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ADVIA Centaur®
Immunoassay Systems

ELF ™ Test

Current revision and date [a]	Rev. E, 2020-07	
Product Name	ADVIA Centaur® ELF Test	
Systems	ADVIA Centaur XP system ADVIA Centaur XPT system ADVIA Centaur CP system	
Materials Required but Not Provided	ADVIA Centaur HA assay ADVIA Centaur PIIINP assay ADVIA Centaur TIMP-1 assay	**REF** 10493157 **REF** 10492440 **REF** 10492065

a In Rev. B or later, a vertical bar in the margin indicates a technical update to the previous version.

Intended Use

The ADVIA Centaur® systems Enhanced Liver Fibrosis (ELF) test is an *in vitro* diagnostic multivariate index assay intended to provide a single ELF score by combining in an algorithm the quantitative measurements of hyaluronic acid (HA), amino-terminal propeptide of type III procollagen (PIIINP) and tissue inhibitor of metalloproteinase 1 (TIMP-1) in human serum using the ADVIA Centaur systems.

The ADVIA Centaur ELF test is indicated, in conjunction with other laboratory findings and clinical assessments, as an aid in the diagnosis and assessment of the severity of liver fibrosis in patients with signs and symptoms of chronic liver disease. This test is not intended for use on any other system.

Summary and Explanation

Progressive liver fibrosis is a major cause of morbidity and mortality throughout the world.[1] Liver biopsy, the reference method used to assess severity and progression of liver disease, has limitations including small but significant morbidity and mortality rates,[2,3,4] sampling error,[5,6] and inter- and intra-observer variation in pathology reporting.[7] Furthermore, liver biopsy provides only a static picture of liver architecture despite the clinical need to assess a dynamic disease process. Serum assays for products of matrix synthesis or degradation and the enzymes involved in these processes have been investigated as surrogate markers of liver fibrosis as a result of this clinical need.[8,9,10] Surrogate markers of liver fibrosis could be used as an initial screen to rule out biopsy in some patients, or they could be used in conjunction with a single liver biopsy to follow progression or regression of fibrosis and response to changes in lifestyle and antifibrotic, antiviral, or other therapies.[9]

The ADVIA Centaur ELF test quantifies analytes that are direct measures of liver fibrosis. PIIINP is a marker of early fibrogenesis and inflammation, TIMP-1 is the circulating inhibitor of MMP enzymes that can enhance fibrogenesis, and HA is a glycosaminoglycan that is produced by hepatic stellate cells. Together, these assays measure qualitative and quantitative changes in the extracellular matrix (ECM). The ECM refers to a set of macromolecules that comprise 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 results for HA, PIIINP and TIMP-1, may be useful to assess the status of liver fibrosis in patients who have been diagnosed with chronic liver disease.[10] The ELF score may be useful as a baseline determination of liver fibrosis; for monitoring changes in fibrosis over time (natural history); before, during, and after therapy or life-style modification; and as an aid in determining prognosis.

Specimen Collection and Handling

Collecting the Specimen

Serum is the only recommended sample type for these assays. The following recommendations for handling and processing blood samples are provided by the Clinical and Laboratory Standards Institute (CLSI):[11]

- Collect all blood samples observing universal precautions for venipuncture. Handle all samples as if capable of transmitting disease.
- Allow samples to clot adequately before centrifugation.
- Keep tubes stoppered at all times.
- Test samples as soon as possible after collecting.
- Do not use specimens with obvious microbial contamination.

Storing the Specimen

Store specimens according to the following recommendations:

- Do not use samples that have been stored at room temperature for longer than 48 hours.
- Tightly cap and refrigerate samples at 2–8°C if the assay is not completed within 48 hours. Specimens may be stored on the clot.
- Freeze samples at or below -20°C, if the sample is not assayed within 7 days.
- Do not store in frost-free freezer.
- Freeze samples devoid of red blood cells up to 4 times, and mix thoroughly after thawing.
- Centrifuge thawed samples at 1000 x g for 10 minutes before using.

The purpose of handling and storage information is to provide guidance to users. 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 samples for shipment in compliance with applicable federal and international regulations covering the transport of clinical samples and etiological agents.

Procedure

Materials Required but Not Provided

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

Item	Description
REF 10493157	ADVIA Centaur HA assay
REF 10492440	ADVIA Centaur PIIINP assay
REF 10492065	ADVIA Centaur TIMP-1 assay

Assay Procedure

Note To schedule multi-component testing, refer to the system operating instructions.

All component assays of the ADVIA Centaur ELF test (HA, PIIINP, and TIMP-1) must result within 8 hours of one another for the ELF score to be valid.

To schedule multi-component testing on the ADVIA Centaur XPT system, the ADVIA Centaur XP system software version 7.0 or higher, and the ADVIA Centaur CP system software version 6.0 or higher, refer to the system operating instructions.

When using earlier software versions of the ADVIA Centaur XP and ADVIA Centaur CP systems, or when using the ADVIA Centaur system, you can determine the ELF score as follows:

1. Order the component tests (TIMP-1, PIIINP, HA) individually. Refer to the system operating instructions.
2. Calculate the ELF score manually. See *Calculation of Results* for instructions.

Preparing the Samples

The ADVIA Centaur ELF test requires 65 µL of serum. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. The sample required to perform onboard dilution may differ from the sample volume required to perform a single determination. Refer to the individual analyte instructions for use for volume requirements.

For detailed information about determining the minimum required volume, see the system operating instructions.

Before placing samples on the system, ensure that samples have the following characteristics:

- Samples are free of fibrin or other particulate matter.
- Samples are free of bubbles.

Performing Quality Control

Follow government regulations or accreditation requirements for quality control frequency.

To monitor system performance and chart trends, as a minimum requirement, assay 3 levels of quality control material on each day that samples are analyzed. Assay quality control samples when performing a two-point calibration. Treat all quality control samples the same as patient samples.

For quality control, use ADVIA Centaur ELF quality control material. Refer to the quality control product insert for the suggested expected values.

For detailed information about entering quality control values, see the system operating instructions.

Taking Corrective Action

If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

1. Determine and correct the cause of the unacceptable control results:
   a. Verify that the materials are not expired.
   b. Verify that required maintenance was performed.
   c. Verify that the assay was performed according to the instructions for use.
   d. Rerun the assay with fresh quality control samples, and confirm that quality control results are within acceptable limits before running patient samples.
   e. If the quality control results are not within acceptable limits, recalibrate the assay, and repeat step d.
   f. If necessary, contact your local technical support provider or distributor for assistance.
2. Repeat testing of patient samples before reporting results.

Perform corrective actions in accordance with your established laboratory protocol.

Results

Calculation of Results

The ELF score can be calculated manually or by the ADVIA Centaur systems. For detailed information about how the system calculates results, refer to the system operating instructions.

Note The auto-calculation feature is only available for the ADVIA Centaur XPT system, for the ADVIA Centaur XP system software version 7.0 or higher, and for the ADVIA Centaur CP system software version 6.0 or higher. For the earlier versions of the ADVIA Centaur XP and ADVIA Centaur CP systems, calculate the ELF score manually.

To calculate the ELF score manually for the ADVIA Centaur XP systems, first obtain results for the ADVIA Centaur HA, PIIINP, and TIMP-1 assays, and then 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.

To calculate the ELF score manually for the ADVIA Centaur CP system, first obtain results for the HA, PIIINP, and TIMP-1 assays on the ADVIA Centaur CP system, and then use the following equation to calculate the ELF score:

$$\text{ELF score} = 2.494 + 0.846 \ln(C_{HA}) + 0.735 \ln(C_{PIIINP}) + 0.391 \ln(C_{TIMP\text{-}1})$$

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

Note The ELF score is a unitless numerical value.

Interpretation of Results

Interpretation of the ELF score is as follows:

ELF Score	Severity of Liver Fibrosis
< 7.7	None to mild
≥ 7.7– < 9.8	Moderate
≥ 9.8	Severe

Always interpret results in conjunction with the patient's medical history, clinical presentation, and other findings.

Limitations

The following information pertains to limitations of the assay:

- See the HA, PIIINP, and TIMP-1 instructions for use for limitations of the component assays.
- Results of component assays are only for use in determining ELF scores on ADVIA Centaur systems.
- All component assays of the ELF score (HA, PIIINP, and TIMP-1) must result within 8 hours of one another for the ELF score to be valid.
- The ADVIA Centaur ELF test is limited to the detection of HA, PIIINP, and TIMP-1 in human serum.
- Only use assay results obtained on ADVIA Centaur systems to calculate ELF scores.
- Hemolyzed samples have been shown to decrease the apparent concentration of PIIINP in samples using the ADVIA Centaur PIIINP assay.
- Always interpret results in conjunction with the patient's medical history, clinical presentation, and other findings.

Expected Values

The ELF Test was performed using the Bayer Immuno 1 automated analyzer on 921 serum samples from subjects undergoing liver biopsy for the investigation of chronic liver disease. Biopsies were evaluated by a central pathologist using the Ishak staging system. Subjects were excluded for having an age outside the range of 18 to 75, for any disorder associated with extra-hepatic fibrosis, for cardiovascular disease or cancer, for advanced cirrhosis, for consumption of regular aspirin, or for hepatocellular carcinoma or drug-induced liver disease. The samples were divided into 3 bins based on their associated biopsy stage: 0 to 2 (no to mild fibrosis), 3 to 4 (moderate fibrosis) and 5 to 6 (severe fibrosis). Descriptive statistics for these 3 bins are shown in the table below.

	Ishak Stage 0 to 2	**Ishak Stage 3 to 4**	**Ishak Stage 5 to 6**
N	561	198	162
Median	8.07	8.83	10.30
Interquartile Range	1.31	1.55	2.25
Mean	8.11	8.97	10.47
Standard Deviation	1.09	1.31	1.69
Minimum	4.16	5.62	5.84
Maximum	14.07	13.54	16.67

Two cutoffs were established. The lower cutoff was defined to discriminate Ishak biopsy stages 0 to 2 from 3 to 6 with a sensitivity of about 90%. The upper cutoff was defined to discriminate Ishak biopsy stages 0 to 4 from 5 to 6 with a specificity of about 90%. A summary of the samples defined by these cutoffs and their associated biopsy stage are shown in the table below.

ELF Score	**Ishak Stage 0 to 2**	**Ishak Stage 3 to 4**	**Ishak Stage 5 to 6**	**Total**
< 7.7	194	32	9	235
7.7 to < 9.8	334	121	47	502
≥ 9.8	33	45	106	184
Total	**561**	**198**	**162**	**921**

For the lower cutoff:

Area Under the Receiver Operating Characteristic Plot (AUROC) = 0.786 (95% CI: 0.755 to 0.817)
Sensitivity = 88.6% (319/360, 95% CI: 84.9 to 91.7%)
Specificity = 34.6% (194/561, 95% CI: 30.6 to 38.7%)

For the upper cutoff:

AUROC = 0.859 (95% CI: 0.826 to 0.893)
Sensitivity = 65.4% (106/162, 95% CI: 57.6 to 72.7%)
Specificity = 89.7% (681/759, 95% CI: 87.3 to 91.8%)

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[12]

Performance Characteristics

Precision

Precision was evaluated according to the CLSI protocol EP5-A2.[13] Samples were assayed in 3 replicates 2 times a day for 20 days (n = 120 replicates per sample). Each of the component assays were calibrated according to their individual calibration intervals.

The following results were obtained from testing performed on 1 ADVIA Centaur XP system using 1 reagent lot and 1 calibrator lot:

Mean ELF Score	Within-Run SD	Between-Run SD	Total SD
6.98	0.07	0.04	0.11
7.12	0.04	0.03	0.08
8.95	0.03	0.04	0.09
11.05	0.03	0.04	0.08
14.51	0.04	0.03	0.08

The following results were obtained from testing performed on 1 ADVIA Centaur CP system using 1 reagent lot and 1 calibrator lot:

Mean ELF Score	Within-Run SD	Between-Run SD	Total SD
7.09	0.04	0.07	0.11
7.33	0.06	0.03	0.08
9.08	0.05	0.04	0.08
11.15	0.05	0.04	0.08
14.59	0.06	0.04	0.09

Accuracy / Method Comparison

For 167 serum samples with an ELF score of 6.49 to 14.87, the relationship between the results of the ADVIA Centaur ELF test performed on the ADVIA Centaur XP system (y) and the Bayer Immuno 1 system (x) is described by the equation:

ADVIA Centaur ELF = 1.01 (Bayer Immuno 1 ELF) - 0.04
Correlation Coefficient (r) = 0.971

For 358 serum samples with an ELF score of 5.8 to 15.0, the relationship between the results of the ADVIA Centaur ELF test performed on the ADVIA Centaur CP system (y) and ADVIA Centaur XP system (x) is described by the equation:

ADVIA Centaur CP ELF = 0.99 (ADVIA Centaur ELF) - 0.05
Correlation Coefficient (r) = 0.996

Technical Assistance

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

www.siemens.com/diagnostics

References

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12. Clinical and Laboratory Standards Institute (formerly NCCLS). *How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline - Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2000. NCCLS Document C28-A2.
13. Clinical and Laboratory Standards Institute (formerly NCCLS). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2004. NCCLS Document EP5-A2.

Definition of Symbols

The following symbols may appear on the product labeling:

Symbol	Definition	Symbol	Definition
IVD	*In vitro* diagnostic medical device	REF REF	Catalog number
	Legal manufacturer	EC REP	Authorized Representative in the European Community
CE	CE Mark	CE 0088	CE Mark with identification number of notified body
	Consult instructions for use		Biological risk
	Do not freeze (> 0°C)		Temperature limitation
	Lower limit of temperature		Upper limit of temperature
	Keep away from sunlight and heat	UP	Up
	Use by	Σ (n)	Contains sufficient for (n) tests
LOT	Batch code		Shake the reagent pack vigorously. Refer to *Preparing Reagents* in the assay-specific ADVIA Centaur product instructions for detailed information.
YYYY-MM-DD	Date format (year-month-day)	Rev.	Revision
MC DEF	Master Curve Definition	CHECKSUM	Variable hexadecimal number that ensures the Master Curve and Calibrator definition values entered are valid.
LOT DTL	Lot Details	DER GRÜNE PUNKT	Green dot
	Recycle	PRINTED WITH SOY INK	Printed with soy ink

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