

Tools and Advanced Imaging Technologies for Assessing Intestinal Epithelial Barrier Integrity: A Systematic Review

Sophie Vieujean¹, Raja Atreya², Andrea Buda³, Josselin Caradec⁴, Sandra Citi⁵, Silvio Danese⁶, Olivier Dewit¹, Matthias Friedrich⁷, Subrata Ghosh⁸, Marietta Iacucci⁸, Vipul Jairath⁹, Arthur Kaser^{10,11}, Rupert W Leong¹², Markus F Neurath¹³, Nicolas Pierre¹⁴, Mathilde Pohin¹⁵, Timo Rath¹⁶, Pauline Rivière¹⁷, Simon Travis¹⁸, James Westcott⁴, Sebastian Zeissig¹⁹, Laurent Peyrin-Biroulet²⁰

¹ Department of Gastroenterology, Cliniques Universitaires Saint-Luc, Brussels, Belgium

² Medical Department 1, University Hospital Erlangen, Friedrich-Alexander-University of Erlangen-Nürnberg, Ulmenweg 18, 91054 Erlangen, Germany

³ Gastroenterology Unit, Department of Gastrointestinal Oncological Surgery, S. Maria del Prato Hospital, Feltre, Italy

⁴ ThirtyFiveBio, Milton, Abingdon OX14 4RY, UK

⁵ Department of Molecular and Cellular Biology, University of Geneva, Geneva, Switzerland

⁶ Gastroenterology and Endoscopy, IRCCS Ospedale San Raffaele and University Vita-Salute San Raffaele, Milano, Italy

⁷ Translational Gastroenterology and Liver Unit and NIHR Biomedical Research Centre, University of Oxford, Oxford, UK

⁸ APC Microbiome Ireland, College of Medicine and Health, University College Cork, Cork, Ireland

⁹ Department of Medicine, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada

25 ¹⁰ Cambridge Institute of Therapeutic Immunology and Infectious Disease, University of
26 Cambridge, Cambridge, CB2 0AW, UK

27 ¹¹ Division of Gastroenterology and Hepatology, Department of Medicine, University of
28 Cambridge, Cambridge, CB2 0QQ, UK

29 ¹² Department of Gastroenterology, Macquarie University Hospital, Sydney, Australia

30 ¹³ MFN Department of Medicine 1, Ludwig Demling Center of Excellence in Endoscopy,
31 Deutsches Zentrum Immuntherapie DZI, FAU University Erlangen-Nürnberg, Germany

32 ¹⁴ Laboratory of Translational Gastroenterology, GIGA-institute, University of Liege, Liege,
33 Belgium

34 ¹⁵ Immunoconcept UMR 5164 University of Bordeaux – Immunology and immunogenetic
35 Unit, CHU de Bordeaux, France

36 ¹⁶ University Hospital Erlangen, Department of Gastroenterology, Ludwig Demling
37 Endoscopy Center, Erlangen, Germany

38 ¹⁷ Service d'Hépto-gastroentérologie et oncologie digestive, CHU de Bordeaux.
39 ImmunoConcept UMR5164, University of Bordeaux France

40 ¹⁸ Kennedy Institute of Rheumatology, Translational Gastroenterology and Liver Unit and
41 NIHR Biomedical Research Centre, University of Oxford, Oxford, UK

42 ¹⁹ Department of Visceral Surgery and Medicine, Inselspital - Bern University Hospital,
43 University of Bern, Switzerland

44 ²⁰ Department of Gastroenterology, CHRU Nancy, INSERM NGERE, Université de Lorraine,
45 F-54500 Vandœuvre-lès-Nancy, France

46

47 **Shortened version of the title:** Tools to Assess Intestinal Epithelial Barrier Integrity

48

49

50 **Corresponding author :**

51 Sophie Vieujean

52 Clinique Universitaire Saint-Luc

53 Avenue Hippocrate, 10

54 1200 Brussels

55 sophie.vieujean@saintluc.uclouvain.be

56 **References:** 215 references

57

58 **Figures and tables:** 4 Tables, 4 Figures, 2 Supplementary Tables

59

60 **Word count:** 5876 words

61

62

63 **Abbreviations:** α -GST, α -glutathione-S-transferase; AJC, apical junctional complex; CLE,
64 confocal laser endomicroscopy; CMOS, complementary metal oxide semiconductor; DAO,
65 Diamine oxidase; DCE-MRI, Dynamic contrast-enhanced magnetic resonance imaging; EC,
66 endocytoscopy; FITC, fluorescein isothiocyanate; HD-MEAs, high-density microelectrode
67 arrays; HPLC–MS, high-performance liquid chromatography–tandem mass spectrometry;
68 IBD, inflammatory bowel disease; I-FABP, intestinal fatty acid–binding protein; Ig,
69 immunoglobulin; LBP, LPS-binding protein; LCN-2, Lipocalin-2; LPS, lipopolysaccharide;
70 NMR, nuclear magnetic resonance; OECT, organic electrochemical transistor; pCLE, probe-
71 based confocal laser endomicroscopy; PEG, polyethylene glycols; PET, Positron emission
72 tomography; sCD14, soluble cluster of differentiation 14; TEER, transepithelial electrical
73 resistance; TJs, tight junctions; ZO, zonula occludens.

74

75

76 **Abstract**

77 The intestinal epithelium is a key component of the intestinal barrier, which is the largest and
78 most complex barrier of the human body, regulating nutrients absorption while restricting the
79 entry of harmful antigens. Breakdown of this barrier facilitates microbial and dietary
80 antigenic translocation, triggering local immune system activation and inflammation.
81 Although barrier alterations alone may not be sufficient to initiate disease, accumulating
82 evidence highlights its critical role in the pathogenesis and progression of a wide range of
83 gastrointestinal and systemic disorders. Early identification of intestinal epithelium and
84 barrier alterations could enable timely therapeutic approaches. This systematic review
85 provides an overview of current in vivo (both non-invasive and invasive) and ex vivo/in vitro
86 approaches used to assess intestinal epithelial barrier alterations. Non-invasive in vivo
87 approaches rely mainly on urinary detection of orally ingested probes, but their clinical utility
88 is limited by lack of standardization and specificity. Circulating and fecal constitutive
89 markers derived from the intestinal barrier, which reflect epithelial alterations, together with
90 indicators of microbial translocation, provide complementary insights but remain
91 insufficiently validated. Advanced invasive endoscopic modalities such as confocal laser
92 endomicroscopy enable near-histologic, real-time visualization but are costly and largely used
93 as research tools in specialist centers. In vitro, transepithelial electrical resistance assessment
94 remains the reference standard, though novel technologies (including impedance
95 spectroscopy and organic electrochemical transistors) offer enhanced sensitivity and
96 resolution. Despite progress, major gaps remain, including the absence of a standardized
97 definition of epithelial barrier breakdown, the lack of a practical diagnostic tool,
98 methodological heterogeneity, unvalidated thresholds, and limited prospective validation.

99

100 **Keywords:** intestinal epithelium, intestinal barrier, tool, in vivo, in vitro, advanced imaging,
101 probe confocal endomicroscopy, endocytoscopy.

102

Introduction

The intestinal epithelium is a key component of the intestinal barrier, which is the largest barrier separating our body from the external environment and also the most functionally complex^{1,2}. By permitting the absorption of electrolytes, water, and nutrients while restricting the entry of pathogens and harmful substances, it acts as both a selective gateway for molecular exchange and a frontline defense against continuous environmental and microbial challenges, while simultaneously shaping appropriate immune responses to luminal compounds^{2,3}.

Repeated exposure to dietary antigens, microbial agents and environmental insults can compromise the integrity of the intestinal epithelial barrier⁴⁻⁷. This results in impaired epithelial barrier function, commonly referred to as “leaky gut”, which facilitates the translocation of luminal contents into the underlying mucosa and systemic circulation, thereby activating the gut immune system and promoting inflammation, which can in turn exacerbate barrier dysfunction and support a self-perpetuating disruptive cycle^{8,9}. While barrier dysfunction alone may not be sufficient to initiate disease^{10,11}, a growing body of evidence underscores its pivotal role in the onset and progression of a broad spectrum of digestive and extra-digestive diseases (**Figure 1**)¹².

Building Blocks and Functions of the Intestinal Epithelial Barrier

Forming crypts and villi in the small intestine, and crypts only in the colon, the intestinal barrier is composed of a mucus layer that overlies a single layer of epithelial cells, within which intraepithelial lymphocytes and myeloid cells are embedded. These cells rest on a basement membrane and are supported by the underlying lamina propria. The colon contains a two-layered mucus structure (an inner, firmly adherent layer and an outer, loose layer),

whereas in the small intestine the mucus is single-layered and typically thinner¹³. In quantitative terms, the combined thickness of the mucus and unstirred water layer exceeds the height of a single epithelial cell by more than twenty-fold, highlighting the substantial diffusion barrier luminal molecules must traverse before reaching the epithelium¹³. This mucus layer functions as a chemical barrier, owing to its composition, and as a physical shield. The underlying epithelial lining carries out numerous functions: beyond serving as a physical barrier and providing protection^{14,15}, notably through the production of antimicrobial substances¹⁶, it plays essential roles in nutrients absorption^{17,18}, bile salt reabsorption¹⁹, secretion of digestive enzymes^{20–22}, hormonal control, environmental sensing, and immune system development and regulation^{23–27}. This wide functional repertoire relies on the diversity of specialized epithelial cell types, all derived from a shared intestinal stem cell pool, including absorptive enterocytes, mucus-producing goblet cells, Paneth cells, enteroendocrine cells, M cells, and tuft cells^{16,28–35}.

To function as an effective barrier, these epithelial cells must be tightly connected. This cohesion is ensured by the apical junctional complex (AJC), located at the apical part of the lateral membrane, closest to the external environment³⁶. The AJC is composed of tight junctions (TJs) and adherens junctions (zonula adherens), the latter comprising two spatially separated domains, the apical containing PLEKHA7, nectins and afadin, the basal containing E-cadherin and catenins^{37,38}. The integrity and function of the AJC is regulated by the actomyosin cytoskeleton, which is connected to the AJC through interaction of actin and myosin with cytoplasmic scaffolding and adaptor proteins, including α -catenin, afadin, ZO-1 and cingulin^{39–42}. Additional junctions present on the lateral membrane, but not associated with the AJC are desmosomes and gap junctions, involved in cell-cell adhesion and communication^{43,44} (**Figure 2**). There are three main pathways through which molecules can

cross the intestinal epithelium: the transcellular, the paracellular and unrestricted routes⁴⁵. The transcellular route involves transport directly across the plasma membrane of epithelial cells, often through specific channels or transporters⁴⁵. The paracellular route (decreasing from small to large intestine), primarily regulated by TJs, can be subdivided into two distinct pathways: (1) the pore pathway, a high-conductance route that is both charge- and size-selective, permitting the passage of small substances with an upper size limit of 6–8 Å^{11,46,47}; and (2) the leak pathway, a low-conductance, charge-nonselective route that allows the passage of larger substances with an approximate diameter of ~100 Å⁴⁶. Lastly, the unrestricted pathway arises in the context of epithelial damages, allowing nonspecific passage of luminal contents¹².

Making Sense of Intestinal Barrier Terminology

The intestinal barrier is a complex, multilayered system composed not only of the mucus and epithelial layer, but also of essential additional components including the microbiota, vascular network, immune system and mesenchyme. It is therefore important to recognize that *intestinal epithelial barrier* alteration or dysfunction is not synonymous with global *intestinal barrier* impairment, which encompasses these other structural and immunological elements. Terminological precision is thus crucial when comparing available tools, evaluating their ability to discriminate between epithelial versus global barrier alterations, and when interpreting results across studies. The terms *barrier defect*, *dysfunction*, *disruption*, and *damage* are often used interchangeably in the literature, yet they refer to distinct biological states. *Barrier defect* may imply an intrinsic, sometimes genetically determined abnormality in barrier structure or assembly. *Barrier dysfunction* refers to impaired function (for example, increased paracellular or transcellular permeability), while the epithelial structure may remain macroscopically intact. Notably, dysfunction can arise not only in disease but also as part of a

physiological, adaptive response to infection or injury. In contrast, *disruption* or *damage* denotes a loss of epithelial continuity, such as erosions or ulcerations, allowing unrestricted passage of luminal contents. Distinguishing these concepts matters, as the underlying mechanisms, assessment methods, biomarkers, and therapeutic strategies may differ substantially between defect, dysfunction, and true tissue disruption.

Measurement of intestinal permeability is often used as a proxy for epithelial barrier integrity and is widely applied to detect or quantify epithelial injury. However, it is important to emphasize that these two concepts are distinct and should not be used interchangeably^{12,48}. This measurement of intestinal permeability is also influenced by components of the barrier beyond the epithelium. Mucus permeability, for example, differs substantially between the small and large intestine, and the vascular endothelium represents another critical determinant of intestinal permeability. Under physiological conditions, paracellular diffusion across capillaries is limited to molecules smaller than approximately 4 kDa; larger solutes cannot cross directly and must instead be cleared via the lymphatic system or enter the bloodstream only when the vascular barrier is compromised⁴⁹. A distinction is typically made between *physiological permeability*—observed under normal conditions without inflammation or toxic injury, when the selective passage of molecules is preserved—and *increased or pathological permeability*, which arises in disease states^{9,50}. For instance, the size and nature of the molecules or metabolites measured in permeability assays can help distinguish between a moderately “leaky” but continuous barrier from a barrier showing overt epithelial loss.

A wide range of tools is now available to assess intestinal epithelial barrier alterations and the resulting changes in permeability; however, only a few have been systematically validated against direct morphological evaluation of the epithelium or other assessment methods¹². In

this context, the present review provides an comprehensive overview of the currently available approaches—non-invasive and invasive in vivo, as well as in vitro and ex vivo—with particular emphasis on methods whose ability to detect barrier alterations has been directly compared against other techniques⁵¹.

Methods

Search strategy

A systematic literature search was conducted in MEDLINE and Scopus up to July 25, 2025, to identify studies evaluating methods for assessing the intestinal epithelial barrier or permeability. Search terms combined concepts of the *intestinal epithelium* or *intestinal barrier* with those of *diagnostic methods* and *tools*, and the specific combinations used in the literature search are summarized in **Supplemental Table 1**. No language restrictions were initially applied, and no limits were set regarding the publication date to ensure comprehensive retrieval of relevant studies.

Eligibility criteria

Studies were eligible for inclusion if they met the following criteria: (1) involved humans, animals, or preclinical models; (2) assessed the intestinal epithelial barrier (structure, permeability, function) using any measurement method; (3) included a comparison with another method or reference standard for barrier or permeability assessment; and (4) reported at least one relevant outcome, such as diagnostic accuracy, reliability, sensitivity/specificity, reproducibility, or concordance between methods, regardless of whether the approach was applied in humans, animals, or ex vivo/in vitro, and whether it was invasive or non-invasive, and irrespective of the type of tool used. Studies were excluded if they were narrative reviews, investigated non-intestinal epithelia, lacked a comparator group, were published in a

language other than English, or did not address barrier or permeability assessment (e.g., intervention-only studies).

Study selection, outcomes and data extraction

Titles and abstracts were screened independently by two reviewers (SV and LBP). Full texts of potentially relevant studies were retrieved for eligibility assessment. Disagreements were resolved by discussion and consensus. The primary outcomes of interest were the diagnostic accuracy of methods used to evaluate the intestinal epithelial barrier, including assessments of intestinal barrier alterations and permeability. From each eligible study, we systematically extracted detailed information on the method or intervention applied to assess the intestinal epithelium, together with the comparative method against which it was evaluated. We also documented the study population, specifying whether the investigation was conducted in humans or in preclinical models. For each study, we recorded the type of specimen analyzed, the degree of correlation between the tested method and its comparator, and any reported measures of diagnostic performance, reliability, sensitivity/specificity, or reproducibility.

Results

Literature search

A total of 10,302 records were identified through electronic databases and manual searching (**Figure 3**). After removing duplicates, 7,261 titles and abstracts were reviewed. Of these, 375 full-text articles and conference abstracts were assessed for eligibility. Research has demonstrated that intestinal barrier can be evaluated using **in vivo** tools (in humans or animal models) as well as **ex vivo/in vitro** approaches, the latter referring to techniques performed outside a living organism, typically on cultured cell lines, tissue explants or organoids maintained under controlled laboratory conditions (e.g., culture dishes, multi-well plates,

Ussing chambers, organ-on-chip systems). When applied **in vivo**, assessment methods generally fall into two main categories: (1) **Non-invasive approaches**, such as orally administered permeability probes recovered in urine or constitutive intestinal barrier-derived markers detectable in blood or stool, which infer barrier alteration without requiring tissue collection; (2) **Invasive approaches**, which require direct access to the intestinal lumen or mucosa—usually via endoscopy—and therefore involve invasive entry into the gastrointestinal tract. In total, 60 studies met the predefined inclusion and exclusion criteria and, importantly, included a comparison with another method or reference standard for assessing barrier function or permeability. Among them, 47 were *in vivo* studies (38 in humans and 9 in animal models), of which 43 evaluated *non-invasive in vivo* methods and 4 investigated *invasive in vivo* approaches. An additional 13 studies employed *in vitro* techniques.

Non-Invasive Tools to Assess Intestinal Barrier Function *In Vivo*

Tables 1 and **2** summarize the probes and constitutive intestinal barrier-derived markers used to assess intestinal barrier alterations *in vivo* in humans and animal models, respectively. **Figure 4** illustrates the biological samples in which they are measured, as well as the corresponding regions of the barrier defects, which they reflect.

Urinary Detection of Orally ingested Probes

A classic and widely used approach to indirectly assess intestinal permeability *in vivo*, both in humans and in animals, is to measure the fractional urinary excretion of orally ingested probes. When epithelial barrier integrity is compromised, increased amounts of these probes cross the mucosa, enter the bloodstream, are then filtered by the glomeruli, and subsequently are excreted in urine^{52,53}. It is important to keep in mind that permeability tests can be

affected by epithelial barrier alterations, but they are also influenced by other components of the intestinal barrier. The probes can be quantified in urine using enzymatic assays, high-performance liquid chromatography (HPLC), HPLC–tandem mass spectrometry (HPLC–MS)⁵¹, ion-exchange chromatography combined with mass spectrometry, or nuclear magnetic resonance (NMR) spectroscopy^{53–55}. The choice of probe is critical and should reflect the specific intestinal segment under investigation. For permeability assessment of the small intestine or colon, the probe should be absorbed in the corresponding intestinal region, rather than in the stomach⁵³. To assess colonic barrier integrity, the probe must also be resistant to degradation by colonic bacteria⁸. Ideally, probes should not be present in the diet to avoid background interference. Finally, the timing of urine collection enables regional discrimination of permeability⁵⁶.

There are four main types of probes: sugars, isotopes, and polyethylene glycols (PEGs) and fluorophores. Different non-metabolized sugars can be used as permeability probes, including monosaccharides (e.g., mannitol, rhamnose) and disaccharides (e.g., lactulose, cellobiose, sucralose). To minimize confounding factors such as gastric emptying, intestinal transit time, renal clearance, incomplete urine recovery, and intra-individual variability, these sugars are typically co-administered, as both are equally influenced by non-mucosal factors —the sole difference being their route of permeation⁵³. The disaccharide acts as a large-pore marker, crossing the epithelium only through predominantly paracellular pathways or at sites of epithelial injury (thereby reflecting barrier dysfunction)⁵⁴. In contrast, the monosaccharide serves as a small-pore marker, absorbed by passive transcellular diffusion through enterocytes (reflecting mucosal surface area)⁵⁷. This dual administration enables calculation of the intestinal permeability index, usually expressed as the urinary disaccharide-to-

monosaccharide ratio, which integrates both paracellular leakiness and epithelial damage relative to mucosal surface area^{12,58}.

For the small intestine, the lactulose/mannitol ratio has been the most widely used permeability test over the past three decades^{59,60}. The cellobiose/mannitol ratio is also employed⁶¹. It is worth noting that cellobiose can be metabolized by intestinal lactase; however, lactase has a low affinity for this disaccharide, making this effect negligible for test interpretation^{61,62}. In contrast, lactulose is not metabolized by human disaccharidases^{61,62}. Mannitol, commonly used as the monosaccharide denominator, presents several limitations, including a baseline urinary excretion of mannitol exists, complicating interpretation^{61,63}, mannitol can cross certain cell membranes and undergo a degradation by intracellular enzymes^{61,64}, and urinary excretion may not fully reflect the intact fraction⁶⁵, reducing analytical accuracy. Furthermore, both lactulose/mannitol and cellobiose/mannitol tests rely on hyperosmolar sugar solutions. This hyperosmolar stress increases the permeability of abnormal epithelia to disaccharides, enhancing discrimination but also complicating interpretation^{61,66,67}. As an alternative to mannitol, L-rhamnose is sometimes used as the monosaccharide probe because it appears to have better transport characteristics than mannitol across certain cell membranes^{61,68,69}. Additionally, the commonly used lactulose/L-rhamnose test is formulated to be iso-osmolar, thereby avoiding the hyperosmolar stress induced by lactulose/mannitol or cellobiose/mannitol solutions^{61,70–72}.

To assess colonic permeability, sucralose is commonly used as the disaccharide probe because it is resistant to colonic fermentation (unlike lactulose), with colonic permeability typically evaluated using the sucralose/mannitol ratio⁷³. More recently, multi-sugar permeability assays have been developed to enable simultaneous, site-specific assessment of

intestinal permeability along the gastrointestinal tract^{58,74,75}. In the protocol described by van Wijck *et al.*, five probes—sucrose, lactulose, L-rhamnose, erythritol, and sucralose—are administered and their timed urinary recoveries quantified⁵⁸. Gastroduodenal, small-intestinal, and colonic permeability can respectively be assessed by sucrose excretion (0–5 h urine; sucrose is normally rapidly hydrolyzed by duodenal sucrase)^{76–78}, the lactulose/L-rhamnose ratio (0–5 h), and the sucralose/erythritol ratio (5–24 h), reflecting region-specific absorption and bacterial resistance profiles^{58,73,79,80}.

Beyond sugar probes, alternative markers such as polyethylene glycols (PEG) of various molecular weights (PEG 400, 1000, or 4000) and isotopic tracers (e.g., ⁵¹Cr-EDTA) have also been employed^{81–85}. Their main advantage lies in their stability, as they are neither metabolized by enzymes nor degraded by intestinal bacteria. However, the use of isotopes is limited by their inherent radioactivity. Furthermore, recent advances include the use of orally administered fluorophores—MB-402 and MB-301 in rats with indomethacin-induced intestinal injury, and MB-102 (relmapirazin) in patients with Crohn’s disease—which have been shown to closely parallel lactulose excretion and the lactulose/rhamnose urinary ratio^{86,87}. Notably, these fluorophores can also be detected transcutaneously, paving the way for a new noninvasive assessment approach⁸⁷.

The detection of probes in urine has several drawbacks. A range of factors (outlined above) can influence urinary excretion^{54,56,88,89}. In addition, these tests are burdensome for participants: urine collection is cumbersome, requires prolonged fasting, and ingestion of sugar may induce gastrointestinal symptoms⁹⁰. From an analytical perspective, urine samples must be transported to specialized laboratories under conditions that prevent contamination and bacterial growth, and the sophisticated equipment required for probe measurement is

often not readily available⁹¹. These limitations—together with the unreliable specificity of these measurements for the intestinal barrier, the lack of global standardization of test procedures and the absence of clearly defined cut-off values to distinguish normal from abnormal results for each assay—have limited the widespread adoption of probe-based assessment of the intestinal epithelial barrier^{59,92,93}.

Barrier-Derived Biomarkers Detected in Blood, Urine, and Stool as Indicators of Intestinal Epithelial Barrier Alterations

A variety of alternative barrier-derived biomarkers can be detected in blood, feces, or urine as indicators of intestinal epithelial barrier damage (**Supplemental Table 2**). Epithelial cell-derived proteins such as intestinal fatty acid-binding protein (I-FABP), diamine oxidase (DAO), α -glutathione-S-transferase (α -GST), claudin-3, and occludin reflect epithelial injury, since these proteins are normally expressed within epithelial cells and their damage results in their release and subsequent elevation in the circulation^{90,94–96}. Other proteins, such as citrulline—synthesized predominantly in the intestinal mucosa of small intestine from glutamine—decrease when the epithelial barrier is damaged, as their synthesis is impaired^{97,98}. Citrulline levels are also reduced in cases of short bowel syndrome or after intestinal resection⁹⁹. Zonulin, another enterocyte-derived protein released in response to luminal stimuli, regulates TJs dynamics and increases paracellular permeability^{94,100}. Elevated zonulin levels in plasma, stool, or urine have been proposed as markers of intestinal permeability^{94,100}. Still, their specificity is limited by stimulus-dependent secretion, high intra-individual variability, and poor concordance with standard permeability tests. Furthermore, concerns regarding the accuracy of commercial enzyme-linked immunosorbent assay (ELISA) kits—many of which target non-authentic sequences—further limit its reliability as a biomarker^{100–102}. Recently, studies have shown that circulating

immunoglobulin (Ig)G and IgA antibodies against zonulin can be quantified, and they may offer greater temporal stability and assay reliability than measuring zonulin itself to evaluate the loss of intestinal barrier integrity¹⁰³.

Because barrier defects can also facilitate microbial translocation, additional circulating markers can be detected, including bacterial products such as D-lactate and lipopolysaccharide (LPS), or host response proteins like LPS-binding protein (LBP) and soluble CD14 (sCD14), which offer more stable surrogates of Gram-negative translocation^{48,104–106}. Finally, some of the biomarkers mentioned above, such as zonulin, as well as calprotectin, lactoferrin and lipocalin-2, can also be measured in feces⁴⁸. Calprotectin, lactoferrin and lipocalin-2 are predominantly produced by neutrophils; their presence in the stool may therefore reflect epithelial injury, as neutrophils accumulate at sites of mucosal damage. However, their levels increase markedly in the context of mucosal inflammation and are thus considered primarily markers of inflammation rather than specific indicators of intestinal epithelial barrier alterations¹⁰⁷. Finally, a recent study explored the use of metabolomics to identify biomarkers of intestinal permeability. The combination of two fecal metabolites, 3-methyladipic acid and adenine, significantly improved diagnostic accuracy, compared with single-metabolite analyses⁹⁰.

There are notable discrepancies across studies regarding the diagnostic accuracy, reliability, sensitivity, specificity, and reproducibility of these markers¹⁰⁸. Several factors may explain these inconsistencies, including the small sample sizes often used, the heterogeneity in the way these biomarkers are measured, and the variation in the samples collected¹⁰⁹. Moreover, the reference (normal) threshold values for each biomarker remain poorly defined, and there is currently no standardized test procedure, further limiting comparability between studies¹⁰⁹.

402

403 *Non-invasive Imaging Modalities for the Evaluation of the Intestinal Barrier*

404 Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) uses intravenous low–
405 molecular-weight contrast agents that diffuse into the extravascular–extracellular space,
406 influenced by blood flow and microvascular permeability¹¹⁰. Changes in signal intensity are
407 captured by rapid T1-weighted imaging¹¹⁰. As proof-of-concept, Towner *et al.* demonstrated
408 that, in rats, DCE-MRI detected bladder and colonic barrier disruption after protamine sulfate
409 exposure, highlighting its translational potential, though evidence remains preclinical^{111,112}.
410 Positron emission tomography (PET) could offer another noninvasive approach. In the study
411 by Yamashita *et al.*, oral administration of 18F-fluorodesoxyglucose (FDG) was tracked in
412 conscious and anesthetized rats, allowing real-time visualization of gastric emptying,
413 intestinal transit, and absorption into the bloodstream¹¹³. By comparing uptake kinetics, the
414 authors showed that in conscious rats, absorption was limited primarily by intestinal
415 membrane permeability. At the same time, in anesthetized animals, the limiting factor shifted
416 to delayed gastric emptying. These findings suggest that PET imaging could potentially
417 provide a dynamic assessment of intestinal permeability. However, its clinical application
418 remains limited by the need for radiotracers, reliance on indirect readouts, radiation exposure,
419 high cost, and restricted availability. Nevertheless, neither DCE-MRI nor PET imaging has
420 yet been validated against established methods to confirm their accuracy in detecting or
421 monitoring epithelial barrier defects.

422

423 **Invasive tools to assess intestinal epithelium defects in vivo**

424 *Cutting-edge endoscopic imaging techniques*

425 The methods mentioned above provide only indirect information about epithelial barrier
426 integrity, do not capture the morphological alterations that underlie increased permeability,

and do not allow real-time assessment of the barrier¹¹⁴. By contrast, cutting-edge endoscopic imaging techniques, such as probe-based confocal laser endomicroscopy (pCLE) and ultra-high magnification endocytoscopy in combination with specific tools, are new technologies allowing real-time exploration of the 'cellular' intestinal barrier (Figure 5)¹¹⁴. These tools allow assessment of the intestinal barrier with near-histologic resolution ($\approx 1000\times$ with CLE), which is why the term *optical biopsy* is frequently used to describe this approach^{115,116}. Table 3 summarizes studies that have evaluated invasive methods for assessing intestinal epithelial barrier integrity in comparison with other approaches.

In practice, pCLE requires the intravenous administration of a fluorescent agent (fluorescein sodium 10%, $\approx 2.5\text{--}5$ mL) immediately before the examination¹¹⁵. A probe is then advanced through the working channel of a standard gastroscope or colonoscope and positioned in close contact with the mucosal surface, where a 488-nm excitation laser illuminates the region of interest¹¹⁶. The emitted fluorescence is collected through the same lens, with only the refocused light passing through the pinhole being detected. This selective detection minimizes scattered light and enables the generation of high-resolution two-dimensional grayscale images¹¹⁶. By visualizing fluorescein leakage through epithelial gaps, pCLE provides a functional assessment of intestinal barrier, while simultaneously allowing detailed evaluation of microvascular features (such as vessel tortuosity) and crypt architecture^{114,117}. Fluorescein leakage in the crypt lumen can indeed be observed even in colonic crypts with normal architecture (not dilated) in the presence of barrier alteration/dysfunction.

pCLE provides a more standardized assessment of the intestinal barrier, with lower inter- and intra-observer variability compared to histology^{114,118,119}. However, software-engineered machine learning allows for automated detection of these features with high accuracy,

removing the need for the endoscopist to learn the interpretation of images¹²⁰. This technique enables the detection of residual permeability beyond apparent endoscopic healing¹²¹, which correlated with ongoing symptoms in patients despite endoscopic remission¹²². In particular, the Watson grade has been proposed to characterize small-bowel barrier dysfunction in vivo and shows strong correlation with histological findings^{114,123}. Although, pCLE was primarily designed to evaluate intestinal inflammation¹²⁴ and to grade remission in inflammatory bowel disease (IBD), beyond the endoscopic and histologic remission^{125,126}, it can successfully assess epithelial barrier integrity with high sensitivity and specificity. In most pCLE-based indices, barrier-related features account for only one or two items among several parameters used to characterize inflammation or remission status (e.g., fluorescein leakage in the ENHANCE index)^{127,128}. Furthermore, several limitations currently restrict wider adoption: platform availability, cost and lack of reimbursement, a learning curve for both performance and image interpretation, and heterogeneity of scores/protocols in the absence of formal guidance¹²⁹. Finally, although intravenous fluorescein is generally well tolerated, its use nonetheless requires attention to safety, as it can on rare occasions trigger allergic reactions or even anaphylaxis^{114,130,131}.

Endocytoscopy (EC) is an advanced endoscopic imaging technique that involves topical application of a mucolytic agent such as N-acetylcysteine, followed by absorptive staining of the mucosa with dyes including methylene blue, toluidine blue, or cresyl violet—either alone or in combination to optimize tissue contrast. After approximately 60 seconds of dye exposure, the mucosa is thoroughly rinsed to remove excess stain. Imaging is then performed at ultra-high magnification (approximately 520-fold magnification), yielding images that closely resemble histological sections, as well^{132,133}. This enables direct visualization of the intestinal barrier, including crypt architecture, cellular and nuclear morphology, and

inflammatory infiltrates¹³⁴. Although EC does not allow direct visualization of the epithelial barrier, Majumder *et al.* demonstrated that combining EC with machine-learning–assisted multispectral spatial imaging (MSI) of intestinal barrier proteins can substantially enhance the assessment of epithelial barrier integrity^{135,136}. In patients with ulcerative colitis in clinical remission, the authors showed that integrating EC findings with claudin-2 expression profiling accurately predicted major adverse outcomes over a 12-month period¹³⁶. Multispectral immunofluorescence analysis of TJ protein expression therefore represents an emerging and promising tool for evaluating epithelial barrier alteration¹¹⁴. Although EC has yielded promising diagnostic and monitoring results, its implementation requires specific operator training to ensure adequate proficiency. Moreover, the high cost of the equipment remains a major barrier to its widespread adoption in routine clinical practice¹³⁷.

Ex vivo (and in vitro) assessment and measurement of tissue marker expression

Finally, intestinal tissues can also be collected from humans or animal models and maintained viable for a short period of time. For example, they can be mounted in an Ussing chamber, positioned between two compartments filled with physiological buffer continuously aerated with carbogen (95% O₂, 5% CO₂) and maintained at 37 °C^{138,139}. Several tools exist to assess intestinal epithelial barrier alterations *ex vivo*, and these approaches are also widely used in *in vitro* models (**Table 4**).

Tracer-Based Permeability Assays

Epithelial barrier function can be evaluated by introducing traceable macromolecules and measuring their passage across the epithelial monolayer. The molecules used may overlap with those employed in *in vivo* human and animal assessments or differ depending on the experimental design. Commonly applied probes include hydrophilic tracers of defined

molecular weight (e.g., sucrose, dextrans), radioactively labeled sugars, or fluorescently labeled molecules such as fluorescein isothiocyanate (FITC)–dextran or lucifer yellow¹⁴⁰. Typically, after addition of the tracer to the apical (donor) chamber, samples are collected from the basolateral (receiver) chamber at defined time points under physiological conditions. For fluorescent tracers, aliquots are transferred to black-walled plates and fluorescence intensity is measured using a microplate fluorometer (e.g., excitation/emission 490/520 nm for FITC)¹⁴⁰. This approach provides a global measure of intestinal epithelial barrier alterations but lacks the sensitivity to detect subcellular alterations or transient, localized disruptions. Moreover, it does not offer spatial information on the sites of increased permeability and is suboptimal for capturing rapid or dynamic changes in barrier function.

Transepithelial Electrical Resistance

Another widely used method to evaluate epithelial barrier integrity is transepithelial electrical resistance (TEER), which quantifies ion flux across a cell monolayer or a tissue¹⁴¹. Electrical resistance results from the restricted passage of ions through TJs, and TEER can measure barrier integrity¹⁴². A high TEER value indicates a tight, low-permeability barrier, whereas a low value reflects impaired epithelial integrity with increased ionic permeability. Standard TEER measurements are obtained using a voltohmmeter connected to chopstick-like electrodes¹⁴³. The instrument applies a defined current across the monolayer via external electrodes, while internal electrodes record the potential difference; resistance is then calculated using Ohm's law ($R = U/I$). This direct-current method measures the combined resistance of the culture medium, support, and cell layer. Instruments such as the Millicell®-ERS Voltohmmeter enable normalization of resistance to the surface area, expressed in $\Omega \cdot \text{cm}^2$ ^{144,145}. TEER measurement remains the reference technique in most *in vitro* studies of epithelial barrier and can also be measured in an Ussing chamber¹⁴⁶. It is simple, non-

invasive, rapid, and provides a favorable signal-to-noise ratio. TEER values generally correlate well with other barrier function assays, show high sensitivity for detecting integrity loss, and exhibit good reproducibility. However, TEER provides only an averaged resistance value across the entire surface, limiting spatial resolution and precluding detection of localized or transient barrier defects.

Electric cell-substrate impedance sensing (ECIS) or impedance spectroscopy

To overcome the limitations of direct current methods, alternating current-based approaches such as electric cell-substrate impedance sensing (ECIS) or impedance spectroscopy have been developed. These techniques enable continuous, non-invasive, real-time monitoring of both TEER and membrane capacitance, the latter reflecting membrane surface area and composition¹⁴⁷. Beyond assessing barrier integrity, they also allow investigation of key aspects of cellular behavior—including growth, differentiation, viability, and migration—in vitro^{148–150}. By analyzing resistance and capacitance together, these methods can distinguish between paracellular TJs opening (characterized by decreased TEER with stable capacitance) and membrane perturbations (decreased TEER with increased capacitance), thereby providing more specific insights into barrier dysfunction. Two main systems are available. The cellZscope device uses standard culture inserts with semi-permeable membranes, preserving apical and basolateral compartments and enabling simultaneous transport studies¹⁴⁹. By contrast, the ECIS system cultures cells directly on thin gold electrodes, which eliminates the basolateral compartment but provides access to the α parameter, a measure of cell–matrix interactions¹⁴⁹. The xCELLigence system offers high-throughput impedance monitoring of intestinal epithelial barrier function in 16- or 96-well plate formats, making it well-suited for screening applications. It outputs a Cell Index that increases or decreases in accordance with epithelial monolayer integrity¹⁵¹. These impedance-based platforms thus

provide complementary advantages, with their suitability determined by the research objective. Importantly, they can be applied not only to immortalized cell lines but also to two-dimensional monolayers derived from patient-derived intestinal organoids¹⁵². Evidence suggests that ECIS exhibits higher sensitivity than TEER in detecting disruptions of intestinal epithelial barrier integrity¹⁴⁸.

Recently, small electrodes arranged in dense patterns on a chip, known as high-density microelectrode arrays (HD-MEAs), have been shown to enhance spatial resolution¹⁵³. When integrated with complementary metal oxide semiconductor (CMOS) technology, they allow multiplexed, rapid impedance measurements, enabling high-resolution, continuous spatiotemporal monitoring of epithelial tissues¹⁵³. This approach can track the dynamic evolution of intestinal epithelial cells, including proliferation, barrier formation, and 3D structural development on-chip¹⁵³.

Organic electrochemical transistor

The organic electrochemical transistor (OECT) is a powerful tool for dynamically monitoring ionic flux across epithelial barriers, enabling real-time, label-free, and low-cost detection of barrier breaches¹⁵⁴. The device consists of source and drain electrodes positioned on either side of a stable, biocompatible conducting polymer channel capable of transporting both electronic and ionic carriers, upon which the cell layer is cultured¹⁵⁴. The polymer is in contact with an electrolyte, and when a gate electrode is immersed in this medium, cations are driven into the channel, thereby modulating the source–drain current. Because the transistor amplifies these changes, even minute variations in ionic flux can be detected with high sensitivity. An intact epithelial layer acts as a barrier to cation entry, leading to reduced current, whereas barrier disruption permits cations to enter the polymer channel, resulting in

an increased drain current^{155,156}. Thus, modulation of the drain current directly reflects alterations in ionic flux across the monolayer. OECTs offer very high sensitivity and temporal resolution, detecting barrier alterations within seconds¹⁵⁵. These systems can discriminate between gradual and abrupt epithelial disruption, allowing identification of subtle, early paracellular barrier defects, though they lack spatial resolution to localize the site of barrier impairment^{155,156}.

Specific markers expression

The expression of specific markers within the barrier can also be investigated through complementary approaches such as immunohistochemistry, western blotting, or quantitative PCR, in addition to multispectral IF mentioned above. A comprehensive overview of all available methods for evaluating the tissue expression of epithelial barrier proteins, along with an exhaustive description of these proteins, is beyond the scope of this systematic review^{114,136}.

Models to Assess Intestinal Epithelial Barrier In Vitro

It is important to distinguish between intestinal epithelial barrier *tools* and the *models* used to assess their integrity. A wide range of *in vitro* models is available, spanning from simple culture systems to sophisticated microphysiological platforms. These include two-dimensional monolayers of epithelial cell lines grown on porous semi-permeable membranes¹⁵⁷; three-dimensional cultures developed on collagen scaffolds that recapitulate the villus-crypt architecture¹⁵⁸—sometimes incorporating multiple cell types and an endothelial compartment^{159,160}; ex vivo explants and organoids^{161–164}; and advanced gut-on-chip systems that mimic physiological microenvironments^{165–167}. Most of the tools can be applied to these different models. However, the analysis of barrier function in *in vitro* models

(primary epithelial cultures and organoids) may serve to mechanistically dissect genetic/cytokine-dependent pathways involved in the regulation of barrier function, but in contrast to ex-vivo-measurements of barrier function using biopsies (e.g. in Ussing chambers), such in vitro assays do not predict barrier function in vivo.

Outlook

Despite significant advances, important gaps remain in our understanding and assessment of intestinal epithelial barrier alterations and dysfunction. First, there is no clear or universally accepted definition of what constitutes intestinal barrier alterations or a loss of function. Some authors suggest that such a definition should include the presence of an underlying condition capable of impairing intestinal epithelial barrier, associated clinical symptoms, elevated plasma endotoxin levels, and evidence of increased permeability or reduced intestinal perfusion^{109,168}. However, this proposed framework remains vague, inconsistently applied across studies, and a more precise and standardized definition is therefore needed. The term “*leaky gut*” is often used to describe any form of epithelial barrier impairment, even though it encompasses a wide spectrum of conditions ranging from subtle TJs dysfunction to overt mucosal injury. These stages of gut permeability should be stratified from mild barrier dysfunction to severe epithelial cells damage, and combining specific diagnostic tools may help distinguish between these different levels of impairment. It is also possible that barrier assessments may need to be tailored to different diseases (e.g., IBD vs. liver cirrhosis) or to different disease locations within the gastrointestinal tract (such as isolated small-intestinal vs. colonic for gastrointestinal disease). Furthermore, there is a lack of consensus regarding how these tests should be performed (such as fasting requirements or test duration) as well as on the cut-off values that should be applied to define intestinal barrier alterations as pathological. This limits inter-study comparisons and inter-laboratory

627 reproducibility^{108,169,170}. Emerging *in vivo* tools for assessing the intestinal epithelial barrier
628 show promise but require more targeted investigation for the characterization of barrier
629 alterations, rather than being used predominantly to assess residual inflammation or remission
630 status. For example, pCLE can be combined with molecular imaging. The local
631 administration of fluorescently labeled antibodies targeting therapeutic molecules—such as
632 membrane-bound tumor necrosis factor (mTNF)—has enabled the *in vivo* detection of
633 intestinal mTNF-positive immune cells during CLE and has been used to predict response to
634 anti-TNF therapy¹¹⁵. This approach could ultimately be adapted for the development of
635 fluorescent antibodies directed against intercellular junction proteins or other molecular
636 targets indicative of epithelial barrier alterations. Finally, it is unclear whether single
637 biomarkers or combinations of approaches provide better diagnostic performance, as most
638 candidate markers lack disease specificity.

639
640 The development of reliable tools to detect intestinal epithelial barrier abnormalities could
641 enable timely intervention before the onset of overt pathological processes such as IBD^{171,172}.
642 These tools would also make it possible, for example in patients with established IBD, to
643 assess the degree of remission, determine whether the epithelial barrier has been restored^{173–}
644 ¹⁷⁵. Conversely, they can also be used to evaluate both the extent and the progression of
645 epithelial damage once the disease is established¹⁷⁶. There is a need for novel tools and
646 approaches that extend beyond simple detection of increased intestinal permeability and
647 instead provide detailed characterization of the nature, severity, and spatial distribution of
648 epithelial barrier alterations. Such advances could help distinguish patients who may benefit
649 from barrier-stabilizing strategies from those requiring therapies that actively promote
650 mucosal healing and re-epithelialization. Several emerging therapies aim to promote
651 epithelial barrier repair as stand-alone agents. Therefore, achieving standardization and

652 consensus on how to measure barrier restoration is essential to demonstrate proof-of-concept
653 and evaluate therapeutic response for these future treatments^{8,12,177}.
654

Funding

None

Acknowledgements

ChatGPT (version 5.2), a large language model, was used to assist with data extraction from published articles and to help rephrase selected sentences for clarity. The tool was used in accordance with APS ethical policies, and the authors take full responsibility for the content of the manuscript.

Supplemental Material

Supplemental Tables 1-2: <https://doi.org/10.6084/m9.figshare.31224541>

Declaration of interests

S Vieujean received speaker's fees from Abbvie, Celltrion, Ferring, Janssen, Takeda and support travel from Abbvie, Celltrion, Ferring, Galapagos, Janssen, Lilly, Pfizer and Takeda.

R Atreya: AbbVie, Abivax, AlfaSigma, Amgen, AstraZeneca, Biogen, Boehringer Ingelheim, Bristol-Myers Squibb, CED Service GmbH, Celltrion Healthcare, Falk Foundation, Galapagos, Gilead, InDex Pharmaceuticals, Johnson&Johnson, Lilly, MSD Sharp & Dohme, Pfizer, Pandion Therapeutics, Roche Pharma, Takeda Pharma, Viartis

A Buda: Lecture fees from Abbvie, Takeda, Janssen, Alfasigma, Pfizer, GSK

J Caradec has no conflicts of interest related to this manuscript.

S Citi has no conflicts of interest related to this manuscript.

S Danese declares consultancy/advisory fees from AbbVie, Allergan, Amgen, AstraZeneca, Biogen, Boehringer Ingelheim, Celgene, Celltrion, Ferring Pharmaceuticals, Gilead Sciences, Hospira, Janssen, Johnson & Johnson, MSD, Mundipharma, Pfizer Inc, Roche, Sandoz, Takeda, TiGenix, UCB and Vifor; lecture/speaker fees: AbbVie, Amgen, Ferring

678 Pharmaceuticals, Gilead Sciences, Janssen, Mylan, Pfizer Inc, Takeda; and has
679 directorship/ownership interests in Gastroenterology and Endoscopy.

680 O Dewit has received consulting and/ or speaker fees from AbbVie, Biogen, Celltrion, Eli
681 Lilly, Galapagos, Janssen Pharmaceuticals, Pfizer, and Takeda Pharmaceuticals.

682 M Friedrich received speaker and/or consulting fees from EliLilly, OnoPharma, Quell
683 Therapeutics and Foghorn, and grant support from EliLilly.

684 Subrata Ghosh has received research grants from Eli Lilly and Company; served on steering
685 committees for AbbVie and Bristol Myers Squibb; and received consulting fees from
686 AbbVie, Celltrion, Ferring Pharmaceuticals, Galapagos NV, Janssen, Pfizer, and Takeda.

687 M Iacucci -Grant research support the Helmsley charitable trust, Eli Lilly, Olympus- Speaker
688 fees: Pentax, Pfizer, Eli Lilly, Janssen

689 V Jairath has received has received consulting/advisory board fees from AbbVie, Alimentiv,
690 Asieris, Astra Zeneca, Biomebank, Boehringer Ingelheim, Caldera Therapeutics, Calluna,
691 Celltrion, Domain Therapeutics, Eli Lilly, Entera, Ensho, Exeliom Biosciences, Ferring
692 Ventures, Flagship Pioneering, Fresenius Kabi, Galapagos, GlaxoSmithKline, Genentech,
693 Gilead, Granite Bio, Janssen, Merck, Metacrine, Mountainfield, MRM Health, Nxera,
694 Organon, OSE Immunotherapeutics, Pandion, Pendopharm, Pfizer, Prometheus Biosciences,
695 Roche, Salvina, Sanofi, Shattuck Labs, Sorriso, Spyre, Synedgen, Takeda, Teva, Thabor
696 Therapeutics, Union Therapeutics, Ventus, Ventyx, Vividion, Xencor, Zealand Pharma
697 speaker's fees from, Abbvie, Ferring, Eli Lilly, Fresenius Kabi, Janssen, Pfizer, Takeda,
698 Tillotts

699 A Kaser has no conflicts of interest related to this manuscript.

700 R Leong declares advisory board membership: AbbVie, Aspen, BMS, Celgene, Celltrion,
701 Chiesi, Ferring, Glutagen, Hospira, Janssen, Lilly, MSD, Novartis, Pfizer, Prometheus

702 Biosciences, and Takeda; research grant recipient from: Joanna Tiddy University of Sydney,
 703 McKusker Charitable Foundation, Celltrion, Shire, Janssen, Takeda, Gastroenterological
 704 Society of Australia, NHMRC, Gutsy Group, Pfizer, and Medical Research Future Fund
 705 Australia.

706 M Neurath has served as advisor or speaker for Pentax, Roche Pharma, Takeda Pharma,
 707 Pfizer, MSD, PPM, Janssen, Gilead, Dr Falk Pharma, Boehringer Ingelheim, Amgen and
 708 AbbVie.

709 N Pierre has no conflicts of interest related to this manuscript.

710 M Pohin has no conflicts of interest related to this manuscript.

711 T Rath: Consultant fees : Takeda Pharma GmbH, Lilly, Olympus Medical, Pentax Medical,
 712 Mauna Kea Technologies. Speaking fees : Olympus Medical, Pentax Medical, Mauna Kea
 713 Technologies, Medtronic, Takeda, Galapagos, Falk, Janssen, Abbvie, Repha, Medical
 714 Tribune, Lilly, Janssen.

715 P Rivière: Consultancy fees from Abbvie, Celltrion, Johnson and Johnson and Takeda.

716 S Travis Grants/Research Support: AbbVie, Celgene, Celsius, ECCO, Galapagos, GSK,
 717 Helmsley Trust, IOIBD, Janssen, Lilly, Pfizer, Takeda, UKIERI, 35Bio, Vifor, and Norman
 718 Collisson Foundation. Consulting Fees: Alimentiv; Apexian; Apollo; Arcturis; AstraZeneca;
 719 BMS; Clario; Cosmo; Dova Health; Endpoint Health; EQrX; Equillium; Ferring; Galapagos;
 720 Genentech/Roche; Gilead; GSK; Janssen; Iterative Health; Lilly; Mestag; Microbiotica;
 721 ONO; Pfizer; Phesi; Protagonist; Sanofi; Satisfai; Sensyne Health; Spyre; Takeda; Teva;
 722 Theravance; TrlX Bio Speaker fees: BMS, Ferring, GSK, Janssen, Lilly, Pfizer, Sun Pharma,
 723 Takeda. Share options: Dova Health Inc .

724 S Zeissig: Consultant fees: AbbVie Deutschland GmbH & Co KG, Amgen GmbH, Biogen
 725 GmbH, Bristol-Myers Squibb GmbH & Co KG, Celgene GmbH, Celltrion Healthcare,
 726 Ferring Arzneimittel GmbH, Galapagos Biopharma Germany GmbH, Hogg Robinson GmbH,
 727 Janssen Pharmaceutica NV, Janssen Cilag GmbH, MICE Service GmbH, Mylan, Pfizer
 728 Pharma GmbH, Takeda Pharma GmbH. Speaker fees: AbbVie Deutschland GmbH & Co
 729 KG, Amgen GmbH, Biogen GmbH, Bristol-Myers Squibb GmbH & Co KG, CED Service
 730 GmbH, Falk Foundation e.V., Ferring Arzneimittel GmbH, FORGA Solutions GmbH, GWT-
 731 TUD GmbH, Janssen Cilag GmbH, Janssen Pharmaceutica NV, MSD Sharp & Dome,
 732 Roche, Pfizer Pharma GmbH, Pharma Zentrale GmbH, Takeda Pharma GmbH, WebMD
 733 Global.

734 J Westcott has no conflicts of interest related to this manuscript.

735 L Peyrin-Biroulet declares consulting fees from Abbvie, Abivax, Adacyte, Alimentiv,
 736 Amgen, Applied Molecular Transport, Arena, Banook, Biogen, BMS, Celltrion, Connect
 737 Biopharm, Cytoki Pharma, Enthera, Ferring, Fresenius Kabi, Galapagos, Genentech, Gilead,
 738 Gossamer Bio, GSK, IAC Image Analysis, Index Pharmaceuticals, Inotrem, Janssen, Lilly,
 739 Medac, Mopac, Morphic, MSD, Nordic Pharma, Novartis, Oncodesign Precision Medicine,
 740 ONO Pharma, OSE Immunotherapeutics, Pandion Therapeutics, Par' Immune, Pfizer,
 741 Prometheus, Protagonist, Roche, Samsung, Sandoz, Sanofi, Satisfay, Takeda, Telavant,
 742 Theravance, Thermo Fischer, Tigenix, Tillots, Viatris, Vectivbio, Ventyx, Ysopia; ; receives
 743 grant from Celltrion, Fresenius Kabi, Medac, MSD, Takeda; gives lecture for Abbvie,
 744 Alfasigma, Amgen, Arena, Biogen, Celltrion, Ferring, Galapagos, Genentech, Gilead,
 745 Janssen, Kern Pharma, Lilly, Medac, MSD, Nordic Pharma, Pfizer, Sandoz, Takeda, Tillots,
 746 Viatris; received support travel from Abbvie, Alfasigma, Amgen, Celltrion, Connect
 747 Biopharm, Ferring, Galapagos, Genentech, Gilead, Gossamer Bio, Janssen, Lilly, Medac,
 748 Morphic, MSD, Pfizer, Sandoz, Takeda, Thermo Fischer, Tillots.

749

750 **Authors' Contributions:** LPB and SV conceived the study. SV and LBP collected the data,
751 wrote the article and created tables. All authors critically reviewed the content of the paper.
752 The manuscript was approved by all authors.

753

754 **Data availability statement:** The data underlying this article are available in the article.

755

References

1. Citi S. Intestinal barriers protect against disease: Leaky cell-cell junctions contribute to inflammatory and autoimmune diseases. *Science* (80-) 2018;1097–8. Doi: 10.1126/science.aat0835.
2. Neurath MF., Artis D., Becker C. The intestinal barrier: a pivotal role in health, inflammation, and cancer. *Lancet Gastroenterol Hepatol* 2025;573–92. Doi: 10.1016/S2468-1253(24)00390-X.
3. Meng J., Xiao H., Xu F., She X., Liu C., Canonica GW. Systemic barrier dysfunction in type 2 inflammation diseases: perspective in the skin, airways, and gastrointestinal tract. *Immunol Res* 2025. Doi: 10.1007/s12026-025-09606-9.
4. Buckley A., Turner JR. Cell biology of tight junction barrier regulation and mucosal disease. *Cold Spring Harb Perspect Biol* 2018;10(1). Doi: 10.1101/cshperspect.a029314.
5. Krug SM., Schulzke JD., Fromm M. Tight junction, selective permeability, and related diseases. *Semin Cell Dev Biol* 2014;166–76. Doi: 10.1016/j.semcdb.2014.09.002.
6. Luissint AC., Parkos CA., Nusrat A. Inflammation and the Intestinal Barrier: Leukocyte–Epithelial Cell Interactions, Cell Junction Remodeling, and Mucosal Repair. *Gastroenterology* 2016;616–32. Doi: 10.1053/j.gastro.2016.07.008.
7. Khor B., Gardet A., Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature* 2011;307–17. Doi: 10.1038/nature10209.
8. Camilleri M. Leaky gut: mechanisms, measurement and clinical implications in humans. *Gut* 2019;1516–26. Doi: 10.1136/gutjnl-2019-318427.
9. Vancamelbeke M., Vermeire S. The intestinal barrier: a fundamental role in health and disease. *Expert Rev Gastroenterol Hepatol* 2017;821–34. Doi: 10.1080/17474124.2017.1343143.
10. Edogawa S., Peters SA., Jenkins GD., Gurunathan S V., Sundt WJ., Johnson S., et al. Sex differences in NSAID-induced perturbation of human intestinal barrier function and microbiota. *FASEB J* 2018;32(12):6615–25. Doi: 10.1096/fj.201800560R.
11. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* 2009;799–809. Doi: 10.1038/nri2653.
12. Perez-Diaz-del-Campo N., Castelnuevo G., Ribaldone DG., Caviglia GP. Fecal and Circulating Biomarkers for the Non-Invasive Assessment of Intestinal Permeability. *Diagnostics* 2023. Doi: 10.3390/diagnostics13111976.
13. Valibeknejad M., Abdoli SM., Alizadeh R., Mihăilă SM., Raoof A. Insights into transport in mucus barrier: Exploring particle penetration through the intestinal mucus layer. *J Drug Deliv Sci Technol* 2023;86:104752. Doi: 10.1016/j.jddst.2023.104752.
14. Liang GH., Weber CR. Molecular aspects of tight junction barrier function. *Curr Opin Pharmacol* 2014;84–9. Doi: 10.1016/j.coph.2014.07.017.
15. Koch S., Nusrat A. Dynamic regulation of epithelial cell fate and barrier function by

- intercellular junctions. *Ann N Y Acad Sci* 2009;**1165**:220–7. Doi: 10.1111/j.1749-6632.2009.04025.x.
16. Clevers HC., Bevins CL. Paneth cells: Maestros of the small intestinal crypts. *Annu Rev Physiol* 2013;289–311. Doi: 10.1146/annurev-physiol-030212-183744.
17. Chen L., Tuo B., Dong H. Regulation of intestinal glucose absorption by ion channels and transporters. *Nutrients* 2016. Doi: 10.3390/nu8010043.
18. Kato A., Romero MF. Regulation of electroneutral NaCl absorption by the small intestine. *Annu Rev Physiol* 2011;**73**:261–81. Doi: 10.1146/annurev-physiol-012110-142244.
19. Fleishman JS., Kumar S. Bile acid metabolism and signaling in health and disease: molecular mechanisms and therapeutic targets. *Signal Transduct Target Ther* 2024. Doi: 10.1038/s41392-024-01811-6.
20. Turnberg LA. Absorption and secretion of salt and water by the small intestine. *Digestion* 1973;357–81. Doi: 10.1159/000197461.
21. Binder HJ., Rajendran V., Sadasivan V., Geibel JP. Bicarbonate secretion: A neglected aspect of colonic ion transport. *J Clin Gastroenterol* 2005. Doi: 10.1097/01.mcg.0000155521.81382.3a.
22. Barrett KE., Keely SJ. Chloride secretion by the intestinal epithelium: Molecular basis and regulatory aspects. *Annu Rev Physiol* 2000;535–72. Doi: 10.1146/annurev.physiol.62.1.535.
23. Pacheco AR., Munera D., Waldor MK., Sperandio V., Ritchie JM. Fucose sensing regulates bacterial intestinal colonization. *Nature* 2012;113–7. Doi: 10.1038/nature11623.
24. Oh JZ., Ravindran R., Chassaing B., Carvalho FA., Maddur MS., Bower M., et al. TLR5-mediated sensing of gut microbiota is necessary for antibody responses to seasonal influenza vaccination. *Immunity* 2014;**41**(3):478–92. Doi: 10.1016/j.immuni.2014.08.009.
25. Knoop KA., McDonald KG., McCrate S., McDole JR., Newberry RD. Microbial sensing by goblet cells controls immune surveillance of luminal antigens in the colon. *Mucosal Immunol* 2015;**8**(1):198–210. Doi: 10.1038/mi.2014.58.
26. Wang S., Charbonnier LM., Noval Rivas M., Georgiev P., Li N., Gerber G., et al. MyD88 Adaptor-Dependent Microbial Sensing by Regulatory T Cells Promotes Mucosal Tolerance and Enforces Commensalism. *Immunity* 2015;**43**(2):289–303. Doi: 10.1016/j.immuni.2015.06.014.
27. Smith K., Karimian Azari E., LaMoia TE., Hussain T., Vargova V., Karolyi K., et al. T1R2 receptor-mediated glucose sensing in the upper intestine potentiates glucose absorption through activation of local regulatory pathways. *Mol Metab* 2018;**17**:98–111. Doi: 10.1016/j.molmet.2018.08.009.
28. Martini E., Krug SM., Siegmund B., Neurath MF., Becker C. Mend Your Fences: The Epithelial Barrier and its Relationship With Mucosal Immunity in Inflammatory Bowel Disease. *CMGH* 2017;33–46. Doi: 10.1016/j.jcmgh.2017.03.007.
29. Yang Q., Bermingham NA., Finegold MJ., Zoghbi HY. Requirement of Math1 for

- secretory cell lineage commitment in the mouse intestine. *Science* (80-) 2001;**294**(5549):2155–8. Doi: 10.1126/science.1065718.
30. Pelaseyed T., Bergström JH., Gustafsson JK., Ermund A., Birchenough GMH., Schütte A., et al. The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. *Immunol Rev* 2014;8–20. Doi: 10.1111/imr.12182.
31. Van Der Flier LG., Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol* 2009;241–60. Doi: 10.1146/annurev.physiol.010908.163145.
32. van der Flier LG., van Gijn ME., Hatzis P., Kujala P., Haegebarth A., Stange DE., et al. Transcription Factor Achaete Scute-Like 2 Controls Intestinal Stem Cell Fate. *Cell* 2009;**136**(5):903–12. Doi: 10.1016/j.cell.2009.01.031.
33. Adams SH., Won WB., Schonhoff SE., Leiter AB., Paterniti JR. Effects of peptide YY[3-36] on short-term food intake in mice are not affected by prevailing plasma ghrelin levels. *Endocrinology* 2004;**145**(11):4967–75. Doi: 10.1210/en.2004-0518.
34. Kraehenbuhl JP., Neutra MR. Epithelial M cells: Differentiation and function. *Annu Rev Cell Dev Biol* 2000;301–32. Doi: 10.1146/annurev.cellbio.16.1.301.
35. Schneider C., O’Leary CE., Locksley RM. Regulation of immune responses by tuft cells. *Nat Rev Immunol* 2019;584–93. Doi: 10.1038/s41577-019-0176-x.
36. Ivanov AI. Structure and regulation of intestinal epithelial tight junctions current concepts and unanswered questions. *Adv Exp Med Biol* 2013;**763**:132–48. Doi: 10.1007/978-1-4614-4711-5_6.
37. Pulimeno P., Bauer C., Stutz J., Citi S. PLEKHA7 is an adherens junction protein with a tissue distribution and subcellular localization distinct from ZO-1 and E-Cadherin. *PLoS One* 2010;**5**(8). Doi: 10.1371/journal.pone.0012207.
38. Mangeol P., Massey-Harroche D., Sebbagh M., Richard F., Le Bivic A., Lenne PF. The zonula adherens matura redefines the apical junction of intestinal epithelia. *Proc Natl Acad Sci U S A* 2024;**121**(9):2316722121. Doi: 10.1073/pnas.2316722121.
39. Rimm DL., Koslov ER., Kebriaei P., Cianci CD., Morrow JS. α 1(E)-catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex. *Proc Natl Acad Sci U S A* 1995;**92**(19):8813–7. Doi: 10.1073/pnas.92.19.8813.
40. Mandai K., Nakanishi H., Satoh A., Obaishi H., Wada M., Nishioka H., et al. Afadin: A novel actin filament-binding protein with one PDZ domain localized at cadherin-based cell-to-cell adherens junction. *J Cell Biol* 1997;**139**(2):517–28. Doi: 10.1083/jcb.139.2.517.
41. Fanning AS., Jameson BJ., Jesaitis LA., Anderson JM. The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. *J Biol Chem* 1998;**273**(45):29745–53. Doi: 10.1074/jbc.273.45.29745.
42. Rouaud F., Huang W., Flinois A., Jain K., Vasileva E., Di Mattia T., et al. Cingulin and paracingulin tether myosins-2 to junctions to mechanoregulate the plasma membrane. *J Cell Biol* 2023;**222**(7). Doi: 10.1083/jcb.202208065.

- 880 43. Vanslebrouck B., Chen JH., Larabell C., van Hengel J. Microscopic Visualization of
881 Cell-Cell Adhesion Complexes at Micro and Nanoscale. *Front Cell Dev Biol* 2022.
882 Doi: 10.3389/fcell.2022.819534.
- 883 44. Odenwald MA., Turner JR. The intestinal epithelial barrier: A therapeutic target? *Nat*
884 *Rev Gastroenterol Hepatol* 2017;9–21. Doi: 10.1038/nrgastro.2016.169.
- 885 45. Horowitz A., Chanez-Paredes SD., Haest X., Turner JR. Paracellular permeability and
886 tight junction regulation in gut health and disease. *Nat Rev Gastroenterol Hepatol*
887 2023;417–32. Doi: 10.1038/s41575-023-00766-3.
- 888 46. Zuo L., Kuo WT., Turner JR. Tight Junctions as Targets and Effectors of Mucosal
889 Immune Homeostasis. *CMGH* 2020;327–40. Doi: 10.1016/j.jcmgh.2020.04.001.
- 890 47. Anderson JM., Van Itallie CM. Physiology and function of the tight junction. *Cold*
891 *Spring Harb Perspect Biol* 2009. Doi: 10.1101/cshperspect.a002584.
- 892 48. Seethaler B., Basrai M., Neyrinck AM., Nazare JA., Walter J., Delzenne NM., et al.
893 Biomarkers for assessment of intestinal permeability in clinical practice. *Am J Physiol*
894 *- Gastrointest Liver Physiol* 2021;**321**(1):G11–7. Doi: 10.1152/AJPGI.00113.2021.
- 895 49. Brescia P., Rescigno M. The gut vascular barrier: a new player in the gut–liver–brain
896 axis. *Trends Mol Med* 2021;844–55. Doi: 10.1016/j.molmed.2021.06.007.
- 897 50. Bischoff SC., Barbara G., Buurman W., Ockhuizen T., Schulzke JD., Serino M., et al.
898 Intestinal permeability - a new target for disease prevention and therapy. *BMC*
899 *Gastroenterol* 2014. Doi: 10.1186/s12876-014-0189-7.
- 900 51. Vanuytsel T., Tack J., Farre R. The Role of Intestinal Permeability in Gastrointestinal
901 Disorders and Current Methods of Evaluation. *Front Nutr* 2021. Doi:
902 10.3389/fnut.2021.717925.
- 903 52. Del Valle-Pinero AY., Van Deventer HE., Fourie NH., Martino AC., Patel NS.,
904 Remaley AT., et al. Gastrointestinal permeability in patients with irritable bowel
905 syndrome assessed using a four probe permeability solution. *Clin Chim Acta*
906 2013;**418**:97–101. Doi: 10.1016/j.cca.2012.12.032.
- 907 53. Mishra A., Makharia GK. Techniques of functional and motility test: How to perform
908 and interpret intestinal permeability. *J Neurogastroenterol Motil* 2012;**18**(4):443–7.
909 Doi: 10.5056/jnm.2012.18.4.443.
- 910 54. Odenwald MA., Turner JR. Intestinal Permeability Defects: Is It Time to Treat? *Clin*
911 *Gastroenterol Hepatol* 2013;**11**(9):1075–83. Doi: 10.1016/j.cgh.2013.07.001.
- 912 55. Stryeck S., Horvath A., Leber B., Stadlbauer V., Madl T. NMR spectroscopy enables
913 simultaneous quantification of carbohydrates for diagnosis of intestinal and gastric
914 permeability. *Sci Rep* 2018;**8**(1). Doi: 10.1038/s41598-018-33104-8.
- 915 56. Rao AS., Camilleri M., Eckert DJ., Busciglio I., Burton DD., Ryks M., et al. Urine
916 sugars for in vivo gut permeability: Validation and comparisons in irritable bowel
917 syndrome-diarrhea and controls. *Am J Physiol - Gastrointest Liver Physiol*
918 2011;**301**(5):919–28. Doi: 10.1152/ajpgi.00168.2011.
- 919 57. Galipeau HJ., Verdu EF. The complex task of measuring intestinal permeability in
920 basic and clinical science. *Neurogastroenterol Motil* 2016;957–65. Doi:
921 10.1111/nmo.12871.

58. van Wijck K., Verlinden TJM., van Eijk HMH., Dekker J., Buurman WA., Dejong CHC., et al. Novel multi-sugar assay for site-specific gastrointestinal permeability analysis: A randomized controlled crossover trial. *Clin Nutr* 2013;**32**(2):245–51. Doi: 10.1016/j.clnu.2012.06.014.
59. Camilleri M., Nadeau A., Lamsam J., Linker Nord S., Ryks M., Burton D., et al. Understanding measurements of intestinal permeability in healthy humans with urine lactulose and mannitol excretion. *Neurogastroenterol Motil* 2010;**22**(1). Doi: 10.1111/j.1365-2982.2009.01361.x.
60. Dastyh M., Dastyh M., Novotná H., Číhalová J. Lactulose/mannitol test and specificity, sensitivity, and area under curve of intestinal permeability parameters in patients with liver cirrhosis and Crohn's disease. *Dig Dis Sci* 2008;**53**(10):2789–92. Doi: 10.1007/s10620-007-0184-8.
61. Travis S., Menzies I. Intestinal permeability: Functional assessment and significance. *Clin Sci* 1992:471–88. Doi: 10.1042/cs0820471.
62. Dahlqvist A. Method for assay of intestinal disaccharidases. *Anal Biochem* 1964;**7**(1):18–25. Doi: 10.1016/0003-2697(64)90115-0.
63. LAKER MF., BULL HJ., MENZIES IS. Evaluation of mannitol for use as a probe marker of gastrointestinal permeability in man. *Eur J Clin Invest* 1982;**12**(6):485–91. Doi: 10.1111/j.1365-2362.1982.tb02230.x.
64. Pappenheimer JR. Paracellular intestinal absorption of glucose, creatinine, and mannitol in normal animals: Relation to body size. *Am J Physiol - Gastrointest Liver Physiol* 1990;**259**(2 22-2). Doi: 10.1152/ajpgi.1990.259.2.g290.
65. Cobden I., Hamilton I., Rothwell J., Axon ATR. Cellobiose/mannitol test: physiological properties of probe molecules and influence of extraneous factors. *Clin Chim Acta* 1985;**148**(1):53–62. Doi: 10.1016/0009-8981(85)90300-6.
66. Menzies IS. Alimentary disacchariduria in adults related to osmolality of ingested solutions. *Biochem J* 1972;**126**(3). Doi: 10.1042/bj1260019Pb.
67. Laker MF., Menzies IS. Increase in human intestinal permeability following ingestion of hypertonic solutions. *J Physiol* 1977;**265**(3):881–94. Doi: 10.1113/jphysiol.1977.sp011750.
68. Menzies, I.S. Transmucosal passage of inert molecules in health and disease. In: Skadhauge, E. & Heintze, K., eds. Intestinal absorption and secretion. Falk Symposium 36. Lancaster: MTP Press Ltd, 1984: 527-43 n.d.
69. Crowther, R.S. & Wen, G. Physicochemical properties of probe molecules used to assess intestinal permeability. *Gastroenterology* 1991; 100, A205 n.d.
70. Maxton DG., Menzies IS., Slavin B., Thompson RP. Small-intestinal function during enteral feeding and starvation in man. *Clin Sci (Lond)* 1989;**77**(4):401–6. Doi: 10.1042/cs0770401.
71. Erickson RA., Epstein RM. Oral Chenodeoxycholic Acid Increases Small Intestinal Permeability to Lactulose in Humans. *Am J Gastroenterol* 1988;**83**(5):541–4. Doi: 10.1111/j.1572-0241.1988.tb02411.x.
72. Ford RP., Menzies S., Phillips AD., Walker-Smith JA., Turner MW. Intestinal sugar

- 964 permeability: Relationship to diarrhoeal disease and small bowel morphology. *J*
 965 *Pediatr Gastroenterol Nutr* 1985;**4**(4):568–74. Doi: 10.1097/00005176-198508000-
 966 00012.
- 967 73. Meddings JB., Gibbons I. Discrimination of site-specific alterations in gastrointestinal
 968 permeability in the rat. *Gastroenterology* 1998;**114**(1):83–92. Doi: 10.1016/S0016-
 969 5085(98)70636-5.
- 970 74. Li L., Xiong L., Yao J., Zhuang X., Zhang S., Yu Q., et al. Increased small intestinal
 971 permeability and RNA expression profiles of mucosa from terminal ileum in patients
 972 with diarrhoea-predominant irritable bowel syndrome. *Dig Liver Dis* 2016;**48**(8):880–
 973 7. Doi: 10.1016/j.dld.2016.05.002.
- 974 75. Mujagic Z., Ludidi S., Keszthelyi D., Hesselink MAM., Kruimel JW., Lenaerts K., et
 975 al. Small intestinal permeability is increased in diarrhoea predominant IBS, while
 976 alterations in gastroduodenal permeability in all IBS subtypes are largely attributable
 977 to confounders. *Aliment Pharmacol Ther* 2014;**40**(3):288–97. Doi: 10.1111/apt.12829.
- 978 76. Meddings JB., Sutherland LR., Byles NI., Wallace JL. Sucrose: A novel permeability
 979 marker for gastroduodenal disease. *Gastroenterology* 1993;**104**(6):1619–26. Doi:
 980 10.1016/0016-5085(93)90637-R.
- 981 77. Sutherland LR., Verhoef M., Wallace JL., van Rosendaal G., Crutcher R., Meddings
 982 JB. A simple, non-invasive marker of gastric damage: sucrose permeability. *Lancet*
 983 1994;**343**(8904):998–1000. Doi: 10.1016/S0140-6736(94)90125-2.
- 984 78. Dahlqvist A., Borgström B. Digestion and absorption of disaccharides in man. *Biochem*
 985 *J* 1961;**81**(2):411–8. Doi: 10.1042/bj0810411.
- 986 79. Hiele M., Ghooys Y., Rutgeerts P., Vantrappen G. Metabolism of erythritol in humans:
 987 Comparison with glucose and lactitol. *Br J Nutr* 1993;**69**(1):169–76. Doi:
 988 10.1079/bjn19930019.
- 989 80. Farhadi A., Keshavarzian A., Holmes EW., Fields J., Zhang L., Banan A. Gas
 990 chromatographic method for detection of urinary sucralose: Application to the
 991 assessment of intestinal permeability. *J Chromatogr B Anal Technol Biomed Life Sci*
 992 2003;**784**(1):145–54. Doi: 10.1016/S1570-0232(02)00787-0.
- 993 81. Martinez D., Morris AI., Gilmore IT., Williams A., Stockdale H., Critchley M., et al.
 994 Comparison between the cellobiose/mannitol and ⁵¹Cr-labelled ethylenediaminetetra-
 995 acetate absorption tests in the detection of coeliac disease. *Clin Sci* 1988;**75**(4):375–8.
 996 Doi: 10.1042/cs0750375.
- 997 82. Howden CW., Robertson C., Duncan A., Morris AJ., Russell RI. Comparison of
 998 Different Measurements of Intestinal Permeability in Inflammatory Bowel Disease.
 999 *Am J Gastroenterol* 1991;**86**(10):1445–9. Doi: 10.1111/j.1572-0241.1991.tb06870.x.
- 1000 83. Blomquist L., Bark T., Hedenborg G., Svenberg T., Norman A. Comparison between
 1001 the lactulose/mannitol and ⁵¹Cr-ethylenediaminetetraacetic acid/¹⁴C-mannitol methods
 1002 for intestinal permeability frequency distribution pattern and variability of markers and
 1003 marker ratios in healthy subjects. *Scand J Gastroenterol* 1993;**28**(3):274–80. Doi:
 1004 10.3109/00365529309096085.
- 1005 84. Bjarnason I., Maxton D., Reynolds AP., Catt S., Peters TJ., Menzies IS. Comparison
 1006 of four markers of intestinal permeability in control subjects and patients with coeliac

- 1007 disease. *Scand J Gastroenterol* 1994;**29**(7):630–9. Doi: 10.3109/00365529409092484.
- 1008 85. van Wijck K., Bessems BAFM., van Eijk HMH., Buurman WA., Dejong CHC.,
1009 Lenaerts K. Polyethylene glycol versus dual sugar assay for gastrointestinal
1010 permeability analysis: Is it time to choose? *Clin Exp Gastroenterol* 2012;**5**(1):139–50.
1011 Doi: 10.2147/CEG.S31799.
- 1012 86. Dorshow RB., Hall-Moore C., Shaikh N., Talcott MR., Faubion WA., Rogers TE., et
1013 al. Measurement of gut permeability using fluorescent tracer agent technology. *Sci Rep*
1014 2017;**7**(1). Doi: 10.1038/s41598-017-09971-y.
- 1015 87. Holtz LR., Nix BD., Akuse SE., Hall-Moore C., Newberry RD., Ciorba MA., et al.
1016 First-in-Human Assessment of Gut Permeability in Crohn’s Disease Patients Using
1017 Fluorophore Technology. *Gastro Hep Adv* 2024;**3**(4):491–7. Doi:
1018 10.1016/j.gastha.2024.02.003.
- 1019 88. Camilleri M., Lasch K., Zhou W. Irritable bowel syndrome: Methods, mechanisms,
1020 and pathophysiology. the confluence of increased permeability, inflammation, and pain
1021 in irritable bowel syndrome. *Am J Physiol - Gastrointest Liver Physiol* 2012. Doi:
1022 10.1152/ajpgi.00155.2012.
- 1023 89. Addobbati R., Pascolo L., Di Toro N., Sebastiani GB., Martellosi S., Not T. Influence
1024 of urine volume on the assessment of intestinal permeability in affected children by
1025 multiple sugar probes. *Clin Chem Lab Med* 2014;**52**(2):227–35. Doi: 10.1515/cclm-
1026 2013-0626.
- 1027 90. Morifuji M., Yasuda M., Takahashi S., Sato A. Search for new biomarkers using
1028 metabolomic analysis reflective of intestinal permeability in healthy Japanese
1029 participants. *Metabolomics* 2025;**21**(3). Doi: 10.1007/s11306-025-02270-y.
- 1030 91. Dorshow RB., Johnson JR., Debreczeny MP., Riley IR., Shieh J-J., Rogers TE., et al.
1031 Noninvasive point-of-care measurement of gastrointestinal permeability. 2019. p. 8.
- 1032 92. Michel K., Zarogiannis SG. Clinical tests for intestinal permeability: “sweet” transition
1033 from clinical to basic physiology. *Clin Exp Pharmacol Physiol* 2014;**41**(2):105–6. Doi:
1034 10.1111/1440-1681.12192.
- 1035 93. Camilleri M., Madsen K., Spiller R., Van Meerveld BG., Verne GN. Intestinal barrier
1036 function in health and gastrointestinal disease. *Neurogastroenterol Motil* 2012:503–12.
1037 Doi: 10.1111/j.1365-2982.2012.01921.x.
- 1038 94. Fasano A. Intestinal Permeability and Its Regulation by Zonulin: Diagnostic and
1039 Therapeutic Implications. *Clin Gastroenterol Hepatol* 2012;**10**(10):1096–100. Doi:
1040 10.1016/j.cgh.2012.08.012.
- 1041 95. Bodelier AGL., Pierik MJ., Lenaerts K., De Boer E., Olde Damink SW., Hameeteman
1042 WM., et al. Plasma intestinal fatty acid-binding protein fails to predict endoscopic
1043 disease activity in inflammatory bowel disease patients. *Eur J Gastroenterol Hepatol*
1044 2016;**28**(7):807–13. Doi: 10.1097/MEG.0000000000000616.
- 1045 96. Luk GD., Bayless TM., Baylin SB. Diamine oxidase (histaminase). A circulating
1046 marker for rat intestinal mucosal maturation and integrity. *J Clin Invest*
1047 1980;**66**(1):66–70. Doi: 10.1172/JCI109836.
- 1048 97. Hoisington AJ., Stamper CE., Stearns-Yoder KA., Haghighi F., Lowry CA., Brenner
1049 LA. A cross-sectional study of correlations among blood-based biomarkers for

1050 intestinal permeability: A pilot study of United States veterans with posttraumatic
 1051 stress disorder symptoms. *Brain, Behav Immun - Heal* 2023;**34**. Doi:
 1052 10.1016/j.bbih.2023.100702.

1053 98. Shivakumar N., Huq S., Paredes-Olortegui M., Konyole SO., Devi S., Yazbeck R., et
 1054 al. A cross-sectional study of associations between the ¹³C-sucrose breath test, the
 1055 lactulose rhamnose assay, and growth in children at high risk of environmental
 1056 enteropathy. *Am J Clin Nutr* 2024;**120**(6):1354–63. Doi: 10.1016/j.ajcnut.2024.10.001.

1057 99. Jianfeng G., Weiming Z., Ning L., Fangnan L., Li T., Nan L., et al. Serum citrulline is
 1058 a simple quantitative marker for small intestinal enterocytes mass and absorption
 1059 function in short bowel patients. *J Surg Res* 2005;**127**(2):177–82. Doi:
 1060 10.1016/j.jss.2005.04.004.

1061 100. Tripathi A., Lammers KM., Goldblum S., Shea-Donohue T., Netzel-Arnett S., Buzza
 1062 MS., et al. Identification of human zonulin, a physiological modulator of tight
 1063 junctions, as prehaptoglobin-2. *Proc Natl Acad Sci U S A* 2009;**106**(39):16799–804.
 1064 Doi: 10.1073/pnas.0906773106.

1065 101. Massier L., Chakaroun R., Kovacs P., Heiker JT. Blurring the picture in leaky gut
 1066 research: How shortcomings of zonulin as a biomarker mislead the field of intestinal
 1067 permeability. *Gut* 2021:1801–2. Doi: 10.1136/gutjnl-2020-323026.

1068 102. Ajamian M., Steer D., Rosella G., Gibson PR. Serum zonulin as a marker of intestinal
 1069 mucosal barrier function: May not be what it seems. *PLoS One* 2019;**14**(1). Doi:
 1070 10.1371/journal.pone.0210728.

1071 103. Vojdani A., Vojdani E., Kharrazian D. Fluctuation of zonulin levels in blood vs
 1072 stability of antibodies. *World J Gastroenterol* 2017;**23**(31):5669–79. Doi:
 1073 10.3748/wjg.v23.i31.5669.

1074 104. Cohen J. The detection and interpretation of endotoxaemia. *Intensive Care Medicine*,
 1075 *Supplement*, vol. 26. Intensive Care Med; 2000.

1076 105. Derkinderen P., Cossais F., Kulcsárová K., Škorvánek M., Sellier-Montaigne L.,
 1077 Coron E., et al. How leaky is the gut in Parkinson's disease? *EBioMedicine* 2025. Doi:
 1078 10.1016/j.ebiom.2025.105796.

1079 106. Sun XQ., Fu XB., Zhang R., Lü Y., Deng Q., Jiang XG., et al. Relationship between
 1080 plasma D(-)-lactate and intestinal damage after severe injuries in rats. *World J*
 1081 *Gastroenterol* 2001;**7**(4):555–8. Doi: 10.3748/wjg.v7.i4.555.

1082 107. Magro F., Lopes J., Borralho P., Lopes S., Coelho R., Cotter J., et al. Comparison of
 1083 different histological indexes in the assessment of UC activity and their accuracy
 1084 regarding endoscopic outcomes and faecal calprotectin levels. *Gut* 2019;**68**(4):594–
 1085 603. Doi: 10.1136/gutjnl-2017-315545.

1086 108. Linsalata M., Riezzo G., Clemente C., D'Attoma B., Russo F. Noninvasive
 1087 Biomarkers of Gut Barrier Function in Patients Suffering from Diarrhea Predominant-
 1088 IBS: An Update. *Dis Markers* 2020;**2020**. Doi: 10.1155/2020/2886268.

1089 109. Kong C., Li S-M., Yang H., Xiao W-D., Cen Y-Y., Wu Y., et al. Screening and
 1090 combining serum biomarkers to improve their diagnostic performance in the detection
 1091 of intestinal barrier dysfunction in patients after major abdominal surgery. *Ann Transl*
 1092 *Med* 2019;**7**(16):388–388. Doi: 10.21037/atm.2019.07.102.

- 1093 110. Viewjean S., Coibion C., Seidel L., Louis E., Meunier P. Magnetic resonance
1094 enterography perfusion parameters reveal complex changes in affected and unaffected
1095 segments in Crohn's disease. *Scand J Gastroenterol* 2020;**55**(9):1041–8. Doi:
1096 10.1080/00365521.2020.1802773.
- 1097 111. Towner RA., Smith N., Saunders D., Van Gordon SB., Wisniewski AB., Tyler KR., et
1098 al. Contrast enhanced magnetic resonance imaging as a diagnostic tool to assess
1099 bladder permeability and associated colon cross talk: Preclinical studies in a rat model.
1100 *J Urol* 2015;**193**(4):1394–400. Doi: 10.1016/J.JURO.2014.10.120,.
- 1101 112. Greenwood-Van Meerveld B., Mohammadi E., Tyler K., Van Gordon S., Parker A.,
1102 Towner R., et al. Mechanisms of visceral organ crosstalk: Importance of alterations in
1103 permeability in rodent models. *J Urol* 2015;**194**(3):804–11. Doi:
1104 10.1016/j.juro.2015.02.2944.
- 1105 113. Yamashita S., Takashima T., Kataoka M., Oh H., Sakuma S., Takahashi M., et al. PET
1106 imaging of the gastrointestinal absorption of orally administered drugs in conscious
1107 and anesthetized rats. *J Nucl Med* 2011;**52**(2):249–56. Doi:
1108 10.2967/jnumed.110.081539.
- 1109 114. Iacucci M., Santacroce G., Majumder S., Morael J., Zammarchi I., Maeda Y., et al.
1110 Opening the doors of precision medicine: Novel tools to assess intestinal barrier in
1111 inflammatory bowel disease and colitis-associated neoplasia. *Gut* 2024;**73**(10):1749–
1112 62. Doi: 10.1136/gutjnl-2023-331579.
- 1113 115. Liu J., Dlugosz A., Neumann H. Beyond white light endoscopy: The role of optical
1114 biopsy in inflammatory bowel disease. *World J Gastroenterol* 2013;**19**(43):7544–51.
1115 Doi: 10.3748/wjg.v19.i43.7544.
- 1116 116. Neumann H., Kiesslich R., Wallace MB., Neurath MF. Confocal laser
1117 endomicroscopy: Technical advances and clinical applications. *Gastroenterology*
1118 2010;**139**(2). Doi: 10.1053/j.gastro.2010.06.029.
- 1119 117. Iacucci M., Jeffery L., Acharjee A., Grisan E., Buda A., Nardone OM., et al.
1120 Computer-Aided Imaging Analysis of Probe-Based Confocal Laser Endomicroscopy
1121 with Molecular Labeling and Gene Expression Identifies Markers of Response to
1122 Biological Therapy in IBD Patients: The Endo-Omics Study. *Inflamm Bowel Dis*
1123 2023;**29**(9):1409–20. Doi: 10.1093/ibd/izac233.
- 1124 118. Chang J., Ip M., Yang M., Wong B., Power T., Lin L., et al. The learning curve,
1125 interobserver, and intraobserver agreement of endoscopic confocal laser
1126 endomicroscopy in the assessment of mucosal barrier defects. *Gastrointest Endosc*
1127 2016;**83**(4):785–791.e1. Doi: 10.1016/j.gie.2015.08.045.
- 1128 119. Huynh R., Ip M., Chang J., Haifer C., Leong R. Expert-led didactic versus self-
1129 directed audiovisual training of confocal laser endomicroscopy in evaluation of
1130 mucosal barrier defects. *Endosc Int Open* 2018;**06**(01):E115–22. Doi: 10.1055/s-0043-
1131 114664.
- 1132 120. Boschetto D., Mirzaei H., Leong RWL., Tarroni G., Grisan E. Semiautomatic
1133 detection of villi in confocal endoscopy for the evaluation of celiac disease.
1134 *Proceedings of the Annual International Conference of the IEEE Engineering in*
1135 *Medicine and Biology Society, EMBS*, vol. 2015- Novem. Annu Int Conf IEEE Eng
1136 Med Biol Soc; 2015. p. 8143–6.

- 1137 121. Buda A., Hatem G., Neumann H., D'Inca R., Mescoli C., Piselli P., et al. Confocal
1138 laser endomicroscopy for prediction of disease relapse in ulcerative colitis: A pilot
1139 study. *J Crohn's Colitis* 2014;**8**(4):304–11. Doi: 10.1016/j.crohns.2013.09.005.
- 1140 122. Chang J., Leong RW., Wasinger VC., Ip M., Yang M., Phan TG. Impaired Intestinal
1141 Permeability Contributes to Ongoing Bowel Symptoms in Patients With Inflammatory
1142 Bowel Disease and Mucosal Healing. *Gastroenterology* 2017;**153**(3):723-731.e1. Doi:
1143 10.1053/j.gastro.2017.05.056.
- 1144 123. Kiesslich R., Duckworth CA., Moussata D., Gloeckner A., Lim LG., Goetz M., et al.
1145 Local barrier dysfunction identified by confocal laser endomicroscopy predicts relapse
1146 in inflammatory bowel disease. *Gut* 2012;**61**(8):1146–53. Doi: 10.1136/gutjnl-2011-
1147 300695.
- 1148 124. Li CQ., Xie XJ., Yu T., Gu XM., Zuo XL., Zhou CJ., et al. Classification of
1149 inflammation activity in ulcerative colitis by confocal laser endomicroscopy. *Am J*
1150 *Gastroenterol* 2010;**105**(6):1391–6. Doi: 10.1038/ajg.2009.664.
- 1151 125. Iacucci M., Cannatelli R., Gui X., Zardo D., Bazarova A., Gkoutos G V., et al.
1152 Assessment of endoscopic healing by using advanced technologies reflects histological
1153 healing in ulcerative colitis. *J Crohn's Colitis* 2020;**14**(9):1282–9. Doi: 10.1093/ecco-
1154 jcc/jjaa056.
- 1155 126. Iacucci M., Daperno M., Lazarev M., Arsenascu R., Tontini GE., Akinola O., et al.
1156 Development and reliability of the new endoscopic virtual chromoendoscopy score:
1157 the PICaSSO (Paddington International Virtual ChromoendoScopy ScOre) in
1158 ulcerative colitis. *Gastrointest Endosc* 2017;**86**(6):1118-1127.e5. Doi:
1159 10.1016/j.gie.2017.03.012.
- 1160 127. Santacroce G., Zammarchi I., Tan CK., Coppola G., Varley R., Ghosh S., et al. Present
1161 and future of endoscopy precision for inflammatory bowel disease. *Dig Endosc*
1162 2024;292–304. Doi: 10.1111/den.14672.
- 1163 128. Macé V., Ahluwalia A., Coron E., Le Rhun M., Boureille A., Bossard C., et al.
1164 Confocal laser endomicroscopy: A new gold standard for the assessment of mucosal
1165 healing in ulcerative colitis. *J Gastroenterol Hepatol* 2015;**30**(S1):85–92. Doi:
1166 10.1111/jgh.12748.
- 1167 129. Chauhan SS., Abu Dayyeh BK., Bhat YM., Gottlieb KT., Hwang JH., Komanduri S.,
1168 et al. Confocal laser endomicroscopy. *Gastrointest Endosc* 2014;**80**(6):928–38. Doi:
1169 10.1016/j.gie.2014.06.021.
- 1170 130. Robles-Medrand C. Confocal endomicroscopy: Is it time to move on? *World J*
1171 *Gastrointest Endosc* 2016;**8**(1):1. Doi: 10.4253/wjge.v8.i1.1.
- 1172 131. Waldner MJ., Rath T., Schürmann S., Bojarski C., Atreya R. Imaging of mucosal
1173 inflammation: Current technological developments, clinical implications, and future
1174 perspectives. *Front Immunol* 2017. Doi: 10.3389/fimmu.2017.01256.
- 1175 132. Nardone OM., Cannatelli R., Zardo D., Ghosh S., Iacucci M. Can advanced
1176 endoscopic techniques for assessment of mucosal inflammation and healing
1177 approximate histology in inflammatory bowel disease? *Therap Adv Gastroenterol*
1178 2019. Doi: 10.1177/1756284819863015.
- 1179 133. Neumann H., Fuchs FS., Vieth M., Atreya R., Siebler J., Kiesslich R., et al. Review

- 1180 article: In vivo imaging by endocytoscopy. *Aliment Pharmacol Ther* 2011;1183–93.
1181 Doi: 10.1111/j.1365-2036.2011.04647.x.
- 1182 134. Iacucci M., Jeffery L., Acharjee A., Nardone OM., Zardo D., Smith SCL., et al. Ultra-
1183 high Magnification Endocytoscopy and Molecular Markers for Defining Endoscopic
1184 and Histologic Remission in Ulcerative Colitis-An Exploratory Study to Define Deep
1185 Remission. *Inflamm Bowel Dis* 2021;**27**(11):1719–30. Doi: 10.1093/ibd/izab059.
- 1186 135. Abtahi S., Sailer A., Roland JT., Haest X., Chanez-Paredes SD., Ahmad K., et al.
1187 Intestinal Epithelial Digestive, Transport, and Barrier Protein Expression Is Increased
1188 in Environmental Enteric Dysfunction. *Lab Invest* 2023;**103**(4). Doi:
1189 10.1016/j.labinv.2022.100036.
- 1190 136. Majumder S., Santacroce G., Maeda Y., Zammarchi I., Puga-Tejada M., Ditunno I., et
1191 al. Endocytoscopy with automated multispectral intestinal barrier pathology imaging
1192 for assessment of deep healing to predict outcomes in ulcerative colitis. *Gut*
1193 2024;**73**(10):1603–6. Doi: 10.1136/gutjnl-2024-332894.
- 1194 137. Nardone OM., Cannatelli R., Ghosh S., Iacucci M. New endoscopic tools in
1195 inflammatory bowel disease. *United Eur Gastroenterol J* 2022;1103–12. Doi:
1196 10.1002/ueg2.12316.
- 1197 138. Nunes R., Silva C., Chaves L. Tissue-based in vitro and ex vivo models for intestinal
1198 permeability studies. *Concepts and Models for Drug Permeability Studies: Cell and*
1199 *Tissue based in Vitro Culture Models*. Woodhead Publishing; 2015. p. 203–36.
- 1200 139. Wallon C., Braaf Y., Wolving M., Olaison G., Söderholm JD. Endoscopic biopsies in
1201 Ussing chambers evaluated for studies of macromolecular permeability in the human
1202 colon. *Scand J Gastroenterol* 2005;**40**(5):586–95. Doi: 10.1080/00365520510012235.
- 1203 140. Volynets V., Reichold A., Bárdos G., Rings A., Bleich A., Bischoff SC. Assessment of
1204 the Intestinal Barrier with Five Different Permeability Tests in Healthy C57BL/6J and
1205 BALB/cJ Mice. *Dig Dis Sci* 2016;**61**(3):737–46. Doi: 10.1007/s10620-015-3935-y.
- 1206 141. Zeissig S., Bojarski C., Buergel N., Mankertz J., Zeitz M., Fromm M., et al.
1207 Downregulation of epithelial apoptosis and barrier repair in active Crohn's disease by
1208 tumour necrosis factor α antibody treatment. *Gut* 2004;**53**(9):1295–302. Doi:
1209 10.1136/gut.2003.036632.
- 1210 142. Theile M., Wiora L., Russ D., Reuter J., Ishikawa H., Schwerk C., et al. A simple
1211 approach to perform teer measurements using a self-made volt-amperemeter with
1212 programmable output frequency. *J Vis Exp* 2019;**2019**(152). Doi: 10.3791/60087.
- 1213 143. Yeste J., Illa X., Alvarez M., Villa R. Engineering and monitoring cellular barrier
1214 models. *J Biol Eng* 2018;18. Doi: 10.1186/s13036-018-0108-5.
- 1215 144. Li L., Wang Y., Pei Y., Chen J., Zhou J., Gong Y., et al. Lactoferrin prevents heat
1216 stroke-induced intestinal barrier damage by reducing ferroptosis via regulating MAPK
1217 signaling pathway: In vitro and in vivo studies. *Int J Biol Macromol* 2025;**319**(Pt 2).
1218 Doi: 10.1016/j.ijbiomac.2025.145454.
- 1219 145. Chen Y., Li Y., Li X., Fang Q., Li F., Chen S., et al. Indole-3-propionic acid alleviates
1220 intestinal epithelial cell injury via regulation of the TLR4/NF- κ B pathway to improve
1221 intestinal barrier function. *Mol Med Rep* 2024;**30**(4). Doi: 10.3892/mmr.2024.13313.
- 1222 146. Sandle GI., Higgs N., Crowe P., Marsh MN., Venkatesan S., Peters TJ. Cellular basis

- 1223 for defective electrolyte transport in inflamed human colon. *Gastroenterology*
1224 1990;**99**(1):97–105. Doi: 10.1016/0016-5085(90)91235-X.
- 1225 147. Zeissig S., Bürgel N., Günzel D., Richter J., Mankertz J., Wahnschaffe U., et al.
1226 Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight
1227 junctions and barrier dysfunction in active Crohn's disease. *Gut* 2007;**56**(1):61–72.
1228 Doi: 10.1136/gut.2006.094375.
- 1229 148. Linz G., Djeljadini S., Steinbeck L., Köse G., Kiessling F., Wessling M. Cell barrier
1230 characterization in transwell inserts by electrical impedance spectroscopy. *Biosens*
1231 *Bioelectron* 2020;**165**. Doi: 10.1016/j.bios.2020.112345.
- 1232 149. Benson K., Cramer S., Galla HJ. Impedance-based cell monitoring: Barrier properties
1233 and beyond. *Fluids Barriers CNS* 2013. Doi: 10.1186/2045-8118-10-5.
- 1234 150. Nakagawa S., Deli MA., Kawaguchi H., Shimizudani T., Shimono T., Kittel Á., et al.
1235 A new blood-brain barrier model using primary rat brain endothelial cells, pericytes
1236 and astrocytes. *Neurochem Int* 2009;**54**(3–4):253–63. Doi:
1237 10.1016/j.neuint.2008.12.002.
- 1238 151. Sun M., Fu H., Cheng H., Cao Q., Zhao Y., Mou X., et al. A dynamic real-time
1239 method for monitoring epithelial barrier function in vitro. *Anal Biochem*
1240 2012;**425**(2):96–103. Doi: 10.1016/j.ab.2012.03.010.
- 1241 152. Ouahoud S., Giugliano FP., Muncan V. Monitoring Intestinal Organoid-Derived
1242 Monolayer Barrier Functions with Electric Cell-Substrate Impedance Sensing (ECIS).
1243 *Bio-Protocol* 2024;**14**(5). Doi: 10.21769/BioProtoc.4947.
- 1244 153. Venz A., Duckert B., Lagae L., Takaloo SE., Braeken D. Impedance mapping with
1245 high-density microelectrode array chips reveals dynamic heterogeneity of in vitro
1246 epithelial barriers. *Sci Rep* 2025;**15**(1). Doi: 10.1038/s41598-025-85783-9.
- 1247 154. Tria SA., Ramuz M., Jimison LH., Hama A., Owens RM. Sensing of barrier tissue
1248 disruption with an organic electrochemical transistor. *J Vis Exp* 2014;(84). Doi:
1249 10.3791/51102.
- 1250 155. Jimison LH., Tria SA., Khodagholy D., Gurfinkel M., Lanzarini E., Hama A., et al.
1251 Measurement of barrier tissue integrity with an organic electrochemical transistor. *Adv*
1252 *Mater* 2012;**24**(44):5919–23. Doi: 10.1002/adma.201202612.
- 1253 156. Yeung SY., Gu X., Tsang CM., Tsao SW., Hsing I ming. Engineering organic
1254 electrochemical transistor (OECT) to be sensitive cell-based biosensor through tuning
1255 of channel area. *Sensors Actuators, A Phys* 2019;**287**:185–93. Doi:
1256 10.1016/j.sna.2018.12.032.
- 1257 157. Supjaroen P., Niamsi W., Thirabowonkitphithan P., Thummarati P., Laiwattanapaisal
1258 W. A customizable and low-cost 3D-printed transwell device coupled with 3D cell
1259 culture for permeability assay. *HardwareX* 2024;**20**. Doi: 10.1016/j.ohx.2024.e00603.
- 1260 158. Nakajima T., Sasaki K., Yamamori A., Sakurai K., Miyata K., Watanabe T., et al. A
1261 simple three-dimensional gut model constructed in a restricted ductal microspace
1262 induces intestinal epithelial cell integrity and facilitates absorption assays. *Biomater*
1263 *Sci* 2020;**8**(20):5615–27. Doi: 10.1039/d0bm00763c.
- 1264 159. Macedo MH., Martínez E., Barrias CC., Sarmiento B. Development of an Improved 3D
1265 in vitro Intestinal Model to Perform Permeability Studies of Paracellular Compounds.

- 1266 *Front Bioeng Biotechnol* 2020;**8**. Doi: 10.3389/fbioe.2020.524018.
- 1267 160. Macedo MH., Barros AS., Martínez E., Barrias CC., Sarmiento B. All layers matter:
1268 Innovative three-dimensional epithelium-stroma-endothelium intestinal model for
1269 reliable permeability outcomes. *J Control Release* 2022;**341**:414–30. Doi:
1270 10.1016/j.jconrel.2021.11.048.
- 1271 161. Tsai YH., Czerwinski M., Wu A., Dame MK., Attili D., Hill E., et al. A Method for
1272 Cryogenic Preservation of Human Biopsy Specimens and Subsequent Organoid
1273 Culture. *CMGH* 2018;**6**(2):218–222.e7. Doi: 10.1016/j.jcmgh.2018.04.008.
- 1274 162. He A., Powell S., Kyle M., Rose M., Masmila E., Estrada V., et al. Cryopreservation
1275 of Viable Human Tissues: Renewable Resource for Viable Tissue, Cell Lines, and
1276 Organoid Development. *Biopreserv Biobank* 2020;**18**(3):222–7. Doi:
1277 10.1089/bio.2019.0062.
- 1278 163. Dykstra GD., Kawasaki M., Burbick CR., McConnel CS., Ambrosini YM. From in
1279 vitro development to accessible luminal interface of neonatal bovine-derived intestinal
1280 organoids. *BMC Vet Res* 2025;**21**(1). Doi: 10.1186/s12917-025-04773-1.
- 1281 164. Jelinsky SA., Derksen M., Bauman E., Verissimo CS., van Dooremalen WTM., Roos
1282 JL., et al. Molecular and Functional Characterization of Human Intestinal Organoids
1283 and Monolayers for Modeling Epithelial Barrier. *Inflamm Bowel Dis* 2023;**29**(2):195–
1284 206. Doi: 10.1093/ibd/izac212.
- 1285 165. Eslami Amirabadi H., Donkers JM., Wierenga E., Ingenhut B., Pieters L., Stevens L.,
1286 et al. Intestinal explant barrier chip: Long-term intestinal absorption screening in a
1287 novel microphysiological system using tissue explants. *Lab Chip* 2022;**22**(2):326–42.
1288 Doi: 10.1039/d1lc00669j.
- 1289 166. Xiang Y., Wen H., Yu Y., Li M., Fu X., Huang S. Gut-on-chip: Recreating human
1290 intestine in vitro. *J Tissue Eng* 2020. Doi: 10.1177/2041731420965318.
- 1291 167. Beaurivage C., Naumovska E., Chang YX., Elstak ED., Nicolas A., Wouters H., et al.
1292 Development of a gut-on-a-chip model for high throughput disease modeling and drug
1293 discovery. *Int J Mol Sci* 2019;**20**(22). Doi: 10.3390/ijms20225661.
- 1294 168. Guo Y., Liu M-L., He X., Jiang C., Liu R. Functional changes of intestinal mucosal
1295 barrier in surgically critical patients. *World J Emerg Med* 2010;**1**(3):205–8.
- 1296 169. Rusticeanu M., Zimmer V., Lammert F. Visualising and quantifying intestinal
1297 permeability -where do we stand. *Ann Hepatol* 2021. Doi:
1298 10.1016/j.aohp.2020.09.010.
- 1299 170. Martínez C., Vicario M., Ramos L., Lobo B., Mosquera JL., Alonso C., et al. The
1300 jejunum of diarrhea-predominant irritable bowel syndrome shows molecular
1301 alterations in the tight junction signaling pathway that are associated with mucosal
1302 pathobiology and clinical manifestations. *Am J Gastroenterol* 2012;**107**(5):736–46.
1303 Doi: 10.1038/ajg.2011.472.
- 1304 171. Turpin W., Lee SH., Raygoza Garay JA., Madsen KL., Meddings JB., Bedrani L., et
1305 al. Increased Intestinal Permeability Is Associated With Later Development of Crohn's
1306 Disease. *Gastroenterology* 2020;**159**(6):2092–2100.e5. Doi:
1307 10.1053/j.gastro.2020.08.005.
- 1308 172. Teshima CW., Goodman KJ., El-Kalla M., Turk S., El-Matary W., Valcheva R., et al.

- 1309 Increased Intestinal Permeability in Relatives of Patients With Crohn's Disease Is Not
1310 Associated With Small Bowel Ulcerations. *Clin Gastroenterol Hepatol*
1311 2017;**15**(9):1413-1418.e1. Doi: 10.1016/j.cgh.2017.02.028.
- 1312 173. Rath T., Atreya R., Bodenschatz J., Uter W., Geppert CE., Vitali F., et al. Intestinal
1313 Barrier Healing Is Superior to Endoscopic and Histologic Remission for Predicting
1314 Major Adverse Outcomes in Inflammatory Bowel Disease: The Prospective ERICA
1315 Trial. *Gastroenterology* 2023;**164**(2):241-55. Doi: 10.1053/j.gastro.2022.10.014.
- 1316 174. Liu CY., Cham CM., Chang EB. Epithelial wound healing in inflammatory bowel
1317 diseases: the next therapeutic frontier. *Transl Res* 2021:35-51. Doi:
1318 10.1016/j.trsl.2021.06.001.
- 1319 175. Rettura F., Lambiase C., Tedeschi R., Grosso A., Cancelli L., Ricchiuti A., et al.
1320 Mucoprotectants and gut barrier: mechanisms of action and clinical applications in
1321 IBS. Is there a possible role? *Front Pharmacol* 2025. Doi:
1322 10.3389/fphar.2025.1538791.
- 1323 176. Friedrich M., Pohin M., Jackson MA., Korsunsky I., Bullers SJ., Rue-Albrecht K., et
1324 al. IL-1-driven stromal-neutrophil interactions define a subset of patients with
1325 inflammatory bowel disease that does not respond to therapies. *Nat Med*
1326 2021;**27**(11):1970-81. Doi: 10.1038/s41591-021-01520-5.
- 1327 177. Villablanca EJ., Selin K., Hedin CRH. Mechanisms of mucosal healing: treating
1328 inflammatory bowel disease without immunosuppression? *Nat Rev Gastroenterol*
1329 *Hepatol* 2022:493-507. Doi: 10.1038/s41575-022-00604-y.
- 1330 178. Akinbami FO., Brown GA., McNeish AS. Intestinal permeability as a measure of
1331 small intestinal mucosal integrity: Correlation with jejunal biopsy. *Afr J Med Med Sci*
1332 1989;**18**(3):187-92.
- 1333 179. Juby LD., Rothwell J., Axon ATR. Lactulose/mannitol test: An ideal screen for celiac
1334 disease. *Gastroenterology* 1989;**96**(1):79-85. Doi: 10.1016/0016-5085(89)90767-1.
- 1335 180. Juby LD., Rothwell J., Axon ATR. Cellobiose/mannitol sugar test - A sensitive
1336 tubeless test for coeliac disease: Results on 1010 unselected patients. *Gut*
1337 1989;**30**(4):476-80. Doi: 10.1136/gut.30.4.476.
- 1338 181. Willems D., Cadranet S., Jacobs W. Measurement of urinary sugars by HPLC in the
1339 estimation of intestinal permeability evaluation in pediatric clinical practice. *Clin*
1340 *Chem* 1993;**39**(5):888-90. Doi: 10.1093/clinchem/39.5.888.
- 1341 182. Öman H., Blomquist L., Henriksson AEK., Johansson SGO. Comparison of
1342 polysucrose 15000, 51cr-labelled ethylenediaminetetraacetic acid, and 14c-mannitol as
1343 markers of intestinal permeability in man. *Scand J Gastroenterol* 1995;**30**(12):1172-7.
1344 Doi: 10.3109/00365529509101627.
- 1345 183. Troncone R., Mayer M., Mugione P., Cucciardi M., Abete A., Greco L.
1346 Cellobiose/mannitol sugar permeability test in children in relation to jejunal
1347 morphometry. *Ital J Gastroenterol* 1995;**27**(9):489-93.
- 1348 184. Cox MA., Lewis KO., Cooper BT. Measurement of small intestinal permeability
1349 markers, lactulose, and mannitol in serum: Results in celiac disease. *Dig Dis Sci*
1350 1999;**44**(2):402-6. Doi: 10.1023/A:1026679123148.
- 1351 185. Van Nieuwenhoven MA., Geerling BJ., Deutz NEP., Brouns F., Brummer RJM. The

- 1352 sensitivity of the lactulose/rhamnose gut permeability test. *Eur J Clin Invest*
1353 1999;**29**(2):160–5. Doi: 10.1046/j.1365-2362.1999.00421.x.
- 1354 186. Hessels J., Eidhof HHM., Steggink J., Roeloffzen WWH., Wu K., Tan G., et al.
1355 Assessment of hypolactasia and site-specific intestinal permeability of differential
1356 sugar absorption of raffinose, lactose, sucrose and mannitol. *Clin Chem Lab Med*
1357 2003;**41**(8):1056–63. Doi: 10.1515/CCLM.2003.163.
- 1358 187. Anderson ADG., Jain PK., Fleming S., Poon P., Mitchell CJ., MacFie J. Evaluation of
1359 a triple sugar test of colonic permeability in humans. *Acta Physiol Scand*
1360 2004;**182**(2):171–7. Doi: 10.1111/j.1365-201X.2004.01347.x.
- 1361 188. Blomquist L., Bark T., Hedenborg G., Norman A. Evaluation of the lactulose/mannitol
1362 and 51Cr- ethylenediaminetetraacetic acid/14C-mannitol methods for intestinal
1363 permeability. Influence of urinary volume, sex, age, smoking, and intestinal disease on
1364 marker excretion. *Scand J Gastroenterol* 1997;**32**(8):805–12. Doi:
1365 10.3109/00365529708996538.
- 1366 189. Haas V., Büning C., Buhner S., Von Heymann C., Valentini L., Lochs H. Clinical
1367 relevance of measuring colonic permeability. *Eur J Clin Invest* 2009;**39**(2):139–44.
1368 Doi: 10.1111/j.1365-2362.2008.02075.x.
- 1369 190. Shaikh M., Rajan K., Forsyth CB., Voigt RM., Keshavarzian A. Simultaneous gas-
1370 chromatographic urinary measurement of sugar probes to assess intestinal
1371 permeability: Use of time course analysis to optimize its use to assess regional gut
1372 permeability. *Clin Chim Acta* 2015;**442**:24–32. Doi: 10.1016/j.cca.2014.12.040.
- 1373 191. Grover M., Camilleri M., Hines J., Burton D., Ryks M., Wadhwa A., et al. 13C
1374 mannitol as a novel biomarker for measurement of intestinal permeability.
1375 *Neurogastroenterol Motil* 2016;**28**(7):1114–9. Doi: 10.1111/nmo.12802.
- 1376 192. Heitkemper MM., Cain KC., Shulman RJ., Burr RL., Ko C., Hollister EB., et al. Stool
1377 and urine trefoil factor 3 levels: Associations with symptoms, intestinal permeability,
1378 and microbial diversity in irritable bowel syndrome. *Benef Microbes* 2018;**9**(3):345–
1379 55. Doi: 10.3920/bm2017.0059.
- 1380 193. Wegh CAM., De Roos NM., Hovenier R., Meijerink J., Besseling-Van Der Vaart I.,
1381 Van Hemert S., et al. Intestinal Permeability Measured by Urinary Sucrose Excretion
1382 Correlates with Serum Zonulin and Faecal Calprotectin Concentrations in UC Patients
1383 in Remission. *J Nutr Metab* 2019;**2019**. Doi: 10.1155/2019/2472754.
- 1384 194. Tulkens J., Vergauwen G., Van Deun J., Geeurickx E., Dhondt B., Lippens L., et al.
1385 Increased levels of systemic LPS-positive bacterial extracellular vesicles in patients
1386 with intestinal barrier dysfunction. *Gut* 2020:191–3. Doi: 10.1136/gutjnl-2018-317726.
- 1387 195. Tatucu-Babet OA., Forsyth A., Owen E., Navarro-Perez D., Radcliffe J., Benheim D.,
1388 et al. Serum zonulin measured by enzyme-linked immunosorbent assay may not be a
1389 reliable marker of small intestinal permeability in healthy adults. *Nutr Res*
1390 2020;**78**:82–92. Doi: 10.1016/j.nutres.2020.05.003.
- 1391 196. Power N., Turpin W., Espin-Garcia O., Smith MI., Croitoru K., Abreu M., et al. Serum
1392 Zonulin Measured by Commercial Kit Fails to Correlate With Physiologic Measures of
1393 Altered Gut Permeability in First Degree Relatives of Crohn’s Disease Patients. *Front*
1394 *Physiol* 2021;**12**. Doi: 10.3389/fphys.2021.645303.

- 1395 197. Piras C., Mussap M., Noto A., De Giacomo A., Cristofori F., Spada M., et al.
1396 Alteration of the Intestinal Permeability Are Reflected by Changes in the Urine
1397 Metabolome of Young Autistic Children: Preliminary Results. *Metabolites* 2022;**12**(2).
1398 Doi: 10.3390/metabo12020104.
- 1399 198. Holtz LR., Hoffmann J., Linneman L., He M., Smyrk TC., Liu TC., et al. Rhamnose Is
1400 Superior to Mannitol as a Monosaccharide in the Dual Sugar Absorption Test: A
1401 Prospective Randomized Study in Children With Treatment-Naïve Celiac Disease.
1402 *Front Pediatr* 2022;**10**. Doi: 10.3389/fped.2022.874116.
- 1403 199. Rodríguez-Ramírez R., Fernández Peralbo MA., Mendía I., Long JCD., Sousa C.,
1404 Cebolla Á. Urinary excretion of gluten immunoreactive peptides as an indicator of
1405 gastrointestinal function after fasting and dietary provocation in healthy volunteers.
1406 *Front Immunol* 2024;**15**. Doi: 10.3389/fimmu.2024.1433304.
- 1407 200. Barbeiro HV., Barbeiro DF., de Souza HP., Soriano FG., Machado MC., Hajjar LA.
1408 LBP and iFABP mismatch in the evaluation of intestinal barrier dysfunction due to
1409 SARS-CoV-2 infection. *Clinics* 2025;**80**. Doi: 10.1016/j.clinsp.2025.100642.
- 1410 201. Frias R., Steiner JM., Williams DA., Sankari S., Westermarck E. Urinary recovery of
1411 orally administered chromium 51-labeled EDTA, lactulose, rhamnose, D-xylose, 3-O-
1412 methyl-D-glucose, and sucrose in healthy adult male Beagles. *Am J Vet Res*
1413 2012;**73**(5):654–8. Doi: 10.2460/ajvr.73.5.654.
- 1414 202. Gianolio E., Boffa C., Orecchia V., Bardini P., Catanzaro V., Poli V., et al. A
1415 relaxometric method for the assessment of intestinal permeability based on the oral
1416 administration of gadolinium-based MRI contrast agents. *NMR Biomed*
1417 2016;**29**(4):475–82. Doi: 10.1002/nbm.3471.
- 1418 203. Erkens T., Bueters R., van Heerden M., Cuyckens F., Vreeken R., Goeminne N., et al.
1419 Translational safety biomarkers of colonic barrier integrity in the rat. *J Appl Toxicol*
1420 2018;**38**(10):1282–92. Doi: 10.1002/jat.3639.
- 1421 204. Wiersema ML., Kerr BJ., Koltjes DA. Comparison of Intestinal Permeability Methods
1422 in Broilers over a 6-Week Growth Period. *Poultry* 2023;**2**(3):383–94. Doi:
1423 10.3390/poultry2030028.
- 1424 205. Tsai K., Ma C., Han X., Allaire J., Lunken GR., Crowley SM., et al. Highly Sensitive,
1425 Flow Cytometry-Based Measurement of Intestinal Permeability in Models of
1426 Experimental Colitis. *CMGH* 2023;**15**(2):425–38. Doi: 10.1016/j.jcmgh.2022.10.004.
- 1427 206. Hu SX., Benner CP., Fuller TE. Correlation of lactulose-to-mannitol ratios in plasma
1428 and urine for intestinal permeability assessment in pigs. *Am J Vet Res* 2023;**84**(8). Doi:
1429 10.2460/ajvr.23.01.0002.
- 1430 207. Lim LG., Neumann J., Hansen T., Goetz M., Hoffman A., Neurath MF., et al.
1431 Confocal endomicroscopy identifies loss of local barrier function in the duodenum of
1432 patients with Crohn's disease and ulcerative colitis. *Inflamm Bowel Dis*
1433 2014;**20**(5):892–900. Doi: 10.1097/MIB.0000000000000027.
- 1434 208. Neuhaus W., Bogner E., Wirth M., Trzeciak J., Lachmann B., Gabor F., et al. A novel
1435 tool to characterize paracellular transport: The APTS-dextran ladder. *Pharm Res*
1436 2006;**23**(7):1491–501. Doi: 10.1007/s11095-006-0256-z.
- 1437 209. Muendoerfer M., Schaefer UF., Koenig P., Walk JS., Loos P., Balbach S., et al. Online

- 1438 monitoring of transepithelial electrical resistance (TEER) in an apparatus for combined
 1439 dissolution and permeation testing. *Int J Pharm* 2010;**392**(1–2):134–40. Doi:
 1440 10.1016/j.ijpharm.2010.03.046.
- 1441 210. Felix K., Tobias S., Jan H., Nicolas S., Michael M. Measurements of transepithelial
 1442 electrical resistance (TEER) are affected by junctional length in immature epithelial
 1443 monolayers. *Histochem Cell Biol* 2021;**156**(6):609–16. Doi: 10.1007/s00418-021-
 1444 02026-4.
- 1445 211. Fernandes J., Karra N., Bowring J., Reale R., James J., Blume C., et al. Real-time
 1446 monitoring of epithelial barrier function by impedance spectroscopy in a microfluidic
 1447 platform. *Lab Chip* 2022;**22**(10):2041–54. Doi: 10.1039/d1lc01046h.
- 1448 212. Sciurti E., Blasi L., Prontera CT., Barca A., Giampetruzzi L., Verri T., et al. TEER and
 1449 Ion Selective Transwell-Integrated Sensors System for Caco-2 Cell Model.
 1450 *Micromachines* 2023;**14**(3):496. Doi: 10.3390/mi14030496.
- 1451 213. Kurosu J., Sakamaki T., Kanai K., Morishita K., Sumaru K., Tsutsumi J.
 1452 Spatiotemporal dynamics of microscopic biological barrier visualized by electric-
 1453 double-layer modulation imaging. *Biosens Bioelectron* 2024;**266**. Doi:
 1454 10.1016/j.bios.2024.116721.
- 1455 214. Schimetz J., Shah P., Keese C., Dehnert C., Detweiler M., Michael S., et al.
 1456 Automated measurement of transepithelial electrical resistance (TEER) in 96-well
 1457 transwells using ECIS TEER96: Single and multiple time point assessments. *SLAS*
 1458 *Technol* 2024;**29**(1). Doi: 10.1016/j.slas.2023.10.008.
- 1459 215. Lucchetti M., Werr G., Johansson S., Barbe L., Grandmougin L., Wilmes P., et al.
 1460 Integration of multiple flexible electrodes for real-time detection of barrier formation
 1461 with spatial resolution in a gut-on-chip system. *Microsystems Nanoeng* 2024;**10**(1).
 1462 Doi: 10.1038/s41378-023-00640-x.

1463

1464

Figure Legends

Figure 1. Conditions in which alterations of the intestinal barrier have been identified.

Created in BioRender. Vieujean, S. (2026) <https://BioRender.com/jmybxu4>.

Figure 2. Intercellular junctions and pathways of molecular passage across the intestinal epithelium.

The intestinal epithelial barrier regulates the selective passage of electrolytes, water, and nutrients, while restricting the translocation of microbial or dietary antigens. This function is primarily mediated by the apical junctional complex (AJC), located at the apical end of the lateral membrane, in close contact with the external environment. From apical to basal, the AJC is composed of tight junctions (TJs) and adherens junctions (zonula adherens). TJs consist of transmembrane proteins such as occludins, claudins, and junctional adhesion molecules, which are anchored to cytoplasmic scaffolding and adaptor proteins (ZO-1, ZO-2, ZO-3 and cingulin) that link the complex to the actin cytoskeleton, thereby regulating permeability and maintaining barrier integrity. Adherens junctions (cadherins and nectins together with catenins and afadin) provide strong intercellular adhesion. Additional cell-cell junctions on the lateral membrane are desmosomes and gap junctions, involved in maintaining strong adhesion and direct cell-cell communication. Molecules may cross the epithelium through transcellular transport (across epithelial cell membranes) or paracellular transport (between cells), the latter mainly regulated by TJs via the “pore pathway” or the “leak pathway.” In the case of epithelial injury, substances can also cross the barrier through an unrestricted route. Barrier defects are typically associated with altered expression of key junctional proteins, including reduced levels of claudins (1, 3, 14), occludin, ZO-1, ZO-2, N-cadherin, β -catenin, and zonulin. Created in BioRender. Vieujean, S. (2026) <https://BioRender.com/821o1j7>.

Figure 3. Results of the literature search.

Figure 4. Probes and constitutive intestinal barrier-derived markers.

Different probes and constitutive intestinal barrier-derived markers can be used to study the intestinal epithelial barrier, depending on the site where they can be detected and the specific region of the barrier defect, they reflect. Created in BioRender. Vieujean, S. (2026) <https://BioRender.com/5qese8f>.

DAO, Diamine oxidase; EDTA, ethylenediaminetetraacetic acid; I-FABP, Intestinal fatty acid binding protein; Ig, immunoglobulin; LCN-2, Lipocalin-2; LPS, Lipopolysaccharide; LBP, LPS-binding protein; PEG, polyethylene glycol; sCD14, soluble cluster of differentiation 14.

Figure 5. Advanced endoscopic imaging of intestinal barrier integrity using probe-based confocal laser endomicroscopy (pCLE) and endocytoscopy (ECS) in the colon and ileum.

The figure shows the main features of intestinal barrier impairment, comparing them across pCLE and ECS modalities. In the healthy colon, an intact crypt architecture with preserved vascularisation can be seen. In contrast, impaired colonic mucosa shows both epithelial disruption with crypt architectural distortion, and vascular barrier involvement expressed by tortuous vessels with fluorescein leakage on pCLE. Similarly, normal ileal mucosa displays

1509 well-organized villi architecture with intact epithelium and a regular vascular pattern.
1510 Impaired ileal mucosa demonstrates villous inflammation with epithelial breaks and vascular
1511 barrier impairment characterised by dilated, tortuous vessels with fluorescein leakage on
1512 pCLE.
1513
1514

Neuropsychiatric

Autism spectrum, bipolar, depressive disorders, schizophrenia

Neurologic

Alzheimer's disease, cognitive impairment, gliomas, multiple sclerosis, Parkinson's disease, Spina bifida

Dermatologic

Acne rosacea, alopecia areata, atopic dermatitis, dermatitis herpetiformis, eczema, prurigo nodularis, psoriasis, rosacea

Respiratory

Allergies, asthma, chronic rhinosinusitis with nasal polyps

Infectious diseases

Bacteremic sepsis, HIV, malaria

Cardiovascular

Hypertension, coronary artery disease, ischemic heart disease, heart failure

Liver

Cirrhosis, hepatocellular carcinoma, non-alcoholic fatty liver disease, primary biliary cholangitis, primary sclerosing cholangitis

Endocrine/Metabolic

Acute pancreatitis, Hashimoto thyroiditis, hyperlipemia, insulin resistance, obesity, type I, type II and gestational diabetes

Musculoskeletal

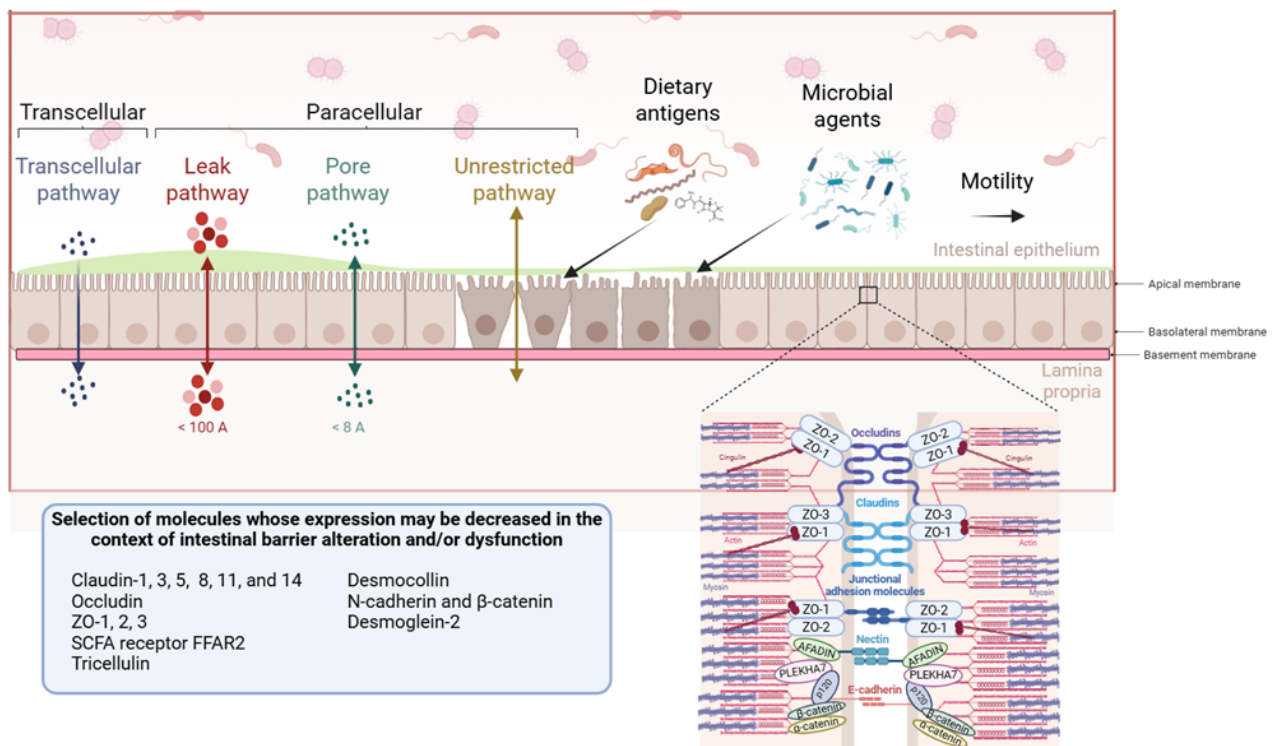
Ankylosing spondylitis, fibromyalgia, osteoarthritis, rheumatoid arthritis

Gastrointestinal

Celiac disease, colorectal cancer, eosinophilic esophagitis, inflammatory bowel disease (Crohn's disease and ulcerative colitis), intestinal ischemia, irritable bowel syndrome, necrotizing enterocolitis, non-celiac gluten sensitivity, short bowel syndrome

Hematological

Graft versus host disease, leukemia (acute myeloid leukemia), Sickle cell disease

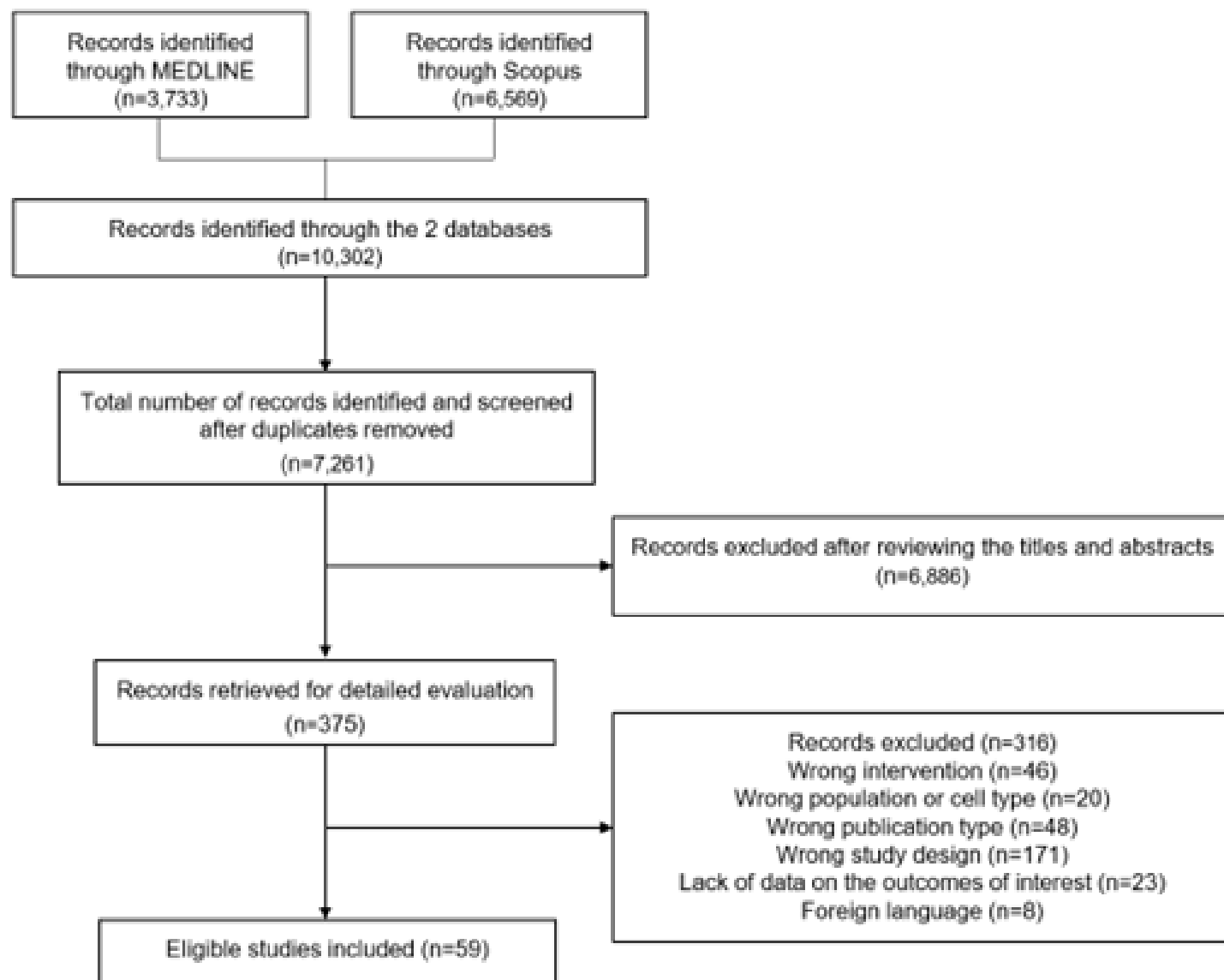


Identification

Screening

Eligibility

Included



Constitutive intestinal barrier-derived markers

Blood biomarkers

Small intestine
Citrulline, DAO, I-FABP, zonulin, IgG and IgA antibodies against zonulin

Whole intestine
Claudin-3, D-lactate, Glucagon-like peptide-2, Glycoprotein VI platelet; LPS, LBP, occludin, sCD14

Urinary biomarkers

Small intestine
I-FABP, zonulin

Whole intestine
Claudin-3

Fecal biomarkers

Small intestine
Zonulin

Colon
Short-chain fatty acid

Whole intestine
 α -1-antitrypsin, calprotectin, LCN-2

Probe-based analyses



Probes orally ingested

Bloodstream

Intestinal absorption

Urinary excretion

Urinary biomarkers

Stomach-Duodenum
Sucrose, sucrose/rhamnose

Small intestine
Cellobiose, lactulose, Lactulose/Mannitol, Lactulose/L-Rhamnose

Colon
Erythritol, Erythritol/sucralose, Sucralose, Sucralose/Mannitol

Small intestine + colon
(3C-)Mannitol, rhamnose

Whole intestine
 ^{51}Cr -EDTA, ^{51}Cr -EDTA/mannitol, PEG 1000 Da, PEG 4000/400 kDa, sucrose/L-rhamnose

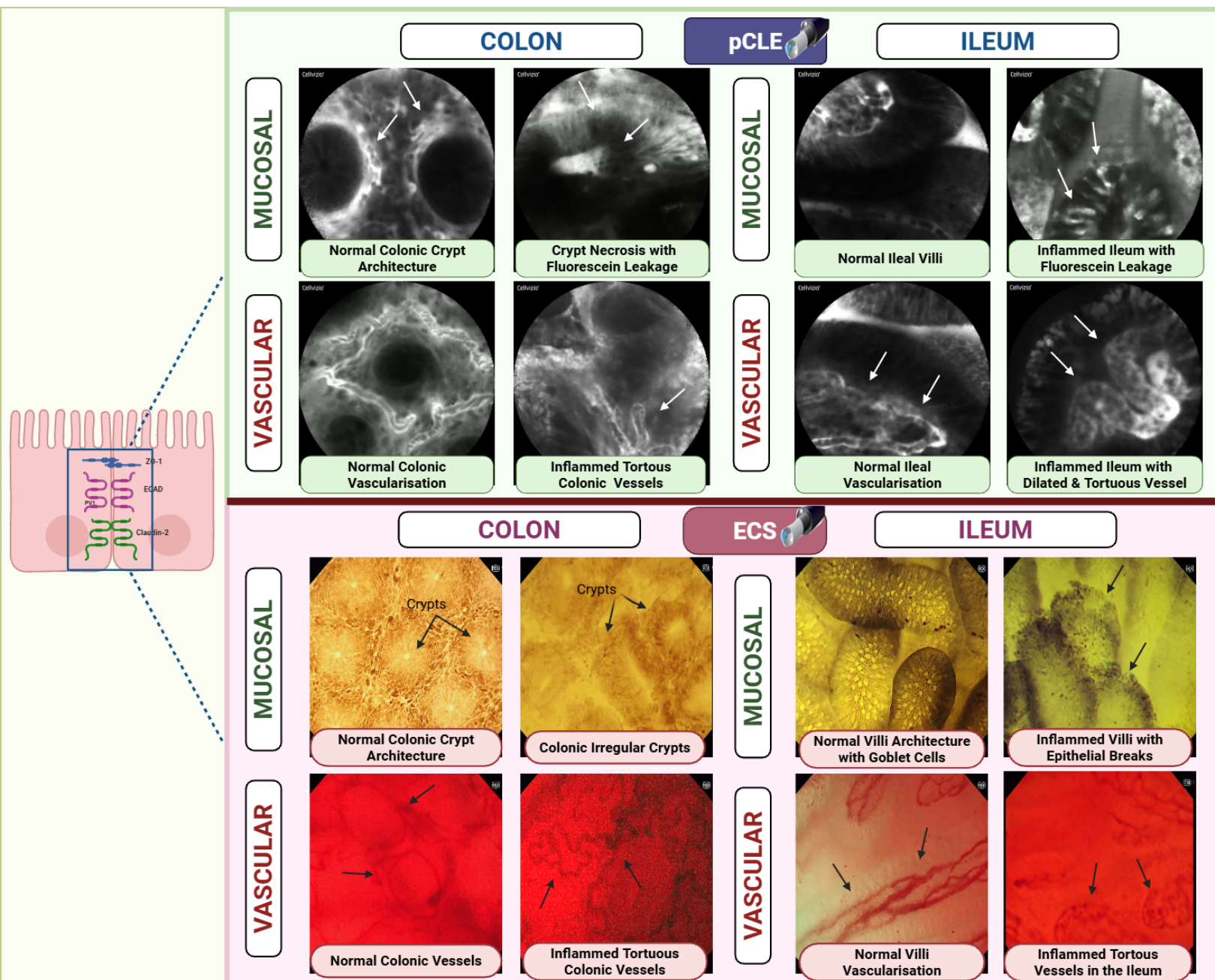


Table 1. Non-invasive in vivo tools for assessing the intestinal epithelial barrier integrity in humans.

Non-invasive <i>in vivo</i> tools for assessing the intestinal epithelial barrier in humans	Authors and year of publication	Population (number of patients and condition)	Comparative method	Correlation	Diagnostic accuracy	Reliability	Sensitivity/specificity	Reproducibility
Mannitol (urine)	Laker MF, et al. ¹⁶ (1982)	- 13 healthy volunteers (no GI disease)	- Lactulose (extracellular distribution marker). - 3-O-methyl-D-glucose (actively transported, intracellular marker)	Distribution and clearance patterns resembled lactulose, not 3-O-methyl-D-glucose	Moderate — absorption measurable, but confounded by endogenous urinary mannitol and bacterial degradation	Reliable excretion after intravenous; but oral test confounded by: - Natural urinary mannitol - Variable absorption (8–40%) - Bacterial metabolism in stool cultures	Not determined (healthy volunteers only, no disease/control contrast)	Not established; variability due to endogenous presence and bacterial breakdown
Cellobiose/mannitol ratio (urine; 5h collection) ⁵¹ Cr-EDTA (urine; 24h collection)	Marines D, et al. ³⁹ (1988)	Cellobiose/mannitol ratio: - 15 patients with celiac disease - 17 healthy volunteers ⁵¹ Cr-EDTA: - 12 celiac patients - 27 healthy volunteers	Jejunal biopsy	- Abnormal Cellobiose/mannitol ratio associated with celiac villous atrophy - Poor correlation between ⁵¹ Cr-EDTA and cellobiose/mannitol ratio (no direct correlation found)	- Cellobiose/mannitol ratio abnormal: detected 93% of celiac patients (13/14) - ⁵¹ Cr-EDTA: detected only 42% of celiac patients (5/12)	Cellobiose/mannitol ratio more reliable than ⁵¹ Cr-EDTA; ⁵¹ Cr-EDTA affected by colonic absorption and possibly chemical instability	- Sensitivity: Cellobiose/mannitol ratio 93% and ⁵¹ Cr-EDTA 42%. - Specificity for both not formally given, but normal ranges established in controls	Not directly reported; Cellobiose/mannitol ratio more consistent across studies; ⁵¹ Cr-EDTA stability questioned
Lactulose/mannitol ratio (urine; 5h collection)	Akinbami FO, et al. ¹⁷⁸ (1989)	- 39 children with GI disorders	Jejunal biopsy	Strong correlation with jejunal biopsy results (abnormal vs normal mucosa)	- Lactulose/mannitol ratio significantly higher in abnormal mucosa (0.98) vs normal mucosa (0.2, $p < 0.001$) - Clear separation: all abnormal mucosa group ratios above the defined upper limit of normal	Described as a "sensitive, non-invasive screening test"; reliability supported by consistency across abnormal mucosa group	- Sensitivity high (all abnormal mucosa cases detected using the defined threshold) - Specificity not explicitly quantified, but implied by the cut-off	Not directly reported
Lactulose/mannitol ratio (urine; 5h collection)	Juby LD, et al. ¹⁷⁹ (1989)	- 17 untreated celiac patients (new diagnosis, biopsy-proven) - 12 healthy controls - 82 symptomatic patients referred for possible celiac	- Jejunal biopsy - Cellobiose/mannitol ratio	- Lactulose/mannitol ratio discriminated celiacs vs controls (mean 0.163 vs 0.016; $p < 0.001$) - Comparable to cellobiose/mannitol test (no significant difference)	- Strong separation between celiacs vs controls ($p < 0.001$) - Lactulose/mannitol ratio > 0.028 = abnormal	Good reproducibility shown; ratio compensates for incomplete urine collections and extraneous factors (e.g. motility, bladder emptying).	- Sensitivity: 89–94%. - Specificity: 54–100% (depending on population and cut-off) - Negative predictive value: 94–95%	Improved over cellobiose/mannitol test due to simpler enzymatic assay and better patient tolerance
Cellobiose/mannitol ratio (urine; 5h collection)	Juby LD, et al. ¹⁸⁰ (1989)	1010 unselected patients presenting to gastroenterology clinic with symptoms or labs compatible with celiac disease	Jejunal biopsy	Abnormal cellobiose/mannitol ratio correlated with biopsy-proven celiac disease and other small bowel abnormalities (Crohn's disease, giardiasis, NSAID enteropathy, etc.)	In biopsied subgroup: Sensitivity 96%, Specificity 70%, PPV 36%, NPV 99%. - Lactulose/mannitol ratio: Sensitivity 91%, Specificity 88%, PPV 43%, NPV 99%.	Considered highly sensitive, practical for outpatient use, simple specimen collection; occasional false positives linked to other intestinal pathology	Sensitivity: 91–96%; Specificity: 70–88%; NPV consistently ~99%	Reproducible across >1000 patients; normal cut-off (0.004–0.023) cellobiose/mannitol ratio) confirmed in 50 biopsy-normal controls.
Multiple probes (⁵¹ Cr-EDTA, lactulose, cellobiose, rhamnose, mannitol) (urine; 0–6h collection; 6–24h collection)	Howden CW, et al. ¹⁸¹ (1991)	- 28 patients with Crohn's disease (small intestine involvement) - 14 patients with ulcerative colitis - 17 healthy controls	None (comparison across groups + correlations with inflammatory markers)	- ⁵¹ Cr-EDTA recovery correlated with lactulose in all groups - Rhamnose recovery correlated with mannitol	- ⁵¹ Cr-EDTA (0–6 h) discriminated Crohn's from ulcerative colitis and controls ($p = 0.013$ and $p = 0.032$) - Other probes did not differentiate groups	Correlations across probes suggest internal consistency	Not directly reported, but ⁵¹ Cr-EDTA recovery at 0–6 h had discriminatory value	Not reported
Lactulose/mannitol ratio (urine; 6h collection) ⁵¹ Cr-EDTA/14C-mannitol (urine; 6h collection)	Blomquist L, et al. ¹⁸² (1992)	- 28 healthy volunteers - 12 underwent the tests on 3 occasions (for intra-individual reproducibility)	Lactulose/mannitol ratio (chemical) vs. ⁵¹ Cr-EDTA/14C-mannitol ratio (isotopic)	- Lactulose excretion correlated with ⁵¹ Cr-EDTA ($p < 0.01$). - Mannitol correlated strongly with 14C-mannitol ($p < 0.001$). - Ratios (lactulose/mannitol ratio vs ⁵¹ Cr-EDTA/14C-mannitol) did not significantly correlate ($p = 0.11$).	- Study in healthy subjects <i>only</i> , so no diagnostic performance against disease. - But authors note isotopic markers less variable and more robust	- Better for isotope-labeled probes than chemically determined sugars - Paracellular markers (lactulose, ⁵¹ Cr-EDTA) more variable than transcellular (mannitol, 14C-mannitol)	Not assessed (no patient vs. control comparison)	- Inter- and intra-individual variability: higher for chemically measured sugars, lower for isotopic probes. - Ratios showed greater variability than single markers.
Lactulose/mannitol (urine)	Willems D, et al. ¹⁸³ (1993)	20 children - 5 with celiac disease - 1 with Crohn's disease - 4 with GI pathology - 4 allergic - 6 normal biopsy	Intestinal biopsy	Abnormal biopsy → 1 mannitol; Allergic symptoms → 1 lactulose; normalization after gluten-free diet	Clear separation between normal vs abnormal biopsy groups; complete discrimination	- Mean recovery of spiked standards: 99–100%. - Intra-assay CVs: 2.9% (mannitol), 3% (lactulose). - Inter-assay CVs: 4.9% (mannitol), 6.9% (lactulose).	- Sensitivity: high (all abnormal biopsies detected by 1 mannitol recovery). - Specificity: implied high, as no overlap between normal and abnormal biopsy groups.	High reproducibility (99–100% recovery in spiked samples)
Polyethylene glycol (PEG) 400, L-rhamnose, lactulose, and ⁵¹ Cr-EDTA (urine; 0–2.5, 2.5–5, 5–10, 10–24 h collection)	Bjarnason I, et al. ¹⁸⁴ (1994)	- 10 healthy controls - 25 with celiac disease • 9 untreated (symptomatic), • 16 treated (on gluten-free diet for 8 months–7 years)	- Direct head-to-head comparison of probe recoveries and ratios. - Correlation with jejunal biopsy	- High correlation between lactulose and ⁵¹ Cr-EDTA ($r = 0.94$). - No significant correlation of markers with jejunal histology	- Lactulose/rhamnose ratio and ⁵¹ Cr-EDTA distinguished untreated celiac patients from controls - PEG 400 and rhamnose excretion decreased in celiac (less discriminative). - Sensitivity increased with longer urine collection and hyperosmolar challenge	- Intravenous recoveries confirmed high renal excretion efficiency for rhamnose, lactulose, and ⁵¹ Cr-EDTA, but lower for PEG (~40%). - Variation in PEG polymer recovery reduces reliability	- Lactulose/rhamnose ratio and ⁵¹ Cr-EDTA had comparable sensitivity; both superior to PEG and rhamnose alone. - No specific % values reported, but untreated celiac consistently distinguished from controls	- Intravenous vs oral studies showed consistency in renal excretion - However, osmolality of test solution strongly influenced reproducibility of results
Polysucrose 15,000 (urine; 0–4 h, 4–8 h, and 8–12 h collection)	Oman H, et al. ¹⁸⁵ (1995)	- 20 healthy adults	- ⁵¹ Cr-EDTA (paracellular marker) - ¹⁴ C-mannitol (transcellular marker).	- Polysucrose 15,000 excretion highly correlated with ⁵¹ Cr-EDTA - No correlation with ¹⁴ C-mannitol	- Both polysucrose 15,000 and ⁵¹ Cr-EDTA detected increased permeability after NSAID treatment; ¹⁴ C-mannitol did not. - Polysucrose 15,000 behaved like a paracellular marker (similar to ⁵¹ Cr-EDTA)	- Variability reduced by standard meal. - Highest test variability for polysucrose 15,000, intermediate for ⁵¹ Cr-EDTA, lowest for ¹⁴ C-mannitol	- Sensitive to NSAID-induced permeability changes (similar to EDTA) - Not influenced by urinary volume (better specificity than mannitol)	- Non-symmetrical distribution of excretion values (non-Gaussian) - Inter-individual variability improved with standardized meal
Cellobiose/mannitol ratio (urine)	Troncone R, et al. ¹⁸⁷ (1995)	Children undergoing biopsy for suspected intestinal disease (e.g., celiac disease, malabsorption, chronic diarrhea)	Jejunal biopsies	Increased cellobiose/mannitol ratio correlates with abnormal jejunal morphology (villous atrophy, crypt hyperplasia)	Demonstrates ability to distinguish between children with abnormal vs normal jejunal histology	Non-invasive, reproducible under controlled conditions, though affected by urine collection accuracy	Not reported	Generally reproducible, but requires standardized sugar dosing and urine collection.
Lactulose/mannitol ratio (serum)	Cox MB, et al. ¹⁸⁶ (1999)	- 24 newly diagnosed untreated celiac patients - 10 treated celiac patients (gluten-free diet) - 10 healthy controls	Standard urinary lactulose/mannitol excretion ratio over 6 h	Significant correlation between Lactulose/mannitol ratio (serum) and Lactulose/mannitol ratio (urine) (Spearman $R = 0.81$, $p < 0.0001$)	- Untreated celiac: median lactulose/mannitol ratio (serum) 0.42 vs. 0.039 in normals ($p < 0.001$) - Treated celiac: median lactulose/mannitol ratio (serum) 0.08 (improved, closer to controls) → Clear differentiation between untreated celiac, treated celiac, and controls	Consistent separation between groups; single 1-h blood sample adequate	Not explicitly quantified, but lactulose/mannitol ratio (serum) effectively distinguished untreated celiac from controls (high sensitivity for untreated celiac enteropathy)	Serum high-performance liquid chromatography method validated; comparable results to standard urinary recovery

Lactulose/rhamnose ratio (urine; 2h collection)	van Nieuwenhoven MA, et al. ¹⁶⁶ (1999)	10 healthy adult volunteers (two groups of 10) Group 1: controls Group 2: permeability increased pharmacologically with chondrocytic acid	Within-subject comparison of different lactulose/rhamnose ratio doses; comparison between normal vs. induced increased permeability	Higher lactulose/rhamnose ratio observed with increased permeability after chondrocytic acid vs. controls. Dose-dependent differences observed only under increased permeability	- Lactulose: 0.6 g, rhamnose: coefficient to permeability normal vs. increased (p < 0.001). - Lower dose (1 g + 0.1 g) less sensitive	Increased lactulose/rhamnose ratio consistently decreased for chondrocytic, dose-response effect reproducible	Not compared for permeability, but sensitivity demonstrated (determination between normal and increased permeability possible at 5 g/0.5 g and 10 g/1 g)	Good reproducibility within groups; consistent lactulose/rhamnose for control vs. chondrocytic-induced increase and permeability
Raffinose:mannitol, lactulose/rhamnose, sucrose/rhamnose ratio (urine)	Hessels J, et al. ¹⁶⁷ (2003)	- 39 healthy volunteers - 70 patients with nonspecific GI symptoms - 70 patients investigated for hypolactasia (via lactose tolerance test) - 40 patients with localized small intestinal damage (21 Crohn's, 19 celiac disease with duodenal damage)	- Duodenal biopsy - Histological diagnosis of Crohn's and celiac disease	- Raffinose:mannitol ratio correlated with gradation of duodenal damage - Sucrose/rhamnose suggested as marker of small intestine deficiency	- Raffinose:mannitol: sensitivity 93%, specificity 91% for intestinal damage - Lactulose/rhamnose: sensitivity 81%, specificity 89% - Sucrose/rhamnose: 92% specificity in celiac patients (though limited data)	Ratio allowed discrimination between healthy controls, celiac disease, Crohn's, and functional GI patients; suggested robust across groups	- Raffinose:mannitol: sensitivity 93%, specificity 91% for intestinal damage - Lactulose/rhamnose: sensitivity 81%, specificity 89% - Sucrose/rhamnose: 92% specificity in celiac patients (though limited data)	Reference ranges established in 39 healthy volunteers (suggests repeatability, but no explicit reproducibility testing described)
Triple sugar test (lactulose, rhamnose, sucrose) (urine; 0-5 h and 5-24 h collection)	Anderson AD, et al. ¹⁶⁸ (2004)	- 21 healthy volunteers - 18 ileostomies	- ¹⁴ C-EDTA test (established colonic permeability probe; resistant to fermentation) - ¹⁴ C-EDTA/ ¹⁴ C-mannitol test	Positive (but not statistically significant) correlation between sucrose and ¹⁴ C-EDTA (R = 0.284, P = 0.082). - Lactulose unexpectedly absorbed in celiac → confounding. - Test distinguishes healthy vs. ileostomies.	- Both sucrose and ¹⁴ C-EDTA detected colonic absorption. - Lactulose unexpectedly absorbed in celiac → confounding. - Test distinguishes healthy vs. ileostomies.	- Not explicitly reported as a diagnostic accuracy measure. - Shown significant group difference (e.g., mannitol 24h excretion higher in controls than ileostomies, P=0.003).	- Not explicitly reported as a diagnostic accuracy measure. - Shown significant group difference (e.g., mannitol 24h excretion higher in controls than ileostomies, P=0.003).	- Analytical reproducibility demonstrated (duplicate assays, recovery ~103%). - Clinical reproducibility not fully assessed (single study, limited sample size)
Lactulose:mannitol test (urine; 6h collection) and 5h-24h collection	Blomquist L, et al. ¹⁶⁹ (2009)	- 65 controls subjects - 70 patients: acute leukemia post-chemotherapy, carcinoma of female genital tract, post-radiation, Crohn's disease, indeterminate colitis, ulcerative colitis post-colectomy with pouch, untreated celiac disease, chronic radiation enteritis, small intestinal diverticula with bacterial overgrowth.	- ¹⁴ C-EDTA/ ¹⁴ C-mannitol test	- Strong correlation between lactulose and ¹⁴ C-EDTA (large-pore). - Strong correlation between mannitol and ¹⁴ C-EDTA (small-pore). - Strong correlation between lactulose-mannitol and ¹⁴ C-EDTA/ ¹⁴ C-mannitol excretion ratios	- Large-pore marker excretion higher, small-pore lower in patients vs. controls. - Ratios discriminated patients from controls better than single markers.	- Not directly reported as diagnostic performance values (study mainly methodological). - Discrimination compared to single markers.	- Not directly reported as diagnostic performance values (study mainly methodological). - Ratios improved discrimination compared to single markers.	- Better for wide-spread labeled mannitol (°C than for unlabeled mannitol (food contamination issues). - Lactulose: mannitol: sensitivity 81%, specificity 89% - EDTA (skewed distributions, occasional outliers).
Oral multi-sugar permeability test (sucrose for colonic permeability, lactulose/mannitol for small intestine, saccharose for gastrointestinal)	Haus V, et al. ¹⁷⁰ (2009)	- 100 healthy controls - 29 Crohn's disease patients - 35 coronary artery bypass graft patients	Classic dual sugar test (lactulose/mannitol mix, saccharose) vs. sucrose excretion.	No correlation between sucrose and lactulose:mannitol ratio or saccharose	Cut-off for abnormal saccharose excretion = 2.11% (90th percentile of controls)	High variability in controls and patients; not stable	Not explicitly provided	Not tested; high variability suggests poor reproducibility
PEG excretion (400, 1500, 3350) (urine; 2h and 5h-24h collection)	van Wijk K, et al. ¹⁷¹ (2012)	- 9 healthy volunteers - Tested under basal (pneumo) and induced (acidified) conditions (NSAD to induce bowel dysfunction)	- Comparison between PEG assay and dual sugar (lactulose/rhamnose ratio) within the same individuals. - Biomarker validation with plasma FxABP and calprotectin	- PEG 3350 excretion positively correlated with plasma FxABP (intestine damage) - Lactulose/rhamnose ratio increased in parallel with lactulose and rhamnose. - All showed good ability to detect intestine inflammation	- ROC curve analysis • PEG 400 excretion (0-2h): AUC 0.84, sensitivity 94%, specificity 81% • PEG 3350/400 ratio (0-2h): AUC 0.85, sensitivity 94%, specificity 81% • Lactulose/rhamnose ratio (0-2h): AUC 0.87, sensitivity 94%, specificity 81% All showed good ability to detect intestine inflammation	- Consistency between PEG and sugar assays. - Sensitivity of early urinary collection (0-2h) as best diagnostic window - Lactulose/rhamnose ratio (0-2h): Sp 78%, Sp 100%.	- Single-center, crossover design assessed within-subject reproducibility - Analytical methods (HPLC, LC-MS), validated with good CVs (1-4%).	- High reproducibility of 6h sample, within-day and between-day CV ≤ 2.9%.
Sucrose: lactulose ratio (urine)	Shahid M, et al. ¹⁷² (2015)	- Male and 40 healthy adults - Validation cohort: 9 Parkinson's disease patients vs. 10 healthy controls (previously published, reanalyzed here)	- Traditional 1h lactulose:mannitol ratio - Proposed sucrose:lactulose ratio.	- Mannitol kinetics significantly different from lactulose/sucrose - Sucrose and lactulose exhibited nearly identical kinetics - stable ratios - In Parkinson's disease patients: urinary mannitol excretion was significantly higher than controls - Better than sucrose alone	- Not directly assessed in disease, but ¹⁴ C-mannitol showed 2h:old lower baseline excretion and higher specificity and potential accuracy	- Effect of age, sex, body mass index, renal function, smoking on sugar excretion in healthy subjects. - Sucrose:lactulose ratio had less overlap between Parkinson's disease and controls → higher discriminatory capacity	- Not formally quantified; higher sensitivity for detecting early small-bowel permeability expected due to low baseline noise.	- Analytical reproducibility demonstrated (LoD < 0.1 pg/mL, CV ~14-17% interday validation not yet done).
¹⁴ C-mannitol (urine; 2-2h, 2-4h and 8-24h urine collection)	Grover M, et al. ¹⁷³ (2016)	10 healthy adult volunteers (no GI disease)	Standard ¹⁴ C-mannitol and lactulose:mannitol ratio	- ¹⁴ C-mannitol excretion followed expected absorption/excretion kinetics; ¹⁴ C-mannitol excretion was significantly higher in Crohn's disease patients (likely due to dietary intake or microbial production)	- Not formally quantified; higher sensitivity for detecting early small-bowel permeability expected due to low baseline noise.	- Not formally quantified; higher sensitivity for detecting early small-bowel permeability expected due to low baseline noise.	- Analytical reproducibility demonstrated (LoD < 0.1 pg/mL, CV ~14-17% interday validation not yet done).	- High reproducibility of 6h sample, within-day and between-day CV ≤ 2.9%.
IgG/IgA antibodies against zonulin, occludin, zonulin, aquaporin-4, (serum)	Voliani A, et al. ¹⁷⁴ (2017)	- 18 healthy volunteers - 18 patients with celiac disease - 30 healthy controls	Zonulin (serum)	- Zonulin IgA vs serum zonulin: r = 0.53 (p = 0.02, p < 0.01) - Occludin IgG: r = 0.69 (<0.001) - Other correlations positive, most significant (except AQP4 IgA)	- Zonulin elevated in 33% of celiac disease patients - Antibodies elevated in up to 86% of celiac disease patients	- Sensitivity: Zonulin 33%, antibodies 63-70% - Specificity based on control ZSD above control mean; not explicitly reported, but implied high healthy controls largely negative	- Zonulin: poor (duplicate variability >10% if <2 h urine; large inter-day variation) - Antibodies: reproducible (variation <10%)	- Moderate — consistent correlations with diarrhea symptoms and permeability; but influenced by hormone contraceptives.
Trehalose factor 2 (stool and urine)	Hickmott MM, et al. ¹⁷⁵ (2018)	82 adult women with Rome III-defined irritable bowel syndrome	Lactulose:mannitol ratio	- Urinary increase correlated with serum zonulin (r = 0.62, p < 0.05) - Urinary increase correlated with fecal zonulin (r = 0.65, p = 0.014) - No correlation between serum and fecal zonulin - No correlation with other sugars (lactulose, sucrose, mannitol, fructose)	- Not reported as ROC sensitivity/specificity. The study reports associations rather than diagnostic thresholds	- ELISA with acceptable intra-assay variability (10-12%). Results reproducible across multiple irritable bowel syndrome subtypes.	- Not determined.	- Authors note urine collection completeness may affect reproducibility; sucrose particularly sensitive to collection accuracy.
Sugar absorption test (urinary sucrose, lactulose/rhamnose, sucrose:xythitol ratio)	Wright CAM, et al. ¹⁷⁶ (2019)	25 ulcerative colitis patients in remission (13 probiotics, 12 placebo); 23 completed 12 weeks	Compared with fecal serum zonulin and fecal calprotectin	- Urinary sucrose correlated with serum zonulin (r = 0.62, p < 0.01) - Urinary sucrose correlated with fecal zonulin (r = 0.65, p = 0.014) - No correlation between serum and fecal zonulin - No correlation with other sugars (lactulose, sucrose, mannitol, fructose)	- No diagnostic thresholds established (patients in remission; permeability not elevated)	- Stable over 12 weeks; values comparable to healthy controls; little variability over time	- Not reported	- Authors note urine collection completeness may affect reproducibility; sucrose particularly sensitive to collection accuracy.
Bacterial culture of ileal vesicle-associated lipopolysaccharide in plasma	Talavera J, et al. ¹⁷⁷ (2020)	- 49 human subjects - 25 controls (12 healthy + 13 cancer patients) - 24 patients with intestinal barrier dysfunction (13 IBD, 5 cancer with radiation/chemotherapy-induced mucositis, 6 cancer with active human immunodeficiency virus)	Soluble lipopolysaccharide activity in plasma from (some cases) - Plasma zonulin as a marker of barrier integrity	- Soluble lipopolysaccharide activity in plasma from (some cases) - Plasma zonulin as a marker of barrier integrity	- Not formally reported (no ROC, AUC, sensitivity/specificity). - Extra-cellular vesicles-associated lipopolysaccharide distinguished barrier dysfunction patients vs controls better than soluble lipopolysaccharide.	- Consistency between assays (LAL assay, TL84 reporter assay, and EM confirmation). - Small n; no replication cohort.	- Not assessed.	- Reproducibility across labs; patients not tested.
Zonulin (serum)	Tanaka-Babst OA, et al. ¹⁷⁸ (2021)	34 healthy adults	Lactulose/rhamnose ratio Lipopolysaccharide	- No correlation between serum zonulin and lactulose/rhamnose ratio (r = -0.016, p = 0.929) - Serum zonulin ratio correlated positively with lipopolysaccharide (r = 0.536, p = 0.018)	- Serum zonulin failed to reflect small intestinal permeability compared to lactulose/rhamnose ratio - Suggests zonulin may reflect inflammation/deposit rather than permeability	- Zonulin ELISA did not have intra-assay CV 3-4.6%, inter-assay CV 13.1-13.6%, consistent trends	- Not defined in diagnostic, but zonulin showed low sensitivity/specificity for intestinal permeability detection.	- Lactulose/rhamnose test reproducible, but zonulin showed high intra-individual variation in zonulin assays noted in literature.

Zonulin/ZO-1 (serum) I-FABP (serum)	Power N, et al. ¹⁸⁶ (2021)	39 healthy first-degree relatives of Crohn's disease patients	Lactulose/mannitol ratio (urine)	- No significant correlation between ZO-1 IDK® and lactulose/hamnose ratio ($r^2 = 0.004$, $p = 0.71$). - No correlation between I-FABP and lactulose/hamnose ratio ($r^2 = 0$, $p = 0.99$)	Poor; biomarkers failed to distinguish abnormal vs. normal permeability as defined by lactulose/hamnose ratio.	ELISA: run in duplicate; however, commercial ZO-1 IDK® kit criticized for lack of specificity (may detect unrelated proteins)	Not established (no significant discrimination between abnormal vs. Normal lactulose/hamnose ratio).	Limited; conflicting results reported across studies using different ZO-1 assays
Albumin (stool) Calprotectin (stool) Zonulin (stool, plasma) I-FABP (plasma) Lipopolysaccharide-binding protein (plasma)	Seethaler B, et al. ¹⁸⁷ (2021)	- Healthy cohort: 51 adults (31 normal weight, 20 overweight). - Obesity cohort: 27 adults with obesity.	Lactulose/mannitol ratio (urine)	- Plasma lipopolysaccharide-binding protein: consistently correlated with lactulose/mannitol ratio across cohorts. - Fecal zonulin: correlated with lactulose/mannitol ratio in overweight and obese groups (but not in normal weight). - No consistent correlation for fecal albumin, fecal calprotectin, plasma I-FABP, plasma zonulin.	- Plasma LBP: independent of age, BMI, sex → suggested as a robust biomarker. - Fecal zonulin: predictive only in overweight/obese; weight-dependent. - Others: low or inconsistent accuracy	- Plasma LBP described as having low intra-individual variability (from external supporting studies). - Zonulin measurement questioned due to ELISA specificity issues.	Not explicitly quantified (no AUC, Sensibility, Specificity reported). Evaluation based on correlation strength and regression models.	- Associations consistent across two independent cohorts (Healthy and Obesity). - Plasma LBP robust; fecal zonulin partially reproducible but BMI-dependent.
Metabolomics (urine)	Piras C, et al. ¹⁸⁸ (2022)	- 13 autistic children - 14 unaffected siblings	Lactulose/mannitol test	Significant correlation between L/M ratio and urine metabolic profile ($R^2 = 0.791$)	ROC AUC = 0.737 (9 discriminant metabolites)	Not reported	Not reported explicitly	Reproducible statistical models (validated by permutation test)
Dual Sugar Absorption Test (urine; 1h collection) Lactulose/hamnose (urine) Lactulose/mannitol (urine)	Holtz LR, et al. ¹⁸⁹ (2022)	- 64 children undergoing endoscopy - 54 with elevated celiac serology - 10 controls	- Duodenal biopsy	- Lactulose/hamnose correlated significantly with duodenal biopsies ($p=0.01$) - Lactulose/hamnose weaker correlation (non significant with duodenal biopsies)	- ROC AUC: lactulose/hamnose = 0.89 vs lactulose/mannitol (urine) = 0.78 - Lactulose/hamnose distinguished Marsh ≥3a from controls better than lactulose/mannitol	Single-timepoint urine collection (1h post-ingestion); reproducibility across patients not longitudinally assessed	- At cutoff 0.148 lactulose/hamnose ratio: Sensitivity 89%, Specificity 75% - At cutoff 0.262 lactulose/hamnose ratio: Sensitivity 78%, Specificity 100% - Lactulose/mannitol ratio values generally lower diagnostic performance	- Single-center study; urine assays standardized by HPLC-MS/MS - No inter-lab reproducibility tested
Citrulline, DAO, GLP-2, I-FABP, LBP, LPS, zonulin (blood)	Hoisington AJ, et al. ¹⁹⁰ (2023)	17 Veterans; mixed health background (2 with IBD noted, some with clinically meaningful post-traumatic stress disorder symptoms, others without GI disorders)	Only internal biomarker-to-biomarker correlations (Spearman).	No significant correlations among biomarkers.	Not reported	Reported intra-assay CVs: GLP-2 highest (mean 24.8%), DAO (13.1%), citrulline (6.2%), LBP (5.4%), I-FABP (3.3%), zonulin (2.0%).	Not assessed	Limited; high intra-assay variability for some assays (esp. GLP-2)
Gluten Immunogenic Peptides (u-GIP) (urine; 15 collection)	Rodríguez-Ramírez R, et al. ¹⁹¹ (2024)	15 healthy adult volunteers, no GI disease, no celiac, no food allergies.	Lactulose/mannitol ratio	- Moderate correlation between u-GIP and L/M ratio in 2–15 h interval ($R^2 = 0.52$; weaker when outliers excluded). - Similar kinetics between u-GIP and lactulose.	- Exploratory; study not in patients, so diagnostic accuracy for disease not assessed. - Demonstrated feasibility of u-GIP as a permeability marker.	- Duplicate runs, aliquot testing; consistent within individuals. - Reproducible timing patterns (peak ~4 h for GIP, ~5 h for lactulose).	- Not directly calculated (healthy volunteers only). - Detection limit for u-GIP assay: 2.5 ng/mL urine.	- Double-peak excretion observed in ~40% of volunteers (similar to lactulose/mannitol patterns). - Consistent findings across individuals, though interindividual variability noted.
MB-102 (relmapirazin), a fluorescent tracer (urine; 12h collection)	Holtz LR, et al. ¹⁹² (2024)	19 adults: 9 with active small bowel Crohn's disease (confirmed by MRE), 10 healthy controls	Lactulose/Rhamnose ratio	- Strong correlation with lactulose (% recovery, $R^2=0.83$, Spearman $r=0.75$, $p=0.0002$) - Moderate correlation with L/R ratio ($R^2=0.57$, $r=0.46$, $p=0.045$)	No formal diagnostic thresholds, but CD patients showed higher recovery of MB-102 and lactulose than controls (not statistically significant due to small sample)	Well tolerated, no serious adverse events, urine recovery tracked consistently	Not formally assessed in this pilot trial.	Urine recovery reproducible across doses (1.5 vs 3.0 mg/kg) in controls; plateau patterns in colectomy/ileostomy case suggest small vs colonic permeability discrimination
¹³ C-sucrose breath test	Shivakumar N, et al. ¹⁹³ (2024)	- 158 children (121 children had paired ¹³ C-sucrose breath test and lactulose/hamnose test results)	Lactulose/hamnose ratio Citrulline, kynurenine/tryptophan ratio (plasma)	- No significant correlation between ¹³ C-sucrose breath test (cPDR90, T50, p) and lactulose/hamnose ratio - No correlation with plasma citrulline or kynurenine/tryptophan	- No evidence of diagnostic validity relative to lactulose/hamnose	- Test-retest reliability low - High coefficients of variation (37–45%).	Not calculated (no ROC/AUC analysis).	- Between sites: large variability (Bangladesh vs. India vs Peru vs Kenya). - Within sites: inconsistent breath enrichment in Kenya → data excluded.
I-FABP (plasma) LBS (plasma)	Barbeiro HV, et al. ¹⁹⁴ (2025)	87 patients with severe SARS-CoV-2	Internal comparison of I-FABP vs LBP	No strong correlation between I-FABP and LBP	Both markers discriminated subgroups (LBP ↑ in bacterial co-infection; I-FABP ↑ in elderly); cytokines associated with severity	Not formally assessed	Not formally calculated	Not reported
Candidate metabolites: - Guanidineacetic acid, theophylline (plasma) - Uracil, guanidinosuccinic acid, N-acetyl-L-cysteine (urine) - 3-methyladipic acid, adenine, acetylcholine (stool)	Morifuji M, et al. ¹⁹⁵ (2025)	- Analysis cohort: 115 screened, 108 included (healthy Japanese adults, age 20–60). - Validation cohort: 149 healthy Japanese participants	Lactulose/mannitol ratio	Some biomarkers correlated with high lactulose/mannitol ratio test values	ROC AUC values: - Plasma: 0.78 (guanidineacetic acid + theophylline). - Urine: ~0.65. - Feces: up to 0.85 (3-methyladipic acid + adenine combination)	Validation performed in a second independent cohort (n=149). Consistent detection of several overlapping metabolites	Not reported as raw percentages; ROC curves used. Example: fecal metabolite combo AUC = 0.853	Cross-validation within discovery cohort; replication in validation cohort supports reproducibility

AUC, area under the curve; CD, Crohn's disease; DAO, diamine oxidase; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; EV, extracellular vesicle; GI, gastrointestinal; GLP-2, glucagon-like peptide-2; HPLC, high-ance liquid chromatography; IBS, irritable bowel syndrome; IEL, intraepithelial lymphocytes; I-FABP, Intestinal fatty acid-binding protein; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide; NPV, negative predictive value; NSAID, nonsteroidal anti-inflammatory drugs; PEG, polyethylene glycol; PPV, positive predictive value; ROC, receiver operating characteristic curve; SBT, sucrose breath test; SD, standard deviation; u-GIP, urinary gluten immunogenic peptides.

Table 2. Non-invasive in vivo tools for assessing the intestinal epithelial barrier integrity in animal models.

Non-invasive <i>in vivo</i> tools for assessing the intestinal epithelial barrier in animal model	Authors and year of publication	Population (number of animals and condition)	Comparative method	Correlation	Diagnostic accuracy	Reliability	Sensitivity/specificity	Reproducibility
⁵¹ Cr-EDTA (urine) Lactulose (urine) Rhamnose (urine) D-xylose(urine) 3-O-methyl-D-glucose (urine) Sucrose (urine)	Frias R, et al. ²⁰¹ (2012)	Beagle dogs (n=19) - Healthy dogs	Correlations between probes (e.g., ⁵¹ Cr-EDTA vs lactulose, lactulose/rhamnose ratio vs ⁵¹ Cr-EDTA/rhamnose ratio, d-xylose vs 3-O-methyl-D-glucose).	- ⁵¹ Cr-EDTA vs lactulose: r = 0.64 (P=0.003) (single markers), r = 0.50 (ratios). - Lactulose vs rhamnose: r = 0.63. - Rhamnose vs 3-O-methyl-D-glucose: r = 0.90 (P<0.001). - Rhamnose vs d-xylose: r = 0.63 (P=0.004). - Lower correlation for ⁵¹ Cr-EDTA and lactulose than reported in humans/cats.	Not tested in disease; reference values in healthy dogs established.	Correlations confirm internal consistency across sugar probes.	Not directly assessed	Ratios and recoveries in healthy dogs consistent with published canine data; reproducibility suggested but not formally quantified
FITC-D4000 Ovalbumin PEG400 and PEG4000 Lactulose/mannitol ratio LPS (endogenous bacterial endotoxin)	Volynets V, et al. ¹⁴⁰ (2016)	Mice (n=192) - Healthy mice	Tests compared against each other within same population.	- Limited: LPS weakly correlated with L/M ratio (but invalid since many LPS values < detection). - No valid correlation between PEG400 and PEG4000.	- Not evaluated in terms of disease (all animals healthy). - Defined reference ranges for each marker.	- PEG400, FITC-D4000, and L/M yielded consistent detectable signals. - PEG4000 and OVA at low dose (100 µg) often undetectable → poor reliability unless high doses used.	- Not formally calculated (no diseased vs. control). - Sensitivity of OVA improved with 10× higher dose. - PEG4000 insensitive in mice.	- Variability reported between animals, but consistent trends by sex and strain. - Suggested requirement for larger cohorts to overcome inter-animal variability.
Gadolinium complexes (urine)	Gianolio E, et al. ²⁰² (2016)	BALB/c mice (n=40). Conditions: - DSS-induced colitis - Healthy controls	- Lactulose/mannitol ratio (small bowel) - Sucralose (colonic permeability)	Strong correlation with sugar tests (L/M ratio and sucralose) — Pearson r = 0.91, R ² = 0.83, p<0.0001	- ProHance and Magnevist (gadolinium complexes): 2.5–3× higher urinary excretion in colitis mice vs. controls (good discrimination). - MultiHance: not discriminative (due to biliary excretion).	Consistent results across relaxometry, ICP-MS, and sugar tests.	Not formally reported; indirect evidence of good sensitivity (detects colitis-related barrier damage) and high specificity (no false positives in controls).	Validated by two methods (relaxometry and ICP-MS); reproducible across two timepoints (acute vs. recovery).
Fluorescent tracers MB-402 and MB-301 (urine)	Dorshow RB, et al. ⁸⁶ (2017)	Rats (n=68) Condition: - NSAID (indomethacin)-induced small bowel injury (low, intermediate, high dose (n=36) - 32 vehicle-only controls (n=32)	Lactulose/rhamnose ratio (urine)	Both fluorophore and sugar ratios detected intestinal injury in a dose-dependent manner. Fluorophore ratios had smoother, more consistent trajectories than sugar ratios.	- Not reported as ROC/AUC. - But fluorophores clearly distinguished injured vs control animals and graded severity better than sugars.	- Fluorophore ratios produced smooth, curvilinear, reproducible trajectories. - Sugar ratios were more erratic with inter-sample variability.	- Not explicitly quantified. - Dynamic range of fluorophores (1.3x, 9.3x, 28.4x control) exceeded that of sugars (1.4x, 2.2x, 5.9x), suggesting greater sensitivity.	- Within each indomethacin dose group, fluorophore ratios were consistent across animals. - Sugar ratios varied more widely and often intersected across timepoints.
- FITC-D4000 (FD4, plasma/urine) - Triple sugar test (lactulose, mannitol, sucralose (urine) - PEG 400/1000 (urine) - Endogenous biomarkers (D-lactate, α2-macroglobulin, calprotectin, cytokines, prostaglandins, metabolomics).	Erkens T, et al. ²⁰³ (2018)	Male Sprague–Dawley rats Condition: experimental colonic barrier perturbation using sodium caprate or ibuprofen.	- Histopathology of colon and rectum (H&E stained “swiss rolls”)	- FD4 plasma increase correlated with barrier dysfunction (even when histology minimal). - Intracolonic probe delivery gave stronger signal than oral. - Sucrose/mannitol/sucralose mixture also increased after barrier disruption.	FD4 and triple sugar test (intracolonic) detected barrier dysfunction earlier and more sensitively than histopathology or endogenous biomarkers.	- Reproducible FD4 plasma peaks within 10–20 min - Consistent increases in urine sugars after intracolonic administration.	- FD4: very sensitive (up to 100-fold increase within minutes). - Triple sugar test: 10–50-fold increases in urine after intracolonic dosing. - Endogenous biomarkers (d-lactate, α2-macroglobulin, cytokines, calprotectin): not sensitive or inconsistent.	- Consistent FD4 signal across animals and timepoints, less variability than sugar probes. - Urinary sugars showed some inter-animal variation, especially with oral administration.
- Lactulose (serum) - FITC-D (serum) - Mannitol (serum) - Lactulose/mannitol ratio	Wiersma M, et al. ²⁰⁴ (2023)	Broiler chickens (n=960) (2 × 480 birds across 2 trials). - Healthy, non-disease state	- Internal comparison: serum FITC-D vs serum sugars (lactulose, mannitol, sucralose). - No gold-standard histology or permeability gold standard	- Lactulose showed consistent increase with fasting, aligning with expected permeability ↑. - FITC-D less consistent (high background autofluorescence). - Mannitol unreliable due to co-elution with glucose in serum. - Lactulose/mannitol ratio	- Lactulose assay: better specificity (no detectable lactulose in controls). - FITC-D: lower accuracy (high autofluorescence background). - Mannitol: inaccurate in serum due to glucose interference.	- Lactulose reproducible across trials and ages. - FITC-D less reliable due to variation and background noise. - Mannitol unreliable.	- Lactulose: ↑ sensitivity to fed vs fasted, clear background = higher specificity. - FITC-D: sensitive to fed vs fasted but low specificity (background signal). - Mannitol: poor sensitivity/specificity.	- Lactulose: consistent across two trials - FITC-D: variable - Mannitol: poor reproducibility

				confounded by mannitol unreliability				
Flow cytometry–based assay detecting orally gavaged ovalbumin (OVA) (plasma)	Tsai K, et al. ²⁰⁵ (2023)	Mice Conditions: - DSS-induced colitis - Genetic mucus barrier defects - Citrobacter rodentium infection	- FITC-dextran permeability assay. - In some experiments: histology (OVA localization, tissue pathology).	OVA assay showed stronger discrimination than FITC–dextran to detect permeability defect	- OVA assay detected permeability defects in Muc2 ^{-/-} mice and subtle glycosylation defects not measurable by FITC–dextran.	Low intra- and inter-experiment error (<5%); consistent results across replicates.	- Sensitivity: Superior to FITC–dextran (detects small permeability changes). - Specificity: High (negligible background compared to FITC–dextran).	Assay reproducible across multiple experiments, mouse cohorts, and disease models.
Lactulose/mannitol ratio (plasma)	Hu SX, et al. ²⁰⁶ (2024)	Weaned piglets (n=10)	- Lactulose/mannitol ratio in plasma vs urine - Pharmacokinetics: AUC0–6h, AUCextrap, Cmax - Correlation analysis (R ² values)	Strong correlation: - AUC0–6h R ² = 0.995 - AUCextrap R ² = 0.965 - Cmax R ² = 0.990 - Plasma ratios at 2–6h also correlated with urinary ratios	Plasma ratios at 2–6h accurately predicted urinary ratios → reliable surrogate for intestinal permeability	Within-animal correlation consistent; variation across pigs noted due to stress effects in metabolism cages	Not explicitly reported (study focused on correlation, not diagnostic cutoffs in disease models)	Variation across pigs noted due to stress effects in metabolism cages

AUC, Area under the curve; Cmax, maximum concentration; EDTA, ethylenediaminetetraacetic acid; DSS, dextran sodium sulfate; FITC, fluorescein isothiocyanate; H&E, hematoxylin and eosin; IBS, irritable bowel syndrome; ICP-MS, inductively coupled plasma-mass spectrometry; L/M, Lactulose:mannitol; LPS, lipopolysaccharide; NSAID, nonsteroidal anti-inflammatory drugs; OVA, ovalbumin; PEG, polyethylene glycols

Table 3. Invasive in vivo tools for assessing the intestinal epithelial barrier integrity.

Non-invasive tools evaluated <i>in vivo</i> in animal models	Authors and year of publication	Population (number of participants and condition)	Comparative method	Correlation	Diagnostic accuracy	Reliability	Sensitivity/specificity	Reproducibility
CLE with fluorescein	Kiesslich R, et al. ²⁰⁷ (2011)	Mice, small intestine (n not explicitly given, ~4 per group for flow direction/osmolarity experiments).	In vivo mouse CLE compared with fluorescent dextran assays (FITC-dextran for luminal influx, Alexa-dextran IV for vascular efflux).	CLE fluorescein leakage correlated with presence of epithelial shedding and microerosions	Not reported in formal accuracy terms (proof-of-concept).	High agreement between CLE leakage detection and tracer movement.	Not quantified in animals	Repeatable across mice; showed reproducible inward/outward/bidirectional flows depending on osmolarity.
CLE with Confocal Leak Score (CLS)	Chang J et al. ¹²² (2017)	110 subjects (22 controls, 31 UC, 57 CD; 49 IBD in mucosal healing analyzed for primary endpoint)	CLS vs Watson grade	CLS correlated with diarrhea frequency (each +1.82 CLS → +1 diarrheal stool/day, $p = 0.008$) and with Watson grade (Spearman $r = 0.40$, $p = 0.03$)	ROC AUC = 0.88 for ongoing bowel symptoms in IBD with mucosal healing	CLS described as previously validated (learning curve, inter-/intra-observer agreement shown in earlier work), not re-estimated here	CLS $\geq 13.1 \rightarrow$ sensitivity 95.2%, specificity 97.6% for ongoing bowel symptoms in IBD with mucosal healing	Not reported
CLE	Rath T, et al. ¹⁷³ (2023)	181 patients with IBD in clinical remission (100 Crohn's disease, 81 ulcerative colitis)	Histologic remission (RHI, NHI, mRiley)	Barrier healing superior to endoscopic and histologic remission for predicting major adverse outcomes in both UC & CD	UC: Accuracy 85% (barrier healing-colon) vs 70–75% (endoscopy) and 67–69% (histology) CD: Accuracy 88.7% (barrier healing-ileum) vs 54–62% (endoscopy) and 56% (histology)	CLE interpretation reproducible across blinded readers using semiquantitative Watson score; requires expertise but shown feasible with training	UC: Sensitivity 68%, Specificity 92.7%, PPV 81%, NPV 86.4% (colon barrier healing) CD (ileum): Sensitivity 69.4%, Specificity 100%, PPV 100%, NPV 84.7%	CLE shown in prior studies to have high inter/intraobserver agreement; no formal reproducibility metrics in ER1ca but barrier scoring standardized
Ultra-high magnification endocytoscopy (EC) + automated multispectral imaging (barrier protein analysis)	Iacucci M, et al. (2024)	23 PSC-colitis patients in clinical remission, surveillance colonoscopy	- Barrier protein expression (Claudin-2, Occludin, JAM-A)	Moderate correlation with endoscopic scores ($r=0.44-0.57$); Strong correlation with histological scores ($r=0.56-0.62$), esp. right colon	Barrier healing (EC <3 ileum, <5 colon) predicted fewer adverse outcomes	Not reported directly, but EC scoring feasible and combined with protein markers	Not reported numerically; predictive value of EC+Claudin-2 significant ($p=0.02-0.04$)	Not reported

CD, Crohn's disease; CLE, confocal laser endomicroscopy; EC: endocytoscopy; IBD, inflammatory bowel disease; JAM-A, junctional adhesion molecule A; mRiley, modified Riley histological score; NPV, negative predictive value ; PSC: primary sclerosing cholangitis-colitis; PPV, positive predictive Value; RHI, Robarts histopathology index; NHI, Nancy histological index; UC, ulcerative colitis.

Table 4. Tools for in vitro assessment of the intestinal epithelial barrier.

Tool tested IN VITRO	Reference	Cell line	Comparative method	Correlation	Diagnostic accuracy	Reliability	Sensitivity/specificity	Reproducibility
APTS–dextran ladder (fluorescent dextran oligomers labeled with 8-aminopyrene-1,3,6-trisulfonate)	Neuhaus W, et al. ²⁰⁸ (2006)	Caco-2	- TEER - Conventional paracellular markers (sucrose, Evans blue, FITC–dextrans)	- Strong exponential correlation between TEER and APTS–dextran permeability ($R^2 = 0.8958$) - Confocal microscopy confirmed paracellular route	- Higher precision than FITC–dextrans (fraction-by-fraction resolution) - Can detect subtle changes in barrier leakiness	- Showed consistent size-dependent permeability patterns - Reproducible correlation with TEER	- Sensitive to detect paracellular vs. transcellular transport (diazepam unaffected by TEER) - More specific than bulk FITC–dextran assays because it separates each molecular fraction.	- Confirmed across three different cell models
Online monitoring of TEER in a combined dissolution–permeation apparatus	Muendoerfer M, et al. ²⁰⁹ (2010)	Caco-2	- Offline TEER measurements (before/after experiment). - Sodium fluorescein permeability as functional comparator	TEER changes corresponded with sodium fluorescein permeability, but not always linearly (TEER more sensitive, especially in short-term changes)	High sensitivity for detecting integrity loss	Consistent TEER profiles under repeated EDTA challenges; reproducible permeability trends	Specific to paracellular permeability (TEER reflects ionic permeability; fluorescein flux reflects solute permeability).	Good reproducibility across replicate runs; consistent correlation between TEER drop and permeability increase
Organic electrochemical transistor (OECT) integrated with epithelial barrier tissue	Jimison, L, et al. ¹⁵⁵ (2012)	Caco-2	- Lucifer Yellow paracellular permeability assay - TEER	- OECT responses paralleled barrier integrity changes detected by permeability assays and TEER - More sensitive than Lucifer Yellow assay	- Very high sensitivity and temporal resolution (<30 s for H ₂ O ₂ effects, compared to 30–60 min with conventional assays) - Specific discrimination between gradual (ethanol) vs rapid (H ₂ O ₂) barrier disruption	- Reproducible drain current/normalized response signals across replicates - Compatible with complex cell culture media without interference	- Detects subtle and early paracellular barrier defects at lower concentrations than standard assays - Specific to barrier integrity (validated with controls showing no parasitic chemical effects on electrodes/polymer)	- Consistent dynamic responses across conditions; normalization allows cross-device comparisons - Device-to-device variation acknowledged, but minimized with standardized fabrication and normalization
Real-time xCELLigence impedance-based monitoring	Sun M, et al. ¹⁵¹ (2012)	Caco-2	- TEER - Phenol red permeability assay - Immunofluorescence (tight junction proteins, F-actin, E-cadherin)	Strong correlation with TEER and permeability assays. Impedance curves paralleled barrier disruption/recovery	Demonstrated sensitivity to detect barrier disruption and recovery	Repeated experiments, reproducible CI profiles across multiple wells	Sensitive to dose- and time-dependent barrier dysfunction; specific in identifying effects of inhibitors	Reproducible CI profiles in multiple replicates; consistent with TEER results
Organic Electrochemical Transistor (OECT)-based biosensor with variable channel geometries	Yeung S, et al. ¹⁵⁶ (2019)	Caco-2	- Standard TEER measurement by EVOM volt-ohmmeter	- OECT responses correlated with TEER values: - Caco-2 (tight) → high TEER, strong impedance plateau, slower response - NPC43 which is another cell type (leaky) → low TEER, weaker impedance, faster response	Not expressed in clinical terms but clear discrimination between tight vs. leaky epithelia shown	- Sensitivity of OECT depends on geometry (channel area) and cell type - Repeated measurements consistent across devices	Not reported in quantitative diagnostic terms	Demonstrated across multiple OECT sizes and two independent cell lines
Electrical impedance spectroscopy (EIS) using a 4-electrode “chopstick” setup	Linz G, et al. ¹⁴⁸ (2020)	Caco-2	- Standard TEER by epithelial volttohmmeter (EVOM/Millicell)	TEER values from EIS closely matched standard volttohmmeter measurements at all stages of barrier maturation.	EIS differentiated effects of barrier-disrupting agents (EGTA vs saponin vs forskolin), which standard TEER could not fully distinguish (e.g., capacitance changes with saponin only).	- EIS detected consistent barrier formation trends across coatings (Matrigel, collagen, uncoated). - Capacitance reliably reflected morphological changes	- High sensitivity: TEER drop detected within minutes after EGTA, saponin, or forskolin exposure. - Specificity: capacitance changes specific to saponin, indicating morphological damage vs junction-only disruption.	- Experiments performed in triplicate, consistent TEER and capacitance trajectories across replicates. - Daily monitoring over 14 days showed reproducible barrier development profiles.
Transepithelial electrical resistance (TEER) measurement	Felix K, et al. ²¹⁰ (2021)	Caco-2	- Paracellular permeability using FITC–dextran (4 kDa) flux - Immunocytochemistry for junctional proteins (E-cadherin, claudins, desmoglein-2)	TEER changes did not fully correlate with FITC–dextran permeability; early TEER fluctuations reflected junctional length rather than barrier tightness.	Not directly quantified (no AUC, sensitivity/specificity), but TEER shown to be influenced by junctional morphology rather than solely barrier function	TEER measurements reproducible within experiments; variability arises depending on cell maturation stage and junctional length.	Not formally assessed; TEER sensitive to morphological dynamics, but less specific for barrier integrity compared with permeability assays	Moderate; absolute TEER values varied widely between clones and conditions, limiting cross-study comparability.

Microfluidic barrier-on-chip platform with integrated electrical impedance spectroscopy (EIS)	Fernandes J, et al. ²¹¹ (2022)	Caco-2	- Standard Transwell® TEER measurements (EVOM voltohmmeter). - Immunofluorescence (occludin tight junction staining, actin cytoskeleton)	- TEER values correlated with barrier formation and polarization - Barrier disruption measured by EIS corresponded with occludin redistribution and actin changes. - Chip vs. Transwell showed consistent trends (lower TER under flow, as reported in prior studies)	- Not clinical; proof-of-concept accuracy demonstrated by detecting: - Immediate barrier disruption with detergent. - Time-dependent TEER decrease with dsRNA (viral mimetic). - Protective effect of corticosteroid	- 8 chips run in parallel with consistent baseline medium resistance and circuit parameters. - Robust detection of barrier formation and challenge response across experiments	- High sensitivity: detected rapid TEER collapse (Triton X-100) and gradual decline (dsRNA). - Specificity not quantified in diagnostic terms, but biological readouts supported functional interpretation.	- Parallel chips (n=8) gave comparable TEER trends. - Results reproducible across repeats and consistent with literature (TEER lower under flow vs static).
Integrated ion-selective electrochemical sensors (zinc & copper) + TEER and impedance spectroscopy	Sciurte E, et al. ²¹² (2023)	Caco-2	- TEER (standard ohmic and impedance-based) - Immunofluorescence (E-cadherin staining)	- Ion flux changes correlated with TEER decrease and loss of E-cadherin staining after EGTA.	- Not reported (exploratory feasibility study).	- Reported sensitivity to 10⁻¹¹ M ion concentration. - TEER reproducibility demonstrated across experiments	- Sensitivity: LOD ~10⁻¹² M for Zn²⁺ and Cu²⁺. - Specificity: Improved by ion-selective membranes (ionophores)	- Calibration curves highly linear (R > 0.99). - Relative standard deviation: < 10.63% (Cu²⁺), < 8% (Zn²⁺).
Electric-double-layer modulation imaging (EDLMI), a label-free live-cell imaging technique for tight junction integrity and intestinal barrier function	Kurosu J, et al. ²¹³ (2024)	Caco-2	- TEER (gold standard). - Discussed vs. paracellular permeability assays and scanning ion conductance microscopy (SICM)	- Strong correlation with TEER (deviation <12%)	- Demonstrates higher spatial/temporal resolution than TEER (can distinguish local vs global permeability changes). - Detected both paracellular (local, tricellular junctions) and transcellular (global, slower) pathways under sodium caprate exposure.	- Consistent results across multiple imaging sessions. - TEER-EDLMI correlation supports reliability.	- Not explicitly quantified, but described as higher sensitivity than TEER/SICM for detecting dynamic/local permeability changes.	- Proof-of-concept with repeated imaging on Caco-2 monolayers. - No inter-laboratory or large-sample reproducibility data yet.
Automated TEER measurement using ECIS TEER96 in 96-well transwells	Schimet J, et al. ²¹⁴ (2024)	Caco-2	- EVOM (manual TEER probe)	- Good correlation with EVOM at low/moderate TEER (R² = 0.979 for Caco-2). - Poorer correlation at high TEER due to EVOM upper quantification limits	- Demonstrated ability to detect concentration-dependent barrier disruption by DMSO and BEC. Outperformed EVOM in high TEER range (>1500 Ω cm²)	- Consistent intrawell reproducibility (Caco-2 average CV% < 1%).	- Not directly reported in clinical terms, but device detected subtle concentration-dependent TEER changes more sensitively than EVOM, especially at high TEER values.	- High — automated pin electrodes reduced operator variability; repeated readings stable (<1% CV).
Multiple flexible thin-film platinum electrodes into the HuMiX gut-on-chip system for real-time, spatially resolved TEER	Lucchetti M, et al. ²¹⁵ (2024)	Caco-2	- Standard Transwell TEER measurements with chopstick electrodes. - Immunofluorescence staining of occludin (tight junction protein) as biological confirmation.	- TEER increase correlated with: occludin immunostaining (tight junction formation).	- Not clinical — but proof-of-concept accuracy demonstrated by detecting: - Barrier formation timeline (days 2–12). - Rapid drop in TEER upon calcium removal (within 1 h). - Recovery upon calcium reintroduction (within 2 h)	- Consistent detection of barrier disruption and recovery. - Geometrical correction factor applied to normalize TEER values	- Not quantified in clinical diagnostic terms. Sensitivity demonstrated by ability to detect rapid Ca²⁺-dependent barrier disruption and recovery	- Multiple independent experiments (n=3) confirmed trends. - TEER variability across positions showed spatial reproducibility but also highlighted heterogeneity of barrier formation.
High-density microelectrode arrays (HD-MEAs) integrated with CMOS technology	Venz A, et al. ¹⁵⁹ (2025)	Caco-2	- Brightfield and fluorescence microscopy (cell membrane staining, DAPI, F-actin, ZO-1)	- Good correlation with microscopy (fluorescence/confocal). - Impedance changes matched dome formation, tight junction development, and barrier disruption seen by imaging.	- Demonstrated capacity to distinguish subtle differences in barrier disruption (e.g., stronger impedance decrease under domes vs flat monolayers with EGTA treatment).	- Consistent impedance patterns across multiple chips and clusters. - Daily monitoring up to 14 days reproducibly captured three life-cycle phases of Caco-2 barriers.	- High sensitivity: detects localized, transient events - Specificity: improved differentiation of tight junction loosening vs cell detachment compared to conventional TEER - Exact % sensitivity/specificity not quantified.	- Biological replicates (n=3 chips, ~49,000 electrodes) showed reproducible life-cycle dynamics. - Consistent EGTA disruption/recovery patterns.

APTS, 1-aminopyrene-3,6,8-trisulfonate; AUC, area under the curve; BEC, benzethonium chloride; CI, cell index; CV, coefficient of variation; DAPI, 4',6-diamidino-2-phenylindole; DMSO, dimethyl sulfoxide; dsRNA, double-stranded ribonucleic acid; EDLMI, Electric-double-layer modulation imaging; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid; EIS, Electrical impedance spectroscopy; FITC, fluorescein isothiocyanate; HD-MEAs, high-density microelectrode arrays; OECT, Organic electrochemical transistor; SICM, scanning ion conductance microscopy; TEER, transepithelial electrical resistance; ZO-1, zonula occludens-1.