KAISER PERMANENTE®
COLORADO LABORATORY

HCG IN SERUM AND URINE
OSOM® hCG Combo Test

PRINCIPLE

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 towards 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 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. The CLIA Complexity for this test is considered as waived for urine and moderate for serum.

SPECIMEN

Urine and serum are the recommended specimens. ***Plasma specimens are not suitable and should not be used.*** No filtration or centrifugation of urine or serum specimens is required for testing with the OSOM® hCG Combo Test.

Urine

Urine specimens may be collected in any, clean, dry, plastic, paper, 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°–30 °C, for up to 8 hours, or refrigerated at 2º to 8º C for up to 72 hours.

Serum

Serum specimens should be obtained aseptically in tubes without anticoagulants. Serum specimens may be stored 2º to 8º C 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 -20º C or colder. Frozen specimens may be stored for up to 1 year. The frozen specimens should be thawed, and mixed, and brought to room temperature, 15°– 30 °C, before testing.

EQUIPMENT AND MATERIALS

- OSOM® hCG Combo Test Devices individually pouched, each containing a disposable pipette.
- Clock or timer
- Sample collection cups or tubes
- Positive and negative controls

PREPARATION

- ✓ 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 test to avoid contamination errors.
- ✓ No filtration or centrifugation of urine or serum specimens is required for testing

QUALITY CONTROL

Internal Controls

Several controls are incorporated into each OSOM® hCG Combo Test for routine quality checks. These procedural controls are documented in the LIS for each sample as part of daily quality control. 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. Appearance of the control band should be documented as part of the daily quality control.

Procedural: The clearing of the background in the results area is 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.

External Controls

The external quality control for this procedure is recorded on the Preg QC log and performed:

1. With each new lot number
2. With each new Shipment
3. Once per month

SERUM – Osom HCG serum control set - positive & negative serum control Controls are stored at 2-8˚ C and stable until expiration date indicated on the bottles.

URINE - BIO-RAD qUAntify Control System, Negative urine control, Level 1 and
BIO-RAD qUAntify Control System**,** Positive urine control, Level 2

- The unopened urine controls are stable at **2 - 8° C (35° - 46°F)** up to the expiration date printed on the label. After opening, the controls are stable for 31 days at **2 to 25°C (40° -80° F).**
- Immediately upon opening a new set of controls:
    1. Write the "date opened" on each bottle
    2. Determine the expiration date, (31 days from "date opened"), and write the new "expiration date" on each bottle.

Positive and negative controls for both urine and serum must be run with each new lot and with each new shipment. Refer to the Test Procedure for interpretation of the control results. The control results must be recorded on the new lot or shipment external quality control log. If the controls do not work, repeat using new test packs or controls until the cause of the failure is identified.

New reagent lots must be checked against old reagent lots before being placed into service. Crosscheck by running the same lot number of external negative and positive control with both old and new reagent lots, ensuring that the same results are obtained with the new lot. Document results on the new lot or shipment external quality control log and indicate that the cross-check is OK.

TEST PROCEDURE

- DO NOT remove the Reaction Unit from the foil pouch until you are ready to perform the test.
- Use a new disposable dropper for each sample to avoid cross-contamination.
- Remove the Reaction Unit from the pouch. Label with patient's small accn label.
- Using the disposable pipette in the pouch, squeeze the bulb and draw up enough sample to fill the barrel to the line indicated on the pipette. Do not overfill
- Expel the entire contents of the barrel (135µL) into the sample well marked w/ an "S" of the Test Device. No drop counting required. Discard the pipette in a suitable biohazardous waste container.
- **Read result at 3 minutes for urine and 5 minutes for serum. Cartridges should never be held longer than 5 minutes for review at a later time.**
    Notes:
    1. Some positive result may appear sooner than stated time
    2. Results are invalid after the stated read time. The use of a timer is required
- See result interpretation below
- Pour off aliquot of urine specimen and hold for 1 week at 2 - 8°C

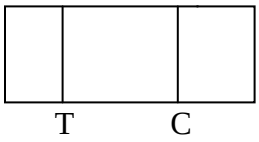
RESULT INTERPRETATION

Computer Codes: P = positive N= negative I = Inconclusive

(NOTE: A faint distinct line should be reported as positive)

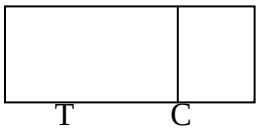
Positive

The two distinct black or gray bands-one at "T": Test and the other a "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 two distinct bands should be interpreted as a positive result.



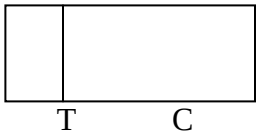
Negative

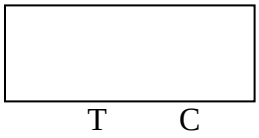
Only a black or gray band at the "C": Control position, indicating that a detectable level of hCG is not present



Invalid

If no band appears at the "C": Control or incomplete or beaded bands appear at the either the "T: Test or "C": Control the test is invalid. The test should be repeated using another Contrast ® II hCG 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 sample window.

REPORTING RESULTS

1. Carry the test cartridge to the computer
2. Login and navigate to ARE
3. Enter the ACCN and click Retrieve
4. Using the keyboard, choose the key that matches the result you want to enter:

P = Positive
I = Inconclusive
N = Negative

5. Proceed with entering the QC and Background checks using the keyboard.
6. Click on Perform.
7. Retrieve the ACCN.
8. Confirm the result. Click on Verify.

REFERENCE RANGES

Expected Values

hCG is not normally detected in the urine and serum specimens of healthy men and non-pregnant women. In normal pregnancy, 20 mlU/mL hCG is reported to be present in both urine and serum 2 to 3 days before the first missed menstrual period. The levels of hCG continue to increase up to 200.000mlU/mL at the end of the first trimester.

STORAGE and DISPOSAL REQUIREMENTS

- Store OSOM® hCG Combo Tests at room temperature, 15˚ to 30˚ C (59˚ - 86˚ F)
- Keep 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, the Test may have been stored or handled improperly or the foil pouch may not be intact.
- Dispose of used OSOM® hCG Combo Test Devices, pipette and specimens in suitable waste containers and in accordance with Kaiser Permanente Infection Control guidelines.

PERFORMANCE CHARACTERISTICS

Sensitivity

The OSOM® hCG Combo Test will detect down to 10mlU/mL hCG for serum and to 20mlU/mL for urine.
The expected sensitivity of urine samples at a read time of 3 minutes is 20 mIU/ml.
The expected sensitivity of serum samples at a read time of 5 minutes is 10 mIU/ml.
Note: Samples containing minute quantities of hCG (below 10mlU/mL) may develop very faint test bands.

Cross Reactivity

When luteinzing hormone (300mIU/mL of LH), follicle stimulating hormone (1000mIU/mL of FSH) or thyroid stimulating hormone (1000μIU/mL of TSH) was added to negative urine and serum specimens, **the results remained negative.**

Interfering Substances

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

<u>Urine</u>

Acetaminophen 20mg/dl Desipramine 20mg/dl Methadone 10mg/dl
Acetoacetic acid 2000 mg/dl Diazepam 2mg/dl Morphine 6 ug/ml
Acetyl salicylic acid 20mg/dl Ephedrine 20mg/dl Nortriptyline 100 mg/dl
Amitriptyline 100mg/dl Estradiol 25ng/ml Phenobarbital 15mg/dl
Amphetamines 10ug/ml Estriol 1mg/dl Phenylpropanolamine 20mg/dl
Ascorbic acid 20mg/dl Ethanol 200mg/dl Hydroxybutyrate 2000mg/dl
Atropine 20mg/dl Gentisic acid 20mg/dl Pregnanediol 1500 μg/dl
Benzoylecogonine 10mg/dl Glucose 2000mg/dl Progesterone 40ng/ml
Bilirubin 2mg/dl Hemoglobin 250 mg/dl Proteins 2000mg/dl

Caffeine 20mg/dl Human albumin 2000mg/dl Salicylic acid 20mg/dl
Cannabinol 10mg/dl Ibuprofen 40mg/dl Tetracycline 20mg/dl
Chlorpromazine 5mg/dl Imipramine 100mg/dl Thioridazine 2mg/dl
Codeine 10mg/dl Lithium 3.5mg/dl Mesoridazine 1mg/dl Methadone 10mg/dl

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

LIMITATIONS OF TESTING

1. This assay is capable of detecting only whole molecule (intact) hCG. It cannot detect the presence of free hCG subunits. Therefore, this test should only be used for the qualitative detection of human chorionic gonadotropin in urine or serum for the early determination of pregnancy.
2. 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. Ectopic pregnancy cannot be distinguished from normal pregnancy by hCG alone.
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. The absence of urinary hCG may suggest a falsely elevated serum result. Additionally, results may be confirmed by performing serial dilutions of the sample 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.
4. Interfering substances may falsely depress of 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 reactions, unknown interference from medications or endogenous substances may affect results.
5. Infrequently, hCG levels may appear consistently elevated and could be due to, but not limited to, the presence of the following:
    - 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. This test should not be used in the diagnosis of these conditions.

- nonspecific protein binding
- hCG like substances

6. Specimens from patients who have received preparations of Mouse Mono-clonal 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. These specimens should not be tested with the OSOM® hCG Combo Test Device.
7. 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. In the presence of weakly positive results, it is good laboratory practice to repeat testing after 48 hrs.
8. If the test band is indistinct (the line is broken) the test should be reported as inconclusive and it is recommended that a new sample be collected 48 hours later and tested using another OSOM® hCG Combo Test Device. If the line is distinct (the line is faint, but is unbroken) the test should resulted as positive.
9. Dilute urine specimens may not have representative levels of hCG.
10. Detection of very low levels of hCG does not rule out pregnancy, as low levels of hCG can occur in apparently healthy, nonpregnant subjects. 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. Patients with very low levels of hCG should be sampled and tested again after 48 hours, or tested with alternative method.
11. Some antipsychotic agents/drugs are known to cause false positive results in pregnancy tests.