

Mass spectrometry in wastewater-based epidemiology (WBE) for the determination of small and large molecules as biomarkers of exposure. Needs for COVID-19 testing with environmental proteomics (EP-WBE). **Damiâ Barceló**^{1,2}, Yolanda Picó³, Manish Kumar⁶ Carlos Perez-Lopez¹, Antoni Ginebreda¹, Roma Tauler¹, Roberto Parra ⁵ Montserrat Carrascal⁴, Joaquin Abian⁴

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6Indian Inst.of tech Gandhingar, Gujarat, India



C.G. Daughton. Pharmaceuticals and Personal Care Products in the Environment, Scientific and Regulatory Issues, C.G. Daughton, T.L. Jones-Lepp (Eds.), American Chemical Society, Washington (2001), pp. 348-364



E. Zuccato, C. Chiabrando, S. Castiglioni, D. Calamari, R. Bagnati, S. Schiarea, R. Fanelli, Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse, Environ. Health, 4 (2005), p. 14





Barcelona esnifa cada dia 70.000 dosis de cocaïna

INFORME • Les aigües residuals porten les restes de droga a la depuradora del Baix Llobregat



AUGMENT • Els caps de setmana l'arribada dels residus es dobla respecte als altres dies **P23**



CrossMark

Five-year monitoring of 19 illicit and legal substances of abuse at the inlet of a wastewater treatment plant in Barcelona (NE Spain) and estimation of drug consumption patterns and trends

Nicola Mastroianni^a, Ester López-García^a, Cristina Postigo^{a,*}, Damià Barceló^{a,b}, Miren López de Alda^a



- Drug use in Barcelona during 5 years assessed using WBE.
- Alcohol, cannabis and cocaine were the most consumed drugs.
- Drug use increased from 2011 to 2015, contrary to national official data.
- Temporal trends of drug use were in agreement with regional official data.
- Consumption of alcohol, cocaine and MDMA increased during the weekend.



Contents lists available at ScienceDirect

Science of the Total Environment



journal homepage: www.elsevier.com/locate/scitotenv

Monitoring wastewater for assessing community health: Sewage Chemical-Information Mining (SCIM)



Christian G. Daughton

Environmental Futures Analysis Branch, Systems Exposure Division, National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, 944 East Harmon Avenue, Las Vegas 89119, NV, USA



- Update on Sewage Chemical-Information Mining (SCIM) for assessing public health
- Additional endogenous biomarkers are proposed for targeted monitoring with SCIM.
- Challenges for SCIM include confounding aspects of sewage and data normalization.
- A new concept is proposed that avoids the need for data normalization.
- Biomarkers are needed that measure not just disease but also health.



Pandemics timeline WBE timeline

Biosensors for the detection of disease outbreaks through Wastewater-based Epidemiology

Mildred G. Jiménez-Rodríguez¹, Fernando Silva-Lance¹, D. Alejandra Medina-Salaza Manuel Martínez-Ruíz¹, Elda M. Melchor-Martinez¹, María Adriana Martínez-Prato M.N. Iqbal¹, Roberto Parra-Saldivar^{1,*}, Damià Barcelo^{3,4,5} and Juan Eduardo Sosa-Hernández^{1,*}

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I TrAC

Read full aims & score G CheScore 2

12.296

CN US BR

15.5

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³Department of Environmental Chemistry, Institute of Envir (IDAEA-CSIC), Jordi Girona, 18-26, 08034, Barcelona, Spa ⁴Catalan Institute for Water Research (ICRA-CERCA), Par Girona, c/Emili Grahit, 101, Edifici H2O, 17003, Girona, Sp Editor-in-Chief > Editorial board ⁵College of Environmental and Resources Sciences, Zhe Invisor Pawliszyn China.



FIRST STUDY ON WBE IN BELFAST (UK)

ISOLATION OF B. THYPOSUS FROM SEWAGE AND SHELFISH W.James Wilson, M.D, D .Sc. British Medical Journal, (1928)1,1061

The success obtained by Wilson (1928) in demonstrating B. typhosus in Belfast sewage by his method of selective culture marks a great advance in this branch of bacteriological investigation. I have applied Wilson's technique along with other methods in the examination of sewage in Edinburgh, and the object of this communication is to

PARTICLES. Prevalence WBE ALSO FOR VIRUS CASE STUDY COVID-19







WBE using MS (PCR, Sensors): Lab-based surveillance method totally independent of health-care access

Detection of small molecules as biomarkers

Detection of large molecules as biomarkers

Detection of viral proteins of SARS CoV2 by HRMS

Detection of small molecules



Top-down approach





Biomarkers

Compounds	Biomarkers
Consumption of addictive substances	
Illicit drugs	
Cocainics	Human metabolites (> benzoylecgonine)
Opiods	Human metabolites and parent compounds
Cannabis	Human metabolites (> tretrahydrocannabinoic acid)
Amphetaminics	Parent compounds
Торассо	Parent compound (Nicotine) and HM (cotinine, 3'-hydroxycotinine, anabasine, anatabine)
Alcohol	Human metabolites (ethyl sulfate)
Caffeine	Parent compound (caffeine) and human metabolites (1,7-dimethyluric acid)
New psychoactive compounds	Mostly parent compounds
Consumption of pharmaceuticals	
Pharmaceuticals (NSAIDs, antibiotics,	Mostly parent compounds and transformation products, human metabolites in very few cases
anticancerigens, etc)	
Food Consumption	
Artificial sweeteners	As parent compounds (they are neither, metabolized or degraded).
Exposure to contaminants	
Organophosphorus flame retardants	Human metabolites (hydroxylated)
Plasticizers and phthalates	Human metabolites
Pesticides (Triazines, organophosphorus,	Human metabolites (hydroxylated, dealkyl and acids)
organochlorine and pyrethroids)	
Mycotoxins	Specific metabolites
Prevalence of disease	
Hepatitis B	Lamivuline (pharmaceutical)
Stress	8-iso-prostaglandin F2 α (PGF2 α), its metabolite dinor-11 β -Prostaglandin F2 α (dnPGF2 α) and
	Prostaglandin E2 (PGE2)
Diabetes	Metformin (pharmaceutical)
Gout	Oxyallopurinol (metabolite of a pharmaceutical allopurinol)



WBE to assess drug consumption: "Fallas of Valencia" as study case

Daily consumption of the investigated drugs of abuse in 2014 in the influent from the WWTPs in the city of Valencia during Fallas 2014. 6000, 500, March 19 18 **MDMA** Cocaine 19 4500, 18 375, 16 mg/day/g000 inh. mg/day/1000 inh. 16 17 20 3000, 6 20 13 10 1500, 125, 0, 0. Monday Tuesday Friday Thursday Sunday Tuesday Friday wonday Thursday Sunday Wednesday Wednesday Pinedo | 2014 Pinedo II 2014 Quart-Benager 2014





Mass spectrometry criterion for Megestrol identification:



Journal of Chromatography A, 1461 (2016) 98-106



Analysis of psychoactive substances in water by information dependent acquisition on a hybrid quadrupole time-of-flight mass spectrometer

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(CrossMark



Chemical pollutants (inorganic, pesticides, pharnaceuticals...)



Detection of large molecules

Science of the Total Environment 747 (2020) 141145



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journal homepage: www.elsevier.com/locate/scitotenv



Discovery of large molecules as new biomarkers in wastewater using environmental proteomics and suitable polymer probes



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HIGHLIGHTS

GRAPHICAL ABSTRACT

- Large-scale proteomic analysis of sewage and treated water.
- Proteomics for Wastewater Based Epidemiology.
- Profiling of human proteins in sewage waters for WBE.
- Human uromodulin, α-amylase, and S100A8 identified in urban sewage.



Objectives: Environmental Proteomics-WBE

• To design a probe for the evaluation of synthetic organic matter degradation in natural and engineered systems based on a previously selected polymer.

Rivas et al. **STOTEN**, (2016), 566–567:27–33

- To study the degradation patterns of the polymer and its transformation products by MALDI-TOF MS IMAGING and UPLC-HR-MS.
- To test the polymer probe in the different aquatic environments found in a WWTP :
 - Specifically, to examine chemical degradation and microbial community characterization in the influent flow (IN), in the anaerobic/anoxic bioreactor (ANA) and in the oxygen aeration tank (AER).
- To characterize the taxonomical composition of microbial communities present in those aquatic environments, comparing the free-living with the polymer-attached ones.
- To perform Proteomic discovery of the key peptides and related pollution tracers

Methods: Experimental setup



POLYCAPROLACTONEDIOL 1250





3 replicates per site 5 days + 1 blank (sterile conditions) 5 WWTPs









LC-MS/MS SEARCH RESULTS

Uniprot



BACTERIAL				
name	abundance			
60 kDa chaperonin	1175			
Elongation factor Tu	1152			
ATP synthase subunit beta	415			
60 kDa chaperonin 2	285			
Elongation factor Tu 2	148			
ATP synthase subunit alpha	129			
60 kDa chaperonin 1	126			
Elongation factor Tu 1	89			
Malate dehydrogenase	74			
Phosphoglycerate kinase	65			
RNA-binding protein Hfq	64			
Glyceraldehyde-3-phosphate dehydrogenase	52			
Enolase	50			
Major outer membrane lipoprotein	46			
ATP synthase subunit c	42			
60 kDa chaperonin 4	41			
60 kDa chaperonin 3	39			
Outer membrane protein Omp38	36			
60 kDa chaperonin 5	34			
K(+)-insensitive pyrophosphate-energized proto	34			
30S ribosomal protein S5	33			
Thioredoxin 1	33			
Glycerol kinase	31			
Elongation factor Tu-A	31			
ATP synthase subunit alpha 1	31			
50S ribosomal protein L17	27			
50S ribosomal protein L29	ක			
Elongation factor Tu-B	25			
Phosphoenolpyruvate carboxykinase [GTP]	25			
Acetoacetyl-CoA reductase	24			
30S ribosomal protein S2	24			
60 kDa chaperonin 6	22			
50S ribosomal protein L5	19			
50S ribosomal protein L19	19			
30S ribosomal protein S7	16			
50S ribosomal protein L7/L12	15			

abundance = int(100000 * peptides / mass)

Most Abundant Proteins PEAKS search

HUMAN					
name	abundance				
Keratin type II cytoskeletal 1	84				
Chymotrypsin-like elastase family member 3A	74				
Keratin type II cytoskeletal 2 epidermal	68				
Keratin type I cytoskeletal 10	67				
Keratin type I cytoskeletal 9	56				
Keratin type II cytoskeletal 5	40				
Neutrophil defensin 3	39				
Keratin type I cytoskeletal 14	34				
Keratin type II cytoskeletal 6A	31				
Chymotrypsin-C	27				
Keratin type I cytoskeletal 16	27				
Keratin type II cytoskeletal 6B	26				
Histone H4	26				
Small proline-rich protein 2E	ක				
Chymotrypsin-like elastase family member 3B	23				
Protein S100-A8	18				
Keratin type II cytoskeletal 2 oral	15				
Histone H2B type 1-K	14				
Keratin type II cytoskeletal 75	13				
_ysozyme C	12				
Keratin type II cytoskeletal 79	12				
Keratin type II cytoskeletal 78	10				
Keratin type II cytoskeletal 1b	9				
Defensin-5	9				
Keratin type II cytoskeletal 4	8				
Dermcidin	8				
Keratin type II cytoskeletal 73	8				
Keratin type I cytoskeletal 13	8				
Trypsin-2	7				
Serum albumin	7				

60 KDa chaperonin (GroEL)

GroEL belongs to the chaperonin family of molecular chaperones, and is found in a large number of bacteria. It is required for the proper folding of many proteins. To function properly, GroEL requires the lid-like cochaperonin protein complex GroES.



In eukaryotes the proteins Hsp60 and Hsp10 are structurally and functionally nearly identical to GroEL and GroES, respectively.

ATP synthase

ATP synthase is an enzyme that creates the energy storage molecule adenosine triphosphate (ATP). ATP is the most commonly used "energy currency" of cells for all organisms. It is formed from adenosine diphosphate (ADP) and inorganic phosphate (Pi).

E. coli ATP synthase is the simplest known form of ATP synthase, with 8 different subunit types.

elongation factor Tu

In bacteria, elongation factor Tu plays a central role during the selection of the correct amino acids throughout the elongation phase of translation.

The amount of EF-Tu in the cell is equimolar to that of tRNA, while the total number of EF-Tu molecules is 8–14 times the number of ribosomes depending on growth conditions.2

wikipedia

ASSESSMENT OF HEALTH STATUS, HABITS, AND BEHAVIORS OF THE POPULATION THROUGH WASTEWATER-BASED EPIDEMIOLOGY (WBE) BASED ON PROTEINS.

M.Carrascal, A. Ginebreda, J. Abián, D. Barceló. IIBB/IDAEA-CSIC.

Characterization of human protein biomarkers in sewage waters: presence in urban sewage waters of proteins from diverse origins, including human proteins such as uromodulin, α -amylase, and S100A8, which have been proposed as health \rightarrow Wastewater Based Epidemiology (WBE).



(Carrascal et al. Stoten,2020 747:141145)



Data analysis (chemometrics) workflow



ROIMCR-Regions of Interest-Multivariate Curve Resolution

50% of the m/z MS1 spectra from ROIMCR resolution matched MS1 signals- MS2 were taken. When no MS2 fragments were present, no identification by-Protein Discovery 523 Peptides corresponding to 35 different proteins from different eukaryotic species: Human, Chicken, Rat or Mouse, bacterial species

Proteins	Protein name	Organism name	Time 1	Time 2	Time 5
P35908	Keratin, type II cytoskeletal 2 epidermal	Homo sapiens	7	10	5
Q6IFZ6	Keratin, type II cytoskeletal 1b	Mus musculus	7	9	5
P04264	Keratin, type II cytoskeletal 1	Homo sapiens	12	17	6
Q981J9	60 kDa chaperonin 5	Mesorhizobium japonicum	1	1	1
B5YJN3	60 kDa chaperonin	Thermodesulfovibrio yellowstonii	1	1	1
A1K436	60 kDa chaperonin 1	Azoarcus sp.	1	1	1
Q5P7G2	60 kDa chaperonin	Aromatoleum aromaticum	1	1	1
A4G837	60 kDa chaperonin	Herminiimonas arsenicoxydans	1	1	1
Q1H4F2	60 kDa chaperonin	Methylobacillus flagellatus	1	1	1
A4SZV4	60 kDa chaperonin	Polynucleobacter asymbioticus	1	1	1
Q3A0V2	60 kDa chaperonin	Pelobacter carbinolicus	1	1	1
P13645	Keratin, type I cytoskeletal 10	Homo sapiens	1	9	2
P04259	Keratin, type II cytoskeletal 6B	Homo sapiens	2	4	0
A8EV70	ATP synthase subunit beta	Arcobacter butzleri	0	1	0
Q99895	Chymotrypsin-C	Homo sapiens	0	6	0
P01876	Immunoglobulin heavy constant alpha 1	Homo sapiens	0	7	2
Q6FF97	Elongation factor Tu	Acinetobacter baylyi	0	4	0
A3M1F6	Elongation factor Tu	Acinetobacter baumannii	0	4	0
P09093	Chymotrypsin-like elastase family member 3A	Homo sapiens	0	14	0
A5FLS1	ATP synthase subunit beta	Flavobacterium johnsoniae	0	1	0
A1ALL7	ATP synthase subunit beta 1	Pelobacter propionicus	0	1	0
Q82XP8	ATP synthase subunit beta	Nitrosomonas europaea	0	1	0
Q9Y6R7	IgGFc-binding protein	Homo sapiens	0	1	0
Q14533	Keratin, type II cuticular Hb1	Homo sapiens	0	0	1
P78385	Keratin, type II cuticular Hb3	Homo sapiens	0	0	1
P78386	Keratin, type II cuticular Hb5	Homo sapiens	0	0	1
O43790	Keratin, type II cuticular Hb6	Homo sapiens	0	0	1
A5A6M5	Keratin, type I cuticular Ha1	Pan troglodytes	0	0	1
O76009	Keratin, type I cuticular Ha3-I	Homo sapiens	0	0	1



WASTEWATER-BASED EPIDEMIOLOGY (WBE) BASED ON PROTEINS IN 10 CATALAN WWTPs





WASTEWATER-BASED EPIDEMIOLOGY (WBE) BASED ON PROTEINS IN 10 CATALAN WWTPs.



MALDI-TOF of Besos and Olot WWTP

10 (Besos)peaks and 7 (Olot(peaks) match the most abundant proteins



Figure 8. The spectra of MALDI – ToF of Besós and Olot. Each number represents the molecular weight of each corresponding peptide, the height of peaks indicates the abundance of the corresponding peptide detected in the sample.

LC/MS/MS of Besos and Olot WWTP: Confirmation of the Proteins detected by MALDI-TOF

 Table 4. The analytical results of LC – MS/MS of Besós (top) and Olot (bottom). PSMs indicate the abundance of the corresponding proteins detected in the sample.

Description	Score	Coverage	# Proteins	Unique Peptid	# Peptides	# PSMs
Pancreatic alpha-amylase OS=Homo sapiens C	1689.54	73.97	3	5	34	427
Alpha-amylase 1 OS=Homo sapiens OX=9606	1572.32	72.80	3	7	34	395
Alpha-amylase 2B OS=Homo sapiens OX=960	1375.19	72.80	4	1	33	361
Maltase-glucoamylase, intestinal OS=Homo sa	532.97	36.51	3	48	52	152
Albumin OS=Homo sapiens OX=9606 GN=AL	390.23	67.16	6	29	37	107

Score	Coverage	# Proteins	Unique Peptid	# Peptides	# PSMs
1519.02	76.11	1	45	58	410
481.28	64.25	1	16	39	129
454.32	67.00	1	31	38	125
475.58	64.19	4	5	25	121
451.86	64.19	3	6	25	115
	Score 1519.02 481.28 454.32 475.58 451.86	ScoreCoverage1519.0276.11481.2864.25454.3267.00475.5864.19451.8664.19	ScoreCoverage# Proteins1519.0276.111481.2864.251454.3267.001475.5864.194451.8664.193	ScoreCoverage# ProteinsUnique Peptid1519.0276.11145481.2864.25116454.3267.00131475.5864.1945451.8664.1936	ScoreCoverage# ProteinsUnique Peptid# Peptides1519.0276.1114558481.2864.2511639454.3267.0013138475.5864.194525451.8664.193625

WASTEWATER-BASED EPIDEMIOLOGY (WBE) BASED ON PROTEINS IN 10 CATALAN WWTPs.

Species distribution according to **albumin protein**. Presence of blood from main species (pigs, chicken, cows) in wastewater-illegal discharges to the WWTP . Sampling December 2020.



WASTEWATER-BASED EPIDEMIOLOGY (WBE) BASED ON PROTEINS IN 10 CATALAN WWTPs: IMMUNOGLOBULIN.

Immunoglobulin in feces : tentative biomarker for people infected by SARS-CoV-2 or vaccinated (1 Million vaccinated in Catalonia April 2021) or bacteria,

Accession 💌	immunodeficiency myeloma	Species	Ŧ
P01834	Immunoglobulin kappa constant OS=Homo sapiens OX=9606 GN=IGKC PE=1 SV=2 - [IGK0	Homo sapiens	
P0DOX7	Immunoglobulin kappa light chain OS=Homo sapiens OX=9606 PE=1 SV=1 - [IGK_HUMAN]	Homo sapiens	
P01876	Immunoglobulin heavy constant alpha 1 OS=Homo sapiens OX=9606 GN=IGHA1 PE=1 SV=	Homo sapiens	
P0DOX2	Immunoglobulin alpha-2 heavy chain OS=Homo sapiens OX=9606 PE=1 SV=2 - [IGA2_HUN	Homo sapiens	
P01833	Polymeric immunoglobulin receptor OS=Homo sapiens OX=9606 GN=PIGR PE=1 SV=4 - [P	Homo sapiens	
P0DOY2	Immunoglobulin lambda constant 2 OS=Homo sapiens OX=9606 GN=IGLC2 PE=1 SV=1 - [Homo sapiens	
P0CG04	Immunoglobulin lambda constant 1 OS=Homo sapiens OX=9606 GN=IGLC1 PE=1 SV=1 - [Homo sapiens	
P01857	Immunoglobulin heavy constant gamma 1 OS=Homo sapiens OX=9606 GN=IGHG1 PE=1 S	Homo sapiens	
A0A075B6P5	Immunoglobulin kappa variable 2-28 OS=Homo sapiens OX=9606 GN=IGKV2-28 PE=3 SV=	Homo sapiens	
P01591	Immunoglobulin J chain OS=Homo sapiens OX=9606 GN=JCHAIN PE=1 SV=4 - [IGJ_HUMA	Homo sapiens	
P06310	Immunoglobulin kappa variable 2-30 OS=Homo sapiens OX=9606 GN=IGKV2-30 PE=3 SV=	Homo sapiens	
P01859	Immunoglobulin heavy constant gamma 2 OS=Homo sapiens OX=9606 GN=IGHG2 PE=1 S	Homo sapiens	
P06312	Immunoglobulin kappa variable 4-1 OS=Homo sapiens OX=9606 GN=IGKV4-1 PE=1 SV=1 -	Homo sapiens	
P01764	Immunoglobulin heavy variable 3-23 OS=Homo sapiens OX=9606 GN=IGHV3-23 PE=1 SV=	Homo sapiens	
P01871	Immunoglobulin heavy constant mu OS=Homo sapiens OX=9606 GN=IGHM PE=1 SV=4 - [Homo sapiens	
P01619	Immunoglobulin kappa variable 3-20 OS=Homo sapiens OX=9606 GN=IGKV3-20 PE=1 SV=	Homo sapiens	
P01782	Immunoglobulin heavy variable 3-9 OS=Homo sapiens OX=9606 GN=IGHV3-9 PE=1 SV=2	Homo sapiens	
A0A075B6R9	Probable non-functional immunoglobulin kappa variable 2D-24 OS=Homo sapiens OX=9606	Homo sapiens	
A0A0C4DH31	Immunoglobulin heavy variable 1-18 OS=Homo sapiens OX=9606 GN=IGHV1-18 PE=3 SV=	Homo sapiens	
A0A0C4DH72	Immunoglobulin kappa variable 1-6 OS=Homo sapiens OX=9606 GN=IGKV1-6 PE=3 SV=1 -	Homo sapiens	
A0A0A0MT36	Immunoglobulin kappa variable 6D-21 OS=Homo sapiens OX=9606 GN=IGKV6D-21 PE=3 S	Homo sapiens	
A0A0C4DH24	Immunoglobulin kappa variable 6-21 OS=Homo sapiens OX=9606 GN=IGKV6-21 PE=3 SV=	Homo sapiens	
A0A0B4J2H0	Immunoglobulin heavy variable 1-69D OS=Homo sapiens OX=9606 GN=IGHV1-69D PE=1	Homo sapiens	
A0A075B6H9	Immunoglobulin lambda variable 4-69 OS=Homo sapiens OX=9606 GN=IGLV4-69 PE=1 SV	Homo sapiens	
P01624	Immunoglobulin kappa variable 3-15 OS=Homo sapiens OX=9606 GN=IGKV3-15 PE=1 SV=	Homo sapiens	

WBE of SARS-CoV-2 in India using PCR by, Manish Kumar,Indian Institute of Technology,Gandhinagar, Gujarat, India, IDAEA-CSIC,ICRA, et al. *Chemical Engineering Journal*, 425 (2021)130635.



WBE can definitely help in city zonation for effective COVID-19 pandemic preparedness powered by early warning !!

What about effects of population density and condition of sewer systems?



Location from which samples tested for a Location from which samples tested for \$



Antidrug resistance in the Indian ambient waters of Ahmedabad during the COVID-19 pandemic

Manish Kumar^{a,*}, Kiran Dhangar^a, Alok Kumar Thakur^a, Bhagwana Ram^b, Tushara Chaminda^c, Pradeep Sharma^d, Abhay Kumar^e, Nirav Raval^f, Vaibhav Srivastava^f, Jörg Rinklebe^{g, h}, Keisuke Kurodaⁱ, Christian Sonne^j, Damia Barcelo^{k,1}

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Detection of viral proteins SARS COVID



Non-structural proteins (present only in host cells) o Enzymes o Transcription factors

Structural proteins

(in the virion particle)

- o S (spike)
- o E (envelope)
- o M (membrane)
- N (nucleocapsid)

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			-		

Mass Spectrometry Techniques in Emerging Pathogens Studies: Journal of the American Society for Mass Spectrometry **COVID-19** Perspectives pubs.acs.org/jasms Account & Perspective Igbal Mahmud and Timothy J. Garrett* COVID-19 patients **Protein Extraction** MS-based proteomic Techniques Data analysis Specimens Virus deactivation Healthy Treatment with alkylating Serum agent for peptide mapping Asymptomatic COVID-19 Protein solubilization MS Plasma Spectral comparison Blood Mild infected 6 Severe infected PBMCs Proteome digestion Using Trypsin Urine LC-MS Recovery Swab Saliva Multivariate analysis **Digestion termination** biomarker and peptides desaltation Cell model Infected Dried and reconstitution MALDI-MS rotein for MS run Uninfected Cell pellets

Figure 1. MS-based proteomics to study COVID-19. Workflow developed based on recent proteomic analysis on COVID-19 pandemic. SARS-CoV-2 virus with different symptomatic patient conditions or infected cell model are shown. Typically, serum, plasma, and PBMCs from blood as well as urine, saliva, and nasal swabs have been utilized for targeted or untargeted proteomic analysis in COVID-19 patients. MS spectral comparison followed by multivariate statistical analysis can be applied for biomarker analysis with different levels of COVID-19 severities. For details about different COVID-19 proteomic sample preparation, data collection, and analysis, see Table 1.





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Article

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N- and O-Glycosylation of the SARS-CoV-2 Spike Protein

Miloslav Sanda,* Lindsay Morrison, and Radoslav Goldman



HCD fragmentation of the N165 glycopeptide carrying an asymmetric biantennary glycan with a sialylated LacdiNAc structural motif.



cIMS of the *m*/*z* 657 fragment produced by fragmentation of the AGC(cam)LIGAEHVNN(dea)SYEC(cam)DIPIGAGIC(cam)ASYQTQTNSPR O-glycopeptide.





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Article

oteotyping SARS-CoV-2 Virus from Nasopharyngeal Swabs: A pof-of-Concept Focused on a 3 Min Mass Spectrometry Window

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TRACT: Rapid but yet sensitive, specific, tion of the severe acute respiratory syndrome 2) in clinical samples is key to diagnose infecte of the spread of the virus. Alternative metho anodiagnostics that would not require specific tigate not only for fighting the COVID-19 pand emergent pathogenic threats. Here, we propspectrometry to detect SARS-CoV-2 marker al swabs. We documented that the signal from ch samples is low and can be overlooked whe omic data acquired on a restricted windo cape. In this proof-of-concept study, simili d with different quantities of purified SARS-Co



Figure 4. Heatmap of peptide intensities in the clinical nasopharyngeal swabs. Cell color corresponds to MS1 peak area, red be

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stablishing a mass spectrometry-based system for apid detection of SARS-CoV-2 in large clinical ample cohorts

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bulent flow chromatography (TFC) setup in Transcend TLX-4 system coupled to TSQ Altis triple quadrupole as four independent channels (channel 1: blue, channel 2: orange, channel 3: green and channel 4: red) that can be and IGM (IGMEVTPSGTWLTYTGAIK) from nucleoprotein; HSG (HSGFEDELSEVLENQSSQAELK) from 15N-labeled surrogate standard chromogra

(in Tier 3) and SYE (SYELPDGQVITIGNER) from human beta actin (in Tier 1).

a Qualitative (Tier 3) assay; b Quantitative (Tier 1) assay. The first three residues of each peptide are used to label peptide peaks: DGI (DGIIWVATEC



Detection of SARS-CoV-2 in nasal swabs using MALDI-MS

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General scheme for SARS-CoV-2 detection by MALDI-MS and ML MALDI-MS analysis Sample collection Spectra ML m/zSARS-CoV-2 Control Prediction of SARS-CoV-2 using ML PCA 5 7: 0 ML (•••• ••• Control mass spectra SARS-CoV-2 mass spectra -5 Predicted class -5 5 10 Р Ν Spectral preprocessing TP FN Actual class P Intensity matrix N FP TN Feature selection



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Rapid Detection of COVID-19 Using MALDI-TOF-Based Serum Peptidome Profiling

Ling Yan, $^{\nabla}$ Jia Yi, $^{\nabla}$ Changwu Huang, $^{\nabla}$ Jian Zhang, $^{\nabla}$ Shuhui Fu, Zhijie Li, Qian Lyu, Yuan Xu, Kun Wang, Huan Yang, Qingwei Ma, Xiaoping Cui, Liang Qiao,* Wei Sun,* and Pu Liao*

Scheme of establishing a diagnostic model for rapid screening of COVID-19 patients.



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Step/parameter	RT-PCR detection ³¹	DNA amplicon detection by MS ^{32, 33}	Viral peptide detection by MALDI-MS ³⁴	Viral peptide sequencing by LC- ESI-MS/MS ³⁵
Viral component recovery	RNA	RNA	protein	protein
Recovery time	minutes	minutes	1–2 h	1–2 h
Steps pre-analysis	reverse transcription, denaturation, annealing, amplification ^b	reverse transcription, denaturation, annealing, amplification ^b	proteolytic digestion with/without reduction/alkylation	proteolytic digestion with/without reduction/alkylation
Pre-analysis time	30 min	30 min	4–14 h°	4–14 h°
Detection time	2–4 h	few minutes	few minutes	30 min – 1 h
Detection limit (copies)	~10	>10-10 ^{2 32}	> 10 ^{5d}	10 ⁵ -10 ⁶
Reliability/confidence	up to 95%	high (with multiple amplicons detected)	high (with multiple peptides detected)	high (with multiple peptides sequenced)
Analysis cost/sample (USD)	\$10	\$10-50	\$100	\$250
Instrument cost (USD)	\$20 K+	\$100 K+	\$100–1000 K+	\$250–500 K+

^a All times and figures are approximate only and depend on specific protocols and equipment employed. Citations are: 1. Da Silva et al. ACS Infect. Dis. 2020, 6, 2319-2336.

2. Xiu et al, J., Front. Microbiol. 2017, 8, 1510.

3. Wandernoth et al. Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by Mass Spectrometry. 2020, 12, 849.

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5. Nikolaev et al. J.Proteome Res. 2020, 19, 4393-4397.

^b According to real-time RT-PCR detection of SARS-CoV-2 protocol, Institute Pasteur, Paris (https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institutpasteur-paris.pdf).

^cImprove using immobilized enzyme digestion to 1–2 h.

dImprove by one or two orders of magnitude with selected ion monitoring (SIM).

Conclusions and Challenges

- 1. The **identification** of small molecules used as **biomarkers** is linked to the search of **HBM studies** and to **the advances in MS**
- 2. Introduction of **HR-MS** and the improvement of raw data **processing**, **identification** systems and applied **bioinformatics** has opened an important door to the discovery of new useful biomarkers directly in wastewater.
- 3. However, this aspect of WBE is not yet resolved and the lack of human-specific metabolites for some compounds and additional sources of transformation products need to be addressed.
- 4. Solving these problems can be somewhat **time-consuming** due to the **complexity of wastewater.**



Shall we need to increase/cleanup /sample preparation of the WWTP samples? *Pros and cons to any of the answers should be carefully evaluated on a case-by-case basis.*

Conclusions and Challenges

- 5. Recently, there is an **emerging interest** in complementing the metabolite profile of biomarkers determined in wastewater with **proteomics**.
- 6. Although the studies are still scarce, **WBE of large molecules using MS** got a lot of possibilities using **HRMS** with **non-target** approaches and **chemometrics**
- 7. Problems in Proteomics -WBE remain, such as the **prevalence of the bacterial proteome versus human proteomes**, being more difficult for the later.
- 8. Nevertheless, despite the limited number of studies that exist, several **proteins of human and animal origin** have been **identified** that are related illegal discharges of livestock blood (**Albumin**), Covid-19 infection (**immunoglobulin**), human proteins identified (**Keratins,pancreatic enzymes,alpha-amylase, uromodulin,S100A8**)
- **9. MALDI-TOF is a** fast method to detect the most abundant proteins in WBE with confirmation by LC/MS/MS

Proteins and peptides related to humans as well as proteins and peptides from SARSther viruses will be soon identified

Conclusions and Challenges

- 9. Virus identification is the only field of WBE where MS is not prevalent
- 10. Sensitivity still needs to be resolved → the identification of these proteins in clinical analysis is a reality of great help in diagnosis.
- **11. Biosensors- paper based** and other devices, Little Middle Income Countries (LMICs) A cking WWTP facilities- today's problem of more than 1 billion people
 - Should be able to find specific peptides from the nucleocapsid protein responsible of oV-2 infection in WBE?
 - This would always help to improve identification and, **why not?** Future is always going a and this would be of help.



Do we need a more sensitive LC-MS/MS for SARS peptide specific detection in WWTP? Clearly yes, but the ADVANCES IN MS DOES NOT STOP.

e.g. Ion mobility mass spectrometry and improve workflows in non-target analysis are g sensitivity to unexpected extremes.



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Paper and other fibrous materials for biosensing applications



Schematic representation of a paper-based analytical device (PAD) and its primary function as a matrix for biomolecules immobilization, functional materials, and microfluidic control.



a) Two-dimensional flow design for sequential delivery of reagents or mixing; (**b**) threedimensional flow configurations; (**c**) open-channel microfluidic omniphobic paper; (**d**) Separation membrane; (**e**)"on/off" fluidic switch; (**f**) hydrogel-driven paperbased microfluidics; (**g**) flow time delays using dissolvable bridges and absorbent pads ; (**h**) slip device for one-step point-of-care testing.

Paper-based devices for SARS-CoV-2 in WBF



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Paper-based devices for rapid diagnostics and testing sewage for early warning of COVID-19 outbreak

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Fig. 3. The schematic diagram of the paper device for the detection of BoHV-1, Brucella and Leptospira [33]



Fig. 4. The schematic illustration of SARS-CoV-2 IgG and IgM test [41]. (A) The detection device. (B) Different test results.

Case Studies in Chemical and Environmental Engineer



Electrochemical sensing of SARS-CoV-2 amplicons with printed circuit board (PCB) electrodes



SARS-CoV-2 nucleic acid detection workflow. RNA extracted from wastewater is amplified using a low cost thermo cycler and then placed onto PCB electrodes.



Field-Effect TransistorBased Biosensor



COVID-19 FET sensor

Schematic diagram of COVID-19 FET sensor operation procedure. Graphene as a sensing material is selected, and SARS-CoV-2 spike antibody is conjugated onto the graphene sheet via 1-pyrenebutyric acid N-hydroxysuccinimide ester, which is an interfacing molecule as a probe linker.

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Many thanks for your attention