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| **SUBJECT: Urine Pregnancy Test, Sofia** |

**PRINCIPLE**

The Sofia hCG FIA is an immunofluorescence-based lateral flow assay intended for the qualitative detection of human Chorionic Gonadotropin (hCG) in urine specimens and is designed to aid early detection of pregnancy. The test uses a pair of monoclonal murine antibodies specific to the beta subunit of hCG to capture and detect hCG. The beta subunit was chosen to ensure specificity as the alpha subunit is nearly identical to the alpha subunit found in LH, FSH and TSH. To perform the test, a urine specimen is collected and dispensed into the Sample Well on the test Cassette. The Cassette is placed inside of Sofia for an automatically defined development time. Sofia then scans the test strip and analyzes the fluorescent signal, using method-specific algorithms. Sofia then displays the test result (Positive, Negative, or Invalid) on the screen, and optionally prints the results on an integrated printer.

**CLINCIAL SIGNIFICANCE**

Human Chorionic Gonadotropin is a hormone produced by the placenta shortly after implantation. Since hCG is present in the urine of pregnant women, it is an excellent marker for confirming pregnancy.

**SPECIMEN COLLECTION AND STORAGE**

Collect a urine specimen in a clean container. First morning specimens generally contain the highest

concentrations of hCG and are recommended for early detection of pregnancy. However, any urine sample is suitable for testing. If testing will not be performed immediately, the specimens may be kept at room temperature (15°C to 30°C) or refrigerated (2°C to 8°C) for up to 72 hours. Ensure that specimens are at room temperature and well mixed before beginning the assay.

**REAGENTS AND MATERIALS SUPPLIED**

50-Test Kit:

. Individually Packaged Cassettes (50): Mouse monoclonal anti-hCG antibodies

. Fixed Volume Pipettes

. Package Insert

. Quick Reference Instructions

. QC Card (located on kit box)

. Printer Paper

MATERIALS REQUIRED BUT NOT SUPPLIED IN KIT

. Specimen collection containers

. Sofia analyzer

. Calibration Cassette (supplied with the Sofia Installation Pack)

. External hCG urine controls (stored in refrigerator 2-8 C)

**WARNINGS AND PRECAUTIONS**

. For in vitro diagnostic use.

. Do not use the kit contents beyond the expiration date printed on the outside of the box.

. Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.1

. Use of Nitrile or Latex (or equivalent) gloves is recommended when handling patient samples.

. Dispose of containers and used contents in Biohazardous containers.

. Do not reuse the used Cassette or Fixed Volume Pipettes.

. The user should never open the foil pouch of the test Cassette exposing it to the ambient environment

until the Cassette is ready for immediate use.

. Discard and do not use any damaged Cassette.

. To obtain accurate results, the Package Insert instructions must be followed.

. The Sofia hCG FIA will automatically be forced into WALK AWAY Mode when inserted into Sofia. Do NOT allow the Test Cassette to develop on the bench or counter top prior to placing the Cassette into Sofia.

. The Calibration Cassette must be kept in the provided storage pouch between uses.

. Inadequate or inappropriate specimen collection, storage, and transport may yield false test results.

. Do not write on the barcode of the Cassette. This is used by Sofia to identify the type of test being run and to identify the individual Cassette so as to prevent a second read of the Cassette by the same Sofia.

. As the detection reagent is a fluorescent compound, no visible results will form on the test strip. Sofia

Analyzer must be used for result interpretation.

**KIT STORAGE AND STABILITY**

Store the kit at room temperature, 59°F to 86°F (15°C to 30°C), out of direct sunlight. Kit contents are stable until the expiration date printed on the outer box. Do not freeze.

**QUALITY CONTROL**

There are three types of Quality Control for Sofia and Cassette: Sofia Calibration Check procedure, built-in procedural control features, and External Controls.

**Sofia Calibration Check Procedure**

The Calibration Check Procedure should be performed every 30 days. Sofia can be set to remind the user to complete the Calibration Check Procedure. The Calibration Check is a required function that checks the Sofia optics and calculation systems using a specific Calibration Cassette. This Calibration Cassette is shipped with the Sofia Installation Pack. Refer to the Sofia User Manual for details regarding the Calibration Check Procedure. Important: Ensure that the Calibration Cassette is stored in the provided storage pouch between uses in order to protect it from exposure to light.

1. To check the calibration of Sofia, select “Calibration” from the Main Menu.

2. Following the prompts, insert the Calibration Cassette into Sofia and close the drawer. Sofia performs the Calibration Check automatically with no user input required.

3. Sofia indicates when the Calibration Check is completed. Select OK to return to the Main Menu.

NOTE: If the calibration cannot be completed successfully, notify the on-site Supervisor or contact Quidel Technical Support for assistance from 7:00 a.m. to 5:00 p.m. Pacific Time at 800.874.1517

 **Built-in Procedural Control**

The Sofia hCG FIA test strip contains a built-in procedural control feature. Each time a test is run, Sofia scans this part of the test strip, and the result is displayed on the Sofia screen as “valid” or “invalid.” These built-in procedural control results will be documented daily for the first sample tested each day. This documentation is also automatically logged into Sofia with each test result.

A valid result obtained with this procedural control demonstrates that the test flowed correctly and the

functional integrity of the Cassette was maintained. The procedural control is interpreted by Sofia

simultaneously with the end of the assay. If the test does not flow correctly, Sofia will indicate that the result is invalid. Should this occur, review the procedure and repeat the test with a new test Cassette.

**External Quality Control**

External controls are used to demonstrate that the reagents and assay procedure perform properly. External controls will be tested when opening each new box of kits and every 31 days thereafter. To test external controls:

1. Remove controls from refrigerator & allow to warm to room temperature
2. The user must first select Run QC on the main Menu of Sofia.
3. When prompted, scan the QC card (located on kit box). This card provides information specific to the kit lot, including lot number and expiration date.
4. Sofia will then prompt the user to run the external controls. The positive control test must be run prior to the negative control test.
5. When preparing the test Cassettes, ensure 120 µL of the well mixed control solution is added to the Cassette sample well.
6. When the QC test is complete, each result will be displayed as “Passed” or “Failed” for the positive control and the negative control.
7. After successful control testing mark test kit box lid with date & initials indicating acceptable QC and ready to patient testing use. Save printed QC results on Sofia log sheet & record in LIS.

Do not perform patient tests or report patient test results if either of the QC test results fail. Repeat the test or contact Supervisor or Quidel Technical Support before testing patient specimens.

**TEST PROCEDURE**

DO NOT open the foil pouch containing the test Cassette until ready to test the specimen. Place test

cassette on a clean and level surface. Urine specimens must be at room temperature and well mixed before beginning the assay. Check expiration date on each individual test package or outer box before using. Do not use any test past the expiration date on the label.

**Using Sofia: Forced WALK AWAY Mode**

The Sofia hCG FIA will automatically be run in the WALK AWAY Mode in Sofia. Once a prepared Test Cassette is inserted into the Sofia drawer and the drawer closed (Step 7 of the Test Procedure), Sofia will be forced into WALK AWAY Mode***. Do not allow the Cassette to develop on the bench or counter top prior to placing the Cassette into Sofia.***

*Set Up Sofia*

1. Select “Run Test” from the main menu on Sofia.

2. Input User ID using the barcode scanner or manually enter the data using the key pad.

3. Input Patient ID or Order # using the barcode scanner or manually enter the data using the key pad.

4. Press Start Test and the Sofia drawer will automatically open.

*Prepare the test Cassette*

5. Fill the provided Fixed Volume Pipette (120 µL) with the patient sample from the specimen collection container. To fill the Fixed Volume Pipette with the patient sample:

a) FIRMLY squeeze the top bulb.

b) Still squeezing, place the Pipette tip into the patient sample.

c) With the Pipette tip still in the patient sample, release pressure on bulb to fill the Pipette.

6. Firmly squeeze the top bulb to empty the contents of the Fixed Volume Pipette into the Cassette sample well. Extra liquid in the overflow bulb is OK. Do not attempt to dispense it too. NOTE: The Fixed Volume Pipette is designed to collect and dispense the correct amount of liquid sample. Discard the pipette in biohazard waste. NOTE: Proceed promptly to the next step.

*Insert the Cassette into Sofia*

7. Carefully lift the test Cassette and insert the Cassette into Sofia. Gently close the drawer.

8. Sofia will start automatically and display the progress. The test results will be displayed on the screen in approximately 3 minutes.

9. Discard used cassette in biohazard waste after completion of test.

**INTERPRETATION OF RESULTS**

When the test is complete, the results will be displayed on the Sofia screen. The results will be automatically printed on the integrated printer. Test Lines, which are fluorescent, cannot be seen with the naked eye. The Sofia screen will display results for the procedural control as being “valid” or “invalid,” and will provide a positive or negative result for the detection of hCG. If the procedural control is “invalid,” retest the patient’s sample with a new Cassette.

Positive Results: Negative Results:

 

Invalid Results:



**EXPECTED VALUES**

Specimens containing as low as 20 mIU/mL hCG for urine will yield positive results when tested with Sofia hCG FIA. For some patients, an hCG level of 25 mIU/mL can be detected as early as 2-3 days before expected menses. 7

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| REFERENCE RANGE: |
| NEGATIVE **-** Specimens from healthy men and healthy nonpregnant women should not contain detectable levels of hCG. Other conditions mayexist which cause positive results. See Limitations section.  |
|  REPORTING RESULTS: Positive or Negative in the LIS under correct patient order. |
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| **PROCEDURAL NOTES:**BACKUP FOR INOPERABLE SYSTEM |
| Send properly labeled specimen to main lab. Order in LIS. |

**LIMITATIONS**

. The contents of this kit are to be used for the qualitative detection of hCG in urine specimens.

. While pregnancy is the most likely reason for the presence of hCG in urine, elevated hCG concentrations unrelated to pregnancy have been reported in some patients.2,3 Conditions other than normal pregnancy may be associated with detectable hCG, including, for example, ectopic pregnancy or molar pregnancy.4

. hCG may remain detectable for a few days to several weeks after delivery, abortion, natural termination or hCG injections.5,6

. Abnormal pregnancies cannot be diagnosed by qualitative hCG results. The above conditions should be

ruled out when diagnosing pregnancy.

. If a negative result is obtained but pregnancy is suspected, hCG levels may be too low or urine may be too dilute for detection. Another specimen should be collected after 48-72 hours and tested. If waiting 48 hours is not medically advisable, the test result should be confirmed with a more sensitive quantitative hCG test.

. A negative test result may occur if the level of hCG in a sample is below the clinical threshold of 20 mIU/mL of the test or if the sample was collected or transported improperly.

. Failure to follow the Test Procedure may adversely affect test performance and/or invalidate the test

result.

. Test results must be evaluated in conjunction with other clinical data available to the physician.

Analytical Specificity

The following substances, hormones, and microorganisms listed in the Table were tested and did not show interference or cross-reactivity in the assay at the quantities indicated.

Subtances Concentration

Acetaminophen 20 mg/dL

Acetoacetic Acid 1,600 mg/dL

Acetylsalicylic Acid 20 mg/dL

Ampicillin 2 mg/dL

Ascorbic Acid 20 mg/dL

Atropine 20 mg/dL

ß-Hydroxybutyrate 2,000 mg/dL

Benzoylecgonine 8 mg/dL

Bovine Serum 10 mg/dL

Caffeine 20 mg/dL

Cannabinol 10 mg/dL

Cellulose 500 mg/dL

Citric Acid 500 mg/dL

Clomiphene 100 mg/dL

Cow’s Milk 9 mg/dL

DMSO 0.90%

EDTA 80 mg/dL

Ephedrine 18 mg/dL

Ethanol 0.80%

Gentisic Acid 20 mg/dL

Methanol 0.90%

Phenothiazine 20 mg/dL

Phenylpropanolamine 20 mg/dL

Salicylic Acid 20 mg/dL

Tetracycline 20 mg/dL

Theophylline 20 mg/mL

Uric Acid 18 mg/dL

Urine substances

Albumin (serum) 2,000 mg/dL

Bilirubin 1 mg/dL

Glucose 2,000 mg/dL

Haptoglobin 1 mg/dL

Hemoglobin 1 mg/dL

Human Anti-Mouse Antibodies 2.85 ng/mL

Myoglobin 1 mg/dL

Rheumatoid Factor 1.08 IU/mL

Serum (negative human) 1%

Urine Peroxide 10 mg/dL

Urine pH 5–9

Urine Specific Gravity 1.005-1.037

Hormones

hLH 450 mIU/mL

hFSH 900 mIU/mL

hTSH 1,000 mIU/mL

Estriol 17-beta 28,000 µg/dL

Pregnanediol glucuronide 45,000 µg/dL

ß-core fragment, hCG 5.1 x 105 pmol/L

Microorganisms

E. coli 2.61 x 108 CFU/mL

Streptococcus agalactiae (Group B) 2.50 x 107 CFU/mL

Chlamydia trachomatis 1.00 x 107 IFU/mL

Candida albicans 1.07 x 107 CFU/mL

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