PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

FilmArray® Respiratory Panel

Department of Microbiology

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1.0 Purpose and Test Principle

The FilmArray Respiratory Panel (RP) is a multiplexed nucleic acid test intended for use with the FilmArray Instrument for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs obtained from individuals suspected of respiratory tract infections. The following organism types and subtypes are identified using the FilmArray RP: Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype H1-2009, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Human Rhinovirus/Enterovirus, Respiratory Syncytial Virus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of a respiratory infection aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test or, lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen. Positive results do not rule out co-infection with other organisms: the agent(s) detected by the FilmArray RP may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.

The FilmArray RP pouch is a closed system disposable that houses all the chemistry required to isolate, amplify and detect nucleic acid from multiple respiratory pathogens within a single nasopharyngeal swab specimen. The rigid plastic component (fitment) of the FilmArray RP pouch contains reagents in freeze-dried form. The flexible plastic portion of the pouch is divided into discrete segments (blisters) which, through interactions with actuators and sensors in the FilmArray Instrument, are where the required chemical processes are carried out. The user of the FilmArray RP system loads the sample into the FilmArray RP pouch, places the pouch into the FilmArray Instrument, and starts the run. All other operations are automated.

The following is an overview of the testing procedure:

1. Remove the FilmArray pouch from its vacuum-sealed package. Since solutions are drawn into the FilmArray RP pouch by vacuum, it is important to keep pouches in their protective packaging until the time of use.
2. Place the FilmArray RP pouch into the FilmArray Pouch Loading Station. The FilmArray Pouch Loading Station has been designed to prevent errors by providing instructions and visual cues in the form of color-coded arrows to ensure that the pouch is properly loaded.
3. Load Hydration Solution into the FilmArray RP pouch using the Pouch Hydration Syringe provided in the FilmArray RP Kit. The syringe is fitted with a blunt stainless steel cannula, which is used to deliver the solution into the pouch. Loading the pouch with Hydration Solution rehydrates the freeze-dried reagents contained in the pouch fitment.
4. Mix nasopharyngeal swab specimen with Sample Buffer, using a provided Transfer Pipette. The Sample Buffer contains reagents that inactivate RNases in the sample and promote binding of nucleic acids to magnetic beads for isolation.
5. Load the sample/buffer mixture into the FilmArray RP pouch using the Sample Loading Syringe provided. When the sample mixture is loaded, a process control contained in the fitment of the pouch is introduced into the sample. The process control monitors all of the critical processes that occur in the pouch.
6. Transfer the pouch to the instrument and initiate a run. To aid in proper insertion of the pouch in the instrument, the FilmArray Instrument Control application provides on-screen animations illustrating the steps needed to start the run.
7. View results on the test report at the completion of the run.

The following is an overview of the operations and processes that occur during a FilmArray run:

1. **Nucleic Acid Purification** - Nucleic acid purification occurs in the first three blisters of the pouch. The sample is lysed by agitation (bead beating), and the liberated nucleic acid is captured, washed and eluted using magnetic bead technology. These steps require about ten minutes, and the bead-beater apparatus can be heard as a high-pitched whine during the first minute of operation.
2. **Reverse Transcription and 1st Stage Multiplex PCR** - Since many pathogens identified by the FilmArray RP pouch are RNA viruses, a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a preheated master mix to initiate the RT step and subsequent thermocycling for multiplex PCR. The effect of 1st stage PCR is to enrich for the target nucleic acids present in the sample.
3. **2nd Stage PCR** - The products of 1st stage PCR are diluted and mixed with fresh PCR reagents containing an intercalating fluorescent DNA dye (LCGreen® Plus, BioFire Diagnostics, Inc.). This solution is distributed over the 2nd stage PCR array. The individual wells of the array contain primers for different assays (each present in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material. These primers are 'nested' or internal to the specific products of the 1st stage multiplex reaction, which enhances both the sensitivity and specificity of the reactions.
8. **DNA Melting Analysis** – After 2nd stage PCR, the temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melting curve. The temperature at which a specific PCR product melts (melting temperature or Tm) is consistent and predictable, and the FilmArray Software automatically evaluates the data from replicate wells for each assay to report results.

The FilmArray Software controls the operation of the instrument, collects and analyzes data and automatically generates a test report at the end of the run. The entire process takes about an hour.

2.0 Clinical Significance

Respiratory pathogens cause acute local and systemic disease of varying severity, with the most severe cases occurring in children, the elderly and immunocompromised individuals. Respiratory symptoms can include coughing, nasal discharge, congestion, fever, wheezing, headache and myalgia. Due to the similarity of diseases caused by many viruses and bacteria, diagnosis based on clinical symptoms alone is difficult. Identification of potential causative agents provides data to aid the physician in determining appropriate patient treatment and public health response for disease containment.

Adenoviruses are a diverse group of non-enveloped DNA viruses with seven species (A to G) categorized by hemagglutination and approximately 55 serotypes. All serotypes have been associated with human disease. Adenovirus species B, C, and E cause acute respiratory disease. Outbreaks occur in institutional settings such as military training, long-term care facilities, and pediatric tertiary-care hospitals, due to high rates of transmission in closed populations. Adenoviruses (species A, D, F and G) can cause a variety of illnesses, including cystitis, gastroenteritis, and conjunctivitis. Adenoviruses are shed for long periods of time and persist on surfaces in an infective state.

Coronaviruses 229E, HKU1, NL63, and OC43. Human Coronaviruses were established as respiratory pathogens in the 1960's. Initially, two serologic variants were characterized (229E and OC43) and recently, two additional human coronaviruses (HKU1 and NL63) have been identified. These viruses are most commonly associated with upper respiratory tract infections; however, they have also been detected in individuals with lower respiratory tract infections. Coronaviruses have been associated with croup and exacerbation of asthma. Coronavirus infection occurs more often in the winter, and there appears to be a periodicity of epidemics for strains 229E and OC43 of every two to three years.

Human Metapneumovirus was discovered in 2001 as a respiratory pathogen in children. Further studies confirmed hMPV infections in persons of all ages. Human Metapneumoviruses

are in the family *Paramyxoviridae*. Infection in infants and young children is commonly associated with bronchiolitis. The two genotypes, A and B, can circulate at the same time and do not appear to differ in the severity of illness.

Influenza A and B are RNA viruses in the *Orthomyxoviridae* family. During annual Influenza epidemics, 5-20% of the population is affected with upper respiratory tract infections with rapid onset of fever. The dominant type of Influenza virus varies often due to antigenic drift and shift. During the 2009-10 Influenza season, Influenza A H1-2009 was the dominant circulating Influenza virus, accounting for approximately 99% of reported Influenza infections. Influenza A can be subtyped by the hemagglutinin (H) and neuraminidase (N) genes; subtypes H1N1 and H3N2 are the strains that most commonly infect humans. More severe disease and increased mortality are associated with H3N2 subtype. Currently, at least four antiviral medications are available for Influenza treatment – amantadine, rimantadine, zanamivir and oseltamivir – with type-specific efficacy and drug resistance arising with the spread of new strains of the virus. Complications with viral or bacterial pneumonia increase mortality from Influenza infections.

Parainfluenza Viruses are RNA viruses in the *Paramyxoviridae* family. In the 1950's, Parainfluenza viruses were determined to be respiratory pathogens different from influenza viruses. Parainfluenza viruses are divided into four types antigenically and genetically, with type 4 further subtyped as A and B. Parainfluenza Virus 1 causes biennial epidemics in the fall, with 50% of croup cases attributed to this virus. Parainfluenza Virus 2 has a periodicity of epidemics of one to two years that may alternate with Parainfluenza 1 outbreaks. Children less than six months old are particularly susceptible to Parainfluenza Virus 3 infection, with outbreaks occurring in neonatal intensive care units, and epidemics are most common in the spring and summer. Parainfluenza Virus 4 infection affects all age groups, and a periodicity of infection has not been established.

Respiratory Syncytial Virus is a member of the RNA viruses in the *Paramyxoviridae* family, related to human metapneumoviruses and parainfluenza viruses. RSV is the most common cause of severe respiratory disease in infants, with acute bronchiolitis as the major cause of hospitalization.

Rhinoviruses and **Enteroviruses** are closely related RNA viruses in the *Picornavirus* family. There are more than 100 serotypes of Human Rhinovirus based on the serology of the capsid protein. Rhinovirus is noted as causing the "common cold" but may also be involved in precipitating asthma attacks and severe complications. Enteroviruses are divided into four species that include a total of 89 serotypes. Individual serotypes can be associated with different clinical manifestations, including nonspecific respiratory illnesses in infants or adults.

Bordetella pertussis is the causative agent of whooping cough or pertussis, a vaccine-preventable disease that is reportable to public health organizations. *B. pertussis* is a gram-negative bacterium with a high infectivity rate that is treatable with several antibiotics. Vaccine-induced immunity has been shown to decrease after 5-10 years. Pertussis occurs most commonly in children but also occurs in adolescents and adults. Outbreaks have been documented in fully vaccinated populations due to waning immunity. The highest mortality from pertussis occurs with infants and the elderly. Early (catarrhal) pertussis disease is non-specific, and classic signs of pertussis (paroxysmal coughing, inspiratory 'whoop', post-tussive emesis, as well as apnea or cyanosis in infants) do not arise until approximately 2 weeks after the initial onset of symptoms. Symptoms of *B. pertussis* infection are known to vary due to a number of factors including: age, previous immunization or infection, passively acquired antibody, and antibiotic treatment. No peak season has been defined for *B. pertussis* infection.

Chlamydophila pneumoniae is an obligate intracellular bacterium that causes acute respiratory infections and is a common cause of community-acquired pneumonia. Outbreaks occur in schools, military barracks, and nursing homes. No peak season has been identified for *C. pneumoniae* infections.

Mycoplasma pneumoniae is a causative agent of community-acquired atypical pneumonia, frequently in outbreak situations. Incubation time for *M. pneumoniae* infection is approximately 1

to 4 weeks. *M*. *pneumoniae* respiratory disease does not have a defined season of highest incidence, but epidemics have a periodicity of 3-7 years.

3.0 Scope

This procedure is classified under CLIA as Moderately Complex. It should be carried out by technical personnel familiarized and trained on the operation of the FilmArray. Testing includes but is not limited to: instrument start up, shutdown, routine maintenance, basic troubleshooting, QC checks, technical proficiency. Records of employee proficiency testing are kept in the department. Performance reviews of technical personnel are to be carried out annually.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

The reagents and/or chemicals that are used in this procedure may be hazardous to your health if handled incorrectly. A brief listing of precautions for each chemical hazard is included in the reagent section of this procedure.

More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (SDS/MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Airborne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves – must be worn when handling specimens and reagents.
- Laboratory Coat – must be worn when handling specimens and reagents.
- Biological Safety Cabinet – must be used when processing specimens.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination:

- SDS/MSDS
- Chemical hygiene plan

5.0 Materials

5.1 Equipment

- FilmArray Instrument
- FilmArray Pouch Loading Station

5.2 FilmArray Respiratory Panel Kit

Each FilmArray Respiratory Panel Kit contains sufficient reagents to test 30 specimens. Store the test kit, including reagent pouches and buffers, at room temperature (18–30 °C). **DO NOT REFRIGERATE.** Always check the expiration date, and do not use reagents beyond the expiration date printed on the pouch or kit. Do not use pouches if the outer packaging has been damaged or if the vacuum is not intact. Do not remove pouches from their packaging until a sample is ready to be tested. Once the pouch packaging has been opened, the pouch should be loaded as soon as possible (within approximately 30 min). Once a pouch has been loaded, the test run should be started as soon as possible (within 60 min).

Kit Components:

- Individually packaged FilmArray RP pouches

- Single-use (0.5 mL) Sample Buffer vials (red lid)
  Sample Buffer is assigned the following classifications: Acute toxicity (Category 4), Serious Eye damage (Category 1), and Skin irritation (Category 2). Accordingly, the Sample Buffer is harmful if swallowed, causes serious eye damage, and causes skin irritation. Sample Buffer will form hazardous compounds and fumes when mixed with bleach or other disinfectants. Bleach should never be added to Sample Buffer or sample waste.
- Single-use (1.5 mL) Hydration Solution Vials (blue lid)
- Individually packaged Transfer Pipettes
- Individually packaged Sample Loading Syringes with attached cannula (red cap)
- Individually packaged Pouch Hydration Syringes with attached cannula (blue cap)

5.3 Control Materials and Usage

- NATtrol™ RP Multimarker Controls, Catalog #: NATRPC-BIO, 3 x 0.6 mL vials of RP Multi 1 and 3 x 0.6 mL vials of RP Multi 2, store at 2-8 °C

6.0 Interfering Substances

The manufacturer performed studies with the FilmArray RP Assay in the presence of a variety of potential biological and chemical substances in order to characterize the ability of the assay to detect target DNA under these conditions. A complete description of the studies can be found in the manufacture's package insert. Results demonstrated no reportable interference or inhibition with any substances tested.

7.0 Warnings and Precautions

7.1 Preventing Organism Contamination

Due to the sensitive nature of the FilmArray RP system, it is important to guard against contamination of the work area by following these guidelines:

- Laboratory workers can be infected with common respiratory pathogens and can inadvertently contaminate the sample while it is being processed. To avoid this, specimens should be processed and pouches should be loaded in a biosafety cabinet.
- Work surfaces within the biosafety cabinet may become contaminated with respiratory pathogens during specimen processing. Decontaminate work surfaces prior to beginning work and then again after processing has been completed. Wipe down with 1:10 diluted bleach followed by another wipe down with 70% alcohol. The alcohol cleanup helps remove residual bleach that could inhibit PCR reactions.
- After processing specimens, thoroughly clean the FilmArray Pouch Loading Station by wiping with 10% bleach or a similar disinfectant. To avoid residue build-up and potential PCR inhibition, wipe disinfected surfaces with water.
- The pouch loading stations should be switched out each day so that one can be decontaminated by submersion in freshly-prepared 10% bleach. Fill a sink or bin with enough 10% bleach to cover the loading station. Submerge the pouch loading station completely and soak for 15 min. After 15 min, rinse the loading station under running water and then rinse by submerging it completely in water two times. Allow the station to air dry until the following day.
- Specimens and pouches should be handled one-at-a-time.
- Change gloves and clean the work area between preparations of each patient specimen.
- Personnel with active respiratory symptoms (runny nose, cough) should wear a standard surgical mask and avoid touching the mask while preparing specimens.

7.2 Preventing Amplicon Contamination

A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicons. Because the FilmArray RP pouch is a closed system, the risk of amplicon contamination is low, provided that pouches remain intact after the test is completed. Adhere to the following guidelines to prevent amplicon contamination:

- Discard used pouches in an appropriate biohazard container immediately after the run has completed.
- Avoid excessive handling of pouches after test runs.
- Avoid exposing pouches to sharp edges or anything that might cause a puncture.
- Never wear gloves or lab coats worn in amplification/detection area, where the instrument is located, out into the main lab.
- When unloading completed test pouches, examine the exterior of the film for drips or liquid leaking.

WARNING: If liquid is observed on the exterior of a pouch, the liquid and pouch should be immediately contained and discarded in a biohazard container. The instrument and work space must be decontaminated as described in the FilmArray Operator's Manual. DO NOT PERFORM ADDITIONAL TESTING UNTIL THE AREA HAS BEEN DECONTAMINATED.

8.0 Specimen Collection, Handling and Storage

Nasopharyngeal Swab Collection - Specimens are collected by inserting a small swab on a flexible (plastic or aluminum wire) shaft through the nostril. The swab should be inserted back into the posterior nasopharynx. Once in place, the swab should be rotated gently for several seconds before withdrawal in order to absorb secretions. Specimens should be immediately placed in viral transport media (VTM).

Minimum Sample Volume - 300 µL of sample is required for testing.

Transport and Storage - Specimens in VTM should be processed and tested as soon as possible. If storage is required, specimens in VTM can be held at room temperature (18–30 ºC) for up to 4 h, at refrigerator temperature (2-8 °C) for up to 3 d, or at freezer temperature (< -15 °C) for up to 30 d.

9.0 Procedure

Refer to the FilmArray Respiratory Panel Quick Guide, the FilmArray Training Video or the FilmArray Operator's Manual for more detail and pictorial representations of these instructions.

Gloves and other Personal Protective Equipment (PPE) should be used when handling pouches and specimens. Only one FilmArray RP pouch should be loaded at a time. Once the pouch is loaded, it should be promptly transferred to the instrument to start the run. After the run is complete, the pouch should be discarded in a biohazard container.

9.1 Pouch Preparation

1. Thoroughly clean the work area with 10% bleach (or suitable disinfectant) followed by 70% alcohol.
2. Remove the FilmArray RP pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.

   NOTE: The outer packaging should be tight around the canister prior to opening. DO NOT use the pouch if the vacuum has been lost.
3. Place an accession label near the barcode label on the pouch. Do not cover the barcode on the pouch.
4. Place the FilmArray Pouch into the FilmArray Pouch Loading Station by holding the pouch so that the barcoded label is upright and readable, and then slide the flexible film portion of the pouch into the slot at the base of the loading station until the rigid plastic part of the pouch clicks into place. In the correct configuration, the inlet ports on both ends of the rigid plastic part of the pouch will point up, and the red and blue labels on the pouch will align with the red and blue arrows on the base of the FilmArray Pouch Loading Station.
5. Place a blue-capped Hydration Solution vial in the blue well of the FilmArray Pouch Loading Station.
6. Place a red-capped Sample Buffer vial in the red well of the FilmArray Pouch Loading Station.

9.2 Pouch Hydration

1. Remove the blue labeled Pouch Hydration Syringe from the packaging.
2. Remove the cap from the Hydration Solution vial.
3. Using the Pouch Hydration Syringe (blue cap), draw Hydration Solution to the 1 mL mark on the syringe, taking care to avoid the formation of bubbles. If you notice bubbles at the base of the syringe, leave the tip of the cannula in the Hydration Solution vial and dislodge the bubbles by gently tapping the side of the syringe with your finger. The bubbles will float up to the plunger.

   NOTE: DO NOT remove air bubbles by inverting the syringe and expelling liquid.

4. Insert the cannula tip into the port in the pouch fitment located directly below the blue arrow of the FilmArray Pouch Loading Station. While holding the body of the syringe, push down forcefully in a firm and quick motion until you hear a faint "pop" and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum; there is no need to use the plunger.

   NOTE: DO NOT push the syringe plunger. Injecting liquid will cause the pouch to overfill.

5. Verify that the pouch has been hydrated.
   Most of the liquid will have been drawn out of the syringe. Also, check to see that fluid has entered and hydrated reagents in the reagent wells (eleven wells located at the base of the rigid plastic part of the pouch). Flip the barcode label down to see the reagent wells. Small air bubbles may be seen. If the pouch fails to hydrate (dry reagents appear as white pellets), repeat Step 4 to verify that the seal of the port was broken or retrieve a new pouch and repeat from Step 2 of the Pouch Preparation section.

9.3 Sample Loading

1. Vortex the specimen for 30 s just prior to removing sample.
2. Remove the cap from the Sample Buffer vial.
3. Using the Transfer Pipette provided in the test kit, draw sample to the third line (approximately 0.3 mL). Add sample to the red-capped Sample Buffer vial and gently pipette up and down to mix. Discard the Transfer Pipette in a biohazard waste container.
4. Remove the red-labeled Sample Loading Syringe from the packaging.
5. Using the Sample Loading Syringe, draw approximately 0.3 mL of sample/sample buffer mix (to the 0.3 mL mark on the syringe), taking care to avoid the formation of bubbles. If you notice bubbles at the base of the syringe, leave the tip of the cannula in the Sample Buffer vial and dislodge the bubbles by gently tapping the side of the syringe with your finger. The bubbles will float up to the plunger.

   NOTE: To avoid contaminating the work area, DO NOT remove air bubbles by inverting the syringe and expressing liquid.

6. Insert the cannula tip into the port in the pouch fitment located directly below the red arrow of the FilmArray Pouch Loading Station. While holding the body of the syringe, push down forcefully in a firm and quick motion until you hear a faint "pop" and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum; there is no need to use the plunger.

   NOTE: DO NOT push the syringe plunger. Injecting liquid will cause the pouch to overfill.

7. Verify that the sample has been loaded.
   Most of the liquid will have been drawn out of the syringe. Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port. If the pouch fails to pull sample from the Sample Loading Syringe, the pouch should be discarded. Retrieve a new pouch and repeat from Step 2 of the Pouch Preparation section.

   NOTE: To reduce the risk of exposure to hazardous or potentially infectious material, DO NOT re-cap the syringes.

8. Dispose of syringes in a biohazard sharps container.

9. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the FilmArray Pouch Loading Station.

9.4 Using the FilmArray Instrument to Perform the Test

The FilmArray Instrument Control Software includes a step-by-step on-screen tutor that shows each step of the test.

1. Ensure that the laptop and FilmArray Instrument have been turned on. Launch the FilmArray Instrument Control Software by double clicking on the desktop icon.
2. Open the instrument lid.
3. Insert the loaded FilmArray pouch into the instrument.
   Position the pouch so that the array is on the right and the film is inserted first. The red and blue labels on the FilmArray pouch should align with the red and blue arrows on the FilmArray Instrument. There is a 'click' when the FilmArray pouch has been placed securely in the instrument. If inserted correctly, the pouch barcode is visible. If the FilmArray pouch is not completely in place, the instrument will not continue to the next step.

   NOTE: If the pouch does not slide into the instrument easily, gently push the lid of the instrument back to be sure that it is completely open.
4. Scan the barcode on the FilmArray pouch using the barcode scanner.
   Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol are preprogrammed in the rectangular barcode located on the FilmArray pouch. The information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label into the appropriate fields.

   NOTE: The barcode cannot be scanned prior to placing the pouch in the instrument. A "Cannot scan now" message will be displayed.
5. Enter the Sample ID.
   The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID (accession label) is used.
6. If necessary, select a protocol from the Protocol drop down list.
7. Enter a user name and password in the Name and Password fields.
8. Close the FilmArray Instrument lid.
9. Click Start Run.
   Once the run has started, the screen displays a list of the steps being performed by the instrument and the number of minutes remaining in the run.

   NOTE: The bead-beater apparatus can be heard as a high-pitched noise (whine) during the first minute of operation.
10. When the run is finished, follow the on-screen instructions to open the instrument and remove the pouch.
11. Immediately discard the pouch in a biohazard container.
12. Results are automatically displayed in the report section of the screen.
13. Select Print to print the report or Save to save the report as a file.

10.0 Interpretation of Results

When 2nd stage PCR is complete, the FilmArray Instrument performs a high resolution DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well. The FilmArray Software then performs several analyses and assigns a final assay result.

10.1 Analysis of Melting Curves

The FilmArray Software evaluates the DNA melting curve for each well of the 2nd stage PCR array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve. The Tm value is then compared against the expected Tm range for the assay. If the software determines that the melt is positive and the melt peak falls inside the assay-specific

Tm range, the curve is called positive. If the software determines that the melt is negative or is not in the appropriate Tm range, the curve is called negative.

10.2 Analysis of Replicates

Once melt curves have been identified, the software evaluates the three replicates for each assay to determine the assay result. For an assay to be called positive, at least two of the three associated melt curves must be called positive, and the Tm for at least two of the three positive curves must be similar (within 1°C). Assays that do not meet these criteria are called negative.

10.3 Organism Interpretation

For most organisms detected by the FilmArray RP, the organism is considered to be detected if a single corresponding assay is positive. For example, Human Metapneumovirus will have a test report result of Human Metapneumovirus Detected if at least two of the three replicates of the one Human Metapneumovirus assay have similar positive melt peaks with Tm values that are within the assay-specific Tm range. The test results for Adenovirus, the Human Rhinovirus/Enterovirus group, and Influenza A depend on the interpretation of results from several assays. Interpretation and follow-up testing for these three results are provided below.

10.3.1 Rhinovirus/Enterovirus Group

The FilmArray RP pouch contains six different assays (HRV1, HRV2, HRV3, HRV4, Entero 1, Entero 2) for the detection of Rhinoviruses and Enteroviruses. Though these viruses are both very diverse, they are also closely related. Therefore, the six assays are not able to reliably differentiate Rhinovirus and Enterovirus. The FilmArray Software interprets each of the six assays independently (as described above) and the results are combined as a final test result for the virus(es). If any of the six assays are positive, the test report result will be Human Rhinovirus/Enterovirus Detected. If all six assays are negative, the test report result will be Human Rhinovirus/Enterovirus Not Detected.

NOTE: Despite the names, the HRV (1-4) and Entero (1-2) assays are not specific for detection of Human Rhinovirus or Enterovirus, respectively. Individual assay results cannot be used to differentiate these two viruses.

10.3.2 Adenovirus

The FilmArray RP pouch contains two different assays (Adeno, Adeno2) for the detection of Adenovirus. The FilmArray Software interprets each of these assays independently (as described above), and the results are combined as a final test result for the virus. If either or both assays are positive, the test report result will be Adenovirus Detected. If both the Adeno and Adeno2 assays are negative, the test report result will be Adenovirus Not Detected.

10.3.3 Influenza A

The assays in the FilmArray RP are designed to both detect Influenza A and to differentiate commonly occurring hemagglutinin subtypes. To accomplish this, the FilmArray RP uses two Influenza A assays, (FluApan-1 and FluApan-2) and three subtyping assays directed at the hemagglutinin gene (FluA-H1-pan, FluAH1-2009 and FluA-H3). The FluA-H1-pan assay is designed to detect both Influenza A H1 and the Influenza A H1-2009 variant. Each of the individual assays is interpreted independently (as described above) and the test result reported for Influenza A is based on the combined results of the five assays.

In general, Influenza A is determined to be detected if at least one of the two FluA-pan assays is positive and a subtyping assay is also positive. If neither of the FluA-pan assays is positive, but a subtyping assay is positive, then the result is considered equivocal for that specific subtype. If one of the FluA-pan assays is positive and none of the subtyping assays are positive, the result is equivocal for Influenza A. When equivocal results for Influenza A are encountered, leave the FilmArray report for the technical specialist for follow up. The run files will be sent to BioFire for further analysis.

10.3.4 Influenza A (no subtype detected)

If both of the FluA-pan assays are positive, but none of the hemagglutinin subtyping assays are positive, then the interpretation is Influenza A (no subtype detected). This result could occur when the titer of the virus in the specimen is low and not detected by the subtyping assays. This result could also indicate the presence of a novel Influenza A strain. When no subtype is detected for Influenza A, leave the FilmArray report for the technical specialist for follow up. The run files will be sent to BioFire for further analysis.

11.0 Reporting

11.1 FilmArray RP Test Report

The FilmArray RP test report is automatically displayed upon completion of a run and contains three sections, the Run Summary, the Results Summary, and the Run Details. The test report can be saved as a file or printed.

11.1.1 Run Summary

The Run Summary section of the test report provides the Sample ID, time and date of the run, control results and an overall summary of the test results. Any target with a Detected or Equivocal result will be listed in the corresponding field of the summary. If all of the tests were negative then None will be displayed in the Detected field. Controls are listed as Passed, Failed or Invalid. See the Control Field section below for detailed information about the interpretation of controls and appropriate follow-up in the case of control failures.

Run Summary			
Sample ID:	1-214	**Run Date:**	23 Sep 2010 11:30 AM
Detected:	Human Rhinovirus/Enterovirus	**Controls:**	Passed
Equivocal:	↔ Influenza A H1-2009		

11.1.2 Result Summary

The **Results Summary** section of the test report lists the result for each target tested by the panel. Possible results are Detected, Not Detected, Equivocal (see Organism Interpretation for Influenza A above) or Invalid (control failure).

Result Summary		
	Not Detected	Adenovirus
	Not Detected	Coronavirus HKU1
	Not Detected	Coronavirus NL63
	Not Detected	Human Metapneumovirus
✓	Detected	Human Rhinovirius /Enterovirus
↔	Equivocal	Influenza A H1 2009

11.1.3 Run Details

The **Run Details** section provides additional information about the run including: pouch information (type, lot number, and serial number), run status (Completed, Incomplete, Aborted, Instrument Error, Instrument Communication Error, or Software Error), the protocols that were used to perform the test, the identity of the operator that performed the test, and the instrument used to perform the test.

Run Details			
Pouch	Respiratory Panel v1.6	**Protocol:**	RPP_v.5
Run Status:	Completed	**Operator:**	Sample
Serial No.:	00026577	**Instrument:**	ITI FA "AFA17"
Lot No.:	100302A		

Once a run has completed, it is possible to edit the Sample ID. If this information has been changed, an additional section called **Change History** will be added to the test report. This Change History section lists the field that was changed, the original entry, the revised entry, the operator that made the change and the date that the change was made. Sample ID is the only field of the report that can be changed.

11.2 Result Reporting in LIS

11.2.1 Positive Result(s)

Enter **Positive for:** and the name of the organism detected.

The following comment should be added to the report to describe all of the pathogens that are included in the panel: **The following organisms were tested for by PCR: Adenovirus, *Bordetella pertussis, Chlamydophila pneumoniae*, Coronavirus (HKU1, NL63, 229E, and OC43), Influenza A (H1, H1 2009, and H3), Influenza B, Metapneumovirus, *Mycoplasma pneumoniae*, Parainfluenza (1, 2, 3, and 4), Respiratory Syncytial Virus, and Rhinovirus/Enterovirus.**

11.2.2 Negative Result

Enter **Negative for all organisms tested.**

The following comment should be added to the report to describe all of the pathogens that are included in the panel: **The following organisms were tested for by PCR: Adenovirus, *Bordetella pertussis, Chlamydophila pneumoniae*, Coronavirus (HKU1, NL63, 229E, and OC43), Influenza A (H1, H1 2009, and H3), Influenza B, Metapneumovirus, *Mycoplasma pneumoniae*, Parainfluenza (1, 2, 3, and 4), Respiratory Syncytial Virus, and Rhinovirus/Enterovirus.**

11.2.3 Equivocal Result

Enter **Equivocal for:** and the name of the organism detected (Influenza A, Influenza A H1, Influenza A H3, or Influenza A H1-2009).

Enter the comment: **An equivocal result may be due to viral nucleic acid below the limit of detection of the test or the presence of inhibitors in the sample. It may also be caused by suboptimal specimen collection or handling. [EQVCL]**

The following comment should be added to the report to describe all of the pathogens that are included in the panel: **The following organisms were tested for by PCR: Adenovirus, *Bordetella pertussis, Chlamydophila pneumoniae*, Coronavirus (HKU1, NL63, 229E, and OC43), Influenza A (H1, H1 2009, and H3), Influenza B, Metapneumovirus, *Mycoplasma pneumoniae*, Parainfluenza (1, 2, 3, and 4), Respiratory Syncytial Virus, and Rhinovirus/Enterovirus.**

12.0 Quality Control & Quality Assurance

12.1 Process Controls

Two process controls are included in each pouch:

1. **RNA Process Control**
   The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, 1st stage PCR, dilution, 2nd stage PCR and DNA melting. A positive control result indicates that all steps carried out in the FilmArray RP pouch were successful.
2. **PCR2 Control**
   The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that 2nd stage PCR was successful.

Both control assays must be positive for the test run to pass. The Control field on the test report will display Passed, Failed, or Invalid. The Control field will display Passed only if the run completed successfully (no instrument or software errors) and both of the pouch control assays (RNA Process Control and PCR2 Control) were successful. The Control field will display Failed if the run was completed successfully (no instrument or software errors) but one or both of the pouch control assays failed (0 or 1 positive replicates for either of the controls, each of which is tested in triplicate). If the control result is Failed, then the result for all of the tests on the panel are displayed as Invalid, and the specimen will need to be retested with a new pouch.

The FilmArray Instrument monitors each run to ensure that the instrument is working within specification and to detect hardware or software errors that might compromise the accuracy of the test result. If the instrument detects an out-of-specification condition, or a significant error, it will automatically abort the run. If this happens, or if a run is aborted by the user, then the Control field on the report will display Invalid, and all test results in the Result Summary of the report will also be displayed as Invalid. To determine why a run failed to complete, note any specific error codes that are displayed on the screen, and refer to the Run Status in the Run Details section of the report. The Run Status will display Incomplete, Aborted, Software Error, Instrument Error, or Instrument Communication Error. Refer to the FilmArray Operator's Manual or call Technical Support for further instruction. The specimen should be retested after the error is corrected or by using an alternate FilmArray Instrument, if available.

12.2 External Controls

External control materials are available commercially from ZeptoMetrix Corporation. NATtrol™ RP Multimarker External Run Controls (NATRPC-BIO) are designed to monitor the performance of FilmArray® RP assay**.** These controls are formulated with purified, intact organisms that have been chemically modified to render them non-infectious and refrigerator stable. Each control pack contains 3 x 0.6 mL vials of RP Multi 1 and 3 x 0.6 mL vials of RP Multi 2. NATtrol™ RP Multimarker controls are formulated in a purified protein matrix that is fully commutable with true clinical specimens. Quality Control testing using the NATtrol™ RP Multimarker is performed in the same manner as for clinical specimens, except that 0.3 mL of the control material is used instead of a specimen in VTM. Control materials should be vortexed for 30 s just prior to use. Table 1 lists the respiratory targets and expected results when tested on FilmArray® RP assay.

Table 1

Target		RP Multi 1	RP Multi 2
Adenovirus		Positive	Negative
Coronavirus 229E		Negative	Positive
Coronavirus HKU1		Negative	Positive
Coronavirus NL63		Negative	Positive
Coronavirus OC43		Negative	Positive
Human Metapneumovirus		Positive	Negative
Human Rhinovirus/ Enterovirus	Entero 1	Positive	Negative
	Entero 2	Positive	Negative
	HRV1	Positive	Negative
	HRV2	Positive	Negative
	HRV3	Positive	Negative
	HRV4	Positive	Negative
Influenza AH1-2009	Flu A-H1-2009	Positive	Negative
Influenza AH1	Flu A-H1-pan	Positive	Positive
Influenza AH3	Flu A-H3	Positive	Negative
	Flu A-pan1	Positive	Positive
	Flu A-pan2	Positive	Positive
Influenza B		Negative	Positive
Parainfluenza Virus 1		Positive	Negative
Parainfluenza Virus 2		Negative	Positive
Parainfluenza Virus 3		Negative	Positive
Parainfluenza Virus 4		Positive	Negative
Respiratory Syncytial Virus		Negative	Positive
Bordetella pertussis		Negative	Positive
Chlamydophila pneumoniae		Positive	Negative
Mycoplasma pneumoniae		Positive	Negative

External control materials must be used to evaluate each new lot or shipment of FilmArray Respiratory Panels. External controls must be tested every 30 d while a lot is in use. Controls should be tested on alternate instruments each time in order to correlate results from each instrument. Quality control results should be entered into the LIS. Notify technical specialist or supervisor if results are not as expected, and do not report any patient results until the issue has been resolved. Repeat testing using new external controls.

12.3 Report Review

All test results entered into LIS should be reviewed by a second technologist on the same shift or the beginning of the next shift. Results should be compared to the printed results from the BioFire computer. The review should be documented on the BioFire Report with date and initials.

12.4 Environmental Surveillance Testing

Due to the nature and sensitivity of nucleic acid amplification testing, practices are necessary to safeguard against contamination of laboratory equipment and surfaces used to process and perform the PCR assays. Environmental contamination with organisms, target nucleic acids, or amplicons may lead to contamination of patient samples that could result in false-positive test results. Improper decontamination can lead to false-negative test results.

1. Surfaces to be Tested
    - The biosafety cabinet work surfaces and the pouch loading station should be sampled and tested at least once per month or following a potential issue with contamination. More surfaces may be tested following a potential contamination event. The instrument and surrounding surfaces may be tested if a leaking pouch is discovered.

2. Sample Collection and Preparation
    - Use one tube of viral transport medium and one swab to sample both the biosafety cabinet and the pouch loading station.
    - Dip the swab into the viral transport medium (VTM). Swab the areas by rotating the swab 2 to 3 times across the surfaces (~ 10 cm^2).
    - After sampling the surface areas, place the swab into the VTM and break off the upper portion of the shaft.
    - Recap the VTM tube and briefly vortex the sample for 30 s.
3. Sample Testing
    - Load and test one RP Panel following the procedure described above.
4. Interpretation of Test Results
    - A negative assay result indicates that no nucleic acids for any of the targets were detected.
    - A positive assay result indicates that target material was detected, and environmental contamination has occurred.
    - An unresolved assay result indicates the presence of an inhibitory substance or internal control amplicon.
5. Decontamination
    - Surfaces that test positive for any of the targets should be decontaminated. Surface samples that yield unresolved results should also be decontaminated and cleaned.
    - If an environmental surface tests positive for any of the targets, additional surveillance testing should be performed after decontamination to verify that decontamination was successful. Consult with the director or technical specialist.

13.0 Limitations

1. This test is a qualitative test and does not provide a quantitative value for the virus(es) and/or bacteria detected in the specimen.
2. This test has not been validated for testing specimens other than nasopharyngeal swab specimens. It is important to note that nasopharyngeal specimens contain columnar epithelial cells, and nasal swabs sample only squamous epithelial cells. It is essential that columnar epithelial cells be obtained for optimal sensitivity.
3. The performance of this test has not been established for immunocompromised individuals.
4. The performance of this test has not been established for patients without signs and symptoms of respiratory infection.
5. Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
6. Viral and bacterial nucleic acids may persist *in vivo* independent of organism viability. Detection of organism target(s) does not imply that the corresponding organisms are infectious or are the causative agents for clinical symptoms.
7. The detection of viral and bacterial nucleic acid is dependent upon proper specimen collection, handling, transportation, storage and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive or false negative values resulting from improperly collected, transported, or handled specimens.
8. A negative FilmArray RP result does not exclude the possibility of viral or bacterial infection. Negative test results may occur from the presence of sequence variants in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up or an infection caused by an organism not detected by the panel. Test results may also be affected by concurrent antiviral/antibacterial therapy or levels of organism in the specimen that are below the limit of detection for the test. Negative results should not be used as the sole basis for diagnosis, treatment, or other management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen.

9. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods when prevalence is moderate to low.
10. Organism and amplicon contamination may produce erroneous results for this test. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.
11. There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
12. Cross-reactivity with respiratory tract organisms other than those listed in the Analytical Specificity section below may lead to erroneous results.
13. The FilmArray RP Adenovirus assay may show variable detection with non-respiratory serotypes within species A, D, F and G.
14. The FilmArray RP Influenza A subtyping assays target the Influenza A hemagglutinin gene only. The FilmArray RP does not detect or differentiate the Influenza A neuraminidase gene.
15. Clinical specificity was established when Influenza A H1-2009 was the predominant Influenza A virus in circulation. When other Influenza A viruses are emerging, clinical specificity may vary.
16. Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Bordetella pertussis,* Coronavirus 229E, Coronavirus OC43, Influenza A H1, Influenza A H3, Influenza A H1-2009, Influenza B, *Mycoplasma pneumoniae*, Parainfluenza Virus 1, Parainfluenza Virus 2, and Parainfluenza Virus 4, were established primarily with retrospective clinical specimens. Performance characteristics for *Chlamydophila pneumoniae* were established primarily with spiked clinical specimens. The performance of this test has not been established for monitoring treatment of seasonal Influenza A H1, A H3, A H1-2009, or RSV infections.
17. The performance of this test has not been established for screening of blood or blood products for the presence of seasonal Influenza A H1, A H3, or A H1-2009.
18. The performance of this test has not been established with potentially interfering medications for the treatment of influenza or cold viruses. The effect of interfering substances has only been evaluated for those listed in the labeling. Interference by substances other than those described in the “Interference” section could lead to erroneous results.
19. The performance of the FilmArray RP has not been established in individuals who received influenza vaccine. Recent administration of a nasal influenza vaccine may cause false positive results for Influenza A and/or Influenza B.
20. Due to the genetic similarity between Human Rhinovirus and Enterovirus, the FilmArray RP cannot reliably differentiate them.
21. The Coronavirus OC43 assay may cross-react with Coronavirus HKU1. As a result, when both HKU1 and OC43 are detected in the same patient specimen, the result may be due to assay cross-reactivity. A coinfection with these two viruses is also possible.
22. The FilmArray RP may not be able to distinguish between existing viral strains and new variants as they emerge. For example, the FilmArray RP can detect Influenza A H3N2v (first recognized in August, 2011), but will not be able to distinguish this variant from Influenza A H3N2 seasonal.
23. Results of the FilmArray RP *B. pertussis* assay may not be concordant with the results of commonly used *Bordetella* PCR assays that target the multi-copy insertion sequence (IS481) due to differences in sensitivity and specificity. IS481 is a multi-copy target and is present in several *Bordetella* species (*B. pertussis, B. holmesii and B. bronchiseptica*). The FilmArray RP *B. pertussis* assay targets the single-copy promoter region of the pertussis toxin gene and is designed to be highly specific for detection of *B. pertussis.* Crossreactivity with other closely related *Bordetella* species (e.g. *B. parapertussis, B. holmesii, and B. bronchiseptica*) has not been observed with the FilmArray *B. pertussis* assay. However, cross reactivity has been observed when testing at concentrations above $1x10^6$ CFU/mL

14.0 Verification Information

The BioFire FilmArray Respiratory Panel Assay has been cleared by the FDA for clinical diagnostic testing. No modifications have been made to the FDA-cleared assay. The manufacturer's published findings for analytical sensitivity and specificity can be found in the package insert. In this evaluation, clinical nasopharyngeal swab specimens and commercial materials were used to verify the accuracy and precision of the assay.

Assay Accuracy

The clinical material used to evaluate the performance of the FilmArray RP were samples that previously tested positive by culture, DFA, EIA, or PCR. The samples were collected and frozen at -70 °C prior to this evaluation. All of the samples were nasopharyngeal specimens except for the *Mycoplasma pneumoniae* sample. Since no positive nasopharyngeal samples were available, a bronchial specimen that was PCR-positive for *M. pneumoniae* was used.

The NATtrol™ Respiratory Verification Panel, produced by ZeptoMetrix Corporation, was also used. This verification panel consists of 19 vials, each containing a single target in a purified protein matrix. The contents of each vial used were combined with an equal portion of a patient sample, positive for a different target, in Viral Transport Medium. This provided test samples with multiple targets and provided a matrix that would resemble that of patient samples.

A total of 27 samples were tested. These 27 samples contained a total of 52 targets. Fifty-one (98%) of the targets were detected with the FilmArray RP. Table 2 below summarizes the results.

Table 2	**Positive Clinical Samples**		**Zeptometrix**	**Total**
	Original Test	FilmArray +		
Adenovirus	3 DFA, 2 PCR	5	-	5
Bordetella pertussis	2 PCR	2	-	2
Chlamydophila pneumoniae	1 PCR	1	1	2
Coronavirus HKU1	2 PCR	2	1	3
Coronavirus NL63	1 PCR	1	1	2
Coronavirus 229E	1 PCR	1	1	2
Coronavirus OC43	1 PCR	1	1	2
Influenza A	1 Cult, 1 DFA, 1 EIA	3	2	5
Influenza A/H1	N/A	N/A	1	1
Influenza A/H1-2009	1 Culture	1	1	2
Influenza A/H3	1 EIA, 1 DFA	2	-	2
Influenza B	2 EIA	2	-	2
Metapneumovirus	2 DFA	2	1	3
Mycoplasma pneumoniae	2 PCR	1	1	2
Parainfluenza Virus 1	2 DFA	2	-	2
Parainfluenza Virus 2	1 DFA	1	1	2
Parainfluenza Virus 3	2 DFA	2	1	3
Parainfluenza Virus 4	1 PCR	1	1	2
Respiratory Syncytial Virus	2 DFA, 3 PCR	5	-	5
Rhinovirus/Enterovirus	2 PCR	2	-	2
Total	36	**37**	**14**	**51**

One of the *Mycoplasma pneumoniae* targets was not detected by the FilmArray RP. A review of the individual PCR analysis of this sample revealed that it had a late crossing point, suggesting that it was a low-level positive. The individual *M. pneumoniae* PCR assay is likely more sensitive

than that of the FilmArray multi-plex assay. The overall sensitivity of the assay was 98%, with a specificity of 100%.

Precision

Due to the cost of individual panels and the fact it is an FDA-cleared assay, precision testing was limited to testing one sample in triplicate. The sample used was a combination of a clinical sample positive for *Bordetella pertussis* mixed with an equal amount of the ZeptoMetrix Influenza A/H1 material. The mixed sample was tested by 3 different users and produced the expected results each time.

Additional precision data was gathered using external control materials (RP Multi 1 and RP Multi 2). Quality control testing was performed daily for 20 days with no failures detected.

15.0 References

1. FilmArray® Respiratory Panel (RP) Instruction Booklet, RFIT-PRT-0103 Feb 2013.

16.0 Document Control

Microbiology Director Approval: Dr. Ann Robinson 10/16/2013

Microbiology Supervisor Reviews: Jerry Claridge 10/17/2013, Jason Ammons 12/18/2015

12/17/2013 Added instructions to vortex control material and clinical specimens just prior to sample testing. AR/MM 1/8/2014 Defined time for vortexing control material and clinical specimens to 30 s. 3/9/14 Added: Controls should be tested on alternate instruments each time in order to correlate results from each instrument. 3/19/14 Added instructions for decontaminating pouch loading station by 10% bleach submersion once daily. 05/13/2014 Added precision data gathered from testing external controls. 1/14/2015 Added comment to report with equivocal Influenza A results. Influenza A with no subtype should be sent to the state lab. Added instructions for sending out samples. 02/20/2015 the procedure was updated for follow-up on equivocal and no subtype detected influenza A results. Samples will not be sent to the state for further testing. However, the run files will be sent to BioFire for further analysis.