Reporting Results of Molecular Tests
A Retrospective Examination of BRAF Mutation Reporting

Amanda L. Treece, MD; Margaret L. Gulley, MD; Patricia Vasalos, BS; Cherie Paquette, MD; Neal I. Lindeman, MD; Lawrence J. Jennings, MD; Angela N. Bartley, MD

Context.—With enormous growth in the field of molecular pathology, the reporting of results gleaned from this testing is essential to guide patient care.

Objective.—To examine molecular reports from laboratories participating in proficiency testing for required elements to convey molecular laboratory test results to clinicians and patients.

Design.—Molecular laboratories participating in the College of American Pathologists (CAP) proficiency testing program for BRAF mutation analysis were solicited to submit examples of final reports from 2 separate proficiency testing reporting cycles. Reports were reviewed for the presence or absence of relevant components.

Results.—A total of 107 evaluable reports were received (57 demonstrating a positive result for the BRAF V600E mutation and 50 negative). Methods for BRAF testing varied, with 95% (102 of 107) of reports adequately describing their assay methods and 87% (93 of 107) of reports adequately describing the target(s) of their assays. Information on the analytic sensitivity of the assay was present in 74% (79 of 107) of reports and 83% (89 of 107) reported at least 1 assay limitation, though only 34% (36 of 107) reported on variants not detected by their assays. Analytic and clinical interpretive comments were included in 99% (106 of 107) and 90% (96 of 107) of reports, respectively. Of participants that perform a laboratory-developed test, 88% (88 of 100) included language addressing the development of the assay.

Conclusions.—Laboratories participating in BRAF proficiency testing through the CAP are including most of the required reporting elements to unambiguously convey molecular results. Laboratories should continue to strive to report these results in a concise and comprehensive manner.

**Table 1. Reporting Characteristics Evaluated in This Study, With Stringency and Source of Guidelines**

<table>
<thead>
<tr>
<th>Reporting Characteristic</th>
<th>Reporting Guidelines</th>
<th>Source (Phase of CAP Checklist Deficiency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory name</td>
<td>Required</td>
<td>GEN.41096 (Phase II)7</td>
</tr>
<tr>
<td>Date specimen collected</td>
<td>Required</td>
<td>GEN.41096 (Phase II)7</td>
</tr>
<tr>
<td>Date specimen received</td>
<td>Recommended</td>
<td>Gulley et al6</td>
</tr>
<tr>
<td>Date results reported</td>
<td>Required</td>
<td>GEN.41096 (Phase II)7</td>
</tr>
<tr>
<td>Specimen type (FFPE, frozen tissue, etc)</td>
<td>Recommended</td>
<td>Gulley et al6</td>
</tr>
<tr>
<td>Ordering physician</td>
<td>Required</td>
<td>GEN.41096 (Phase II)7</td>
</tr>
<tr>
<td>Results with test name</td>
<td>Recommended</td>
<td>Gulley et al6</td>
</tr>
<tr>
<td>Reference range</td>
<td>Required, when applicable</td>
<td>GEN.41096 (Phase II)7</td>
</tr>
<tr>
<td>Standard gene nomenclature</td>
<td>Required</td>
<td>COM.29950 (Phase II)5</td>
</tr>
<tr>
<td>Methodology</td>
<td>Required</td>
<td>MOL.49630 (Phase I)6</td>
</tr>
<tr>
<td>Target description</td>
<td>Required</td>
<td>MOL.49570 (Phase II)6</td>
</tr>
<tr>
<td>Specimen-enrichment method</td>
<td>Not required</td>
<td></td>
</tr>
<tr>
<td>Variants not detected</td>
<td>Required</td>
<td>MOL.49570 (Phase II)6</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>Required for sequencing assays</td>
<td>MOL.35790 (Phase II)5</td>
</tr>
<tr>
<td></td>
<td>Recommended for all</td>
<td>Gulley et al2</td>
</tr>
<tr>
<td>Additional assay limitations</td>
<td>Required</td>
<td>MOL.49570 (Phase II)6</td>
</tr>
<tr>
<td>Analytic interpretive comment</td>
<td>Required</td>
<td>MOL.49570 (Phase II)6</td>
</tr>
<tr>
<td>Clinical interpretive comment</td>
<td>Required, when appropriate</td>
<td>MOL.49570 (Phase II)6</td>
</tr>
<tr>
<td>Pathologist/designee signature</td>
<td>Required</td>
<td>MOL.49585 (Phase II)6</td>
</tr>
<tr>
<td>LDT reporting language</td>
<td>Required</td>
<td>MOL.31705 (Phase I)6</td>
</tr>
<tr>
<td>ASR language</td>
<td>Required, if applicable</td>
<td>MOL.49580 (Phase II)6</td>
</tr>
</tbody>
</table>

Abbreviations: ASR, analyte-specific reagent; CAP, College of American Pathologists; FFPE, formalin-fixed paraffin-embedded; LDT, laboratory-developed test.

**METHODS**

Sample Report Acquisition and Review

Laboratories participating in the CAP proficiency testing program for Braf mutation analysis were solicited to submit examples of final reports during 2 separate proficiency testing reporting cycles in 2013 and 2014. One of the challenges was positive for a Braf V600E mutation, and the other was negative. Instructions indicated that reports should be presented as if they were actual patient results reported from the laboratory, based on the tumor type for which they most commonly perform Braf testing. Reports were reviewed initially by a CAP staff person and all laboratory-identifying information was deidentified. Reports were then reviewed independently by 2 molecular pathologists and scored for the presence or absence of multiple components listed in the “Scoring Criteria” section. These components were selected from requirements set forth in the CAP All Common Checklist regarding use of a laboratory-developed test (LDT; COM.40630), those set forth in the CAP Molecular Pathology Checklist regarding gene nomenclature, final report content, LDT reporting, analyte-specific reagents, report sign-off (MOL.49630, MOL.49570, MOL.31705, MOL.49580, and MOL.49585), and those in the Laboratory General Checklist regarding basic reporting criteria.5–7 Additional criteria were evaluated from recommendations for molecular pathology reporting by Gulley et al.2 All reporting characteristics evaluated in this study and their sources of reporting guidelines are listed in Table 1.

Scoring Criteria

Gulley et al2 recommend general information in all reports, including laboratory name and contact information, patient name, and an additional identifier, date of specimen collection, date of receipt by laboratory, specimen type, and ordering physician, most of which are also required by the CAP General Checklist (GEN.41096).7 These components were scored as either present or absent. Though specimen source is required (GEN.41096), it was not scored here owing to the nature of the proficiency testing samples. Gulley et al2 also recommend clearly listing the test name as part of the result by using standardized nomenclature and official symbols (ie, Braf V600E mutation) was listed as its own result field with a separate positive or negative result or as part of the result line. Incorrect gene nomenclature was also noted. A reference range is also required by CAP (GEN.41096), when applicable, and was scored as present if a separate reference range field was included or if an interpretation of normal or abnormal was included in the report.7

The CAP Molecular Pathology Checklist final report criteria require the report to contain a summary of methods and the loci or variants tested. The methods included in each report were evaluated for a clear description of the technology used. For example, methods including, but not limited to, a specific kit, Sanger sequencing, or allele-specific primers were considered acceptable, while “a polymerase chain reaction (PCR)–based assay” was considered incomplete information. The target locus or loci were evaluated in the context of the assay(s) being performed, regardless of whether testing encompassed only the Braf c.1799T>G mutation, or whether it tested for multiple specific mutations or sequenced the whole exon or more. The description of variants tested was considered appropriate if it was clear which loci were and were not covered by the assay. Inappropriate reporting included reference to the target(s) as “Braf mutation(s)” without further clarification or a mention of the region/mutations analyzed that was inconsistent with the described methods. Additionally, reports were evaluated for inclusion of information regarding dissection of tumor from sections on slides or other tumor-enrichment procedures, if performed. Though the method of enrichment is not required in the report by CAP, laboratories performing sequencing assays are required to discuss tumor cellularity and enrichment thereof in the setting of the limits of detection of the assay (MOL.35795), and assessment of tumor cell content is required for all paraffin-embedded tumor specimens (MOL.32395). Examination of tumor cellularity was not scored in this study owing to the nature of the specimen received by the laboratories for proficiency testing.

The limitations of the assay, specifically variants not detected, were also evaluated. Gulley et al2 suggest reporting the analytic sensitivity of the assay and other caveats that may affect results, and the lower limit of detection is a report requirement for sequencing assays (MOL.35795). Reports were evaluated for mention of variants not detected by the assay that may have clinical relevance, such as when a laboratory performs an assay for

(ie, Braf V600E mutation) was listed as its own result field with a separate positive or negative result or as part of the result line. Incorrect gene nomenclature was also noted. A reference range is also required by CAP (GEN.41096), when applicable, and was scored as present if a separate reference range field was included or if an interpretation of normal or abnormal was included in the report.7

The CAP Molecular Pathology Checklist final report criteria require the report to contain a summary of methods and the loci or variants tested. The methods included in each report were evaluated for a clear description of the technology used. For example, methods including, but not limited to, a specific kit, Sanger sequencing, or allele-specific primers were considered acceptable, while “a polymerase chain reaction (PCR)–based assay” was considered incomplete information. The target locus or loci were evaluated in the context of the assay(s) being performed, regardless of whether testing encompassed only the Braf c.1799T>G mutation, or whether it tested for multiple specific mutations or sequenced the whole exon or more. The description of variants tested was considered appropriate if it was clear which loci were and were not covered by the assay. Inappropriate reporting included reference to the target(s) as “Braf mutation(s)” without further clarification or a mention of the region/mutations analyzed that was inconsistent with the described methods. Additionally, reports were evaluated for inclusion of information regarding dissection of tumor from sections on slides or other tumor-enrichment procedures, if performed. Though the method of enrichment is not required in the report by CAP, laboratories performing sequencing assays are required to discuss tumor cellularity and enrichment thereof in the setting of the limits of detection of the assay (MOL.35795), and assessment of tumor cell content is required for all paraffin-embedded tumor specimens (MOL.32395). Examination of tumor cellularity was not scored in this study owing to the nature of the specimen received by the laboratories for proficiency testing.

The limitations of the assay, specifically variants not detected, were also evaluated. Gulley et al2 suggest reporting the analytic sensitivity of the assay and other caveats that may affect results, and the lower limit of detection is a report requirement for sequencing assays (MOL.35795). Reports were evaluated for mention of variants not detected by the assay that may have clinical relevance, such as when a laboratory performs an assay for
the BRAF V600E mutation that does not detect other mutations at V600 that have been shown to be clinically significant. The presence of a limit of detection was documented, as well as the allele fraction detectable by the assay. If the limit of detection was documented based upon the percentage of tumor cells present, the number was converted to allele fraction with the assumption of a heterozygous mutation. The presence of additional factors affecting assay performance was also noted.

The presence or absence of the responsible professional’s signature or the laboratory director name or designee was also evaluated, as required by MOL.49585. The exception to this requirement is when reports are generated automatically by computer without a subjective or interpretive component. Owing to the nature of BRAF mutation testing, the general need for interpretation in the context of the input specimen and the clinical implications of results, and our inability to identify computer-generated reports, all reports were evaluated for the presence of a signature.

An analytic interpretation is required by MOL.49570, and this term implies there was an examination of raw data for the patient and for all pertinent controls to reach a conclusion about the result that was obtained. A clinical interpretation was scored as present if there was any mention of the implications of the result of positive reports and 4 negative reports), leaving 107 for evaluation. Not all laboratories submitted reports for both rounds of evaluation, and those that did submit reports for both rounds were not specifically identified.

### Basic Reporting Information

Basic reporting information, including laboratory identity and specimen information, is shown in Table 2. Each of the basic information categories were present in greater than 75% of reports, while 46% (49 of 107) of reports contained all 6 criteria. The specimen type was reported according to the specimen preparation (eg, paraffin block, unstained slide, scrolls) but was counted as present if there was a field in the report for specimen source/type or material or specimen received (some laboratories left the field empty or entered the name of the proficiency testing sample). Few laboratories reported the anatomic site from which the tissue was collected or the type of cancer for which testing was selected. This information was not analyzed owing to the absence of site and diagnosis information accompanying the proficiency testing sample.

Overall, 98% (105 of 107) of reports contained the test name as a header or part of the results. Two reports did not contain a clear heading for test name and only reported “positive” or “negative” in the Results section, though the gene and mutation name were included in the further interpretation or comments: This is considered inadequate, since the purpose of this criterion is to easily identify the test name on the report. None of the reports contained full gene nomenclature as defined by the Human Genome Organization (HUGO) Gene Nomenclature Committee (”v-raf murine sarcoma viral oncogene homolog B” at the time of this survey), which is reasonable since the gene symbol (BRAF) is the accepted term. Four reports did include a full name but used the outdated nomenclature, “v-raf murine sarcoma viral oncogene homolog B1.” It should be noted that the HUGO nomenclature has since been updated again, with the current approved name “B-Raf proto-oncogene, serine/threonine kinase.” All reports used the approved symbol “BRAF,” which has not changed.

### Reporting of Methods and Gene Targets

Methods used for BRAF mutation testing were widely variable. The most common method was real-time PCR with a probe discrimination method. The reported technologies are summarized in Figure 1. Ninety-five percent (102 of 107) of reports demonstrated a clear description of the technology used. Four of those reports contained 2 different technologies but clearly explained their utilization. While scored as adequate, 7 reports used a real-time PCR-based assay without clarification of whether the specificity was based on the primers or the probes. Of the 5 reports that were scored as inadequate, 2 did not specify a technology, 2 reported using a “PCR-based assay” without further

---

**Table 2. Percentage of Laboratories Reporting Basic Information**

<table>
<thead>
<tr>
<th>Report Component</th>
<th>Positive Reports, % (n = 107)</th>
<th>Negative Reports, % (n = 107)</th>
<th>All Reports, % (n = 107)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory name</td>
<td>93</td>
<td>82</td>
<td>88</td>
</tr>
<tr>
<td>Date specimen collected</td>
<td>84</td>
<td>68</td>
<td>77</td>
</tr>
<tr>
<td>Date specimen received</td>
<td>86</td>
<td>80</td>
<td>83</td>
</tr>
<tr>
<td>Date results reported</td>
<td>82</td>
<td>86</td>
<td>84</td>
</tr>
<tr>
<td>Specimen type</td>
<td>86</td>
<td>78</td>
<td>82</td>
</tr>
<tr>
<td>Ordering physician</td>
<td>84</td>
<td>86</td>
<td>85</td>
</tr>
</tbody>
</table>
explanation, and 1 contained methods and performance information for 3 platforms, including the cobas BRAF V600 Mutation Assay (Roche, Indianapolis, Indiana) on the 4800 platform, but was unclear as to what was actually performed (counted as 1 assay in the unclear category in Figure 1).

Most reports adequately defined the targets of all BRAF assays they described (87%, 93 of 107), such as the nucleotide positions within the gene or the codon positions for the predicted amino acid. The 107 reports included 113 identifiable described assays (including those with incomplete reporting of methods). The following situations were scored as inadequate for target description: (1) unclear target based on incomplete information (0.9%, 1 of 113); (2) mentioning possible detection of additional mutations other than V600E, while reporting any positive result as a V600E mutation (8%, 9 of 113); (3) stating that the assay identified the V600E mutation, using a sequencing method without documenting other mutations, such as encodes V600K, that would likely be detected by that method (0.9%, 1 of 113); or (4) using a kit that identifies multiple mutations, but only mentioning the V600E mutation (2.7%, 3 of 113). Additionally, 3 reports used next-generation sequencing assays with targeted gene panels. Though the exact regions targeted were not listed in the report, they were stated to be available on a Web site or by request.

Assay targets were further characterized by how mutations were reported in the results, including V600E only, more than 1 specific mutation, any mutation within specific codons, any mutation within all of exon 15, or cannot be determined. Again, the 107 reports included 113 identifiable assays described. Of those, 109 (96%) had an identifiable target or region reported as positive or negative in the results (see Figure 2). The 41 assays reporting only V600E included those targeted to only the V600E mutation, as well as those that showed or were known to have cross-reactivity with other mutations, but still reported any positive result as a V600E mutation.

Some form of tumor enrichment was mentioned in 42 of 107 reports (39%). These included microdissection (n = 21), macrodissection (n = 15), dissection otherwise undesignated (n = 4), and use of a core taken from a paraffin block (n = 2). Thresholds for using enrichment were not clearly stated in most reports, which seems reasonable as long as specimen requirements for the test are clearly defined for those personnel who select and submit specimens for testing.
Assay Limitations

Even though the CAP Molecular Pathology Checklist specifically recommends including a statement about clinically significant variants not detected by the assay, only one-third of reports included such a statement (34%, 36 of 107). The limit of detection of the assay, or analytic sensitivity, was the most commonly reported assay limitation documented and was included in 74% (79 of 107) of reports and 83 total assays (73%, 83 of 113). The clinical importance of the limit of detection is greater when a given mutation is not detected than when it is detected. Limit of detection depended on assay technology and ranged from 0.15% to 30%, reported as mutant allele fraction (MAF, see Figure 3). Some laboratories included caveats, such as better limits of detection for the V600E mutation or "hotspots" compared to other mutations detectable by the assay. Laboratories using real-time PCR with a probe detection method and melt curve analysis reported limits of detection between 1% and 5% MAF. Laboratories using allele-specific PCR with mutation-specific primers reported between 0.15% and 5% MAF limit of detection. For pyrosequencing, limit of detection ranged from 5% to 12.5% MAF. It was noted that some laboratories performing Sanger sequencing report limit of detection below what is generally accepted for the technology (20% MAF). The range for Sanger sequencing was 10% to 30% MAF. If the limit of detection was documented on the basis of percentage tumor cells present, the number was converted to allele fraction with the assumption of a heterozygous mutation for scoring. Some of those laboratories reporting a limit of detection of less than 40% percentage tumor cells, which indicates an allele fraction below 20%, may have actually intended to report allele fraction, emphasizing the need for clarity when reporting assay performance data and assay limitations.

Additional assay limitations were described in 42% (45 of 107) of reports and included general statements that a negative result does not rule out the presence of a mutation, as well as specific factors, such as rare polymorphisms, tumor heterogeneity, sample preservation and integrity, presence of inhibitors, and technical errors.

No assay limitations were mentioned in 17% (18 of 107) of reports.

Interpretive Comments

Analytic interpretations were included in all but 1 report (106 of 107). That report gave a result of "negative" without clear mention of the mutation for which the test was negative or how they arrived at that result. Additionally, 19% (20 of 107) of reports contained a reference range. Most reports included at least a brief clinical interpretive comment (90%, 96 of 107). The comments varied depending on what cancer types were tested by the laboratory. They were largely generalized to the disease process most commonly tested by each laboratory and not the specific patient (since the specimen type and specific patient information were not provided to survey participants).

All reports were evaluated for the presence of a signature, which was present on 86% (92 of 107) of reports. We recognize that the signature may not have shown up in some reports if they were printed from reporting software and not "signed out" as would be feasible with standard clinical laboratory workflow.

Laboratory-Developed Test and Analyte-Specific Reagent Reporting

Most reports used a laboratory-developed procedure only (94% of reports, 100 of 107). The remaining 7 used the FDA-approved "Roche cobas 4800 BRAF V600 Mutation Test" alone or in combination with another assay. Of those that used an LDT only, 12% (12 of 100) failed to include any language addressing the development of the assay and the fact that it was not FDA approved. It should be mentioned that participating laboratories outside the United States are subject to different regulations and FDA guidance than are US testing laboratories, but owing to deidentification of reports, those laboratories could not be separated in this study. Most of those who did address the LDT used the mandated Class-I ASR statement or a modified version of it. While these laboratories addressed the laboratory-developed nature of their procedure, not all of them included everything required for LDT reporting as outlined in CAP checklists, as the ASR statement alone does not fulfill the CAP's LDT reporting requirement. However not all survey
Participants are CAP accredited, and some may be research laboratories or may be validating test procedures.

Reports for LDTs are required to document appropriate methods and performance characteristics. While the CAP checklist does not list specific criteria, we applied our scoring criteria and found that 26 of the 88 reports containing the LDT language (30%) did not contain all necessary components, with 10 lacking adequate methods, 15 lacking performance characteristics (defined here as presence of limit of detection, listing pertinent variants not detected, or other assay limitations as described in the “Assay Limitations” section), and 1 lacking both methods and performance characteristics. Interestingly, all laboratories performing the FDA-approved cobas BRAF V600 Mutation Assay also included LDT language. This inclusion is likely due to testing nonmelanoma specimens. We did not attempt to determine which laboratories were required to use the mandatory ASR Class-I reporting language; however, most reports (83%, 83/100) of LDTs only included it.

Overall, 15% (16 of 107) of reports included all explicitly required elements from the CAP checklists evaluated in this study (laboratory name, date specimen collected, report date, ordering physician, methodology, standard gene nomenclature, variants not detected, assay limitations, analytic interpretive comment, clinical interpretive comment, pathologist/designee signature, and LDT reporting language). The remaining components evaluated in this study were either recommended, but not required, or included ambiguity as to whether they were appropriate and/or applicable.

**DISCUSSION**

The goals of this study were to evaluate the quality and completeness of molecular reporting by laboratories participating in CAP proficiency testing. Greater than 75% of reports contained the laboratory name, specimen information including date collected, date received, date reported, and type, and the ordering physician, when analyzed separately (see Table 2), but only 46% (49 of 107) contained all 6. In some cases, it is possible that the report copy sent for evaluation was printed directly from the reporting software and does not reflect a full, “signed out” report as would appear in a patient’s medical record. In general, reporting failures are likely overestimated because proficiency surveys do not precisely mimic actual patient care owing to the artificial nature of the proficiency testing specimen. While laboratories are instructed to treat the specimen the same as a clinical specimen, the lack of information supplied likely led to laboratories not including all information in these sample reports that would normally be included in a clinical report. Despite this weakness of the current study design, it should be noted that these criteria remain important for test reporting. For example, only 77% (82 of 107) of reports contained date of specimen collection, but collection date is not provided in the instructions accompanying CAP surveys. Date of collection is important for evaluating specimen stability, turnaround time, preanalytic quality issues, and clinical status at the time of specimen acquisition, which may be days to years before testing.

While almost all reports included the test name as an easily identifiable heading or as part of the results, no reports used the full name of the BRAF gene. Use of the abbreviated BRAF symbol is appropriate as based on the CAP Molecular Pathology Checklist, and laboratories should periodically check gene name and gene symbol to ensure it matches current HUGO gene nomenclature (BRAF, B-Raf proto-oncogene, serine/threonine kinase). The authors advocate that official gene symbols should never change so as not to confuse people and to promote patient safety in clinical settings.

Figure 3. Percentage of assays reporting different ranges of limit of detection. Limit of detection was not reported for all assays, n = 83.
important in assays that query the entire exon 15 or multiple exons in the BRAF gene, where there is the increased possibility of polymorphisms, variants of undetermined significance, or actionable variants.

While real-time PCR with a probe-based detection was the single most common method used, numerous technologies are being used for BRAF testing. The description of the methods used also varied widely, from step-by-step procedures to basic statements of the broad technology used. In some cases, it was unclear from the description what method was being used. A clear description is important for the interpretation of the report by others, as well as for purposes of billing and reimbursement based on procedural method. While the methods statement should be brief, it should be sufficient for an informed reader to understand the technology used, which helps judge inherent limitations of the assay beyond those specifically outlined in the report. A clear description of the methods allows for better understanding of what loci are targeted and pertinent variants that are not detectable by the assay.

Owing to the wide variety of assays in use, the detectable BRAF mutations also varied across testing laboratories. The CAP Molecular Pathology Checklist requires inclusion of the loci or variants detectable, which was omitted or incomplete in 13% (14 of 107) of reports. All participants reported the assay could detect the nucleic acid alterations underlying the V600E mutation, but not all were able to detect other codon 600 mutations such as V600K, and fewer were able to detect other exons 15 mutations outside of codon 600. Though V600E alone was the most commonly reported mutation, multiple laboratories mentioned the possibility of cross-reactivity for other codon 600 mutations or used a technology in which this is a known possibility without mentioning it in the report. Many of these laboratories still reported their results as positive or negative for a V600E mutation. An interpretation of positive or negative for a mutation predicted to alter codon 600 may be more appropriate in this scenario. A clear statement of the loci detected is vital for clear communication of results to the health care team and to clarify the potential need for additional testing.

Though 39% (42 of 107) of reports contained a tumor-enrichment technique such as macrodissection or microdissection, it is likely that additional laboratories are not including this step as part of their report. The use of tumor-enrichment procedures could impact billing and reimbursement, and the threshold for performing enrichment, and the percentage of malignant cells required for performance of the assay (either overall or in the enriched area) are useful factors impacting specimen selection that should be included in the laboratory’s description of specimen requirements. The CAP Molecular Pathology Checklist requires that information about the tumor cell percentage from the area of the slide used for sequencing assays and the analytic sensitivity of the assay be included in the report as appropriate (MOL.35797). An assessment and record of tumor cellularity is also required for any testing on DNA extracted from paraffin-embedded tissue, though it does not explicitly have to be included in the report (MOL.32395). Hematoxylin–eosin–stained slides were not distributed as a part of the proficiency testing, so this component was not evaluated in this study.

A description of assay limitations, specifically clinically significant factors such as variants not detected, is required by the CAP Molecular Pathology Checklist and is important information for clinician interpretation. Seventeen percent of reports did not contain any assay limitations and only 34% (36 of 107) included a statement regarding variants not detected. While a clear description of methods and targeted loci may be enough for specialists to infer what variants were and were not detected, generalist physicians should also feel confident that the report is sufficient for appropriate downstream decision making based on the stated indication for testing. The limit of detection of the assay is an important limitation to include in the report, particularly for a negative BRAF mutation result, and is required for sequencing assays (MOL.25790).

Interestingly, it was noted during evaluation that some laboratories reported a limit of detection well below the generally accepted sensitivity for the technology they were using. This was particularly noticeable with Sanger sequencing, which is commonly reported to have a limit of detection of 20%, where the reported minimum detectable mutant allele fraction ranged from 10% to 30%. Close attention should be paid to the difference between mutant allele fraction and tumor percentage. With the assumption of a heterozygous BRAF mutation, an assay with a validated limit of detection of 20% mutant allele fraction could detect mutation in a specimen with at least 40% tumor cells. It is also possible that laboratories reporting limits of detection below 40% tumor cellularity were using high-sensitivity Sanger sequencing without specifying it in their methods.

Almost all laboratories provided an analytic interpretation. In the case of BRAF mutation testing, this implies a clear statement of the results was provided. The clinical interpretive comments were much more varied, though they were included to some extent in 90% (96 of 107) of reports. Most were disease-specific, while others were more general with regard to availability of targeted therapy. It should be noted that laboratories were not told what type of tumor the proficiency testing sample represented, which may have affected how the analytic and clinical interpretations were written. The extent of a clinical interpretation likely depends not only on validated uses of the assay in the testing laboratory, but also on the extent of clinicopathologic information available to the laboratorian who performs the assay. For molecular tests such as BRAF, which requires consideration of clinical history, specimen characteristics and limitations, and interpretation of raw data to determine the final result and clinical implications, an analytic and a clinical interpretation are required. Utilization of a computer-generated report would only be appropriate for an FDA-approved test performed on samples with rigid clinical indications and preanalytic procedures, with appropriate built-in quality checks and straightforward interpretation.

The types of criteria more commonly omitted by laboratories in this study, while required by CAP, are unlikely to have a direct impact on patient care. Results were clearly stated in most reports, with analytic and clinical interpretive comments included in 99% (106 of 107) and 90% (96 of 107) of reports, respectively. Though 44% (47 of 107) of reports omitted at least 1 of the CAP-required general reporting criteria (laboratory name, collection date, report date, ordering physician, and pathologist/designee signature), only 17% (18 of 107) omitted more than 1 of the criteria. These criteria are also the most likely to be affected by the artificial nature of the proficiency testing specimen and the configuration of the laboratory information system, which may be outside the control of the reporting laboratory.
The criteria most likely to affect patient care are those that affect the clinician’s interpretation of the result, which include a clear summary of the methodology, the targeted loci, the variants not detected, and other assay limitations. Of these, variants not detected was the component most commonly omitted (66% of reports, 71 of 107). Though explicitly required by the CAP checklist, the wording is in the comment for the checklist item, which may explain the common failure to include it. When considering only summary of the methodology, the targeted loci, and other assay limitations, the noncompliance rate drops to 33% (35 of 107) and only 2 of those reports were lacking more than 1 of the criteria. These data should be readily available from the laboratories’ testing validation and should be included in the final report.

Factors outside of the CAP requirements also may affect how laboratories structure their reports and what they include. A recent CAP KRAS proficiency test mailing contained a survey question to laboratories that do not include an interpretation related to therapy, which highlighted local variation in what clinicians want to see in reports. The most common reason for the omission of this type of interpretation was that it was not required/requested by the client or that it was the clinician’s responsibility. The CAP checklist does not give clear criteria for when a clinical interpretation is appropriate, so it is up to individual laboratories to determine what is appropriate for their patient population and their clinical clients. In the United States, the Clinical Laboratory Improvement Amendments of 1988 (CLIA) require that each high-complexity laboratory director must “… ensure that reports of test results include pertinent information required for interpretation; … ensure that consultation is available to the laboratory’s clients on matters relating to the quality of the test results reported and their interpretation concerning specific patient conditions” (Sec. 493.1445). Section 493.1457 states that “the clinical consultant provides consultation regarding the appropriateness of the testing ordered and interpretation of test results. The clinical consultant must— (a) be available to provide consultation to the laboratory’s clients; (b) be available to assist the laboratory’s clients in ensuring that appropriate tests are ordered to meet the clinical expectations; (c) ensure that reports of test results include pertinent information required for specific patient interpretation; and (d) ensure that consultation is available and communicated to the laboratory’s clients on matters related to the quality of the test results reported and their interpretation concerning specific patient conditions.”

Though the reporting requirements for LDTs are clearly stated in the CAP checklists, 12% (12 of 100) of reports using an LDT only did not contain a clear statement that the test was an LDT and an additional 26% (26 of 100) did not contain all of the additional required components as scored in this study, such as description of methods and performance characteristics. Most reports used the recommended Class-I ASR wording verbatim or an adaptation of it in their description of an LDT; however, the ASR wording alone does not satisfy the CAP requirement for LDT reporting.

This study was limited by a relatively small sample size (107 evaluable reports) and the fact that it evaluated only 1 molecular test. Because the reports were deidentified, laboratories performing testing outside of the United States could not be excluded from analysis. Also, because all of the reports were deidentified before data collection and analysis, it was not possible to report our data as percentage of laboratories, necessitating the use of the number of reports instead. Responder bias may have contributed to the overall high rates of reporting compliance. Though most of the criteria used in this study were taken directly from CAP inspection checklists, some were based on published recommendations of best practices. Laboratory professionals may adapt the recommendations to their own practice settings to help produce clear, concise, and complete reports.

In general, reports for BRAF mutation tests demonstrated high rates of inclusion for required and recommended elements; however, only 16 of 107 reports (15%) included all criteria explicitly required by the CAP checklists. We highlight a number of recurrent reporting concerns. While the requirements discussed here may seem extensive, all report components can be easily included with clear and concise wording. A brief, but well-defined Methods section is acceptable as long as the analytic and clinical interpretations account for the clinical implications of the result and the interpretation of the raw data, including assay limitations. Accredited laboratories should adhere to all applicable CAP checklist requirements and strongly consider published recommendations for reporting.

As technology continues to evolve and technologies such as next-generation sequencing of DNA or RNA become more widespread, potentially detecting translocations and expression profiles that complement or extend beyond the single nucleotide variants emphasized in this study, a description of the methods underlying analytic and clinical interpretation will continue to be critical for conveying pertinent positive and negative findings in light of indications for testing and specimen characteristics.

References