Thyroid Cancers - Next Generation Sequencing and Pyrosequencing

Overview

Thyroid cancer (TC) is a rare cancer affecting the thyroid gland, situated at the base of the neck. There are around 135 new diagnosis of thyroid and endocrine cancers every year in Wales. Thyroid cancer is the most common type of endocrine cancer.

Differentiated thyroid carcinomas (DTC) (papillary and follicular thyroid carcinomas, including hurtle cell carcinoma) are the most common types, comprising 90% of diagnoses. Other thyroid carcinomas include medullary carcinoma, poorly differentiated thyroid carcinoma and anaplastic carcinoma.

Table 1: Types of Thyroid Cancer and their Prevalence

Type of thyroid carcinoma	Prevalence %
Papillary Thyroid Carcinoma (PTC)	80-85
Follicular Thyroid Carcinoma (FTC)	5-10
Thyroid Medullary Carcinoma	5-9
Anaplastic Thyroid Carcinoma (ATC)	1-10

Test Information

Next Generation Sequencing

The All Wales Genomics Laboratory utilises the Illumina TruSight Oncology 500 (TSO500) High Throughput DNA/RNA assay for next generation sequencing using the Illumina NovaSeq 6000TM to identify nucleotide variants and gene rearrangements (fusions) in patients with solid tumours, including TC. More information on this service is available on the AWMGS website:
https://medicalgenomicswales.co.uk/index.php/download-services

Note: If <40ng of DNA is obtained from the FFPE sample provided, an alternative NGS panel will be utilised for testing. This provides hotspot analysis of the majority of the same clinically relevant genes to the tumour type, but overall is a less comprehensive gene analysis.

This service aims to offer a 14-calendar day turnaround time.

Table 2: Thyroid Cancer DNA NGS Gene Panel

Gene	Hotspots/Screen	Regions covered
BRAF	Hotspots	Exon 15 (covers p.599, p.600 and p.601 which account for ~98% of known BRAF variants)
KRAS	Hotspots	Exons 2, 3 and 4 (covers p.12, p.13, p.59, p.61, p.117, p.146)
NRAS	Hotspots	Exons 2, 3 and 4 (covers p.12, p.13, p.59, p.61, p.146)
TP53	Screen	Whole gene sequence
RET	Screen	Whole gene sequence
HRAS	Hotspot	Exons 2 and 3

Table 3: Thyroid Cancer RNA NGS Gene Panel

Gene	Region covered
RET	Whole gene
EGFR	Exon 1-8 EGFR vIII structural variant
NTRK1 NTRK2 NTRK3	Whole Gene

NTRK1 (OMIM 191315), **NTRK2** (OMIM 600456), and **NTRK3** (OMIM 191316) genes fusion testing is also available by RNA NGS.

Next Generation Sequencing Interpretation

For thyroid cancer samples, the AWMGL report is non-interpretive and provides only genetic variant information with no diagnostic, prognostic or treatment implications highlighted.

This test is performed to evaluate somatic variants within tumour samples and is not designed to assess for germline variants within the targeted genes.

DNA assay sensitivity/specificity may be reduced in specimens containing <10% tumour nuclei.
RNA assay sensitivity/specificity may be reduced in specimens containing <30% tumour nuclei.

RET (OMIM 164761) variants have been found in 10-20% of people with papillary thyroid cancer [(1)], and are shown to exhibit a more aggressive phenotype when compared to other gene variant PTC's [(2)]. NICE guidelines for treatment of advanced RET fusion-positive thyroid cancer and advanced RET mutation positive medullary thyroid cancer (MTC) are currently in development. This is a changing landscape however, with guidance expected to be published in the near future as studies are ongoing.

NTRK fusions have been detected in 2.28-6.7% of thyroid cancers [(3,4)].

Variants in the BRAF gene (OMIM 164757) occur in about 40-45% of papillary thyroid cancer cases and such variants are linked to poorer prognosis [(5)].

URGENT Pyrosequencing

FFPE

Please note that clinically urgent thyroid samples for patients diagnosed with **anaplastic thyroid carcinoma (ATC)** can be referred to the laboratory for rapid BRAF testing by pyrosequencing upon request. This test will be performed specifically to detect the variant V600E but pyrosequencing will generally cover the hotspot region at codons p.599, p.600, and p.601. The turnaround time is 7 calendar days and this is to guide treatment specifically for BRAF-targeted therapies [(6,7)].

Specimen Requirements

For information on sending FFPE samples, refer to the CYSGODI service information sheet.

Please use the FFPE solid tumour request form and complete all fields.

Links for Further Information

Orphanet: http://www.orpha.net/
OMIM: http://www.omim.org/
Genetic Test Registry: http://www.ncbi.nlm.nih.gov/gtr
Cancer Research UK: http://www.cancerresearchuk.org/

All Wales Genetics Laboratory (AWGL)
Phone: 029 207 42641; Fax: 029 207 44043
Email: lab.genetics@wales.nhs.uk
Website: http://www.medicalgenomicswales.co.uk

References

(1) Romei, C., & Elisei, R. (2012). RET/PTC translocations and clinico-pathological features in human papillary thyroid carcinoma. *Frontiers in endocrinology*, *3*, 54.

(2) Ullmann, T. M., Thiesmeyer, J. W., Lee, Y. J., Beg, S., Mosquera, J. M., Elemento, O., ... & Houvras, Y. (2022). RET Fusion-Positive Papillary Thyroid Cancers are Associated with a More Aggressive Phenotype. *Annals of surgical oncology*, 1-8.

(3) Pekova, B., Sykorova, V., Mastnikova, K., Vaclavikova, E., Moravcova, J., Vlcek, P., ... & Bendlova, B. (2021). NTRK fusion genes in thyroid carcinomas: clinicopathological characteristics and their impacts on prognosis. *Cancers*, *13*(8), 1932.

(4) Solomon, J. P., Linkov, I., Rosado, A., Mullaney, K., Rosen, E. Y., Frosina, D., ... & Hechtman, J. F. (2020). NTRK fusion detection across multiple assays and 33,997 cases: diagnostic implications and pitfalls. *Modern pathology*, *33*(1), 38-46.

(5) Liu, C., Chen, T., & Liu, Z. (2016). Associations between BRAFV600E and prognostic factors and poor outcomes in papillary thyroid carcinoma: a meta-analysis. *World journal of surgical oncology*, *14*(1), 1-12.

(6) Subbiah. V., Kreitman. R. J., Wainberg, Z. A., Cho J. Y., Schellens J. H. M., Soria, J. C., … & Keam, B. (2018). Dabrafenib and Trametinib Treatment in Patients With Locally Advanced or Metastatic BRAF V600-Mutant Anaplastic Thyroid Cancer. *J Clin Oncol*, 1;36(1), 7-13.

(7) Lim AM, Taylor GR, Fellowes A, Cameron L, Lee B, Hicks RJ, McArthur GA, Angel C, Solomon B, Rischin D. BRAF Inhibition in BRAFV600E-Positive Anaplastic Thyroid Carcinoma. J Natl Compr Canc Netw. 2016 Mar;14(3):249-54.

Consent for genetic testing and DNA storage us assumed when a test request and samples are received.

Please note, that the NGS DNA and RNA panels are not accredited by UKAS.