



TULAREMIA

GUIDELINES FOR ACTION IN THE EVENT OF A DELIBERATE RELEASE

Contents:	page:
1 Background and Clinical Information	2
1.1 Introduction	2
1.2 Epidemiology	2
1.3 Clinical features	3
1.4 Mortality	4
1.5 Organism survival	4
1.6 Antimicrobial susceptibilities	4
2 Clinical procedures	5
2.1 Diagnosis and collection of samples	5
2.2 Treatment	6
2.3 Infection control practice	7
2.4 Prophylaxis	9
2.5 Environmental decontamination	10
2.6 Protection of healthcare workers	10
2.7 Patient, visitor and public information	11
3 Laboratory procedures	12
3.1 Risk assessment	12
3.2 Isolation and identification	12
3.3 Confirmation	13
3.4 Waste disposal	13
3.5 Reference laboratories	13
3.6 Transport of samples	13
3.7 Protection of laboratory staff	15
4 Public Health procedures	16
4.1 Surveillance and detection of deliberate releases	16
4.2 Case definitions	16
4.3 Public health action	17
4.4 Epidemiological investigations	18
5 List of national specialists	19
6 References	20
7 Appendix	22

Note: Comments are welcome from healthcare, laboratory and public health professionals, and should be sent to DRcomments@hpa.org.uk. These guidelines may be subject to change as comments are received, so please ensure that you have the latest version available through the HPA website at: [http://www.hpa.org.uk/deliberate accidental releases/biological](http://www.hpa.org.uk/deliberate_accidental_releases/biological)

For this version of the guidelines changes were made to the following sections of the previous version:

3.2	5
3.5	6
3.7	

1 BACKGROUND

These guidelines are intended for healthcare, laboratory and public health professionals to guide clinical and public health action in the event of a deliberate release of tularemia.

1.1 Introduction

The discovery of tularemia is attributed to McCoy who reported a plague-like illness in ground squirrels from Tulare County, California, in 1911. The disease is caused by the Gram-negative pleomorphic bacterium, *Francisella tularensis*. There are two main subspecies, type A, (*F. tularensis* subspecies *tularensis*) and type B (*F. tularensis* subspecies *holarctica*) that cause disease in man and have been considered as potential biological agents. The SCHU S-4 strain of type A is one of the most infectious pathogenic agents known, requiring inoculation or inhalation of fewer than ten organisms to cause disease.

Humans become naturally infected through diverse environmental and animal exposures but there has been no documented person-to-person transmission. Six forms of tularemia are recognised:

- Ulceroglandular)
- Oropharyngeal) 45%-85% of naturally occurring cases
- Oculoglandular)
- Pneumonic <5% of cases
- Septicaemic <5% of cases
- Typhoidal up to 25% of cases

1.1.1 Deliberate release of *Francisella tularensis*

F. tularensis does not occur naturally in the United Kingdom and the very few numbers of cases seen have all been acquired abroad.

The World Health Organisation (WHO) and Centres for Disease Control and Prevention (CDC) in the United States anticipate that the greatest impact in terms of mortality and morbidity following intentional release of *F. tularensis* would be achieved through aerosolisation of a virulent strain, making inhalation into the lungs the most likely route of infection. Monkeys that inhaled the SCHU S-4 strain developed acute bronchiolitis within 24 hours of exposure to 1- μ m particles and within 48 hours of exposure to 8- μ m particles. Bronchopneumonia was most pronounced in animals exposed to the smaller particles.

1.2 Epidemiology

F. tularensis infects more than 100 species of wild mammals, birds and insects worldwide. A variety of small mammals, including voles, mice, water rats, squirrels, rabbits and hares, are natural reservoirs of infection. They acquire infection through bites by ticks, flies and mosquitoes, and by contact with contaminated environments. Although enzootic cycles of *F. tularensis* typically occur without notice, epizootics with extensive 'die-offs' of animal hosts may herald outbreaks of tularemia in humans. The ecosystems depend on the subspecies and locality.

1.2.1 Transmission

Infection with type B strains occurs across northern Europe (including Scandinavia), Russia and Japan and large outbreaks have occurred. Disease due to type B *F. tularensis*, by comparison with type A infection, is relatively mild, with negligible mortality. Naturally occurring infection with type A is more sporadic and often severe. It is restricted to defined geographical foci in North America, where it accounts for 90% of reported tularemia. People of all ages and both sexes appear to be equally susceptible to tularemia. **Person-to-person transmission has not been documented.**

Naturally acquired human infection occurs through a variety of mechanisms:

- Bites of infected arthropods including *Dermacentor* and *Ixodes* ticks (summer months)
- Contact with infected animals, including cats
- Handling infectious animal tissues or fluids
- Direct contact or ingestion of contaminated water, food, or soil
- Inhalation of infective aerosols e.g. from handling damp hay

1.2.2 Infectious dose

The infectious dose is very low and depends upon the portal of entry and type of *F. tularensis*. Approximately 10-50 type A organisms can initiate infection by the inhalational route. If SHU S-4 strain is used <10 organisms would be required.

1.2.3 Incubation period

Symptoms usually appear between 2-5 days (range: 1-21 days) post exposure.

1.2.4 Period of communicability

Human-to-human spread has not been reported following casual contact. Handling of infectious secretions or tissues may pose a risk to health care workers due to the low infectious dose.

1.3 Clinical features

Tularemia classically presents as one of six clinical syndromes, depending on the route of infection and biotype of the infecting organism. Both inoculum size and host immune status influence the severity and extent of disease. Onset of infection is usually acute and is heralded by fever, chills, headache and myalgias. Following a deliberate airborne release the most likely presentation would be pneumonic or septicaemic tularemia.

1.3.1 Pneumonic tularemia

This usually results from the direct inhalation of contaminated aerosols but can also follow haematogenous spread from another site. In primary inhalation disease the common presentation is an **acute flu-like illness with or without clinical pneumonia/pneumonitis**. Features include fever, non-productive cough, pharyngitis, pleuritic chest pain and hilar lymphadenopathy. This can progress to a severe pleuropneumonitis with moderate sized pleural effusions. When consolidation occurs this is usually non-lobar, with patchy infiltrates. Chest signs may, however, be minimal or absent. Volunteers challenged with aerosols of virulent *F. tularensis* type A regularly developed systemic symptoms of acute illness 3-5 days following exposure and 25-50% showed radiological evidence of pneumonitis early in the infection. In the largest airborne outbreak involving type B organisms, which occurred in Sweden in 1966/7, only 10% of serologically confirmed cases had symptoms suggestive of pneumonia. In most cases of established pulmonary disease, progression tends to be less dramatic than that seen with anthrax or plague, although mortality rates in excess of 30% may occur.

1.3.2 Septicaemic tularemia

The patient presents as an acute 'Gram-negative' sepsis with fever, abdominal pain, diarrhoea, and vomiting which may be prominent early in the course of illness. The patient typically appears toxic and may progress to septic shock, disseminated intravascular coagulation, haemorrhage, acute respiratory distress, confusion and coma.

1.3.3 Ulceroglandular tularemia

Naturally occurring ulceroglandular tularemia usually arises from handling a contaminated carcass or following an arthropod bite. Typically a local papule arises at the site of inoculation accompanied by generalised symptoms including fever and aches. The lesion may be pruritic and enlarges to form a pustule, which ruptures and develops into a painful, indolent ulcer. This may

or may not be accompanied by eschar formation. Differential diagnosis includes cutaneous anthrax (surrounding oedema is usually not as prominent as in cutaneous anthrax), plague, lymphogranuloma venereum, granuloma inguinale, and cat-scratch fever. A localised vesiculopapular eruption may also occur. As the lesion progresses it is accompanied by tender enlargement of one or more regional lymph nodes, which may become fluctuant and rupture releasing caseous material. Local disease often continues to progress despite appropriate antibiotic therapy.

Glandular tularemia may occur in the absence of an obvious site of inoculation.

Clinical pictures of tularemia ulcers are available on the HPA website at:

<http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1204707135610?p=1204707135610>

1.3.4 Oculoglandular tularemia

This follows airborne exposure, autoinoculation or after cleaning infected animal carcasses. Ulceration of the cornea produces chemosis and pain and is accompanied by tender preauricular lymphadenopathy.

1.3.5 Oropharyngeal tularemia

This is acquired by drinking contaminated water or food, direct inoculation from the hands to the mouth and sometimes by inhaling contaminated droplets or aerosols. Affected persons may develop a stomatitis, but more commonly an exudative pharyngitis or tonsillitis ensues with or without painful mucosal ulceration.

1.3.6 Typhoidal tularemia

An acute flu-like illness, often with diarrhoea and vomiting may follow ingestion or inhalation of *F. tularensis*. Pneumonic changes, mucocutaneous lesions and regional lymphadenopathy are usually absent.

1.4 Mortality

Untreated, the overall mortality for all types of tularemia is 8%, 4% for ulceroglandular and 30-50% for typhoidal, septicaemic and pneumonic types. With appropriate treatment, mortality is reduced to 1%.

1.5 Organism Survival

The survival of *F. tularensis* in aerosols is short and infective doses are not likely to persist in air for more than a few hours. Survival in non-chlorinated water can occur for up to 90 days. Studies suggest that *F. tularensis* can persist in the environment perhaps by surviving within protozoa such as *Acanthamoeba*.

1.6 Antimicrobial Susceptibilities

Aminoglycoside antibiotics (eg gentamicin), are bactericidal against *F. tularensis* and are currently the treatment of choice for pneumonic or typhoidal tularemia (and severe forms of glandular disease). Fluoroquinolones have shown promise because of their low toxicity and potential for oral therapy. Chloramphenicol and tetracyclines are associated with high relapse rates. The beta-lactams, except for carbapenems, are considered ineffective. Macrolide antibiotics are not recommended. If doxycycline or ciprofloxacin are given 48 hours before challenge and continued for 5 days after challenge in a murine model, these antibiotics protected against intraperitoneal infection. However all mice succumbed once the antibiotics were stopped. If treatment was continued for 10 days after challenge, then fewer relapses occurred.

CLINICAL PROCEDURES

2.1 Diagnosis and collection of samples

The first indication of a deliberate release via aerosol will be a cluster of acute, severe, flu-like illness with respiratory symptoms and unusual epidemiological features eg occurring in previously healthy young adults.

Type B strains released into water or food would probably present as a typhoidal illness or stomatitis/ pharyngitis/ tonsillitis with mucosal ulceration and tender cervical lymphadenopathy. In the highly unlikely event of release through direct contact eg via contaminated mail package, an ulceroglandular presentation would probably predominate.

Identification of *F. tularensis* in clinical specimens (detailed in 3.2) may be delayed for some time or missed altogether when procedures for routine microbiological screening of bacterial pathogens are followed, and it is unlikely that laboratory identification would be the initial event that alerted authorities to a deliberate release of this organism unless cysteine enriched media such as BCYE are used.

2.1.1 Precautions for sampling

The organism is most infectious by the inhalation route as fewer than 10 organisms of certain strains of type A *F. tularensis* may result in pulmonary tularemia. Infection may occur through the skin, mucous membranes, gastrointestinal tract, and lungs via infectious animal tissues or fluids, direct contact with or ingestion of contaminated water, food, or soil, and inhalation of infective aerosols. During the taking of clinical samples healthcare workers should observe universal contact precautions i.e., wearing gloves and plastic aprons followed by thorough handwashing. Healthcare workers should protect skin lesions with a water-proof dressing. Although person-to-person transmission has not been documented following casual contact, the use of the best available (highest efficiency) face and eye protection is recommended if infected secretions are likely to be aerosolised during sampling. A disposable surgical face mask will reduce the risk from large respiratory droplets, however, where available at least a medium efficiency FFP2 mask should be used. All laboratory work must be performed in a Class I safety cabinet in a containment level 3 facility. Exposure during examination of an open culture plate can cause infection and in several outbreaks the identity of the pathogen first became apparent after laboratory staff became infected after handling cultures on the open bench.

2.1.2 Samples to be taken from acutely ill humans

In suspected inhalational tularemia specimens of sputum, pharyngeal washings, fasting gastric aspirates, pleural fluid and blood may be culture positive for *F. tularensis*. Both radiometric and non-radiometric blood culture systems can detect *F. tularensis*, but subculture to cysteine rich medium such as BCYE is necessary for isolation. Other samples include exudates from lesions and biopsies of lymph nodes or cutaneous lesions. The procedure for transport of specimens to the laboratory is outlined in section 3.6. The laboratory must be informed in advance. Chain of evidence documentation should also accompany all specimens; however in larger incidents this would only be required for several of the initial cases.

2.1.3 Post-mortem specimens

Samples may be taken from dead humans to assist diagnosis, including body fluids or tissues. However, full post-mortem examinations are strongly discouraged if tularemia is suspected (see 2.3.4).

2.1.4 Samples to be taken from the environment

F. tularensis does not produce spores. Type A strains survive for only a few hours following deliberate release due to desiccation, UV radiation and oxidation. Type B strains may survive for

months in surface water and soil. If samples of exposed water, soil, dust and clothing are taken they should be processed as described in section 3.2.

2.1.5 Samples to be taken from others who have or may have been exposed

The decision to issue antibiotic prophylaxis following deliberate or accidental release should be taken following a risk assessment of likelihood and extent of exposure. Appropriate antibiotics should be started, if possible, within 24 hours of such an exposure (see section 2.4). Serological screening of exposed individuals may be useful in establishing the extent of exposure (2-4 weeks) following covert or overt release.

2.1.5 Transport of samples

Strict procedures should be followed for the transport of samples of suspected tularemia, both from the clinical environment to the laboratory, and from local laboratories on to the reference laboratory. These are outlined in section 3.6. *F. tularensis* cultures fall into category A for the purposes of transport. All samples should be transported as per UN 602 as described in "Appendix 1.2 Transport of infectious substances" in "Biological agents: Managing the risks in laboratories and healthcare premises." Advisory Committee on Dangerous Pathogens (ACDP), Health and Safety Executive (HSE) May 2005 accessed at:

<http://www.advisorybodies.doh.gov.uk/acdp/managingtherisks.pdf>

2.2 Treatment

F. tularensis is generally susceptible to a range of antibiotics. The antibiotic susceptibilities of 50 *F. tularensis* type B strains isolated from human patients and hares from Austria against 24 antibiotics were ascertained (Tomaso *et al.* 2005) and all isolates were sensitive to tetracyclines, aminoglycosides, quinolones, chloramphenicol and rifampicin.

Gentamicin for 10 days is effective in the treatment of tularemia (see Table 1) and is considered first line therapy. Gentamicin is administered as a 1 hour infusion and therefore antibiotic levels should be taken between 6 and 14 hours after the start of the first infusion to ascertain future dosing intervals in accordance with the Hartford nomogram (see Appendix). The Hartford nomogram suggests that at 6 hours the gentamicin level should be < 7.5 mg/litre, and at 14 hours ≤ 2 mg/litre. For all stages in between the Hartford nomogram can be used.

Streptomycin was regarded as the drug of choice for the treatment of non-meningeal tularemia in both adults and children but it is no longer widely available in the UK.

It is recognised that these agents are not routinely used as monotherapy in the treatment of acute febrile illnesses, including pneumonia. If tularemia is suspected but not confirmed microbiologically or serologically, an aminoglycoside should be added to an appropriate antibiotic regimen and **not used** as a single agent. Other antibiotics can be stopped when the identity of *F. tularensis* is confirmed and antibiotic susceptibility data available.

Ciprofloxacin and other fluoroquinolones have been used effectively to treat tularemia, although experience with these medications is somewhat limited (Johansson 2000). Ciprofloxacin and other fluoroquinolones are active *in vitro* and animal studies suggest that they are candidates for the treatment of pneumonic tularemia (Steward *et al.* 2006). Ciprofloxacin has been used successfully to treat a number of cases in both children and adults, including a large non-randomised study in Spain where 21 of 22 patients with predominantly ulceroglandular disease were cured. In one outbreak, ciprofloxacin showed a lower treatment failure rate than streptomycin or doxycycline (Perez-Castrillon *et al.* 2001). However, ciprofloxacin is not licensed for use in children or pregnant women.

Tetracycline and chloramphenicol are bacteriostatic against *F. tularensis* and if they are used in treatment at least 21 days therapy is required to reduce the chance of relapse. Due to the poor penetration of aminoglycosides into CSF, chloramphenicol may be added to gentamicin in the treatment of patients with clinical features of meningitis.

Table 1. Recommended treatment for tularemia

	Antimicrobial agent	Duration
Adults (including pregnant women)	Gentamicin (first choice in pregnancy*) 5mg/kg IM or IV once daily or Ciprofloxacin 400mg IV twice daily (change to oral 500mg twice daily when appropriate)	14 days
Children >1month†	Gentamicin 2.5 mg/kg IM or IV three times daily or Streptomycin 15/mg/kg IM twice daily (not to exceed 2g per day) or Ciprofloxacin 15mg/kg PO bd (maximum 500mg) – not to exceed 1g per day Ciprofloxacin dose depends on age and weight, as a guide: newborn – 6 months 100mg/day 1 year – <3 years 200mg/day 3 years – <5 years 300mg/day 5 years – <7 years 400mg/day 7 years – <12 years 500mg/day 12 years+(adult dose)1000mg/day	10 days

* The Hartford nomogram for monitoring is not applicable to pregnant women due to their altered volume of distribution and a single daily dose may not be appropriate – multiple dosing may be needed (seek specialist advice).

† For children < 1month seek specialist advice re dosing of aminoglycosides.

2.2.1 Treatment of mass casualties

Treatment of large numbers of casualties following a deliberate release may warrant the use of oral ciprofloxacin and doxycycline (see Table 2). The risk of side effects from medication would not preclude their use in children, in such a situation.

In a major incident information on how to access stocks of antibiotics for treatment or prophylaxis can be found on the DH website at:

http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_081038

2.3 Infection control practice

2.3.1 Decontamination of exposed persons

The number of viable organisms that may be re-aerosolised following the handling of contaminated clothing of exposed individuals is probably low. However, even low numbers of organisms could potentially lead to infection in any person having contact. An incident specific risk assessment will be required.

In situations where the threat of exposure to *F. tularensis* exists, cleansing of skin and potentially contaminated fomites such as clothing, personal possessions or environmental surfaces should take place. Decontamination of exposed individuals should include:

- Removal of contaminated clothing and possessions, which should be placed, with minimum agitation, in labelled and sealed double plastic bags and stored until exposure to *F. tularensis* has been ruled out.
- Contaminated material must be incinerated or autoclaved.
- Instructing exposed persons to shower thoroughly with soap and water- appropriate facilities will be provided at the scene as necessary.

Attending personnel should wear full PPE including standard Universal Precautions when handling contaminated clothing and other fomites.

2.3.2 Isolation of patients

- Standard universal precautions including gloves, gowns and hand washing are recommended for patients with tularemia.
- Although person-to-person spread of *F. tularensis* has not been reported following casual contact, isolation of patients in a side room, or as a cohort on a ward, is recommended for pulmonary disease or where there is a danger of aerosolisation from lesions during sampling.
- All persons entering or leaving the room should wear the best available (highest efficiency) face and eye protection in addition to standard Universal Precautions when attending to isolated patients. Lesions should be covered with a waterproof dressing.

2.3.3 Cleaning, disinfection and waste disposal

Normal procedures for standard isolation are appropriate. Contaminated environmental surfaces should be cleaned with hypochlorite solution (10,000 ppm available chlorine). NB: This should not be applied to body surfaces. Laundry and garments should be placed in plastic bags and laundered through a hot cycle (>70°C) when *F. tularensis* is confirmed.

2.3.4 Post-mortem

Autopsy

The risk of acquiring tularemia following contact with the body of a person who has died from the disease is minimal because, although there is no person-to-person transmission, there is evidence of autopsy transmission.

Autopsy examinations are strongly discouraged if tularemia infection is suspected, as the body fluids and tissues of a patient who has died of tularemia are likely to have *F. tularensis* bacteria present in them. If an autopsy is necessary expert advice must be sought from the HPA. The Pathologist must be informed of the known or suspected diagnosis. Standard precautions for post-mortem examinations on patients infected with Containment Level 3 organisms are appropriate. Instruments should be autoclaved.

Similarly, body preparation should be carried out with normal control of infection procedures. Standard precautions for the disposal of bodies infected with Containment Level 3 pathogens should be observed, and the undertaker should be informed. **Cremation** is the preferred method of disposal for the deceased. **Embalming** of bodies should not be undertaken because the body fluids are likely to contain large numbers of the causative bacteria and therefore the process of embalming exposes the embalmer to an unacceptable risk.

Pacemaker removal

Pacemaker removal is permitted. Pacemaker should be treated with hypochlorite solution (10,000 ppm available chlorine), bagged and disposed of appropriately (not by incineration).

2.4 Prophylaxis

In the event of a known exposure to *F. tularensis* antibiotic prophylaxis should be initiated as soon as possible – as described in Table 2. Prophylaxis should continue until exposure has been excluded. Ciprofloxacin is the prophylactic agent of choice when there is a credible threat of exposure to a biological agent. Ciprofloxacin has the added advantage that it is also effective prophylactic treatment for other potential bioterrorist agents such as anthrax and plague. The risk of adverse effects from antibiotic prophylaxis must be weighed against the risk of developing serious disease.

After an initial five day treatment with ciprofloxacin, doxycycline may be substituted to complete the 14 days prophylaxis. Only the initial course of antibiotics for prophylaxis is held in pods as part of the antibiotic stockpile. Arrangements for the supply of the remainder of the prophylactic course are being developed and individuals should be advised to report to their own GP. See DH Patient Group Directions for the initial and further supply of ciprofloxacin and the further supply of doxycycline in the event of exposure to a suspect biological agent

http://www.dh.gov.uk/en/Managingyourorganisation/Emergencyplanning/DH_4069610

In a major incident information on how to access stocks of antibiotics for initial treatment or prophylaxis can be found on the DH website at:

http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_081038

The regimen may be modified when information regarding the identity and/or antibiotic susceptibility of an organism becomes available. One small study, in which volunteers were exposed to an aerosol of a virulent strain of *F. tularensis*, showed that oral tetracycline 1g/day for 24 days or 2g/day for 14 days were effective in preventing clinical infection if given within 24 hours of exposure. A shorter course was associated with symptomatic tularemia in two of ten exposed individuals.

In the case of significant exposure of a laboratory worker, via aerosol or autoinoculation of infected material from a patient with tularemia, ciprofloxacin or doxycycline should be started immediately and given for 14-21 days.

Although ciprofloxacin is not licensed for use in children or pregnant women and there have been no formal studies of the use of ciprofloxacin during pregnancy, it is unlikely to be associated with a high risk of abnormalities of foetal development.

Paediatric use of fluoroquinolones and tetracyclines may be associated with adverse effects and their use must be weighed against the risk of developing an infectious disease with significant morbidity and mortality. In general doxycycline is not recommended for use in children under 12 years unless no alternative anti-bacterial can be given.

2.4.1 Immunisation

A live attenuated vaccine was developed and has been used to immunise laboratory staff who work with *F. tularensis*. The vaccine is unlicensed and provides incomplete protection, particularly against inhalational tularemia. It is not currently recommended for use as post-exposure prophylaxis.

2.4.2 Contacts of cases

There is no need to provide antibiotic prophylaxis to contacts of patients unless there is concern that they were also exposed to the initial release.

Table 2: Recommended prophylaxis after exposure to *F. tularensis*

	Antimicrobial agent	Duration
Adults (including pregnant women)	Initial (5 day) treatment Ciprofloxacin 500mg PO bd Further (9 day) treatment Ciprofloxacin 500mg PO bd or Doxycycline 100mg PO bd	14 days
Children	Initial (5 day) treatment Ciprofloxacin 500mg PO bd Further (9 day) treatment Ciprofloxacin 15mg/kg PO bd (maximum 500mg) – not to exceed 1g per day Ciprofloxacin dose depends on age and weight, as a guide: newborn – 6 months 100mg/day 1 year – <3 years 200mg/day 3 years – <5 years 300mg/day 5 years – <7 years 400mg/day 7 years – <12 years 500mg/day 12 years+(adult dose)1000mg/day or Doxycycline only for children >8y and >45kg adult dose 100mg PO bd	14 days

2.5 Environmental decontamination

The greatest risk of acquiring tularemia from deliberate airborne release follows exposure to the primary aerosol in the exposure zone. The duration and scale of the infectious risk depends on the duration for which organisms remain airborne and the distance they travel before falling to the ground. This depends on meteorological conditions and the aerobiological properties of the aerosol. The aerosol is likely to have completely dispersed before the first cases appear. The more pathogenic type A strains are unlikely to survive for long on environmental surfaces due to desiccation, solar radiation and oxidation and the risk of developing infection following secondary dispersal is likely to be low. If there is visible contamination, such as in the case of laboratory spillage, surfaces should be cleaned with hypochlorite solution (10,000 ppm available chlorine). The current level of chlorination used in UK mains water supplies, ie 0.5-1 ppm available chlorine, kills 99-100% of *F. tularensis* organisms within five minutes at 10°C. Boiling rapidly kills *F. tularensis*.

2.6 Protection of frontline workers

This includes all emergency staff involved in management at the scene of a release and staff involved in the care of patients.

2.6.1 Protective clothing

Following an overt release of *F. tularensis* the area affected by primary aerosolisation will depend on the time and place of release. This **exposed zone** presents a high risk of infection, and anyone entering it should wear a biologically-resistant suit with outer gloves and boots (for

example a CR1, PRPS or gas-tight suit) and a correctly fitting high-efficacy respirator of FFP3 standard AT ALL TIMES.

Healthcare workers will not normally be asked to enter this zone, however it is possible that they may be called into it to treat casualties, for example if an explosive device has accompanied the release of a biological agent. In this case the appropriate protective clothing should be worn.

Exposed persons will normally be moved from the exposed zone, through decontamination if necessary, and into a place of safety or holding area for medical assessment. Frontline workers involved in the decontamination of exposed individuals and handling of contaminated clothing and fomites should observe standard universal precautions – gloves, plastic aprons along with face masks (as described in section 2.3.2) and eye protection if splashing is likely. Hands should always be washed after the removal of gloves.

For healthcare workers involved in the management of hospitalised patients with tularemia Universal Precautions provide sufficient protection. Mortuary staff should use similar barrier protection.

2.6.2 Antibiotic prophylaxis and immunisation

Frontline workers entering the exposed zone should be offered antibiotic prophylaxis as outlined in Table 2 and section 2.4. Prophylactic antibiotics may also be considered for frontline workers involved in other activities including:

- Decontamination of exposed persons
- Handling of exposed persons
- Management of patients or disposal of bodies of patients dying of tularemia

Decisions about who should receive prophylaxis should be taken on an individual basis according to duration and degree of exposure and taking into account the availability and side effects of prophylactic treatments available. The live attenuated vaccine currently used for the protection of laboratory staff who work with live *F. tularensis* provides incomplete protection against inhalational tularemia and is not currently recommended for use as post-exposure prophylaxis.

2.7 Patient visitor and public information

Information sheets have been prepared for distribution in the event of an incident.

3 LABORATORY PROCEDURES

3.1 Risk assessment

F. tularensis, particularly type A is an extremely hazardous pathogen and has been associated with severe infection in exposed laboratory personnel. Although currently regarded as an ACDP category 3 pathogen and covered by existing risk assessments, extreme caution should be exercised when handling such organisms in diagnostic laboratories. Any exposure through leakage or breakage of clinical specimens, from patients known or thought to have tularemia, should be reported immediately to the laboratory safety officer, infection control team and occupation health physician so that a risk assessment can be performed and appropriate action taken.

3.1.1 Receipt of samples

All clinical specimens from patients known or thought to have tularemia should be labelled 'High risk' by the submitting staff and further work, including unpacking, conducted in a properly maintained Class I protective safety cabinet within a containment level 3 facility.

3.2 Isolation and identification

In the event of there being a credible risk of tularemia arising from a deliberate release it is recommended that a senior member of the clinical or laboratory staff contact one of the national specialists listed at the end of this document to discuss processing of specimens, along with appropriate Consultants in Communicable Disease Control (CCDCs) and the duty doctor at HPA CfI. The handling of material that is likely to contain viable *F. tularensis*, by inexperienced personnel is strongly discouraged.

Laboratory pictures of *F. tularensis* are available on the HPA website at:
<http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/Page/1204707135624>

3.2.1 Culture

F. tularensis type A is a small (0.2x0.2-0.7µm) encapsulated, pleomorphic Gram-negative bacterium. It can be readily distinguished from the Gram-positive rod *Bacillus anthracis* and does not show the characteristic bipolar staining of *Yersinia pestis*. It is a fastidious organism requiring cysteine-enriched media such as cysteine-glucose blood agar, or BCYE *Legionella* medium, for growth. Positive blood cultures showing small Gram-negative bacilli that fail to grow on conventional media should be subcultured to such media. Small, 1-2mm grey-white colonies appear after 24-72 hours in CO₂ enriched air at 37°C although growth may be delayed and cultures should be held for at least 10 days before discarding.

The organism is non-motile, a slow catalase producer, oxidase negative, H₂S positive and produces acid but not gas from glucose, maltose and mannose. It can be differentiated from other Gram-negative organisms except *Legionella* spp due to cysteine dependence. *Legionella* spp are motile and do not produce H₂S. **Attempts to isolate *F. tularensis* strains should be avoided in the absence of adequate containment facilities.**

F. tularensis type B grows poorly on primary culture and is best isolated by subcutaneous injection into white mice.

3.2.2 Antibiotic sensitivity

This should be performed by a laboratory experienced in handling *F. tularensis*. (See list of national specialists at the end of this document).

3.2.3 Serology

Serodiagnosis of tularemia can be performed using ELISA. A significant rise in antibody titre demonstrated on samples of acute and convalescent sera (2-4 weeks later) offer a confirmatory diagnosis of tularemia. This would be unlikely to provide useful information for initial management of an outbreak although a single high titre offers a presumptive diagnosis in a patient who has not been previously vaccinated (CDC criteria).

3.2.4 Molecular methods

Primers specific for *F. tularensis* have been developed and PCR may offer the best means of rapid diagnosis in samples from patients with suspected tularemia. Specimen collection and transport should be discussed with the reference laboratory (See 3.5).

3.3 Confirmation

This should be performed by a laboratory experienced in handling *F. tularensis*. (See list of national specialists at the end of this document).

3.4 Waste disposal

In the laboratory, surfaces that have been contaminated with *F. tularensis* should be disinfected with hypochlorite solution (10,000 ppm). Hands should be washed after removing gloves. All waste containers should be autoclaved.

3.5 Reference laboratory

All positive isolates and cultures should be sent to the reference laboratory for confirmation. In addition, samples may be sent there directly if local laboratories lack the facilities for dealing with them. All samples and cultures must be packaged appropriately, taking care to observe the procedures outlined in section 3.6. The sender's name and address should be clearly marked. The reference laboratory should be telephoned prior to sending to expect the sample. Samples should be forwarded urgently to:

Dr Tim Brooks
HPA Centre for Emergency Preparedness and Response
Special Pathogens Reference Unit
Porton Down
Salisbury SP4 0JG
Tel: (+44) 01980 612100 (24hours)

Further information on SPRU and referral of specimens and samples:

<http://www.hpa.org.uk/HPA/ProductsServices/InfectiousDiseases/SpecialPathogensReferenceUnit/>

3.6 Transportation of samples

Strict procedures apply for transport of samples to the laboratory. Biological agents, or materials that contain them, are allocated to UN Division 6.2 – infectious substances. Infectious substances are divided into Category A or Category B. Full details are given in Appendix 1.2 Transport of infectious substances in *Biological agents: Managing the risks in laboratories and healthcare premises*. ACDP HSE May 2005, available at:

<http://www.advisorybodies.doh.gov.uk/acdp/managingtherisks.pdf>

and in the Department of Health's guidance, available at:

http://www.dh.gov.uk/en/PublicationsAndStatistics/Publications/PublicationsPolicyAndGuidance/DH_075439

Cultures of *F. tularensis* are Category A infectious substance capable of causing disease in humans or animals and are therefore assigned to UN2814 and must be packaged in accordance with UN Packaging Instructions PI620 (road/rail) /PI602 (air). P620 and P602 are identical specifications but given different codes in ADR and ICAO regulations respectively (full description of PI see <http://www.unece.org/trans/welcome.html>). Category A transfers should be individually requested through an approved courier. The service will be a next day, tracked door-to-door delivery, which must be signed for at collection and receipt.

Clinical samples are generally classified as Category B and are assigned to UN3373 ("Biological Substance, Category B") and should be packaged in accordance with UN PI650. Clinical samples may be posted.

Packaging must meet with UN performance requirements i.e. UN-type approved packaging for Class 6.2 substances. The packaging should consist of an inner package (watertight receptacle, watertight secondary packaging, an absorbent material in sufficient quantity to absorb the entire contents placed between the receptacle and the secondary packaging) and a rigid outer package of adequate strength for capacity, mass and intended use. Packages should be marked with the proper shipping name i.e. "Infectious substance affecting humans", the appropriate UN number (i.e. UN 2814), and the appropriate warning label (i.e. the danger sign for infectious substances).

The following procedures should be adopted for the transport of all specimens and also all cultures for confirmation. These apply within hospitals and laboratories as well as for specimens sent to the reference laboratory:

- The primary container (bijoux or similar) should be screwed tight, labelled and placed in an intact plastic bag.
- A 'High Risk' label should be affixed to both specimen and request form. The latter should include any other relevant information and include adequate clinical details to indicate level of suspicion.
- Under no circumstances should the request form be placed in the same bag or compartment as the specimen.
- The bag should be sealed, using tape or heat sealer. Pins, staples and metal clips should not be used. A separate bag should be used for each specimen.
- Each specimen must then be placed in a leak-proof secondary container with sufficient absorbent material to absorb all the contents should leakage occur.
- Each specimen must be packaged individually - i.e. three specimens, three separate packages.
- The secondary container should be externally disinfected – e.g. by wiping with hypochlorite (1,000ppm available chlorine).

3.6.1 Samples sent to the reference laboratory

Samples should be transported according to local arrangements for High Risk specimens.

Precautions should include:

- Secondary containers should be placed within a final outer tertiary packaging.
- This packaging **must** comply with the UN-type approved packaging for the transport of infectious substances.
- The package should be certified to this standard and carry the appropriate UN certification numbers on the tertiary packaging along with the following information:
 - 1 BIOHAZARD – danger of infection symbol Class UN 6.2.
 - 2 Instructions not to open if found.
 - 3 Telephone number of a responsible person - e.g. Consultant Microbiologist, Laboratory Manager.

- The container should be transported either by an approved courier for cultures (UN 2814) or by post for clinical samples (UN3373), without delay, directly to the reference laboratory.

All enquiries regarding the sending of specimens from a patient with suspected tularemia should be discussed with one of the national specialists listed in section 5

3.6.2 Samples sent within hospitals and laboratories

Samples should be transported according to local arrangements for High Risk specimens.

Precautions should include:

- Secondary containers should be placed in a good quality box, which is well taped up and clearly labelled "Pathological Specimen – Open only in Laboratory".
- Specimens should be transported by hand by a responsible person using the above packaging.
- Vacuum-tube systems should **not** be used for transportation of specimens within hospitals or laboratories.
- Extra care should be taken to ensure that laboratory records are kept to a high standard.

3.7 Protection of laboratory staff

All laboratory procedures must be performed in a Containment Level 3 facility using a Class 1 biological safety cabinet. Under these circumstances there is no indication for antibiotic prophylaxis for laboratory staff unless there is an inoculation injury or a spillage releasing aerosols containing the organism.

Any member of laboratory staff, working with specimens or cultures of *F. tularensis*, who develops a febrile/ respiratory illness, should seek urgent medical attention.

4 PUBLIC HEALTH PROCEDURES

4.1 Surveillance and detection of deliberate releases of tularemia

A deliberate release may be overt with an announcement and/or confirmation by environmental sampling. However, a deliberate release may be covert and will not be identified until the first cases of disease arise. Indigenous tularemia has never been recorded in the UK, but cases are occasionally imported. Person-to-person spread of tularemia has not been documented.

Surveillance is based on identification of 'suspected' and/or 'confirmed' cases by clinicians and/or medical microbiologists, who should notify the CCDC at the local HPU immediately. In case of 'suspected deliberate release' the duty doctor of HPA Centre for Infections should be notified immediately (tel. 0208 200 6868). Case definitions, including those for 'suspected deliberate release' are listed in the next paragraph (4.2).

Various mammals including rabbits and domestic cats are susceptible to tularemia, so close coordination with veterinary colleagues is essential. Infected animals could also act as an ongoing source of potential human infection.

4.2 Case Definitions

4.2.1 Suspected case

- A severe, unexplained febrile illness or febrile death in a previously healthy person.
- Severe unexplained respiratory illness in an otherwise healthy person.
- Severe unexplained sepsis or respiratory failure not due to a predisposing illness.
- Severe sepsis with unknown Gram-negative coccobacillary species that fails to grow on standard blood agar, identified in the blood or cerebrospinal fluid.

If tularemia is suspected, microbiological specimens should be sent to the reference laboratory, and consideration should be given to initiating empirical treatment pending results. Obviously the level of suspicion of tularemia depends on local circumstances at the time – in the event of a known or suspected deliberate release the threshold for suspecting tularemia should be lower.

As discussed in section 3.3, clinical microbiology laboratories should also be alert to the possibility of tularemia. If the clinical syndrome is suggestive of tularemia the sending of samples should be discussed with one of the national specialists (section 5).

4.2.2 Confirmed case

A case that clinically fits the criteria for suspected tularemia, and in addition, definitive positive results are obtained on one or more pathological specimens by the reference laboratory (see section 3.2 and 3.3).

4.2.3 Suspected deliberate release.

Two or more **suspected** cases of tularemia that are linked in time and place, especially geographical related groups of illness following a wind direction pattern (analogous to legionnaire's disease).

4.2.4 Deliberate release.

Single confirmed case of indigenously acquired tularemia not explained by occupational exposure.

4.3 Public Health Action

4.3.1 Procedure for handling exposed persons

Depending on the site and method of release, tularemia bacteria may be dispersed over a wide area. In contrast to anthrax, inhalation of fewer than 10 organisms can cause disease. If an incident is regarded as having 'credible risk' for release of any biological agent, including *F. tularensis*, expert advice will be provided by the responding authority ie police, to define the **exposed zone** in time and space. Definition of the exposed zone may need reviewing if cases arise in persons who were not present within it. All individuals who have been present in the exposed zone need to be identified. Some of them will still be at the scene when emergency services respond to the incident. This group will be decontaminated and then referred to health workers at a nearby place of safety for assessment and prophylaxis (this will be a clinical area just outside the exposed zone and within the cordon that will be established at the scene of the incident). Others will have left the scene before emergency services arrive and will be identified later when they approach GPs and Emergency Departments after details of the incident have been made public. Procedures need to ensure that these individuals are appropriately decontaminated, receive prophylaxis, and have their details collected for follow-up.

4.3.2 Post-exposure prophylaxis

There are two groups of individuals for which prophylaxis is indicated:

I **Individuals who have been present in the exposed zone** should be offered post-exposure prophylaxis as outlined in Table 2.

II **Healthcare workers** may require prophylaxis as described in section 2.6.2.

If suspected or confirmed cases of tularemia arise among persons who have been outside but in close proximity to the exposed zone in time or space, the defined parameters of the exposed zone should be reviewed with a view to extending post-exposure prophylaxis.

Prophylaxis for other groups may be considered in the event of an incident. However, it is not advisable to give antibiotics to people who do not have a clear history of having been present at the time and site of release. It is inappropriate to provide antibiotics to large numbers of people who have not been exposed, but who are generally concerned or have non-specific mild illnesses.

4.3.3 Follow-up of exposed persons

After an overt release, a basic set of personal details needs to be collected from all persons present in the exposed zone.

4.3.4 Case finding

If cases of tularemia arise and a covert release is suspected, health services should be contacted to determine whether other possible cases have presented.

4.3.5 Preventing secondary spread

As previously mentioned, person-to-person spread of *F. tularensis* does not occur therefore there is no specific treatment or advice required for secondary contacts. There is no requirement for quarantine of infected patients. There is no need to provide antibiotic prophylaxis to contacts of patients unless there is concern that they were also exposed to the initial release.

4.4. Epidemiological investigation

If a case is strongly suspected or confirmed notify the CCDC at the local HPU (or relevant out-of-hours cover) and the HPA Centre for Infections (020 8200 6868, 24 hours) immediately.

If cases arise due to a covert release, or following an overt release but in people who have not been present in the exposed zone, it is important to collect some epidemiological details in addition to a basic set of personal details. This is in order to define or redefine the exposed zone and aid identification of others at risk of infection. Details should be as thorough as possible, whilst recognising that in the event of a large release with multiple exposed persons or cases, it may not be possible to collect comprehensive information from everyone.

The aim of epidemiological investigations may be:

- Following a covert release, to assist definition and ongoing review of the temporal and spatial parameters of the exposed zone so that post exposure prophylaxis can be distributed appropriately.
- Following an overt release, to guide review of the exposed zone if cases arise in persons who were not present within it.

4.4.1 Epidemiological sampling

Microbiological samples will be taken from the environment by the police. These will be tested in designated laboratories.

5. LIST OF NATIONAL SPECIALISTS

Advice on tularemia including, diagnosis, management and public health aspects can be obtained from:

Dr Tim Brooks
HPA Centre for Emergency Preparedness and Response
Novel and Dangerous Pathogens
Porton Down
Salisbury SP4 0JG
Tel: (+44) 01980 612774
e-mail: tim.brooks@hpa.org.uk

Dr Robert Spencer
HPA South West
Level 8
Bristol Royal Infirmary
Marlborough Street
Bristol BS2 8HW
Tel: (+44) 0117 342 3242
e-mail: robert.spencer@UHBristol.nhs.uk

Dr Andrew Simpson
Consultant Clinical Microbiologist
Dstl Porton Down
Salisbury SP4 0JQ
Tel: (+44) 01980 613102
e-mail: ajsimpson@dstl.gov.uk

Diagnostic laboratory:

Dr Tim Brooks
HPA Centre for Emergency Preparedness and Response
Special Pathogens Reference Unit
Porton Down
Salisbury SP4 0JG
Tel: (+44) 01980 612774
(+44) 01980 612100 (24hours)
e-mail: tim.brooks@hpa.org.uk

Out of hours contact details are held at HPA Centre for Infections by the 24 hr on call duty doctor; Tel: (+44) 020 8200 6868

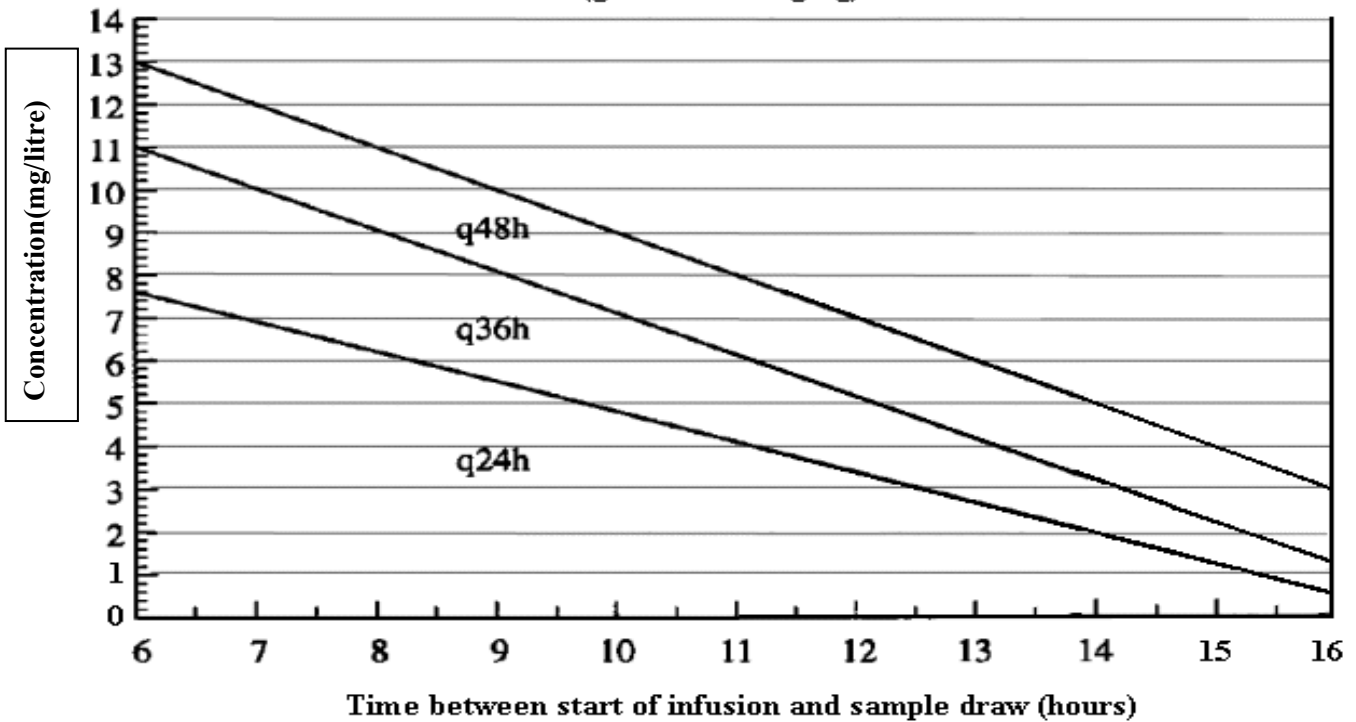
6. REFERENCES

1. Abd H, Johansson T, Golovliov I, Sandström G, Forsman M. Survival and growth of *Francisella tularensis* in *Acanthamoeba castellanii*. *Appl Environ Microbiol* 2003; **69**(1): 600-606.
2. Centre for infectious disease research and policy (CIDRAP) University of Minnesota. Tularemia: Current, comprehensive information on pathogenesis, microbiology, epidemiology, diagnosis, treatment, and prophylaxis. (Last updated April 2, 2009). <http://www.cidrap.umn.edu/cidrap/content/bt/tularemia/biofacts/tularemiafactsheet.html>
3. Dennis DT, Inglesby TV, Henderson DA. Tularemia as a Biological Weapon. Medical and public health management. *JAMA* 2001; **285**(21): 2763-73.
4. Feldman KA, Stiles-Enos D, Julian K, Matyas BT, Telford SR, Chu MC, Petersen LR, Hayes EB. Tularemia on Martha's Vineyard: Seroprevalence and occupational risk. *Emerg Infect Dis* 2003; **9**(3): 350-354.
5. Griffin KF, Oyston PCF, Titball RW. *Francisella tularensis* vaccines. *FEMS Immunol Microbiol* 2007; **49**: 315-323.
6. Health aspects of chemical and biological weapons. Geneva, Switzerland: World Health Organization; 1970. p105-7.
7. Johansson A, Berglund L, Gothefors L, *et al*. Ciprofloxacin for treatment of tularemia in children. *Pediatr Infect Dis J* 2000; **19**(5): 449-53.
8. Kaufmann AF, Meltzer MI, Schmid GP. The economic impact of a bioterrorist attack: are prevention and post-attack intervention programs justifiable? *Emerg Infect Dis* 1997;**3**: 83-94.
9. McCrumb FR Jr. Aerosol infection in man with *Pasteurella tularensis*. *Bacteriol Rev* 1961;**25**: 262-7.
10. Oyston PCF. *Francisella tularensis*: unravelling the secrets of an intracellular pathogen. *J Med Microbiol* 2008; **57**: 921-30.
11. Pearson AP. Tularemia. In: Palmer FR, Lord Soubry Simpson DIH, editors. Zoonoses: biology, clinical practice and public health control. Oxford University Press, 1998. p267-80.
12. Pearson AP. *Francisella* and tularemia . In: Smith GR and Easmon CSF, editors. Topley and Wilson's Microbiology and Microbial infections. 8th ed. London; Arnold; 1990. Vol 2 p595-7, Vol 3 p389-92.
13. Pearson AP. Yersiniosis, Pasteurellosis and Tularemia. In: Weatherall DJ, Ledingham JGG, Warrell DA, editors. Oxford Textbook of Medicine. 3rd edition, Oxford University Press; Oxford; 1996. p599-612.
14. Perez-Castrillon, JL, Bachiller-luque P, Martin-Luquero M, Mena-Martin J, Herreros V. Tularemia epidemic in Northwestern Spain: clinical description and therapeutic response. *CID* 2001; **33**:573-6.
15. Sawyer WD, Dangerfield HG, Hogge AL, Crozier D. Antibiotic prophylaxis and therapy of airborne tularemia. *Bacteriol Rev* 1966; **30**:542-8.

16. Steward J, Piercy T, Lever MS, Simpson AJH, Brooks TJG. Treatment of murine pneumonic *Francisella tularensis* infection with gatifloxacin, moxifloxacin or ciprofloxacin. *Int J Antimicrob Agents* 2006; **27**:439-43.
17. Tomaso H, Al Dahouk S, Hofer E, Splettstoesser WD, Treu TM, Dierich MP, Neubauer H. Antimicrobial susceptibilities of Austrian *Francisella tularensis* holarctica biovar II strains. *Int J Antimicrob Agents* 2005; **26**: 279-84.

7. APPENDIX

**Hartford ODA Dose Adjustment Nomogram
(gent/tobra 7mg/kg)**



The 'Hartford Nomogram' was developed and validated by Dr David Nicolau *et al*/Division of Infectious Diseases Hartford Hospital Hartford Connecticut USA

Reference:

Nicolau DP, Freeman CD, Belliveau PP, Nightingale CH, Ross JW, Quintiliani R. Experience with a once daily aminoglycoside program administered to 2184 adult patients. *Antimicrobial Agents and Chemotherapy* 1995; **35**:650-5.