PROCEDURE
Corewell Health East - Rapid fFN (Fetal Fibronectin) TLi-IQ System - Royal Oak

This Procedure is Applicable to the following Corewell Health sites:
Corewell Health William Beaumont University Hospital (Royal Oak)

Applicability Limited to: N/A

Reference #: 34307

Version #: 2

Effective Date: 12/03/2025

Functional Area: Clinical Operations, Laboratory

Lab Department Area: Lab - Chemistry

1. PURPOSE AND PRINCIPLE:

A. Detection of fFN in cervicovaginal secretions is associated with preterm delivery in symptomatic pregnant women between 24 weeks and 34 weeks, 6 days gestation (1-6), and in asymptomatic pregnant women between 22 weeks and 30 weeks, 6 days gestation (7-10).

B. Rapid fFN is a qualitative test for the detection of fetal fibronectin and consists of the Rapid fFN Cassette used in the TLiIQ analyzer. The Rapid fFN Cassette is a lateral flow, solid-phase immunosorbent device designed to qualitatively detect fetal fibronectin in cervicovaginal secretions collected with the Hologic Biomedical Specimen Collection Kit. The cervicovaginal specimen is extracted into a buffer and filtered with a plunger filter. Filtered sample (200 µL) is dispensed onto the sample application well of the Rapid fFN Cassette. The sample flows from an absorbent pad across a nitrocellulose membrane via capillary action through a reaction zone containing murine monoclonal anti-fFN antibody conjugated to blue microspheres (conjugate); the monoclonal antibody is FDC-6, specific for fFN (1). The conjugate, embedded in the membrane, is mobilized by the flow of the sample. The sample then flows through a zone containing goat polyclonal anti-human fibronectin antibody which captures the fibronectin-conjugate complexes. The remaining sample flows through a zone containing goat polyclonal anti-mouse IgG antibody which captures unbound conjugate, resulting in a control line. After 20 minutes of reaction time, the intensities of the test line and control line are interpreted with the TLiIQ analyzer.

2. SPECIMEN COLLECTION AND HANDLING:

A. Specimen must be obtained using the Hologic Biomedical Specimen Collection Kit.
   1. Specimen Storage and Transport
      a. Specimens that are not tested within eight (8) hours of collection must be stored refrigerated at 2 to 8C and assayed within three (3) days of collection to avoid degradation of fetal fibronectin. Store appropriately and avoid extreme temperatures.
      b. Transport specimens at 2 to 25C. It is ideal to transport specimens at 2 to 8C. Exposure of the specimen to heat for even short periods of time can be detrimental. It is best to keep the specimen cool whenever possible.
   2. Unacceptable Specimens
      a. Specimens collected in or by any sample device other than the Adeza Biomedical Specimen Collection Kit.

b. Specimens with insufficient volume for testing.
c. Specimens received > 3 days after the sampling date.
d. Specimens received at temperatures >25C.

3. REAGENTS/SUPPLIES:

A. Rapid fFN for the TLiIQ System, analyzer and printer.
B. Rapid fFN Cassettes: 26 cassettes containing all necessary reagents (murine monoclonal anti-fFN antibody conjugated to blue microspheres, goat polyclonal anti-human fibronectin antibody, and goat polyclonal anti-mouse IgG antibody) dried onto membranes. Each cassette contains a desiccant and is sealed in a foil pouch. The shelf life of the Rapid fFN Cassette is 18 months from the date of manufacture. Unopened cassettes may be used until the expiration date printed on the foil pouch and the box containing the pouched cassettes. Once the foil pouch is opened, the Rapid fFN Cassette should be used immediately. The Rapid fFN Cassette should be stored at room temperature (15to 30C).
C. TLiIQ QCette. Store at room temperature (15 to 30C).
D. Rapid fFN Control Kit.
   1. Rapid fFN Positive Control: 1.2 mL (0.090-0.100 µg/mL fFN)
   2. Rapid fFN Negative Control: 1.2 mL (<0.020 µg/mL fFN)
      a. The shelf life of the Rapid fFN Control Kit is one year from the date of manufacture. Unopened controls may be used until the expiration date printed on the bottle.
      b. Once opened, expiration is 6 months from opened date.
      c. Control Kit should be stored refrigerated (2to 8C).
      d. Controls should be discarded if they are cloudy or discolored.
E. 200 µL Micropipettor and appropriate tips.
F. Labels for printer.

4. PRECAUTIONS:

A. Do not use glass tubes or glass pipettes, as fetal fibronectin binds to glass. Tubes and pipettes of polypropylene or polyethylene are acceptable.
B. Test results may not be interpreted visually and must be based on the use of the TLiIQ analyzer.
C. Do not mix materials from different kit lots.
D. Do not use cassettes or controls past their expiration dates.
E. Do not use controls if they are cloudy or discolored.
F. Handle cassettes with care: do not touch, scratch, or compress membrane materials in the Rapid fFN Cassette.
G. Avoid cross-contamination of reagents. When using a micropipettor, change tips between each control and patient sample. Recap controls tightly with the correct color-coded caps.
H. Source material used to prepare the controls is of human origin. The donors were tested and found to be negative for HIV1, HIV2, HCV antibody and hepatitis B surface antigen (HBsAg) using established methods. No known test method can offer total assurance that HIV, hepatitis C virus, hepatitis B virus or other infectious agents are absent. Handle the controls and all patient specimens as if potentially infectious.
I. Labels (e.g., bar code labels) can be placed on the thumb grip area of the cassette. Do not place labels on an area of the cassette that will be inserted into the TLIIQ analyzer.

5. CALIBRATION:

Calibration must be set with each new cassette lot. (See procedure section).

6. QUALITY CONTROL (QC):

See Automated Chemistry IQCP Fetal Fibronectin for further requirements in the Individualized Quality Control Plan for this test.

A. Q-Cette: This QC is performed once every 24 hours on day shift. The TLiIQ QCette™ is a quality control device used to verify that the TLiIQ™ Analyzer performs within specification. The TLiIQ QCette is a Rapid fFN Cassette replica containing a membrane with printed test and control lines,

which is read by the TLiIQ analyzer. Three different levels of response are measured with this QC device.

1. High Level: The blue line at the procedural control position, which is in the high positive range, must be above a minimum threshold value for QC to pass.
2. Low Level: The blue printed line at the test line position is in the cutoff range. This line is measured and compared with a value established during instrument setup and must be within 5% of that value for QC to pass.
3. Negative: The white space between the blue lines is measured and should always be in the negative range for QC to pass.
4. The result should be SYSTEM PASS. A label will print with QC results. Attach this printout to the FF worksheet. A FAIL or INVALID result should be repeated.
5. Note on Maintenance Log when completed.

B. Liquid QC Negative and Positive
   1. The frequency of use of the liquid controls is with each new lot or new shipment of Rapid fFN cassettes, with each newly trained operator, and at least monthly. The Rapid fFN Control Kit contains two liquid controls: one Rapid fFN Positive Control and one Rapid fFN Negative Control for use in monitoring the performance of the Rapid fFN Cassette. The control results should PASS. Results will be displayed and printed in 23 minutes each.
   2. A FAIL or INVALID result should be repeated. To ensure accurate and reliable test results, performance testing should be done only with the controls from the Rapid fFN Control Kit.
   3. Note on Maintenance Log and in Laboratory's QC program when completed.

C. Internal Controls
   1. These controls are part of the TLiIQ System and are performed automatically with every test. These internal controls check for 4 items:
      a. A threshold level of signal at the procedural control position.
      b. Proper sample flow across the Rapid fFN Cassette.
      c. Absence of conjugate aggregation (Cassette: Pass/Fail).
      d. Proper function of analyzer hardware (Analyzer: Pass/Fail).
   2. These QC results will print with the patient results. Attach this printout to the FF worksheet

7. PROCEDURE:

A. Calibration: Setting Calibration for a new Rapid fFN Cassette Lot. Must be set with every New Lot #.
   1. Select Option 2 (SET CALIBRATION) from the Main Menu of the analyzer.
   2. Enter the User ID and press ENTER.
   3. Enter the cassette lot number. All letters and numbers must be entered. Enter the number that corresponds to the correct letter and use the up and down arrows to scroll to the correct letter. Press ENTER when completed.
   4. Enter the Calibration Code provided on each box of cassettes. The calibration code consists of two letters followed by two numbers. If the code is not entered correctly or does not match the cassette lot number that has been entered, the analyzer will request that the code be re-entered. This calibration code will be used for all cassettes of that lot number.
   5. When calibration is complete, the result will be displayed on the analyzer screen and the printed label as SYSTEM CALIBRATED. Press ESC to return to the Main Menu.
   6. Liquid QC must be performed after each calibration. (See Quality Control section).
   7. Save the calibration and QC printouts in the Fetal Fibronectin binder.

B. Performing Daily Analyzer Quality Control
   1. Perform once every 24 hours.
   2. Select Option 3 (DAILY QC) from the Main Menu of the analyzer.
   3. Enter the User ID and press ENTER.
   4. Enter the QCette Serial Number and press ENTER.
   5. Insert the QCette and press ENTER.
   6. When analysis is complete, the result will be displayed on the analyzer screen and the printed label as SYSTEM PASS. A FAIL or INVALID result should be repeated. Results will print in 23 minutes.

7. Save the printed QC results in the Fetal Fibronectin binder.
8. NOTE: Choose ESC to go back to Main Menu at anytime.

C. Specimen Preparation
1. Allow all Specimen Transport Tubes to come to room temperature before testing.
2. Gently mix, by inversion, the Specimen Transport Tube prior to removing the swab.
3. Open the Specimen Transport Tube cap and swab assembly. The swab shaft should be seated in the cap. Express as much liquid as possible from the swab by rolling the tip against the inside of the tube. Dispose of the used swab in a manner consistent with handling of bio-hazardous materials.
4. Test patient samples on the TLiIQ analyzer using internal incubation mode.
   a. Incubation Modes
      Note: The TLiIQ analyzer has two incubation modes for testing samples: INTERNAL and EXTERNAL. The incubation mode refers to the timing of the incubation process and the initiation of the cassette analysis. In the internal mode, the analyzer times the incubation and starts the analysis. In the external mode, the user will be responsible for timing the incubation and for starting the analysis.
      i. Internal Incubation Mode: Use this mode for testing patient samples and Quality Control.
   a. Turn on the TLiIQ analyzer and the attached printer. Select option 6 from the Main Menu for CHANGE SETUP by pressing the down arrow once and then entering the number 6.
   b. Select option 3 from the Setup Menu for INCUBATION MODE. Then select option 1 for INTERNAL. Press ESC to return to Main Menu.
      1) External Incubation Mode; Only to be used with Manager or Medical Technologist (MT) Lead Approval.
   a. Turn on the TLiIQ analyzer and the attached printer. Select option 6 from the Main Menu for CHANGE SETUP by pressing the down arrow once and then entering the number 6.
   b. Select option 3 from the Setup Menu for INCUBATION MODE. Then select option 2 for EXTERNAL. Press ESC to return to Main Menu

D. Procedure for Patient and Liquid QC testing
1. From Main Menu Choose #1 Test Patient or # 3 for Liquid Controls
2. Enter User ID and press ENTER
3. Enter the cassette lot number (printed on the foil pouch and the box containing the pouched cassettes). The first three characters of the lot number will be displayed because the calibration has been set for a specific cassette lot number. Enter the remaining numbers of the lot number and press ENTER.
4. Enter control lot numbers in the same manner.
5. Remove one Rapid fFN Cassette from the foil pouch being careful not to touch the sample well or the reaction area. When prompted, insert the cassette into the analyzer. Push it in until it clicks and press ENTER.
6. Invert patient sample (or liquid control) to mix before testing. Using a calibrated pipette, add 200 µL of patient sample (or liquid control) to sample well and immediately press ENTER.
7. The analyzer times the 20 minute incubation of the cassette and starts the analysis. When the analysis is complete, the result will be displayed on the analyzer screen and the printed label. Press ESC to return to the Main Menu.
8. Remove the cassette from the analyzer.
9. A FAIL or INVALID control result must be repeated before any further patient testing can be done.
10. A FAIL or INVALID on a patient must be repeated.
    a. If repeat is FAIL or INVALID, call instrument hotline for an instrument replacement.
    b. Corewell Troy Chemistry is the backup for this test. Make arrangements for test to be transported to Corewell Troy.
11. Attach the label which displays patient results to the worksheet and file in the Fetal Fibronectin binder.

12. Results of patient, Negative or Positive will be entered into the Laboratory Information System (LIS).
13. Results of Liquid QC will be entered into the LIS.
14. Choose ESC to go back to Main Menu anytime.
15. If you are using the last cartridge in the box, check the storeroom for another box. If it is a new lot #, immediately perform a Set Calibration and run Liquid QC

8. INTERPRETATIONS OF RESULTS:

A. Rapid fFN is a qualitative test. The result is positive if the value derived from the patient sample is greater than or equal to the reference calibration value specified by the calibration code. The result is negative if the value derived from the patient sample is less than the reference calibration value specified by the calibration code. The test must pass Analyzer and Cassette internal controls to be valid. The result is invalid if the test does not meet internal quality controls.

B. If the assay does not meet internal acceptance criteria for a valid test, (FAIL or INVALID) retest with 200 µL of additional sample, if available, on a new Rapid fFN Cassette. If the problem is not corrected, contact Adeza Biomedical for technical assistance.

C. The Rapid fFN result should not be interpreted as absolute evidence for the presence or absence of a process that will result in delivery in less than or equal to 7 or 14 days from specimen collection. A positive fFN result may be observed for patients who have experienced cervical disruption caused by, but not limited to, events such as sexual intercourse, digital cervical examination, or vaginal probe ultrasound. The Rapid fFN result should always be used in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures such as cervical examination, cervical microbiological culture, assessment of uterine activity, and evaluation of other risk factors.

9. LIMITATIONS AND INTERFERING SUBSTANCES:

A. Assay interference from the following components has not been ruled out: douches, white blood cells, red blood cells, bacteria and bilirubin.

B. The presence of infections has not been ruled out as a confounding factor to risk of preterm delivery.

C. Assay interference from semen has not been ruled out. Specimens should not be collected less than 24 hours after intercourse. However, even when a patient reports having had intercourse in the previous 24 hours. A negative fFN test result is valid.

D. Manipulation of the cervix may lead to false positive results. Specimens should be obtained prior to digital examination or manipulation of the cervix.

E. Care must be taken not to contaminate the swab or cervicovaginal secretions with lubricants, soaps, disinfectants, or creams (e.g. K-Y Jelly lubricant, Betadine® disinfectant, Monistat® cream, hexachlorophene.) These substances may interfere with absorption of the specimen by the swab or with the antibody-antigen reaction of the Rapid fFN test.

F. Patients with suspected or known placental abruption, placenta previa, or moderate or gross vaginal bleeding should not be tested for fFN.

10. REPORTING:

A. Patient results are manually entered into the LIS.

B. Liquid QC is manually entered into the laboratory's QC program.

C. Report as Negative or Positive.

11. Revisions

Corewell Health reserves the right to alter, amend, modify, or eliminate this document at any time without prior written notice.

12. REFERENCES:

A. Lockwood CJ, AE Senyei, MR Dische, DC Casal, et al. Fetal fibronectin in cervical and vaginal secretions as a predictor of preterm delivery. New Engl J Med 325:669-674, 1991.

B. Morrison JC, JR Allbert, BN McLaughlin, NS Whitworth, et al. Oncofetal fibronectin in patients with false labor as a predictor of preterm delivery. Am J Obstet Gynecol 168:538-542,
C. Inglis SR, J Jeremias, K Kuno, K Lescale, et al. Detection of tumor necrosis factor-a, interleukin-6, and fetal fibronectin in the lower genital tract during pregnancy: Relation to outcome. Am J Obstet Gynecol 171:5-10, 1994.
D. Iams J, DC Casal, TM Goodwin, US Kreaden, et al. Fetal fibronectin improves the accuracy of diagnosis of preterm delivery. Am J Obstet Gynecol 173:141-145, 1995.
E. Burrus DR, JM Ernest and JC Veille. Fetal fibronectin, interluekin-6, and C-reactive protein are useful in establishing prognostic sub-categories of idiopathic preterm labor. Am J Obstet Gynecol 173:1258- 1262, 1995.
F. Bartnicki J, D Casal, US Kreaden, E Saling, and K Vetter. Fetal fibronectin in vaginal specimens predicts preterm delivery and very low birth weight infants. Am J Obstet Gynecol 174:971-974, 1996.
G. Lockwood CJ, R Wein, R Lapinski, D Casal, et al. The presence of cervical and vaginal fetal fibronectin predicts preterm delivery in an inner-city obstetric population. Am J Obstet Gynecol 169:798-804, 1993.
H. Leeson SC, MJA Maresh, EA Martindale, T Mahmood, et al. Detection of fetal fibronectin as a predictor of preterm delivery in high risk asymptomatic pregnancies. Br J Obstet Gynecol 103:48-53, 1996.
I. Goldenberg RL, BM Mercer, PJ Meis, RL Copper, et al. The Preterm Prediction Study: Fetal fibronectin testing and spontaneous preterm birth. Obstet Gynecol 87:643-648, 1996.
J. Morrison JC, RW Naef, JJ Botti, M Katz, et al. Prediction of spontaneous preterm birth by fetal fibronectin and uterine activity. Obstet Gynecol 87:649-655, 1996.

13. Procedure Development and Approval

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14. Keywords

Not Set