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Note

Chlamydia trachomatis antibody detection in home-collected blood samples for use in epidemiological studies

Hoenderboom B.M.[a,b,*,1], van Ess E.F.[b,1], van den Broek I.V.F.[a], van Loo I.H.M.[c], Hoebe C.J.P.A.[c,d], Ouburg S.[b], Morré S.A.[b,e]

[a] *Epidemiology and Surveillance Unit, Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands*
[b] *Laboratory of Immunogenetics, Department Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, The Netherlands*
[c] *Department of Medical Microbiology, Care and Public Health Research Institute (CAPHRI), Maastricht University Medical Centre (MUMC+), Maastricht, The Netherlands*
[d] *Department of Sexual Health, Infectious Diseases and Environmental Health, South Limburg Public Health Service (GGD South Limburg), Geleen, The Netherlands*
[e] *Institute for Public Health Genomics (IPHG), Department of Genetics and Cell Biology, Research School GROW (School for Oncology & Developmental Biology), Faculty of Health, Medicine & Life Sciences, University of Maastricht, Maastricht, The Netherlands*

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ABSTRACT

Capillary blood collected in serum tubes was subjected to centrifugation delay while stored at room temperature. *Chlamydia trachomatis* (CT) IgG concentrations in aliquoted serum of these blood samples remained stable for seven days after collection. CT IgG concentrations can reliably be measured in mailed blood samples in epidemiological studies.

Implementing *Chlamydia trachomatis* (CT) antibody testing in large cohort studies increases insight in CT infection history. CT-infections are often missed due to the asymptomatic nature of the infection (Center for Disease Control and Prevention, 2014; Davies et al., 2016; Low et al., 2007), however 40–100% of women test positive for CT antibodies after CT-infection (Clad et al., 2000; Gijsen et al., 2002; Horner et al., 2013; Morre et al., 2002). Using antibody testing increases insight in CT infection history compared to only using self-reported CT infection history or test for prevalent infection (Johnson and Horner, 2008). Therefore, the Netherlands Chlamydia Cohort Study (NECCST), a large cohort study determining the risk of CT related complications, uses antibody testing to determine CT infection history (Hoenderboom et al., 2017). To test for CT antibodies in NECCST a low cost, practical and reliable blood collection method was required (Clark et al., 2003).

The most practical collection method is capillary blood drawing by participants at home. Collected blood can be sent back to the laboratory by regular mail (Hoenderboom et al., 2017; Holland et al., 2003). Blood can either be collected on filter paper or in capillary blood collection tubes with serum separator additive. Previously CT IgG was tested for validation using dried blood spots (DBS) versus clinician derived regular serum collected for a study to validate HIV, HBV and syphilis screening. DBS were validated for HIV, HBV and syphilis (van Loo et al., 2017), but failed when we tested for CT IgG. High background distortion gave false positive results (89–100%) when compared to regular serum in assays from two different manufacturers (Medac, Wedel, Germany and Savyon Diagnostics, Israel). By varying the dilution factor, failure rates were reduced but still up to 25%. Because of the high failure rates in DBS, we switched to an evaluation of collecting capillary blood in collection tubes as we were then able to perform the CT IgG test with regular serum (Liu et al., 2017).

Liquid capillary whole blood sent by regular mail cannot be separated from cells within the recommend 2 h after blood is collected. Separation delay could result in clot-induced changes, possibly altering the analyte concentration in serum (Holland et al., 2003). Previous studies on delayed separation of whole blood showed that for a variety of analytes, samples could be stored for up to a week with only slight concentration alterations (Clark et al., 2003; Ikeda et al., 2015).

We determined CT IgG stability in three paired capillary blood samples newly collected in serum gel tubes (BD Microtainers with clot activator and serum separator gel) exposed to room temperature for 2 h, four days and seven days prior to centrifugation to simulate mail times. If CT IgG stability remained stable after four and seven days at room temperature, CT IgG concentrations could reliably be used in epidemiological studies on *Chlamydia trachomatis*.

Samples were collected from NECCST's participants. In NECCST,

* Corresponding author at: Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands.
E-mail address: Bernice.Hoenderboom@rivm.nl (B.M. Hoenderboom).
[1] Shared first authorship.

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Table 1
Baseline sample CT IgG outcomes compared to CT IgG outcomes from samples stored for four and seven days.

Baseline sample (t0)	Four days delay (t1) *n*(%)			*N*	Seven days delay (t2) *n*(%)			*N*
	Positive	Grey-zone	Negative	Total	Positive	Grey-zone	Negative	Total
Positive	13 (100.0)	0 (0.0)	0 (0.0)	13	14 (93.3)	1 (6.7)	0 (0.0)	15
Grey-zone	2 (50.0)	2 (50.0)	0 (0.0)	4	1 (25.0)	3 (75.0)	0 (0.0)	4
Negative	0 (0.0)	1 (7.7)	12 (92.3)	13	0 (0.0)	0 (0.0)	14 (100.0)	14
Total	15 (50.0)	3 (10.0)	12 (40.0)	30	15 (45.5)	4 (12.1)	14 (42.4)	33

women with and without a positive CT infection history are prospectively followed for at least 10 years until 2022 (Hoenderboom et al., 2017). A preselected subset of women with known CT infection history (positive/negative) was asked to participate in this validation study. We aimed for at least 20 respondents. The study was approved by the Medical Ethical Committee Noord-Holland, Alkmaar (NL 51553.094.14/M014-042).

To test CT IgG stability, three paired samples were collected during one appointment by a medical professional at the VU University medical center in Amsterdam, The Netherlands, or at participant's home. Blood was collected via finger prick in BD Microtainer® blood collection tubes with clot activator and serum separator additive (SST) (Ref. 365967). We aimed for at least five droplets of whole blood per collection tube. The collected blood was stored at room temperature (21 °C) before centrifugation for different time intervals based on (delayed) mail delivery time. The first sample (the baseline sample, t0) was centrifuged in accordance with the guideline standard of 2 h (t0), a second sample was stored for either 3, 4 or 5 days (t1), and the last sample was stored for seven days (t2). Following centrifugation, aliquoted serum was stored at − 20 °C until analysis. Prior to analysis, frozen serum samples were thawed at room temperature and inverted several times. To avoid run-to-run variability, serum samples from all time points (t0, t1 and t2) per participant were analyzed together in one batch and all samples were tested in duplicate.

The CT IgG ELISA plus (CT IgG ELISA plus; Medac, Wedel, Germany) assay was used to test for CT antibodies. This is a quantitative peptide based serological assay. The ELISA was used according to the manufacturer's instructions. The mean outcome of the duplicates per sample was used in further analyses. Outcomes were reported as negative (IgG concentration < 22 AU/ml), grey-zone (IgG concentration 22–28 AU/ml), or positive (IgG concentration ≥ 28 AU/ml), and quantitative in IgG concentration (AU/ml).

Laboratory results were entered in Microsoft Excel and analyzed using STATA, (version 14.2; StataCorp, College Station, TX, USA). Samples centrifuged at different time intervals (t1 and t2) were compared to the baseline sample (t0). Results were first analyzed as ordinal (i.e. negative, grey-zone and positive) using Kappa values, which indicates the level of agreement. And for calculation of the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), grey-zones were classified as positive in accordance with NECCST protocol (Hoenderboom et al., 2017). Second, IgG concentrations from paired samples were analyzed using r2's to indicate the proportion of the variance between the baseline samples versus other time points. Additionally, Bland-Altman plots were constructed to analyze the agreement between the different time points in CT IgG concentration (Altman and Bland, 1983). For the Bland Altman plots upper and lower agreement limits were predefined as the margin of the grey-zone, which is − 6 and 6 AU/ml (Bland and Altman, 1999; Giavarina, 2015). We chose 6 AU/ml because paired samples may not differ > 6 AU/ml otherwise negative samples could turn positive and vice versa.

Due to the association between the difference of paired samples and the size of the measurements, raw data (including upper and lower agreement limits) were log transformed for use in Bland Altman plots (Scott et al., 2003).

In total, we obtained blood from 35 women. The average age was 31.6 years (range 24–37 years) and the majority was of Western ethnicity, 77%. Twenty-one women (60%) had a positive CT infection history either by previous positive test result or self-reported. One woman had a gonorrhoea infection in the past. We collected 98 samples, an average of 2.8 per woman. Seven women provided only two paired samples instead of three; the second sample was stored for either four days (n = 2) or seven days (n = 5). The amount of whole blood collected was generally small resulting in samples with a median of 40 μl serum (IQR 28.5–50.0 μl) per collection tube. Of the 35 baseline samples, 15 (42.9%) were CT IgG positive, 5 (14.3%) were grey-zones and 15 (42.9%) were negative.

Kappa values between t0 samples and, t1 and t2 samples indicated high level of agreement: 0.83 and 0.90 respectively. Three samples from the t1 and two samples from the t2 differed in outcome from t0 (Table 1). When grey-zones were classified as positive, only one sample was discordant. Using the latter definition sensitivity, specificity, PPV and NPV were calculated (Table 2).

Fig. 1 shows the concentration of the baseline samples compared to the concentration of the t1 and t2 samples, r2's were both 0.99. Bland Altman plots show all values to be within the predefined limits of agreement.

This is the first study to investigate stability of CT IgG concentration in samples with separation delay stored in BD Microtainers. The SST tubes cause blood to clot rapidly, enabling the separation of the blood clot from serum (Bowen and Remaley, 2014). The high sensitivity, specificity, PPV, and NPV and r2's of almost one showed that CT IgG concentrations in serum remain stable up to seven days after whole blood collection. Therefore, this method can be used in epidemiological studies.

In this study, samples were stored in the laboratory to simulate delayed mail times, but kept in temperature controlled rooms. In the actual NECCST cohort, samples are sent using regular mail; this means

Table 2
Sensitivity, specificity, positive predictive value and negative predictive value.

[a]	*N*	True positive (*n*)	False positive	True negative	False negative	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
t1	30	17	1	12	0	94	100	100	92
t2	33	19	0	14	0	100	100	100	100

[a] For these analyses grey zone values were considered as positive values. PPV = positive predictive value. NPV = negative predictive value.

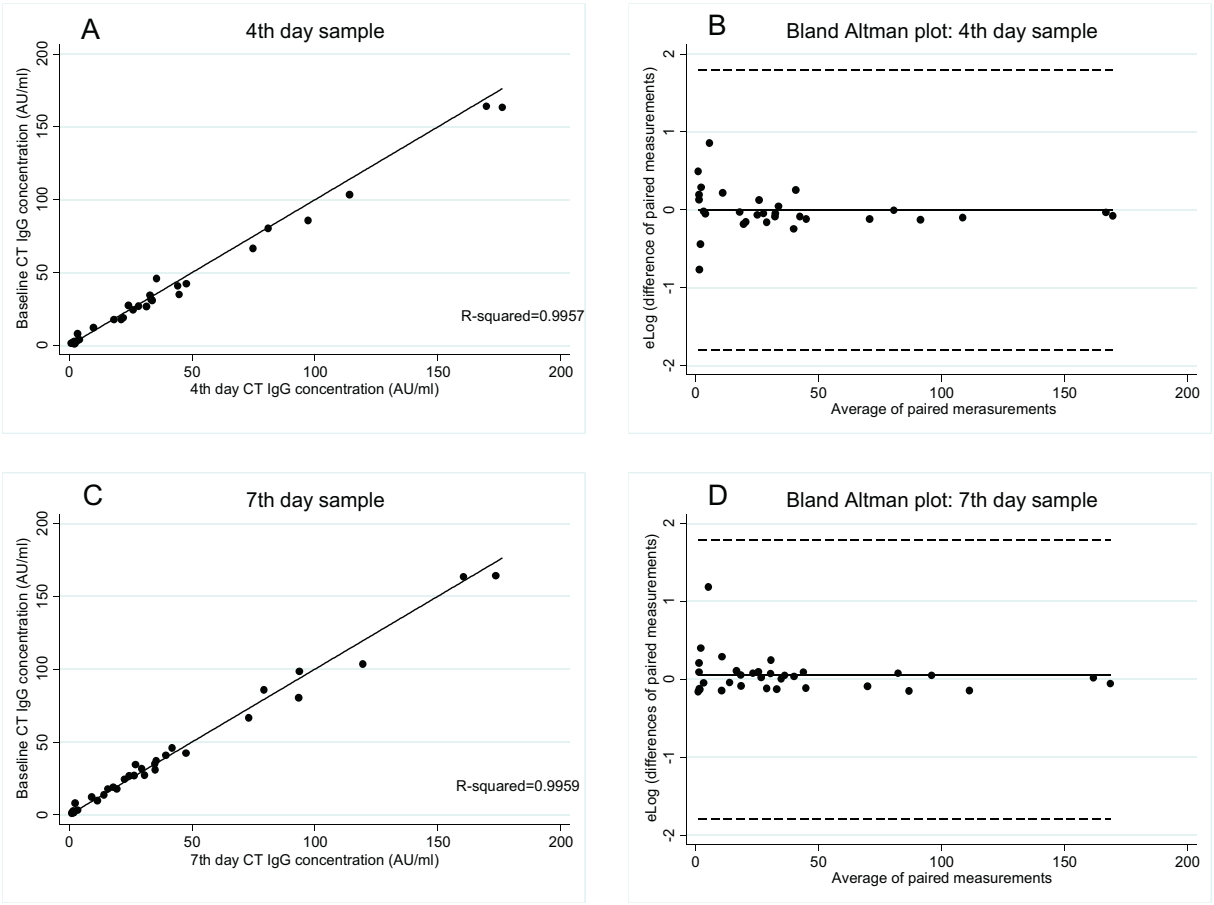

Fig. 1. Graphs A and C show scatterplots with regression lines of baseline sample concentrations plotted against 4th day (A) and 7th day (C) delayed samples including r-squared coefficients. Graphs B and D show Bland-Altman plots, based on log-transformed data, in which elog difference between the baseline sample and 4th day (B) or 7th day (D) sample concentration were plotted against the average of paired measurements. Upper and lower limits (dashed lines) in the Bland-Altman plots were based on clinically relevant elog(− 6 and 6 AU/ml) values.

samples will be exposed to more variable temperatures, including day/night and day-to-day differences, which may result in lower stability. However, other studies determining the effect of centrifugation delay on a variety of analytes that compared various storage temperatures, also showed that total protein levels, including IgG levels, often only change by a few percentages after 24 h to seven days of delay (Clark et al., 2003; Tanner et al., 2008).

Home-collected biological materials such as urine samples and vaginal swab samples are already implemented in CT studies and screening (Doshi et al., 2008; Hocking et al., 2013; Morre et al., 1999; van den Broek et al., 2012). Home-sampling increases participation rates and provides a less costly alternative for clinic-testing. Even though only small amounts of blood can be collected, in general this proved to be enough to perform reliable serology tests for detection of CT IgG antibodies. Following results of this study, we conclude blood collected at home in collection tubes and sent to laboratories can reliably be used for *Chlamydia trachomatis* IgG measurements.

Conflicts of interest

None.

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Availability of data and materials

On request, (anonymized) data and available biological material can be provided for research related to STI, after approval by an advisory committee.

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