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A reconstructive workflow for integrated clinical and translational handling of rectal cancer surgical specimens

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Method Article

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Abstract

Colorectal cancer (CRC) is the third most common cancer worldwide and accounts for 9.6% of cancer-related deaths. Rectal cancer (RC) is a major contributor to CRC-related morbidity and mortality and total mesorectal excision (TME) has become the foundation for its treatment, both upfront surgery or after neoadjuvant therapy in selected/non-responding patients. Standard processing of TME specimens in pathology department does not permit fresh-state luminal assessment or tissue procurement, limiting the translational value of surgical material. Here, we describe an integrated specimen-handling workflow that enables longitudinal opening, direct tumor visualization, optical interrogation, and biobanking prior to anatomical reconstruction and conventional formalin fixation. Overall, this approach enables the optimal use of TME samples for research without compromising their integrity for clinical assessment.

INTRODUCTION

Rectal cancer requires a highly coordinated multidisciplinary approach, as clinical, radiological, and pathological variables directly influence therapeutic decision-making and patient prognosis. Despite the availability of several therapeutic strategies for rectal tumors, surgery remains the cornerstone of management, and the quality of total mesorectal excision (TME) is a critical determinant of local control and long-term outcomes[1,2]. However, the effectiveness of surgical treatment relies on accurate preoperative characterization of the disease. High-resolution rectal magnetic resonance imaging (MRI) has therefore become a fundamental tool for preoperative staging and risk stratification. MRI allows detailed assessment of tumor extent, circumferential resection margin (CRM) involvement, and other imaging features associated with oncological outcomes, thereby contributing to treatment selection and patient stratification[3–6].

The concept of TME, introduced by Heald and colleagues in the 1980s[7], and subsequently refined through the pathological grading system proposed by Quirke[8,9], established the importance of preserving an intact mesorectal envelope. In parallel, high-resolution rectal MRI helped translate these anatomical principles into the preoperative setting by enabling assessment of the relationship between tumor and the mesorectal fascia, as well as other imaging features associated with short- and long-term oncological outcomes. Within this framework, pathological evaluation serves as the definitive *ex vivo* assessment of surgical quality and complements both surgical execution and radiologic prediction.

Traditionally, rectal cancer surgical specimens are processed in pathology departments without violating their native cylindrical configuration. Standard protocols recommend opening the proximal and distal ends of the specimen to insert a formalin-soaked gauze within the lumen, thereby facilitating adequate fixation of the mucosal surface[10]. After fixation, specimens are serially sectioned in the transverse plane using the bread-loaf technique, which allows optimal evaluation of circumferential margin status and supports topographical correlation between gross sections and preoperative MRI findings. However, a key methodological limitation is that both macroscopic evaluation and measurement of tumor-to-margin distances are performed after fixation, when formalin-induced tissue shrinkage may alter anatomical

relationships[9,11–15]. Moreover, because the lumen remains closed throughout standard processing, the mucosal surface cannot be accessed in the fresh state. While this has no impact on routine diagnostic evaluation, it prevents the procurement of fresh tissue for biobanking and limits the implementation of research protocols that require direct exposure of unfixed mucosa.

The growing emphasis on precision oncology has further amplified the need to identify robust biomarkers capable of stratifying patients and guiding individualized treatment strategies[16]. In this context, biobanking has become a critical component of modern cancer research, enabling standardized preservation of high-quality biological material for molecular, cellular, and translational studies[17]. Fresh tissue aliquots are particularly valuable, supporting applications such as RNA sequencing, metabolomic profiling, xenograft generation, *ex vivo* functional assays, and a range of optical and spectroscopic technologies - including fluorescence lifetime imaging and hyperspectral analysis - which benefit from uncompromised biochemical integrity[18–20].

Although several alternative grossing strategies have been described - such as semi-opened approaches and longitudinal slicing techniques[21–23]- these methods involve inherent trade-offs. Consequently, existing workflows, whether conventional or modified, rarely accommodate both the requirements of advanced translational research and the preservation of specimen geometry necessary for standard histopathological evaluation. Furthermore, while high-resolution MRI is the gold standard for monitoring treatment response in rectal cancer, particularly following neoadjuvant chemoradiotherapy, distinguishing residual viable tumor from dense fibrosis remains a major diagnostic challenge. In this setting, direct macroscopic inspection of the fresh specimen can reveal subtle mucosal and tumor-bed features that may be obscured when the rectum remains closed or is fixed in its entirety.

In summary, there is no currently available workflow that reconciles these competing requirements within a single, reproducible protocol. In this technical report, we propose a unique and novel specimen-handling workflow that integrates comprehensive fresh-state luminal assessment and tissue procurement with subsequent reconstruction of the rectum's native cylindrical configuration. Developed by an interdisciplinary team comprising colorectal surgeons, pathologists, pathology grossing technicians and biobank specialists, this approach preserves compatibility with standard fixation and transverse slicing protocols while maximizing the investigational value of rectal cancer surgical specimens.

METHODS

Written informed consent for clinical data handling, photographic documentation, and tissue sample collection for the Champalimaud Foundation Biobank (CFB) was obtained prior to surgery. These procedures do not interfere with standard medical practice or diagnostic efficiency and in accordance with institutional ethics policies and ISBER Best Practices[24]. The overall integrated workflow is summarized in **Fig. 1.**

The proposed workflow was developed and implemented at our institution as part of routine specimen handling and translational research activities. The present report provides a descriptive account of the procedural steps, illustrated by representative photographic documentation. No prospective case registry or systematic comparative evaluation was undertaken.

Specimen acquisition and initial handling

After completion of TME, the specimen is transported fresh to the Service of Anatomic Pathology with a cold ischemia time of less than 10 minutes. In accordance with College of American Pathologists (CAP)[25], the specimen is photographed with patient identifiers and orientation markers and mesorectal status is assessed (Fig. 2A-B). The distal staple line is transected, and the same procedure is performed proximally when applicable. The anterior and posterior non-peritonealized mesorectal resection surfaces (circumferential resection margins- CRM) are inked with blue and black tissue-marking dyes respectively, and briefly fixed with acetic acid to stabilize the ink[26] (Fig. 2C).

Longitudinal opening, cleaning, and fresh-state macroscopic assessment

Digital palpation through the distal end is used to identify the axial position of the tumor (Fig. 2E). A longitudinal incision starting from the proximal end through the anterior wall until the peritoneal reflection and then along the side opposite to the tumor is performed to avoid transgressing the lesion. After opening the lumen, fecal material is gently removed using isotonic rinsing, ensuring preservation of mucosal architecture. Macroscopic evaluation is then performed prior to fixation, documenting tumor size and morphology, invasion of deeper layers and distance to both proximal and distal margins (Fig. 2D-F). Standardized photographic documentation is obtained as suggested by Rampy *et al*[27].

When feasible, rectal specimens processed using this workflow are included in an institutional optical imaging protocol[19] and fresh tissue fragments are collected and processed following biobank standards for different research projects without compromising subsequent histopathological diagnosis[17,24].

Reconstruction of cylindrical morphology and fixation

A tubular roll of gauze soaked in 10% buffered formalin is inserted along the mucosal surface, ensuring uniform fixation of the tumor-bearing mucosa by extending at least 3cm beyond the macroscopic tumor boundaries in both directions[28]. The longitudinal edges are realigned and secured with three interrupted 4 – 0 polypropylene sutures at the distal, mid-rectal, and proximal levels (Fig. 3A-B). A running continuous suture approximates the mucosal and muscular layers from distal to proximal, completing the first-layer closure (Fig. 3C). The mesorectal fat and fascia are subsequently reapproximated with a second running suture from proximal to distal, restoring cylindrical morphology (Fig. 3D). Any remaining sigmoid colon is

left open and pinned onto a polypropylene board using 18G needles to reduce deformation and shrinkage during fixation.

Fixation, grossing, and systematic slice mapping

The reconstructed specimen is completely immersed in 10% buffered formalin for up to 48hours (Fig. 4A). After fixation, transverse slices approximately 3-4mm thick are obtained using the bread-loaf technique[10,29] (Fig. 4B-D). Slices are sequentially positioned in 3 to 4 columns, following the natural cranial-to-caudal axis of the rectum (Fig. 4E) and photographed following recommended practices for standardized specimen mapping[10,27].

Histopathological assessment and diagnostic compatibility

Histological evaluation demonstrates well-preserved architecture, with no evidence of tissue compression, tearing, stitch-related artifacts, or mesorectal misalignment (Fig. 5A) and allows for close correlation with pre-surgical MRI (Fig. 5B). Tumor morphology, depth of invasion, margin status and other required histological tumor features (e.g. tumor budding) are all computable according to CAP standards and the reconstructed configuration does not compromise the assessment of the circumferential resection margin (Fig. 5C-D). This anatomical reconstruction preserves the spatial integrity required for multimodal correlation, without imposing technical constraints on standard histopathological processing.

Research capabilities with this technique

Fresh-state fluorescence spectroscopic measurements are acquired without interference from blood, fecal residue, or fixation-related changes (Fig. 6A)[19]. Additionally, tissue fragments of approximately 5mm³ (tumor and adjacent non-tumor mucosa) can be collected (Fig. 6B) and delivered fresh to different researchers at our institution for subsequent use, and also kept as frozen aliquots (embedded in OCT, snap-frozen in cryovial and cryopreserved) in the CFB for future research.

Our initial experience illustrates the technical applicability of this workflow, including fresh-tissue procurement, optical interrogation, and subsequent conventional histopathological processing. Further systematic evaluation is required to establish its reproducibility and diagnostic equivalence to standard specimen-handling protocols.

DISCUSSION

To the best of our knowledge, the specimen-handling workflow implemented in our institution is original and has not previously been described. Opening the rectum in the fresh state for direct luminal inspection, margin assessment, and tissue procurement, followed by full geometric reconstruction of the specimen, allows the integration of multiple ongoing research projects while minimally altering the

standard pathology workflow. This hybrid approach enhances the clinical and translational utility of the surgical specimen, enabling fresh-tissue sampling, non-destructive optical interrogation, and subsequent conventional histopathological processing.

Although rectal cancer specimen handling is relatively standardized[30], several groups have proposed modifications to the traditional protocol to address specific diagnostic needs. Takemasa and Ishii[21,22] described a semi-open technique in which most of the specimen is opened while the cylindrical configuration is preserved only in the tumor-bearing segment. Their objective was to optimize interpretation of the circumferential resection margin (CRM). Conversely, Matsunaga et al.[23] demonstrated the clinical utility of a fully open, longitudinal slicing approach, particularly for inter-sphincteric resections, where aligning grossing with the surgical dissection plane facilitates histological interpretation. However, none of these methods allow full reconstruction of the rectum, nor do they support comprehensive fresh-state tumor evaluation or systematic procurement of fresh biological material. In contrast, the method presented here broadens the utility of the rectal specimen by enabling direct fresh-state assessment before fixation, while preserving compatibility with standard pathological processing thereafter.

Opening the rectum allows more accurate macroscopic evaluation of tumor size, morphology, wall involvement, and distance to margins than when the specimen remains closed. This is particularly relevant in post-neoadjuvant specimens, in which ulceration, fibrosis, and partial response may be difficult to interpret without full mucosal exposure. Beyond visual inspection, fresh-state examination also provides tactile and spatial information - such as tumor consistency and the degree of mobility or adherence between layers - that may offer additional clues regarding depth of invasion. This direct interaction with the specimen also has substantial educational value, as it allows trainees, whether pathology grossing technicians or physicians, to develop practical and experiential understanding in the handling and interpretation of rectal cancer specimens. At the same time, the open fresh specimen creates a unique opportunity for non-destructive research applications, including non-destructive optical interrogation and procurement of tissue for biobanking when applicable. Importantly, these gains do not come at the expense of conventional pathological assessment. After reconstruction, restoration of the native cylindrical anatomy preserves compatibility with the traditional transverse bread-loaf protocol for circumferential margin evaluation and supports radiologic–pathologic correlation with preoperative MRI. Such correlation is valuable not only from an academic perspective, but also as a framework for critical comparison between imaging and pathology that may help identify additional features of potential relevance for the management of rectal cancer patients. In this way, the proposed workflow combines the advantages of fresh-state luminal assessment with the structural preservation required for standard CRM analysis, thereby extending both the investigational and diagnostic utility of the specimen.

Despite the observed advantages, several limitations warrant consideration. First, successful implementation requires close coordination among the surgical, pathology, biobanking, and research teams, which may necessitate an initial adaptation period. Second, the workflow modestly increases specimen processing time, as fresh-state macroscopic evaluation, optical interrogation, and tissue

procurement must be completed before reconstruction. Nevertheless, efficient communication among personnel allows smooth integration of the method into the routine workflow, which in turn benefits from the added academic and translational value. Third, reconstruction of the rectal cylinder requires basic suturing skills; although the learning curve is short, variability in technique may affect early reproducibility. Finally, as with any fresh manipulation of surgical specimens, careful handling is essential to avoid introducing artifacts, though in our experience the technique does not compromise CRM assessment or the integrity of the mesorectal envelope. Although the workflow has been implemented in a limited number of specimens, its diagnostic equivalence to conventional specimen handling has not been formally assessed. Therefore, the present report should be interpreted as a technical description of a novel specimen-processing approach rather than a validation study.

CONCLUSION

Overall, this method provides a practical and reproducible solution for integrating fresh-state evaluation, tissue procurement, and advanced imaging research with conventional rectal cancer pathology. Its implementation expands the scientific value of each specimen while maintaining adherence to established macroscopic and histopathological standards.

Declarations

Conflict of interest:

Authors declare no conflicts of interest

Competing Interests

Laura M. Fernández serves as an Editor of Techniques in Coloproctology. The remaining authors declare no competing interests.

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Author Contribution

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