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

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Keywords:

Serology

Sub-fertility

Chlamydia

Tubal factor infertility

ELISA

Sero-epidemiology

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.

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.

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.*, 2016).

Numerous studies have shown that chlamydial sero-positivity, 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.*, 2016, Menon *et al.*, 2016).

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.*, 2016, Menon *et al.*, 2016). 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.*, 2016). 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 one year to conceive (Menon *et al.*, 2016). 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.*, 2016) (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.*, 2016). 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.*, 2016). 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) (12099); UC Health HREC (1221); Prince Charles Hospital HREC (EC2809); Ipswich and West Moreton Health Services District HREC (10-09), Gold Coast Hospital District HREC (200893); Cairns Sexual Health HREC (09/QCH/4–554); Queensland University of Technology HREC (080000268); and University of Technology Sydney HREC (2015000699), 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, 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 infertile, fertile, and current infection groups. We report that the PGP3 ELISA absorbance levels were significantly different in chlamydial infertile 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 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 chlamydia infertile 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 infertile) 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. AIn 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.*, 2016) (Stansfield *et al.*, 2013) (Rodgers *et al.*, 2011, 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.

ACKNOWLEDGEMENTS

The authors acknowledge previous contributions to the collection and handling of the serum samples by Shruti Menon, Benignus Logan, and Scott Stansfield. Patrick Horner is a member of the NIHR Health Protection Research Unit in Evaluation of Interventions, University of Bristol, UK.

FUNDING

This work was funded by University of Technology Sydney, Faculty of Science funding awarded to W Huston.

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Table 1. PGP3 ELISA absorbance in subfertile, infected and fertile groups in Australia and Samoa

	Groups	Fertility status, serological status or infection status	Age (Range)	p-value*	Absorbance (SEM)	p-adj[j]	Fertile controls (Group 2) p-adj[j]	Chlamydial infection (Group 3) p-adj[j]
Australia (n=302)	Infertile Group 1[a] n=97	**Chlamydial tubal infertile** TFI and CT MIF[b] + (n=11)	36.6 (27-45)	0.539	1.79 (0.35)	0.05	p<0.0001*	1.0
		Other infertility tubal patency **and/or** MIF- (n=86)	36.6 (26-48)		0.25 (0.046)		0.003	p<0.0001*
	Infertile Group 4[c] n=73	**Chlamydial tubal infertile** TFI and CT MIF[b] + (n=5)	36.2 (30-42)	0.715	0.90 (0.55)	1.0	0.344	0.124
		Other infertility (n=68)	36.3 (28-45)		0.24 (0.049)		0.005	p<0.0001*
		Fertile controls (Group 2) Pregnant fertile women (n=53)	34.9 (27-43)	p<0.01*	0.18 (0.062)	p<0.0001*		
		Chlamydial infection (group 3) NAAT (n=79)	29.6 (20-52)		2.10 (0.18)			

						Samoa Chlamydia sero-positive[h]	**Samoa NAAT positive**
Samoa Group 5 (n=239)	**Samoa sub-fertile – Chlamydia sero-positive[h]** (n=29)	23.2 (20-29)	0.068	2.904 (0.228)	0.016*		1.0
	Others[h] Sub-fertile and sero-negative or fertile (n= 134)	23.6 (18-29)		2.124 (0.101)			p<0.0001*
	Samoa– Chlamydia NAAT-positive [i] (n=86)	23.3 (18-29)	0.265	3.160 (0.086)	p<0.0001*	p<0.0001*	
	NAAT negative [j] (n=153)	23.8 (18-29)		1.677 (0.119)			

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