



SAMPLE PROCEDURE

This “Sample Procedure” is not intended as a substitute for your facility’s Procedure Manual or reagent labeling, but rather as a model for your use in customizing for your laboratory’s needs.

Space has been provided within the document to allow you to update this template with information specific to your facility. It is suggested that a current version of the manufacturer’s directional insert be maintained as a supplement.

I. TEST NAME

OSOM[®] hCG Combo
CLIA Complexity: Waived for urine, Non-waived for serum

II. INTENDED USE

The OSOM[®] hCG Combo Test is a rapid immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in urine or serum as an aid in the early determination of pregnancy. This test is for professional use in physicians' offices and clinical laboratories.

III. SUMMARY AND EXPLANATION OF TEST

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by the developing placenta.⁽¹⁾ After fertilization, the concentration of hCG rapidly rises in both the urine and serum of pregnant women. The detection of hCG in these fluids is an excellent marker for confirming pregnancy.

The OSOM hCG Combo Test is a rapid test which can detect the presence of hCG in urine or serum. The test utilizes monoclonal and polyclonal antibodies to hCG.

IV. PRINCIPLE OF THE TEST

OSOM hCG Combo Test is a solid phase, sandwich-format immunochromatographic assay for the qualitative detection of hCG. Urine or serum is added to the sample well of the Test Device using the pipette provided. The sample migrates through reaction pads where hCG, if present in the sample, binds to a monoclonal anti-hCG dye conjugate. The sample then migrates across a membrane toward the results window, where the labeled hCG complex is captured at a test line region containing immobilized rabbit anti-hCG. Excess conjugate will flow past the test line region and be captured at a control line region containing an immobilized antibody directed against the anti-hCG dye conjugate (with or without hCG complexed to it).

The appearance of 2 black bands in the results window – one at “T: Test” and the other at “C: Control” – indicates the presence of hCG in the sample. If a detectable level of hCG is not present, only the control band will appear in the result window.

V. KIT CONTENTS AND STORAGE

25 OSOM hCG Combo Test Devices individually pouched, each containing a disposable pipette

- Membrane coated with rabbit polyclonal anti-alpha hCG
- Conjugate pad containing mouse monoclonal anti-beta hCG

1 Directional Insert

STORAGE CONDITIONS

Store OSOM hCG Combo Tests at room temperature, 15° to 30°C (59° to 86°F), out of direct sunlight. Test Devices are stable until the expiration date printed on the kit or foil pouch. **DO NOT FREEZE.**

If the control band does not appear when running the test, the Test Cassette or kit may have been stored or handled improperly or the foil pouch may not have been intact.

At this facility, kits are stored: _____.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

- Clock or timer
- Sample collection cups or tubes
- Positive and Negative Controls (Sekisui Diagnostics recommends the OSOM hCG Urine Control (Catalog # 134) and the OSOM hCG Serum Control (Catalog # 138)).

VII. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use. Federal law restricts this device to sale by or on the order of a physician.
- Do not use beyond the expiration date printed on the kit or foil pouch.
- The lot numbers may be different on the foil pouch and the kit.
- Use appropriate precautions for the collection, handling, and storage of specimens. All human blood products should be treated as potentially infectious and handled with good laboratory practices using appropriate precautions recommended in the Centers for Disease Control / National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1993.
- Dispose of all used Test Devices, pipettes and specimens in suitable biohazardous waste containers.
- Test Devices are stable in the unopened foil pouches until the expiration date. Do not remove the Test Device from the pouch until needed.

VIII. SPECIMEN COLLECTION & PREPARATION

Specimen Collection and Handling

No filtration or centrifugation of urine or serum specimens is required for testing with the OSOM hCG Combo Test.

This facility's procedure for patient preparation is: _____
_____.

This facility's procedure for sample labeling is: _____
_____.

Urine

Urine specimens may be collected in any clean, dry, plastic or glass container. For early determination of pregnancy, the first morning specimen of urine is recommended since it usually contains the highest concentration of hCG. Urine specimens may be stored at room temperature 15° to 30°C (59° to 86°F) for up to 8 hours, or refrigerated at 2° to 8°C (35° to 46°F) for up to 72 hours.

Serum

Serum specimens should be obtained aseptically in tubes without anticoagulants. Plasma specimens are not suitable and should not be used for the OSOM hCG Combo Test. Serum specimens may be stored at 2° to 8°C (35° to 46°F) for up to 48 hours before testing. However, if testing is delayed beyond 48 hours, the serum specimens (separated from the clot) should be frozen at -20°C (-4°F) or colder. Frozen specimens may be stored for up to 1 year. The frozen specimens should be thawed, mixed, and brought to room temperature 15° to 30°C (59° to 86°F) before testing.

This facility's procedure for transporting specimens is: _____

This facility's procedure for rejected specimens is: _____

IX. QUALITY CONTROL

Internal Quality Control

Several procedural controls are incorporated into each OSOM hCG Combo Test for routine quality checks.

The same labeled conjugate antibody results in the appearance of both the test and the control bands. The appearance of the control band in the results window is an internal positive procedural control which validates the following:

Test System: The appearance of the control band assures that the detection component of both the test line and control line is intact, that adequate volume was added and that adequate capillary migration of the sample has occurred. It also verifies proper assembly of the Test Device.

Operator: The appearance of the control band indicates that an adequate volume of fluid was added to the sample well for capillary migration to occur. If the control band does not appear at the read time, the test is invalid.

The clearing of the background in the results area may be documented as a negative procedural control. It also serves as an additional capillary flow control. At the read time, the background should appear white to light gray and not interfere with the reading of the test. The test is invalid if the background fails to clear and obscures the formation of a distinct control band.

If the Control band fails to appear with a repeat assay do not report patient results. Contact Sekisui Diagnostics for technical service: Tel 800-332-1042 (U.S. customers only)

External Quality Control

Sekisui Diagnostics recommends that external hCG controls are run with each new lot, and with each new untrained operator. The OSOM hCG Urine Control (Catalog Number 134) or the OSOM hCG Serum Control (Catalog Number 138) are designed for this purpose. Quality Control requirements should be established in accordance with local, state and federal regulations or accreditation requirements.

QC Testing Frequency and Documentation

For this facility, External QC is run: _____

Results of External QC and action(s) taken when control results are unacceptable are documented:

X. TEST PROCEDURE

- Patient specimens and control material must be brought to room temperature (15°-30°C; 59°-86°F) prior to testing.
 1. Remove the Test Device and the pipette from the pouch. Place the Device on a flat surface.
 2. Squeeze the bulb of the pipette and insert the barrel into the patient sample. Release the bulb and draw up enough sample **to fill the barrel to the line indicated on the pipette. Do not overfill.**
 3. Expel the **entire** contents of the barrel (135 µL) into the sample well of the Test Device. No drop counting required.
 4. Discard the pipette in a suitable biohazardous waste container.

For this facility, sample swabs, used test tubes and Test Sticks are disposed: _____

5. Read the results at 3 minutes for urine or 5 minutes for serum. Strong positive results may be observed sooner.
- **Results are invalid after the stated read time. The use of a timer is recommended.**
 - Procedural Notes:
 - If specimen has been stored refrigerated, allow it to warm to room temperature before use.
 - Several tests can be run at the same time. Use a new pipette with each new test to avoid contamination errors.

XI. INTERPRETATION OF RESULTS

POSITIVE

Two separate black or gray bands- one at "T: Test" and the other at "C: Control" are visible in the results window, indicating that the specimen contains detectable levels of hCG. While the intensity of the test band may vary with different specimens, the appearance of 2 distinct bands should be interpreted as a positive result.

NEGATIVE

If no band appears at “T” and a black or gray band is visible at the “C: Control” position the test can be considered negative, indicating that a detectable level of hCG is not present.

INVALID

If no band appears at the “C: Control” the test is invalid. The test is also invalid if incomplete or beaded bands appear at either the “T: Test” or “C: Control”. The test should be repeated using another Test Device.

Note: The test is valid if the control line appears by the stated read time, regardless of whether the sample has migrated all the way to the end of the sample window.

In the event this test becomes inoperable, this facility’s course of action for patient samples is:

XII. RESULT REPORTING

This facility’s procedure for patient result reporting is: _____

XIII. LIMITATIONS

- This assay is capable of detecting only whole molecule (intact) hCG, which is the predominant form of hCG in early pregnancy. It cannot detect the presence of hCG fragments or free subunits.
- In later term pregnancies (generally beyond the first trimester), occasional urine samples can contain very high levels of hCG fragments. Therefore for urine testing, the OSOM hCG Combo Test is most effective when used for the detection of pregnancy in its earlier stages.
- For diagnostic purposes, hCG test results should always be used in conjunction with other methods and in the context of the patient’s clinical information (e.g., medical history, symptoms, results of other tests, clinical impression, etc.). Ectopic pregnancy cannot be distinguished from normal pregnancy by hCG measurements alone.^(2,3)
- If the hCG level is inconsistent with, or unsupported by, clinical evidence, results should also be confirmed by an alternative hCG method. If a serum specimen is initially tested qualitatively, alternative methods may include the quantitative testing of serum or the qualitative testing of urine.⁽⁴⁾ The absence of urinary hCG may suggest a falsely elevated serum result. Additionally, results may be confirmed by performing serial dilutions of the sample as usually, but not always, samples that contain interfering substances exhibit nonlinear results when diluted. **Test results should be confirmed using a quantitative hCG assay prior to the performance of any critical medical procedure.**
- Interfering substances may falsely depress or falsely elevate results. These interfering substances may cause false results over the entire range of the assay, not just at low levels, and may indicate the presence of hCG when there is none. As with any immunochemical reaction, unknown interferences from medications or endogenous substances may affect results.

- Infrequently, hCG levels may appear consistently elevated and could be due to, but not limited to, the presence of the following: ⁽⁵⁻⁸⁾
 - heterophilic antibodies: Patients routinely exposed to animals or to animal serum products, can be prone to this interference and anomalous values may be observed
 - trophoblastic or nontrophoblastic neoplasms: abnormal physiological states that may falsely elevate hCG levels ^(9,10) This test should not be used in the diagnosis of these conditions.
 - nonspecific protein binding
 - hCG like substances
- Specimens from patients who have received preparations of Mouse Monoclonal Antibodies for diagnosis or therapy may contain Human Anti-Mouse Antibodies (HAMA). Such specimens may demonstrate either falsely elevated or falsely depressed results when tested with assay kits which employ Mouse Monoclonal Antibodies. ^(11,12)
- Because of the high degree of sensitivity of the assay, specimens tested as positive during the initial days after conception may later be negative due to natural termination of the pregnancy. Overall, natural termination occurs in 22% of clinically unrecognized pregnancies and 31% of other pregnancies. ⁽¹³⁾ In the presence of weakly positive results, it is good laboratory practice to sample and test again after 48 hours.
- If the test band appears very faint, it is recommended that a new sample be collected 48 hours later and tested using another OSOM[®] hCG Combo Test Device.
- Dilute urine specimens may not have representative levels of hCG.
- Detection of very low levels of hCG does not necessarily indicate pregnancy, ⁽⁵⁾ as low levels of hCG can occur in apparently healthy, nonpregnant subjects. ^(14,15) Additionally, post-menopausal specimens may elicit weak positive results due to low hCG levels unrelated to pregnancy. In a normal pregnancy, hCG values double approximately every 48 hours. ⁽¹⁶⁾
- Patients with very low levels of hCG should be sampled and tested again after 48 hours, or tested with an alternative method.
- Some antipsychotic agents/drugs are known to cause false positive results in pregnancy tests. ⁽¹⁷⁾

XIV. EXPECTED RESULTS

hCG is not normally detected in the urine and serum specimens of healthy men and non-pregnant women. In normal pregnancy, 20 mIU/mL hCG is reported to be present in both urine and serum 2 to 3 days before the first missed menstrual period ^(17,18). The levels of hCG continue to increase up to 200,000 mIU/mL at the end of the first trimester.

XV. CROSS REACTIVITY

The addition of luteinizing hormone (300 mIU/mL of LH), follicle stimulating hormone (1000 mIU/mL of FSH), or thyroid stimulating hormone (1000 μ IU/mL of TSH) to negative urine and serum specimens gives negative results in the OSOM hCG Combo Test.

XVI. INTERFERING SUBSTANCES

The following substances were added to urine and serum specimens containing 0 or 20 mIU/mL (urine) or 10 mIU/mL (serum) hCG. The substances at the concentrations listed below were not found to affect the performance of the test.

Urine					
Acetaminophen	20	mg/dL	Gentisic acid	20	mg/dL
Acetoacetic acid	2000	mg/dL	Glucose	2000	mg/dL
Acetyl salicylic acid	20	mg/dL	Hemoglobin	250	mg/dL
Amitriptyline	100	mg/dL	Human albumin	2000	mg/dL
Amphetamines	10	ug/mL	Ibuprofen	40	mg/dL
Ascorbic acid	20	mg/dL	Imipramine	100	mg/dL
Atropine	20	mg/dL	Lithium	3.5	mg/dL
Benzoyllecgonine	10	mg/dL	Mesoridazine	1	mg/dL
Bilirubin	2	mg/dL	Methadone	10	mg/dL
Caffeine	20	mg/dL	Morphine	6	ug/mL
Cannabinol	10	mg/dL	Nortriptyline	100	mg/dL
Chlorpromazine	5	mg/dL	Phenobarbital	15	mg/dL
Codeine	10	mg/dL	Phenylpropanolamine	20	mg/dL
Desipramine	20	mg/dL	Pregnanediol	1500	ug/dL
Diazepam	2	mg/dL	Progesterone	40	ng/mL
Ephedrine	20	mg/dL	Proteins	2000	mg/dL
Estradiol	25	ng/mL	Salicylic acid	20	mg/dL
Estriol	1	mg/dL	Tetracycline	20	mg/dL
Hydroxybutyrate	2000	mg/dL	Thioridazine	2	mg/dL
Ethanol	200	mg/dL			

Serum					
Amitriptyline	100	mg/dL	Lithium	3.5	mg/dL
Amphetamines	10	ug/mL	Mesoridazine	1	mg/dL
Benzoyllecgonine	10	mg/dL	Methadone	10	mg/dL
Bilirubin	30	mg/dL	Morphine	6	ug/ml
Cannabinol	10	mg/dL	Nortriptyline	100	mg/dL
Chlorpromazine	5	mg/dL	Phenobarbital	15	mg/dL
Codeine	10	mg/dL	Phenothiazine	2	mg/dL
Desipramine	20	mg/dL	Pregnanediol	1500	ug/ml
Diazepam	2	mg/dL	Progesterone	40	ng/mL
Estradiol	25	mg/dL	RF factor	40	IU/mL
Estriol	1	mg/dL	Tetracycline	20	mg/dL
Hemoglobin	500	mg/dL	Thioridazine	2	mg/dL
Ibuprofen	40	mg/dL	Triglycerides	2000	g/dL
Imipramine	100	mg/dL			

XVII. PERFORMANCE CHARACTERISTICS & POL STUDIES

Refer to directional insert – OSOM[®] hCG Combo Test

XVIII. REFERENCES

Refer to directional insert – OSOM[®] hCG Combo Test

XIX. ASSISTANCE

For technical assistance contact Sekisui Diagnostics Technical Service at (800) 332-1042.

OSOM[®] is a registered U.S. trademark of Sekisui Diagnostics, LLC.