

Guiding Principles: Reporting Elements for Gastrointestinal Organoid Research



Kelli L. VanDussen,^{1,2,*} Tatiana A. Karakasheva,^{3,*} Maxime M. Mahe,^{4,5} Joseph Burclaff,^{6,7} Luis F. Arrieta-Viana,⁸ Ian Williamson,⁹ Heather A. McCauley,^{7,10} Andrés J. García,⁸ Scott T. Magness,^{6,7} and Kathryn E. Hamilton^{11,12}

¹Division of Gastroenterology, Hepatology, and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; ²Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, Ohio; ³Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; ⁴Nantes Université, Inserm, TENS UMR1235, The Enteric Nervous System in Gut and Brain Diseases, IMAD, Nantes, France; ⁵Center for Stem Cell and Organoid Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; ⁶Lamé Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill/North Carolina State University, Chapel Hill, North Carolina; ⁷Center for Gastrointestinal Biology and Disease, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; ⁸Woodruff School of Mechanical Engineering, Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia; ⁹Department of Molecular and Biomedical Sciences, North Carolina State University, Raleigh, North Carolina; ¹⁰Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; ¹¹Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania; and ¹²Institute for Regenerative Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Gastrointestinal (GI) organoids are now widely used for disease modeling, drug discovery, regenerative-, and precision medicine. As publications using GI organoids have increased exponentially, methodological reporting across GI organoid studies remains inconsistent, making results difficult to interpret, compare, and reproduce. To address this, we present community-informed guidance for reporting key experimental details in GI organoid research. Our recommendations were developed by integrating established biomedical reporting frameworks, organoid-focused resources from international organizations, and structured input from the GI organoid community through an expert panel survey. These inputs informed a core set of reporting items, including stem cell source and donor context, media and supplement composition, extracellular matrix type, culture configuration, and other parameters essential for replication. We provide an "Organoid Reporting Toolkit" containing practical checklists and templates intended to help authors, reviewers, editors, and journals. Together, these resources aim to improve transparency and reproducibility, facilitate cross-study comparison, and streamline communication in the GI organoid field while preserving flexibility for methodological innovation. (*Cell Mol Gastroenterol Hepatol* 2026;20:101772; <https://doi.org/10.1016/j.jcmgh.2026.101772>)

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The use of gastrointestinal (GI) organoids has evolved from a stem cell biology model into an essential experimental system for human disease modeling, drug development and testing, regenerative medicine, and precision medicine (reviewed in¹⁻⁷). GI organoids are self-

organizing, 3-dimensional (3D) epithelial (and, in some contexts multi-lineage) structures generated from adult tissue stem cells or pluripotent stem cells. They recreate key features of intestinal tissue, including stem cell-driven renewal and multilineage differentiation. These attributes distinguish organoids from conventional cell lines, which are robust and cost-effective but often reflect transformed or adaptation-prone states and typically lack native tissue architecture and differentiation programs.

Because conventional cell culture systems capture only a subset of human biology, researchers frequently turn to animal models to test whether in vitro findings hold in vivo. However, species-specific differences in development, physiology, immune responses, and environmental exposures can limit direct translation to humans. GI organoids help bridge this gap, and the field accelerated through several key advances, including the establishment of adult mouse intestinal stem cell-derived organoid culture

*Authors share co-first authorship.

Abbreviations used in this paper: 3D, 3-dimensional; ALI, air-liquid interface; BME, basement membrane extract; BMP, bone morphogenetic protein; CNV, copy number variation; ECM, extracellular matrix; EGF, epidermal growth factor; ES, embryonic stem cell; FASEB, Federation of American Societies for Experimental Biology; GI, gastrointestinal; HIO, human intestinal organoid; iPSC, induced pluripotent stem cell; MOI, multiplicity of infection; MPS, microphysiological system; PBMC, peripheral blood mononuclear cell; PSC, pluripotent stem cell; STR, short tandem repeat; tHIO, transplanted human intestinal organoid; VIP, vasoactive intestinal polypeptide.



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systems in 2009, long-term expansion of adult human GI epithelial organoids in 2011, and the development of pluripotent stem cell (PSC)-derived human intestinal organoids (HIOs; often termed tHIOs when transplanted as xenografts) in 2010 to 2011.^{8–12}

With the rapid expansion of GI organoid research, the number of publications has grown exponentially, yet methodological reporting remains highly variable, limiting cross-study comparison, interpretation, and reproducibility. For example, a researcher may read a study reporting that a candidate therapeutic restores barrier function in intestinal organoids, but replication fails. Only after extensive troubleshooting do they learn that a key media component, used in the original work, was omitted from the published methods. This scenario reflects a recurring challenge in the field. When critical, and sometimes nuanced, variables go unreported, time and resources are wasted, findings are harder to reproduce, and the translational potential of organoid research is weakened. These recurring and avoidable failures underscore the need for a shared, practical approach to reporting that is rigorous enough to support reproducibility, yet flexible enough to accommodate diverse organoid systems and experimental goals for groups ranging from new adopters to long-established organoid programs and cores.

To address this gap, this commentary highlights the need for clear, consistent, and community-aligned reporting guidance for GI organoid studies to strengthen rigor, reproducibility, and cross-study comparability. We drew on established reporting frameworks in biomedical research (eg, ARRIVE,¹³ host-microbe guidance,¹⁴ and single-cell RNA sequencing standards¹⁵), as well as organoid-focused resources from international organizations (eg, International Society for Stem Cell Research [ISSCR; <https://www.isscr.org/guidelines>] and the European Union-funded HYBRIDA project [<https://hybrida-project.eu>]), prior commentaries, and input from the GI organoid community.^{16–18} In contrast to initiatives focused on organoid manufacturing requirements or translational readiness across organ systems, our focus is publication-ready reporting guidance for GI organoid research, paired with a practical toolkit, to ensure that key culture variables and experimental units are disclosed transparently. By making these details explicit, authors can communicate methods more clearly and reviewers, editors, and readers can more consistently interpret findings, compare studies, and reproduce results across laboratories, all while keeping the guidance practical enough for broad adoption without slowing research progress.

To ground these recommendations in real-world practice, we also solicited feedback via an expert panel survey administered from June through September 2025 (see [Supplementary Appendices 1 and 2](#) for full anonymized survey results). The survey was advertised through the American Gastroenterological Association's social media platforms and a targeted email campaign, and 70% of respondents self-identified as routine organoid users at academic medical centers or research-intensive universities. Input from government and industry researchers represents an important opportunity for future iterations.

Survey responses highlighted areas where inconsistent reporting most strongly limits reproducibility, including stem cell source (eg, adult, fetal, pediatric, embryonic stem cell [ES] or induced PSC [iPSC]), media composition, extracellular matrix (ECM) type and composition, culture platform, and passage number ([Figure 1](#)). Respondents further emphasized reporting of practical details that frequently drive variability: passaging protocols (method, frequency, ratio), donor/source information (age, sex, anatomical region, disease status), and quality control measures (eg, mycoplasma testing and contamination screening). Although many priorities were shared across participants, defining and standardizing minimum experimental units ("N") remains a point of ongoing debate. In addition to surveying researchers prior to the development of this article, the initial article draft was reviewed by a panel of experts followed by a reviewer group discussion to provide consensus feedback to authors. Together, this community-driven process ensured that resulting recommendations reflect both practical realities and shared priorities of researchers actively working in the GI organoid field. These inputs inform the guiding principles presented in this article to support best practices in reporting GI organoid research.

This effort is intended as a practical first step toward greater alignment in how GI organoid methods are communicated, enabling more consistent interpretation, comparison, and replication across studies. We recognize that meaningful standardization will take time, will evolve with the field, and will ultimately depend on community buy-in across laboratories, core facilities, reviewers, editors, and journals. Accordingly, we provide a 'scaffold' for each recommended reporting element and an "Organoid Reporting Toolkit" ([Supplementary Appendices 3–6](#)) containing checklists, templates, and example language designed to be accessible and actionable for researchers at all levels, whether new to organoid systems, coordinating multi-site studies, or leading a GI research center core. We propose that a subset of essential reporting elements be adopted broadly by the community to improve transparency and reproducibility, whereas additional elements are well-suited for implementation at the individual laboratory level to streamline internal documentation, record keeping, and troubleshooting within and between research groups. Future follow-up articles are planned to update and refine these principles as organoid technologies and experimental applications continue to change.

Reporting Elements for GI Organoids

As GI organoid use has expanded, the field has also become increasingly aware that relatively small differences in culture conditions can produce meaningful changes in organoid morphology, cellular composition, and function. Because experimental outcomes are strongly influenced by factors such as stem cell source, media formulation, ECM type/composition, passage number and culture history, growth platform configuration, and other study-specific methods, transparent reporting of these elements is essential for accurate interpretation and reproducibility

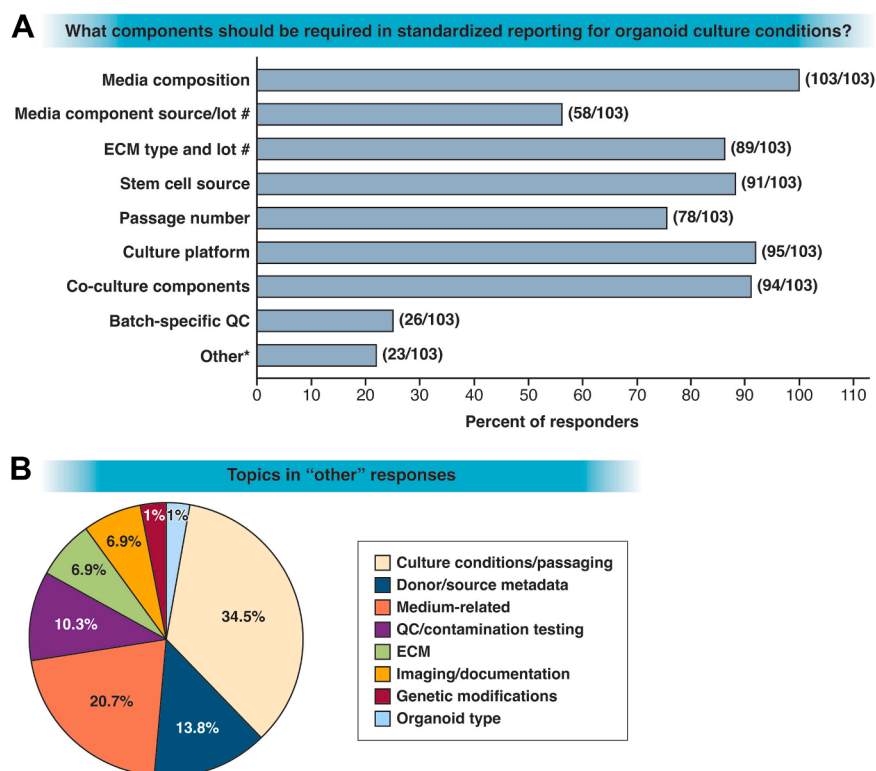


Figure 1. Survey respondent priorities for organoid reporting standards. (A) Percentage of 103 respondents indicating each of the listed components are essential for inclusion in organoid experiment reporting. (B) Summarized responses for 23 respondents who included "Other" components in the open answer field. Survey data is provided in [Supplementary Appendix 1](#). QC, quality control.

across laboratories. In the following section, we outline the key information that should be included in submitted manuscripts and, where appropriate, provided as supplementary material. Importantly, these reporting elements are not intended as rigid "rules," but as a practical framework to improve consistency and reproducibility while preserving flexibility for new methods, creativity, and discovery. We therefore provide broad categories of reporting elements below in Guiding Principles Boxes 1 to 5 with a corresponding Organoid Reporting Toolkit ([Supplementary Appendices 3–6](#)) meant to remove barriers to implementation for investigators.

What We Call an 'Organoid' Influences Experimental Meaning and Reproducibility

Intestinal "organoid" is often used as a catch-all label, but what investigators mean by the term is central to interpreting experimental context, biological goals, and readouts. Culture origin and format influence differentiation state, architecture, and function, so inconsistent terminology can obscure what was actually studied and complicate cross-laboratory comparisons. GI organoid nomenclature has evolved inconsistently since these systems were first established, creating confusion that can impede cross-laboratory replication.¹⁹ In 2012, the United States National Institutes of Health Intestinal Stem Cell Consortium proposed systematic terminology to distinguish organoid cultures. Adult tissue-derived epithelial stem cell cultures, defined as long-term self-renewing, multipotent stem cells capable of differentiating into all functional cell types while retaining self-organizing capacity that reflects the *in vivo* state, are commonly termed

tissue-derived small intestinal organoids or enteroids, and tissue-derived colon organoids or colonoids. Separately, pluripotent stem cell-derived intestinal organoids, are commonly termed human intestinal organoids or HIOs. Despite this prior framework, terminology remains variably applied across the literature. In addition, the term "organoid" is sometimes used to describe primary 3D cultures that solely contain progenitors with a limited half-life, other primary 3D cultures, or explants. Despite efforts to unify nomenclature, terminology remains inconsistently applied, and organoid nomenclature is still evolving and, at times, debated. A full resolution is beyond the scope of this commentary, but acknowledging this unsettled landscape is important because imprecise labels can obscure experimental context and complicate replication. Compounding this issue, "organoid" is sometimes used for biologically distinct culture formats, including 3D epithelial structures embedded in ECM, 2D monolayers derived from organoids (eg, on coated surfaces or Transwells), and other engineered architectures (eg, air-liquid interface [ALI], microfluidic chips, or scaffold-based systems), each of which can influence differentiation state and functional readouts. Within these guidelines, the term organoid covers all cultures made from primary tissues, tissue-resident stem cells, and PSCs, regardless of culture technique. To improve clarity without constraining innovation, it is essential that investigators define their system at first mention.

Stem Cell Source and Characterization

The biological origin and baseline state of the cells used to establish GI organoids are among the strongest

Box 1. Guiding Principle: Defining Organoid Origin, History, Propagation.

Specify the starting material, derivation, and quality checks needed for interpretation and replication

- Species and organism background
- GI anatomical region of origin
- Starting material category and tissue source type
- Tissue state and disease context
- Donor/source metadata and clinical context
- Derivation and isolation workflow
- Seeding density, handling, and cryopreservation details
- Baseline phenotypic and molecular characterization for validation
- Culture and passaging history
- Definition of experimental replicates
- Contamination and identity/stability testing
- Protocol transparency and deviations from published methods
- Experimental design and data analysis practices
- Reporting of sample sizes, replicates, and statistics

determinants of organoid architecture, cellular composition, differentiation capacity, growth kinetics, and experimental responsiveness. Variation introduced at the point of tissue acquisition (or cell line selection), donor attributes, handling/processing conditions, and derivation methods can propagate through culture and meaningfully shift phenotypes and functional readouts. For these reasons, transparent reporting of stem cell source and characterization is essential for accurate interpretation, reproducibility, and cross-study comparison, and it aligns with broader organoid reporting efforts (Box 1). Authors are encouraged to specify the anatomical origin of starting material reflecting regional differences (eg, gastric subregion, small intestinal segment, proximal vs distal colon) and to state whether the epithelium is normal, diseased, inflamed, dysplastic, or tumor. In the future, more precise data regarding the location of organoid generation, such as the actual distance from anatomical landmarks, will be valuable as tissue sampling technology evolves. The term “Control” should be qualified by context (eg, normal-appearing tissue from diseased or previously injured donors). For primary human or animal-derived material, providing information that include species, strain (if applicable), age, sex, and relevant disease status/clinical indication, and the number of independent donors per analysis strengthens transparency.

Authors should clearly define the starting population (primary GI tissue, tumor tissue, or PSC-derived lines) and provide identifiers for PSC lines where available. For iPSCs, including the somatic cell source (eg, peripheral blood mononuclear cells [PBMCs], fibroblasts),

reprogramming factors, and method (viral vs non-viral; integrating vs non-integrating) improves reproducibility. If crypts from transplanted PSC organoids are used as source material for tissue stem cell-derived epithelial organoid culture, all parameters relevant for primary tissue organoids should also apply here. Because derivation and maintenance strategies influence clonal composition and drift,^{20–24} manuscripts should include a detailed description of isolation/derivation methods, the passage number (or time in culture) at experimental use, and whether identity/characteristics were reassessed at defined passages. Finally, quality control reporting benefits from inclusion of contamination testing (eg, mycoplasma) and, when performed, genetic identity or stability assays (eg, short tandem repeat [STR] profiling, karyotype, copy number variation [CNV]), with the passage or time point of testing specified.

A persistent challenge in GI organoid research is that “N” can refer to different experimental units, including donors or animals, independent organoid lines, wells or domes, or technical repeats, and the field has not converged on a single replication standard across use cases. Community polling at the 2025 Federation of American Societies for Experimental Biology (FASEB) GI meeting highlighted this variability and reinforced a key takeaway: clearly defining the experimental unit and replication strategy is more informative than imposing a universal minimum. For example, when participants were asked whether adult stem cell-derived organoids could be treated like transformed cell lines, for which $N = 3$ independent rounds of passaging, defined here as splitting, re-establishing, and regrowing the same donor-derived organoid culture, has historically been considered sufficient, opinions were markedly divided: 29% indicated this is never acceptable, 15% stated it is always acceptable, and 56% endorsed context-dependent use (Figure 2). In this setting, those 3 replicates reflect repeated propagation of 1 biological source rather than sampling of multiple subjects. Thus, the significance of this design is that it can demonstrate reproducibility across independently maintained iterations of the same donor-derived line, rather than biological variation across donors. This level of replication may support conclusions about stable, line-intrinsic behavior or treatment response within a single subject-derived culture, but it should not be interpreted as evidence of broader human generalizability. Acceptable contexts for $N = 3$ independent passages from a single human subject included research on rare genetic variants (24% of respondents) or homeostatic processes in non-disease organoids (18%), where the goal is to assess line-intrinsic behavior rather than population-level variability. Accordingly, manuscripts should explicitly define what constitutes biological vs technical replication in each figure or experiment and clearly state the unit of inference used for statistical comparisons. At minimum, authors should report: how raw data were processed or normalized (eg, per organoid, per well, per unit area, or relative to a within-experiment control); the structure of controls and comparisons (eg, whether a vehicle control was included in each experimental run, or whether a reference organoid

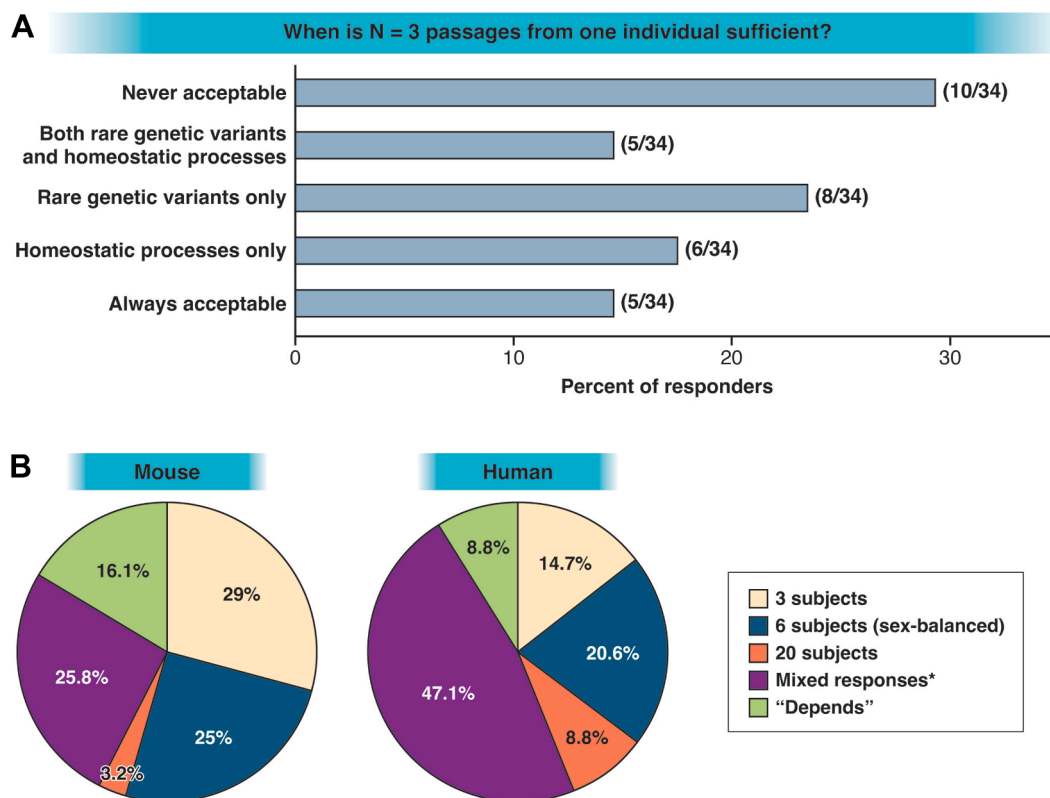


Figure 2. Survey responses about minimum experimental ‘N’ for organoid experiments. (A) Percentage of 34 respondents indicating sufficiency of N = 3 passages from one individual subject for organoid experiments in different scenarios. (B) Percentage of survey respondents indicating minimum number of individual subjects required for experiments using either mouse (*left*) or human (*right*) organoids. Survey data is provided in [Supplementary Appendix 2](#). *Denotes respondents who selected multiple answer options indicating those who determine sample size on a case-by-case basis.

line was used across batches); how data were combined across independent experiments or batches, including whether experiments were pooled or analyzed separately before aggregation; and any factors known or likely to influence results that were tracked but not formally modeled, such as passage number, plate position, operator, or donor characteristics. More extensive statistical guidance—including formal approaches to hierarchical and nested data structures, mixed-effects modeling, pseudoreplication avoidance, and variance component reporting—reflects genuinely important considerations that merit dedicated attention as the field matures, and we anticipate addressing these in future iterations of these guidelines.

Media Composition

GI organoid growth and self-renewal depend on culture media that provide key niche cues, making media composition one of the most consequential, and most variably reported, drivers of organoid morphology, cellular composition, and experimental responsiveness. For PSC-derived systems, sequential factor cocktails are used to guide differentiation toward GI lineages, whereas established epithelial organoids typically rely on WNT pathway agonism (eg, WNT3a and/or R-spondins) coupled to bone morphogenetic protein (BMP) inhibition (eg, Noggin), often alongside additional growth

factors, small-molecule inhibitors, and supplements that tune proliferation vs differentiation and can be modified to promote specific lineages, maturation or regeneration states, defined environmental conditions, or tumor organoid growth.^{24–30} Likely stemming from this complexity and the large effect of media composition on downstream experimental outcomes, 100% of survey respondents supported a requirement for standardized reporting of media composition for GI organoid experiments.

Because closely related reagents can produce meaningfully different outcomes (eg, different R-spondin or epidermal growth factor [EGF] family members, or growth-factor modifications that alter activity),^{31–36} the most actionable reporting priority identified by the community was clear disclosure of the identity, source, and concentration of media components. This includes whether factors are supplied as recombinant proteins, small molecules, conditioned media, or proprietary commercial formulations; and when conditioned media are used, the producer cell line identifiers,^{37–39} final percentage used, and the production protocol (or a citation plus any deviations). When relevant, authors should also report any batch-validation steps used to directly confirm media activity, such as the TOPflash reporter system,⁴⁰ or assessments of resulting organoids, such as visual inspection by microscopy, cell growth assays, or gene expression analysis of

Box 2. Guiding Principle: Reporting Organoid Media Composition.

Specify factors, concentrations, and delivery methods that shape organoid behavior

- Growth factor delivery method
- Basal medium composition and supplements
- Recombinant proteins and small-molecule additives
- Conditioned medium source and production details
- Conditioned medium concentration and batch/activity validation

WNT signaling targets, stem cell markers, or differentiated cell markers. Such assays were compared to determine best practices for assessing batch-to-batch replicability of L-WRN conditioned medium.⁴¹ Of note, in this study, a WNT reporter assay did not distinguish between medium batches that did and did not support organoid growth, cautioning against the use of a single assay to validate medium activity. Finally, it is important to recognize that media storage conditions, reagent diluents, and lot-to-lot variation among other variables produce biological differences in organoid experiment outcomes and should be recorded as part of best laboratory practices and be available to share upon request. Guiding principles for reporting media composition are listed in [Box 2](#).

ECM Specifications

ECM is a major yet often underreported source of variability in GI organoid experiments. Across the literature, differences in matrix biochemical composition and mechanical properties can meaningfully shift organoid growth, morphology, differentiation state, and pathway responsiveness, making ECM reporting essential for interpretation and reproducibility. This concern was strongly echoed by our community survey, in which >85% of respondents supported standardized reporting of ECM details for GI organoid studies. Standardized ECM reporting not

only supports cross-study comparison but also enables investigators to troubleshoot culture variability more efficiently and accelerates adoption of organoid methods by new laboratories.

Most GI organoid cultures rely on animal-derived basement membrane extracts (BMEs) (eg, Matrigel, Cultrex BME, Geltrex), which have enabled broad adoption of organoid methods but inherently exhibit batch-to-batch variability due to biological source material and manufacturing processes.^{24,42,43} At minimum, manuscripts should specify the ECM type and formulation (eg, BME vs collagen vs synthetic hydrogel; standard vs growth factor-reduced), the commercial source and catalog number, and the working concentration used. Proteomic analyses identified over 1000 proteins in different formulations with substantial variation in abundance between production lots.⁴⁴ This compositional heterogeneity manifests in both biochemical signaling activity and mechanical properties, which are critical determinants of intestinal organoid fate.^{45,46} Rheological measurements, typically comprising elastic and viscous moduli, have reported divergent elastic moduli under comparable conditions, with values ranging from approximately 30 Pa to 600 Pa depending on the batch and analytical methodology.^{47,48} Manufacturers have responded to reproducibility concerns by developing optimized formulations with enhanced lot-to-lot consistency and qualified mechanical properties for organoid applications, although fundamental variability inherent to animal-derived products cannot be entirely eliminated.⁴⁹ As described above for media composition, storage conditions and lot number should be recorded as part of best laboratory practices and available to share with others in the field upon request.

Authors should also report the working concentration (eg, mg/mL or % v/v), whether stock material was diluted, and the diluent used, because concentration directly affects gel mechanics and the effective levels of matrix-associated bioactive factors. We recommend that ECM handling and polymerization conditions should be described with enough detail for others to recreate the scaffold environment, including storage/thawing practices (and freeze-thaw cycles if relevant), gelation temperature and time, whether material was pre-warmed vs transferred directly from ice, and the volume/geometry used (eg, dome/droplet volume, inverted domes, plate format). Not all laboratories routinely track these parameters; however, reporting them when available strengthens reproducibility and is therefore recommended. For collagen and other polymerizing matrices, key preparation steps that affect gelation (eg, neutralization method, pH, dilution ratios) should be reported. Similarly, mechanical and biophysical characterization of the matrix (eg, rheological measurements or certificate-of-analysis values) provides valuable context for data interpretation; although this information is critical, we recommend that these characterization parameters are reported while recognizing that many laboratories do not have access to rheometric instrumentation.

Finally, as defined synthetic matrices become more common and hold the promise of greater reproducibility than biological matrices,^{45,46,50} authors should report the

Box 3. Guiding Principle: Reporting Extracellular Matrix.

Document ECM composition and mechanics that shape organoid behavior

- Matrix composition and class/type
- Vendor and identifier information
- Matrix formulation features
- Working concentration and preparation
- Matrix additives and modifications
- Gelation and polymerization conditions
- Matrix handling and storage history
- Mechanical and biophysical characterization
- Synthetic matrix design and fabrication parameters

Box 4. Guiding Principle: Reporting Organoid Platform Configuration.

Specify physical platform properties that determine gradients, forces, and downstream biology

- Culture platform and/or vessel
- Matrix geometry and volume
- Media volume and exposed surface
- Incubation conditions

specific chemical composition and fabrication parameters (eg, polymer identity and density, functional peptides, crosslinkers/degradability, polymerization conditions, and mechanical properties), because these parameters collectively define the biochemical and biophysical environment that governs cell-matrix interactions and experimental outcomes (see [Box 3](#) for matrix reporting standards).

Platform Configuration

GI organoid systems now span 3D ECM-embedded cultures, 2D monolayers, ALI, bioreactors, and microphysiological systems (MPSs). Even modest platform changes, such as culture vessel material or surface treatment, can alter hydrogel geometry and polymerization, and thereby shift growth factor diffusion, nutrient gradients, and downstream biology.^{51,52} To support interpretation and cross-lab reproducibility, manuscripts should therefore report the physical culture configuration alongside the biological inputs.

Key baseline parameters include vessel type/manufacturer, matrix contact mode (embedded vs superficial), matrix and media volumes, dome/droplet geometry, and seeding concentration, because these collectively shape diffusion and signaling landscapes in culture. Incubator conditions are equally important—most cultures are maintained near ambient oxygen (~20% O₂), which differs substantially from physiological hypoxia in vivo (~1%–7% O₂), and altered oxygen exposure can shift proliferation and lineage composition.^{53–55} For planar barrier/transport

formats, Transwell membrane and coating specifications (material, porosity, coating composition/cross-linking) should be reported because these features influence epithelial physiology and gene expression. If ALI is used, note how it is established and any ion-transport manipulations (eg, vasoactive intestinal polypeptide [VIP]) that can change apical secretions and barrier phenotypes.⁵⁶ Finally, for specialized platforms (bioreactors, perfusion, microfabricated diffusion-limited devices, microfluidic MPS), procedural details that set physical forces and transport (mixing, flow, shear, bubble control) should be disclosed, because they can be major drivers of phenotype and may not be directly comparable across systems.^{57–62} Highly customized MPS systems use microfluidic manipulation in microfabricated culture vessels to improve data collection and tissue modeling^{63,64}; however, these advanced platforms are not widely available, complicating comparisons between MPS approaches and more typical culture conditions.⁶⁵ A list of key reporting elements for platform configuration are listed in [Box 4](#).

Custom Manipulations, Exposure Conditions, and Workflows

As GI organoid studies increasingly incorporate customized workflows, ranging from genetic engineering and multi-step differentiation to co-culture and pathogen exposure, methods sections must report the study-specific details that determine experimental exposure and outcomes. Although no single template fits every application, clearly documenting these common elements allows readers to reproduce the approach and interpret results in the correct experimental context ([Box 5](#)).

If genetically modified cells are used, authors should describe the modification in a way that is reproducible and interpretable, including the genetic material introduced (eg, construct identity and key sequence features or guide RNA/target site), the delivery method (eg, electroporation, viral transduction, chemical transfection), and the selection/enrichment strategy. Key technical parameters should be reported (eg, electroporation voltage, pulse number and duration, and kit/device; or for viral workflows, the packaging system, harvest/concentration method, and multiplicity of infection [MOI] or equivalent dosing). Finally, authors should state how each engineered line was validated (eg, genotyping, copy number, expression at RNA/protein level, and/or functional assays) and the passage range at which validation and experiments were performed.

For every assay, the full experimental timeline should be described from plating through endpoint collection, including any media transitions, differentiation steps, treatments, and sampling points. For multi-step protocols (eg, expansion followed by differentiation, then treatment and analysis), reporting should remove ambiguity about “day” definitions by clearly anchoring time to a reference point (eg, days post-plating vs days after medium switch). When timelines are complex, a simple visual schematic is strongly encouraged. All media and buffers used during the

Box 5. Guiding Principle: Reporting Study-Specific Methods.

Disclose manipulations and timelines that shape organoid responses

- Organoid manipulation, genetic modification, and validation
- Culture workflow and treatment schedule
- Assay window, sampling, and collection procedures
- Media composition, exposures, and reagent preparation
- Co-culture components and microbial or pathogen exposures

workflow should be specified or referenced with complete component lists and final concentrations, and any wash steps, media exchanges, or handling steps that could affect exposure should be reported.

Finally, studies involving treatment with biological agents or co-cultures require additional transparency. If organoids are exposed to live pathogens, microbiota-derived products, stromal/immune cells, or feeder populations, authors should report the source and preparation of the added component (including genetic modifications, activation state, or culture conditions where relevant), how it was introduced (eg, apical vs basal, microinjection, overlay, embedding, or co-seeding), dosing (eg, MOI, cell ratios, concentration, or exposure volume), and duration of exposure. For pharmacologic agents, reporting key preparation details (solvent/vehicle, stock concentration, dilution scheme, storage, and any special steps such as sonication or heating) helps explain variability in potency and supports reproducibility across laboratories.

Conclusion

GI organoids have rapidly transitioned from a niche stem cell biology tool to a broadly used, human-relevant experimental platform, and the field is now large enough that variability in methodological reporting has become a major barrier to interpretation and reproducibility. Improving rigor therefore requires more than proposing a checklist, it depends on shared expectations for what constitutes sufficiently transparent reporting. Academic investigators, core facilities, biotechnology and pharmaceutical partners, funding agencies, and critically, journals, editors, and reviewers, all shape incentives and norms for how organoid studies are designed, documented, and evaluated. Without clearer and more consistently applied reporting expectations, avoidable sources of technical variability will continue to drive irreproducible outcomes and slow progress.

The guiding principles presented here aim to make GI organoid studies more interpretable across laboratories by promoting clear, consistent reporting across the major domains outlined, including system definitions, tissue and donor context, essential culture inputs and configuration, experimental timelines and exposures, and validation, replication, and analysis practices. Importantly, these principles are intended as practical guidance rather than rigid requirements, so transparency can increase without constraining innovation or slowing adoption of new approaches. The accompanying Organoid Reporting Toolkit ([Supplementary Appendices 3–6](#)) supports implementation with templates and examples that can be readily used by authors, reviewers, editors, and journals to enable more consistent evaluation and cross-study comparison.

We view this manuscript as an initial step toward greater alignment in how GI organoid methods are communicated. Any movement toward standardization will be iterative and will need to keep pace with a rapidly changing experimental landscape. Accordingly, we propose that a subset of reporting elements be treated as

community-wide expectations for publication, whereas additional elements are well-suited for laboratory-level adoption to streamline internal documentation, record keeping, and troubleshooting within and between research groups. In this way, the framework supports both external transparency and internal reproducibility, helping investigators distinguish biological signals from technical variation before discrepancies surface across laboratories.

The benefits of broad uptake are substantial. More consistent reporting can reduce trial-and-error repetition by helping groups identify when divergent outcomes arise from culture conditions rather than biology, saving time and resources and easing onboarding of laboratories new to organoid systems. In parallel, improved transparency strengthens confidence in conclusions, supports meaningful replication, and facilitates benchmarking, reference-line sharing, and cross-lab validation. Over time, these advances should also improve the signal-to-noise ratio for translational organoid applications, where subtle technical differences can otherwise masquerade as therapeutic effects.

We also recognize that parts of this framework will remain debated. Community feedback underscores that defining minimum experimental units (“N”) and replication expectations is not settled across use cases, and some recommendations, such as intensive benchmarking or validation steps, may be burdensome for smaller or resource-limited groups. For these reasons, we present these guiding principles as a starting point intended to encourage broader discussion and ongoing refinement rather than as a final prescription. We anticipate future updates to revise and extend these recommendations as organoid technologies, analytical approaches, and experimental applications continue to evolve. Ultimately, reproducibility is a shared responsibility, and transparent reporting, supported by practical tools and aligned incentives, will help ensure that organoid-based insights are robust enough to guide our work.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the full text version at <https://doi.org/10.1016/j.jcmgh.2026.101772>.

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Correspondence

Address correspondence to: Kathryn E. Hamilton, PhD, University of Pennsylvania Perelman School of Medicine, Department of Pediatrics, Division of Gastroenterology, Hepatology, and Nutrition; Children's Hospital of Philadelphia, 3615 Civic Center Blvd, Abramson 902F, Philadelphia, Pennsylvania 19104. e-mail: hamiltonk1@chop.edu; or Scott T. Magness, PhD, UNC/NCSU Lampe Joint Departments of Biomedical Engineering, UNC Departments of Medicine, Cell Biology, & Physiology, 111 Mason Farm Rd., Rm. 4337 MBRB, CB#7032, Chapel Hill, North Carolina 27599. e-mail: magness@med.unc.edu.

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CRedit Authorship Contributions

Kelli L. VanDussen (Conceptualization: Supporting; Writing – original draft: Equal; Writing – review & editing: Equal)
 Tatiana A. Karakasheva (Conceptualization: Supporting; Writing – original draft: Equal; Writing – review & editing: Equal)
 Maxime M. Mahe (Conceptualization: Supporting; Writing – original draft: Equal; Writing – review & editing: Equal)
 Joseph Burclaff (Conceptualization: Supporting; Writing – original draft: Equal; Writing – review & editing: Equal)
 Luis F. Arrieta Viana (Conceptualization: Supporting; Writing – original draft: Equal; Writing – review & editing: Supporting)
 Ian Williamson (Conceptualization: Supporting; Writing – original draft: Equal; Writing – review & editing: Supporting)
 Heather A. McCauley (Writing – original draft: Equal; Writing – review & editing: Equal)
 Andres J. Garcia (Conceptualization: Supporting; Writing – original draft: Equal; Writing – review & editing: Equal)
 Scott T. Magness (Conceptualization: Lead; Funding acquisition: Lead; Project administration: Lead; Writing – original draft: Equal; Writing – review & editing: Lead)
 Kathryn E. Hamilton, PhD (Conceptualization: Lead; Data curation: Lead; Funding acquisition: Lead; Project administration: Lead; Writing – original draft: Lead; Writing – review & editing: Lead)

Review Panel

Sarah Elizabeth Blutt, PhD, Katlynn Bugda-Gwilt, PhD, Koen Cornelis Oost, PhD, Joana F. Neves, PhD, Audrey Ferrand, PhD, Nicholas Zachos, PhD, Tamas Korcsmaros, PhD.

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