

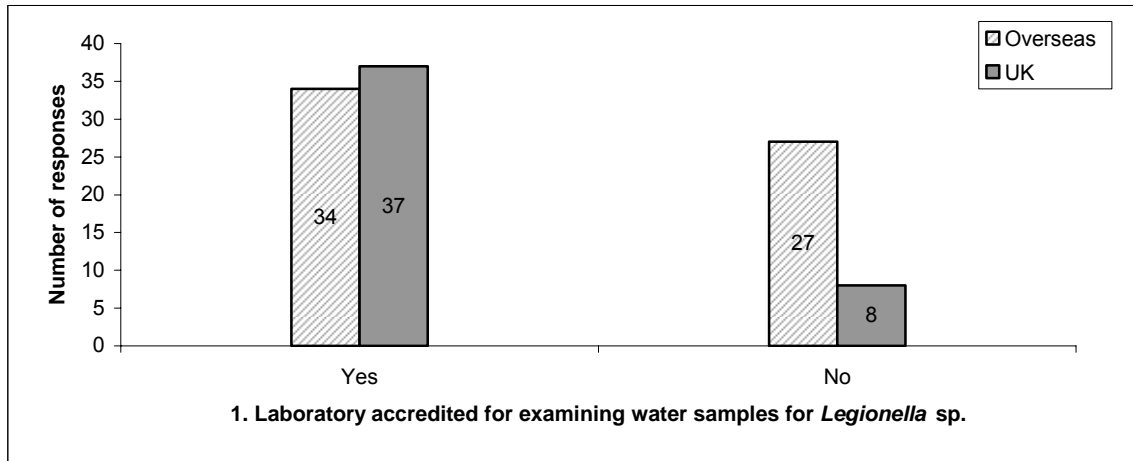
## HPA Water EQA Scheme for Legionella Isolation Participant Method Questionnaire

### Isolation of *Legionella* spp. from Environmental Waters

1. Is your laboratory accredited for examining water samples for *Legionella* spp.? **Total response = 106**

Yes  71/106 (67%)

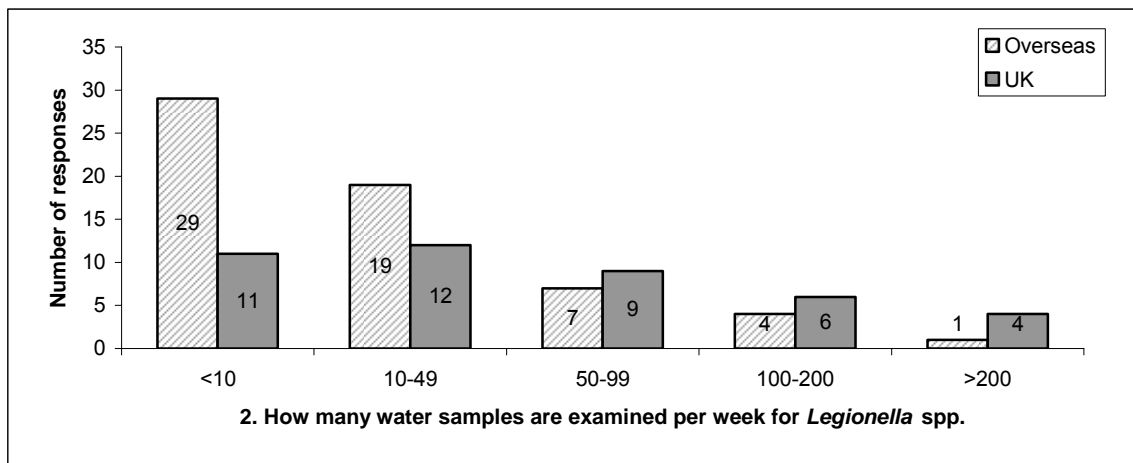
No  35/106 (33%)



*if yes please give the name of your accreditation body*

|   |           |
|---|-----------|
| AZLA - The American Association for Laboratory Accreditation                | 1         |
| BELAC – Belgian Accreditation Structure                                     | 1         |
| CPA – Clinical Pathology Accreditation                                      | 2         |
| DANAK – Danish National Body for Accreditation                              | 3         |
| Duetsche Accreditationing Gesellschaft                                      | 1         |
| ENAC (Entidad Nacional de Acreditacion) Espana                              | 2         |
| Federal ministry of economics and labour Austria                            | 2         |
| Final state of UKAS accreditation   | 1         |
| Hellenic Accreditation System ESYD  | 1         |
| INAB – Irish National Accreditation Body                                    | 1         |
| Irish national accreditation board  | 1         |
| Metas – Federal office of metrology   | 1         |
| Not yet (but fall 2007)   | 1         |
| SAC – SINGLAS – Singapore Laboratory Accreditation Scheme                   | 2         |
| Sinal – National body for the accreditation of testing Laboratories         | 3         |
| Singapore Accreditation council - Singapore laboratory accreditation scheme | 1         |
| SQS, Swiss association for quality and management systems                   | 1         |
| Swedac  | 2         |
| Swiss accreditation service (SAS)   | 1         |
| The Czech accreditation institute   | 1         |
| UKAS  | 38        |
| ZLGI Germany  | 1         |
| Not recorded  | 3         |
| <b>Total</b>  | <b>71</b> |

2. How many water samples do you examine per week for *Legionella* spp.? **Total response = 102**



Less than 10

40/102 (39%)

10-49

31/102 (30%)

50-99

16/102 (16%)

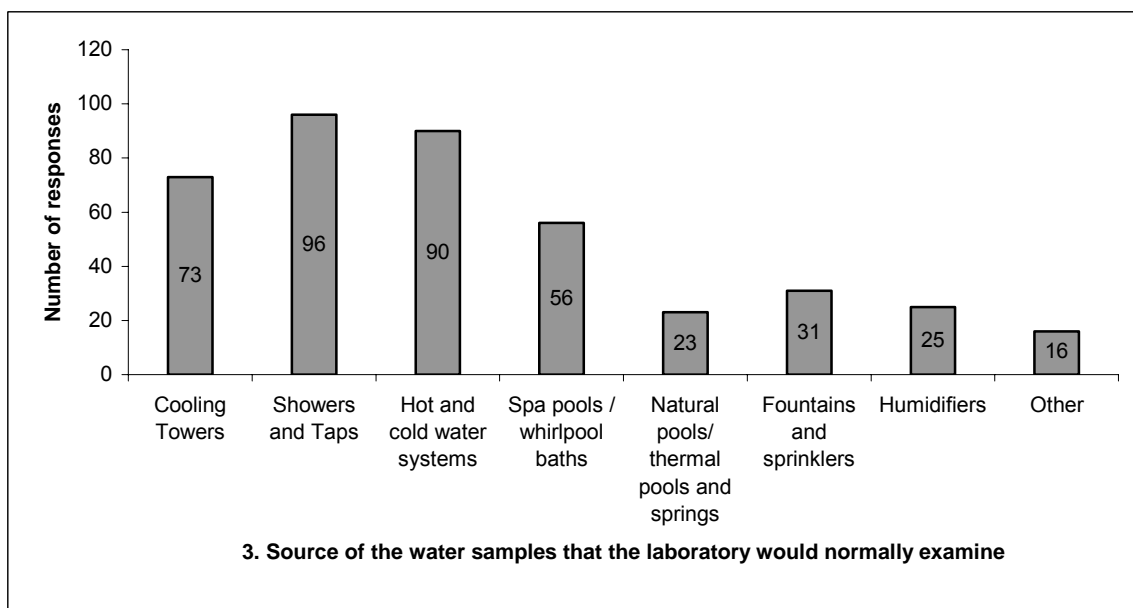
100-200

10/102 (10%)

More than 200

5/102 (5%)

3. What is the source of the water samples that you normally examine? **Total response = 104**  
please tick all that apply



Cooling towers

73

Showers and taps

96

Hot and cold water systems (including air-conditioning units)

90

Spa pools / whirlpool baths

56

Natural pools / thermal pools and springs

23

Fountains and sprinklers

31

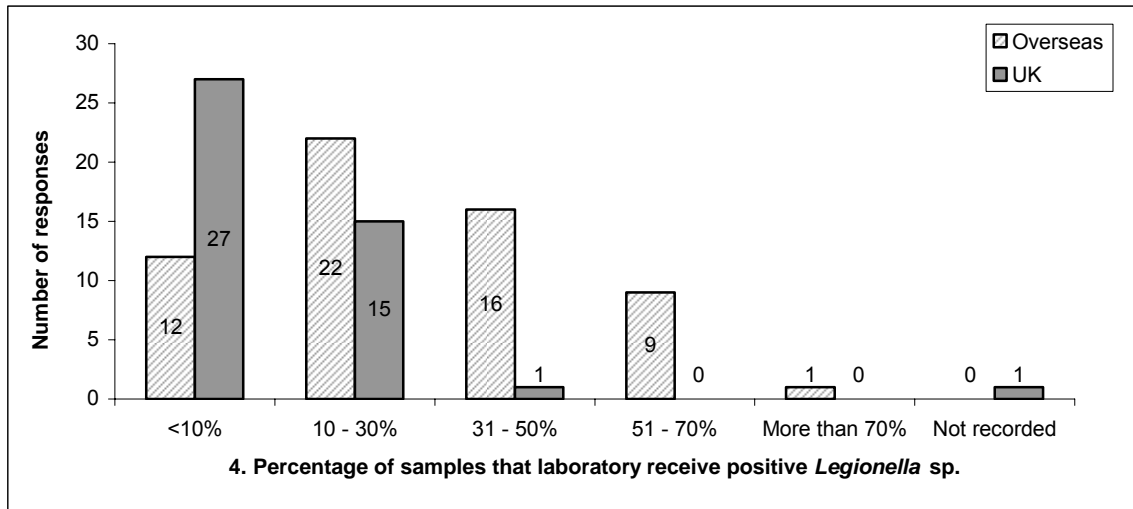
Humidifiers

25

Other please give details below

|   |   |
|---|---|
| Biofilm   | 1 |
| Swabs   | 2 |
| Scrubbers   | 1 |
| Biological Filters  | 1 |
| Industrial Water  | 1 |
| Dental unit water system                                    | 1 |
| Garden Ponds  | 1 |
| Piping  | 1 |
| Gas rigs – storage tanks                                    | 1 |
| Industrial devices  | 1 |
| Swimming Pool   | 1 |
| Turkish Bath  | 1 |
| Reservoir   | 1 |
| Water supplies for dialysis units and home dialysis systems | 1 |
| Disinfected plumbing systems                                | 1 |

4. What percentage of the samples that you receive are positive for *Legionella* spp.? **Total response = 104**



- |               |                                       |
|---------------|---------------------------------------|
| Less than 10% | <input type="checkbox"/> 39/104 (38%) |
| 10-30%        | <input type="checkbox"/> 37/104 (36%) |
| 31-50%        | <input type="checkbox"/> 17/104 (16%) |
| 51-70%        | <input type="checkbox"/> 9/104 (8%)   |
| More than 70% | <input type="checkbox"/> 1/104 (1%)   |
| Not recorded  | <input type="checkbox"/> 1/104 (1%)   |

5. Please indicate below which type of water samples usually give positive results for *Legionella* spp. in your laboratory (for example, water from spa pools, water from cooling towers etc.) **Total response = 92**

|                           |    |
|---------------------------|----|
| Cooling towers            | 29 |
| Hot water system          | 17 |
| Domestic water system     | 2  |
| Hospital                  | 5  |
| Hot and Cold water system | 16 |
| Showers                   | 44 |

|                          |    |
|--------------------------|----|
| Taps                     | 28 |
| Spa pools                | 11 |
| Fountains and sprinklers | 1  |
| Oil Rig                  | 1  |
| Swimming pool            | 1  |
| Bath Water               | 1  |
| Portable hot water taps  | 1  |
| Nursing home             | 1  |
| Whirlpool baths          | 1  |
| Turkish Bath             | 1  |
| Swabs                    | 1  |
| Boilers                  | 1  |
| Calorifiers              | 1  |
| Industrial devices       | 1  |

6. What is the basis of the methods that you use for isolation of *Legionella* spp. from water samples?  
please tick all that apply **Total response = 102**

|   |    |
|---|----|
| APHA Standard methods for the examination of water and wastewater, etl 20th 1998, 92605                                       | 1  |
| AFNOR: T90431.  | 1  |
| AS/NZS 3896:93  | 2  |
| AS3896: 1998  | 1  |
| centrifugation stage is omitted   | 1  |
| Doc. 4/4/2000 G.U.103 del 5/5/2000 (Gazzeita Ufficiale Repubblica Italiana)   | 1  |
| DS30292001  | 2  |
| guide for prevention legionellosis  | 1  |
| HPA National Method W12   | 21 |
| HPA standard method W13 (Issue 1)   | 1  |
| hybrid membrane filtration, resuspension in 10mL using stomacher bag, GVPC to isolate ETC.                                    | 1  |
| ISO 11731: 1998 (BS6068-4.12:1998)  | 81 |
| ISO/ IEC 17025, ISO 15189   | 1  |
| ISO1173-Part 2:2004   | 12 |
| method from Italian guideline (G.U. no 103/2000)  | 1  |
| National Guidelines (Italy)   | 1  |
| National guidelines from SMI in Stockholm   | 1  |
| other CDC methods   | 1  |
| our method is transposed from the method which is from Prof. Dr. V. L. Yu Legionella Lab in VA medical centre Pittsburgh, PA. | 1  |
| VNICHIN 1037 2002   | 1  |

7. Do you regularly test non-water samples (e.g. sediments, deposits or slimes) for *Legionella* spp.?  
**Total response = 106**

Yes  10/106 (10%)

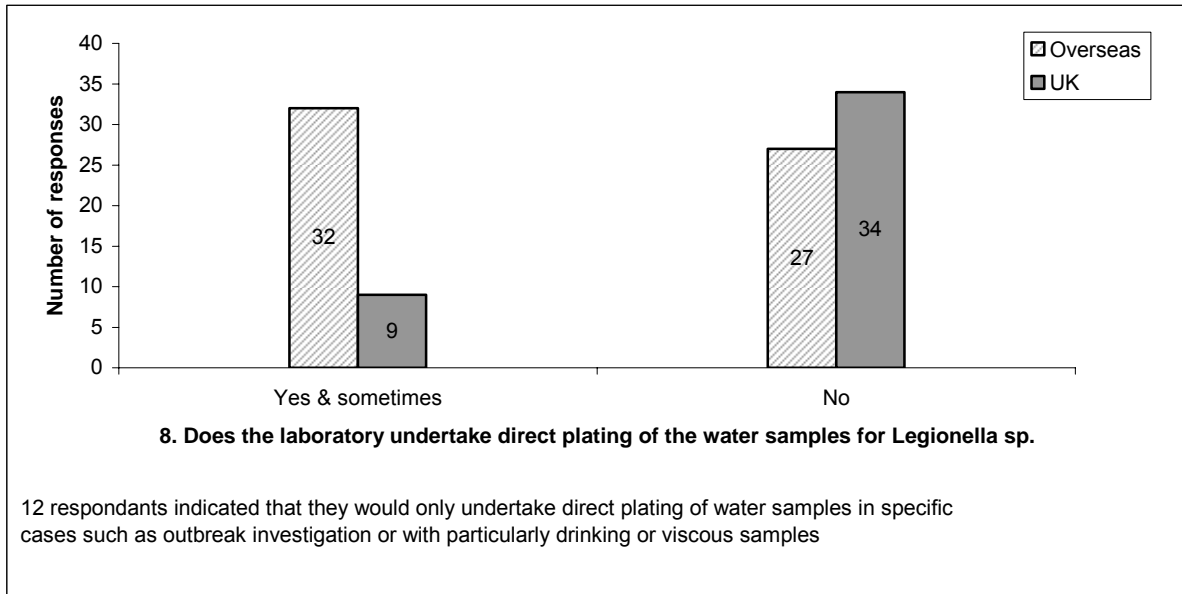
No  96/106 (90%)

*if yes please give further details below*

|  |
|--|
| Yes  |
| Yes, Bio film  |
| Yes, from shower heads and hoses - culture bio film assays with fish + culture |

|  |
|--|
| Yes, meal working fluids   |
| Yes, sediments and slimes of a cooling tower, filters and swabs of a bath water system |
| Yes, sediments from phone company manholes filters - air                               |
| Yes, some companies send us deposit material from the cooling system                   |
| Yes, swab of tank wall   |
| Yes, Swabs of shower heads   |
| Yes, Swabs of taps and showers   |

8. Do you undertake direct plating of your water samples for *Legionella* spp.? **Total response = 102**



Yes  29/102 (28%)

No  61/102 (60%)

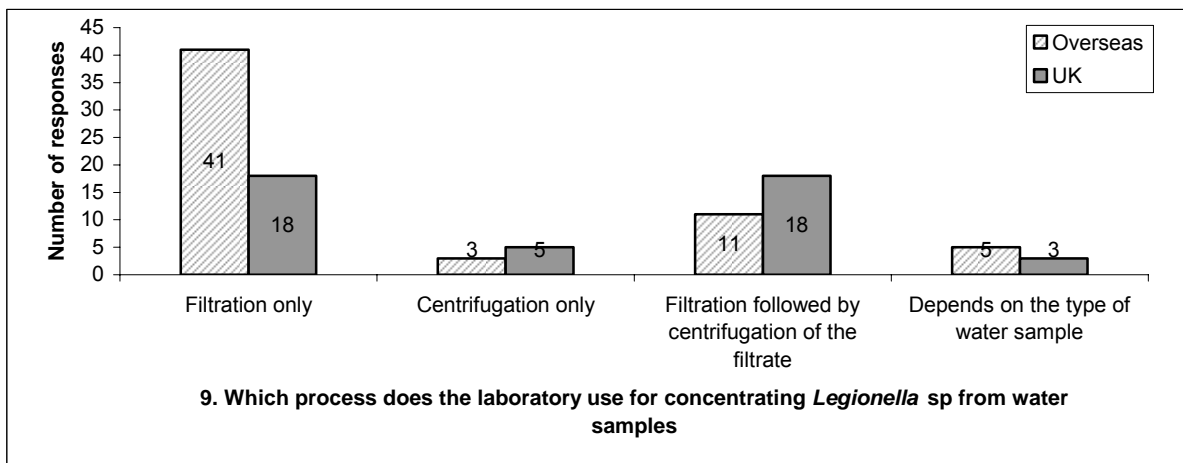
Sometimes – it depends on the type of water sample *please give details below*  12/102 (12%)

|  |
|--|
| Sometimes - it depends on the type of water sample   |
| Sometimes - it depends on the type of water sample, we use direct plating when we know the sample is positive because previously analysed and when in first instance the sample is positive  |
| Sometimes - it depends on the type of water sample, we make direct plating for example in case of samples from conditioning system   |
| Sometimes - it depends on the type of water sample, water samples (0.1mL) from cooling towers and water tanks are plated directly for <i>Legionella</i> spp. Because those kind of samples may have an adequate amount of bacteria to recover it |
| Sometimes - it depends on the type of water sample, samples with expected high counts and swabs of bio film. Some neat samples need to be pre-treated with acid combined with heat treatment followed with dilution down to 10 <sup>-6</sup> .   |
| Sometimes - it depends on the type of water sample, outbreak samples only  |

|   |
|---|
| Sometimes - it depends on the type of water sample, It is performed from: water from cooling towers, Industrial devices and swabs |
| Sometimes - it depends on the type of water sample, If there's knowledge of previous samples and results                          |
| Sometimes - it depends on the type of water sample, If the water sample is implicated in an outbreak of Legionnaires disease      |
| Sometimes - it depends on the type of water sample, if the sample is too dirty or viscous to filter we plate it directly          |
| Sometimes - it depends on the type of water sample, if AS method is used  |
| Sometimes - it depends on the type of water sample, during outbreak investigation or when previous results suggest high count     |

## Concentration of Samples

9. Which processes do you use for concentrating *Legionella* spp. from water samples? **Total response = 104**



- |   |                                       |
|---|---------------------------------------|
| Filtration only   | <input type="checkbox"/> 59/104 (57%) |
| Centrifugation only   | <input type="checkbox"/> 8/104 (7%)   |
| Filtration followed by centrifugation of the filtrate                 | <input type="checkbox"/> 29/104 (28%) |
| Depends on the type of water sample. <i>Please give details below</i> | <input type="checkbox"/> 8/104 (7%)   |

|  |
|--|
| Depends on the type of water sample  |
| Depends on the type of water sample, 1) filtration alone routinely 2) filtration + centrifugation for increased sensitivity 3) centrifugation alone when water too colloidal or turbid to allow filtration                 |
| Depends on the type of water sample, centrifugation method is used when a sample included a lot of sediments such as a cooling towers water, filtration method is used to other samples such as a bath water and tap water |
| Depends on the type of water sample, filtration if possible, if too contaminated with solid matter centrifugation is used  |
| Depends on the type of water sample, mainly direct membrane filtration acc. To 11731:2 in combination with direct planting of samples with high expected counts.   |

|   |
|---|
| Depends on the type of water sample, mainly filtration centrifugation if V. directly or lots of biosides which stops it filtering |
| Depends on the type of water sample, normally filtration but we use centrifugation for turbid samples                             |
| Depends on the type of water sample, samples that will not pass through 0.2µm filter are centrifuged                              |

## Preparation of Sample Concentrate

### Filtration

10. Do you routinely filter your water samples to concentrate the micro-organisms when examining for *Legionella* spp.? **Total response = 103**

- Yes  92/103 (89%)
- Sometimes – it depends on the type of water sample  6/103 (6%)
- No  5/103 (5%)
- If no please go to Q.16 on page 5*

11. What volume of water do you normally filter? **Total response = 98**

1 Litre  70/98 (71%)

Other  
*please give details below*

|  |                                    |
|--|------------------------------------|
| 2 litres   | <input type="checkbox"/> 3/98 (3%) |
| 100ml  | <input type="checkbox"/> 9/98 (9%) |
| 250ml  | <input type="checkbox"/> 3/98 (3%) |
| 500ml  | <input type="checkbox"/> 6/98 (6%) |
| 200mL ~ 300mL  | <input type="checkbox"/> 7/98 (7%) |
| 5 litres for tap water, 1 litre for shower heads and cooling towers  |                                    |
| 50mL. (Approx. 100mL water is sampled and 50mL is used for filtration and culture. Residual part is used for test repeats if necessary.) |                                    |
| According to 11731-2 filter volume is dependent on type of water e.g. shower 250 acid; 100+acid, 10mL                                    |                                    |
| Between 0.01L, depending on the type of water sample   |                                    |
| Normally we filter 10 and 100mL of sample  |                                    |
| Preferably 1 litre but may be a lot less   |                                    |

12. What is the pore size of membrane that you use for filtering your samples? **Total response = 98**

- 0.2 µm  68
- 0.45 µm  32
- Other  
*please give details below*

3  3

956;m

13. Do you treat the filtrate directly with acid buffer? **Total response = 99**

Yes  29/99 (29%)

No  65/99 (66%)

*please give details below*

|   |
|---|
| Yes = 14  |
| Yes, 1:1 mix filtration + acid buffer, leave 5 minutes, inoculate plates with 0.5mL       |
| Yes, 1mL aliquot, centrifuge, remove 0.5mL, add 0.5mL buffer 5 min, plate                 |
| Yes, 1mL concentrate + 1mL acid buffer ph2.2 for 5 mins                                   |
| Yes, 20mL acid buffer 5min 10ml "acid test" (not sure what last word says can only guess) |
| Yes, After elution from filter, centrifugation, and resuspension                          |
| Yes, as per ISO document  |
| Yes, cooling towers water   |
| Yes, filtrate - 10mL pages saline. 0.2mL of suspending treated with ph2.2 buffer. (0.2mL) |
| Yes, filtrate supernatant int 3 aliquots, 1) not treated, 2) acid treated 3) heat treated |
| Yes, filter then centrifuge, deposit (aliquot) treated and acid buffer (as in W12)        |
| Yes, for samples with overgrown untreated and heat-treated plates                         |
| Yes, if the sample is contaminated  |
| Yes, KCL-HCL  |
| Yes, method of Bops. J Clin microbiol 1981, 13, 714.                                      |
| Yes directly into the funnel 5' ~ 30mL  |

Other:

|   |
|---|
| Centrifuge 5mL of filtrate, no more than 2.5mL supernatant, add 2.5mL acid buffer |
| Depends on sample type / quality  |
| Do you mean filtrate? The resuspended is split into 3 and half is acid treated    |
| Filtrate is concentrated before acid treatment                                    |
| We treat the filtrate with heat and acid and neat analysis                        |

5/99 (5%)

14. How do you wash the micro-organisms from the filter? **Total response = 96**

Not applicable because the membrane is placed directly on the plating culture medium

By cutting the filter with sterile scissors, adding 5 to 25 mL diluent to the filtrate and shaking vigorously for at least 2 mins

13/96 (13%)

By placing the membrane in a stomacher bag (or similar) with 10 mL diluent and rubbing it by hand for at least 30 s.

24/96 (25%)

By transferring the membrane to 10 mL diluent and vortexing for a minimum of 30 s.

19/96 (20%)

N/A

8/96 (8%)

Other

*please give details below*

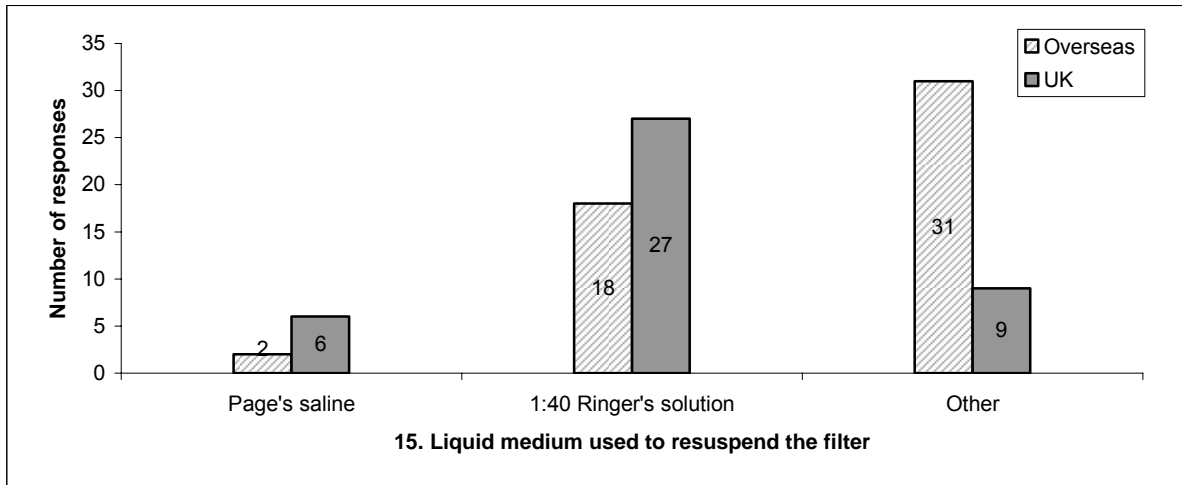
|  |
|--|
| After filtration, filter is transferred to the 5mL sterile or original water |
|--|

|  |
|--|
| and it is vigorously shaken at least 30s   |
| By filtering with phosphate buffer   |
| By placing membrane in 20mL diluent & shaking for 30secs with glass balls  |
| By placing membrane in 4mL diluent and scrapping gridded area  |
| By placing the membrane in 10mL of the water sample and remove from the filter with loop   |
| By placing the membrane in a sterile bottle (with 50mm of diameter) containing 20mL of sterile diluent.<br>The membrane is scraped with a sterile cell scraper, then vortexed for 10 mins, at 200±5 osc/min          |
| By placing the membrane in a stomacher bag (or similar) with 10mL diluent and rubbing it by hand for at least 2mins  |
| By placing the membrane in a stomacher bag (or similar) with 10mL diluent and rubbing it by hand for at least 3 minutes  |
| By scrapping the membrane in 25mL diluent with an inoculation loop (sterile one). * diluent = 25mL unfiltrate water  |
| By transferring the membrane to 10mL of the sample and scour the surface of the membrane   |
| By transferring membrane filter to 5mL sterile water then cutting the filter with sterile scissors and vortexing for at least 2 mins   |
| By transferring the membrane to 10mL diluent and by scratching the membrane with a sterile plastic loop and  |
| By transferring the membrane to 5 mL diluent and sonicating it for 8 mins  |
| By transferring the membrane to 5 mL diluent and vortexing for a minimum of 30s  |
| By transferring the membrane to 50mL centrifuge tube with screw cap, adding 5mL sterile deionised water and vortexing for 5 mins, after being vortexed for 5 min, the tube is shaken strongly by hand about 10 times |
| By transferring the membrane to a test tube with 1mL collection buffer   |
| By transferring the membrane to 10 mL diluent and vortexing for 2mins  |
| By transferring the membrane to 5-10 mL diluent placing in an ultrasound tray 5 min and vortexing for 2-5 mins   |
| By transferring the membrane to 10mL diluent and shaking for 1 hour  |
| By transferring the membrane to 10mL diluent, scraping with swab and homogenising, and then vortexing for at least 30secs  |
| By transferring the membrane to 10mL water sample, vortexing for 2mins and scraping the membrane by scalpel  |
| By transferring the membrane to a bag containing 10mL diluent, scraping with sterile pipette, then place the bag in an ultrasound tank for 2 mins and vortexing for 15 seconds                                       |
| place filter in honey jar with 20mL sterile water and glass beads<br>Shake vigorously for 2 mins   |
| Place sample in 10mL diluent and shake for 2 mins  |
| Split filter to two 5mL diluent vortex mix with beads for 15 secs  |
| The filtering 10mL water from the sample, then the filter is washed by ultrasounds 25HZ 45seconds  |
| Transfer the membrane to a 10mL diluent, rub the surface of the membrane with a swab to move the organisms to the diluent then vortex  |
| Transferring membrane to 20mL diluent and shaking for 2 minutes  |
| Transferring the membrane to 10mL of the sample and shaking (20rpm/min.)   |

□ 32/96 (33%)

|   |
|---|
| Transferring the membrane to 10 mL diluent and vortexing 5min<br>ultrasound for 10min |
| Ultrasound-base in 20mL diluent   |
| We use ultrasound - bath for 3 mins   |

15. Which liquid medium do you use to resuspend the filter? **Total response = 93**



Page's saline

8/93 (9%)

1:40 Ringer's solution

45/93 (48%)

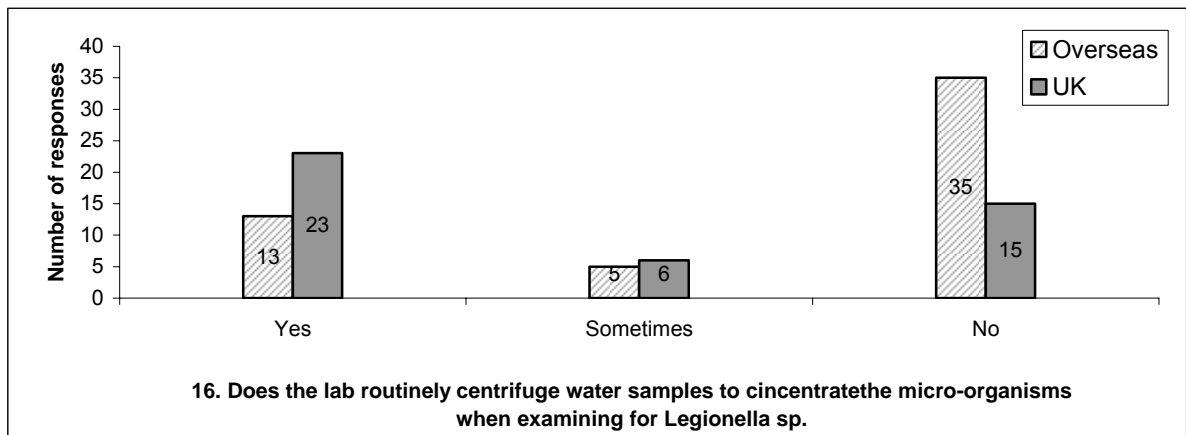
Other - *please give details below*

|  |
|--|
| 0.85% saline peptone = 3                             |
| 10mL from water sample itself = 3                    |
| 10mL sterile deionised water                         |
| 15mL of sample taken prior to filtration             |
| 20mL distilled water                                 |
| 20mL of sample                                       |
| 5 mL sterile deionised water                         |
| Collection buffer with surfactant                    |
| Deionised water = 3                                  |
| Diluted water  |
| Filtered water                                       |
| Not applicable                                       |
| Original sample                                      |
| Phosphate buffer saline (pH 7.4)                     |
| Tap water  |
| The sample itself (a 50mL portion)                   |
| Sterile water = 2                                    |
| The sample water = 8                                 |
| Unfiltrated water (25mL)                             |
| We resuspend the filter using 10mL of sample         |
| We use 10 mL of water sample recovered by filtration |

40/93 (43%)

## Centrifugation

16. Do you routinely centrifuge your water samples to concentrate the micro-organisms when examining for *Legionella* spp.? **Total response = 97**



- Yes  36/97 (37%)  
 Sometimes – it depends on the type of water sample  11/97 (11%)  
 No - If no please go to Q.20 on page xx  50/97 (52%)

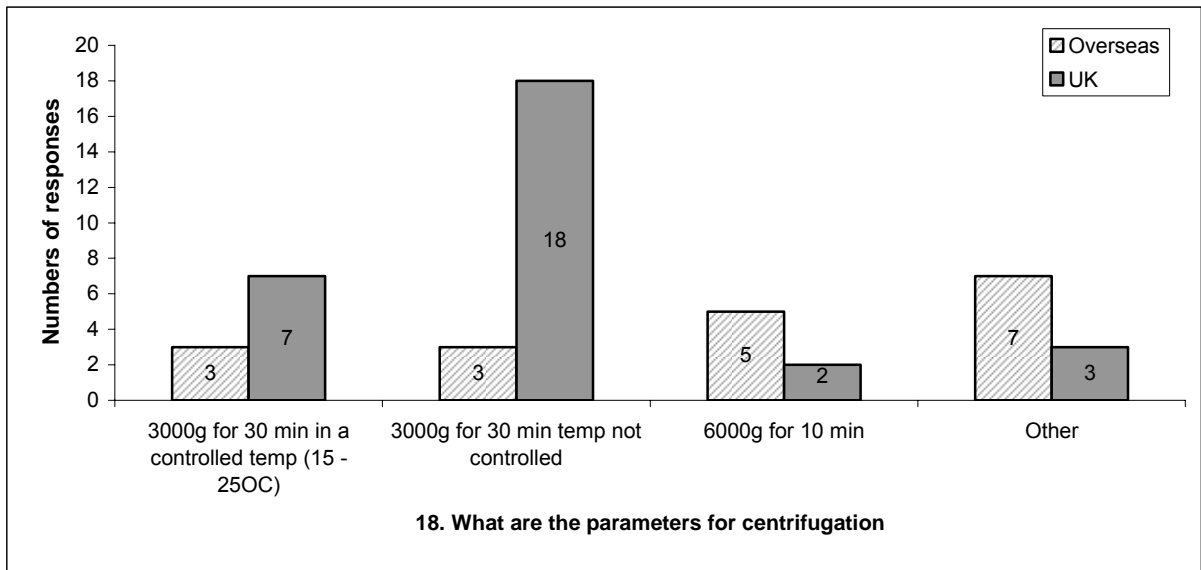
17. What volume of water do you normally centrifuge? **Total response = 47**

- 200 mL of the original water sample  6/47 (12%)  
 10 mL from a concentrate produced by filtering the sample prior to centrifugation  21/47 (45%)  
 Other - please give details below

|   |
|---|
| 1000mL  |
| 100mL of the original water sample  |
| 15mL  |
| 1L then remove supernatant and centrifuge again 2x30mL                          |
| 2 x 50mL = 100mL in total   |
| 20mL = 2  |
| 250mL   |
| 2mL from a concentrate produced by filtering the sample prior to centrifugation |
| 2mL only for acid treatment   |
| 2x1 1/2mL concentrate produced by filtering the sample prior to centrifugation  |
| 3x2mL   |
| 400mL of the original water sample  |
| 50 - 100mL  |
| 500mL   |
| 50mL = 2  |
| 5mL from concentrate  |
| 5mL from concentrate produced by filter   |
| All the sample  |

- 20/47 (43%)

18. What are your parameters for centrifugation? **Total response = 48**



3000 g for 30 min in a controlled temperature (15-25°C)

10/48 (21%)

3000 g for 30 min – temperature not controlled

21/48 (44%)

6000 g for 10 min

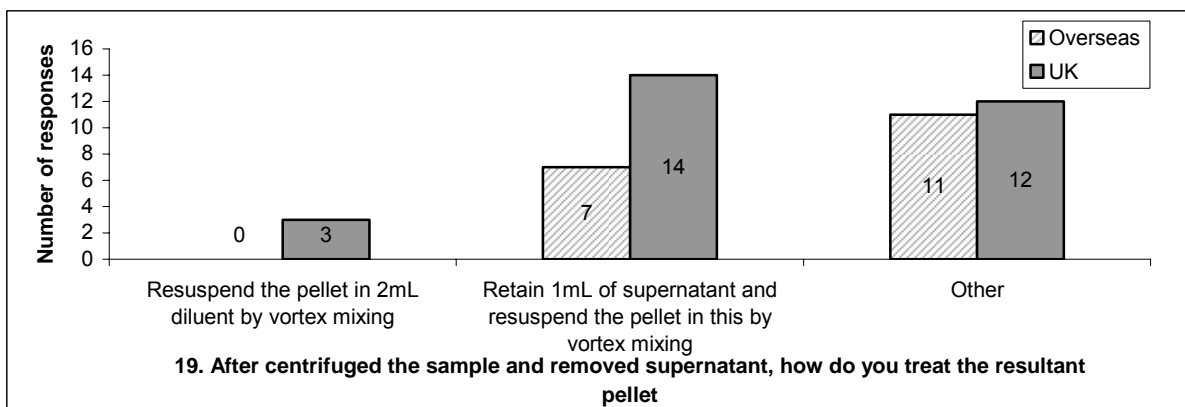
7/48 (14%)

Other - please give details below

|   |
|---|
| 2000g for 5min                                      |
| 3000g for 20mins                                    |
| 3000g for 30min at 40C                              |
| 4500 rpm for 30 min - temperature not controlled    |
| 4500g for 10 mins in a controlled temperature (50C) |
| 4600 for 13 mins                                    |
| 5500 x g 30 mins 12OC                               |
| 6400g for 30min at 15OC                             |
| 8000g for 10 mins                                   |
| 8000g for 5 mins                                    |

10/48 (21%)

19. After you have centrifuged the sample and removed the supernatant, how do you treat the resultant pellet? **Total response = 47**



Resuspend the pellet in 2 mL diluent by vortex mixing

□ 3/47 (6%)

Retain 1 mL of supernatant and resuspend the pellet in this by vortex mixing

□ 21/47 (45%)

Other - *please give details below*

|   |
|---|
| 0.5mL KCl pH2.2   |
| 2 x 1300µL is removed and the pellets are resuspended and pooled<br>2 x 200µL       |
| Remove 1.8mL of supernatant - resuspend the pellet in the left<br>0.2mL supernatant |
| Resuspend in 0.5mL pages saline   |
| Resuspend in 10mL sterile distilled water, vortex                                   |
| Resuspend pellet in 2mL of supernatant, + mix (and vortex)                          |
| Resuspend pellet in retained 7.5mL of supernatant                                   |
| Resuspend the pellet in 10mL diluent by vortex mixing = 3                           |
| Resuspend the pellet in 2.5mL diluent by vortex mixing                              |
| Resuspend the pellet in 4 mL of sterile deionised water by pipeting                 |
| Resuspend the pellet in 5mL diluent   |
| Resuspend the pellet in 5mL of supernatant by vortex mixing                         |
| Retain ~2mL of supernatant and resuspend the peller manual                          |
| Retain 0.2 mL of supernatant and resuspend the pellet in this by<br>vortex mixing   |
| Retain 1-5mL vortex   |
| Retain 1mL of supernatant + resuspend by hand mixing = 2                            |
| Retain 2.5mL of supernatant and resuspending pellet by vortex<br>mixing             |
| Retain 5 mL of supernatant and resuspend by vortex mixing = 2                       |
| Retain 5mL of supernatant and resuspend pellet by vortex for acid<br>treatment      |

□ 23/47 (49%)

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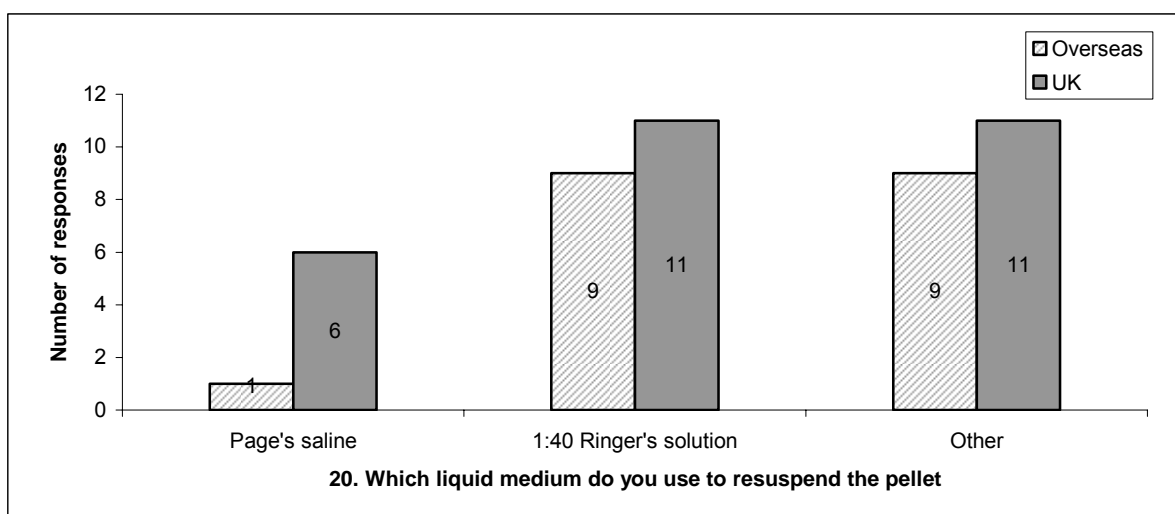
If you do not resuspend the pellet by vortex mixing please note below the process that you use (e.g. shaking by hand etc.)

---

|  |
|--|
| Hand shake   |
| Mix by pipetting in + out of pipettes several times throw hand shake |
| Repeat forced pipetting using 1 mL pastettes                         |
| Resuspending using sterile pastaur pipette in up and down motion     |
| Retain 1mL of supernatant and shake by hand                          |
| Shaking by hand = 2  |

---

20. Which liquid medium do you use to resuspend the pellet? **Total response = 47**



Page's saline

7/47 (14%)

1:40 Ringer's solution

20/47 (43%)

Other - *please give details below*

|                                      |  |
|--------------------------------------|--|
| Deionised water = 6                  |  |
| KCl pH2.2                            |  |
| NaCl 0.9% solution                   |  |
| Phosphate buffer (acc. To 150 8199)  |  |
| Phosphate buffer saline (pH 7'4)     |  |
| Supernatant = 4                      |  |
| Supernatant = 1:40 Ringer's solution |  |
| Supernatant = H2O = 2                |  |
| Supernatant remaining                |  |
| Ultra-pure water                     |  |

20/47 (43%)

## Sample Concentrates

21. How do you examine the sample concentrates? **Total response = 101**

*Please tick all that apply*

Untreated

91

After heat-treatment

24

(0.2 mL concentrate treated at 50°C for 30 min)

After heat-treatment

50

(1 mL concentrate treated at 50°C for 30 min)

After acid-treatment

73

(0.2 mL concentrate treated in an equal volume of acid buffer, pH 2.2) for 5 min)

Other - *please give details below*

62 participants done all three (Untreated, heat-treatment and acid-treatment) 31 overseas and 31 UK

22. Do you store your sample concentrates? **Total response = 103**

Yes  74/103 (72%)

No  29/103 (28%)

*if yes please give further details below* **Total response = 73**

6 °C in the dark

52/73 (71%)

-20 °C (frozen)

Room temperature in dark

17/73 (23%)

Other

*please give details below*

|   |
|---|
| (-5 ±3) °C  |
| 2 <sup>o</sup> -8 <sup>o</sup> C in the dark for a maximum of 14 days |
| 4 <sup>o</sup> C  |
| Frozen -70 <sup>o</sup> C   |

4/73 (5%)

### Selective Plating Media

23. How much sample do you inoculate onto your selective plating media?

Not applicable because the entire membrane is placed directly (upside-down) on the plating culture media  3/96 (3%)

Untreated (96)

0.1 ml  63/96 (66%)

0.2 ml  14/96 (15%)

0.5 ml  9/96 (9%)

Other = 7/96 (7%)

|                   |
|-------------------|
| 0.15mL            |
| 0.1mL, 0.5mL      |
| 0.1mL, 0.5mL      |
| 0.25mL            |
| 1 mL respectively |
| 1mL (0.3+0.3+0.4) |
| onto 3 plates     |
| Untreated 4mL     |

Heat-treated (84)

0.1 ml  60/84 (71%)

0.2 ml  13/84 (15%)

0.5 ml  8/84 (10%)

Other = 3/84 (4%)

|              |
|--------------|
| 0.15mL       |
| 0.1mL, 0.5mL |
| 0.25mL       |

Acid-treated (75)

0.1 ml  33/75 (44%)

0.2 ml  32/75 (43%)

0.5 ml  8/75 (11%)

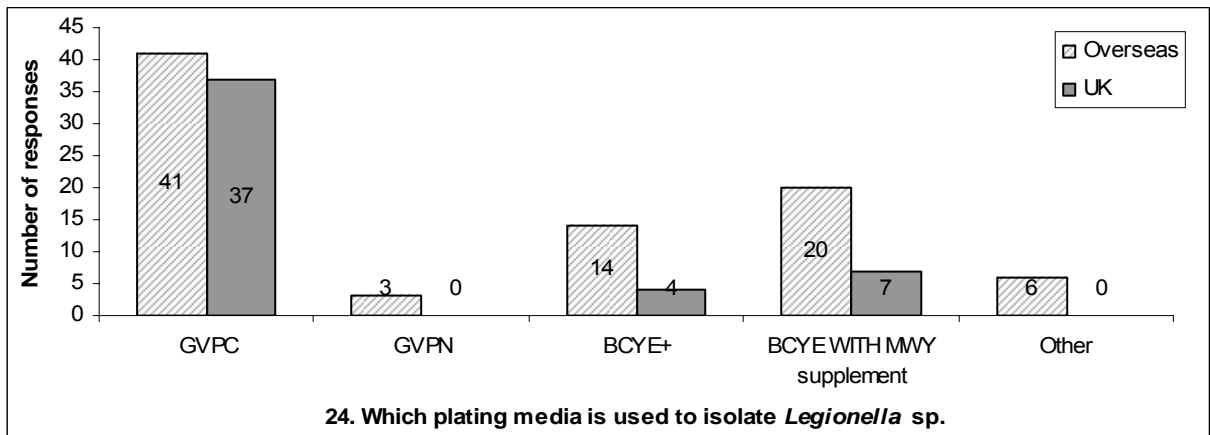
Other = 2 (2%)

|   |
|---|
| 0.2mL, in 0.1mL sample and 0.1mL buffer |
| 0.3mL                                   |

Other  
 please give details below

|  |
|--|
| 0.1mL for each plate medium used   |
| 0.25mL   |
| 0.2mL was used for plating the acid treated concentrate (diluted 1:2 in acid buffer) so as to achieve the same detection limit as that for heat-treated or untreated concentrate |
| 0.4mL for all  |
| Acid treated inoculate is a half dilution of suspension  |
| Additionally inoculation of 1mL out of selective plating media   |
| Cooling towers are heat-treated (5mL not concentrated sample), then inoculated onto the media (0.1mL)  |
| For cooling towers we also plate heat-treated dilutions (-1 and -2)  |
| The filter is placed upside-up for direct membrane filtration (culture)  |
| Untreated 1mL  |
| We also inoculate 0.1mL of untreated 1/10 and heat-treated 1/10  |

24. Which plating media do you use to isolate the *Legionella* spp. **Total response = 104**



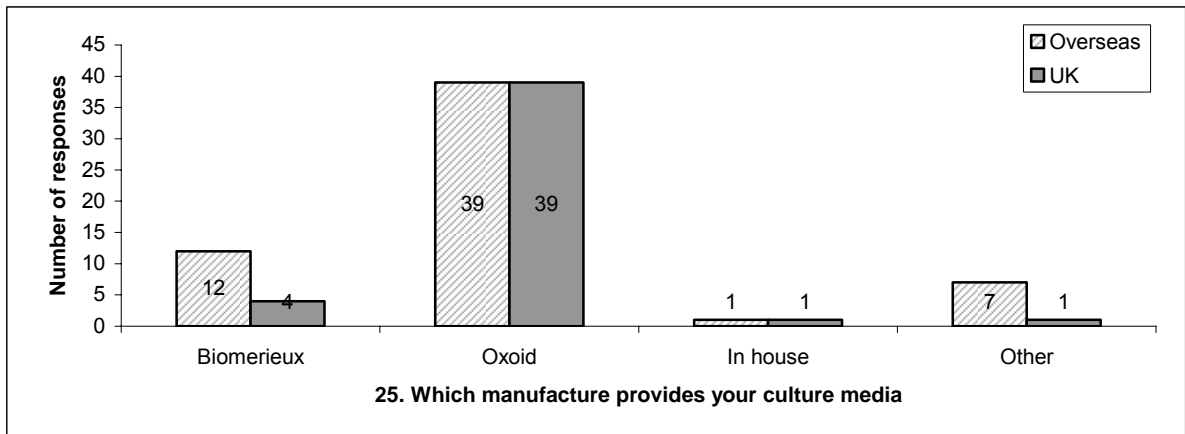
- Glycine vancomycin polymyxin cycloheximide (GVPC) agar  78
- Glycine vancomycin polymyxin natamycin (GVPN) agar  3
- Buffered charcoal yeast extract agar with cysteine (BCYE+)  18
- BCYE agar with MWY supplement - (containing glycine, polymyxin B, anisomycin and vancomycin)  27

Other - please give details below

|   |
|---|
| BCYE agar with BMPA supplement = 2  |
| MWY   |
| BMPA  |
| DGVP agar (BCYE+ dye, Glycine, Vancomycine, Polumyxin B), CCVC agar (BCYE+ cycloheximid, colistine, Vancomycine, Cephalothine)              |
| Glycine VCM PLB Cycloheximide Amphotericin B Thiabendazole (CAT) agar, This medium is our original selective agar plate. (high selectivity) |

25. Which manufacture provides your culture media? **Total response = 104**

Please indicate the name and the product number below



Biomerieux (Product number = 43031)

16/104 (15%)

Oxoid (Product number = Base - CM0655, Supplements - SR0110C, SR0110A, SR0118, SR0215E, SR0152E, SR0111E, P05074A and PO5072A)

78/104 (75%)

In house

2/104 (2%)

Other

|   |
|---|
| "Bio germ" from Oxoid   |
| DID   |
| GUPC: Heipha (Biotest) or Biomerieux cd 43031, MWY: Abtek (Bio life) (Indicated for cooling towers) cod 4215LGZ |
| GVPC agar is MERCK  |
| Hardy diagnostics W169 - GVPC 607 - BCYE  |
| Healthlink, Inc.  |
| Helpa diagnostika (BIOTEST), Legionella MWY Selektivagar  |
| LAB M.(base) LAB 195, LAB M: BCYE Growth supplement. No. X196, LAB M: GVPC Supplement. No.X195                  |

8/104 (8%)

26. How do you incubate your culture plates? **Total response = 106**

36°C, aerobic, for 10 days

22/106 (21%)

36°C with increased humidity for 10 days

41/106 (39%)

36°C with 2.5% CO<sub>2</sub> for 10 days

14/106 (13%)

Other

*please give details below*

|  |
|--|
| 35°C aerobic for 10 days   |
| 35°C aerobic, for 7 days   |
| 36°C with increased humidity and 50C CO <sub>2</sub> for 10 days   |
| 36°C with increased humidity with 2.5% CO <sub>2</sub> for 10 days |
| 36°C with 2.5% CO <sub>2</sub> and increased humidity for 10 days  |
| 36°C with 2.5% CO <sub>2</sub> for 12 days                         |
| 36°C with 2.5% CO <sub>2</sub> for 15 days                         |
| 36°C with 2.5% CO <sub>2</sub> for 7 days = 2                      |

29/106 (27%)

|   |
|---|
| 36°C with 2.5% CO2 with increased humidity for 8 to 10 days                                     |
| 36°C with 5% CO2 for 10 days  |
| 36°C with increased humidity for 10 days, (sometimes for 10 days, normally for 7 days)          |
| 36°C with increased humidity for 10 days, 36°C with 2.5% CO2 for 10 days = 3                    |
| 36°C with increased humidity for 10 days, 36°C with increased humidity for 7 days for AS method |
| 36°C aerobic, for 7 days = 2  |
| 37°C for 10 days  |
| 37°C±10C Aerobic for 10 days  |
| 37°C + or - 1.00C aerobic, for 10 days  |
| 37°C aerobic with increased humidity for 10 days  |
| 37°C in plastic box with increased humidity for 10 days   |
| 37°C with 2.5% CO2 for 10 days  |
| 37°C with increased humidity for 10 days = 2  |
| 37°C with increased humidity for 8 days   |
| 37°C aerobic, for 10 days   |
| 37°C aerobic, for 7 days  |

27. When do you examine the plates and count the colonies? **Total response = 105**

- After 3 days, 6 days and 10 days  52/105 (50%)
- After 4 days and 10 days  10/105 (9%)
- After 10 days only  1/105 (1%)

Other

*please give details below*

|   |
|---|
| 2, 3, 6, and 10 days                                      |
| 2, 4, 6, 8 and 10 days                                    |
| 2, 4, 7, and 10 days                                      |
| 3, 5, 7 and 10 days = 4                                   |
| 4, 7, 10 days = 2   |
| 5 and 10 days   |
| After 2, 4, 6, 8, and 10 days = 2                         |
| After 2-4 5-9 10-14 days                                  |
| After 3 and 5 days, negative samples are kept for 15 days |
| After 3 and 7 days = 3                                    |
| After 3 days, 5, 7 days and 10 days                       |
| After 3 days, 6 days, 10 days and 15 days                 |
| After 3 days, 6 days, 10 days and after 12 days           |
| After 3 days, 7 days and 10 days = 2                      |
| After 3, 5, 7 and 10 days = 4                             |
| After 3, 5, and 7 days                                    |
| After 3-7 days, 7days and 10 days                         |
| After 5 and 10 days                                       |
| After 6 and 10 days                                       |
| After 6 and 8 days  |

42/105 (40%)

|  |
|--|
| After 7 days only = 2  |
| At least 3 times during 10 days  |
| Every day  |
| Every other day = 2  |
| Examine the plates at intervals of 2-4 days during the 10 day period incubation  |
| Examine on 3 different occasions at intervals of 2 to 4 days, right up to 10 days for BS method and up to 7 days for AS method |
| First 3 (or 4, 5) second 7 (10)  |
| Mondays, Wednesdays, Fridays   |
| Read C dg 4, 6, 8 and 10 days  |

28. Do you routinely determine the recovery rate for your *Legionella* isolation method? **Total response = 101**

Yes  24/101 (24%)

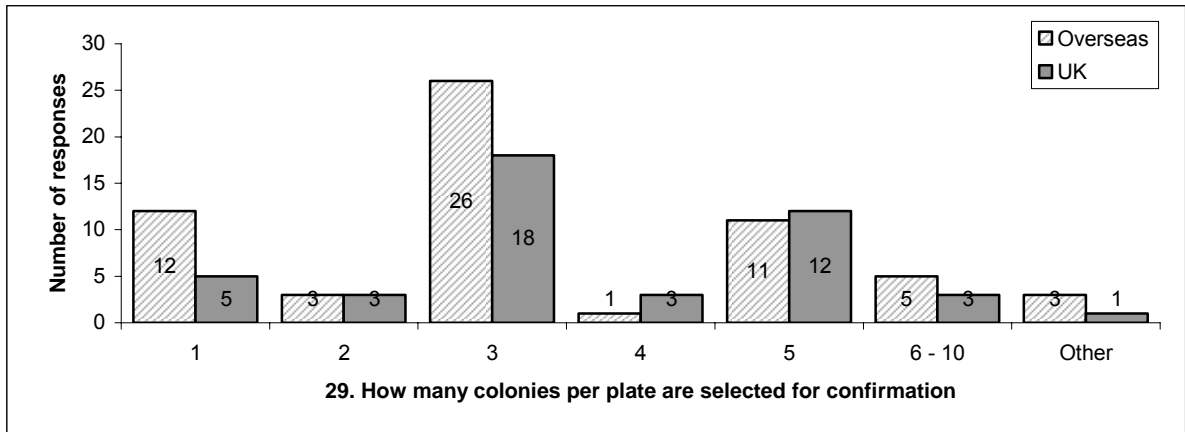
No  77/101 (76%)

*if yes please give further details below such as your average recovery rates*

|   |
|---|
| Yes, 40% recovery   |
| Yes average 24%   |
| Yes, 20-30%   |
| Yes, 27%  |
| Yes, 30-60% recovery  |
| Yes, 8-80%  |
| Yes, Average recovery rate: 30%   |
| Yes, between 20-50% typically   |
| Yes, EQA recovery rates against participants mean = 158%, IQC recovery rates against spiked water = 63% |
| Yes, from IQA and EQA results   |
| Yes, IQA = 60% recovery   |
| Yes, IQC  |
| Yes, only use of Lenticules discs   |
| Yes, our average recovery rates are between 50 - 60%  |
| Yes, recovery after centrifugation compared to direct plating is typically 20-50%                       |
| Yes, Untreated = 60-80%   |
| Yes, we are currently validating this method  |

**Confirmation of *Legionella* spp.**

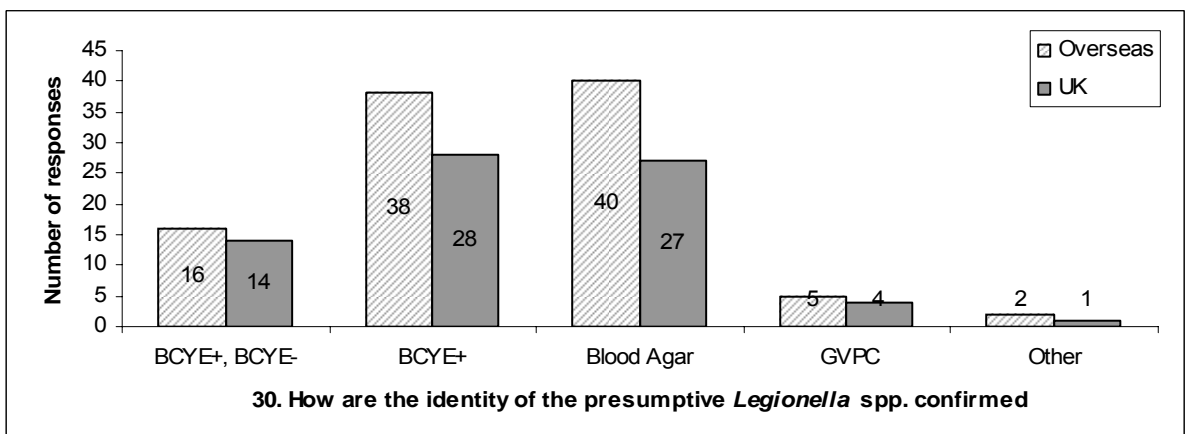
29. How many colonies per plate to you select for confirmation? **Total response = 106**



- One □ 17/106 (16%)
  - Two □ 6/106 (6%)
  - Three □ 44/106 (42%)
  - Four □ 4/106 (4%)
  - Five □ 23/106 (22%)
  - 6-10 □ 8/106 (8%)
  - Other □ 4/106 (4%)
- please give details below*

|  |              |
|--|--------------|
| According to the size, colour fluorescence   |              |
| Colony selection is relied on the appearance of the colonies under colony microscope |              |
| Dependant on the number of the plates and number of colonies on plate                | □ 4/106 (4%) |
| Depends on the type and number of colonies   |              |

30. How do you confirm the identity of the presumptive *Legionella* spp.? **Total response = 106**

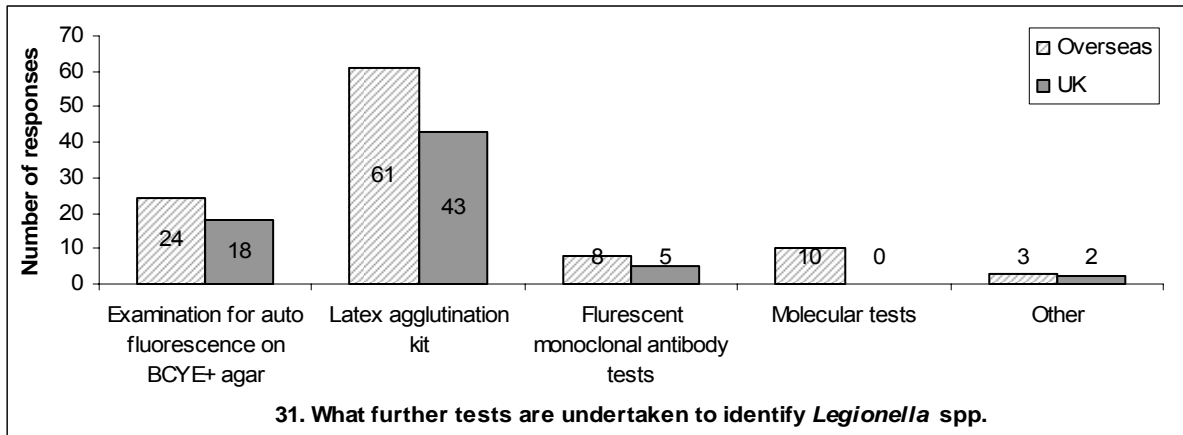


- Sub-culture to buffered charcoal yeast extract agar plates with and without cysteine (BCYE+, BCYE-) □ 30
- Sub-culture to buffered charcoal yeast extract agar plates with cysteine (BCYE+) and nutrient agar/blood agar □ 66

Other - please give details below

|                 |
|-----------------|
| Blood Agar = 67 |
| GVPC = 9        |
| CLED = 1        |
| MWY = 1         |
| BCYE- = 1       |

31. What further tests do you undertake to identify the *Legionella* spp.? **Total response = 105**



- Examination for auto fluorescence on BCYE+ agar  42
- Latex agglutination kit  104
- Fluorescent monoclonal antibody tests  13
- Molecular tests  10
- Other - please give details below

|   |
|---|
| Slide agglutination using Denka seiken reagents |
| Plate microscope                                |
| Biogenetics (Antisera for slide agglutination)  |
| Catalase, gram's stain and B-Lactamase          |

32. Which manufacturer provides your kit/monoclonal antibodies or other reagents for identifying *Legionella* spp.? **Total response = 105**

Please indicate the name(s) and the product number(s) below

|  |
|--|
| Oxoid = 98/105 (93%) (Lg1 - DR0801M, Lg2-14 – DR0802M, Lag sp. – DR0803M, DR0200M, DR0210M, DR0220M, DR0800M and Oxoid dry spot) |
| Microgen = 4/105 (4%)  |
| Prolab = 1/105 (1%)  |
| Remel = 1/105 (1%)   |
| Biogenetics = 1/105 (1%)   |

## Control Strains

33. Which control strains do you use for your culture media and process controls?

Please enter the level of micro-organisms (where appropriate) that you use routinely next to the controls you have indicated

**Total response = 95**

None

*Legionella pneumophila* NCTC 12821

*Legionella pneumophila* NCTC 11191

*Legionella pneumophila* NCTC 11192

*Escherichia coli* NCTC 9001

*Pseudomonas aeruginosa* NCTC 10662

**Other - please give details below**

*Escherichia coli* NCTC 12241 (ATCC 25922)

*Pseudomonas aeruginosa* NCTC 12934 (ATCC 27853), NCTC 10332, NCTC 12951

*Legionella bozemanii* NCTC 11368, ATCC 33545, ATCC 33217

*Legionella anisa* ATCC 35292

*Legionella pneumophila* sg6 NCTC 12082 (ATCC 33215), ATCC 43111, non-O1 NCTC 11230, NCTC 11404

*Legionella micdadei* ATCC 33218

*Staphylococcus aureus* ATCC 12228, NCTC 12981 (ATCC 25923)

*Candida albicans* ATCC 14053

*B.bronchisephila* ATCC 16580

Culture media

7

32

3

28

14

5

9

3, 2, 1

4, 1, 1

1

1, 2, 1, 1

2

2, 3

1

1

|   |
|---|
| <i>E.coli</i> DSN, <i>P.aeruginosa</i> DSN 46358  |
| il controllo dei terrelli di coltura e' fatto con un ceppo di <i>Legionella Pneumophila</i> sierotib 1 isolato in laboratorio |
| <i>L.pneumophila</i> NCIMB 50008, <i>E coli</i> NCIMB 50109   |
| <i>Legionella micdadei</i> VAMC594 BCYE, DGVP, CCVC, PAV, <i>Legionella bozemanii</i> VAMC14 BCYE, DGVP, CCVC, PAV, PAC       |
| <i>Legionella Pneumophila</i> C 3950L   |
| <i>Legionella Pneumophila</i> CCUG 9568 process   |
| <i>E.coli</i> CCM 3954, <i>Pseudomonas aeruginosa</i> CCM 1960 (culture media and process)                                    |
| Stain corby in the process control, media are ready plates from the manufactures and were not controlled                      |
| We use a "wild" strain, isolated from routine sample  |
| We use a strain of <i>Legionella</i> isolated in lab  |
| Wild type confirmed <i>Legionella</i> (gel) in future NCTC - strains  |

**Total response = 61**

None

*Legionella pneumophila* NCTC 12821

*Legionella pneumophila* NCTC 11191

*Legionella pneumophila* NCTC 11192

*Escherichia coli* NCTC 9001

*Pseudomonas aeruginosa* NCTC 10662

**Other - please give details below**

Process control

4

31

1

12

9

1

*Legionella bozemanii* ATCC 33217  
*Pseudomonas aeruginosa* NCTC 10332  
*Legionella bozemanii* NCTC 11368  
*Escherichia coli* NCTC 12241 (ATCC 25922)

1  
 1  
 1  
 1

|  |
|--|
| 10 <sup>4</sup> -10 <sup>5</sup> cfu   |
| 1mL aliquots of water sample (sorted at -80°C) previously positive (>25,000 cfu (L)                                  |
| BCYE agar  |
| <i>L.bozemanii</i>   |
| Wild strain <i>Legionella pneumophila</i> serogroup 1  |
| L.p sg 2-14 - isolate from G46 sample c Colon. XIII, <i>L.erythra</i> ATCC 35303                                     |
| <i>Legionella pneumophila</i> C 3950L  |
| None, we use Pre-owned selective agar plates from external suppliers, who have performed the QC check for the plates |
| Process controls not carried out following risk assessment   |
| <i>S.aureus</i> ATCC G538 - COS, <i>Klebsiella pneumonia</i> NCTC 7761 - COS   |

Thank you for completing this questionnaire – if you can think of any other comments or information that might be useful please enter the details below:

|   |
|---|
| As you might notice we are using the 11731-2 method on most of the samples in combination with direct plating it works out fine.  |
| May include what control procedures are used, controlling media and pre-treatment e.g. Lenticules, microcosm  |
| Recovering data using EQA samples for acid and heat treatment as well as untreated would be useful  |
| We are not currently carrying out routine Legionella testing. We partake in EQA scheme to maintain expertise in case the situation changes.   |
| We are observing that heat treatment of agar from Legionella (reference or wild strains) in markedly more bactericidal than Legionella isolated from direct water sample post heat treatment  |
| We have examined Legionella detection before now more than 80000 samples, the method of Legionella detection has some problems, and we must clarify it. Thank you for your cooperation  |
| We have recently modified our method to bring it in line with the HPA W12, Answers given reflect new method rather than method until very recently.   |
| We routinely undertake direct plating (0.5mL) and plating (0.1mL) from the filter as well as 0.1mL of the concentrate from the centrifugation. We report the highest reliable count for these tree investigations as the result. As we often find these values for direct plating or for the filtrate our results most often are from one of these. According to the Legionella EQA scheme we should report results from the concentrate, but as this is not our routine reporting method we report the highest reliable count, and this count is often higher than the count for the concentrate. We would like you to consider this to be an option when reporting EQA results to the HPA, we think that other laboratories also test and report in a similar way as we do. |