Abbott AXSYM® SYSTEM

E

Cortisol
REF 2G98
217-232 6/05

Cortisol

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

This package insert must be read carefully prior to 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.

Note Changes Highlighted

Key to symbols used

REF	List Number	LOT	Lot Number
IVD	For In Vitro Diagnostic Use	STANDARD CAL A	Standard Calibrator (A-F)
2°C – 8°C	Store at 2-8°C	CONTROL L	Control Low, Medium, High (L, M, H)
15°C – 30°C	Store at 15-30°C	REAGENT PACK	Reagent Pack
	Expiration Date	REACTION VESSELS	Reaction Vessels
CE ABBOTT LABORATORIES Abbott Park, IL 60064 USA	Legal Manufacturer	SAMPLE CUPS	Sample Cups
EC REP	Authorized Representative		Consult instructions for use

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

CE ABBOTT LABORATORIES Abbott Park, IL 60064 USA

EC REP ABBOTT
Max-Planck-Ring 2
65205 Wiesbaden
Germany
+49-6122-580

Produced for
ABBOTT
Diagnostics Division

NAME

AXSYM® Cortisol

INTENDED USE

The Cortisol assay is a Fluorescence Polarization Immunoassay (FPIA) for the quantitative measurement of cortisol in human serum, plasma or urine on the AxSYM System to aid in the diagnosis and treatment of adrenal disorders.

SUMMARY AND EXPLANATION OF THE TEST

The AxSYM Cortisol assay utilizes Fluorescence Polarization Immunoassay (FPIA) technology. Refer to the AxSYM System Operations Manual, Section 3, under Principles of Operation for a discussion of this technology.

Cortisol is the major glucocorticoid hormone secreted by the adrenal cortex. Its physiological functions include regulation of carbohydrate metabolism and electrolyte and water distribution. Cortisol also has immunosuppressive and anti-inflammatory activity. In normal individuals, cortisol levels are regulated through a negative feedback loop in which the adrenal cortex responds to increased adrenocorticotropic hormone (ACTH) levels by increasing cortisol secretion, and the pituitary responds to elevated cortisol levels by down-regulation of ACTH production. Plasma cortisol levels are highest in the morning, and concentrations decrease by about half toward evening.[1] Pregnancy or estrogen treatment markedly elevates cortisol levels. Other stimuli such as severe stress may also lead to increased cortisol production.

Cortisol measurements are used as a direct monitor of adrenal status and an indirect measure of pituitary hyper or hypofunction. Elevated cortisol levels are associated with adrenal tumors, pituitary tumors or ectopic ACTH-producing tumors.[2] Subnormal cortisol concentrations may indicate generalized adrenal hypofunction or a defect in the metabolic pathway for cortisol biosynthesis.[3] Cortisol measurements are often performed in conjunction with certain "challenge" tests designed to measure whether regulation of the hypothalamic-pituitary-adrenal axis is intact. These include the dexamethasone suppression test (DST), ACTH stimulation and insulin challenge tests.[4-6] Such challenge tests aid in the differential diagnosis of Cushing's syndrome (cortisol overproduction) and the assessment of Addison's disease (cortisol underproduction).

Dexamethasone Suppression Test (DST)

The DST in serum has been used in the diagnosis of endogenous depression (neuroendocrine dysfunction).[7] Serial DST's have also been used to determine if neuroendocrine function has normalized so that antidepressant or electroconvulsive therapy can be terminated.[8]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The AxSYM Cortisol assay is based on Fluorescence Polarization Immunoassay (FPIA) technology. The AxSYM Cortisol Reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

- Sample and all AxSYM Cortisol Reagents required for one test are pipetted by the sampling probe into various wells of a Reaction Vessel (RV).
- Sample, the Cortisol Antiserum (antibody), pretreatment solution and Solution 4 (Line Diluent) are pipetted into one well of the RV to make up the predilution mixture.
- Additional pretreatment solution and Solution 4 (Line Diluent) are pipetted into the cuvette of the RV.
- The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center by the Processing Probe.

PROCESSING CENTER

- An aliquot of the predilution mixture and Solution 4 (Line Diluent) are transferred to the cuvette of the RV and the blank intensity of the sample is measured.
- A second aliquot of the predilution mixture is transferred to the cuvette along with the Cortisol Fluorescein Tracer.
- Cortisol from the sample and the Cortisol Fluorescein Tracer compete for binding sites on the antibody molecule.
- The intensity of polarized fluorescent light is measured by the FPIA optical assembly.

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

REAGENTS

REAGENT PACK, 100 TESTS

AxSYM Cortisol Reagent Pack (2G98-20)

- 1 Bottle (10 mL) Cortisol Fluorescein Tracer in buffer containing surfactant and stabilizers. Concentration <0.01%. Preservative: Sodium Azide. (Reagent Bottle 1)
- 1 Bottle (5 mL) Cortisol Antiserum (Mouse Monoclonal and Goat Polyclonal) in buffer with protein stabilizers. Concentration <1%. Preservative: Sodium Azide. (Reagent Bottle 2)
- 1 Bottle (6 mL) Pretreatment Solution. Surfactant in TRIS buffer. Preservative: Sodium Azide. (Reagent Bottle 3)

CALIBRATORS

AxSYM Cortisol Standard Calibrators (2G98-01)

6 Bottles (2.5 mL each A-F) of AxSYM Cortisol Standard Calibrators. Calibrator A contains buffer and Calibrators B through F contain cortisol prepared in buffer to yield the following concentrations:

Bottle	Cortisol Concentration (µg/dL)*
STANDARD CAL A	0.0
STANDARD CAL B	2.5
STANDARD CAL C	5.0
STANDARD CAL D	10.0
STANDARD CAL E	25.0
STANDARD CAL F	60.0

Preservative: Sodium Azide

CONTROLS

AxSYM Cortisol Controls (2G98-10)

3 Bottles (2.5 mL each) of AxSYM Cortisol Controls contain cortisol prepared in buffer to yield the following target concentrations:

Bottle	Cortisol Concentration (µg/dL)*	Range (µg/dL)*
CONTROL L	4.0	1.9 - 6.1
CONTROL M	15.0	11.7 - 18.3
CONTROL H	40.0	30.9 - 49.1

Preservative: Sodium Azide

* An alternate unit (nmol/L) may be selected for reporting results (Assay Parameter 45). The conversion factor used by the AxSYM System is 27.6.

Concentration in nmol/L = Concentration in µg/dL x 27.6

STANDARDIZATION

The calibrators and controls are standardized to Institute for Reference Materials and Measurements / International Federation of Clinical Chemists (IRMM/IFCC-451) Cortisol Serum Reference Panels.

OTHER REAGENTS

PROBE CLEANING SOLUTION (9A35-05)

PROBE CLEANING SOLUTION 2 Bottles (220 mL each) AxSYM Probe Cleaning Solution containing 2% Tetraethylammonium Hydroxide (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 Agent.

WARNINGS AND PRECAUTIONS

IVD For In Vitro Diagnostic Use.

SAFETY PRECAUTIONS

- CAUTION: This product requires the handling of human specimens. It is recommended that all human sourced materials are considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[9] Biosafety Level 2[10] or other appropriate biosafety practices [11,12] should be used for materials that contain or are suspected of containing infectious agents.
- The AxSYM Probe Cleaning Solution (2%TEAH) may cause mild eye irritation. If this solution comes in contact with eyes, rinse immediately with water. If irritation persists, seek medical attention.
- All components of this product contain Sodium Azide. For a specific listing, refer to the **REAGENTS** section of this package insert. The components containing Sodium Azide are classified per applicable European Community Directives as: Harmful (Xn). The following are the appropriate Risk(R) and Safety (S) phrases.

R22	Harmful if swallowed.
R32	Contact with acids liberates very toxic gas.
S35	This material and its container must be disposed of in a safe way.
S36	Wear suitable protective clothing.
S46	If swallowed, seek medical advice immediately and show this container or label.

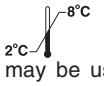
For product not classified as dangerous per European Directive 1999/45/EC - Safety data sheet available for professional user on request.

HANDLING PRECAUTIONS

- Do not use Reagent Packs beyond the expiration date or a maximum of 336 cumulative hours on-board the AxSYM System.
- Do not mix reagents from different Reagent Packs.
- The Fluorescein Tracer, Reagent Bottle 1, is sensitive to light and is packaged in a black bottle to protect the solution.

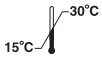
Refer to the AxSYM System Operations Manual, Sections 7 and 8, for a more detailed discussion of the safety and handling precautions during system operation.

STORAGE INSTRUCTIONS



The AxSYM Cortisol Reagent Pack and Cortisol Calibrators and Controls must be stored at 2-8°C. They may be used immediately after removal from the refrigerator. Calibrators and controls should be returned to 2-8°C storage immediately after use. Reagents are stable until the expiration date when stored and handled as directed.

The AxSYM Cortisol Reagent Pack may be on-board the AxSYM System for a maximum of 336 cumulative hours; for example, 42 eight hour shifts. After 336 hours, the reagent pack must be discarded. Refer to the AxSYM System Operations Manual, Sections 2, 5, and Appendix C, for further information on tracking on-board time.



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

INSTRUMENT PROCEDURE

ASSAY FILE INSTALLATION

The AxSYM Cortisol Assay File must be installed on the AxSYM System from the following assay disk prior to performing AxSYM Cortisol assays.

- AxSYM Cortisol Assay Disk 4J63-01 or higher

Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures. NOTE: AxSYM Cortisol assay must only be run with AxSYM System software version 3.60 or higher.

AxSYM CORTISOL ASSAY PARAMETERS

The default values for the assay parameters used for the AxSYM Cortisol 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 Parameters screen. Press PRINT to print the assay parameters.

	Assay Parameters
1	Long Assay Name (English): Cortisol
6	Abbrev Assay Name (English): Cortisol
11	Assay Number: 848
12	Assay Version: *
13	Calibration Version: *
14	Assay File Revision: *
15	Assay Enabled > ON
17	Assay Type: FPIA
18	Standard Cal Reps > 2
21	Cal A Concentration: 0.0
22	Cal B Concentration: 2.5
23	Cal C Concentration: 5.0
24	Cal D Concentration: 10.0
25	Cal E Concentration: 25.0
26	Cal F Concentration: 60.0
43	Default Dilution Protocol > UNDILUTED
44	Default Calibration Method > Standard Cal
45	Selected Result Concentration Units > µg/dL
46	Selected Result Decimal Places > 1
62	Blank I-Max background intensity: *
63	Min Tracer-Min net intensity: *
91	Low Range Undiluted: 0.0
92	High Range Undiluted: 60.0

NOTE: Parameter 45 can be edited to the alternate result unit, nmol/L.

It is recommended that you set General Configuration Parameter, Release Mode, to the “Manual” or “Hold” release mode to ensure that all flagged results are reviewed prior to reporting assay results. Refer to the AxSYM System Operations Manual, Section 2, for a detailed description of Instrument Procedures. If General Configuration Parameter, Release Mode, is configured to the “Automatic” or “Auto With Exceptions” release modes, ensure that all flagged results are reviewed prior to reporting assay results.

For details on Automatic Sample Retest Configuration, refer to the AxSYM System Operations Manual, Section 2.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- To minimize the effects of evaporation, all samples (patient specimens, controls, and calibrators) should be tested within 3 hours of being placed on-board the AxSYM System. Refer to the AxSYM System Operations Manual, Section 5, for a more detailed discussion of on-board sample storage constraints.
- The AxSYM System does not provide the capability to verify sample type. It is the responsibility of the operator to verify the correct sample type(s) is (are) used in the AxSYM Cortisol assay.
- Inspect all samples for bubbles. Remove bubbles prior to analysis.
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter.
- Mix specimens thoroughly after thawing by LOW speed vortexing or by gently inverting to ensure consistency of the results. Avoid repeated freezing and thawing. Specimens showing particulate matter, erythrocytes, or turbidity should be centrifuged before testing.

- When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and etiologic agents. Specimens may be shipped at 2-8°C. Prior to shipment, blood specimens must be removed from the clot or red blood cells.

Serum and Plasma

- Serum (including serum collected in serum separator tubes [SST®]) and plasma (collected in sodium heparin, lithium heparin, or tripotassium EDTA collection tubes) may be used in the AxSYM Cortisol assay; however sodium heparin samples may give slightly lower values. Other anticoagulants and specimen collection tubes have not been validated with the AxSYM Cortisol assay. Follow the manufacturer's processing instructions for serum or plasma collection tubes.
- Samples may be stored for up to 24 hours at 2-8°C prior to being tested. If testing will be delayed more than 24 hours, the serum or plasma should be separated from the clot or red blood cells and stored frozen at -20°C.[1]
- Ensure that complete clot formation has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

Urine

- The AxSYM Cortisol assay can also be used to determine cortisol levels in urine. The urine sample must be collected in a clean container, void of any residue from former samples, reagents, or cleaning compounds.
- Preservatives are not required; however, ten grams of boric acid per liter of urine may be added as a preservative, if desired. Store urine samples at 2-8°C until collection is complete. Freeze an aliquot of sample at -20°C for prolonged storage.[1]
- Frozen urine samples must be brought to room temperature prior to testing. Complete mixing of thawed sample is required before analysis.
- Urine samples that appear cloudy or contain particulate matters, should be centrifuged before testing (recommended 8,000 – 10,000 Relative Centrifugal Force X 10 minutes).

SAMPLE VOLUME

The sample volume required to perform a single undiluted Cortisol test on the AxSYM System varies depending on the type of sample container used. For sample cups, a ROUTINE test requires 150 µL and a STAT test requires 94 µL. For every additional AxSYM Cortisol test performed (ROUTINE or STAT) from the same sample container, an additional 44 µL of sample is required.

The sample cup minimum volumes for both STAT and ROUTINE tests are calculated by the AxSYM System. They are displayed on the Order screen at the time the test(s)is (are) ordered and printed on the Orderlist Report. When using Host Order Query, the Order screen information and the Orderlist Report are not available. Refer to the AxSYM System Operations Manual, Section 5, for a description of the Host Order Query option.

If the assay is configured for Auto Retest, the additional sample volume needed for the retest will not be displayed on the Order screen at the time the test(s) is (are) ordered. Therefore, the total sample volume should include an additional 44 µL of sample. Refer to the AxSYM System Operations Manual, Section 2, for details on Automatic Sample Retest Configuration.

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

The volume requirement for the AxSYM Cortisol Calibrators is 138 µL for each respective sample cup.

The volume requirement for the AxSYM Cortisol Controls is 150 µL (ROUTINE) or 94 µL (STAT) for each respective sample cup.

AxSYM CORTISOL PROCEDURE

Materials Provided

- 2G98-20 AxSYM Cortisol **REAGENT PACK**

Materials Required But Not Provided

- 2G98-01 AxSYM Cortisol Standard Calibrators
- 2G98-10 AxSYM Cortisol Controls
- 8A46 **SOLUTION 4 LINE DILUENT**
- 9A35-05 AxSYM **PROBE CLEANING SOLUTION**
- 8A76-01 **SAMPLE CUPS**
- Pipettes or pipette tips (optional) to deliver the volumes specified on the Order screen.

CAUTION:

- AxSYM Cortisol Calibrators and Controls should be mixed by gentle inversion prior to use.
- When manually dispensing sample into sample cup, verify that dispensing equipment does not introduce cross contamination or bubbles and delivers the specified sample volume. Use a separate pipette tip for each sample. Use accurately calibrated equipment.
- For optimal performance it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Assay Procedure

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 (RVs), bulk solutions and waste levels are acceptable.

The Orderlist Report contains sample placement information and sample cup volume requirements for all ordered tests. 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, 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 (RV's).
- An "Error Code 5066 Matrix cell not detected, trap door, processing center" may be displayed when the instrument homes the motors. If performing only 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 Cortisol Reagent Pack are removed from the Sampling Center to maximize the on-board reagent pack use. Store at 2-8°C.

SPECIMEN DILUTION PROCEDURES

Automated Dilution Protocol

Cortisol samples CANNOT be diluted automatically on the AxSYM System. Patient specimens with cortisol values exceeding 60 µg/dL (HIGH RANGE, Assay Parameter 92) will be flagged as ">60.0". To quantitate the concentration of these specimens, perform Manual Dilution as follows:

Manual Dilution Protocol

Patient samples with Cortisol concentrations reported as greater than 60.0 µg/dL may be diluted using a manual dilution of 1:2. Add one part of the patient sample to one part of the AxSYM Line Diluent. Repeat the test using this manually diluted sample. 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 =

$$\frac{\text{(Volume of Sample + Volume of Dilution Reagent)}}{\text{(Volume of Sample)}}$$

QUALITY CONTROL PROCEDURES

CALIBRATION

The AxSYM Cortisol assay must be calibrated using a Standard Calibration (6-point) procedure.

Standard Calibration

To perform an AxSYM Cortisol Standard Calibration, test the Cortisol Standard Calibrators A, B, C, D, E, and F in duplicate. A single sample of all levels of Cortisol controls must be tested as a means of evaluating the assay calibration.

Once the AxSYM Cortisol 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
- Controls are out of 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, Appendix E, for an explanation of the calibration validity parameters that may be used by the AxSYM System.

Operator Verification

An acceptable Cortisol calibration curve should meet the following criteria:

a) Polarization Error (PERR) -3.00 to +3.00 for all calibrators.

b) Root Mean Squared Error (RMSE) less than or equal to 3.00.

c) All controls are within the acceptable ranges.

NOTE: PERR's and RMSE's are to be used as guidelines only. If controls are within specified ranges, the calibration curve is acceptable.

QUALITY CONTROL

The recommended control requirement for an AxSYM Cortisol assay is a single sample of all Cortisol control levels tested once every 24 hours each day of use for each reagent lot. Controls may be placed in any position in the Sample Carousel.

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 this package insert. Refer to the **REAGENTS**, CONTROLS section of this package insert for Cortisol 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, 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. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

RESULTS

Calculation

The AxSYM Cortisol assay utilizes a Four-Parameter Logistic Curve Fit method (4PLC, Y weighted) to generate a standard calibration curve. Refer to the AxSYM System Operations Manual, Appendix F, for further information.

Alternate Result Unit

The default result unit for AxSYM Cortisol is µg/dL. When selecting the alternate result unit, nmol/L, the conversion factor used by the AxSYM System is 27.6.[13] The equation to convert Cortisol concentration from µg/dL to nmol/L is as follows:

µg/dL Cortisol x 27.6 = nmol/L Cortisol

Urine Conversion of Results to µg/24 hours

For urine samples, conversion from µg/dL to µg/24 hours, use the following conversion factor:

AxSYM result (µg/dL) x 10(dL/L) x V = µg/24 hours

V = Volume of urine excreted in liters per 24 hours

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, Sections 1 and 2.

LIMITATIONS OF THE PROCEDURE

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

- Due to the diurnal variation of cortisol levels in normal subjects, all serum/plasma cortisol measurements should be referenced to the time of day of sample collection.
- Patients receiving prednisolone or prednisone (which is converted to prednisolone in vivo) therapy may show artificially elevated cortisol values due to cross-reactivity to prednisolone. Cross-reactivity to endogenous and synthetic steroids are reported in the **SPECIFIC PERFORMANCE CHARACTERISTICS**, SPECIFICITY section in this package insert.
- Fludrocortisone Acetate is an adrenocortical steroid that may result in artificially elevated cortisol values due to cross-reactivity. For diagnostic purposes, cortisol results should be used in conjunction with other data; e.g., patients medical history, symptoms, results of other tests, clinical impressions, etc.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibody (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.[14,15] These specimens should not be tested with the AxSYM Cortisol assay.

EXPECTED VALUES

Normal Range: Serum, AM, PM

Serum cortisol levels were determined by assaying samples drawn from 50 apparently healthy individuals between the hours of 7:00 to 9:00 AM and 4:00 to 6:00 PM.

The 95% confidence limit for the normal range of AM and PM cortisol in the serum specimens was determined to be 4.2 to 38.4 µg/dL (median value 10.8 µg/dL) and 1.7 to 16.6 µg/dL (median value 6.7 µg/dL), AM and PM respectively.

It is recommended that each laboratory establish its own expected range.

Normal Range: Direct Urine (24 hr)

Cortisol levels in urine were determined by assaying 24 hour urine samples from 49 apparently healthy individuals. The 95% confidence limit for the normal range of cortisol in urine specimens was determined to be 32 to 243 µg/24 hours (median value 88 µg/24 hours).

It is recommended that each laboratory establish its own expected range.

SPECIFIC PERFORMANCE CHARACTERISTICS

PRECISION

Precision was determined as described in the National Committee for Clinical Laboratory Standards (NCCLS) protocol EP5-A[16]. Three buffer-based panels (1,2, and 3) were assayed in replicates of two, at two separate times per day, for 20 days. Three reagent lots were tested using two instruments, with a single standard calibration per reagent. Data from this study are summarized below.

Panel 1

Reagent		Mean	Within Run		Total	
Lot	n	(µg/dL)	SD	%CV	SD	%CV
1	80	5.0	0.58	11.7	0.67	13.5
2	80	4.5	0.49	10.8	0.57	12.8
3	80	4.6	0.63	13.9	0.69	15.0

Panel 2

Reagent		Mean	Within Run		Total	
Lot	n	(µg/dL)	SD	%CV	SD	%CV
1	80	15.2	0.75	5.0	1.01	6.6
2	80	15.2	0.87	5.7	0.98	6.5
3	80	14.9	0.85	5.7	0.95	6.4

Panel 3

Reagent		Mean	Within Run		Total	
Lot	n	(µg/dL)	SD	%CV	SD	%CV
1	80	39.8	2.57	6.5	2.60	6.5
2	80	40.9	2.47	6.0	2.52	6.1
3	80	38.1	2.02	5.3	2.16	5.7

SPECIFICITY

The specificity of the AxSYM Cortisol assay was determined by performing cross-reactivity testing for compounds whose chemical structure or concurrent usage may potentially interfere with the AxSYM Cortisol assay. Representative data follows.

Compound	**Concentration (µg/dL)**	**% Crossreactivity**
11-Deoxycortisol	100	13.6
Cortisone	1000	1.4
Dexamethasone	5000	2.0
Corticosterone	500	8.0
Prednisolone	100	32.2

SENSITIVITY

The sensitivity of the AxSYM Cortisol assay was calculated to be equal to or less than 1.1 µg/dL by testing the AxSYM Cortisol Calibrator A (n=24 runs) in replicates of 10. This sensitivity represents the lowest measurable concentration of Cortisol that can be distinguished from zero with 95% confidence.

ACCURACY BY RECOVERY

Recovery was determined by adding known concentrations of Cortisol to human serum samples to produce expected values ranging from 10.5 µg/dL to 62.3 µg/dL. The concentration of Cortisol was determined using the AxSYM Cortisol assay, and the resulting percent recovery was calculated according to the following equation:

$$\% \text{ Recovery} = \frac{\text{Mean Observed (µg/dL)}}{\text{Expected (µg/dL)}} \times 100$$

Representative data are shown in the following table.

Number of Samples	Cortisol Added	Average % Recovery
10	5.7	100.4
10	11.4	97.4
10	22.7	92.6
5	45.5	95.5
5	48.0	97.4

DILUTION LINEARITY

The AxSYM Cortisol assay was designed to have a mean dilution linearity of 100 ± 15%. Dilution Linearity was evaluated by 1:2 dilutions of 5 human serum specimens with known cortisol concentrations. All specimens were diluted with AxSYM Line Diluent. The concentration of cortisol was determined using the AxSYM Cortisol assay and the resulting percent recovery was calculated. Data from this study are shown in the table below.*

Specimen	Dilution Factor	Expected (µg/dL)	Mean Observed (µg/dL)	%Recovery**
1	Undiluted	49.1	49.1	-
	1:2	24.6	23.8	96.7
2	Undiluted	43.1	43.1	-
	1:2	21.6	20.5	94.9
3	Undiluted	57.0	57.0	-
	1:2	28.5	30.9	108.4
4	Undiluted	38.0	38.0	-
	1:2	19.0	21.0	110.5
5	Undiluted	41.3	41.3	-
	1:2	20.7	21.5	103.9

Average recovery across the five diluted samples above = 102.9%

*Representative data; results in individual laboratories may vary from these data.

** $\% \text{ Recovery} = \frac{\text{Mean Observed (µg/dL)}}{\text{Expected (µg/dL)}} \times 100$

INTERFERENCE

Potential interference from bilirubin, hemoglobin, triglycerides, total protein and red blood cells was evaluated in the AxSYM Cortisol assay. The AxSYM Cortisol assay demonstrated less than 10% interference in the presence of each of the following potentially interfering substances when added to a normal human serum matrix: 10 mg/dL bilirubin, 500 mg/dL hemoglobin, 1500 mg/dL triglycerides, 12 g/dL total protein, and 0.4% red blood cells.

ACCURACY BY CORRELATION

The AxSYM Cortisol assay was compared to a commercially available cortisol assay. The results of the Least Squares[17] and Passing-Bablok[18] Linear Regression analyses follow.

In this evaluation, endogenous and cortisol-spiked serum and sodium heparin plasma samples tested ranged from 1.9 to 54.8 μg/dL with the AxSYM Cortisol assay and 3.0 to 67.1 μg/dL with a commercially available Cortisol assay.

Abbott AxSYM Cortisol vs. Commercial Cortisol Assay

Regression Analysis	n	Intercept	Slope	Correlation Coefficient
Least Squares	130	-0.74	0.87	0.96
Passing-Bablok*	130	-2.39	0.93	0.96

* A linear regression method with no special assumptions regarding distribution of specimens and measurement errors.[18]

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Abbott Laboratories
Diagnostics Division
Abbott Park, IL 60064 USA

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