# Hepatitis C Q80K prevalence in BC, Canada determined by a public domain assay

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## Background

HCV genotype 1a (GT1a) infections harbouring a baseline Q80K polymorphism in the NS3 gene have reduced virologic response to IFN-based HCV treatments containing simeprevir. We aimed to develop, validate, and freely disseminate a NS3 clinical sequencing assay to detect Q80K and other NS3 mutations, and establish their prevalence in British Columbia (BC), Canada.

## Methods

RNA was extracted using a NucliSens easyMag, amplified by nested RT-PCR and sequenced by Sanger or Illumina sequencing. Sanger chromatogram interpretations were performed automatically using in-house analysis software (ReCall). HCV genotypes were identified using INNO-LiPA HCV 2.0 (5'UTR); sequence-based genotype assignment was verified phylogenetically in comparison with reference sequences. Our assay was validated via comparison with 70 sequences from an external laboratory and relative to consensus sequences from next-generation sequencing (MiSeq). To establish the prevalence of Q80K in BC, samples from 376 GT1 LiPA HCV+ individuals diagnosed in 2011 were examined. Sequences were also screened for mutations associated with boceprevir/telaprevir resistance.

## Results

Comparison of sequences generated by an external lab and with MiSeq consensus sequences revealed > 98% sequence concordance, and no discordant calls of Q80K. LiPA identified 231 (61%) individuals as GT1a, 91 (24%) as GT1b, while subtype could not be resolved in 54 cases (14%). The prevalence of Q80K was 61%, 11%, and 50% in LiPA GT1a, 1b and unresolved, respectively. Sequence analysis suggested that 52/54 individuals (96%) whose LiPA genotype was unresolved were actually GT1a, as were 28/91 (44%) cases that LiPA identified as 1b. Using a sequence-based assessment of HCV genotype, the prevalence of Q80K was 176/309 (57%) in GT1a compared to 1/66 (2%) in GT1b **(Figure 1)**. Mutations associated with boceprevir or telaprevir resistance in this newly diagnosed population were also observed (V36M, N=2; T54S, N=5; and R155K, N=3).

## Conclusions

Our results suggest the overall prevalence of Q80K in GT1 in BC is 47%. Q80K was highly prevalent where LiPA results could not resolve GT1a vs 1b. Inconsistent HCV genotype 1a vs 1b assignment by LiPA UTR in comparison to sequence-based genotyping suggests that all individuals with GT1 HCV infection in BC considering simeprevir with pegylated interferon/ribavirin should be screened for the Q80K polymorphism. Assay details and software are freely available to academic labs.

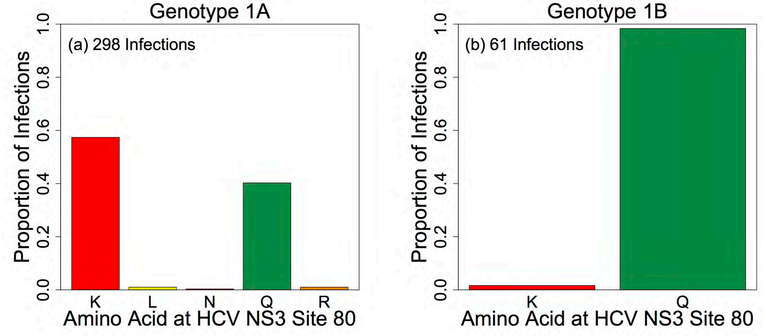



**Figure 1 | Proportion of HCV genotype 1A and 1B infections from British Columbia, Canada which may display reduced susceptibility to treatments containing simeprevir. Overall prevalence of Q80K in BC HCV genotype 1 infections was 47%.**

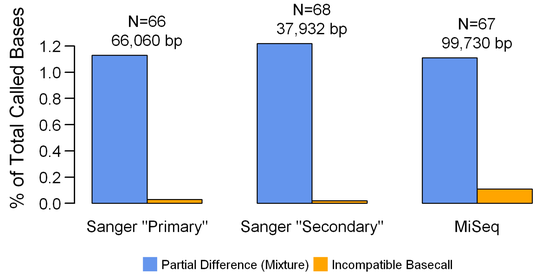



**Figure 2 | Summary of nucleotide sequence discordances between HCV NS3 sequencing assays developed by BCCfE and results obtained by Janssen Diagnostics**.

Three versions of the BCCfE assay were investigated: A "primary" Sanger sequencing method yielding 1200-bp sequence; A "secondary" Sanger method yielding 564-bp (backup where primary assay fails); A next-generation sequencing assay in which nearly-whole-genome HCV amplicons are generated and sequenced on a MiSeq after Nextera XT library preparation. For the MiSeq assay consensus sequences were generated, where all nucleotides observed at >20% frequency at each position were called as mixtures. Sequences obtained by BCCfE methods were compared to Sanger sequences obtained by Janssen. All assays achieved ≥98.8% concordance in nucleotide basecalls when compared to Sanger sequences obtained by Janssen Diagnostics **(Figure 2)**. Nearly all discordant basecalls (>97.5% Sanger, 91.7% MiSeq) were due to differences in mixture calling between laboratories. All instances of the Q80K (N=30) polymorphism detected by the Janssen method were also detected by all three BCCfE methods. No false positive Q80K calls were reported by any BCCfE method.

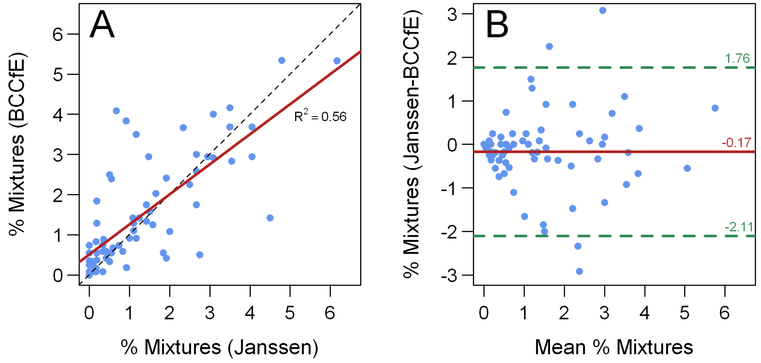



**Figure 3 | Analysis of the number of mixed bases per sequence suggests no systematic bias in amplification of minority species.**

A portion of HCV NS3 was successfully amplified by at least one of the "primary" or "secondary" Sanger sequencing methods in all 70 samples previously sequenced by Janssen BVBA. To demonstrate that neither method systematically missed minority HCV variants, the percentage of ambiguous nucleotides ("mixed" bases) in the longest fragment sequenced by both laboratories (543, 564 or 1200-bp) was calculated and compared **(Figure 3)**. A modest, but not perfect correlation (**panel A**), consistent with variation in RNA extraction, RT-PCR amplification and basecalling, was observed between sequences collected in the two laboratories. However, inspection of the Bland-Altman plot (**panel B**) revealed no systematic over- or under-calling of mixtures by either laboratory.

## Acknowledgements

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