# Results of Proficiency Test
# AZO Dyes in Finger Paint
# June 2020


Organized by: Institute for Interlaboratory Studies
Spijkenisse, the Netherlands

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**CONTENTS**

# 1 INTRODUCTION

Some Azo Dyes are known to metabolise in aromatic amines. These primary aromatic amines have been considered mutagenic and/or carcinogenic for many years. Fingerpaint is especially important as a matrix to avoid Azo colorants that break down to aromatic amines, because fingerpaint is mainly used by children, with direct skin contact and possibility of ingestion of the paint. Method EN71-7 Finger Paints – Requirements and test methods describes a limit of 10 mg/kg of every primary aromatic amine present and a limit of 20 mg/kg for the total of primary aromatic amines.

During the annual proficiency testing program 2019/2020 the Institute for Interlaboratory Studies (iis) decided to organize a proficiency test scheme for the determination of banned aromatic amines derived from AZO dyes in Fingerpaint.
In this interlaboratory study 7 laboratories in 5 different countries registered for participation. See appendix 4 for the number of participants per country. In this report the results of this 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 analyzes for fit-for-use and homogeneity testing were subcontracted to an ISO/IEC17025 accredited laboratory. It was decided to send one sample of approximately 8 mL of fingerpaint, labelled #20625, positive on banned Aromatic Amines derived from AZO dyes. The participants were requested 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 can be downloaded from 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 yellow fingerpaint was obtained from a local supplier and made positive on 3,3'-Dimethoxybenzidine with Direct Blue 15, resulting in a dark green colored fingerpaint. After homogenization the batch was divided over 21 subsamples in small vials of approximately 8 mL each and labelled #20625. The homogeneity of the subsamples was checked by determination of 3,3'-Dimethoxybenzidine using test method ISO14362-1 on five stratified randomly selected subsamples.

| | 3,3'-Dimethoxybenzidine in mg/kg |
|---|---|
| sample #20625-1 | 117.2 |
| sample #20625-2 | 115.0 |
| sample #20625-3 | 110.6 |
| sample #20625-4 | 124.1 |
| sample #20625-5 | 120.5 |

Table 1: homogeneity test results of subsamples #20625

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

| | 3,3'-Dimethoxybenzidine in mg/kg |
|---|---|
| r (observed) | 14.5 |
| reference test method | ISO14362-1:17 |
| 0.3 * R (reference test method) | 13.6 |

Table 2: evaluation of the repeatability of subsamples #20625

The calculated repeatability was in agreement with 0.3 times the reproducibility of the reference test method. Therefore, homogeneity of the subsamples was assumed.

To each of the participating laboratories one sample labelled #20625 was sent on May 20, 2020.

## 2.5 ANALYZES

The participants were asked to determine on sample #20625 the concentrations of the following aromatic amines:

Benzidine (CASno. 92-87-5)
2-Naphtylamine (CASno. 91-59-8)
4-Chloro-2-methyl-aniline (CASno. 95-69-2)
4-Aminodiphenyl (CASno. 92-67-1)
o-Aminoazotoluene (CASno. 97-56-3)
2-Amino-4-nitrotoluene (CASno. 99-55-8)
4-Chloroaniline (CASno. 106-47-8)
2,4-Diaminoanisole (CASno. 615-05-4)
4,4'-Diaminodiphenylmethane (CASno. 101-77-9)
3,3'-Dichlorobenzidine (CASno. 91-94-1)
3,3'-Dimethoxybenzidine (CASno. 119-90-4)
3,3'-Dimethylbenzidine (Casno. 119-93-7)
3,3'-Dimethyl-4,4'-Diaminodiphenylmethane (CASno. 838-88-0)
p-Cresidine (CASno. 120-71-8)
2,2'-Dichloro-4,4'-methylenedianiline (CASno. 101-14-4)
4,4'-Oxydianiline (CASno. 101-80-4)
4,4'-Thiodianiline (CASno. 139-65-1)
o-Toluidine (CASno. 95-53-4)
2,4-Xylidine (CASno. 95-68-1)
2,6-Xylidine (CASno. 87-62-7)
4-Amino-3-fluorophenol (CASno. 399-95-1)
6-Amino-2-ethoxynaphthalene (CASno. 293733-21-8)
2-Methoxyaniline or o-Anisidine (CASno. 90-04-0)
4-Aminoazobenzene (CASno. 60-09-3)
4-Methyl-m-phenylenediamine (CASno. 95.80-7)
2,4,5-Trimethylaniline (CASno. 137-17-7)
Aniline (CASno. 62-53-3)

It was also requested to report if the laboratory was accredited to determine the reported components and to report some analytical details.

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 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 results cannot be used for meaningful statistical evaluations.

To get comparable 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 appropriate reference test method (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 are 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 appendix 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 reanalysis). Additional or corrected test results are used for data analysis and original test results are placed under 'Remarks' in the test result tables in appendix 1.
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 dataset does not have a normal distribution, the (results of the) statistical evaluation should be used with due care.

According to ISO5725 the original test results per determination were submitted to Dixon’s, Grubbs’ and/or Rosner’s outlier tests. 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.

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. This 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 was projected over the Kernel Density Graph for reference.

### 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, e.g. ISO reproducibilities, 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. In some cases, a reproducibility based on former iis proficiency tests could be used.

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 should be done in order to evaluate whether the reported test results are fit-for-purpose.

The z-scores were calculated in accordance with:

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

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:

| | | |
|---|---|---|
| $\lvert z\rvert$ | $< 1$ | good |
| $1 <$ $\lvert z\rvert$ | $< 2$ | satisfactory |
| $2 <$ $\lvert z\rvert$ | $< 3$ | questionable |
| $3 <$ $\lvert z\rvert$ | | unsatisfactory |

## 4 EVALUATION

In this proficiency test no severe problems were encountered with the dispatch of the samples. Two participants did not report at all and one of the participants reported the test results after the final reporting date. Not all laboratories were able to report all components requested.
A total of five participants reported 5 numerical test results. No outlying results were observed. In proficiency studies outlier percentages of 3% - 7.5% are quite normal.

### 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 methods are also in the tables together with the original data in appendix 1. The abbreviations, used in these tables, are explained in appendix 5.

All reporting participants performed test method EN71-7. Unfortunately, this method only states a precision for 3,3'-Dimethoxybenzidine with data from two laboratories (a mean and an uncertainty) at a concentration of 1 mg/kg. This is a much lower than the concentration found in this PT and not representative for this concentration. However, ISO14362-1:17 does have a precision statement for aromatic amines in Annex B, Table B.2 for concentrations around 25 mg/kg. This is a method for testing Azo Dyes in textile, but the determination of the Aromatic Amines in the solution will be similar. Therefore, the precision of this method may also apply to this PT. Regretfully, not for all listed Aromatic Amines precision data are available, but for the component 3,3'-Dimethoxy-benzidine, which is present in sample #20625 a precision statement is mentioned.

**Sample #20625**

3,3'-Dimethoxybenzidine: The determination of this aromatic amine at a concentration level of 127 mg/kg may not be problematic. No statistical outliers were observed. The calculated reproducibility is in good agreement with the requirements of ISO14362-1:2017.

The majority of the participants agreed on a concentration near or below the limit of detection for all other aromatic amines mentioned in paragraph 2.5. Therefore, no z-scores were calculated for these aromatic amines. The test results of these components are given in appendix 2.

### 4.2 PERFORMANCE EVALUATION FOR THE GROUP OF LABORATORIES

A comparison has been made between the reproducibilities as declared by the relevant reference test method and the reproducibilities as found for the group of participating laboratories. The number of significant test results, the average, the calculated reproducibilities (2.8 * standard deviation) and the target reproducibility, derived from literature reference test methods are presented in the next table.

| Component | unit | n | average | 2.8 * sd | R(target) |
|---|---|---|---|---|---|
| 3,3'-Dimethoxybenzidine | mg/kg | 5 | 129.2 | 13.9 | 45.3 |

Table 3: reproducibility on sample #20625

Without further statistical calculations, it can be concluded that the group of participating laboratories has no problem with the analysis of 3,3'-Dimethoxybenzidine in Fingerpaint at the given concentration level.

### 4.3 OVERVIEW OF THE PROFICIENCY TEST OF JUNE 2020

The evolution of the uncertainty expressed as relative standard deviation for Azo Dyes in Fingerpaint as observed in this proficiency scheme is listed in table 4.

| Year | Component | Observed RSD% | Target RSD% | Concentration mg/kg |
|---|---|---|---|---|
| 2020 | 3,3'-Dimethoxybenzidine | 4% | 13% | 129 |

Table 4: uncertainty in % for Azo Dyes in Fingerpaint

### 4.4 EVALUATION ANALYTICAL DETAILS

For this PT also some analytical details were requested and are listed in appendix 3.
Based on the answers given by the participants the following can be summarized:
- Four out of five reporting participants mentioned that they are accredited for the determination of aromatic amine components.
- Four out of five reporting participants used around 0.5 grams or more sample intake, one used 0.2 grams.
- Only two participants reported measuring a pH of the fingerpaint when tested.

Because the amount of analytical details and participating laboratories is small, no conclusions could be drawn from these analytical details.

## 5 DISCUSSION

All reporting participants were able to detect 3,3'-Dimethoxybenzidine in sample #20625. No other aromatic amines were detected.
The sample of #20625 was well above the limit of 10 mg/kg for a single aromatic amine as mentioned in EN71-7.

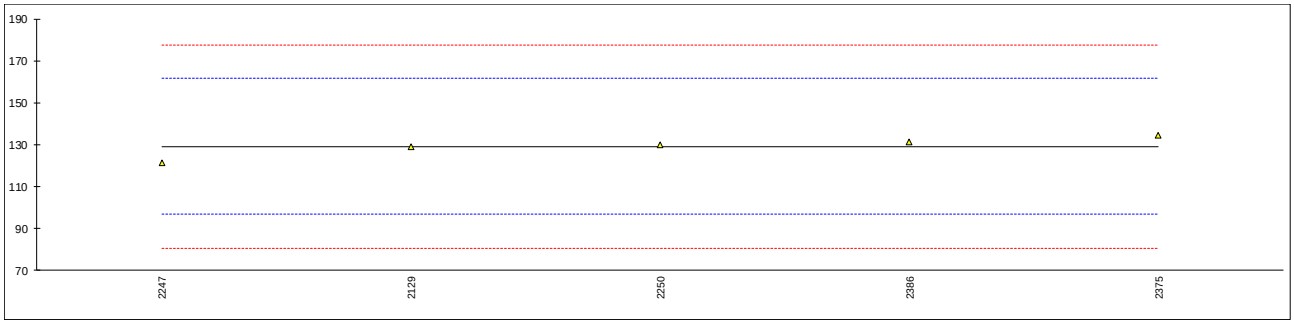
## 6 CONCLUSION

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 3,3'-Dimethoxybenzidine (CAS no. 119-90-4) in sample #20625; results in mg/kg

| lab | method | value | mark | z(targ) | remarks |
|---|---|---|---|---|---|
| 2102 | | ----- | | ----- | |
| 2129 | EN71-7 | 129.0 | | -0.01 | |
| 2247 | EN71-7 | 121.16 | | -0.49 | |
| 2250 | EN71-7 | 130 | | 0.05 | |
| 2375 | EN71-7 | 134.6 | | 0.34 | |
| 2386 | EN71-7 | 131.1 | | 0.12 | |
| 3172 | | ----- | | ----- | |
| | | | | | |
| | normality | unknown | | | |
| | n | 5 | | | |
| | outliers | 0 | | | |
| | mean (n) | 129.172 | | | |
| | st.dev. (n) | 4.9520 | | | |
| | R(calc.) | 13.865 | RSD = 4% | | |
| | st.dev.(ISO14362-1:17) | 16.1926 | | | |
| | R(ISO14362-1:17) | 45.339 | | | Compare R(EN71-7) = 1.764 |



## APPENDIX 2 Other reported aromatic amines

**Abbreviations**

BD = Benzidine (CASno. 92-87-5)
2NA = 2-Naphtylamine (CASno. 91-59-8)
4CoT = 4-Chloro-o-toluidine / 4-Chloro-2-methyl-aniline (CASno. 95-69-2)
4AD = 4-Aminodiphenyl (CASno. 92-67-1)
oAAT = o-Aminoazotoluene (CASno. 97-56-3)
ANT = 2-Amino-4-nitrotoluene (CASno. 99-55-8)
4CA = 4-Chloraniline (CASno. 106-47-8)
DAA = 2,4-Diaminoanisol (CASno. 615-05-4)
DADM = 4,4'-Diaminodiphenylmethane (CASno. 101-77-9)
DCB = 3,3'-Dichlorobenzidine (CASno. 91-94-1)
DMB = 3,3'-Dimethylbenzidine (Casno. 119-93-7)
DDDM = 3,3'-Dimethyl-4,4'-Diaminodiphenylmethane (CASno. 838-88-0)
pC = p-Cresidine (CASno. 120-71-8)
DMD = 2,2'-Dichloro-4,4'-methylenedianiline (CASno. 101-14-4)
OA = 4,4'-Oxydianiline (CASno. 101-80-4)
TA = 4,4'-Thiodianiline (CASno. 139-65-1)
oT = o-Toluidine (CASno. 95-53-4)
24X = 2,4-Xylidine (CASno. 95-68-1)
26X = 2,6-Xylidine (CASno. 87-62-7)
AFP = 4-Amino-3-fluorophenol (CAS No. 339-95-1)
AEN = 6-Amino-2-ethoxynaphthalene (CAS No. 293733-21-8)
2MA = 2-Methoxyaniline (CAS No. 90-04-0)
4AAB = 4-Aminoazobenzene (CAS No. 60-09-3)
4MPD = 4-Methyl-m-phenylenediamine (CAS No. 95-80-7)
TMA = 2,4,5-Trimethylaniline (CAS No. 137-17-7)
AL = Aniline (CAS No. 62-53-3)

Sample #20625; abbreviations explained above

| lab | BD | 2NA | 4CoT | 4AD | oAAT | ANT | 4CA | DAA | DADM | DCB | DMB | DDDM | pC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2102 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| 2129 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| 2247 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 2250 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| 2375 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| 2386 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 |
| 3172 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |

Sample #20625 -continued; abbreviations explained above

| lab | DMD | OA | TA | oT | 24X | 26X | AFP | AEN | 2MA | 4AAB | 4MPD | TMA | AL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2102 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| 2129 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 2.9 | ----- | ----- | ----- | ----- |
| 2247 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 2250 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 3.2 | ----- | ----- | ----- | ----- |
| 2375 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| 2386 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 |
| 3172 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |

## APPENDIX 3 Analytical details

| lab | ISO/IEC17025 accredited | sample intake (g) | pH of fingerpaint | remarks |
|---|---|---|---|---|
| 2102 | ----- | ----- | ----- | |
| 2129 | Yes | 0.5 | not tested | |
| 2247 | Yes | 1 | 6.15 | N.D: Not Detected (<5mg/kg) |
| 2250 | Yes | 0,2 | ----- | |
| 2375 | No | 1.0016 | ----- | |
| 2386 | Yes | 0,5 | ph > 7 | |
| 3172 | ----- | ----- | ----- | |

## APPENDIX 4

## Number of participants per country

| | |
|---|---|
| 3 labs in | GERMANY |
| 1 lab in | INDIA |
| 1 lab in | ITALY |
| 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
W = test result withdrawn on request of participant
ex = test result excluded from statistical evaluation
fr. = first reported
f-? = possibly a false negative test result?
f+? = possibly a false positive test result?
n.a. = not applicable
n.e. = not evaluated
n.d. = not detected

**Literature**

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