TABLE I

Results of sensitivity and specificity of singleplex assays for the detection of IgG anti-*Toxoplasma gondii* and *Rubella virus*

| *T. gondii* Ags | Coupling buffer | Antigen concentration (µg/mL) | Sensitivity (specificity 100%) | AUC |
|---|---|---|---|---|
| SAG-1 | PBS (pH 7.2) | 50 | 3% | 0.619 |
| GRA-7 | PBS (pH 7.2) | 100 | 7% | 0.630 |
| GRA-1 | MES (pH 5.5) | 50 | 7% | 0.757 |
| | MES (pH 5.0) | 50 | 15% | 0.751 |
| MIC-3 | MES (pH 6.5) | 125 | 86% | 0.999 |
| | MES (pH 5.5) | 100 | 87% | 0.970 |
| | MES (pH 5.5) | 125 | 66% | 0.999 |
| | MES (pH 5.5) | 150 | 85% | 0.970 |
| | MES (pH 5.0) | 100 | 93% | 0.985 |
| | MES (pH 5.0) | 125 | 99% | 0.999 |
| *T. gondii* lysate | PBS (pH 7.2) | 100 | 100% | 1.0 |
| | PBS (pH 7.2) | 150 | 100% | 1.0 |
| | PBS (pH 7.2) | 200 | 100% | 1.0 |
| | PBS (pH 7.2) | 250 | 100% | 1.0 |
| | PBS (pH 7.2) | 500 | 100% | 1.0 |
| TOX 8122 | PBS (pH 7.2) | 100 | 97% | 0.997 |
| | PBS (pH 7.2) | 150 | 97% | 0.998 |
| | PBS (pH 7.2) | 200 | 96% | 0.997 |
| TOX 8131 | PBS (pH 7.2) | 100 | 99% | 0.997 |
| | PBS (pH 7.2) | 150 | 100% | 1.0 |
| | PBS (pH 7.2) | 200 | 100% | 1.0 |
| CTOX | MES (pH 6.5) | 100 | 99% | 0.987 |
| | PBS (pH 7.2) | 100 | 99% | 0.997 |
| | $NaHCO_3$ (pH 8.0) | 100 | 99% | 0.994 |
| CTOXH | MES (pH 6.5) | 100 | 99% | 0.995 |
| | PBS (pH 7.2) | 100 | 99% | 0.995 |
| | $NaHCO_3$ (pH 8.0) | 100 | 99% | 0.996 |

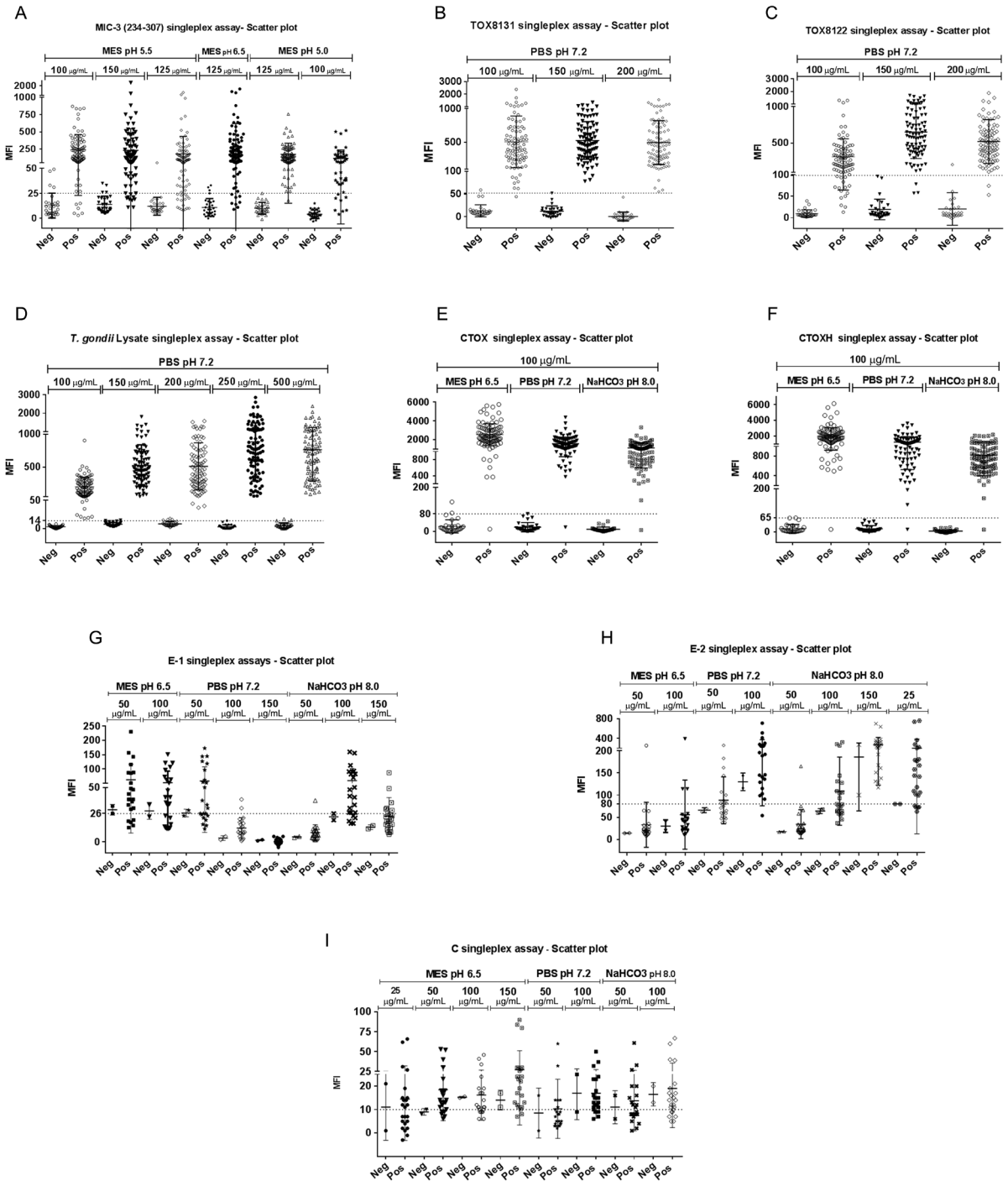
| *R. virus* Ags | Coupling buffer | Antigen concentration (µg/mL) | Sensitivity (specificity 100%) | AUC |
|---|---|---|---|---|
| E-1 | MES (pH 6.5) | 50 | 70% | 0.739 |
| | PBS (pH 7.2) | 50 | 57% | 0.663 |
| | $NaHCO_3$ (pH 8.0) | 50 | 70% | 0.728 |
| | MES (pH 6.5) | 100 | 61% | 0.652 |
| | PBS (pH 7.2) | 100 | 82% | 0.870 |
| | $NaHCO_3$ (pH 8.0) | 100 | 78% | 0.826 |
| | $NaHCO_3$ (pH 8.0) | 150 | 70% | 0.739 |
| E-2 | MES (pH 6.5) | 50 | 70% | 0.717 |
| | PBS (pH 7.2) | 50 | 57% | 0.587 |
| | $NaHCO_3$ (pH 8.0) | 50 | 78% | 0.793 |
| | MES (pH 6.5) | 100 | 48% | 0.674 |
| | PBS (pH 7.2) | 100 | 61% | 0.717 |
| | $NaHCO_3$ (pH 8.0) | 100 | 65% | 0.717 |
| | $NaHCO_3$ (pH 8.0) | 150 | 43% | 0.717 |
| | $NaHCO_3$ (pH 8.0) | 25 | 78% | 0.783 |
| C | MES (pH 6.5) | 50 | 57% | 0.717 |
| | PBS (pH 7.2) | 50 | 9% | 0.543 |
| | $NaHCO_3$ (pH 8.0) | 50 | 23% | 0.533 |
| | MES (pH 6.5) | 100 | 39% | 0.609 |
| | PBS (pH 7.2) | 100 | 13% | 0.520 |
| | $NaHCO_3$ (pH 8.0) | 100 | 22% | 0.533 |
| | MES (pH 6.5) | 150 | 19% | 0.707 |
| | MES (pH 6.5) | 25 | 57% | 0.583 |

The best coupling conditions are marked in gray. AUC: area under the curve.

TABLE II

Optimisation of sera and R-phycoerythrin (R-PE) conjugate dilutions in Rubplex assay for specific IgG detection

| | Dilution (serum and R-PE conjugate) | Antigen | Coupling buffer | Antigen concentration (µg/mL) | Sensitivity | Specificity | Overall sensitivity and specificity |
|---|---|---|---|---|---|---|---|
| Multiplex assay | Serum: 1:200 conjugate:1:500 | E-1 | $NaHCO_3$ (pH 8.0) | 100 | 74% | 100% | 96-100% |
| | | E-2 | $NaHCO_3$ (pH 8.0) | 25 | 65% | 100% | |
| | | C | MES (pH 6.5) | 50 | 65% | 100% | |
| | Serum: 1:200 conjugate:1:100 | E-1 | $NaHCO_3$ (pH 8.0) | 100 | 74% | 100% | 91-100% |
| | | E-2 | $NaHCO_3$ (pH 8.0) | 25 | 61% | 100% | |
| | | C | MES (pH 6.5) | 50 | 43% | 100% | |
| | Serum: 1:100 conjugate:1:1000 | E-1 | $NaHCO_3$ (pH 8.0) | 100 | 48% | 100% | 87-100% |
| | | E-2 | $NaHCO_3$ (pH 8.0) | 25 | 57% | 100% | |
| | | C | MES (pH 6.5) | 50 | 45% | 100% | |
| | Serum: 1:100 conjugate:1:500 | E-1 | $NaHCO_3$ (pH 8.0) | 100 | 76% | 100% | 91-100% |
| | | E-2 | $NaHCO_3$ (pH 8.0) | 25 | 57% | 100% | |
| | | C | MES (pH 6.5) | 50 | 30% | 100% | |
| | Serum: 1:100 conjugate:1:100 | E-1 | $NaHCO_3$ (pH 8.0) | 100 | 91% | 100% | 100-100% |
| | | E-2 | $NaHCO_3$ (pH 8.0) | 25 | 96% | 100% | |
| | | C | MES (pH 6.5) | 50 | 35% | 100% | |




Scatter plots of singleplex assays with *Toxoplasma gondii* and *Rubella virus* antigens. Line represents cutoff value of the best assay. MFI: median of fluorescence intensity; Neg: negative; Pos: positive. (A) Results of MIC-3 antigen coupling in MES buffer, pH 5.5, at 100 µg/mL, 125 µg/mL and 150 µg/mL; MES, pH 6.5, at 125 µg/mL; and MES, pH 5.0, at 100 µg/mL and 125 µg/mL. (B) TOX8131 antigen coupled in PBS buffer, pH 7.2, at 100 µg/mL, 150 µg/mL and 200 µg/mL. (C) TOX8122 antigen coupled in PBS buffer at 100 µg/mL, 150 µg/mL, and 200 µg/mL. (D) Results for the *T. gondii* lysate coupled in PBS buffer, pH 7.2, at 100 µg/mL, 150 µg/mL, 200 µg/mL, 350 µg/mL, and 500 µg/mL. (E) CTOX antigen coupled at 100 µg/mL in MES, pH 6.5, PBS, pH 7.2, and $NaHCO_3$, pH 8.0. (F) CTOXH antigen coupled in 100 µg/mL in MES, pH 6.5, PBS, pH 7.2, and $NaHCO_3$, pH 8.0. (G) E-1 antigen coupled in MES buffer, pH 6.5, at 50 µg/mL and 100 µg/mL; in PBS, pH 7.2, at 50 µg/mL, 100 µg/mL, and 150 µg/mL; and in $NaHCO_3$, pH 8.0, at 50 µg/mL, 100 µg/mL, and 150 µg/mL. (H) E-2 antigen coupled in MES buffer, pH 6.5, at 50 µg/mL and 100 µg/mL; in PBS at 50 µg/mL and 100 µg/mL; and $NaHCO_3$, pH 8.0, at 50 µg/mL, 100 µg/mL, 150 µg/mL, and 25 µg/mL. (I) C antigen coupled in MES buffer, pH 6.5, at 25 µg/mL, 50 µg/mL, 100 µg/mL, and 150 µg/mL, in PBS, pH 7.2, at 50 µg/mL, 100 µg/mL, and in $NaHCO_3$ at 50 µg/mL and 100 µg/mL.