



# A Comparison of Grossing Procedures for Hirschsprung's Disease

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## Abstract

Hirschsprung's Disease is congenital cause of neonatal and less commonly adult obstructive intestinal dysfunction. Hirschsprung's results from the absence of enteric ganglion cells in sections of the bowel preventing normal peristaltic motion required for excretion. Treatment of the disease requires surgical resection of the impacted bowel and an anastomosis of functioning bowel to the anus.<sup>1</sup> Proper grossing of Hirschsprung's Disease allows for accurate mapping of aganglionic, euganglionic and transitional bowel for optimal surgical outcomes. This poster compares two grossing procedures and their outcome on pathological findings. Utilization of the new grossing procedure by Pathologists' Assistants allows for proper mapping of the transition zone which provides assurance that aganglionic, transition, and euganglionic bowel are present. The new procedure also allows for histologic determination of whether surgical transition zone pull through occurred during the operation.

## Background

Hirschsprung's Disease results from failure in the development of the enteric nervous system in the distal colon. Peristaltic contraction regulated by the enteric nervous system is essential to normal gastrointestinal motility.<sup>2</sup> Individual cases show variation in segment length described as short (10%), classic (75%), long (10%) and total (5%). Hirschsprung's Disease occurs in approximately 1 in 5,000 live births in the United States with a 4:1 higher rate in males.<sup>1</sup> While females represent less cases they tend to present with longer aganglionic segments.<sup>3</sup> Chromosomal abnormalities are commonly seen in association with the disease with Down Syndrome or trisomy 21 being the most common.<sup>1</sup>

Treatment consists of a surgical resection of the bowel with a "pull through" procedure anastomosing euganglionic bowel to aganglionic above the pectinate line. Frozen sections obtained during surgery are used to find the most distal euganglionic bowel for anastomosis.

The "transition zone" is the area of bowel occurring between the histologically normal bowel and aganglionic bowel. The transition zone is typically marked by myenteric hypoganglionosis, incomplete circumferential ganglion cells, and hypertrophied nerves in the submucosal plexus. The transition zone is thought to be limited to bowel within 3 cm - 5 cm of the aganglionic section.<sup>4</sup> Improper resection of the transition zone during a pull through surgery is suspected as the cause of poor post-surgical outcomes and persistent obstructive symptoms.

## Procedures

### Old Grossing Procedure

Resected bowel specimens were differentially inked to indicate proximal and distal resection margins. The specimen was rolled and pinned using forceps and a dissection pin creating a "swiss roll". The forceps were removed from the "swiss roll" with the roll maintained by the pin. A cut was then made on either side of the pin providing a longitudinal section along the entire length of the specimen with the proximal and distal ends differentially inked. The section was submitted in a whole mount cassette (Fig. 1).

### New Grossing Procedure

Resected bowel specimens are differentially inked with blue denoting the proximal margin and black denoting distal margin per institutional standards. The distal end typically features a mucosal sleeve extending past the transmural distal margin. Enface margins are submitted for the proximal and distal ends, taking care to submit the entire wall distally, not the mucosal sleeve. A longitudinal section of the entire length of the specimen is removed from the specimen and serially sectioned allowing for two sections per cassette, where non inked margins represent contiguous sections. Cross sections are then submitted sequentially from the remainder of the specimen at 1 cm intervals for transitional zone extent documentation (Fig. 1).

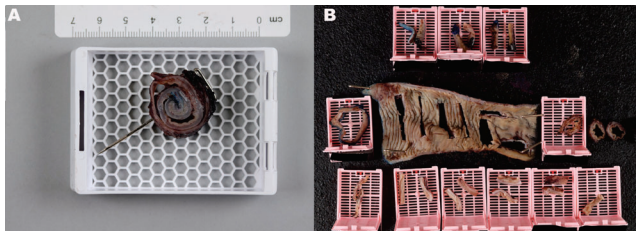


Figure 1. A) Old grossing procedure cassette B) New grossing procedure cassettes

## Results

### Old Grossing Procedure

Histologic slides feature the resected bowel along the entire length in a "swiss roll" orientation. Slides can demonstrate the presence of transmural aganglionic and euganglionic bowel. Features of the transition zone may be demonstrated in some areas. However, this technique did not provide enough sampling to effectively map, or more accurately demonstrate, the length of the transitional zone and its proximity to the euganglionic and aganglionic segment of colon (Fig. 2).

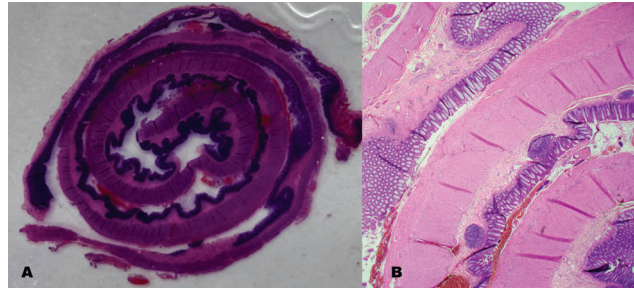


Figure 2. A) Whole mount from Old grossing procedure B) Old grossing procedure showing aganglionic exterior layers and middle layer with ganglion cells present.

### New Grossing Procedure

Histologic slides feature serially sectioned contiguous sections along the entire length of the resected bowel, circumferential proximal and distal surgical margins with a full wall thickness and transmural cross sections along the specimen documenting the transitional zone. The slides were evaluated for the presence of aganglionic and euganglionic segments, and defining features of the transition zone, (e.g. hypertrophied submucosal nerves, myenteric hypoganglionosis, and absent ganglion cells) (Fig. 3).

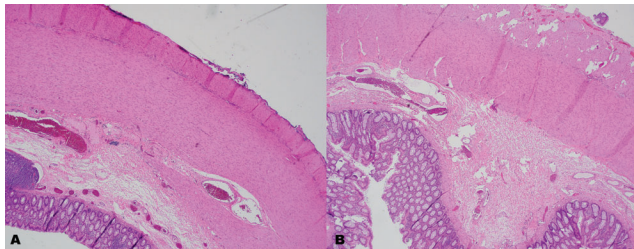


Figure 3. New grossing procedure histology showing: A) proximal margin with myenteric ganglion cells and no submucosal nerve hypertrophy B) Transition zone with slightly prominent submucosal nerves and rare ganglion cells.

## Discussion

Removal of all aganglionic bowel and the transition zone is essential for patients to have normal bowel function post-operatively. Pre-operative diagnostic biopsy and intraoperative frozen section provide an initial picture of the impacted area, but post-operative grossing of the surgical resection is needed for assurance of successful surgery. Post-operative confirmation of full wall euganglionic bowel around the entire circumference at the surgical anastomosis of proximal and distal bowel provides this.

Utilization of the new grossing procedure by Pathologists' Assistants allows for proper mapping of the transition zone which provides assurance that aganglionic, transition, and euganglionic bowel are present. The old grossing procedure makes orientation complicated when tracking what area of the bowel nerves of interest appear in. When utilizing the new grossing procedure the pathologist is able to move through the slides documenting the transition zone ensuring transmural normal bowel. The new procedure also allows for histologic determination of whether surgical transition zone pull through occurred during the operation. Differences in innervation circumferentially of the transitional zone can easily be missed by intraoperative sampling and frozen sections due to the unpredictable patterns. Post-operative confirmation allows the surgeon to assess if the patient requires further surgery, and provides more information to the patient. While the new grossing procedure is more time consuming at the bench, it reduces the need for further specimen sampling. The previous grossing procedure, while less time consuming, does not yield an accurate map of the transition zone. Another benefit of the new procedure is that the histotechnologist embedding and cutting the tissues at microtomy does not have to accommodate the technical challenges that come with whole mount processing and slide storage.

Understanding the variety in presentation of the affected colonic segments and the importance of the transition zone better empowers Pathologists' Assistants in grossing specimens of this type. Understanding epidemiologic patterns such as the potential for longer affected bowel segments in females also allows the Pathologists' Assistant to approach these cases with additional care to optimize transition zone mapping. Providing a map of the transition zone and documentation of circumferential euganglionic bowel increases surgical confidence and patient outcome. By using the new grossing procedure Pathologists' Assistants provide greater information for determining postsurgical outcomes and future patient quality of life.

## Conclusion

The new grossing procedure provides more informative histology slides used to determine post-surgical outcomes. This new procedure produces multiple slides mapping the transition from euganglionic to aganglionic resected bowel. By using the new grossing procedure Pathologists' Assistants provide greater information for determining postsurgical outcomes and future patient quality of life.

## References

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