

Validation of Dry Flocked Swabs after Storage for the Detection of Influenza A using Real Time PCR

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REVISED ABSTRACT

Background: Detection of influenza is usually done on swabs transported in viral transport medium. Dry swabs are preserving and stabilizing nucleic acids for molecular diagnosis.

Objectives: To validate Copan dry flocked swabs to stabilize influenza A viral RNA using real time PCR.

Methods: Flocked throat swabs (TS) and nasopharyngeal swabs (NPS) were compared to Dacron TS and NPS swabs. An Influenza ATCC strain was titrated in cell culture and by PCR. Dilutions above and at the limit of detection were used as contrived specimens and dispensed in one ml aliquots in sterile tubes. Each swab type was dipped to simulate a TS and NPS, then placed into a sterile dry tube and stored at room temperature (RT) or 4° C for 3 weeks. Clinical nasopharyngeal swabs (50), stored dry, were also tested. 300 microliters of PBS was added to each tube, vortexed for 15 sec and 250 microliters was used to extract viral RNA using the Qiagen RNeasy Mini Kit, and tested in the Arrow Diagnostics Fast set real time assay on the Rotor Genie.

Results: The contrived specimens prepared with flocked TS and NPS were positive for influenza A RNA up to 3 weeks when stored at 4° C and RT. Instead the Dacron TS and NPS were positive up to 3 week at 4° C, but negative at RT after 2 weeks. Fifteen of the 50 patient flocked NPS transported dry were positive after 3 weeks at RT.

Conclusions: The Copan flocked TS and NPS demonstrated the ability to stabilize influenza A viral RNA when stored dry at RT or 4° C up to 3 weeks.

BACKGROUND

With the current public concern over the possible occurrence of a pandemic influenza outbreak, wide surveillance programs have been implemented for the prevention and spreading of this sometime lethal disease.

Conventional surveillance specimens for the detection of influenza are collected and transported in viral transport medium that may not be available in rural places and specimens in liquid format cannot be sent in the mail.

Molecular methods are now routinely used in most diagnostic laboratories, and has been reported that nucleic acids from respiratory viruses are stable when nasopharyngeal or throat specimens are collected with swabs and the swabs are transported dry to the laboratory.

OBJECTIVE

To validate the ability of the Copan flocked swabs to stabilize viral RNA for collecting and transporting respiratory viruses without the aid of transport medium using real time PCR assays

METHODS

For this validation the regular flocked swabs (throat swabs) and the pernasal flocked swabs (nasopharyngeal or nasal) were compared to regular and nasopharyngeal Dacron swabs for the ability to preserve influenza RNA when stored in dry swabs for different times and conditions.

ATCC strain of influenza A at 0 time, 48h, 1, 2, 3 weeks at 4° C and RT and 50 clinical specimens, 15 defined positive and 35 negative, were used for this validation.

The following swabs were used for this validation:

- Copan pernasal flocked swabs (used for nasopharyngeal or nasal collection) and regular flocked swabs (used for throat swab collection)
- Copan pernasal rayon swabs (used for nasopharyngeal or nasal collection) and regular rayon swabs (used for throat swab collection)

The ATCC strain of influenza A diluted from 10-1 to 10-5 in UTM. 250 microliters were inoculated in shell vial cell cultures and 250 microliters were used for nucleic acid extraction in order to find the limit of detection.

A dilutions above and one close to the limit of detection were used for the preparation of contrived specimens.

Influenza A dilutions in UTM were dispensed in one ml aliquots in sterile tubes for the preparation of the swabs.

Each swab type was dipped, to simulate a TS and NPS, for a count of ten in the one ml aliquot of influenza dilutions, then placed into a sterile dry tube and stored at room temperature (RT) or 4° C for 3 weeks.

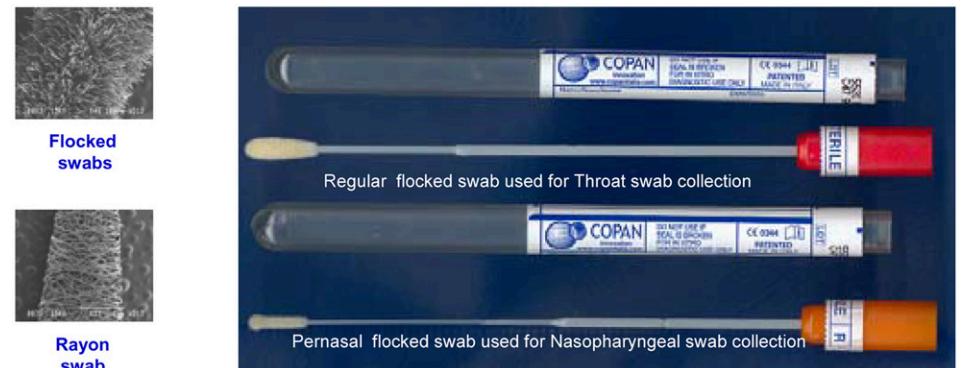
Clinical nasopharyngeal swabs (50), stored dry at room temperature, were also tested.

All swab specimens were tested as follows: 300 ml of PBS was added to each tube containing a swab, after vortexing for 15 second, 250 microliters of each swab suspension was used to extract viral RNA using the Qiagen RNeasy Mini Kit as per extraction protocol.

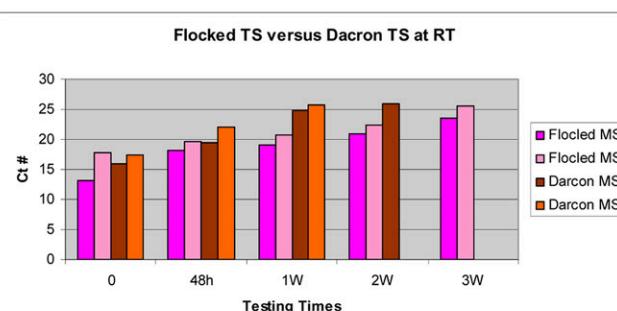
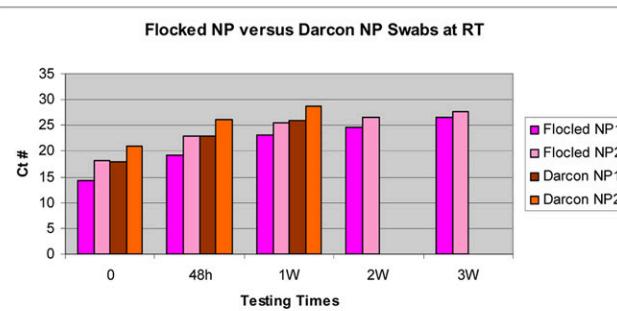
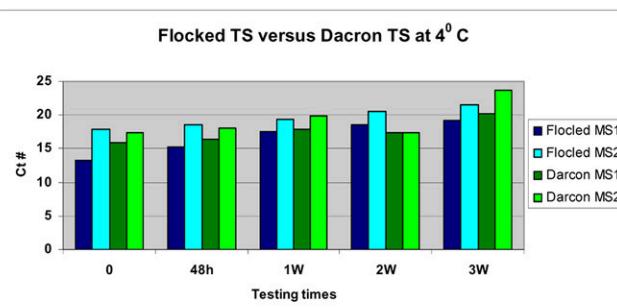
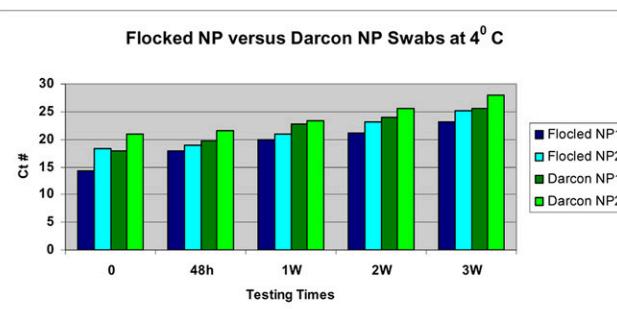
The extracted nucleic acid for each swab type was stored frozen until all the extraction for all the storage time and conditions were done.

The Arrow Diagnostics Fast set Influenza A and Influenza B real time assay on the Rotor Genie was used for the real time PCR testing. 10 microliters of each extracted nucleic acid were amplified as per assay protocol.

METHODS



RESULTS



The contrived specimens prepared with regular flocked swabs (TS) and with pernasal flocked swabs (NPS) were positive for influenza A RNA up to 3 weeks when stored at 4° C. and RT.

Instead the specimens prepared with Dacron TS and NPS were positive up to 3 week at 4° C, but negative at RT after 2 weeks.

Fifteen of the 50 clinical specimens, collected with flocked NP Swabs and transported dry, were positive after 3 weeks at RT as per original testing done with swabs transported in transport medium.

CONCLUSIONS

The Copan regular flocked (TS) and pernasal flocked swabs (NPS) demonstrated the ability to stabilize influenza A viral RNA when stored dry at RT or 4° C up to 3 weeks.

The Copan flocked swabs can be used dry to collect clinical specimens for molecular amplification assays during for the diagnosis of single cases of influenza or for the surveillance of pandemic outbreak of influenza

Specimens collected with flocked swabs can be placed back in their plastic container and can be sent to the laboratory by mail.