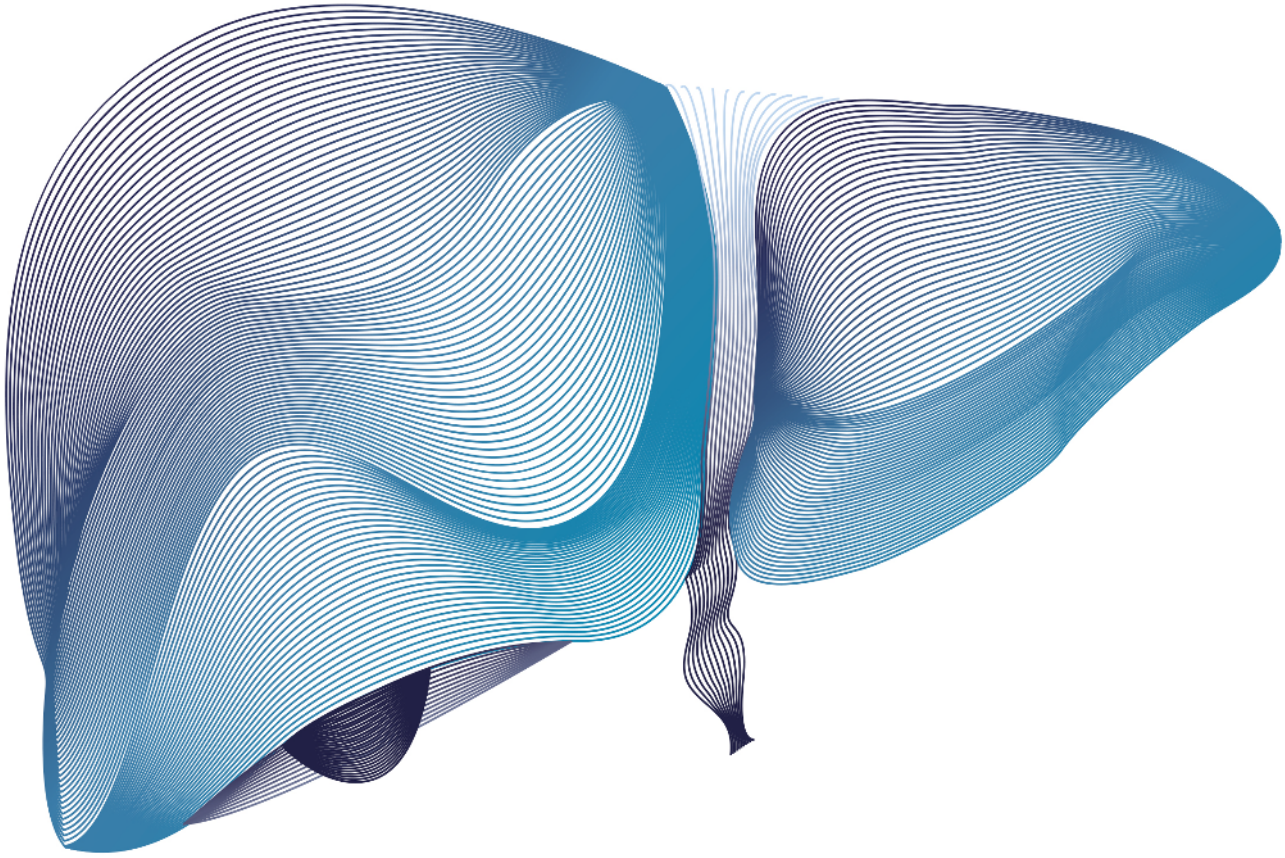
LINC Medical



Shaping the Future of Noninvasive Diagnostics with Artificial Intelligence

Clinical Management with

LIVERFASt™

In Use with Other Noninvasive Tests

What LIVERFASt™ is

LIVERFASt™ is a noninvasive panel of tests, combining patient's age, gender and body mass index with 10 blood biomarkers; correlated with liver conditions to generate three scores for estimating fibrosis, activity and steatosis.

For each condition, LIVERFASt™ evaluates a quantitative score (0.00 to 1.00) and its estimated grade or stage, similar to the liver biopsy classification.

LIVERFASt™ algorithm uses AI neural networks to optimize the clinical diagnosis according to the latest NAFLD histopathologic SAF scoring system, that covers the entire spectrum of lesions (from S0-A0-F0 to S3-A4-F4) and provides an activity score not biased by steatosis.

LIVERFASt™ vs other Standards of Care (SOC)

Only Test Specific to Three Lesions, able to Discriminate Fibrosis from Steatosis & Activity Without Bias

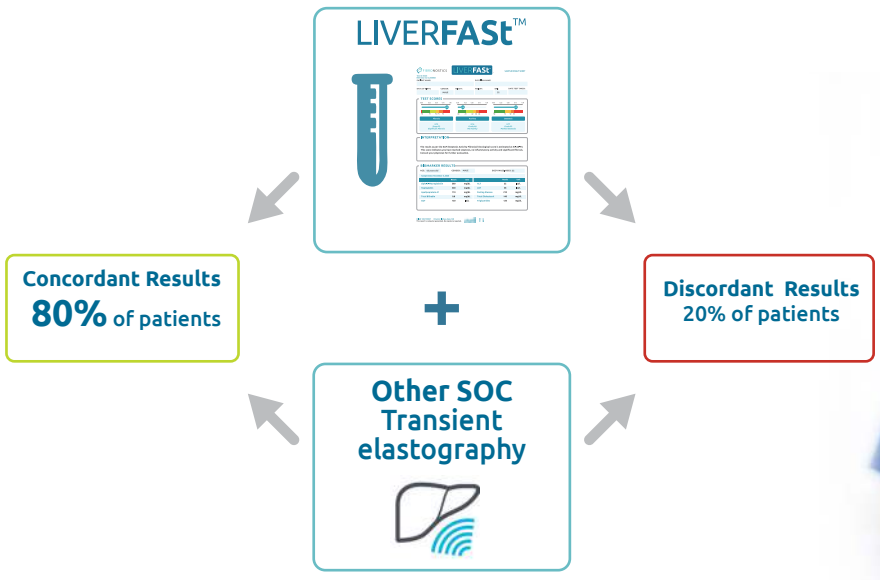
	LIVERFASt™	FIB-4	Vibration Controlled Transient Elastography (VCTE)	Liver Ultrasound (US)
Specific for **Fibrosis**	✓	✓	✓ (biased by BMI, liver inflammation and operator dependent)	✗ (detects only end-stage cirrhosis complication)
Specific for **Activity**	✓	✗	✗	✗
Specific for **Steatosis**	✓	✗	✓	✓ (raw semi-quantitative evaluation, operator dependent)
NASH Staging	✓	✗	✗	✗
No. of **Biomarkers**	**10**	**3**	**N/A**	**N/A**
Assessment **Towards fibrosis** unbiased by steatosis and liver inflammation	✓	✗ (AST/ALT)	✗ (biased by liver inflammation)	-

- Performance for cirrhosis similar to VCTE with better applicability and no failure
- Superior to VCTE for early fibrosis staging
- Superior to FIB-4 in diabetics
- Better steatosis grading than CAP and US for obese patients (BMI > 35)
- Provides activity grading to distinguish NASH from steatosis only
- High performance for identifying NASH vs no NASH

  AUROC (95% CI)
  0.88 (0.75-0.94)

LIVERFASt™ in use with other Standards of Care (SOC)

Noninvasive tests (NITs) are quantitative estimators (scores) for the severity of liver lesions. Specific cut-offs are used to convert scores in stages of the disease. Differences of two or more stages between NITs are called discordances



All Standards of Care (SOC) liver diagnosis including liver biopsy have risks of false positives or false negatives (RFPN) that should be taken into account when interpreting a result

LIVERFASt™ RFPN could be related to[1]:

- **Clinical conditions associated with extreme values of one of the biomarkers (2% of cases):**
  - Hemolysis (e.g. thalassemia, mechanical cardiac valvular prosthesis) could decrease haptoglobin serum levels with a risk of overestimating fibrosis
  - Inflammatory syndrome (e.g. infected ulcerated diabetic foot, urinary tract infection, etc) could increase haptoglobin serum levels with a risk of underestimating fibrosis
  - Massive hepatic cytolysis (e.g. acute hepatitis, severe DILI) could lead to RFPN
- **Not following instructions on the required pre-analytical conditions of the blood sample:**
  - Non-fasting blood drawn could increase serum glucose and triglycerides, resulting in overestimated steatosis
  - Improper storage of blood samples prior to blood analysis (e.g. shipping at room temperature) could lead to underestimation of liver enzymes (ALT and AST) and activity scores

Liver stiffness measurement (LSM) by Transient Elastography Pitfalls (RFPN)[2-3]:

- **Failure for one of the three quality criteria (18% of cases)** :
  - IQR/median LSM RATIO <30% / Success rate (SR)*≥ 60% / At least 10 valid measurements

    **SR: number of valid LSM on the total number of LSM*
- **Any condition that increases the liver stiffness could lead to LSM overestimation of fibrosis**
  - Liver inflammatory activity (cytolysis with ALT > 3x ULN)
  - Having MetS factors: T2D, high-blood pressure, BMI>30Kg/m²
  - Cholestasis, congestive hepatopathy (eg. tricuspid regurgitation, mitral stenosis) non-fasting patient, small intercostal spaces
- **Operator dependent (novice versus experienced)**

Liver Biopsy RFPN related to[1]:

- Poor quality: small sample size (≤20 mm) and fragmentation
- Important steatosis (could lead to underestimation of fibrosis)
- Sample and inter-pathologist variability

FIB-4 RFPN related to[4-5]:

- Age range (≥65 years, resulting in a high false positive rate)
- Overestimation of fibrosis with cytolysis, underestimation in patients with normal liver enzymes
- Dehydration (increases platelet count)

Example of Analysis of Discordance

CHECK	TRANSIENT ELASTOGRAPHY	LIVERFASt™
Reliability criteria RFPN	IQR/median ratio, SR, valid LSM	Extreme values of biomarkers (Fibronostics' security warnings)
Other factors that could impact noninvasive tests	• Liver enzyme level >x3 ULN • High BMI, Severe steatosis • Cholestasis, congestive hepatopathy, non-fasting	Lab error (values, units), non-fasting
Severe fibrosis indicators*	• Platelet count, Prothrombin index • Other NIT: FIB-4, AST/ALT ratio • Imaging (e.g ultrasound signs of portal hypertension or cirrhosis) • Endoscopy: esophageal varices	
Repeated non-invasive tests	Fluctuation of NITs or stable results (fluctuations indicate a less reliable result)	
Liver biopsy	Good quality sample ≥20mm	

In few cases, the analysis of discordance cannot identify the attributability of failure to LIVERFASt™ or to elastography. As a precaution, the most severe score should be taken into account.

* Independent criteria for the attributability of failure. Physicians should check for all clinical evidence supporting diagnosis.

Why does the LIVERFASt™ indicate severe fibrosis despite a normal range of all the biomarkers?

LIVER**FASt**™ combines age and gender with five blood biomarkers that may provide a score indicating advanced fibrosis or cirrhosis despite being in the normal range of the laboratory.

What happens if LIVERFASt™ is performed without fasting?

Fasting is mandatory for most of blood determinatons in order to obtain a lipid serum, especially when it comes to the lipid panel and fasting glucose determinations. If the LIVER**FASt**™ was performed in a patient without fasting (at least for 8 hours), serum triglycerides and glucose levels could be elevated and so, could lead to overestimation of the steatosis score.

Why does the LIVERFASt™ test indicate severe fibrosis when the liver enzymes SGPT (ALT) and SGOT (AST) are in the normal range?

Serum liver enzymes level is increasing with cytolysis and inflammatory activity in the liver. Liver enzymes, when elevated, could be an indicator of liver disease. LIVER**FASt**™ combines only biomarkers specific for liver fibrosis and therefore, it could detect liver fibrosis even in patients having normal ALT and AST. One third of diabetic patients could have normal liver enzymes despite advanced fibrosis. Liver enzymes are decreasing with age and with the severity of liver fibrosis and therefore should not be used to screen for liver fibrosis.

Should I rely on liver biopsy or on LIVERFASt™ result?

80% of patients having had both LIVER**FASt**™ and liver biopsy have concordant results. In 20% of cases, differences of 2 stages or more could occur. The attributability of the flaw could be either in the test or in the liver biopsy mainly related to biopsy sample variability, small size or inter-pathologist variability. A study performed using double biopsy in the left and right lobe of the liver in patients with NAFLD concluded: "a 41% variability rate for one fibrosis stage was observed, as histologic lesions of steatohepatitis are unevenly distributed throughout the liver parenchyma." (Gastroenterology. 2005)

My patient's BMI is very high, is LIVERFASt™ reliable?

Yes, contrary to liver ultrasound and transient elastography measurements that are impacted by high BMI, LIVER**FASt**™ is based on blood biomarkers that are not related to the BMI. Moreover, the BMI itself is used as a surrogate for steatosis evaluation along with other blood biomarkers.

ABBREVIATIONS

CAP	**Controlled Attenuation Parameter**
CHD	**Coronary Heart Disease**
DILI	**Drug Induced Liver Injury**
IQR	**Inter Quantile Range**
LSM	**Liver stiffness measurement**
MetS	**Metabolic syndrome**
RFPN	**Risk of false positives or false negatives**
SOC	**Standard of care**
SR	**Success rate**
T2D	**Type 2 Diabetes**
ULN	**Upper limit of the normal (Lab range)**

REFERENCES

1 de Ledinghen V, Hepatology 2020.72;1:906A.
2 Castera L, Hepatology 2010 51:828-35.
3 Nascimbeni F, Clin Gastroenterol Hepatol 2014;13: 763-71.
4 Bedossa P, Hepatology 2003, 38:1449-57.
5 Ratziu V, Gastroenterology 2005, 128:1898-1906.
6 McPherson S, Am J Gastroenterol. 2017;112:740-51.
7 Bedossa P. and al. Hepatology 2012;56:1751-9.
8 Decraecker M, Aliment Pharmacol Ther 2022.

LINC Medical

UK Distributor
LINC Medical Systems LTD
7 Kingsley Street
Leicester
LE2 6DY
UK

Telephone:
+44 (0)1572 717515

Email:
sales@linc-medical.co.uk

Website:
www.linc-medical.co.uk

CE