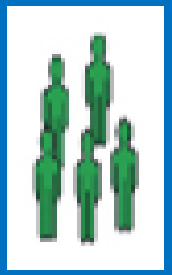
Stable isotope or unlabeled-probe breath tests vs endogenous VOC's breath tests - a review

Anil S. Modak, PhD

FDA/EMA approved breath tests

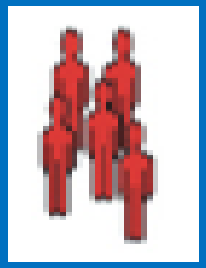
- Urea-^{13}C breath test - Otsuka Pharm -1997
- Spirolina-^{13}C breath test - Cairn Diagnostics - 2016
- Methacetin-^{13}C breath test by Humedics GmbH- 2017-18
- NO breath test for asthma - Aerocrine AB - 2003
- Heartsbreath test - Mensanna Research Inc - 2004

Drug prescription outcome



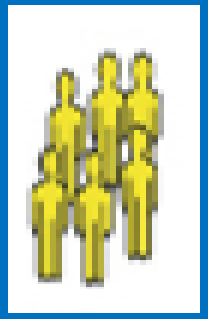
Drug toxic but beneficial

Patient group



Drug toxic and not beneficial

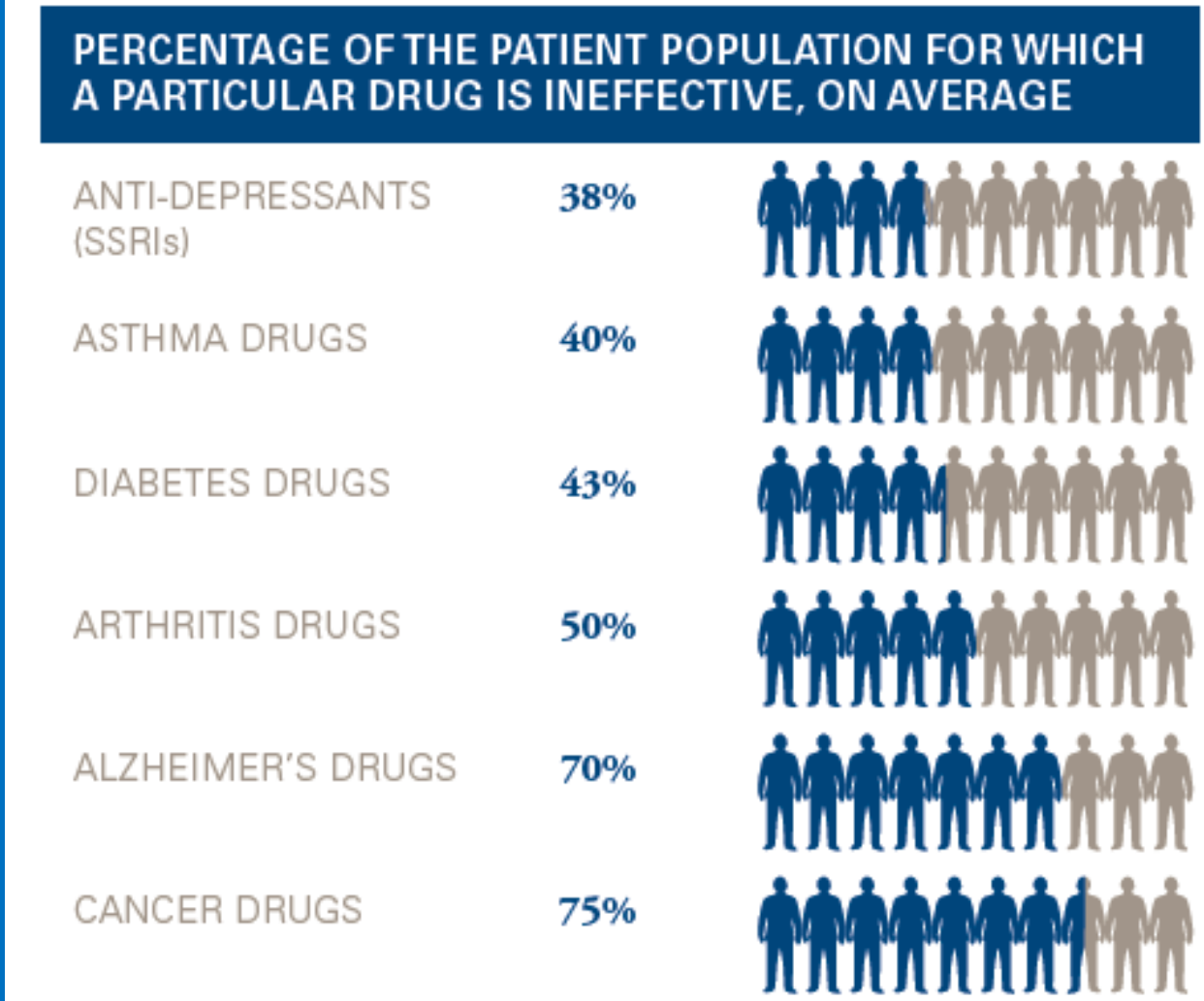
Same diagnosis
Same drug prescription



Drug not toxic and not beneficial

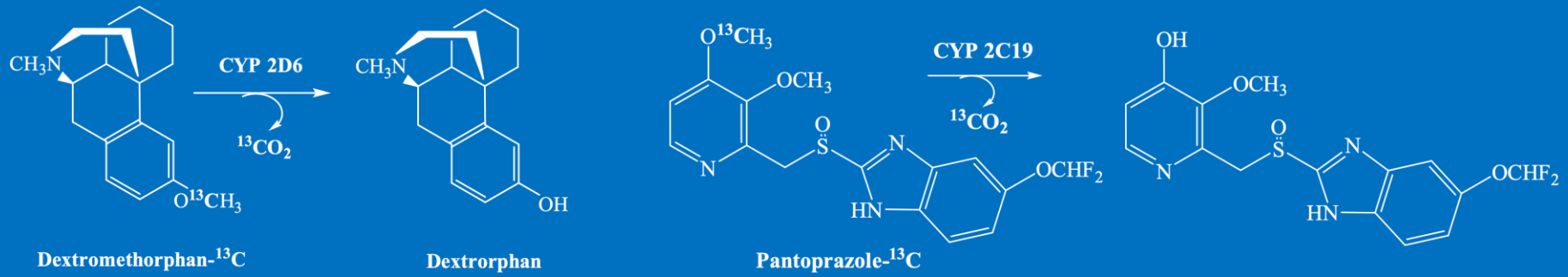
Drug not toxic and beneficial

ONE SIZE DOES NOT FIT ALL



Source of data: Brian B. Spear, Margo Heath-Chiozzi, Jeffery Huff, "Clinical Trends in Molecular Medicine," Volume 7, Issue 5, 1 May 2001, Pages 201-204.

Dextromethorphan-^{13}C breath test (DM-BT) & Pantoprazole-^{13}C breath test (Ptz-BT) for personalizing meds



- **Salient features of Breath Tests:**
  - Noninvasive, nonradioactive
  - Rapid (30 (Ptz-BT) & 50 (DM-BT) minutes), point of care
  - Easy to administer, patient friendly
  - *in vivo* phenotype

Breath Tests are vastly superior to:

- Existing static genetic tests, which cannot detect phenoconversion
- Laborious, expensive therapeutic drug monitoring (TDM)

Modak AS Point-of-care companion diagnostic tests for personalizing psychiatric medications: fulfilling an unmet clinical need *J. Breath Research* 2018, 12: 017101

Prevalence of mental illness in US

- One in four adults−approximately 74 million Americans −experiences mental illness in a given year[1]
- $150 billion in direct healthcare costs
- Spending on antidepressants is $ 15 billion[2]
- 314 million prescriptions were filled for antidepressants[3], higher than any therapeutic class
- Global Depression Drug Market Poised to Surge from $ 14.5 Billion in 2014 to $ 16.8 Billion by 2020[4]

[1]NIH, NIMH. Statistics: Any Disorder Among Adults. Retrieved March 5, 2013, from http://www.nimh.nih.gov/statistics/1ANYDIS_ADULT.shtml

[2-3]IMS Health National sales Dec 2016

[4] http://www.marketresearchstore.com/news/global-depression-drug-market-215

Background & Rationale

- Prescribing safe and effective **medications** is a challenge in **psychiatry**.

- While clinical use of pharmacogenomic testing for individual genes has provided some clinical benefit, it has largely failed to show clinical utility.

- The neuroscience market is characterized by subjective diagnosis and **trial and error** approaches to prescription drugs.

Benitez et al The clinical validity and utility of combinatorial pharmacogenomics: Enhancing patient outcomes *Applied & Translational Genomics* 5, 47–49, 2015

Pharmacogenomic Biomarkers in Drug Labeling - CYP2D6

Psychiatry

Abilify®
Amitriptyline
Aripeperizole Lauroxil
Atomoxetine
Brexpiperizole
Citalopram
Clomipramine
Clozapine
Desipramine
Doxepin
Duloxetine
Escitalopram
Fluoxetine
Fluoxamine
Galantamine
Illoperidone
Imipramine
Modafinil
Nefadozone
Nortiptyline
Neudexta ®
Paroxetine

Psychiatry

Perphenazine
Pimozide
Protriptyline
Risperidone
Thioridazine
Trimipramine
Venlaflaxine
Vortioxetine

Neurology

Nuedexta
Tetrabenazine

Analgesics

Codeine
Tramadol

Infectious disease

Quinine sulfate
Terfinabine

Other

Arformoterol – Pulmonary
Cevimeline – Dental
Eliglustat – Inborn metabolic errors

Cardiology

Carvediol
Metoprolol
Propafenone
Propranolol
Quinidine

http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm

Pharmacogenomic Biomarkers in Drug Labeling - CYP2C19

Psychiatry

Citalopram
Clobazam
Diazepam
Doxepin

Proton Pump Inhibitors

Omeprazole
Esomeprazole
Pantoprazole
Lansoprazole
Deslanzoprazole
Rabeprazole

Cardiology

Clopidogrel
Prasugrel
Ticagrelor

Other

Carisoprodol
Voriconazole
Drospirenone
Ethinylestradiol

http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm

List of genetic tests approved by the FDA for CYP2D6 and CYP2C19

Genetic test	Diagnostic company	Year	510 application
Roche AmpliChip CYP450 microarray CYP2D6	Roche Molecular Systems, Inc	2004	K042259
Roche AmpliChip CYP450 microarray CYP2C19	Roche Molecular Systems, Inc	2005	K043576
XTAG CYP2D6 Kit V3	Luminex Molecular Diagnostics, Inc	2010	K093420
INFINITI CYP2C19 Assay	Autogenomics, Inc	2010	K101683
Verigene CYP2C19 Nucleic Acid Test	Nanosphere, Inc	2012	K120466
Spartan RX *CYP2C19 Test*	Spartan Bioscience, Inc	2013	K123891
XTAG CYP2D6 Kit V3 (Inc. software)	Luminex Molecular Diagnostics, Inc	2013	K130189

Predicting phenotype from genotype

- Pharmacokinetic Pharmacogenetic Prescribing Guidelines for Antidepressants: A Template for Psychiatric Precision Medicine *Mayo Clinic Proceedings*, 2016: 91(7), 897-907

- Utility of integrated pharmacogenomic testing to support the treatment of major depressive disorder in a psychiatric outpatient setting. *Pharmacogenet Genomics* 2013: 23(10) 535-48

- Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6 and CYP2C19 genotypes and dosing of selective serotonin reuptake inhibitors. *Clinical Pharmacology & Therapeutics*, 2015: *98*(2), 127-134

- Clinical Pharmacogenetics Implementation Consortium guideline for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants. *Clinical Pharmacology & Therapeutics*, 2013: *93*(5), 402-408

- Prediction of CYP2D6 phenotype from genotype across world populations *Genetics in Medicine* 2017: 19, 69–76

Predicting phenotype from genotype

Table 1a Assignment of CYP2D6 predicted phenotypes

Likely phenotype	Activity score	Genotypes	Examples of *CYP2D6* diplotypes
Ultrarapid metabolizer (~1–2% of patients)[a]	> 2.0	An individual carrying duplications of functional alleles	**1/*1xN, *1/*2xN, *2/*2xN*[b]
Extensive metabolizer (~77–92% of patients)	2.0-1.0[c]	An individual carrying two normal function alleles or two decreased function alleles or one normal function and one no function allele or one normal function and one decreased function allele	**1/*1, *1/*2, *1/*4, *1/*5, *1/*9, *1/*41, *2/*2,*41/*41*
Intermediate metabolizer (~2–11% of patients)	0.5	An individual carrying one decreased function and one no function allele	**4/*10, *4/*41, *5/*9*
Poor metabolizers (~5–10% of patients)	0	An individual carrying only no functional alleles	**3/*4, *4/*4, *5/*5, *5/*6*

Table 1b Assignment of CYP2C19 predicted phenotypes

Likely phenotype	Genotypes	Examples of *CYP2C19* diplotypes
Ultrarapid metabolizer (~5–30% of patients)[d]	An individual carrying two increased function alleles or one normal function allele and one increased function allele	**17/*17, *1/*17*
Extensive metabolizer (~35–50% of patients)	An individual carrying two normal function alleles	**1/*1*
Intermediate metabolizer (~18–45% of patients)	An individual carrying one normal function allele or one increased function allele and one no function allele	**1/*2, *1/*3, *2/*17*[e]
Poor metabolizer (~2–15% of patients)	An individual carrying two no function alleles	**2/*2, *2/*3, *3/*3*

[a]CYP2D6 metabolizer status frequencies are based on data from Caucasians and may differ from other ethnicities. See **Supplemental Tables S3** and **S6** note for information on the chances of observing specific diplotypes in different major race/ethnic groups. [b]Where *xN* represents the number of *CYP2D6* gene copies. For individuals with *CYP2D6* duplications or multiplications, see **Supplemental Data** for additional information on how to translate diplotypes into phenotypes. [c]Patients with an activity score of 1.0 may be classified as intermediate metabolizers by some reference laboratories. [d]CYP2C19 metabolizer status frequencies are based on average multiethnic frequency. [e]The predicted metabolizer phenotype for the**2/*17* diplotypes is a provisional classification. The currently available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the no function *CYP2C19*2* allele.[36] See **Supplemental Materials** for a more comprehensive list of predicted metabolizer phenotypes.

Alleles for CYP2D6 and CYP2C19

List of most relevant (42 of 105) alleles for CYP2D6 enzyme activity

Null alleles (no function) *3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *16, *18, *19, *20, *21, * 31, *38, *40, *42, *44, *56, *62, *68, *92, *100, *101

Partially functional alleles *9, *10, *17, *29 *36, *41, *69

Fully functional alleles *1, *2, * 27, *33, * 35, *39

Duplication or multiplication alleles *1XN, *2XN

List of most relevant (9 of 35) alleles for CYP2C19 enzyme activity

Null alleles (no function) *2, *3, *4, *5, *6, *7 &*8

Fully functional allele *1

Increased activity allele *17

Why are existing tests not being used?

Genotype test	**Therapeutic drug monitoring**
Expensive	Expensive
Not point of care, 2-3 days	Not point of care, 7 day turnaround
Invasive	Invasive
Difficult to interpret	Easier to interpret
Predicts phenotype	Evaluates phenotype

Phenoconversion!

- Addressing phenoconversion: the Achilles' heel of personalized medicine *Br J Clin Pharmacol* 2015;79(2):222-40.
- Inflammation-induced phenoconversion of polymorphic drug metabolizing enzymes: hypothesis with implications for personalized medicine *Drug Metab Dispos*. 2015;43(3):400-10
- Cytochrome P450 2D6 phenoconversion is common in patients being treated for depression: implications for personalized medicine *J Clin Psychiatry*. 2013 ;74(6):614-21
- Phenoconversion and therapeutic drug monitoring *Br J Clin Pharmacol* 2015; 80(4): 777–778
- Mass Spectrometry in Precision Medicine: Phenotypic Measurements Alongside Pharmacogenomics *Clinical Chemistry* 2016; 62:1 70–76

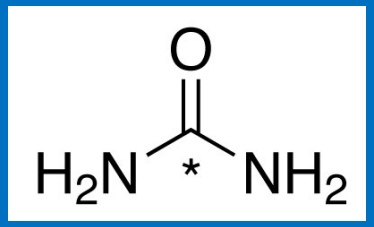
Avisa Breath Test

Patient inhales nebulized AV-U13, ^{13}C-urea

Bacteria convert AV-U13 to $^{13}CO_2$

Avisar spec measures $^{13}CO_2$

Point of care analysis in seconds

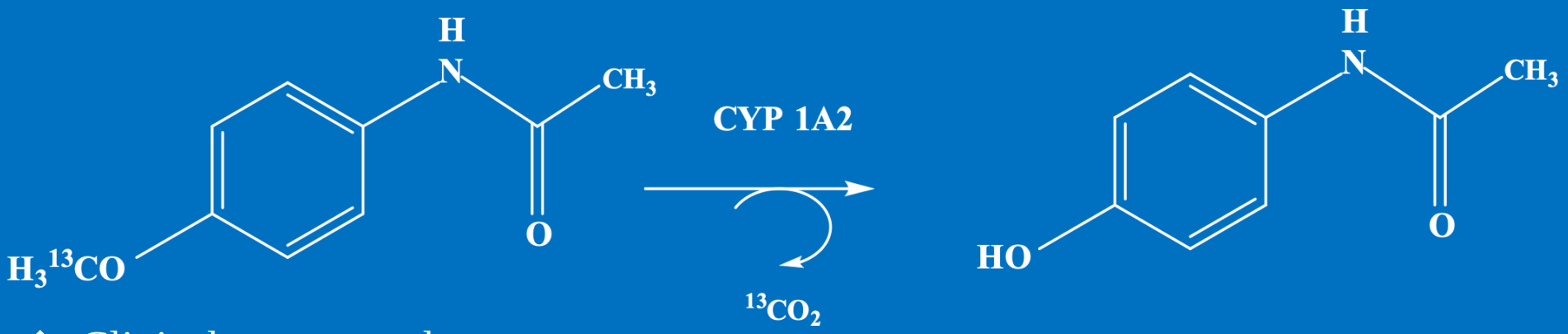


Urease

$^{13}CO_2 + 2NH_3$

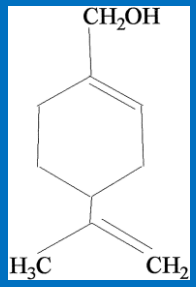
Quantitative, point-of-care test for rapidly detecting pulmonary infections

Methacetin breath test - Exalenz Biosciences

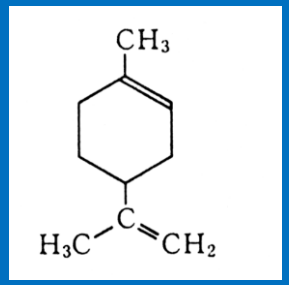


- Clinical unmet need
- Humedics LiMAx (MBT) is intravenous
- Monitoring Hep C patients after treatment with Olysio, Sovaldi, Harvoni
- Monitoring patients with NASH/NAFLD on drugs in clinical trials - Elafibranor, Emricasan, Obeticholic acid, Pioglitazone etc
- Monitor patients on liver transplant lists as well a liver diseases like ALF and CSPH
- Evaluate CYP1A2 enzyme activity

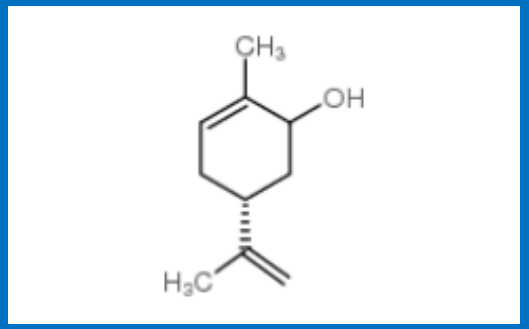
Limonene breath test



CYP2C19



CYP2C9



Perillyl alcohol | Limonene | Carveol

- Clinical unmet need
- Monitor clinical efficacy of new drugs for NASH
- Monitor patients on liver transplant lists
- Evaluate CYP2C19 and CYP2C9 enzyme activity

O'Hara ME et al. Limonene in exhaled breath is elevated in hepatic encephalopathy. *J Breath Res.* 2016;10(4):046010.

E-VOC probes

EVOC Probe	Metabolized by enzyme(s):
Eucalyptol	CYP3A4
Limonene	CYP2C9, 2C19
Menthol	CYP2A6
Linalool	CYP2C19, 2D6

Endogenous VOC breath tests

➢Lack of specificity

- Origins of VOC's
- Study design of protocols
- Standardization of breath sampling, storage and analytical tools
- No uninimity on VOC lists for detection of disease

In a review of 10 studies regarding VOCs in LC, 170 different VOCs were detected in total; however, only 17 of them appeared in at least two different studies

Corrado Di Natale et al Interpretation of **exhaled** VOC's *Eur Respir Mon*. 2010;49:115–129

Procedures for breath sampling, storage, and analysis in the reviewed studies

D Marzorati et al *J. Breath Res*. 13 (2019) 034001

Author year	Analysis method	Sample	Storage
Phillips 2003	GC-MS	ME	1 L sorbent traps
Poli 2005	SPME GC-MS	Alveolar	150 ml Bio-VOC sample
Machado 2005	Cyranose 320	ME	Mylar bags
Wehinger 2007	PTR-MS	ME	3 L Tedlar bags cleaned with N_2
Phillips 2007	GC-MS	Alveolar	Activated carbon sorbent traps
Dragonieri 2009	Cyranose 320	ME	20 ml sealed vials
Fuchs 2010	SPME GC-MS	Alveolar	20 ml sealed vials
Ulanowska 2011	SPME GC-MS	Alveolar	Tedlar bags cleaned with Ar
Buszewski 2012	GC-MS	Alveolar	1 L Tedlar bags cleaned with Ar
Gasparri 2016	QMB	ME	Tedlar bags
Schallschmidt 2016	SPME GC-MS	ME	1 L gas bulbs cleaned with MeOH
Kou 2017	Type-different GS	ME	Tedlar bags cleaned with N_2
Li 2017	Type-different GS	ME	Tedlar bags cleaned with N_2
Chang 2018	MOSSensors	Alveolar	3 L Tedlar bags
Tirzite 2018	Cyranose 320	ME	Tedlar Bags

VOC's for detection of lung cancer

VOCs found significant for the discrimination between lung cancer patients and healthy controls reported in studies that used exhaled breath to perform lung cancer diagnosis.

Author and Year	VOCs
Phillips 2003	Butane; 3-methyl Tridecane; 7-methyl Tridecane; 4-methyl Octane; 3-methyl Hexane; Heptane; 2-methyl Hexane; Pentane; 5-methyl Decane
Poli 2005	Isoprene; Methylpentane; Pentane; Ethylbenzene; Xylenes; Trimethylbenzene; Toluene; Benzene; Heptane; Decane, Styrene; Octane; Pentamethylheptane
Machado 2005	Isobutane; Methanol; Ethanol; Acetone; Pentane; Isoprene; Isopropanol; Dimethylsuflide; Carbon disulfide; Benzene; Toluene
Phillips 2007	Cyclododecatriene; Pentane; Benzoic acid; Propanoic acid; azepine; Cyclohexadiene; Benzene; Furan; Biphenyl; Pentanone; Caryophyllene; Indene; Propanol, Decane, Benzenedicarboxylic acid; Hexadiene
Bajtarevic 2009	Butanone; Benzaldehyde; Butanedione;Propanol; Butane; Butene; Acetophenone; Cyclopentene, Pentanal; Cyclopentadiene; Butanol; Isoquinoline; Undecane; Benzene; Butyl acetate; Ethylenimine
Fuchs 2010	Pentanal; Hexanal; Octanal; Nonanal
Ulanowska 2011	Ehtanol; Acetone; Butane; Dimethyl sulfide; Isoprene; Propanal; 1-propanol; 2-pentanone; Furan; o-xylene; Ethylbenzene; Pentanal; Hexanal; Nonane
Rudnicka 2011.	Propane; 2-Propenal; Carbon disulfide; Isoprophyl alcohol; Ethylbenze; Styrene
Wang 2012	Hexadecanal; Eicosane; 5-(2-methyl-)propylnonane; 7-methylhexadecane; 8-methylheptadecane; 2,6-di-tertbutyl, 4-methylphenol; 2,6,11-trimethyldodecane; 3,7-dimethylpentadecane; nonadecane; 8-hexylpentadecane; 2,6,10-trimethyltetradecane; 5-(1-methyl-)propylnonane; 2-methylnapthalene; 2-methylhendecanal; nonadecanol, 2-pentadecanone; tridecanone, 5-propyltridecane; 2,6-dimethylnaphthalene; tridecane, 2,8-dimethylhendecane, 5-butylnonane
Handa 2014	Dodecane; Butanol; Metylbutylacetat or Hexanol; Cyclohexanon; Iso-propylamin; n-Nonal or Cyclohexanon; Ethylbenzol; Hexanal; Heptanal
Kumar 2015	Pentanoic acid; Hexanoic acid; Phenol; Methyl phenol; Ethyl phenol; Butanal; Pentanal; Hexanal; Heptanal; Octanal; Nonanal; Decanal
Ligor 2015	Butane ; 2-methyl-Butane ; 4-methyl-Octane ; Propane; 2-Pentanone; Propanal; 2,4-dimethyl-Heptane; Propene
Schallschmidt 2016	Pentane; Hexane; Heptane; Octane; Dodecane; Methylpentane; 2-Methylpentane; 3-Methylpentane; Cyclohexane; Benzene; Ethylbenzene; Propylbenzene; Propanal; Butanal; Pentanal; Hexanal; Octanal; Nonanal; Decanal; Butanol; Butanone; Pentanone; Isoprene; Acetone; 2-Propanol
Phillips 2019	1,4-Butanediol; 4-Methyl-2-pentanamine; 2-Propanamine; 3-Butenamide; 4 -Penten-2-ol; 2-Cyano alanine acetamide; N-Methyl glycine; Octodrine

Impediments for transition of breath tests from research phase to clinic

- Financial resources for regulatory approval
- Market tests to insurance companies and patients
- Overcome lack of specificity for VOC breath tests

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