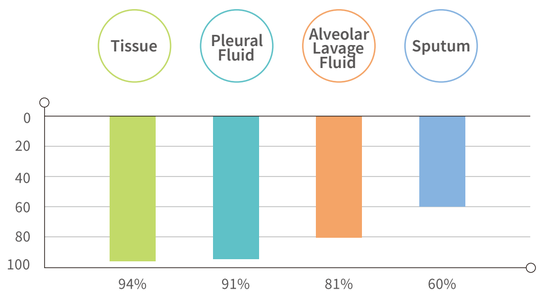
Lung-Me™ Clinical Diagnostics Performance

Lung-Me™ Diagnostic Capability using Different Sample Types



Lung-Me™ Applicable Population

- Patients with negative biopsy examination
- Patients with unexplained pleural effusion
- Patients with negative cytology examination
- Patients with small nodules and shadow in lungs
- Patients with positive tumor marker examination

Lung-Me™ DNA Methylation Detection Kit

LungMe™

CFDA CE Certified

Clinical Application	Lung cancer high-risk population: Diagnose early-stage lung cancer and differentiate malignant and benign lung small nodules		
Sample Type	Alveolar lavage fluid/Rinsing fluid/Brush eluent (3-5mL)		
Detection Method	Methylation specific real-time PCR method		
Applicable Instruments	Real-time PCR Instrument (Including FAM, VIC/HEX, Cy5 channels)		
Specification	20 tests/box	Storage Conditions	-20°C and below for 9 months
Components	PCR reaction solution; DNA polymerase; Positive and negative control		
Sample Storage	Fresh samples: Store at room temp. (Process sample within 24 hrs); Centrifuge sample and resuspend in Tellgen cell preservation solution: Store at room temp. (7 days), 2-8°C (2 months), ≤-20°C (6 months)		

Lung-Me™ Detection Process and Results Analysis

Detection Process: Results in 6 hrs in any standard laboratory

Detection Results: Sensitivity with

S. No.	Sample Type	DNA Conc. (ng/ul)	AC CY5(Ct) 18≦Ct≦23	RASSF1A FAM(Ct) <35	RASSF1A Result (POS and NEG)	SHOX2 VIC(Ct) <32	SHOX2 Result (POS and NEG)	Methylation Result	Cytology Diagnosis Result	Other Pathological Diagnostic Results
1	Brush eluent	30	20.08	26.7	POS	22.5	POS	POS	Cancer cells	Poorly differentiated adenocarcinoma
2	Alveolar lavage fluid	8.2	20.69	28.02	POS	NoCt	NEG	POS	Abnormal cells	Right lung squamous cell carcinoma
3	Pleural fluid	157.2	19.89	NoCt	NEG	26.24	POS	POS	Abnormal cells not oberserved	Squamous cell carcinoma
4	Alveolar lavage fluid	15.2	20.12	NoCt	NEG	34.84	NEG	NEG	Dust cell and red blood cells observed	Chronic inflammation

Note: If either RASFF1A or SHOX2 is POS, then sample is determined to be DNA methylated.

★ If DNA methylation is detected in a sample, this hints that there is an increased possibility of malignant lung lesions; if DNA methylation is not detected in a sample, this hints that there is an increased possibility of benign lung lesions, but does not exclude the possibility of malignancy.

★ Patients are recommended to undergo cytology examination aside DNA methylation detection. If sample is negative in both alveolar lavage fluid cytology and DNA methylation detection, follow-up checks and monitoring is strongly recommended. If sample is positive in either alveolar lavage fluid or DNA methylation detection, patients are recommended to undergo further examination to diagnose condition at the soonest.

References

[1] Dietrich ,et al. Diagnostic Molecular Pathology, 2012,21(2):93-104.
[2] Wu X M, et al. Asian Pacific Journal of Cancer Prevention Apjcp, 2014, 15(19):8451-4.
[3] Zhang Y.M., et al. Journal of Chinese Oncology, 22(2016).12.1032-1036.
[4] Ren M.P., et al. Annals of Diagnostic Pathology 27(2017). 57-61.
[5] Zhang C.Z., et al. Journal of Cancer 8(2017) 17. 3585-3591.

Tellgen

LungMe™ Lung Cancer DNA Methylation Detection Kit

SHOX2 + RASSF1A Real-Time PCR Method

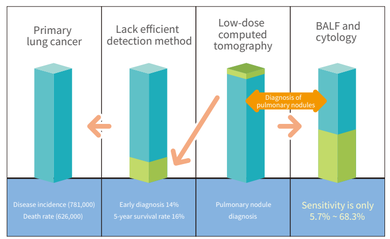
Early Diagnosis of Lung Cancer, Differentiation of benign and malignant pulmonary nodules

More Sensitive, More Stable

CFDA CE | Exclusive Patent

A new era in lung cancer molecular diagnostics

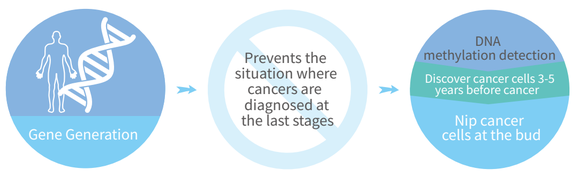
Current situation in lung cancer diagnostics



Auxiliary diagnosis of malignant/benign pulmonary nodules in high-risk population

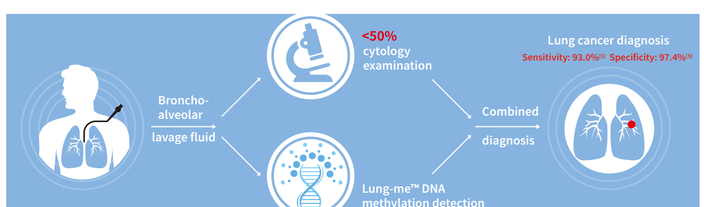
- Bronchoalveolar lavage fluid (BALF) is a non-invasive fluid cytology for diagnosing lung diseases
- BALF cytology testing is greatly affected by sujective factors, therefore sensitivity ranges from 5.7% to 68.3%
- BALF DNA methylation detection is more sensitive and stable, a solid backup for cytology. (Sensitivity can reach 71.5% ~ 83.2%, specificity can reach 90% ~ 97.4%

Cancer and DNA Methylation



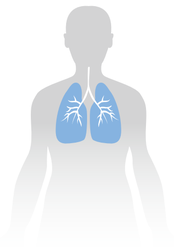
DNA methylation happens when a methyl group (CH3) is added to the fifth carbon of the cytosine ring by a DNA methyltransferase.

Lung-Me™ DNA methylation detection can be used for cancer diagnostics



Lung-Me™ DNA Methylation Detection

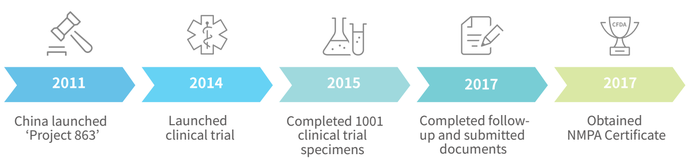
Double Marker Detection



SHOX2	RASSF1A
Short Stature Homeobox2	Ras-association domain family 1A
Transcription factor in growth and reproduction	Tumor supressor gene
Can regulate growth and drives embryonic development processes and organ development	Regulate cell cycle arrest, migration, microtubular stabilization, apoptosis promotion and so on
Excellent marker in early-stage cancer detection[1]	Closely related to lung adenocarcinoma[2]

Combining SHOX2 and RASSF1A double gene methylation detection, patient diagnostics can be much more efficient

Research Process



Diagnostic capability is significantly higher than traditional cytology examination

Clinical Trial Information

Clinical trial location:

Shanghai Chest Hospital

Sir Run Run Shaw Hospital

The First Affiliated Hospital of Zhengzhou University

Clinical trial specimens:

Diagnosed lung cancer 592 specimens

Unclear lung cancer 409 specimens

	Lung-Me™ (Sensitivity)	Cytology (Sensitivity)
Lung cancer group (558)	78%	53%
Cancer staging (508)		
I (100)	74%	27%
II (81)	79%	49%
III (182)	79%	64%
IV (145)	81%	66%
Cancer typing (455)		
Adenocarcinoma (214)	66%	39%
Squamous cell carcinoma (163)	88%	69%
Small cell carcinoma(78)	94%	60%
Tumor location (532)		
Centralized (279)	84%	66%
Peripheral (253)	74%	41%
Tumor size (466)		
⩽1.0cm (182)	76%	50%
1.0~5.0cm (183)	78%	47%
⩾5.0cm (101)	79%	56%

Lung-Me™ Clinical Diagnostic Ability

An early warning for lung cancer

In the 82 follow-up patients with positive DNA methylation but no clinical diagnosis of lung cancer, 29 patients have been diagnosed with lung cancer since August 2018 (43 months post initial test for DNA methylation)

Hospital	Single Positive Methylation (number)	Follow-up lung cancer diagnosis (number)
Shanghai Chest Hospital	31	17 (55%)
Sir Run Run Shaw Hospital	20	7 (35%)
The First Affiliated Hospital of Zhengzhou University	31	5 (16%)
Total	82	29 (35%)

Lung-Me™ comparison with cytology study

DNA methylation detection alone is more sensitive and stable compared to conventional cytology studies as it is less affected by sample and operational deviations.

Combining Lung-Me™ with cytology study increases sensitivity in diagnosis