ARCHITECT SYSTEM

en

Cortisol

REF 8D15

840607/R4

Cortisol i

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

This package insert must be read carefully prior to product use. Package insert instructions must be carefully followed. 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	SN	Serial Number
IVD	*In Vitro* Diagnostic Medical Device	REAGENT LOT	Reagent Lot
LOT	Lot Number	CAL A	Calibrator (A-F)
	Expiration Date	CONTROL NO.	Control Number
2°C 8°C	Store at 2-8°C	ASSAY CD-ROM	Assay CD-ROM
	Consult instructions for use	REACTION VESSELS	Reaction Vessels
		SAMPLE CUPS	Sample Cups
EC REP	Authorized Representative	SEPTUM	Septum
	Manufacturer	REPLACEMENT CAPS	Replacements Caps

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

NAME

ARCHITECT Cortisol

INTENDED USE

ARCHITECT Cortisol is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of cortisol in human serum, plasma or urine on the ARCHITECT *i* System. The ARCHITECT Cortisol assay is intended for use as an aid in the diagnosis and treatment of adrenal disorders.

SUMMARY AND EXPLANATION OF THE TEST

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] The majority of cortisol in plasma is bound to proteins and approximately 1% is excreted unchanged into the urine.[4] Urinary cortisol is generally thought to reflect the level of unbound (free) plasma cortisol, which is biologically active. In cases of cortisol overproduction, cortisol-binding globulin becomes saturated, such that unbound plasma cortisol increases disproportionately, as does urinary excretion. The measurement of urinary cortisol is a sensitive means of determining adrenocortical hyperfunction such as Cushing's syndrome.[5,6] Urinary cortisol from 24-hour collections represent integration over a full day and are not affected by the diurnal variation evident in plasma cortisol levels.

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 test and insulin tolerance test.[7-11] Such challenge tests aid in the differential diagnosis of Cushing's syndrome (cortisol overproduction) and the assessment of Addison's disease (cortisol underproduction).

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT Cortisol assay is a delayed one-step immunoassay for the quantitative determination of cortisol in human serum, plasma or urine using CMIA technology with flexible assay protocols, referred to as Chemiflex.

Sample and anti-cortisol coated paramagnetic microparticles are combined to create a reaction mixture. Cortisol present in the sample binds to the anti-cortisol coated microparticles. After incubation, cortisol acridinium-labeled conjugate is added to the reaction mixture. The cortisol acridinium-labeled conjugate competes for the available binding sites on the anti-cortisol coated microparticles. Following a second incubation, the microparticles are washed, and pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of cortisol in the sample and the RLUs detected by the ARCHITECT *i* System optics.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Reagent Kit, 100 Tests/500 Tests

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT *i* Systems. Please contact your local distributor.

ARCHITECT Cortisol Reagent Kit (8D15)

- **MICROPARTICLES** 1 Bottle (6.6 mL/27.0 mL) Anti-cortisol (mouse, monoclonal) coated microparticles in TRIS/BIS-TRIS buffer with protein (bovine) stabilizer. Minimum concentration: 0.09% solids. Preservatives: sodium azide and ProClin 300.
- **CONJUGATE** 1 Bottle (5.9 mL/26.3 mL) Cortisol acridinium-labeled conjugate in citrate buffer with surfactant stabilizer. Minimum concentration: 0.7 ng/mL. Preservative: ProClin 300.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger Solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

- **WASH BUFFER** Wash Buffer containing phosphate buffered saline solution. Preservative: antimicrobial agent.

WARNINGS AND PRECAUTIONS

- **IVD**

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens[12]. Biosafety Level 2[13] or other appropriate biosafety practices[14,15] should be used for materials that contain or are suspected of containing infectious agents.
- The microparticles and conjugate contain methylisothiazolones, which are components of ProClin, 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.
- For information on the safe disposal of sodium azide and a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents between reagent kits.**
- Before loading the ARCHITECT Cortisol Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- **To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.**
  - Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts, which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

- The ARCHITECT Cortisol Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT Cortisol Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded**. For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

- When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT Cortisol assay file must be installed on the ARCHITECT *i* System from the ARCHITECT *i* Assay CD-ROM Addition B prior to performing the assay. For detailed information on assay file installation and on viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.
- The default result unit for the ARCHITECT Cortisol assay is µg/dL. When the alternate result unit, nmol/L, is selected, the conversion factor used by the system is 27.59.[16] When the alternate result unit, µmol/L, is selected, the conversion factor used by the system is 0.02759.
  - Conversion Formula: (Concentration in µg/dL) x (27.59) = nmol/L
  - Conversion Formula: (Concentration in µg/dL) x (0.02759) = µmol/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify the correct specimen types are used in the ARCHITECT Cortisol assay.

Serum and Plasma

- Human serum (including serum collected in serum separator tubes) or plasma (collected in lithium heparin, plasma separator tubes with lithium heparin, sodium heparin or potassium EDTA) may be used in the ARCHITECT Cortisol assay. Other anticoagulants have not been tested for use with the ARCHITECT Cortisol assay. Follow the manufacturer's processing instructions for collection tubes.

Urine

- Human urine may be used in the ARCHITECT Cortisol assay. The urine sample must be collected in a clean, previously unused container. Preservatives are not required; however, ten grams of boric acid per liter of urine may be used.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated specimens
  - cadaver specimens or body fluids other than human serum, plasma or urine
  - obvious microbial contamination
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Plasma and serum specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

Preparation for Analysis

- Serum, plasma or urine specimens that appear cloudy or contain particulate matter should be centrifuged before testing.
- Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.
- Specimens must be mixed THOROUGHLY after thawing by LOW speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze/thaw cycles of specimens should be avoided.

Storage

- If testing will be delayed for more than eight hours, remove plasma or serum from the serum or plasma separator, red blood cells or clot. Specimens removed from the separator gel, cells or clot may be stored up to 14 days at 2-8°C.
- Urine samples may be stored up to 14 days at 2-8°C.
- Serum, plasma or urine specimens can be stored up to 30 days at -10°C or colder.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the serum or plasma separator, red blood cells or clot.
- When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped ambient or on wet or dry ice. Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

- 8D15 ARCHITECT Cortisol Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- 3K51 ARCHITECT *i* **ASSAY CD-ROM** -US- Addition B
- 3K53 ARCHITECT *i* **ASSAY CD-ROM** -WW (excluding US)- Addition B
- 8D15-01 ARCHITECT Cortisol Calibrators
- 6E20-10 Abbott Immunoassay Multi-Constituent Controls or other commercial controls
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional)

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the ARCHITECT Cortisol Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - Invert the microparticle bottle 30 times.
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles remain adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions on placing septums on bottles, refer to the **Handling Precautions** section of this package insert.
- Load the ARCHITECT Cortisol Reagent Kit on the ARCHITECT *i* System.
  - Verify that all necessary assay reagents are present. Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls, refer to the ARCHITECT System Operations Manual, Section 5.
- The minimum sample volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. Verify adequate sample cup volume is present prior to running the test.
  - Priority: 70 µL for the first ARCHITECT Cortisol test plus 20 µL for each additional ARCHITECT Cortisol test from the same sample cup.
  - ≤ 3 hours on board: 150 µL for the first ARCHITECT Cortisol test plus 20 µL for each additional ARCHITECT Cortisol test from the same sample cup.
  - > 3 hours on board: additional sample volume is required. For information on sample evaporation and volumes, refer to the ARCHITECT System Operations Manual, Section 5.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare calibrators and controls.
  - ARCHITECT Cortisol Calibrators and Abbott Immunoassay Multi-Constituent Controls should be prepared according to their respective package inserts.
  - To obtain the recommended volume requirements for the ARCHITECT Cortisol Calibrators, hold the bottles **vertically** and dispense 5 drops of each calibrator into each respective sample cup. Dispense 150 µL of each control into each respective sample cup.
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the ARCHITECT System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedures

Specimens with a cortisol value exceeding 59.8 µg/dL are flagged with the code ">59.8" and may be diluted with the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

- If using the Automated Dilution Protocol, the system performs a 1:2 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.
- Specimens with a cortisol value exceeding 119.6 µg/dL are flagged with the code ">119.6" when run using the Automated Dilution Protocol. These specimens may be diluted by following the Manual Dilution Procedure.

Manual Dilution Procedure

- Manual dilutions should be performed as follows:
  - The suggested dilution for a cortisol test is 1:4.
  - Prior to diluting the specimen, dispense approximately 7 drops of ARCHITECT Cortisol Calibrator A into a clean test tube for use in the next step.
  - Transfer 150 µL of ARCHITECT Cortisol Calibrator A from the test tube prepared in the prior step into another clean test tube and add 50 µL of the patient specimen.
  - The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result. The dilution should be performed so that the diluted result (before the dilution factor is applied) reads greater than 3.0 µg/dL.
  - For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT Cortisol calibration, test Calibrators A, B, C, D, E, and F in duplicate. A single sample of each cortisol control level must be tested to evaluate the assay calibration. Ensure that assay control values are within established ranges. Calibrators should be priority loaded.
- Calibration Range: 0.0 - 59.8 µg/dL.
- Once an ARCHITECT Cortisol calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT Cortisol assay is that a single sample of each control level be tested once every 24 hours each day of use. Commercial controls such as the Abbott Immunoassay Multi-Constituent Controls are suitable for this purpose. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures.

Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT Cortisol assay belongs to method group 1.

Use ARCHITECT Cortisol Calibrators in place of MasterCheck as described in the ARCHITECT System Operations Manual, Appendix B.

RESULTS

Calculation

The ARCHITECT Cortisol assay uses a 4 Parameter Logistic Curve Fit (4PLC, Y-weighted) data reduction method to generate a calibration curve.

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 ARCHITECT System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- 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 fludrocortisone, prednisolone or prednisone (which is converted to prednisolone *in vivo*) may show artificially elevated cortisol values due to cross-reactivity. Cross-reactivity to endogenous and synthetic steroids is reported in the **SPECIFIC PERFORMANCE CHARACTERISTICS, Specificity** section in this package insert.
- If the cortisol results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with other data; e.g., 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 antibodies (HAMA).[17] Such specimens may show either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies.[17,18] Assay results that are not consistent with other clinical observations may require additional information for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[19] The presence of heterophilic antibodies in a patient specimen may cause anomalous values to be observed.[19] Additional information may be required for diagnosis.
- The concentration of cortisol in a given specimen, determined with assays from different manufacturers, can vary due to differences in assay methods, calibration, and reagent specificity.

EXPECTED VALUES

Reference Range: Serum

Serum cortisol levels were determined by assaying samples drawn from apparently healthy individuals collected before 10 a.m. and collected after 5 p.m. The 95% reference interval of the a.m. and p.m. populations was determined. Data from this study are summarized in the following table.*

Specimen Type	Specimen Collection	n	95% Reference Interval µg/dL	95% Reference Interval nmol/L
Serum	Before 10 a.m.	150	3.7 - 19.4	101.2 - 535.7
Serum	After 5 p.m.	150	2.9 - 17.3	79.0 - 477.8

* Representative data; results in individual laboratories may vary from these data. It is recommended that each laboratory establish its own reference range.

Reference Range: Urine

Cortisol levels in urine were determined by assaying 24-hour urine samples from apparently healthy individuals. The 95% reference interval was determined. Data from this study are summarized in the following table.*

Specimen Type	n	95% Reference Interval µg/24 hour[a]	95% Reference Interval nmol/24 hour[b]
Urine	128	4.3 - 176.0	11.8 - 485.6

a µg/24 hour = (Concentration in µg/dL) x (10) x (Volume of urine excreted in liters per 24 hours)

b nmol/24 hour = (Concentration in nmol/L) x (Volume of urine excreted in liters per 24 hours)

* Representative data; results in individual laboratories may vary from these data. It is recommended that each laboratory establish its own reference range.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT Cortisol assay is designed to have an assay precision of ≤ 10% total CV for serum samples ≥ 3 to ≤ 35 µg/dL and ≤ 20% total CV for urine samples ≥ 3 to ≤ 35 µg/dL.

A study was performed with guidance from the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Protocol EP5-A2.[20] Abbott Immunoassay Multi-Constituent Controls (Levels 1, 2 and 3) and seven panels were assayed using two lots of reagents in replicates of two at two separate times per day for 20 days on two instruments. Each reagent lot used a single calibration curve throughout the study. Data from this study are summarized in the following table.*

Sample	Instr.	Reagent Lot	n	Mean Conc. (µg/dL)	Within Run SD	Within Run % CV	Total SD	Total % CV
Level 1	1	A	80	3.8	0.14	3.6	0.19	5.0
	2	B	80	4.0	0.19	4.8	0.23	5.8
Level 2	1	A	80	16.6	0.43	2.6	0.62	3.7
	2	B	80	17.3	0.40	2.3	1.32	7.7
Level 3	1	A	80	30.3	0.87	2.9	1.17	3.9
	2	B	80	31.0	0.63	2.1	1.32	4.3
Serum Panel 1	1	A	80	2.9	0.08	2.9	0.11	4.0
	2	B	80	2.9	0.16	5.5	0.18	6.2
Serum Panel 2	1	A	80	39.8	0.95	2.4	1.01	2.5
	2	B	80	41.0	1.08	2.6	1.29	3.2
Serum Panel 3	1	A	80	53.3	1.71	3.2	1.73	3.3
	2	B	80	55.8	1.50	2.7	1.87	3.4
Urine Panel 1	1	A	80	2.4	0.13	5.3	0.15	6.2
	2	B	80	2.7	0.16	6.1	0.17	6.4
Urine Panel 2	1	A	80	14.5	0.39	2.7	0.59	4.1
	2	B	80	15.9	0.60	3.8	0.72	4.5
Urine Panel 3	1	A	80	36.8	1.05	2.9	1.39	3.8
	2	B	80	40.6	1.56	3.9	1.59	3.9
Urine Panel 4	1	A	80	49.0	2.84	5.8	2.84	5.8
	2	B	80	53.7	3.18	5.9	3.18	5.9

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

Sensitivity

Functional Sensitivity

The ARCHITECT Cortisol assay is designed to have a functional sensitivity of ≤ 1 µg/dL.

In a study, serum and urine panels ranging in concentration from 0.1 - 2.1 µg/dL were tested in replicates of two over 10 days on two instruments using two reagent lots and two calibrations for a total of 40 replicates per panel. The total %CVs were calculated and plotted against the mean concentration. A reciprocal curve was fitted through the data and the functional sensitivity value was calculated as the concentration corresponding to the 20% CV level of the fitted curve. At the upper 95% confidence limit, the lowest ARCHITECT Cortisol assay value exhibiting a 20% CV was calculated to be 0.8 µg/dL for serum samples and 1 µg/dL for urine samples.*

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

Limit of Detection

The ARCHITECT Cortisol assay is designed to have a limit of detection (LoD) of ≤ 0.8 µg/dL. The limit of blank (LoB) and LoD of the ARCHITECT Cortisol assay were determined with guidance from CLSI Protocol EP17-A[21] using proportions of false positives (α) less than 5% and false negatives (β) less than 5%. These determinations were performed using 60 blank and 120 low level samples; LoB = 0.23 µg/dL and LoD = 0.40 µg/dL.*

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

Linearity

The ARCHITECT Cortisol assay is linear between 1 and 59.8 µg/dL based on a study performed with guidance from CLSI Protocol EP6-A.[22]

Specificity

The specificity of the ARCHITECT Cortisol assay was determined by studying the cross-reactivity of compounds whose chemical structure or concurrent usage may potentially interfere with the ARCHITECT Cortisol assay. Specificity of the assay was determined by spiking each compound into human serum specimens with cortisol levels spiked between 11.4 and 12.0 µg/dL.*

Compound	Concentration (µg/dL)	% Cross-Reactivity
Aldosterone	1000	0.0
Beclomethasone	1000	0.0
Budesonide	1000	0.0
Canrenone	1000	0.1
Corticosterone	1000	0.9
Cortisol 21-glucuronide	1000	0.2
Cortisone	1000	2.7
β-Cortol	1000	0.0
β-Cortolone	1000	0.0
11-Deoxycorticosterone	100	0.0
11-Deoxycortisol	100	1.9
Dexamethasone	1000	0.0
DHEA	1000	0.0
DHEA-S	1000	0.0
β-Estradiol	1000	0.0
Estriol	1000	0.0
Estrone	1000	0.0
Fludrocortisone	100	36.6
Fluticasone Propionate	1000	0.0
6β-Hydroxycortisol	1000	0.2
17α-Hydroxypregnenolone	1000	0.1
11β-Hydroxyprogesterone	1000	0.2
17-Hydroxyprogesterone	1000	0.6
Medroxyprogesterone Acetate	1000	0.0
6-Methylprednisolone	1000	0.1
Mometasone	1000	0.0
Prednisolone	100	12.3
Prednisone	1000	0.6
Pregnanediol	1000	0.0
Pregnanetriol	1000	0.0
Pregnenolone	1000	0.0
Progesterone	1000	0.0
β-Sitosterol	1000	0.0
Spironolactone	1000	0.0
Testosterone	1000	0.0
Tetracycline	1000	0.0
Tetrahydrocortisol	1000	0.5
Triamcinolone	1000	0.5

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

Interference

Potential interference in the ARCHITECT Cortisol assay from the following compounds is designed to be ≤ 15% at the levels indicated.

A study based on guidance from the CLSI Protocol EP7-A2[23] was performed for the ARCHITECT Cortisol assay. Serum specimens with cortisol levels between 5.1 and 34.2 µg/dL and urine specimens with cortisol levels between 4.6 and 37.9 µg/dL were supplemented with the following potentially interfering compounds. The average amount of interference observed during the study ranged from -7.8% to 13.2%.*

Specimen Type	Potentially Interfering Substance	Potentially Interfering Substance Concentration
Serum	Bilirubin	20 mg/dL
	Hemoglobin	500 mg/dL
	Total Protein (Low)	3 g/dL
	Total Protein (High)	10 g/dL
	Triglycerides	2000 mg/dL
Urine	Creatinine	5 mmol/L
	Urea	350 mmol/L
	Glucose	5 mmol/L
	Sodium Chloride	1000 mmol/L
	Total Protein (High)	1000 mg/dL

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

Evaluation of Other Potential Interferents

Potential interference in the ARCHITECT Cortisol assay from HAMA and rheumatoid factor (RF) is designed to be ≤ 15%. In a study, the ARCHITECT Cortisol assay was evaluated by testing specimens with HAMA and RF to further assess the clinical specificity. Specimens positive for HAMA and specimens positive for RF were evaluated for % interference with cortisol levels spiked between 9.0 and 44.1 µg/dL. Mean absolute % interference is summarized in the following table.*

Other Potential Interferents	n	Mean Absolute % Interference
HAMA Positive	10	1.0
RF Positive	10	5.9

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

Correlation

The ARCHITECT Cortisol assay is designed to have a slope of 1.0 ± 0.1 and a correlation coefficient (r) of ≥ 0.95 for serum samples when compared to Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LC-MS/MS). The ARCHITECT Cortisol assay is also designed to have a slope of 1.0 ± 0.2 and a correlation coefficient (r) of ≥ 0.85 for urine samples when compared to LC-MS/MS. In a study the ARCHITECT Cortisol assay was compared to LC-MS/MS. Data from this study were analyzed using the Passing-Bablok[a] regression method and are summarized in the following table.*

ARCHITECT Cortisol vs. LC-MS/MS

Specimen Type	n	Slope	Intercept	Correlation Coefficient (r)
Serum	125	1.08	-0.02	0.996
Urine	81	1.06	0.84	0.997

Serum Sample Range (ARCHITECT): 1.5 – 52.5 µg/dL

Serum Sample Range (LC-MS/MS): 1.4 – 49.4 µg/dL

Urine Sample Range (ARCHITECT): 0.8 – 51.1 µg/dL

Urine Sample Range (LC-MS/MS): 0.1 – 49.5 µg/dL

The ARCHITECT Cortisol assay is designed to have a correlation coefficient (r) of ≥ 0.90 for serum samples and ≥ 0.80 for urine samples when compared to the AxSYM Cortisol assay. In a study the ARCHITECT Cortisol assay was compared to the AxSYM Cortisol assay. Data from this study were analyzed using the Passing-Bablok[a] regression method and are summarized in the following table.*

ARCHITECT Cortisol vs. AxSYM Cortisol

Specimen Type	n	Slope	Intercept	Correlation Coefficient (r)
Serum	121	0.91	0.92	0.983
Urine	74	0.54	-1.14	0.980

Serum Sample Range (ARCHITECT): 1.5 – 52.5 µg/dL

Serum Sample Range (AxSYM): 1.3 – 59.1 µg/dL

Urine Sample Range (ARCHITECT): 0.8 – 46.8 µg/dL

Urine Sample Range (AxSYM): 2.1 – 60.0 µg/dL

[a] A linear regression method with no special assumptions regarding the distribution of the samples and measurement errors.[24]

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

BIBLIOGRAPHY

1. Whitley RJ, Meikle AW, Watts NB. Endocrinology. In: Burtis CA, Ashwood ER, editors. *Tietz Textbook of Clinical Chemistry*. 2nd ed. Philadelphia, PA: W.B. Saunders Company; 1994;1825-6.
2. Gold EM. The Cushing syndromes: changing views of diagnosis and treatment. *Ann Intern Med* 1979;90:829-44.
3. Hsu T-H. The pituitary-adrenal axis: clinical considerations. *Journal of Clinical Immunoassay* 1983;6(4):277-87.
4. Stewart PM. The adrenal cortex. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, editors. *Williams Textbook of Endocrinology*. 10th ed. Saunders Company, 2003;502-3.
5. Newell-Price J, Trainer P, Besser M, *et al*. The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. *Endocr Rev* 1998;19(5):647-72.
6. Contreras LN, Hane S, Tyrrell JB. Urinary cortisol in the assessment of pituitary-adrenal function: utility of 24-hour and spot determinations. *J Clin Endocrinol Metab* 1986;62(5):965-9.
7. Liddle GW. Tests of the pituitary-adrenal suppressibility in the diagnosis of Cushing's syndrome. *J Clin Endocrinol Metab* 1960;20(12):1539-60.
8. Henry JB. Evaluation of endocrine function. In: Davidsohn I, Henry JB, editors. *Clinical Diagnosis and Management by Laboratory Methods*. Philadelphia, PA: W.B. Saunders, 1979;408-9.
9. Kehlet H, Binder C. Value of an ACTH test in assessing hypothalamic-pituitary-adrenocortical function in glucocorticoid-treated patients. *Br Med J* 1973;2:147-9.
10. Gwirtsman H, Gerner RH, Sternbach H. The overnight dexamethasone suppression test: clinical and theoretical review. *J Clin Psychiatry* 1982;43(8):321-7.
11. Orsulak PJ, Rush AJ. The dexamethasone suppression test in depression. *Journal of Clinical Immunoassay* 1983;6(4):302-7.
12. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
13. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; January 2007.
14. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
15. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline-Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2005.
16. Kratz A, Lewandrowski KB. MGH case records: normal reference laboratory values. *N Engl J Med* 1998;339(15):1063-72.
17. Schroff RW, Foon KA, Beatty SM, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985;45:879-85.
18. Primus FJ, Kelley EA, Hansen HJ, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem* 1988;34(2):261-4.
19. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin Chem* 1988;34(1):27-33.
20. National Committee for Clinical Laboratory Standards. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition*. NCCLS document EP5-A2. Wayne, PA: NCCLS, 2004.
21. National Committee for Clinical Laboratory Standards. *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*. NCCLS document EP17-A. Wayne, PA: NCCLS, 2004.
22. National Committee for Clinical Laboratory Standards. *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*. NCCLS document EP6-A. Wayne, PA: NCCLS, 2003.
23. Clinical and Laboratory Standards Institute. *Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition*. CLSI document EP7-A2. Wayne, PA: Clinical and Laboratory Standards Institute, 2005.
24. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. *J Clin Chem Clin Biochem* 1983;21(11):709-20.

Abbott Laboratories
Diagnostics Division
Abbott Park, IL 60064 USA

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

Produced by Fisher Diagnostics
a division of Fisher Scientific Company LLC
a part of Thermo Fisher Scientific Inc.
8365 Valley Pike
Middletown, VA 22645-1905 USA
for Abbott Diagnostics Division

Distributed by Abbott Laboratories, Abbott Park, IL 60064 USA

ABBOTT
Diagnostics Division

April 2009