

CORRESPONDENCE

The importance of neoplastic cell content assessment and enrichment by macrodissection in cancer pharmacogenetic testing

Formalin-fixed paraffin embedded samples for actionable somatic pathogenic variant (SPV) testing should undergo review in order to ensure that the appropriate material has been submitted and the neoplastic cell content (NCC) is sufficient for SPV detection. In samples with suboptimal NCC, light microscopy identification of areas of higher NCC and the provision of a marked H&E slide for precise macrodissection increase the likelihood of detecting an actionable SPV.

While this should reflect common practice, despite requesting this information, our experience is that a significant number of referrals arrive at the laboratory with no indication of NCC and/or are unmarked for macrodissection, necessary on samples with an NCC of less than 10%. This increases the risk of a false-negative result.

Using malignant melanoma as a representative for actionable SPV testing, we audited 231 samples obtained by the laboratory between August and October 2018. Eight per cent (n=19) of the samples were obtained with no estimate of NCC and 7% (n=17) of the samples stated to have <10%NCC were not marked for macrodissection.

Three exemplar cases of malignant melanoma referred for clinical testing of actionable *BRAF* SPVs are presented

to underline the importance of careful review and identification of neoplastic cell-enriched areas in suboptimal samples.

BRAF testing in malignant melanoma samples is routinely performed using a pyrosequencing assay in this laboratory, which is validated to detect *BRAF* codon 600 variants down to a level of 10% variant allele fraction (VAF) reflecting at least 20% NCC.

Additionally, the Roche cobas 4800 *BRAF* p.V600 (cobas) assay is used for samples with less than 20% NCC. This kit is validated to detect the *BRAF* c.1799T>Ap.V600E variant down to 5% VAF.

CASE 1

A wax block from a metastatic melanoma was obtained from a 67-year-old man for *BRAF* codon 600 testing. No NCC estimate was provided. DNA extracted from unstained whole sections was tested using pyrosequencing and the cobas assay according to locally validated protocols.

Pyrosequencing was suggestive of the *BRAF* c.1799T>Ap.V600E mutation at an approximate VAF of 2% (figure 1A), below the validated detection level for this test. The variant was not detected by the cobas assay.

An in-house review of an H&E stained section identified an area suitable for macrodissection of enriched NCC up to 20%–30%. Additional DNA was then extracted from the selected areas.

Subsequently, pyrosequencing detected *BRAF* c.1799T>Ap.V600E at a reportable VAF of 14% (figure 1B); a codon 600 variant was also detected by the cobas assay in this sample.

CASE 2

Four unstained slide-mounted sections from a pigmented lesion were obtained from a 45 year old man. The overall NCC was stated to be 10%–20%. No H&E slide was obtained.

Pyrosequencing was suggestive of the *BRAF* c.1799T>Ap.V600E mutation at an approximate VAF of 2%, below the validated detection level for this test. No codon 600 variants were detected by the cobas assay.

In response to this finding, a marked H&E slide and further unstained sections were requested and obtained from the referring pathology laboratory. Although the NCC in the marked area was not stated, enrichment by macrodissection resulted in pyrosequencing detecting *BRAF* c.1799T>Ap.V600E at a reportable VAF of 9%, and a codon 600 variant was detected by the cobas assay.

CASE 3

Slide-mounted paraffin sections from a metastatic melanoma were obtained from a 59-year-old man for *BRAF* codon 600 testing. The request form indicated an NCC of 10%–20%, presumed to be over the whole section. Pyrosequencing and the cobas assay were performed on DNA extracted from the unstained whole sections.

Pyrosequencing was suggestive of the *BRAF* c.1798_1799delinsAA p.V600K mutation at an approximate VAF of 3%, but a codon 600 variant was not detected by the cobas assay.

Assessment of an H&E slide identified two small areas of neoplasia; these were highlighted for macrodissection (figure 2), yielding an NCC greater than 30%. Pyrosequencing then detected *BRAF* p.V600K at a reportable allele fraction of 5%; a codon 600 variant was also detected by the cobas assay on the macrodissected DNA sample.

Experienced scientists can spot visual cues on a pyrogram that may reflect the presence of a low-level variant in a sample. Steps can then be taken to check and enrich NCC (if possible) and thus confirm the result. Molecular testing for actionable SPVs, however, is progressing to high-throughput next-generation sequencing (NGS)-based techniques, which do not always provide these visual cues. This is already the case in colorectal cancer, which in our laboratory was formerly tested by pyrosequencing but is now tested by NGS. The importance of correct NCC assessment and identification of areas for macrodissection thus becomes even

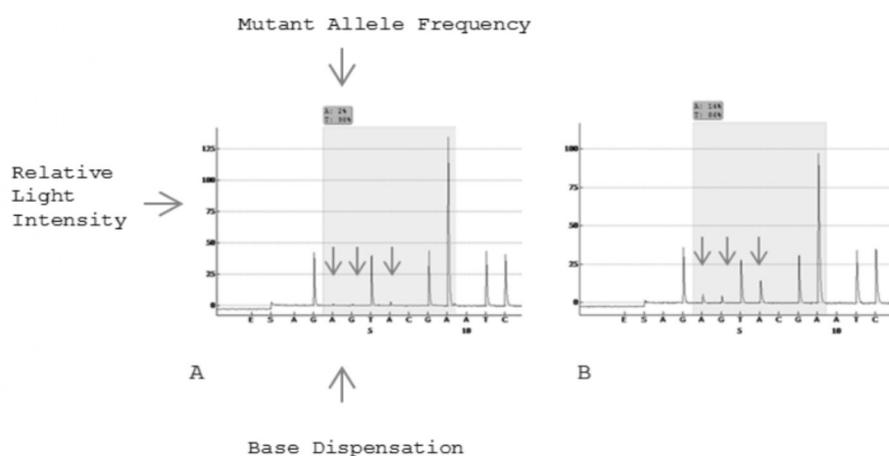


Figure 1 Case 1 pyrosequencing result. Premacrodissection (A) and postmacrodissection (B) pyrosequencing extension products from the *BRAF* c.1799T>Ap.V600E variant are shown (arrows).

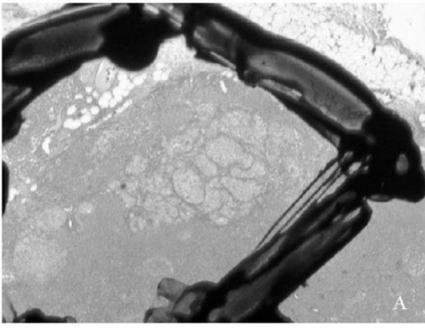


Figure 2 Case 3, metastatic malignant melanoma marked for macrodissection (×2).

more important when ‘visual’ techniques become obsolete.

Microscopic identification of areas of higher NCC and the provision of a marked H&E slide for precise macrodissection increase the likelihood of detecting an actionable SPV. Overestimation of NCC can result from inclusion of desmoplastic stroma, in addition to the true neoplastic cell population. Additionally, a prominent inflammatory cell population can significantly dilute the level of variant

DNA available and should be taken into account when estimating the percentage of neoplasia in an formalin-fixed paraffin-embedded tissue specimen.

As the outcome of genomic testing increasingly influences clinical management and treatment of cancer patients,¹ it is of high importance that the adequacy of all samples for genomic testing is assessed by appropriately experienced and competent professionals. Competency assessment for this important function is already available from external quality assessment providers, for example, GenQA (www.genqa.org).

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Handling editor Professor Runjan Chetty.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

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GJB, AMQ and PS contributed equally.



To cite Burghel GJ, Quinn AM, Smith P, *et al.* *J Clin Pathol* 2019;**72**:721–722.

Received 12 July 2019

Accepted 24 July 2019

Published Online First 2 August 2019

J Clin Pathol 2019;**72**:721–722.

doi:10.1136/jclinpath-2019-206094

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