

**Table 1 Establishment of a threshold for the multi-peptide ELISA using MIF serology and tubal status to define chlamydial infertility**

Assay	Absorbance Threshold(s) criteria <sup>a</sup>	True pos <sup>b</sup>	False neg	False pos <sup>c</sup>	True neg	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Odds Ratio (95% ci)	Fisher's exact P value (unadjusted)	Adjusted Odds Ratio (95% CI)
<b>MEDAC Infertile</b>		5	6	0	78	0.45 (0.17-0.77)	1 (0.93-1)	1 (0.36-1)	0.93 (0.85-0.97)	0	<0.001	5.3e+17 (0-Inf)
<b>MIF.CT</b>		11	0	4	74	1 (0.62-1)	0.95 (0.87-0.99)	0.73 (0.45-0.92)	1 (0.93-1)	0	<0.001	5.8e+40 (0-Inf)
<b>MIF.CP</b>		7	4	20	58	0.64 (0.31-0.89)	0.74 (0.63-0.84)	0.26 (0.11-0.46)	0.94 (0.84-0.98)	5.08 (1.34-19.18)	0.016	5.9 (1.3-28)
<b>MIF.CS</b>		4	7	1	77	0.36 (0.11-0.69)	0.99 (0.93-1)	0.8 (0.28-0.99)	0.92 (0.84-0.97)	44 (4.31-449.48)	0.001	32 (2.3-440)
<b>P 11</b>	P 11 >= 0.411	1	10	4	74	0.09 (0-0.41)	0.95 (0.87-0.99)	0.2 (0.01-0.72)	0.88 (0.79-0.94)	1.85 (0.19-18.24)	0.491	0.72 (0.053-10)
<b>P 47</b>	P 47 >= 0.522	1	10	4	74	0.09 (0-0.41)	0.95 (0.87-0.99)	0.2 (0.01-0.72)	0.88 (0.79-0.94)	1.85 (0.19-18.24)	0.491	3.2 (0.26-40)
<b>P 50</b>	P 50 >= 0.539	1	10	4	74	0.09 (0-0.41)	0.95 (0.87-0.99)	0.2 (0.01-0.72)	0.88 (0.79-0.94)	1.85 (0.19-18.24)	0.491	0.89 (0.055-12)
<b>COMBI</b>	P 11 >=	3	8	4	74	0.27 (0.06-	0.95 (0.87-	0.43 (0.1-	0.9 (0.82-	6.94 (1.31-	0.038	4.7 (0.6-

<b>NED_P 11_or_P 47_or_P 50</b>	0.686 or P 47 >= 0.559 or P 50 >= 0.5715					0.61)	0.99)	0.82)	0.96)	36.68)		36)
<b>COMBI NED_P 11_and_ P47_an d_P 50</b>	P 11 >= 0.1425 and P 47 >= 0.106 and P 50 >= 0.4685	2	9	4	74	0.18 (0.02- 0.52)	0.95 (0.87- 0.99)	0.33 (0.04- 0.78)	0.89 (0.8- 0.95)	4.11 (0.66- 25.7)	0.158	1.5 (0.18-13)
<b>COMBI NED_P 11_and_ P 50</b>	P 11 >= 0.1425 and P e 50 >= 0.4685	2	9	4	74	0.18 (0.02- 0.52)	0.95 (0.87- 0.99)	0.33 (0.04- 0.78)	0.89 (0.8- 0.95)	4.11 (0.66- 25.7)	0.158	1.5 (0.18-13)

<sup>a</sup> Analysis used to set the peptide assay threshold for all subsequent evaluations (Group 1 participants only)

<sup>b</sup> Positives were defined by being Group 1 (infertile women seeking IVF) with both confirmed Tubal factor and MIF CT positive (all participants from Group 1) (n=11)

<sup>c</sup> Negatives are infertile women seeking IVF (can be tubal factor or not, can be MIF positive or not, but cannot be positive for both) (Group 1) (n=80)

**Table 2. Testing the multi-peptide assays for detection of tubal factor infertility**

Assay	Absorbance Threshold (s) criteria	True pos <sup>a</sup>	False neg	False pos <sup>b</sup>	True neg	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Odds Ratio (95% CI)	Fisher's Exact P value (unadjusted)	Adjusted Odds Ratio (95% CI)
<b>MEDACInfertile</b>		5 <sup>c</sup>	38	0	46	0.12 (0.04-0.25)	1 (0.89-1)	1 (0.36-1)	0.55 (0.44-0.66)	0	0.022	4.2e+07 (0-Inf)
<b>MIF.CT</b>		11	33	5	42	0.25 (0.13-0.4)	0.89 (0.77-0.96)	0.69 (0.41-0.89)	0.56 (0.44-0.67)	2.8 (0.89-8.85)	0.099	3.9 (0.95-16)
<b>MIF.CP</b>		12	32	16	31	0.27 (0.15-0.43)	0.66 (0.51-0.79)	0.43 (0.24-0.63)	0.49 (0.36-0.62)	0.73 (0.3-1.78)	0.505	0.61 (0.23-1.6)
<b>COMBINED_P11_or_P47_or_P50</b>	P 11 >= 0.686 or P 47 >= 0.559 or P50 >= 0.5715	5	39	2	45	0.11 (0.04-0.25)	0.96 (0.85-0.99)	0.71 (0.29-0.96)	0.54 (0.42-0.65)	2.88 (0.53-15.71)	0.257	3.3 (0.5-21)
<b>COMBINED_P11_and_P47_and_P50</b>	P 11 >= 0.1425 and P 47 >= 0.106 and P 50 >= 0.4685	6	38	0	47	0.14 (0.05-0.27)	1 (0.89-1)	1 (0.42-1)	0.55 (0.44-0.66)	0	0.011	3.5e+07 (0-Inf)

<sup>a</sup> Positives were participants with confirmed tubal factor status and seeking IVF treatment (Group 1) (n=44)

<sup>b</sup> Negatives were participants with confirmed no detected tubal factor and seeking IVF treatment (Group 1) (n=47)

c The MEDAC assay had an 'unequivocal and repeat with new sample' result repeatedly in two samples therefore, for this assay alone there are two results that could not be determined, however, these samples recorded reproducible results in all other assays tested.

**Table 3. Testing the performance of the multi-peptide ELISA against MIF positive tubal infertile women compared to infertile, sexual heath, and fertile negative cohorts**

Assay	Absorbance Threshold (s) criteria	True pos <sup>a</sup>	False neg	False pos <sup>b</sup>	True neg	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Odds Ratio (95% CI)	Fisher's exact P value (unadjusted)
<b>MEDAC Infertile</b>		5	6	25	196	0.45 (0.17-0.77)	0.89 (0.84-0.93)	0.17 (0.06-0.35)	0.97 (0.94-0.99)	6.53 (1.86-22.98)	0.007
<b>MIF.CT</b>		11	0	84	164	1 (0.62-1)	0.66 (0.6-0.72)	0.12 (0.06-0.2)	1 (0.97-1)	0	<0.001
<b>MIF.CP</b>		7	4	110	138	0.64 (0.31-0.89)	0.56 (0.49-0.62)	0.06 (0.02-0.12)	0.97 (0.93-0.99)	2.2 (0.63-7.69)	0.231
<b>MIF.CS</b>		4	7	4	244	0.36 (0.11-0.69)	0.98 (0.96-1)	0.5 (0.16-0.84)	0.97 (0.94-0.99)	34.86 (7.21-168.63)	<0.001
<b>COMBI NED_P 11_or_P 47_or_P 50</b>	P 11 >= 0.686 or P 47 >= 0.559 or P 50 >= 0.5715	3	8	18	228	0.27 (0.06-0.61)	0.93 (0.89-0.96)	0.14 (0.03-0.36)	0.97 (0.93-0.99)	4.75 (1.16-19.48)	0.051
<b>COMBI NED_P 11_and_P 47_and_P 50</b>	P 11 >= 0.1425 and P 47 >= 0.106 and P 50 >= 0.4685	2	9	12	237	0.18 (0.02-0.52)	0.95 (0.92-0.97)	0.14 (0.02-0.43)	0.96 (0.93-0.98)	4.39 (0.85-22.59)	0.112

<sup>a</sup> Positives were defined as Group 1 Infertile women with MIF CT positive and confirmed Tubal factor status (n=11)

<sup>b</sup> Negatives were either Group 1 infertile not MIF CT positive or not tubal factor (n=80), acute infection (n=112) (Group 2), or fertile currently pregnant (n=53) (Group 3)