Report of the SoHT Proficiency Test - EtG 2013

1. Organization

6th ethyl glucuronide proficiency test organized by the **University Center of Legal Medicine Lausanne-Geneva** in co-operation with **MEDICHEM** and the **BAM** (Federal Institute for Materials Research and Testing)

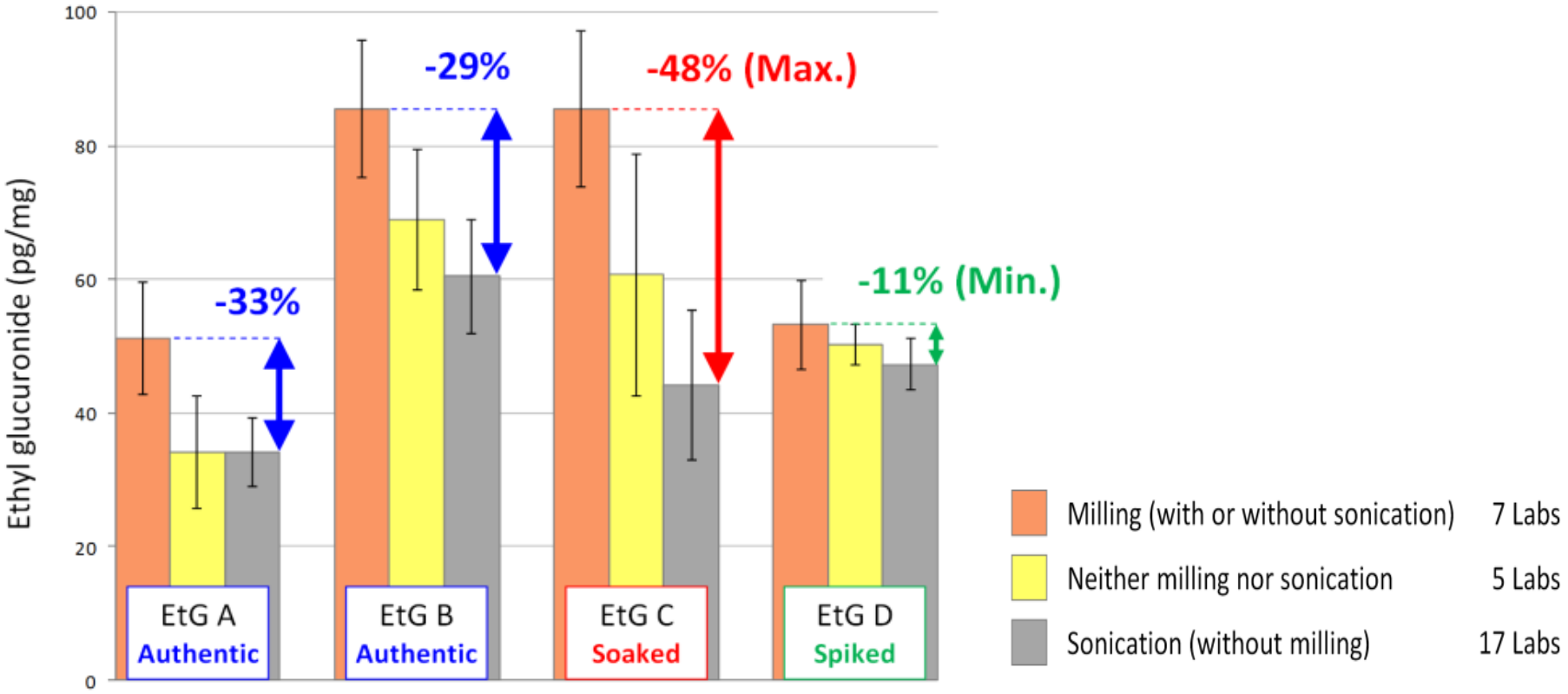
Examination period: Dec. 20th 2013 – Jan. 31st 2014

2. Conception

2.1 Planning

The previous proficiency testing results (2012) demonstrated that extraction significantly contributes to the overall scatter of EtG results between laboratories:

PT EtG 2012 - Comparison of Extraction Methods and Sample Types



- EtG concentrations obtained after pulverization have been significantly higher than those obtained from cut hair.

- Pulverization improves the reproducibility as observed for all types of material.

- Suggesting a decreasing quantity of easily extractable EtG, starting from the spiked sample over both authentic samples to the soaked sample, which has been washed repeatedly after incorporation to eliminate remaining EtG on the hair surface, the difficulties of extraction appear to increase analogously.

- Sonication obviously does not guarantee a complete extraction of EtG. Pulverization appears to improve the EtG recovery in accordance with recent literature reports [Albermann et al. 2012, Mönch et al. 2013].

- For the 2013 EtG PT, 4 authentic hair samples have been transmitted for analysis not only in cut but also in powdered form. That allowed laboratories without milling possibility to determine EtG in powdered hair.

2.2 Preparation of Samples

The preparation has been realized with special emphasis on

- EtG concentrations in the range of 10 pg/mg to 100 pg/mg
- comparable homogeneity
- preserving the structural integrity of the hair fibers

The homogenization has been performed **by cutting the hair strands very precisely into pieces of 1 mm length** by using a cut technique developed by MEDICHEM.

Homogeneity of the cut samples was assessed as relative standard deviation (RSD) under repeatability conditions (n=20). The inter-sample variation was calculated using analysis of variance (ANOVA).

Samples and their homogeneity

	Sample Type	LOT N°	Homogeneity	Sample Title	Characteristics	Size
1	spiked	BJ412	4,8%	13-**A**	cut (pieces of 1 mm)	1 x 100 mg (vial)
				13-**J**	milled	1 x 100 mg (vial)
2	authentic	BJ425	4,5%	13-**B**	cut (pieces of 1 mm)	1 x 100 mg (vial)
				13-**I**	milled	1 x 100 mg (vial)
3	authentic	AI050	13,0%	13-**C**	cut (pieces of 1 mm)	1 x 100 mg (vial)
				13-**G**	milled	1 x 100 mg (vial)
4	authentic	AI047	5,9%	13-**D**	cut (pieces of 1 mm)	1 x 100 mg (vial)
				13-**F**	milled	1 x 100 mg (vial)
5	authentic	AI051	7,0%	13-**E**	cut (pieces of 1 mm)	1 x 100 mg (vial)
				13-**H**	milled	1 x 100 mg (vial)

2.3 Required Procedure of Determination

- Do not wash the hair samples!
- Determine all cut EtG samples (A – E) according to your routinely performed method.
- Determine all powdered EtG samples (F – J) in the same way, but without further pulverization.
- Please perform 2 independent measurements (two different sample intakes) for each sample.
- A sample size of 50 mg hair per analysis was recommended.
- **Analysis of cut hair samples on voluntary basis only**

2.4 Proficiency assessment and Statistical Analysis

- Processing of results using the certified software PROLab^{TM} Plus (Quodata GmbH, Germany)
- Proficiency assessment of laboratories according to ISO 5725-5 (based on the robust consensus values derived from the participants` results)
- Z-Score based on the **Horwitz target standard deviation:** A range of $\chi - 2 * Z < x < \chi + 2 * Z$ was defined as decision criteria for considering a test result x for ethyl glucuronide positive.
- **Certificate** of successful participation **based on the results of the powdered hair samples only!**

Participants

- Total : 31 laboratories
- EtG: 26
- FAEE: 9
- EtG + FAEE: 5

4. Results

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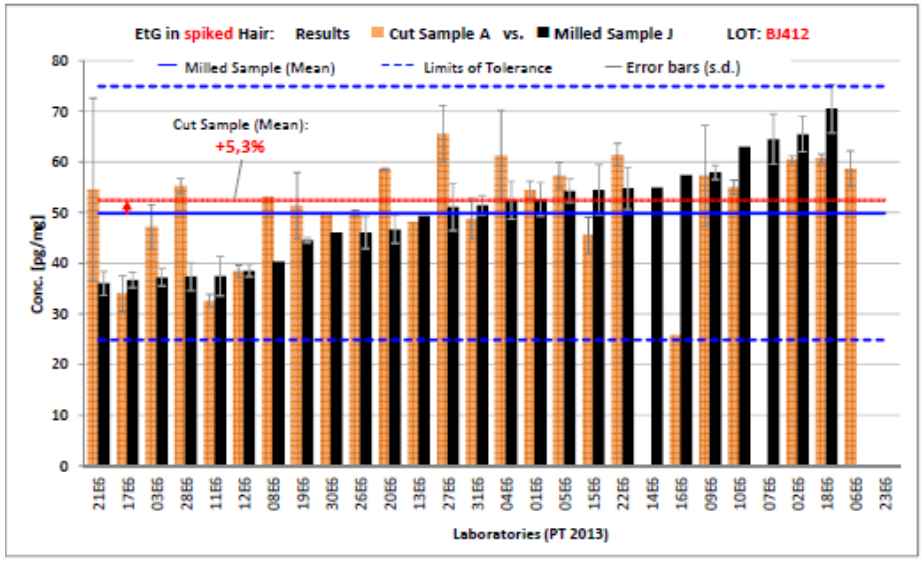
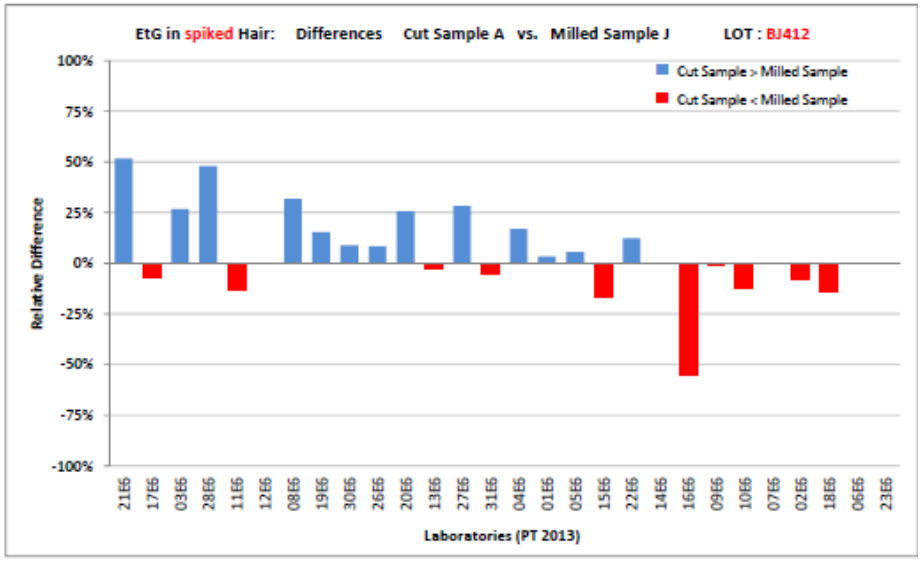
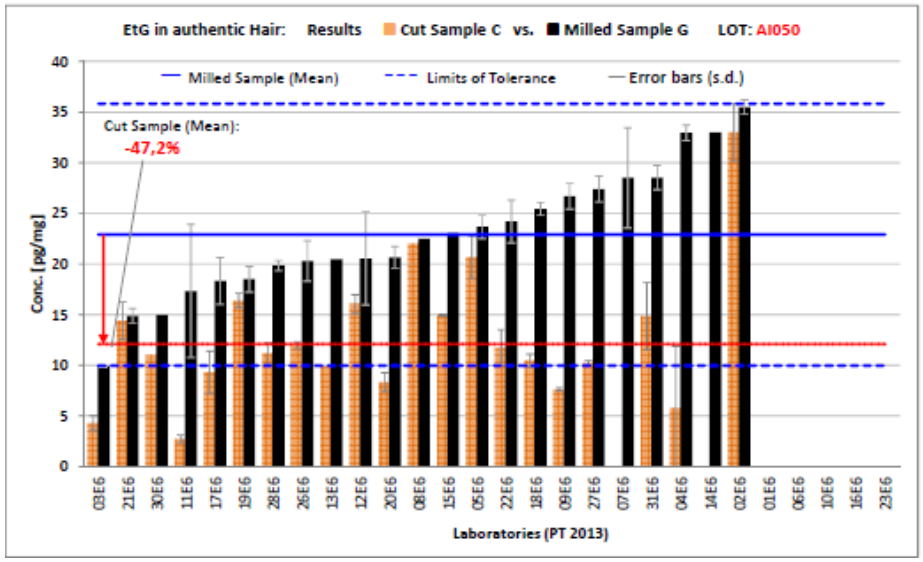
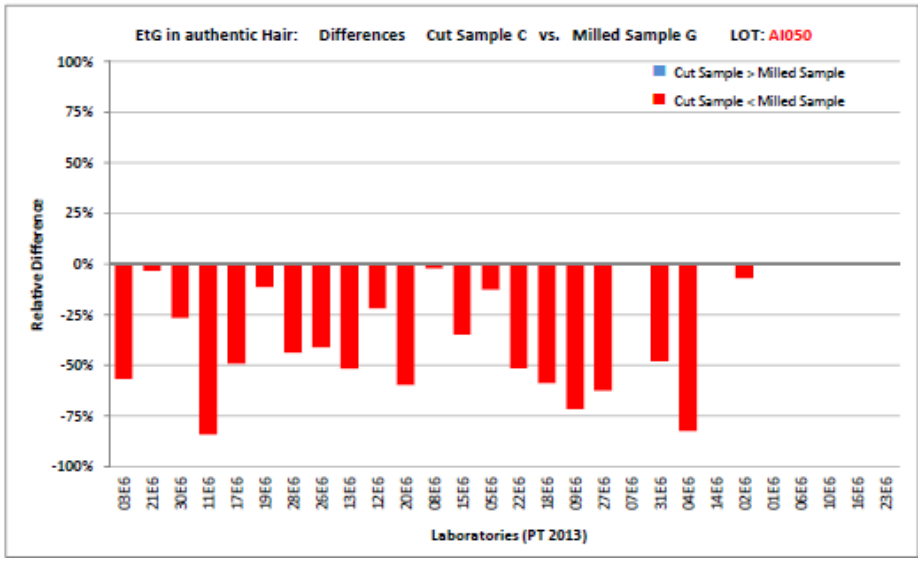
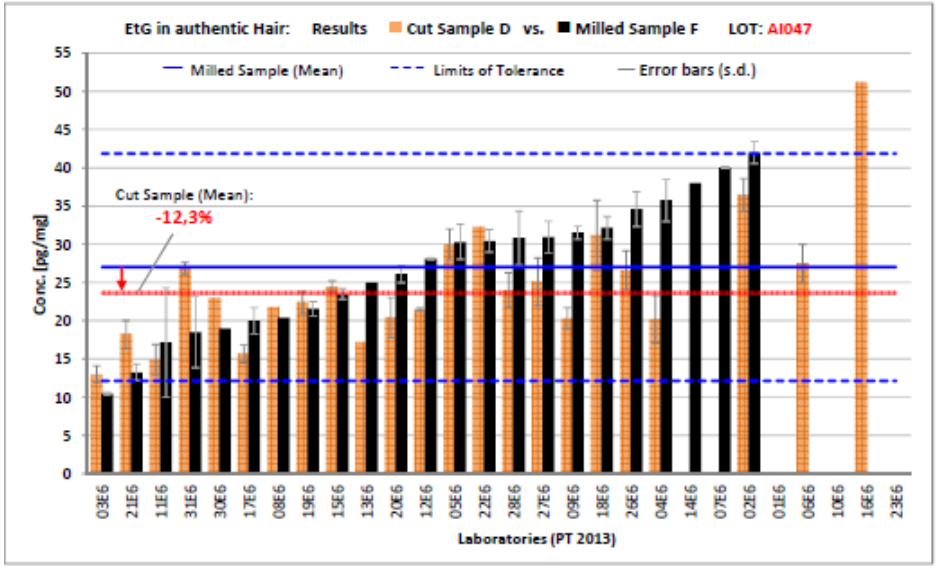
4. Results – Cut vs. powdered hair

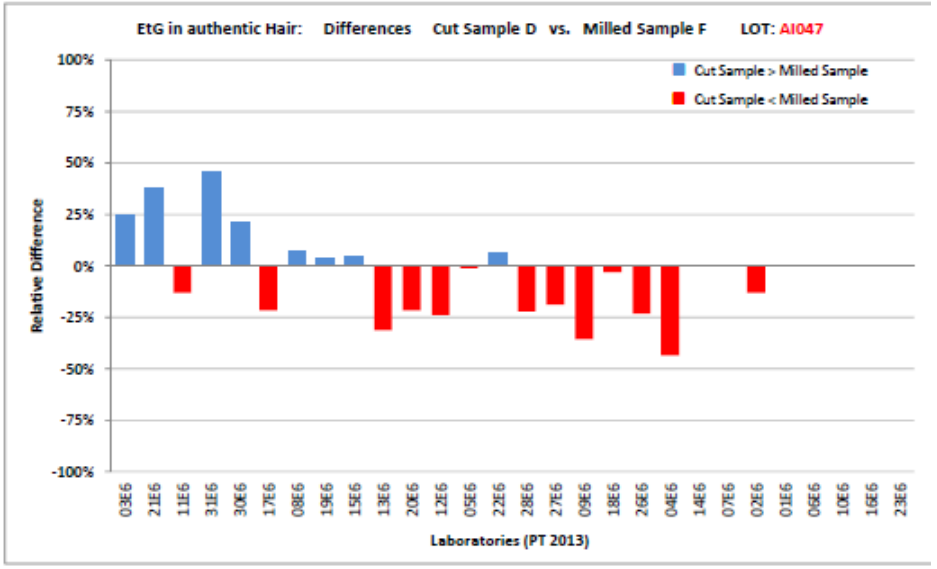


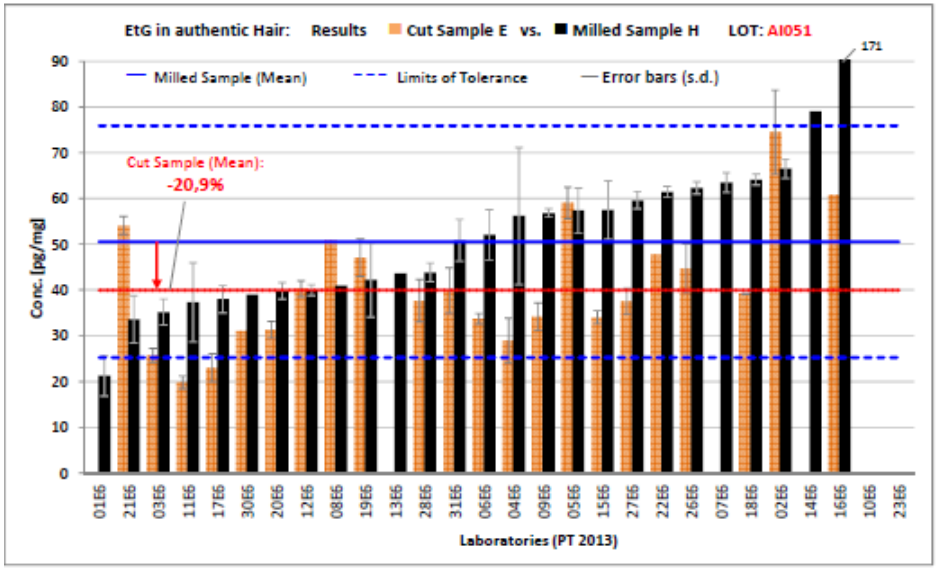


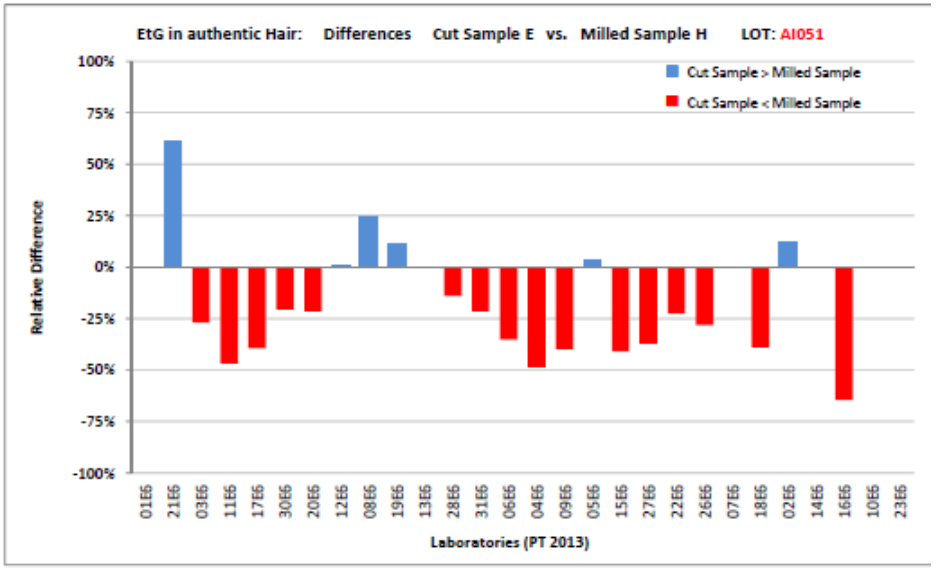





EtG in authentic Hair: Results Cut Sample D vs. Milled Sample F LOT: AI047
Milled Sample (Mean)
Limits of Tolerance
Error bars (s.d.)
Cut Sample (Mean):
-12,3%
Conc. [pg/mg]
Laboratories (PT 2013)


EtG in authentic Hair: Differences Cut Sample D vs. Milled Sample F LOT: AI047
Cut Sample > Milled Sample
Cut Sample < Milled Sample
Relative Difference
Laboratories (PT 2013)


EtG in authentic Hair: Results Cut Sample E vs. Milled Sample H LOT: AI051
Milled Sample (Mean)
Limits of Tolerance
Error bars (s.d.)
Cut Sample (Mean):
-20,9%
Conc. [pg/mg]
Laboratories (PT 2013)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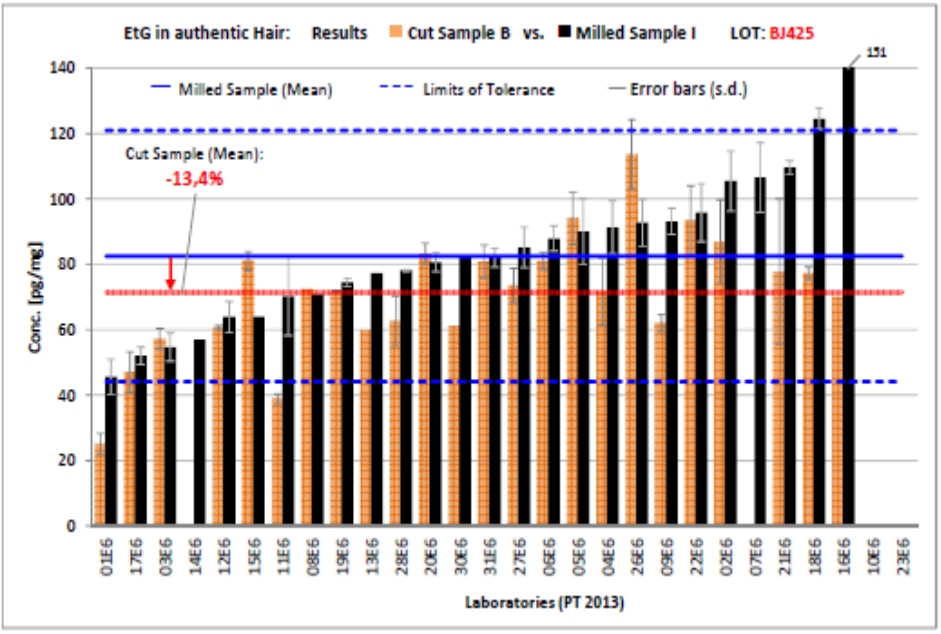
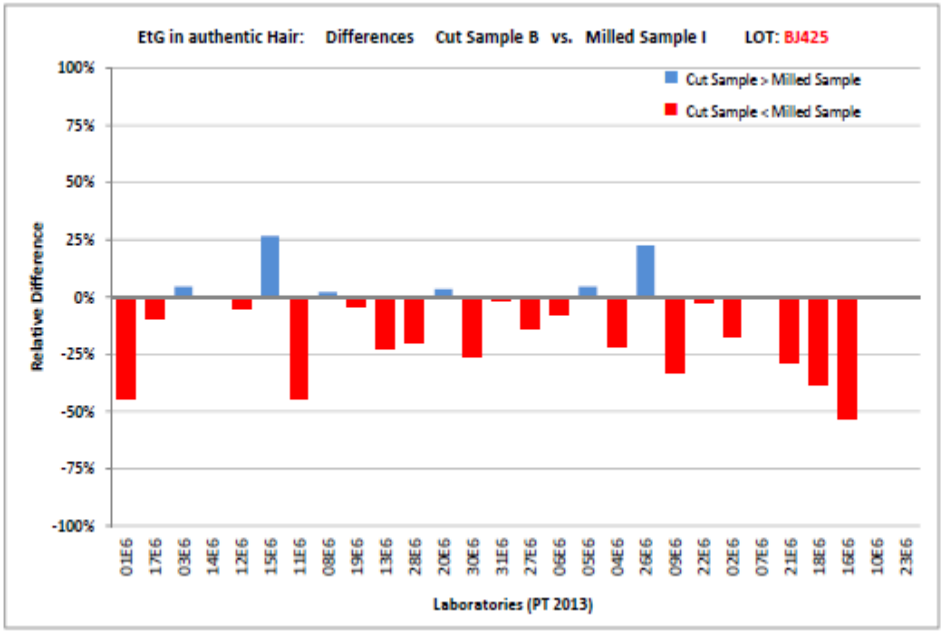
Cut Sample > Milled Sample
Cut Sample < Milled Sample
Relative Difference
Laboratories (PT 2013)


EtG in authentic Hair: Results Cut Sample B vs. Milled Sample I LOT: BJ425
Milled Sample (Mean)
Limits of Tolerance
Error bars (s.d.)
Cut Sample (Mean):
-13,4%
151
Conc. [pg/mg]
Laboratories (PT 2013)
01E6 17E6 03E6 14E6 12E6 15E6 11E6 08E6 19E6 13E6 28E6 20E6 30E6 31E6 27E6 06E6 05E6 04E6 26E6 09E6 22E6 02E6 07E6 21E6 18E6 16E6 10E6 23E6


EtG in authentic Hair: Differences Cut Sample B vs. Milled Sample I LOT: BJ425
Cut Sample > Milled Sample
Cut Sample < Milled Sample
Relative Difference
Laboratories (PT 2013)
01E6 17E6 03E6 14E6 12E6 15E6 11E6 08E6 19E6 13E6 28E6 20E6 30E6 31E6 27E6 06E6 05E6 04E6 26E6 09E6 22E6 02E6 07E6 21E6 18E6 16E6 10E6 23E6

Indications of Insufficient Extraction

Overview of Results II

EtG Results [pg/mg]	LOT	AI050			AI047			AI051			BJ425			BJ412			
	Hair Typ	authentic			authentic			authentic			authentic			spiked			
	Assigned value	22,9			27,0			50,6			82,5			49,9			
Method: ISO 5725-5	Preparation	cut	milled	Diff.	cut	milled	Diff.	cut	milled	Diff.	cut	milled	Diff.	cut	milled	Diff.	Diff. Mean
Assessment: |Z|<=2,000	Samples	C	G		D	F		E	H		B	I		A	J		
No. of laboratories that submitted results		21	23		23	23		22	26		24	26		25	26		
Mean		**12,1**	**22,9**	-47%	**23,7**	**27,0**	-12%	**40,0**	**50,6**	-21%	**71,5**	**82,5**	-13%	**52,5**	**49,9**	5%	
Rel. reproducibility s.d.		47,7%	28,5%		28,9%	34,7%		33,9%	28,8%		24,0%	26,5%		17,2%	22,0%		
Rel. repeatability s.d.		12,0%	6,6%		8,6%	6,7%		8,3%	7,7%		9,0%	7,1%		6,8%	6,3%		
Lower limit of tolerance		4,6	10,0		10,4	12,1		19,2	25,2		37,4	44,1		26,3	24,8		
Upper limit of tolerance		19,6	35,8		37,0	41,9		60,7	75,9		105,5	120,9		78,7	74,9		
Indication of **Insufficient Extraction?** -->	**Groups**																Mean **Bias**
Mean of the group	no	21,3	23,0		25,8	25,5		57,1	48,1		80,5	90,1		55,4	48,1		
n Labs	no	5	5		5	5		5	5		5	5		5	5		
Bias (relative)	no	-7%	0%	-8%	-5%	-6%	1%	13%	-5%	18%	-2%	9%	-12%	11%	-3%	15%	0%
Mean of the group	?	12,4	24,4		29,0	28,6		40,7	53,2		75,8	89,6		49,6	53,0		
n Labs	?	5	7		7	7		7	8		7	9		8	9		
Bias (relative)	?	-46%	6%	-52%	7%	6%	1%	-20%	5%	-25%	-8%	9%	-17%	-1%	6%	-7%	-23%
Mean of the group	yes	9,0	22,0		21,1	26,5		32,5	48,0		64,2	77,4		51,0	48,6		
n Labs	yes	11	11		11	11		10	12		12	12		12	12		
Bias (relative)	yes	-61%	-4%	-57%	-22%	-2%	-20%	-36%	-5%	-31%	-22%	-6%	-16%	2%	-3%	5%	-31%

Comparison of the analyzed samples

LOT	Analyt	PT	Sample		Data	Labs	Mean	Diff.	Uncertainty		Diff.	Reproducibility		Repeatability		Diff.	Homogeneity
	EtG	Year	No.	Prep.	n	n	pg/mg		U, k=2	$U_{rel.}$		SD	RSD	SD	RSD		(n=20) RSD
AI050	authentic	2013	13-G	**milled**	44	**23**	**22,9**		± 2,7	**11,7%**		6,5	**28,5%**	1,5	**6,6%**		x%
		2013	13-C	cut	41	21	12,1	**-47,2%**	± 2,5	20,5%	**8,7%**	5,8	47,7%	1,5	12,0%	**5,5%**	13,0%
		2011	11-C	cut		28	12,4	**-45,9%**	± 2,6	21,0%	**9,2%**	7,0	56,3%				
AI047	authentic	2013	13-F	**milled**	44	**23**	**27,0**		± 3,9	**14,3%**		9,4	**34,7%**	1,8	**6,7%**		
		2013	13-D	cut	43	23	23,7	**-12,3%**	± 2,8	11,8%	-2,6%	6,8	28,9%	2,0	8,6%	1,8%	5,9%
		2011	11-B	cut		32	21,6	**-20,0%**	± 2,5	11,6%	-2,8%	7,4	34,3%				
AI051	authentic	2013	13-H	**milled**	51	**26**	**50,6**		± 5,6	**11,1%**		14,6	**28,8%**	3,9	**7,7%**		
		2013	13-E	cut	42	22	40,0	**-20,9%**	± 5,7	14,2%	3,1%	13,5	33,9%	3,3	8,3%	0,6%	
		2012	12-A	cut		28	38,7	**-23,5%**	± 5,2	13,4%	2,4%	13,8	35,7%	4,3	11,0%	3,3%	7,0%
		2012	12-A	milled*		7*	51,2	1,3%	± 8,4	16,4%	5,3%	11,5	22,5%	4,4	8,7%	1,0%	
BJ425	authentic	2013	13-I	**milled**	49	**26**	**82,5**		± 8,4	**10,2%**		21,8	**26,5%**	5,8	**7,1%**		
		2013	13-B	cut	46	24	71,5	**-13,4%**	± 6,8	9,5%	-0,7%	17,2	24,0%	6,4	9,0%	1,9%	
		2012	12-B	cut		28	68,6	**-16,8%**	± 7,2	10,5%	0,3%	19,1	27,8%	3,3	4,8%	-2,3%	4,5%
		2012	12-B	milled*		7*	85,4	3,6%	± 10,2	12,0%	1,8%	13,6	16,0%	2,0	2,3%	-4,8%	
BJ412	spiked	2013	13-J	**milled**	50	**26**	**49,9**		± 4,2	**8,5%**		11,0	**22,0%**	3,1	**6,3%**		
		2013	13-A	cut	49	25	52,5	5,3%	± 3,5	6,6%	-1,9%	9,0	17,1%	3,6	6,8%	0,5%	
		2012	12-D	cut		29	49,3	-1,2%	± 2,7	5,5%	-3,0%	7,2	14,6%	3,6	7,4%	1,1%	4,8%
		2012	12-D	milled*		7*	53,2	6,7%	± 6,6	12,4%	4,0%	9,0	16,9%	3,0	5,6%	-0,7%	

Summary - EtG

- No lab working normally with cut hair reported any problem with the powdered hair
- No significant differences in the results for cut and powdered hair in the spiked sample
- Significant differences in the results for cut and powdered hair in the authentic samples
- Authentic samples can show considerable differences regarding their extractability.
- Only authentic hair samples can be used for extraction optimization
- Possible revision of the target values for the reference materials based on the results after powdering
- Publication ?

Summary - FAEE

- Authentic samples A and B
- One lab with probably analytical problems
- Washing decreased FAEE concentrations 30 – 50 %
- Lower repeatability compared to EtG
- Overall SD comparable to the EtG SD
- Sum of FAEE preferable
- Only Palmitate and Oleate ?

Next Proficiency test

- Inscription: September 2014
- Enrolement: October - November 2014
- Samples: 2 authentic samples for EtG and FAEE in cut form
- Only results after powdering will be considered in the case of EtG
- For FAEE, only the sum of the 4 FAEE after washing has to be calculated