Water sources and potential \textit{Pseudomonas aeruginosa} infection of taps and water systems

\textit{Advice for augmented care units}
This guidance provides advice on what actions healthcare organisations should take (within augmented care units):  
• to assess the risk to their patients if their water systems become contaminated with P. aeruginosa or other opportunistic pathogens;  
• if their water systems become contaminated with P. aeruginosa; and  
A sampling and recording protocol for routine monitoring of P. aeruginosa contamination.
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*Advice for augmented care units*
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Executive summary

1. This best practice technical guidance supplements the advice provided in the CMO “dear colleague” letter issued on 6 February 2012 and provides advice for health care providers across the UK on:

   i) assessing the risk to patients if water systems become contaminated with *P. aeruginosa*;

   ii) what actions to take if water systems become contaminated with *P. aeruginosa*;

   iii) protocols for sampling, testing and monitoring water for *P. aeruginosa*; and

   iv) developing local water safety plans.

2. This guidance is aimed at those healthcare organisations providing patient care in augmented care units (primarily paediatric and adult critical care, neonatal and burns units), but as case mix and patient susceptibility varies between units a local risk assessment is required to establish if these measures are appropriate in other areas such as renal, transplant and haematology units. For neonatal care this should focus on those areas where the more vulnerable babies are cared for. In some units this may mean establishing a water testing regimen; in other units water testing could be guided by clinical surveillance of patients.

3. It is recommended that all registered providers put in place systems to manage and monitor the prevention and control of infection. Providers can use risk assessment to determine susceptible patient groups and any risks that their environment and other patients may pose to them.

4. This guidance provides advice on how to systematically assess water systems for *P. aeruginosa* and similar opportunistic pathogens other than *Legionella* and apply a suitable risk assessment approach in order to reduce the risk to patient safety. The guidance is particularly intended for those professionals engaged in infection prevention and control, estates and facilities and the Responsible Person (Water).

5. *P. aeruginosa* is not the only risk from contaminated water systems and adopting the approach set out here will help reduce the risk from other opportunistic pathogens by providing baseline information on the water system.

Context

6. The current state of knowledge on *P. aeruginosa*, taps and water systems is not extensive and is based on limited scientific evidence. This guidance is based on current expert opinion and will develop as the knowledge base expands. At present, evidence suggests that the microbial ecology of *P. aeruginosa* within water distribution and delivery systems
may not be identical to that of Legionella, so testing regimes appropriate for Legionella may not be appropriate for P. aeruginosa.

7. Although this guidance focuses on P. aeruginosa, other opportunistic pathogens may behave in a similar manner but appear less frequently as clinical problems. Sampling could also identify pathogens such as Stenotrophomonas and Burkholderia or other opportunistic pathogens if clinical surveillance suggests investigation is needed.

8. This guidance builds on that issued by the Department of Health in England in August 2010 and additionally on 6th February 2012. Similar advice was provided by the Devolved Administrations. The Department seeks to provide pragmatic guidance on how to control the risk from P. aeruginosa in water systems. This guidance will be subject to review as and when new evidence becomes available.

9. This guidance takes account of recent advice from the Department of Health Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection (ARHAI) and the Health Protection Agency (HPA).

10. This guidance does not cover sampling for Legionella, as this is covered elsewhere (see HTM 04-01).

11. An addendum to Health Technical Memorandum (HTM) 04-01 “ The control of Legionella, hygiene, “ safe” hot water, cold water and drinking water systems” to cover P. aeruginosa and water quality in augmented care is expected to be available by end of March 2013.

12. This guidance neither provides advice on the clinical management of patients nor guidance on the safe use of water outside of augmented care, e.g. for care of leg ulcers and in hydrotherapy pools.

Introduction

13. Correct maintenance of hot and cold water supply systems is vital for patient safety. The continuous delivery of microbiologically safe water requires effective management and operation throughout the water supply and delivery system.

14. P. aeruginosa are opportunistic pathogens, particularly in patients who are compromised. There can be an association between the presence of P. aeruginosa in water from taps and other outlets and infection or colonisation in patients in augmented care units. Many of the risks associated with P. aeruginosa in water can be mitigated by adhering to good infection prevention and control.

15. Infection prevention and control teams should:
   • Ensure application of and compliance with the National evidence-based guidelines for preventing healthcare-associated infections in NHS hospitals in England
   • Ensure best practice advice relating to hand wash stations is followed to minimise the risk of P. aeruginosa contamination (see paragraph 16).
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Continue to monitor clinical isolates of *P. aeruginosa* as an alert organism and be aware of possible outbreaks of infection with this micro-organism

**Best practice advice relating to hand wash stations**

16. Only use the hand wash station for hand washing
   
   i. Do not dispose of body fluids at the wash-hand basin – use the dirty utility area.
   
   ii. Do not wash any patient equipment in wash-hand basins.
   
   iii. Do not use wash-hand basins for storing used equipment awaiting decontamination.
   
   iv. Taps should be cleaned before the rest of the handbasin as set out the NHS Cleaning Manual.
   
   v. Wash patients, including neonates, on augmented care units with water from outlets demonstrated by risk assessments and if necessary by water sampling as safe.
   
   vi. Do not dispose of used environmental cleaning fluids at wash-hand basins.

17. Flush **all** taps that are used infrequently on augmented care units regularly (at least daily in the morning for 1 min) (manually) (HTM 04-01 Part B chapter 5, paragraph 5.12). Some taps can be programmed to flush automatically; such flushing will be recorded on the building management system. Keep a record of when they were flushed (HTM 04-01 Part B chapter 5).

18. Identify any problems or concerns relating to safety, maintenance and cleanliness of hand wash stations to the Infection Prevention & Control Team and the Estates and Facilities Department. Unresolved issues should be escalated to the Infection Prevention Control Team/Committee as appropriate.

19. Use pre-filled single-use bottles for alcohol based handrubs or cleaning solutions. Do not top-up cleaning spray, alcohol or other containers.

**Risk Assessment and Management Approach**

20. A risk assessment should be undertaken to identify actions to mitigate risks and ensure appropriate sampling, monitoring and clinical surveillance arrangements are being implemented and adhered to. The initial risk assessment should be undertaken by June 2012 with appropriate water sampling where required by the end of 2012.

21. To assist with understanding and mitigating risks associated with bacterial contamination of water distribution and supply systems, it is recommended that organisations should develop a Water Safety Plan (WSP) which provides a risk-management approach to the microbiological safety of water and establish good practices in local water distribution and supply. Those organisations with existing robust water management policies for *Legionella* will already have in place much of the integral requirements for developing a WSP.

22. The risk assessment will consider:
   
   • the susceptibility of patient groups
   
   • clinical practice and ongoing care of invasive devices
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- the cleaning of patient equipment
- engineering assessment of water systems, including correct installation, commissioning and maintenance
- sampling and monitoring programme that needs to be put in place

Who to involve in Risk Assessment and Management

23. It can be helpful to convene a multi-disciplinary group to undertake the risk assessment comprising:
- The Infection Prevention and Control Team
- Consultant Medical Microbiologist
- The Estates and Facilities Team
- Senior nurses from relevant augmented care units
- Hotel/cleaning services
- The Director of Infection Prevention and Control (DIPC.)

24. The group can be a sub-group of the organization's infection control committee or other relevant forum.

25. The risk assessment should be led by the DIPC, a consultant microbiologist or the Infection Prevention and Control Team.

26. Whoever leads the group will be responsible for ensuring it identifies microbiological hazards, assessing risks, identifies and monitors control measures, and develops incident protocols. The group will be accountable to the DIPC.

Water Safety Plans (WSP)

27. WSPs should identify potential microbiological hazards caused by Legionella, P. aeruginosa and other opportunistic pathogens, consider practical aspects and detail appropriate control measures. They are working documents that are kept up to date and reviewed annually and when there are major changes to water supplies and uses. If a WSP is developed, it should be led by the Responsible Person (Water) who will be responsible for ensuring it is implemented.

28. Development of the WSP will complement the existing Operational Management requirements of HTM 04-01 and the work that has to be undertaken to fulfill the statutory requirement for a Legionella risk assessment and written scheme for the control and management of Legionella. Those organisations with existing robust water management policies will already have in place much of the integral requirements for developing a WSP.

29. A WSP should identify units with at risk patients and include:
- Risk assessment to identify those units where patients are at significant risk from water-borne P. aeruginosa contamination associated with the water supply system.
- Engineering assessment of the water system, including correct installation, commissioning and maintenance.
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- Operational monitoring of control measures.
- Links to clinical surveillance which can offer an early warning regarding microbiological safety.
- Plans for the sampling and microbiological testing in identified at risk units (see Annex 1 and 2).
- Changes to the water system to remedy high counts for *P. aeruginosa*, and other opportunistic pathogens where appropriate.
- Adjustments to clinical practice until remedial actions have been demonstrated to be effective.
- Cleaning/descaling or replacement of the water outlets/shower heads where there may be direct or indirect water contact with patients (see HTM 04-01).
- Annual review, including the inclusion of new builds, refurbishments and recently decommissioned clinical departments or units.
- Documentation and record keeping (best practice examples of the types of documentation and record keeping required are given in HTM 04-01).
- To review the results of any water testing regimen undertaken.

30. The multidisciplinary group that developed the WSP also has a role in advising should one or more outlets be found to be contaminated. These should be based on the risks to patients (see paragraphs 49-52).
**P. aeruginosa biofilms in water supply systems**

31. *P. aeruginosa* is usually present in biofilms (mixed populations of bacteria adherent to specific surfaces within the water storage, distribution and delivery systems), although they may also be present in low numbers on occasion in the water supplied to the hospital. Biofilms will be present on the plumbing materials in the water system but in most cases, *P. aeruginosa* will be concentrated within 2 metres of the point of water delivery at the outlet, i.e. after the water has left the circulation system.

32. Whilst the majority of bacteria are trapped within a biofilm, the biofilm will constantly generate bacteria that are released as free-floating individual cells (“planktonic” forms) and parts of the biofilm may slough off in clumps. The concentration of these planktonic bacteria will build up over time in the water adjacent to a biofilm when the water is static, but will be diluted as water is used and flows through the pipework or tap containing the biofilm.

33. During cleaning, there is a risk of contaminating tap outlets with microorganisms, if the same cloth is used to clean the bowl of the handbasin before the tap. These bacteria may be of patient origin, so it is possible that bacteria, including antibiotic resistant organisms, could seed the outlet, become resident in any biofilm and have the potential to be transmitted to other patients.

**Water Sampling and Testing**

34. The **sampling protocol** (Annex 1) is intended to help healthcare providers establish if the water from taps in at-risk units is contaminated with *P. aeruginosa* and, if it is, to help locate its origin and to monitor the efficacy of remedial measures.

35. The same water outlet¹ can give very different results if sampled at times of normal use and may be negative if water from the tap is used before a sample is collected.

36. To maximise the recovery of these free floating planktonic bacteria it is essential that water samples are taken:
   a) during a time of (preferably) no use (at least 2 hours or preferably longer); or
   b) low use

   The first water to be delivered from the outlet should be collected to assess the microbial contamination in the outlet.

37. In order to be able to carry out the appropriate microbiological examinations on a sample and provide a meaningful interpretation of test results, it is essential that samples are

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¹ In this document, “water outlet/outlet” is generally used in the context of a tap. The guidance given to other outlets such as showerheads and other water outlets, which may be used in augmented care units.
Water sources and potential Pseudomonas aeruginosa infection of taps and water systems collected in a suitable manner using the correct equipment and that the sampling protocol (Annex 1) is adhered to.

Protocols for microbiological examination of samples are at Annex 2

Where to sample water outlets

38. The water outlets to be sampled should be those that supply water that has direct contact with patients, used to wash staff hands or used to clean equipment that will have contact with patients as determined by local risk assessment.

When and How to Sample Water Outlets

39. All such outlets should be sampled to provide an initial assessment of contamination levels. There is no necessity to sample all taps that are due to be sampled on the same occasion; samples can be taken in batches on separate occasions. It may assist the receiving laboratory if the sampling schedule is agreed beforehand.

Interpretation of pre- or post-flush water sample test results

40. The range of levels of *P. aeruginosa* which may be found in water samples are outlined in Table 1 together with the actions which should be taken.

<table>
<thead>
<tr>
<th>Hazard</th>
<th>CFU in 100ml</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0</td>
<td>Satisfactory</td>
</tr>
<tr>
<td></td>
<td>1 – 10</td>
<td>Retest and refer back to those responsible for the WSP to determine what actions may be required.</td>
</tr>
<tr>
<td></td>
<td>&gt;10</td>
<td>Investigate cause and put corrective actions in place</td>
</tr>
</tbody>
</table>

NB: This table will be reviewed during the review of HTM 04-01.

41. Experience to date has shown no meaningful correlation between the presence and level of *P. aeruginosa* and the level of total viable count of bacteria.

42. If water sample test results are satisfactory (0 cfu per 100ml) there is no need to repeat such sampling for a period of 6 months unless there are changes in the water distribution and delivery systems components or system configuration (e.g. refurbishments that could lead to the creation of dead legs). Water sampling could be undertaken earlier than at 6 months if there are clinical suspicions that the water may be linked with patient colonization or infection.
If water sample tests show counts between 1-10 cfu per 100ml then refer to those responsible for the water safety plan who would risk assess the use of water in the unit. Simultaneously, retesting of the water outlet should be undertaken.

If water sample test results are not satisfactory (> 10 cfu per 100ml), further sampling, along with a survey of the water system, could be used to identify problems areas and modifications that may be implemented to improve water quality.

After such interventions, the water should be re-sampled after three weeks (to allow possible biofilm to re-establish). If this sampling shows satisfactory results, no further sampling is necessary for six months unless indicated by patient colonisation or infection.

**Interpretation of results**

Considering the variation in counts that can occur depending on the type of sample collected for augmented care units/high risk areas then a threshold level of <1 cfu *P.aeruginosa* per 100ml of water would be appropriate (see Table 1) and represents the possibility of the presence of biofilm.

High counts in preflush samples but with low or zero counts post flush would indicate that areas at or near the outlets are the source of contamination. In addition a few positive outlets where the majority of outlets are negative would also indicate that the individual outlets are contaminated.

If the samples indicate that the circulating water is the problem, then most outlets would possibly be positive and other points in the water system could then be sampled to assess the extent of the problem.

<table>
<thead>
<tr>
<th>Interpretation of pre and post flush counts</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>High <em>P.aeruginosa</em> count pre-flush (&gt;10cfu/100ml) and low post flush count (&lt;10cfu/100ml)</td>
<td>Suggestive of a local outlet problem</td>
</tr>
<tr>
<td>High <em>P.aeruginosa</em> count preflush (&gt;10cfu/100ml) and high post flush count (&gt;10cfu/100ml)</td>
<td>Suggestive of a systemic problem</td>
</tr>
</tbody>
</table>
What to do in the event of a *P. aeruginosa* in water contamination problem in units with at risk patients

49. Should the risk assessment or sampling measures identify contamination with *P. aeruginosa*, the following risk reduction and preventive measures should be considered:

50. Protecting the patient

i. Use water of a known satisfactory quality (Table 1) (sterile, filtered or a source shown to be free of *P. aeruginosa*), for direct contact with patients.

ii. Review water outlets/showers where there may be direct or non direct contact with patients.

iii. For patient hygiene also consider using single-use wipes. Sterile or filtered water can be used for “top & tailing” neonates.

iv. Supplement hand washing with the use of alcohol hand rub

v. Rigorous attention should be given to standard infection control practices

vi. Review cleaning of patient contact equipment e.g. incubators, humidifiers, nebulisers and respiratory equipment. Options would be to:
   a) use single use equipment,
   b) if locally reprocessed, even if used on the same patient, clean equipment with water of a known satisfactory quality (e.g. sterile, filtered or a source shown to be free of *P. aeruginosa*),
   c) use single-use detergent wipes for cleaning incubators. If a disinfectant is used, it is important that will not cause damage to the material of the incubator. Manufacturers instructions should be followed. Disinfectants should not be used to clean incubators while occupied.

vii. All other uses of water on an affected unit should be considered in the risk assessment and risk reduction plans e.g. the use of ice machines, wet shaving of male patients with a central venous catheter inserted into the jugular vein and washing patients with indwelling devices.

viii. All reusable patient equipment should be stored clean, dry and away from potential splashing with water.

51. Remediation of water quality delivery

i. Check for underused outlets - assess frequency of usage and if necessary remove under used outlet(s). The branch hot and cold water pipes should also be removed back to the main distribution pipework in order to eliminate blind ends.

ii. Assess the water distribution system for non-metallic materials such as flexible hoses and replace as in safety alert (DH (2010) 03 : Flexible water supply hoses).
iii. Assess the water system for blind ends (section of plumbing pipe that have previously been capped off but not removed) and dead legs (sections of stagnant water in pipes in underused outlets, e.g. where water is supplied to both the cold water outlet and to a thermostatic mixing valve (TMV) supplying an adjacent blended water outlet. Such cold water outlets in augmented care units are commonly underused).

iv. Point of use filters, where they can be fitted, may be used to provide water of a satisfactory standard, 0 cfu per 100ml (Table 1). Where fitted, filters should primarily be regarded as a temporary measure until a permanent safe engineering solution is developed, although long term use of such filters may be required in some cases.

v. Where point of use filters are fitted to taps, the same cleaning regimen applies to the wash-hand basin but the filter itself should be cleaned according to the manufacturer’s instructions. Care should be taken to avoid contaminating the external surface of the filter.

vi. It may be necessary to carry out a disinfection of the hot and cold water distribution systems that supply the unit to ensure that contaminated outlets are treated. Guidance on how to carry out the disinfection procedure can be found in HTM 04-01 (Part A Chapter 17). However, this may not be effective against established biofilms.

vii. If the TMV is not integral to the body of the tap/shower ensure that the TMV providing the “safe” hot water is located as close to the tap/shower outlet as possible.

viii. Consider replacing contaminated taps with new taps, however, there is currently a lack of scientific evidence to suggest that this will provide a long term solution. Consider fitting taps that can be readily dismantled for ease of use of cleaning and disinfection. In line with HTM 04-01 Part A (Chapter 9, 9.54) thermostatic mixer valves are required to be fitted where there is a risk of patient scalding.

52. Further advice on the management of *P. aeruginosa* contamination in water systems can be sought from the Health Protection Agency via Regional Microbiologists or Health Protection Units.
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Annexes 1 & 2 have been developed to provide technical guidance for a range of laboratories, including NHS, HPA Food, Water and Environment and commercial laboratories that have the capability and capacity to undertake water sampling and testing.
Annex 1 - Water Sampling Procedure

Water sampling procedure

1.1 This sampling and testing protocol provides provisional guidance until the addendum to HTM 04-01 is produced (March 2013).

1.2 Sampling should be undertaken by staff trained in the appropriate technique for taking water samples, particularly in the method outlined in this guidance, which may differ from the collection of water samples for other purposes, e.g. for sampling Legionella, and the use of aseptic technique to avoid extraneous contamination.

1.3 Samples should be carefully labeled such that the outlet can be clearly identified; diagrammatic maps indicating each numbered outlet to be sampled can be helpful in this respect.

1.4 Sampling should take place during a period of no use (at least 2 hours or preferably longer) of that outlet or, if that is not possible, during a time of its lowest usage. This will normally mean sampling in the early morning, though a variety of use patterns may need to be taken into account.

1.5 Disinfectants in the water, such as chlorine or chlorine dioxide, will have residual activity after taking the sample and may inactivate bacteria in the sample prior to its processing. To preserve the microbial content of the sample the disinfectant should be neutralised as the sample is taken in order to assess the microbial content of the water at the time of sampling.

1.6 Neutralisers should be present in the sterile sampling containers and where appropriate, advice on these can be obtained from the testing laboratory prior to sampling. The most commonly used neutraliser, which is appropriate for chlorinated or brominated water systems and those using ozone or hydrogen peroxide, is sodium thiosulphate. If these disinfectants are to be neutralised, 18 mg/L sodium thiosulphate equating to 18mg/l in the final sample should be in each sampling bottle (sterile bottles are normally purchased containing the neutralizer). EDTA may be used as a neutralizer for systems treated with copper and silver ions (BS 7592: 2008). Where disinfectants are being applied to the water system advice should be taken as to the appropriate neutralisers to use.

1.7 The tap should not be disinfected by heat or chemicals before sampling, nor should it be cleaned immediately before sampling.

1.8 Label a sterile collection vessel (500 – 1,000ml volume) containing a suitable neutraliser for any biocide the water may contain. The labeling information should contain details of the tap location, sender’s reference, pre or post flush (see later), person sampling, date and time of sampling.
The main strategy for sampling is to take the first sample of water ("pre-flush") delivered from a tap at a time of no use (at least 2 hours or preferably longer) or low use. If *P. aeruginosa* has been found in a pre-flush, a second paired set of samples should be taken. The first would be a pre-flush sample as before. The tap should then be run for two minutes (or more if the flow rate is particularly low) and a second identical post-flush taken. Bacteria in this second sample (termed "post-flush") are more likely to originate further back in the water system. A substantially higher bacterial count in the pre-flush sample, compared to the post-flush, should direct remedial measures towards the tap, associated pipework and fittings near to that outlet. A similar bacterial count in pre-flush and post-flush samples indicates that attention should focus on the whole water supply, storage and distribution system and more extensive sampling regimen should be considered throughout the water distribution system, particularly if that result is obtained from a number of outlets.

Whilst water sampling is the principal means of sampling there may be occasions when water samples cannot be obtained immediately for analysis. In the event of a suspected outbreak, swabbing water outlets to obtain strains for typing may provide a means of assessing a water outlet, but this does not replace water sampling.

**Obtaining the samples**

1.12 **Pre-flush sample:** Aseptically (i.e. without touching the screw thread, inside of the cap or inside of the collection vessel) hold the container under tap and collect approximately the first 450 – 500 ml water into a sterile water container containing neutraliser. Replace the cap and invert or shake to mix the neutraliser with the collected water.

1.13 Dependent upon the water distribution system design, and the type of tap/water outlet, the water feed to the outlet may be provided by:

   a) a separate cold water supply and hot water supply (which may, or may not have its final temperature controlled by the use of an integral thermostatic mixing valve within the outlet), or

   b) a separate cold water and a pre-blended hot water supply that has had its temperature reduced by a thermostatic mixing valve prior to delivery to the outlet.

1.14 For either system it is recommended that the container is filled with half the sample by running the cold water into the container first. The rest of the sample should then be collected from the hot or blended outlet. (It is recommended to run the hot/blended and cold separately as there are occasions when if both are opened simultaneously the flow will drop off on one side and that water will not be sampled).

1.15 **Post-flush sample:** where this is required, allow the water to flow from the tap for 2 minutes (see above) before collecting 450 – 500 ml water into a sterile water container containing neutraliser. Replace the cap and invert or shake to mix the neutraliser with the collected water. This sample, when taken together with the pre-flush sample, will indicate whether the tap outlet and its associated components is contaminated or if the contamination is remote from the point of delivery (see Table 2).
1.16 If a sample from a shower is required then place a sterile bag over the outlet, and using sterile scissors cut a small section of the corner and collect the sample as above.

1.17 The collected water should be processed within 2 hours or refrigerated within 2 hours at 2-8°C and processed within 24 hours. Transport may be aided by the use of a temperature controlled box.

1.18 Swabbing: Use the sterile swab to take a sample of the tap’s aerator/flow-straightener (the device immediately inside the spout) and spout’s metal collar. Place the swab in transport medium or maximum recovery diluents and send to the laboratory.
Annex 2 - Microbiological examination of water samples for *P. aeruginosa*

**Definition**

2.1 *P. aeruginosa* are Gram-negative, oxidase-positive bacteria which, in the context of this method, grow on selective media containing cetrimide (cetyl trimethylammonium bromide), usually produce pyocyanin, fluoresce under UV light 360 ± 20 nm, hydrolyse casein.

**Sampling principle**

2.2 A measured volume of the sample or a dilution of the sample is filtered through a membrane filter (0.45 microns or smaller) to retain bacteria and the filter is then placed on a solid selective and differential medium (commercially available).

2.3 The medium (CN agar) is made selective by the addition of cetyl trimethylammonium bromide and nalidixic acid to levels that will inhibit the growth of bacteria other than *P. aeruginosa*. Other selective agars are available and acceptable if validated.

2.4 The membrane is incubated on a selective/differential agar and characteristic colonies are counted. Confirmatory tests are carried out where necessary (see 2.16) and the result is calculated as the colony count per 100 ml waters.

2.5 *P. aeruginosa* usually produces characteristic blue-green or brown coloured colonies when incubated at 37°C for up to 48 hours (indicative growth may exist after 18 hours). Confirmation of isolates is by sub-culture to milk agar supplemented with cetyl trimethylammonium bromide (commercially available) to demonstrate hydrolysis of casein.

**Sample preparation and dilutions**

2.6 Water samples should be received and handled as described in: Standing Committee of Analysts. The Microbiology of Drinking Water (2002) - Part 3 - Practices and procedures for laboratories. Environment Agency). For example samples should be examined as soon as is practicable on the day of collection. In exceptional circumstances, if there is a delay, store at 2-8°C and not exceed 24 hours before the commencement of analysis.

**Filtration and incubation**

2.7 Aseptically measure and dispense 100ml of test solution into the sterile filter-holder funnel. If the funnel is graduated to indicate volume, this can also serve to measure the volume.
2.8 If high counts are expected, 100ml of a 10 or 1% dilution (i.e. a 1 in 10 dilution) of the original solution diluted in sterile distilled water, maximum recovery diluent (MRD) or other suitable diluent can be filtered in parallel. Instead of a 10% dilution, 10ml of the neat solution could be filtered.

2.10 Draw the test solution through the filter by application of negative pressure on the filtrate collection vessel.

2.11 Aseptically place the membrane onto the Pseudomonas selective agar and incubate aerobically at 37°C.

**Preparation of Swabs**

2.12 In the laboratory, the swab should be used to inoculate a portion of an agar plate selective and differential for *P. aeruginosa* (see above) and that inoculum streaked on the plate as for a clinical sample. Incubate as described for filter samples above. Alternatively after sampling, the swab could be placed in 10ml MRD containing neutraliser, vortexed then plated out, using serial dilution, on the appropriate media and incubated as above.

**Counting of Colonies**

2.13 Examine plates after 22 hours ± 4 hours and 44 hours ± 4 hours incubation.

2.14 Count all colonies that produce a green/blue (demonstrating pyocyanin production), or reddish brown pigment and those which fluoresce with UV light (optional). Exposure of colonies to daylight for 2-4 hours enhances pigment production. When there is a moderately heavy growth of *P. aeruginosa* and other organisms on the membrane, colonies adjacent to pyocyanin producing colonies of *P. aeruginosa* can also appear green after 44 hours ± 4 hours incubation making the interpretation of the count difficult. Observing the plates after 22 hours ± 4 hours assists in the interpretation in these instances. If in doubt, all green colonies can be confirmed.

**Confirmatory Tests**

2.15 Colonies that clearly produce pyocyanin (green/blue pigmented) on the membrane are considered to be *P. aeruginosa* and require no further testing. Other colonies which fluoresce or are red/brown require confirmation. If more than one volume or dilution has been filtered, proceed if possible with the membrane yielding 20 - 80 colonies.

2.16 To confirm other colonies, sub-culture from the membrane onto a milk cetrimide agar (MCA) plate and incubate at 37°C for 22 hours ± 4 hours. Examine the plates for growth, pigment, fluorescence and casein hydrolysis (clearing medium’s opacity around the colonies). If pigment production is poor, expose the MCA to daylight at room temperature for 2 - 4 hours to enhance pigment production and re-examine.

2.17 Perform an oxidase test using colonies from the MCA plate. A positive reaction is indicated by the appearance of a dark purple colour within 10 seconds. No colour change or a purplish colour which develops later are both negative reactions.
2.18 *P. aeruginosa* is oxidase positive, hydrolyses casein and produces pyocyanin and/or fluorescence. Occasionally atypical non-pigmented variants of *P. aeruginosa* occur. A pyocyanin negative, casein hydrolysis positive, fluorescence positive culture shall be regarded as *P. aeruginosa*. Additional tests may be necessary to differentiate non-pigmented *P. aeruginosa* from *P. fluorescens* (such as growth at 42°C or resistance to C-390, 9-chloro-9-(4-diethylaminophenyl)-10-phenylacridan or phenanthroline or more extensive biochemical tests.

### Table 3

<table>
<thead>
<tr>
<th>Colony on CN agar</th>
<th>Oxidase test</th>
<th>Fluorescing on MCA</th>
<th>Caseinolytic on MCA</th>
<th>Confirmed <em>P. aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue or Green</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>Yes</td>
</tr>
<tr>
<td>Fluorescing and not pigmented</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Yes</td>
</tr>
<tr>
<td>Reddish brown non fluorescing</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Yes</td>
</tr>
</tbody>
</table>

NT – Not tested

**Retention of *P. aeruginosa* strains**

2.19 Where an investigation into clinical infections is underway then the testing laboratory should be informed that the isolates of *P. aeruginosa* and associated sampling location information should be retained for a minimum of three months as they may be required for typing at a later date.

2.20 It will then be the responsibility of the testing laboratory to ensure that these strains are supplied to the typing laboratory (e.g. HPA Colindale) when requested and this should be written into the contract for testing.

**Calculation of results**

2.21 The results shall be expressed as the colonies of *P. aeruginosa* per 100 ml of the undiluted sample (e.g. for 100 ml sample, the count on the membrane; for 10 ml of sample, the count on the membrane multiplied by 10; for 1 ml of sample, the count on the membrane multiplied by 100).

**Reporting**

2.22 If *P. aeruginosa* is not detected, report as: ‘Not detected in 100 ml’.

2.23 If the test organism is present, report as the number of confirmed *P. aeruginosa* per 100 ml.

2.24 The sample reference originally submitted should be reported with each result.
Water sources and potential Pseudomonas aeruginosa infection of taps and water systems

Microbiological typing

2.25 Environmental and water samples being sent to the HPA Laboratory of Healthcare Associated Infection (LHCAI) for molecular analysis of *P. aeruginosa* should only be referred if the isolates have been confirmed to be *P. aeruginosa* and if there is a possible epidemiological link to the outbreak strain under investigation.

2.26 Referrals of *P. aeruginosa* isolates for typing should only be sent after consultation with the typing laboratory.

2.27 Where many taps are positive for *P. aeruginosa* send one colony of the *P. aeruginosa* from each water sample. Save the primary isolation plate for possible further examination once the results of typing are known and have been discussed with the typing laboratory.

2.28 If only two or three taps are positive for *P. aeruginosa*, then send two separate colony picks of confirmed *P. aeruginosa* from the primary plate per water sample to LHCAI (taking the stipulations in para 2.25 into account). Please label these clearly as being from the same water sample. This is so that LHCAI can accumulate data for you on how common mixed strains are seen in the same tap water.

2.29 It is important that the request forms have information about the links between tap water and cases as illustrated in the following examples.

   a. Water from tap in room “A” ref patient “X”

   b. Water from tap in sluice room.

   c. Tap water from room “C” with no cases.
Transport to laboratory at 2°C – 8°C out of direct sunlight in suitable containers

Store at 2°C – 8°C in the dark and examine on the day of collection if possible otherwise within 24 hours of collection

Mix sample well and make any necessary dilutions

Filter

Place membrane on Pseudomonas agar containing cetrimide and nalidixic acid

Incubate at 37°C for 22 hours ± 2 hours. Count green or blue colonies and re-incubate for a further 22 hours ± 2 hours

Count colonies that produce a green, blue or reddish brown pigment and those which fluoresce under the ultra violet lamp

Sub-culture non-pyocyanin producing (blue/green) colonies to MCA. Incubate at 37°C for 22 ± 2 hours. Examine for growth, casein hydrolysis and pigment production and/or fluorescence under UV light. (optional). Perform an oxidase test on non-pyocyanin producing colonies

Calculate confirmed count for *P. aeruginosa*
Bibliography


Health Technical Memorandum 04-01 – The control of Legionella, hygiene, “safe” hot water, cold water and drinking water systems’. 2006


Water sources and potential Pseudomonas aeruginosa infection of taps and water systems


Willis C, D Lamph, K Nye, E Youngs, H Aird, A Fox and S Surman-Lee. 2010. Guidelines for the Collection, Microbiological Examination and Interpretation of Results from Food, Water and Environmental Samples taken from the Healthcare Environment. Available from the HPA.