Rapid Foetal Fibronectin (fFN) Using the Hologic Perilynx System

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Changes from last version of this document

Updated to new template

Updated from 10Q to Perilynx analyser

Contents:

1 Purpose and Principle

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. 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 centimetres) and uterine activity occur normally and are not necessarily diagnostic of imminent preterm delivery.

Detection of foetal fibronectin (fFN) in cervicovaginal secretions is associated with preterm delivery in symptomatic pregnant women between 22-35+6 weeks gestation.

The Hologic PeriLynx™ system is a quantitative test for the detection of foetal fibronectin and consists of the Rapid fFN 10Q cassette, the Rapid fFN® control kit, and the PeriLynx™ QCette used in the Perilynx analyser. The PeriLynx™ analyser is an electronic optical reflectance device that converts a colorimetric reaction from a cassette into a digitized format.

The cervicovaginal specimen is extracted into a supplied buffer and a 200 µL sample is dispensed onto 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-fFN antibody conjugated to blue microspheres (conjugate); the monoclonal antibody is FDC-6, specific for fFN. 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.

Once the sample is added, the analyzer will begin a 10-minute countdown. Upon completion of the 7-minute incubation period, the analyzer will begin the analysis of the cassette. The analysis will take approximately 2–3 minutes.

The Rapid fFN Perilynx System is to be used as an aid in assessing the risk of preterm delivery in pregnant women with signs and symptoms of early preterm labour, intact amniotic membranes and minimal cervical dilatation (< 3 cm), sampled between 22 weeks and 35weeks, 6 days gestation.

Women with a result of <50ng/ml are discharged and those with a result of >50ng/ml are admitted and monitored.

fFN Level (ng/ml)	% of patients	≤7 days	≤14 days	≤34 weeks
<10	57%	1%	1.8%	1.5%
10 – 49	21%	0%	1.6%	8.2%
50 – 199	14%	0%	7.7%	11.5%
200 – 499	5%	14%	29%	33%
≥500	4%	38%	46%	75%

2 References

- Hologic Perilynx User Guide (PC-INF-FIBRO)
- Hologic Perilynx Verification (PC-VERI-FIBRO)

3 Equipment

Analyser

Rapid fFN Perilynx System,

This analyser is under a service contract and in the event of breakdown please contact Hologic Technical Support telephone: 0800 032 3318

Printer
The Rapid Perilynx Analyzer and Printer should be operated at room temperature (15 to 30C).

Analyser Cassettes
Rapid fFN 10Q Cassettes: Catalog No: PRD-01018

26 cassettes containing all necessary reagents dried onto membranes. Each cassette contains a desiccant and is sealed in a foil pouch

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.

The Rapid fFN 10Q Cassette should be stored at room temperature (15 to 30C).

Liquid controls
Rapid fFN Control Kit, Catalog No: PRD-01019

1. Rapid fFN Positive Control: 2.5 mL (> 50ng/ml fFN)

2. Rapid fFN Negative Control: 2.5 mL (< 50ng/ml fFN).

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. Once opened, they should be used within 6 months. Upon opening a bottle of control, label with the new expiry date (i.e. 6months time). Controls should not be used if they are cloudy or discoloured.

The Rapid fFN 10Q liquid control kit should be stored in the refrigerated (2 to 8C).

Sample Collection Kit
Rapid fFN 10Q Specimen Collection Kit Catalog No: PRD-01020

25 sterile swabs

25 transport tubes containing 1ml extraction buffer

The Rapid fFN Test Specimen Collection Kit should be stored at 2 to 25C

Internal Control
Rapid fFN 10Q Qcette : Catalog No: PRD-01021

The Rapid fFN 10Q Qcette should be stored at room temperature (15 to 30C) in the container provided.

If a new Qcette is required refer to the user manual (Software Functions-Change set up-Qcette set up) for set up details.

Pipette
200µL fixed volume transfer pipette replaced every 6 months to ensure precision.

4 Personnel Authorised to Perform Procedure

These procedures must only be carried out by staff members who have received face-to-face Perilynx fibronectin training with POCT, the manufacturer or with an approved link trainer and completed competency paperwork. Evidence is recorded in Cobas IT and paperwork is stored in the X drive>Biochemistry >POCT >Training Logs. Competency is recertified every 2 years.

5 Sample Requirements (including COSHH Risk Assessment & First Aid)

A cervico-vaginal swab is obtained using the Rapid fFN 10Q System Specimen Collection Kit.

The swab should be taken from the posterior fornix of the vagina during a speculum examination. The polyester-tipped applicator provided in the Specimen Collection Kit should be inserted into the vagina and lightly rotated across the posterior fornix for approximately 10 seconds to absorb the cervico-vaginal secretions.

Once obtained the swab should be immersed in the tube of buffer provided with the Specimen Collection Kit and sealed. The tube should be labelled with the patient's name, unit number and NHS number.

Samples should be tested as soon as possible – preferably straight after collection.

Specimens that are not tested within eight hours of collection must be stored refrigerated at 2 to 8C and assayed within three days of collection.

Disclaimer – collection of the sample is classed as a medical procedure and therefore this text should be taken as a guide. The decision regarding how and whether to take a sample in individual patients is a matter for the patient’s consultant or other medical staff. For full details please refer to sampling kit insert.

Unacceptable Specimens

- PLEASE BE AWARE that in the following circumstances samples MAY be unsuitable: bleeding, presence of semen, ruptured membranes etc.
- Specimens collected in or by any sample device other than the Rapid fFN Perilynx System Specimen Collection Kit.
- Specimens with insufficient volume for testing.
- Unlabelled specimens.
- Specimen not tested within 8 hours of collection and specimens not stored refrigerated (2°–8°C) and tested within 72 hours.

- All human blood samples must be treated as potentially BIO-HAZARDOUS.
- Approved Personal Protective Equipment (PPE) including lab coats, gloves and eye-protection must be worn when handling open blood samples or derivatives thereof.

	When performed according to the protocol detailed in this SOP, and in conjunction with adherence to Trust Policies and Good Laboratory Practice, the handling of patient samples represents minimal risk to staff.

Exposure to Bio-Hazardous Material

In the event of a needle stick injury or accidental blood splashes to eyes or mouth:

- If skin has been punctured encourage bleeding by gently squeezing. Wash with soap and running warm water then dry and dress the wound.
- Splashes to the eyes: irrigate eyes thoroughly with eye wash / saline
- Splashes to the mouth: gargle with drinking water (avoid swallowing)

Contact the Occupational Health Department / Emergency Department for guidance and report all adverse incidents to your line manager / complete a DATIX form.

Disposal of Patient Samples

Ensure compliance with the Laboratory Medicine Policy for the Retention, Storage and Disposal of Laboratory Samples: LM-POL-RSDS.

6 Chemicals (including COSHH Risk Assessment & First Aid)

Name	COSHH Ref. No.	Classification & Specific Instructions
Extraction Buffer	 fFN safety SCK_MSDS .pdf	**Description:** Extraction buffer for Fibronectin sample extraction **Preparation:** Aqueous solution **Storage:** store between 2 -25°C **Supplier:** Hologic Risk Statement & Control Measures Use appropriate PPE Hazard Identification & First Aid Measures  HAZARDOUS/ IRRITANT

		Eye contact: flush eyes with running water for 15-20 minutes. Seek medical attention **Skin contact:** Flush skin with soap and water for 15-20 minutes. If irritation persists seek medical attention **Inhalation:** Remove to fresh air **Ingestion:** do not induce vomiting. Seek medical attention Liquid Waste Disposal- refer to local guidelines

GENERAL FIRST AID

The following first aid guidelines may be applied to all the substances detailed in this SOP.

Eyes: Irrigate thoroughly with water. At least 10 minutes is the recommended duration. Sterile saline is also available at the eye wash stations.

Lungs: Remove from exposure, rest and keep warm.

Skin: Wash substance off skin thoroughly with water. Remove contaminated clothing and wash before re-use.

Mouth: Wash out mouth thoroughly with water and give plenty of water to drink.

Remember – If at all concerned about the nature or severity of the problem, SEEK MEDICAL ADVICE.

7 Risk Assessment

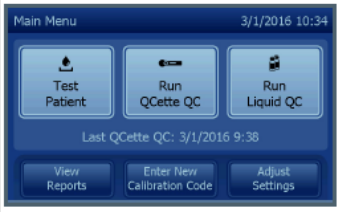
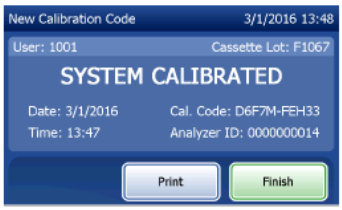
See PC-HSR-FIBRO for full risk assessment

COSHH and Risk Assessment

This SOP and the associated risk assessment(s) have considered all hazards and necessary precautions required to control any risks identified. Where appropriate this is detailed in the COSHH assessment and Risk Assessment. Any risk; where possible is mitigated and or monitored with health surveillance to ensure health and safety for all those affected by this procedure

8 Calibration

This should be performed with each new Cassette lot:

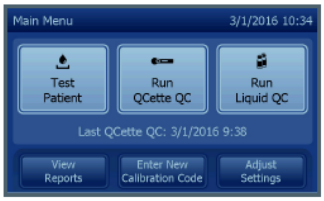
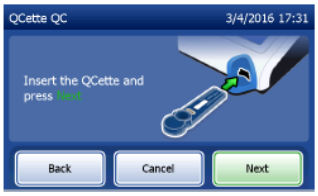
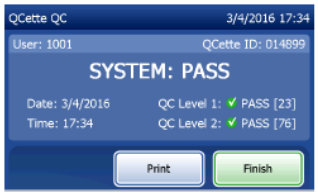
<table>
<tr><td>From the Main Menu, select Enter New Calibration Code.</td><td>Enter User ID and press Next.</td><td>Enter the cassette lot number (on cassette pouch) and press Next.</td></tr>
<tr><td> 
</td><td> 
</td><td> 
</td></tr>
<tr><td>Enter the Calibration Code (on the cassette box) and press Next.</td><td>Calibration results will be displayed and can be printed.
Press Finish to return to the Main Menu.</td><td></td></tr>
<tr><td> 
</td><td> 
</td><td></td></tr>
</table>

9 Quality Control

Internal controls monitor all components of the Rapid fFN Perilynx 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: Pass/Fail), and (4) proper function of analyser hardware (Analyzer: Pass/Fail).

9.1 Daily Quality Control

The Rapid fFN 10Q QCette is a quality control replica of a Cassette with printed test and control lines on its membrane. It is used to verify that the Rapid fFN Perilynx analyser performs within specification. Three different levels of response are measured with this QC device. It is performed as follows:

<table>
<tr><td>From the Main Menu select Run QCette QC.</td><td>Enter User ID and press Next.</td><td></td></tr>
<tr><td> 
</td><td> 
</td><td></td></tr>
<tr><td>Enter the QCette ID, or verify, if it is already entered. Press Next.</td><td>Insert QCette and press Next.</td><td>Results will be displayed and printed in 3 minutes.</td></tr>
<tr><td> 
</td><td> 
</td><td> 
</td></tr>
</table>

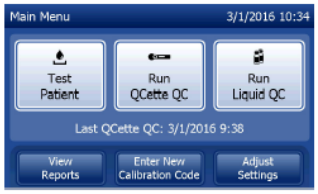
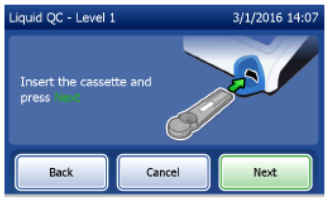
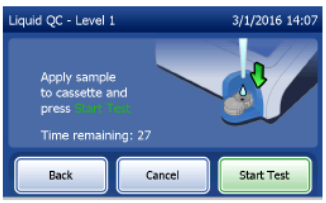
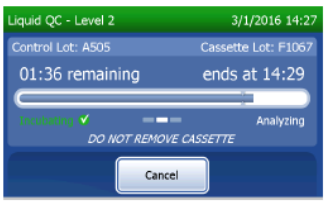
- When analysis is complete, the result will be displayed on the analyser screen and the printed label as “SYSTEM PASS & Level 1 PASS (numeric value), Level 2 PASS (numeric value)”. Place sticker with results in the Results Log.
- A FAIL or INVALID result should be repeated. If it fails again DO NOT USE the analyzer for a patient test – contact the POCT team on York 772 5890 Scarborough 771 2659
- Press ESC to return to the main menu.

9.2 Liquid Quality Control

Liquid Quality Control MUST be performed each time a new lot number or a new delivery of Rapid fFN 10Q Cassettes is received. This procedure is carried out by the POCT team. This is recorded

on the side of the Cassette box and on the delivery/lot verification forms which are then stored in on the X-drive-Biochemistry-POCT-Fibronectin-Lot verification..

The Rapid fFN Control Kit contains two liquid controls (Low & High) for use in monitoring the performance of the Rapid fFn 10Q Cassette. To ensure accurate and reliable test results, performance testing should be done only with the controls from the Rapid fFN Control Kit as follows:

From the Main Menu, select **Run Liquid QC**.	Enter the User ID and press **Next**.	Enter the Cassette Lot number (on cassette pouch) and press **Next**.
		
Select **Level 1** or **Level 2** and press **Next**.	Enter the Control Lot number (on bottle label and controls' box) and press **Next**.	Insert cassette and press **Next**.
		
The analyzer will check that a Rapid fFN 10Q cassette is properly inserted.	The instrument will beep repeatedly and the display will read, "Add sample to cassette and press Start Test." Add 200 µL of control sample and press **Start Test**.	The analyzer will begin a 10-minute countdown, with 7 minutes of incubation and 2–3 minutes of analysis of the cassette.
		
When testing is complete, the system will display and print the result. Select **Finish** to run the next control sample.	**Note:** Both a Level 1 and a Level 2 control sample must be run.	Repeat the test with the other control sample and a fresh cassette.
		

- Place sticker with the QC results in the Results logbook.
- The result will be displayed and printed as a numerical value which to PASS must be within the stated range for that level of QC. In the event of QC failure, the test should be repeated. If it fails again DO NOT USE the analyser for a patient test.

10 EQA

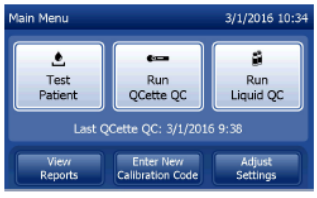
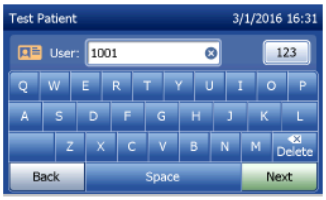
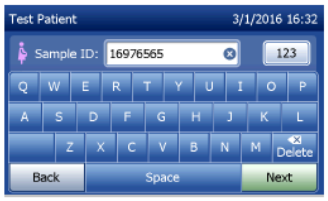
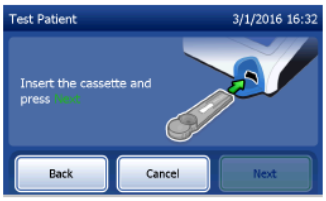
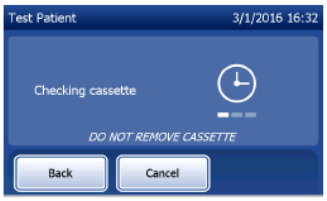
The analyser has been enrolled on the WEQAS EQA scheme. Samples will be sent out every 2 months and will be analysed by staff on the unit. EQA is monitored by the POCT Coordinator

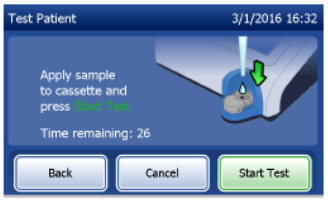
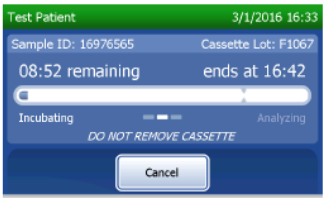
11 Method

11.1 Specimen Collection and Preparation

- The polyester-tipped swab from the Rapid fFN Perilynx System Specimen Collection Kit should be used. During speculum examination, obtain specimen by inserting the swab into the vagina and lightly rotating across the posterior fornix of the vagina for around 10 seconds to collect the cervico-vaginal secretions.
- Carefully remove swab from the vagina and immerse the tip in the tube of buffer provided with the Specimen Collection Kit. Break the shaft (at the score) even with the top of the tube. Align the shaft with the hole inside the tube cap and push down tightly over the shaft, sealing the tube. Gently mix the specimen tube.
- Label the tube with the patient's name, unit number and NHS number.
- Open the specimen tube cap and applicator assembly. The applicator shaft should be seated in the cap. Express as much liquid as possible from the applicator by rolling the tip against the inside of the tube. Dispose of the used applicator in clinical waste.

11.2 Patient Test

<table>
<tr><td>From the Main Menu select Test Patient.</td><td>Enter User ID and press Next.</td><td>Enter the cassette lot number (on cassette pouch) and press Next.</td></tr>
<tr><td> 
</td><td> 
</td><td> 
</td></tr>
<tr><td>Enter the patient accession number and press Next.</td><td>Remove the patient cassette from its pouch.
Insert the cassette into the analyzer and press Next.</td><td>The analyzer will check that a Rapid fFN 10Q Cassette is properly inserted.</td></tr>
<tr><td> 
</td><td> 
</td><td> 
</td></tr>
</table>

The instrument will beep repeatedly and the display will read, "Apply sample to cassette and press Start Test." Add 200 µL of patient sample and press **Start Test**.	The analyzer will begin a 10-minute countdown, with 7 minutes of incubation and 2–3 minutes of analysis of the cassette.	When testing is complete, the system will display and print the result.
		

12 Reporting of Results

- The fFN result for the patient sample will be displayed on the Rapid fFN Perilynx analyzer display screen as a quantitative value in ng/ml.
- The result will be reported as INVALID if the test does not meet internal quality controls. If the assay is reported as INVALID then retest with 200µL of additional sample, if available, on a new Rapid fFN Cassette.
- The interpretation of the result MUST be done by the Medical staff treating the patient in conjunction with the clinical presentation and all other available test information.

13 Reference Ranges

The ranges have been set by the Obstetrics and Gynecology consultants at York Hospital

Result	Action
1-50ng/ml	Patient allowed home. Monitor.
>50ng/ml	Patient should be admitted.

14 Assay Performance & Known 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 less than or equal to 7 or 14 days from specimen collection in symptomatic women or delivery in less than or equal to 34 weeks, 6 days in asymptomatic women evaluated between 22 weeks, 0 days and 30 weeks, 6 days of gestation. A raised level of fFN 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.

The assay has been optimized with specimens taken from the posterior fornix of the vagina. Samples obtained from other locations should not be used.

The safety and effectiveness of using a cut-off other than that provided by the Rapid fFN 10Q Cassette Calibration Code has not been established.

Assay interference from the following components has not been ruled out:

- Douches
- White blood cells
- Red blood cells
- Bacteria
- Bilirubin
- Semen (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 fetal fibronectin test result is valid).

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

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

Care must be taken not to contaminate the applicator 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 applicator or with the antibody-antigen reaction of the Rapid fFN test.

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

15 General maintenance

15.1 Cleaning the Analyser

If required, the analyser can be cleaned with a damp cloth and any of the following cleaning agents

- 70% ethanol
- General laboratory cleaning detergent with disinfectant properties

Take care that no liquids enter interior of the analyser.

15.2 Loading the Printer Labels

When the printer is out of labels the power light will flash. Open the cover if the last label is still attached to the roll core cut the tape and feed the last labels through the printer using the form feed button.

- Open the printer cover
- Remove the label spool from the printer. There are distinct left and right sides.
- Remove the right side of the spool by sliding it off the right end
- Slide the roll of labels over the spool.
- Re attach the right side of the spool making sure there is no gap between the roll and the spool. The labels will feed from the bottom of the roll
- Turn the printer on (if off) and feed the labels into the slot on the inside of the printer. Continue pushing gently until the printer begins to feed the labels through automatically.

- Insert the spool into the printer
- Close the printer lid

16 Troubleshooting

Please see manual or contact the POCT team on

York 772 5890

Scarborough 771 2659