Alinity c Triglyceride (Trig)-21

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity c Triglyceride assay is used for the quantitation of triglyceride in human serum or plasma on the Alinity c analyzer.

SUMMARY AND EXPLANATION OF THE TEST

Triglycerides are a family of lipids absorbed from the diet and produced endogenously from carbohydrates and fatty acids. Measurement of triglyceride is important in the diagnosis and management of hyperlipidemia. These diseases can be genetic or secondary to other disorders including nephrosis, diabetes mellitus, and endocrine disturbances. The National Cholesterol

Education Program (NCEP) cites evidence that triglycerides are an independent risk factor for atherosclerosis.[1] Individuals with hypertension, obesity, and/or diabetes are at greater risk than are those without these conditions.[2, 3]

The Adult Treatment Panel of the NCEP recommends that all adults 20 years of age and over should have a fasting lipoprotein profile (total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride) once every five years to screen for coronary heart disease risk.[1]

PRINCIPLES OF THE PROCEDURE

Triglycerides are enzymatically hydrolyzed by lipase to free fatty acids and glycerol. The glycerol is phosphorylated by adenosine triphosphate (ATP) with glycerol kinase (GK) to produce glycerol-3-phosphate and adenosine diphosphate (ADP). Glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate (DAP) by glycerol phosphate oxidase (GPO) producing hydrogen peroxide (H_2O_2). In a color reaction catalyzed by peroxidase, the H_2O_2 reacts with 4-aminoantipyrine (4-AAP) and 4-chlorophenol (4-CP) to produce a red colored dye. The absorbance of this dye is proportional to the concentration of triglyceride present in the sample. This analytical methodology is based on the reaction sequence described by Fossati et al.[4] and by McGowan et al.[5] In this reagent, 4-chlorophenol is used rather than 2-hydroxy-3,5-dichlorobenzenesulfonate, used in the Fossati and McGowan studies.

Methodology: Glycerol Phosphate Oxidase

For additional information on system and assay technology, **refer to the Alinity ci-series Operations Manual, Section 3.**

REAGENTS

Kit Contents

Alinity c Triglyceride Reagent Kit 07P77

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	**07P7720**
Tests per cartridge	400
Number of cartridges per kit	10
Tests per kit	4000
R1	67.3 mL
R1 Active ingredients: ATP (2.5 mmol/L), Mg^{2+} (2.5 mmol/L), 4-aminoantipyrine (0.4 mmol/L), 4-chlorophenol (2 mmol/L), Peroxidase (horseradish) (> 2000 U/L), GK (microbial) (> 600 U/L), GPO (microbial) (> 6000 U/L), Lipoprotein lipase (microbial) (> 3000 U/L). Preservative: sodium azide (0.05%).	

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY
- Certain disease states may cause endogenous serum triglyceride values to be grossly elevated. Samples that are grossly lipemic by visual examination should be diluted prior to analysis.

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[6], [7], [8], [9]

The following warnings and precautions apply to: R1	
Contains sodium azide.	
EUH032	Contact with acids liberates very toxic gas.
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or/and SDS folder

For a detailed discussion of safety precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 8.**

Reagent Handling

- Upon receipt, place reagent cartridges in an upright position for 48 hours before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 8 hours before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 7.

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position.
Onboard	System Temperature	42 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, **refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Alinity c Triglyceride assay file must be installed on the Alinity c analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 2.**

For information on printing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 5.**

For a detailed description of system procedures, **refer to the Alinity ci-series Operations Manual.**

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit
mg/dL	0.0113	mmol/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Other specimen types and anticoagulants have not been verified with this assay.

Specimen Type	Collection Vessel	Special Conditions
Serum	Serum tubes (with or without gel barrier)	
Plasma	Collection tubes Acceptable anticoagulants are: Lithium heparin (with or without gel barrier) Sodium heparin	To ensure accurate results, the plasma specimen tube should be filled with the prescribed minimum volume for an appropriate anticoagulant to specimen ratio.

- The National Cholesterol Education Program (NCEP) recommends using fasting specimens.[1]

Specimen Conditions

- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells,

and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.

- For accurate results, plasma specimens should be free of platelets and other particulate matter. Ensure centrifugation is adequate to remove platelets.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross-contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time
Serum/Plasma	Room temperature (20 to 25°C)	2 days[10]
	2 to 8°C	7 days[10], [11]
	-20°C	> 1 year[10]

Specimens may be stored for up to 7 days refrigerated at 2-8°C prior to being tested. If testing will be delayed more than 7 days, store frozen (-20°C).

Avoid multiple freeze/thaw cycles.

Guder et al. suggest storage of frozen specimens at -20°C for no longer than the time intervals cited above.[10]

Stored specimens must be inspected for particulates. If present, mix with a low speed vortex or by inversion and centrifuge the specimen to remove particulates prior to testing.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

PROCEDURE

Materials Provided

07P77 Alinity c Triglyceride Reagent Kit

Materials Required but not Provided

- Alinity c Triglyceride assay file
- 08P6001 Alinity c Multiconstituent Calibrator Kit
- Commercially available controls containing triglyceride
- Saline (0.85% to 0.90% NaCl) for specimen dilution

For information on materials required for operation of the instrument, **refer to the Alinity ci-series Operations Manual, Section 1.**

For information on materials required for maintenance procedures, **refer to the Alinity ci-series Operations Manual, Section 9.**

Assay Procedure

For a detailed description of how to run an assay, **refer to the Alinity ci-series Operations Manual, Section 5.**

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
- Minimum sample volume requirements:
  - Sample volume for single test: 1.5 µL.

    NOTE: This amount does not include the dead volume plus the additional over-aspiration volume. **For total sample volume requirements, refer to the Alinity ci-series Operations Manual, Section 4.**
- Refer to the Alinity c Multiconstituent Calibrator Kit package insert and/or commercially available control package insert for preparation and usage.

- For general operating procedures, **refer to the Alinity ci-series Operations Manual, Section 5.**
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, **Section 9. Perform maintenance more frequently when required by laboratory procedures.**

Sample Dilution Procedures

Samples with a triglyceride value exceeding 1420 mg/dL (16.05 mmol/L) are flagged with the code "> 1420 mg/dL" ("> 16.05 mmol/L") and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a **1:4** dilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor.

If using an automated dilution protocol, the system performs a dilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor. For details on configuring automated dilutions, refer to the Alinity ci-series Operations Manual, Section 2.

Manual Dilution Procedure

Dilute the sample with saline (0.85% to 0.90% NaCl).

The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result.

If the operator does not enter the dilution factor, the result must be manually multiplied by the appropriate dilution factor before reporting the result. If a diluted sample result is less than the lower value of the measuring interval of 7 mg/dL (0.08 mmol/L), do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to the Alinity ci-series Operations Manual, Section 5.

Calibration

For instructions on performing a calibration, **refer to the Alinity ci-series Operations Manual, Section 5.**

Calibration is stable for approximately **41 days (984 hours),** but is required with each change in reagent lot. Verify calibration with at least 2 levels of controls according to the laboratory quality control procedure. If control results fall outside acceptable ranges, recalibration may be necessary.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

- At least two levels of controls (normal and abnormal) are to be run every day testing performed.
- If quality control results do not meet the acceptance criteria defined by laboratory procedure, sample results may be suspect. Follow the laboratory quality control procedures to troubleshoot. Recalibration may be necessary. For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10**.
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Commercial controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Quality Control Guidance

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[12]

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

The Alinity c Triglyceride assay utilizes the Linear data reduction method to generate a calibration and results.

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.

Measuring Interval

Measuring interval is defined as the range of values in mg/dL (mmol/L) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity c Triglyceride assay is **7 to 1420** mg/dL (0.08 to 16.05 mmol/L).

LIMITATIONS OF THE PROCEDURE

N-Acetyl-L-Cysteine at therapeutically achieved concentrations may lead to falsely low results.

Refer to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert.

EXPECTED VALUES

The National Cholesterol Education Program (NCEP) Adult Treatment Panel III suggested reference ranges as it is present in this policy will be adopted.

Reference Range

Serum/Plasma[1]

	Range (mg/dL)	**Range (mmol/L)**
Normal	< 150	< 1.70
Borderline High	150 to 199	1.70 to 2.25
High	200 to 499	2.26 to 5.64
Very High	≥ 500	≥ 5.65

The National Cholesterol Education Program (NCEP) Adult Treatment Panel III Report recommends the classification shown above. Laboratories should follow recommendations for lipid ranges effective in their locale if they differ from those of the NCEP.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity c analyzer, and the ARCHITECT c System and AEROSET System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity c analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2. Testing was conducted using 1 lot of the Alinity c Triglyceride Reagent Kit, 1 lot of the Alinity c Multiconstituent

Calibrator Kit, and 1 lot of commercially available controls and 1 instrument. Three controls were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.[13]

Sample	n	Mean (mg/dL)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Control Level 1	120	78	0.5	0.7	0.8	1.1
Control Level 2	119	151	0.6	0.4	1.3	0.9
Control Level 3	119	259	1.2	0.5	2.0	0.8

[a] Includes within-run, between-run, and between-day variability.

Sample	n	Mean (mmol/L)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Control Level 1	120	0.88	0.005	0.6	0.008	1.0
Control Level 2	119	1.71	0.008	0.5	0.016	0.9
Control Level 3	119	2.93	0.014	0.5	0.023	0.8

[a] Includes within-run, between-run, and between-day variability.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2. Testing was conducted using 3 lots of the Alinity c Triglyceride Reagent Kit on each of 2 instruments over a minimum of 3 days. The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below. These representative data support the lower limit of the measuring interval.[14]

	mg/dL	mmol/L
LoB[a]	1	0.01

	mg/dL	mmol/L
LoD[b]	2	0.02
LoQ[c, d]	**6.2**	0.071

[a] The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b] The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ is defined as the lowest concentration at which a maximum allowable precision of **20 %CV** was met.

[d] This value represents the observed LoQ on the ARCHITECT System. The LoQ observed on the Alinity c analyzer supports this LoQ.

Linearity

A study was performed based on guidance from CLSI EP06-A.*15*

This assay is linear across the measuring interval of **7 to 1420** mg/dL (0.08 to 16.05 mmol/L).

Interference

This study was performed using Triglyceride reagents on the AEROSET System.

Potentially Interfering Substances

Interference studies were conducted using CLSI protocol NCCLS EP7-P. Interference effects were assessed by Dose Response and Paired Difference methods, at the medical decision level of the analyte.*16*

Potentially Interfering Substance	Interferent Level		Target Level (mg/dL)	Recovery (% of Target)
	Default Units	Alternate Units		
Bilirubin	7.5 mg/dL	128 µmol/L	211.0	106.6
	15 mg/dL	257 µmol/L	211.0	111.3
Hemoglobin	750 mg/dL	7.5 g/L	193.1	109.6
	1000 mg/dL	10.0 g/L	193.1	111.2
Ascorbate	1.5 mg/dL	85 µmol/L	220.4	97.0
	3.0 mg/dL	170 µmol/L	220.4	94.0

The following drugs were tested on the ARCHITECT system for interference at the concentrations indicated using an acceptance criteria of ± 10% from the target value.

Potentially Interfering Substance	Interferent Level		Target Level (mg/dL)	Recovery (% of Target)
	Default Units	Alternate Units		
Acetaminophen	200 mg/L	1324.5 µmol/L	79.0	99.4
Dipyrone	100 mg/L	300.3 µmol/L	78.6	100.0
N-Acetyl-L-Cysteine	800 mg/L	4908.0 µmol/L	50.1	**34.1**

Interferences from medication or endogenous substances may affect results.[17]

Method Comparison

A study was performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method.[18]

		Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity c Triglyceride vs ARCHITECT Triglyceride	Serum	mg/dL	128	1.00	1.50	1.00	8 - 1374
		mmol/L	128	1.00	0.02	1.00	0.09 - 15.52

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