HOLOGIC®

Rapid fFN® 10Q Cassette Kit

REF PRD-01018 REF PRD-05607

For *in vitro* diagnostic use only.
Store at room temperature (15° to 30°C / 59° to 86°F).

CE

To be used by trained medical personnel only

INTENDED USE

The Rapid fFN® 10Q Cassette for use in the PeriLynx™ System or the Rapid fFN® 10Q System (the Rapid fFN 10Q Test) is an *in vitro* diagnostic device for the quantitative detection of fetal fibronectin in cervicovaginal secretions to be used as an aid to rapidly assess the risk of preterm delivery before ≤ 7 and ≤ 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 22 weeks, 0 days and 35 weeks, 6 days of gestation.

The Rapid fFN® 10Q Test is further indicated for use as an aid to rapidly assess the risk of preterm delivery in less than 34 weeks, 0 days of gestation in pregnant women with signs and symptoms of early preterm labor, intact amniotic membranes, and minimal cervical dilatation (< 3 cm), sampled between 22 weeks, 0 days and 33 weeks, 0 days of gestation.

The Rapid fFN® 10Q Test is further indicated for use as an aid to rapidly assess the risk of preterm delivery in <30 weeks, <34 weeks, and <37 weeks of gestation in pregnant women at risk of preterm birth sampled between 18 weeks, 0 days and 27 weeks, 6 days of gestation. Women at risk of preterm birth include patients with:

- PPTB or prior PROM <37 weeks
- previous spontaneous second trimester miscarriage
- previous cervical surgery [large loop excision of the transformation zone (LLETZ), loop electrosurgical excision procedure (LEEP), laser or cone excision]
- incidental finding of a cervical length of 25 mm or less in the index pregnancy

The Rapid fFN 10Q 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 10Q Test should not be used in 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 imminently 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.

SUMMARY AND EXPLANATION OF THE TEST

Approximately 15 million babies are born prematurely every year worldwide. Preterm delivery, defined by the World Health Organization as delivery prior to the 37th week of gestation, is responsible for the majority of non-chromosomal perinatal morbidity and mortality (1). Symptoms of threatened preterm delivery include uterine contractions, change of vaginal discharge, vaginal bleeding, backache, abdominal discomfort, pelvic pressure, and cramping. Diagnostic modalities for the identification of threatened preterm delivery include uterine activity monitoring, performance of a digital cervical examination and the measurement of cervical length via transvaginal ultrasound, 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 (2, 3, 4). Transvaginal ultrasound equipment may not always be available and accuracy of cervical length measurements are dependent on skill (5). While several serum biochemical markers have been evaluated, none have been widely accepted for practical clinical use (6, 7, 8).

RapidfFN® 10Q Cassette

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Fetal fibronectin (fFN), an isoform of fibronectin, is a complex adhesive glycoprotein with a molecular weight of approximately 500,000 daltons (9, 10). 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 (9, 10). Immunohistochemical studies of placentae 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 (2, 11).

Fetal Fibronectin can be detected in cervicovaginal secretions of women throughout pregnancy by use of a monoclonal antibody-based immunoassay. The association between increasing levels of fFN measured in vaginal fluid and increased preterm birth risk is well documented (12, 13, 14, 15).

PRINCIPLE OF THE TEST

The Rapid fFN 10Q Cassette is a lateral flow, solid-phase immuno-chromatographic quantitative 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 10Q Cassette. The sample flows from an absorbent pad across a nitrocellulose membrane via capillary action through a reaction zone containing murine 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 7 minutes of reaction time, the intensities of the test line and control line are measured by the analyzer. The measured intensity of the specimen is automatically interpreted with the calibration code that is established for each lot of cassettes. The test result is displayed within 10 minutes of sample addition.

Note: In these instructions for use, the term “analyzer” refers to either the PeriLynx Analyzer or the Rapid fFN 10Q Analyzer. Unless specifically noted, the instructions for using the Rapid fFN 10Q Cassettes are the same for the two kinds of analyzers.

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).

1. For *in vitro* diagnostic use only.
2. Test results cannot be interpreted visually and must be interpreted by the analyzer.
3. **Do not use glass tubes or glass pipettes, as fetal fibronectin binds to glass. Tubes and pipettes of polypropylene or polyethylene are acceptable.**
4. **Do not use cassettes past their expiration dates.**
5. Handle cassettes with care; do not touch, scratch, or compress membrane materials in the Rapid fFN 10Q Cassette.
6. 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.**
7. Labels (e.g., barcode 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 analyzer.
8. Each cassette is a single-use device. Do not reuse.
9. Use a new pipette tip for each control or patient sample.

STORAGE

The Rapid fFN 10Q Cassettes should be stored at room temperature (15° to 30°C / 59° to 86°F).

STABILITY

The shelf life of the Rapid fFN 10Q 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 10Q Cassette should be used immediately.

Rapid fFN® 10Q Cassette Kit

MATERIALS PROVIDED

Rapid fFN 10Q Cassette Kit (Kit number PRD-01018 includes 26 cassettes and directional insert. Kit number PRD-05607 includes 12 cassettes and directional insert.)

MATERIALS REQUIRED BUT NOT PROVIDED

1. PeriLynx Analyzer, Printer, User Manual, and PeriLynx QCette
   or
   Rapid fFN 10Q Analyzer, Printer, User Manual, and Rapid fFN 10Q QCette
2. Rapid fFN Control Kit
3. 200 µL pipette

SPECIMEN COLLECTION

The Hologic Rapid fFN® 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 analyzer QCette to ensure proper function of the analyzer. See the directional insert for the PeriLynx QCette or for the Rapid fFN 10Q QCette for complete instructions.

Setting Calibration for a Rapid fFN Cassette Lot

Select **Enter New Calibration Code** or **SET CALIBRATION** from the analyzer Main Menu and enter the information requested (user ID, cassette lot # and calibration code). The cassette lot # is located on the cassette pouch and on the cassette box. The calibration code is located on the cassette box. See the analyzer's user manual for details. The calibration code must be set for each lot of Rapid fFN 10Q Cassettes used for testing.

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

1. Prepare patient sample according to the Specimen Preparation section. Mix the patient sample before testing.
2. Remove one Rapid fFN 10Q Cassette from the foil pouch.
3. Select **Test Patient** from the analyzer Main Menu and enter the necessary information until the analyzer prompts for cassette insertion.
4. Insert the cassette into the analyzer and press **Next** or **ENTER**.
5. When prompted by the analyzer, pipette 200 µL of patient sample into the sample application well of the Rapid fFN 10Q Cassette. Immediately press **Start Test** or **ENTER** to activate the analyzer.
6. The analyzer will begin a countdown, with 7 minutes of incubation and 2–3 minutes of analysis of the cassette.
7. The fFN concentration will be displayed.

Interpretation of Results

The fFN concentration result indicates the level of fFN in the clinical specimen. Quantitative fFN assay results are reported in units of ng/mL and the result is standardized using purified fFN and A280 measurement with a ε = 1.28 (16). The analyzer reports fFN concentrations ranging from 0 to 500 ng/mL. Concentrations greater than 500 ng/mL will be displayed as > 500 ng/mL. The result is INVALID if the test does not meet internal quality controls. See Quality Control Procedures below.

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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 contains two liquid controls: one Rapid fFN Negative/Level 1 Control and one Rapid fFN Positive/Level 2 Control. These controls are recommended for use in monitoring the performance of the Rapid fFN 10Q Cassette. The recommended frequency of use of the controls is once each time a new lot or a new shipment of cassettes is received, or whenever there is uncertainty about the cassettes. The control testing may be performed more frequently, in accordance with your local applicable requirements. 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. See the Rapid fFN Control Kit directional insert for complete instructions.

The analyzer's QCette is a quality control device used to verify that the analyzer performs within specification. The QCette device is a cassette replica, containing a membrane with printed test and control lines, which is read by the analyzer. Two different levels of response are measured with this QC device. See the analyzer QCette directional insert for complete instructions.

Internal controls monitor all components of the analyzer 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 10Q Cassette,
3. absence of conjugate aggregation (Cassette QC: Pass/Fail), and
4. proper function of analyzer hardware (Analyzer QC: Pass/Fail).

The result is invalid if the test does not meet internal quality controls.

- An INVALID test result caused by Cassette QC failure may occur if the dispensed sample volume is less than or greater than 200 µL. If an INVALID result is obtained, retest with 200 µL of additional sample on a new cassette. If the problem is not corrected, see the analyzer's user manual for details, or contact Technical Support.
- An INVALID test result due to Cassette QC failure can also occur when there is an abnormal flow rate across the cassette membrane. This can be due to characteristics inherent to the sample. Highly mucoid samples as well as samples contaminated with lubricants, soaps, disinfectants, or creams may cause this issue. Retest specimen on a new cassette. If the problem is not corrected, contact the physician and recommend recollection in 24 hours.
- An INVALID test result caused by Analyzer QC failure can occur when there is an analyzer malfunction. Turn the analyzer off and back on to reinitialize the system. Rerun the QCette. If the QCette fails, contact Technical Support. If the QCette passes, retest with 200 µL of additional sample on a new cassette. If the problem is not corrected, see the analyzer's user manual for details, or contact Technical Support.

LIMITATIONS

The Rapid fFN 10Q test result should not be interpreted as absolute evidence for the presence or absence of a process that will result in preterm delivery. The fFN concentration may be influenced by cervical disruption caused by, but not limited to, events such as sexual intercourse, digital cervical examination, or vaginal probe ultrasound. The Rapid fFN 10Q test 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 cannot be interpreted visually and must be interpreted by the analyzer.**
- 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. Samples obtained from other locations should not be used.
- Assay interference from the following components has not been ruled out: douches, white blood cells, red blood cells, bacteria, and bilirubin.
- The presence of infections has not been ruled out as a confounding factor to risk of preterm delivery.

- A fetal fibronectin sample can be collected in patients who report having had sexual intercourse in the prior 24 hours, but healthcare providers should be aware of the following information relevant to these patients:

  A sample contaminated with semen may lead to a falsely elevated fFN result. However, healthcare providers can be assured that interference from semen will not cause a falsely lowered fFN result. For example, a result of less than 10 ng/mL can be relied on to be a valid result of less than 10 ng/mL, even if the patient has had sexual intercourse in the prior 24 hours.

  The above example is also applicable to higher management thresholds used by some facilities.
- Specimens should be obtained prior to digital examination or manipulation of the cervix. Manipulations of the cervix may lead to falsely elevated fFN results.
- Patients with suspected or known placental abruption, placenta previa, or moderate or gross vaginal bleeding should not be tested.
- A sample contaminated with blood may lead to a falsely elevated fFN result. However, healthcare providers can be assured that interference from blood will not cause a falsely lowered fFN result. For example, a result of less than 10 ng/mL can be relied on to be a valid result of less than 10 ng/mL, even if the sample is contaminated with blood.

  The above example is also applicable to higher management thresholds used by some facilities.
- The performance data associated with the fFN concentration is only for pregnant women with signs and symptoms of preterm labor. There are no performance data associated with the fFN concentration value for asymptomatic women at this time.

EXPECTED VALUES

Symptomatic Population

A multi-center study was conducted in the United Kingdom from October 2010 through April 2012 to evaluate the utility of the fFN concentration in predicting preterm birth risk. This prospective, observational blinded study included 300 symptomatic women with singleton pregnancies who were sampled between 22 weeks, 0 days and 35 weeks, 6 days gestation (15). Among women with signs and symptoms of preterm labor, the increasing concentration of fFN measured in cervicovaginal specimens collected between 22 weeks, 0 days and 35 weeks, 6 days correlated with increased risk of delivery in ≤ 7 or ≤ 14 days from sample collection. Similarly, an increasing concentration of fFN in cervicovaginal specimens collected between 22 weeks, 0 days and 33 weeks, 0 days correlated with increased risk of delivery before 34 weeks, 0 days of gestation.

The risk of delivery within 7 and 14 days of sampling and the risk of delivery before 34 weeks, 0 days gestation is shown in Table 1. The level of risk increases with increasing fFN concentration.

Table 1. Stratification of Preterm Birth Risk by fFN Concentration

fFN Level	N (%)	Delivery ≤ 7 days	Delivery ≤ 14 days	Delivery before 34 wks, 0 days
< 10 ng/mL	170 (57%)	1%	1.8%	1.5%
10 to 49 ng/mL	62 (21%)	0%	1.6%	8.2%
50 to 199 ng/mL	41 (14%)	0%	7.7%	11.5%
200 to 499 ng/mL	14 (5%)	14%	29%	33%
≥ 500 ng/mL	13 (4%)	38%	46%	75%

The fFN concentration corresponds to various relative risk levels at relevant delivery time points as shown in Table 2. Relative risk increases with increasing concentrations of fFN.

Table 2. Relative Risk[1] of Preterm Birth Stratified by fFN Concentration

fFN Level	N (%)	Delivery ≤ 7 days	Delivery ≤ 14 days	Delivery before 34 wks, 0 days
< 10 ng/mL	170 (57%)	—	—	—
10 to 49 ng/mL	62 (21%)	0.0	0.9	5.6[3]
50 to 199 ng/mL	41 (14%)	0.0	4.3	7.9[4]
200 to 499 ng/mL	14 (5%)	12.1[2]	16.1[2]	22.8[4]
≥ 500 ng/mL	13 (4%)	32.5[4]	26.0[2]	51.3[4]

1. Relative risk compared to fFN < 10 ng/mL
2. Chi-square, p < 0.01
3. Chi-square, p < 0.05
4. Chi-square, p < 0.001

The above performance data associated with the fFN concentration is only for pregnant women with signs and symptoms of preterm labor.

High Risk Population

A multi-center study was conducted in the United Kingdom from October 2010 through September 2013 to evaluate the utility of the fFN concentration in predicting preterm birth risk. This prospective, observational blinded study included 1448 high risk women with singleton pregnancies who were sampled between 22 weeks, 0 days and 27 weeks, 6 days gestation (17). This population included patients with PPTB or prior PROM <37 weeks, patients with a previous spontaneous second trimester miscarriage; patients with previous cervical surgery [large loop excision of the transformation zone (LLETZ), loop electrosurgical excision procedure (LEEP), laser or cone excision]; and patients with an incidental finding of a cervical length of 25 mm or less in the index pregnancy. Among women at high risk of preterm birth, the increasing concentration of fFN measured in cervicovaginal specimens collected between 22 weeks, 0 days and 27 weeks, 6 days correlated with increased risk of delivery in <30 weeks, <34 weeks, and < 37 weeks of gestation. A subsequent study demonstrated that quantitative cervicovaginal fetal fibronectin measured from 18–21 weeks of gestation has similar predictive value as measurement at 22–27 weeks of gestation for the prediction of spontaneous preterm birth (18).

Table 3. Spontaneous Preterm Birth Rates in Asymptomatic High Risk Women According to Quantitative Fetal Fibronectin Categories

fFN Category (ng/mL)	n (%)	sPTB <30 weeks n (%)	sPTB <34 weeks n (%)	sPTB <37 weeks n (%)
<10	1000 (69.1)	10 (1.0)	27 (2.7)	81 (8.1)
10–49	249 (17.2)	8 (3.2)	27 (11.0)	50 (20.1)
50–199	121 (8.4)	6 (5.0)	18 (14.9)	32 (26.4)
200–499	57 (3.9)	13 (22.8)	19 (33.9)	26 (45.6)
≥500	21 (1.5)	8 (38.1)	10 (47.6)	11 (52.4)
Total**	1448 (100)	45 (3.1)	101 (7.0)	200 (13.8)

*All comparisons for each gestational end point are statistically significant (p<0.01) except 10–49 ng/mL vs. 50–199 ng/mL and 200–499 vs. ≥500+ (p>0.1 for all gestational end points).
** Women with iatrogenic deliveries before the gestation of analysis were excluded (n=7 <30 weeks, n=15 <34 weeks, n=41 <37 weeks).

Table 4. Prediction of Spontaneous Preterm Birth Before 30 Weeks of Gestation According to Quantitative Fetal Fibronectin Threshold (n=1441)

Predictive variable (95% CI)	Fetal fibronectin threshold (ng/mL)			
	≥10	≥50	≥200	≥500
Sensitivity (%)	77.8 (62.9–88.8)	60.0 (44.3–74.3)	46.7 (31.7–62.1)	17.8 (8.0–32.1)
Specificity (%)	70.5 (68.0–72.9	87.7 (85.5–89.4)	95.9 (94.7–96.9)	99.1 (98.4–99.5)
PPV (%)	7.8 (5.5–10.7)	13.6 (9.1–19.1)	26.9 (17.5–38.2)	38.1 (18.1–61.6)
NPV (%)	99.0 (98.2–99.5)	98.6 (97.7–99.1)	98.2 (97.4–98.9)	97.4 (96.4–98.2)
LR +	2.64 (2.21–3.14)	4.9 (3.7–6.4)	11.4 (7.6–17.1)	19.1 (8.3–43.8)
LR –	0.32 (0.18–0.55)	0.5 (0.3–0.7)	0.6 (0.4–0.7)	0.83 (0.72–0.95)
ROC area	0.81 (0.73–0.89)			

NPV: negative predictive value
PPV: positive predictive value
LR: likelihood ratio
ROC: receiver operating curve

Table 5. Prediction of Spontaneous Preterm Birth Before 34 Weeks of Gestation According to Quantitative Fetal Fibronectin Threshold (n=1433)

Predictive variable (95% CI)	Fetal fibronectin threshold (ng/mL)			
	≥10	≥50	≥200	≥500
Sensitivity (%)	73.3 (63.5–81.6)	46.5 (36.5–56.7)	28.7 (20.1–38.6)	9.9 (4.9–17.5)
Specificity (%)	72.2 (69.7–74.6)	88.7 (86.8–90.3)	96.4 (95.3–97.3)	99.2 (98.5–99.6)
*PPV (%)	16.7 (13.3–20.5)	23.7 (18.0–30.3)	37.7 (26.9–49.4)	47.6 (25.7–70.2)
*NPV (%)	97.3 (96.1–98.2)	95.6 (94.3–96.7)	94.7 (93.4–95.8)	93.6 (92.1–94.8)
*LR +	2.64 (2.28–3.05)	4.10 (3.17–5.31)	7.97 (5.27–12.1)	12.0 (5.20–27.6)
*LR –	0.37 (0.27–0.51)	0.60 (0.50–0.72)	0.74 (0.65–0.84)	0.91 (0.85–0.97)
ROC area	0.78 (0.73–0.84)			

*All comparisons for each gestational end point are statistically significant ($p<0.01$) except 10–49 ng/mL vs 50–199 ng/mL and 200–499 vs. ≥500+ ($p>0.1$ for all gestational end points).
NPV: negative predictive value
PPV: positive predictive value
LR: likelihood ratio
ROC: receiver operating curve

Table 6. Prediction of Spontaneous Preterm Birth Before 37 Weeks of Gestation According to Quantitative Fetal Fibronectin Threshold (n=1407)

Predictive variable (95% CI)	Fetal fibronectin threshold (ng/mL)			
	≥10	≥50	≥200	≥500
Sensitivity (%)	59.3 (52.1–66.2)	34.7 (27.1–41.7)	18.6 (13.4–24.7)	5.5 (2.8–9.7)
Specificity (%)	73.7 (71.1–76.2)	89.7 (87.9–91.4)	96.8 (95.6–97.7)	99.2 (98.5–99.6)
PPV (%)	27.1 (23.0–31.6)	35.8 (29.0–43.0)	48.7 (37.0–60.4)	52.4 (29.8–74.3)
NPV (%)	91.6 (89.7–93.3)	89.3 (87.4–91.0)	87.8 (85.9–89.5)	86.4 (84.5–88.2)
LR +	2.26 (1.94–2.62)	3.37 (2.62–4.34)	5.75 (3.76–8.79)	6.67 (2.87–15.49)
LR –	0.55 (0.47–0.66)	0.73 (0.66–0.81)	0.84 (0.79–0.90)	0.95 (0.92–0.99)
ROC area	0.70 (0.66–0.75)			

NPV: negative predictive value
PPV: positive predictive value
LR: likelihood ratio
ROC: receiver operating curve

Table 7. Relative Risk of Spontaneous Preterm Birth According to Quantitative Fetal Fibronectin Concentration

fFN Category (ng/mL)	sPTB <30 weeks RR	sPTB <34 weeks RR	sPTB <37 weeks RR
<10	1	1	1
10–49	3.2 (1.3–8.0)	4.0 (2.5–6.4)	2.5 (1.8–3.4)
50–199	4.9 (1.8–13.3)	5.5 (3.3–9.1)	3.3 (2.3–4.7)
200–499	22.7 (10.4–49.5)	10.1 (6.2–16.6)	5.7 (4.0–8.0)
>500	37.9 (16.6–86.2)	15.6 (9.2– 26.5)	6.3 (4.0–9.9)

RR: relative risk

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PERFORMANCE CHARACTERISTICS

Precision

Within-day and total precision were determined by testing two levels of control materials containing fetal fibronectin. The study was conducted on three cassette lots and tested over three weeks. Ten replicates were tested six times per lot using different combinations of Rapid fFN 10Q Analyzers. Precision results are shown in Table 8.

Table 8. Precision

Within-day Precision	Level 1 (53 ng/mL)	Level 2 (156 ng/mL)
Lot 1		
N	10	10
Average	54	170
SD	3.1	12.4
CV (%)	5.8	7.3
Lot 2		
N	10	10
Average	55	167
SD	3.1	11.0
CV (%)	5.7	6.6
Lot 3		
N	fFN Method Comparison	10
Average	53	163
SD	3.1	11.5
CV (%)	5.9	7.1
Total Precision		
N	180	180
Average	54	167
SD	3.2	12.5
CV (%)	5.9	7.5

Accuracy

The accuracy of the Rapid fFN 10Q System was determined by testing two levels of control material containing known amounts of fetal fibronectin on three cassette lots. Accuracy results are shown in Table 9.

Table 9. Accuracy

Cassette Lot Number	[fFN] (ng/mL)	Rapid fFN 10Q System (ng/mL)	Accuracy (%)
Lot 1	53	54	98.1
Lot 2	53	55	96.2
Lot 3	53	53	100
Lot 1	156	170	91.0
Lot 2	156	167	92.9
Lot 3	156	163	95.5

Similar precision and accuracy data were obtained for the Rapid fFN 10Q test run on the PeriLynx System.

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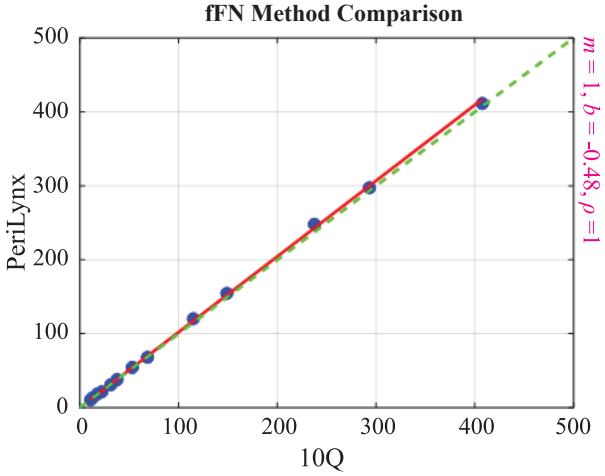
Comparison Study

A method comparison study was carried out to demonstrate the equivalence of the quantitative measurement of fFN concentration on the Rapid fFN 10Q Cassette, comparing the PeriLynx System and the Rapid fFN 10Q System. Solutions were prepared over a range of fFN concentrations and tested in replicates across multiple PeriLynx Systems and multiple Rapid fFN 10Q Systems. Thirteen (13) fFN concentrations were tested on ten (10) each of PeriLynx Systems and Rapid fFN 10Q Systems, and six (6) replicate measurements were made per analyzer. The chart below shows the data from the study. The "10Q" axis shows the fFN concentrations in ng/mL measured on the Rapid fFN 10Q System (averaged across instruments and replicates), and the "PeriLynx" axis shows the averaged results for the same solution measured on the PeriLynx System.

Figure 1. fFN Regression Plot



Deming regression analysis calculates the slope of the regression line as 1.02 with 95% CI: 1.01 to 1.04. The results of this study establish that the PeriLynx System produces fFN measurement results equivalent to those of the Rapid fFN 10Q System, when used with the Rapid fFN 10Q Test.

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, vaginal progesterone gel, Betadine® disinfectant, Monistat® cream). Lubricants or creams may physically interfere with 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: ampicillin (up to 100 µg/mL), cephalexin (up to 18 µg/mL), dexamethasone (up to 200 µg/mL), erythromycin (up to 10 µg/mL), gentamycin (up to 4 µg/mL), magnesium sulfate (up to 50 µg/mL), oxytocin (up to 100 µg/mL), prostaglandin E2 (up to 10 µg/mL), ritodrine (up to 10 µg/mL), and terbutaline (up to 100 µg/mL). These drugs did not interfere with the assay at the concentration limits cited above.

Earlier software version (1.0) of the Rapid fFN 10Q Analyzer

An earlier version of the Rapid fFN 10Q Analyzer reports positive and negative results in addition to the fFN concentration. If the fFN concentration is ≥ 50 ng/mL the result is POSITIVE. If the fFN concentration is < 50 ng/mL, the result is NEGATIVE. For more information about the quantitative result, see the Expected Values section above.

A multi-center study was conducted in the United Kingdom from October 2010 through April 2012 to evaluate the utility of the fFN concentration in predicting preterm birth risk. This prospective observational blinded study included 300 symptomatic women with singleton pregnancies who were sampled between 22 weeks, 0 days and 35 weeks, 6 days gestation (15). The results of this study were analyzed qualitatively using a 50 ng/mL cut-off. The prediction of risk of delivery within 7 and 14 days of sampling for the qualitative result are summarized in Table 10. The risk of delivery within the next 7 and 14 days in symptomatic women who test negative for fFN was 0.9% and 1.7%, respectively.

Table 10. Preterm Birth Risk by Qualitative Fetal Fibronectin Result

fFN Level	N (%)	Delivery ≤ 7 days	Delivery ≤ 14 days
Negative	231 (77.3%)	0.9%	1.7%
Positive	68 (22.7%)	10.3%	19.7%

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