Development of ELISA Assay for d1327 Adenovirus

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Adenoviruses are a common cause of upper respiratory tract infections in children and the source of acute respiratory disease outbreaks among military recruits. Named after the human adenoid tissue it was first isolated in, the adenoviridae are non-enveloped, double-stranded DNA viruses with over 50 serotypes. Yet to date vaccines have been developed for only two serotypes, and no adenovirus antiviral drugs have been approved by the FDA, highlighting the need for more research.

In virus research, detection the presence of a particular virus is of especial interest when developing animal or cell models. As human pathogens often do not infect common lab animals or certain cell types, researchers must alter either the host or the virus in order to produce a working model. This may involve genetically engineering host cells to express a need receptor protein, or passing cells multiple times in the presence of the virus, anticipating that the virus will evolve pathogenicity. Thus, the ability to detect if a virus has replicated in a host is key to determining the viability of a cell or animal model. Here we attempt to develop an ELISA detection assay for adenovirus d1327. ELISA assay is especially useful for virus detection because of its specificity and easily identifiable positive result. Antibodies specific to the virus of interest bind the virus to the plate, while enzyme conjugates provide a visible signal if the virus is present.

I. Introduction

II. Methods

A plate layout as well as needed reagents and blocking times were determined the previous year. However, in order to select the correct antibodies to be used in the assay, the type of the virus had to be determined. There is little information in the literature which confirms the type of d1327, with the type determined to be either AdV 1 or AdV 5. In order to remedy this, the virus needed to be identified by treating a plaque assay of d1327 with antibodies towards type 1 and type 5. The proper dilution and incubation times for the plaque assay must first be determined, and then the virus sample can be challenged with the different antibodies. Once the virus type is determined, the assay can be constructed using the correct antibodies.

III. Results

We had difficulty getting definitive results from the plaque assay. The first difficulty was removing the agarose plug without disturbing the monolayer. Removal by spatula or vacuum have the potential to be messy. We then attempted to do a live staining with neutral red added directly to the agarose, but this failed to visualize any plaques. We ultimately settled on using a crystal violet stain with careful removal of the monolayer with a fine-tipped vacuum attachment. Once the monolayer was cleanly removed and the wells stained, cytopathic effect was observed in the monolayer. However, the CPE was diffuse and did not form individual plaques.

IV. Conclusions

Development of the assay was not limited by the inability to produce a potential protocol for the ELISA, but rather from difficulty identifying the type of virus being tested. In order to remedy the diffuse CPE, a higher concentration agar could be used to cover the monolayer, which should help restrict the virus from infecting neighboring cells. However, an alternative to the plaque assay, the virus neutralization assay, may be a better option. Once the virus type is determined, our ELISA protocol can be tested and optimized.

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