**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION SUMMARY**
**ASSAY ONLY TEMPLATE**

**A. 510(k) Number:**

K110605

**B. Purpose for Submission:**

New assay

**C. Measurand:**

AFP (alpha-fetoprotein) and PP12 (placental protein 12)

**D. Type of Test:**

Qualitative immunochromatographic test

**E. Applicant:**

Clinical Innovations LLC

**F. Proprietary and Established Names:**

ROM Plus Fetal Membranes Rupture Rest; Rom Plus Quality Control Kit

**G. Regulatory Information:**

1. Regulation section:

   21 CFR 862.1550, Urinary pH;

   21 CFR 862.1660, Quality Control Material

2. Classification: Class I, meets limitations of exemptions under 21 CFR 862.9 for near-patient testing, Class I, reserved

3. Product code:

   NQM; JJX

4. Panel:

Chemistry (75)

**H. Intended Use:**

1. Intended use(s):
   See indications for use.

2. Indication(s) for use:
   The Clinical Innovations ROM Plus fetal membrane rupture test is a rapid, qualitative immunochromatographic test for the in vitro detection of amniotic fluid in vaginal secretions of pregnant women with signs and symptoms of ROM. The test detects AFP (alpha-fetoprotein) and PP12 (placental protein 12 or insulin growth factor binding protein) from amniotic fluid in vaginal secretion. The test is for prescription use by health care professionals to aid in the detection of rupture of membranes (ROM) in pregnant women in conjunction with other signs and symptoms.

   The ROM Plus Quality Control Kit monitors the performance of the ROM Plus Fetal Membranes Rupture Test for the purposes of external quality control. The lyophilized human positive protein control is an assayed control material for qualitative testing.

3. Special conditions for use statement(s):

   The package insert contains the following warning:
   **The test may report positive results in patients with intact membranes (see specificity in the performance section) and therefore decisions to induce labor should not be based solely on the ROM Plus test results.**

4. Special instrument requirements:

   No instrument required; this is a visually-read test.

**I. Device Description:**

Each individual test pack contains a sterile polyester swab, specimen extraction buffer solution in a plastic vial and a cassette with integral timer containing the lateral flow strip packed in a foil pouch with desiccant.

The ROM Plus Controls contain one vial each of negative, and positive controls, and reconstitution solution.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

AmniSure™ ROM (Rupture Of fetal Membranes) Test

2. Predicate 510(k) number(s):

k081767

3. Comparison with predicate:

Both devices are qualitative, lateral flow immunochromatographic assays intended to aid in detecting rupture of fetal membranes in pregnant women. The specimen collection and extraction, test procedure, and reading and interpretation of results are similar between devices. Detection of results is by visual inspection for both tests. Both devices are for use in prescription use point-of-care settings.

| | New device | Predicate |
|---|---|---|
| **Intended use/Indications** | The Clinical Innovations ROM Plus fetal membrane rupture test is a rapid, qualitative immunochromatographic test for the in vitro detection of amniotic fluid in vaginal secretions of pregnant women. The test is for use by health care professionals in conjunction with other clinical evaluations to aid in the detection of ROM when rupture is suspected. | Same |
| **Analytes** | AFP (alpha fetoprotein) and PP-12 (also referred to by the sponsor as IGFBP-1, or insulin growth factor binding protein 1) | PAMG-1 protein |
| **Reading/Result** | Visually-read qualitative test | Same |
| **Detection limits** | Detects PP-12 at 5 ng/mL and AFP at 150 ng/mL | Detects PAMG-1 at 5-7 ng/mL. |
| **Methodology** | Lateral flow immunochromatographic method | same |
| **Timing mechanism** | Integrated timer | External timer. |
| **Control Solutions** | Positive and negative controls available | Positive and negative controls available |

**K. Standard/Guidance Document Referenced (if applicable):**

None were referenced.

**L. Test Principle:**

The ROM Plus test uses a combination of antibodies to the PP12 and AFP proteins, including monoclonal mouse anti-human (anti-AFP and PP-12) and polyclonal sheep anti-PP-12 and goat anti-AFP. Patient vaginal secretion sample is collected and extracted using the extraction buffer. The sample is collected by placing the swab on the vaginal mucosal lining for 15 seconds. The swab is then mixed into a vial containing 400 µL of buffer solution, and the diluted sample is applied to the sample pad of the test strip via the sample well on the cassette. The liquid moves chromatographically and unidirectionally towards the absorbent pad. During migration, the sample reacts with the antibodies bound to the test strip membrane In addition, as the membrane absorbs the liquid sample, a control line will appear, indicating a sample was applied. If the sample contains the PP12 and/or AFP above the detection limit, it binds to the antibody of the test line, causing the test line to appear and indicating a positive result. If the sample does not contain the PP12 and/or AFP above the detection limit, only the control line will be visible, indicating a negative result.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

*a. Precision/Reproducibility:*

See Sensitivity section below for reproducibility evaluations near the LoD.

*b. Linearity/assay reportable range:*

The test was evaluated for hook effect up to PP12 of 400 ug/mL, and AFP of 200 ug/mL. These high concentrations still showed positive results.

*c. Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability:
The device is traceable to an ELISA method that uses purified PP-12 and AFP as standards.

Stability:
The methods for stability testing was reviewed and found to be adequate to support the claims for control material storage and dating.
Control materials:

Control materials are prepared from amniotic fluid. Concentrations are determined based on an ELISA method and contain 20 ng/mL PP12 and 600 ng/mL AFP. Each control kit contains: One Positive Control Vial (self-contained glass ampoule of buffer) and One Negative Control Vial (self-contained glass ampoule of buffer).

*d. Detection limit:*

Samples were prepared from purified AFP and PP-12 in a matrix with the same salinity, pH, and a total protein concentration designed to be similar to amniotic fluid. Protein concentrations determined were based on protein analytical methods.

Testing was performed at external sites. Each protein separately and a combined mixture (AFP and PP12 together) were evaluated at five concentrations near (above and below) the cutoff.

Testing at concentrations near C50, was carried out using 3 lots, under two lighting conditions (fluorescent light and light from window), by three operators. All sample concentrations were masked. A summary of results is shown below:

| **PP-12 concen-tration (ng/mL)** | **AFP concen-tration (ng/mL)** | **ROM Plus Lot #** | **Lighting** | **Fraction Test Positive** | | |
|---|---|---|---|---|---|---|
| | | | | **PP 12** | **AFP** | **mixture** |
| 20 | 600 | 1-3 | Fluorescent | 6/6 | 6/6 | 6/6 |
| 20 | 600 | 1-3 | outdoor window | 6/6 | 6/6 | 6/6 |
| 10 | 300 | 1-3 | Fluorescent | 6/6 | 6/6 | 6/6 |
| 10 | 300 | 1-3 | outdoor window | 6/6 | 6/6 | 6/6 |
| 5 | 150 | 1-3 | Fluorescent | 6/6 | 6/6 | 6/6 |
| 5 | 150 | 1-3 | outdoor window | 6/6 | 6/6 | 6/6 |
| 2.5 | 75 | 1-3 | Fluorescent | 0/6 | 1/6 | 0/6 |
| 2.5 | 75 | 1-3 | outdoor window | 0/6 | 1/6 | 0/6 |
| 1.25 | 37.5 | 1-3 | Fluorescent | 0/6 | 0/6 | 0/6 |
| 1.25 | 37.5 | 1-3 | outdoor window | 0/6 | 0/6 | 0/6 |

Using analyses recommended in CLSI EP12 the following results were determined:
2.5ng/mL< C50 < 5ng/mL for PP-12.
75 ng/mL <C50 <150 ng/mL for AFP.

All results were positive for concentrations 5 ng/mL and above; and all results were negative for concentrations of 2.5 ng/mL and below for PP-12. All results were positive for concentrations of 150 ng/mL and above; and all results, except for one operator reading one sample were negative for concentrations of 75 ng/mL and below for AFP.

*e. Analytical specificity:*
Blood, urine and semen were spiked into a low positive sample and the negative control at a high level (10% of sample volume) and run in the ROM Plus test. There was no effect on either the positive control or the negative control. In addition, common potentially interfering substances including aspirin, Tylenol, Pert shampoo, Noxzema skin moisturizer and Lever bath soap were spiked into both the low positive and negative control at a final concentration of 0.1% with no effect observed. Higher levels of blood or urine may interfere.

Samples from subjects with vaginal infections were tested using the ROM Plus tests. Infections tested were Bacterial Vaginosis (Candidiasis, Trichomoniasis, Gardnerella), STS infections (Chlamydia, syphilis, Gonorrhea and Herpes and HIV). All gave non-positive results. The sponsor concluded that samples with vaginal infections did not produce positive results.

*f. Assay cut-off:*

The cutoff concentration for PP-12 is set at 5 ng/mL, and the cutoff concentration for AFP is set at 150 ng/mL.

2. <u>Comparison studies:</u>

*a. Method comparison with predicate device:*

See "other clinical supportive data" below.

*b Matrix comparison*

Not applicable.

3. Clinical studies:

*a. Clinical Sensitivity:*

Not applicable.

*b. Clinical specificity:*

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

Study Design:

Prospective clinical studies were performed at 3 US sites.
Inclusion Criteria were healthy pregnant women with signs or symptoms of rupture of membranes. Exclusion Criteria were known placental previa or active vaginal bleeding

Women in the study were evaluated by sterile speculum exam. Afterward, a second examiner (nurse or physician) who did not know the results of the conventional diagnosis read the results of the ROM Plus test. The test was compared to visual leaking from cervical os, or (if this leaking was not observed) pooling of amniotic fluid in the posterior fornix, nitrazine testing, and ferning. Visual leaking or two out of three positive results for, the ferning, pooling and nitrazine tests were considered positive. Results are shown below for the three study sites combined:

**RESULTS**

| Combined | Clin-Assess | positive | negative |
|---|---|---|---|
| ROM Plus | positive | 153 | 28 |
| | negative | 1 | 82 |
| Total: 264 | | | |
| Sens | 0.99 | CI: 0.96 to 1.00 | |
| Spec | 0.75 | CI: 0.66 to 0.82 | |
| PPV | 0.85 | CI: 0.79 to 0.90 | |
| NPV | 0.99 | CI: 0.94 to 1.00 | |

| ≥ 37 | Clin-Assess | positive | negative |
|---|---|---|---|
| ROM Plus | positive | 125 | 23 |
| | negative | 1 | 33 |
| Total: 182 | | | |
| Sens | 0.99 | CI: 0.96 to 1.00 | |
| Spec | 0.58 | CI: 0.46 to 0.71 | |
| PPV | 0.84 | CI: 0.78 to 0.89 | |
| NPV | 0.97 | CI: 0.85 to 0.99 | |

| 34-37 EGA | Clin-Assess | positive | negative |
|---|---|---|---|
| ROM Plus | positive | 18 | 3 |
| | negative | 0 | 16 |
| Total: 37 | | | |
| Sens | 1.00 | CI: 0.82 to 1.00 | |
| Spec | 0.85 | CI: 0.64 to 0.95 | |
| PPV | 0.87 | CI: 0.65 to 0.95 | |
| NPV | 1.00 | CI: 0.81 to 1.00 | |

| < 34 | Clin-Assess | positive | negative |
|---|---|---|---|
| ROM Plus | positive | 10 | 2 |
| | negative | 0 | 33 |
| Total: 45 | | | |
| Sens | 1.00 | CI: 0.72 to 1.00 | |
| Spec | 0.94 | CI: 0.81 to 0.98 | |
| PPV | 0.83 | CI: 0.55 to 0.95 | |
| NPV | 1.00 | CI: 0.90 to 1.00 | |

Additional evaluations, including patients ≤ 34 full weeks are shown below. (The table contains some supplementary samples not included in the initial study shown above):

| Gestational Age ≤ 34 weeks | | | | |
|---|---|---|---|---|
| | | Initial clinical assessment (visual leaking or 2/3 ferning pooling and nitrazine) | | |
| | | Pos | Neg | Total |
| ROM Plus | Pos | 20 | 4 | 24 |
| | Neg | 0 | 39 | 39 |
| | Total | 20 | 43 | 63 |
| | | | | |
| Positive percent agreement [95% confidence intervals]<br>100% [84 to 100%] | | | | |
| Negative percent agreement [95% confidence intervals]<br>91% [78 to 96%] | | | | |
| | | | | |

4. Clinical cut-off**:**

5. Expected values/Reference range:

The sponsor includes the following in the expected range section in the package insert: IGFBP-1 concentration in amniotic fluid as determined in literature studies in amniotic fluid is between 10,500 and 350,000 ng/ml and for AFP from 2,800 to 26,000 ng/ml and in serum from 55 to 242 U/ml (equivalent to 33 to 290 ng/ml). Concentrations of IGFBP-12 (PP12) in amniotic fluid observed PP12 to be 100 to 1000 times higher than that in maternal serum. These studies also showed that concentrations of these proteins in urine to be negligible.

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.