluding that the EP tests on reproducible performance, level of precision and laboratory-to-laboratory variation are valid. The test was based on the assessment of expression of twelve genes (eight informative, three reference and one gene that determined the presence of genomic DNA in RNA) from formalin-fixed, paraffin-embedded (FFPE) tissue obtained from biopsies or surgical specimen using reverse transcription-quantitative real-time PCR (qRT-PCR). The relative gene expression levels were used to calculate the Endo-Predict score (EP score) ranging from 0 to 15 for distant recurrence under endocrine therapy; 0-5 indicating low risk, 5 and above indicating medium to high risk. The key variables are thoroughly tested and the appropriate controls are utilized throughout the assay protocols as are the statistical analyses used to express the resulting data.

The research described is important since molecular diagnostics is a rapidly
growing testing field that holds exciting promise for information solution and healthcare providers. The results are appropriately discussed in the context of moving from analytical validation of the performance characteristics of the assay to its verification in a molecular-pathological routine laboratory. The results of both the proficiency testing to test the clinical validation studies and the verification of the performance characteristics in an independent laboratory added further support as to the robustness of the test. The authors provide adequate rationale as to the advantages of their test as compared to those of competing tests.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'