

SUPPLEMENTARY FILES

Methodological Approaches towards a Gastrointestinal Cancer (MAGIC) breath test in primary care

Authors:

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SUPPLEMENTARY FILE 1: Names of 26 GP practices participating in the MAGIC breath test study:

Phase 1:

1. Aksyr Medical Practice, NW10 8RY
2. Oxgate Gardens Surgery NW26EA
3. Hillcrest surgery, W3 9RA
4. Dr Jefferies and Partners, Fulham, SW6 6BQ
5. The Law Medical Group practice Willesden NW10 5UY
6. The Law Medical group practice Harrow HA96QQ
7. The Gill medical practice, Feltham TW14 0AB
8. Grove Park Terrace Surgery Chiswick W4
9. The Bush Doctors W12 8PP
10. Twickenham Park Medical Centre, TW13 6HD
11. Buckingham Road Surgery NW10 4RR
12. Fulham Medical Centre, SW6 1BG
13. Acre Surgery, HA6 1TQ
14. Gladstone Medical Centre, NW2 6JH
15. Cuckoo Lane Practice, Hanwell, W7 1DR
16. Wembley Park Medical Centre, Wembley, HA9 8HD

Phase 2:

17. Pimlico Health, SW1V 3EB
18. Lonsdale Medical Centre, NW6 6RR
19. The Good practice, SW10 0LR

7 practices as part of Central London Healthcare (CLH) GP federation:

20. Woodfield Road Medical Centre, W9 3XZ
21. Covent Garden Medical Centre, WC2H 9AA
22. Cavendish Health Centre, W1G 9TG
23. Marylebone Health centre, NW1 5LT
24. Fitzrovia Medical Centre, W1T 6EU
25. Newton Medical Centre, W2 5LT
26. Crawford Street Surgery, W1H 2HJ

SUPPLEMENTARY FILE 2: Summary of National Institute for Health and Care Excellence (NICE) guidelines for gastrointestinal (GI) cancer referral 2016, available at:

<https://www.nice.org.uk/guidance/ng12/chapter/1-Recommendations-organised-by-site-of-cancer#upper-gastrointestinal-tract-cancers>

Upper GI cancers

Two week wait (2WW) direct access oesophago-gastro-duodenoscopy (OGD) for:

- 1) Dysphagia
- 2) Age >55 years with weight loss AND upper abdominal pain/reflux/dyspepsia

Non urgent direct access OGD:

- 1) Haematemesis
- 2) Age >55 years with
 - persistent dyspepsia OR
 - upper abdominal pain WITH anaemia OR
 - raised platelets with nausea/vomiting/weight loss/reflux/dyspepsia/upper abdominal pain OR
 - nausea/vomiting with weight loss/reflux/dyspepsia/upper abdominal pain

2WW computerised tomography scan/abdominal ultrasound scan for:

-Abdominal mass
(stomach and gallbladder and liver cancers)

Pancreatic cancer

2WW appointment for:

- Age >40 years with new jaundice

2WW CT scan/Ultrasound scan:

- Age >60 years AND weight loss AND diarrhoea/back pain/abdominal pain/nausea/vomiting/constipation/diabetes


Colorectal cancers

2WW appointment for:

- 1) Age >40 years with weight loss and abdominal pain
- 2) Age >50 years with rectal bleeding
- 3) Age >60 years with anaemia/change in bowel habit/positive faecal occult blood test
- 4) Abdominal mass
- 5) Age <50 years with rectal bleeding AND abdominal pain/ change in bowel habit /weight loss/anaemia

SUPPLEMENTARY FILE 3: Poster for GPs

Imperial College
London

Imperial College Healthcare 

Breath Testing for Gastrointestinal Disease 2016- 2017

- Please ask all patients with **current/recent** (within 2months) **gastrointestinal symptoms** if they would consider speaking to a research nurse about this study.
- Patients are also eligible with **chronic gastrointestinal problems**, even if controlled on medication.
- Patients must be >18 or <90 years of age, and be able to speak to a research nurse in order to consent to giving a breath sample.

We are testing the feasibility of breath testing for the diagnosis of GI disease.

- **ANY/ALL gastrointestinal symptoms are accepted in this study.** These could include abdominal pain, diarrhoea, constipation, reflux/dyspepsia, dysphagia, change in bowel habit, nausea, vomiting, weight loss, anaemia, GI bleeding, jaundice, or others.

- The study involves giving a breath sample and will take 5-10minutes.
- A research nurse will explain the breath test to the patient and consent them.

Please advise patients to ask at reception to see the clinical research nurses who will be based in the GP practice, or please email: ichc-tr.breathtest@nhs.net with the patient details.

Primary Care Poster v.3.0 16/12/2016. Dr G Woodfield, Prof G Hanna

SUPPLEMENTARY FILE 4: Letter for GPs

Imperial College
London


**National Institute for
Health Research**

Clinical Research Network
North West London

Division of Surgery
Department of Surgery and Cancer
10th Floor, QEQM Building
St Mary's Hospital
Praed Street, London, W2 1NY

Tel: +44 (0)20 3312 2125
Fax: +44 (0) 020 3312 6309

g.hanna@imperial.ac.uk
www.imperial.ac.uk

Professor George Hanna PhD FRCS
Head of Division of Surgery

14th December 2016

Dear North West London General Practitioners,

Thank you very much for agreeing to participate in the study:

Non-invasive testing for the diagnosis and assessment of gastro-intestinal disease – Primary Care feasibility Study. November 2016 to March 2017.

The study aims to trial the use of a breath test in patients with gastrointestinal symptoms, as a future device for detecting oesophago-gastric, pancreatic and colorectal cancer. This initial trial is a feasibility study of 500 patients. The breath test is not currently a diagnostic tool, as there is no validated "positive" or "negative" result to be gained currently, GPs and patients therefore will not be informed of results. It therefore should not influence patient management or referral pathways in any way.

Recommendation for GPs:

- Please look out for patients who have/have had GI symptoms. Symptoms include **dysphagia, weight loss, abdominal pain, reflux, dyspepsia, nausea, vomiting, diarrhoea, constipation, change in bowel habit, abdominal mass, GI blood loss, jaundice** or any other variation of GI symptoms. Patients are eligible if they have had **current/recent symptoms (within 2 months)**, OR if they have ever consulted the GP with GI symptoms, where the symptom is **chronic or requires medication to control it**. This includes all patients on frequent anti-reflux medications, laxatives or anti diarrhoeals for example. The symptom does not have to be present on the day of testing.
- Please ask these patients if they would mind talking to a research nurse about performing a breath test as part of our research study. If it is outwith the time that the research nurse is in attendance, please ask them if they would mind being contacted by phone.
- Research nurses will be stationed in practices for 1-2 week blocks. If a research nurse is in your practice that week, please send the patient to see the nurse straight away.
- If it is out of hours or on a week where the research nurse is not present, please email their Name, Phone number and Practice name to ichc-tr.breathtest@nhs.net
- The research nurse will then contact them in a few days time to ask them to come in to perform the breath test.
- Research nurses will give an information leaflet to patients, consent them, document basic medical history and perform the breath test. It should take about 5-10 minutes.
- Practices will be compensated financially for their time in sending patients to see the research nurse for a breath test, and for every patient they highlight via email for the study. (£5 per patient referred)
- Please be aware that neither GPs nor patients will get any feedback/results after their breath test, as both the technology and analysis are still in development. This is not currently a diagnostic tool.

Should you have any questions please don't hesitate to contact me. Thanks again for your cooperation with this study.

Yours sincerely,

Dr Georgia Woodfield, Clinical Research Fellow Imperial College. (g.woodfield@imperial.ac.uk)

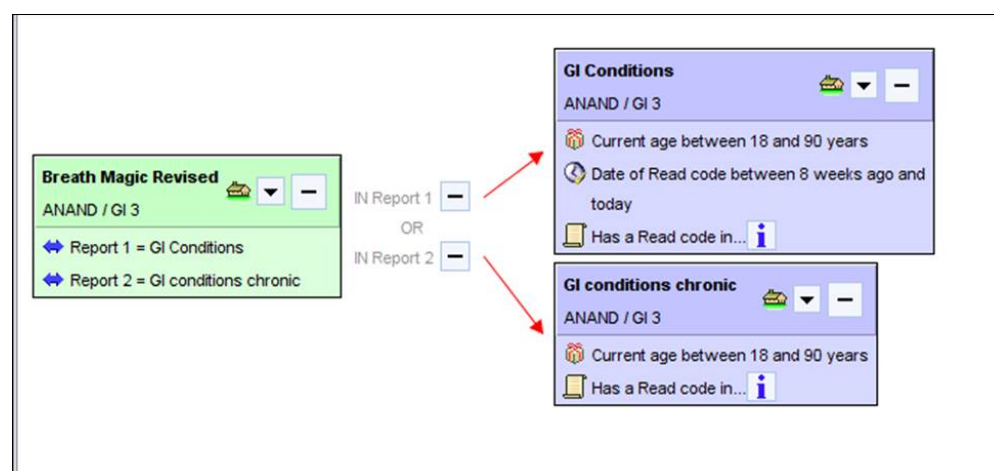
Professor George Hanna PhD FRCS, Principle Investigator

SUPPLEMENTARY FILE 5: Example of GP database searches

The following database searches were performed in order to identify patients for potential text (SMS) recruitment during phase 2 of Breath MAGIC, as part of the hub and spoke model of sampling. Of note, this search example is the most complex and thorough search done within the Breath MAGIC study. In phase 1, local GP practices did simple local database searches performed by local GP receptionists or GPs themselves based on specific symptoms or medication use. This is because only small numbers of patients were needed per practice in phase 1. The aim of this more complex search was to reach as many eligible patients as possible from 7 GP practices in the Central London Healthcare (CLH) GP federation for General Practices in Westminster, to be breath-tested at the central hub (Marylebone Health Centre). The database search was performed by central CLH administrative staff at CCG level (Ahmed Hosny and Anand Bhundia- GP Network Support Officers CLH), neither of whom worked directly in the participating practices. The large searches of CLH records for the hub and spoke sampling strategy was done as follows:

Search 1**Gastrointestinal symptoms recorded in the past eight weeks (including those coded as chronic) AND age 18-90 years inclusive**

Search 1 therefore picked up patients who fulfilled the 1st and 2nd inclusion criteria for the study (gastrointestinal symptoms today or within last 8 weeks).



gastrointestinal symptoms are represented by “i” in the diagram above. Symptoms (with database read codes) included:

Indigestion (1954.)
Abdominal pain (1969.)
Altered bowel function (19EA.)
Diarrhoea (19F2.)
Viral gastroenteritis (A07y0)
Gastro-oesophageal reflux disease with ulceration (J1020)
[D]Dysphagia (R072.)
[D]Change in bowel habit (R078.)
[D]Abdominal pain (R090.)
Gastric reflux (Ua1kQ)
Gastro-oesophageal reflux disease (X3003)
Gastritis (X301N)
Gastroenteritis (X30BN)
Nausea (X75qw)
Jaundice (X769z)
Weight loss (X76CA)
Flatulent dyspepsia (X76d5)
Campylobacter gastrointestinal tract infection (XE0QI)
Gastro-oesophageal reflux disease with oesophagitis (XE0aL)
Gastro-oesophageal reflux disease without oesophagitis (XE0aO)
Irritable bowel syndrome (XE0as)
Biliary tract disorders NOS (XE0dR)
Constipation (XE0rD)
[D]Abdominal mass (XE2nV)
Dysphagia (XM08J)
Abdominal mass (XM097)
Bacterial gastroenteritis (XM0pJ)
Nausea and vomiting (Xa1pJ)
Moderate gastric reflux (Xa7Ta)
Minimal gastric reflux (Xa7Tb)
Gastric aspirate containing blood (Xa7Tj)

Chronic conditions:

Chronic gastric ulcer (J111.)
Chronic gastrojejunal ulcer (J141.)
Chronic gastritis (J151.)
Chronic constipation with overflow (J5201)
Chronic nonspecific abdominal pain (X3062)
Chronic constipation (X30BI)
Chronic diarrhoea (X30Bn)

Search 2

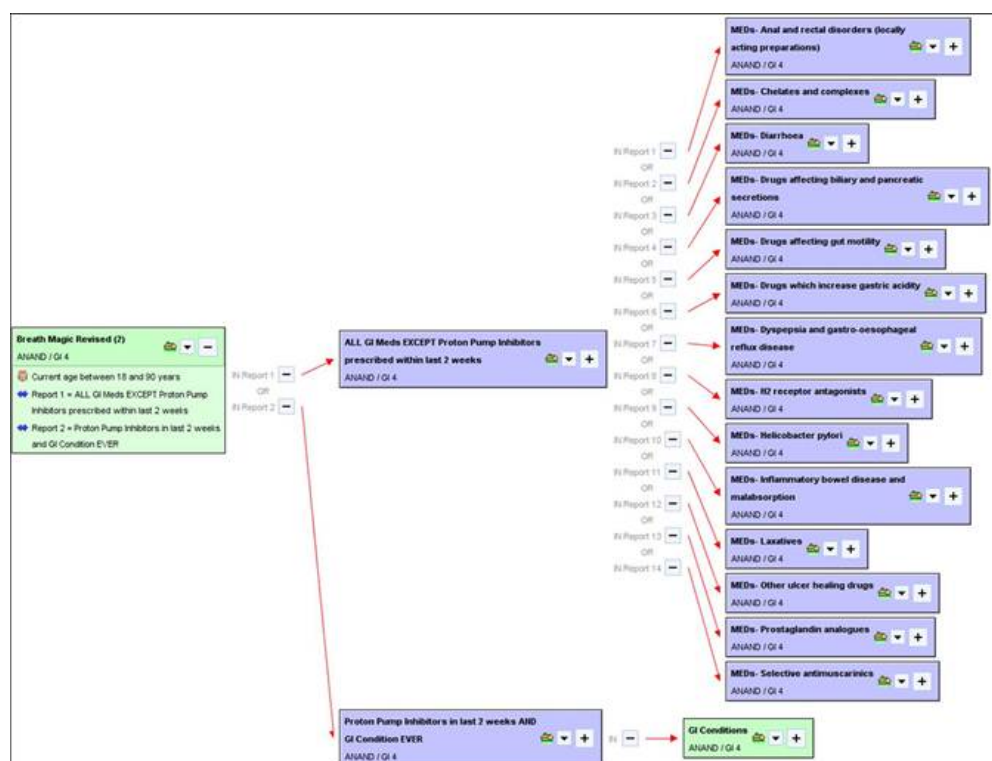
Currently on gastrointestinal medications except proton pump inhibitors (PPIs) prescribed within last two weeks AND age 18-90 years inclusive.

OR

Patients prescribed PPIs (within last two weeks) AND a recorded gastrointestinal condition at any point in their records.

Search 2 therefore picked up patients who fulfilled the 3rd inclusion criteria for the study (chronic gastrointestinal condition controlled on medication).

The caveat with PPIs was that we did not want patients who were on PPIs for non-gastrointestinal reasons; e.g. for patients who were on steroids. For this reason the search started with “All GI meds EXCEPT PPIs prescribed in last two weeks” AND “PPIs in last two weeks AND GI condition EVER”.



Gastrointestinal medications that were included as part of this search are encoded by the headings on the right of the diagram above.

Included categories and subcategories of gastrointestinal medications were:

Actions

- ↳ Gastro-intestinal
 - ↳ Dyspepsia and gastro-oesophageal reflux disease
 - ↳ Antacids and simeticone
 - ↳ Aluminium- and magnesium- containing antacids
 - ↳ Hydrotalcite
 - ↳ Antacid preparations containing simeticone or local
 - ↳ Simeticone
 - ↳ Sodium citrate
 - ↳ Raft-forming indigestion remedies
 - ↳ Alginate preparations
 - ↳ Indigestion remedies
 - ↳ Drugs affecting gut motility
 - ↳ Antimuscarinics
 - ↳ Other antispasmodics
 - ↳ Motility stimulants
 - ↳ Gastroprotection
 - ↳ H2 receptor antagonists
 - ↳ Selective antimuscarinics
 - ↳ Chelates and complexes
 - ↳ Prostaglandin analogues
 - ↳ Proton pump inhibitors
 - ↳ Other ulcer healing drugs
 - ↳ Helicobacter pylori
 - ↳ Diarrhoea
 - ↳ Adsorbent & bulk-forming drugs
 - ↳ Antimotility drugs
 - ↳ Intestinal antisecretory agents
 - ↳ Inflammatory bowel disease and malabsorption
 - ↳ Aminosalicylates
 - ↳ Corticosteroids (in chronic bowel disorders)
 - ↳ Immunosuppressants (in chronic bowel disorders)
 - ↳ Food allergy
 - ↳ Other drugs to treat inflammatory bowel disease
 - ↳ Laxatives
 - ↳ Bulking agents
 - ↳ Stimulant laxatives
 - ↳ Stool softeners
 - ↳ Osmotic laxatives
 - ↳ Colonic evacuation
 - ↳ Peripheral opioid-receptor antagonists
 - ↳ Other laxatives
 - ↳ Anal and rectal disorders (locally acting preparations)
 - ↳ Soothing haemorrhoidal preparations
 - ↳ Corticosteroid haemorrhoidal preparations
 - ↳ Haemorrhoidal sclerosants
 - ↳ Anal fissures

SUPPLEMENTARY FILE 6: Text (SMS) recruitment

Text wording was as follows (one practice used personalised text messaging as this was their usual method of text communication):

“Are you available to donate your breath for cancer research? (..Name..) Surgery are asking our patients to help develop a new breath-testing device. A sample of breath will be collected at the practice during a 15-minute visit with a researcher. You will be helping to develop a new tool that could potentially be used for early cancer diagnosis in the future. Are you interested in hearing more? Text YES for a callback”

5. Would a breath test to detect/rule out cancer be a useful future tool?
(please circle)

a. Point of care device with instant results

0 1 2 3

b. Breath test done in same way as a blood test, with results electronically

0 1 2 3

0= Not useful 1= Not sure 2= Useful 3= Very useful

6. If you think breath testing could be useful, which patient groups do you think it would particularly benefit?

.....

7. What do you think is a reasonable cost for GP surgeries to pay for one patient to have a breath test?

.....

8. How many patients complaining of general gastrointestinal symptoms do you see on average per day as a GP?

.....

9. Did your request to recruit patients for the study have any impact on the quality of your consultation? (please circle)

0= negative impact

1= no impact

2= minimal impact

3= Positive impact

0 1 2 3

Please elaborate on any reasons for your answer.....

10. Did patients raise questions or concerns when you mentioned the study? If so what were their concerns?

.....

11. How could we have improved the organisation of the study?

.....

.....

Please do not hesitate to contact me with further comments or questions:
g.woodfield@imperial.ac.uk

GP Questionnaire Version 5.0 16/1/17

SUPPLEMENTARY FILE 8: Patient acceptability questionnaireNon-invasive testing for the diagnosis and assessment of gastro-intestinal disease-
Patient Acceptability Questionnaire

General Practice name..... Study ID..... Date.....

Please tick the box corresponding to your level of agreement with the following statements:

1.	Yes, today	Yes, in the past 2 months	Yes, over 2 months ago	No, not within the past 5 years	Never
Have you seen a doctor because of stomach/ bowel/abdominal symptoms in the past 5 years?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

2.	More than 6 months	Between 2-6 months	Less than 2 months	Less than 1 week	Not applicable
How long did you have your most troubling symptom before seeing a doctor?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

3.	Extremely	Quite a bit	Moderately	Slightly	Not at all	Not applicable
How worried were you about your abdominal symptoms when you had them?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

4.	Very satisfied	Satisfied	Dissatisfied	Very Dissatisfied	Not applicable
How satisfied were you with the explanation given for how to do the breath test?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

5.	Very easy	Easy	Difficult	Very Difficult	Not applicable
How easy was it to do the breath test?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

If you found it difficult/very difficult, please explain why.....

.....

6.	Very comfortable	Comfortable	Uncomfortable	Very uncomfortable	Not applicable
How comfortable were you whilst wearing the face mask?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Patient Questionnaire Version 9.0 5/7/17

7.	Took too long	Acceptable amount of time	Too quick	Not applicable
What did you think about the time it took to give a breath sample?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

8.	Very comfortable	Comfortable	Uncomfortable	Very uncomfortable	Not applicable
How did you find the experience of holding the device during the test?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

9.	Very comfortable	Comfortable	Uncomfortable	Very uncomfortable	Not applicable
Would you be comfortable to do the breath test again, if recommended by a doctor?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

10.	Strongly encourage	Encourage	Discourage	Strongly discourage	Not applicable
Would you encourage family and friends who were offered a breath test to complete it?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

11. How could the breath test be improved?

.....

.....

.....

.....

Please do not hesitate to contact me with further comments or questions:

Dr Georgia Woodfield
 Clinical Research Fellow, Imperial College London
 g.woodfield@imperial.ac.uk

SUPPLEMENTARY FILE 9: Quality control (QC) process for lab instruments

Two types of QC were performed daily for the proton transfer reaction time-of-flight mass spectrometer (PTR-ToF-MS) (1). A first *instrument QC* evaluated instrument stability with the three ionisation modes (H_3O^+ , NO^+ and O_2^+) against four parameters: impurities, fragmentation, mass resolution and accuracy. These were measured by using a permeation unit (a unit generating a constant flow of gaseous standard VOCs with known concentrations) which was connected directly to the PTR-ToF-MS for five minutes. Impurities levels below 10% were considered acceptable. Accuracy was evaluated through quantification of a benzene certified standard permeation tube (Kin-Tek Analytical Inc., La Marque TX). The PTR-ToF-MS quantitative measurement had to be within 20% of the certified standard in order to pass the QC. Fragmentation had to be above 60% to pass the QC. Butyric acid fragmentation was used as the check for H_3O^+ , where the ratio of diagnostic ions was used m/z 89 / (m/z 43 +71+89). For NO^+ butanal fragmentation was used, where the ratio of diagnostic ions was used m/z 71 / (m/z 43 +71+89). Resolution had to be above 1500 $m/\Delta m$. Our lab has a standard operating procedure for measuring instrument reproducibility with an action plan of what to do when parameters are not reaching the appropriate levels. No one can use the instrument for analysis until it passes the QC.

The second *standard QC* check evaluated the recovery of VOCs from TD tubes loaded from the permeation unit (2). Tube loading was performed at a flow of 0.910 (+-0.010) L/min at a temperature of 30 degrees Celsius. This was done by connecting a pocket pump to the permeation tube inlet via a TD tube. Flow across the tube was achieved by exploiting the permeation unit flow with the addition of the pocket pump. VOCs from the permeation unit were passed through the tube for 2.5 minutes. The VOC recovery was then measured by analysing the TD tubes using the PTR-ToF-MS. This test is performed daily before laboratory users can use the instrument.

For the gas chromatography mass spectrometer (GC-MS), five TD tubes loaded with a standard mixture (as explained above) were analysed daily. Retention time, peak shape and peak area were used to assess consistency and accuracy of the instrument response.

SUPPLEMENTARY FILE 10: Quality control process for TD tubes

Our lab follows a simple threshold system to identify whether there is breath present on a TD tube in sufficient quantities for it to be allowed to proceed to analysis. The full paper presenting this method is pending publication.

The QC system works by checking the VOC data from each TD tube to see that it reaches the minimum level for concentration of a particular reference compound (compound differs depending on whether the TD tube was analysed by PTR-ToF-MS H_3O^+ , NO^+ or O_2^+ ionisation or GC-MS). VOC data from TD tubes with inadequate levels of the reference compound, and therefore inadequate levels of breath within them, are discarded before data analysis.

This QC system is required because when collecting a breath sample in TD tubes it may not be immediately obvious that the full 500ml of breath has passed through the TD tube, as even where the ReCIVA software indicates the correct volume collected, breath can be lost if the caps are not tightened adequately on the tube post collection, or if the TD tube ends were not tightly sealed during breath transfer.

Our lab identified thresholds for particular reference compounds:

- **Acetone >45 ppb for PTR-ToF-MS (H_3O^+ ionisation)**
- **Isoprene >2.5 ppb for PTR-ToF-MS (NO^+ ionisation)**
- **Isoprene >5 ppb for PTR-ToF-MS (O_2^+ ionisation)**
- **Acetone >7,500,000 area counts for GC-MS**

These thresholds were identified by comparing breath samples to a control group of TD tubes with non-biological samples that consisted of (i) empty conditioned TD tubes; (ii) 500 ml of room air samples collected onto TD tubes using ReCIVA, following a procedure similar to that adopted for patient breath and (iii) TD tubes, previously conditioned and then loaded with a standard mixture of benzene (63 ppb, certified standard, Kin-Tek Analytical Inc., La Marque TX), phenol (90 ppb), butyric acid (20 ppb), pentanoic acid (5 ppb), hexanoic acid (5 ppb), decanal (4 ppb) and butanal (5 ppb), generated by a permeation unit (ES 4050P, Eco Scientific, Gloucestershire UK).

Over 100 breath samples were compared to over 100 controls for each ionisation of the PTR-ToF-MS and for the GC-MS, 1097 samples in total.

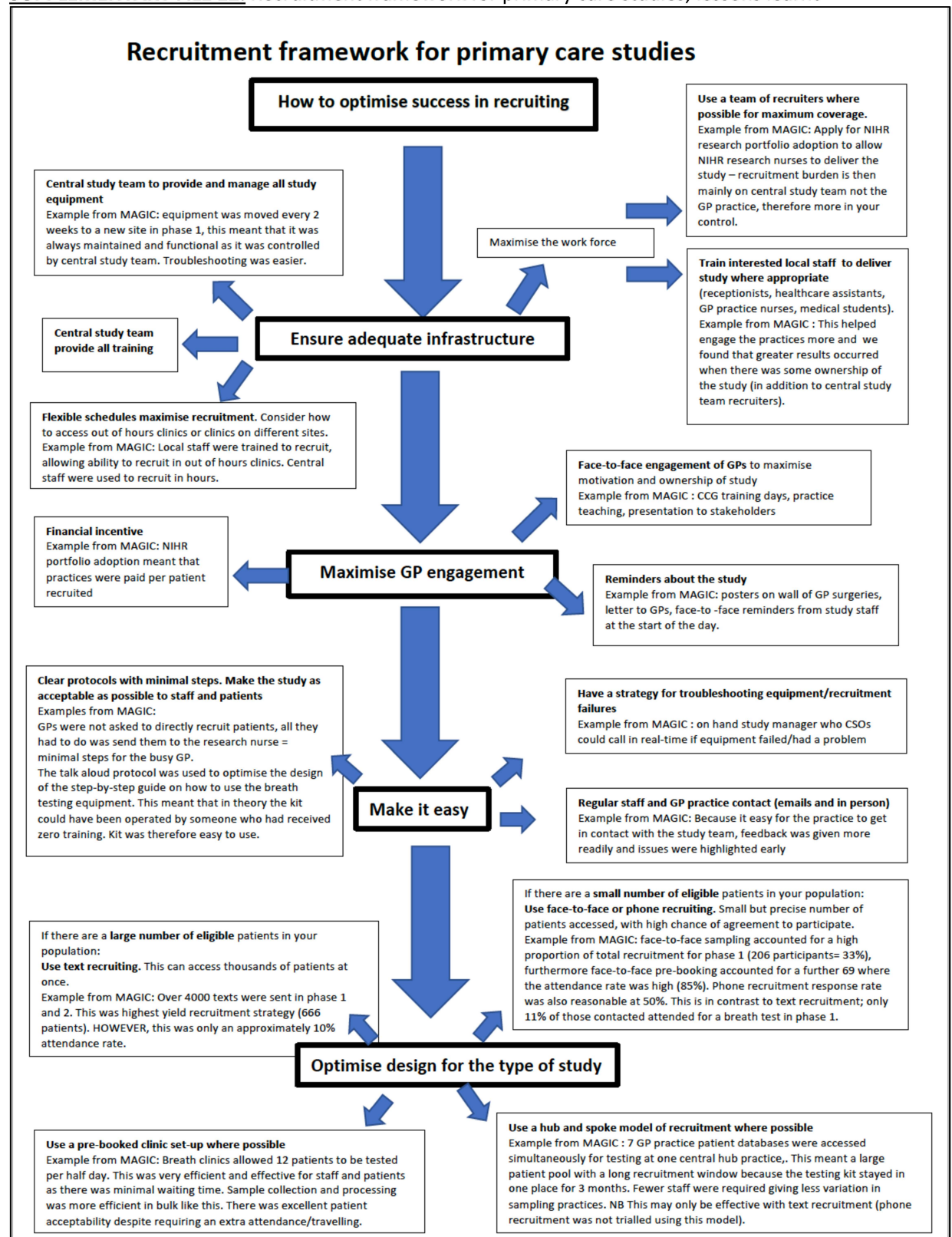
Based on this work, TD tubes which contain high levels of the appropriate reference compound are assumed to have high enough concentrations of breath collected onto the TD tube. The sample is then deemed to be adequate for inclusion in our group's study data.

SUPPLEMENTARY FILE 11: Summary of barriers to recruitment and subsequent implementations, during Phase 1 of Breath MAGIC study (from Field notes, teleconference and focus group of CSOs)

Recruitment Barrier	Cause of this problem (CSO view point)	Implementation made	Effect of implementation	Resolved?
1. Slow referral of patients, leading to slow recruitment and inefficient use of time for CSOs (6 of the first 10 days of sampling had 0 or 1 referrals per day only)	-Busy GP practices: GPs didn't have time - GPs varied in their interest levels in research -Locums were less likely to refer -Some had not heard about the study <i>"Nurses and HCAs would refer patients but GPs had to be prompted every session."</i> <i>"6 patients were found in the system to have GI symptoms, but none were referred. When asked directly, GPs and nurses said they hadn't remembered."</i>	-Emphasis on daily interaction of CSOs with GPs, reminding them of study and inclusion criteria - New GP poster and information leaflet was made	-Improved experience of CSOs meant they engaged more with GPs - Poster and leaflet helped guide discussion and acted as a reminder for GPs	Partially... - Still a marked variation between engagement levels of different individuals, often heightened by very busy practices
2. Persistence of above problem, Face-to-face sampling therefore inconsistent between practices	- As above, problem not fully solved	-Addition of phone and text bookings as well as face-to-face pre-booking. -Inclusion of referrals from other healthcare staff e.g specialist/practice nurses and HCAs doing clinics alongside GPs	-Tailored to practice resources; played to strengths of each practice -Engaged receptionists and HCAs in the recruitment task- drastically increased numbers - Efficient use of CSO time with dedicated breath clinics -However relied on staff time to pre-identify and call/text patients	Recruitment increased exponentially Target reached 6 weeks ahead of schedule
3.Smaller practices had lower recruitment	Some small practices had fewer GPs and fewer sessions. This meant that on days that there were baby clinics/other specialist clinics, no sampling could occur	-Combine practices that are close together, to boost recruitment and make CSOs time more efficient.	We combined Carepoint into Acre surgery in Northwood, so that patients could be referred from either site to see the same nurse. This helped recruitment.	Good solution for these practices, but not possible in every location.
4.Labour intensive sampling: requires a dedicated CSO present all day for sampling. Costly from central research team staff perspective	-The study design relied on central research team doing all the recruitment and breath testing.	-We trained practice staff to deliver the breath test instead of central study team. HCAs, nurse practitioners, local research nurses and a medical student (after GCP training) were trained in 4 practices, which covered the sampling over 10 study weeks	This was a huge success. It saved a lot of central study team time/resources. Local teams found it easy to recruit as they knew their colleagues and often knew the patients, familiar with the computer systems and had their own clinic rooms. They also recruited during evening clinics as this was their expected working hours at the GP practice.	Yes – This also showed how a breath test could be used in future by multiple different staff members. There was also the added bonus of great collaboration with sometimes new GP practices with great engagement from many different staff members.
5.Study timings of 9-5pm not always matching GP clinic hours	Some GPs have evening clinics and have admin time in the afternoons-not good for patient sampling	-CSO staff hours were adjusted where possible -Addition of face-to-face pre-booking enrolment so	This strategy allowed sampling outside our planned sampling hours.	Yes- recruitment was excellent during the weeks where we used local staff, and it had other positive effects

		that patients could return within hours. -Local practice staff were trained (see above) which meant they could sample in late clinics if this was their usual working pattern	Other out of hours patients could also still be recruited at a later date using face-to-face pre-booking	such as cost saving and increased engagement with local practices and different allied health professionals.
6. Equipment shortages and delays	-Mask supply ran out at one point in the study -GC Mass spectrometer malfunctioned at one point, leading to backlog of TD tubes and therefore a supply issue	-For problematic weeks, sampling was limited to 20 patients per week. This affected 8 of the sampling weeks (6 practices affected). - Samples were processed on the PTR-ToF-MS where possible, allowing clearance of back log	-20 per week cap was not a problem for most GP practices as long as they knew in advance -Only one GP practice was negatively affected where 3 days of sampling had to be cancelled -3 weeks of recruitment was significantly affected by this issue because phone bookings were not made in practices where this would have been the recruitment method.	Resolved at the time with back-up alternative instrument -Instrument malfunctions are unavoidable but this could also be mitigated by a larger back-up supply of tubes - Masks will now be ordered a year in advance for next time.
7. Problems with rooms/space	Room availability- <i>"There was a pressure on rooms today, and I had to move rooms mid-course during recruitment and find a suitable computer to work from. The recruitment was fairly low as a result".</i> Upstairs rooms – <i>"being based upstairs as opposed to downstairs where most patients were being seen impacted on recruitment"</i>	Requesting downstairs rooms where possible/rooms near where the patients were being seen.	Not clear what the effect of the intervention was, as room availability could often not be controlled, but anecdotally the nurses felt that more patients came when the sampling room was easy/accessible.	Partially resolved by enquiring and requesting certain rooms, but room availability was often largely not within our control.

SUPPLEMENTARY FILE 12: Recruitment framework for primary care studies; lessons learnt



SUPPLEMENTARY FILE 13: GP opinions about place of the breath test in future care.

Twenty-one GPs, from 10 of the 26 participating practices, answered the GP specific questionnaire. GPs felt the breath test would be best placed as a point of care test with instant results, considering this *“very useful”* (88%) or *“useful”* (8%). 10 GPs (48%) felt testing would still be useful if results were available electronically at a later date. There were varied views about which groups of patients would benefit most from a breath test, ranging from *“all age, any group”* to *“low risk cancer patients without red flag symptoms”*, to *“at risk groups”*. Of the eleven GPs who said *“at risk groups”*, six of them qualified this with specific symptoms (*“dyspepsia/weight loss”, “chronic reflux”, “chronic dyspepsia”, “elderly and frail”, “elderly with weight loss”, “lower abdominal or upper GI symptoms”*) and three others gave age cut-offs (*“>30”, “>45”* and *“45-74”*). Only two GPs gave an opinion about cost per test (*“£10”* and *“£14”*) where the others indicated *“don’t know”* or said that the CCG should decide. From the GPs’ perspective it appeared the breath test was feasible, but that its cost and place in a future referral pathway was yet to be determined.

SUPPLEMENTARY FILE 14: Summary of themes regarding feasibility and acceptability of the sampling process (from Field notes, teleconference and focus group of CSOs)

Theme	Examples of representative comments
Patient based limiting factors	<p><i>"Small print of the information sheet was an issue for one patient"</i></p> <p><i>"One patient had a bad cold and felt like they couldn't exhale properly into the mask"</i></p> <p><i>"One patient was anxious as said she was really claustrophobic, but she managed to do it in the end without a problem"</i></p> <p><i>"There were really no issues with patients, all were happy to help"</i></p>
Equipment (computer) based limiting factors	<p><i>"Computer very haphazard. Flowometer not working. About half of the patients were timed samples in the end. Cutting out (going blue) which responded to ctrl alt delete."</i></p> <p><i>"Flowometer responding to turning off and on but not always. I think it is a mask connection problem,"</i></p> <p><i>"Large number of timed samples because of flowometer issues"</i></p> <p><i>"Screen went blue"</i></p> <p><i>"Screen froze and wouldn't respond"</i></p> <p><i>"The machine displayed an error message and the breath monitor did not increase. Solved when system was rebooted but patient had gone by then."</i></p> <p><i>"Computer fault again, not recognising mask and not reading, had to do timed sample. This corrected itself the next day."</i></p>
Equipment (ReCIVA device) based limiting factors	<p>CSOs felt that the equipment was "fiddly" but "ok once they got the hang of it", particularly the spanners used to tighten screws.</p> <p>They commented on the time it took to set up the equipment at the start of the day and said they found it easier if there was a desk/workspace to lay out equipment and documents.</p> <p>When commenting on their perceptions of patient acceptability, CSOs said that they observed that patients didn't always breathe "normally" when wearing the masks, and some held their breath. This was not reported by patients in the acceptability questionnaires.</p>
Equipment (TD tube) based limiting factors	<p>1 tube in phase 1 arrived with black soot (sorbent) coming out of one end. This was sent to the company for repacking. Breath data was discarded.</p>
Training	<p>CSOs commented in the focus group that training was vital to performing the breath test because "preparation is key", "if you prepare before then it runs like clockwork and you can sample patients back to back".</p> <p>The troubleshooting manual was "useful" but the training allowed "hands on practice".</p> <p><i>"The fiddliness and multiple steps required made it not very obvious what to do next unless you had had the training."</i></p>
Human errors in sampling	<p>Early in phase 1 of the study 13 tubes in a batch arrived with no caps on, meaning that the samples would have been very contaminated. This was solved by contacting the CSO individually, (who had mistakenly forgotten to do this). This was an easily solved problem that did not recur. Breath data was discarded.</p> <p>Tubes occasionally arrived with loose caps on. However, these were likely tight enough to have held in the sample, but were easily removed by hand. This was solved by sending a reminder to CSOs and including this as a point in all subsequent training. Breath data was not necessarily discarded, but quality was checked as per all samples.</p> <p>Some tubes were overly tightened with the spanners, which could potentially damage the tubes. This was solved by supplying handheld spanners only, rather than conventional long spanners.</p>

References

1. Romano A, Hanna GB. Identification and quantification of VOCs by proton transfer reaction time of flight mass spectrometry: An experimental workflow for the optimization of specificity, sensitivity, and accuracy. . *J Mass Spectrom*. 2018;53(4):287-95.
2. Romano A, Doran S, Belluomo I, Hanna GB. High-Throughput Breath Volatile Organic Compound Analysis Using Thermal Desorption Proton Transfer Reaction Time-of-Flight Mass Spectrometry. *Analytical chemistry*. 2018;90(17):10204-10.