Research Article

Cancer Epidemiology, Biomarkers & Prevention

Hepatitis C Virus Seroprevalence in Mongolian Women Assessed by a Novel Multiplex Antibody Detection Assay

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Abstract

Background: Hepatitis C virus (HCV) infection causes hepatocellular carcinoma and is an important cause of mortality in both industrialized and developing countries. We developed a single-step high-throughput multiplex serology assay for HCV antibody detection and determined HCV prevalence in a highly endemic country.

Methods: Five proteins (Core, NS3, NS4A, NS5A, NS5B) each from the three most common subtypes of HCV (1a, 1b, 2a) were recombinantly expressed and used as antigens in a multiplexed antibody detection assay. Multiplex HCV serology was validated with 432 reference sera whose HCV status was established by commercial ELISA, Western blot, and RNA assays. HCV antibodies were determined in 1,023 sera representative for the adult female population of Mongolia.

Results: In reference sera, detection of HCV (mostly Core and NS3) antibodies by multiplex serology showed 100% sensitivity and 99.6% specificity, and was in very good agreement with the commercial diagnostic assays (kappa, 0.96; 95% confidence interval, 0.92–0.99). The role of antibodies to NS4 and NS5 remains to be evaluated. In Mongolia, overall HCV antibody prevalence was 18.9% (17.8% when age-standardized to the world population). HCV seroprevalence increased with age from 10% in women <30 years to 32% in women ≥50 years, but was not related to sexual risk factors.

Conclusions: The single-step high-throughput multiplex HCV serology assay performs similarly to conventional HCV antibody screening followed by secondary confirmation assays. A very high HCV seroprevalence was confirmed across all socio-economic groups in the female population of Mongolia.

Impact: Multiplex HCV serology facilitates large seroepidemiologic studies of HCV infection. *Cancer Epidemiol Biomarkers Prev; 24(9); 1360–5. ©2015 AACR.*

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Introduction

Global prevalence of hepatitis C virus (HCV) infection is estimated to be approximately 3% (1), with a particularly high burden in many developing countries, including Mongolia (2). The majority of individuals with acute HCV infection are asymptomatic but are unable to clear the virus, resulting in 80% chronic infections. Chronic HCV infection progresses to cirrhosis in about 20% of the patients, who then have a 1% to 4% annual risk of further developing hepatocellular carcinoma (3). However, new HCV treatment paradigms offer very high cure rates with short treatment durations and few side effects, and have the potential to revolutionize the global management of HCV (4).

HCV is divided into six major genotypes which share approximately 65% nucleic acid sequence homology (5), and 80 subtypes based on its extensive genetic heterogeneity. Genotypes 1, 2, and 3 are found throughout the world, genotype 4 is common in North Africa and the Middle East, genotype 5 is common in South Africa, and genotype 6 is endemic in South East Asia (6).

The initial test to screen for HCV infection is based on the use of third-generation enzyme immunoassays (EIA) to detect anti-HCV antibodies against recombinant HCV proteins of the Core, NS3, NS4, and NS5 regions (7) in plasma or serum. Serologic assays cannot discriminate acute from chronic or resolved infection (8), so that EIA positives require the detection of HCV RNA to define active HCV infection. This confirmation step complicates the diagnostic algorithm, especially for limited resource settings. Rapid tests for point of care testing have been developed (9).

We have developed multiplex serology to simultaneously quantify antibodies to arrays of human papillomaviruses (10) and other viral and bacterial infections (11) using Luminex technology. Antigens used are bacterially expressed, affinity-purified glutathione S-transferase (GST) fusion proteins presenting conformational epitopes (12). They are bound to individual sets of fluorescent polystyrene beads, and antigen-loaded bead mixtures are exposed to human serum in a single reaction. For each bead set, the antibody bound to the respective antigen is quantified separately. This assay allows the concurrent analysis of antibodies to up to 100 different antigens and thus provides a high-throughput platform for detection of antibody patterns in large epidemiologic studies.

The present study describes the specific development and validation of a single-step multiplex assay for HCV serology. Five recombinantly expressed, single-step affinity-purified proteins (Core, NS3, NS4A, NS5A, and NS5B) each from the most

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common subtypes of HCV (1a, 1b, 2a) are used as antigens. After validation with reference sera of known HCV status, HCV seroprevalence was determined in a seroepidemiologic study from Mongolia, a country known to have one of the highest HCV prevalences in the world and where hepatocellular carcinoma is the leading cause of cancer death (13).

Materials and Methods

Human sera

Reference sera ($n = 432$) were selected from the diagnostic unit of the Department of Infectious Diseases, University Clinics Heidelberg, Germany. HCV antibody status had been determined first by screening with the AxSYM HCV version 3.0 microparticle enzyme immune assay (MEIA; Abbott Diagnostics) according to the manufacturer's instructions. Sera that were positive or borderline in MEIA (signal/cutoff value between 0.8 and 0.99) were subjected to qualitative RT-PCR for HCV RNA. Samples were regarded as positive only if both MEIA and RNA assay were positive. Sera that were positive or borderline in MEIA and negative in the RNA assay were further investigated by Western blot (INNO-LIA HCV SNS3; Innogenetics). If the INNO-LIA was negative, the sample was regarded as false positive. False-positive (MEIA only positive) sera were deliberately overrepresented among the reference sera.

For the Mongolian study, sera of 1,023 women (median age, 36 years; range, 16 to 63) participating in a previously conducted cross-sectional population-based study in Ulaanbaatar (14) were analyzed. This collection of sera is expected to be well representative of the adult female population of Mongolia. The study was approved by the ethical review committees of the International Agency for Research on Cancer (IARC) and the Ministry of Health in Mongolia, and all study participants signed an informed consent form.

Expression of recombinant GST-HCV-tag proteins in *E. coli*

Full-length coding sequences of Core and nonstructural proteins NS3, NS4A, NS4B, NS5A, NS5B of the three HCV subtypes 1a, 1b, and 2a were expressed as double fusion proteins with an N-terminal GST and a C-terminal tag epitope derived from the large T-antigen of SV40 in *E. coli* (15). Open reading frames were amplified by PCR and cloned into a modified pGEX4T3 vector (15). The PCR primers are listed in Table 1. Plasmids pCV-H77(c) subtype 1a (16), pFK1-9605Con1 subtype 1b (17), and pJ6CF subtype 2a (18) were used as templates.

All clones were verified by commercial sequence analysis (MWG).

Fusion proteins of NS4A and NS5A were expressed in *E. coli* strain BL21 and fusion proteins of Core, NS3, and NS5B in BL21 Rosetta (Invitrogen). Successful full-length antigen expression was verified by anti-tag ELISA as described previously (15).

Despite confirmed correct sequence, no protein expression for all three NS4B constructs was detectable. This lack of expression is probably caused by the very hydrophobic nature of the NS4B proteins (19).

Multiplex serology

Multiplex serology was performed as described previously (10). Briefly, fusion proteins were loaded and affinity-purified on glutathione-casein coupled spectrally distinct fluorescence-labeled polystyrene beads (SeroMap; Luminex). Serum samples were incubated at 1:100 final dilution with pooled antigen-loaded bead sets. Bound antibodies were quantified with biotinylated goat anti-human IgA, IgM, IgG (Dianova), and R-phycoerythrin–labeled streptavidin in a Luminex 100 analyzer as the median R-phycoerythrin fluorescence intensity (MFI) from at least 100 beads of the same bead set. Antigen-specific MFI values were calculated as described (10).

Cutoff definitions for multiplex HCV serology

For each antigen, cutoff values (Table 2) were defined as the mean MFI plus three SDs from 235 reference sera negative in commercial HCV antibody screening assay (MEIA). For NS3 of subtype 2a, one positive outlier was excluded from the cutoff calculation. For all three NS5B proteins, the calculated cutoff was very low and arbitrarily raised to 100 MFI.

A serum showing any subtype-concordant seropositivity for NS3 + Core or NS3 + two other NS proteins was classified as HCV seropositive.

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Statistical analysis

After classification of HCV seropositive and seronegative status, kappa values with their corresponding 95% confidence intervals (CI) were computed to estimate assay concordance and reproducibility. Mann–Whitney nonparametric tests were used to evaluate differences in antibody reactivity (MFI values) with individual antigens between HCV seropositive and seronegative samples.

Odds ratios (OR) for HCV seropositivity and corresponding 95% CI were calculated using unconditional logistic regression adjusted for age, number of births, and number of induced abortions.

All statistical analyses were performed with the software package SAS (SAS Institute Inc.). Graphical representations for overall distributions of net MFI values obtained by multiplex HCV serology were performed with SigmaPlot 11 (SPSS). All tests were performed two-sided, and P values below 0.05 were considered statistically significant.

Results

Assay validation, agreement with commercial ELISA and confirmatory Western blot analysis, and assay reproducibility

A panel of 432 reference sera classified by screening MEIA, qualitative RT-PCR for HCV RNA, and Western blot was used for validation of multiplex HCV serology. Of these sera, 235 had been classified as HCV negative (screening MEIA negative), 64 as confirmed HCV positive (MEIA and RNA positive), and 132 as HCV false positive (MEIA positive but INNO-LIA and RNA negative).

Antibody reactivity against Core and NS3 antigens in multiplex HCV serology showed clear separation between positives versus false positives and negatives (Fig. 1), and the MFI differences were highly significant ($P < 0.0001$). Elevated antibody reactivity against the other nonstructural proteins NS4A, NS5A, and NS5B was also frequent but not consistently present among positive sera, resulting in a substantial overlap of MFI values of the positive group with those of the false positive and negative groups. Despite the overlap, these differences were statistically significant ($P < 0.001$) for all 3 subtypes.

Of the 64 HCV-positive reference sera, all reacted with NS3 of all three subtypes, and 63 with Core of all three subtypes (Table 3).

Table 1. Primers used for generation of recombinant GST-HCV-tag proteins[a,b]

HCV protein	Subtype[c]	Sense	Antisense
Core	1a	CGAGTCGGATCCATGAGCACGAATCCTAAACCTC	CAGGGAACCTTCCTGGTTGCGTCGACTCATGC
	1b	Same as above	Same as above
	2a	Same as above	Same as above
NS3	1a	CGAGTCGGATCCGCGCCCATCACGGCGTAC	GACCTGGAGGTCGTCACGAATTCTCATGC
	1b	Same as above	Same as above
	2a	CGAGTCGGATCCGCCCCCATCACTGCTTAC	CGACCTTGAGGTCATGACGAATTCTCATGC
NS4A	1a	CGAGTCGGATCCAGCACCTGGGTGCTCGTTG	CGATGAGATGGAAGAGTGCGTCGACTCATGC
	1b	CGAGTCGGATCCAGCACCTGGGTGCTGGTAG	Same as above
	2a	CGAGTCGGATCCAGCACATGGGTCTTGGCAG	TGATGAGATGGAGGAATGTGTCGACTCATGC
NS4B	1a	CGAGTCGGATCCTCTCAGCACTTACCGTACA	GGAGTGTACCACTCCATGCGTCGACTCATGC
	1b	CGAGTCGGATCCGCCTCACACCTCCCTTACA	GGACTGCTCCACGCCATGCGTCGACTCATGC
	2a	CGAGTCGGATCCGCCTCTAGGGCGGCTCTCA	GGACTGCCCCATCCCATGCGTCGACTCATGC
NS5A	1a	CGAGTCGAATTCGTCCGGTTCCTGGCTAAGGG	CACGGAAGATGTCGTGTGCTGGAATTCTCATGC
	1b	CAGTCCCCGGGAGTCCGGCTCGTGGCTAAGAG	AGTGAGGACGTCGTCTGCTGCACCCGGGTCATGC
	2a	CGAGTCGGATCCGGCGGCTCGTGGCTCCG	CGACTCCGTCGTGTGCTGCGTCGACTCATGC
NS5B	1a	CGAGTCGGATCCTCAATGTCTTATTCCTGGACAG	CTACCTCCTCCCCAACCGAGTCGACTCATGC
	1b	CGAGTCGGATCCTCGATGTCCTACACATGGACAG	CTATCTACTCCCCAACCGAGTCGACTCATGC
	2a	CGAGTCGGATCCTCCATGTCATACTCCTGGACC	TTCCTACTCCCCGCTCGGGTCGACTCATGC

[a]Restriction sites used for cloning are underlined.
[b]Because all three core constructs were generated with PCR primers matching the 1a sequences, the 1b core construct has three (T845C, G848T, C851T) and the 2a core construct four nucleotide exchanges (A349G, T845C, A847T, C850T), but all of these are silent point mutations.
[c]Isolate pCV-H77C (Accession number AF009606) was used for subtype 1a, Con1 (AJ238799) for 1b, and pJ6CF (AF177036) for 2a.

Decreasing fractions reacted also with NS5A, NS4A, and NS5B. None of these NS proteins reached the sensitivity of NS3 or Core, even when combined for all three proteins and/or subtypes. Five of the sera reacting with Core and NS3 did not react with any of the other NS proteins. The single serum not reacting with Core but with NS3 also recognized NS4A and NS5A of 2 subtypes. Positivity with NS3 and either Core or two other NS proteins of the same subtype was defined as criterion for HCV seropositivity by multiplex serology and yielded 100% sensitivity (98% for dual positivity with NS3 and Core of the same subtype).

Among the negative reference sera, positive reactions with any of the HCV proteins were rare, with 5.1% for any of the three NS5A proteins, and both 3.0% for any of the three NS3 and NS4A proteins. The false-positive reference sera showed higher seroprevalence than the negative sera for each of the proteins, with the highest value for NS5A subtype 1a (24.1%).

Four of the 133 false positive (3.0%) and one of the 235 negative reference sera (0.4%) were discordantly classified by multiplex serology as HCV seropositive (Table 3) yielding specificities of 97% and 99.6%, respectively, for these two groups of reference sera. When the false-positive and negative reference sera were combined as true negative group, the concordance of HCV serostatus classification by multiplex serology versus the commercial screening (EIA) and validation assays (RNA analysis and Western blot) was very high with a kappa value of 0.96 (95% CI, 0.92–0.99).

When 185 sera from the reference panel containing 67 multiplex serology positive sera were retested a day later with identical batches of antigen-loaded beads, 183 were concordantly classified (98.9%) resulting in a kappa value of 0.98 (95% CI, 0.94–1.00), which indicated very good reproducibility of HCV multiplex serology. Both sera discordant upon reproduction belonged to

Table 2. Characteristics of recombinant HCV proteins expressed for multiplex HCV serology

HCV protein	Subtype	Nucleotide[a]	AA homology[b] (%)	R^2[c]	Cutoff[d] (MFI)
Core	1a	342–857	Ref	Ref	1,492
	1b	342–857	(98.3)	0.81	1,456
	2a	341–858	(91.6)	0.79	1,422
NS3	1a	3420–5312	Ref	Ref	371
	1b	3420–5312	(92.6)	0.54	214
	2a	3431–5323	(81.4)	0.82	493
NS4A	1a	5313–5474	Ref	Ref	373
	1b	5313–5474	(92.6)	0.45	356
	2a	5324–5485	(66.7)	0.15	1,249
NS5A	1a	6258–7601	Ref	Ref	287
	1b	6258–7598	(82.1)	0.60	510
	2a	6269–7666	(58.1)	0.64	219
NS5B	1a	7602–9374	Ref	Ref	100
	1b	7599–9371	(88.0)	0.56	100
	2a	7667–9439	(74.8)	0.47	100

[a]Nucleotide: refers to localization in HCV genome of respective subtype.
[b]Percent identical amino acids (AA) compared with subtype 1a as reference (ref).
[c]Square of Pearson correlation coefficient (R^2) of median fluorescence intensities (MFI) in 64 HCV-positive reference sera compared with corresponding protein of subtype 1a.
[d]Cutoff values for each antigen derived from 235 sera HCV antibody negative in commercial MEIA.

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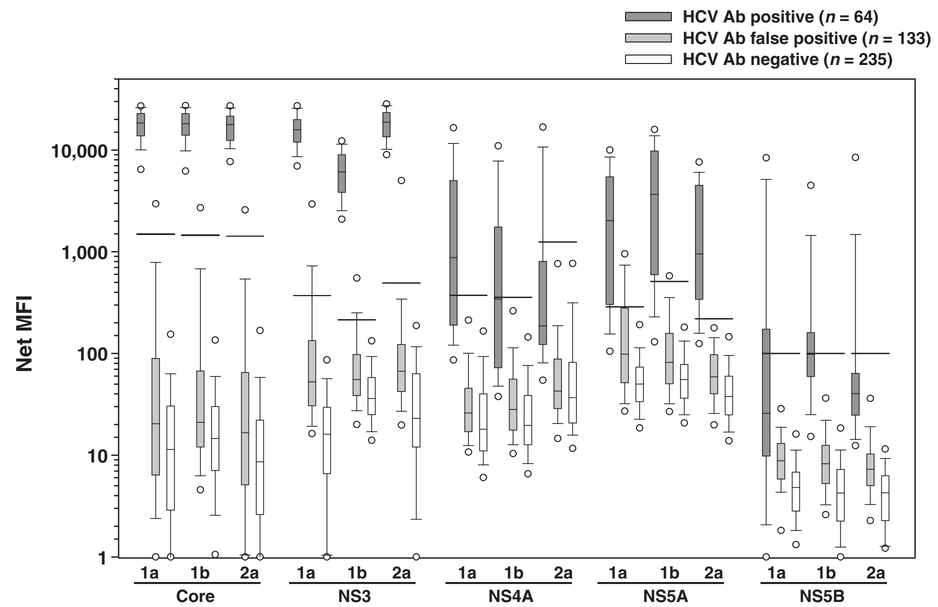

Figure 1.
Box plot of net MFI values obtained by multiplex HCV serology. The line inside the boxes represents the median, and the boxes are delimited by the first and third quartile. Whiskers show the 10th and 90th, and circles the 5th and 95th percentiles, respectively. The distribution of MFI values of the 64 sera positive by commercial HCV serology (MEIA and PCR) are shown in dark grey, of the 133 sera false positive by MEIA in light gray, and of the 235 MEIA-negative sera in white boxes. Horizontal lines indicate the cutoff values for each antigen. All individual antigens showed highly significant (at least $P < 0.001$) differences of positive versus false-positive and -negative sera.

the group of 4 false-positive reference sera that were initially classified positive by multiplex serology.

HCV seropositivity in Mongolian women

HCV serostatus was determined by multiplex HCV serology in 1,023 sera representative for the adult female population of Mongolia. Overall, HCV seropositivity was 18.9% (Table 3), and 17.8% when age-standardized to the world population. All of the 193 HCV seropositive women had antibodies against NS3 and Core, none was defined by the alternative HCV serostatus algorithm using positivity for NS3 and two other NS proteins. None of the three subtypes alone offered 100% sensitivity.

HCV seropositivity sharply increased with age from 10.0% in Mongolian women younger than 30 years to 32.0% in women ≥50 years (OR, 4.15; 95% CI, 2.15–7.99; Table 4). The same age pattern was seen for antibody positivity with individual HCV antigens (P values from <0.0001 to 0.016, data not shown). After adjustment for age, HCV seropositivity showed

Table 3. Antigen specific, type-specific, and overall seropositivity in multiplex HCV serology in the three groups of reference sera and in the female population of Mongolia

		Reference sera classified by commercial assays			Female population of Mongolia
		HCV positive ($N = 64$)	HCV false positive ($N = 133$)	HCV negative ($N = 235$)	All ($N = 1{,}023$)
HCV proteins	Subtype	n (%)	n (%)	n (%)	n (%)
Core	1a	63 (98.4)	8 (6.0)	3 (1.3)	196 (19.2)
	1b	63 (98.4)	7 (5.3)	3 (1.3)	177 (17.3)
	2a	63 (98.4)	9 (6.8)	3 (1.3)	198 (19.4)
	Any	63 (98.4)	9 (6.8)	3 (1.3)	202 (19.7)
NS3	1a	64 (100)	20 (15.0)	4 (1.7)	283 (27.7)
	1b	64 (100)	14 (10.5)	3 (1.3)	217 (21.2)
	2a	64 (100)	10 (7.5)	2 (0.9)	274 (26.8)
	Any	64 (100)	23 (17.3)	7 (3.0)	306 (29.9)
NS4A	1a	37 (57.8)	3 (2.3)	2 (0.9)	66 (6.5)
	1b	30 (46.9)	4 (3.0)	2 (0.9)	55 (5.4)
	2a	13 (20.3)	3 (2.3)	7 (3.0)	23 (2.2)
	Any	39 (60.9)	6 (4.5)	7 (3.0)	72 (7.0)
NS5A	1a	50 (78.1)	32 (24.1)	6 (2.6)	46 (4.5)
	1b	49 (76.6)	7 (5.3)	4 (1.7)	47 (4.6)
	2a	55 (85.9)	3 (2.3)	6 (2.6)	57 (5.6)
	Any	57 (89.1)	39 (29.3)	12 (5.1)	78 (7.6)
NS5B	1a	17 (26.6)	0 (0.0)	0 (0.0)	27 (2.6)
	1b	29 (45.3)	1 (0.8)	0 (0.0)	31 (3.0)
	2a	11 (17.2)	0 (0.0)	0 (0.0)	14 (1.4)
	Any	31 (48.4)	1 (0.8)	0 (0.0)	34 (3.3)
NS3 and core of same subtype	1a	63 (98.4)	2 (1.5)	1 (0.4)	185 (18.1)
	1b	63 (98.4)	2 (1.5)	0 (0.0)	154 (15.1)
	2a	63 (98.4)	4 (3.0)	0 (0.0)	183 (17.9)
	Any	63 (98.4)	4 (3.0)	1 (0.4)	193 (18.9)

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Table 4. Risk factor assessment for HCV seropositivity in Mongolian women

	All N	HCV positive n (%)	OR[a] (95% CI)
Age group			
≤29	350	35 (10.0)	Ref
30–39	265	46 (17.4)	1.62 (0.92–2.83)
40–49	261	66 (25.3)	**2.76 (1.54–4.97)**
≥50	147	47 (32.0)	**4.15 (2.15–7.99)**
Education			
≤8 years	35	5 (14.3)	Ref
10 years	398	83 (20.9)	1.31 (0.48–3.57)
College or higher	585	106 (18.1)	1.13 (0.41–3.10)
Number of births			
0	138	14 (10.1)	Ref
1 to 2	492	96 (19.5)	1.24 (0.68–2.28)
>2	300	75 (25.0)	1.01 (0.50–2.06)
Number of induced abortions			
0	162	20 (12.4)	Ref
1	238	38 (16.0)	0.89 (0.56–1.41)
≥2	391	93 (23.8)	1.12 (0.76–1.67)
Lifetime number of sexual partners			
≤1	431	82 (19.0)	Ref
2	254	48 (18.9)	1.13 (0.75–1.70)
≥3	315	63 (20.0)	1.14 (0.77–1.69)

[a]OR adjusted for age, number of births, and number of induced abortions, when applicable. Statistically significant associations are displayed in bold font.

no association with education level, number of births, number of induced abortions, or life-time number of sexual partners (Table 4).

Discussion

Assay development and validation

We developed and validated a multiplex HCV serology assay comprising 5 antigens from each of the 3 most prevalent HCV subtypes, 1a, 1b, and 2a. Due to the multiplex format, antibodies against these 15 HCV antigens could be determined and quantified simultaneously. Antibody prevalence for individual proteins differed strongly across reference sera, with Core and NS3 being clearly the most immunogenic. Importantly, cross-reactivity to homologous proteins of all three investigated subtypes of Core and NS3 was very high, which may reflect the higher amino acid sequence conservation of these proteins across HCV subtypes as well as the higher antibody titers (indicated by the several-fold higher MFI values, Fig. 1) as compared with the NS4 and NS5 proteins. Indeed, an extreme predominance of HCV genotype 1b in Mongolia (20) is known, but no clear differences could be seen in seroprevalences by subtype. Hence, there was no strong evidence for HCV serotypes, and this assay can be expected to be relevant also for populations infected with HCV genotypes 3 to 6, although direct comparison of the genetic subtype involved in current infections with antibody responses would be needed to fully address this question.

Defining overall HCV seropositivity by including multiple proteins of the same subtype resulted in higher screening specificity in comparison with the commercial HCV antibody screening assay (EIA). However, the exact increase in specificity remains to be determined as the strong reduction of false positives observed in our study is largely based on oversampling this particular group of reference sera. The assay performs very similar to results following confirmation by immunoblot and RNA detection, while still retaining absolute sensitivity (98% when restricting the antigens to Core and NS3 only). Improved specificity over EIA could be due to the fact that multiplex HCV serology includes full-length soluble, non-denatured proteins, in which conformational epitopes are likely present, whereas EIA includes peptide antigens in denatured form. An extended study with concurrent HCV RNA determination as indicator of active infection and inclusion of seroconversion panels should be the next steps to evaluate in detail the sensitivity of this new assay.

Reactivity to NS4 and NS5 proteins varied strongly among HCV seropositives, but it remains to be determined whether specific NS4 and/or NS5 antibody patterns may be associated with infection or disease status.

In conclusion, this HCV multiplex serology assay appears to be a sensitive, specific, and robust assay to detect individuals with current or past HCV infection in a single step. Due to its high-throughput potential and reduced need for confirmatory testing, it provides a useful and cost-effective tool for large epidemiologic studies assessing HCV status. Furthermore, the assay has also been shown to be applicable to dried blood spots (11).

HCV seropositivity and risk factors in Mongolian women

HCV seropositivity in Mongolian women was high and increased with age, in agreement with a recently reported meta-analysis of HCV seroprevalence [9% in the current study vs. 10% (2) for 20–29 years, 17% vs. 12% for 30–39, 25% vs. 21% for 40–49, and 32% vs. 34% for ≥50, respectively). Age-specific prevalences in our study were also similar to the prevalence of HCV RNA in a single large national survey (ref. 20; 10% for 20–29, 12% for 30–39, 24% for 40–49, and 30% for ≥50, respectively).

A strong association between lifetime number of sexual partners and seropositivity to human papillomavirus infection has been previously demonstrated in this study population (14), but was not apparent for HCV seropositivity, suggesting that sexual transmission of HCV is not common in Mongolia. Furthermore, little difference in HCV prevalence was found by education, occupation, or reproductive factors, suggesting that the spread of HCV has been rather similar in different sociocultural groups.

Molecular clock tracing studies suggest that the spread of HCV in Mongolia started with the first mass efforts of vaccination against smallpox in 1927 (21). Iatrogenic transmission of HCV in Mongolia was further sustained by other mass vaccination programs, e.g., Polio (21), blood transfusions (22–24), and extensive use of injection treatments (25). Nosocomial HCV transmission has also been linked to surgery, tattooing, and dental care (20, 26, 27). Part of the steep increase of HCV seropositivity with age is likely to be a birth cohort effect. Compared with the younger generations, women born before 1960 were more heavily exposed to iatrogenic HCV transmission. Screening of blood donations for HCV was introduced in Mongolia in 1997, but is not done systematically, especially in case of emergencies.

The strength of the epidemiologic part of our study is a relatively large sample size with sociodemographic and sexual behavior data, which was absent in previous studies in Mongolia and the use of a highly specific high-throughput HCV serological screening technology. The main limitations are the restriction to the adult female population only, and the absence of information on infection and blood transfusion history.

In summary, our results reemphasize the need for Mongolia to continue to engage in strict measures to prevent the transmission of HCV (i.e., safety of injections and blood transfusions and

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avoidance of unnecessary injections) and to consider the cost effectiveness of the use of new antiviral treatments to cure HCV infection and the avoidance of progression to cirrhosis and HCC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: B. Dondog, P. Schnitzler, M. Pawlita, T. Waterboer
Development of methodology: B. Dondog, K.M. Michael, M. Pawlita, T. Waterboer
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B. Dondog, M. Pawlita, T. Waterboer
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B. Dondog, P. Schnitzler, G. Clifford, M. Pawlita, T. Waterboer
Writing, review, and/or revision of the manuscript: B. Dondog, P. Schnitzler, K.M. Michael, G. Clifford, S. Franceschi, M. Pawlita, T. Waterboer
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