



Technical Recommendations for FibroTest and FibroMax assays

A guide for biologists and laboratories

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Table of contents

History of document	2
Table of contents	3
Introduction	4
FibroTest and ActiTest	4
FibroMax	4
Conditions of use	5
The different biochemical tests	5
A - Pre-analytical phase : conditions to respect.....	6
Blood sample	6
Storage and transport conditions, pretreatment before analysis	6
Interference	6
B - FibroTest and ActiTest parameter assays	7
Specific proteins assays	7
<i>Immunonephelometric methods:</i>	7
<i>Immunoturbidimetric methods:</i>	7
<i>Gamma-GT, ALT and AST measurements</i>	9
<i>Total bilirubin assay</i>	10
Table 1: Analytical methods used for the FibroTest and FibroMax assay.....	12
Table 2: Analytical methods used for the complementary FibroMax assay	17
C – References	20
D – Precautions for use.....	24
E – Contact Us	25

Introduction

FibroTest and ActiTest

FibroTest and ActiTest enable the assessment of liver fibrosis and necroinflammatory activity, respectively. They are considered as an alternative to liver biopsy in patients with chronic C hepatitis (1)(2).

They are computed from the results of six biochemical parameter assays.

The algorithms used to calculate FibroTest and ActiTest, have been defined and validated in different clinical studies. They have been patented.

Prior to use and develop combinations of these tests as tools in the biological world, it is important to ensure transferability of test results between laboratories. In the absence of an effective analytical standardization, the results remain dependent on the analytical system used. The described IFCC reference methods for the determination of different parameters and methods appropriate to the various machines must be in agreement with these reference methods, in order to respect the rules of international standards and meet the EU directives.

Assays of biochemical parameters of FibroTest and ActiTest are performed using methods which have been standardized with respect to reference methods and/or reference material.

FibroMax

The FibroMax is a biomarker offering the FibroTest and the SteatoTest with, according to risk factors, the NashTest and/or AshTest and/or ActiTest.

- The ActiTest for patients with chronic hepatitis C and B
- The NashTest for patients with metabolic steatosis
- The AshTest for patients with alcoholic steatosis

Conditions of use

Biologists working in biological analysis laboratories and wishing to benefit from calculated FibroTest and/or FibroMax should respect the conditions indicated for performing the different tests. These recommendations are related to the blood sample itself, the analytical method, calibration, and the criteria for reliability of the assays.

These recommendations are described in the present document.

The different biochemical tests

Fibrotest associates 5 biochemical tests:

- alpha2-macroglobulin
- haptoglobin
- apolipoprotein A1
- GGT (gamma glutamyl transpeptidase)
- total bilirubin

ActiTest associates these 5 components and ALT (alanine aminotransferase) .

If ActiTest is not prescribed, the ALT's value will be obligatory 3333.

Results of the six components, adjusted for gender and age, are used to calculate FibroTest and ActiTest (1)(2).

FibroMax associates components of FibroTest-ActiTest plus 4 biochemical tests:

- AST
- fasting glucose
- cholesterol
- triglycerides

Results of the components are adjusted for patient's age, gender, weight and height to calculate FibroMax.

A - Pre-analytical phase : conditions to respect

Blood sample

- Blood is obtained by venipuncture; the classical requested 12-h patient fast is not necessary.
- Blood samples are collected in 5-ml or 7-ml tubes, without anticoagulant.
- Blood sample is centrifugated within 2 h after the blood collection.
- The centrifugation conditions (speed and time) must be in conformity with recommendations of tube manufacturer.

Storage and transport conditions, pretreatment before analysis

- Biochemical assays are usually performed on fresh serum.
- Serum can be decanted and stored no more than 72 h at + 2°C/ + 8°C, protected from light to avoid bilirubin degradation.
- The assays of the specific proteins (alpha2-macroglobulin, haptoglobin, and apolipoprotein A1) can be carried out on serum stored at +2°C / +8°C no more than 5 days.
- If you intend to defer analysis beyond the recommended time, serum must be frozen at – 80°C from the start.
- Freezing and thawing can be done only once.
- After thawing at the ambient (laboratory) temperature, serum must be centrifuged at 1500 g for 10 min.

Interference

Lipids and hemolysis interfere with measurements.

It is always possible to dilute a lightly lipemic serum according to the recommendations of the standardized methods.

Hemolysed and/or hyperlipemic serum must be rejected.

B - FibroTest and ActiTest parameter assays

The performances of the assay methods of the FibroTest and ActiTest have been verified on analyzers and under the conditions mentioned below. They are a guarantee of the quality of the results of FibroTest.

Specific proteins assays

Immunonephelometric methods:

Assays of alpha2-macroglobulin, haptoglobin, and apolipoprotein A1

- **BN2 or BN Prospec analyzers** (Dade Behring). Reagents are commercialized by the analyzer manufacturer. Internal quality control Dade Behring and external quality control.
- **Image analyzer** (Beckman Coulter) [see *Remark C*]. New antiserum of the provider company is available for alpha2-macroglobulin and a specific adjustment [see *Remark C*] is needed for the apolipoprotein A1 result. Internal and external quality controls.

Immunturbidimetric methods:

Assay of alpha2-macroglobulin

- **Modular and Integra analyzers** (Roche Diagnostics). Reagent Diagam [see *Remarque D*] or reagent Dako Cytomation marketed by Elitech France; needs specific adjustment [see *Remark B*] for alpha2-macroglobulin assay.
- **AU400, AU600, AU640 and AU2700 analyzers** (Olympus). Reagent Dako Cytomation marketed by Elitech France.
- **Konelab 20, 20XT, 30, 60, Prime analyzers** (Thermo Fisher Scientific). Reagent Diagam [see *Remarque D*] or reagent Dako Cytomation marketed by Elitech France.
- **T20, T20XT, T30, T60 New Generation analyzers** (Siemens Medical Solutions Diagnostics). Reagent Diagam [see *Remarque D*] or reagent Dako Cytomation marketed by Elitech France.

- **Architect c8000** (Abbott). Reagent Diagam. [see *Remarque D*]
- **Advia** (Siemens Medical Solutions Diagnostics). Reagent Diagam [see *Remarque D*] or reagent Dako Cytomation marketed by Elitech France.

Assays of haptoglobin and apolipoprotein A1

- **Modular and Integra 400 analyzers** (Roche Diagnostics). Reagents marketed by Roche Diagnostics. Internal quality control Roche Diagnostics and external quality control.
- **RxL and ArX analyzers** (Dade Behring) [see *Remark A*]. Reagents marketed by Dade Behring. Internal quality control Dade Behring and external quality control.
- **AU400, AU600, AU640 and AU2700 analyzers** (Olympus). [see *Remark E*]. Reagent marketed by Olympus. Internal quality control Olympus and external quality control.
- **Konelab 20, 20XT, 30, 60, Prime analyzers** (Thermo Fisher Scientific). Reagent marketed by Thermo Fisher Scientific. Internal quality control Thermo Fisher Scientific and external quality control.
- **T20, T20XT, T30, T60 New Generation analyzers** (Siemens Medical Solutions Diagnostics). Reagent marketed by Siemens Medical Solutions Diagnostics. Internal quality control Siemens Medical Solutions Diagnostics and external quality control.
- **Architect c8000** (Abbott). Reagent marketed by Abbott. Internal quality control Abbott and external quality control.
- **Advia** (Siemens Medical Solutions Diagnostics). [see *Remark F*]. Reagent marketed by Siemens Medical Solutions Diagnostics. Internal quality control Siemens Medical Solutions Diagnostics and external quality control.

Analytical measurements of alpha2-macroglobulin and haptoglobin are standardized compared to the CRM 470 reference material. **CRM 470** is certified by the Community Bureau of Reference (**BCR**), the International Federation of Clinical Chemistry (**IFCC**), and the College of American Pathologists (**CAP**)(3)(4).

The apolipoprotein A1 assay is standardized compared to WHO- IFCC SP1-01 reference material (World Health Organization- International Federation of Clinical Chemistry SP1-01) (5).

Gamma-GT, ALT and AST measurements

To secure the transferability of the enzyme results activities (GGT, ALT, AST) between the analytical system used to record validating FibroTest and other analytical systems, it is necessary to calibrate using the CFAS (IFCC value for AST, ALT and GGT - use GGT liquid value, standardization compared to the method IFCC).

- **Hitachi 917, Modular, Integra 400 analysers** (Roche Diagnostics). Reagents are provided by Roche Diagnostics.

Enzymatic activity measurements are performed at 37°C and calibrated with a CFAS (calibrator for automated systems- Roche Diagnostics Company) (6)(7).

Internal quality control Roche Diagnostics and external quality control ASQUALAB.

- **RxL, ArX and XPAND** (Dade Behring). Reagents are provided by Dade Behring.
- AU400, AU600, AU640 and AU2700 analyzers (Olympus). Reagents are provided by Olympus. *[Remark E]*.
- **Konelab 20, 20XT, 30, 60, Prime analyzers** (Thermo Fisher Scientific). Reagent marketed by Thermo Fisher Scientific.
- **T20, T20XT, T30, T60 New Generation analyzers** (Siemens Medical Solutions Diagnostics). Reagent marketed by Siemens Medical Solutions Diagnostics.
- **Architect c8000** (Abbott). Reagents are provided by Abbott. Cfas calibration from Roche is needed for GGT, ALT and AST
- **Advia** (Siemens Medical Solutions Diagnostics). Reagent marketed by Siemens Medical Solutions Diagnostics.

Remarks:

- For GGT: IFCC standardized enzymatic method according to the reference method of IFCC.

- For ALT: IFCC standardized enzymatic method according to the reference method of IFCC with **pyridoxal phosphate** (9).
- In order to make safe the transferability of the results of the enzymatic activities (GGT, ALT, AST) between the analytical system used for the analytical validation of FibroTest and the other systems, it is necessary:
 - either calibrate using the CFAS (IFCC value for ALT and AST and Szasz value for GGT)
 - either include this calibrator in the series of the samples to be analyzed in order to be determine, if needed, a factor allowing to correct the results
- Enzymatic activities of GGT and ALT are expressed in International Units per liter (IU/l).

Total bilirubin assay

- **Hitachi 917, Integra 400 and Modular analyzers** (Roche Diagnostic). Reagents are provided by the manufacturer. Internal quality control Roche Diagnostics and external quality control ASQUALAB. The different methods consist of diazoreactions according to Jendrassik Grof (10) for Hitachi 917, Malloy-Evelyn (11) for Integra 400 and Wahlefeld (12) for Modular. The different methods were initially calibrated using the CFAS.
- RxL, ArX and XPAND (Dade Behring).
Analytical Method: Doumas modified. Biochemists must check with manufacturer that the traceability according to reference systems was verified for their analyzers.
- AU400, AU600, AU640 and AU2700 analyzers (Olympus).
Analytical Method: diazoreaction (DPD). Biochemists must check with manufacturer that the traceability according to reference systems was verified for their analyzers.

- Konelab 20, 20XT, 30, 60, Prime analyzers (Thermo Fisher Scientific).
Analytical Method : diazoreaction (DPD). Biochemists must check with manufacturer that the traceability according to reference systems was verified for their analyzers.
- T20, T20XT, T30, T60 New Generation analyzers (Siemens Medical Solutions Diagnostics).
Analytical Method : diazoreaction (DPD). Biochemists must check with manufacturer that the traceability according to reference systems was verified for their analyzers.
- Architect c8000 (Abbott).
Analytical Method : diazoreaction (surfactant accelerator not described by the provider). Biochemists must check with manufacturer that the traceability according to reference systems was verified for their analyzers.
- **Advia** (Siemens Medical Solutions Diagnostics).
Analytical Method : diazoreaction (DPD). Biochemists must check with manufacturer that the traceability according to reference systems was verified for their analyzers.

Results are expressed in micromoles per liter ($\mu\text{mol/l}$).

Details of the assays and of enzymatic activity measurements are summarized in the table 1 (1)(2).

Table 1: Analytical methods used for the FibroTest and FibroMax assay

This is a temporary list. Several studies are running on to extend to other analysers and reagents proposed by different constructors. Call BioPredictive for details.

Reproducibility CV must be less than 5% for all parameters.

Parameter	Analyser	Reagents	Material or Reference method	Analytical method	Units
Alpha-2 macroglobulin	BN2	Dade-Behring	CRM 470	Nephelometry	g/l
	BN Prospec Modular	Diagam or Dako Cytomation Elitech France	CRM 470	Turbidimetry <i>No correction for Diagam</i> [Remark D] Turbidimetry <i>Correction factor for Dako</i> [Remark B]	g/l
	Integra utility channel	Diagam or Dako Cytomation Elitech France	CRM 470	Turbidimetry <i>No correction for Diagam</i> [Remark D] Turbidimetry <i>Correction factor</i> [Remark B]	g/l
	Image	Beckman Coulter	CRM 470	Nephelometry [Remark C]	g/l
	AU400, AU600, AU640, AU2700	Dako Cytomation Elitech France	CRM 470	Turbidimetry	g/l
	Konelab 20, 20XT, 30, 60, Prime	Diagam or Dako Cytomation Elitech France	CRM 470	Turbidimetry <i>No correction for Diagam</i> [Remark D]	g/l
	T20, T20XT, T30, T60, T60 New Generation	Diagam or Dako Cytomation Elitech France	CRM 470	Turbidimetry <i>No correction for Diagam</i> [Remark D]	g/l

Parameter	Analyser	Reagents	Material or Reference method	Analytical method	Units
Alpha-2 Macroglobulin (continued)	Architect c8000	Diagam	CRM 470	Turbidimetry <i>No correction</i> [Remark D]	g/l
	Advia	Diagam or Dako Cytomation Elitech France	CRM 470	Turbidimetry <i>No correction for Diagam</i> [Remark D]	g/l
Haptoglobin	BN2	Dade-Behring	CRM 470	Nephelometry	g/l
	BN Prospec				
	RxL	Dade-Behring	CRM 470	Turbidimetry	g/l
	ArX				
	Modular Integra	Roche Diagnostics or Diagam	CRM 470	Turbidimetry <i>No correction for Diagam</i> [Remark D]	g/l
	Image	Beckman Coulter	CRM 470	Nephelometry [Remark C]	g/l
	AU400, AU600 AU640, AU2700	Olympus	CRM 470	Turbidimetry [Remark E]	g/l
	Konelab 20, 20XT, 30, 60, Prime	Thermo Fisher Scientific	CRM 470	Turbidimetry	g/l
	T20, T20XT, T30, T60, T60 New Generation	Siemens Medical Solutions Diagnostics	CRM 470	Turbidimetry	g/l
	Architect c8000	Abbott	CRM 470	Turbidimetry	g/l
Advia	Siemens Medical Solutions Diagnostics	CRM 470	Turbidimetry	g/l	
Apolipoprotein A1	BN2	Dade-Behring	WHO-IFCC SP1-01	Nephelometry	g/l
	BN Prospec				
	RxL	Dade-Behring	WHO-IFCC SP1-01	Turbidimetry	g/l
	ArX				
	Modular Integra	Roche Diagnostics ou Diagam	WHO-IFCC SP1-01	Turbidimetry <i>No correction for Diagam</i> [Remark D]	g/l

Parameter	Analyser	Reagents	Material or Reference method	Analytical method	Units
Apolipoprotein A1 (continued)	Immage	Beckman Coulter	WHO-IFCC SP1-01	Nephelometry [Remark C]	g/l
	AU400 AU600 AU640 AU2700	Olympus	WHO-IFCC SP1-01	Turbidimetry [Remark E]	g/l
	Konelab 20, 20XT, 30, 60, Prime	Thermo Fisher Scientific	WHO-IFCC SP1-01	Turbidimetry	g/l
	T20, T20XT, T30, T60, T60 New Generation	Siemens Medical Solutions Diagnostics	WHO-IFCC SP1-01	Turbidimetry	g/l
	Architect c8000	Abbott	WHO-IFCC SP1-01	Turbidimetry	g/l
	Advia	Siemens Medical Solutions Diagnostics	WHO-IFCC SP1-01	Turbidimetry Correction factor [Remark F]	g/l
GGT	Hitachi 917 Modular Integra	Roche Diagnostics	method according to the reference method of IFCC (8)	method according to the reference method of IFCC (8)	IU/l
	RxL ArX XPAND	Dade-Behring	Reference method IFCC (4)	Method according to the IFCC	IU/l
	AU400, AU600, AU640, AU2700	Olympus	Reference method IFCC (4)	Method according to the IFCC	IU/l
	Konelab 20, 20XT, 30, 60, Prime	Thermo Fisher Scientific	Reference method IFCC (4)	Method according to the IFCC	IU/l
	T20, T20XT, T30, T60, T60 New Generation	Siemens Medical Solutions Diagnostics	Reference method IFCC (4)	Method according to the IFCC	IU/l
	Architect c8000	Abbott	Reference method IFCC (4)	Method according to the IFCC Calibration Cfas Roche	IU/l

Parameter	Analyser	Reagents	Material or Reference method	Analytical method	Units
GGT(continue)	Advia	Siemens Medical Solutions Diagnostics	Reference method IFCC modified	Method according to the IFCC	IU/l
Total Bilirubin	Hitachi 917		Reference method of Doumas (13)	Diazoreactions: Jendrassik Grof (10) modified by Wahlefeld (12)	µmol/l
	Modular	Roche Diagnostics	Standard reference of SRM	Malloy-Evelyn (11)	
	Integra				
	RxL		Reference method of Doumas (13)	Modified Doumas method	µmol/l
	ArX	Dade-Behring			
	XPAND				
	AU400 AU600 AU640 AU2700	Olympus	Reference method of Doumas (13) Standard reference of SRM	Diazoreactions (DPD)	µmol/l
	Konelab 20, 20XT, 30, 60, Prime	Thermo Fisher Scientific	Malloy Evelyn	Diazoréactions (DPD)	µmol/l
T20, T20XT, T30, T60, T60 New Generation	Siemens Medical Solutions Diagnostics	Malloy Evelyn	Diazoréactions (DPD)	µmol/l	
Architect c8000	Abbott	NIST SRM 916a	Diazoréactions	µmol/l	
Advia	Siemens Medical Solutions Diagnostics	Oxydation by VANADATE	AACC Reference method	µmol/l	
ALT	Hitachi 917		Reference method IFCC with pyridoxal phosphate (9)	Enzymatic method according to the reference method IFCC.	IU/l
	Modular	Roche Diagnostics			
	Integra				

Parameter	Analyser	Reagents	Material or Reference method	Analytical method	Units
ALT (continue)	RxL ArX XPAND	Dade-Behring	Reference method IFCC with pyridoxal phosphate (9)	Enzymatic method according to the reference method IFCC.	IU/l
	AU400 AU600 AU640 AU2700	Olympus	Reference method IFCC with pyridoxal phosphate (9)	Enzymatic method according to the reference method IFCC.	IU/l
	Konelab 20, 20XT, 30, 60, Prime	Thermo Fisher Scientific	Reference method IFCC with pyridoxal phosphate (9)	Enzymatic method according to the reference method IFCC.	IU/l
	T20, T20XT, T30, T60, T60 New Generation	Siemens Medical Solutions Diagnostics	Reference method IFCC with pyridoxal phosphate (9)	Enzymatic method according to the reference method IFCC.	IU/l
	Architect c8000	Abbott	Reference method IFCC with pyridoxal phosphate (9)	Enzymatic method according to the reference method IFCC. Calibration Cfas Roche	IU/l
	Advia	Siemens Medical Solutions Diagnostics	Reference method IFCC with pyridoxal phosphate (9)	Enzymatic method according to the reference method IFCC.	IU/l

Table 2: Analytical methods used for the complementary FibroMAX assay

Parameter	Material or Reference method	Analytical method	Units
AST	Reference method IFCC with pyridoxal phosphate	Enzymatic method according to the reference method IFCC. (Calibration Cfas Roche for Architect c8000 of Abbott)	IU/l
CHOLESTEROL	Enzymatic colorimetric test (20)	Trinder endpoint reaction	mmol/l
TRIGLYCERIDES	Enzymatic colorimetric test (20)	Trinder endpoint reaction	mmol/l
FASTING GLUCOSE	Enzymatic test (21)	The hexokinase method is a recognised reference method	mmol/l

CRM: Certified Reference Material for 14 serum proteins, Bureau Communautaire de référence à Bruxelles (BCR), International Federation of Clinical Chemistry (IFCC), College of American Pathologists-(CAP); **SRM:** Standard Reference Material; **CFAS** (Roche Diagnostics): Calibrator For Automated Systems; **WHO-IFCC SP1-01** : World Health Organization and International Reference Material.

For the analyzers already in place in the laboratories, the biologist itself need to verify with the manufacturer that the traceability of the results to the reference systems was checked in agreement with European Directives DIVD starting at the end 2003. If corrections are to be done to the programming tests, check with the manufacturer that they were already made on the used analyzers.

Remark A: A study of 150 chronic hepatitis C patients validated the use of FibroTest on DIMENSION analysers product (haptoglobin, apolipoprotein A1, GGT, ALT and total bilirubin).

For more details and a study report please contact **Dade-Behring Company** (phone no. 00 33 1 42 91 24 00). (18)

Remark B: A study comparing 146 patients made it possible to validate the turbidimetric assay of the alpha2-macroglobulin and FibroTest on Roche Diagnostics

analysers (19). A 15 % difference between the nephelometry and turbidimetry for these assays was observed. For FibroTest calculation, the introduction of a correction factor for alpha2-macroglobulin results for Modular and for Cobas Intégra are necessary to ensure concordant results between the system taken as reference (nephelometry Behring) and the system DakoCytomation-Roche Diagnostics (turbidimetry) using the DakoCytomation reagent.

These correction factors were established under very strict analytical conditions and are available from BioPredictive. The biologist eager to carry out these alpha2-macroglobulin assays on analyzer Roche Diagnostics in order to do FibroTest, will have to conform to the following recommendations:

- To use DakoCytomation reagents, calibrator and controls.
- To conform to the adaptation chart provided by the distributor of the reagent for the Modular P analyzer and provided by Roche Diagnoses for the Cobas Integra analyzer.
- To introduce with each series of assay the two different level controls provided by Dako Cytomation. (distributed by ELITECH France)
- The values of controls must imperatively range between more or less 5 % of the target value announced by the manufacturer.
- Validate the series, and then apply the correction factor.

Phone no. Roche Diagnostics: 00 33 4 76 76 30 00 or 00 33 4 76 76 46 63.

Phone no. DAKO CYTOMATION: (French provider J2L ELITECH): 00 33 5 61 88 59 00.

Remark C: Various comparative studies of the results of three proteins of FibroTest (alpha2-macroglobulin, apolipoproteinA1, haptoglobin) were carried out in order to compare results between the Immage nephelometers of the Beckman Coulter Company and nephelometers BN2 and BNProspec of the Dade Behring Company. The results of these studies led to modifications concerning the assay of alpha2-macroglobulin (new antiserum) in order to obtain comparable results with those obtained in the laboratory having validated FibroTest. Currently, the results of apolipoproteineA1 obtained on Immage analyzer require to be corrected by a factor established during two different studies.

You may contact the **Beckman Coulter Company from France (Mrs. Isabelle PELISSOLO, phone 00 33 1 49 90 91 55)** for more information on the reagents to be used for the alpha2-macroglobulin and the factor to be applied for the results of ApolipoproteinA1.

Remark D: The reagents of the Diagam Company were evaluated on Modular analyzers (Roche Diagnostics)-turbidimetric assay-for all three specific proteins of FibroTest (alpha2-macroglobulin, haptoglobin, apolipoproteinA1), their application is from now available for many analyzers and the results don't need a correction factor. **Call for details Diagam Company (Mr.Ameryckx 00 32 49 52 55 080)**

Remark E: A study of 150 chronic hepatitis C patients validated the use of FibroTest on Olympus analyser product (haptoglobin, apolipoprotein A1, GGT, ALT and total bilirubin). For more details please contact **Olympus Company, phone 0 810 00 28 48.**

Remark F: Regarding the Apolipoprotein A1 on Advia (reference 03050910) a correction of -0.20 g / l should be applied to the results of Apolipoprotein A1. For more details please contact Siemens Healthcare Diagnostics (Technical Service: +33 1 34 40 40 50)

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D – Precautions for use

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

- Follow the pre-analytical and analytical recommendations for analyzer and reagent use.
- Defer the test in transient situations which could modify the components of the FibroTest, such as:
 - Acute hemolysis (malarial attack; medications causing hemolysis such as ribavirin, azathioprine), which could decrease haptoglobin and increase nonconjugated bilirubin.
 - Acute hepatitis whether drug-induced, viral (super infection 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.
 - Extra hepatic cholestasis, such as gallstones.
- The advice of a liver disease specialist should be sought for interpretation in chronic states where the components of the test could be modified, such as:
 - Chronic hemolysis, particularly in patients with a cardiac valvular prosthesis
 - Gilbert's disease, where FibroTest and ActiTest should be calculated using the median of the observed total bilirubin in the original study (10 μ moles/L).
 - 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, where hemolysis should be ruled out.
 - Haptoglobin higher than 3.2 g/L where acute inflammation or sepsis should be ruled out.
 - Transaminases higher than 622 IU/l, where acute hepatitis should be ruled out.
 - Bilirubin higher than 30 μ moles/L and GGT lower than 50 IU/l, where Gilbert's syndrome should be suspected.
 - Alpha2-macroglobulin higher than 5.0 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. 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%).

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