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Evaluation of the ESwab Transport System to Collect, Transport and Maintain RSV, FluA and FluB Viruses for Rapid Antigen and Molecular Tests

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Abstract

One of the most significant advances in the detection of respiratory viruses is the recent introduction of novel swab types such as flocked swabs. Studies comparing nasal flocked swabs to nasal aspirates showed that the sensitivity of flocked swabs was at least equivalent for the detection of a variety of respiratory viruses.

The aims of this study were: (a) to evaluate the performance of the ESwab™ (Copan, USA), a transport system normally used for bacteriology, for the transport and maintenance of FluA, FluB and RSV for rapid antigen and molecular testing, and (b) to compare its performance with the standard of care UTM™ Virus Collection System (Copan).

Methods: A 10^5 U/mL pool solution of FluA, FluB and RSV were prepared (Zeptometrix, NY) followed by two 10-fold dilutions (10^4 and 10^3) of the same pool of organisms. Each swab type was inoculated in duplicate with 100µL of the dilutions prepared. Swabs were held for 0h, 24h, 48h and 72h at refrigerated temperature (4°C). After incubation, the swab transport media were tested for FluA, FluB and RSV using both Real Time PCR (Simplexa™ FluA/B & RSV Direct RT PCR Kit – Focus Diagnostics) and Rapid Antigen Tests (QuicKVue® Influenza A+B and QuickVue® RSV Tests – Quidel), accordingly to manufacture instructions. In the end, results obtained from ESwabs and UTM swabs were analyzed and compared using the Limit of Detection (LOD) values, PCR Ct values and time of incubation at 4°C.

Results: PCR was able to detect all the samples tested from different swab types, dilutions and time of incubations. Similar PCR Ct values were observed in specimens from different swab types but same organism dilution and time of incubation. Rapid Antigen Tests also presented similar results when different swab types from same dilution and incubation time where compared. However, its LOD was inferior to PCR and none of the specimens inoculated with the 10^3U/mL dilution were detected by this test.

Conclusions: The performance of both ESwab Transport System and UTM Virus Collection System was excellent to maintain the viability of FluA, FluB and RSV for up to 72h at a refrigerated temperature. The PCR test was more sensitive than the rapid antigen test for the organisms tested. Finally, this study demonstrates that the ESwab Transport System performed equally well to UTM Virus Collection System to transport and maintain FluA, FluB and RSV for rapid antigen and molecular testing. This extends the multipurpose use of ESwab beyond bacteriological investigations.

Material & Methods

Organisms:

- Influenza A H3N2 Virus
- Influenza B Virus Infectious Culture Fluid
- Respiratory Syncytial Virus (RSV-A) Type A

From ZeptoMetrix Corporation, titers >10^5 U/mL.

Swab Transport Systems tested

- Copan UTM™ Virus Collection System
- Copan eSwab Collection System

Methods:

1. FluA, FluB and RSV were evaluated for survival after incubation at refrigerator (4°C) temperature for 0h, 24h, 48h and 72h using two swab transport systems.
2. FluA, FluB and RSV pool solution were prepared by mixing up the 10^5U/mL dilution of each one of the three viruses
3. A 10^4U/mL dilution were prepared by adding a 200µL of the 10^5U/mL in a 1800µL of saline
4. A 10^3U/mL dilution were prepared by adding 200µL of the 10^4U/mL solution in a 1800µL of saline
5. 100µL of each dilution prepared in step 1, 2 and 3 were added into 16 wells of a microtiter plate.
6. Eswabs and UTM transport system were inoculated by placing each swab into one of the 16 wells of the microtiter plate.
7. In the end, we will have 8 eSwabs and 8 UTM swabs
8. Two of each swab type and dilution were held for 0h, 24h, 48h and 72h in refrigerated temperature (4°C)
9. Tests for FluA, FluB and RSV were performed using the following methodologies and assays, accordingly to manufacture's instructions:

- Real Time PCR: Simplexa™ FluA/B & RSV Direct RT PCR Kit
- Rapid Antigen Tests: QuicKVue® Influenza A+B Test and QuickVue® RSV Tests.

Results

Table 1. Ct Values Flu A PCR:

Temp	eSwab						UTM swab					
4°C	10^5		10^4		10^3		10^5		10^4		10^3	
0h	27	27	31	31	36	36	28	27	31	31	36	34
24h	27	28	31	32	35	35	28	28	32	32	36	36
48h	28	27	31	30	36	35	35	30	35	35	33	35
72h	28	27	31	31	35	35	28	28	31	32	35	37

Table 2. Ct Values Flu B PCR :

Temp	eSwab						UTM swab					
4°C	10^5		10^4		10^3		10^5		10^4		10^3	
0h	23	24	27	27	31	31	27	27	30	29	34	34
24h	23	24	27	28	31	30	27	28	30	30	34	34
48h	24	24	27	27	31	31	27	26	30	30	35	34
72h	24	24	27	27	31	31	27	27	31	30	35	34

Table 3. Ct Values RSV PCR:

Temp	eSwab						UTM swab					
4°C	10^5		10^4		10^3		10^5		10^4		10^3	
0h	17	17	21	20	25	25	19	18	22	22	25	26
24h	17	18	21	21	25	25	19	19	22	22	26	26
48h	17	17	21	21	25	24	19	19	23	23	26	25
72h	18	18	21	20	25	24	19	19	23	22	25	26

Table 4. FluA Antigen Test Results:

Temp	eSwab						UTM swab					
4°C	10^5		10^4		10^3		10^5		10^4		10^3	
0h	+	+	+	+	-	-	+	+	+	+	-	-
24h	+	+	+	+	-	-	+	+	+	+	-	-
48h	+	+	+	+	-	-	+	+	+	+	-	-
72h	+	+	+	+	-	-	+	+	+	+	-	-

Table 5. FluB Antigen Test Results:

Temp	eSwab						UTM swab					
4°C	10^5		10^4		10^3		10^5		10^4		10^3	
0h	+	+	+	+	-	-	+	+	+	+	-	-
24h	+	+	+	+	-	-	+	+	+	+	-	-
48h	+	+	+	+	-	-	+	+	+	+	-	-
72h	+	+	+	+	-	-	+	+	+	+	-	-

Table 6. RSV Antigen Test Results:

Temp	eSwab						UTM swab					
4°C	10^5		10^4		10^3		10^5		10^4		10^3	
0h	+	+	+	+	-	-	+	+	+	+	-	-
24h	+	+	+	+	-	-	+	+	+	+	-	-
48h	+	+	+	+	-	-	+	+	+	+	-	-
72h	+	+	+	+	-	-	+	+	+	+	-	-

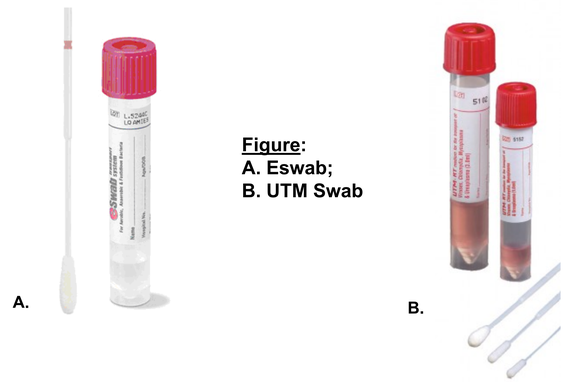

Figure:
A. Eswab;
B. UTM Swab

Conclusions

1. PCR was able to detect all the samples tested from different swab types, dilutions and time of incubations.
2. Similar PCR Ct values were observed in specimens from different swab types but same organism dilution and time of incubation.
4. Rapid Antigen Tests also presented similar results when different swab types from same dilution and incubation time where compared.
5. The performance of both ESwab Transport System and UTM Virus Collection System was excellent, maintaining the viability of FluA, FluB and RSV for up to 72h at a refrigerated temperature.
6. The PCR test was more sensitive than the rapid antigen test for the organisms tested.
7. Finally, this study demonstrates that the ESwab Transport System performed equally well to UTM Virus Collection System to transport and maintain FluA, FluB and RSV for rapid antigen and molecular testing.
8. This study demonstrates a broader capability of ESwab, beyond its intended bacteriological uses. It's ability to maintain virus increases ESwab multipurpose appeal and can help simplify clinical and laboratory investigations for respiratory pathogens. In this scenario ESwab could be used for bacteriology culture for organisms such as Strep A as well as virology investigations.