

Contents

List of Contributors	xix
Foreword	xxvii

PART A INTERPRETATION OF BREATH ANALYSIS DATA

CHAPTER 1 Mathematical and Statistical Approaches for Interpreting Biomarker Compounds in Exhaled Human Breath 3

Joachim D. Pleil and Jon R. Sobus

1.1 Introduction.....	3
1.2 Data interpretation	4
1.2.1 Data visualization and summary statistics	4
1.2.2 Variable independence and clustering	6
1.2.3 Population statistics and variance components.....	8
1.2.4 Stochastic models and meta-data	10
1.2.5 Dynamic models and longitudinal data.....	12
1.3 Conclusions and recommendations	15
References	17

CHAPTER 2 Issues and Challenges in Human Breath Research: Perspectives from Our Experience 19

Steven F. Solga and Terence H. Risby

2.1 Introduction.....	19
2.2 Defining normal in clinical practice: the case of a routine liver blood test	20
2.3 Developing a breath test: can the blood assay be unseated?	21
2.4 Convincing clinicians.....	22
2.5 Breath markers.....	22
2.6 Conclusion	23
References	24

PART B REAL-TIME ANALYSIS OF EXHALED BREATH

CHAPTER 3 Physiological Modeling for Analysis of Exhaled Breath..... 27

Julian King, Helin Koc, Karl Unterkofler, Gerald Teschl, Susanne Teschl, Pawel Mochalski, Hartmann Hinterhuber, and Anton Amann

3.1 Introduction.....	27
3.2 Real-time measurements: experimental basics	29
3.3 Modeling.....	29
3.3.1 Isoprene.....	34
3.3.2 Acetone	37
3.4 Concluding remarks.....	42
References	43

PART C PHYSIOLOGICAL AND CLINICAL STUDIES

CHAPTER 4 Recent SIFT-MS Studies of Volatile Compounds in Physiology, Medicine and Cell Biology..... 49

Patrik Španěl and David Smith

4.1 Introduction.....	49
4.2 Direct breath analysis	50
4.2.1 Inter-individual and intra-individual concentration distributions of mouth-exhaled breath metabolites in the healthy population	50
4.2.2 Variation with age of mouth-exhaled breath concentrations of common metabolites for healthy individuals and the influence of diet.....	51
4.2.3 Intra-individual studies of mouth-exhaled and nose-exhaled breath; volatile compounds deliberately generated in the oral cavity	54
4.2.4 The special case of exhaled hydrogen cyanide.....	57
4.2.5 On-line breath analysis in the operating theatre.....	58
4.3 VOC emission from skin; comparison with VOCs in breath.....	60
4.4 Exhaled breath condensate and broncoalveolar lavage.....	61
4.5 VOCs in urine headspace; ketones and ovulation; 3-HBA.....	62
4.6 Volatile biomarkers of cancer cells, <i>in vitro</i> and <i>in vivo</i>	64
4.7 Alcohol ingestion and detection and cannabis.....	65

4.8 Flowing afterglow mass spectrometry, FA-MS, and total body water.....	67
4.9 Summary remarks; future prospects for SIFT-MS and FA-MS in medicine.....	70
References	70

CHAPTER 5 The Analysis of Oral Air by Selected Ion Flow Tube Mass Spectrometry Using Indole and Methylindole as Examples 77

Brian M. Ross and Ashley Esarik

5.1 Introduction.....	77
5.2 Oral malodor	77
5.3 Oral air sampling considerations.....	79
5.4 Indoles	81
5.5 Summary and conclusion	85
References	86

CHAPTER 6 Smokers Breath as Seen by Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-TOF-MS) 89

*Ingrid Kohl, Jens Herbig, Jürgen Dunkl, Armin Hansel,
Martin Daniaux, and Michael Hubalek*

6.1 Introduction	89
6.2 Materials and methods.....	90
6.2.1 Human subjects	90
6.2.2 Statement.....	91
6.2.3 Sampling procedure	91
6.2.4 VOC sensor: proton-transfer-reaction time-of-flight mass spectrometer.....	92
6.2.5 Data acquisition and breath sample analysis.....	93
6.2.6 Species identification	93
6.2.7 Determination of the sensitivity and LOD	93
6.2.8 Mass scale calibration	94
6.2.9 Raw data analysis	95
6.3 Results and discussion.....	96
6.3.1 Sensitivity and LOD.....	96
6.3.2 Mass accuracy	97
6.3.3 The exhaled breath composition of the women volunteers	98
6.3.4 Breath compounds enhanced by cigarette smoking.....	101
6.3.5 Structure of mass spectral peaks	109

6.4 Conclusion.....	112
References	112

CHAPTER 7 Exhaled Breath Analysis in Occupational Medicine 117

Massimo Corradi and Antonio Mutti

7.1 Rationale for the use of exhaled breath analysis in occupational medicine.....	117
7.2 Exhaled nitric oxide.....	119
7.2.1 Exhaled NO as diagnostic tool in occupational respiratory diseases	120
7.3 Exhaled breath condensate (EBC).....	121
7.3.1 EBC in occupational settings	122
7.4 Exhaled volatile organic compounds.....	123
7.4.1 Exhaled volatile organic compounds in occupational medicine.....	124
References	125

CHAPTER 8 Volatile Organic Compounds in Human Breath: Biogenic Origin and Point-of-Care Analysis Approaches..... 129

*Alexander A. Aksenov, Michael Schivo, Hamzeh Bardaweel,
Yuriy Zrodnikov, Alice M. Kwan, Konstantin Zamuruyev,
William H.K. Cheung, Daniel J. Peirano, and Cristina E. Davis*

8.1 Biogenic origin of volatile compounds in human exhaled breath	129
8.1.1 VOC production by neoplastic cells	131
8.1.2 VOC production on cellular level by non-neoplastic cells	134
8.1.3 Viral and bacterial single cell VOCs	135
8.2 Miniature mobile point-of-care diagnostic systems for VOC breath biomarkers: trends and future requirements.....	137
8.2.1 Point-of-care devices: requirements and challenges.....	140
8.3 Advances in data analysis required for breath sensor technologies	141
8.3.1 Data preprocessing	142
8.3.2 Unsupervised learning methodology	143
8.3.3 Supervised data analysis	144
References	147

CHAPTER 9 Breath Analysis in Critically Ill Patients— Potential and Limitations 155

Jochen K. Schubert and Wolfram Miekisch

9.1	Introduction.....	155
9.2	Technical aspects of breath analysis in critically ill patients	156
9.2.1	Sampling.....	156
9.2.2	Pre-concentration and desorption	157
9.2.3	Analysis and detection	159
9.2.4	Technical development—trends in breath analysis.....	160
9.3	Methodological aspects	161
9.3.1	Normalization; expression of data	161
9.3.2	Inspired concentrations.....	161
9.3.3	Data interpretation	162
9.3.4	Physiological meaning, biochemical pathways	164
9.3.5	Study design.....	164
9.4	Clinical aspects of breath analysis in critically ill patients.....	164
9.4.1	Oxidative stress; inflammation	164
9.4.2	Lung diseases.....	166
9.4.3	Metabolism	167
9.4.4	Organ dysfunction.....	168
9.4.5	Transplantation	168
9.4.6	Drug monitoring	169
9.5	Conclusions.....	169
	References.....	170

CHAPTER 10 Analysis of Cancer Biomarkers in Exhaled Breath and Comparison with Sensory Indications by Dogs 177

*Bogusław Buszewski, Tomasz Ligor, Joanna Rudnicka,
Tadeusz Jezierski, Marta Walczak, and Anna Wenda-Piesik*

10.1	Introduction.....	177
10.2	Experimental	179
10.2.1	GC-TOF/MS experiments.....	179
10.2.2	Breath collection for GC/MS analysis	179
10.2.3	Calibration.....	180
10.2.4	Experiments with dogs.....	180
10.2.5	Odor samples	180
10.2.6	Training procedure	181

10.2.7 Breath samples	182
10.2.8 Statistical evaluation	182
10.3 Results and discussion.....	182
10.3.1 Calibration and methods	182
10.3.2 Exhaled air	183
10.3.3 Data from the experiments with dogs	186
10.3.4 Correlation between dog indications and chemical analyses.....	188
10.4 Conclusions	189
References.....	190

PART D NITRIC OXIDE, NO, AND CARBON MONOXIDE, CO

CHAPTER 11 Added Value with Extended NO Analysis 195

Marieann Högman and Pekka Meriläinen

11.1 Background	195
11.2 A two compartment model.....	197
11.3 Different NO models.....	199
11.3.1 The Tsoukias and George two compartment model (2CM).....	199
11.3.2 The Silkoff technique (ST).....	199
11.3.3 The Pietropaoli technique (PT).....	200
11.3.4 The Högman and Meriläinen algorithm (HMA).....	200
11.4 Corrections for axial back diffusion.....	202
11.5 Limitations	204
11.6 Values from non-smoking healthy subjects.....	205
11.7 The usefulness of extended NO analysis	205
11.8 Conclusions	206
References	206

CHAPTER 12 Carbon Monoxide as an Exhaled Biomarker of Pulmonary Diseases 211

Stefan W. Ryter

12.1 Introduction.....	211
12.2 Chemical and biochemical properties of CO	211
12.3 Environmental sources of CO	212
12.4 Endogenous sources of CO: the heme oxygenase enzyme system	213
12.5 Signaling properties of CO.....	214

12.6	Cyto- and tissue-protective effects of CO	215
12.7	Methods for breath CO detection	215
12.8	Exhaled CO in human diseases	216
12.8.1	Asthma	216
12.8.2	Chronic obstructive pulmonary disease, COPD	217
12.8.3	Cystic fibrosis, CF	220
12.8.4	Infectious disease	220
12.8.5	Lung transplantation	220
12.8.6	Critical care medicine	221
12.9	Conclusions	221
	References	222

CHAPTER 13 Exhaled Nitric Oxide in Clinical Practice: Recent Advances and New Challenges 231

Ilidkó Horváth and Peter J. Barnes

13.1	Introduction	231
13.2	Exhaled nitric oxide	231
13.3	Technical aspects of F _E NO measurement	232
13.4	F _E NO in clinical decision making	234
13.4.1	Asthma diagnosis and monitoring	234
13.4.2	Chronic obstructive pulmonary disease (COPD)	235
13.4.3	Primary ciliary dyskinesia	236
13.4.4	Cystic fibrosis	236
13.4.5	Lung transplantation	236
13.4.6	Chronic cough	236
13.5	Conclusions and directions for future areas of research	236
	References	237

PART E CLINICAL BREATH TESTS

CHAPTER 14 An Update on ¹³C-Breath Tests: The Transition to Acceptability into Clinical Practice 245

Anil S. Modak

14.1	Introduction	245
14.2	History of ¹³ C breath tests	246

18.6	Gastro-intestinal illness	353
18.7	Bladder cancer	353
18.8	Concluding remarks	354
	References	355

PART G EXHALED BREATH CONDENSATE (EBC) AND PARTICULATES

CHAPTER 19 Measurement of Biomarkers of Oxidative Stress and Airway Inflammation in Exhaled Breath Condensate: Methodology and Potential Applications in Patients with COPD and Healthy Smokers..... 361

Paolo Montuschi

19.1	Introduction	361
19.2	EBC analysis: methodology	362
	19.2.1 Experimental setup.....	362
	19.2.2 Analytical techniques	364
	19.2.3 Methodological aspects of EBC analysis.....	364
19.3	Analysis of EBC from patients with COPD and healthy smokers	370
	19.3.1 Eicosanoids	370
	19.3.2 Hydrogen peroxide.....	371
	19.3.3 NO-derived products.....	372
	19.3.4 pH.....	373
	19.3.5 Aldehydes.....	373
	19.3.6 Adenosine triphosphate.....	373
	19.3.7 Other markers.....	374
19.4	Advantages and limitations of the EBC technique.....	374
19.5	Future research	375
	References	376

CHAPTER 20 Particles in Exhaled Air—A Novel Method of Sampling Non-Volatiles in Exhaled Air 383

Anna-Carin Olin

20.1	Introduction	383
20.2	Number of particles in exhaled breath	383
20.3	Formation and origin of exhaled particles.....	385

20.4	Composition of exhaled particles.....	387
20.4.1	Phospholipids in asthma	387
20.4.2	Proteins	388
20.5	Sampling of exhaled particles in occupational medicine.....	390
20.6	Conclusions.....	390
	References	391

PART H VOLATILES OF MICROBIAL ORIGIN: URINE, STOOL AND *IN VITRO* CULTURES

CHAPTER 21 Challenges in the Investigation of Volatile Disease Biomarkers in Urine..... 395

Jae Kwak and George Preti

21.1	Introduction.....	395
21.2	Distinctive odors and/or volatile profiles associated with diseases are present in urine and detected by animals and analytical instruments	396
21.3	Challenges in monitoring volatile disease biomarkers in urine	397
21.3.1	Intrinsic and extrinsic factors.....	397
21.3.2	Analytical aspects.....	399
21.4	Discussion	400
	References	401

CHAPTER 22 Volatile Organic Compounds (VOCs) Found in Urine and Stool 405

Ben de Lacy Costello and Norman M. Ratcliffe

22.1	Introduction.....	405
22.2	Urine VOCs and disease.....	405
22.2.1	Alcoholic hepatitis	437
22.2.2	Alcohols, short-chain fatty acids.....	437
22.2.3	Ammonia, amines, trimethylaminuria.....	438
22.2.4	Cystinuria.....	439
22.2.5	Diabetes.....	439
22.2.6	Diagnosis of gastro-intestinal conditions via the analysis of VOCs from urine.....	439

22.2.7 Isovaleric acidemia (isovaleric acid CoA dehydrogenase deficiency).....	440
22.2.8 Maple syrup urine disease (MSUD)	440
22.2.9 Methionine malabsorption syndrome (Oast-house syndrome also called Smith-Strang disease).....	440
22.2.10 Ovulation and volatiles	440
22.2.11 Phenylketonuria	441
22.2.12 Pre-renal azotemia and volatiles	441
22.2.13 Tuberculosis (TB)	441
22.2.14 Putative volatile biomarker(s) for urinary tract cancers	442
22.3 Bacteria present in human urine, current test methods, and VOC analyses	443
22.3.1 Electronic nose technology for VOC detection of bacterial metabolites in urine	445
22.4 Stool VOCs and disease	446
22.4.1 Volatiles from stool	447
22.4.2 Metabolism of acid in stool.....	454
22.4.3 Specific changes in VOCs in gastrointestinal disease	454
22.5 A comparison of VOCs found in urine and stool	455
22.6 Summary	456
References	456

CHAPTER 23 Volatile Organic Compounds (VOCs) Released by Pathogenic Microorganisms *in vitro*: Potential Breath Biomarkers for Early-Stage Diagnosis of Disease 463

Wojciech Filipiak, Andreas Sponring, Anna Filipiak, Maria Baur, Clemens Ager, Helmut Wiesenhofer, Rosa Margesin, Markus Nagl, Jakob Troppmair, and Anton Amann

23.1 Introduction.....	463
23.2 Methodology	467
23.2.1 Bacterial and fungal cultures	467
23.2.2 Cultivation of microorganisms in a custom-made system for headspace sampling.....	468
23.2.3 Preconcentration of VOCs on multibed tubes.....	469
23.2.4 Thermal desorption	469

23.2.5 Gas chromatography mass spectrometry (GC-MS) analysis	469
23.2.6 Substance identification	474
23.2.7 Preparation of standard mixtures	474
23.2.8 Statistics and determination of detection limits	475
23.3 Results	475
23.3.1 Limits of detection	476
23.3.2 Linearity and precision of the sampling method.....	476
23.3.3 Reduction of the matrix effect	480
23.3.4 Proliferation of microorganisms	482
23.3.5 Volatile metabolites released by microorganisms	482
23.4 Discussion	494
23.5 Summary	507
References	508

PART I URBAN SEARCH AND RESCUE OPERATIONS

CHAPTER 24 Potential Applications of Volatile Organic Compounds in Safety and Security..... 515

*Agapios Agapiou, Pawel Mochalski, Alex Schmid,
and Anton Amann*

24.1 Introduction	515
24.2 Chemical analysis of breath	516
24.3 Analytical instrumentation; field technology	517
24.4 Factors affecting VOCs	519
24.5 Volatiles in safety and security applications	520
24.5.1 Safety (exposure); attack exposure	521
24.5.2 Safety (exposure); attack scenarios.....	521
24.5.3 Security (natural disasters); human life signals	523
24.6 Urine as a potential source of markers of human presence	526
24.6.1 Background	526
24.6.2 Volatiles spontaneously emitted by human urine.....	526
24.6.3 Evolution of urine scent profile during sample storage at room temperature.....	527
24.6.4 Preliminary study on interactions of urine-borne species with common debris materials	530

24.7	Evaluation of IMS-based portable technologies for the detection of urine-borne human scent constituents	538
24.7.1	Background	538
24.7.2	Application of the MCC-IMS for the detection and quantification of urine-borne volatiles	538
24.7.3	Application of the VAMPPI multi-sensor detector for the detection of selected volatile constituents of human scent	543
24.8	Spatio-temporal measurements of VOCs	546
24.9	Real-time measurement of exhaled breath and skin emanations	547
24.10	A hit list of compounds for urban search and rescue operations	551
24.11	Summary	551
	References	553
Index		559