

# Multiplex fluorescence-based PCR assay to detect pathogens in blood

A. Selvapandiyan

LBPUA, OBRR, CBER, FDA,

Bethesda, MD

July 13 2006

## Background:

- Transfusion blood safety has improved with pathogen testing
- Increasing number of known potential infectious agents and emerging threats, including bioterrorism increases the burden of testing
- Urgent need for methods to streamline and consolidate testing

## Aim:

- Design a method that can detect rapidly for many pathogens simultaneously using Multiplex Fluorescence-based PCR
- Performing in a convenient portable instrument SmartCycler



## Pathogens

## Assay conducted with

*Bacillus anthracis*

pa gene

*B. anthracis* (vaccine strain)

*Yersinia sp.*

16S rRNA

*Y. pseudotuberculosis*

*Leishmania sp.*

18S rRNA

*L. donovani*

*Trypanosoma sp.*



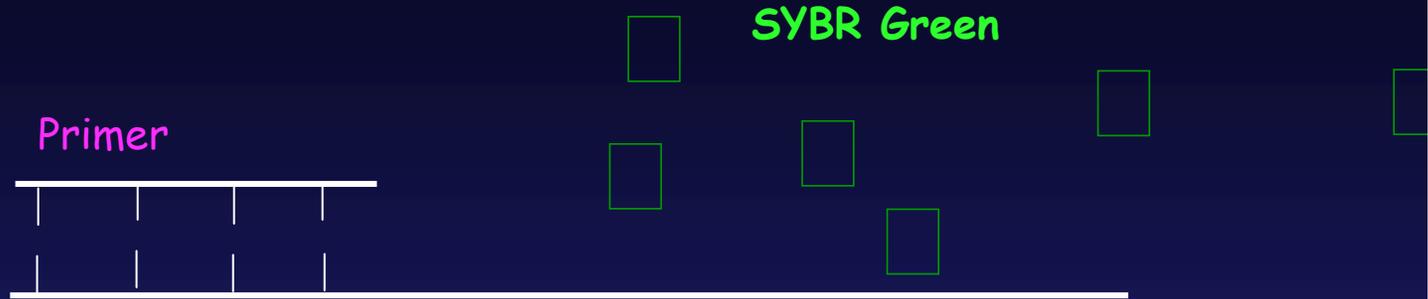
Internal control:

Human

18S rRNA

# How SYBR Green based fluorescence PCR works

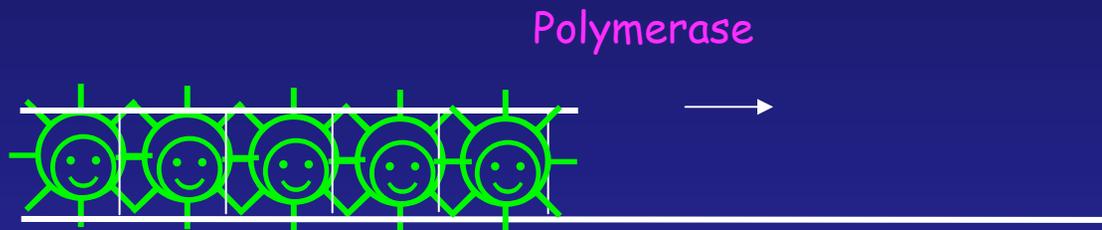
1) Denaturation



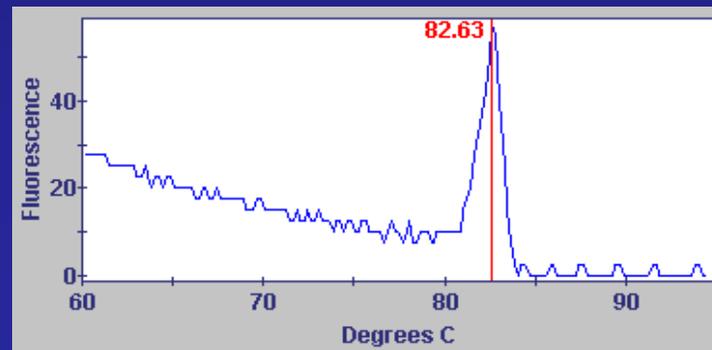
2) Hybridization



3) Extension

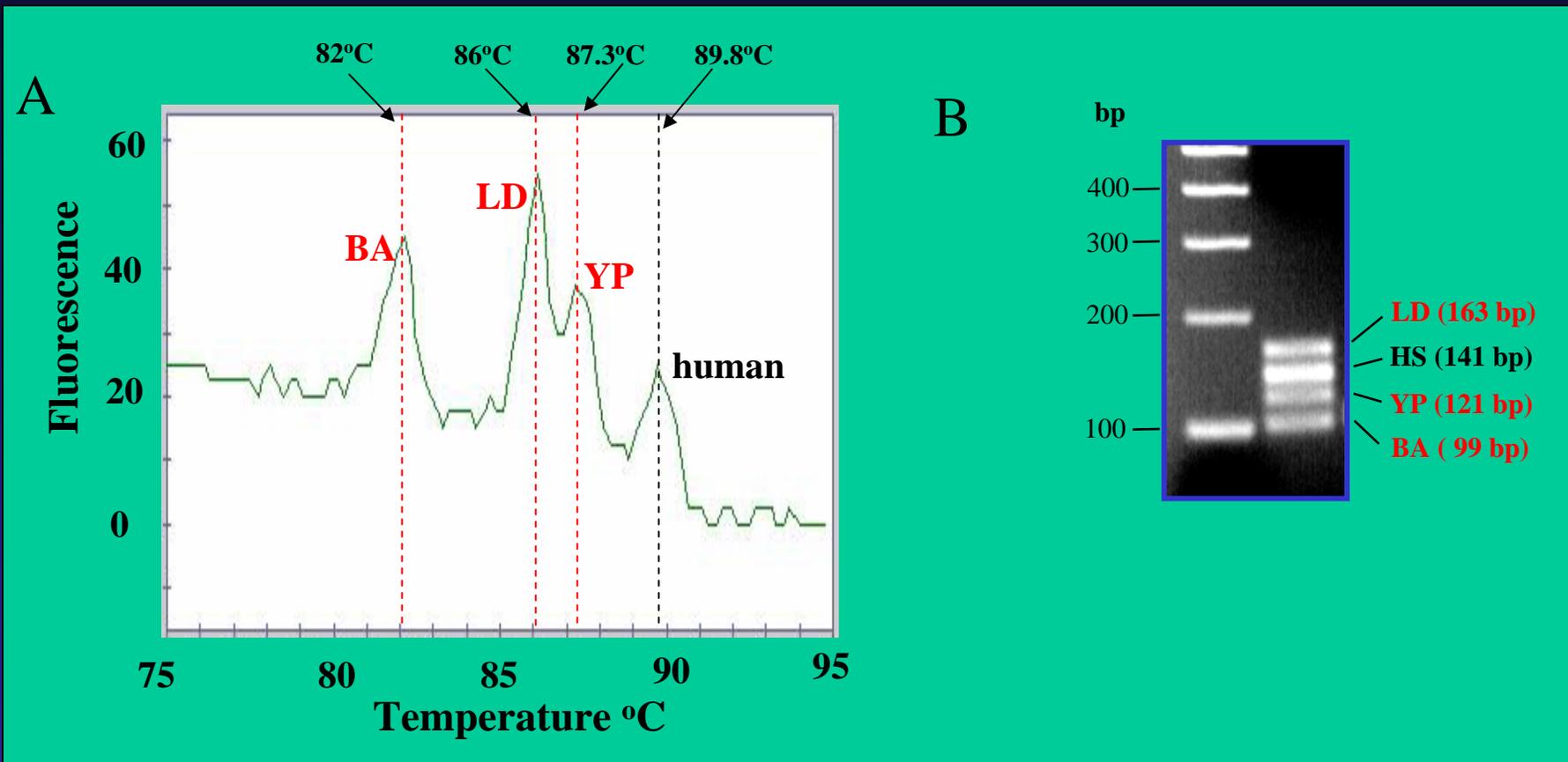


Readout



Melt Derivative

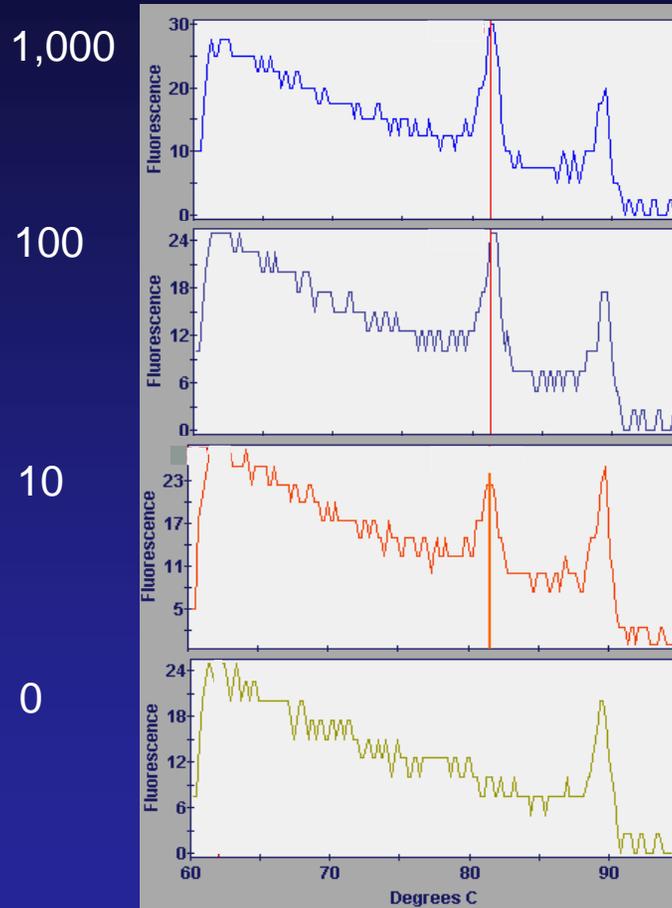
## Fluorescence-based Multiplex PCR Assay for Simultaneous Detection of Bacterial and Parasitic Pathogens



✓ This study outlines the development of a multiplex PCR for the simultaneous detection of the bacterial and parasitic pathogens.

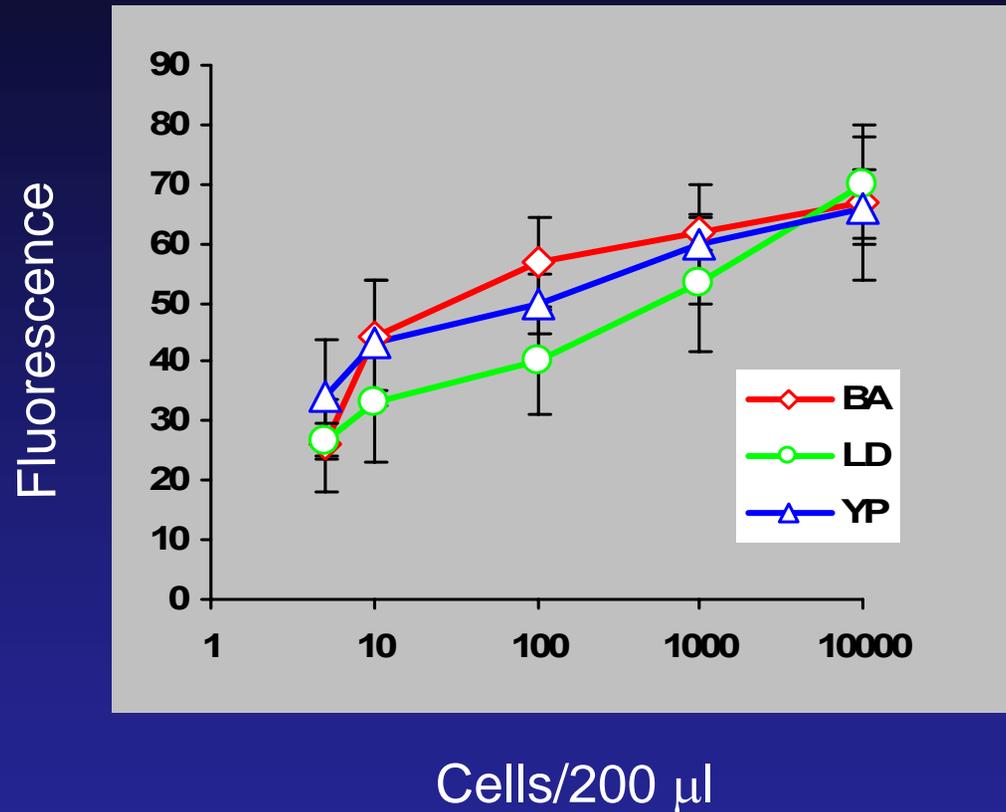
# Multiplex fluorescence PCR on the DNA isolated from the blood spiked with the serially diluted *B. anthracis* cells (200 $\mu$ l blood)

*B. anthracis*  
cells



- The  $T_m$  peak height seems as a novel measure to quantitate pathogens in blood
- For this pathogen we could detect as low as 10 cells /200 $\mu$ l blood.

Quantitation graph showing the relationship between the height of fluorescence peak and number of pathogen cells in blood



For all the pathogens we could detect as low as 50 cells/ ml of blood

## Multiplex PCR with *in vivo* infected blood samples

### Human leishmaniasis

Source of blood Sample	Total number of samples	Number of PCR positive
Patients with VL	11	11 (50 –1000 cells/ml)
Post treated VL patients	3	0

### Mouse anthrax

Blood sample by hour	Total number of samples	Number of PCR positive
0	5	0
12	5	0
24	5	1 ( <b>25%</b> ) (200-1000 cells/ml)
36	4	3 ( <b>66%</b> ) (1X10 <sup>4</sup> –10 <sup>5</sup> cells/ml)
48	2	2 ( <b>100%</b> ) (1X10 <sup>4</sup> –10 <sup>5</sup> cells/ml)

Assay accurately detected pathogens in the samples from leishmaniasis patients as well as mice infected with *B. anthracis*.

## Summary

- ✓ This study outlines the development and evaluation of a single-tube multiplex real-time PCR for the simultaneous detection of the bacterial and parasitic pathogens.
- ✓ For all the three pathogens we could detect as low as 50 cells /ml blood.
- ✓ *Leishmania* primers recognized DNA sequences from other *Leishmania* sp as well. Similarly *Yersinia* primers could identify sequences from *Y. enterocolitica*.
- ✓ Assay accurately detected pathogens in the blood samples from leishmaniasis patients as well as mice infected with *B. anthracis*.
- ✓ This assay takes less than one and half hours and hence could be useful for rapid identification purposes.