ThermoFisher SCIENTIFIC

Sample prep

# Targeting gastrointestinal microbes from fecal and rectal swab specimens without bead beating

## Introduction

Bead beating is a mechanical lysis method commonly used in sample preparation of microbiological targets in stool derivatives to disrupt cells for the extraction of biomolecules like DNA, RNA, proteins, and metabolites.

**While mechanical lysis by bead beating offers several advantages, it also comes with known hurdles within the sample preparation workflow:**

- **Inconsistent results:** Across different sample specimens, mechanical disruption can be variable, leading to inconsistent results. Variability can arise due to differences in sample properties (e.g., cell wall thickness, sample thickness, solid debris) and bead-beating conditions (e.g., duration, speed, bead-beating vessels such as tubes or plates).
- **Cross-contamination risk:** Mechanical lysis requires vigorous shaking methods with a bead beater. Contamination risk during the process of mechanical lysis is higher because it can cause samples to migrate from well to well, especially in a bead-beating plate. This can compromise the integrity of experiments and produce unreliable results.
- **Equipment wear and tear:** The mechanical forces generated during bead beating can cause wear and tear on equipment, leading to increased maintenance costs and potential equipment failure over time.
- **High energy consumption:** Bead beating can be energy-intensive, especially when large numbers of samples need to be processed. This can increase operational costs and environmental impact.
- **Time-consuming and laborious:** Labeling, decapping, and recapping individual tubes come with long processing times and laborious effort. For medium- to high-throughput laboratories, these processes could be a significant risk for efficiency in microbial research.

To address the hurdles of up-front mechanical lysis by bead beating, we evaluated alternative lysis methods and workflows for fecal swabs. Successful implementation of an alternative approach to bead beating with fecal swabs could provide clinical researchers in molecular and microbiology applications with increased consistency, reduced risk of cross-contamination, less equipment and energy usage, and less time spent on sample preparation.

**Advantages of fecal swab sample preparation without mechanical lysis by bead beating:**

- **Gentle:** Removing bead beating minimizes mechanical shearing and harsh treatment of samples, helping to preserve the integrity of nucleic acids and reducing the risk of degradation.
- **Streamlined workflow:** By eliminating bead beating, concerns associated with heat generation, sample loss, cross-contamination, and a high labor requirement are avoided. Less equipment is necessary, streamlining general workflows.
- **No centrifugation necessary:** Unlike stool samples, fecal swab samples have minimal solid debris, and the commonly required centrifugation step can be bypassed.
- **Sample type versatility:** The method can provide more consistent results across a variety of sample types because of the controlled enzymatic digestion and gentle extraction.
- **Eco-friendly:** The method may require less energy and generate less waste (plastic and hazardous chemicals) than the method with bead beating.

Here we report an evaluation of a diverse range of fecal and rectal swab samples. The samples chosen encompassed various characteristics, such as fresh nonfrozen samples, samples with different levels of inhibition, frozen samples, and samples exhibiting a wide range of color and consistency. Our primary objective was to evaluate the effectiveness of workflows with bead beating compared to those without bead beating or mechanical lysis preprocessing. For a more complete study, we evaluated contrived and naturally occuring samples with various types of microbes, including gram-negative and gram-positive bacteria, as well as a virus, fungus, and parasite.

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## Experimental methods

### Contrived and clinical fecal swabs

Fecal swabs were prepared from raw stool by suspending them in 2 mL of Cary Blair medium [1]. This process was carried out across four healthy donor sample specimens. The detailed breakdown of the number of healthy donors assessed for both standard and difficult swab types, along with the pathogens used to contrive the sample, can be found in Table 1. Standard fecal swabs were created by combining three different diarrheal stool specimens, while difficult fecal swabs were derived from a specimen containing a substantial amount of solid debris and exhibiting a high inhibition level.

The amount of pathogen input was determined based on the sensitivity of the downstream qPCR assay utilized. To further enhance the study, we evaluated over 30 fecal swab samples naturally containing pathogens.

**Table 1. Experimental details of contrived fecal swab specimens from raw stool.**

| | Extraction products | Workflow methods (Pub. No. MAN0029683) | Sample volumes evaluated |
|---|---|---|---|
| **Workflows evaluated** | MagMAX Prime Viral/Pathogen NA Isolation Kit, KingFisher Apex Purification System | Advanced stool workflow (bead beating), basic workflow (no bead beating), advanced lysis workflow (enzymatic digestion)* | 200 µL (advanced stool workflow); 200, 300, 400 µL (basic and advanced lysis workflows) |
| | **Fecal swab types** | **Number of healthy donors** | **Contrived pathogens** |
| **Samples contrived** | Standard, difficult | 3 (standard), 1 (difficult) | *Campylobacter coli, Shigella sonnei, Salmonella enterica* serotype typhi, *Listeria monocytogenes, Candida albicans*, norovirus G1 |

* The basic and advanced workflows of the MagMAX Prime Viral/Pathogen kit included 200 µL elution volumes, matching the advanced stool workflow of the same kit.

### Workflows evaluated

Table 2 and Figure 1 summarize the experimental parameters employed in the evaluation of workflows utilizing the Applied Biosystems™ MagMAX™ Prime Viral/Pathogen NA Isolation Kit on the Thermo Scientific™ KingFisher™ Apex Purification System. Detailed descriptions of all three evaluated workflows of the MagMAX Prime Viral/Pathogen kit can be found in the user guide (Pub. No. MAN0029683).

All automation protocols used for the advanced stool, basic, and advanced lysis workflows of the MagMAX Prime Viral/Pathogen kit can be found at thermofisher.com/order/catalog/product/A58145.

**Table 2. MagMAX Prime Viral/Pathogen NA Isolation Kit workflows evaluated using contrived fecal swab specimens.**

| Workflow | Experiment | Preprocessing | Type of preprocessing |
|---|---|---|---|
| **Advanced stool** | Control | Yes | Bead beating |
| **Basic** | No preprocessing | No | NA |
| **Advanced lysis** | Enzyme digestion | Yes | Onboard heating |

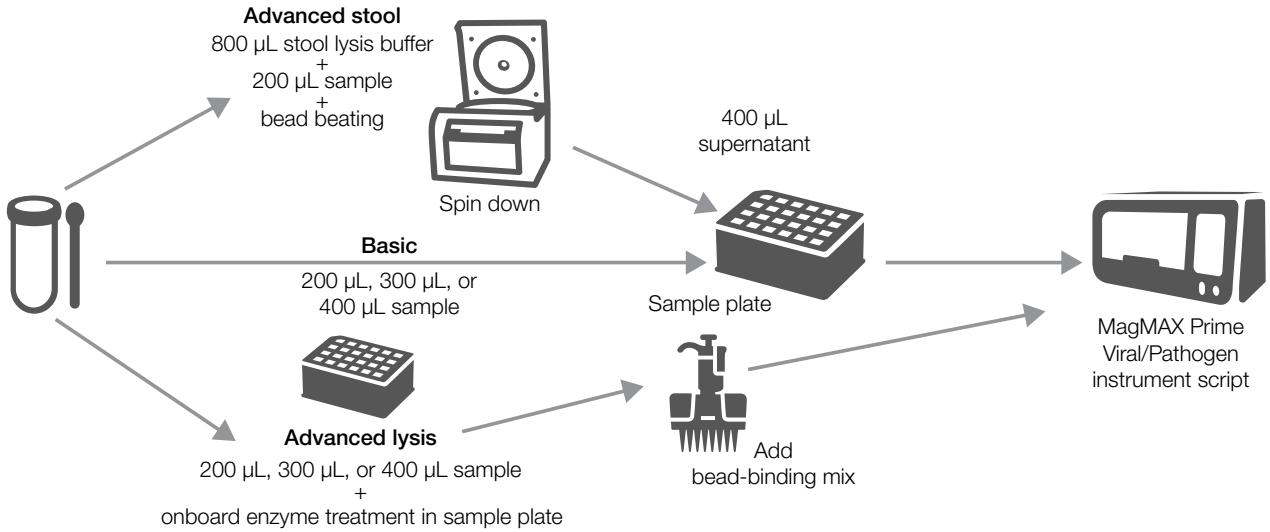


**Figure 1. Visual representation of three different workflows evaluated using fecal swab specimens.** Bead beating was performed with Applied Biosystems™ MagMAX™ Prime Bead Beating Tubes and MagMAX™ Prime Stool Lysis Buffer. Enzyme treatment was performed with Applied Biosystems™ MagMAX™ Prime G+ Bacterial and Fungal Lysis Buffer.

The advanced stool workflow incorporates the up-front mechanical lysis procedure using MagMAX Prime Bead Beating Tubes and MagMAX Prime Stool Lysis Buffer. The advanced stool workflow was executed on the KingFisher Apex instrument using the Prime_GI_APX.kfx protocol. The basic workflow was utilized to compare an omission of preprocessing. Here we utilized a modified elution volume of 200 µL on the KingFisher Apex instrument using the Prime_APX.kfx protocol.

To evaluate enzymatic lysis, we utilized the advanced lysis workflow with the MagMAX Prime G+ Bacterial and Fungal Lysis Buffer with a modified elution volume of 200 µL. This workflow was executed on the KingFisher Apex platform using the Prime_GPB_Fungi_APX.kfx protocol.

To assess sample inhibition levels associated with no mechanical lysis, sample input volumes of 200 µL, 300 µL, and 400 µL were employed during the modified extraction process.

### Molecular analysis

Functional analysis of the nucleic acids obtained from fecal swab extractions using the workflows previously described was performed using the Applied Biosystems™ TrueMark™ Enteric Bacterial Select Panel I. This panel is designed to detect *Campylobacter, Shigella,* and *Salmonella* species. For samples containing *Candida albicans*, *Listeria monocytogenes*, and norovirus G1, Applied Biosystems™ TaqMan™ Assays were used. All assays were run on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System, 0.2 mL, following the appropriate workflow described in the user guide (Pub. No. MAN0029144).

## Results and discussion

### qPCR analysis of contrived samples

Figures 2A and 2B illustrate the $C_t$ values obtained from two distinct sample sets, namely difficult and standard samples, which were contrived with *Campylobacter, Salmonella,* and *Shigella*. The findings indicate that utilizing the basic workflow of the MagMAX Prime Viral/Pathogen kit with a sample input of 300 µL, without any preprocessing, yields results comparable to those from the advanced stool workflow incorporating bead beating (Figures 2A and 2B). Notably, across all three targets, the amplification of the 200 µL sample input typically has later $C_t$ values than with higher sample inputs, indicating that lower specimen inputs yield later amplification of the target specified. Additionally, at a sample input of 400 µL, there is an increased risk of inhibition, particularly evident in the difficult fecal swab sample set for samples without preprocessing (Figure 2A). The inhibitory effect of 400 µL of input from supernatant from difficult fecal swab samples is due to general sample type and donor-to-donor variation. The same impact is not observed with standard fecal swab samples (Figure 2B) because there is

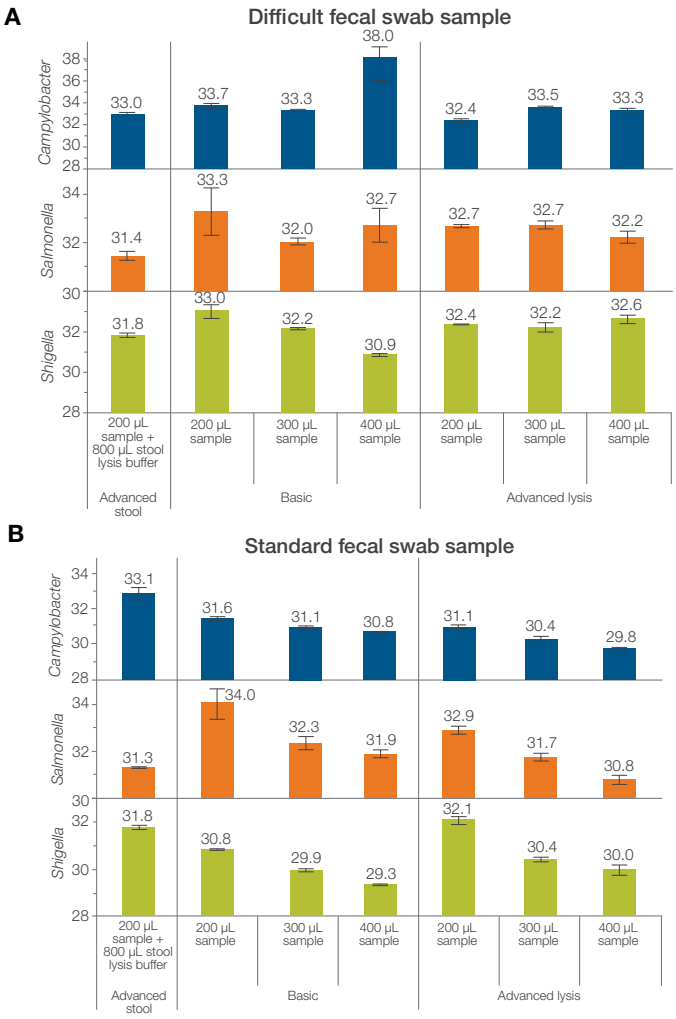


**Figure 2. qPCR results for *Campylobacter, Salmonella,* and *Shigella*.** $C_t$ values are on the y-axis and evaluated workflows are on the x-axis. **(A)** Results from a difficult sample type (qPCR inhibitory). **(B)** Results from standard sample pools (less qPCR inhibition expected).

less risk of debris, discoloration, and other factors that could affect downstream applications. Based on these observations, a sample input of 300 µL appears to be the most optimal choice. Furthermore, it is evident that the performance of the basic workflow without preprocessing is comparable to the mechanical lysis and enzymatic preprocessing methods.

Figures 3A and 3B display the more difficult-to-lyse targets, *C. albicans*, *Listeria*, and norovirus G1. Detection of gram-positive *Listeria* and fungal *C. albicans* is improved in workflows with preprocessing compared to the basic workflow without preprocessing; this finding holds particularly true for *C. albicans.* Interestingly, for norovirus in standard samples, the results clearly indicate that omitting bead beating yields the best outcomes. Consistent with the results presented in Figure 2, the utilization of 300 µL of sample input typically delivers the most favorable results for both standard and challenging fecal swab samples.

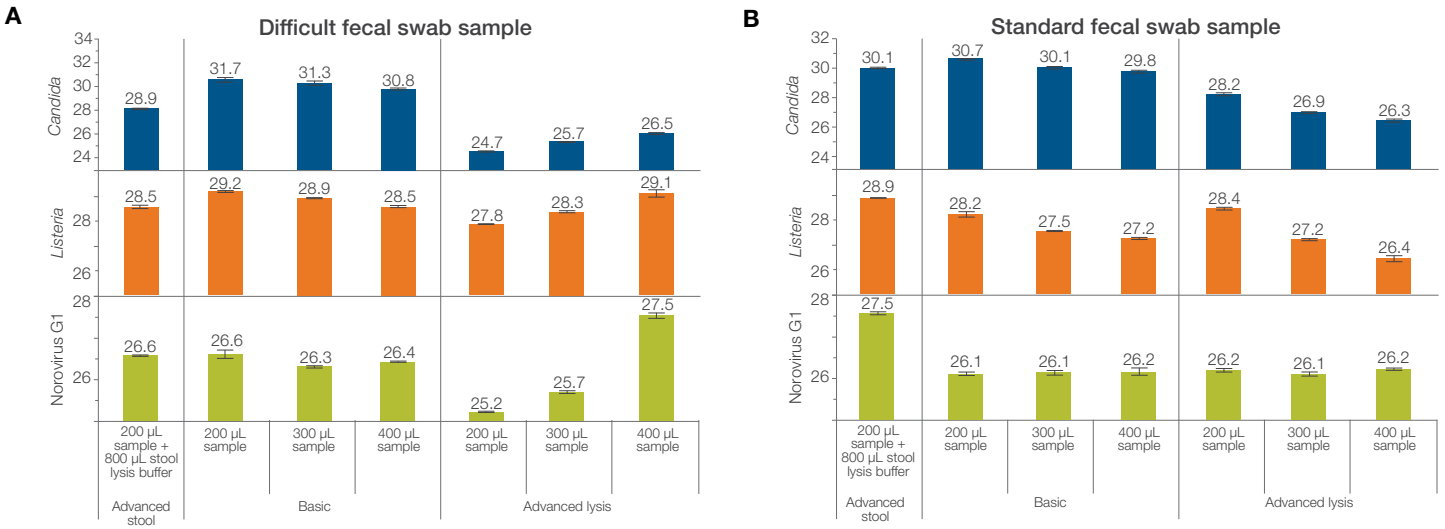


**Figure 3. qPCR results for *C. albicans, L. monocytogenes***, and norovirus G1. $C_t$ values are on the y-axis and evaluated workflows are on the x-axis. **(A)** Results from a difficult sample type (qPCR inhibitory). **(B)** Results from standard sample pools (less qPCR inhibition expected).

## qPCR analysis of clinical samples

Evaluation of five samples with naturally occurring (non-contrived) *Salmonella* and *Cryptosporidium* (a parasite) targets confirmed that the basic workflow of the MagMAX Prime Viral/Pathogen kit without bead beating and using 300 µL of sample input provides better results than the advanced stool workflow with bead beating (Figure 4).

## Bead-beating trade-off

Compared to fecal swab samples, stool samples typically contain more solid debris, making bead beating followed by centrifugation crucial for effective processing. Bead beating involves mixing the sample with stool lysis buffer to liquefy the sample and aid in the subsequent lysis step. As depicted in Table 3, the mixing of the stool sample with lysis buffer results in dilution of the pathogen/microbiome population within the sample. In contrast, fecal swab specimens collected in transport medium or stabilization solution are relatively cleaner than stool samples, rendering the mixing with stool lysis buffer and bead beating unnecessary.

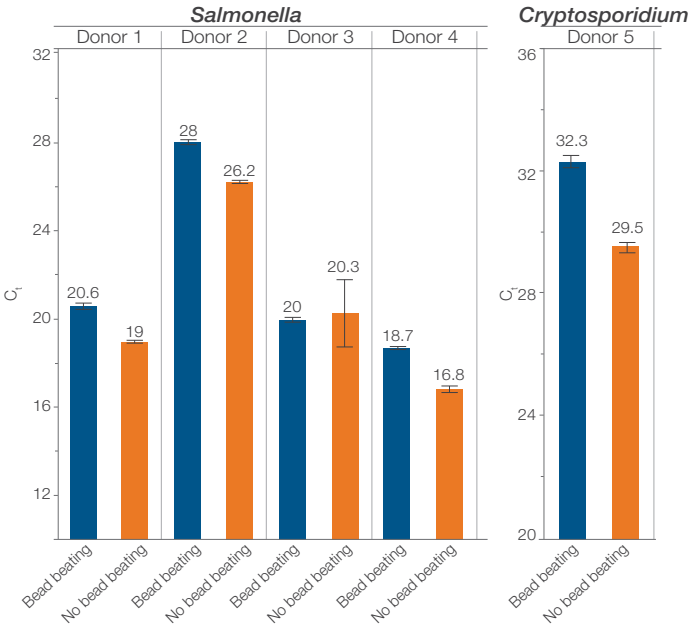


**Figure 4. qPCR results for clinical samples containing *Salmonella* and *Cryptosporidium*.** $C_t$ values are shown for nucleic acid isolated using the advanced stool workflow of the MagMAX Prime Viral/Pathogen kit with up-front mechanical lysis by bead beating, and the basic workflow without bead beading (no preprocessing). Error bars indicate standard deviation.

**Table 3. Hypothetical example showing dilution of the pathogen population in workflows with and without bead beating.**

| Hypothetical pathogen concentration: 1 copy/µL | | | | | |
|---|---|---|---|---|---|
| | **Fecal swab sample** | **Stool lysis buffer for bead beating** | **Sample dilution** | **Volume per extraction** | **Copies into extraction** |
| **No bead beating** | Up to 400 µL | 0 µL | NA (1 copy/µL) | 400 µL | 400 |
| | | | | 300 µL | 300 |
| **Bead beating** | 200 µL | 800 µL | 5x dilution (0.2 copies/µL) | 400 µL | 80 |

5x more copies in the workflow with no bead beating (~2.32 $C_t$ gain for gram-negative bacteria and viruses)

The dilution resulting from mixing with stool lysis buffer leads to delayed $C_t$ values, particularly for gram-negative bacteria and viral pathogens. However, bead beating is advantageous primarily for the detection of gram-positive bacteria and fungi. Figures 2 and 3 demonstrate earlier $C_t$ values for certain gram-negative bacteria and viral pathogens in fecal swab samples without any preprocessing with the modified basic workflow of the MagMAX Prime Viral/Pathogen kit, as opposed to the advanced workflow.

Overall, understanding the differences between stool samples and fecal swab samples highlights the importance of bead beating for effective processing of stool samples, while emphasizing the potential impact on $C_t$ values for different types of pathogens.

## Conclusions

In this study, we have demonstrated efficient extraction of microbial nucleic acid from fecal and rectal swab samples without the need for mechanical lysis by bead beating. While mechanical lysis is essential for stool samples, it is not required for fecal swab sample types. Interestingly, in certain cases such as *Campylobacter coli, Shigella sonnei*, and norovirus G1, performance could improve by omitting the bead-beating procedure. It is worth noting that *C. albicans*, a fungal pathogen, was the only pathogen type that required enzyme treatment or preprocessing within this study. Research on fungal pathogens in fecal samples is relatively uncommon because laboratories primarily working with fecal or rectal swab samples typically focus on bacteria, viruses, and parasitic pathogens.

We have conducted tests on the workflow without bead beating for downstream Applied Biosystems™ OpenArray™ plate applications and observed results comparable to those of the bead-beating workflow. However, to enhance the outcomes, particularly when dealing with microbes that have dsRNA genomes, we have discovered that incubating the eluate at 95°C for 5 minutes and subsequently cooling it down to 4°C before using it for qPCR or OpenArray plate applications yields significantly improved results.

We recommend utilizing 300 µL of fecal or rectal swab sample for extraction using the basic workflow of the MagMAX Prime Viral/Pathogen kit, with a 200 µL elution volume and no preprocessing steps. For high-throughput laboratories processing a large number of samples per day, eliminating the bead-beating process not only saves time but also reduces costs. It eliminates the need to prepare bead-beating tubes, avoids the requirement for centrifugation after bead beating, and eliminates the cost associated with bead-beating tubes or plates.

All workflows for the MagMAX Prime Viral/Pathogen kit, including basic, advanced lysis, and advanced stool, can be performed on KingFisher instruments with automation enabled for convenience and ease of use.

## Authors

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## Ordering information

| Product | Cat. No. |
|---|---|
| MagMAX Prime Viral/Pathogen NA Isolation Kit | A58145 |
| MagMAX Prime Stool Lysis Buffer | A58154 |
| MagMAX Prime Bead Beating Tubes | A58155 |
| MagMAX Prime G+ Bacterial and Fungal Lysis Buffer | A58153 |

### References

1. Copan Diagnostics. Fecal swab procedure. **https://www.copanusa.com/fecalswab-frequently-asked-questions/#How-is-FecalSwab%C2%AE-Used**
2. Puritan Medical Products. Fecal swabs: what to look for in a fecal swab test kit. **https://blog.puritanmedproducts.com/fecal-swab-test-kit**

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