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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

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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

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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.

P206

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

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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

be assessed to demonstrate whether these sensitivity differences may have clinical implications.

P207

Detection of Noroviruses from environmental surfaces

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Objective: Noroviruses are the most common cause of non-bacterial gastroenteritis world wide. Surface and environmental contamination with norovirus is an important source for outbreaks of norovirus infection. The goal of this study was to develop and evaluate a method for detection of noroviruses from swabs collected from environmental surfaces.

Methods: A fecal suspension containing genogroup II norovirus was allowed to dry onto a tile surface. Virus was recovered by swabbing with a moist swab, and viral RNA extracted and detected using real time reverse transcription-PCR. The efficiency of recovery was compared using different swab types and diluents. The effect of dilution, drying, duration left at room conditions, and disinfection on the recovery of norovirus from dried fecal samples was investigated.

Results: Swab type (cotton vs flocked swab) did not affect recovery of norovirus. Swabs moistened with water yielded higher recovery than swabs moistened with either PBS or virus transport medium. Norovirus could be detected in repeated samples swabbed from the same spot, even after no visible fecal material remained. Norovirus was detectable for at least 14 days after application to tile surfaces, without a significant increase in Ct. When diluted samples were dried on to the tile surface, recovery was reduced by at least 2 log dilutions. Treatment of dried virus spots with laboratory disinfectants or with household cleaner for up to 30 minutes did not reduce detection by PCR, but no virus was detected after treatment with bleach.

Conclusion: An existing method for detection of norovirus in fecal specimens was applied to the detection of norovirus on swabs collected from tiled surfaces. Norovirus was detectable for at least 14 days after drying onto tiled surfaces. Limitations of this study include both the lack of quantitation and the inability to confirm whether norovirus was viable or not. This method can be applied in future studies to investigate environmental norovirus contamination associated with outbreaks.

Diagnostic laboratory methods and studies – Molecular: Molecular mycology

P208

Molecular genetic analysis supports recognition of new species among *Emmonsia* and *Blastomyces* isolates

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Objectives: Recent molecular studies have confirmed that Emmonsia species are closest relatives of Blastomyces dermatitidis, Histoplasma capsulatum and Paracoccidiodes brasiliensis. The typical disease presentation caused by Emmonsia crescens and E. parva is adiaspiromycosis, in which inhaled conidia enlarge to form thick-walled spherule-like cells called adiaspores. The adiaspores neither bud nor produce internal spores, thus differentiating them from the budding yeast cells of Blastomyces and Histoplasma and from the endosporulating spherules of Cocciodioides. E. pasteuriana, causing disseminated infection in an immunocompromised patient, differs in producing a yeast stage. Our objectives were to examine genetic diversity among Emmonsia isolates, some of which produce yeast stages, and to assess relationships of isolates identified as atypical Blastomyces dermatitidis based on absence of conidia and characteristic yeast cells.

Methods: DNA was extracted from killed cultures. The ID fragment (ITS1, 5.8S rDNA, ITS2 and partial LSU r-DNA) was amplified via PCR using primers ITS5 and D2R. DNA fragments were aligned using CLUSTALW and checked visually. Phylogenetic analysis was performed using PAUP. Isolates were examined for morphological features and expression of a yeast stage.

Results: Emmonsia species and B. dermatitidis isolates grouped in a strongly supported clade. Isolates were placed in nine genotype groups, of which two corresponded to E. crescens and B. dermatitidis. The atypical B. dermatitidis isolates grouped separately from typical isolates of B. dermatitidis from North America and Africa. Isolates classified as E. parva were placed in three subgroups and were morphologically distinct. E. pasteuriana was sister to two subgroups of isolates also producing yeast stages.

Conclusion: The close relationship of Emmonsia and B. dermatitidis species is strongly supported. Non-budding E. parva isolates are closer to B. dermatitidis than they are to E. crescens. Yeast stages occur on several branches of the phylogenetic tree and are not significant above the species level. Molecular and morphological data support recognition of several additional species, four of which are pathogens. These results will be useful in developing diagnostic methods for the accurate identification of these pathogens.

Diagnostic laboratory methods and studies – Molecular: Molecular parasitology

P209

Genotyping and phylogentic analysis of a hypervirulent outbreak strain of *Entamoeba histolytica*

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Objective: Only 10% of those infected with *Entamoeba histolytica* develop symptomatic disease. The factors determining whether a person infected with *E. histolytica* develops disease are poorly understood. Molecular genotyping by targeting polymorphic genetic loci may aid in the close examination of the population structure of *E. histolytica* clinical isolates.

Method: In the present study, we analyzed six tRNA-linked polymorphic short tandem repeats loci among stool isolates received at TPHL from seven patients who were either asymptomatic, had diarrhea/dysentery, or had developed a liver abscess. The study included a case of a hypervirulent outbreak strain in a traveler returning from Italy. The genetic relatedness among the isolates was analyzed by performing the pars parsimony analysis using the Phylip 3.68 software.

Results: The genotyping analysis revealed six different *E. histolytica* genotypes. The most remarkable and extensive variations among the six t RNA STR loci was found in the outbreak strain which means that it was genetically distinct from *E. histolytica* sensu stricto strains obtained from patients with either diarrhea or who were asymptomatic. Two isolates from asymptomatic individuals residing at the same household were epidemiologically linked. Interestingly, the outbreak strain which was responsible for highly virulent disease (ALA, elevated serology) was most genetically similar to HM1: IMSS, a reference strain originally isolated from a patient with invasive colitis.

Conclusion: The results showed that the hypervirulent outbreak strain was a genetically distinct outlier. This genotyping schema may be useful to distinguish virulent strains from less virulent strains of *E. histolytica* sensu stricto.

P210

Validating quantitative real-time PCR (RTPCR) for the detection of Plasmodia spp. in returning travelers

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Objective: Malaria is a mosquito-borne disease caused by the parasite of genus Plasmodia spp. There are at least 4 species that infect humans of which *P. falciparum* can be fatal if not treated immediately. It is endemic in countries in Africa, South America, India and South Asia. In