For Professional Use Only

**INTENDED USE**

The Rapid fFN Cassette for use in the TLiIQ® System is an in vitro diagnostic device for the detection of fetal fibronectin in cervicovaginal secretions to be used as an aid to rapidly assess the risk of preterm delivery in ≤ 7 or ≤ 14 days from the time of cervicovaginal sample collection in pregnant women with signs and symptoms of early preterm labor, intact amniotic membranes and minimal cervical dilatation (< 3 cm), sampled between 24 weeks, 0 days and 34 weeks, 6 days of gestation.

The Rapid fFN test is further indicated for use in conjunction with other clinical information as an aid to rapidly assess the risk of preterm delivery in ≤ 34 weeks, 6 days when a cervicovaginal sample is obtained during a routine prenatal visit between 22 weeks, 0 days and 30 weeks, 6 days of gestation in women with a singleton pregnancy.

The Rapid fFN test represents a significant and critically needed improvement in the ability to manage preterm labor that may result in preterm delivery.

**CONTRAINDICATIONS**

The Rapid fFN test should not be used for asymptomatic women with one or more of the following conditions:

- advanced cervical dilatation (≥ 3 centimeters)
- rupture of amniotic membranes
- cervical cerclage
- moderate or gross vaginal bleeding

Delivery typically occurs imminent when the cervix is dilated more than 3 centimeters or if the amniotic membranes are ruptured. Additional diagnostic testing is usually not necessary to confirm risk for women with advanced cervical dilatation or rupture of amniotic membranes. Moderate or gross vaginal bleeding is an independent risk factor for preterm delivery and may be associated with other severe obstetrical or medical problems. Clinical attention should be focused on identification of the origin of bleeding rather than immediate assessment of delivery risk. At this time, information is insufficient regarding the association of vaginal fetal fibronectin expression to delivery for women with cervical cerclage.

The Rapid fFN test should not be used for asymptomatic women with one or more of the following conditions:

- multiple gestations, e.g., twins
- cervical cerclage
- placenta previa (partial or complete)
- sexual intercourse in the preceding 24 hours

At this time, information is insufficient regarding the association of cervicovaginal fetal fibronectin expression to delivery for asymptomatic women with HIV/AIDS, multiple gestations or cervical cerclage.

**SUMMARY AND EXPLANATION OF THE TEST**

Of the approximately 4,000,000 deliveries that occur annually in the United States, about 400,000 are premature. Preterm delivery, defined by the American College of Obstetricians and Gynecologists as delivery prior to the 37th week of gestation, is responsible for the majority of non-chronic fetal perinatal morbidity and mortality (1-4). Symptoms of threatened preterm delivery include uterine contractions, change of vaginal discharge, vaginal bleeding, backache, abdominal discomfort, pelvic pressure, and cramping. Diagnostic modalities for identification of threatened preterm delivery include uterine activity monitoring and performance of a digital cervical examination, which allows estimation of cervical dimensions. These methods have been shown to be limited, as minimal cervical dilatation (< 3 centimeters) and uterine activity occur normally and are not necessarily diagnostic of imminent preterm delivery (5,11,13). While several serum biochemical markers have been evaluated, none have been widely accepted for practical clinical use (6,7,20).

Fetal fibronectin (fFN), an isofrom of fibronectin, is a complex adhesive glycoprotein with a molecular weight of approximately 500,000 daltons (8,9). Matsuura and co-workers have described a monoclonal antibody called FDC-6, which specifically recognizes III-CS, the region defining the fetal isoform of fibronectin (8,9). Immunohistochemical studies of placenta have shown that fFN is confined to the extracellular matrix of the region defining the junction of the maternal and fetal units within the uterus (5,10).

Fetal fibronectin can be detected in cervicovaginal secretions of women throughout pregnancy by use of a monoclonal antibody-based immunoassay. Fetal fibronectin is elevated in cervicovaginal secretions during the first 24 weeks of pregnancy but is diminished between 24 and 34 weeks in normal pregnancies. The significance of its presence in the vagina during the first 24 weeks of pregnancy is not understood. However, it may simply reflect the normal growth of the extravillous trophoblast population and the placenta. Detection of fFN in cervicovaginal secretions between 24 and 34 completed weeks gestation is reported to be associated with preterm delivery in symptomatic (5,11-15) and asymptomatic pregnant women (16-19).

**PRINCIPLE OF THE TEST**

The Rapid fFN Cassette is a lateral flow, solid-phase immuno-chromatographic assay. The cervicovaginal specimen is extracted into a buffer and a 200 μL sample is dispensed into 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 one monoclonal anti-fetal fibronectin antibody conjugated to blue microspheres (conjugate). 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.

**PRECAUTIONS AND WARNINGS**

**Note:** Transport specimens at 2° to 25°C, or frozen. Specimens are stable for up to eight (8) hours at room temperature. Specimens not tested within eight hours of collection must be stored refrigerated at 2° to 8°C and assayed within three (3) days of collection, or frozen and assayed within three (3) months to avoid degradation of the analyte. Specimens arriving frozen may be tested as described below (subject to a single freeze-thaw cycle only).

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**Manufacturer**

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**Caution, consult accompanying documents**

- In Vitro Diagnostic Medical Device
- Use by
- Batch code
- Authorized Representative in the European Community
- Temperature limitation: 15°–30°C
- Catalogue Number
- Do not reuse
- Manufacturer

**For In Vitro Diagnostic Use Only**

Store at room temperature (15° to 30° C / 59° to 86°F).

**Rapid fFN™ Cassette Kit**

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1. For in vitro diagnostic use only.
2. Carefully follow the instructions and procedures described in this insert.
3. Test results may not be interpreted visually and must be based on the use of the TLiIQ® System.
4. Do not use glass tubes or glass pipettes, as fetal fibronectin binds to glass. Tubes and pipettes of polypropylene or polyethylene are acceptable.
5. Do not mix materials from different kit lots.
6. Do not use cassettes or controls past their expiration dates.
7. Do not use controls if they are cloudy or discolored. Avoid cross-contamination of reagents. Use a new pipette tip for each control or patient sample. Recap controls tightly with the correct color-coded caps.
8. Handle cassettes with care; do not touch, scratch, or compress membrane materials in the Rapid fFN Cassette.
9. Source material used to prepare the controls is of human origin. The donors were tested and found to be negative for HIV 1, HIV 2, and 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.
10. 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.

STORAGE
The Rapid fFN Cassette should be stored at room temperature (15° to 30°C / 59° to 86°F).

STABILITY
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.

MATERIALS PROVIDED
Rapid fFN for the TLiIQ® System, REF 01200 (includes Cassettes and Directional Insert).

MATERIALS REQUIRED BUT NOT PROVIDED
1. TLiIQ System, REF 01202 (includes Analyzer, Printer, User Manual, and TLiIQ® QCette®)
2. Rapid fFN Control Kit, REF 01166 (includes Positive Control, Negative Control, and Directional Insert)
3. 200 μL pipette

SPECIMEN COLLECTION
The Hologic Specimen Collection Kit for fetal fibronectin testing is the only acceptable specimen collection system that can be used to collect specimens for this assay. See the Specimen Collection Kit directional insert for complete instructions.

PROCEDURE
PERFORMING ANALYZER QUALITY CONTROL
Use the TLiIQ® QCette to ensure proper function of the TLiIQ® Analyzer. See the TLiIQ® QCette directional insert for complete instructions.

SETTING CALIBRATION FOR A RAPID fFN CASSETTE LOT
Select SET CALIBRATION from the TLiIQ® Analyzer Main Menu and enter the information requested (Cassette Lot # and Calibration Code #). The Cassette Lot # is located on the cassette pouch. The Calibration Code # is located on the cassette box. See the TLiIQ® System User Manual for details.

RUNNING RAPID fFN CONTROL KIT
The Rapid fFN Control Kit must be run each time a new lot or a new shipment of Rapid fFN Cassettes is received. Run the liquid controls as if testing patient samples. See the Rapid fFN Control Kit directional insert for complete instructions.

Note: For your convenience, space is provided on the Rapid fFN Cassette Kit box for control testing documentation.

SPECIMEN PREPARATION
Note: Handle the Specimen Transport Tube and all Patient Specimens as if potentially infectious.

1. Allow all Specimen Transport Tubes to come to room temperature before testing.
2. Gently mix 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 biohazardous materials.

TESTING PATIENT SAMPLES
Incubation Mode – Internal
Note: The default setting for the TLiIQ® Analyzer is Internal Incubation Mode. In this mode, the analyzer will time the incubation and automatically initiate reading of the cassette when incubation is complete.

1. Prepare Patient Samples according to the Specimen Preparation section. Mix patient samples before testing.
2. Remove one Rapid fFN Cassette from the foil pouch.
3. Select VIEW SETUP from the TLiIQ® Analyzer Main Menu to determine if the analyzer is set to Internal Incubation Mode. If Internal Mode is indicated, proceed to step 4. If the analyzer is not set to Internal Mode, select CHANGE SETUP from the Main Menu and change to Internal Incubation Mode. See the TLiIQ® System User Manual for details.
4. Select TEST PATIENT from the TLiIQ® Analyzer Main Menu and enter the necessary information until the analyzer prompts for cassette insertion.
5. Insert the cassette into the analyzer and press ENTER.
6. When prompted by the analyzer, pipette 200 μL of patient sample into the sample application well of the Rapid fFN Cassette. Immediately press ENTER to activate the analyzer.
7. The analyzer will count down for 20 minutes and analyze the Rapid fFN Cassette.
8. The fFN result for the patient sample will be displayed on the TLiIQ® Analyzer display screen as POSITIVE, NEGATIVE, or INVALID.
9. If an INVALID result is obtained, retest with 200 μL of additional sample, if available, on a new cassette. If the problem is not corrected, see the TLiIQ® System User Manual for details, or contact Hologic for technical assistance.

Incubation Mode – External
Note: In External Incubation Mode, the user is responsible for timing the incubation and starting the analysis. If additional cassettes are run, wait 5 minutes before adding sample to the next cassette to allow for the analysis of the previous cassette (approximately 3 minutes) and for entering menu information required by the analyzer for the next cassette.

1. Prepare Patient Samples according to the Specimen Preparation section. Mix patient samples before testing.
2. Remove one Rapid fFN Cassette from the foil pouch.
3. Select VIEW SETUP from the TLiIQ® Analyzer Main Menu to determine if the analyzer is set to External Incubation Mode. If External Mode is indicated, proceed to step 4. If the analyzer is not set to External Mode, select CHANGE SETUP from the Main Menu and change to External Incubation Mode. See the TLiIQ® System User Manual for details.
4. Select TEST PATIENT from the TLiIQ® Analyzer Main Menu and enter the necessary information until the analyzer prompts for cassette insertion.
5. Pipette 200 μL of patient sample into the sample application well of the Rapid fFN Cassette and allow to incubate at room temperature for 20 minutes.

6. When the incubation time is complete, insert the cassette into the analyzer and press ENTER. The analyzer will complete the analysis of the Rapid fFN Cassette.

7. The IFN result for the patient sample will be displayed on the TLIq Analyzer display screen as POSITIVE, NEGATIVE, or INVALID.

8. If an INVALID result is obtained, retest with 200 μL of additional sample, if available, on a new cassette. If the problem is not corrected, see the TLIq System User Manual for details, or contact Hologic for technical assistance.

INTERPRETATION OF RESULTS

The IFN result for the patient sample will be displayed on the TLIq Analyzer display screen as POSITIVE, NEGATIVE, or INVALID. The result is positive if the value derived from the patient sample is greater than 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 result is invalid if the test does not meet internal quality controls.

QUALITY CONTROL PROCEDURES

Current Good Laboratory Practice includes the daily use and documentation of either liquid controls or electronic (internal) controls to assure that the calibration of the diagnostic device is maintained within acceptable limits.

The Rapid fFN Control Kit (REF 01166) contains two liquid controls: one Rapid fFN Positive Control and one Rapid fFN Negative Control. The controls are recommended for use in monitoring the performance of the Rapid fFN Cassette. The recommended frequency of use of the controls is once a week or when new lots or new shipments of cassettes are received, or whenever there is uncertainty about Rapid fFN Cassettes. Deviation from the recommended frequency of quality control testing must be validated by the laboratory. If the criteria for controls are not met, do not test patient samples until acceptable results are obtained.

The TLIq QCette is a quality control device used to verify that the TLIq Analyzer performs within specification. The TLIq QCette is a Rapid fFN Cassette replica, containing a membrane with printed test and control lines, which is read by the TLIq 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.

Internal controls monitor all components of the TLIq System and are performed automatically with every test. These internal controls check for: (1) a threshold level of signal at the procedural control position, (2) proper sample flow across the Rapid fFN Cassette, (3) absence of conjugate aggregation (Cassette: Pass/Fail), and (4) proper function of analyzer hardware (Analyzer: Pass/Fail).

LIMITATIONS

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 ≤ 7 days or ≤ 14 days from specimen collection in symptomatic women or delivery in ≤ 34 weeks, 6 days in asymptomatic women evaluated between 22 weeks, 0 days and 30 weeks, 6 days of gestation. 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.

- Test results may not be interpreted visually and must be based on the use of the TLIq System.
- Modification of the assay protocol described herein may yield erroneous results.
- The assay has been optimized with specimens taken from the posterior fornix of the vagina or the ectocervical region of the external cervical os. Samples obtained from other locations should not be used.

EXPECTED VALUES

Among symptomatic women, elevated levels (> 0.050 μg/mL) of fFN between 24 weeks, 0 days and 34 weeks, 6 days indicate increased risk of delivery in ≤ 7 or ≤ 14 days from sample collection. Similarly, among asymptomatic women, elevated levels of fFN between 22 weeks, 0 days and 30 weeks, 6 days indicate increased risk of delivery in ≤ 34 weeks, 6 days of gestation. The cutoff of 0.050 μg/mL fFN was established in a multicenter study conducted to evaluate the association between fetal fibronectin expression during pregnancy and preterm delivery (5).

Only subjects with symptoms of preterm labor or preterm rupture of membranes were eligible for this study. Of the total study population, the association of vaginal fetal fibronectin expression to preterm delivery was evaluated for 117 symptomatic women with intact amniotic membranes. The strength of this association was determined at a variety of cutoffs using receiver operator characteristic (ROC) curves. These results show that the optimal sensitivity and specificity is provided at a cutoff of 0.050 μg/mL fFN. Subsequent studies evaluating the fFN Enzyme Immunoassay test as a predictor of preterm delivery among symptomatic women and asymptomatic women with singleton pregnancies have confirmed that the optimal cutoff is 0.050 μg/mL fFN (5,13,15,18). Laboratory studies have confirmed the optimal cutoff of 0.050 μg/mL fFN in the Rapid fFN. This analytical cutoff was used to show equivalency between the fFN Enzyme Immunoassay and the Rapid fFN.

PERFORMANCE CHARACTERISTICS

Accuracy

A comparison of the Fetal Fibronectin Enzyme Immunoassay to the Rapid fFN was assessed in 587 cervicovaginal samples. The two test formats were equivalent 94.9% of the time. The Kappa coefficient for inter-test agreement was 0.81 with 95% confidence interval of [0.75, 0.88].

Table

Analytical Concordance Between fFN Enzyme Immunoassay and Rapid fFN (n=587) (Symptomatic and Asymptomatic Women)

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<thead>
<tr>
<th>fFN Enzyme Immunoassay (+)</th>
<th>Rapid fFN (+)</th>
<th>Rapid fFN (-)</th>
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<tbody>
<tr>
<td>IFN Enzyme Immunoassay (+)</td>
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<td>13</td>
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<td>497</td>
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Within-Run Reproducibility

Within-run reproducibility was determined using three lots of Rapid fFN Cassettes. Twenty replicates each of the Rapid fFN Negative Control (fFN concentration approximately 0.015 μg/mL) and of the Rapid fFN Positive Control (fFN concentration approximately 0.080 μg/mL) were tested on three lots of Rapid fFN Cassettes using three different TLIq Analyzers. (One lot of cassettes was run on one analyzer on one day, on three different occasions.) The results from these studies show that the Rapid fFN correctly identified all specimens 100% of the time.
Between-Run Reproducibility

Between-run reproducibility was determined in 36 independent assays, using different lot combinations of Rapid fFN Cassette (three lots) and TLiIQ Analyzers (six each). Each assay consisted of the Rapid fFN Negative Control and assay of the Rapid fFN Positive Control. The Rapid fFN correctly identified all samples 100% of the time.

Interfering Substances

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). Lubricants or creams may physically interfere with the absorption of the specimen onto the swab. Soaps or disinfectants may interfere with the antibody-antigen reaction.

Various concentrations of pharmacologic agents were added to specimens containing approximately 0.015 μg/mL to 0.080 μg/mL fFN and assayed in triplicate. The drugs added were: prostaglandin E₂ (up to 250 μg/mL), ampicillin (up to 100 μg/mL), cephalaxin (up to 18 μg/mL), erythromycin (up to 10 μg/mL), gentamicin (up to 4 μg/mL), dexamethasone (up to 200 μg/mL), magnesium sulfate (up to 50 μg/mL), oxytocin (up to 100 μg/mL), terbutaline (up to 100 μg/mL), and ritodrine (up to 10 μg/mL). These drugs did not interfere with the assay at the concentration limits cited above.

BIBLIOGRAPHY