Development And Validation Of A Multiplexed Assay For The Detection Of Respiratory Viruses

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Abstract:

Background: Many viruses cause respiratory infections. However, only a handful of these viruses routinely cause dangerous pathologies. For these viruses, rapid detection is important for patient management. Infection may be diagnosed by viral culture, direct immunofluorescent assay (DFA), or enzyme immunoassay. It is the objective of our research to develop a sandwich ELISA based microarray assay which can simultaneously detect the presence of influenza A (all types) and B virus; respiratory syncytial virus; parainfluenza 1,2,3; and adenovirus (all types) particles in nasal wash samples.

Methods: The assay was developed by printing monoclonal antibodies to the viruses noted above in a defined pattern in 96-well plates. The plates were incubated with 30 microliters of sample and the presence of particles detected by a second biotinylated antibody, with subsequent streptavidin-HRP treatment. The plate was then flooded with chemiluminescent substrate and imaged using a digital camera (Alpha Innotech 8900).

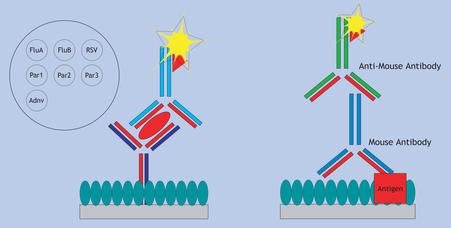
In developing the assay we tested multiple monoclonal and polyclonal antibodies specific to each virus. Initial screening of monoclonal antibodies was performed using gamma irradiated samples of the most common research strains. We then followed up by testing 15 nasal wash specimens previously shown positive to the viruses listed above.

Results: When assayed individually, 4/4 influenza A, 1/1 influenza B, 2/2 parainfluenza 1, 2/3 parainfluenza 2, 1/1 parainfluenza 3, 2/2 adenovirus, and 2/2 respiratory syncytial virus samples consistently showed a positive response over background. However, when a mix of detection antibodies was used, 2/4 influenza A, 0/1 influenza B, 1/2 parainfluenza 1, 0/3 parainfluenza 2, 1/1 parainfluenza 3, 0/2 adenovirus, and 0/2 respiratory syncytial virus samples consistently showed a positive response over background.

Conclusion: 14/15 samples were accurately detected when tested individually, while only 4/15 were accurately detected in a multiplexed format. When multiple detection antibodies were mixed, the cross-reactivity between detection and capture antibodies was too great to distinguish the response from the background. We showed that it is possible do detect respiratory viruses from nasal wash specimens in a multiplexed format, though application requires greater control of cross-reactivity. Future research includes the development of new antibodies with greater specificity and less cross reactivity.

Development process:

1. Acquire multiple clones to each virus.
2. Confirm specificity and determine match pairs using gamma irradiated stocks.
3. Confirm match pairs with live virus.
4. Determine assay sensitivity.
5. Multiplex all match pairs and identify cross-reactivity among antibodies and viruses.
6. Validate using clinical samples.



Sandwich ELISA Direct Assay

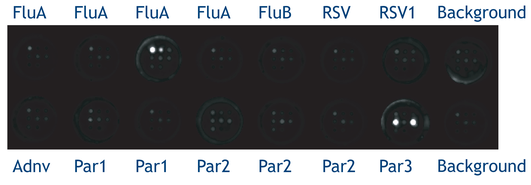
1. Print Antigen or Antibody
2. Run ELISA Assay
3. Image with CCD Based Imager
4. Analyze with Quansys Array Analysis Software

Micro-ELISA Process

Direct Assay

Individual Tests

Multiplexed Tests



Conclusion:

- 14/15 samples accurately detected when tested individually.
- 4/15 accurately detected in a multiplex format.
- Cross-reactivity between dectection and capture too great to distinguish response from background when multiplexed.