Enhanced Liver Fibrosis (ELF™)

Current Revision and Date[a]	Rev. 02, 2022-10	
Product Name	ADVIA Centaur Enhanced Liver Fibrosis (ELF)	REF 11207300
Abbreviated Product Name	ADVIA Centaur ELF	
Test Name/ID	ELF	
Systems	ADVIA Centaur XP system ADVIA Centaur XPT system	
Materials Provided	ADVIA Centaur Hyaluronic Acid (HA)	50 tests
	ADVIA Centaur Amino-Terminal Propeptide of Type III Procollagen (PIIINP)	50 tests
	ADVIA Centaur Tissue Inhibitor of Matrix Metalloproteinase 1 (TIMP-1)	50 tests
	ADVIA Centaur ELF CAL	
Materials Required but Not Provided	ADVIA Centaur ELF QC	REF 10492342
	ADVIA Centaur Ancillary Probe Wash 1 (for HA)	REF 03395373
	ADVIA Centaur Probe Wash 3 (for PIIINP)	REF 03333963
	ADVIA Centaur Wash 1 (2 x 1500 mL)	REF 01137199
	ADVIA Centaur Wash 1 (2 x 2500 mL)	REF 03773025
Optional Materials	ADVIA Centaur ELF MCM	REF 11208709
	ADVIA Centaur Multi-Diluent 10 (diluent) (for TIMP-1)	REF 05440554
	ADVIA Centaur Multi-Diluent 13 (diluent) (for HA and PIIINP)	REF 10492364
Specimen Types	Serum	
Sample Volume	ADVIA Centaur HA: 20 µL ADVIA Centaur PIIINP: 20 µL ADVIA Centaur TIMP-1: 25 µL	
Measuring Interval	ADVIA Centaur HA: 3.00–1000.00 ng/mL ADVIA Centaur PIIINP: 1.00–150.00 ng/mL ADVIA Centaur TIMP-1: 5.0–1300.0 ng/mL	

[a] A vertical bar in the page margin indicates technical content that differs from the previous version.

Intended Use

ADVIA Centaur® Enhanced Liver Fibrosis (ELF™) is for *in vitro* diagnostic use in the determination of an ELF score based on the combined quantitative measurements of hyaluronic acid, amino-terminal propeptide of type III procollagen, and tissue inhibitor of matrix metalloproteinase 1 in human serum using the ADVIA Centaur® XP and ADVIA Centaur® XPT systems.

ADVIA Centaur ELF is indicated as a prognostic marker in conjunction with other laboratory findings and clinical assessments in patients with advanced fibrosis (F3 or F4) due to non-alcoholic steatohepatitis (NASH) to assess the likelihood of progression to cirrhosis and liver-related clinical events.

Summary and Explanation

Chronic liver disease is a major cause of morbidity and mortality throughout the world.[1-3] Typical disease etiologies that lead to chronic liver disease include non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease, primary sclerosing cholangitis, primary biliary cholangitis, and viral hepatitis due to chronic infection with hepatitis B virus and hepatitis C virus.

NAFLD is now the most common underlying cause of chronic liver disease.[4,5] In 1994, NAFLD accounted for < 50% of chronic liver disease cases, but due to the increasing rate of obesity, the proportion of NAFLD of chronic liver disease cases jumped to 75% by 2008.[4] Furthermore, it is anticipated that the incidence of NAFLD will continue to rise in parallel with the increasing incidence of obesity and type 2 diabetes mellitus.[6]

Within NAFLD, nonalcoholic steatohepatitis (NASH) is the more severe form, characterized by hepatic steatosis (fatty infiltration of the liver), inflammation, and hepatocyte injury (ballooning).[7] Prevalence in the United States is estimated to range from 3–5% in the general population and up to 56% in obese individuals.[8] Over the next 20 years, it is estimated that the 6.4 million individuals living with NASH and type 2 diabetes mellitus in the U.S. will account for 65,000 transplants, 1.37 million cardiovascular-related deaths, and 812,000 liver-related deaths.[9]

The clinical course of chronic liver disease is highly variable, but the typical progression involves the advancement of fibrosis, leading to cirrhosis, followed by either decompensation or hepatocellular carcinoma (HCC), and ultimately liver transplantation or death.[10] In the U.S., waitlist registrations for transplants due to NASH have increased 170% from 2004 to 2013.[11]

Serum assays for products of extracellular matrix (ECM) synthesis or degradation and the enzymes involved in these processes have been investigated as markers of liver fibrosis.[12-14]

ADVIA Centaur ELF quantifies analytes that are components of the ECM, which directly contribute to liver fibrosis. Hyaluronic acid (HA), a glycosaminoglycan produced by hepatic stellate cells, is an essential component within the connective matrix. Amino-terminal propeptide of type III procollagen (PIIINP), produced by fibroblasts in the liver, is a marker of fibrogenesis and inflammation. Tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) is a circulating inhibitor of matrix metalloproteinase (MMP) enzymes that can inhibit fibrolysis and fibrosis repair. Together, these analytes reflect qualitative and quantitative changes in the ECM associated with progression of liver disease.

The ECM refers to a set of macromolecules that comprises the extracellular scaffolding of the liver. Some ECM markers reflect fibrogenesis and others reflect fibrosis regression, allowing for a dynamic evaluation of ECM activity.

The ELF score, derived from an algorithm that combines the individual measurements of HA, PIIINP, and TIMP-1, has been shown to be useful as a prognostic marker in patients with advanced fibrosis due to NASH to assess the risk of progressing to cirrhosis and the occurrence of future liver-related clinical events.[15-21]

A prognostic risk assessment using ADVIA Centaur ELF, in conjunction with other laboratory findings and clinical assessments, may be useful in determining which patients could benefit from additional examinations, from increased monitoring, and from potential lifestyle changes and treatment interventions.

Principles of the Procedure

The reagents that comprise ADVIA Centaur ELF are ADVIA Centaur HA, ADVIA Centaur PIIINP, and ADVIA Centaur TIMP-1. These reagents are based on 2-site sandwich assay methodology and direct chemiluminescent technology, and are run on fully automated ADVIA Centaur instruments.

ADVIA Centaur HA reagents use hyaluronic acid binding protein (HABP) for both capture and detection of HA. The Lite Reagent contains HABP conjugated with acridinium ester, and the Ancillary Well Reagent contains HABP conjugated with fluorescein isothiocyanate (FITC). The Solid Phase contains an anti-FITC mouse monoclonal antibody covalently bound to paramagnetic particles.

ADVIA Centaur PIIINP reagents use 2 mouse monoclonal antibodies (MAb). The antibody in the Lite Reagent is an acridinium-ester-labeled anti-PIIINP antibody. The antibody in the Ancillary Well Reagent is a biotin-labeled anti-PIIINP antibody. The Solid Phase contains streptavidin-coated paramagnetic particles.

ADVIA Centaur TIMP-1 reagents use 2 mouse monoclonal antibodies (MAb) that bind to TIMP-1 in the Lite Reagent: an anti-TIMP-1 F(ab)2 antibody labeled with acridinium ester and an FITC-labeled anti-TIMP-1 antibody. The Solid Phase contains an anti-FITC mouse monoclonal antibody covalently bound to paramagnetic particles.

A direct relationship exists between the amount of HA, PIIINP, and TIMP-1 present in the patient sample and the amount of relative light units (RLUs) detected by the system.

For ADVIA Centaur XP and ADVIA Centaur XPT systems, the ELF score is calculated using the following equation:

$$\text{ELF score} = 2.278 + 0.851 \ln(C_{HA}) + 0.751 \ln(C_{PIIINP}) + 0.394 \ln(C_{TIMP\text{-}1})$$

Concentrations (C) of each of the constituents are in ng/mL.

Reagents

Material Description	Storage	Stability
ADVIA Centaur HA ReadyPack® primary reagent pack[a, b]	Unopened at 2–8°C	Until expiration date on product
Lite Reagent 10.0 mL/reagent pack Hyaluronic acid binding protein (bovine) labeled with acridinium ester (~0.5 µg/mL) in buffer; bovine serum albumin; surfactant; preservatives **Solid Phase** 10.0 mL/reagent pack Paramagnetic particles coated with mouse monoclonal anti-FITC (~37.8 µg/mL) in buffer; bovine serum albumin; surfactant; sodium azide (< 0.1%) **Ancillary Well Reagent** 5.0 mL/reagent pack Hyaluronic acid binding protein (bovine) labeled with FITC (~0.55 µg/mL) in buffer; bovine serum albumin; surfactant	Onboard	28 days

Material Description	Storage	Stability
ADVIA Centaur PIIINP ReadyPack® primary reagent pack[a, b] **Lite Reagent** 5.0 mL/reagent pack Mouse monoclonal anti-PIIINP labeled with acridinium ester (~2.0 µg/mL) in buffer; bovine serum albumin; mouse IgG; surfactant; sodium azide (< 0.1%) **Solid Phase** 15.0 mL/reagent pack Streptavidin-coated paramagnetic microparticles (~0.225 mg/mL) in buffer; bovine serum albumin; surfactant; sodium azide (< 0.1%) **Ancillary Well Reagent** 5.0 mL/reagent pack Mouse monoclonal anti-PIIINP labeled with biotin (~0.7 µg/mL) in buffer; bovine serum albumin; mouse IgG; surfactant; sodium azide (< 0.1%)	Unopened at 2–8°C Onboard	Until expiration date on product 28 days
ADVIA Centaur TIMP-1 ReadyPack® primary reagent pack[a, b] **Lite Reagent** 10.0 mL/reagent pack Mouse monoclonal anti-TIMP-1 labeled with acridinium ester (~0.1 µg/mL) and mouse monoclonal anti-TIMP-1 labeled with FITC (~1.8 µg/mL) in buffer; goat serum; bovine serum albumin; bovine gamma globulin; mouse IgG; surfactant; preservatives **Solid Phase** 17.5 mL/reagent pack Paramagnetic particles coated with mouse monoclonal anti-FITC (~26.3 µg/mL) in buffer; goat serum; bovine serum albumin; bovine gamma globulin; surfactant; preservatives	Unopened at 2–8°C Onboard	Until expiration date on product 28 days
ADVIA Centaur ELF CAL[a] 2.0 mL/vial After reconstitution, low or high levels of HA, PIIINP (bovine), TIMP-1 (human); bovine serum albumin, buffer; sodium azide (< 0.1%); preservative	Lyophilized at 2–8°C Reconstituted at 2–8°C Reconstituted at room temperature	Until the expiration date on the vial label 60 days 8 hours
ADVIA Centaur Multi-Diluent 10 ReadyPack ancillary reagent pack[b, c] 5.0 mL/pack Tris buffer; bovine serum albumin; goat serum; mouse serum; sodium azide (< 0.1%); preservatives	Unopened at 2–8°C Onboard	Until expiration date on product 28 days
ADVIA Centaur Multi-Diluent 13 ReadyPack ancillary reagent pack[b, c] 10.0 mL/pack Buffer; surfactant; sodium azide (< 0.1%)	Unopened at 2–8°C Onboard	Until expiration date on product 28 days
ADVIA Centaur Ancillary Probe Wash 1 ReadyPack ancillary reagent pack[a, d] 25.0 mL/pack 0.4 N sodium hydroxide	Unopened at 2–8°C Onboard	Until expiration date on product 14 days

Material Description	Storage	Stability
ADVIA Centaur Probe Wash 3[b, d] 50.0 mL/pack Sodium hypochlorite (0.5%), sodium hydroxide (< 0.5%), pH 11.0	Unopened at 2–8°C	Until expiration date on product
	Onboard	100 days
ADVIA Centaur Wash 1[b, d] 1500 mL/pack Phosphate-buffered saline with sodium azide (< 0.1%); surfactant	Unopened at 2–25°C	Until expiration date on product
	Onboard	30 days
ADVIA Centaur Wash 1[b, d] 2500 mL/pack Phosphate-buffered saline with sodium azide (< 0.1%); surfactant	Unopened at 2–25°C	Until expiration date on product
	Onboard	30 days

[a] Store in an upright position.
[b] Prevent exposure to sunlight and heat.
[c] Refer to *Optional Materials*.
[d] Refer to *Materials Required but Not Provided*.

Warnings and Precautions

For *in vitro* diagnostic use.

For Professional Use.

For Prescription Use Only.

CAUTION

Federal (USA) law restricts this device to sale by or on the order of a licensed healthcare professional.

Safety data sheets (SDS) available on siemens-healthineers.com.

H312, H412
P280, P273, P312, P501

Warning!
Harmful in contact with skin. Harmful to aquatic life with long lasting effects.
Wear protective gloves/protective clothing/eye protection/face protection. Avoid release to the environment. Call a POISON CENTER or doctor/physician if you feel unwell. Dispose of contents and container in accordance with all local, regional, and national regulations.
Contains: Sodium azide (ADVIA Centaur ELF CAL).

H290, H319, H315
P234, P264, P280, P337+P313, P390, P501

Warning!
May be corrosive to metals. Causes serious eye irritation. Causes skin irritation.
Keep only in original container. Wash hands thoroughly after handling. Wear protective gloves/protective clothing/eye protection/face protection. If eye irritation persists: Get medical advice/attention. Absorb spillage to prevent material damage. Dispose of contents and container in accordance with all local, regional, and national regulations.
Contains: sodium hydroxide (ADVIA Centaur Ancillary Probe Wash 1 for ADVIA Centaur HA assay).

H412	Harmful to aquatic life with long lasting effects.
P273, P501	Avoid release to the environment. Dispose of contents and container in accordance with all local, regional, and national regulations. **Contains:** Sodium hypochlorite (ADVIA Centaur Probe Wash 3 for ADVIA Centaur PIIINP assay).

Warning! Potential Biohazard

Contains human source material.

No known test method can ensure that products derived from human source materials will not transmit infection. These materials should be handled using good laboratory practices and universal precautions.[22-24]

CAUTION

This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.

Storage and Stability

Store all reagents in an upright position, away from light and heat. Do not use products beyond the expiration date printed on the product labeling.

For information about storage and stability, refer to *Reagents*.

Onboard Stability

Discard products at the end of the onboard stability interval. Do not use products beyond the expiration date printed on the product labeling.

For information about product onboard stability, refer to *Reagents*.

Specimen Collection and Handling

Serum is the recommended specimen type.

Collecting the Specimen

- Observe universal precautions when collecting specimens. Handle all specimens as if they are capable of transmitting disease.[24]
- Follow recommended procedures for collection of diagnostic blood specimens by venipuncture.[25]
- Follow the instructions provided with your specimen collection device for use and processing.[26]
- Allow blood specimens to clot completely before centrifugation.[27]
- Keep tubes capped at all times.[27]

Storing the Specimen

- Specimens may be stored on the clot for up to 48 hours at 2–8°C.
- Samples may be stored at room temperature for up to 24 hours prior to centrifugation.
- Separated samples are stable for 8 hours onboard the system, for 48 hours at room temperature, and for 7 days at 2–8°C.
- Separated samples are stable at ≤ -20°C for up to 12 months. Avoid more than 4 freeze-thaw cycles. Do not store in a frost-free freezer. Thoroughly mix thawed samples and centrifuge them before using.

The handling and storage information provided here is based on data or references maintained by the manufacturer. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria to meet specific needs.

Transporting the Specimen

Package and label specimens for shipment in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiological agents.

If during shipment, specimens may be subjected to temperatures > 25°C, then ship specimens frozen.

Preparing the Samples

For a single determination, measurement of HA requires 20 µL of sample, measurement of PIIINP requires 20 µL of sample, and measurement of TIMP-1 requires 25 µL of sample. These volumes do not include the unusable volume in the sample container, or the additional volume required when performing duplicates or other tests on the same sample. For a complete list of appropriate sample containers and information about determining the minimum required volume, refer to the system online help.

The sample volume required to perform onboard dilution may differ from the sample volume required to perform a single determination on an undiluted sample. Refer to *Dilutions*.

Do not use specimens with apparent contamination.

Before placing samples on the system, ensure that samples are free of:

- Bubbles or foam.
- Fibrin or other particulate matter.

Remove particulates by centrifugation according to CLSI guidance and the collection device manufacturer's recommendations.[27]

Procedures

Materials Provided

The following materials are provided:

REF	Contents	Number of Tests
11207300	1 ReadyPack primary reagent pack containing ADVIA Centaur HA Lite Reagent, Solid Phase, and Ancillary Well Reagent 1 ReadyPack primary reagent pack containing ADVIA Centaur PIIINP Lite Reagent, Solid Phase, and Ancillary Well Reagent 1 ReadyPack primary reagent pack containing ADVIA Centaur TIMP-1 Lite Reagent and Solid Phase ADVIA Centaur HA master curve card ADVIA Centaur PIIINP master curve card ADVIA Centaur TIMP-1 master curve card 1 vial of ADVIA Centaur ELF CAL low calibrator CAL L 1 vial of ADVIA Centaur ELF CAL high calibrator CAL H ADVIA Centaur ELF CAL calibrator assigned value cards and barcode labels CAL LOT VAL	50

Materials Required but Not Provided

The following materials are required to perform ADVIA Centaur ELF, but are not provided:

REF	Description
	ADVIA Centaur XP system[a] ADVIA Centaur XPT system[a]
10492342	ADVIA Centaur ELF™ QC (quality control material)
03395373	ADVIA Centaur Ancillary Probe Wash 1 ReadyPack ancillary reagent pack APW 1 (For ADVIA Centaur HA)
03333963	ADVIA Centaur Probe Wash 3 ReadyPack reagent pack PW 3 (For ADVIA Centaur PIIINP)
01137199 (112351)	ADVIA Centaur Wash 1 WASH 1
03773025	ADVIA Centaur Wash 1 WASH 1

[a] Additional system fluids are required to operate the system: ADVIA Centaur Wash, ADVIA Centaur Acid, ADVIA Centaur Base, and ADVIA Centaur Cleaner. For system fluid instructions for use, refer to the Document Library.

Optional Materials

The following materials may be used to perform ADVIA Centaur ELF, but are not provided:

REF	Description
11208709	ADVIA Centaur ELF™ MCM (master curve material)
05440554	ADVIA Centaur Multi-Diluent 10 (diluent) DIL (For ADVIA Centaur TIMP-1)
10492364	ADVIA Centaur Multi-Diluent 13 (diluent) DIL (For ADVIA Centaur HA and ADVIA Centaur PIIINP)

Assay Procedure

Measurements for HA, PIIINP, and TIMP-1 must be obtained within 8 hours of one another for the ELF score to be valid.

The ADVIA Centaur ELF score is automatically calculated by the ADVIA Centaur XP system with software version 7.0 or higher and is reported along with results for HA, PIIINP, and TIMP-1.

For the ADVIA Centaur XP system with software versions earlier than version 7.0, only HA, PIIINP, and TIMP-1 results are reported. The ELF score must be calculated manually.

Refer to the system online help to schedule multi-component testing on the ADVIA Centaur XP system, software version 7.0 or higher. When using earlier software versions for the ADVIA Centaur XP system, you can determine the ELF score using the following instructions:

1. Schedule measurements of HA, PIIINP and TIMP-1 individually. Refer to the system online help.
2. Calculate the ELF score manually.

Refer to *Calculation of Results* for more information.

Measurement of HA

The system automatically performs the following steps:

1. Dispenses 20 µL of sample into a cuvette.
2. Dispenses 100 µL of Ancillary Well Reagent and 200 µL of Solid Phase, then incubates for 18 minutes at 37°C.
3. Separates, aspirates, then washes the cuvette with ADVIA Centaur Wash 1.
4. Resuspends the particles in 115 µL of ADVIA Centaur Wash 1 and incubates for approximately 7 minutes.
5. Dispenses 200 µL of Lite Reagent, then incubates for 18 minutes at 37°C.
6. Separates, aspirates, then washes the cuvette with ADVIA Centaur Wash 1.
7. Dispenses 300 µL each of ADVIA Centaur Acid and ADVIA Centaur Base to initiate the chemiluminescent reaction.
8. Reports results.

Measurement of PIIINP

The system automatically performs the following steps:

1. Dispenses 20 µL of sample into a cuvette.
2. Dispenses 300 µL of Solid Phase and 100 µL of Ancillary Well Reagent, then incubates for 3 minutes at 37°C.

3. Dispenses 100 µL of Lite Reagent, then incubates for 6 minutes at 37°C.
4. Separates, aspirates, then washes the cuvette with ADVIA Centaur Wash 1.
5. Dispenses 300 µL each of ADVIA Centaur Acid and ADVIA Centaur Base to initiate the chemiluminescent reaction.
6. Reports results.

Measurement of TIMP-1

The system automatically performs the following steps:

1. Dispenses 25 µL of sample into a cuvette.
2. Dispenses 350 µL of Solid Phase and 200 µL of Lite Reagent, then incubates for 3 minutes at 37°C.
3. Separates, aspirates, then washes the cuvette with ADVIA Centaur Wash 1.
4. Dispenses 300 µL each of ADVIA Centaur Acid and ADVIA Centaur Base to initiate the chemiluminescent reaction.
5. Reports results.

Preparing the Reagents

All reagents are liquid and ready to use. Before loading the packs onto the system, reagents require mixing. For information about mixing the reagents, refer to the system online help.

Preparing the System

Ensure that sufficient reagent packs are loaded onto the system. Refer to *Materials Provided* and *Materials Required but Not Provided* for guidance about required reagents.

For information about loading products, refer to the system online help.

Master Curve Definition

Before initiating calibration on each new lot of reagent, enter the master curve values by scanning the master curve cards. For information about defining the master curve, refer to the system online help.

Performing Calibration

For calibration of ADVIA Centaur ELF, use the calibrators provided with each kit to calibrate measurements of HA, PIIINP and TIMP-1.

Note Calibrators provided in an assay kit must only be used with the reagent lot provided in the same kit.

Calibration Frequency

Perform a calibration if one or more of the following conditions exist:

- At the end of the 14-day calibration interval.
- When changing lot numbers of primary reagent packs.
- When indicated by quality control results.
- After major maintenance or service, if indicated by quality control results.

Follow government regulations or accreditation requirements for calibration frequency. Individual laboratory quality control programs and procedures may require more frequent calibration.

Preparing the Calibrators

Prepare calibrators using the following steps:

1. Add 2.0 mL of reagent water into each vial. Replace cap.

   Note For information about reagent water requirements, refer to the system online help.

2. Let the vials stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve.
3. Gently swirl and invert the vials to ensure homogeneity of the material.

Note Use calibrators within the stability limits specified in *Reagents* and discard any remaining material.

Calibration Procedure

Perform the calibration procedure using the following steps:

1. Ensure that the appropriate master curve and calibrator assigned values are entered on the system. For information about defining the master curve and entering calibrator values, refer to the system online help.
2. Load the required reagents.
3. Schedule the calibrators.
4. Label two sample containers with barcode labels: one container for the low calibrator and one container for the high calibrator. Place the barcode labels on the sample containers with the readable characters oriented vertically.

   Note Barcode labels are lot-specific. Do not use barcode labels from one lot of calibrators with any other lot of calibrators.

5. Gently mix the product and dispense a sufficient volume of each calibrator into the appropriate sample containers. Avoid bubbles.

   The required sample volume for testing depends on several factors. For information about sample volume requirements, refer to the system online help.

6. Load the samples according to the system online help.

Note Dispose of any calibrator that remains in the sample container after 8 hours. Do not refill or reuse sample containers. Do not return any calibrator material back into the original container.

Performing Quality Control

For quality control of ADVIA Centaur ELF, use the ADVIA Centaur ELF QC at least once during each day that samples are analyzed. Use the quality control material in accordance with the quality control instructions for use. For the assigned values, refer to the quality control assigned value sheet provided.

Additional quality control material can be used at the discretion of the laboratory. Use the quality control material in accordance with the quality control instructions for use.

In addition, perform quality control:

- Following a valid calibration
- With use of a new lot of reagent
- When troubleshooting test results that do not match clinical conditions or symptoms

Follow government regulations or accreditation requirements for quality control frequency. Individual laboratory quality control programs and procedures may require more frequent quality control testing.

Acceptable performance is achieved when the analyte values obtained are within the expected control interval for the system, as indicated by the manufacturer of the control material or within the interval determined by an internal laboratory quality control procedure.

Follow your laboratory's quality control procedures if the results obtained do not fall within the acceptable limits. For information about entering quality control definitions, refer to the system online help.

Taking Corrective Action

If the quality control results do not fall within the expected control interval, do not report results. Perform corrective actions in accordance with established laboratory protocol. For suggested protocol, refer to the system online help.

Results

Calculation of Results

The ELF score is a unitless numerical value, derived from an algorithm that combines the individual quantitative measurements of HA, PIIINP, and TIMP-1.

The ELF score is automatically calculated by the ADVIA Centaur XP system with software version 7.0 or higher and is reported along with results for HA, PIIINP, and TIMP-1.

For the ADVIA Centaur XP system with software versions earlier than version 7.0, only HA, PIIINP, and TIMP-1 results are reported. The ELF score must be calculated manually. Results for HA, PIIINP, and TIMP-1 must be obtained within 8 hours of one another for the ELF score to be valid. For detailed information about how the system calculates results, refer to the system online help.

Note The auto-calculation feature is only available for the ADVIA Centaur XP system, software version 7.0 or higher.

For the earlier versions of the ADVIA Centaur XP system, calculate the ELF score manually according to the following steps:

1. Obtain measurements for HA, PIIINP, and TIMP-1.
2. Use the following equation to calculate the ELF score:

   $$\text{ELF score} = 2.278 + 0.851 \ln(C_{HA}) + 0.751 \ln(C_{PIIINP}) + 0.394 \ln(C_{TIMP\text{-}1})$$

   Concentrations (C) of each of the constituents are in ng/mL.

Dilutions

HA

Serum HA measurements performed using ADVIA Centaur ELF are validated from 3.00–1000.00 ng/mL. For information about dilution options, refer to the system online help.

Dilute and retest serum samples with HA levels > 1000.00 ng/mL to obtain accurate results.

For automated dilutions, perform the following activities:

1. Load ADVIA Centaur Multi-Diluent 13.
2. Ensure that sufficient sample volume is available.
3. Select the appropriate dilution factor.

For automatic dilutions, enter a dilution setpoint ≤ 1000.00 ng/mL.

Sample	Dilution	Sample Volume (µL)
Serum	1:5	40

PIIINP

Serum PIIINP measurements performed using ADVIA Centaur ELF are validated from 1.00–150.00 ng/mL. For information about dilution options, refer to the system online help.

Dilute and retest serum samples with PIIINP levels > 150.00 ng/mL to obtain accurate results.

For automated dilutions, perform the following activities:

1. Load ADVIA Centaur Multi-Diluent 13.
2. Ensure that sufficient sample volume is available.
3. Select the appropriate dilution factor.

For automatic dilutions, enter a dilution setpoint ≤ 150.00 ng/mL.

Sample	Dilution	Sample Volume (µL)
Serum	1:5	30

ADVIA Centaur TIMP-1

Serum TIMP-1 measurements performed using ADVIA Centaur ELF are validated from 5.0–1300.0 ng/mL. For information about dilution options, refer to the system online help.

Dilute and retest serum samples with TIMP-1 levels > 1300.0 ng/mL to obtain accurate results.

For automated dilutions, perform the following activities:

1. Load ADVIA Centaur Multi-Diluent 10.
2. Ensure that sufficient sample volume is available.
3. Select the appropriate dilution factor.

For automatic dilutions, enter a dilution setpoint ≤ 1300.0 ng/mL.

Sample	Dilution	Sample Volume (µL)
Serum	1:5	25

Interpretation of Results

Interpret the ELF score using the following guidelines:

ELF Score	Risk of Disease Progression (Development of Cirrhosis or Liver-Related Events)
< 9.80	Lower
≥ 9.80 – < 11.30	Mid[a]
≥ 11.30	Higher

[a] In the Mid group, the risk of disease progression is similar to the pre-test risk. Pre-test risk refers to the likelihood of disease progression in the overall intended use population without considering the ELF score.

Results should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.

Limitations

- Measurements of HA, PIIINP and TIMP-1 must be obtained within 8 hours of one another for the ELF score to be valid.
- ADVIA Centaur ELF is limited to the detection of HA, PIIINP, and TIMP-1 in human serum.

- Only use results obtained on the ADVIA Centaur XP and XPT systems to calculate ELF scores.
- Do not use hemolyzed samples.
- Do not use in patients taking biotin supplements.
- Do not use samples that contain fluorescein. Samples with fluorescein may cause falsely depressed results. Evidence suggests that patients undergoing retinal fluorescein angiography can retain amounts of fluorescein in the body for up to 72 hours post-treatment. In cases of patients with renal insufficiency, including many diabetics, retention could be longer.[28]
- Patient samples may contain heterophilic antibodies that could react in immunoassays and cause falsely elevated or depressed results. This assay is designed to minimize interference from heterophilic antibodies.[29,30] Additional information may be required for diagnosis.
- An ELF score < 9.80 is associated with a lower prognostic risk, but disease progression is still possible for patients with ELF measurements below this threshold.
- An ELF score ≥ 11.30 is associated with a higher prognostic risk, but disease progression may not occur in patients with ELF measurements above this threshold.
- The reagents for HA, PIIINP, and TIMP-1 have only been clinically validated in terms of their combined contribution to the ELF score. Clinical decisions should not be based on individual measurements of HA, PIIINP, or TIMP-1.
- ADVIA Centaur ELF is not for use in the diagnosis of NASH or for the staging of fibrosis.
- ADVIA Centaur ELF is not for use in the serial monitoring of disease progression or for the monitoring of effects of therapeutic products.
- Test results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including information obtained by alternative methods, and clinical evaluation as appropriate.
- Always interpret results in conjunction with the patient's medical history, clinical presentation, and other findings.

Expected Values

Expected values of ADVIA Centaur ELF were established on the ADVIA Centaur XP system with 594 samples from U.S. blood donors of known gender, ethnicity, and age. Study subjects were selected to be representative of the U.S. population. Although subjects were apparently healthy and presumably free of viral hepatitis, the absence of liver disease (including cryptic fibrosis) and other conditions associated with liver fibrosis (including metabolic syndrome) were not confirmed.

Reference intervals were determined by calculating the 5th and 95th percentiles of the distribution of values accordance with the CLSI guideline EP28-A3c.[31] In populations with n < 120, a robust method was used to determine reference intervals.

Gender	Ethnicity	N	Age Range	Mean	Median	5th Percentile	95th Percentile
Female	African American	68	27–62	7.62	7.66	6.38	8.92
Female	Caucasian	86	18–64	7.74	7.77	6.63	8.81
Female	All	154	18–64	7.69	7.75	6.53	8.69
Male	African American	236	18–65	7.64	7.66	6.19	8.92
Male	Caucasian	198	19–66	7.85	7.81	6.87	9.01
Male	All[a]	440	24–53	7.74	7.74	6.46	8.94

Gender	Ethnicity	N	Age Range	Mean	Median	5th Percentile	95th Percentile
Both	African American	304	18–65	7.64	7.68	6.21	8.83
Both	Caucasian	284	18–66	7.82	7.79	6.83	8.97
Both	All[a]	594	18–66	7.72	7.74	6.51	8.90

[a] Includes an additional 6 Hispanic male subjects.

A published study conducted using 183 apparently healthy East Asian female and male subjects demonstrated the following ELF scores: mean of 7.75, median of 7.82, 5th percentile of 5.95, and 95th percentile of 8.73.[32]

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference interval for the diagnostic evaluation of patient results.[31] Consider these values as guidance only.

Performance Characteristics

The reagent formulations used on the ADVIA Centaur XPT system are the same as those used on the ADVIA Centaur XP system.

Measuring Interval

HA

3.00–1000.00 ng/mL

The lower limit of the measuring interval is defined by the limit of quantitation (LoQ). Report results below the measuring interval as < 3.00 ng/mL. When sample results exceed the measuring interval, refer to *Dilutions*.

PIIINP

1.00–150.00 ng/mL

The lower limit of the measuring interval is defined by the LoQ. Report results below the measuring interval as < 1.00 ng/mL. When sample results exceed the measuring interval, refer to *Dilutions*.

TIMP-1

5.0–1300.0 ng/mL

The lower limit of the measuring interval is defined by the LoQ. Report results below the measuring interval as < 5.0 ng/mL. When sample results exceed the measuring interval, refer to *Dilutions*.

Clinical Performance

Prognostic performance of ADVIA Centaur ELF in subjects with advanced fibrosis due to NASH was analyzed using data generated in the placebo arms of 5 prospective clinical trials of investigational drugs.

The information provided should not be used to infer risk in patient populations other than those described for patients with advanced fibrosis due to NASH.

NASH F3 (Bridging Fibrosis)

Two prospective multicenter international clinical trials were performed in NASH subjects with biopsy-confirmed bridging fibrosis (F3; Ishak 3-4) to evaluate the safety and efficacy of investigational drugs (NCT01672866; NCT03053050).[18,21,33,34]

Study A (NCT01672866) included subjects with chronic liver disease due to NASH defined as macrovesicular steatosis involving > 5% of hepatocytes on a liver biopsy with associated lobular inflammation and Stage 3-4 fibrosis by Ishak score. The intended follow-up period of this trial was 240 weeks, but it was discontinued after the Week 96 interim analysis. For the placebo arm subjects included in the analysis, the median follow-up time was 27.4 months (Interquartile range (IQR): 22.2–31.7 months).

Study B (NCT03053050) included subjects with liver biopsy consistent with NASH and bridging (F3) fibrosis according to the NASH Clinical Research Network (CRN) classification. The intended follow-up period of this trial was 240 weeks, but it was discontinued after the Week 48 interim analysis. For the placebo arm subjects included in the analysis, the median follow-up time was 16.2 months (IQR: 13.9–18.7 months).

Baseline clinical characteristics for the clinical trial placebo subjects included in the analysis are described in the following table.

	Study A (N = 68)	**Study B (N = 144)**
Age in years (median [IQR])	56 (48-59)[a]	59 (51-63)[a]
Sex (N [%]) Female: Male:	 43 (65%) 25 (35%)	 69 (48%) 75 (52%)
Diabetes mellitus (N [%])	44 (68%)	103 (72%)
BMI in kg/m^2 (median [IQR])	32.7 (29.0–37.3)[a]	32.2 (27.5–37.5)[a]
Ishak score (N [%]) Ishak 3: Ishak 4:	 36 (53%) 32 (47%)	 79 (55%) 65 (45%)
ELF score, median (median [IQR])	9.7 (9.3-10.4)	9.9 (9.3-10.6)

[a] Calculations were performed using the enrolled placebo groups (N=74 for Study A; N=159 for Study B) which included subjects without ELF scores and subjects without follow-up biopsy.

An analysis of 212 subjects pooled from the placebo arms of these studies estimated the prognostic ability of ADVIA Centaur ELF at baseline to predict the risk of progression to cirrhosis (defined histologically as Ishak 5 or 6) up to 3.9 years following enrollment (median: 16.8 months; IQR: 14.0-22.4 months). Although the data presented below relates to a pooled analysis from multiple clinical trials, it is representative of the totality of the information provided in the NASH F3 population.

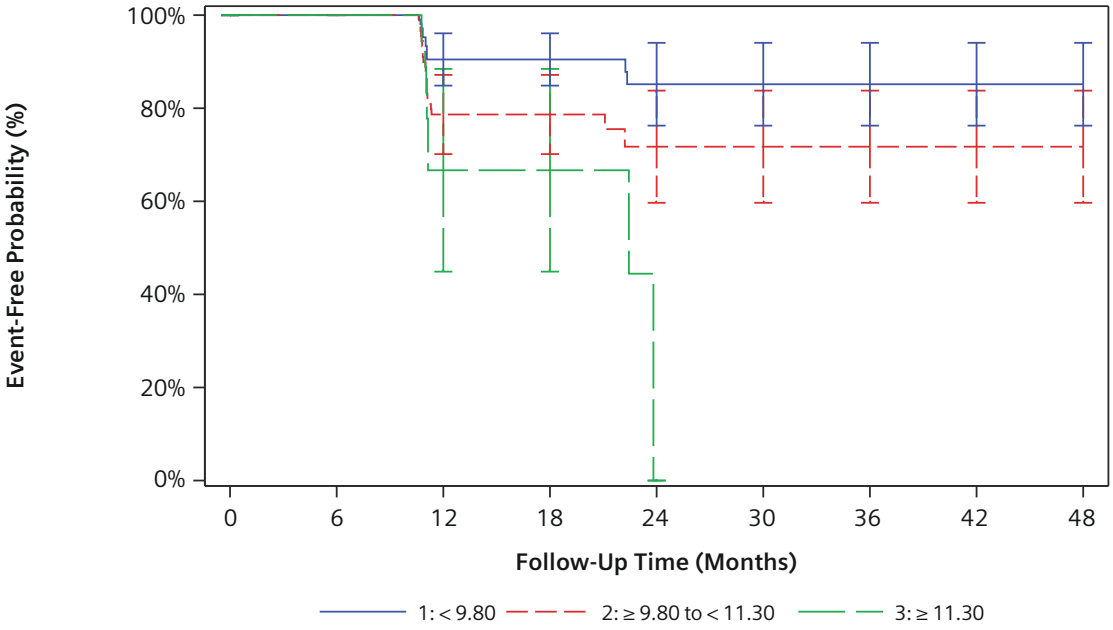
Baseline ELF measurements were performed on an ADVIA Centaur XP system. The risk of progression to cirrhosis was assessed according to absolute risk, likelihood ratio (LR) and Cox proportional hazard ratio.

Risk Group (ELF Score)	**N[a]**	**Progression to Cirrhosis**		**Absolute Risk (95% CI)[b]**	**Likelihood Ratio (95% CI)**	**Cox Proportional Hazard Ratio (95% CI)**
		Yes	**No**			
Lower (< 9.80)	105	12	93	11.4% (5.3%, 17.5%)	0.54 (0.33, 0.88)	1.00
Mid (≥ 9.80 to < 11.30)	89	21	68	23.6% (14.8%, 32.4%)	1.29 (0.91, 1.83)	2.30 (1.13, 4.68)

Risk Group (ELF Score)	N[a]	Progression to Cirrhosis		Absolute Risk (95% CI)[b]	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI)
		Yes	No			
Higher (≥ 11.30)	18	8	10	44.4% (21.5%, 67.4%)	3.34 (1.40, 7.92)	4.58 (1.87, 11.24)
All	212	41	171	19.3% (14.2%, 25.3%)	—	—

[a] Number of subjects.
[b] Confidence interval.

Kaplan-Meier curves up to 48 months were plotted for each of the risk groups to display the proportion of subjects without a liver-related event over time.



	Number at Risk								
1: < 9.80	105	105	94	50	29	16	11	1	0
2: ≥ 9.80 to < 11.30	89	89	70	41	16	5	1	1	0
3: ≥ 11.30	18	18	12	5	0				

Note Confidence intervals were calculated using data pooled across studies without the ability to adjust for differences in study population or length. Confidence intervals may overstate the statistical confidence of these measurements.

Note Study B did not display strong predictive ability over its shorter timeframe, with similar absolute risks across all ELF scores. It is possible the shorter timeframe for this trial is responsible for the lesser prognostic effect shown here.

NASH F4 (Compensated Cirrhosis)

Three prospective multicenter international clinical trials were performed in NASH subjects with biopsy-confirmed compensated cirrhosis (F4; Ishak 5-6) to evaluate the safety and efficacy of investigational drugs (NCT01672879; NCT02462967; NCT03053063).[18,20,21,33-35]

Study C (NCT01672879) included subjects with liver biopsy consistent with NASH (N = 151; 60%) or cryptogenic cirrhosis (N = 99; 40%). The intended follow-up period of this trial was 244 weeks, but it was discontinued after the Week 96 interim analysis. For the placebo arm subjects included in the analysis, the median follow-up time was 30.0 months (IQR: 25.3–34.4 months).

Study D (NCT03053063) included subjects with liver biopsy consistent with NASH and cirrhosis. The intended follow-up period of this trial was 240 weeks, but it was discontinued after the Week 48 interim analysis. For the placebo arm subjects included in the analysis, the median follow-up time was 15.9 months (IQR: 13.9–18.1 months).

Study E (NCT02462967) included subjects with HVPG measurement ≥ 6 mm Hg and liver biopsy consistent with cirrhosis and NASH categorized as:

1. Definitive NASH diagnosis (N = 145; 90%);
2. Biopsy containing fat > 5% or ballooning hepatocytes with no evidence of viral hepatitis or other liver disease (N = 6; 4%); or
3. No evidence of viral hepatitis or other liver disease in a subject with at least a 5-year history of obesity (BMI ≥ 30) or a 5-year history of diabetes mellitus (N = 10; 6%). Half of the study participants had esophageal varices at baseline (N = 81; 50%). The trial was performed over 52 weeks. For the placebo arm subjects included in the analysis, the median follow-up time was 11.5 months (IQR: 11.5–11.6 months).

Baseline clinical characteristics for the clinical trial placebo subjects included in the analysis are described in the following table.

	Study C (N = 79)	**Study D (N = 172)**	**Study E (N = 54)**
Age in years (median [IQR])	57 (51-61)[a]	61 (55-67)	59 (53-64)
Sex (N [%]) Female: Male:	 52 (66%) 27 (34%)	 101 (59%) 71 (41%)	 36 (67%) 18 (33%)
Diabetes Mellitus (N [%])	54 (68%)	135 (78%)	32 (59%)
BMI in kg/m^2 (median [IQR])	33.8 (29.8-38.0)[a]	32.9 (27.9-37.5)	33.6 (30.2-38.1)
Small esophageal varices (N [%])[b]	N/A	N/A	21 (39%)
HVPG in mm Hg (median [IQR])	12.0 (9.3-15.8)[a]	Not evaluated	10.8 (9.0-15.0)
Ishak score (N [%]) Ishak 5: Ishak 6:	 25 (32%) 54 (68%)	 74 (43%) 97 (56%)	 13 (24%) 54 (76%)
ELF score, median (median [IQR])	10.8 (10.1-11.5)	10.7 (10.1-11.2)	10.8 (10.0-11.4)

[a] Calculations were performed using the enrolled placebo group (N=85) which included subjects without ELF scores.

[b] For Study C and Study D, subjects with varices of any size at baseline were excluded. For Study E, small varices at baseline were defined by veins that occupied < 25% of the distal one third of the esophageal lumen when insufflated.

An analysis of 305 subjects estimated the prognostic ability of ADVIA Centaur ELF at baseline to predict the risk of liver-related events up to 3.8 years following enrollment (median: 15.8 months; IQR: 12.6–21.5 months). The clinical endpoints were:

	Study C	Study D	Study E
Death	✓	✓	✓[a]
Liver transplantation	✓	✓	✓
Qualification for liver transplantation (Model for End-Stage Liver Disease (MELD) ≥ 15)	✓	✓	✓
Esophageal variceal bleeding requiring hospitalization	✓	✓	✓
Clinically apparent ascites requiring treatment	✓	✓	✓
Hepatic encephalopathy of at least Grade 2 (West Haven criteria) requiring treatment	✓	✓	✓
Newly diagnosed varices in a subject without prior varices	✓	---	✓
Progression from small to medium or large varices	---	---	✓

[a] For Study E, this endpoint was based on liver-related mortality. No deaths were recorded for the analysis population in this study.

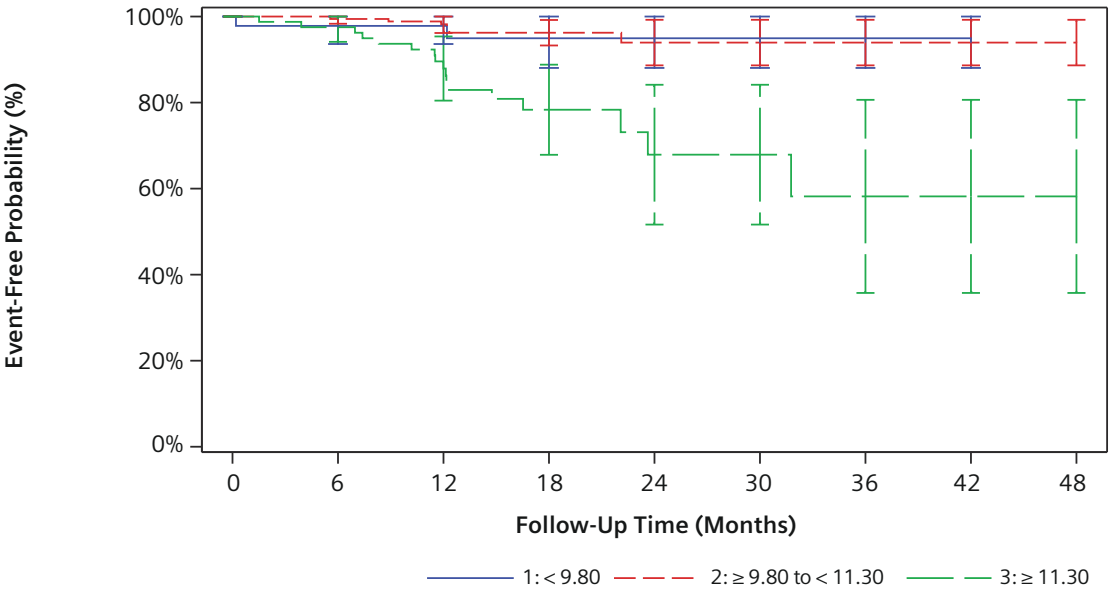
Although the data presented below relates to a pooled analysis from multiple clinical trials, it is representative of the totality of the information provided in the NASH F4 population.

Baseline ELF measurements were performed on an ADVIA Centaur XP system. The risk of liver-related events was assessed according to absolute risk, likelihood ratio (LR) and Cox proportional hazard ratio.

Risk Group (ELF Score)	N[a]	Liver Related Event		Absolute Risk (95% CI)[b]	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI)
		Yes	No			
Lower (< 9.80)	47	2	45	4.3% (0.0%, 10.0%)	0.48 (0.12, 1.85)	1.00
Mid (≥ 9.80 to < 11.30)	177	7	170	4.0% (1.1%, 6.8%)	0.44 (0.23, 0.84)	0.93 (0.19, 4.49)
Higher (≥ 11.30)	81	17	64	21.0% (12.1%, 29.9%)	2.85 (2.00, 4.06)	5.84 (1.35, 25.37)
All	305	26	279	8.5% (5.6%, 12.2%)	—	—

[a] Number of subjects.
[b] Confidence interval.

Kaplan-Meier curves up to 48 months were plotted for each of the risk groups to display the proportion of subjects without a liver-related event over time.



	Number at Risk								
1: < 9.80	47	44	34	19	12	10	1	0	
2: ≥ 9.80 to < 11.30	177	174	150	67	35	20	9	3	0
3: ≥ 11.30	81	77	53	24	13	8	2	1	0

Note Confidence intervals were calculated using data pooled across studies without the ability to adjust for differences in study population or length. Confidence intervals may overstate the statistical confidence of these measurements.

Detection Capability

Detection capability was determined in accordance with CLSI Document EP17-A2.[36]

Method	HA (ng/mL)	PIIINP (ng/mL)	TIMP-1 (ng/mL)	ELF Score[a]
Limit of Blank (LoB)	1.00	0.50	3.0	2.19
Limit of Detection (LoD)	2.00	0.75	4.0	3.20
Limit of Quantitation (LoQ)	3.00	1.00	5.0	3.85

[a] The detection capability limits for the ELF score are based on a theoretical calculation incorporating the established detection capability limits derived individually for HA, PIIINP and TIMP-1.

Results obtained at individual laboratories may vary from the data presented.

The LoB corresponds to the highest measurement that is likely to be observed for a blank sample with a probability of 95%.

The LoD corresponds to the lowest concentration of HA, PIIINP, or TIMP-1 that can be detected with a probability of 95%.

The LoQ corresponds to the lowest amount of HA, PIIINP, or TIMP-1 in a sample at which the total CV is ≤ 20%.

Precision

Single-Site Precision

Precision was determined in accordance with CLSI Document EP05-A3.[37] For determination of an ELF score, measurements were obtained for HA, PIIINP, and TIMP-1 using samples that were tested in 3 replicates, 2 times per day, for 20 days (n = 120).

ADVIA Centaur ELF

		Repeatability		Within-Laboratory Precision	
Sample	Mean ELF Score	SD[a] (ELF Score)	CV[b] (%)	SD[a] (ELF Score)	CV[b] (%)
1	7.49	0.03	0.4	0.05	0.7
2	11.61	0.03	0.2	0.04	0.4
3	12.70	0.03	0.2	0.05	0.4
4	9.91	0.03	0.3	0.04	0.4
5	12.53	0.02	0.2	0.04	0.3
6	13.78	0.04	0.3	0.05	0.3
7	9.27	0.03	0.3	0.04	0.4
8	7.99	0.02	0.3	0.04	0.6

[a] Standard deviation.
[b] Coefficient of variation.

The maximum observed coefficients of variation for the measurements of HA, PIIINP and TIMP-1 in this study are shown below.

ADVIA Centaur Reagent	Repeatability CV[a] (%)	Within-Laboratory Precision CV (%)
HA	3.5	4.5
PIIINP	1.8	3.3
TIMP-1	2.6	3.5

[a] Coefficient of variation.

ADVIA Centaur ELF is designed to have the following precision:

	Precision	
ELF Score	Repeatability (Within-Run)	Within-Laboratory (Total Precision)
≥ 5.00	≤ 0.15 SD[a]	≤ 0.20 SD

[a] Standard deviation.

Reproducibility

Reproducibility was evaluated according to CLSI document EP05-A3.[37] A reproducibility study was conducted at 3 sites using 1 reagent lot. The study was run over 5 days, 2 runs per day, and 3 replicates per run for serum pools. Reproducibility data was pooled across 3 sites.

			Repeatability		Between-Run		Between-Day		Between-Site		Reproducibility	
Sample	N[a]	Mean ELF Score	SD[b] (ELF Score)	CV[c] (%)	SD (ELF Score)	CV (%)	SD (ELF Score)	CV (%)	SD (ELF Score)	CV (%)	SD (ELF Score)	CV (%)
Serum 1	90	10.13	0.04	0.4	0.03	0.2	0.01	0.1	0.03	0.3	0.06	0.6
Serum 2	90	13.59	0.05	0.4	0.02	0.2	0.00	0.0	0.01	0.1	0.06	0.4
Serum 3	89[d]	12.85	0.06	0.4	0.02	0.1	0.01	0.1	0.01	0.1	0.06	0.5
Serum 4	90	12.06	0.05	0.4	0.00	0.0	0.02	0.2	0.00	0.0	0.06	0.5
Serum 5	90	11.35	0.05	0.5	0.00	0.0	0.01	0.1	0.01	0.1	0.06	0.5
Serum 6	90	10.90	0.05	0.4	0.02	0.2	0.00	0.0	0.01	0.1	0.05	0.5
Serum 7	90	7.87	0.06	0.7	0.00	0.0	0.02	0.2	0.01	0.1	0.06	0.8
Serum 8	90	5.98	0.05	0.9	0.06	1.0	0.02	0.3	0.02	0.4	0.09	1.4

[a] Number of measurements.
[b] Standard deviation.
[c] Coefficient of variation.
[d] One replicate read above the analytical measuring range for HA and was removed from the analysis.

Precision data presented above are representative of results obtained by testing of multiple reagent lots. Results obtained at individual laboratories may vary from the data presented.

Interferences

Interference testing was performed in accordance with CLSI Documents EP07-ed3[38] and EP37-ed1.[39] Testing demonstrated ≤ 10.0% change in results in the measurements of HA, PIIINP, and TIMP-1 and ≤ 0.30 change in ELF score for each of the following substances.

	Substance Test Concentration	
Therapeutic Substance	Common Units	SI Units
Acetaminophen	200 µg/mL	1.32 mmol/L
Acetylsalicylic acid	652 µg/mL	3.62 mmol/L
Azathioprine	2.58 µg/mL	9.31 µmol/L
Biotin[a]	150 ng/mL	0.61 µmol/L
Cetirizine	4.35 µg/mL	11.18 µmol/L
Diphenhydramine	774 ng/mL	3.03 µmol/L
Disulfiram	1.14 µg/mL	3.84 µmol/L
Fluorescein[b]	850 ng/mL	2.56 µmol/L
Glyburide	720 ng/mL	1.45 µmol/L
Hydroxyzine	267 ng/mL	0.71 µmol/L
Ibuprofen	219 µg/mL	1.06 mmol/L
Interferon α2a	10 ng/mL	N/A
Interferon α2b	10 ng/mL	N/A

	Substance Test Concentration	
Therapeutic Substance	**Common Units**	**SI Units**
Ledipasvir	969 ng/mL	1.09 µmol/L
Liraglutide	168 ng/mL	44.86 nmol/L
Loratadine	87 ng/mL	0.27 µmol/L
Mesalamine	20.4 µg/mL	133.42 µmol/L
Metformin	12 µg/mL	92.88 µmol/L
Methotrexate	1.36 mg/mL	2.99 mmol/L
Obeticholic Acid	540 ng/mL	1.28 µmol/L
Pioglitazone	4.76 µg/mL	13.38 µmol/L
Ribavirin	25 µg/mL	102.37 µmol/L
Rifampicin	48 µg/mL	58.56 µmol/L
Sofosbuvir	1.85 µg/mL	3.50 µmol/L
Tenofovir	978 ng/mL	3.40 µmol/L
Tolazamide	45 µg/mL	144.45 µmol/L
Ursodiol (UDCA)	169 µg/mL	430.51 µmol/L

[a] The concentration listed reflects the lowest concentration of the drug that caused a ≤ 0.30 change in the ELF score. For biotin, there was ≤ 10.0% change in results in the measurements of HA, PIIINP, and TIMP-1 at 3500 ng/mL, 65 ng/mL, and 3500 ng/mL, respectively.

[b] For fluorescein, there was ≤ 10.0% change in the measurements of HA, PIIINP, and TIMP-1 at 500 ng/mL, 300,000 ng/mL, and 250 ng/mL, respectively.

Hemolysis, Icterus, Lipemia (HIL)

Substance	Substance Test Concentration
Hemoglobin	1000 mg/dL
Bilirubin, conjugated	60 mg/dL
Bilirubin, unconjugated	60 mg/dL
Lipemia (Intralipid)	3500 mg/dL

Endogenous Substances

Substance	Substance Test Concentration
Cholesterol	400 mg/dL
Fructose	18 mg/dL
Glucose	1000 mg/dL
Protein (Albumin)	6 g/dL
Protein (Total)	15 g/dL

Biotin Interference

The following information summarizes the effect of biotin interference on the ELF score.

	Biotin Test Level (ng/mL)					
	50	65	75	150	300	1200
ELF Score	Bias (change in ELF score)					
7.44	-0.08	-0.10	-0.12	-0.28	-0.53	-1.95
9.90	-0.05	-0.10	-0.13	-0.28	-0.42	-1.59
12.51	-0.07	-0.05	-0.08	-0.20	-0.38	-1.28
13.80	-0.13	-0.10	-0.11	-0.28	-0.60	-1.71

Biotin interference was observed for the measurement of PIIINP, but not for HA or TIMP-1. The following information summarizes biotin interference for measurement of PIIINP:

	Biotin Test Level (ng/mL)					
	50	65	75	150	300	1200
PIIINP (ng/mL)	% Bias					
6.97	-8.0	-6.9	-10.9	-26.0	-49.6	-92.2
10.20	-4.5	-6.5	-9.2	-22.0	-43.7	-87.9
18.64	-5.7	-5.7	-9.7	-21.1	-37.6	-81.4
105.23	-9.3	-8.0	-9.2	-23.6	-53.0	-88.5

Specimens that contain biotin at a concentration of 150 ng/mL demonstrate a less than or equal to 0.30 change in results for ADVIA Centaur ELF. Biotin concentrations greater than this may lead to falsely depressed results for patient samples.

The recommended adult daily dietary intake for biotin is 30 µg/day. Over-the-counter dietary supplements promoted for use in hair, skin, and nail health may contain 5–100 mg of biotin, with recommendations to take multiple pills per day. Pharmacokinetic studies in healthy adults have shown that, in subjects ingesting 5 mg, 10 mg, and 20 mg of biotin, serum concentrations of biotin can reach up to 73 ng/mL, 141 ng/mL, and 355 ng/mL, respectively.[40] Subjects who take up to 300 mg of biotin per day may have plasma biotin levels as high as 1160 ng/mL.[41] These studies were performed in a small number of apparently healthy subjects. Clearance of biotin could be different in other patient populations, such as in patients with impaired renal function, which could lead to higher concentrations of biotin in serum or plasma.

Results were established using the ADVIA Centaur XP system. Results obtained at individual laboratories may vary from the data presented.

Specificity

Cross-reactivity was determined in accordance with CLSI Document EP07-ed3.[38] The potential cross reactant was added to a human serum-based sample at the concentrations indicated and were evaluated for potential interference on the ADVIA Centaur XP system. Cross-reactivity was calculated as follows:

$$\% \text{ cross reactivity} = \frac{(\text{concentration of spiked sample - concentration of unspiked sample})}{\text{concentration of compound}} \times 100$$

Testing was performed using the ADVIA Centaur XP system. No significant cross reactivity was observed in the presence of the following substances:

Test Protein	ADVIA Centaur Reagent	Test Concentration (µg/mL)	Cross-Reactivity (%)
Chondroitin Sulfate A	HA	1000	≤ 0.07%
Chondroitin Sulfate B	HA	1000	≤ 0.01%
Chondroitin Sulfate C	HA	1000	≤ 0.01%
Fibronectin	HA	500	≤ 0.01%
Heparin Sulfate	HA	200	≤ 0.01%
Laminin	PIIINP	500	≤ 0.15%
Type I Collagen	PIIINP	2000	≤ 0.07%
Type IV Collagen	PIIINP	200	≤ 0.23%
Type VI Collagen	PIIINP	50	≤ 0.31%
Matrix metalloproteinase-1 (MMP-1) (activated)	TIMP-1	2.5	≤ 0.79%
MMP-2 (activated)[a]	TIMP-1	5	≤ 1.55%
MMP-2 (latent)	TIMP-1	5	≤ 0.19%
MMP-3 (latent)	TIMP-1	5	≤ 0.13%
MMP-9 (activated)	TIMP-1	5	≤ 0.45%
MMP-9 (latent)	TIMP-1	3.5	≤ 0.97%
TIMP-2	TIMP-1	4	≤ 0.08%

[a] Activated MMP-2 does not have significant interference with the ELF score. In the presence of 5 µg/mL activated MMP-2 change in ELF score was ≤ 0.30.

Linearity

Linearity was evaluated according to CLSI document EP06-A.[42] For individual measurements of HA, PIIINP, and TIMP-1, a sample containing high levels of analyte was mixed in various proportions with a sample containing low levels of analyte. The resulting mixtures were tested. ADVIA Centaur ELF components of HA, PIIINP, and TIMP-1 were all found to be linear within their respective measuring intervals.

ADVIA Centaur Reagent	Measuring Interval
HA	3.00–1000.00 ng/mL
PIIINP	1.00–150.00 ng/mL
TIMP-1	5.0–1300.0 ng/mL

High-Dose Hook Effect

High HA levels can cause a paradoxical decrease in the RLUs (high-dose hook effect). For measurements of HA, levels as high as 25,000.00 ng/mL will report greater than 1000.00 ng/mL on ADVIA Centaur systems.

High PIIINP levels can cause a paradoxical decrease in the RLUs (high-dose hook effect). For measurements of PIIINP, levels as high as 2500.00 ng/mL will report greater than 150.00 ng/mL on ADVIA Centaur systems.

High TIMP-1 levels can cause a paradoxical decrease in the RLUs (high-dose hook effect). For measurements of TIMP-1, levels as high as 30,000.0 ng/mL will report greater than 1300.0 ng/mL on ADVIA Centaur systems.

Standardization

HA, PIIINP, and TIMP-1 reagents that comprise ADVIA Centaur ELF are traceable to internal standards. Currently no reference standards are available for these reagents. Assigned values of calibrators and controls are traceable to these internal standards.

Technical Assistance

For customer support, contact your local technical support provider or distributor.

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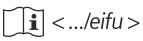
Definition of Symbols

The following symbols may appear on the product labeling:

Symbol	Symbol Title	Source	Symbol	Symbol Title	Source
	Manufacturer	5.1.1[a]		Authorized representative in the European Community	5.1.2[a]
	Use-by date	5.1.4[a]		Authorized representative in Switzerland	Proprietary
	Catalog number	5.1.6[a]		Batch code	5.1.5[a]
	Consult Instructions for Use	5.4.3[a]		Contains sufficient for <n> tests	5.5.5[a]
	Internet URL address to access the electronic instructions for use	Proprietary		Version of Instructions for Use	Proprietary

Symbol	Symbol Title	Source	Symbol	Symbol Title	Source
IVD	*In vitro* diagnostic medical device	5.5.1[a]	Rev. REVISION	Revision	Proprietary
RxOnly	Prescription device (US only)	FDA[b]	UDI	Unique Device Identifier	5.7.10[c]
CE XXXX	CE Marking with Notified Body	EU IVDR[d]	CE	CE Marking	EU IVDR[d]
	Temperature limit	5.3.7[a]		Keep away from sunlight	5.3.2[a]
	Upper limit of temperature	5.3.6[a]		Lower limit of temperature	5.3.5[a]
	Do not re-use	5.4.2[a]		Do not freeze	Proprietary
	Recycle	1135[e]		This way up	0623[e]
	Biological risks	5.4.1[a]		Caution	5.4.4[a]
UNITS C	Common Units	Proprietary	UNITS SI	International System of Units	Proprietary
YYYY-MM-DD	Date format (year-month-day)	N/A	YYYY-MM	Date format (year-month)	N/A
	Document face up[f]	1952[e]		Target	Proprietary
	Handheld barcode scanner	Proprietary		Interval	Proprietary
LOT DTL	Lot details	Proprietary	CHECKSUM	Variable hexadecimal number that ensures the Master Curve and Calibrator definition values entered are valid.	Proprietary
CAL LOT VAL	Calibrator lot value	Proprietary	MC DEF	Master Curve definition	Proprietary
CONTROL LOT VAL	Quality control lot value	Proprietary			

[a] International Standard Organization (ISO). ISO 15223-1 Medical Devices- Symbols to be used with medical device labels, labelling and information to be supplied.
[b] Federal Register. Vol. 81, No 115. Wednesday, June 15, 2016. Rules and Regulations: 38911.
[c] ISO 15223-1:2020-04
[d] IVDR REGULATION (EU) 2017/746
[e] International Standard Organization (ISO). ISO 7000 Graphical symbols for use on equipment.
[f] Indicates Assay-*e*Note

Legal Information

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