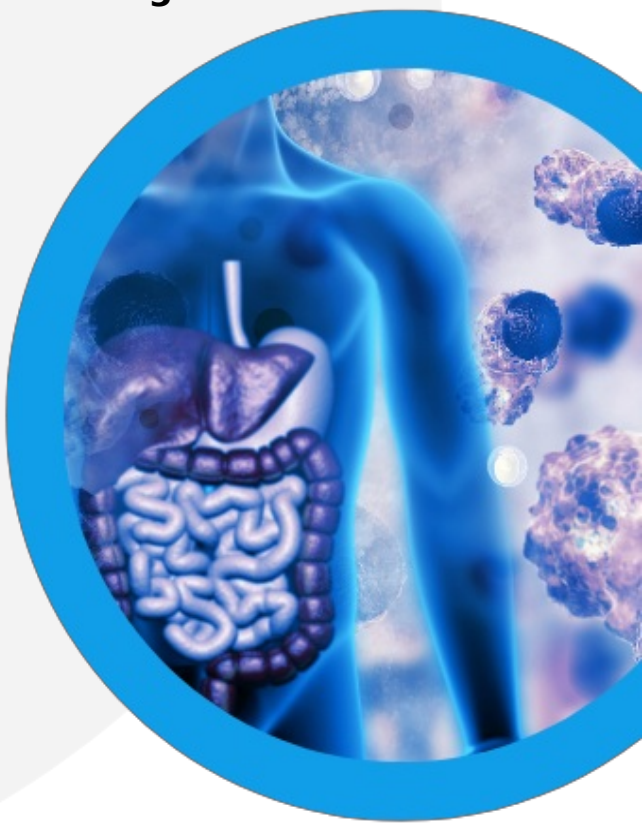
HKG epi THERAPEUTICS Ltd.
Harnessing the epigenome

YOUR EPIPANCANCER TEST RESULTS

epiPancancer a Proprietary Novel Blood Test For Accurate Early Detection of **Cancer From 33 Different Origins** Using a BCD-Next Generation Method



Your Barcode: _____

Date: _____

Testing Laboratory: HKG epiTherapeutics Limited
Address: Unit 313-315, Biotech Centre 2,
11 Science Park West Avenue,
Hong Kong Science Park,
Shatin, Hong Kong
Contact us: info@hkgepitherapeutics.com
+852 2354 8297

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YOUR INFORMATION

Customer ID :	
Sample Type :	
Sample Collected on :	
Sample Received on :	
Name of ordering physician:	
Order No :	
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. The test was not yet validated by CAP and is considered a research state product.

YOUR RESULT

Your M-score

NEGATIVE

Test Result

Negative M-score Range: 0 - 7.13

M-score - 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. Based on our analysis of public DNA methylation data from thousands of sample, the test should detect 19 common cancers at high accuracy. The test is new and has not yet completed full clinical trial and has not been approved yet by FDA. The test detects cancer DNA but does not detect its origin. This will require specific tests.

What should I do:
If your **M-score** is **above 7.13**, 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 epiPancancer test as well in 3 months.

SENSITIVITY FOR DETECTION OF DIFFERENT TYPES OF CANCER IN TCGA DATA (SPECIFICITY FOR ALL CANCERS IS SET AT 95%)

Cancer	AUC	Sensitivity
BLCA	0.99	97
BRCA	0.99	97
CESC	1.0	99
GBM	1.0	99
ESCA	1.0	99
HNSC	1.0	99
CHOL	0.98	94
LUSC	0.99	98
OV	0.97	90
PRAD	0.98	95
LGG	0.99	97
LIHC	0.99	96
LUAD	1.0	100
UCS	1.0	100
COAD	1.0	100
UCEC	1.0	100
READ	1.0	100
STAD	1.0	100
PAAD	1.0	99

Cancer	AUC	Sensitivity
KIRC	0.94	75
DLBC	0.94	79
MESO	0.95	80
SKCM	0.93	76

Cancer	AUC	Sensitivity
ACC	0.83	50
LAML	0.81	51
SARC	0.9	67

Cancer	AUC	Sensitivity
THCA	0.8	33
THYM	0.75	25
PCPG	0.7	10
UVM	0.8	30
KICH	0.82	30
KIRP	0.82	38
TGCT	0.69	17

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.

BCD METHOD FOR DETECTING TUMOR DNA

A new way to detect tumor DNA in blood

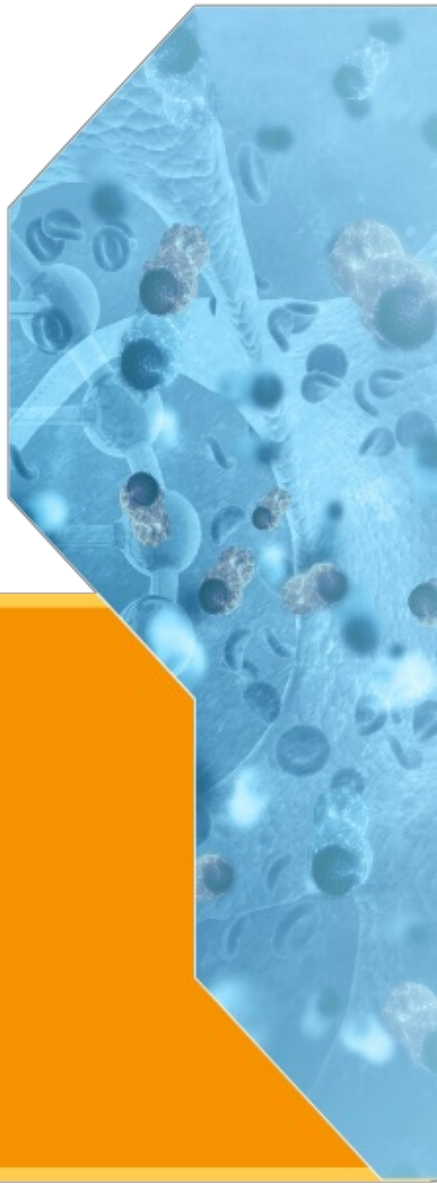
However, there is a unique and novel way to detect few molecules of cancer DNA on the background of normal DNA, it is not by examining the genetic sequence but rather by examining the “epigenetic” profile of DNA.

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**.

Our team has identified a set of regions that are methylated in almost all cancers and are not methylated in any normal tissue examined and are therefore used to detect cancer in our assay.

THE NOVEL EPIPANCANCER 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 thousands of people and used **proprietary methods** to discover methylation profiles that are categorically different between 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

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.

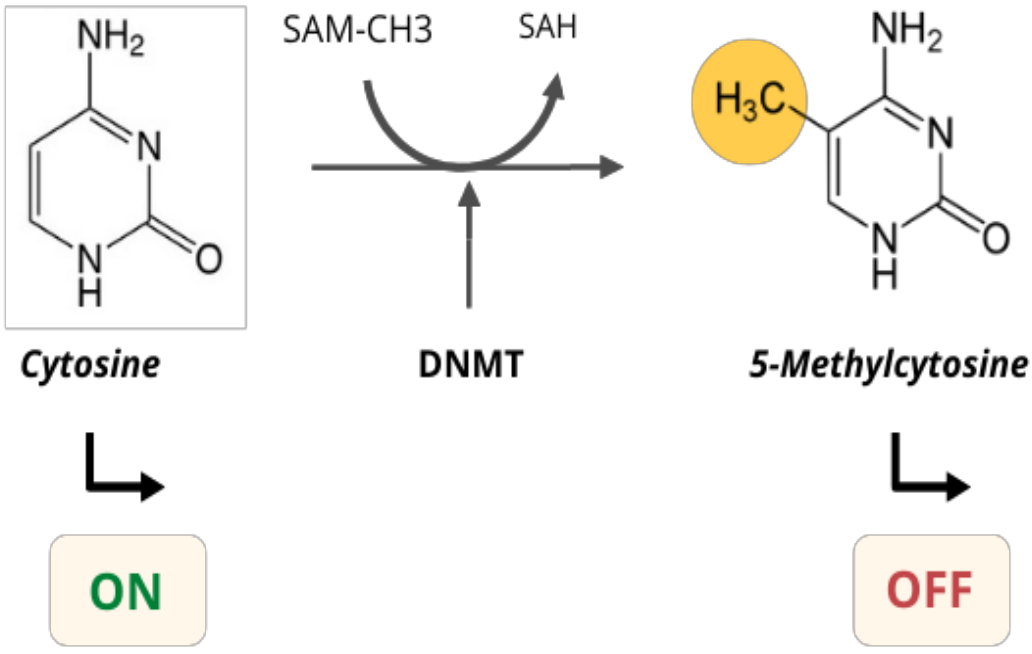
WHAT IS DNA METHYLATION?

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.

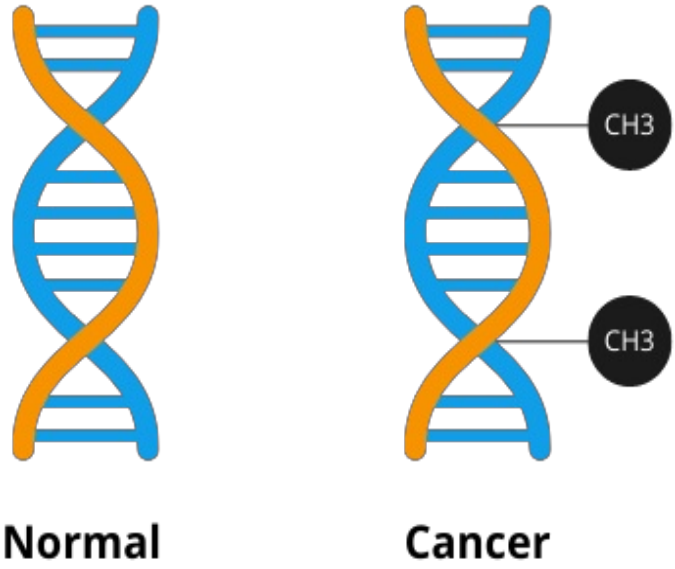
WHAT IS DNA METHYLATION?

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.



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



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.

DISCOVERY OF EPIPANCANCER MARKERS

Using our **proprietary BCD method**, we discovered methylation profiles that are so unique that are totally unmethylated in healthy blood and methylated in many cancers by examining publicly available DNA methylation data from **169 Healthy blood Samples and 130 Cancer** (13 Cancer type, 10 each).

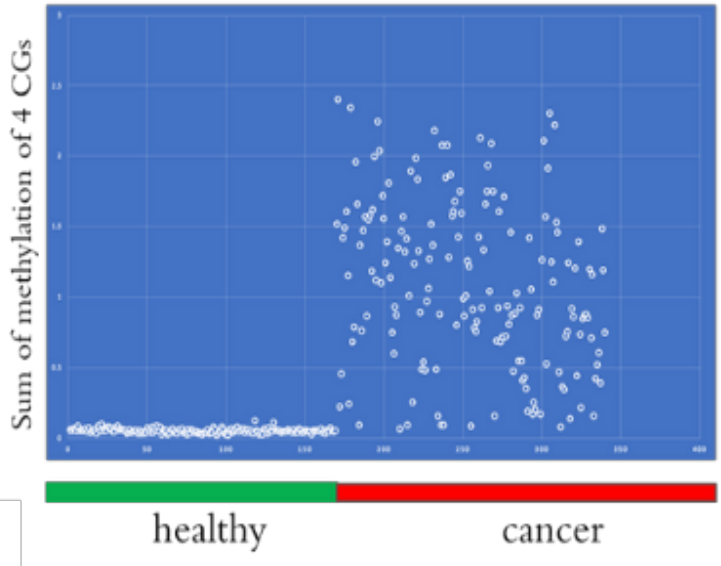
Discovery of epiPancancer Markers

In **Figure A** below each sample received an average epiPancancer methylation score; the cancer samples methylation scores are much higher than the healthy controls which are close to 0.

In **Figure B**, we present the methylation scores for each of the samples for the 4 genes that together form the epiPancancer marker. Red indicates high methylation and blue no methylation. While all healthy are blue many reds are seen in the cancer samples.

In **Figure C** is a list of the different cancers that were examined here.

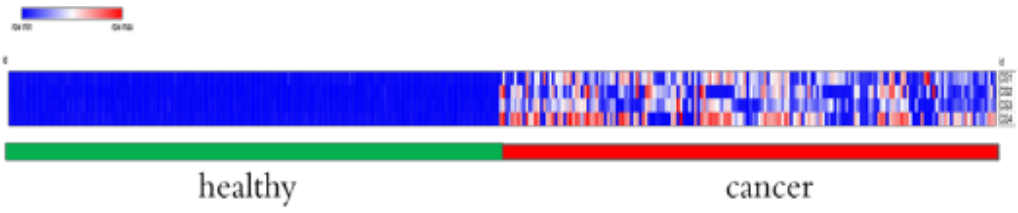
A



C

- Bladder Urothelial Carcinoma
- Breast invasive carcinoma
- Cervical squamous cell carcinoma and endocervical adenocarcinoma
- Colon adenocarcinoma
- Esophageal carcinoma
- Glioblastoma multiforme
- Head and Neck squamous cell carcinoma
- Brain Lower Grade Glioma
- Liver hepatocellular carcinoma
- Lung adenocarcinoma
- Lung squamous cell carcinoma
- Ovarian serous cystadenocarcinoma
- Pancreatic adenocarcinoma
- Prostate adenocarcinoma
- Stomach adenocarcinoma

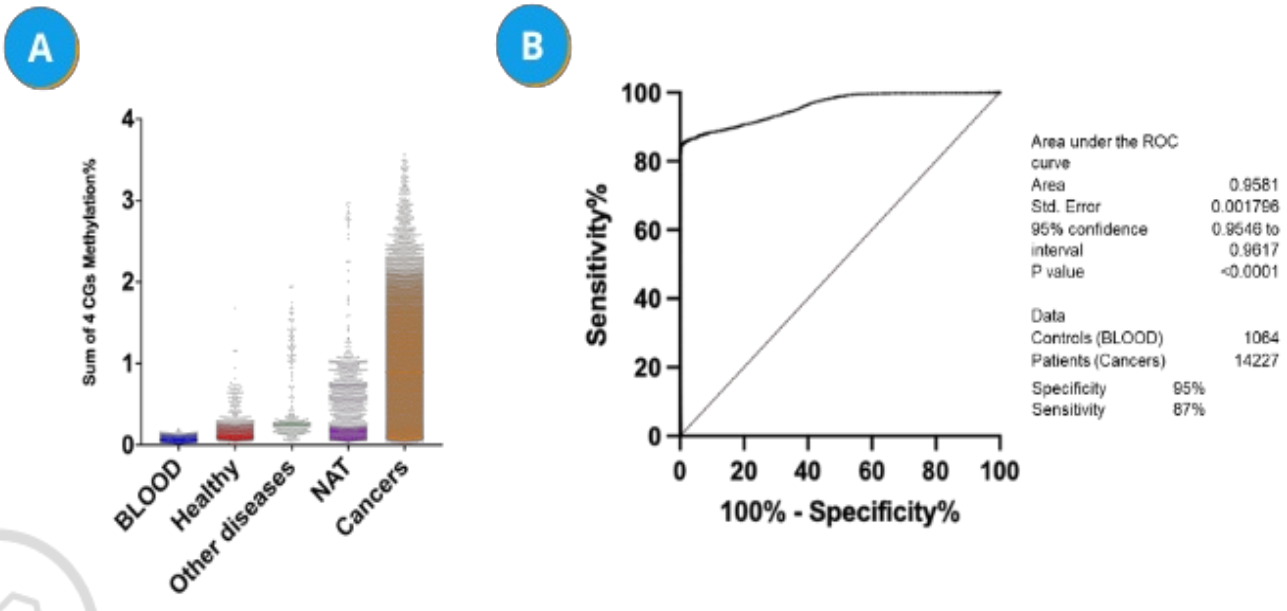
B



VALIDATION OF EPIPANCANCER MARKERS ON 18,752 INDIVIDUALS FROM PUBLIC DATA

We then verified that DNA methylation profiles that we discovered in the 130 cancer samples using DNA methylation profiles from more than 18,000 people. These DNA methylation profiles are publicly available.

The figure below shows that the **methylation score** that we developed **detects cancer** in numerous cancer patients (Figure A) (in orange) but not in blood from healthy people (blue) and very few healthy tissues (red) and other diseases (green) (each spot represents one person).
There is some **detection in tissues** which are adjacent to the **tumor** (purple NAT). The specificity is above 95% (only 5% false positives) and area under the curve is 0.9581 (95% specificity and 87% sensitivity across 31 different cancers).



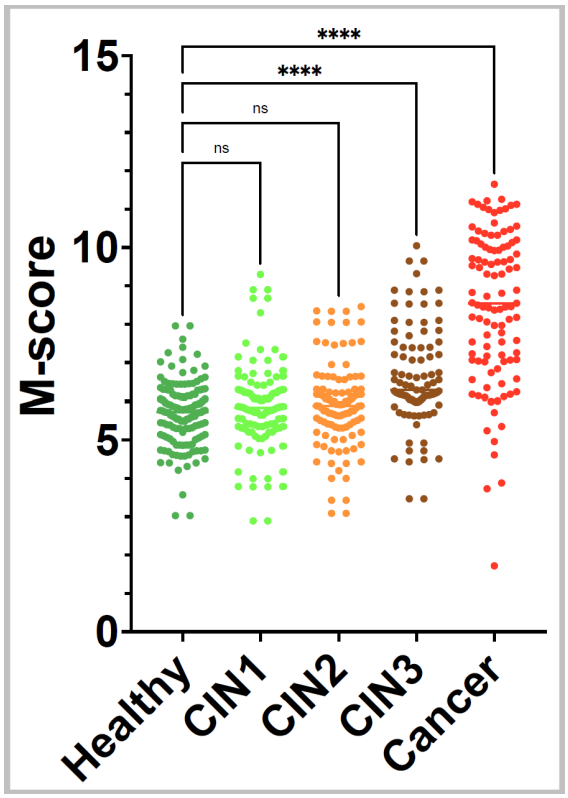
WE USED TCGA PUBLIC DATA FROM THE FOLLOWING 14128 TUMOR SAMPLES TO TEST EPIPANCANCER MARKERS

Study Abbreviation	Study Name	Cancer Subject number
ACC	Adrenocortical carcinoma	80
BLCA	Bladder Urothelial Carcinoma	418
BRCA	Breast invasive carcinoma	796
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma	309
CHOL	Cholangiocarcinoma	36
COAD	Colon adenocarcinoma	315
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma	48
ESCA	Esophageal carcinoma	186
GBM	Glioblastoma multiforme	153
HNSC	Head and Neck squamous cell carcinoma	530
KICH	Kidney Chromophobe	66
KIRC	Kidney renal clear cell carcinoma	325
KIRP	Kidney renal papillary cell carcinoma	276
LAML	Acute Myeloid Leukemia	140
LGG	Brain Lower Grade Glioma	534
LIHC	Liver hepatocellular carcinoma	380
LUAD	Lung adenocarcinoma	411
LUSC	Lung squamous cell carcinoma	337
MESO	Mesothelioma	87
OV	Ovarian serous cystadenocarcinoma	10
PAAD	Pancreatic adenocarcinoma	185
PCPG	Pheochromocytoma and Paraganglioma	184
PRAD	Prostate adenocarcinoma	503
READ	Rectum adenocarcinoma	99
SARC	Sarcoma	265
SKCM	Skin Cutaneous Melanoma	473
STAD	Stomach adenocarcinoma	394
TGCT	Testicular Germ Cell Tumors	156
THCA	Thyroid carcinoma	515
THYM	Thymoma	124
UCEC	Uterine Corpus Endometrial Carcinoma	439
UCS	Uterine Carcinosarcoma	57
UVM	Uveal Melanoma	80
	Different Cancers from GEO NCBI	5217
Total number		14128

DETECTION OF CERVICAL CANCER BY EPIPANCANCER

We further validated our epiPancancer next generation sequencing test in our lab in HK on a cohort of 549 clinical cervical specimens from McGill University (136 normal, 106 CIN1, 111 CIN2, 93 CIN3, 103 cancers (50 ADC and 53 SSC). The figure below shows that the **methylation score** that we developed **detects cancer** in numerous cancer patients (Figure A) but not in cervical samples from healthy people (each spot represents one person). **The specificity** is **95%** and **sensitivity** of cervical cancer detection is **78%** and area under the curve is 0.9.

A



B

Healthy vs	AUC	Sensitivity with 95% specificity
CIN1	0.55	12.26%
CIN2	0.56	12.73%
CIN3	0.74	34.41%
Cancer	0.9	78%

DETECTION OF LIVER CANCER BY EPIPANCANCER

We also validated our epiPancancer next generation sequencing test on a cohort of 403 plasma blood samples from icddr, b, one of the leading research institutes of the Global South (an international Health research organisation located in Dhaka, Bangladesh).

- 49 healthy (cohort#2)
- 50 patients with hepatitis B,
- 34 patients with Stage A of liver cancer (HCC-A),
- 86 with stage B,
- 106 (HCC-B) stage C (HCC-C) and
- 77 stage D (HCC-D).

Healthy cohort (“Healthy”) is a commercially available set of blood samples with confirmed healthy status of individuals purchased from a US company.

Cohort#1 are the people that used our epiPancancer test service.

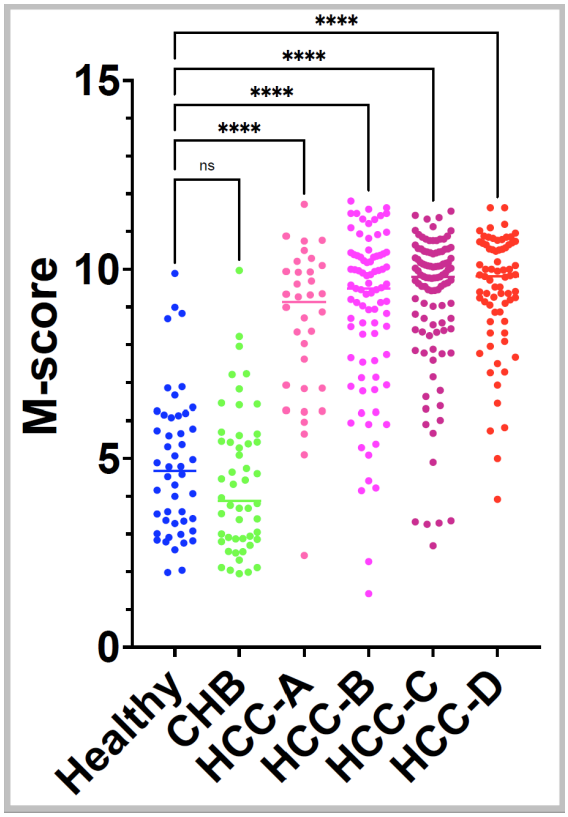
Cohort#2 are individuals who participated in clinical study in icddr, b.

Detection of Liver Cancer by epiPancancer

The figure A below shows that the **methylation score** that we developed detects **cancer** in numerous cancer patients (HCC-A, HCC-B, HCC-C and HCC-D) (each spot represents one person).

Table B demonstrates overall performance of our epiPancancer test in people with confirmed liver cancer with stages 1 to 4.

A



B

Healthy vs	AUC	Sensitivity with 95% specificity
HCC-A	0.98	88%
HCC-B	0.97	88%
HCC-C	0.98	92%
HCC-D	0.99	94.5%

EPIPANCANCER TEST DETECTS CANCER

1. However it doesn’t detect the specific origin of the cancer
2. The result of epiPancancer means that there is cancer DNA from any of the types listed in the table
3. But it isn't a diagnosis of cancer. This test needs to be followed up with specific cancer test or careful examination by your physician
4. Our analysis is based on thousands of DNA methylation samples from clinical data that originated in tumors
5. Needs to be repeated on clinical data from our own patients that originates in cell free tumor DNA in blood
6. epiPancancer is not a definitive test and the final conclusion should be done if needed by your physician and would be based on a biopsy pathology report.

EPIPANCANCER REPORT

- We will provide a **methylation score**
- Using test results from a cohort of healthy patients, we will let you know whether **your sample score** is beyond normal healthy samples and possibly in the **cancer range**