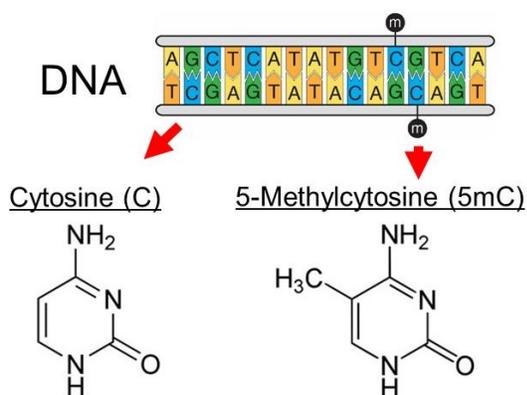


## Performance Characteristics of the IvyGene® Test

The IvyGene® test is an *in vitro* blood test intended to measure the methylation status of cell-free DNA extracted from blood samples at target sites within specific genes. These target sites have been demonstrated to be hypermethylated when certain cancers are present. These cancers include: breast, colon, liver and lung. Test results are reported as a quantitative IvyGene® Score, which indicates the methylation status of the target sites. The IvyGene® Score is calculated as a composite average of cell-free DNA that is methylated at the target sites as a fraction of the total cell-free DNA present.

### Introduction

DNA methylation is an epigenetic regulator of gene expression. In mammals, the DNA base cytosine can be methylated at the C5 position of the cytosine ring to form 5-methylcytosine (5mC). The dynamic methylation of cytosine to 5mC often occurs at sites in the genome where cytosine is immediately adjacent to guanosine, which are called CpG sites (5'-CG-3'). These CpG sites are frequently found in clusters in the promoter regions of genes. Hypermethylation of the CpG sites within a gene promoter is associated with gene silencing.



**Figure 1.** Cytosine and 5-methylcytosine

Extensive alterations in CpG methylation profiles have been noted during malignant

transformation. These alterations in CpG methylation occur concurrently with changes in gene expression which promote oncogenesis. Alterations in the methylation of select CpG sites consistently occur in nearly all cases of specific cancer types.

Circulating tumor DNA (ctDNA) consists of extracellular DNA fragments that are shed into circulation through cancer cell death (apoptosis and necrosis) or active release. Importantly, ctDNA retains the CpG methylation profile of the malignant cell from which it was shed. Therefore, ctDNA may be used as an analyte for the detection of cancer-specific DNA methylation patterns.

### IvyGene® Test Description

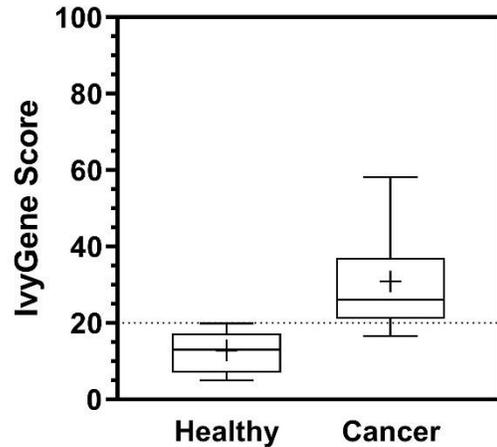
The IvyGene Test measures the methylation status of cell-free DNA extracted from blood samples at target sites. These target sites have been demonstrated to be hypermethylated when certain cancers are present (Hao et al. Proc Natl Acad. Sci. 2017). Test results are reported as a quantitative IvyGene Score, which indicates the methylation status of the target sites. The IvyGene Score is calculated as a composite average of cell-free DNA that is methylated at the target sites as a fraction of the total cell-free DNA present.

### IvyGene® Test Performance Characteristics

LAM's CLIA registered and CAP accredited laboratory ran a validation data set performed on samples drawn from 197 subjects with either no history of cancer or a diagnosis of breast, colon, lung and liver cancer. Cancer patients were diagnosed according to current clinical practices. The IvyGene scores of these subjects are presented in Figure 2.

By using a pre-established clinical cutoff of 20 (IvyGene Score  $\geq 20$  is positive), the IvyGene test was determined to possess a calculated sensitivity of 84% (95%CI: 75-93), and a specificity of 90% (95%CI: 85-95).

Additional test performance characteristics are summarized in Table 1.



**Figure 2.** IvyGene Scores of Validation Cohort

**Table 1.** Summary of IvyGene Test Performance Characteristics

Performance Characteristic	Value or range	Definition
Sensitivity	84% (95%CI: 75-93)	$Sensitivity = TP/(TP+FN)$
Specificity	90% (95%CI: 85-95)	$Specificity = TN/(TN+FP)$
Positive Predictive Value	81% (95%CI: 72-90)	$PPV = TP/(TP+FP)$
Positive Predictive Value*	If prevalence = 0.168% PPV = 1.36%	$PPV = \frac{Sensitivity \cdot Prevalence}{Sensitivity \cdot Prevalence + (1 - Specificity) \cdot (1 - Prevalence)}$
Negative Predictive Value	91% (95%CI: 87-96)	$NPV = TN/(TN+FN)$
Negative Predictive Value*	If prevalence = 0.168% NPV = 99.97%	$NPV = \frac{Specificity \cdot (1 - Prevalence)}{(1 - Sensitivity) \cdot Prevalence + Specificity \cdot (1 - Prevalence)}$
Precision	IvyGene Score +/- 2	Standard Deviation
Reference Interval	IvyGene Score 0-20	One-sided 95th percentile 95% of non-cancer subjects are in this range
Reportable Range	IvyGene Score 0-100	Range of IvyGene Scores
Analytical Sensitivity	LoQ = 20 ng	Limit of Quantification (LoQ), the lowest amount of cfDNA that can provide an accurate result ( $SD \leq 3$ )
Analytical Specificity	Hemolyzed samples are predicted to contain genomic DNA from white blood cells	Known interfering substances

\*Positive and negative predictive values are dependent upon prevalence. The estimated, combined incidence of breast, colon, liver can lung cancers in the general US population is 168 per 100,000