Fecal Calprotectin

From Clinical Need to Laboratory Validation

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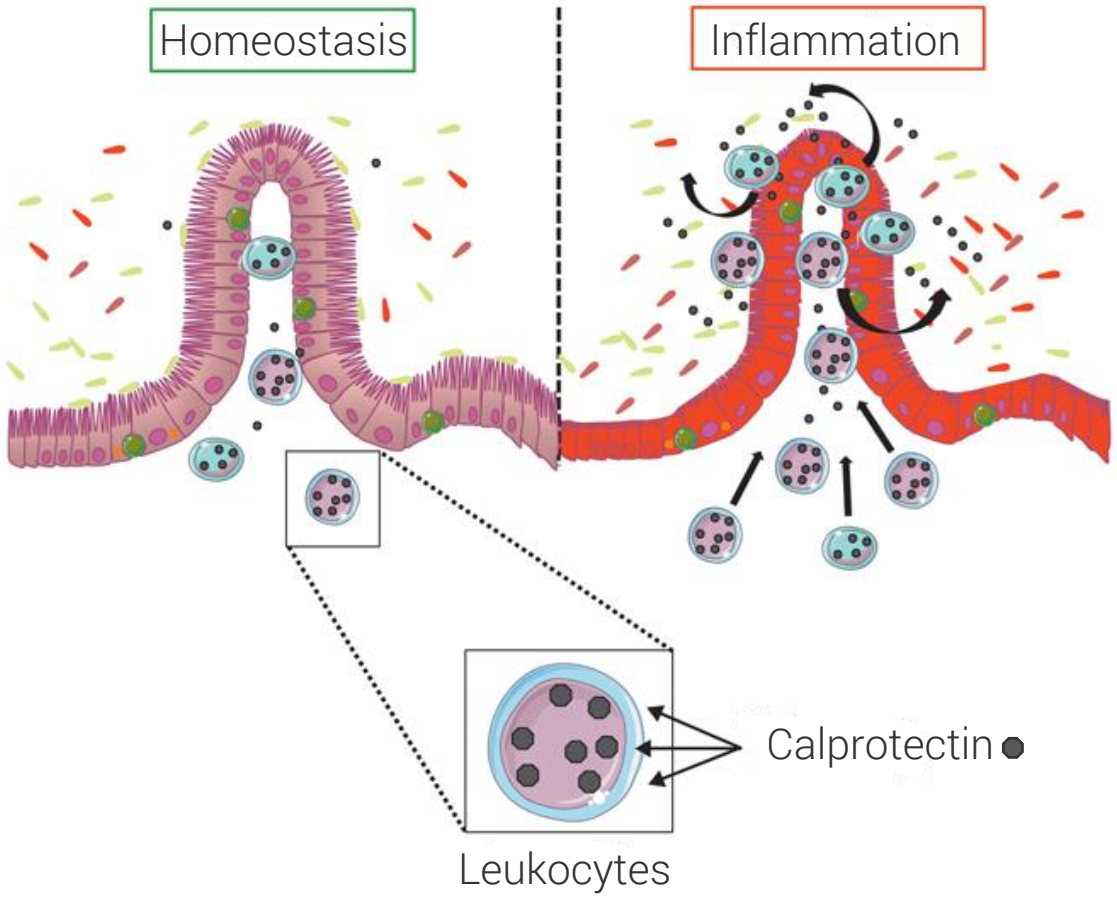
Learning Objectives

1. Explain the clinical utility of fecal calprotectin.
2. Discuss extraction methods and available fecal calprotectin assays.
3. Create an assay validation plan for laboratory testing of feacl calprotectin using a FDA-approved assay

Outline

1. Clinical Utility
2. Analytical considerations
3. Validating the assay
4. Benefits of in-house testing

Calprotectin

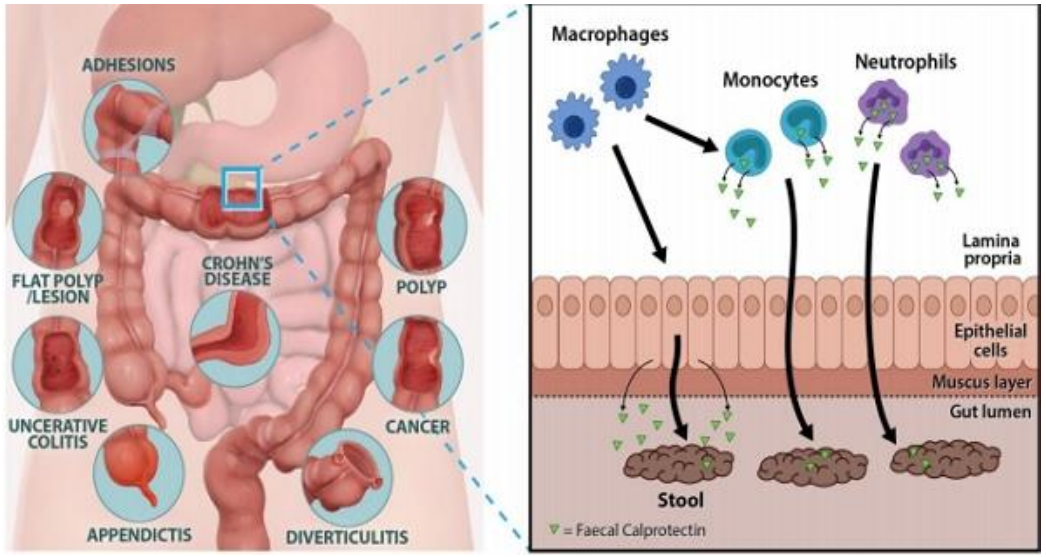


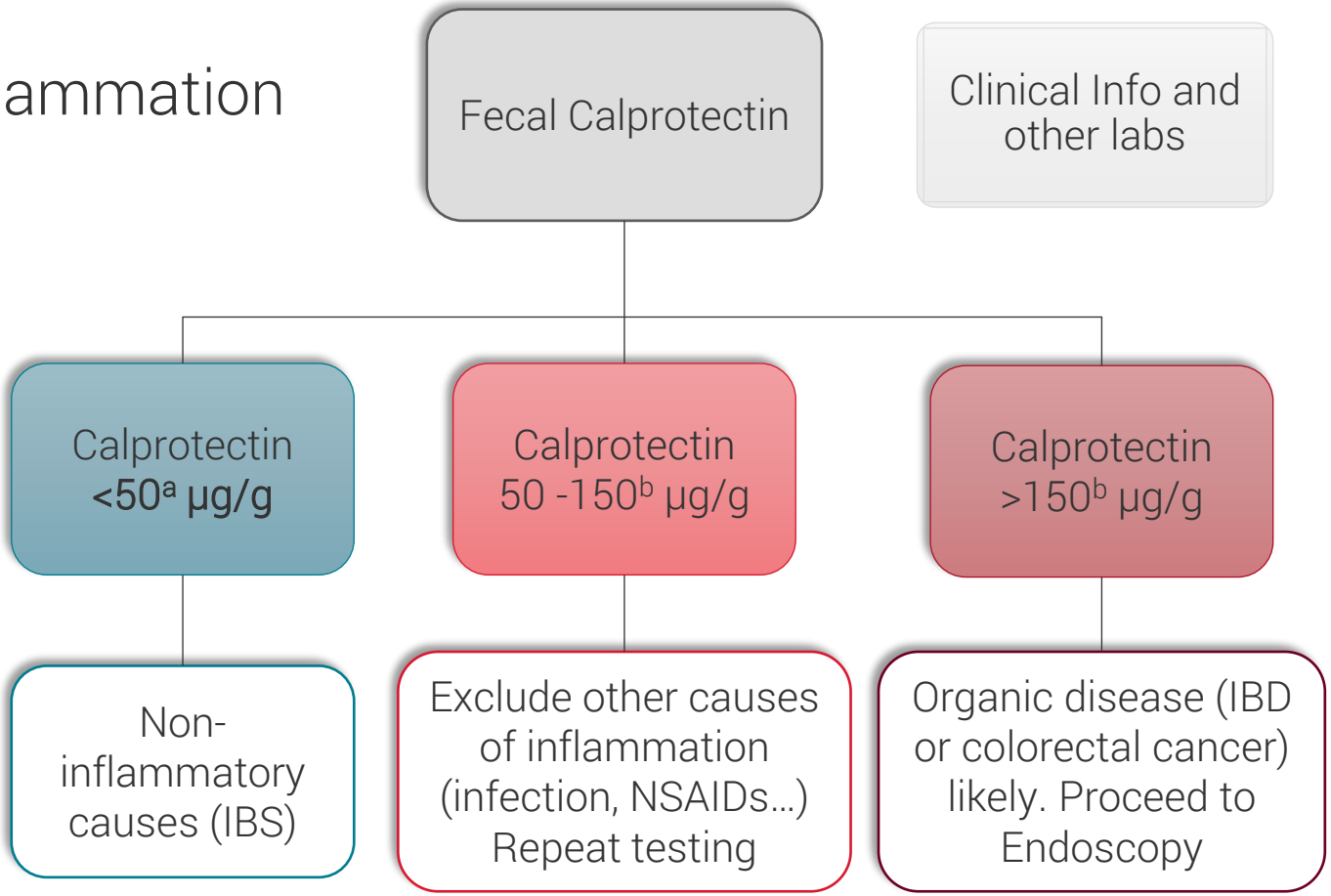
- Calcium- and zinc-binding protein
- Predominant protein in cytosol of neutrophils (~60%) and other leukocytes
- Activation of leukocytes → release calprotectin
- Accumulates in feces
  - » Stable several days after excretion

Figure adapted from: Nancey S, et. al. Hépato-Gastro & Oncologie Digestive. 2015;22(6):477-487.

Clinical Utility of Fecal Calprotectin

- Highly sensitive biomarker for inflammation
  - » Not specific



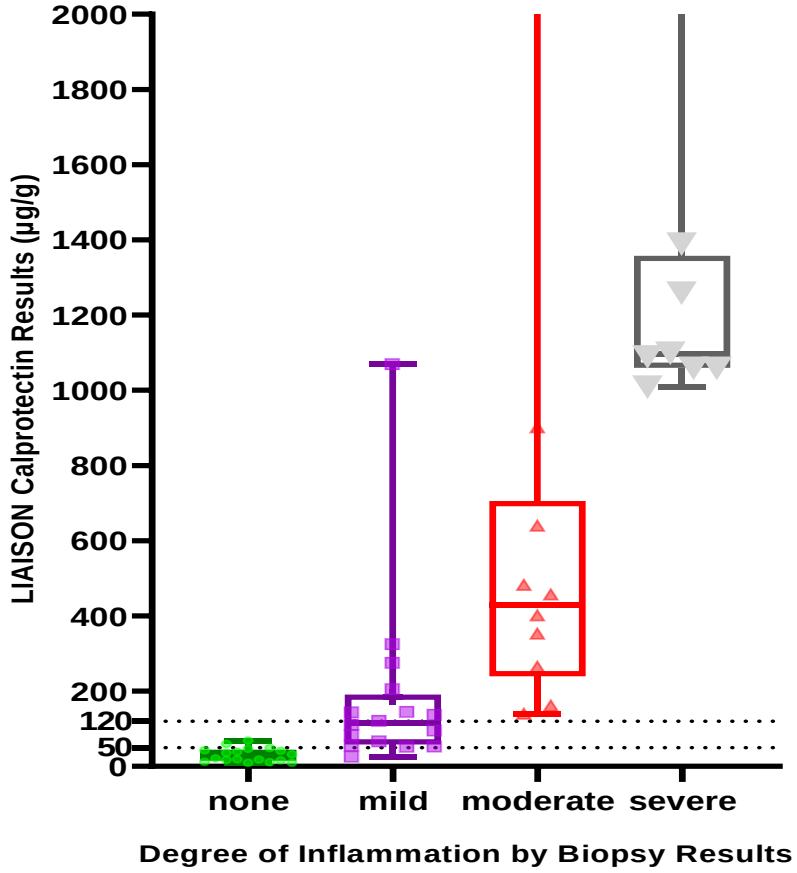


Jeong SJ. *Korean J Pediatr*. 2019. 62(8):287-291

[a]Widely adopted
[b]Variable, refer to assay manufacturer

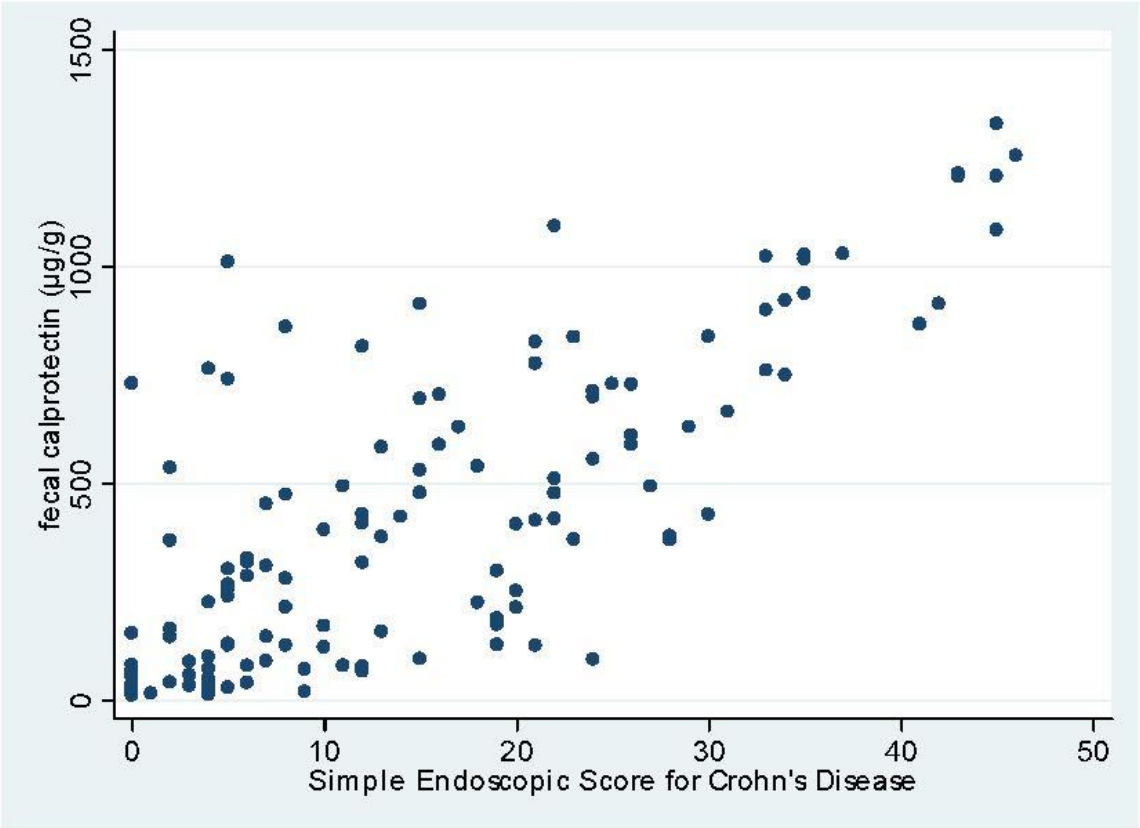
Fecal Calprotectin Correlates with Disease Activity

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



Fecal Calprotectin for Monitoring Disease

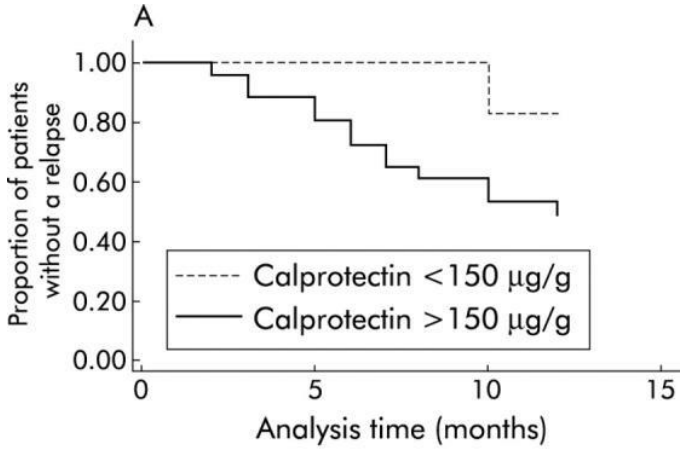
- Simple Endoscopic Score for Crohn Disease (SES-CD)
  - » Inactive (remission): 0 – 3
  - » Mild activity: 4 – 10
  - » Moderate activity: 11 – 19
  - » High activity: ≥20
- 140 CD patients; 40 control



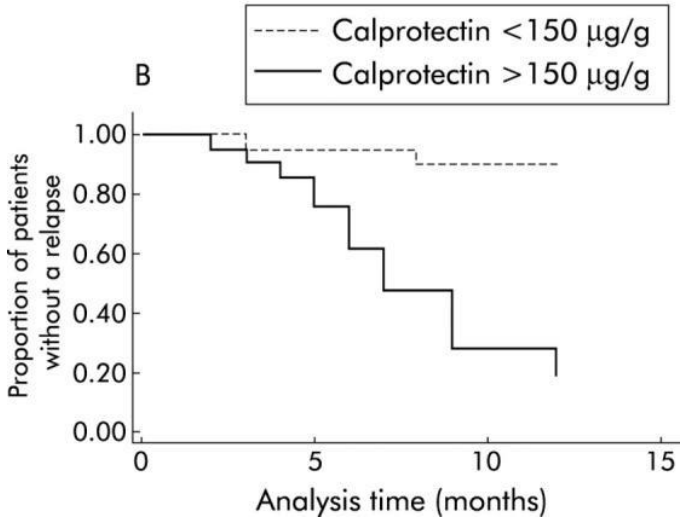
Schoepfer AM, *et. al. Am J Gastroenterol.* 2010, 105(1):162-9.

Fecal Calprotectin to Predict Relapse

Crohn's Disease

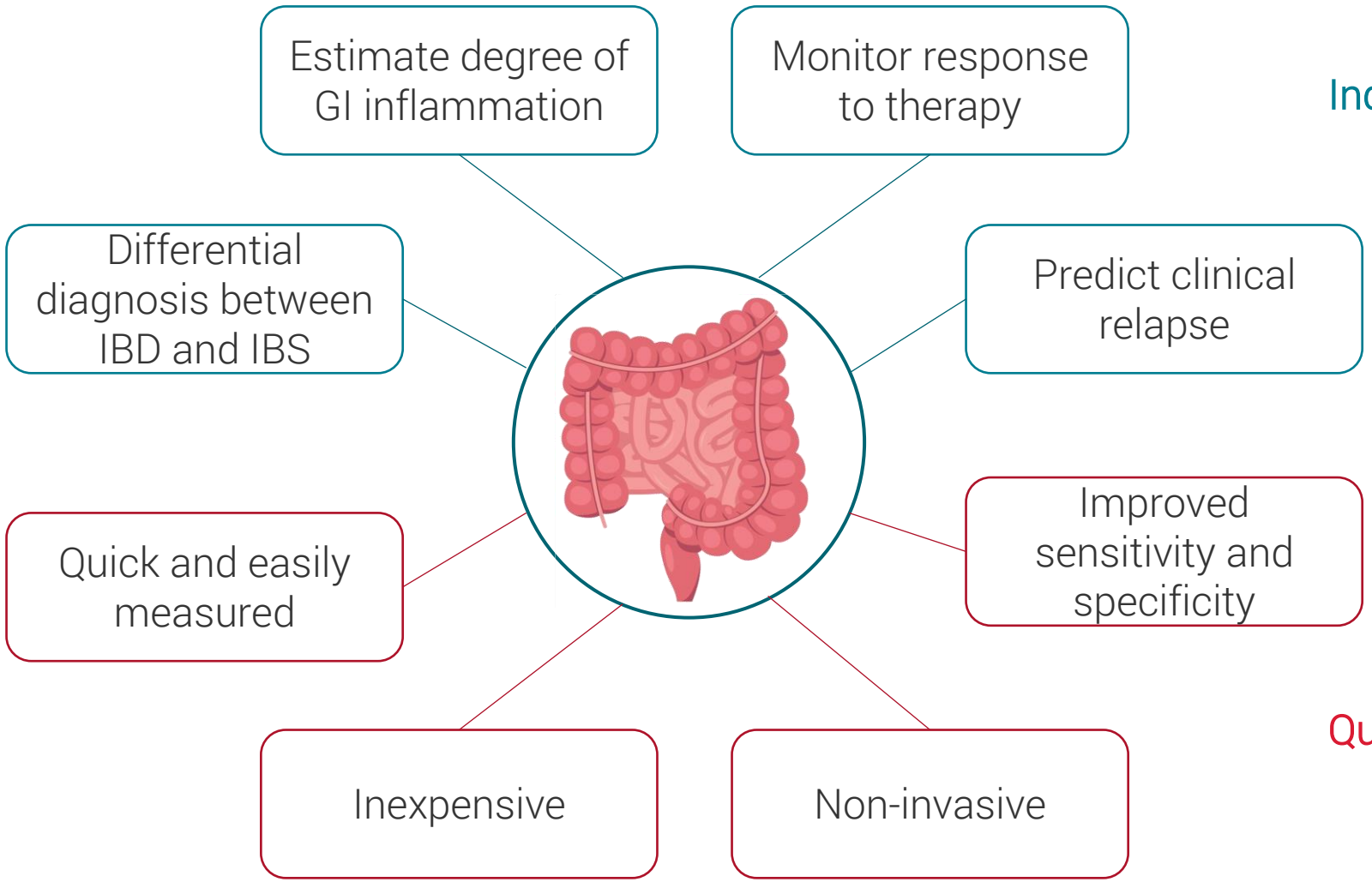


Ulcerative Colitis



- Calprotectin > 150 µg/g stool is a predictor of relapse for both CD and UC
- ESR and CRP – not useful predictors of relapse

Clinical Utility of Fecal Calprotectin



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

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 µg/g suggests significant inflammation in colon or small intestine
  - » Serum CRP >5mg/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

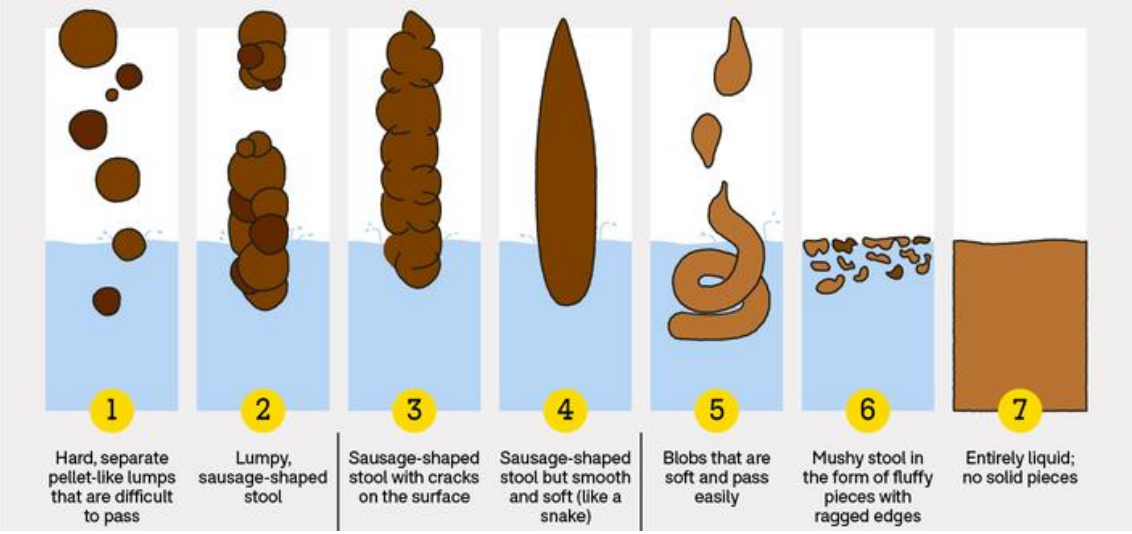
AGA, American Gastroenterological Association

Analytical Considerations

Measuring Fecal Calprotectin

Sample

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



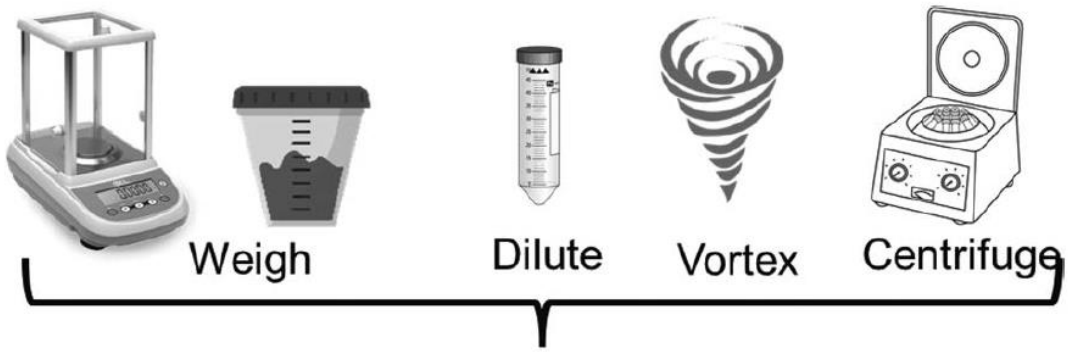
Challenges

- Heterogeneous
  - » Undigested food, mucus, fibers
- Bristol Stool Types
  - » Variable water content (65 – 75%)[1]
    - No normalization
- Day-to-day variability
  - » Within subject CV 30 - 40%[2,3]
- Reference change value: 87 – 115%[3]

[1] *Aliment Pharmacol Ther*. 2016; 44: 693-703.
[2] *Inflamm Bowel Dis*. 2015; 21(5):1072–1076.
[3] *Clin Chem Lab Med*. 2018; 56(11):1926-1935.

Extraction of Calprotectin

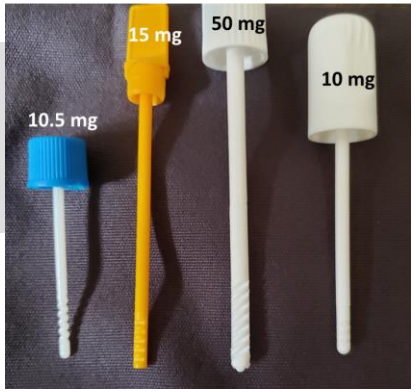
Manual

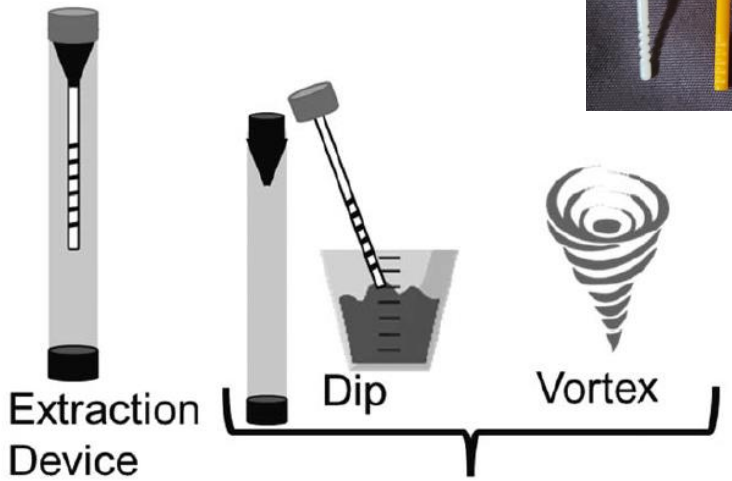


Manual Extraction Components

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

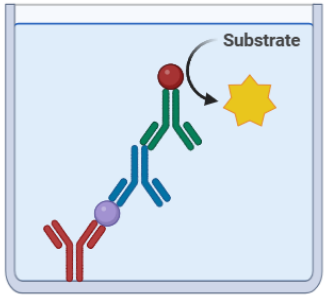
Device

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

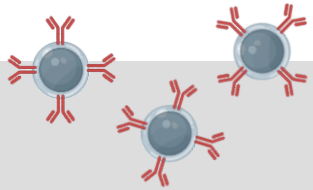
Assays

ELISA

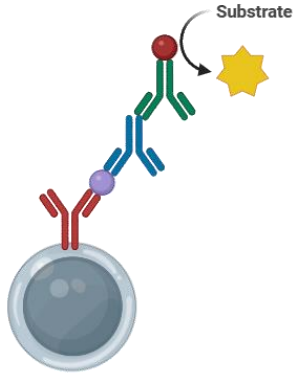
- Commercial assays available
- Batch
- Dilution for higher concentrations
- Steps can be performed manually
- Plate reader



Bead



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

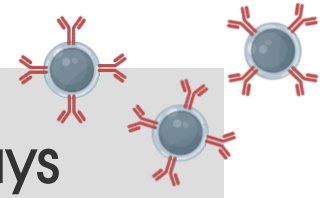


FDA-Approved Fecal Calprotectin Assays

ELISA

- PhiCal™ ELISA (Genova Diag.)
- QUANTA Lite® Calprotectin ELISA (Inova Diag.)
- fCal® ELISA (Bühlmann)
- Calprest®NG ELISA (Eurospital)
- Calprotectin Chemiluminescence ELISA (ALPCO)

Automated Bead Assays



- LIAISON® Calprotectin CLIA (DiaSorin)
- QUANTA Flash® Calprotectin (Inova Diag.)
- fCal Turbo turbidimetric immunoassay (Bühlmann)
- Calprotectin Immunoturbidimetric (ALPCO)

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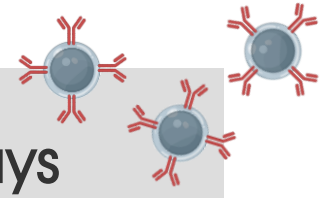

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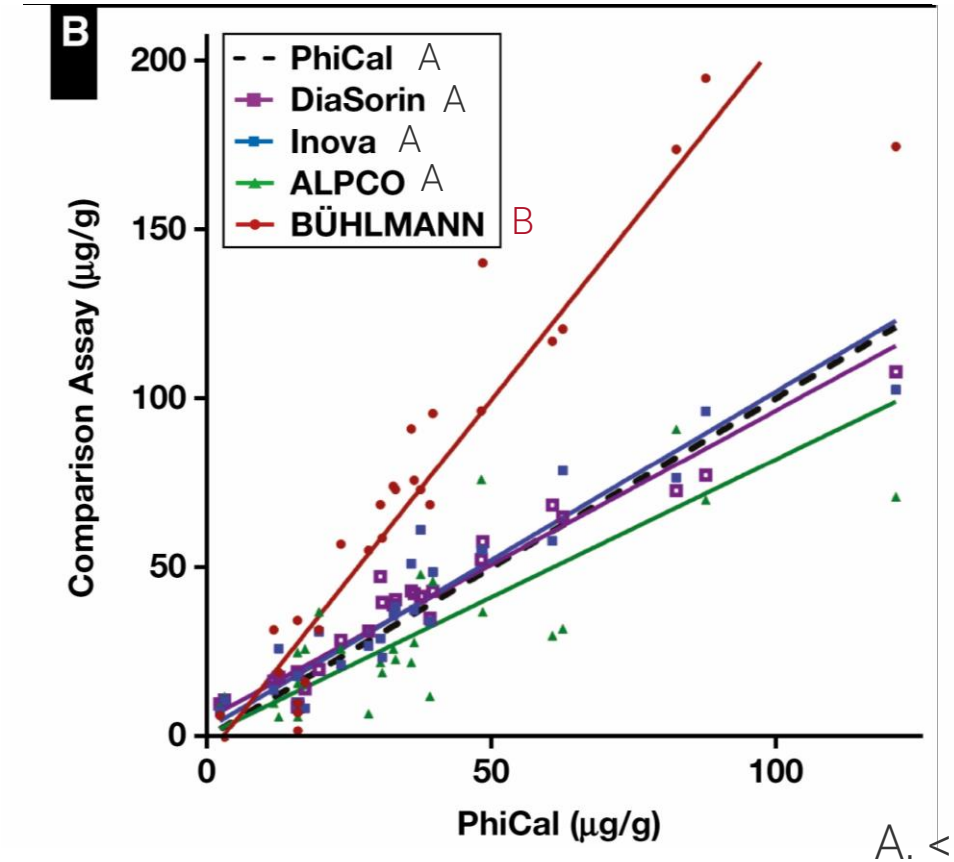
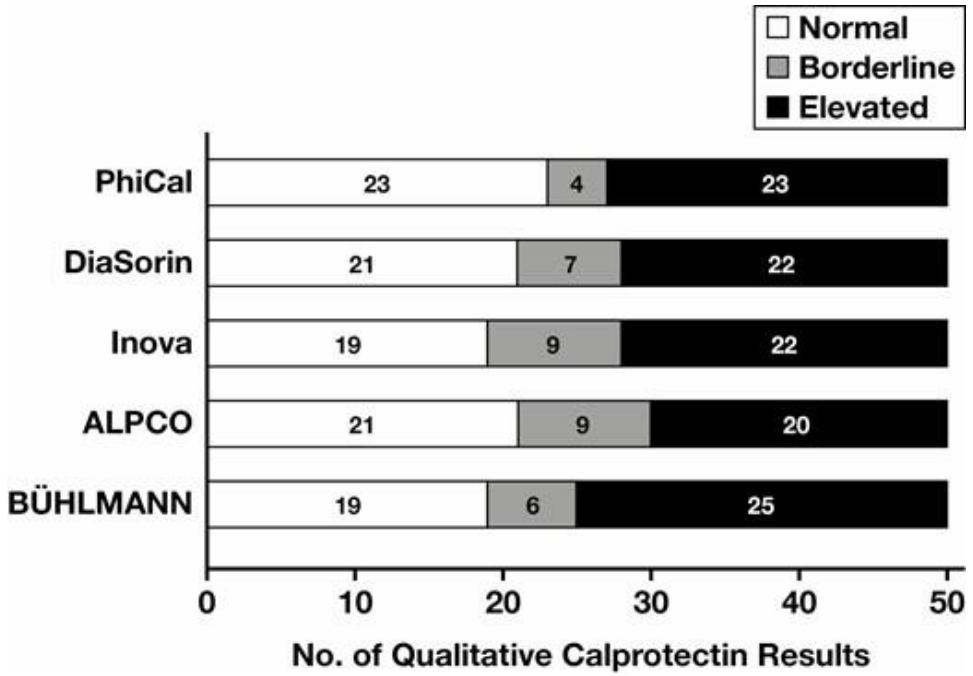
Lack of Standardization for Calprotectin





A. <50µg/g = normal, 51-120µg/g = borderline, >120 = elevated

B. <80µg/g = normal, 81-160µg/g = borderline, >160 = elevated

Assay Selection

- Diagnostic accuracy
- Expected test volumes
- Turn around time required
- Equipment required
  - » What is already available in lab
- Ease of use

Assay Validation

FDA Approved Assay Verification

- Accuracy
- Precision
- Linearity / Reportable Range
- Reference range

Accuracy

- Patient samples tested on new method and comparator method
  - » Minimum 20 specimens spanning the AMR
  - » Linear regression and bias plots
- Extraction method – assess all methods that will be used
  - » We tested 50 samples on each the extraction device and manual
- Acceptability criteria
  - » Slope (0.80 – 1.20), bias (±20%), correlation (≥0.99)
- Qualitative agreement
  - » >80% agreement in classification of result: normal, borderline, abnormal

*Parameters in this color text are example acceptability criteria, not required

Precision

- Material
  - » Kit controls - test precision of assay
  - » Extract control - test precision of whole process – extraction and assay
- Within run: 10 – 20 replicates at 2 – 3 concentrations
- Between run: Minimum 20 results at 2 – 3 concentrations, in duplicate
  - » 2x2x10 (2 levels/twice per day/10 days) – 20 days preferable
- Extraction method – assess precision of all methods that will be used
- Acceptability
  - » ≤20% CV Kit controls
  - » ≤30% CV Extract control (patient pools)

Linearity / Reportable Range

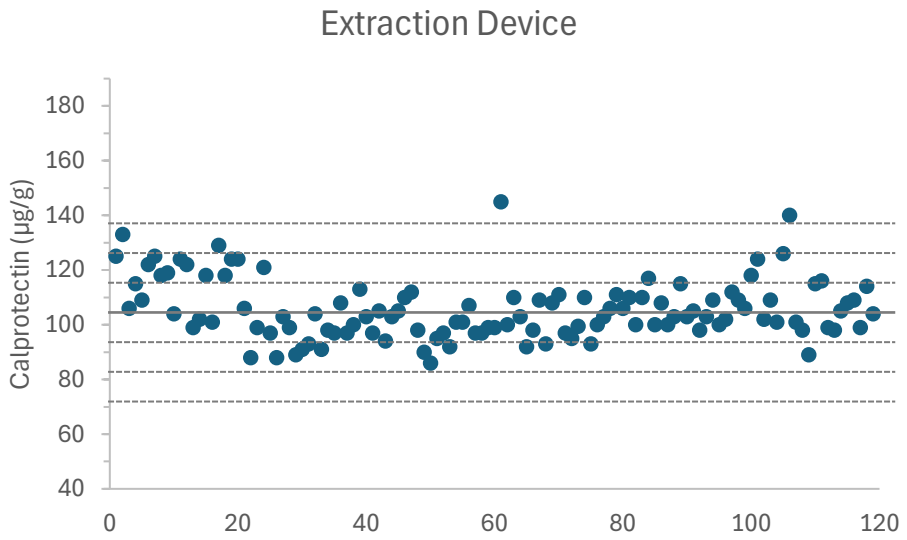
- Minimum 5 concentrations spanning the AMR
  - » One run, at least duplicate testing
  - » 85 – 115% recovery
- Material
  - » Many manufacturers provide linearity material
  - » Extract a high patient sample and then dilute – determine % recovery

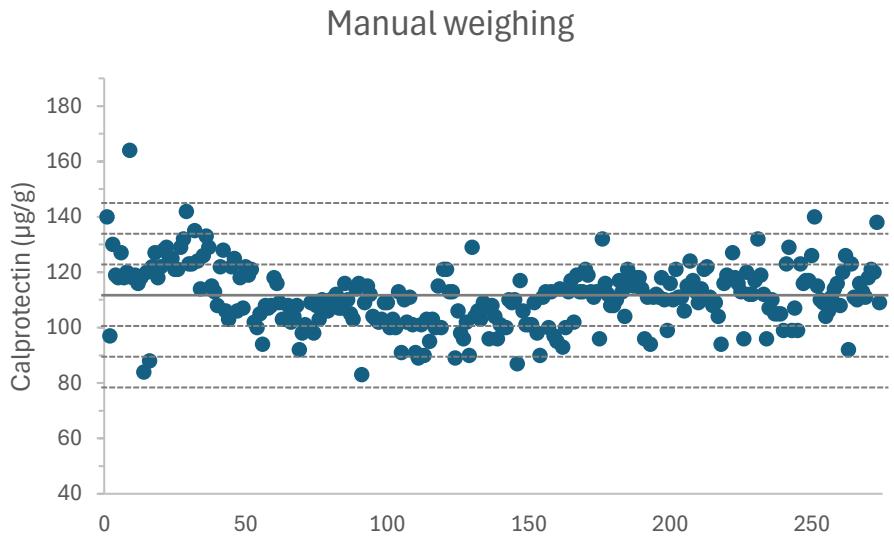
Reference Range

- Verify PI claims
  - » Minimum 20 specimens
  - » 90% of values must fall within proposed interval
- We verified lower cutoff (≤50 µg/g) using specimens from healthy donors
  - » Donor criteria: No history of IBD or pancreatic insufficiency, no recent abdominal pain or diarrhea, not taking PPIs or NSAIDs in last 5 days

Monitoring the Testing Process After Go-Live

- Kit controls – monitor assay performance
- **Optional**: Pooled stool extract control QC – monitor extraction





Benefits of in-house testing

Faster Turn-Around Times

Lab	Published TAT
A	1 – 4 days
B	3 – 5 days
C	4 – 5 days
D	4 – 6 days
Average	**3 – 5 days**

- Providers say* it takes 7 -10 days.
- Based on guidelines, symptomatic patients with elevated biomarkers are candidates for treatment changes.
  - » Send-outs could result in 1–2-week delay of optimizing treatments for patients

* Based on direct physician (GI specialist) interviews

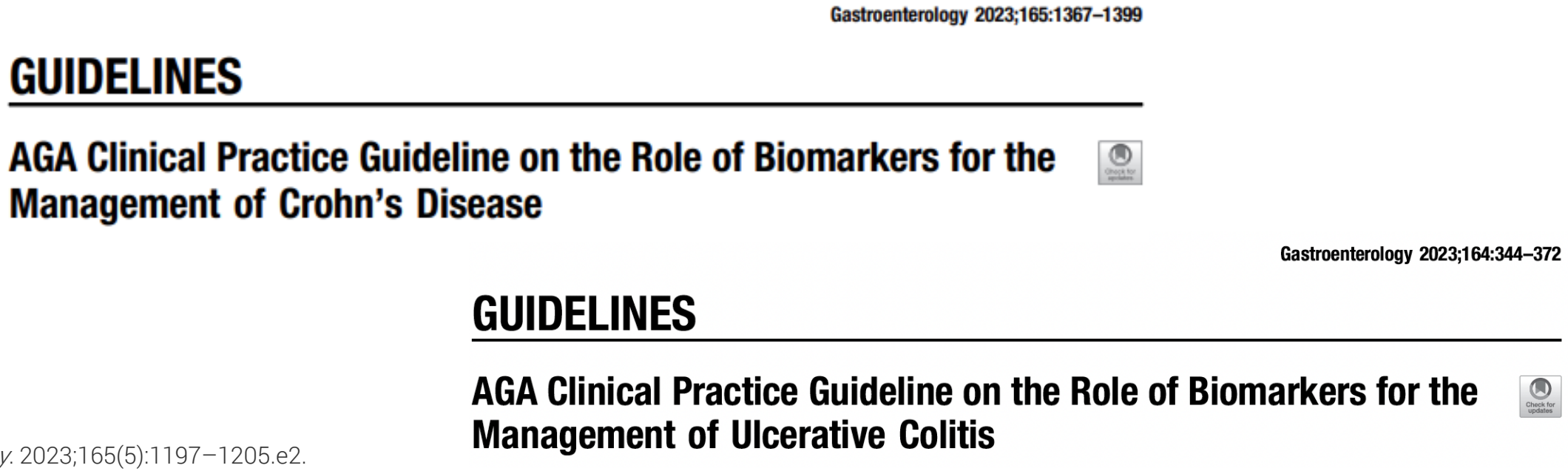
Cost Effectiveness

- CPT Code: 83993 Medicare reimbursement: $19.63 (private insurance is higher)
- Send-out (reference lab costs): $100 – $200[1]
- Assay Costs for running in-house: $5 – $15 based on methodology & equipment used
  - » Additional 25 – 30% for labor and overhead ($6 – $20)
- Diagnostic Endoscopy Cost: $1000 - $1500 not including histology[2]

[1]Based on reference lab test menus
[2]https://cost.sidecarhealth.com/c/diagnostic-colonoscopy-cost

Growing Market

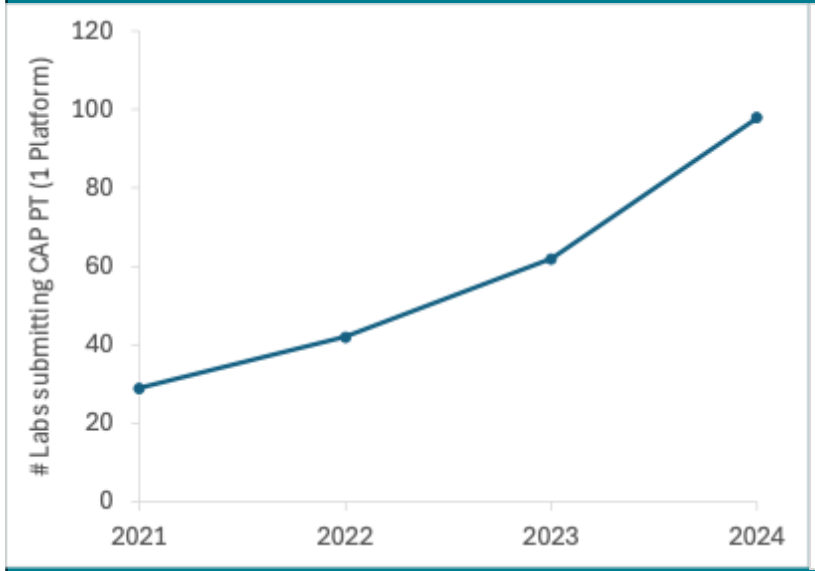
- Prevalence of IBD is nearly 1 in 100 people in the United States[1]
  - » ~2.4 million Americans are diagnosed with IBDs
- Calprotectin is included in clinical guidelines for diagnosis and monitoring in IBD (AGA)

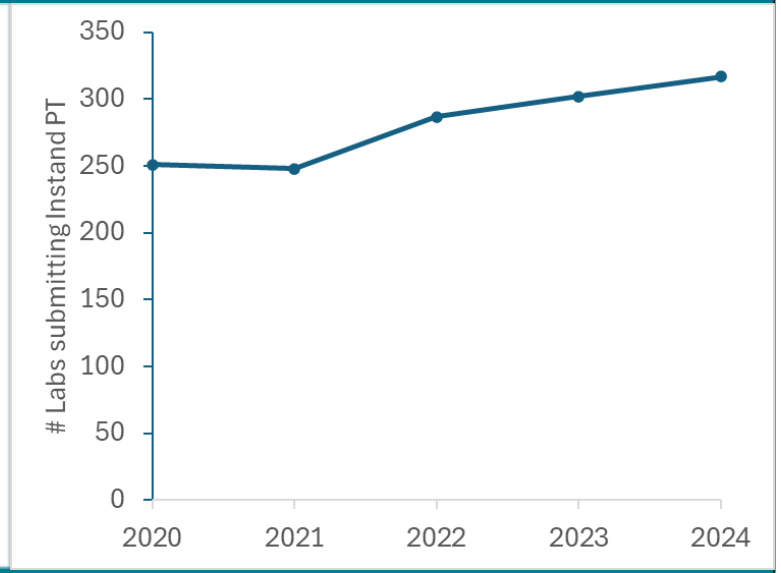


[1] *Gastroenterology*. 2023;165(5):1197–1205.e2.

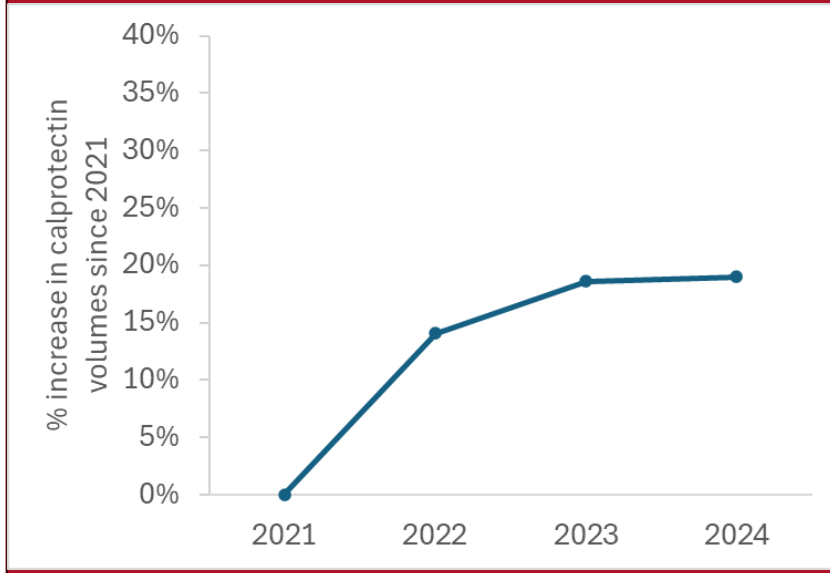
Growing Market

Laboratory growth based on PT surveys





Growth at ARUP



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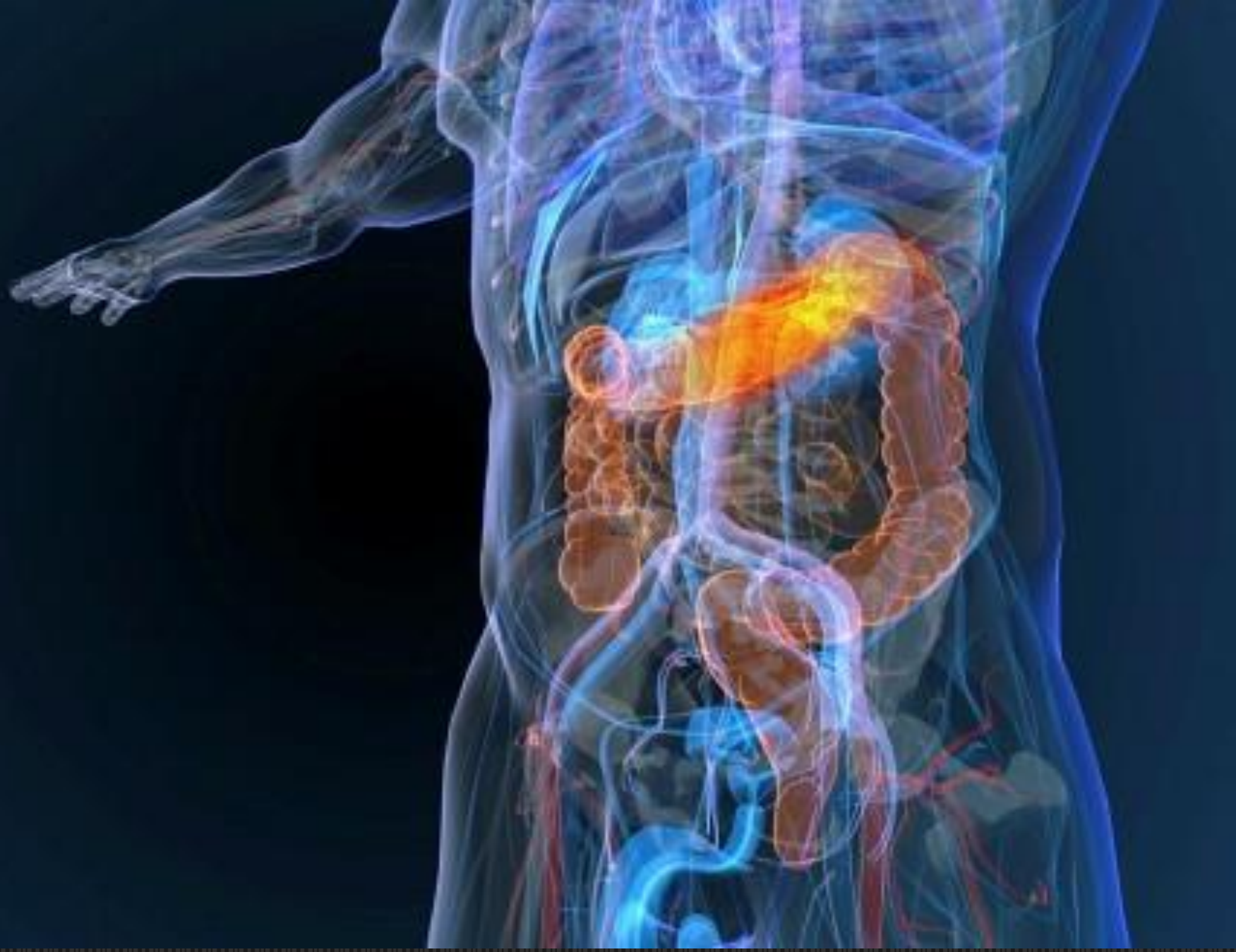

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