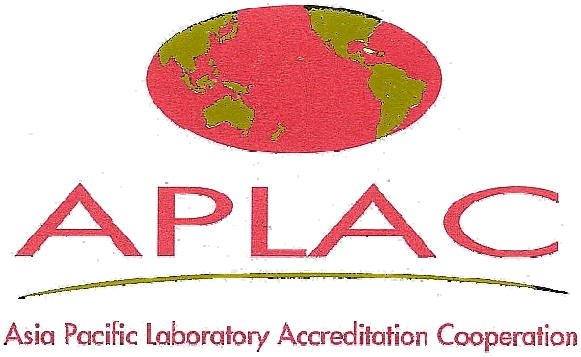


MALACHITE GREEN & LEUCOMALACHITE GREEN IN SWAMP EELS PROFICIENCY TESTING PROGRAMME

APLAC T058

FINAL REPORT

January 2008

CONTENTS

INDEX OF APPENDICES:

Summary of Results

1. The proficiency testing programme (APLAC T058) aimed at evaluating the testing capability of participants on the quantitative analysis of MG and LMG in swamp eels.

2. A total of 48 laboratories from 21 different economies participated in the programme. 45 participants (93.8%) returned their results on or before the deadline of the programme.

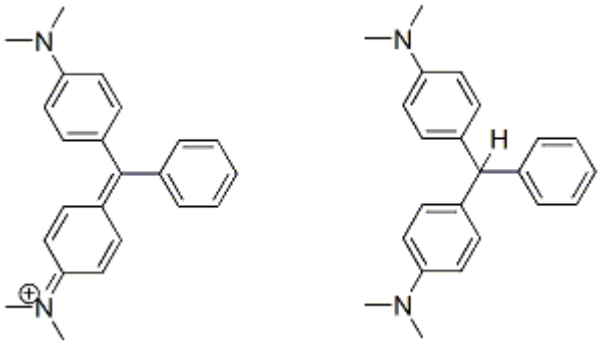
3. The assigned values used for z-score assessment were based on a reverse isotope dilution liquid chromatography-mass spectrometric (ID-LC-MS) method. The assigned values were 28.2 µg/kg for MG and 2.21 µg/kg for LMG respectively.

4. Results of APLAC T058 are as follows:

Performance Achieved	Number of Participants		Percentage of Participants	
	MG	LMG	MG	LMG
$\|z\| \leq 2$	20	18	48 %	62 %
$2 < \|z\| < 3$	9	3	21 %	10 %
$\|z\| \geq 3$	13	8	31 %	28 %
Total:	45	29	100 %	100 %

1. Introduction

1.1. Malachite green (MG), a triphenylmethane synthetic compound, had traditionally been used as an anti-fungal agent in aquaculture for the treatment of external fungal, parasitic and protozoan infections on fish eggs, fish, shellfish and as a general hatchery disinfectant since the 1930s. When animals treated with MG, the substance will be absorbed and rapidly metabolised in tissues. One of its major metabolites, leucomalachite green (LMG), was reported to persist in animal tissues for a long period of time. Owing to the potential carcinogenic and tetragenic properties of MG and its metabolites, most countries (Canada, China, EU, Japan, Korea, Singapore, UK, USA, etc.) had already banned the use of the chemical as a veterinary drug on any food animals for human consumption.



MG LMG

1.2. This report summarised the results of the proficiency testing programme (APLAC T058) on the quantitative analysis of MG and LMG in swamp eels. APLAC T058 was organised by the Hong Kong Government Laboratory (HKGL), in collaboration with Hong Kong Accreditation Service (HKAS), under the auspices of the Asia-Pacific Laboratory Accreditation Co-operation (APLAC).

1.3. A total of 48 laboratories from 21 different economies participated in the programme. 45 participants returned their results on or before the deadline on 31 July 2007 (APPENDIX I). Each participant was confidentially and randomly assigned with a unique laboratory code.

2. Objectives

2.1. The main objective of this programme is to offer an exercise for participants to assess their performance in the analysis of trace quantity of MG and LMG in eel samples through interlaboratory comparison.

2.2. Test results from participants were evaluated according to the statistical techniques that outlined in ISO13528:2005[8.1] "Statistical methods for use in proficiency testing by interlaboratory comparisons" and the international harmonized protocol for the proficiency testing of analytical chemistry laboratories[8.2]. z-score was provided as a numerical indicator to reveal individual participant's analytical competence relative to others in the programme.

3. Test Material

3.1. Sample Preparation: about 5 kg of live swamp eels (*Monopterus Albus*) were purchased from local market. The eels were divided into 2 groups: one group (seed) consisted of 1 kg of eels was placed in a 40 L water containing appropriate 0.5 ppm MG; whereas the second group (blank) of 4 kg of eels was placed in a 40 L water and containing no MG. After 24 hours, the eels in both groups were washed with water to remove the presence of residual MG in the skin. The eels were then slaughtered, deboned, freeze-dried, powdered and passed through 250 μm sieve. The concentration of MG and LMG in the dried powder of the seed group was estimated using an accredited LC-MS/MS method. Appropriate quantity of the powder taken from both groups was thoroughly mixed in order to produce the concentration of MG and LMG at the desirable μg/kg level. The powder, with an aliquot of 5 g was independently dispensed into clean amber bottles and disinfected by γ-irradiation at a dose of about 10 kGy. A total of about 150 bottled samples were prepared.

3.2. Homogeneity (October 2006) and stability tests (at 25ºC and 37ºC from December 2006 to August 2007) of the samples were determined in accordance with the procedures stipulated in APPENDICES II and III and the results were found to be satisfactory. Since the objectives of the tests were to ensure the homogeneous and stability status of the prepared bottled samples, selected samples used in those tests were not subject to reconstitute in distilled water as stipulated in APPENDIX VIII "Procedure for Reconstitution of Sample".

3.3. Samples together with "Instructions to Accreditation Bodies", "Receipt Form", "Instructions to Participating Laboratories", "Procedure for Reconstitution of Sample" and "Results Proforma" were sent to participating accreditation bodies (AB) in May 2007. A specimen copy of these instructions was given in APPENDICES IV to IX.

3.4. All AB were informed to read the "Instructions to Accreditation Bodies" and to redirect the sample(s) and the attached documents to their nominated laboratories as soon as possible.

3.5. Each participant was provided with one bottle of 5 g of test sample in the programme.

4. Test Procedures

4.1. Participants were required to determine the concentration of MG and LMG in the test sample according to their appropriate validated methods.

4.2. Prior to analysis, participants were instructed to follow the procedure of reconstituting sample in distilled water in the dark at 4°C overnight as stipulated in APPENDIX VIII.

4.3. Results of triplicate analyses in μg/kg with three significant figures or three decimal place, whichever was appropriate, should be reported.

4.4. Relative expanded uncertainty measurement of the analysis and technical information of method used by participants were also requested.

5. Statistical Evaluation

5.1. Participants' test results

5.1.1. Test results received from participants were presented in APPENDICES IX to XI. Their methodologies and techniques employed; and accreditation status were tabulated in APPENDIX XII to XIII.

5.2. Performance evaluation

5.2.1. The performance of each participant was evaluated by means of a performance index, z-score which was calculated as:

$$z = \frac{x_i - m}{S}$$

where x_i = reported mean of individual participant
m = assigned value (determined using isotope dilution mass spectrometry)
S = SD estimated from Horwitz Equation[8.3]

5.2.2. z-score is interpreted as follows:

(a)	$\|z\| \leq 2$	Satisfactory
(b)	$2 < \|z\| < 3$	Questionable
(c)	$\|z\| \geq 3$	Unsatisfactory

5.2.3. Participants having z-score(s) ≥ 3 or ≤ -3 should thoroughly investigate their results and reported to their AB. Participants having z-score(s) in the range $2 < |z| < 3$ are encouraged to review their results.

6. Results

6.1. Interim report was issued by HKGL on 7 September 2007 and later forwarded by HKAS to participants through their AB in mid September 2007. Participants were requested to check their analytical data before 25 September 2007 to reaffirm the reported data were correct. The number of participants' data compiled was as follow:

	MG	**LMG**
Data submitted	44	41
Data used for z-score evaluation	42	29

6.2. Assigned values

6.2.1. Determination of assigned values for MG and LMG was based on a reverse isotope dilution liquid chromatography-mass spectrometric (ID-LC-MS) method. Portions of 0.5 g sample and calibration blend (sample free of analytes) were separately reconstituted in distilled water overnight as stipulated in APPENDIX VIII "Procedure for Reconstitution of Sample". Then

appropriate quantities of deuteriated MG and LMG were spiked into the sample and the calibration blend. After equilibration, the samples were preceded to extraction, cleanup and analysed. Purity of MG oxalate and LMG standards used were cross validated using two different sources. Preparations of standards were performed using a gravimetric method. The assigned values were the average of three independent analyses that performed in different periods.

6.2.2. Concentrations (C_x) of MG and LMG (in µg/kg) were calculated using the following equation:

$$C_x = C_z \times \frac{m_{zc}}{m_{yc}} \times \frac{m_y}{m_x} \times \frac{R_B}{R_{BC}}$$

where:

C_z	=	mass fraction (µg/kg) of analytes in calibration solution
m_{zc}	=	mass of analytes in the calibration blend
m_{yc}	=	mass of deuteriated analytes in the calibration blend
m_y	=	mass of deuteriated analytes spiked into the sample
m_x	=	mass of sample used
R_B	=	isotope ratios in sample
R_{BC}	=	isotope ratios in calibration blend

6.2.3. Expanded uncertainty (at k = 2) was estimated as:

$$2 \times \sqrt{(C_z)^2 + (m_{zc})^2 + (m_{yc})^2 + (m_y)^2 + (m_x)^2 + (R_B)^2 + (R_{BC})^2}$$

6.2.4. Results of assigned values of MG and LMG:

	MG	**LMG**
Assigned Value (µg/kg)	28.2	2.21
Expanded Uncertainty (µg/kg)	± 3.38	± 0.35
Horwitz SD (µg/kg)	7.65	0.88

6.3. The distributions of participants' results were very wide, ranging from 0.4 to 712 µg/kg for MG; and 0.23 to 92 µg/kg for LMG respectively. The robust mean ± standard deviation were 19.0 ± 14.9 µg/kg for MG and 4.65 ± 5.08 µg/kg for LMG respectively.

6.4. Further data analysis indicated the precision and/or accuracy of the results were influenced by the choice of method being used.

As summarized in the following table, the relative standard deviation of the analysis of MG and the accuracy of the analysis of LMG (relative to the assigned value) were found to be better when using LC-MS/MS than that of the LC-UV technique. With the use of isotopic internal standards such as MG-d5 and LMG-d6 in LC-MS/MS analysis, the precision for both analyses were further improved.

Analytical Techniques	**Mean Concentration ± SD (µg/kg)**	
	MG	**LMG**
LC-UV	18.7 ± 20.9 (n=11)	46.5 ± 31.8 (n=6)
LC-MS/MS	17.3 ± 16.5 (n=13)	2.37 ± 2.11 (n=9)
LC-MS/MS (with isotopic internal standard)	21.1 ± 11.1 (n=18)	2.02 ± 0.57 (n=14)

n is the number of valid data submitted

6.5. The within-laboratory variations of triplicate analysis for MG were 0 – 33% (an average of 7.1%) for MG; and 0 – 25% (an average of 7.4%) for LMG.

6.6. z-scores of individual participant were presented in APPENDIX XIV and illustrated graphically in APPENDICES XV to XVI. The performance of participants was summarized as follows:

Performance Achieved	**Number of Participants (percentage)**	
	MG	**LMG**
$\|z\| \leq 2$	20 (48 %)	18 (62 %)
$2 < \|z\| < 3$	9 (21 %)	3 (10 %)
$\|z\| \geq 3$	13 (31 %)	8 (28 %)

In summary, 19 (Lab. Code: 2, 4, 8, 10, 12, 13, 14, 15, 17, 19, 25, 28, 29, 32, 36, 37, 38, 3 and 43) out of the 45 participants (42.2%) were identified as having reported one or more unsatisfactory results; and 21 out of the 71 results submitted were identified as unsatisfactory results (29.6 %).

6.7. As one of the technical requirements for method validation that stated in ISO/IEC 17025, MU was required to be reported.

However, those MU were not used in the performance assessment in the programme. Similar to those of the two previous APLAC PT programmes (APLAC T049 and APLAC T057), large variation of reported MU was found amongst participants (APPENDICES X to XI) and a proportion of participants (~17%) did not report the MU of their analyses. The information might indicate that some participants have difficulties to estimate MU and more training should be recommended.

7. Remarks

7.1. Efforts and supports from all AB and participants to this programme are gratefully noted. Participants are welcome to forward any comments on this PT to the following address:

Dr. Yiu-chung WONG
The Proficiency Test Advisory Board
Hong Kong Government Laboratory
7/F. Homantin Government Offices,
88 Chung Hau Street, KLN,
Hong Kong.

Email: ycwong@govtlab.gov.hk

8. References

8.1. ISO 13528 “Statistical methods for use in proficiency testing by interlaboratory comparisons”, **2005**, Geneva, Switzerland.

8.2. M. Thompson, S.L. Ellison, R. Wood. The International harmonized protocol for the proficiency testing of analytical chemistry laboratories (IUPAC technical report), *Pure Appl. Chem.* **2006**, 78:146-196.

8.3. W. Horwitz. Evaluation of analytical methods used for regulations of food and drugs, *Anal. Chem.* **1982**, 54:67A-76A.

APPENDIX I: Geographical Distribution of Participants

ECONOMIES	ENROLLED	RETURNED RESULTS
AUSTRALIA	4	4
AUSTRIA	2	2
BELGIUM	2	1
BRUNEI	1	1
CANADA	4	4
CHILE	2	2
CHINA	4	4
CYPRUS	1	1
GERMANY	2	2
HONG KONG	5	5
INDONESIA	3	3
JAPAN	2	2
KOREA	1	1
NEW ZEALAND	1	1
NORWAY	1	1
POLAND	2	2
ROMANIA	2	0
SINGAPORE	3	3
SWITZERLAND	3	3
TAIWAN	2	2
USA	1	1
TOTAL	**48**	**45**

APPENDIX II: Homogeneity Test

a. 10 bottles of powder were randomly selected from a total of 150 bottles. Two test portions of 0.5 g each were taken from each bottle for duplicate analysis of MG and LMG on 18 October 2006.

b. 20 determinations in a random order were as follows:

Sample Code	Concentration (µg/kg) of duplicate analysis					
	MG			LMG		
	#1	#2	Average	#1	#2	Average
1	33.08	29.90	31.49	2.30	2.19	2.25
13	32.53	32.86	32.70	2.21	2.31	2.26
23	32.44	33.08	32.76	1.92	1.85	1.89
37	32.75	28.99	30.87	1.83	2.15	1.99
52	28.56	32.09	30.33	2.00	2.20	2.10
68	30.72	28.44	29.58	1.96	2.28	2.12
79	32.71	28.59	30.65	1.92	2.00	1.96
92	28.42	30.01	29.22	2.21	1.88	2.05
102	28.38	28.30	28.34	1.90	1.96	1.93
130	30.01	33.12	31.57	1.9	2.10	2.00
		Mean ($\bar{x}$)	30.75		Mean ($\bar{x}$)	2.05
		SD (S_x)	1.37		SD (S_x)	0.12
		RSD (%)	4.46		RSD (%)	5.9

c. According to ISO13528:2005, the samples may be considered to be adequately homogeneous if:

$S_s \leq 0.3\sigma_R$

where S_s = Between samples standard deviation
σ_R = Standard deviation for proficiency assessment

σ_R is estimated using Horwitz equation of $0.02c^{0.8495}$ where c was the mean concentration (in percent) of analyte from homogeneity test:

Analyte	$\bar{x}$, µg/kg	σ_R (%)	$0.3\sigma_R$ (%)	$0.3\sigma_R$ (µg/kg)
MG	30.75	26.8	8.03	2.47
LMG	2.05	40.2	12.1	0.25

S_s is calculated as:

$$\sqrt{S_x^2 - (S_w^2/2)}$$

where S_x = Standard deviation of sample averages
S_w = Within-samples standard deviation

S_w is calculated as:

$$\sqrt{\sum w_t^{\,2}/(2n)}$$

where w_t = Absolute difference of the duplicate sample
n = Number tested samples

The results were summarized as follows:

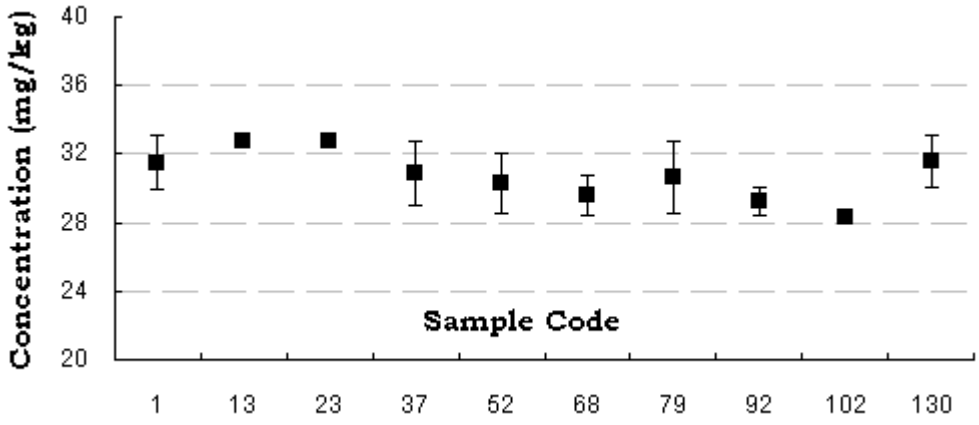
Analyte	**n**	**Σw_t (µg/kg)**	**S_w (µg/kg)**	**S_x (µg/kg)**	**S_s (µg/kg)**
MG	10	71.6	1.89	1.37	0.30
LMG	10	0.43	0.15	0.12	0.06

d. Since the between samples standard deviations (S_s) from the homogeneity test for both MG and LMG were found to be less than that of the $0.3\sigma_R$, the materials were considered homogeneous.

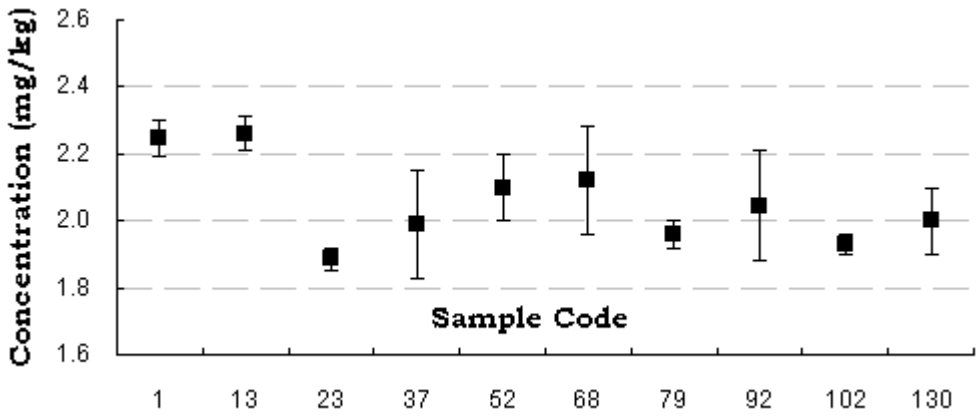
APPENDIX II: Homogeneity Test (Cont'd)

e. Graphical illustrations of selected samples (n =10) in homogeneity test

(i) MG



(ii) LMG



APPENDIX III: Stability Test

a. Stability test was monitored regularly on a monthly basis from December 2006 to August 2007, which adequately covered the time when the samples were analyzed by participants.

b. Samples selected for the stability test were stored at (i) a room temperature of 25 °C and (ii) an elevated temperature of 37 °C.

c. One sample from each temperature set was randomly selected and analyzed in duplicate or triplicate under the same operational conditions as those in homogeneity test.

d. Stability of samples was evaluated from the absolute difference between the mean value obtained in homogeneity test ($\bar{x}$) and the mean value ($\bar{yi}$) of each stability test. According to ISO13528:2005, the absolute difference should not be more than 30% of the standard deviation of the proficiency test (σ_R).

ie. $\left|\bar{x} - \bar{yi}\right| \leq 0.3\sigma_R$

e. Results of the stability tests at 25 °C were summarized below:

(i) MG at 25°C

Period	Sample Code	Concentration (mg/kg)					$\left\|\bar{x} - \bar{yi}\right\|$	Status
		#1	#2	#3	$\bar{yi}$	$\bar{x}$		
Dec 06	129	29.69	30.11	28.83	29.55	30.75	1.20	Pass
Jan 07	42	28.91	29.83	29.32	29.36	30.75	1.39	Pass
Feb 07	85	29.34	28.55	28.86	28.92	30.75	1.83	Pass
Mar 07	135	29.53	29.04	31.26	29.94	30.75	0.81	Pass
Jun 07	59	29.73	28.51	---	29.12	30.75	1.63	Pass
Aug 07	65	31.55	31.33	31.24	31.37	30.75	0.62	Pass

(ii) MG at 37°C

Period	Sample Code	Concentration (mg/kg)					$\left\|\bar{x} - \bar{yi}\right\|$	Status
		#1	#2	#3	$\bar{yi}$	$\bar{x}$		
Dec 06	128	28.71	28.60	28.45	28.59	30.75	2.16	Pass
Jan 07	66	29.10	28.66	28.81	28.86	30.75	1.89	Pass
Feb 07	81	29.11	28.52	29.01	28.88	30.75	1.87	Pass
Mar 07	26	28.53	28.70	28.66	28.63	30.75	2.129	Pass
Jun 07	48	31.08	30.03	---	30.55	30.75	0.20	Pass
Aug 07	43	31.59	31.73	31.77	31.70	30.75	0.95	Pass

APPENDIX III: Stability Test (Cont'd)

(iii) LMG at 25°C

Period	Sample Code	Concentration (mg/kg)					$\lvert\bar{x} - \bar{yi}\rvert$	Status
		#1	#2	#3	$\bar{yi}$	$\bar{x}$		
Dec 06	129	1.99	2.02	1.90	1.97	2.05	0.08	Pass
Jan 07	42	2.18	2.07	1.96	2.07	2.05	0.02	Pass
Feb 07	82	1.89	1.95	1.86	1.90	2.05	0.15	Pass
Mar 07	135	2.00	1.89	1.98	1.96	2.05	0.09	Pass
Jun 07	59	1.94	1.93	---	1.94	2.05	0.11	Pass
Aug 07	65	2.01	1.92	2.10	2.01	2.05	0.04	Pass

(iv) LMG at 37°C

Period	Sample Code	Concentration (mg/kg)					$\lvert\bar{x} - \bar{yi}\rvert$	Status
		#1	#2	#3	$\bar{yi}$	$\bar{x}$		
Dec 06	128	2.00	1.94	2.02	1.97	2.05	0.08	Pass
Jan 07	66	1.92	1.91	1.99	1.94	2.05	0.11	Pass
Feb 07	81	1.84	1.87	1.87	1.86	2.05	0.19	Pass
Mar 07	26	1.89	2.08	1.86	1.94	2.05	0.11	Pass
Jun 07	48	1.92	1.90	---	1.91	2.05	0.14	Pass
Aug 07	43	2.18	2.06	2.09	2.11	2.05	0.06	Pass

f. Since the absolute differences between the mean value from homogeneity test ($\bar{x}$) and the mean value of the stability test ($\bar{yi}$) were found to be less than $0.3\sigma_R$ for both MG and LMG, the materials were considered to be stable for six-month at 25°C and 37°C.

APPENDIX IV: Instructions to Accreditation Bodies

INSTRUCTIONS
(FOR ACCREDITATION BODIES)

1. ORGANIZATION

The PT programme on "Malachite green in swamp eels (APLAC T058)" is organized by the Hong Kong Government Laboratory (HKGL) in collaboration with Hong Kong Accreditation Service (HKAS) under the auspices of the Asia-Pacific Laboratory Accreditation Cooperation (APLAC). The main objective of the programme is to evaluate the performance of participating laboratories on the concerned tests through inter-laboratory comparison.

2. SAMPLE

Accreditation bodies (AB) should receive the PT samples and instruction information (hard copy and soft copy) from the organizers. The samples that each contains about 5 g of dried swamp eel powder (≤ 250 μm) are properly packaged in amber bottles. Upon receipt, AB should carefully check the samples (number of bottles, physical conditions, etc.) and shall promptly acknowledge the HKGL by returning the "Receipt Form (for Accreditation Bodies)" electronically to T58@govtlab.gov.hk. New sample(s) will be re-sent by the HKGL for any damaged claims.

AB are recommended to promptly distribute the samples, together with the "Instructions for Participating Laboratories" and "Results Proforma" (hard and soft copy), to their respective participating laboratories. The samples shall be stored in a secure environment at room temperature (around 25°C) before dispatch.

AB shall remind their respective participating laboratories carefully check the sample upon receipt and promptly acknowledge the HKGL by returning the "Receipt Form (for Participating Laboratories)" electronically to T58@govtlab.gov.hk.

3. RESULTS

AB shall inform their respective participating laboratories complete Part I and II of the "Result Proforma" and submit electronically to the HKGL on or before **31 July 2007** to T58@govtlab.gov.hk. (Notes: 1. Unless with special reasons, results submitted after the deadline might be rejected by the organizers. 2. To avoid poor transmission quality, results by fax are not accepted.)

4. ENQUIRIES

Please contact the HKGL for further enquiries at T58@govtlab.gov.hk

APPENDIX V: Receipt Form for Accreditation Bodies

RECEIPT FORM
(FOR ACCREDITATION BODIES)

From: .....
(Name of AB)

.....
(Contact Person)

To: Dr. Siu-kay WONG
Secretary of the PT Advisory Board
HKGL,
Hong Kong.

Email: .....

Email: T58@govtlab.gov.hk

Date:
.....

I hereby acknowledge the receipt of _____ # sample(s) for the APLAC T058 programme.

The sample(s) is/are found *(intact / damaged) and should be *(suitable / not suitable) for laboratory testing.

Remark(s) : .....
.....
.....
.....
.....

Number of samples
* Delete as appropriate

APPENDIX VI: Instruction to Participating Laboratories

INSTRUCTIONS
(FOR PARTICIPATING LABORATORIES)

1. ORGANIZATION

The PT programme on "Malachite green in swamp eels (APLAC T058)" is organized by the Hong Kong Government Laboratory (HKGL) in collaboration with Hong Kong Accreditation Service (HKAS) under the auspices of the Asia-Pacific Laboratory Accreditation Cooperation (APLAC). The main objective of the programme is to evaluate the performance of participating laboratories on the concerned tests through inter-laboratory comparison.

2. SAMPLE

(a) Participating laboratories will independently receive an amber sample bottle that containing about 5 g of dried swamp eel powder from their respective accreditation bodies (AB).

(b) Upon receipt of the sample, participating laboratories should carefully inspect the sample for any physical damages and defects.

(c) Participating laboratories shall promptly acknowledge receipt of the sample by returning the "Receipt Form (for Participating Laboratories)" electronically to the HKGL to T58@govtlab.gov.hk. New sample will be re-sent by the HKGL for any damaged claims.

(d) Intact sample is recommended to store in a secure environment at room temperature around 20 to 25°C before use (Please be informed that analytes in samples was found to be gradually degraded at 37°C). Sample should be immediately capped after used and be kept in a dry box when not in use.

3. ANALYSIS

(a) Participating laboratories are required to determine the concentration (in **μg/kg**) of malachite green (MG) and leucomalachite green (LMG) in the sample. Determination should be conducted in triplicate.

(b) Participating laboratories should use their preferable test methods (could be accredited, in-house, modified, ad hoc methods, etc.) which are normally used to test their customer samples and record the method used on the results sheet.

4. RESULTS

(a) Report **ALL** the triplicate data and the arithmetic mean in Part I of the "Results Proforma".

(b) Estimate and report the relative expanded uncertainty (± %) for individual analyte.

(c) Fill in the information of your performed analytical method in Part II of the "Results Proforma".

(d) Submit the results electronically to the HKGL on or before **31 July 2007** to T58@govtlab.gov.hk using the soft copy of the "Results Proforma" provided.

(Notes: 1. Unless with special reasons, results submitted after the deadline might be rejected by the organizers. 2. To avoid poor transmission quality, results by fax are not accepted.)

5. ENQURIES

Please contact the HKGL for further enquiries at T58@govtlab.gov.hk

APPENDIX VII: Receipt Form for Participating Laboratories

RECEIPT FORM
(FOR PARTICIPATING LABORATORIES)

From: .....
(Name of Participating Laboratory)

.....
(Contact Person)

Email: .....

Date: .....

To: Dr. Siu-kay WONG
Secretary of the PT Advisory Board
HKGL,
Hong Kong.

Email: T58@govtlab.gov.hk

I hereby acknowledge the receipt of ONE sample (S/N: _____) for the APLAC T058 programme.

The sample(s) is/are found (intact / damaged)* and should be (suitable / not suitable)* for laboratory testing.

Remark(s) : .....
.....
.....
.....
.....

* Delete as appropriate

APPENDIX VIII: Procedure for Reconstitution of Sample

PROCEDURE FOR RECONSTITUTION OF SAMPLE

1. Open the bottle and weigh accurately a portion of approximately 0.5 g of the eel powder sample. The weighing should be performed immediately to minimize water uptake by the freeze dried sample.

2. Add 2 mL of distilled water, well mix and capped.

3. Allow the sample mixture to stand overnight (16 to 24 hrs.) at **4°C in the dark**.

4. Analyse the sample immediately.

5. Concentration determined (in μg/kg) should be based on the 0.5 g weighed sample.

APPENDIX IX: Results Proforma

RESULTS PROFORMA

<Both Part I & II **MUST** be completed by Participating Laboratory>

Name of Participating Laboratory: _____

_____

Contact Person: (Prof/Dr/Mr/Ms)* _____

Email: _____

Part I: Analytical Results

Analyte	Concentration (in **µg/kg**, based on as-received weight)				Measurement Uncertainty	
	Data 1	Data 2	Data 3	Mean	Rel. Expanded Uncertainty (%)	Coverage Factor (k)
MG						
LMG						

* Delete as appropriate

- Please submit this "Results Proforma" electronically to the HKGL at T58@govtlab.gov.hk
- § It is the responsibility of the participants to avoid collusion and falsification of result so as to ensure a reliable assessment to be made in this proficiency testing programme

To be continued …

APPENDIX IX: Results Proforma (Cont'd)

RESULTS PROFORMA

<Both Part I & II **MUST** be completed by Participating Laboratory>

Part II: Test Parameters

1. Analytical Instrument(s): HPLC-UV / LC-MS / LC-MS/MS
(*delete as appropriate)

Other: .....
(please specify)

2. Analytical Column(s):
(Type and dimensions) .....
.....

3. Mobile Phase:
(Flow rate, gradient elution, etc) .....
.....
.....
.....

4. Internal Standard(s):
(If Yes, pls. specify) .....
.....
.....

5. Extraction Solvent & Method: .....
.....
.....
.....

6. Cleanup:
(If Yes, pls. specify) .....
.....
.....
.....

7. Method Accredited? YES / NO (*delete as appropriate)

8. Other Additional Information: .....
.....
.....
(please specify)

Date: _____

APPENDIX X: Participants' Results for MG

Lab. Code	Concentration (µg/kg)				Measurement Uncertainty	
	Replicate 1	Replicate 2	Replicate 3	Mean	Relative Expanded Uncertainty (%)	Coverage Factor
1	16.5	16.2	15.9	16	28	2
2	3.4	5.4	4.2	4.3	23	---
3^	12.6	12.6	---	12.6	---	---
4	<15	<15	<15	<15	---	---
5	20.47	21.19	19.09	20.250	10.5	2
6	11.6	11.2	11.4	11.4	40.0	2
7	11.67	11.34	11.53	11.51	5.990	2
8	0.74	0.73	1.16	0.88	27.37	---
9	24.3	25.6	24.0	24.6	21	3
10#	3.06	---	---	3.06	15.00	3.0
11	---	---	---	---	---	---
12	1.86	2.03	2.03	1.97	5.00	
13	4.45	4.23	4.81	4.50	3.10	2
14	3.4300	3.1597	3.1076	3.2324	---	---
15	3.92	4.09	4.03	4.01	0.13	2
16	22.6	25.1	28.9	25.5	18.00	2
17^	25.4	27.5	---	26.5	7.94	2
18	33.3	33.4	32.3	33.0	3.55	2
19	ND	ND	ND	ND	5.72	2
20	6.30	5.22	7.09	6.20	15.1	2
21	9.56	8.68	10.59	9.61	10.20	2
22	22.17	23.31	23.18	22.89	2.35	2.26
23	10	11	11	11	20	2
24	46.6	42.9	46.6	45.4	---	---
25	37.8	31.5	28.7	32.6	17	2
26	20.06	20.56	20.33	20.32	20	2
27	35	37	35	35	20	2
28	20.99	22.08	21.21	21.43	0.59	2
29	94.3	97.5	95.0	96	12	2
30	11.0	9.40	8.50	9.63	---	---
31	33	30	32	32	17	2
32	0.245	0.3798	0.490	0.3716	$2.7x10^{-8}$	2
33	23.9	26.6	30.4	27.0	30	2
34	40.5	39.5	37.7	39.2	18	2
35	23	20	22	22	30	2
36	752	684	700	712	4.02	2
37	70.3	64.2	65.9	66.8	16.1	2
38	12.8	8.9	10.5	10.7	18.6	85.5
39	3.05	3.35	3.05	3.15	---	---
40	7.0	6.9	7.1	7.0	---	---
41	24.5	25.3	25.1	25.0	4.24	2
42	15.95	16.65	16.10	16.2	95	2
43	80.2	79.5	75.1	78.3	0.704	2
44	20	20	20	20	14	2
45	18.3	18.0	17.9	18.07	10.000	2

" ^ "denotes duplicate data submitted by participants

" # " denotes single data submitted by participants

"---" denotes data/information was not provided by participants

"ND" denotes not detected

APPENDIX XI: Participants' Results for LMG

Lab. Code	Concentration (µg/kg)				Measurement Uncertainty	
	Replicate 1	Replicate 2	Replicate 3	Mean	Relative Expanded Uncertainty (%)	Coverage Factor
1	1.9	1.9	1.9	1.9	30	2
2	---	---	---	---	---	---
3^	0.62	0.54	---	0.58	---	---
4	50	59	68	59	15.9	1.393
5	ND	ND	ND	ND	---	---
6	<10	<10	<10	<10	---	---
7	3.80	3.96	3.92	3.89	12.610	2
8	≤0.5	≤0.5	≤0.5	≤0.5	63.46	---
9	3.1	3.3	2.9	3.1	28	3
10	1.43	1.41	1.41	1.42	2.40	3.0
11	<5	<5	<5	<5	70.0	80%
12	<1.0	<1.0	<1.0	<1.0	27.000	---
13	ND	ND	ND	ND	1.3	2
14	---	---	---	---	---	---
15	0.284	0.293	0.301	0.293	0.31	2
16	3.29	2.38	2.04	2.56	60.00	2
17^	14.1	8.3	---	11.2	16	2
18	3.8	5.0	3.6	4.1	36.50	2
19	76	82	70	76	7.21	2
20	1.30	1.56	1.160	1.34	15.1	2
21	1.84	1.77	1.84	1.82	10.8	2
22	1.90	1.82	1.98	1.90	0.5	2.26
23	<5	<5	<5	<5	30	2
24	2.4	2.3	2.4	2.4	---	---
25	92.8	82.3	101.3	92.1	26	2
26	ND	ND	ND	ND	---	---
27	2.1	2.1	2.0	2.1	20	2
28	46.22	37.7	41.89	41.94	4.35	2
29	20.3	19.5	20.0	20	15	2
30	<2	<2	<2	<2	---	---
31	1.2	1.3	1.2	1.2	15	2
32	9.658	8.994	6.984	8.545	$2.16X\ 10^{-7}$	2
33	2.0	2.0	2.2	2.1	30	2
34	---	---	---	---	---	---
35	1.7	2.0	2.1	1.9	34	2
36	ND	ND	ND	ND	---	---
37	2.0	2.0	2.0	2.0	6.7	2
38	69.1	75.2	76.3	73.5	5.3	112.4
39	0.22	0.23	0.23	0.23	---	---
40	ND	ND	ND	ND	---	---
41	1.58	1.68	1.63	1.63	6.14	2
42	---	---	---	---	---	---
43	2.04	2.16	2.13	2.11	24.1	2
44	<10	<10	<10	<10	10	2
45	4.1	3.1	3.8	3.65	30.000	2

" ^ " denotes duplicate data submitted by participants
" # " denotes single data submitted by participants
"---" denotes data/information was not provided by participants
"ND" denotes not detected

APPENDIX XII: Participants' Methodologies & Techniques

Lab Code	Method	Internal Standard	Extraction Medium	Clean Up
1	LC-MS/MS	MG-d5, LMG-d6	MeCN	SCX SPE
2	HPLC-UV	---	MeCN + DCM + $HClO_4$	Hexane wash
3	LC-MS/MS	---	MeCN + DCM	C18 SPE
4	HPLC-VIS	NO	MeCN + $HClO_4$	NO
5	LC-MS/MS	MG-d5 picrate, LMG-d5	MeCN + buffer mixture	SCX SPE
6	HPLC-UV	Brilliant Green	McIlvaine buffer + MeCN + TMPD + DCM	SCX SPE
7	LC-MS/MS	---	TMPD + McIlvaine solution	MCX SPE
8	LC-MS/MS	Brilliant Green	MeCN + $HClO_4$	SPE
9	LC-MS/MS	---	MeCN	Hexane wash
10	LC-MS/MS	LMG-d5	TMPD + McIlvaine solution	MCX SPE
11	HPLC-UV	---	MeCN	Hexane
12	HPLC-DAD	---	DCM + $HClO_4$	SCX SPE
13	LC-MS/MS	MG-d5, LMG-d6	MeCN + citrate buffer + DCM	NO
14	HPLC-VIS	NO	Na Acetate + MeCN + Chloroform + 5% NH_3	C18 SPE
15	LC-MS/MS	NO	Disodium phosphate + citric acid + MeCN + DCM	Aromatic acid sulphonic SPE
16	LC-MS/MS	LMG-d6	MeCN + citrate buffer	SCX SPE
17	LC-MS/MS	MG-d5, LMG-d6	MeCN + citrate buffer	---
18	LC-MS/MS	MG-d5, picrate.	MeCN + McIlvaine buffer pH3 + p-TSA + TMPD + DCM	SCX SPE
19	HPLC-UV	NO	MeCH + H_2O	Filtration
20	LC-MS/MS	MG-d5, LMG-d6	GB/T 19857-2005	Alumina SPE
21	LC-MS/MS	MG-d5, LMG-d6	0.1% formic acid in MeCN + DCM	SCX SPE
22	LC-MS/MS	MG-d5 oxalate, LMG-d5	Hydroxylamine Hydrochloride + MeCN	NO
23	LC-MS/MS	MG-d5, LMG-d6	MeCN + citric acid-phosphoric acid buffer + DCM	SCX SPE
24	LC-MS/MS	LMG-d6	Ethyl acetate	PRS SPE
25	LC-MS	NO	Acetonitrile-acetate buffer + MeCN	Alumina SPE
26	LC-MS/MS	Brilliant Green	MeCN + ammonium acetate buffer	NO
27	LC-MS/MS	MG-d5, LMG-d5	DCM + MeCN + $HClO_4$	SCX SPE
28	HPLC-UV	NO	Hydroxylamine sulphate + p-TSA + acetic buffer + MeCN	Sep-Pak® C2t
29	HPLC-UV	NO	MeCN + ammonium acetate buffer	NO
30	LC-MS/MS	Crystal violet	Acidic MeCN	Hexane wash
31	LC-MS/MS	MG-d5, LMG-d5	DCM + MeCN + $HClO_4$	SCX SPE
32	HPLC-VIS/FLD	NO	Hhydroxylamine hydrochloride + p-TSA + acetate buffer + MeCN + DCM	Alumina SPE
33	LC-MS/MS	MG-d5, LMG-d6	McIlvaine solution + p-TSA + TMPD + MeCN + DCM	MCX SPE
34	HPLC-UV/FLD	NO	p-TSA + Hydroxylamine chloride + Ascorbic acid + DCM + MeCN + NH_3	SCX SPE
35	LC-MS/MS	MG-d5, LMG-d6	MeCN + ammonium acetate buffer	MCX SPE
36	HPLC-UV	NO	Hydroxylamine chloride + p-TSA + acetate buffer + MeCN + NH_3	Alumina + PRS SPE
37	LC-MS/MS	MG-d5, picrate. LMG-d6	Ammonium acetate + MeCN + citrate buffer	Partition with DCM
38	HPLC-UV	NO	MacIlvaine-Buffer	Aromatic sulfonic acid SPE
39	LC-MS/MS	---	---	---
40	LC-MS/MS	NO	MeCN + $HClO_4$	C18 SPE
41	LC-MS / LC-MS/MS	MG and LMG standard	Citrate buffer + MeCN	SCX SPE
42	HPLC-UV	NO	Laboratory Information Bulletin No. 4334, Andersen et al. US FDA	Alumina + PRS SPE
43	LC-MS/MS	NO	Acidic MeCN + DCM	C18 SPE
44	LC-MS/MS	MG-d5, LMG-d6	MeCN + citric acid-phosphoric acid buffer + hexane wash + DCM + NaCl	SCX SPE
45	LC-MS/MS	MG-d5, LMG-d5	MacIlavine buffer + MeOH	SPE

"---" denotes data/information was not provided by participants

DCM = dichloromethane; MeCN = acetonitrile; MeOH = methanol; p-TSA = para-toluene sulphonic acid; TMPD = N,N,N,N-tetramethyl-1,4-phenylenediamine dihydrochloride

APPENDIX XIII: Accreditation Status of Participants' Analytical Methods

Lab. Code	Accreditation
1	YES
2	NO
3	YES
4	YES
5	NO
6	YES
7	NO
8	YES
9	YES
10	NO
11	YES
12	NO
13	NO
14	NO
15	YES
16	YES
17	---
18	NO
19	NO
20	YES
21	NO
22	---
23	NO
24	YES
25	YES
26	NO
27	NO
28	NO
29	NO
30	YES
31	NO
32	NO
33	NO
34	NO
35	YES
36	NO
37	NO
38	NO
39	NA
40	YES
41	NO
42	YES
43	YES
44	NO
45	YES

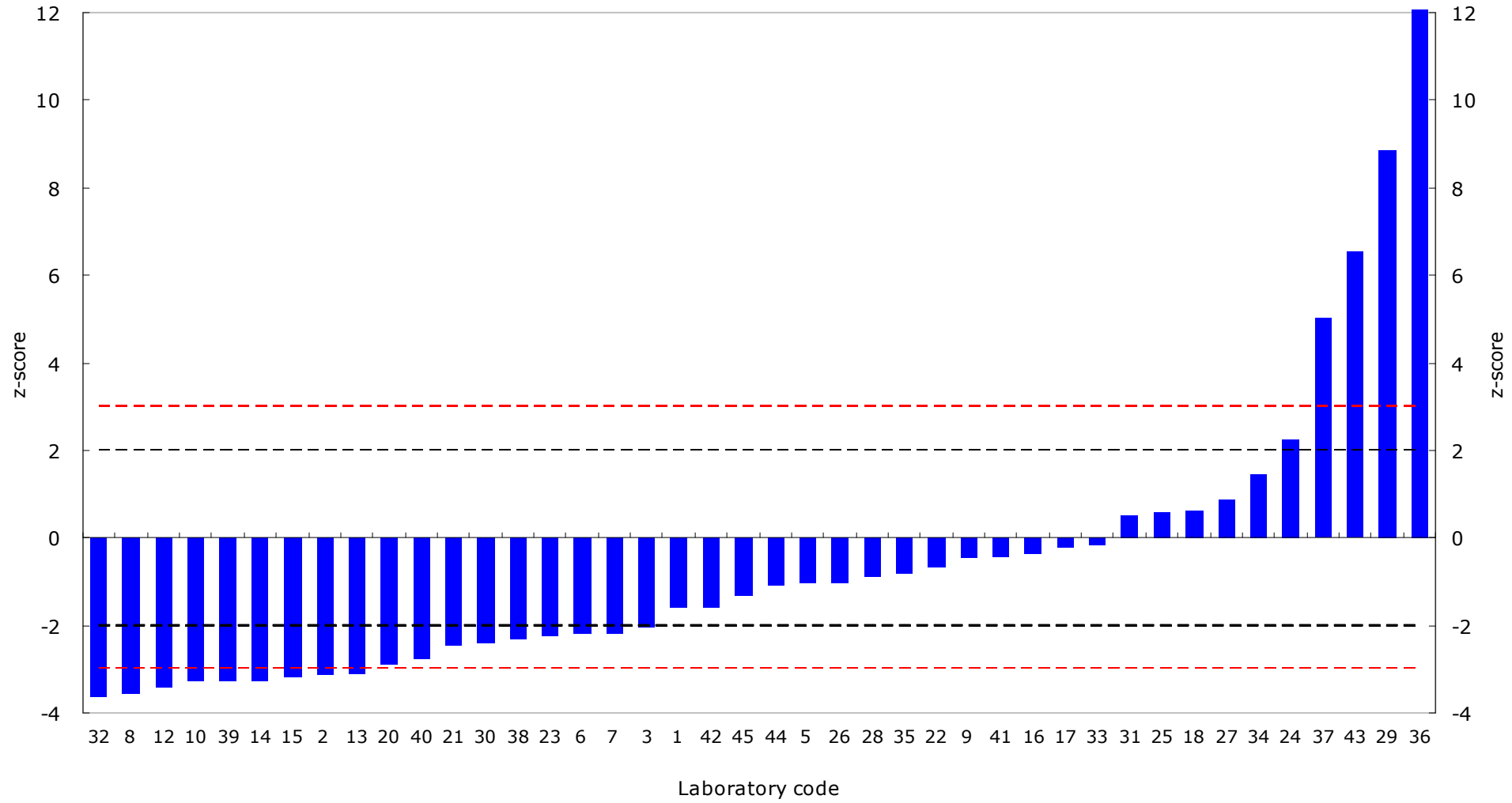
"---" denotes data/information was not provided by participants

APPENDIX XIV: z-Scores of Participants

Lab. Code	MG		LMG	
	Reported mean	z-score	Reported mean	z-score
1	16	-1.6	1.9	-0.36
2	4.3	**-3.1**	---	NA
3	12.6^	-2.0	0.58^	-1.9
4	<15	NA	59	**64**
5	20.250	-1.0	ND	NA
6	11.4	-2.2	<10	NA
7	11.51	-2.2	3.89	1.9
8	0.88	**-3.6**	≤0.5	NA
9	24.6	-0.47	3.1	1.0
10	3.06#	**-3.3**	1.42	-0.91
11	---	NA	<5	NA
12	1.97	**-3.4**	<1.0	NA
13	4.50	**-3.1**	ND	NA
14	3.2324	**-3.3**	---	NA
15	4.01	**-3.2**	0.293	-2.2
16	25.5	-0.35	2.56	0.39
17	26.5^	-0.23	11.2^	**10**
18	33.0	0.63	4.1	2.2
19	ND	NA	76	**84**
20	6.20	-2.9	1.34	-1.0
21	9.61	-2.4	1.82	-0.46
22	22.89	-0.69	1.90	-0.36
23	11	-2.3	<5	NA
24	45.4	2.2	2.4	0.17
25	32.6	0.58	92.1	**102**
26	20.32	-1.0	ND	NA
27	35	0.89	2.1	-0.17
28	21.43	-0.89	41.94	**45**
29	96	**8.8**	20	**20**
30	9.63	-2.4	<2	NA
31	32	0.45	1.2	-1.1
32	0.3716	**-3.6**	8.545	**7.2**
33	27.0	-0.16	2.1	-0.17
34	39.2	1.4	---	NA
35	22	-0.85	1.9	-0.33
36	712	**89**	ND	NA
37	66.8	**5.0**	2.0	0
38	10.7	-2.3	73.5	**81**
39	3.15	-3.3	0.23	-2.3
40	7.0	-2.8	ND	NA
41	25.0	-0.42	1.63	-0.67
42	16.2	-1.6	---	NA
43	78.3	**6.5**	2.11	-0.12
44	20	-1.1	<10	NA
45	18.07	-1.3	3.65	1.6

"---" denotes data/information was not provided by participants
"ND" denotes not detected
"NA" denotes z-score not assessed
Unsatisfactory results are bolded

Appendix XV: Histogram of z-scores of participants for MG



Appendix XVI: Histogram of *z*-scores of participants for LMG