

Rapid identification of respiratory syncytial virus

Respiratory syncytial virus (RSV) is a major cause of severe lower respiratory tract diseases in infancy and early childhood. A rapid, simple, and accurate plaque assay for RSV identification is one of the most important procedures in RSV studies. Here we report a new RSV plaque assay method using immunostaining. This assay can be completed at 2 days post-infection with an earlier observation of plaque than crystal violet stain. The method presented here not only is simple, rapid and repetitive, but also can be used in plaque reduction assay to evaluate drug effect. This method can be used to sensitively and rapidly titer virus from mouse lung samples. This assay would be the discrimination of cell death between virus-induced or mediators (e.g. cytokine/chemokine) induced cell lysis in biological samples, e.g. in the mouse lung homogenate.

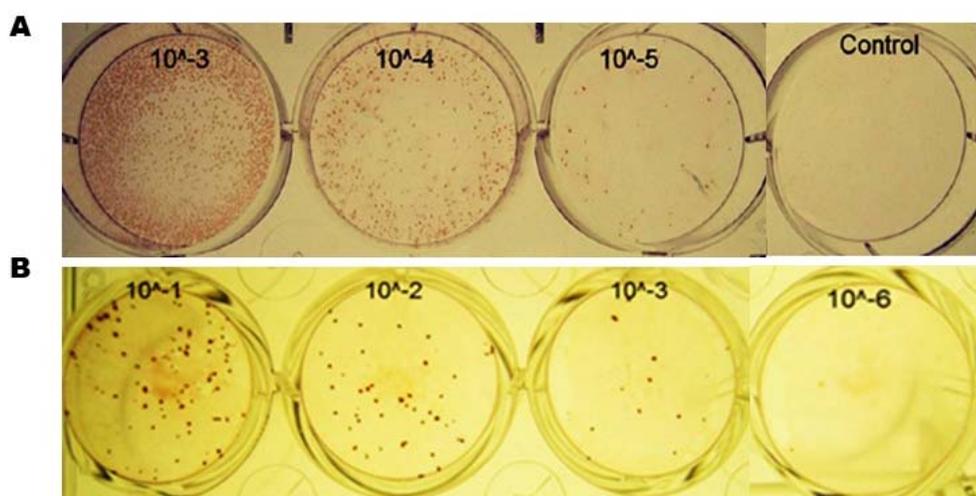


Fig. 1. Plaques from Hep-2 cells or mouse lungs. A. HEp-2 cells were grown in 6-well plates and infected with serially diluted virus (10^{-3} , 10^{-4} , 10^{-5}). Plates were incubated with virus at 37°C for 1 h, after which 2 mL of overlay was added to each well. After 2 days of incubation, cells were immunostained, observed, and photographed. Control: non-infected cells. B. For plaques from mouse lungs, mice were infected with RSV A2 and lung tissue was harvested at day 4 post-infection. The individual lung was minced completely between two glass slides in 1 mL of DMEM media. The homogenates were spun by centrifugation at 1000 rpm for 10 min and the supernatants were serially diluted (10^{-1} , 10^{-2} , 10^{-3} to 10^{-3} , 10^{-6}). The plaques from lung samples was observed at day 2 after incubation of Hep-2 cells with virus.

Obtaining rapid and accurate plaque counts is a very important aspect of RSV-related studies. The RSV A2 virus grows relatively slowly and typically requires 4-7 days for visible plaques to form. These visible plaques are from lysed cells. When the plaques are large enough, the surviving cells surrounding the lysed cells can be stained with various dyes Both HEp-2 and Vero cells have been used in plaque assays for RSV. HEp-2 cells have been shown to produce larger plaques than Vero cells; moreover, Vero cells take longer (7 – 9 days) to produce pinpoint plaques. Clinical isolates of RSV have been tested in Vero and HEp-2 cells and yielded poor plaques in Vero cells. Thus, we used HEp-2 cells to optimize our plaque assay protocol. We found that only 75% – 95% of all cells

were lysed by 4 – 6 days postinfection. Thus, plaque assays employing neutral red or crystal violet would be expected to exhibit only 75% – 95% accuracy. With our immunostaining of the current method, infected cells (plaques) could be accurately observed by eye by day 2. We found that plaque count wasn't accurate after longer incubation time for virus since 2 or 3 plaques nearby merged into one plaque which caused plaque bigger following the longer virus incubation, resulting in reduced plaque counts.

Our plaque assay is rapid and simple, in which only 2 days are required for virus incubation and 50 min for immunostaining, whereas previously described assays require around 4-7 days for virus incubation and anywhere from 1 h to multiple days for staining. Our overlay consisted of only 0.8% agar, whereas other reported overlays require complicated reagents such as FBS, FCS, or methylcellulose. Hence, our assay is extremely fast and simple. Our plaque assay also can be counted by naked eye. It is reported that a flow cytometry-based RSV-specific neutralization assay is rapid, highly sensitive and reproducible. However, it needs instrument for flow cytometry which is not available for every laboratory. Overall, our plaque assay is more rapid and simpler than other published methods. In our current study, monoclonal antibody against anti-RSV fusion protein was used for immunostaining, which seems expensive. However, newly published work indicates, using immunostaining with anti-RV P monoclonal antibody, the plaque shape induced by rabies virus is clearer than that observed by crystal violet staining, supporting the advantage of immunostaining plaque assay we presented in this study.

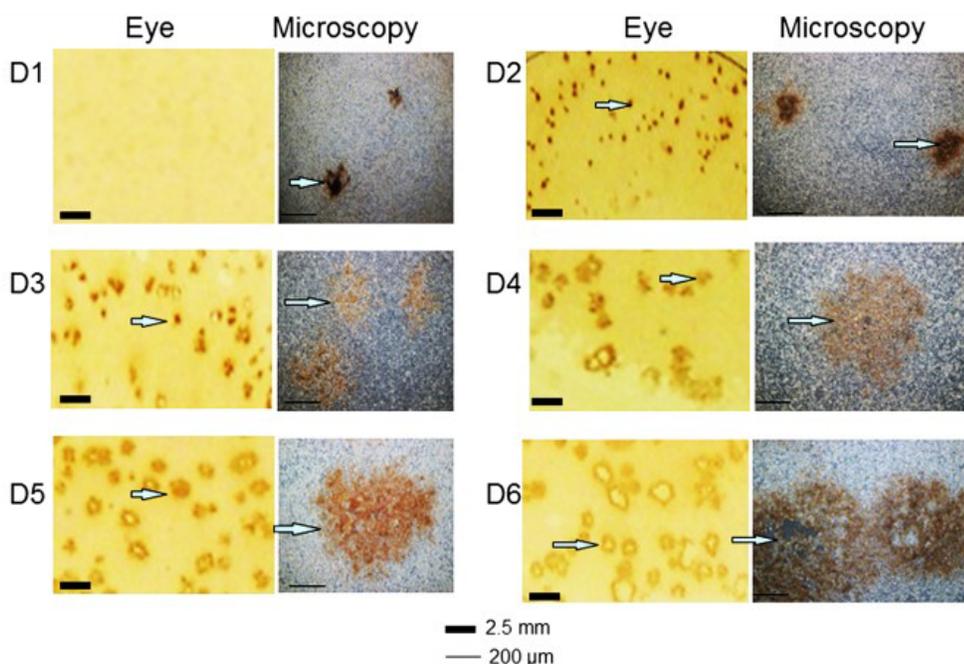


Fig. 2. Virus identification using plaque assay. The plaques generated on different days (days 1, 2, 3, 4, 5, and 6) were immunostained and compared. HEp-2 cells were grown in 6-well plates and infected with 10^3 serially diluted virus. Plaques were observed on days 2, 3, 4, 5, and 6 post-infection by eye and under a microscope.

In conclusion, we have developed a rapid, simple, and sensitive plaque assay for identification of HRSV. In this assay, plaques can be directly visualized by the naked eye at day 2 post-infection. Stained plates can be stored permanently for future reference. This method has a significant impact on all studies related to RSV disease.

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Publication

[A rapid, simple, and accurate plaque assay for human respiratory syncytial virus \(HRSV\).](#)

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