Suppression and Stimulation

PROTOCOLS FOR PERFORMING SUPPRESSION TESTS

I. INTRODUCTION

Described in this section are the sample requirements, patient preparation instructions, dosages of substances to be administered, protocols for collecting specimens, time intervals for collecting specimens etc for Suppression and Absorption tests. The performance and accuracy of these tests is directly dependent upon the protocols herein described. Therefore, it is extremely important that the integrity of these protocols be maintained. The best analytical procedures and techniques cannot compensate for poor patient preparation or deviation from the standard operating procedure. By not following the protocols and directions described will lead to inaccurate results at best or harm to the patient at worst.

The protocols for performing Suppression Tests outlined in this manual are considered by the Department of Pathology to be the standard procedures. If a physician wishes to modify these protocols to suit his own needs, he may do so at his discretion. However, if the physician does make some modifications in a particular protocol, it is in his and the patient?s best interest that the physician describes these changes as clearly as possible, in writing, so that the laboratory is well informed of the changes and is thus able to respond without confusion as to the physician?s wishes. The proper communication between the physician and the laboratory is of paramount importance if the test is to be conducted properly and in accordance with the physician?s wishes.

II. SUPPRESSION TESTS

A. Dexamethasone Adrenal Suppression Test ? Method I: Screening

   THIS PROCEDURE IS RECOMMENDED FOR CUSHING’S DISEASE

   Rationale:

   This test may be used as a rapid screening test to confirm suspicion of adrenal hyperplasia (i.e. Cushing’s disease) or neoplasia. This test does not differentiate between the two, as does the Method III protocol, but is a good rapid screening test as an indicator of abnormal cell proliferation of the adrenal cortex.

   Dexamethasone (9a-fluro-16a-methylprednisolone) is a potent steroid analogue, which inhibits the production of cortisol by the adrenal cortex in normal individuals through its action on the anterior pituitary and suppression of ACTH synthesis. The primary purpose of this test is to differentiate hyperplasia of the adrenal cortex (e.g. Cushing’s Disease). It may also be used to confirm a suspected hyperplasia or neoplasia of the adrenal cortex.

   In patients with either hyperplasia or neoplasia, low doses of dexamethasone do not inhibit cortisol production. With higher doses of dexamethasone, patients with normal or hyperplastic adrenal cortex, demonstrate suppression of cortisol production while those individuals with tumors of the adrenal cortex (i.e. adenoma or adenocarcinoma) are unaffected.

   Recommended Protocol:

   1. This test must be made by appointment with the laboratory and must be confirmed before 1500 p.m. on the day prior to the test.

   2. On Day 1, the laboratory will draw a plasma specimen at 0800 (baseline). This step is optional. Please refer to physician instructions.
3. At 2300 on Day 1, the patient is to take a 2 mg, oral dose of Dexamethasone.

4. On Day 2, the laboratory will draw a plasma specimen at 0800. The test is now complete.

In normal patients, cortisol concentration is suppressed to 2 ug/dl or less.

**B. B. Dexamethasone Suppression Test - Method II: Diagnosis of Depression**

**DO THIS METHOD FOR PSYCH UNIT AND FOR DEPRESSION**

**Rationale:**

Depression refers to either normal or pathological states of sadness or loss of normal mood. The word depression is commonly used to describe the normal mood state of unhappiness, which might occur in a wide variety of everyday situations such as disappointment, failure, or mourning. Depression is pathological when sadness is so intense and of such duration, or is accompanied by other specific signs and symptoms, that usual day-to-day functioning is impaired.

Depression is one of the more distressing and pervasive problems confronting society today. According to publications from the National Institute of Mental Health, between 8 and 15 million people in the United States suffer from a severe episode of affective (depressive or manic) illness each year, and at least 50% of these will have more than one attack in their lifetime. More than 25,000 people commit suicide each year in the United States, and 80% of these deaths can be attributed to depression.

Over the past 20 years, major advances in psychiatric treatment of depression have ensued largely from basic laboratory findings and from techniques developed to better recognize, diagnose, and select treatments for specific disorders. During the past decade, biological measures have provided methods to validate independently the existence of particular depressive subtypes and help identify which criterion signs and symptoms are indeed essential to making a particular diagnosis.

One of the most well-developed and clinically useful "biological markers" yet identified in psychiatry, namely the dexamethasone suppression test (DST), is a provocative pharmacologic challenge test which was developed originally as a screening test for patients with Cushing's syndrome. When hypersecretion of cortisol and other abnormalities in the hypothalamic-pituitary-adrenocortical axis were identified in patients with severe depression, Dr. Bernard Carroll at the University of Michigan applied the DST in this population as well. In the past several years, many reports have confirmed that depressed patients, especially those with endogenous depression or melancholia, have abnormal DST results. Recent investigations have focused on standardization of the test for psychiatric use and on its clinical applications.

**Recommended Protocol:**

1. This test must be made by appointment with the laboratory and must be confirmed before 1500 hours on the day prior to the test.

2. On Day 1, the laboratory will draw a serum or plasma specimen at 0800 a.m. (baseline).

3. At 2300 on Day 1, the patient is to take a one (1) mg, oral dose of Dexamethasone.

4. On Day 2, the laboratory will draw a serum or plasma specimen at 1600 and a second specimen at 2300.

5. The test is now complete. If either level is >3.2 ug/dL, the test is abnormal and demonstrates non-suppression.
C. Dexamethasone Adrenal Suppression Test?Method III: Cushing's Syndromes

PERFORM METHOD I IF DOCTOR IS NOT SURE

Rationale:

Dexamethasone (9a-fluro-16a-methylprednisolone) is a potent steroid analogue, which inhibits the production of cortisol by the adrenal cortex in normal individuals through its action on the anterior pituitary and suppression of ACTH synthesis. The primary purpose of this test is to differentiate hyperplasia of the adrenal cortex (e.g. Cushing's Disease). It may also be used to confirm a suspected hyperplasia or neoplasia of the adrenal cortex.

In patients with either hyperplasia or neoplasia, low doses of dexamethasone do not inhibit cortisol production. With higher doses of dexamethasone, patients with normal or hyperplastic adrenal cortex, demonstrate suppression of cortisol production while those individuals with tumors of the adrenal cortex (i.e. adenoma or adenocarcinoma) are unaffected.

Recommended Protocol

1. This test must be made by appointment with the laboratory and must be confirmed before 1500 (i.e. 3:00 PM) on the day prior to the test. The patient or the floor is to obtain the proper urine collection containers from the laboratory.

2. On Day 1 (0700), have the patient void his overnight urine and begin a 24-hour urine collection.

3. On Day 2, the first (baseline) 24-hour collection is complete. The patient is now to receive 0.5 mg of Dexamethasone, orally, every six hours for two days.

4. On Day 3, begin another 24-hour urine collection as previously described. The patient is to be maintained on 0.5 mg of Dexamethasone, orally, every six hours during the collection period.

5. On Day 4, the second 24-hour collection is complete. The patient is now to receive 2 mg of Dexamethasone every six hours for two days.

6. On Day 5, begin another 24-hour urine collection as previously described. The patient is to be maintained on 2 mg Dexamethasone, orally, every six hours during the collection period.

7. On Day 6, the third 24-hour urine collection is complete. The test is now complete and Dexamethasone is discontinued, unless otherwise informed by the laboratory. In some cases, the physician may wish to continue the test, giving 8 mg Dexamethasone over an additional two-day period and collecting another 24-hour urine.

8. All urine collections are to be brought to the laboratory immediately upon completion of each collection.

PROTOCOLS FOR PERFORMING STIMULATION TESTS

A. ACTH STIMULATION OF CORTISOL (Addison's)

THIS METHOD IS THE RECOMMENDED METHOD

Rationale:

Stimulation tests using ACTH are most often used in differentiating between Addison's Disease (primary adrenal cortical insufficiency), and hypopituitarism (lack of ACTH synthesis by the anterior pituitary gland). In Addison's Disease, the adrenal cortex is incapable of responding to ACTH stimulation and thus
providing cortisol. This insufficiency of the adrenal cortex may be caused by two major disease processes. One is a chronic granulatous process brought on by fibrocaseous tuberculosis or fungal infections of the adrenal gland. The second cause is an idiopathic process leading to bilateral adrenal cortical atrophy.

In primary adrenal insufficiency, the adrenal gland is not capable of responding to ACTH stimulation, even though the pituitary is secreting large quantities of the hormone as a result of low circulating cortisol levels. As a consequence, circulating ACTH levels are increased. In secondary adrenal insufficiency (hypopituitarism), the pituitary is incapable of secreting ACTH, as demanded by low circulating cortisol levels. In tertiary adrenal insufficiency, the hypothalamus is diseased and does not secrete adequate levels of Corticotropin Releasing Hormone to stimulate release of ACTH by the anterior pituitary. As a consequence, both plasma cortisol levels and circulating ACTH levels are low.

The ACTH Stimulation Test also has some diagnostic importance in differentiating hyperadrenalism, that is Cushing's syndrome (adrenal hyperplasia) from adenomas or adenocarcinomas.

The principle of this test is based on the feedback control mechanism normally existing between the release of pituitary ACTH and the circulating blood level of unbound cortisol. When the cortisol levels in blood are increased in the normal individual, the pituitary release of ACTH diminishes, with a consequent decrease of steroid output by the adrenal glands. In a diseased state, such as adrenal carcinoma, this feedback mechanism is completely deranged, and consequently the lack of suppression of adrenal activity, even after administration of large doses of cortisol, or its potent synthetic analogues, is a good indication of the autonomous functioning of the adrenal tumor. Under these circumstances, circulating levels of ACTH are low.

However, Method I test does not have the capabilities of being able to differentiate between primary and secondary adrenal insufficiency as does the more prolonged Method II. It does offer the advantage of offering a quick screen for adrenal insufficiency. If this test is negative for adrenal insufficiency, further work up for adrenal insufficiency is not necessary. If the test is positive for adrenal insufficiency, the more rigorous Method I may be used to determine the exact etiology of the disease.

Recommended Protocol:

1. Outpatients- This test must be scheduled for the PCC in able to coordinate efforts between the PCC, Pharmacy and Infusion Area.

   Inpatients may be done any day on 1st shift. Lab will need to coordinate with the nurse to ensure correct timing of the test.

2. On the day of the test, just prior to the administration of ACTH, the laboratory will draw a blood sample for a baseline plasma cortisol level.

3. Immediately upon drawing, the sample, 25 units of CORTROSYN (ACTH; Organon, INC) in 2 mL saline, is administered intramuscularly. Note the time of the injection.

4. At precisely 30 minutes following the administration of ACTH, a second blood specimen is drawn for plasma cortisol determination.

5. At precisely 60 minutes following the administration of ACTH, another blood specimen is drawn for plasma cortisol determination. The test is now complete.

   In normal individuals the administration of exogenous ACTH rapidly increases the secretion of cortisol by 2-3 times baseline within 60 minutes.
A peak cortisol concentration greater than 20 ug/dl within 60 minutes is considered normal.

Patients who are tested at times of stress may not show an incremental change in cortisol due to their adrenal output of cortisol is at the maximum level due to endogenous ACTH.

B. ACTH STIMULATION OF CORTISOL Method II

DO METHOD I IF DOCTOR IS UNSURE

Recommended Protocol:

1. This test must be made by appointment with the laboratory and must be confirmed before 1500 Hour on the day prior to the start of the test. Obtain the proper collection containers from the laboratory.

2. On Day 1 (7:00 a.m.), have the patient void his overnight urine and begin a 24-hour urine collection (baseline).

3. Inform Pharmacy sometime during the course of Day 1 that CORTROSYN (ACTH; Organon, Inc) is required for administration the next morning.

4. On Day 2, the first (baseline) 24-hour collection is complete. The patient is immediately to receive 25 International Units of Cortrosyn in 500 ml of saline over an 8-hour period. Start a second 24-hour urine collection at this time.

5. On Day 3, the second 24-hour urine collection is complete. The test is now complete.

6. Under certain circumstances (in cases of suspected adrenal insufficiency secondary to pituitary hypofunction), the physician may wish to continue the test an additional day. If the physician does leave such instructions, inform Pharmacy that ACTH is to be administered on two consecutive days. On Day 3, as soon as the second 24-hour urine collection is complete, the patient is to receive another I.V. drip of 25 International Units of ACTH in 500 ml of saline over an 8-hour period. A third 24-hour urine collection is started at this time, and is completed on the following day.

7. All urine collections are to be brought to the laboratory immediately upon completion of each collection for urinary free cortisol.

C. Thyrotropin Releasing Hormone (TRH) Stimulation Test

Rationale:

The thyroid gland, and the release of the active thyroid hormones thyroxine (T-4) and lyothyronine (T-3) is under the control of a complex metabolic negative feedback system, which involves the thyroid gland, the anterior pituitary, and the hypothalamus (HPT axis). The pituitary produces Thyroid Stimulating Hormone (TSH), the primary metabolic function of which is to act upon the thyroid gland to stimulate it to initiate the synthesis and release of the thyroid hormones thyroxine (T-4) and lyothyronine (T-3). The synthesis and secretion of TSH by the anterior pituitary is stimulated by thyroid releasing hormone (TRH), a tripeptide produced by the hypothalamus. Under normal metabolic conditions, TRH secretion by the hypothalamus is in direct response to the circulating levels of T-3 and/or T-4. Under normal conditions, elevated levels of circulating T-3 and/or T-4 suppress TRH, thus suppressing TSH, through the classical negative feedback mechanism. When circulating levels of T-3 and T-4 are low, hypothalamic production of TRH is induced thus raising circulating TRH levels, which in turn stimulate the anterior pituitary to produce TSH. The rise in circulating TSH has a stimulatory effect on the thyroid to synthesize and release T-4 and T-3. Failure at any point in the feedback regulation of the hypothalamic-anterior pituitary-thyroid axis, including the effects of nonthyroidal disease, drug therapy, etc., will result in either under production of thyroid hormones (i.e. hypothyroidism) or overproduction of thyroid hormones (i.e. hyperthyroidism).
In primary hypothyroidism, (i.e. failure of the thyroid gland itself), TSH levels arise above normal, from moderately elevated to highly elevated levels. In secondary and tertiary hypothyroidism, TSH levels remain within the reference range or may drop to very low levels (e.g. panhypopituitarism, Simmond’s disease). Decreased levels of both TSH and Total T-4 or Free T-4 are indicative of secondary or tertiary hypothyroidism. Stimulation of the pituitary by exogenous TRH (Thyrel TRH; Ferring Pharmaceuticals) is helpful in verifying secondary or tertiary hypothyroidism and elucidating whether the failure is pituitary or hypothalamic. If there is an increase in TSH, especially a delayed response with peak values at 60 minutes, the problem is hypothalamic. If there is little to no response, the problem lies in the pituitary, most likely panhypopituitarism (Simmond’s Disease).

Recommended Protocol:

1. This test must be ordered by appointment with the laboratory and must be confirmed 48 hours prior to the test. Also, inform the Pharmacy 48 hours prior to the test so that the TRH can be delivered. Indicate the time the test is to be performed on the requisition.

2. Nursing Service is to be notified on the day prior to the test so that a nurse is available to give the injection and monitor the patient during the course of the test.

3. Just prior to the administration of TRH, Laboratory personnel will draw a pre-injection blood sample for the baseline TSH level.

4. After the baseline specimen has been taken, the patient is prepared as follows:

   a. The patient is to void his/her urine to avoid involuntary urination during the course of the test.

   b. The patient is to assume a supine position on the bed in the drawing room (or own bed if on the floor) and remain in that position until the test is complete.

   c. Once the patient is comfortable, the nurse is to take and record the patient’s blood pressure.

   d. The TRH is to be delivered as a bolus (500 mg), I.V., over a period of 15 to 30 seconds.

   e. After the TRH is delivered, the patient is to be closely monitored and blood pressure readings are to be
taken every five minutes for the first 15 minutes of the test. If there is a significant change in blood pressure, monitor more frequently until it returns to baseline. Call a code if severe hypotension is observed.

f. Specimens are to be drawn at 30 and 60 minutes following the administration of the TRH.

5. At the end of the test, again monitor the patient's blood pressure. Have them get up slowly and walk around. If they notice no ill effects (e.g. dizziness, syncope, etc.), they may be discharged or remain in their room unobserved.

6. Interpretation of the laboratory results will appear on the report.

D. Corticotrophin Releasing Hormone (CRH) Stimulation Test

Rationale:

Circulating cortisol levels are regulated by a negative feedback mechanism. As cortisol levels rise, a sensory mechanism in the hypothalamus senses the increased levels. As a result, the hypothalamus shuts down the production of CRH. The function of CRH is to stimulate the production of ACTH by the anterior pituitary. As CRH levels fall, there is a decrease in the stimulation of ACTH production in the pituitary. The function of ACTH is to stimulate the synthesis of cortisol by the adrenal cortex. As ACTH levels fall, there is a decrease in stimulation of the adrenal cortex with a parallel decrease in cortisol synthesis. When cortisol decreases to a certain level, the process is reversed by increased release of CRH, which in turn increases the release of ACTH, which in turn increases the synthesis and release of cortisol. Failure at any point in the feedback regulation of the hypothalamic-anterior pituitary-adrenal cortical axis, including the effects of nonadrenal disease, drug therapy, etc., will result in either under production of cortisol as manifested in Addison's Disease or overproduction of cortisol as manifested by Cushing's Disease (pituitary) or Cushing's Syndrome (adrenal cortex).

The CRH Stimulation Test can be used both in the differentiation of Cushing's Disease (pituitary hyperfunction due to an ACTH secreting adenoma), or Cushing's Syndrome due to a tumor of the adrenal gland or a nonendocrine ACTH-producing tumor as well as differentiate primary adrenal insufficiency from secondary or tertiary adrenal insufficiency.

Addison's Disease, or adrenal insufficiency, is manifested by hyposecretion of cortisol as demonstrated by low levels of circulating cortisol. This is most frequently due to failure of the adrenal cortex as a result of an idiopathic process leading to bilateral adrenal cortical atrophy or some other etiology such as chronic granulomatous process brought on by fibrocaseous tuberculosis or fungal infections of the adrenal
gland. Secondary adrenal insufficiency is due to failure of the pituitary to secrete ACTH (panhypopituitarism, Simmond’s Disease) or a failure of the hypothalmus to secrete Corticotropin Releasing Hormone (CRH).

Recommended Protocol:

1. This test must be ordered by appointment with the laboratory and must be confirmed 48 hours prior to the test. Also, inform the Pharmacy 48 hours prior to the test so that the CRH (ACTHREL, Ferring Pharmaceutical) can be delivered on time. Indicate the time the test is to be performed on the requisition.

2. Nursing Service is to be notified on the day prior to the test so that a nurse is available to give the injection and monitor the patient, at least through the first 15 minutes.

3. Baseline specimens are to be drawn 15 minutes prior to beginning the test. The ACTH specimen is to be drawn in a pre-chilled EDTA (Lavender Top) tube and the Cortisol is to be drawn in a serum tube (Gold Top or Plain Red).

3. Just prior to the administration of CRH, Laboratory personnel will draw another set of tubes as described in Step 3. The ACTH and Cortisol levels on these two specimens are to be averaged to calculate the baseline level for each analyte.

4. After the baseline specimen has been taken, the patient is prepared as follows:
   a. The patient is to void his/her urine to avoid involuntary urination during the course of the test.
   b. The patient is to assume a sitting position in the drawing room and remain in that position for at least the first 15 minutes.
   c. Once the patient is comfortable, the nurse is to take and record the patient’s blood pressure.
   d. The CRH is to be delivered as a bolus (1 ug/kg of body weight), I.V., over a period of 30 to 60 seconds.
   e. After the CRH is delivered, the patient is to be closely monitored and blood pressure readings are to be taken every five minutes for the first 15 minutes of the test. If there is a significant change in blood
pressure, monitor more frequently until it returns to baseline. Call a code if severe hypotension is observed.

f. Specimens as described in Step 3., above, are to be drawn at 15, 30, and 60 minutes following the administration of the CRH.

5. At the end of 15 minutes, again monitor the patient’s blood pressure. Have them get up slowly and walk around. If they notice no ill effects (e.g. dizziness, syncope, etc.), they may be wait in the Out Patient Waiting Room or remain in their room unobserved.

6. Interpretation of the laboratory results will appear on the report.

E. Water Loading (Stimulation) Test (SIADH)

Rationale

The role of ADH in the regulation of water balance was discussed above as part of the explanation for the Water Deprivation Test described under Stimulation Tests in this Appendix. An inappropriate autonomous, sustained production of ADH (Syndrome of Inappropriate Antidiuretic Hormone Secretion; SIADH) will decrease elimination of water by the kidney thus increasing the water content of the blood, lower serum osmolality, and lower serum sodium levels. The etiology of SIADH is varied. SIADH may be the result of a malignancy such as small-cell carcinoma of the lung; the presence of acute or chronic disease of the central nervous system; pulmonary disorders; certain drugs (e.g. chlorpromide, vincristine, clofibrate, carbamazepine, nicotine, phenothiazines, and cyclophosphamide).

Recommended Protocol:

1. The patient is to eat a light breakfast on the morning of the test at around 0600 to 0700.

2. The Water Loading Test is to commence two hours after breakfast. Prior to beginning the test, blood is drawn (Li Heparin; Green PST or Plain Green) and a sodium and osmolality are run STAT. If the sodium is <130 mmol/L, the test is canceled.

3. At this time, the patient is to void his/her urine and the urine osmolality is determined.
4. If the sodium is $^{130}$mmol/L, proceed with the test by giving the patient a measured volume of water to drink, calculated as 20 mL of water/kg of body weight. Use the follow formula to calculate the correct volume:

$$\text{Volume water} = \frac{\text{weight in pounds}}{2.2} \times 20$$

Example: Vol. $= \frac{220}{2.2} \times 20 = 100 \times 20 = 2000$ mL

5. The patient must drink the entire volume of water over 15 - 30 minutes.

6. Once all of the water is consumed, the patient is to assume a recumbent position (lying down) during the course of the next four (4) hours.

7. At the end of each hour, the patient must get up and void his/her urine. The volume of each void is measured and recorded. An aliquot of the urine is taken for measuring the osmolality and sodium.

8. At the end of each hour, the patient must have his/her blood drawn (Li Heparin; Green PST or Plain Green) and a plasma osmolality is run along with the urine osmolality.

9. At the end of the second hour, a sample is drawn for ADH and Renin levels:

   Antidiuretic Hormone and Renin Levels: 6 EDTA (Lavender Top) Tubes (pre-chilled; centrifuge immediately using the refrigerated centrifuge; remove plasma; aliquot 3 mL into each of four plastic tubes; and freeze immediately. Send to SKBCL frozen).

10. At the end of four (4) hours, the test is complete and the patient may be released.

Interpretation

Patient?s with SIADH will yield the following results during the course of the test:

Total Urine Volume Collected (4 Hours): $<90\%$ of the volume administered.
Urine Osmolality: >250 mOsm/kg

Serum Osmolality: <270 mOsm/kg

Serum Osmolality/Urine Osmolality: ≤1.0

Serum Sodium: ≤130 mmol/L

Urine Sodium: 40 ? 80 mmol/L (Usually >60 mmol/L)

ADH: Elevated

Renin: Decreased

ADH and Renin Both Decreased: Primary renal defect (within the kidney) in renal water excretion.