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A Guide to North West GLH Cancer Reports

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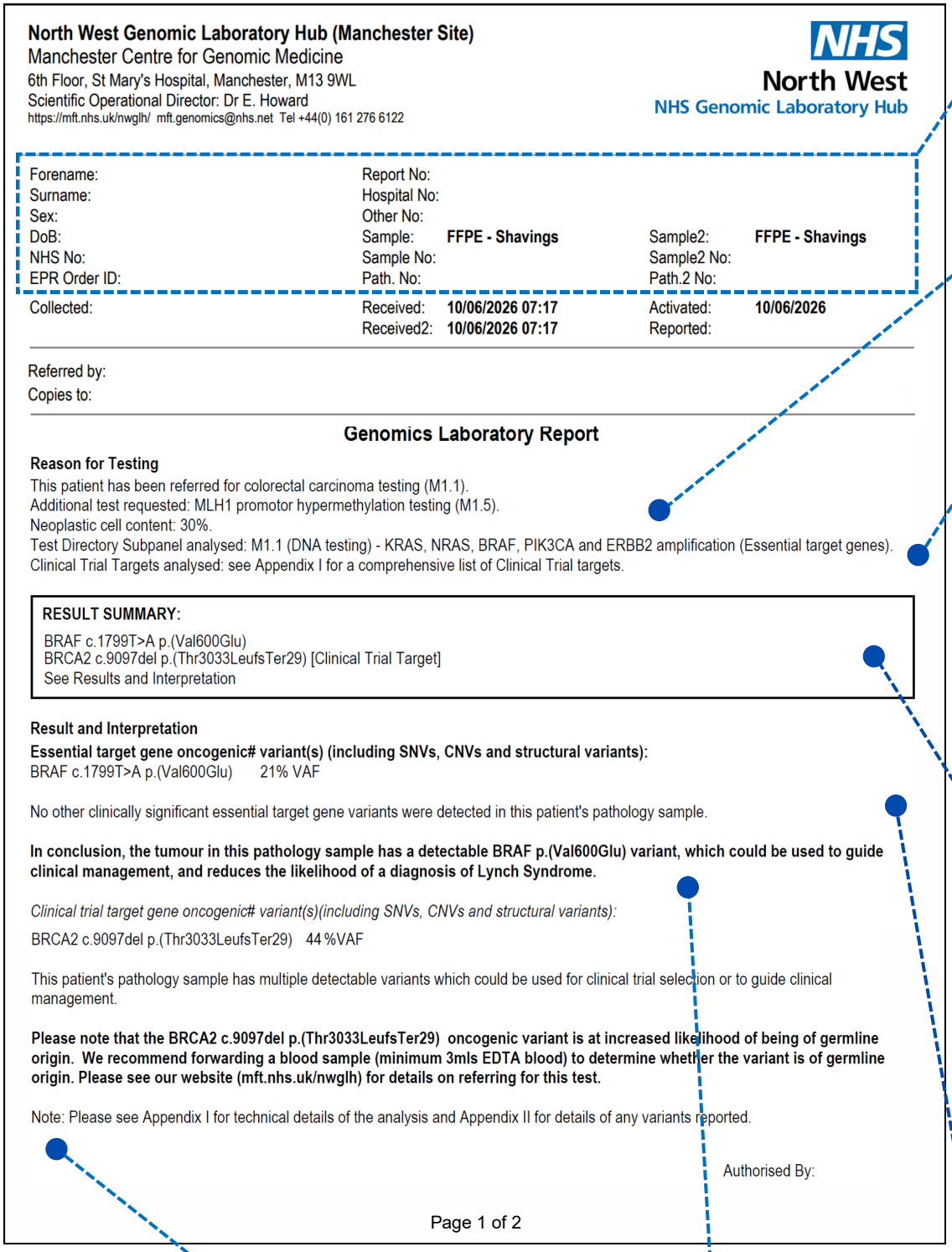

North West Genomic Laboratory Hub (Manchester Site)
Manchester Centre for Genomic Medicine
6th Floor, St Mary's Hospital, Manchester, M13 9WL
Scientific Operational Director: Dr E. Howard
https://mft.nhs.uk/nwglh/ mft.genomics@nhs.net Tel +44(0) 161 276 6122

NHS North West NHS Genomic Laboratory Hub

Forename:	Report No:			
Surname:	Hospital No:			
Sex:	Other No:			
DoB:	Sample:	**FFPE - Shavings**	Sample2:	**FFPE - Shavings**
NHS No:	Sample No:		Sample2 No:	
EPR Order ID:	Path. No:		Path.2 No:	
Collected:	Received:	**10/06/2026 07:17**	Activated:	**10/06/2026**
	Received2:	**10/06/2026 07:17**	Reported:	

Referred by:
Copies to:

Genomics Laboratory Report

Reason for Testing
This patient has been referred for colorectal carcinoma testing (M1.1).
Additional test requested: MLH1 promotor hypermethylation testing (M1.5).
Neoplastic cell content: 30%.
Test Directory Subpanel analysed: M1.1 (DNA testing) - KRAS, NRAS, BRAF, PIK3CA and ERBB2 amplification (Essential target genes).
Clinical Trial Targets analysed: see Appendix I for a comprehensive list of Clinical Trial targets.

RESULT SUMMARY:
BRAF c.1799T>A p.(Val600Glu)
BRCA2 c.9097del p.(Thr3033LeufsTer29) [Clinical Trial Target]
See Results and Interpretation

Result and Interpretation
Essential target gene oncogenic# variant(s) (including SNVs, CNVs and structural variants):
BRAF c.1799T>A p.(Val600Glu) 21% VAF

No other clinically significant essential target gene variants were detected in this patient's pathology sample.

In conclusion, the tumour in this pathology sample has a detectable BRAF p.(Val600Glu) variant, which could be used to guide clinical management, and reduces the likelihood of a diagnosis of Lynch Syndrome.

Clinical trial target gene oncogenic# variant(s)(including SNVs, CNVs and structural variants):
BRCA2 c.9097del p.(Thr3033LeufsTer29) 44 %VAF

This patient's pathology sample has multiple detectable variants which could be used for clinical trial selection or to guide clinical management.

Please note that the BRCA2 c.9097del p.(Thr3033LeufsTer29) oncogenic variant is at increased likelihood of being of germline origin. We recommend forwarding a blood sample (minimum 3mls EDTA blood) to determine whether the variant is of germline origin. Please see our website (mft.nhs.uk/nwglh) for details on referring for this test.

Note: Please see Appendix I for technical details of the analysis and Appendix II for details of any variants reported.

Authorised By:

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Patient demographics: Includes patient identifiers and the pathology reference number of the sample tested

Test Directory subpanel: Lists the test(s) performed and the genes analysed according to the Test Directory.

Clinical Trial Targets: Additional genomic targets are now analysed to support emerging treatments and clinical trials.

Results Summary: An overview of the key findings. If further details need considering, the note "See Result and Interpretation" should prompt a thorough review of the report to ensure appropriate interpretation. Variants detected in Clinical Trial Target genes will be annotated with [Clinical Trial Target].

Result and Interpretation: Includes details of pertinent variant(s) identified within the relevant test directory panel and any clinical trial targets. Supporting information on the variant and the clinical significance in the context of the referral will also be summarised. This may include recommendations regarding putative germline findings.

Notes: Directs the reader to the appendices (see page 2), where information on reference sequences used and any regions not successfully analysed is provided. Relevant references and resources are cited.

Conclusion: Summarises identified variants, their clinical implications, and potential treatment options, with links to relevant resources (where applicable)

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Forename:
Surname:

Report No:
DoB:

APPENDIX I - Test Methodology:

DNA extraction: *QiaSymphony extraction*
Test Methodology: *Single-primed PCR enrichment using a QIAseq Targeted DNA custom panel with Unique Molecular Identifiers (UMIs) and Illumina Next Generation Sequencing on NovaSeq6000*
Test directory standard of care genes targeted: *This enrichment targets 212 commonly mutated genes (contact laboratory for further details). Please note that we only analysed KRAS, NRAS, BRAF and PIK3CA and significant amplifications in ERBB2 for this referral. Re-analysis of other genes on the panel is available on request.*
Reference sequences: *NM_004985.5 (KRAS), NM_002524.5 (NRAS), NM_004333.6 (BRAF), NM_006218.4 (PIK3CA), NM_004448.4 (ERBB2).*
Clinically relevant regions (hotspots): *The clinically relevant regions are: KRAS codons 12, 13, 59-61, 117 & 146, NRAS codons 12, 13, 59-61, 117 & 146, BRAF codons 599-601.*
Clinical Trials Targets: *Please note we have analysed additional genomic targets to support emerging treatments and clinical trials. SNVs: NM_004333.6 (BRAF), NM_007294.3 (BRCA1), NM_000059.3 (BRCA2), NM_016507.4 (CDK12), NM_005228.5 (EGFR), NM_004448.4 (ERBB2), NM_001174067.1/NM_023110.2/NM_001174064.2 (FGFR1), NM_001144915.1/NM_000141.4/NM_001144913.1 (FGFR2), NM_001163213.1/NM_000142.4 (FGFR3), NM_005896.3 (IDH1), NM_002168.3 (IDH2), NM_004985.5 (KRAS), NM_001127500.3 (MET exon14 skipping), NM_000249.3 (MLH1), NM_000251.2 (MSH2), NM_000179.2 (MSH6), NM_000267.3 (NF1), NM_000268.3/NM_016418.5 (NF2), NM_002524.5 (NRAS), NM_024675.3 (PALB2), NM_006218.4 (PIK3CA), NM_058216.3 (RAD51C), NM_001142571.2 (RAD51D), NM_000546.5 (TP53), NM_020975.6 (RET). CNVs: Amplification ERBB2, FGFR1, FGFR2, FGFR3 and MET; Loss BRCA1, BRCA2, CDK12, MLH1, MSH2, MSH6, NF1, NF2, PALB2, RAD51C, RAD51D, TP53 and CDKN2A.*
Please note coverage of the following clinical trial targets was suboptimal and therefore potentially targetable variants may not have been detected: MSH2 exon 5.
If further discussion is required about a variant then consider referral to the Regional Genomic Tumour Advisory Board (GTAB) email: mdt-coordinators.nwglh@mft.nhs.uk. Potential clinical trials may be found on the Experimental Cancer Medicine Centre trial finder (https://www.ecmcnetwork.org.uk/ec-trial-finder) or the Cancer Research UK clinical trials finder (https://www.cancerresearchuk.org/about-cancer/find-a-clinical-trial).
Bioinformatics and quality control: *Variant calling against hg38 human genome by custom bioinformatic analysis pipeline validated to detect SNVs, small insertion/deletion variants (<40bp), CNVs and LOH using Qiagen CLC software. SNVs are called in the coding regions of the genes and the flanking 5bp. SNVs are called down to 4% variant allele frequency (VAF) for the entire region of interest and to 2% VAF at known clinically relevant regions (hotspots). CNV fold change for gains and losses is calculated by a custom bioinformatics analysis which divides observed coverage by expected coverage. A fold change of 1x is classed as normal. Fold change thresholds for classifying CNV regions as amplifications and deletions are 2.8 and -1.25, respectively. Samples with a signal-adjusted noise metric of >1 are sub-optimal for CNV analysis. Copy number estimation is performed based on the neoplastic cell content provided. Where a range of neoplastic cell content has been provided, the higher value is used. Where no neoplastic cell content has been provided, copy number calculation is based on the assumption there is 100% neoplastic cell content.*
Coverage: *SNVs/INDELs: The limit of detection for this assay varies as a product of multiple factors including sequencing depth. The assay aims to achieve a minimum UMI depth of 138x which statistically gives a >99.5% cumulative probability of detecting a variant at >10% VAF. A minimum UMI depth of at least 60x was achieved for any clinically relevant codons in oncogenes (hotspots), which statistically gives a 98.6% cumulative probability of detecting a variant at >10% VAF. A minimum UMI depth of at least 60x was achieved across this panel including any clinically relevant codons.*
Assay Sensitivity: *SNV/Indels - Validation of this test has determined a sensitivity of 98.82% (95% CI: 97.27% - 99.62%) to detect variants to a 4% VAF and 99.50% (95% CI: 98.21% to 99.94%) to a 10% VAF. CNVs - the sensitivity and specificity compared to whole genome sequencing on diploid samples for detection of amplifications are 100% (95% CI: 54.1-100) and 100% (95% CI: 97.9-100), and for detection of deletions are 95.1% (95% CI: 87.8-98.6) and 99.7% (95% CI: 98.6-100), however test accuracy may be reduced for non-diploid samples. Sensitivity and specificity for detection of LOH compared to fragment PCR are 100% (95% CI: 87.2-100) and 100% (95% CI: 82.4-100).*

Limitations: *SNVs/Indels - A neoplastic cell content of 20% or greater is required for optimum somatic variant detection. Variant detection can be affected by regions of homology elsewhere in the genome, allele drop out due to a co-occurring variant under a primer binding site or the variants being within or adjacent to a homopolymer tract. CNVs - The test does not detect haploidy or polyploidy when present, which may impact copy number calculations. Data from experiments with DNA mixtures shows that the limit of detection for amplifications is 12 additional copies at 30% NCC, excluding the MYC gene; for deletions the limit of detection for biallelic losses is 40% NCC, and 60% NCC for monoallelic losses. However there can be variation between genes and the true limit of detection is impacted by the exact NCC of the sample, the number of gene copies in the tumour, the coverage across the locus of interest, the quantity of DNA available, the size and sequence content of the gene, intra-genic CNVs, the ploidy of the sample, the uncertainty of measurement, and the presence of intra-tumoral heterogeneity in gene copy number. The sensitivity metrics quoted above are for CNVs impacting the whole gene region; CNVs impacting only part of the gene region may not be detected. Other variants may have been identified that are not considered clinically relevant and/or are low confidence e.g. passenger variants in regions of low sequence coverage or variants in problematic sequence contexts (contact laboratory for details).*
Confirmation of findings: *Variants where identified are not routinely confirmed by other methods.*
Regulation and accreditations: *This test is not UKAS accredited. The test has been validated within the laboratory.*
Variant Nomenclature: *#Oncogenic includes oncogenic and likely oncogenic variants. Variant classification has been performed as per the relevant guidance (see below references). Only clinically relevant results are shown; full details of methods and results, including benign/likely benign variants and variants of uncertain clinical significance, are stored on file and are available on request. Variant nomenclature follows HGVS guidelines (https://varnomen.hgvs.org/) with nucleotide 1 counted as the first nucleotide of translation initiation codon using Genbank accession numbers listed above and therefore nomenclature may differ from that given in the quoted literature.*
References:
1) https://www.england.nhs.uk/cancer/cdf/cancer-drugs-fund-list/
2) Kuzbari, Z., C. Bandlamudi, C. Loveday, et al. (2023). Ann Oncol. 34(3): 215-227 (PMID: 36529447)
3) McVeigh, T. P., Hanson, H., et al. (2025). J Med Genet. 62: 709–719 (PMID: 40750343)
4) Garrett et al, (2020). J Med Genet. 57(12): 829-834 (PMID: 32170000)
5) https://www.cangene-canvaruk.org/canvig-uk-guidance
6) Burghel, G. J., Mason, J., et al. (2025). J Med Genet. 63(3): 174-156 (PMID: 41339070)
7) Newton et al. (2014). Tumour MLH1 promoter region methylation testing is an effective pre-screen for Lynch Syndrome (HNPCC). J Med Genet. 51(12):789-96.
8) https://www.nice.org.uk/guidance/dg27.

APPENDIX II - Variant Details and Classification Evidence:

HGVS description: *BRAF c.1799T>A p.(Val600Glu)*	***Classification:*** *Oncogenic*
Genomic coordinates: *null*	***Variant allele frequency (%):*** *21*

HGVS description: *BRCA2 c.9097del p.(Thr3033LeufsTer29)*	***Classification:*** *Oncogenic*
Genomic coordinates: *Chr13(GRCh38):g.32379893del*	***Variant allele frequency (%):*** 44

Genes targeted:
Lists the genes analysed with their associated reference sequences, along with any hotspot regions included.

Clinical trial targets:
Provides details of additional clinical trial targets analysed as standard with the referral.

Coverage:
Sequencing coverage is a key quality metric that indicates how many times each base within a targeted region has been sequenced. This section outlines the coverage requirements for adequacy and lists any failed regions or hotspots.

References:
A list of references are provided, including the Cancer Drug Fund, any guidelines or recommendations utilised, and any references relating to the specific findings identified, where appropriate.

Appendix II:
Gives the final description of variant nomenclature, classification, genomic coordinates (if applicable), and variant allele frequency.

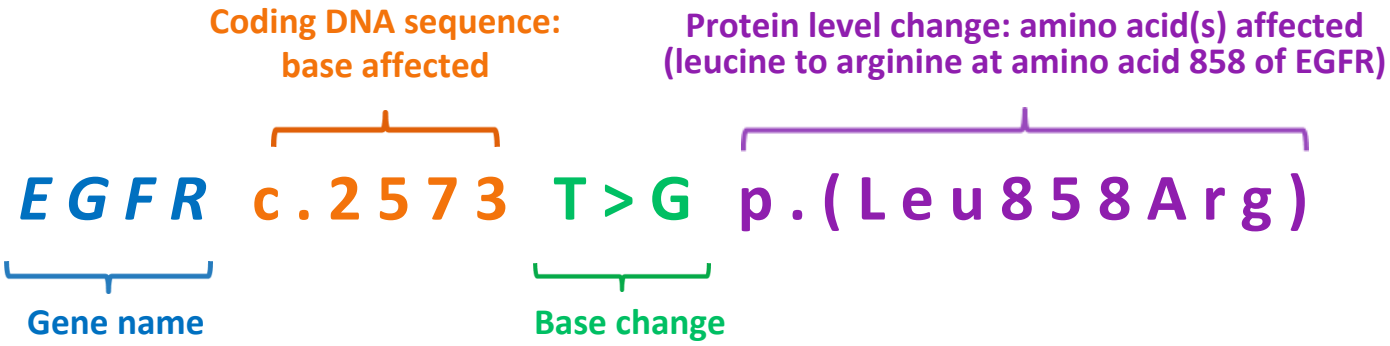
Key terms and statements

Neoplastic cell content (NCC)	*This is the proportion of neoplastic/tumour cells in the sample submitted, as a percentage of total nucleated cells. This value is used for the analysis and interpretation of the results.*
Variant allele frequency (VAF)	*This is the proportion of sequencing reads that carry the variant, as a percentage of the total number of reads that have been sequenced for that exact position.*
Low NCC / No NCC caveats	*For most tests, **NCC above 20%** is required for accurate and informative interpretation of the results. If the sample submitted falls below this level or if no NCC is provided, a comment will be added which highlights the risk of false negative results and to recommend that another sample (with a higher NCC) should be sent if possible and clinically indicated.*
See results and interpretation	*If any important further details need considering, the note "See Results and Interpretation" will appear in the **Results Summary** box as a prompt to carefully review the full report, e.g. if there is a risk of a false negative result, or potential germline implications.*
Partial fails	*It is possible for a sample to have failed a number of regions and/or hotspots even in the context of a pathogenic variant being detected. This will be communicated in the report and detailed in **Appendix I** in case additional testing is indicated to cover the failed genes.*
Variant classification	*This will be detailed in the body of the report and in **Appendix II**. If there are any relevant caveats or difficulties in variant analysis then this will be highlighted in the 'Results and Interpretation' section, such as specific elements which made interpretation difficult, or for any variants which have been regarded as reportable variants of uncertain significance.*
Minority clone variants	*A variant detected at a significantly lower allele frequency than is predicted based on the NCC estimate may indicate that it is present in a minority clone. In such cases, predicting treatment response is more difficult, therefore clinical judgement should be applied.*
Germline risks and referral recommendation	*If there are any germline implications to consider, recommendations for further testing (including sample requirements) will be highlighted in 'Results and Interpretation', in bold. The comment 'See Results and Interpretation' will appear in the Results Summary box to flag to the clinical team there is more information to impart other than just the variant identified.*
Low-level gene amplification	*If low-level gene amplification (approx. 5 to 9 copies per cell) is detected, the following comment will be included: 'This level of amplification is considered borderline and should be correlated with any IHC findings and NCC assessment. The clinical significance of this finding is uncertain and should be treated with caution. If repeat testing is required, please forward additional pathology material with higher neoplastic cell content if available.'*
Bold text	*Any information of particular importance. such as details relating to a variant's actionability, or any potential germline testing implications, will **appear in bold** on the report.*
Coverage information	*This indicates the overall quality of the sample. Any key hotspots or regions which fall below the reportable threshold will be indicated to allow the clinical team to make decisions regarding further testing, if appropriate. Failed hotspots will be mentioned in 'Results and Interpretation'. Other failed regions will be recorded in the coverage section of **Appendix I**.*

Variant nomenclature

The NWGLH follows the internationally recognised Human Genome Variation Society (HGVS) standard nomenclature for variant descriptions.

The example below highlight the different parts of a full variant descriptor as per HGVS standards. This full description provides a unique identifier for variants to avoid misinterpretation / miscalling. For more information, please see the HGVS Recommendations for the Description of Sequence Variant or the HGVS nomenclature website.



Please note that in the **protein level** description, we apply the **3 -letter amino acid abbreviations**. However, some clinical guidance, databases or alternative reports may refer to the variant with a **single-letter amino acid abbreviation**.

The above variant, *EGFR c.2573T>G p.(Leu858Arg)*, is also commonly referred to as *L858R* where L is equivalent to Leu (leucine) and R is equivalent to Arg (arginine). This can cause confusion, particular for amino acids where the single-letter and three-letter abbreviations are less intuitive. Other common examples include:

- *KRAS c.35G>A p.(Gly12Asp) = KRAS G12D*
- *KRAS c.34G>T p.(Gly12Cys) = KRAS G12C*
- *BRAF c.1799T>A p.(Val600Glu) = BRAF V600E*
- *EGFR c.2369C>T p.(Thr790Met) = EGFR T790M*

The table (shown right) below provides a complete list of amino acids, including their three-letter and single-letter codes, to support interpretation of full HGVS descriptions.

Amino acid	3-letter	1-letter
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V