

Figure 1. Molecular Phylogenetic analysis of HN gene by Maximum Likelihood method
The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 15 nucleotide sequences. There were a total of 1718 positions in the final dataset. Evolutionary analyses were conducted in MEGA7. BMT – bone marrow transplant patient. H – haematology ward patient. P – Pediatric general ward patient.

Disclosures. All authors: No reported disclosures.

1174. Molecular Investigation of an Ontario Mumps Outbreak using Whole Genome Sequencing

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Background. In early 2017 an outbreak of Mumps virus affected over 100 individuals in the province of Ontario, concurrent with multiple mumps virus outbreaks across North America. Traditional genotyping of mumps outbreaks relies on sequencing a portion of the small hydrophobic (SH) gene, but has limited capability to distinguish between strains of the same genotype. Most mumps cases in Ontario in recent years are of genotype G. We used a novel whole genome sequencing (WGS) protocol to perform a molecular epidemiological investigation of the outbreak.

Methods. Throat ($n = 5$) and buccal ($n = 15$) swabs positive by RT-PCR for SH or Fusion (F) gene targets were cultured in primary Rhesus monkey kidney cells. Cell free viral extract underwent RT-PCR and subsequent PCR amplification using overlapping primer pairs to cover the entire 15 kilobase (kb) genome. The first 8 samples were amplified with 18 pairs of overlapping primers, which was reduced to 9 sets (average fragment size 1.9 kb, range 1.6–2.8 kb) for the final 12 samples. Mumps cDNA libraries were prepared with Nextera XT kit and WGS of the indexed fragments was performed with V2 reagent kits on the Illumina MiSeq instrument. Reference based genome assembly was performed using samtools version 1.4. Phylogenetic analysis was performed by maximum likelihood method in MEGA7.

Results. We identified two distinct genotype G lineages comprised of 9 patients each and closely related to a 2009–2010 outbreak in Ontario and New York (Figure 1). Inter-lineage single nucleotide polymorphism (SNP) differences ranged from 25 to 31, whereas intra-lineage SNPs ranged from 0 to 8 SNPs. Two outlying sequences, of genotype C and G respectively, may represent sporadic introduction of virus from other areas. Time from virus isolation to SNP based analysis was approximately 4 days.

Conclusion. WGS of Mumps virus culture isolates using the PCR fragment method identified two distinct genotype G lineages in a large provincial outbreak. This method may aid public health authorities identify separate transmission chains in the case of large outbreaks.

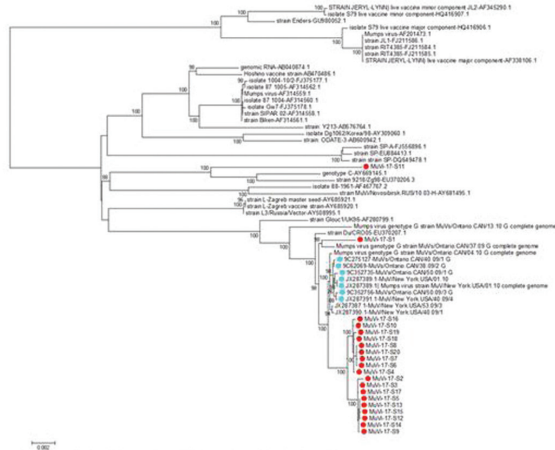


Figure 1. Molecular Phylogenetic Analysis by Maximum Likelihood Method
Red markers indicate 2017 outbreak strains and blue markers represent 2009–2010 outbreak strains. The evolutionary history for mumps whole genome sequences was inferred using the Maximum Likelihood method based on the Tamura-Nei model with gamma distributed rates. Bootstrap values greater than 75% based on 50 pseudoreplicates are displayed. The tree is rooted on the midpoint for clarity. Evolutionary analyses were conducted in MEGA7.

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1175. Optimization of a Polymerase Chain Reaction System for Whole Genome Amplification of Human Immunodeficiency Virus Type 2 (HIV-2)

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Background. In this study, attempts were made to amplify the whole genome of HIV-2 using polymerase chain reaction (PCR) from viral RNA and proviral DNA extracted from archived human plasma and whole blood, respectively. The aim this study is to develop a PCR system that can be used to amplify the entire genome of all known subtypes and recombinant forms of HIV-2

Methods. Proviral DNA and viral RNA were extracted from archived human whole blood and plasma using Zymo Research Viral DNA kit and Zymo Research Viral nucleic acids kit, respectively. Primers that target the conserved sites of the 3' and 5' long terminal repeat were selected based on *in silico* analysis using Snapgene® tool version 2.1.0. Eight overlapping Primers for PCR whole genome amplification of HIV-2 were also selected and used for overlap PCR amplification of whole genome of HIV-2. Long range and overlapping PCR of whole genome of HIV-2 were carried out using long range Kit (New England Biolabs Inc., Ipswich, MA, USA) and cDNA synthesized using NEB ProtoScript II for proviral DNA and viral RNA templates. Amplified whole genome of HIV-2 was gel purified and PCR confirmed using two sets of primers ENVF/ENVG and EB2/EB5 and gel electrophoresis.

Results. Proviral DNA and viral RNA were successfully extracted from archived whole blood and plasma. Six primers were selected out of the 68 primer sequences retrieved using *in silico* analysis for long range single PCR amplification of HIV-2 whole genome. Primers P1/P8 and HIV2upA/HIV2lowA were successfully used in the whole genome amplification of HIV-2. Overlapping primers P1/P4, P6/P8, PolF/EnvG and Pol4F/EnvG covering the entire genome of HIV-2 were also successfully used in the whole genome amplification of HIV-2. The amplified whole genome fragment was confirmed to be HIV-2 by PCR using primers EB2/EB5 and EnvF/EnvG.

Conclusion. The techniques of long-range and overlapping amplification of HIV-2 whole genome may be useful in HIV-2 genotyping using viral RNA and proviral DNA. This study has led to the selection and shown novel primer combinations of already existing primers which can be used to amplify the entire genome of HIV-2.

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1176. Comparison of Roche TaqMan v.2.0, Abbott RealTime and Modified Abbott Residual Viremia Assays in Testing of Routine Samples for Viremia Monitoring in HIV Care

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Background. Routine HIV RNA clinical monitoring frequently reports viremia (VL) as detectable, quantifiable <50 or blips >50 c/mL. To assess whether the results represent true increases or just assay variability, we designed comparisons of the Roche COBAS AmpliPrep / COBAS TaqMan HIV-1 Test version 2.0 (TM) and the Abbott RealTime HIV-1 assay (RT) to a residual viremia (RV) assay with a limit of detection of 3 c/mL, the Modified Abbott Residual Viremia assay (MARV).

Methods. HIV suppressed patients on stable therapy presenting for routine visits at the HIV clinic in Henry Ford Hospital (HFH) were prospectively enrolled. Blood samples and questionnaires were obtained at each visit. Paired plasma samples were processed and analyzed per manufacturer instructions; HFH clinical laboratory processed the samples using the TM assay and the Mckinnon Research Laboratory performed the FDA approved RT assay and RV testing using the MARV assay. Parametric and non-parametric analyses were conducted as indicated.

Results. 124 HIV patients are reported with a mean age of 51, mostly male (84%) and African American (64%). Mean CD4 cell counts were 661 cells/mm³. TM assay results for 196 plasma samples were not detectable (ND) 119 (61%), detectable (DT) 61 (31%) and quantifiable viremia (QV) >20 c/mL 16 (8%). 5 patients had QV over 50 c/mL. 60 tested patients (49.5%) had at least one sample either DT or QV. TM results were not correlated to CD4 cell counts, antiretrovirals, medical conditions or reported adherence (avg. 97%). 174 RT and 187 MARV paired tests were ND on 142 (81%) / 121 (65%), DT on 34 (17%) RT, and quantifiable in 0% / 34 (33%) of the samples respectively. Higher VL detection by the TM was shown compared with the RT ($P = 0.005$) and MARV ($P < 0.001$) assays. Mean QV by the MARV was 5.0 vs. 55 c/mL on the TM assay ($P < 0.001$). VL for all assays trends correlated when compared for ND, DT and >20 copies/mL ($P < 0.001$). Bland-Altman plots show higher VL detected by TM as compared with MARV ($P < 0.001$) and good correlation between RT and MARV assays ($P = 0.6$).

Conclusion. HIV viremia is more frequently reported by TM assay as compared with the RT assay and was significantly higher than VL detected by the MARV assay. Over detection of VL by the TM assay may impact clinical decision making and increase cost of care.

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