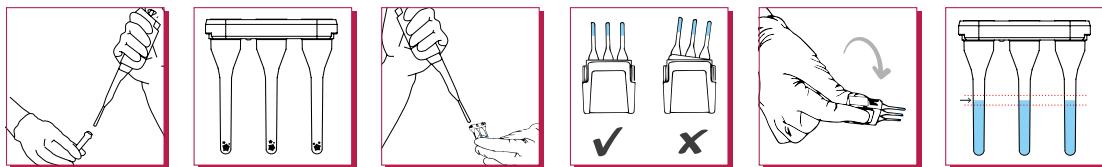


SUPPLEMENTARY DATA

Supplementary Figure 1. Assay workflow

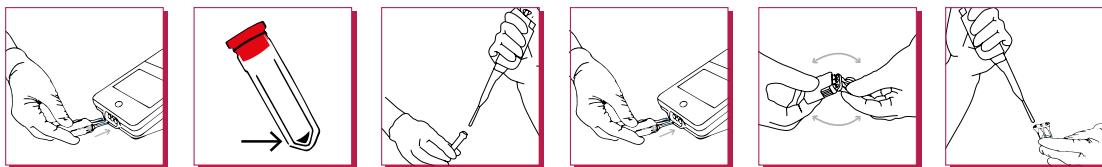


Step 1
Dilute plasma sample using 25 μ L of sample and 50 μ L of Nuclease Free Water.

Step 2
Check for lyophilised reagent at base of each channel. Add 15 μ L diluted plasma to each channel.

Step 3
Attach cartridge lid.
✓ Correct fit. Lid sits flush with cartridge top. Lid tabs fully clipped down.
✗ Incorrect fit. Left lid tab NOT fully clipped down.

Step 4
Flick cartridge downwards and check fill levels in each channel.



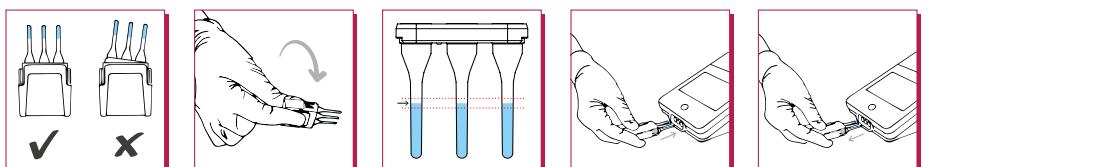
Step 5
Immediately insert cartridge into Genedrive. Press and hold function button.

Step 6
Once the plasma preparation reaction is complete, remove HCV ID Assay tube from pouch and check for lyophilised reagents.

Step 7
Open tube and add 100 μ L Nuclease Free Water.

Step 8
Remove cartridge from Genedrive. Flick downwards and remove lid.

Step 9
Immediately transfer 30 μ L HCV Assay RT-PCR reagents to each channel.



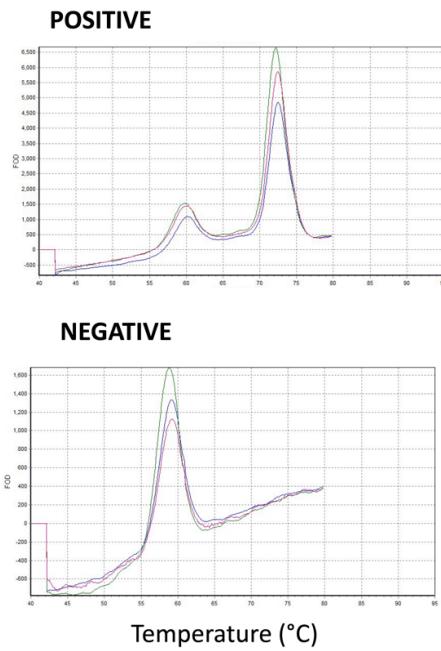
Step 10
Attach cartridge lid.

Step 11
Flick cartridge downwards and check fill levels in each channel.

Step 12
Insert cartridge into Genedrive. Press and hold function button.

Step 13
Upon completion, record the test result. Remove the cartridge from the Genedrive and dispose of as clinical waste material. **Do not attempt to remove the cartridge lid before disposal.**

Supplementary Figure 2. Positive and negative outcomes



IPC		HCV	
Peak Tm	Peak height	Peak Tm	Peak height
58.9	341	71.4	1650
58.5	428	71	2388
58.7	419	71.2	2017

IPC		HCV	
Peak Tm	Peak height	Peak Tm	Peak height
57.5	501	-	-
57.7	660	-	-
57.9	440	-	-

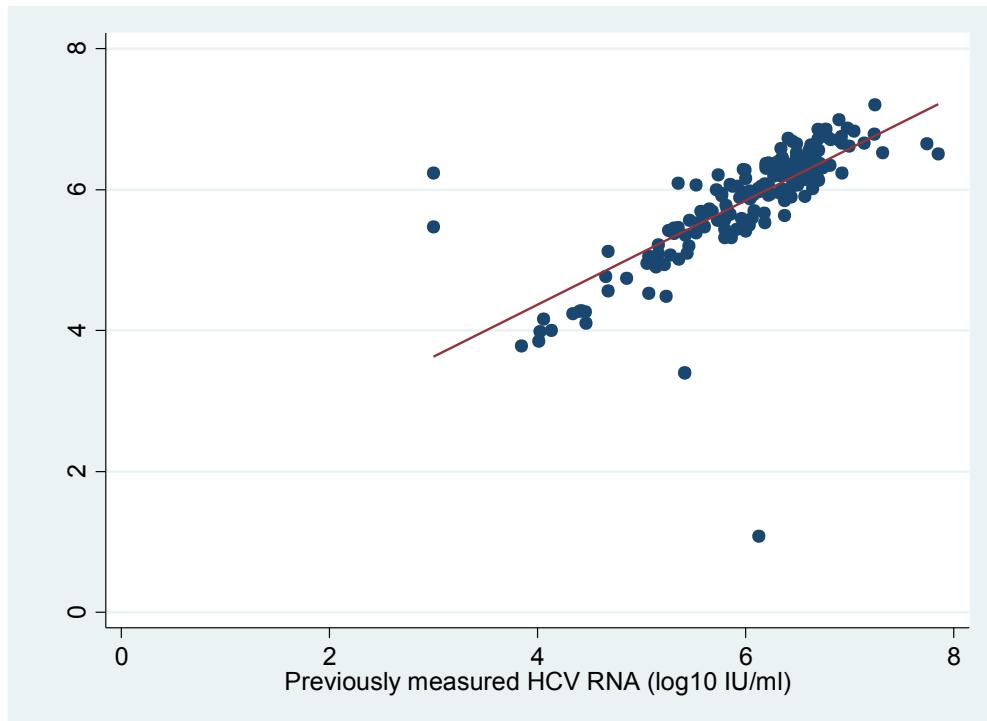
Supplementary Figure 3. Calling Logic

		Internal Positive Control (IPC)				
		Frequency of Channel detection	3/3	2/3	1/3	0/3
HCV Target	3/3	POSITIVE	POSITIVE	POSITIVE	POSITIVE	
	2/3	POSITIVE	POSITIVE	POSITIVE	POSITIVE	
	1/3	INDETERMINATE - RETEST	INDETERMINATE - RETEST	INDETERMINATE - RETEST	INDETERMINATE - RETEST	
	0/3	NEGATIVE	NEGATIVE	CONTROL FAIL - RETEST	CONTROL FAIL - RETEST	

Supplementary Figure 4. Examples of Genedrive outcomes

Result	Significance
Detected, Positive 	Hepatitis C virus has been detected. The internal positive control has been detected.
Result	Significance
Undetected, Negative 	Hepatitis C virus has NOT been detected. The internal positive control has been detected.
Result	Significance
Indeterminate, Retest 	Hepatitis C virus has been detected in one of the channels, however for confirmation a retest is required. The internal positive control has been detected.
Result	Significance
Control Failed, Retest 	Hepatitis C virus and the internal positive control have not been detected. This may be due to the sample containing PCR inhibitors.

Supplementary Figure 5. Correlation between previously and currently measured HCV RNA levels (log₁₀ IU/ml)



Pearson's correlation coefficient: $r = 0.7343$ ($p < 0.0001$)

Supplementary Table 1. Target selection (HCV 5-UTR sequences) and Genedrive testing of all six major genotypes.

A

Genotype	Number of sequences obtained
1a	36
1b	127
Other 1-subtypes	4
2	45
3	49
4	7
5	7
6	64

B

Sample number	Genotype	HCV Titre (IU/ml)	Result1	Result2
6814099626	1b	144320	Detected	Positive
7314100242	1a	1119800	Detected	Positive
62140471781	1b	341000	Detected	Positive
68140567959	1a	156640	Detected	Positive
68141084588	1b	100760	Detected	Positive
BM216846	1b	1799600	Detected	Positive
9222515	1b	1067000	Detected	Positive
9116009	1a	831600	Detected	Positive
BM205275	1a	188760	Detected	Positive
BM204883	1a	159280	Detected	Positive
72141013570	2	2354000	Detected	Positive
73131068070	2	2552000	Detected	Positive
2015004HCV2-03	2	6710000	Detected	Positive
2015004HCV2-08	2	6710000	Detected	Positive
72140673593	2	510400	Detected	Positive
2015004HCV2-02	2	3190000	Detected	Positive
2015004HCV2-04	2	858000	Detected	Positive

9224788	2	25960000	Detected	Positive
9255089	2	26620000	Detected	Positive
BM217520	2	4708000	Detected	Positive
62005681	3	4576000	Detected	Positive
62020478	3a	2222000	Detected	Positive
7214077479	3	38060	Detected	Positive
71130236459	3	126280	Detected	Positive
72141175131	3	2134000	Detected	Positive
73130505136	3	212520	Detected	Positive
9161466	3	2288000	Detected	Positive
BM217113	3	8580000	Detected	Positive
9216276	3	4422000	Detected	Positive
9267755	3	121880	Detected	Positive
72140053677	4	325600	Detected	Positive
0215A334	4	294800	Detected	Positive
0315A093	4	1001000	Detected	Positive
0315A081	4	719400	Detected	Positive
0716A006	4	376200	Detected	Positive
0716A011	4	2706000	Detected	Positive
0716A004	4	3102000	Detected	Positive
0114A026	4	215820	Detected	Positive
9219510	4	34980	Detected	Positive
9135525	4	35860	Detected	Positive
1051-HCV-0020	5	1731400	Detected	Positive
1051-HCV-0018	5	3762000	Detected	Positive
1051-HCV-0001	5	1161600	Detected	Positive
1051-HCV-0002	5	3102000	Detected	Positive
1051-HCV-0006	5	1344200	Detected	Positive
1051-HCV-0008	5	3352800	Detected	Positive
1051-HCV-0009	5	6446000	Detected	Positive
1051-HCV-0016	5	668800	Detected	Positive
1051-HCV-0004	5	13329411	Detected	Positive
1051-HCV-0005	5	3542000	Detected	Positive
HCV6140425-24	6e	182160	Detected	Positive
HCV6140619-01	6e	101640	Detected	Positive
HCV6151029-15	6	8162	Detected	Positive
HCV6140425-22	6e	13816	Detected	Positive
10007228	6	875600	Detected	Positive
10007222	6	11638000	Detected	Positive
10007225	6	886600	Detected	Positive
10007224	6	13288000	Detected	Positive
10007221	6	181060	Detected	Positive
9262137	6	27500000	Detected	Positive

Supplementary Table 2. Test samples and controls run daily on both study sites

	Institut Pasteur (Paris)	Queen's Medical Centre (Nottingham)
Test samples	12	24
HCV positive controls	1	4
HCV negative controls	3	4

Supplementary Table 3. Interfering substances

Reagent	Class	Concentration (µg/ml)	Detection Frequency (HCV Positive/Total Tests)
Peginterferon 2a	Anti-viral (HCV)	15.12	8/8
Ribavirin		11.04	8/8
Saquinavir	Anti-retroviral, protease inhibitor (HIV)	15.62	8/8
Ritonavir		44.40	8/8
Amprenavir		18.24	8/8
Lopinavir		42.00	8/8
Indinavir sulfate		35.52	4/4
Nelfinavir		12.00	8/8
Telaprevir	HCV Direct Acting Antiviral	3.51	4/4
Sofosbuvir		0.71	4/4
Ledipasvir		0.62	4/4
Danoprevir		0.08	4/4
Daclatasvir		1.50	4/4
VX-222		0.70	4/4
Zidovudine	Nucleoside Reverse Transcriptase Inhibitor	6.87	4/4
Tenofovir disoproxil fumarate		0.98	8/8
Lamivudine		35.52	8/8
Stavudine		2.05	8/8
Didanosine		3.00	8/8
Ganciclovir	Anti-viral (CMV)	27.00	4/4
Valganciclovir hydrochloride		20.19	8/8
Abacavir sulfate	Anti-viral, Polymease Inhibitors	12.90	8/8

Adefovir dipivoxil	(HBV)	0.30	4/4
Efavirenz	Non-nucleoside Reverse Transcriptase Inhibitor (HIV)	12.21	8/8
Nevirapine		17.22	8/8
Clarithromycin	Macrolide Antibiotic	30.00	8/8
Azithromycin		15.69	8/8
Acyclovir	Anti-viral, nucleoside analog	62.10	8/8
Valacyclovir		24.06	4/4
Fluoxetine (HCl)	Anti-depressive (Prozac)	0.18	4/4
Ciprofloxacin	DNA gyrase inhibitor	2.40	4/4
Enfuvirtide	HIV fusion inhibitor	13.80	4/4
Bilirubin	Endogenous substance	312.50	4/4
Haemoglobin		1250	8/8
Whole blood		1%	4/4
Human DNA		10^5 cells/ml equivalence	4/4
IgG from human serum		17 mg/ml	4/4

Supplementary Table 4. Analytical sensitivity

Genotype	Number of samples	Limit of detection (IU/ml)
1	3	1885
2	2	2637
3	2	2387
4	3	2755
5	3	3203
6	3	1406
Overall	16	2362 (95% CI 1966 – 2758)

Supplementary Table 5. Analytical specificity

		# donors	# tests	Result frequency (Positive/Total)
Mono-infections	HBV	57	68	0/68
	HIV	63	71	0/71
	Dengue	6	14	0/14
	HDV	5	10	0/10
	Yellow Fever	3	6	0/6
	Zika	4	16	0/16
	GBV-C	5	10	1/10*
	HAV	2	8	0/8
	HTLV-I	1	4	0/4
	HTLV-II	1	4	0/4
	Lyme	1	4	0/4
	Mycoplasma	2	8	0/8
	Helicobacter pylori	2	8	0/8
	Rubella	2	8	0/8
	Toxoplasma	2	8	0/8
Non-HCV co-infections	Syphilis (RPR)	2	8	0/8
	CMV	1	4	0/4
	HDV/HBV	2	8	0/8
	Dengue/HAV	1	4	0/4
	HTLV-I/HAV	1	4	0/4

	Lyme/HAV	1	4	0/4
	HTLV-II/Chagas	1	4	0/4
	HIV/HAV	1	4	0/4
	CMV/Mononucleosis	1	4	0/4
	Chagas/HAV	2	8	0/8
HCV co-infections	HCV/HIV	5	20	16/20**
	HCV/HBV	1	4	4/4
	HCV/HBV/HIV	1	4	4/4

* One of two duplicate samples from one donor returned a positive result on Genedrive.

Repeated test was negative.

** One of four HCV/HIV coinfecte samples consistently exhibited assay inhibition.

Supplementary Table 6. Factors associated with the lack of result at the first attempt

< 3.0	8 (1.9)	5 (55.6)	<0.001	N/A		
3.0-6.0	223 (54.0)	4 (44.4)				
≥ 6.0	182 (44.1)	0				
HCV genotype (%)						
1	297 (76.0)	4 (66.7)	0.06	N/A		
2	4 (1.0)	0				
3	58 (14.8)	1 (16.7)				
4	31 (7.9)	0				
5	1 (0.3)	1 (16.7)				

* P-values were obtained using Wilcoxon rank-sum test for the continuous variables and Fisher's exact test for the categorical variables.

** Of the variables associated with the lack of result at the 1st attempt in the univariable analysis (age, study site, sample type and HCV RNA levels), only HCV RNA was found to be associated with the lack of result ($p<0.001$) in multivariable logistic model that included all these variables.

*** Because there was no lack of result in Paris Cohort, the multivariable model was restricted to the Nottingham cohort, and fitted by including all the variable associated with the lack of result at the 1st attempt in the univariable analysis (age and sample type)

Supplementary Table 7. Factors associated with false-negative results

	True positive (n=412)	False-negative (n=6)	p-value*
Median age (IQR)	51 (41-61)	49 (46-61)	0.9
Sex (%)			
Male	247 (66.8)	5 (83.3)	0.4
Female	123 (33.2)	1 (16.7)	
Study site			
Paris	219 (53.2)	2 (33.3)	0.3
Nottingham	193 (46.8)	4 (66.7)	
Sample type			
Heparin plasma	219 (53.1)	2 (33.3)	0.3
EDTA plasma	119 (28.9)	2 (33.3)	
Serum	74 (18.0)	2 (33.3)	
Median HCV RNA (log₁₀ IU/ml) (IQR)	5.9 (5.4-6.3)	2.2 (1.7-2.7)	<0.0001
HCV RNA (log₁₀ IU/ml) (%)			
< 3.0	6 (1.5)	5 (83.3)	<0.001
3.0-6.0	224 (54.4)	1 (16.7)	
≥ 6.0	182 (44.1)	0	
HCV genotype (%)			
1	297 (76.1)	3 (75.0)	0.6
2	4 (1.0)	0	

3	57 (14.6)	1 (25.0)	
4	31 (8.0)	0	
5	1 (0.3)	0	

* P-values were obtained using Wilcoxon rank-sum test for the continuous variables and Fisher's exact test for the categorical variables.

Supplementary Table 8. Factors associated with non-agreement*

	Agreed (<1 log ₁₀ IU/ml difference) (n=369)	Not agreed (>1 log ₁₀ IU/ml difference)(n=33)	p-value**
Median age (IQR)	52 (41-61)	44 (40-55)	0.1
Sex (%)			
Male	219 (66.2)	23 (76.7)	0.2
Female	112 (33.8)	7 (23.3)	
Median HCV RNA (log₁₀ IU/ml) (IQR)	5.9 (5.4-6.3)	5.1 (4.1-6.5)	0.06
HCV RNA (log₁₀ IU/ml) (%)			
< 3.0	3 (0.8)	3 (9.1)	0.001
3.0-6.0	198 (53.7)	18 (54.5)	
≥ 6.0	168 (45.5)	12 (36.4)	
HCV genotype (%)			
1	275 (77.5)	19 (76.0)	0.06
2	3 (0.9)	0	
3	47 (13.2)	5 (20.0)	
4	30 (8.4)	0	
5	0	1 (4.0)	

* Non-agreement was defined as >1 log₁₀ IU/ml difference between the viral load estimated by Genedrive and viral load observed by Abbott RealTime.

** P-values were obtained using Wilcoxon rank-sum test for the continuous variables and Chi-squared test for the categorical variables.