

Cunningham Plaque Assay

Materials

- Glass slides 3 x 1" non-frosted (Clay Adams #3010)
- Double sided tape (Minnesota, Mining & Manufacturing Co., Product #410) available locally through Blake, Moffit & Towne)
- 50:50 mixture of vaseline petroleum jelly and paraffin (Paraplast tissue embedding medium (available through SP). When sealing the slides, the mixture is kept melted on a hot plate (setting #3 on our hot plate)
- Microtiter plate - "U" well (Cooke Engr. Co.- available through SP)
- Dissecting microscope
- Plastic cup, about 150 ml capacity, that has the bottom cut out of it.

Slide Chambers

- 1) Scrub slides with 95% ETOH, wipe dry.
- 2) Apply tape to each 1" edge of slide, then center a third strip between them. Lay a second slide on top of the first and press down firmly.

Set-up

To mix dil. later on a 5-10 minutes basis making cell suspensions in microtiter plates
Reagents are kept on ice until mixtures are made in microtiter plates immediately before plaquing.

The following reagents are added together in the order that they appear below in the wells of the microtiter plate. The total volume will be 100 λ of which 50 λ is pipetted onto the slide. All dilutions are done in MEM. Each point in the assay is done in duplicate or triplicate.

- 1) Mouse spleen cells: 25 λ . Concentration adjusted to give 100-200 plaques/slide chamber. Take into account that the number of cells finally counted is 1/2 the number put into the microtiter well.
HINT: To obtain concentration (cells/ml) needed, multiply number of cells wanted in chamber times 80. Example:
$$\begin{array}{r} 1 \times 10^5 \text{ cells wanted in chamber to give } 100-200 \text{ plaques} \\ 1 \times 10^5 \\ \hline \times 80 \\ \hline 8 \times 10^6 \text{ cells/ml concentration} \end{array}$$

1 x 10⁶ / chamber
(1 x 10⁴ min for at least 30 plaques)
- 2) Developing antisera: 25 λ . Concentration determined by titration. The rabbit anti-mouse-immunoglobulin antisera currently in use (R-65) is diluted to give a final concentration of 1/200-1/300 in the well.
- 3) Complement: 25 λ . Concentration determined by titration. Presently 25 λ of a 1/5 or 1/6 dilution of SRBC-absorbed guinea pig serum is being used. (Final concentration = 1/20 or 1/24).
- 4) SRBC - 25 λ of a 5% dilution is added to each well, 24 wells at a time. Just prior to pipetting the assay mixtures into the slide chambers.
- 5) Mix contents of each well by aspirating with a 50 λ pipette, then transfer 50 λ of the mixture to the slide chamber. Slight pressure may be needed to get the contents to flow between the slides.
- 6) The open sides of each slide are sealed in the melted paraffin-vaseline mixture. Slides are then put on a tray and incubated at 37°C for 45-60 minutes before being read. NB? Developed plaques may take longer.
- 7) Count the plaques under a dissecting microscope using indirect light. Set the slide on top of the plastic cup and adjust light source and mirror so that the plaques appear as dark spots against the SRBC background. Often it is helpful to mark a grid on the slide with a green Pentel Sign Pen to make counting easier.

Rationale: far less reagents necessary; smaller plaques visible because the SRBC are