

URINE PREGNANCY TEST LAB EXERCISE

Skill: 20 points

Objectives:

1. State the substance being detected in the urine pregnancy test.
2. State the principle of the urine pregnancy test.
3. State the samples of choice for a pregnancy test.
4. Describe the temperature and amount of time a urine sample may be stored for a pregnancy test.
5. Describe the quality control which must be performed on pregnancy test kits and the expected results.
6. Describe the course of action which must be taken if the controls do not give the expected results.
7. Define “false positive” as it relates to pregnancy testing and list two causes.
8. Define “false negative” as it relates to pregnancy testing and list two causes.
9. Describe the proper handling of specimens for urine pregnancy tests which cannot be tested right away.
10. List five (5) precautions of storage, usage and disposal of pregnancy test kits
11. Properly interpret the results of pregnancy test for Positive, Negative and Invalid test results
12. Perform urine pregnancy testing on two samples and evaluate the results for validity based on the results of the quality control.

Discussion

Human chorionic gonadotropin (hCG) is a hormone produced by the placenta shortly after implantation of the embryo into the uterine wall and functions to maintain the pregnancy during the early stages. Since hCG is present in the urine of pregnant women, it is an excellent marker for confirming pregnancy.

Principle of the Test

Modern pregnancy tests are based on Enzyme Linked Immunoassay (ELISA) methodology and use monoclonal antibodies selective for the *beta* area of the hCG molecule. The test method uses a membrane that contains all necessary antibodies and reagents to perform the test. **The principle** of the test is based on the sample migrating by capillary action along the membrane and encountering a monoclonal anti-hCG antibody. If the sample contains hCG, a complex is formed with the anti-hCG antibody, and continues to migrate along the membrane to the Test (T) line region, where the complex becomes bound to a “fixed” polyclonal anti-hCG antibody. The result will be the production of a colored marker if the level of hCG in the sample is 25 mIU/mL or greater. Unbound monoclonal anti-hCG migrates to the Control (C) line, where it binds to a different “fixed” polyclonal anti-hCG that will produce a color regardless of the amount of hCG in the sample. Color development at the Control line indicates that the test system is working properly.

The test membrane may be attached to a supporting strip or contained in a cassette. A test sample is introduced to the membrane by briefly dipping the strip in urine or urine is applied to a specific location on the test cassette. The manufacturer’s instructions for how much sample to add must be followed, as well as specific instructions on how long to wait before reading the test results.

As a procedural control, a colored line will always appear at the Control (C) line region. It confirms sufficient specimen volume has been added and that the test has been performed properly. The absence of the Control line invalidates the test and patient results cannot be released based on that test cassette. The test must be repeated using a fresh test cassette.

A clear background is an internal negative background control. A white to light colored background in the test area that does not interfere with reading the test result indicates that the test is working properly.

Specimen Collection and Preparation

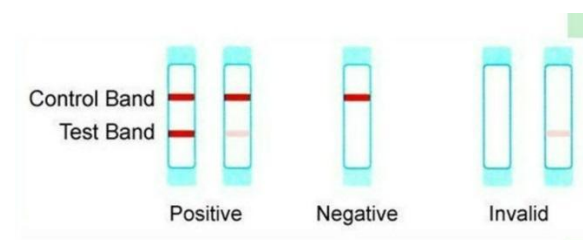
Collect at least one mL of urine in a clean, dry container with no preservatives. While specimens may be collected at any time of the day, the specimen of choice is the first morning sample as it has the highest concentration of hCG, and is less likely to produce false negative results.

If testing cannot be performed immediately, ***urine samples may be refrigerated and stored up to 72 hours prior to testing.*** Samples may be frozen for long term storage. If samples are refrigerated or frozen, they must be brought to room temperature before testing.

Quality Control

External control: Pregnancy test kits must be periodically tested with commercially prepared positive and negative control samples and must produce the expected results. If positive and negative controls do not give the expected reactions, the test kits in that lot number cannot be used for patient testing until the problem is resolved. If the controls are repeated and invalid results are still produced, the manufacturer must be notified and documentation of these actions must be performed. Patient testing must be performed using a different lot number or test type that does produce acceptable quality control.

Internal control: If the Control (C) line does not give the expected reaction (internal positive control) or the background does not remain colorless (internal negative control) on any sample, the test is invalid, and must be repeated. If the internal control fails to work properly a second time, patient testing must be performed using a different lot number or test methodology for which all controls work correctly.



Interpretation of the Test Results

Positive: Color change visible at both Control (C) line and Test (T) line, background remains colorless.

Negative: Color change visible only at Control (C) line and not at Test (T) line, background remains colorless.

Invalid: Uncertain results. No color change visible at Control (C) line, regardless of the absence or presence of color at Test (T) line, and/ or the background is darkly colored, not white.

Precautions

1. Test kits are stored at room temperature, and must be kept in their sealed pouch until testing.
2. Test kits should be kept away from direct sunlight, moisture and heat.
3. Test kits should not be used past the expiration date.
4. Various manufacturers will require different amounts of patient sample, and may require a different amount of time after starting the test to interpret test results. Always consult the specific package instructions for the test kit being used.
5. Dispose of all specimens and used cassettes as potentially biohazardous.
6. Refer to the specific package instructions for proper storage time and temperature of urine samples prior to testing.
7. Urine samples with visible precipitates should be filtered, centrifuged or allowed to settle so that clear aliquots can be used for testing.

Limitations of the Procedure

A “**false positive**” pregnancy test is one in which the pregnancy test is positive, but the **patient is not pregnant**. HCG has been found in patients with both gestational and non-gestational trophoblastic disease. HCG of trophoblastic neoplasm is similar to that found in pregnancy, and includes conditions such as choriocarcinoma and hydatidiform moles. Test methods using monoclonal and polyclonal antibodies result in fewer false positive tests. A physician must make a diagnosis of pregnancy using the results of the urine pregnancy test and other data.

A “**false negative**” pregnancy test is one in which **the patient is pregnant**, but the result of the pregnancy test is negative. False negative results are more common and are especially undesirable. They delay onset of proper care for women after being falsely reassured that they are not pregnant, who may then use potentially hazardous medications or have other medical procedures performed.

One cause of false negative pregnancy tests is inaccuracies in performing the test procedure. It is important to follow the specific manufacturer's instructions, especially the amount of urine to use and the amount of time you must wait before recording the absence or presence of color development. A second cause of a false negative test is that the sample was not a first morning urine and the patient is recently pregnant (hCG levels are too low). If pregnancy is suspected, the test should be repeated 48 hours later using a first morning urine sample.

A normal pregnancy cannot be distinguished from an ectopic pregnancy based on hCG levels alone. Also, a spontaneous miscarriage may cause confusion in interpreting test results. If negative or questionable results are obtained and pregnancy is suspected, the test should be repeated on a fresh first morning urine specimen at least 48 hours after initial testing. As early as 7 – 10 days after conception, hCG can be detected in urine.

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URINE PREGNANCY TEST STUDY QUESTIONS

Name: _____ Date: _____ Points: _____ 12

1. State the principle of the urine pregnancy test and the substance being detected. (2 points)

Substance detected:

Principle (What reacts with what to give a positive reaction):

2. State the specimen of choice for pregnancy testing and why it is recommended. (2 points)

Specimen of choice:

Advantage of this specimen:

3. Interpret the following pregnancy test results. (4 points)

Color development at the Control (C) line	Color Development at the Test (T) Line	Test Interpretation
Yes	Yes	
No	Yes	
Yes	No	
No	No	

4. Define “false positive” as it relates to pregnancy testing and list two causes. (2 points)

5. Define “false negative” as it relates to pregnancy testing and list two causes. (2 points)

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LAB EXERCISE: URINE PREGNANCY TEST

Name: _____ Date: _____ Points _____/28

Follow the instructions on the reagent package insert provided by the instructor or on the cassette pouch to properly perform the test. The instructor will demonstrate the procedure using the Negative and Positive Urine Controls. Record those results on the appropriate lines in the Quality Control Section.

Record **“Yes” or “No”** for the visual development of color at the Control and Test Lines on the cassette.

Record **“Clear” or “Color”** for the color of the Background.

Record **“Yes” or “No”** for the acceptability of results based on the control line and background color.

Record results as **“Positive” or “Negative” or “Invalid”** based on the Test line and Results Acceptable.

Pregnancy Cassette Lot Number _____ **Expiration Date** _____

QUALITY CONTROL	Color Development: YES or NO		Background: CLEAR or COLORED	Results Acceptable: YES or NO	Results: POSITIVE or NEGATIVE
	Control Line	Test Line			
Positive Control					
Lot Number:	yes	yes	clear	yes	positive
Expiration Date:					
Negative Control					
Lot Number:	yes	no	clear	yes	negative
Expiration Date:					

Patient Testing	Color Development: YES or NO		Background: CLEAR or COLORED	Results Acceptable: YES or NO	Results: POSITIVE or NEGATIVE
	Control Line	Test Line			
Patient Name:					
ID or DOB:					
Patient Name:					
ID or DOB:					

When finished, turn in this sheet, patient samples, and test cassettes to your instructor.

EVALUATION OF RESULTS: For Instructor Use Only

Award 1 point for each lot number, expiration date, patient name, identification number, color development, background, acceptability of results and test result. (7 points possible for each line)

Count off 0.5 points for each instance where symbols are used in place of Yes, No, Positive, Negative, or Invalid.

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