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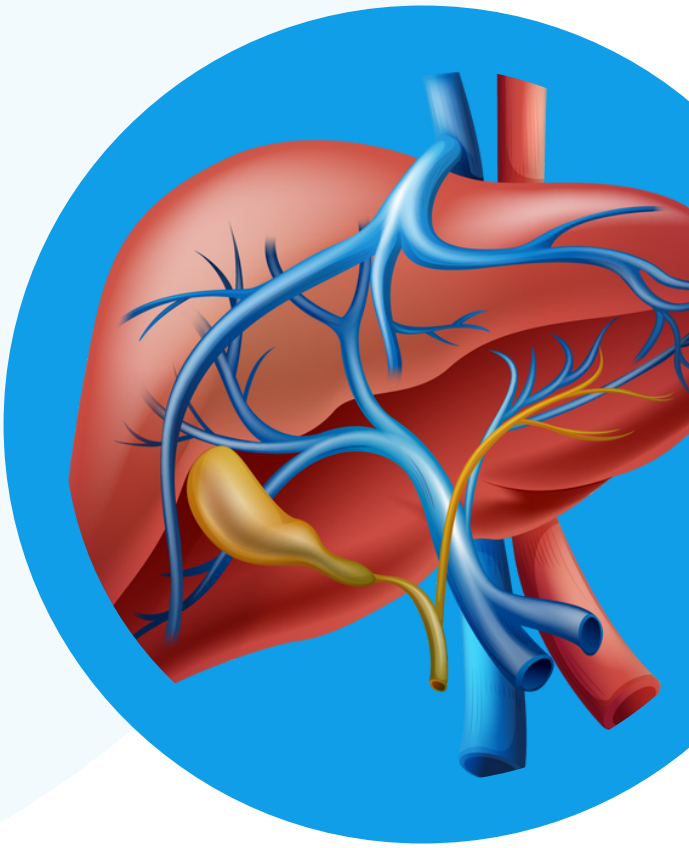
HKG *epi* THERAPEUTICS Ltd.
Harnessing the epigenome

YOUR EPILIVER TEST RESULTS

A proprietary novel blood test for accurate and early detection of **liver cancer** using a **BCD - next generation method**

Your Barcode: .....

Date: .....



Testing Laboratory: **HKG epiTherapeutics Limited**

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TABLE OF CONTENTS

YOUR INFORMATION

Customer ID:	
Sample Type:	
Sample Collected on :	
Sample Received on :	
Name of ordering physician :	
Order ID :	
Sample ID	
Report ID :	
Report Released on :	
Clinic :	
Results Reviewed by:	

Disclaimer:

This test was developed and its performance characteristics determined by HKG epiTherapeutics Limited. It has not been cleared or approved by the US Food and Drug Administration. HKG epiTherapeutics Limited is committed to maintain the accuracy, security and confidentiality of your personal information.

YOUR RESULT

96% specificity

M score spec (liver)	Test result spec	M score detect	Test result detect
XX	Negative	XX	Negative

Detect Negative Range: 0 - 6.27

Spec Negative Range: 0-6.44

M-score detect is the composite score of methylation of 4 genes calculated by a proprietary equation that we developed, based on our clinical data. A higher score means a higher detection of cancer DNA.
M-score spec is a score of methylation of a gene that is specifically methylated in DNA from liver origin. A higher score means a higher detection of DNA from the liver. Positive M score in both "detect" and "spec" indicates presence of liver cancer DNA in the blood.

What should I do:

If ylf your detect M-score is above 6.27, you might consult your physician on whether you should do a traditional lab test or other kind of tests to confirm the diagnosis. You might consider to repeat the epiLiver test as well in 3 months.

WHY IS THE EARLY DETECTION OF LIVER CANCER SO IMPORTANT?

Liver cancer Hepatocellular Carcinoma (HCC) is the fifth most common cancer world-wide. It is particularly prevalent in Asia, and its occurrence is highest in areas where hepatitis B is prevalent.

Hundreds of millions of people in Asia are at risk for developing liver cancer particularly people who suffer from liver disease, chronic liver inflammation high alcohol uptake.

Follow up of high-risk populations such as chronic hepatitis patients and early diagnosis of transitions from chronic hepatitis to HCC would improve cure rates. However, current diagnostic methods, which include imaging and immunoassays with single proteins such as alpha-fetoprotein often fail to diagnose HCC early (Flores & Marrero, 2014).

The main challenge is that solid tumors hide in internal organs and evolve long before they exhibit clinical symptoms.

Many studies showed that it is possible to find tumor DNA in one of the most accessible and commonly used biological sample in clinical medicine, blood

Additional information on the test and how it was developed

CELL FREE TUMOR - DERIVED DNA IN PLASMA

It is widely established by now that **tumor DNA** is shed into the body and could be found in the plasma portion of whole blood even before the onset of clinical symptoms (Warton & Samimi, 2015).

Isolating plasma from blood is a simple procedure that could be performed by any clinical lab in almost any hospital or nurse/doctor clinic. The main challenge is how to tell apart tumor DNA from all other normal DNA that finds itself in plasma. And moreover, how do we know if we detect cancer whether it is a liver cancer or some other cancer. It is like finding a needle in a haystack.

Although screening for sequence mutations has held high promise, it appears now that it is not a highly effective way for detecting tumor DNA in blood.

A NEW WAY TO DETECT TUMOR DNA IN BLOOD AND DETERMINE THAT IT IS SPECIFICALLY LIVER CANCER

However, there is a unique and novel way to detect few molecules of cancer DNA on the background of normal DNA, it is not by examining the genetic sequence but rather by examining the “epigenetic” profile of DNA.

Research pioneered by **Moshe Szyf**, a professor at McGill University and a fellow of the Royal Society of Canada has taken almost **three decades** to establish the **unique epigenetic characteristics of cancer cells and their DNA**.

The fruit of this research is a robust test for early detection of liver cancer that requires just 5 ml of blood (2 ml of plasma) and could be used for early detection of liver cancer in healthy people and people who are at a higher risk for liver cancer.

The **test** does not only detect cancer but can **determine that the cancer originates in the liver**.
This is a proprietary invention that is under patent protection process across the world (PCT/IB2019/055855).

WHAT IS DNA METHYLATION?

The DNA that we have in every cell of our body is like a minicomputer that is managing our cells so that our body functions properly to perform all the tasks that are required throughout our lives.

We inherit from our parents our DNA (the genes) which is the operating system of this minicomputer. We have the same DNA in every cell of our body. However, our DNA operates differently in different cells and different organs of our body.

Like any computer, our DNA needs software to program it and we need different programs in different kinds of cells. Part of the programming is achieved by a chemical coating of DNA called **DNA methylation**. This programming, the software of our DNA is laid down when we develop in the womb of our mother. A very precise plan that evolved during evolution ensures a highly accurate DNA methylation program for each cell in our body so that all our organs function accurately.

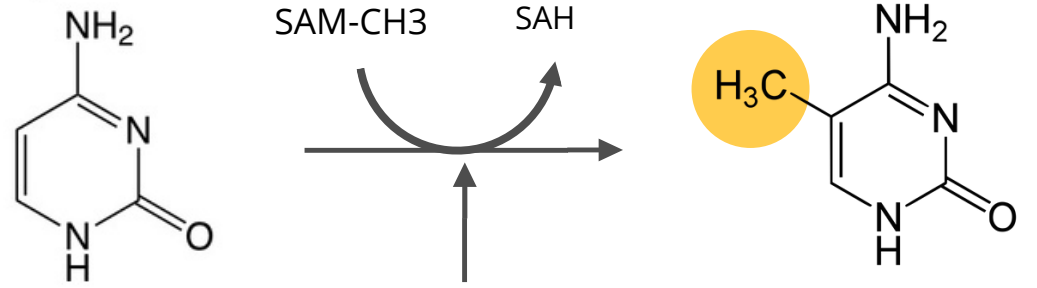
WHAT IS DNA METHYLATION?

Like any software, a **DNA methylation** profile of a cell is a script. As a proper script guarantees proper functioning of an app, correct DNA methylation profiling guarantees proper function of our organs and thus, our health and well being.

WHAT IS DNA METHYLATION?

The proper positioning of DNA methylation on genes serves as an on/off switch for genes.

Our company is using the most advanced next generation sequencing methods to map at high accuracy methylation profiles from as little as 1 ng (1 gram divided by a billion) of DNA.



Cytosine **DNMT** ***5-Methylcytosine***

ON **OFF**

DNA METHYLATION IS DISRUPTED IN CANCER. COULD BE USED FOR DETECTION OF CANCER DNA IN BLOOD

Normal **Cancer**

Moshe Szyf was amongst the first pioneers that suggested thirty-six years ago that the **prime force driving cancer** is **a change in DNA methylation**.

Moshe Szyf and others have shown since that the changes in DNA methylation are broad in cancer. DNA that is derived from cancer cells has **a different methylation profile** than DNA that comes from normal tissues (in the figure above the balloons represents methylation). DNA methylation guides us toward a revolution in early detection of cancer.

THE NOVEL EPILIVER TEST: ROBUST BINARY CATEGORICAL DIFFERENTIATION BETWEEN LIVER CANCER AND NORMAL DNA (BCD)

Our scientists **investigated DNA methylation results** from normal tissues, blood and cancer from dozens of people and used proprietary methods to discover methylation profiles that are categorically different between liver cancer, blood and normal tissues.

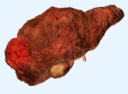
We called this new method BCD: Binary Categorical Differentiation. The method identifies Black and white differences between cancer and normal.

Our test has two components:

1 DETECT

Detects cancer

2 SPEC



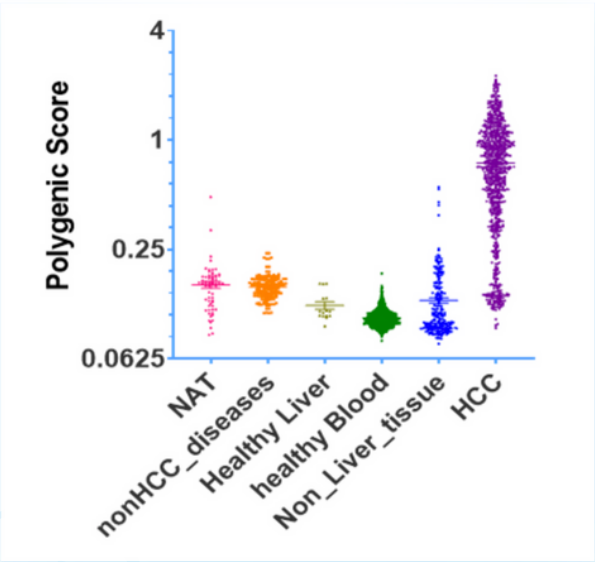
Determines that the cancer is liver cancer {HCC}

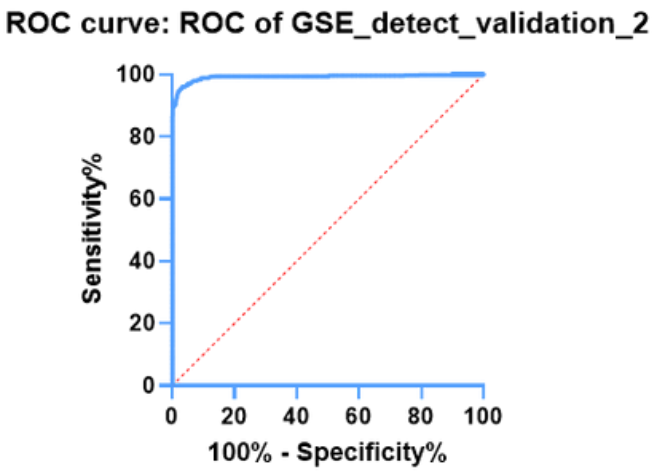
DETECT FOR DETECTION OF CANCER

Using our proprietary BCD method, we discovered HCC methylation profiles that are so unique that even if we have only few copies of DNA from cancer mixed with tens of thousand of copies from other cells we could detect it using our method of analysis called next generation sequencing.

We then verified that these DNA methylation profiles that we discovered would indeed discover cancer when we examined DNA methylation profiles from more than 10000 people.

The figure below shows that the methylation score that we developed detects HCC in 789 HCC patients (in purple) but not in a variety of normal liver tissue and blood from 1442 people (each spot represents one person) in publicly available databases. The specificity and sensitivity are above 95% and area under the curve is 0.9926. (Note that the polygenic score below was developed for public data which is based on a different method of mapping DNA methylation than the M score developed later for our clinical Next Generation Sequencing test).



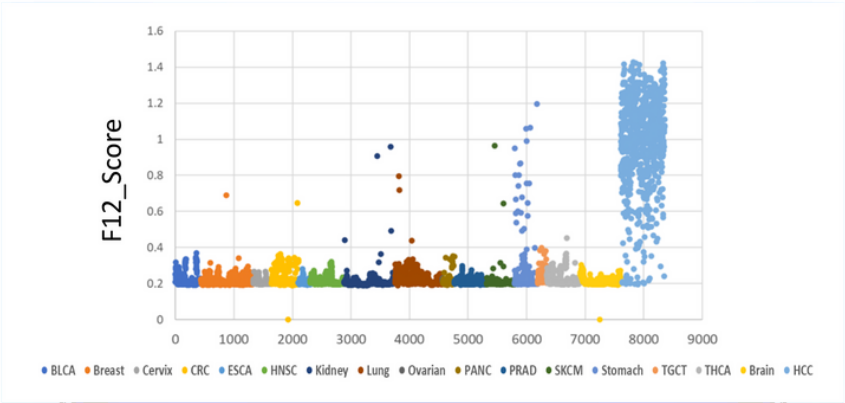


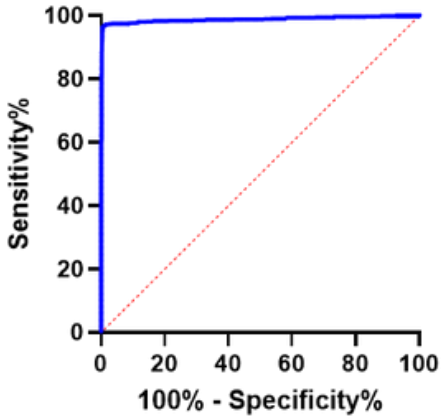
SPEC DETECTS SPECIFICALLY LIVER CANCER

However, what makes our test truly unique in the field is that we have also discovered DNA methylation profiles that can tell that the DNA is not just cancer DNA but **liver cancer DNA** with a potential specificity of close to 95%.

Most available test don't differentiate between liver and other cancers. The figure below shows the scores for the Spec test across DNA methylation results from close to 8000 different tumors.

Each spot represent a sample from a single person. The test clearly differentiates between liver cancer and other 17 cancers (the liver cancers are in blue in the far right. Each spot is one sample, other 17 cancers are in different colors).





The AUC (area under the curve) is 0.99

CLINICAL VALIDATION OF BCD-NEXT GENERATION SPEC AND DETECT FOR LIVER CANCER DETECTION IN PLASMA FROM 401 PEOPLE

In summary, we validated that our **detect** and **spec** markers reveal a profile that is **highly accurate** for liver cancer DNA at any stage on thousands of samples and tissues using DNA methylation.

In **HKG epiTherapeutics** we developed a lab test using a proprietary next generation sequencing based method that can reveal the profiles of DNA methylation of thousands of copies of DNA in people plasma and then searches for the few copies that have cancer-specific and liver-specific profiles.

Our method counts copies that have cancer-specific and liver-cancer-specific profiles and issues a result. We validated that the **background of other DNA in the blood is very low** as we had predicted, providing a “black and white” difference between cancer and normal DNA.

CLINICAL VALIDATION OF BCD-NEXT GENERATION SPEC AND DETECT FOR LIVER CANCER DETECTION IN PLASMA FROM 401 PEOPLE

The “heatmap below shows **the results of a study on 401people** from Dhaka Bangladesh.

- 49 healthy controls
- 50 chronic hepatitis B
- 302 cancers from stages A to D

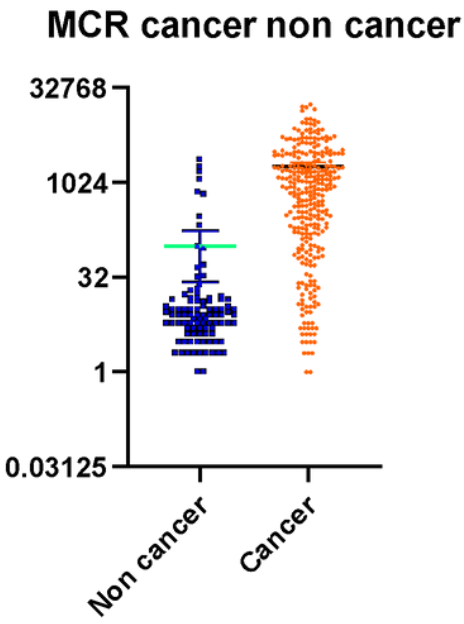
Each column is a different patient. For each patient we sequence 5 genes. The level of methylation is indicated by the color. Red is most methylated and blue is not methylated. You can see a clear red and blue difference between the samples. All the **control** and **chronic hepatitis** are almost totally **blue** while a lot of **red** is seen in the **cancer patients**.
The boundary between cancer and non-cancer is sharp and clear.

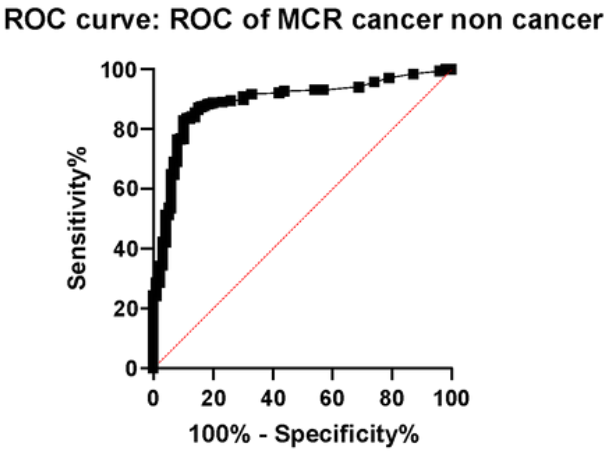
most methylated *not methylated*

CLINICAL VALIDATION OF BCD-NEXT GENERATION SPEC AND DETECT FOR LIVER CANCER DETECTION IN PLASMA FROM 401 PEOPLE

We developed a combined score that takes into account the **number of methylated copies (MCR)** in a cancer patient's plasma.

The chart below shows that the number of methylated copies (MCR) in cancer patient plasma in our Bangladesh trial is dramatically different than in healthy and chronic hepatitis B patients. Most people have between 2 and 10 methylated copies, while in cancer patients it could reach more than 10000.





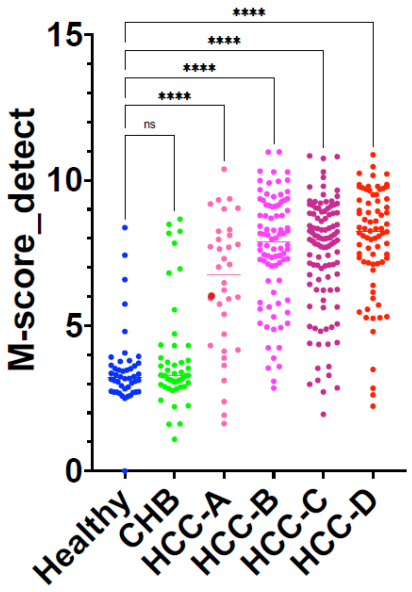
The 4 gene "detect" M-Score predicts cancer
The single gene "spec" M-score predicts liver origin

Our team then developed a proprietary equation to quantify the methylation levels across the 4 genes for "detect" and 1 gene for "spec" for each patient, which we call **M-score**.

We then calculated the M-scores for "detect" and "spec" markers for healthy and cancer patients from our Bangladesh clinical trial.

The chart below shows that the "detect" and "spec" M-scores for liver cancer (HCC) patients are much higher than in controls.

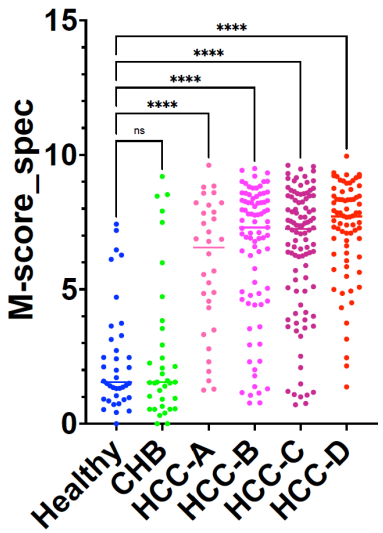
A



In chart A each spot is the detect M-score for a different patient from the Bangladesh study at different stages of HCC, healthy people, and Hepatitis B patients. You can see the large difference between the cancer and healthy groups.

In chart B each spot is the spec M-score for a different patient from the Bangladesh study at different stages of HCC, healthy and Hepatitis B patients.

B

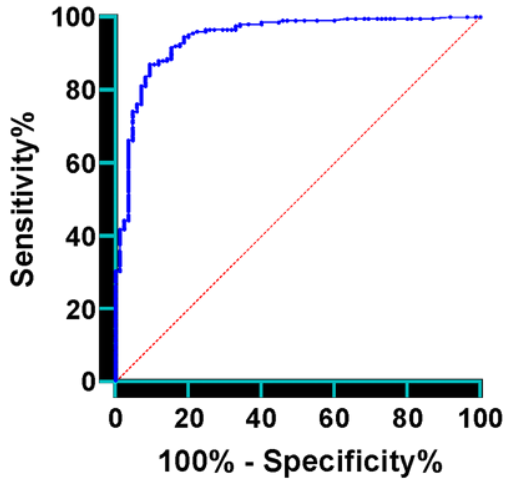


THE 5 GENE M-SCORE PREDICTS HCC

In chart C we examined how well the M-score can differentiate between the cancer and healthy people.

The ROC curve measures **sensitivity** and **specificity** of the test.
The area under the curve (AUC) defines the accuracy of the test integrating both specificity and sensitivity where 1 is a perfect score, the AUC of the epiLiver test in this study was 0.9437.

C



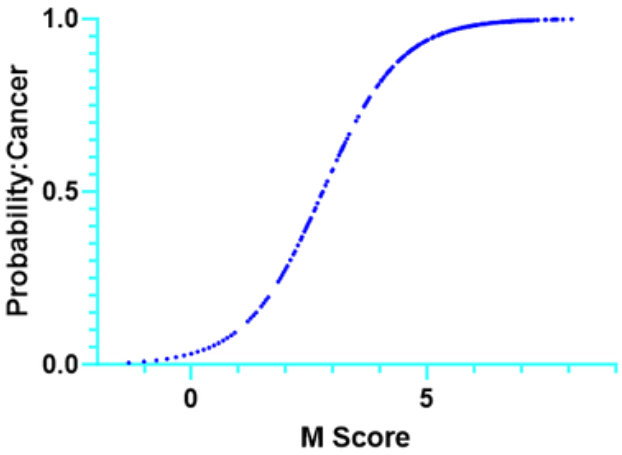
Area under the ROC curve

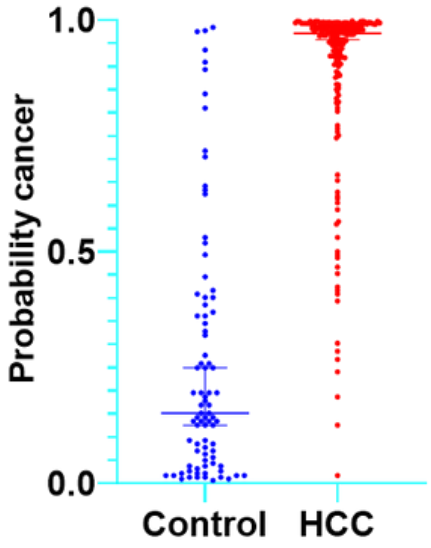
Area	0.9437
Std.Error	0.01518
95% confidence interval	0.9140 to 0.9735
P value	< 0.0001

WHAT DO THE RESULTS OF EPILIVER TEST MEAN?

The results disclose presence-of liver cancer in the blood.

The results disclose a probability of having liver cancer.
We used our clinical data to compute the probability that a person with a certain M-score has cancer (HCC).
Chart A below shows that the probability of cancer increases from 0 as the M- score is increased reaching close to 1 (100% chance) at very high scores.
Chart B computed the probability of cancer from the M-score for each patient from our Bangladesh clinical trial.
Each spot represents a patient (red - cancer, blue - healthy). You can see that most of the cancer patients cluster close to probability of 1 while the control patients cluster close to probability of 0. We used the same equation to calculate your probability of having liver cancer.

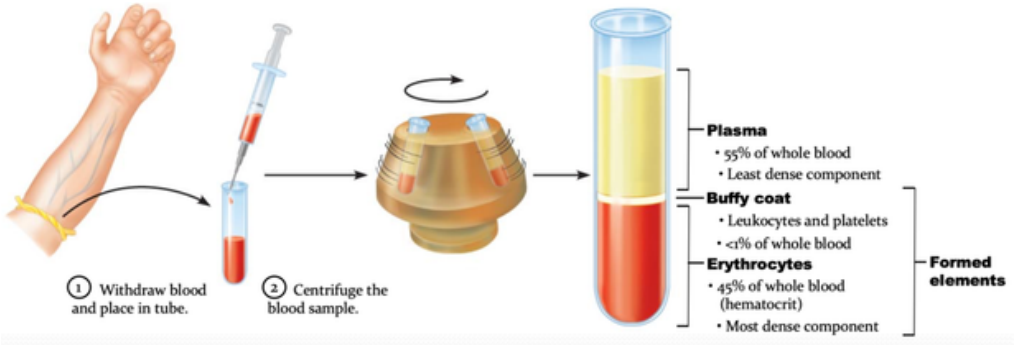




HOW DOES THE TEST WORK?

1. A sample of 5 ml of blood is drawn from the patient and stored in an EDTA tube.

2. Plasma is separated by a simple lab procedure called centrifugation.



3. Sample is shipped to our lab frozen

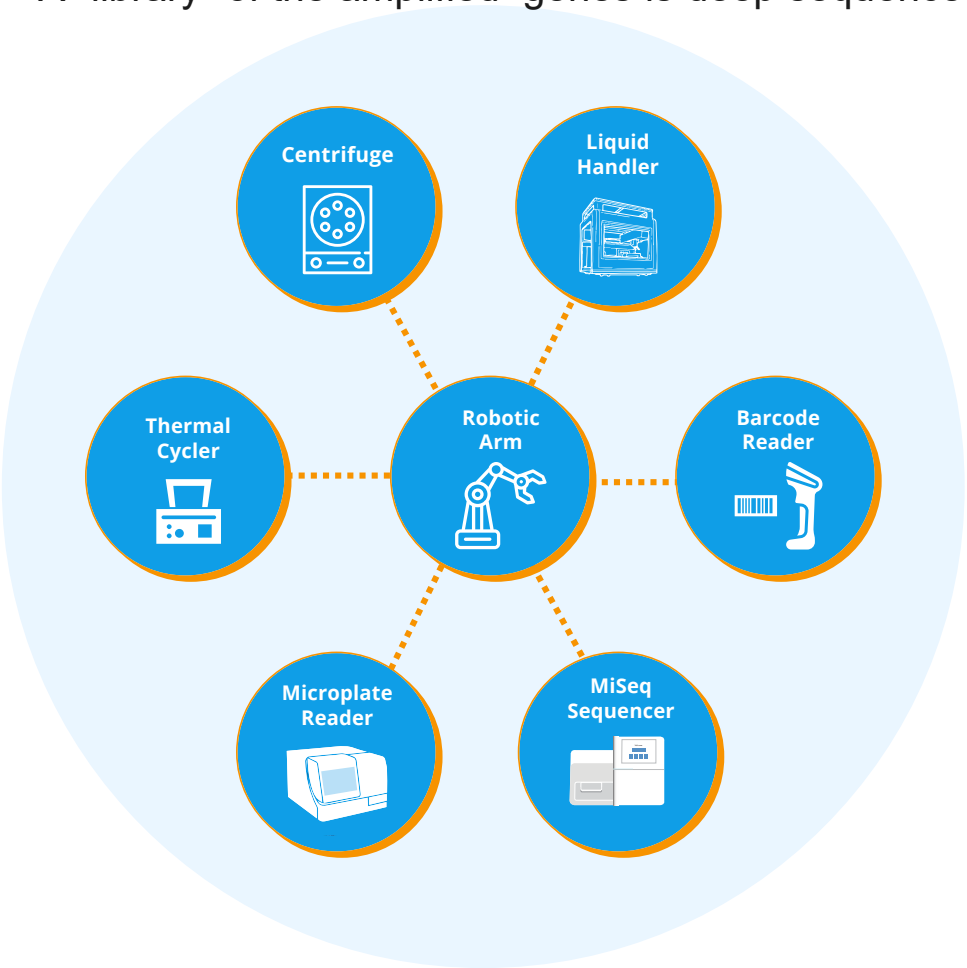
4. Using a proprietary method, the lab extracts DNA from the sample.

HOW DOES THE TEST WORK?

5 DNA is chemically converted for methylation mapping (bisulfite conversion).

6 Five genes used in the analysis are amplified.

A "library" of the amplified genes is deep sequenced.



HOW DOES THE TEST WORK?

7 Sequencing data is analyzed in the "cloud" and a report is issued to your doctor.

8 It will inform whether HCC DNA was detected in blood and the M scores for the "spec" and "detect" markers.

9 If test results are positive the doctor might recommend repeating the test and if positive again the doctor might perform additional tests including imaging such as ultrasound or MRI.

The test serves as a red flag but does not diagnose cancer.

10 The doctor might advise different treatment modalities based on best clinical practice.

11

WHO SHOULD BE TESTED?

- People with chronic liver inflammation such as Hepatitis B or Hepatitis C
- People with known chronic liver disease such as NAFLD (nonalcoholic fatty liver disease) and cirrhosis or fibrosis from any cause
- Alcoholics
- People who are morbidly obese
- People who have diabetes

HOW FREQUENTLY SHOULD I BE TESTED?

The method is very new and more clinical results are needed to determine the most effective frequency of testing. However, it is believed that liver cancer can grow significantly in 6 months. So, it is advisable to be tested **every 6 months**.