BACKGROUND AND PURPOSE
Germline mutations that increase the susceptibility of an individual to certain cancers have been identified in numerous genes, and individuals can be tested for mutations in these genes to understand their inherited lifetime risk for developing cancer.1,2 Traditional approaches, using Sanger sequencing or qPCR, focus on small groups of genes and therefore present difficulty in screening for mutations in numerous genes from multiple individuals synchronically. As the landscape of genes that are important to hereditary cancer risk grows, it is becoming increasingly important to develop methods to interrogate multiple genes that may carry mutations. With rapid advances in next-generation sequencing (NGS) technology and bioinformatics, testing of multiple genes from multiple individuals synchronically with panel-based tests is becoming increasingly common.

The CalMax DNA Genetic Cancer Risk Test was designed to allow the analysis of genes and genetic variants associated with predisposition to cancer. The test targets germline variants detectable in white blood cell DNA. The 98 gene panel was selected based on systematic, expert review of the scientific literature to identify genes that predispose to cancer, and focuses on genes for which there is already strong evidence of such predisposition. The focus of this study was to evaluate the analytical and clinical performance of the CalMax DNA Genetic Cancer Risk Test, and to observe the prevalence of highly-penetrant, rare, pathogenic germline variants strongly linked to predisposition of cancers in patient populations from Taiwan and USA.

METHODS AND STUDY DESIGN
The CalMax DNA Genetic Cancer Risk Test covers all coding exons, nearly flanking regions (±1-20bp), and intrinsic splice sites in the 98 gene panel. The test detects single nucleotide substitutions, insertions and deletions in genomic DNA with a mean coverage of ≥10X for each sample.

As part of this study, we validated the 98 gene panel prior to sequencing the patient samples from the study cohorts. The assay validation design completely took advantage of the availability of gold standard reference samples. The lab performed end-to-end sample processing and data analysis with 19 unique gold standard reference samples run over 38 different library preparations throughout the analytical validation. After programmatically calling the data, the product is compared to the high-quality reference calls to assess the assay’s analytical performance. A concordance study to compare genotyping performance between blood and saliva-derived DNA was also performed.

We collected 2,259 patient samples from two clinical centers for testing on the CalMax DNA Genetic Cancer Risk Test (98 gene panel) - 1,885 patients from CellMax’s CAP laboratory in Taiwan, and 374 patients from CellMax’s CLIA laboratory in California, many of whom had been referred for hereditary cancer risk counseling or assessment. Blood or saliva specimens were accepted for sample processing. Information on demographics, personal and family history of cancer was collected at time of sample collection.

The mean age of the Taiwan cohort was 43, with 58% males and 42% females, while the US cohort had a mean age of 58 with 40% males and 60% females.

Sequence variant classification as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign and benign was performed according to the American College of Medical Genetics and Genomics (ACMG) sequence variant interpretation guidelines.3 All classifications were evaluated by a board-certified pathologist and only pathogenic variants were clinically reported.

RESULTS
The analytical sensitivity, specificity and accuracy of the assay panel were 99.95%, 100% and 99.95%, respectively. Similarly, the reportable variants for 30 DNA samples comprised of important SNVs in BRCA1/2, RET, APC, MEN1 and PTEN were called with no false positives. Paired blood (Reference lab) and saliva (CellMax CLIA lab) samples were run to validate the sample collection protocol. The concordance for the paired blood saliva/saliva analysis was found to be 99.95%.

Summary of Results
- Germline pathogenic variants were detected in 47/1885 (2.5%) and 25/374 (6.7%) of individuals in the Taiwan and US cohort respectively.
- Pathogenic BRCA1 and BRCA2 mutations comprised 85.3% and 75% of total positive cases in Taiwan, and 4% and 0% of total positive cases in the US.
- Traditional approaches, using Sanger sequencing or qPCR, focus on small groups of genes and therefore present a difficulty to screen for mutations in numerous genes from multiple individuals synchronically.

CONCLUSIONS
We have demonstrated in 2 clinical cohorts the analytical and clinical validity of a 98 gene panel for the detection of rare pathogenic germline variants associated with cancer predisposition. High sensitivity and specificity of this panel was demonstrated irrespective of the use of blood or saliva as samples. This study provides useful data of the prevalence for many cancer predisposition genes in two diverse ethnic groups. The frequencies of pathogenic mutations within these cohorts revealed a disparity between racial/ethnic groups with similar risks. Multi-gene panel testing using next-generation sequencing provides more comprehensive, cost-effective, and clinically actionable assessment compared to traditional single gene hotspot testing for hereditary cancer risk.