



Technical Recommendations for FibroTest and FibroMax assays

A guide for biologists and laboratories

Version 2.3

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History of document

Author	Date	Version	Modifications
MM	2018-03-12	2.3	Add Optilite analyzer & reagents (A2M, Hapto), update manufacturer's contact details
FD, MM	2018-01-31	2.2	Add Vitros 4600
MM, FD, FIB	2017-06-13	2.1	Update Beckman AU analyzers and reagents for ApoA1 (OSR6241) - Corrections Remark E – ApoA1 for AU analyzers – Hitachi 917 hapto and ApoA1 added in the summary table
FIB, FD	2016-03-23	2.0	General review - updated methods and analyzers
MM, FD	2016-01-13	1.19	Corrections for Ortho method for ApoA1, GGT, Bilirubin
MM, FD	2015-12-15	1.18	Add Remark A Siemens RXL
MM, FIB	2014-10-20	1.17	Update Vitros 5600 5100 (Fusion 5.1) (Ortho CD) - Update AU5800, AU480, AU680 analyzers (Beckman, <i>former Olympus</i>) Update Synchron LX / DXC (Beckman) analyzers for A2M with Diagam reagents Eliminate Dako for AU and Advia 1650 Corrections
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FD	2006-06-02	1.5	Update Olympus AU400, 640 et 2700 analyzers
FD	2006-05-31	1.4	Update Olympus AU600 analyzer
FD	2006-01-27	1.3	Update Diagam reagent for Modular
MM	2005-12-20	1.2	Update Precautions for use

Table of contents

History of document	2
Table of contents	4
Introduction	5
FibroTest and ActiTest	5
FibroMax	5
Conditions of use	6
A - Pre-analytical phase	7
Blood Samples	7
Interferences	7
B – Analytical methods and analyzers used for the FibroTest and FibroMax assays	8
Methods	8
Manufacturers and analyzers	9
Analyzers and analytical methods evaluated for FibroTest and FibroMax transferability of results	9
Protein assays	9
a) Immunonephelometric methods	10
b) Immunoturbidimetric methods	10
Gamma-GT, ALT and AST assays	12
Total bilirubin assay	13
Table 1: Analyzers and FibroTest/FibroMax assays	15
Table 2: Analytical methods used for the complementary FibroMax assays: AST, total cholesterol, triglycerides, fasting glucose	22
C – References	25
D – Precautions for use	28
E – Contact Us	29

Introduction

FibroTest and 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 and ActiTest are computed from the results of five and six biochemical parameter assays (alpha2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, GGT, ALT), respectively, 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).

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 and fasting glucose are added to the FibroTest- ActiTest components for assessing liver steatosis.

- 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 steatosis (acute alcoholic hepatitis).

Conditions of use

Biologists working in biomedical laboratories and wishing to benefit from calculated FibroTest and/or FibroMax 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 be associated with the external quality assessment** (accuracy control) in accordance with the biomedical laboratories accreditation. This is mandatory in France.

A list of analyzers for which test result transferability has been verified will be presented later.

The different biochemical 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 these 5 components with alanine aminotransferase (ALT in IU/L).

If ActiTest is not prescribed, the default value to be entered for ALT is 1.

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 associates the parameters of FibroTest and ActiTest plus 4 additional parameters:

- AST (aspartate aminotransferase) (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. (6)

For each parameter result, the **units** indicated in brackets need to **be adhered to for the calculations of FibroTest and FibroMax**.

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, it is *mandatory that the patient has fasted for 12 hours* at the time of the blood sample. 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.
- 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 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 always 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 and FibroMax assays

Transferability of FibroTest and FibroMax results 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 quality** of the FibroTest and FibroMax results.

Methods

In summary

- Enzyme activity measurements must be performed in accordance with the reference methods at 37°C, using **pyridoxal phosphate** for ASAT and ALAT (**IFCC method**) (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 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 results are expressed with SI units (IU/L)

- Total bilirubin ($\mu\text{mol/L}$): 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

Some companies have now been grouped.

The marked analyzers cited and underlined below have been tested for the transferability of FibroTest-FibroMax results. 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
Image nephelometer
and AU, formerly from Olympus
(AU400, AU480, AU600, AU640, AU680, AU2700, AU5800)

SIEMENS HEALTHCARE DIAGNOSTICS:

BN2, BNProspec, nephelometers, formerly from Dade Behring
RxL, ArX, XPand, VISTA, formerly from Dade Behring

and ADVIA 1650, formerly from Bayer Diagnostics

ABBOTT

ARCHITECT c8000

ROCHE DIAGNOSTICS

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

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 and FibroMax 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 or BN Prospec 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 Society (mail@diagam.com) 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 and Cobas 8000 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: sprinzis@beckman.com*
- **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: thierry.diradourian@thermofischer.com*
- **Advia 1650 (Siemens Healthcare Diagnostics, formerly Bayer Diagnostics)**. Diagam reagents with standards and controls are used. [see **Remark D**].

- **Architect c8000** analyzer (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 and Cobas 8000 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. *Contact sprinzis@beckman.com*.
- **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) [see *Remark F*]. Reagents, standards and internal controls are marketed by Siemens Healthcare Diagnostics.
- **Architect c8000 analyzer** (Abbott). Reagent, 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 apolipoprotein A1 assays performed on the different analyzers, the external quality assessment is highly recommended. This is mandatory in France.

Protein results are expressed in 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 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 and Cobas 8000 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 with pyridoxal phosphate at 37°C and calibrated using C.f.a.s (Calibrator For Automated Systems- Roche Diagnostics).

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 [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 analyzer** (Abbott). Reagents, standards and internal controls are provided by Abbott. Calibration with C.f.a.s from Roche Diagnostics is needed for GGT, ALT and AST activity measurements.
- **Advia 1650 analyzer** (Siemens Healthcare Diagnostics, formerly Bayer Diagnostics). 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, and with **pyridoxal phosphate** (20-21).
- In order to secure the transferability of the enzymatic activity results (GGT, ALT, AST) between the analyzers used for FibroTest/FibroMax validations and the other automated systems, it is necessary to either:
 - calibrate using the C.f.a.s (IFCC target value for ALT, AST and GGT)
 - or include this calibrator in the sample batches in order to determine, if needed, a factor that corrects the results.

In all cases and before performing FibroTest/FibroMax, it is important to check the traceability of results obtained on the analyzer with the manufacturer.

- 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 and Cobas 8000 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 (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 analyzer (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 Medical Solutions Diagnostics)**. Analytical Method: diazo reaction (DPD). 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 ($\mu\text{mol/L}$).

Table 1: Analyzers and FibroTest/FibroMax 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	<i>Siemens Healthcare Diagnostics (formerly Dade Behring)</i> BN2 BN-Prospec Vista	Siemens	ERM DA 470k (CRM 470)	Nephelometry	g/L
	<i>Beckman Coulter</i> Immage	Beckman	ERM DA 470k (CRM 470)	Nephelometry [Remark C]	g/L
	<i>Roche Diagnostics</i> Hitachi 917 Modular P or Integra 400 utility channel	Diagam or Dako Cytomation	ERM DA 470k (CRM 470) ERM DA 470k (CRM 470)	Turbidimetry No correction for Diagam [Remark D] Turbidimetry Correction for Dako [Remark B]	g/L
	Cobas 6000 Cobas 8000	Diagam	ERM DA 470k (CRM 470)	Turbidimetry No correction for Diagam [Remark D]	
	<i>Beckman Coulter (formerly Olympus)</i> AU5800 (AU400, AU480, AU600, AU640, AU680, AU2700)	Diagam	ERM DA 470k (CRM 470)	Turbidimetry	g/L
	Synchron LX DXC	Diagam	ERM DA 470k (CRM 470)	Turbidimetry	
<i>Thermo Fischer Scientific (Konelab)</i> 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 <i>No correction</i>	g/L
	Abbott Architect c8000	Diagam	ERM DA 470k (CRM 470)	Turbidimetry <i>No correction</i>	g/L
	Ortho-Clinical Diagnostics Vitros 4600, Vitros 5600 Vitros 5.1 (Fusion)	Diagam	ERM DA 470k (CRM 470)	Turbidimetry <i>No correction</i>	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	Siemens	ERM DA 470k (CRM 470)	Nephelometry	g/L
	Beckman Coulter Image	Beckman Coulter	ERM DA 470k (CRM 470)	Nephelometry [Remark C]	g/L
	Roche Diagnostics Hitachi 917 Modular P Integra 400 Cobas 6000 Cobas 8000	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	<i>Thermo Scientific (Konelab)</i> T20, T20XT, T30, T60, T60 New Generation	Thermo Scientific	ERM DA 470k (CRM 470)	Turbidimetry	g/L
	<i>Siemens Healthcare Diagnostics (formerly Bayer)</i> Advia 1650	Siemens	ERM DA 470k (CRM 470)	Turbidimetry	g/L
	<i>Abbott</i> Architect c8000	Abbott	ERM DA 470 (CRM 470)	Turbidimetry	g/L
	<i>Ortho-Clinical Diagnostics</i> Vitros 4600, Vitros 5600 Vitros 5.1 (Fusion)	Ortho-CD	ERM DA 470 (CRM 470)	Turbidimetry	g/L
	<i>The Binding Site Group LTD</i> Optilite – Optimised Protein System	The Binding Site Group LTD	ERM DA 470 (CRM 470)	Turbidimetry	g/L
	Apolipoprotein A1	<i>Siemens Healthcare Diagnostics (formerly Dade- Behring)</i> BN2 BN-Prospec Vista	Siemens	WHO-IFCC SP1-01	Nephelometry
<i>Beckman Coulter</i> Image		Beckman Coulter	WHO-IFCC SP1-01	Nephelometry [Remark C]	g/L
<i>Roche Diagnostics</i> Hitachi 917 Modular P Integra 400		Roche Diagnostics Or Diagam	WHO-IFCC SP1-01	Turbidimetry No correction [Remark D]	g/L
Cobas 6000 Cobas 8000		Roche Diagnostics			
<i>Siemens Healthcare Diagnostics (formerly Dade- Behring)</i> 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	<i>Beckman Coulter (formerly Olympus)</i> AU400 / AU600 / AU640 / AU2700	Beckman (OSR6142)	WHO-IFCC SP1-01	Turbidimetry	g/L
	AU5800 (AU480, AU680) et AU400, AU600, AU640, AU2700	Diagam	WHO-IFCC SP1-01 et SP1-03	Turbidimetry [Remark E]	
	<i>Thermo Fischer scientific (Konelab)</i> T20, T20XT, T30, T60, T60 New Generation	Thermo Fisher Scientific	WHO-IFCC SP1-01	Turbidimetry	g/L
	<i>Siemens Healthcare Diagnostics (formerly Bayer)</i> Advia 1650	Siemens	WHO-IFCC SP1-01	Turbidimetry Correction factor [Remark F]	g/L
	<i>Abbott</i> Architect c8000	Abbott	WHO-IFCC SP1-01	Turbidimetry	g/L
	<i>Ortho-Clinical Diagnostics</i> Vitros 4600, Vitros 5600 Vitros 5.1 (Fusion)	Ortho-CD	WHO-IFCC SP1-01	Turbidimetry	g/L
GGT	<i>Roche Diagnostics</i> Hitachi 917 Modular P Integra 400 Cobas 6000 Cobas 8000	Roche	IFCC reference method (19)	Szasz method calibrated in accordance with IFCC method C.f.a.s – IFCC GGT value	IU/L
	<i>Siemens Healthcare Diagnostics (formerly Dade-Behring)</i> Dimension-RXL, ArX and XPAND Vista	Siemens	IFCC reference method (19)	According to IFCC method [Remark A]	IU/L
	<i>Beckman Coulter (formerly Olympus)</i> 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	<i>Thermo Scientific (Konelab)</i> T20, T20XT, T30, T60, T60 New Generation	Thermo Fisher Scientific	IFCC reference method (19)	Method in accordance with IFCC method	IU/L
	<i>Siemens Healthcare Diagnostics (formerly Bayer)</i> Advia 1650	Siemens	Modified IFCC method	According to IFCC method	IU/L
	<i>Abbott</i> Architect c8000	Abbott	IFCC reference method (19)	According to IFCC method. Calibration C.f.a.s Roche	IU/L
	<i>Ortho-Clinical Diagnostics</i> Vitros 4600, Vitros 5600 Vitros 5.1 (Fusion)	Ortho-CD	IFCC reference method (19)	Method in accordance with IFCC method	IU/L
Total Bilirubin	<i>Roche Diagnostics</i> Hitachi 917 Modular P Integra 400 Cobas 6000 Cobas 8000	Roche Diagnostics	Doumas reference method (25) Reference standard SRM	Diazo reaction: Jendrassik Grof (22) modified by Wahlefeld (24); Malloy-Evelyn (23)	µmol/L
	<i>Siemens Healthcare Diagnostics (formerly Dade Behring)</i> Dimension-RXL, ArX and XPAND Vista	Siemens	Doumas reference method (25) Reference standard SRM	Diazo reaction Modified Doumas method [Remark A]	µmol/L
	<i>Beckman Coulter (formerly Olympus)</i> AU5800 (AU400, AU480 AU600 AU640, AU680 AU2700)	Beckman	Doumas reference method (25) Reference standard SRM	Diazo reaction (DPD)	µmol/L
	<i>Thermo Fischer Diagnostics (Konelab)</i> 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	<i>Siemens Healthcare Diagnostics (formerly Bayer)</i> Advia 1650	Siemens	AACC Reference method Vanadium Oxidation	Oxidation by vanadate	µmol/L
	<i>Abbott</i> Architect c8000	Abbott	NIST SRM 916a	Diazo reaction	µmol/L
	<i>Ortho-Clinical Diagnostics</i> Vitros 4600, Vitros 5600 Vitros 5.1 (Fusion)	Ortho-CD	Standard Reference Material (SRM)	Diazo reaction	µmol/L
ALT	<i>Roche Diagnostics</i> Hitachi 917 Modular Integra Cobas 6000 Cobas 8000	Roche Diagnostics	IFCC reference method with pyridoxal phosphate (20)	Enzymatic method according to the IFCC reference method	IU/L
	<i>Siemens (formerly Dade Behring)</i> RxL Dimension RXL ArX XPAND Vista	Siemens	IFCC reference method with pyridoxal phosphate (20)	Enzymatic method according to the IFCC reference method	IU/L
	<i>Beckman Coulter (formerly Olympus)</i> AU5800 (AU400, AU480, AU600, AU640, AU680, AU2700)	Beckman	IFCC reference method with pyridoxal phosphate (20)	Enzymatic method according to the IFCC reference method	IU/L
	<i>Thermo Fisher Scientific (Konelab)</i> T20, T20XT, T30, T60, T60 New Generation	Thermo Fisher Scientific	IFCC reference method with pyridoxal phosphate (20)	Enzymatic method according to the IFCC reference method	IU/L
	<i>Siemens Healthcare Diagnostics (formerly Bayer Diagnostics)</i> Advia 1650	Siemens	IFCC reference method with pyridoxal phosphate (20)	Enzymatic method according to the IFCC reference method	IU/L

Parameter	Analyzer	Reagents/ Manufacturer	Material or Reference method	Analytical method	Units
ALT	<u>Abbott</u> Architect c8000	Abbott	IFCC reference method with pyridoxal phosphate (20)	Enzymatic method according to the reference method IFCC. Calibration C.f.a.s Roche	IU/L
	<u>Ortho-CD</u> Vitros 4600, Vitros 5600 Vitros 5.1 (Fusion)	Ortho-CD	IFCC reference method with pyridoxal phosphate (20)	Enzymatic method according to the reference method IFCC.	IU/L

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

Parameter	Material or Reference method	Analytical method	Units
AST	IFCC reference method with pyridoxal phosphate	Enzymatic method according to the IFCC reference method. (Initial calibration with C.f.a.s Roche for Architect c8000 of Abbott)	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 above.

In the case of use of different analyzers than those listed above, please contact BioPredictive company (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 Siemens Healthcare Diagnostics: Mr. Didier HEULS -Head of Sales Siemens Healthcare SA/NV;
Tel.: +32 253 69510; <mailto:didier.heuls@siemens.com>

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.

Roche Diagnostics:

Hotline France: +33 4 76 76 30 65; mailto : diagassistance@roche.com

World: Mr. Didier GARRY -*Product Manager SWA*- Tel. [+33 4 76 76 31 44](tel:+33476763144); mailto: didier.garry@roche.com

DAKO CYTOMATION: (French provider ELITechGroup SOLUTIONS):

Contact France: Tel [+33 4 83 36 10 82](tel:+33483361082); mailto: commandes@elitechgroup.com

World (export): Mr Romain BERGEAUT Tel [+33 1 41 45 07 10](tel:+33141450710); mailto: r.bergeaut@elitechgroup.com

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 apolipoprotein A1 results; as a result, the correction factor is no longer useful.

You may contact **Beckman Coulter (Mrs.Susi PRINZIS -*Clinical Chemistry Product Manager*- Tel: +33 1 49 90 91 70 and standard +33 1 49 90 90 00; mailto: sprinzis@beckman.com)** for more information on the reagents used for alpha2-macroglobulin and apolipoprotein A1.

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 **Diagam for details (Mr. Gilbert Ameryckx -CEO Diagam- Tel: +32 495 255 080 or Mob +32 684 439 068; mailto: mail@diagam.com)**

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

A study of 150 chronic liver diseases patients validated the use of FibroTest and FibroMax 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). For more details please contact **Beckman** (formerly Olympus) **Mrs. Susi PRINZIS-Clinical Chemistry Product Manager-Tel: +33 1 49 90 91 70 and standard +33 1 49 90 90 00; mailto: sprinzis@beckman.com.**

Remark F - About Apolipoprotein A1 on Advia 1650

For the Apolipoprotein A1 on Advia 1650 (reference 03050910), 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 Siemens Healthcare Diagnostics: Mr. Didier HEULS, *Head of Sales Siemens Healthcare SA/NV*; Tel.: +32 253 69510; mailto:didier.heuls@siemens.com

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 on the OPTILITE (Optimized Protein System) analyzer. Apolipoprotein A1 is not currently available on OPTILITE analyzers; transferability work is ongoing for apolipoprotein A1.

Contact The Binding Site Group LTD: Mr. Mark Perkins *-International Product Manager-Assays -* **Tel: [+44 \(0\) 121 456 9651](tel:+44201214569651) mailto: mark.perkins@bindingsite.co.uk**

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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 disease
 - 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
- In patients with renal insufficiency or on dialysis, FibroTest had an acceptable diagnostic value, though lower than in transplanted patients. More studies must therefore be performed.
- 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 anhaptoalbuminemia (more frequent in western African 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 a FibroTest result, it is advisable to seek the advice of a liver disease specialist. The causes of these discordances could be due to a flaw of the FibroTest, as stated previously, or to a flaw in the biopsy. Liver biopsy, even under optimal conditions (a single fragment, greater than 15 mm in length with at least five portal tracts) has a very high intrinsic variability (between two biopsies from the same liver). There is a variability of at least one fibrosis stage in 33% of cases and at least one activity grade in 24% of cases. (30) A prospective study has observed that most of the significant discordant results of at least two stages or two activity grades (28.7%) were attributable to biopsy failures (18.1%). (31)

E – Contact Us

Please contact us for any additional information:

Website <http://biopredictive.com>

E-mail contact@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