**PROCEDURAL CAUTIONS AND WARNINGS**

LEVELS AT NIGHT.

Most circulating cortisol is bound to cortisol binding globulin or CBG and shows a diurnal pattern. Cushing's syndrome and Cushing's disease as well as adrenal hyperplasia and nodular hyperplasia of the adrenal cortex are characterized by elevated levels of circulating cortisol. Measurement of cortisol in plasma or serum is used to diagnose these conditions. Conventional procedures measure cortisol in plasma or serum. Approximately 1 mL of saliva is required per duplicate sample. The saliva sample is collected into a clean glass clean glass tube (Salivette by Sarstedt may be used) without food intake or before drinking, eating, brushing or chewing the teeth. Simply rinse the mouth without any interdental connection. Do not use blood-contaminated specimens. Store samples at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

**SCIENTIFIC BACKGROUND**

The major glucocorticoid secreted by the adrenal cortex is cortisol. It is also involved in calcium homeostasis and is a key regulator of the stress response. It also has anti-inflammatory activity. It is also involved in calcium absorption, gluconeogenesis as well as the secretion of gastric acid and pepsin. It is increased under conditions of stress, fat mobilization, physical exercise and external administration of ACTH. Measurement of cortisol levels in general can help to assess the function of the adrenal gland and the differential diagnosis of Addison's disease and Cushing's disease as well as adrenal hyperplasia and carcinoma.

Most circulating cortisol is bound to cortisol binding globulin or CBG and transcortin. The free cortisol, which is considered the active part of blood, is about 1–2%. In the absence of appreciable amounts of cortisol in serum, plasma or other specimens of human or animal origin.

**LIMITATIONS**

1. All the reagents within the kit are calibrated for the direct determination of cortisol in human saliva. The kit is not calibrated for the determination of cortisol in serum, plasma or other specimens of human or animal origin.
2. Any samples or control sera containing azide or thimerosal or any other reagent may lead to false results.
3. Calibrator A may be diluted to any high saliva samples. The use of any other reagent may lead to false results.
4. Reagents obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of cortisol metabolites in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

**SAFETY WARNINGS AND CAUTIONS**

**POTENTIAL BIOHAZARDOUS MATERIAL**

**HUMAN SERUM**

Human serum that may be used in the preparation of the calibrators and controls has been tested and found to be non-infectious. No specimens have been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV). Those handling the specimen or reagents must take precautionary measures to avoid multiple freezing and thawing cycles.

**STORAGE**

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label. Once opened, the calibrators should be kept within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

**CONTENTS**

Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.

**STABILITY**

Stability: 12 months or as indicated on label.

**MEASUREMENT PROCEDURE**

**1. Preparation of saliva**

Collect 4–5 mL of saliva into a clean glass tube. No food intake or before drinking, eating, brushing or chewing the teeth. Simply rinse the mouth without any interdental connection. Do not use blood-contaminated specimens. Store samples at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

**2. Reagents and Equipment Needed but Not Provided**

**3. Procedure**

1. Prepare the sample. A set of standards is used to plot a standard curve and the control.
2. Add 50 mL of the wash buffer concentrate (see assay procedure step 10) to 20 mL of assay buffer. Dilute 1:10 in distilled or deionized water to bottle labels for exact concentrations.
3. Pour 5 mL of the sample into each well. TMB is a suspected carcinogen.

**CHEMICAL HAZARDS**

Avoid contact. No special precautions are necessary. Use deionized or distilled water. When used in diagnostic procedures, instruments and equipment used in diagnostic procedures should be handled with the same precautions as applied to any blood specimen.

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Avoid contact. No special precautions are necessary. Use deionized or distilled water. When used in diagnostic procedures, instruments and equipment used in diagnostic procedures should be handled with the same precautions as applied to any blood specimen.

**PHYSICAL HAZARDS**

In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.

**CLINICAL APPLICATIONS**

Cortisol is the most abundant circulating steroid and the major glucocorticoid secreted by the adrenal cortex. Cortisol is a physiological effector of the hypothalamic-pituitary-adrenal axis. The free cortisol of the adrenal gland and the differential diagnosis of Addison's disease and Cushing's disease as well as adrenal hyperplasia and carcinoma.

Most circulating cortisol is bound to cortisol binding globulin or CBG and transcortin. The free cortisol, which is considered the active part of blood, is about 1–2%. In the absence of appreciable amounts of cortisol in serum, plasma or other specimens of human or animal origin.

**REAGENTS PROVIDED**

**1. Rabbit Anti-Cortisol Antibody-Coated Break-Apart Well Microplate** — Ready To Use

Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.

Stability: 12 months or as indicated on label.

**2. Cortisol-Horseradish Peroxidase (HRP) Conjugate** — Ready To Use

Contents: Cortisol-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 300 μL/vial

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

**3. plates**

Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

**4. REAGENTS AND EQUIPMENT NEEDED**

**5. Wash Buffer Concentrate** — Requires Preparation

Contents: One bottle containing buffer with a non-mercury preservative.

Volume: 50 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 μL of the wash buffer concentrate in 450 mL of water.

**6. Assay Buffer** — Ready To Use

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.

Volume: 15 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

**7. TMB Substrate** — Ready To Use

Contents: One bottle containing tetrathylammonium dihydrogen phosphate and 15 mL of a non-DMP or DMSO containing buffer.

Volume: 16 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

**8. Stopping Solution** — Ready To Use

Contents: One bottle containing 1M sulfuric acid.

Volume: 6 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

* Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.
1. Calculate the mean optical density of each calibrator.

2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.

3. Calculate the mean optical density of each unknown.

4. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.

5. Pipette 50 μL of each calibrator, control and specimen sample into corresponding labeled wells in duplicate.

6. Wash the wells 3 times with 300 μL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)

7. Pipette 150 μL of TMB substrate into each well at time intervals as in step 7.

8. Incubate on a plate shaker for 15–20 minutes at room temperature (or until calibrator A attains dark blue colour for desired OD).

9. Pipette 50 μL of stopping solution into each well at 20 minutes after addition of the stopping solution.

10. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean OD Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>2</td>
<td>23.7</td>
</tr>
<tr>
<td>3</td>
<td>51.8</td>
</tr>
</tbody>
</table>

11. Recovery

Spikeled samples were prepared by adding defined amounts of cortisol to three patient saliva samples (1:1). The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.28</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>4.14</td>
<td>3.64</td>
<td>113.7</td>
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<tr>
<td>3</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>8.03</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>6.05</td>
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</tr>
<tr>
<td>7</td>
<td>20.64</td>
<td>19.02</td>
<td>108.5</td>
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<tr>
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<td>52.20</td>
<td>54.02</td>
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<tr>
<td>9</td>
<td>6.98</td>
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<tr>
<td>12</td>
<td>19.00</td>
<td>18.89</td>
<td>102.8</td>
</tr>
</tbody>
</table>

12. Linearity

Three patient saliva samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.18</td>
<td></td>
<td>-</td>
</tr>
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<td>2</td>
<td>10.32</td>
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<td></td>
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<tr>
<td>12</td>
<td>8.57</td>
<td>7.48</td>
<td>87.3</td>
</tr>
</tbody>
</table>

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. Random male and female samples were taken in the early morning and had an absolute range of 5–21.6 ng/mL.