BSN
Biological Sales Network

FloMass Ethylglucuronide in Hairs

Reagents for 200 assays

Instruction Manual

REF **EUM04200**

IVD **For *in vitro* diagnostic use**

CE

PROVIDE PERSONAL SOLUTIONS

B.S.N. S.r.l. | Biological Sales Network
Tel. +39.0374.351005
Via Coelli, 18 26012 Castelleone (CR)

contact@bsn-srl.it
bsn-srl.it

B.S.N. BIOLOGICAL SALES NETWORK S.R.L.
26012 Castelleone (CR) Italy - Via Coelli, 18
Tel. +39 0374 351005 - Fax +39 0374 57965 - e-mail: info@bsn-srl.it – bsn@postecert.it
Reg. Imprese / C.F. / P.IVA: 11317290150 - R.E.A. di Cremona n. 143395

REF **EUM04200**

IVD **For *in vitro* diagnostic use**

CE

Document Version: 0
Date of revision: 06.05.2022
File name: M-EUM04200 (eng)

CONTENTS

TABLES INDEX

FIGURES INDEX

1 INTRODUCTION

1.1 IVD SYMBOLS

IVD	In vitro diagnostic medical device /Dispositif médical de diagnostique en vitro/ In-Vitro-Diagnostikum / Producto sanitario para diagnóstico in vitro / Dispositivo medico-diagnostico in vitro / Dispositivo médico para in til in vitro diagnostik
LOT	Batch code / Code du lot / Chargenbezeichnung / Código de lote / Codice del lotto / Código do lote / Número do lote / Lotnummer
PN	Packing number / Numéro d'emballage / Packnummer / Número de envase / Numero confezioni / Número de embalagem / Número de embalagem / Emballagenummer
REF	Catalog number / Référence du catalogue / Bestellnummer / Número de catálogo / Numero di catalogo / Referência de catálogo / Código / Katalognummer
	Use by / Utiliser jusqu'au / Verwendbar bis / Fecha de caducidad / Utilizzare entro / Prazo de validade / Data limite de utilização / Holdbar til
	Temperature limitation / Limites de température / Temperaturbegrenzung / Limite de temperatura / Limiti di temperatura / Limites de temperatura / Limite de temperatura / Temperaturbegrænsning
	Add liquid / Ajout de liquide / Flüssigkeit zugeben / Añadir líquido / Aggiungi liquido / Adicionar líquido / Adicionar líquido / Tilføj væske
	Store in the dark / Conserver à l'abri de la lumière / Dunkel aufbewahren / Almacenar en ambiente oscuro / Conservare al buio / Armazenar no escuro / Guardar longe da luz / Opbevares mørkt
	Contains sufficient for <n> tests / Contenu suffisant pour "n" tests / Inhalt ausreichend für <n> Prüfungen / Contenido suficiente para <n> ensayos /Contenuto sufficiente per "n" saggi / Conteúdo suficiente para "n" ensaios / Conteúdo suficiente para <n> testes / Indeholder tilstrækkeligt til "n" test
	Consult instructions for use / Consulter les instructions d'utilisation / Gebrauchsanweisung beachten / Consulte las instrucciones de uso / Consultare le istruzioni per l'uso / Consulte as instruções de utilização / Consultar Instruções de uso / Se brugsanvisning
	Manufacturer / Fabricant / Hersteller / Fabricante / Fabbricante / Fabricante / Fabricado por / Producent
	This way up / Haut / Diese Seite oben / Este lado arriba / Questo lato in alto / Este lado para cima / Este lado para cima / Denne side op
	Recyclable / Recyclable / Recyclebar / Reciclable / Riciclabile / Reciclável / Reciclável / Genanvendeligt
	Brittle / Fragile / Zerbrechilich / Fragile / Fragil / Skrøbelig

1.2 ABBREVIATIONS

CAD: Collision Gas Fragmentation
CE: Collision Energy
CLSI: Clinical and Laboratory Standards Institute
CUR: Curtain Gas
CV: Coefficient of Variation
CXP: Collision Cell Exit Potential
DP: Desolvation Potential
EP: Entrance Potential
ESI: Electrospray Ionization
EtG: Ethylglucuronide
GS1: Gas 1
GS2: Gas 2
HPLC-MS/MS: High Performance Liquid chromatography coupled with tandem mass spectrometry
IS: Ion Spray Voltage
LLOD: Lower Limit of Detection
LLOQ: Lower Limit of Quantification
M/Z: Mass/Charge ratio
MPA: Mobile Phase A
MPB: Mobile Phase B
MRM: Multiple Reaction Monitoring
PP: Polypropylene
Q1: Quadrupole 1
Q3: Quadrupole 3
RT: Retention Time
S/N: Signal/Noise ratio
TEM: Source Gas Temperature

1.3 CLINICAL APPLICATION

FloMass Ethylglucuronide in Hairs is an in vitro diagnostic kit intended for the quantitative determination of Ethylglucuronide in human hairs samples using high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS).
The important and often devastating clinical and social effects determined in modern society by alcohol abuse have made evident since long the need to replace generic investigations for the determination of alcoholic substances with more specific biochemical markers.
Diagnosing alcohol abuse is complicated. In addition to the classic hematological and biochemical indexes usually considered, recently the use of CDT (desialated transferrin) as a biomarker has become increasingly popular. Particular attention was also paid to the search for effective biomarkers to reveal situations of binge drinking (the consumption of more alcoholic beverages in a more or less short time interval), very common among young people.

Ethylglucuronide (EtG), a metabolite of ethanol, is a sensitive and specific marker present in serum and urine up to 80 hours after the last intake. Therefore, it becomes an interesting marker of alcohol consumption with a higher and more specific sensitivity. It is present in hairs and other tissues, and this particularity further increases its diagnostic value. EtG is a non-volatile, polar, relatively stable molecule formed by the conjugation of ethanol with glucuronic acid, with the mediation of UDP-glucuronyl transferase (UGT).
Recently, the need to have an enough sensitive and specific analysis for the diagnosis of chronic alcohol abuse led to an increasingly in-depth study of this metabolite in hairs. The piliferous matrix, indeed, has the undoubted advantage of being able to broaden the surveillance window, theoretically allowing to identify not only a recent continuous abuse, but also an abuse in previous times. Unlike the blood parameters ordinarily used for this type of diagnosis (such as Aspartate Transaminase (AST), Alanine Amino Transferase (ALT), Gamma Glutamyl Transferase (GGT), CDT, and the mean corpuscular volume), EtG is a direct marker, therefore present in hairs only after an alcohol consumption. Furthermore, the keratin matrix has many practical advantages compared to other biological samples, and among these it is worth to highlight the greater ease of collection that can also be carried out by non-medical personnel, the non-invasiveness of sampling, the easy storage of the material and the intramatrix stability of analytes, much higher than other biological samples [1-5].

2 PRINCIPLE OF THE METHOD

The kit is intended for the quantitative determination of Ethylglucuronide using high performance liquid chromatography technique coupled with tandem mass spectrometry (HPLC-MS/MS).
The preparation of the sample described in Paragraph 7.1 involves a first phase of washing hairs, followed by a reconditioning and finally the extraction of the analyte from the hair matrix.
At the beginning of the preparatory phase, to normalize sample preparation and instrumental variability, the internal standard marked with stable isotope is added (Table 1).

ANALYTE	INTERNAL STANDARD
Ethylglucuronide	Ethylglucuronide $^{2}H_{5}$

Table 1: Analytes measured by kit EUM04200 and relative internal standard

Once extracted, the samples are injected in the HPLC-MS/MS system.
Subsequently, they enter in ESI source where they are ionized. Then ions enter in the triple quadrupole mass spectrometer, where they are measured in MRM mode.
Thus, only selected ions with a defined mass/charge ratio (m/z) are isolated in the first quadrupole and subsequently transferred into the collision cell where they are fragmented by impact with an inert gas (nitrogen or argon). Among the fragments, only those with a defined m/z ratio are isolated in the third quadrupole for subsequent detection.

Measurement in MRM mode with HPLC chromatographic separation ensures high selective and sensitive analyte identification and quantification [1-9].

3 COMPONENTS AND ACCESSORIES

3.1 KIT CONTENTS

Components for sample preparation included in the kit are shown in Table 2.

CATALOG NUMBER	DESCRIPTION	QUANTITY	STORAGE
EUM04011	Mobile Phase A	800 mL	Room Temperature
EUM04012	Mobile Phase B	600 mL	Room Temperature
EUM04021	Wash Solution	850 mL	Room Temperature
EUM04022	Reconditioning Solution	850 mL	Room Temperature
EUM04023	Extraction Solution	60 mL	Room Temperature
EUM04031	Internal Standard	1.1 mL	-20°C

Table 2: Components, description, quantity and storage of the kit EUM04200

The kit consists of reagents for 200 assays.
The expiry date of the intact kit is shown on the external product label. Follow storage conditions given on the product label of each component of the kit and keep it away from light and/or heat.

3.2 KITS SUPPORT ACCESSORIES

CATALOG NUMBER	DESCRIPTION	QUANTITY	STORAGE
EUM04041	6-Levels Calibrators, lyophil.	3 x 6 x 0.2 mL	-20°C
EUM04051	2-Levels Controls, lyophil.	3 x 2 x 0.2 mL	-20°C
EUM00C04	Chromatographic Column	1 pc	Room Temperature
EUM00A17	Precolumn	2 pcs	Room Temperature
EUM00A18	Holder + precolumn	1 pc	Room Temperature

Table 3: Accessories, description, quantity and storage of kit EUM04200

3.3 CONTROLS AND CALIBRATION OF ANALYTICAL SYSTEM

Calibration should be done using a 6-Levels Calibrators (EUM04041) containing the analytes. Calibrators should be prepared by reconstituting the lyophil with 0.2 mL of water and then following the sample preparation from step 15 for mill preparation (Paragraph 7.1) and from step 14 for preparation without mill (Paragraph 7.2). A new calibrator series should be prepared for each analytical run.

BSN supplies quality control sets at two different concentration levels (EUM04051). Lyophilized controls from extracted hairs are useful to verify the accuracy and precision of analytical procedures and to determine the analysis in the matrix. Controls must be prepared by reconstituting the lyophil with 0.2 mL of water and then following the sample preparation from step 15 for mill preparation (Paragraph 7.1) and from step 14 for preparation without mill (Paragraph 7.2).
For analytes concentrations, stability and accessories preparation, refer to package leaflets.

3.4 CHROMATOGRAPHIC SYSTEM

The kit has been validated using analytical column (EUM00C04) coupled to the precolumn (EUM00A18) and its holder (EUM00A19).

4 REQUIRED INSTRUMENTS

The kit requires a HPLC system with a tandem mass spectrometer and dedicated software. Triple quadrupole mass spectrometers should be either medium or medium-high level.

4.1 REQUIRED HPLC MODULES

1. Binary pump able to support a backpressure of 400 bar or more
2. Autosampler with cooling function and at least 50 µL loop (8°C)
3. Column Heater (25°C)
4. Degasser

4.2 REQUIRED EQUIPMENT AND MATERIALS FOR SAMPLE PREPARATION

1. Centrifuge (10000-13000 rpm) for 1.5- or 2-mL vials
2. Vortex
3. Pipettes and tips
4. 1.5- or 2-mL PP vials
5. Autosampler vials with plastic adapter for 200 µL
6. Vortex
7. Thermostated ultrasonic bath
8. Nitrogen evaporator
9. 7-mL tubes and balls for ball mill (Paragraph 7.1)
10. Ball mill (Paragraph 7.1)
11. 10-15-mL tubes (Paragraph 7.2)
12. Hairs scissors (Paragraph 7.2)
13. Chemical hood

5 HPLC-MS/MS SYSTEM CONDITIONS

Ionization: ESI negative mode
MS/MS: specific MRM
Injection volume: 10-20 µL (variable according to instrumental sensitivity)
Running time: 11 min
Column heater: 30°C
Analytical colum flow: 0.3 mL/min

Chromatographic gradient

TIME (min)	%MPB	FLOW (mL/min)
0.00	0.1	0.30
1.00	0.1	
1.50	6	
6.50	95	
8.50	98	
8.51	0.1	
8.52		0.30
8.53		0.40
11.0	Stop	

Table 4: Chromatographic gradient of kit EUM04200

Column conditioning: column should be conditioned for 10 min at the chromatography gradient initial condition. Then run 3 blank injections (MPA only) using the gradient as above.

Backpressure: at a flow rate of 0.3 mL/min, chromatographic system backpressure should not exceed 200 bar.

Column storage: if it is required to disconnect the analytical column and/or the precolumn, to preserve them, it is recommended to store them in the starting condition of the gradient program and to close it tightly.

Example of chromatogram

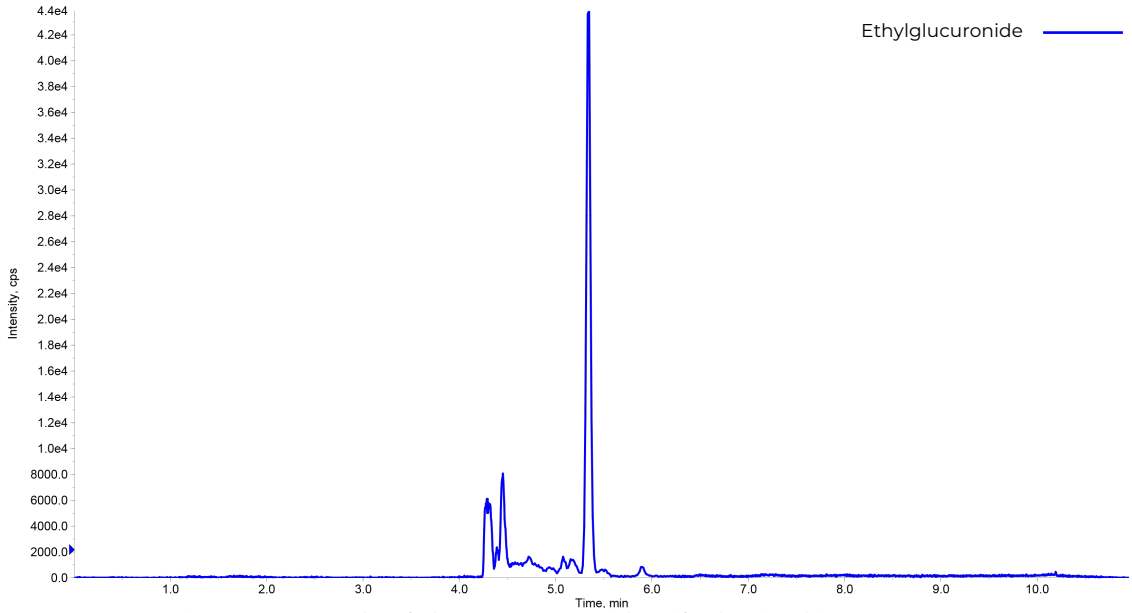

Figure 1: Example of chromatogram identified using kit EUM04200

6 SOURCE PARAMETERS AND TRANSITIONS

6.1 SOURCE PARAMETERS

Source parameters used in MS Method of kit EUM19100 with a Sciex series X500 triple quadrupole mass spectrometer are given below.

Curtain Gas (CUR): 20 psi
Collision Gas Pressure (CAD): Medium
Ion Spray Voltage (IS): -4500 V
Temperature (TEM): 600°C
Gas 1 (GS1): 60 psi
Gas 2 (GS2): 40 psi

6.2 TRANSITIONS

Monitored (1) and confirmatory (2) transitions and the MS parameters for each analyte using a HPLC Shimadzu Nexera combined with the Sciex 6500 triple quadrupole mass spectrometer are shown in Table 5. ESI positive mode.

ANALYTE	RT	Q1	Q3	DP	EP	CE	CXP
Ethylglucuronide 1	4.8*	221.0	85.0	-30	-6	-20	-10
Ethylglucuronide 2	4.8*	221.0	75.0	-30	-6	-17	-10
Ethylglucuronide IS	4.8*	226.0	75.0	-30	-6	-17	-10

Table 5: Detected transitions, retention times and potentials using HPLC Shimadzu + Sciex mass spectrometer

(*) Due to the characteristics of the EUM00C04 analytical column, the retention time TR can deviate from the value indicated with a larger window than other LC columns. However, this behavior does not affect the quality of the analytical data.

7 SAMPLE PREPARATION

Calibrators and controls follow the same samples preparation starting from step 15 for the preparation with a mill (Paragraph 7.1) and from step 14 for the preparation without a mill (Paragraph 7.2).

7.1 SAMPLE PREPARATION WITH MILL (CALIBRATORS/CONTROLS)

1. Insert the sample segment (approximately 100 mg) into a 7 mL tube
2. Add 4 mL of Wash Solution (EUM04021) ensuring that the entire sample is immersed
3. Vortex for 5 sec
4. Place in the ultrasonic bath for 10 min
5. Remove the Wash Solution and add 4 mL of the Reconditioning Solution (EUM04022), ensuring that the entire sample is immersed
6. Vortex per 30 sec
7. Discard the supernatant
8. Evaporate the residual supernatant with nitrogen flow
9. Add 20 steel balls to the 7 mL tube
10. Grind the entire quantity using the mill with the following operating conditions: rate = 5.3 m/s, cycle time = 3 min, nr cyles = 3, waiting time among two cycles = 20 sec
11. After trituration, weigh exactly about 20 mg of chopped hairs
12. Add 200 µL of Extraction Solution to the sample (EUM04023)
13. Incubate at 60°C for 2 hours
14. Prepare an Internal Standard Solution sufficient for the number of samples to be analyzed by mixing 5 µL Internal Standard (EUM04031) + 70 µL of Extraction Solution (EUM04023) for each sample
15. Add 30 µL of Internal Standard Solution*
16. Vortex for 30 sec
17. Place in the ultrasonic bath for 2 hours at 60°C
18. Centrifuge for 20 min at 12000 rpm
19. Pipette the supernatant in an autosampler vial with low volume insert
20. Inject in the HPLC-MS/MS system

* For calibrators and controls add the Internal Standard Solution directly in the vial after reconstitution.

7.2 SAMPLE PREPARATION WITHOUT MILL (CALIBRATORS/CONTROLS)

1. Insert the sample segment (approximately 100 mg) into a 7 mL tube
2. Add 4 mL of Wash Solution (EUM04021) ensuring that the entire sample is absorbed
3. Vortex for 5 sec
4. Place in the ultrasonic bath for 10 min
5. Remove the Wash Solution and add 4 mL of the Reconditioning Solution (EUM04022), ensuring that the entire sample is absorbed
6. Vortex for 30 sec
7. Discard the supernatant
8. Evaporate the residual supernatant with nitrogen flow
9. Finely grind the hairs
10. After grinding, weigh exactly about 20 mg of shredded hairs
11. Add 200 µL of Extraction Solution to the sample (EUM04023)
12. Incubate at 60°C for 2 hours
13. Prepare an Internal Standard Solution sufficient for the number of samples to be analyzed by mixing 5 µL Internal Standard (EUM04031) + 70 µL of Extraction Solution (EUM04023) for each sample
14. Add 30 µL of Internal Standard Solution*
15. Vortex for 30 sec
16. Place in the ultrasonic bath for 2 hours at 60°C
17. Centrifuge for 20 min at 12000 rpm
18. Pipette the surnatant in an autosampler vial with low volume insert
19. Inject in the HPLC-MS/MS system

* For calibrators and controls add the Internal Standard Solution directly in the vial after reconstitution.

8 COLLECTION AND STORAGE OF SAMPLES

The kit is intended for the analysis of human hairs samples collected with standard methods, such as those described in document GP16-A3 of the Clinical and Laboratory Standards Institute (CLSI) [10-11].

Stability of the samples: the concentration of Ethylglucuronide in hairs samples remains stable for up to 2 years if stored at room temperature in a moisture-free environment [12].

8.1 EXPECTED VALUES AND RESULTS INTERPRETATION

Each laboratory should conduct a pilot study to determine the distribution of analytes in relation to its population. To establish the population dimension study, it is recommended to check CLSI document EP28-A3C [13].
Reference values and normal ranges are set according to the distribution.

8.2 REFERENCES RANGES

Reference values to assess chronic alcohol abuse of Ethylglucuronide are listed in table 7.

ANALYTE	CUT-OFF (pg/mg)
Ethilglucuronide	<30

Table 6: Analyte reference values

Note: reference cut-off is taken from selected and updated scientific literature. Their update corresponds to the date of revision of this document [5,8].
Reference ranges are not a recommendation by the manufacturer, but they can be used as guideline for reference ranges evaluation of each clinical laboratory.

9 VALIDATION DATA

Validation data have been obtained with an HPLC-MS/MS system consisting of a HPLC Shimadzu Nexera coupled to a Sciex 6500 QTrap triple quadrupole mass spectrometer.
Refer to Paragraph 4.2 for the materials and equipment used in the sample preparation.

9.1 LINEARITY, DETECTION LIMITS AND QUANTIFICATION

A linear regression analysis of real values concentration has been completed to evaluate linearity of calibration curve for each analytic session.
Linearity range of acceptability corresponds to $R^2 \geq 0.98$. All values obtained are higher than the above-mentioned value.
Detection limit (LLOD) and quantification limit (LLOQ), which concentration provide a peak with S/N>3 and S/N>10 respectively, are reported in the table below (Table 8).

ANALYTE	LLOD (pg/mg)	LLOQ (pg/mg)	LINEARITY (pg/mg)
Ethylglucuronide	0.370	1.23	1.23 – 500

Table 7: LLOD, LLOQ and linearity

9.2 RECOVERY

Increasing amount of standard has been added to 3 blank extracted matrix pools to evaluate the analytical recovery characteristics. Three different levels of enriched urine (low, medium and high level) have been obtained.

Recovery = (Measured quantity on enriched matrix - Measured quantity on non-enriched matrix) / Added quantity

Average recovery range of acceptability = ±20%, all the values obtained are higher than the above-mentioned value.

ANALYTE	AVERAGE RECOVERY (%)	MIN RECOVERY (%)	MAX RECOVERY (%)
Ethylglucuronide	96.8	81.5	113.3

Table 8: Average, minimum and maximum recovery values

9.3 PRECISION

Average concentration values (pg/mg) measured in the 3 pools enriched with increasing concentrations of analytes (low,medium and high level) are reported in Table 10.
Precision has been evaluated as intra-assay, inter-assay and total coefficient of variation.

Intra-assay precision has been determined assaying 10 replicates (n=10) of each sample.
Inter-assay precision has been determined assaying 3 repetitions in 8 analytical series (n=24) for each sample.

Total CV% = $(CV\%Intra^2 + CV\%Inter^{2})^{1/2}$

Range of acceptability used for each variation coefficient are reported below.
Range of acceptability CV% Intra-assay = 10%
Range of acceptability CV% Inter-assay = 20%
Range of acceptability CV% Total = 20%

Obtained results respect the imposed ranges of acceptability.

ANALYTE	AVERAGE CONC. (pg/mg)			CV% INTRA			CV% INTER			CV% TOTAL		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
EtG	8.28	32.1	47.4	11.6%	11.3%	9.5%	14.0%	8.2%	12.0%	18.2%	14.0%	15.4%

Table 9: Intra-assay, inter-assay and total precision

10 GENERAL LIMITATIONS

- Kit must be used with the calibrators and the internal standard indicated in the kit instructions. The use of other standards or materials with this kit has not been validated.
- The use of other mobile phases, solutions or reagents other than those indicated in Paragraph 3.1 "KIT CONTENTS" has not been validated.
- The kit has been validated with the configuration described in Chapter 9 "VALIDATION DATA".
  The use of other triple quadrupole systems, HPLC systems and columns, which may require further development of the method, has not been validated.
- Do not use the kit after the expiry date of its components

11 REFERENCES

[1] Mueller A., Jungen H., Iwersen-Bergmann S., Raduenz L., Lezius S., Andresen-Streichert H. (2016): **Determination of Ethyl Glucuronide in Human Hair Samples: A Multivariate Analysis of the Impact of Extraction Conditions on Quantitative Results**. *Forensic Science International* 271:43-48

[2] Mönch B., Becker R., Nehls I. (2014): **Determination of Ethyl Glucuronide in Hair: A Rapid Sample Pretreatment Involving Simultaneous Milling and Extraction.** *Int J Legal Med* 128:69–72

[3] C.L. Crunelle C.L., Yegles M., A.L.N. van Nuijs A.L.N., Covaci A., De Doncker M., K.E. Maudens K.E., Sabbe B., Dom G., W.E. Lambert W.E., Michielsen P., Neels H. (2014): **Hair Ethyl Glucuronide Levels as a Marker for Alcohol Use and Abuse: A Review of the Current State of the Art.** *Drug and Alcohol Dependence* 134:1–11

[4] Morini L., Politi L., Groppi A., Stramesi C. and Polettini A. (2006): **Determination of Ethyl Glucuronide in Hair Samples by Liquid Chromatography/Electrospray Tandem Mass Spectrometry.** *J. Mass Spectrom.* 41:34-42

[5] Concheiro M., Cruz A., Mon M., de Castro A., Quintela O., Lorenzo A., López-Rivadulla M. (2009): **Ethylglucuronide Determination in Urine and Hair from Alcohol Withdrawal Patients.** *Journal of Analytical Toxicology,* 33:155-161

[6] Bastiani M.F., Lizot L.L.F., Da Silva A.C.C., Hahn R.Z., Dries S.S., Perassolo M.S., Antunes M.V., Linden R. (2020): **Improved Measurement of Ethyl Glucuronide Concentrations in Hair Using UPLC-MS/MS for the Evaluation of Chronic Ethanol Consumption.** *Forensic Science International* 306:110071

[7] Binz T.M., Baumgartner M.R., Kraemer T. (2014): **The influence of cleansing shampoos on ethyl glucuronide concentration in hair analyzed with an optimized and validated LC–MS/MS method.** *Forensic Science International* 244:20–24

[8] Pascali J.P., Palumbo D., Ronchi F.U., Mari F., Fioravanti A., Bertol E. (2019): **Ethyl Glucuronide Concentration in Hair of Detainees: A Preliminary Study.** J. *Forensic Sci.* 1-4

[9] Politi L., Morini L. **Marcatori Diagnostici dell'Abuso Alcolico e Ruolo dell'Etil Glucuronide.** *Chimica Clinica 6*

[10] Clinical and Laboratory Standards Institute (CLSI) (2021): **Toxicology and Drug Testing in the Medical Laboratory – Third Edition.** *CLSI document C52*

[11] Pichini S., Pacifici R.: **Linee Guida per la Determinazione di Sostanze d'Abuso nella Matrice Pilifera.**

[12] Ammann D., Becker R., Nehls I. (2015): **Stability of Ethyl Glucuronide in Hair Reference Materials After Accelerated Aging.** *Forensic Science International* 257:337–340

[13] Clinical and Laboratory Standards Institute (2010): **Defining, Establishing and verifying reference intervals in the clinical laboratory – Approved Guideline Third Edition** *CLSI Document EP28-A3C*

ANNEX 1: EC DECLARATION OF CONFORMITY

BSN Srl as Manufacturer and the only responsible for in-vitro diagnostic medical devices placed on the market under his own name, declares that these products meet all the provisions of the Legislative Decree n. 332 of the 8th September 2000, directive of in vitro diagnostic medical device 98/79/EC (in particular with regard to annex I) and subsequent amendments and additions. According to article 9 of the Legislative Decree 332/2000 and similar, this device belongs to the fourth class of devices, GENERIC IN VITRO DIAGNOSTIC MEDICAL DEVICES (all the other in vitro diagnostic medical devices except those in annex II and self-diagnostic tests).

COMPONENT	CODE	CERTIFICATION
FloMass Ethylglucuronide in Hairs	EUM04200	CE-IVD marked medical device according to Annex III
Mobile Phase A	EUM04011	CE-IVD marked medical device according to Annex III
Mobile Phase B	EUM04012	CE-IVD marked medical device according to Annex III
Wash Solution	EUM04021	CE-IVD marked medical device according to Annex III
Reconditioning Solution	EUM04022	CE-IVD marked medical device according to Annex III
Extraction Solution	EUM04023	CE-IVD marked medical device according to Annex III
Internal Standard	EUM04031	CE-IVD marked medical device according to Annex III
6-Levels Calibrators, lyophil.	EUM04041	CE-IVD marked medical device according to Annex III
2-Levels Controls, lyophil.	EUM04051	CE-IVD marked medical device according to Annex III
Chromatographic column	EUM00C04	CE-IVD marked medical device according to Annex III
Precolumn	EUM00A17	CE-IVD marked medical device according to Annex III
Holder + Precolumn	EUM00A18	CE-IVD marked medical device according to Annex III

Quality assurance system complying with the following directive:
UNI CEI EN ISO 13485:2016
UNI EN ISO 9001:2015

This declaration becomes invalid if modifications are introduced without B.S.N. Srl consent.
It is declared that the product is placed on the market in non-sterile package.
It is declared that B.S.N. Srl will keep all documents referred to in Annex III of the European Directive 98/79/EC at the disposal of the competent authorities for a 5-year period from the last date of production of the kit.
After the placing on the market of the product in question, it is declared that the Manufacturer has notified the competent authority of the application of post-market surveillance as requested from the European Directive 98/79/CE.

Castelleone (CR), 06 May 2022

Director