Challenges in clinical validation studies

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JAMA Oncology | Original Investigation

Accuracy and Methodologic Challenges of Volatile Organic Compound–Based Exhaled Breath Tests for Cancer Diagnosis

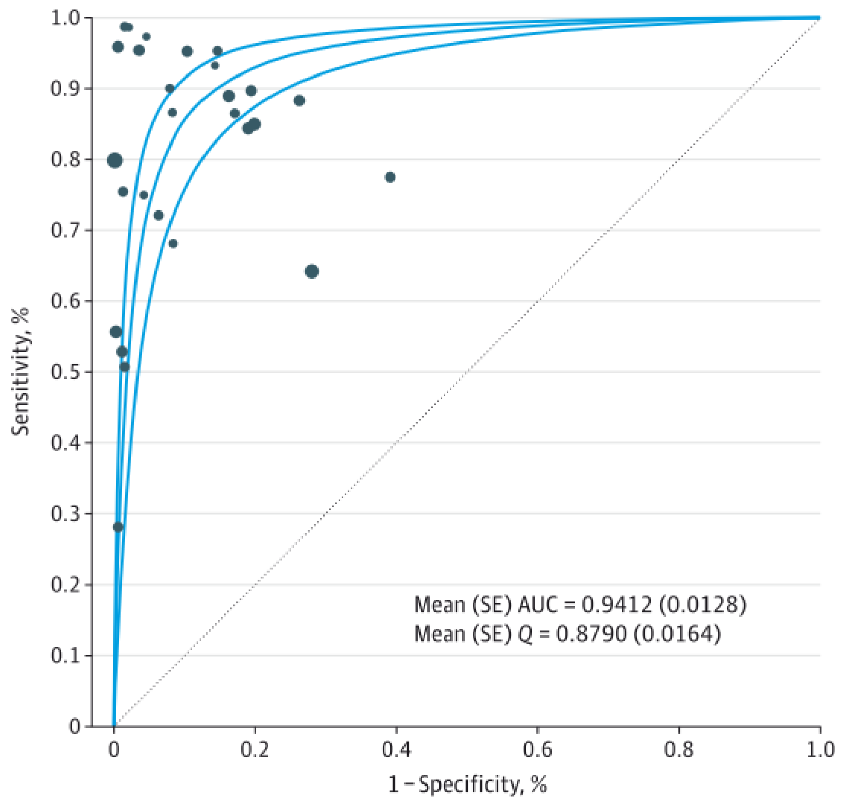
A Systematic Review and Pooled Analysis

George B. Hanna, FRCS, PhD; Piers R. Boshier, MRCS, PhD; Sheraz R. Markar, MRCS, PhD; Andrea Romano, PhD

574 abstracts screened

87 Relevant Articles

Patients related factors	**55**
Sampling Methods	**29**
Environmental Considerations	**24**
Instrument variability	**24**



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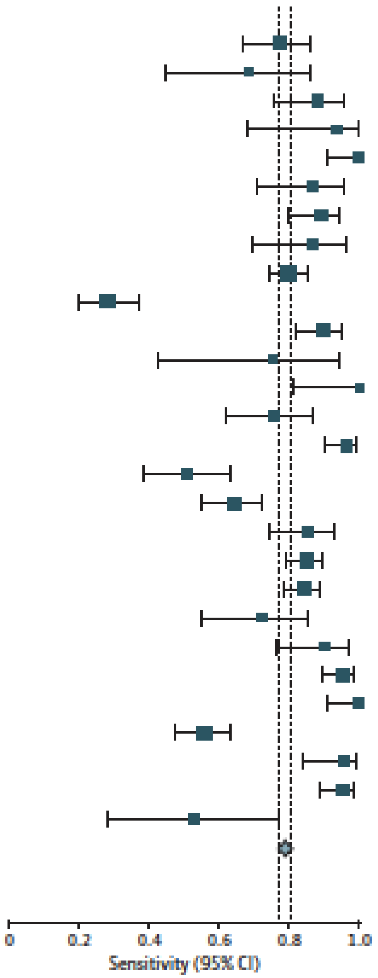
Pooled Sensitivity 79% (0.77 – 0.81)

Figure 2. Forest Plot of Pooled Sensitivity Analysis

Source	Type of Cancer	Sensitivity (95% CI)
Barash et al,[14] 2015	Breast	0.78 (0.67-0.86)
Li et al,[15] 2014	Breast	0.68 (0.45-0.86)
Phillips et al,[18] 2003	Breast	0.88 (0.76-0.96)
Phillips et al,[19] 2006	Breast	0.93 (0.68-1.00)
Guo et al,[22] 2015	Thyroid	1.00 (0.91-1.00)
Altomare et al,[27] 2013	Colorectal	0.86 (0.71-0.95)
Kumar et al,[32] 2015	Gastroesophageal	0.89 (0.80-0.95)
Qin et al,[36] 2010	Liver	0.87 (0.69-0.96)
Bajtarevic et al,[38] 2009	Lung	0.80 (0.74-0.85)
Bousamra et al,[39] 2014	Lung	0.28 (0.20-0.38)
Fu et al,[48] 2014	Lung	0.90 (0.82-0.95)
Fuchs et al,[49] 2010	Lung	0.75 (0.43-0.95)
Gaspar et al,[50] 2009	Lung	1.00 (0.81-1.00)
Handa et al,[51] 2014	Lung	0.76 (0.62-0.87)
Li et al,[53] 2015	Lung	0.96 (0.90-0.99)
Ligor et al,[54] 2009	Lung	0.51 (0.38-0.63)
Ligor et al,[55] 2015	Lung	0.64 (0.55-0.73)
Phillips et al,[60] 2003	Lung	0.85 (0.74-0.93)
Phillips et al,[61] 2007	Lung	0.85 (0.79-0.90)
Phillips et al,[62] 2008	Lung	0.84 (0.79-0.89)
Poli et al,[63] 2005	Lung	0.72 (0.55-0.86)
Poli et al,[64] 2010	Lung	0.90 (0.76-0.97)
Sakamura et al,[66] 2017	Lung	0.95 (0.89-0.98)
Schallschmidt et al,[67] 2016	Lung	1.00 (0.91-1.00)
Schumer et al,[68] 2015	Lung	0.56 (0.48-0.64)
Song et al,[71] 2010	Lung	0.95 (0.84-0.99)
Wang et al,[73] 2012	Lung	0.95 (0.89-0.99)
Wehinger et al,[74] 2007	Lung	0.53 (0.28-0.77)
Pooled Sensitivity		0.79 (0.77-0.81)

χ^2_{27} = 371.07; P < .001

Inconsistency (I^2) = 92.7%



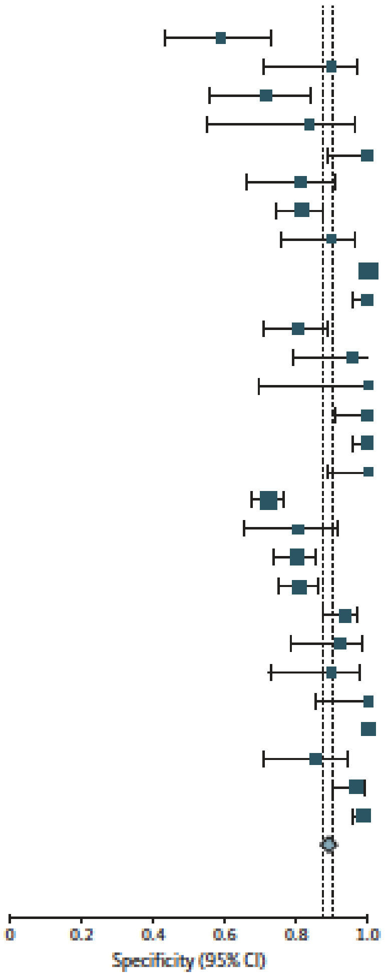
Pooled Specificity 89% (0.88 – 0.90)

Figure 3. Forest Plot of Pooled Specificity Analysis

Source	Type of Cancer	Specificity (95% CI)
Barash et al,[14] 2015	Breast	0.61 (0.45-0.75)
Li et al,[15] 2014	Breast	0.92 (0.73-0.99)
Phillips et al,[18] 2003	Breast	0.74 (0.58-0.86)
Phillips et al,[19] 2006	Breast	0.86 (0.57-0.98)
Guo et al,[22] 2015	Thyroid	1.00 (0.89-1.00)
Altomare et al,[27] 2013	Colorectal	0.83 (0.68-0.93)
Kumar et al,[32] 2015	Gastroesophageal	0.84 (0.76-0.90)
Qin et al,[36] 2010	Liver	0.92 (0.78-0.98)
Bajtarevic et al,[38] 2009	Liver	1.00 (0.99-1.00)
Bousamra et al,[39] 2014	Liver	1.00 (0.96-1.00)
Fu et al,[48] 2014	Liver	0.81 (0.71-0.88)
Fuchs et al,[49] 2010	Liver	0.96 (0.79-1.00)
Gaspar et al,[50] 2009	Liver	1.00 (0.69-1.00)
Handa et al,[51] 2014	Liver	1.00 (0.91-1.00)
Li et al,[53] 2015	Liver	1.00 (0.96-1.00)
Ligor et al,[54] 2009	Liver	1.00 (0.89-1.00)
Ligor et al,[55] 2015	Liver	0.72 (0.67-0.77)
Phillips et al,[60] 2003	Liver	0.80 (0.65-0.91)
Phillips et al,[61] 2007	Lung	0.80 (0.74-0.85)
Phillips et al,[62] 2008	Lung	0.81 (0.75-0.86)
Poli et al,[63] 2005	Lung	0.94 (0.87-0.97)
Poli et al,[64] 2010	Lung	0.92 (0.79-0.98)
Sakamura et al,[66] 2017	Lung	0.90 (0.73-0.98)
Schallschmidt et al,[67] 2016	Lung	1.00 (0.85-1.00)
Schumer et al,[68] 2015	Lung	1.00 (0.98-1.00)
Song et al,[71] 2010	Lung	0.85 (0.71-0.94)
Wang et al,[73] 2012	Lung	0.96 (0.90-0.99)
Wehinger et al,[74] 2007	Lung	0.99 (0.96-1.00)
Pooled Sensitivity		0.89 (0.88-0.90)

χ^2_{27} = 406.66; P < .001

Inconsistency (I^2) = 93.4%



Lack of external validation and crucial factors consideration in clinical trials

Patients related factors

Clinical confounding factors

Patient Demographics

Medical Conditions

Medications

Smoking

Patients physiologic conditions

Diet

Oral Hygiene

Exercise & rest time

Patients related factors

Smoking

Volatile Organic Compounds in Exhaled Breath in a Healthy Population: Effect of Tobacco Smoking

José Javier Jareño-Esteban[a,??], M. Ángeles Muñoz-Lucas[b], Belén Carrillo-Aranda[c], José Ángel Maldonado-Sanz[d], Ignacio de Granda-Orive[e], Antonio Aguilar-Ros[f], Concepción Civera-Tejuca[g], Carlos Gutiérrez-Ortega[b], Luis Miguel Callol-Sánchez[h], on behalf of the Study Group [◊]

Chromatographic analysis of VOC patterns in exhaled breath from smokers and nonsmokers

Simonetta Capone[1] | Maria Tufariello[2] | Angiola Forleo[1] | Valentina Longo[1] | Lucia Giampetruzzi[1] | Antonio Vincenzo Radogna[1] | Flavio Casino[1] | Pietro Siciliano[1]

A rapid method for the chromatographic analysis of volatile organic compounds in exhaled breath of tobacco cigarette and electronic cigarette smokers[☆]

Esther Marco, Joan O. Grimalt*

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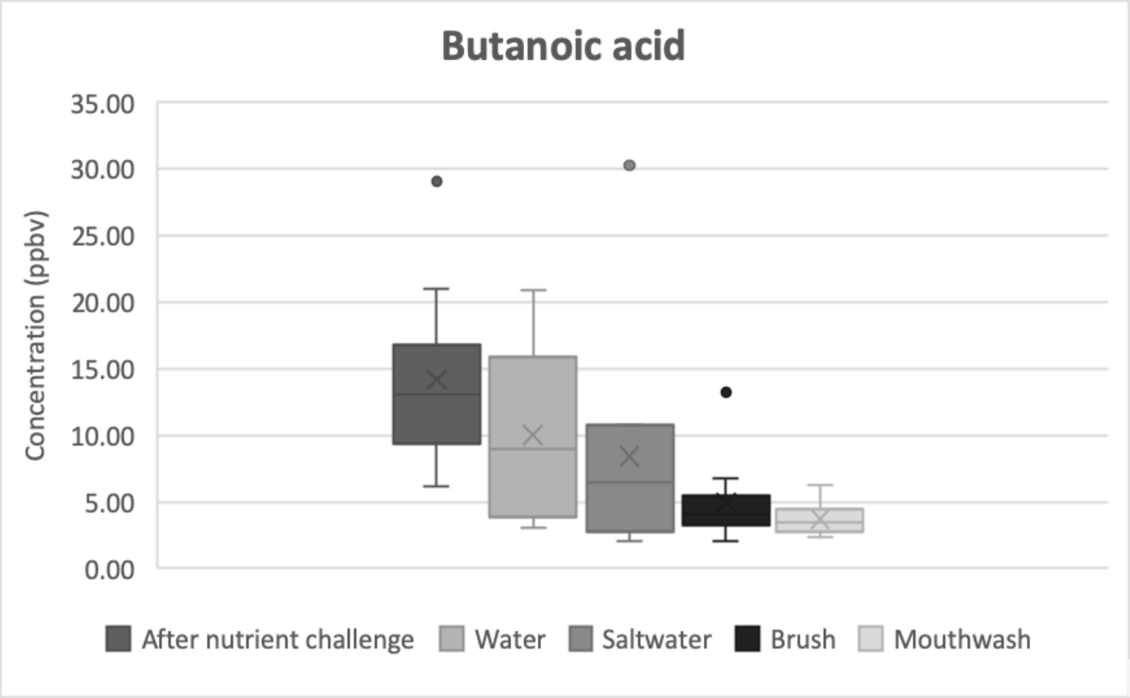
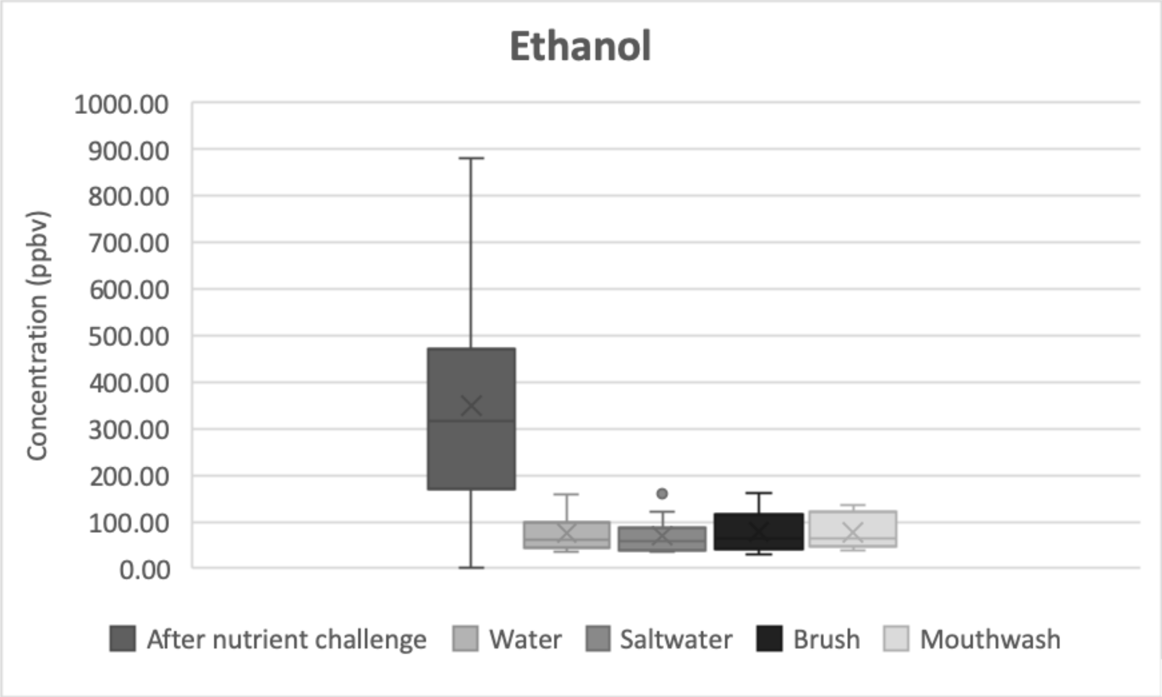
Patients related factors

Oral cleaning





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Environment

Air VOC measurement in laboratory and clinical environment

IOP PUBLISHING JOURNAL OF BREATH RESEARCH
J. Breath Res. 4 (2010) 031001 (6pp) doi:10.1088/1752-7155/4/3/031001

COMMUNICATION

Variation in the levels of volatile trace gases within three hospital environments: implications for clinical breath testing

P R Boshier[1], J R Cushnir[1], O H Priest[1], N Marczin[2] and G B Hanna[1,3]

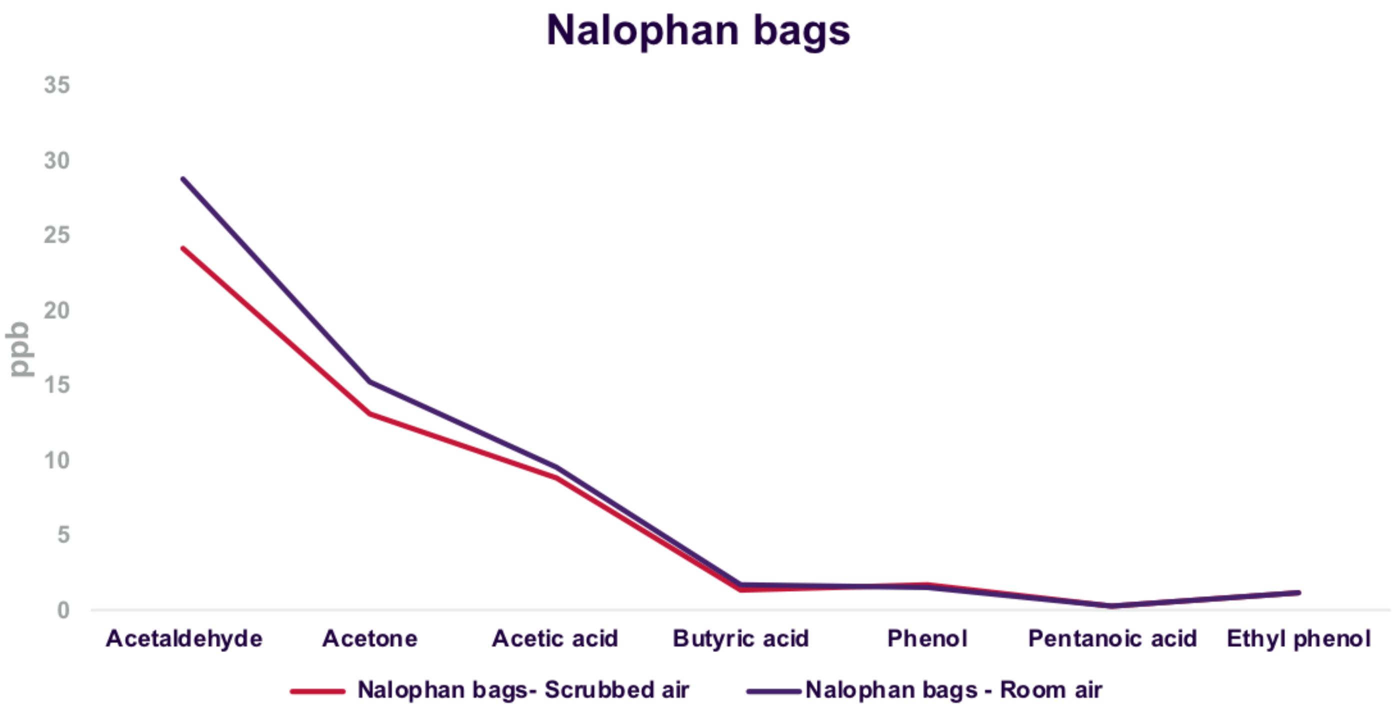
	Hospital ward ($n = 17$)[a]		Outpatient clinic ($n = 17$)		Operating theatre ($n = 17$)		
	Median[b]	IQR[c]	Median	IQR	Median	IQR	P value[d]
Acetone	17	[12–20]	11	[10–13]	5	[4–6]	<0.01
Ethanol	2547	[2149–2931]	424	[210–549]	89	[55–272]	<0.01
Propanol	158	[91–228]	236	[112–302]	3	[2–3]	<0.01
Hydrogen cyanide	1	[1–2]	1	[1–2]	1	[<1–2]	0.61
Isoprene	<2	[<2–2]	<2	[<2–4]	<2	[<2–2]	0.20
Acetic acid	44	[30–60]	44	[31–47]	21	[15–52]	0.11
Ammonia	77	[62–83]	68	[66–82]	57	[52–63]	<0.01

Need to evaluate target VOCs in the air in **clinical environments** where patients are sampled and **laboratory** where samples are analysed

Breath collection

Bags

Bags



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Breath collection

Devices

Journal of Breath Research

PAPER

Optimisation of sampling parameters for standardised exhaled breath sampling

Sophie L F Doran, Andrea Romano and George B Hanna
Department of Surgery and Cancer, Imperial College, London, United Kingdom

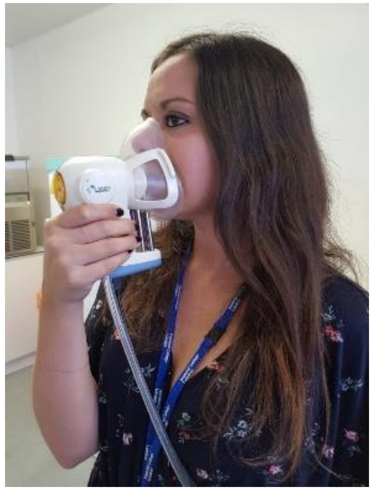


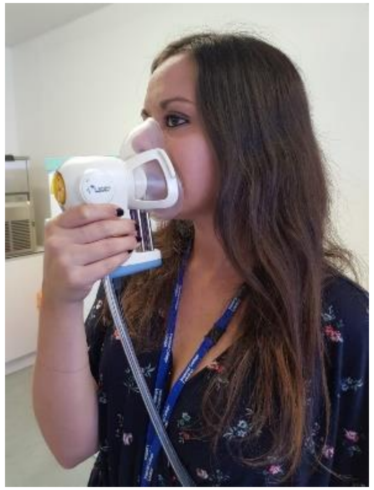
Table 4. VOCs elevated in exhaled breath collection system (ReCIVA and CASPER) compared to room air samples.

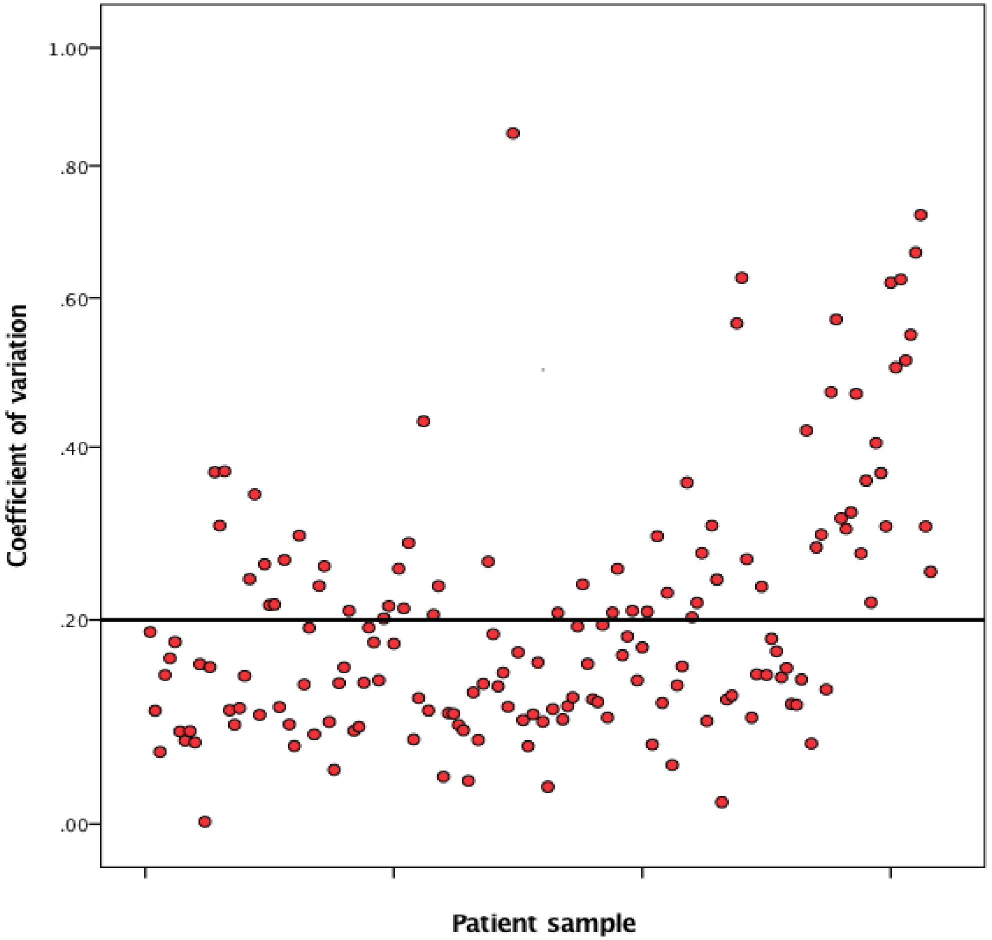
	Room air sample, peak area[a]		Mask sample, peak area[a]		
VOC	Median	IQR	Median	IQR	Mann–Whitney *U*-test
Cyclopentane	12 051	11 153–13 941	138 112	80 145–367 963	<0.001[b]
Disiloxane, hexamethyl-	10 420	9022–11 983	19 556 638	880 170–25 499 373	<0.001[b]
2-Oxa-1,3-disilacyclohexane, 1,1,3,3-tetramethyl-	14 338	12 755–16 536	68 011	24 101–1201 545	<0.001[b]
1,3-bis[(2Z)-Hex-2-en-1-yloxy]-1,1,3,3-tetramethyldisiloxane	20 755	17 645–29 569	343 046	165 585–1069 703	<0.001[b]
Trisiloxane, octamethyl-	13 430	10 753–17 306	1752 287	815 687–6127 763	<0.001[b]
Heptane, 2,2,4,6,6-pentamethyl-	17 660	13 278–25 093	367 240	166 605–677 080	<0.001[b]
Cyclotrisiloxane, hexamethyl-	20 804	18 057–25 539	53 059	41 728–116 568	<0.001[b]
2-Propenamide	15 866	12 790–17 465	39 700	27 199–52 062	<0.001[b]

Note. Mask sample: TD tube sample collected from ReCIVA and CASPER attached to the glass head.
[a] Mass area units IQR (inter-quartile range).
[b] Denotes statistical significance at <0.05 level.

Breath collection

Devices





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Breath collection

Standardisation of sampling parameters

Journal of Breath Research

PAPER

Optimisation of sampling parameters for standardised exhaled breath sampling

Sophie L F Doran , Andrea Romano and George B Hanna
Department of Surgery and Cancer, Imperial College, London, United Kingdom

- Breath **fraction** sampled
- **Route** for breath collection
- Sampling factors (**volume and flow rate**)
- **Stability** of target VOC and samples **storage**
- **Human factor** of collection devices

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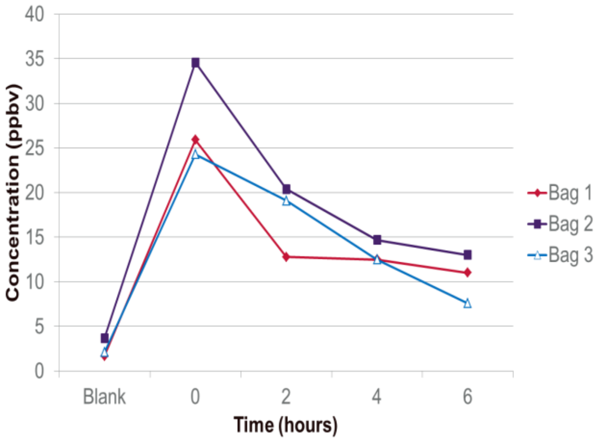
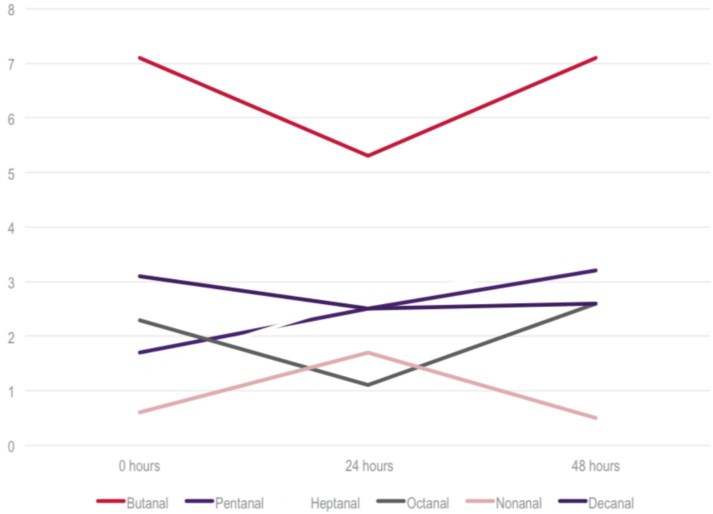
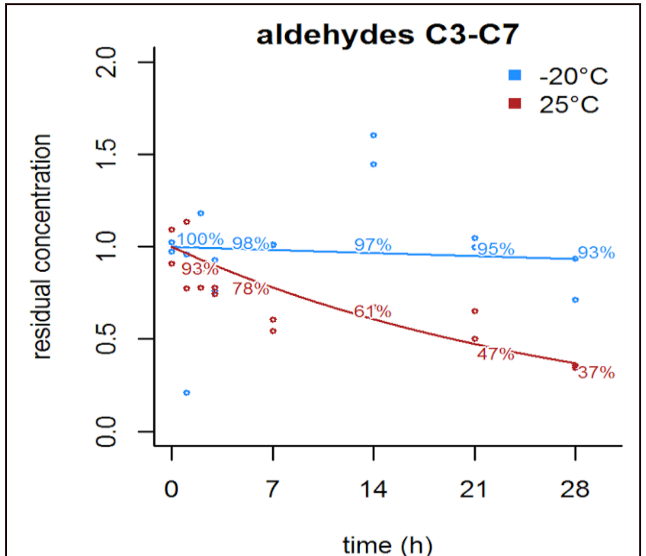
Breath collection

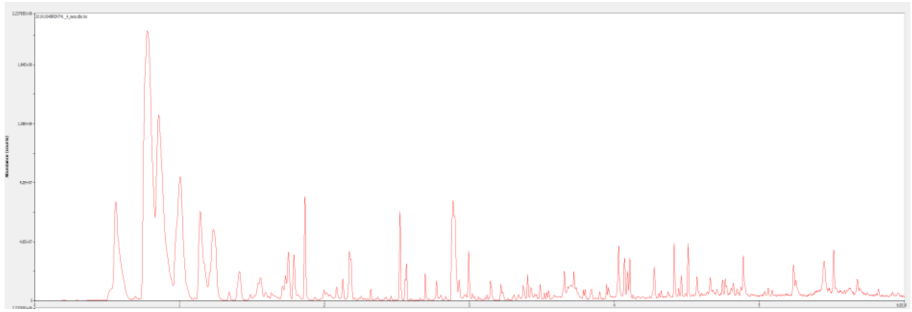


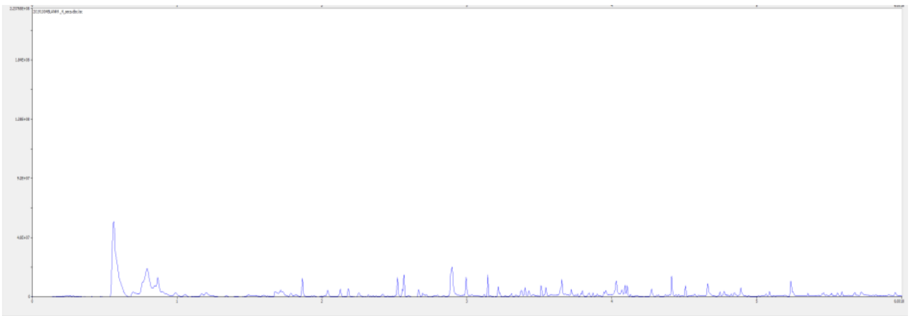


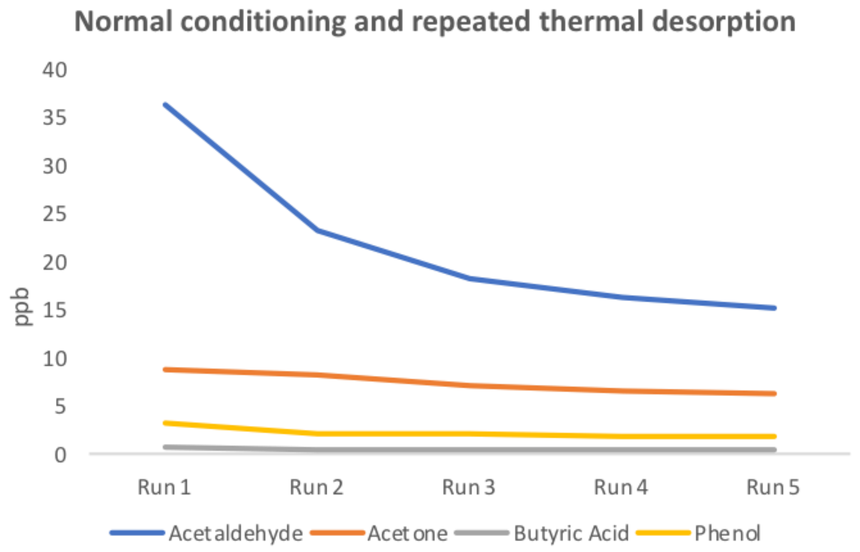


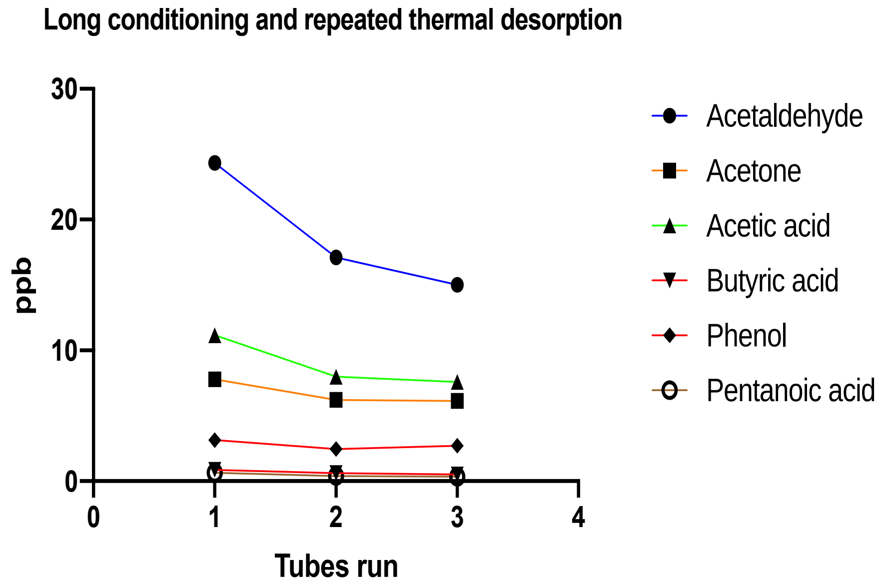
Breath collection

TD tubes



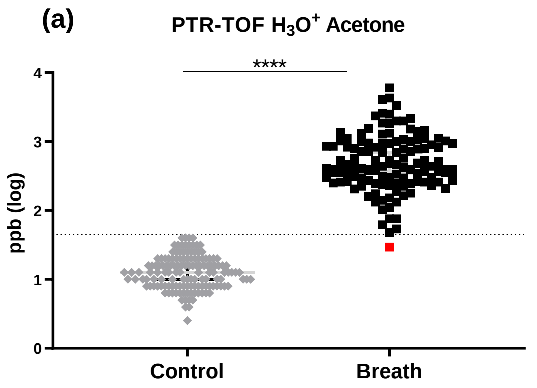


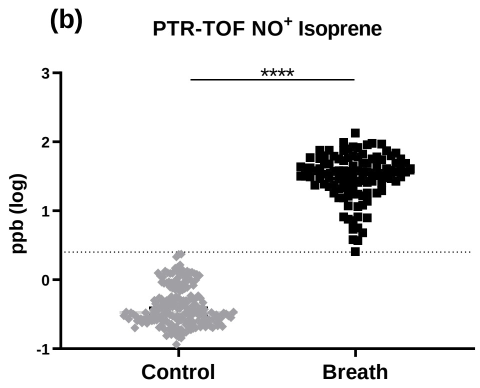


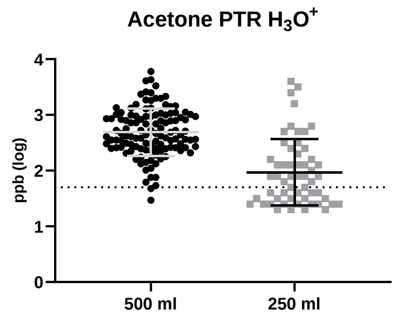


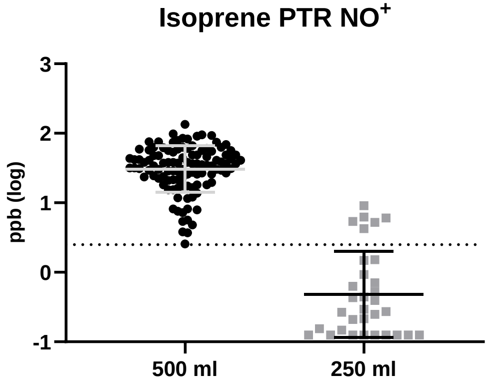
Quality control system

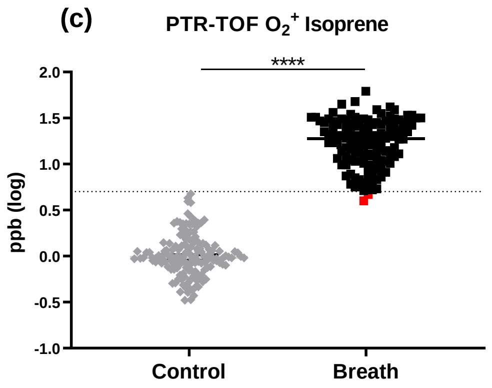
Presence of breath

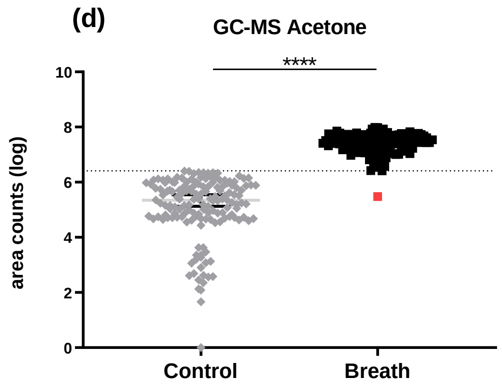


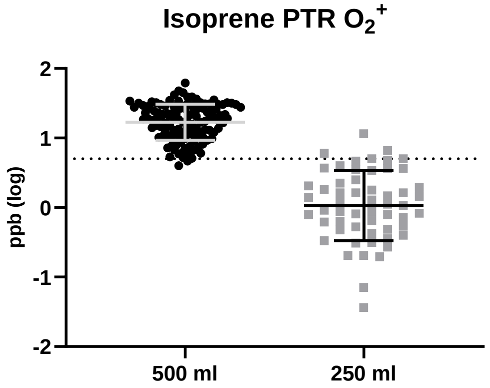


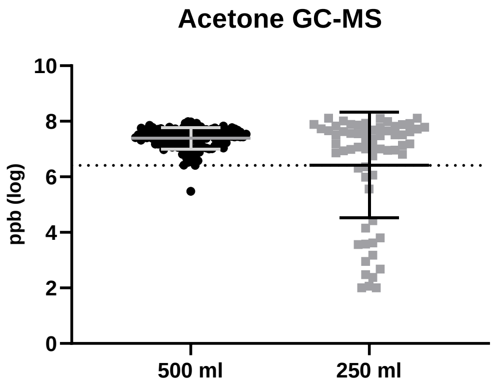












Quality control using original biological matrix

Gas standards can be used as quality control but breath is a difficult matrix to replicate (composition, humidity..)

There is no pooled breath samples to analyse periodically as quality controls (gold standard in metabolomics)

Batch effect

Biomarker discovery

Direct Sampling

PTR-ToF-MS

SIFT-MS

Chromatographic

GC-FoF-MS

GCxGC-ToF-MS

- Limits of detection
- Limits of quantification
- Reproducibility
- Carry over effect

Model validation

- Selection of patients among the target population
- Appropriate control group
- Appropriate sample size
- Patients should have a standard diagnostic test
- Tests performed before therapeutic intervention
- Knowledge about confounding factors in the target population

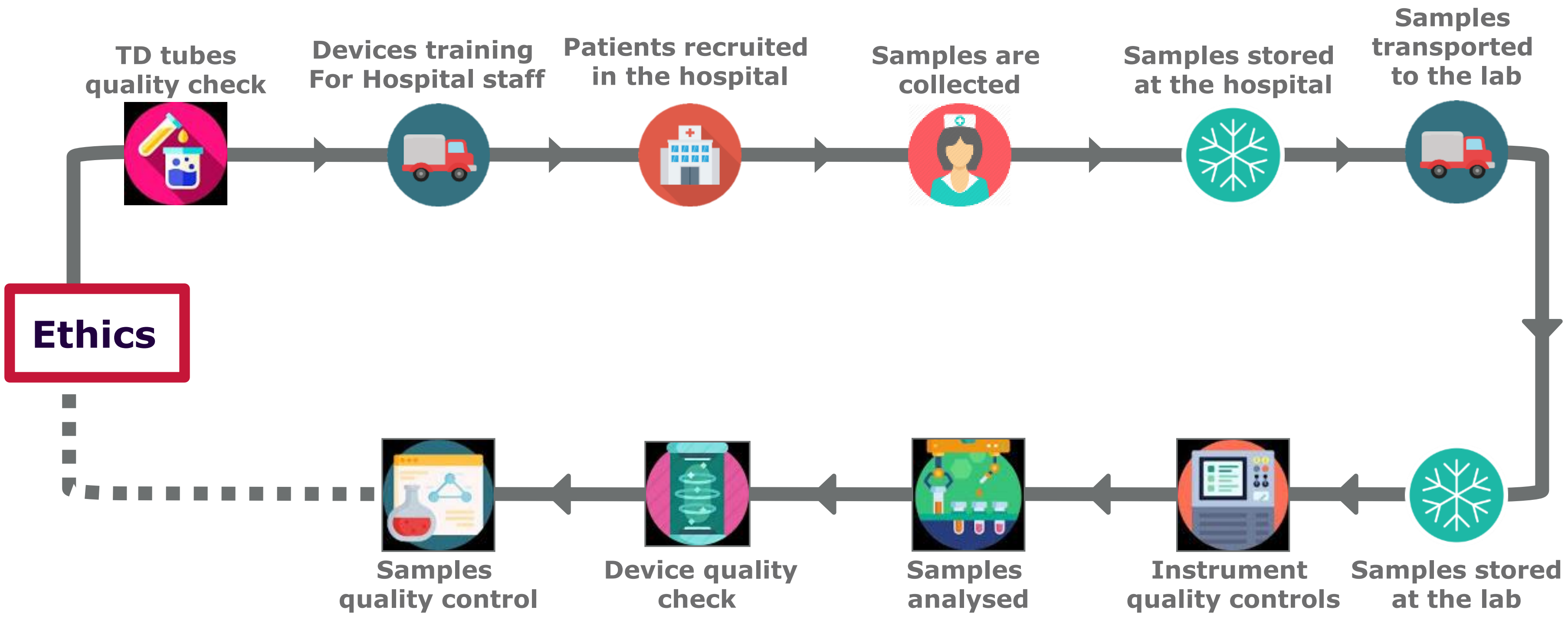
Validation studies

- External validation
- Multiple centres studies
- Multiple laboratory analysis
- Double blind operators (processing, analysis)
- Adaptive design for clinical trials

High throughput system

- Targeted VOC methods for analysis
- Thermal desorption automated system
- Auto-deconvolution of GC-MS data analysis sing molecular networking of volatiles
- Quality management system

Quality control system



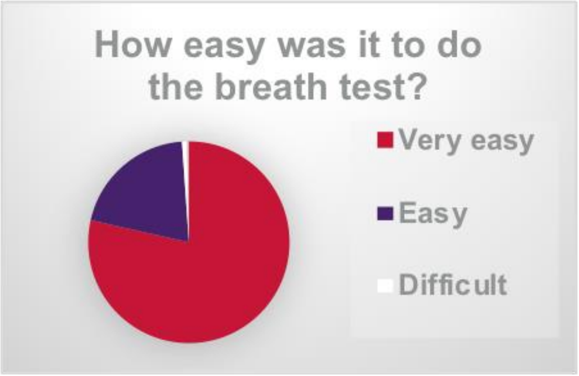
QC for each step of workflow in multi-centre clinical studies

Breath MAGIC

Breath Models for Assessment of GI Cancer in Primary Care

Is it **feasible and acceptable** in Primary Care
to use breath testing in patients with gastrointestinal symptoms?

1002 patients



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analytical chemistry

Selected Ion Flow Tube-MS Analysis of Headspace Vapor from Gastric Content for the Diagnosis of Gastro-Esophageal Cancer

Sacheen Kumar,[†,‡] Juzheng Huang,[†,‡] Julia R. Cushnir,[†] Patrik Španěl,[¶] David Smith,[§] and George B. Hanna*[,†]

Anal. Chem. 2012, 84, 9550−9557

Selected Ion Flow Tube Mass Spectrometry Analysis of Volatile Metabolites in Urine Headspace for the Profiling of Gastro-Esophageal Cancer

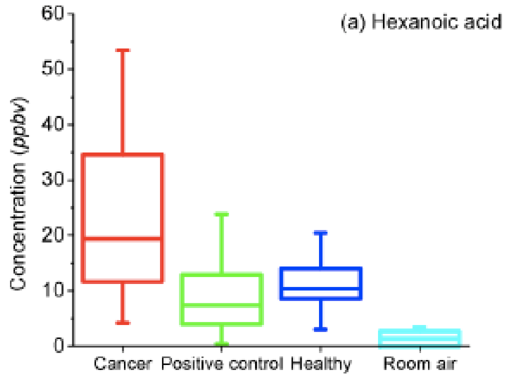
Juzheng Huang,[†,∥] Sacheen Kumar,[†,∥] Nima Abbassi-Ghadi,[†] Patrik Španěl,[‡] David Smith,[§] and George B. Hanna*[,†]

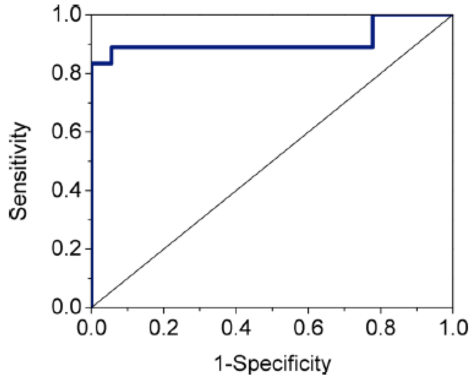
Anal. Chem. 2013, 85, 3409−3416

Selected Ion Flow Tube Mass Spectrometry Analysis of Exhaled Breath for Volatile Organic Compound Profiling of Esophago-Gastric Cancer

Sacheen Kumar,[†,‡] Juzheng Huang,[†,‡] Nima Abbassi-Ghadi,[†] Patrik Španěl,[¶] David Smith,[§] and George B. Hanna*[,†]

Anal. Chem. 2013, 85, 6121−6128





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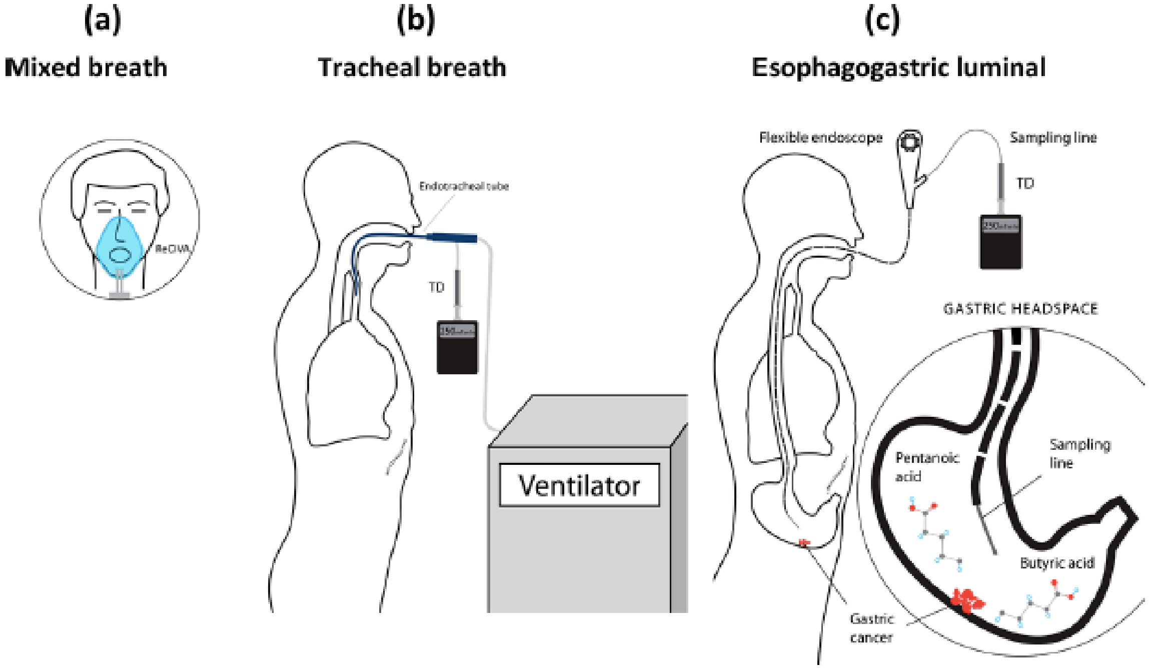
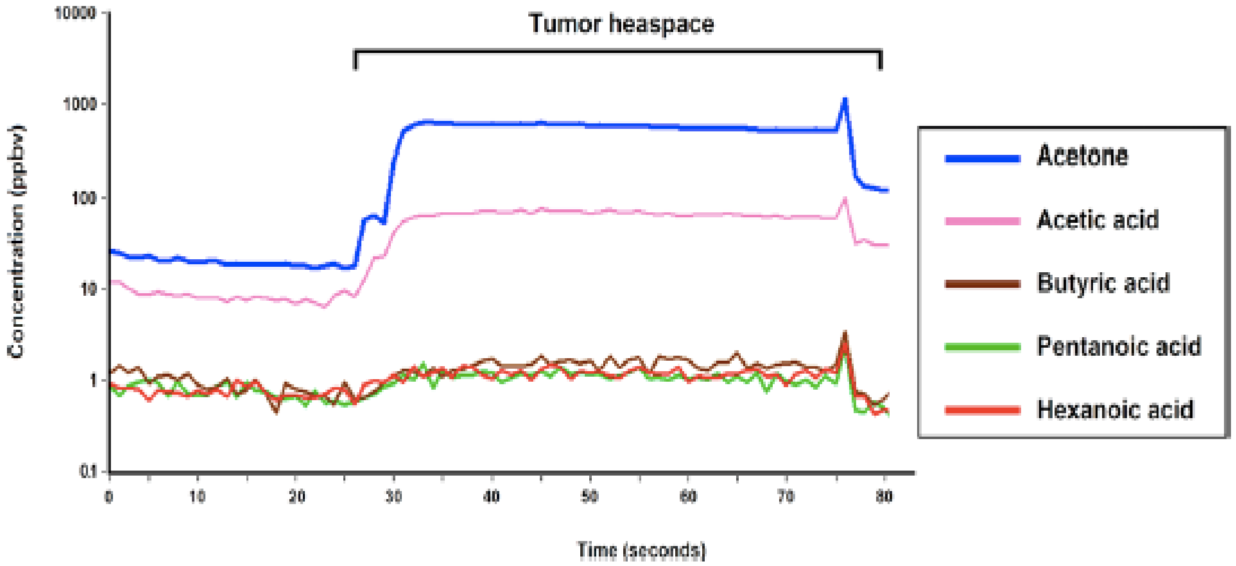
analytical chemistry

Mass-Spectrometry Analysis of Mixed-Breath, Isolated-Bronchial-Breath, and Gastric-Endoluminal-Air Volatile Fatty Acids in Esophagogastric Cancer

Mina E. Adam,[†,§] Matyas Fehervari,[†,§] Piers R. Boshier,[†] Sung-Tong Chin,[†] Geng-Ping Lin,[†] Andrea Romano,[†] Sacheen Kumar,[†,‡] and George B. Hanna*[,†]

Anal. Chem. 2019, 91, 3740–3746



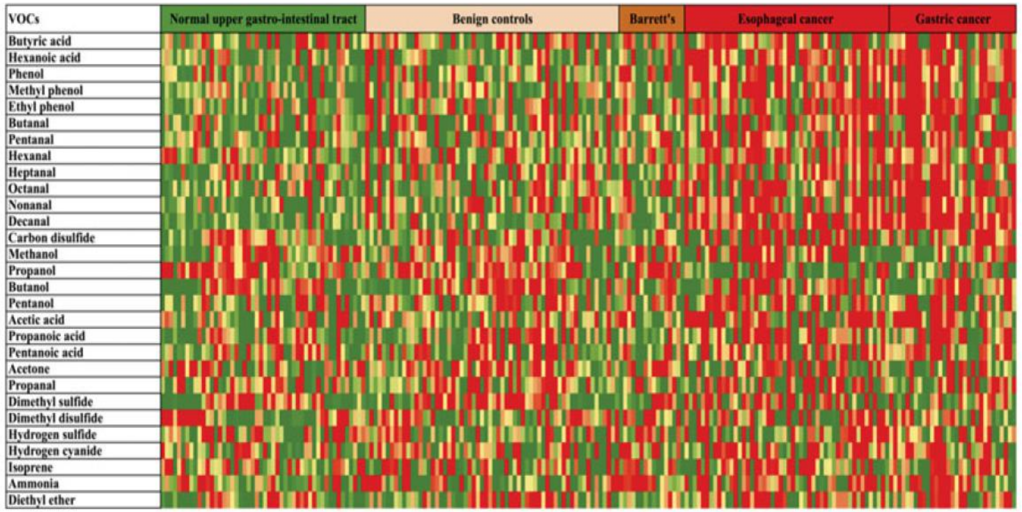


Mass Spectrometric Analysis of Exhaled Breath for the Identification of Volatile Organic Compound Biomarkers in Esophageal and Gastric Adenocarcinoma

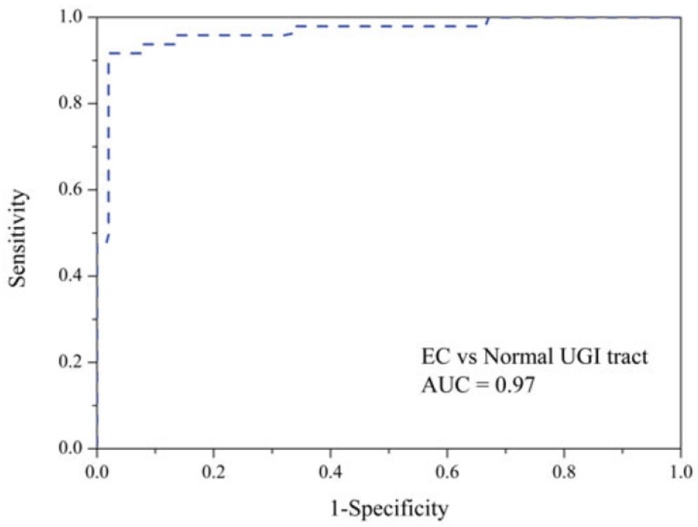
Sacheen Kumar, MRCS, Juzheng Huang, PhD,* Nima Abbassi-Ghadi, MRCS,* Hugh A. Mackenzie, MRCS,* Kirill A. Veselkov, PhD,* Jonathan M. Hoare, PhD, FRCP,† Laurence B. Lovat, PhD, FRCP,‡ Patrik Španěl, PhD,§ David Smith, PhD, FRS,¶ and George B. Hanna, PhD, FRCS**

(*Ann Surg* 2015;00:1–10)

A



B



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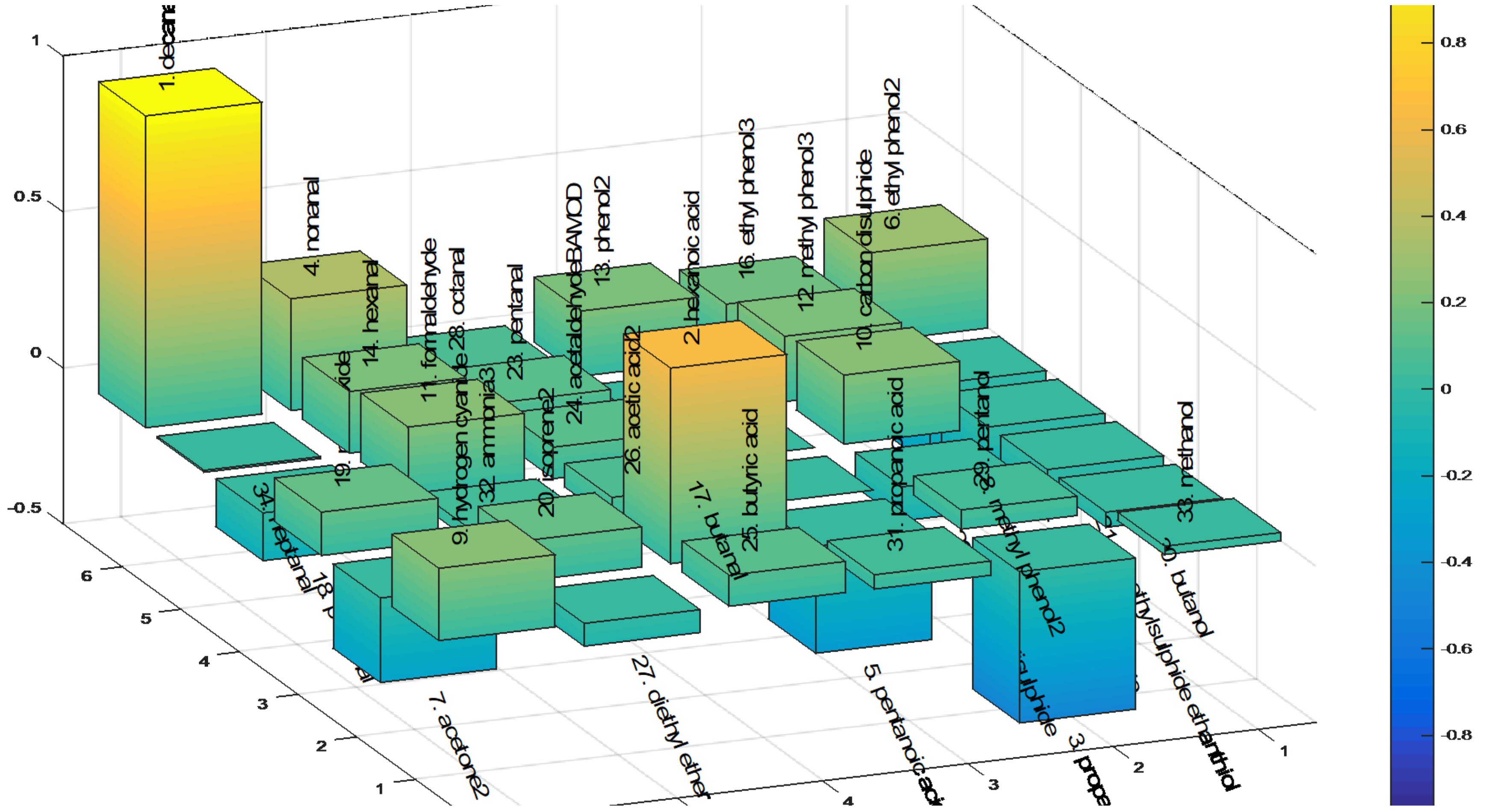
Vector-based analysis



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JAMA Oncology | Original Investigation

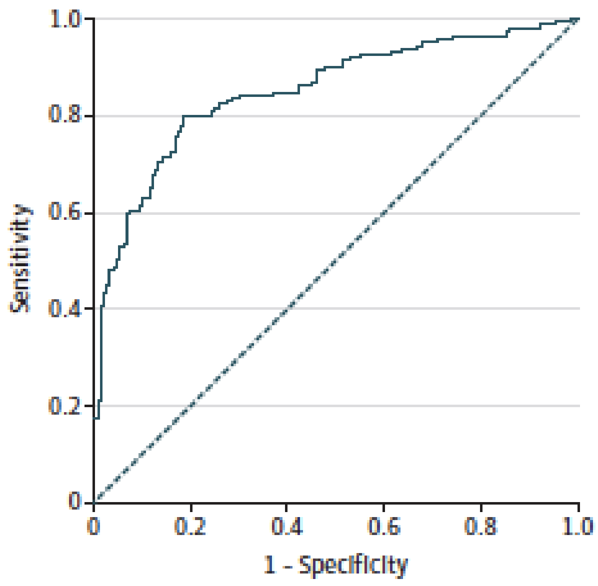
Assessment of a Noninvasive Exhaled Breath Test for the Diagnosis of Oesophagogastric Cancer

Sheraz R. Markar, PhD; Tom Wiggins, PhD; Stefan Antonowicz, PhD; Sung-Tong Chin, PhD; Andrea Romano, PhD; Konstantin Nikolic, PhD; Benjamin Evans, PhD; David Cunningham, PhD; Muntzer Mughal, MD; Jesper Lagergren, PhD; George B. Hanna, PhD

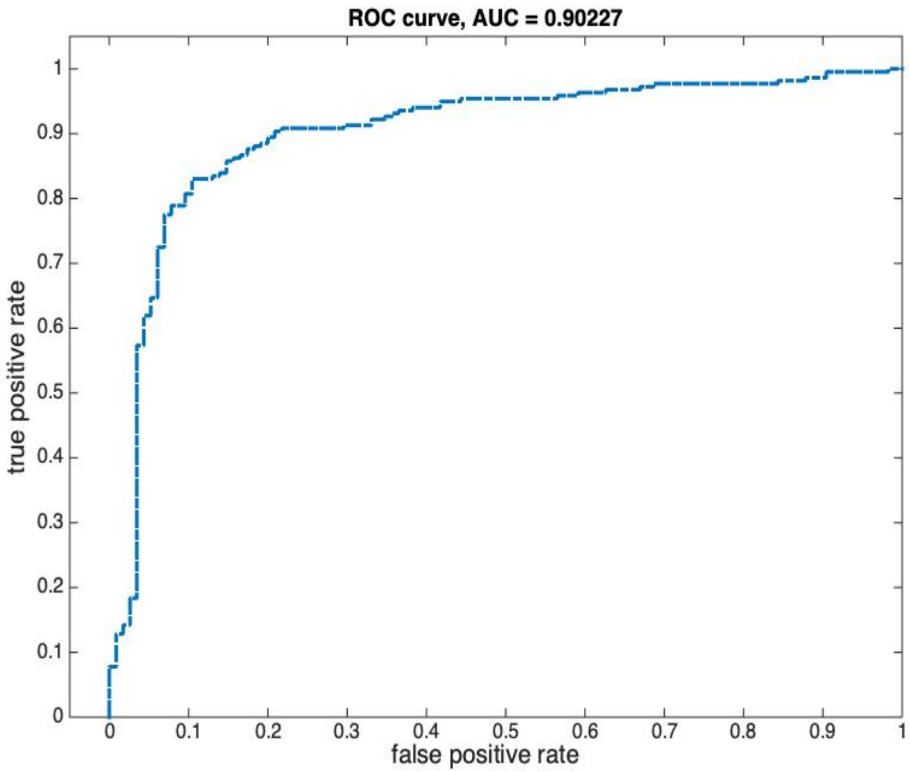
Table 2. Description of Cancer-Specific Factors

Tumor-Related Factor	Patients, No. (%)
Tumor location	
Gastric	72 (44.2)
Gastroesophageal junction	36 (22.1)
Oesophageal	55 (33.7)
Clinical T stage	
1	18 (11.0)
2	32 (19.6)
3	61 (37.4)
4	52 (31.9)
Clinical N stage	
0	57 (35.0)
1	58 (35.6)
2	22 (13.5)
3	26 (16.0)

Figure. ROC Curve for the 5-VOC Breath Model in the Diagnosis of Esophagogastric Cancer in the Multicenter Clinical Trial[a]



Multicentre study
(GC-MS, molecular network analysis)



AUC 0.91

Imperial College London

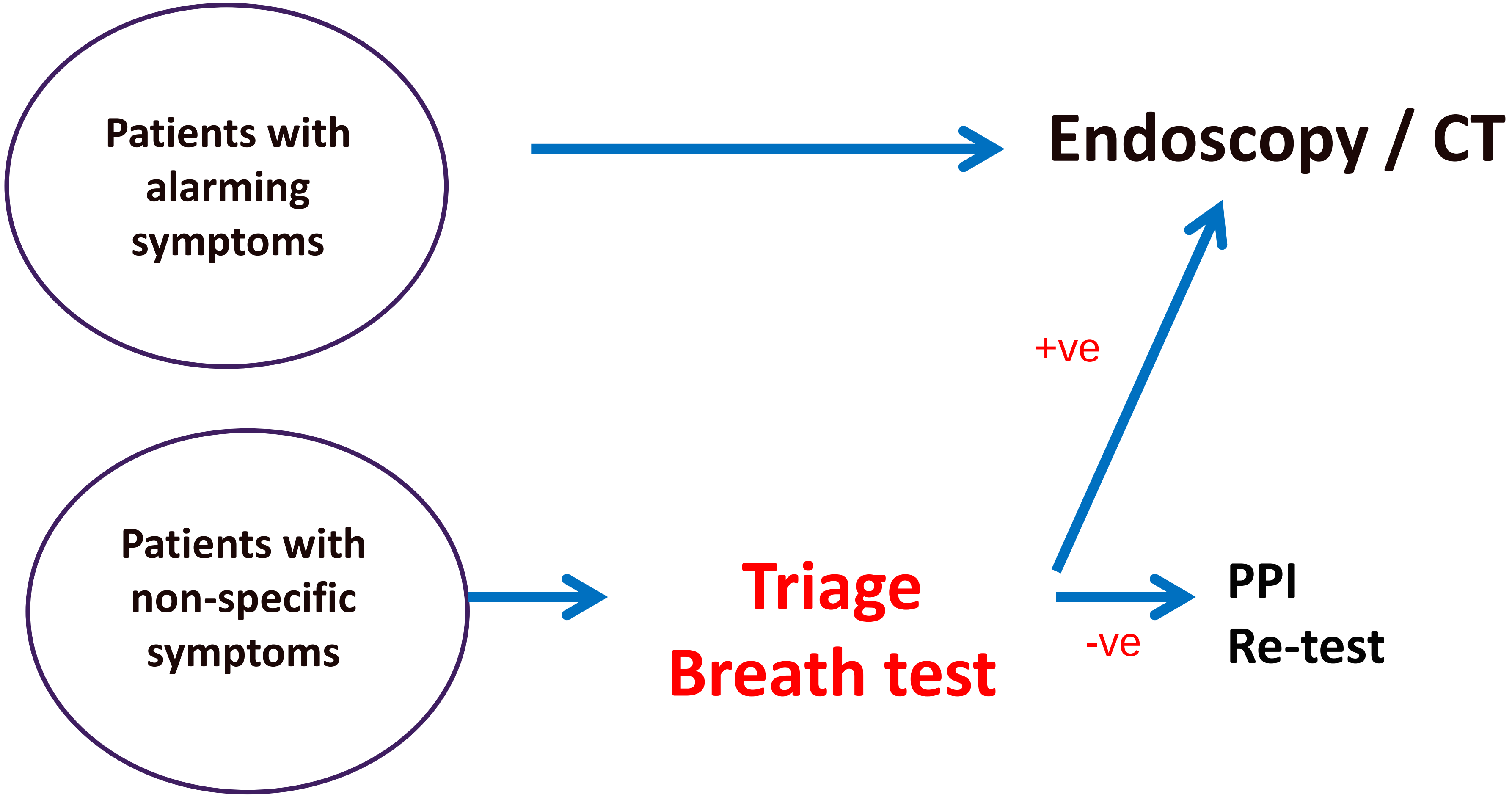
3 current oesophago-gastric multicentre trials

- **Validity in Barrett’s dysplasia early oesophageal cancer**
- **Developing breath test for squamous oesophageal cancer**
- **Augmenting VOC response to detect oesophagogastric cancer**





Breath test assisted pathway



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