Toxoplasma Modified Agglutination Test (MAT)

Materials:

a). TgMAT antigens, 2x10^8/ml. *Toxoplasma* whole-cell antigen, formalin-fixed tachyzoites.

b). Alkaline Buffer. (Dissolve 7.01g NaCl, 3.09g boric acid (H_3BO_3), and 1g sodium azide in 950 ml distilled water, add 20 ml of 1N NaOH and adjust pH to 8.95. Add 4g of bovine plasma albumin, bring volume to 1 liter using distilled water.)

c). Phosphate Buffered Saline (PBS). (dissolve 7.20g NaCl, 1.48g Na_2HPO_4 (anhydrous), 0.43g KH_2PO_4 (anhydrous) in 1 liter of distilled water).

d). Positive control serum

e). Negative control serum

f). 2% Evans blue dye (2g in 100 ml distilled H_2O)

g). 2-mercaptoethanol.

h). 96-well U-bottom microtiter plates

i). Serum samples. Note: The minimum amount of serum for a sample is 3 µl.

Note: All reagents should be stored at 4ºC, except serum at -20ºC.

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Procedure:

1. Make 1:25 dilution of serum samples and the controls in PBS.

PBS	72.0 µl	
Serum sample or control	3.0 µl	
Total volume	75.0 µl	Mix well.

2. To a 96-well U-bottom microtiter plate, transfer 50 µl of diluted samples to the first and fifth rows from column 1 to column 10 of the plate. Transfer 50 µl of diluted negative and positive controls to the first wells of columns 11 and 12, respectively (20 samples, 1 negative and 1 positive control).
   See **Template 1** for sample layout.

3. Add 25 µl of PBS to the rest of the wells.

4. Using a multichannel pipette, take 25 µl of diluted samples (10 samples) from row 1, make serial dilution to row 4, remove 25 µl from the last dilution and discard. Repeat the procedure for the 10 samples in row 5. For negative and positive controls, make serial dilution to 1:3,200 and remove 25 µl from the last dilutions.

5. Prepare antigen mixture (each 96-well plate):

Alkaline Buffer	2.5 ml
2-mercaptoethanol	35 µl
Evans blue dye (2 mg/ml in H_2O)	50 µl
TgMAT antigen	150 µl
Total	2.735 ml

6. Mix antigen well by pipetting, immediately transfer 25 µl antigen mixture to each well using multichannel pipette. To prevent carryover of serum, the pipette tips should not touch the bottom of wells. Tap the plate lightly to bring the liquid to the bottom of the wells.
   Note: Each well has 3x10^5 tachyzoites.

7. Cover the plate with sealing tape and incubate at 37°C for 16-24 hours. A pellet at the bottom of the well means negative. Samples without pellets are positive.

8. For positive samples with titers >= 1:200, further test can be performed to determine titers. Serial dilutions include 1:25, 50, 100, 200, 400, 800, 1600, and 3200. See **Template 2** for sample layout.

Reference:

Desmonts G., Remington JS. Direct agglutination test for diagnosis of *Toxoplasma* infection: Method for increasing sensitivity and specificity. J. Clin. Microbiol. 1980. 11:562-568.

Dubey JP, Desmonts G. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. 1987. 19:337-339.

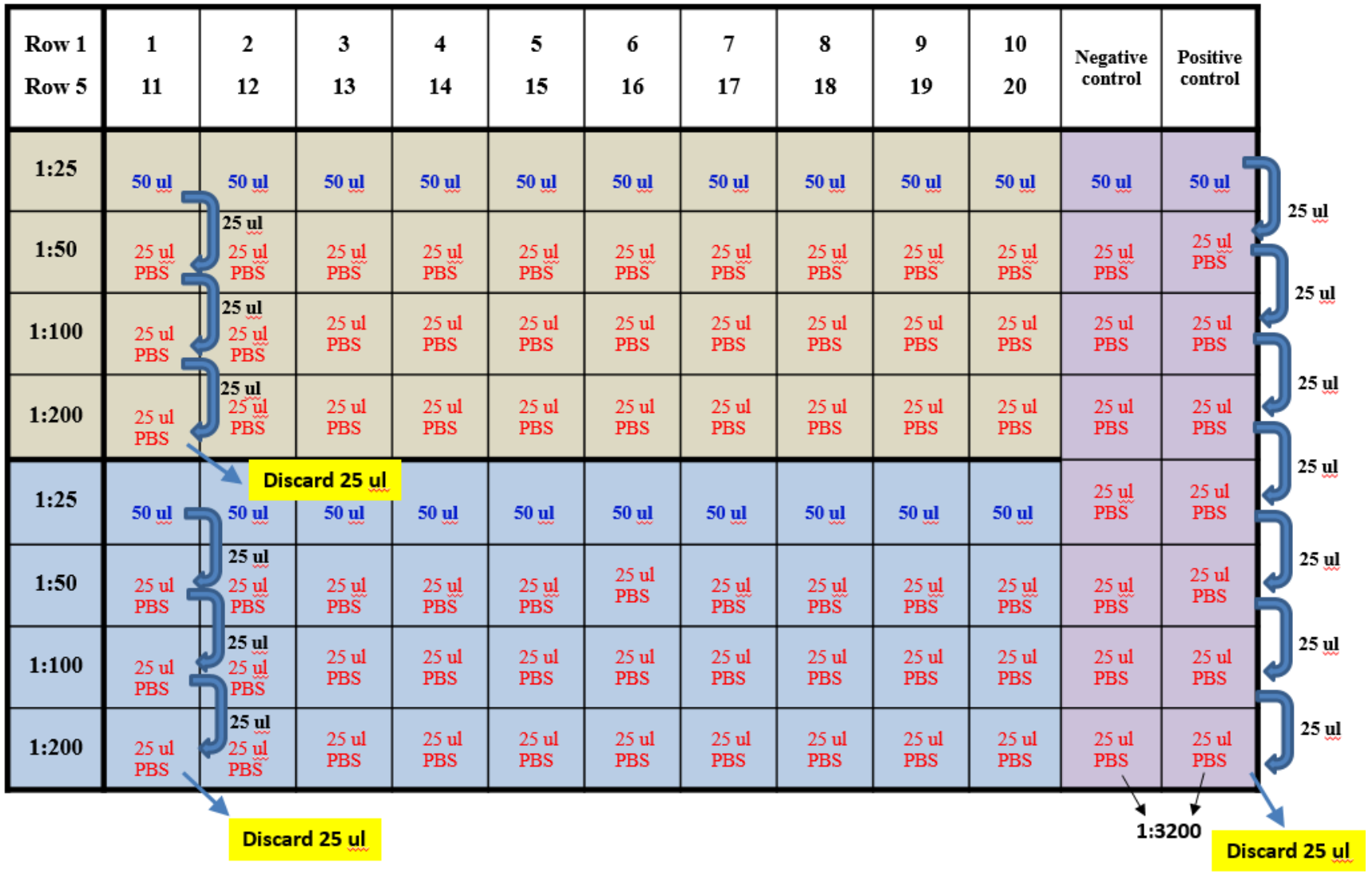
Note:

One milliliter TgMAT antigens can test six and half of 96-well plates. Each plate can screen 20 serum samples. Therefore, a total of 130 samples can be screened.

The recommended protocol for MAT test is also provided online:

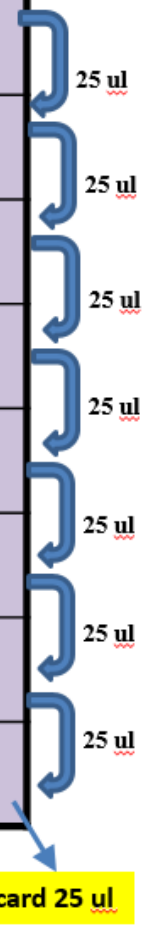
http://web.utk.edu/~csu1/MATprotocol.pdf

Template 1. Serial dilution of serum samples for MAT screening (20 samples/plate)



Template 2. Serial dilution of serum samples for titration (10 samples per plate)

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Negative control	Positive control
1:25	50 ul	50 ul	50 ul	50 ul	50 ul	50 ul	50 ul	50 ul	50 ul	50 ul	50 ul	50 ul
1:50	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS
1:100	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS
1:200	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS
1:400	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS
1:800	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS
1:1600	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS
1:3200	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS	25 ul PBS



Example of MAT test results (8 titers for each sample):