Statistical Analysis Plan (SAP)

TEMPO Trial

A Randomised, Double-Blind, Placebo-Controlled Two-Period Cross-Over Study of the Effect of Chronic Feeding of Methylcellulose and Inulin on Inulin Fermentation in People with IBS-C

SAP Version: 1.0
Date: 3 November 2026
Protocol Version: 5.0 (8 December 2025)
Trial Acronym: TEMPO
Chief Investigator: Prof Robin Spiller
Statistician: Dr Colin Crooks

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1. Introduction

TEMPO is a single-centre, randomised, double-blind, placebo-controlled, 2×2 crossover mechanistic study investigating whether chronic consumption of methylcellulose (MC) alters fermentation of inulin in patients with IBS-C when compared with maltodextrin placebo. Primary outcomes are based on MRI assessment of colonic gas production following a standardised 15 g inulin challenge.

The SAP provides detailed procedures for efficacy, mechanistic and safety analyses and supersedes the abbreviated statistical section included within the protocol.

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2. Study Objectives

Primary Objective

To test whether 3 weeks of methylcellulose plus inulin reduces colonic gas production (AUC T180-360) compared with placebo plus inulin in patients with IBS-C.

Secondary Objectives

To assess the effects of chronic methylcellulose versus placebo on:

- Colonic (gel; AUC T180-360) and small bowel water content (T180)
- Colonic volumes (AUC T180-360)
- Ascending colon T1 as a measure of chyme wateriness (AUC T180-360)
- Breath hydrogen production (AUCs 06h, 6-12h and 12-24h)
- Gut microbiota composition

- Orocaecal transit time
- Whole gut transit time
- In vitro fermentation outcomes
- Stool SCFA production
- Bowel habit
- IBS symptoms
- Intervention tolerability.

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3. Study Design

Design

- Randomised, Double-blind, Placebo-controlled, Two-period crossover study
- Single-centre
- Mechanistic physiological study.

Treatment Sequences

Sequence 1:

- Period 1: Methylcellulose
- Washout
- Period 2: Placebo

Sequence 2:

- Period 1: Placebo
- Washout
- Period 2: Methylcellulose

Participants receive both interventions separated by a 4-8 week washout period.

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4. Sample Size

Target completion:

22 participants

The sample size is pragmatic and based on previous MRI studies demonstrating detectable differences in colonic gas responses after acute fibre administration. No

formal power calculation was possible because of limited prior longitudinal data in IBS-C patients.

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5. Analysis Populations

Full Analysis Set (FAS)

All randomised participants who:

- Received study intervention
- Have at least three post-baseline primary endpoint measurements available.

Per-Protocol Population (PP)

Participants from the FAS who:

- Complete both intervention periods
- Have no major protocol deviations
- Demonstrate acceptable compliance with intervention diaries.

This will be the primary analysis population.

Safety Population

All randomised participants receiving any quantity of study intervention.

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6. General Statistical Principles

Statistical Software

Primary analysis will be undertaken using:

- R (Version ≥4.3)
- Relevant validated packages

as specified by the trial statistician.

Significance Level

Two-sided alpha: $\alpha = 0.05$

Confidence Intervals: 95% confidence intervals will be reported.

Multiplicity

No formal correction for multiplicity will be applied because all secondary analyses are exploratory and mechanistic.

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7. Baseline Characteristics

The following will be summarised by randomised sequence:

- Age
- Sex
- BMI
- IBS-SSS
- HAD-S
- Habitual FODMAP intake
- Baseline MRI variables
- Baseline breath testing variables.

Continuous variables:

- Mean (SD)
- Median (IQR)
- Range

Categorical variables:

- Frequency (%)

No significance tests of baseline characteristics will be performed.

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8. Derivation of Variables

MRI Area-Under-the-Curve Measures

AUC will be calculated using the trapezoidal rule.

Outcomes include:

- Colon gas volume from time 180-360 post ingestion
- Colon volume from time 180-360 post ingestion
- Colon water content from time 180-360 post ingestion

- T1 ascending colon from time 180-360 post ingestion

Breath Hydrogen

Measurements will generate:

AUC0-24h using all available timepoints over 24 hours.

Units: ppm·hour

Orocaecal Transit Time (OCTT)

Defined as: Time from challenge ingestion to sustained rise in breath hydrogen ≥10 ppm above baseline.

Reported in hours.

Whole Gut Transit Time

Defined as: Time between ingestion of blue muffins and first observed stool colour change.

Reported in hours.

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9. Primary Endpoint Analysis

Endpoint

MRI-measured:

Colonic Gas AUC 3-6h at the end of each 3-week intervention period.

Estimand

Difference in post-treatment colonic gas AUC between:

- Methylcellulose
- Placebo

in participants with IBS-C.

Primary Statistical Model

Two-way repeated measures ANOVA

Where:

- Treatment = MC or Placebo
- Study Visit = within-subject factor (repeated measure)

Participant = random effect

Outputs:

- 95% CI
- p-value

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10. Secondary Endpoint Analyses

The same repeated measures-model framework will be used whenever appropriate.

MRI Outcomes

Colonic Gas Adaptation

Comparison:

- Baseline versus Week 3

Within treatment periods.

Small Bowel Water Content

AUC 3-6h.

Colon Water Content

AUC 3-6h.

Ascending Colon T1

AUC 3-6h.

Colon Volumes

AUC 3-6h.

Breath Testing

Hydrogen Production

AUC0-24h.

Continuous outcomes analysed using mixed models.

Transit

OCTT

Primary analysis:

- Repeated measures model

Sensitivity:

- Wilcoxon signed-rank test if non-normal.

Whole Gut Transit

Mixed-effects model.

Stool Fermentation Outcomes

Outcomes:

- Total gas production
- SCFA production

Analysis:

- Paired crossover mixed models
- Log transformation if required.

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11. Microbiome Analysis

Alpha Diversity:

Within arm change from baseline and between arm differences in Shannon index tested by repeated measures linear mixed effects model

Beta Diversity: PERMANOVA test on Bray-Curtis dissimilarities by treatment arm, time and interaction.

Univariate: Species level differences in relative abundance between arms at each time point tested by LMM

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12. Clinical Outcomes

Bristol Stool Form Score (BSFS)

The protocol identifies BSFS as the primary clinical endpoint.

Analysis

Weekly mean BSFS during:

- MC period
- Placebo period

Mixed-effects ordinal regression.

Secondary presentation:

- Mean difference with 95% CI.

Stool Frequency

Outcomes:

- Daily bowel movements
- Complete spontaneous bowel movements

Analysed using:

- Poisson mixed models

or

- Negative binomial models if overdispersion occurs.

Symptoms

Daily symptom scores:

- Bloating
- Wind/flatulence
- Abdominal pain

and IBS-SSS scores.

Analysed using mixed-effects models with repeated measurements.

Outputs:

- Mean difference
- 95% CI
- p-value

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13. Missing Data

The protocol specifies: **Single Missing Timepoint**

Impute as:

$$\frac{Previous + Next}{2}$$

Two Consecutive Missing Timepoints

The affected study-day dataset for that endpoint will be excluded.

Missing Baseline Values

Imputed using available baseline values from alternative study days.

Sensitivity Analysis

Primary endpoint analyses will additionally be repeated:

- Without imputation
- Complete-case only

to assess robustness.

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14. Protocol Deviations

Major deviations include:

- Failure to complete intervention period
- Non-adherence to MRI protocol
- Unblinding
- Major dietary violations
- Use of prohibited antibiotics/probiotics
- Missing primary endpoint data

All deviations will be reviewed before database lock.

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15. Safety Analysis

No formal safety endpoints are defined in the protocol.

Safety summaries will include:

- Number of AEs
- Number of SAEs
- Relationship to intervention
- Withdrawals due to adverse events

Presented descriptively by treatment.

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16. Planned Tables, Listings and Figures

Tables

1. Participant disposition
2. Baseline characteristics
3. Compliance
4. Primary endpoint analysis
5. MRI secondary outcomes
6. Breath test outcomes
7. Transit outcomes
8. Stool fermentation results
9. Microbiome summary analyses
10. Stool and symptom outcomes
11. Adverse events

Figures

1. CONSORT crossover flow diagram
2. Individual participant treatment sequences
3. Mean colonic gas curves

4. Small bowel water curves
5. Colon volume curves
6. Breath hydrogen profiles
7. Breath methane profiles
8. Stool frequency plots
9. Forest plot of treatment effects
10. Microbiome diversity plots

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17. Reporting Conventions

All estimates will be reported as:

- Mean difference (or ratio where appropriate)
- 95% confidence interval
- Exact p-value

Interpretation will focus on effect size estimation and biological plausibility rather than statistical significance alone, reflecting the mechanistic and pilot nature of TEMPO.

This SAP expands substantially on the protocol's statistical section and would be suitable as a standalone SAP for ethics submission, publication planning, and statistical sign-off.