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How Long Can It Sit? The Stability of Viruses in Primary Specimens Over Time and Temperature

Erik Rist, Emaly Leak, Gabriel Solis, Rama Ramani, Amy B. Dean, Michael Popowich, Meghan Fuschino, Kirsten St. George
Virology Laboratory, Wadsworth Center, New York State Department of Health, Albany, NY

Introduction

CLIA requires clinical laboratories to have documented policies on acceptable storage conditions for specimens prior to testing. To generate data for these policies, our laboratory conducted viral stability studies to determine the effects of storage time and temperature on the outcome of PCR and culture-based tests. We receive samples in conditions ranging from frozen on dry ice, to room temperature. Time between collection date and receipt can also be variable. A variety of RNA, DNA, enveloped, and non-enveloped viruses were tested to ensure the laboratory's acceptance/rejection criteria are suitable for a wide variety of viruses in several sample matrices.

Hypotheses

- Temperature: Viral stability may decline with increased storage temperatures.
- Time: Viral stability may decline with increased time between specimen collection and testing.
- Concentration: Viral load may have an impact on stability.
- Virus type: Viral nucleic acid type (RNA rather than DNA) and viral envelope presence, may predispose a virus to instability.
- Matrix type: Specimen matrix and collection fluid may influence virus stability.
- Culture: Virus stability for culture viability may differ from stability for PCR testing.

Methods

- Matrix selection included swabs in viral transport media (VTM), cerebrospinal fluid (CSF), serum, urine, stool, and gelatin–tris-Hanks (GTH).

Viruses Used for Study

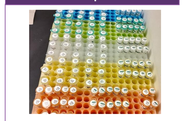
Virus	Family	Genome	Enveloped
Adenovirus (AV)	*adenoviridae*	DNA	No
Enterovirus (EV)	*picornaviridae*	RNA	No
Influenza A (Flu A)	*orthomyxoviridae*	RNA	Yes
Herpes Simplex Virus 2 (HSV2)	*herpesviridae*	DNA	Yes
SARS CoV-2 (SC2)	*coronaviridae*	RNA	Yes
West Nile (WNV)	*flaviviridae*	RNA	Yes

Step 1

High titer isolate is spiked into pooled negative patient samples and diluted to three titers (strong, medium, weak), then aliquoted and placed in assigned temperature condition (-80°C, -20°C, 4°C, 15°C, room temp (RT)).

Photo: Wren, Michael, 3/10/20, Wadsworth Center NYSDOH

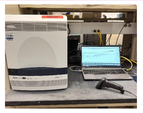
Step 2



Days 1, 2, 3, 4, and 7, aliquots taken out of storage. One set of aliquots (from each of three titers) extracted on bioMerieux easyMAG™, while another set (strong and medium titers only) is sent for culture.

Photo: Rist, Erik, 3/3/23, Wadsworth Center NYSDOH

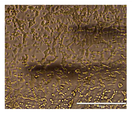
Step 3a



Extracted nucleic acid from aliquots are then RT-PCR tested on Applied Biosystems 7500 FAST Dx™ Real-Time PCR system in duplicate. Cycle threshold (Ct) values are recorded, and resulting curves analyzed across all time points.

Photo: Rist, Erik, 8/8/23, Wadsworth Center NYSDOH

Step 3b



For selected virus/matrix combinations, aliquots are inoculated into culture in duplicate; cytopathic effect (CPE) scores recorded at harvest.

Photo: Church, Theresa, 8/11/22, Wadsworth Center NYSDOH

PCR Results

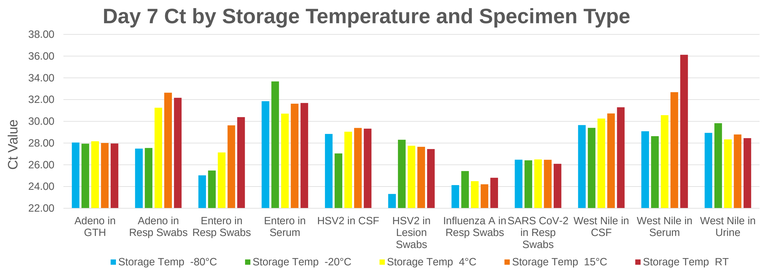


Figure 1. Day 7 data showing effect of storage temperature and specimen type on Ct values for all virus/matrix combinations.

PCR Results Continued

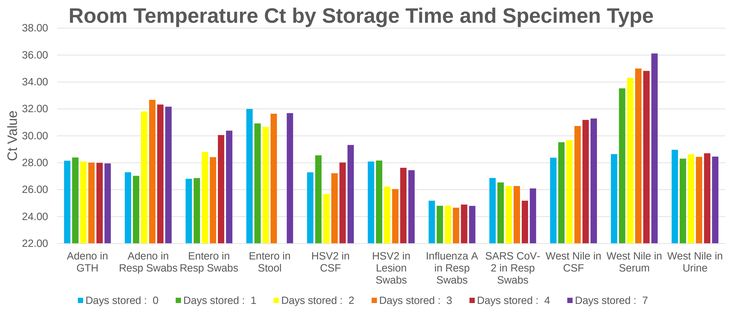


Figure 2. Room temperature data showing effect of time (Day 0-7) and specimen type on Ct values for all virus/matrix combinations.

Table 1. Difference in Ct values for temperature and time.

Virus and matrix	Temperature: Delta Ct between -80°C and room temp at Day 7	Time: Delta Ct between Day 0 and Day 7 at room temperature
Adenovirus in GTH	-0.10	-0.20
Adenovirus in Respiratory Swabs	4.68	4.87
Enterovirus in Respiratory Swabs	5.36	3.58
Enterovirus in Stool	-0.17	-0.31
Herpes Simplex 2 in CSF	0.48	2.04
Herpes Simplex 2 in Lesion Swabs	4.14	-0.64
Influenza A in Respiratory Swabs	0.68	-0.38
SARS-CoV-2 in Respiratory Swabs	-0.38	-0.78
West Nile Virus in CSF	1.64	2.92
West Nile Virus in Serum	7.04	7.48
West Nile Virus in Urine	-0.49	-0.51

Viral Concentration

Table 2. Difference in Ct between Day 0 and Day 7 at room temperature for each viral titer, and all virus/matrix combinations.

Virus and matrix	Strong	Medium	Weak
Adeno in Resp. Swabs	4.69	4.87	4.16
Entero in Resp. Swabs	3.58	3.35	3.35
Entero in Stool	-0.31	-0.47	N/A*
HSV2 in CSF	-3.97	2.04	1.72
HSV2 in Lesion Swabs	3.93	-0.64	-0.28
Flu A in Resp. Swabs	-0.18	-0.38	-0.14
SC2 in Resp. Swabs	-0.28	-0.78	0.54
West Nile in CSF	2.31	2.24	2.92
West Nile in Serum	6.86	7.48	N/A*
West Nile in Urine	0.57	0.52	-0.51

* N/A: PCR negative on day 7

Virus Type

Table 3. Stability effects in PCR and culture correlated with viral type and viral envelope presence.

Virus and matrix	Genome	Enveloped	PCR Stability Effected	Culture Stability Effected
Adeno in sterile VTM	DNA	No	No	N/A*
Adeno in Resp. Swabs	DNA	No	Yes	No
Entero in Resp. Swabs	RNA	No	Yes	No
Entero in Stool	RNA	No	No	N/A*
HSV2 in CSF	DNA	Yes	No	N/A*
HSV2 in Lesion Swabs	DNA	Yes	No	No
Flu A in Resp. Swabs	RNA	Yes	No	Yes
SC2 in Resp. Swabs	RNA	Yes	No	N/A*
West Nile in CSF	RNA	Yes	Yes	N/A*
West Nile in Serum	RNA	Yes	Yes	N/A*
West Nile in Urine	RNA	Yes	No	N/A*

* N/A: Condition not tested in culture

Sample Type

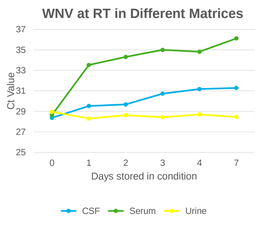


Figure 3. Effect of matrix type on Ct for West Nile virus at room temperature.

Negative sample matrix vs. sterile GTH

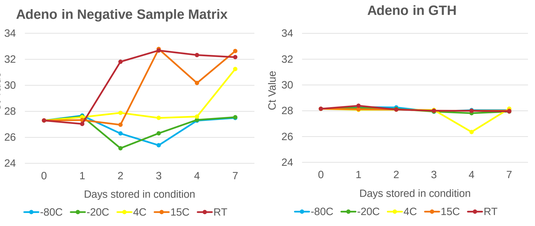


Figure 4a. Ct values over time for adenovirus in a negative sample pool of respiratory swabs.

Figure 4b. Ct values over time for adenovirus in sterile GTH.

Culture Results

CPE score at harvest

Table 4. Culture results for all conditions tested, from viral titers in the Ct range 25-29.

Adeno Swab 27.3 Ct

Days at storage condition

	0	1	2	3	4	7
-80°C	4	4	4	4	4	3
-20°C	4	4	4	4	4	3
4°C	4	4	4	4	3	3
15°C	4	4	4	4	4	4
RT	4	4	4	4	4	4

Entero Swab 26.8 Ct

Days at storage condition

	0	1	2	3	4	7
-80°C	4	4	4	4	4	3
-20°C	4	4	4	4	4	3
4°C	4	4	4	4	3	3
15°C	4	4	4	4	4	4
RT	4	4	4	4	4	4

Influenza A Swab 25.3 Ct

Days at storage condition

	0	1	2	3	4	7
-80°C	4	4	4	4	4	4
-20°C	4	4	4	4	0	0
4°C	4	4	0	4	0	0
15°C	4	0	0	0	0	0
RT	4	0	0	0	0	0

HSV2 Swab 28.1 Ct

Days at storage condition

	0	1	2	3	4	7
-80°C	4	3	2	3	3	4
-20°C	4	3	2	3	3	4
4°C	4	3	2	3	3	4
15°C	4	3	2	3	3	3.5
RT	4	3	2	3	3	3

Red highlights indicate no CPE detected.

Influenza A in culture

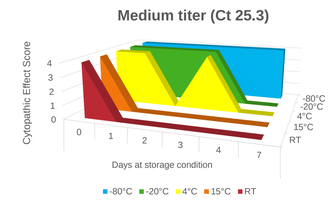


Figure 5a. Cytopathic effect of influenza A virus of medium titer on cell culture.

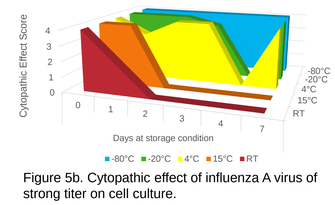


Figure 5b. Cytopathic effect of influenza A virus of strong titer on cell culture.

Conclusions

- Adenovirus and enterovirus in respiratory swabs, as well as West Nile Virus in CSF and serum, showed decreased detectability in PCR with increased storage temperatures and time (Figs 1&2). The overall change in Ct values for both temperature and time effects are condensed in Table 1, and show a possible temperature effect for HSV2 in lesion swabs and a lesser time effect for HSV2 in CSF. The HSV2 effects were not considered reliable, as the data for these was more erratic (Figs 1&2).
- Viral concentration had minimal impact on stability, although weak concentrations produced less precise results overall (Table 2).
- There was no demonstrated effect of viral type (RNA *vs.* DNA, enveloped *vs.* non-enveloped) on stability as measured by PCR or in culture (Table 3).
- Sample matrix type did impact PCR stability, as demonstrated by WNV in urine, serum, and CSF (Figure 3). There was also a clear effect noted for adenovirus, with Cts increasing in negative sample matrix but not in sterile culture media (Figs 4a, 4b).
- Adenovirus and enterovirus stability for PCR detection (Table 1) may have been affected by contaminants in the negative sample pool. The 15°C and room temperature aliquots turned yellow and cloudy as the days progressed, indicating a decrease in pH and possible growth of microbes. This is relevant to clinical laboratories because primary viral samples received for testing may have one or more co-infections, which could impact the stability of the primary viral target at higher storage temperatures and times.
- Influenza A showed decreased culture viability with increased time and temperature (Table 4), a result that was not mirrored by the PCR results (Table 1). Culture results for adeno and enteroviruses in respiratory swabs (Table 4) also did not correspond with the PCR data, failing to parallel the decreased PCR stability (Table 1).
- There was a possible effect of initial viral titer on subsequent influenza A viability in culture (Figs 5a, 5b), but additional work would be needed to clarify this trend.
- The rapid deterioration of some aliquots at 4°C and warmer, for detection by both PCR and virus culture, reinforces the laboratory's requirement for samples to be shipped frozen on dry ice or refrigerated with frozen cold packs. If specimens will be delayed more than one week from collection to receipt, they must be frozen at -80°C immediately after collection and shipped on dry ice. While no impact of time or temperature was found for some viruses, it is important to follow procedures that protect the most sensitive sample types and viruses.

References

1) CLIA standard for specimen submission handling and referral 42 CFR 493.1242

2) Dupuis M, Hull R, Wang H, Nattanmai S, Glasheen B, Fusco H, Dzigua L, Markey K, Tavakoli NP. Molecular detection of viral causes of encephalitis and meningitis in New York State. J Med Virol. 2011 Dec;83(12):2172-81. doi: 10.1002/jmv.22169. PMID: 22012726.

3) Shu B, Wu KH, Emery S, Villanueva J, Johnson R, Guthrie E, Berman L, Warnes C, Barnes N, Klimov A, Lindstrom S. Design and performance of the CDC real-time reverse transcriptase PCR swine flu panel for detection of 2009 A (H1N1) pandemic influenza virus. J Clin Microbiol. 2011 Jul;49(7):2614-9. doi: 10.1128/JCM.02636-10. Epub 2011 May 18. PMID: 21593260; PMCID: PMC3147828.