

Keywords: Bacterial detection, *E. coli* O157, functionalised μ Beads, Colligo Mag μ Beads

Colligo Mag μ Beads: functionalised magnetic μ Beads for detection and concentration of *E. coli* O157

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Abstract

Rapid and reliable detection of pathogenic bacteria in food, water, and environmental samples remains a critical challenge in public health and food safety, as conventional culture- and PCR-based methods are time-consuming and require specialised equipment. FluIDect® Colligo Mag μ Beads — anti-*E. coli* O157 functionalised magnetic microBeads (μ Beads) — offer a fast, selective alternative, using immunomagnetic separation (IMS) to concentrate *E. coli* O157 directly from unenriched samples. Antibody-functionalised polystyrene μ Beads bind *E. coli* O157 strains and the resulting bead–bacteria complexes are isolated with a simple magnetic platform, enabling detection from as low as 10 CFU/mL after only 60 minutes of incubation, with capture rates of up to 88%.

Once captured, bacteria bound to the μ Beads can be read out by multiple detection modalities, including flow cytometry with nucleic acid staining, allowing flexible integration into existing laboratory workflows. The platform demonstrated high, specific capture of *E. coli* O157 with very low cross-reactivity against *Salmonella enterica* serovar Typhimurium, confirming target selectivity. Robust and reproducible capture performance was achieved across a range of sample matrices, including phosphate-buffered saline (PBS), water, artificial urine, mud-spiked water and 1% milk. Furthermore, the functionalised μ Beads retained full activity for at least eight months of storage. This was confirmed by a monthly flow cytometric assessment of both the presence of the antibody on the bead surface (using a fluorescently labelled secondary antibody) and the capture functionality (using SYTO-stained *E. coli*). This underscores their suitability for routine, real-world diagnostic and monitoring applications.

Contamination of food and water supplies with pathogenic *Escherichia (E.) coli* O157:H7 represents a serious public health threat, associated with haemorrhagic colitis and haemolytic uraemic syndrome. Rapid and accurate detection of this pathogen is essential for timely intervention, yet traditional microbiological methods — including culture-based assays and PCR — typically require 24–72 hours to yield actionable results and demand specialised laboratory infrastructure. Immunological approaches such as ELISA offer greater speed but are often constrained by multi-step protocols, limited sensitivity in complex or turbid matrices, and reduced adaptability across sample types.

µBead-based capture platforms offer a compelling alternative, combining the high surface-to-volume ratio of functionalised particles with the specificity of antibody or protein-ligand interactions to concentrate and present target bacteria for downstream detection. Once loaded, the µBeads are compatible with multiple readout modalities, including flow cytometry with nucleic acid staining or other µBead-compatible detection assays, allowing seamless integration into diverse laboratory workflows.

In this application note, we evaluate the performance of the functionalised Colligo Mag µBeads for the specific capture of *E. coli* O157 across a range of relevant sample matrices, including PBS, water, artificial urine, mud-spiked water, and 1% milk. Capture specificity is confirmed by the high selectivity of the µBeads for *E. coli* O157 over the closely related *Salmonella enterica* serovar Typhimurium.

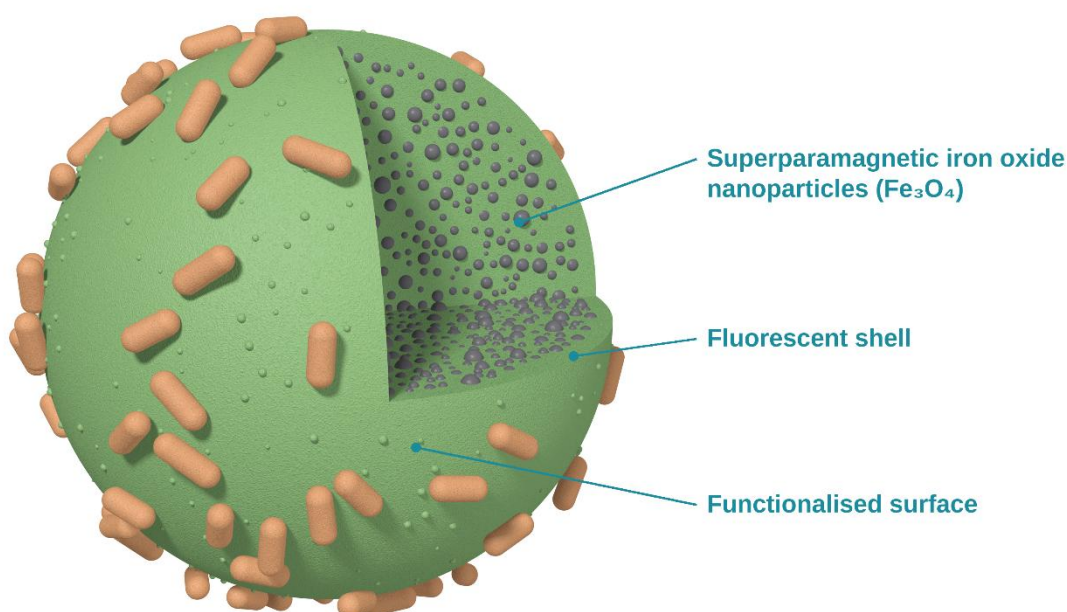


Figure 1. Schematic of a fluorescent, superparamagnetic FluIDect Colligo Mag µBead bearing captured *E. coli* bacteria on its surface. The cut-away section shows the polymer shell containing an embedded fluorescent dye and dispersed superparamagnetic iron oxide (Fe_3O_4) nanoparticles. These nanoparticles enable the bead to be separated using magnetic forces. The functionalised surface binds *E. coli* cells (shown in orange), enabling the bacteria to be selectively captured and concentrated from the sample. Schematic, not to scale.

Experimental Design

Bacterial Capture & Detection

Colligo Mag µBeads were functionalised with antibodies specific for *E. coli* O157 using established in-house protocols. The functionalised µBeads were then incubated with suspensions of *E. coli* O157 or *Salmonella enterica* serovar Typhimurium prepared in the respective test matrix. Captured bacteria were recovered and plated on tryptic soy agar (TSA), and colonies were counted to determine CFU/mL.

Design of the Experiment

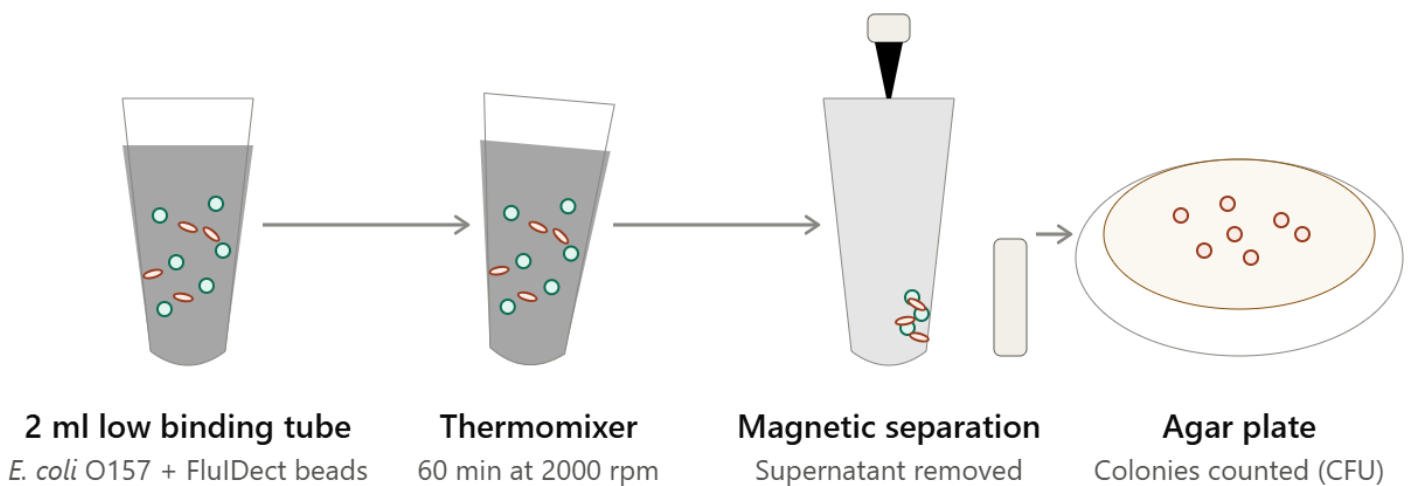


Figure 2: Workflow for the magnetic capture of *E. coli* O157 using FlulDect Colligo Mag μ Beads. Combine *E. coli* O157 and Colligo Mag μ Beads in a 2 ml low-binding tube. Incubate in a thermomixer for 60 minutes at 2000 rpm to allow the bacteria to bind to the bead surface. The Colligo Mag μ Bead-*E. coli* complexes were then pulled against the tube wall using a permanent magnet and the supernatant was removed. The captured bacteria were then plated on agar, and the number of colony-forming units (CFUs) was used to quantify the capture efficiency.

Assessment Conditions

- **Specificity:** anti-*E. coli* O157 μ Beads challenged with *E. coli* O157 and *S. enterica* Typhimurium
- **Matrix robustness:** *E. coli* O157 (100 CFU/mL) spiked into PBS, deionised water, artificial urine, mud-spiked water and 1% milk
- **Sensitivity:** *E. coli* O157 at 10^1 to 10^4 CFU/mL
- **pH tolerance:** PBS at pH 3, 5, 7.4 and 8.5
- **Protocol optimisation:** sample volumes of 300 μ L to 1 mL and incubation times of 15 to 60 minutes

Results

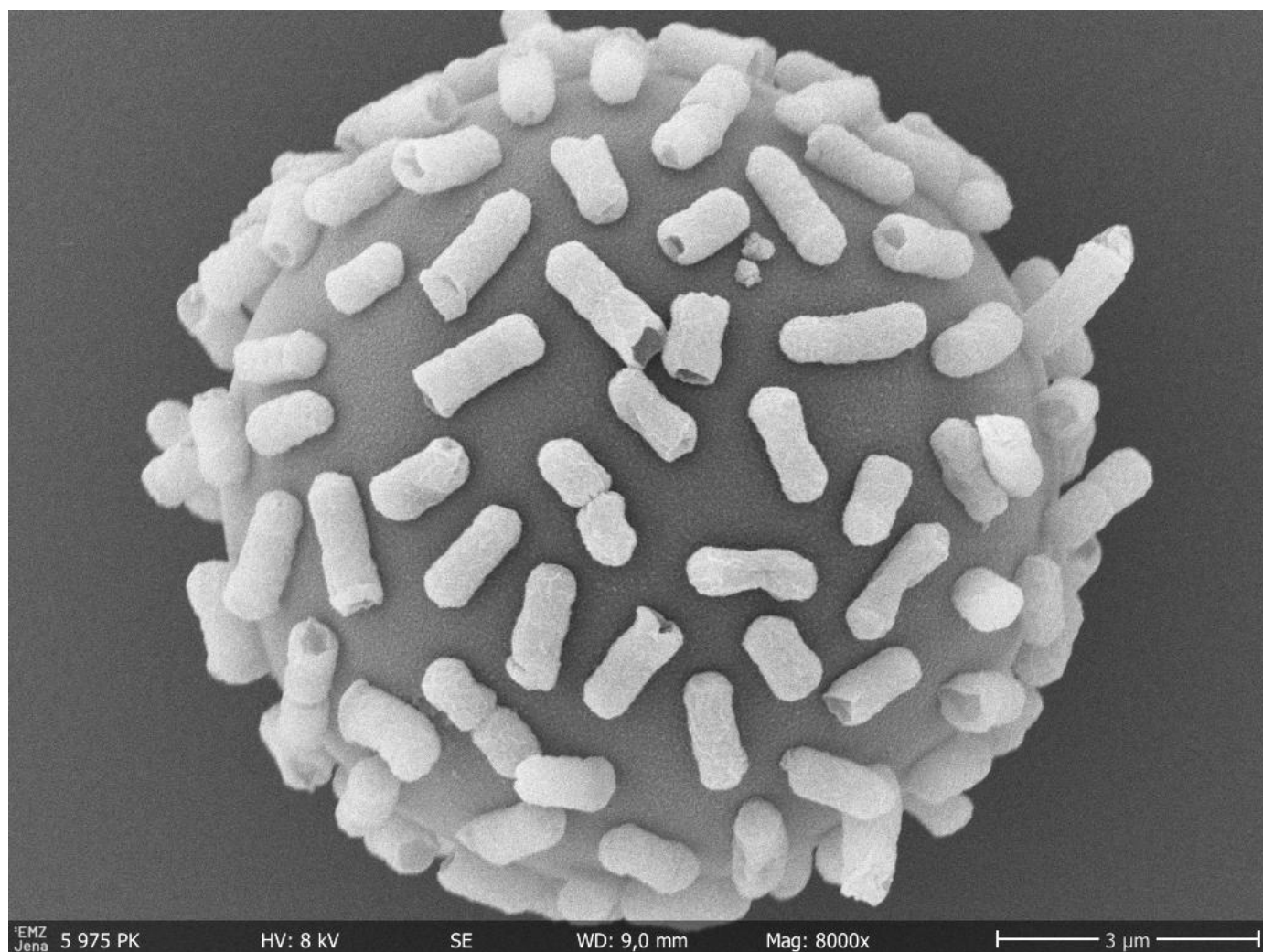


Figure 3. Scanning electron micrograph of a FluIDect Colligo Mag μ Bead with captured *E. coli* O157. Numerous rod-shaped bacteria are attached across the functionalised bead surface, showing efficient binding of the target organism. Imaging conditions: secondary electron (SE) detector, 8 kV accelerating voltage, 9.0 mm working distance, 8000 $\times$ magnification. Scale bar: 3 μ m.

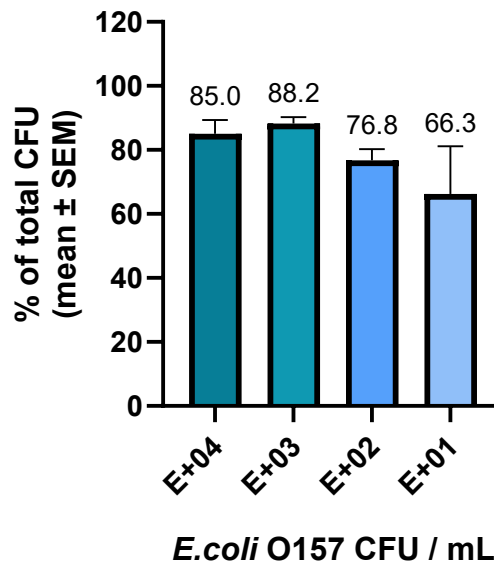


Figure 4. Capture efficiency of FluiDect Colligo Mag μ Beads (anti-*E. coli* O157) across a range of bacterial concentrations. Antibody-functionalised μ Beads were incubated with *E. coli* O157 at four concentrations spanning 10^4 to 10^1 CFU/mL. Capture efficiency, expressed as the percentage of total CFU recovered on the beads (mean $\pm$ SEM), remained high and consistent at the higher concentrations, reaching 85.0% at 10^4 CFU/mL, 88.2% at 10^3 CFU/mL, and 76.8% at 10^2 CFU/mL. At the lowest concentration tested (10^1 CFU/mL), mean capture efficiency was 66.3%, with greater variability between replicates (larger SEM), consistent with the increased stochastic effects expected when working near single-digit CFU inputs. These results demonstrate that the FluiDect Colligo Mag μ Beads (anti-*E. coli* O157) maintain robust, high-efficiency capture performance down to at least 100 CFU/mL, with reliable detection extending to as low as 10 CFU/mL.

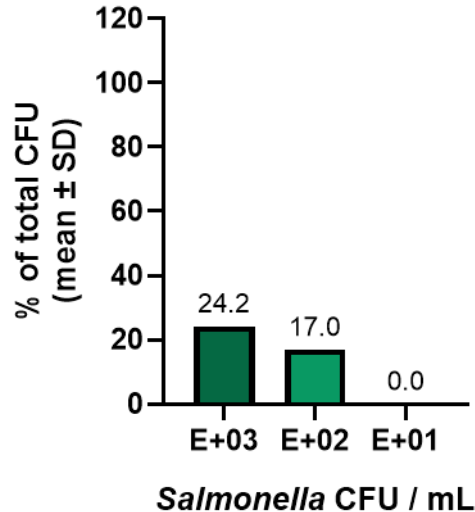


Figure 5. Cross-reactivity assessment against *Salmonella enterica* serovar Typhimurium. To confirm target specificity, FluiDect Colligo Mag μ Beads (anti-*E. coli* O157) were challenged with *Salmonella enterica* serovar Typhimurium, a Gram-negative enteric bacterium closely related to *E. coli* at the genomic level (~84% DNA sequence identity between orthologous genes; McClelland et al., 2000) and of comparable size and morphology. Despite this similarity, cross-reactivity remained low across the concentration range tested. At 10^3 CFU/mL, only 24.2% of the total *Salmonella* population was non-specifically captured, decreasing further to 17.0% at 10^2 CFU/mL, and dropping to undetectable levels (0.0%) at 10^1 CFU/mL. These values contrast markedly with the 88.2% and 76.8% capture efficiency observed for *E. coli* O157 at the equivalent 10^3 and 10^2 CFU/mL concentrations, respectively — corresponding to a 3.6- to 4.5-fold higher capture rate for the intended target over *Salmonella*. Taken together, these results confirm that the antibody-functionalised μ Beads retain high target selectivity, even against a genomically similar, morphologically comparable organism.

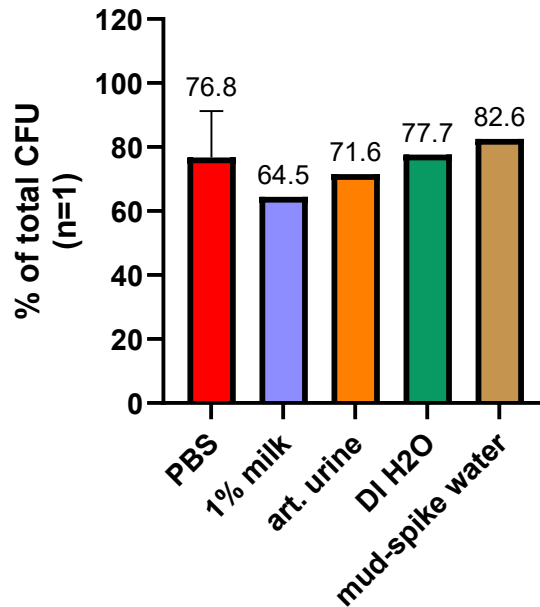


Figure 6. Capture efficiency of FluiDect Colligo Mag μ Beads (anti-*E. coli* O157) across diverse sample matrices. *E. coli* O157 was added at a concentration of 100 CFU/mL to a range of sample types and captured using antibody-functionalised magnetic μ Beads under standard assay conditions. The bacteria that were captured on the beads were then quantified by plating. Recovery was consistently high across all tested matrices, with a mean of 76.8% in PBS serving as the reference condition. Similar performance was achieved in 1% milk (64.5%), artificial urine (71.6%) and deionised water (77.7%), while the turbid, mud-spiked water matrix yielded the highest recovery rate of 82.6%. These results demonstrate that Colligo Mag μ Beads robustly and reproducibly capture *E. coli* O157 irrespective of sample composition, including complex, turbid, and protein-containing matrices. This supports their application in food safety, water quality, and environmental monitoring workflows.

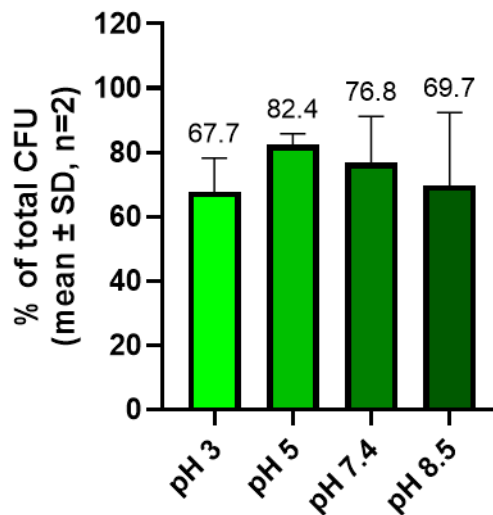


Figure 7. Effect of sample pH on the capture of *E. coli* O157 by FluiDect Colligo Mag μ Beads. To assess the pH tolerance of the assay, *E. coli* O157 was added to PBS with a pH of 3, 5, 7.4 or 8.5. The bacteria were then captured using antibody-functionalised magnetic μ Beads under standard assay conditions and the recovered bacteria were quantified by plating. Capture efficiency remained high across the entire tested pH range, with mean recoveries of 67.7% at pH 3, 82.4% at pH 5, 76.8% at pH 7.4, and 69.7% at pH 8.5 (mean $\pm$ SD, n = 2). Although optimal performance was observed under mildly acidic to neutral conditions (pH 5–7.4), recovery at the extremes of the range remained above 65%. Overlapping standard deviations indicate that there was no substantial loss of antibody function or bead–bacteria binding. These results demonstrate that the μ Beads tolerate a broad pH range, enabling their use with acidic and alkaline samples without the need for prior pH adjustment.

Protocol Optimisation: Sample Volume and Incubation Time

The standard assay conditions established in this study comprise combining 300 μ L of the sample with 2 μ L of FluIDect Colligo Mag μ Beads and incubating the mixture at 2000 rpm in a thermomixer for 60 minutes. These conditions were selected following the systematic optimisation of both parameters. The protocol is flexible with respect to sample volume and can be scaled up to at least 1 mL without a substantial loss of capture efficiency. Larger volumes were not evaluated here as the maximum capacity of the thermomixer tube adapter used is 1.5 mL (see Figure 8). Shorter incubation times were also tested, but 60 minutes was found to be optimal, providing the highest and most consistent recovery. This incubation time was validated across all sample volumes tested, confirming that a single set of conditions can be applied throughout the working volume range (see Figure 9).

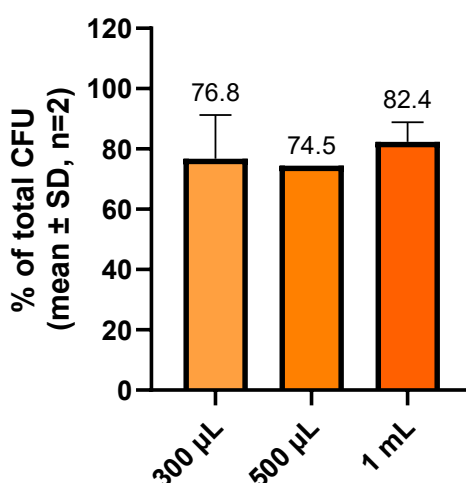


Figure 8. Effect of sample volume on the capture of *E. coli* O157 by FluIDect Colligo Mag μ Beads. *E. coli* O157 was added to increasing volumes of PBS (300 μ L, 500 μ L, and 1 mL) and processed with antibody-functionalised magnetic μ Beads under standard assay conditions. The recovered bacteria were then quantified by plating. Capture efficiency remained consistently high across the tested range, with mean recoveries of 76.8%, 74.5% and 82.4% at 300 μ L, 500 μ L and 1 mL, respectively (mean $\pm$ SD, n = 2). This indicates that the bead-to-sample ratio and magnetic separation are effective throughout this range. Volumes above 1 mL were not evaluated as the maximum capacity of the thermomixer tube adapter used is 1.5 mL, and mixing efficiency at this limit cannot be guaranteed. These results confirm reliable capture performance for sample volumes of up to 1 mL.

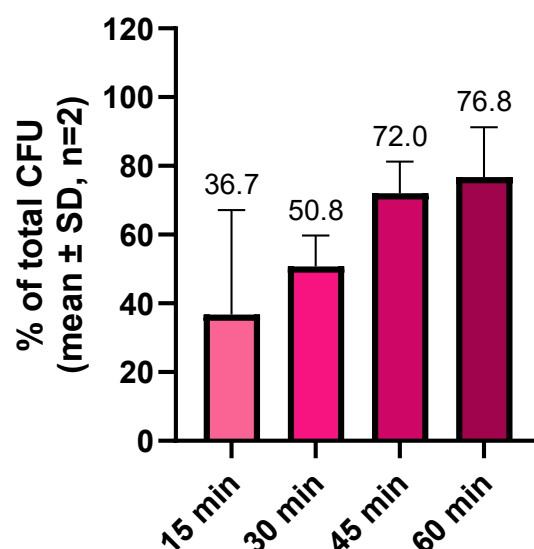


Figure 9. Optimisation of incubation time for the capture of *E. coli* O157 by FluidDect Colligo Mag μ Beads. To determine the optimal incubation period, *E. coli* O157 was spiked at 100 CFU/mL into 300 μ L of PBS and incubated with the antibody-functionalised magnetic μ Beads at 2000 rpm in a thermomixer for 15, 30, 45 or 60 minutes; captured bacteria were subsequently quantified by plating. Capture efficiency increased progressively with incubation time, from 36.7% at 15 minutes to 50.8% at 30 minutes, 72.0% at 45 minutes and 76.8% at 60 minutes (mean $\pm$ SD, $n = 2$). Recovery at the shorter time points was not only lower but also markedly more variable, as reflected in the large standard deviation at 15 minutes, indicating that binding equilibrium had not yet been reached. The increase between 45 and 60 minutes was comparatively modest, suggesting that capture approaches saturation towards the end of the time course. Based on these data, an incubation time of 60 minutes was selected as the standard assay condition, providing the highest and most reproducible recovery of *E. coli* O157.

Conclusions

- ✓ **High capture efficiency:** *E. coli* O157 was captured at a rate of 77–88% across concentrations of 10^2 to 10^4 CFU/mL, with detection still achievable at 10 CFU/mL.
- ✓ **Rapid workflow:** Target capture achieved after only 60 minutes of incubation, with no pre-enrichment required.
- ✓ **High target selectivity:** Low cross-reactivity against *Salmonella enterica* serovar Typhimurium, a Gram-negative rod of comparable size, morphology and cell-envelope structure.
- ✓ **Matrix robustness:** Consistent performance in artificial urine, deionised water, turbid/sediment-spiked environmental water and diluted milk (1%).
- ✓ **Storage stability:** Full activity retained after at least eight months of storage.
- ✓ **Flexible readout:** Compatible with plating, flow cytometry with nucleic acid staining, and downstream DNA extraction, allowing integration into existing laboratory workflows.

Technical Summary

Parameter	Value / Description
Target organism	<i>Escherichia coli</i> O157
µBead material	Magnetic polystyrene
Detection method	Plating (CFU), flow cytometry (SYTO 85 nucleic acid stain), ELISA
Tested matrices	PBS, water, artificial urine, mud-spiked water, and 1% milk
Storage stability	≥ 8 months, PBS + 0.02% NaN ₃ , 4 °C
Cross-reactivity	Low vs. <i>Salmonella enterica</i> Typhimurium (3.6–4.5-fold selectivity for <i>E. coli</i> O157)
Limit of detection	10–100 CFU/mL
Capture time	60 min
Sample input volume	300 µL to 1 mL
Bead size	3.1–7.2 µm

Ordering Information

FluIDect® Colligo Mag µBeads – anti-*E. coli* O157

Package sizes can be provided according to customer requests.

Find out more at <https://fluidect.com/en/functionalised-beads>

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