Technical Recommendations for FibroTest-ActiTest, FibroMax and NASH-FibroTest assays

A guide for biologists and medical laboratories

BioPredictive
218 Boulevard Saint-Germain
75007 PARIS
FRANCE

Version 3.5.3
Last Modification: 2022-01-06

Table of contents

Introduction

BioPredictive is an independent bio-pharmaceutical company, based in France, which produces and markets diagnostic tests for liver diseases.

Understanding of BioPredictive's products line

Liver Tests

BioPredictive product line is made of easy to perform and easy to interpret blood-based diagnoses:

- **FibroTest**, assessing liver fibrosis and cirrhosis,
- **ActiTest**, assessing liver necroinflammatory,
- **SteatoTest** (versions 1 and 2), assessing liver fat overload (steatosis or fatty liver),
- **NashTest** (versions 1 and 2), assessing liver inflammation (NASH) for metabolic diseases,
- **AshTest**, assessing liver inflammation for alcoholic diseases,
- **HCV-GenoFibroTest**, combining genomics with FibroTest to predict SVR for some HCV treatments,
- **SteatoScreen**, a screening for metabolic diseases,
- **Elasto-FibroTest**, combining FibroTest and elastometry results for an improved assessment of liver fibrosis using elastography devices.

Each test comes with a score, and its translation in stage/grade to ease interpretation.

Bundle Tests

Most BioPredictive tests are offered in bundles. These packages allow physicians to benefit from a clear and enlarged insight of the diagnostic necessary for the adequate medical decision, using a single prescription.

BioPredictive offers the following bundles:

- ***FibroTest-ActiTest***, including FibroTest and ActiTest
- ***FibroMax***, including FibroTest, ActiTest, SteatoTest, NashTest and AshTest
- ***NASH-FibroTest***, including FibroTest, SteatoTest 2, NashTest 2, ActiTest and AshTest

FibroTest-ActiTest

FibroTest and ActiTest are used for the assessment of liver fibrosis and necroinflammatory lesions, respectively. They are considered an alternative to liver biopsy in patients with the most frequent chronic liver diseases (1-3).

FibroTest-ActiTest are computed from the results of six biochemical parameter assays (alpha2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, GGT, ALT), adjusted for age and gender. The algorithms used to calculate FibroTest and ActiTest have been defined and validated in different clinical studies and have been patented.

FibroTest-ActiTest are biological tools now developed worldwide and the quality of their determinations depends on the transferability of results of their component assays between laboratories (4)(5). Assay results are still dependent on the analytical system used. To ensure the homogeneity of the results between laboratories, the International Federation of Clinical Chemistry (IFCC) has described reference methods for the different parameter measurements. The analytical methods set up on the various analyzers must be in accordance with the reference methods and/or reference materials (traceability) in order to comply with the rules of international standardization and European directives.

The same recommendations are applied to the **HCV Geno-FibroTest** (combining FibroTest-ActiTest with *IL28B genotype,* HCV genotype, HCV viral load) and the **Elasto-FibroTest** (combining FibroTest with liver stiffness measurement). (32-33)

FibroMax

Five different combinations of tests are included under the name **FibroMax** (6): FibroTest, ActiTest, SteatoTest, NashTest, AshTest. In the three last combinations, cholesterol, triglycerides, AST and fasting glucose are added to the FibroTest-ActiTest components for assessing liver steatosis, metabolic steatohepatitis and alcoholic liver disease.

- FibroTest is proposed for patients with chronic viral hepatitis C and B, alcoholic liver disease and metabolic steatohepatitis (overweight, diabetes, hyperlipidaemia).
- SteatoTest is for patients with chronic viral hepatitis B and C, alcoholic liver disease and metabolic steatohepatitis (overweight, diabetes, hyperlipidaemia).
- ActiTest is for patients with chronic viral hepatitis C and B
- NashTest is for patients with metabolic steatosis (overweight, diabetes, hyperlipidaemia).
- AshTest is for patients with alcoholic liver disease (acute alcoholic hepatitis).

The same recommendations are applied to the **SteatoScreen** (combining FibroTest and SteatoTest). (34)

NASH-FibroTest

Five different combinations of tests are part of the **NASH-FibroTest**: FibroTest, SteatoTest 2, NashTest 2, ActiTest and AshTest. NASH-FibroTest is proposed for patients with metabolic liver diseases (metabolic steatohepatitis) with the following risk factors: overweight, diabetes or hyperlipidemia. (35,36).

- SteatoTest 2 is computed from the results of several biochemical parameter assays: alpha2-macroglobulin, haptoglobin, apolipoprotein A1, GGT, ALT, AST, total cholesterol, triglycerides and fasting glucose adjusted for age and gender. Compared to the first generation of SteatoTest, the weight, height and total bilirubin are no longer required for SteatoTest 2.
- NashTest 2 is computed from the results of several biochemical parameter assays: alpha2-macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, GGT, ALT, AST, total cholesterol, triglycerides and fasting glucose adjusted for age and gender. Compared to the first generation of NashTest, the weight and height are no longer required for NashTest 2.

- ActiTest and AshTest are also provided since they may be useful when NAFLD is associated with chronic viral hepatitis (ActiTest) or excessive alcohol consumption (AshTest).

Conditions of use

Biologists working in biomedical laboratories and wishing to benefit from calculated FibroTest, FibroMax, NASH-FibroTest and/or other unit tests mentioned above should respect the conditions indicated for performing the various parameter assays. These recommendations concern the **blood sample** itself, **the analytical methods to be used, calibration and control conditions**, and **reliability criteria of the assays** (reproducibility CV must be less than 5% for all parameters).

It is highly recommended that **the internal quality control should be associated with the external quality assessment** (accuracy control) in accordance with the biomedical laboratory's accreditation. This is mandatory in France.

A list of analyzers for which test result transferability has been verified is available. (Page 11)

The different biochemical tests

For each parameter result, the **units** indicated in brackets need to **be adhered to for the calculations of BioPredictive's tests**.

FibroTest combines 5 biochemical tests:

- Alpha2-macroglobulin (g/L)
- Haptoglobin (g/L)
- Apolipoprotein A1 (g/L)
- GGT (gamma glutamyl transpeptidase) (IU/L)
- Total bilirubin (micromoles/L)

ActiTest combines 6 biochemical tests:

- Alpha2-macroglobulin (g/L)
- Haptoglobin (g/L)
- Apolipoprotein A1 (g/L)
- GGT (gamma glutamyl transpeptidase) (IU/L)
- Total bilirubin (micromoles/L)
- ALT (Alanine Aminotransferase) (IU/L)

The results of the six components, adjusted for **gender** and **age**, are used to determine the FibroTest scores and ActiTest stages (1-3,6).

FibroMax combines the following biochemical assays:

- Alpha2-macroglobulin (g/L)
- Haptoglobin (g/L)

- Apolipoprotein A1 (g/L)
- GGT (gamma glutamyl transpeptidase) (IU/L)
- Total bilirubin (micromoles/L)
- ALT (alanine aminotransferase) (IU/L)
- AST (aspartate aminotransferase) I(U/L)
- Fasting glucose (mmol/L)
- Total cholesterol (mmol/L)
- Triglycerides (mmol/L)

The results of the assays are adjusted for patients' **age**, **gender**, **weight** (kg) and **height** (meters) for the calculation of FibroMax tests. (6)

NASH-FibroTest combines the following biochemical assays:

- Alpha2-macroglobulin (g/L)
- Haptoglobin (g/L)
- Apolipoprotein A1 (g/L)
- GGT (gamma glutamyl transpeptidase) (IU/L)
- Total bilirubin (micromoles/L)
- ALT (alanine aminotransferase) (IU/L)
- AST (aspartate aminotransferase) (IU/L)
- Fasting glucose (mmol/L)
- Total cholesterol (mmol/L)
- Triglycerides (mmol/L)

The results of the assays are adjusted for patients' **age** and **gender** for the calculation of NASH-FibroTest tests. (35-36)

A - Pre-analytical phase

Blood Samples

- Assays are performed on serum or plasma.
- For FibroTest-ActiTest, a blood sample is collected in a dry tube or one containing lithium heparin, preferably from a fasting patient or after a light meal. (7)
- For FibroMax and NASH-FibroTest it is *mandatory that the patient has had a fasting period of 12 hours* prior to the blood sample test. The blood is collected in a dry tube or one containing lithium heparin. An additional blood sample on glycolytic inhibitor (sodium fluoride and potassium oxalate) is requested for the fasting glucose assay.
- In the event of an isolated request for SteatoTest or NashTest, the blood samples are identical to those of FibroMax.
- In the event of an isolated request for SteatoTest 2 or NashTest 2, the blood samples are identical to those of NASH-FibroTest; except for SteatoTest 2 if ordered alone, which does not require bilirubin assay.
- The blood sample is centrifuged within 2 hours of being collected.
- The centrifugation conditions (speed and time) must conform to the recommendations of the tube manufacturer.
- For the FibroMax and NASH-FibroTest assays, a 500-microlitre volume of plasma or serum is necessary. For the fasting glucose assay, 200 microlitres are sufficient.
- Biochemical assays are preferentially done on ***fresh*** serum/plasma. Serum or plasma can be decanted and stored no more than 72 h at + 2°C/ + 8°C, protected from light (to avoid bilirubin degradation) (8).
- The measurement of the specific proteins (alpha2-macroglobulin, haptoglobin and apolipoprotein A1) can be carried out after centrifugation on serum stored at +2°C / +8°C for no more than 5 days (8–9).
- If the parameters cannot be measured within the required time interval,
  *- the serum / plasma must be frozen at – **80°C**.* (8)
  - frozen samples must not be thawed more than once.
  - after thawing in the laboratory temperature, the sera must be homogenized and then centrifuged at 1500 g for 10 min before performing the assays.

Interferences

Lipids and hemolysis interfere with the measurements.

It is possible to dilute a slightly lipemic serum that has already been centrifuged. Any milky and /or haemolysed serum must be rejected.

B – Analytical methods and analyzers used for the FibroTest-ActiTest, FibroMax and NASH-FibroTest assays

Transferability of FibroTest-ActiTest, FibroMax and NASH-FibroTest assays between analytical systems (i.e. analyzer-method-reagents together) has been verified while respecting the analytical conditions and on the analyzers mentioned below.

These conditions are a **guarantee of the reliability** of the FibroTest-ActiTest, FibroMax and NASH-FibroTest results.

Methods

- Enzyme activity measurements must be performed in accordance with the reference methods at 37°C, using **IFCC reference method** for AST and ALT. (19-20).

For GGT activity, when the IFCC method is not set up on the analyzer (for example, if the Szasz method is set up on Roche Diagnostics analyzers), the activity measurement has to be calibrated using C.f.a.s (Calibrator for automated systems), and the GGT value should be indicated for the IFCC method and not the Szasz method. **The C.f.a.s. calibration** of enzyme activity measurements is **highly recommended** (for example C.f.a.s Roche Diagnostics), as it corrects the automated analytical system variations and secures homogeneity of the results between laboratories (21). Enzyme activity, GGT, ALT, AST results are expressed in International Units per Liter (IU/L).

- Total bilirubin (µmol/L): diazo reaction is used in accordance with the Doumas method or is calibrated with a standard traceable to a Standard Reference Material (SRM).
- Cholesterol, triglycerides (mmol/L): analytical method traceable to a reference method or calibrator titrated against reference material.
- Fasting glucose (mmol/L): method with hexokinase, which limits bilirubin interference. Result assays must be traceable to a reference method.
- Alpha2-macroglobin, haptoglobin (g/L): nephelometric or turbidimetric method, calibrated using a traceable standard to the ERM-DA470k/IFCC reference material.
- Apolipoprotein A1 (g/L): nephelometric or turbidimetric method, calibrated using a traceable standard to the WHO-IFCC SP1-01 reference material.

Manufacturers and analyzers

Attention the name of some suppliers may be brought to change depending on possible mergers.

The marked analyzers cited and underlined below have been tested for the transferability of FibroTest-ActiTest, FibroMax and NASH-FibroTest assays. The others are from the same manufacturer and belong to the same range of analyzers. The manufacturers have confirmed that these analyzers share the same reagents, analytical method principles and traceability.

BECKMAN COULTER:

LX, DXC
Immage
and AU, formerly from Olympus
(AU400, AU480, AU600, AU640, AU680, AU2700, AU5800)

SIEMENS HEALTHCARE DIAGNOSTICS:

BN2, BNProspec, (formerly from Dade Behring), Atellica NEPH 630 and Atellica CH
RxL, ArX, XPand, VISTA (formerly from Dade Behring),
ADVIA 1650 (formerly from Bayer Diagnostics)

ABBOTT

ARCHITECT c8000
ALINITY c (only or integrated with other Alinity modules c or i)

ROCHE DIAGNOSTICS

HITACHI 917, INTEGRA 400,
MODULAR P, COBAS 6000 and 8000
COBAS Pro c503

THERMO FISCHER SCIENTIFIC

KONELAB 20, 20XT, 30, 60 prime analyzers
also named T20, T20XT, T30, T60 and T60 new Generation

ORTHO-CLINICAL DIAGNOSTICS

VITROS 4600, VITROS 5600, VITROS 5.1 (FUSION)

THE BINDING SITE GROUP LTD

OPTILITE – OPTIMISED PROTEIN SYSTEM

Analyzers and analytical methods evaluated for FibroTest-ActiTest, FibroMax and NASH-FibroTest transferability of results

Protein assays

Alpha2-macroglobulin and haptoglobin assays are standardized against the ERM-DA470k reference material (formerly CRM 470). **ERM-DA470k** is certified by the European Commission, Joint Research Centre, the Institute for Reference Materials and Measurements (Belgium) and selected by the International Federation of Clinical Chemistry (**IFCC).** The apolipoprotein A1 assay is standardized relative to the **WHO-IFCC SP1-01** reference material (World Health Organization-International Federation of Clinical Chemistry SP1-01). (13 - 15)

a) Immunonephelometric methods

Assays of alpha2-macroglobulin, haptoglobin, and apolipoprotein A1

- **BN2, BN Prospec, Atellica NEPH 630 analyzers** (Siemens Healthcare Diagnostics). Reagents and internal quality controls are commercialized by Siemens.
- **Immage analyzer** (Beckman Coulter) [see ***Remark C***]. New antiserum of the provider company is available for alpha2-macroglobulin. Since 2007 no more specific adjustment [see ***Remark C***] is needed for the apolipoprotein A1 results (9-10). Internal quality controls are commercialized by Beckman Coulter.

External quality assessment is highly recommended and mandatory in France.
For alpha2macroglobulin (if external controls are not available), the external quality assessment can be verified by a comparative study of results with another laboratory using the same method and analyzer.

b) Immunoturbidimetric methods

Assays of alpha2-macroglobulin

Alpha2macroglobulin assays were only performed for a long time using the nephelometric method on BN2, BN Prospec and Immage analyzers. Since 2005, the turbidimetric method has been developed and set up on various chemical analyzers (11). Reagents from Diagam are tested and used on most of them, with standards and internal controls requiring no correction.
Turbidimetric assay results using Diagam reagents were compared to those obtained on BN2 and BN Prospec nephelometers. Results obtained on Beckman analyzers were compared to those obtained on Immage.

- Modular P, Hitachi 917, Integra 400, Cobas 6000, Cobas 8000 and Cobas Pro c503 analyzers (Roche Diagnostics). Diagam reagents [see *Remark D*] or Dako Cytomation reagents. When Dako reagents are used, a specific adjustment [see *Remark B*] for the alpha2-macroglobulin result is needed. Dako Cytomation reagents are not validated on Cobas 6000 and Cobas 8000 (11).
- AU5800, AU400, AU480, AU600, AU640, AU680, AU2700 analyzers, formerly Olympus. Diagam reagents, standards and internal controls are used (contact BioPredictive Ref#D).
- Konelab 20, 20XT, 30, 60, (Thermo Fischer Scientific). Since 2007, these analyzers have been commercialized under the respective names T20, T20XT, T30, T60 and T60 New Generation. Diagam reagents with standards and internal controls are used [see *Remark D*] or Dako Cytomation reagents (contact BioPredictive Ref#G).
- Advia 1650 (Siemens Healthcare Diagnostics, formerly Bayer Diagnostics) and Atellica CH. Diagam reagents with standards and controls are used. [see *Remark D*].
- Architect c8000 and Alinity C analyzers (Abbott). Diagam reagents, standards and controls are used. [see *Remark D*].
- Vitros 4600, Vitros 5600, Vitros 5.1 Fusion (Ortho Clinical Diagnostics). Diagam reagents with standards and controls are used.
- Synchron LX / DXC (Beckman Coulter). Diagam reagents, standards and controls are used.
- Optilite – Optimised Protein System analyzer (The Binding Site Group LTD). Reagents, standards and internal quality controls are commercialized by The Binding Site Group LTD. [see *Remark G*]

External quality assessment is highly recommended and mandatory in France.

For alpha2macroglobulin (if external controls are not available), the external quality assessment can be verified by a comparative study of results with another laboratory using the same method and analyzer.

Haptoglobin and apolipoprotein A1 assays

- Modular P, Hitachi 917, Integra 400, Cobas 6000, Cobas 8000 and Cobas Pro c503 analyzers (Roche Diagnostics). Reagents, standard and internal controls are marketed by Roche Diagnostics (11).
- Dimension-RxL, ArX and Xpand analyzers (Siemens Healthcare Diagnostics, formerly Dade Behring) [see *Remark A*]. Reagents, standards and internal controls are marketed by Siemens Healthcare Diagnostics (12).
- AU 400, AU 480, AU 600, AU 640, AU 680 and AU 2700, AU 5800 analyzers (Beckman Coulter, formerly Olympus) [see *Remark E*]. Reagents are marketed by Beckman Coulter, *except for the* apolipoprotein A1 assay using Diagam reagents instead of manufacturer reagents, which have been validated. Internal quality control is marketed by Beckman Coulter and Diagam.
- Konelab 20, 20XT, 30, 60, Prime analyzer (Thermo Fisher Scientific). Reagents, standards and controls are marketed by Thermo Fisher Scientific. More recently, the analyzers have been marketed under the respective names T20, T20XT, T30, T60 and T60 New Generation.

- Advia 1650 analyzer (Siemens Healthcare Diagnostics, formerly Bayer Diagnostics) and Atellica CH. Reagents, standards and internal controls are marketed by Siemens Healthcare Diagnostics. [see *Remark F*].
- Architect c8000 and Alinity c analyzers (Abbott). Reagents, standards and controls are marketed by Abbott.
- Vitros 4600, Vitros 5600, Vitros 5.1 Fusion (Ortho Clinical Diagnostics). Reagents, standards, and controls are marketed by Ortho Clinical Diagnostics.
- Optilite – Optimised Protein System (The Binding Site Group LTD) (haptoglobin only). Reagents, standards and internal quality controls for haptoglobin are commercialized by The Binding Site Group LTD. [see *Remark G*]

For haptoglobin and apoliprotein A1 assays performed on the different analyzers, the external quality assessment is highly recommended. This is mandatory in France.

Protein results are expressed in grams per liter g/L.

Gamma-GT, ALT and AST assays

The homogeneity of the enzyme activity results (GGT, ALT, AST) between the analytical systems used for the validation of FibroTest / FibroMax / NASH-FibroTest must be secured. The best way to achieve this is to calibrate the activity measurement and to use the same C.f.a.s calibrator (Roche Diagnostics: IFCC value for AST, ALT and for GGT). Independent of this, for the automated analytical system used and for each parameter, the biologist needs to check with the manufacturer the traceability of results to a reference method and /or to reference material (16 – 22).

- Hitachi 917, Modular P, Integra 400, Cobas 6000, Cobas 8000 and Cobas Pro c503 analyzers (Roche Diagnostics). Reagents are provided by Roche Diagnostics.

Methods used are standardized against the reference IFCC method. AST and ALT activity measurements are performed at 37°C and calibrated using C.f.a.s (Calibrator for automated systems - Roche Diagnostics). [*see Remark H*]

GGT activity is measured according to the Szasz method on Roche Diagnostics analyzers. In order to comply with the standardisation rules, GGT activity measurement needs to be calibrated with C.f.a.s using the GGT target value indicated for the IFCC method. Internal quality controls are marketed by Roche Diagnostics.

- Dimension-RxL, ArX and XPAND analyzers (Siemens Healthcare Diagnostics, formerly Dade Behring) Reagents, standards and internal controls are provided by Siemens (12).

- AU5800, AU400, AU480, AU600, AU640, AU680 and AU2700 analyzers (Beckman Coulter, formerly Olympus). Reagents, standards and internal controls are provided by Beckman *[see Remark E]*.

- Konelab 20, 20XT, 30, 60, Prime analyzers (Thermo Fisher Scientific). Reagents, standards and internal controls are marketed by Thermo Fisher Scientific. These analyzers have recently been commercialized under the respective names T20, T20XT, T30, T60 and T60 New Generation.

- Architect c8000 and Alinity c analyzers (Abbott). Reagents, standards and internal controls are provided by Abbott.

- Advia 1650 analyzer (Siemens Healthcare Diagnostics, formerly Bayer Diagnostics) and Atellica CH. Reagents, standards and controls are marketed by Siemens.

- Vitros 4600, Vitros 5600, Vitros 5.1 Fusion (Ortho Clinical Diagnostics). Reagents, standards and internal controls are marketed by the manufacturer.

In summary

- For GGT: enzymatic method according to the IFCC reference method (19). For GGT on Roche analyzers, the Szasz method is used, which is very close to the IFCC method. The GGT enzymatic activity measurement needs to be calibrated using C.f.a.s with the IFCC target value indicated for the GGT (and not the Szasz value).
- For ALT and AST: enzymatic method in accordance with the IFCC reference method at 37°C (20-21) [*see Remark H*].

In order to make the transferability of the results of the enzymatic activities (GGT, ALAT, ASAT) reliable between the analytical system used for the validation of FibroTest/FibroMax/NASH-FibroTest and the other analytical systems referenced in these recommendations, it is necessary to :

  - calibrate using CFAS (IFCC value for AST, ALT and GGT),
  - or include this calibrator in the series of samples to be analyzed to determine, if a correction factor is needed to correct the results.

In all cases and before calculating a FibroTest / FibroMax / NASH-FibroTest, it is important to check the traceability of the results obtained with the supplier of the analyzer.

- Accuracy is controlled with the external quality assessment and is highly recommended. It is mandatory in France.
- Enzymatic activities of GGT, ALT and AST are expressed in International Units per litre (IU/L).

Total bilirubin assay

- Hitachi 917, Integra 400, Modular P, Cobas 6000, Cobas 8000 and Cobas Pro c503 analyzers (Roche Diagnostics). Reagents, standard and internal controls are provided by Roche Diagnostics. Measurement methods: diazo reactions according to Jendrassik Grof (23) for Hitachi 917, Malloy-Evelyn (24) for Integra 400 and Wahlefeld (25) for Modular. The different methods were initially calibrated using the C.f.a.s (Roche Diagnostics). Internal quality controls are marketed by Roche Diagnostics.
- Dimension-RxL, ArX and XPAND analyzers (Siemens Healthcare Diagnostics, formerly Dade Behring). Analytical Method: diazo reaction according to Doumas modified (26). Reagents, standards and internal controls are provided by Siemens.
- AU5800, AU400, AU600, AU640 and AU2700 analyzers (Beckman, formerly Olympus). Analytical Method: diazo reaction using DPD. Reagents, standards and internal controls are provided by Beckman Coulter.
- Konelab 20, 20XT, 30, 60, Prime analyzers (Thermo Fisher Scientific). Analytical Method: diazo reactions using DPD. Reagents, standards and internal controls are provided by Thermo Fischer. Since 2007, these analyzers have been commercialized by Siemens under the respective names T20, T20XT, T30, T60 and T60 New Generation.
- Architect c8000 and Alinity c analyzers (Abbott). Analytical Method: diazo reactions (surfactant accelerator not described by the manufacturer). Reagents, standards and internal controls are provided by Abbott.
- Advia 1650 analyzer (Siemens Healthcare Diagnostics). Analytical Method: diazo reaction using DPD. Reagents, standards and internal controls are provided by Siemens.
- Atellica CH analyzer (Siemens Healthineers). Analytical Method: Vanadate oxydation. Reagents, standards and internal controls are provided by Siemens.
- Vitros 4600, Vitros 5600, Vitros 5.1 Fusion (Ortho Clinical Diagnostics). Reagents, standards and internal controls are provided by Ortho Clinical Diagnostics.

Remarks

As previously recommended and regardless of the analyzer used, the biologist needs to check with the manufacturer the traceability of results to a reference method or reference material. The external quality assessment is highly recommended and mandatory in France.

Results are expressed in micromoles per litre (µmol/L).

Table 1: Analyzers and FibroTest-ActiTest / FibroMax / NASH-FibroTest assays

This is a temporary list. Several studies are underway to include other analyzers and reagents proposed by different manufacturers.

Parameter	Analyzer	Reagents/ Manufacturer	Material or Reference method	Analytical Method	Units
Alpha-2 macroglobulin	*Siemens Healthcare Diagnostics (formerly Dade Behring)* **BN2** **BN-Prospec** **Vista** **Atellica NEPH 630** **Atellica CH**	Siemens Diagam	ERM DA 470k (CRM 470) ERM DA 470k (CRM 470)	Nephelometry Turbidimetry	g/L
	Beckman Coulter **Immage**	Beckman	ERM DA 470k (CRM 470)	Nephelometry ***[Remark C]***	g/L
	Roche Diagnostics **Hitachi 917** **Modular P** or **Integra 400** *utility channel* **Cobas 6000** **Cobas 8000** **Cobas Pro c503**	Diagam or Dako Cytomation Diagam	ERM DA 470k (CRM 470) ERM DA 470k (CRM 470) ERM DA 470k (CRM 470)	Turbidimetry *No correction for Diagam* ***[Remark D]*** Turbidimetry Correction for Dako ***[Remark B]*** Turbidimetry *No correction for Diagam* ***[Remark D]***	g/L
	Beckman Coulter (formerly Olympus) **AU5800 (AU400, AU480, AU600, AU640, AU680, AU2700)** **Synchron LX** **DXC**	Diagam Diagam	ERM DA 470k (CRM 470) ERM DA 470k (CRM 470)	Turbidimetry Turbidimetry	g/L
	Thermo Fischer Scientific (Konelab) **T20, T20XT, T30, T60, T60 New Generation**	Diagam or Dako Cytomation	ERM DA 470k (CRM 470)	Turbidimetry *No correction for Diagam* ***[Remark D]***	g/L

Parameter	Analyzer	Reagents/ Manufacturer	Material or Reference method	Analytical method	Units
Alpha-2 macroglobulin	*Siemens Healthcare Diagnostics (Formerly Bayer Diagnostics)* **Advia 1650**	Diagam	ERM DA 470k (CRM 470)	Turbidimetry *No correction*	g/L
	Abbott **Architect c8000 Alinity c**	Diagam	ERM DA 470k (CRM 470)	Turbidimetry *No correction*	g/L
	Ortho-Clinical Diagnostics **Vitros 4600, Vitros 5600 Vitros 5.1 (Fusion)**	Diagam	ERM DA 470k (CRM 470)	Turbidimetry *No correction*	g/L
	The Binding Site Group LTD **Optilite – Optimised Protein System**	The Binding Site Group LTD	ERM DA 470k (CRM 470)	Turbidimetry	g/L
Haptoglobin	*Siemens Healthcare Diagnostics (formerly Dade- Behring)* **BN2 BN-Prospec Vista Atellica NEPH 630 Atellica CH**	Siemens	ERM DA 470k (CRM 470) IRMM CRM 470	Nephelometry Turbidimetry	g/L
	Beckman Coulter **Immage**	Beckman Coulter	ERM DA 470k (CRM 470)	Nephelometry ***[Remark C]***	g/L
	Roche Diagnostics **Hitachi 917 Modular P Integra 400** **Cobas 6000 Cobas 8000 Cobas Pro c503**	Roche Diagnostics or Diagam Roche Diagnostics	ERM DA 470k (CRM 470) ERM DA 470k (CRM 470)	Turbidimetry ***[Remark D]*** Turbidimetry	g/L
	Siemens Healthcare Diagnostics (formerly Dade- Behring) **Dimension-RXL, ArX and XPand**	Siemens	ERM DA 470k (CRM 470)	Turbidimetry ***[Remark A]***	g/L
	Beckman Coulter (formerly Olympus) **AU5800 (AU400, AU480, AU600, AU680, AU640, AU2700)**	Beckman	ERM DA 470k (CRM 470)	Turbidimetry ***[Remark E]***	g/L

Parameter	Analyzer	Reagents/ Manufacturer	Material or Reference method	Analytical method	Units
Haptoglobin	*Thermo Scientific (Konelab)* **T20, T20XT, T30, T60, T60 New Generation**	Thermo Scientific	ERM DA 470k (CRM 470)	Turbidimetry	g/L
	Siemens Healthcare Diagnostics (formerly Bayer) **Advia 1650**	Siemens	ERM DA 470k (CRM 470)	Turbidimetry	g/L
	Abbott **Architect c8000** **Alinity c**	Abbott	ERM DA 470 (CRM 470)	Turbidimetry	g/L
	Ortho-Clinical Diagnostics **Vitros 4600, Vitros 5600** **Vitros 5.1 (Fusion)**	Ortho-CD	ERM DA 470 (CRM 470)	Turbidimetry	g/L
	The Binding Site Group LTD **Optilite – Optimised Protein System**	The Binding Site Group LTD	ERM DA 470 (CRM 470)	Turbidimetry	g/L
Apolipoprotein A1	*Siemens Healthcare Diagnostics (formerly Dade- Behring)* **BN2** **BN-Prospec** **Vista** **Atellica NEPH 630** **Atellica CH**	Siemens	WHO-IFCC SP1-01	Nephelometry Turbidimetry	g/L
	Beckman Coulter **Immage**	Beckman Coulter	WHO-IFCC SP1-01	Nephelometry ***[Remark C]***	g/L
	Roche Diagnostics **Hitachi 917** **Modular P** **Integra 400** **Cobas 6000** **Cobas 8000** **Cobas Pro c503**	Roche Diagnostics Or Diagam Roche Diagnostics	WHO-IFCC SP1-01	Turbidimetry *No correction* ***[Remark D]***	g/L
	Siemens Healthcare Diagnostics (formerly Dade- Behring) **Dimension RXL, ArX and Xpand**	Siemens	WHO-IFCC SP1-01	Turbidimetry ***[Remark A]***	g/L

Parameter	Analyzer	Reagents/ Manufacturer	Material or Reference method	Analytical method	Units
Apolipoprotein A1	*Beckman Coulter (formerly Olympus)* **AU400 / AU600 / AU640 / AU2700** **AU5800 (AU480, AU680) et AU400, AU600, AU640, AU2700**	Beckman (OSR6142) Diagam	WHO-IFCC SP1-01 WHO-IFCC SP1-01 et SP1-03	Turbidimetry Turbidimetry ***[Remark E]***	g/L
	Thermo Fischer scientific (Konelab) **T20, T20XT, T30, T60, T60 New Generation**	Thermo Fisher Scientific	WHO-IFCC SP1-01	Turbidimetry	g/L
	Siemens Healthcare Diagnostics (formerly Bayer) **Advia 1650**	Siemens	WHO-IFCC SP1-01	Turbidimetry Correction factor ***[Remark F]***	g/L
	Abbott **Architect c8000 Alinity c**	Abbott	WHO-IFCC SP1-01	Turbidimetry	g/L
	Ortho-Clinical Diagnostics **Vitros 4600, Vitros 5600 Vitros 5.1 (Fusion)**	Ortho-CD	WHO-IFCC SP1-01	Turbidimetry	g/L
GGT	*Roche Diagnostics* **Hitachi 917 Modular P Integra 400 Cobas 6000 Cobas 8000 Cobas Pro c503**	Roche	IFCC reference method (19)	Szasz method calibrated in accordance with IFCC method C.f.a.s – IFCC GGT value	IU/L
	Siemens Healthcare Diagnostics (formerly Dade-Behring) **Dimension-RXL, ArX and XPAND Vista**	Siemens	IFCC reference method (19)	According to IFCC method ***[Remark A]***	IU/L
	Beckman Coulter (formerly Olympus) **AU400, AU480, AU600, AU640, AU680, AU2700, AU5800**	Beckman	IFCC reference method (19)	Method in accordance with IFCC method	IU/L

Parameter	Analyzer	Reagents/ Manufacturer	Material or Reference method	Analytical method	Units
GGT	*Thermo Scientific (Konelab)* **T20, T20XT, T30, T60, T60 New Generation**	Thermo Fisher Scientific	IFCC reference method (19)	Method in accordance with IFCC method	IU/L
	Siemens Healthcare Diagnostics (formerly Bayer) **Advia 1650 Atellica CH**	Siemens	Modified IFCC method JCCLS_SOP CRM-001	According to IFCC method IFCC modified	IU/L
	Abbott **Architect c8000**	Abbott	IFCC reference method (19)	According to IFCC method**. Calibration C.f.a.s Roche**	IU/L
	Ortho-Clinical Diagnostics **Vitros 4600, Vitros 5600 Vitros 5.1 (Fusion)**	Ortho-CD	IFCC reference method (19)	Method in accordance with IFCC method	IU/L
Total Bilirubin	*Roche Diagnostics* **Hitachi 917 Modular P Integra 400 Cobas 6000 Cobas 8000 Cobas Pro c503**	Roche Diagnostics	Doumas reference method (25) Reference standard SRM	Diazo reaction: Jendrassik Grof (22) modified by Wahlefeld (24); Malloy-Evelyn (23)	µmol/L
	Siemens Healthcare Diagnostics (formerly Dade Behring) **Dimension-RXL, ArX and XPAND Vista**	Siemens	Doumas reference method (25) Reference standard SRM	Diazo reaction Modified Doumas method ***[Remark A]***	µmol/L
	Beckman Coulter (formerly Olympus) **AU5800 (AU400, AU480 AU600 AU640, AU680 AU2700)**	Beckman	Doumas reference method (25) Reference standard SRM	Diazo reaction (DPD)	µmol/L
	Thermo Fischer Diagnostics (Konelab) **T20, T20XT, T30, T60, T60 New Generation**	Thermo Fisher Scientific	Malloy Evelyn	Diazo reaction (DPD)	µmol/L

Parameter	Analyzer	Reagents/ Manufacturer	Material or Reference method	Analytical Method	Units
Total Bilirubin	*Siemens Healthcare Diagnostics (formerly Bayer)* **Advia 1650** **Atellica CH**	Siemens	AACC Reference method SRM 916	Oxidation by vanadate	µmol/L
	Abbott **Architect c8000** **Alinity c**	Abbott	NIST SRM 916a	Diazo reaction	µmol/L
	Ortho-Clinical Diagnostics **Vitros 4600, Vitros 5600** **Vitros 5.1 (Fusion)**	Ortho-CD	Standard Reference Material (SRM)	Diazo reaction	µmol/L
ALT	*Roche Diagnostics* **Hitachi 917** **Modular** **Integra** **Cobas 6000** **Cobas 8000** **Cobas Pro c503**	Roche Diagnostics	IFCC reference method (20) ***[Remark H]***	Enzymatic method according to the IFCC reference method	IU/L
	Siemens (formerly Dade Behring) **RxL** **Dimension RXL** **ArX** **XPAND** **Vista**	Siemens	IFCC reference method (20) ***[Remark H]***	Enzymatic method according to the IFCC reference method	IU/L
	Beckman Coulter (formerly Olympus) **AU5800 (AU400, AU480, AU600, AU640, AU680, AU2700)**	Beckman	IFCC reference method (20) [***Remark H]***	Enzymatic method according to the IFCC reference method	IU/L
	Thermo Fisher Scientific (Konelab) **T20, T20XT, T30, T60, T60 New Generation**	Thermo Fisher Scientific	IFCC reference method (20) ***[Remark H]***	Enzymatic method according to the IFCC reference method	IU/L
	Siemens Healthcare Diagnostics (formerly Bayer Diagnostics) **Advia 1650** **Atellica CH**	Siemens	IFCC reference method (20) ***[Remark H]*** IFCC reference method (20)	Enzymatic method according to the IFCC reference method IFCC modified P5P	IU/L
	Abbott **Architect c8000** **Alinity c**	Abbott	IFCC reference method (20) ***[Remark H]***	Enzymatic method with P5P according to the reference method IFCC.	IU/L
	Ortho-Clinical Diagnostics **Vitros 4600, Vitros 5600** **Vitros 5.1 (Fusion)**	Ortho-CD	IFCC reference method (20) ***[Remark H]***	Enzymatic method according to the reference method IFCC.	IU/L

Table 2: Analytical methods used for the complementary FibroMax and NASH-FibroTest assays: AST, total cholesterol, triglycerides, fasting glucose

Parameter	Material or Reference method	Analytical method	Units
AST	IFCC reference method (19) ***[Remark H]***	Enzymatic method according to the IFCC reference method. Enzymatic method with P5P according to the IFCC reference method for ARCHITECT c8000 and Alinity c. IFCC P5P modified method for Atellica CH	IU/L
CHOLESTEROL	Colorimetric test (27)	Standardised enzymatic method by enzymatic hydrolysis of cholesterol esters followed by a Trinder endpoint reaction	mmol/L
TRIGLYCERIDES	Colorimetric test (27)	Standardised enzymatic method by enzymatic hydrolysis of cholesterol esters followed by a Trinder endpoint reaction	mmol/L
FASTING GLUCOSE	UV test (28)	Hexokinase reference method	mmol/L

For these parameters, good transferability of the results was verified on the automated analyzers listed in Table 1.

In the case of use of different analyzers than those listed in Table 1, please contact BioPredictive (contact@biopredictive.com).

For the analyzers already in place in the laboratories, the biologist needs to check with the manufacturer the traceability of the results to the reference system, which must be in accordance with the European Directives DIVD instituted at the end of 2003.
If corrections need to be made to the programming tests, verify the corrections already done on the used analyzers with the manufacturers.

Remark A – About Dimension RXL, ARX and XPAND

A study of 150 chronic hepatitis C patients validated the use of FibroTest on the DIMENSION line analyzers. Alpha2macroglobulin is not available on DIMENSION analyzers. (contact BioPredictive Ref#A)

Remark B – About Dako Cytomation Reagents

A study on 146 patients validated the turbidimetric assay of alpha2-macroglobulin using Dako Cytomation reagents on Roche Diagnostics analyzers (11). An approximate 15% difference between nephelometry (BN2) and turbidimetry (Roche analyzers) was observed for the alpha2macroglobulin assays using Dako Cytomation reagent. For the FibroTest calculation, the introduction of *a correction factor for Modular and Cobas Integra* is necessary to ensure concordant results with those obtained with the system used as a reference (BN2 nephelometry).

These correction factors were established under very strict analytical conditions and are available from BioPredictive.

Biologists wishing to carry out these alpha2-macroglobulin assays as part of FibroTest on Roche Diagnostics analyzers must comply with the following recommendations:

- Use of DakoCytomation reagents, calibrators and controls.
- Conformance to the adaptation chart provided by Roche Diagnostics for Modular P and Cobas Integra analyzers.
- For each batch of assays, two levels of controls must be introduced.
- The values of controls must imperatively be within about a 5% range of the target value reported by the manufacturer.
- Application of the correction factor after batch validation.

(contact BioPredictive Ref#B and C)

Remark C- About Beckman Coulter

For the three proteins of FibroTest-ActiTest (alpha2-macroglobulin, apolipoprotein A1, haptoglobin), various studies were carried out in order to compare results between the Immage nephelometer of Beckman Coulter and the BN2 and BN Prospec nephelometers of Siemens Healthcare Diagnostics (formerly Dade- Behring). The results of these studies led to modifications concerning the alpha2-macroglobulin assay (new antiserum) in order to obtain results comparable to those in the laboratory that validated FibroTest. Currently, the results of apolipoprotein A1 obtained on the Immage analyzer require correction by a factor established during two different studies. Nevertheless, this problem was resolved in 2007 as per an internal study (Beckman Coulter) showing good transferability between Immage versus BN2 apolipoprotein A1 results and LX versus Immage apoliprotein A1 results; as a result, the correction factor is no longer useful. (contact BioPredictive Ref#D)

Remark D- About Diagam reagents

The reagents from the Diagam company were evaluated on Modular analyzers (Roche Diagnostics) for the turbidimetric assays of FibroTest proteins (alpha2-macroglobulin, haptoglobin, apolipoprotein A1). Their application is now available for various analyzers with good precision criteria, and the results do not require a correction factor. (contact BioPredictive Ref#E)

Remark E – About AU analyzers (Beckman Coulter, formerly Olympus)

A study of 150 chronic liver diseases patients validated the use of FibroTest, FibroMax, NASH-FibroTest on AU5800 and other AU analyzers sharing the same reagents and analytical methods. The alpha2macroglobulin assay is performed with Diagam reagents. The apolipoprotein A1 assay is performed with Diagam reagents on AU5800 and similar analyzers (AU480 and AU680). (contact BioPredictive Ref#D)

Remark F - About Apolipoprotein A1 on Advia 1650

For the Apolipoprotein A1 on Advia 1650, a correction factor must be applied to the results. This correction factor has been established under very strict analytical conditions and is available from BioPredictive. (contact BioPredictive Ref#A)

Remark G - About Apolipoprotein A1 on Optilite (Optimized Protein System) analyzer

A transferability study has validated Alpha2-Macroglobulin and Haptoglobin reagents (The Binding Site Group LTD) for the use of FibroTest, FibroMax and NASH-FibroTest on the OPTILITE (Optimized Protein System) analyzer. Apolipoprotein A1 is not currently available on OPTILITE analyzers; transferability work is ongoing for apolipoprotein A1. (contact BioPredictive Ref#F)

Remark H – About the use of pyridoxal-5-phosphate (P-5-P) for ALT/AST assays

For ActiTest interpretation, the use of the IFCC reference method with P-5-P for ALT assay is recommended in patients with B6 vitamin deficiency. (37-38)

For SteatoTest, SteatoTest 2, NashTest, NashTest 2, FibroMax and NASH-FibroTest interpretations, the use of the IFCC reference method with P-5-P for ALT and AST assays is recommended in patients with B6 vitamin deficiency. (37-38)

C – References

1. Poynard T, Morra R, Ingiliz P, Imbert-Bismut F, Thabut D, Messous D, Munteanu M, Massard J, Benhamou Y, Ratziu V. Biomarkers of liver fibrosis. Adv Clin Chem. 2008;46:131-60. Review.
2. Halfon P, Munteanu M, Poynard T. FibroTest-ActiTest as a non-invasive marker of liver fibrosis. Gastroenterol Clin Biol. 2008;32:22-39.
3. Poynard T, Morra R, Halfon P, Castera L, Ratziu V, Imbert-Bismut F, Naveau S, Thabut D, Lebrec D, Zoulim F, Bourliere M, Cacoub P, Messous D, Munteanu M, de Ledinghen V. Meta-analyses of FibroTest diagnostic value in chronic liver disease. BMC Gastroenterol. 2007;7:40.
4. Halfon P, Imbert-Bismut F, Messous D, Antoniotti G, Benchetrit D, Cart-Lamy P, Delaporte G, Doutheau D, Klump T, Sala M, Thibaud D, Trepo E, Thabut D, Myers RP, Poynard T. A prospective assessment of the inter-laboratory variability of biochemical markers of fibrosis (FibroTest) and activity (ActiTest) in patients with chronic liver disease. Comp Hepatol. 2002;1:3.
5. Imbert-Bismut F, Messous D, Thibaut V, Myers RB, Piton A, Thabut D, Devers L, Hainque B, Mercadier A, Poynard T. Intralaboratory analytical variability of biochemical markers of fibrosis (FibroTest) and activity (ActiTest) and reference ranges in healthy blood donors. Clin Chem Lab Med. 2004;42:323-33.
6. Morra R, Munteanu M, Imbert-Bismut F, Messous D, Ratziu V, Poynard T. FibroMax: towards a new universal biomarker of liver disease? Expert Rev Mol Diagn. 2007;7:481-90. Review.
7. Munteanu M, Messous D, Thabut D, Imbert-Bismut F, Jouys M, Massard J, Piton A, Bonyhay L, Ratziu V, Hainque B, Poynard T. Intra-individual fasting versus postprandial variation of biochemical markers of liver fibrosis (FibroTest) and activity (ActiTest). Comp Hepatol. 2004;3:3.
8. Messous D, Munteanu M, Morra R, Piton A, Poynard T, Hainque B, Imbert-Bismut F. Time impact of serum storage temperature on stability of important liver enzymes and proteins, and on FibroTest-ActiTest results. Clinica Chimica Acta. 2005;335:400.
9. Rosenthal-Allieri MA, Peritore M, Tran A, Halfon P, Benzakena S, Bernada A. Analytical variability of the FibroTest proteins. Clin Biochem 2005;38;473-8.
10. Rosenthal-Allieri MA, Tran A, Halfon P, Imbert-Bismut F, Munteanu M, Messous D, Peritore ML, Poynard T, Bernard A. Optimal correlation between different instruments for FibroTest-ActiTest protein measurement in patients with chronic hepatitis C. Gastroenterol Clin Biol. 2007;31:815-21.
11. Piton A, Messous D, Imbert-Bismut F, Berges J, Munteanu M, Poynard T, Hainque B. Alpha2-macroglobulin immunoturbidimetric assays (DakoCytomation reagents) on Roche Diagnostic analyzers (Modular P, Cobas Integra). Application to FibroTest-ActiTest (FT-AT). Ann Biol Clin. 2005;63:385-95.
12. Imbert-Bismut F, Messous D, Raoult A, Poynard T, Bertrand JJ, Marie PA, Louis V, Audy C, Thouy JM, Hainque B, Piton A. Results transferability on RXL, ARX, X-Pand, BN2 (Dade Behring) and modular DP (Roche Diagnostics) analyzers: application to component assays of FibroTest and ActiTest. Ann Biol Clin. 2005;63:305-13.
13. Bienvenu J, Doche C, Later R, Manceaux J.C, Pontet E, Pressac M, Vassault A, Dumont G. Améliorations apportées par le matériau de référence CRM 470 dans la standardisation du dosage des protéines sériques. Ann Bio Clin. 1997;55:37-40.
14. Dati F, Schumann G, Thomas L, Aguzzi F, Baudner S, Bienvenu J, Blaabjerg O, Blirup- Jensen S, Carlstrom A, Petersen PH, Johnson AM, Milford- Ward A, Ritchie RF, Svendsen PJ, Whicher J. Consensus of a group of professional societies and diagnostic companies for interim reference

ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP Reference Material (CRM 470). International Federation of Clinical Chemistry. Community Bureau of Reference of the commission of the European Communities. College of American Pathologists. Eur J Clin Chem Clin Biochem. 1996;34:517-20.

15. Albers J.J, Marcovina S.M, and H. Kennedy International Federation of Clinical Chemistry Standardization Project for Measurement of Apolipoproteins A1 and B. II Evaluation and Selection of Candidate Reference Materials. Clin Chem. 1992;38:658-62.
16. Myara A, Ferard G, Guéchot J, Imbert-Bismut F, Lasnier E, Piton A, Voitot H, Dhumeaux D. Calibration to achieve standardization in enzymology, example of ALT. Clin Chem Lab Med. 2001;39, Special Supplement pp S1-S448.
17. Groupe de Travail SFBC (Lessinger J.M, Doffoel S, Ferard G, Grafmeyer D, Schiele F, Vassault A, Vialle A) -Assurance de Qualité en Enzymologie Clinique- et Hépatites-(Myara A, Guéchot J, Imbert-Bismut F, Lasnier E, Piton A, Voitot H). Le calibrage en enzymologie clinique : principe, conditions d'application et résultats. Ann Biol Clin. 2002;60:281-6.
18. Ferard G, Piton A, Messous D, Imbert-Bismut F, Frairi A, Poynard T, Lessinger JM. Intermethod calibration of alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) results: application to FibroTest and ActiTest scores. Clin Chem Lab Med. 2006; 44: 400-6.
19. Schumann G, Bonora R, Ceriotti F, Férard G, Ferrero CA, Franck PF, Gella FJ, Hoelzel W, Jørgensen PJ, Kanno T, Kessner A, Klauke R, Kristiansen N, Lessinger JM, Linsinger TP, Misaki H, Panteghini M, Pauwels J, Schiele F, Schimmel HG, Weidemann G, Siekmann L; International Federation of Clinical Chemistry and Laboratory Medicine. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 6. Reference procedure for the measurement of catalytic concentration of gamma-glutamyltransferase. Clin Chem Lab Med. 2002;40:734-8.
20. Schumann G, Bonora R, Ceriotti F, Férard G, Ferrero CA, Franck PF, Gella FJ, Hoelzel W, Jørgensen PJ, Kanno T, Kessner A, Klauke R, Kristiansen N, Lessinger JM, Linsinger TP, Misaki H, Panteghini M, Pauwels J, Schiele F, Schimmel HG, Weidemann G, Siekmann L; International Federation of Clinical Chemistry and Laboratory Medicine. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 4. Reference procedure for the measurement of catalytic concentration of alanine aminotransferase. Clin Chem Lab Med. 2002;40:718-724.
21. Schumann G, Bonora R, Ceriotti F, Férard G, Ferrero CA, Franck PF, Gella FJ, Hoelzel W, Jørgensen PJ, Kanno T, Kessner A, Klauke R, Kristiansen N, Lessinger JM, Linsinger TP, Misaki H, Panteghini M, Pauwels J, Schiele F, Schimmel HG, Weidemann G, Siekmann L; International Federation of Clinical Chemistry and Laboratory Medicine. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 5. Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. Clin Chem Lab Med. 2002;40:725-731.
22. Férard G, Imbert-Bismut F, Messous D, Piton A, Abella A, Burnat P, Hainque B, Glasser N, Lessinger JM. Influence of pyridoxal phosphate in measuring aminotransferases activities in patients with viral hepatitis. Ann Biol Clin. 2004;62:717-20. French.
23. Jendrassik Grof. Vereinfachte Photometrische Methoden zur Bestimmung des Blutbilirubins. Biochem Z. 1938;297:8189.
24. Malloy HT, Evelyn KA. The determination of bilirubin with photoelectric colorimeter J Biol Chem. 1937;19:984-93.

25. Wahlefeld AW, Herz G, Bernt E. Modification of the Malloy-Evelyn method for a simple, reliable determination of total bilirubin in serum. Scand J Clin Lab Invest. 1972;29,supp 26, Abstract 11-12.
26. Doumas BT, Perry BW, Sasse EA, Straumfjord JV Jr. Standardization in bilirubin assays: evaluation of selected methods and stability of bilirubin solutions. Clin Chem. 1973;19:984-93.
27. Trinder P. Ann Clin Biochem. 1969;6:24.
28. Peterson JL and Young DS. Anal Biochemistry. 1958;23:301.
29. Thierry Poynard, Mona Munteanu, Olivier Deckmyn, Yen Ngo, Fabienne Drane, Djamila Messous, Jean Marie Castille, Chantal Housset, Vlad Ratziu, Françoise Imbert-Bismut. Applicability and precautions of use of liver injury biomarker FibroTest. A reappraisal at 7 years of age. BMC Gastroenterol. 2011;11:39.
30. Bedossa P, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology. 2003;38:1449-57.
31. Poynard T, Munteanu M, Imbert-Bismut F, Charlotte F, Thabut D, Le Calvez S, Messous D, Thibault V, Benhamou Y, Moussalli J, Ratziu V. Prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C. Clin Chem. 2004;50:1344-55.
32. Costa JM, Telehin D, Munteanu M, Kobryn T, Ngo Y, Thibault V, Joseph M, Ratziu V, Benhamou Y, Koz'ko V, Dubins'ka G, Poveda JD, Poynard T. HCV-GenoFibrotest: a combination of viral, liver and genomic (IL28b, ITPA, UGT1A1) biomarkers for predicting treatment response in patients with chronic hepatitis C. Clin Res Hepatol Gastroenterol. 2011;35:204-13.
33. Poynard T, de Ledinghen V, Zarski JP, Stanciu C, Munteanu M, Vergniol J, France J, Trifan A, Lenaour G, Vaillant JC, Ratziu V, Charlotte F; Fibrosis-TAGS group. Performances of Elasto-FibroTest(®), a combination between FibroTest(®) and liver stiffness measurements for assessing the stage of liver fibrosis in patients with chronic hepatitis C. Clin Res Hepatol Gastroenterol. 2012;36:455-63.
34. Deckmyn O. BioPredictive 2010. SteatoScreen internal evaluation.
35. Poynard T, Peta V, Munteanu M, Charlotte F, Ngo Y, Ngo A, Perazzo H, Deckmyn O, Pais R, Mathurin P, Myers R, Loomba R, Ratziu V; FLIP consortium, the FibroFrance-CPAM group, the FibroFrance-Obese group, and the Selonsertib group. The diagnostic performance of a simplified blood test (SteatoTest-2) for the prediction of liver steatosis. Eur J Gastroenterol Hepatol. 2019;31:393-402.
36. Poynard T, Munteanu M, Charlotte F, Perazzo H, Ngo Y, Deckmyn O, Pais R, Merrouche W, de Ledinghen V, Mathurin P, Ratziu V; FLIP Consortium, the FibroFrance-CPAM Group, the FibroFrance-Obese Group. Diagnostic performance of a new noninvasive test for nonalcoholic steatohepatitis using a simplified histological reference. Eur J Gastroenterol Hepatol. 2018;30:569-77.
37. Gossens K, Van Uytfanghe K, Thienpont LM. Trueness and comparability assessment of widely used assays for 5 common enzymes and 3 electrolytes. Clin Chim Acta. 2015;442:44-5.
38. Infusino I, Frusciante E, Braga F, Panteghini M. Progress and impact of enzyme measurement standardization. Clin Chem Lab Med. 2017;55:334-40.
39. Varaut A, Fontaine H, Serpaggi J, Verkarre V, Vallet-Pichard A, Nalpas B, Imbertbismuth F, Lebray P, Pol S. Diagnostic accuracy of the fibrotest in hemodialysis and renal transplant patients with chronic hepatitis C virus. Transplantation. 2005;80:1550-5.
40. Alric L, Kamar N, Bonnet D, Danjoux M, Abravanel F, Lauwers-Cances V, Rostaing L. Comparison of liver stiffness, fibrotest and liver biopsy for assessment of liver fibrosis in kidney-transplant patients with chronic viral hepatitis. Transpl Int. 2009;22:568-73.
41. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A,Capron F, Poynard T; LIDO Study Group. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology. 2005;128:1898-906.

D – Precautions for use

Taking into account the different risk factors for false positives and false negatives (29), the users of the tests should be aware of the following precautions.

- Follow the analytical recommendations for analyzer and reagent use.
- Defer the test in transient situations that could modify the components of FibroTest, such as:
  - Acute haemolysis (malarial attack; medications causing haemolysis such as ribavirin, azathioprine), which could decrease haptoglobin and increase unconjugated bilirubin.
  - Acute hepatitis, whether drug-induced, viral (superinfection by HAV, HBV, EBV) or autoimmune. Massive hepatic necrosis leads to a large increase of transaminases and total bilirubin.
  - Acute inflammation, as with concomitant bacterial or acute viral infection: bronchopulmonary or urinary tract infection. The large increase of haptoglobin can lead to false negatives.
  - Extrahepatic cholestasis, such as gallstones.

- The advice of a liver disease specialist should be sought for interpretation in chronic states, in which the components of the test could be modified, such as:
  - Chronic haemolysis, particularly in patients with a cardiac valvular prosthesis
  - Gilbert's syndrom
  - Protease inhibitors used in HIV treatment, which can increase unconjugated bilirubin (Indinavir, Atazanavir) or GGT and ALT (Ritonavir).

- The interpretation of FibroTest has been validated in renal transplant patients and renal insufficiency or on dialysis patients (39, 40).

- As a general rule, isolated extreme values of one of the six components should signal caution in interpreting the results, particularly in the following cases:
  - Haptoglobin less than 0.12g/L, in which haemolysis or anhaptoglobinemia (more frequent in sub-Saharan patients) must be ruled out.
  - Haptoglobin greater than 3.2 g/L, in which acute inflammation must be ruled out.
  - Transaminases greater than 622 IU/L, in which acute hepatitis must be ruled out.
  - Bilirubin greater than 30 micromoles/L, and GGT less than 50 IU/L, in which Gilbert's syndrome must be suspected.
  - Alpha2-macroglobulin greater than 5.9 g/L.

In case of discordance between a biopsy result and one of BioPredictive's test result, advise should be asked to a liver disease specialist. The causes of these discordances could be due to a flaw of one of BioPredictive's test, as stated previously, or to a flaw in the liver biopsy. Liver biopsy, even under optimal conditions (a single fragment, greater than 15 mm in length with at least five portal tracts) is limited by sampling error and by non-homogeneous distribution of liver lesions. A study, including a large surgical biopsy as a reference, showed a discordance rate of 35% for the presence of liver fibrosis. (30) A discordance of 41% of at least one fibrosis stage was observed between two biopsies made in the same patient on the same day. (41) A discordance of at least two stages or two grades of activity between FibroTest and liver biopsy was observed in 29% of cases. (29) The cause of these discrepancies was attributed in the 2.4% of cases to FibroTest, in the 18% of cases to the liver biopsy, and no attribution has been given to the remaining 8.2% of cases.

E – Contact Us

Please contact us for any additional information:

Website	https://www.biopredictive.com
E-mail	contact@biopredictive.com or experts@biopredictive.com
Bibliography	http://library.biopredictive.com
Tel	+33 1 84 79 23 90
Fax	+33 1 84 79 03 07
Postal Address	218 Boulevard Saint Germain 75007 PARIS FRANCE