RECEDE

Reducing Colonoscopies in patients without significant bowEl DiseasE

PROTOCOL

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SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the UK Policy Framework for Health and Social Care Research, the ICH Good Clinical Practice guidelines and the Sponsor's SOPs.
I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.
I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

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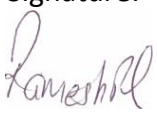
Signature: 		Date: 11.02.2022
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STUDY SUMMARY

Study title	Reducing Colonoscopies in patients without significant bowEl DiseasE **- RECEDE** study	
Research question	Can the addition of urine VOC to stool FIT testing rule out significant bowel disease (SBD) and by so doing, reduce unnecessary invasive tests (colonoscopy/CTC) – the right test for the right participant?	
Study aim	To investigate the diagnostic accuracy of stool FIT plus urine VOC compared to stool FIT alone for detection of SBD*. If stool FIT plus urine VOC is sufficiently accurate it will enable a reduction in the number of colonoscopies and Computed Tomography Colonography (CTC) in those without SBD. *Significant Bowel Disease (SBD) is defined as those with colorectal cancer, significant pre-malignant polyps (adenomas with size >10mm or with high grade dysplasia)or inflammatory bowel disease	
Study design	Multicentre – Prospective Diagnostic Accuracy Study	
Study participants	Participants referred from primary care to NHS Trusts that undertake colonoscopy or CTC will be approached to participate.	
Reference standard	Final report from the CTC or colonoscopy examination and confirmatory histology report	
Index tests	Stool FIT alone Stool FIT and urine VOC	
Study arms	All participants will receive the index tests: stool FIT and urine VOCs; and reference standard: full colonoscopy examination or CTC.	
Sample size	1915	
Planned study period	40 months	
	Objectives	**Outcome Measures**

Primary	Diagnostic accuracy of stool FIT plus urine VOC compared to stool FIT alone to improve detection of SBD.	Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of stool FIT plus urine VOC in detection of SBD, using CTC or colonoscopy histology findings.
Secondary	Diagnostic accuracy of stool FIT plus urine VOC in detection of SBD Impact on number of colonoscopies and CTCs undertaken Cost effectiveness and colonoscopy/CTC disutility	Receiver operating characteristic (ROC) curve of stool FIT plus urine VOC in detection of SBD. Calculation of potential number of colonoscopies and CTCs avoided in those without SBD. Cost calculation, Quality of life (EQ-5D-5L) and quality adjusted life years (QALY) associated with utility of each diagnostic strategy (stool FIT and urine VOC)

Key Words:

FIT, urine VOC, diagnostic, colorectal cancer, adenoma, IBD, colonoscopy

STUDY FLOW CHART

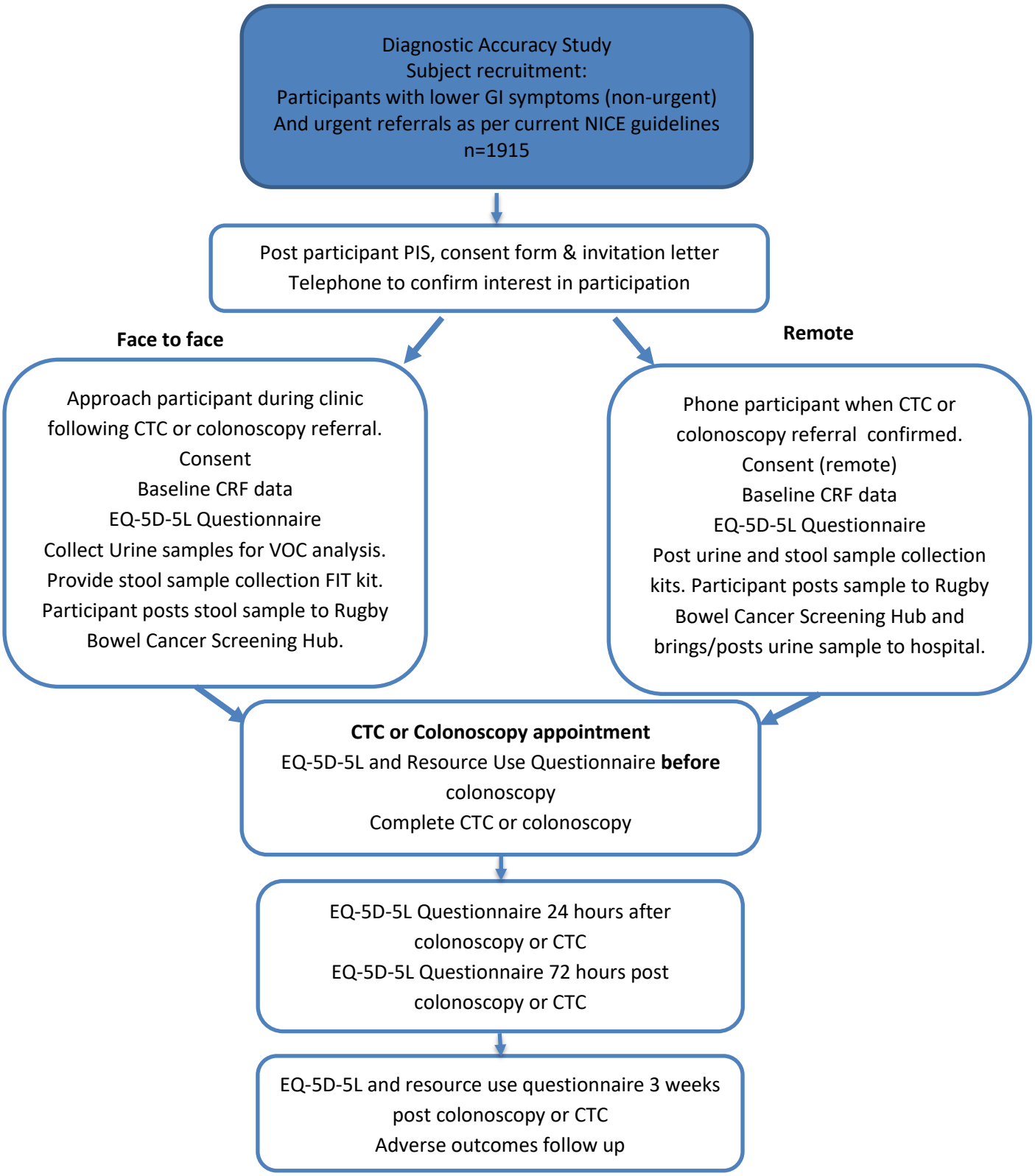


Figure 1 *Study flow chart.*

Note – EQ-5D-5L questionnaire only to be requested at sponsor site (UHCW)

Abbreviations: GI, Gastrointestinal; PIS, Participant Information Sheet; CRF, Case Report Form; FIT, Faecal Immunochemical Test for haemoglobin; VOC, Volatile Organic Compounds.

SCHEDULE OF EVENTS

Table 1 Schedule of events for collection of stool FIT and urine VOC for both face-to-face and remote recruitment scenarios.

Face to Face:

Observation	Baseline (referral visit)	Day of colonoscopy or CTC (before procedure)	24hrs & 72hrs post colonoscopy or CTC	3 weeks post colonoscopy or CTC	Analysis complete and results available
Eligibility screening	X				
Informed consent	X				
Baseline data	X				
Urine sample for VOC analysis	X[a]				
Stool sample for FIT test	X[b]				
Participant questionnaires: EQ-5D*	X[c]	X	X[c]	X[c]	
Resource use questionnaire*		X		X[c]	
Colonoscopy or CTC results and adverse outcomes				X	
Follow Up in CTC negative patients					X

Remote:

Observation	Baseline (remotely)	Pre-colonoscopy or CTC (participant at home)	Day of colonoscopy or CTC (before procedure)	24hrs and 72hrs post colonoscopy or CTC	3 weeks post colonoscopy or CTC	Analysis complete and results available
Eligibility screening	X					
Informed consent	X (via telephone)					
Baseline data	X					
Urine sample for VOC analysis (kit to be posted to participant)		X[d]				
Stool sample for FIT test (kit to be posted to participant)		X[b]				

Participant questionnaires: EQ-5D*	X[c]		X	X[c]	X[c]	
Resource use questionnaire*			X		X[c]	
Colonoscopy or CTC results and adverse outcomes					X	
Follow Up in CTC negative patients						X

Note - all sample collections are designed to be timed, if possible, with participant's attendance to clinic or treatment to minimise additional visits. If this is not feasible, there is a postal option or delivery to local GP for transport to central site only*

(a) If participant is unable to provide a urine sample at Baseline, they can return sample to their GP surgery* or drop it off at the hospital. If this is not possible, participants also have the option to collect a urine sample, 24 hours prior to bowel prep, and bring it with them on the day of colonoscopy/CTC (participant will have to keep the sample in the freezer and, if required, place in a provided cool bag when bringing back to the hospital if sample is likely to be out the freezer for over 1 hour)

(b) Only provide participant with FIT testing kit and pre-paid envelope for stool sample collection at home if it will be 4 weeks between their first referral FIT test and their colonoscopy/CTC appointment.

(c) Both paper, electronic and telephone versions of questionnaires will be made available depending on site and participant preference. If a paper option is preferred, provide the participant with a pre-paid envelope to return questionnaire(s) or collect on the day of colonoscopy or CTC if possible.

(d) Provide participant with VOC testing kit and optional cool bag (if required) for urine sample collection at home. Also provide pre-labelled envelope if returning their sample via their GP courier service.

(e) Participants medical records will be checked prior to contacting participant for follow up

***Note –questionnaires only to be requested at sponsor site (UHCW)**

LIST OF ABBREVIATIONS

BCSP	Bowel Cancer Screening Programme
CI	Confidence interval
CRC	Colorectal cancer
CRF	Case Report Form
CTC	Computed Tomography Colonography
DAS	Diagnostic accuracy study
FCP	Faecal Calprotectin
FIT	Faecal Immunochemical Testing
GCP	Good Clinical Practice
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
ISF	Investigator Site File
NHS R&D	National Health Service Research & Development
PI	Principal Investigator
PIC	Participant Identification Centre
PIS	Participant Information Sheet
REC	Research Ethics Committee
SDV	Source Data Verification
SBD	Significant bowel disease
SOP	Standard Operating Procedure
SSI	Site Specific Information
SMF	Study Master File
TMG	Study Management Group
TSC	Trial Steering Committee
VOC	Volatile organic compounds

STUDY PROTOCOL

RECEDE study (Reducing Colonoscopies in those without bowEl DiseasE)

1 SUMMARY OF RESEARCH

1.1 Lay Summary

Investigating people with bowel symptoms uses a test that detects traces of blood in the stools, the FIT test. There are many possible reasons for positive tests. A few people have cancer. However, most participants with symptoms don't have any serious bowel disease but have benign problems such as piles or irritable bowel syndrome (IBS). It is very difficult to diagnose on symptoms alone, those participants who have serious bowel disease and those who do not.

After a positive test, people are invited for colonoscopy– a sort of articulated tube that is passed up the bowel, or a computed tomography colonography (CTC) – a radiological technique used to image the large bowel.. Most people invited for colonoscopy or CTC don't have cancer. Only about 5% of those with positive FIT tests have cancer. About 25% have other serious bowel diseases (pre-cancerous polyps or inflammatory bowel disease), but most have nothing serious wrong at all. So they have the inconvenience and discomfort of having a CTC or colonoscopy but don't get any benefit from it.

We want to try adding another test, the volatile organic compound (VOC) test, to see if we can separate those with positive FIT tests who do have something serious, from those who don't. The VOC test uses a urine sample. Using both tests might also be better for detecting cancer and other serious bowel diseases. FIT alone misses about 20%.

So we think that using both tests might not only be better for detecting cancer, but also might mean that a lot of people will avoid having to have an invasive colonoscopy or CTC.

We will recruit 1,915 participants with bowel symptoms from NHS trusts in the UK. They will provide stool samples for FIT and urine for VOC analysis. They will have colonoscopy or CTC to get a definite diagnosis. Then we will look at their FIT and VOC test results to see if in future, people with both tests negative don't need invasive colon investigations.

1.2 Scientific Abstract

Research Question: Can the addition of urine VOC to stool FIT testing rule out significant bowel disease (SBD) and by so doing, reduce unnecessary invasive tests (colonoscopy/CTC) – the right test for the right participant?

Background: There is currently disparity between demand and available resources for colonoscopy. At present around 300,000 participants (and rising) are being referred annually to NHS trusts suspected of colorectal cancer (CRC) (1). These participants are offered invasive colonic examinations (colonoscopy or CT colonography) but only 30% will have significant bowel disease (2). Significant bowel disease (SBD) includes neoplasia (cancer and benign tumours) and significant treatable benign conditions such as inflammatory bowel disease (3). Of the remaining 70%, 40% have completely normal colonic investigations (2) and 30% have functional bowel conditions such as irritable bowel syndrome or diverticular disease.

Set against this there is and will remain for the foreseeable future a capacity shortfall for colonoscopy and CTC. This limits the ability of the NHS to extend colorectal cancer detection within the Bowel Cancer Screening Programme (BCSP) or to target those participants that present for the first time through the Emergency Department (25% of all colorectal cancer diagnoses).

The increasing demand, limited capacity and lack of a triage tests have left NHS trusts with a conundrum of how best to stratify those with symptoms and at risk of SBD including CRC. The National Institute for Health and Care Excellence (NICE) has recommended a stool test (faecal immunochemical testing for haemoglobin, known as FIT) in the assessment of those suspected of CRC (4). NICE have recommended 10 μgHb/g faeces as the cut off for investigation of people with low risk symptoms who, account for only 10% of those referred with suspected CRC[5]. When applied to high risk symptom groups, FIT will *miss* a significant number of participants with CRC (~10%) if used on its own at the threshold recommended by NICE (10 μgHb/g faeces). FIT will also *miss* a large number of significant potentially pre-cancerous polyps (~40%) (6, 7). Early detection and removal of such polyps will reduce risk of CRC (8).

The Bowel Cancer Screening Programme (BCSP) has set a FIT cut off of >120 μgHb/g faeces (9, 10), compared to the NICE threshold of 10 μgHb/g faeces, but even at the lower threshold recommended by NICE some SBDs will be missed (2, 7, 11, 12). Whilst a lower threshold might improve detection of SBD, it will increase the number of colonoscopies and CTCs that find no abnormality. Consequently, we have been investigating a urine test (in addition to FIT) to improve detection of participants with SBD with a view to reducing unnecessary colonoscopies and CTCs. The urine test analyses volatile organic compounds (VOCs) that originate from the body and provides a chemical 'fingerprint' that is disease specific. Stool FIT and urine VOCs identify different biological characteristics of SBD – haemoglobin (as a marker of excess blood loss) versus metabolic response to inflammation. In a preliminary study of 562 participants, stool FIT on its own (at a threshold determined from the data) detected 80% of those with colorectal cancer. However, the addition of urine chemical testing improved this to 97% (6). The number of CRC cases missed by combined FIT and urine chemical testing is similar to that of colonoscopy, the current gold standard.

Aim: To **investigate the diagnostic accuracy of stool FIT plus urine VOC compared to stool FIT alone to improve detection of SBD.**

This may enable reduction in number of colonoscopies and CTCs in those without SBD.

Methods, study population and design

RECEDE study will be a multicentre diagnostic accuracy study involving secondary care centres, recruiting 1,915 participants referred for colonic investigations.

The study will compare single index testing with FIT with dual testing with FIT plus VOC. The reference standard will be colonoscopy (complete examination) or CTC.

Primary outcome

Primary outcome will be diagnostic accuracy for sensitivity, specificity, NPV and PPV of stool FIT plus urine VOC compared to stool FIT alone in the detection of SBD.

Secondary outcome

Secondary outcome measures will include:

I. ROC curve of stool FIT plus urine VOC in detection of SBD.
II. The potential number of colonoscopies and CTCs avoided in those without SBD,
III. Total NHS & Personal Social Services (PSS) costs, and
IV. Total quality-adjusted life years (QALYs) associated with each option.

Exploratory outcome measures will include:

- Calculation of the number of CTC negative participants who later present with SBD or microscopic colitis

- Sensitivity, specificity, NPV and PPV of stool FIT plus urine VOC in the detection of SBD + microscopic colitis

1.3 Knowledge gap this research will address

RECEDE study will address the following knowledge gaps:

I. Whilst FIT is valuable for CRC detection, it will still miss some SBD at the cut offs recommended. Hence, we need to understand whether urine VOC added to FIT can improve detection of SBD such that those without SBD can avoid a colonoscopy or CTC.
II. With implementation of non-invasive triage testing, what are the cost savings to the NHS?

1.4 Proposed study

RECEDE study will be a multicentre prospective diagnostic accuracy study involving secondary care centres to recruit 1,915 participants referred for colonic investigations.

1.5 Study population

All participants referred from primary care with lower gastrointestinal symptoms either routinely or urgently in accordance with NICE NG12 criteria. Sites will include NHS Trusts where colonoscopy or CTC is offered.

1.6 Impact of COVID-19 on Patient pathways

COVID-19 has brought about a change in the way people who are experiencing lower GI symptoms are referred into secondary care (illustrated in Appendix C Figure 3). Previously, FIT testing would be used in some regions, and only on low risk patients (~10% of all patients) before they were referred to secondary care. They would then be referred for colonoscopy only if the FIT test was positive. However, in most regions and patients, a person presenting to their GP with GI symptoms would be referred either routinely or urgently in accordance with the NICE guidelines (4) without the need for a prior FIT test.

Since the outbreak of COVID-19 within the UK, hospitals have seen a drastic fall in the number of patients being referred with GI symptoms. However since lockdown measures have eased and at time of writing this protocol, we are now beginning to see a rise in the number of referrals, and there is a backlog to be cleared. To manage limited colonoscopy capacity due to the impact of COVID-19 and colonoscopy (which is a potential aerosol generating procedure), FIT may be used (outwith) NICE guidance to triage referrals to colonoscopy.

In addition there has also been a stark increase in the number of patients being referred for a CT scan instead of colonoscopy. Our preliminary data shows that over 25% of all GI referrals to secondary are currently having CT scans. CT scan is a radiological procedure that was often favoured for older patients with comorbidities as they are more at risk of adverse events during a colonoscopy. However, COVID-19 has caused a shift in practice, whereby more people are now being referred for CT scans, and this shift appears to be here to stay for the long term. CT scans are split into regular CTs and CTC, the latter of which has been shown to have similar sensitivity to colonoscopy for the detection of colorectal cancer (Halligan et al., 2005) and large polyps (Pickhardt et al., 2003). It is common for CTC positive patients to then have a colonoscopy to receive histological confirmation of any diagnosis. Some NHS Trusts have already adopted CT scans for 100% of referrals, using colonoscopy as only a confirmatory diagnostic follow up.

NHS England has suggested that FIT may be used in secondary care to triage timing of colonoscopy or CTC rather than need for colonoscopy or CTC, or to discharge patients if FIT is returned negative. This is because there is recognition of the lack of evidence to guide referral for colonoscopy or CTC. This is the very reason for undertaking the RECEDE study. NHS England guidance also actively encourages primary care physicians to continue to refer to secondary care all those with lower GI symptoms and not to delay or defer such referrals.

In summary, it is likely there will be a population (unable to estimate proportion at this time) that will have had a FIT test prior to their colonoscopy or CTC. The time interval from FIT test to colon investigation will be highly variable hence for participants into RECEDE, we will still re-test FIT alongside urine VOC as originally planned if the participant has a wait of over 4 weeks between their first FIT test and their colonoscopy or CTC. If their colon investigation is scheduled within 4 weeks of the first FIT test (i.e. for urgent referrals) then we will be able to use the first test alongside VOC analysis for the primary outcome analysis and not need to request a second test. The initial FIT test will be purely to triage ***timing*** of colonoscopy or CTC rather than *need* for colonoscopy or CTC. Due to limited capacity, initial recruitment rate will be slower, possibly only half the rate of what was previously predicted for the next 12 months but the aims of RECEDE are still achievable.

FIT kits are fairly uniform across practices - both HMJac-K and OC-Sensor are most commonly used, however these kits collect different quantities of faecal matter into different buffer volumes and so cannot be directly compared. However a conversion factor is available in order to equalise the tests (71). Therefore if a recruiting site uses a FIT test other than the HMJac-k to triage their patients we are able to accurately compare the two.

Further, as the waiting list for secondary care appointments continues to grow we are seeing an increase in the number of primary care lower GI referrals sent directly to A&E. These patients are then referred for colonoscopy or CTC from here. These patients are eligible for RECEDE however currently their medical history does not become available on the hospital system making them difficult to recruit. If this happens, once a patient consents into the study we will approach their GP to request the participant's medical history be sent to the study team. The current consent form already requests permission to access the patients' health records.

1.7 Index Tests

The index tests will be stool FIT and urine VOCs.

1.8 Reference Standard

The reference standard will be complete colonoscopy or CTC.

1.9 Clinical data

Both FIT and urine VOC have potential to detect SBD but their diagnostic accuracy may be improved if used in combination; either in series (VOC in FIT negatives) or both in parallel.

Evidence of stool FIT in SBD detection

Our previous data and that of others have shown that stool FIT on its own has sensitivities of 80%, 53% and 86% for colon cancer, adenomatous polyps and inflammatory bowel disease respectively (2, 11, 13). Importantly, the alternative stool marker faecal calprotectin (FCP) offers no added advantage for the added cost (2, 11). The sensitivity of FCP for colon cancer is lower at 68% (vs 80%) and 43% (vs 53%) for adenomatous polyps compared to FIT but comparable for diagnosis of inflammatory bowel disease (for which it is intended) (3). This has meant that NICE has not recommended FCP for diagnosis of SBD

except inflammatory bowel disease. Note that blood or stool based genomic markers e.g. mSEPTIN 9, Cologuard (methylated DNA markers) whilst specific for CRC perform less well to identify colonic polyps (sensitivity 60% with specificity of 46%) (14). Moreover, these markers cannot identify other SBD such as inflammatory bowel disease. Their high cost (15) makes it prohibitive for routine use with almost comparable cost to that of a colonoscopy. Conversely, urine VOCs on their own can detect SBD with sensitivities for colon cancer, adenomatous polyps and inflammatory bowel disease at 80%, 92% and 86% respectively (6, 16),(17). Of further note, concordance between FIT and urine VOC is low to moderate indicating a potential synergistic effect when combining results from the two tests.

FIT (£18 per test) is recognised to be superior to the guaiac faecal occult blood test for detecting CRC due its ability to detect human haemoglobin (18). There is good evidence for its use within the screening population. In those with symptoms, there has been increasing evidence supporting its use in primary and secondary care. However, significant limitations as outlined earlier are the number of missed CRC cases, and lack of sensitivity and specificity to detect pre-malignant lesions (polyps) (6, 7, and 12). Thus, it is apparent that FIT on its own is not likely to be sufficient to detect SBD, but rather a second complementary test is required to improve SBD detection.

Existing evidence suggests that FIT on its own can miss up to 10% of CRC (applying threshold recommended by NICE), but adding urine VOC testing can reduce this to 3% (similar to colonoscopy) (6).

Studies to date in those with lower gastrointestinal symptoms utilising FIT for detection of CRC provides sensitivities ranging from 80-92%. Some of these studies included testing in primary care and others in secondary care. A systematic review undertaken in 2017(7) and subsequently updated by our group in 2019(19), of studies in 6755 patients with lower gastrointestinal symptoms concluded that FIT (at a threshold of 20µgHb/g faeces) would achieve a 90% sensitivity (95% confidence interval, CI: 87 to 92) and 86% specificity (95% CI: 83 to 90) for CRC detection – Figure 2. The meta-analysis reviewed studies applying varying thresholds from 7 to 97 μg Hb/g faeces as well as evaluated different diagnostic kits used to measure FIT.

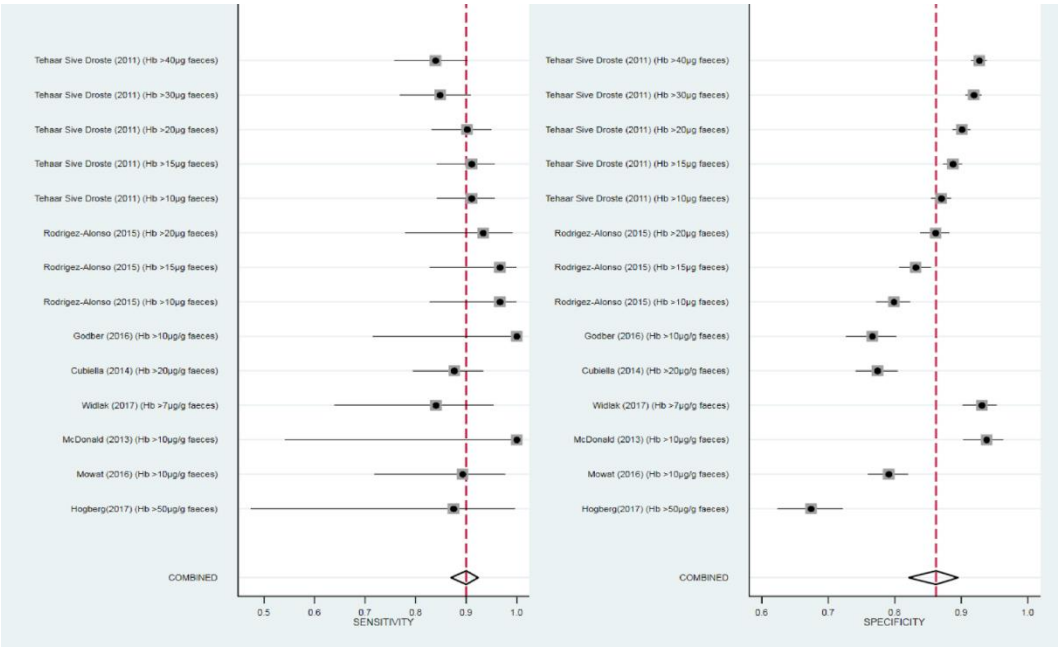


Figure 2 Forest plot of sensitivity and specificity of FIT for detection of CRC in 6755 symptomatic participants. The pooled sensitivity was 0.90 (95%CI: 0.87 to 0.92) and specificity 0.86 (95% CI: 0.83 to 0.90) represented by the red dashed line.

Evidence of urine VOC in SBD detection

VOCs are organic molecules that have a low boiling point, which results in a large number of these molecules evaporating from a liquid (or even sublimating from a solid form) to the surrounding air. Large numbers of VOCs are generated from natural or artificial sources. The detection of VOCs is a mature technology and has been used successfully in a range of applications from security, food and drug quality/freshness and pollution monitoring (14).

VOCs vary in abundance and mobility in health and disease. Measurement from biological samples provide an indirect reflection of cellular/tissue health based on the metabolic output profile measured as a 'chemical fingerprint'. They are as equally informative as genetic tests in terms of probability of having a disease and able to provide this information in real time. VOCs were first reported in the 1970s by Nobel Laureate Linus Pauling who detected inflammatory conditions in urine samples. (21) Urine VOC analysis has had resurgence of interest in the last decade largely due to its ease in measurement (on the spot collection) and advancement in sensor technology, which is now available at relatively low cost, £25 per test. VOCs have a strong translational component from *in vitro* application to measure cellular differentiation (22) to clinical diagnosis of respiratory, gastrointestinal diseases, wound infection and cancers (including non-gastrointestinal) (23, 24).

Unlike faeces, urine collection for VOC measurement can be collected at time of point of care testing. Subsequently, it can be sent to a central laboratory for analysis (as with FIT) allowing contemporaneous results to guide clinicians with decision making. Thus, it lends itself as an ideal clinical test.

VOCs detected in urine of those with SBD reflect metabolic processes either directly from the tumour/inflamed mucosa, from the distinct microbiome dysbiosis commonly associated with neoplasia and IBD (17, 25, 26) or more likely, from an interaction between both entities.

We and others have studied VOC detection for CRC using different modalities – faeces, urine and breath (16, 27-29). The sensitivities range from 80-92% (95% CI: 63 to 94). More recently our group has also shown utility of urine VOC in CRC detection in those within the BCSP population (30). Our group has optimised methods for VOC detection in urine from sample collection, storage and stability as well as specific algorithm for analysis (6, 31, 32). This both allows for VOC analysis to be introduced into conventional secondary care laboratory setting and for its diagnostic utility to be optimised. We have already identified four potential key chemicals that are associated with CRC detection (cyclooctatetraene, propanediamine, methylbenzoic acid and isothiocyanate) (33) that contributes to 20 discriminant features providing a score that can be quantified and threshold adjusted accordingly – see Appendix A Table 1.

Dual Testing

Our further recent study has shown that dual testing (stool FIT followed by urine VOCs in the FIT negatives) improves the detection of colorectal cancer from 80% (95% CI: 0.63 to 0.93) to 97% (95% CI: 0.90 to 1.0) (6). Stool FIT detects the presence of human haemoglobin moiety thus in conditions where there is no overt bleeding (e.g. diminutive adenomatous polyps, microscopic colitis) the test if used on its own becomes less sensitive. Volatile organic compounds (VOCs) on the other hand are compounds produced by interaction of the host in the presence of illness and are therefore *disease* specific. These compounds are of low molecular weights and result from cellular interaction with the gut microbiome in the presence of disease.

If used in combination, the diagnostic accuracy of these tests (stool FIT and urine VOC) should improve the detection of SBD as shown with colon cancer and adenomatous polyps (Figure 3). Based on our previous study, 37% of those with SBD test FIT negative. In this preliminary study, of the 7 individuals with cancers missed by FIT alone (FIT negative CRC), 6 of them were subsequently identified when urine VOCs were added. Existing studies have demonstrated the utility of urine VOC to detect not just colon

cancer or inflammatory bowel disease but other gastrointestinal diseases including coeliac disease and bile acid diarrhoea, but be negative in benign conditions such as irritable bowel syndrome (IBS) (34, 35). The importance of this is the premise that the triage tests (urine volatile markers) can distinguish SBD from those without SBD (e.g. IBS). NICE recommends that IBS can safely be managed in primary care without recourse to referral to secondary care for invasive and expensive investigations (36).

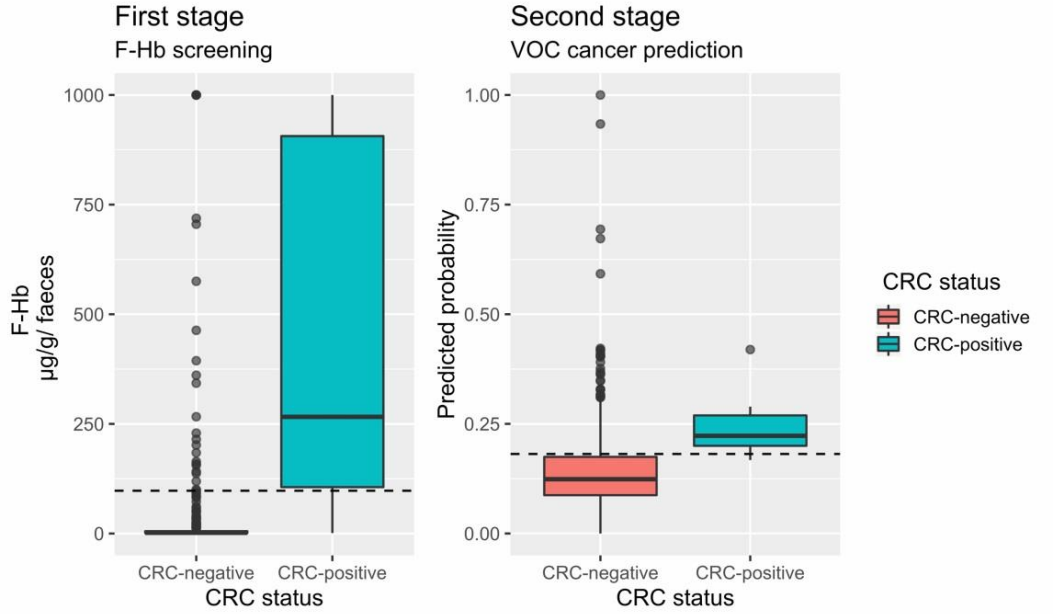


Figure 3: Two plots showing the stages of the FIT/VOC two-stage test. The left-hand plot shows the distribution of FIT measurements for CRC-positive and CRC-negative participants. The dotted line shows the threshold for the first stage test. Participants above this threshold are classed as positive for the test, while participants below this threshold go on to the second stage of VOC screening, shown in the right-hand plot. For participants who are screened using VOCs, the distribution of predicted probabilities of CRC are significantly reduced as shown in the right-hand plot, with the decision threshold again shown by a dotted line. Participants above the line are positive for the test, while participants below the line are negative (6).

Evidence of urine VOC in other diseases

Urine VOCs on their own can detect SBD with sensitivities for colon cancer, adenomatous polyps and inflammatory bowel disease at 80%, 92% and 86% respectively (6, 17, 18).

Our previous work has identified key urinary metabolites in 'inflammatory' gastrointestinal diseases other than CRC. Table 2 below demonstrates key urinary VOCs that are identified in specific conditions including relative abundance (19). The importance of these findings are that it highlights that the technique applied for urinary VOC detection is feasible in separating CRC including that of pre-cancerous lesions such as adenomas from other overlap gastrointestinal conditions.

Table 2 Specific compounds identified in urine by relative incidence in different disease groups – CRC, colorectal polyps (adenoma), inflammatory bowel disease, irritable bowel syndrome, coeliac disease and healthy volunteers. Key volatile metabolites can be identified in urine of those with CRC and are sufficiently diverse from other disease groups including pre-cancerous lesions.

Colorectal Cancer	
High Incidence	**Low Incidence**

1,3,5,7-Cyclooctatetraene	Oxime-, methoxy-phenyl-
	1,3-Propanediamine
Irritable Bowel Syndrome	
Isothiocyanate	
1,3,5,7-Cyclooctatetraene	
Phenol, 2,4-bis(1,1-dimethylethyl)-	
Inflammatory Bowel Disease	
1,3-Propanediamine	Phenol, 2,4-bis(1,1-dimethylethyl)-
4-Heptanone	1,3,5,7-Cyclooctatetraene
	2-Pentanone
Healthy Volunteers	
Oxime-, methoxy-phenyl-	Ethanone, 1,1'-(1,4-phenylene)bis
	1,3,5,7-Cyclooctatetraene
	1,3-Propanediamine
Colorectal Polyps (Adenomas)	
	Phenol, 2,4-bis(1,1-dimethylethyl)-
	Oxime-, methoxy-phenyl-
	1,3,5,7-Cyclooctatetraene
	4-Heptanone
	Acetone
Coeliac Disease	
1,3-Propanediamine	Phenol, 2,4-bis(1,1-dimethylethyl)-
Oxime-, methoxy-phenyl-	2-Pentanone
1,3,5,7-Cyclooctatetraene	

2 RATIONALE

We hypothesise that the dual testing with stool FIT and urine VOC tests is more sensitive than stool FIT alone in detecting SBD using colonoscopy (complete examination) or CTC as the reference standard.

2.1 Aims and Objectives

The overarching aim of RECEDE study is to investigate the diagnostic accuracy of dual testing with stool FIT plus urine VOC compared to stool FIT alone to improve detection of SBD. If sufficiently accurate this has the potential to reduce the number colonoscopies and CTCs in those without SBD.

Detailed list of research objectives include:

Clinical

- Comparative sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of (i) FIT, (ii) urine VOC and (iii) FIT plus urine VOC for detection of SBD.Developing an optimisation of sensitivity threshold for FIT, VOC and FIT + VOC when tests are either performed in parallel or sequentially as an exploratory outcome.
- Potential number of colonoscopies and CTCs avoided in those without SBD
- Survey of NHS stakeholders and policy decision makers as to acceptable sensitivity of diagnostic strategies before implementation into clinical practice. These will include members of the Cancer Alliance Network who will be asked to complete an online survey when results are available.
- Return rate of stool and urine samples

Economics

- Estimation of per-participant NHS & Personal Social Services (PSS) costs associated with each of the compared options (FIT plus urine VOC compared to FIT alone, as indicators for colonoscopy)
- Impact of colonoscopy and CTC on participants' health-related quality of life (colonoscopy disutility including bowel preparation).
- Number of potentially avoidable colonoscopies, CTCs and resulting savings. We will also consider opportunity benefits such as reduced waiting times for other participants
- Estimation of quality-adjusted life years (QALYs) associated with each of the compared options (FIT plus urine VOC compared to FIT alone)
- Overall cost-effectiveness of adding VOC testing in the diagnostic pathway.

All 1,915 participants clinical data and surplus samples associated metadata will be stored in the Arden Tissue Bank (hosted at UHCW) and will be made available (upon written application and approval by the TSC and Chief Investigator) for Universities, NHS and commercial researchers worldwide for further research.

3 STUDY DESIGN

This is a prospective diagnostic accuracy study to compare dual testing of stool FIT with urine VOC markers compared to single testing with FIT, in the detection of SBD – see Figure 1.

4 STUDY SETTING

RECEDE will be a multicentre diagnostic accuracy study, in centres that receive referrals from primary care for those with lower gastrointestinal symptoms.

5 ELIGIBILITY CRITERIA

5.1 Inclusion criteria

- All participants referred either routinely with lower gastrointestinal symptoms or urgently (fulfilling the national criteria for referral - NICE NG12) for colonoscopy or CTC that is determined by their overseeing clinician
- Minimum age of 18
- Able to provide informed consent
- Have the ability to return both stool and urine samples

5.2 Exclusion criteria

- Those who are pregnant

6 STUDY PROCEDURES

Recruitment options will be tailored to the respective site either remotely or at time of clinic visit. This is shown in Appendix B Figures 1 and 2. If the clinician decides that colonoscopy or CTC is required the participant will then be invited to participate in the RECEDE study – see Figure 1.

Informed consent will be obtained and Case Report Forms (CRFs) completed and questionnaires (EQ-5D) handed out at the same time (questionnaires will only be requested at the sponsor site - UHCW). The participant will be requested to provide a stool sample from home and post for analysis, which must be at least 24 hours before starting bowel cleansing medication. Urine samples can be collected either during a clinic visit (preferential) or, if this is not possible, at home and either sent via GP courier back to the hospital or hand delivered by the participant on a future visit to the hospital that aligns with standard care at the recruiting site. Participants are requested to record date and time when samples are collected. Participants may be provided with cool bags if collecting urine from home, if participant needs to freeze their sample at home and the sample is likely to be out of the freezer for longer than 1 hour before returned to the hospital.

For stool samples, a pre-prepared stool collection kit is provided together with return envelope to be posted directly to the Rugby Bowel Cancer Screening Hub – see Figure 4A below. The kit can either be posted to participants or given directly at time of clinic visit (site dependant). Stool samples are required at least 24hrs **prior** to commencing bowel preparation for colonoscopy.

For urine samples (sample collection kit shown in Figure 4B), this can be collected either at the time of a hospital visit or at home (at least 24hrs prior bowel preparation) and kept in a freezer until returned if the sample cannot be returned the same day as it is produced. At site level, the PI may adapt based on local policy. If the sample is frozen at home by the participant, cool bags with ice blocks can be provided to maintain cold chain until deposit at the site freezer. Depending on the site, participants may have the option of returning samples to their respective GPs for transfer to the site. We will aim for the time between the sample being produced and the sample being frozen at -80^{o}c to be <4 hours, unless the participant freezes their sample at their home first.

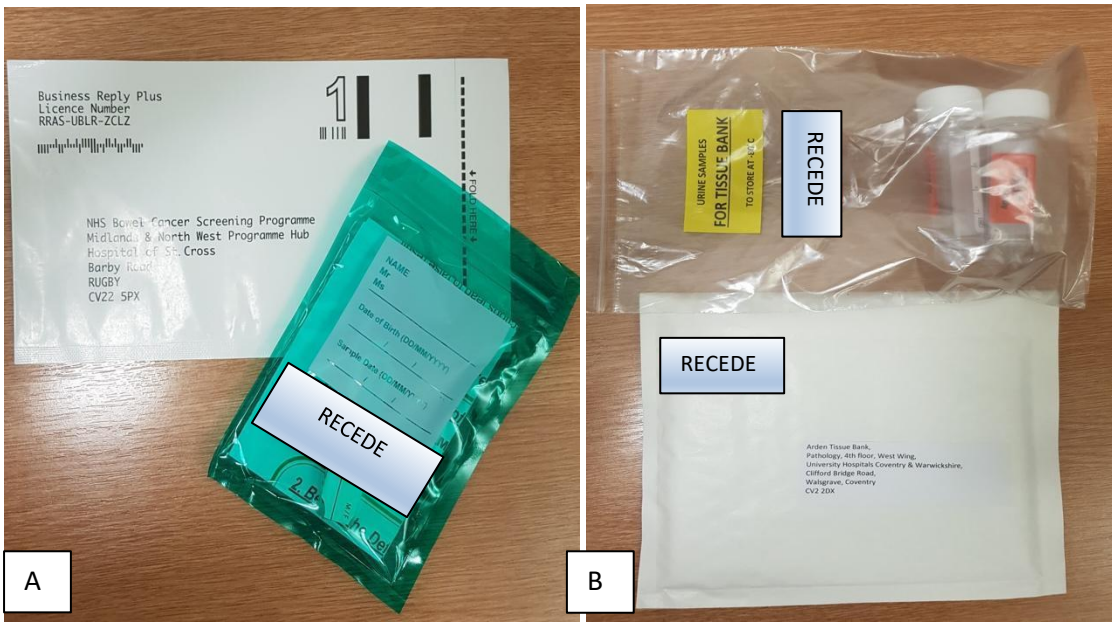


Figure 4 A: Stool FIT sampling and return kit. This is similar to that used in the bowel cancer screening programme nationally. **B**: Urine sample kit.

6.1 Screening and Recruitment

Screening will be undertaken by each site once referral from primary care has been received and date of appointment in secondary care is known. An invitation letter, PIS, and consent form will then be sent or given to participants accordingly and participation will be requested in the event that the clinician organises a colonoscopy or CTC. It is important for each site to ensure that participants are approached sequentially (unless they decline) to avoid risk of bias.

Two potential pathways are highlighted in Appendix B Figure 1 and 2.

6.2 Participant identification

At each site, a dedicated clinical research team member will identify potential participants through screening methods described above.

6.3 Assessment for eligibility

The PI or Research/Clinical staff at each site will have oversight and confirm eligibility if referral criteria are met and participant has agreed to undergo a colonoscopy or CTC (requested by overseeing clinician).

6.4 Payment

It is envisaged that recruitment can be undertaken as part of participant visit to clinic or colonoscopy. Hence no additional visits or inconvenience are anticipated for potential recruits, unless participants test CTC negative, but FIT or VOC positive. At the end of the study, this cohort (imagined to be a small percentage) will be recommended for further investigation with colonoscopy. For samples that will be posted, a pre-paid self-addressed envelope will be provided. No payment will be made to participants.

6.5 Consent

It is envisaged that the PIS and invitation letter can be sent by post to the participant ahead of their colonoscopy or CTC appointment (site dependant). The study team will telephone participants after they receive this information to confirm they are interested in taking part in the study, with the study details being explained to them at this point. Verbal consent can be given and recorded at this time. Written consent only needs collecting if recruiting the participant face to face. Participants will be encouraged to ask questions and they will have the right to decline to take part in the study without giving any reasons. They will also remain free to withdraw at any time from the study. Participants will be enrolled into the study once verbal consent is given. If telephone consent is obtained, a copy of the consent form signed by the staff member obtaining consent will be sent to the participant.

If unable to get hold of a potential participant before a clinic visit, consent can be obtained from them during a pre-colonoscopy clinic visit. The PIS and invitation letter can be given to them by a research nurse on that day with the study details being explained to them at that point. Participants will still be encouraged to ask questions and have the right to decline to participate in the study at that time. If the participant agrees to participate then written consent can be recorded on that day, with the participant then becoming enrolled in the study, or they can be contacted by telephone at a later date.

The Principal Investigator (PI) retains overall responsibility for the conduct of research at their site; this includes the taking of informed consent of participants at their site. They must ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained and competent. If verbal translation is needed, this will be via a hospital interpreter or an independent interpreter or telephone translation services where available.

6.6 Follow Up

Participants referred to CTC will be followed up for a longer duration, at least 52 weeks post-CTC procedure or until the study results are un-blinded. The follow-up check is on the participant's medical records to determine if they have a lower GI diagnosis later on that was not picked up by CTC.

It is anticipated that a small number of participants may return false negative results from their CTC as CTC is not able to pick up microscopic colitis, a treatable serious bowel condition. Whereas it is predicted that dual testing FIT + VOC may be able to detect if microscopic colitis is present in this population. Therefore those who return a negative CTC, but following analysis show a FIT or VOC positive result and did not have any further specialist lower GI care following checks on their medical records, will be re-contacted and recommended for further investigation with colonoscopy. The prevalence of microscopic colitis in those referred for further bowel investigation is small (<0.5%) and so we predict that this will only occur in a small number of participants.

7 DATA MANAGEMENT

7.1 Baseline data

This will include demographic data (age, sex, and ethnicity). Clinical data will include lifestyle and medical history, including medications. This data collection is part of standard care for those suspected of SBD but may vary according to local site procedures.

7.2 EuroQoL EQ-5D-5L

Questionnaires for the Health Economics analysis will include the EuroQoL EQ-5D-5L instrument. This is the most widely used tool for the collection and calculation of preference-based health related quality of life, and is required for the calculation of quality-adjusted life years (QALY). The EQ-5D is preferred in economic evaluations in health care (47, 48). The questionnaires will only be completed at the sponsor site (UHCW).

7.3 Stool FIT

Samples with traces of haemoglobin in the faeces at and above the cut off value 10μgHb/g are defined as suspected SBD. Any previous FIT results collected to triage patients can be sourced from patient records.

7.4 Urine VOC

Duplicate samples are collected in universal sterilin pots (2/3rd full only) for each participant. These will be frozen at -80^{o}c at sites and when ready transported to the central site on dry ice. Duplicate samples will be sent separately to avoid loss of samples. Thereafter samples will be sent for analysis on dry ice to the Manchester analytical lab and the UK National Measurement Laboratory. Once in the lab, they will be frozen until analysis. Records of the cold chain will be kept by individual and central sites up to transfer.

A subset of urine samples (n = 100) from the main recruiting site (UHCW NHS Trust) will be transferred directly to the National Measurement Laboratory (NML) in Middlesex for development of a standardised operating procedure for measurement in the future within accredited NHS laboratory networks.

The urine VOC analysis using established reference methods will be undertaken at the UK National Measurement Laboratory, Middlesex which is the national measurement laboratory for chemical and bio-measurement. Gas-chromatography mass spectroscopy system (GCMS) is considered the gold standard for VOC analysis and is commonly used to identify unknown chemicals. It comprises of a GC front-end, which separates complex mixtures of chemical based on their interaction with a retentive layer, resulting in chemical eluding out of the GC at different times (called the retention time). These individual chemicals are then ionised and the mass of the resultant fragments measured. As each chemical always fragments the same way, we can use these to identify specific chemicals. This will be used to validate the headspace sampling methods to be used and to provide an independent analysis of potential volatile biomarkers for the medical conditions of interest in this study. This will feed into subsequent “electronic nose” analysis.

The urinary volatiles will be analysed in Manchester using "electronic nose" techniques using an array of gas sensors coupled with an appropriate polymeric pre-concentrator. Appendix 3 provides a detailed description of the technique being utilised at Manchester for VOC analysis. This technique provides multivariate data documenting differences in the chemical composition of the urinary volatiles that may contain volatile biomarkers for a condition. This allows screening of populations rapidly using chemometric data analysis, neural networks and receiver operator curves to distinguish one population from another, with receiver operating curves being used to measure the appropriate thresholds to be set to distinguish samples as having colon cancer, colorectal adenomas, inflammatory bowel disease or microscopic colitis. Unlike GCMS – this technique does not provide identities of individual biomarkers but considers the data derived from the entire complex mixture of volatile chemicals that are present in urine headspace.

A receiver characteristic curve (ROC) with an area under the curve (AUC) of 0.63 or greater will be applied for the detection of SBD (colon cancer, colorectal adenomas or, inflammatory bowel disease). The threshold for participants samples being defined as having SBD will be >0.63 (see Appendix D).

To determine the relationship to the stool test (which is a quantitative continuous variable) a canonical correlation test will be applied. The canonical correlation tool is often used in a multivariate analysis of correlation. Canonical is the statistical term for analysing latent variables (which are not directly observed) that represent multiple variables (which are directly observed). Canonical correlation analysis is the analysis of multiple-X multiple-Y correlation. The canonical correlation coefficient measures the strength of association between two canonical variates. Canonical correlation analysis is preferable in analysing the strength of association between two constructs. This is because it creates an internal structure containing different importance of single item scores that make up the overall score.

7.5 Reference test

The reference test is the final report from the CTC or colonoscopy examination and confirmatory histology report. Biopsies are required as per national guidance (4) in the investigation of participants with lower gastrointestinal symptoms even in the absence of macroscopic abnormalities.

7.6 Data collection and management

The research team will make every effort to contact participants who have failed to submit the samples or attend the endoscopic procedure. They will schedule a maximum of 3 reminders over a 3 week period for the participant. Attempts to contact such participants will be documented in their records (e.g. times and dates of attempted telephone contact). To ensure questionnaires are completed at the correct time points research nurses will call participants to remind them or schedule email reminders if the participant has chosen to complete their questionnaire online.

The diagnosis from the colonoscopy or CTC examination, stool FIT and urine VOC will be collected by the research team once a diagnosis is confirmed.

7.7 Data collection tools and source document identification

Quantitative data on FIT and urine VOC scores will be collected together upon analysis on the eCRF with demographics, baseline characteristics and meta-data. Data will be collected from participants while they are in the clinic, GP referral letters, hospital records, CTC and colonoscopy and histology reports.

A case report form (CRF) either electronic or paper with individual (anonymised) participant data will be used to record data as required for the study protocol. The data can be collected on the paper CRF first and then entered on the database, or entered directly on the online validated, GCP compliant, electronic data capture (EDC) system by each research team at their respective site. Individual user log-

in access to this database will be granted to only those in the study team that require it for the performance of their role. Screening and recruitment logs of all participants approached to take part, and enrolled in the study will be held at each site. This will be stored in a password protected NHS computer. No participant personal data will be shared between sites. The collected data is used for the statistical analysis of the RECEDE study.

Each PI/recruiting site must ensure to keep records of all participating participants (sufficient information to link records e.g., CRFs, hospital records and samples), all original signed informed consent forms and all copies of the CRF pages which will be kept in the site file in a locked cabinet. Equally to ensure use of unambiguous participant identification codes that allows identification of all the data reported for each participant.

7.8 Data handling and record keeping

All data will be kept on a central server system hosted by the sponsor and supported by the IT department to include security measures and back up of all files. Individual sites will be required to send scanned copies of their paper CRFs to the lead site periodically. Original copies will be are to be kept at the site and archived following study close out.

The sponsor will use an unambiguous participant identification code that allows identification of all the data reported for each participant.

Database validation and data entry checking procedures will be detailed in the Data Management Plan.

7.9 Access to Data

Direct access will be granted to authorised representatives from the Chief Investigator, Sponsor, host institution and the regulatory authorities to permit study-related monitoring, audits and inspections - in line with participant consent. Participant personal data will only be accessed by those members of the study team that require it for the performance of their role.

7.10 Archiving

Following the resolution of queries and confirmation of study close-out by the Chief Investigator, all essential documentation will transferred to a third party archiving service, which provides suitable fire and water-resistant facilities. Study files will be archived for a period of 25 years. Access to the study documentation will be restricted to named individuals within the study team with express permission from the Chief Investigator.

7.11 Study assessments

See details and schedule events as detailed in Table 1.

7.12 Storage and analysis of samples

Stool samples will be posted directly to the Rugby Bowel Cancer Screening Hub. Urine sample storage and transfer is described above in section 6.

7.13 End of study definition

The study will end once the full 1915 participants have been recruited when the last participant completes their last follow-up assessment. The Sponsor and Chief Investigator reserve the right to terminate the research on safety grounds at any time. Before terminating the research, the sponsor and investigators will ensure that a review of the overall benefit-risk analysis confirms the balance to be no longer acceptable. Should termination be necessary both parties will arrange the relevant procedures which include informing the Research Ethics Committee. On termination of the research, the sponsor

and CI's will ensure that adequate consideration is given to the protection of enrolled participants interests.

The study will be stopped prematurely if:

- Mandated by the Ethics Committee
- Following recommendations from the Trial Steering Committee (TSC)
- Funding for the study ceases

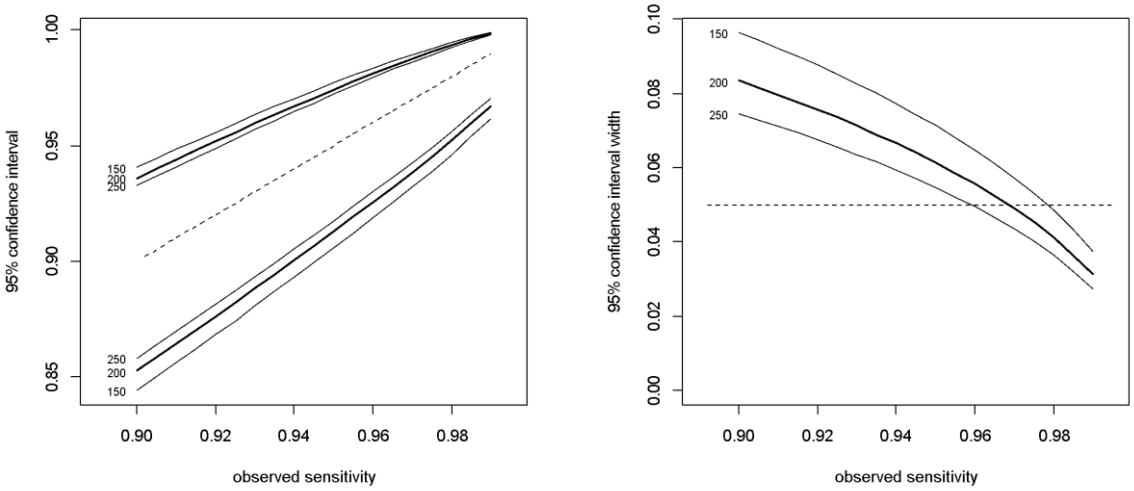
The Research Ethics Committee will be notified in writing if the study has been concluded or terminated early. As this is an observational study with no planned intervention it is thought that there won't be any compelling circumstances which require early termination.

8 STATISTICS AND DATA ANALYSIS

8.1 Sample size calculation

To replace colonoscopy and CTC the new testing strategy requires very high sensitivity to avoid false negative results and missed disease. False positive results are also undesirable, but these participants would simply follow the colonoscopy testing pathway that all participants follow currently. Therefore, the sample size calculations are based on achieving acceptable sensitivity. There are some data on the utility of FIT alone and urine VOC alone in the detection of SBD(6, 10, 37). Our recent study (n=562) is the only published study that evaluated the combination of both FIT and urine VOC for the detection of colon cancer, which was 97% (95% CI: 90 to 100), similar to that for colonoscopy(6). The sensitivity of FIT alone in detecting IBD is 0.85, with the combined accuracy of FIT and VOC unknown. On the basis of these previous studies, and the necessity for high sensitivity to replace colonoscopy, we assumed that the sensitivity (Se) of the FIT and urine VOC tests to detect SBD would be at least 0.97 which is very near 1.0. The confidence interval for such high sensitivity has to be adjusted such that the interval does not exceed 1.0. One recommended method is to use the Zhou-Li method as described in equations 4.5 to 4.10(38). The confidence interval is $\left(\frac{e^{LL}}{1+e^{LL}}, \frac{e^{UL}}{1+e^{UL}}\right)\left(\frac{e^{LL}}{1+e^{LL}}, \frac{e^{UL}}{1+e^{UL}}\right)$ where $LL = \ln\left(\frac{\tilde{Se}}{1-\tilde{Se}}\right) - \frac{1}{\sqrt{n\tilde{Se}(1-\tilde{Se})}} g^{-1}(z_{1-\alpha/2})$, $UL = \ln\left(\frac{\tilde{Se}}{1-\tilde{Se}}\right) - \frac{1}{\sqrt{n\tilde{Se}(1-\tilde{Se})}} g^{-1}(z_{\alpha/2})$, $g^{-1}(x) = -\sqrt{n}\left(\frac{6}{\hat{\gamma}}\right)\left\{\left[1 - \frac{\hat{\gamma}}{2}\left(\frac{x}{\sqrt{n}} - \frac{\hat{\gamma}}{6n}\right)\right]^{1/3} - 1\right\}$ and $\hat{\gamma} = \frac{1-2\tilde{Se}}{\tilde{Se}(1-\tilde{Se})}$. As we are planning a study, $\tilde{Se}$ will be replaced by Se. Assuming that the sensitivity of FIT and VOC to be at least 97% then for Se = 0.97 and a 95% CI width of 0.05 (ensuring a high precision around the sensitivity estimate) the required number of participants with SBD is 200. Further assuming that the prevalence of SBD in the population with bowel symptoms is 19% (likely underestimate), then 1053 participants are required. Based on previous study(6) about 55% of the participants would return both stool and urine samples and sufficient for FIT and VOC analyses. Thus, the total sample size required is 1,915.

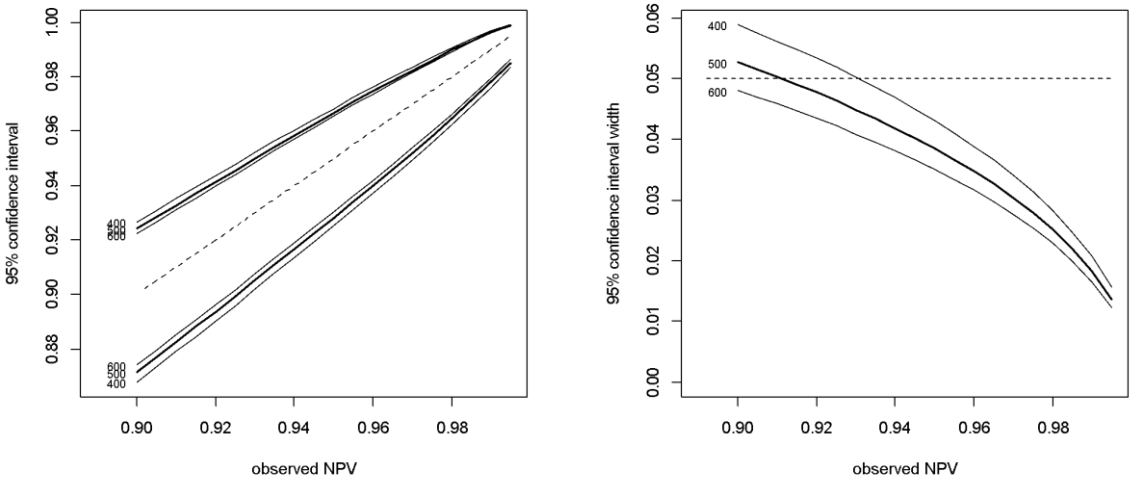
This strategy is robust to changes in the underlying assumptions of test accuracy. Confidence intervals for sensitivity for different observed sensitivity for 150, 200 and 250 cases of SBD are shown in Figure 5(a). For the target sensitivity of 0.97, the width of the confidence interval is 0.05 with 200 SBD participants, with narrower and wider confidence interval limits, when the observed sensitivity is above and below this value, respectively (Figure 5(b)).



(a) (b)

Figure 5 Confidence intervals for different estimates of sensitivity for 150, 200 and 250 cases of SBD.

This sample size is also robust to NPV (Figure 6), an outcome that is of interest as a high NPV suggests that participants without SBD would avoid having colonoscopy examination.



(a) (b)

Figure 6 Confidence intervals for different estimates of NPV for 400, 500 and 600 negative cases of SBD from 1000 samples.

8.2 Statistical analysis

8.2.1 Summary of baseline data and flow of participants

The flow of participants through the study with outcome of the index tests (stool FIT and urine VOC) will be produced. The diagram will follow the template from STARD (Standards for Reporting of Diagnostic Accuracy Studies)(39). Descriptive statistics for the population will be presented in tables with comparisons made between those who do and do not consent to participate, and those who do and do not fully participate by providing a complete set of samples. Full details will be given for exclusions from analysis with reasons where available.

Demographics and baseline characteristics will be summarised as mean and standard deviation, median and interquartile range (for continuous outcome) or frequency and percentage (for categorical outcome). The final diagnosis including histology where available from colonoscopy (the reference test) specifically diagnosis of SBD will be summarised as frequency and percentage.

8.2.2 Primary outcome analysis

The primary outcome is diagnostic accuracy - sensitivity, specificity, NPV and PPV of the dual index test, FIT and VOC. A FIT result that is >10 µgHb/g faeces OR a VOC result with predicted probability >0.63 implies that the participant is defined as having SBD and need to be referred for colonoscopy. We will report 2x2 tables of test accuracy of the dual test by the reference standard (final report from the CTC or colonoscopy examination and confirmatory histology report of SBD which is either colon cancer, colorectal adenomas or inflammatory bowel disease), alongside summary measures of sensitivity, specificity, NPV and PPV, and their 95% confidence intervals. Confidence intervals for sensitivity and specificity estimates that are very close to 1.0 will be according to the Zhou-Li method and the intervals for NPV and PPV estimates that are very close to 1.0 will be according to the Bayesian method (38). These test accuracy estimates will be used in the economic model.

We will analyse the combined FIT+VOC test results as both parallel (results from both tests are interpreted in combination) and serial testing (VOC performed only if FIT negative, i.e. <10 µgHb/g faeces). The "or" rule will be considered in the interpretation of both parallel and serial tests where the diagnosis is positive when either test is positive (FIT >10 µgHb/g faeces and/or VOC predicted probability >0.63), and negative when both are negative (FIT <10 µgHb/g faeces and VOC predicted probability <0.63). In the serial test, if the FIT is positive (FIT >10 µgHb/g faeces) then the diagnosis is positive. Otherwise, use the result from VOC and if it is positive (VOC predicted probability >0.63) then the diagnosis is positive. If the VOC result is also negative then the diagnosis is negative. Mathematically, both methods would give the same diagnostic results but would make a difference in the economic model.

A 2x2 table of test accuracy for each individual index test by the reference test will also be presented. Sensitivity, specificity, PPV and NPV will also be presented with their 95% confidence intervals for FIT only and VOC only. Participants with missing data for either of the index tests (FIT or VOC) or the reference standard (colonoscopy) will be excluded from this analysis.

8.2.3 Secondary outcome analysis

To find the optimal threshold of FIT and VOC we will first plot the receiver operating characteristic (ROC) curves of both tests individually. The individual optimal threshold is the one that gives the greatest specificity whilst achieving the required 97% sensitivity to avoid colonoscopy or CTC. Secondly, we will plot combinations of the two tests in the ROC space and similarly, choose the combination of thresholds which has greatest specificity whilst achieving the required 97% sensitivity to avoid colonoscopy or CTC. The combined thresholds of FIT and VOC in the ROC space is done by combining every unit of FIT (from 7 to 120 µgHb/g faeces) with every unit of the predicted probability of VOC (from 0.63 to 1). Both the "or" and "and" rules will be used in combining the FIT and VOC results to define a case as positive (having SBD and requires colonoscopy) and negative (no SBD and does not require colonoscopy or CTC). The "or" rule is as described in Section 8.2.2. The "and" rule is when both FIT and VOC tests define a case as positive then the diagnosis is positive. If both tests are in discordant, i.e. only one of them defines a case as positive and the other defines it as negative then the diagnosis is negative. For each of the thresholds that we derive from the ROC curves, we will also report the NPV estimates. This will produce an overestimate of test accuracy, which we will use as a sensitivity analysis in the economic model, as a

more optimistic assumption to demonstrate the range of uncertainty. Similarly, the reference standard is the final report from the CTC or colonoscopy examination and confirmatory histology report of SBD.

Other secondary analyses include sensitivity, specificity, PPV and NPV estimates and their corresponding 95% CI for each individual condition of SBD (colorectal cancer, colorectal adenomasand inflammatory bowel disease) by each individual test and combination rules.

We will also estimate the compliance rates – return of stool and urine samples – by presence of SBD or not and individual conditions comprising SBD, as well as if samples are missing at random or not.
All accuracy tests will be adjusted by covariates (demographics and/or clinical parameters) if there is an association between each covariate and the test results.

Appropriate procedures to account for the type of missing data will be performed for sensitivity analyses.

We will investigate the types of SBD and microscopic colitis, and the number of cases missed by CTC, FIT and VOC analyses. Depending on the number of microscopic colitis cases, we may explore the accuracy of FIT and VOC in detecting microscopic colitis with the other SBDs.

A full statistical analysis plan will be written and signed off prior to conducting the final analyses.

8.3 Economic evaluation

An economic analysis will be carried out to determine the costs, benefits and overall cost-effectiveness of FIT + VOC versus FIT followed by colonoscopy or CTC in participants with gastrointestinal symptoms referred for investigation.

As recommended, and in order to meet the needs of NICE for a future technology appraisal, the analysis will be carried out principally from the perspective of the NHS and Personal Social Services (PSS) and it will employ a decision analytic model built as part of this study. Should relevant data be available, additional analyses will consider wider costs that are likely to differ between the compared options, including productivity loss resulting from time taken off paid employment (40, 41).

A review of the literature will be carried out to identify existing decision models used in the broader clinical area, insights from which will inform the structure of a de novo decision model tailored to address the particular decision problem. A preliminary review of the literature has identified only a few relevant decision models, the key one being the model developed as part of NICE Guidance on recognition and referral of suspected cancers (NICE Guideline NG12) (42). This work has informed two more recent models investigating the cost-effectiveness of FIT test as a diagnostic aid for colorectal cancer referrals in primary care commissioned by NICE [15] and the NIHR Health Technology Assessment programme (7). All of these models have employed a multi-part structure, and at this stage, it is envisaged that our model will follow a similar structure, as this is suitable for delineating and capturing short and long-term costs and consequences (accruing over a lifetime horizon). In particular, it is possible that the model will consist of the following three parts:

(i) a decision tree delineating and modelling the short-term cost and consequences (life years and QALYs) resulting from the possible findings (diagnoses) following the use of each diagnostic option
(ii) a simple state-transition model capturing the long-term costs and consequences accruing over a life-time horizon, associated with the treatment and progression of SBD, and
(iii) a simple state-transition model reflecting long-term costs and consequences in participants without SBD.

The first two parts of the model will be run for each of the conditions classified as SBD (i.e. colorectal cancer, colorectal adenomas and inflammatory bowel disease).

Calculations will consider all key costs and consequences, including the costs and 'disutility' associated with having a colonoscopy or CTC. Model input will be drawn from relevant sources, including the RECEDE study and the available literature. For example, parameters related to the diagnostic characteristics of the compared options—which will be a key input in the first part of the model and a crucial parameter for the overall analysis—will be collected directly from RECEDE (we will model the accuracy at the thresholds pre-specified in the primary outcome, and the optimal thresholds from the exploratory analysis) . Further information for the economic model, such as estimates of the rate of adverse outcomes associated with colonoscopies (e.g. bowel perforation and bleeding) or CTCs, preference-based health-related quality of life (utility) values and primary and secondary care resource use for the diagnostic part of the model will be collected from a sample of 370 RECEDE participants (20% of the total intended sample) recruited at UHCW. The questionnaires will continue to be administered until 370 participants have completed all five of their questionnaires.

In planning the study, we considered carefully whether collecting information on the 'disutility' of colonoscopy from patients at one site would introduce any unanticipated bias. We believe that the nature of the data we are interested in (impact of colonoscopy or CTC on patient's quality of life over the short time period before and after the procedure) and the fact that colonoscopy and CTC procedures and the preparation stage preceding them is well defined and standardised across NHS settings, makes it unlikely that single site data collection will results in any unanticipated bias or unrepresentativeness. Given this, and the fact that other sites indicated that further data collection (quality of life questionnaires) might be a barrier to recruitment, we felt that collecting data from a single site is sensible and justifiable.

Costs associated with the compared diagnostic options (including the costs of the diagnostic tests, colonoscopies, CTCs and subsequent staging and treatment in primary and secondary care) will be obtained from manufacturers, the available literature and published sources (including the most recent NHS Reference Cost Schedules, the latest Unit Cost of Health and Social Care report and the British National Formulary). Additional preference-based quality of life ('utility') estimates to be used in the model (e.g. long-term 'utility' values associated with different stages of colorectal cancer or SBD in part (ii) (43) and age and gender specific 'utility' values for the healthy general population in part (iii)(44,45) will be obtained from the relevant literature.

Undergoing a colonoscopy, including the bowel preparation and the procedure itself, will usually mean loss of usual activities (typically for 2-3 days) in addition to experiencing unpleasant symptoms. Participants undergoing a colonoscopy need to follow a low fibre diet for two days prior to the examination and are required to take laxatives the day before. This will lead to frequent, watery diarrhoea and occasional incontinence; thus the process is associated with discomfort, anxiety and affects participants' ability to go about their usual activities. Though CTC is often preferred by patients, it still requires bowel preparation to be completed, and the procedure itself can still be invasive and uncomfortable. Whilst evidence suggests that people are averse to having a colonoscopy, specific evidence on the 'disutility' of a colonoscopy or CTC examination, presented in the form of decrements in preference-based quality of life which can be used in an economic evaluation is scarce. Searches in the literature have highlighted a paucity of evidence, thus the RECEDE study will offer an opportunity to collect individual participant data.

Information on the disutility of colonoscopy and CTC will be captured alongside the collection of HRQoL and resource use for the diagnostic part of the model. This will involve administering the EQ-5D-5L questionnaire to the sample of 370 participants scheduled to undergo colonoscopy or CTC. The collected information will enable us to obtain insights into the impact of the procedure (including the preparation) on participants' quality of life, which is an important parameter in the cost-effectiveness analysis. The

EQ-5D is a widely used generic measure of health-related quality of life that enables the calculation of QALYs and is recommended for use in economic evaluations in health care (47,48). The descriptive part of the EQ-5D-5L questionnaire (which comprises questions on mobility, self-care, usual activities, pain/discomfort, anxiety/depression) and the accompanying visual analogue scale ask participants about their quality of life on the particular day and will be administered at (i) baseline (i.e. when participant consent is received) (ii) immediately prior to colonoscopy or CTC once the participant has fully completed bowel preparation, (iii) 24 hours after the colonoscopy or CTC examination, (iv) 72 hours post colonoscopy or CTC and (v) 3 weeks post colonoscopy or CTC. Collected data from the EQ-5D-5L (5-digit numbers describing the respondent's health state at different points in time) will be subsequently translated into preference-based health related quality of life indices using available value sets (49, 50).

In line with recommendations, final results will be presented in terms of total costs (discounted at 3.5% per annum) per additional QALY associated with each of the compared options (51). Deterministic and probabilistic sensitivity analyses will be undertaken to explore the robustness of the obtained results to sample variability and plausible variations in key assumptions and employed analytical methods [14 20]. The model will also form the basis for conducting value of information analysis, which will quantify the total expected cost due to the remaining uncertainty around the decision problem (53, 54).

9 STUDY OVERSIGHT

9.1 Role and responsibilities of the Sponsor

UHCW has agreed to act as sponsor for this study and will undertake the responsibilities of sponsor as defined by the UK Policy Framework for Health and Social Care Research and ICH Good Clinical Practice. An authorised representative of the Sponsor has approved the final version of this protocol with respect to the study design, conduct, data analysis and interpretation and plans for publication and dissemination of results. As sponsor, UHCW provides indemnity for this study and, as such, will be responsible for claims for any negligent harm suffered by anyone as a result of participating in this study. The indemnity is renewed on an annual basis and will continue for the duration of this study.

9.1.1 Trial Steering Committee

Name	**Affiliation**	**Expertise**
Professor John McLaughlin (clinician)	University of Manchester	Professor of Gastroenterology
Mr Baljit Singh (chair/clinician)	University Hospitals Leicester NSH Trust	Chair of Specialised Colorectal Services CRG NHS England
Dr Fay Cafferty	University College London	Statistician
Dr Rui Duarte	University of Liverpool	Senior Research Fellow and Health Technology Assessment Lead working for the Liverpool Reviews and Implementation Group. Health Economics
Mr Charles Noble	Noble Software Ltd	Software Engineer – Lay member
Professor Ramesh Arasaradnam*	University of Warwick/UHCW	Consultant Gastroenterology

Mr Chris Bradley*	UHCW	Clinical Trial Officer
Mrs Becky Haley*	UHCW	Sponsor Representative

***non-voting members**

The role of the Trial Steering Committee (TSC) will be to provide overall supervision of the study, in particular with respect to the progress of the study, adherence to the protocol, participant safety and the review of new information. The TSC has reviewed and agreed the final version of the protocol. Meetings will be held at regular intervals determined by need, but no less than twice a year or at times determined by stages such as recruitment targets. A TSC Charter will be agreed at the first meeting which will detail how it will conduct its business.

9.1.2 Study Co-ordinator/Manager

The Study Co-ordinator/Manager will have responsibility for overseeing day to day coordination of the study and reporting regularly to the TSC. The Study Manager's responsibilities include, but are not limited to:

- Coordinating protocol development, participant and study management documents;
- Correspondence with study funder and tracking of progress against agreed milestones;
- Setting up and maintaining the Study Master File;
- Ensuring necessary approvals are in place before the start of the study at each site;
- Providing training to study personnel;
- Providing data management support; including data input, maintenance of the study database and raising of queries;
- Producing study progress reports, in particular recruitment against targets, and coordinating TSC meetings and minutes;
- Ensuring data security and quality and ensuring data protection laws are adhered to;
- Ensuring complete records are in place for audit and monitoring purposes;
- Ensuring the study is conducted in accordance with the ICH GCP;
- Archiving all original study documents including the data forms in line with UHCW NHS Trust policy; and
- Visiting study centres and liaison with centre PIs and other staff.

9.1.3 Principal Investigators

Site Principal Investigator responsibilities include, but are not limited to:

- Ensuring that the study is conducted as set out in the protocol and supporting documents;
- Delegating study related responsibilities only to suitably trained and qualified personnel and ensuring that those with delegated responsibilities fully understand and agree to the duties being delegated to them;
- Ensuring that CVs and evidence of appropriate training for all Site staff are available in the Study Site File;
- Ensuring that all delegated duties are captured in the study Delegation Log;
- Ensuring all Adverse Events (that are specifically as a result of study-related activity) are documented and reported promptly to the Study Manager;
- Accountability for study treatments at their site;
- Ensuring the study is conducted in accordance with ICH GCP principles;
- Allowing access to source data for monitoring, audit and inspection; and
- Ensuring that all source data is complete and provided to the Study Manager at regular intervals.

10 MONITORING, AUDIT & INSPECTION

The study may be monitored by the Research & Development Department at UHCW as representatives of the Sponsor, to ensure that the study is being conducted as per protocol, adhering to Research Governance and GCP. The approach to, and extent of, monitoring may be specified in a study monitoring plan determined by the risk assessment undertaken prior to the start of the study.

11 ETHICAL AND REGULATORY CONSIDERATIONS

11.1 Ethical approval and research governance

The study will be conducted in compliance with the principles of the ICH GCP guidelines and in accordance with all applicable regulatory guidance, including, but not limited to, the UK policy framework for health and social care research. Ethical approval for this study will be sought from the Research Ethics Committee combined with Health Research Authority (HRA) approval. No study activities will commence until favourable ethical opinion and HRA approval has been obtained. Progress reports and a final report at the conclusion of the study will be submitted to the approving REC within the timelines defined by the committee. Confirmation of capacity and capability will be obtained from the R&D department prior to commencement of the study at all participating sites.

11.2 Peer review

RECEDE study has been peer reviewed by the NIHR HSD&R grant awards committee. This protocol has also been reviewed externally by the Trial Steering Committee and internally by the Trial Management Group.

11.3 Public and Participant Involvement

RECEDE study was reviewed by members of the GUT club (survivors of gastrointestinal related cancer) and UHCW participant research advisory group at outline stage and again at Stage 2 of the application. The main theme from these consultations were support for non-invasive tests to diagnose SBD and thereby avoid need for a colonoscopy. The group agreed that further evidence for utility of FIT and urine VOC compared to colonoscopy was required hence very supportive of this study. Consequently, we have a Public Co-applicant who will contribute through overseeing of conduct and progress of the study from inception and design to delivery throughout the duration of RECEDE study. Specifically, the Public Co-applicant will review the PPI strategy throughout the project, support public dissemination of findings, writing of material that is appropriate for the target audience, co-author on manuscripts as well as being part of the TMG. Our Public Co-applicant also has first-hand experience of colonoscopies and will provide guidance in developing participant facing materials. He has been involved in developing other NIHR projects and has attended 'Being Part of a Research Team' workshop at Warwick CTU. Together with other PPI contributors, they will be funded to attend conferences as part of the research team and contribute to delivery of presentations to ensure participant/public perspective is included. Specific training needs will be identified and supported by UHCW (sponsor) accordingly.

11.4 Data protection and participant confidentiality

The study will comply with the current Data Protection regulations and regular checks and monitoring will be undertaken by the Study Manger to ensure compliance. Participants will be assigned a unique identifier upon enrolment into the study to allow pseudonymisation of participant-identifiable data. Access to participant identifiable data will be restricted to members of the site study co-ordination team who require it for the performance of their role. Electronic data will be stored on password protected encrypted drives and hard copies of study documents will be stored in locked filing cabinets in secure entry-card protected sites.

12 DISSEMINATION POLICY

It is anticipated that broadly two important findings will emerge. From a clinical stand point, the utility of FIT plus urine VOC testing for SBD detection. This will enable us to determine if there is a group without SBD that can potentially avoid the need for a colonoscopy or CTC with quantification of potential cost savings. Naturally results will be of profound interest to clinicians worldwide that investigate lower gastrointestinal symptoms. Results can also be used by NICE to inform a revised pathway for managing participants with lower gastrointestinal symptoms – to categorise high risk i.e. those with SBD versus low risk (without SBD). Those with very low risk of SBD could potentially be managed in primary care without need to refer to secondary care.

This will lead to high level presentations at Gastrointestinal and Oncological meetings both nationally and internationally. It will also benefit from production of high quality open access manuscripts.

We will also evaluate the health economic impact as well as savings to the NHS which will be of interest to NICE. Hence results from RECEDE will form key evidence base for review of guidance on how to manage participants with lower gastrointestinal symptoms referred from primary care. Additionally, some co-applicants (NW, RA and STP) have been involved in technology assessments by the NICE Diagnostics Programme, including the appraisal of faecal calprotectin. Care would be taken to produce the sort of evidence required by NICE, including costs per QALY.

A range of dissemination products to include annual reports, national publications, press releases through UHCW's Communications Department, participant safety collaborations, presentation and talks as well as videos will be included to ensure that all audiences can be updated. The team has close links with the West Midlands Academic Health Science Network (AHSN) and will make use of the Meridian Health Innovation Exchange Platform (https://meridian.wmahsn.org) to disseminate the results of the study. Through this, the wider national network of AHSN's will also be exploited for this purpose together with UHCW Communications and other online stakeholder case study platforms, including MidTECH and NHS Innovation Hub for the West Midlands region.

Finally, the disutility of colonoscopy and CTC will be evaluated in participants with symptoms – previous evaluation in 2010 was undertaken in those undergoing screening colonoscopies, but none in the larger group of those with symptoms referred for investigations.

Dissemination of results to participants will be led by our PPI co-applicant and facilitated through GUTs UK which is the partner charity of the British Society of Gastroenterology. We will also engage with key stakeholders including PPIE groups, local specialised colorectal Clinical Research Group (CRG), as well as Cancer Alliance groups.

All data arising from RECEDE will lie with the sponsor. Authorship in the final report will be granted (order to be agree) and in line with ICMJE guidance; http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html for those that contributed directly to the study.

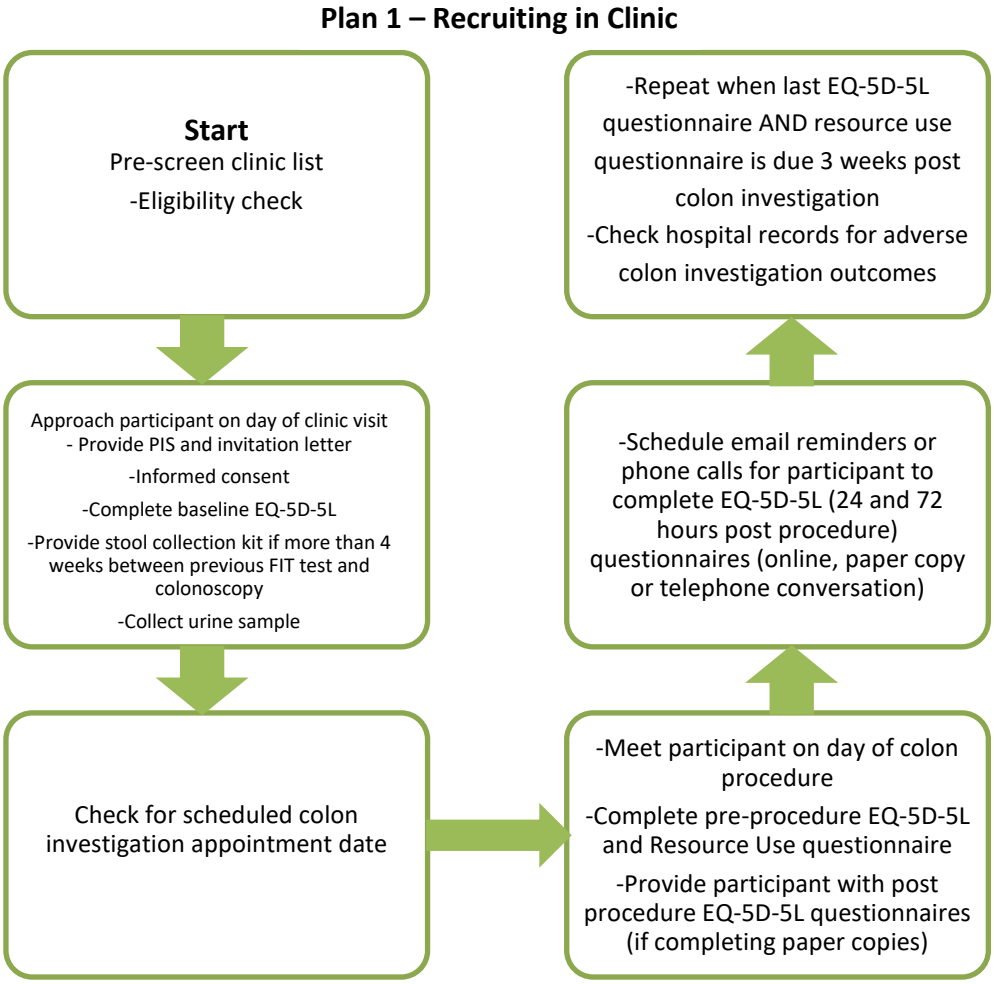
13 APPENDICES

Appendix A Volatile compounds in urine

Appendix A Table 1 Summary of key VOCs identified in urine in those with SBD. Note that individually these compounds are non-discriminatory but collectively in their specific relative abundance these chemicals are discriminatory in the diagnosis of SBD.

VOLATILE COMPOUNDS (33,55,56)	**DESCRIPTION**
ACETONE	Acetone has been shown to contribute to pH regulation within the mammals. It also has the potential to be used as a cellular fuel source to aid metabolic processes in the liver. It is produced by the oxidation of fatty acids by gut bacteria(57-59).
2-PENTANONE	Common urinary volatile metabolite noted in different diseases. Also found in some foods (yoghurt, honey) with higher levels found in faeces (60).
4-HEPTANONE	4-heptanone is found in healthy control groups compared to leukaemia, CRC and lymphoma(61, 62).
1,3,5,7-CYCLOOCTATETRAENE	This chemical has not been noted in studies investigating the products of metabolic processes. It is linked with fermented wheat germ and provides a potential connection with the metabolic products being absorbed in the gut. We have noted its presence in coeliac disease and filed a patent (34).
ALLYL ISOTHIOCYANATE	This chemical has been found to inhibit the growth of many strains of pathogenic intestinal bacteria associated with inflammation(63, 64).
OXIME-, METHOXY-PHENYL-	Production linked with Myxobacteria such as *Sorangium cellulosum*(64)
1,3-PROPANEDIAMINE	Common metabolic molecule produced by certain bacteria. Also noted in the human gut(65, 66).
CARVONE	Carvone is a food additive and natural product in mint and can inhibit the growth of fungal and bacterial microbes. It also inhibits quorum sensing which bacteria use to aid virulence(67).
ETHANONE, 1,1'-(1,4-PHENYLENE)BIS	This compound has been linked to interactions between gut bacteria metabolism and host physiology in response to disease(68, 69).
PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-	Common urinary volatile metabolite noted in health and disease. It is also thought to inhibit quorum sensing utilised by bacteria(70).

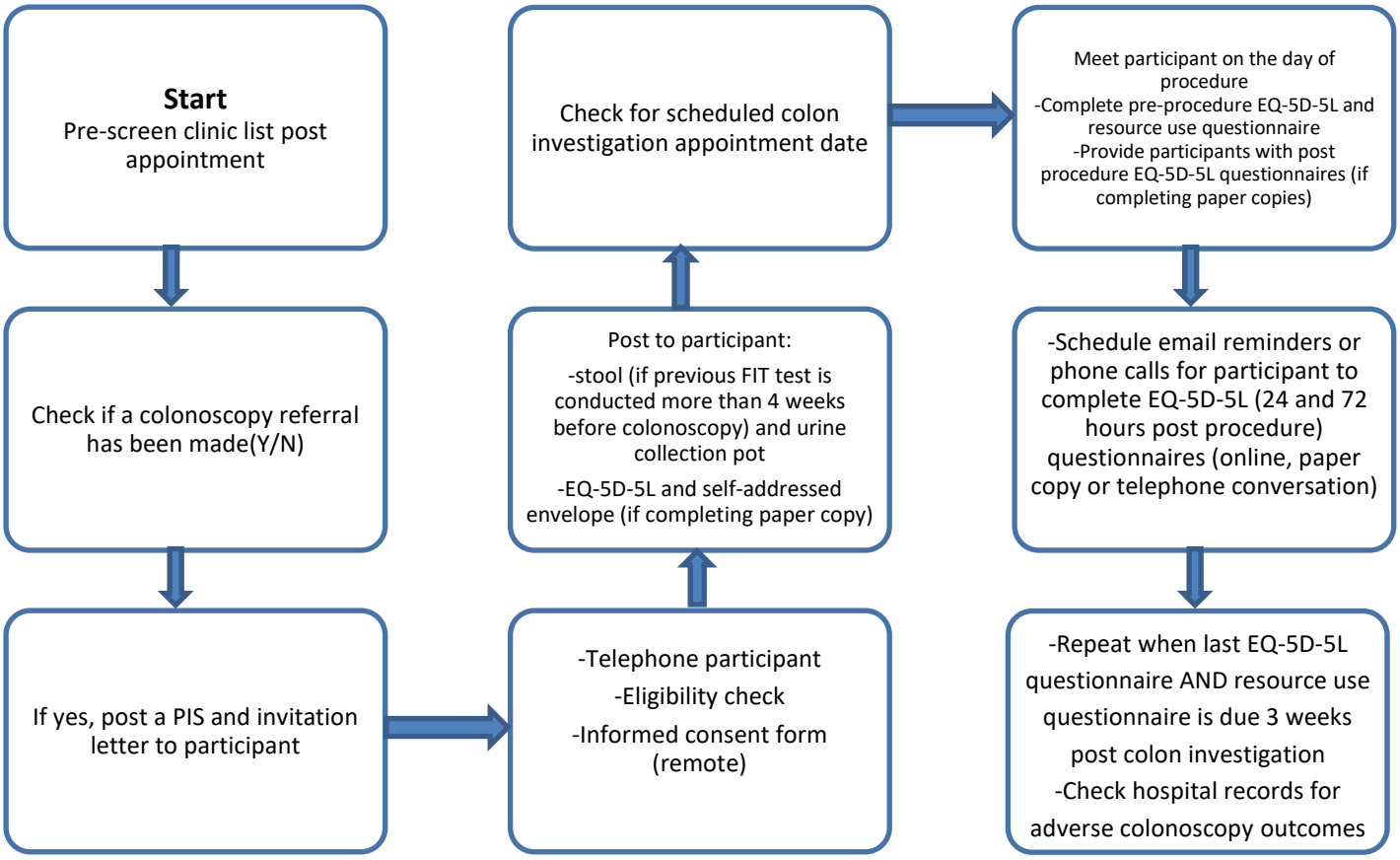
Appendix B RECEDE recruitment and assessments process



NOTE: Q*uestionnaires are only being completed at sponsor site (UHCW).*

Appendix B Figure 1 RECEDE clinic recruitment and assessment flow chart.

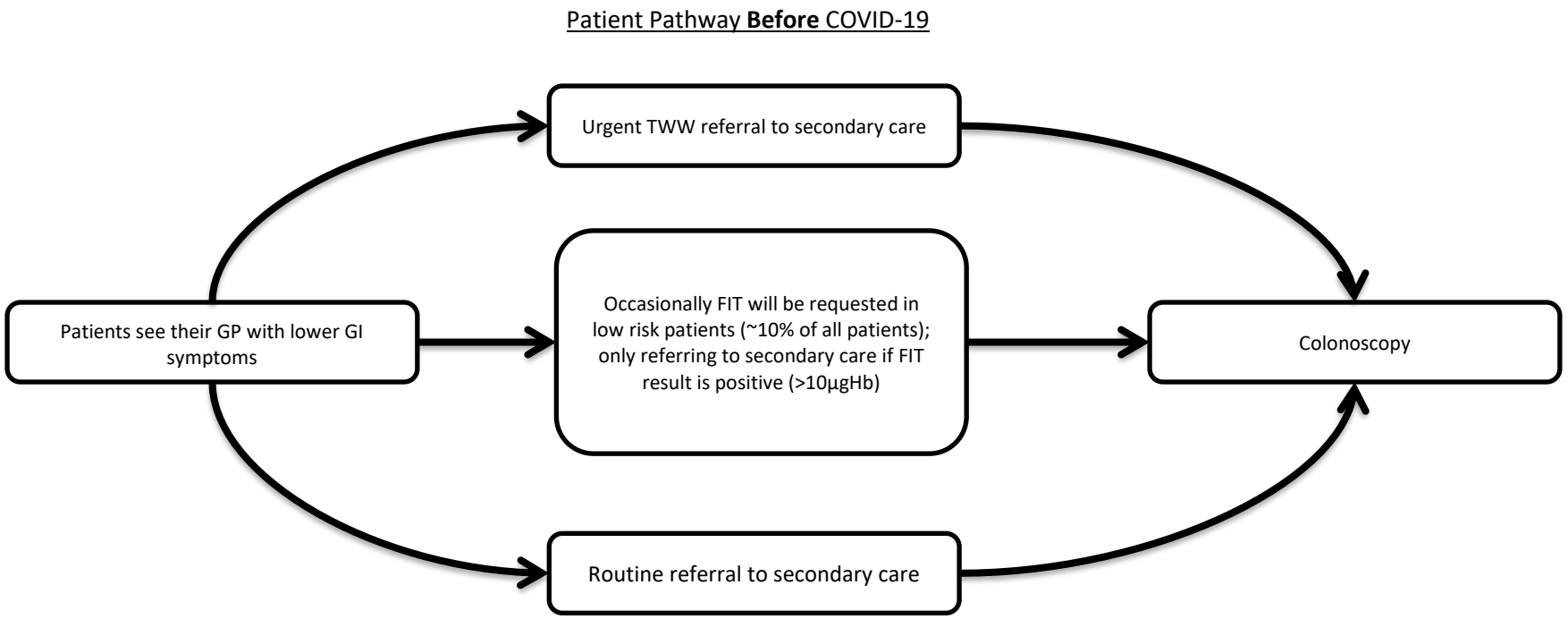
Plan 2 – Recruiting Remotely

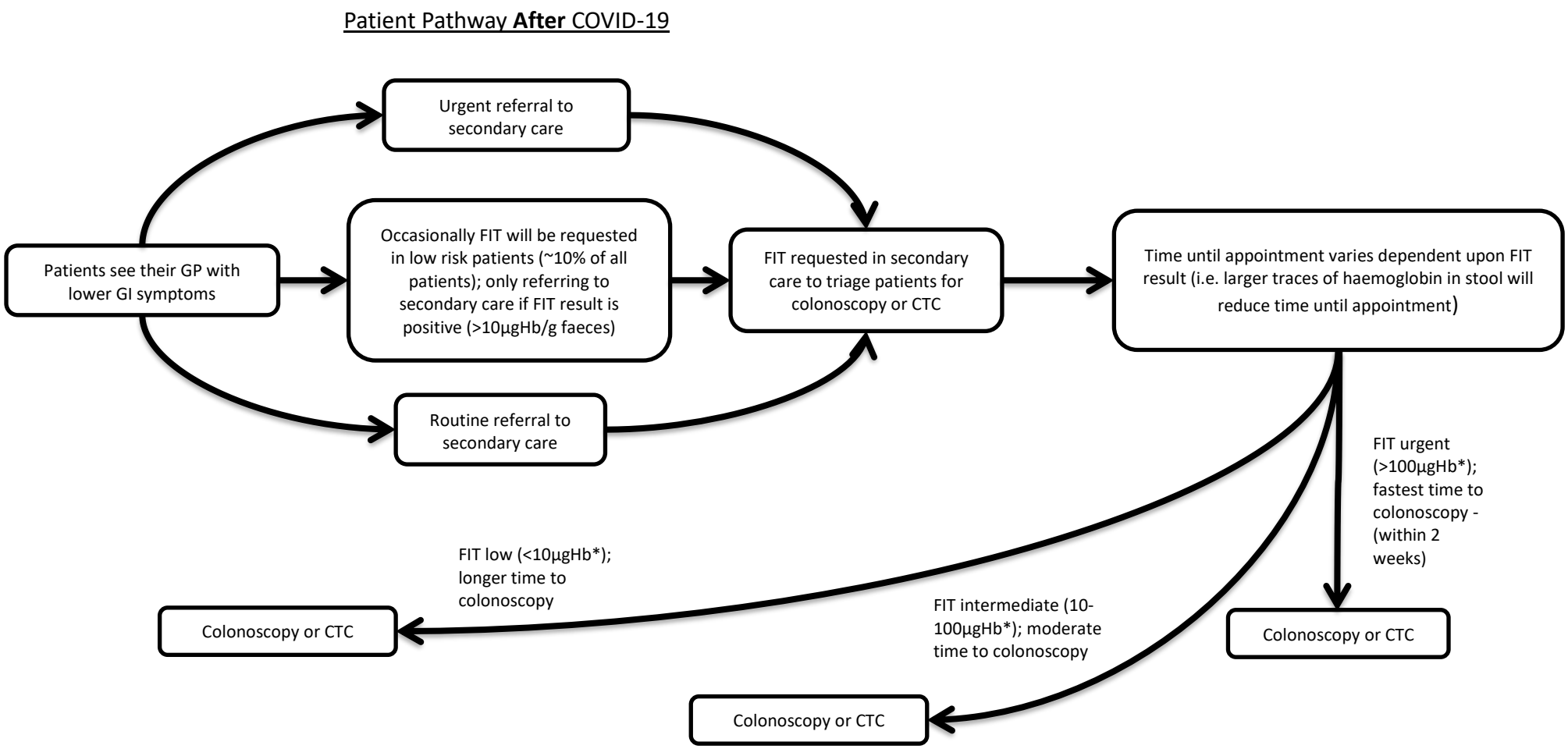


NOTE: Q*uestionnaires are only being completed at sponsor site (UHCW).*

Appendix B Figure 2 RECEDE remote recruitment and assessment flow chart.

Appendix C Participant referral pathways





*values are estimates and will vary regionally based on demand and available capacity

Appendix C Figure 3. *The changes caused by COVID 19 to patient referral pathways for colonoscopy*

Appendix D Volatile Organic Compounds (VOC) Sampling

VOCs are detected by applying a solid-phase micro extraction (SPME) technique to the urine samples. SPME is a technique which is established as a means for preconcentrating samples for analysis by gas chromatography and has been adapted for this study to capture and present VOCS to a gas sensor array used as a detector. The technique is applicable for gas and liquid samples. Special absorptive or adsorptive 'sorptive' materials are used to extract analytes of interest by capturing them in their matrices. The sorptive materials are then heated to a suitable temperature to cause the analyte to desorb from the coating and pass directly into the analytical part of the instrument. In this case proprietary SPME polymer tabs manufactured by Sensam Ltd are used to capture urine VOCs.

1. Specimen bottles with frozen urine samples are placed in water bath, set to 25C, for 1 hour.

2. Bottle with thawed samples is shaken briefly to mix the contents.

3. Sample ID is written two sealable plastic bags with suffixes a and b respectively.

4. A thawed sample is transferred to a larger sample bottle (big enough to accommodate SensAm Tabs).

5. A lid with two slots is screwed onto the larger sample bottle.

6. Two SensAm Tabs are removed from the packaging and inserted into the slots in the lid of the bigger sample bottle.

7. Tabs are left in the head space above the sample for 5 minutes.

8. After 5 minutes Tabs are removed from the slot in the lid, placed it into the sealable bags, labelled in step 3 above, which are then sealed.

9. Sample and the bottles are disposed of according to appropriate procedures for the disposal of such materials and items.

10. Sealed bags with exposed Tabs are taken to SensAm's instrument.

Exposed Tabs Processing

When the Sensor Tabs are inserted in to the SensAm device for VOCs analysis, they are then automatically heated in the device to 120°C to desorb captured VOCs. Responses from the array of gas sensors to the desorbed vapours are captured over a period of 180 seconds, digitized and stored.

1. When SensAm's instrument shows "Ready, Insert Sensor Tab" message, one exposed tab is removed from the sealed plastic bag.

3. The data logging file is named after the label on the bag, including the suffix.

3. The Tab is inserted into the input port of the instrument.

4. Tab processing is started by touching the screen when invited to do so.

5. When instrument indicates that the Tab processing is completed, the file with the logged data is saved.

6. The used Tab is removed from the input port of the instrument when instrument issues an instruction do so and discarded in the appropriate waste bin.

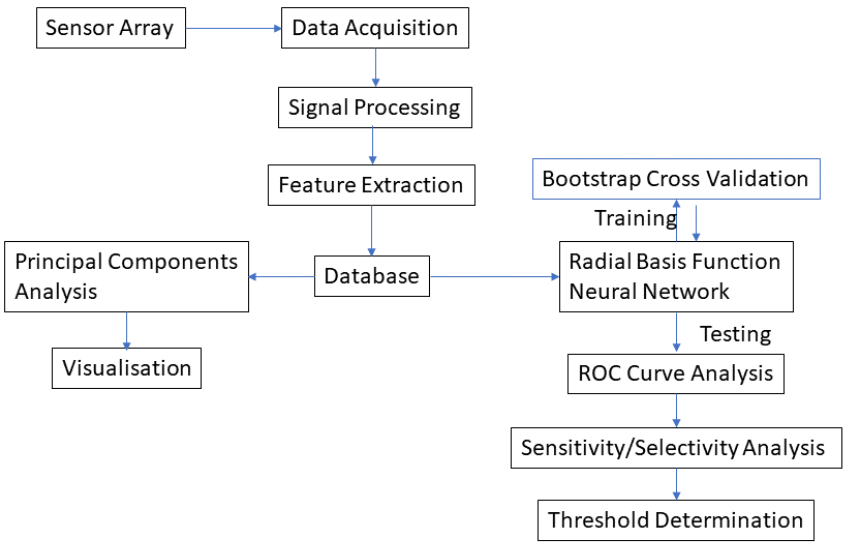
7. Steps 1 to 7 are repeated until all exposed Tabs are processed.

Data Analysis

1. Each logged data file is transferred to the SENSAMSNIFFER program.

2. Appropriate data base containing key points from captured profiles is generated.

3. Analysis of the data base is performed, as required, using the SENSAMSNIFFER program.



Appendix D Figure 4 Data processing protocol.

Appendix D Figure 4 shows an outline of the data processing protocol. The portion of the gas sensor response corresponding to the detection of desorbed volatiles from an individual sample is extracted and averaged to produce n=5 samples straddling the response profile. For each patient sample, databases of the patterns are created, and these form the basis for further data processing. To visualise the data from all the samples that are processed, the method of principal components analysis (PCA) is used to provide an unbiased means of observing clusters or patterns when amalgamating samples from a patient population and a control population.

To discriminate between different classes of data a neural network based on radial basis functions is employed. Radial basis function networks (RBFN) are a variant of three-layer feedforward neural networks. They contain an input layer, a hidden layer and an output layer where the transfer function in the hidden layer is called a radial basis function (RBF). To each individual pattern in the databases a class

name is assigned. The neural network is trained against a subset of sample patterns and a bootstrap method used for validation and testing the prediction accuracy of the neural network against previously unseen samples. The output nodes of the neural network are scaled to provide an output scaled between 0 to 1 representing the probability of an input sample pattern belonging to a certain class.

Positive predictive value is the proportion of positive results that are true positives (i.e. have the target condition) whereas negative predictive value is the proportion of negative results that are true negatives (i.e. do not have the target condition). This approach usually requires the creation of a cut-off point from continuous data and depending on the cut-off selected, the sensitivity and specificity of a test will vary. If the cut-off is selected so that the sensitivity increases, the specificity will decrease. ROC curves are a way of graphically displaying true positives versus false-positives across a range of cut-offs and of selecting the optimal cut-off for clinical use to be selected. To generate Receiver Operating Curves (ROC), the neural network is trained on a series of test cases versus controls, and ROC are generated for each of the test cases from the outputs of the neural network when tested against previously unseen patterns, using established algorithms. The ROC curves are analysed to determine appropriate thresholds to optimise sensitivity and selectivity of the VOC analysis tests. These studies address how well the VOC test identifies the target condition of interest. Appendix D Table 2 below shows the data generated by the neural network and ROC analysis.

Appendix D Table 2 Data generated by the neural network and ROC analysis

Result	**Disease Present**	**Disease Absent**	**Total**
	True Positive (TP)	False Positive (FP)	TP+FP
	False Negative (FN)	True Negative (TN)	TN+FN
Total	TP+FN	TN+FP	
Sensitivity	TP/(TP+FN)		
Specificity	TN/(TN+FP)		
Positive predictive value (PPV)	TP/(TP+FP)		
Negative predictive value (NPV)	TN/(TN+FN)		
Positive likelihood ratio (LR+)	Sensitivity/(1-specificity)		
Negative likelihood	(1-		

ratio (LR-)	sensitivity)/specificity		

(a) Sensitivity, specificity, predictive values and likelihood ratios (LRs) are all different ways of expressing test performance.
(b) Receiver operating characteristic (ROC) curves compare sensitivity versus specificity across a range of values for the ability to predict a dichotomous outcome. Area under the ROC curve is another measure of test performance.
(c) All of these parameters are not intrinsic to the test and are determined by the clinical context in which the test is employed.
(d) High sensitivity corresponds to high negative predictive value and is the ideal property of a "rule-out" test.
(e) High specificity corresponds to high positive predictive value and is the ideal property of a "rule-in" test.

From previous work [6] it has been shown that the use of FIT alone for CRC detection revealed a sensitivity of 0.80 [95% confidence interval (CI) 0.66– 0.93] and specificity of 0.93 (95%: CI 0.91–0.95). The use of urinary VOC's for the detection of CRC gave a sensitivity of 0.63 (95% CI: 0.46–0.79) and specificity of 0.63 (95% CI: 0.59–0.67). The AUC was 0.67 (95% CI: 0.57–0.77) and the NPV was 0.96 (95% CI: 0.94–0.98). For high-risk adenoma and all adenomas, using urinary VOCs the sensitivity was 0.93 (95% CI: 0.81–1.0) and 0.91 (95% CI: 0.85–0.97), respectively, with specificity of 0.16 (95% CI: 0.13–0.20) and 0.15 (95% CI: 0.12–0.19), respectively. These data give a guide for fixing a sensitivity threshold for the VOC analysis which we propose as a probability of **0.63** on the basis of the above data. This would be refined as the study progresses to optimise the sensitivity and selectivity for the VOC analysis and to examine the effects of combining FIT plus VOC analysis.

An assignment of pre-test probability is a major factor influencing any decision to undertake a diagnostic test or not and it presupposes that there is diagnostic uncertainty that is the reason for this study. Related to this are the concepts of test and treatment thresholds. If the probability of a condition is very unlikely (i.e. below the test threshold), it can be eliminated from the differential diagnosis. If the probability is sufficiently high for treatment to be initiated (above the treatment threshold), then testing is not really required. Where the probability lies between the two thresholds, further diagnostic testing is indicated. Where the thresholds are set depends upon the clinical context and clinician preference which are all part of the RECEDE study where the combination of FIT and VOC measurements would tend to enhance the certainty of a diagnosis.

Appendix E Costs and Consequences

Appendix E Table 3: Investigation of People with Lower Bowel Symptoms – Costs and Consequences. (Blank boxes indicate areas which RECEDE will attempt to answer)

Strategy	**Benefits**	**Costs**
No filter – all people with symptoms get colonoscopy or CTC	Very high sensitivity	Very high numbers of negative colonoscopies Morbidity to patients and less QALY
Filter by symptoms only		
Filter by red flag symptoms* – no FIT testing	High detection of CRC in this group? Where high = 20% or more?	What % of all CRCs have red flag symptoms? Minority? What % CRC missed?
TWW overall – see NICE DG for criteria		Only 9% have CRC, and only 12% of all CRCs detected via this pathway (5)
Add single testing with FIT		
UHCW study (5) TWW (all n= 612) TWW FIT positive (≥10μgHb/g) 135 people, 30 cancers TWW Fit negative (<10μgHb/g) 477 people, 5 cancers	CRC 6% CRC 22% CRC 1%	Scoping only FIT positives would reduce scopes by 78% - major cost savings and disutility's reduced. 95 colonoscopies saved for each CRC missed, almost £70,000. NICE DG30: symptoms not enough for TWW but if FIT > 10μgHb/g, qualify for referral via TWW
FIT > 100μgHb/g go to colonoscopy or CTC	High proportion of CRC in this group where "high" might be 20%	Fewer colonoscopies and/or CTCs and lower costs than with lower cut-off but % of CRC missed
FIT > 50μgHb/g to colonoscopy or CTC	Higher % of CRC detected	Far more colonoscopies and/or CTCs with costs and disutility's

		than with higher cut-off
FIT ≥ 10μgHb/g (NICE) or 7μgHb/g to colonoscopy	Highest % of CRC detected but marginal compared to cut-off of 50?	Huge number colonoscopies or CTCswith cost and disutility's
FIT 50 to 99μgHb/g	What % have CRC?	
FIT 7 to 49μgHb/g	Low prevalence CRC? Avoid colonoscopies	Missed cancers
FIT negative (<7μgHb/g)	Avoid colonoscopy or CTC	Review if symptoms persist? About 10% have cancer
Single testing with VOC		
As above, VOC but no FIT	80% Sensitivity	
Dual testing FIT and VOC		
FIT positive (≥10μgHb/g) to scope, FIT negatives to VOC (5)	97% Sensitivity	Most positives not CRC so lot of negative endoscopies. What % FIT negative cancers would be VOC positive?
FIT 50-99μgHb/g, VOC negative		
FIT 50-99μgHb/g, VOC positive	How many cancers detected?	
FIT 7-47μgHb/g, VOC negative		
FIT 7-49μgHb/g, VOC positive		
FIT negative (<7μgHb/g) but VOC positive	Missed cancers reduced from 10% to 3%?	

*Red flag symptoms include rectal bleeding, a change in bowel habits for at least 6 weeks, weight loss, anaemia, abdominal pain or mass.

NICE's guideline DG 30 2017 on suspected cancer (so ignoring other serious bowel disease) on assessing people presenting to primary care with clinical signs and symptoms that may suggest colorectal cancer makes the following recommendations:

Refer people using a suspected cancer pathway referral (TWW) for colorectal cancer if:

- they are aged 40 or over with unexplained weight loss and abdominal pain or
- they are aged 50 or over with unexplained rectal bleeding or
- they are aged 60 or over with:

- iron-deficiency anaemia or
- changes in their bowel habit, or

- Tests show occult blood in their faeces.

A suspected cancer referral should also be considered for:

- People with a rectal or abdominal mass
- Adults aged under 50 with rectal bleeding and any of the following unexplained symptoms or findings:
  - abdominal pain
  - change in bowel habit
  - weight loss
  - Iron-deficiency anaemia.

NICE guidance DG30

> 1.1 The OC Sensor, HM-JACKarc and FOB Gold quantitative faecal immunochemical tests are recommended in primary care to guide referral for suspected colorectal cancer in people without rectal bleeding who have unexplained symptoms but do not meet the criteria for a suspected cancer pathway referral outlined in NICE's guideline on suspected cancer .
>
> 1.2 Results should be reported using a threshold of 10 micrograms of haemoglobin per gram of faeces. Companies should provide advice about the performance characteristics of their assays to laboratories and ensure standardisation of results.

Key thing is diagnosis at a stage when surgically resectable

Dukes A – 5-year survival >95%

Dukes D – 5-year survival <10%

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