REPORT NO. 1138

DIALYSIS WATERS

PROFICIENCY TESTING PROGRAM

ROUND 4

MAY 2019

ACKNOWLEDGMENTS

PTA gratefully acknowledges the technical advice provided for this program by Dr M Hodge of PathWest Laboratory Medicine WA.

Also thank you to Ms N Patel from the Food and Environmental Proficiency Testing Unit (FEPTU) of Public Health England (PHE) who supplied the samples and Ms S Giannoulidis of Global Proficiency Pty Ltd who distributed the samples

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CONTENTS Page

1. FOREWORD

This report summarises the results of a microbiological proficiency testing program on dialysis waters, i.e. waters used to prepare dialysis fluids. This program is accredited to ISO/IEC 17043:2010 “Conformity assessment - General requirements for proficiency testing” by International Accreditation New Zealand (IANZ).

The program was conducted in February/March 2019 by Proficiency Testing Australia (PTA). The Program Coordinator was Mrs K Weller and the technical adviser was Dr M Hodge of PathWest Laboratory Medicine WA. This is the fourth round in a series of on-going dialysis waters proficiency testing programs. This report was authorised by Mrs K Cividin, PTA Quality Manager.

The aim of the program was to assess laboratories’ ability to competently perform the tests examined.

2. FEATURES OF THE PROGRAM

(a) A total of three laboratories received samples for this program all of which were located in Australia. All laboratories returned results for inclusion in the report.

(b) A code number was randomly allocated to each participant. All reference to the participants in this report is via the code numbers they were allocated, thus ensuring the confidential treatment of results.

(c) Each participant was sent three lenticule discs, labelled DW16B, DW17A and DW17B

(d) Participants were requested to test the samples for the total viable count (TVC), giving quantitative results (in cfu/mL).

(e) Laboratories were requested to perform the tests according to the *Instructions to Participants* provided and to record the results on the accompanying *Results Sheet*, both of which were distributed to participants with their samples. (See Appendix C of this report).

(f) Along with their result for each sample, laboratories were requested to report a Measurement Uncertainty (MU) and details of the test method used. These are presented in Appendix A, together with calculated z-scores.

As is the convention with microbiological count data, the raw results were transformed ($\log_{10}$) before being analysed statistically.

3. FORMAT OF THE APPENDICES

(a) Appendix A contains the following (where relevant):

- a table of the results reported by laboratories,
- the transformed (log_{10}) results and calculated z-scores,
- tables of summary statistics,
- ordered robust z-score charts.

(b) Appendix B contains details of the samples homogeneity and stability testing used in the program.

(c) Appendix C contains copies of the *Instructions to Participants* and *Results Sheet*, as supplied to participants.

4. STATISTICAL DESIGN OF THE PROGRAM

For this proficiency testing program, samples were provided by the Food and Environmental Proficiency Testing Unit (FEPTU) of Public Health England (PHE) in the form of lenticule discs.

For this round z-scores have been calculated using the total viable count (TVC) results provided by FEPTU. The below table shows the sample results and contents.

TABLE A - SAMPLE RESULTS AND CONTENTS

SAMPLE	**DW16B**	**DW17A**	**DW17B**
FEPTU Participants' Median TVC (cfu/mL)	47	N/A	152
Uncertainty of the Median	5	N/A	5
Species	*Micrococcus luteus*	No micro-organisms	*Enterobacter cloacae* and *Enterococcus faecalis*

Z-scores have been generated using the following calculation:

$Z = (x – X) / \sigma$

Where x = participant's result (expressed as a $\log_{10}$ value)

X = FEPTU Participants' Median result (expressed as a $\log_{10}$ value) from FEPTU/PHE Report No's. DW16 and DW17

σ = the fixed standard deviation for the examination (calculated by FEPTU)

The σ-value used for calculating z-scores for all parameters in this round is 0.35.

5. OUTLIER RESULTS

Any result which has an absolute z-score value greater than or equal to three (i.e. $Z \leq -3.0$ or $Z \geq 3.0$) is classified as an outlier. For further details on the calculation and interpretation of the robust z-scores, please see the *Guide to Proficiency Testing Australia* [1].

In addition to statistical outliers, other types of outlier results are false positives/negatives - i.e. when a laboratory erroneously reports the presence/absence of an organism or species which is/is not present.

SUMMARY OF OUTLIER RESULTS

Code numbers of the laboratories whose results have been identified as outliers appear in Tables B and C, i.e. these laboratories have either statistical outliers (identified by the robust z-scores technique) or false negatives.

TABLE B - FALSE NEGATIVE RESULTS

Test	Sample DW16B Laboratory Code Number	Sample DW17A Laboratory Code Number	Sample DW17B Laboratory Code Number
Total Viable Count FALSE NEGATIVE	2	-	-

TABLE C - STATISTICAL OUTLIERS - TOTAL VIABLE COUNT

Total Viable Count	Sample DW16B	Sample DW17A	Sample DW17B
Robust Z-Score	1	-	-

6. PTA AND TECHNICAL ADVISER'S COMMENTS

Complete details of the results received and the statistical analyses appear in Appendix A. Commentary on each of the tests is presented below.

Round 4 of the Dialysis Waters program included samples with both gram positive organisms (*Micrococcus luteus*) suggestive of skin contamination of the sample, and mixed gram positive/gram negative (*Enterococcus* and *Enterobacter*) suggestive of a faecal contamination picture. There was also a negative sample.

Membrane Filtration (MF) was used by all laboratories, with two laboratories also performing spread plates. In this round, all laboratories used R2A at 22°C incubation temperature for 7 days. Varying dilutions were employed . Only one participant (Laboratory code 2) reported values of <1 cfu/ml, so this laboratory would not be able to satisfy the requirement for Ultrapure dialysis water should clients require this detection level (ie <0.1 cfu/ml).

One participant (Laboratory code 2) reported a false negative result for Sample DW16B, with Laboratory code 1 reporting an outlier (low) for the same sample. Laboratories with outlying results and false negatives should review their procedures. Particular care should be taken to reconstitute the lenticule discs according to the *Instructions to Participants*, adhering to the specified holding conditions, and using the correct volumes of diluent and temperatures for rehydration steps. Transfer of all the sample into 1L sterile or distilled water with the final rinse of the sample bottle must occur, with adequate homogenisation. Low counts could occur if these procedures are not followed. Other sporadic errors, such as failure to inoculate media, could account for false negatives. Further discs can be requested if laboratories wish to pursue a repeat test.

The ISO 11663:2009 standard has been superseded by ISO 11663:2014, therefore it would be prudent that laboratories quoting the ISO 11663 method have reviewed the 2014 version.

Total Viable Count (TVC) Enumeration Results
(refer Appendix A: pages A-1 to A-3)

Laboratories were also requested to report MU for each reported result. These values are tabulated in Appendix 1 as reported by the participants. Two laboratories reported MU estimations as a range in cfu/L values.

Analysis of Grouped Methods
In order for methods to be grouped for analysis, PTA requires at least 11 sets of results from the same method group. As there were less than 11 sets of results submitted, and due to the statistical analysis employed, the analysis of grouped methods does not apply for this round.

Metrological Traceability and Measurement Uncertainty of Assigned Values
Samples (lenticule discs) used for this program were provided by FEPTU of PHE and were prepared according to their standard operating procedures. Participants' median and the standard deviation provided by FEPTU were used to determine z-scores for this round. The uncertainty of the assigned value as determined by FEPTU for each of the samples DW16B, and DW17B is 5 cfu/mL. FEPTU is accredited to ISO/IEC 17043:2010 by the United Kingdom Accreditation Service (UKAS).

7. REFERENCES

[1] *Guide to Proficiency Testing Australia (2016)*. [This document is located on the PTA website at www.pta.asn.au, under Programs/Documents.]

[2] *Summary of Results – External Quality Assessment of Water Microbiology, Dialysis Water Scheme.* Distribution Number: DW12. Food and Environmental Proficiency Testing Unit (FEPTU).

[3] *Summary of Results – External Quality Assessment of Water Microbiology, Dialysis Water Scheme.* Distribution Number: DW13. Food and Environmental Proficiency Testing Unit (FEPTU).

[4] *Summary of Results – External Quality Assessment of Water Microbiology, Dialysis Water Scheme.* Distribution Number: DW14. Food and Environmental Proficiency Testing Unit (FEPTU).

[5] ISO 11663:2014 *Quality of dialysis fluid for haemodialyis and related therapies.*

[6] ISO/IEC 17043:2010 *Conformity assessment - General requirements for proficiency testing.*

APPENDIX A

Tables of Results and Z-Scores

Total Viable Count Results

RESULTS SUBMITTED (cfu/mL)

Lab Code	Total Viable Count					
	DW16B	MU	DW17A	MU	DW17B	MU
1	2	1.2 - 2.5	<0.02	N/A	70	48 - 100
2	<1	-	<1	-	120	-
3	28	16 to 50	<0.01	-	160	90 to 280

TOTAL VIABLE COUNT (Sample DW16B)
TRANSFORMED RESULTS (log_{10}cfu/mL) and Z-SCORES

Lab Code	Total Viable Count [log_{10} (cfu/mL)]	Z-Score
	Sample DW16B	
1	0.23	-4.12 §
2	N/A	†
3	1.45	-0.64

Notes:

1. N/A - not applicable.
2. † - denotes a false negative.
3. § - denotes an outlier result (i.e |z-score| ≥ 3.0)

Summary Statistics (Sample DW16B)

No. of Results	57
Median (FEPTU)	1.672
Fixed Standard Deviation	0.35
Robust CV	14.5%
Minimum	0.00
Maximum	2.23
Range	2.23
Uncertainty (Median)	0.042

Note:

1. For this round z-scores have been calculated using the total viable count (TVC cfu/mL) results provided by FEPTU.

TOTAL VIABLE COUNT (DW17B)
TRANSFORMED RESULTS (log_{10}cfu/mL) and Z-SCORES

Lab Code	Total Viable Count [log_{10} (cfu/mL)]	Z-Score
	Sample DW17B	
1	1.85	-0.96
2	2.08	-0.29
3	2.20	0.06

Summary Statistics (Sample DW17B)

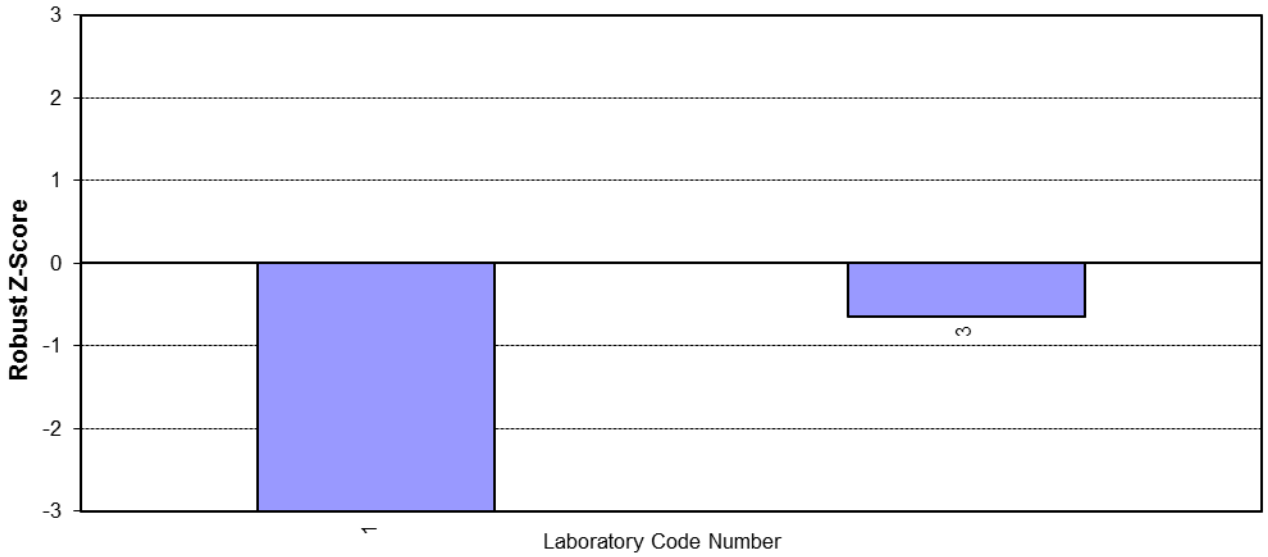
No. of Results	62
Median (FEPTU)	2.182
Fixed Standard Deviation	0.35
Robust CV	4.1%
Minimum	1.18
Maximum	2.36
Range	1.18
Uncertainty (Median)	0.014

Note:

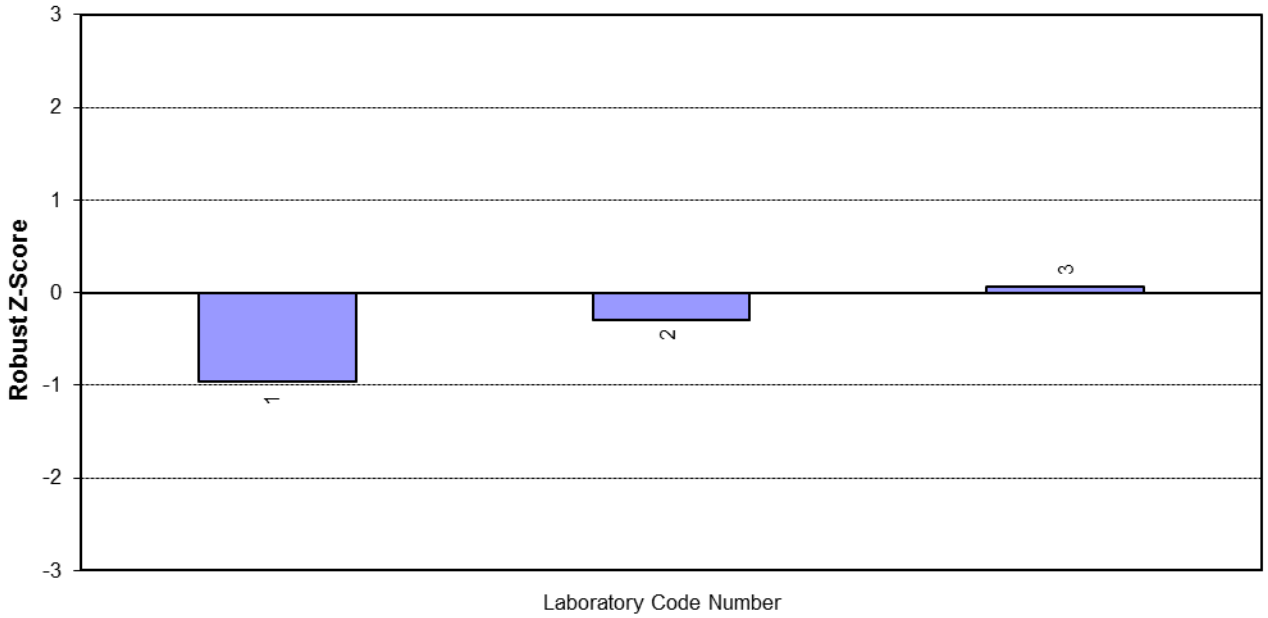
1. For this round z-scores have been calculated using the total viable count (TVC cfu/mL) results provided by FEPTU.

Total Viable Count (cfu/mL)
Ordered Robust Z-Score Charts

Sample - DW16B



Sample - DW17B



APPENDIX B

Homogeneity and Stability Testing

HOMOGENEITY AND STABILITY TESTING

The PHE Water Scheme for Dialysis is accredited by the United Kingdom Accreditation Service (UKAS) to ISO/IEC 17043:2010. Samples provided for this round are simulated samples. PHE conducted testing which found the samples to be homogeneous and stable for the duration of this round.

APPENDIX C

Instructions to Participants
and
Results Sheet

Proficiency Testing Australia

Dialysis Waters Proficiency Testing Program

Round 4 (Lenticule discs)

INSTRUCTIONS TO PARTICIPANTS

To ensure that results obtained from this program can be analysed properly, participants are asked to adhere carefully to the following instructions.

1. Each participant is supplied with three LENTICULE discs in screw cap plastic vials (with desiccant). The desiccant should be orange in colour, please contact PTA if this is not the case. The LENTICULE discs require reconstitution by a process of re-hydration and dispersion prior to examination, as described below. The Safety Data Sheet for lenticules can be found at the following website: www.gov.uk/government/publications/safety-data-sheet-for-lenticules

2. **Storage:**
   a) Store the samples at **-20 ± 5ºC** on receipt.
   b) Allow the LENTICULE discs to reach ambient temperature (5 – 10 minutes) **before** reconstituting in diluent.

3. **Reconstitution:**
   a) Open the sample container and transfer the LENTICULE disc into approximately **9mL** 0.1% peptone saline (maximum recovery diluent (MRD)) that has been allowed to reach ambient temperature. Please note that normal saline is an acceptable alternative.
   b) Leave at ambient temperature for 10-12 minutes to rehydrate.
   c) Tighten the cap of the bottle and shake to disperse the micro-organisms.
   d) Transfer all the inoculated MRD to a sample bottle containing 1L sterile deionised or distilled water at ambient temperature.
   e) Rinse with approximately 2mL from the sample bottle, ensuring all liquid is transferred back to the 1L sample.
   c) Disperse the inoculum by inverting approximately 30 times.

4. **Examination:**
   a) Each reconstituted sample is equivalent to 1L water.
   b) Undertake the sample examinations within 45 minutes of reconstitution.
   c) Examine in accordance with your routine procedures.

   Participants are also requested to provide details of the test methods used.

   To aid us with the statistical analyses of the results we ask that all laboratories set up methods such that you can report actual numerical results.

5. Laboratories are requested to calculate and report an estimate of measurement uncertainty (MU) for each reported measurement result. All estimates of MU must be given as a 95% confidence interval (coverage factor

k ≈ 2). Submitted MU information will not form part of the evaluation of performance, and is for information purposes only.

6. Your laboratory has been allocated the code number shown on the attached Results Sheet. All reference to your laboratory in the final report for this program will be through this code number, thus ensuring the confidentiality of your results.

7. All laboratories must return the Results Sheet no later than **Tuesday 12th March 2019** to:

Kathy Weller
Proficiency Testing Australia
PO Box 1122
Archerfield BC QLD 4108 Australia
phone: +61 7 3721 7373
fax: +61 7 3217 1844
email: Kathy.Weller@pta.asn.au

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Proficiency Testing Australia

Dialysis Waters Proficiency Testing Program – Round 4

Results Sheet

Laboratory Code: ____

Samples received at _____ (time) on _____ (date) Temperature of samples on arrival _____

Testing commenced on: _____ (date)

Sample Number	Enumeration	Result	MU
DW16B	TVC 17ºC - 23ºC for 7 days per mL		
DW17A	TVC 17ºC - 23ºC for 7 days per mL		
DW17B	TVC 17ºC - 23ºC for 7 days per mL		

Method used	
Medium used	
Sample Volume used	
Incubation conditions (time/temperature/atmosphere)	
Standard/Guideline followed (please include title, Standard/Guideline unique number and date/edition)	
Additional comments (include any deviations from Standard method/guideline)	

Signature: _____ Date: _____

Please return results **NO LATER THAN TUESDAY 12TH MARCH 2019** to:

Kathy Weller, Proficiency Testing Australia
PO Box 1122, Archerfield BC QLD 4108 Australia
phone: +61 7 3721 7373, fax: +61 7 3217 1844, email: Kathy.Weller@pta.asn.au

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