

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 As of June 23, 2010, the EUAs have been terminated and this information is no longer current.



H1N1-09 INFLUENZA TEST PACKAGE INSERT

Emergency Use Authorization for *in vitro* Diagnostic Use

INTENDED USE

The DIATHERIX Laboratories H1N1-09 Influenza Test is a multiplex RT-PCR panel intended for the *in vitro* qualitative detection of the 2009 H1N1 influenza A viral RNA in nasopharyngeal swabs, nasal swabs, throat swabs, nasal aspirates, and nasopharyngeal aspirates from human patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors. Specimens are collected using the DIATHERIX collection pack (swab with collection tube) and sent to DIATHERIX Laboratories for testing. Test results are returned on a laboratory report within two days (usually the next day). This test is not intended for the detection of seasonal influenza A, B, or influenza C viruses.

Testing with the DIATHERIX H1N1-09 Influenza Test should only be performed on human patients who meet the clinical and epidemiologic criteria for testing suspect specimens.

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

EXPLANATION OF THE TEST

The DIATHERIX H1N1-09 Influenza Test detects the presence of the HA (hemagglutinin) and NA (neuraminidase) gene sequences specifically found in the novel 2009 H1N1 influenza virus. The presence or absence of these gene sequences is the basis of differentiation between novel 2009 H1N1 influenza and seasonal influenza A.

MATERIALS PROVIDED FOR THE TEST

Explanation of Primers, Probes and Controls:

There is one set of nested primers for each target in the test, and one set of probes that is complementary to the amplicon produced by each set of nested primers. Following RT-PCR, amplicons are hybridized to complementary probes for detection.

Nested Primers	Complementary Probes
H109C Fo	H109C De
H109C Fi	
H109C Ri	
H109C Ro	
N109B Fo	N109B De
N109B Fi	
N109B Ri	
N109B Ro	

There are three controls used in the test system: a negative control (PCR water blank), a PCR positive control (a combination of RNA extracted from Human Metapneumovirus, Parainfluenza Virus type 2 and Respiratory Syncytial Virus A, and an extraction control (a diluted culture of *Acinetobacter baumannii*).

Control	Purpose
Negative Control	To demonstrate that the PCR reaction did not generate non specific binding resulting in false amplification products or products from contamination.
PCR Positive Control	To demonstrate that the PCR reaction produced amplicons as expected.
Extraction Positive Control	To demonstrate that the extraction process was successful and that no carryover contamination was present in the control.

All test runs are batched on 96 well plates and each batch includes one negative control, one PCR positive control and one extraction control. The controls are the first samples run on each plate. If any one of the controls fails to perform according to its algorithm, (see section 8 under “In House Test Procedure” below), the run fails and the LIS will not report the data for the run. In this event of run failure, hybridization will be repeated first, if this run still fails the run is repeated starting from the original sample.

MATERIALS PROVIDED FOR COLLECTION OF SPECIMENS

The DIATHERIX specimen collection pack (part number SP132FL75) includes one sterile swab and one specimen tube containing a sterile medium that stabilizes and inhibits growth of microorganisms. The swab and collection tube are contained within a small bag.

The DIATHERIX shipping pack includes a small biohazard bag and a small cardboard box. Collected specimens should be placed inside the biohazard bag and test requisitions should be placed in the outer pocket on the bag. The bag should be placed inside the cardboard box. The box should be shipped to DIATHERIX via FedEx in a FedEx Clinical Pack.

All collection and shipping materials may be stored at room temperature.

PRECAUTIONS

- Follow universal precautions while collecting specimens.
- Use aseptic technique while collecting specimens.

- Do not leave swabs inside collection containers.
- Be certain that the collection tube cap is tightly sealed to prevent leakage.
- Be sure to label the tube and completely fill out the laboratory requisition.
- Do not use if the outer packaging or containers are damaged upon arrival.

INSTRUCTIONS FOR USE OF THE COLLECTION PACK

Nasal Swab Collection & Transport Procedure

1. The specimen should be collected using a Starplex swab kit containing a Standard Flocked swab and a blue top transport tube. Please read instructions thoroughly before collection.
2. If the patient has nasal discharge, ask the patient to attempt to clear the discharge by “blowing” his/her nose into non-scented tissue paper. Do not try to clear the discharge with swabs, as this might be excessively traumatic. Assist children whenever necessary.
3. Open the swab package and set the blue top transport tube in a vertical position.
4. Remove the swab, being careful to prevent contamination.
5. Carefully insert the swab into the patient’s nostril up to 2.5 cm (1 inch) from the edge of the nare.
6. Roll the swab 5 times against the surface of the nare. Insert the same swab into the second nostril and roll the swab 5 times against the surface of the nare.
7. Without contaminating the swab, place the swab in the blue top transport tube and roll the swab 5 times in the liquid in the bottom of the transport tube.
8. Raise the swab about 1 inch above the level of the liquid and roll against the side of the tube to press any remaining liquid out of the swab. **DISCARD THE SWAB** and screw the top **tightly** on the transport tube.
9. Label the transport tube with the patient’s first and last name and date of collection. Completely fill out a DIATHERIX Laboratories requisition.
10. Place the transport tube and absorbent gauze in the front of the biohazard bag and seal the bag. Place the completed requisition in the pocket in the back of the biohazard transport bag. Each biohazard bag must contain only one patient’s sample.
11. The biohazard bag containing the blue top transport tube and requisition should be placed in a cardboard container. The container should be placed in a Clinical Pak envelope. The Clinical Pak envelope should be sealed and labeled with a FedEx address stamp and sent overnight to DIATHERIX Laboratories.
12. The specimen cannot be over 5 days old when received at DIATHERIX Laboratories.

Nasopharyngeal Swab Collection & Transport Procedure

1. The specimen should be collected using a nasopharyngeal flocked swab and a blue top transport tube. Please read instructions thoroughly before collection.
2. Open the swab package, discard the Standard Flocked swab and set the blue top transport tube in a vertical position. Ask the patient to cough twice if possible.
3. Insert a nasopharyngeal swab through one nostril straight back (not upwards), along the floor of the nasal passage until reaching the posterior wall of the nasopharynx. The distance from the nose to the ear gives an estimate of the distance the swab should be inserted.
4. Rotate the swab for a few seconds and then leave the swab in place for a few seconds. Slowly withdraw the swab with a rotating motion.
5. Hold the transport tube in a vertical position and without contaminating the swab, place the swab in the blue top transport tube and roll the swab 5 times in the liquid in the bottom of the transport tube.
6. Raise the swab about 1 inch above the level of the liquid and roll against the side of the tube to press any remaining liquid out of the swab.
7. **DISCARD THE SWAB** and screw the top **tightly** on the transport tube.
8. Label the transport tube with the patient’s first and last name and date of collection. Completely fill out a DIATHERIX Laboratories requisition.
9. Place the blue top transport tube in the front of the biohazard transport bag with an absorbent gauze and seal the bag. Place the completed requisition in the pocket in the back of the biohazard transport bag. Each biohazard bag must contain only one patient’s sample.
10. The biohazard bag containing the transport tube and requisition should be placed in a cardboard container. The container should be placed in a Clinical Pak envelope.
11. The Clinical Pak should be sealed and labeled with a FedEx address stamp and sent overnight to DIATHERIX Laboratories.
12. The specimen cannot be over 5 days old when received at DIATHERIX Laboratories.

Nasopharyngeal Wash/Aspirates or Nasal Wash/Aspirates Collection and Transport Procedure

1. Collect the specimen (Nasopharyngeal Wash/Aspirates or Nasal Wash/Aspirates) using standard protocol.
2. Open the swab package, discard the Standard Flocked swab and open the blue top transport tube.
3. Add approximately 3 to 4 ml of the Nasopharyngeal Wash/Aspirates or Nasal Wash/Aspirate to the blue top transport tube.
4. Screw the top **tightly** on the blue top transport tube and invert a few times to mix the specimen and buffer.
5. Label the transport tube with the patient's first and last name and date of collection. Completely fill out a DIATHERIX Laboratories requisition.
6. Place the blue top transport tube in the front of the biohazard transport bag with an absorbent gauze and seal the bag. Place the completed requisition in the pocket in the back of the biohazard transport bag. Each biohazard bag must contain only one patient's sample.
7. The biohazard bag containing the blue top transport tube and requisition should be placed in a cardboard container. The container should be placed in a Clinical Pak envelope.
8. The Clinical Pak envelope should be sealed and labeled with a FedEx address stamp and sent overnight to DIATHERIX Laboratories.
9. The specimen cannot be over 5 days old when received at DIATHERIX Laboratories.

IN-HOUSE TEST PROCEDURE

During all steps of the procedure, record all required information on the batch worksheet.

1. BEGINNING OF THE DAY

- Clean all hoods and workstations according to PR-080501. UV all hoods. Note: After completing this procedure, clean according to PR-080501, except for any special cleaning/discarding steps noted in the procedure.
- Print the number of accession ID labels needed for the day. Print duplicate labels for all samples.
- Thaw an appropriate amount of extraction controls and nuclease free water for the day's testing.
- Turn on all heat sealers to preheat before needed.
- Warm up, start up and calibrate the Qiagen Luminex LiquiChip 100 platform if calibration is needed that day.
- Turn on all thermocyclers needed and initialize the panel specific program under the CLIA user profile.

2. REAGENT PREPARATION

- Following the document "Daily Reagent Worksheet for MagnetX Extraction", prepare all reagents required for the day's tests. Prepare them in the order listed on the worksheet and have them ready before they are needed.

3. RECEIPT OF SAMPLES, LABELING, AND BATCH BUILDING

- Receive the samples from the delivery person. Open and label the samples.
- One bag at a time, remove the sample and the requisition form from the biohazard bag and apply labels.
- Ensure the sample tube is labeled and the sample is not past the expiration time. Otherwise, reject the sample.
- Using duplicate accession labels with a unique barcode for each sample, place one of the accession labels on the sample tube and the other on the sample's requisition form.
- Place the labeled sample tube in a rack according to panel type.
- Place the requisition form in the accessioning inbox.
- Select labeled samples from the blue rack. Spin the sample tubes at 3500 rpm for 1 minute.
- Place the centrifuged samples in a new rack by panel type without disrupting the liquid.
- Build the batches into the DIATHERIX LIS using the Batch Builder program.
- Select the appropriate panel from the panel list for the current batch being built.
- Select a sample from a blue rack and scan it into the Batch Builder.
- Remove the lid from the sample and discard the lid in a biohazard trash bag.
- Place the sample into a batch rack in the SAME POSITION that it was entered into the Batch Builder. The Batch Builder enters samples from top to bottom, then left to right.
- When a full rack has been scanned (or when all samples are added), select transfer to prep. Write the batch ID number on a piece of tape and attach it to the rack. Double check that the number is correct.
- **IMPORTANT:** At this point the samples **MUST NOT** be rearranged for any reason.

- **IMPORTANT:** When working with multiple batches, DO NOT mix up the racks and their corresponding barcodes and batch worksheets.
- Transfer 250 µL of sample to a Kingfisher deep well plate, ensuring that the position of the samples is NOT changed.
- Add 250 µL of the Extraction Control to the third well (C1).
- Label the plate with the “SPECIMEN” batch barcode.
- Pass the plate along with the corresponding barcodes and batch worksheet to extraction.
- Cover the samples and place the rack in the processed samples area.

4. **KINGFISHER EXTRACTION**

- **IMPORTANT:** Ensure that the position of well A1 is ALWAYS in the correct orientation when loading plates into the King Fisher, in the beginning and after adding reagents during the protocol.
- Add 100 µL of prepared buffer MB1 + lysozyme + lysostaphin mixture to each sample.
- Select the MagnetX_GPos_Bac program on a Kingfisher 96 robot and press start.
- Label a prepared elution plate with the “DNA” batch barcode.
- Load the sample plate, prepared wash and elution plates, and tip comb into the Kingfisher as prompted by the program (press start after loading each plate to go to the next plate, the final start begins the program).
- At the first pause step in the program, mix the buffer MSL + proteinase K + magnetic beads mixture by pipetting up and down. Pour the mixture into a reservoir. Add 280 µL of this mixture to each sample. Press start to continue.
- At the second pause step in the program, add 340 µL of 200 proof ethanol to each sample. Press start to continue.
- When the program is finished, remove the plates from the Kingfisher as prompted by the program (press start after removing each plate to go to the next plate). Once all the plates are removed, press stop to end the program.
- Heat seal the elution plate with an easy pierce foil seal
- Place a dot sticker on the foil seal over wells A1 and B1 of the DNA elution plate.
- Place the elution plate in the freezer for 30 seconds.
- Pass the DNA elution plate along with the corresponding barcodes and batch worksheet to template addition.
- Discard the sample plate, tip comb, and wash plates in a biohazard trash can.

5. **TEMPLATE ADDITION**

- Keep the DNA elution plate and PCR plate on PCR cooler blocks.
- In a PCR hood, transfer 5 µL of nucleic acid from each sample in the DNA elution plate to a panel specific PCR master mix plate. Pierce the foil of the elution plate with the pipette to attain the RNA. **DO NOT** change the positions of the samples.
- Add 5 µL of the panel specific PCR Positive Control to well B1 of the PCR master mix plate.
- Heat seal the PCR master mix plate with an easy pierce foil seal.
- Label the PCR plate with the “PCR PRODUCT” batch barcode, then quick mix and quick spin the PCR plate.
- Place the PCR plate into the thermocycler and run the panel specific program.
- The PCR cycling conditions are as follows:
- Re-seal the DNA elution plate and store the plate in a -20°C freezer.

RT Reaction	50°C for 40 minutes
Initial Activation	95°C, 15 min
Enrichment	94°C, 30 sec; 52°C, 1 min; 72°C, 1 min for 15 cycles
Tagging	94°C, 15 sec; 70°C, 1 min 30 sec; for 6 cycles
Amplification	94°C, 15 sec; 52°C, 15 sec; 72°C, 15 sec for 30 cycles
Extension	72°C, 3 min

- After all extractions and template additions are done for the day, turn off the heat sealers and clean up work areas.

- During the PCR step, warm up and start up the Qiagen Luminex LiquiChip 100 platform.

6. ACCESSIONING

- Accession the samples into the DIATHERIX LIS using the CLIA Lab program.
- Select file and new requisition.
- With the cursor blinking in the accession ID text box, scan the accession barcode on the requisition form.
- Enter the given information into the new requisition form. Select save. Initial the form.
- Place the accessioned requisition form in the accessioning “to double check” inbox.
- Double check the accessioned requisition form by viewing the requisition in the LIS and checking it against the hardcopy of the requisition. Do not double check your own accessioning.
- Once the accessioned requisition form has been double checked and all information is correct, initial the requisition form and place it in the accessioning “to file” box, upside-down. Once complete, all filed requisitions should have two sets of initials. Repeat for all samples.

7. HYBRIDIZATION AND DETECTION

- Note: During all hybridization steps, protect beads and SAPE from light.
- Re-warm the Luminex if it is near the time for the lasers to turn off.
- Open the panel specific program in the Luminex software. Clear the file name field. With the cursor blinking in the file name field, scan the batch ID barcode. Type your name into the created by field. Select load patient list.
- Select the folder containing the patient lists created by the Batch Builder program.
- With the cursor blinking in the file name field, scan the batch ID barcode.
- The batch patient list loads into the Luminex software. Select finish.
- In the run screen, ensure that the XYP plate is set to 52°C, the dCal temperature is within range, the sheath fluid pressure is within range, and that there is sufficient sheath fluid.
- Vortex a tube of panel specific 1X bead mix. Add 45 µL of the panel specific 1X bead mix to each well of a cone bottom hybridization plate that corresponds to a PCR product.
- Transfer 5 µL of each PCR product from the PCR plate to the hybridization plate. DO NOT change the positions of the samples. Mix by pipetting and cover the plate.
- Label the hybridization plate with the “DETECTION” batch barcode.
- Incubate the hybridization plate at 52°C for 10 minutes.
- As soon as the 10 minute incubation begins, add 120 µL of filtered distilled water to each well of a flat bottom 96 well plate that corresponds to a sample. Cover the water plate and place it on a 52°C heat block to pre-warm.
- Re-seal the PCR product plate with a self-adhesive thick foil seal and store the plate in a 4°C refrigerator.
- When the incubation is complete, add 10 µL of 1X SAPE to each sample in the hybridization plate. Mix by pipetting.
- Incubate the hybridization plate at 52°C for 5 minutes.
- Transfer the entire hybridization solution for each sample to the pre-warmed filtered water plate. DO NOT change the positions of the samples. Mix by pipetting.
- Insert the flat bottom plate WITHOUT the lid into the XYP platform (in the CORRECT orientation) and begin reading the plate using the Luminex.
- When the Luminex has finished reading the plate, check the Luminex software diagnostics to ensure that there were no equipment or reagent failures during the run (LBC, dCal temperature out of range, etc.).

8. VERIFY AND RELEASE REPORTS

- Open the CLIA Lab program. Select reports and release reports. Select refresh.
- The accession numbers just processed will load into the release reports screen. Select an accession number.
- Review the data to make sure the correct results are given.
- Once completed, select accept. Then select release. Repeat for all samples.
- Once all the results for a particular batch have been released, the specimen tubes can be discarded in a biohazard trash can or queued for storage, and the PCR products can be discarded.
- File the batch records in the appropriate place and fill out the batch log sheet.

- Format data by batch and save on the server under the panel specific results. Email the results to the General Manager and Technical Supervisor.

Algorithm for test results:

- If H109C > 109 and N109B > 116, then;
- Result: 2009 H1N1 Influenza A viral RNA detected.
- Otherwise, result: 2009 H1N1 influenza A viral RNA not detected.
 - All negative control results must be below the relevant cut-off value on file, otherwise the run fails.
 - All PCR positive controls must be positive above the relevant cut-off values on file, otherwise the run fails.
 - The extraction control must be positive for ABMR and negative for all other targets.
 - If any one of the controls fails to perform according to its algorithm, the run fails and the LIS will not report the data for the run. In this event of run failure, hybridization will be repeated first, if this run still fails the run is repeated starting from the original sample.

9. STORAGE OF SPECIMENS

- Label a 1.5 screwcap tube with the specimen accession number or accession label.
- Pipette the leftover specimen from the sample tube to its correctly labeled screwcap tube either manually or using the liquid handler. Store the samples at -80°C. Update the specimen database on the server.

INTERPRETATION OF RESULTS

Test results are interpreted in-house by an automated algorithm within the LIMS (see above), which generates a Laboratory Report that is sent to clients via email, fax and/or web interface.

The Laboratory Report states whether 2009 H1N1 influenza A viral RNA was detected or not detected. The Laboratory Report states that this test is not authorized for the detection of seasonal influenza A, B, or influenza C viruses and that negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.

LIMITATIONS

- 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.
- False negative results may occur when the infecting organism has genomic mutations, insertions, deletions, or rearrangements or when performed very early in the course of illness.
- False negative results may occur if inadequate numbers of organisms are present in the specimen due to improper collection, transport or handling.
- As with other tests, false positive results may occur. Repeat testing or testing with a different device may be indicated in some settings.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- The performance of this test has not been established for patients without symptoms of Influenza infection.
- The performance of this test has not been established for monitoring treatment of Influenza A or 2009 H1N1 influenza infection.
- The performance of this test has not been established for screening of blood or blood products for the presence of Influenza A or 2009 H1N1 influenza.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- This test is not intended for the detection of seasonal influenza A, B, or influenza C viruses. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.

ANALYTICAL PERFORMANCE CHARACTERISTICS

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The Limit of Detection was determined for the DIATHERIX H1N1-09 test using three viral samples: the 2009 novel H1N1 influenza virus (A/California/04/2009), a seasonal strain of H1N1 (A/WS/33) and a seasonal strain of H3N2

(A/Victoria/3/75). Viral stocks were titered, serially diluted and extracted in 5 replicates of each dilution to determine a preliminary LoD TCID₅₀ dilution that produces positive values 95% of the time. Once a preliminary LoD dilution was found, 20 replicates of refined dilutions were tested. From this information, a final dilution for confirmation of LoD was run in 20 replicates (Tables 1-6). Positive detection is indicated by an MFI of 117 or higher and by an MFI of 110 or higher for the N1 and H1 targets of the 2009 H1N1 influenza virus, respectively.

Table 1. Target H1-- Influenza A/California/04/2009 (H1N1) Virus Initial LoD Estimation. Stock = 1.23x10⁹ TCID₅₀/mL

Dilution	TCID ₅₀ /mL	# Pos.	MFI 1	MFI 2	MFI 3	MFI 4	MFI 5	Mean	%CV
1.00E01	1.23x10 ⁴	5	4710	4381	4542	4424	4334	4478	3
1.00E02	1.23x10 ³	5	3217	3503	3495	3207	3539	3392	5
1.00E03	1.23x10 ²	5	2520	2239	2209	2132	2411	2302	7
1.00E04	1.23x10 ¹	5	863	791	885	712	1104	871	17
1.00E05	1.23	5	276	174	229	236	118	206	30
1.00E06	1.23x10 ⁻¹	0	22	55	27	27	6	27	64

Table 2. Target H1-- Influenza A/California/04/2009 (H1N1) Virus LoD Refinement

	6.15 TCID ₅₀ /mL				1.23 TCID ₅₀ /mL		
	Sample	Pos/Neg	MFI		Sample	Pos/Neg	MFI
	1	Pos	752	1	Pos	385	
	2	Pos	811	2	Pos	474	
	3	Pos	832	3	Pos	373	
	4	Pos	588	4	Pos	516	
	5	Pos	518	5	Pos	615	
	6	Pos	472	6	Pos	445	
	7	Pos	850	7	Pos	730	
	8	Pos	573	8	Pos	521	
	9	Pos	956	9	Pos	428	
	10	Pos	735	10	Pos	413	
	11	Pos	905	11	Pos	301	
	12	Pos	483	12	Pos	408	
	13	Pos	531	13	Pos	389	
	14	Pos	527	14	Pos	446	
	15	Pos	882	15	Pos	451	
	16	Pos	961	16	Pos	452	
	17	Pos	1050	17	Pos	516	
	18	Pos	837	18	Pos	520	
	19	Pos	452	19	Pos	463	
	20	Pos	609	20	Pos	506	
Results: 20/20 Positive				Results: 20/20 Positive			
Mean: 716				Mean: 467			
%CV: 27				%CV: 29			

Table 3. Target H1-- Influenza A/California/04/2009 (H1N1) Virus LoD Confirmation at 6.15x10⁻¹ TCID₅₀/mL

Sample	Pos/Neg	MFI
1	Pos	267

2	Pos	244
3	Pos	203
4	Pos	327
5	Pos	188
6	Pos	189
7	Pos	150
8	Pos	204
9	Pos	282
10	Pos	256
11	Pos	210
12	Pos	164
13	Pos	267
14	Pos	360
15	Pos	179
16	Pos	147
17	Pos	229
18	Pos	258
19	Pos	173
20	Neg	108
Results: 19/20 Positive		
Mean: 220		
%CV: 29		

Table 4. Target N1-- Influenza A/California/04/2009 (H1N1) Virus Initial LoD Estimation. Stock = 1.23×10^9 TCID₅₀/mL

Dilution	TCID ₅₀ /mL	# Pos.	MFI 1	MFI 2	MFI 3	MFI 4	MFI 5	Mean	%CV
1.00E01	1.23×10^4	5	5709	5894	6042	5920	6089	5931	3
1.00E02	1.23×10^3	5	4497	4761	4691	4504	4498	4590	3
1.00E03	1.23×10^2	5	3389	3225	3386	3200	3500	3340	4
1.00E04	1.23×10^1	5	1553	1395	1444	1355	1623	1474	8
1.00E05	1.23	5	390	336	493	310	301	366	22
1.00E06	1.23×10^{-1}	0	33	50	19	85	55	48	52

Table 5. Target N1-- Influenza A/California/04/2009 (H1N1) Virus LoD Refinement

6.15 TCID ₅₀ /mL	Sample	Pos/Neg	MFI	1.23 TCID ₅₀ /mL	Sample	Pos/Neg	MFI
	1	Pos	903		1	Pos	422
2	Pos	791	2	Pos	549		
3	Pos	683	3	Pos	353		
4	Pos	783	4	Pos	558		
5	Pos	592	5	Pos	628		
6	Pos	448	6	Pos	595		
7	Pos	707	7	Pos	645		

	8	Pos	586		8	Pos	573
	9	Pos	681		9	Pos	476
	10	Pos	702		10	Pos	456
	11	Pos	844		11	Pos	368
	12	Pos	533		12	Pos	361
	13	Pos	598		13	Pos	381
	14	Pos	441		14	Pos	391
	15	Pos	780		15	Pos	397
	16	Pos	634		16	Pos	523
	17	Pos	765		17	Pos	520
	18	Pos	823		18	Pos	505
	19	Pos	497		19	Pos	505
	20	Pos	710		20	Pos	614
Results: 20/20 Positive				Results: 20/20 Positive			
Mean: 675				Mean: 491			
%CV: 20				%CV: 20			

Table 6. Target N1-- Influenza A/California/04/2009 (H1N1) Virus LoD Confirmation at 6.15×10^{-1} TCID₅₀/mL

Sample	Pos/Neg	MFI
1	Pos	305
2	Pos	397
3	Pos	228
4	Pos	383
5	Pos	250
6	Pos	234
7	Pos	234
8	Pos	185
9	Pos	186
10	Pos	335
11	Pos	267
12	Pos	177
13	Pos	225
14	Pos	247
15	Pos	338
16	Pos	200
17	Neg	80
18	Pos	238
19	Pos	117
20	Pos	135
Results Pos/Neg: 19/20		
Mean: 238		
%CV: 35		

The Limit of Detection was found to be 6.15×10^{-1} TCID₅₀/mL for 2009 H1N1 influenza virus.

ANALYTICAL SPECIFICITY/CROSS REACTIVITY

Nucleic acids extracted from a variety of bacterial and viral respiratory samples were tested on the DIATHERIX H1N1-09 panel at the CFU or TCID₅₀ shown below. The results showed no cross reactivity between the non-panel specimens and the 2009 H1 or N1 targets or the seasonal influenza A target. See table 7 below for data.

Sample	CFU/mL OR TCID ₅₀ /mL	Source	H1 Target MFI	N1 Target MFI
<i>H. influenzae</i> type B	2.60×10^6	MBI 0441v	-	-
<i>H. influenzae</i> type F	3.90×10^6	ATCC 700222	-	-
<i>N. meningitidis</i>	5.90×10^4	MBI 0454V	-	-
<i>S. pneumoniae</i>	1.50×10^5	MBI 0947V	-	-
<i>P. aeruginosa</i>	3.40×10^9	ATCC 27853	-	-
<i>S. pyogenes</i>	8.9×10^3	ATCC 14289	-	-
<i>C. pneumoniae</i>	1.11×10^6	ATCC VR-1360	-	-
<i>K. pneumoniae</i>	2.60×10^{10}	ATCC 35555	-	-
<i>S. aureus</i>	1.50×10^9	ATCC BAA40	-	-
<i>L. pneumophila</i>	3.60×10^5	MBI 0211V	-	-
<i>S. maltophilia</i>	2.25×10^9	ATCC 49130	-	-
<i>A. baumannii</i>	3.2×10^9	ATCC 19606	-	-
RSVA	1.78×10^6	ATCC VR-1540	-	-
RSVB	3.16×10^4	ATCC VR-1580	-	-
Influenza A H3N2	1.78×10^7	ATCC VR-822	-	-
Influenza B	5.62×10^6	ATCC VR-101	-	-
Parainfluenza virus 1	1.97×10^4	ATCC VR-94	-	-
Parainfluenza virus 2	1.97×10^6	ATCC VR-92	-	-
Parainfluenza virus 3	1.11×10^8	ATCC VR-93	-	-
Parainfluenza virus 4	6.22×10^3	ATCC VR-1377	-	-
Coxsackie virus B	5.62×10^6	ATCC VR-184	-	-
Echovirus	1.78×10^6	ATCC VR-41	-	-
Rhinovirus	3.16×10^4	ATCC VR-489	-	-
Adenovirus 3	3.16×10^5	ATCC VR-847	-	-
Adenovirus 4	3.16×10^5	ATCC VR-1572	-	-
CDC H1N1-09 Sample	$>5 \times 10^4$	2009/A/CALIFORNIA	+	+

REPEATABILITY

Four clinical samples that were confirmed positive on the CDC rRT-PCR for 2009 H1N1 Influenza test were tested using the DIATHERIX H1N1-09 Test. Each sample was tested in five replicates. All samples were tested in a single run. See table 8 below for data.

Sample	H1 target		N1 target			
	H1 MFI	N1 MFI	MFI mean	%CV	MFI mean	%CV
#2194-1	1783	2146	1839	4.0	1689	20.8

#2194-2	1957	1981				
#2194-3	1823	1378				
#2194-4	1856	1415				
#2194-5	1776	1525				
#2225-1	1185	829	1288	7.9	1124	31.4
#2225-2	1301	788				
#2225-3	1192	1615				
#2225-4	1425	1043				
#2225-5	1339	1346				
#2320-1	1483	1021	1432	4.1	950	6.0
#2320-2	1332	873				
#2320-3	1458	988				
#2320-4	1425	937				
#2320-5	1460	933				
#2322-1	526	425	592	18.1	496	14.9
#2322-2	513	451				
#2322-3	553	476				
#2322-4	592	615				
#2322-5	776	512				

REPRODUCIBILITY

One CDC viral specimen (A/CALIFORNIA/04/09) and one positive H1N1-09 influenza patient sample confirmed by the Alabama State Health Department were used as test materials for this study. The samples were run on the DIATHERIX assay in sets, for a total of nineteen runs. The results showed that for all nineteen runs, all expected H1 and N1 positive targets were positive and there were no false negatives for the H1 and N1 targets. See table 9 below for data.

Run	A/California/04/09 Target H109C	A/California/04/09 Target N109B
1	3196	5288
2	3640	5782
3	3915	6161
4	3950	5884
5	4637	6224
6	5454	5747
7	5431	5642
8	5236	6070
9	4990	5943
10	5398	5675
11	3245	5843
12	3384	5611
13	3662	6115
14	3961	5944

15	4196	6272
16	5468	6086
17	5739	6911
18	5822	6827
19	5779	6465
mean	4584	6026
%CV	20.6	6.7

ANALYTICAL REACTIVITY

Analytical reactivity was tested using three influenza A viral strains: the 2009 novel H1N1 influenza virus (A/California/04/2009), a seasonal strain of H1N1 (A/WS/33) and a seasonal strain of H3N2 (A/Victoria/3/75). Each strain was tested in five replicates. Two out of the three strains were tested at high concentrations, as Tem-PCR has more potential for nonspecific PCR interactions when high, rather than low, concentration of sample is used. See table 10 below for results. All replicates produced the same pass/fail results.

Strain type	Titer	Target: H1N1-09
H1N1-09 (A/California/04/09)	Unknown	+
H1N1 (A/WS/33)	5.62×10^6	-
H3N2 (A/Victoria/3/75)	1.78×10^7	-

CLINICAL PERFORMANCE CHARACTERISTICS

Seventy patient samples were received from the Louisiana State Health Department. These samples had been tested for the H1N1 2009 virus using the CDC rRT-PCR for 2009 H1N1 Influenza test. The test samples included twenty positive samples and fifty negative samples. The samples were randomized and run blindly on the DIATHERIX H1N1 test. Results of comparison of the DIATHERIX H1N1-09 Test and the CDC rRT-PCR for 2009 H1N1 Influenza test following all protocols described above are presented in table 11.

		CDC Real Time RT-PCR H1N1-09 Test			
		2009 H1N1 Positive	2009 H1N1 Negative	Total	Positive Agreement
DIATHERIX H1N1-09 Test	2009 H1N1 Positive	22	0	22	95.7% (22/23) 95% CI: 78.1 – 99.9
	2009 H1N1 Negative	1	47	48	Negative Agreement 100% (47/47)
	Total	23	47	N = 70	95% CI: 92.5 - 100

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