Supplemental Methods:

Specimens: Retrospective CMV and EBV positive serum samples, *N.meningitidis* positive EDTA Blood samples and influenza A positive nasopharyngeal washes (NPW), were maintained at -80^{o}C following initial testing for the respective viruses at the Microbiology and Virology Laboratory of BC Children's and Women's Hospitals (Vancouver, BC, Canada). For spiking experiments, EDTA blood, serum, cerebrospinal fluid (CSF) and NPW samples were used. Only residual samples were used. To maintain patient anonymity, each sample was coded and all patient identifiers removed.

Spiking: To spike samples with bacteria, a *N.meningitidis* B strain (ATCC 13090) was grown in chocolate agar plate (Oxoid) overnight at 37^{o}C in a 5% CO_2 atmosphere, and a bacterial suspension was prepared in phosphate buffered saline (PBS) to a turbidity equivalent to a 0.5 McFarland standard. The suspension was further diluted 1:100 in PBS and 40 µl of diluted preparation was added to 960 µl of EDTA blood samples. To spike samples with a DNA-virus, 60 µl of a CMV patient isolate (10^4 PFU/ml) was added to 940 µl of serum and EDTA blood samples. To spike samples with RNA-virus, a human enterovirus 70 strain (ATCC VR-836 at $8.89X10^6$ $TCID_{50}$/ml) was diluted to 1:100 in TE8 buffer (10 mM Tris; 1 mM EDTA; pH:8.0) and 10 µl of diluted preparation was added to 990 µl of CSF samples. A patient isolate of influenza A virus (C_T=24) was diluted to 1:10 in TE8 buffer and 20 µl of diluted preparation was added to 980 µl of NPW samples.

Nucleic acid extraction and sample storage: Viral nucleic acids and bacterial DNA were extracted from 200 µl of undiluted serum and NPW samples. The purified eluates were divided into 50 µl aliquots and stored at RT, 4^{o}C and -80^{o}C for 0-30 days. Spiked samples were divided into 300 µl aliquots and stored at RT, 4^{o}C and -80^{o}C for 0-16 days. 50 µl of serum, CSF and NPW samples were diluted to 1:6 in TE8 buffer and nucleic acids were extracted from 200 µl of diluted samples. For extraction of bacterial DNA from spiked EDTA blood samples, 50 µl samples were diluted to 1:6 in TE8 buffer followed by manual extraction of 200 µl of samples using QIAamp DNA Blood Mini Kit according to manufacturer's instructions (Qiagen).

Taqman assays: Laboratory standard methods used for routine diagnostic purposes were employed for the detection of each pathogen. For EBV, CMV and *Neisseria meningitidis* detection, 5µl of sample extract was mixed with 20µl of a master mix containing 12.5µl of Taqman Universal PCR Master Mix (Applied Biosystems), and primers and probes to final concentrations shown in Supplemental Table 1. Thermal cycling was performed with 1 cycle of 95°C-10min, followed by 45 cycles consisting of 95°C-15s and 60°C-60s. For influenza A detection, 5 µl of sample extract was mixed with 20µl of a master mix containing 12.5 µl of 2X Master Mix without UNG, 0.625 µl of 40X MultiScribe and RNase Inhibitor Mix (Applied Biosystems) and primers and probes to final concentrations shown in Supplemental Table 1. Thermal cycling was performed with 1 cycle of 48^{o}C-30 min, 1 cycle of 95°C-10min, followed by 40 cycles consisting of 95°C-15s and 60°C-60s. *N.meningitidis* assay was performed in SmartCycler (Cepheid) and all other assays were performed in a ABI7500 Fast instrument (Applied Biosystems). General PCR precautions and procedures were followed; separate areas and bio-safety cabinets were maintained and dedicated equipment and consumables were used for sample preparation, Master mix preparation, PCR setup and PCR amplification.

Half life calculation: C_T values (y-axes) for each pathogen were plotted against the number of days (x-axes) the samples were stored at different temperature by using scatter chart in Microsoft Excel. A trend line was drawn and linear equation (y=mx+c; m=slope of the curve, c=intercept) was derived. Half life (x) was calculated assuming, y=(C_T value at day '0' + 1), because an increase of 1 C_T indicates 0.5 fold or 50% decrease of target.