INTENDED USE

FebriDx is a rapid immunoassay for the visual, qualitative, \textit{in vitro} detection of elevated levels of both MxA and CRP directly from peripheral whole blood. The test measures a clinically significant immune response to a suspected invasive viral and/or bacterial infection in patients older than 2 years that present within 3 days of an acute onset fever (exhibited or reported) and within 7 days of new onset respiratory symptoms consistent with a community-acquired upper respiratory infection.

The FebriDx test aids in the clinical identification of patients with an underlying invasive viral infection from either Influenza A/B, Adenovirus, Respiratory Syncytial Virus, Metapneumovirus, Parainfluenza Virus, or Epstein-Barr Virus; and/or patients with a clinically significant immune response consistent with an underlying bacterial infection.

The test is intended for professional use in an outpatient setting and should be used in conjunction with other clinical evidence including laboratory, radiographic, and epidemiological information.

Negative results do not preclude respiratory infection (e.g. rhinovirus, coronavirus) and should not be used as the sole basis for diagnosis, treatment, or other clinical and patient management decisions. In addition to utilizing radiography and clinical presentation to aid in diagnosis, additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and polymerase chain reaction [PCR]) may be used to confirm whether a specific respiratory pathogen exists.

SUMMARY AND EXPLANATION OF THE TEST

Viral and bacterial acute respiratory infections (ARIs) are highly contagious and represent a major source of morbidity, mortality, and healthcare costs. Due to the significant overlap in symptoms and signs, clinically differentiating the etiology of these infections is challenging, often leading to frequent and unnecessary antibiotic use. This is especially true in the outpatient setting where access to laboratory diagnostics is expensive, time consuming, and requires several days to produce a result. Bacterial cultures are typically only performed in cases of severe infection, such as pneumonia, or when the consequence of missing a diagnosis can lead to severe complications, such as with Strep throat. New viral screening PCR panels are useful but they are costly, and do not provide clinically relevant information at the point of care.

The FebriDx test is a rapid, point-of-care lateral flow immunoassay that uses a fingerstick blood sample to identify and differentiate a clinically significant immune response to viral and/or bacterial acute febrile respiratory infection.

BIOMARKERS

MxA

Myxovirus resistance A (MxA) is a derivative of interferon alpha/beta cells that becomes elevated in the presence of acute viral infection, but is not specific to a particular type of virus. MxA has a low basal concentration of less than 50 ng/ml, a fast induction time of 1-2 hours, and long half-life of 2.3 days, making it an ideal marker for viral infection.\textsuperscript{1,2} MxA levels peak at 16 hours and remain elevated in the presence of elevated interferon.\textsuperscript{3} Viral infections elevate MxA levels while only having a modest increase in CRP levels.\textsuperscript{4,7} MxA protein expression in peripheral blood has been shown to be a sensitive and specific marker for viral infection.\textsuperscript{8-13}
CRP

C-reactive protein (CRP) is a nonspecific indicator for the presence of acute inflammation and is elevated in the presence of bacterial infection. CRP is an acute-phase protein with normal serum concentrations of less than 3 mg/L that increases during an inflammatory process, especially following severe infection. In the presence of severe infection or inflammation, CRP levels can rise above 500 mg/L. Bacterial infection is a potent stimulus of marked CRP elevation, which occurs within 4-6 hours after stimulation and peaks after 36 hours. The administration of antibiotic treatment causes CRP levels to fall rapidly.

Multiplexed Pattern of Results

In isolation, neither MxA nor CRP alone is sensitive or specific enough to differentiate viral from bacterial infection. However, by simultaneously examining low and high levels of CRP, each in combination with the presence of elevated MxA, it is possible to classify patients with viral versus bacterial etiology of infection.

The FebriDx test produces a multiplexed pattern of results consisting of clinically relevant cut-off levels of low CRP, high CRP, and MxA, together providing a sensitive and specific way to identify patients with a clinically significant immune response consistent with a suspected respiratory infection from those patients with a microbiologically unconfirmed respiratory illness (MURI). As such, the test will also help aid in the differentiation of viral and/or bacterial acute febrile respiratory infection.

PRINCIPLES OF THE TEST

The FebriDx test is a lateral flow immunoassay that utilizes direct sampling micro-filtration technology. The test utilizes two lateral flow test strips contained in the same plastic housing and monoclonal anti-MxA and anti-CRP antibodies. One test strip contains a control line and two result lines (MxA and low CRP). The cut-off value for MxA is 40 ng/ml and the cut-off value for low CRP is 20 mg/L serum equivalent. The second test strip contains a control line and a single result line (high CRP). The cut-off value for high CRP is 65 mg/L serum equivalent.

If the fingerstick blood samples contain elevated levels of MxA, low CRP, or high CRP, above their respective cut-off levels, the appropriate test line will appear in the result window. FebriDx is a disposable, rapid test requiring at least 15 minutes for a result.

Materials Provided

- 20 test cards
- 22 accessory kits (1 lancet, 2 pipettes)
- 22 buffer solution tubes
- 1 package insert
- 1 quick reference card

Materials Not Provided

- Timer
- Gloves
- Alcohol pad
- Gauze

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use only.
2. Keep the FebriDx test card in the sealed foil pouch until just before use.
3. Do not use the FebriDx test card or the buffer solution past the expiration date.
4. Use standard precautions for collecting and handling a blood sample.
5. All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent. Proper handling and disposal methods should be established according to local, state, and federal regulations.
6. Wear disposable gloves while handling samples and wash hands after the test is complete.
7. The lancet is sterile until the protective cap is removed. Do not use the lancet if the protective cap is not secured in place.
8. After the two (2) blood samples are applied to the test card and it is snapped closed, buffer solution should be applied immediately into the blue activation window. The test must be run within one (1) minute after closing the flap.
9. The FebriDx test card, pipettes, lancet, and buffer solution are single use items. Do not reuse with multiple specimens.
10. The FebriDx test requires a visual readout. Do not interpret the test result if you have color-impaired vision.
11. Result interpretation requires a brightly lit environment.
STORAGE AND STABILITY

Store the FebriDx test between 77ºF/25ºC and 39ºF/4ºC. The FebriDx test card and the buffer solution are stable until the expiration dates printed on their packaging.

TEST COMPONENTS

Check the expiration date on all packaging. Discontinue use if any components are expired.

Preparing the Test

1. Tear open the foil pouch at the indicated perforation and remove the test card.
2. Place the test card on a flat surface with the FebriDx logo facing upright.
3. Grasp the flap at the red arrow and lift the flap to the left to expose the sample application zone.
   
   Note: Gently bend the flap to the left to ensure it is fully open. Do not close the flap.

Collecting and Transferring the Blood Sample

   Note: Use standard precautions for collecting and handling a blood sample.

1. Cleanse the fingertip with an alcohol pad and allow it to air dry.
2. Locate the lancet and remove the protective cap. Firmly press the lancet to puncture the skin. Wipe away the first drop of blood with gauze and gently massage towards the puncture site to encourage blood flow.
   
   Note: The provided lancet was specifically chosen for use with the FebriDx test. Other lancets may not produce an adequate amount of blood required to run the FebriDx test and a second fingerstick sample on an alternate puncture site may be needed.
3. Hold one of the pipettes horizontally and collect 5 μl of blood from the puncture site.
   
   Note: Do NOT squeeze the bulb of the pipette. Capillary action will automatically draw the blood sample into the pipette in the required amount (5 μl) to reach the black fill line.
4. Position the pipette over the blue strip in the sample application zone. Touch the tip of the pipette to the strip and squeeze the pipette bulb to transfer the full amount of blood. After the sample is transferred, carefully discard the contaminated pipette into a medical waste container.
5. Hold the second pipette horizontally and collect another 5 μl of blood from the puncture site. If an adequate amount of blood sample is not present, it may be necessary to gently massage towards the puncture site to encourage blood flow.
   
   Note: Do NOT squeeze the bulb of the pipette. Capillary action will automatically draw the blood sample into the pipette in the required amount (5 μl) to reach the black fill line.
6. Position the pipette over the purple strip in the sample application zone. Touch the tip of the pipette to the strip and squeeze the pipette bulb to transfer the full amount of blood. After the sample is transferred, carefully discard the contaminated pipette into a medical waste container.
7. Close the flap. Using two thumbs, press down on the black fingerprints to snap the pegs into place.
   
   Note: Do not attempt to re-open the test card after it is snapped closed.
Running the Test

1. Locate the buffer solution. Hold the tube upright and twist off the cap.

2. Slowly squeeze the buffer solution into the blue activation window until all the liquid has been emptied, ensuring that none of the liquid spills onto the test card.

   Note: Squeeze the tube several times to ensure all of the liquid has been emptied.

3. Lay the test card on a flat surface for 15 minutes.

TEST RESULTS

A blood fluid wave is observed moving across the result windows while the test is running. If a blood fluid wave is not visible after approximately one (1) minute, locate another buffer solution tube and slowly squeeze to add more liquid (4-5 drops) into the blue activation window. Once the majority of the background in each result window has cleared (aside from minor peripheral blood streaks along the sides of each result window) and at least 15 minutes have elapsed, the test may be accurately read.

Note: If the majority of the background has not cleared after 15 minutes, allow additional development time prior to interpretation. If the majority of the background has not cleared sufficiently for interpretation of results after 30 minutes of development time, the test cannot be accurately read and must be discarded. Use a new FebriDx test card to retest the patient.

FebriDx test results are stable for up to three (3) hours. Do not interpret the test results after this period of time.

A blue control line must appear in each result window for the test to be valid.

Positive Result

The positive result lines appear as red or black lines in the result windows. An uneven or incomplete result line is due to an uneven sample distribution on the test strip. Even if the result line is faint in color, incomplete over the width of the test strip, or uneven in color, it should be interpreted as positive. A positive result indicates the presence of elevated MxA and/or CRP antigens.

Negative Result

If only a blue control line is visible in both result windows, the test should be interpreted as negative. A negative result indicates a lack of elevated MxA and CRP antigens.

Invalid Result

A blue control line must appear in each result window for the test to be valid. The absence of two blue control lines indicates an invalid result.

If an invalid result occurs, the test must be discarded and the patient retested using a new FebriDx test card. Choose an alternative puncture site on a different finger when retesting the patient.

*During the FebriDx multicenter, prospective clinical trial, 10 patients were categorized as viral infection with a combined MxA elevation in the presence of both elevated low and high CRP. Of these 10 patients, one was confirmed to have elevated Procalcitonin (PCT) consistent with a bacterial infection and a high MxA, but there was no associated bacterial cell culture growth and no viral pathogen detected by PCR. Nine out of 10 patients were either confirmed viral by PCR or were confirmed negative for a clinically significant infection.
Procedural Controls

The FebriDx test contains the following built-in procedural controls. For daily quality control, RPS recommends documenting that these internal procedural controls are checked for the first sample tested each day.

Unused test

An unused FebriDx test card has faint orange lines in each result window, indicating the potential appearance of control and result lines.

Fluid wave

A blood fluid wave is observed moving across the result windows while the test is running. Once the majority of the background in each result window has cleared and at least 15 minutes have elapsed, the test may be accurately read.

Note: If the majority of the background has not cleared after 15 minutes, allow additional development time prior to interpretation. If the majority of the background has not cleared sufficiently for interpretation of results after 30 minutes of development time, the test cannot be accurately read and must be discarded. Use a new FebriDx test card to retest the patient.

Control lines

A blue control line must appear in each result window for the test to be valid. The absence of two blue control lines indicates an invalid result.

LIMITATIONS

1. The FebriDx test is best used within three (3) days of acute onset fever and seven (7) days of new onset respiratory symptoms.

2. Fresh capillary blood (fingerstick) must be used on the FebriDx test.

3. Five (5) μl of blood must be applied to each test strip in order for the test to run properly. To ensure delivery of adequate volume (5 μl) on each test strip, the blood sample must reach the black fill line of the pipette. An erroneous result may occur if an insufficient blood sample is applied to the test.

4. The following conditions may lead to erroneous results:
   - Current immunosuppressive state or use of immunosuppressive drugs
   - Current use of oral anti-infective drugs
   - Current use of interferon therapy (e.g. for multiple sclerosis, HIV, HBV, HCV)
   - Live viral immunization within the last 30 days
   - Major trauma, major surgical intervention, and severe burns within the preceding 30 days
   - Chronic fevers lasting more than 7 days

5. Many bacteria, including Streptococcus species, Staphylococcus species, Hemophilus species, and enterobacteria species, as well as viruses such as rhinovirus, coronaviruses, Herpes Simplex Virus, Epstein-Barr Virus, and Cytomegalovirus are known to colonize the respiratory tract and their significance is unknown. Colonization of viral or bacterial pathogens or periodic viral shedding without an invasive systemic response will not be detected.

EXPECTED VALUES

The prevalence of ARI varies during the year and from region to region, with outbreaks typically occurring during fall and winter. In the United States, there are approximately 76 million physician office visits annually for ARI. Additionally, fever has been shown to represent 19-30% of all pediatric office visits, making it the most common presenting sign of illness. In developed countries, ARIs are the leading cause of morbidity accounting for: 20% of medical consultations, 30% of absenteeism, and 75% of all antibiotic prescriptions.

PERFORMANCE CHARACTERISTICS

371 patients were enrolled as part of a prospective, multicenter, blinded clinical trial performed with trained and untrained operators to determine the positive and negative agreement of the FebriDx test at identifying a clinically significant immune response to viral and/or bacterial community-acquired acute febrile respiratory infection as compared to the evaluation of expert clinical reviewers and the results of clinical standardized microbiologic, laboratory, and/or radiological testing. This study was performed on patients older than 1 year that presented within 3 days of an acute onset fever and within 7 days of new onset respiratory symptoms consistent with a community-acquired acute febrile respiratory infection to primary care and urgent care outpatient offices and within 3 days of new onset respiratory symptoms to emergency departments.
FebriDx test results were compared against the microbiological and laboratory testing listed below.

**Viral testing:**
- BioFire FilmArray® Respiratory Panel PCR: Influenza A/B, Adenovirus, Respiratory Syncytial Virus 1-2, Parainfluenza Virus 1-4, Metapneumovirus
- Supplemental real-time PCR for Epstein-Barr Virus

**Bacterial testing:**
- BioFire FilmArray® Respiratory Panel PCR testing for atypical bacteria: *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Bordetella pertussis*
- Oropharyngeal cultures (blood, chocolate, and MacConkey plates)

**Laboratory testing:** Procalcitonin (PCT) and white blood cell (WBC) count

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**Upper Respiratory Infection (URI)**

All microbiological and laboratory testing (except MxA and CRP) will be evaluated in conjunction with history, physical exam, and other tests as part of standard of care, etc. by 2 clinical medical experts.

**NP + OP Sample for PCR**

- (+) Real-time PCR EBV
- (+) Real-time PCR EBV
- (+) EBV
- (+) EBV
- (+) Influenza A/B, Parainfluenza 1-4, Metapneumovirus, Adenovirus, RSV
- (+) Influenza A/B, Parainfluenza 1-4, Metapneumovirus, Adenovirus, RSV

**OP Sample for Cell Culture**

- (+) Bordetella, Chlamydia, Mycoplasma
- (+) Group A Strep
- (+) Group C Strep
- Other primary bacteria
- Any bacteria
- Group A Strep
- Group C Strep
- Other primary bacteria
- Any bacteria except Group A or Group B Strep

**Microbiological Confirmation**

- Viral
- Bacterial

**Colonization**

- PCT ≥ 0.1 ng/ml
- 0.15 ng/ml ≤ PCT < 0.25 ng/ml
- AND WBC < 12,000
- AND no bands

- PCT ≥ 0.25 ng/ml or 0.15 ng/ml ≤ PCT < 0.25 ng/ml
- AND WBC ≥ 12,000 or the presence of bands

**Microbiologically Unconfirmed**

- PCT < 0.15 ng/ml

**Final Clinical Diagnosis**

- (Negative, Viral, Bacterial)

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[1] Rhinovirus and coronavirus positive BioFire PCR results will be treated as colonization and defined as microbiologically unconfirmed.

[2] Since PCR is highly sensitive, it is unlikely that a viral or atypical bacteria will not be detected; thus any elevated PCT ≥ 0.1 ng/ml is more likely bacterial.

*Neisseria gonorrheae, Corynebacterium diphtheria, Arcanobacterium haemolyticum*

Pharyngeal bacterial colonization was differentiated from true infections if cell culture growth occurred in association with an elevated PCT level. Patients without microbiological confirmation were further categorized as bacterial if the patients demonstrated an elevated PCT. Any patients without microbiologically confirmed respiratory infection and a normal PCT were deemed negative for clinically significant infection.

### FEBRIDX PERFORMANCE DATA

**N = 371 patients enrolled**

- 165 asymptomatic patients
- 205 URI patients
  - 38% confirmed infectious by gold standards
    - 12% Bacterial
    - 26% Viral
  - 62% MURI

Two invalid tests occurred while testing asymptomatic patients. One URI patient had no cultures or blood work performed so this patient data was excluded.
FEBRIDX PERFORMANCE VALUES

Asymptomatic patients
- Negative Agreement = 99% (161/163)

Symptomatic patients

- **Bacterial**
  - Positive Agreement = 80% (20/25)
    - 95% CI [60.8 to 91.1]
  - Negative Agreement = 94% (169/180)
    - 95% CI [89.4 to 96.6]

- **Viral**
  - Positive Agreement = 87% (46/53)
    - 95% CI [75.1 to 93.5]
  - Negative Agreement = 83%* (126/152)
    - 95% CI [76.1 to 88.1]

*Colonization of viral or bacterial pathogens or periodic viral shedding without an invasive systemic response was not detected. The presence of elevated PCT and/or WBC in association with known pathogens was required to differentiate bacterial colonization from active infection. Since rhinovirus and coronavirus are frequent colonizers of the respiratory tract and only cause a clinically significant active infection in approximately 10% of patients, these two viruses are not included in the intended use. During the FebriDx prospective, multicenter clinical trial, rhinovirus was confirmed present by PCR in 52 subjects; however, only 8/52 patients actually demonstrated an elevation in MxA. Of those patients with confirmed rhinovirus and elevated MxA, FebriDx correctly identified 5/8. Because rhinovirus is not included in the intended use and 8 patients had an elevated MxA, these patients were deemed “false positive,” despite being correct, which led to an artificially lower viral specificity.

**INTERFERING SUBSTANCES**

The analytical specificity of the FebriDx test was determined by evaluating a series of interfering substances mixed with samples with and without MxA and CRP antigens at their C₉₀ (LoD) and C₅₀ concentrations. Positive and negative interference of the following substances was examined. The list includes typical medications that febrile patients may be taking and the proteins and other substances normally found in blood.

<table>
<thead>
<tr>
<th>Analyte Concentration</th>
<th>Number Read as Positive</th>
<th>Total tests</th>
<th>% Read as Positive</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>MxA Low CRP</td>
<td>High CRP</td>
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<tr>
<td>MxA ng/ml CRP mg/L</td>
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<td>0</td>
<td>42</td>
<td>0</td>
<td>118</td>
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</tbody>
</table>

Among these, only Rheumatoid factor at concentrations higher than 200 IU/mL produced a positive test line for low CRP when added to the C₅₀ concentration of low CRP. Rheumatoid factor at concentrations lower than 150 IU/mL did not show any interference. There was no interference with the MxA or high CRP test lines at any of the concentration’s tested.

**PRECISION AND REPRODUCIBILITY STUDY**

Samples were prepared in fresh EDTA whole blood with recombinant MxA and CRP proteins. Eight samples, consisting of a combination of C₅₀ and C₉₀ concentrations of the three analytes namely, MxA, low CRP, and high CRP were tested. A total of 960 determinations were performed by a combination of laboratory technicians, nurses, and clinicians at three different sites over five contiguous days during a two-week period. The study demonstrates overall reproducibility among three lots of material, among three separate sites, and among six separate users.

**AN RPS DIAGNOSTIC SOLUTION**
REFERENCES


ORDERING AND CONTACT INFORMATION

REF RPS-FDX – FebriDx (20 pack)

Manufacturer and United States Representative

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