



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

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.

P204

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

S. Castriciano^{1*}, S. Carruthers¹, M. Ackerman¹, S. Chong¹, K. Luinstra¹, C. Robinson¹, G. So¹, A. Petrich¹, J. Mahony¹, M. Smieja¹. ¹Department 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 µl 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. Chong¹, J. Mahony¹, M. Smieja², A. Petrich¹. ¹St. Joseph's Healthcare, Hamilton, Canada, ²McMaster 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 H1N1 and 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.

P206

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

K. Luinstra^{1*}, S. Chong¹, J. Mahony¹, M. Smieja², A. Petrich^{1*}. ¹St. Joseph's Healthcare, Hamilton, Canada, ²McMaster 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