UK Health Security Agency

UK NEQAS Microbiology

ECDC External Quality Assessment (EQA) scheme supporting the surveillance of Legionnaires' disease at European level

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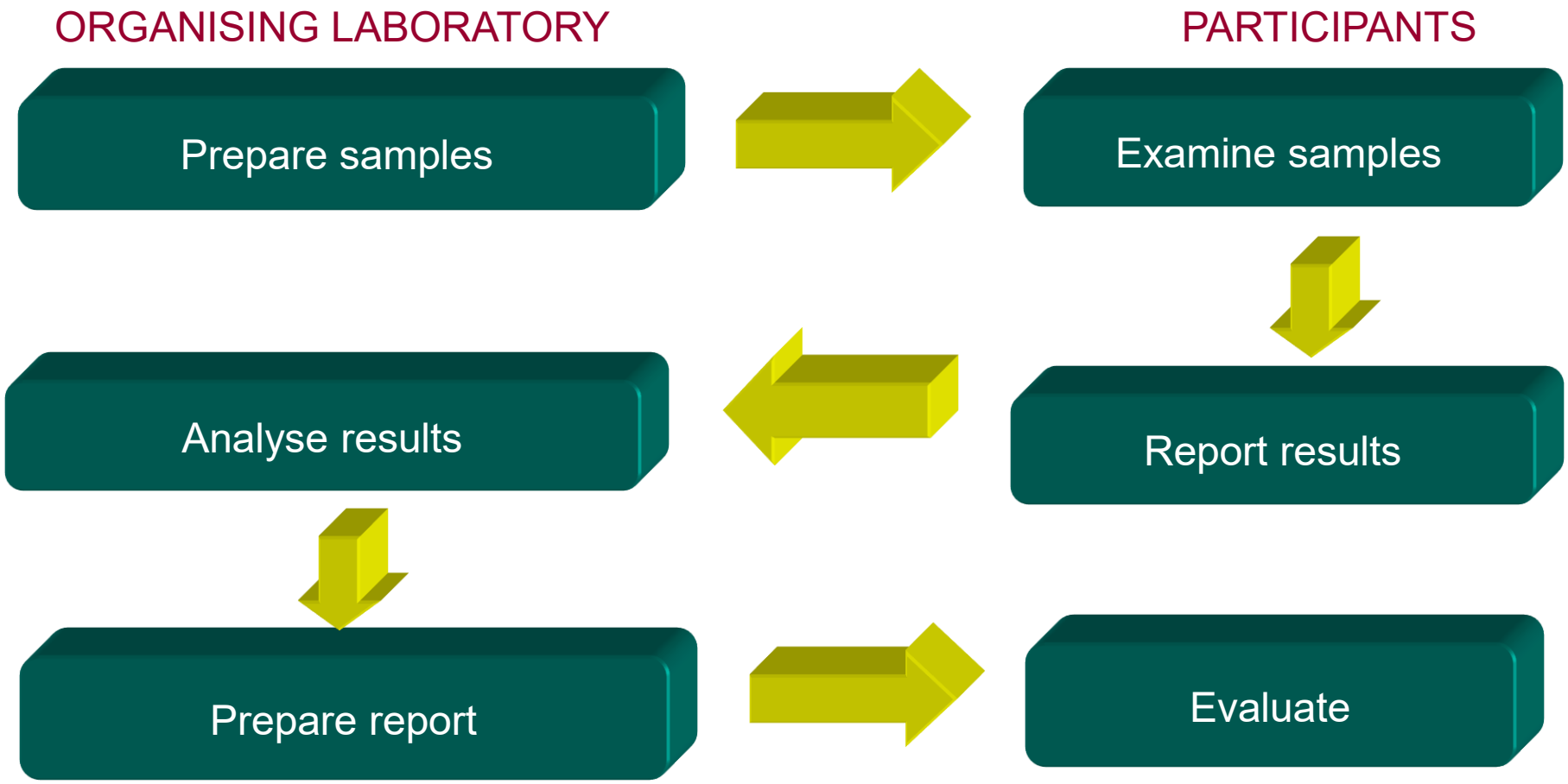
External Quality Assessment/Proficiency Testing

Simulated samples/specimens of known but undisclosed content are introduced into a laboratory's routine testing procedure



Purpose of this EQA

- Continue to determine the accuracy of *Legionella* testing and results reported by individual laboratories (both clinical and environmental)
- Continue with inter and intra- laboratory comparison of results from participating countries across Europe
- The information collated demonstrates and highlights:

1. Capabilities of laboratories to accurately perform *Legionella* testing
2. Confidence in data submitted for surveillance
3. Which laboratories that need further support
4. Which countries that need further assistance
5. Allows laboratories to understand their own capabilities, if testing demand increases due to an outbreak

Objectives for March's 2021 EQA

For both clinical and environmental simulated specimens:

- to continue understanding the baseline level of testing undertaken by laboratories in response to routine outbreak scenarios
- to continually assess general performance issues and
- to provide subsequent individual technical support to laboratories as a follow up to the exercises, (if requested by the countries)

This project was delivered jointly by the Food and Environmental Proficiency Testing Unit (FEPTU) and United Kingdom National External Quality Assessment Service (UK NEQAS)

Sample/specimen format

Clinical samples were sent as freeze dried vials or urine in plastic tubes. These samples had to be stored at 4°C until ready for processing.

Water and environmental samples were sent as LENTICULE® discs in plastic tubes, dry cotton swabs were also included. These samples had to be stored at -20°C until ready for processing.

Analysis of participants' results for the March 2021 EQA exercise as part the ECDC tender application

Outbreak strain details

Legionella pneumophila Sg1 ST62 – outbreak strain

- This sequence type of *Legionella pneumophila* has been isolated from clinical and environmental samples
- Associated with community acquired, nosocomial and travel-related cases
- Isolated from several countries across Europe, Japan, Canada and USA
- Majority of *L. pneumophila* ST62 strains identified to date belong to serogroup 1

Clinical EQA exercise

Scheme Information

- A total of 27 registered laboratories were sent this distribution, with 23 examining the specimens
- A total of 27 countries participated in this distribution, with 23 participants returning results within the specified period

Specimen design

- Ten simulated specimens from six patients were dispatched. The simulated specimens included:
  - Five lyophilised sputum specimens requesting organism identification, serogroup and sequence type determination
  - Five liquid urine specimens for the detection of *L. pneumophila* urinary antigen (LUA)
  - Three patient sets of samples (both sputum and urine) were designed to represent an outbreak

Specimen design - other pathogens chosen

Legionella pneumophila Sg 6 ST2923

- This sequence type of *Legionella pneumophila* is unique and has only been identified once from an environmental sample in the UK
- This ST is a single locus variant of other *L. pneumophila* non-sg1 STs: 1745,1356 and 1322

Legionella bozemanii

- *Legionella bozemanii* was first isolated from lung tissue in 1959. The isolate came from a healthy scuba diver in Florida with fatal bronchopneumonia
- The second isolate was recovered in 1979 from lung tissue from a patient with lymphatic leukaemia in whom a fulminant pneumonia developed after submersion in brackish swamp water
- *Legionella bozemanii* is within the top five most common *Legionella* species isolated from water distribution systems

Intended results 2021: outbreak specimens

Patient	Specimen number	Clinical site	Clinical Details	Specimen contents
1	7034	Sputum	Muscle aches and headache following recent stay on orthopaedic ward	*Legionella pneumophila* Sg1 ST62 *Streptococcus oralis*
	7035	Urine		*Legionella pneumophila* Sg1 ST62 antigen
2	7036	Sputum	Previous hospitalisation following bone fracture repair. Patient now presenting with shortness of breath.	*Moraxella catarrhalis* *Streptococcus oralis*
	7037	Urine		*Legionella pneumophila* Sg1 ST62 antigen
3	7038	Sputum	Persistent cough, fever and headache following hospital stay 10 days previous	*Legionella pneumophila* Sg1 ST62 *Streptococcus mitis* *Streptococcus oralis*
	7039	Urine		*Legionella pneumophila* Sg1 ST62 antigen

Intended results 2021: non out break

Patient	Specimen number	Clinical site	Clinical Details	Specimen contents
4	7040	Sputum	Chest pain and shortness of breath since discharge from hospital 3 days ago	*Legionella pneumophila* Sg6 ST2923 *Streptococcus mitis* *Streptococcus salivarius*
	7041	Urine		*Legionella pneumophila* Sg6 ST2923 antigen
5	7042	Urine	Fever of 104F (40C) and confusion. Query secondary infection following discharge from hospital	Negative for *L. pneumpohila* Sg1 antigen
6	7043	Sputum	Cough, bringing up mucus and muscle pains for 5 days	*Legionella bozemanii*

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Overall concordance

Comparison of outbreak associated specimens

Test performed	Patient 1 (%)	Patient 2 (%)	Patient 3 (%)
Identification	100	87	100
Serogroup	100	-	100
Sequence type	100	-	100
Urinary antigen	100	77.3	100

Patient 1

Specimen 7034

Intended result: *Legionella pneumophila* serogroup 1: ST62

Participant concordance

- Identification: Excellent concordance, 100% (23/23)
- Serogroup: Excellent concordance, 100% (21/21)
- Sequence type: Excellent concordance, 100% (10/10)

Specimen 7035

Intended result :Urinary antigen: positive for *L. pneumophila*

Participant concordance

- Excellent performance with 100% (22/22) of participants reporting the correct result

Patient 2

Specimen 7036

Intended result: Sample negative *for Legionella pneumophila*

Participant concordance*:*

- Identification: Good performance 87% (20/23)

Specimen 7037

Intended result :Urinary antigen: positive for *L. pneumophila*

Participant concordance:

- Only 77.3% (17/22) of participants reported the correct result

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Patient 3

Specimen 7038

Intended result: *Legionella pneumophila* serogroup 1: ST62

Participant concordance:

- Identification: Excellent performance 100% (23/23)
- Serogroup: Very good concordance 100% (21/21)
- Sequence type: 100% performance (10/10)

Specimen 7039

Intended result: Urinary antigen: positive for *L. pneumophila*

Participant concordance:

- An excellent performance with 100% (22/22) of participants reporting the correct result

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Patient 4

Specimen 7040

- Intended result: *Legionella pneumophila* serogroup 6: ST2923

Participant concordance:

- Identification: Identification: Very good performance 95.7% (22/23)
- Serogroup: Excellent concordance 100% (20/20)
- Sequence type: 100% performance (6/6)

Specimen 7041

- Intended result: Urinary antigen: negative for *L. pneumophila*

Participant concordance:

- An excellent performance with 100% (22/22) of participants reporting the correct result

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Patient 5

Specimen 7042

Intended result: Urinary antigen: negative for *L. pneumophila*

Participant concordance:

- An excellent performance with 100% (22/22) of participants reporting the correct result

Patient 6

Specimen 7043

Intended result: *Legionella bozemanii*

Participant concordance:

- Identification: Only 73.9% (17/23) of participants reporting the correct result
- Reports of ‘Not *L. pneumophila* isolated' was considered correct

Conclusions for clinical specimens for November 2020 and March 2021 EQA

Identification:

- Overall concordance was very good to excellent, across both distributions for identification of *L. pneumophila*
- Concordance with intended results was reduced for specimens not containing a *Legionella* spp., and those containing non-*Legionella pneumophila*

Serogroup:

- Overall concordance was very good to excellent, for both distributions for reporting the correct serogroup
- The majority of laboratories performed serogrouping, with only two participants not reporting a result in distribution 5113 sent out in March 2021

Conclusions for clinical specimens for November 2020 and March 2021 EQA

Sequence type:

- A reduced number of laboratories reported a result for sequence type for both distributions
- From those reporting a result, concordance was lower for a non-*L. pneumophila* Sg1 (included in distribution 4895, November 2020)
- One laboratory listed several possible sequence types for two out of three outbreak associated specimens

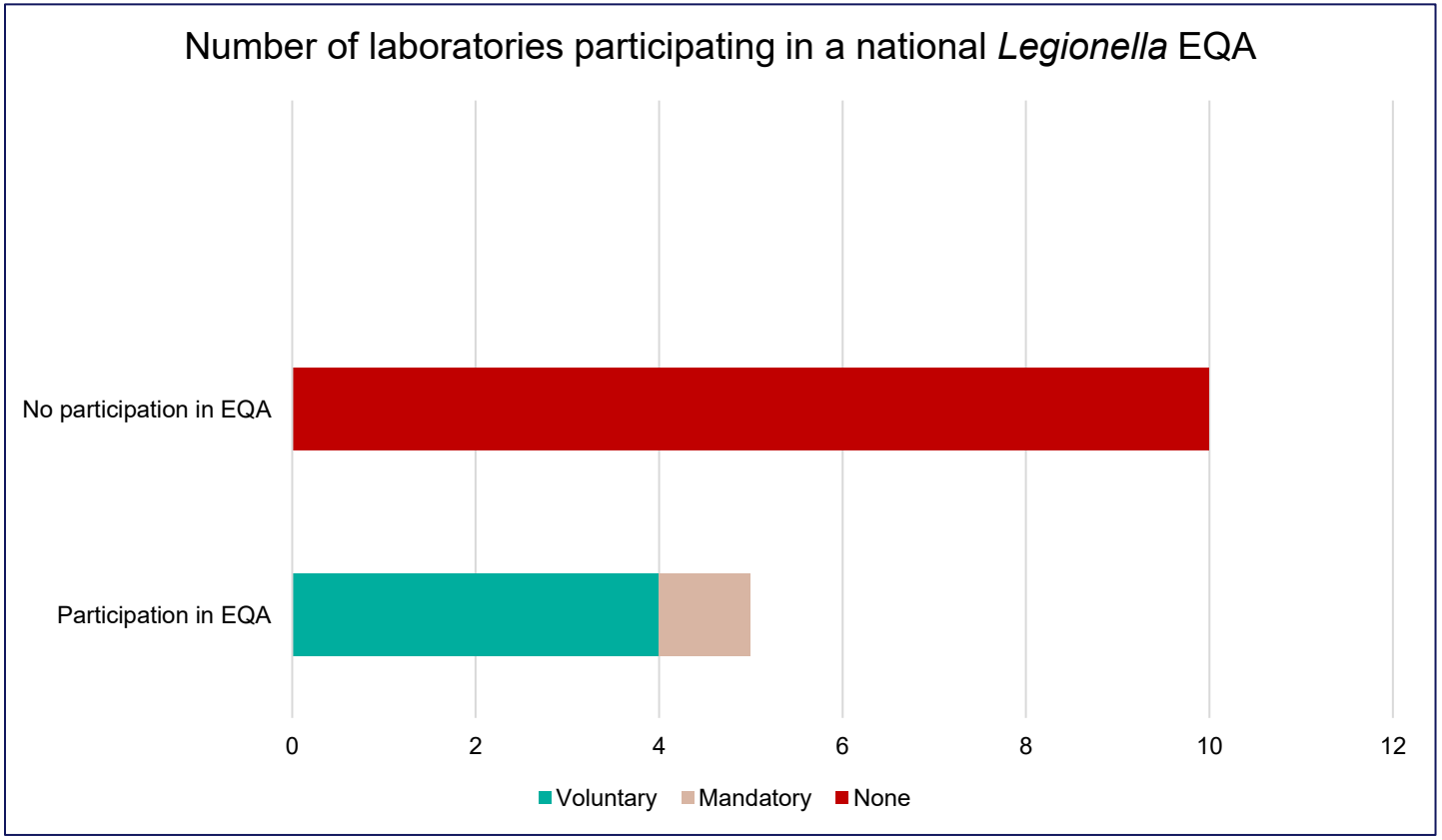
Urinary antigen detection:

- Distribution 5113 (March 2021), specimen number 7037 attained a low concordance, with only 77.3% (17/22) reporting the correct positive intended result. The paired sputum specimen contained no *Legionella* species

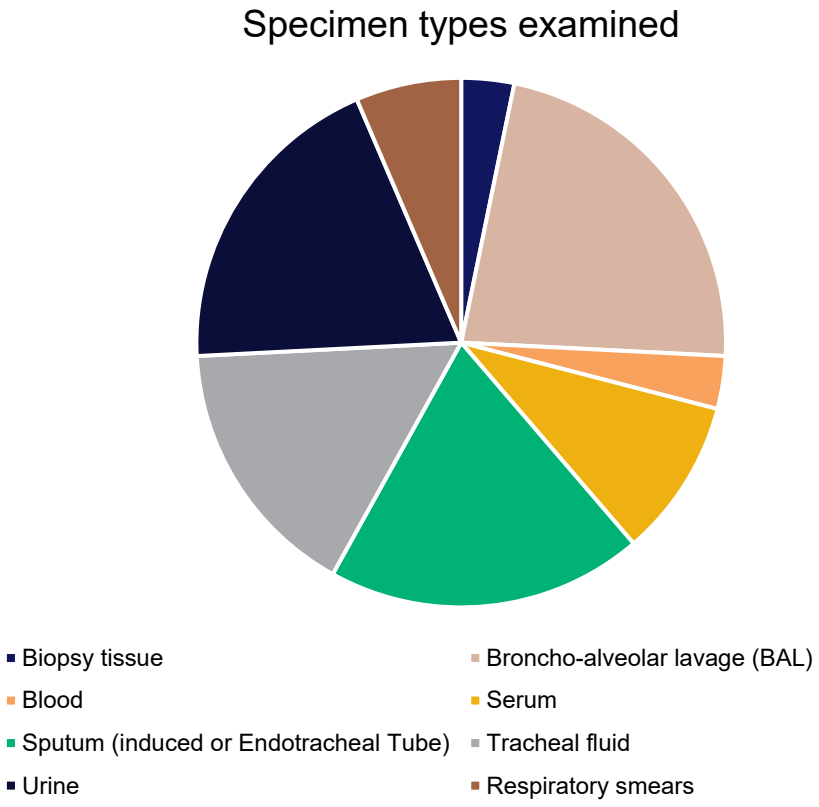
Methods questionnaire

- A questionnaire was sent out to all participants taking part in the March 2021 EQA
- Only 65.2% (15 /23) participants provided a response
- From the laboratories returning a result, 14 were clinical diagnostic laboratories
- A total of 9 of these laboratories were also reference labs
- From the 5 that were not reference labs, 3 stated they did have access to a reference lab

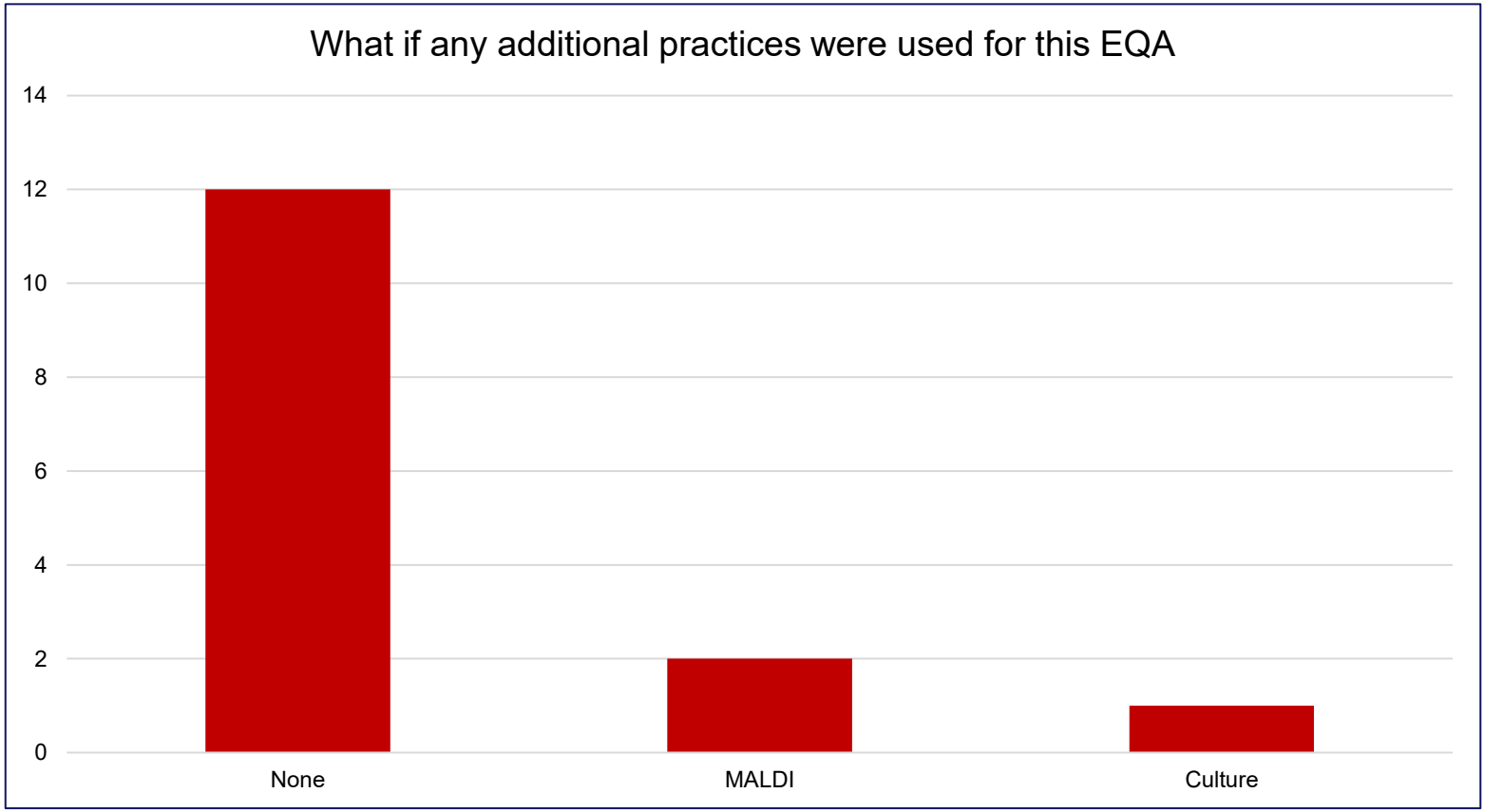
Does your laboratory participate in a National EQA scheme?



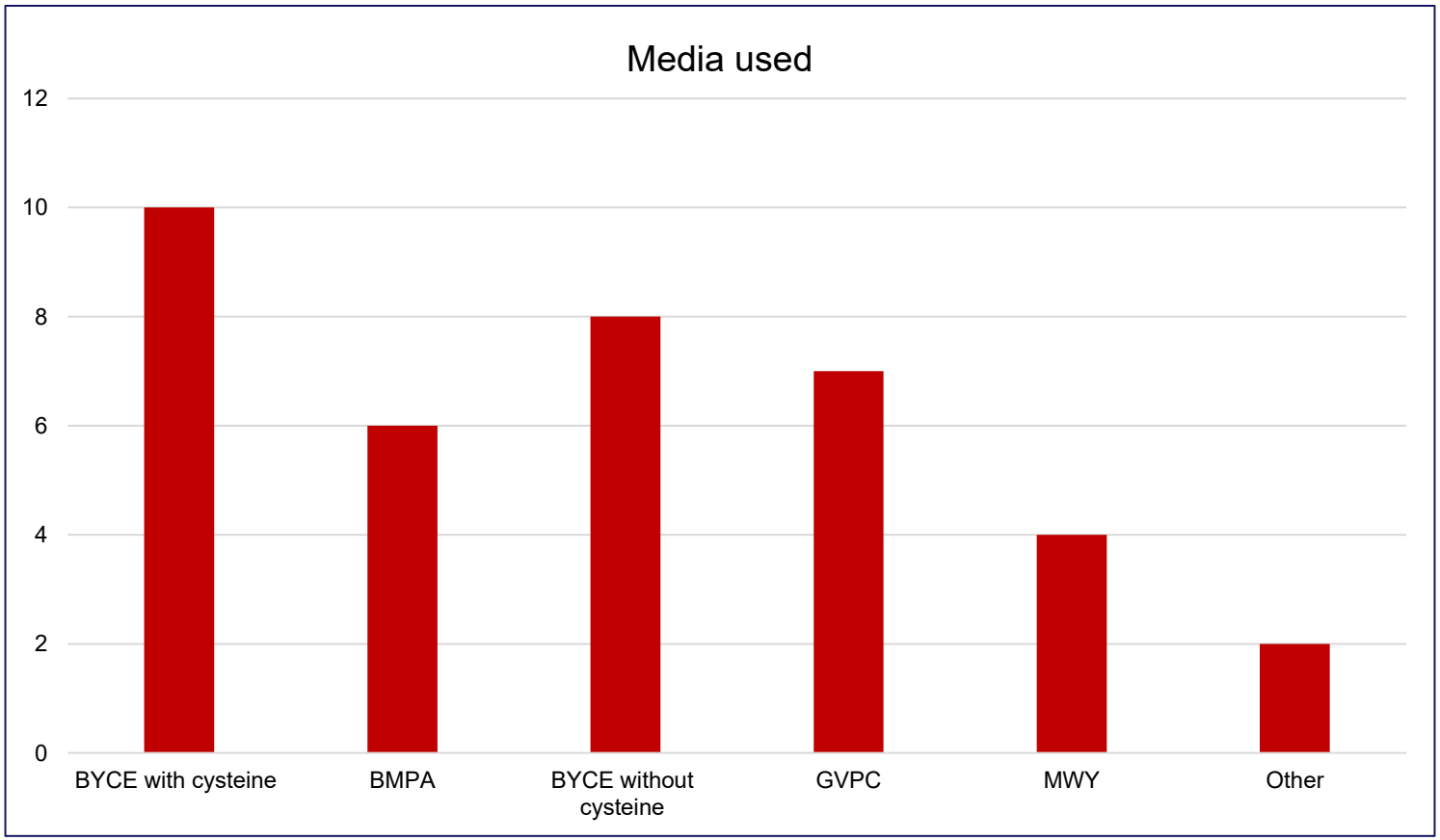
Type of specimens received for *Legionella* investigations



Methods used other than the routine protocol



Media used in participating laboratories

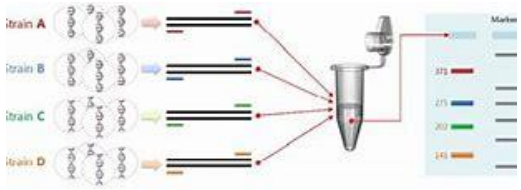


Urinary antigen testing kit and manufacturer used

Urinary antigen testing kit	No of participants
BinaxNOW Abbott	5
Nal von minden	1
ImmuViewÂ® *S. pneumoniae* and *L. pneumophila* Urinary Antigen	1
Diagnostic automation	1
Legionella pneumophila rapid test	1
Sofia *Legionella* FIA, quidel corporation	1
Microgen *Legionella* Latex agglutination kit	1

Multiplex PCR- other respiratory pathogens targeted in the assay used

Additional respiratory targets in multiplex PCR	No of participants
Legionella species	2
Chlamydophila pneumoniae, Chlamydia pneumoniae	3
Legionella pneumophila	3
Bordetella pertussis/ parapertussis	3
Streptococcus pneumoniae	2
Haemophilus influenzae	2
L. pneumophila serogroup 1	2
Chlamydophila psittaci	1
Mycoplasma pneumoniae	3



PCR methods used by participants

PCR methods kit	No of participants
ARGENE Legio pneumo/Cc R-GENEÂ® (BioMerieux)	1
"in-house" method adapted from the literature	4
Allplex RP4, Seegene	2
L.spp / *L. pneumophila* : R-DiaLeg kit (Diagenode)	1

L. pneumophila serogroups

Serogroup	No of participants
1-6	2
1-14	8
1-15	1

Approximate number of tests performed each year

Number of tests performed	None	<10	10-99	100-499	500-1000
Culture	1	2	6	2	2
PCR tests	2	1	2	6	1
7 alleles ESGLI method	6	2	2	1	0
Direct Fluorescent Antibody (DFA) staining	9	2	0	0	0
Mip sequencing	5	6	0	0	0
Serotyping	4	1	4	2	0
Monoclonal antibody	6	2	2	0	0
Latex agglutination	3	2	4	2	0
Sequencing (sanger & WGS)	5	2	2	2	0

Environmental EQA

Environmental 7044 - 7048

Sample number	Sample type	Sample contents	Serogroup	Sequence base type	Comments
7044	Water from hot and cold water system from orthopaedic ward	*L. pneumophila* ($2.9x10^3$) *Brevundimonas vesicularis* *Aerococcus viridans*	1	62	Samples taken as part of one outbreak investigation
7045	Shower water from orthopaedic ward	*L. pneumophila* ($5.7x10^4$) *Pseudomonas putida* *Staphylococcus epidermidis*	1	62	
7046	Water from hot and cold water system from estates department	*Acinetobacter junii* *Pseudomonas fluorescens*			
7047	Biofilm swab from hot and cold water system	*Legionella anisa* ($9.1x10^2$) *Citrobacter braakii*			
7048	Water from a cooling tower located in the hospital ground	*L. pneumophila* ($6.8x10^4$) *Roseomonas aestuarii* *Pseudomonas aeruginosa*	1	62	

Environmental 7049 - 7053

Sample number	Sample type	Sample contents	Serogroup	Sequence base type	Comments
7049	Potable water	*L. pneumophila* (8.1×10^3) *Microbacterium luteolum*	1	1	Samples taken as part of routine quality monitoring of water
7050	Water from cooling tower	*Staphylococcus saprophyticus*			
7051	Pool Water from a spa pool	*L. pneumophila* (1.4×10^4) *Klebsiella pneumoniae* *Staphylococcus haemolyticus* *Enterococcus faecium*	1	1	
7052	Water from hot and cold system	*Enterococcus faecalis*			
7053	Biofilm swab from hot and cold water system	*L. pneumophila* (2.8×10^2) *Klebsiella pneumoniae* *Staphylococcus haemolyticus* *Enterococcus faecium*	6	2923	

Scheme information

Ten samples were dispatched

A total of 18 countries participated in this distribution

22 participants were sent this distribution. Two laboratories informed the organisers that they would not be able to examine the samples and two laboratories did not return a result.

Sample numbers: 7044 – 7048: these samples were prepared as part of one outbreak investigation

Sample numbers: 7049 – 7053: these samples were prepared as part of routine quality monitoring of water

Sample numbers: 7047 and 7053 were swab samples

Additional *Legionella* species details

Legionella anisa

- This species was first isolated from water during a nosocomial outbreak in the United States between March 1980 and June 1981
- *Legionella anisa* is one of the most frequent species of *Legionella* other than *Legionella pneumophila* present in the environment and may be hospital acquired in rare cases
- A distinguishing characteristic is the ability of colonies to exhibit blue-white autofluorescence when examined under ultraviolet light. Thus, *L. anisa*, along with several other species of *Legionella*, is sometimes referred to as "blue-white" *Legionella*.

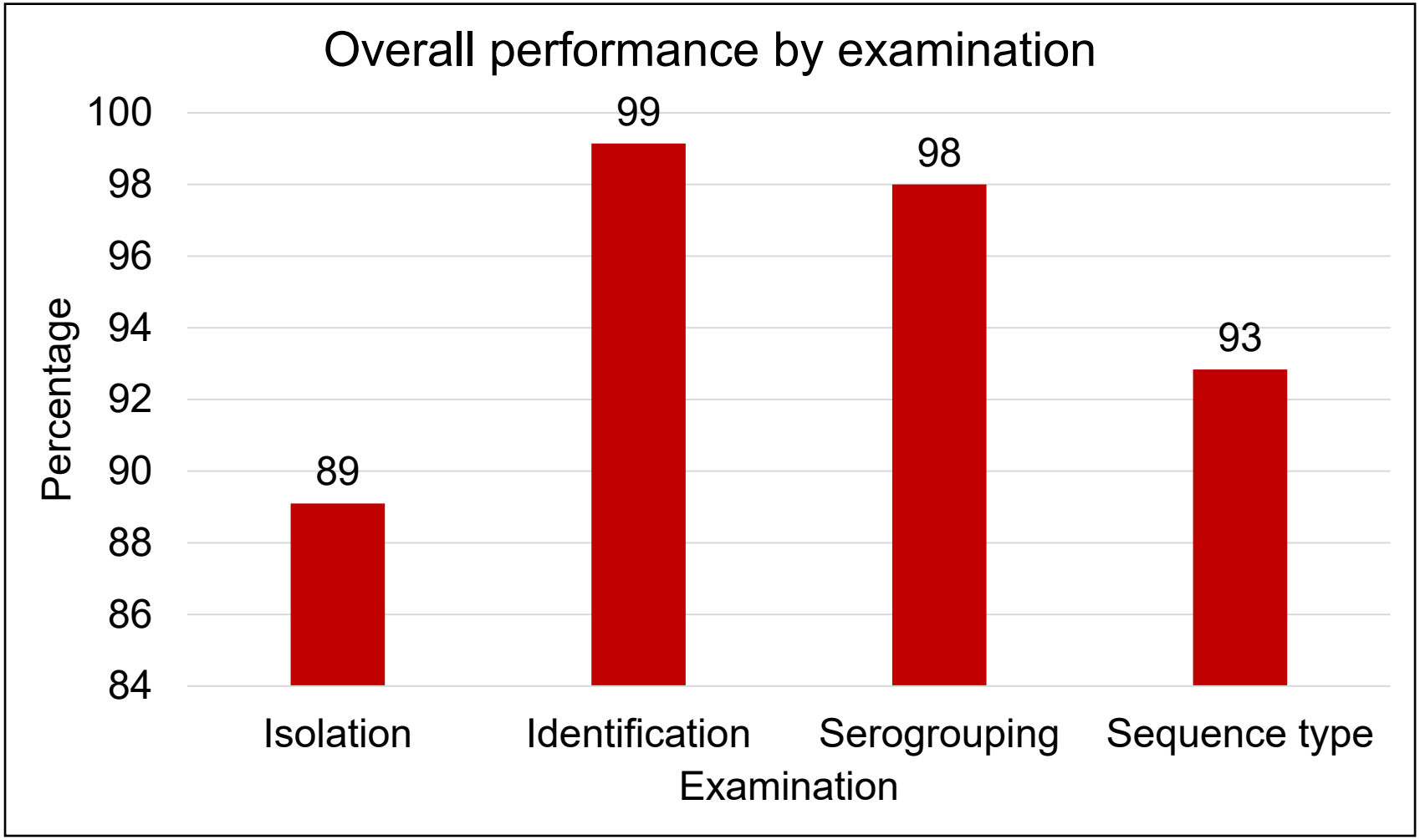
Performance – outbreak (isolation)

Sample number	Content	Performance n (%)
7044	*Legionella pneumophila* serogroup 1, ST62	14/18 (79)
7045	*Legionella pneumophila* serogroup 1, ST62	18/18 (100)
7046	No legionellae	16/18 (89)
7047	*Legionella anisa*	17/18 (94)
7048	*Legionella pneumophila* serogroup 1, ST62	15/17 (88)

Performance – routine monitoring (isolation)

Sample number	Content	Performance n (%)
7049	*Legionella pneumophila* serogroup, ST1	17/18 (94)
7050	No legionellae	13/16 (81)
7051	*Legionella pneumophila* serogroup 1, ST1	16/17 (94)
7052	No legionellae	17/18 (94)
7053	*Legionella pneumophila* serogroup 6, ST2923	14/18 (79)

Overall performance by examination



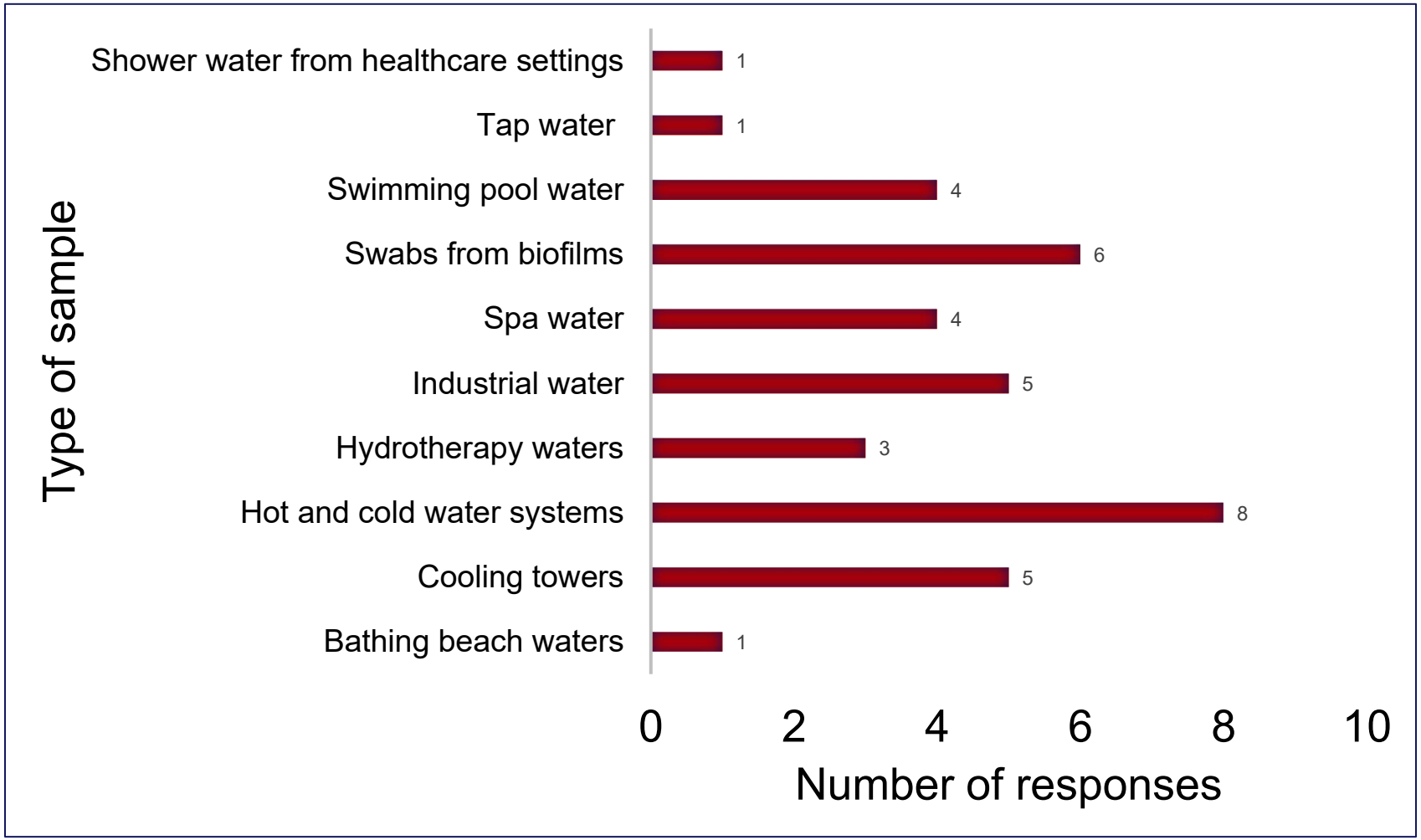
Enumeration performance

Sample number	No. of labs	Expected range cfu per litre	Number of outliers	Median cfu per litre	% within expected range
7044	13	$1.1x10^2 – 3.9x10^3$	2 (1 low, 1 high)	$1.1x10^3$	84.6
7045	15	$1.2x10^3 – 1.1x10^5$	1 (1 high)	$2.5x10^4$	93.3
7048	13	$2.3x10^2 – 6.4x10^5$	1 (1 low)	$1.6x10^4$	92.3
7049	15	$6.1x10^2 – 1.9x10^4$	-	$3.8x10^3$	100
7051	15	$8.7x10^2 – 7.9x10^4$	2 (1 low, 1 high)	$5.0x10^3$	86.6

Only one laboratory reported an outlying count twice in this distribution.

Method information (n = 10)

Types of samples processed



Number of examinations done in a year?

	Culture isolation and identification	Serotyping	Molecular	Sequencing (sanger & WGS)
None		2	1	3
<10				1
10-99	1	2	5	2
100-499	3	3		1
500-1000	3		1	
>1000	2	1		

Method details

Did you outsource any tests used in this distribution 5114 to another laboratory?

- Of the 10 responses, nine stated they did not outsource any of the tests in this EQA exercise, one laboratory outsourced the test for sequenced based typing of *L. pneumophila* and WGS of *L. pneumophila*

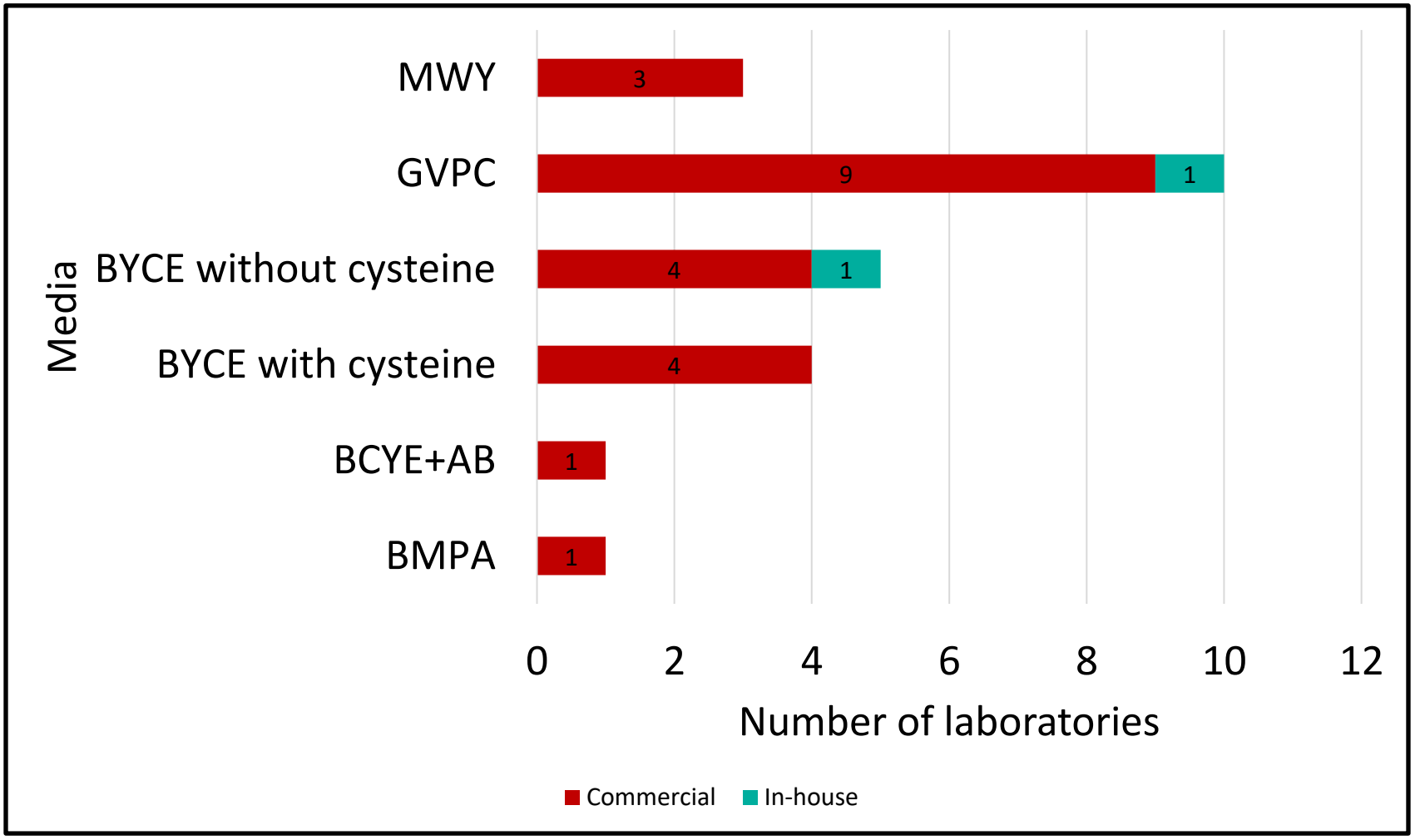
Did you have any difference in protocol for processing routine samples outbreak samples in this EQA distribution?

- Of the 10 responses received, eight stated they did not process the routine and outbreak samples differently

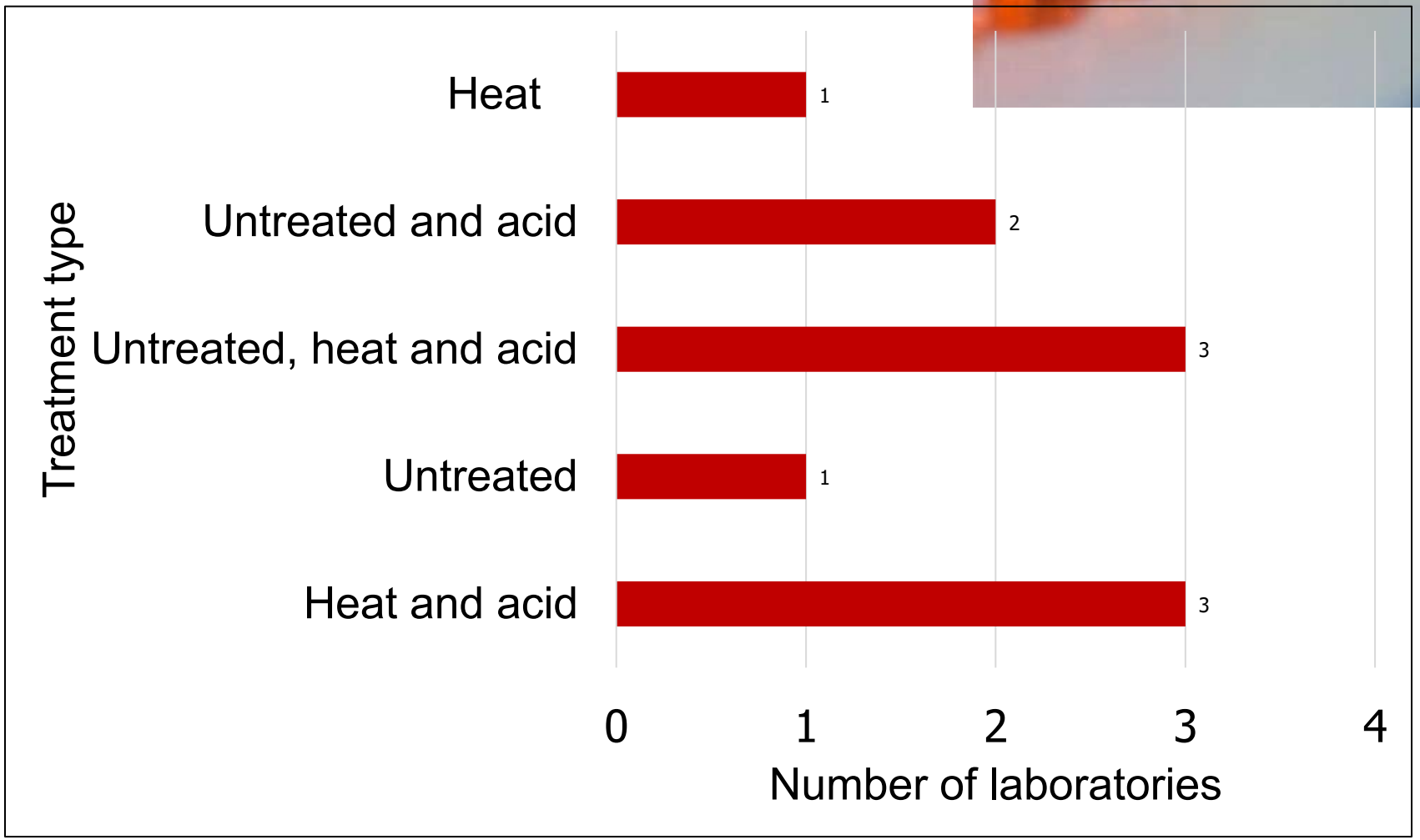
Which published method does your laboratory follow for analysing water/swab samples

- Of the 10 responses received nine used ISO 11731: 2017 for both water and swab samples, one laboratory used NF T90-431 v.2017 for water and an in-house method for swab samples

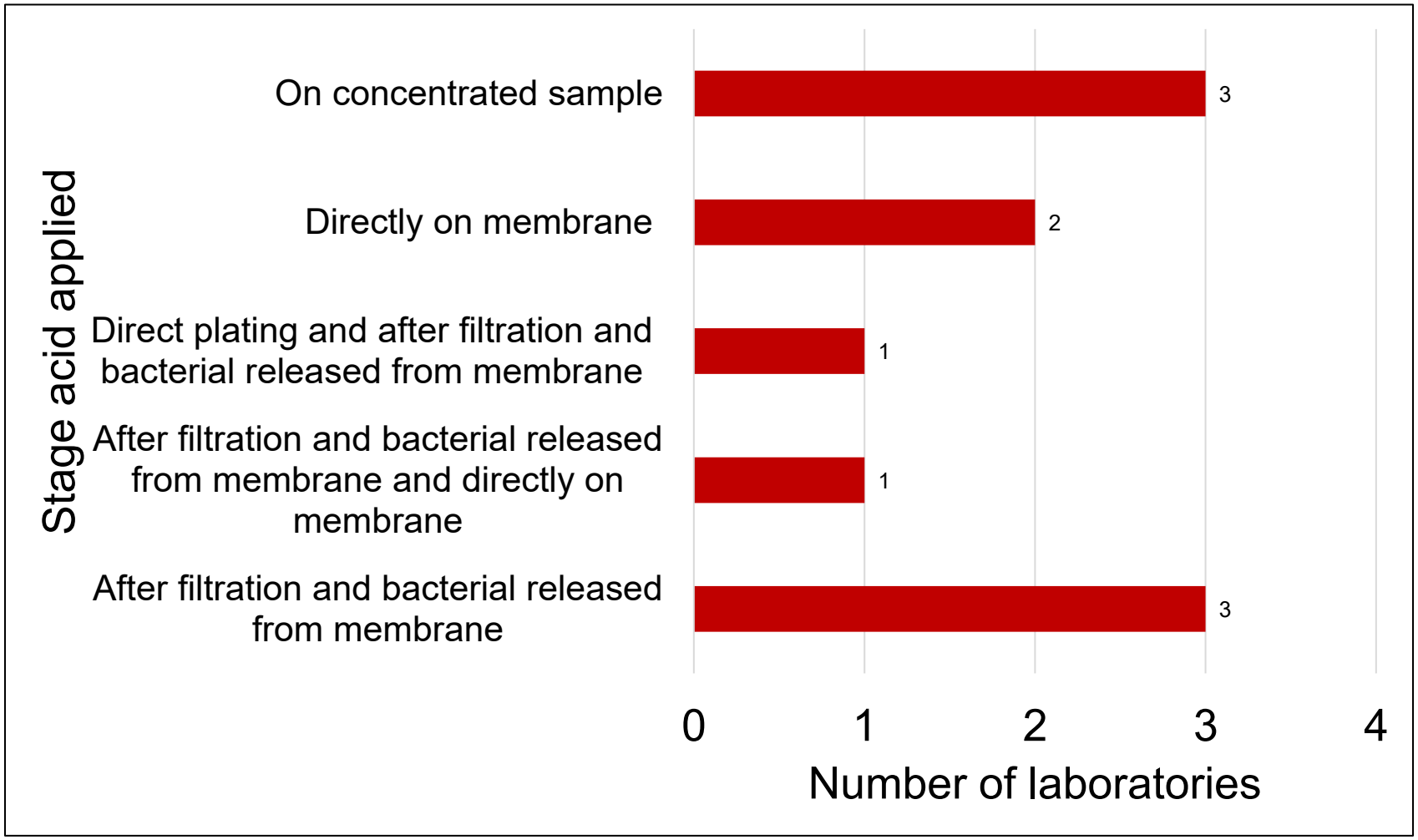
Media information



Treatment of the sample?



Stage treatment applied to sample



Conclusion for environmental samples for November 2020 and March's distribution

- Overall performance was very good with isolation, serogroup, enumeration and sequence typing undertaken by the laboratories
- The lower detection limit for culture methods varied between countries
- Filtration of water samples is the method of choice
- Using a molecular method for examination of samples is still uncommon

Benefits of taking part in EQA

Some of the benefits of participating in this EQA are to:

- Provide laboratory management with an insight of their performance
- Help to improves local standards
- Reveal unsuspected areas of difficulty
- Provide an educational stimulus for improvement
- Act as a check on the efficacy of internal quality control procedures
- Demonstrate to colleagues and customers a commitment to quality
- Provide method performance evaluation
- Provide independent evidence of performance for accreditation bodies
- Allow participants to monitor, evaluate and improve their own performance and training needs

Recommendations - 1

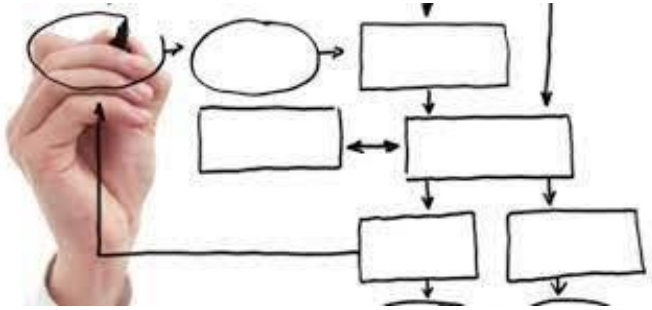
<u>Sample/specimen design:</u>

- To continue including different *L. pneumophila* serogroups (Sgs), sequence types (STs) and *Legionella* species
- To identify if there are issues when less commonly encountered species, Sg or ST are included. A single EQA distribution cannot confirm this
- For environmental samples to include levels of *Legionella* spp. that are on the lower end of the detection limit for culture methods
- Design of the simulated samples/specimens with to include more than one species of *Legionella* spp.

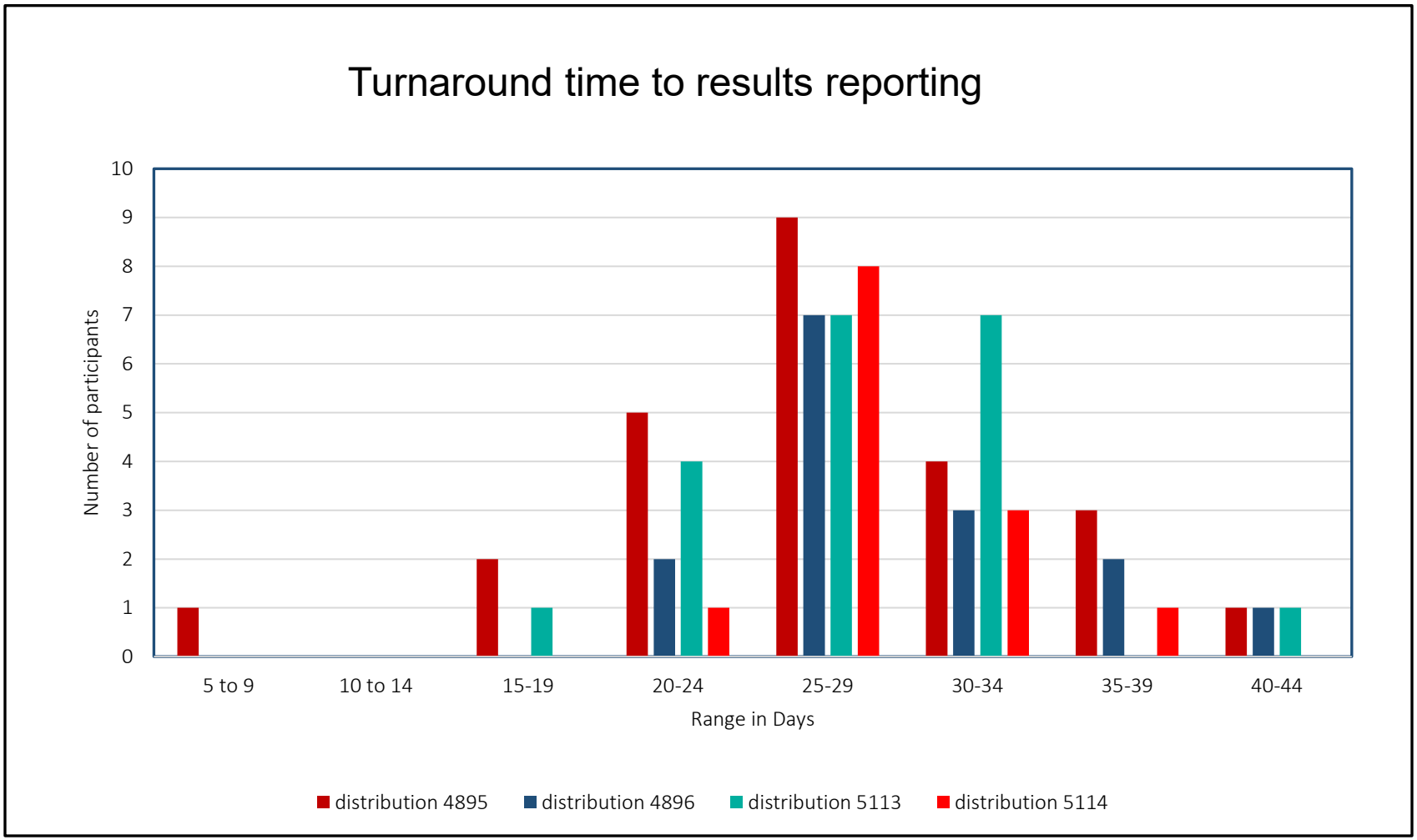
Recommendations - 2

Methods:

- To continue gathering exact methods used by participants to report results on the samples/specimens
- To improve the link between the method information with results reported and identify tests that laboratories perform routinely but did not report a result upon



Turnaround time for reporting results



Discussion points- for virtual meeting 19 October 2021

1. Why is molecular testing not performed on outbreak samples?
2. Some laboratories have obtained poor results with molecular tests when done – unsure how the EQA was used to assess their own ability
3. Sequence type results are reported even less on non serogroup 1 strains, why?
4. For clinical we observed that for positive urine specimen which is paired with a negative sputum specimen a lower concordance for performance was achieved, why?
5. It is evident that some laboratories need further support, however unclear how this is being done?

Acknowledgements

Lara Payne Hallstrom and Tessa Foronda-Pettersson at ECDC

All the laboratories who participated in this EQA exercise

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National ECDC correspondents in Western Balkans and Turkey

EQA services staff

Shipping agents