Supplementary Online Content

Kim AS, Bartley AN, Bridge JA, et al. Comparison of Laboratory-Developed Tests and FDA-Approved Assays for *BRAF*, *EGFR*, *KRAS* Testing. *JAMA Oncol*. Published online December 14, 2017. doi:10.1001/jamaoncol.2017.4021

eMethods

This supplementary material has been provided by the authors to give readers additional information about their work.

Methods.

Sample Definition and Timeframe.

All CAP proficiency testing (PT) for *BRAF* from 2011B through 2015A was included in this study. For *EGFR*, data from 2013A through 2015A were examined. For *KRAS*, only data from 2013B through 2015A were examined, because the specific option for respondents to indicate the use of the FDA-approved platform was not available until 2013B. For all three analytes, two PTs (A and B) are provided each year. During this time frame, PTs 2011B through 2013B contained only a single specimen. Beginning with 2014A, each mailing contained three specimens. In total, we included data from fourteen samples for *BRAF,* eleven samples for *EGFR*, and ten samples for *KRAS* (ranging from 33% to 100% neoplastic cellularity, the latter in cell lines carrying the variant). Samples for *BRAF* and *EGFR* were composed of neoplastic tissue prior to the 2013A survey, and FFPE cell lines thereafter. Samples for *KRAS* were composed of FFPE cell lines for the entirety of the period of this study.

Method Definition.

Although attempts were made to determine if laboratories using a kit produced by a manufacturer with an FDA-CD were actually using the FDA-CD or a research use only (RUO) version, too few laboratories responded to enable assignment of laboratories to the proper category. Therefore, for all three surveys, all laboratories using kits purchased from a vendor with an FDA-CD were considered in the FDA cohort for the purpose of this study, acknowledging that some laboratories (in particular for *EGFR* and *KRAS*) may have been using the RUO version (and therefore should have been categorized as an LDT).

Data Definition.

Results on CAP PT are considered either good, acceptable, or unacceptable. These terms are defined differently depending upon the type of sample used for the PT. For tissue samples used prior to 2013, a good result (detected or not detected) was one that matched the consensus response, defined as concordance of 80% or greater of respondents. Beginning with the 2013A survey, when cell lines with defined mutations were introduced for the PTs, a good result was defined as the identification of the correct result. Supplementary Table 1 lists the good responses for each of the PTs. The responses, "Does not detect" and "Does not discriminate," were also considered acceptable. However, only laboratories that reported results for a specific variant were included in the subsequent studies on the specific variants while laboratories reporting "does not discriminate variants" or “test not performed” were excluded from further consideration. Therefore, if a laboratory reported "Not detected" for a *BRAF* p.V600K variant when using an FDA-CD that does not discriminate p.V600K from p.V600E, this was counted in the analysis as unacceptable. For the purposes of this study, all "good" and "acceptable" results are considered "acceptable" and are included in the overall rates of acceptability that are utilized throughout the manuscript.

For the assessment of laboratory practice, select qualitative and quantitative pre-analytic considerations were also surveyed. Of note, not all participants answered all questions; only respondents were considered in our analysis. For the determination of whether or not an FDA-CD laboratory followed the appropriate FDA-approved procedure, the following steps were pulled from the manuals accompanying the kits: specimen preparation (including tissue preparation and type of tumor tested), whether or not a pathologist review is required, the stated

minimal neoplastic cellularity for testing, whether or not tissue dissection is performed, whether or not DNA quantification is performed, the method of DNA quantification, and whether or not an interpretive comment was included. For the type of tumor tested, each FDA-CD is limited to a specific tumor type. The PT survey questions only directly interrogated this point on the *BRAF* survey. For the *BRAF* assays, an FDA-CD can only test melanomas to be within the FDA-approved procedure. The PT survey questions named specific other tumor types for *BRAF*, and testing of non-melanoma tumors was considered a formal deviation from the FDA-approved procedure. In the case of neoplastic cellularity, the FDA-approved procedure either specified a minimum tumor content to be used or the minimum tumor content was calculated as twice the minimum detectable variant allele fraction (for bioMerieux THxID® BRAF Test and the therascreen® *EGFR* RGQ PCR Kit). In the latter therascreen® *EGFR* assay, there is such a variant-specific range that it was not possible to assign a single minimum tumor cellularity cutoff that a laboratory should accept for testing, rendering an assessment of off-label use of the assay impossible to determine.