**GPT-4o (Model 1) – Patch Level Prompts**  
  
Patch Level Prompt #1: Q1: Please provide a summary of the factors found in whole slide images of intestinal tissue that may indicate the presence or absence of plexus regions relevant to Hirschsprung disease diagnosis, along with a description of their image features in short terms, separated by semicolons. Please avoid using diagnostic terms in your response.

Answer 1:

* **Cellular density**: High or low cell concentration in specific regions.
* **Nuclear arrangement**: Clusters or dispersed patterns of nuclei.
* **Tissue architecture**: Organized or disorganized structural patterns.
* **Fibrous content**: Presence or absence of dense connective fibers.
* **Vascular structures**: Abundant or scarce blood vessels.
* **Ganglion-like formations**: Well-defined or indistinct cellular groupings.
* **Extracellular matrix patterns**: Compact or loose arrangement of surrounding tissue components.

Patch Level Prompt #2: Q2: Suppose we group intestinal tissue samples without identifiable plexus regions into one category. Please provide a summary of the factors found in whole slide images that may indicate this category, along with a description of their image features in short terms, separated by semicolons.

Answer 2:

* **Cellular composition**: Predominance of uniform or undifferentiated cells.
* **Nuclear distribution**: Evenly spaced or irregularly scattered nuclei.
* **Fibrous density**: Increased connective tissue with minimal cellular structures.
* **Vascular presence**: Reduced or inconsistent blood vessel patterns.
* **Tissue homogeneity**: Lack of distinct cellular clusters or structures.
* **Extracellular matrix organization**: Densely packed or irregularly arranged fibers.
* **Structural uniformity**: Absence of complex or layered organization.

Patch Level Prompt #3: Q3: How do image features of plexus regions within the muscle layer of the colon differ from surrounding tissue structures in whole slide images? Please summarize key distinguishing factors in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 3: Higher cellular density; distinct vascular organization; increased nerve fiber presence; altered collagen distribution; unique staining patterns; different textural homogeneity; varied anisotropy in fiber orientation; distinct glandular arrangement; sharper contrast in immunohistochemical markers.

Patch Level Prompt #4: Q4: Please provide a summary of the factors found in colon or the muscularis layer whole slide images that may indicate Hirschsprung’s disease, along with a description of their image features in short terms, separated by semicolons. Please avoid using subtype names in your response.

Answer 4:

* **Cellular composition:** Absence or scarcity of specific cell clusters.
* **Nuclear distribution:** Uniformly spaced nuclei without distinct groupings.
* **Fibrous density:** Increased connective tissue replacing expected structures.
* **Vascular presence:** Irregular or reduced blood vessel patterns.
* **Tissue homogeneity:** Uniform appearance lacking expected cellular diversity.
* **Extracellular matrix organization:** Densely packed with minimal structural variation.
* **Structural uniformity**: Consistent smooth texture without distinct cellular formations.

**GPT-o3-mini-high (Model 2) – Patch Level Prompts**  
Patch Level Prompt #1: Q1: Please provide a summary of the factors found in whole slide images of intestinal tissue that may indicate the presence or absence of plexus regions relevant to Hirschsprung disease diagnosis, along with a description of their image features in short terms, separated by semicolons. Please avoid using diagnostic terms in your response.

Answer1: Dense, well-organized cell clusters: distinct, tightly grouped regions with uniform staining; Sparse cell areas: irregular, low-density groups with weaker staining intensity; Clear network patterns: sharply demarcated, interconnected regions with high contrast; Diffuse arrangements: gradual transitions with blurred, less-defined borders.

Patch Level Prompt #2: Q2: Suppose we group intestinal tissue samples without identifiable plexus regions into one category. Please provide a summary of the factors found in whole slide images that may indicate this category, along with a description of their image features in short terms, separated by semicolons.

Answer 2: Indistinct network formations: absence of clearly demarcated cell clusters; diffuse cell dispersion: scattered, low-density cellular arrangement; blurred structural borders: gradual transitions without sharp demarcation; reduced intercellular connectivity: minimal grouping of cells into organized patterns.

Patch Level Prompt #3: Q3: How do image features of plexus regions within the muscle layer of the colon differ from surrounding tissue structures in whole slide images? Please summarize key distinguishing factors in short terms, separated by semicolons.

Answer 3: Clustered neural cell aggregates; irregular, network-like architecture; heterogeneous staining intensity; distinct textural contrast; clear demarcation from smooth muscle fibers.

Patch Level Prompt #4: Q4: Please provide a summary of the factors found in colon or the muscularis layer whole slide images that may indicate Hirschsprung’s disease, along with a description of their image features in short terms, separated by semicolons. Please avoid using subtype names in your response.

Answer 4: Absence of neuronal clusters: missing ganglion cells in expected regions; enlarged nerve bundles: thick, dark‐staining nerve fibers; increased neural staining: accentuated signal of nerve elements; disordered architecture: irregular and chaotic arrangement of neural structures.

**DeepSeek-R1 (Model 3) – Patch Level Prompts**  
  
Patch Level Prompt #1: Q1: Please provide a summary of the factors found in whole slide images of intestinal tissue that may indicate the presence or absence of plexus regions relevant to Hirschsprung disease diagnosis, along with a description of their image features in short terms, separated by semicolons. Please avoid using diagnostic terms in your response.

Answer 1: **Factors indicating presence:** clustered large nuclei with prominent nucleoli; fine, wavy fibrous mesh structures; organized cellular clusters surrounded by supporting cells; thickened nerve bundles adjacent to cellular aggregates; specific immunohistochemical staining patterns.

**Factors indicating absence:** enlarged, densely packed nerve fibers without clustered nuclei; increased density of linear, disorganized fibers; absence of organized cellular clusters; thickened muscular layers with sparse cellularity; inflammatory cell aggregates obscuring tissue architecture; submucosal layer thinning or reduced cellularity; excessive connective tissue deposition; lack of immunohistochemical staining in expected regions.

Patch Level Prompt #2: Q2: Suppose we group intestinal tissue samples without identifiable plexus regions into one category. Please provide a summary of the factors found in whole slide images that may indicate this category, along with a description of their image features in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 2: Absence of clustered ganglion cells; uniform smooth muscle layers lacking neural structures; sparse vascular networks without branching complexity; homogeneous mucosal architecture lacking specialized zones; minimal inflammatory infiltrates disrupting tissue organization; fibrosis obscuring normal stromal patterns; thickened muscularis propria without interspersed neural elements; lack of spindle-shaped interstitial cells between muscle fibers; reduced neuronal process density in submucosal and myenteric regions.

Patch Level Prompt #3: Q3: How do image features of plexus regions within the muscle layer of the colon differ from surrounding tissue structures in whole slide images? Please summarize key distinguishing factors in short terms, separated by semicolons.

Answer 3: Ganglion cell clusters (large nuclei, prominent nucleoli); looser stromal matrix vs. dense muscle bundles; lower cellular density with interspersed nerve fibers; eosinophilic cytoplasm contrast to spindle-shaped myocytes; nodular/network architecture vs. linear muscle alignment; distinct nuclear morphology (round vs. elongated); supporting glial cell presence; vascularity variations; staining contrast in H&E (lighter vs. uniform).

Patch Level Prompt #4: Q4: Please provide a summary of the factors found in the colon or the muscularis layer whole slide images that may indicate Hirschsprung’s disease, along with a description of their image features in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 4: Absence of ganglion cells: no clusters of large, pale-staining cells between smooth muscle layers; hypertrophic nerve fibers: thickened, eosinophilic strands in submucosa and muscularis; increased nerve bundle size: enlarged, irregularly contoured bundles within intermuscular regions; smooth muscle hypertrophy: thickened inner circular and outer longitudinal muscle layers; inflammatory infiltrates: lymphocytes or eosinophils in muscularis or submucosa; fibrosis: collagen deposition in muscularis, appearing as dense, acellular areas; mucosal alterations: crypt atrophy or ulceration secondary to obstruction; transition zone: abrupt histological shift from aganglionic to normal bowel with sudden nerve density reduction.

**Grok-3 (Model 4) – Patch Level Prompts**  
  
Patch Level Prompt #1: Q1: Please provide a summary of the factors found in whole slide images of intestinal tissue that may indicate the presence or absence of plexus regions relevant to Hirschsprung disease diagnosis, along with a description of their image features in short terms, separated by semicolons. Please avoid using diagnostic terms in your response.

Answer 1:

* **Presence of cell clusters in specific locations**: clusters of cells with large, round nuclei and prominent nucleoli located between the circular and longitudinal muscle layers or in the submucosa.
* **Absence of cell clusters in these areas**: no such clusters of cells with large, round nuclei and prominent nucleoli in the expected regions between muscle layers or in the submucosa.
* **Presence of thick linear structures**: thick, stained, elongated elements appearing in the submucosa or mucosal layers.

Patch Level Prompt #2: Q2: Suppose we group intestinal tissue samples without identifiable plexus regions into one category. Please provide a summary of the factors found in whole slide images that may indicate this category, along with a description of their image features in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 2:

* **Incomplete tissue representation:** only mucosa present, lacking submucosa and muscularis externa.
* **Poor staining quality:** indistinct cellular details due to faint or uneven staining.
* **Sectioning artifacts:** irregular or distorted tissue architecture from oblique cuts or folds.
* **Absence of characteristic cell clusters:** no large cells with pale nuclei in the submucosa or between muscle layers.
* **Low image resolution:** pixelated or blurry details preventing clear identification.

Patch Level Prompt #3: Q3: How do image features of plexus regions within the muscle layer of the colon differ from surrounding tissue structures in whole slide images? Please summarize key distinguishing factors in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 3:

* **Clustered round cells:** These areas contain groups of round cells, differing from the elongated, spindle-shaped muscle cells.
* **Thin eosinophilic fibers:** Fine, pink-staining linear structures are present, contrasting with the uniform muscle fiber arrangement.
* **High nuclear density:** These regions show a higher concentration of nuclei in clusters, unlike the more evenly spaced nuclei in muscle tissue.

Patch Level Prompt #4: Q4: Please provide a summary of the factors found in colon or the muscularis layer whole slide images that may indicate Hirschsprung’s disease, along with a description of their image features in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 4:

* **Absence of ganglion cells:** no nerve cell clusters visible in the intermuscular or submucosal layers.
* **Hypertrophy of nerve fibers:** thick nerve bundles present in the muscularis or submucosal layers.

**GPT-4o (Model 1) – Slide Level Prompts**  
Slide Level Prompt #1: Q1 Summary of the appearance of whole slide images showing plexus regions within the muscle layer of the colon in short terms, separated by semicolons.

Answer1: Dense nerve networks; located within the muscle layer; elongated and interconnected structures; varying fiber thickness; embedded within smooth muscle bundles; stained for neural markers; distinguishable from surrounding tissues; sometimes associated with blood vessels.

Slide Level Prompt #2: Q2: Suppose we group colon muscle layer regions where plexus structures are not identifiable into one category. Summarize the appearance of whole slide images of this group in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 2: Disorganized muscle fibers; increased tissue density; reduced structural definition; absence of distinct neural elements; homogenized texture; irregular staining patterns.

Slide Level Prompt #3: Q3: How do image features of whole slide images displaying plexus regions within the muscle layer of the colon differ from those without plexus regions? Please summarize key distinguishing factors in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 3: Structural complexity; increased cellular density; distinct texture patterns; higher vessel density; unique morphological arrangements; altered intensity distributions; increased edge sharpness; differentiated spatial organization; variation in feature heterogeneity.

Slide Level Prompt #4: Q4: Please provide a summary of the factors observed across the entire whole slide images of the colon or its muscle layer that may indicate Hirschsprung’s disease, along with a description of their image features in short terms, separated by semicolons. Please avoid using subtype names in your response.

Answer 4: Absence or reduction of specific cellular structures; thickened nerve fibers; abnormal clustering of cells in certain regions; irregular or disrupted muscle layer organization; increased connective tissue density; altered tissue staining patterns; enlarged or misshapen cellular components; regions with reduced cellular density.

**GPT-o3-mini-high (Model 2) – Slide Level Prompts**  
Slide Level Prompt #1: Q1 Summary of the appearance of whole slide images showing plexus regions within the muscle layer of the colon in short terms, separated by semicolons.

Answer1: Ganglion cell clusters; interwoven nerve fibers; pale eosinophilic staining; patchy distribution; distinct boundaries from smooth muscle; subtle architectural irregularities.

Slide Level Prompt #2: Q2: Suppose we group colon muscle layer regions where plexus structures are not identifiable into one category. Summarize the appearance of whole slide images of this group in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 2: Uniform smooth muscle bundles; well-defined laminar architecture; homogeneous staining; absence of neural aggregates; minimal interstitial variation.

Slide Level Prompt #3: Q3: How do image features of whole slide images displaying plexus regions within the muscle layer of the colon differ from those without plexus regions? Please summarize key distinguishing factors in short terms, separated by semicolons.

Answer 3: Reticular nerve network; clustered ganglion cells; heterogeneous tissue texture; interrupted smooth muscle bundles; distinct staining contrast.

Slide Level Prompt #4: Q4: Please provide a summary of the factors observed across the entire whole slide images of the colon or its muscle layer that may indicate Hirschsprung’s disease, along with a description of their image features in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 4: Absence of ganglion cells: Missing neural cell bodies in regions where they are normally present; Nerve fiber hypertrophy: Enlarged, densely stained nerve bundles within the muscle layer; Elevated neural marker reactivity: Intense staining of nerve elements on histochemical assays; Disrupted smooth muscle architecture: Irregular, fragmented arrangement of muscle fibers adjacent to abnormal neural elements.

**DeepSeek-R1 (Model 3) – Slide Level Prompts**  
Slide Level Prompt #1: Q1 Summary of the appearance of whole slide images showing plexus regions within the muscle layer of the colon in short terms, separated by semicolons.

Answer1: Ganglion clusters with large, pale nuclei; interspersed nerve fibers (wavy, eosinophilic); supporting glial cells (small, dark nuclei); located between inner circular and outer longitudinal muscle layers; sparse connective tissue matrix; distinct from densely packed smooth muscle surroundings.

Slide Level Prompt #2: Q2: Suppose we group colon muscle layer regions where plexus structures are not identifiable into one category. Summarize the appearance of whole slide images of this group in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 2: Uniform, continuous muscular layers with indistinct connective tissue borders; absence of neural aggregates or ganglionic clusters between smooth muscle fibers; homogeneous stromal composition lacking specialized cellular networks; smooth, uninterrupted interfaces between longitudinal and circular muscle bands; inconspicuous interstitial cell populations; unremarkable vascular patterns without adjacent organized cellular arrangements.

Slide Level Prompt #3: Q3: How do image features of whole slide images displaying plexus regions within the muscle layer of the colon differ from those without plexus regions? Please summarize key distinguishing factors in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 3: Clustered large neuronal cell bodies with prominent nucleoli; reticular stromal fiber networks; heterogeneous tissue texture with mixed cell densities; focal disruption of smooth muscle continuity; localized presence of glial support cells; distinct basophilic or eosinophilic staining variations; nodular architecture interrupting muscular uniformity.

Slide Level Prompt #4: Q4: Please provide a summary of the factors observed across the entire whole slide images of the colon or its muscle layer that may indicate Hirschsprung’s disease, along with a description of their image features in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 4: Absence of ganglion cells in submucosa and muscularis propria; large neuronal cells missing in typical locations; hypertrophic nerve fibers; thickened, eosinophilic nerve bundles within the submucosa or muscular layers; increased density of small nerve bundles; clusters of irregular nerve structures in affected areas; thickened muscularis propria; hyperplastic smooth muscle layers with altered orientation; abrupt transition from aganglionic to ganglionic segments; narrow lumen proximal to the affected zone with dilated normal colon distally; secondary mucosal inflammation; neutrophilic infiltrates or cryptitis near aganglionic regions; mucosal ulceration or erosions due to obstruction.

**Grok-3 (Model 4) – Slide Level Prompts**  
Slide Level Prompt #1: Q1 Summary of the appearance of whole slide images showing plexus regions within the muscle layer of the colon in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer1:

* nerve ganglia.
* between muscle layers.
* basophilic cells.

Slide Level Prompt #2: Q2: Suppose we group colon muscle layer regions where plexus structures are not identifiable into one category. Summarize the appearance of whole slide images of this group in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 2:

* Uniform smooth muscle.
* Absence of ganglia.
* No visible nerve structures.
* Lacking basophilic clusters.
* Homogeneous muscle architecture.

Slide Level Prompt #3: Q3: How do image features of whole slide images displaying plexus regions within the muscle layer of the colon differ from those without plexus regions? Please summarize key distinguishing factors in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 3:

* **Ganglionic structures**: Clusters of neuronal cell bodies with large, pale nuclei appear in regions with nerve networks, contrasting with the uniform spindle-shaped muscle cells elsewhere.
* **Nerve fiber networks**: Eosinophilic, fibrillar structures representing nerve bundles are present, differing from the smooth muscle fiber arrangement.
* **Heterogeneous tissue composition**: A mix of cell types and structures creates varied texture and complexity, unlike the more homogeneous muscle tissue in areas lacking these networks.

Slide Level Prompt #4: Q4: Please provide a summary of the factors observed across the entire whole slide images of the colon or its muscle layer that may indicate Hirschsprung’s disease, along with a description of their image features in short terms, separated by semicolons. Please avoid using the word "plexus" in your response.

Answer 4:

* **Absence of ganglion cells**: No large neurons with prominent nucleoli in the submucosa and between the inner circular and outer longitudinal muscle layers.
* **Presence of hypertrophic nerve fibers**: Enlarged, prominent eosinophilic nerve bundles in the submucosa and between the muscle layers.