PLAGUE RAPID TESTS: Development, implementation, use at primary health care level

International meeting of Latin American experts on plague

Lima Peru, January 22-24, 2013
Geographical distribution

Reported Cases

- > 90
- 40-90
- 20-40
- 10-20
- 01-10

Sources: OCHA, Institut Pasteur de Madagascar
Rural cycle

- *Rattus rattus*
- *Xenopsylla cheopis*
- *Yersinia pestis*
- *Synospsyllus fonquerniei*
Bubonic Plague: sudden onset of fever, chills, headache, very painful swelling of lymph nodes, severe malaise, prostration (incubation 5-6d, without treatment = death in <1 week)

Pneumonic Plague: fever, cough with blood stained sputum, chest pain, and difficulty in breathing (incubation 24 h, without treatment = death in 2d)
• Bacteriology (Gold Standard)  
  *Y. pestis* isolation/acute phase specimen or post-mortem organs, **Time 6-15d**

• Detection of F1 Ag (ELISA)  
  Acute phase specimen (threshold 2ng/ml)  
  Specificity 100%, Sensitivity: 100%  **Time 6h**

• Serology anti-F1 IgG (ELISA)  
  (serum > 7 days after first clinical sign)  
  Specificity 98%, Sensitivity 91%, **Time 4h**
Problems in endemic countries

- Late clinical diagnosis (high mortality)
- Late responses
- Biological confirmation long

Strategy

- Early detection, treatment
- Suspect cases
- Chemoprophylaxis of contacts
- Urgent confirmation
- Fleas control (insecticides)
- Rodents control
Need for health workers: A rapid, easy test, used at primary health care level
RDT for F1 antigen detection: PLAGUE DIAGNOSIS
Rapid Diagnosis Test for F1 Ag detection

- **Development (2000):** IP Madagascar - IP Paris
- **Evaluation (2001):** IP Madagascar, IP Paris (lab. and field)
- **Validation, Diffusion (2001, 2002):** Madagascar
- **Diffusion (2003...):** Diagnostic use in other countries

Capsular Glycoprotein - coded by plasmidic DNA (pFra)

*Y. pestis* specific

Abundant (secreted at 37°C), heat stable

Different kinds samples (human, rodent)

Not influenced contaminants, treatment
Principle:
immunochromatography colloïdal gold particles (vertical flow)

Samples: bubo, sputum, serum, urine, post-mortem, spleen / liver (rats)

One-step, 15 mn, cut-off : 0.5 ng/ml F1
Internal validation, evaluation

- Reference tests
  Microscopy Gram stain
  Bacteriology
  ELISA for F1 Antigen Detection
  PCR

- Samples:
  rodent and patients’ samples’ strains
Internal validation...

- **Validation of F1 Dips on control clinical samples**
  - Specificity 100% (on 420 specimen from plague free and healthy individuals)
  - Sensitivity 100% (on 166 sputa, sera, urine and bubo)

- **Validation of F1 Dips on rodent samples**
  - Specificity 100% (on 78 healthy rats and mice specimen)
  - Sensitivity 96-100% (on 64 dead rats and mice)
Internal validation

Validation of F1 Dips on other *Yersinia* cultures

Specificity 100% (on 134 strains of *Y. enterocolitica*, *Y. pseudo-tuberculosis*, non pathogenic *Yersinia*) (E. Carniel IPP)

Validation on *Y. pestis* cultures

93-100% positive

(70 strains from Madagascar, African and Asian countries)
1. Pilot Health Care Centres (6 hospitals, 20 primary health care level): Diagnostic Tool
2. Central Plague Laboratory Surveillance and Diagnostic Tool
3. General diffusion in Madagascar
Lecture of RDT results
Pilot site: use of Dips on primary level care

Sample collection, test on the bedside, treatment

Result, notification, report

Remaining sample to be sent to the CLP for comparison
### Comparison Pilot sites vs Central Lab

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<th>Central Lab.</th>
<th>Pilot Centres</th>
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<tr>
<td></td>
<td>Pos.</td>
<td>Neg.</td>
<td>Ind.</td>
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<tr>
<td>Pos.</td>
<td>49</td>
<td>5</td>
<td>1</td>
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<tr>
<td>Neg.</td>
<td>4</td>
<td>66</td>
<td>3</td>
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<tr>
<td>Total</td>
<td>53</td>
<td>71</td>
<td>4</td>
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**Agreement** 90%

**Disagreement** 7% (weak pos.)

**Indeterminate** 3% (no control line)

**Concordance F1 Dips / Bact. = 78.2%**
Pilot study: users’ satisfaction

Physician, Nurse, Technician, Health agent

Bar chart showing:
- Impact on morbidity/fatality
- Availability
- Lecture easy
- Test easy
- Training satisfaction

Scale from 0 to 100
Training 100 trainers from 42 District Health (SSD): Plague (clinical sign, differential diagnosis, use RDT, information...)

Diffusion:
kits in 300 plague endemic foci centres in Madagascar (SSD, CHD, CSB)

~ 3500 kits and ~ 300 supplies cases
Diffusion of the RDT in Madagascar

42 Districts in plague area (100 staffs)
Composition of a kit

- Syringe · Needle (18G)
- Eppendorf PBS
- Cary Blair tube · Swab
- Calibrated plastic tubes for assay
- Alcohol wipe
- Disinfectant wipe
- Elastoplast
Diffusion: kit preparation
Plague kits and supplies cases
Dispatching to plague area
Confirmation rate: <25%

Plague situation before RDT use

Year

Nb notified cases
Use RDT in Health Centre

Use RDT: Confirmation rate ++

Situation from 2002 to 2012

Fatality Rate, RC: Reported cases

FR : Fatality Rate, RC: Reported cases
Excellent internal value (Sp, Se ~ 100%)

bubonic / pneumonic plague, epizootic

Validated in programme conditions

Substantial contribution in Central Lab

Nevertheless, 5% false negative (low F1)

† treat every suspect patients
Detection at primary level (human, rat) → Alerte rapid → Prevention of human cases

Use RDT F1: Interregional meeting on plague on April 2006, WHO took into account the latest scientific and technical advances: case definition
SUSPECTED CASE: compatible clinical presentation; and consistent epidemiological features

PRESUMPTIVE CASE: (definition of suspected case +) Putative new or re-emerging focus: at least two of the tests positive (microscopy, F1 antigen, single anti-F1 serology, PCR); Known endemic focus: at least one

CONFIRMED CASE: (the definition of suspected case +) Y. pestis isolated, or fourfold rise in anti-F1 antibody titre in paired serum samples F1 Ag + (in endemic areas when no other confirmatory test can be performed)
RDT CANNOT REPLACE THE BACTERIOLOGY

IMPORTANCE OF STRAIN FOR THE SURVEILLANCE OF THE SENSITIVITY ON ATB USE FOR THE TREATMENT AND CHIOMIOPROPHYLAXIE
RDT for antibodies anti-F1 detection
PLAGUE SURVEILLANCE


Evaluation cont'd (2007....) Madagascar, Iran, DRC, Peru, other countries

Internal value:

Humans Se=84.6%, Spe=98%.

Rodents, other small mammals:
Se=87.8%, Spe = 90.3%

Dog: Se=93%, Spe=98%
Seroprevalence in dogs varies seasonally

Effect of month: \( \chi^2 = 19.56 \) df=4, \( p=0.006 \)

Seasonal pattern in dog seroprevalence is consistent with a loss of antibodies during the low season

Human cases of plague (Oct-Mar)
Use of dog on plague surveillance

Surveillance: low/high season

Presence of seroconverted dog (negative-positive): a clue of plague circulation in the area

Presence of rodent carrier of plague bacilli

Alert, measure preventive,

Few Nb dog in a village vs rodent: save a significant cost on assay
RDT Implementation in Peru: Use in pilote sites Production RDT for OPS
AGREEMENT N° E – 2009-OPD/INS

COLLABORATION AGREEMENT BETWEEN THE NATIONAL INSTITUTE OF HEALTH AND THE INSTITUT PASTEUR DE MADAGASCAR.

The present document consists of the Agreement established between

General:
To create strategic alliances between the 2 Institutes, for the exchange of scientific and technological knowledge related to plague epidemiological surveillance and control, including the utilization of rapid tests for the diagnosis of plague in the field.
August 2010 (Investigation of PP)

Training of Lab Technician, Biologist, MD

Health Centers: INS, GERESA La Libertad, LRR Lambayeque

Supplies: RDT, video for instruction use in spanish version (WHO-PAHO-INS)
Pilot site: RDT training

Use of rapid test on human samples
Importance of sample in plague diagnosis

Samples collection according to clinical form
(kits for Peru Health Center, instruction use sheet in spanish)

Sample testing process on rodent
Lecture of RDT results
Practices (field condition for rodent surveillance)
Training on sample collection
Training in field condition
Mab used for RDT production

Patent not exclusive (Conservation Matériels Scientifique IP Paris)

Technical assistance (IPM –Paris PF5)
Conclusion, perspectives

Improve the technical capacities of the National lab reference INS Peru

Serology, RDT, Bacteriology (IPM)
Molecular biology (IP Paris)

Include the use of RDT (Ag F1; Ab) in the National system surveillance in Peru: training of staff from plague foci (pilot) in 2010

Next step:
Training of Peru lab staff (in Paris, Madagascar)
To strengthen plague epidemiological system: share experience Peru vs Madagascar (meeting in Madagascar)
Laboratory support for outbreak response:

✓ Essential functions and Roles of laboratoires: rapid detection = alerte rapid

✓ During the investigation: to confirm the identification of disease.
Acknowledgment:

Financial support
World Bank, IP, OMS, MoH, IP, Wellcome-Trust, OMS, PAHO, INS, IP

Staffs involved in these studies
Merci de votre aimable attention
Importance du réseau de laboratoire: maintient / amélioration de la qualité (technique, personnel, ....) pour pouvoir confirmer à temps une épidémie

Définition des cas selon critères OMS

Quelles sont les tendances spatiales et temporelles dans les nombres des rats et des puces ?

Quels facteurs influencent les tendances spatio-temporelles?
Climat, végétation,
Déplacements (situation habituelle, forte pression=feux dans les champs de canne à sucre)

Est-ce que les rats des différents endroits dans la zone endémique ont une sensibilité différente à Y. pestis?

Quels facteurs influencent la sensibilité ?

Adaptater les mesures de lutte

Peste une maladie multifactorielle: (socio-economique, politique, comportement humain, .......)