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To

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Department of General Pediatrics
(General Pediatrics, Neurology, Metabolism, Gastroenterology, Nephrology)
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Chairman
Center for Metabolic Diseases Heidelberg

Metabolic Laboratory

Heidelberg, 03rd May 2012

ERNDIM QA Scheme for qualitative urinary organic acid analysis

Annual Report 2012

Participation

The geographical distribution of the active participants of the quality assurance scheme organized and distributed through the centre of Heidelberg in 2012 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	2		Lithuania	1
Belgium	1		Luxembourg	1
Bulgaria	1		New Zealand	1
Canada	7		Norway	1
Croatia	1		Philippines	1
Cyprus	1		Poland	2
Czech Republic	2		Slovakia	2
Denmark	1		Slovenia	1
Estonia	1		Spain	2
France	4		Sweden	2
Germany	13		Switzerland	4
Greece	1		Turkey	1
Hungary	1		The Netherlands	9
India	3		Ukraine	1
Italy	12		United Arab Emirates	1
Kingdom of Saudi Arabia	1		United Kingdom	1
Latvia	1		USA	11

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Samples and results

Three sets of three samples (total 9; sample number 196 - 204) were distributed to 95 laboratories.

Table 2 shows 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	87	4	91
2. circulation	82	4	86
3. circulation	88	1	89

Eighty-nine percent of the participants returned results for all three circulations. Four laboratories (4%) did not respond to any of the circulations (see also table 3)

Table 3: returned results

Circulations	Number of laboratories	%
3	85	89
2	5	5
1	1	1
0	4	4

Shipment of the samples

As the years before we sent out the samples for all three circulations 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 report the results near the closing date!

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

		-2	-1	0	1	2
Sample 196	Normal pattern			2	6	83
Sample 197	Methylmalonic aciduria					91
Sample 198	Maple syrup urine disease	1			1	89
Sample 199	Aminoacylase I deficiency *)			17	6	63
Sample 200	Normal pattern			3		83
Sample 201	Normal pattern			2	1	83
Sample 202	Normal pattern					89
Sample 203	Phenylketonuria (untreated)	1				88
Sample 204	Glutaric aciduria type I		1			88

*) updated scores of the individual report

Scoring scheme

Individual returns for each sample were scored on the scale

2	Correct/satisfactory
1	helpful but incomplete
0	unhelpful
-2	misleading

The ERNDIM organisation is moving towards providing a single "Certificate" to cover participation and performance in all its schemes. The scheme organizers of the "Qualitative Organic Acid Scheme" in Sheffield and Heidelberg agreed on criteria to define "Participation" and "Satisfactory Performance".
We are aware that these criteria are rather arbitrary but we are convinced that they will represent the different contexts in which the participants are working.
So "Participation" will be defined as requiring at least two returns during a year and "Satisfactory Performance" as obtaining a score of 11 or more based on three returns (out of maximum 18). When two returns have been received a score of 7 or more (in this case possible maximum score 12) is satisfactory.

Comments on performance

Sample 196:

Patient details: 12-month-old boy with hypotonia and seizures

Known diagnosis: Normal pattern

Analytical details: The chromatogram showed an unremarkable organic acid profile except for a prominent peak of lactic acid. Such moderate high concentrations could be assessed in different ways according to individual practice of the laboratories. Helpful approaches to clarify the diagnosis should be repeating organic acid analysis with a newly ordered sample and determining lactate in a blood sample.

Overall Performance: 92% regarded this sample to be normal.

Sample 197:

Patient details: 6-days-old neonate with hyperammonaemia and acidosis

Known diagnosis: methylmalonic aciduria

Overall Performance: all laboratories clearly identified the essential metabolites methylmalonic acid and methylcitric acid.
Analytical and interpretative performance was 100 %.

Sample 198:

Patient details: 10-month old girl with recurrent generalized convulsions, at present under medication

Known diagnosis: Maple syrup urine disease

Analytical details: In this sample several branched-chain oxo- and hydroxyacids could be identified. Nearly all participants reported these metabolites. One laboratory detected only 2-hydroxyisovaleric acid and another one had difficulties in identifying any abnormal metabolite.

Diagnosis: 98% diagnosed MSUD.

Chromatogram:

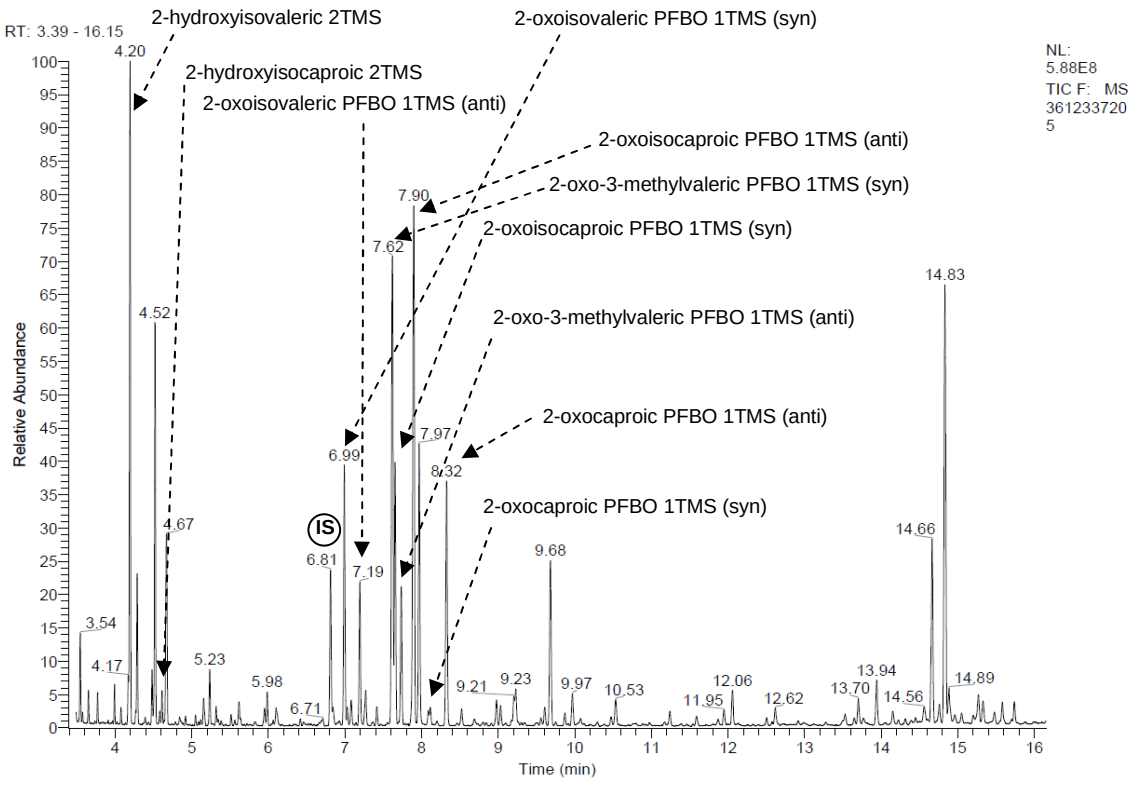


Fig 1: Organic acid profile of MSUD [IS: 4-Nitrophenol-d4 125mmol/mol creatinine]

Sample 199:

Patient details: 5-year-old male with recurrent episodes of seizures

Known diagnosis: Aminoacylase I deficiency

Analytical details: The main analytical challenge was identifying N-acetylated amino acids like alanine, glycine, valine, leucine, serine, methionine, aspartic acid and glutamic acid.
73% of the participants detected these metabolites, 22% did not.

Overall Performance: The identification or N-acetylated amino acids lead in all cases to the correct diagnosis.

Samples from the same urine were already circulated in 2009 (sample #171). At that time only 43% of the participants diagnosed aminoacylase I deficiency. Seventy laboratories participated in 2009 and 2012. Thirty laboratories (43%) gave the correct diagnosis in both years, twenty-six participants (37%) improved their performance from 2009 to 2012. Twelve participants (17%) missed the diagnosis in both years. Surprisingly, two laboratories gave the correct diagnosis in 2009 but missed it in 2012 (table 5).
Compared to 2009 nineteen participants registered newly in 2012. Forteen participants sent results for sample #199. Seven of them diagnosed correctly aminoacylase I deficiency.

Table 5: Performance of the laboratories which participated in 2009 and 2012

Correct diagnosis	**participants**	**%**
in both years (2009 + 2012)	30	43
Only in 2012	26	37
Neither in 2009 nor in 2012	12	17
Only in 2009	2	3
	70	**100**

Chromatogram:

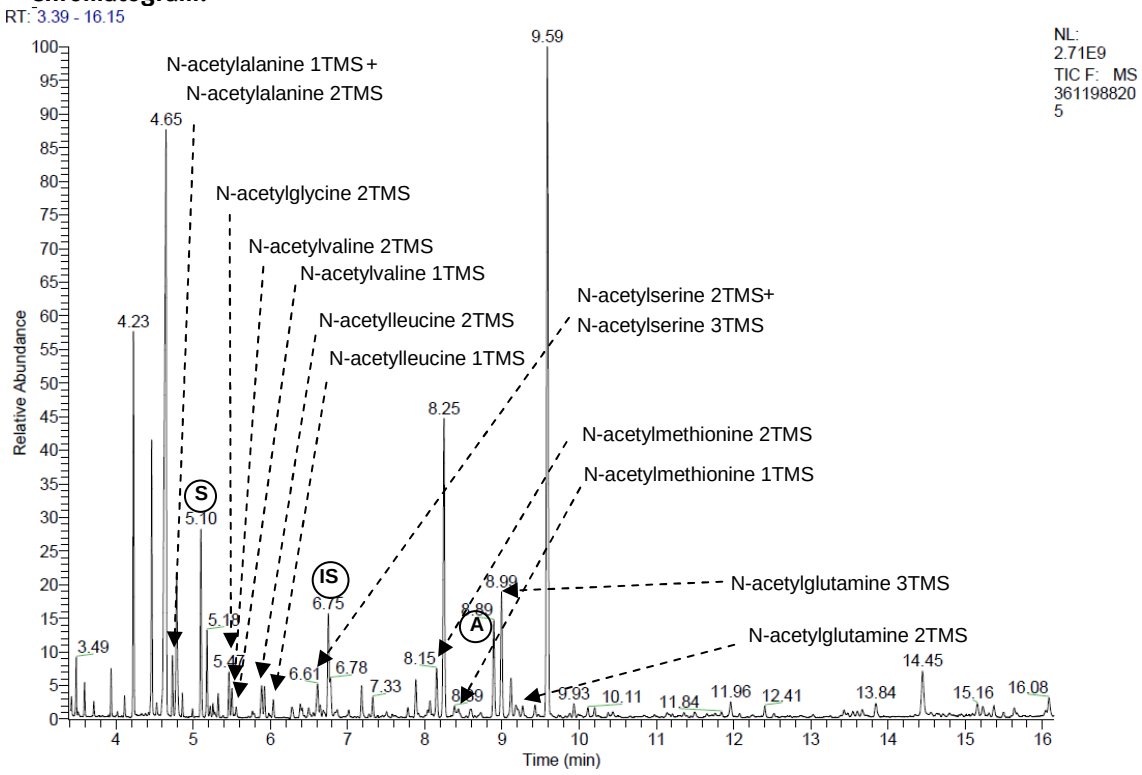


Fig 2: Organic acid profile of Aminoacylase Ideficiency [IS: 4-Nitrophenol-d4 125mmol/mol creatinine; S: succinic acid; A: aconitic acid]

Mass spectra of trimethylsilylated N-acetylated amino acids can be found in

Gerlo-E; Van Coster-R; Lissens-W; Winckelmans-G; De Meirleir-L; Wevers-R
*Gas chromatographic-mass spectrometric analysis of N-acetylated amino acids: the first case of aminoacylase I deficiency. Analytica chimica acta 2006; **571**(2): 191-9.*

Sample 200:

Patient details:	2-year old girl with failure to thrive
Known diagnosis:	Normal pattern
Overall Performance:	97 % reported a normal organic acid profile

Sample 201:

Patient details:	8-month-old boy with acute ataxia
Known diagnosis:	Normal pattern
Overall Performance:	97 % reported a normal organic acid profile

Sample 202:

Patient details: 4-year-old girl with poor appetite and growth failure

Known diagnosis: Normal pattern

Overall Performance: 100 % reported a normal organic acid profile

Sample 203:

Patient details: 2.5-year-old girl with mental retardation and microcephaly

Known diagnosis: untreated phenylketonuria

Analytical details: Several aromatic and hydroxylated aromatic acids were detected in this urine sample. Phenylacetic acid, mandelic acid, 2-hydroxyphenylacetic acid, phenyllactic acid, 4-hydroxyphenylacetic acid, 4-hydroxyphenyllactic acid, and phenylpyruvic acid were moderately/highly elevated. These metabolites were reported by nearly all laboratories.

Diagnosis: Phenylketonuria / hyperphenylalaninaemia was diagnosed by 99% of the participants.

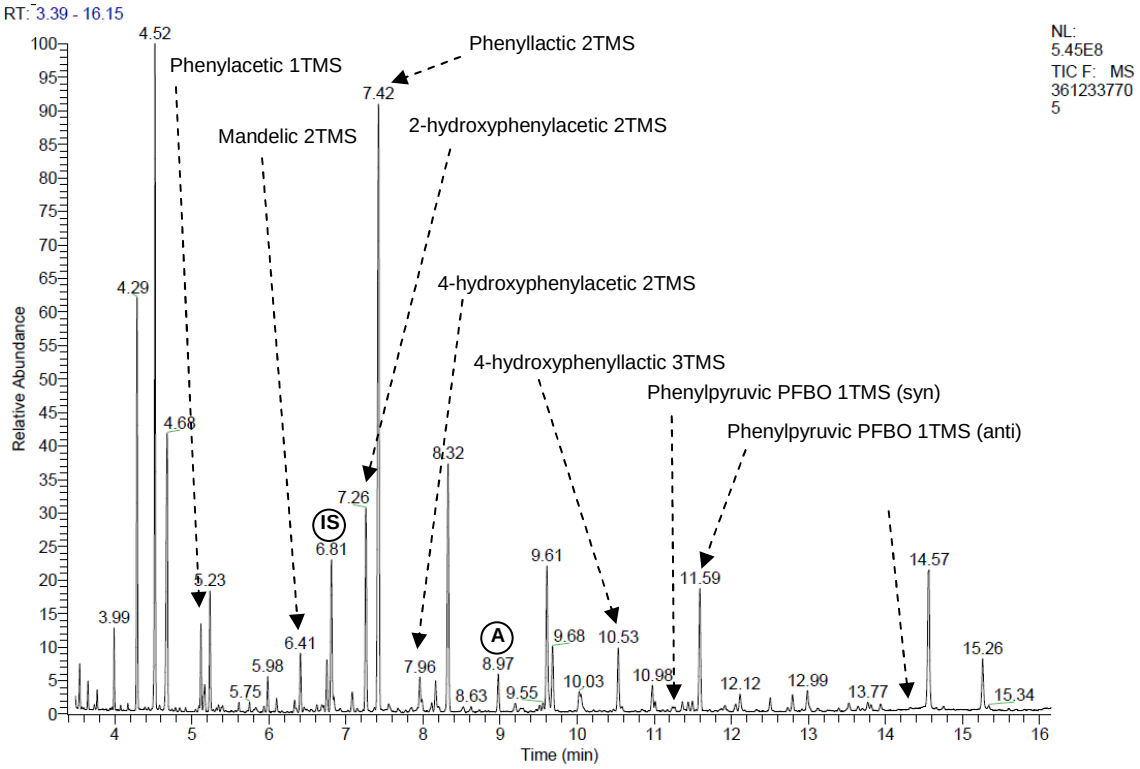


Fig 3: Organic acid profile of phenylketonuria [IS: 4-Nitrophenol-d4 125mmol/mol creatinine; A: aconitic acid]

Sample 204:

Patient details: 10-month-old boy with large head and developmental delay

Known diagnosis: glutaric aciduria type I

Analytical details: Increased concentration of glutaric acid was reported by all participants. For satisfactory analytical performance laboratories should be able to clearly differentiate between 2-hydroxyglutaric acid and 3-hydroxyglutaric acid. 96% reported the latter metabolite.

Diagnosis: The diagnostic performance for glutaric aciduria type I was at 99%. One participant diagnosed glutaric aciduria type II.

Chromatogram:

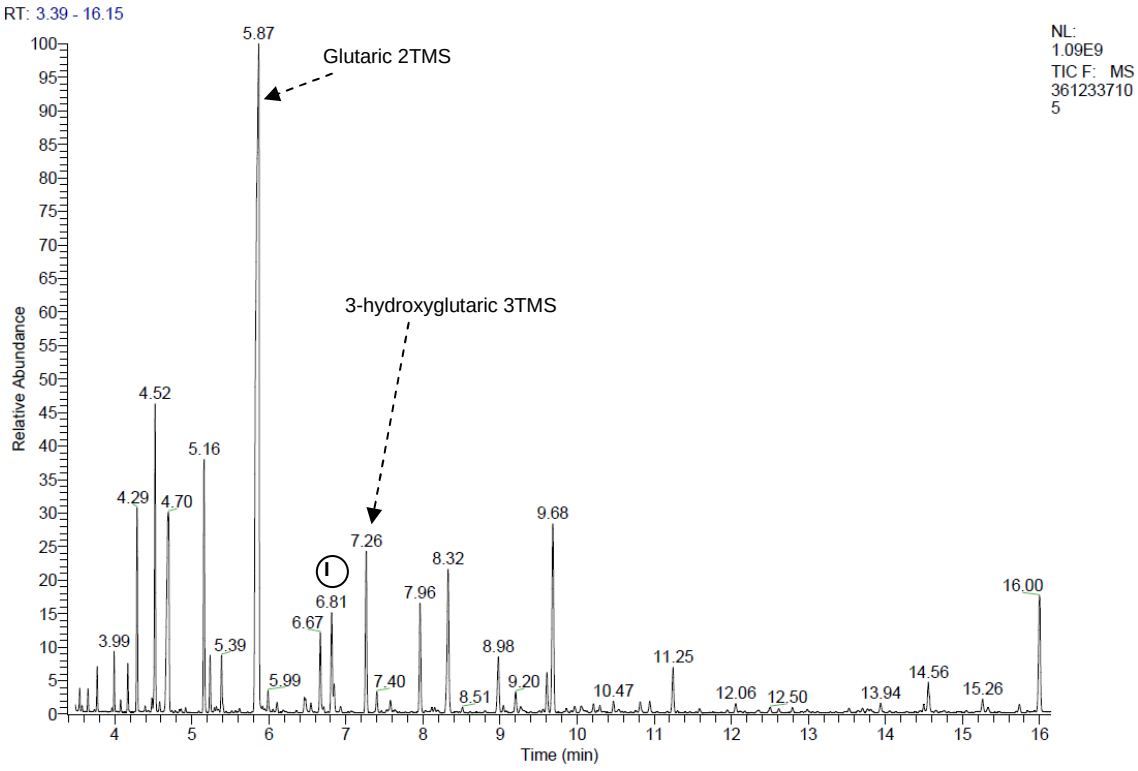


Fig 4: Organic acid profile of glutaric aciduria type I [IS: 4-Nitrophenol-d4 125mmol/mol creatinine]

The participants´ cumulative scores are shown in table 6 and in figure 5.
Cumulative scores are the scores for the whole year. In 2012 fifty-eight participants (61%) got full marks!

Table 6: Cumulative total scores 2012 – 2005
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	**2012**	**2011**	**2010**	**2009**	**2008**	**2007**	**2006**	**2005**
18	61	69	53	32	25	71	23	37
17	7	11	2	4	31	5	14	6
16	14	4	5	2	6	-	14	18
15	3	-	-	-	1	-	6	1
14	2	4	22	38	13	3	17	9
13	-	-	-	1	2	-	3	3
12	5	4	3	7	7	9	7	9
11	-	1	-	-	4	3	0	1
10	1	1	3	1	1	1	6	4
9	-	-	2	1	-	-	1	1
8	-	1	3	4	1	1	-	-
7	-	1	-	-	1	-	-	1
6	1	1	-	2	1	4	4	-
5	-	-	-	-	-	-	-	-
4	1	-	3	1	-	-	-	-
3	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	3
1	-	-	-	-	-	-	-	-
0	4	1	2	7	5	4	6	4
Number of all participants	95	90	87	85	83	78	71	67
Number of Nonresponders	4	1	2	6	3	3	4	3

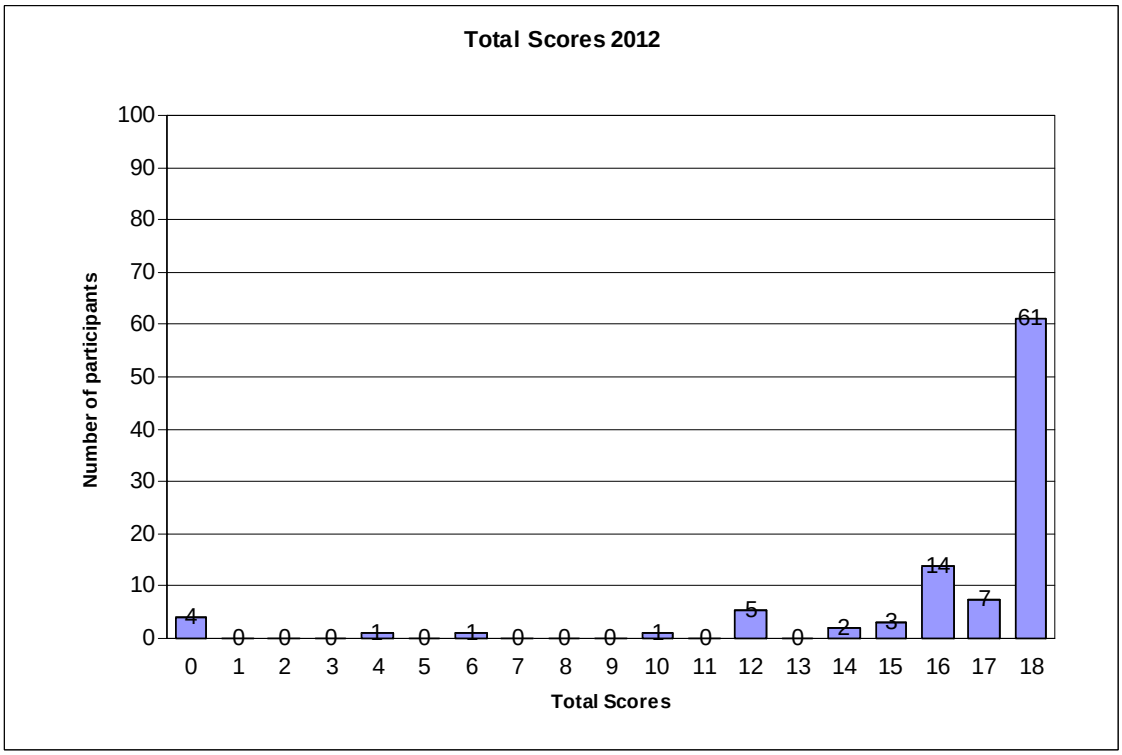


Fig. 6: Cumulative scores 2012

Your total score 2012

Your total score for 2012 was:

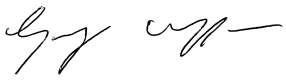
Your number of returns in 2012 was:

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,



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