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Laboratory Diagnosis of Plague

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The World Health Organization mandates that all countries report the occurrence of human plague cases.

Both human and animal plague diagnosis must follow the case definition criteria :

Identification

A case is diagnosed as suspected plague if one of the following conditions are met :

1. Clinical symptoms are compatible with plague
2. If an initial identification of gram negative or bi-polar bacteria is seen.

A case is only diagnosed as presumptive plague if one or both of the following conditions are met :

1. Only if both Wayson and Immunofluorescence stain is positive.
2. If only one serum specimen was tested and the result was positive (> 1 : 10 for serum).

A case is diagnosed as confirmed plague if one or both of the following conditions are met :

1. In addition to Wayson and Immunofluorescence positivity, a culture was isolated and was positive by both bacteriophage and biochemical reactions.

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2. Two sera samples were collected with appropriate time difference (usually two weeks apart) to demonstrate a four-fold difference in end point titters.

The absolute confirmation of plague infection in human beings, rodents or fleas requires the isolation and identification of *Yersinia pestis.*

Plague is a proven laboratory hazard. Four laboratory acquired infections have been reported even in the United States.

The agent may be present in bubo fluid, blood, sputum, cerebrospinal fluid (CSF), feces, and urine from humans depending on the clinical form and the stage of the disease.

Primary hazards to laboratory personal include :

1. direct contact with cultures and infectious materials from humans;
2. Infectious aerosols or droplets generated during the manipulation of the cultures, infected tissues;
3. accidental autoinoculation
4. ingestion.

Specimen For Diagnosis[1,2,3]

Material for laboratory diagnosis may be obtained from Human beings suffering from infection;

(a) Aspirate from bubo; (b) Blood for culture; (c) blood for smear (two slides); (d) Sputum; (e) Throat swab.

1. If lymph nodes are enlarged perform the following steps :

The puncture site is first disinfected with tincture of iodine. A 10 ml syringe in armed with 19 SWG needle and a few ml of sterile saline is drawn into the syringe. The lymph is then punctured and suction is applied. If the aspiration doesn't produce any fluid, saline can slowly injected into the lymph node and aspirated back into the syringe.

Bend the needle, block it with a sterile rubber bunk and dispatch it to the laboratory without any delay.

2 Blood should be collected in Brain Heart Infusion broth in all cases. Collect 2 to 5 ml of blood, inoculate the blood culture bottle and dispatch to the laboratory.

3. Make two thin peripheral blood slides and fix with methanol for 4 minutes.

4. In cases of suspected pneumonia plague, request for expectorate sputum (not saliva) into a sterile MacConkey bottle.

5. It may be worthwhile to collect 1 to 2 ml of serum which could be tested for presence of antibodies to *Y. pestis, Legionella pneumophila, Mycoplasma pneumonia* and Hantavirus among others.

It delay in dispatching the samples to the lab is anticipated, keep the samples in refrigerator as the bacteria not only survives but also multiplies at 4°C.

Isolation and Identification

Smear Examination

Gram stain. Gram negative, coccobacilli with polymorphic forms more common as culture ages. Chains with age in broth . But this method doesn't bring out the characteristic bipolar staining.

Wayson stain. This stain brings out the characteristic bipolar staining. The bacteria gives the appearance of a safety pin.

(i) Prepare smear on clean new slide; (ii) Fix it with alcohol for 4 '(this will kill the bacteria); Stain the smear a few seconds; (iii) Wash with water, blot and dry.

Composition of Wayson's stain. Dissolve 0.2 grams of basic fuchsin and 0.75 grams of methylene blue in 20 ml absolute ethanol. Add the dye solution to 200 ml of a 5% solution of phenol in distilled water. Filter.

Fluorescent antibody. Positive on original tissue and any subsequent test (FAT) cultures polyclonal rabbit antibodies to the F 1 antigen are used. Since F 1 antigen is present in only *Y. Pestis* this can be a very rapid yet specific test for the presumptive diagnosis of plague.

Culture Examination[2]

Culture should be attempted whenever possible, samples must be collected before administration of antibiotics. Culture the sample onto two blood agar, Brain Heart Infusion and MacConkey agar plates. Incubate one set at 28°C and the other at 37°C.

Rest of the sample should be kept at +4°C for cold enrichment either per se or in PBS, pH 7.2 and subculture as and when required, usually after 2 weeks.

Examine the culture plates each day. Colonies may take 48 to 72 hrs to appear. Make smears from suspicious colonies.

Obtain pure cultures and set biochemical tests. *Yersinia pestis* is catalase +, oxides -, Nitrate +, Acid but no gas is seen.

Other biochemicals/examinations including cxides, colony > 1mm in 24 hrs/37°C,

motility at 37°C, gas from glucose, citrate VP and hydrogen peroxide are negative.

Examinations	*Y. pestis*	*Y. pseudo*	*Y. entero.*
Motility at 25°C.	-	+	+
Ornithine	-	-	+
Ureas	-	+	-
Indole	-	-	+/-
Sucrose	-	-	+
Sorbitol	-	-	+
Mice pathogenicity	+ Rapid	+ Slow	+/-
Lysis by plague sp. phage	+	+/-	-

Y. pestis can be further subdivided into three subspecies depending upon

	Fermentation of Glycerol	Reduction of Nitrate
Antigua	+	+
Mediavalis	+	-
Orientalist	-	+

Serological Methods[3]

Antibodies to F 1 antigens would appear 8 to 14 days after clinical disease. The detection of F 1 antibodies should provide presumptive diagnosis of plague, though titre movement is required to confirm the occurrence of plague. The main advantages of serological methods for making a diagnosis are :

(1) Important method of making a laboratory diagnosis especially if antibiotic has been taken
(2) To make retrospective diagnosis
(3) For prevalence studies.

However, serological methods have a greater utility in rodent infections, where they may help in

(i) Active Foci in rodents; (ii) In sentinel animals; (iii) Evaluation of control measures.

The methods are:

(i) Bacterial agglutination tests; (ii) Compliment fixation test; (iii) Passive haemagglutination test; (iv) ELISA for anti F 1 antibodies; (v) Antigen detection (FAT).

The rapid methods using state of the are techniques are :

1. Fluorescent antibody test (FAT) for detection of F 1 antigen in the smear from clinical samples.

This test can provide microbiologically confirmed diagnosis in 45 minutes.

The main drawback is that it is not very sensitive test and would be positive in the later stages of illness.

2. *Automaton for rapid growth detection.* Blood culture by Bac T/Alert : This is a technique for isolation of bacteria (any bacteria) from the blood or any sterile body fluids. Positive signals indicating growth of bacteria may be available in as little as 8 hours based on detection of bacterial growth by release of CO_2. Yersinia spp., are known to take nearly 24 hours for growth in this system.

Polymerase Chain Reaction.[5]

A 4 hours nested PCR for detection of the plasminogen activator gene of *Y. pestis* has been described. The primer used are
Outer primers, which amplify a DNA fragment of 928 bp

YP 1 5'-AGTTCTATTGTGGCAACC-3'
and YP 2 5'-GAAGCGATATTGCAGACC-3'

the inner nested primers, which amplify a DNA fragment of 458 bp

YP 1a 5'-AAGTTCTATTGTGGCAACC-3'
and YP 2a 5'-CACTCCTTTCGGGAAGTTTCCG-3'

another set of primers also based on the nucleotide sequence of the *Y. pestis* plasminogen activator (pla) gene has also shown promising results

YP 1 5'-ATCTTACTTTCCGTGAGAAG-3'
and YP 2 5'-CTTGGATGTTGAGCTTCCTA-3'

corresponding to the nucleotide 971 to 990 and 1431 to 1450, respectively, of the pla locus sequence and would amplify a 478 bp segment. This gene encodes as outer membrane protease that is responsible for two well-known properties of virulent *Y. pestis*, including a plasminogen activator activity, resulting in the lysis of fibrin clots and weak coagulase activity.

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HELPING TO SOLVE READING PROBLEMS

The tricky business of learning to read is made even more difficult for children with dyslexia, a conditions that interferes with their perception of words and letters. A new system has been developed that helps people with dyslexia and other reading difficulties. The equipment, enables a patient to select an individual precision ophthalmic tint that will improve perception. Glasses can then the made as non-prescription lenses or within a individual's refractive prescription.

Studies throughout the world have shown that at least two pupils per class room have a form of reading difficulty, and it is considered that the wearing of glasses with an individually chosen colour tint will help to improve some children's ability. The yenses could also help those suffering from reading-induced migraine and photosensitive epilepsy. The system, known as calorimeter, was devised after extensive collaborative research by Dr. Arnold Wilkins at the British Medical Research Council's Applied Psychology Unit in Cambridge, eastern England and Cerium Visual Technologies, a company based in Kent, southern England.

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