Peer Review File

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Reviewer A

The authors compared HERE the accuracy rate in detecting EGFR mutations using the automated cartridge-based RT-PCR assay Idylla versus ARMS and NGS in 146 and 86 formalin-fixed paraffin-embedded samples of nsclc (surgical specimens and cell bloks), respectively, derived from the same block.

The overall concordance between Idylla and ARMS was 89.51% (95% CI 83.31% to 93.64%) and the specificity of Idylla was 88.68% (95% CI 80.69% to 93.76%). A concordance of 97.67% (95% CI 91.41% to 99.86%) was obtained between Idylla and NGS, the specificity of Idylla was 96.30% (95% CI 86.16% to 99.36%). Compared to the ARMS and NGS, the Idylla system significantly reduces the turnaround time. Combining labor, equipment, reagents and time costs, Idylla is considered more affordable.
Clinically urgent cases with adequate cellularity, can first do Idylla to detect critical 33 markers, then do NGS for a comprehensive mutation analysis. Besides, with very little molecular expertise or infrastructure, the Idylla has the potential to extend EGFR testing to more pathology Laboratories in primary hospitals.

I would suggest the authors to include among the refs and discuss some studies from the Swansea University showing similar results, as the following one:

Finall A, et AL. Integration of rapid PCR testing as an adjunct to NGS in diagnostic pathology services within the UK: evidence from a case series of non-squamous, non-small cell lung cancer (NSCLC) patients with follow-up J Clin Pathol 2023 Jun;76(6):391-399. doi: 10.1136/jclinpath-2021-207987. Epub 2022 Jan 18.

Another point of discussion arises from this author reply concerning the impossibility to detect all EGFR mutations in Idylla, as follows:
Bennett P, Finall A, Medeiros F, Gerrard G, Taniere P. Re: Inadequacy of PCR genotyping in advanced non-small cell lung cancer: EGFR L747_A755delinsSS Exon 19 deletion is not detected by the real-time PCR IdyllaTM EGFR mutation test but is detected by ctDNA NGS and responds to osimertinib. Eur J Cancer. 2022 Oct;174:315-317. doi: 10.1016/j.ejca.2022.06.039.
Reply: We have discussed the studies recommended by the reviewer in the manuscript.
Changes in the text: Line379-404.

Reviewer B

In this study, the authors compared sensitivity and specificity of EGFR testing in NSCLC between Idylla, ARMS, and NGS. The authors conclude, that due to very little molecular expertise and due to speed Idylla testing provides significant clinical benefit to patients over NGS.
I completely disagree with the authors´ conclusions and think that the recommendations are not valid.
(1) Concordance of 97% and specificity of 96% comparing Idylla and NGS is far from optimal. What it means is that 1 out of 40 cases with EGFR mutations is missed and 1 out of 30 falsely reported positiv. Since EGFR-TKIs provide significant clinical benefit, these patients would certainly suffer from disadvantageous treatment.
Reply: We realized that Idylla is not highly accurate in detecting the FFPE cell blocks samples and samples with low tumor cell percentage. So Idylla is not the only detection method, just a supplement to NGS. Clinically urgent cases with adequate cellularity can first do Idylla to detect critical markers, then do NGS for a comprehensive mutation analysis. For patients with advanced lung cancer who would not survive to see the potential beneficial repercussions of NGS-based mutation detection, NHS allows local testing to continue by rapid PCR methods in a context of genomic testing in centralised laboratory hubs.
Changes in the text: Line 399-404.

(2) I further believe that the time of single gene testing is over. In fact more comprehensive NGS test include a minimal set of testing for mutations in EGFR, BRAF, KRAS, C-mat ex14 skipping, all of which are actionable mutations and would be missed by simply testing EGFR by Idyllla.
Reply: Each methodology has its own advantages and disadvantages. Choosing between single-gene testing and NGS depends on clinical, sample quality, the main purpose for the molecular testing and economic factors. The ordering clinician should communicate with the lab to determine the most suitable technique.
Changes in the text: Line 298-303.

(3) The authors make a big deal of speeding up turn-around times (TAT). However, most NGS technologies provide comprehensive mutation and gene fusion (ALK, ROS1, RET, and others) results in less than 10 days. They state "The faster the better". Very rarely, clinical need requires start of therapy in less than 10 days. Thus, the loss in quality and comprehensive testing is clearly larger than the gain in speed.
Reply: Timeliness of reporting genetic mutations in solid malignancies is important for ensuring that patients get the most appropriate treatment available at a time when they are sufficiently well enough to tolerate side effects and achieve a progression free survival advantage. Several studies have found Idylla EGFR testing was, on average, 9-12 days faster than NGS. Although most patients would be expected to be negative for EGFR mutations, the pre-test probability of harbouring an EGFR-mutation-positive tumor is higher in certain patient populations such as non-smokers. The true value of rapid EGFR testing in these patient subsets is the prompt identification of a targetable mutation and

subsequent quick match to the appropriate treatment.
Changes in the text: Line 346-354.

(4) The cost discussion completely misses the fact that most costs do not result from testing, but rather result from therapies. Indeed, ineffective therapies as a result of inadequate testing would significantly increase the total cost burden to health care providers. Along these lines, some diagnostic samples are so small that careful decisions in testing is required and this means mandatory NGS testing, as a single test such as Idylla could force clinicians to rebiopsies for further testing.
Reply: Idylla is not the only detection method, just a supplement to NGS. Given its limited sensitivity in detecting cell block samples, clinically urgent cases with adequate cellularity can first do Idylla to detect critical markers, then do NGS for a comprehensive mutation analysis. We would communicate with the ordering clinician, and determine the most suitable technique according to clinical, sample quality, the main purpose for the molecular testing and economic factors.
Changes in the text: Line 301-303.