Microbiota Profiling in FFPE Archival Tumour Tissue from Early-Stage Triple-Negative Breast Cancer

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Introduction

Emerging evidence highlights the role of the microbiota in breast cancer prognosis and treatment response, with tissue-resident bacteria potentially influencing the tumour microenvironment and immune modulation[1]. Formalin-fixed paraffin-embedded (FFPE) tissue represents a vast, clinically annotated resource in pathology archives, offering a unique opportunity for retrospective microbiome studies. However, its suitability remains uncertain due to DNA fragmentation, low bacterial biomass, and background contamination[2]. We assessed the feasibility of using FFPE breast tumour tissue for bacterial DNA analysis in patients with early-stage triple-negative breast cancer (TNBC) receiving neoadjuvant chemotherapy (NAC).

Methods

- **Samples:** Breast tissue specimens obtained from 23 participants with early stage TNBC enrolled in an observational biobank (NCT01840293). 38 FFPE samples were analysed (18 baseline diagnostic biopsies, 20 primary surgical resections), with paired samples available for 70% of patients treated between 2018-2023.
- **DNA extraction:** Performed using a protocol optimised for FFPE tissue. DNA concentration was measured by Qubit fluorometry.
- **Bacterial DNA detection:** PCR targeting 16S rRNA V1–V2 and V3–V4 regions; qPCR targeting the V6 region using a synthetic gene block as standard.
- **Human DNA quantification:** qPCR for the RNase P gene, standardised with human genomic DNA.
- **Controls:** Each qPCR plate included water (no-template control), tissue-free FFPE shaves to monitor contamination, and agar only FFPE blocks to capture contamination introduced during paraffin embedding.
- **Contamination control:** Laboratory procedures were designed to minimise background contamination, though archival FFPE specimens were not originally prepared for microbial analysis.

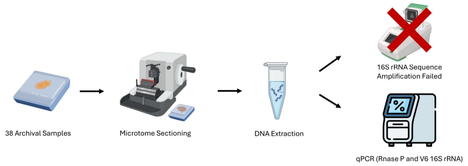


Figure 1. Workflow for human and bacterial DNA quantification in archival FFPE breast tissue.

Acknowledgements

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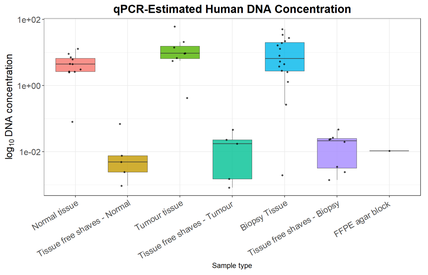
Results

Baseline biopsies yielded an average DNA concentration of 95 µg/µl; primary surgical resections averaged 85.75 µg/µl, consistent with FFPE expectations. Tissue-free negative controls yielded ~0.1 µg/µl, confirming minimal background DNA from the paraffin matrix. As bacterial identification by conventional PCR was unsuccessful, qPCR was used to measure both human and bacterial DNA concentrations.

Human DNA concentration analysis

- The extraction and quantification workflow reliably detected human DNA, reflecting the presence of tissue material in FFPE sections (FDR adjusted $p < 0.01$).

Figure 2. qPCR-estimated human DNA concentration across FFPE sample types. Human DNA concentrations were quantified using RNase P qPCR.



Bacterial DNA concentration analysis

- Bacterial DNA levels in tissue samples were not significantly different from those in agar FFPE blocks (FDR adjusted $p > 0.05$).
- This pattern suggests that most of the bacterial DNA detected originates from background contamination introduced during FFPE processing rather than from true tissue-associated microbiota.

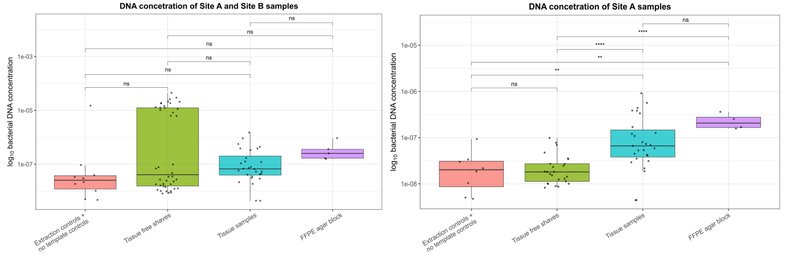


Figure 3. Bacterial DNA concentration across FFPE sample types from Site A and Site B. Bacterial DNA concentrations were estimated using 16S rRNA V6 qPCR. Results are shown for samples from both sites combined (left) and for Site A alone (right).

FFPE matrix contains off-target bacterial DNA

- FFPE agar blocks and small biopsy samples, both of which contain little or no tissue but a relatively large proportion of embedding matrix, yielded bacterial DNA concentrations comparable to or higher than those from tissue-rich blocks.
- Shaves taken from the edge of tissue blocks, which include less paraffin, showed DNA levels similar to extraction controls.
- This pattern indicates that the apparent bacterial DNA signal in FFPE material is largely driven by the proportion of matrix rather than biological content.
- These findings highlight the need to consider the matrix-associated background signal when interpreting bacterial DNA measurements from FFPE material.

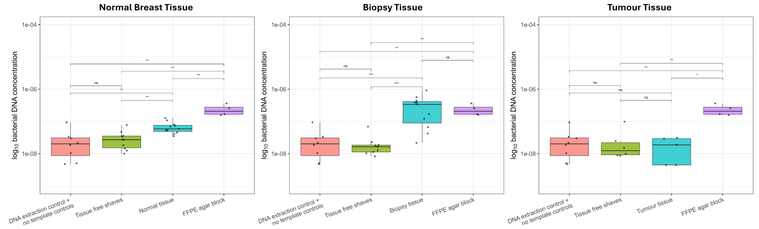


Figure 4. Bacterial DNA concentration by sample type for normal, biopsy, and tumour FFPE blocks from site A. Bacterial DNA concentrations were quantified using 16S rRNA V6 qPCR and compared across sample types within each block category (normal, biopsy, and tumour).

Conclusion

- Routinely archived FFPE specimens are not optimally prepared for microbiota analysis.
- Despite contamination-aware protocols, bacterial DNA levels in tissue sections were comparable to agar block controls, indicating background contamination, probably from non-sterile embedding.
- Human DNA was reliably detected and present in much higher quantity.
- These findings highlight the limitations of FFPE tissue for tumour microbiota studies.
- Results have informed workflow refinement of our prospective translational protocol (MINA trial, NCT06709651), which now prioritises fresh-frozen tissue collected under sterile conditions.

References

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