IMPROVING PRE-ANALYTIC COLLECTION SYSTEMS: INACTIVATION AND PRESERVATION OF INFLUENZA A FOR RAPID TESTING

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Abstract

Objective: An alternative sample collection system that would render a virus non-infectious but permit rapid identification by direct immunofluorescence (DFA) or molecular methods would be beneficial during a pandemic. In this study, the CyMol collection system was evaluated for its ability to inactivate influenza A and preserve the sample for molecular testing.

Methods: CyMol (Copan, Italia) is an alcohol-based medium that preserves cells for DFA. Flocked nasopharyngeal swabs (NPS) collected in universal transport media (UTM) were compared to NPS collected in CyMol. Aliquots of Influenza A viral lysate were absorbed onto duplicate flocked swabs and placed into the UTM and CyMol collection systems. Inactivation of the virus after exposure to each collection medium was assessed by inoculation of the mocked samples into R-mix shell vial culture followed by immunofluorescent staining at 48 hours. Inactivation was assessed for 5 influenza A subtypes. The stability and recovery of influenza A nucleic acid (NA) was also assessed after 1, 7, 14 and 21 days at 4ºC, -20ºC, room temperature (RT) and 37ºC. Aliquots of mocked specimens were extracted by easyMAG (bioMérieux) and 5 uL of purified NA tested by quantitative RT-PCR on the Roche LightCycler.

Results: In contrast to the UTM, the influenza A virus was inactivated on exposure to the CyMol collection medium and failed to grow in shell vial culture. Influenza A RNA levels were stable for up to 14 days in both the UTM and CyMol collection systems at -20ºC, 4ºC and RT. The stability of Influenza A RNA declined in both systems at 37ºC.

Conclusions: The Copan CyMol medium inactivates influenza A virus while stabilizing RNA for molecular testing for up to 14 days at -20ºC, 4ºC and RT. CyMol is a potential alternative for safe sample collection during an influenza pandemic situation.

Objective

The ability of the CyMol collection system (Copan, Italy) to inactivate influenza A and preserve the sample for molecular testing was evaluated in this study.

Methods

Influenza A Virus Inactivation

- Viral lysate (Influenza A/Victoria/3/1975 (H3N2)) was diluted in pooled negative respiratory samples to obtain a dilution representative of an influenza A positive sample (10^4-10^5 copies/5uL; Crossing Point 25-27)
- 50 uL of the diluted viral lysate was adsorbed onto a flocked nasopharyngeal swab and then placed in a CyMol or UTM collection tube
- After 30 minutes exposure to the media, the mock sample collections were vortexed for 20 seconds, swabs removed and collection media spun for 10 minutes at 2000 rpm
- 200 uL of the neat supernatant and 200 uL of 1:10 diluted supernatant were inoculated in duplicate into R-Mix shell vials with coverslips
- Immunofluorescent staining of the shell vials occurred after 48 hours incubation at 37ºC
- Virus inactivation was monitored by shell vial culture at time zero and after 5, 10 and 20 minute exposures to CyMol or UTM Collection Media
- Viral lysate from 4 other influenza A subtypes (H1, H8, H10 and H15) was exposed to CyMol or UTM for 30 minutes, inoculated into shell vial culture and stained after 48 hours incubation at 37ºC

Methods

Influenza A RNA Stability and Recovery

- Viral lysate was adsorbed onto duplicate swabs and placed in CyMol and UTM Collection tubes
- The stability and recovery of influenza A nucleic acid was assessed in duplicate after 1, 7, 14 and 21 days exposure to CyMol or UTM at -20ºC, 4ºC, room temperature and 37ºC.
- 200 uL aliquots of mocked collected samples were extracted by easyMAG (bioMérieux) and eluted in 60 uL
- 5 uL of purified NA was tested by a quantitative Influenza A RT-PCR (Matrix gene, CDC) on the Roche LightCycler 2.0.

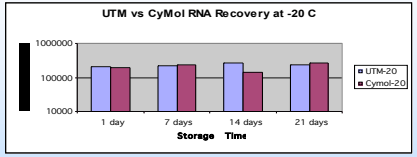
Results

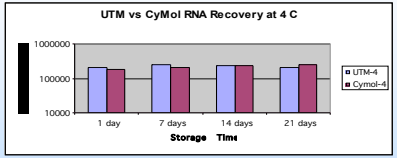
Inactivation of Infectivity

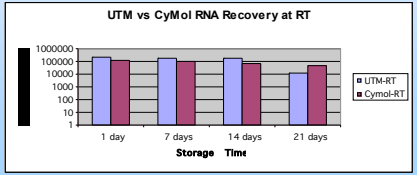
- In contrast to the UTM, Influenza A/ Victoria/3/75 H3N2 was inactivated after 30 minutes exposure to the CyMol Collection Medium at RT and failed to grow in shell vial culture.
- 4 other influenza A subtypes (H1, H8, H10 and H15) were also inactivated by a 30 minute exposure to the CyMol Collection Medium at RT and failed to grow in shell vial culture.
- Influenza A virus inactivation was observed to occur after CyMol Collection Medium exposure at time zero.

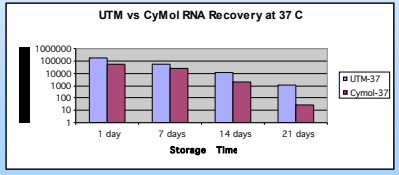
Influenza A RNA Stability and Recovery

- Influenza A RNA levels were stable for up to 14 days in both the UTM and CyMol collection systems at -20ºC, 4ºC and RT.
- The stability of Influenza A RNA declined in both systems at 37ºC.









Average RNA Copies Recovered/5uL

Sample	1 day	7 days	14 days	21 days
UTM-20	2.13E+05	2.26E+05	2.62E+05	2.33E+05
Cymol-20	1.92E+05	2.36E+05	1.45E+05	2.59E+05
UTM-4	2.09E+05	2.49E+05	2.29E+05	2.11E+05
Cymol-4	1.80E+05	2.07E+05	2.31E+05	2.47E+05
UTM-RT	2.05E+05	1.95E+05	1.86E+05	1.21E+04
Cymol-RT	1.18E+05	1.10E+05	7.18E+04	4.24E+04
UTM-37	1.69E+05	5.10E+04	1.08E+04	1.19E+03
Cymol-37	5.80E+04	2.29E+04	1.91E+03	2.90E+01

Average Crossing Points

Sample	1 day	7 days	14 days	21 days
UTM-20	23.86	23.82	23.75	23.90
Cymol-20	24.35	23.88	24.62	23.75
UTM-4	23.94	23.67	23.94	24.05
Cymol-4	24.44	24.07	23.93	23.83
UTM-RT	23.98	24.08	24.30	28.38
Cymol-RT	25.06	24.99	25.64	26.41
UTM-37	24.25	25.98	28.40	31.55
Cymol-37	26.09	27.28	31.47	36.13

Conclusions

- The Copan CyMol medium inactivates influenza A virus infectivity on exposure to the media.
- Influenza A RNA was stabilized for molecular testing for up to 14 days at -20ºC, 4ºC and RT.
- CyMol is a potential alternative for safe sample collection during an influenza pandemic situation.