Supplementary Materials: Antibodies against *Chlamydia trachomatis* and ovarian cancer risk in two independent populations

Supplementary Methods:

***C. trachomatis* pGP3 assay validation**

C. trachomatis Multiplex Serology based on antigen pGP3 (aa 1-267, NCBI Reference Sequence YP_328713.1) was validated against a combination of two enzyme-linked immunosorbent assays (ELISA) also based on pGP3, an indirect ELISA (1) and a double-antigen sandwich ELISA (2). Reference assay *C. trachomatis* serostatus of all specimens was determined by a combination of both pGP3 ELISAs (3).

In total, 192 reference sera were kindly provided by Dr. Sarah Woodhall (PHE) through UK Biobank, represented by Dr. Alexander Mentzer (University of Oxford) acting as a trusted third party.

For validation of pGP3 Multiplex Serology, reference samples were tested at serum dilutions 1:100 and 1:1000 at the German Cancer Research Center (DKFZ) as previously described (4). Testing took place while DKFZ was still blinded for reference assay serostatus. After independently transferring both gold-standard (PHE) and DKFZ results to Oxford, all parties were unblinded with regard to reference assay serostatus (n_{pos} = 99, n_{neg} = 93). Statistical analysis included graphical representation of results (Supplementary Figure 1), contingency tables and calculation of sensitivity, specificity, and agreement (Supplementary Table 2).

Development and validation of *M. genitalium* multiplex serology

M. genitalium multiplex serology was validated against an enzyme-linked immunosorbent assay (ELISA) based on lipid-associated membrane proteins (LAMP) extracted from *M. genitalium* (5). Antigens for *M. genitalium* multiplex serology were selected based on known immunogenicity of adhesion protein MgPa (NCBI reference sequence WP_010869366) (6). Two unconserved regions of the protein were selected to avoid cross-reactive antibody responses originating from the homologous protein P1 of *M. pneumonia* (6): MgPaN (aa 31-370) and rMgPa (aa 1,075-1,352).

In total, 71 reference sera were kindly provided by Dr. Annika Idahl (Umeå University, Sweden).

For validation, reference samples were tested at serum dilution 1:100 as described elsewhere (4). Testing and first data evaluation took place blinded for reference assay serostatus. For further statistical analysis, reference assay serostatus (n_{pos} = 35, n_{neg} = 36) was unblinded.

Both, MgPaN and rMgPa discriminate well between LAMP-ELISA positive and negative reference samples (Supplementary Figure 2). Optimal cut-offs (500 MFI) were identified by ROC-curve analysis (Prism 6, GraphPad Software Inc., La Jolla, CA, USA). Applying these cut-offs, sensitivity of MgPaN and rMgPa was high in comparison to LAMP-ELISA with 97.1% and 100%, respectively (Supplementary Table 3). Specificity was lower with 88.9% and 77.8%. Positivity to both proteins increased specificity to 94.4%, while sensitivity remained high with 97.1% and a very good agreement with LAMP-ELISA (kappa: 0.92; 95% CI: 0.82-100).

Assay quality control

For quality control, we used a bead set with the viral capsid (VP1) of the BK virus, a ubiquitous polyomavirus with almost universal positive antibody status and a bead set without antigens for background determination.

Cases and matched controls from each study were included on the same plate, which included 92 study samples. Sera were tested at 1:100 serum dilution and there were no issues with assay detection limits. To evaluate assay performance, we included blinded duplicate samples from three study participants within and across each plate. The average concordance in marker positivity within and across plates was high (mean 97.7%), ranging from 87.5% to 100%. The intraclass correlation coefficients (ICC) for the continuous MFI levels were also high (mean 92.2%), ranging from 86.3 to 100%.

REFERENCES

1. Wills GS, Horner PJ, Reynolds R*, et al.* Pgp3 antibody enzyme-linked immunosorbent assay, a sensitive and specific assay for seroepidemiological analysis of Chlamydia trachomatis infection. *Clin Vaccine Immunol.* 2009;16(6):835-43.
2. Horner PJ, Wills GS, Righarts A*, et al.* Chlamydia trachomatis Pgp3 Antibody Persists and Correlates with Self-Reported Infection and Behavioural Risks in a Blinded Cohort Study. *PLoS One.* 2016;11(3):e0151497.
3. Woodhall SC, Wills GS, Horner PJ*, et al.* Chlamydia trachomatis Pgp3 Antibody Population Seroprevalence before and during an Era of Widespread Opportunistic Chlamydia Screening in England (1994-2012). *PLoS One.* 2017;12(1):e0152810.
4. Waterboer T, Sehr P, Michael KM*, et al.* Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. *Clin Chem.* 2005;51(10):1845-53.
5. Idahl A, Jurstrand M, Olofsson JI*, et al.* Mycoplasma genitalium serum antibodies in infertile couples and fertile women. *Sex Transm Infect.* 2015;91(8):589-91.
6. Svenstrup HF, Jensen JS, Gevaert K*, et al.* Identification and characterization of immunogenic proteins of mycoplasma genitalium. *Clin Vaccine Immunol.* 2006;13(8):913-22.

Supplementary Table 1. Serologic marker cutpoints for sero-positivity; Pearson correlations between serologic markers and with Chlamydia marker Pgp3 by study population (Polish case-control study and a nested case-control study conducted within the PLCO Cancer Screening Trial).

		Poland		PLCO		Poland	PLCO
Infectious agent	marker cutpoint	Correlated markers	Correlations (min-max)	Correlated markers	Correlations (min-max)	Correlation with Pgp3	
Chlamydia trachomatis							
Pgp3	300	MOMP-A, MOMP-D, MOMP-L2, Tarp-F1, Tarp-F2	0.50-0.63	MOMP-D, MOMP-L2, Tarp-F1, Tarp-F2	0.62-0.72		
MOMP-A	165	Pgp3, MOMP-D, MOMP-L2, HSP60-1	0.55-0.74	MOMP-D, MOMP-L2, HSP60-1	0.51-0.78	0.50	0.48
MOMP-D	165	Pgp3, MOMP-A, MOMP-L2, HSP60-1	0.51-0.96	Pgp3, MOMP-A, MOMP-L2, Tarp-F1,HSP60-1	0.55-0.95	0.62	0.62
MOMP-L2	165	Pgp3, MOMP-A, MOMP-D, Tarp-F1, Tarp-F2, HSP60-1	0.52-0.96	Pgp3, MOMP-A, MOMP-D, Tarp-F1, HSP60-1	0.55-0.95	0.63	0.62
Tarp-F1	250	Pgp3, MOMP-L2, Tarp-F2	0.52-0.65	Pgp3, MOMP-L2, Tarp-F2	0.52-0.72	0.60	0.72
Tarp-F2	150	Pgp3, MOMP-L2, Tarp-F1	0.52-0.65	Pgp3, Tarp-F1	0.65, 0.65	0.55	0.65
HSP60-1	200	MOMP-A, MOMP-D, MOMP-L2	0.51-0.55	MOMP-A, MOMP-D, MOMP-L2	0.51-0.55	0.47	0.43
Mycoplasma genitalium							
MgPaN	400	rMgPa	0.55			0.19	0.25
rMgPa	400	MgPaN	0.55			0.24	0.35
Herpes Simplex Virus-2 (HSV-2)							
mgGunique	200					0.30	0.21
Human Papillomavirus (HPV)							
11 L1	500					0.19	0.23
16 L1	422					0.20	0.11
18 L1	394	45 L1	0.58	45 L1	0.58	0.10	0.06
31 L1	712					0.06	0.08
33 L1	515	45 L1	0.52	45 L1	0.52	0.10	0.11
45 L1	368	18 L1, 33 L1	0.52-0.58	18 L1, 33 L1	0.52-0.58	0.18	0.05
Herpes Simplex Virus-1 (HSV-1)							
gD	250					0.15	0.09
Polyomavirus							
BK VP1	400					0.00	0.00
JC VP1	400					0.07	0.09
HPyV9 VP1	400					0.06	-0.01
Hepatitis C Virus							
Core	1000	NS3	0.93			0.01	-0.06
NS3	500	Core	0.93			0.02	-0.02
Hepatitis B Virus							
HBc	800	HBVAe	0.97	HBVAe	0.88	0.10	0.04
HBe	800	HBVAc	0.97	HBVAc	0.88	0.09	0.01
Epstein Barr Virus (EBV)							
Zebra	300	EA-D	0.53	EA-D	0.58	0.11	0.10
EA-D	700	Zebra	0.53	Zebra	0.58	0.08	0.05
EBNA-1	350					0.05	0.04
Cytomegalovirus (CMV)							
pp150N	400	pp52, pp28	0.60-0.61	pp52, pp28	0.76-0.78	0.09	0.18
pp52	400	pp150N, pp28	0.60-0.63	pp150N, pp28	0.73-0.76	0.13	0.16
pp28	400	pp150N, pp52	0.61-0.63	pp150N, pp52	0.73-0.78	0.07	0.15

Supplementary Table 2. Contingency tables and summary statistics showing concordantly and disconcordantly reacting sera in pGP3 Multiplex Serology in comparison to reference assay serostatus tested at serum dilution 1:100 (A) [MFI cut-off = 500] and 1:1000 (B) [MFI cut-off = 95].*

	Reference assay				
Multiplex serology	Positive (n=99)	Negative (n=93)	Sensitivity (95% CI)	Specificity (95% CI)	Agreement *kappa* (95% CI)
A					
pGP3+	97	3			
pGP3-	2	90	98.0 (92.9-99.8)	96.8 (90.9-99.3)	0.95 (0.90-0.99)
B					
pGP3+	97	4			
pGP3-	2	89	98.0 (92.9-99.8)	95.7 (89.4-98.8)	0.94 (0.89-0.99)

*Statistical analysis was conducted using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). For sensitivity and specificity, exact 95 % confidence intervals (CI) are reported.

Supplementary Table 3. Contingency tables and summary statistics showing concordantly and disconcordantly reacting sera in *M. genitalium* multiplex serology in comparison to reference assay serostatus (LAMP-ELISA) tested at serum dilution 1:100 and MFI cut-off of 500.*

	LAMP-ELISA				
Multiplex serology	Positive (n=35)	Negative (n=36)	Sensitivity (95% CI)	Specificity (95% CI)	Agreement *kappa* (95% CI)
MgPaN +	34	4			
MgPaN -	1	32	97.1 (85.1-99.9)	88.9 (73.9-96.9)	0.86 (0.74-0.98)
rMgPa +	35	8			
rMgPa -	0	28	100 (90.0-100)	77.8 (60.9-89.9)	0.77 (0.62-0.92)
Both +	34	2			
≤ 1 +	1	34	97.1 (85.1-99.9)	94.4 (81.3-99.3)	0.92 (0.82-1.00)

*Statistical analysis was conducted using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). For sensitivity and specificity, exact 95 % confidence intervals (CI) are reported.

Supplementary Table 4. Serologic marker associations by serous/non-serous ovarian histotype in a Polish case-control study and a nested case-control study conducted within the PLCO Cancer Screening Trial. Chlamydia markers include evaluation at laboratory cutpoint for positivity and with increasing marker titer (established in Poland and tested in PLCO) to evaluate association with PID-like infection.

		Poland										PLCO									
	marker	controls		serous cases (n=112)				non-serous cases (n=132)				controls		serous cases (n=84)				non-serous cases (n=68)			
Infectious agent	cutpoint	n +	% +	n +	% +	OR*	(95% CI)	n +	% +	OR*	(95% CI)	P het†	n +	% +	n +	% +	OR*	(95% CI)	n +	% +	OR*	(95% CI)	P het†
Chlamydia trachomatis																							
Pgp3																							
Lab cutpoint (cutpoint 1)	300	192	34.5	47	42.0	1.30	(0.84-2.01)	64	48.5	1.79	(1.20-2.66)	0.25	34	21.4	19	22.6	1.16	(0.58-2.34)	18	26.5	1.59	(0.78-3.25)	0.80
Increase OR from Poland (cutpoint 2)	3878	97	17.4	33	29.5	1.85	(1.13-3.03)	37	28.0	2.01	(1.26-3.19)	0.85	17	10.7	13	15.5	1.98	(0.82-4.75)	14	20.6	2.99	(1.24-7.17)	0.70
Max OR from Poland (cutpoint 3)	8839	36	6.5	14	12.5	2.24	(1.12-4.46)	14	10.6	2.06	(1.04-4.08)	0.88	3	1.9	4	4.8	3.04	(0.61-15.27)	3	4.4	2.73	(0.51-14.71)	0.63
MOMP-A																							
Lab cutpoint	165	146	26.3	36	32.1	1.38	(0.87-2.17)	44	33.3	1.39	(0.91-2.12)	0.96	41	25.8	19	22.6	0.89	(0.46-1.74)	13	19.1	0.75	(0.36-1.58)	0.45
Increase OR from Poland	386	78	14.0	24	21.4	1.81	(1.07-3.08)	28	21.2	1.73	(1.05-2.85)	0.88	25	15.7	13	15.5	1.15	(0.53-2.54)	10	14.7	1.06	(0.46-2.47)	0.87
Max OR from Poland	1727	22	4.0	11	9.8	2.95	(1.33-6.54)	11	8.3	2.80	(1.27-6.18)	0.69	6	3.8	1	1.2	0.43	(0.05-3.82)	5	7.4	2.46	(0.67-8.97)	0.09
MOMP-D																							
Lab cutpoint	165	186	33.5	38	33.9	1.01	(0.64-1.58)	51	38.6	1.25	(0.83-1.88)	0.57	38	23.9	20	23.8	1.19	(0.61-2.34)	18	26.5	1.37	(0.67-2.77)	0.92
Increase OR from Poland	638	81	14.6	25	22.3	1.71	(1.01-2.89)	31	23.5	1.83	(1.12-2.99)	0.98	21	13.2	10	11.9	1.12	(0.46-2.70)	11	16.2	1.52	(0.64-3.65)	0.83
Max OR from Poland	1042	59	10.6	23	20.5	2.16	(1.23-3.78)	24	18.2	1.93	(1.12-3.32)	0.60	13	8.2	7	8.3	1.32	(0.47-3.72)	9	13.2	2.31	(0.86-6.19)	0.63
MOMP-L2																							
Lab cutpoint	250	127	22.8	37	33.0	1.55	(0.97-2.47)	45	34.1	1.78	(1.16-2.73)	0.74	32	20.1	17	20.2	1.30	(0.63-2.70)	13	19.1	1.10	(0.50-2.41)	0.52
Increase OR from Poland	602	77	13.8	24	21.4	1.70	(0.99-2.91)	35	26.5	2.26	(1.40-3.66)	0.49	21	13.2	10	11.9	1.20	(0.49-2.92)	10	14.7	1.36	(0.56-3.34)	0.93
Max OR from Poland	834	65	11.7	24	21.4	2.05	(1.19-3.54)	31	23.5	2.35	(1.41-3.90)	0.86	16	10.1	9	10.7	1.66	(0.63-4.36)	9	13.2	1.70	(0.65-4.46)	0.98
Tarp-F1																							
Lab cutpoint	265	185	33.3	47	42.0	1.48	(0.96-2.29)	47	35.6	1.05	(0.69-1.59)	0.18	41	25.8	26	31.0	1.45	(0.78-2.72)	21	30.9	1.36	(0.71-2.61)	0.60
Increase OR from Poland	1326	78	14.0	23	20.5	1.42	(0.82-2.46)	25	18.9	1.44	(0.86-2.41)	0.88	19	11.9	9	10.7	1.03	(0.41-2.59)	12	17.6	1.91	(0.81-4.50)	0.58
Max OR from Poland	1689	62	11.2	19	17.0	1.45	(0.80-2.64)	22	16.7	1.57	(0.91-2.72)	0.74	17	10.7	7	8.3	0.89	(0.32-2.47)	11	16.2	2.00	(0.81-4.96)	0.41
Tarp-F2																							
Lab cutpoint	165	261	46.9	54	48.2	1.01	(0.66-1.54)	72	54.5	1.37	(0.92-2.03)	0.26	61	38.4	33	39.3	1.15	(0.64-2.06)	29	42.6	1.33	(0.71-2.47)	0.90
Increase OR from Poland	1667	50	9.0	19	17.0	2.03	(1.11-3.74)	18	13.6	1.72	(0.94-3.12)	0.87	23	14.5	11	13.1	0.92	(0.40-2.14)	10	14.7	1.21	(0.50-2.89)	0.71
Max OR from Poland	2106	38	6.8	17	15.2	2.49	(1.30-4.80)	15	11.4	1.97	(1.02-3.81)	0.77	21	13.2	9	10.7	0.80	(0.32-1.98)	9	13.2	1.20	(0.48-2.98)	0.61
HSP60-1																							
Lab cutpoint	200	192	34.5	47	42.0	1.30	(0.84-2.01)	64	48.5	1.79	(1.20-2.66)	0.25	57	35.8	32	38.1	1.22	(0.68-2.19)	30	44.1	1.62	(0.88-2.99)	0.36
Increase OR from Poland	1308	157	28.2	42	37.5	1.46	(0.94-2.29)	53	40.2	1.70	(1.13-2.57)	0.75	15	9.4	10	11.9	1.23	(0.49-3.11)	8	11.8	1.49	(0.56-3.95)	0.82
Max OR from Poland	3407	110	19.8	35	31.3	1.70	(1.05-2.75)	39	29.5	1.82	(1.16-2.86)	0.94	6	3.8	3	3.6	0.81	(0.18-3.70)	2	2.9	0.87	(0.16-4.66)	0.81
Seropositive‡		158	28.4	38	33.9	1.22	(0.77-1.92)	51	38.6	2.42	(1.06-2.42)	0.35	34	21.4	21	25.0	1.40	(0.71-2.78)	15	22.1	1.24	(0.59-2.61)	0.50
Mycoplasma genitalium																							
MgPaN	400	166	29.9	36	32.1	1.08	(0.69-1.71)	48	36.4	1.38	(0.91-2.08)	0.31	36	22.6	12	14.3	0.60	(0.28-1.30)	12	17.6	0.87	(0.40-1.90)	0.49
rMgPa	400	183	32.9	41	36.6	1.12	(0.72-1.74)	53	40.2	1.48	(0.99-2.22)	0.22	50	31.4	22	26.2	0.83	(0.44-1.57)	17	25.0	0.81	(0.41-1.59)	0.92
Seropositive‡		103	18.5	23	20.5	1.06	(0.62-1.81)	35	26.5	2.68	(1.07-2.68)	0.09	19	11.9	8	9.5	0.83	(0.32-2.16)	4	5.9	0.52	(0.16-1.71)	0.53
Herpes Simplex Virus-2 (HSV-2)																							
mgGunique	200	115	20.7	23	20.5	0.96	(0.56-1.64)	32	24.2	1.20	(0.75-1.93)	0.48	28	17.6	22	26.2	1.98	(0.98-3.99)	12	17.6	1.16	(0.52-2.60)	0.23
Human Papillomavirus (HPV)																							
11 L1	500	25	4.5	5	4.5	1.09	(0.40-2.99)	6	4.5	1.02	(0.40-2.60)	0.81	3	1.9	3	3.6	1.82	(0.34-9.71)	7	10.3	7.71	(1.81-32.87)	0.02
16 L1	422	23	4.1	10	8.9	2.48	(1.11-5.54)	7	5.3	1.23	(0.51-3.00)	0.29	3	1.9	0	0.0	--	--	2	2.9	1.31	(0.17-10.12)	0.99
18 L1	394	34	6.1	6	5.4	0.94	(0.38-2.35)	11	8.3	1.48	(0.71-3.08)	0.58	6	3.8	3	3.6	1.33	(0.30-6.00)	2	2.9	0.83	(0.15-4.48)	0.80
31 L1	712	23	4.1	6	5.4	1.27	(0.49-3.27)	4	3.0	0.62	(0.20-1.87)	0.21	6	3.8	3	3.6	1.35	(0.29-6.25)	1	1.5	0.31	(0.03-2.72)	0.15
33 L1	515	13	2.3	6	5.4	2.23	(0.80-6.19)	4	3.0	1.17	(0.36-3.76)	0.34	1	0.6	0	0.0	--	--	0	0.0	--	--	
45 L1	368	32	5.8	7	6.3	1.27	(0.53-3.02)	10	7.6	1.40	(0.65-3.03)	0.91	2	1.3	2	2.4	1.92	(0.25-14.93)	3	4.4	4.20	(0.62-28.59)	0.30
Seropositive‡		69	12.4	20	17.9	1.70	(0.96-2.99)	20	15.2	1.25	(0.72-2.19)	0.36	14	8.8	7	8.3	1.22	(0.43-3.46)	6	8.8	0.90	(0.30-2.68)	0.88
Herpes Simplex Virus-1 (HSV-1)																							
gD	250	540	97.1	106	94.6	0.43	(0.15-1.21)	129	97.7	0.87	(0.24-3.21)	0.38	131	82.4	62	73.8	0.68	(0.34-1.35)	48	70.6	0.53	(0.26-1.09)	0.64
Polyomavirus																							
BK VP1	400	520	93.5	95	84.8	0.39	(0.21-0.74)	122	92.4	0.90	(0.43-1.89)	0.04	145	91.2	78	92.9	1.42	(0.42-4.81)	62	91.2	1.60	(0.42-6.10)	0.76
JC VP1	400	377	67.8	70	62.5	0.81	(0.52-1.26)	79	59.8	0.71	(0.48-1.07)	0.70	94	59.1	51	60.7	1.21	(0.68-2.14)	38	55.9	0.89	(0.49-1.63)	0.42
HPyV9 VP1	400	314	56.5	55	49.1	0.77	(0.51-1.19)	59	44.7	0.65	(0.44-0.96)	0.50	53	33.3	31	36.9	1.33	(0.74-2.39)	21	30.9	0.91	(0.48-1.73)	0.32
Hepatitis C Virus																							
Core	1000	22	4.0	4	3.6	1.02	(0.33-3.14)	10	7.6	2.22	(0.98-5.03)	0.19	3	1.9	4	4.8	3.78	(0.70-20.49)	0	0.0	--	--	0.98
NS3	500	49	8.8	5	4.5	0.49	(0.19-1.29)	10	7.6	0.87	(0.42-1.80)	0.24	1	0.6	1	1.2	2.28	(0.12-41.76)	0	0.0	--	--	0.99
Seropositive‡		18	3.2	3	2.7	0.97	(0.27-3.46)	7	5.3	5.22	(0.79-5.22)	0.27	0	0.0	1	1.2	--	--	0	0.0	--	--	0.99
Hepatitis B Virus																							
HBc	800	100	18.0	21	18.8	1.10	(0.64-1.90)	15	11.4	0.57	(0.31-1.02)	0.11	3	1.9	0	0.0	--	--	1	1.5	0.79	(0.06-10.79)	0.99
HBe	800	113	20.3	22	19.6	1.00	(0.59-1.71)	16	12.1	0.54	(0.30-0.95)	0.12	5	3.1	3	3.6	1.04	(0.23-4.81)	2	2.9	0.86	(0.15-4.91)	0.70
Seropositive‡		99	17.8	21	18.8	1.13	(0.65-1.94)	15	11.4	1.04	(0.32-1.04)	0.11	3	1.9	0	0.0	--	--	1	1.5	0.79	(0.06-10.79)	0.99
Epstein Barr Virus (EBV)																							
Zebra	300	531	95.5	107	95.5	0.98	(0.36-2.69)	124	93.9	0.80	(0.33-1.94)	0.38	149	93.7	77	91.7	0.82	(0.25-2.71)	61	89.7	0.85	(0.24-3.04)	0.49
EA-D	700	403	72.5	85	75.9	1.18	(0.72-1.92)	102	77.3	1.27	(0.80-2.01)	0.98	130	81.8	70	83.3	1.25	(0.58-2.70)	51	75.0	0.78	(0.37-1.63)	0.42
EBNA-1	350	534	96.0	106	94.6	0.80	(0.29-2.18)	122	92.4	0.48	(0.22-1.05)	0.51	141	88.7	78	92.9	2.17	(0.68-6.92)	56	82.4	0.74	(0.30-1.83)	0.11
Seropositive‡		531	95.5	108	96.4	1.22	(0.41-3.64)	124	93.9	1.63	(0.30-1.63)	0.36	148	93.1	77	91.7	0.94	(0.29-3.05)	60	88.2	0.72	(0.22-2.33)	0.92
Cytomegalovirus (CMV)																							
pp150N	400	526	94.6	106	94.6	1.07	(0.42-2.74)	120	90.9	0.55	(0.27-1.15)	0.17	116	73.0	57	67.9	0.88	(0.47-1.66)	54	79.4	1.82	(0.85-3.91)	0.08
pp52	400	537	96.6	106	94.6	0.84	(0.29-2.43)	127	96.2	0.83	(0.29-2.36)	0.68	129	81.1	66	78.6	1.03	(0.50-2.14)	56	82.4	1.33	(0.57-3.08)	0.51
pp28	400	536	96.4	107	95.5	0.83	(0.30-2.35)	123	93.2	0.46	(0.20-1.07)	0.29	127	79.9	62	73.8	0.75	(0.38-1.50)	57	83.8	1.77	(0.75-4.21)	0.05
Seropositive‡		533	95.9	107	95.5	1.05	(0.37-2.96)	123	93.2	1.26	(0.24-1.26)	0.16	122	76.7	60	71.4	0.86	(0.45-1.67)	55	80.9	1.62	(0.73-3.58)	0.12

*Baseline category logistic regression models adjusting for matching factors (Poland: age and study site; PLCO: age, race, time of blood draw, month of blood draw) and adjusted for nulliparity, duration of oral contraceptive use, and duration of menopausal hormone therapy use.

†P values for heterogeneity are based on the likelihood ratio test from a model limited to cases with serous histotype as the reference category, P values are two-sided.

‡The definition for seropositive for a specific antigen was based on the following: C. trachomatis ≥3 markers positive (MOMP-A, MOMP-D, MOMP-L2, Tarp-F1, Tarp-F2, HSP60-1); M. genitalium, Hepatitis B, Hepatitis C, both markers positive; HPV if any of high risk types are positive (16, 18, 31, 33, 45); EBV and CMV at least 2 of 3 markers positive.

Frequency (n) and percent (%) reported based on seropositive (+) for individual markers or the combined marker ("Seropositive"). The reference category is seronegative for an individual marker or negative for the combined marker.

Supplementary Table 5. Serologic marker associations by time between blood draw and diagnosis in a nested case-control study conducted within the PLCO Cancer Screening Trial. Adjusted arithmetic mean serologic marker levels by time between blood draw and diagnosis among ovarian cancer cases in PLCO.

	Time between blood draw and diagnosis									Time between blood draw and diagnosis				
	2-<5 years (n=94)				5+ years (n=66)					2-<5 years		5+ years		
Infectious agent	**n +**	**% +**	**OR***	**(95% CI)**	**n +**	**% +**	**OR***	**(95% CI)**	**P het†**	**Mean‡**	**(95% CI)**	**Mean‡**	**(95% CI)**	**P§**
Chlamydia trachomatis														
Pgp3 - lab cutpoint (1)	24	25.5	1.50	(0.78-2.88)	16	24.2	1.27	(0.61-2.66)	0.72	1434	(860, 2008)	1627	(931, 2323)	0.25
Increase OR from Poland (cutpoint 2)	17	18.1	2.48	(1.11-5.56)	11	16.7	1.99	(0.81-4.91)	0.71					
Max OR from Poland (cutpoint 3)	4	4.3	2.71	(0.56-13.1)	3	4.5	2.74	(0.50-15.0)	0.89					
MOMP-A - lab cutpoint	17	18.1	0.67	(0.34-1.30)	16	24.2	0.99	(0.49-2.01)	0.31	231	(110, 351)	345	(199, 491)	0.40
Increase OR from Poland	13	13.8	1.01	(0.47-2.17)	11	16.7	1.15	(0.50-2.62)	0.74					
Max OR from Poland	3	3.2	0.98	(0.23-4.24)	3	4.5	1.86	(0.41-8.40)	0.41					
MOMP-D - lab cutpoint	24	25.5	1.36	(0.72-2.59)	16	24.2	1.14	(0.55-2.36)	0.70	337	(184, 490)	506	(320, 691)	0.11
Increase OR from Poland	11	11.7	1.07	(0.46-2.51)	11	16.7	1.54	(0.63-3.73)	0.41					
Max OR from Poland	7	7.4	1.16	(0.42-3.19)	9	13.6	2.33	(0.87-6.24)	0.19					
MOMP-L2 - lab cutpoint	24	25.5	1.27	(0.67-2.40)	17	25.8	1.19	(0.58-2.43)	0.87	339	(195, 483)	448	(273, 623)	0.19
Increase OR from Poland	11	11.7	1.17	(0.50-2.75)	11	16.7	1.58	(0.65-3.82)	0.52					
Max OR from Poland	10	10.6	1.56	(0.62-3.93)	10	15.2	2.07	(0.80-5.36)	0.53					
Tarp-F1 - lab cutpoint	34	36.2	2.01	(1.11-3.62)	16	24.2	0.89	(0.45-1.78)	0.03	757	(497, 1016)	576	(261, 891)	0.17
Increase OR from Poland	13	13.8	1.41	(0.62-3.19)	8	12.1	1.08	(0.42-2.80)	0.87					
Max OR from Poland	10	10.6	1.25	(0.51-3.05)	8	12.1	1.21	(0.46-3.22)	0.73					
Tarp-F2 - lab cutpoint	37	39.4	1.04	(0.59-1.82)	30	45.5	1.34	(0.72-2.49)	0.55	960	(596, 1324)	639	(198, 1081)	0.54
Increase OR from Poland	16	17.0	1.35	(0.63-2.89)	6	9.1	0.58	(0.21-1.60)	0.13					
Max OR from Poland	13	13.8	1.14	(0.51-2.56)	5	7.6	0.53	(0.18-1.57)	0.34					
HSP60-1 - lab cutpoint	42	44.7	1.61	(0.92-2.79)	22	33.3	0.96	(0.51-1.82)	0.08	644	(407, 881)	540	(253, 827)	0.61
Increase OR from Poland	10	10.6	1.10	(0.44-2.73)	8	12.1	1.41	(0.53-3.74)	0.70					
Max OR from Poland	3	3.2	0.75	(0.17-3.33)	2	3.0	0.75	(0.14-4.15)	0.84					
Seropositive‖	23	24.5	1.45	(0.75-2.80)	14	21.2	1.03	(0.49-2.18)	0.49					
Mycoplasma genitalium														
MgPaN	16	17.0	0.83	(0.41-1.65)	10	15.2	0.61	(0.27-1.37)	0.44	319	(168, 470)	305	(122, 488)	0.82
rMgPa	31	33.0	1.26	(0.71-2.27)	10	15.2	0.39	(0.18-0.85)	0.004	553	(376, 730)	258	(43.8, 473)	0.06
Seropositive‖	10	10.6	0.99	(0.41-2.40)	3	4.5	0.34	(0.09-1.26)	0.13					
Herpes Simplex Virus-2 (HSV-2)														
mgGunique	24	25.5	1.96	(1.00-3.86)	13	19.7	1.29	(0.59-2.86)	0.41	600	(304, 896)	726	(367, 1085)	0.50
Human Papillomavirus (HPV)														
11 L1	10	10.6	6.15	(1.56-24.24)	1	1.5	0.93	(0.09-9.46)	0.11	145	(113, 177)	85.4	(46.6, 124)	<0.01
16 L1	0	0.0	--	--	2	3.0	1.38	(0.18-10.74)	0.99	63.0	(25.2, 101)	82.2	(36.5, 128)	0.64
18 L1	3	3.2	1.02	(0.23-4.47)	2	3.0	0.92	(0.17-4.98)	0.95	114	(80.0, 148)	91.5	(50.2, 133)	0.70
31 L1	4	4.3	1.09	(0.27-4.31)	0	0.0	--	--	0.98	156	(104, 208)	129	(66.0, 193)	0.79
33 L1	0	0.0	--	--	0	0.0	--	--		55.2	(43.3, 67.1)	56.8	(42.4, 71.2)	0.72
45 L1	2	2.1	1.58	(0.20-12.44)	3	4.5	4.27	(0.63-28.99)	0.39	92.1	(59.4, 125)	87.1	(47.5, 127)	0.80
Seropositive‖	8	8.5	1.00	(0.37-2.69)	5	7.6	0.98	(0.31-3.14)	0.96					
Herpes Simplex Virus-1 (HSV-1)														
gD	70	74.5	0.63	(0.33-1.22)	44	66.7	0.46	(0.23-0.94)	0.64	3355	(2668, 4042)	2934	(2102, 3767)	0.06
Polyomavirus														
BK VP1	89	94.7	2.05	(0.54-7.71)	58	87.9	0.89	(0.29-2.77)	0.30	4550	(3909, 5191)	4101	(3324, 4878)	0.34
JC VP1	54	57.4	0.93	(0.54-1.59)	41	62.1	1.31	(0.70-2.47)	0.32	1105	(853, 1357)	1167	(861, 1472)	0.89
HPyV9 VP1	37	39.4	1.36	(0.78-2.37)	18	27.3	0.87	(0.45-1.68)	0.19	626	(231, 1022)	507	(27.8, 985)	0.09
Hepatitis C Virus														
Core	4	4.3	2.16	(0.43-10.93)	0	0.0	--	--	0.98	166	(68.8, 264)	68.0	(-50.0, 186)	0.39
NS3	1	1.1	1.13	(0.06-20.63)	0	0.0	--	--	0.99	154	(46.2, 263)	71.6	(-59.7, 203)	0.45
Seropositive‖	1	1.1	--	--	0	0.0	--	--						
Hepatitis B Virus														
HBc	1	1.1	0.37	(0.03-4.14)	0	0.0	--	--	0.99	82.6	(-87.0, 252)	15.3	(-190, 221)	0.42
HBe	5	5.3	1.64	(0.43-6.21)	0	0.0	--	--	0.98	207	(-24.4, 438)	75.5	(-204, 355)	0.55
Seropositive‖	1	1.1	--	--	0	0.0	--	--						
Epstein Barr Virus (EBV)														
Zebra	86	91.5	0.75	(0.24-2.34)	60	90.9	1.13	(0.28-4.57)	0.30	5158	(4477, 5839)	4653	(3828, 5479)	0.65
EAD	75	79.8	0.91	(0.46-1.83)	53	80.3	1.16	(0.51-2.64)	0.32	5082	(4286, 5877)	5427	(4464, 6391)	0.43
EBNA1	79	84.0	0.61	(0.27-1.37)	62	93.9	8.40	(1.07-65.95)	0.01	5633	(4847, 6418)	6477	(5526, 7429)	0.41
Seropositive‖	86	91.5	0.77	(0.25-2.32)	59	89.4	1.10	(0.32-3.87)	0.40					
Cytomegalovirus (CMV)														
pp150N	68	72.3	1.04	(0.56-1.93)	51	77.3	1.60	(0.75-3.42)	0.45	3741	(3101, 4381)	3595	(2820, 4370)	0.82
pp52	77	81.9	1.15	(0.55-2.37)	53	80.3	1.23	(0.54-2.83)	0.92	7313	(6309, 8317)	7349	(6132, 8566)	0.81
pp28	74	78.7	0.96	(0.48-1.90)	53	80.3	1.32	(0.58-2.98)	0.59	4844	(3945, 5743)	5185	(4096, 6274)	0.80
Seropositive‖	72	76.6	1.06	(0.55-2.03)	51	77.3	1.31	(0.61-2.82)	0.80					

*Baseline category logistic regression models adjusting for matching factors (age, race, time of blood draw, month of blood draw) and adjusted for nulliparity, duration of oral contraceptive use, and duration of menopausal hormone therapy use.

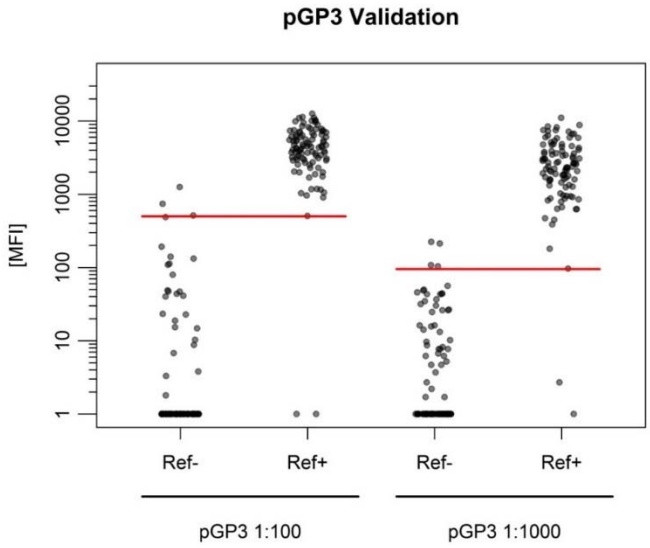
†P values for heterogeneity are based on the likelihood ratio test from a model limited to cases with 2-<5 years between blood draw and diagnosis as the reference category, P values are two-sided.

‡Adjusted for age, race (white vs. non-white), time of blood draw (AM vs. PM), and month of blood draw.

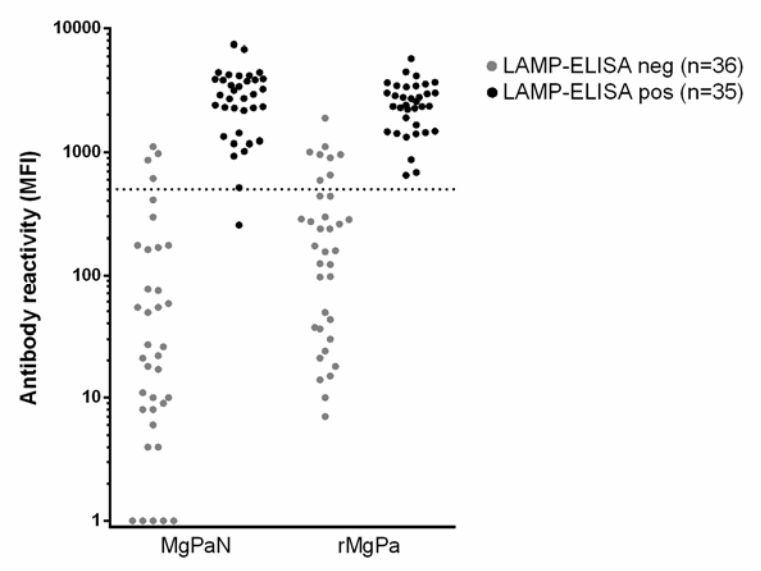
§P values calculated using a two-tailed Wald test with an F-distribution as the reference distribution at alpha=0.05.

‖The definition for seropositive for a specific antigen was based on the following: C. trachomatis ≥3 markers positive (MOMP-A, MOMP-D, MOMP-L2, Tarp-F1, Tarp-F2, HSP60-1); M. genitalium, Hepatitis B, Hepatitis C, both markers positive; HPV if any of high risk types are positive (16, 18, 31, 33, 45); EBV and CMV at least 2 of 3 markers positive.

Frequency (n) and percent (%) reported based on seropositive (+) for individual markers or the combined marker ("Seropositive"). The reference category is seronegative for an individual marker or negative for the combined marker.



Supplementary Figure 1. pGP3 seroreactivity [median fluorescence intensity (MFI)] by reference assay serostatus. Sera were tested at two dilutions (1:100, 1:1000) for antibody reactivity against pGP3. The solid red lines indicate the cut-offs maximizing sensitivity and specificity (1:100, 500 MFI; 1:1000, 95 MFI). Ref+: reference assay seropositive, Ref-: reference assay seronegative. In total, 99 reference assay seropositive and 93 reference assay seronegative sera were analyzed.



Supplementary Figure 2. Antibody reactivity [MFI] to *M. genitalium* proteins MgPaN and rMgPa stratified by reference assay serostatus. Sera were tested at dilution 1:100. The dashed line indicates the optimum cut-off (500 MFI).