Frequently Asked Questions

**General**

**How can I cut down on the chances of contamination in the lab?**
Our assays are very sensitive and each lab needs to take all precautions to avoid carry-over and residual contamination. Be sure to clean well with 10% bleach, use UV light, work in a uni-directional manner, change tips between each sample, use barrier tips and change gloves often.

**What is the precision of the Lyra Influenza A+B Assay?**
The reproducibility of the Lyra Influenza A+B Assay was evaluated at three laboratory sites. Each panel and controls were extracted using the bioMérieux easyMAG system while using two panels (one panel for Cepheid SmartCycler® II and a different panel for the ABI® 7500 Fast Dx) and both were found to be reproducible. Additional information regarding the performance characteristics for the reproducibility study can be found in the Lyra Influenza A+B Assay Package Insert.

**How accurate is the Lyra Influenza A+B Assay?**
In recent clinical studies, the positive percent agreement with fresh nasal/nasopharyngeal swab samples was 100% for Type A and 98% with Type B on the Cepheid SmartCycler II. The negative percent agreement with the SmartCycler was 99% with Type A and 95% with Type B. These results were in comparison with an FDA-cleared RT-PCR device. Additional clinical performance characteristics with the ABI 7500 Fast Dx and with frozen specimens are listed as follows:

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<th>Cepheid SmartCycler II</th>
<th>ABI 7500 Fast Dx</th>
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<tbody>
<tr>
<td>Positive Percent Agreement (Fresh N/NP swabs)</td>
<td>A-100%, B-98%</td>
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<td>Negative Percent Agreement (Fresh N/NP swabs)</td>
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<tr>
<td>Positive Percent Agreement (Frozen N/NP swabs)</td>
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</table>
**What is the limit of detection (LOD) for Influenza A and B using this kit?**
The LOD varies by strain but is generally in the range of 2 to 100 TCID₅₀/mL.

**Refer to our website at quidel.com for additional performance claims.**

**Is the Lyra Influenza A+B Assay FDA cleared?**
Yes. It has been 510k-cleared.

**What are the targets for the Lyra Influenza A+B Assay?**
The conserved regions of the Influenza A matrix protein gene and Influenza B neuraminidase gene.

**What detection technology does Quidel use?**
The Lyra Influenza A+B assay is based on TaqMan® chemistry, and uses an enzyme with reverse transcriptase, DNA polymerase, and 5'-3' exonuclease activities.

**Specimen Collection, Storage and Handling**

**What sample types can I use with the Lyra Influenza A+B Assay?**
Each Package Insert describes the sample types that were used in validation of the product. The Influenza A+B test can be used for nasal swabs and nasopharyngeal swabs.

**Can the Eswab be used with the Lyra assay?**
The Eswab is a bacterial collection swab and should not be used for collection of samples for viral detection.

**What types of transport media are acceptable for use with the Lyra Assay?**
Quidel has validated the following media types for use with the Lyra Assay: Universal Transport Media (UTM), M4, M4-RT, M5, and M6.

**How should specimens be handled and stored prior to the extraction process?**
Specimens should be transported to the lab in viral transport media at 2°C to 8°C or on ice within 72 hours. If specimens cannot be tested within 72 hours of collection, they should be frozen at −70°C or colder.

**What are the storage conditions for specimen eluates?**
The user is responsible for validation of the storage procedures and conditions used in their own laboratory. Eluates can typically be stored at room temperature (20°C to 25°C) for 2 hours, at 2°C to 8°C for 8 hours, and 1 month at −20°C to −70°C.

**Kit Formats**

**What kit formats are available and what does the kit include?**
The kit comes in one format which allows you to run as few as 3 (1 patient, 2 controls) but up to 96 reactions. The kit includes:
- Rehydration Solution – 1 vial/kit 1.9 mL
- Lyra Influenza A+B Master Mix – 12 vials/kit, 8 reactions/vial
- Process Control – 1 vial/kit 2.0 mL
**Kit Storage**

What are the storage conditions for the kit and reconstituted components?
The kit is stored at 2°C to 8°C until expiration; the rehydrated Master Mix can be stored at room temperature (20°C to 25°C) for up to 24 hours. For longer storage the rehydrated Master Mix should be recapped, sealed with parafilm and stored in an upright position at ≤–20°C for up to 14 days. Protect the Master Mix from light during storage.

What is the shelf life of the kit?
The kit shelf life is 24 months from the date of manufacture with a 12 month minimum shelf life at time of shipment.

**Master Mix**

Which enzyme is used in the Master Mix?
The master mix is a proprietary mix with excipients chosen to enhance shelf life.

**Quality Control (External Control)**

What is Quidel’s quality control recommendation for these tests?
Quidel recommends that each thermocycler run includes a reaction well for an Influenza A/Influenza B positive processing/extraction control (i.e., Quidel Molecular Influenza A+B Control Set #M106 or previously characterized positive sample) and Negative Control (this can be transport media or a previously identified negative sample). Controls should be run and interpreted in accordance with your lab practices and policies.

I would like to run Limits of Detection. Are your controls quantified?
No. The controls are provided in a qualitative format. Quantification of controls is the responsibility of the end user.

**Process Control (PRC)**

What is the PRC in the kit?
The RNA bacteriophage MS2.

What is the concentration of the PRC?
That is proprietary information.

Can I use a process control (internal control) other than what is provided in the kit?
No. We do not recommend deviating from the Package Insert.

Can a sample extracted with another manufacturer's process control be used in your assays?
No. The sample must have Quidel’s PRC in it, therefore, you should not use a sample extracted using another kit (another MS2 could have a different concentration).

Can I add the PRC to the Master Mix instead of adding it to the samples prior to extraction?
No. We do not recommend deviating from the Package Insert. The PRC acts as a control for the extraction process as well.
How long can I leave my sample and the PRC mixed together before adding it to the easyMAG?
The samples should be processed on the easyMAG as soon as possible after mixing the PRC and sample. The Package Insert does not state that it is acceptable to allow samples to sit after they are mixed.

My PRC is coming up too early. What should I do?
There are no set Ct values for the PRC. As long as the Ct values fall within 10 to 45 on the SmartCycler and 5 to 35 on the ABI they are valid.

My PRC did not show up. What should I do?
If the specimen is positive, the PRC does not need be detected for the specimen to be called a positive. If the specimen is negative and the PRC is not detected, it is considered an invalid result.

Reagents

My kit was placed in the freezer (–20°C or –80°C) upon arrival, can I still use it?
It is recommended that the kit not be used. The proper storage conditions of the kit are 2°C to 8°C.

Can I purchase the reagents individually?
The reagents are produced in a kit format. Individual reagents are not available for purchase. If a vial is damaged upon receipt, please contact Quidel Technical Support for replacement arrangements.

Extraction

What extraction system can I use to run this assay?
The bioMérieux NucliSENS easyMAG is the only extraction system that has been validated for this assay.

How long does it take to extract the nucleic acid on the bioMérieux NucliSENS easyMAG?
The extraction time is dependent on the number of samples. A full 24 samples would take approximately 40 minutes.

Thermocycler

What thermocycler can I use to run this assay?
The Applied Biosystems 7500 Fast Dx, Life Technologies QuantStudio™ Dx and the Cepheid SmartCycler II have been validated for this assay.

Which SmartCycler or ABI software version is compatible with the templates provided?
Version 3.0b for the SmartCycler, SDS version 1.4 software for the ABI. The test cannot be run on other software versions.

Does the ABI need to be calibrated in a certain manner to perform appropriately with the dye sets used in the assay?
Calibration for influenza A+B is not required.
Can you send me the supplemental instructions for the ABI?
They are incorporated into the Package Insert.

On the SmartCycler what does it mean when my channel result says Fail, Pass, NA, or ND?
For interpretation of the results on the SmartCycler, refer to the Lyra Influenza A+B Assay Package Insert.

What is the approximate test time for the ABI 7500 Fast Dx, Life Technologies QuantStudio Dx and/or the Cepheid SmartCycler II?
Once specimens are loaded, the cycling program time for ABI 7500 Fast Dx and the Cepheid SmartCycler II is 70-90 minutes.

**Reporting**

Can I use the results if I forgot to centrifuge my plate/tubes?
Failure to centrifuge the plate/tube is a major deviation from the PI. It is recommended the run be repeated.

What does the amplification curve tell me?
In general it allows you to visualize the PCR reaction. If the amplification of DNA crosses over the specified threshold during the appropriate cycling time, then the sample is positive for that virus.

What do I do if I get an invalid result for the positive control?
We suggest that the run be repeated. Controls should be run and interpreted in accordance with your lab practices and policies.

What can cause a false positive with a negative control?
This could be caused by contamination from the target virus. Controls should be run and interpreted in accordance with your lab practices and policies.

What can cause a false positive with a patient sample?
This could be caused by a cross contamination from another sample.

What can cause a false negative with a patient sample?
Some suggested causes are the presence of sequence variants in the viral target, improper collection, storage, or transport of the specimen, inhibitors present in the specimen or not following the assay procedure correctly.

What is the protocol for reporting a result which is not ordered by the physician? For example, the physician only orders influenza A and the Quidel assay includes influenza B as well.
Reporting would be in accordance with your lab practices and policies.
Additional Information

Does the Lyra Influenza A+B Assay detect H5N1 or other strains of “avian” influenza viruses?
The Lyra Influenza A+B Assay has been shown to detect cultured avian influenza; as with other tests for influenza, the ability of the assay to detect Influenza Type A in patients infected with H5N1 has not been established.

Will the Lyra Influenza A+B Assay specify that a patient has avian influenza?
No. The Lyra Influenza A+B Assay can distinguish between Influenza A and B viruses, but it will not differentiate influenza subtypes.

Does the Lyra Influenza A+B Assay detect the 2009 H1N1 Influenza A virus?
The Lyra Influenza A+B Assay has been shown to detect the 2009 H1N1 virus cultured from a positive human respiratory specimen and with clinical specimens that are positive for the 2009 H1N1 Influenza virus.

Will the Lyra Influenza A+B Assay specify that a patient has the 2009 H1N1 Influenza A virus?
No. The Lyra Influenza A+B Assay can distinguish between Influenza A and B viruses, but it will not differentiate influenza subtypes.

Where can I find up-to-date news and information on avian influenza and 2009 H1N1 influenza A virus?
The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) post information on their websites:
http://www.cdc.gov/flu/avian
http://www.cdc.gov/h1n1flu/update.htm