Focus Diagnostics' 2009 H1N1 Influenza Tests
Frequently Asked Questions

What Focus Diagnostics tests are available for 2009 H1N1 influenza testing?

Focus Diagnostics offers two real-time PCR assays authorized by the FDA for emergency use (EUA):

The first EUA was issued July 23, 2009 for the Influenza A H1N1 (2009) Real-Time RT-PCR test. The EUA for this test was subsequently amended to allow test kits to be distributed to CLIA high complexity laboratories using specified equipment. The test detects 2009 H1N1 influenza virus and differentiates it from seasonal influenza A. This test is currently performed as a referral test at Focus Diagnostics and Quest Diagnostics.

The second EUA was issued October 16, 2009 for the Simplexa™ Influenza A H1N1 (2009) test, which is now available as a test kit for use in CLIA high complexity laboratories using the Integrated Cycler from 3M Health Care. The Simplexa test is a real-time PCR assay that uses different technology to detect 2009 H1N1 influenza virus and differentiate it from seasonal influenza A virus.

Where are test kits sold?

Test kits are labeled for FDA emergency use for distribution in the US and CE marked for distribution in Europe.

What is an emergency use authorization (EUA)?

The U.S. Food and Drug Administration, in response to requests from the U.S. Centers for Disease Control and Prevention, has issued emergency use authorizations (EUAs) to make available to public health and medical personnel important diagnostic and therapeutic tools to identify and respond to the 2009 H1N1 influenza virus under certain circumstances. In April 2009, the agency issued EUAs for the use of certain antiviral products, and for a CDC RT-PCR test for the 2009 H1N1 influenza virus, which is used in public health labs.
There are currently no FDA cleared or approved tests for the identification of the 2009 H1N1 influenza virus. The EUA authority allows the FDA, based on the evaluation of available data, to authorize the use of unapproved or uncleared medical products or unapproved or uncleared uses of approved or cleared medical products following a determination and declaration of emergency, provided certain criteria are met.

Does an EUA mean the test is now FDA approved?

No. The FDA has not cleared or approved the test. The test has been authorized by FDA under an Emergency Use Authorization, and is only authorized for the duration of the declaration of emergency {under section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1)}. The declaration of emergency will expire on April 26, 2010, unless it is terminated or revoked sooner or renewed.

How is the Simplexa test different than the first EUA test?

Both assays have been shown to consistently detect 2009 H1N1 influenza virus. The tests differ primarily in the PCR technology used and the equipment required by the laboratory.

How will physicians use the test results?

Physicians will use the test results to help establish or rule out the presence of 2009 H1N1 influenza or seasonal influenza A. They may order a PCR test in a high risk patient or when a rapid test flu result was negative.

What type of physicians will order PCR testing?

Pediatricians, general practitioners, OB/Gyns, or physicians who treat patients with compromised respiratory or immune systems.

What are the different PCR technologies used by the Focus tests?

- **First EUA – Influenza A H1N1 (2009) Real-Time RT-PCR (TaqMan®)**TaqMan is the PCR technology from Roche Molecular. It is still currently the most commonly used PCR technology. The Focus EUA TaqMan kit is indicated for use on Applied Biosystems 7500 and Roche MagNa Pure instrumentation. Real-time PCR technology allows amplification of the target nucleic acid concurrent with detection so the overall reaction time is typically shorter than PCR not performed in real time.

- **Second EUA - Simplexa™ Influenza A H1N1 (2009)** The Simplexa™ chemistry is also a real-time PCR test that uses a different technology (Scorpions®*), one of several technologies available for performing PCR. Simplexa chemistries are designed to run on the 3M Integrated Cycler, a compact thermocycler marketed in collaboration with 3M Health Care, Infection and Prevention Division. **The use of Scorpions® Probes for human in vitro diagnostic purposes is covered by a license to Focus Diagnostics, Inc. from DxS, Ltd.*

What is real-time PCR?

Real-time PCR is a process where amplification (replication of RNA or DNA) and detection occur simultaneously. Once the minimum detectable amount of amplified nucleic acid is present, the results can be reported. The more nucleic acid in the original specimen, the more rapid the detection and the reporting of results compared to non real-time PCR platforms. Negative specimens with no detectable nucleic acid take the longest time to report.

Are there competitive H1N1 test kits on the market?

There are other products that can detect influenza A virus, however, most current laboratory tests are limited in their ability to differentiate the pandemic flu from the seasonal flu. Rapid antigen formats, direct fluorescent antigen (DFA) tests, rapid influenza virus culture tests and many PCRs can detect influenza A viruses but cannot differentiate the influenza A H1N1 (2009) virus from the seasonal influenza A virus. Multiple studies have shown that although specificity is generally high for rapid tests, many rapid influenza tests suffer from poor sensitivity, meaning a negative test does not exclude influenza virus infection.

What can you tell me about Focus Diagnostics' Simplexa Molecular products?

Simplexa™ is a new line of molecular diagnostic test kits that employ real-time PCR (polymerase chain reaction) to detect the presence of an infectious agent (e.g. bacterial or viral nucleic acid). Simplexa Molecular test Diagnostic kits contain the reagent components a lab would need to test for the presence of specific pathogen nucleic acid (DNA or RNA), and provide an answer using the laboratory's real-time detection system. Simplexa kits are designed to be run on the 3M Integrated Cycler, a compact thermocycler marketed in collaboration with 3M Health Care.

What Simplexa test kits are available?

The first Simplexa kit is the Influenza A H1N1 (2009) test, authorized by the FDA for emergency use with the 3M Integrated Cycler. Focus expects to launch additional Simplexa test kits in 2010.

1. The purchase of this product grants the purchaser rights under certain Roche patents to use it solely for providing human in vitro diagnostic services. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.
2. The use of Scorpions® Probes for human in vitro diagnostic purposes is covered by a license to Focus Diagnostics, Inc. from DxS, Ltd.
3. CAL Fluor™ and Quasar™ dyes are trademarks of Biosearch Technologies, Inc. ('BTI'). CAL Fluor and Quasar dye technology is licensed pursuant to an agreement with BTI, and these products are sold exclusively for clinical, diagnostic, or research and development purposes.

How do Simplexa kits work?

Simplexa kits provide the reagents required to perform a real-time PCR test on a sample of nucleic acid that has been extracted from a clinical specimen, such as a throat swab or sputum. Extraction is a chemical process that exposes even a small amount of nucleic acid in the specimen and makes it available for binding or hybridizing to the specific DNA or RNA probes contained in the Simplexa kit. Once the specific binding or hybridization has occurred, the targeted pathogenic nucleic acid is amplified or multiplied many times until it is detectable and can be reported.

What is different about the Simplexa test kits?

Simplexa test kits are designed as a system with the 3M Integrated Cycler. The system has a small footprint and can report results fast – 30 to 75 minutes once the extraction step is complete.

Who will purchase and use the Simplexa test kits?

Any CLIA high complexity laboratory that is currently performing molecular diagnostic testing or plans to begin testing in the future is a prospect for Simplexa and the Integrated Cycler. The testing will be performed in the laboratory and results reported to the physician.

How quickly can a physician get test results using the Simplexa test?
The test can be performed by the laboratory in less than three hours, which allows the laboratory to report results the same day. This is comparable with other molecular tests.

The CDC guidelines seem to suggest that testing is not that important to clinical care. Is this accurate, and what do you say to someone who questions the value of testing for the H1N1 virus?
To the contrary, the CDC guidelines identify those who should be tested, not who should not be tested. Current recommendations for testing:

- Most patients with clinical illness consistent with uncomplicated influenza who reside in an area where influenza viruses are circulating do not require diagnostic influenza testing for clinical management.
- Patients who should be considered for influenza diagnostic testing include:
  - Hospitalized patients with suspected influenza
  - Patients for whom a diagnosis of influenza will inform decisions regarding clinical care, infection control, or management of close contacts.
  - Patients who died of an acute illness in which influenza was suspected.
- When a decision is made to use antiviral treatment for influenza, treatment should be initiated as soon as possible without waiting for influenza test results. Antiviral treatment is most effective when administered as early as possible in the course of illness. http://www.cdc.gov/h1n1flu/recommendations.htm

How have the guidelines changed for testing since FDA first granted the EUA on July 23?
The CDC and ASM both issued updated their guidelines with in the last month. Both indicated that reliance on the rapid tests should be limited, and that rt-PCR (or viral culture) is the preferred methods to correctly diagnose H1N1.

An Excerpt from the CDC Guidance:
"Nucleic acid amplification tests, including rRT-PCR, are the most sensitive and specific influenza diagnostic tests, but they may not be readily available, obtaining test results may take one to several days, and test performance depends on the individual rRT-PCR assay. As with any assay, false negatives can occur. Not all nucleic acid amplification assays can specifically differentiate 2009 H1N1 influenza virus from other influenza A viruses. If specific testing for 2009 H1N1 influenza virus is required, testing with an rRT-PCR assay specific for 2009 H1N1 influenza or viral culture should be performed. "
http://www.cdc.gov/h1n1flu/guidance/diagnostic_tests.htm