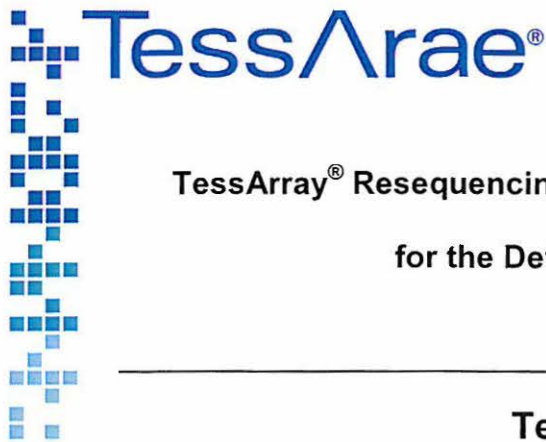
The information previously contained on these webpages were authorized under the 2009 H1N1 Influenza Emergency Use Authorizations (EUAs). As of June 23, 2010, the EUAs have been terminated and this information is no longer current.



TessArray® Resequencing Influenza A Microarray Detection Panel

for the Detection of 2009 H1N1 Virus

TessArray RM-Flu

A single test intended for the *in vitro* qualitative detection and differentiation of the 2009 H1N1 influenza A virus aided by an algorithm that relies on seasonal A/H1N1 and seasonal A/H3N2 influenza virus results.

Emergency Use Authorization (EUA)
For *in vitro* Diagnostic Use (IVD)

INTENDED USE

The TessArray RM-Flu test is a resequencing microarray assay intended for use in CLIA High Complexity Laboratories with access to an Affymetrix® GeneChip® Microarray Instrumentation System for the *in vitro* qualitative detection of 2009 H1N1 influenza A viral RNA, aided by an algorithm that relies on seasonal influenza A/H1 virus and seasonal influenza A/H3 virus results in throat swabs (TS) from patients with signs and symptoms of respiratory infection.

Testing with the TessArray RM-Flu should not be performed unless the patient meets clinical and epidemiologic criteria for testing suspect specimens. Similarly, the identification of 2009 H1N1 influenza virus should be made in conjunction with an appropriate clinical and epidemiological assessment.

Negative results from the TessArray RM-Flu test do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.

OVERVIEW OF THE ASSAY

Introduction:

Influenza viruses belong to the taxonomic group of Orthomyxoviruses, characterized by their segmented, single-stranded, negative-sense viral genomes. Influenza viruses are serologically classified as types A, B and C. The serologically distinct hemagglutinin (HA) and neuraminidase (NA) proteins and their similarly distinctive gene sequences provide the basis for further classification of the type A influenza viruses by HA (H1 through H16) and NA (N1 through N9) subtypes.

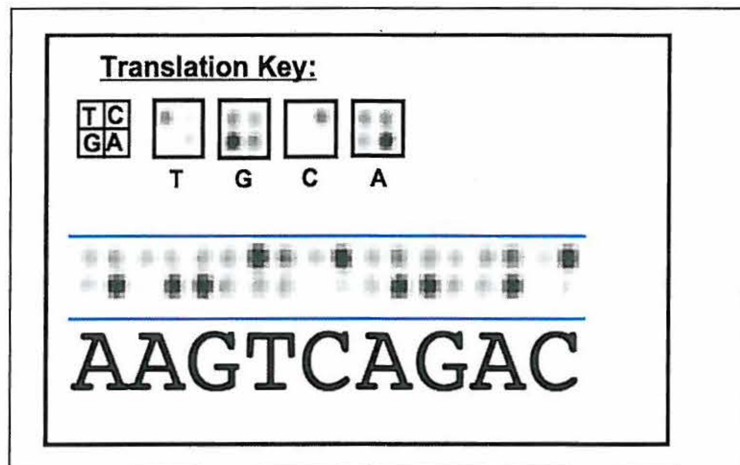
In recent years the seasonal epidemic outbreaks of influenza in humans have been associated with infections by seasonal A/H1N1 or seasonal A/H3N2 influenza virus strains. In the spring of 2009, infections by a new A/H1N1 strain (namely 2009 H1N1 influenza virus, also referred to as novel 2009 H1N1 virus, swine influenza virus, or Pandemic (H1N1) 2009 virus), were first reported in Mexico. Because of the rapid and global spread of infections by this 2009 H1N1 influenza A strain, the World Health Organization declared an influenza pandemic in June 2009.

Genomes of type A influenza viruses are comprised of eight segments, each encoding genes that are critical for the virus to successfully infect and replicate within host cells. The similarities and differences among nucleotide sequences of different viral genes from different influenza virus types, subtypes and strains provide the molecular basis for both detection and differential identification of 2009 H1N1 influenza A virus by the TessArray RM-Flu assay.

Principle of the procedure:

The TessArray RM-Flu uses a resequencing microarray (a DNA sequencing device) together with reverse transcriptase (RT) and polymerase chain reaction (PCR), to simultaneously determine nucleotide sequences of multiple influenza virus gene segments. The test detects 2009 H1N1 influenza A virus based upon successful determination of the sequences of one or more influenza viral genes that may be present in a specimen, those sequences need to have at least 80% similarity to the sequences represented as microarray detector tiles. The assay-generated gene sequences are compared to more than 90,000 known influenza virus gene sequences in a validated sequence record database (VSRD). The VSRD contains more than 4,000 sequence records representing the unique gene sequences from different isolates of the 2009 H1N1 influenza A virus; more than 10,000 gene sequence records from different isolates of seasonal A/H1N1 strains; and more than 25,000 gene sequence records from different isolates of seasonal A/H3N2 strains. Identification of the particular strain of influenza virus that may have been detected in the test is based upon the strain identity of those sequence records found to be most similar to the assay-generated gene sequences. The tested specimen is reported DETECTED if the differential identification of assay-generated influenza virus gene sequences corresponds to the 2009 H1N1 influenza A virus.

Sample preparations from clinical specimens and the laboratory protocol of the TessArray RM-Flu are performed in the diagnostic laboratory (CLIA High Complexity Laboratory).



The Affymetrix GeneChip Microarray Instrumentation System automatically scans the finished TessArray RM-Flu microarrays and generates a resequencing data text file (FASTA format) representing called (A, C, G or T) and uncalled (N) nucleotides of targeted gene sequences.

After performance of the assay's laboratory protocol, the operator/client uploads one or batches of more than one FASTA resequencing data text file(s) through a client-dedicated secure web portal for near real-time analysis by the TessArray RM Data Analysis Server. High-level reports are returned by e-mail to a client-authorized e-mail address, indicating individual assay results as ANALYTE NOT DETECTED, INDETERMINATE, or ANALYTE DETECTED for 2009 H1N1 influenza virus. An attached text file report summarizes assay data supporting the result. The TessArray RM Data Analysis Server can analyze data and return results from client venues that use either the Affymetrix GCS 3000 7G (RUO) system or the Affymetrix GCS 3000 Dx/Dx.2 (IVD) system. In the latter case a different software is implemented; the scanner-generated image data files (.CEL files) are uploaded and the Data Analysis Server automatically generates a resequencing data text file for analysis. The resequencing data text file is returned to the client as a second e-mail file attachment with the supporting assay results data file. As an alternative to e-

mail, the client may elect to access assay results and assay data reports exclusively by downloading through the secure web portal.

Array Content:
The TessArray RM-Flu assay uses nine different resequencing microarray detector tiles to identify the 2009 H1N1 influenza and differentiate it from seasonal A/H1N1 and seasonal A/H3N2 influenza viruses. The nine detector tiles are identified in Table 1 below. The average nucleotide sequence length of viral gene sequences that are directly targeted by the resequencing detector tiles is 1,100 nucleotides (representing an average 80% of the full-length sequence of each corresponding influenza virus genome segment).

Table 1: Description of the Nine Analyte-Specific Resequencing Detector Tiles Used in the TessArray RM-Flu Assay Data Analysis

TessArray RM-Flu Analyte-Specific Detector Tiles		
Analyte	**Detector Gene**	**Detector Tile(s)***
2009 H1N1 flu	**Neuraminidase**	**NA1av**
2009 H1N1 flu	**Non-Structural**	**NSav**
2009 H1N1 flu	**Matrix**	**M1hu, M3hu, M5av**
Seasonal A/H1N1	**Hemagglutinin**	**HA1hu**
Seasonal A/H1N1	**Neuraminidase**	**NA1hu**
Seasonal A/H1N1	**Matrix**	**M1hu, M3hu, M5av**
Seasonal A/H3N2	**Hemagglutinin**	**HA3hu**
Seasonal A/H3N2	**Neuraminidase**	**NA2hu**
Seasonal A/H3N2	**Matrix**	**M1hu, M3hu, M5av**

*** hu – indicates target gene from seasonal type A human influenza virus**
av – indicates target gene from avian type A influenza virus

The viral gene resequencing detector tiles of TessArray RM-Flu represent:

- 2009 H1N1 influenza virus
  - **NA1av** – an avian type A influenza virus neuraminidase gene sequence
  - **NSav** – an avian type A influenza virus non-structural gene sequence
  - **M1hu** - a representative matrix gene sequence from seasonal A/H1N1
  - **M3hu** – a representative matrix gene sequence from seasonal A/H3N2
  - **M5Av** – an avian type A influenza virus matrix gene sequence

- Seasonal A/H1N1
  - **HA1hu** – a representative hemagglutinin gene sequence from A/H1N
  - **NA1hu** – a representative neuraminidase gene sequence from A/H1N1
  - **M1hu** - a representative matrix gene sequence from seasonal A/H1N1
  - **M3hu** – a representative matrix gene sequence from seasonal A/H3N2
  - **M5Av** – an avian type A influenza virus matrix gene sequence

- Seasonal A/H3N2
  - **HA3hu** – a representative hemagglutinin gene sequence from A/H1N1
  - **NA2hu** – a representative neuraminidase gene sequence from A/H1N1
  - **M1hu** - a representative matrix gene sequence from seasonal A/H1N1
  - **M3hu** – a representative matrix gene sequence from seasonal A/H3N2
  - **M5Av** – an avian type A influenza virus matrix gene sequence

The three different and independent matrix gene detector tiles contribute together to detection and identification of each of the three analytes. Only one of the targets is ultimately identified by the matrix gene sequence homology analysis. The two non-matrix gene detector tiles for each analyte are more specific than the matrix gene detector tiles for detection and identification of the respective analyte.

Quality Control:

Negative/Background Controls: For each assay, the TessArray RM-Flu data analysis uses 25 non-analyte resequencing detector tiles as background control detector tiles. These background control detector tiles represent a variety of different type A influenza virus HA and NA genes, from subtypes that rarely infect humans and have little sequence similarity with the HA and NA genes of the analyte subtypes A/H1N1 and A/H3N2. They represent matched non-analyte type A influenza virus gene sequences as negative background controls to set a threshold for detection of any of the assay's targeted influenza viruses.

Positive/Protocol Controls: Two additional resequencing detector tiles used by the TessArray RM-Flu represent over 1,000 nucleotide base pairs (bp) of sequences of the TIM and NAC1 genes from *Arabidopsis thaliana* (wild mustard weed). The four Multiplex PCR Mix Tubes (described in the assay protocol, below) each contain oligonucleotide primers and template to support amplification of sequences representing the left (5') or right (3') half of one of these two control detector tiles. The control template for the NAC1 detector tile is plasmid-cloned DNA. The left and right half of the NAC1 gene sequence represent 495 bp and 456 bp amplicons in Multiplex PCR Tubes C and D, respectively. The control template for the TIM detector tile is RNA transcribed *in vitro* from a plasmid-cloned gene sequence. If the single-tube TessArray RM-Flu reverse transcription (RT) step succeeds, resulting TIM-gene cDNA products may be amplified in the following multiplex PCR step of the protocol. The left and right half of the TIM gene sequence represent 482 bp and 446 bp amplicons in Multiplex PCR Tubes A and B, respectively. In addition to providing assurance of successful protocol execution of the critical RT (TIM) and multiplex PCR (NAC1) steps, positive protocol control detector tile scores provide assurance that the pooled products after the multiplex PCR step have successfully passed through the amplicon fragmentation, end-labeling, hybridization and antibody-based fluorescent staining steps of the protocol.

Nucleic Acid Extraction Controls: Each batch of specimens to be tested should include a known reference sample as a positive control for RNA extraction and subsequent protocol steps. Trivalent seasonal influenza virus vaccines are suitable as positive nucleic acid extraction controls. If the manufacturer's recommended storage conditions of the neat vaccine stock have been sustained, the utility of the vaccine as a positive control extends at least three years beyond the expiration date for clinical use. We recommend preparation of the positive control sample by dilution of 1 µl to 10 µl of neat vaccine stock with virus transport medium (as used for clinical TS specimen collection) to a total volume of 150 µl. Total nucleic acid extraction from the positive control sample follows the same assay protocol (below) that is used for analysis of individual 150 µl TS specimens. This dilution of vaccine stock should be freshly prepared at the time of each batch assay requiring a positive nucleic acid extraction control sample and not stored for later use.

DATA ANALYSIS: ANALYTE DETECTION AND REPORTING

Analyte target gene resequencing:

The TessArray RM Data Analysis Server automatically processes the FASTA sequence file resulting from TessArray RM-Flu assay of a specimen, with respect to specific base calls (A, C, G

or T) that are identified by GSEQ at specific positions within each of the different resequencing microarray detector tiles.

Simple enumeration of the number of called bases reported from a detector tile does not reflect any specific gene sequence-context of consecutive base calls, therefore the TessArray RM-Flu uses the "**C3 Score**" as a quantitative metric of targeted gene sequences generated from a resequencing microarray detector tile. **The C3 Score is the number of called bases from the detector tile that appear within consecutive runs of three or more base calls, expressed as a percentage of the detector tile length.**

Background and Threshold C3 Scores for Analyte Target Gene Detection:

The average C3 Score from the 25 background control detector tiles was determined from a set of 607 clinical and laboratory specimens (227 influenza-virus positive and 380 influenza-virus negative). The mean and standard deviation of the 15,175 (= 25 * 607) individual background control detector tile C3 Scores was

$$\textbf{C3 Score}_{average} = \textbf{3.37} \pm \textbf{2.11}$$

For each individual TessArray RM-Flu assay, the mean and standard deviation of C3 Scores from the set of 25 background control detector tiles ($mean_{(Assay25)}$, $\sigma_{(Assay25)}$) are used to set the detection threshold (**$C3_{detect}$**) for targeted influenza virus gene sequences from the analyte-specific detector tiles. A lower limit for acceptable detection thresholds (**$C3_{detect}$**) is the average of the $mean_{(Assay25)}$ C3 Scores for the sampled ensemble of 607 assay results (**$C3_{detect}$** $\geq$ 3.37).

Analysis of assay-generated analyte gene sequence(s):

A statistically significant ($p < 0.001$) C3 Score > **$C3_{detect}$** from one or more of the analyte-specific detector tiles implies detection of template nucleic acid from the specimen that is related to the identity of the corresponding detector tile sequence. However, the C3 Score reported from a resequencing detector tile is a quantitative metric for the targeted viral gene sequence generated from that detector, and alone the C3 Score conveys no qualitative or specific sequence information. Specific sequence information generated by the assay detector tile(s) is analyzed by sequence alignment similarity search as described below.

The TessArray RM Data Analysis Server automatically evaluates assay-generated target gene sequences using the NCBI Basic Local Alignment Search Tool (BLAST) to execute a systematic alignment-similarity search across the TessArray Validated Reference Sequence Database (VSRD). The BLAST-VSRD analysis executed by the TessArray RM Data Analysis Server specifies the identities of the most similar sequence record(s) matching assay-generated target gene sequences.

As of September 2009 there are 39,876 VSRD sequence records representing known strains corresponding to the TessArray RM-Flu analytes:

- 2009 H1N1 influenza virus (N = 4,453)
- seasonal A/H1N1 strains (N = 9,880)
- seasonal A/H3N2 strains (N = 25,143)

There are also 43,561 VSRD viral gene sequence records representing known type A influenza virus strains that are **not** analytes of the TessArray RM-Flu. Most of these other known type A influenza viruses represent subtypes that are prevalent in or exclusively associated with infections of other vertebrate species, in particular avian, equine and swine populations.

Quality Assurance:

Background Controls Results: Background levels of resequencing from the set of 25 non-analyte control detector tiles are evaluated in every TessArray RM-Flu assay. If one or more of the 25 background control detector tiles reports a C3 Score > 7, then a background control "FLAG" is set.

The assay data quality FLAG indicates if there may be any issue of background control tile C3 Scores and detector threshold settings that might adversely lead to a false report of analyte detection based upon (a low) C3 score. If the maximum analyte-specific detector tile C3 Score is < 7, and the Assay Data Quality FLAG is set, then the assay result is NEGATIVE (reported as Not Detected).

Protocol Controls Results (TIM and NAC1 genes from *Arabidopsis thaliana*): Failure of any of the four control detector tiles to report a C3 Score > 30 is indication that the assay protocol has failed to amplify those target genes associated with the particular multiplex PCR reaction tube(s). In this case the assay result is reported **Indeterminate**. Retesting or new sampling is recommended. If a TessArray RM-Flu assay report is either **Indeterminate** or **Analyte Not Detected,** the operator should note if the Assay Protocol Control is indicated PASS or FAIL. If it indicates FAIL, then it is recommended to repeat the test with another aliquot of the sample. If the replicate assay also fails, this may reflect deterioration of assay reagents or issues with adherence to the recommended assay protocol.

Nucleic Acid Extraction Controls: When using seasonal trivalent influenza vaccines as positive nucleic acid extraction controls, assay results from vaccine control specimens should report:

- Assay outcome as **Analyte Not Detected** (as for the analyte 2009 H1N1 Influenza A Virus)
- Positive C3 Scores > $\mathbf{C3}_{detect}$ threshold from the HA1hu, NA1hu, HA3hu, NA2hu, M1hu, and M3hu detector tiles
- Positive C3 Scores Scores > $\mathbf{C3}_{detect}$ threshold may also be reported from NA1av, NSav and M5av detector tiles.
- The lists of most similar sequence records returned by BLAST-VSRD analysis of assay-generated sequences from M1hu, M3hu, M5av, and/or NSav detector tiles should include the vaccine master donor strains A/Puerto Rico/8/1934(H1N1) or A/Ann Arbor/6/1960 (H2N2) for inactivated or live viral vaccine samples, respectively.
- The lists of most similar sequence records returned by BLAST-VRSD analysis of assay-generated sequences from the HA1hu, NA1hu, HA3hu and NA2hu detector tiles should correspond to the seasonal A/H1N1 and seasonal A/H3N2 configurations of the vaccine sample.

The following pages 8 through 20 are page images from the TessArray RM-Flu User Manual, with sections

- **OVERVIEW OF THE PROCEDURE**
- **TESSARRAY REQUIRED MATERIALS**
- **STARTING MATERIAL**
- **ADDITIONAL REAGENTS REQUIRED**
- **RECOMMENDED INSTRUMENTS**
- **GENERAL LABORATORY REAGENTS/EQUIPMENT**
- **EXTRACTION AND PURIFICATION OF TOTAL NUCLEIC ACIDS FROM CLINICAL SPECIMENS**
- **OVERVIEW OF THE RM-FLU MULTIPLEX PROTOCOL**
- **SAMPLE AMPLIFICATION**
- **REVERSE TRANSCRIPTION**
- **MULTIPLEX PCR**
- **SAMPLE POOLING AND PURIFICATION**
- **SAMPLE FRAGMENTATION AND LABELING**
- **SAMPLE HYBRIDIZATION AND MICROARRAY WASHING AND SCANNING**
- **APPENDIX – RECOMMENDED GENERAL LABORATORY SUPPLIES**

LIMITATIONS	begins on page 21
PROTOCOL USE LIMITATIONS	begins on page 22
INTERPRETATION OF RESULTS	begins on page 22
ANALYTICAL PERFORMANCE CHARACTERISTICS	begins on page 23
CLINICAL PERFORMANCE CHARACTERISTICS	begins on page 29

Overview of the Procedure

The TessArray RM-Flu multiplex protocol is shown in Figure 2 on page 10.

1. **Reverse Transcription step** converts any viral RNA to cDNA using random primers.
2. **Multiplex PCR step** amplifies target influenza gene sequences with specific primers in four separate reactions.
3. **Sample pooling and DNA purification** combines the four multiplex reactions, removes any primers, enzymes, salts, dNTPs, and serves to concentrate the amplified DNA.
4. **Fragmentation and labeling** prepares the amplified targets to be hybridized to the RM-Flu array.
5. **Hybridization, staining and washing** allows for the detection of the target sequences on the RM-Flu array.
6. **Internal Controls** monitor the efficiency of amplification and hybridization steps.

TessArray Required Materials and Storage Conditions

1. RM-Flu Kit (P/N 900-009), 1 specimen; comprised of:
   a. RT Mix tube (P/N 500-009); store at -20°C
   b. RM-Flu PCR Mix tubes (P/N 510-009), set of 4; store at -20°C
   c. RM-Flu Array (P/N 520-447); store at +4°C
2. RPM Wash-Stain-Hybridization (WSH) Kit (P/N 900-010), 50 rxns; store at +4°C
3. RM-Flu Library File for array scanning protocols; download from the TessArae website: www.tessarae.com/h1n1flu
4. RM-Flu Alternative Data Analysis Package for Affymetrix GCS 3000 7G (RUO) Scanner, secure portal for GSEQ-generated FASTA file transfer; contact TessArae directly for implementation
5. RM-Flu Alternative Data Analysis Package for Affymetrix GCS 3000Dx or Dx2 (IVD) Scanner, secure portal for GSEQ-generated FASTA file transfer; contact TessArae directly for implementation

Starting Material

We recommend using the Epicentre® Masterpure™ Complete DNA and RNA isolation kit (cat# MC89010) for purification of total nucleic acids. This kit produces a high yield of nucleic acids and it gives excellent reproducibility in the assay.

NOTE: Other nucleic acid extraction/purification products or methods that produce high yield of high-quality PCR templates have not been tested for compatibility with this assay.

CAUTION: Inclusion of large quantities of carrier RNA will negatively impact the performance of the assay. Therefore we do not recommend protocols requiring the addition of carrier RNA .

Additional Reagents Required for this Protocol (not supplied by TessArae)

1. **Epicentre Masterpure Complete DNA and RNA isolation Kit** (Cat# MC89010)
2. **Life Technologies™ Superscript™ III Reverse Transcriptase**, includes 5x First Strand Buffer and 0.1 M DTT; 200 rxns (Cat# 18080-085, 40,000 U), 10 rxns (Cat# 18080-093, 2,000 U) or 50 rxns (Cat# 18080-044, 10,000 U)
3. **Life Technologies RNaseOUT™ Recombinant Ribonuclease Inhibitor** (Cat# 10777-019, 5,000 U), 125 rxns
4. **Promega®, GoTaq® Flexi DNA Polymerase** (Cat# M8298, 10,000 U), 50 rxns
5. **USB (Affymetrix) Uracil-DNA Glycosylase (UDG), Heat-Labile** (Cat# 78310, 100 U), 100 rxns
6. **Qiagen® QIAquick® PCR Purification Kit** (Cat#28104), 50 rxns
7. **Affymetrix GeneChip Resequencing Assay Kit** (P/N 900447), 30 rxns

Recommended Instruments

1. Thermal cyclers that were tested with the RM-Flu Multiplex PCR:
   a. Bio-Rad MJ Mini,
   b. Bio-Rad MyCycler,
   c. Bio-Rad Peltier DNA Engine Tetrad
2. Affymetrix GeneChip Microarray Instrumentation System
   a. Model 640 Hybridization Oven
   b. Fluidics Station 450 (up to 8 Fluidics Station 450 modules per System)
   c. Scanner
      i. GCS 3000 7G (RUO)
      ii. GCS 3000Dx (IVD)
      iii. GCS 3000Dx2 (IVD)
   d. Workstation and Software
      i. GCOS/GSEQ
      ii. AGCC/GSEQ
      iii. AGCC-Dx or AGCC-Dx2

General Laboratory Reagents/Equipment (detailed list with suppliers and catalog numbers in the Appendix).

NOTE: For the best performance and to guard against contamination it is not suitable to use repacked, autoclaved tips, as autoclaving does not remove RNases. Also use only barrier or filter tips to prevent cross contamination of samples and reagents.

1. Microcentrifuge, capable of speeds up to 13,000 rpm for spinning 1.5 ml tubes
2. Microcentrifuge capable of spinning down 96-well plates at low speed (for high-throughput experiments) or 0.2 ml PCR tubes and strips (for smaller scale experiments)
3. Pipettes P2.5, P10, P20, P200 and P1000
4. Filter tips suitable for the pipettes
5. Nuclease-Free water
6. 100% Ethanol
7. 100% Isopropanol
8. Life Technologies DNAZap® or Molecular BioProducts DNAaway™
9. Life Technologies RNaseZap® or Molecular BioProducts RNaseAWAY™
10. Tough Spots or equivalent for sealing the septa of the RM-Flu arrays
11. Microcentrifuge tube racks for 0.2 and 1.5 ml tubes
12. Nuclease-free microcentrifuge tubes 1.5 ml
13. Additional PB buffer (Qiagen, Cat# 19066)
14. Additional 2 ml Collection Tubes (Qiagen, Cat# 19201)
15. Nuclease-free 0.2 ml PCR tubes
16. Powder-free gloves

Extraction and Purification of Total Nucleic Acids from Clinical Specimens

The TessArray RM-Flu assay has been validated using throat swabs. The swabs should be collected in MicroTest™ M4 transport tubes (REF: R12500 or R12502, Remel, Lenexa, KS). Collected specimens should be transported at 4ºC and can be stored at 4ºC for up to 72 hours. For prolonged storage, specimens should be aliquoted into smaller volumes and frozen at -20ºC, and care should be exercised to minimize subjecting archived specimens to unnecessary freeze-thaw cycles.

Stored purified nucleic acids can be unsuitable for analysis after prolonged storage due to potential degradation of the sample. Therefore fresh isolation of total nucleic acids is recommended.

Each TessArray RM-Flu assay begins with 0.15 ml of a clinical specimen (throat swab in transport media, see above). Extraction and purification of total nucleic acid (RNA+DNA) from each 0.15 ml specimen aliquot is performed using the Epicentre Masterpure Complete DNA and RNA Isolation Kit, following the recommended procedures of the manufacturer. The final nucleic acid product from an initial 0.15 ml specimen aliquot is resuspended in 25 µl RNase-free water.

A total of 4 µl of the resuspended total nucleic acid is used for each TessArray RM-Flu assay, and the remainder may be stored at -20°C for use in optional replicate assays.

Several commercial kits for total nucleic acid extraction and purification, commonly used in some RT-PCR assay protocols, are **incompatible** with TessArray RPM (including RM-Flu) assay protocols, so TessArae strongly recommends exclusive use of the Epicentre Masterpure kit for nucleic acid extraction and purification for the TessArray RM-Flu test.

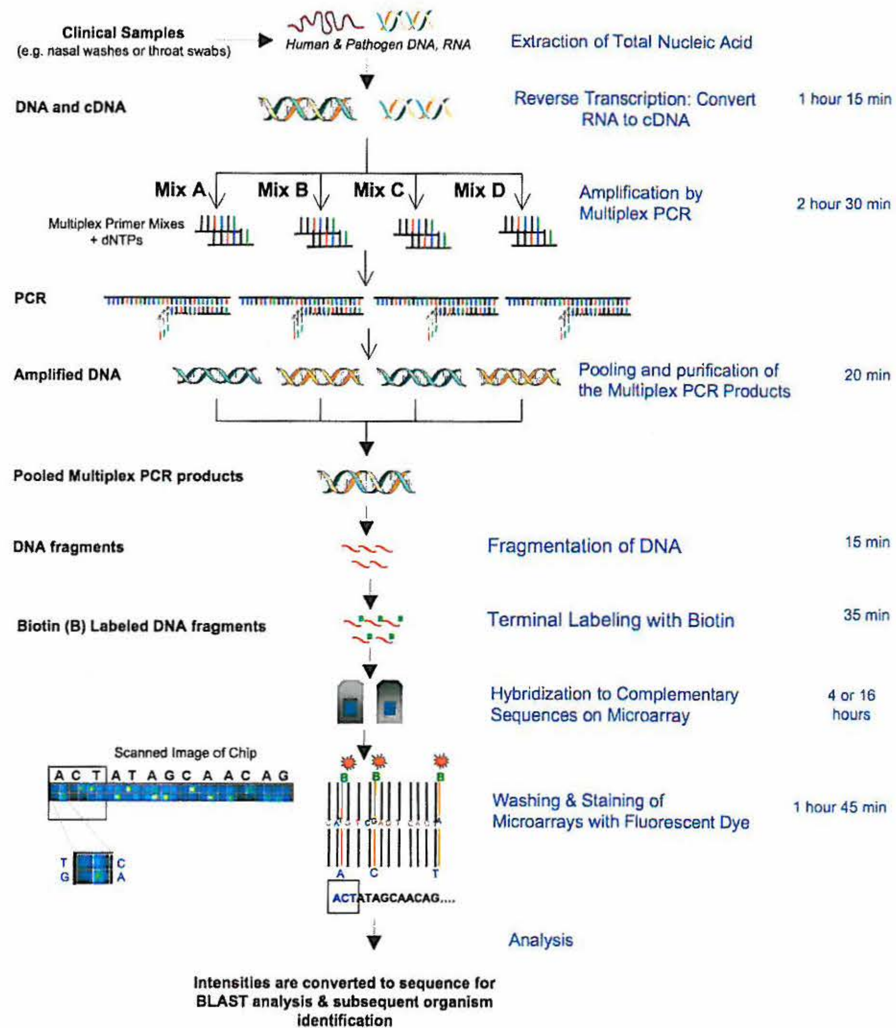


Figure 2. Overview of the RM-Flu multiplex protocol.

Sample Amplification

I. Reverse Transcription (RT)

When using the Epicentre Masterpure Complete DNA and RNA isolation kit, the final volume of purified nucleic acids is 25 µl and 4 µl of that final volume is used in the RT step. When using other nucleic acid extraction methods or products, the input volume may need to be adjusted appropriately.

1. Add 4 µl of purified total nucleic acid to one RM-Flu RT tube

a. Before proceeding spin down the **RM-Flu RT tubes.** After that place them on ice or in a PCR cooler.

b. Place a maximum of 4 µl of purified total nucleic acid into one **RM-Flu RT tube** (containing 8 µl of initial volume) to give a final volume of 12 µl. Supplement with nuclease-free water if less than 4 µl of total nucleic acids is used.

c. Vortex briefly and centrifuge to bring the contents to the bottom of the tube.

2. Incubate for 5 min at 65°C, then cool on ice

a. Incubate the reaction at 65°C for 5 minutes in a thermal cycler, then cool on ice for 5 minutes.

3. Add 8 µl of Reverse Transcription Master Mix and incubate at 25°C, then 50°C with inactivation at 85°C

a. In a 1.5 ml nuclease-free tube on ice prepare **Reverse Transcription Master Mix** as shown below:

Reagent	Volume	Volume	Volume
RM-Flu RT tube with nucleic acid sample	12 µl	12 µl	12 µl
RT Master Mix for	**1 sample**	**4 samples***	**10 samples***
5x First Strand Buffer	4 µl	17.6 µl	44 µl
0.1 M DTT	2 µl	8.8 µl	22 µl
RNaseOUT	1 µl	4.4 µl	11 µl
Superscript III	1 µl	4.4 µl	11 µl
Total volume	8 µl	35.2 µl	88 µl
Volume to be added to a single sample	**8 µl**	**8 µl**	**8 µl**
Total reaction volume	20 µl	20 µl	20 µl

* Includes 10% extra volume

b. Mix the reagents by pipetting up and down. Place on ice or in a benchtop cooler until ready to use.

c. Transfer 8 µl of **Reverse Transcription Master Mix** to each **RM-Flu RT tube** containing nucleic acid sample and mix by pipetting up and down.

d. Place the samples in a thermal cycler set to the following program:

25°C - 10 minutes,
50°C - 50 minutes,
85°C - 5 minutes.

Place the resulting **RM-Flu RT samples** from the RT step on ice while preparing **Multiplex PCR Master Mix**. Zip spin the tubes before proceeding to the next step. **Option:** Alternatively the **RM-Flu RT samples** can be kept at 4°C in the thermal cycler or frozen at -20°C until ready to use.

II. Multiplex PCR Step

Each **RM-Flu RT sample** must be amplified with 4 different multiplex primer cocktails, so each is split into 4 PCR reactions: A, B, C and D with 5 µl in each.

1. **Aliquot 5 µl of a RM-Flu RT sample into each of the 4 RM-Flu PCR Mix tubes**

   a. Thaw the 4 **RM-Flu PCR Mix tubes** (containing 24 µl of initial volume) provided with the RM-Flu kit and place immediately on ice or in a PCR cooler, when thawed spin briefly.

   b. Aliquot 5 µl of a single **RM-Flu RT sample** into each **RM-Flu PCR Mix tube** labeled A, B, C and D.

2. **Add 21 µl of Multiplex PCR Master Mix to each tube**

   a. On ice prepare **Multiplex PCR Master Mix** in a 1.5 ml nuclease-free tube as shown below:

Reagent	Volume	Volume	Volume
RM-Flu RT sample	5 µl	5 µl	5 µl
Multiplex PCR Master Mix for	1 sample* (4 PCR rxns)	4 samples* (16 PCR rxns)	10 samples* (40 PCR rxns)
5x GoTaq Flexi Buffer	44 µl	176 µl	440 µl
25 mM $MgCl_2$	35.2 µl	140.8 µl	352 µl
GoTaq (50U/µl)	8.8 µl	35.2 µl	88 µl
UDG	4.4 µl	17.6 µl	44 µl
Total volume	92.4 µl	369.6 µl	924 µl
Volume to be added to a single sample	21 µl	21 µl	21 µl
Total reaction volume	50 µl	50 µl	50 µl

* Includes 10% extra volume

   b. Mix the reagents by pipetting up and down. Place on ice or in a benchtop cooler until ready to use.

   c. Transfer 21 µl of **Multiplex PCR Master Mix** to each **RM-Flu PCR Mix tube** (A, B, C or D). Mix the tubes by vortexing and centrifuge briefly to bring the contents to the bottom of the tubes.

3. **Perform PCR amplification**

   a. Place the **RM-Flu PCR Mix tubes** in a thermal cycler and use the following program:

      24°C – 10 minutes
      94°C – 2 minutes
      16 cycles of:
      94°C – 30 seconds
      45°C – 30 seconds (increase by 1°C per cycle, up to 60°C)
      72°C – 90 seconds
      24 cycles of:
      94°C – 30 seconds
      60°C – 2 minutes

   b. When completed place the resulting **RM-Flu PCR**

Products from the amplification step on ice while preparing the next step. Zip spin the tubes before proceeding to the next step

Option 1: Alternatively the **RM-Flu PCR Products** can be kept at 4°C in the thermal cycler or frozen at -20°C until ready to use.

Option 2: PCR products from the 4 **RM-Flu PCR Products** per sample can be pooled into a single new 1.5 ml nuclease-free tube and frozen at -20°C if they cannot be processed immediately.

Sample Pooling and Purification

The sample pooling and purification is performed using Qiagen QIAQuick PCR purification kit.

When using the 50 rxn kit, only 25 **RM-Flu PCR Mix tubes** can be processed using the PB included in this Qiagen kit. Therefore we recommend obtaining additional PB buffer from Qiagen if you want to purify more samples. The kit also provides only a single 2 ml collection tube per column, so additional tubes will be required for changing the tubes during washes.

When using Qiagen QIAQuick PCR purification kit ensure that the columns are brought to room temperature before use and that none of the solutions has precipitated.

1. **Pool the 4 RM-Flu PCR Products and add 1000 µl of PB buffer to each pool**
   a. At room temperature prepare one 1.5 ml nuclease-free tube per sample. Combine all 4 **RM-Flu PCR Products** into this single tube.
   b. Add 1000 µl of DNA binding buffer (PB) to each tube, mix the **RM-Flu PCR Products** with PB buffer by pipetting up and down.
2. **Centrifuge sample through Qiagen QIAQuick PCR purification columns**
   a. Pipette 700 µl of each sample onto a column and spin the column at 13,000 rpm for 30 sec at room temperature, place the column in a new 2 ml tube.
   b. Repeat the previous step with the remaining sample.
3. **Wash the column with 730 µl of PE buffer**
   a. Transfer the column to a new 2 ml elution tube. Pipette 730 µl of Wash Buffer (PE) onto the column and spin the column at 13,000 rpm for 30 sec at room temperature.
4. **Spin to dry the column**
   a. Transfer the column to a new tube and spin the column again at 13,000 rpm for 30 sec at room temperature to remove all traces of the wash buffer.
5. **Elute the DNA with 25 µl of Elution Buffer (EB)**
   a. Place each column in a new nuclease-free 1.5 ml tube and add 25 µl of Elution Buffer (EB). Let the column stand for 1 minute.
   b. Spin the column at 13,000 rpm for 30 sec at room temperature.
   c. Discard the column and place the tube with the **eluted pooled Multiplex PCR products** on ice until ready to continue.

Option: eluted pooled Multiplex PCR products can be frozen at -20°C if they cannot be processed immediately.

Option: It is recommended that 1-2 µl of eluted PCR product is quantified using a NanoDrop or a similar instrument to check amplification of the nucleic acids.

Sample Fragmentation and Labeling

The reagents used for fragmentation and labeling are a part of the **Affymetrix Resequencing Assay Kit** (P/N 900447).

1. On ice aliquot 2.6 µl of Fragmentation Master Mix to a new 0.2 ml PCR tube, add sample and mix

a. In a new nuclease-free 1.5 ml tube prepare the following Fragmentation Master Mix on ice:

Reagent	Volume	Volume	Volume
Eluted pooled Multiplex PCR products	23 µl	23 µl	23 µl
Fragmentation Master Mix for	**1 sample**	**4 samples***	**10 samples***
10x Affymetrix Fragmentation Buffer	2.5 µl	12 µl	30 µl
Affymetrix Fragmentation Reagent	0.1 µl	0.48 µl	1.2 µl
Total volume	2.6 µl	12.48 µl	31.2 µl
Volume to be added to a single sample	**2.6 µl**	**2.6 µl**	**2.6 µl**
Total reaction volume	25.6 µl	25.6 µl	25.6 µl

* Includes 20% extra volume

b. Mix the reagents by pipetting up and down.

c. Aliquot 2.6 µl of **Fragmentation Master Mix** to new nuclease-free 0.2 ml tubes on ice.

d. Add the **eluted pooled Multiplex PCR products** and mix with the Fragmentation mix by pipetting up and down.

2. Incubate the eluted pooled Multiplex PCR products for 5 min at 37°C followed by 10 minutes at 95°C

a. Place the tubes in a thermal cycler and use the following program:

37°C – 5 minutes
95°C – 10 minutes

b. Place the resulting **Fragmented Multiplex PCR products** from the fragmentation step on ice while preparing the labeling Master Mix.

3. On ice add 10.4 µl of Labeling Master Mix to each sample and mix

a. In a new 1.5 ml nuclease-free tube prepare the following **Labeling Master Mix**:

Reagent	Volume	Volume	Volume
Fragmented Multiplex PCR products	25.6 µl	25.6 µl	25.6 µl
Labeling Master Mix for	**1 sample**	**4 samples***	**10 samples***
5x Affymetrix TdT Buffer	7.2 µl	31.7 µl	79.2 µl
Affymetrix Labeling Reagent	1.2 µl	5.28 µl	13.2 µl
Affymetrix TdT (30U/µl)	2 µl	8.8 µl	22 µl
Total volume	10.4 µl	45.8 µl	114.4 µl
Volume to be added to a single sample	**10.4 µl**	**10.4 µl**	**10.4 µl**
Total reaction volume	36 µl	36 µl	36 µl

* Includes 10% extra volume

b. Mix the reagents by pipetting up and down. Place on ice until ready to use.

c. Add 10.4 µl of the Master Mix to each tube containing **Fragmented Multiplex PCR products**.

4. Incubate the Fragmented Multiplex PCR products for 30 min at 37°C followed by 5 minutes at 95°C

a. Place the tubes in a thermal cycler and use the following program:

37°C – 30 minutes
95°C – 5 minutes

b. Place the resulting **Labeled Multiplex PCR products** from the labeling step on ice for at least 5 minutes before adding them to the hybridization mix.

Sample Hybridization, Washing and Scanning

The following steps can all be performed using the TessArray WSH kit (P/N 900-010).

It is recommended that hybridization is performed for 16 hours.

1. Prepare the arrays and prepare the experiment files in GCOS

a. Warm the arrays to room temperature for a minimum of 10 - 15 minutes.

b. While the arrays are warming, open GCOS on your computer and create a new experiment file for each of your samples.

c. Enter the barcodes for the arrays into GCOS experiment files either by typing or scanning with a barcode scanner and note which samples will be put on which array.

d. **Please note that filenames must only consist of letters, numbers, dashes, and underscores.**

2. Add 96 µl of Hybridization Master Mix to each Labeled Multiplex PCR product

a. In a new 1.5 ml nuclease-free tube prepare the following **Hybridization Master Mix**:

Reagent	Volume	Volume	Volume
Labeled Multiplex PCR products	36 µl	36 µl	36 µl
Labeling Master Mix for	**1 sample**	**4 samples***	**10 samples***
Affymetrix Hybridization solution	94.7 µl	416.7 µl	1041.7 µl
130x B2 oligo control	1.3 µl	5.72 µl	14.3 µl
Total volume	96 µl	422.42 µl	1056 µl
Volume to be added to a single sample	**96 µl**	**96 µl**	**96 µl**
Total reaction volume	132 µl	132 µl	132 µl

* Includes 10% extra volume

b. Mix the **Hybridization Master Mix** by vortexing. Place on ice until ready to use.

c. Add 96 µl of the **Hybridization Master Mix** to each **Labeled Multiplex PCR** product to create the **Hybridization cocktail**.

3. Denature the probe by incubating the Hybridization cocktails at 95°C for 5 minutes and 49°C for 5 minutes

a. Place the tubes in a thermal cycler and use the following program:

95°C – 5 minutes
49°C – 5 minutes

4. Prehybridize the RM-Flu arrays with 120 µl of prehybridization for 10 minutes at 49°C

a. Fill the **RM-Flu arrays** with 120 µl of prehybridization solution

b. Place the **RM-Flu arrays** in the array holders and into the Affymetrix Hybridization Oven and rotate for 10 minutes at 49°C and 60 rpm.

5. Add 120 µl of each Hybridization cocktail to the RM-Flu arrays

a. Remove the prehybridization solution from the **RM-Flu arrays** and replace with 120 µl of the **Hybridization cocktail**.

b. Place tough spots on the septa to seal and prevent sample leakage Replace the **RM-Flu arrays** in the holders and into the Affymetrix Hybridization Oven.

6. Hybridize the RM-Flu arrays for 16 hours

a. Rotate at 49°C and 60 rpm for 16 hours.

7. Prepare staining solutions

a. Prepare 3 new 1.5 ml nuclease-free tubes; one clear and two amber or dark tubes.

b. Aliquot 500 µl of SAPE Solution into one amber tube

c. Aliquot 500 µl of Antibody solution into a clear tube

d. Aliquot 800 µl of Array Holding Buffer into the second amber tube.

NOTE: if any of the solutions develop a precipitate, then place them in a 37°C waterbath until they dissolve (5 minutes should suffice).

8. Wash the RM-Flu array in the Fluidics Station 450 using the Midi_DNAarray_WS5_450 protocol

a. Turn on the Fluidics Station. In GCOS, select the Wash Station Modules to be primed and follow the instructions on the LCD screens

b. Meanwhile remove the **RM-Flu arrays** from the Hybridization Oven.

c. Remove the tough spots from the back of the **RM-Flu arrays.** Remove the **Hybridization cocktail** from the array and return to the original tubes and store at -20ºC.

d. Immediately replace the **Hybridization cocktail** with 120 µl of Array Holding Buffer. If more than 4 arrays are to be processed refrigerate any additional arrays till ready to process them. They will need to be warmed up to room temperature 10 minutes before use.

e. After Priming is complete on each selected module, Select the the **Midi_DNAarray_WS5_450** protocol for each module and follow the instructions on the LCD screens.

f. Place the **RM-Flu arrays** in the cartridge holders and select run from Fluidics Station Menu in the appropriate module.

g. Place the prepared stains and Array Holding Buffer in the fluidics station in the following order: Stain 1, Stain 2, Array Holding Buffer.

h. Turn on the scanner while washing the **RM-Flu arrays** as it takes approximately 15 minutes to warm up before you can scan arrays.

9. Scan the RM-Flu array

a. After washing, check the **RM-Flu arrays** for air bubbles, if an air bubble is present remove the Array Holding Buffer and replace with 150 µl of the same buffer manually. Place tough spots on the septa to prevent spillage of the liquid into the scanner.

b. Place the array in scanner and using GCOS software scan it.

c. If using a single array scanner, continue scanning arrays one by one till finished. If using a scanner with an autoloader, the scanner will automatically continue till all arrays have been scanned.

d. When scanning of all **RM-Flu arrays** is complete remove the **RM-Flu arrays** from the scanner and turn the scanner off. The **RM-Flu arrays** should be stored at 4ºC if required.

e. Analyze the output .CEL file using GSEQ Software from Affymetrix and refer to the Affymetrix GeneChip Sequence Analysis Software Users Guide (can be downloaded from the Affymetrix website, P/N 702084).

f. Perform a Shutdown protocol on the Fluidics Station modules used and turn it off.

Notes when using AGCC instead of GCOS

1. Open the Affymetrix Launcher and then AGCC Portal
2. In order to enter samples they have to be registered and there are 4 options: register each sample one by one, quick register, batch register or HT register.
3. **When performing the RPM assays DO NOT USE "QUICK REGISTER" OPTION**, it automatically adds array type in brackets to the filename thus causing an error in data analysis using TSEQ software. If using manual one by one registration that option can be turned off by un-selecting the box "Derive array name from the sample file's name." It means that you need to re-enter the sample name you typed at the top of that page. When using batch or HT register, the sample file name remains as entered by the user.
4. Barcodes can be entered into AGCC when doing one by one registration or into the spreadsheet created for the user by AGCC.
5. To use the Fluidics Station AGCC Fluidics Control has to be selected from the Affymetrix Launcher. In order to assign individual samples to particular modules on a Fluidics Station do not use "Master Control" tab but select the individual tab for the Fluidics Station that is to be used.
6. Once the Fluidics Station is running follow the instructions on the LCD screen.
7. When the washing and staining is complete, open AGCC Scan Control in the Affymetrix Launcher. Place the arrays in the autoloader and begin scanning.
8. NOTE: When using the AGCC there is no visualization of the array during scanning
9. In order to visualize the scanned arrays, AGCC Viewer has to be opened.
10. In order to be able to open multiple files, select "Search and Open", then in the dialog box select the project and samples to be opened or you can use the filters to open recently scanned arrays. When done selecting press "Open Selected" button and the files will be opened and visual QC (or gridding) can be checked and adjusted if necessary as described in the Analysis Manual.

APPENDIX

Recommended General Laboratory Supplies.

1. Microcentrifuge, capable of speeds up to 13,000 rpm for spinning 1.5 ml tubes
    a. Eppendorf - MiniSpin, MiniSpin plus,
    b. Sigma - SIGMA 1-15P, 1-14, 1-15PK
    c. Heraeus - Pico and Fresco Microcentrifuges
    d. Beckman Coulter - microfuge 16, 18, 22R
2. Microcentrifuge capable of spinning down 96-well plates at low speed (for high-throughput experiments) or 0.2 ml PCR tubes and strips (for smaller scale experiments)
    a. Tomy MicroONE Mini Centrifuge (for small scale)
    b. VWR Galaxy™ MiniStar Microcentrifuge (for small scale)
    c. Spectrafuge Mini-Centrifuge, Spectrafuge 7M and 16M can be fitted with 0.2 ml strip adaptors
    d. Sigma - SIGMA 3-16PK, 4K15, 2-16PK, 3-18K, SIGMA 4-16 (true high throughput)
    e. Eppendorf - MiniSpin, MiniSpin plus, 5424, 5430, 5417R , 5804/5804R, 5810/5810R (additional rotors needed for spinning 0.2 ml strips, or microplate rotors can be used with 0.2 ml tube racks.
3. Pipettes P2.5, P10, P20, P200 and P1000, all pipettes have to be calibrated at least once every 6 months.
    a. Gilson
    b. Eppendorf
    c. Nichiryo Nichipet
    d. Biohit
4. Filter tips suitable for the pipettes, optimally the brand selected should match the pipette brand used, however, some other brands offer adequately good tips and those should be used for calibration if they will be used as a standard.
    a. ART Barrier tips - ART 10 Reach (0.1-10 µl), ART 200 (1-200 µl), ART 1000 (100-1000 µl)
    b. Eppendorf tips - ep Dualfilter: #022491296 (2-200 µl), #022491211 (10 µl) and #022491253 (100-1000µl)
    c. Axygen filter tips - TXLF-10 (0.5-10 µl), TF20 (0.5-20 µl), TF-200 (1-200 µl) and TF-1000 (100-1000 µl) fit most major pipette brands but **the compatibility should be checked before ordering on www.axygen.com.**
    d. Biohit - #783201 (0.1-10 µl), #790021F (0.5-20 µl), #790201 (5-200 µl) and #783208 (50-1000 µl)
5. Nuclease-Free water
    a. Ambion - AM9932 (1L non-DEPC treated), AM9922 (1L DEPC treated)
    b. Invitrogen - 10977-015 (0.5L nuclease-free), 750023 (1L DEPC treated)
6. 100% Ethanol
    a. Sigma - E7023-500ML
7. 100% Isopropanol
    a. Sigma - I9516-500ML
    b. Sigma - P9416-50ML
8. Life Technologies DNAZap or Molecular BioProducts DNAaway
9. Life Technologies RNaseZap or Molecular BioProducts RNaseAWAY
10. Tough Spots® or equivalent for sealing the septa of the RPM arrays (Diversified Biotech) - 3/8" Tough-Spots cat # T-SPOTS
11. Microcentrifuge tube racks for 0.2 and 1.5 ml tubes
    a. VWR - rack series, Reversible microfuge racks (hold 0.2 ml, 0.5 ml and 1.5 ml tubes)
    b. Molecular BioProducts - FlipStrip Microtube Rack with Lid
12. Benchtop coolers
    a. SBS Compliant PCR chillers (0.2 ml PCR tubes, plates, strips) (Phenix Research Products)
    b. Eppendorf PCR cooler
    c. Stratagene - StrataCooler LP - for 1.5 ml tubes (storing enzymes required for RPM assays)
13. Nuclease-free microcentrifuge tubes 1.5 ml
    a. Axygen tubes clear - Axygen catalog number MCT-175-C
    b. Axygen tubes amber - Axygen catalog number MCT-175-X
14. Nuclelase-free 0.2 ml PCR tubes
    a. Axygen tubes - Axygen catalog number PCR-02-L-C or PCR-02-C
15. Powder-free gloves

LIMITATIONS

1. Analysts should be familiar with and follow Good Laboratory Practice standards, with special attention to the topics covered in the TessArray RM-Flu User Manual (pages 11-13).
2. Analysts should be trained and familiar with testing procedures and with interpretation of results prior to performing the assay.
3. All results from this and other tests must be correlated with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
4. The prevalence of infection will affect the test's predictive value.
5. Negative results do not rule out infection by 2009 H1N1 influenza A virus, by Seasonal A/H1N1 or A/H3N2 influenza virus strains, or by other influenza A viruses.
6. False negative results may occur:
    a. when the infecting virus has genomic mutations, insertions, deletions, or rearrangements, or
    b. when the infecting virus may be present at low viral load, as late in the course of illness (convalescence), or
    c. if inadequate quantity of undegraded virus or viral genome components may be present in the specimen due to improper collection, transport or handling.
    d. Repeat testing of the original specimen, or testing after attempts to culture the virus from the specimen is recommended.
7. False positive results may occur; the test attempts to mitigate risk of false positive tests through direct sequencing of multiple targeted viral genes in every specimen: Repeat testing or testing with a different device is recommended.
8. This qualitative molecular diagnostic test does not provide direct evidence that detected virus strain(s) is/are infectious. Viral infectivity tests may be performed using host cell and virus culture-based procedures that are standard for this determination.
9. This qualitative molecular diagnostic test does not report an absolute or relative quantitative estimate of the concentration (load) of a detected influenza virus in a specimen. The concentration of detected influenza virus in a specimen may be estimated using cell-culture based methods (viral plaque assay, endpoint dilution CPE assay), or using real-time RT-PCR assays.
10. The performance of this test has not been established for monitoring treatment of influenza A or 2009 H1N1 influenza A virus infection.
11. The performance of this test has not been established for screening of blood or blood product for the presence of influenza A or 2009 H1N1 influenza virus.
12. This test does not rule out diseases caused by bacterial or viral pathogens other than 2009 H1N1, Seasonal A/H1N1 and Seasonal A/H3N2 influenza viruses.

PROTOCOL USE LIMITATIONS

The TessArray RM-Flu assay protocol has been optimized for analysis of total nucleic acid from throat swab specimens extracted and purified using the Epicentre Masterpure Complete DNA and RNA Isolation kit. Alternative nucleic acid preparation kits are not recommended.
The TessArray RM-Flu assay protocol reverse transcription and multiplex PCR amplification steps have been optimized using Life Technologies Superscript III Reverse Transcriptase and

Promega GoTaq Flexi DNA Polymerase, respectively. Alternative enzymatic reagents are not recommended.

The TessArray RM-Flu resequencing microarray analysis has been optimized using the Affymetrix GeneChip Instrumentation System, specifically with the Affymetrix GCS 3000 7G microarray scanner, bundled with Affymetrix GCOS/GSEQ or AGCC/GSEQ operating/sequence analysis software.

INTERPRETATION OF TEST RESULTS

Scoring TessArray RM-Flu detector tile results as analyte-positive or analyte-negative:

The C3 Score of each analyte-associated detector tile (Table 1) is assessed with respect to the average C3 Score of 25 background control tiles, as follows.

1. A TessArray RM-Flu resequencing microarray detector tile is scored as statistically positive (pos) if
   a. C3 detection Score > average background detector C3 Score + 3.29* standard deviation of background detector C3 Scores,
   (this threshold corresponds to $p < 0.001$ for the positive result to arise within the random distribution of background detector tile C3 Scores)

   b. and if the C3 Score is > 3.37
   ($C3_{detect} = 3.37$ is the lower bound for acceptable detection thresholds established as the average of 25 background detector tile C3 Scores from assays of a sampled ensemble of 607 influenza A virus-positive and influenza A virus-negative specimens.)

2. If assay results from a TessArray RM-Flu detector tile fail to meet criteria above (1a. and 1b.) then the detector tile is scored negative (neg).

Interpretation of a POSITIVE analyte detection and identification by TessArray RM-Flu

The outcome of each TessArray RM-Flu assay is reported as either **Analyte Detected, Indeterminate, or Analyte Not Detected**, for the 2009 H1N1 influenza A virus, as follows:

1. A specimen is reported as **Analyte Detected** only if one or more of the analyte-associated resequencing detector tiles (Table 1) is scored as positive (C3 Score is > $C3_{detect}$) and if the automated BLAST-VSRD analysis of assay-generated sequences from the detector tile(s) returns most similar sequence records that represent that particular analyte.
2. A specimen is reported **Indeterminate** if one or more of the analyte-associated resequencing detector tiles (Table 1) has C3 Score > $C3_{detect}$ but the automated BLAST-VSRD analysis of assay-generated sequences from the detector tile(s) fails to return sequence records. Retesting or new sampling is recommended.
3. A specimen is reported **Analyte Not Detected** if there are no analyte-associated resequencing detector tiles (Table 1) with C3 Score > $C3_{detect}$.
4. A specimen is also reported **Analyte Not Detected** if one or more of the analyte-associated resequencing detector tiles (Table 1) has C3 Score > $C3_{detect}$ but the BLAST-VSRD analysis of the generated sequence(s) returns most similar sequence records that do not represent the particular analyte strain(s) of influenza A virus.

ANALYTICAL PERFORMANCE CHARACTERISTICS

Analytical Sensitivity - Limit of Detection (LoD)

The Limit of Detection (LoD) of the TessArray RM-Flu was determined for three influenza virus stocks representing 2008-2009 season isolates of clinical specimens (throat swabs, TS). Collaborators from the Naval Health Research Center (San Diego) selected three isolates that had previously been confirmed to represent the three target analytes of the TessArray RM-Flu assay

2009 H1N1 influenza Strain	A/CA/NHRC-BRD40116N/2009(H1N1)
Seasonal A/H1N1 Strain	A/CA/NHRC-BRD10622N/2009(H1N1)
Seasonal A/H3N2 Strain	A/CA-/NHRC-BRD10601N/2009(H3N2)

Aliquots from original TS specimens or from low-passage shell vial MDCK cell culture lysates were used to infect MDCK cell cultures to produce high titer stocks. Replicate shell vial cultures of MDCK cells were then inoculated with serially diluted stock lysate in order to determine the infectious titer as $TCID_{50}$/ml. The resulting influenza virus titers of the three neat stocks were determined to be:

2009 H1N1 influenza virus	5.63×10^7 $TCID_{50}$/ml
Seasonal A/H1N1 Strain	3.16×10^7 $TCID_{50}$/ml
Seasonal A/H3N2 Strain	1.78×10^7 $TCID_{50}$/ml

Neat stocks were first diluted 1:1,000 using cell culture medium (DMEM plus glutamine and antibiotic/antimycotic solution, TCPK trypsin). After preparing triplicate 3-fold dilutions of each 1:1,000 preparation of each stock to final 1:729,000 the aggregate of 63 samples was blind-labeled by another laboratorian. The identification key was secured until completion of all assays and initial review of assay results.

Nucleic acid was independently extracted and purified from each of the 63 samples using the standard TessArray protocol (Epicentre MasterPure kits), followed by the remaining steps of the TessArray RM-Flu assay protocol.

Table 2, Table 3 and Table 4 present summaries of unblinded sample identifications and corresponding assay results for the TessArray RM-Flu assays of serially diluted 2009 H1N1 flu strain, seasonal A/H1N1 strain and seasonal A/H3N2 strain, respectively.

Note that after the first round of assays was competed, two additional sets of triplicate sample dilutions were prepared (as 1:2,187,000), representing further dilutions of the Seasonal A/H1N1 and Seasonal A/H3N2 stocks to be assayed. The results from these assays are included as the last lines of Table 4 and Table 5, respectively.

The first set and the second set of triplicate assays were each performed blindly with respect to individual sample analyte identity and dilution tier.

To define a 95% detection rate, confirmation of endpoint LoD was performed using assays of 20-fold replicates from selected triplicate dilution tiers.

Table 2. LoD Estimation from Diluted Neat Stock of 2009 H1N1 Strain (BRD40116N).

Diluted Titer ($TCID_{50}$/mL)	2009 H1N1 influenza Strain Analyte Detector Tile Results (pos/total)					Assay Result	Confirmation
	NA1av	NSav	M1hu	M3hu	M5av	POS/NEG	
56,300	3/3	3/3	3/3	3/3	3/3	POS	NA
18,767	3/3	3/3	3/3	3/3	3/3	POS	NA
6,256	2/3	3/3	3/3	3/3	3/3	POS	NA
2,085	0/3	2/3	3/3	3/3	3/3	POS	20/20
695	0/3	0/3	2/3	2/3	2/3	POS	14/20
232	0/3	0/3	0/3	0/3	1/3	POS	NA
77	0/3	0/3	0/3	0/3	0/3	NEG	NA

Table 3. LoD Estimation for Seasonal A/H1N1 Strain (BRD10622N).

Diluted Titer ($TCID_{50}$/mL)	Seasonal A/H1N1 Strain Analyte Detector Tile Results (pos/total)					Assay Result	Confirmation
	HA1hu	NA1hu	M1hu	M3hu	M5av	POS/NEG	
31,600	3/3	3/3	3/3	3/3	3/3	POS	NA
10,533	3/3	3/3	3/3	3/3	3/3	POS	NA
3,511	3/3	3/3	3/3	3/3	3/3	POS	NA
1,170	3/3	3/3	3/3	3/3	3/3	POS	NA
390	2/3	2/3	3/3	3/3	3/3	POS	NA
130	2/3	1/3	3/3	1/3	1/3	POS	20/20
43	2/3	0/3	0/3	0/3	0/3	POS	NA
14	1/3	0/3	1/3	0/3	0/3	POS	NA

Table 4. LoD Estimation for Seasonal A/H3N2 Strain (BRD10601N).

Diluted Titer ($TCID_{50}$/mL)	Seasonal A/H3N2 Strain Analyte Detector Tile Results (pos/total)					Assay Result	Confirmation
	HA3hu	NA2hu	M1hu	M3hu	M5av	POS/NEG	
17,800	3/3	3/3	3/3	3/3	3/3	POS	NA
5,933	3/3	3/3	3/3	3/3	3/3	POS	NA
1,978	3/3	3/3	3/3	3/3	3/3	POS	NA
659	3/3	2/3	1/3	2/3	2/3	POS	NA
220	2/3	1/3	0/3	1/3	1/3	POS	19/20
73	2/3	1/3	1/3	1/3	0/3	POS	10/20
24	1/3	1/3	0/3	0/3	0/3	POS	NA
8	0/3	0/3	0/3	0/3	0/3	NEG	NA

Data presented in Table 2 through Table 4 confirm the Analytical Sensitivity of the TessArray RM-Flu as LoD (minimum concentration of analyte at which > 95% of assays results are likely to report positive detection of the analyte) for each of the three analyte strains:

2009 H1N1 influenza Strain	**20/20 positive**	**LoD = 2,085 $TCID_{50}$/mL**
Seasonal A/H1N1 Strain	**20/20 positive**	**LoD = 130 $TCID_{50}$/mL**
Seasonal A/H3N2 Strain	**19/20 positive**	**LoD = 220 $TCID_{50}$/mL**

Assessing possible interference from non-analyte influenza viruses

The 25 background control detector tiles used in the TessArray RM-Flu assay represent various HA and NA genes of non-analyte type A influenza viruses. Twenty specimens containing validated reference strains of non-analyte type A influenza viruses were assayed using TessArray RM-Flu in order to demonstrate that the presence of a non-analyte type A influenza virus does not lead to false positive detection and identification of a target analyte.

The reference strain samples for which assay results are shown in Table 5, were provided by the USDA/ARS SouthEast Poultry Research Laboratory (SEPRL, Athens, GA) and by the Naval Medical Research Unit-3 (NAMRU3, Cairo, Egypt) through the Naval Health Research Center (NHRC, San Diego, CA). Reference strain subtype identification and genotypes were determined by the laboratories providing the specimens using validated serological and/or PCR-based assays and *de novo* viral gene sequencing.

Results summarized in Table 5 demonstrate negative analyte-detection outcomes in all samples tested with non-analyte Influenza A virus subtypes. Although individual analyte-associated detector tiles (Table 1) and or background control detector tiles were scored positive in each of these assays, the automated BLAST-VSRD sequence alignment-similarity analysis returned most similar sequences that only shared identities with the corresponding non-analyte influenza A viruses. Thus all of the assays described in Table 5 reported **Analyte Not Detected**.

Table 5. Various Non-Analyte (Non 2009 H1N1, non seasonal A/H1, A/H3) Type A Influenza Virus Subtypes Do Not Lead to False Positive Detection and Identification of Analyte Strains

Non-Analyte Strain	Most Similar Sequence Records		2009 H1N1 Seasonal H1 Seasonal H3 POS/NEG	Non-Analyte POS/NEG (seq)
	Analyte	Non-Analyte		
A/H1N1(av)	0	13	NEG	POS
A/H2N8	0	24	NEG	POS
A/H3N2(av)	0	13	NEG	POS
A/H4N6	0	7	NEG	POS
A/H5N1*	0	20	NEG	POS
A/H5N2	0	19	NEG	POS
A/H5N3	0	8	NEG	POS
A/H7N1	0	39	NEG	POS
A/H7N2	0	7	NEG	POS
A/H7N3	0	37	NEG	POS
A/H7N3	0	9	NEG	POS
A/H7N7	0	25	NEG	POS
A/H8N4	0	16	NEG	POS
A/H10N7	0	9	NEG	POS
A/H11N3	0	12	NEG	POS
A/H11N9	0	10	NEG	POS
A/H12N5	0	25	NEG	POS
A/H13N6	0	10	NEG	POS
A/H14N5	0	10	NEG	POS
A/H15N9	0	32	NEG	POS

NEG = assay outcome was **Analyte Not Detected**

POS = BLAST-VSRD analysis returned only non-analyte influenza A virus sequence records

* This specimen (A/H5N1) is one of ten human subjects confirmed by CDC rRT-PCR comparator test to be infected by A/H5N1 avian influenza virus. The TessArray RM-Flu assay outcomes for this specimen are representative of all ten of these A/H5N1-positive specimens. The performance characteristics of the TessArray RM-Flu with clinical specimens positive for the avian H5N1 virus have not been established.

These results demonstrate that elevated assay detector thresholds and BLAST-VSRD return of non-analyte sequence records together assure that the intended performance of the TessArray RM-Flu assay is not compromised by the presence in a sample of non-analyte influenza viruses.

Analytical Reactivity (inclusivity)

Five different seasonal configurations of live or inactivated trivalent influenza virus vaccines, including the current 2009-2010 season configurations, were tested with the TessArray RM-Flu assay. The results described in Table 6 and Table 7 below demonstrate the capability of TessArray RM-Flu to discern mixtures of seasonal A/H1N1 and seasonal A/H3N2 in individual specimens, and indicate the capability of TessArray RM-Flu to determine vaccine strains from circulating seasonal strains of A/H1N1 and/or A/H3N2, based upon distinctive sequences of assay-generated specimen-specific HA and NA sequences, and the distinctive sequences of master donor strain-specific M and NS gene sequences of the vaccine configurations.

Table 6. Evaluation of Analytical Reactivity of TessArray RM-Flu using 2009-2010 Trivalent Inactivated Vaccine and 2009-2010 Tri-valent Live Virus Vaccine

2009-2010 Seasonal Inactivated Influenza Virus Vaccine (Fluvirin)			2009-2010 Seasonal Live Influenza Virus Vaccine (FluMist)		
Strain Configuration of Vaccine: HA1hu and NA1hu - A/South Dakota/6/2007(H1N1) HA3hu and NA2hu – A/Uruguay/716/2007(H3N2) M1hu, M3hu, M5av – A/Puerto Rico/8/1934(H1N1) (master donor virus) NSav – A/Puerto Rico/8/1934(H1N1) (master donor virus)			Strain Configuration of Vaccine: HA1hu and NA1hu - A/South Dakota/6/2007(H1N1) HA3hu and NA2hu – A/Uruguay/716/2007(H3N2) M1hu, M3hu, M5av – A/Ann Arbor/6/1960(H2N2) (master donor virus) NSav – A/Ann Arbor/6/1960(H2N2) (master donor virus)		
Analyte: Detector Tile	**BLAST results include configuration strain(s) among most sequence records (pos/neg)**	**Analyte Outcome**	**Analyte: Detector Tile**	**BLAST results include configuration strain(s) among most sequence records (pos/neg)**	**Analyte Outcome**
2009 H1N1			2009 H1N1		
NA1av	(neg)	2009 H1N1-NEGATIVE*	NA1av	no matching sequence records (neg)	2009 H1N1-NEGATIVE*
NSav	A/Puerto Rico/8/1934(H1N1) (neg)		NSav	A/Puerto Rico/8/1934(H1N1) (neg)	
M1hu	A/Puerto Rico/8/1934(H1N1) (neg)		M1hu	A/Puerto Rico/8/1934(H1N1) (neg)	
M3hu	A/Puerto Rico/8/1934(H1N1) (neg)		M3hu	A/Puerto Rico/8/1934(H1N1) (neg)	
M5av	A/Puerto Rico/8/1934(H1N1) (neg)		M5av	A/Puerto Rico/8/1934(H1N1) (neg)	
Seasonal A/H1N1			Seasonal A/H1N1		
HA1hu	A/South Dakota/6/2007(H1N1) (pos)	Seasonal A/H1N1-POSITIVE (Vaccine Strain)	HA1hu	A/South Dakota/6/2007(H1N1) (pos)	Seasonal A/H1N1-POSITIVE (Vaccine Strain)
NA1hu	A/South Dakota/6/2007(H1N1) (pos)		NA1hu	A/South Dakota/6/2007(H1N1) (pos)	
M1hu	A/Puerto Rico/8/1934(H1N1) (pos)		M1hu	A/Puerto Rico/8/1934(H1N1) (pos)	
M3hu	A/Puerto Rico/8/1934(H1N1) (pos)		M3hu	A/Puerto Rico/8/1934(H1N1) (pos)	
M5av	A/Puerto Rico/8/1934(H1N1) (pos)		M5av	A/Puerto Rico/8/1934(H1N1) (pos)	
Seasonal A/H3N2			Seasonal A/H3N2		
HA3hu	A/Uruguay/716/2007(H3N2) (pos)	Seasonal A/H3N2-POSITIVE (Vaccine Strain)	HA3hu	A/Uruguay/716/2007(H3N2) (pos)	Seasonal A/H3N2-POSITIVE (Vaccine Strain)
NA2hu	A/Uruguay/716/2007(H3N2) (pos)		NA2hu	A/Uruguay/716/2007(H3N2) (pos)	
M1hu	A/Puerto Rico/8/1934(H1N1) (pos)		M1hu	A/Puerto Rico/8/1934(H1N1) (pos)	
M3hu	A/Puerto Rico/8/1934(H1N1) (pos)		M3hu	A/Puerto Rico/8/1934(H1N1) (pos)	
M5av	A/Puerto Rico/8/1934(H1N1) (pos)		M5av	A/Puerto Rico/8/1934(H1N1) (pos)	

Table 7. Evaluation of Analytical Reactivity of TessArray RM-Flu using 2006-2007 Trivalent Inactivated Vaccine and 2004-2005 Tri-valent Live Virus Vaccine

2006-2007 Seasonal Inactivated Influenza Virus Vaccine (Fluzone)			2004-2005 Season Live Influenza Virus Vaccine (FluMist)		
Strain Configuration of Vaccine: HA1hu and NA1hu - A/New Caledonia/20/1999(H1N1) HA3hu and NA2hu – A/Wisconsin/67/2005(H3N2) M1hu, M3hu, M5av – A/Puerto Rico/8/1934(H1N1) (master donor virus) NSav – A/Puerto Rico/8/1934(H1N1) (master donor virus)			Strain Configuration of Vaccine: HA1hu and NA1hu - A/New Caledonia/20/1999(H1N1) HA3hu and NA2hu – A/Wyoming/03/2003(H3N2) M1hu, M3hu, M5av – A/Ann Arbor/6/1960(H2N2) (master donor virus) NSav – A/Ann Arbor/6/1960(H2N2) (master donor virus)		
Analyte: Detector Tile	**BLAST results include configuration strain(s) among most sequence records (pos/neg)**	**Analyte Outcome**	**Analyte: Detector Tile**	**BLAST results include configuration strain(s) among most sequence records (pos/neg)**	**Analyte Outcome**
2009 H1N1			2009 H1N1		
NA1av	(neg)	2009 H1N1 NOT DETECTED*	NA1av	(neg)	2009 H1N1 NOT DETECTED*
NSav	A/Puerto Rico/8/1934(H1N1) (neg)		NSav	A/Ann Arbor/6/1960(H2N2) (neg)	
M1hu	A/Puerto Rico/8/1934(H1N1) (neg)		M1hu	A/Ann Arbor/6/1960(H2N2) (neg)	
M3hu	A/Puerto Rico/8/1934(H1N1) (neg)		M3hu	A/Ann Arbor/6/1960(H2N2) (neg)	
M5av	A/Puerto Rico/8/1934(H1N1) (neg)		M5av	A/Ann Arbor/6/1960(H2N2) (neg)	
Seasonal A/H1N1			Seasonal A/H1N1		
HA1hu	A/New Caledonia/20/1999(H1N1) (pos)	Seasonal A/H1N1-DETECTED (Vaccine Strain)	HA1hu	A/New Caledonia/20/1999(H1N1) (pos)	Seasonal A/H1N1-DETECTED (Vaccine Strain)
NA1hu	A/New Caledonia/20/1999(H1N1) (pos)		NA1hu	A/New Caledonia/20/1999(H1N1) (pos)	
M1hu	A/Puerto Rico/8/1934(H1N1) (pos)		M1hu	A/Ann Arbor/6/1960(H2N2) (pos)	
M3hu	A/Puerto Rico/8/1934(H1N1) (pos)		M3hu	A/Ann Arbor/6/1960(H2N2) (pos)	
M5av	A/Puerto Rico/8/1934(H1N1) (pos)		M5av	A/Ann Arbor/6/1960(H2N2) (pos)	
Seasonal A/H3N2			Seasonal A/H3N2		
HA3hu	A/Wisconsin/67/2005(H3N2) (pos)	Seasonal A/H3N2-DETECTED (Vaccine Strain)	HA3hu	A/Wyoming/03/2003(H3N2) (pos)	Seasonal A/H3N2-DETECTED (Vaccine Strain)
NA2hu	A/Wisconsin/67/2005(H3N2) (pos)		NA2hu	A/Wyoming/03/2003(H3N2) (pos)	
M1hu	A/Puerto Rico/8/1934(H1N1) (pos)		M1hu	A/Ann Arbor/6/1960(H2N2) (pos)	
M3hu	A/Puerto Rico/8/1934(H1N1) (pos)		M3hu	A/Ann Arbor/6/1960(H2N2) (pos)	
M5av	A/Puerto Rico/8/1934(H1N1) (pos)		M5av	A/Ann Arbor/6/1960(H2N2) (pos)	

Similar assay results as shown in Table 6 and Table 7 were obtained for the seasonal 2005-2006 Inactivated Influenza Virus Vaccine (Fluzone), which has the following strain configuration:

- HA1hu and NA1hu - A/New Caledonia/20/1999(H1N1)
- HA3hu and NA2hu – A/New York/55/2004(H3N2)
- M1hu, M3hu, M5av – A/Puerto Rico/8/1934(H1N1) (master donor virus)
- NSav – A/Puerto Rico/8/1934(H1N1) (master donor virus)

Performance of the TessArray RM-Flu assay on the 2009-2010 monovalent vaccine for the 2009 H1N1 influenza virus has not been established.

CLINICAL PERFORMANCE CHARACTERISTICS

A total of 27 different specimens independently confirmed to be positive for the 2009 H1N1 influenza A virus were provided for testing by the Department of Respiratory Disease Research, Naval Health Research Center (NHRC); the Walter Reed Army Institute of Research (WRAIR), and the Molecular Diagnostics Laboratory at Children's National Medical Center (CNMC). Twenty two of these 27 specimens (81.5%) were reported by TessArray RM-Flu assays to be positive (**Analyte Detected**) for the 2009 H1N1 virus.

Additionally, a set of 282 2009 H1N1 virus negative clinical specimens were collected from multiple U S military training facilities during 2006-2009 seasons, as throat swabs (TS), from healthy consented volunteers (19) as well as individuals with diagnosed febrile respiratory infection (191) or pneumonia confirmed by X-ray (72), under NHRC IRB review and oversight of clinical research and surveillance. These 282 samples included 9 positive seasonal flu A H1N1 specimens and 3 seasonal flu A H3N2 specimens confirmed by a validated comparator.

RM-Flu testing results for these specimens are summarized in the two panels of Table 8: assay outcome results are presented in the upper panel; percent positive agreement (PPA, by analyte) and percent negative agreement (PNA, by analyte) are presented with 95% confidence intervals in the lower panel.

Table 8. Outcomes from TessArray RM-Flu assays of 27 different specimens that were independently confirmed to be positive for the 2009 H1N1 flu virus and 282 confirmed 2009 H1N1 negative specimens. Upper panel: assay results by analyte. Lower panel: percent positive agreement and percent negative agreement.

	Comparator Assay Outcomes				
RM-Flu Outcomes	2009 H1N1 Influenza-Positive	Seasonal A/H1N1 Influenza-Positive	Seasonal A/H3N2 Influenza-Positive	Influenza A Virus - Negative	Total
2009 H1N1-Positive	22	0	0	0	22
2009 H1N1-Indeterminate	1 [a]	0	0	0	1
Seasonal A/H1N1-Positive	0	9	0	0	9
SeasonalA/H3N2-Positive	0	0	6	0	6
2009 H1N1 Negative	4 [a]	NA	NA	267	267
Total	27	9	6	267	309

Assay Performance	2009 H1N1 Influenza A Virus	Seasonal A/H1N1 Influenza A Virus	Seasonal A/H3N2 Influenza A Virus
Percent Positive Agreement	81.5% (22/27) 95%CI [b]: 61.9% - 93.7%	100% (9/9) 95%CI [b]: 66.4% - 100%	100% (6/6) 95%CI [b]: 54.1% - 100%
Percent Negative Agreement	100% (267/267) 95%CI [b]: 98.6% - 100%	100% (267/267) 95%CI [b]: 98.6% - 100%	100% (267/267) 95%CI [b]: 98.6% - 100%

[a] The four RM-Flu 2009 H1N1 influenza A-negative and single **Indeterminate** assay results were obtained from one series of assays performed by NHRC. Potential low viral load in these specimens (as from convalescent patients) or state of possible virus aggregation, precipitation or deterioration due to successive freeze/thawing of specimen samples for other tests may have affected these test results. The **Indeterminate** result (one detector tile reported statistically positive C3 Score but no most similar sequence record matches were returned from BLAST-VSRD analysis) was tallied against the RM-Flu assay in the evaluation of performance.
[b] The 95% confidence interval is estimated by the Exact method.

Cross-reactivity Studies

Twenty four clinical specimens confirmed by both validated RT-PCR comparator assay and by TessArray RM-Flu 3.1 (Parent Panel RUO) and TessArray RM-Flu to be influenza virus-negative, were tested in the cross-reactivity studies. These samples were demonstrated by the parent TessArray RM-Flu 3.1 (RUO Respiratory Pathogen Panel) to be positive (C3 Score > 75) for the particular non-influenza A, viral or bacterial respiratory pathogen; a subset of 9 samples were confirmed positive for the respective non-influenza pathogen by the validated comparator assay. All samples were clinical specimens. Although load was considered to be high, there was no data available on the respective titer of non-analyte virus or bacteria in these specimens.

Results from this series of assays are presented in Table 9.

There was no detection reported of 2009 H1N1, seasonal A/H1N1 or seasonal A/H3N2 influenza A virus strains for any of the 24 specimens tested, demonstrating no cross-reactivity of TessArray RM-Flu for specimens containing common non-analyte pathogens.

Table 9. Cross-reactivity. Negative Controls: Assays in the Presence of Other Respiratory Pathogens

	2009 H1N1 RM	Seasonal A/H1N1 RM	Seasonal A/H3N2 RM	RM-Flu Outcome [c]
Viral Respiratory Pathogens				
Adenovirus Type 4 [a]	**NEG**	**NEG**	**NEG**	**NEG**
Adenovirus Type 14 [a]	**NEG**	**NEG**	**NEG**	**NEG**
Human Rhinovirus [a]	**NEG**	**NEG**	**NEG**	**NEG**
Adenovirus Type 7 [a]	**NEG**	**NEG**	**NEG**	**NEG**
Type A Influenza A/H5N1 [a]	**NEG**	**NEG**	**NEG**	**NEG** [d]
Coronavirus OC43 [b]	**NEG**	**NEG**	**NEG**	**NEG**
Coronavirus NL63 [b]	**NEG**	**NEG**	**NEG**	**NEG**
Rhinovirus Type A [b]	**NEG**	**NEG**	**NEG**	**NEG**
Metapneumovirus A/B [b]	**NEG**	**NEG**	**NEG**	**NEG**
Parainfluenzavirus 1,2,3 [b]	**NEG**	**NEG**	**NEG**	**NEG**
Respiratory Syncytial Virus [b]	**NEG**	**NEG**	**NEG**	**NEG**
Bacterial Respiratory Pathogens				
Haemophilus influenzae [a]	**NEG**	**NEG**	**NEG**	**NEG**
Moraxella catarrhalis [a]	**NEG**	**NEG**	**NEG**	**NEG**
Mycoplasma pneumoniae [a]	**NEG**	**NEG**	**NEG**	**NEG**
Non-A, B *Streptococcus* [a]	**NEG**	**NEG**	**NEG**	**NEG**
Chlamydophila pneumoniae [b]	**NEG**	**NEG**	**NEG**	**NEG**
Klebsiella pneumoniae [b]	**NEG**	**NEG**	**NEG**	**NEG**
Mycoplasma pneumoniae [b]	**NEG**	**NEG**	**NEG**	**NEG**
Neisseria meningitidis [b]	**NEG**	**NEG**	**NEG**	**NEG**
Pseudomonas aeruginosa [b]	**NEG**	**NEG**	**NEG**	**NEG**
Staphylococcus aureus [b]	**NEG**	**NEG**	**NEG**	**NEG**
S. aureus (MRSA) [b]	**NEG**	**NEG**	**NEG**	**NEG**
Streptococcus pneumoniae [b]	**NEG**	**NEG**	**NEG**	**NEG**

[a] Presence of non-analyte pathogen confirmed by RT-PCR panel comparator.

[b] Presence of non-analyte pathogen not confirmed by RT-PCR panel comparator.

[c] With the exception of the assay in the presence of Avian Influenza A/H5N1, assay outcomes for these samples were reported NEG (**Analyte Not Detected**) because none of the analyte-associated detector tiles (Table 1) met or exceeded the individual assay $C3_{detect}$ threshold.

[d] The reported assay outcome for the specimen with Avian Influenza A/H5N1-positive specimen was NEG (**Analyte Not Detected**): although multiple analyte detector tiles had C3 Score greater than the $C3_{detect}$ threshold, the most similar sequence records from BLAST-VSRD analysis were exclusively from A/H5N1 strains and not from analyte influenza A virus strains. The performance characteristics of the TessArray RM-Flu with clinical specimens positive for the avian H5N1 virus have not been established.

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TECHNICAL ASSISTANCE - If questions arise concerning the kit, its reagents, assay data processing, or assay data analysis please contact TessArray, LLC Technical Services personnel

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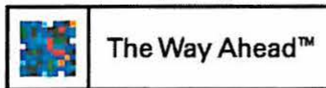

TessArray RM-Flu

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