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Commentary on Current Changes of the SoHT 2016 Consensus on Alcohol Markers in Hair and Further Background Information Fritz Pragst[a,*], Silke Suesse[b], Alberto Salomone[c], Marco Vincenti[d], Vincent Cirimele[e], Jayne Hazon[f], Lolita Tsanaclis[g], Robert Kingston[h], Frank Sporkert[i], Markus R. Baumgartner[j]

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Abstract

The consensus on alcohol markers in hair was revised for the fourth time by an expert group of the Society of Hair Testing based on current state of research. This revision was adopted by the members of the Society during the business meeting in Brisbane on August 29th 2016. For both markers, ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs), two cut-off values for discrimination between teetotalers or occasional low amount consumption and moderate alcohol drinking (low cut-off), and between non-excessive (abstinence up to moderate alcohol intake) and chronic excessive drinking (high cut-off value) were critically examined. For the current revision, the cut-off values for EtG (7 pg/mg and 30 pg/mg, respectively) remained unchanged despite different findings or discussions published in the meantime. This was mainly due to the lack of broader data collections from new studies with great numbers of volunteers following thorough study concepts. In contrast, an essential change of the consensus was accepted for the FAEEs, where the concentration of ethyl palmitate (E16:0) can be used autonomously for interpretation instead of the concentration sum (ΣFAEE) of the four esters ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate, as previously applied. After evaluation of the data from seven laboratories, the E16:0 cut-off for abstinence assessment was defined at 0.12 ng/mg for the 0-3 cm segment and at 0.15 ng/mg for the 0-6 cm segment. The cut-off for chronic excessive drinking was fixed at 0.35 ng/mg for the 0-3 cm segment and at 0.45 ng/mg for the 0-6 cm segment. The

use of E16:0 with these cut-offs in place of ΣFAEE for alcohol intake assessment produces only a minor loss in discrimination power, leads to no essential difference in the interpretation concerning chronic excessive alcohol consumption and is suitable to confirm EtG results in abstinence assessment if ethanol containing hair sprays or lotions are excluded.

Keywords: Alcohol biomarker; Ethyl glucuronide; Ethyl palmitate; Fatty Acid ethyl esters; Hair analysis; SoHT consensus

1. Introduction

The current revision of the SoHT consensus about the use of alcohol biomarkers in hair for assessment of both abstinence and chronic excessive alcohol consumption was adopted by the members of the Society of Hair Testing during the business meeting (general assembly 2016) in Brisbane, Australia on August 29th 2016 [1]. A draft of this revision was prepared by an expert group of SoHT members with large practical experience in determination of ethyl glucuronide (EtG) and/or fatty acid ethyl esters (FAEEs) as alcohol biomarkers not only for research but also for forensic or clinical purposes. The paragraphs of the preceding 2014 consensus [2] were critically discussed and clarified with emphasis on definitions, pre-analytical and analytical conditions, and cut-offs. A particularly essential change was finally accepted for the FAEEs, where only the concentration of ethyl palmitate shall be used for interpretation instead of the concentration sum of four esters previously applied. In this context, also the previous ΣFAEE cut-offs for 0-3 cm and 0-6 cm segmental lengths were adjusted as described in a previous paper [3].

In this paper, the background and the reasons of the changes in the 2016 consensus are elucidated and discussed based on the current literature.

2. General aspects

Quantification of direct biomarkers by hair testing is nowadays a widely accepted tool for the long-term monitoring of the alcohol consumption behaviour [4]. The target molecules are the minor metabolites of ethanol: ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs). Both markers are efficient and reliable biomarkers of alcohol consumption with a high diagnostic performance to evaluate drinking habits by applying two distinct cut-off values each. Both cut-offs of both biomarkers follow similar definitions, namely to differentiate between teetotalers or occasional low amount consumption and moderate alcohol drinking (low cut-off), and between non-excessive (abstinence up to moderate alcohol intake) and chronic excessive drinking (high cut-off value), respectively. The current consensus retains this concept with two cut-off values for each marker. All applications referring to the SoHT consensus must adopt cogently these definitions of the respective cut-off value.

The differentiation between these drinking categories refers mainly to the average ethanol consumption in g/day. Whereas the terms “abstinence” (0 g/day) and “occasional low amount consumption” (<1 g/day) are easily understood, it is difficult to define a strict limit between “moderate alcohol intake” and “chronic excessive drinking”.

Paragraph 1.7 of the consensus says: “Chronic excessive alcohol drinking corresponds to an average consumption of 60 g or more of pure ethanol per day over several months.” This is in agreement with the literature, where the term “chronic excessive drinking” was defined according to risk categories specified in the WHO International guide for monitoring alcohol consumption and related harm [5]. An average consumption of more than 60 g ethanol per

day roughly corresponds to 5 standard drinks (each of 12 g) or 6 standard drinks (each of 10 g). Other equivalent terms referring to the same drinking amount have been in use such as “high risk drinking” [5,6], “higher risk drinking” [7], “heavy regular drinking” [8], “average volume drinking category III” [9], “Chronic heavy drinking” [10] or “High alcohol intake” [11]. These varying expressions document that it is only a very rough approach and that the detrimental effects of chronic alcohol consumption on physical, mental and social health depend on several additional individual conditions beside the average daily dose, primarily on gender and drinking pattern. For example, a limit of 40 g/day is generally recommended for women, because of their lower average body weight and their lower volume of distribution. Concerning the drinking pattern applies: The fewer occasions on which a given amount of alcohol is consumed, the more detrimental are the consequences [9].

There is a large biological variability in the correlation between daily ethanol dose and adverse health effects, as well as the concentrations of EtG and FAEEs in hair. From the toxicokinetic point of view, it would be preferable to use a parameter such as the mean area under the ethanol concentration in blood vs. time curve per day (AUC_{EtOH}/day) rather than the ethanol dose per day. The AUC_{EtOH}/day parameter would widely compensate the influence of gender and drinking pattern [12], but unfortunately is rarely available. Therefore, the average daily dose remains the most suitable parameter for the assessment of drinking behaviour. The threshold of 60 g ethanol per day should be seen only as a rough reference value and cannot be taken as unchallengeable. This is also recollected in paragraph 1.3 of the consensus: “It is not advisable to use the results of hair testing for alcohol markers in isolation; all relevant factors surrounding a case must be considered when providing expert interpretation and opinion.”

3. Ethyl glucuronide (EtG)

EtG determined in the hair matrix is a widely used and accepted direct marker for monitoring alcohol consumption for clinical and forensic purposes. Different review articles published in the last two years present a convincing evidence of its applicability. However, a critical appraisal of the cut-off values 7 pg/mg and 30 pg/mg, respectively, is an ongoing topic of the current debate even within the expert group. In addition, new studies and improved new insights document that EtG as a marker for alcohol intake behavior might be subject to different influences. This information will be summarized in the following chapters.

3.1. Low cut-off value (7 pg/mg)

Paragraph 3.3.1 of the SoHT consensus states that a concentration of less than 7 pg/mg EtG does not contradict self-reported abstinence of a person during the corresponding time period before sampling. However, it is shown by different reports that EtG is either not detectable in hair of long-term teetotallers [13,14] or is present at very low level (<1 pg/mg) [15,16]. Moreover, recent study results have shown that numerous individuals undergoing a controlled drinking protocol of a moderate alcohol intake have often hair EtG-levels considerably under 7 pg/mg. The alcohol intake in these studies was limited to 16 or 32 g of ethanol per day over three months [17], or 100 or 150 g of ethanol per week over 12 consecutive weeks [18], or an added up total intake of 10 up to 3300 g ethanol during 3 months [19]. Based on these data and the increased sensitivity of the methods with LOQs down to < 1 pg/mg [16], some authors propose to lower the current hair EtG cut-off value for abstinence assessment. The expert group of SoHT will continuously monitor new

investigations and developments and adjust the recommendations outlined in the consensus in due time. Given the often severe legal consequences of hair testing results, lowering of the cut-off should be based on unquestionable evidences and very broad data collections.

3.2. High cut-off value (30 pg/mg)

One of the most crucial points of any hair testing protocol is the extraction of the analyte from the keratinized matrix. During the last few years, the extraction of powdered hair instead of hair snippets has gained great acceptance as a methodical development for the quantification of EtG in hair [17,20-23]. Accordingly, the paragraph 2.3 was adjusted with the expression “powdering hair for the extraction of EtG is best practice”. However, the initial hair treatment technique affects not only the time to achieve an extraction plateau but also the overall extraction efficiency. Therefore, a critical appraisal of the cut-off values is indicated.

The general study concept for determining such a cut-off value, to be subsequently used as indicative for a certain alcohol consumption behaviour, should ideally be based on a questionnaire completed by the participants of the study with retrospective comments on his/her drinking habits or with a prospective drinking protocol to establish EDI (ethanol daily intake) values. However, a prospective drinking protocol at a level defined as “harmful” or “excessive” is not grant capable due to ethical reasons. The second important aspect is a state-of-the-art analytical method.

The 30 pg/mg cut-off for EtG in hair, found in different studies and established in 2009 by the SoHT, can serve as a screening test for excessive chronic drinking behaviour. It provides high sensitivity, specificity, likelihood ratios, as well as high positive and negative predictive values. This summary assessment was also the result of an elaborate meta-analysis [24]. A similar outcome is described in different reviews [4,14]. The above mentioned development of novel or modified sample pre-treatment or extraction protocols have launched again the discussion about the ongoing validity of the 30 pg/mg cut-off. Retrospective statistical data evaluation is an excellent way to reveal a certain trend and could help establishing a reliable cut-off value [25]. Currently, new studies with great numbers of volunteers following the thorough study concepts mentioned before and comparing the different extraction methods (extraction of hair snippets vs powdered hair, combined with variable temperatures and extraction solvents) are still rare [26]. Here again, the expert group of SoHT will continuously monitor these developments together with the recognition of other intrinsic sources of variability. The result of the critical discussion in the expert group was that at the moment it is not advisable to modify the current high cut-off value of 30 pg/mg.

3.3. Factors influencing EtG concentration in hair (external influence, cosmetic treatment, medical conditions)

SoHT strongly recommends that analytical biomarkers quantified in hair samples should never be used in isolation (paragraph 1.3 of the SoHT consensus). All relevant factors surrounding a case must be considered. This may not only include long and short term markers (direct and indirect ones) for ethanol drinking such as EtG, FAEE, PEth, CDT, and others, but also reports from physicians, clinical examinations, and therapeutic institutions, to name just a few. However, the distinct diagnostic power of each parameter should be interpreted with caution.

In addition, knowledge of the aspects that might have an impact on the different markers is crucial. In the case of direct markers determined by hair analysis there are three main potential factors of bias. Degradation of incorporated substances on the one hand and false positive or increased concentration levels due to external contamination on the other hand are main pitfalls in hair analysis. For EtG, a third potential source of error, determining elevated levels, is the occurrence of certain medical conditions.

Degradation is mainly a consequence of cosmetic treatments. For EtG, this aspect was investigated in different studies. In summary, no effect was observed for semi-permanent coloring [27], , use of cleansing shampoos [29] or EtOH-containing lotions [30]. In contrast, strong decrease in EtG concentration was observed after hair bleaching [31], dyeing with use of H_2O_2 or perming [27,32]. According to in-vitro experiments, hair straightening may decrease (mainly dark hair) or increase (mainly blond hair) the EtG concentration [28]. False positive EtG results can be produced by EtG containing hair lotions [33]. These contaminating hair tonics are based on ethanolic plant extracts [34,35]. To avoid any potential misclassification of an individual's drinking habit, recommendations for the collection of hair samples mandatorily include a comprehensive questionnaire or a detailed chain of custody form [36,37].

Certain medical conditions might represent a third source for altered EtG concentrations in hair. For instance, alopecia leads to an altered hair growth cycle which has a strong influence on the represented time frame. In a recent study, it was shown that renal failure can result in an increased EtG content, because the excretion rate might be lowered [38]. The corresponding information must be made available in order to avoid misclassification. The additional determination of ethyl palmitate in hair or of other alcohol markers can be helpful in such cases.

4. Fatty acid ethyl esters (FAEEs)

4.1. Advantages and disadvantages of the concentration sum of ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate

FAEEs are a group of more than 20 compounds, which are enzymatically formed from free fatty acids, triglycerides, lipoproteins and phospholipids in almost all human body fluids and tissues in the presence of ethanol. From these, the concentration sum of ethyl myristate (E14:0), ethyl palmitate (E16:0), ethyl oleate (E18:1) and ethyl stearate (E18:0) in hair was used to evaluate alcohol intake, since the first publication about FAEEs in hair in 2001 [39]. The selection of four representative FAEEs added together seemed to be reasonable in order to (a) compensate variations in fatty acid composition caused by individual dietary and metabolic differences, (b) provide mutual confirmation within a typical concentration ratio range of the four esters, and (c) exclude false positive results from external sources in case of strongly deviating concentration ratio.

However, the most disturbing external source of false positive results are not the FAEEs themselves, but on the contrary ethanol contained in hair sprays or hair lotions. Their use leads to external formation of FAEEs and incorporation in hair from sebum and skin surface lipids [40-42]. It was shown that the concentration ratio of the four esters produced from external ethanol in such samples is not significantly different from that measured after excessive drinking. Therefore, it cannot be used to identify false positive results from ethanol-containing hair care products.

The mean concentration ratio E14:0 / E16:0 / E18:1 / E18:0 was found to be 8:45:36:9 (N=644 [43]), 8:45:37:10 (N=78 [44]), 8:37:47:10 (N=160 [45]) and 14:45:34:7 (N=1657, Suesse et al., unpublished results) with a strong variation of the percentage of the single esters as shown in Fig. 1. The predominant esters are always E16:0 and E18:1. This is in agreement with FAEE findings in blood [45] and the fatty acid profile in human adipose tissue and blood [47-49].

The fatty acid composition of sebum and skin surface lipids as the main sources of FAEEs in hair differs significantly from adipose tissue and blood [50,51]. Nevertheless, E16:0 and E18:1 were found to be the most abundant esters also in this study [52].

Besides the advantages (a)-(c) mentioned above, the use of the added up concentration of the four esters for interpretation appeared to have also some disadvantages in the practical application. For social drinkers, the concentrations of E14:0 and E18:0 were often below the limits of quantification and could therefore not be included in the sum, which might result in false negative classification.

The analytical quantification of E18:1 showed lower sensitivity than of the saturated esters, and higher standard deviation. This might be a consequence of the double bond which leads to a stronger and more variable fragmentation [43,44,53]. In addition, the double bond of E18:1 is susceptible to autoxidation and photo-oxidation. Therefore, E18:1 exhibits a lower stability in hair compared to saturated esters and was sometimes excluded or discarded because of its negligible concentration in hair samples [41].

The sum of four analytical values as a marker was compared with a single result from the viewpoint of error calculation. According to the rules of error propagation, the variance SD^2 of the sum ΣFAEE is the sum of the variances of the four esters:

$$\mathrm{SD}^2_{\sum \mathrm{FAEE}} = \mathrm{SD}^2_{E14} + \mathrm{SD}^2_{E16} + \mathrm{SD}^2_{\mathrm{E18:1}} + \mathrm{SD}^2_{\mathrm{E18}} \quad (1)$$

The standard deviation SD of the sum of the four esters is obtained as the square root of its variance:

$$\mathrm{SD}_{\sum \mathrm{FAEE}} = \sqrt{\mathrm{SD}^2_{E14} + \mathrm{SD}^2_{E16} + \mathrm{SD}^2_{\mathrm{E18:1}} + \mathrm{SD}^2_{\mathrm{E18}}} \quad (2)$$

In Table 1 the standard deviations of the single esters obtained from a control sample (positive hair pool) in 30 measurement series are compared with that of ΣFAEE calculated according to equations (1) and (2). It can be seen that the absolute standard deviation SD of the sum is clearly higher than of the single esters, but the relative standard deviation RSD is significantly lower, due to error compensation. Therefore, from the viewpoint of error propagation, ΣFAEE has an advantage as compared to a single ester.

In summary, the advantage of using ΣFAEE to monitor alcohol drinking behaviour was not overwhelming and E16:0 alone can be used as a suitable alternative.

4.2. Choice and validation of cut-offs of ethyl palmitate E16:0 for abstinence assessment and excessive alcohol consumption

In practice, FAEEs are determined in the proximal 0-3 or 0-6 cm hair segment. Because of their accumulation from sebum, different cut-offs must be used for the two segment lengths [3]. In principle, the cut-offs for E16:0 could be derived from those of ΣFAEE using the E16:0/ΣFAEE concentration ratio. However, this ratio strongly varies, as it is shown by the scattering plot for the 0-6 cm segment of 1006 samples in Fig. 2. Examination of single cases

shows that extreme deviations from the mean are almost always caused by exceptionally high or low concentrations of E18:1.

Colleagues from eight laboratories were asked to evaluate their FAEE data and to enter their results in a questionnaire and answer some questions about details and performance of their methods. Responses were obtained from seven laboratories. Only two of them used alcohol markers in hair for abstinence assessment. Table 2 shows the E16:0/ΣFAEE ratios (means, standard deviations, medians and interquartile ranges) of the 0-3 cm and/or 0-6 cm hair segment from the seven laboratories. The means and medians of the ratio vary between 0.4 and 0.5 with some differences between the laboratories, also in standard deviation and interquartile range.

Based on the experimental results from the seven laboratories and in consideration of the E16:0/ΣFAEE ratio of 0.47 (mean of all 7353 samples in Table 2) and of the most probable segmental lengths 0-3 cm/0-6 cm concentration ratio of about 0.80 [3] the following final cut-offs were accepted in the consensus.

Abstinence assessment:	0.12 ng/mg (0-3 cm) and 0.15 ng/mg (0-6 cm)
Excessive alcohol consumption:	0.35 ng/mg (0-3 cm) and 0.45 ng/mg (0-6 cm)

The cut-offs for abstinence assessment take into account the results of Albermann et al. who thoroughly investigated this aspect for the 0-3 cm segment [53,54] and concluded that ΣFAEE = 0.2 ng/mg may represent a reasonable cut-off for this purpose. Using the mean ratio E16:0/ΣFAEE = 0.47, this corresponds to 0.094 ng/mg for E16:0. The cut-off of 0.12 ng/mg chosen for E16:0 is less restrictive and, as an example, was also confirmed by the results reported in Fig. 3 for 29 hair samples from children routinely controlled within family matters with a range of 0.01-0.11 ng/mg, a mean of 0.040±0.021 ng/mg, and a median of 0.040 ng/mg. Further studies with more strict abstainers are necessary to corroborate this cut-off.

The discrimination power and the ΣFAEE cut-offs for excessive drinking were repeatedly evaluated by receiver operation characteristics analysis (ROC analysis) [55-57] and recently reviewed [4]. The ROC curves from ref. [55] data are compared between ΣFAEE and E16:0 in Fig. 4 for the 0-3 cm segment and in Fig. 5 for the 0-6 cm segment. It is evident that the discrimination power of E16:0 with AUC 0.858 (0-3 cm) and 0.923 (0-6 cm) is slightly lower than that of ΣFAEE with AUC 0.873 and 0.955 respectively. Sensitivity and specificity for E16:0 obtained from these data at the cut-offs of the 2016 consensus are given in Table 3. Also for detection of excessive drinking, more controlled data are needed to further optimize the cut-offs.

In order to examine the effect of the change from ΣFAEE to E16:0 on result interpretation, the data from the seven laboratories were evaluated using the previous and the new cut-offs. The number of agreeing (E16:0-ΣFAEE = Neg-Neg and Pos-Pos) and disagreeing (E16:0-ΣFAEE = Neg-Pos and Pos-Neg) cases and the percentage of agreement are shown in Table 4 for 0-6 cm as well as for 0-3 cm segmental lengths. For the studies 1-5 concerning the 0-6 cm segmental length with the cut-offs of E16:0 = 0.45 ng/mg and ΣFAEE = 1.00 ng/mg a relatively good agreement of 90.3 to 97.2% (mean 94.3%) was found with higher numbers of Neg-Pos cases in three studies (1.9 to 5.1%) and of Pos-Neg cases in two studies (4.6 to 7.9%). This shows that for the 0-6 cm segment both ways of interpretation are almost equally restrictive. The relative low percentage of varying interpretation is acceptable since it concerns borderline cases with results close to the cut-offs, where the shifts caused by biological variability and measurement errors are at least in the same order.

In the studies 6a, 7a, 8 and 9 involving the 0-3 cm segment, concurrent interpretation is found only between 82.6% and 88.2% of the samples if the cut-off of E16:0 0.35 ng/mg and the previously recommended of ΣFAEE 0.50 ng/mg are applied.

In two of these studies (6a and 7a), all differently interpreted samples (11.8% and 14.1%) refer to the combination E16:0-ΣFAEE = Neg-Pos, while no cases are recorded with E16:0-ΣFAEE = Pos-Neg. In the other two studies (8 and 9), the combination Neg-Pos clearly predominates with 12.9% and 14.0% over Pos-Neg with 4.5% and 0.3%. The reason for this divergence is the missing coherence between the 0-3m and the 0-6 cm segments of the previous ΣFAEE cut-offs, which was analysed in detail in a previous paper [3] and is adjusted with the proposition of new cut-offs for E16:0.

As a matter of fact, the previous 0-3 cm cut-off of 0.50 ng/mg for ΣFAEE appears to be too restrictive, while the new 0-3 cm cut-off of 0.35 ng/mg for E16:0 better corresponds to ΣFAEE 0.80 ng/mg and is congruent with 0.45 ng/mg value chosen for the 0-6 cm segment. The comparison between the 0-3 cm cut-offs of ΣFAEE 0.80 ng/mg and E16:0 0.35 ng/mg in the studies 6b, 7b and 10-12 of Table 4 results in 94.9% to 98.1% concurrent interpretations and only 0.7% to 1.8% Neg-Pos and 1.3% to 2.4% Pos-Neg for E16:0-ΣFAEE, respectively.

In summary, the use of E16:0 in place of ΣFAEE for alcohol intake assessment apparently produces only a small loss in discrimination power. The application of the new 0.45 ng/mg cut-off for E16:0 in the 0-6 cm segment leads to no essential difference in the interpretation concerning chronic excessive alcohol consumption with respect to the previous cut-off for ΣFAEE = 1.00 ng/mg. The incongruity of the previous cut-off for the 0-3 cm and the 0-6 cm segments was adjusted by adopting an E16:0 cut-off of 0.35 ng/mg for the 0-3 cm segment. This is bound to a less restrictive interpretation of the results from the 0-3 cm segment than the previously used cut-off of ΣFAEE = 0.50 ng/mg. The E16:0 cut-offs for abstinence assessment of 0.12 ng/mg and 0.15 ng/mg proved to be sufficiently high to avoid false positive results when this analysis is used to confirm EtG results, provided that the use of ethanol containing hair lotions or hair sprays by the investigated subject can be excluded.

The expert group recommends the further harmonization of the analytical methods used by the different laboratories and the implementation of regular proficiency tests for ethyl palmitate. In the same way as for EtG, new results about this marker in relation to amount and pattern of alcohol consumption and to other influencing factors will be attentively followed and considered in upcoming revisions of the consensus.

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Table 1. Mean, absolute and relative standard deviations and variances of the concentrations of the four FAEEs obtained for a control sample (positive hair pool) in 30 measurement series. The data for the sum of the four esters were calculated according to eq. (1) and (2).

FAEE	Mean ng/mg	SD ng/mg	RSD %	Variance $(ng/mg)^2$
E14:0	0.043	0.011	25	0.000121
E16:0	0.169	0.023	13.4	0.000529
E18:1	0.22	0.034	15.3	0.001156
E18:0	0.05	0.014	27.7	0.000196
ΣFAEE	0.482	0.045	9.3	0.002002

Table 2. Concentration ratio E16:0/ΣFAEE in in hair different studies.

Study	Number of samples	Segment length, cm	Range of ΣFAEE, ng/mg	Ratio E16:0/Σ FAEE			
				Mean	SD	Median	Interquartil range
Suesse et al. 2010 [43]	644	0-6 or less	0.11-31.3	0.382	0.082	0.388	0.330-0.428
Suesse et al. 2012 [55]	1006	0-6	0.06-55.0	0.422	0.086	0.433	0.375-0.481
Suesse et al. 2012 [55]	376	0-3 or less	0.03-25.1	0.425	0.078	0.430	0.388-0.471
Pragst et al. 2015*	175	0-3 or less	0.20-34.7	0.428	0.096	0.426	0.373-0.480
Salomone and Vincenti 2016*	155	0-3	0.05-12.00	0.486	0.138	0.483	0.421-0.547
Cirimele et al. 2016*	195	0-3	0.00-14.4	0.490	0.191	0.437	0.367-0.514
Cirimele et al. 2016*	334	0-6	0.00-26.2	0.475	0.350	0.440	0.372-0.512
Kayongo et al. 2016*	824	0-3 or 0-6	0.10-18.8	0.551	0.265	0.499	0.418-0.631
Tsanaclis et al. 2016*	491	0-3 or less	0.20-78.9	0.561	0.576	0.482	0.267-0.702
Tsanaclis et al. 2016*	216	0-6 and 0-3 or less	0.20-37.9	0.595	0.893	0.443	0.254-0.629
Kingston et al., 2016*	1646	0-3 (Only)	0.2-147.6	0.479	0.134	0.467	0.467-0.530
Kingston et al., 2016*	1291	0-6 (Only)	0.2-209.0	0.468	0.125	0.458	0.458-0.522

* Unpublished data

Table 3. Discrimination power obtained by receiver operation characteristics (ROC) analysis and sensitivities and specificities of the detection of chronic excessive alcohol consumption using E16:0 with the cut-offs of the 2016 SoHT consensus at the example of the data from Suesse et al. [19a].

Hair segment	AUC (ROC analysis)	Cut-off, ng/mg	Sensitivity, %	Specificity, %
0-3 cm	0.858	0.35	79	88
0-6 cm	0.923	0.45	76	91

Table 4. Comparison of the interpretation results of E16:0 and ΣFAEE for chronic excessive alcohol consumption.

Study No.	Reference	Samples N	Segmental length, cm *Cut-offs*	Number of samples, E16:0 vs. ΣFAEE				Percentage agreement
				Neg-Neg	Neg-Pos	Pos-Neg	Pos-Pos	
1	Suesse et al. [55]	1006	0-6 *0.45 /1.00*	573	20	14	399	96.6
2	Cirimele et al. 2016*	334	0-6 *0.45/1.00*	210	17	6	101	93.1
3	Kayongo et al. 2016*	568	0-6 *0.48/1.00*	453	11	5	99	97.2
4	Tsanaclis et al. 2016*	381	0-6 *0.45/1.00*	268	7	30	76	90.3
5	Kingston et al., 2016*	1291	0-6 *0.45/1.00*	506	44	34	707	94.0
6a	Pragst et al. 2016*	305	0-3 *0.35/0.50*	204	36	0	65	88.2
6b	Pragst et al. 2016*	305	0-3 *0.35/0.80*	236	5	4	60	97.0
7a	Suesse et al. [55]	376	0-3 *0.35/0.50*	166	53	0	157	85,9
7b	Suesse et al. [55]	376	0-3 *0.35/0.80*	215	3	5	153	97.9
8	Tsanaclis et al. 2016*	961	0-3 *0.35/0.50*	563	124	43	231	82.6
9	Kingston et al., 2016*	1646	0-3 *0.35/0.50*	356	230	5	1055	85.7
10	Salomone and Vincenti 2016*	155	0-3 *0.36/0.80*	115	1	2	37	98.1
11	Cirimele et al., 2016*	195	0-3 *0.32/0.80*	110	3	7	75	94.9
12	Kayongo et al. 2016*	1207	0-3 *0.38/0.80*	906	22	29	250	95.8

*Unpublished data

Captions of the figures

Fig. 1. Distribution of the percentage of ethyl myristate (E14:0), ethyl palmitate (E16:0), ethyl oleate (E18:1) and ethyl stearate (E18:0) of the sum of the four esters in 1658 hair samples. Data from Suesse et al. [55].

Fig. 2. Scatter plot of the concentration ratio E16:0/ΣFAEE in the 0-6 cm segments of 1006 hair samples in the order of increasing ΣFAEE from 0.06 to 55 ng/mg. Data from Suesse et al. 2012 [55].

Fig.3. Concentrations of ethyl palmitate in the 0-3 cm hair segment of 29 children, age 1 to 12 years. Range 0.01-0.11 ng/mg, mean: 0.040±0.021 ng/mg, median: 0.04 ng/mg.

Fig. 4. Evaluation of the discrimination power of ΣFAEE and of E16:0 in the 0-3 cm hair segment for detection of chronic excessive alcohol consumption by ROC analysis. True negative N = 33, true positive N = 38, data from Suesse et al. [55].

Fig 5. Evaluation of the discrimination power of ΣFAEE and of E16:0 in the 0-6 cm hair segment for detection of chronic excessive alcohol consumption by ROC analysis. True negative N = 92, true positive N = 137, data from Suesse et al. [55].