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P200

Improving pre-analytic collection systems: inactivation and preservation of influenza for rapid testing

K. Luinstra[1]*, S. Castriciano[2], M. Ackerman[3], A. Petrich[1], S. Chong[1], J. Mahony[1], M. Smieja[4]. [1]*St. Joseph's Healthcare, Hamilton, Canada,* [2]*Copan, Brescia, Italy,* [3]*St.Joseph's Healthcare, Hamilton, Canada,* [4]*McMaster University, Hamilton, Canada*

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 a 30 minute exposure to each collection medium was assessed by duplicate inoculation of the mocked samples into R-mix shell vial culture followed by immunofluorescent staining at 48 hours. The stability and recovery of influenza A nucleic acid (NA) was also assessed after 1, 7, 14 and 21 days at 4°C, −20°C, room temperature (RT) and 37°C. 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, after a 30 minute exposure to the CyMol collection medium influenza A virus was inactivated and did not grow in shell vial culture. Influenza A RNA levels were stable for up to 21 days in both the UTM and CyMol collection systems at −20°C, 4°C and RT. The stability of Influenza A RNA declined in both systems at 37°C.

Conclusions: The Copan CyMol medium inactivates influenza A virus while stabilizing RNA for molecular testing for up to 21 days at −20°C, 4°C and RT. CyMol is a potential alternative for safe sample collection during an influenza pandemic situation.

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Practical considerations for implementing a quantitative EBV PCR assay: a critical comparison of two commercial LightCycler EBV assays

D. Johnson[1], C. Rutherford[1], S. Dale[1]*. [1]*Hamilton Regional Laboratory Medicine Program, Hamilton, Canada*

Objectives: Significant challenges face laboratories during the validation and implementation of quantitative PCR assays for transplant-associated viruses including CMV, EBV and BK. The objective of this study was to compare the artus LightCycler EBV PCR with the Roche LightCycler EBV PCR with respect to analytical and clinical performance in a population of primarily solid organ transplant recipients.

Methods: Whole virus EBV standards (AcroMetrix) were diluted in normal human plasma and extracted using a bioMérieux EasyMag platform to assess analytical performance characteristics of the artus PCR and the Roche PCR. The Roche PCR was validated using plasma and whole blood specimens from solid organ transplant recipients.

Results: The limit of detection for the artus PCR and the Roche PCR were calculated at ~1000 copies/ml by PROBIT analysis and each assay was linear from 2×10^4 to greater than 10^7 copies/ml. The precision of the artus PCR was substantially poorer than the Roche PCR at the lower limits of the linear range and the artus PCR portrayed abnormal melt curve profiles with various concentrations of input DNA. In the clinical evaluation, 32 plasma and whole blood specimens were collected from 27 unique patients and tested in the Roche PCR. The overall correlation for plasma compared to whole blood was 72%. The remaining 9 specimens were positive for EBV in whole blood only. These specimens were submitted to a reference laboratory for EBV PCR and 3 were reported as negative, 2 were reported as <600 copies/ml and 4 were quantified between 1901 and 33,275 copies/ml. None of the patients in our study were diagnosed with PTLD. Further, in terms of the specificity of the Roche PCR, 1 of the specimens quantified from both whole blood and plasma exhibited a shift in the melting temperature of the probe from 62°C to 55°C. Given the variability in the melt curve of the artus PCR, this polymorphism would not be detected.

Conclusions: In this study, we show that the Roche EBV PCR has similar analytical characteristics compared to the artus PCR, however, the Roche PCR exhibits a superior melt curve. Moreover, the Roche EBV PCR has a high negative predictive value to exclude the diagnosis of PTLD using either plasma or whole blood in our patient population.

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Implementation of a pan-viral microarray for detection of viruses in clinical samples

V. Montoya[1]*, D. Eisler[2], A. McNabb[2], J. Isaac-Renton[1], P. Tang[1]. [1]*University of British Columbia, Vancouver, Canada,* [2]*British Columbia Centre for Disease Control, Vancouver, Canada*

Background: Most clinical virology laboratories are only capable of detecting a limited range of viral pathogens. Multiple molecular assays are often required for each sample. We propose the use of a pan-viral microarray, the Virochip, for detection of a broad range of viruses from clinical specimens. The Virochip, currently in its fourth generation, contains approximately 16,000 oligonucleotides representing all known viruses and has the capability of detecting novel viruses.

Methods: Samples positive for viruses by PCR or Luminex were selected from respiratory and stool samples submitted to the BCCDC for virus detection. Total nucleic acids were extracted from the samples and amplified by random RT-PCR. Samples were then labeled with Cy3 and hybridized to the Virochip overnight. Microarray data were analyzed for the presence of viruses using E-predict, hierarchical clustering and Z-score analysis. The Virochip results were compared to the PCR and Luminex results.

Results: The Virochip was able to detect a wide range of common viruses including: influenza A, influenza B, human metapneumovirus, enterovirus, rhinovirus, adenovirus and norovirus. In addition, the Virochip was able to subtype Influenza A, enterovirus and adenovirus based on the subtype-specific oligonucleotides on the array. The limit of detection of the Virochip was approximately 10^3 virus genomes corresponding to a qPCR cycle threshold of approximately 23 to 28.

Discussion: The Virochip is able to detect a broad range of viruses in clinical samples with high accuracy. Although the analytical sensitivity of the Virochip is lower than that of specific PCR, the majority of respiratory and gastrointestinal samples from patients with viral illnesses contain viral titres above the limit of detection of the Virochip. Overall, the Virochip is an efficient tool for viral diagnosis, reducing the number of assays required for detecting a wide spectrum of viral pathogens as well as providing simultaneous subtyping information for certain viruses such as influenza A.

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Earlier identification of human immunodeficiency virus and hepatitis C virus in high-risk emergency departments

J. Fenton[1]*, B. Lee[1], L. Mashinter[2], S. Plitt[3], S. Houston[4], B. Rowe[4], J. Preiksaitis[1]. [1]*Provincial Public Health Laboratory, Edmonton, Canada,* [2]*Alberta Health Services, Edmonton, Canada,* [3]*Public Health Agency of Canada, Edmonton, Canada,* [4]*University of Alberta, Edmonton, Canada*

Objectives: Identification of acute HIV and HCV infection is important in high risk emergency departments (ED) patients. We examined the utility of pooled nucleic acid test (pNAT) for early seroconverter identification [RNA positive (pos) but antibody (Ab) negative (neg)] in the ED of three hospitals in Edmonton.

Materials and Methods: Between Jan and Mar 2006, 3484 consecutive patients aged 15–54 years who had a complete blood count in the ED were included in the study. Patients known to be HIV or HCV Ab

pos (by cross-referencing to provincial databases) were removed from pooled Ab test and pNAT but were included in the prevalence analysis. Samples were anonymized and pooled Ab test was performed with MONOLISA® anti-HCV PLUS and GS HIV-1/HIV-2 PLUS O assay (Bio-Rad Laboratories). Anti-HIV neg samples pooled in groups of 75 were tested for HIV RNA with the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test. Anti-HCV neg samples were pooled in groups of 25 and were tested with the Roche COBAS AmpliPrep/COBAS AMPLICOR™ HCV Test. NAT pos pools were broken down to identify pos individuals.

Results: One HIV and 2 HCV cases were detected by pNAT only representing 25.0% and 3.0% of all newly identified cases respectively. All 3 cases were from the inner-city hospital (Site 2), representing 50.0% and 5.9% of newly identified HIV and HCV cases, respectively at this site.

	Site 1 (%)	Site 2* (%)	Site 3 (%)	Total (%)
HIV seroprevalence (Ab only)	7/1181 (0.6)	27/1159 (2.3)	19/1144 (1.7)	53/3484 (1.5)
New HIV by Ab/All HIV pos†	2/7 (28.6)	1/28 (3.6)	0/19 (0.0)	3/54 (5.6)
New HIV by NAT/All New HIV†	0/2 (0.0)	1/2 (50%)	0/0 (0.0)	1/4 (25.0)
HCV seroprevalence (Ab only)	54/1181 (4.6)	158/1159 (13.6)	88/1143 (7.7)	300/3483 (8.6)
New HCV by Ab/All HCV pos†	18/54 (33.3)	32/160 (20.0)	15/88 (17.0)	65/302 (21.5)
New HCV by NAT/All New HCV†	0/18 (0.0)	2/34 (5.9)	0/15 (0.0)	2/67 (3.0)

*Inner-city hospital. † Included cases detected by Ab and NAT.

Conclusions: pNAT testing identified acute seroconverters that were not detected by pooled antibody testing. pNAT of Ab neg samples was feasible and proved to be an effective approach for identifying early acute HIV and HCV infection using plasma samples in a large high-risk population.

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Self-collected nasal mid-turbinate flocked swabs for molecular respiratory virus diagnosis in symptomatic volunteers

S. Castriciano[1]*, S. Carruthers[1], M. Ackerman[1], S. Chong[1], K. Luinstra[1], C. Robinson[1], G. So[1], A. Petrich[1], J. Mahony[1], M. Smieja[1]. *[1]Department of Pathology and Molecular Medicine, Hamilton, Canada*

Background: We previously tested the new Copan mid-turbinate nasal flocked swab in asymptomatic volunteers, and demonstrated superiority to both rayon nasopharyngeal (NPS) or nasal swabs, and equivalence to flocked NPS, for sampling respiratory tract epithelial cells. The objective of this study was to validate nasal self-collection for detecting respiratory viruses in symptomatic volunteers, and to determine whether two nasal swabs improved viral yield over a single swab.

Methods: Seventy-four symptomatic volunteers followed written and illustrated instructions to self-swab one or two nasal mid-turbinate swabs within 72 hours of any future acute respiratory tract infection. Swabs were placed in 1.0 mL of UTM, and returned to the laboratory. 500 ul of each specimen was used for nucleic acid extraction with the EasyMag extractor. DNA from 33 extracted specimens was quantitated using a beta-actin real time PCR on the Lightcycler. All specimens were tested for respiratory viruses using the Luminex xTAG Respiratory Virus Panel, a multiplex PCR which detects 17 respiratory viruses.

Results: Beta-actin quantitation average from 33 specimens was 5.7 ± 0.6 log genomic equivalents or cells) ml. In 22 of 74 (29.7%) symptomatic volunteers, a virus was detected in their self-collected nasal swab, including 16 entero/rhinovirus, 1 influenza B, 1 parainfluenza-2, 2 coronavirus NL63, and 2 human metapneumovirus. For volunteers who submitted two swabs, virus infections were detected with both swabs in 13 out of 14 cases ($P=1.0$, McNemar test).

Conclusions: The Copan flocked nasal mid-turbinate swab enabled self-collection and molecular detection of virus in one-third of the subjects, and a single specimen was adequate for diagnosis. Self-collection has many advantages over NPS including feasibility of collecting serial specimens, eliminating biohazard for clinical staff, and facilitating outbreak investigation.

P205

Development and inter-laboratory analysis of a quantitative influenza A RT-PCR

K. Luinstra[1]*, S. Chong[1], J. Mahony[1], M. Smieja[2], A. Petrich[1]. *[1]St. Joseph's Healthcare, Hamilton, Canada, [2]McMaster University, Hamilton, Canada*

Objectives: Standardization of a quantitative assay for detection of Influenza A virus is necessary for determining whether concentration of the virus in clinical samples can be an important marker for prognosis and drug response/resistance. The objectives of this study were: to develop, standardize, and optimize the quantitation of influenza A virus by quantitative RT-PCR and to analyze inter-laboratory variability using this assay on a panel of H1 and H3 purified RNA samples.

Methods: A quantitative Influenza A assay was developed based on the CDC matrix RT-PCR using the Roche LightCycler Platform. An inter-laboratory analysis between 5 laboratories was performed to assess variability. A panel of 18 specimens of extracted H1N1and H3N2 replicates in serial 2 log dilutions plus 5 standards was generated. All samples were coded and the order randomized. All reagents necessary for testing including standards and the protocol were sent with the panels to participating laboratories. Participants performed the assay according to a standardized protocol and reported results for the panel specimens quantitatively.

Results: No significant differences in genome copy number of H1N1 or H3N2 RNA was seen between the five laboratories across the dynamic range of the assay. All laboratories were able to discriminate 2–3 log differences in viral copy number between samples. A prognostic marker based on a drop in viral load of 3 to 6 logs in 48 hours would be achievable with this assay.

Conclusions: Quantitation of influenza A RNA from mock clinical samples by a standardized RT-PCR assay was reproducible across 5 centres. Real clinical specimens collected serially from patients will be required to determine the clinical utility of the assay.

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Results from the testing of a national influenza A RNA sensitivity panel

K. Luinstra[1], S. Chong[1], J. Mahony[1], M. Smieja[2], A. Petrich[1]*. *[1]St. Joseph's Healthcare, Hamilton, Canada, [2]McMaster University, Hamilton, Canada*

Objective: Proficiency testing is an important component of quality management in clinical microbiology laboratories. Most proficiency panels do not rigorously evaluate the sensitivity of assays to allow assay improvement. In this study a proficiency panel was set up to challenge the sensitivity of assays beyond the lowest level of detection of influenza A RNA in a national survey. Differences due to extraction were eliminated as the panels were composed of purified RNA.

Methods: A central laboratory generated panels containing 36 specimens of extracted RNA from mocked respiratory specimens. The panel included H3N2 replicates of 4 serial 10-fold dilutions, H5N2 replicates of 4 serial 10-fold dilutions, Influenza B, and specificity controls. Specimens were coded and the order randomized. Panels were sent to five centres for amplification and the laboratories were instructed to report the results qualitatively and to provide data describing their RT-PCR assay(s).

Results: Ten laboratories participated with 16 different assays. Five laboratories performed the CDC recommended real time PCR assay on either the ABI or LightCycler. Three commercial assays, one in-house NASBA and 3 in-house RT-PCRs were also evaluated. Probit (estimate of 50% detection) varied by 2–3 logs for detection of H3N2 and H5N2-influenza A RNA. Inter-assay variability was significant and due to differences in input volumes, primers, and detection methods. Specificity of the assays was good with only 1 false positive detected out of a possible 96 specimens.

Conclusions: Influenza A assays currently being used across Canada demonstrated significant differences in sensitivity with the mocked specimens tested in this study. Real clinical specimens will need to