HIV-1 and HCV Sequence-based Genotyping Methods Validated Against TRUGENE Commercial Kits.

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ABSTRACT

The HCV and HIV-1 genotype determination have clinical importance because the response to antiviral treatment varies with the genotype. Furthermore, the classification of HIV-1 subtype and HCV genotypes provides a scientific opportunity to study the worldwide spread of these viruses. Sequence-based genotyping is the method that is most widely used to genotype. Some laboratories use in-house sequencing, but a number of commercial assays are also extensively used.

Two genotyping assays were validated: one for HIV-1 and another for HCV genotyping in human plasma samples. For both assays, the viral RNA was extracted using the Abbott molecular m2000 sample preparation instrument. The HIV-1 genotyping assay included two regions of the HIV-1 virus RNA: *env* gp41 immunodominant region (IDR) and *pol* integrase (*pol* IN). The sequences were amplified by RT-PCR using specific primers for each one. The *env* gp41 IDR was amplified using the forward primer, JH35F, and the reverse primer, JH38R. The sequences from the *pol* IN region were obtained using primers poli5_OF and poli8_OR, followed by a nested PCR using primers poli7_IF and poli6_IR. Samples with low viral loads (< 500 copies/ml) were analyzed using an alternative protocol designed to amplify the *env* gp41 IDR. Reverse transcription and primary PCR were performed using primers GP40F1 and GP41R1. Primers GP46F2 and GP47R2 were then used for the nested PCR. The HCV genotyping assay was carried out using sequences obtained from the NS5b region. The sequences were amplified by RT-PCR using the specific primers Pr1 and Pr2. Alternatively a hemi-nested PCR was carried out using primers Pr3, Pr4 and Pr5. The obtained sequences were blasted against available HIV and HCV sequence databases.

Results were validated against previously obtained results from the TRUGENE HIV-1 genotyping kit and TRUGENE HCV 5'NC genotyping kit. All 20 samples (100%) for the HIV-1 subtypes showed concordant results in both methods. However, at sub-subtype level, one sample was not coincident showing an F sub-subtype with TRUGENE method and an F2 sub-subtype in this study. The HCV genotypes determined by the two methods were concordant in 100% (21/21) of the samples but the results differed at the subtype level (15/21). On the other hand, the NS5b method was able to classify all samples at the subtype level whereas the TRUGENE HCV 5'NC genotyping kit did not in four cases (20%).

It is concluded that these methods are reliable and convenient for HIV-1 and HCV genotyping. Moreover, the HIV-1 genotyping assay permitted subtype identification in samples with a low viral load and the HCV genotyping assay showed more resolution at the subtype level in comparison with TRUGENE methods.

INTRODUCTION

Genotyping of HIV-1 and HCV provides scientific opportunities to study the worldwide spread of these viruses. Additionally, it is of clinical importance because studies have shown that the probability of a sustained response to antiviral treatment varies with the genotype, suggesting that the therapeutic schedule should be adapted to it. HIV-1, the predominant HIV type in the United States and worldwide, produces chronic infection damaging the host's immune system. The clinical course of HIV -1 infection is remarkable for its great variability. This heterogeneity is the result of the relatively high frequency of viral replication errors. When the virus replicates, it can produce genetic variants that may be compatible with continued viral growth and survival, while resistant to antiviral drugs. Antiretroviral drug therapy has been directed at reducing plasma HIV-1 RNA concentration to below levels of detection, thus limiting the potential for the selection of drug resistant variants and delaying the onset of therapeutic failure. Hepatitis C is a positive-stranded RNA virus with a linear genome of approximately 940 nucleotides. The coding region of the HCV genome is comprised of the core (C), envelope (E), and nonstructural (NS) domains, flanked at both the 5' and 3' ends by highly conserved untranslated regions (UTR). The HCV genome is extremely heterogeneous; it is divided into six main distinct genotypes throughout the world with multiple subtypes in each genotype class. This procedure focused on the NS5b region to determine genotype. Numerous studies have shown that genotyping of HCV by nucleotide sequence analysis of NS5b is efficient because it allows for the accurate discrimination of subtypes and is an effective tool for studying the molecular epidemiology of HCV.

This work aims to validate different genotyping procedures for HIV-1 and HCV against renowned commercial kits.

METHODS & PROCEDURES

Extraction

RNA was extracted using the Abbott Molecular m2000 sample preparation instrument

HIV-1

PCR

- *env* gp41 IDR
  - ❖ cDNA and PCR primers: JH35F and JH38R [1,5]
- *pol* IN region
  - ❖ cDNA and PCR primers: Poli5_OF and Poli8_OR [5]
  - ❖ Nested PCR and sequencing primers: Poli6_IF and Poli7_IR [5]
- *env* gp41 IDR (low viral load)
  - ❖ cDNA and PCR primers: GP40F1 and GP41R1 [6]
  - ❖ Nested PCR and sequencing primers: GP46F2 and GP47R2 [6]

HCV

PCR

- Sequences were amplified with primers Pr1 and Pr2 [4].
- If there was no c-DNA amplification, a hemi-nested PCR was carried out with primers Pr3, Pr4, and Pr5 [4].

Gel Electrophoresis

The PCR products (DNA) were visualized and quantified in 1.2% agarose gel using a Bio-rad electrophoresis chamber

Sequencing

- The PCR products were sent to Macrogen Corp for purification and sequencing.
- For both assays sequences were blasted against the NIH and Los Alamos databases. Additionally HIV-1 *pol* IN sequences were blasted against the Stanford University HIV Drug resistance database and the Geno2pheno resistance database .
- In order to confirm the identity of HIV-1 and HCV sequences, neighbor-joining trees were drawn using the most related sequences obtained from databases by MEGA software [3].

RESULTS

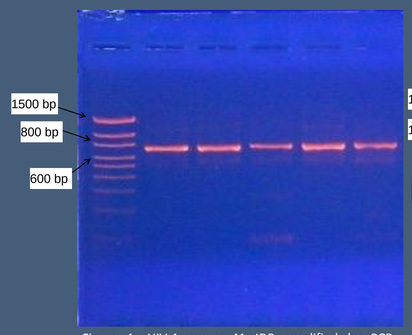

Figure 1. HIV-1 *env* gp41 IDR amplified by PCR (primers JH35F and JH38R) yielding an expected band of 715 bp. A positive and a negative control were run, results are not shown.

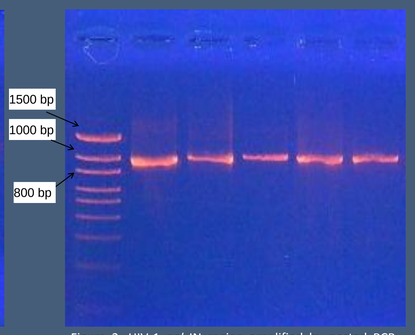

Figure 2. HIV-1 *pol* IN region amplified by nested PCR (primers Poli6_IF and Poli7_IR) yielding an expected band of 944 bp. A positive and a negative control were run, results are not shown.

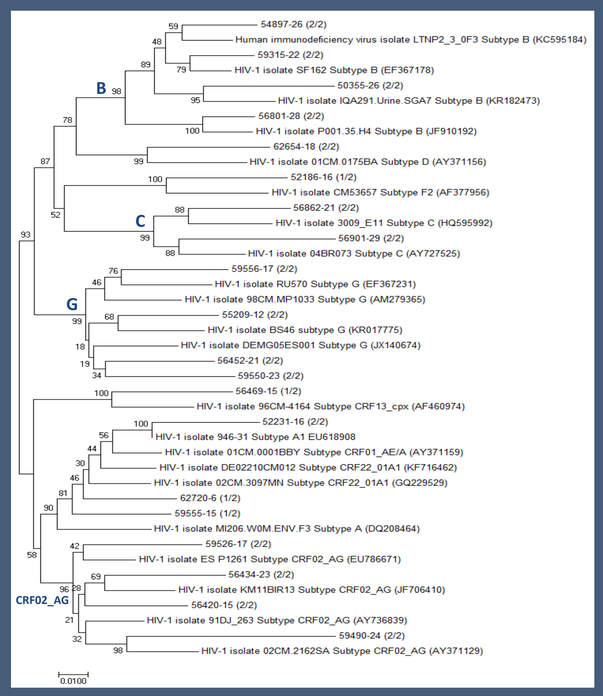

Figure 3. Neighbor-joining tree for HIV-1 *env* gp41 IDR sequences (715 bp). The numbers at each node represent the percent bootstrap support for 1,000 replicates. The evolutionary distances were computed using the Jukes-Cantor method [2] and are in the units of the number of base substitutions per site. The main subtypes analyzed are indicated next to their corresponding node. Samples are identified by numbers next to each branch. The coincidences in database results between TRUGENE and the method being validated are shown in parenthesis next to each sample number.

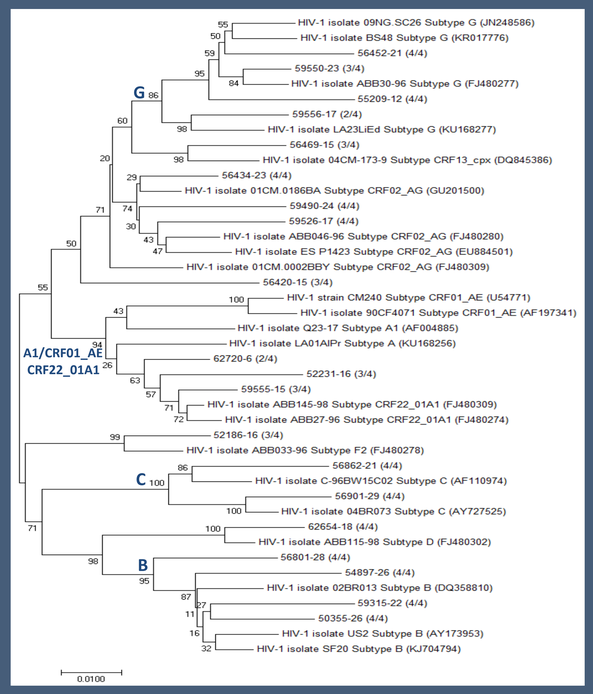

Figure 4. Neighbor-joining tree for HIV-1 *pol* IN sequences (944 bp). The numbers at each node represent the percent bootstrap support for 1,000 replicates. The evolutionary distances were computed using the Jukes-Cantor method [2] and are in the units of the number of base substitutions per site. The main subtypes analyzed are indicated next to their corresponding node. Samples are identified by numbers next to each branch. The coincidences in database results between TRUGENE and the method being validated are shown in parenthesis next to each sample number.

RESULTS

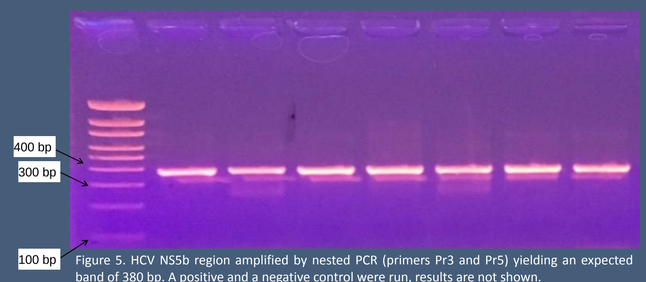

Figure 5. HCV NS5b region amplified by nested PCR (primers Pr3 and Pr5) yielding an expected band of 380 bp. A positive and a negative control were run, results are not shown.

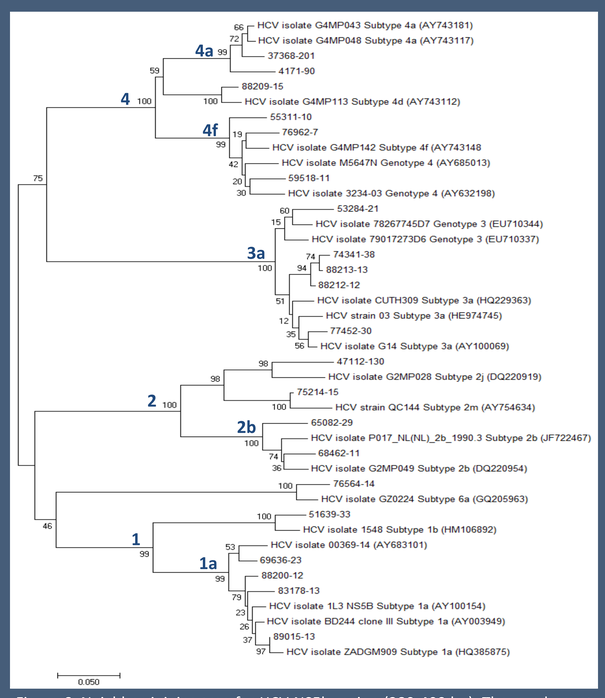

Figure 6. Neighbor-joining tree for HCV NS5b region (380-400 bp). The numbers at each node represent the percent bootstrap support for 1,000 replicates. The evolutionary distances were computed using the Jukes-Cantor method [2] and are in the units of the number of base substitutions per site. The main subtypes analyzed are indicated next to their corresponding node. Samples are identified by numbers next to each branch. The coincidences in database results between TRUGENE and the method being validated are shown in parenthesis next to each sample number.

Table 1. HCV Results

Sample #	Siemens	NS5b
4171	4	**4a**
37368	4a	4a
47112	2a	2j
51639	1b	1b
53284	3d	3a
55311	4f	4f
59518	4f	4f

Sample #	Siemens	NS5b
65082	2b	2b
68462	2b	2b
69636	1a	1a
74341	3	**3a**
75214	2a	2m
76564	6a	6a
76962	4f	4f

Sample #	Siemens	NS5b
77452	3a	3a
83178	1	**1a**
88200	1b	1a
88209	4c	4d
88212	3	**3a**
88213	3c	3a
89015	1a	1a

The 20 HIV-1 samples that were validated against the TRUGENE HIV-1 genotyping kit showed concordant results at the subtype level. One sample however, was not coincident at sub-subtype level, showing an F sub-subtype with the TRUGENE method and an F2 sub-subtype in this study. Furthermore, the alternative procedure developed to genotype samples with low viral loads was successful in detecting HIV-1 *env* gp41 IDR sequences from samples with viral loads as low as 45 copies/ml.

The 21 HCV genotypes that were validated against the TRUGENE HCV 5'NC genotyping kit were consistent. However, at subtype level, the results differed in 6 of the 21 samples. It is also noted that the NS5b method was able to classify all samples at subtype level, whereas the TRUGENE HCV 5'NC genotyping kit did not in four cases (20%), shown in yellow in Table 1.

CONCLUSIONS

Results were validated against previously obtained results from the TRUGENE HIV-1 genotyping kit and TRUGENE HCV 5'NC genotyping kit. It is concluded that these methods are reliable and convenient for HIV-1 and HCV genotyping. Moreover, the HIV-1 genotyping assay permitted subtype identification in samples with a low viral load and the HCV genotyping assay showed more resolution at the subtype level in comparison with TRUGENE methods. Determination of genotype and subtype can be helpful because the selection of therapy and its duration can be more specifically designed to target that particular strain of the virus.

REFERENCES

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