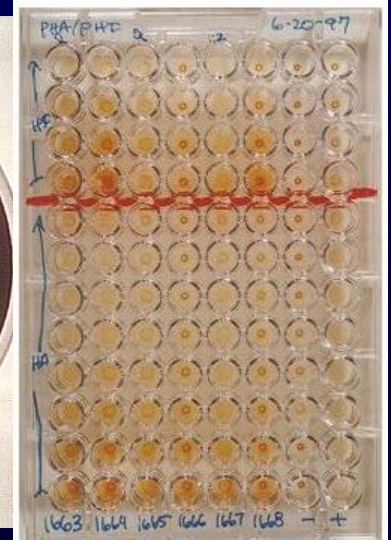
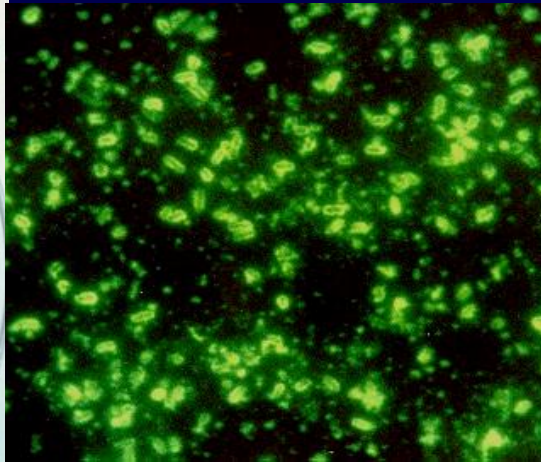


Outline

- Review of US national strategy for diagnosis of plague
- Application of diagnostics in US and other countries
- New diagnostics in development and evaluation



Laboratory Diagnostic Criteria For Plague

Presumptive

- Positive staining by direct fluorescent antibody (DFA) assay
- Positive PCR
- Positive serum titer by passive hemagglutination/inhibition assay (PHA/PHI)

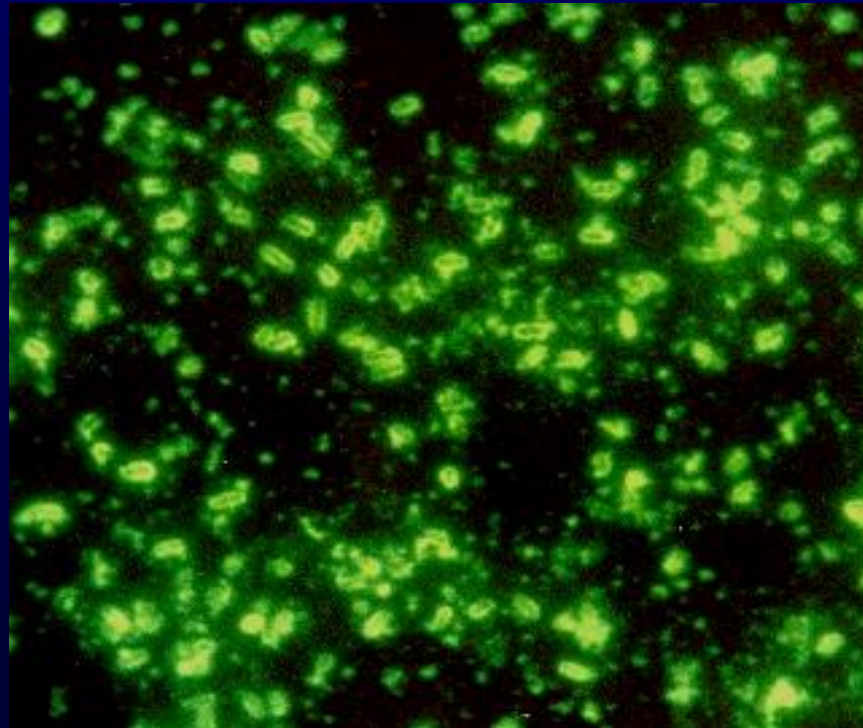
Laboratory Diagnostic Criteria For Plague

Confirmed

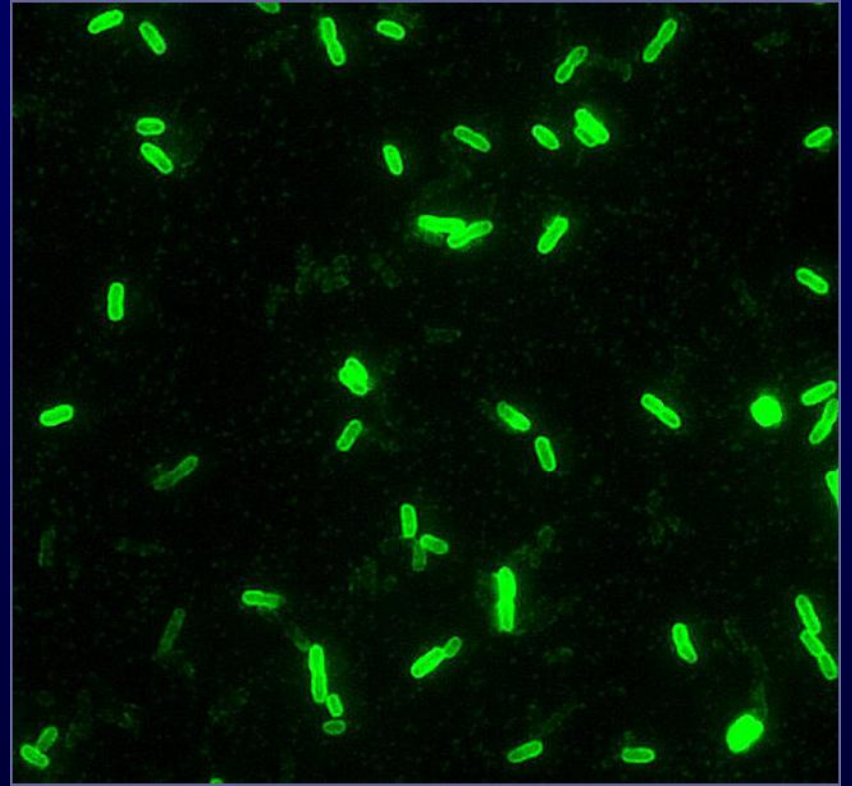
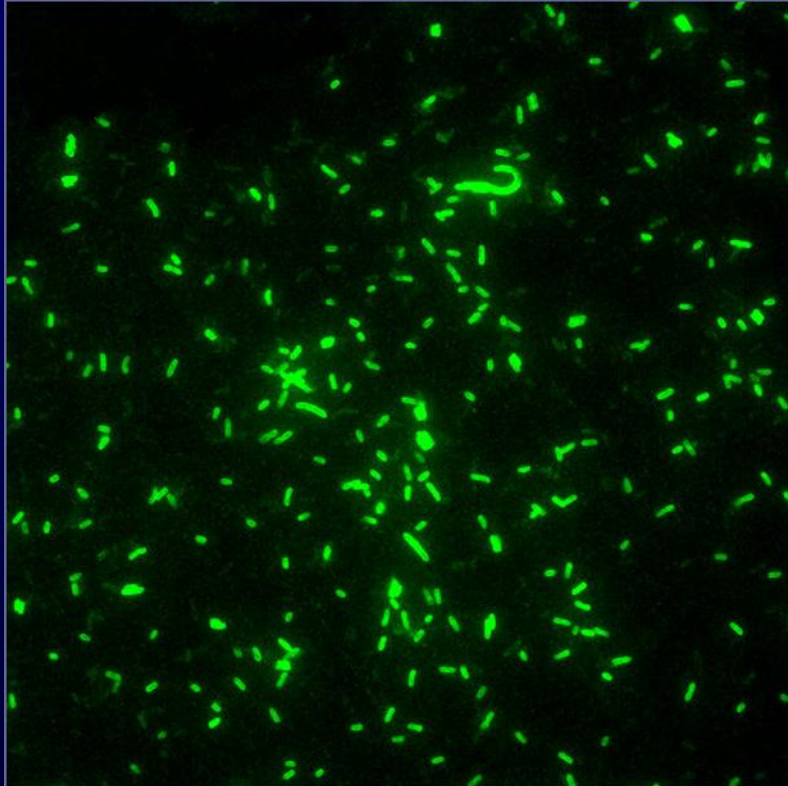
- Isolate identified as *Yersinia pestis* by positive bacteriophage lysis test
- Paired serum specimens that differ by a 4-fold change in titer, with one serum having a positive titer

Direct Fluorescent Antibody (DFA)

- Rapid presumptive assay for primary specimens
- FITC labeled rabbit polyclonal antibody against F1 antigen



Direct Fluorescent Antibody (DFA)



Culture of *Yersinia pestis*

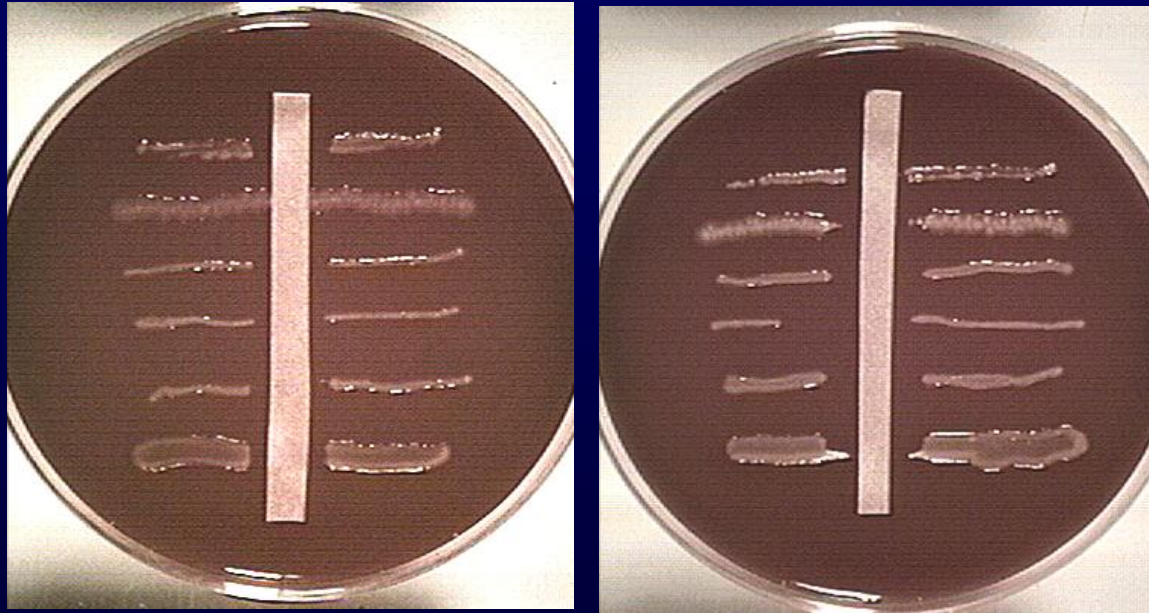
Sheep blood agar

- Sheep blood agar with 6% sheep blood
- Inoculate two plates, incubate one at 35°C and one at 22-25°C, for more rapid growth of *Y. pestis*
- At 24 hours
Tiny, almost invisible growth
- At 48 hours
1 to 2 mm irregular colonies



Bacteriophage Lysis for Confirmation of *Yersinia pestis*

Confirmatory test for recovered isolates



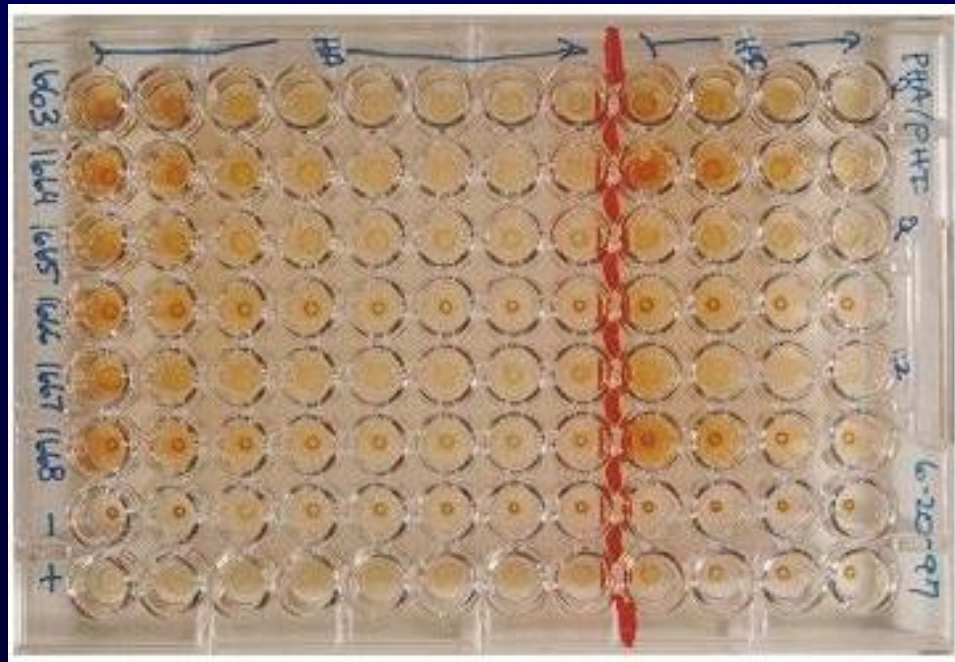
22-25°C

37°C

Test results: *Y. pestis* is lysed at both $< 28^{\circ}\text{C}$ and at 37°C
Y. pseudotuberculosis is lysed only at 37°C

Passive Hemagglutination (PHA) and Inhibition (PHI) Assay

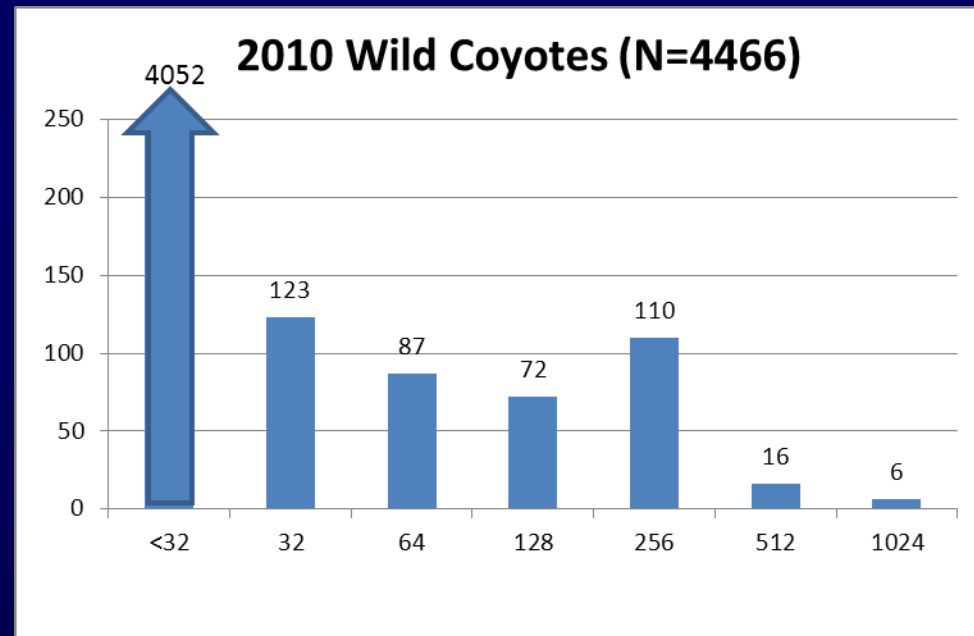
- Detects antibody to F1 antigen
- Presumptive: Single positive serum titer $\geq 1:10$
- Confirmatory: Paired serum specimens that differ by a 4-fold change in titer, with one sera having a positive titer



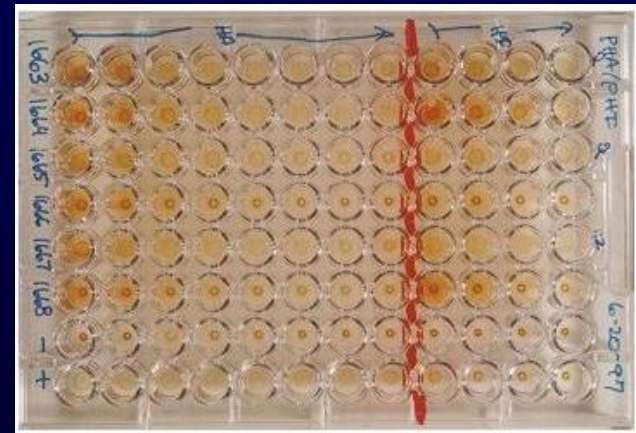
Plague Serosurveillance US

SEROLOGY

- Passive Hemagglutination
 - Serosurveillance (Carnivores, rodents, etc.)
 - Human diagnosis
- ELISA
 - Total antibody
 - Tests for anti *Y. pestis* IgM or IgG antibodies

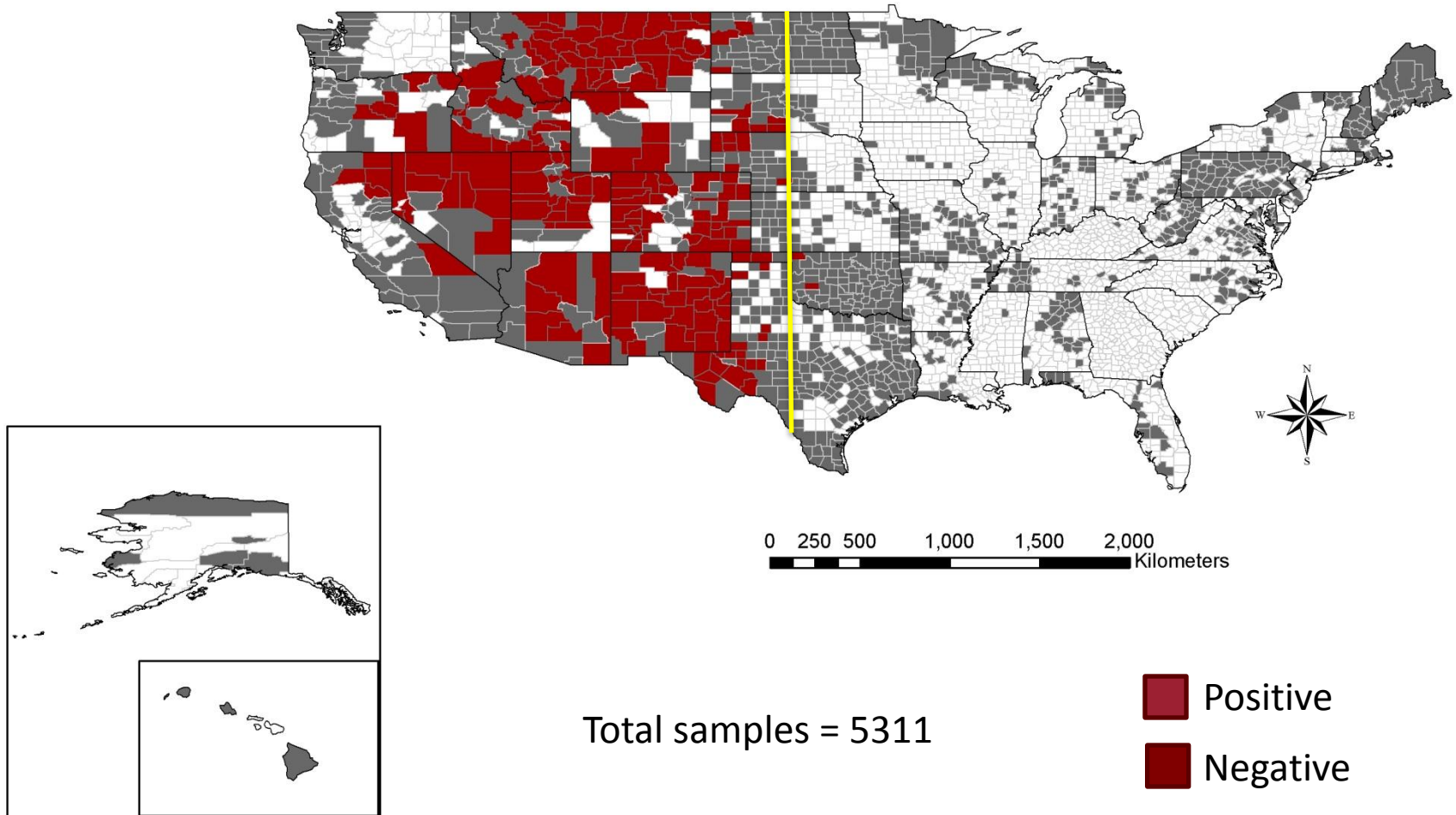


PHA titer of $\geq 1:32$ considered positive



Passive Hemagglutination Assay

Plague Sero-surveillance, 2010



Standardized Reagent Distribution

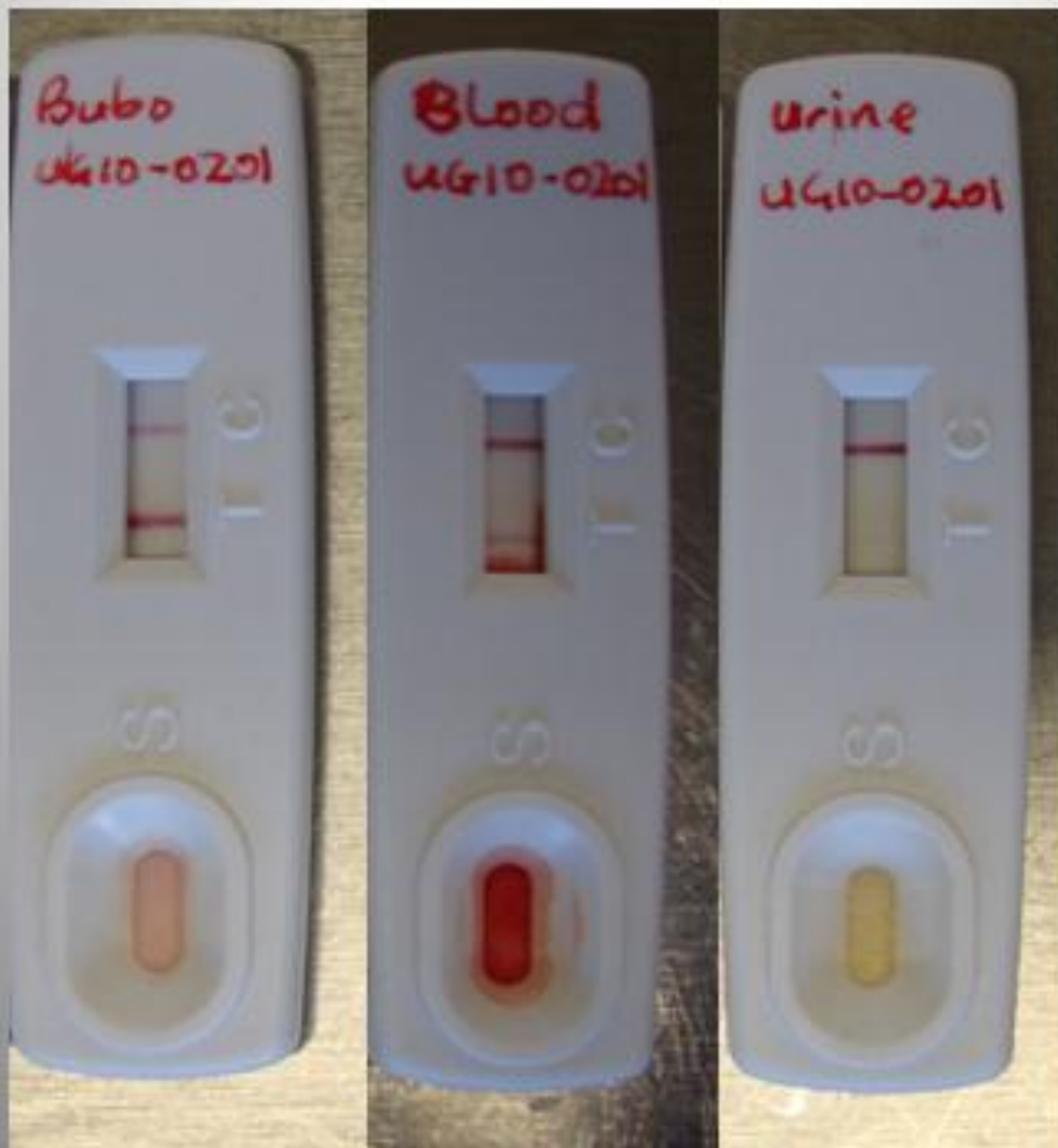
- DFA reagent
- Control slides for DFA
- Bacteriophage strips
- sSRBCs for PHA/PHI
- Positive and negative controls for PHA/PHI
- F1 antigen

Performance of Diagnostics in the United States 1998-2005

Year	# confirmed cases	Confirmed by culture	Confirmed by serology
1998-2005	38	27(71%)	14(37%)

- Of 27 culture confirmed cases: 23(85%) isolates were from blood, 4(15%) isolates were from bubo aspirates





CDC RDT

2011

Pt 201

LN aspirate

Blood

Urine

Sensitivity and Specificity of Hand-Held Assays

Specimen	% Sensitivity	% Specificity
Bubo aspirate	80-91	95-98
Sputum	100	93-100
Blood *	38-54	92-100
Urine **	45-6***	100

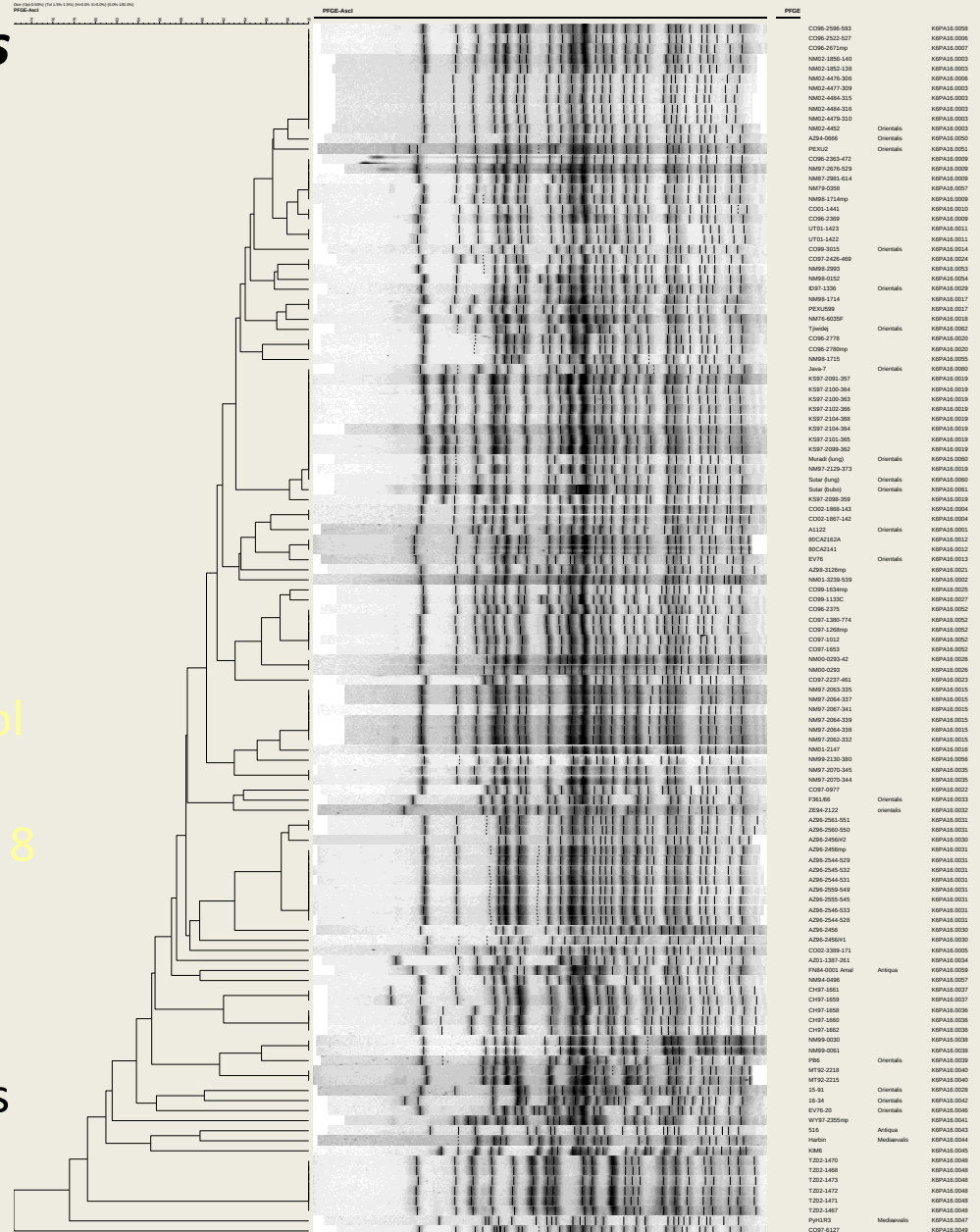
*Small vol. (0.1-3 ml) sample

** collected d1-7 post antibiotic treatment

*** declining sensitivity as a function of days post treatment

PFGE subtyping of *Yersinia pestis*

- Goal: Develop a pulsed-field gel electrophoresis (PFGE) method to identify epidemiologically linked isolates
- Significant discrimination seen among isolates subtyped
- To date, over 600 *Yersinia* isolates typed
 - Human, mammal, and flea pool isolates
 - Isolates from 15 countries and 8 U.S. States
 - Over 250 unique patterns in database
- Real-time sharing of PFGE patterns among laboratories



Molecular typing of *Yersinia pestis* during human case investigations

New York Cases

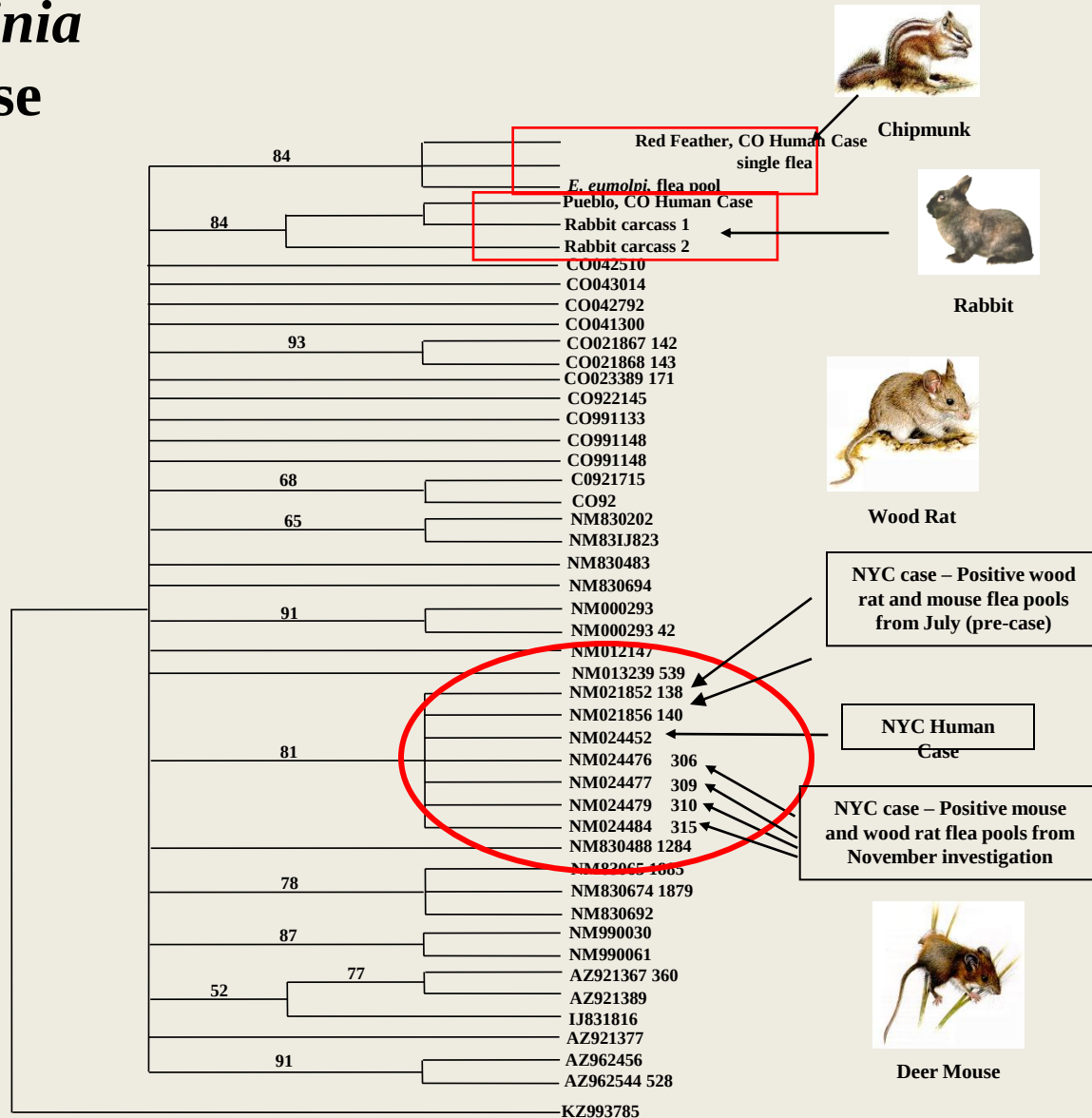
-Case isolate identical to ones recovered from *Neotoma* (wood rat) and *Peromyscus* (white-footed mice/deer mice) fleas at or near case home

Colorado Rabbit Hunter

-Case isolate identical or highly similar to ones recovered from rabbits shot by human case

Red Feather Lakes Case

-Case isolate identical to ones recovered from chipmunk fleas at suspected exposure site



(Lowell et al. J. Clin. Microbiol. 2005 and unpubl. data)