For Hepatocellular Carcinoma

MethyLiverDx-Sigma™

Find it Early, Treat it Better

Physician Brochure

Why Current Surveillance Falls Short for HCC

Current Problems of HCC Surveillance

The Unmet Need: Challenges in HCC Diagnosis

Hepatocellular carcinoma (HCC) ranks among the most prevalent and lethal cancers globally:

- 6th most common cancer worldwide
- 4th leading cause of cancer-related death
- 800,000 deaths annually, with 80% of cases in Asia and Africa

Major risk factors:

- Chronic Hepatitis B (predominant in Asia)
- Hepatitis C (prevalent in Japan, Europe)
- Liver cirrhosis, alcohol abuse, obesity, and smoking

800,000+ deaths/year

6th most common

Limitations of Current Surveillance

Standard HCC surveillance includes serum AFP testing and abdominal ultrasound (US) every 6 months. However, these approaches have considerable drawbacks:

Alpha-fetoprotein (AFP):

- At a 20 ng/mL cutoff, sensitivity is 43% for HCC overall, but only ~30% in early-stage (stage I) HCC.
- Prone to false positives due to benign liver inflammation

Ultrasound:

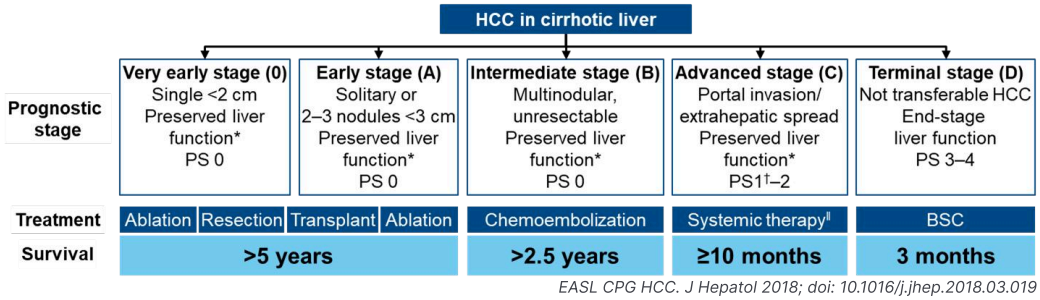
- Highly operator-dependent
- Less effective for detecting lesions < 2 cm
- Challenged by patient factors such as obesity or bowel gas

Despite improvements in treatment, the 5-year survival rate for HCC remains modest and recurrence is frequent, occurring in ~50% of patients.

AFP Sensitivity for Stage I HCC:

~30%

The Advantages of Regular Surveillance for HCC



EASL CPG HCC. J Hepatol 2018; doi: 10.1016/j.jhep.2018.03.019

Benefits of Regular Surveillance

- **Early Detection:** Improves survival rates, which can reach up to 75%.
- **More Treatment Options:** Increases eligibility for curative therapies.
- **Better Outcomes:** Lowers mortality and recurrence through timely intervention.

Guidelines from the European Association for the Study of the Liver (EASL) recommend surveillance every 6 months in high-risk populations.

What is MethyLiverDx-Sigma™ (MLDx-Sigma™)?

MLDx-Sigma™: A Novel Non-Invasive Approach to HCC Detection

A reliable and accessible methylation-based assay for HCC detection

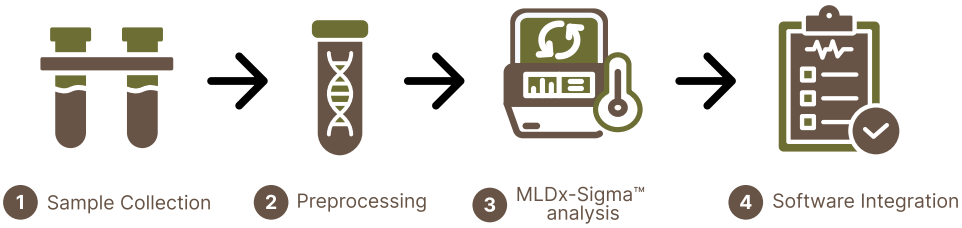
Sample Type

The test uses plasma extracted from whole blood. Blood can be collected in:

- K_2EDTA tube, a minimum of 10 mL of whole blood

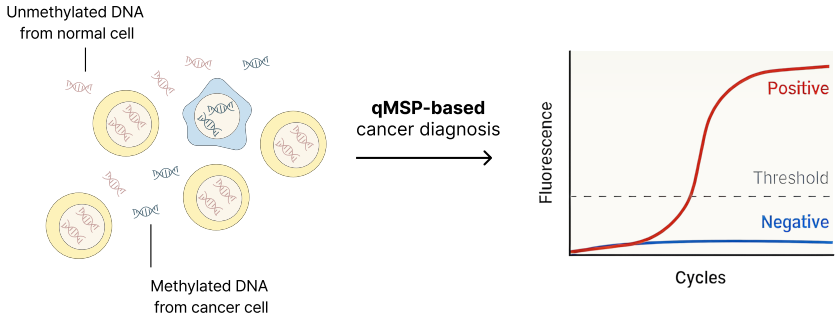
Components

Primers: RNF135 (2D), DNMT3A (3D), GAPDH (internal control)
Fluorescent Dye
Rapid Red PCR Master Mix
Nuclease-Free Water
Positive and Negative Controls
Software
Kit Manual



Method

1. **Sample Collection**
2. **Preprocessing**
   - **cfDNA Extraction:** Plasma samples are processed to isolate cfDNA.
   - **Bisulfite Conversion:** Unmethylated cytosines are converted to uracil, while methylated cytosines remain unchanged.
3. **MLDx-Sigma™ analysis**
   - **quantitative methylation-specific PCR (qMSP) analysis**
     qMSP uses primers designed to specifically amplify bisulfite-converted methylated DNA sequences. The amount of methylated DNA is quantified in real time through fluorescence signal intensity, allowing sensitive and specific detection of methylation levels in the target genes.



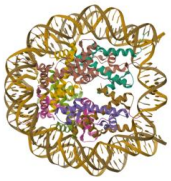
4. **Software Integration and Result Reporting**

Science behind MLDx-Sigma™

Target Biomarkers

Biomarkers were identified through large-scale datasets and precision epigenetic analysis

RNF135 (2D): RNF135 is specifically altered in HCC, linked to reduced expression, increased cell migration, immune microenvironment changes, and poorer prognosis.

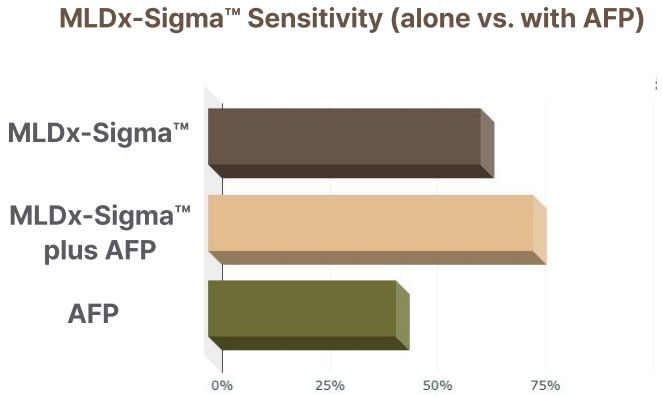


DNMT3A (3D): DNMT3A shows aberrant activity in HCC, associated with epigenetic dysregulation, aggressive tumor behavior, drug resistance, and adverse clinical outcomes.

Why These Markers?

- Selected due to exceptionally high methylation in HCC, with minimal methylation in normal blood, liver, and other tissues.

Clinical Performance of MLDx-Sigma™



Sensitivity: 63%
Specificity: 97%

- MLDx-Sigma™ demonstrates strong clinical performance in distinguishing between healthy individuals, at-risk populations, and patients with HCC.
- MLDx-Sigma™ shows 63% sensitivity on its own and 75% when combined with AFP.
- Specificity reaches 97% on its own and 96% when combined with AFP.
- MLDx-Sigma™ is more sensitive than AFP alone (43%) and provides reliable results across all stages of liver cancer.

Application of MLDx-Sigma™

Who Should be Tested?

MLDx-Sigma™ is recommended for high-risk individuals requiring routine HCC surveillance, consistent with established clinical guidelines

MLDx-Sigma™ accurately identifies patients at high risk of HCC

MLDx-Sigma™ is recommended for individuals at high risk of developing HCC. It accurately identifies high-risk patients, supporting routine surveillance and earlier detection.

High-risk groups include:

- Individuals with **chronic Hepatitis B (HBV)** or **Hepatitis C (HCV)** infections
- Patients with **liver cirrhosis** of any cause
- Those with a family history of liver cancer
- Individuals with a history of **heavy alcohol use** or **non-alcoholic fatty liver disease.**

MLDx-Sigma™ is for people with

Chronic hepatitis B or C
Liver cirrhosis
Family history of liver cancer
Heavy alcohol user
Fatty Liver

Interpreting the Results

Results are interpreted by comparing peak providing a clear positive or negative result for HCC detection.

Positive

May indicate the presence of HCC

If the result is **Positive (+)**

MLDx-Sigma™ detected liver cancer–specific DNA in blood. The patient is more likely to have liver cancer. Arrange follow-up diagnostic imaging in consultation with a specialist.

Negative

Indicates a lower likelihood of presence of HCC

If the result is **Negative (-)**

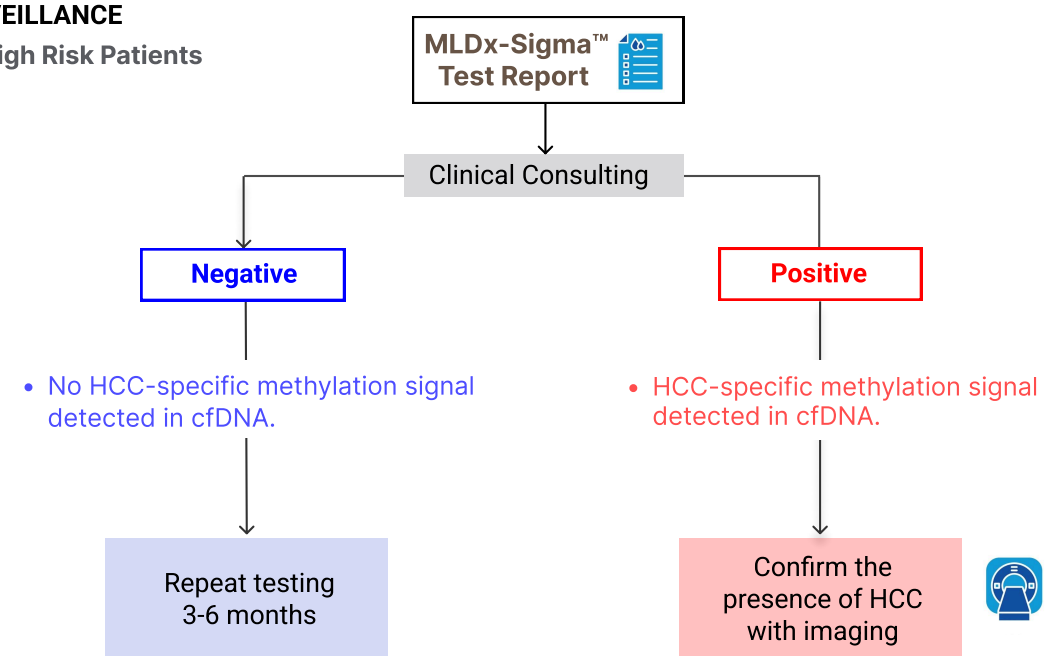
MLDx-Sigma™ did not detect liver cancer–specific DNA in blood. The patient is unlikely to have liver cancer. Continue regular surveillance per guidelines.

Where MLDx-Sigma™ Fits in the Diagnostic Pathway

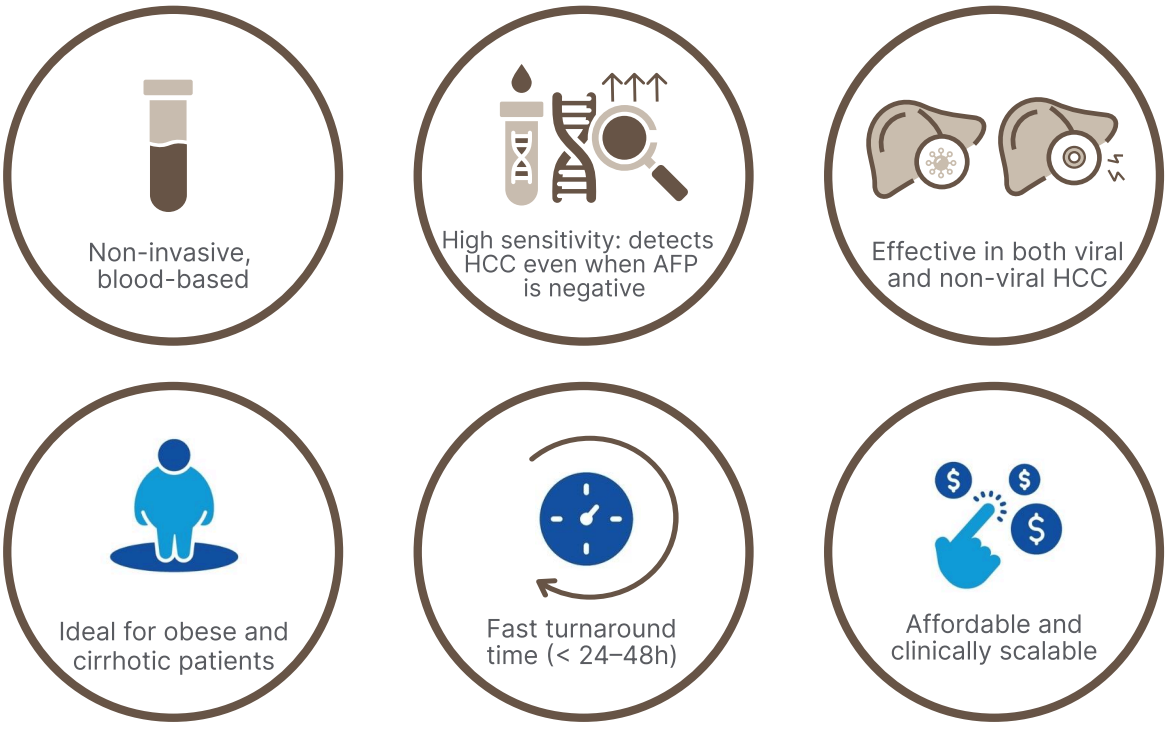
With high specificity, MLDx-Sigma™ boosts diagnostic confidence in high-risk patients and supports timely, curative intervention.

Diagnostic Algorithm for MLDx-Sigma™

SURVEILLANCE
For High Risk Patients

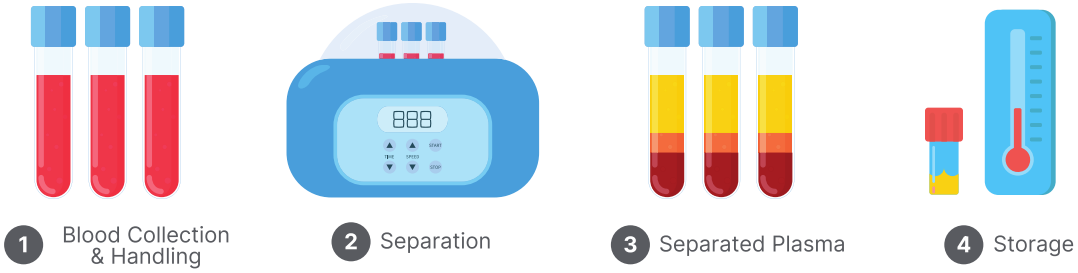


Why Choose MLDx-Sigma™?



Plasma Preparation and Storage

Blood Collection: Collect 10 mL blood in a K2 EDTA tube.
Handling: Gently mix; process samples within 4 hours.
Plasma Separation: Plasma should be separated by centrifugation, carefully avoiding the buffy coat.
Storage: Aliquot plasma into labeled cryovials and store at –70 °C to –90 °C if not processed immediately.



1 Blood Collection & Handling
2 Separation
3 Separated Plasma
4 Storage

Limitations & Clinical Considerations

- **A negative MLDx-Sigma™ result suggests no detectable liver cancer–associated methylation signals at the time of testing.** However, as methylation signals may be below the detection limit in cases with low tumor-derived cfDNA, repeat testing and continued routine HCC surveillance are recommended according to clinical guidelines.
- **A positive MLDx-Sigma™ result indicates the detection of liver cancer–associated methylation signals.**
  To confirm the presence of HCC, follow-up diagnostic imaging (e.g., contrast-enhanced CT or MRI) is strongly recommended as part of the diagnostic process.
- MLDx-Sigma™ does not replace diagnostic imaging or histological confirmation. It should be used as part of a multimodal surveillance or diagnostic approach.
- Sample quality (e.g. hemolysis in blood, degradation of cfDNA) can significantly affect results. To ensure the integrity of the sample, the laboratory must follow the SOP for the specimens.

References:

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Learn more about MethyLiverDx-Sigma™:
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