Laboratory diagnosis for a clinical case of Legionnaires' disease

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Legionnaires' disease (Last updated on 27 February 2008)

Description

Legionnaires' disease is characterized by fever, myalgia, cough and pneumonia. It is caused by *Legionella pneumophila* and other *Legionella* species. At least 46 species and 70 serogroups have been identified so far. *L. pneumophila* serogroup 1 is most commonly associated with disease.

Laboratory criteria

Any one of the following:

- Isolation of *Legionella* species from respiratory secretions, lung tissue, pleural fluid
- Demonstration of a four-fold or greater rise in antibody titre to ≥ 1:64 against *Legionella pneumophila* serogroup 1 between paired acute- and convalescent-phases serum specimens
- Detection of *L. pneumophila* serogroup 1 in respiratory secretions, lung tissue or pleural fluid by direct fluorescent antibody staining
- Demonstration of *L. pneumophila* serogroup 1 antigens in urine

Confirmed case

A clinically compatible case that is laboratory confirmed.

Probable case

A clinically compatible case with a single antibody titre of ≥ 1:128 against *L. pneumophila* serogroup 1.

Diagnostic tests

- Direct detection
- Culture
- Serology

TABLE 1. USEFULNESS OF SPECIALIZED LABORATORY TESTS FOR THE DIAGNOSIS OF LEGIONNAIRES' DISEASE.

TEST	SENSITIVITY	SPECIFICITY
	percent	
Sputum culture*	80	100
Direct fluorescent-antibody stain of sputum	33–70	96–99
Urinary antigen assay†	70	100
Serologic tests for antibody‡	40–60	96–99

*Multiple selective mediums that contain dyes and have been pretreated with acid or heat to minimize overgrowth of competing microorganisms should be used.

†This test is useful only for *L. pneumophila* serogroup 1.

‡This approach requires IgG and IgM testing of serum samples obtained during both the acute phase and convalescence. A single titer of ≥1:128 in a patient with pneumonia is considered presumptive evidence of infection, and a single titer of ≥1:256 or a fourfold increase in antibody titer is considered definitive evidence.

Stout and Yu. NEJM 1997; 337: 682.

Direct detection

- Antigen detection
  - Urine
  - Respiratory tract specimens
- Molecular detection

Urinary antigen detection

- *Legionella pneumophila* serogroup 1 soluble antigen
- Becomes positive 3 days after onset
- Commercial kits
  - Immunochromatography
    - Rapid but more costly
  - ELISA

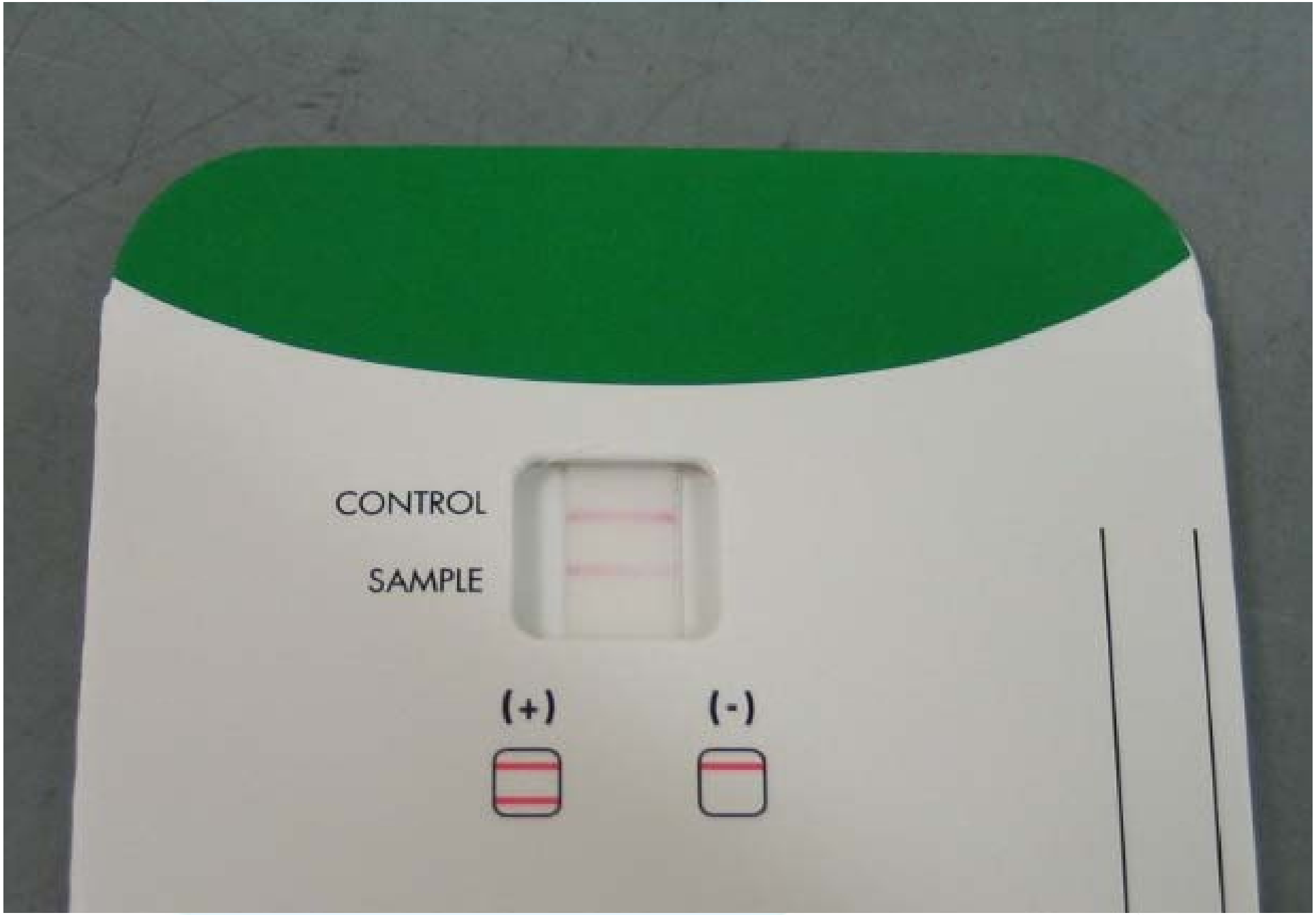
CONTROL
SAMPLE
(+)
(-)

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TABLE 1. Results of Binax NOW and Oxoid Xpect tests after 15 min and 1 h of incubation

Test and incubation time (min)	% Sensitivity	% Specificity
Xpect		
15	81 (69/85)[a]	100 (0/86)
60	89 (76/85)	98 (2/86)
NOW		
15	86 (74/86)	100 (0/87)
60	93 (80/86)	100 (0/87)

[a] The values in parentheses are the number of samples positive/total.

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DIAGNOSTIC MICROBIOLOGY

Detection of *Legionella pneumophila* antigen in urine samples by the BinaxNOW immunochromatographic assay and comparison with both Binax *Legionella* Urinary Enzyme Immunoassay (EIA) and Biotest *Legionella* Urin Antigen EIA

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Immunochromatography test

- Specificity (279 specimens):
  - 97.1%
  - 100% after discounting “false positive” results with “very weak bands” which did not increase in intensity from 15 to 60 minutes of incubation
- Sensitivity (117 specimens):
  - 79.7%

Respiratory tract antigen detection

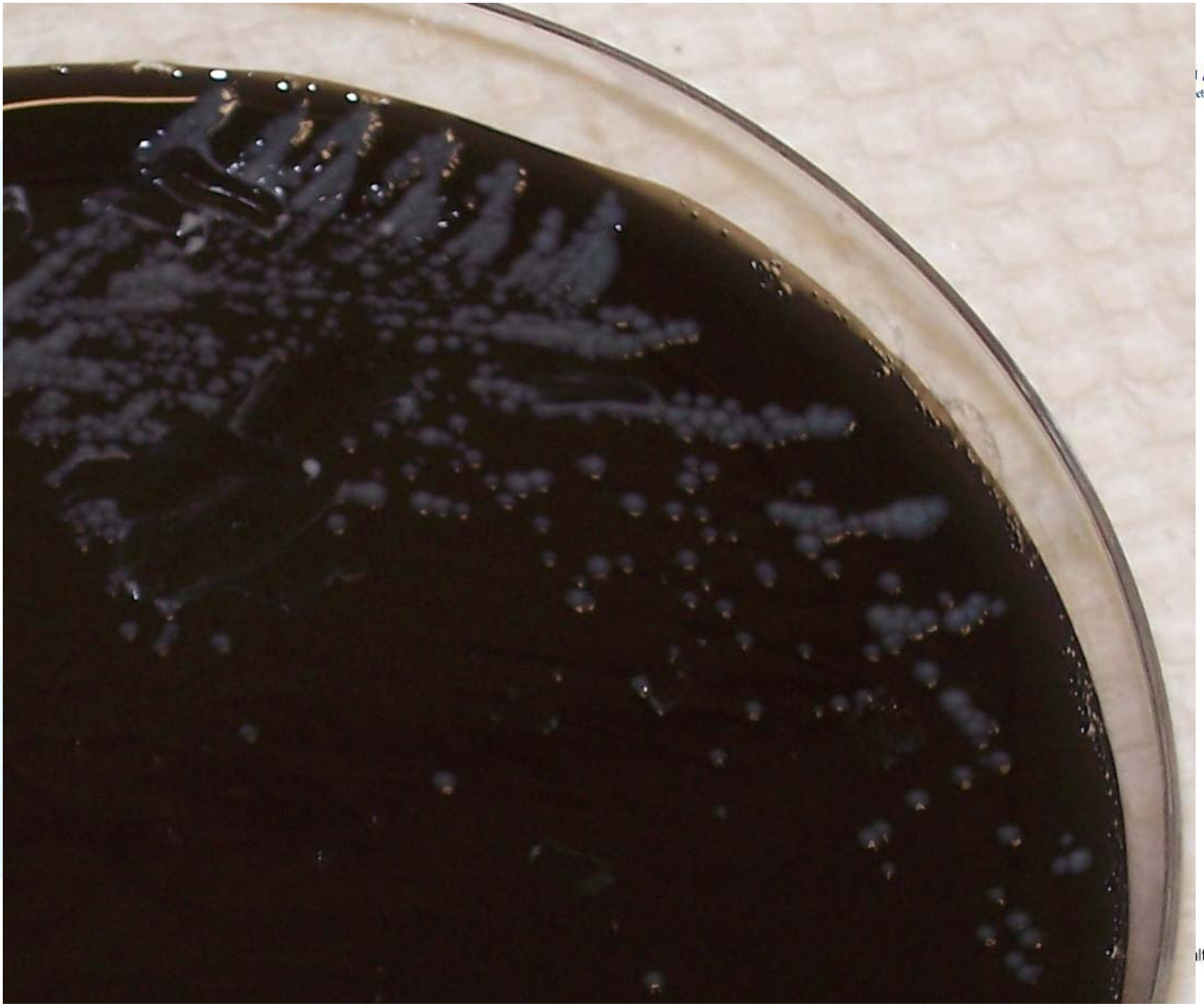
- Immunofluorescence test for *L. pneumophila* serogroup 1 or more serogroups
- Low sensitivity
- May cross-reacts with other bacteria, e.g. *Pseudomonas* spp.
- Requires expertise to maximize sensitivity and specificity
- Not generally performed in diagnostic laboratories

Molecular detection

- Lower respiratory specimens
- PCR followed by sequencing: e.g.
  - Macrophage infectivity potentiator (mip)
  - 16S rRNA
  - 23S-5S rRNA spacer region
- Can differentiate different Legionella species
- PCR on LPS gene cluster serogroup-specific
- High sensitivity
- More labour intensive and time-consuming than urinary antigen test for primary diagnosis

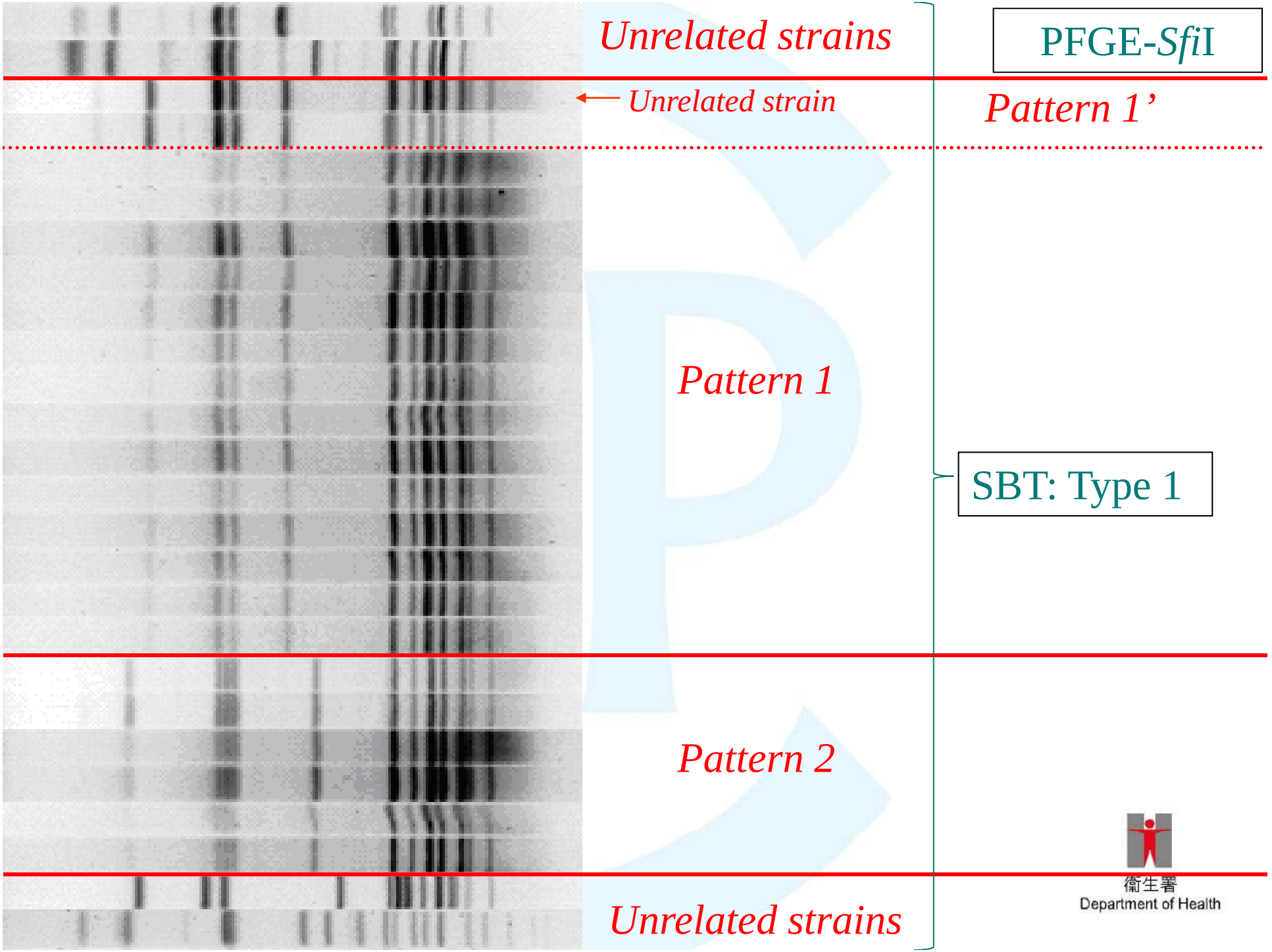
Culture

- Obligate aerobe, depending on L-cysteine, with growth enhancement by iron
- Heat-/acid-treatment to reduce contaminating flora
- Enrichment and selective media (BCYE, BMPA)
- Incubation for up to 2 weeks
- Identification of isolates
  - Colony morphology and phenotypic characteristics
  - Serology
  - Molecular



Typing of isolates

- Clinical and environmental isolates: Determine clonality
- Methods
  - Sequence-based typing (can be attempted on direct specimen when culture negative)
  - Pulsed-field gel electrophoresis


Unrelated strains
PFGE-SfiI
Unrelated strain
Pattern 1'
Pattern 1
SBT: Type 1
Pattern 2
Unrelated strains
衞生署
Department of Health

Typing of isolates

- Result interpretation requires epidemiological correlation
- SBT type 1 (1,4,3,1,1,1,1):
  - One of the known large common complexes in Europe
  - Majority of environmental samples in Hong Kong
  - Minority of patient samples in Hong Kong

Serology testing

- Seroconversion: Takes weeks to months after infection
- Sensitivity: Around 75%
- Specificity: May cross-react with other antibodies (heat-treated agar culture vs. formalin-treated infected egg yolk antigens)
- Methods:
  - Rapid microagglutination test
  - Immunofluorescence test

Rapid microagglutination test

- For *L. pneumophila* serogroup 1 only
- More non-specific cross-reactions (e.g. *Pseudomonas* spp., *Proteus* spp. and rickettsiae) than immunofluorescence test
- Technically simpler than immunofluorescence test
- Four-fold rise in titre to ≥ 16 usually taken as positive

Immunofluorescence test

- For *L. pneumophila* serogroup 1 and other serogroups
- May cross-react among different serogroups
- For confirmation of rapid microagglutination test result
- Four-fold rise in titre to ≥ 64 usually taken as positive

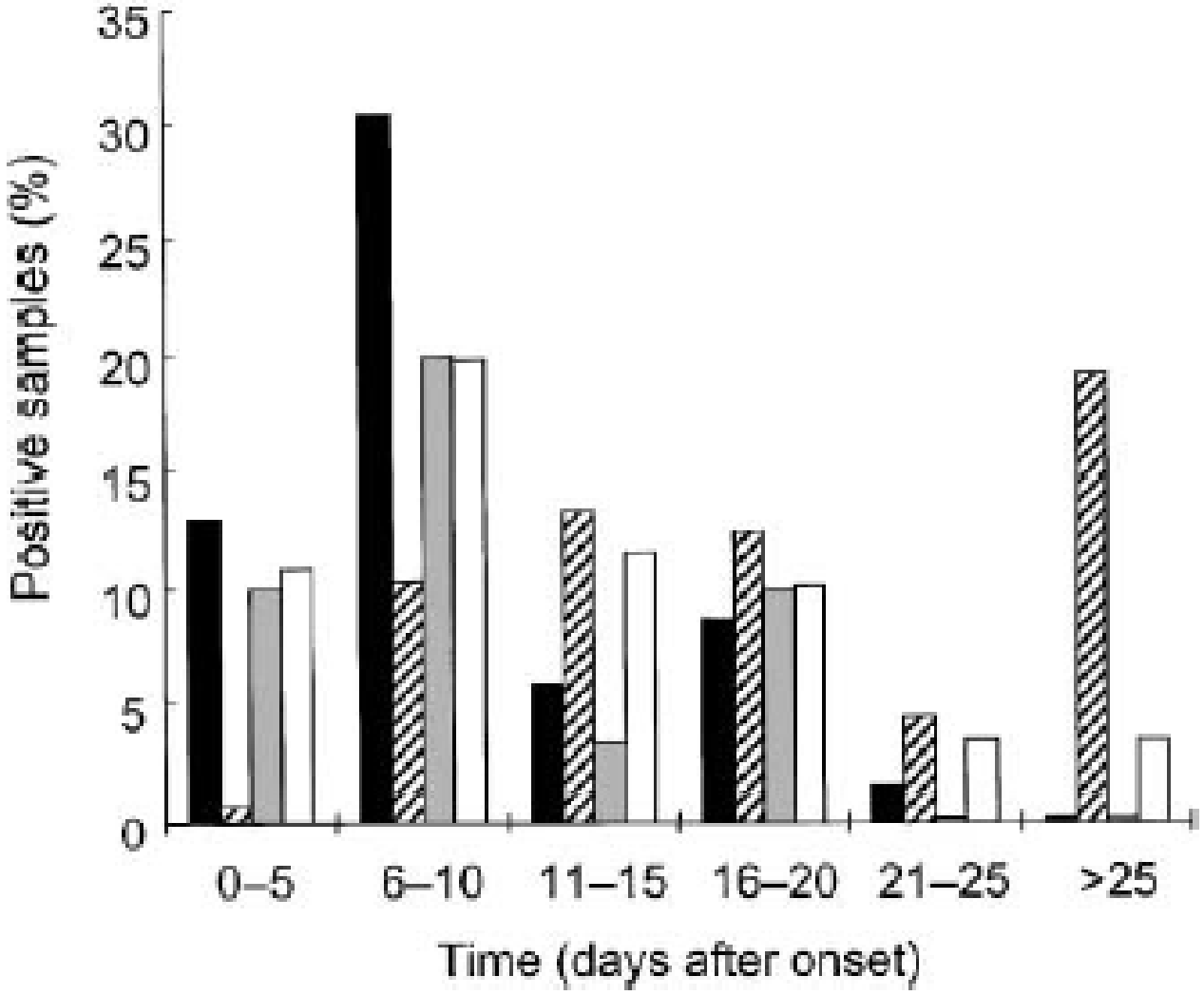


Fig. 1. Effect of sample timing after disease onset on positive outcome of the urinary antigen EIA (filled bars), IFA (hatched bars), culture (shaded bars) and 5S rRNA PCR (open bars). *JMM 2004; 53: 183*

Testing strategy

- Acute infection
  - Urinary antigen test (*L. pneumophila* serogroup 1)
- Determine source of infection
  - Sputum culture
  - Environmental sample culture
  - Typing of culture isolates
- Retrospective diagnosis
  - Serology

Thank you

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