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RESEARCH ARTICLE

Evaluation of a PGP3 ELISA for surveillance of the burden of Chlamydia infection in women from Australia and Samoa

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One sentence summary: The PGP3 ELISA has potential for sero-epidemiological studies of current and/or past chlamydial infection of women in a variety of settings, including high prevalence.

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ABSTRACT

Serological assays can be used to investigate the population burden of infection and potentially sequelae from Chlamydia. We investigated the PGP3 ELISA as a sero-epidemiological tool for infection or sub-fertility in Australian and Samoan women. The PGP3 ELISA absorbance levels were compared between groups of women with infertility, fertile, and current chlamydial infections. In the Australian groups, women with chlamydial tubal factor infertility had significantly higher...
absorbance levels in the PGP3 ELISA compared to fertile women ($P < 0.0001$), but not when compared to women with current chlamydial infection ($P = 0.44$). In the Samoan study, where the prevalence of chlamydial infections is much higher there were significant differences in the PGP3 ELISA absorbance levels between chlamydial sub-fertile women and fertile women ($P = 0.003$). There was no difference between chlamydial sub-fertile women and women with a current infection ($P = 0.829$). The results support that the PGP3 assay is effective for sero-epidemiological analysis of burden of infection, but not for evaluation of chlamydial pathological sequelae such as infertility.

**Keywords:** chlamydia; ELISA; Sero-epidemiology; serology; Sub-fertility; tubal factor infertility

**INTRODUCTION**

*Chlamydia trachomatis* is the most common bacterial sexually transmitted infection worldwide. Infections can result in serious sequelae such as pelvic inflammatory disease, tubal factor infertility (TFI) and ectopic pregnancy (reviewed (Menon et al. 2015)). Estimating the population attributable risk of these sequelae is difficult as they have multiple aetiologies and are often not diagnosed until sometime after infection (Menon et al. 2015). Serological assays have the potential to be used in population studies to estimate the burden of sequelae attributable to chlamydia, and to evaluate and monitor health interventions.

Ades et al. 2017 evaluated whole organism immunofluorescence titres (WIF) across a 10-year period, for women undergoing care for tubal factor infertility (Ades et al. 2017). Titres were analysed using a finite mixture models approach to estimate the population excess fraction of chlamydial TFI (case-control study of TFI compared with female infertility from other causes) to be 28% (95% credible interval [CrI]: 5%–95%) and maximum of 46.8% (95% CrI: 23.3%–64.1%) (Ades et al. 2017). The population burden beyond the infertility setting remains less well characterised, and relatively few sero-epidemiological studies have included fertile women as controls (Menon et al. 2016a).

Numerous studies have shown that chlamydial seropositivity, in infertile women has been significantly associated with laparoscopically diagnosed TFI (Gijsen et al. 2002; Akande et al. 2003; Land et al. 2010). However, the sensitivity and specificity of the assays used vary considerably. Recent studies in the UK have demonstrated that the Pgp3 ELISA (enzyme linked immunosorbent assay) (Wills et al. 2009; Horner et al. 2016) could be used for such monitoring and evaluating (Horner et al. 2013; Horner et al. 2016; Woodhall et al. 2017). Here, we explored the Pgp3 ELISA as a surveillance assay in women from Australia and Samoa who have infertility/sub-fertility, fertility or current infection (Huston et al. 2010; Walsh et al. 2015; Menon et al. 2016a; Menon et al. 2016b).

**MATERIALS AND METHODS**

**Study design and participant groups**

The study aimed to evaluate if the PGP3 ELISA could be used for estimating the burdens of chlamydial infertility and/or infection in women from Australia and Samoa. We tested absorbance levels in the PGP3 ELISA using a 1/100 serum dilution, from previously described samples (Huston et al. 2010; Walsh et al. 2015; Menon et al. 2016a; Menon et al. 2016b). Participants from Australia ($n = 302$) were categorised into four groups. Group 1 was infertile women with greater than a year of trying to conceive, who all had laparoscopic investigations for tubal occlusion (Menon et al. 2016a). Past chlamydial infection measured by MIF was used to define chlamydial TFI. Group 2 were fertile women attending antenatal care, who had never had assisted reproductive technologies and whose current pregnancy took less than 1 year to conceive (Menon et al. 2016a). Group 3 were women attending University General Practice or Sexual Health Clinics with a NAAT confirmed chlamydial infection (Huston et al. 2010; Menon et al. 2016a) (serum was typically collected when participants returned for treatment, within 1 week). Group 4 was a separate group of infertile women, in this group chlamydial sero-positivity (MIF) and tubal factor infertility diagnosis were used to define chlamydial TFI (Menon et al. 2016a). Group 5 ($n = 239$) were from a previously described study in Samoa (high chlamydia prevalence) where urine for PCR and blood for serum was collected at the same time (Walsh et al. 2015; Menon et al. 2016b). The serological results from Samoan women were analysed using two groupings (Table 1). First, based on epidemiological data, and previous serological results (MIF), they were categorised into chlamydial sub-fertile or fertile. The second analysis was by grouping the participants from Samoa into women who had a infection confirmed by NAAT compared to currently NAAT negative women.

**ELISA Protocol and analysis**

PGP3 ELISA was conducted as previously described (Wills et al. 2009), with the exception that the blocking agent was skim milk powder (0.5%). Samples were re-tested or excluded when the standard error deviated by more than 5% within the replicates (mean of replicates were analysed). All analysis was conducted in IBM SPSS V 25.

**Ethics statement**

The study was reviewed by Human Research Ethics Committees and each participant provided informed written consent. Human Research Committee Ethical Approvals include: Monash Private Surgical Human Research Ethics Committee (HREC) (12 099); UC Health HREC (1221); Prince Charles Hospital HREC (EC2809); Ipswich and West Moreton Health Services District HREC (10-09), Gold Coast Hospital District HREC (200 893); Cairns Sexual Health HREC (09/QCH/4–554); Queensland University of Technology HREC (080000268); and University of Technology Sydney HREC (201500699), and initial ethical approval was from National University of Samoa, Samoa National Health Service Board approval for the use of the Laboratory and staff, and the Samoa Ministry of Women approved the village based survey, and the Samoan Ministry of Health.

**RESULTS**

The PGP3 ELISA absorbance level was significantly higher when chlamydia infertile (or sub-fertile) women were compared with women who were infertile (or sub-fertile) for other reasons ($n = 11, n = 86$ respectively, Group 1: $P < 0.0001$) in only one of two groups in Australia (Table 1). Women who were categorized as chlamydia sub-fertile from Samoa had a significantly higher absorbance than the rest of that population ($n = 29 A = 2.9,
## Table 1. PGP3 ELISA absorbance in subfertile, infected and fertile groups in Australia and Samoa.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fertility status, serological status or infection status</th>
<th>Age (Range)</th>
<th>P-value*</th>
<th>Absorbance (SEM)</th>
<th>p-adjj</th>
<th>Fertile controls (Group 2) p-adjj</th>
<th>Chlamydial infection (Group 3) p-adjj</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Australia (n = 302)</strong></td>
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<tr>
<td>Infertile Group 1**</td>
<td>Chlamydial tubal infertility TFI and CT MIF⁺ (n = 11)</td>
<td>36.6 (27–45)</td>
<td>0.539</td>
<td>1.79 (0.35)</td>
<td>0.05</td>
<td>P &lt; 0.0001*</td>
<td>1.0</td>
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<td></td>
<td>Other infertility tubal patency and/or MIF⁻ (n = 86)</td>
<td>36.6 (26–48)</td>
<td></td>
<td>0.25 (0.046)</td>
<td>0.003</td>
<td>P &lt; 0.0001*</td>
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<tr>
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<td>Chlamydial tubal infertility TFI and CT MIF⁻ (n = 5)</td>
<td>36.2 (30–42)</td>
<td>0.715</td>
<td>0.90 (0.55)</td>
<td>1.0</td>
<td>0.344</td>
<td>0.124</td>
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<tr>
<td></td>
<td>Other infertility (n = 68)</td>
<td>36.3 (28–45)</td>
<td></td>
<td>0.24 (0.049)</td>
<td>0.005</td>
<td>P &lt; 0.0001*</td>
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<td></td>
<td>Fertile controls (Group 2)</td>
<td>34.9 (27–43)</td>
<td>P &lt; 0.01*</td>
<td>0.18 (0.062)</td>
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<td></td>
<td>Pregnant fertile women (n = 53)</td>
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<td>Chlamydial infection (group 3)</td>
<td>29.6 (20–52)</td>
<td></td>
<td>2.10 (0.18)</td>
<td>P &lt; 0.0001*</td>
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<td>Samoa NAAT (n = 79)</td>
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<tr>
<td>Samoa Group 5**</td>
<td>Samoa sub-fertile—Chlamydia sero-positiveb (n = 29)</td>
<td>23.2 (20–29)</td>
<td>0.068</td>
<td>2.904 (0.228)</td>
<td>0.016*</td>
<td></td>
<td>1.0</td>
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<td></td>
<td>Othersb Sub-fertile and sero-negative or fertile (n = 134)</td>
<td>23.6 (18–29)</td>
<td></td>
<td>2.124 (0.101)</td>
<td></td>
<td>P &lt; 0.0001*</td>
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<tr>
<td></td>
<td>Samoa—Chlamydia NAAT-positivec (n = 86)</td>
<td>23.3 (18–29)</td>
<td>0.265</td>
<td>3.160 (0.086)</td>
<td>P &lt; 0.0001*</td>
<td>P &lt; 0.0001*</td>
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<td></td>
<td>NAAT-negatived (n = 153)</td>
<td>23.8 (18–29)</td>
<td></td>
<td>1.677 (0.119)</td>
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<tr>
<td><strong>Samoa (n = 239)</strong></td>
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*Age was tested using Wilcoxon signed-rank test, the difference in absorbance between the groups was tested adjusting for age using linear univariate analysis with Bonferroni post test.

**Group 1: Fertility clinic attendees all undergoing IVF or other ART treatments with greater than 1 year of trying to achieve pregnancy. All have had tubal investigations using either laparoscopy or HSG (hysterosalpingogram) Defined as chlamydial tubal factor infertility by confirmed tubal infertility and MIF IgG positive serology ≥1:64 titre (Menon et al. 2016a). Negatives were either tubal patent and/or chlamydial MIF sero-negative.

bMIF- microimmunofluorescence serology positive at >1:64.

cGroup 4: Women attending a fertility clinic for IVF or other ART treatments with past history of greater than 1 year of trying achieve pregnancy. Chlamydial infertility was defined by tubal factor confirmation (HSG or laparoscopy) and MIF positive serology. Negative where other known infertility factors or tubal factor status unknown and/or MIF negative.

dGroup 2: Fertile controls women who were pregnant within less than a year of trying with no previous history of assisted reproductive technologies, recruited from an antenatal clinic.

eGroup 3: Women recruited from sexual health clinics or university general practice with positive diagnosis for Chlamydia trachomatis, confirmed by Nucleic Acid Amplification Test (NAAT).

fGroup 5: Women defined as sub-fertile or fertile based on nurse-administered questionnaire of whether or not have tried to become pregnant and success or not (Menon et al. 2016b). Only women sexually active for over a year without contraceptive included in the study.

gGroup 5: Women recruited during a sero-epidemiological study of sub-fertility and chlamydial infection in Samoa (Walsh, Hope et al. 2015; Menon et al. 2016a). Defined as sub-fertile or fertile based on nurse-administered questionnaire of whether or not have tried to become pregnant and success or not (Menon et al. 2016b), only women sexually active for over a year without contraceptive included in the study.

hGroup 5: Women defined as sub-fertile or fertile as described above, however, here we tested for sub-fertile and sero-positive in the MEDAC Infertile ELISA (cHSP60 and MOMP) to differentiate from fertile and sub-fertile but not sero-positive. Not all women had reliable serological assays in this group for the MEDAC tests, where as NAATs were conducted on all women in this group (see i).

iGroup 5: Sub-fertile and NAAT positive for current infection were compared to sub-fertile and NAAT negative or fertile.

jP-value has been adjusted using the Bonferroni method for false discovery rate based on age differentiation.
n = 134 A = 2.12, respectively; P = 0.016) (Table 1). In both Australian and Samoan women, the PGP3 ELISA absorbance levels in women with chlamydial sero-positivity by MIF and who had TFI or sub-fertility were not significantly different from women with current infections (Table 1). However, the PGP3 ELISA absorbance level was significantly higher in women in Australia with a confirmed infection (Group 3, n = 79) compared to all groups except chlamydial tubal factor infertility (Table 1). Similarly in the participants recruited in Samoa, women with a current infection had high PGP3 ELISA absorbance levels, that were significantly different from women who were currently infected (mean Abs 3.16, P < 0.0001, n = 86, n = 153). Furthermore, this was also higher than infected participants in Australia (mean Abs: 2.10, P < 0.0001) likely due to the higher prevalence of Chlamydia and lower access to testing and treatment in Samoa.

**DISCUSSION**

Whilst diagnosis of current chlamydial infections is effective using NAATs, detection of and population level understanding of the burden of infection and disease sequelae is not possible using NAATs. Here, we present an evaluation of the PGP3 ELISA in the Australian and Samoan context comparing chlamydial infertility, fertile and current infection groups. We report that the PGP3 ELISA absorbance levels were significantly different in chlamydial infertility or sub-fertile women compared with fertile women in Australia, but only in one fertility clinic setting and not in another. However, the numbers were small in one group (n = 5). Additionally, as we used MIF to define Chlamydia infertility, which is reported to have variability in the sensitivity and specificity depending on the reagent preparation and which have Chlamydia pneumoniae cross-reactivity could mean some of these participants are mis-assigned (Clad et al. 1994; Akande et al. 2003). Previously, such cross-reactivity has been resolved using multiple assays, such as PGP3, but given we are assessing PGP3 in this context we can only interpret this result with caution (Ades et al. 2017). There was also significant difference when comparing chlamydial sub-fertile women in Samoa with fertile or sub-fertile for other reasons (P = 0.016). However, in both settings chlamydial infertility or sub-fertile women had PGP3 ELISA absorbance levels that were not significantly different from women with current infections. The groups of women with current infections in both Samoa and Australia had significantly higher absorbance levels than all other groups (apart from chlamydial infertility) supporting that infectious burden can be successfully evaluated using this assay in population studies.

One limitation of this study is that we used a single antibody dilution and conducted all measures on absorbance levels, rather than antibody titres. This method is likely to underestimate the differences in serum antibody levels because antibody responses are not linear. It is important that more work is conducted in high prevalence setting such as Samoa, as our study is too small to draw conclusions, although the findings support implementation of the PGP3 ELISA as a sero-epidemiological tool. Aln neither the higher or lower prevalence groups of women tested here, did we see a consistent significant difference in the absorbance between women with a current infection and chlamydial infertility or sub-fertility, and in future serological studies using this ELISA it should be considered that positive result could indicate current or recent infection. However, there are several studies that report specific, but not sensitive antigens for chlamydial infertility or pathology (e.g. CT117/CT223 for Ct and cancer; HtRa and OmcB for infertility; HtRa and TroA for pathology; and CT443 and CT381 for infertility; and 11 different peptides for infertility), and it could be that a double or multiple antigen approach including PGP3 with these antigens could be implemented to measure population burdens of chlamydial infertility (Menon et al. 2016b) (Stansfield et al. 2013) (Rodgers et al. 2013; Hokynar et al. 2017; Hufnagel et al. 2018; Rahman et al. 2018). Overall, the data presented here add to the evidence that the PGP3 ELISA could be an effective sero-epidemiological tool to evaluate the burden of chlamydial infection in women in a population.

**CONCLUSIONS**

The data presented here means we now have a global dataset (in conjunction with the recent UK studies (Horner et al. 2013; Woodhall et al. 2017)) supporting that the PGP3 ELISA has potential for sero-epidemiological studies of current and/or past chlamydial infection of women. We propose the assay could be used for longitudinal sero-epidemiological monitoring of public health intervention programs to control and reduce Chlamydia.

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**Conflict of interest.** None declared.

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