The potential role of exhaled breath analysis in the diagnostic process of pneumonia - a systematic review

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1 The Potential Role of Exhaled Breath Analysis in the Diagnostic Process
2 of Pneumonia – A Systematic Review

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37 **Abstract**

38 Diagnostic strategies currently used for pneumonia are time-consuming, lack
39 accuracy and suffer from large inter-observer variability. Exhaled breath contains
40 thousands of volatile organic compounds (VOCs), which include products of host and
41 pathogen metabolism. In this systematic review we investigated the use of so-called
42 ‘breathomics’ for diagnosing pneumonia.

43 A Medline search yielded 18 manuscripts reporting on animal and human studies
44 using organic and inorganic molecules in exhaled breath, that all could be used to
45 answer whether analysis of VOC profiles could potentially improve the diagnostic
46 process of pneumonia. Papers were categorised based on their specific aims; the
47 exclusion of pneumonia; the detection of specific respiratory pathogens; and whether
48 targeted or untargeted VOC analysis was used.

49 Ten studies reported on the association between VOCs and presence of pneumonia.
50 Eight studies demonstrated a difference in exhaled VOCs between pneumonia and
51 controls; in the individual studies this discrimination was based on unique sets of
52 VOCs. Eight studies reported on the accuracy of a breath test for a specific
53 respiratory pathogen: five of these concerned preclinical studies in animals. All
54 studies were valued as having a high risk of bias, except for one study that used an
55 external validation cohort.

56 The findings in the identified studies are promising. However, as yet no breath test
57 has been shown to have sufficient diagnostic accuracy for pneumonia. We are in
58 need of studies that further translate the knowledge from discovery studies to clinical
59 practice.

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62 **Key words:** Exhaled breath analysis; Pneumonia; Diagnosis; Breathomics; Volatile
63 Organic Compounds

64

65 Introduction

66 The prevalence of community-acquired pneumonia (CAP)¹ and nosocomial
67 pneumonia^{2,3} is high with substantial impact on morbidity and mortality⁴⁻¹⁰. Treatment
68 of pneumonia is a balance between optimal antibiotic therapy for the patient (e.g. a
69 combination of antibiotics that effectively target the causative pathogen) and for the
70 community (e.g. minimizing the duration of exposure to broad spectrum antibiotics to
71 limit antimicrobial resistance)¹¹. In the ideal world, this balance would be met through
72 a diagnostic test that is not only quick, non-invasive, reliable and available in real-
73 time at the bedside, but most of all (1) excludes pneumonia in order to withhold
74 antibiotic treatment from patients without an infection; (2) enables targeting of
75 antibiotic treatment of the causative pathogen; and (3) facilitates evaluation of the
76 treatment response aiming to refine antibiotic de-escalation and duration of antibiotic
77 treatment.

78 'Breathomics' refers to the analysis of volatile compounds in exhaled breath
79 that resulted from, or are affected by metabolism¹². The complete human breathome
80 consists of thousands of compounds¹³⁻¹⁷. The volatile organic compounds (VOCs)
81 that are present in the exhaled breath have various origins. Exogenous VOCs are
82 derived from the environment and are taken in through inhalation or ingestion (e.g.
83 via food or drugs). VOCs that are produced within the body can emerge as products
84 of physiological metabolic processes from the host, as products of metabolic
85 processes from microbial pathogens, or results from of a host response to
86 pathological processes such as infection or inflammation¹⁸⁻²⁰. Changes, therefore, in
87 host or microbial metabolism might lead to an impact on the composition of the
88 exhaled breath profile.

89 In this systematic review we aim to investigate the potential role of exhaled
90 breath analysis for diagnosing pneumonia, by providing: (1) sensitive detection of
91 pneumonia; (2) specific detection of the causative organism(s); and (3) a tool to
92 monitor the treatment response after the initiation of antibiotics (see Figure 1). We

93 hypothesize that changed concentrations of VOCs in exhaled breath can be used to
94 accurately discriminate patients with pneumonia from patients without pneumonia
95 and may be used for specific identification of the causative pathogen.

96

97 **Methods**

98 Search

99 This is a systematic review following PRISMA (Preferred Reporting Items for
100 Systematic Reviews and Meta-Analyses) guidelines, performed by two independent
101 researchers. We searched Medline for potentially relevant articles up to March 7th
102 2017, using the following search terms: “(((Chromatography OR Spectrometry OR
103 MS OR (Volatile AND Organic) OR Metabol*) AND breath) OR (volatile fingerprint*)
104 OR (breathprin*) OR (electronic AND nose)) AND (pneumonia OR (lung infection*)
105 OR (respiratory infection*) OR (lung bacteria*) OR (respirator* bacteria*))”. There
106 was no restriction with respect to human or animal studies; but articles written in a
107 language other than English and studies performed in vitro were excluded. Two
108 authors (PvO and LB) reviewed the abstracts and/or full-text manuscripts
109 independently and selected those that were regarded to be relevant. No
110 disagreement on selection of articles was seen between the two reviewers.

111 Selection criteria

112 Inclusion criteria were (1) human or animal studies that (2) studied volatiles in
113 exhaled breath to (3) diagnose bacterial pneumonia or identify the causative
114 organism of pneumonia. Objective 3 as mentioned in the introduction (the evaluation
115 of the treatment effect in patients with pneumonia) was let go, due to a lack of
116 studies specifically investigating this. We excluded in vitro studies and studies that
117 focused on very specific atypical causative organisms (such as Aspergillus).

118 Reference test

119 The diagnosis of pneumonia could be based on clinical symptoms alone, or could be
120 supported by chest radiography and/or microbiology testing (cultures of endotracheal

121 aspirate (ETA), nondirected bronchial lavage (NBL or mini-BAL) or bronchoalveolar
122 lavage (BAL)). For community-acquired pneumonia, the combination of clinical signs
123 and symptoms with an evident infiltrate on the chest radiograph was considered a
124 good reference test, while anything less was considered too nonspecific. For
125 ventilator-associated pneumonia, clinical signs, laboratory parameters, an infiltrate
126 on chest radiography and quantitative cultures of BAL or NBL were considered an
127 appropriate reference standard.

128 Index test

129 Advances in chemical analytics have enabled the measurement of inorganic^{21,22} and
130 organic compounds^{23–25} in biological matrices such as exhaled breath. Volatile
131 molecules in breath can be studied via a targeted and an untargeted approach¹².
132 With the targeted approach the researcher identifies the molecules of interest
133 beforehand and uses analytical assays to measure those compounds quantitatively.
134 The untargeted approach entails analytical techniques that measure multiple
135 molecules present in the breath. Untargeted analysis can be performed with mass-
136 spectrometry based techniques aimed to identify a variety of VOCs²⁶ or with so-
137 called electronic nose technology that is based on pattern recognition^{14,27,28}. The
138 analytical details of these techniques are discussed in detail in previous
139 publications^{27,29}. Figure 2 summarises the analytical methods that will be referred to
140 in this systematic review. No single method is superior to the others, they provide
141 different types of information, therefore the quality of the index test was assessed
142 based on the use of an independent validation cohort, which has been shown to limit
143 bias²⁷.

144 Methodological assessment and categorisation

145 The methodological quality of each selected full manuscript was evaluated using the
146 QUADAS-2 tool by the same authors as describes above³⁰. Risk of bias was
147 assessed concerning patient selection, the interpretation or conduct of the index test,
148 the interpretation or conduct of the reference standard and the patient flow. The

papers were classified as either (1) studies concerning sensitive detection of the presence of pathogenic bacteria, either concerning studies investigating inorganic compounds, untargeted analysis of VOCs or eNose technology for discrimination between pneumonia and no pneumonia; or (2) studies investigating the use of VOC analysis for specific detection of pathogenic bacteria, in animals or in humans.

Results

The search was last updated on March 7th 2017 and yielded 321 articles, of which 18 were selected after screening on title/abstract and full text (Figure 3). Of these, 13 studies were in humans and five were performed in murine models. Eight studies dealt with the detection of specific pathogenic bacteria, the others focused on discrimination between patients with and without pneumonia. One of the studies discussed treatment response. Table 1 demonstrates the areas of interest for each study and summarises the methodology used.

The studies were critically appraised and risk of bias was assessed regarding patient selection, index test, reference standard and flow and timing (Table 2). The domain 'patient selection' was considered not applicable in the five animal studies. For most studies the risk of bias was valued as high, except for one that used a validation cohort³¹, resulting in a low risk of bias regarding the index test.

Discrimination between patients with and without pneumonia

Detection of volatile inorganic compounds

NO was not increased in the breath of a small group of patients admitted with pneumonia, when compared with control patients³². As expected it was elevated in patients with an exacerbation of asthma. This result was in contrast to the results of a larger study at less risk of bias (Table 2) in which exhaled NO was measured in tracheal and nasal gas in patients ventilated within 72 hours of ICU admission³¹.

Some of these patients were later diagnosed with VAP and this was used as the reference standard. A validation cohort consisting of similar patients to the first group was used to determine sensitivity and specificity of the NO threshold that was calculated in the preceding group. NO concentrations were measured at multiple sampling points in the airway as well as in the nasal cavity, and significantly higher NO levels were found at all points in patients with pneumonia. Of these, the maximum (end-expiratory) tracheal NO values resulted in the highest sensitivity and specificity for the diagnosis of pneumonia: 88% and 76% respectively (see Table 1). Results from one study with an imperfect reference test, namely subjective symptoms of lower respiratory infection, suggested a possible relationship between elevated exhaled CO levels and the clinical presence of pneumonia³³. Notably, the exhaled CO concentration followed similar trends as the patients' symptoms after antimicrobial treatment.

Untargeted analysis of VOCs

The abundance of particular VOCs seems to be different in the breath of mechanically ventilated patients with pneumonia compared with those without pneumonia³⁴⁻³⁶. The results of studies using gas chromatography and mass spectrometry (GC-MS, see Figure 2), however, were not uniform. The described VOCs differed between studies and two compounds that were identified as being associated with VAP (ethanol and heptane) showed conflicting results in two studies (as shown in Table 3). Differences between studies regarding investigated cohorts, reference standards and outcome measures (sensitivity, specificity and/or accuracy) can be found in Table 1. Nevertheless, breath tests showed promising discrimination between patients with and without pneumonia in the included clinical studies. The most frequently isolated pathogens in these studies were *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*³⁴⁻³⁶.

205

206 *Electronic nose technology*

207 Preliminary results indicated a potential correlation between chest CT scans³⁷ or
208 Clinical Pulmonary Infection Score (CPIS)³⁸ and the subsequent eNose sensor
209 responses in mechanically ventilated patients. The eNose (see Figure 2) seemed to
210 distinguish patients with and without bacterial infection³⁹ (Table 1). When specifically
211 focusing on diagnosis of VAP, the eNose appeared to have good accuracy,
212 moderate sensitivity and a rather poor specificity⁴⁰.

213

214 Specific detection of pathogens by VOC analysis

215 Secondary electrospray ionization – mass spectrometry (SESI-MS, see
216 Figure 2) breathprint analysis was used to investigate the ability to identify respiratory
217 infection caused by strains of *Haemophilus influenzae*, *Klebsiella pneumoniae*,
218 *Legionella pneumophila*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*,
219 *Staphylococcus aureus* or *Streptococcus pneumoniae* in mice^{41–44}. Overall SESI-MS
220 breathprints seemed to be able to distinguish between respiratory infection and no
221 infection in mice and enabled differentiation between strains of aforementioned
222 pathogens. A high degree of variation was seen when translating *in vitro* experiments
223 to the *in vivo* VOC fingerprints⁴². The relative contribution of bacterial metabolism and
224 host response on the exhaled breath profile could be inferred through an experiment
225 in which mice were exposed to bacterial cell lysates⁴⁵. This experimental set-up,
226 using these bacterial cell lysates, allows for host and pathogen derived metabolites
227 to be differentiated. The obtained SESI-MS breathprints changed over time after
228 lysate exposure and appeared to 1) correlate to the host immune response; and 2)
229 distinguish active infections of *P. aeruginosa* or *S. aureus* from cell lysate exposure.
230 Also using GC-MS specific VOCs in the exhaled breath seemed to reflect the
231 presence of particular microorganisms in the respiratory tract and, in line with the use
232 of SESI-MS, direct translation of biomarkers from the *in vitro* to the *in vivo* setting

proved difficult^{46,47}. In clinical studies, the evidence for specific detection of particular causative pathogens seemed speculative; as the published papers did not provide data on the accuracy of such measurements⁴⁶. The only study that reported a high diagnostic accuracy for the identification of a causative pathogen focused on *Acinetobacter baumannii*. A set of eight compounds resulted in excellent separation of patients with *A. baumannii* pneumonia, colonization with the same bacterium and controls. The major limitation of the described studies was that they did not evaluate the diagnostic accuracy of a breath test in the clinical scenario where such a test would be used; e.g. in patients with a clinical suspicion of VAP.

Discussion

Based on our systematic review, the presence of certain profiles or patterns of volatile molecules in the exhaled breath appeared to be associated with pneumonia. However, the precise identity of these volatile biomarkers remains largely unknown. Furthermore, none of the studied breath tests delivered results with sufficient clinical diagnostic accuracy that would likely impact on clinical decisions. Most of the available studies provided feasibility or proof of concept data with a substantial risk of bias and did not test a clear, pre-defined hypothesis.

There are two leads to follow in the diagnosis of pneumonia: measurement of the host response or direct identification of the pathogen^{48,49}, both important establishing the ideal diagnostic test. In vitro results suggested that different pathogenic bacteria produced different volatile molecules, which might be used for identification²⁰. One of the major challenges is that bacterial growth and metabolism are influenced by the chosen culture media, timing and the selection of particular strains and, therefore, may not be representative of growth in vivo²⁰. A sterile inflammatory response altered the VOC release in several animal models of lung injury²¹. Thus, pneumonia may be recognized through exhaled breath analysis by detection of molecules produced either directly by the pathogen or through an altered host metabolism associated with the host response. Animal studies might offer

261 advantages enabling the investigation of 1) a single bacterial infection, 2) the
262 influence of timing on disease progression and 3) post-mortem histology for the gold-
263 standard diagnosis of respiratory infection.

264 This systematic literature review demonstrates that certain volatile molecules
265 could be useful as possible biomarkers for the diagnosis of pneumonia. One of them
266 is nitric oxide (NO), a compound that has a bronchodilating and vasodilating effect in
267 the respiratory tract and plays a key role in local inflammatory response²². NO is
268 relatively easy to measure and thus forms an attractive candidate for diagnostic
269 purposes^{50,51}. In the airways NO is produced by endothelial, epithelial and
270 inflammatory cells. Generation of NO involves the oxidation of the aminoacid L-
271 arginine, a process that is catalysed by the enzyme NO synthase⁵². An increased
272 concentration of exhaled NO is seen in asthma, bronchiectasis and sepsis⁵³, and has
273 also been associated with rhinitis, active pulmonary sarcoidosis and viral respiratory
274 illnesses⁵². Table 3 shows other biomarkers of potential interest regarding the
275 discrimination of patients with and without pneumonia. However, hardly any overlap
276 is seen between the different VOCs reported in separate studies and they also show
277 conflicting results for heptane and ethanol. Two studies found an association
278 between pneumonia and a decrease in exhaled breath acetone. Generally, acetone
279 is present in large quantities in the exhaled breath. Its decrease in the breath of
280 pneumonia patients might be explained by a reduced ketogenesis that is seen during
281 inflammation or infection³⁴.

282 As soon as a breath test fulfils the requirements for a diagnostic test for
283 pneumonia, it shall be able to fulfil a role alongside the currently available and
284 frequently used alternatives^{54,55} and can either compete with them, or complement
285 them. The diagnosis of pneumonia relies on a combination of physical examination
286 and chest radiography⁵⁶, potentially accompanied by measurement of inflammatory
287 markers in plasma, urinary antigen testing⁵⁴, repeated determination of C-reactive
288 protein (CRP)⁵⁷ and collecting airway samples for microbiology cultures⁵⁸. Current

diagnostics lack clinical accuracy⁵⁹ and have high inter-observer variability⁶⁰. Microbiology results take 48-72 hours to become positive. The unnecessary prescription of antimicrobial treatment increases antimicrobial resistance⁶¹⁻⁶³, whereas applying the wrong antibiotics is likely to increase mortality⁶⁴⁻⁶⁶. In order to withhold antibiotics, the CPIS⁶⁷ combines clinical and physiological data, pulmonary radiography and microbiology results into a numeric score that can be used to exclude pneumonia with moderate accuracy due to substantial inter-observer variability^{68,69}. Additionally, biomarkers like pulmonary interleukin-1 β (IL-1 β) and interleukin-8 (IL-8) measured in BAL fluid have shown promising results as discriminators for VAP^{70,71}. In the near future Polymerase Chain Reaction (PCR) of respiratory samples might be used to identify the causative pathogen rapidly and specifically⁷²⁻⁷⁴ and serum procalcitonin has been proposed as an attractive candidate for determining antibiotic duration^{75,76}. How would exhaled breath analysis compete with these alternatives? In contrast to blood or BAL samples, breath can be collected completely non-invasive and it is continuously available. A breath test could also provide results rapidly and cost-effectively, which is important in the setting of pneumonia. A breath test with the right test characteristics could thus provide real opportunities for improved real-time diagnostic utility, patient acceptability and cost effectiveness.

Many different methods for breath sampling have been described in literature, including but not limited to: glass syringes, needle traps⁷⁷ steel or glass tubes filled with sorbent material and/or breath gas bags (e.g. Tedlar bags). Pre-concentration of the breath sample could be established through the absorption of the VOCs using for instance organic polymers (e.g. Tenax TA), graphitized carbon, activated charcoal or carbon molecular sieves⁷⁸. A challenge in the process of breath sampling is the humidity of exhaled breath – especially true for mechanically ventilated patients – which possibly affects pre-concentration, separation and detection of individual compounds¹⁶. The use of storage containers such as Tedlar Bags has been linked to

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3 317 loss of analytes or contamination of samples⁷⁷. The lack of standardization of
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5 318 analytical methods leads to a wide variation of results among studies. Application of
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7 319 a standardised method of exhaled breath analysis would lead to comparable results,
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9 320 thereby facilitating the potential use of breath biomarkers in the future⁷⁹.

11 321 Based on the results from the studies included in this review, we can
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13 322 conclude that the VOCs that are measurable in exhaled breath are altered during
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15 323 pneumonia and can derive from the bacterial metabolism as well as the host
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17 324 response. However, these results do not yet allow us to link specific compounds to
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19 325 particular pathogens or disease states, nor does it allow us to pool data from different
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21 326 experiments or studies due to bias and heterogeneity in experimental procedures.
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23 327 Future studies should utilize this understanding and not only focus on VOCs
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25 328 produced by bacteria or the host, but should also combine these two for optimal
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27 329 diagnostic accuracy. Additionally, a more stringent approach towards the
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29 330 methodological design of the studies is recommended. This includes following the
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31 331 STARD guidelines for reporting studies on diagnostic accuracy to limit the amount of
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33 332 bias^{80,81}. Previous reviews^{27,82} properly summarised the necessary steps to validate
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35 333 preliminary results in breath research. Importantly, future studies should focus more
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37 334 on the clinical application of a breath test. As advocated in this review such a test
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39 335 would 1) exclude pneumonia in order to withhold antibiotic treatment from patients
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41 336 without an infection; 2) enable targeting of antibiotic treatment to the causative
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43 337 pathogen; and/or 3) facilitate evaluation of the treatment response aiming to refine or
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45 338 stopping antibiotics. To date, most focus has been on VAP rather than on
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47 339 community-acquired pneumonia, implicating that currently most evidence is available
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49 340 for this particular respiratory infection aetiology. Therefore, this might also be the
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51 341 clinical problem that might require direct focus in the forthcoming years of breath
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53 342 research.

54 343 This systematic review of the literature has several strengths and
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56 344 weaknesses. We chose to apply wide inclusion criteria in order to fully cover the
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literature in this relatively nascent field of research. Naturally, this resulted in a wide diversity of selected articles and made it impossible to pool data due to the underlying heterogeneity, which can be seen as a limitation of our review. In general one can also wonder to what extent the results provided by animal experiments can be translated to the human situation. This study also has several strengths: clinical and pre-clinical studies with multiple analytical devices were included and the results were clustered into the clinical perspective of three scenarios where a biomarker could alter clinical decision-making.

This review demonstrates that a relationship exists between respiratory infection and the presence of particular VOCs in the exhaled breath. Presently, no available breath test is accurate enough to qualify for a role within the diagnostic process of pneumonia. Future studies should focus on clinical scenarios in which a breath test could impact on antimicrobial stewardship and should limit bias by strictly adhering to the latest guidelines.

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integrity of the work as a whole, from inception to published article.

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References

1. Waterer G, Rello J, Wunderink R. Management of community-acquired pneumonia in adults. *Am J Respir Crit Care Med* 2011;183:157–164.
2. American Thoracic Society. Guidelines for the Management of Adults with Hospital-acquired, Ventilator-associated, and Healthcare-associated Pneumonia. *Am J Respir Crit Care Med* [Internet] 2005;171(4):388–416. Available from: <http://www.atsjournals.org/doi/abs/10.1164/rccm.200405-644ST>
3. Ego A, Preiser J-C, Vincent J-L. Impact of diagnostic criteria on the incidence of ventilator-associated pneumonia. *Chest* [Internet] 2014;1–28. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25340476>
4. Klevens RM, Edwards JR, Richards Jr. CL, et al. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. *Public Heal Rep* [Internet] 2007;122(2):160–166. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17357358>
5. Restrepo MI, Faverio P, Anzueto A. Long-term prognosis in community-acquired pneumonia. *Curr Opin Infect Dis* [Internet] 2013;26(2):151–8. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4066634&tool=pmc-entrez&rendertype=abstract>
6. Rotstein C, Evans G, Born A, et al. Clinical practice guidelines for hospital-acquired pneumonia and ventilator-associated pneumonia in adults. *Can J Infect Dis Med Microbiol = J Can des Mal Infect la Microbiol médicale / AMMI Canada* [Internet] 2008;19(1):19–53. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19145262> <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2610276>
7. Kalanuria AA, Zai W, Mirski M. Ventilator-associated pneumonia in the ICU. *Crit Care* [Internet] 2014;18(2):208. Available from:

- 406 <http://ccforum.com/content/18/2/208>
- 407 8. Chastre J, Fagon J. State of the Art Ventilator-associated Pneumonia. *Am J*
408 *Respir Crit Care Med* 2002;165(23):867–903.
- 409 9. Browne E, Hellyer TP, Baudouin S V, et al. A national survey of the diagnosis
410 and management of suspected ventilator-associated pneumonia. *BMJ open*
411 *Respir Res* [Internet] 2014;1(1):e000066. Available from:
412 <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4275666&tool=pmc>
413 [entrez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4275666&tool=pmc&rendertype=abstract)
- 414 10. Melsen W, Rovers M, Groenwold R, Bonten M. Attributable mortality of
415 ventilator-associated pneumonia: a meta-analysis of individual patient data
416 from randomised prevention studies. *Lancet Infect Dis* [Internet]
417 2013;13(8):665–671. Available from: [http://dx.doi.org/10.1016/S1473-](http://dx.doi.org/10.1016/S1473-3099(13)70081-1)
418 [3099\(13\)70081-1](http://dx.doi.org/10.1016/S1473-3099(13)70081-1)
419 [3099\(13\)70081-1](http://dx.doi.org/10.1016/S1473-3099(13)70081-1)
- 420 11. Mandell L, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of
421 America/American Thoracic Society consensus guidelines on the
422 management of community-acquired pneumonia in adults. *Clin Infect Dis*
423 2007;44 Suppl 2(Suppl 2):S27-72.
- 424 12. Bos LD, Sterk PJ, Fowler SJ. Breathomics in the setting of asthma and chronic
425 obstructive pulmonary disease. *J Allergy Clin Immunol* [Internet]
426 2016;138(4):970–976. Available from:
427 <http://linkinghub.elsevier.com/retrieve/pii/S009167491630882X>
- 428 13. Phillips MHJ. Variation in volatile organic compounds in the breath of normal
429 humans. *J Chromatogr* 1999;(729):75–88.
- 430 14. Schee MP van der, Paff T, Brinkman P, Aalderen WMC van, Haarman EG,
431 Sterk PJ. Breathomics in Lung Disease. *CHEST J* [Internet] 2015;147(1):224.
432 Available from:
433 <http://journal.publications.chestnet.org/article.aspx?doi=10.1378/chest.14->

- 434 0781
- 435 15. Lacy Costello B de, Amann A, Al-Kateb H, et al. A review of the volatiles from
436 the healthy human body. *J Breath Res* [Internet] 2014;8(1):14001. Available
437 from: <http://www.ncbi.nlm.nih.gov/pubmed/24421258>
- 438 16. Cao W. Breath Analysis: Potential for Clinical Diagnosis and Exposure
439 Assessment. *Clin Chem* [Internet] 2006;52(5):800–811. Available from:
440 <http://www.clinchem.org/cgi/doi/10.1373/clinchem.2005.063545>
- 441 17. Fens N, Schee MP van der, Brinkman P, Sterk PJ. Exhaled breath analysis by
442 electronic nose in airways disease. Established issues and key questions. *Clin*
443 *Exp Allergy* [Internet] 2013;43(7):705–15. Available from:
444 <http://www.ncbi.nlm.nih.gov/pubmed/23786277>
- 445 18. Sethi S, Nanda R, Chakraborty T. Clinical application of volatile organic
446 compound analysis for detecting infectious diseases. *Clin Microbiol Rev*
447 2013;26(3):462–475.
- 448 19. Boots AW, Berkel JJBN van, Dallinga JW, Smolinska A, Wouters EF,
449 Schooten FJ van. The versatile use of exhaled volatile organic compounds in
450 human health and disease. *J Breath Res* 2012;6:27108.
- 451 20. Bos LDJ, Sterk PJ, Schultz MJ. Volatile Metabolites of Pathogens: A
452 Systematic Review. *PLoS Pathog* [Internet] 2013;9(5):e1003311. Available
453 from: <http://dx.plos.org/10.1371/journal.ppat.1003311>
- 454 21. Miekisch W, Schubert JK, Noeldge-Schomburg GF. Diagnostic potential of
455 breath analysis—focus on volatile organic compounds. *Clin Chim Acta*
456 [Internet] 2004;347(1–2):25–39. Available from:
457 <http://linkinghub.elsevier.com/retrieve/pii/S0009898104002256>
- 458 22. Yates DH. Role of exhaled nitric oxide in asthma. *Immunol Cell Biol*
459 2001;79(2):178–190.
- 460 23. Bos LDJ, Sterk PJ, Schultz MJ. Metabolomics in critically ill patients: focus on
461 exhaled air. In: Vincent J.-L. (Ed.): Annual update in intensive care and

- emergency medicine. Springer; 2012. p. 53–62.
24. Moser B, Bodrogi F, Eibl G, Lechner M, Rieder J, Lirk P. Mass spectrometric profile of exhaled breath—field study by PTR-MS. *Respir Physiol Neurobiol* [Internet] 2005;145(2–3):295–300. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S1569904804000266>
25. Wilson A. Advances in Electronic-Nose Technologies for the Detection of Volatile Biomarker Metabolites in the Human Breath. *Metabolites* [Internet] 2015;5(1):140–163. Available from: <http://www.mdpi.com/2218-1989/5/1/140/>
26. Filipiak W, Sponring A, Baur MM, et al. Molecular analysis of volatile metabolites released specifically by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *BMC Microbiol* 2012;12:113.
27. Leopold JH, Bos LDJ, Sterk PJ, et al. Comparison of classification methods in breath analysis by electronic nose. *J Breath Res* [Internet] 2015;9(4):46002. Available from: <http://iopscience.iop.org/article/10.1088/1752-7155/9/4/046002>
28. Kant KD van de, Sande LJ van der, Jöbsis Q, Schayck OC van, Dompeling E. Clinical use of exhaled volatile organic compounds in pulmonary diseases: a systematic review. *Respir Res* [Internet] 2012;13(1):117. Available from: <http://respiratory-research.com/content/13/1/117>
29. Bos L, Schultz M, Sterk P. A simple breath sampling method in intubated and mechanically ventilated critically ill patients. *Respir Physiol Neurobiol* [Internet] 2014;191:67–74. Available from: <http://www.sciencedirect.com/science/article/pii/S1569904813003674#>
30. Whiting PF, Rutjes AWS, Westwood ME, et al. QUADAS-2: A revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011;155(4):529–536.
31. Adrie C, Monchi M, Dinh-Xuan AT, Dall'Ava-Santucci J, Dhainaut JF, Pinsky MR. Exhaled and nasal nitric oxide as a marker of pneumonia in ventilated patients. *Am J Respir Crit Care Med* 2001;163(5):1143–1149.

- 490 32. Al-Ali MK, Howarth PH. Exhaled nitric oxide levels in exacerbations of asthma,
 491 chronic obstructive pulmonary disease and pneumonia. *Saudi Med J* [Internet]
 492 2001;22(3):249–253. Available from:
 493 [http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med4&NEWS](http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med4&NEWS=N&AN=11307112)
 494 [=N&AN=11307112](http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med4&NEWS=N&AN=11307112)
- 495 33. Biernacki WA, Kharitonov SA, Barnes PJ. Exhaled carbon monoxide in
 496 patients with lower respiratory tract infection. *Respir Med* 2001;95(12):1003–
 497 1005.
- 498 34. Schnabel R, Fijten R, Smolinska A, et al. Analysis of volatile organic
 499 compounds in exhaled breath to diagnose ventilator-associated pneumonia.
 500 *Sci Rep* [Internet] 2015;5(October):17179. Available from:
 501 <http://www.nature.com/articles/srep17179>
- 502 35. Fowler SJ, Basanta-Sanchez M, Xu Y, Goodacre R, Dark PM. Surveillance for
 503 lower airway pathogens in mechanically ventilated patients by metabolomic
 504 analysis of exhaled breath: a case-control study. *Thorax* [Internet] 2015;320–
 505 325. Available from: [http://thorax.bmj.com/cgi/doi/10.1136/thoraxjnl-2014-](http://thorax.bmj.com/cgi/doi/10.1136/thoraxjnl-2014-206273)
 506 [206273](http://thorax.bmj.com/cgi/doi/10.1136/thoraxjnl-2014-206273)
- 507 36. Oort PMP Van, Bruin S De, Weda H, Knobel HH, Schultz MJ, Bos LD.
 508 Exhaled Breath Metabolomics for the Diagnosis of Pneumonia in Intubated
 509 and Mechanically-Ventilated Intensive Care Unit (ICU) -Patients. *Int J Mol Sci*
 510 2017;18:1–14.
- 511 37. Hockstein NG, Thaler ER, Torigian D, Miller WT, Deffenderfer O, Hanson CW.
 512 Diagnosis of pneumonia with an electronic nose: correlation of vapor signature
 513 with chest computed tomography scan findings. *Laryngoscope* [Internet]
 514 2004;114(10):1701–5. Available from:
 515 <http://www.ncbi.nlm.nih.gov/pubmed/15454757>
- 516 38. Hockstein NG, Thaler ER, Lin Y, Lee DD, Hanson CW. Correlation of
 517 pneumonia score with electronic nose signature: A prospective study. *Ann*

- 518 *Otol Rhinol Laryngol* 2005;114(7):504–508.
- 519 39. Geffen WH van, Bruins M, Kerstjens HAM. Diagnosing viral and bacterial
520 respiratory infections in acute COPD exacerbations by an electronic nose: a
521 pilot study. *J Breath Res* 2016;10(3):36001.
- 522 40. Schnabel RM, Boumans MLL, Smolinska A, et al. Electronic nose analysis of
523 exhaled breath to diagnose ventilator-associated pneumonia. *Respir Med*
524 [Internet] 2015;Available from:
525 <http://linkinghub.elsevier.com/retrieve/pii/S0954611115300615>
- 526 41. Zhu J, Bean HD, Jimenez-Diaz J, Hill JE. Secondary electrospray ionization-
527 mass spectrometry (SESI-MS) breathprinting of multiple bacterial lung
528 pathogens, a mouse model study. *J Appl Physiol* 2013;114(11):1544–1549.
- 529 42. Zhu J, Bean HD, Wargo MJ, Leclair LW, Hill JE. Detecting bacterial lung
530 infections: in vivo evaluation of in vitro volatile fingerprints. *J Breath Res*
531 2013;7(1):16003.
- 532 43. Zhu J, Jimenez-Diaz J, Bean HD, et al. Robust detection of *P. aeruginosa* and
533 *S. aureus* acute lung infections by secondary electrospray ionization-mass
534 spectrometry (SESI-MS) breathprinting: from initial infection to clearance. *J*
535 *Breath Res* 2013;7(3):37106.
- 536 44. Bean HD, Zhu J, Sengle JC, Hill JE. Identifying methicillin-resistant
537 *Staphylococcus aureus* (MRSA) lung infections in mice via breath analysis
538 using secondary electrospray ionization-mass spectrometry (SESI-MS). *J*
539 *Breath Res* 2014;8(4):41001.
- 540 45. Bean HD, Jimenez-Diaz J, Zhu J, Hill JE. Breathprints of model murine
541 bacterial lung infections are linked with immune response. *Eur Respir J*
542 2015;45(1):181–190.
- 543 46. Filipiak W, Beer R, Sponring A, et al. Breath analysis for in vivo detection of
544 pathogens related to ventilator-associated pneumonia in intensive care
545 patients: a prospective pilot study. *J Breath Res* [Internet] 2015;9(1):16004.

- Available from: <http://stacks.iop.org/1752-7163/9/i=1/a=016004?key=crossref.798f068dedadbc97df4cca8b842a921c>
47. Gao J, Zou Y, Wang Y, et al. Breath analysis for noninvasively differentiating *Acinetobacter baumannii* ventilator-associated pneumonia from its respiratory tract colonization of ventilated patients. *J Breath Res* 2016;10(2):27102.
 48. Murdoch DR. How best to determine causative pathogens of pneumonia. *Pneumonia* [Internet] 2016;8(1):1. Available from: <http://pneumonia.biomedcentral.com/articles/10.1186/s41479-016-0004-z>
 49. Ginsburg GS, Woods CW. The host response to infection: advancing a novel diagnostic paradigm. *Crit Care* [Internet] 2012;16(6):168. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23134694>
 50. Cameli P, Bargagli E, Refini RM, Pieroni MG, Bennett D, Rottoli P. Exhaled nitric oxide in interstitial lung diseases. *Respir Physiol Neurobiol* 2014;197:46–52.
 51. Cameli P, Bargagli E, Fossi A, et al. Exhaled nitric oxide and carbon monoxide in lung transplanted patients. *Respir Med* 2015;109(9):1224–1229.
 52. Ricciardolo FLM, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. *Physiol Rev* 2004;84(3):731–765.
 53. Hyde RW, Geigel EJ, Olszowka AJ, et al. Determination of production of nitric oxide by lower airways of humans--theory. *J Appl Physiol* [Internet] 1997;82(4):1290–1296. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9104867>
 54. Sinclair A, Xie X, Teltscher M, Dendukuri N. Systematic review and meta-analysis of a urine-based pneumococcal antigen test for diagnosis of community-acquired pneumonia caused by *Streptococcus pneumoniae*. *J Clin Microbiol* 2013;51(7):2303–2310.
 55. Badoux P, Euser SM, Bruin JP, Mulder PPG, Yzerman EPF. Evaluation of the bioNexia *Legionella* test including the impact of incubation time extension for

- the detection of *Legionella pneumophila* serogroup 1 antigen in urine. *J Clin Microbiol* [Internet] 2017;(March):JCM.02448-16. Available from: <http://jcm.asm.org/lookup/doi/10.1128/JCM.02448-16>
56. Lim WS, Baudouin S V, George RC, et al. BTS guidelines for the management of community acquired pneumonia in adults: update 2009. *Thorax* [Internet] 2009;64 Suppl 3(6):iii1-55. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21502103> <http://www.ncbi.nlm.nih.gov/pubmed/19783532>
57. Póvoa P, Martin-Loeches I, Ramirez P, et al. Biomarker kinetics in the prediction of VAP diagnosis: results from the BioVAP study. *Ann Intensive Care* 2016;6(1):32.
58. Kalil AC, Metersky ML, Klompas M, et al. Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin Infect Dis* [Internet] 2016;ciw353. Available from: <http://cid.oxfordjournals.org/lookup/doi/10.1093/cid/ciw353>
59. Fàbregas N, Ewig S, Torres A, et al. Clinical diagnosis of ventilator associated pneumonia revisited: comparative validation using immediate post-mortem lung biopsies. *Thorax* [Internet] 1999;54(10):867–73. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1745365&tool=pmc&rendertype=abstract>
60. Masterton RG, Galloway A, French G, et al. Guidelines for the management of hospital-acquired pneumonia in the UK: Report of the working party on hospital-acquired pneumonia of the british society for antimicrobial chemotherapy. *J Antimicrob Chemother* 2008;62(1):5–34.
61. Neu HC. The crisis in antibiotic resistance. *Science* [Internet] 1992;257(5073):1064–73. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/1509257>

- 602 62. Goossens H, Ferech M, Stichele R Vander, Elseviers M. Outpatient antibiotic
603 use in Europe and association with resistance: A cross-national database
604 study. *Lancet* 2005;365(9459):579–587.
- 605 63. Lipsitch M, Samore MH. Antimicrobial use and antimicrobial resistance: A
606 population perspective. *Emerg Infect Dis* 2002;8(4):347–354.
- 607 64. Melsen WG, Rovers MM, Bonten MJ. Ventilator-associated pneumonia and
608 mortality: a systematic review of observational studies. *Crit Care Med*
609 [Internet] 2009;37(10):2709–2718. Available from:
610 <http://www.ncbi.nlm.nih.gov/pubmed/19885994>
611 <http://graphics.tx.ovid.com/ovftpdfs/FPDDNCLBABFAPH00/fs046/ovft/live/gv025/00003246/00003246-200910000-00005.pdf>
- 613 65. Iregui M, Ward S, Sherman G, Fraser VJ, Kollef MH. Clinical importance of
614 delays in the initiation of appropriate antibiotic treatment for ventilator-
615 associated pneumonia. *Chest* [Internet] 2002;122(1):262–268. Available from:
616 <http://dx.doi.org/10.1378/chest.122.1.262>
- 617 66. Kuti EL, Patel AA, Coleman CI. Impact of inappropriate antibiotic therapy on
618 mortality in patients with ventilator-associated pneumonia and blood stream
619 infection: A meta-analysis. *J Crit Care* 2008;23(1):91–100.
- 620 67. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM. Diagnosis
621 of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic
622 and nonbronchoscopic “blind” bronchoalveolar lavage fluid. *Am Rev Respir*
623 *Dis* [Internet] 1991;143(5 Pt 1):1121–9. Available from:
624 <http://www.ncbi.nlm.nih.gov/pubmed/2024824>
- 625 68. Shan J, Chen H-L, Zhu J-H. Diagnostic accuracy of clinical pulmonary
626 infection score for ventilator-associated pneumonia: a meta-analysis. *Respir*
627 *Care* 2011;56(8):1087–1094.
- 628 69. Zilberberg MD, Shorr AF. Ventilator-Associated Pneumonia: The Clinical

- 629 Pulmonary Infection Score as a Surrogate for Diagnostics and Outcome. *Clin*
630 *Infect Dis* [Internet] 2010;51(S1):S131–S135. Available from:
631 <http://cid.oxfordjournals.org/lookup/doi/10.1086/653062>
- 632 70. Conway Morris A, Kefala K, Wilkinson TS, et al. Diagnostic importance of
633 pulmonary interleukin-1beta and interleukin-8 in ventilator-associated
634 pneumonia. *Thorax* 2010;65(3):201–207.
- 635 71. Hellyer TP, Conway Morris A, McAuley DF, et al. Diagnostic accuracy of
636 pulmonary host inflammatory mediators in the exclusion of ventilator-acquired
637 pneumonia. *Thorax* [Internet] 2015;70(1):41–47. Available from:
638 <http://thorax.bmj.com/lookup/doi/10.1136/thoraxjnl-2014-205766>
- 639 72. Schulte B, Eickmeyer H, Heininger A, et al. Detection of pneumonia
640 associated pathogens using a prototype multiplexed pneumonia test in
641 hospitalized patients with severe pneumonia. *PLoS One* 2014;9(11).
- 642 73. Ost DE, Poch D, Fadel A, Wettimuny S, Ginocchio C, Wang X-P. Mini-
643 bronchoalveolar lavage quantitative polymerase chain reaction for diagnosis of
644 methicillin-resistant *Staphylococcus aureus* pneumonia. *Crit Care Med*
645 [Internet] 2010;38(7):1536–41. Available from:
646 <http://www.ncbi.nlm.nih.gov/pubmed/20473148>
- 647 74. Gadsby N, Hellyer T, McMullan R, et al. 16S pan-bacterial PCR can accurately
648 identify patients with ventilator associated pneumonia. *Thorax* [Internet]
649 2016; Available from:
650 [http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L71630018%5Cnhttp://dx.doi.org/10.1007/s00134-013-3451-5%5Cnhttp://elvis.ubvu.vu.nl:9003/vulink?sid=EMBASE&issn=03424642&id=d](http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L71630018%5Cnhttp://dx.doi.org/10.1007/s00134-013-3451-5%5Cnhttp://elvis.ubvu.vu.nl:9003/vulink?sid=EMBASE&issn=03424642&id=doi:10.1007%2Fs00134-013-3451-5&atitle=16S+pan-bacterial+P)
651 [http://dx.doi.org/10.1007/s00134-013-3451-](http://dx.doi.org/10.1007/s00134-013-3451-5)
652 [5%5Cnhttp://elvis.ubvu.vu.nl:9003/vulink?sid=EMBASE&issn=03424642&id=d](http://elvis.ubvu.vu.nl:9003/vulink?sid=EMBASE&issn=03424642&id=doi:10.1007%2Fs00134-013-3451-5&atitle=16S+pan-bacterial+P)
653 [oi:10.1007%2Fs00134-013-3451-5&atitle=16S+pan-bacterial+P](http://dx.doi.org/10.1007/s00134-013-3451-5&atitle=16S+pan-bacterial+P)
- 654 75. Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High
655 serum procalcitonin concentrations in patients with sepsis and infection.
656 *Lancet (London, England)* [Internet] 1993;341(8844):515–8. Available from:

- 657 <http://www.ncbi.nlm.nih.gov/pubmed/8094770>
- 658 76. Becker KL, Snider R, Nylen ES. Procalcitonin in sepsis and systemic
659 inflammation: A harmful biomarker and a therapeutic target. *Br J Pharmacol*
660 2010;159(2):253–264.
- 661 77. Filipiak W, Filipiak A, Ager C, Wiesenhofer H, Amann A. Optimization of
662 sampling parameters for collection and preconcentration of alveolar air by
663 needle traps. *J Breath Res* [Internet] 2012;6(2):27107. Available from:
664 [http://stacks.iop.org/1752-](http://stacks.iop.org/1752-7163/6/i=2/a=027107?key=crossref.4c1053321294512bd66677cb52135658)
665 [7163/6/i=2/a=027107?key=crossref.4c1053321294512bd66677cb52135658](http://stacks.iop.org/1752-7163/6/i=2/a=027107?key=crossref.4c1053321294512bd66677cb52135658)
- 666 78. Schubert JK, Miekisch W. Breath Analysis in Critically Ill Patients—Potential
667 and Limitations. *Volatile Biomarkers* [Internet] 2013;155–176. Available from:
668 <http://linkinghub.elsevier.com/retrieve/pii/B978044462613400009X>
- 669 79. Horváth I, Barnes PJ, Loukides S, et al. A European Respiratory Society
670 technical standard: exhaled biomarkers in lung disease. *Eur Respir J* [Internet]
671 2017;49(4):1600965. Available from:
672 <http://erj.ersjournals.com/lookup/doi/10.1183/13993003.00965-2016>
- 673 80. Bossuyt P, Reitsma J, Bruns D, et al. Towards complete and accurate
674 reporting of studies of diagnostic accuracy : the STARD initiative. *BMJ Br Med*
675 *J* 2003;326:41–44.
- 676 81. Bossuyt PM, Reitsma JB, Bruns DE, et al. STARD 2015: An updated list of
677 essential items for reporting diagnostic accuracy studies. *Clin Chem*
678 2015;61(12):1446–1452.
- 679 82. Boots AW, Bos LD, Schee MP van der, Schooten FJ van, Sterk PJ. Exhaled
680 Molecular Fingerprinting in Diagnosis and Monitoring: Validating Volatile
681 Promises. *Trends Mol. Med.* 2015;21(10).
- 682 83. Chiu S-W, Wang J-H, Chang K-H, et al. A fully integrated nose-on-a-chip for
683 rapid diagnosis of ventilator-associated pneumonia. *IEEE Trans Biomed*
684 *Circuits Syst* 2014;8(6):765–778.

- 685 84. Rock F, Barsan N, Weimar U, Röck F, Barsan N, Weimar U. Electronic nose:
686 Current status and future trends. *Chem Rev* [Internet] 2008;108(2):705–725.
687 Available from:
688 http://pubs3.acs.org/acs/journals/doilookup?in_doi=10.1021/cr068121q%5Cnpapers://316bc3e6-3d1b-427b-a4e3-80bd1b4d480e/Paper/p1578
689
690 85. Bean HD, Zhu J, Hill JE. Characterizing bacterial volatiles using secondary
691 electrospray ionization mass spectrometry (SESI-MS). *J Vis Exp*
692 2011;2(52):3–6.

Table Legends

Table 1. Full text selected articles

The selected articles either concern clinical (C) or pre-clinical (P) studies.

Investigated inorganic compounds include nitric oxide (NO) and carbon monoxide (CO). The investigated study population could be on ICU patients in general, or could involve a specific target group, e.g. patients with COPD (Chronic obstructive pulmonary disease), Community-acquired pneumonia (CAP) or Ventilator-associated pneumonia (VAP). As a reference test a protected specimen brush (PSB), bronchoalveolar lavage (BAL) or endotracheal aspirate (ETA) was used. The used index test to analyse Volatile Organic Compounds (VOCs) could involve secondary electrospray ionization – mass spectrometry (SESI-MS), Clinical Pulmonary Infection Score (CPIS), eNose technology or gas chromatography – mass spectrometry (GC-MS): either sensitivity (sens) and specificity (spec), or accuracy has been displayed in the Table.

*For details: see Table 3.

Table 3. VOCs identified by GC-MS: The VOCs are either increased (↑) or

decreased (↓) in the exhaled breath of pneumonia patients when compared to patients without pneumonia.

Table 1. Full text selected articles

Type	Study	Population	Sample size	Index test	Disease under study; Reference test	Sens	Spec	Accuracy	Comments
C	31	ICU patients	49	NO analyser	VAP; Clinical signs + culture of unknown specimen	88%	76%		
C	32	General ward	42	NO analyser	CAP; Clinical signs + X-ray				Quantitative; lower NO concentration
C	47	Outpatient clinic	72	CO analyser	CAP; Clinical signs			~90%	Accuracy estimated from figure
C	80	ICU patients	76	eNose	VAP; Clinical signs			~90-100%	
C	45	ICU patients	28	GC-MS	VAP; Culture of BAL / ETA			Unknown	Quantitative
C*	34	ICU patients	46	GC-MS	VAP; Culture of ETA	98%	98%		Invasively ventilated patients at risk for VAP
C	46	ICU patients	60	GC-MS	VAP; Clinical signs + radiology + culture of BAL / PSB / ETA			88-89%	
C	36	ICU patients	25	eNose	VAP; Chest CT scan			91-100%	
C	37	ICU patients	44	eNose	VAP; CPIS > 6			70%	
C*	33	ICU patients	100	GC-MS	VAP; Clinical signs + radiology + culture of BAL	76%	73%		Only patients that were suspected of VAP
C	39	ICU patients	125	eNose	VAP; Clinical signs + radiology + culture of BAL	76%	56%		Only patients that were suspected of VAP
C	38	COPD patients	43	eNose	CAP; Sputum cultures	73%	76%		
C*	35	ICU patients	93	GC-MS	VAP; Clinical signs + radiology + culture of ETA	75%	72%	73%	
P	43	Mice	12	SESI-MS	Instillation of MRSA or MSSA			100%	Experimental model with <i>Staphylococcus</i>
P	44	Mice	87	SESI-MS	Instillation of alive or lysated bacteria			100%	Experimental model with alive or dead bacteria
P	41	Mice	?	SESI-MS	Instillation of <i>Pseudomonas</i> or <i>Staphylococcus</i>			Unknown	Quantification of difference between in vitro and in vivo
P	40	Mice	42	SESI-MS	Instillation of seven different bacterial strains			Unknown	Quantification of difference between pathogens
P	42	Mice	?	SESI-MS	Instillation of <i>Pseudomonas</i> or <i>Staphylococcus</i>			Unknown	Quantification of dynamic changes in breath VOCs

Table 2. QUADAS-2 (adapted version)

Study	Risk of Bias				
	Year	Patient selection	Index test	Reference standard	Flow and timing
Adrie ³¹	2001	No	No	No	No
Ali-Ali ³²	2001	Yes	Yes	Yes	No
Bean ⁴⁴	2014	N/A	Yes	No	No
Bean ⁴⁵	2015	N/A	Yes	No	No
Biernacki ³³	2001	Yes	Yes	Yes	No
Chiu ⁸³	2014	Yes	Yes	Yes	Unclear
Filipiak ⁴⁶	2015	Yes	Yes	No	No
Fowler ³⁵	2015	Unclear	Yes	No	No
Gao ⁴⁷	2016	Yes	Yes	No	No
Hockstein ³⁷	2004	Yes	Yes	Yes	No
Hockstein ³⁸	2005	Yes	Yes	Yes	No
Schnabel ³⁴	2015	Unclear	Yes	No	No
Schnabel ⁴⁰	2015	No	Yes	No	No
Van Geffen ³⁹	2016	Yes	Yes	No	No
Van Oort ³⁶	2017	No	Yes	No	No
Zhu ⁴²	2013	N/A	Yes	No	No
Zhu ⁴¹	2013	N/A	Yes	No	No
Zhu ⁴³	2013	N/A	Yes	No	No

Table 3. VOCs identified by GC-MS: increased (↑) or decreased (↓) in breath of pneumonia vs. no pneumonia patients

Classification	Volatile Organic Compound	Reporting article		
		Schnabel ³⁴	Fowler ³⁵	Van Oort ³⁶
Ketone	Acetone	↓		↓
	2-methyl cyclopentanone		↓	
	Methylisobutylketone			↓
Aldehyde	Acrolein	↓		
	Nonanal		↑	
	Tetradecanal	↑		
Ether	Sevoflurane			↓
	Tetrahydrofuran	↓		
Alkane	2-methyl butane	↑		
	2-ethoxy-2-methyl propane			↓
	Carane	↑		
	Dodecane	↓		
	Heptane	↑	↓	
	Tetradecane	↑		
	2,6,11,15-tetramethyl-hexadecane		↑	
Alkene	Cyclohexene			↓
Terpene	3-carene		↑	
Alcohol	Ethanol	↑	↓	
	Isopropyl Alcohol	↓		
	Hexafluoroisopropanol			↓
	1-propanol			↓
Arene	Ethylbenzene	↑		
Ester	N-butyric acid 2-ethylhexyl ester		↑	
Sulfide	Carbon disulfide			↓
Amide	N-cyclohexyl-N'(2-hydroxyethyl)thio-urea		↓	

Figure Legends

Figure 1. The aspired contribution of breath analysis regarding antibiotic stewardship for pneumonia (↑: increase)

Figure 2.

Exhaled breath analysis for the prediction of pneumonia: several available techniques and accompanying analytical principles.

eNose: Conventional electronic noses consist of an array of gas sensors using transducer principles (e.g. through metal oxide sensors, conducting polymer sensors or surface or bulk acoustic wave sensors)⁸⁴, whereas new approaches involve optical sensor systems and colorimetric sensors¹⁷; GC-MS: Gas chromatography – mass spectrometry is currently seen as the preferred method for separation, detection and identification of individual VOCs; SESI-MS: Secondary electrospray ionization – mass spectrometry enables rapid detection of VOCs without the need for sample pretreatment⁸⁵. After introduction into the SESI reaction chamber the sample passes through an electrospray cloud that ionizes the volatiles, after which the ionized VOCs are detected in the mass spectrometer. The method cannot be used to quantify individual VOCs, as many volatiles have similar molecular weights and the methodology relies on pattern recognition. GC-MS and SESI-MS can both be used for targeted and untargeted analysis.

Figure 3. Flow diagram of article selection.

VOC: Volatile Organic Compound; GC-MS: Gas chromatography – mass spectrometry; SESI-MS: Secondary electrospray ionization – mass spectrometry.

Figure 1

Figure 2.

Figure 3.