

# Intestinal Disease

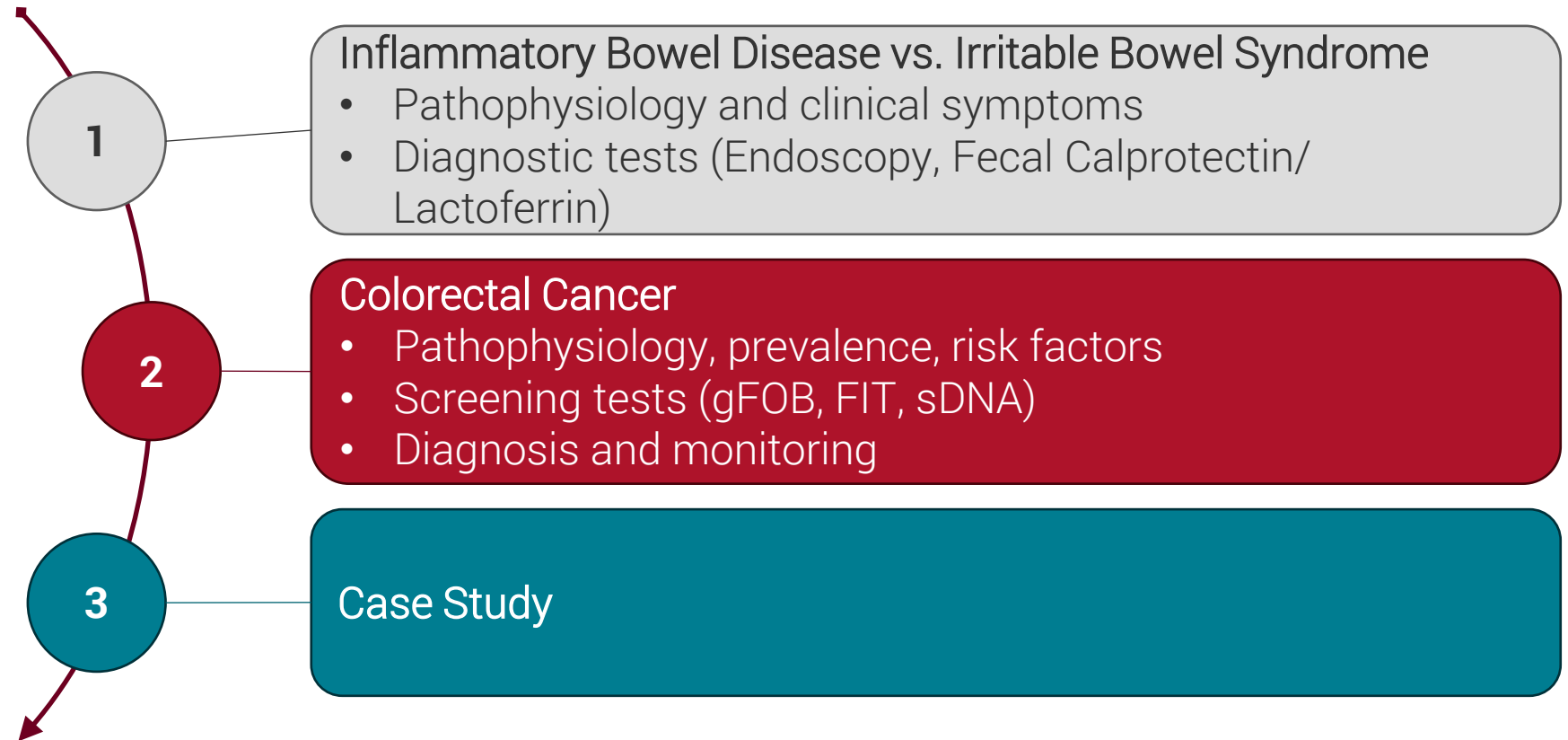
Laboratory Screening and Diagnosis

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Assistant Professor (Clinical)

DECEMBER 6, 2023

# Outline





# Inflammatory Bowel Disease (IBD) and Irritable Bowel Syndrome (IBS)

Abdominal pain/discomfort with diarrhea or constipation

# Inflammatory Bowel Disease (IBD)

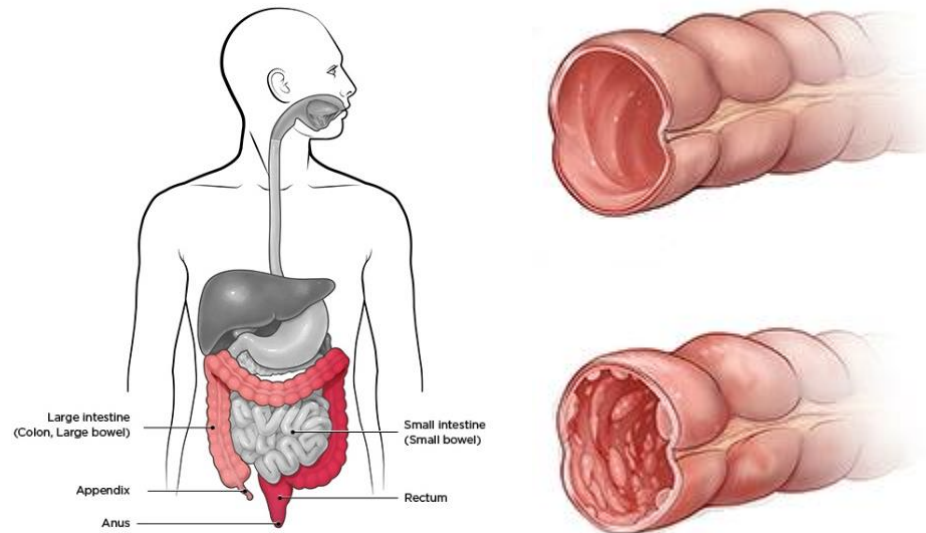
- Non-infectious chronic inflammation of the GI tract
- Affects ~1.6 million Americans
- Most diagnosed before 35-years-old
- Includes Ulcerative Colitis (UC) and Crohn's disease (CD)



# Inflammatory Bowel Disease (IBD)

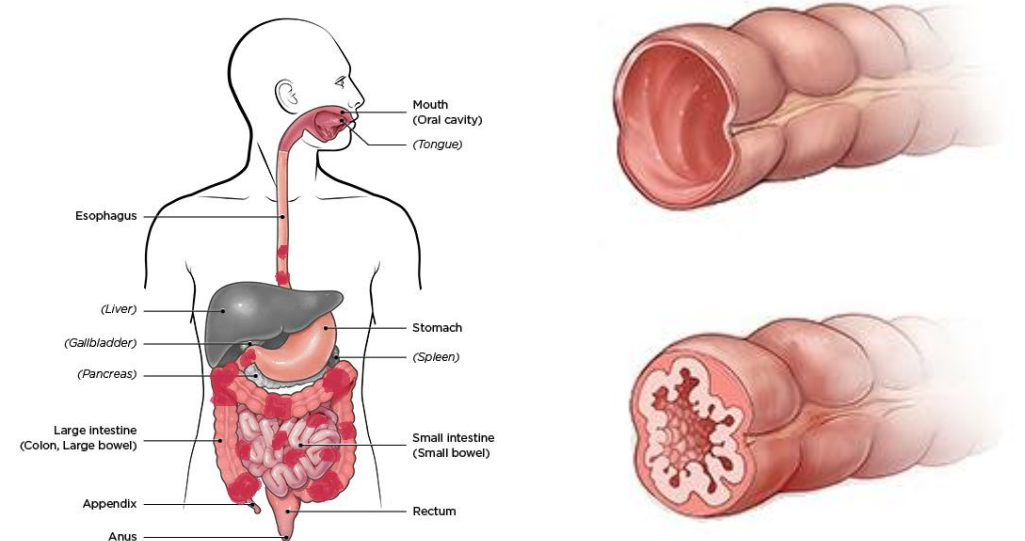
## Ulcerative Colitis (UC)

- Colon to rectum
- Continuous, diffuse inflammation of the mucosal layer



## Crohn's disease (CD)

- From “gum to bum”
- Discontinuous, patchy lesions with cobblestone appearance



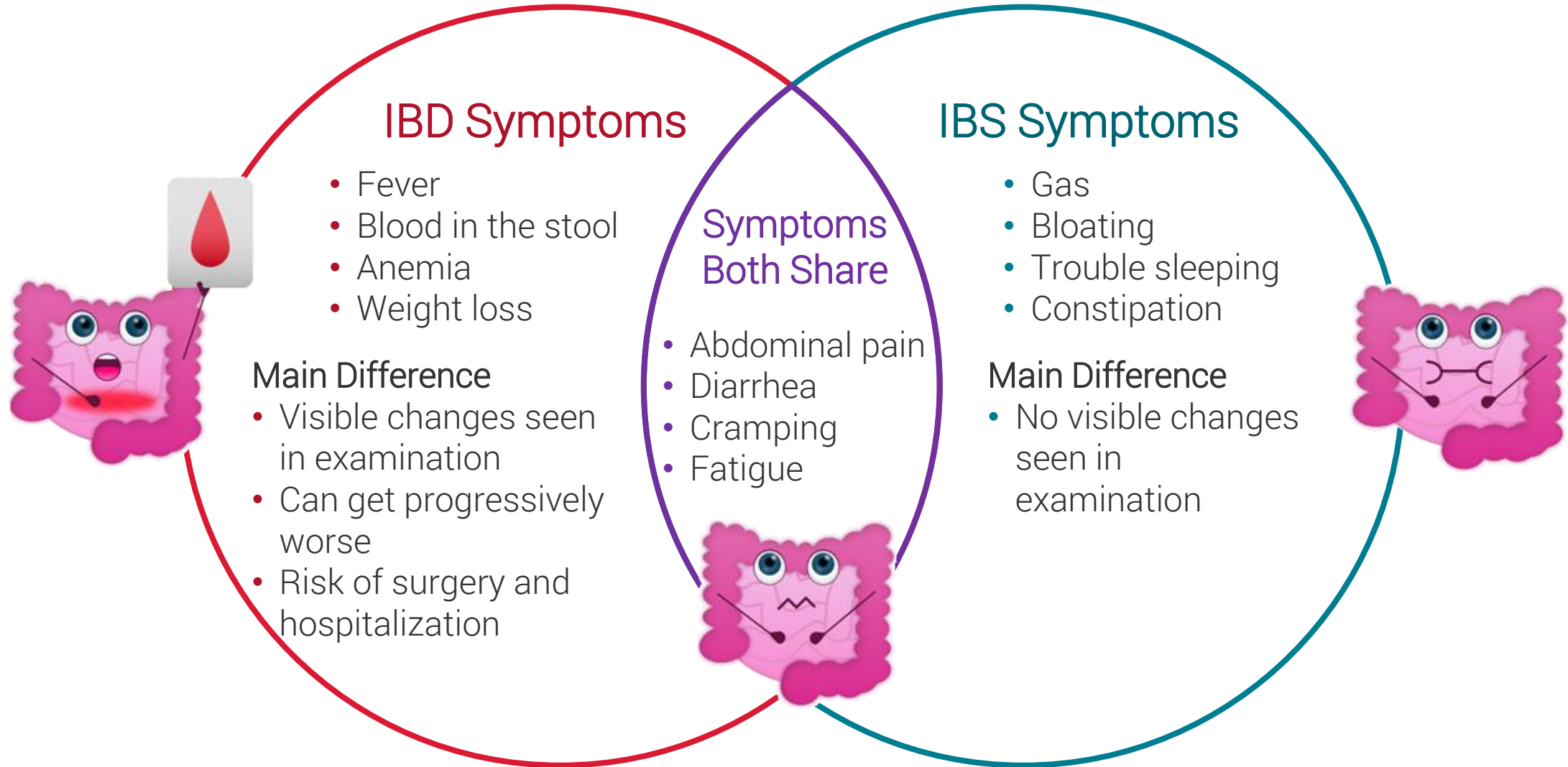
# Irritable Bowel Syndrome (IBS)

- Functional bowel disorder (clinical diagnosis)
- Prevalence: 10 – 15%
- Onset: 20 – 30 years old
- Rome IV Diagnostic Criteria
  - » **Recurrent abdominal pain, on average, at least 1 day per week in the last 3 months, associated with  $\geq 2$  of the following:**
    - Related to defecation
    - Associated with change in frequency of stool
    - Associated with change in form (appearance) of stool

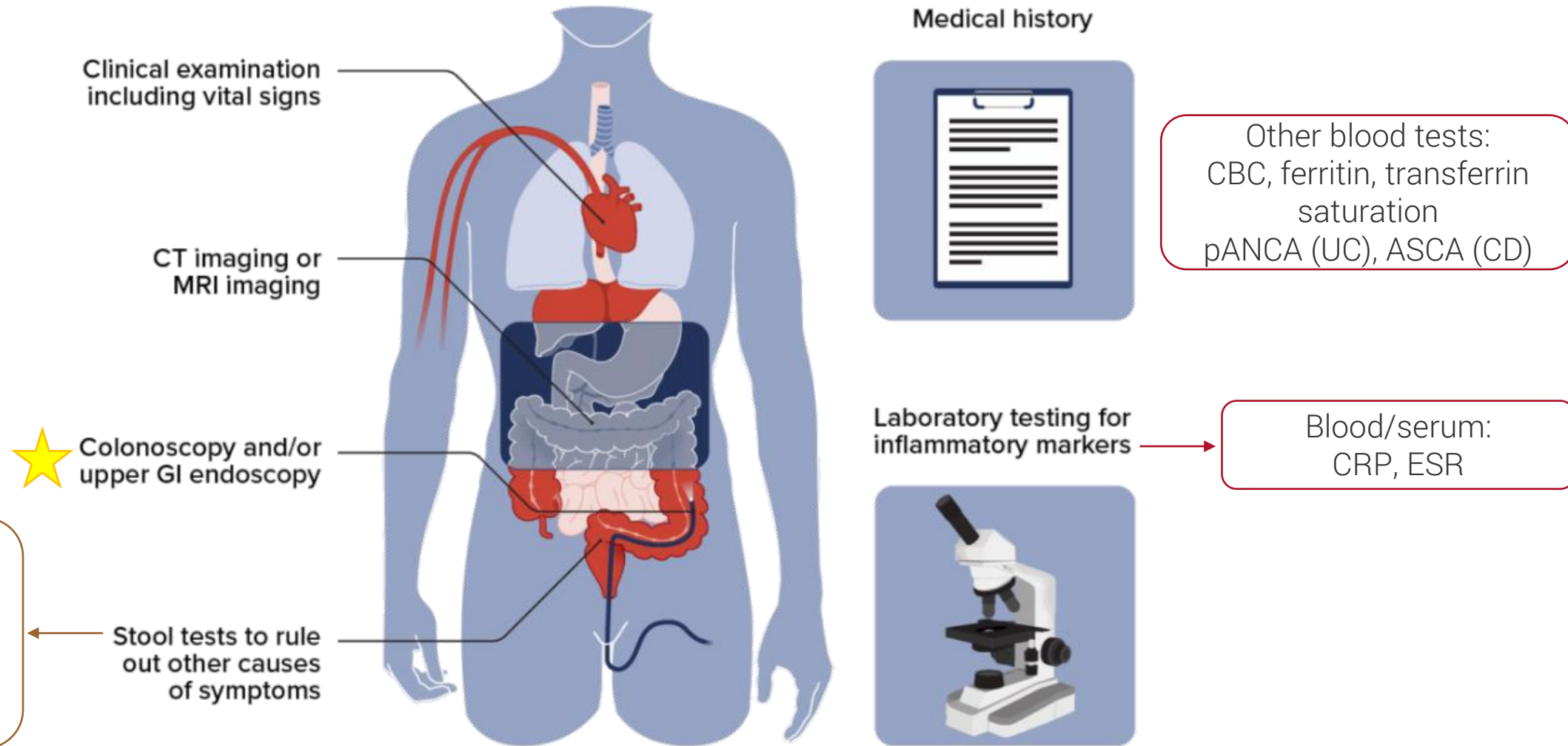


Image: The Awkward Yeti

# Symptoms of IBD vs. IBS



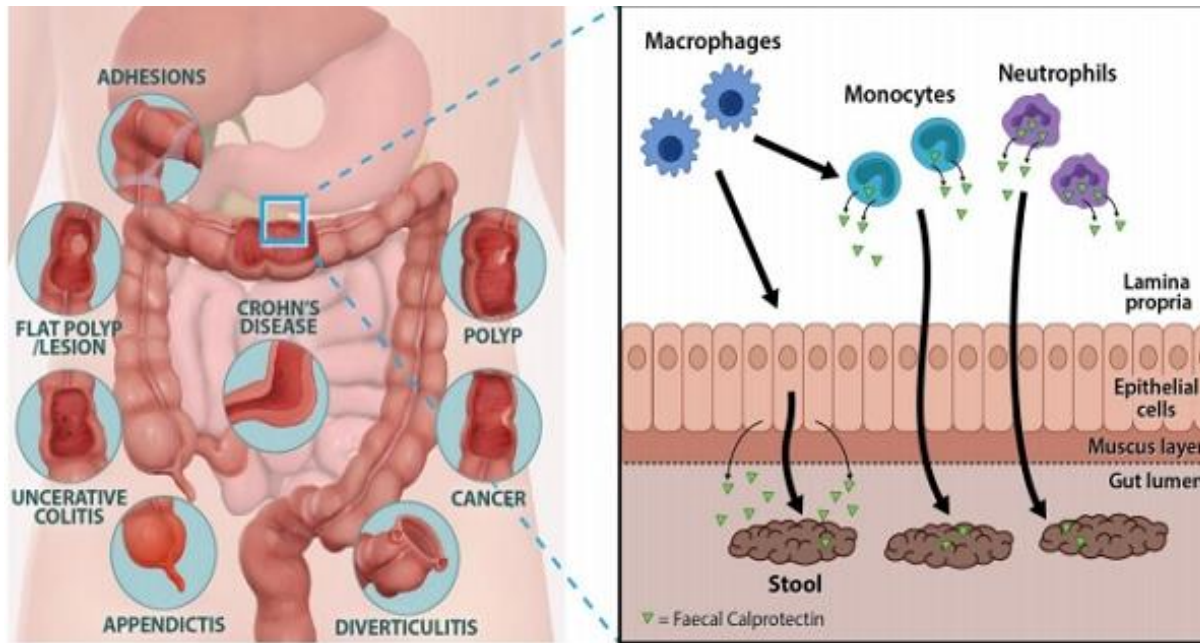
# Diagnosis of IBD



ESR, erythrocyte sedimentation rate; CRP, c-reactive protein; pANCA, perinuclear antineutrophil cytoplasmic antibody; ASCA, anti-Saccharomyces cerevisiae antibody



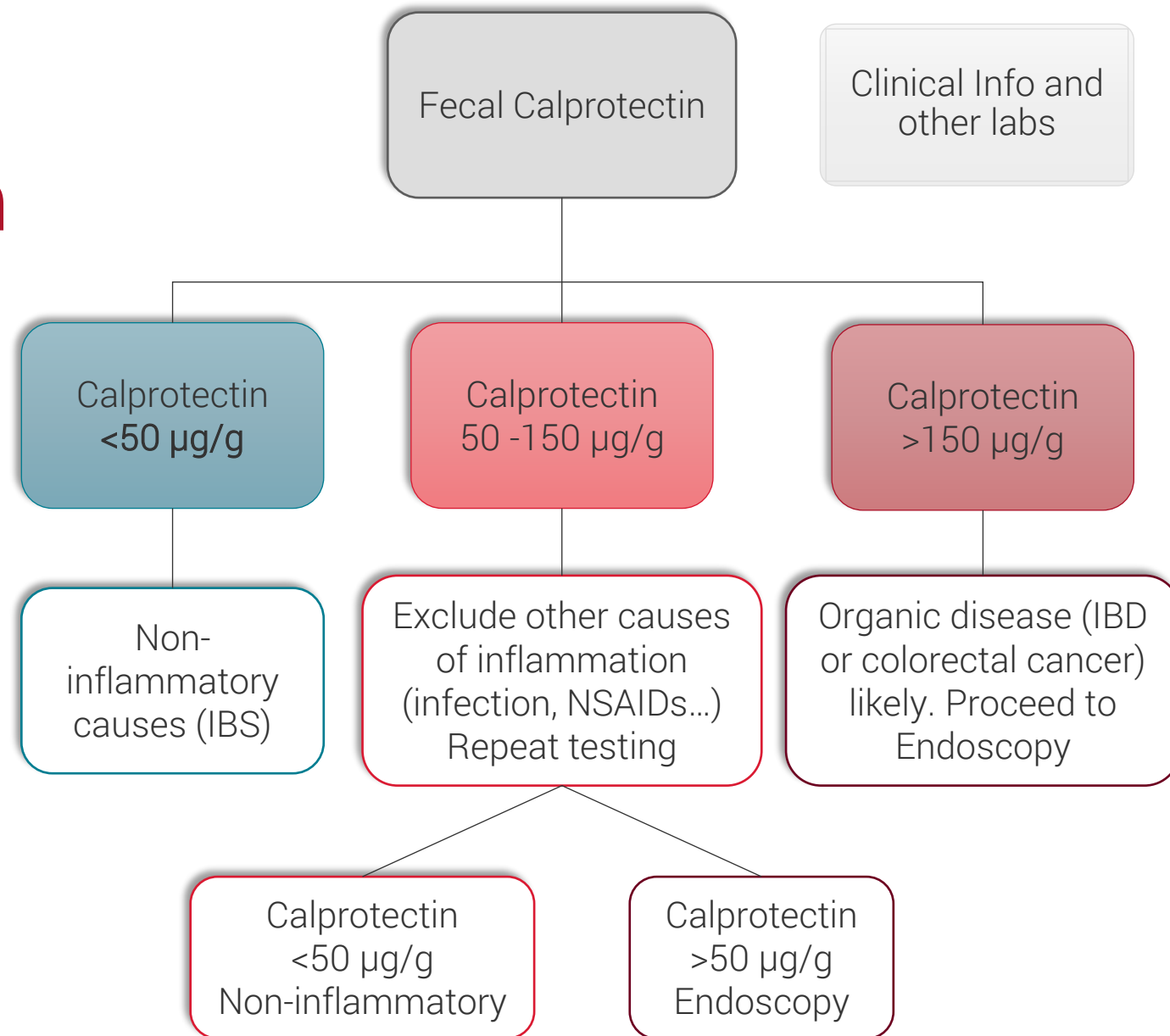
# Calprotectin: A Marker of Inflammation



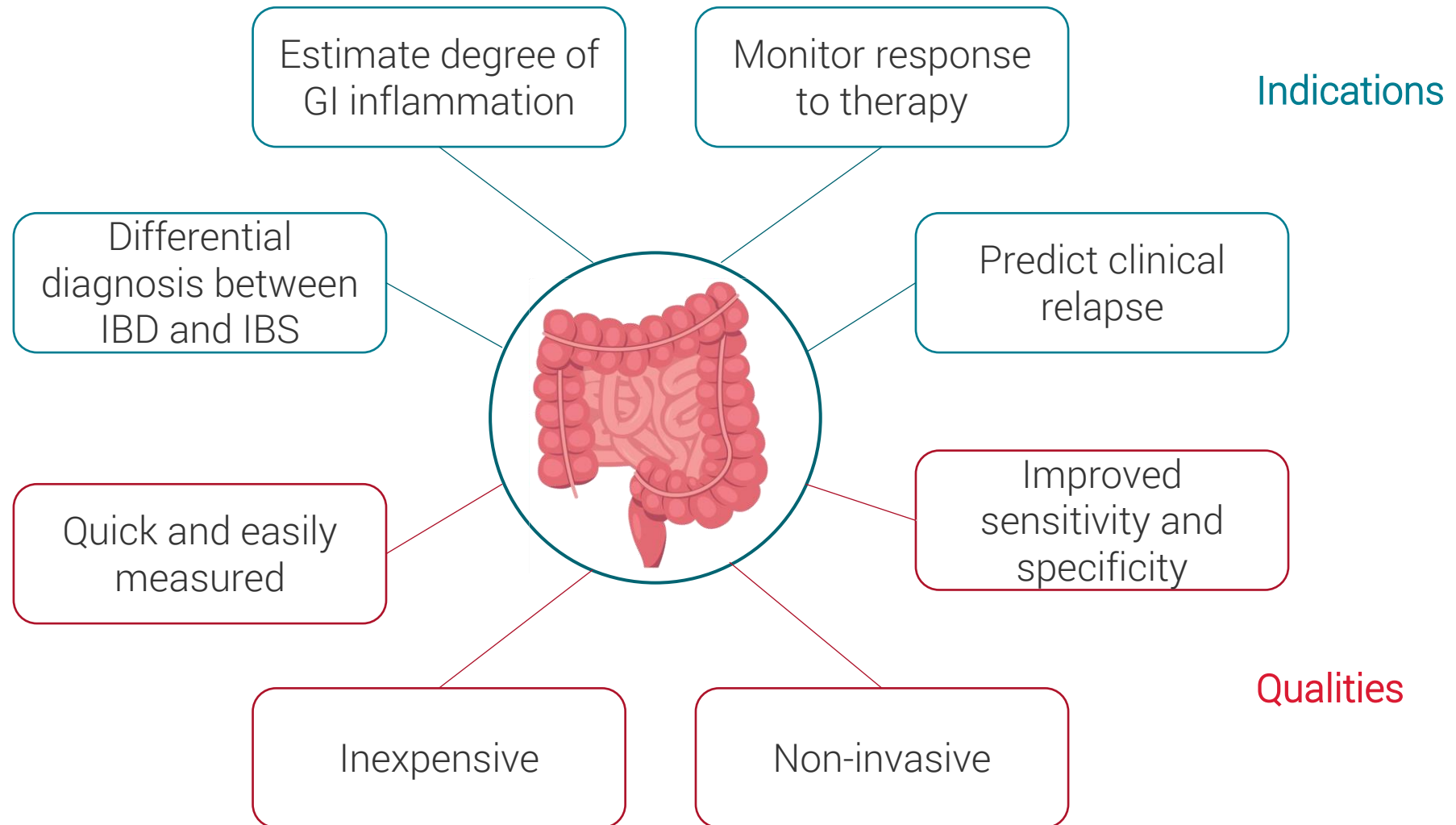
- Calcium- and zinc-binding protein
- Predominant protein in cytosol of neutrophils (~60%)
- Activation of neutrophils → release calprotectin
- Accumulates in feces
  - » Stable several days after excretion

# Clinical Utility of Fecal Calprotectin

- Sensitive biomarker for inflammation
  - » Not specific for IBD
- 50 µg/g – upper limit of the reference range in adults
  - » Assays are not standardized
  - » Higher in infants and adults >60 years old

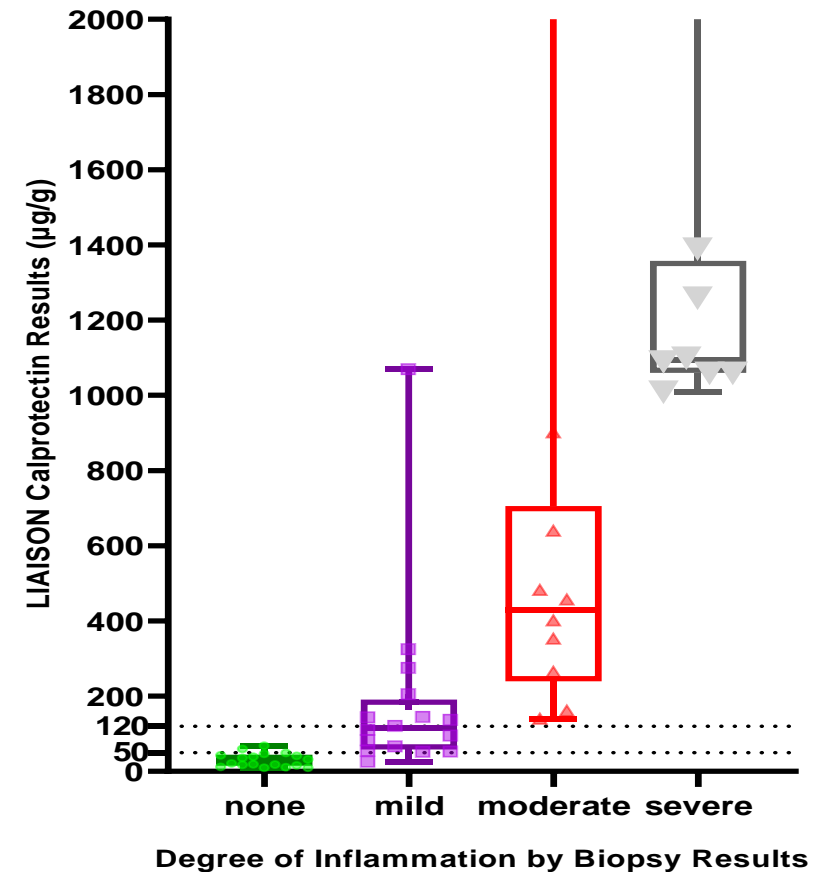


# Clinical Utility of Fecal Calprotectin



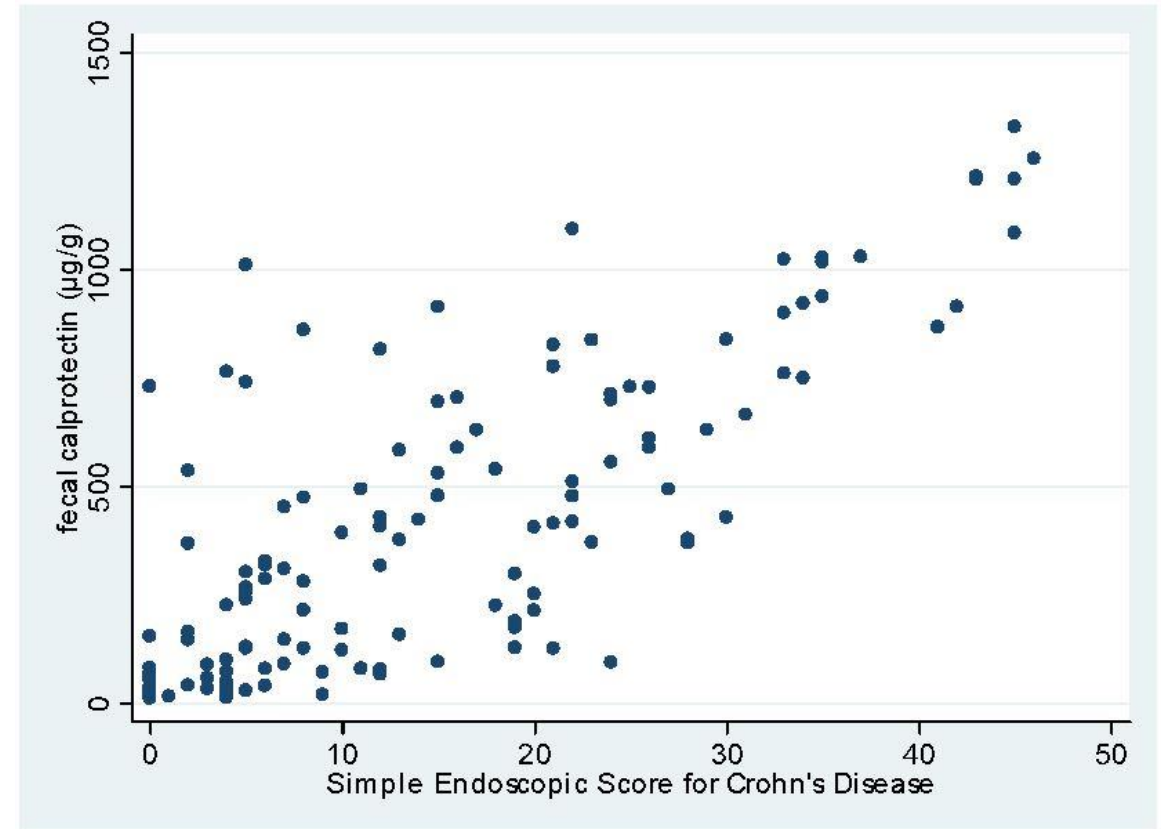
# Fecal Calprotectin Correlates with Disease Activity

- Compared calprotectin result to gold standard
  - » 52 stool samples clinically characterized (endoscopy with biopsy)



# Fecal Calprotectin for Monitoring Disease

- Simple Endoscopic Score for Crohn Disease (SES-CD)
  - » Inactive (remission): 0 – 3
  - » Mild activity: 4 – 10
  - » Moderate activity: 11 – 19
  - » High activity:  $\geq 20$
- 140 CD patients; 40 control





# Management of Crohn's Disease

Gastroenterology 2023;165:1367–1399

## GUIDELINES

### AGA Clinical Practice Guideline on the Role of Biomarkers for the Management of Crohn's Disease



- Use combination of biomarkers and symptoms
- Fecal calprotectin and serum CRP used to assess disease status

- » Fecal calprotectin  $>150 \mu\text{g/g}$  suggests significant inflammation in colon or small intestine
- » Serum CRP  $>5\text{mg/L}$ , inflammation
- » Reduces more invasive endoscopies

- When biomarkers and symptoms are discordant → Endoscopy

Patient status	Biomarkers checked
Remission	Every 6 – 12 months
Active symptoms	Every 2 – 4 months

AGA, American Gastroenterological Association

# Management of Ulcerative Colitis

Gastroenterology 2023;164:344–372

## GUIDELINES

### AGA Clinical Practice Guideline on the Role of Biomarkers for the Management of Ulcerative Colitis



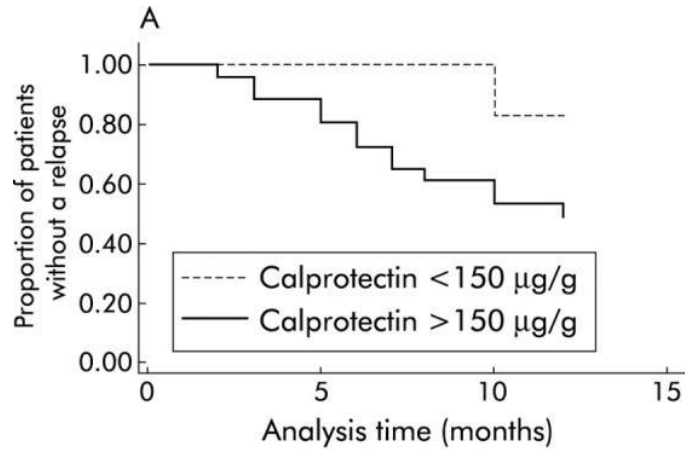
- Use combination of biomarkers and symptoms
- Fecal calprotectin or fecal lactoferrin and serum CRP used to assess disease status
  - » Suggestive of active inflammation:
    - Fecal calprotectin >150 µg/g
    - Abnormal fecal lactoferrin
    - Abnormal serum CRP
  - » Reduces more invasive endoscopies
- When biomarkers and symptoms are discordant → Endoscopy

Patient status	Biomarkers checked
Remission	Every 6 – 12 months
Active symptoms	Every 3 – 6 months

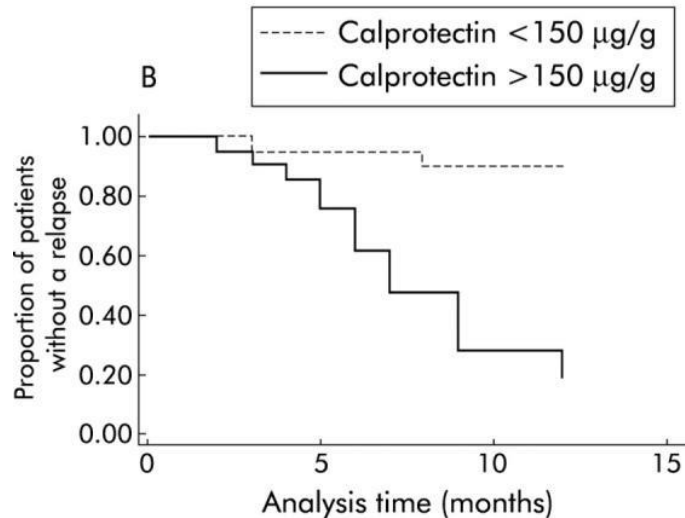
AGA, American Gastroenterological Association

# Fecal Calprotectin to Predict Relapse

Crohn's  
Disease



Ulcerative  
Colitis



- Calprotectin > 150  $\mu\text{g/g}$  stool is a predictor of relapse for both CD and UC
- ESR and CRP – not useful predictors of relapse

# Fecal Calprotectin Test Characteristics

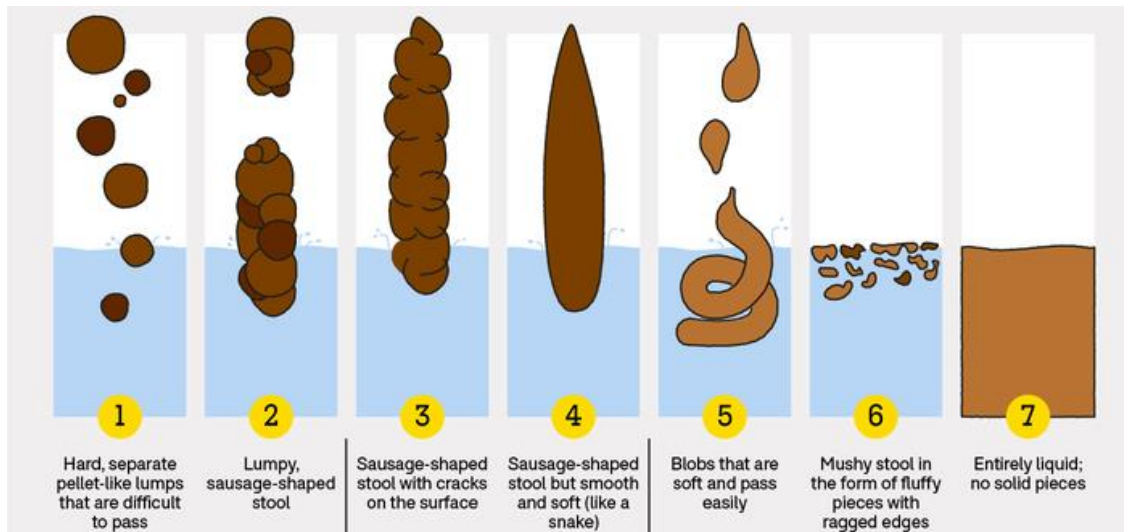
Test	Sensitivity	Specificity	PPV (%)	NPV (%)	Accuracy (%)
Calprotectin $\geq 50\mu\text{g/g}$	89	59	89	61	84
Calprotectin $\geq 100\mu\text{g/g}$	84	74	83	77	82
CRP $\geq 5\text{mg/L}$	68	58	88	29	66
WBC $\geq 7.9\text{G/L}$	55	50	83	21	54
CDAI $\geq 150$	33	68	80	20	40

CRP, C-reactive protein; WBC, white blood cells; CDAI, Crohn Disease Activity Index; PPV, positive predictive value; NPV, negative predictive value

# Measuring Fecal Calprotectin

## Sample

- Random stool
- Stable at room temperature 3 – 7 days

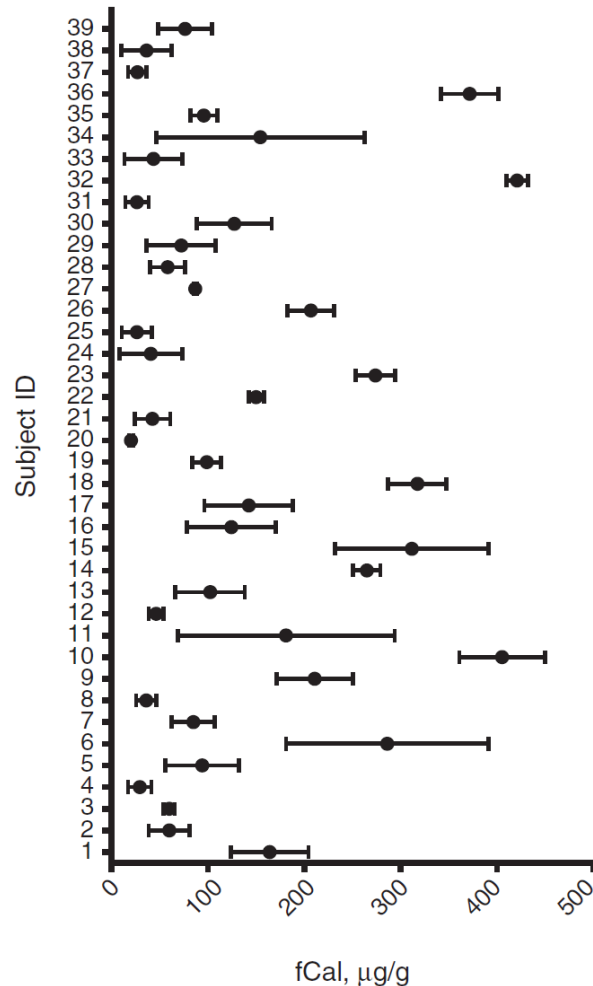


## Challenges

- Heterogeneous
  - » Undigested food
  - » Mucus
  - » Fibers
- Bristol Stool Types
  - » Variable water content
    - No normalization
- Day-to-day variability



# Intra- and Inter-Individual Variability



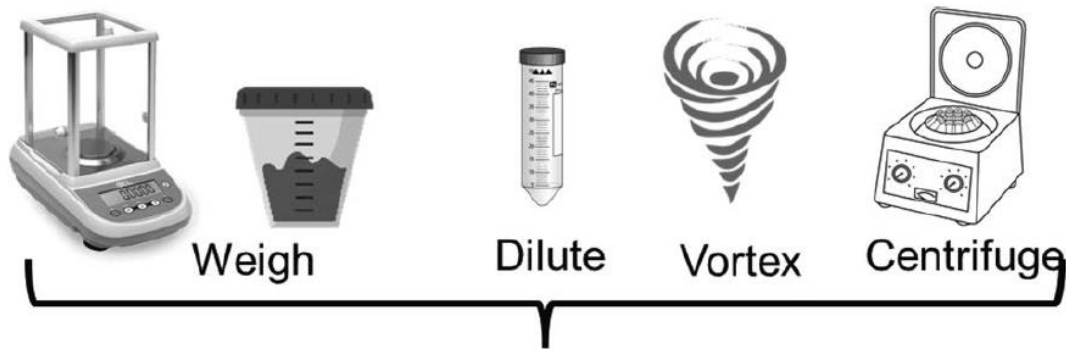
## Study

- 50 UC individuals  
» CV: 5 – 114%
- 39 Healthy individuals  
» CV: 30 – 40%

	Calprotectin ELISA
Intra-individual (CVi)	37.7%
Inter-individual (CVg)	78.0%
Reference Change Value	118%

# Extraction of Calprotectin

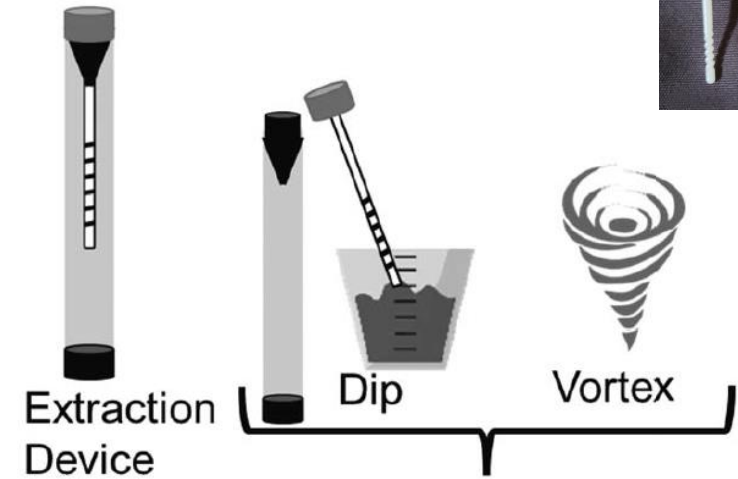
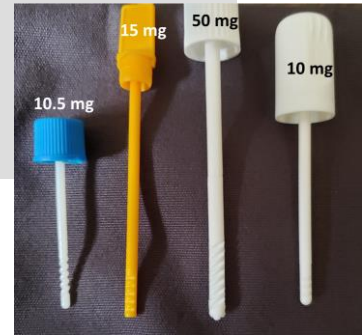
## Manual



Manual Extraction Components

Weigh 50 – 100 mg of stool

## Device



Device Extraction Components

Grooves collect 10 – 50 mg of stool

# Comparison of Extraction Methods

## Manual

- Uses more stool
- Heterogenous stool samples
- Liquid stool samples
- Requires more time and effort



## Device

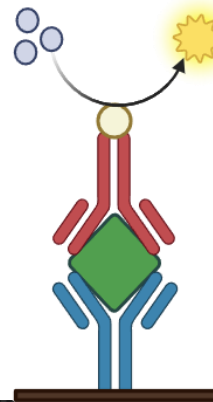
- Uses less stool
- Best for homogenous samples
- More efficient
- Differences in extract stability



# Assays

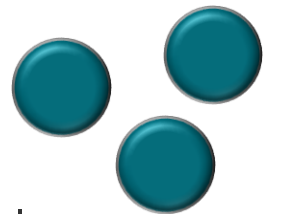
## ELISA

- Commercial assays available
- Batch
- Dilution for higher concentrations
- Steps can be performed manually
- Plate reader

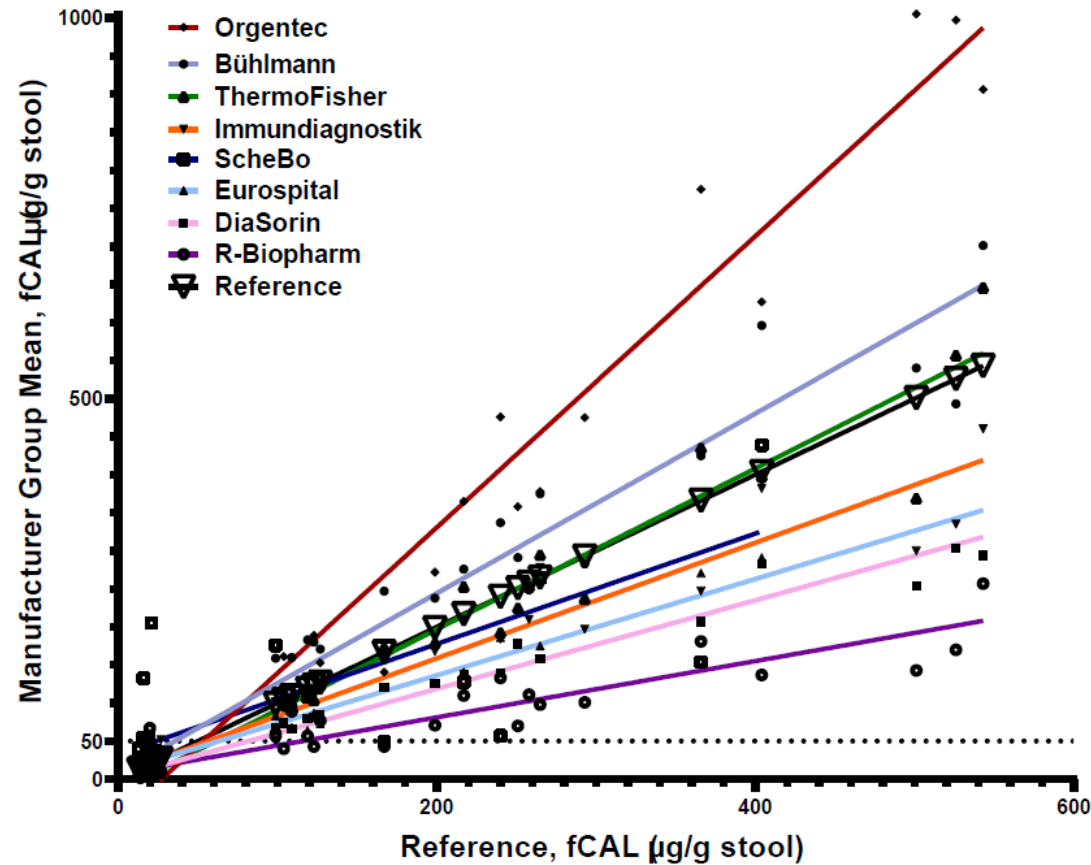


## Bead

- Commercial assays available
- Random access
- Chemiluminescence, fluorescence, immunoturbidimetry
- Dilutions performed on instrument
- Requires immunoassay analyzer



# Lack of Standardization for Calprotectin

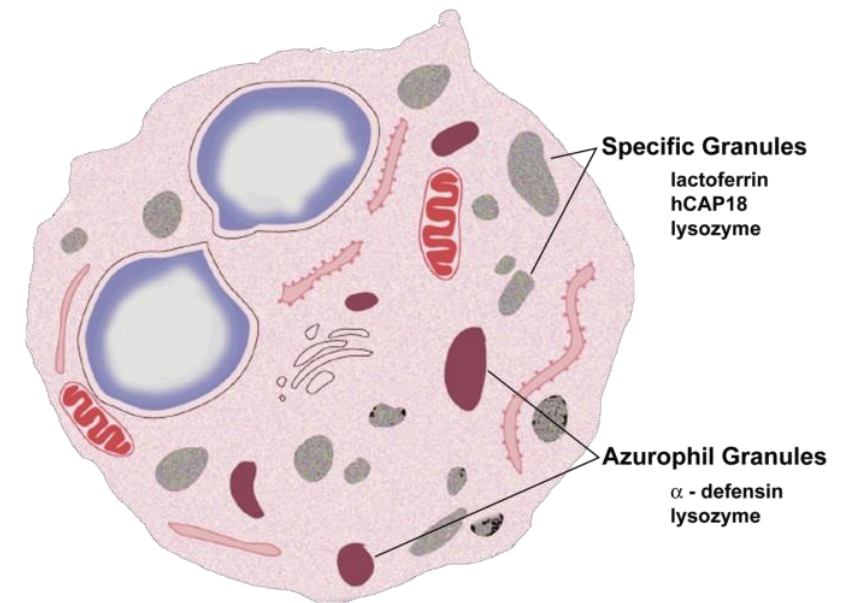


- INSTAND fecal diagnostics proficiency testing surveys (2015 – 2020)
  - » Average result of submitted responses for each manufacturer



# Lactoferrin

- 78 kDa iron binding glycoprotein of transferrin family
- Major component of secondary granules of neutrophils
  - » Secreted in gut during intestinal inflammation
  - » Stable in feces at room temperature for days
- Quantitative and qualitative commercial assays available
- Normal range: <7.25 µg/g



# Lactoferrin Clinical Performance

- Correlates strongly with Calprotectin
- Similar diagnostic sensitivity (67 – 91%) and specificity (90 – 100%) as calprotectin (78 - 100% and 76 - 100%) for IBD
- Useful to identify inflammatory process, monitor response to therapy, and predict relapse

# Conclusions

## IBD Versus IBS

- Similar clinical presentation: abdominal pain, diarrhea, cramping and fatigue
- IBD is an inflammatory disease
  - » UC – Colon to rectum, continuous
  - » CD – Gum to bum, patches
- IBS is a functional disorder

## Calprotectin/Lactoferrin

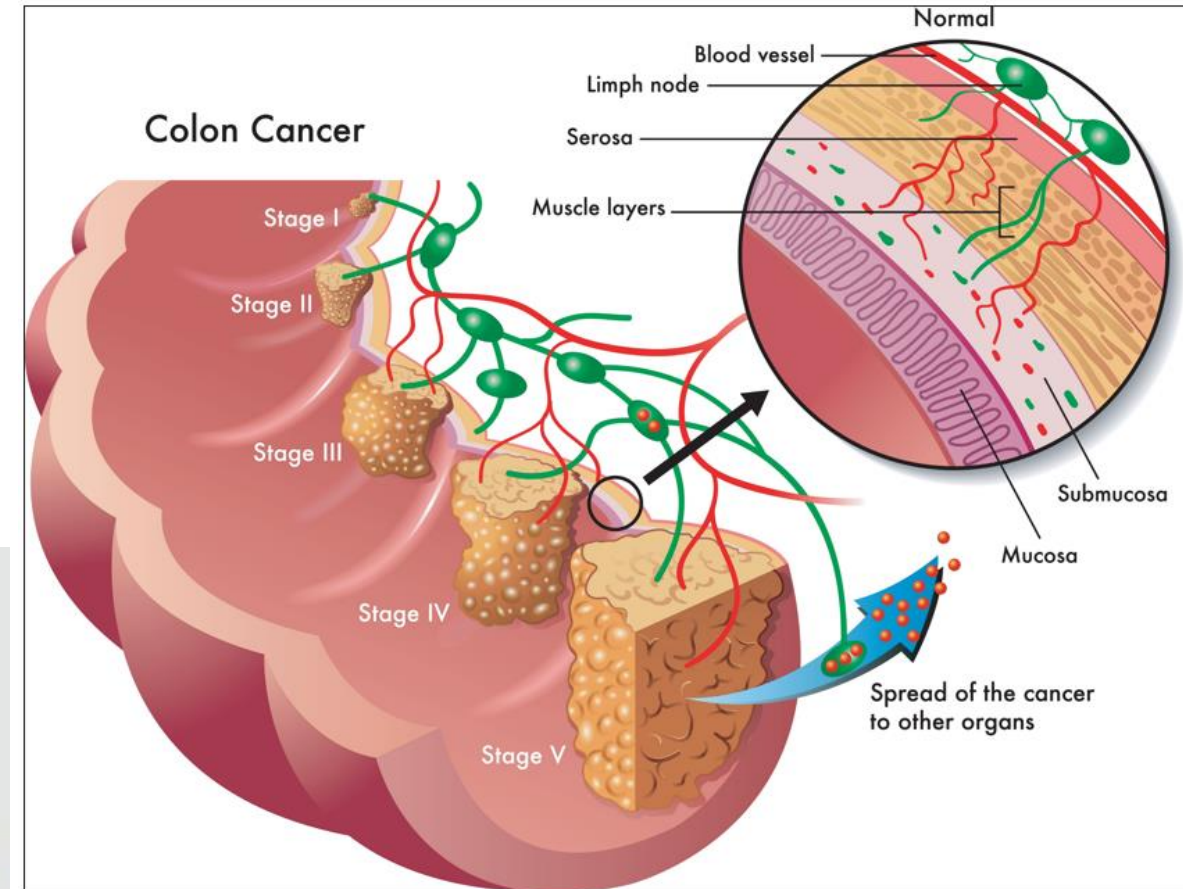
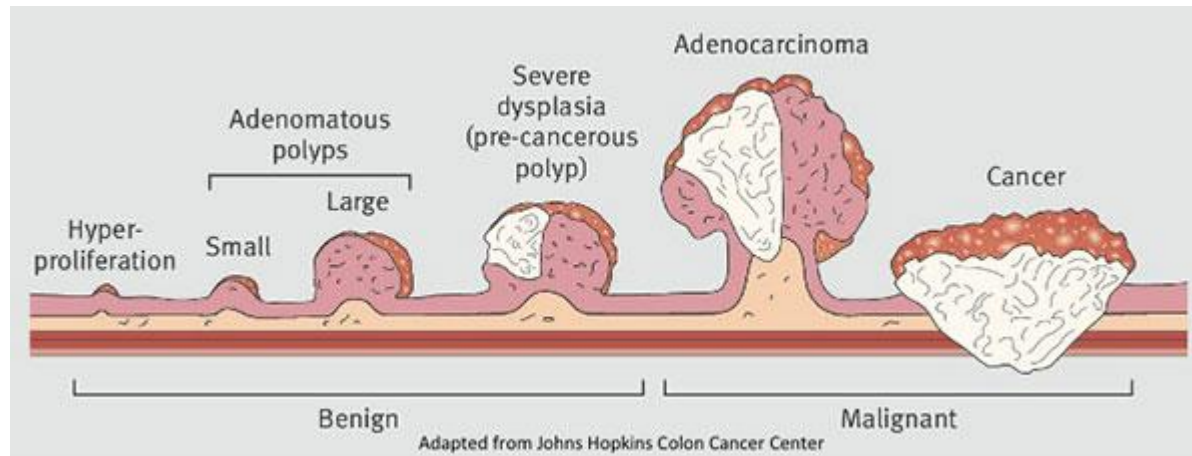
- High sensitivity for detecting patients with IBD
  - » Abnormal in IBD, nonsteroidal enteropathy, and **colorectal carcinoma**
- Correlates with disease severity and mucosal healing
- Predict relapse



# ■ Colorectal Cancer

# Colorectal Cancer

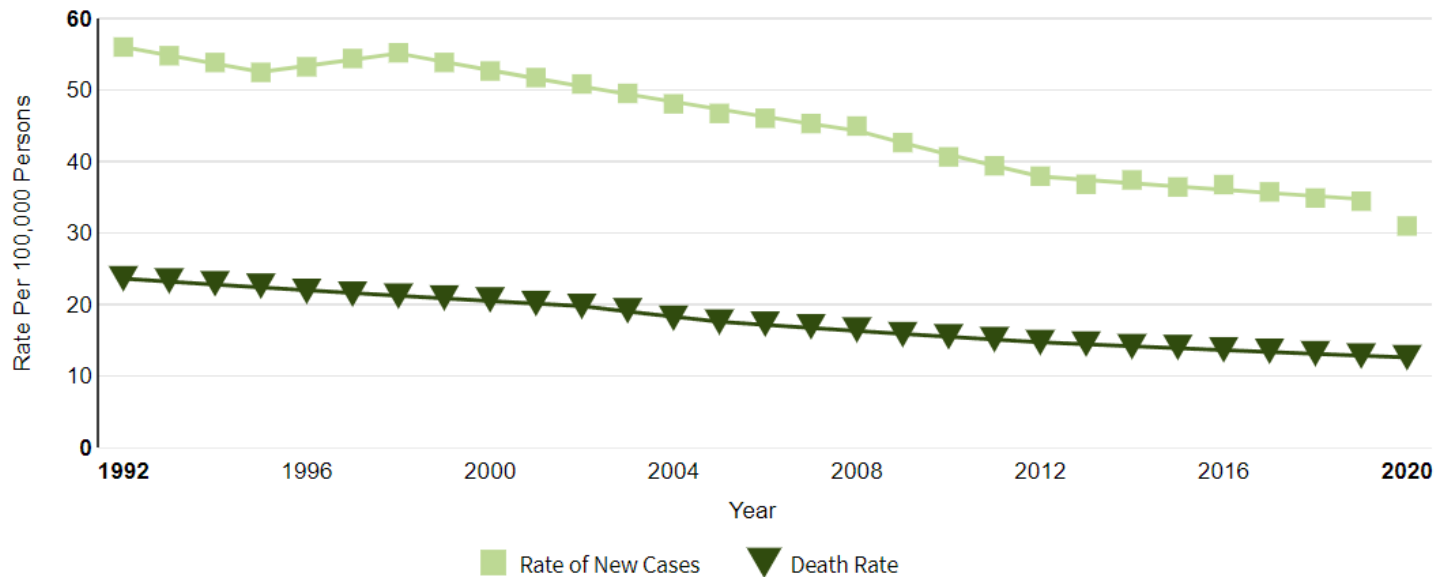
- Cancer starting in the colon or rectum
  - » Typically adenocarcinomas
  - » Most start as polyp on inner lining
  - » Genetic and epigenetic alterations





# Prevalence

- 4<sup>th</sup> most common cancer in US
  - » 2<sup>nd</sup> for cancer-related deaths



Estimated New Cases in 2023	153,020
% of All New Cancer Cases	7.8%
Estimated Deaths in 2023	52,550
% of All Cancer Deaths	8.6%

# Risk Factors

## Nonmodifiable



\*Age\*  
>50 years



Family  
History



Inflammatory  
Bowel Disease

## Modifiable



Obesity



Physical  
Inactivity



Diet  
(↑ red/processed meat)



Excess  
Alcohol



Smoking

# Screening Guidelines

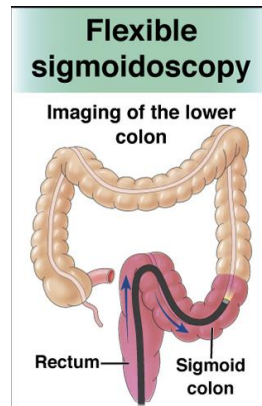
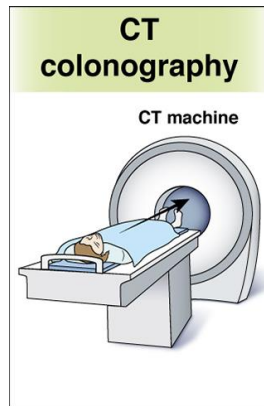
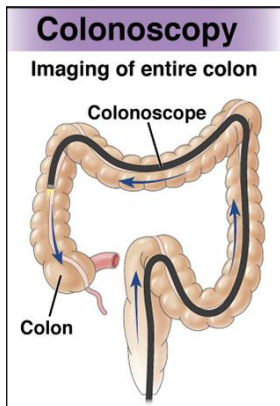
Risk Level	Age	Recommendation	Grade
Average	45 – 49 years	Start screening	B
	50 – 75 years	Screen	A
	> 75 years	Selective screening	C
High Risk	40 years	Start screening	B

US Preventative Services Task Force. 2021  
American College of Gastroenterology. 2022  
American Cancer Society

# Screening Tests

## Direct Visualization

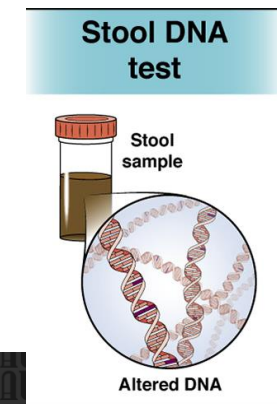
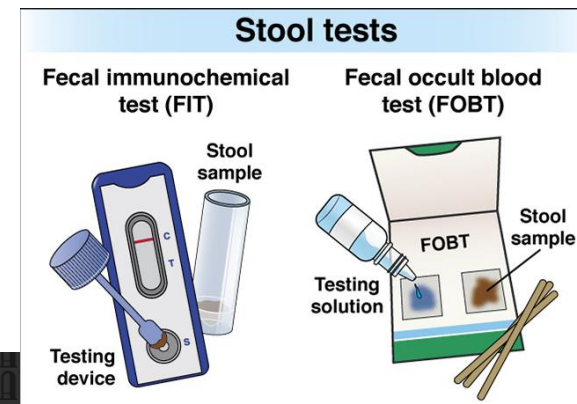
Test	Frequency
Colonoscopy	Every 10 years
CT colonography	Every 5 years
Flexible sigmoidoscopy	Every 5 years



## Stool-based

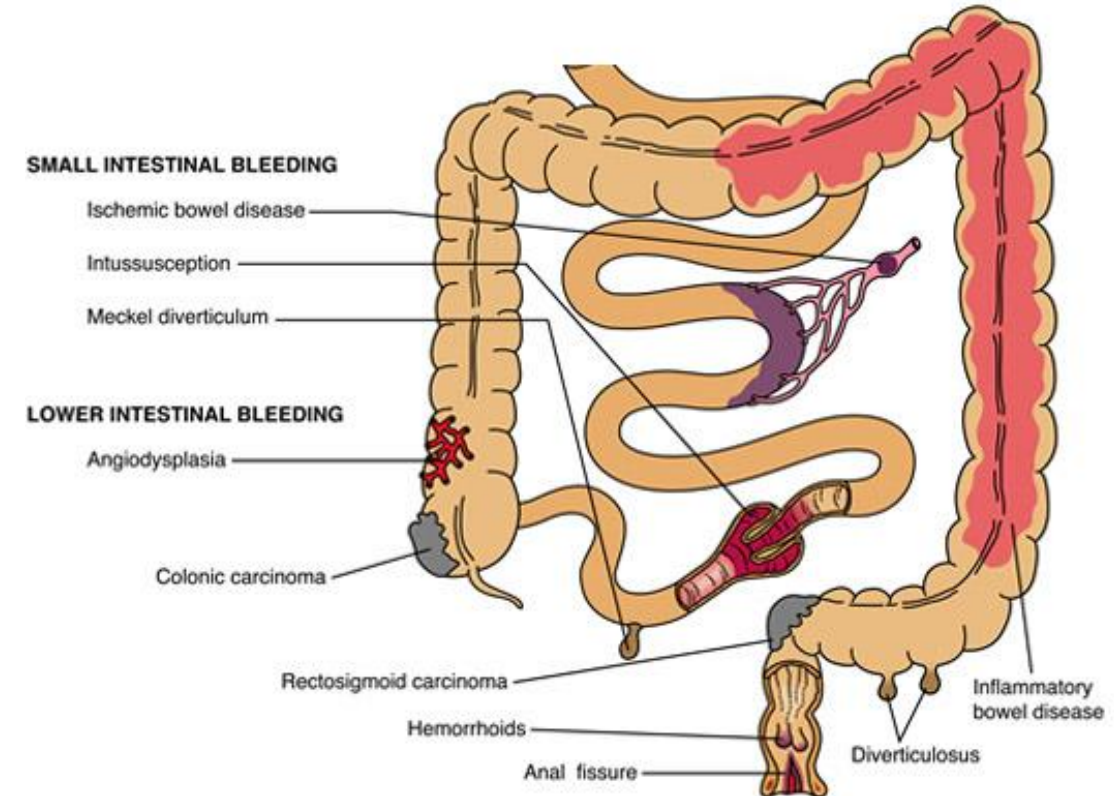
Test	Frequency
High-sensitivity gFOBT	Every year
FIT	Every year
sDNA-FIT	Every 3 years

gFOBT, guaiac fecal occult blood test; FIT, fecal immunochemical test; sDNA-FIT, stool DNA test with fecal immunochemical test



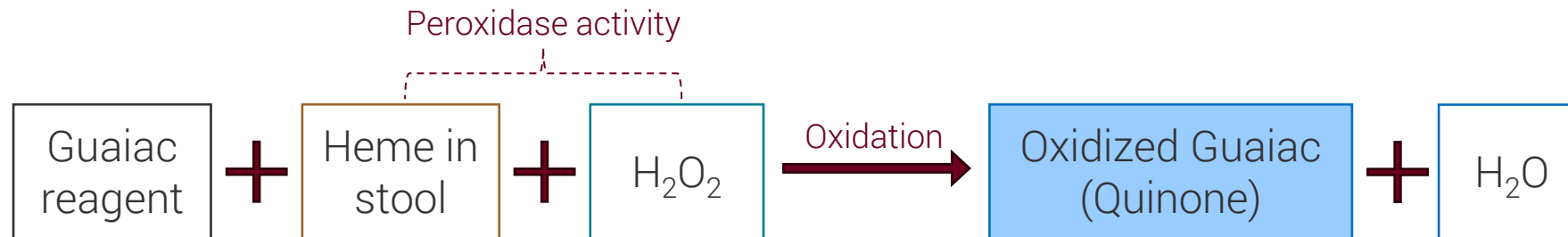
# Fecal Occult Blood (FOB) Test

- Test for hidden (occult) blood in stool
- Indications:
  - » Colon Cancer screening
  - » Anemia
  - » Suspected GI bleeding
  - » IBD vs IBS
- Tests to detect fecal occult blood:
  - » Guaiac (gFOBT)
  - » Immunochemical (FIT)

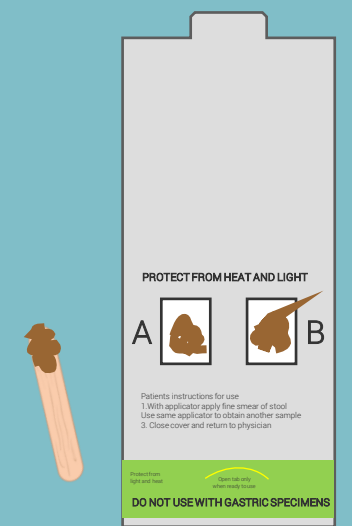


# Guaiac-based Tests (gFOB)

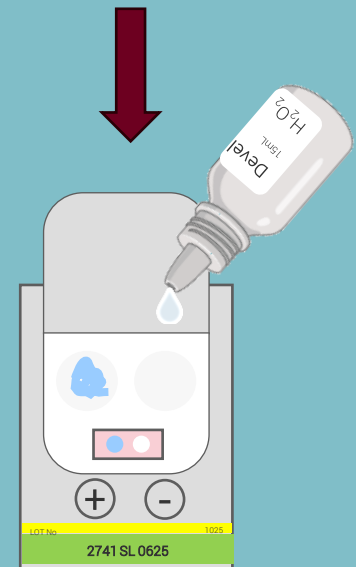
- Detect heme in stool on guaiac paper



- High-sensitivity gFOB: Added enhancer to developer to lower detection limit



Apply thin smear of stool on card



Drop developer on sample



# Guaiac-based Tests

## ADVANTAGES

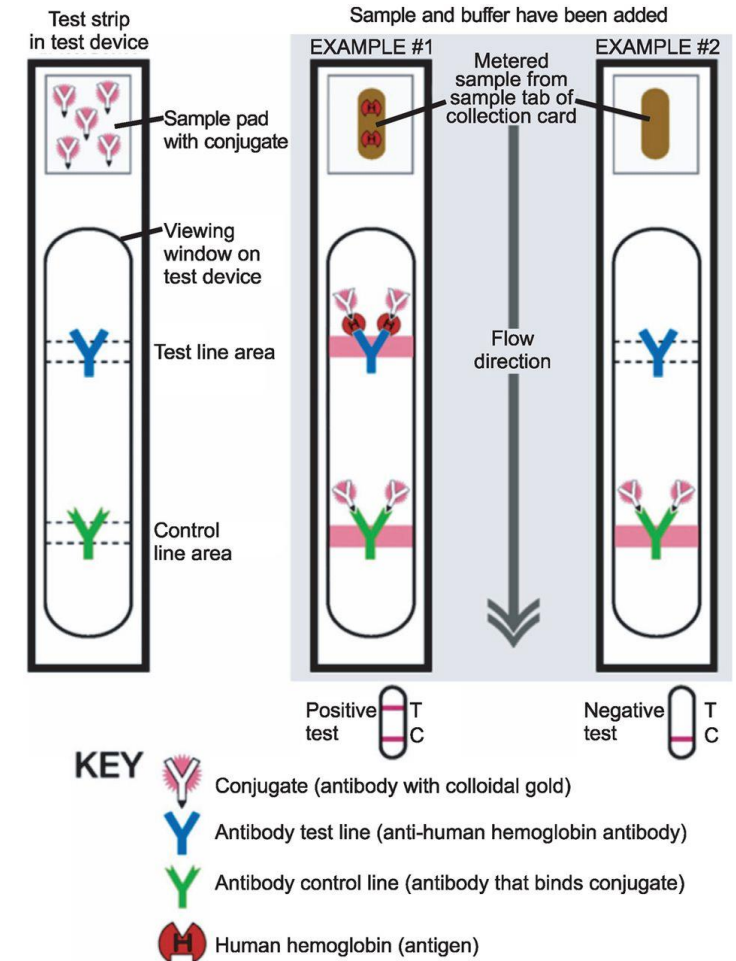
- CLIA-waived
- Inexpensive
- Fast and simple

## LIMITATIONS

- Prone to interference (requires restricted diet)
  - » Peroxidase activity in raw fruits and vegetables, red meat → False Positive
  - » Vitamin C → False negative
- Requires testing 3 stool samples

# Fecal Immunochemical Tests (FIT)

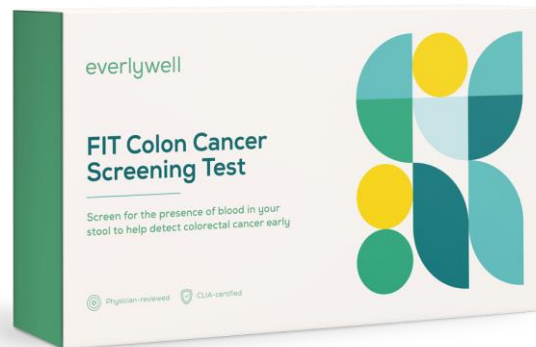
- Uses antibodies specific to human globin
- Manual format
  - » Qualitative result
  - » CLIA-waived
- Automated format
  - » Quantitative or Qualitative
    - Positivity cutoffs differ by manufacturer
  - » Not CLIA-waived
  - » Requires instrumentation
  - » Better reproducibility



# FIT Advantages

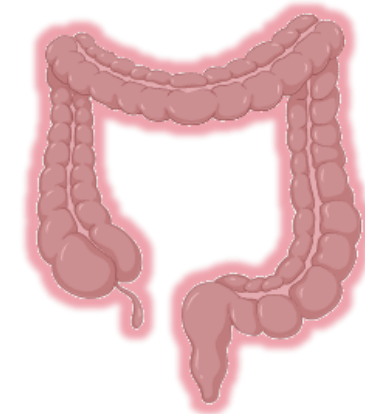
## ADVANTAGES

- No dietary or drug restrictions
- Improved sensitivity and specificity compared to guaiac methods

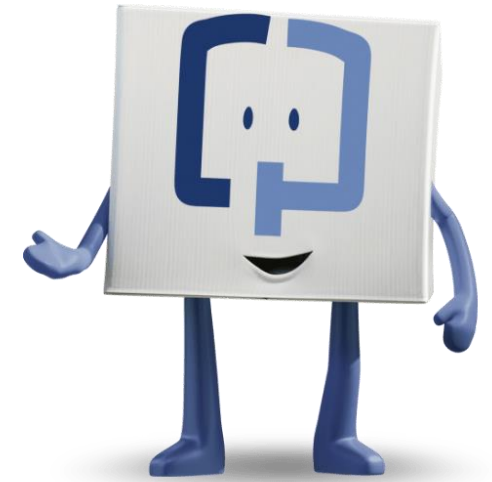


## LIMITATION?

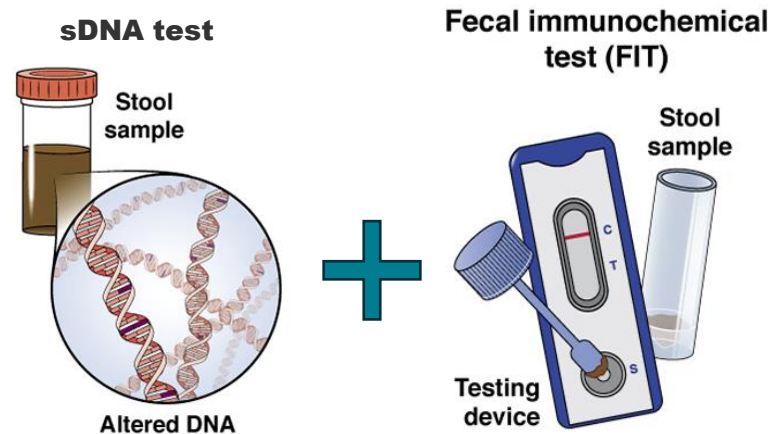
- Only sensitive for blood from lower GI tract (colon specific!)
  - » Globin in upper GI is hydrolyzed



# Stool DNA with FIT (Cologuard)



- Multitarget stool DNA (MT-sDNA) testing
  - » Blood (FIT)
  - » Altered DNA from cells shed in stool
    - 2 methylated DNA markers, mutant *KRAS* (7 point mutations),  $\beta$ -actin
- Approved by FDA in 2014 for colorectal cancer screening in adults 45 – 75 years of age at average risk



# Cologaurd vs FIT

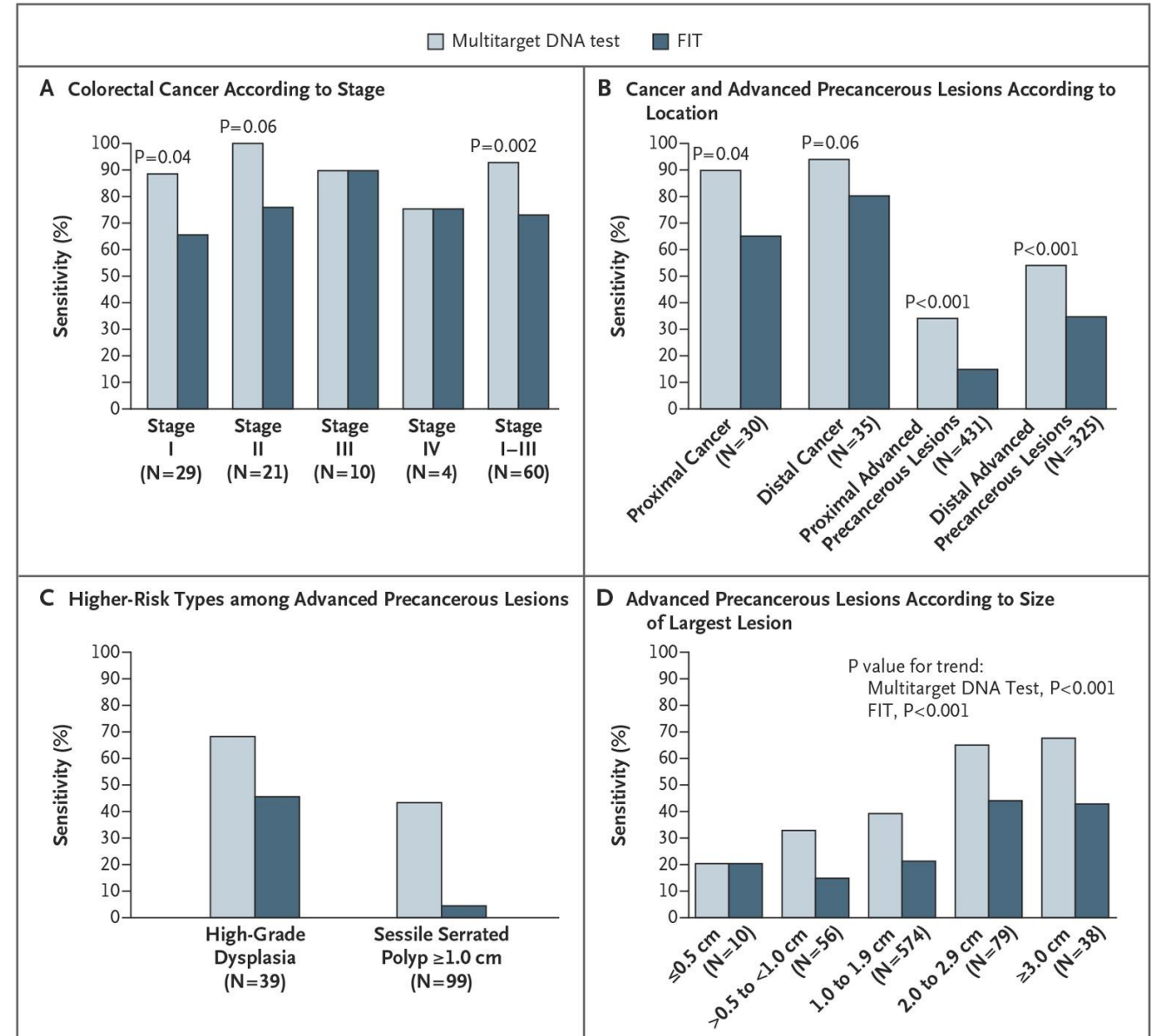
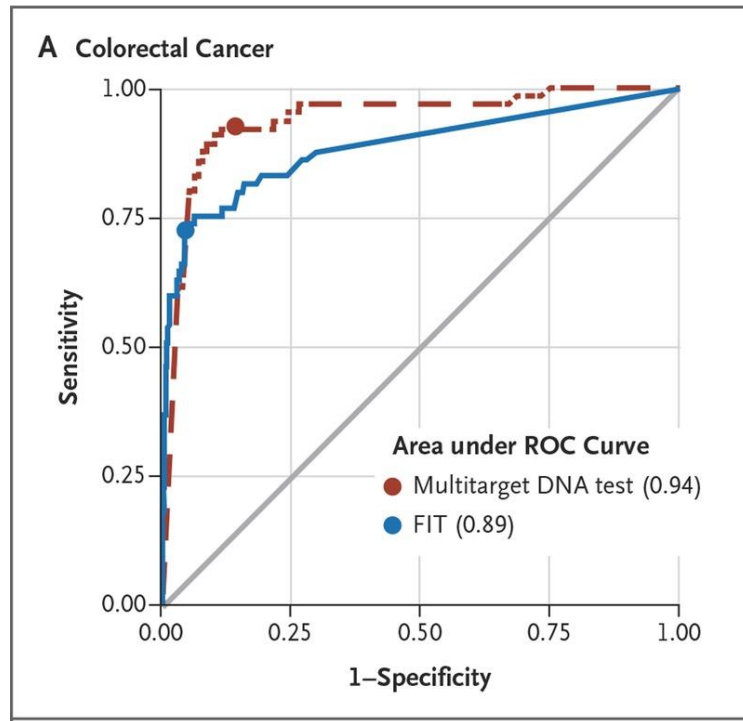
- 9989 asymptomatic study participants
  - » 65 – colorectal cancer
  - » 757 – Advanced precancerous lesions
- **Specificity** among those with negative results on colonoscopy
  - » Cologuard: 89.8%
  - » FIT: 96.4%

Most Advanced Finding	Colonoscopy (N = 9989)  no.	Multitarget DNA Test (N = 9989)		FIT (N = 9989)	
		Positive Results	Sensitivity (95% CI)	Positive Results	Sensitivity (95% CI)
		no.	%	no.	%
Colorectal cancer					
Any	65	60	92.3 (83.0–97.5)	48	73.8 (61.5–84.0)
Stage I to III*	60	56	93.3 (83.8–98.2)	44	73.3 (60.3–83.9)
Colorectal cancer and high-grade dysplasia	104	87	83.7 (75.1–90.2)	66	63.5 (53.5–72.7)
Advanced precancerous lesions†	757	321	42.4 (38.9–46.0)	180	23.8 (20.8–27.0)
Nonadvanced adenoma	2893	498	17.2 (15.9–18.6)	220	7.6 (6.7–8.6)
			Specificity (95% CI)		Specificity (95% CI)
All nonadvanced adenomas, non-neoplastic findings, and negative results on colonoscopy	9167	1231	86.6 (85.9–87.2)	472	94.9 (94.4–95.3)
Negative results on colonoscopy	4457	455	89.8 (88.9–90.7)	162	96.4 (95.8–96.9)

\* These stages of colorectal cancer, as defined by the system recommended by the American Joint Committee on Cancer, are associated with an increased rate of cure.

† Advanced precancerous lesions include advanced adenomas and sessile serrated polyps measuring 1 cm or more.

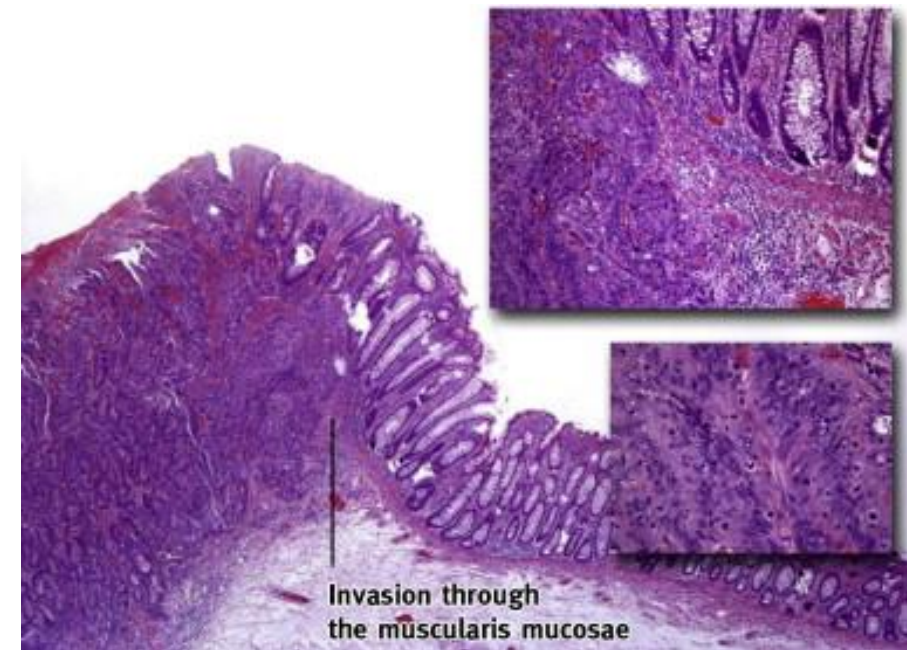
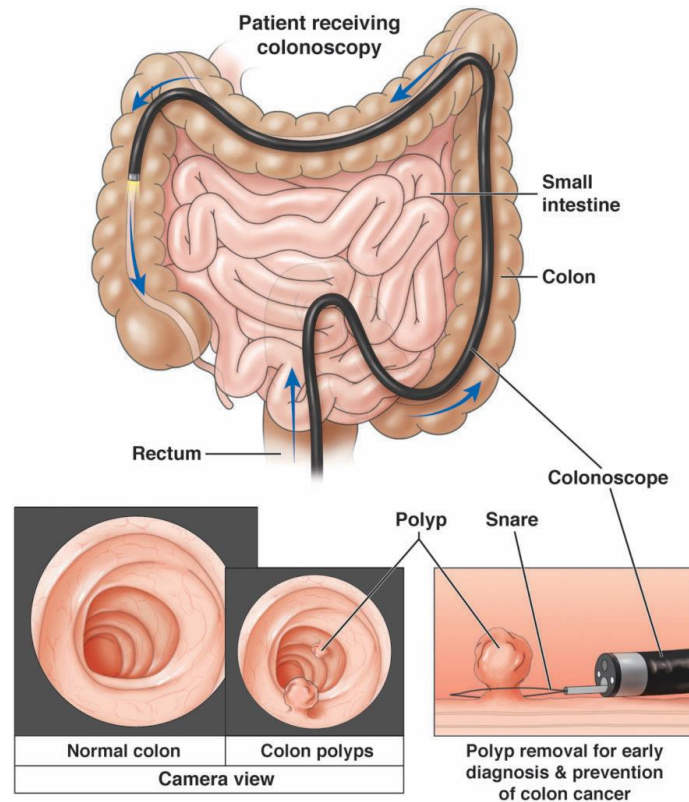
# Cologuard vs FIT





# Diagnosing Colon Cancer

- Colonoscopy with biopsy



Invasion of neoplastic cells through the muscularis mucosae into submucosa

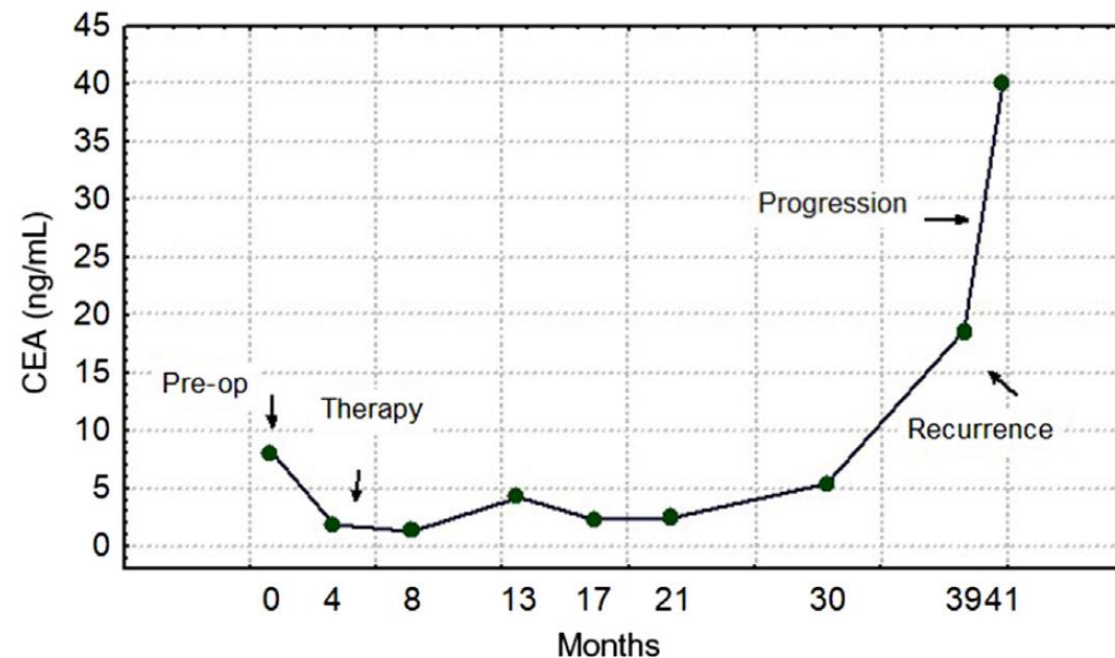
# Carcinoembryonic Antigen (CEA)

- Tumor marker
  - » Glycoprotein with molecular mass of 150 – 300 kDa, 45 – 55% carbohydrate
- Upper limit of **~3 ng/mL** (nonsmokers) and **~5 ng/mL** (smokers)\*
- Marker for:
  - » Colorectal cancer—monitor throughout therapy (elevated in 70%)
  - » GI cancers
    - Gastric—elevated in 50%
    - Pancreatic—elevated in 55%
  - » Lung—associated with non-small cell carcinoma (65% positive)
  - » Breast—associated with metastatic disease (elevated in 40%)
  - » Uterine—elevated in 40%
  - » Ovarian—elevated in 20%

\*Contemporary Practice in Clinical Chemistry, 4<sup>th</sup> Ed

# Carcinoembryonic Antigen (CEA)

- Monitor clinical course and therapy for colorectal cancer
  - » Baseline measurement then at 2 – 3 months for 3 years following surgery
  - » Continue to measure every 6 months until 5 years



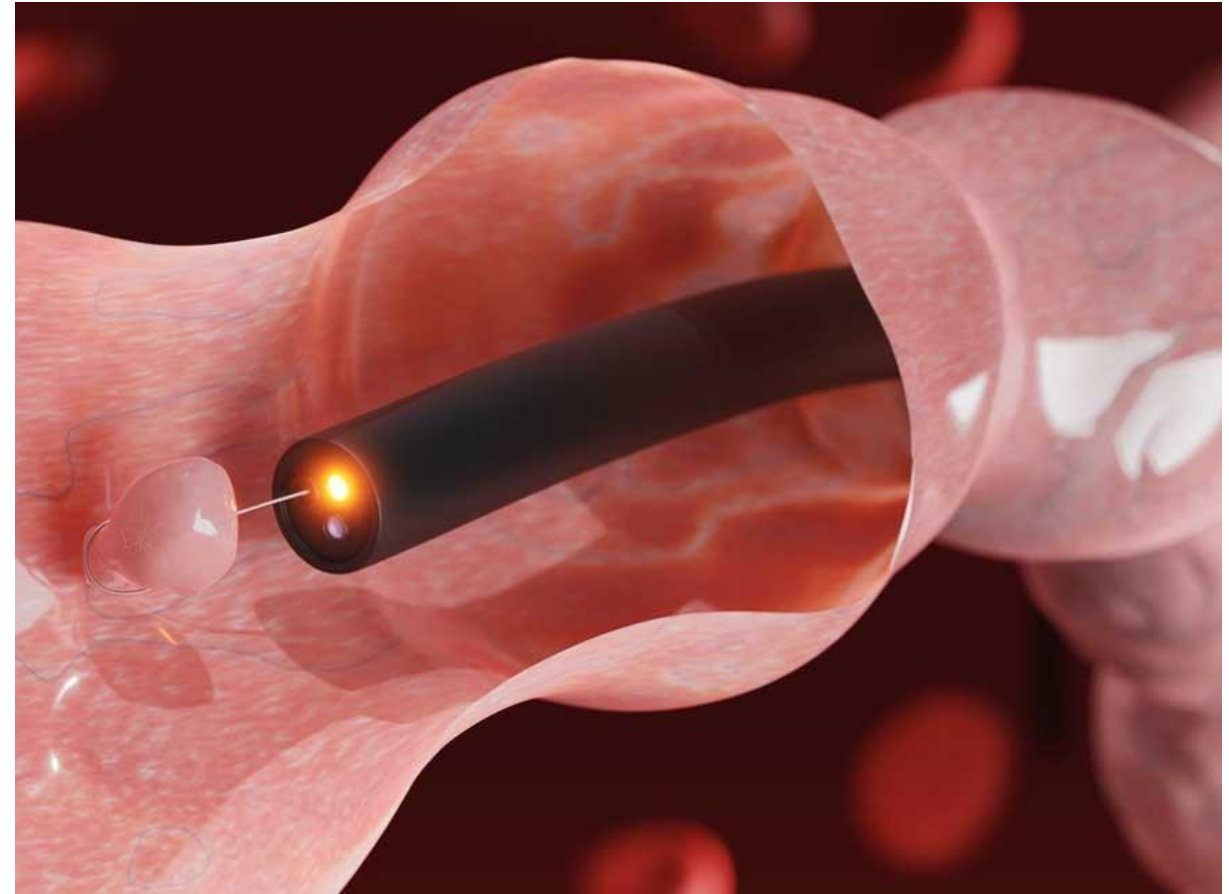
*Contemporary Practice in Clinical Chemistry, 4<sup>th</sup> Ed.*

# Conclusions

- Screening for colorectal cancer
  - » Begin at 45-years-old
  - » gFOB, FIT, or Cologuard (sDNA)



- Diagnosis
  - » Colonoscopy with biopsy
- Monitor
  - » Carcinoembryonic Antigen (CEA)



# Case Study

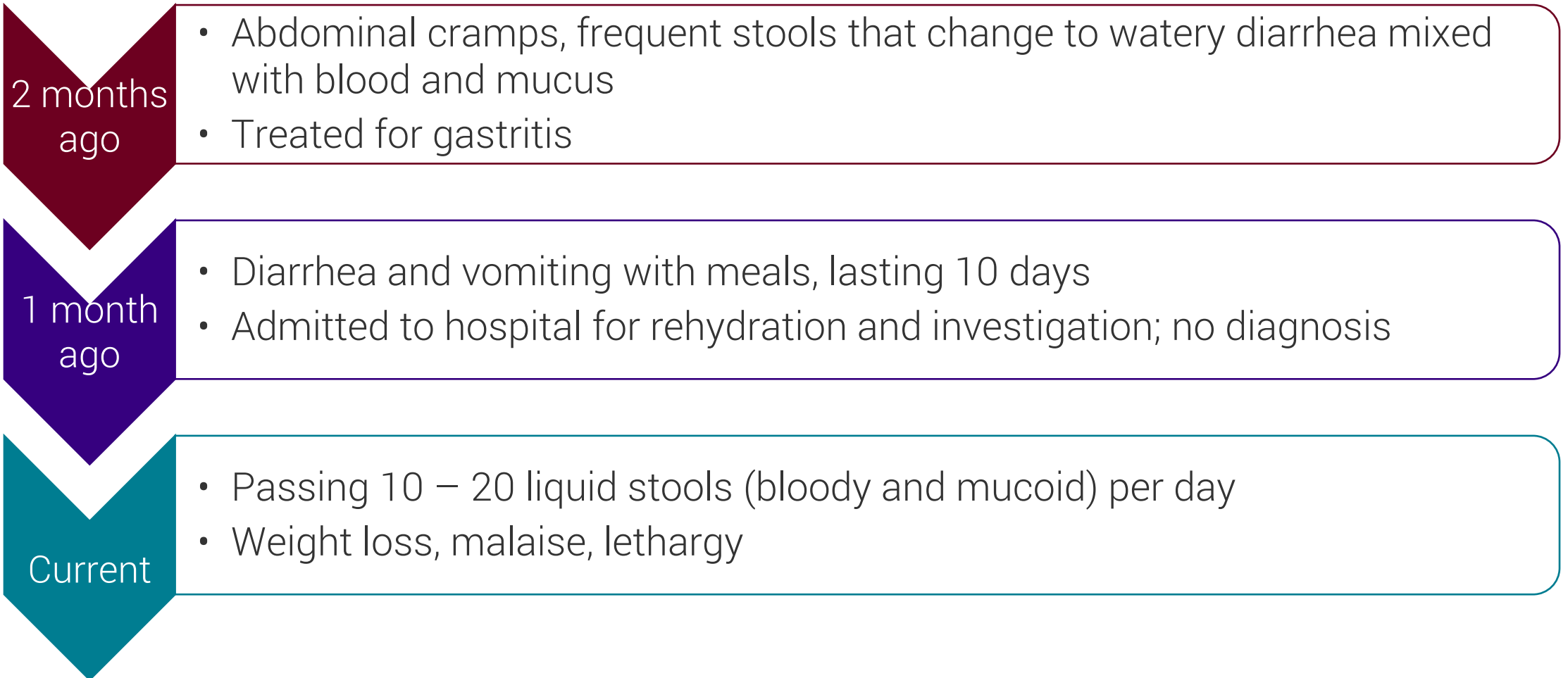
## Presentation

- 22-year-old female
- Presented to clinic with 2-month history of severe abdominal cramps, persistent bloody and mucoid diarrhea, weight loss, and fatigue
- No significant medical history or surgery
- No family medical history

## Physical Exam

- Thin and ill appearance
- Heart Rate: 80 bpm
- Blood Pressure: 120/70
- Temperature 37 °C
- Abdominal tenderness

# Case Study – Recent History





# Case Study - Differential Diagnosis?

## Infectious Disease

- E. Coli
- Salmonella
- Clostridium difficile
- Shigella

## Non-inflammatory

- IBS
- Malabsorption
- Celiac disease

## IBD

- Ulcerative Colitis
- Crohn's Disease

# Case Study – Investigations

Urea and Electrolytes		
Na	137 mmol/l	(135 – 147 mmol/l)
K	3.5 mmol/l	(3.3 – 5.0 mmol/l)
Cl	96 mmol/l <b>L</b>	(99 – 113 mmol/l)
CO <sub>2</sub>	31 mmol/l <b>H</b>	(18 – 29 mmol/l)
Urea	3.3 mmol/l	(2.5 – 7.0 mmol/l)
Creat	32 umol/l <b>L</b>	(60 – 120 umol/l)

CBC		
WBCs	5.9 x 10 <sup>9</sup> /l	(4.00 – 10.00)
Hb	9.0 g/dl <b>L</b>	(12.1 – 15.1 g/dl)
Platelets	748 x 10 <sup>9</sup> /l <b>H</b>	(150 – 400)
CRP	17.4 mmol/l <b>H</b>	(0 – 10 mmol/l)

Liver Function Tests	Normal
Thyroid Function Tests	Normal

# Case Study – Investigations

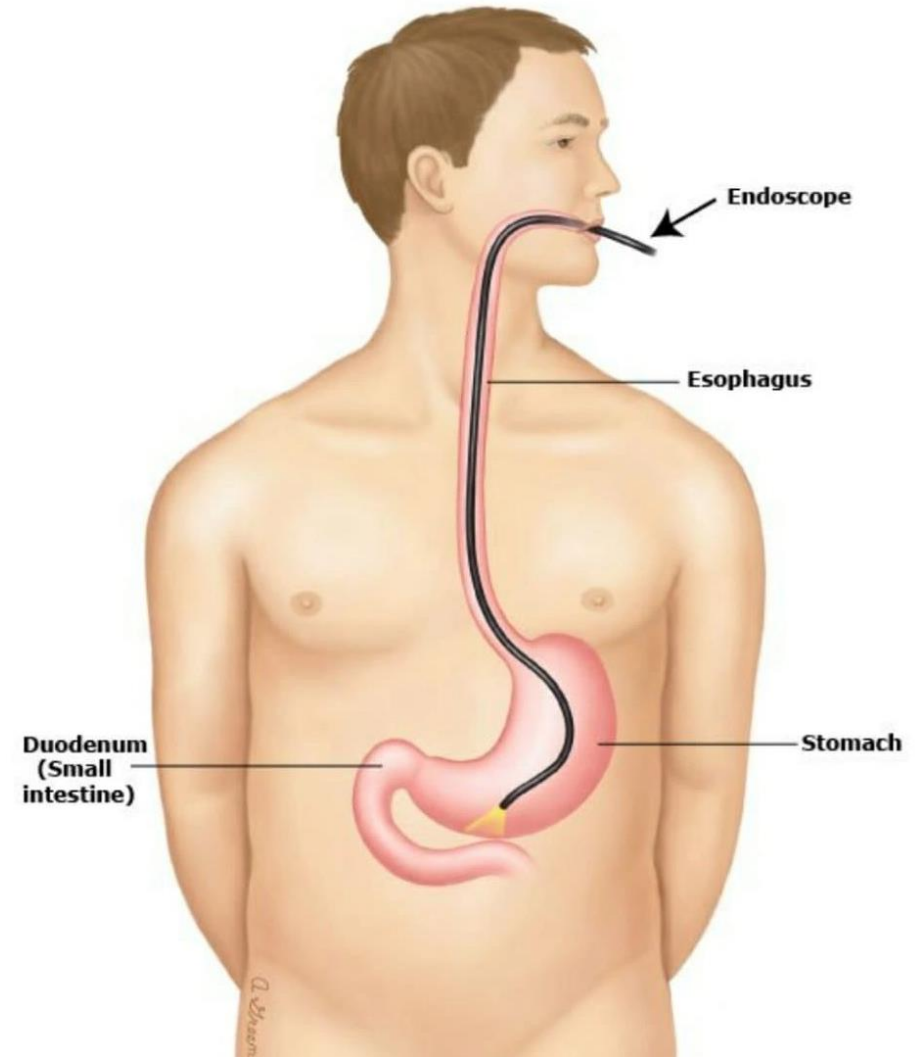
Stool	
Brown	Unformed
FIT	Positive
Parasites	Not observed
Aerobic organisms	Not observed
C difficile toxin	Negative
Calprotectin	1050 µg/g

What does this testing suggest?  
What next?

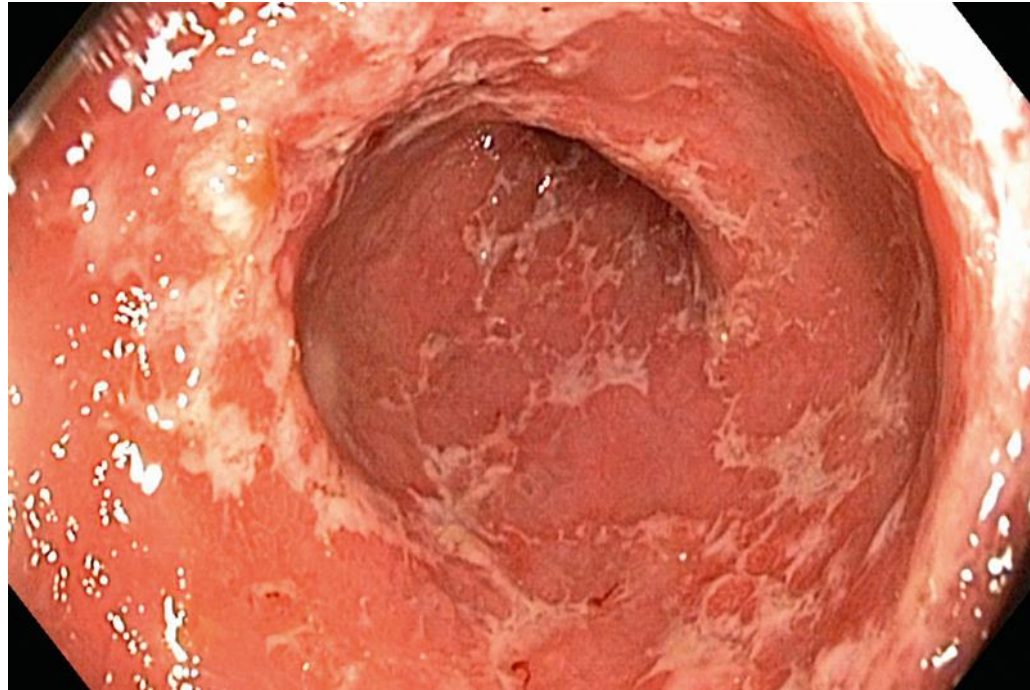
# Case Study – Investigations

## Upper Endoscopy

- Normal esophagus
- Stomach mucosa intact and normal
  - » No gastritis or ulceration
- Pylorus and duodenum normal



# Case Study – Investigations

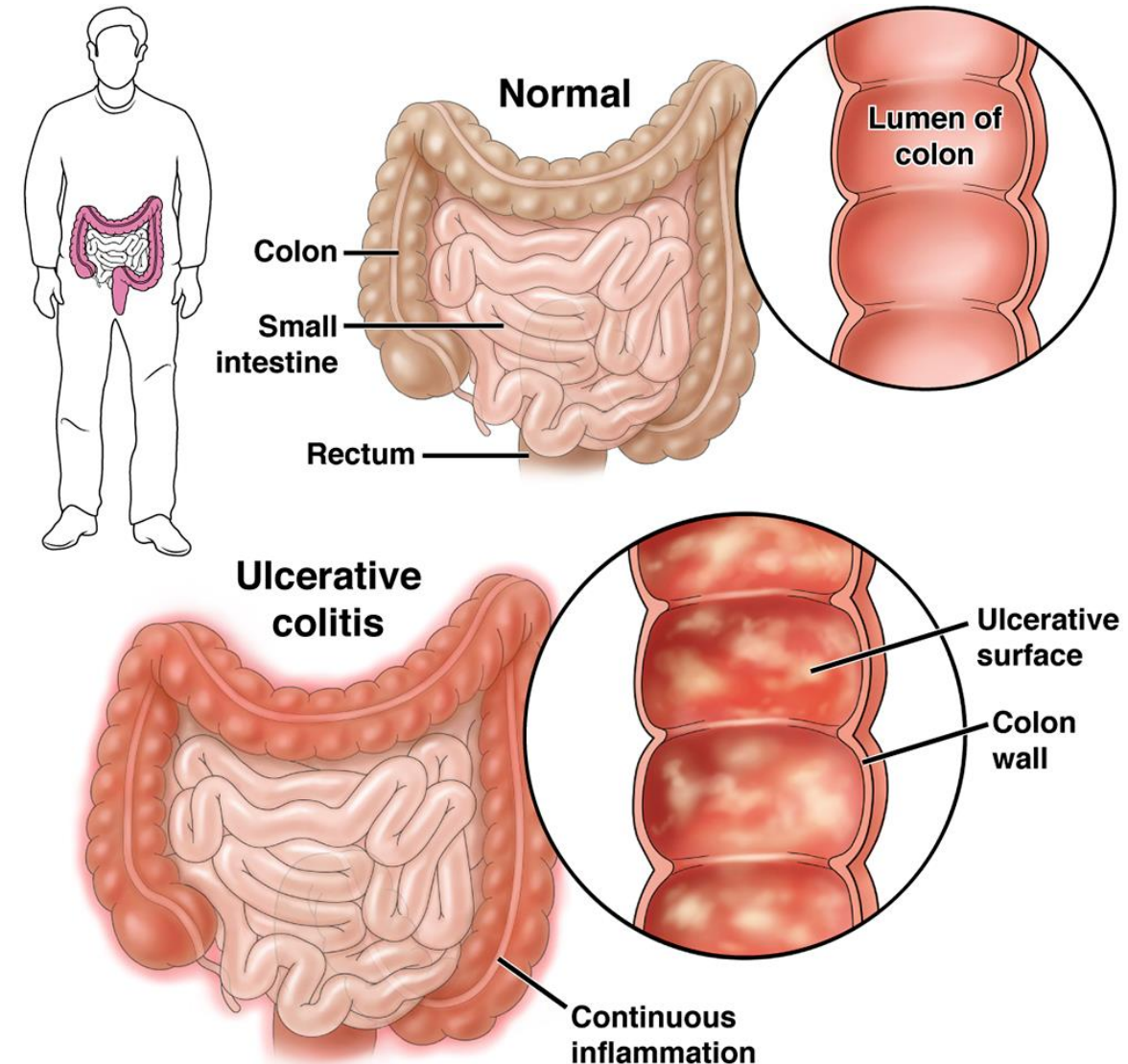


## Colonoscopy

- Friable mucosa
- Extensive ulceration with pseudopolyps in rectum, sigmoid and left colon
- Tissue collected for biopsy
  - » Pathology limited to mucosa

# Case Study - Diagnosis

## Ulcerative Colitis







■ Questions?

Thank you!



*ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.*