







REA Ethanol

Customer Service
 United States: 1-877-4ABBOTT
 International: Call your Abbott Representative

Read Highlighted Changes
 Revised November, 2006

This package insert must be read carefully prior to product use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF List Number	 Expiration Date
IVD <i>In Vitro</i> Diagnostic Medical Device	LOT Lot Number
 Store at 2-8°C	CAL A Calibrator (A-F)
 Store at 15-30°C	CONTROL L Control Low, Medium, High (L, M, H)
 CAUTION: Consult accompanying documents.	SAMPLE CUPS Sample Cups
 Consult instructions for use	REAGENT PACK Reagent Pack
 Abbott Laboratories Diagnostics Division Abbott Park, IL 60064 USA	REACTION VESSELS Reaction Vessels
Manufacturer	EC REP Authorized Representative

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

NAME

Ethanol

INTENDED USE

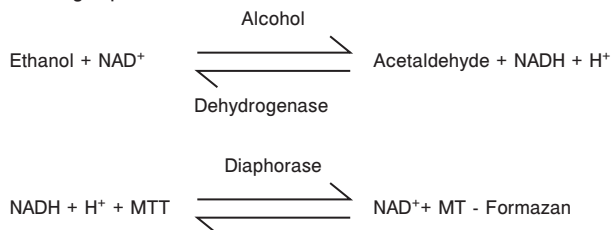
The AxSYM REA Ethanol assay is a quantitative reagent system for the measurement of ethanol in human whole blood, serum, plasma and urine. Measurements obtained are used in the diagnosis and treatment of alcohol intoxication and poisoning.

SUMMARY AND EXPLANATION OF TEST

The AxSYM REA Ethanol assay utilizes Radiative Energy Attenuation technology.¹ Radiative Energy Attenuation assays involve color development reactions. Their reaction systems use analyte to convert a chromogen (unreacted dye) to a chromophore (colored dye). A stable fluorescent substance (fluorophore) is also included in the reaction mixture. The light-absorbing properties of the chromophore produced cause a decrease of measured fluorescent light intensity (attenuates radiative energy) from the fluorophore. The FPIA Optics are used to measure changes in fluorescent intensity. This is different from FPIA assays where the optics measure changes in fluorescence polarization. Changes in radiant energy intensity follow the principles of Beer, Lambert and Bouguer (often called Beer's Law).

REA is used quantitatively to measure specific analytes based on the principle that the logarithm of measured fluorescent light intensity is inversely proportional to the amount of chromophore present. Production of chromophore is linked by the reaction system to consumption of analyte, so development of fluorescence attenuation can be calibrated to measure the concentration of analyte in the sample.

The ethanol concentration is determined by the combined catalytic reactions of alcohol dehydrogenase and diaphorase to generate a color change in a dye. The reaction scheme is shown by the following equation:



where: NAD(H) = Nicotinamide Adenine Dinucleotide
(reduced)
MTT = Monotetrazolium Dye

The relationship between the concentration of ethanol and the measured fluorescence intensity is established by generating a calibration curve. Ethanol calibrators of known concentration are run and the resulting attenuated fluorescence signal is measured. When an unknown is read, its concentration is calculated from the stored calibration curve.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The AxSYM REA Ethanol assay is based on Radiative Energy Attenuation technology. The AxSYM REA Ethanol Reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

- Sample and AxSYM REA Ethanol reagents required for one test are pipetted by the sampling probe into various wells of the Reaction Vessel (RV).

The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center with the processing probe.

PROCESSING CENTER

- An aliquot of a sample-reagent mixture containing sample, alcohol dehydrogenase with diaphorase, MTT with fluorescein and Solution 4 (Line Diluent) is transferred to the cuvette.
- A baseline fluorescence measurement is taken followed by the transfer of a second aliquot of the sample-reagent mixture, NAD and Solution 4 (Line Diluent) to the cuvette.
- The enzymatic reaction uses ethanol and NAD to convert the chromogen (MTT) to a chromophore.
- The changes in fluorescent intensity are measured by the FPIA optical assembly.

For further information, refer to the AxSYM System Operations Manual, Section 3.

REAGENTS

REAGENT PACK, 100 TESTS

AxSYM REA Ethanol Reagent Pack (3B32-20)*

- 1 Bottle (7.4 mL) < 5% Nicotinamide adenine dinucleotide in sodium citrate buffer. Preservative: Sodium Azide. (Reagent Bottle 1)
- 1 Bottle (7.9 mL) < 5% Yeast alcohol dehydrogenase and <1% diaphorase in a protein stabilizer solution containing human serum, nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2, in buffer. Preservative: Sodium Azide. (Reagent Bottle 2)
- 1 Bottle (8.8 mL) < 1% Monotetrazolium dye and < 0.01% fluorescein in solvent. (Reagent Bottle 3)

* 3B32-99 includes an AxSYM REA Ethanol Reagent Pack (100 tests) and reaction vessels (100 each). 3B32-20 includes these items for international shipment.

CALIBRATORS

XSYSTEMS REA Ethanol Calibrators (9545-02)

6 Bottles (2.5 mL each) of XSYSTEMS REA Ethanol Calibrators contain ethanol in an aqueous solution to yield the following concentrations:

Bottle	Ethanol Concentration	
	(mg/dL)	(mmol/L)
CAL A	0	0.00
CAL B	25	5.43
CAL C	50	10.85
CAL D	100	21.71
CAL E	200	43.41
CAL F	300	65.12

Preservative: Sodium Azide.

Abbott Ethanol calibrators are manufactured volumetrically and are matched to NIST (National Institute of Standards and Technology) Standard Reference Material Ethanol-Water solutions.

CONTROLS

For the quantitative determination of ethanol in plasma, serum, whole blood or urine, either the XSYSTEMS REA Ethanol Serum Controls or the XSYSTEMS REA Ethanol Whole Blood Controls may be used for the verification of the AxSYM REA Ethanol calibration curve.

XSYSTEMS REA Ethanol Whole Blood Controls (9545-12)

3 Bottles (4 mL each) of XSYSTEMS REA Ethanol Controls contain ethanol in human whole blood, nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2, to read within the following ranges:

Bottle	Ethanol Concentration		Range	
	(mg/dL)	(mmol/L)	(mg/dL)	(mmol/L)
CONTROL L	50	10.85	31.86 - 68.14	6.92 - 14.79
CONTROL M	100	21.71	69.95 - 122.00	15.18 - 26.48
CONTROL H	150	32.56	123.00 - 186.90	26.70 - 40.57

Preservative: sodium azide and antimicrobial agents.

XSYSTEMS REA Ethanol Serum Controls (9545-13)

3 Bottles (4 mL each) of XSYSTEMS REA Ethanol Controls contain ethanol in human serum, nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2, to read within the following ranges:

Bottle	Ethanol Concentration		Range	
	(mg/dL)	(mmol/L)	(mg/dL)	(mmol/L)
CONTROL L	50	10.85	34.02 - 65.98	7.38 - 14.32
CONTROL M	100	21.71	78.05 - 121.95	16.94 - 26.47
CONTROL H	250	54.27	200.39 - 299.61	43.50 - 65.03

Preservative: Sodium Azide.

OTHER REAGENTS

AxSYM Probe Cleaning Solution (9A35-05)

PROBE CLEANING SOLUTION 2 Bottles (220 mL each) AxSYM Probe Cleaning Solution containing 2% Tetraethylammoniumhydroxide (TEAH).


Solution 4 (Line Diluent) (8A46)

SOLUTION 4 LINE DILUENT 1 Bottle (10 L) Solution 4 (Line Diluent) containing 0.1 M Phosphate Buffer. Preservatives: Sodium Azide and Antimicrobial Agents.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use.

SAFETY PRECAUTIONS

-  **CAUTION:** This product contains human sourced and/or potentially infectious components. For a specific listing, refer to the **REAGENTS** section of this package insert. Components sourced from human blood have been tested and found to be nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2 by FDA licensed tests. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens². Biosafety Level 2³ or other appropriate biosafety practices^{4,5} should be used for materials that contain or are suspected of containing infectious agents.
- This product contains Sodium Azide; for a specific listing, refer to the REAGENTS section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

- The XSYSTEMS REA Ethanol Whole Blood Controls contain methylisothiazolones and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases:



- R43 May cause sensitization by skin contact.
- S24 Avoid contact with skin.
- S35 This material and its container must be disposed of in a safe way.
- S37 Wear suitable gloves.
- S46 If swallowed, seek medical advice immediately and show this container or label.


For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.

HANDLING PRECAUTIONS

- Ethanol is a volatile analyte. Handle as described in the Assay Procedure section of this insert.**
- Open XSYSTEMS REA Ethanol Whole Blood and Serum Controls with care, contents under pressure.**
- AxSYM REA Ethanol reagent packs are shipped frozen. Ensure reagents are completely thawed before using. Do not shake or invert the reagent pack. Shaking or inverting the reagents may cause bubbles which can interfere with the liquid level sense capabilities of the AxSYM System.
- Do not use kits beyond the expiration date or a maximum of 112 cumulative hours on-board the AxSYM System.
- Do not mix reagents from different reagent packs regardless of lot number.


Refer to the AxSYM System Operations Manual, Sections 7 and 8, for a more detailed discussion of safety and handling precautions during system operation.

STORAGE INSTRUCTION

 The AxSYM REA Ethanol Reagent Pack, XSYSTEMS REA Ethanol Calibrators and XSYSTEMS REA Ethanol Controls must be stored at 2-8°C. The AxSYM Reagent Pack, XSYSTEMS Calibrators and XSYSTEMS Controls may be used immediately after removing them from the refrigerator (ensure reagents are thawed). XSYSTEMS Calibrators and XSYSTEMS Controls should be returned to 2-8°C storage immediately after use.

The AxSYM REA Ethanol Reagent Pack may be on-board the AxSYM System for a maximum of 112 cumulative hours; for example, 14 eight hour shifts. Refer to the AxSYM System Operations Manual, Sections 2 and 5, for further information on tracking on-board time.

Reagents are stable until the expiration date when stored and handled as directed.

 The AxSYM Probe Cleaning Solution and Solution 4 (Line Diluent) must be stored at 15-30°C.

INSTRUMENT PROCEDURE

Assay File Installation

The AxSYM REA Ethanol Assay File must be installed on the AxSYM System from one of the following software disks, prior to performing the REA Ethanol assay:

- 9B73-02, or higher (112 hours on-board Stability)
- 3D54-02, or higher (112 hours on-board Stability)

Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

AxSYM REA Ethanol Assay Parameters

The default values for the assay parameters used for the AxSYM REA Ethanol assay are listed below. Assay parameters that can be edited contain a (>) symbol. These parameters can be displayed and edited according to the procedure in the AxSYM System Operations Manual, Section 2. In order to obtain values for the parameters with an asterisk (*), review the specific Assay Parameter Screen. Press PRINT to print the assay parameters.

Assay Parameters

1	Long Assay Name (English): Ethanol
6	Abbrev Assay Name (English): Ethanol
11	Assay Number: 544
12	Assay Version: *
13	Calibration Version: *
14	Assay file Revision: *
15	Assay Enabled > ON
17	Assay Type: REA
18	Standard Cal Reps > 2
21	Cal A Concentration: 0.00
22	Cal B Concentration: 25.00
23	Cal C Concentration: 50.00
24	Cal D Concentration: 100.00
25	Cal E Concentration: 200.00
26	Cal F Concentration: 300.00
43	Default Dilution Protocol > UNDILUTED
44	Default Calibration Method > Standard Cal
45	Selected Result Concentration Units > mg/dL
46	Selected Result Decimal Places > 2
73	Low Limit - Normal/Therapeutic Range lower limit > 0.00
74	High Limit - Normal/Therapeutic Range upper limit > 0.00
75	Low Extreme Value > 0.00
76	High Extreme Value > 0.00
91	Low Range Undiluted: *
92	High Range Undiluted: *
112	Max End-Point Deviation: *
113	Max Baseline Intensity: *
114	Min Baseline Intensity: *
115	Max Percent Transmission: *

Note: Parameter 45 can be edited to the alternate result unit mmol/L or %.

Refer to the AxSYM System Operations Manual for a detailed description of Instrument Procedures.

SAMPLE COLLECTION AND PREPARATION FOR ANALYSIS

- Serum, whole blood and plasma (collected in heparin, EDTA, fluoride, citrate or oxalate collection tubes) should be used in the AxSYM REA Ethanol assay. Follow the manufacturer's processing instructions for serum or plasma collection tubes.
- The AxSYM System does not provide the capability of verifying sample type. It is the responsibility of the operator to verify the correct sample type(s) is (are) used in the Ethanol assay.
- Inspect all samples for bubbles. Remove bubbles prior to analysis.
- When shipped, samples must be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical samples and etiologic agents.

Refer to the Assay Procedure section of this insert for a detailed discussion of on-board sample storage constraints.

- Care must be taken in blood collection for the determination of ethanol.⁶ Make sure that skin is cleaned with a non-alcohol disinfectant.
- **DO NOT USE ALCOHOL WIPES FOR PATIENT PREPARATION**

Due to the volatility of ethanol in patient samples, follow the special handling requirements listed below:

- Urine may also be used in the AxSYM REA Ethanol assay. Immediately after collection, urine samples for ethanol analysis should be secured in evaporation-tight containers to minimize loss due to head-space evaporation.
- The collection tube or container must be tightly sealed immediately after sample collection.
- The sample should be analyzed immediately after the sample tube is opened.
- Sample segments must be loaded as rapidly as possible and run immediately thereafter.

SAMPLE VOLUME

The AxSYM REA Ethanol assay must be requested as STAT on the AxSYM System. The minimum sample volume required to perform a STAT ethanol test from a sample cup is 150 µL on the AxSYM System. An additional 100 µL of sample is requested for every ethanol test performed (STAT) from the same sample cup.

CAUTION: Due to the volatility of ethanol, do not use the Auto Retest feature on the AxSYM System with this assay.

The sample cup minimum volume for STAT tests is calculated by the AxSYM System. It is displayed on the Order screen at the time the test(s) is (are) ordered. The STAT sample cup minimum volume is printed on the Orderlist Report.

When using Host Order Query, the Order screen information and Orderlist Report are not available. Refer to the AxSYM System Operations Manual, Section 5: Ordering Patient Samples, for a description of the Host Order Query option.

For sample volume requirements for primary or aliquot tubes, or calibrator and control requirements for multiple reagent lots, refer to the AxSYM System Operations Manual, Section 5.

AxSYM REA ETHANOL PROCEDURE

Materials Provided

- 3B32-99 AxSYM REA Ethanol Reagent Kit containing:
 - AxSYM REA Ethanol **REAGENT PACK**
 - 100 **REACTION VESSELS**

Materials Required But Not Provided

- 9545-02 XSYSTEMS REA Ethanol Calibrators
- 9545-12 XSYSTEMS REA Ethanol Whole Blood Controls
- 9545-13 XSYSTEMS REA Ethanol Serum Controls
- 8A46 **SOLUTION 4 LINE DILUENT**
- 9A35-05 AxSYM **PROBE CLEANING SOLUTION**
- 8A76-01 **SAMPLE CUPS**
- Pipettes/Pipette tips (optional) to deliver the volume specified on the order screen

CAUTION:

- For optimal performance it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 9. If the laboratory requires more frequent maintenance, follow those procedures.
- **When cleaning the outside of the Probe and the Wash Stations, you must use only 95% Methanol.**
- **Do not use ethanol or ethanol-containing agents to clean the outside of the AxSYM System or lab areas where ethanol samples are being handled.**

Assay Procedure

Before using XSYSTEMS REA Ethanol Calibrators and Serum Controls, mix by inversion. Due to naturally occurring lipids in human serum, particulate material may be seen in the XSYSTEMS REA Ethanol Serum Controls. Ethanol serum control results and instrument functions are not impacted by the presence of lipid material. Before using XSYSTEMS Ethanol Whole Blood Controls, mix well to insure a homogenous mixture. To store XSYSTEMS Ethanol Calibrators and Controls, tightly cover the stopper with plastic film immediately after each use. Failure to properly reseal vials will result in decreased stability of calibrators and controls. When re-opening the vials, place a gauze over the stopper to control pressurized escape of the contents.

As an alternative to pouring, an adequate amount of calibrator or control may be withdrawn from the vial using a syringe and needle. Sharps with engineered sharps injury protection should be used where possible. To avoid potential injury, handle with caution. To prevent contamination, the same syringe and needle must not be used with more than one vial.

Ethanol is a volatile analyte. **To minimize the potential for sample evaporation on-board the AxSYM System, all Ethanol tests must be requested STAT.**

If a panel includes an ethanol test, the panel must be run as a STAT.

Before running an ethanol test, update the ORDER STATUS screen by selecting, F4, REFRESH DISPLAY. Determine the number of STAT tests and calibrators pending in the ORDER STATUS screen. Confirm that this number, when added to the desired ethanol tests, is ≤ 30 .

Pending STATS + Pending Calibrators + Desired Ethanol tests ≤ 30 .

Do not request any assay calibrations until STAT ethanol test requests have completed sampling on the AxSYM System.

Sections 5 and 6 of the AxSYM System Operations Manual contain detailed steps for performing assay calibration and sample testing procedures.

Prior to ordering tests, confirm that the System inventory of Reaction Vessels and Solution 4 (Line Diluent) is sufficient.

The operator may obtain an Orderlist Report by pressing PRINT. The printout contains sample placement information and minimum STAT sample cup volume requirements for all tests ordered. It is recommended that this report be referenced when loading samples into sample segments.

When using Host Order Query, the Orderlist Report is not available. Refer to the AxSYM System Operations Manual, Section 5: Ordering Patient Samples, for a description of the Host Order Query option.

Caution: When operating the AxSYM System, always observe the following:

- The System status must be WARMING, PAUSED, READY, or STOPPED before adding or removing sample segments, reagent packs or Reaction Vessels (RVs).
- An "Error Code 5066 Matrix cell not detected, trap door, processing center" may be displayed when the instrument homes the motors. If performing only REA (and/or FPIA) assays, select OK to proceed with testing.
- Do not open the Interior Waste Door or the AxSYM Processing Center Cover while any test is in process. If opened, all processing will stop. Tests in process will be terminated and must be repeated.
- When testing is completed, it is recommended that samples and the AxSYM REA Ethanol Reagent Pack are removed from the Sampling Center to maximize the on-board reagent pack use. Store Reagent Pack at 2-8°C.

QUALITY CONTROL PROCEDURES

CALIBRATION

To review the detailed results of a calibration curve, refer to the AxSYM System Operations Manual, Section 6.

To perform an AxSYM REA Ethanol Calibration, test XSYSTEMS REA Ethanol Calibrators A, B, C, D, E and F in duplicate. A single sample of all levels of controls must be tested to evaluate the assay calibration.

Once the AxSYM REA Ethanol calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent pack with a new lot number is used
- Control values are out of their specific range

Refer to the AxSYM System Operations Manual, Section 6, for:

- Setting up an assay calibration
- When recalibration may be necessary
- Calibration verification

The AxSYM System verifies that the results of an assay calibration meet the specifications assigned to selected validity parameters. An error message occurs when the calibration fails to meet a specification. Refer to the AxSYM System Operations Manual, Section 10, for an explanation of the corrective actions for the error code. Refer to the AxSYM System Operations Manual, Appendices, for an explanation of the calibration validity parameters that may be used by the AxSYM System.

Operator Verification

- An acceptable AxSYM REA Ethanol calibration curve must have all controls within the acceptable ranges.

QUALITY CONTROL

The recommended control requirement for an AxSYM REA Ethanol assay is a single sample of at least two control levels tested once every 24 hours, each day of use. Controls may be placed in any position in the Sample Carousel. Controls are to be requested as STAT tests.

If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow those procedures.

Ensure that assay control values are within the concentration ranges specified in the package insert. Refer to the **REAGENTS, CONTROLS** section of this package insert for XSYSTEMS REA Ethanol Serum and Whole Blood Control ranges.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and require retesting. Assay recalibration may be indicated. Refer to the AxSYM System Operations Manual, Section 10, Subsection: Observed Problems, for further troubleshooting information.

The AxSYM System has the capability to generate a Levey-Jennings plot of each assay's quality control performance. Refer to the AxSYM System Operations Manual, Section 5, for further information. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

RESULTS

A polynomial curve fit method is used to generate the AxSYM REA Ethanol Calibration curve. This curve is stored in memory and concentrations of ethanol in controls and unknown samples are calculated from this curve using normalized intensity (%T) values generated.

Alternate Result Units

The default unit for AxSYM REA Ethanol is mg/dL. When selecting the alternate unit mmol/L, the conversion factor used by the AxSYM System is 0.217061. When selecting the alternate unit %, the conversion factor used by the AxSYM System is 0.001.

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 AxSYM System Operations Manual, Section 1.

AxSYM REA Ethanol Specific Error Codes

For error codes generic to all AxSYM Assays refer to the AxSYM System Operations Manual, Section 10. AxSYM REA Ethanol assay specific error codes are shown below:

1122 Invalid test result, baseline intensity too low, (#).	
1123 Invalid test result, baseline intensity too high, (#).	
<u>Probable Cause(s)</u>	<u>Corrective Action</u>
Interferent is present in sample.	Dilute sample and rerun.
Dispense System malfunction.	<ol style="list-style-type: none"> 1. Ensure all connections are finger-tight. 2. Check for dried buffer or current leaks. 3. Check for air bubbles in Solution 4 (Line Diluent) tubing and Sample and Process center Probe Link tubing. Perform flush to remove air bubbles present. 4. Check for damaged or dirty probes. Replace if damaged. Perform Probe Calibration to ensure proper alignment. 5. Perform Fluidics Check. For more details, refer to Section 9: <p>Service and Maintenance, Subsection: Additional Maintenance.</p>
Poor probe alignment.	Perform Sampling and Processing Probe Calibration.

1124 Invalid test result, end-point not reached, deviation, (#).	
<u>Probable Cause(s)</u>	<u>Corrective Action</u>
Interferent is present in sample.	Dilute sample and rerun along with a control. If control is within range, but error 1124 recurs on retested sample, an interferent is present and sample must be tested by a different methodology.

LIMITATIONS OF THE PROCEDURE

The reportable range of the AxSYM REA Ethanol assay is 13-300 mg/dL. Samples with concentrations < 13 mg/dL should be reported as none detected. Samples with concentrations > 300 mg/dL may be manually diluted (refer to the Manual Dilution Protocol of this insert).

Factors that may affect ethanol results are microbial contamination of the sample, the use of an alcohol-containing disinfectant for cleansing the venipuncture site or testing area, and evaporation caused by improper sample handling. The patient's ingestion of alcohol-containing medicinal liquids, e.g. elixirs and mouthwashes, may affect the ethanol result.

For interferences related to physiological conditions, refer to the Interference section of this assay insert.

As with all analyte determinations, the ethanol value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

SAMPLE DILUTION PROCEDURES

CAUTION: The automated dilution protocol, as described in the AxSYM System Operations Manual, Section 5, CANNOT BE USED with the AxSYM REA Ethanol assay.

Manual Dilution Protocol

If a numerical value is desired for patient samples reported as > 300 mg/dL, the sample may be manually diluted (do not exceed a 1:4 dilution) with the XSYSTEMS REA Ethanol Calibrator A and repeated on the AxSYM System. The concentration reported by the AxSYM System must be multiplied by the manual dilution factor to obtain the final sample concentration.

Final Sample Concentration = Reported Concentration x Manual Dilution Factor

Manual Dilution Factor = $\frac{\text{Volume of Sample} + \text{Volume of Dilution Reagent}}{\text{Volume of Sample}}$

EXPECTED VALUES

The principal pharmacological action of ethanol is depression of the central nervous system (CNS). The CNS effects vary, depending on the blood ethanol concentration, from euphoria and decreased inhibitions (≤ 50 mg/dL) to increased disorientation and incoordination (100-300 mg/dL) and then to coma and death (> 400 mg/dL). Not all individuals experience the same degree of CNS dysfunction at similar blood alcohol levels.⁷

The AxSYM REA Ethanol assay is capable of measuring the concentration of ethanol in various body fluids. The ethanol concentration will be higher in serum than in a sample of whole blood.⁸

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

Precision was determined as described in National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP5-T2.⁹ Three member serum and whole blood panels were assayed, using a single lot of reagents and a single calibration, in replicates of 2 at two separate times each day for 20 days. Data from this study yielded %CVs of less than 10%. Representative data are shown in the following table.

Serum Control Levels	Target Concentration (mg/dL)	Mean Conc. Value (mg/dL)	Within Run		Total Run	
			SD	%CV	SD	%CV
Low	50	49.29	1.68	3.41	4.01	8.14
Medium	100	96.82	3.60	3.72	5.02	5.19
High	250	249.30	9.60	3.85	8.98	3.60

Whole Blood Control Levels	Target Concentration (mg/dL)	Mean Conc. Value (mg/dL)	Within Run		Total Run	
			SD	%CV	SD	%CV
Low	50	54.89	2.61	4.75	4.07	7.41
Medium	100	103.57	4.25	4.11	5.83	5.63
High	150	161.94	8.81	5.44	8.99	5.55

Accuracy by Recovery

Two sets of calibrators were prepared by adding known quantities of ethanol to calibrator diluent and to human serum to levels of 25, 50, 100, 200 and 250 mg/dL. The analyzer was calibrated with the calibrator diluent calibrators. Both sets of calibrators were assayed relative to this calibration. Percent recovery = 100 x ("concentration in serum" divided by "concentration in calibrator diluent").

Representative data are shown in the following table.

Added Concentration (mg/dL)	Concentration in Calibrator Diluent (mg/dL)	Concentration in Human Serum (mg/dL)	Percent (%) Recovery
25	22.85	23.95	104.82
50	47.33	52.79	111.54
100	103.09	99.94	96.95
200	191.76	209.49	109.25
250	260.52	234.37	89.96

Average Recovery = 102.5 ± 8.95%

Sensitivity

The sensitivity of the AxSYM REA Ethanol assay was calculated to be 13.00 mg/dL for serum, plasma, whole blood or urine. This sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with at least 95% confidence.

Specificity

Specificity of the AxSYM REA Ethanol assay was determined by varying the concentrations of the following compounds and measuring them as samples. These compounds were tested with concentrations of 1000 mg/dL unless otherwise indicated.

Test Compound	% Reactivity
Acetone	< 1
n-Butanol	27.4
Ethylene Glycol	< 1
Isopropanol	< 5
Methanol	< 1
*n-Propanol	63.6
Propylene Glycol	< 1

* Tested at 100 mg/dL

Interference

The compounds listed below resulted in less than 10% error in detecting analyte when assayed with the AxSYM REA Ethanol assay.

Compound	Sample Matrix	Concentration Tested
Anticoagulants		
Citrate	Serum	4 mg/mL
EDTA	Serum	2 mg/mL
Heparin	Serum	20 Units/mL
Oxalate	Serum	2 mg/mL
Sodium Fluoride	Serum	8 mg/mL
Ascorbic Acid	Urine	0.1 g/dL
Bilirubin	Serum, Plasma, Whole Blood, Urine	25 mg/dL
Creatinine	Urine	0.5 g/dL
Glucose	Urine	2 g/dL
Hemoglobin	Serum, Plasma	1 g/dL
	Urine	115 mg/dL
Lactic Acid	Serum, Whole Blood	270 mg/dL
Oxalic Acid	Urine	100 mg/dL
Protein	Serum, Whole Blood, Plasma	10 g/dL
	Urine	0.05 g/dL
Riboflavin	Urine	7.5 mg/dL
Sodium Chloride	Urine	6 g/dL
Triglycerides	Serum, Plasma, Whole Blood, Urine	0.5 g/dL
Urea	Urine	6 g/dL

Accuracy by Correlation with Reference Assays

The AxSYM REA Ethanol assay was compared to TDxFLx and GC (headspace analysis) by assaying human samples suspected of having elevated ethanol levels. Samples were analyzed on-site at Abbott Laboratories on AxSYM and TDxFLx. GC (headspace analysis) was performed at a reference lab in Nashville, Tennessee.

AxSYM REA Ethanol vs.	Sample Type	Number of Observations	Intercept	Slope	Correlation Coefficient
TDxFLx REA Ethanol	All Samples	235	5.48	0.92	0.994
TDxFLx REA Ethanol	Plasma	73	8.97	0.90	0.992
TDxFLx REA Ethanol	Serum	64	2.85	0.90	0.997
TDxFLx REA Ethanol	Whole Blood	49	6.95	0.99	0.993
TDxFLx REA Ethanol	Urine	49	3.76	0.90	0.997

AxSYM REA Ethanol vs.	Sample Type	Number of Observations	Intercept	Slope	Correlation Coefficient
GC (headspace analysis)	All Samples	235	2.05	0.97	0.972
GC (headspace analysis)	Plasma	73	5.11	0.90	0.978
GC (headspace analysis)	Serum	64	-8.78	0.99	0.994
GC (headspace analysis)	Whole Blood	49	18.55	0.92	0.972
GC (headspace analysis)	Urine	49	-1.40	1.07	0.974

Plasma samples were within the following ranges:

15.03-267.90 mg/dL on AxSYM, 15.09-269.00 on TDxFLx and 10-273 mg/dL with GC (headspace analysis).

Serum samples were within the following ranges: 14.79-248.44 on AxSYM, 11.82-255.47 on TDxFLx and 19-256 mg/dL with GC (headspace analysis).

Whole Blood samples were within the following ranges: 20.74-288.53 on AxSYM, 11.43-275.28 on TDxFLx and 11-273 mg/dL with GC (headspace analysis).

Urine samples were within the following ranges: 13.01-250.89 on AxSYM, 12.14-265.57 on TDxFLx and 13-277 mg/dL with GC (headspace analysis).

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