Experimental operation of *P. vivax* serology testing

(Version 1.0; Dec 30 2020)

1. Chip preparation

1. Mixing SA-Cy3 and SA-Cy5, then diluted by 1000 times and used as positioning points; serum of P. vivax clinical patient with 100 times diluted as positive control points; PBS and CBS spotting buffers are used as negative control points;

2. Dilute the sample with spotting buffer at 0.025, 0.05, 0.1, 0.2, 0.4, 0.8 ug/uL;

3. Take 30 uL each of the prepared working samples and transfer them to a 384-well plate, and place the 384-well plate on the sample stage of the spotting instrument.

4. Place the slide at room temperature for 1 hour.

5. Use the sciFLEX S1 spotter to perform dot system according to the settings; dot setting: 16 x Block, matrix 16 x 16 = 256, dot diameter 300 um, dot spacing 300 um, spray dot setting 1drop x 500pL.

6. After the spotting is completed, the chip is placed on the substrate stage and fixed overnight; take it out and store in a refrigerator at 4°C.

2. Chip testing

1) Sample preparation

1. preparing POS and negative control;

2. Tested serum samples were diluted 100 times with 3% BSA.

2) Reagent preparation:

1. Blocking solution: Weigh 3.0g BSA into a 15ml centrifuge tube containing 100 mL PBST (pH=7.2), vortex and shake, mix well, and set aside.

2. Washing solution: 1x PBST (pH=7.2).

3. Secondary antibody: Anti-Human IgG-Cy3 and Anti-Human IgM-Cy5 are mixed and diluted 1,000 times with 1x PBST.

3) Chip inspection

5. Take the chip out of the refrigerator at 4°C, return to room temperature, attach the front of the chip to the side of the fence rubber pad, put the fixing clip, and the chip assembly is completed.

6. Use a pipette to slowly add 100 uL of 3% BSA blocking solution to the fence hole; place the chip on a horizontal shaker, 70 rpm, room temperature, and seal for 1 hour.

7. Remove the blocking solution, pipette 100uL of the diluted sample and quickly add it to the corresponding well, place the chip on a horizontal shaker, 70 rpm, room temperature, and react for 2 hours.

8. Use 1x PBST to wash 3 times, 5 min each time; place on a horizontal shaker at 70 rpm.

9. Remove the cleaning solution, add 100 uL of the diluted secondary antibody to the well, place the chip on a horizontal shaker, 70 rpm, room temperature, and dark, and react for 1 hour.

10. Use 1x PBST to wash 3 times, 5 min each time; place on a horizontal shaker at 70 rpm.

11. Remove the fence, wash 2 times with 1x PBST and ddH2O for 5 minutes each time, and spin dry the chip.

12. Avoid light, scan the chip with Axon scanner at PMT350, Power 100%.