FAPAS® Report 15109

Sodium Nitrate and Sodium Nitrite in Meat

January-February 2016

PARTICIPANT LABORATORY NUMBER

Participants can log in to FAPAS SecureWeb at any time to obtain their laboratory number for this proficiency test.

Laboratory numbers are displayed in SecureWeb next to the download link for this report.

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Fera is an ISO 9001 certified organisation.

UKAS PROFICIENCY TESTING
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SUMMARY

1. The test material for FAPAS® proficiency test 15109 was dispatched in January 2016. Each participant received a meat test material to be analysed for nitrate and nitrite as the sodium salts, $NaNO_3$ and $NaNO_2$ respectively.

2. An assigned value (x_a) was determined for each analyte and in conjunction with the standard deviation for proficiency (σ_p) was used to calculate a z-score for each result.

3. Results for this proficiency test are summarised as follows:

analyte	assigned value, x_a mg/kg	number of scores, $\|z\| \leq 2$	total number of scores	% $\|z\| \leq 2$
nitrate (as $NaNO_3$)	573	33	39	85
nitrite (as $NaNO_2$)	15.6	16	40	40

CONTENTS

1. INTRODUCTION

1.1. Proficiency Testing

Proficiency testing aims to provide an independent assessment of the competence of participating laboratories. Together with the use of validated methods, proficiency testing is an essential element of laboratory quality assurance.

Further details of the FAPAS® proficiency testing scheme are available in our protocols [3, 4].

2. TEST MATERIAL

2.1. Preparation

Preparation of the samples for this proficiency test was sub-contracted to a laboratory meeting the quality requirements of the scheme's accreditation [2].

A quantity of gammon was purchased from a retail source, minced, freeze-dried, milled, sieved and mixed. The freeze-dried gammon was screened to establish the levels of nitrate and nitrite present; which was then left at the natural levels.

Samples were stored at -20˚C until dispatch.

2.2. Homogeneity

To test for homogeneity, randomly selected test materials were analysed in duplicate. Testing was sub-contracted to a laboratory meeting the quality requirements of the scheme's accreditation [2].

These data showed sufficient homogeneity and were not included in the subsequent calculation of the assigned values.

2.3. Dispatch

The start date was 6 January 2016. Test materials were sent to 52 participants.

3. RESULTS

The instructions for reporting results were as follows:

> Determine the level of nitrate and nitrite present in the test material, in mg/kg, as received. Results should be expressed as the sodium salts ($NaNO_3$ and $NaNO_2$). It is important that you report the results in this way so that we can include as many results as possible in the statistical analysis.

Results were submitted by 46 participants (88%) before the closing date for this test, 4 February 2016.

Each participant was given a laboratory number, assigned in order of receipt of results. The reported analyte concentrations are given in Table 1.

Participants' comments are given in Table 2.

The analytical methods used by each participant are summarised in APPENDIX I.

4. STATISTICAL EVALUATION OF RESULTS

The results submitted by participants were statistically analysed in order to provide an assigned value for each analyte. The assigned values were then used in combination with the standard deviation for proficiency, σ_p, to calculate a z-score for each result. The procedure follows that recommended in the IUPAC International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [5].

Further details on the procedure followed can be found in the relevant protocols [3, 4].

4.1. Calculation of the Assigned Value, x_a

The assigned value, x_a, for each analyte was derived from the consensus of the results submitted by participants.

The following results were excluded from the calculation of the assigned value:

i) non numerical results i.e. qualitative or semi-quantitative results,

ii) results reported as approximately 10, 100 or 1000 × greater or smaller than the majority of submitted results (as these were considered to be reporting errors).

For sodium nitrate, this procedure was straightforward and the robust mean was chosen as the assigned value.

For sodium nitrite, the major mode was chosen as the assigned value because the distribution of results was skewed towards the high end. A plot showing the distribution of results can be seen as an insert to Figure 2.

The assigned values for all analytes are shown in Table 3.

4.2. Standard Deviation for Proficiency, σ_p

The standard deviation for proficiency, σ_p, was set at a value that reflects best practice for the analyses in question.

For each analyte, σ_p was derived from collaborative trial data [6].

The values for σ_p used to calculate z-scores from the reported results of this test are given in Table 3.

4.3. Individual z-Scores

Participants' z-scores were calculated as:

$$z = \frac{(x - x_a)}{\sigma_p}$$

where x = the participant's reported result,
x_a = the assigned value
and σ_p = the standard deviation for proficiency.

Participants' z-scores for all analytes are given in Table 1 and shown as histograms in Figures 1–2. It is possible for the z-scores published in this report to differ slightly from the z-score that can be calculated using the formula given above. These differences arise from the necessary rounding of the actual assigned values and standard deviations for proficiency prior to their publication in Table 3.

The number and percentage of z-scores in the range $-2 \leq z \leq 2$ for all analytes are given in Table 4.

5. INTERPRETATION OF SCORES

In normal circumstances, over time, about 95% of z-scores will lie in the range $-2 \leq z \leq 2$. Occasional scores in the range $2 < |z| < 3$ are to be expected, at a rate of 1 in 20. Whether or not such scores are of importance can only be decided by considering them in the context of the other scores obtained by that laboratory.

Scores where $|z| > 3$ are to be expected at a rate of about 1 in 300. Given this rarity, such z-scores very strongly indicate that the result is not fit-for-purpose and almost certainly requires investigation.

The consideration of a set or sequence of z-scores over time provides more useful information than a single z-score. Examples of suitable methods of comparison are provided in the IUPAC International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [5].

6. REFERENCES

1 Adobe Certified Document Services, http://www.adobe.com/misc/pki/cds_cp.html, accessed 10/05/2015.

2 ISO/IEC 17043:2010, Conformity assessment – General requirements for proficiency testing.

3 FAPAS, 2014, Protocol for Proficiency Testing Schemes, Part 1 – Common Principles, Version 4, Issued May 2014.

4 FAPAS, 2014, Protocol for Proficiency Testing Schemes, Part 2 – FAPAS®, Version 3, Issued May 2014.

5 Thompson, M., Ellison, S.L.R. and Wood, R., 2006, The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, *Pure Appl. Chem.*, **78**, No. 1, 145–196.

6 Fiddler, R.N., 1977, Collaborative Study of Modified AOAC Method of Analysis for Nitrite in Meat and Meat Products, *J. Assoc. Off. Anal. Chem.*, **60(3)**, 594-599.

Table 1: Results and z-Scores

laboratory number	analyte			
	nitrate (as $NaNO_3$) assigned value 573 mg/kg		nitrite (as $NaNO_2$) assigned value 15.6 mg/kg	
	result mg/kg	z-score	result mg/kg	z-score
001	530	-0.6	18	1.2
002			14.9	-0.4
003	602	0.4	<15	
004	615	0.6	38	**11.7**
005	539	-0.5	22	**3.3**
006	579	0.1	23	**3.9**
007			16.33	0.4
008	580	0.1	<15.0	
009	637	0.9	17.01	0.7
010	682	1.6	18	1.2
011	445.98	-1.8	11.08	**-2.4**
012	558	-0.2	17	0.7
013	623	0.7	32	**8.6**
014			635.5	**325.7**
015	650.11	1.1	28.50	**6.8**
016			14.95	-0.4
017	660	1.3	7	**-4.5**
018	600	0.4	16.5	0.5
019	1332	**10.9**	14	-0.9
020	629	0.8		
021	93.4	**-6.9**	617.8	**316.4**
022	261	**-4.5**	16.6	0.5
023	507.6	-0.9	4.035	**-6.1**
024	623.84	0.7	15.6	0.0
025	185.9	**-5.5**	14.6	-0.5
026	627.21	0.8	47.01	**16.5**
027			9.45	**-3.3**
028	563.8	-0.1	47.6	**16.8**

z-scores outside |z| >2 are shown in **bold,** see Section 5

Table 1: Results and z-Scores

laboratory number	analyte			
	nitrate (as $NaNO_3$) assigned value 573 mg/kg		nitrite (as $NaNO_2$) assigned value 15.6 mg/kg	
	result mg/kg	z-score	result mg/kg	z-score
029	532.42	-0.6	13.43	-1.2
030	481.9	-1.3	6.1	**-5.0**
031	576	0.0	<40	
032	578	0.1	12	-1.9
033	579.6	0.1	27.6	**6.3**
034	599	0.4	10.1	**-2.9**
035	649.9	1.1	29.7	**7.4**
036	513	-0.9	16	0.2
037	590	0.2	30	**7.5**
038			36.88	**11.2**
039	400	**-2.5**	<5	
040	541	-0.5	13.83	-1.0
041	>400		9.2	**-3.4**
042	606	0.5	24.6	**4.7**
043	625.8	0.8	22	**3.3**
044	607	0.5	<10	
045	609.14	0.5	10.74	**-2.6**
046	418.0	**-2.2**	76.1	**31.8**

z-scores outside |z| >2 are shown in **bold,** see Section 5

Table 2: Participants' Comments

participant number	comments
008	sodium nitrite determined below limit of quantification
020	sodium nitrite not reported due to interference.
035	corrected for recovery

comments are as submitted by participants

Table 3: Assigned Values and Standard Deviations for Proficiency

analyte	data points, *n*	assigned value, x_a mg/kg	uncertainty, *u*	standard deviation for proficiency, σ_p	
nitrate (as $NaNO_3$)	39	573	12.2	Coll. Trial	69.7
nitrite (as $NaNO_2$)	38	15.6	0.52	Coll. Trial	1.90

Table 4: Number and Percentage of z-Scores where |z| ≤2

analyte	number of scores, |z| ≤2	total number of scores	% |z| ≤2
nitrate (as $NaNO_3$)	33	39	85
nitrite (as $NaNO_2$)	16	40	40

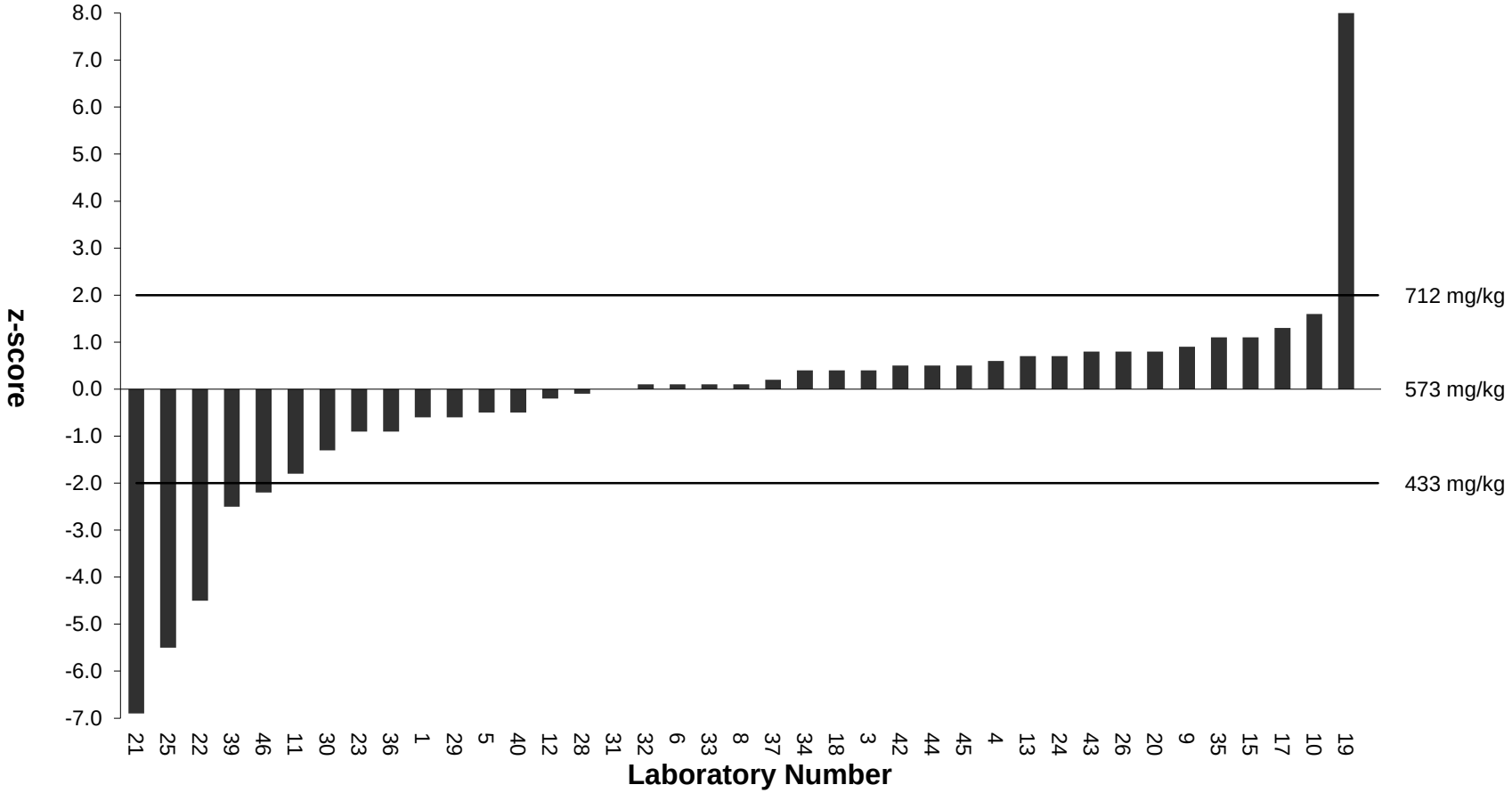

Figure 1: z-Scores for Nitrate (as $NaNO_3$)

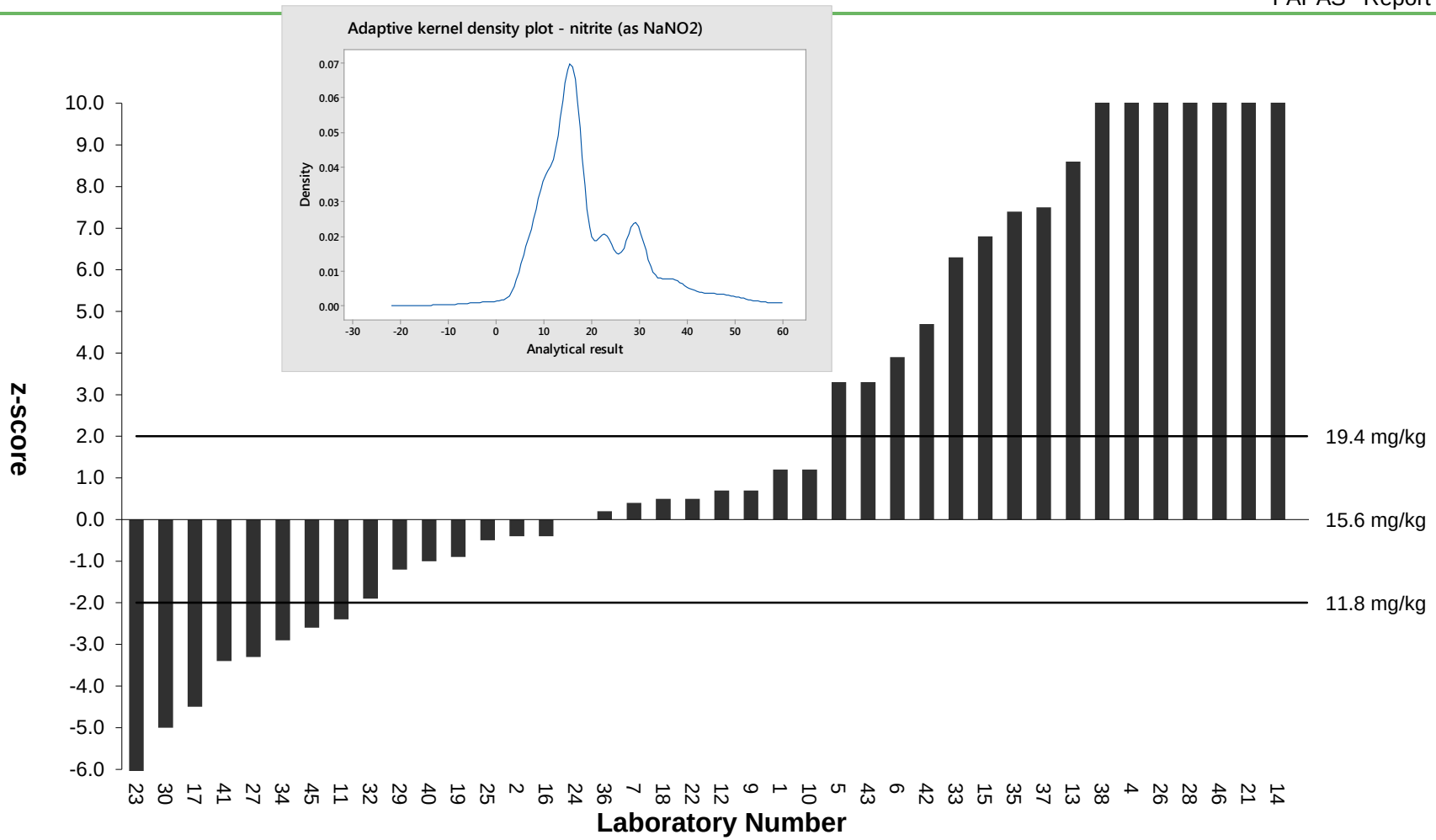

Figure 2: z-Scores for Nitrite (as $NaNO_2$)

APPENDIX I: Analytical Methods Used by Participants

Methods are tabulated according to the information supplied by participants, but some responses may have been combined or edited for clarity.

Method Used Accredited	laboratory number
yes	002 008 012 013 015 016 019 021 022 023 025 027 028 034 035 037 040 041 044
no	003 004 005 024 039

Method Based On	laboratory number
International Standard	003 013 015 027 034 035 044
National Standard	002 004 016 023 025 037
Paper Published In An International Journal	021
Manufacturer/Kit Instructions/Technical Note	019
In house method	005 008 022 028 040 041

Sample Weight (g)	laboratory number
<1	037
≥1 - <2	004
≥2 - <5	008 013 015 021 022 034 035 044
≥5 - <10	012 016 024 025 027 028
≥10 - <25	002 003 005 019 040 041

Extraction Procedure	laboratory number
cold water extraction	037
heat	037
hot borax	004 016 025 041
hot water extraction	002 003 005 008 019 021 024 025 027 028 034 040 044
sonicate/ultrasonic bath	023
Ultra Turrax	012 035
ultrasonic extraction	022
vortex mix	023

Protein Precipitation?	laboratory number
Carrez I & II	004 005 008 012 022 024 025 027 028 035 040 041
no	003 013 016 019 021 034
yes	002 023 025 037 044

Extraction pH	laboratory number
<6.0	021
≥6.0 - <7.0	019 023 025 034
≥7.0 - <8.0	004
≥8.0 - <9.0	013 024 035
≥9.0 - <10.0	040

Filtration Procedure	laboratory number
glass fibre	021
membrane	003 012 022 035
nitrate free paper	004 005 008 023 028 044
paper	002 013 016 019 024 025 027 034 037 040 041

Methodology	laboratory number
colorimetric method	002 004 005 015 016 024 025 027 040 044
HPLC	003 008 012 022 034
Ion Chromatography (IC)	019 021 023 028 035 037
FIA / colorimetric	041

Clean-up Prior to HPLC/IC	laboratory number
C18 Sep-Pak	008
solid phase C18	023 037

HPLC Column Packing	laboratory number
Ion Exchange	003 008 021 022 023 028 034 035
NH2	012

HPLC Column Temperature (°C)	laboratory number
ambient	003 008 023 028 034
>ambient - <50	012 019 021 022 035

HPLC Guard Column Used?	laboratory number
yes	003 019 021 022 023 028 034 035
no	005 008

Mobile Phase Components	laboratory number
acetonitrile	022
phosphate	008
sodium hydroxide	023 028
water	023
10mM KOH	021
Potassium Hydroxide	019
Sodium perchlorate	022
Water lithiumborate glukonate	034

Mobile Phase pH	laboratory number
<6.0	021 022
≥6.0 - <7.0	003 034
≥7.0 - <8.0	008
≥9.0 - <10.0	023

Isocratic Mobile Phase?	laboratory number
yes	003 008 012 019 021 022 028 035
no (gradient)	023 034

Mobile Phase Flow Rate (ml/min)	laboratory number
≥0.25 - <0.75	012 022
≥0.75 - <1.25	008 019 021 023 028 035
≥1.75 - <2.25	003 034

HPLC Injection Volume (µl)	laboratory number
≥10 - <25	008 019 022 028 034 035
≥25 - <50	012 021 023
≥50 - <100	003

HPLC Detector Type	laboratory number
conductivity	019 021 023
Diode Array Detector	003 008 012 022 034
UV	028
UV/Vis	035

HPLC Wavelength (nm)	laboratory number
<210	003 012 034 035
≥210 - <214	008 022
≥214 - <220	028

Colorimetric / Enzymatic Reduction Procedure	laboratory number
cadmium column	004 005 024 040 041

Colorimetric / Enzymatic Colour Reagents	laboratory number
N-(1-naphthyl)ethylenediamine dihydrochloride	004 016 025 027 040 041 044
sulfanilamide	005 013 024 025 027 044

Colorimetric / Enzymatic Detector Type	laboratory number
spectrophotometric	002 004 005 013 016 024 025 027 040 041 044

Colorimetric / Enzymatic Wavelength (nm)	laboratory number
≥450 - <500	005
≥520 - <540	002 004 024 025
≥540 - <560	016 027 040 041

APPENDIX II: FAPAS SecureWeb, Protocol and Contact Details

1. FAPAS SECUREWEB

Access to the secure area of our website is only available to participants in our proficiency tests. Please contact us if you require a UserID and Password. FAPAS SecureWeb allows participants to:

- Obtain their laboratory numbers for the proficiency tests in which they have participated.
- View the results they submitted in past and current proficiency tests.
- Submit their results and methods for current tests.
- Review future tests they have ordered.
- Order proficiency tests, reference materials and quality control materials.
- Freely download copies of reports (PDF file), of proficiency tests in which they have participated.
- View charts of their z-scores obtained in previous FAPAS® proficiency tests.

2. PROTOCOL

The Protocols [3, 4] set out how FAPAS® is organised. Copies can be downloaded from our website.

3. CONTACT DETAILS

This report was prepared and authorised on behalf of FAPAS by Elaine Leach (Round Coordinator). Participants with any comments or concerns about this proficiency test should contact:

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