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Figure 1.

Cellular-Linked (Cloud-Based) System for Near Real-Time Surveillance of Influenza Viruses A and B Using the Sofia® Fluorescence Immunoassay Platform

[1]S Barlow, [2]L Brammer, [3]D Booker, [2]A Fowlkes, [2]A Giorgi, [3]L Mimms, [4]E Reisdorf, [4]P Shult, [3]J Tamerius, [1]J Temte

[1]University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, United States; [2]Centers for Disease Control and Prevention, Atlanta, Georgia, United States; [3]Quidel Corporation, San Diego, California, United States; [4]Wisconsin State Laboratory of Hygiene, Madison, Wisconsin, United States.

Abstract

Background: The Sofia Analyzer is an immunofluorescence, point-of-care instrument for use with assays developed for the rapid detection of viral and bacterial pathogens, including the Sofia Influenza A+B Fluorescence Immunoassay (FIA). The system was waived by the Clinical Laboratory Improvement Amendments (CLIA) and available for clinical use in October 2011. In November 2012, a cellular wireless capability was added to the Sofia Analyzer with the primary purpose of providing near real-time, patient de-identified influenza surveillance data to public health laboratories and other organizations.

Materials And Methods: Outpatients presenting at two small clinics in southern Wisconsin with at least two respiratory symptoms (fever, cough, sore throat, rhinitis, and congestion) had paired nasal (NS) and nasopharyngeal (NP) swab specimens collected. The Sofia Analyzer, Sofia Influenza A+B FIA kits, and wireless router were provided by Quidel (San Diego, CA). The NS was tested using Sofia, and the NP specimen was promptly transported to the Wisconsin State Laboratory of Hygiene for testing by PCR using the Centers for Disease Control and Prevention (CDC) human influenza virus real-time RT-PCR diagnostic panel. Sensitivity, specificity and overall accuracy were calculated. The Sofia Analyzer and router enabled transmission of the doubly encrypted, patient de-identified Sofia test result within one minute of testing to a cloud-based server. The data were received wirelessly at Quidel and uploaded to a secure FTP site supported by the CDC each night between midnight and 1:00 AM Pacific time. The Sofia Analyzer data (hard copy printout) were compared to the data received wirelessly by the CDC for data transmission accuracy, and assay results from Sofia were compared to those obtained from the PCR assay.

Results: Of the 100 patients tested, all test results were shown to have been transmitted and received correctly. Software validation identified no anomalies in the transmission of patient results from the Sofia Analyzer to the authorized recipients. In addition to test results, patient age and patient status (i.e. in- or out-patient) were optional fields for entry by the operator and, when entered, were transmitted with 100% accuracy for all patients. The test cassette lot number, instrument serial number (traceable to location), internal quality control results, and calibration results were transmitted automatically without operator action. Influenza A or B was detected by both Sofia and PCR from 25 specimens, including 31 by PCR and 30 by Sofia. Thus, compared to PCR Sofia yielded a sensitivity of 81% (25/31), a specificity of 93% (64/69) and an overall accuracy of 89%.

Conclusions: The Sofia Analyzer with cellular-wireless-based reporting capability was demonstrated to require minimal input by users in the point of care, outpatient clinics and to reliably transmit data to public health. The wireless capacity provided more immediate, extensive data to public health authorities and has the potential to report to additional organizations for whom rapid surveillance information is valued.

Introduction

The Sofia FIA Analyzer is an easy-to-use instrument for worldwide use with the Sofia Influenza A+B FIA—an in vitro diagnostic test. Recently, a cellular wireless capability was added to the Sofia Analyzer primarily to provide for the near real-time delivery of influenza surveillance data to public health agencies and other healthcare institutions. A cellular wireless Router was interfaced to the device to enable communication with cloud based servers that enable ready access of health agencies and other interested parties to the appropriate influenza surveillance data. This Sofia-based surveillance system, recently coined Virena™ (**Figure 1**), is designed to transmit incidence rates of influenza A & B by precise location of the testing site. This capability requires minimal input from the person running the assay and provides more comprehensive and near-real-time data to public health authorities.

System Overview

The overall system, shown in **Figure 2**, pictorially displays how the surveillance network functions from the Sofia Analyzer to the Router and ultimately through the two "clouds" to the final recipient of the results.

The surveillance data exported from the Sofia Analyzer are fully encrypted and maintained encrypted until prepared for conveyance to the designated recipient. It is well-known that wireless networks can incur transmission delays and outages. For this reason steps were taken to develop a specially customized Router that provides custom programming, configurable routing and switching capability, dual Ethernet ports and worldwide cellular wireless compatibility (now certified in 57 countries). One of the Router's two Ethernet ports is dedicated to communicating with Sofia and the other is used for Internet connectivity to Cloud #1. If connectivity is not available, the Router automatically buffers the encrypted data and sends it when the connection is restored. The cellular wireless mode was used for the study presented below. To ensure data security, the communications from the Router to Cloud #1 uses Secure Socket Layer (SSL) over TCP/IP. The non-encrypted part of the incoming messages includes the serial number of the Sofia Analyzer. Cloud #1 files these messages by owner and then by serial number. If a particular organization requires that its surveillance data be segregated, i.e. confidential, this can readily be accomplished using pre-programmed features within Cloud #1. At a predetermined time, Cloud #2 interrogates Cloud #1 and pulls all the new result messages, as depicted (**Figure 2**). Currently this task runs daily at 08h00 UTC, but the time and frequency can be adjusted if circumstances warrant, e.g. during a pandemic. The result messages are checked for transmission integrity and for consistency of the Sofia and Router serial numbers. Additional data including location, ownership and LOINC as well as SNOMED codes are added to the result message and it is saved to the database.

The Sofia Analyzer and Router are pre-configured to require minimum set up time and effort at the user's facility. Once the system has been installed by the user and activated by Quidel, there is only one change to the operator work flow. For each patient test to be run, an additional screen appears. It allows for entry of demographic data. Age may be set by the site's Supervisor as a compulsory entry and the Operator may enter it in Years, Months or Weeks as appropriate. Ages over 89 years are shown as ">89" to meet patient confidentiality requirements in some countries such as the US. A list of the possible information transmitted with each test is shown in **Figure 3**; the items numbered 8 through 11 are available for customized data entry at the request of the institution, public health agency or country officials using the surveillance system.

Study Design

The goal of the study was to determine if the software developed for the Virena system and guiding the network connections and performance worked as intended. One small rural clinic and one small urban clinic in Madison, Wisconsin—both CLIA waived—were selected for participation in the study. Both clinics were supplied with the Virena system (**Figure 2**) and agreed to enroll patients of any age that presented with influenza like illness (ILI). **Figure 3** presents a flow chart showing the overall study design. As shown, nasal swab (NS) and nasopharyngeal swab (NPS) specimens were collected from each patient. The nasal swab specimen was tested directly in the Sofia Influenza A+B FIA (Quidel Corporation). The nasopharyngeal swab specimens were placed in VTM and transported to the Wisconsin State Laboratory of Hygiene where they were tested by PCR for influenza A and B and other respiratory viruses. Two PCRs were used for their analyses: (1) CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel; and, (2) Qiagen ResPlexII v2.0 Respiratory Virus Panel. As shown in **Figure 4**, the Sofia test results were automatically transmitted from Wisconsin to San Diego within seconds and later pushed nightly to the U.S. CDC.

Figure 2. Sofia Wireless system

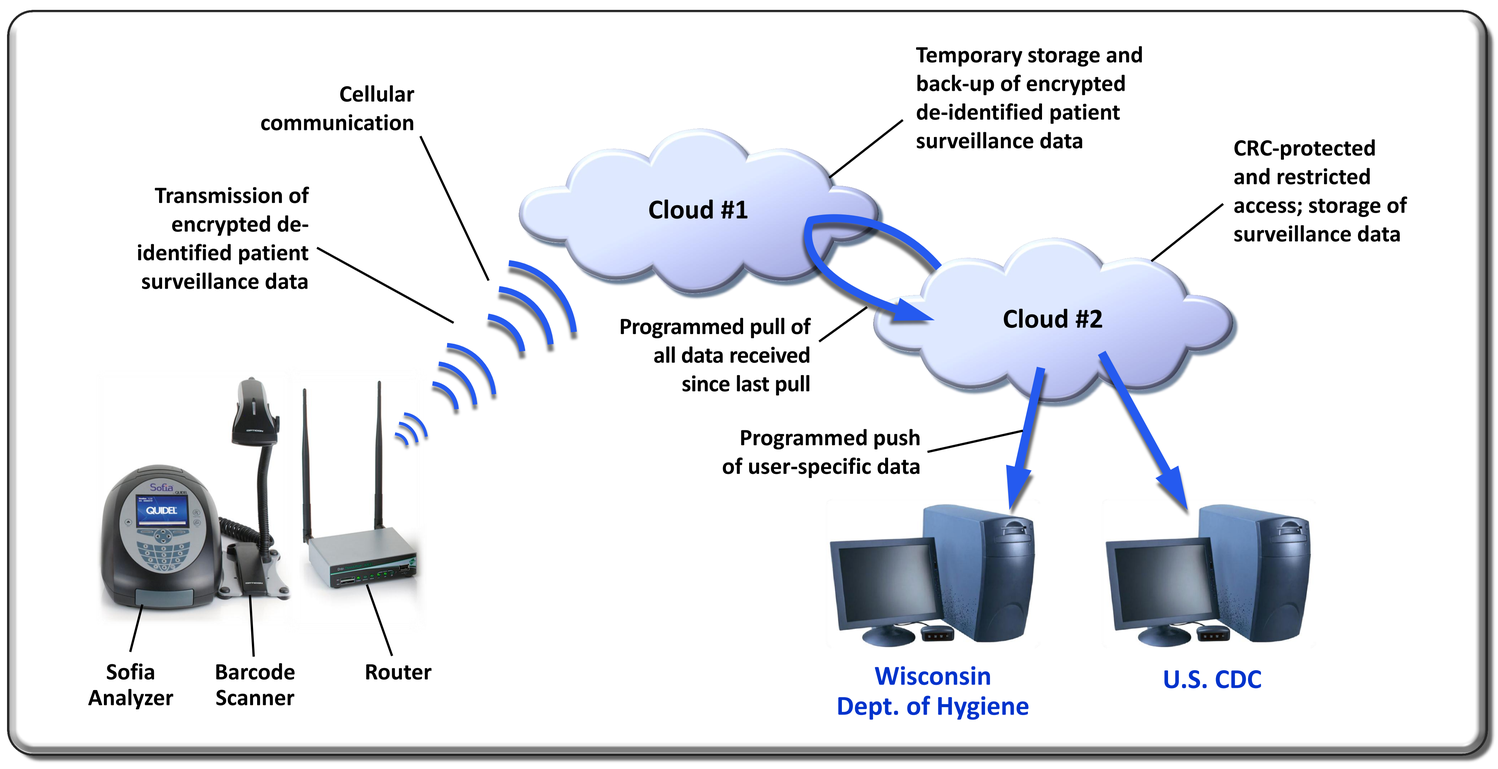
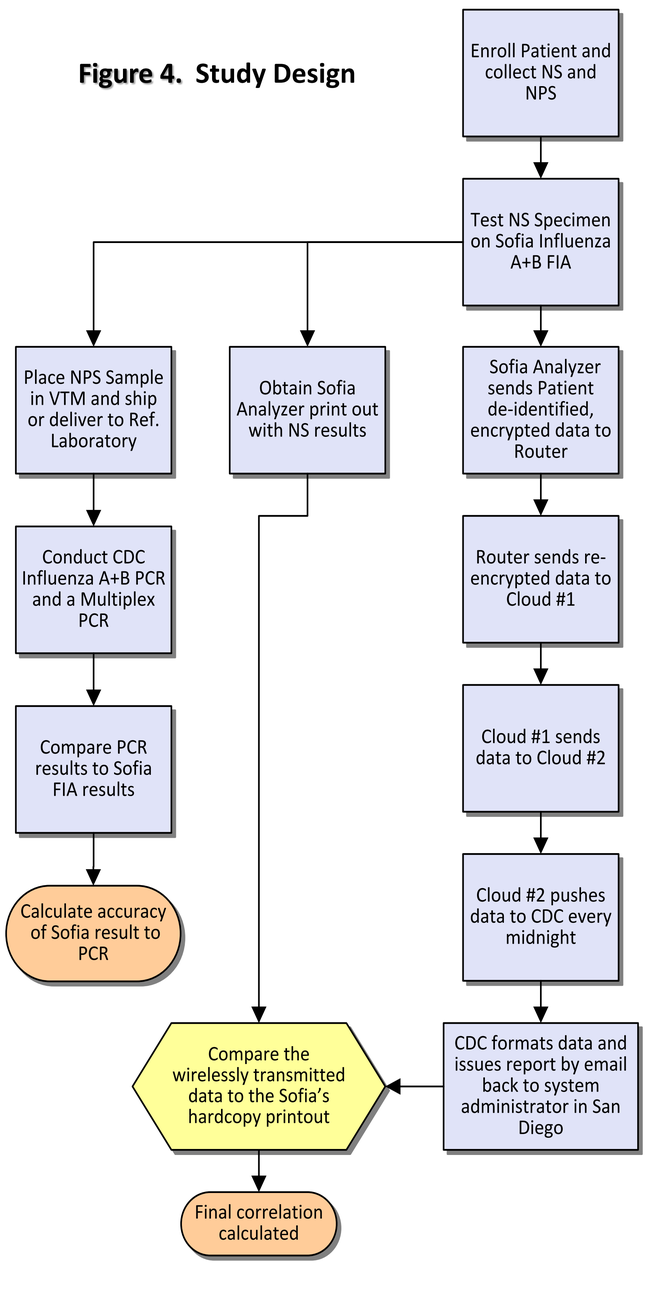


Figure 3. Sofia Output Messages

Field#	Description	Example
1	Instrument Name	Sofia
2	Firmware Revision No.	01.01.00
3	Site name	TriCore ED
4	Current date/time	20130618/801235
5	Patient ID	--
6	Demographics – Age	Weeks, months, years
7	Demographics - Patient Status	In- or out-patient
8	Demographics #1	*customizable*
9	Demographics #2	*customizable*
10	Demographics #3	*customizable*
11	Demographics #4	*customizable*
12	Order #	A1234567890
13	Operator ID	B1234567865
14	Test Type Name	FLU A + B
14a	Assay Type	90
15	Sample Type	P
16	Kit lot#	12345678
17	Cassette lot #	123456
18	Cassette number	127865
19	Test flag	F
20	Date/time of test completion	20130618/801235
21	# of results to follow	2
22	Test name	Flu A
23	Test value	Positive *(e.g.)*
24	Units	Analyte-dependent

Figure 4. Study Design



Near real-time: *The Sofia Analyzer automatically transmits results within 60 seconds to the Clouds. The data can then be pushed to the receiving site any time thereafter, depending upon the preferences of the institution or agency receiving the surveillance data.*

Results

One hundred (100) patients with ILI were enrolled in total by the two clinical study sites. The ages of the patients ranged from 35 weeks to 82 years. All the patients were out-patients. 31% (31/100) of the patients were infected with influenza A or B as shown by the CDC's in vitro diagnostic PCR for influenza. Using the Qiagen multiplex PCR, no virus was detected in 41% (41/100) of the patients. The following viruses were detected: 20 influenza B; 14 influenza A; 8 RSV A; 7 CoV OC43; 4 RSV B; 3 Rhino; 2 hMPV; and 2 parainfluenza viruses. When compared to CDC's PCR, the performance of the Sofia Influenza A+B FIA is shown in **Figure 5**.

The hardcopy results printed out by Sofia at both clinics at the end of each assay were collected and the results on the printouts were compared to the data received by the CDC via the Virena system's network. Nearly 2,300 different data points were transmitted, including the Sofia FIA test results, demographic and other information (see **Figure 3**). 100% of the nearly 2,300 results were faithfully and accurately transmitted from Wisconsin to the CDC with no deletions or errors. This confirmed that the Virena system's Software and Clouds all worked perfectly as designed.

Figure 5. Transmission of Surveillance Data

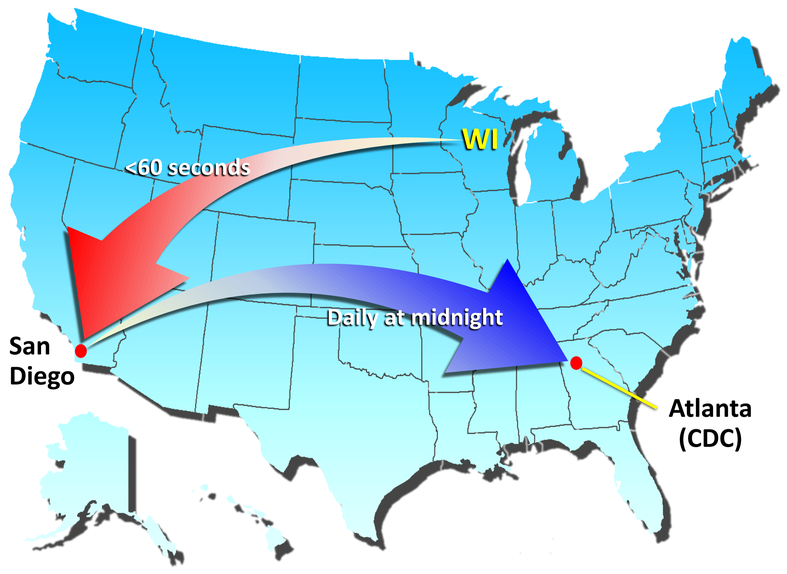


Figure 6. Sofia Influenza FIA Test Performance Versus PCR

Influenza A and B Combined				
Fresh NS/NPS (N=100)		PCR +	PCR –	Total:
Sofia FIA	+	25	5	30
	–	6	64	70
Total:		31	69	100

Sensitivity: 25/31= 80.6% *PPV: 25/30 = 83.3%*
Specificity: 64/69 = 92.8% *NPV: 64/70 = 91.4%*

Overall Accuracy = 89%

Conclusion

The cellular-linked, Sofia-based surveillance system, Virena, allowed near real-time tracking of influenza A and B virus incidence by geography. This pilot study demonstrated that Sofia test results together with demographic information could be wirelessly transmitted from the two Wisconsin test sites to the appropriate dual cloud system within seconds after the patients' samples were tested. And these results could be automatically pushed to the CDC at a pre-agreed time on a daily basis. The Virena system shows significant potential as a tool for near real-time surveillance for influenza A and B.