

Human-Relevant Non-Animal Methodologies (NAMs)

A practical catalogue for researchers, institutions, reviewers, and anyone mapping alternatives

Version 2.0 • September 2026

180 methods in 18 sections

*Original compilation by Georgina Rednall
for The Alliance for Cruelty Free Science*

Changes since Version 1.0

Version 2.0 is the first published revision since Version 1.0 (August 2026). It brings together updates made during September 2026 as UK Home Office project licences were mapped against this catalogue for the Licence Watch series, together with new external regulatory evidence. These changes were developed in interim working revisions 1.1 to 1.6; any Licence Watch report citing one of those numbers refers to content now contained in Version 2.0. No entry was removed, and no existing entry's readiness tag changed.

New numbering by section

Entries are now numbered within their section (for example, 4.1 for Retinal organoids, formerly Entry 37), replacing the single running sequence used in Version 1.0. Each entry shows its former number beside its title, so citations made against Version 1.0 or the interim revisions can still be traced. From Version 2.0, a new entry is added at the end of its section, and existing entries are never moved or renumbered.

New entry

16.5, Cell-based prion / protein-misfolding bioassays (scrapie cell assay-class methods, RT-QuIC, PMCA), in Section 16 (Infection and microbiome systems); numbered 180 in the interim revisions. The gap was identified while auditing a prion-disease project licence (Licence Watch, Licence 73). Its Case-by-case tag rests on independent evidence rather than the compiler's assessment alone, and it carries a Provenance line. The catalogue now lists 180 methods.

New appendix

"External regulatory validation tracking", recording three items of external evidence, each with an explicit verification status: FDA ISTAND pipeline figures (pathway and database confirmed against FDA's programme page); a reported FDA resource on non-animal methods used in named approved drugs (unverified, not to be cited as settled); and a human iPSC-derived neuromuscular junction assay for botulinum toxin potency (Curi Bio, ISTAND Letter of Intent accepted September 2026; independently corroborated).

Entries updated

1.12 and 10.1 (dated ISTAND figures); 14.10 (the NMJ modality, at its own earlier readiness level); 17.3 and 18.8 (dated FDA CDER statements); 11.1, 11.2, 11.3, 11.6, and 11.8 (pointers to appendix item 2, marked pending verification). None of these updates changed a readiness tag.

Cross-references and standing rule

"See also" lines added where an entry filed by method class also serves another section's endpoint or organ, with a Cross-reference index at the back. A "Gap-driven additions" rule in "How to use this catalogue" sets how entries prompted by Licence Watch audits are added and evidenced.

Corrections

Entry 1.12's ISTAND sentence is reworded to match appendix item 1: organ-chip and engineered 3D tissue NAMs had 11 of 11 decided Letters of Intent accepted, out of 26 accepted across all technology classes.

Disclaimer

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How to use this catalogue

Use it as a directory. Identify the scientific question first, then search by organ, endpoint, or readiness tag. Cite a method only for the question it can actually answer. Confirm current status with FDA, EMA, MHRA, OECD, Ph. Eur., USP, ISO, or the relevant national guidance before a licence, dossier, grant, or protocol depends on it.

In the United Kingdom, section 2A(a) of the Animals (Scientific Procedures) Act 1986 requires a scientifically satisfactory method or testing strategy that does not use protected animals, wherever one exists. That duty is endpoint-specific. The same logic appears in EU Directive 2010/63 and in FDA/EMA context-of-use practice.

Intended users include experimental scientists, study directors, AWERB and ethics-committee members, project-licence holders, regulatory-affairs staff, agency reviewers, funders, students, and anyone else who needs a single working list.

Cross-references (new in Version 2.0): where a method is filed under one section by its technology class but serves an endpoint covered by another section (for example, a chip filed under Oncology that is also relevant to Immunology), a “See also” line appears at both the entry and the relevant section heading. Cross-references do not change an entry's primary section or number.

Numbering (from Version 2.0): entries are numbered within their section (for example, 4.1 is the first entry in Section 4). The Version 1.0 number is shown beside each title. Entries are never moved or renumbered; a new entry is added at the end of its section.

Gap-driven additions (from Version 2.0): when a Licence Watch audit report identifies a scientific question with no matching Catalogue entry, a new entry may be added to close that gap, added at the end of the relevant section (for example, 16.6 after 16.5), so no existing entry's number changes. Such an entry must cite independent, externally verifiable evidence (published literature, or adoption by a regulatory or clinical-practice body) for its readiness tag, not the compiler's assessment alone, and must carry a Provenance line naming the report that identified the gap. An entry that cannot yet meet the evidence requirement is marked “proposed” in its tag and must not be cited in any report as a settled method until it is upgraded.

Readiness-tag legend

Accepted / routine: In standing regulatory or industry use for a defined purpose, with no live dispute about applicability.

Accepted (qualifier in parentheses): Accepted, but only within the scope named in the qualifier: a specific endpoint, framework, product class, or agency context. Reading past the qualifier and treating it as general acceptance is the single most common misuse risk in this catalogue.

Case-by-case: Genuinely used in real submissions and decisions, but whether it is scientifically satisfactory for a given question has to be argued each time. No blanket rule covers it.

Emerging / validation / guidance: Moving through an OECD, ECVAM, or equivalent validation process; not yet a finalised guideline or accepted method.

Research / enabling: Not used for safety or efficacy decisions. Either exploratory science, or infrastructure/technology that other NAMs are built on.

Observational: Human data gathered outside an experimental protocol (imaging, epidemiology). Evidentiary weight, not a test.

Enabling: Knowledge or data infrastructure (repositories, knowledge bases) rather than a method that produces a result.

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Document scope and version history

1. General in vitro, stem-cell, chip, and enabling methods

1.1 Biochemical assays [Accepted / routine] *(formerly Entry 1)*

Cell-free or simple cell-based tests of binding, enzyme activity, receptor occupancy, or biomarkers. Used for early target engagement and mechanistic work. Do not model organ physiology.

1.2 2D cell cultures [Accepted / routine] *(formerly Entry 2)*

Monolayer human cells, frequently iPSC-derived, for high-throughput cytotoxicity and basic function. High throughput, limited architecture and polarity.

1.3 High-content screening (HCS) [Accepted / routine] *(formerly Entry 3)*

Automated multiparametric imaging of morphology, viability, organelles, and reporter readouts. Core early-safety and phenotypic screening tool.

1.4 Pluripotent stem cells [Research / enabling] *(formerly Entry 4)*

hESC or equivalent starting material for generating differentiated human lineages used in downstream NAM platforms.

1.5 Induced pluripotent stem cells (iPSCs) [Case-by-case] *(formerly Entry 5)*

Reprogrammed adult cells differentiated into disease-relevant lineages. Foundation for patient-specific efficacy, safety, and rare-disease models.

1.6 3D cell cultures and spheroids [Case-by-case] *(formerly Entry 6)*

Simple aggregates that restore cell–cell contact and gradients. Better than 2D for penetration and some toxicity endpoints; still lack vasculature and immune context.

1.7 Brain spheres / neurospheres [Case-by-case] *(formerly Entry 7)*

3D neural aggregates used for proliferation, migration, and early network formation, including developmental neurotoxicity screens.

1.8 Organoids [Case-by-case] *(formerly Entry 8)*

Self-organising 3D structures that recapitulate aspects of tissue architecture and cell diversity. Widely used in disease modelling; regulatory use is context-specific, not generic replacement.

1.9 Gastruloids (post-implantation embryo-like models) [Research] *(formerly Entry 9)*

PSC aggregates that model gastrulation, axial elongation, and germ-layer specification. Research models for early development and teratogen hypotheses. Not a qualified whole-embryo replacement.

1.10 Blastoids (blastocyst-like models) [Research] *(formerly Entry 10)*

Stem-cell structures mimicking pre-implantation lineage segregation. Used for implantation biology. Ethical and regulatory status remains restricted.

1.11 3D bioprinting [Research / enabling] *(formerly Entry 11)*

Layer-by-layer placement of cells and biomaterials to control geometry and, in advanced systems, vascular channels. Enabling technology rather than a single assay.

1.12 Organs-on-chips [Case-by-case] *(formerly Entry 12)*

Microfluidic devices with flow, stretch, and tissue–tissue interfaces. Growing IND use under defined contexts of use. As of September 2026, organ-chip and engineered 3D tissue NAMs had 11 of 11 decided Letters of Intent accepted in FDA's public IStand database (a 100% LOI acceptance rate for this technology class to date, out of 26 LOIs accepted across all classes), though IStand letters of intent are not the same as full qualification, and no IStand project of any technology class had reached full qualification as of that date. See the External regulatory validation tracking appendix for the source and its limits.

1.13 Microphysiological systems (MPS) [Case-by-case] *(formerly Entry 13)*

Broader class of dynamic multi-cell platforms that mimic organ-level physiology. Includes multi-organ coupling for ADME and selected toxicities.

1.14 Multi-electrode array (MEA) neural cultures [Case-by-case] *(formerly Entry 14)*

Electrical recordings from neuronal networks or organoids. Used for seizurogenic risk, network maturation, and parts of the DNT battery.

1.15 CRISPR-based functional genomics [Research / enabling] *(formerly Entry 15)*

Gene editing in human cultured systems to map targets, resistance, and genetically driven toxicity. Enabling method, not a standalone safety test.

1.16 Gut–brain assembloids [Research] *(formerly Entry 16)*

Fused or coupled gut and neural components for microbiome–brain or systemic neuroimmune questions. Early-stage complexity; limited duration and immune completeness.

1.17 Multi-regional assembloids [Research] (formerly Entry 17)

Fused regional organoids to study connectivity and region-specific drug effects. Research tool for circuit-level hypotheses.

1.18 Patient-derived iPSC models [Case-by-case] (formerly Entry 18)

Donor-specific lineages for precision disease, pharmacogenetics, and selected safety risks. Strength is genotype; weakness is often immature phenotype.

1.19 Vessel-on-chip and thrombosis-on-chip [Case-by-case] (formerly Entry 19)

Endothelialised microfluidics under shear for platelet function, thrombus formation, and antithrombotic testing with human blood cells.

1.20 Muscle-on-chip and 3D skeletal muscle organoids [Research] (formerly Entry 20)

Engineered myotubes or iPSC muscle constructs for wasting, cachexia, sarcopenia, and contractile pharmacology.

1.21 Extracellular vesicle testing platforms [Research] (formerly Entry 21)

Isolated human EVs applied to 3D tissues or multi-organ chips to study inter-tissue signalling without a whole animal.

1.22 Phage display and recombinant antibody production [Accepted / routine] (formerly Entry 22)

Fully in vitro selection and recombinant expression of sequence-defined antibodies. Replaces animal immunisation for many research and diagnostic reagents.

1.23 Impedance and real-time cell analysis [Accepted / routine] (formerly Entry 23)

Label-free platforms (e.g. xCELLigence-class) tracking attachment, cytotoxicity, and barrier function over time. Common HTS complement to imaging.

1.24 Defined, animal-free extracellular matrices [Research / enabling] (formerly Entry 24)

Synthetic or human-derived hydrogels that avoid Engelbreth–Holm–Swarm (Matrigel) mouse sarcoma extracts. Important if the claim is animal-component-free, not only animal-test-free.

2. Specialised oncology models

2.1 Breast cancer organoids [Case-by-case] (formerly Entry 25)

Patient-derived 3D cultures preserving subtype, histology, and often drug response. Used for pharmacotyping and discovery; not a validated substitute for all in vivo efficacy packages.

2.2 Leukemia-on-chip / bone marrow-on-chip [Research] (formerly Entry 26)

Marrow-niche MPS with stroma and endothelium for leukaemia, resistance, and selected immunotherapy readouts including CAR-T interaction studies.

See also: Section 13 (Immunology and lymphoid models) for marrow-niche immune interactions, and Entry 14.12 (CFU-GM myelotoxicity assay, Section 14) for marrow-suppression endpoints on the same tissue.

2.3 Patient-derived PDAC organoids [Case-by-case] (formerly Entry 27)

Biopsy- or FNA-derived pancreatic ductal adenocarcinoma organoids for pharmacotyping and resistance. Replacement claim versus xenografts is programme-specific.

2.4 Tumour-on-chip (vascularised / immune-competent) [Case-by-case] (formerly Entry 28)

General class including vascularised tumour–immune microenvironment (vTIME) platforms. A 2025 oncology IND used such data for efficacy/PoC (SillaJen/Qureator). Efficacy precedent is not a full safety waiver.

2.5 Patient-derived explants (PDE) [Case-by-case] (formerly Entry 29)

Short-term culture of intact human tumour fragments retaining stroma. Used for ex vivo drug response; limited culture life.

2.6 CTC- and organoid-derived tumour panels [Research] (formerly Entry 30)

Circulating-tumour-cell or multi-indication organoid libraries (colorectal, lung, GBM, ovarian, prostate, etc.) for panel screening beyond single-indication models.

3. Specialised respiratory models

See also Entry 16.2 (Airway ALI infection models, Section 16) for infection applications of these platforms.

3.1 Lung / airway organoids [Case-by-case] (formerly Entry 31)

Patient- or iPSC-derived structures modelling mucociliary biology and inherited airway disease (CF, PCD) for gene and delivery studies.

3.2 Lung-on-chip / airway-on-chip [Case-by-case] (formerly Entry 32)

ALI epithelium with optional breathing stretch, endothelium, and mucus transport. Used for inhaled delivery, barrier, and infection work.

3.3 Precision-cut lung slices (PCLS) [Case-by-case] (formerly Entry 33)

Viable human lung sections preserving architecture and mucociliary function for short- to medium-term delivery, infection, and pharmacology studies.

3.4 3D bioprinted lung / airway constructs [Research] (formerly Entry 34)

Engineered airway–alveolar geometries with controlled architecture. Still largely research-grade.

3.5 Patient iPSC lung epithelium (ALI and assembloids) [Case-by-case] (formerly Entry 35)

Donor-specific airway lineages at air–liquid interface for gene-editing and formulation testing.

3.6 Commercial reconstructed airway tissues [Case-by-case] (formerly Entry 36)

Standardised ALI products such as MucilAir, EpiAirway, and EpiAlveolar used in inhalation toxicology and antiviral work.

4. Specialised ocular models

4.1 Retinal organoids [Case-by-case] (formerly Entry 37)

iPSC or patient-derived 3D retina used for inherited dystrophies and RNA/gene therapies (including CEP290/LCA packages). Strong where the human allele is the point.

4.2 Cornea-on-chip and reconstructed corneal epithelium [Accepted (selected endpoints)] (formerly Entry 38)

Barrier, wound, and irritation models. RhCE methods underpin OECD eye-irritation guidelines and replace rabbit Draize for defined chemical classes.

4.3 Ocular multi-organ and assembloid platforms [Research] (formerly Entry 39)

Retina–RPE–choroid or cornea–conjunctiva systems for AMD, glaucoma, and immune ocular toxicity hypotheses.

4.4 Patient iPSC ocular disease models [Case-by-case] (formerly Entry 40)

Retinal, RPE, corneal, and trabecular lineages for precision screening.

4.5 3D bioprinted ocular constructs [Research] (formerly Entry 41)

Engineered corneal or retinal tissues for device and implant biocompatibility research.

4.6 Blood–retinal barrier models [Research] (formerly Entry 42)

Transwell and chip models of inner/outer BRB for ocular PK and permeability.

5. Specialised cardiovascular models

5.1 Cardiac organoids [Case-by-case] (formerly Entry 43)

PSC-derived 3D cardiac tissue for contractility, electrophysiology, and cardiomyopathy modelling.

5.2 Heart-on-chip / cardiac MPS [Case-by-case] (formerly Entry 44)

Flowing or stretched cardiac microtissues with real-time force and field-potential readouts. Used in cardiotoxicity triage.

5.3 3D bioprinted cardiac and vascular constructs [Research] (formerly Entry 45)

Printed myocardium or grafts for regenerative and device research.

5.4 Ex vivo human heart perfusion [Case-by-case] (formerly Entry 46)

Langendorff or working-heart human preparations. High physiological fidelity, scarce tissue, short duration.

5.5 Patient iPSC cardiovascular models [Case-by-case] (formerly Entry 47)

Cardiomyocyte, endothelial, and fibroblast assemblies for genetic cardiomyopathy and personalised QT/contractile risk.

5.6 Pumping cardiac organoid chambers (“heart-in-a-jar”) [Case-by-case] (formerly Entry 48)

Fluid-ejecting human ventricular chambers (e.g. Novoheart hvCOC / MyHeart) reporting EF-like and pressure–volume metrics. Used as supporting IND data, including HFpEF gene-therapy programmes.

5.7 Comprehensive in vitro proarrhythmia assay (CiPA) [Accepted (context-specific)] (formerly Entry 49)

Integrated hERG / ion-channel panel, human iPSC-cardiomyocyte electrophysiology, and in silico action-potential modelling. Recognised FDA/CDER NAM class for TdP risk, replacing over-reliance on hERG plus TQT alone.

6. Specialised musculoskeletal and joint models

6.1 Synovium-on-chip [Research] (formerly Entry 50)

Synoviocyte and immune-cell lining under flow for RA cytokine and hyperplasia studies.

6.2 Joint-on-chip / cartilage-on-chip / osteochondral units [Research] (formerly Entry 51)

Loaded cartilage–bone–synovium interfaces for OA and regenerative testing.

6.3 Immune-competent RA joint-on-chip [Research] (formerly Entry 52)

Adds macrophages and T cells to joint MPS for immune-driven matrix loss.

6.4 Instrumented synovial joint-on-chip [Research] (formerly Entry 53)

Biosensors plus controllable mechanical load and synovial-fluid dynamics.

6.5 3D synovial organoids and fibroblast–macrophage co-cultures [Research] (formerly Entry 54)

Self-organising synovial models of inflammatory remodelling.

6.6 Patient iPSC musculoskeletal / joint assembloids [Research] (formerly Entry 55)

Cartilage, bone, synovium, and muscle constructs for genetic MSK disease.

6.7 Multi-organ musculoskeletal MPS [Research] (formerly Entry 56)

Joint modules coupled to immune or vascular compartments.

7. Specialised gastrointestinal models

See also Entries 16.1 and 16.3 (Infection and microbiome systems, Section 16) for infection and microbiome applications of these platforms.

7.1 Intestinal / gut organoids [Case-by-case] (formerly Entry 57)

Crypt–villus structures from biopsies or iPSCs for IBD, coeliac disease, CRC, absorption, and infection (including viruses poorly modelled in mice).

7.2 Gut-on-chip / intestine-on-chip / colon-on-chip [Case-by-case] (formerly Entry 58)

Peristalsis-capable epithelium with optional endothelium, immune cells, and microbiota.

7.3 Precision-cut intestinal slices (PCIS) [Case-by-case] (formerly Entry 59)

Human small-bowel or colon slices for permeability, metabolism, and local toxicity.

7.4 Vascularised intestinal assembloids and immune gut MPS [Research] (formerly Entry 60)

Adds stroma, endothelium, and immune cells for gut–liver axis and chronic inflammation studies.

7.5 Patient iPSC gastrointestinal disease models [Case-by-case] (formerly Entry 61)

Monogenic GI disorders and host–microbiome interaction models.

7.6 Caco-2 and MDCK-MDR1 permeability assays [Accepted / routine] (formerly Entry 62)

Standard human (or human-transporter) epithelial monolayers for oral absorption, efflux, and BCS-type decisions. Industry workhorse omitted from many “organoid-first” lists.

8. Specialised pancreatic, islet, and diabetes models

8.1 iPSC pancreatic organoids [Research] (formerly Entry 63)

Branched acinar–ductal–endocrine structures for development and diabetes modelling.

8.2 Vascularised islet organoids / islet assembloids [Research] (formerly Entry 64)

Islets with endothelium and stroma for glucose-responsive hormone secretion and beta-cell replacement testing in vitro.

8.3 Pancreas / islet-on-chip [Research] (formerly Entry 65)

Perfused islets or iPSC islet cells with multiparametric sensing (insulin, Ca²⁺, oxygen).

8.4 Multi-organ glucose-homeostasis MPS [Research] (formerly Entry 66)

Islet–liver–muscle–adipose coupling for insulin resistance and systemic glucose control.

8.5 Pancreatic digital twins and iPSC pancreatic disease models [Research] (formerly Entry 67)

Computational beta-cell fate models plus patient iPSC lines for monogenic diabetes and protocol optimisation.

9. Nervous system, blood–brain barrier, and DNT

See also Entry 16.5 (Cell-based prion / protein-misfolding bioassays, Section 16), a detection and titre-screening assay class relevant to neurodegeneration and proteopathic disease work, filed under Infection systems because the licence programmes it was drafted against treat prion inoculation as analogous to infection.

9.1 Brain-on-chip and neurovascular-unit chips [Research] (formerly Entry 68)

Microfluidic brain tissue with endothelium and optional glia under flow. Addresses a gap left by closed organoids (no controlled PK exposure).

9.2 Blood–brain barrier Transwells and BBB-on-chip [Case-by-case] (formerly Entry 69)

TEER and permeability models using primary or iPSC brain endothelial cells, pericytes, and astrocytes. Used for CNS exposure ranking.

9.3 Myelinating neuron–oligodendrocyte co-cultures [Research] (formerly Entry 70)

In vitro myelination and remyelination assays for MS-class and neurotoxic questions.

9.4 Neuromuscular-junction chips [Research] (formerly Entry 71)

Motor neuron–muscle pairings for ALS, MG, and neuromuscular drug effects.

9.5 iPSC nociceptor and pain-on-chip models [Research] (formerly Entry 72)

Sensory-neuron excitability and inflammatory pain circuitry without in vivo pain assays.

9.6 Developmental neurotoxicity in vitro battery (DNT-IVB) [Accepted (guidance / screening)] (formerly Entry 73)

OECD Guidance Document 377 battery covering proliferation, migration, neurite outgrowth, synaptogenesis, and MEA network formation. Listed by EPA as a NAM for DNT screening.

10. Toxicology MPS and hepatic ADME stack

10.1 Liver-on-chip / hepatotoxicity MPS [Case-by-case (ISTAND pathway)] (formerly Entry 74)

Primary or iPSC hepatocytes with endothelium and immune cells under flow. Emulate Liver-Chip entered FDA ISTAND (LOI accepted 2024, reported as 217 days from submission to decision; a Qualification Plan is reported accepted as of September 2026, per third-party tracking of FDA's public ISTAND database; see the External regulatory validation tracking appendix). Published DILI sensitivity ~87% in a defined 27-drug set is not a universal qualification, and an accepted Qualification Plan is not the same as full qualification.

10.2 Kidney-on-chip / nephrotoxicity models [Case-by-case] (formerly Entry 75)

Glomerular and proximal-tubule compartments for transporter and injury endpoints.

10.3 Skin-on-chip and reconstructed human epidermis [Accepted (selected endpoints)] (formerly Entry 76)

RhE models underpin OECD dermal irritation, corrosion, and some phototoxicity guidelines. Chips add flow and immune complexity.

10.4 Multi-organ toxicity MPS [Research / case-by-case] (formerly Entry 77)

Linked liver–kidney–heart–lung–immune modules for selected ADME and off-target questions. Cannot yet reproduce a full repeat-dose GLP package.

See also: Section 13 (Immunology and lymphoid models) for the immune-module component.

10.5 Patient iPSC multi-endpoint toxicity screens [Case-by-case] (formerly Entry 78)

Donor panels for cardio, neuro, and developmental liability. Useful triage, not a two-species tox replacement.

10.6 AOP-integrated NAM strategies [Accepted (as IATA logic)] (formerly Entry 79)

Combine in vitro, omics, and computational evidence along an adverse outcome pathway for IATA and read-across. Framework, not a single test.

10.7 High-throughput / high-content toxicology [Accepted / routine] (formerly Entry 80)

Multiwell human-cell panels for cytotoxicity, mitochondria, oxidative stress, and endocrine flags. Supports prioritisation.

10.8 Precision-cut liver slices (liver PCLS) [Case-by-case] (formerly Entry 81)

Human liver explant slices preserving zonation and multicellular architecture. Classic NAM for metabolism and DILI mechanisms; missing from many organoid-centric lists.

10.9 Sandwich-cultured hepatocytes, HepaRG, and micropatterned co-cultures [Accepted / routine] (formerly Entry 82)

HepatoPac-class and HepaRG systems for CYP induction, clearance, and cholestasis flags. Standard industry ADME/tox tools.

10.10 Transporter and BSEP vesicle / sandwich assays [Accepted / routine] (formerly Entry 83)

Human BSEP, OATP, OCT, MATE assays for cholestatic and DDI risk. Directly inform labelling and interaction packages.

10.11 Precision-cut kidney and lymphoid slices [Research] (formerly Entry 84)

Human kidney, spleen, or lymph-node slices for local pharmacology and immunotoxicity hypotheses.

See also: Section 13 (Immunology and lymphoid models). The lymphoid-slice component of this entry is relevant to immune/lymphoid endpoints as well as renal ones.

11. Regulatory NAM batteries

11.1 In chemico skin-sensitisation KE1 assays [Accepted] (formerly Entry 85)

DPRA, ADRA, and kDPRA (OECD TG 442C) measuring covalent peptide binding. First key event of the skin-sensitisation AOP.

See also: External regulatory validation tracking appendix, item 2, for reported use of DPRA in named FDA-approved drug applications (pending independent verification).

11.2 Keratinocyte skin-sensitisation KE2 assays [Accepted] (formerly Entry 86)

KeratinoSens, LuSens, EpiSensA, and α -Sens (OECD TG 442D, including 2025–26 updates). Nrf2/ARE or equivalent keratinocyte activation.

See also: External regulatory validation tracking appendix, item 2, for reported use of KeratinoSens in named FDA-approved drug applications (pending independent verification).

11.3 Dendritic-cell skin-sensitisation KE3 assays [Accepted] (formerly Entry 87)

h-CLAT, U-SENS, IL-8 Luc, GARDskin (OECD TG 442E). Third key event; used inside defined approaches.

See also: External regulatory validation tracking appendix, item 2, for reported use of h-CLAT in named FDA-approved drug applications (pending independent verification).

11.4 OECD TG 497 defined approaches on skin sensitisation [Accepted] (formerly Entry 88)

2 out of 3 and ITS defined approaches, with 2025–26 updates adding further information sources and SARA-ICE point-of-departure logic. International replacement for guinea-pig and, for many substances, LLNA hazard calls.

11.5 Transcutaneous electrical resistance (OECD TG 430) [Accepted] (formerly Entry 89)

In vitro skin-corrosion method.

11.6 Reconstructed human epidermis corrosion (OECD TG 431) [Accepted] (formerly Entry 90)

EpiDerm, EpiSkin, SkinEthic, LabCyte and equivalents for UN GHS skin corrosion.

See also: External regulatory validation tracking appendix, item 2, for reported use of EpiSkin in named FDA-approved drug applications (pending independent verification).

11.7 Reconstructed human epidermis irritation (OECD TG 439) [Accepted] (formerly Entry 91)

RhE irritation as a Draize-replacement for defined applicability domains.

11.8 Bovine corneal opacity and permeability (BCOP, OECD TG 437) [Accepted] *(formerly Entry 92)*

Ex vivo bovine cornea. Uses slaughterhouse tissue; animal-test replacement for serious eye damage classification, not a live-animal test.

See also: External regulatory validation tracking appendix, item 2, for reported use of BCOP in named FDA-approved drug applications (pending independent verification).

11.9 Isolated chicken eye (ICE, OECD TG 438) [Accepted] *(formerly Entry 93)*

Ex vivo enucleated chicken eye for eye-damage classification.

11.10 Short-time exposure test (STE / STE0.5, OECD TG 491) [Accepted] *(formerly Entry 94)*

SIRC cell viability method used alone or inside OECD 467 defined approaches, including surfactant updates.

11.11 Reconstructed human cornea-like epithelium (OECD TG 492 / 492B) [Accepted] *(formerly Entry 95)*

EpiOcular and similar RhCE models for eye irritation / no-classification decisions.

11.12 OECD TG 467 defined approaches for eye irritation [Accepted] *(formerly Entry 96)*

Rule-based combinations of in vitro eye methods, updated to widen surfactant applicability.

11.13 OptiSafe macromolecular eye test (OECD TG 496) [Accepted] *(formerly Entry 97)*

In chemico macromolecular matrix for selected eye-irritation questions.

11.14 3T3 Neutral Red Uptake phototoxicity (OECD TG 432) [Accepted] *(formerly Entry 98)*

In vitro phototox screen aligned with ICH S10 thinking for many pharmaceuticals.

11.15 RhE phototoxicity (OECD TG 498) [Accepted] *(formerly Entry 99)*

Reconstructed epidermis phototoxicity method.

11.16 In vitro skin absorption (OECD TG 428) [Accepted] *(formerly Entry 100)*

Franz-cell diffusion through human or reconstructed skin for dermal PK and risk assessment.

11.17 Bacterial reverse mutation (Ames, OECD TG 471) [Accepted] *(formerly Entry 101)*

Core mutagenicity NAM in ICH S2 batteries. Bacterial, not human, but animal-free and regulatorily required.

11.18 In vitro micronucleus (OECD TG 487) [Accepted] *(formerly Entry 102)*

Human or mammalian cell chromosomal-damage assay. Standard ICH S2 component.

11.19 In vitro mammalian gene-mutation assays [Accepted] *(formerly Entry 103)*

Mouse lymphoma Tk and related assays in the genotoxicity battery.

11.20 ToxTracker reporter assay (OECD TG 446A, 2026) [Accepted] *(formerly Entry 104)*

Stem-cell reporter panel distinguishing genotoxic versus non-genotoxic modes of action. Now an OECD test guideline, not only a commercial kit.

11.21 3D reconstructed-skin comet and micronucleus [Emerging / guidance] *(formerly Entry 105)*

Dermal-route genotoxicity follow-up (OECD project 4.139 / ECVAM evaluation). Weight-of-evidence for topically exposed substances.

11.22 Estrogen receptor transactivation (OECD TG 455) [Accepted] *(formerly Entry 106)*

In vitro ER agonist/antagonist detection for endocrine assessment.

11.23 Androgen receptor transactivation (OECD TG 458) [Accepted] *(formerly Entry 107)*

In vitro AR activity method.

11.24 H295R steroidogenesis (OECD TG 456) [Accepted] *(formerly Entry 108)*

Human adrenal cell assay for hormone-production disruption.

11.25 ToxCast / Tox21 ER and AR pathway models [Accepted (EPA screening)] *(formerly Entry 109)*

Multi-assay computational pathway models used by EPA for endocrine bioactivity.

11.26 Thyroid-disruption in vitro battery [Emerging / validation] *(formerly Entry 110)*

EURL ECVAM / EU-NETVAL methods covering thyroid-gland and peripheral TH mechanisms. Validation chemical set published; moving toward regulatory use.

11.27 IATA and defined approaches (framework) [Accepted (as framework)] *(formerly Entry 111)*

Pre-specified combinations of NAMs that yield a classification without expert “black box” judgement. Already adopted for skin and eye; template for other endpoints.

11.28 Next Generation Risk Assessment (NGRA) [Accepted (cosmetics / case-by-case chemicals)] *(formerly Entry 112)*

Exposure-led, hypothesis-driven chemical safety assessment using NAM evidence instead of apical animal studies. Standard in modern cosmetics safety; expanding to chemicals.

11.29 ICH S1 weight-of-evidence carcinogenicity assessment [Accepted (ICH)] *(formerly Entry 113)*

Allows waiver or reduction of 2-year rodent bioassays when mode of action, pharmacology, and NAM evidence support the case.

11.30 Structured read-across with OECD QSAR Toolbox [Accepted] *(formerly Entry 114)*

Analogue and category approaches using mechanistic profiling. Regulatory staple for chemicals; quality depends on analogue justification.

12. Reproductive, placental, and developmental models

12.1 Endometrial organoids [Research] *(formerly Entry 115)*

Glandular hormone-responsive cultures for implantation, endometriosis, and uterine pharmacology.

12.2 Trophoblast and placental organoids [Research] *(formerly Entry 116)*

Maternal–fetal interface models for preeclampsia, transfer, and developmental-toxicity hypotheses.

12.3 Ovarian and testicular organoids / gonadal MPS [Research] *(formerly Entry 117)*

Follicle or tubule models for gametogenesis, hormones, and reprotox screening.

12.4 Multi-organ reproductive / developmental MPS [Research] *(formerly Entry 118)*

Placenta–endometrium–embryo or gonad–liver couplings. Holistic ambition; limited validation.

12.5 BeWo and other placental barrier transport models [Case-by-case] *(formerly Entry 119)*

Classic trophoblast monolayers and dual-chamber systems for transplacental transfer ranking.

12.6 Embryonic stem-cell test and ReProGlo-class assays [Case-by-case] *(formerly Entry 120)*

Differentiation-based developmental-toxicity screens. Useful flags; not a full EFD study replacement.

13. Immunology and lymphoid models

See also Entry 2.2 (Leukemia-on-chip / bone marrow-on-chip, Section 2), a marrow-niche MPS relevant to immune-cell and haematopoietic questions, filed under Oncology because its primary use case is leukaemia. See also Entries 14.3–14.7 (Section 14, Biologics, vaccines, pyrogen testing, and devices): cytokine release, MHC-presentation, immunogenicity, and complement assays that are immunological in mechanism, filed under Biologics because their primary regulatory use is biologics safety testing. See also Entry 15.6 (Immune-competent and microbiome-containing skin models, Section 15) for skin-resident immune-cell modelling.

13.1 Lymphoid follicle / lymphoid organ-chip [Research] *(formerly Entry 121)*

PBMC-dense chips that self-assemble germinal-centre-like structures for antibody and vaccine-response work.

13.2 Lymph node-on-chip with lymphatics [Research] *(formerly Entry 122)*

Perfused lymphatic endothelium plus lymphocyte trafficking.

13.3 Human tonsil, adenoid, and spleen immune organoids [Research] *(formerly Entry 123)*

Primary lymphoid-tissue organoids preserving humoral responses for antigen screens.

13.4 Multi-organ immune MPS [Research] *(formerly Entry 124)*

Lymphoid modules coupled to vascular or mucosal compartments.

13.5 Monocyte activation test (MAT) [Accepted] *(formerly Entry 125)*

Human blood-cell cytokine test for endotoxin and non-endotoxin pyrogens. Compendial in Ph. Eur. 2.6.30 as replacement for the rabbit pyrogen test; FDA accepts with product-specific validation.

14. Biologics, vaccines, pyrogen testing, and devices

14.1 Recombinant Factor C (rFC) endotoxin test [Accepted] *(formerly Entry 126)*

Animal-free replacement for horseshoe-crab LAL in many bacterial-endotoxin applications.

14.2 Whole-blood / PBMC / MM6 cytokine pyrogen assays [Accepted / case-by-case] (formerly Entry 127)

Related human-cell pyrogen methods evaluated by ICCVAM and used as MAT variants.

14.3 Cytokine release assays (CRA) [Case-by-case (expected for many biologics)] (formerly Entry 128)

Human whole-blood or PBMC assays for mAb and T-cell engager CRS risk. Essential after TGN1412; format choice (wet/dry, density) is still debated.

See also: Section 13 (Immunology and lymphoid models) for related human immune-cell platforms.

14.4 MAPPs (MHC-associated peptide proteomics) [Case-by-case] (formerly Entry 129)

Identifies peptides presented on human MHC class II for immunogenicity risk ranking.

See also: Section 13 (Immunology and lymphoid models) for related human immune-cell platforms.

14.5 HLA-binding and in silico immunogenicity tools [Case-by-case] (formerly Entry 130)

Sequence-based T-cell epitope prediction used with MAPPs and in vitro T-cell assays.

See also: Section 13 (Immunology and lymphoid models) for related human immune-cell platforms.

14.6 In vitro ADA and T-cell proliferation assays [Case-by-case] (formerly Entry 131)

Human PBMC assays for anti-drug antibody risk and T-cell activation.

See also: Section 13 (Immunology and lymphoid models) for related human immune-cell platforms.

14.7 Complement-activation panels [Case-by-case] (formerly Entry 132)

CH50, C3a/C5a, and related human-serum assays for infusion reactions.

See also: Section 13 (Immunology and lymphoid models) for related human immune-cell platforms.

14.8 ADCC, CDC, and FcγR-binding panels [Accepted / routine] (formerly Entry 133)

Human-cell effector-function characterisation for Fc-engineered antibodies.

14.9 IL-2 lymphocyte transformation test (immunosuppression) [Emerging] (formerly Entry 134)

In vitro immunosuppression method advanced through the OECD TG programme (2025 WNT).

14.10 Cell-based botulinum potency (SNAP-25) [Accepted (many jurisdictions)] (formerly Entry 135)

Enzymatic or cell assays replacing mouse LD50 for botulinum toxin potency. A distinct, newer modality, human iPSC-derived 3D neuromuscular junction (NMJ) assays measuring functional contractility rather than SNAP-25 cleavage, is at an earlier stage: FDA/CDER accepted an ISTD Letter of Intent for one such platform (Curi Bio) in September 2026. It sits at a distinct readiness level from the enzymatic/SNAP-25 methods this entry's tag describes; see the External regulatory validation tracking appendix for sourcing and status.

See also: External regulatory validation tracking appendix, item 3, for the NMJ-based functional modality's specific evidence and current (LOI-stage, not yet qualified) status.

14.11 Vaccine serological potency and consistency approach [Accepted (product-specific)] (formerly Entry 136)

In vitro antigen quantification and consistency lot-release instead of in vivo challenge or serology in animals.

14.12 CFU-GM myelotoxicity assay [Case-by-case] (formerly Entry 137)

Human hematopoietic progenitor colony assay for bone-marrow suppression ranking.

See also: Entry 2.2 (Leukemia-on-chip / bone marrow-on-chip, Section 2) and Section 10 (Toxicology MPS and hepatic ADME stack) for related marrow and organ-toxicity endpoints.

14.13 ISO 10993 in vitro device biocompatibility [Accepted] (formerly Entry 138)

Cytotoxicity (10993-5), reconstructed-tissue irritation (10993-23), and in vitro hemocompatibility as device NAMs.

15. Additional organs and mucosal barriers

15.1 Thyroid follicle organoids and thyroid-on-chip [Research] (formerly Entry 139)

Human thyroid models complementing the biochemical thyroid battery with tissue-level hormone synthesis.

15.2 Adipose-on-chip and white/brown fat organoids [Research] (formerly Entry 140)

Adipose metabolism, endocrine signalling, and obesity–diabetes crosstalk beyond the glucose MPS line.

15.3 Adrenal and HPA-axis models [Research] (formerly Entry 141)

Steroidogenic tissue models extending H295R toward tissue context.

15.4 Urothelium-on-chip and bladder models [Research] (formerly Entry 142)

Barrier and infection models for urology and device contact.

15.5 Prostate organoids [Research] (formerly Entry 143)

Patient or iPSC prostate epithelium for oncology and endocrine questions.

15.6 Immune-competent and microbiome-containing skin models [Research] (formerly Entry 144)

Full-thickness skin with dendritic cells and optional microbiota for sensitisation follow-up and topical microbiome effects.

See also: Section 13 (Immunology and lymphoid models) for the dendritic-cell/immune component of this platform.

15.7 Wound-healing reconstructed skin [Case-by-case] (formerly Entry 145)

Re-epithelialisation and fibroblast-contraction models for devices and topicals.

15.8 Reconstructed oral, gingival, and vaginal mucosa [Case-by-case] (formerly Entry 146)

Standardised mucosal tissues for local tolerance of oral and feminine-care products.

16. Infection and microbiome systems

See also Section 9 (Nervous system, blood–brain barrier, and DNT) for neural and neurodegeneration work related to Entry 16.5.

16.1 Intestinal organoid infection models [Case-by-case] (formerly Entry 147)

Human gut organoids that support norovirus, rotavirus, and selected enteroviruses poorly modelled in standard laboratory animals.

See also: Section 7 (Specialised gastrointestinal models, 7.1–7.6) for the underlying organoid/chip platforms this infection application is built on.

16.2 Airway ALI infection models [Case-by-case] (formerly Entry 148)

Influenza, RSV, SARS-CoV-2, and other respiratory-pathogen systems on human ALI tissues.

See also: Section 3 (Specialised respiratory models, 3.1–3.6) for the underlying ALI/chip platforms.

16.3 Dynamic gut–microbiome simulators (SHIME, TIM, HuMiX) [Case-by-case] (formerly Entry 149)

Multicompartment in vitro guts with human microbiota. Not a whole human, but they replace many rodent microbiome colonisation studies.

See also: Section 7 (Specialised gastrointestinal models, 7.1–7.6).

16.4 Controlled human infection / challenge studies [Accepted (ethics-governed)] (formerly Entry 150)

Experimental medicine in volunteers. Clinical NAM, not preclinical, but directly human-relevant evidence.

16.5 Cell-based prion / protein-misfolding bioassays (e.g. scrapie cell assay-class methods; RT-QuIC, PMCA amplification assays) [Case-by-case] (new in Version 2.0; interim number 180)

Cell-based and cell-free amplification assays that detect misfolded prion protein species. Real-time quaking-induced conversion (RT-QuIC) was adopted as a diagnostic criterion by the European CJD Surveillance Network in 2017 and appears in the CDC's 2018 diagnostic criteria for sporadic Creutzfeldt–Jakob disease, with published sensitivity of roughly 90–98% and specificity of roughly 98–100% for CSF-based detection across multiple independently reproduced validation studies; skin- and other tissue-based RT-QuIC protocols have separate published validation. Automated scrapie cell assays and protein misfolding cyclic amplification (PMCA) are used similarly for infectivity/titre screening in research settings. These methods are recognised, third-party-validated tools for detection and titre screening; none is validated as a replacement for in vivo strain typing by incubation-period and neuropathology signature, which remains the residual for that narrower question.

See also: Section 9 (Nervous system, blood–brain barrier, and DNT, Entries 9.1–9.6) for whole-organism and neural-culture correlates; Entry 1.1 (Biochemical assays) for the generic assay class this specialises; Entry 14.12 (CFU-GM myelotoxicity assay) for an analogous specialised bioassay used as a first-line screen ahead of animal work in a different disease context.

Provenance: Added in Version 2.0 (interim number 180) after a gap identified while auditing a prion-disease project licence (Licence Watch series, Licence 73). Tag and description checked against independent evidence (CDC and European CJD Surveillance Network diagnostic-criteria adoption, and peer-reviewed validation studies) rather than resting on the compiler's assessment alone.

17. In silico models and computational platforms

17.1 Mechanistic computer simulation and systems modelling [Case-by-case] *(formerly Entry 151)*

PK/PD, neural-network dynamics, and multi-scale physiology models used to generate hypotheses and support dosing.

17.2 QSAR models [Accepted (endpoint-specific)] *(formerly Entry 152)*

Structure-based prediction of physicochemical, tox, and ADME properties. OECD principles apply. Accepted for many chemical screening and ICH M7 uses.

17.3 Machine-learning / AI predictive models [Case-by-case] *r(formerly Entry 153)*

Data-driven efficacy and safety ranking. Predictive quality tracks training data; not a substitute for a defined COU validation.

See also: External regulatory validation tracking appendix, item 1: FDA's own CDER statement (3 June 2026) on accepting the first in silico Drug Development Tool under ISTAND, for predicting drug-induced liver injury.

17.4 Digital twins [Research / case-by-case] *(formerly Entry 154)*

Virtual organs or patients for personalised simulation. Includes morphogenesis twins used with gastruloids. Maturity varies widely by organ.

17.5 Virtual clinical trials (in silico trials) [Case-by-case] *(formerly Entry 155)*

Simulated cohorts for dose, enrichment, and trial design. Used to de-risk, not to skip, first-in-human studies.

17.6 Pulmonary digital twins and in silico aerosol deposition [Case-by-case] *(formerly Entry 156)*

Airway CFD and particle-deposition models for inhaled gene/cell therapy and device design.

17.7 AlphaFold and AlphaFold 3 [Research / enabling] *(formerly Entry 157)*

Structure and interaction prediction for proteins, ligands, and nucleic acids. Accelerates design; does not by itself demonstrate safety or efficacy.

17.8 Spatial morphogenesis and symmetry-modelling platforms [Research] *(formerly Entry 158)*

AI 3D developmental modelling used with embryo-like systems.

17.9 PBPK / PBTK and IVIVE [Accepted (as modelling evidence)] *(formerly Entry 159)*

Physiologically based models linking in vitro clearance and binding to human exposure. Central to NGRA and modern MIDD. Tools include Simcyp, GastroPlus, PK-Sim.

17.10 Public computational NAM platforms [Accepted (screening)] *(formerly Entry 160)*

OECD QSAR Toolbox, OPERA, EPA CompTox / ICE, CATMoS acute-toxicity models. Used for screening and analogue finding.

17.11 ICH M7 (Q)SAR mutagenicity pair [Accepted] *(formerly Entry 161)*

Two complementary expert-rule and statistical systems (e.g. DEREK, Sarah Nexus, CASE Ultra, VEGA) for impurity mutagenicity.

17.12 ToxCast / Tox21 high-throughput bioactivity [Accepted (screening)] *(formerly Entry 162)*

Large public in vitro bioactivity libraries used for priority-setting and endocrine models.

17.13 Model-informed drug development and population PK [Accepted] *(formerly Entry 163)*

MIDD packages routinely accepted by FDA/EMA to justify dose, paediatric bridging, and sometimes reduced animal or clinical burden.

17.14 In silico cardiac action-potential models [Accepted (with CiPA)] *(formerly Entry 164)*

The modelling arm of CiPA; virtual tissue and population AP models for proarrhythmia risk.

17.15 AOP knowledge bases [Enabling] *(formerly Entry 165)*

AOP-Wiki and related networks used to structure NAM evidence. Knowledge infrastructure, not a test.

17.16 Organoid intelligence [Research] (formerly Entry 166)

Brain organoids coupled to electronics for network computation studies. Research only; no regulatory safety COU.

18. Hybrid, omics, observational, and clinical items

18.1 Ex vivo tissue slices (general) [Case-by-case] (formerly Entry 167)

Organ-agnostic class covering liver, lung, intestine, kidney, and tumour slices already listed by organ.

18.2 Single-cell RNA-seq and spatial transcriptomics [Enabling] (formerly Entry 168)

High-resolution human-tissue profiling for target, biomarker, and mechanism work.

18.3 Proteomics, phosphoproteomics, and multi-omics integration [Enabling] (formerly Entry 169)

Pathway-level readouts that feed AOPs and digital twins.

18.4 Data repositories and biobanks [Enabling] (formerly Entry 170)

Human Cell Atlas, PeptideAtlas, and tissue banks that supply cells and training data. Enabling infrastructure.

18.5 Human post-mortem imaging [Observational] (formerly Entry 171)

Ex vivo MRI/DTI and related modalities. Observational human data.

18.6 Epidemiological studies [Observational] (formerly Entry 172)

Population evidence on risk and outcomes. Not a preclinical test, but human-relevant evidence under ASPA replacement logic.

18.7 Human volunteer studies [Accepted (clinical)] (formerly Entry 173)

Controlled clinical pharmacology, biomarker, and tolerance studies.

18.8 Wearable sensors and digital biomarkers [Case-by-case / clinical] (formerly Entry 174)

Non-invasive human physiology and behaviour streams.

See also: External regulatory validation tracking appendix, item 1: FDA's own CDER statement (22 September 2026) on accepting wearable sensor-derived foot-drop metrics under ISTAND as a longitudinal outcome measure in a rare neurological condition.

18.9 Synthetic and virtual medical simulators [Accepted (training / devices)] (formerly Entry 175)

SynDaver, TraumaMan, Anatomage and similar trainers. Replace animals in education and some device handling, not in IND tox.

18.10 Microdosing / Phase 0 [Accepted] (formerly Entry 176)

Sub-therapeutic human exposure with AMS or imaging. Direct human PK before full Phase 1.

18.11 Ex vivo human skin and living skin equivalents on biopsies [Case-by-case] (formerly Entry 177)

Volunteer or surgical skin for formulation, irritation, and absorption work beyond OECD 428.

18.12 Experimental medicine and quantitative sensory testing [Case-by-case] (formerly Entry 178)

Hypothesis-testing studies in humans that can replace some animal efficacy models in pain and neuroscience.

18.13 Real-world evidence and foreign human safety data [Accepted (context-specific)] (formerly Entry 179)

Explicitly cited in the FDA April 2025 mAb roadmap as evidence that can reduce duplicative animal studies where the product already has human use.

What this catalogue still cannot do

A numbered list is not a waiver. Organoids and chips remain incomplete relative to a whole organism: partial immunity, limited innervation, immature iPSC phenotypes, finite culture life, batch variability, and incomplete chronic multi-organ coverage. Accepted OECD methods replace specific classification tests, not an entire medicine's nonclinical package. ISTAND or agency "interest" is not qualification. Animal-component-free claims fail if the culture matrix is still mouse sarcoma extract.

Before relying on any entry under ASPA 2A(a) or in an IND/CTA: define the endpoint, state the context of use, check the current OECD/ICH/pharmacopoeia/agency text, and record why the method is scientifically satisfactory for that question, and what residual uncertainty remains.

Appendix: External regulatory validation tracking (new in Version 2.0)

This appendix tracks external, real-world evidence of NAM acceptance by regulators, gathered from outside this catalogue's own entry-by-entry research, so that entries whose "Accepted" or "Case-by-case" tags rest partly on live regulatory pipelines can point to current, dated tracking rather than a static claim. Item 1's underlying pathway and database have been confirmed directly against FDA's own IStand programme page; item 2 remains an unconfirmed report; item 3 is independently corroborated across multiple sources and treated as verified. Figures in item 1 are drawn from a third-party compilation of the primary database, not this compiler's own line-by-line extraction, and specific figures should still be checked at the primary database (linked below) before being relied upon in a submission, dossier, or Licence Watch report.

1. FDA IStand pipeline velocity (as of September 2026)

Source: Elliot Fisher (Curi Bio), LinkedIn post "iStand is accelerating – Sept 2026 Update," which states it compiles every project in FDA's public Drug Development Tool (DDT) Qualification Project Search database (the IStand pathway), retrieved by the poster on 21 September 2026.

Primary-source confirmation: the IStand programme itself, and the existence of the underlying database, are confirmed directly against FDA's own programme page, content current as of 22 September 2026: <https://www.fda.gov/drugs/drug-development-tool-ddt-qualification-programs/innovative-science-and-technology-approaches-new-drugs-istand-program>. That page names and links the primary database directly (CDER & CBER's DDT Qualification Project Search, at <https://force-dsc.my.site.com/ddt/s/>), which is the correct place to independently re-verify any individual figure below. This confirms the database and pathway are real and current; it does not itself re-derive or independently check each specific number reported by the secondary source, which a reader should still do at the primary database before relying on any single figure.

- 37 IStand projects are now public: 26 Letters of Intent (LOIs) accepted, 7 not accepted, 4 pending decision (33 decided in total), up from 20 disclosed technologies as of January 2026 (per the same poster's earlier count, attributed to Michael Phelan, PhD).
- Median time from LOI submission to acceptance has fallen from 267 days (submissions before 2025, n=8) to 146 days (submissions from 2025 onward, n=18). FDA's own stated target remains 90 days, so the reported figure, while improved, has not yet reached it.
- By technology class: organ-chip and engineered 3D tissue NAMs are reported at 11 accepted out of 11 decided (100%); this is the figure cited at Entry 1.12. Cell-based assays, spheroids, and other NAMs are reported at 5 accepted, 4 not accepted, 3 pending. In silico NAMs and clinical AI tools are reported at 10 accepted, 3 not accepted, 1 pending.
- 3 of the 37 projects have an accepted Qualification Plan (the stage after LOI acceptance); none has reached full qualification. The three reported are: Integral Molecular (in silico/clinical AI class, reported 410 days LOI-to-decision), AstraZeneca (in silico/clinical AI class, reported 205 days), and Emulate (organ-chip class, the Liver-Chip programme cited at Entry 10.1, reported 217 days).
- By sponsor type across all 37 projects: 24 NAM/tool developers, 8 academic/hospital/non-profit bodies, 3 pharmaceutical companies, 1 contract research organisation, and 1 not listed. The poster's own reading, that tool developers and platform companies rather than drug sponsors are driving most IStand submissions, is noted here as the source's interpretation, not independently assessed by this compiler.

Two individual, dated FDA announcements found directly on the primary IStand page corroborate specific, current activity in this pipeline and are cited at the relevant entries:

- "FDA Accepts Wearable Sensor-Derived Foot Drop Metrics Under IStand as Longitudinal Outcome Measures in Rare Neurological Condition," CDER Statement, 22 September 2026. Cited at Entry 18.8.

- “FDA Accepts First In Silico Drug Development Tool Under IStand Program to Help Predict Drug-Induced Liver Injury,” CDER Statement, 3 June 2026. Cited at Entry 17.3, and relevant by endpoint to the hepatotoxicity entries in Section 10 (10.1, 10.8, 10.9).

This data is cited at Entries 1.12, 10.1, 17.3, and 18.8, where it updates and dates claims already present in those entries. It does not change any readiness tag in this catalogue: “Case-by-case” already reflected that IStand interest and even an accepted Qualification Plan fall short of full qualification, and that remains true on this evidence.

2. FDA-documented NAM use in approved drug applications

Reported to the compiler as an FDA public database or resource listing documented cases where specific non-animal methods were used and accepted within real, approved drug applications, not only validated in principle. As described, it currently lists 23 examples across named approved drugs, including SYMDEKO, VEKLURY (remdesivir), COBENFY, and MYQORZO, with the specific method used in each case (BCOP, DPRA, EpiSkin, h-CLAT, and KeratinoSens all reported as appearing), and is framed by its own text as illustrative rather than an exhaustive list, stating that it “does not constitute FDA guidance or approval.” It is reported as last updated 18 September 2026, referencing FDA guidance from March 2026.

Status: still unverified. The FDA IStand programme page checked above is the correct primary page for the IStand pipeline itself, but it does not host or link a resource matching this description (a database of named approved drugs and the specific non-animal method used in each). This is evidently a separate FDA resource, possibly a CDER webpage on NAM case examples distinct from the IStand/DDT pathway, and checking the IStand page has not confirmed it. It is recorded here, with cross-references at Entries 11.1, 11.2, 11.3, 11.6, and 11.8, so it is not lost. Consistent with this catalogue’s own gap-driven-addition standard (see “How to use this catalogue”), it must not be cited in any Licence Watch report or elsewhere as a settled fact until its actual URL is obtained and the specific drug/method pairings are independently confirmed against it.

3. Botulinum toxin potency: NMJ-based functional replacement (cross-checked, September 2026)

Unlike items 1 and 2, this item is corroborated across independent sources rather than resting on one report, and is treated accordingly: cited directly at Entry 14.10, not held to “unverified” status.

- FDA/CDER accepted a Letter of Intent into the IStand programme (15 September 2026) for Curi Bio’s human iPSC-derived 3D neuromuscular junction (NMJ) assay, as a non-animal-method alternative to the Mouse Lethality Bioassay for botulinum neurotoxin potency testing. Reported directly by Curi Bio (curibio.com) and independently by BioSpace and a Business Wire pickup carried by regional press (Rutland Herald); all three accounts are consistent.
- The underlying platform has independent, peer-reviewed support: Geisse (Curi Bio) and Smith (University of Washington) et al., “An automated platform for simultaneous, longitudinal analysis of engineered neuromuscular tissues for applications in neurotoxin potency testing,” accepted January 2025, published open access (PMC11815696, CC BY), demonstrating dose-dependent loss of function on botulinum toxin exposure and wash-in/wash-out sensitivity to fast-acting neurotoxins.
- Federal research funding independently signals this as a recognised priority, not only a single company’s claim: NIH NCATS and FDA’s Center for Food Safety and Applied Nutrition co-sponsored funding opportunity RFA-TR-22-031, “Botulinum Toxin Potency Assay using Tissue Chips (BoT PATCH),” explicitly aimed at qualifying neuromuscular-junction tissue chips as a Drug Development Tool.
- A named, real-world commercial application exists: Curi Bio, Genetox, and DreamCIS announced a Memorandum of Understanding (February 2024) to use the NMJ model as a non-animal potency assay in advancing Genetox’s botulinum toxin product BOTAONE through FDA approval.

Current status, precisely stated: this NMJ-based functional modality is at the ISTAND Letter-of-Intent stage only: accepted for review, not yet qualified, and not yet the subject of an accepted Qualification Plan. It is a distinct modality from, and at an earlier readiness stage than, the enzymatic/SNAP-25 cell-based assays this catalogue already tags “Accepted (many jurisdictions)” at Entry 14.10. The two should not be conflated: an applicant citing Entry 14.10 for an enzymatic assay stands on settled ground; an applicant wishing to cite the NMJ-functional approach specifically is citing an LOI-stage method, and should say so.

Cross-reference index (new in Version 2.0)

Entries below are filed under one section by method class but carry a “See also” pointer to another section by endpoint or organ. Listed here together for quick lookup; each entry remains under its original section and number.

Entry	Title	Filed under	See also
2.2	Leukemia-on-chip / bone marrow-on-chip	Sec. 2 (Oncology)	Sec. 13 (Immunology); Entry 14.12
10.4	Multi-organ toxicity MPS	Sec. 10 (Toxicology MPS)	Sec. 13 (Immunology)
10.11	Precision-cut kidney and lymphoid slices	Sec. 10 (Toxicology MPS)	Sec. 13 (Immunology)
14.3–14.7	CRA; MAPPs; HLA/in silico immunogenicity; ADA/T-cell proliferation; complement panels	Sec. 14 (Biologics)	Sec. 13 (Immunology)
14.12	CFU-GM myelotoxicity assay	Sec. 14 (Biologics)	Entry 2.2; Sec. 10 (Toxicology MPS)
15.6	Immune-competent and microbiome-containing skin models	Sec. 15 (Additional organs)	Sec. 13 (Immunology)
16.1–16.3	Intestinal organoid infection; Airway ALI infection; gut–microbiome simulators	Sec. 16 (Infection systems)	Sec. 7 (GI models); Sec. 3 (Respiratory)
16.5	Cell-based prion / protein-misfolding bioassays	Sec. 16 (Infection systems)	Sec. 9 (Nervous system); Entry 1.1; Entry 14.12

Document scope and version history

Compiled and maintained by Georgina Rednall for the Alliance for Cruelty Free Science as a single living working catalogue, begun as a research project to support the case for ending animal testing. Add methods, correct status tags, and strike entries that have been superseded as it develops. It is not an official MHRA, FDA, EMA, or OECD list. Status moves quickly; verify before use.

Version 1.0 (August 2026): structural corrections to section placement and a rebuilt index; no entries added, removed, or reworded.

Version 2.0 (September 2026, this revision): entries renumbered within their sections, with former numbers shown beside each entry; added Entry 16.5 and the External regulatory validation tracking appendix; updated Entries 1.12, 10.1, 11.1, 11.2, 11.3, 11.6, 11.8, 14.10, 17.3, and 18.8; added “See also” cross-references and the Cross-reference index; added the gap-driven additions and numbering rules. No entry removed and no existing readiness tag changed. Full details are on the “Changes since Version 1.0” page at the front of this catalogue. Consolidates interim working revisions 1.1 to 1.6 (September 2026).

