

## **Hepatitis C core antigen testing: a reliable, quick and potentially cost-effective alternative to Hepatitis C polymerase chain reaction in diagnosing acute hepatitis C virus infection**

**Cresswell F.V.<sup>1</sup>, Fisher M.<sup>2,1</sup>, Hughes D.J.<sup>1</sup>, Shaw S.G.<sup>1</sup>, Homer G.<sup>3</sup>, Hassan-Ibrahim M.O.<sup>3</sup>**

<sup>1</sup>Lawson Unit, Department of HIV and Sexual Health, Royal Sussex County Hospital, Eastern Road, Brighton, BN2 5BE, United Kingdom

<sup>2</sup>Brighton and Sussex Medical School, Brighton, BN1 9PX, United Kingdom

<sup>3</sup>Department of Virology/Microbiology & Infection, Royal Sussex County Hospital, Eastern Road, Brighton, BN2 5BE, United Kingdom

Corresponding author: Dr Fiona V Cresswell, Lawson Unit, Royal Sussex County Hospital, Eastern road, Brighton, BN2 5BE, Fiona.cresswell@bsuh.nhs.uk, +44 01273 664718, +44 (0)7799605069

Alternative corresponding author: Professor Martin Fisher, Brighton and Sussex Medical School, Brighton, BN1 9PX, United Kingdom, Martin.fisher@bsuh.nhs.uk, +44 (0)1273 664718

### **40-word summary:**

We evaluated the efficacy of HCV core-antigen in diagnosing acute HCV in an HIV-positive cohort. Compared with HCV polymerase-chain-reaction HCV core-antigen proved sensitive (100%) and specific (97.96%). This quick, simple, cost-effective test has considerable utility in screening for acute HCV.

**Abstract**

Hepatitis C (HCV) is increasingly common amongst HIV-positive men-who-have-sex-with-men. We evaluated the efficacy of HCV core-antigen in diagnosing acute HCV in an HIV-positive cohort. Compared with HCV polymerase-chain-reaction core-antigen proved sensitive (100%) and specific (97.9%). As a quick, simple and cost-effective test it has considerable utility in screening for acute HCV.

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## Introduction

Outbreaks of acute hepatitis C are being increasingly reported, almost exclusively amongst HIV-positive men-who-have-sex-with-men (MSM). The incidence is at least 8 fold greater than HIV-uninfected MSM [1], which may be driven by an increase in plasma and semen HCV viral loads in the presence of HIV along with a resurgence of unsafe sexual practices, injecting and other recreational drug use [2].

Less than half of those acutely infected with HCV experience symptoms and, if present, those symptoms are frequently non-specific [3]. It is therefore recommended in American, European and British guidelines that HIV-positive MSM should be screened for HCV at 6-month intervals with serum transaminases and at least annually with anti-HCV antibody [4,5,6]. However, it is well recognized that HCV-antibody seroconversion can be delayed in HIV-infected individuals, with only two-thirds positive at 3 months and 5% remaining negative up to 1 year after infection [7,8]. This has significant implications for timely diagnosis, treatment, partner notification, contact tracing and reduction of onward transmission. Furthermore individuals who have cleared previous HCV infection remain HCV-antibody positive making it an unhelpful marker for screening for re-infection. As HCV treatment expands with wider availability of all-oral therapy a cost-effective screening test for reinfection will be increasingly important.

As such, a nucleic acid amplification test for HCV-RNA by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) is recommended in this group if transaminases are elevated or if there has been a high-risk exposure [4,5,6].

However, HCV qRT-PCR is expensive, time-consuming, and requires advanced technical skills and equipment. A cheaper and quicker assay for initial testing for suspected HCV is needed, particularly in low-resource settings [9].

Less expensive and time-consuming tests against HCV core-antigen have recently become available, which may be useful in diagnosing acute infection. HCV core-antigen can be used as a marker of viraemia as there is good non-linear correlation with HCV-RNA ( $r=0.87$  vs. Abbot Real-Time qRT-PCR) with the lower limit of detection corresponding to HCV viral load of 700-1100 IU/ml [10]. This assay is available in Europe, Canada, Australasia, South and Central America and Asia but FDA-approval has not yet been sought for the USA. To date only three studies have been published in the co-infected population, including only one using the Abbot Architect platform. For this reason, routine use of HCV core-antigen testing in either mono- or co-infected patients cannot be currently recommended and more data is needed [5,6]

In this study we compare the utility of HCV core-antigen compared to qRT-PCR in the diagnosis on acute HCV in an HIV-positive cohort.

## Methods

Subjects were patients attending a dedicated HIV outpatient clinic. Routine liver function tests were performed every 4-6 months in all patients as per British HIV Association monitoring guidelines [11]. Individuals with *newly elevated* alanine transaminase (ALT) levels above the laboratory upper limit of normal (>41 IU men;

>31 IU women) on routine blood testing between April 2012 and December 2013 were screened for HCV infection using:

1. **HCV core-antigen testing (Abbott Architect)**
2. **HCV RNA testing (Abbott Real-Time qRT-PCR)**
3. **HCV Antibody testing (Abbott Architect)**

Those with chronically elevated and stable ALT for an already established diagnosis (e.g steatohepatitis) were excluded. As per manufacturer's guideline, a cut-off of 10 fmol/L for HCV antigen positivity with an indeterminate zone between 3 - 10 fmol/L was adopted. Retrospective testing of stored samples determined acute versus chronic HCV infections, the latter being excluded from the study, consistent with the European recommendations for diagnosing acute infection [5].

Statistical analysis using SPSS was performed; multivariate analysis, sensitivity, specificity, positive and negative predictive value calculations were calculated, and Spearman coefficient calculated for the correlation between qRT-PCR and HCV Ag.

## **Results**

111 (5.4%) of the 2058 patients on routine blood monitoring were found to have a *newly* elevated ALT during the 20-month study. The vast majority (105; 94.6%) were male, 105 (94.6%) were white and 101 (91%) were MSM. The majority (89; 80%) were receiving antiretroviral therapy (ART); the HIV viral load was undetectable (<40copies/ml) in 84 (75.7%) of the patients, with a CD4<sup>+</sup> count of <350 in 23 (20.7%), 350-499 in 26 (23.4%) and >500 in 62 (55.9%) cases.

15 cases of acute HCV were identified by qRT-PCR (HCV viral load range 60,950 – 14,794,746 IU/ml), see table 1. HCV core-antigen testing correctly identified all 15 acute infections, resulting in a sensitivity of 100% (95% confidence interval (CI) = 75% - 100%). Retrospective testing of stored serum samples identified three additional chronic infections (>6 months duration), which were not included in subsequent analyses.

There were no false-positive HCV core-antigen results. However, two 'indeterminate' results were obtained (quantitative value 4.86 and 3.18 fmol/L). The first individual was retested on the same day and did not become positive on re-testing with either assay. His sexual and lifestyle history were explored; there were no HCV risk factors so screening was not repeated again during the study as his ALT normalized. The second individual did become qRT-PCR positive on re-testing 5 months later when he remained antigen positive (1576 fmol/L) with a HCV VL of 694201 IU/ml and had a positive HCV-antibody. It is not clear whether this was a 'false-indeterminate' or whether it was extremely early infection that failed to amplify on qRT-PCR as we had no interim samples which could be analysed. As we are using qRT-PCR as the gold-standard in this analysis we have included that result as a false-indeterminate. Specificity of HCV core antigen is therefore 97.96% (95% CI = 92.1% - 99.6%) with a positive predictive value of 88% (95% CI = 62.2% - 97.9%) and a negative predictive value of 100% (95% CI = 95.2% - 100%).

HCV antibody was positive in 9 of the 15 cases, giving a sensitivity of 60%, with those antibody-negative at the time of acute HCV diagnosis taking up to 112 days to seroconvert.

All 15 acute HCV infections were in MSM and acquired through sexual intercourse; notably 3 were re-infections having previously cleared HCV. HCV genotype 1a predominated (n=11) but genotypes 1b (n=1), 3a (n=1) and 4d (n=2) were also seen as summarized in table 1. No significant associations with age, ethnicity, HIV VL or CD4<sup>+</sup> count were seen when comparing with the total population of those with abnormal ALT.

The non-linear correlation between HCV Ag (fmol/L) and qRT-PCR (IU/mL) in this dataset was extremely good ( $r= 0.943$ ,  $p<0.001$ ), in keeping with previously reported results for this assay in mono-infected studies [10].

Potential cost savings exist by introducing HCV core-antigen testing routinely as a screening tool in place of qRT-PCR. The cost per individual screen would be approximately \$85 less (\$108 for qRT-PCR versus \$23.4 for HCV cAg including kit, staff and laboratory extras, though this will vary between laboratories). We calculate that in our cohort (approximately 2200 patients with HIV infection; 111 with newly raised transaminases, 96 of whom were HCV negative) over the 20 months study period the potential cost savings would have been \$8160 in kits alone. We estimate additional savings in manpower of 14 days – which equates to approximately \$3118 by UK costings (based upon 70 minutes of manpower spared per test).

All except one of the individuals diagnosed with acute HCV during the study period have subsequently been assessed in a dedicated HIV/HCV co-infection clinic. Three (20%) cleared spontaneously; 7 elected to take early treatment with pegylated-interferon and ribavirin for 24-48 weeks (pending rapid virological response), and all of those who completed treatment exhibited a sustained virological response, with an intent-to-treat response rate of 83%.

## Conclusion

In this study HCV core-antigen testing on the Architect platform displayed high sensitivity (100%) and specificity (97.96%) in diagnosing acute HCV in HIV-infected individuals who experience elevated transaminases. It would not have missed any infections if used in place of qRT-PCR.

Similar sensitivity (100%) and specificity (97.7%) has been reported in abstract form by another study using the same platform comparing stored serum panels from HIV-positive patients with known acute HCV, chronic HCV, or negative for HCV [12]. In-keeping with existing data in HIV-negative people HCV core-antigen sensitivity dropped off at low HCV viral loads (625 IU/ml) [10]. Such low viral loads are rarely seen in HIV/HCV co-infection. Furthermore, in our study 3 re-infections occurred within the time period, an increasingly recognized phenomenon, supporting the utility of antigen testing in detecting acute re-infection. In HIV-negative cohorts other studies also report sensitivities in excess of 90% with the Architect platform [10].



We estimated a potential saving of \$8160 in kits and \$3118 in manpower during the study period had HCV core-antigen been used in place of qRT-PCR. This may be a significant underestimate given our inclusion criteria. If we included all HCV screening tests in addition to those with newly abnormal transaminases, as recommended for MSMs after significant sexual risk [6], we believe (based upon historical HCV screening throughout the cohort) these savings may increase, in a cohort of 2200, to \$18,275 in materials and \$6964 in manpower per year.

HCV core-antigen results can usually be issued on the same day versus up to a week for qRT-PCR results due to the necessity to batch samples for cost effectiveness. Such earlier diagnosis would enable earlier commencement of therapy if indicated for the individual, and would facilitate contact tracing and partner notification. We argue therefore that increased use of HCV core-antigen testing in preference to qRT-PCR has potentially substantial benefits to the laboratory, clinician, patient, and to public health.

HCV core-antigen's utility in low-resource settings, where PCR may be unavailable, would be even more significant [9]. Compared with HCV antibody screening alone this test considerably shortens the diagnostic window, as the delay to antibody seroconversion previously reported by others after PCR positivity is confirmed here after antigen positivity [7].

The main limitation of our study is the small sample size. Other general test limitations include potential low-level false positive from antigen carry-over from the

hepatitis C antibody kit if both tests are done on the same instrument and its low sensitivity at low viral loads, as mentioned above.

A larger study with further cost-analysis would be valuable. However in light of our findings and those of Carney *et al.* we believe that national guidelines should now begin to consider HCV core-antigen testing as an integral part of their HCV screening algorithm for acute HCV infection.

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**Table 1 – Summary of acute HCV infection cases**

Number	Age	Ethnicity	HCV Acquisition Risk	HCV Genotype	ART	CD4 count	HIV Viral load	ALT at 1 <sup>st</sup> test	HCV cAg	HCV qRT-PCR level (IU/mL)	HCV cAg level(fmo/L)	HCV Ab	Time to Ab positivity (days)
1	47	White	MSM	1a	TDF FTC ETV	524	<40	901	Detected	60950	181.26	Detected	n/a
2	45	Black Caribbean	MSM	1a	Nil	654	3711	145	Detected	175068	386.16	Detected	13
3	45	White	MSM	3	TDF FTC RAL	168	<40	69	Detected	644269	2820.82	Not Detected	6
4	60	White	MSM	1a	TDF EFV 3TC	613	<40	90	Detected	1017207	2624.27	Detected	n/a
5	38	White	MSM	1a	TDF FTC EFV	969	<40	249	Detected	798414	15171.12	Not detected	42
6	50	White	MSM	1a	TDF FTC DRV/r	369	<40	81	Detected	39345	112.3	Not Detected	112
7	31	White	MSM	4	TDF FTC EFV	468	56	58	Detected	6818724	15230.33	Not Detected	46
8	48	White	MSM	1a	TDF FTC EFV	611	<40	210	Detected	1027129	3172.8	Detected	n/a
9	50	White	MSM	1a	TDF FTC DRV/r	665	<40	1074	Detected	2994028	5186.32	Detected	n/a
10	34	White	MSM	1a	ABC 3TC NVP	592	<40	59	Detected	28654482	>20000	Detected	n/a
11	43	White	MSM	1b	TDF ETV DRV/r RAL	951	<40	78	Detected	1357494	6271.29	Not Detected	62
12	48	White	MSM	1a	TDF FTC DRV/r	126	3631 6	665	Detected	694210	1,576.38	Detected	n/a
13	42	White	MSM	1a	TDF FTC DRV/r	410	<40	1972	Detected	8418803	14198.72	Detected	n/a
14	51	White	MSM	1a	TDF FTC EFV	266	<40	131	Detected	8882949	>20000	Not Detected	90
15	46	White	MSM	4	TDF FTC EFV	602	<40	122	Detected	14794746	>20,000	Detected	n/a

**Abbreviations:**

TDF = tenofovir, EFV = efavirenz, 3TC = lamivudine, FTC = emtricitabine, DRV/r = darunavir / ritonavir, ABC = abacavir, NVP = nevirapine, ETV = etravirine, RAL = raltegravir