Randomised, double-blind, placebo controlled multi-centre study to assess the efficacy, tolerability and safety of Enterosgel® in the treatment of Irritable Bowel Syndrome with Diarrhoea (IBS-D) in adults (RELIEVE IBS-D)

STUDY PROTOCOL

Document Description	Full Study Protocol
Brief Study Title	Enterosgel® in the treatment of Irritable Bowel Syndrome with Diarrhoea
Acronym	RELIEVE IBS-D
Version and Date	Version 6.0 29/06/2020
Protocol Reference (internal)	ENT04UK
IRAS number	230930
National Institute for Health Research (NIHR) portfolio number	GAST 34032
International Standard Randomised Controlled Trial Number (ISRCTN)	ISRCTN17149988
Development Phase	Investigation for CE marking purposes
Sponsor	Enteromed Ltd
Chief Investigator	Professor Yan Yiannakou

Confidentiality Statement

SIGNATURE PAGE

Study Name: Randomised, double-blind, placebo controlled multi-centre study to assess the efficacy, tolerability and safety of Enterosgel® in the treatment of Irritable Bowel Syndrome with Diarrhoea (IBS-D) in adults

Study Number: ENT04UK

The Sponsor and Chief Investigator have approved the protocol version v.6.0 dated 29th June 2020, and confirm hereby to conduct the study according to the protocol and the Sponsor's SOPs, the current version of the World Medical Association Declaration of Helsinki, International Conference of Harmonisation (ICH) Good Clinical Practice (GCP) guidelines and the applicable regulatory requirements.

Chief Investigator:

Professor Yan Yiannakou		3 July 2020
Name	Signature	Date

Sponsor:

Elena Markaryan		3 July 2020
Name	Signature	Date

Statistician:

Professor Victoria Allgar		3 July 2020
Name	Signature	Date

For Local Principal Investigator (PI) at study site:

I agree to conduct this trial as set out in this study protocol, and to comply with the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines and the applicable regulatory requirements.

_____

Site Name

Name	Signature	Date

CONTACT DETAILS

Reporting of Serious Adverse Events (SAEs)
Sites are responsible for submitting SAEs to the Sponsor within 24 hours by email to research@enteromed.co.uk (copy anu@clevercookie.net)

Chief Investigator	Professor Yan Yiannakou (MBChB, MRCP, MD) Consultant Neurogastroenterologist County Durham and Darlington NHS Foundation Trust University Hospital of North Durham, North Road, Durham, DH1 5TW Email: yan.yiannakou@nhs.net Tel: 0191 333 2889
Sponsor	Enteromed Ltd 85 Great Portland Street, First Floor, London, W1W 7LT Email: emarkaryan@enteromed.co.uk Tel: 07860 817365
Funder	Bioline Products s.r.o Krakovska 1338/10 110 00 Praha Czech Republic Email: info@enterosgel.eu Phone: +420 266 317 783
Clinical Trial Manager, Data Manager	Dr Anu Kemppinen Clever Cookie Ltd Email: anu@clevercookie.net Tel: 07826 190018
Statisticians	**Main data analyses:** Dr Victoria Allgar The University of York Email: Victoria.Allgar@hyms.ac.uk Tel: 01904 321321 **GI-MAP™ data analyses:** Dr Clare Lanyon Northumbria University Email: clare.lanyon@northumbria.ac.uk Tel: 0191 2274900
CRF, eDiary and Randomisation tool	Sealed Envelope Ltd 501 Clerkenwell Workshops, 27-31 Clerkenwell Close, Clerkenwell, London, EC1R 0AT Email: contact@sealedenvelope.com Tel: 02031 764242
Steering Committee	Professor Charles Knowles (MBBChir, PhD, FRCS, FRCS (Gen Surg)) Professor of Surgery Barts and the London SMD, Queen Mary University of London Email: c.h.knowles@qmul.ac.uk Professor John McLaughlin (BSc (Hons), MB ChB(Hons), PhD, FRCP) Head of the Division of Diabetes, Endocrinology and Gastroenterology in the Faculty of Biology, Medicine and Health The University of Manchester Email: John.Mclaughlin@manchester.ac.uk

	Professor Peter Whorwell (BSc, MB, BS, MD, PhD, FRCP) Director of the South Manchester Neurogastroenterology Service University Hospital of South Manchester Email: Peter.Whorwell@uhsm.nhs.uk Dr Preeti Pandya (MBBS, MD, DFFP, DRSH, MRCGP) General Practitioner The Village Practice Email: preetipandya@nhs.net
Data Monitoring Committee	Dr Maria Eugenicos (MD, PhD) Senior Lecturer and Specialist in Gastroenterology The University of Edinburgh Email: Maria.Eugenicos@nhslothian.scot.nhs.uk Dr Kasim Adetayo (BSc, MSc, PhD, CStat) Associate Professor in the Wolfson Research Institute for Health and Wellbeing Durham University Email: a.s.kasim@durham.ac.uk

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ADE	Adverse Device Effect
AE	Adverse Event
AxMP	Auxiliary Medicinal Product
BSFS	Bristol Stool Form Scale
DMC	Data Monitoring Committee
eCRF	electronic Case Report Form
EMA	European Medicines Agency
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI-MAP	Gastrointestinal-Microbial Assay Plus
GP	General Practitioner
IBS	Irritable Bowel Syndrome
IBS-D	Irritable Bowel Syndrome with Diarrhoea
IBS-QOL	Irritable Bowel Syndrome Quality of Life Instrument
IBS-SSS	Irritable Bowel Syndrome Severity Scoring System
ICH	International Conference of Harmonisation
ISRCTN	International Standard Randomised Controlled Trial Number
MRI	Magnetic Resonance Imaging
NHS	National Health Service
NICE	The National Institute for Health and Care Excellence
PHQ-15	The Patient Health Questionnaire 15
PHQ-12 SS	PHQ-12 Somatic Symptom scale. A modified version of the PHQ-15 with three questions on gastrointestinal symptoms excluded
PI	Principal Investigator
PIC	Patient Identification Centre
REC	Research Ethics Committee
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
TMG	Trial Management Group
TSC	Trial Steering Committee
UADE	Unanticipated Adverse Device Effect
WPAI	Work Productivity and Activity Impairment
WPAI:IBS	Work Productivity and Activity Impairment (IBS-specific questionnaire)

TRIAL SUMMARY

Title:	Randomised, double-blind, placebo controlled multi-centre study to assess the efficacy, tolerability and safety of Enterosgel® in the treatment of Irritable Bowel Syndrome with Diarrhoea (IBS-D) in adults
Acronym:	RELIEVE IBS-D
Phase:	Medical device study for CE marking purposes
Chief Investigator:	Professor Yan Yiannakou
Sponsor:	Enteromed Ltd
Intervention:	Enterosgel® (Bioline Products s.r.o), which is an intestinal adsorbent categorised as a class IIa medical device
Study Design:	UK multi-centre, parallel arm, randomised, double-blind, placebo controlled trial to evaluate the efficacy, tolerability and safety of Enterosgel® in the treatment of Irritable Bowel Syndrome with diarrhoea (IBS-D) in adults. The study involves a 2-week screening phase, after which eligible patients are randomised 1:1 to receive Enterosgel® or placebo for 8 weeks. After the double-blind treatment phase, all patients will receive open-label Enterosgel® treatment for 8 weeks. The patients then return to standard care. Those patients who responded to open-label treatment are followed up with a phone call at 8 weeks. The study involves four study visits/remote appointments and two follow-up phone calls. Patients will complete a daily study diary during the 2-week screening period and during the 16 weeks of treatment. In addition, patients complete weekly (IBS-SSS, WPAI:IBS) and 4-weekly (IBS-QOL, PHQ-12 SS) paper questionnaires over the 16-week treatment period.
Objectives:	**Primary objective** The primary objective of this study is to test the efficacy (superiority) of Enterosgel® (Bioline Products s.r.o, Czech Republic) compared with placebo in terms of patient reported outcomes for stool consistency and abdominal pain. **Secondary objectives** The secondary objectives for the **double-blind treatment phase** are: 1. To determine the efficacy of Enterosgel® compared with a parallel placebo group in terms of: • patient-reported symptoms (diary, Irritable Bowel Syndrome Severity Scoring System score [IBS-SSS], PHQ-12 Somatic Symptom (PHQ-12 SS scale) • use of loperamide as rescue medication 2. To determine effects of treatment on health-related quality of life (IBS Quality of Life [IBS-QOL]) and IBS-related work productivity and activity impairment (WPAI:IBS). 3. To evaluate safety of Enterosgel® through reported adverse events potentially related to treatment. The secondary objectives for the **open-label treatment phase** are: 1. In patients who had received placebo as blinded treatment, to compare the study outcome measures (same as for double-blind phase above) over the open-label Enterosgel® treatment period to the preceding placebo treatment period. 2. In patients who had received Enterosgel® as the blinded treatment, to test whether the treatment benefits (in terms of study outcome measures; same as

	for double-blind phase above) can be effectively maintained with Enterosgel® in those patients who achieved control in the last 4 weeks (of the double-blind treatment phase) 3. To evaluate safety of Enterosgel® through reported adverse events potentially related to treatment. The secondary objectives for the **follow-up phase** are: In patients who reported adequate relief in the last 4 weeks of open-label period: 1. To assess the percentage of patients who report increased or maintained treatment benefit after cessation of treatment 2. To assess the percentage of patients who used over-the-counter Enterosgel® after cessation of open-label treatment and to assess the frequency of use in these patients 3. To assess the percentage of patients who report reduced loperamide use after cessation of treatment, compared to loperamide use before the trial **<u>Exploratory objective</u>** 1. To investigate faecal microorganisms and biomarkers (GI-MAP™ assay) in a subgroup of 20 patients at baseline and following 8 weeks of double-blind treatment, in order to gain understanding on the potential impact of Enterosgel® on the gut microbiome. 2. To obtain qualitative and quantitative information on gut-related parameters using magnetic resonance imaging (MRI) scan and GIQuant image processing software (Motilent Ltd, UK), in a subgroup of 16 patients at Baseline and after 4 weeks of open-label Enterosgel® treatment, in order to gain understanding on the potential impact of Enterosgel® on intestinal motility, fluid volume, gas content and physiology.
Outcome measures:	**<u>Primary Outcome Measure</u>** Percentage of patients defined as responders for abdominal pain and stool consistency during at least 4 weeks in the 8-week treatment period, where: 1) An *Abdominal Pain Intensity Weekly Responder* is defined as a patient who experiences a decrease in the weekly average abdominal pain score of at least 30 percent compared with baseline. The weekly average abdominal pain score is derived by scoring the worst pain experienced each day and taking the average for one week. AND 2) A *Stool Consistency Weekly Responder* is defined as a patient who experiences a 50 percent or greater reduction in the number of days per week with at least one stool that has a consistency of Bristol Stool Form Scale (BSFS) Type 6 or 7 compared with baseline. **<u>Secondary Outcome Measures</u>** ***Double-blind treatment phase and Open-label treatment phase:*** 1. Stool frequency (mean over 8 weeks and the first and last 4 weeks) 2. Stool consistency assessed as average number of days/week with Bristol Stool Scale type >5 (over 8 weeks and the first and last 4 weeks) and percentage of responders 3. Abdominal pain (mean over 8 weeks and the last 4 weeks, percentage of responders) 4. Bloating (mean weekly score over 8 weeks and the last 4 weeks) 5. Urgency (mean weekly score over 8 weeks and the last 4 weeks) 6. Adequate relief of global IBS symptoms (percentage of patients over 8 weeks and the first and last 4 weeks)

	7. IBS-SSS (weekly score average over 8 weeks and the first and last 4 weeks) 8. WPAI:IBS (weekly score averages over 8 weeks) 9. IBS-QOL (4-weekly score average over 8 weeks and week 4 and week 8 scores) 10. PHQ-12 SS (4-weekly score average over 8 weeks and week 4 and week 8 scores) 11. Use of rescue medication, i.e. loperamide (number of days over 8 weeks and the last 4 weeks) 12. Adverse Events ***Follow-up:*** In patients who reported adequate relief in the last 4 weeks of open-label period: 1. Maintenance of treatment benefit (percentage of patients who report increased or maintained treatment benefit at 8 weeks) 2. Enterosgel® use (percentage of patients who report having used Enterosgel® during the follow-up period; frequency of use in these patients) 3. Loperamide use (percentage of patients who report having used less loperamide during the follow-up period than before the trial) **<u>Exploratory Outcome Measures</u>** 1. Qualitative and quantitative data for faecal microorganisms and biomarkers from the GI-MAP™assay (baseline and at 8 weeks of double-blind treatment period) 2. Qualitative and quantitative data for intestinal motility, fluid volume, gas content and physiology (baseline and at 4 weeks of open-label treatment period)
Sample size:	430 patients will be enrolled
Inclusion criteria:	1. Written informed consent 2. Irritable Bowel Syndrome with diarrhoea (IBS-D) according to Rome IV criteria 3. Aged 16-75 4. Considered suitable to take part in the study by the consenting investigator 5. Diary completed on at least 11 of 14 days (≥75%) during the screening period
Exclusion criteria:	1. Loose stools (BSFS 6 or 7) on less than 3 days during the 14 days after Screening Visit 2. Average abdominal pain <2.5 during the 14 days after Screening Visit (scale 0–10: 0 = no pain; 10 = worst possible pain) 3. Previously diagnosed coeliac disease 4. Previously diagnosed Inflammatory Bowel Disease 5. Previous bowel cancer or bowel resection 6. Other previously known gastrointestinal disorder contributing to the diarrhoea (according to PI's or sub-PI's evaluation) 7. Unexplained weight loss 8. Unexplained rectal bleeding (not including a short history of typical haemorrhoidal bleeding in patients aged <45) 9. Previous use of Enterosgel® 10. Use of antidepressant agents, unless used at a stable dose for at least 6 weeks 11. Use of any probiotic supplements, other intestinal adsorbents (activated charcoal, kaoline, diosmectite), slow-release medications or strong opioids (World Health Organisation Step III) 12. Participation in any research where treatment is provided, or was provided in the last three months 13. Pregnancy or not willing to use contraception for the duration of the study screening and treatment periods

Study schedule:	**Screening appointment (-2 weeks)** • Screening for inclusion/exclusion criteria • Review of medical history and concomitant medications • Informed consent • Provision of study diary (instruct on use of electronic diary, if the patient is able and willing to use the electronic version) • Record Adverse Events **Baseline appointment (0 weeks)** • Confirming eligibility • Review of changes in medical history and concomitant medications • Pregnancy test, if required • Record Adverse Events • Questionnaires (IBS-SSS, IBS-QOL, PHQ-12 SS, WPAI:IBS) • Provision of study diary and questionnaires • Randomisation • Instructing on study treatment use **Follow-up call (2 weeks)** • Ensure participant continuing in the study and discuss any potential issues **Follow-up 1 appointment (8 weeks)** • Review of changes in medical history and concomitant medications • Collect questionnaires and paper diaries/check compliance to using of electronic diary • Record Adverse Events • Questionnaires (IBS-SSS, IBS-QOL, PHQ-12 SS, WPAI:IBS) • Provision of study diary and questionnaires **Follow-up 2 appointment (16 weeks)** • Review of changes in medical history and concomitant medications • Collect questionnaires and paper diaries/check compliance to using of electronic diary • Record Adverse Events • Questionnaires (IBS-SSS, IBS-QOL, PHQ-12 SS, WPAI:IBS) **Follow-up call (24 weeks)** • Record Adverse Events • Review of changes in medical history and concomitant medications • Follow-up questions (maintenance of treatment benefit, Enterosgel® use, loperamide use)

1. BACKGROUND

1.1 IRRITABLE BOWEL SYNDROME

Irritable Bowel Syndrome (IBS) is a common condition with symptoms causing around 1 in 10 people to seek help from their General Practitioner (GP) at some point in their lives [1]. In the UK, the prevalence of IBS is estimated to be between 10% and 20% [2]. It occurs in all age groups, including children and the elderly; however, 50% of patients report having experienced first IBS symptoms before the age of 35 years and prevalence is 25% lower in those aged over 50 years than in those younger than 50 [3]. Internationally, the overall prevalence of IBS in women is 67% higher than in men although there are differences in the sex-specific prevalence between geographic regions [3]. IBS places a significant burden on the NHS, although accurate assessments are difficult due to poor clinical coding [4,5].

IBS is a combination of abdominal discomfort or pain and altered bowel habits, which can affect either frequency of bowel movements or stool form. Diagnosis is made using the Rome IV criteria [6]. Depending on the stool consistency, IBS can be classified as IBS with diarrhoea (IBS-D), IBS with constipation (IBS-C), or mixed IBS (IBS-M). IBS is often a chronic condition that can significantly impair quality of life and increase absences from work and school. The causes of IBS are complex and still not understood, but may include, among other factors, gut immune dysfunction, gut dysbiosis, infective and dietary triggers and changes to gut permeability [7,8,9].

Medications for treating IBS-D related symptoms include antispasmodics and anti-depressants (for abdominal pain). Loperamide can be used for diarrhoea but this often causes rebound constipation and does not improve abdominal pain [6]. There are also safety concerns with chronic high dose loperamide use, which has been associated with ventricular arrhythmias [10]. The emergence of new drugs for IBS has been slow; however, eluxadoline which binds to opioid receptors in the digestive system and slows down the movement of food through the gut [11], was recently approved in the UK for use in patients who do not respond to other treatments. Finally, dietary changes such as the low Fermentable, Oligo-, Di-, Mono-saccharides and Polyols (FODMAP) diet can help symptoms [12], but can be difficult to implement without support from a dietician. There is a need for novel treatment alternatives, including drug-free treatments, which are easy to implement and can safely be used in all patient groups, including in children and pregnant women.

1.2 INTESTINAL ADSORBENTS IN THE TREATMENT OF IBS

Intestinal adsorbents are substances which bind microbial toxins and other molecules in the intestine. Adsorbents such as clay and charcoal have been used for the treatment of intestinal infections for decades, but there is limited data on their use for chronic gastrointestinal conditions like IBS. We found only two published randomised controlled trials looking at efficacy of intestinal adsorbents in IBS-D. Chang *et al*. [13] investigated the natural adsorbent clay, dioctahedral smectite, in 104 subjects with IBS-D and found that it improved pain/discomfort at 4 and 8 weeks of treatment, while no improvement was detected in the placebo group. However, the overall IBS symptoms improved in both treatment groups. Tack *et al*. [14] evaluated the efficacy of the carbon adsorbent AST-120, in 115 subjects with non-constipating IBS. At week 4, the percentage of subjects classified as responders for abdominal pain was significantly higher in the AST-120 group than in the placebo group. More AST-120 treated subjects showed improvement in bloating and stool consistency although the difference to the placebo group was not significant. Although these studies are likely to have been underpowered for many of the outcome measures including bowel movements, the findings suggest that intestinal adsorbents may have benefits in the treatment for IBS-D and sufficiently powered studies should be conducted to further explore their efficacy.

As hypothesised by the authors of the two studies above, there are various possible mechanisms by which intestinal adsorbents could improve IBS-D symptoms, including adsorption of mediators such as histamine and serotonin that are postulated to play a causative role in IBS, and adsorption of bacterial products and bile acids, which also have been implicated in the generation of IBS symptoms [8,9]. There is also some evidence that dioctahedral smectite could enhance the intestinal barrier function and counteract the disruption of the intestinal barrier induced by the release of pro-inflammatory cytokines [15,16]. In rats, diosmectite has also been shown to reduce abdominal contractions induced by water avoidance stress and to improve stress-induced intestinal transit acceleration [17]. While the role of the intestinal barrier in IBS remains to be further studied, there is increasing evidence to suggest that its function is altered in IBS regardless of the subtype [7].

1.3 POLYMETHYLSILOXANE POLYHYDRATE (ENTEROSGEL®)

Although clay and charcoal are very effective adsorbents, as such they may also adsorb beneficial molecules in the gut. Polymethylsiloxane polyhydrate (PP) is an organosilicon based adsorbent, which has been shown to display more selective adsorption characteristics; it effectively binds medium and high molecular weight substances including various bacterial toxins (bacterial endotoxin, *Clostridium difficile* toxins A and B, shiga toxin II), while showing a lower binding capacity than carbon-based adsorbents for smaller molecules such as vitamin B12 and certain drug compounds [18,19]. This selectivity could result from the unique properties of the adsorbent, i.e. its porous structure and the presence of both hydrophilic and hydrophobic groups on the surface.

In Europe, PP is available as a medical device (Enterosgel®), which is sold in the UK and several other countries over-the-counter. It has been used for 30 years in Eastern Europe for various conditions, including infectious diarrhoea, IBS and allergies. There is some evidence suggesting that Enterosgel® could be beneficial in the treatment of symptoms of IBS including diarrhoea and abdominal pain [20]. However, further research is needed to investigate the efficacy and safety of Enterosgel® in the treatment of IBS-D.

This trial will be the first placebo-controlled randomised trial with Enterosgel® in patients with IBS. The study has been designed in line with the current FDA and EMA guidance for clinical trials in IBS [21,22] and in collaboration with a Steering Committee consisting of leading IBS and gastroenterology specialists in the UK.

2. AIMS AND OBJECTIVES

2.1 AIMS

The aims of this study are:

1. To determine the efficacy of Enterosgel® in the treatment of IBS-D
2. To determine the safety and tolerability of use of Enterosgel® in patients with IBS-D
3. To explore typical dose regimen in longer term use
4. To investigate continued effect of Enterosgel® on controlling IBS-D symptoms following cessation of use (i.e. a disease modifying effect)

2.2 OBJECTIVES

2.2.1 Primary objective

The primary objective of this study is to determine the efficacy (superiority) of Enterosgel® (Bioline Products s.r.o, Czech Republic) compared with placebo in terms of patient reported outcomes for stool consistency and abdominal pain.

2.2.2 Secondary objectives

The secondary objectives for the **double-blind treatment phase** are:

1. To determine the efficacy of Enterosgel® compared with a parallel placebo group in terms of:
    - patient-reported symptoms (diary, Irritable Bowel Syndrome Severity Scoring System score [IBS-SSS], PHQ-12 Somatic Symptom [PHQ-12 SS] scale)
    - use of loperamide as rescue medication
2. To determine effects of treatment on health-related quality of life (IBS Quality of Life [IBS-QOL]) and IBS-related work productivity and activity impairment (WPAI:IBS).
3. To evaluate safety of Enterosgel® through reported adverse events potentially related to treatment.

The secondary objectives for the **open-label treatment phase** are:

1. In patients who had received placebo as blinded treatment, to compare the efficacy outcome measures, IBS-QOL and WPAI:IBS over the open-label Enterosgel® treatment period to the preceding placebo treatment period.
2. In patients who had received Enterosgel® as the blinded treatment, to test whether the treatment benefits (in terms of efficacy outcome measures, IBS-QOL, WPAI:IBS) can be effectively maintained with Enterosgel® in those patients who achieved control in the last 4 weeks (of the double-blind treatment phase)
3. To evaluate safety of Enterosgel® through reported adverse events potentially related to treatment.

The secondary objectives for the **follow-up phase** are:

In patients who reported adequate relief in the last 4 weeks of open-label period:

1. To assess the percentage of patients who report increased or maintained treatment benefit after cessation of treatment
2. To assess the percentage of patients who used over-the-counter Enterosgel® after cessation of open-label treatment and to assess the frequency of use in these patients
3. To assess the percentage of patients who report reduced loperamide use after cessation of treatment, compared to loperamide use before the trial

2.2.3 Exploratory objectives

1. To investigate faecal microorganisms and biomarkers (GI-MAP™ assay) in a subgroup of 20 patients at baseline and following 8 weeks of double-blind treatment, in order to gain understanding on the potential impact of Enterosgel® on the gut microbiome.

2. To obtain qualitative and quantitative information on gut-related parameters using magnetic resonance imaging (MRI) scan and GIQuant image processing software (Motilent Ltd, UK), in a subgroup of 16

patients at Baseline and after 4 weeks of open-label Enterosgel® treatment, in order to gain understanding on the potential impact of Enterosgel® on intestinal motility, fluid volume, gas content and physiology.

3. STUDY DESIGN AND OUTCOME MEASURES

3.1 STUDY DESIGN

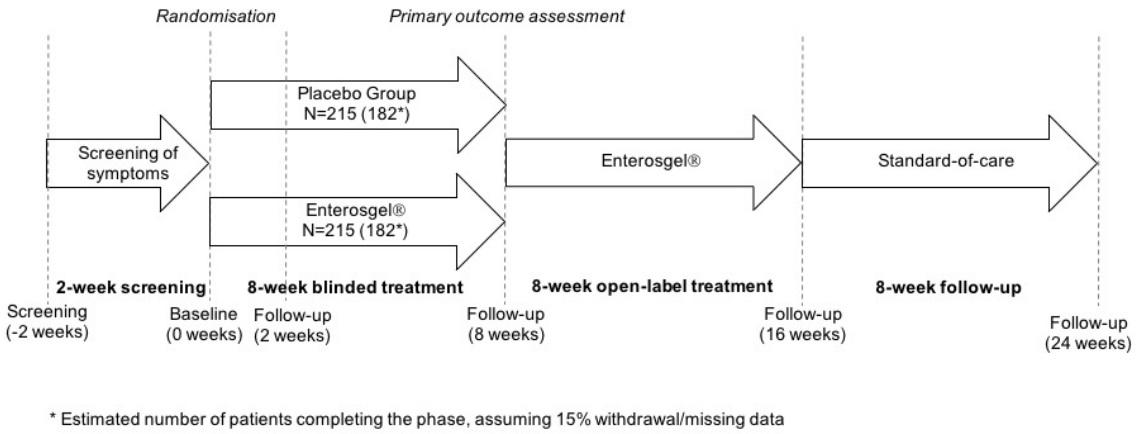
This is a UK multi-centre, parallel arm, randomised, double-blind, placebo controlled trial to evaluate the efficacy, tolerability and safety of a medical device, Enterosgel®, in the treatment of Irritable Bowel Syndrome with diarrhoea (IBS-D) in adults (**Figure 1**).

The study involves a 2-week screening/baseline phase, after which eligible patients are randomised to receive blinded treatment (Enterosgel® or placebo) for 8 weeks. The patients will be allowed to adjust their daily blinded treatment dosage based on their symptoms. The purpose of the dose adjustment is two-fold; first, it will maximise the efficacy of the active treatment by allowing each patient to find the dosage that is most appropriate for their symptoms and second, it will provide us with data to help define the optimal dosage regimen(s) for Enterosgel®.

After the double-blind treatment phase, all patients will receive open-label Enterosgel® treatment for 8 weeks. As in the double-blind phase, patients will be allowed to adjust their dosage based on their symptoms. As the treatment will be open-label, this is a pragmatic part of the trial, allowing us to better understand how Enterosgel® is used in real-life. After 8 weeks of open-label treatment, the patients will return to standard care. Those patients who responded to open-label treatment will receive a follow-up call 8 weeks later.

The study involves 4 study visits/remote appointments and 1-2 follow-up calls: screening visit (-2 weeks), baseline appointment (0 weeks), follow-up call (2 weeks), follow-up appointments at weeks 8 and 16 and a follow-up call at week 24 (only for patients who responded to open-label treatment). Patients will complete a daily study diary during the 2-week screening period and during the 16 weeks of treatment. In addition, patients will complete weekly (IBS-SSS, WPAI:IBS) or 4-weekly (IBS-QOL, PHQ-12 SS) paper questionnaires throughout the 16-week treatment period. These questionnaires will also be completed at baseline and each follow-up appointment (see section 6 of this protocol for a detailed description of the study procedures and schedule).

Figure 1. Study design.



* Estimated number of patients completing the phase, assuming 15% withdrawal/missing data

3.2 STUDY OUTCOME MEASURES

3.2.1 Primary Outcome Measure

The primary outcome measure is the percentage of patients defined as responders for abdominal pain and stool consistency during at least 4 weeks in the 8-week treatment period, where:

1) An *Abdominal Pain Intensity Weekly Responder* is defined as a patient who experiences a decrease in the weekly average abdominal pain score of at least 30 percent compared with baseline. The weekly average abdominal pain score is derived by scoring the worst pain experienced each day and taking the average for one week.

AND

2) A *Stool Consistency Weekly Responder* is defined as a patient who experiences a 50 percent or greater reduction in the number of days per week with at least one stool that has a consistency of Bristol Stool Form Scale (BSFS) Type 6 or 7 compared with baseline.

This primary outcome measure is recommended by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [21,22]. Data will be collected using a daily diary, which will be available as an online version (for use on mobile devices, laptops and computers). Those patients who are not able or willing to use the electronic diary, will be provided with a paper diary. The content of the diary is supplied in **APPENDIX A: STUDY DIARY (DOUBLE-BLIND AND OPEN-LABEL PHASE)**.

3.2.2 Secondary Outcome Measures

3.2.2.1 Double-blind and Open-label treatment phase

The secondary outcome measures used for the double-blind and open-label treatment phase are listed in **Table 1**. Questions included in the daily and weekly diaries are provided in **APPENDIX A: STUDY DIARY (DOUBLE-BLIND AND OPEN-LABEL PHASE)**.

Table 1. Secondary outcome measures for the double-blind and open-label treatment phase

Outcome Measure	Time points and method of assessment	Definition
1. Stool frequency	Daily (diary)	1) Mean number of bowel movements per day over 8 weeks 2) Mean number of bowel movements per day over the first 4 weeks 3) Mean number of bowel movements per day over the last 4 weeks
2. Stool consistency	Daily (diary)	Average number of days/week with Bristol Stool Scale type >5: 1) Mean over 8 weeks 2) Mean over the first 4 weeks 3) Mean over the last 4 weeks 4) Percentage of study period responders. Weekly responder is defined as a patient who experiences a 50 percent or greater reduction in the number of days per week with at least one stool that has a consistency of Bristol Stool Form Scale (BSFS) Type 6 or 7 compared with baseline. Study period responder is defined as a patient who was a weekly responder during at least 4 weeks in the 8-week treatment period.
3. Abdominal pain	Daily (diary)	1) Mean over 8 weeks 2) Mean over the last 4 weeks 3) Percentage of study period responders. Weekly responder defined as a patient who experiences a decrease in the weekly average abdominal pain score of at least 30 percent compared with baseline. The weekly average abdominal pain score is derived by scoring the worst pain experienced each day and taking the average for one week. Study period responder is defined as a patient who was a weekly responder during at least 4 weeks in the 8-week treatment period Scale is from 0 to 10, where 0 means no pain at all and 10 means the worst possible pain the patient can imagine.
4. Bloating	Weekly (diary)	1) Mean over 8 weeks 2) Mean over last 4 weeks Scale is from 0 to 6, where 0 means bloating was not bothersome at all and 6 means bloating was a very great deal bothersome.
5. Urgency	Weekly (diary)	1) Mean over 8 weeks 2) Mean over last 4 weeks Scale is from 0 to 6, where 0 means no urgency at all and 6 means a very great deal of urgency with bowel movements.

6. Adequate relief	Weekly (diary)	Percentage of patients reporting adequate relief (weekly percentages averaged over 8 weeks and the first and last 4 weeks)
7. IBS Severity Scoring System (IBS-SSS) [23]	Weekly (questionnaire) **APPENDIX B: IBS-SSS QUESTIONNAIRE**	Weekly score analysed as: 1) Average over 8 weeks 2) Average over the first 4 weeks 3) Average over the last 4 weeks
8. IBS Quality of Life (IBS-QOL) [24,25]	4-weekly (questionnaire) **APPENDIX C: IBS-QOL QUESTIONNAIRE**	4-weekly score analysed as: 1) Average over 8 weeks 2) Week 4 score 3) Week 8 score
9. Patient Health Questionnaire 12 Somatic Symptom (PHQ-12 SS) scale [26]	4-weekly (questionnaire) **APPENDIX D: PHQ-12**	4-weekly score analysed as: 1) Average over 8 weeks 2) Week 4 score 3) Week 8 score In addition to total score; questions about headache (e.), tiredness (n.) and sleep (o.) to be analysed individually
10. Work Productivity and Activity Impairment (WPAI:IBS) [27,28]	Weekly (questionnaire) **APPENDIX E: WPAI:IBS**	Scores each week (percent work time missed due to IBS, percent impairment while working due to IBS, percent overall work impairment due to IBS, percent activity impairment due to IBS) averaged over 8 weeks
11. Use of rescue medication	Weekly (diary)	Total number of days loperamide used each week averaged over 8 weeks and the last 4 weeks
12. Adverse Events	When reported	1) Percentage of patients reporting SAEs and AEs possibly related to treatment 2) Total number of SAEs and AEs reported

3.2.2.2 Follow-up phase

The secondary outcome measures used for the follow-up phase are listed in **Table 2**. Questions for the follow-up call are provided in **APPENDIX F: FOLLOW-UP CALL INVESTIGATOR QUESTIONNAIRE**.

Only patients who reported adequate relief of IBS symptoms in the last 4 weeks of open-label period, will be included in the follow-up phase.

Table 2. Secondary outcome measures for the follow-up phase

Outcome Measure	**Time points and method of assessment**	**Definition**
1. Maintenance of treatment benefit	At 8 weeks	Percentage of patients who report increased or maintained treatment benefit
2. Enterosgel® use	At 8 weeks	Percentage of patients who report having used Enterosgel® in the last 8 weeks; frequency of use in these patients
3. Loperamide use	At 8 weeks	Percentage of patients who report having used less loperamide in the last 8 weeks than before the trial

3.2.3 Exploratory Outcome Measures

For a subgroup of 20 patients, qualitative and quantitative data for faecal microorganisms and biomarkers will be collected at baseline and at 8 weeks of double-blind treatment period using the GI-MAP™assay (Invivo Clinical Ltd, UK). 10 patients from each treatment group will be assigned for testing. Data will be compared between treatment groups at week 8. Week 8 data will also be compared to baseline in all patients. Depending on the findings, other analyses might be performed in this exploratory dataset.

For a subgroup of 16 patients, qualitative and quantitative data for intestinal motility, fluid volume, gas content and physiology will be collected at baseline and at 4 weeks of open-label treatment period using MRI scanning and novel GIQuant image processing software (Motilent Ltd, UK).Only participants recruited to the main study from the University Hospital of North Durham and Newcastle Upon Tyne Hospitals will be invited to take part in this assessment.

3.3 DEFINITION OF END OF TRIAL

End of trial is defined as the last follow-up call/visit for the last patient. Patients with unresolved SAEs at the last follow-up visit would be followed up until SAE resolution or stabilisation.

4. STUDY PARTICIPANTS

4.1 INCLUSION CRITERIA

1. Written informed consent
2. Irritable Bowel Syndrome with diarrhoea (IBS-D) according to Rome IV criteria [6]*
3. Aged 16-75
4. Considered suitable to take part in the study by the consenting investigator
5. Diary completed on at least 11 of 14 days (≥75%) during the screening period

4.2 EXCLUSION CRITERIA

1. Loose stools (BSFS 6 or 7) on less than 3 days during the 14 days after Screening Visit
2. Average abdominal pain <2.5 during the 14 days after Screening Visit (scale 0–10: 0 = no pain; 10 = worst possible pain)
3. Previously diagnosed coeliac disease† (must be confirmed from medical records before randomisation)
4. Previously diagnosed Inflammatory Bowel Disease (must be confirmed from medical records before randomisation)
5. Previous bowel cancer or bowel resection** (must be confirmed from medical records before randomisation)
6. Other previously known gastrointestinal disorder contributing to the diarrhoea (according to PI's or sub-PI's evaluation) (must be confirmed from medical records before randomisation)
7. Unexplained weight loss
8. Unexplained rectal bleeding (not including a short history of typical haemorrhoidal bleeding in patients aged <45)
9. Previous use of Enterosgel®
10. Use of antidepressant agents, unless used at a stable dose for at least 6 weeks (must be confirmed from medical records before randomisation)
11. Use of any probiotic supplements, other intestinal adsorbents (activated charcoal, kaoline, diosmectite),

slow-release medications or strong opioids (World Health Organisation Step III) (must be confirmed from medical records before randomisation)

12. Participation in any research where treatment is provided, or was provided in the last three months
13. Pregnancy or not willing to use contraception for the duration of the study screening and treatment periods[***]

** According to the Rome IV criteria for IBS, the following three criteria must be met for the last 3 months:*

1. *Recurrent abdominal pain **at least once a week***
2. *Pain is associated with **two or more** of the following criteria:*
    - ***a.*** *Related to defecation on **at least 30% of occasions***
    - ***b.*** *Associated with a change in form (appearance) of stool on **at least 30% of occasions***
    - ***c.*** *Associated with a change in frequency of stool on **at least 30% of occasions***
3. *Symptom onset at least **6 months** prior to diagnosis*

According to the Rome IV criteria, IBS-D is diagnosed based on patient perception of the usual consistency of abnormal stools using the BSFS. The criteria for IBS-D are met if abnormal stools in the last 3 months were usually diarrhoea (BSFS Type 6 or 7).

[**] Any patients aged ≥60 with altered bowel habits, should have had a colonoscopy to exclude bowel cancer since the bowel habits changed.

[***] A pregnancy test will be performed on all female patients of child-bearing potential. Women of childbearing potential (not surgically sterile, 12 months postmenopausal or otherwise incapable of pregnancy) and heterosexually active must use contraception throughout the screening period and the 16 weeks when they are receiving study treatment. Acceptable methods of contraception are methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods include [29]:

- *combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:*
    - *oral*
    - *intravaginal*
    - *transdermal*
- *progestogen-only hormonal contraception associated with inhibition of ovulation:*
    - *oral*
    - *injectable*
    - *implantable*
- *intrauterine device (IUD)*
- *intrauterine hormone-releasing system (IUS)*
- *bilateral tubal occlusion*
- *vasectomised partner (Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the woman of childbearing potential trial participant and that the vasectomised partner has received medical assessment of the surgical success)*
- *sexual abstinence (In the context of this guidance sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.)*

Method of contraception must be documented in the patient's medical records.

4.3 PATIENT RECRUITMENT

4.3.1 Recruitment strategies

4.3.1.1 Research sites

Approximately 30 primary and secondary care sites and private gut clinics in England will be included in the study. GP surgeries can also act as Patient Identification Centres (PIC) referring patients to the participating research sites. Sites have been/will be identified primarily through the National Institute for Health Research (NIHR) site identification service, and selected based on having the capacity to conduct the trial and a sufficient population of patients with IBS-D. Recruitment will be competitive across all sites until the total target of 430 patients has been met. All participating sites will be required to participate in a site initiation and training meeting organised by the study Sponsor.

Sites will identify potential participants opportunistically and through searches of their patient databases, waiting lists, case records and referrals. The study can be advertised with posters and leaflets at the participating practices, hospitals and clinics, and with electronic adverts on their websites, Facebook and Twitter accounts and newsletters. The adverts will advise any interested patients to contact the site research team. Potentially eligible patients will be provided with a Patient Information Sheet either when visiting the GP/hospital/clinic or by post or email together with an Invitation Letter. The research team can also phone or text potentially eligible patients to inform them of the study before sending the Patient Information Sheet to interested patients. The research team can follow-up the approached patients with a phone call to confirm that they have received the Patient Information Sheet and to ask if they would be interested to participate. After having had sufficient time to consider the study information (at least 24 hours), patients who are interested to take part in the trial should contact the site research team to schedule a screening visit/remote appointment.

4.3.1.2 Advertising

The study can be publicly advertised with electronic and/or paper materials approved by the Research Ethics Committee and the Health Research Authority. These include, but are not limited to, posters and/or leaflets at community pharmacies and GP surgeries, social media, and electronic advertising through IBS organisations. There will also be a webpage dedicated to the study on the Sponsor's website (http://enteromed.co.uk/relieve-ibs-d-trial/). The public advertising materials will contain a link to the study webpage, which will have basic information about the study and a list of research sites with their contact details.

Digital advertisements which are targeted for recruitment by The NIHR Patient Recruitment Centre, NIHR, will contain a URL link/QR code which will direct potential participants to an online eligibility questionnaire provided on a secure platform (REDCap survey form) hosted by Newcastle Hospitals NHS Trust. Paper-based advertisement, e.g. posters and newspaper advertisements, will contain a short web-link and QR code to the online questionnaire.

4.3.1.3 ContactME-IBS

Across the country, patients will be enrolled through the national, ethically approved ContactME-IBS registry, which is a registry for patients with IBS to express interest and provide consent to be contacted about research opportunities. ContactME-IBS website is available at: https://www.contactme-ibs.co.uk.

Only ContactME-IBS research team members will have access to search the database for potentially suitable participants based on the basic study eligibility criteria. A member of the ContactME-IBS research team will contact the individual by telephone, letter or email (depending on patient contact preferences) to tell them about the study and ask them if they may be interested in joining. If the individual agrees then further information will be gathered to check eligibility before the Patient Information Sheet is sent out, and

normal protocol related study activity commences.

4.3.2 Screening process

Potentially eligible patients will receive a Patient Information Sheet by email or post. Patients who are interested to take part in the study after having had sufficient time (at least 24 hours) to consider the information will be scheduled for a screening visit appointment at the research site where they are registered as a patient or, in case of patients identified at PICs or through public advertising, at one of the research sites. The NIHR Patient Recruitment Centre, Newcastle, site will conduct remote screening and baseline appointments; all other research sites will conduct the screening and baseline visits onsite. Medical history and list of concomitant medications will be obtained from any patients whose medical notes the research team does not have access to, e.g. if the patient is not registered as a patient at one of the participating research sites or PICs. The information can be requested from the patient's GP by the research site, the patient or through a Site Management Organisation. A specific Medical Information Request document should be used for this purpose to ensure that the correct information is requested. If a patient is referred from a PIC, the PIC will share the relevant medical information of the patient with the research site before the screening visit and after obtaining verbal consent from the patient to share this information. This consent should be documented in the patient's medical notes.

At the screening appointment, written informed consent will be obtained by a delegated member of the research team before any study procedures are conducted. Basic information about all screened patients should be documented in a study screening log and/or eCRF at the research site (age, gender, recruitment strategy, and reason(s) for not enrolled for those who failed screening or declined to take part). Patients who are eligible based on information available at the screening appointment and consent to proceed, will be entered into a 2-week screening phase to monitor the severity of their diarrhoea symptoms and abdominal pain through use of a daily diary (**APPENDIX G: SCREENING DIARY**). The patients should not use any antidiarrhoeal medication (such as loperamide) during the screening period.

If a patient is enrolled into the trial at a secondary care site, private clinic or at a medical practice other than the practice where the patient is registered, the patient's GP will be notified of the patient's participation. The patient's GP practice will maintain the responsibility for the healthcare of the patient outside the research.

At the baseline appointment, the symptoms diary will be assessed and eligibility to proceed to randomisation will be confirmed. A patient must not be randomised before the results from any tests required to exclude other conditions and a negative pregnancy test, if applicable, have been obtained. For patients enrolled at The NIHR Patient Recruitment Centre, Newcastle, female patients of childbearing potential will receive a pregnancy test kit by post and should take the test at home on the day of the baseline appointment. Eligibility must be confirmed against the patient's medical notes as stated in section 4.2. A screening log should be updated to document whether the patient was randomised into the trial, or was not eligible/declined (reasons should be documented).

A patient can be re-screened once if they failed on the diary-based eligibility criteria. In this case, they will not need to attend a second screening visit, and can be enrolled into the screening phase remotely with a phone call. In order not to influence how patients respond to the screening diary questions, the patients should not be informed of the detailed reason(s) they failed screening the first time. A patient should only be enrolled into rescreening if the investigator believes the patient's level of symptoms during the initial screening period were atypical for them and that there is a likelihood of achieving symptom thresholds on a further attempt, or if they failed because they had too many missing diaries (although the sites should avoid enrolling any patients that are likely to remain poorly compliant to study procedures).

A patient can also be re-screened if their vital signs were not within the accepted range at initial screening. (NOTE: checking of vital signs at screening has been removed from study procedures in protocol v.4.0).

Finally, a patient can be re-screened if they failed on the IBS-SSS score (NOTE: IBS-SSS has been removed from the exclusion criteria in protocol v.3.0).

There should be a minimum of 2 weeks from the patient failing screening to re-screening. No re-consenting will be required in order to enter a patient into rescreening, unless patient information has changed. In this case a second screening appointment must be conducted and a new consent form signed. When entered into re-screening, the patient will always receive a new study ID and this should be recorded on their original consent form unless a new consent form was signed.

4.4 DISCONTINUATION AND WITHDRAWAL CRITERIA

Patients can withdraw consent at any time without providing a reason. No further data will be collected after a patient withdraws consent or is withdrawn from the study, but data collected before withdrawal can be used. If a patient refuses to continue the use of study treatment, they can continue with follow-up assessments and visits if willing to do so, but a Protocol Deviation for non-compliance to study treatment should be recorded.

In case of the following the patient should be withdrawn from the study by the Principal Investigator:

- Significant deterioration of the patient's status as assessed by the principle investigator
- Serious Adverse Event (SAE) related to study treatment(s) or procedures
- Pregnancy
- Any other changes in diagnoses or medical conditions that could affect patient safety on the trial and/or scientific quality of the data
- Major protocol violation (affecting patient safety and/or scientific quality of the data)

The reason(s) for withdrawal should be documented in the Continuation Status eCRF as soon as the site becomes aware of the patient's withdrawal. If a patient makes a decision to withdraw consent or is lost to follow-up, this should also be documented in the Continuation Status eCRF without delay. Patients should be informed that they might be contacted in the future if any significant new information becomes available regarding the study treatment.

5. INTERVENTIONS AND STUDY DEVICE

5.1 INTERVENTION

5.1.1 Treatment allocation

5.1.1.1 Double-blind phase

For the double-blind treatment phase, patients will be randomised in 1:1 ratio using a computer-based randomisation tool (Sealed Envelope Ltd, UK) to:

Control Group: will receive placebo for 8 weeks

Interventional Group: will receive Enterosgel® pre-diluted in water for 8 weeks

Randomisation will be based on the minimisation method where treatment allocation will be stratified by study centre. Minimisation allocates subjects to the treatment group that best maintains balance in stratifying factors. It is effective even at small sample sizes and with multiple stratification variables [30].

5.1.1.2 Open-label phase

For the open-label treatment phase, all patients will receive Enterosgel® in standard 15g sachets, which are identical to those available over-the-counter in the UK.

5.1.2 Enterosgel®

5.1.2.1 General description

Enterosgel® is an intestinal adsorbent developed for binding toxins and other harmful substances in the gastrointestinal tract. The commercially available Enterosgel® product contains 30% water and 70% polymethylsiloxane polyhydrate (PP), which is a 3-dimensional crosslinked polymer of methylsiliconic acid formed by polycondensation in which hydroxyl groups form stable siloxane bonds. In solution, these form microglobules via cross-linking of the pentameric cyclic structural units created by the siloxane bonds which connect to each other via strong hydrogen bonds [31]. The microglobules contain porous space filled with water. The overall porous structure of Enterosgel® is formed by connecting the microglobules of approximately 50nm in size, which then associate into larger particles ranging from 5-250μm in size, with over 32% in the 20-50 μm range [18].

Enterosgel® exhibits selective adsorptive activity towards medium and high-molecular weight substances in the gut, including middle-molecular weight toxic metabolites. It effectively binds medium and high molecular weight substances including various bacterial toxins (bacterial endotoxin, *Clostridium difficile* toxins A and B, shiga toxin II), while showing a lower binding capacity than carbon-based adsorbents for smaller molecules such as vitamin B12 and certain drug compounds [18,19]. This selectiveness could result from the unique properties of the adsorbent, i.e. its porous structure and the presence of both hydrophilic and hydrophobic groups on the surface.

Enterosgel® does not cross the intestinal barrier and is therefore classified as a medical device. In Europe, it was certified as a medical device class IIA in 2011 and is currently sold over the counter in 30 countries. According to European database of suspected adverse drug reaction reports (http://www.adrreports.eu/en/index.html) there are no reported adverse reactions in Europe since it was certified in 2011. Enterosgel® is available from pharmacies and health stores in the UK and is available in a tube or in a packet of 10 sachets. It is free from preservatives, sugar and other additives, has no distinct taste and is easily suspended in water and taken orally. Enterosgel® can be used in children and adults, including pregnant and breastfeeding women.

5.1.2.2 Description of study treatment

Although there has been extensive research on Enterosgel® in Eastern Europe, so far none of the conducted trials have included a placebo control arm. Any gel-like substances (for example gelatin or starch based) could potentially have effects in the gastrointestinal tract and their suitability for use as a placebo would require validation. However, since Enterosgel® is taken by diluting 1-1.5 tablespoons of the product in 100-200ml water, a water-based placebo can offer an attractive alternative to a gel-like placebo. In order to allow a water-based placebo to be used as a comparator in this trial, the blinded Enterosgel® will be provided in a pre-diluted form in 90ml tubes containing 15g Enterosgel® in 67.5ml potable water. Diluting the Enterosgel® in water will not alter the structural or absorption properties of Enterosgel® because when placed into a solvent such as water, the larger microgranules in Enterosgel® can be solvated or disperse within the solvent due to the presence of high negative charge on the methyl groups, but they are also able to coalesce and re- form the porous gel structure at reduced alkalinity or by removal of the solvent [31].

For the open-label phase, patients will be provided with the standard over-the-counter Enterosgel® sachets.

5.1.3 Placebo

Each placebo tube will contain 90 ml potable water.

5.1.4 Treatment dosage

5.1.4.1 Double-blind phase

Both the placebo and Enterosgel® will be provided in 90g tubes, each containing a single dose of treatment. Each Enterosgel® tube contains 15g of Enterosgel® (Bioline Products s.r.o., Czech Republic) pre-diluted in 67.5ml of potable water. Each placebo tube contains 90ml water.

Study-specific dosage instructions for the double-blind treatment period are provided in **APPENDIX H: TREATMENT USE INSTRUCTIONS (DOUBLE-BLIND PHASE)**.

5.1.4.2 Open-label phase

For the open-label treatment phase, all patients will receive Enterosgel® in 15g sachets. Study-specific dosage instructions are provided in **APPENDIX I: TREATMENT USE INSTRUCTIONS (OPEN-LABEL PHASE)**.

5.1.5 Labelling, storage, supply and destruction

5.1.5.1 Blinded study treatment

Both the placebo and Enterosgel® dilution will be manufactured in accordance with good manufacturing practice (GMP) by Bioline Products s.r.o. and packed into identical 90g tubes.

In accordance with Annex I of the European Council Directive 93/42/EEC concerning Medical Devices, each study device will be marked with a label containing the text “EXCLUSIVELY FOR CLINICAL INVESTIGATION” and the following information:

1. Content: “This tube contains one dose of blinded treatment”
2. Manufacturer: Bioline Products s.r.o, Krakovská 1338/10, 110 00 Prague 1, Czech Republic
3. Distributor: Enteromed Ltd, UK
4. Study Sponsor: Enteromed Ltd, UK
5. Study reference: RELIEVE IBS-D
6. Batch number
7. Expiry date
8. Instructions for use: “Shake well immediately before use. Empty the contents of the tube into a glass, mix with 100ml room temperature water and drink within 1 minute. Leave at least 2 hours before and after taking this treatment and taking any medications, or eating meals.”
9. Storage: +4C° to +25C°. Keep away from direct sunlight or heat.
10. Disposal: Contents can be safely disposed into the domestic waste. Packaging can be recycled.

The tubes will be stored at an MHRA approved warehouse facility (Wasdell Group, Swindon, UK) contracted by the Sponsor. The study supplies should be clearly separated from any other supplies, and placebo and Enterosgel® will be marked (for example A and B) and separated so that they can be identified by the warehouse staff responsible for coordinating the study supplies. After each randomisation, the site research team will email a supplies request form with the unique randomisation code and the patient’s name, home address and phone number to a dedicated email address, which is only accessed by two unblinded study coordinators at the Sponsor’s research team. A coordinator will check the randomisation code against a pre-generated randomisation code list provided by Sealed Envelope Ltd, stored in a locked safe only accessible by the unblinded coordinators. The coordinator will determine whether the code corresponds to placebo or Enterosgel® and will log onto the secure Wasdell online booking site to request

for a shipment of a box/boxes (A or B) of up to 290 tubes corresponding to the randomisation code to the patient's home address. The online system will generate a unique code which can then be tracked through the system and will confirm dispatch. Supplies will be dispatched within 2 calendar days from receiving the request (next day for orders submitted before 2 pm). All parties should delete the request emails once confirmed received by the next party. However, paper copies of the forms should be kept at the sites, the Sponsor's office and the picking form at the warehouse until the end of the study to allow accountability.

The patients can dispose of the empty tubes at home, as well as any unused tubes remaining at the end of the study. Should the patient run out of study treatment during the 8 weeks, they can request for more supplies by contacting the research nurse, who will submit a request to the Sponsor.

5.1.5.2 Open-label study treatment

After a patient has been entered into the open-label phase, the site research team will email the patient's name, home address and phone number to a dedicated email address, as described above in section 5.1.5.1. The study coordinators receiving this information will then request a shipment of open-label treatment from the warehouse to the patient's home.

Each study device will be marked with a label containing the text "EXCLUSIVELY FOR CLINICAL INVESTIGATION" and the following information:

1. Content: "This sachet contains a single dose (15g) of Enterosgel®"
2. Manufacturer: Bioline Products s.r.o, Krakovská 1338/10, 110 00 Prague 1, Czech Republic
3. Distributor: Enteromed Ltd, UK
4. Study Sponsor: Enteromed Ltd, UK
5. Study reference: RELIEVE IBS-D
6. Batch number
7. Expiry date
8. Instructions for use: "Empty the contents of the sachet into a glass, mix with 200ml room temperature water and drink within 1 minute. Leave at least 2 hours before and after taking this treatment and taking any medications, or eating meals."
9. Storage: +4C° to +25C°. Keep away from direct sunlight or heat.

The label will be placed on the standard outer packaging such that it does not cover any information about the product.

Patients can dispose of the empty sachets at home and keep any remaining unused Enterosgel®. Should the patient run out of study treatment during the 8 weeks, they can request for more supplies by contacting the research nurse, who will submit a request to the Sponsor.

5.2 AUXILIARY MEDICINAL PRODUCTS (AxMP)

Loperamide will be provided to all study participants for use as a rescue medication during the double-blind and open-label treatment phases. Loperamide will be shipped from the warehouse to the patient's home. The patients should record use of rescue medication in the study diary.

5.3 CONCOMITANT TREATMENTS

5.3.1 IBS treatments

Patients will be allowed to continue to take antidepressant agents at a stable dose, provided that they had been taking a stable dose for at least 6 weeks before providing written informed consent.

Use of probiotic supplements, other intestinal adsorbents (activated charcoal, kaoline, diosmectite), slow-release medications or strong opioids during the study will not be permitted and any patients who require slow-release medications, should be withdrawn from the trial. Patients will be advised not to make any changes to their diet while on the trial.

5.3.2 Contraindications

The only listed contraindications for Enterosgel® are intestinal atony, use of slow-release medications and intolerance to Enterosgel® based on previous use. The study eligibility criteria will ensure that no patients with these contraindications will be enrolled into the trial.

5.3.3 Precautions

To minimise the risk that Enterosgel® could adsorb concomitant medications in the gut, it is recommended to leave at least two hours before and after taking the study treatment and taking any medication.

6. STUDY SCHEDULE AND PROCEDURES

6.1 PATIENT IDENTIFICATION AND SCREENING

Patient recruitment strategies and process are described in **4.3 PATIENT RECRUITMENT**. Patients who are interested to take part in the study after having had sufficient time (at least 24 hours) to consider the information will be scheduled for a screening visit appointment at their nearest research site, or for a remote screening appointment with The NIHR Patient Recruitment Centre, Newcastle. Informed consent will be obtained by a member of the research team delegated by the PI before any study procedures are conducted. For patients enrolled by The NIHR Patient Recruitment Centre, Newcastle, an electronic consent form will be completed; other sites will use a paper consent form.

Patients who pass the screening at the screening appointment and consent to proceed, will be entered into a 2-week screening phase to monitor the severity of their diarrhoea symptoms and abdominal pain through use of a daily diary. The symptoms diary will be assessed and eligibility to proceed to randomisation will be confirmed. If after 14 days of completing an electronic diary the patient is not eligible on the diary-based criteria, they will receive an automated notification via email and text message. The notification will say that based on diary data, the patient is not eligible to proceed to the next phase of the study and will not need to attend any further appointments. The notification will advise the patients to contact the research nurse if they have any questions. A screening log should be updated to document whether the patient was randomised into the trial, or was not eligible/declined (reasons should be documented).

6.2 STUDY VISITS AND PROCEDURES

Patients will attend 4 study visits/remote appointments and 1-2 follow-up calls. The schedule of appointments and procedures conducted at each visit are summarised in **Table 3** and described in more detail below. +/- 1 week is allowed around each follow-up appointment. Baseline appointment should take place within 14-28 days from screening appointment. **Table 4** summarises the schedule for completion of study diary and questionnaires. All remote appointments and follow-up calls by the NIHR Patient Recruitment Centre, Newcastle, can be conducted either via telephone or video call, depending on the patient's preference.

Table 3. Schedule of events and appointment procedures.

Procedure	**Screening** **-2 weeks**	**Baseline** **0 weeks**	**Follow-up** **2 weeks**	**Follow-up** **8 weeks**	**MRI** **12 weeks**	**Follow-up** **16 weeks**	**Follow-up*** **24 weeks**
Informed consent	X						
Patient screening	X						
Demographic information	X						
Review of medical history and concomitant medications	X						
Instructions on completion of diary	X						
Confirming eligibility (based on patient diary data; review of medical notes; pregnancy test, where needed)		X					
Instructions on completion of diary		X					
Randomisation		X					
Provision of stool sample kit (for selected patients only)		X		X			
Instructions on use of study treatment		X		X			
Study Questionnaires (IBS-SSS, IBS-QOL, PHQ-12 SS, WPAI:IBS)		X		X		X	
Collection of questionnaires and paper diaries		X		X		X	
Review of changes in medical history or concomitant medications		X		X		X	X
Adverse Events	X	X		X		X	X
MRI (for selected patients only)		X			X		
Checking compliance to study procedures and advising on any issues			X				
Follow-up questions							X

* Only patients who reported having had adequate relief in the last 4 weeks of open-label treatment phase

Table 4. Study diary and questionnaire completion schedule. "X" means that the item is completed once that week, except where otherwise specified.

Visit/study period	Week	Diary	WPAI:IBS	IBS-SSS	IBS-QOL	PHQ-12 SS
Screening appointment	-2	X (daily)		X		
Screening period	-1	X (daily)				
Baseline appointment	0	X (daily)	X	X	X	X
Blinded treatment period	1	X (daily)	X	X		
	2	X (daily)	X	X		
	3	X (daily)	X	X		
	4	X (daily)	X	X	X	X
	5	X (daily)	X	X		
	6	X (daily)	X	X		
	7	X (daily)	X	X		
Follow-up appointment (week 8)	8	X (daily)	X	X	X	X
Open-label treatment period	9	X (daily)	X	X		
	10	X (daily)	X	X		
	11	X (daily)	X	X		
	12	X (daily)	X	X	X	X
	13	X (daily)	X	X		
	14	X (daily)	X	X		
	15	X (daily)	X	X		
Follow-up appointment (week 16)	16	X (daily)	X	X	X	X

6.2.1 Screening appointment

Informed consent should be obtained before any trial-specific procedures take place. Reviewing eligibility based on previously conducted tests or medical history can be done prior to consent.

6.2.1.1 Informed consent

A patient should give their written informed consent to take part in the study before any trial-specific procedures are conducted. The consent can be taken by any team member delegated by the PI, but the consent form must be countersigned by the PI or sub-PI. Countersigning can be done after the day of the visit. All staff delegated by the Principal Investigator (PI) to take informed consent, must have been trained on Good Clinical Practice (GCP) and the study protocol. Before providing consent, the patient should have the opportunity to ask any questions they might have. The signed and dated consent form should be filed in the investigator site file and a copy provided to the patient. Consent must also be documented in the patient's medical notes, in accordance with GCP.

Patients are free to withdraw from the study at any time without giving a reason, without this affecting the treatment they receive. If a patient needs to be re-consented, the PI is responsible for ensuring that this is done in a timely manner.

6.2.1.2 Eligibility screening

The patients' eligibility will be determined against all criteria except for the stool consistency and abdominal pain criteria, which will be determined over the next 2 weeks using an electronic diary or a paper diary (for patients unable or unwilling to use the electronic diary). In addition, if a patient is of childbearing potential, a pregnancy test should be conducted after the 2-week screening period if a patient is confirmed to be eligible. Any criteria related to medical history or medication use that cannot be confirmed from the patient's medical records at screening due to these not being available to the research team, can be initially

assessed based on patient-reported information. However, such eligibility criteria must be confirmed against the patient's medical notes before the patient is randomised. The research team should therefore request for the relevant sections of the patient's medical records from the patient's GP once the patient has provided consent and prior to the Baseline visit unless the patient has themselves requested for the information.

During the screening appointment, the patient should be evaluated against the following study entry criteria:

Age

Patients must be aged 16-75.

IBS-D

Patients must meet the Rome IV criteria for IBS-D [6] (see **4.1 INCLUSION CRITERIA**). IBS-D must be either an existing diagnosis based on Rome IV criteria and recorded in the patient's medical records, or a diagnosis confirmed at screening by the PI or a delegated member of staff. Patients with an IBS diagnosis without subtype classification, or an IBS-D diagnosis according to previous Rome criteria, would not be eligible without confirmation of IBS-D according to the Rome IV criteria.

Exclusion of other conditions

Any patients aged ≥60 with altered bowel habits, should have had a colonoscopy to exclude bowel cancer since the bowel habits changed.

Patients should not be enrolled into the study until any required tests to exclude other conditions have been performed.

Suitable to take part in the study (medical history)

The consenting investigator should assess the patient's suitability to take part, by reviewing their medical history for any conditions or events that could affect the patient's ability to complete the trial. If a copy of medical history from GP records is not available at screening visit, these criteria can be assessed based on patient-reported history. However, in this case, eligibility should be confirmed from the medical records at Baseline appointment.

6.2.1.3 Demographic information

Age, gender and ethnicity should be recorded in the eCRF. If a copy of GP records is not available at screening visit, these can be assessed based on patient-reported information. However, in this case, eligibility should be confirmed from the medical records at Baseline appointment.

6.2.1.4 Medical history and concomitant medications

Patients using probiotic supplements, other intestinal adsorbents (activated charcoal, kaoline, diosmectite), slow-release medications or strong opioids (World Health Organisation Step III) are not be eligible to take part in the study. If a list of concomitant medications from GP records is not available at screening visit, this criteria can be assessed based on patient-reported information. However, in this case, eligibility should be confirmed against the medical records at Baseline appointment. Patients using antidepressant agents are not eligible unless these were used at a stable dose for at least 6 weeks.

All current medical conditions and concomitant medications should be recorded in the eCRF.

6.2.1.5 Instructions for screening period

If the patient passes all the screening steps above, they will be asked to complete a daily diary for the next two weeks to record stool consistency and abdominal pain. The diary will be available as an electronic online version. Training on how to use the electronic diary will be provided to the research teams and the patients and detailed written instructions will be available. If a patient is not able or willing to use an electronic diary, they will receive a paper diary containing identical questions. Copies of the paper diary will also be provided as a back-up to participants using the electronic diary.

The patients will be instructed not to use any antidiarrhoeal medication during the screening period.

6.2.1.6 Adverse Events

Any AEs related to the conduct of the study procedures should be recorded.

6.2.2 Baseline appointment

6.2.2.1 Confirming eligibility

After the 2-week screening period, the following should be reviewed at Baseline appointment to confirm the patient's eligibility:

I. <u>Sufficient completion of diaries over the screening period</u>

The patient must have completed the diary on at least 11 of the last 14 days to be eligible.

II. <u>Diarrhoea symptoms</u>

Over the 14 days after Screening Visit, the patient should have recorded BSFS Type 6 or 7 stools on at least 3 days to be eligible.

III. Abdominal pain

The average abdominal pain over the 14 days after Screening Visit should be ≥2.5 for the patient to be eligible (scale 0–10: 0 = no pain; 10 = worst possible pain).

IV. <u>Pregnancy test for women of childbearing potential</u>

A negative pregnancy test must be obtained from any female patients of childbearing potential. For patients enrolled at The NIHR Patient Recruitment Centre, Newcastle, female patients of childbearing potential will receive a pregnancy test kit by post and should take the test at home on the day of the baseline appointment. They will then be requested to electronically sign a form to confirm that their test was negative.

V. <u>Confirming eligibility and suitability based on medical records</u>

The patient's medical history and medications list from their GP, should be reviewed to confirm the patient's eligibility and suitability to take part in the trial. If any testing was required to exclude other intestinal conditions, the results from these tests must be obtained before the patient can proceed in the study.

Any patients failing at one or more of the assessments above, should not proceed in the trial and will return to standard-of-care.

6.2.2.2 Questionnaires

Patients whose eligibility has been confirmed, will proceed to completing study questionnaires (IBS-SSS, IBS-QOL, PHQ-12 SS, WPAI:IBS).

6.2.2.3 Randomisation

Eligible patients will be randomised to blinded treatment by a delegated member of the research team, who has access to the randomisation tool. The randomisation tool is built into a secure, restricted access web-based eCRF system developed and hosted by Sealed Envelope Ltd. The system will only allow patients to be randomised if they are confirmed to meet all the eligibility criteria listed on the randomisation eCRF.

The randomisation tool will provide a unique randomisation code, which the site research team will record on a treatment request form and email to the Sponsor's unblinded study supplies coordinator. The coordinator will check the code against the randomisation code list to obtain the treatment group allocation, and will then schedule a courier shipment of the corresponding treatment to the patient's home address.

6.2.2.4 Treatment instructions

The investigator will instruct the patient on how to use the blinded study treatment and other IBS treatments during the next 8 weeks (i.e. rescue medication, antidepressants). Each patient will receive a copy of the written study treatment use instructions (**APPENDIX H: TREATMENT USE INSTRUCTIONS (DOUBLE-BLIND PHASE)**). For patients enrolled at The NIHR Patient Recruitment Centre, Newcastle, the treatment instructions will be posted ahead of the appointment.

6.2.2.5 Diary and questionnaires

Patients will receive a pack of paper questionnaires (IBS-SSS, IBS-QOL, PHQ-12 SS, WPAI:IBS) to complete according to the schedule in **Table 4**. The investigator will also explain the study diary and provide paper copies to those patients who are not able or willing to use the electronic diary. Copies of the paper diary will also be provided as a back-up to participants using the electronic diary. The content of the diary is provided in **APPENDIX A: STUDY DIARY (DOUBLE-BLIND AND OPEN-LABEL PHASE)**. For patients enrolled at The NIHR Patient Recruitment Centre, Newcastle, the questionnaire pack will be posted ahead of the appointment together with pre-paid return envelopes.

6.2.2.6 Provision of stool sample kit

In total 40 study participants at selected research sites will be asked to collect a stool sample at home after the Baseline visit and again after the Follow-up 1 visit. These participants will be chosen by the randomisation programme in a way that 10 will be selected from each treatment group. A separate consent will be sought for the provision of stool samples for this study, and for use of anonymised data in future research. The participants will be provided with a stool sample kit and a pre-paid postage envelope to post the sample to the central laboratory where the samples will be analysed using the GI-MAP™ assay (Invivo Clinical Ltd).

6.2.2.7 Magnetic Resonance Imaging

Participants recruited to the main study from the University Hospital of North Durham and Newcastle Upon Tyne Hospitals will be invited to take part in MRI assessment. These participants will be provided with a separate MRI information sheet at Baseline visit and will have the opportunity to discuss the assessment with the research team and ask any questions before deciding whether they wish to consent to MRI by signing a separate written informed consent form. If a participant decides not to take part in the MRI assessment, this will not affect their participation in the main study.

Participants who consent to MRI will undergo two scans: at Baseline (although not necessarily on the same day as the Baseline Visit) and 12 weeks later, i.e. after 4 weeks of open-label Enterosgel® treatment. The scans will take place at Newcastle Upon Tyne Hospitals and will not last longer than 20 minutes involving structural and motility (cine) imaging.

6.2.3 Follow-up appointments

The participants will attend follow-up visits/remote appointments at week 8 (end of blinded treatment period) and week 16 (end of open-label treatment period). At both follow-up visits/remote appointments, the following should be conducted:

- Completion of questionnaires (IBS-SSS, IBS-QOL, PHQ-12 SS, WPAI:IBS)
- Collection of questionnaires completed between visits (for patients enrolled at The NIHR Patient Recruitment Centre, Newcastle, pre-paid envelopes will be sent to the patients to return the questionnaires)
- Review of AEs
- Review of any changes in medical history or medications

Additional, visit-specific procedures are described below.

6.2.3.1 Week 8

In addition to the completion of questionnaires and review of AEs and changes in medical history or medications, the following should be conducted:

I. All participants will receive instructions on how to take Enterosgel® sachets for the next 8 weeks (**APPENDIX I: TREATMENT USE INSTRUCTIONS (OPEN-LABEL PHASE**).
II. The participants will be provided with copies of the paper questionnaires for the next 8 weeks (for patients enrolled at The NIHR Patient Recruitment Centre, Newcastle, paper questionnaires will be sent by post)
III. For those participants using the paper diary, diaries from the double-blind period will be collected and copies provided for the next 8 weeks.
IV. For those participants selected for stool sample testing at Baseline, a stool sample kit will be provided.

6.2.3.2 Week 16

In addition to the completion of questionnaires and review of AEs and changes in medical history or medications, the following should be conducted:

I. The participants will be explained that they will no longer receive any study treatments and are returning to standard of care. The investigator should explain what this means in terms of the IBS treatments.
II. Diaries from the open-label period will be collected from those participants using the paper diary (for patient enrolled at The NIHR Patient Recruitment Centre, Newcastle, pre-paid envelopes will be sent to the patients to return the questionnaires)

Finally, the following question should be asked:

With regard to your IBS symptoms, compared with the way you felt before you started study treatment, have you, in the past 4 weeks, had adequate relief of your IBS symptoms?
a) Yes
b) No

Those participants who respond Yes, will be included in the follow-up period and are advised that there will be a follow-up phone call in 8 weeks. Those who respond No, will be explained that they have completed the study and that there will be no further follow-up from the research team. The plans for disseminating the results should be discussed.

6.2.4 Follow-up calls

At week 2, the research team will contact the participants to ensure that they are continuing in the study and discuss any potential issues with the diary, questionnaires or the study treatments. No data will be recorded on this call.

At week 24, the research team will contact those participants who were selected for follow-up (see 6.2.3.2). The questions asked during the follow-up phone call are provided in **APPENDIX F: FOLLOW-UP CALL INVESTIGATOR QUESTIONNAIRE**. Any AEs and changes in medical history and concomitant medications will be recorded.

After the follow-up questions, the participants will be explained that they have completed the study and that there will be no further follow-up from the research team. The plans for disseminating the results should be discussed.

6.2.5 Unscheduled visits

Any unscheduled clinic visits, hospitalisations or visits to the Accident & Emergency department due to IBS or due to AEs potentially related to study treatment, should be recorded in both medical records and the relevant eCRF.

6.2.6 Recruitment and appointments at The NIHR National Patient Recruitment Centre, Newcastle

Participants enrolled at The NIHR National Patient Recruitment Centre, Newcastle, will attend all study appointments remotely. This will allow patients to take part in the study even if they do not live close to any of the research sites. The process for this remote approach is described below.

Online screening

Digital study advertisements will contain a URL link which will direct potential participants to an online eligibility questionnaire. Paper-based advertisements, e.g. posters and newspaper advertisements, will contain a short web-link and QR code. The online eligibility questionnaire will be provided on a secure platform (REDCap survey form) hosted by Newcastle Hospitals NHS Trust. The web survey will request information relating to key inclusion and exclusion criteria and will determine whether a subject is ineligible or potentially eligible. Answers to questions will include "unsure" responses so that no individual is excluded through lack of certainty. The survey will also ask the individual to provide their email address, telephone number and date of birth (to be used for two factor authentication if they proceed to the consent process) and to tick a box if they agree for the Newcastle research team to contact them.
Individuals who are ineligible will be sent an automated email to explain this result. They will be thanked for expressing interest and completing the survey. Those who are potentially eligible will receive an automated email containing the full patient information sheet . Individuals who are interested to participate in the trial can contact the research team. The research team can also follow-up with the potential participants with a phone call or an email.

Before a Screening appointment is scheduled, the research team will ensure that the patient is able and willing to participate in the trial through the remote process. If they would prefer to have face-to-face appointments, the research team can refer them to their nearest recruiting research site, or with the patient's verbal permission, share the patient's contact details with the research site. The research team will also discuss with the patient if they would like to obtain their relevant medical information from their GP, or if they would prefer for the research team to contact their GP once informed consent has been signed.

The date and time of the screening visit will be agreed by telephone or email, with email confirmation and attached instructions on accessing the Attend Anywhere video consultation. Also attached to the email will be a link to the electronic consent form with instructions that this will be needed at the time of the screening appointment.

Electronic consent

The electronic consent form will be provided by the REDCap system hosted by Newcastle Hospitals NHS Trust. This platform is Health and Social Care Network (HSCN) compliant and has recently been used to manage electronic consent for a clinical trial of an investigation or medicinal product [32]. The potential participant will click a link sent by email which will open up the consent form. Two factor authentication will be used; the consent form will be sent to the email address provided by the participant when they completed the initial eligibility survey and they will also need to put in their date of birth to open the consent form.

The consent process will be undertaken using the Attend Anywhere video consultation platform. If the participant has poor internet access which does not allow video consultation, a telephone consultation will be undertaken instead. The consultation will follow the usual consent process including a discussion about the outline of the study, any questions around the patient information sheet and a talk through each section of the consent form. At this point a template of the consent form can be shared over the video link to help explain it. The participant will tick each of the statement boxes on the electronic consent form and then sign using either a mouse, if working on a PC or laptop, or their finger if working on a touchscreen device (e.g. smartphone or tablet). The system will date and time stamp the signing of the consent form so that this is permanently recorded. The consent form will be printed out, signed by both the consenting researcher and principal investigator and a copy sent to the patient with a second copy filed in the case notes and the original (with researcher and PI ink signatures) in the site file. Having consented to participate, the Screening appointment procedures will be undertaken as described in protocol section 6.2.1.

Pregnancy test

Female participants who are of childbearing potential and pass the screening phase on the diary-based criteria, will be sent a pregnancy test kit ahead of the Baseline appointment, along with a link to an electronic form (REDCap) that must be completed by the participant before randomisation, to confirm that they have taken a pregnancy test and it was negative.

Study questionnaires and other relevant documentation

All study questionnaires and other study documents (i.e. treatment, questionnaire and appointment instructions) will be posted to the participants ahead of the relevant study appointment, along with pre-paid return envelopes to return the completed questionnaires to the research team. An electronic diary guide should be emailed to the participants during or immediately after the screening appointment. £10 study vouchers will also be posted following completion of Screening, Baseline and week 8 and week 16 appointments.

6.2.7 Remote appointments to reduce the risk of COVID-19

All sites will be allowed to conduct Baseline and Follow-up study visits for already enrolled patients remotely if this is required to protect staff and patients from COVID-19. However, new patients cannot be enrolled remotely with the exception of The NIHR National Patient Recruitment Centre, Newcastle. The sites should follow their local policies regarding the conduct of appointments at site. Any visits conducted remotely due to COVID-19 would not be classified as protocol deviations or violations, but should be documented in the Investigator Site File and the eCRF.

6.3 DESCRIPTION OF ASSESSMENTS

6.3.1 Study diary

Data on IBS symptoms and treatment use will be collected using a study-specific diary, which is available as an electronic diary that can be completed online by following a link provided on daily email and text message notifications. Questions included in the diary are provided in **APPENDIX A: STUDY DIARY (DOUBLE-BLIND AND OPEN-LABEL PHASE)**. No patient identifiable data will be collected using the electronic diary.

For patients who are not able or willing to use the electronic diary, a paper diary will be provided. Copies of the paper diary will also be provided as a back-up to participants using the electronic diary and the patients will be advised to contact the research team immediately, should they have any problems in using the electronic diary. The patients should return the paper diaries to the research site at each follow-up visit. Data entry from any paper screening diaries must be done by site before randomising the patient. For double-blind and open-label diaries completed on paper, the site will send copies to the Sponsor's research team, who will have access to enter the data into the same database where the electronic diary data gets stored.

6.3.2 IBS-SSS

The IBS-SSS evaluates the intensity of IBS symptoms during a 10-day period [23]. It considers abdominal pain, distension, stool frequency and consistency, and interference with life in general. Each question is scored on a visual analog scale from 0 to 100, and their sum is then calculated to obtain the total score. Higher scores indicate worse symptoms. Data suggest that the IBS-SSS could be used for selecting symptomatic patients for clinical trials and for measuring response to treatment [33].

A copy of IBS-SSS is provided in **APPENDIX B: IBS-SSS QUESTIONNAIRE**.

6.3.3 IBS-QOL

The IBS-QOL is a self-report quality of life measure specific to IBS that can be used to assess the impact of IBS and its treatment [24,25]. The questionnaire contains 34 items, each with a five-point response scale. The individual responses to the 34 items are summed and averaged for a total score and then transformed to a 0-100 scale using the following formula:

Score = [The sum of the items – lowest possible score]/Possible raw score range x 100

Higher scores indicate a better IBS-specific quality of life.
The IBS-QOL is designed to be self-administered, and takes an average of 10 minutes to complete.

A copy of IBS-QOL is provided in **APPENDIX C: IBS-QOL QUESTIONNAIRE**.

6.3.4 WPAI:IBS

The WPAI is an instrument developed to measure work productivity and activity impairment [27]. An IBS-specific version, WPAI:IBS, has been validated in patients with IBS [28] **(APPENDIX E: WPAI:IBS)**.

6.3.5 PHQ-12 SS

PHQ-12 SS is a modification of the Patient Health Questionnaire 15 (PHQ-15) where the three gastrointestinal items of PHQ-15 (questions a, l and m) are excluded [26] (**APPENDIX D: PHQ-12 QUESTIONNAIRE**). It has been shown to correlate with patient behaviour in IBS [26].

6.3.6 Gastrointestinal-Microbial Assay Plus (GI-MAP)

The GI-MAP™ (Invivo Clinical Ltd, UK) utilises cutting-edge FDA-approved technology to provide a DNA-based assessment of a patient's gut microbiome from a single stool sample. DNA sequencing of 16S and 23S rRNA allows for sensitivity and specificity in the detection of 15 of the most common causes of gastroenteritis, as well as other chronic diseases. It also measures *H. pylori* with virulence factors, while intestinal health markers allow for a comprehensive analysis of the gut microbiome, together with markers of inflammation, mucosal immune system and digestion. For this study, the following will be tested:

- Pathogen bacteria, toxins, parasites and viruses
- Commensal bacteria (including *Bacteroides fragilis*)
- Bacterial autoimmune triggers
- Dysbiotic bacteria
- Opportunistic parasites
- Yeast, fungi and moulds
- Secretory IgA (sIgA)
- Anti-gliadin sIgA
- Steaotocrit
- Beta-glucuronidase
- Faecal zonulin

In total 20 patients from up to four pre-selected research sites will be asked to provide two stool samples for GI-MAP™ testing, i.e. at Baseline and at follow-up visit 1 (8 weeks). The randomisation programme will tell the investigator whether a patient should be selected for testing. This is to ensure that 10 patients from each treatment group are selected. The selected patients will be provided with a stool sample kit containing a collection sleeve, spoon and a bottle containing fixative. A pre-paid postage envelope will also be provided for the patients to ship the sample to the Invivo Clinical central laboratory. The test reports will be sent to the trial manager.

6.3.7 Magnetic Resonance Imaging

Magnetic Resonance Imaging is a scan that uses radio waves and magnetic fields to produce detailed images of the inside of the body. The MRI scanner does not use X-rays so it is considered safe to have multiple scans. The MRI data in this study will be analysed by Motilent Ltd (UK) using their GIQuant image processing software.

6.4 EMERGENCY UNBLINDING

Unblinding (code-break) should only be performed during the trial in a situation where information about the patient's trial treatment is necessary in order to provide the patient with appropriate and optimal medical care. Such emergency unblinding may be requested on the grounds of safety by the Chief Investigator, local PI or authorised delegate or treating physician. A request for unblinding can also come from a patient, carer or GP, for example when an adverse event occurs or there are changes planned to the patient's regular therapy. Requests for unblinding will first be reviewed by the PI or sub-PI who evaluates the information and the importance of unblinding in the given circumstances. If the PI/sub-PI decides that unblinding is necessary to ensure the patient will receive appropriate medical care, emergency unblinding is performed. Unblinding request can be submitted by any local research team member with access to the eCRF. Following the submission of an unblinding request form in the eCRF, the treatment allocation will be emailed, or sent by a text message or fax to the person who had requested unblinding. Following unblinding, a notification will be automatically sent to the trial manager.

In case of emergency unblinding, the PI will be responsible for deciding whether the patient should continue on trial treatment. Unblinded patients should be followed up according to the study protocol until the end of the study. The reason for emergency unblinding should be recorded in the Emergency Unblinding eCRF.

7. SAFETY

7.1 DEFINITIONS

Types of AEs associated with medical devices and applicable for this study are defined in accordance with the European Commission guidelines on medical devices [34].

7.1.1 Adverse Event (AE)

Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) in subjects, users or other persons whether or not related to the investigational medical device.
NOTE 1: This includes events related to the investigational device or the comparator.
NOTE 2: This includes events related to the procedures involved (any procedure in the clinical investigation plan).
NOTE 3: For users or other persons this is restricted to events related to the investigational medical device.

7.1.2 Adverse Device Effect (ADE)

AE related to the use of an investigational medical device.
NOTE 1: This includes any AE resulting from insufficiencies or inadequacies in the instructions for use, the deployment, the implantation, the installation, the operation, or any malfunction of the investigational medical device.
NOTE 2: This includes any event that is a result of a use error or intentional misuse.

7.1.3 Serious Adverse Device Effect (SADE)

Adverse device effect that has resulted in any of the consequences characteristic of a serious AE.

7.1.4 Serious Adverse Event (SAE)

AE that:
a) led to a death,
b) led to a serious deterioration in health that either:
1. resulted in a life-threatening illness or injury, or
2. resulted in a permanent impairment of a body structure or a body function, or
3. required in-patient hospitalisation or prolongation of existing hospitalisation, or
4. resulted in medical or surgical intervention to prevent life threatening illness or injury or permanent impairment to a body structure or a body function.

c) led to fetal distress, fetal death or a congenital abnormality or birth defect.

NOTE 1: This includes device deficiencies that might have led to a SAE if a) suitable action had not been taken or b) intervention had not been made or c) if circumstances had been less fortunate. These are handled under the SAE reporting system.
NOTE 2: A planned hospitalisation for pre-existing condition, or a procedure required by the Clinical

Investigation Plan, without a serious deterioration in health, is not considered to be a SAE.

7.1.5 Unanticipated Serious Adverse Device Effect (USADE)

Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report or product safety information. NOTE: Anticipated: an effect which by its nature, incidence, severity or outcome has been previously identified in the risk analysis report

7.1.6 Expected Adverse Events and Adverse Events of Special Interest

According to the standard Package Information Leaflet, Enterosgel® can cause constipation and nausea. It is normal for patients with IBS-D to feel constipated when diarrhoea symptoms improve. Constipation should only be considered an AE in this study if all of the following are met:

a) patient complains about it to the site research team
b) patient had at least 3 consecutive days without bowel movements
c) clinical intervention is required (i.e. laxative or other treatment)

7.2 RECORDING AND REPORTING OF ADVERSE EVENTS

7.2.1 Investigational procedures and treatment

AEs will be collected throughout the study from screening appointment until week 24. If an AE is reported by the patient, the relationship of the event to the study treatment or procedures should be assessed by the local PI, or a delegated sub-PI or nurse. The following information will be recorded for all AEs:

- Medical term of the AE (SNOMED CT terminology)
- Start date and date of resolution
- Seriousness
- Severity
- Study treatment action
- Outcome
- Relationship with the study treatment
- Expectedness

SADEs, SAEs, and USADEs should <u>always</u> be recorded in the eCRF and reported to the Sponsor using the Sponsor's SAE reporting form. Any SAEs that occur during the screening period that resulted from the administration of any study procedures and are unexpected, should also be reported. The form should be emailed to research@enteromed.co.uk **within 24 hours of the site team becoming aware of the event, and the Trial Manager should be copied in this email (**anu@clevercookie.net**). If the site does not receive an acknowledgement of the receipt of the report within 24 hours, they should immediately contact the Trial Manager.**

The Sponsor should immediately inform the CI of any reported SAEs. The CI should review the SAE to confirm causality and expectedness.

The Sponsor should also report all serious adverse events, whether initially considered to be device related or not, immediately to the MHRA. The REC should be notified of any related and unexpected SAEs within 15 days. Reports of related and unexpected SAEs in double-blind trials should be unblinded. Investigators should only receive information on the code-break if it is necessary for the safety of the patient.

SAEs should be followed-up until resolution or a final outcome. All follow-up information should be emailed to the Sponsor as soon as it becomes available. The SAE form and any email correspondence related to the SAE should be filed in the investigator site file and in the electronic Trial Master File.

7.2.2 Auxiliary Medicinal Products

This section applies to safety reporting requirement of adverse events suspected to be related to the AxMP only (= adverse reaction to AxMP). In case a suspicion of (or interaction with) the investigational treatment cannot be ruled out for this adverse event the reporting rules for the investigational treatment apply. Where an adverse event is suspected to be related only to an authorised AxMP, and does not result from a possible interaction with the investigational treatment, the investigator should report the case through the Yellow Card Scheme. While reporting the suspected adverse reaction, the relevant information regarding the clinical trial (i.e. clinical trial number) must be included in the report.

7.3 REPORTING OF PREGNANCIES

Although according to the package information leaflet Enterosgel® can be used in pregnant women, we have decided to take a conservative approach in this first placebo-controlled Enterosgel® trial in patients with IBS-D, and are excluding any pregnant patients from the trial. Any women of childbearing potential should agree to use a medically accepted method of contraception while they are receiving study treatment (i.e. until the end of the open-label period). Should a patient get pregnant while the patient was receiving study treatment, the site should notify the Sponsor within 24 hours of becoming aware of the pregnancy, by emailing the Sponsor's pregnancy reporting form to research@enteromed.co.uk. The Trial Manager should be copied in this email (anu@clevercookie.net).

In case of a pregnancy while receiving study treatment, a patient should be withdrawn from the study. The pregnancy should be followed for outcome and any adverse outcome of pregnancy should be assessed for causality to the study treatment received. Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/foetus.

8. STATISTICS

8.1 SAMPLE SIZE CALCULATION

The sample size calculation is based on demonstrating superiority for the primary outcome, i.e. response to treatment, with 90% power at 5% significance level. Assuming a response rate of 20% in the placebo group and 35% in the active treatment group, 182 patients per treatment group are required. Assuming 15% drop-out rate, in total 430 patients will need to be enrolled. The response rate of 20% in the placebo group is based on studies by Garsed *et al*. [35] and Lacy *et al*. [36]. We assume that patients with bile acid malabsorption will show an equivalent response rate of 35%, to patients with IBS-D.

Power was calculated using a power calculator for binary outcome superiority trial (Sealed Envelope Ltd. 2012. Power calculator for binary outcome superiority trial. [Online] Available from: https://www.sealedenvelope.com/power/binary-superiority/ [Accessed Tue Aug 08 2017]).

8.2 STATISTICAL ANALYSES

This trial will be reported according to the CONSORT guidelines for clinical trials (Consolidated Standards Of Reporting Trials statement (http://www.consort-statement.org/) [37]. Analyses will be conducted

following the principles of intention-to-treat with patient's outcomes analysed according to their original, randomised group irrespective of deviations based on non-compliance.

Analyses will be undertaken in Stata v14 or later (to be confirmed in the final report). Significance tests will be two-sided at the 5% significance levels unless otherwise stated. The statistician will remain blind to allocation until after the trial is complete and the results have been finalised. Parameter estimates will be presented with associated 95% confidence intervals and p-values as appropriate.

The number of patients screened, eligible and randomised will be reported. The flow of participants through the trial will be presented in a CONSORT diagram.

8.2.1 Baseline data

All participant baseline data will be summarised descriptively overall and by trial arm both as randomised and as analysed in the primary analysis. No formal statistical comparisons will be undertaken. Continuous measures will be reported as means and standard deviations while the categorical data will be reported as counts and percentages.

8.2.2 Primary analysis

The primary outcome measure is the percentage of patients defined as responders for abdominal pain and stool consistency during at least 4 weeks in the 8-week treatment period. The outcome data will be summarised descriptively overall and by trial arm. The proportions will be compared between the placebo and Enterosgel® groups using a logistic regression model with odds ratios and 95% confidence intervals. The model will be adjusted for any baseline variables and other known confounders.

8.2.3 Secondary analysis

Secondary outcome data will be summarised descriptively at each time point, overall and by trial arm. The secondary outcomes will be analyses either by comparing between the trial arms at week 8 by ANCOVA adjusting for baseline scores, or employing covariance pattern model in which each outcome at each time point will be nested within patients and the effect of treatment according to trial arm will be assessed. The outcome at baseline, trial arm, each time point of follow-up, each time point of follow-up by trial arm interaction, stratification factors (fixed effects) and outcome at each time point nested within patient (random effects) will be included in the model. This will allow efficient use of the data collected, and account for potential correlation of repeated measures and within patient correlation.

8.2.4 Handling of missing data

Multiple imputation will be used to impute missing values under a missing at random assumption, to avoid excluding patients from the analysis. Multiple imputation involves creating multiple copies of the data set, with the missing values replaced by imputed values drawn from their predicted distribution by using the observed data [38].

8.3 ANALYSIS POPULATIONS

8.3.1 Intention-to-treat population (ITT)

ITT population will include all randomised patients analysed in the groups to which they were randomised to, regardless of whether they received or adhered to the allocated intervention.

8.3.2 Per-protocol population (PP)

PP population will include all patients who completed the study without reported protocol violations.

8.3.3 Safety population

The safety population is the analysis population based on which AEs will be summarised and reported. This population will include all randomised patients who received at least 1 dose of study treatment. In this population, patients will be analysed according to the treatment they actually received, regardless of the treatment they were randomised to.

9. DATA HANDLING

9.1 DATA COLLECTION AND SOURCE DATA

Source data means any information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents, which are original documents or certified copies of original documents in which data collected for a clinical trial is first recorded. These include paper questionnaires, study diaries, diagnostic questionnaires, hospital or GP records, or laboratory reports.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Data reported in the eCRF derived from source documents, i.e. medical records and the patient diaries, should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

9.2 ELECTRONIC CASE REPORT FORMS (eCRF)

All study data will be entered using a validated electronic Case Report Form (eCRF) system developed by Sealed Envelope Ltd. Data recorded in the eCRFs will not contain any patient identifying information; each participant will be identified in the system by a unique study-specific participant number. The eCRF system can only be accessed by delegated and trained site team personnel, with their individual username and password.

9.3 STUDY DIARY

The patients will be requested to complete a study diary daily during the screening, blinded and open-label treatment periods, and weekly during the follow-up period (content of the diary will vary between the different periods). An electronic diary will be developed for the trial by Sealed Envelope. The diary will be available online for use on any computer or mobile device. No patient identifiable data will be collected using the diary.

Patients who are not able or willing to use the electronic diary, will use a paper version of the diary. The patients should return the paper diaries they have completed at home to the research site for each follow-up appointment, and the site should file these as source documents in the investigator site file. The site will then send copies of the diaries to the Sponsor's research team, who will have access to enter the data into

the same database where the electronic diary data gets stored.

All patients using the electronic diary will receive a paper diary as a back-up.

9.4 PATIENT QUESTIONNAIRES

During the trial, the patients will be asked to complete the IBS-SSS, IBS-QOL, PSQ-12 SS and WPAI:IBS questionnaires at regular intervals and at each follow-up appointment. The patients should return the questionnaires they have completed at home to the research site for each follow-up appointment, either in person during the onsite visit or in the prepaid envelopes provided for patients enrolled at The NIHR Patient Recruitment Centre, Newcastle, and the site should file all completed questionnaires as source documents in the investigator site file.

9.5 ARCHIVING

All essential documents and trial data will be held by the Sponsor for a minimum of 5 years after the end of the trial. Investigator site files should be archived at the participating sites for 5 years and should not be destroyed until authorisation to do so has been received from the Sponsor.

10. QUALITY ASSURANCE

10.1 MONITORING AND QUALITY CONTROL

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures. Regular monitoring will be performed in accordance with the ICH GCP and a risk based Trial Monitoring plan, which will define the monitoring schedule and method (on-site, remote) and the details of targeted monitoring. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. The monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. Any data issues in the eCRF (such as missing data or data discrepancies), should be primarily addressed by raising data queries in the eCRF. Any unresolved or outstanding queries should be discussed with the site during the remote/on-site monitoring visits.

10.2 PROTOCOL DEVIATIONS AND VIOLATIONS

A protocol deviation is any noncompliance with the clinical trial protocol or GCP. The noncompliance may be either on the part of the participant, the investigator, or the study site staff.

Protocol violations are significant deviations that reduce the quality or completeness of the data, make the Informed Consent Form inaccurate, or impact a subject's safety, rights, or welfare [39].

Examples of protocol violations relevant to this study may include the following:

1. Inadequate or delinquent informed consent
2. Inclusion/exclusion criteria not met
3. Unreported SAEs
4. Materially inadequate record keeping
5. Intentional deviation from the protocol, GCP, or regulations by study personnel
6. Subject repeated non-compliance with study requirements
7. Dispensing of incorrect treatment

8. Not using any study treatment
9. Starting on antidepressants, intestinal adsorbents, strong opioids or probiotic supplements
10. Initiating any new treatment during the study that might have a significant impact on stool consistency and/or abdominal pain, according to the PI's opinion

All deviations/violations must be addressed in study source documents and reported to the Sponsor and Research Ethics Committee (REC) and/or the MHRA as per their guidelines and the Medical Device guidelines (MEDDEV 2.7/1). All protocol violations should be recorded in the Protocol Violation eCRF. The site PI/study staff is responsible for knowing and adhering to these requirements. As a result of deviations and violations, corrective actions are to be developed by the Sponsor and/or site as appropriate and implemented promptly.

Patients with protocol violations will be excluded from the PP population.

11. TRIAL MANAGEMENT AND OVERSIGHT

11.1 TRIAL MANAGEMENT

The Trial Management Group (TMG) will be responsible for the set-up and conduct of the trial, including monitoring of recruitment and data. It will include at least one Sponsor's representative, the Chief Investigator, the trial statistician, and the trial manager. The TMG will hold a teleconference once a month and two face-to-face meetings a year.

11.2 TRIAL STEERING COMMITTEE

The Trial Steering Committee consists of three clinicians with specialist expertise in IBS or gastroenterology and a GP. At least one of the TSC members should be independent, i.e. not a PI on the study. The role of the TSC is to give recommendations to the TMG on medical and scientific questions, particularly during the protocol development stage, but also during the conduct of study as requested by the TMG. TSC members will also contribute to the publications.

11.3 DATA MONITORING COMMITTEE

An independent Data Monitoring Committee (DMC) will monitor data collected during the study for efficacy and safety. If any issues emerge, the DMC will make recommendations regarding the continuation of the study. The DMC will consist of a Chair (gastroenterologist) and a statistician.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1 GENERAL CONSIDERATIONS

This study will be conducted in full accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly of Helsinki (1964), revised at Tokyo (1975), Venice (1983), Hong-Kong (1989), Somerset West (1996), Edinburgh (2000) and Seoul (2008), including the Notes of clarification made by the World Medical Assembly of Washington (2002), Tokyo (2004), Seoul (2008) and Fortaleza (2013) as well as in compliance with ICH GCP Consolidated Guideline (E6) and any applicable national and local laws and regulations, including the Clinical Trials

Directive 2001/20/EC and The Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments.

The Investigators and the Sponsor are responsible for ensuring that all activities in relation to this study are conducted in accordance with the protocol, GCP guidelines and any other relevant regulations. The Principal Investigator at each study site has the overall responsibility for the conduct and administration of the study at that site and for ensuring that the site staff conducting any study-related procedures are qualified and appropriately trained to conduct the tasks delegated to them.

The protocol, PIS, informed consent form(s), recruitment materials, and all participant materials will be submitted to the REC, HRA and MHRA for review and approval. Approval of all relevant documents must be obtained before any participant is enrolled. Any substantial amendments to the protocol or other study documents will require review and approval by the relevant authority(ies) before the changes can be implemented to the study.

The Sponsor is responsible for the submissions of SAE reports and annual progress and safety reports to the relevant authority(ies), and for notifying them of the end of the trial.

12.2 PATIENT CONSENT

The participant will sign the informed consent document prior to any study-specific procedures being performed. Participants will have the opportunity to carefully review the study information and ask questions prior to providing consent. A copy of the informed consent document will be provided to the participants for their records.

Participants may withdraw consent at any time throughout the course of the trial. The rights and welfare of the participants will be protected by emphasising to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

12.3 PATIENT CONFIDENTIALITY

The Investigators affirm and uphold the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals. Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers to correspond to treatment data in the computer files.

For data verification purposes, authorised representatives of the Sponsor, a competent authority, or REC may require direct access to parts of the medical records relevant to the study, including participants' medical history.

12.4 PAYMENTS TO PATIENTS

Participants will receive a shopping voucher of £10 per study visit/remote appointment as a compensation for inconvenience. Reasonable travel costs will also be compensated.

13. PUBLICATION AND DISSEMINATION

This study has been registered with International Standard Randomised Controlled Trial Number (ISRCTN17149988).

Study results will be submitted for presentation(s) at gastroenterology conference(s) and for publication in international peer-reviewed scientific journal(s). Authors will acknowledge that the study was funded by Bioline Products s.r.o (Czech Republic). Authorship will be determined in accordance with the International Committee of Medical Journal Editors (ICMJE) guidelines and other contributors will be acknowledged.

Results will be disseminated to the patients and the public through the study website.

Results will be submitted for inclusion into the National Institute for Health and Care Excellence (NICE) guidance if valuable for NHS.

Any data collected by any healthcare provider under this study is the property of the Sponsor and should not be used for any purposes other than the Sponsor's without the Sponsor's written permission.

14. FINANCE AND INSURANCE

This study is funded by Bioline Products s.r.o (Czech Republic). The study Sponsor, EnteroMed Ltd, is the exclusive distributor of Enterosgel® in the UK and holds a Product Liability Insurance for legal liabilities arising from the use of Enterosgel® in the UK.

NHS bodies are legally liable for the negligent acts and omissions of their employees. If a patient is harmed whilst taking part in a clinical study as a result of negligence on the part of a member of the study team this liability cover would apply.

15. CONFLICTS OF INTEREST

The Sponsor of this study (EnteroMed Ltd) is the exclusive distributor of Enterosgel® in the UK.

The Chief Investigator has no conflicts of interest.

16. REFERENCES

1. Lovell RM, Ford AC. 2012. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clinical Gastroenterology and Hepatology. 10:712-21.
2. National Collaborating Centre for Nursing and Supportive Care commissioned by National Institute for Health and Clinical Excellence. 2008. Clinical Practice Guideline. Irritable bowel syndrome in adults: Diagnosis and management of irritable bowel syndrome in primary care. Accessed 15th October 2017. Available at: https://www.nice.org.uk/guidance/cg61/evidence
3. Canavan C, West J, Card T. 2014. The epidemiology of irritable bowel syndrome. Clin Epidemiol. 6:71-80.
4. Soubieres A, Wilson P, Poullis A, Wilkins J, Rance M. 2015. Burden of irritable bowel syndrome in an increasingly cost-aware National Health Service. Frontline Gastroenterol. 6:246-251.
5. Williams M, Barclay Y, Benneyworth R, Gore S, Hamilton Z, Matull R, Phillips I, Seamark L, Staveley K, Thole S, Greig E. 2016. Using best practice to create a pathway to improve management of irritable bowel syndrome: aiming for timely diagnosis, effective treatment and equitable care. Frontline Gastroenterology 0:1–8.

6. Lacy BE, Mearin F, Chang L, Chey WD, Lembo AJ, Simren M, Spiller R. 2016. Bowel disorders. Gastroenterology. 150:1393-407.
7. González-Castro AM, Martínez C, Salvo-Romero E, Fortea M, Pardo-Camacho C, Pérez-Berezo T, Alonso-Cotoner C, Santos J, Vicario M. 2017. Mucosal pathobiology and molecular signature of epithelial barrier dysfunction in the small intestine in irritable bowel syndrome. 32:53-63.
8. Liu HN, Wu H, Chen YZ, Chen YJ, Shen XZ, Liu TT. 2017. Altered molecular signature of intestinal microbiota in irritable bowel syndrome patients compared with healthy controls: A systematic review and meta-analysis. Dig Liver Dis. 49:331-337.
9. Spiller R. 2016. Irritable bowel syndrome: new insights into symptom mechanisms and advances in treatment. F1000Res. 5. pii: F1000 Faculty Rev-780.
10. Spinner HL, Lonardo NW, Mulamalla R, Stehlik J. 2015. Ventricular tachycardia associated with high-dose chronic loperamide use. Pharmacotherapy. 35:234-8.
11. Keating GM. 2017. Eluxadoline: A Review in Diarrhoea-Predominant Irritable Bowel Syndrome. Drugs. 77:1009-1016.
12. Altobelli E, Del Negro V, Angeletti PM, Latella G. 2017. Low-FODMAP Diet Improves Irritable Bowel Syndrome Symptoms: A Meta-Analysis. Nutrients. 9(9). pii: E940.
13. Chang FY, Lu CL, Chen CY, Luo JC. 2007. Efficacy of dioctahedral smectite in treating patients of diarrhea-predominant irritable bowel syndrome. J Gastroenterol Hepatol. 22:2266-72.
14. Tack JF, Miner PB Jr, Fischer L, Harris MS. 2011. Randomised clinical trial: the safety and efficacy of AST-120 in non-constipating irritable bowel syndrome - a double-blind, placebo-controlled study. Aliment Pharmacol Ther. 34:868-77.
15. Leonard AJ, Droy-Lefaix MT, Allen A. 1993. Pepsin damage in rat stomach in vivo: effect of smectite and dimethyl prostaglandin E2. Gastroenterology 104:A132.
16. Mahraoui L, Heyman M, Plique O, Droy-Lefaix MT, Desjeux JF. 1997. Apical effect of diosmectite on damage to the intestinal barrier induced by basal tumour necrosis factor-alpha. Gut 339–43.
17. Eutamene H, Beaufrand C, Mathiex-Fortunet H, Theodorou V. 2017. Diosmectite treatment suppresses gut visceral hypersensitivity induced by chronic stress in rats. Gastroenterology 152:S727.
18. Nikolaev V. Enterosgel: A Novel Organosilicon Enterosorbent with a Wide Range of Medical Applications. In Biodefence - Advanced Materials and Methods for Health Protection. Mikhalovsky, S and Khajibaev, A (Editors). 2011. Springer. Available at: http://www.springer.com/us/book/9789400702165
19. Howell CA, Mikhalovsky SV, Markaryan EN, Khovanov AV. 2019. Investigation of the adsorption capacity of the enterosorbent enterosgel for a range of bacterial toxins, bile acids and pharmaceutical drugs. Scientific reports. 9:5629.
20. Tkachenko E and Avalueva E. 2014. The clinical efficacy and safety of Enterosgel in the treatment of diarrhoea-dominant irritable bowel syndrome. Abstract available at: http://enteromed.co.uk/download/the-clinical-efficacy-and-safety-of-enterosgel-in-the-treatment-of-diarrhoea-dominant-irritable-bowel-syndrome-2014/
21. European Medicines Agency. 2014. Guideline on the evaluation of medicinal products for the treatment of irritable bowel syndrome. 25 September 2014. CPMP/EWP/785/97 Rev. 1.
22. Food and Drug Administration. Guidance for Industry. Irritable Bowel Syndrome - Clinical Evaluation of Drugs for Treatment. May 2012.
23. Francis CY, Morris J, Whorwell PJ. 1997. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther. 11:395-402.
24. Patrick DL, Drossman DA, Frederick IO, DiCesare J, Puder KL. 1998. Quality of life in persons with irritable bowel syndrome: development and validation of a new measure. Dig Dis Sci. 43:400-411.
25. Drossman DA, Patrick DL, Whitehead WE, Toner BB, Diamant NE, Hu Y, Jia H, Bangdiwala SI. 2000. Further validation of the IBS-QOL: a disease-specific quality-of-life questionnaire.
Am J Gastroenterol. 95:999-1007.
26. Spiller RC, Humes DJ, Campbell E, Hastings M, Neal KR, Dukes GE, Whorwell PJ. 2010.
The Patient Health Questionnaire 12 Somatic Symptom scale as a predictor of symptom severity and consulting behaviour in patients with irritable bowel syndrome and symptomatic diverticular disease. Aliment Pharmacol Ther. 32:811-20.
27. Reilly MC, Zbrozek AS, Dukes EM. 1993. The validity and reproducibility of a work productivity and activity impairment instrument. Pharmacoeconomics 4:353-65.

28. Reilly MC, Bracco A, Ricci JF, Santoro J, Stevens T. 2004. The validity and accuracy of the Work Productivity and Activity Impairment questionnaire--irritable bowel syndrome version (WPAI:IBS). Aliment Pharmacol Ther. 20:459-67.
29. Clinical Trial Facilitation Group. 2014. Recommendations related to contraception and pregnancy testing in clinical trials. Final version 2014-09-15. Accessed 30th October 2017. Available at: http://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf
30. Taves DR. 1974. Minimization: a new method of assigning subjects to treatment and control groups. Clin Pharmacol Therapeut. 15:443-53.
31. Yashina NI, Plygan EP, Semenov VG, Martynenko AM, Glushchenko OV. Sol-Gel Methods for Materials Processing. In Sol-Gel Technology of the Mesoporous Methylsilicic Acid Hydrogel: Medicine Aspects of Globular Porous Organosilicon Materials Application. Editors; Plinio Innocenzi, Yuriy L. Zub, Vadim G. Kessle. Springer, Dordrecht 2008. pp 481-488
32. Boulware DR, Pullen MF, Bangdiwala AS, Pastick KA, Lofgren SM, Okafor EC, Skipper CP, Nascene AA, Nicol MR, Abassi M, Engen NW. 2020. A Randomized Trial of Hydroxychloroquine as Postexposure Prophylaxis for Covid-19. New England Journal of Medicine. 2020 Jun 3.
33. Lembo A, Ameen V, Drossman DA. 2005. Irritable bowel syndrome: toward an understanding of severity. Clin Gastroenterol Hepatol 3:717–25.
34. European Commission Directorate General for Health and Consumers. 2010. Guidelines on Medical Devices. MEDDEV 2.7/3. December 2010. Available at: http://ec.europa.eu/consumers/sectors/medical-devices/files/meddev/2_7_3_en.pdf
35. Garsed K, Chernova J, Hastings M, Lam C, Marciani L, Singh G, Henry A, Hall I, Whorwell P, Spiller R. 2014. A randomised trial of ondansetron for the treatment of irritable bowel syndrome with diarrhoea. Gut. 63:1617-25.
36. Lacy BE, Chey WD, Cash BD, Lembo AJ, Dove LS, Covington PS. 2017. Eluxadoline Efficacy in IBS-D Patients Who Report Prior Loperamide Use. Am J Gastroenterol. 112:924-932.
37. Schulz KF, Altman DG, Moher D; CONSORT Group. 2010. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMC Med. 8:18.
38. Little RJ, Rubin DB. 2002. Statistical Analysis With Missing Data, 2nd Ed. Wiley.
39. Bhatt A. 2012. Protocol deviation and violation. Perspect Clin Res. 3:117.

APPENDICES

APPENDIX A: STUDY DIARY (DOUBLE-BLIND AND OPEN-LABEL PHASE)

RELIEVE IBS-D STUDY DIARY

WEEK NO (1-16): _____

STUDY PARTICIPANT ID: _____ CENTRE ID: _____ DATE OF BASELINE VISIT: _____

PLEASE COMPLETE WHEN STUDY TREATMENT DELIVERED:

Date study treatment delivered: _____

PLEASE COMPLETE DAILY:

QUESTION	DAY						
	1	2	3	4	5	6	7
How many bowel movements did you have in the last 24 hours?	___	___	___	___	___	___	___
On a scale of 1–7, what was the score of your least formed bowel movement in the last 24 hours? 1 = Separate hard lumps, like nuts (hard to pass) 2 = Sausage-shaped but lumpy 3 = Like a sausage but with cracks on its surface 4 = Like a sausage or snake, smooth and soft 5 = Soft blobs with clear-cut edges (passed easily) 6 = Fluffy pieces with ragged edges, a mushy stool 7 =Watery stool, no solid pieces; entirely liquid	___	___	___	___	___	___	___
With regard to your IBS symptom of abdominal pain, on a scale of 0–10, what was your worst IBS-related abdominal pain over the last 24 hours? 'Zero' means you have no pain at all; 'Ten' means the worst possible pain you can imagine.	___	___	___	___	___	___	___
How many doses of study treatment did you use today?	___	___	___	___	___	___	___

ENT04UK *Study Diary v.2.0 03/05/2018* 1

PLEASE COMPLETE THE QUESTIONS BELOW ON DAY 7 EACH WEEK:

1. You are now going to be asked to evaluate if you have achieved adequate relief of your IBS symptoms over the last week. Achieving adequate relief is another way of saying that compared with how you felt before receiving any study medication in this study; you feel that the symptoms of IBS have satisfactorily improved during the past 7 days. **With regard to your IBS symptoms, compared with the way you felt before you started study medication, have you, in the past 7 days, had adequate relief of your IBS symptoms (please circle Yes or No below)?**

   Yes No

2. Have you felt or experienced a sense of **urgency** in the last 7 days with any of your bowel movements (please circle a number below)?

   0 = not at all
   1 = hardly
   2 = somewhat
   3 = moderately
   4 = a good deal
   5 = a great deal
   6 = a very great deal

3. With regard to your IBS symptom of **bloating**, on a scale of 0–6, how bothersome was your IBS-related bloating in the last 7 days (please circle a number below)?

   0 = not at all
   1 = hardly
   2 = somewhat
   3 = moderately
   4 = a good deal
   5 = a great deal
   6 = a very great deal

4. On how many days have you used loperamide this week? ____

ENT04UK *Study Diary v.2.0 03/05/2018* 2

APPENDIX B: IBS-SSS QUESTIONNAIRE

IBS Severity Scale

The questions on this page ask about the severity and frequency of your bowel problems. Fill in the appropriate circles beside the best answer to each question. If you answer with a number, please fill in the box.

1. a) Do you currently (in the past 10 days) suffer from abdominal (tummy) pain? ○ No ○ Yes

   b) If yes, how severe was your abdominal (tummy) pain? Please indicate a number from 0 to 100, with 0 meaning "no pain" and 100 meaning "very severe" ☐

   c) Please enter the number of days you had the abdominal pain in the past 10 days. For example, if you enter 4 it means that you had pain 4 out of 10 days. If you have pain every day enter 10. Number of days with pain: ☐

2. a) Do you currently (in the past 10 days) suffer from abdominal distention * (bloating, swollen or tight tummy) ○ No ○ Yes
   **Women, please ignore distention related to your period*

   b) If yes, how severe is your abdominal distention/tightness in the past 10 days? Please indicate a number from 0 to 100, with 0 meaning "no distention" and 100 meaning "very severe" ☐

3. How dissatisfied are you with your bowel functioning in the past 10 days? Please indicate a number from 0 to 100, with 0 meaning "not dissatisfied" and 100 meaning "very dissatisfied" ☐

4. How much did abdominal pain or discomfort or altered bowel functioning affect or interfere with your life in general in the past 10 days. Please indicate a number from 0 to 100, with 0 meaning "not at all" and 100 meaning "completely" ☐

APPENDIX C: IBS-QOL QUESTIONNAIRE

IBS-QOL

RID: _ _ _ _ _

Today's Date: __ __/__ __/__ __ (MM DD YY)

SID: _ _ _ _ _

*Please think about your life over the **past month (30 days)**, and look at the statements below. Each statement has five possible responses. For each statement, please fill in one oval in each row that best describes your feelings.*

		Not at all	Slightly	Moderately	Quite a bit	A great deal
		1	2	3	4	5
1	I feel helpless because of my bowel problems.	O	O	O	O	O
2	I am embarrassed by the smell caused by my bowel problems.	O	O	O	O	O
3	I am bothered by how much time I spend on the toilet.	O	O	O	O	O
4	I feel vulnerable to other illnesses because of my bowel problems	O	O	O	O	O
5	I feel fat because of my bowel problems.	O	O	O	O	O
6	I feel like I'm losing control of my life because of my bowel problems.	O	O	O	O	O
7	I feel my life is less enjoyable because of my bowel problems.	O	O	O	O	O
8	I feel uncomfortable when I talk about my bowel problems.	O	O	O	O	O
9	I feel depressed about my bowel problems.	O	O	O	O	O
10	I feel isolated from others because of my bowel problems.	O	O	O	O	O
11	I have to watch the amount of food I eat because of my bowel problems.	O	O	O	O	O
12	Because of my bowel problems, sexual activity is difficult for me.	O	O	O	O	O
13	I feel angry that I have bowel problems.	O	O	O	O	O
14	I feel like I irritate others because of my bowel problems.	O	O	O	O	O
15	I worry that my bowel problems will get worse.	O	O	O	O	O
16	I feel irritable because of my bowel problems.	O	O	O	O	O

PLEASE CONTINUE ON THE NEXT PAGE

IBS-QOL

RID: _ _ _ _ _

Today's Date: __ __/__ __/__ __ (mm dd yy)

SID: _ _ _ _ _

		Not at all	Slightly	Moderately	Quite a bit	A great deal
		1	2	3	4	5
17	I worry that people think I exaggerate my bowel problems.	O	O	O	O	O
18	I feel I get less done because of my bowel problems.	O	O	O	O	O
19	I have to avoid stressful situations because of my bowel problems.	O	O	O	O	O
20	My bowel problems reduce my sexual desire.	O	O	O	O	O
21	My bowel problems limit what I can wear.	O	O	O	O	O
22	I have to avoid strenuous activity because of my bowel problems.	O	O	O	O	O
23	I have to watch the kind of food I eat because of my bowel problems.	O	O	O	O	O
24	Because of my bowel problems I have difficulty being around people I do not know well.	O	O	O	O	O
25	I feel sluggish because of my bowel problems.	O	O	O	O	O
26	I feel unclean because of my bowel problems.	O	O	O	O	O
27	Long trips are difficult for me because of my bowel problems.	O	O	O	O	O
28	I feel frustrated that I cannot eat when I want because of my bowel problems.	O	O	O	O	O
29	It is important to be near a toilet because of my bowel problems.	O	O	O	O	O
30	My life revolves around my bowel problems.	O	O	O	O	O
31	I worry about losing control of my bowels.	O	O	O	O	O
32	I fear I won't be able to have a bowel movement.	O	O	O	O	O
33	My bowel problems are affecting my closest relationships.	O	O	O	O	O
34	I feel that no one understands my bowel problems.	O	O	O	O	O

Thank you for completing this survey.

APPENDIX D: PHQ-12 QUESTIONNAIRE

PHYSICAL SYMPTOMS (PHQ-15)

During the past 4 weeks, how much have you been bothered by any of the following problems?

	Not bothered at all (0)	Bothered a little (1)	Bothered a lot (2)
[redacted]			
b. Back pain	☐	☐	☐
c. Pain in your arms, legs, or joints (knees, hips, etc.)	☐	☐	☐
d. Menstrual cramps or other problems with your periods *WOMEN ONLY*	☐	☐	☐
e. Headaches	☐	☐	☐
f. Chest pain	☐	☐	☐
g. Dizziness	☐	☐	☐
h. Fainting spells	☐	☐	☐
i. Feeling your heart pound or race	☐	☐	☐
j. Shortness of breath	☐	☐	☐
k. Pain or problems during sexual intercourse	☐	☐	☐
[redacted]	☐	☐	☐
[redacted]			
n. Feeling tired or having low energy	☐	☐	☐
o. Trouble sleeping	☐	☐	☐

(For office coding: Total Score T_____ = _____ + _____)

Developed by Drs. Robert L. Spitzer, Janet B.W. Williams, Kurt Kroenke and colleagues, with an educational grant from Pfizer Inc. No permission required to reproduce, translate, display or distribute.

APPENDIX E: WPAI:IBS

WORK PRODUCTIVITY AND ACTIVITY IMPAIRMENT QUESTIONNAIRE – IRRITABLE BOWEL SYNDROME WITH DIARRHOEA PREDOMINANT SYMPTOMS [WPAI:IBS-D)]

The following questions ask about the effect of your Irritable Bowel Syndrome (IBS) symptoms, e.g. abdominal discomfort, abdominal pain, bloating and diarrhoea, on your ability to work and perform normal daily activities. *Please fill in the blanks or circle a number, as indicated.*

1) Are you currently in paid employment? _____NO _____YES
   If NO, tick "NO" and skip to question 6.

The next questions refer to the **past seven days**, not including today.

2) During the past seven days, how many hours did you miss from work because of problems associated with your IBS symptoms? *Include hours you missed on sick days, times you went in late, left early, etc., because of IBS symptoms. Do not include time you missed to participate in this study.*
   _____HOURS

3) During the past seven days, how many hours did you miss from work because of any other reason, such as annual leave, holidays, time off to participate in this study?
   _____HOURS

4) During the past seven days, how many hours did you actually work?
   _____HOURS *(If "0", skip to question 6)*

5) During the past seven days, how much did IBS Symptoms affect your productivity while you were working? *Think about days you were limited in the amount or kind of work you could do, days you accomplished less than you would like, or days you could not do your work as carefully as usual. If IBS symptoms affected your work only a little, choose a low number. Choose a high number if IBS symptoms affected your work a great deal.*

IBS symptoms had no effect on my work	0	1	2	3	4	5	6	7	8	9	10	IBS symptoms completely prevented me from working

CIRCLE A NUMBER

6) During the past seven days, how much did IBS symptoms affect your ability to perform your normal daily activities, excluding your job? *By normal activities, we mean the usual activities you perform, such as working around the house, shopping, childcare, exercising, studying, etc. Think about times you were limited in the amount or kind of activities you could perform and times you accomplished less than you would like. If IBS symptoms affected your activities only a little, choose a low number. Choose a high number if IBS symptoms affected your activities a great deal.*

IBS symptoms had no effect on my daily activities	0	1	2	3	4	5	6	7	8	9	10	IBS symptoms completely prevented me from doing my daily activities

CIRCLE A NUMBER

APPENDIX F: FOLLOW-UP CALL INVESTIGATOR QUESTIONNAIRE

RELIEVE IBS-D FOLLOW-UP CALL INVESTIGATOR QUESTIONNAIRE

STUDY PARTICIPANT ID: _____ DATE OF FOLLOW-UP: _____

FOLLOW-UP COMPLETED BY (INITIALS): _____

1. Do you feel the benefit obtained during the treatment period has:
    a) increased further
    b) been maintained
    c) reduced somewhat
    d) greatly reduced

2. Have you used any Enterosgel® since the last study visit?
    a) Yes
    b) No

3. If yes to 2., have you used it:
    a) most days
    b) 2-3 days per week
    c) 1-2 days per week
    d) only occasionally

4. In terms of average daily loperamide use, do you feel that in the last 8 weeks you have used loperamide:

    a) More than before the study
    b) Same as before the study
    c) Less than before the study

ENT04UK Follow-up call Investigator Questionnaire v.1.0 13/12/17

APPENDIX G: SCREENING DIARY

RELIEVE IBS-D SCREENING DIARY

STUDY PARTICIPANT ID: _____ **CENTRE ID:** _____ **DATE OF SCREENING VISIT:** _____

PLEASE COMPLETE AT THE END OF EACH DAY UNTIL YOUR NEXT VISIT:

1. On a scale of 1–7, what was the score of your least formed bowel movement in the last 24 hours?

1 = Separate hard lumps, like nuts (hard to pass)
2 = Sausage-shaped but lumpy
3 = Like a sausage but with cracks on its surface
4 = Like a sausage or snake, smooth and soft
5 = Soft blobs with clear-cut edges (passed easily)
6 = Fluffy pieces with ragged edges, a mushy stool
7 =Watery stool, no solid pieces; entirely liquid

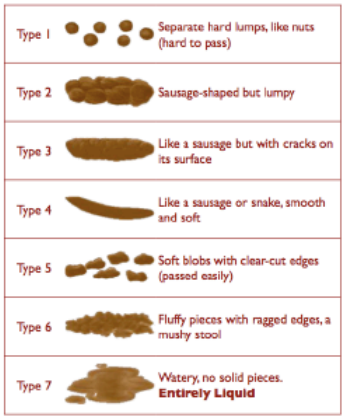
DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

2. With regard to your IBS symptoms of abdominal pain, on a scale of 0-10, what was your worst IBS-related abdominal pain over the last 24 hours?

'Zero' means you have no pain at all; 'Ten' means the worst possible pain you can imagine.

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

Bristol Stool Chart



APPENDIX H: TREATMENT USE INSTRUCTIONS (DOUBLE-BLIND PHASE)

Efficacy, tolerability and safety of Enterosgel® in treatment of IBS-D in adults

Treatment use instructions for research participants (double-blind phase – 8 weeks)

IMPORTANT! PLEASE READ BEFORE STARTING YOUR TREATMENT

Enterosgel® is a safe, drug-free treatment with 30 years of safety history profile without any reports of serious adverse events. There is no risk of overdose. You may contact your research nurse at any time to discuss any questions, but remember that only you know the dose that works best for you.

- **Each patient with IBS is different and only you know what dose works best for your symptoms**
- It is normal that one patient will require a maximum dose while another will feel better with the minimum dose
- Your role in determining your optimal dose is essential. Do not worry about increasing or decreasing your dose to find the dose that works best for you.
- Listen to yourself and your symptoms. Feel confident in adjusting the dose and do not worry about being flexible.
- It is important to continue treatment even if you do not see any improvement after taking the maximum dose. There could be many reasons for not seeing improvement. You might have been randomised to the placebo group, or you might just need more time for the treatment to start working for you. Try to be patient and positive!
- All patients who complete this phase, will receive the active treatment (i.e. Enterosgel®) in the second, open-label phase of this study. Therefore, even if you feel that the blinded treatment does not work for you, do not give up and remember that you will get to try the active treatment soon.
- The recommendations below are only provided as guidance for you to find your individual dose. You may always adjust your dose to suit your stool consistency and bowel movements.
- You can increase treatment to a double dose (two full tubes) up to 3 times a day if your stool is loose, or reduce to a single dose (one full tube) twice a day if your stool is normal
- **It is important to know that your bowel might start changing its habits, including slowing down and not having any stool for 1-2 days.** This is completely normal. If you do not have a bowel movement for 1-2 days, increase your intake of water and continue the treatment after the next bowel movement.

WHAT IS A SINGLE DOSE AND DOUBLE DOSE?

- **SINGLE DOSE** is one full tube
- **DOUBLE DOSE** is two full tubes

HOW TO TAKE THE TREATMENT?

- **Do not eat or take any oral medication within 2 hours from taking the study treatment**
- Shake the tube well before opening
- Open tube just before use
- Mix the contents with approximately 100ml of water (half a glass).
- Make sure to use the entire contents of the tube at one time
- Do not use if package is damaged
- Keep out of the reach of children
- Store at room temperature (between +4 to +30 °C)

HOW MANY TIMES A DAY SHOULD I TAKE THE TREATMENT?

Starting the treatment
Take **A SINGLE DOSE 2 TIMES A DAY** for **5 days**.

If your symptoms improve
Continue on this dosage.

If your symptoms do not improve at all or enough
Take **A SINGLE DOSE 3 TIMES A DAY** for **5 days**.

If your symptoms still do not improve at all or enough
Take **A DOUBLE DOSE 2 TIMES A DAY** for **5 days**.

If your symptoms still do not improve at all or enough
Take **A DOUBLE DOSE 3 TIMES A DAY** for **5 days**.

If your symptoms still do not improve at all
Return to **A DOUBLE DOSE 2 TIMES A DAY** until the rest of the study phase.

NOTE: *If you are satisfied with the improvement on any of the above dosages, stay on that dosage.*

NOTE: *If on any dose you do not have a bowel movement (no stool at all) for 2 days, stop the treatment and increase your intake of water. After the next bowel movement, continue with a single dose once a day. You can then increase step-by-step as described above if your stool is still loose and you are not satisfied with your bowel movements.*

APPENDIX I: TREATMENT USE INSTRUCTIONS (OPEN-LABEL PHASE)

Treatment use instructions for research participants (open-label phase – 8 weeks)

IMPORTANT! PLEASE READ BEFORE STARTING YOUR TREATMENT

Enterosgel® is a safe, drug-free treatment with 30 years of safety history profile without any reports of serious adverse events. There is no risk of overdose. You may contact your research nurse at any time to discuss any questions, but remember that only you know the dose that works best for you.

- **Each patient with IBS is different and only you know what dose works best for your symptoms**
- It is normal that one patient will require a maximum dose while another will feel better with the minimum dose
- Your role in determining your optimal dose is essential. Do not worry about increasing or decreasing your dose to find the dose that works best for you.
- Listen to yourself and your symptoms. Feel confident in adjusting the dose and do not worry about being flexible.
- It is important to continue treatment even if you do not see any improvement after taking the maximum dose. You might just need more time for the treatment to start working for you.
- The recommendations below are only provided as guidance for you to find your individual dose. You may always adjust your dose to suit your stool consistency and bowel movements.
- You can increase treatment to a double dose (two sachets) up to 3 times a day if your stool is loose, or reduce to a single dose (one sachet) twice a day if your stool is normal
- **It is important to know that your bowel might start changing its habits, including slowing down and not having any stool for 1-2 days**. This is completely normal. If you do not have a bowel movement for 1-2 days, increase your intake of water and continue the treatment after the next bowel movement.

WHAT IS A SINGLE DOSE AND DOUBLE DOSE?

- **SINGLE DOSE** is one sachet
- **DOUBLE DOSE** is two sachets

HOW TO TAKE THE TREATMENT?

- **Do not eat or take any oral medication within 2 hours from taking Enterosgel®**
- Each sachet is for single-use only. Make sure to use the entire contents of the sachet at one time.
- Each sachet is disposable and should be opened immediately before use
- Mix the contents of the sachet with approximately 200ml of room temperature water (a full glass)
- Do not use if package is damaged
- Keep out of the reach of children
- Store at room temperature (between +4 to +30 °C)

HOW MANY TIMES A DAY SHOULD I TAKE THE TREATMENT?

Starting the treatment
Take **A SINGLE DOSE 2 TIMES A DAY** for **5 days**.

If your symptoms improve
Continue on this dosage.

If your symptoms do not improve at all or enough
Take **A SINGLE DOSE 3 TIMES A DAY** for **5 days**.

If your symptoms still do not improve at all or enough
Take **A DOUBLE DOSE 2 TIMES A DAY** for **5 days**.

If your symptoms still do not improve at all or enough
Take **A DOUBLE DOSE 3 TIMES A DAY** for **5 days**.

If your symptoms still do not improve at all
Return to **A DOUBLE DOSE 2 TIMES A DAY** until the rest of the study phase.

NOTE: *If you are satisfied with the improvement on any of the above dosages, stay on that dosage.*

NOTE: *If on any dose you do not have a bowel movement (no stool at all) for 2 days, stop the treatment and increase your intake of water. After the next bowel movement, continue with a single dose once a day. You can then increase step-by-step as described above if your stool is still loose and you are not satisfied with your bowel movements.*