CHANG/MOORE LABORATORY

MCPyV VIRUS-LIKE PARTICLES (VLP) PRODUCTION PROTOCOL

From C.B. Buck, C.D. Thompson, Current Protocols in Cell Biology (Unit 26.1, Dec. 2007) with modifications.

1. Reagents and materials:

a. 5M NaCl

b. Dulbecco's Phosphate Buffered Saline (DPBS), Invitrogen (cat. #14140-141)

c. Optiprep density gradient medium, Sigma (cat #D1556-250ML)

d. 293-TT cells

e. Ultracentrifuge

f. Swing-bucket ultracentrifuge rotor (Beckman SW-55)

g. Siliconized collection tubes

h. Ultra-clear centrifuge tubes (1/2x2 in.), Beckman, #344057

i. 5-ml syringe with 21G needle

j. Brij-58 (polyoxyethylene 20 cetyl ether)

2. Protocol

1. Transfection of 293-TT cells. Transfect plasmids with VP1 and VP2 (1:3 proportionally) into confluent 293-TT cells (in 75cm flask) using Lipofectamin 2000 according to manufacturer recommendations. Return cells to the CO2 incubator for 48h.

Note! At this point cells and their products should be treated as an infectious agent.

After incubation collect 293TT cells by trypsinization (we collect about 20 million cells from 75 cm flask), wash cells with DMEM. Spin down the cells and remove supernatant. Resuspend the cells with 0.5 ml of DMEM-Mg. Transfer cell suspension into a siliconized 2ml screw-top tube.

2. **Cell lysis.** Resuspend cells in lysis buffer (10% Brij-58 in DPBS). Use 100 million of cells per 1ml of lysis buffer. Incubate at 37C for 24 hours to allow capsid formation.
3. **Salt extraction.** Place tube with lysate on ice for 5-10 minutes. Add 0.17 volume of 5M NaCl. Incubate on ice for 10 minutes.

Spin cell lysate at 10,000rpm for 10 minutes in refrigerated centrifuge. Collect clarified supernatant to fresh siliconized tube.

4. **Optiprep purification of MCPyV capsids**

   Preparation of gradients. Dilute 46% Optiprep/DPBS with DPBS/0.8M NaCl to 27%, 33%, 39%.

   a. Using the pipet transfer 1.5ml of 27% Optiprep to each ultracentrifuge tube.
   b. Load the syringe with 1.5ml of 33% Optiprep. Insert syringe until the needle is gently touching the bottom of the tube. Eject 33% Optiprep beneath the 27%.
   c. Repeat step b with 39% Optiprep.
   d. Cover the tubes with Parafilm and incubate for 3 hours at room temperature.
   e. Gently overlay 0.5ml of cell lysate (from step 3) onto the top of the gradient. (*Tubes have to be filled to near the rim to avoid tubes collapse during centrifugation*). Place tubes to the ultracentrifuge buckets. Balance the buckets with DPBS/0.8M NaCl to within 5mg.

5. **Ultracentrigugation.** Spin samples for 4 hours at 50,000 rpm (234,000 g) at 16C. Acceleration and deceleration should be set to "slow".
6. **Fraction collection.** Carefully transfer ultracentrifuge tubes from the centrifuge to the tube holder. The VLP band may be visible as a light grey layer a little over a third of the way up the gradient. Puncture the bottom of the tube slightly off center with a syringe needle. Collect fractions in syliconized tubes. Collect first 1ml as one fraction, and then collect 6 drops fractions up to fraction 8.
7. **Screening fractions.** MCPyV VLPs are usually can be found in fractions 3-5. Fraction screening can be performed by silver staining of the gel (load 15-20ul of each fraction to the gel).

MCPyV ELISA ASSAY PROTOCOL

From Tolstov et al., International Journal of Cancer, 2009; 125(6):1250-1256.

1. Reagents and materials:

a. Immulon 2HB (High Binding) flat-bottom 96-well plates (VWR cat. # 62402-972)

b.1x Phosphate-Buffered Saline (PBS) pH 7.4
c. Non-fat dry milk (powder)
d. Secondary antibody (Polyclonal Rabbit anti-human IgG HRP, Dako, cat. # P0214)
e. Tetramethylbenzidine (TMB) substrate (Sigma, cat. # T0440-1L)
f. 2N sulfuric acid, 90 ml (5ml of 32N sulfuric acid and 85ml of Milli-Q water)
g. MCPyV Virus-Like Particles (VLP)
h. Microplate adhesive film (USA scientific, cat. #2920-0000)

2. Protocol

1. Coating Immulon plate with VLPs. Dilute the antigen in PBS to final concentration of 1ug/ml. Use 100ul (100ng) for each well. Cover the plate with microplate adhesive film and incubate overnight at +4C.

2. Next day - wash plate 3X with PBS.

3. Block plate with PBS/0.5% milk (200µl per well) for 2 hours at room temperature.

4. Wash plate 3X with PBS.

5. Dilute human sera in PBS with 0.5% Milk. Use 1:500 dilution (1ul of sera and 499ul of PBS/0.5% milk). Add 100µl per well and incubate for 2 hours at room temperature.

6. Wash plate 3X with PBS.

7. Dilute anti-human-IgG HRP antibody 1:6000 in PBS/0.5% milk. Add 100ul per well and incubate for 1 hour at room temp.

8. Wash plate 3X with PBS.

9. Add 100ul of TMB substrate per well. Incubate for 45 minutes at room temperature.

10. Stop the reaction by adding of 100ul of 2N sulfuric acid in each well.

11. Read the plate on plate reader at OD 405 with a reference of 620nm.

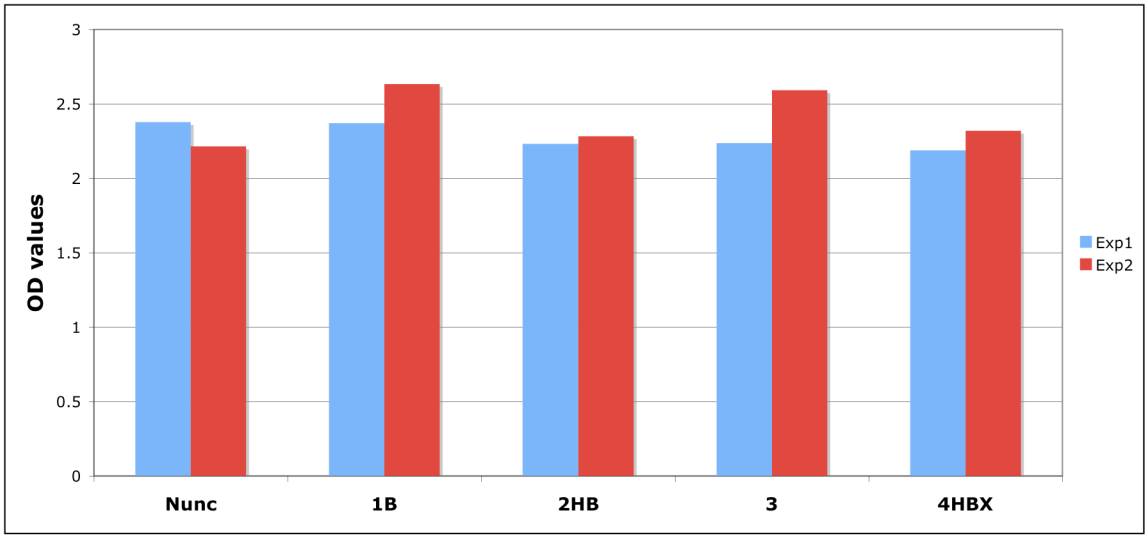

Figure 1. Reactivity of anti-MCPyV VLP antibodies in serum from MCPyV-positive MCC patient. 2HB Immulon plates demonstrated greatest consistency between two tests and were chosen for screening.

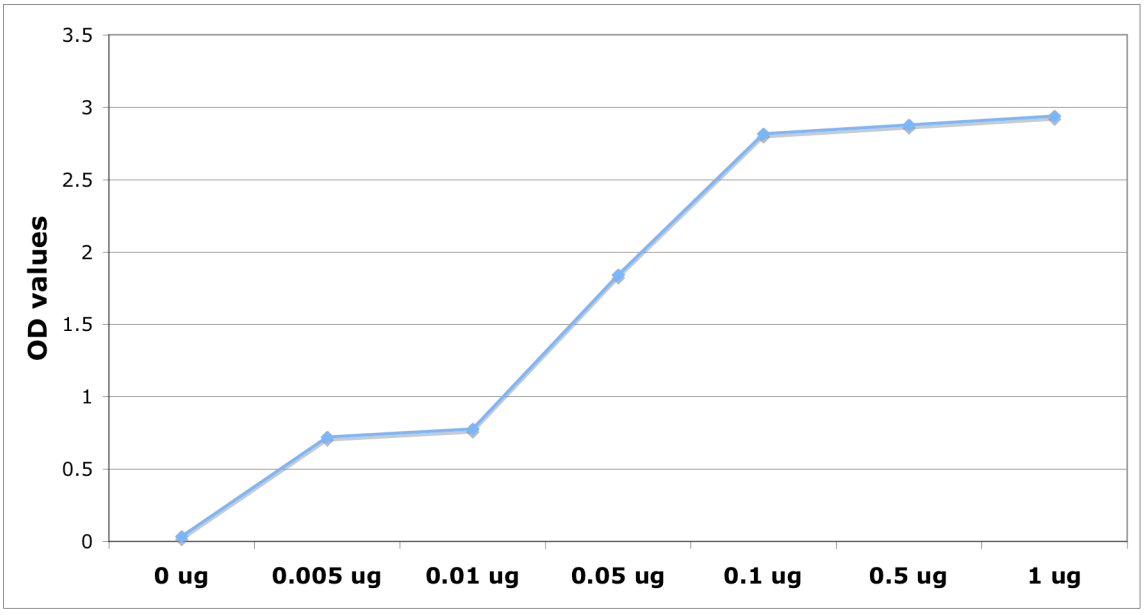

Figure 2. MCPyV saturation chart. Reactivity of mouse anti-VP1 antibodies was measured using MCPyV VLP ELISA.