

# Development of a micro-array based multiplex PCR assay for the differential diagnosis of meningitis and encephalitis

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# *Introduction*

- Important to ID aetiological agent in cases of neurological infection
  - Help physicians to eliminate unnecessary tests and treatments
  - Allows for implementation of a prompt therapeutic approach
- The majority of infective CNS syndromes results from virus infections
  - Majority of cases causal agent is never identified
    - Enteroviruses (80-90% of cases where an agent is identified)
    - Mumps , Herpes viruses and Arboviruses
    - Adenoviruses, Influenza and HIV are less frequently associated with aseptic meningitis

# *Introduction*

- **Problem with diagnostics**
  - Fraction of potential pathogens associated with CNS infection are identified
  
- **Reasons**
  - Large number of potential pathogens involved/ limited testing
  - Small volume of CSF divided up for several tests/ labs

# Aim

- To develop a high throughput diagnostic assay for the differential diagnosis of arboviruses and other aetiologies of neurological infection, that
  - Can easily be incorporated into standard molecular labs
  - Assay for 30 pathogens within 6.5-7.5 hours

# Assay targets

West Nile virus

Rift valley fever

Chikungunya

Sindbis

Rubella

Crimean Congo hemorrhagic fever

Cytomegalovirus

Measles virus

Mumps virus

Herpes simplex virus 1

Herpes simplex virus 2

Varicella zoster virus

Neisseria meningitidis

Flavivirus

Rabies virus

Epstein-barr virus

JC virus

Enterovirus

Dengue

Rickettsia spp.

Borrelia burgdorferi/garinii

Brucella spp

Adenovirus

Coxiella burnetti

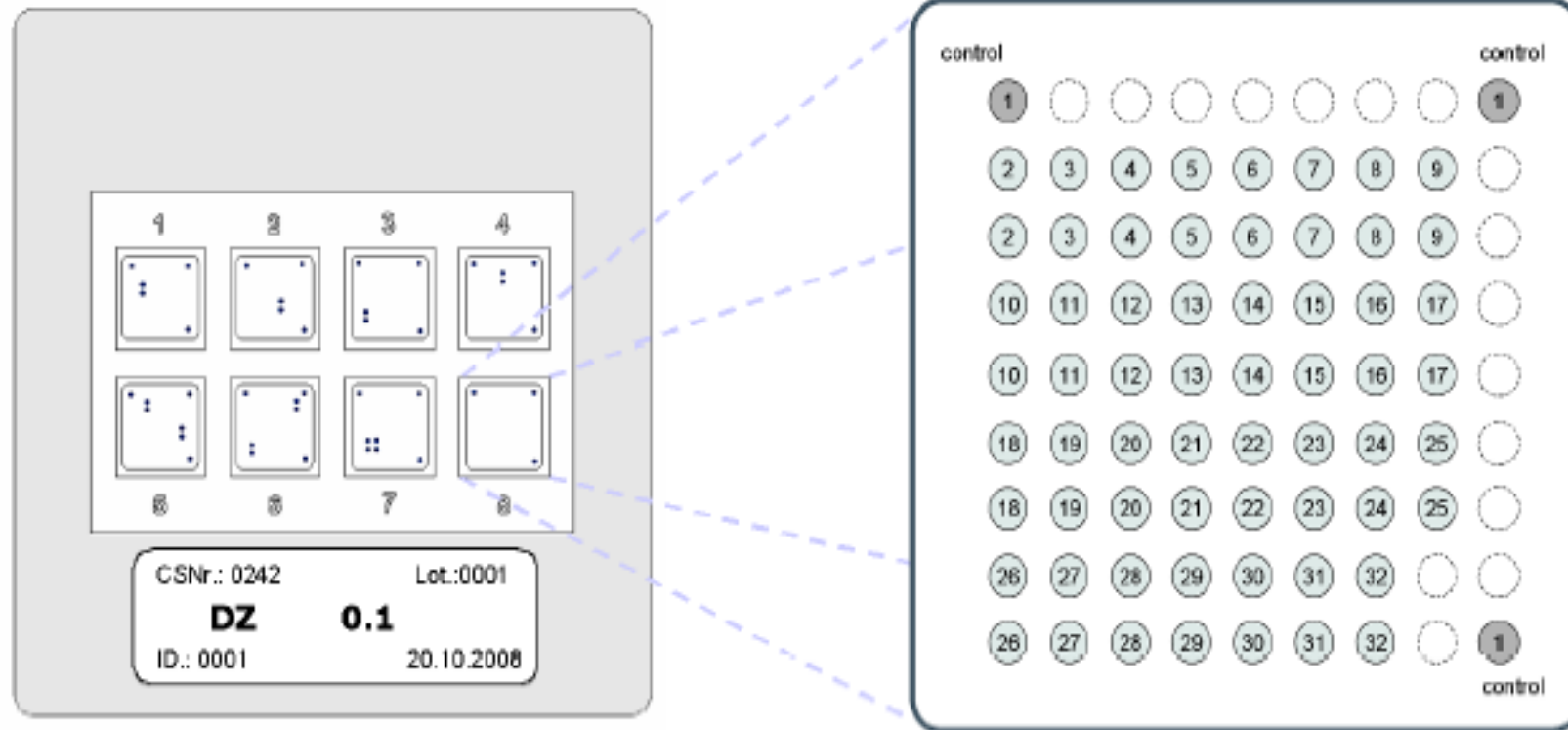
Leptospira spp

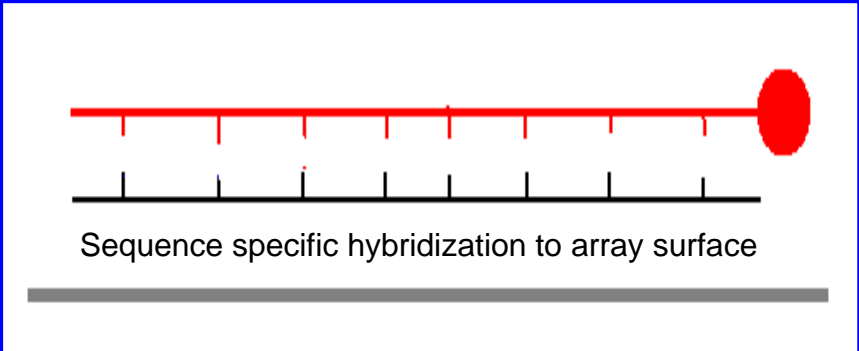
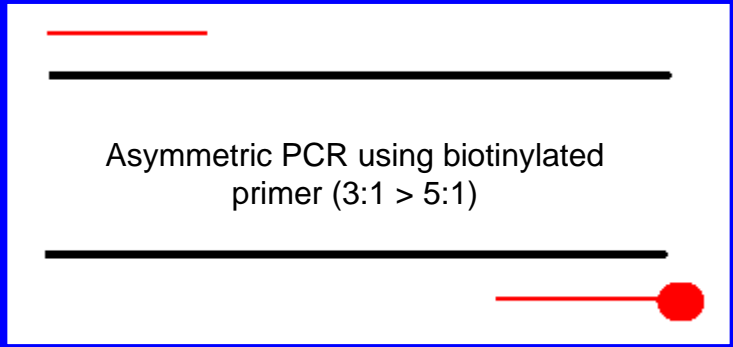
Mycobacterium tuberculosis

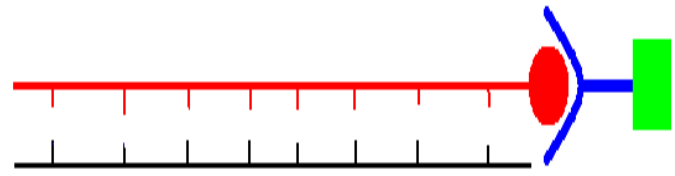
Ehrlichia spp.

Plasmodium  
Hepatitis-A and B

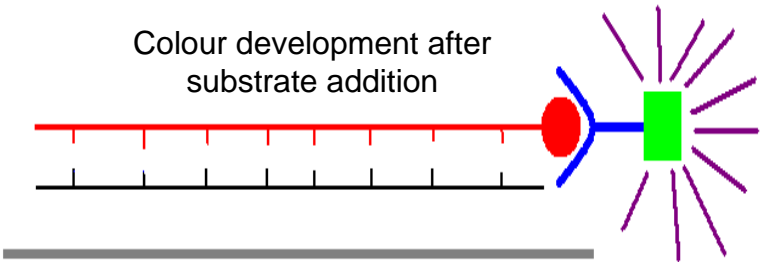
# Array layout



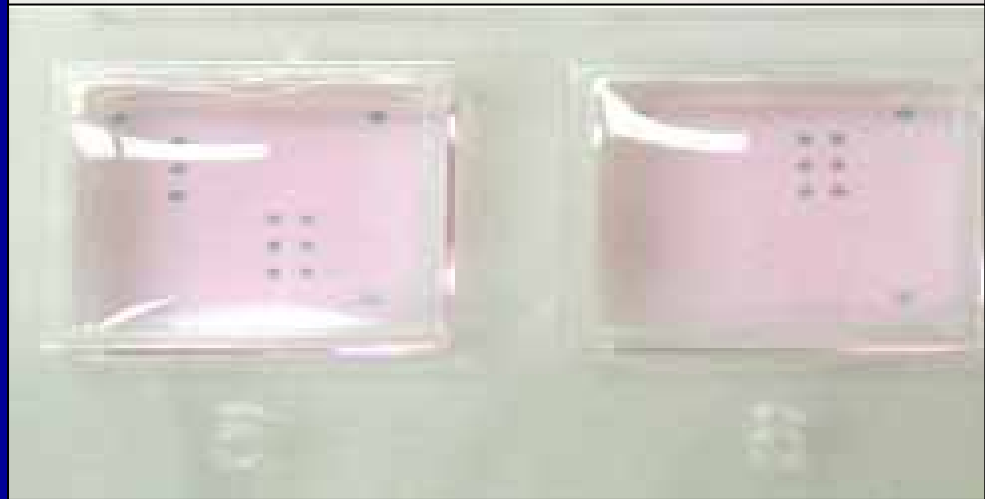




Addition of enzyme-conjugated Streptavidin



Colour development after substrate addition



# Approach

Nucleic acid isolation

30mins-1hour



cDNA synthesis

1hour



2 Multiplex PCRs

4.5hours

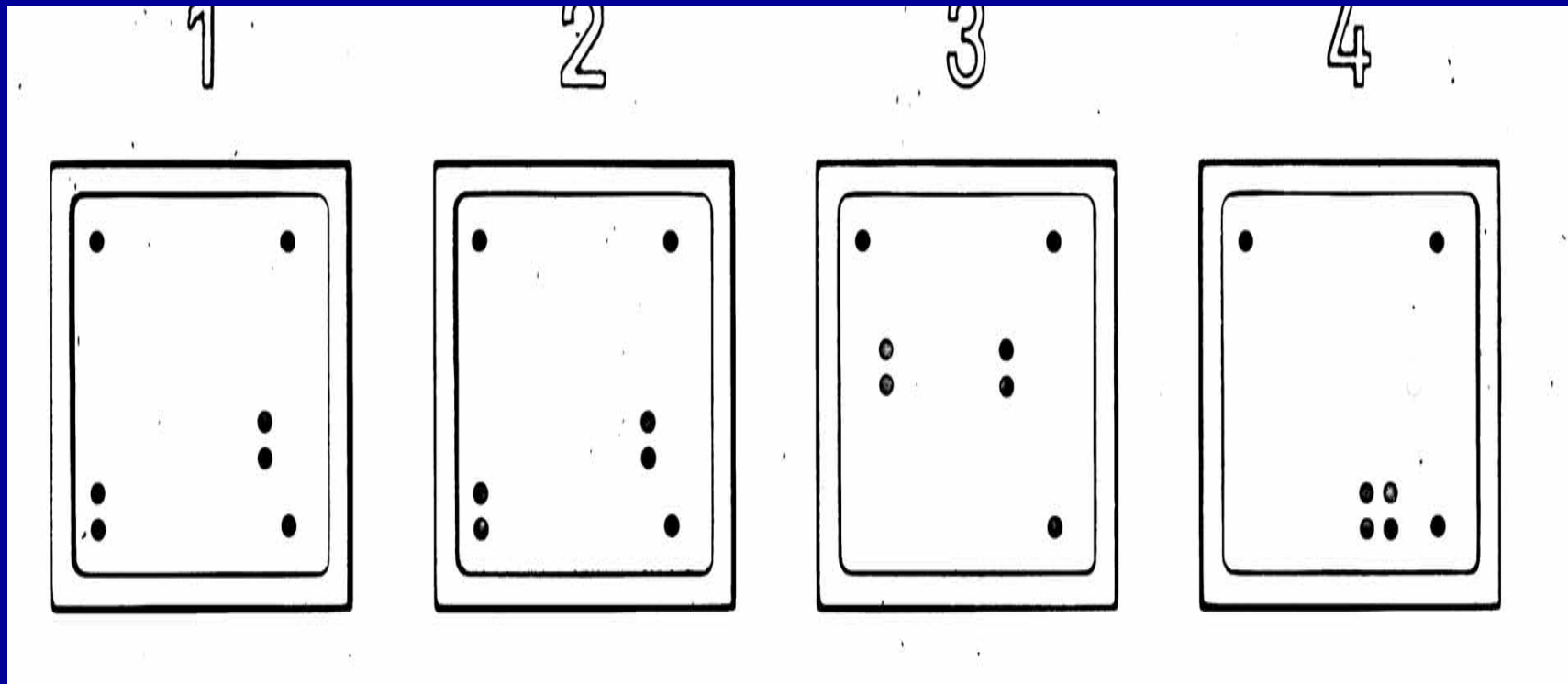


Array hybridization

45mins-1hour

6.5 -7.5 hours

# Hybridization Results



**Mycobacterium and Ehrlichia  
spp.**

**HSV-1 and JCV**

**Hep-A and B**

# *Validation*

- Successfully detect all 30 pathogens in positive control specimens
- Determine sensitivity of the assay
  - Run serial dilutions to calculate assay sensitivity
    - $5 \times 10^{-6}$  –  $5 \times 10^{-7}$  ng plasmid DNA
- Further validation ongoing – Prospective and retrospective screening of clinical specimens
  - Known positive and negative (Unknown) specimens from SPU, NICD
  - Query meningitis (negative) specimens collected from Dept. of Medical Virology diagnostic lab

# Contributors

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