

# Comparison of Multiple Commercial Assays for the Detection of Swine-Origin Influenza A (H1N1) Virus (SOIV)

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## ABSTRACT

### Introduction:

Swine-origin influenza A (H1N1) virus (SOIV) emerged during the tail end of the 2008-2009 influenza season. Initial testing for SOIV was performed with a CDC-primer and probe detection protocol for influenza A matrix gene followed by a SOIV specific sequencing protocol. It was not known whether commercial assays could be employed as a sensitive screen for SOIV in the event that increased surge capacity became necessary. The objective of this study was to evaluate the effectiveness of commercial multiplex respiratory panel assays for the detection of SOIV.

### Methods:

Total nucleic acid was extracted from clinical nasopharyngeal specimens using the NucliSens® easyMAG™ system (Biomérieux). Initial testing for SOIV was carried out by a two-step reference method; 1) real-time reverse transcriptase PCR (rRT-PCR) for influenza A (Centers for Disease Control protocol), and 2) testing of all influenza A-positive specimens with a SOIV specific RT-PCR and sequence analysis protocol (National Microbiology Laboratory protocol). 39 influenza A-negative specimens, 21 seasonal influenza A specimens and 20 SOIV specimens were randomly selected and tested by the following commercial assays; 1. Seeplex RVP “swine flu patch” (Seegene), 2. Luminex xTAG RVP CE (Luminex), and 3. ResPlex II v2.0 (Qiagen).

### Results:

The specificity of commercial methods for influenza A detection was: Seeplex (39/39, 100%), Luminex (38/39, 97%), ResPlex II (39/39, 100%). The sensitivity of commercial methods for seasonal influenza was: Seeplex (16/21, 76%), Luminex (15/21, 71%), and ResPlex II (10/21, 48%). The sensitivity of commercial methods for SOIV detection was: Seeplex (19/20, 95%), Luminex (20/20, 100%), and ResPlex II (11/20, 55%). Decreased sensitivity for SOIV may be due to the inherent differences in detection limits of these commercial assays. We compared the median RT-PCR cycle threshold (Ct value) for Influenza A detection (by the CDC protocol) in SOIV-positive specimens that were missed by the commercial methods. Seeplex missed 1 SOIV+ specimen (FluA Ct of 31.6) and ResPlex II missed 9 SOIV+ specimens (median FluA Ct = 28.39).

### Conclusions:

The Seeplex RVP patch and Luminex RVP were sensitive methods for detecting SOIV in nasopharyngeal specimens. The utility of commercial methods for screening of SOIV may permit increased surge capacity and engagement of additional facilities, in the event that SOIV prevalence increases in the Autumn of 2009. In conclusion, commercial methods may be effective methods for screening for SOIV in laboratories prior to confirmation by SOIV specific assays.

**Keywords:** Influenza A, swine-origin, commercial methods, detection

## OBJECTIVE

To evaluate the effectiveness of three commercial assays, the Luminex xTAG RVP, the Seeplex Respiratory “swine flu patch” and the Qiagen ResPlex II v2.0 as screening methods for SOIV from nasopharyngeal specimens.

## INTRODUCTION

Swine-origin influenza A(H1N1) emerged in Ontario, Canada during the end of the 2008-2009 influenza season. SOIV has now been identified as a pandemic strain and its impact in the upcoming influenza season (2009/10) is still unknown. In Ontario, much of the SOIV testing was undertaken at the Provincial Health Laboratories using a screening test and confirmation assay. Since, it is not yet known what Impact SOIV will have in terms of prevalence and virulence, pandemic planners should investigate ways of expanding laboratory testing on a system-wide basis. One approach is to increase SOIV-screening by implementing commercial methods commonly used in acute care and primary testing laboratories.

## METHODS

**Specimen Collection:** This study utilized nasopharyngeal swab specimens (Starplex, Toronto) submitted to the Ontario Public Health Laboratory-Toronto between April 22 and May 13, 2009 for diagnostic testing of Influenza and SOIV.

**Viral RNA Extraction:** Nucleic acid was extracted from 200µl of primary specimen by the NucliSens® easyMAG™ magnetic extraction method (bioMérieux, NL) for Influenza A and SOIV diagnostic testing. An additional aliquot of specimen (250µl) was spiked with MS-2 bacteriophage internal control and extracted with the easyMag™ method for Luminex, ResPlexII and Seeplex testing.

### Diagnostic Testing Algorithm

**Influenza A detection:** Nucleic acid was tested for the presence of the Influenza A matrix gene by the CDC real-time reverse transcriptase polymerase chain reaction (rRT-PCR) protocol (reference method).

**SOIV detection:** Influenza A-positive specimens were assayed by a SOIV-specific end-point RT-PCR and amplicons were sequenced by Sanger method (ABI 3100, Foster City, CA, USA) to confirm SOIV infection (as specified by the National Microbiology Laboratory protocol).

**Specimen Panel:** Post-diagnostic confirmation 21 Seasonal influenza A (H1 and H3), 20 SOIV and 39 influenza A-negative specimens (RSV, Para, Adeno) were randomly selected and blinded for testing by commercial assays.

**Commercial Assays:** 1) Luminex xTAG RVP, 2) Seeplex Respiratory “swine flu patch”, and 3) Qiagen ResPlex II v2.0

TABLE 1. Assay Features: Targets, Technology and Issues

ASSAY	SEEPLEX RVP	LUMINEX RVP	ResPlex II v2.0
TARGETS	Influenza A/B, RSV-A/B, HMPV, Para1/2/3, Rhinovirus, Coronavirus 229E/NL63/OC43, Adenovirus	Influenza A/B, subtype H1/H3/H5, Para1/2/3/4, RSV-A/B, HMPV, Rhinovirus, Coronavirus NL63/ 229E/ OC43/HKU1/ SARS, Adenovirus	Influenza A/B, Para1/2/3/4, RSV-A/B, HMPV, Rhinovirus, Coxsackie virus, Echovirus, Coronavirus 229E/ NL63/ OC43/HKU1, Adenovirus, Bocavirus
TECHNOLOGY	Dual priming oligo (DPO) technology End-point RT-PCR	End-point RT-PCR, Bead-based detection	End-point RT-PCR Bead based detection
ISSUES	Patch to existing assay Gel-based, Open system Turn-around-time No Influenza A sub-typing	Open system Turn-around-time	Open system Turn-around-time No Influenza A sub-typing

## RESULTS

TABLE 2. Sensitivity and specificity of three commercial methods for detection of Influenza A from seasonal influenza-positive and negative specimens

Test Characteristic	SEEPLEX	LUMINEX	RESPLEX
SENSITIVITY	16/21 (76%)	15/21 (71%)	10/21 (48%)
SPECIFICITY	39/39 (100%)	38/39 (97%)	39/39 (100%)

TABLE 3. Sensitivity and specificity of three commercial methods for screening of SOIV-positive and negative specimens

Test Characteristic	SEEPLEX	LUMINEX	RESPLEX
SENSITIVITY	19/20 (95%)	20/20 (100%)	11/20 (55%)
SPECIFICITY	39/39 (100%)	38/39 (97%)	39/39 (100%)

## CONCLUSIONS

- ❖ The Luminex and Seeplex RVP assays may be effective tools for screening SOIV in nasopharyngeal specimens prior to confirmation by specific assays.
- ❖ Initial screening for SOIV with commercial assays may augment laboratory surge capacity in the event of a pandemic.