UniversitätsKlinikum Heidelberg

Universitätsklinik für Kinder- und Jugendmedizin
Stoffwechselzentrum Heidelberg Stoffwechsellabor
Im Neuenheimer Feld 669 | 69120 Heidelberg

To

**University Children's Hospital**
Angelika-Lautenschläger-Klinik

**Department of General Pediatrics**
(General Pediatrics, Neurology, Metabolism, Gastroenterology, Nephrology)
Prof. Dr. med. G.F. Hoffmann
Chairman
**Center for Metabolic Diseases Heidelberg**

**Metabolic Laboratory**

Heidelberg, 21.April 2016

## ERNDIM QA Scheme for qualitative urinary organic acid analysis

# Annual Report 2015 (updated version)

## Participation

The geographical distribution of the active participants of the quality assurance scheme organized and distributed through the centre of Heidelberg in 2015 is shown in Table 1. Sheffield and Heidelberg participate in each other's scheme and the two centers work closely together under the auspices of the ERNDIM Scientific Advisory Committee.

Table 1: Geographical distribution of participants

| Country | Number of laboratories | | Country | Number of laboratories |
|---|---|---|---|---|
| Austria | 3 | | Latvia | 1 |
| Belgium | 1 | | Lithuania | 1 |
| Bulgaria | 1 | | Luxembourg | 1 |
| Canada | 8 | | New Zealand | 1 |
| Croatia | 1 | | Norway | 1 |
| Cyprus | 1 | | Philippines | 1 |
| Czech Republic | 2 | | Poland | 2 |
| Denmark | 1 | | Serbia | 1 |
| Estonia | 1 | | Slovakia | 2 |
| France | 5 | | Slovenia | 1 |
| Germany | 17 | | Spain | 2 |
| Greece | 1 | | Sweden | 2 |
| Hong Kong | 1 | | Switzerland | 3 |
| Hungary | 1 | | The Netherlands | 9 |
| India | 4 | | Ukraine | 1 |
| Italy | 12 | | United Kingdom | 1 |
| Kingdom of Saudi Arabia | 2 | | USA | 12 |

Im Neuenheimer Feld 669
69120 Heidelberg
Stoffwechsellabor:
Fon +49 (0)6221 56 8276
8423
Fax +49 (0)6221 56 5565

Neugeborenenscreening:
Fon +49 (0)6221 56 8278

stoffwechsellabor@uni-hd.de
www.stoffwechsel.uni-hd.de

## Samples and results

Three sets of three samples (total 9; sample numbers 214 - 222) were distributed to **104 laboratories**.

One laboratory registered as **educational participant** and was not scored.

Table 2 shows the number of returned results for each circulation and the number of late returns.

Table 2: Receipt of results

| Circulation | In time returns | Late returns | Total |
|---|---|---|---|
| 1. circulation | 96 | 2 | 98 |
| 2. circulation | 99 | 1 | 100 |
| 3. circulation | 98 | 0 | 98 |

Ninety-four percent of the participants returned results for all three circulations. Three laboratories (3%) did not respond to any of the circulations (see also table 3)

Table 3: returned results

| Circulations | Number of laboratories | % |
|---|---|---|
| 3 | 97 | 94 |
| 2 | 2 | 2 |
| 1 | 1 | 1 |
| 0 | 3 | 3 |

## Shipment of the samples

Date of sample dispatch: **04 May 2015**

For the first time the nine urine samples were sent out by the **Quality Control Center Switzerland (CSCQ)**.

As the years before the samples for all three circulations were shipped together. This is only for organizational reasons, especially to keep the costs for participating in this scheme as low as possible.

**Please remember, the idea of the scheme is to measure the samples evenly spread over the year and to report the results near to the closing date!**

Table 4: Distribution of scores for individual samples (number of laboratories making returns)

| | | 4 | 3 | 2 | 1 | 0 |
|---|---|---|---|---|---|---|
| **Sample 223** | Canavan disease | 85 | 3 | 7 | | 3 |
| **Sample 224** | Isovaleric aciduria | 98 | 2 | 1 | | |
| **Sample 225** | Normal pattern | 97 | | | | 1 |
| **Sample 226** | Normal pattern | 99 | 1 | | | |
| **Sample 227** | Maple syrup urine disease (MSUD) | 97 | 1 | | | 2 |
| **Sample 228** | Mevalonic aciduria | 100 | | | | |
| **Sample 229** | Normal pattern | 98 | | | | |
| **Sample 230** | Alkaptonuria | 96 | 1 | | | 1 |
| **Sample 231** | 5-oxoprolinuria | 98 | | | | |

## Scoring scheme

In the process of ongoing accreditation of the ERNDIM organization there is a need for harmonization of performance assessment within the qualitative schemes (see ERNDIM 'Newsletter Spring 2013' at www.erndim.org).

In 2013 we changed the scoring system from the former scale (-2, -1, 0, +1, +2) to the four-point system (+1, +2, + 3, +4) which is used also in the DPT schemes. In this system a maximum of two points is given each for analytical results and interpretation, with the latter including suggestions for further testing/actions.

The total score achievable for a single circulation of three samples is twelve and thirty-six for the whole sample set of nine samples per year.

To obtain satisfactory performance a score of 22 or more should be achieved on three returns and 15 or more when two returns have been submitted.

Another criteria for satisfactory performance will be the absence of any "critical error" which is defined as an error resulting from seriously misleading analytical findings and /or interpretations with serious clinical consequences for the patient.

## Comments on performance

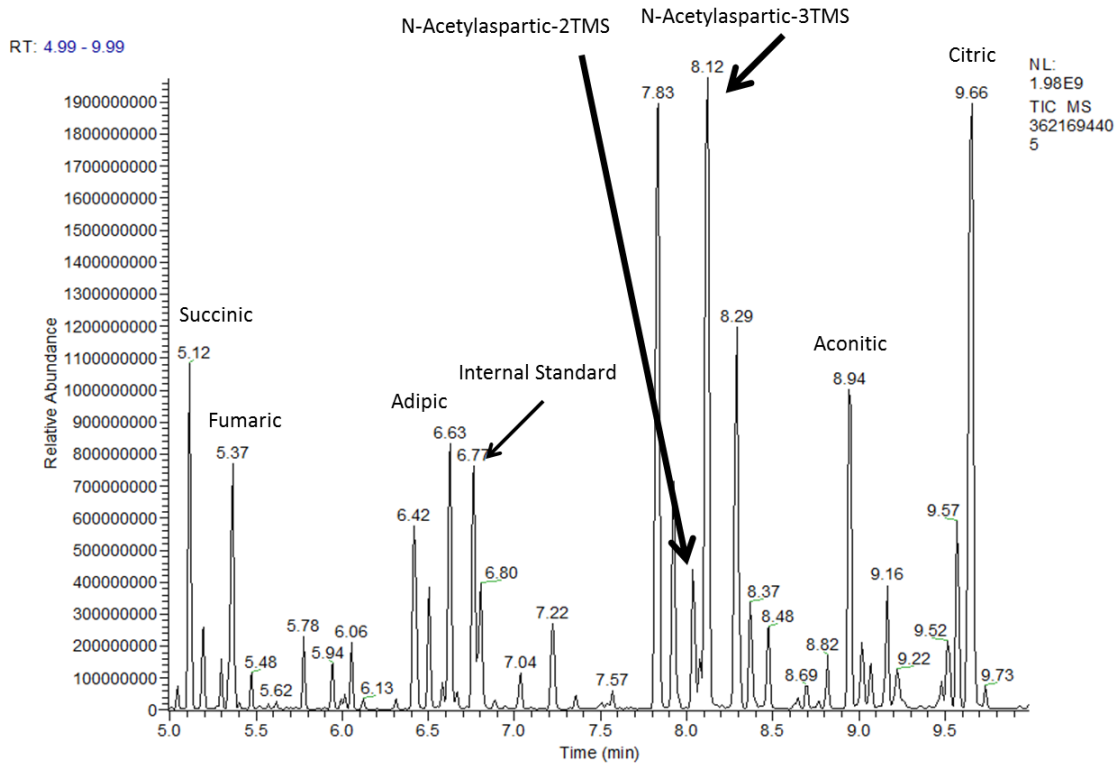
## Sample 223:

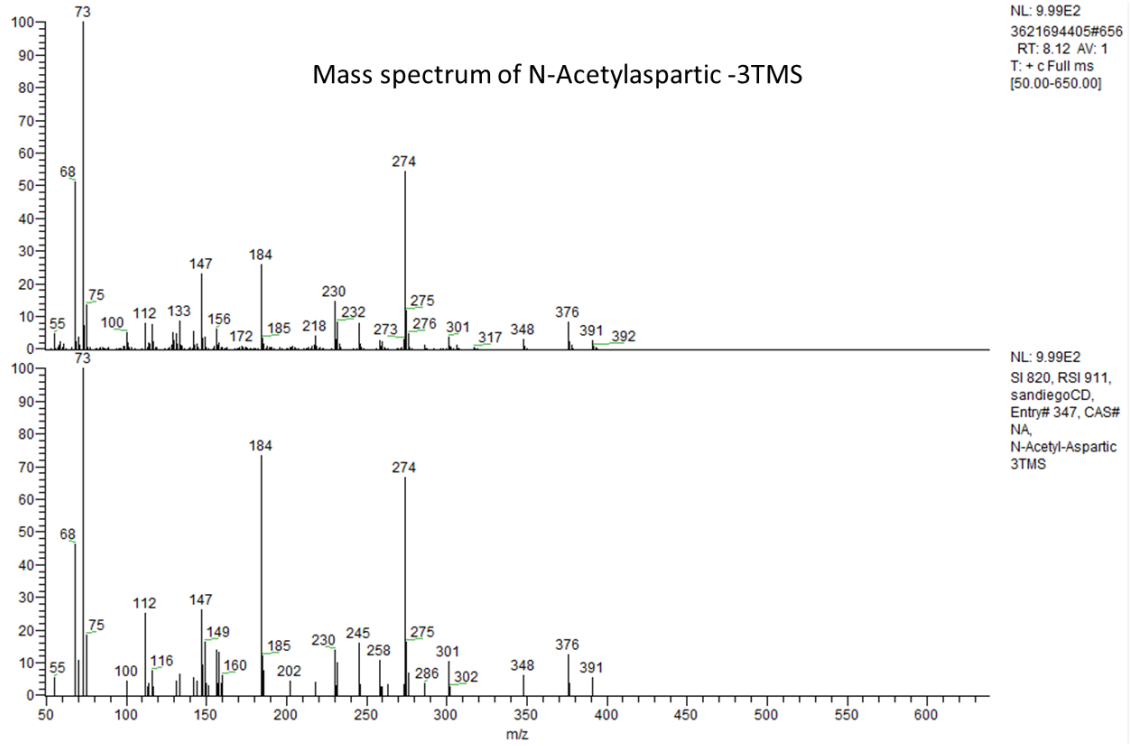
**Patient details:** 15-month old girl with muscle hypotonia and motor retardation

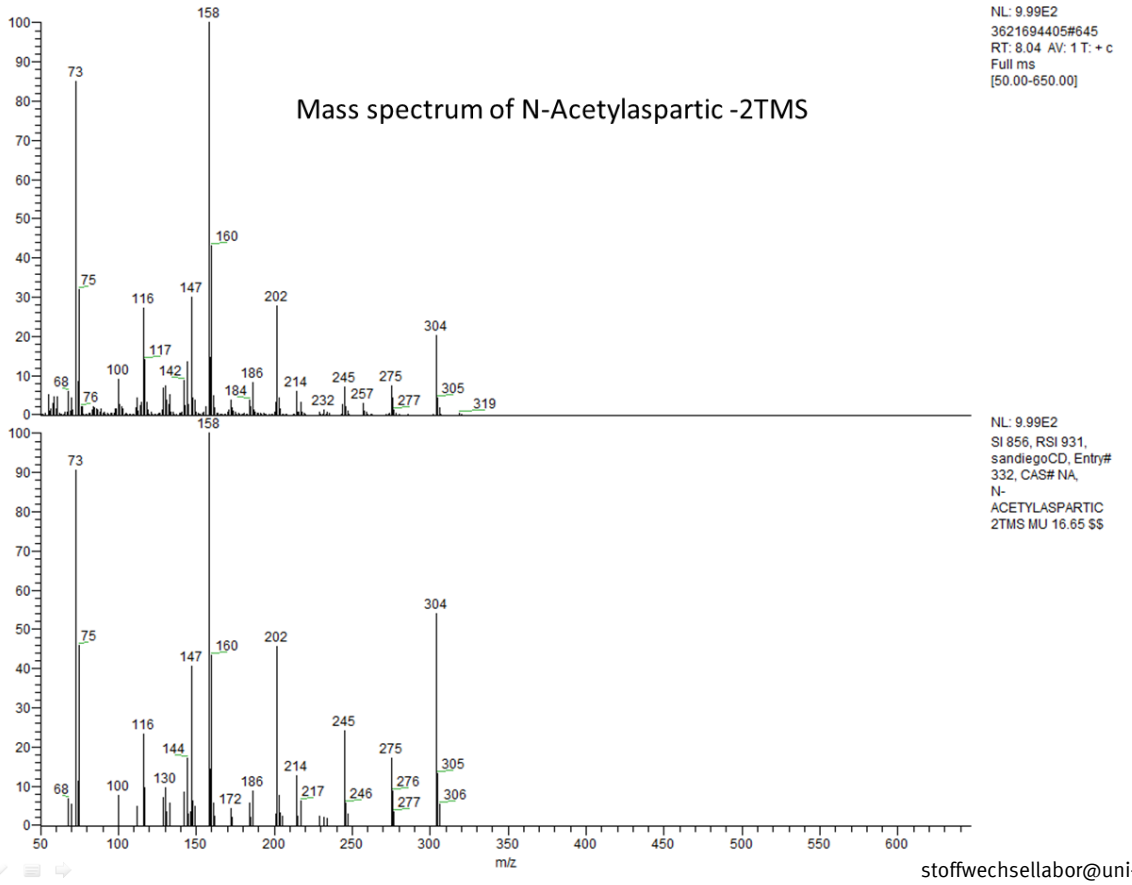
**Known diagnosis:** Canavan disease

**Analytical details** In the chromatogram elevated amounts of 3-hydroxybutyric acid and lactic acid as well as a marked dicarboxyluria could be found.

To make the correct diagnosis the detection of increased amounts of N-acetylaspartic acid was essential. This metabolite forms on trimethylsilylation two derivatives, namely the di-TMS and the tri-TMS derivative.




Mass spectrum of N-Acetylaspartic -3TMS
NL: 9.99E2
3621694405#656
RT: 8.12 AV: 1
T: + c Full ms
[50.00-650.00]
NL: 9.99E2
SI 820, RSI 911,
sandiegoCD,
Entry# 347, CAS#
NA,
N-Acetyl-Aspartic
3TMS
m/z


Mass spectrum of N-Acetylaspartic -2TMS
NL: 9.99E2
3621694405#645
RT: 8.04 AV: 1 T: + c
Full ms
[50.00-650.00]
NL: 9.99E2
SI 856, RSI 931,
sandiegoCD, Entry#
332, CAS# NA,
N-
ACETYLASPARTIC
2TMS MU 16.65 $$
m/z

**Analytical Performance:** 90%. Two points was given for the identification of N-acetylaspartic acid and one point for only 3-hydroxybutyric acid and lactic acid.

**Diagnostic Performance:** 87%. One point was given for the diagnosis of ketosis, lactic acidosis or mitochondrial disease.

**Overall impression:** Ten percent of the laboratories have difficulties in extracting N-acetylaspartic acid

**Critical error:** A critical error was defined if detection of N-acetylaspartic acid was missed or a misleading diagnosis was given with no advice for further investigations.

## Sample 224:

**Patient details:** 3-year old boy presenting with recurrent vomiting, first detected in newbornscreening, at present under medication

**Known diagnosis:** Isovaleric aciduria / isovaleryl-CoA dehydrogenase deficiency

**Analytical details:** A large peak of isovalerylglycine clearly pointed to isovaleric aciduria. Normal 3-hydroxyisovaleric acid indicates a non-crisis situation.

**Overall Performance:** 100%. All participants clearly detected isovalerylglycine and diagnosed isovaleric aciduria.

## Sample 225:

**Patient details:** 3-year old boy with developmental delay

**Known diagnosis:** Normal pattern

**Analytical details:** Nothing specific

**Overall Performance:** 99%

## Sample 226:

**Patient details:** 2-year old girl presented with hypotonia and seizures

**Known diagnosis:** Normal pattern

**Analytical details:** Nothing specific

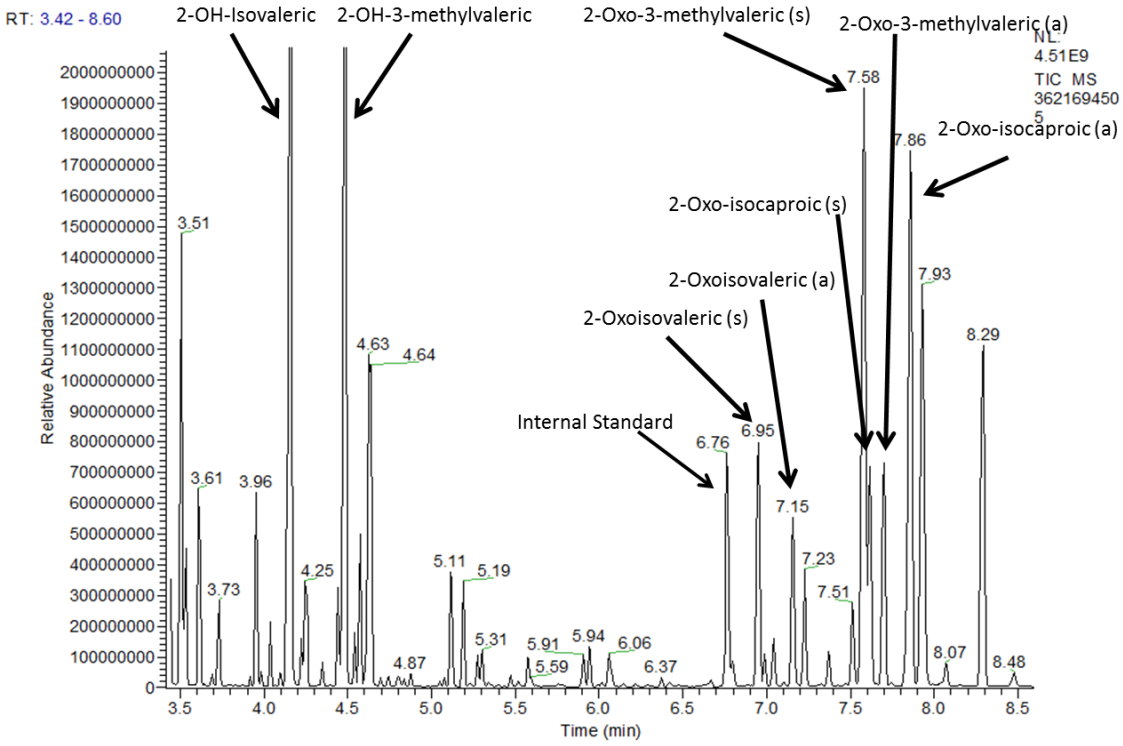
**Overall Performance:** 99%

## Sample 227:

**Patient details:** 4-year old girl with developmental delay, first detected in new-bornscreening, currently under medication

**Known diagnosis:** Maple syrup urine disease (MSUD)

**Analytical details:** The chromatogram showed pathologically elevated amounts of several branched chain oxo- and hydroxy acids, especially 2-hydroxyisovaleric acid, 2-hydroxy-3-methylvaleric acid, 2-oxoisovaleric acid, 2-oxo-3-methylvaleric acid and 2-oxoisocaproic acid.




**Overall Performance:** 98%. Two laboratories had problems in identifying the branched chain oxo- and hydroxy acids

**Critical error:** A critical error was defined if detection of branched chain oxo- and hydroxyacids was missed or a misleading diagnosis was given with no advice for further investigations.

## Sample 228:

**Patient details:** 1-year old boy with hypotonia and dysmorphia

**Known diagnosis:** Mevalonic aciduria

**Analytical details:** **T**he pathognomonic metabolite mevalonic acid manifested in the chromatogram in different derivatives. Beside underivatised dehydromevalonolactone and mevalonolactone one could found the tri-trimethylsilyl derivative of mevalonic acid and the mono-trimethylsilyl derivative of mevalonolactone. The latter is the main product formed under the used sample preparation conditions.

**Overall Performance:** 100%. All participants identified the relevant metabolite and gave the correct diagnosis

## Sample 229:

**Patient details:** 1-year old boy with failure to thrive

**Known diagnosis:** Normal pattern

**Analytical details:** Nothing specific

**Overall Performance:** 100%

## Sample 230:

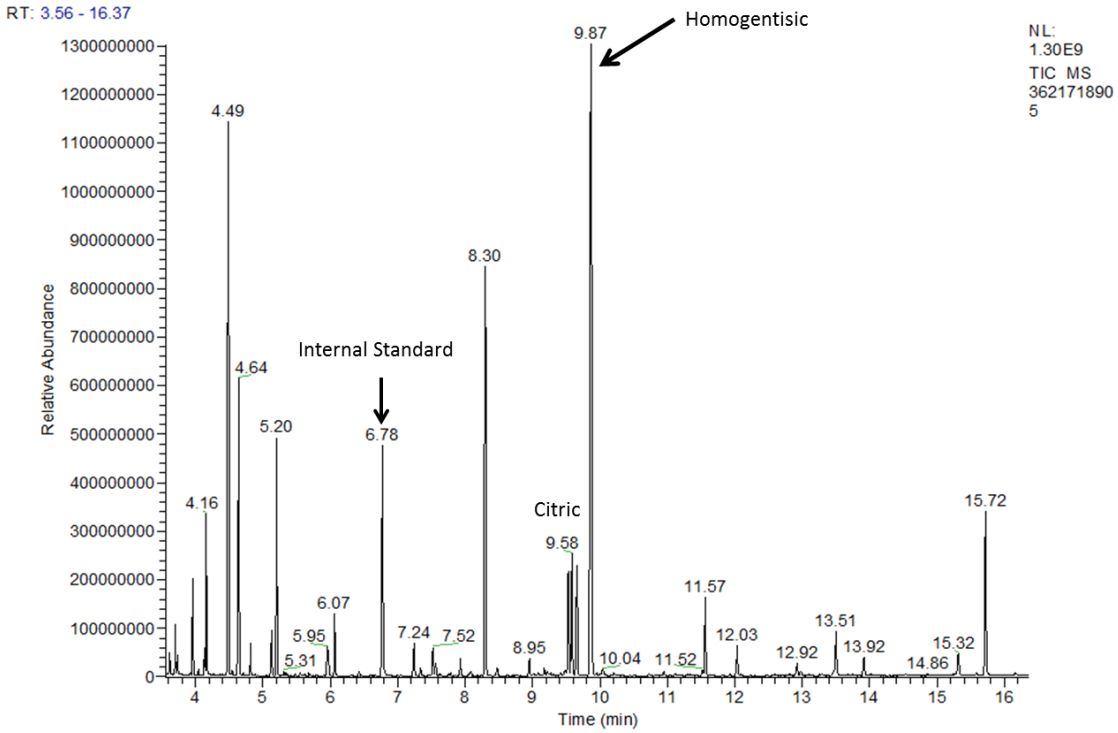
**Patient details:** 16-year old female complain of nausea and drowsiness

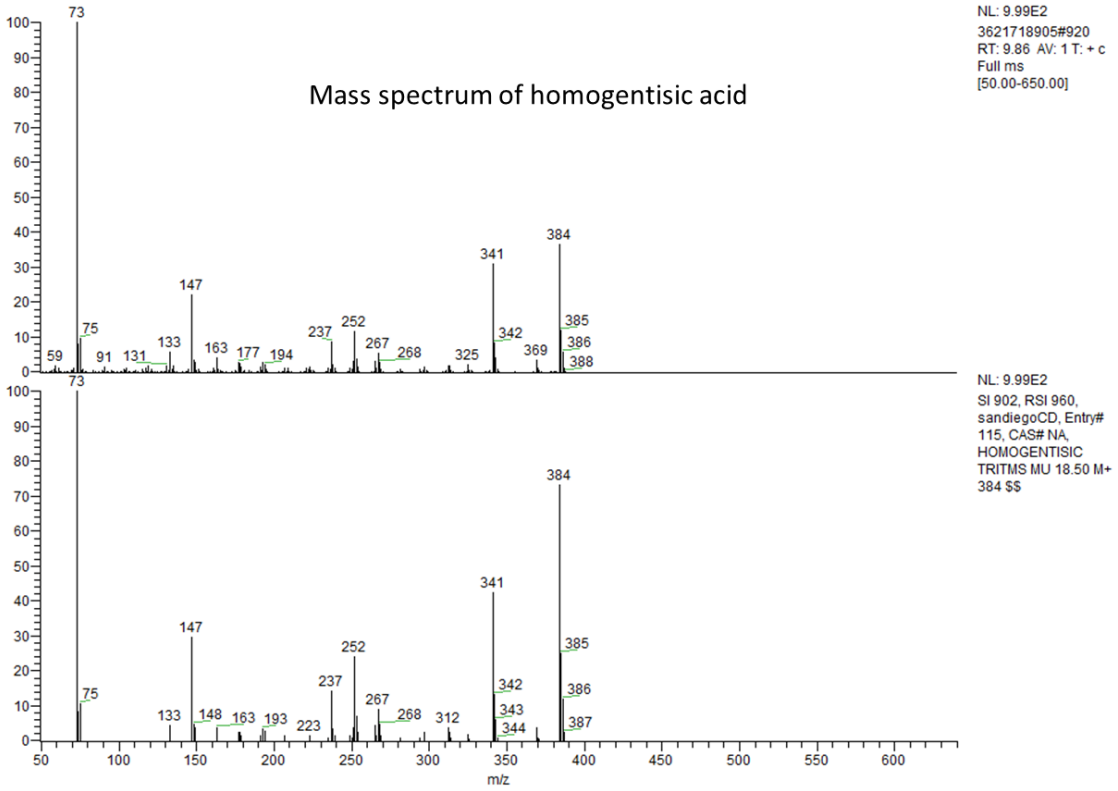
**Known diagnosis:** Alkaptonuria

**Analytical details:** The chromatogram showed a large peak of homogentisic acid

**Analytical Performance:** 99%. One laboratory failed to detect homogentisic acid

**Diagnostic Performance:** 98%


RT: 3.56 - 16.37
NL:
1.30E9
TIC MS
3621718905
Homogentisic
Internal Standard
Citric
Relative Abundance
Time (min)


Mass spectrum of homogentisic acid
NL: 9.99E2
3621718905#920
RT: 9.86 AV: 1 T: + c
Full ms
[50.00-650.00]
NL: 9.99E2
SI 902, RSI 960,
sandiegoCD, Entry#
115, CAS# NA,
HOMOGENTISIC
TRITMS MU 18.50 M+
384 $$
m/z

## Sample 231:

**Patient details:** neonate with psychomotor retardation

**Known diagnosis:** 5-oxoprolinuria

**Analytical details:** The chromatogram showed a large peak of 5-oxoproline

**Overall Performance:** 100%. All participants identified the relevant metabolite and gave the correct diagnosis

The participants´ cumulative scores are shown in table 6 and in figure 5.
Cumulative scores are the scores for the whole year.

In 2015 eighty participants (81%) got full marks!

Table 6: Cumulative total scores 2013 - 2015
Number of all participants: all registered laboratories
Number of nonresponders: no results returned for any of the three circulations

| | Percent of all participants | | |
|---|---|---|---|
| **Cumulative scores** | **2015** | **2014** | **2013** |
| 36 | 78 | 54 | 82 |
| 35 | 3 | 13 | - |
| 34 | 8 | 12 | 7 |
| 33 | - | 1 | - |
| 32 | 5 | 6 | 1 |
| 31 | - | 3 | - |
| 30 | - | 2 | - |
| 29 | - | 1 | - |
| 28 | 1 | 1 | - |
| 27 | - | - | - |
| 26 | - | - | - |
| 25 | - | - | - |
| 24 | 2 | 4 | 5 |
| 23 | - | - | - |
| 22 | - | - | - |
| 21 | - | 1 | - |
| 20 | - | - | - |
| 19 | - | - | - |
| 18 | - | - | - |
| 17 | - | - | - |
| 16 | - | - | - |
| 15 | - | - | - |
| 14 | - | - | - |
| 13 | - | - | - |
| 12 | 1 | - | 1 |
| 11 | - | - | - |
| 10 | - | - | - |
| 9 | - | - | - |
| 8 | - | - | - |
| 7 | - | - | - |
| 6 | - | - | - |
| 5 | - | - | - |
| 4 | - | - | - |
| 3 | - | - | - |
| 2 | - | - | - |
| 1 | - | - | - |
| 0 | 3 | 2 | 3 |
| **Number of all participants** | 103 | 101 | 94 |
| **Number of Nonresponders** | 3 | 2 | 3 |

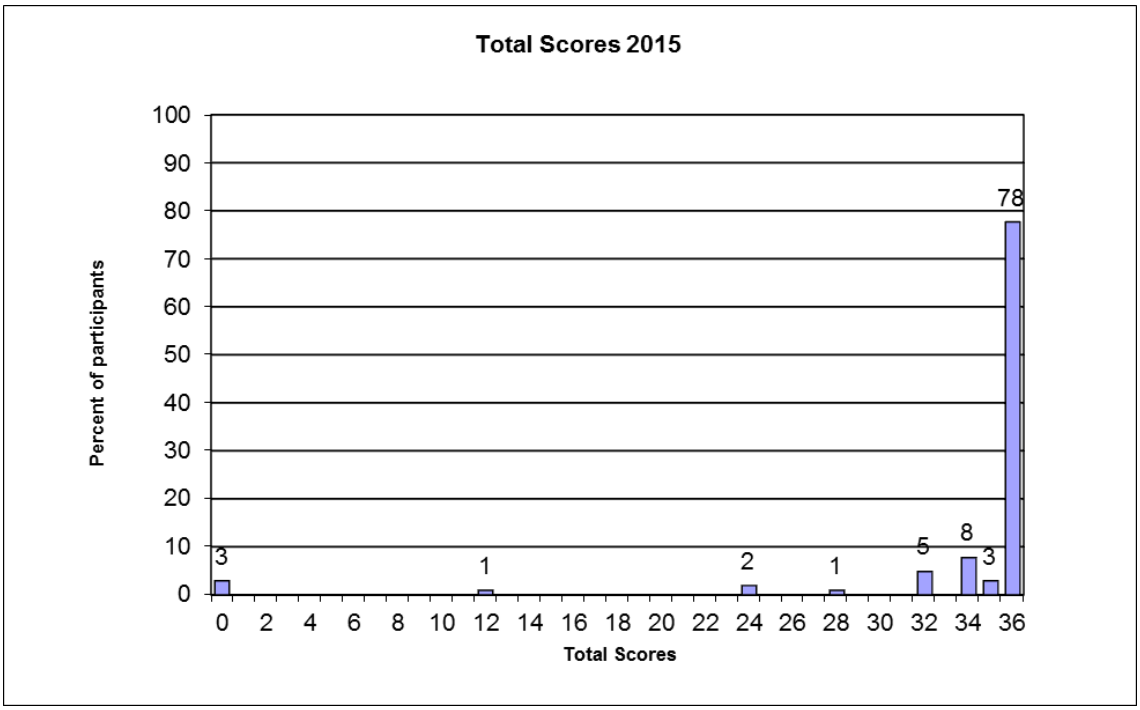



Fig. 6: Cumulative scores 2015

## Your total score 2015

Your total score for 2015 was:

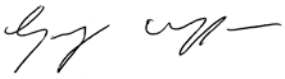
Your number of returns in 2015 was:

## General comments

We would just like to point out here that we are not able to accept returns sent in after the report for the corresponding circulation has been mailed because this would not be compatible with the overall intention of the scheme. We are conscious of the fact that posted results could get lost on a variety of ways. Therefore it would be a good advice to send in results by more than one route (e.g. FAX and email, regular mail and FAX or email).

Special thank for the laboratories that supported us last year with samples. This is critical for the success of the program and will keep the scheme interesting. **It is most appreciated that you will continue to support us with urine from patients. Please send us at least 300 ml urine of any interesting patients you may have. We will cover the costs.**

Yours sincerely,



**Dr. C. D. Langhans**

*Laboratory of Metabolic Diseases*

**Dr. V. Peters**

*Laboratory of Metabolic Diseases*

**Prof. Dr. G. F. Hoffmann**

*Director*
*Department of General Paediatrics*