Author's response to reviews

Title: Analytic performance studies and clinical reproducibility of a real-time PCR assay for the detection of epidermal growth factor receptor gene mutations in formalin-fixed paraffin-embedded tissue specimens of non-small cell lung cancer

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Version: 3 Date: 11 February 2013

Author's response to reviews: see over
Authors response to reviews

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Version: 2 Date: 7 February 2013

Authors response to reviews: see over
Reviewers report

Title: Analytic performance studies and clinical reproducibility of a real-time PCR assay for the detection of epidermal growth factor receptor gene mutations in formalin-fixed paraffin-embedded tissue specimens of non-small cell lung cancer

Version: 2 Date: 22 January 2013

Reviewer: Han-Pin Kuo

We would like to thank Dr. Han-Pin Kuo for his review and comments. Our responses are captured below. No additional changes were made to the manuscript.

Reviewer's report:

Minor Essential Revisions

This manuscript describes the verification of technical performance and clinical reproducibility of the cobas EGFR Mutation Test, in comparison with a widely used Sanger sequencing, to detect 41 mutations in exons 18, 19, 20 and 21 on a panel of 201 formalin-fixed paraffin-embedded tissue specimens of human non-small cell lung cancer. The resolution of discordant specimens was performed by quantitative massively parallel pyrosequencing. The effects on the performance of the cobas EGFR test of endogenous substances and nine therapeutic drugs were evaluated in ten specimens. This manuscript is well written and the results reveal the cobas test is a rapid multiplex real-time PCR assay with 96.7% overall percent agreement with Sanger sequencing, 98% assay repeatability, and was not compromised by endogenous substances, therapeutic drugs, necrosis up to 85%, and common microorganisms.

Comments:

1. The cobas mutation test is able to detect mutations in EGFR exons 18, 19, 20, and 21 using 150ng of total DNA input, and detect mutation at ≥5% mutation level using only 50 ng of DNA. The turnaround time for cobas test is around 6 hours for 1 sample. This rapid and sensitive method may provide opportunity of efficient use of limited specimen in non-small cell lung cancer, where patient samples are difficult to obtain and EGFR mutation testing is being prioritized for treatment decisions.

   We agree with the reviewer that there is a significant clinical need for a rapid and accurate EGFR mutation detection test. We are currently investigating the utility of the cobas test in limited specimens from NSCLC and plan to submit these results for publication in the near future.

2. The readers may be interested in the feasibility and the detection limits of the cobas test for EGFR mutation in cytologic specimens.

   We agree with the reviewers comments. A study in cytological samples has been conducted at an external clinically validated lab and the results have been submitted to ASCO 2013. Detection limits have not been assessed on cytology samples for the cobas® EGFR Test, and studies are currently under consideration.
3. Next generation sequencing has been introduced to assess EGFR mutation with extreme sensitivity. Would the authors comment on its clinical applicability compared to cobas test?

We recognize and agree with the reviewer on the eventuality of next generation sequencing in clinical diagnostics. Though the value of next generation sequencing in a research setting is unparalleled, the clinical utility of next generation sequencing platforms has not yet been validated. We believe that the workflow and analytic performance will require extensive validation and standardization before reduction to clinical practice as not all results lead to clinically actionable decisions. Until such time, we suggest the use of a reproducible, clinically and analytically validated assay for use in routine diagnostics. As this manuscript describes and as discussed by the reviewer, the cobas EGFR test is a highly reproducible, validated test that can provide rapid and accurate results and has been clinically validated in the EURTAC trial (Benlloch, et al, ASCO 2012).
Reviewer's report

Title: Analytic performance studies and clinical reproducibility of a real-time PCR assay for the detection of epidermal growth factor receptor gene mutations in formalin-fixed paraffin-embedded tissue specimens of non-small cell lung cancer

Version: 2 Date: 30 January 2013
Reviewer: Ite A. Laird-Offringa

Reviewer's report:
A well-written and detailed evaluation of a commercial product to measure EGFR mutations in paraffin embedded specimens

We thank Dr. Laird-Offringa for comments on our manuscript. No additional comments or changes were made to address reviewers comments.