Novel colorimetric detection method for the cost-effective identification of influenza on a low-density microarray.

Explore the power of microarray technology without incurring the capital equipment costs associated with fluorescence.

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Abstract

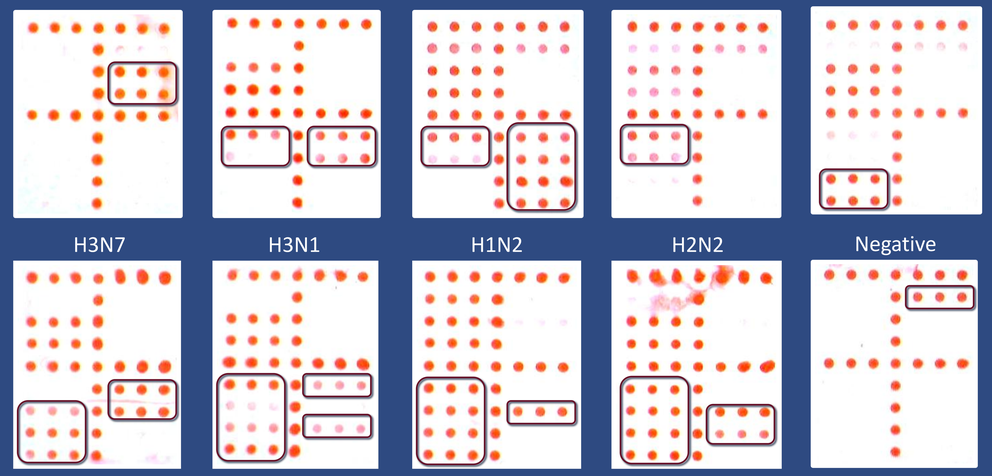
The 2009 H1N1 influenza pandemic highlighted the importance of early detection and response to influenza outbreaks. The presented work explores the use of ampliPHOX, a rapid, cost-effective, field-amenable colorimetric detection method, combined with the FluChip low-density microarray for screening and surveillance of influenza viruses. A DNA microarray was developed using 13 unique synthetic oligonucleotide capture sequences designed to target variable regions within the matrix protein gene (M gene) and nonstructural gene (NS gene). Analysis of microarray data from over 100 influenza samples confirmed the ability to easily distinguish influenza A and B and produce unique array patterns for a wide variety of influenza A subtypes. This work demonstrates that ampliPHOX Detection paired with a low-density microarray can provide a low-cost alternative to methods such as qRT-PCR for surveillance of influenza, particularly in resource-limited settings.

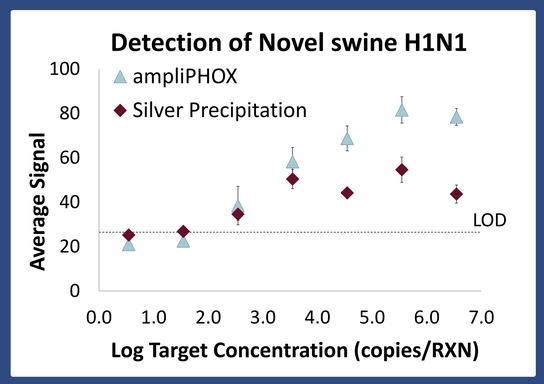
Case Study: Detection of Influenza on a low density microarray

- The M gene segment of influenza A and NS gene segment of influenza B were RT-PCR amplified from 181 Influenza specimens.
- Biotinylated dUTP at an optimized concentration was incorporated during RT-PCR for downstream labeling with a streptavidin conjugated photoinitiator.
- Lambda exonuclease was then used to digest the reverse-complement of the amplified target DNA strand, producing single stranded product for rapid hybridization (60 min).
- Single stranded product was heat fragmented at 95°C prior to hybridization to the microarray (step 2, far right).
- Labeling and photopolymerization steps were then performed (steps 3-6, far right).
- ampliVIEW software package was used for automated image analysis.

Polymer is formed only where positive signal is present

Unique microarray hybridization patterns are produced for a wide variety of Influenza A subtypes.





How do the numbers compare?

ampliPHOX: LOD 50 ± 2 copies/reaction
Silver: LOD 740 ± 70 copies/reaction

***Data is based on 4 separate experiments.**

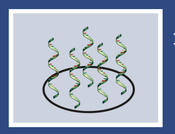
Through both in-house studies and 3rd party beta site testing, ampliPHOX has been shown to have equivalent analytical sensitivity to traditional fluorescence.

Time and Cost Advantages :

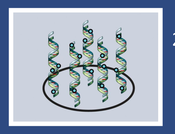
- Inexpensive instrument (< $4k) and low-cost reagents (<$5/assay)
  - For lower density microarray applications, get equivalent analytical sensitivity to fluorescence for a small fraction of the cost of a traditional microarray scanner.
- Ease of use
  - Simple assay is pre-optimized for a range of substrates and targets.
  - Entire labeling/detection procedure can be completed in 20 minutes.
  - High contrast colorimetric results for visual or automated analysis.
- Assay Development
  - Can be used with any biotinylated target for a variety of microarray applications such as DNA, proteins, antibodies, miRNA, peptides, and aptamers.
- Robust and Flexible Instrumentation
  - Instrumentation is lightweight (<3 lbs) and compact (5”H x 5”W x 5”L), ideal for use in resource limited settings.

Basic Principle of ampliPHOX Detection Technology

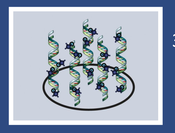
- Although shown here for a nucleic acid application, ampliPHOX is amenable to a variety of biotinylated targets



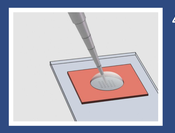
1) Probe is bound to the surface



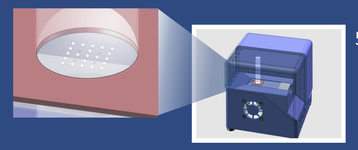
2) Biotinylated target is bound to the probe



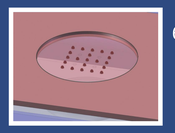
3) Target is labeled with a streptavidin-photoinitiator conjugate



4) A solution of acrylate monomer and cross-linking agents are added to the array

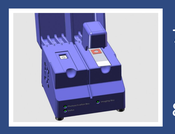


5) Array is exposed to 532 nm light initiating free-radical polymerization
   - Hydrogel is formed only were target has been labeled with initiator.



6) Hydrogel is stained to improve contrast

- Small benchtop-microarray detection platform developed specifically for resource limited settings



7) Microarray is imaged using an inexpensive digital camera.

8) Image is analyzed using intuitive software package