FULLY AUTOMATED RAPID QUANTIFICATION OF HEPATITIS C VIRUS RNA IN HUMAN PLASMA AND SERUM BY INTEGRATED ON-CHIP RT-qPCR AND CAPILLARY ELECTROPHORESIS

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Supplementary Table 1.  Background information of clinical samples from patients with HCV infection for correlation study (n = 202); values shown are median (interquartile range).

|                           | Value                  |
|---------------------------|------------------------|
| Age (years)               | 66 (59–71)             |
| Gender (Male/Female)      | 82 (40.6%) / 120 (59.4%) |
| Alanine aminotransferase (IU/L) | 27 (17–40)        |
| Aspartate aminotransferase (IU/L) | 31 (24–46)        |
| γ-glutamyl transpeptidase (IU/L) | 30 (20–47)        |
| Albumin (g/dL)            | 4.2 (4.0–4.4)          |
| Total bilirubin (mg/dL)   | 0.7 (0.6–1.0)          |
| HCV genotype (1b/2a /2b)  | 126 (62.4%) / 61 (30.2%) / 15 (7.4%) |
| HCV RNA levels - Roche cobas Test (log₁₀ IU/mL) | 3.4 (1.5–5.5) |
### Supplementary Table 2. Pathogens tested for cross-reactivity

| Micro-organism                  | Abbreviation | Description                                           |
|--------------------------------|--------------|-------------------------------------------------------|
| **Virus**                      |              |                                                       |
| *Human immunodeficiency virus* | *HIV-1*      | HIV-1 High Control, viral particles                   |
| *Hepatitis A virus*            | *HAV*        | HAV RNA positive sample, 1st International Standard, viral particles |
| *Hepatitis B virus*            | *HBV*        | HBV DNA Positive Control, viral particles             |
| *Human Adenovirus type 5*      | *AdV-5*      | Human Adenovirus-5 quantitated viral DNA              |
| *Human T-lymphotropic virus 2* | *HTLV-2*     | HTLV-2 Infected host cell DNA                         |
| *Herpes simplex virus Type 1*  | *HSV-1*      | Quantitated viral load control - viral particles      |
| *Herpes simplex virus Type 2*  | *HSV-2*      | HSV-2 quantitated viral DNA                           |
| *Epstein-Barr virus*           | *EBV*        | EBV quantitated viral load control - viral particles  |
| *Human Cytomegalovirus*        | *HCMV*       | HCMV quantitated viral DNA                            |
| *Human herpes virus type 6*    | *HHV-6B*     | HHV-6B (Z-29 strain) quantitated viral DNA           |
| *Influenza A*                  | *IA (H3N2)*  | Genomic RNA from strain A/Hong Kong/8/68              |
| **Bacteria**                   |              |                                                       |
| *Propionibacterium acnes*      | *P. acnes*   | Strain VPI 0389 cell suspension                       |
| Bacteria | *Staphylococcus aureus* | *S. aureus* | Genomic DNA from strain Mu50 |
|----------|------------------------|-------------|-------------------------------|
| Yeast    | *Candida albicans*     | *C. albicans* | Genomic DNA from strain 3147  |
## Supplementary Table 3. Potentially interfering substances (Endogenous Substances)

| Substance                        | Concentration  |
|----------------------------------|----------------|
| Triglycerides                    | 15 mg/mL       |
| Bilirubin, conjugated            | 0.2 mg/mL      |
| Human Serum Albumin              | 50 mg/mL       |
| Hemoglobin                       | 2.4 mg/mL      |
| Human genomic DNA                | 1 µg/mL        |
| EDTA                             | 2 - 10 mM      |

Endogenous substances that are potentially interfering at elevated levels were added to HCV negative and positive (5000 IU/mL) samples at the concentrations shown above. The measurement was done according to the method described in Method section.
### Supplementary Table 4. Drug compounds tested for interference

| Generic Name       | Type of drug         | Target/Action | Concentration (1X Cmax) | Concentration (3X Cmax) |
|--------------------|----------------------|---------------|------------------------|------------------------|
| Clarithromycin     | Antibacterial        | Antibacterial | 10 µg/mL               | 30 µg/mL               |
| Ciprofloxacin      | Antibacterial        | 4.56 µg/mL    | 13.68 µg/mL            |
| Azithromycin       | Antibacterial        | 0.66 µg/mL    | 1.98 µg/mL             |
| Paroxetine HCl     | Antidepressant       | 62 ng/mL      | 186 ng/mL              |
| Fluoxetine HCl     | Antidepressant       | 55 ng/mL      | 165 ng/mL              |
| Sertraline HCl     | Antidepressant       | 165 ng/mL     | 495 ng/mL              |
| Entecavir          | Nucleoside Analogue  | HBV           | 8.2 ng/mL              | 24.6 ng/mL             |
| Lamivudine         | HBV/HIV              | 1.5 µg/mL     | 4.5 µg/mL              |
| Adefovir dipivoxil | HBV                  | 18.4 ng/mL    | 55.2 ng/mL             |
| Telbivudine        | HBV                  | 3.2 µg/mL     | 9.6 µg/mL              |
| Tenofovir          | HBV/HIV              | 326 ng/mL     | 978 ng/mL              |
| Interferon alfa-2b | Interferon           | HBV/HCV       | 273 IU/mL              | 819 IU/mL              |
| Peginterferon alfa-2a | HBV/HCV             | 28 ng/mL      | 84 ng/mL               |
| Telaprevir (VX-950)| NS3/4A Protease Inhibitor | HCV           | 3.51 µg/mL | 10.53 µg/mL |
| Drug Name                  | Description                                | Disease | Concentration 1 | Concentration 2 |
|---------------------------|--------------------------------------------|---------|-----------------|-----------------|
| Simeprevir (TMC435)       |                                            | HCV     | 1.94 µg/mL      | 5.82 µg/mL      |
| Boceprevir                |                                            | HCV     | 1.72 µg/mL      | 5.16 µg/mL      |
| Sofosbuvir (GS-7977)      | Nucleoside and Nucleotide NS5B Polymerase Inhibitor | HCV     | 603 ng/mL       | 1809 ng/mL      |
| Ribavirin                 | Interferon / ribavirin                      | HCV     | 3.7 µg/mL       | 11.1 µg/mL      |
| Peginterferon Alfa-2b     |                                            | HCV     | 320 pg/mL       | 960 pg/mL       |
| Interferon Alfa-2a        |                                            | HCV     | 2.58 ng/mL      | 7.74 ng/mL      |
| Ganciclovir               | Compound for treatment of Herpes Virus      | Herpes  | 9 µg/mL         | 27 µg/mL        |
| Valganciclovir Hydrochloride |                                        | Herpes  | 5.61 µg/mL      | 16.83 µg/mL     |
| Valaciclovir HCl          |                                            | Herpes  | 5.65 µg/mL      | 16.95 µg/mL     |
| Acyclovir                 |                                            | Herpes  | 1.61 µg/mL      | 4.83 µg/mL      |
| Tipranavir, TPV           | Protease Inhibitors (Pis)                  | HIV     | 57.25 µg/mL     | 171.75 µg/mL    |
| Indinavir, IDV,           |                                            | HIV     | 8.98 µg/mL      | 26.94 µg/mL     |
| Saquinavir Mesylate, SQV  |                                            | HIV     | 2.48 µg/mL      | 7.44 µg/mL      |
| Lopinavir                 |                                            | HIV     | 9.6 µg/mL       | 28.8 µg/mL      |
| Drug                          | Type                        | HIV | IC50 | EC50 |
|-------------------------------|-----------------------------|-----|------|------|
| Fosamprenavir Calcium salt    | HIV                         | 7.24 µg/mL | 21.72 µg/mL |
| Ritonavir, RTV                | HIV                         | 11.2 µg/mL | 33.6 µg/mL |
| Darunavir Ethanolate          | HIV                         | 6.5 µg/mL | 19.5 µg/mL |
| Atazanavir Sulfate, ATV       | HIV                         | 3.15 µg/mL | 9.45 µg/mL |
| Nelfinavir Mesylate, NFV      | HIV                         | 4 µg/mL | 12 µg/mL |
| Rilpivirine                   | Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs) | HIV | 79 ng/mL | 237 ng/mL |
| Etravirine (TMC125)           | HIV                         | 1.4 µg/mL | 4.2 µg/mL |
| Delavirdine, DLV (NNRTIs)     | HIV                         | 35 µM | 105 µM |
| Efavirenz, EFV                | HIV                         | 4.07 µg/mL | 12.21 µg/mL |
| Nevirapine                    | HIV                         | 4.5 µg/mL | 13.5 µg/mL |
| Etricitabine, FTC             | Nucleoside Reverse Transcriptase Inhibitor (NRTIs) | HIV | 1.8 µg/mL | 5.4 µg/mL |
| Didanosine                    | HIV                         | 2.32 µg/mL | 6.96 µg/mL |
| Tenofovir Disoproxil Fumarate, TDF | HIV                      | 326 ng/mL | 978 ng/mL |
| Drug Compound | Description | HIV Concentration 1X | HIV Concentration 3X |
|---------------|-------------|----------------------|----------------------|
| Stavudine, D4t | HIV         | 4.15 µg/mL           | 12.45 µg/mL          |
| Abacavir Sulfate, ABC | HIV | 3 µg/mL | 9 µg/mL |
| Zidovudine | HIV | 1.06 µg/mL | 3.18 µg/mL |
| Enfuvirtide, T-20 | Fusion Inhibitor | HIV | 5 µg/mL | 15 µg/mL |
| Raltegravir (MK-0518) | HIV integrase strand transfer inhibitor | HIV | 2.17 µg/mL | 6.51 µg/mL |
| Dolutegravir (GSK1349572) | HIV | 4.15 µg/mL | 12.45 µg/mL |
| Maraviroc | Entry Inhibitor – CCR5 co-receptor antagonist | HIV | 888 ng/mL | 2664 ng/mL |
| Elvitegravir | Integrase Inhibitor | HIV | 1.5 µg/mL | 4.5 µg/mL |
| Cobicistat (GS-9350) | Inhibitor of Cytochrome 450 | HIV | 1.1 µg/mL | 3.3 µg/mL |

Drug compounds at concentrations at 1X or 3X of the peak plasma or serum levels were tested in nine pools. The drug compounds were added to HCV negative and positive (5000 IU/mL) samples at the concentrations shown above. The measurement was done according to the procedure.
**Supplementary Table 5.** Correlation of HCV assay on µTASWako g1 Analyzer and Roche cobas AmpliPrep/cobas TaqMan HCV Test, version 2.0 at low HCV titer

| Roche cobas AmpliPrep/cobas TaqMan HCV Test                                    | µTASWako g1 Analyzer |
|--------------------------------------------------------------------------------|----------------------|
|                                                                                          | Detected | Target not detected |
| Positive and not quantifiable (< 15 IU/mL)                                              | 19        | 12                  | 7       |
| Target not detected                                                                  | 21        | 5                   | 16      |