LC-MS/MS Quantification of Ethyl Glucuronide (and Ethyl Sulphate) (Reference 2014.01.005)

Notice of Assessment

June 2014

1 GENERAL INFORMATION

1.1 Requester: Centre hospitalier de l'Université de Montréal (CHUM)

1.2 Application for Review Submitted to MSSS: September 30, 2013

1.3 Application Received by INESSS: March 1, 2014

1.4 Notice Issued: June 30, 2014

> **Note:**
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> This notice is based on the scientific and commercial information submitted by the requester and on a complementary review of the literature according to the data available at the time that this test was assessed by INESSS.

2 TECHNOLOGY, COMPANY AND LICENCE

2.1 Name of the Technology

Liquid chromatography-tandem mass spectrometry or LC-MS/MS.

2.2 Brief Description of the Technology, and Clinical and Technical Specifications

There are three steps to the technique:

1) sample preparation;
2) liquid chromatography (LC);
3) mass spectrometry (MS).

Preparation of the sample purifies it by removing proteins and other substances before analysis. There are several methods for doing so; the three most common are solid phase extraction (SPE), liquid-liquid extraction (LLE) and protein precipitation (PP) [Adaway and Keevil, 2012]. The requester prepares samples by adding an organic acid to urine samples before LC-MS/MS analysis.

With LC, molecules can be separated from a complex mixture (serum or plasma) based on their physical and chemical properties (molecular weight, hydrophobicity, etc.). The principle behind LC involves a liquid mobile phase and a solid stationary phase (in the column or in a thin layer). The composition of the liquid and solid phases varies based on the type of molecule to be purified.

MS determines the mass of molecules present in the sample of interest. Mass is measured based on the deflection of previously ionized molecules by an electric or magnetic field; a molecule's trajectory is proportional to its mass and charge.

The mass spectrometer consists of the following:

4) An ion source to alter the molecules' charge and convert them to the gas phase (e.g., electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and others);
5) An analyzer that separates ions by their mass-to-charge ratio (m/z).

Analyzers can be coupled sequentially. The mass spectrometry consists of several stages (conducted in tandem in this case, hence "MS/MS"). The first analyzer selects ions based on a given m/z (purification). The purified ion is then fragmented in a collision cell. A second analyzer measures the fragments' m/z [Adaway and Keevil, 2012; Grebe and Singh, 2011].

2.3 Company or Developer

Testing is performed using an in-house method. A urine sample is used for quantification of ethyl glucuronide (EtG) and ethyl sulphate (EtS). The requester[1] prepares samples by adding acetic acid (10%) to urine samples before LC-MS/MS analysis.

2.4 Licence: Not applicable.

2.5 Patent, If Any: Not applicable.

2.6 Approval Status (Health Canada, FDA)

Not applicable. Testing is performed using an in-house method. The requester uses an internal quality control (in-house pool) and an Arvecon external quality control (Proficiency Testing Scheme of the Society of Toxicological and Forensic Chemistry).

2.7 Weighted Value: 44.0

3 CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patient Group

The test targets patients waiting for a liver transplant and patients receiving treatment in addiction clinics.

3.2 Targeted Disease

Alcohol consumption is a significant problem that can cause severe liver disease requiring a liver transplant. The problem has repercussions for the patient both before and after transplantation [Allen et al., 2013].

A review of the literature by Allen et al. [2013] reveals that alcoholic cirrhosis is the indication for liver transplantation in 20% to 30% of cases.

Not all patients with alcoholic cirrhosis are candidates for a liver transplant; consideration is given only to those whose liver condition has not improved with appropriate treatment and prolonged abstinence.[2] For transplant patients, abstinence can stabilize their condition before transplantation and improve their prognosis afterward. However, these patients relapse fairly frequently, despite their commitment to stop drinking. In Canada, patients who do not follow medical advice to stop drinking alcohol are not considered for transplantation.[3] Furthermore, a Quebec publication states that most liver transplant programs require at least six months' abstinence from drugs and alcohol.[4]

[1] Information based on the requester's standard operating procedure (SOP).
[2] Canadian Liver Foundation. Transplant information [Web site]. Available at: http://www.liver.ca/liver-disease/liver-transplants/how-do-transplants-work/ (consulted May 12, 2014).
[3] *Ibid.*
[4] La transplantation. L'Avant-Garde 2009;9(2). Available at: http://www.chumontreal.qc.ca/sites/default/files//documents/A_propos/PDF/ag_vol9_no2.pdf (consulted May 12, 2014).

Alcohol remains in the blood, breath and urine for a short period, which makes monitoring alcohol use difficult. Residual alcohol markers, like ethyl glucuronide, have a longer half-life than ethanol and can determine intake over a longer window of assessment. The information provided by these markers cannot be reliably obtained through patient self-reporting [Allen et al., 2013; Jatlow and O'Malley, 2010].

3.3 Number of Patients Targeted

The requester expects to perform 180 to 200 tests per year.

3.4 Medical Specialties and Other Professions Involved

Gastroenterology, transplant surgery, toxicology and psychology.

3.5 Testing Procedure

Blood, urine and hair can be tested [Albermann et al., 2012; Concheiro et al., 2009]. The requester proposes a urine test. The sample is collected on site by the patient while supervised by the nursing staff to prevent attempts at tampering with samples.

EtG analysis is affected by bacterial degradation and synthesis. Therefore, samples should be stored properly [Helander et al., 2009]. Additionally, because EtG and EtS concentrations are affected by excessive fluid intake prior to sampling leading to urine dilution, a creatinine assay is advised to monitor non-physiological dilution [Helander et al., 2010].

4 TECHNOLOGY BACKGROUND

4.1 Nature of the Diagnostic Technology

The test must be performed with urinary creatinine and glucose quantification.

4.2 Brief Description of the Current Technological Context

There are several methods for measuring alcohol intake. They include the use of biomarkers such as carbohydrate-deficient transferrin (CDT), ethyl glucuronide and phosphatidylethanol (PEth), as well as patient self-reporting [Allen et al., 2013; Walsham and Sherwood, 2012].

Transferrin is a glycoprotein synthesized and secreted by the liver. Moderate to heavy drinking (50 g to 80 g/day) decreases the carbohydrate content of transferrin. Measuring it, together with GGT,[5] increases sensitivity without compromising specificity [Allen et al., 2013]. The INESSS has already issued a favourable notice for CDT quantification for the diagnosis of congenital disorders of glycosylation only, and it has been added to the Index.

Different analytical methods can be used to measure EtG (and EtS): gas or liquid chromatography/mass spectrometry(GC-MS[6], LC-MS[7]) or tandem mass spectrometry or multiple mass spectrometry (LC-MS/MS[8], LC-MS^n [9]), liquid chromatography with pulsed electrochemical detection (LC-PED), CZE[10], EIA[11] and immunochemical tests [Albermann et

[5] Gamma-glutamyltransferase.
[6] Gas chromatography/mass spectrometry.
[7] Liquid chromatography/mass spectrometry.
[8] Liquid chromatography/tandem mass spectrometry.
[9] Liquid chromatography/multiple-stage mass spectrometry.
[10] Capillary zone electrophoresis.
[11] Enzyme immunoassay.

al., 2012; Joya et al., 2012; Staufer et al., 2011]. However, LC-MS/MS seems to be the method most commonly used in studies.

4.3 Brief Description of the Advantages Cited for the New Technology

Most blood biomarkers [Palmer, 2009] of alcohol use (gamma-glutamyltransferase or GGT, carbohydrate-deficient transferrin or CDT, aspartate aminotransferase or AST, alanine aminotransferase or ALT, mean corpuscular volume or MCV) are not sensitive or specific enough to be used alone. A combination is recommended to improve diagnostic performance [Kharbouche et al., 2009].

Ethyl glucuronide (EtG) is a direct metabolite[12] of ethanol. It is specific to alcohol and appears very quickly after consumption, even of very small amounts (10 g of alcohol, or one drink), and can be detected in the urine for several hours (35 to 130 hours) after intake [Concheiro et al., 2009; Helander et al., 2009]. A similar period was noted for ethyl sulphate (EtS) [Helander et al., 2009]. However, there is wide interindividual variability in the detection period for EtG and EtS [Kummer et al., 2013], even after correcting values for urine dilution and estimated times for complete ethanol elimination [Helander et al., 2009].

The presence of EtG in the blood or urine is specific to alcohol use, but the ethanol contained in mouthwashes, for example, can leave detectable traces [Kharbouche et al., 2009]. A good definition for a diagnostic threshold is essential [Imbert et al., 2012].

LC-MS/MS can quantify EtG (and EtS) with greater specificity than immunoassays because it avoids cross-reactions between metabolites that can come from other sources, including medication for patients with liver disease.

4.4 Cost of Technology and Options: Not assessed.

5 EVIDENCE

5.1 Clinical Relevance

5.1.1 Other Tests Replaced: Not applicable.

5.1.2

Morbidity and Mortality

The test is used to document and monitor alcohol use (or abstinence). No evidence was found regarding the relationship between monitoring and morbidity and mortality rates, although the selection of transplant candidates who are abstaining from alcohol can improve the health outcomes of transplant recipients [Staufer et al., 2011] and increase their long-term survival rates [Erim et al., 2007].

Modification of Treatment Based on Test Results

Abstinence from alcohol is a major criterion for receiving a liver transplant. It can reduce severe complications of liver disease, and, in some cases, it may mean that a transplant is no longer required [Erim et al., 2007]. A patient waiting for a liver transplant who obtains a positive result, indicating alcohol consumption, can expect to not move up the waiting list (standby) or to be removed from the transplant list [Erim et al., 2007]. However, there is no evidence.

[12] A small amount (less than 0.1%) of the ethanol ingested is converted into conjugated forms of glucuronic acid and sulfate, ethyl glucuronide (EtG) and ethyl sulfate (EtS) respectively [Helander et al., 2009].

For patients in addiction clinics, the test is used to document alcohol intake to provide objective monitoring and adjust services (psychosocial) and treatment (medication) offered, as needed. However, there is no evidence to show the effects of monitoring on the success of addiction programs.

5.1.3 Therapeutic Value

Optimal selection of transplant candidates and detection of alcohol relapse is necessary to improve long-term health outcomes [Staufer et al., 2011]. Patient alcohol use can be monitored by means of an analytical method that provides an objective and verifiable assessment [Lande and Marin, 2013], unlike a self-reported questionnaire that provides information that is not always reliable or accurate [Wetterling et al., 2014; Stewart et al., 2013; Dahl et al., 2011; Webzell et al., 2011].

There is no consensus on a threshold value (positivity) for a clinical decision [Helander et al., 2010]. However the values most often used for EtG and EtS are 0.5 µg/mL and 0.1 µg/mL, respectively.

5.2 Diagnostic Validity

TERM	PRESENCE	ABSENCE	NOT APPLICABLE
Diagnostic sensitivity	X		
Diagnostic specificity	X		
Positive predictive value (PPV)	X		
Negative predictive value (NPV)	X		
Likelihood ratio (LR)		X	
ROC Curve	X		
Accuracy	X		

Sensitivity, Specificity, PPV, NPV, and Accuracy

Data regarding sensitivity, specificity, positive predictive value, negative predictive value and accuracy are presented in Table 1. Results from two studies [Stewart et al., 2013; Helander et al., 2010] indicate that sensitivity is at least 70% and specificity at least 93% for LC-MS/MS detection of EtG. PPV is at least 81% and NPV is at least 85%. Accuracy ranges from 86% to 99%.

Other Tests

According to the study by Kummer et al. [2013], there is poor correlation between the number of drinks per day before sampling and detected concentrations of EtG ($r = 0.448$, $p < 0.02$) and EtS ($r = 0.406$, $p < 0.04$). In the study by Stewart et al. [2013], logistic regression was performed for age, sex, ethnic origin and the severity of the liver disease. No correlation ($p > 0.25$) was found between these variables and EtG and EtS positivity (three days after drinking).

Table 1: Diagnostic sensitivity and specificity, PPV, NPV and accuracy of urinary EtG and EtS testing

STUDY (COUNTRY)	MARKER AND METHOD	THRESHOLD VALUE (µg/mL)	N	SE (%)	SP (%)	PPV (%)	NPV (%)	OR	ACCURACY
Helander et al., 2010 (Sweden)	EtG LC-MS/MS vs. LC-MS/MS (SPE)¥	0.5							0.1 µg/mL: 86.2% (350/406) 0.3 µg/mL: 95.7% (369/414) 0.5 µg/mL: 98.1% (417/425) 0.75 µg/mL: 99.3% (410/413) 1 µg/mL: 95.8% (411/429)
	EtG LC-MS (SPE) vs. LC-MS/MS (SPE)¥	0.5							0.1 µg/mL: 90.2% (387/429) 0.3 µg/mL: 94.4% (405/429) 0.5 µg/mL: 97.0% (416/429) 0.75 µg/mL: 94.6% (406/429) 1 µg/mL: 96.3% (413/429)
	EtG LC-MS vs. LC-MS/MS (SPE)¥	0.5							0.1 µg/mL: 85.5% (367/429) 0.3 µg/mL: 94.2% (404/429) 0.5 µg/mL: 97.7% (419/429) 0.75 µg/mL: 95.3% (409/429) 1 µg/mL: 96.7% (415/429)
Piano et al., 2014 (Italy)	EtG Homogeneous EIA	0.5	121	89.2	98.9	97.1	95.4	414.5 [61.1, > 999.9], $p < 0.0001$‡ 493.8 [51.3, > 999.9], $p < 0.0001$§	95.9%
Staufer et al., 2011 (Germany)	EtG EIA LC-MS/MS (for confirmation)	0.5	141	89.3	98.9	89.3	98.9	761.1 [145.9, 3,970.5] $p < 0.0001$†	
Stewart et al., 2013 (United States)	EtG (3 days*) LC-MS/MS	0.1	120	76	93	81	91		91.7% (110/120)
	EtG (7 days*) LC-MS/MS		120	70	99	97	85		

STUDY (COUNTRY)	MARKER AND METHOD	THRESHOLD VALUE (µg/mL)	N	SE (%)	SP (%)	PPV (%)	NPV (%)	OR	ACCURACY
	EtS (3 days*) LC-MS/MS	0.025	120	82	86	70	93		91.7% (110/120)
	EtS (7 days*) LC-MS/MS		120	73	89	80	85		

Abbreviations: EIA = enzyme immunoassay; LT = liver transplant; N = number of samples; NPV = negative predictive value; OR = odds ratio; PPV = positive predictive value; Se = sensitivity; Sp = specificity; SPE = solid phase extraction; vs. = versus.

*Number of days after alcohol consumption.

†Estimated risk of alcohol consumption, univariate analysis.

‡ Predictor of alcohol consumption, univariate analysis.

§ Estimated risk of alcohol consumption, multivariate analysis.

¥ Accuracy was calculated manually using LC-MS/MS (SPE) as a reference method. Urine samples came from clinical studies, alcohol consumption experiments and samples from the lab's standard pool.

5.3 Analytical (or Technical) Validity

PARAMETER	PRESENCE	ABSENCE	NOT APPLICABLE
Repeatability	X		
Reproducibility	X		
Analytical sensitivity	X		
Analytical specificity	X		
Matrix effect	X		
Concordance		X	
Correlation between test and comparator	X		

Analytical Sensitivity

Table 3 presents data on analytical sensitivity with respect to detection limits.

Repeatability, Reproducibility

Table 4 provides data on repeatability and reproducibility.

Analytical Specificity, Interference, Recovery and Matrix Effect

Table 5 sets out data regarding analytical specificity. There is little or no interference during LC-MS/MS analysis of concentrations above the threshold value of 0.5 µg/mL. The recovery rate is greater than 75%, except in the study by Concheiro et al. [2009], in which the recovery rate is 55% with an EtG concentration of 5 µg/mL.

Table 2: Validation studies of quantification of ethyl glucuronide (EtG) and ethyl sulphate (EtS) in urine samples

STUDY (COUNTRY)	MARKER	NUMBER OF SPECIMENS	VOLUME (µL)*	ANALYSIS BY LC-MS/MS OR VARIANT						
				PREPARATION METHOD	INTERNAL CONTROL	IONIZATION AND DETECTION	LOD (µg/mL)	LLOQ (µg/mL)	LINEARITY (µg/mL)	COEFFICIENT OF LINEARITY
Albermann et al., 2012 (Germany)	EtG	6	10	PP	EtG-D_5	Negative ion mode ESI, MRM (LC-MS/MS)	0.005	0.019	0.025-2	R = 0.9989
	EtS	6	10		EtS-D_5		0.005	0.015	0.025-2	R^2 = 0.9982
Beyer et al., 2011 (Australia)	EtG and EtS	6 replicates for each of the 7 conc.	10	DI	-	APCI, MRM mode (LC-MS/MS)	-	0.1	0.1-10	$r^2 > 0.99$
Concheiro et al., 2009 (Spain)	EtG	5	10	Formic acid (0.1%)	EtG-D_5	Negative ion mode ESI, SRM (LC-MS/MS)	0.1	0.25	0.25-100	r^2 = 0.9983
Helander et al., 2010 (Sweden)	EtG and EtS	-	-	SPE or DI	EtG-D_5 EtS-D_5	Negative ion mode ESI, SRM (LC/MS/MS)	For EtG SPE: < 0.001 DI: 0.003	EtG: 0.5 EtS: 0.1	EtG: 0.1-100	-
	EtG and EtS	-	10	SPE or DI	EtG-D_5 EtS-D_5	ESI, SIM mode (LC-MS)	For EtG SPE: 0.03 DI: 0.10	-	EtG: 0.1-100 EtS: 0.05-50	-
Kummer et al., 2013 (Belgium)	EtG and EtS Threshold†: 0.1 µg/mL (both metabolites)	27	5	PP	EtG-D_5 EtS-D_5	Negative ion mode ESI, MRM (UPLC-MS/MS)	0.1	-	0.1-10	-
Zheng and Helander, 2008 (Sweden)	EtG Threshold†: 0.5 µg/mL	-	10	SPE	EtG-D_5	ESI (LC-MS)	< 0.1	-	0.1-100	-

Abbreviations: APCI = atmospheric pressure chemical ionization; conc. = concentration; DI = dilution-direct injection; ESI = electrospray ionization; EtG = ethyl glucuronide; EtS = ethyl sulphate; LC-MS = liquid chromatography-mass spectrometry; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; LOD = limit of detection; µg/mL: microgram per millilitre; MRM = multiple reaction monitoring; PP = protein precipitation; SPE = solid phase extraction; SRM = selected reaction monitoring; UPLC-MS/MS = ultra-performance liquid chromatography-tandem mass spectrometry.

*Volume injected into liquid chromatography system.

†Threshold value (cut-off).

Table 3: Repeatability and reproducibility of LC-MS/MS for quantification of ethyl glucuronide (EtG) and ethyl sulphate (EtS)

STUDY	MARKER (METHOD)	NUMBER OF SPECIMENS	NOMINAL CONCENTRATION* (µg/mL)	INTRA-ASSAY		INTER-ASSAY		INTRA-DAY		INTER-DAY	
				Z SCORE	RELATIVE STANDARD DEVIATION (%)	BIAS (%)	RELATIVE STANDARD DEVIATION (%)	CV (%)	RELATIVE STANDARD DEVIATION (%)	CV (%)	RELATIVE STANDARD DEVIATION (%)
Albermann et al., 2012	EtG (LC-MS/MS)	2†	0.1			-7.13			4.5		4.61
		2†	0.35			9.51			3.78		3.78
		2†	1.5			4.44			2.26		3.80
	EtS (LC-MS/MS)	2†	0.1			0.93			3.15		7.31
		2†	0.35			2.68			2.42		2.81
		2†	1.5			3.44			1.84		3.55
Beyer et al., 2011	EtG (LC-MS/MS)	8 consecutive days	0.1			-8.2			7.2		
			0.6			6.4			6.5		
			4.5			8.1			6.9		
			9.0			0.8			4.3		
			50			2.0			6.0		
	EtS (LC-MS/MS)	8 consecutive days	0.1			0.7			7.0		
			0.6			-11.3			6.0		
			4.5			6.9			5.9		
			9.0			-5.0			3.2		
			50			-0.4			5.8		
Concheiro et al., 2009	EtG (LC-MS/MS) SRM transition 221.2 > 221.2/221.2 > 74.7	5 (intra-day) 6 (inter-day)§	0.25					5.5			11.6
		5 (intra-day) 6 (inter-day)§	2.5					5.2			2.6
		5 (intra-day) 6 (inter-day)§	100					2.1			5.4

STUDY	MARKER (METHOD)	NUMBER OF SPECIMENS	NOMINAL CONCENTRATION* (µg/mL)	INTRA-ASSAY		INTER-ASSAY		INTRA-DAY		INTER-DAY	
				Z SCORE	RELATIVE STANDARD DEVIATION (%)	BIAS (%)	RELATIVE STANDARD DEVIATION (%)	CV (%)	RELATIVE STANDARD DEVIATION (%)	CV (%)	RELATIVE STANDARD DEVIATION (%)
	EtG (LC-MS/MS) SRM transition 221.2 > 221.2/221.2 > 84.8	5 (intra-day) 6 (inter-day)§	0.25					13.2			9.1
		5 (intra-day) 6 (inter-day)§	2.5					6.3			5.9
		5 (intra-day) 6 (inter-day)§	100					1.2			4.4
Kummer et al., 2013	EtG (UPLC-MS/MS)	8 different days	0.100			3.70	6.64				
			0.300			-0.74	6.41				
			0.878			-4.18	3.63				
			3.020			-1.41	3.60				
			4.000			-1.00	2.38				
			7.500			0.26	2.29				
	EtS (UPLC-MS/MS)	8 different days	0.100			-4.65	3.59				
			0.300			6.02	4.08				
			0.920			-12.53	2.22				
			1.750			-4.45	3.10				
			4.000			-0.01	3.32				
			7.500			-4.95	2.39				
	EtG (UPLC-MS/MS)	4 (proficiency test)	0.556	0.10							
			0.800	-0.46							
			0.832	0.23							
			1.450	-0.22							

STUDY	MARKER (METHOD)	NUMBER OF SPECIMENS	NOMINAL CONCENTRATION* (µg/mL)	INTRA-ASSAY		INTER-ASSAY		INTRA-DAY		INTER-DAY	
				Z SCORE	RELATIVE STANDARD DEVIATION (%)	BIAS (%)	RELATIVE STANDARD DEVIATION (%)	CV (%)	RELATIVE STANDARD DEVIATION (%)	CV (%)	RELATIVE STANDARD DEVIATION (%)
	EtS (UPLC-MS/MS)	4 (proficiency test)	0.885	0.57							
			0.899	-0.16							
			1.070	0.23							
			1.100	-0.86							
Zheng and Helander, 2008	EtG (LC-MS)	15‡	0.5			4.9					
		15‡	1			4.1					
		15‡	5			5.4					

Abbreviations: CV = coefficient of variation; EtG = ethyl glucuronide; EtS = ethyl sulphate; inter-day = between days; intra-day = within day; LC-MS = liquid chromatography-mass spectrometry; LC-MS/MS = liquid chromatography-tandem mass spectrometry; SRM = selected reaction monitoring; UPLC-MS/MS = ultra-performance liquid chromatography-tandem mass spectroscopy.

* Units from the studies are converted for purposes of uniformity.

† Each concentration was analyzed in duplicate on eight consecutive days.

‡ Analyses were performed in triplicate on five separate days.

§ For intra-day measurements, five replicates were analyzed on the same day, while for inter-day quantification, six samples were analyzed on five different days.

Table 4: Recovery and interference

STUDY	N	MARKER	NOMINAL CONCENTRATION (µg/mL)	RECOVERY (%)	MATRIX EFFECT (%)	INTERFERENCE
Albermann et al., 2012	6	EtG	0.1	95	79	None (n = 10)
	6		1.5	93	69	
	6	EtS	0.1	98	104	-
	6		1.5	92	94	
Beyer et al., 2011	10	EtG	0.6	-	5.6 (RSD)	-
	10		4.5	-	5.6 (RSD)	
	10		9.0	-	5.4 (RSD)	
	10	EtS	0.6	-	6.2(RSD)	-
	10		4.5	-	9.2 (RSD)	
	10		9.0	-	7.9 (RSD)	
Concheiro et al., 2009	10	EtG	5	55	-	None
Kummer et al., 2013	6	EtG	0.3	81	84	-
	6		4	80	80	
	6		7.5	79	76	
	6	EtS	0.3	76	106	-
	6		4	81	95	
	6		7.5	80	88	
Zheng and Helander, 2008	-	EtG	0.5	81-86 (AR)	-	Background noise (interference peaks) for concentrations < 0.5 µg/mL, making quantification of EtG difficult; the threshold value is 0.5 µg/mL.
			5	77-81 (AR)	-	-

Abbreviations: AR = absolute recovery; EtG = ethyl glucuronide; EtS = ethyl sulphate; N = number of specimens; RSD = relative standard deviation.

Correlation Between Test and Comparator

Table 6 provides data on the correlation between the test and the comparator. The value of the coefficients is greater than 0.95, indicating very good correlation between the methods studied.

Table 5: Correlation between LC-MS/MS and a comparator method for urine Samples

STUDY	METHODS	COEFFICIENT OF CORRELATION PASSING-BABLOK REGRESSION	NUMBER OF SAMPLES
Helander et al., 2010	LC-MS/MS vs. LC-MS/MS (SPE)	$r = 0.9594$	348 (< 2 µg/mL EtG)
	LC-MS (SPE) vs. LC-MS/MS (SPE)	$r = 0.9662$	348 (< 2 µg/mL EtG)
	LC-MS vs. LC-MS/MS (SPE)	$r = 0.9562$	348 (< 2 µg/mL EtG)
Zheng and Helander, 2008	LC-MS/MS vs. LC-MS (SPE)	$r^2 = 0.959$, $p < 0.001$	481

Abbreviations: EtG = ethyl glucuronide; LC-MS = liquid chromatography-mass spectrometry; LC-MS/MS = liquid chromatography-tandem mass spectrometry; SPE = solid phase extraction; vs. = versus.

The Bland-Altman plot shows that mean bias is 0.03 [between -0.19 and 0.26]. Most points are within two standard deviations [Zheng and Helander, 2008].

Requester's Data

The positivity threshold is 255 µmol of EtG/mol of creatinine, or 0.5 µg/mL of EtG, assuming 8.85 mmol/L of creatinine.

The limit of detection for EtG is 0.885 mmol/L and for EtS, 0.800 mmol/L, according to the requester's equipment.[13]

5.4 Recommendations from Other Organizations

Abstinence from alcohol is one criterion for access to a liver transplant for patients on the waiting list. Therefore, monitoring of alcohol consumption is recommended.[14] EtG (and EtS) urine testing is one way to objectively assess abstinence from alcohol or to document its consumption.

6 ANTICIPATED OUTCOMES OF INTRODUCING THE TEST

6.1 Impact on Material and Human Resources

Testing is performed using materials and equipment already in place in several hospitals. As the test is conducted in conjunction with other analytical tests, provision should be made for additional resources. Consideration must be given to the availability of qualified staff.

[13] Information based on the requester's standard operating procedure (SOP).

[14] Canadian Liver Foundation. Transplant information [Web site]. Available at: http://www.liver.ca/liver-disease/liver-transplants/how-do-transplants-work/ (consulted May 12, 2014).

6.2 Economic Consequences of Introducing Test Into Quebec's Health Care and Social Services System

Not assessed.

6.3 Main Organizational, Ethical, and Other (Social, Legal, Political) Issues

Results of the test under consideration can have negative clinical, legal and social consequences on people's lives. For example, non-abstinence could mean that a liver transplant is denied, or it could constitute failure to comply with a condition imposed by the courts in a child custody case.

The test's diagnostic specificity is important, given the stakes and possible repercussions on continued treatment (transplant or treatment for addiction) for an individual with a false-positive test result. Caution is required when a positive EtG result is used as primary or sole evidence of drinking for legal or disciplinary action [Helander et al., 2009; Kissack et al., 2008]. In fact, false-positive results are possible through direct contact (with mouth washes, disinfectants) or exposure to ethanol vapour (by inhalation) from a source that is not always recognized or avoidable [Arndt et al., 2014; Hoiseth et al., 2010].

Additionally, prevention of sample falsification through adulteration or dilution is recommended. EtG is sensitive to microbial degradation (but EtS is not) if samples are not stored properly, which can result in false-negative results [Helander et al., 2009]. Therefore, EtG testing requires complementary use of another test with a different biomarker such as creatinine [Helander et al., 2009] to monitor nonphysiological dilution [Helander et al., 2010].

7 IN BRIEF

7.1 Clinical Relevance

The test is used to verify abstinence from alcohol to select liver transplant candidates. It can improve patient health outcomes and long-term survival rates, although no evidence was found to support this. The EtG assay is more reliable and more sensitive than self-report questionnaires and alcohol breath tests.

For patients in addiction clinics, the test is used to document alcohol intake to provide objective and verifiable monitoring to adjust services and treatment offered. However, no evidence is available to show the effects of this monitoring on the success of addiction programs.

7.2 Diagnostic Validity

Values for diagnostic validity parameters are at least 70%. Specificity when testing with LC-MS/MS is at least 93%.

7.3 Analytical Validity

LC-MS/MS testing is sensitive, reproducible and relatively specific. The recovery rate is greater than 75%.

7.4 Recommendations from Other Organizations

Abstinence from alcohol is a criterion for a liver transplant for patients on the waiting list. Therefore, monitoring of alcohol consumption is recommended.[15] EtG (and EtS) urine testing is one way to objectively assess abstinence from alcohol or to document its consumption.

[15] Canadian Liver Foundation. Transplant information [Web site]. Available at: http://www.liver.ca/liver-disease/liver-transplants/how-do-transplants-work/ (consulted May 12, 2014).

8 INESSS NOTICE IN BRIEF

LC-MS/MS Quantification of Ethyl Glucuronide (and Ethyl Sulphate)

Status of the Diagnostic Technology

- ☒ Established
- ☐ Innovative
- ☐ Experimental (for research purposes only)
- ☐ Replacement for technology: _____, which becomes obsolete

INESSS Recommendation

- ☒ Keep test in the Index solely for patients waiting for a liver transplant
- ☐ Remove test from the Index
- ☐ Reassess test

Additional Recommendation

- ☐ Draw connection with listing of drugs, if companion test
- ☐ Produce an optimal use manual
- ☐ Identify indicators, when monitoring is required

Notes

- The number of requisitions could increase dramatically if the tests were used in addiction clinics. The indication should be limited to patients waiting for a liver transplant.
- Informed consent is required so that patients are fully aware of the consequences of a positive result.
- A mechanism to confirm positive results with another laboratory is essential.

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