Results of Proficiency Test
PAA from polyamide kitchenware
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Organized by: Institute for Interlaboratory Studies
Spijkenisse, the Netherlands

Author: ing. C.M. Nijssen-Wester
Correctors: ing. R.J. Starink & ing. G.A. Oosterlaken-Buijs
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CONTENTS

1 INTRODUCTION

Some Primary Aromatic Amines (PAA) are considered to be carcinogenic or suspected to be carcinogenic. PAA can be released from food contact materials, like kitchenware such as spoons, due to impurities or breakdown products present in the polyamide. These PAA together with other precursors present in food can form N-Nitrosamines upon ingestion (through metabolic activation), which are potent carcinogens for animals (and most likely also for humans). In the market some batches of polyamide kitchenware were found to release high levels of PAA in the food. In 2011 the European Commission issued regulation 284/2011 to lay down specific conditions and detailed procedures for the import of polyamide and melamine kitchenware. In support of this, to enhance harmonization of sampling and its testing, EUR24815: Technical Guidelines on testing the migration of primary aromatic amines from polyamide kitchenware was made public (lit. 13), determining PAA after exposing the kitchenware to acidic test conditions. The limits for PAA is that it should not be present, which means the detection limit applies. In EU28415/2011 it is set as 0.01 mg/kg (10 µm/kg) food or food simulants.

In 2020 the Institute of Interlaboratory Studies (iis) has organized this proficiency scheme for the first time. During the annual testing program of 2021/2022 it was decided to continue the proficiency test for the determination of PAA from polyamide kitchenware.

In this interlaboratory study 21 laboratories from 10 different countries registered for participation. See appendix 4 for the number of participants per country. In this report the results of the PAA from polyamide kitchenware proficiency test are presented and discussed. This report is also electronically available through the iis website www.iisnl.com.

2 SET UP

The Institute for Interlaboratory Studies (iis) in Spijkenisse, the Netherlands, was the organizer of this proficiency test (PT). Sample analyses for fit-for-use and homogeneity testing were subcontracted to an ISO/IEC17025 accredited laboratory.
It was decided to send one sample of kitchenware, the nylon part of a spatula, labelled #21725.
Participants were also requested to report some intermediate test results and to report rounded and unrounded test results. The unrounded test results were preferably used for statistical evaluation.

2.1 QUALITY SYSTEM

The Institute for Interlaboratory Studies in Spijkenisse, the Netherlands, has implemented a quality system based on ISO/IEC17043:2010. This ensures strict adherence to protocols for sample preparation and statistical evaluation and 100% confidentiality of participant's data. Feedback from the participants on the reported data is encouraged and customer's satisfaction is measured on regular basis by sending out questionnaires.

2.2 PROTOCOL

The protocol followed in the organization of this proficiency test was the one as described for proficiency testing in the report 'iis Interlaboratory Studies: Protocol for the Organisation, Statistics and Evaluation' of June 2018 (iis protocol, version 3.5). This protocol is electronically available through the iis website www.iisnl.com, from the FAQ page.

2.3 CONFIDENTIALITY STATEMENT

All data presented in this report must be regarded as confidential and for use by the participating companies only. Disclosure of the information in this report is only allowed by means of the entire report. Use of the contents of this report for third parties is only allowed by written permission of the Institute for Interlaboratory Studies. Disclosure of the identity of one or more of the participating companies will be done only after receipt of a written agreement of the companies involved.

2.4 SAMPLES

A batch of 34 black nylon spatulas containing a relevant concentration of Aniline was obtained from a third party. The subsamples were labelled #21725.
The homogeneity of the subsamples was checked by determination of PAA with an in house test method on 5 stratified randomly selected subsamples. Migration conditions were: 3% Acetic Acid and 2 hours at 100°C.

	Aniline in µg/L
Sample #21725-1	0.284
Sample #21725-2	0.331
Sample #21725-3	0.240
Sample #21725-4	0.290
Sample #21725-5	0.350

Table 1: homogeneity test results of the subsamples #21725

From the above test results the repeatability was calculated and compared to 0.3 times the estimated reproducibility calculated with the Horwitz equation in agreement with the procedure of ISO13528, Annex B2 in the next table.

	Aniline in µg/L
r (observed)	0.121
reference method	Horwitz
0.3 x R (reference method)	0.136

Table 2: evaluation of the repeatability of subsamples #21725

The calculated repeatability is in agreement with 0.3 times the estimated reproducibility calculated with the Horwitz equation. Therefore, homogeneity of the subsamples is assumed.

To each of the participating laboratories one spatula labelled #21725 was sent on September 8, 2021.

2.5 ANALYZES

The participants were requested to determine 3 different PAA: Aniline (CAS no. 63-53-3), 4,4'-Methylenedianiline (CAS no. 101-77-9) and 2,4-Toluenediamine (CAS no. 95-80-7) using the prescribed test conditions (total immersion, single use as migration method and 3% Acetic Acid as simulant for 120 minutes at 100°C). In daily practice, not just one item, but more items for testing would have been sent. However, this sample is positive on PAA. This means that one item of the sample is sufficient for the determination of PAA from polyamide kitchenware.
It was requested to report if the laboratory was accredited for the requested components that were determined. Also, some analytical details were requested.

It was explicitly requested to treat the sample as if it was a routine sample and to report the test results using the indicated units on the report form and not to round the test results, but report as much significant figures as possible. It was also requested not to report 'less than' test results, which are above the detection limit, because such test results can't be used for meaningful statistical evaluations.

To get comparable test results a detailed report form and a letter of instructions are prepared. On the report form the reporting units are given as well as the reference test methods (when applicable) that will be used during the evaluation. The detailed report form and the letter of instructions are both made available on the data entry portal www.kpmd.co.uk/sgs-iis-cts/. The participating laboratories were also requested to confirm the sample receipt on this data entry portal. The letter of instructions can also be downloaded from the iis website www.iisnl.com.

3 RESULTS

During five weeks after sample dispatch, the test results of the individual laboratories were gathered via the data entry portal www.kpmd.co.uk/sgs-iis-cts/. The reported test results are tabulated per determination in appendices 1 and 2 of this report. The laboratories are presented by their code numbers.

Directly after the deadline, a reminder was sent to those laboratories that had not reported test results at that moment. Shortly after the deadline, the available test results were screened for suspect data. A test result was called suspect in case the Huber Elimination Rule (a robust outlier test) found it to be an outlier. The laboratories that produced these suspect data were asked to check the reported test results (no reanalyzes). Additional or corrected test results are used for data analysis and the original test results are placed under 'Remarks' in the test result tables in appendices 1 or 2. Test results that came in after the deadline were not taken into account in this screening for suspect data and thus these participants were not requested for checks.

3.1 STATISTICS

The protocol followed in the organization of this proficiency test was the one as described for proficiency testing in the report 'iis Interlaboratory Studies: Protocol for the Organisation, Statistics and Evaluation' of June 2018 (iis-protocol, version 3.5).
For the statistical evaluation the *unrounded* (when available) figures were used instead of the rounded test results. Test results reported as '<…' or '>…' were not used in the statistical evaluation.

First, the normality of the distribution of the various data sets per determination was checked by means of the Lilliefors-test, a variant of the Kolmogorov-Smirnov test and by the calculation of skewness and kurtosis. Evaluation of the three normality indicators in combination with the visual evaluation of the graphic Kernel density plot, lead to judgement of the normality being either 'unknown', 'OK', 'suspect' or 'not OK'. After removal of outliers, this check was repeated. If a data set does not have a normal distribution, the (results of the) statistical evaluation should be used with due care.

The assigned value is determined by consensus based on the test results of the group of participants after rejection of the statistical outliers and/or suspect data.
According to ISO13528 all (original received or corrected) results per determination were submitted to outlier tests. In the iis procedure for proficiency tests, outliers are detected prior to calculation of the mean, standard deviation and reproducibility. For small data sets, Dixon (up to 20 test results) or Grubbs (up to 40 test results) outlier tests can be used. For larger data sets (above 20 test results) Rosner's outlier test can be used. Outliers are marked by D(0.01) for the Dixon's test, by G(0.01) or DG(0.01) for the Grubbs' test and by R(0.01) for the Rosner's test. Stragglers are marked by D(0.05) for the Dixon's test, by G(0.05) or DG(0.05) for the Grubbs' test and by R(0.05) for the Rosner's test. Both outliers and stragglers were not included in the calculations of averages and standard deviations.

For each assigned value the uncertainty was determined in accordance with ISO13528. Subsequently the calculated uncertainty was evaluated against the respective requirement based on the target reproducibility in accordance with ISO13528. In this PT the criterion of ISO13528, paragraph 9.2.1 was met for all evaluated tests, therefore, the uncertainty of all assigned values may be negligible and need not be included in the PT report.

Finally, the reproducibilities were calculated from the standard deviations by multiplying them with a factor of 2.8.

3.2 GRAPHICS

In order to visualize the data against the reproducibilities from literature, Gauss plots were made, using the sorted data for one determination (see appendix 1). On the Y-axis the reported test results are plotted. The corresponding laboratory numbers are on the X-axis. The straight horizontal line presents the consensus value (a trimmed mean). The four striped lines, parallel to the consensus value line, are the +3s, +2s, -2s and -3s target reproducibility limits of the selected reference test method. Outliers and other data, which were excluded from the calculations, are represented as a cross. Accepted data are represented as a triangle.

Furthermore, Kernel Density Graphs were made. The Kernel Density Graph is a method for producing a smooth density approximation to a set of data that avoids some problems associated with histograms. Also, a normal Gauss curve (dotted line) was projected over the Kernel Density Graph (smooth line) for reference. The Gauss curve is calculated from the consensus value and the corresponding standard deviation.

3.3 Z-SCORES

To evaluate the performance of the participating laboratories the z-scores were calculated. As it was decided to evaluate the performance of the participants in this proficiency test (PT) against the literature requirements, the z-scores were calculated using a target standard deviation. This results in an evaluation independent of the variation in this interlaboratory study.

The target standard deviation was calculated from the literature reproducibility by division with 2.8. In case no literature reproducibility was available, other target values were used, like Horwitz or an estimated reproducibility based on former iis proficiency tests.

When a laboratory did use a test method with a reproducibility that is significantly different from the reproducibility of the reference test method used in this report, it is strongly advised to recalculate the z-score, while using the reproducibility of the actual test method used, this in order to evaluate whether the reported test result is fit-for-use.

The z-scores were calculated according to:

$z_{(target)}$ = (test result - average of PT) / target standard deviation

The $z_{(target)}$ scores are listed in the test result tables in appendix 1.

Absolute values for z<2 are very common and absolute values for z>3 are very rare. Therefore, the usual interpretation of z-scores is as follows:

	|z|	< 1	good
1 <	|z|	< 2	satisfactory
2 <	|z|	< 3	questionable
3 <	|z|		unsatisfactory

4 EVALUATION

In this proficiency test no severe problems were encountered with the dispatch of the samples. One participant did not report any test results at all and two of the participants reported the test results after the final reporting date. Not all laboratories were able to report all components requested.

In total 20 participants reported 19 numerical test results for Aniline, one laboratory reported no test result for Aniline but a test result for another PAA. One outlying test result was observed, which is 5.3%. In proficiency studies outlier percentages of 3% - 7.5% are quite normal.

The data set did not prove to have a normal Gaussian distribution. This is referred to as “not OK” or “suspect”. The statistical evaluation of these data sets should be used with due care, see also paragraph 3.1.

4.1 EVALUATION PER COMPONENT

In this section the reported test results are discussed per component. The test methods which were used by the various laboratories were taken into account for explaining the observed differences when possible and applicable. These test methods are also in the table in appendix 1 together with the original data. The abbreviations, used in these tables, are explained in appendix 5.

The Technical Guidelines of EUR28415 (lit. 13) does not have a clear statement that mentions a repeatability and/or reproducibility at the levels of PAA found in this PT. Therefore, it was decided to use an estimated target reproducibility calculated with the Horwitz equation.

Aniline: This determination was not problematic. One statistical outlier was observed. The calculated reproducibility after rejection of the statistical outlier is in agreement with the estimated target reproducibility calculated with the Horwitz equation.

The majority of participants agreed on a concentration near or below the limit of detection for the other requested PAA. The test results are given in appendix 2.

4.2 PERFORMANCE EVALUATION OF THE GROUP OF LABORATORIES

A comparison has been made between the reproducibility as declared by the reference test method and the reproducibility as found for the group of participating laboratories. The number of significant test results, the average, the calculated reproducibility (2.8 * standard deviation) and the estimated reproducibility using the Horwitz equation are presented in the next table.

Component	unit	n	average	2.8 * sd	R(target)
Aniline	µg/dm^2	18	20.1	17.3	16.2

Table 3: Overview of results for sample #21725

Without further statistical calculations, it can be concluded that for the PAA present in the sample there is a good compliance of the group of laboratories with the relevant target reproducibility (see for discussion paragraph 4.1 and 5).

4.3 COMPARISON OF THE PROFICIENCY TEST OF OCTOBER 2021 WITH THE PREVIOUS PT

The uncertainty for PAA from polyamide kitchenware in mg/dm2 as observed in this PT was compared with the former proficiency test in the next table.

Year	Components	Type of migration	Observed RSD%	Target RSD%	Concentration µg/dm^2
2020	4,4'-Methylenedianiline	Immersion	49	22	25
2021	Aniline	Immersion	31	29	20

Table 4: development of the uncertainties over the years

4.4 EVALUATION OF THE ANALYTICAL DETAILS

The reported analytical details that were used by the participants are listed in appendix 3. Sixteen of the reporting laboratories are accredited for the determination of the PAA from polyamide kitchenware.

Eleven participants mentioned to have used the Technical Guidelines of EUR24815. The following methods were only used once or twice by the participants: EN13130-1, EN1186-3 or in house. Three participants did not report a test method for the determination of Aniline.

Almost all participants reported the surface area and volume of simulant used. iis calculated a surface area of the spatula and handle of around 1.8 dm^2. As expected, all participants reported a surface area equal to or smaller than this. The average reported surface area (excluding one very small surface area) is 1.3 dm^2. Some of the participants did use only a part of the blade of the spatula. The average reported volume is 283 mL.

Five participants reported to clean the sample. All participants heated the simulant before adding the sample. The majority of the participants used an oven for the migration step. Others used a hot plate or incubator.
All but two participants sealed the sample container.
It appeared that the effect of the analytical details on the determination of PAA in this PT is small and not statistically significant.

From the intermediate results reported for concentration in mg/L simulant, surface and used volume of simulant, the test results in µg/dm^2 were calculated for the PAA present. It appeared that for one laboratory the reported test value was different than calculated by iis, see appendices 1 and 3 for more details. It appeared that the calculation was done by dividing by the conventional conversion factor of 6 instead of doing a calculation based on the actual dimensions.

5 DISCUSSION

The limits for PAA from 284/2011/EU are stated in mg/kg food. As is mentioned in other Specific Migration methods, such as EN13130-1, the limits expressed in mg/kg can be divided by the conventional conversion factor of 6 in order to express them in mg/dm^2, see table 5.

Component	Specific Migration Limit in µg/kg	Specific Migration Limit in µg/dm^2
Total of PAAs	10	1.7

Table 5: Specific Migration maximum limits according to 287/2011/EU

All reporting participants would reject the sample for PAA based on this limit.

In this PT, the average of the homogeneity test results are not in line with the average (consensus value) from the PT results. There are several reasons for this. First, the goal of the homogeneity testing is very different from the goal of the evaluation of the reported PT results. In order to prove the homogeneity of the PT samples, a test method is selected with a high precision (smallest variation). The accuracy (trueness) of the test method is less relevant.
Secondly, the homogeneity testing is done by one laboratory only. The test results of this (ISO/IEC 17025 accredited) laboratory will have a bias (systematic deviation) depending on the test method used. The desire to detect small variations between the PT samples leads to the use of a sensitive test method with high precision, which may be a test method with significant bias.
Also each test result reported by the laboratories that participate in the PT will have a bias. However, some will have a positive bias and others a negative bias. These different biases compensate each other in the PT average (consensus value). Therefore, the PT consensus value may deviate from the average of the homogeneity test. At the same time the accuracy of the PT consensus value is more reliable than the accuracy of the average of the results of the homogeneity test.

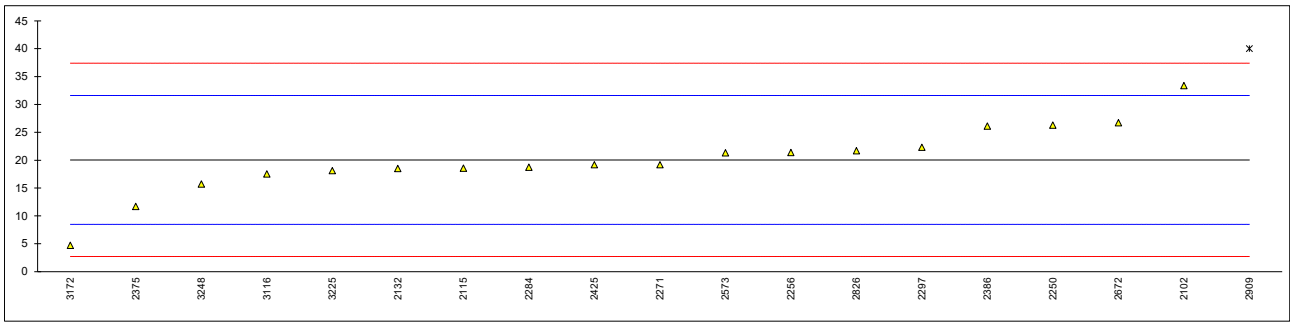
6 CONCLUSION

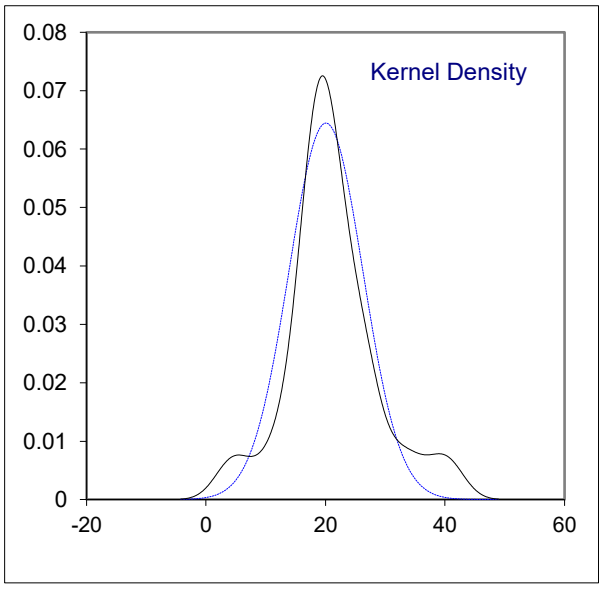
Each laboratory should evaluate its performance in this study and make decisions about necessary corrective actions. Therefore, participation on a regular basis in this scheme could be helpful to improve the performance and the quality of the analytical results.

APPENDIX 1

Determination of Specific Migration of Aniline (CAS No.62-53-3) on sample #21725; results in µg/dm^2 per contact surface

lab	method	value	mark	z(targ)	remarks
2102		33.38		2.31	
2115		18.56		-0.26	
2132	EUR24815 EN2011	18.4978	E	-0.27	Calculation difference, iis calc. 13.99
2250	EUR24815 EN2011	26.27		1.08	
2256	EUR24815 EN2011	21.37		0.23	
2271	EUR24815 EN2011	19.170		-0.15	
2284	EUR24815 EN2011	18.733		-0.23	
2297	EUR24815 EN2011	22.29		0.39	
2375	EUR24815 EN2011	11.679		-1.45	
2379		-----		-----	
2386	In house	26.11		1.05	
2425	In house	19.166		-0.15	
2573	EUR24815 EN2011	21.317		0.22	
2672	In house	26.72		1.15	
2826	EUR24815 EN2011	21.670		0.28	
2901		-----		-----	
2909		40	D(0.05)	3.45	
3116	EN13130-1	17.5		-0.44	
3172	EUR24815 EN2011	4.6981		-2.66	
3225	EN1186-3	18.1		-0.34	
3248	EUR24815 EN2011	15.7188		-0.75	
	normality	suspect			
	n	18			
	outliers	1			
	mean (n)	20.053			
	st.dev. (n)	6.1897	RSD = 31%		
	R(calc.)	17.331			
	st.dev.(Horwitz)	5.779			
	R(Horwitz)	16.181			





APPENDIX 2

Determination of Final concentration of Aniline (CAS No. 62-53-3), 4,4'-Methylenedianiline (CAS No.101-77-9) and 2,4-Toluenediamine (CAS No. 95-80-7) on sample #21725; results in µg/L simulant

lab	Aniline	4,4'-Methylenedianiline	2,4-Toluenediamine
2102	77.27	0	0
2115	148.5	75.4	34.6
2132	84.2152	ND	ND
2250	69.87	< 1	< 1
2256	128.25	-----	-----
2271	115.022	not detected	not detected
2284	112.41	<2	<2
2297	222.9	<2	<2
2375	34.26	ND	ND
2379	-----	2.1780	Not detected
2386	157.78	< 2	< 2
2425	115	Not Detected	Not Detected
2573	127.899	not detected	not detected
2672	160.0	not detected	not detected
2826	63.086	2.5246	Not detected
2901	-----	-----	-----
2909	98.41	<1	<1
3116	105	-----	-----
3172	28.15	n.d.	n.d.
3225	109	<2	<2
3248	52.658	ND	ND

Determination of Specific Migration of 4,4'-Methylenedianiline (CAS No.101-77-9) and 2,4-Toluenediamine (CAS No. 95-80-7) on sample #21725; results in µg/dm^2 per contact surface

lab	4,4'-Methylenedianiline	2,4-Toluenediamine
2102	0	0
2115	9.43	4.33
2132	ND	ND
2250	< 0,376	< 0,376
2256	-----	-----
2271	not detected	not detected
2284	<0.333	<0.333
2297	<0.2	<0.2
2375	ND	ND
2379	0.363	Not detected
2386	< 0,33	< 0,33
2425	Not Detected	Not Detected
2573	not detected	not detected
2672	not detected	not detected
2826	0.87455	Not detected
2901	-----	-----
2909	<1	<1
3116	-----	-----
3172	n.d.	n.d.
3225	<1	<1
3248	ND	ND

APPENDIX 3 ANALYTICAL DETAILS

Details on final concentration, surface area and volume of simulant reported on Aniline

lab	surface area in dm^2	volume simulant in mL	volume to surface ratio in mL/dm^2	final conc. in simulant in µg/L	reported Spec. Migration in $µg/dm^2$	iis calculated Spec. Migration in $µg/dm^2$	difference absolute
2102	1.368	591	432.0	77.27	33.38	33.38	0.00
2115	-----	-----	-----	148.5	18.56	-----	-----
2132	1.15	191	166.1	84.2152	18.4978	13.99	4.51
2250	1.33	500	375.9	69.87	26.27	26.27	0.00
2256	0.6002	100	166.6	128.25	21.37	21.37	0.00
2271	1.72	288	167.4	115.022	19.17	19.26	-0.09
2284	1.32	220	166.7	112.41	18.733	18.74	0.00
2297	1.8	180	100.0	222.9	22.29	22.29	0.00
2375	1.76	600	340.9	34.26	11.679	11.68	0.00
2379	1.02	170	166.7	-----	-----	-----	-----
2386	1.1	182	165.5	157.78	26.11	26.11	0.00
2425	1.26	210	166.7	115	19.166	19.17	0.00
2573	1.68	280	166.7	127.899	21.317	21.32	0.00
2672	0.1412	23.58	167.0	160	26.72	26.72	0.00
2826	1.1645	400	343.5	63.086	21.67	21.67	0.00
2901	-----	-----	-----	-----	-----	-----	-----
2909	1.46	595	407.5	98.41	40	40.11	0.11
3116	1.03	172	167.0	105	17.5	17.53	0.03
3172	1.453	242	166.6	28.15	4.6981	4.69	0.01
3225	1.37	228	166.4	109	18.1	18.14	0.04
3248	1.34	400	298.5	52.658	15.7188	15.72	0.00

ANALYTICAL DETAILS - continued -

Details on the test procedure

lab	accredited for ISO17025	sample cleaned prior to the migration step	part exposed to the simulant
2102	Yes	No	Only the part which comes into contact with food (functional part) upto 1 cm above this part
2115	Yes	No	Spatula without handle
2132	Yes	Yes, by using a lint-free cloth	Functional part
2250	Yes	No	according instructions
2256	Yes	No	food contact part
2271	Yes	No	whole product
2284	No	No	Bottom of Polyamide kitchenware
2297	Yes	Yes	
2375	Yes	No	All
2379	Yes	No	The part of the sample in contact with food
2386	Yes	No	The front part of the sample
2425	Yes	Yes, with lint free cloth	Bottom part of the sample was exposed to the simulant.
2573	Yes	No	all
2672	No	No	sample was cutted in smaller pieces before testing
2826	Yes	No	The blade part of spatula.
2901	---	---	
2909	Yes	No	spatula without handle
3116	No	No	Functional head
3172	---	---	
3225	Yes	Yes, remove the dirt of the surface	Functional Part
3248	Yes	Yes	Spatula without handle

lab	simulant preheated	equipment migration	simulant sealed
2102	Yes	Hot plate	Yes, covered with watch glass during migration
2115	Yes	Oven	Yes, tested in an airtight container
2132	Yes	Oven	Ys, container covered with glass plate and whole container wrapped in plastic
2250	Yes	Oven	Yes, with cling film
2256	Yes	Oven	Yes, tested in an airtight container
2271	Yes	Oven	Yes, the simulant with the sample was sealed with food wrapper (PE)
2284	Yes	Oven	Yes, with aluminum seal
2297	Yes	Incubator	Yes, with aluminum seal
2375	Yes	Oven	Yes, with glass plate
2379	Yes	Oven	Yes, with watch glas
2386	Yes	Oven	Yes, tested in an airtight container
2425	Yes	Oven	No
2573	Yes	Oven	Yes, with aluminum seal
2672	Yes	Oven	Yes, with aluminum seal
2826	Yes	Oven	Yes, with microwave wrap seal
2901	---	---	---
2909	Yes	Heating plate	Yes, with glass plate
3116	Yes	Oven	Yes, with aluminum seal
3172	---	---	---
3225	Yes	Oven	Yes, with aluminum seal
3248	Yes	Oven	Yes, with aluminum seal

APPENDIX 4

Number of participants per country

1 lab in BANGLADESH

1 lab in BRAZIL

3 labs in GERMANY

5 labs in HONG KONG

2 labs in ITALY

1 lab in LUXEMBOURG

5 labs in P.R. of CHINA

1 lab in THAILAND

1 lab in THE NETHERLANDS

1 lab in TURKEY

APPENDIX 5

Abbreviations

C = final test result after checking of first reported suspect test result
D(0.01) = outlier in Dixon's outlier test
D(0.05) = straggler in Dixon's outlier test
G(0.01) = outlier in Grubbs' outlier test
G(0.05) = straggler in Grubbs' outlier test
DG(0.01) = outlier in Double Grubbs' outlier test
DG(0.05) = straggler in Double Grubbs' outlier test
R(0.01) = outlier in Rosner's outlier test
R(0.05) = straggler in Rosner's outlier test
E = calculation difference between reported test result and result calculated by iis
W = test result withdrawn on request of the participant
ex = test result excluded from statistical evaluation
n.a. = not applicable
n.e. = not evaluated
n.d. = not detected
fr. = first reported

Literature

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