Mutations are fundamental to thyroid tumorigenesis and the biological consequences of each specific mutation varies across a spectrum from strong, highly predictive of cancer (BRAF_V600E, RET_T100V, PAX8/PPARGaf), to low drivers being present in both benign and malignant states (RAI, RET_M918T). In addition to strong mutational drivers, multiple microRNA pathways also regulate the development of thyroid tumorigenesis (1, 2).

Molecular diagnostics can provide key insights into the nature of thyroid nodules that can guide patient care and management (3). Currently, the two approaches used for diagnosing thyroid nodule progression to cancer are rule-in and rule-out tests. These tests are either based on the detection of oncogenic variants or altered miRNA and microRNA expression profiles (4, 5).

Previously, we reported a combined test which incorporates the advantages of “rule-in and rule-out” tests. In the combined test, specimens are initially tested using Next Gen Sequencing (NGS) based ThyGenX test to detect single nucleotide variants (SNVs) in 5 genes and 6 fusions transcripts (1-5).

Here we report the clinical validation of an expanded panel of mutations and RNA fusions, known as the ThyGenX test designed to work in concert with a complementary microRNA (ThyraMIR test) profiling assay. These tests when performed together enable a greater understanding of the role of low driver mutational states without diminishing rule in and rule out diagnostic and predictive testing properties. In addition, we also present a novel risk classification and patient care management algorithm using insights derived from testing >10000 clinical thyroid nodule aspirates.

METHODS

Study design: Seventy three cases of thyroid fine need aspirate biopsies with known histopathology diagnosis were retrospectively analysed in this study. Molecular testing of residual total nucleic acids containing gDNA, mRNA and miRNA was carried out by ThyGenX and ThyraMIR assays (Table 1) under CAP/CLIA guidelines as described previously.

Clinical validation: Clinical validation was performed by blinded review of molecular results from the combined ThyGenX + ThyraMIR testing of 73 needle aspirates from nodules with known surgical pathology outcome. Two methods were used to compare diagnostic performance, 2 x 2 contingency table and a tiered classification system were used to compare the positive and negative test results with the gold standard surgical histopathology for these cases with known benign or malignant outcomes.

REFERENCES