Updates on Laboratory Testing and Clinical Diagnosis of Celiac Disease

Vijayalakshmi (Viji) Nandakumar, PhD, DABCC

Assistant Professor, University of Utah School of Medicine, Department of Pathology
Medical Director, Autoimmune & Complement Disease Testing, ARUP Laboratories

OCTOBER 2022

Anti-tTg IgA	• **Positive**
Duodenal Biopsy	• Negative

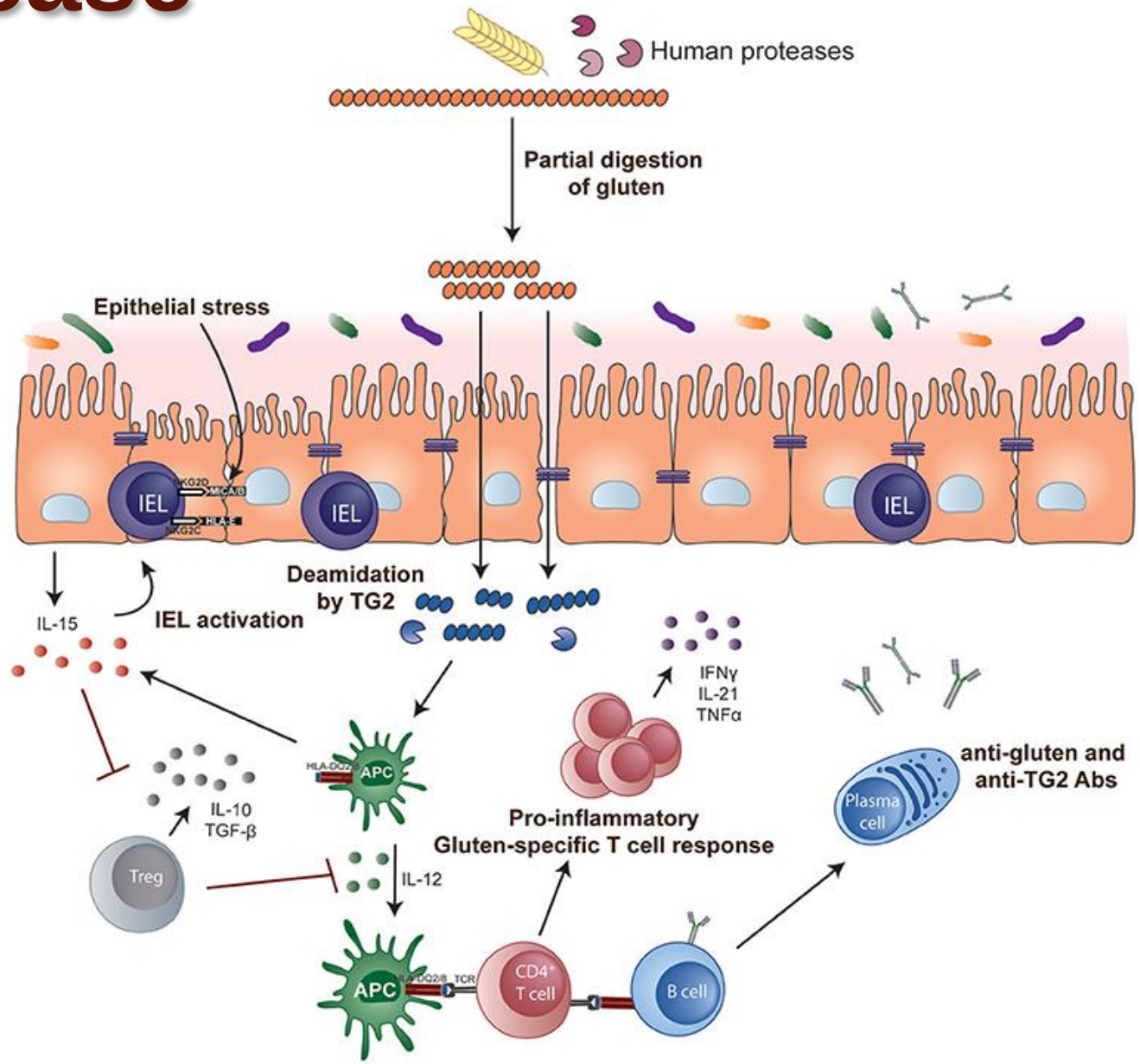
- ❑ **Which of these are correct results?**
- ❑ **What other serology tests can be helpful?**
- ❑ **How will this patient be managed?**

Objectives

- Recognize the utility and limitations of celiac disease markers in the diagnosis.
- Discuss the latest guidelines for serology testing in celiac disease diagnosis including recommendations for biopsy-free diagnosis.
- Discuss the current state of laboratory tests in the treatment and management of celiac disease

Celiac Disease

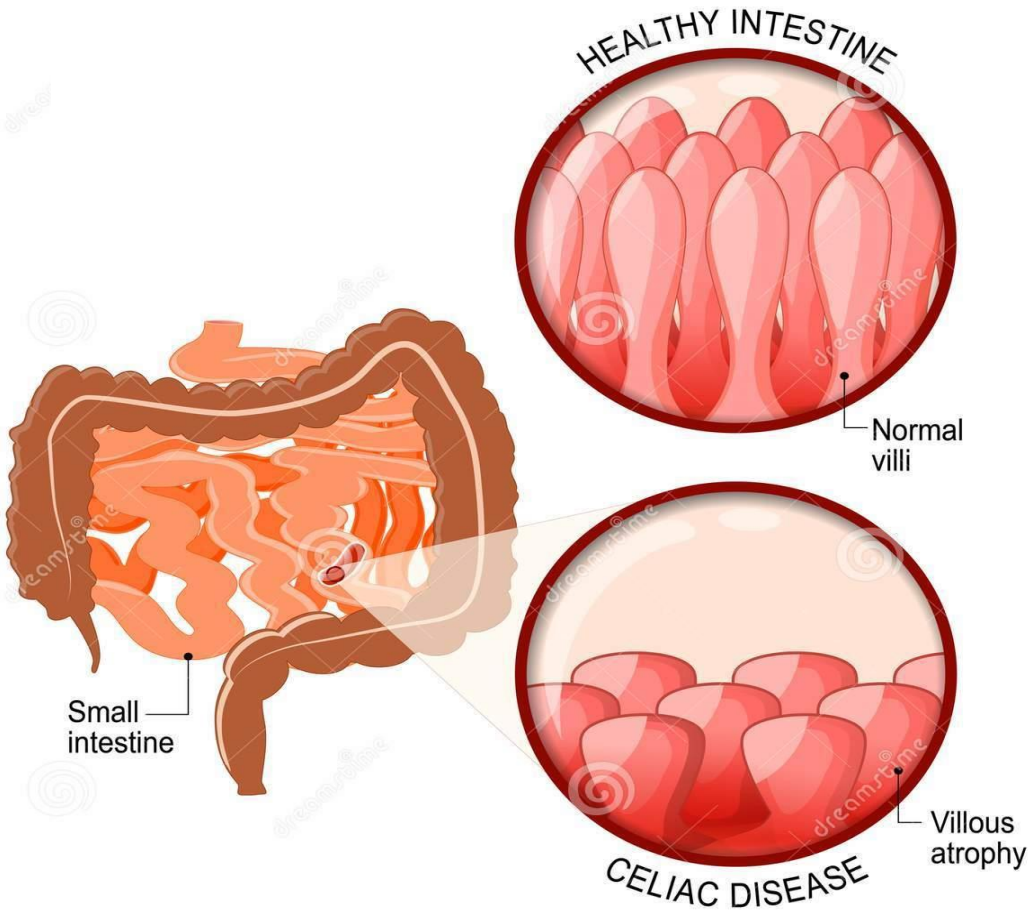
Normal:
Gliadin destroyed by IgA- mediated immune reaction before it crosses the enterocytes

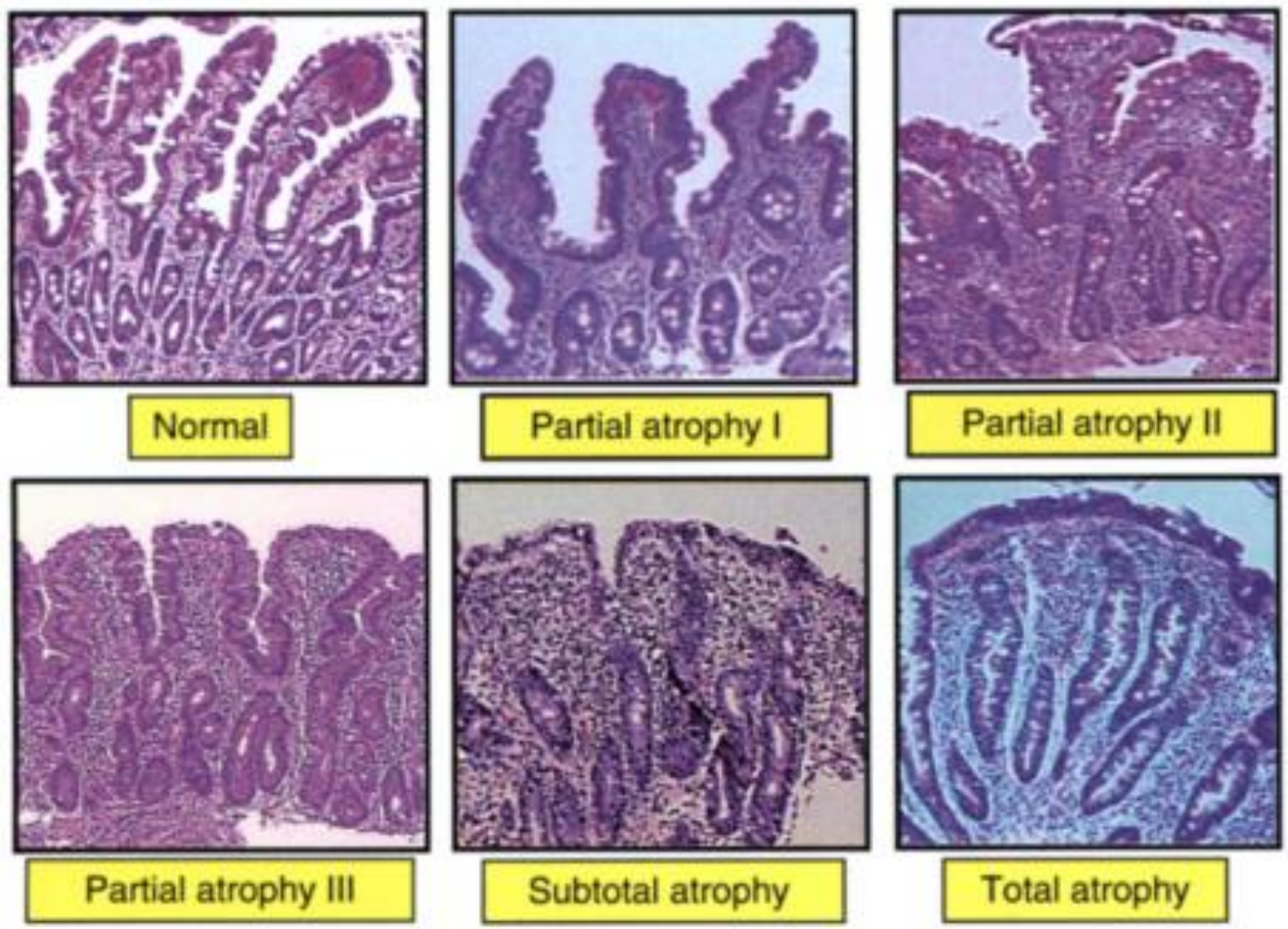


Celiac disease:
Gliadin bound IgA complex enters lamina propria initiating an adaptive immune response

Tye-Din JA, Galipeau HJ, Agardh D. Celiac Disease: A Review of Current Concepts in Pathogenesis, Prevention, and Novel Therapies. *Front Pediatr*. 2018;6:350. Published 2018 Nov 21. doi:10.3389/fped.2018.00350

Normal Villi & Villous Atrophy

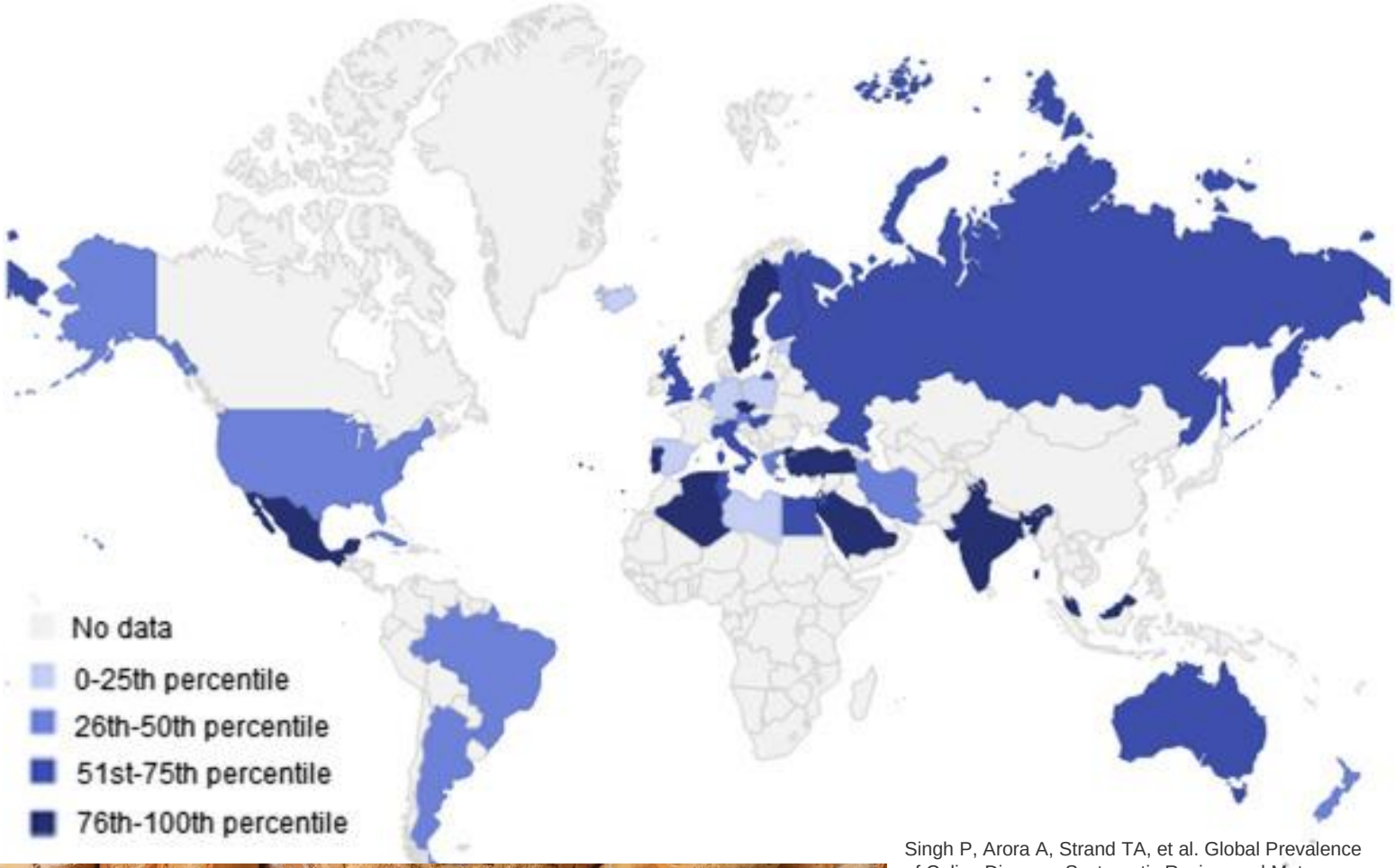




mall intestinal with normal villi, and villous atrophy. Diagram showing changes in intestinal. coeliac disease manifested by blunting of villi.

Carlo Catassi, Alessio Fasano,1 - Celiac disease, Editor(s): Elke K. Arendt, Fabio Dal Bello, In Food Science and Technology, Gluten-Free Cereal Products and Beverages, Academic Press, 2008, Pages 1-I, ISBN 9780123737397, https://doi.org/10.1016/B978-012373739-7.50003-4

Gluten-free Food

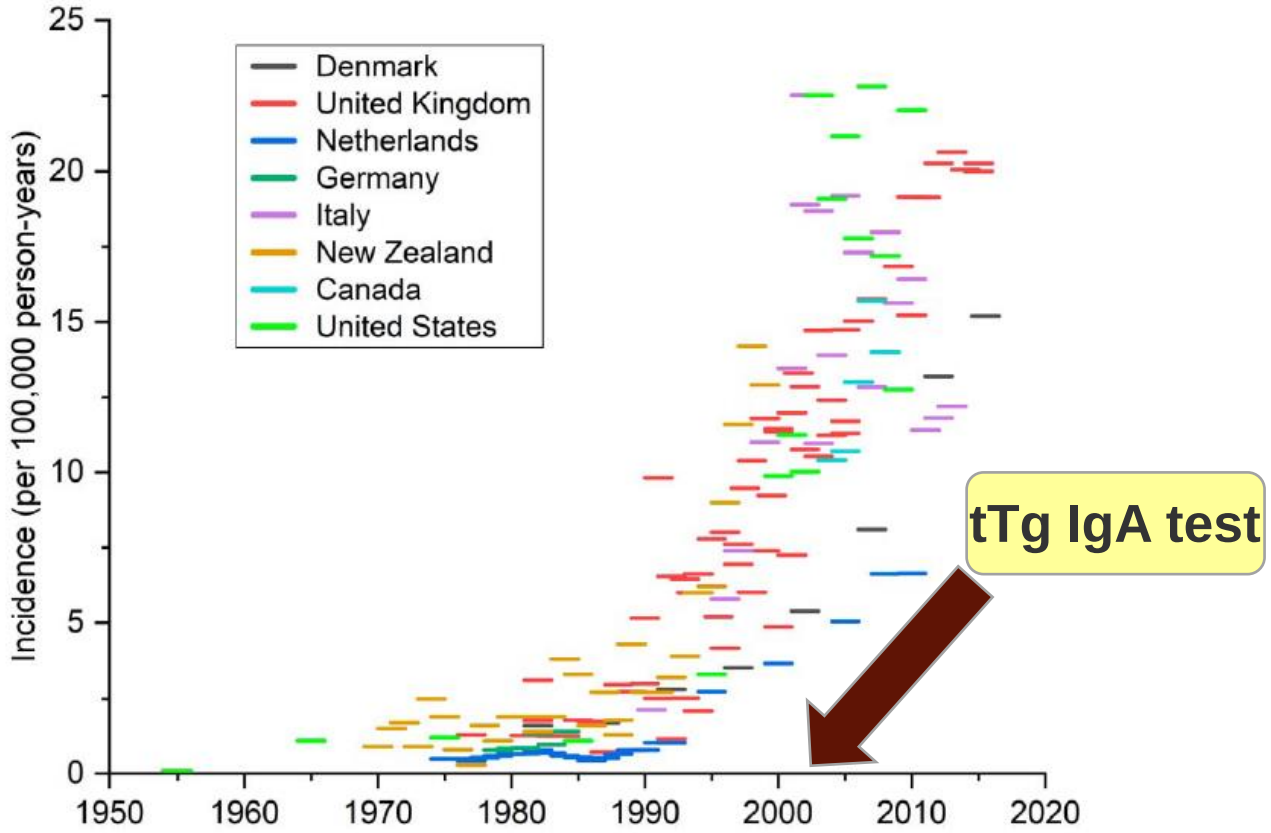


Singh P, Arora A, Strand TA, et al. Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol. 2018;16(6):823-836.e2. doi:10.1016/j.cgh.2017.06.037

Global Prevalence

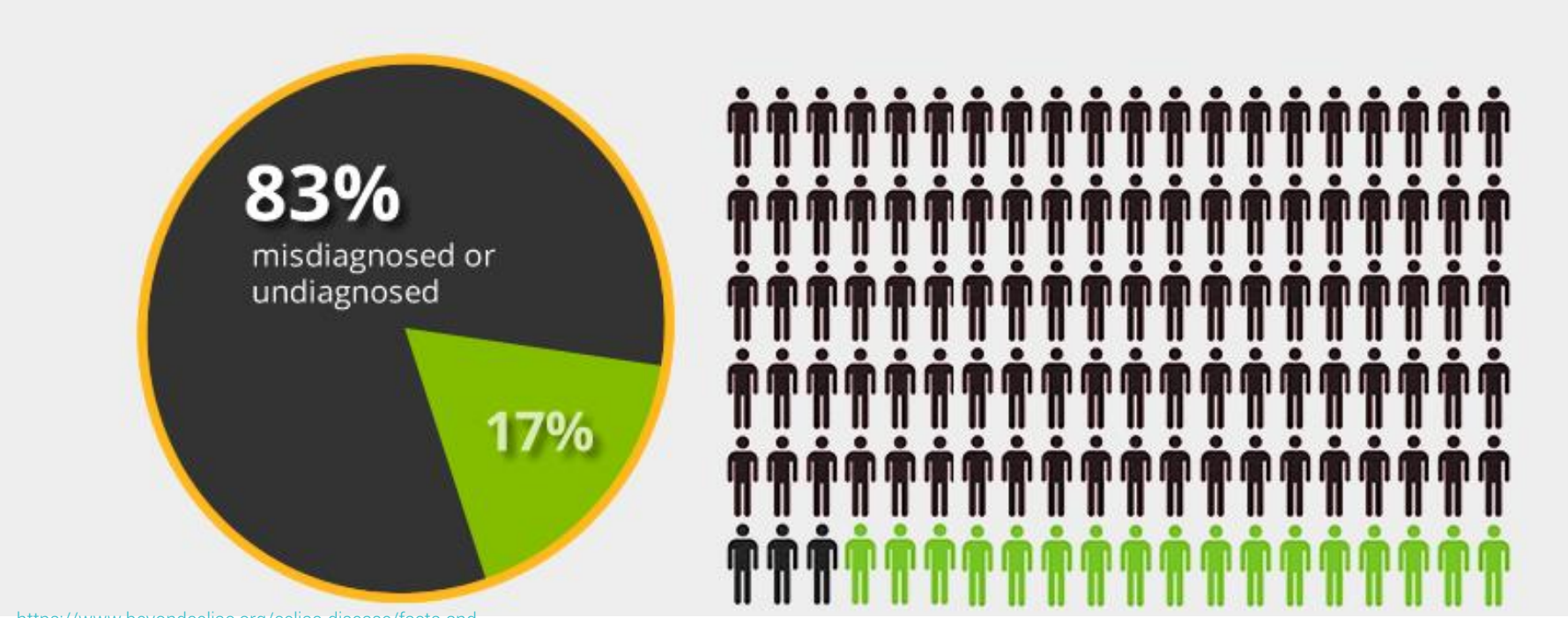
Global Prevalence

1950's: 0.01%
1970's: 0.03%
2020: 1%



King JA, Jeong J, Underwood FE, et al. Incidence of Celiac Disease Is Increasing Over Time: A Systematic Review and Meta-analysis. *Am J Gastroenterol*. 2020;115(4):507-525.

Fast facts



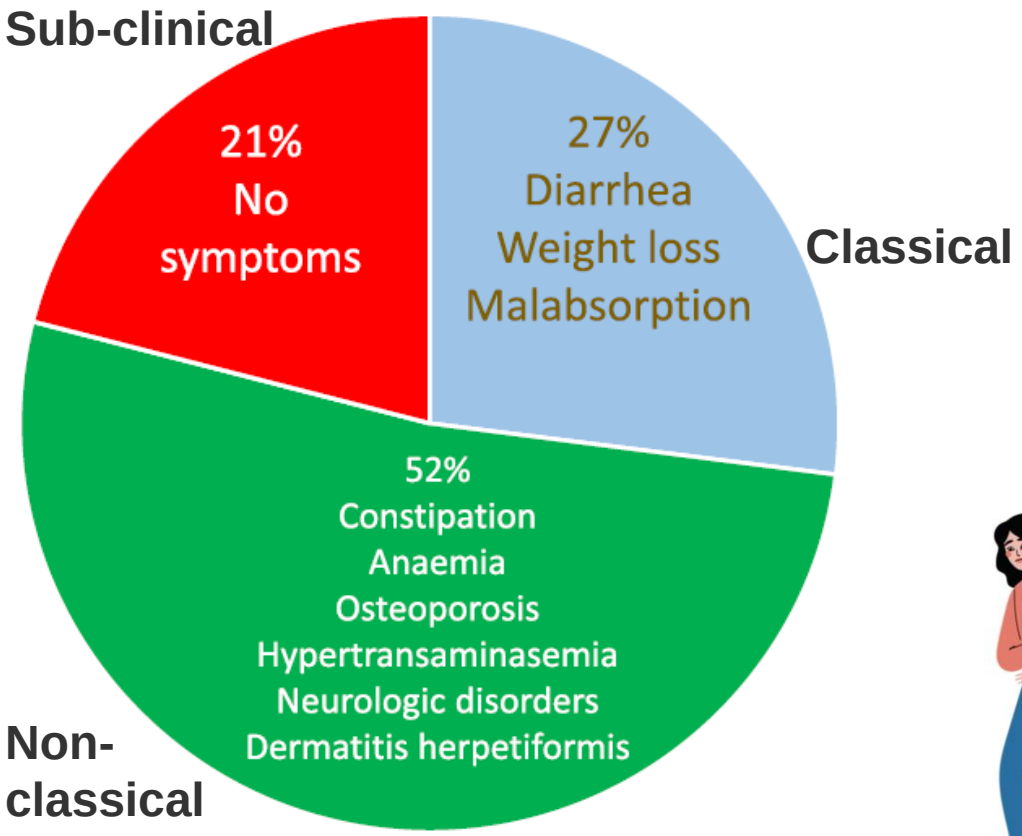
https://www.beyondceliac.org/celiac-disease/facts-and-figures/#:~:text=It%20is%20estimated%20that%20up,or%20misdiagnosed%20with%20other%20conditions.&text=Celiac%20disease%20can%20lead%20to,cancers%2C%20and%20other%20autoimmune%20diseases

- CD is 4X more common than 50 years ago
- 4-fold increased risk of death over 50 yrs in those with undiagnosed CD

Rubio-Tapia A, Kyle RA, Kaplan EL, Johnson DR, Page W, Erdtmann F, Brantner TL, Kim WR, Phelps TK, Lahr BD, Zinsmeister AR, Melton LJ 3rd, Murray JA. Increased prevalence and mortality in undiagnosed celiac disease. Gastroenterology. 2009 Jul;137(1):88-93. doi: 10.1053/j.gastro.2009.03.059. Epub 2009 Apr 10. PMID: 19362553; PMCID: PMC2704247.

Riznik, Petra⁎; De Leo, Luigina†; Dolinsek, Jasmina‡; Gyimesi, Judit§; Klemenak, Martina⁎; Koletzko, Berthold‖,¶; Koletzko, Sibylle¶,#; Koltai, Tunde⁎⁎; Korponay-Szabó, Ilma Rita§,††; Krencnik, Tomaz⁎; Milinovic, Marina‡‡; Not, Tarcisio†; Palcevski, Goran§§; Sblattero, Daniele‖‖; Werkstetter, Katharina Julia¶; Dolinsek, Jernej⁎,¶¶. The Knowledge About Celiac Disease Among Healthcare Professionals and Patients in Central Europe. Journal of Pediatric Gastroenterology and Nutrition: April 2021 - Volume 72 - Issue 4 - p 552-557 doi: 10.1097/MPG.0000000000003019

Spectrum of Clinical Presentations



Common Features	Less Common Features	Associated Conditions	Complications
Adults	General features	Definite associations	Refractory sprue
Iron-deficiency anemi	Short stature	Dermatitis herpetiformis	Enteropathy-associated T-cell lymphoma
Diarrhea	Delayed puberty	IgA deficiency	Carcinoma of the oropharynx, esophagus, and small bowel
Children	Gastrointestinal features	Type 1 diabetes	Ulcerative jejunoileitis
Diarrhea	Recurrent aphthous stomatitis	Autoimmune thyroid disease	Collagenous sprue
Failure to thrive	Recurrent abdominal pain	Sjögren's syndrome	
Abdominal distention	Steatorrhea	Microscopic colitis	
	Extraintestinal features	Rheumatoid arthritis	
	Folate-deficiency anemia	Down's syndrome	
	Osteopenia or osteoporosis	IgA nephropathy	
	Dental-enamel hypoplasia	Possible associations	
	Vitamin K deficiency	Congenital heart disease	
	Hypertransaminasemia	Recurrent pericarditis	
	Thrombocytosis (hyposplenism)	Sarcoidosis	
	Arthralgia or arthropathy	Cystic fibrosis	
	Polyneuropathy	Fibrosing alveolitis	
	Ataxia	Lung cavities	
	Epilepsy (with or without cerebral calcification)	Pulmonary hemosiderosis	
	Infertility	Inflammatory bowel disease	
	Recurrent abortions	Autoimmune hepatitis	
	Anxiety and depression	Primary biliary cirrhosis	
	Follicular keratosis	Addison's disease	
	Alopecia	Systemic lupus erythematosus	
		Vasculitis	
		Polymyositis	
		Myasthenia gravis	
		Schizophrenia	

Farrell RJ, Kelly CP. Celiac sprue. *N Engl J Med*. 2002;346(3):180-188. doi:10.1056/NEJMra010852

Husby S, Murray JA, Katzka DA. AGA Clinical Practice Update on Diagnosis and Monitoring of Celiac Disease-Changing Utility of Serology and Histologic Measures: Expert Review. *Gastroenterology*. 2019;156(4):885-889. doi:10.1053/j.gastro.2018.12.010

Patients with celiac disease get diagnosed at what age on an average in the United States

A. 23 years
B. 45 years
C. 60 years
D. 2 years
E. 10 years

Correct Answer is **B. 45 Years**

Correct Answer is **B. 45 Years**

Current Perspective

- Prevalent across globe; no ethnic preference
- Diverse clinical presentations; symptoms can range for >10 years before an established diagnosis
- Not a pediatric disease; Most diagnosis occurs at 4th decade of life

Diagnosis

- Initial Diagnosis: clinical presentation + **serology** + histology
- Definitive Diagnosis: Resolution of symptoms with gluten free diet

Celiac Disease Testing

- Anti-reticulin
- Anti-Gliadin

→
- First serologic tests
- Low positive predictive value & specificity

- **Anti- tissue transglutaminase(TTG)**
- Anti-endomysial (EMA)

→
- Same target antigens
- **TTG: screening test, high accuracy**
- EMA: High specificity, IFA-based

- Anti-DGP

→
- Moderate sensitivity & specificity
- Indicated only in specific settings

Serology tests

IgA & IgG isotype

- HLA DQ2
- HLA DQ8

→
- High negative predictive value

Genetic tests

A. IgA- deficient or IgA- low individuals

Total IgA and TTG IgA antibody testing superior/primary test for screening

- Anti-DGP (IgA and IgG) recommended against initial screening
- Selective IgA deficiency, IgG tests useful
- IgA isotype more specific than IgG for celiac disease diagnosis
- Anti-DGP IgG antibody more sensitive than TTG IgG in IgA-deficient individuals

Husby S, Koletzko S, Korponay-Szabó I, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. *J Pediatr Gastroenterol Nutr*. 2020;70(1):141-156. doi:10.1097/MPG.0000000000002497

B. Low to Moderate anti-tTg IgA titers

Total IgA and TTG IgA antibody testing <u>superior/primary test</u> for screening

- DGP antibody utility is debatable
  - Recent data suggests limited diagnostic accuracy
  - High false positive in Type 1 Diabetes patients
- Anti-EMA (IgA and IgG) antibody testing recommended only as secondary test
  - IFA subjected to operator-based interpretation
  - Although specific is expensive

Husby S, Koletzko S, Korponay-Szabó I, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. *J Pediatr Gastroenterol Nutr*. 2020;70(1):141-156. doi:10.1097/MPG.0000000000002497

HLA DQ2 and DQ8 testing

- HLA DQ2 and DQ8 is present in 90 to 95% of celiac disease patients
- Present in ~ 40% of most European and US populations
- Not all have celiac disease, only ~1% have celiac disease

High negative predictive value

Negative for HLA

Exclude Celiac Disease Diagnosis

Positive for HLA

Celiac Disease Diagnosis Possible

Leonard MM, Lebwohl B, Rubio-Tapia A, Biagi F. AGA Clinical Practice Update on the Evaluation and Management of Seronegative Enteropathies: Expert Review. *Gastroenterology*. 2021;160(1):437-444. doi:10.1053/j.gastro.2020.08.061

C. When HLA Testing can be Useful?

Not recommended for routine screening

- To rule out celiac disease when seronegative enteropathy is present
- To rule out celiac disease when patient has already initiated gluten-free diet and reports severe symptoms with gluten exposure
- Accuracy to exclude by HLA testing still depends on the method used, awareness required

53 years old female has symptoms of Diarrhea, occasional bloating and weight loss. She has an history of breast cancer; it has been 6 months post-surgery and chemotherapy. She tests positive for TTG, IgA (15 U/mL; Negative, <3 U/mL). She does not want to go through a biopsy procedure due to mental depression from her cancer diagnosis and frequent hospital visits. Which of the following test/(s) would be considered the best to confirm her diagnosis?

A. Anti-EMA,IgA
B. Anti-DGP, IgA
C. Anti-EMA, IgA and Anti-DGP, IgA
D. Anti-TTG, IgG
E. Anti-DGP, IgG

Correct Answer is **A.** **Anti-EMA,IgA**

Correct Answer is **A. Anti-EMA,IgA……**

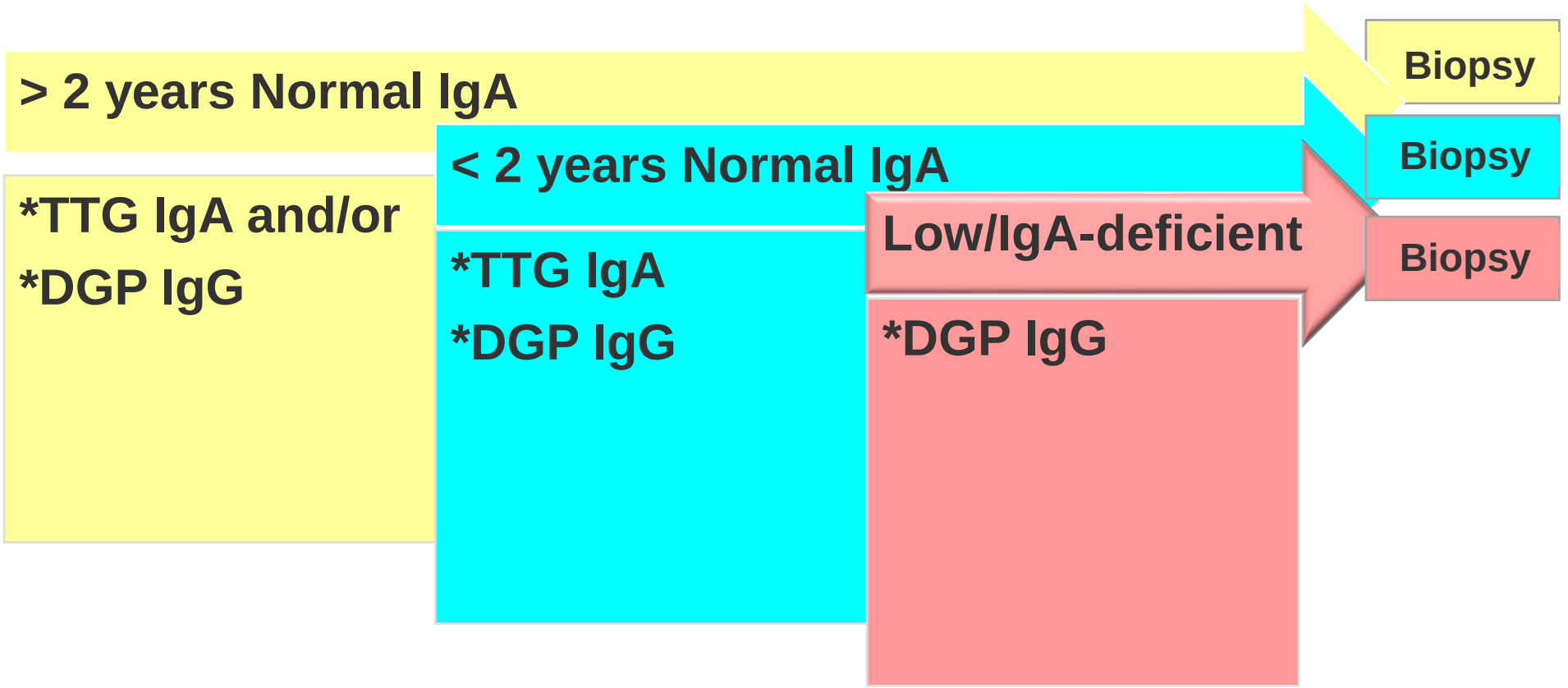
- Biopsy preferred
- Anti-EMA IgA, highly specific
- Caution with the use of Anti-DGP IgA; low predictive power

18 years old female had frequent abdominal pain and bloating after being diagnosed with Autoimmune hepatitis 13 months ago. She comes for a second-opinion in suspicion for celiac disease. She has adhered to a self-prescribed gluten-free diet for 5 months now and has found significant improvements. Her TTG, IgA is negative, and all other labs are normal. Which test can next be helpful with her management ?

A. Gluten challenge
B. Anti-EMA, IgA
C. HLA DQ2/DQ8
D. Anti-DGP, IgA

Correct Answer is **C.** **HLA DQ2/DQ8……**

2013 ACG Guidelines

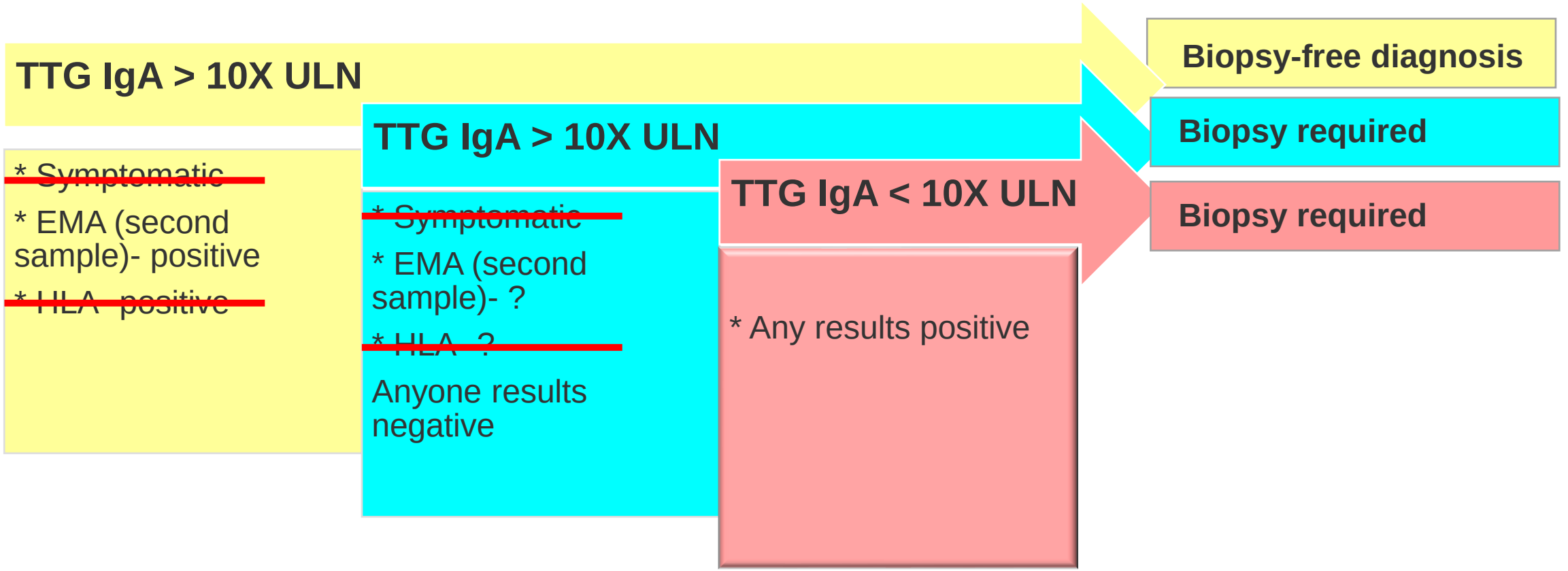


- Antibodies against native gliadin are not recommended anymore
- All tests should be performed on gluten-containing diet
- Combining several tests in lieu of TTG IgA alone is not recommended
- If suspicion is high, biopsy should be pursued even if seronegative

Rubio-Tapia, Alberto; Hill, Ivor D; Kelly, Ciarán P; Calderwood, Audrey H; Murray, Joseph A Official journal of the American College of Gastroenterology| ACG108(5):656-676, May 2013. doi: 10.1038/ajg.2013.79

2012 ESPGHAN Guidelines/2020 Update

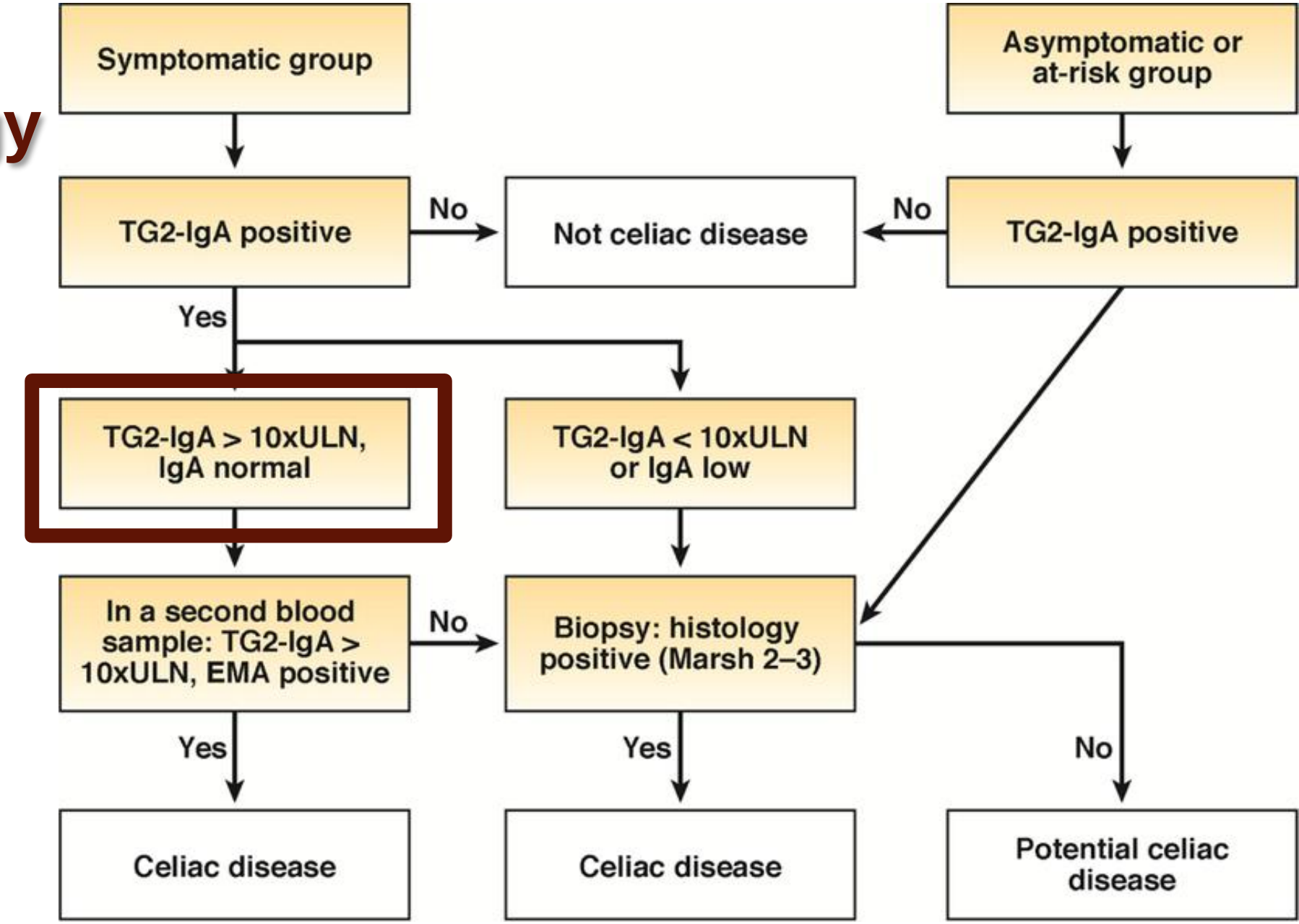
Biopsy-free Diagnosis



Husby S, Koletzko S, Korponay-Szabó I, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. *J Pediatr Gastroenterol Nutr*. 2020;70(1):141-156. doi:10.1097/MPG.0000000000002497

American Gastroenterology Association

- Only North American Update so far
- No consensus yet



Husby S, Murray JA, Katzka DA. AGA Clinical Practice Update on Diagnosis and Monitoring of Celiac Disease-Changing Utility of Serology and Histologic Measures: Expert Review. *Gastroenterology*. 2019;156(4):885-889. doi:10.1053/j.gastro.2018.12.010

Studies: Anti-Tissue Transglutaminase Antibody Levels Are Not Sufficient to Diagnose Celiac Disease

Table 1 tTG IgA titers and the accuracy of celiac disease

tTG titers # patients = 240	Sensitivity % (95 % CI)	Specificity % (95 % CI)	PPV % (95 % CI)	NPV % (95 % CI)	Accuracy %
≥3× ULN (219/240)	93.0 (88.5–96.1)	17.1 (7.2–32.1)	84.5 (79.0–89.0)	33.3 (14.6—57.0)	80.0
≥10× ULN (171/240)	75.4 (68.8–81.2)	48.8 (32.9–64.9)	87.7 (81.8–92.2)	29.0 (18.7–41.1)	70.8
≥100 U/ml (176/240)	75.4 (68.8–81.2)	34.2 (20.1–50.6)	84.8 (78.6–89.7)	22.2 (12.7–34.5)	68.3
≥100 U/ml and ≥10× ULN (134/240)	67.3 (60.4–73.8)	53.7 (37.4–69.3)	87.8 (81.3–92.4)	25.3 (16.6–35.8)	65.0

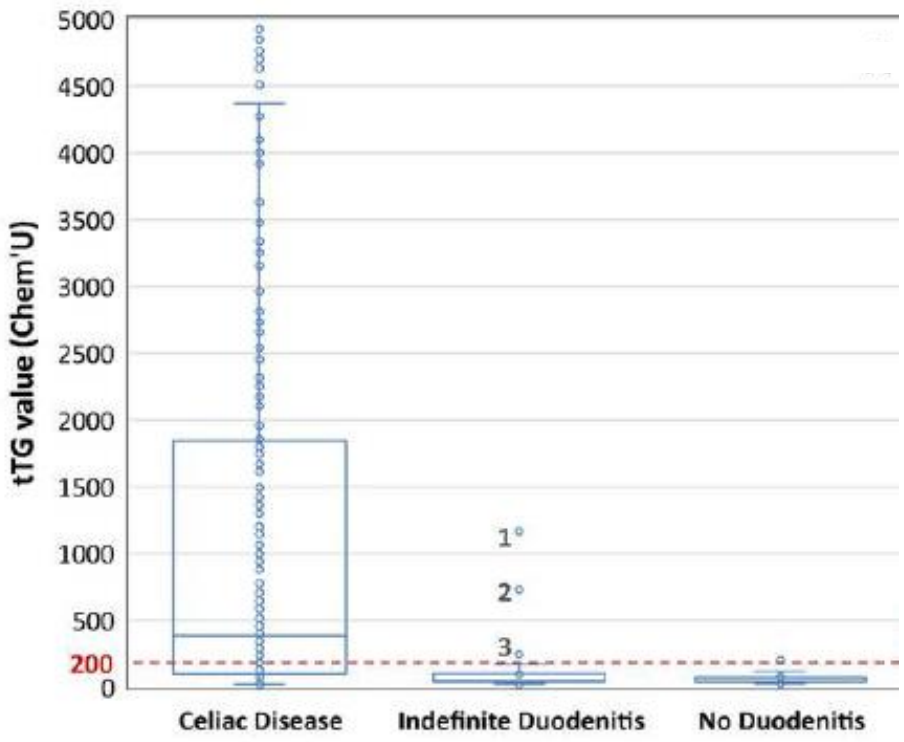
Elitsur Y, Sigman T, Watkins R, et al. Tissue Transglutaminase Levels Are Not Sufficient to Diagnose Celiac Disease in North American Practices Without Intestinal Biopsies. *Dig Dis Sci*. 2017;62(1):175-179. doi:10.1007/s10620-016-4354-4

Studies: Non-biopsy ESPGHAN criteria had a high positive predictive value for CD diagnosis

Table 3. Test performance—PPV, NPV of the TTG, and EMA

	Total	CD+		CD–		PPV			NPV
TTG		All	EMA+	All	EMA+	All	If EMA–	If EMA +	
1–3× ULN	53	16	12	37	11	30.2%	13.3%*	52.2%*	—
3–10× ULN	114	72	56	42	18	63.2%	40.0%**	75.7%**	—
≥10× ULN	270	259	256	11	7	95.9%	42.9%**	97.3%**	—
Normal	574	3	—	571	—				99.4%
Total	1,011								

Gidrewicz D, Potter K, Trevenen CL, Lyon M, Butzner JD. Evaluation of the ESPGHAN Celiac Guidelines in a North American Pediatric Population. *Am J Gastroenterol*. 2015;110(5):760-767. doi:10.1038/ajg.2015.87



Policy differences between Europe and North America guidelines

- **Applying the ESPGHAN cut-off of 200 Chem'U (10x ULN)**
- **289 out of 292 endoscopies (99%) are diagnostic for CD**
- **3 show Indeterminate Duodenitis (on gluten-free diet)**

Badizadegan K, Vanlandingham DM, Hampton W, Thompson KM. Value of biopsy in a cohort of children with high-titer celiac serologies: observation of dynamic policy differences between Europe and North America. *BMC Health Serv Res*. 2020;20(1):962. Published 2020 Oct 20. doi:10.1186/s12913-020-05815-0

- ESPGHAN no-biopsy approach has been evaluated and proved effective in a variety of settings
- Despite debate continues in the US about the adoption of any no- biopsy approach
- Lack of laboratory harmonization as an obstacle in implementation of standard diagnostic algorithms.

Assay-Specific validation of ≥10X ULN cut-off !

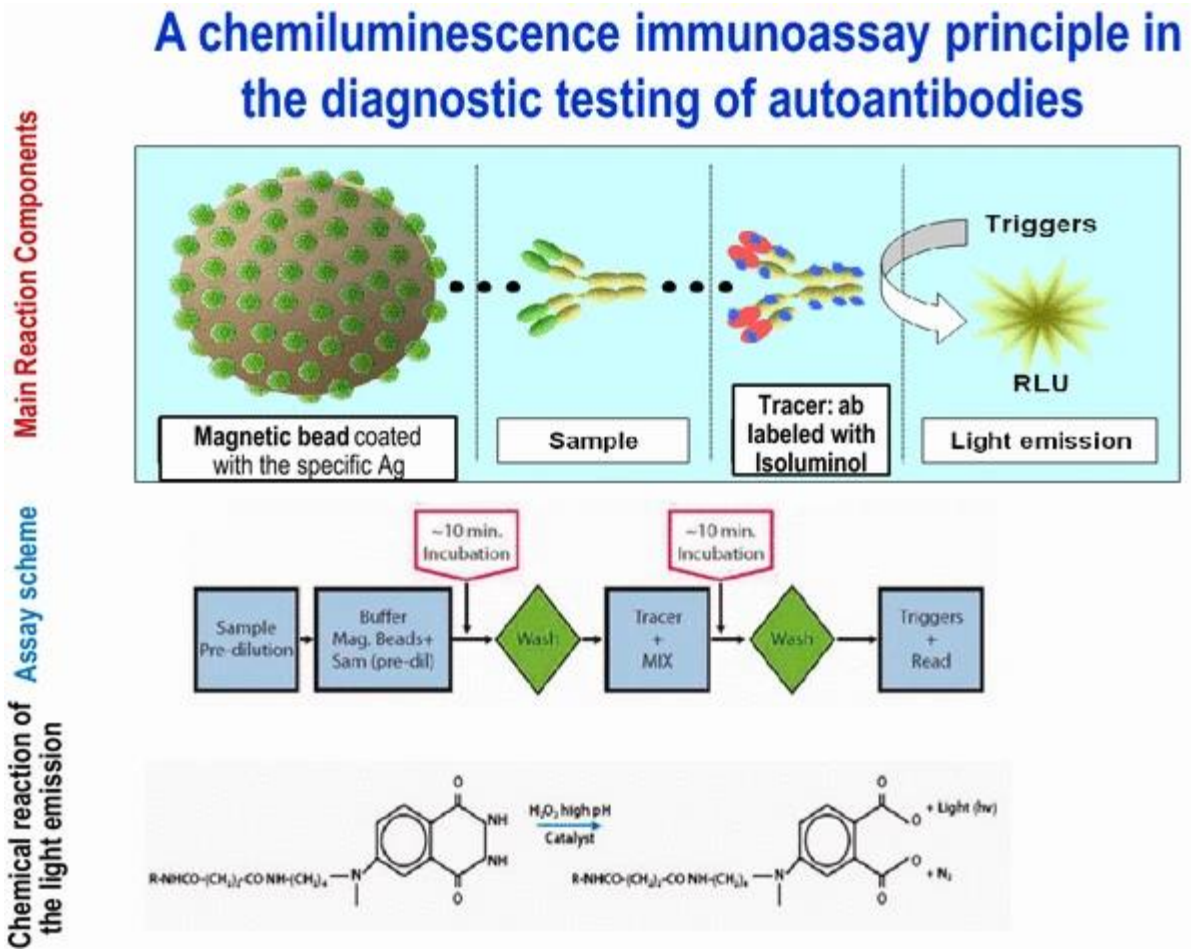
Methods

TTG, IgA Assay methods

- Chemiluminescent immunoassay
- Enzyme-linked immunoassay
- Fluorescence enzyme immunoassay
- Particle-based Multianalyte Fluorescence platforms

- ➢ Lack of standardization
- ➢ Validation using clinically-defined samples
- ➢ Individual labs work-flow needs and logistics

Chemiluminescent immunoassay



Advantages

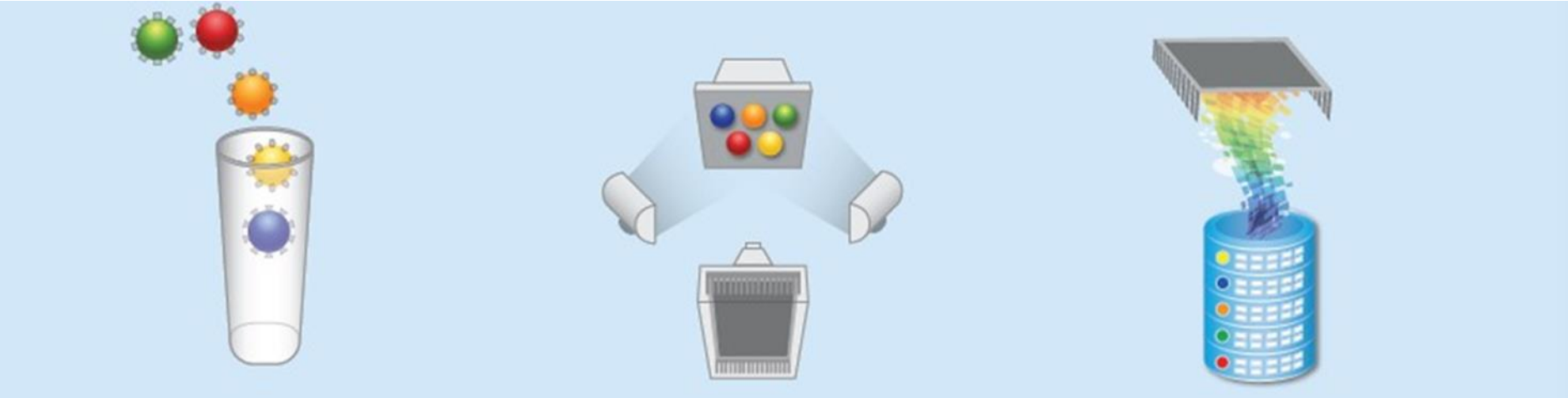
- Wide dynamic range
- High specificity
- Reduced incubation times
- High-throughput and automation

Disadvantages

- Background in the absence of analyte
- Discrete tests

Cinquanta, L., Fontana, D.E. & Bizzaro, N. Chemiluminescent immunoassay technology: what does it change in autoantibody detection?. Autoimmun Highlights 8, 9 (2017). https://doi.org/10.1007/s13317-017-0097-2

Particle-based Multianalyte Fluorescence

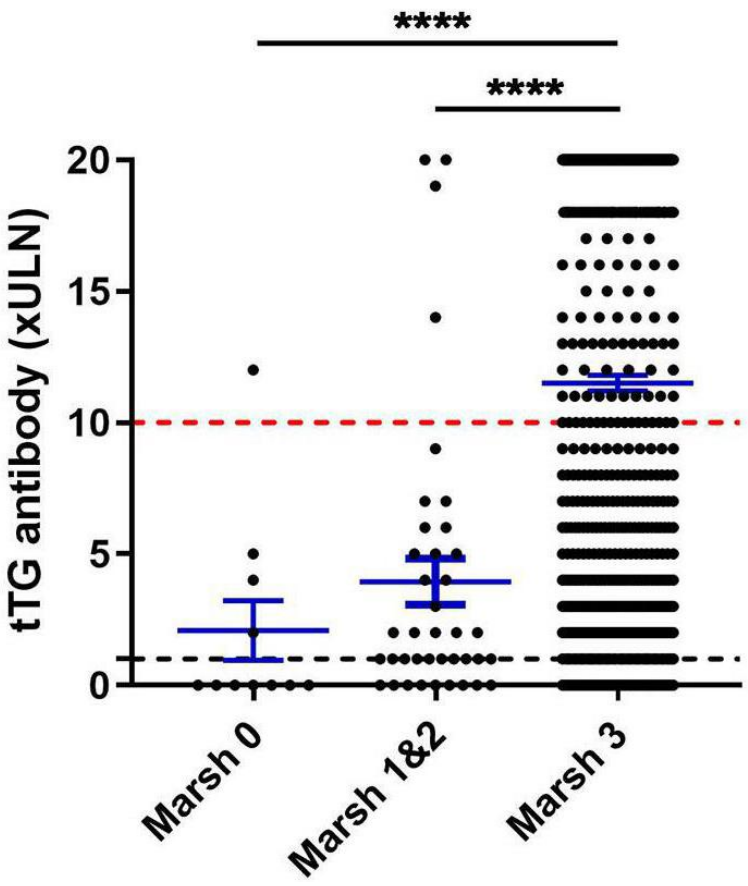


Advantages

- High throughput and automation
- High accuracy
- One tube-multiple analyte detection

Modified from Aptiva (INOVA Diagnostics, Werfen) package insert.

Accuracy of a no biopsy approach in Adults



	≥10x ULN	
	Value	95% CI
Sensitivity	54%	51-58%
Specificity	90%	78-97%
PPV	98.7%	97.0-99.4%
NPV	12.5%	11.2-13.9%

Multicenter study across 8 countries and 11 labs

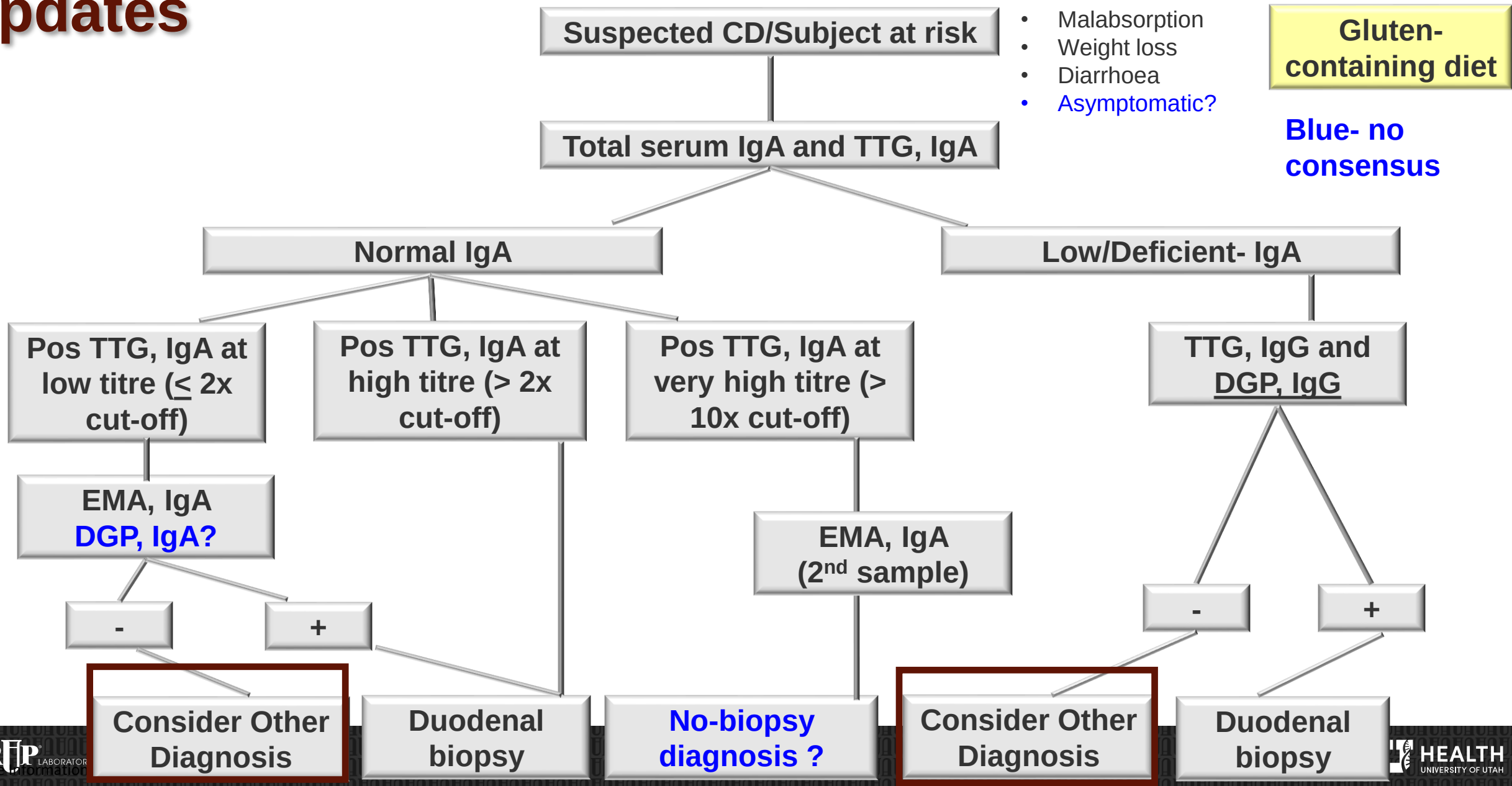
Predictive values ranges from 95.2-98.7%

Penny HA, Raju SA, Lau MS, et alAccuracy of a no-biopsy approach for the diagnosis of coeliac disease across different adult cohortsGut 2021;70:876-883.

Consequences of Avoiding a Biopsy….

- Is the positive predictive value for a high titer TTG IgA high enough?
- Studies show > 95% chance for this group of patients to have celiac disease
- Inaccurate diagnosis can cost major lifestyle changes
- Risk for false diagnosis and management in future
- Potential of missing diagnosis associated with other intestinal enteropathies

Summary: Diagnostic Algorithm based on the current updates



Not Celiac disease, what other diagnoses?

- **Negative confirmatory serology on a gluten-containing diet in the presence of symptoms and/or**
- **No histological improvement after compliance to gluten-free diet**

- Autoimmune enteropathy
- Common variable immunodeficiency
- Whipple disease
- Giardiasis
- Human immunodeficiency virus enteropathy
- Intestinal lymphoma
- Intolerance of foods other than gluten (e.g. milk, soy, etc.)
- Radiation enteritis
- **Crohn's disease (2nd most cause of villous atrophy)**

- Common symptoms: diarrhea, fatigue, weight loss, bloating, and anemia.
- Treatment differs

Pelkowski TD, Viera AJ. Celiac disease: diagnosis and management. *Am Fam Physician*. 2014;89(2):99-105.

Treatment

- Only effective treatment available for CD is **Gluten-free diet**

  - resolution of intestinal and extraintestinal symptoms
  - negativity of autoantibodies
  - regrowth of the intestinal villi

- Upto to **50%** of patients continuing to experience symptoms and/or intestinal damage while on the gluten-free diet

Non-dietary Drugs in Clinical trial

Several **Clinical trials** ongoing but only few have reached later phases

- Larazotide (ALV003) acetate is in phase 3 clinical trial
  - antagonist blocking tight junction disassembly preventing gluten permeability through the epithelial barrier
  - Also degrades gluten to small fragments
  - Not effective with large quantities of gluten.

- IL-15 monoclonal antibodies (AMG 714) are being investigated in phase 2
  - IL15, major mediator of pathophysiological processes of celiac disease
  - inhibits the function of IL-15 and IL-15-induced T cell proliferation

TTG IgA for monitoring

- **Anti-TTG, IgA** although used frequently for monitoring is **not reliable**
  - Titers do not correlate well with histological findings in CD patients on a GFD
  - Due to long half-life of antibodies, titers reflect immune response but not intestinal damage

- **Difficult** to evaluate small and infrequent exposures
  - Exposure may occur due to cross-contamination

Assays for monitoring GFD compliance

- Detection of gluten immunogenic peptides **(GIP) in feces and urine**
  - Direct detection of gluten intake and compliance verification

- Fecal calprotectin, REG Iα, plasma total alkylresorcinols, intestinal-fatty acid binding protein (I-FABP), citrulline, pancreatic secretory-granule membrane glycoprotein 2 (GP2)
  - Not direct measure of gluten intake, measures consequences of dietary transgression

- Self-monitoring device to detect gluten presence in food(E.g. Nemo sensor)
  - Does not detect all forms of gluten
  - Too sensitive and is unnecessary clinically

Summary

- Celiac disease is common(~ 1%) and many remain undiagnosed
- TTG IgA and Total IgA serves as a power screening test for diagnosis
- At high TTG IgA titers biopsy-free approach is supported by European guidelines in children
- Final diagnosis largely dependent on biopsy in adults and in low prevalence settings
- DGP test should be used in a limited manner and with caution.
- Many non-dietary therapies are currently in clinical trial
- Future should be dedicated to development of reliable assays for monitoring compliance to gluten-free diet.

THANK YOU

VIJAYALAKSHMI (VIJI) NANDAKUMAR, PHD, DABCC
Assistant Professor, University of Utah School of Medicine, Department of Pathology
Medical Director, Autoimmune & Complement Disease Testing, ARUP Laboratories
Email: Vijayalakshmi.nandakumar@aruplab.com

ARUP® LABORATORIES

HEALTH
UNIVERSITY OF UTAH

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