A Novel Extraction Methodology for a Novel Biospecimen

The ability to analyse and evaluate collected biospecimens has been integral in the furtherment of clinical and scientific understanding since the advent of medicine. Technology developments have focussed on two main areas, innovative, robust and reproducible sample collection and preservation and optimal recovery of the appropriate analyte from the specimen.

Determining sampling methodologies that enable the investigation of gut health/disease have long been a challenge, with current practices relying on heavily distal sampling, such as blood, or using highly contaminated matrices, such as stool.

Figure 1. OriCol™ sampling device

The OriCol™ sampling device provides an effective minimally invasive solution for the collection of rectal mucus, which provides a diverse and consistent biospecimen for representative gut examination. This biospecimen has broad diagnostic utility in myriad gut health conditions, including; cancer detection, residual disease monitoring, treatment selection and recurrence surveillance. The capabilities and performance of these testing applications is determined by the quality and efficiency of material recovery.

In this report we detail the collaborative development of a highly efficient custom DNA extraction workflow, tailored for mucus matrix bound clinical specimens, harnessing the potential of the sample for a suite of downstream applications.

Market leading extraction solutions show poor performance on mucus sampling

Figure 2. DNA fold loss: RM Exsig Mag vs Extraction kit Q.

8 samples were extracted using three different methods, with fold loss of DNA illustrated per sample and method. Exsig manual (light blue), Exsig CO-PREP automation (orange), Kit Q manual (blue).

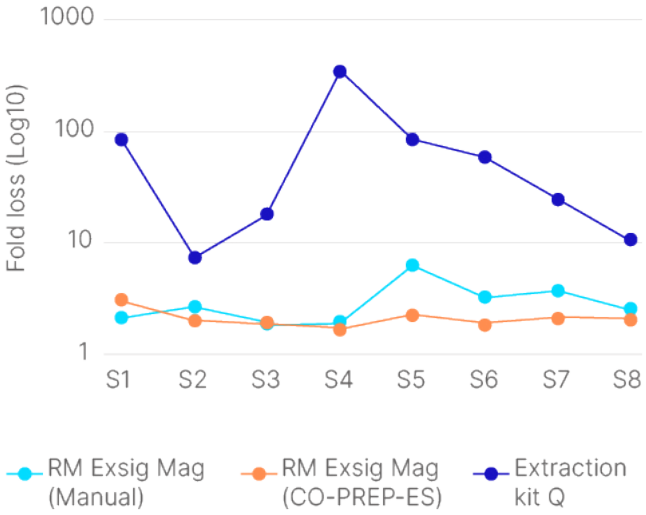


Figure 3. DNA fold loss: RM Exsig Mag vs Extraction kit T.

8 samples were extracted using three different methods, with fold loss of DNA illustrated per sample and method. Exsig manual (light blue), Exsig CO-PREP automation (orange), Kit T manual (blue).

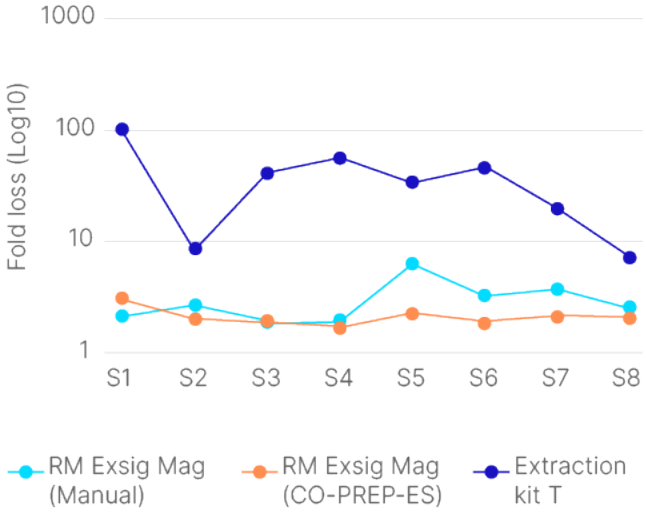


Figure 4. DNA fold loss:
RM Exsig Mag vs Extraction kit P.

8 samples were extracted using three different methods, with fold loss of DNA illustrated per sample and method. Exsig manual (light blue), Exsig CO-PREP automation (orange), Kit P manual (blue).

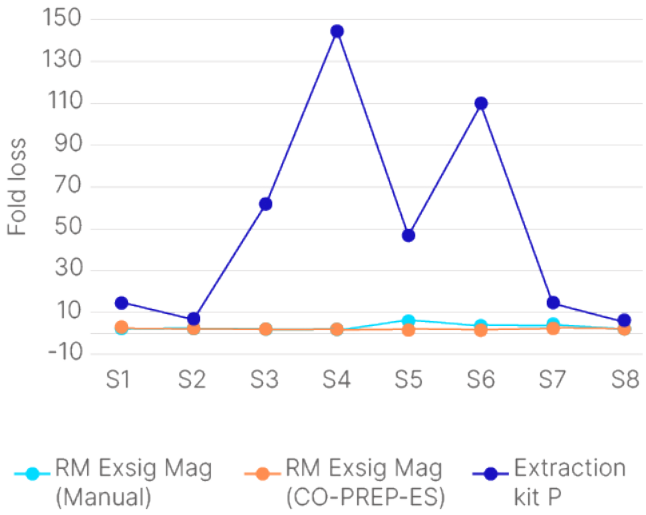


Figure 5. DNA fold loss:
RM Exsig Mag vs Extraction kit N.

8 samples were extracted using three different methods, with fold loss of DNA illustrated per sample and method. Exsig manual (light blue), Exsig CO-PREP automation (orange), Kit N manual (blue).

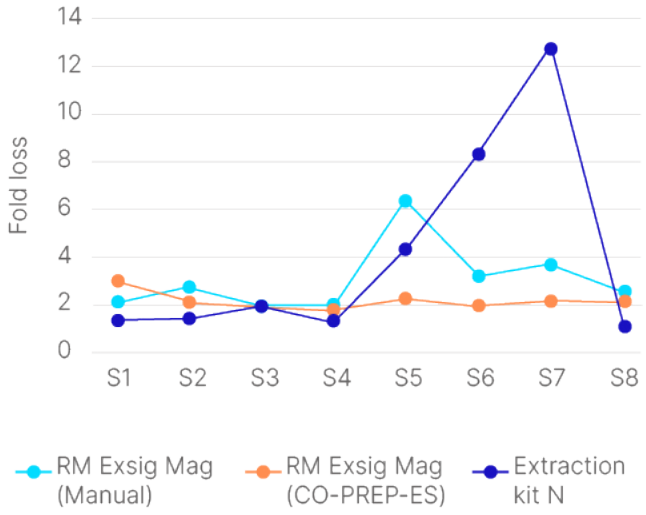
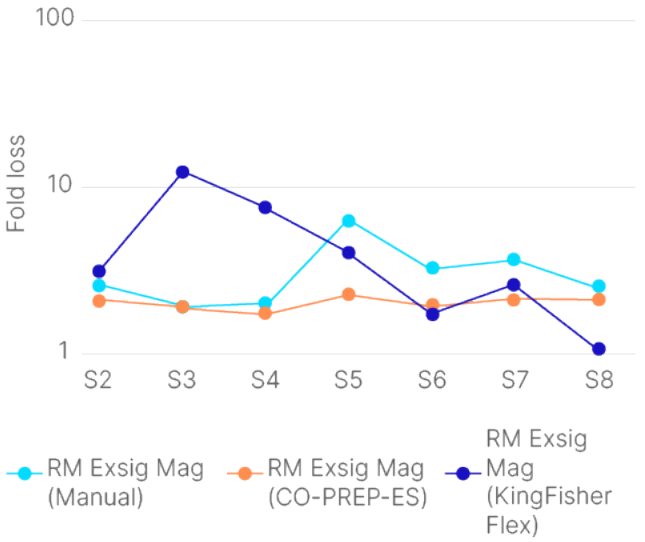


Figure 6. DNA fold loss:
RM Exsig Mag manual vs automation

7 samples were extracted using RM exsig Mag; manually (light blue), CO-PREP Extraction System (orange) and KingFisher Flex automation (blue). Fold loss of DNA is represented per sample and method.



Summary

RM Exsig Mag and four commercially available DNA extraction kits were used to extract Internal Extraction Control material (IEC) from mucus samples, recovery was compared to sample negative controls.

RM Exsig Mag extractions were performed manually and with two forms of automation. RM Exsig Mag consistently maintained minimal DNA loss using all methods, with the CO-PREP Extraction System achieving the most consistent performance and highest yields.

RM Exsig Mag was then compared to four market leading extraction kits designed for more challenging sample matrices (e.g. stool, saliva, sputum). Kits Q and T, showed poor DNA recovery, illustrated by high IEC fold losses, which was consistent across all samples. Kits P and N, showed highly variable performance, with a significant proportion of samples having poor recovery.

Methodology

DNA extraction efficiency from rectal mucus samples was assessed using four commercially available DNA extraction kits and RM Exsig Mag. The ability to extract and recover DNA from samples was proximally assessed by the recovery of a known DNA template (IEC), that is added in a standardised quantity to the required sample extraction input. Additionally, on a per extraction basis, a DNA extraction was performed on input buffer with IEC, as a sample negative control, which was used to benchmark sample independent extraction efficiency. The quantification of the IEC recovered after DNA extractions was measured by qPCR and recorded in Crossing Point (Cp) values. IEC recovery Cp values were compared between mucus samples and the cognate sample negative extraction. The ΔCp for each sample was calculated by subtraction of the corresponding sample negative Cp value from the mucus sample Cp value. Additionally, fold loss was calculated for each sample by calculating ΔCp to the exponent of 2 (ΔCp2). All DNA extraction protocols were conducted as per the manufacturer's instructions for use.

Comparison of Extraction Quality Control by Cp Delta Quantification

Delta Cp values (sample IEC Cp minus sample negative IEC) were calculated from qPCR quantification of IEC. Quality control (QC) thresholds were set as ≤2 optimal and ≤3 Pass, in line with industry QC standard. Delta Cp values for 8 mucus samples were calculated from side by side DNA extractions using RM Exsig Mag and four commercially available DNA extraction kits, with QC outcomes defined.

Figure 7. Delta Cp: RM Exsig Mag vs Extraction kit Q.

8 samples were extracted using three different methods, with delta Cp values compared between sample and method. Exsig manual (light blue), Exsig CO-PREP Extraction System (orange), Kit Q manual (blue). Grey dashed lines represent 2 and 3 Cp thresholds.

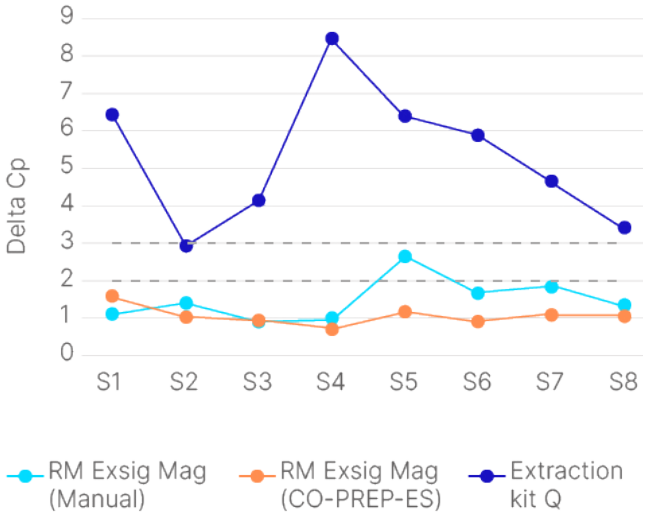


Figure 8. Delta Cp: RM Exsig Mag vs Extraction kit T.

8 samples were extracted using three different methods, with delta Cp values compared between sample and method. Exsig manual (light blue), Exsig CO-PREP Extraction System (orange), Kit T manual (blue). Grey dashed lines represent 2 and 3 Cp thresholds.

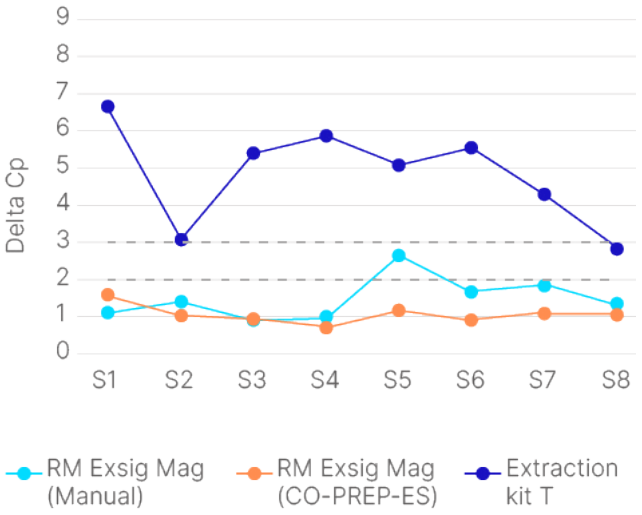


Figure 9. Delta Cp:
RM Exsig Mag vs Extraction kit P.

8 samples were extracted using three different methods, with delta Cp values compared between sample and method. Exsig manual (light blue), Exsig CO-PREP Extraction System (orange), Kit P manual (blue). Grey dashed lines represent 2 and 3 Cp thresholds.

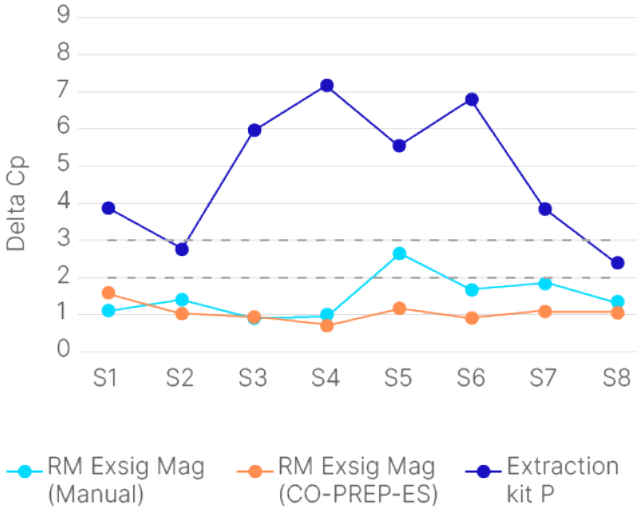


Figure 10. Delta Cp:
RM Exsig Mag vs Extraction kit N.

8 samples were extracted using three different methods, with delta Cp values compared between sample and method. Exsig manual (light blue), Exsig CO-PREP Extraction System (orange), Kit N manual (blue). Grey dashed lines represent 2 and 3 Cp thresholds.

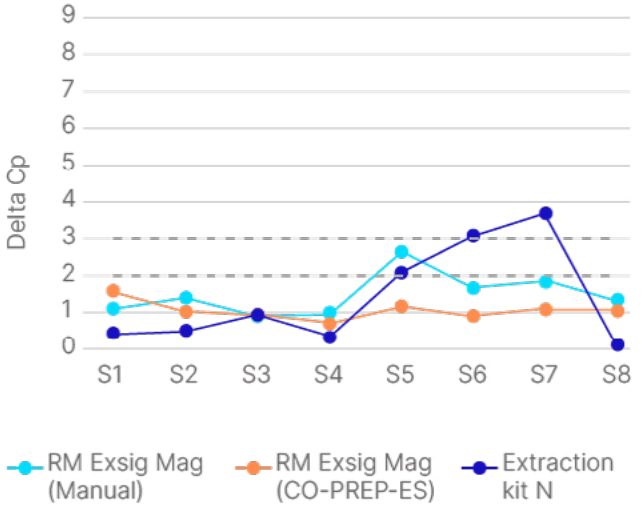


Figure 11. QC Pass Rate:
RM Exsig Mag vs commercial solutions

QC pass rates were determined as "Optimal" (<2 Cp), "Pass" (<3 Cp) and Fail (3> Cp). The QC status of 8 tested mucus samples was assessed and summarised for 4 commercially available extraction kits and RM Exsig Mag.

Extraction kit	Optimal (<2 Cp)	Pass (<3 Cp)	Fail (>3 Cp)
RM exsig Mag	8	8	0
Extraction kit Q	0	1	7
Extraction kit T	0	1	7
Extraction kit P	0	2	6
Extraction kit N	5	6	2

Conclusions

Localised sampling allows for the collection of highly representative biospecimens of a particular organ/ compartment of interest. The composition of locally collected specimens have unique properties and compositions, requiring further consideration for preparation and processing. Analysis and assessment of four market leading DNA extraction solutions have highlighted the lack of readily available extraction methods that are allow for high performance extraction of mucus sampling, in this case intestinal.

We demonstrate that existing technologies assessed have poor consistency across clinical samples and in many cases greatly decreased DNA recovery from the sample. The newly developed RM Exsig Mag extraction methodology overcomes the issues seen in the assessed existing solutions, consistently achieving Cp delta values within QC acceptance parameters and maintains this performance across manual and automated workflows. RM Exsig Mag allows for optimal DNA recovery from mucosal membrane samples, demonstrating substantial potential for myriad diagnostic testing across a range of disease areas.