Advancing the Use and Acceptance of the Human Thyroid Microtissue Assay

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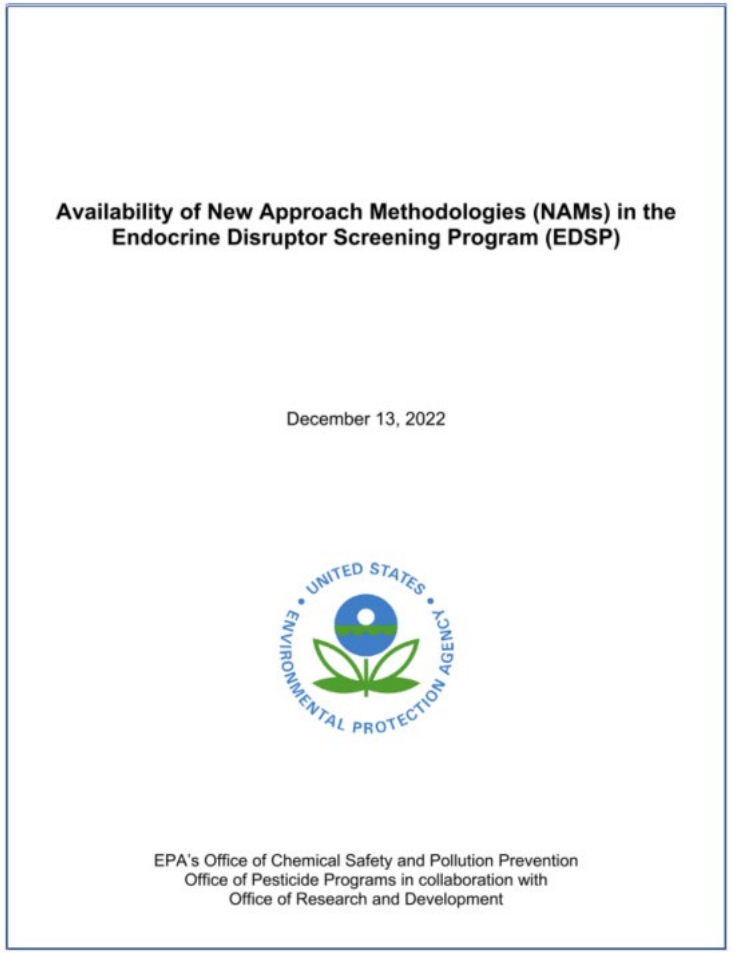
Society of Toxicology 63rd Annual Meeting
Advances in New Approach Methods for Thyroid Toxicity Testing
March 11, 2024

Disclaimer: The views expressed are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. There are no conflicts to declare.

Adoption of New Approach Methods in the EDSP


Availability of New Approach Methodologies (NAMs) in the Endocrine Disruptor Screening Program (EDSP)

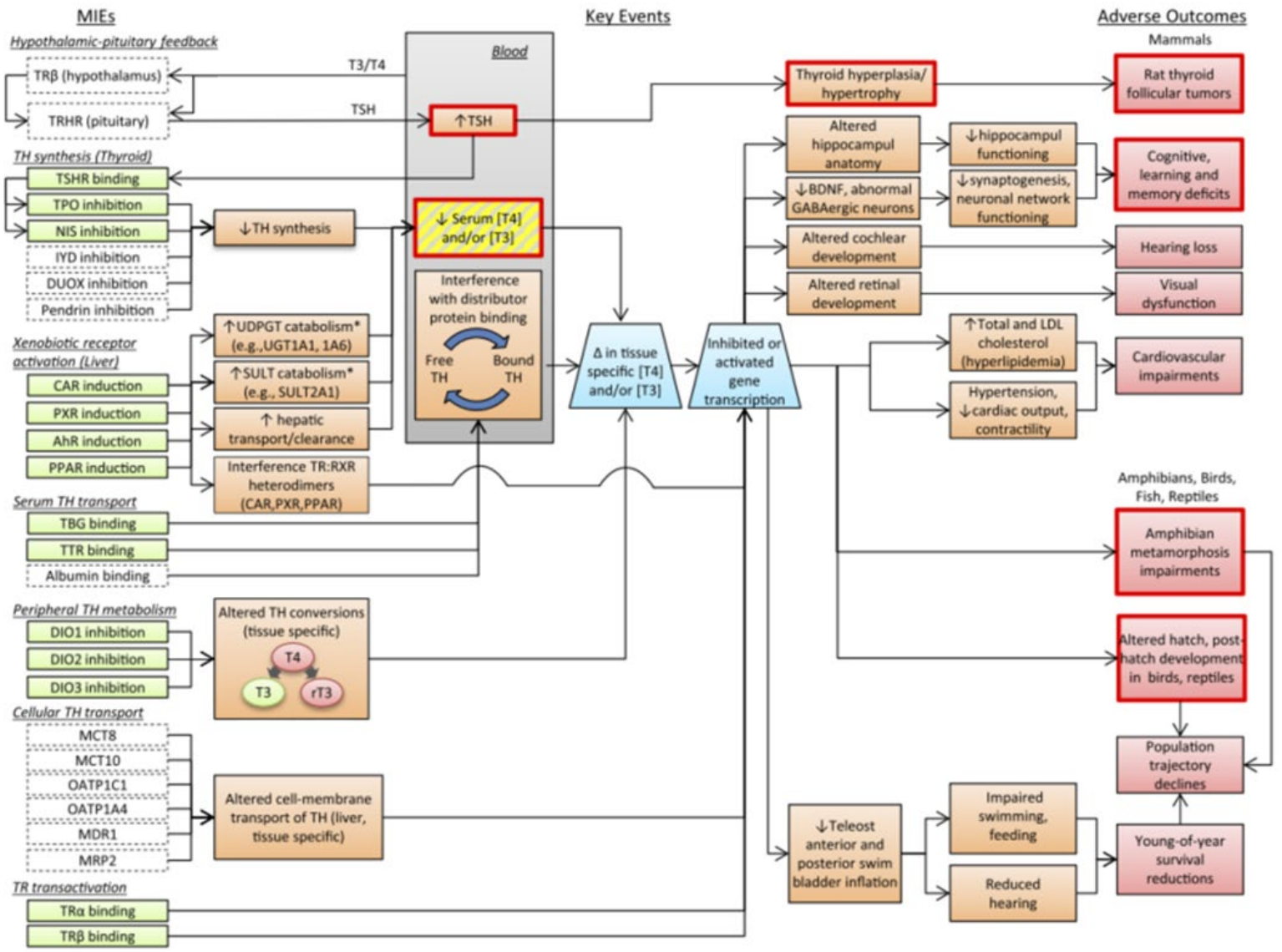
December 13, 2022

EPA's Office of Chemical Safety and Pollution Prevention
Office of Pesticide Programs in collaboration with
Office of Research and Development

- The EDSP evaluates chemical effects on estrogen, androgen, and thyroid endocrine pathways.
- The validated Estrogen Receptor (ER) and Androgen Receptor (AR) pathway models may be used as an alternative to the Tier 1 screening assays.
- Additional NAMs including Integration of Bioactivity Exposure Ratios (IBER), QSAR models for ER and AR activity, and SeqAPASS for cross-species extrapolation may be used as Other Scientifically Relevant Information (OSRI) to prioritize chemicals for screening and hazard assessment.
- **Continue development of a Thyroid Pathway Framework that includes *in vitro* assays for thyroid-relevant targets to produce an integrated prediction model that may be used as OSRI for thyroid system perturbations.**

Thyroid Adverse Outcome Pathway Network

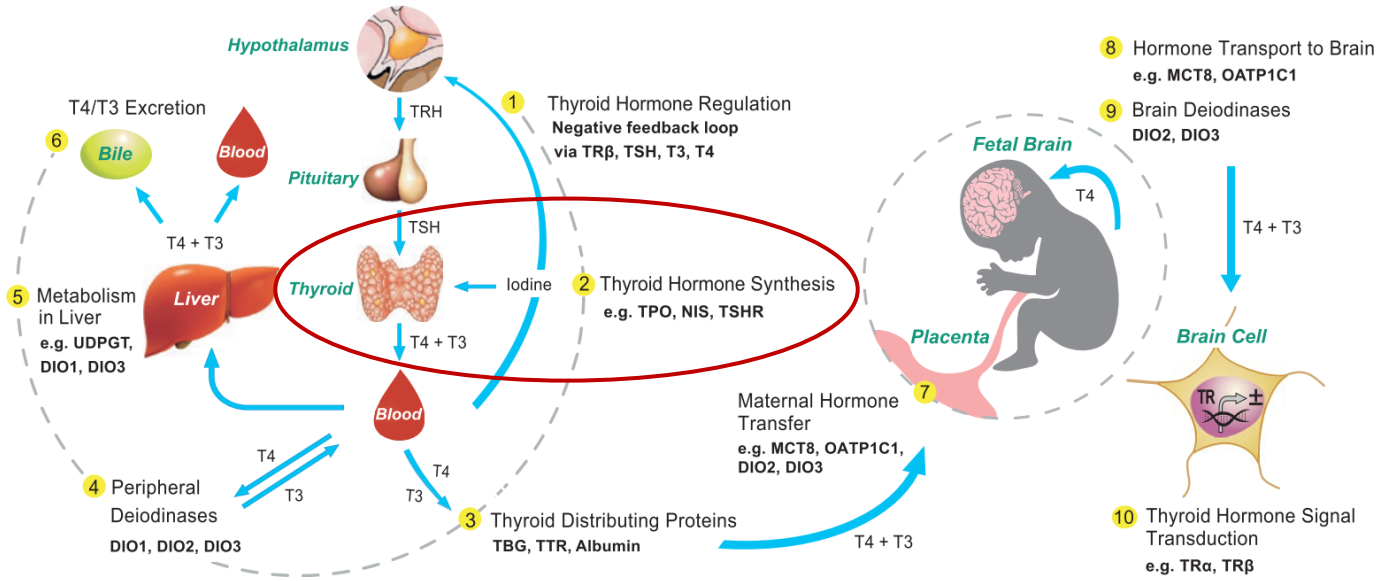
- Most of the *in vitro* assays currently undergoing validation primarily provide coverage of MIEs.
- Human-relevant *in vitro* assays for 'key events' are needed for an integrated Thyroid Pathway prediction model.



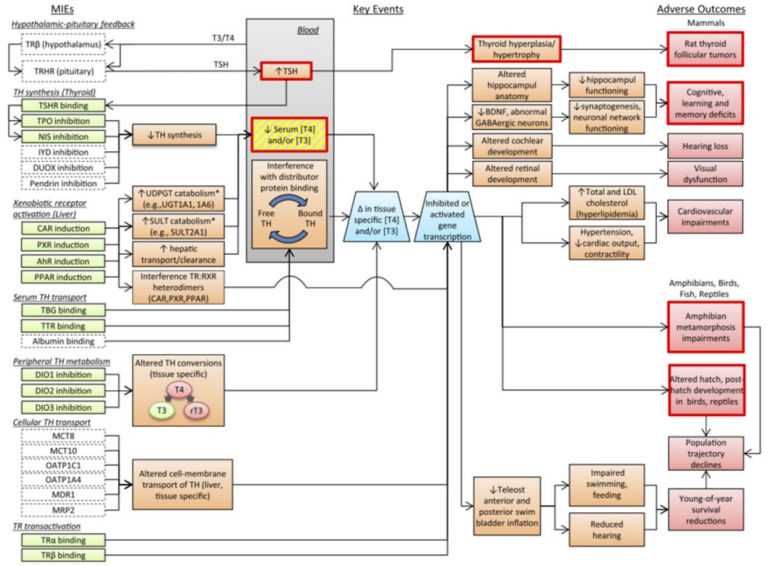
DOI: 10.1289/EHP5297

Thyroid 'MIE' Assays Do Not Directly Measure the 'Key Event' for Thyroid Hormone Synthesis

Sites of Interference for Thyroid Disrupting Chemicals



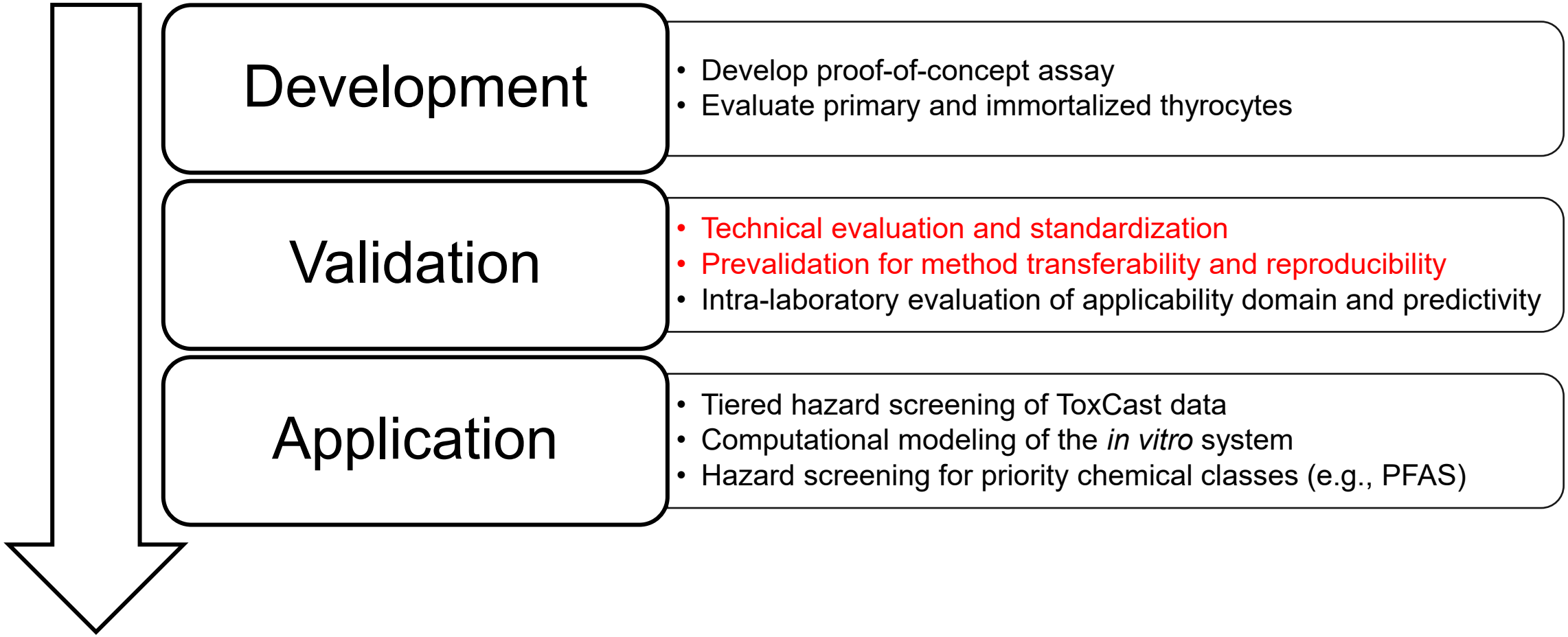
Thyroid AOP Network



Thyroid MIE	Assay	Environmental Chemicals Screened	Active Chemicals	% Active	Reference
TSHR	Engineered Cell Line	7871	825	10	TCPL: TOX21_TSHR_Agonist, TOX21_TSHR_Antagonist
TPO	Microsomal Enzyme	1074	150	14	K. Paul Friedman et al, ToxSci, 151(1), 2016, 160-180
NIS	Engineered Cell Line	293	137	47	J. Wang et al, EnvironSciTechn, 52, 2018, 5417-5426
NIS	Engineered Cell Line	768	167	22	J. Wang et al, Environment International, 126, 2019, 377-386
DIO 1	Recombinant Enzyme	292	18	6	M. Hornung et al, ToxSci, 162(2), 2018, 570–581
DIO 1	Recombinant Enzyme	1819	139	8	J. Olker et al, ToxSci, 168(2), 2019, 430-442
IYD	Recombinant Enzyme	1825	148	8	J. Olker et al, Toxicol In Vitro. 2021 Mar;71:105073.

DOI: 10.1210/endocr/bqaa106; DOI: 10.1289/EHP5297

Human Thyroid Microtissue Assay



Goal: Establish a validated test method for human thyroid hormone disruption.

Filling Technology Gaps for *In Vitro* Thyroid Testing

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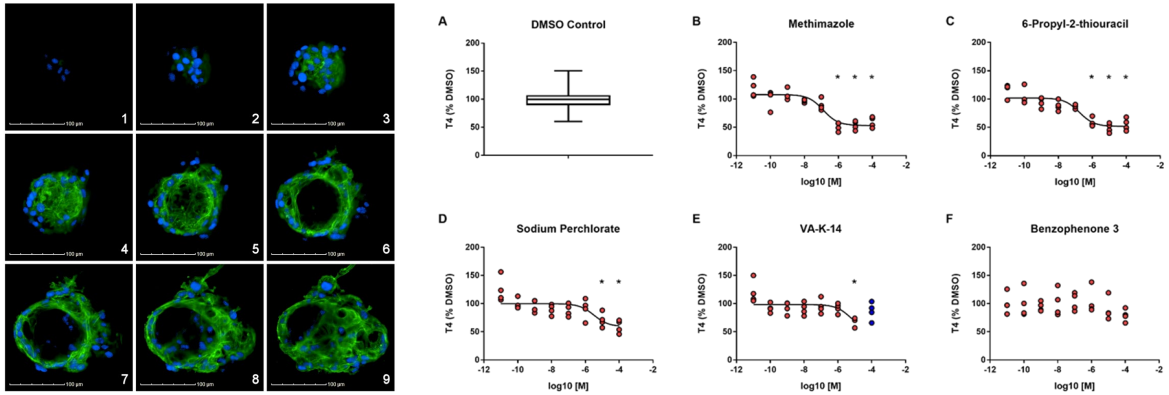
INSCREENEX

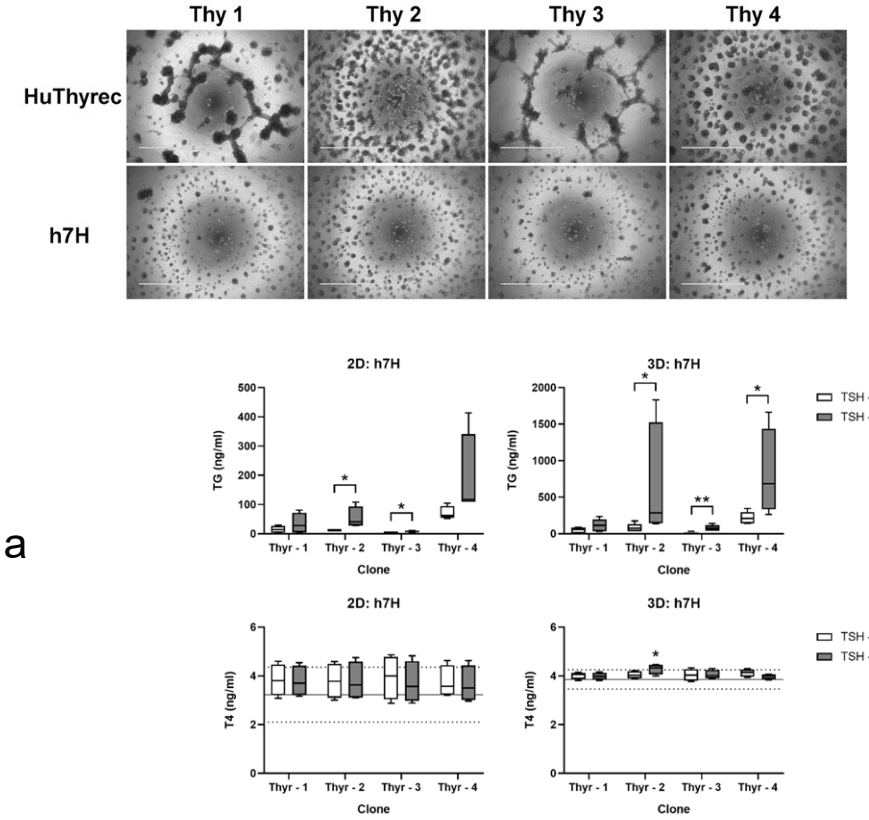
Development of an *In Vitro* Human Thyroid Microtissue Model for Chemical Screening

Chad Deisenroth ,*,[1] Valerie Y. Soldatow,[†] Jermaine Ford,[‡] Wendy Stewart,* Cassandra Brinkman,* Edward L. LeCluyse,[†] Denise K. MacMillan,[‡] and Russell S. Thomas *



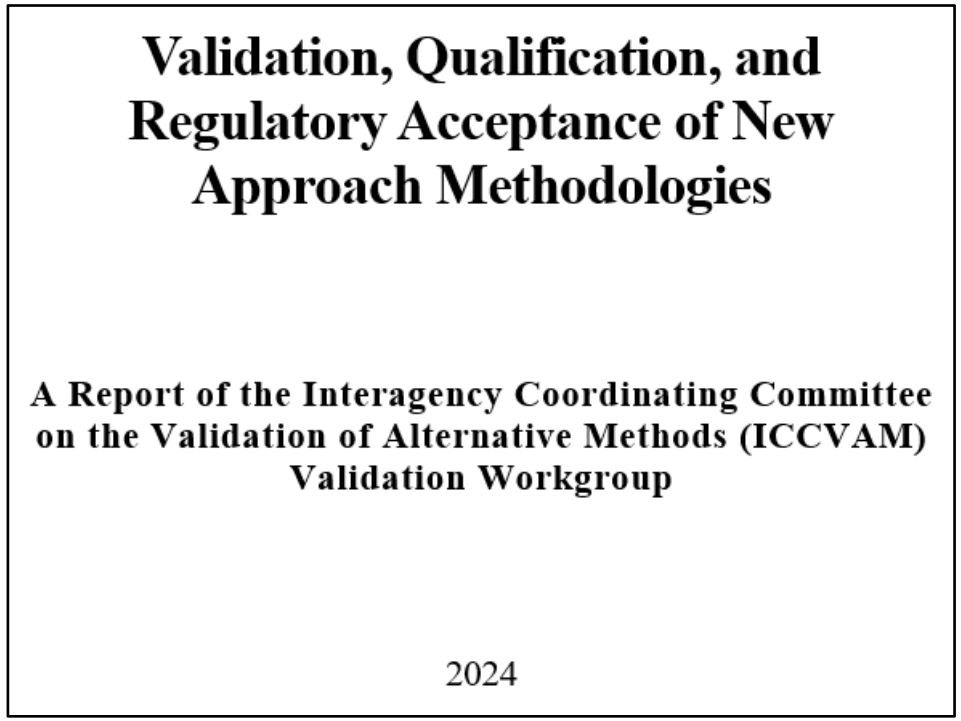
Characterization of Novel Human Immortalized Thyroid Follicular Epithelial Cell Lines

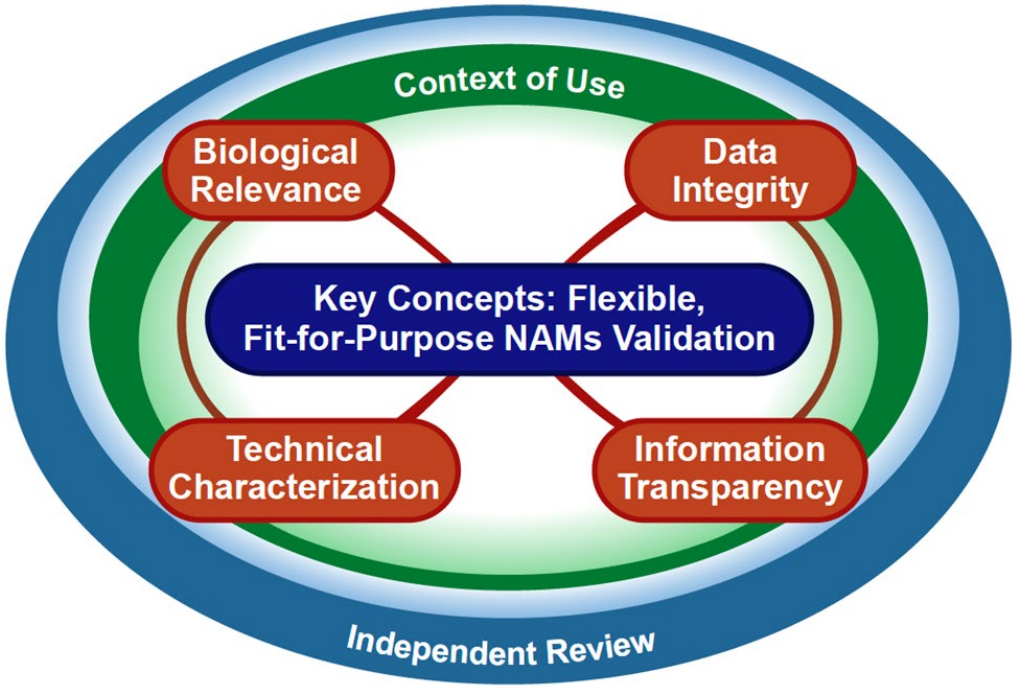
Kristen Hopperstad,[1,*] Theresa Truschel,[2,*] Tom Wahlicht,[2] Wendy Stewart,[1] Andrew Eicher,[1] Tobias May,[2] and Chad Deisenroth[1,†]



- A ‘key event’ assay designed to evaluate thyroid hormone disruption as a mode-of-action for endocrine-related hazard screening.
- Established commercial sources of primary human thyrocytes and immortalized cell lines.

Increasing Confidence in the Human Thyroid Microtissue Assay as a New Approach Method



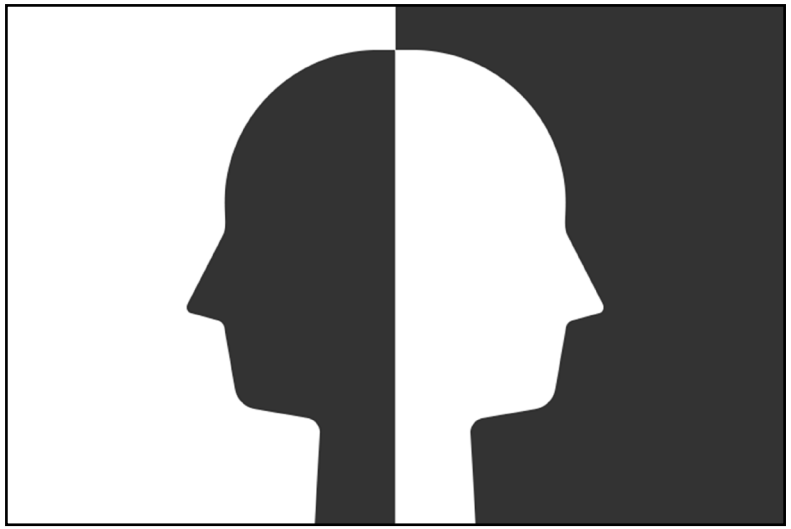


- Guided by underlying principles of OECD GD 34, a framework to validate NAMs that are fit-for-purpose, reliable, and relevant to the species of interest.
- Intended to be a modular and flexible approach to test method validation that accommodates shifting trends in assay technologies and applications.
- Reduce the time and cost of validation to accelerate regulatory adoption and implementation.

https://doi.org/10.22427/NICEATM-2

Standardizing Organotypic Assays is Challenging

"I want an assay that is reproducible"



"I want an assay that predicts a range of human responses"

How do technical precision and biological variability co-exist?

Goal: Minimize technical variability to increase confidence in the 'true' biological performance variability.

Standardization of the Human Thyroid Microtissue Assay

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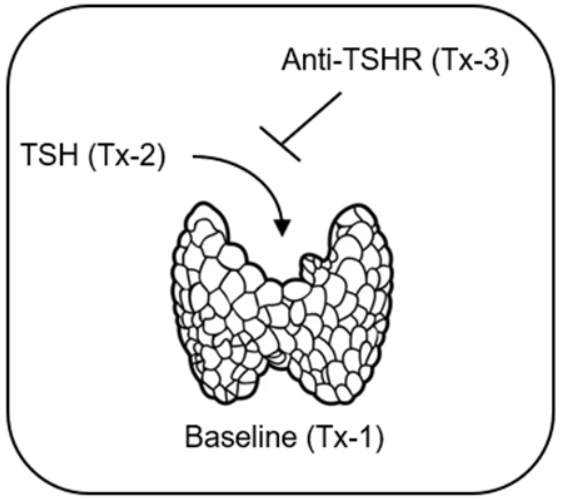
Toxicological Sciences, 2024, 1–19
https://doi.org/10.1093/toxsci/kfae014
Advance Access Publication Date: February 4, 2024
Research article

Technical evaluation and standardization of the human thyroid microtissue assay

Briana Foley,[1] Kristen Hopperstad,[1] John Gamble,[1,2] Scott G. Lynn,[3] Russell S. Thomas,[1] Chad Deisenroth[1,*]



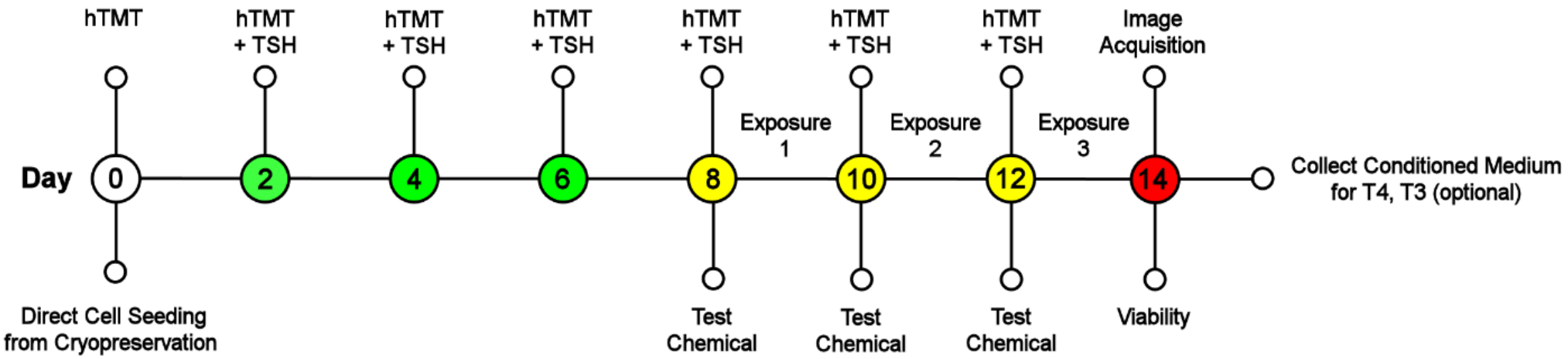
Objectives: 1) Define technical parameters for donor procurement, thyrocyte qualification, and assay performance, 2) Set benchmark ranges for reference chemical responses.

Donor Cohort Demographic Summary

Donors	32
Age	34 (17-61)
Sex	Male (24), Female (8)
Race	Caucasian (25), African American (7)
BMI	28 (18-37)

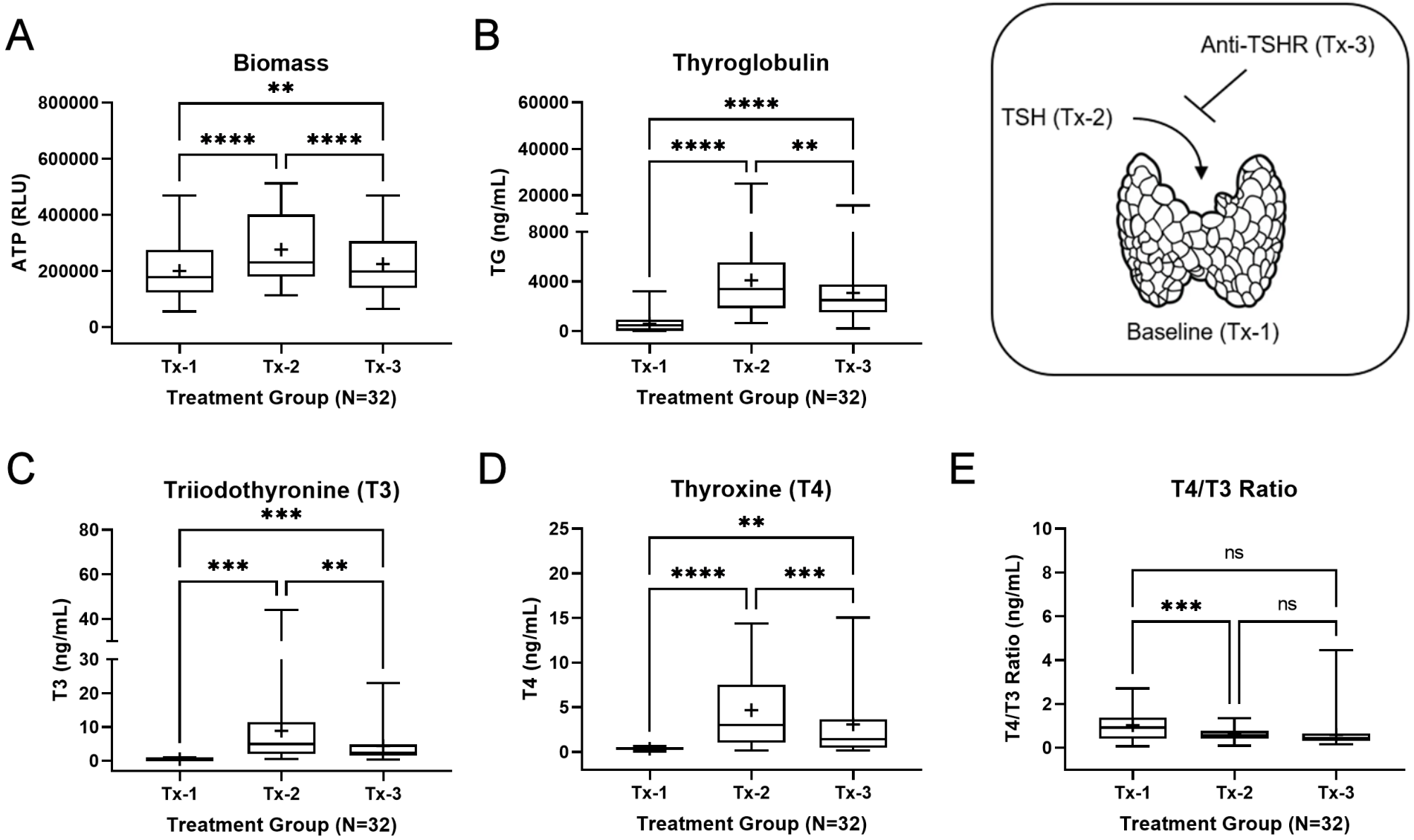
- Microtissue Morphology
- Microtissue Biomass
- TSH Receptor Sensitivity
- Thyroglobulin Synthesis
- Hormone Synthesis
- Reference Chemical Response

Human Thyroid Microtissue Assay v2.0

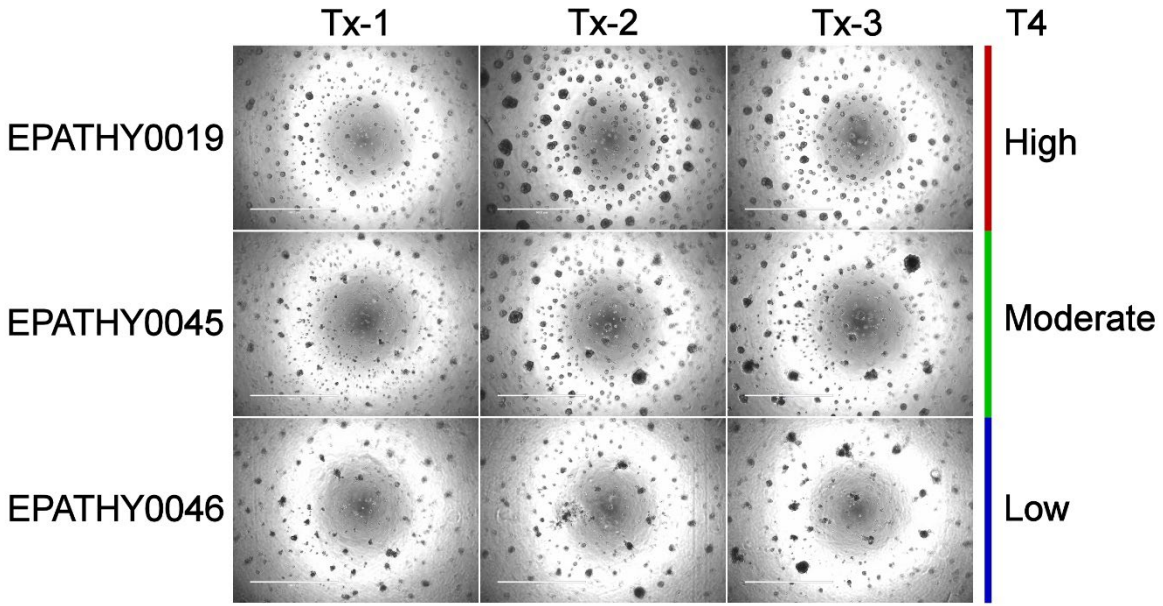


Protocol modified to enhance performance and improve durability for method transfer.

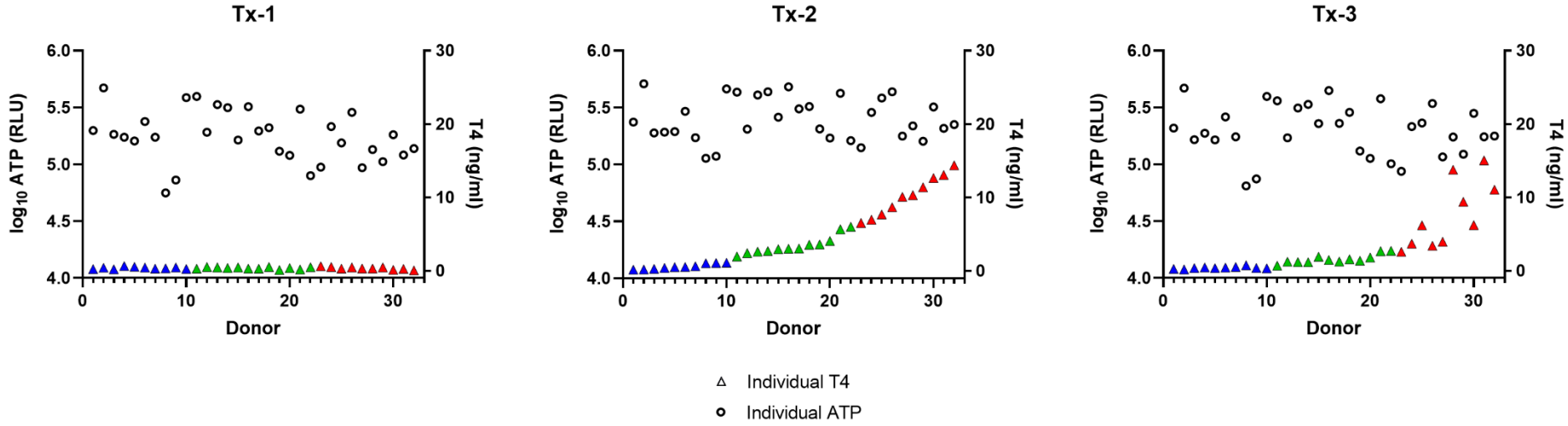
Distinct Treatment Group Effects Across Endpoints



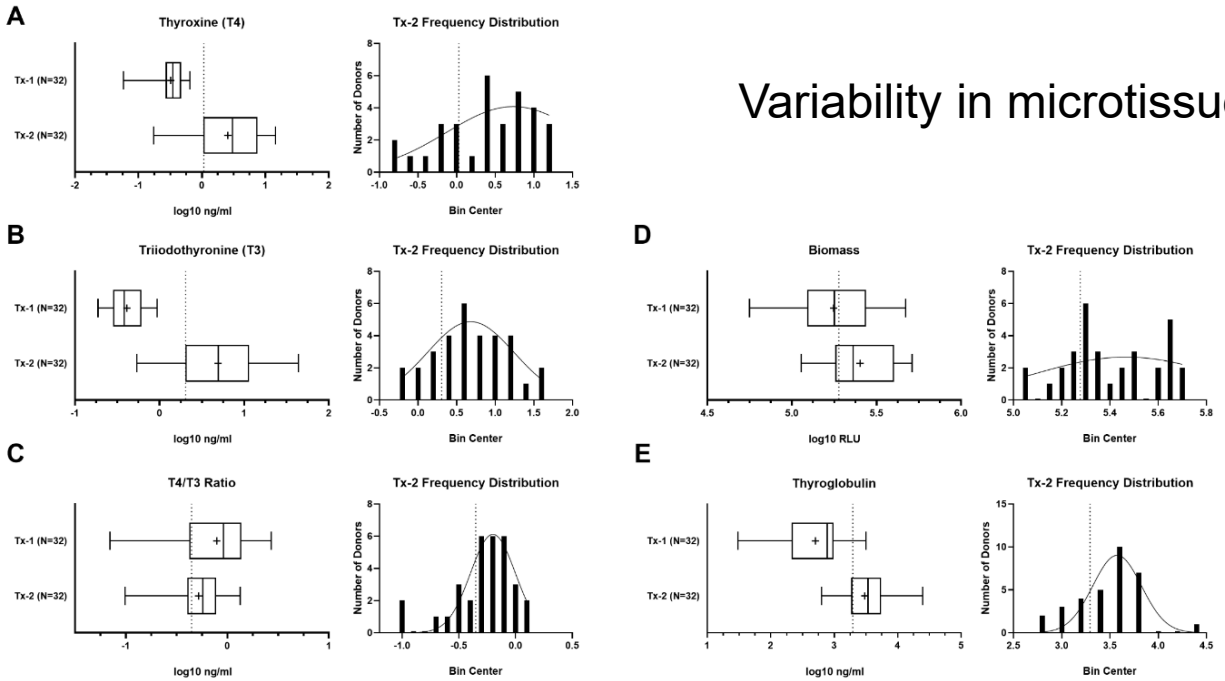
Microtissue Morphology and Biomass



- Donors exhibit a wide range of hormonogenic competence.
- No clear relationship between microtissue size or morphology and hormone synthesis.



Donor Qualification – Setting Minimum Acceptance Criteria for Hormonogenic Competence

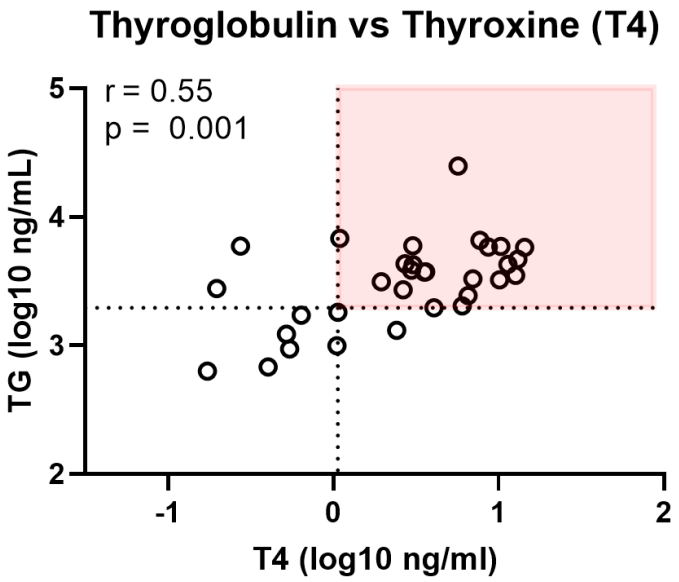


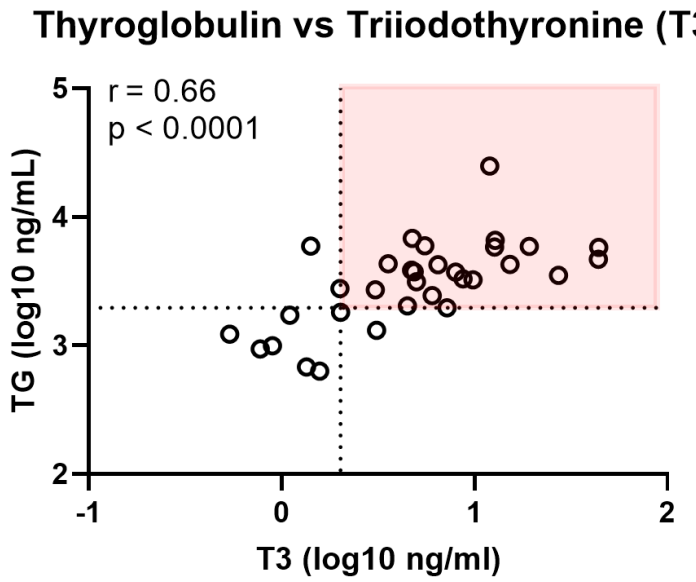
Variability in microtissue performance evaluated.

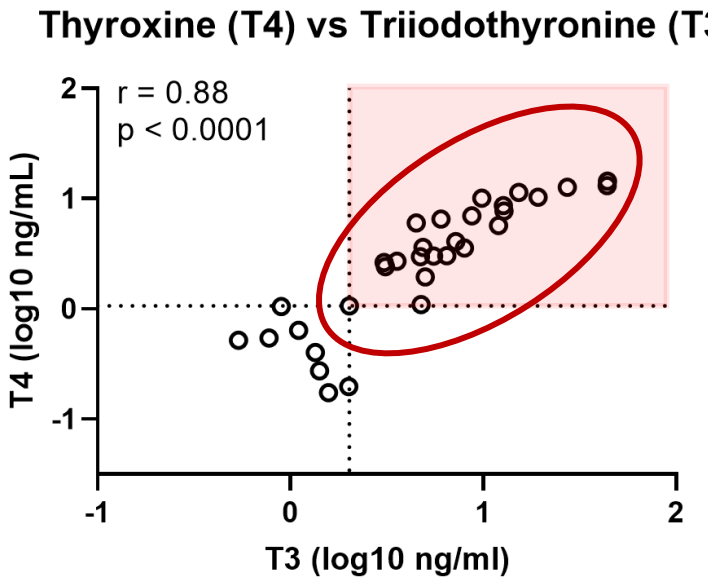
	Donor thyrocyte qualification				
Tx-2 99% CI	**Biomass (RLU)**	**Thyroglobulin (ng/ml)**	**T4 (ng/ml)**	**T3 (ng/ml)**	**T4/T3 ratio (ng/ml)**
Median	230 197	3405	3.03	4.94	0.57
Lower confidence limit	189 321	1961	1.07	2.02	0.45
Criteria	≥180 000	≥1900	≥1.0	≥2.0	≥0.4
Priority	Optional	Optional	Required	Recommended	Optional

Lower confidence limits used to establish minimum donor acceptance criteria.

Donor Qualification - Curation of the Donor Cohort to Emphasize Hormonogenic Competence







- Thyroxine (T4) vs Triiodothyronine (T3) exhibit the cleanest binning for donor-based performance.
- Data suggests up to 25% of donors would not qualify for use in the assay.

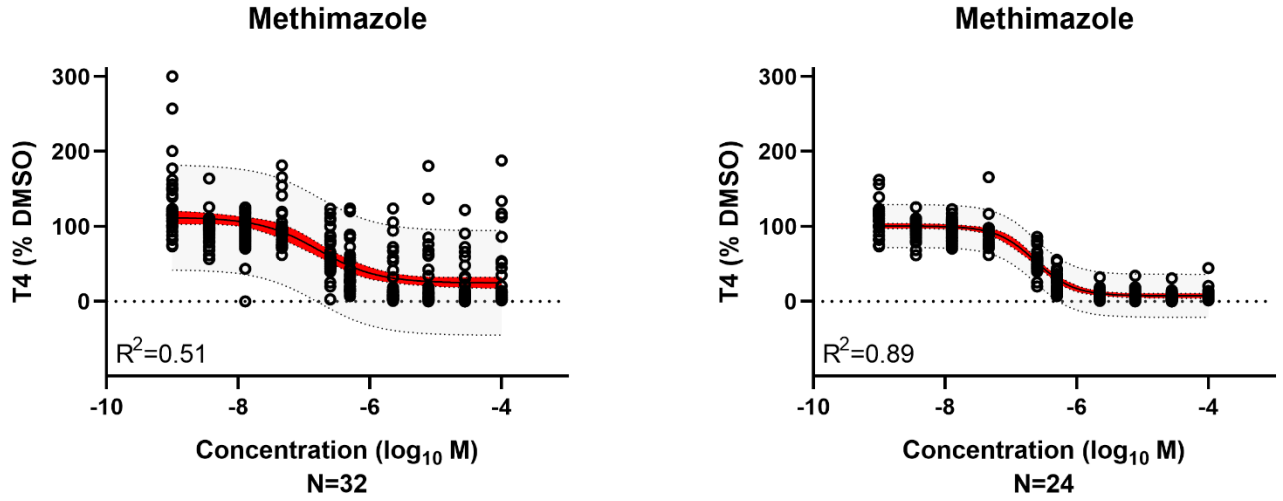
Assay Technical Performance Metrics

		Total cohort ($N = 32$)		Qualified cohort ($N = 24$)	
Solvent (DMSO)	Performance metrics	T4 median (±MAD)	T4 range	T4 median (±MAD)	T4 range
0%	Dynamic range (rS/B)	7.0 (±8.7)	0.5–211.8	9.5 (±6.3)	3.9–211.8
	Precision (rCV)	8.3 (±6.8)	1.2–51.7	7.9 (±6.8)	1.2–21.4
	Screening quality (rZ′-factor)	0.56 (±0.42)	−823.94–0.94	0.64 (±0.35)	−0.57–0.94
0.5%	Dynamic range (rS/B)	9.9 (±8.5)	0.5–508.8	11.6 (±7.0)	2.6–508.8
	Precision (rCV)	11.8 (±6.1)	0.6–60.3	10.6 (±5.4)	0.6–24.0
	Screening quality (rZ′-factor)	0.53 (±0.37)	−17.50–0.94	0.61 (±0.25)	0.00–0.94

Technical reproducibility is more strongly supported in the variable-donor platform when using qualified donors.

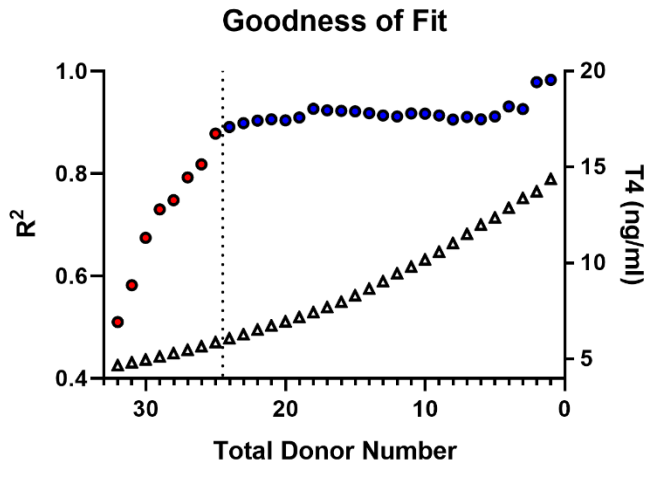
Population Level Modeling - Methimazole

A

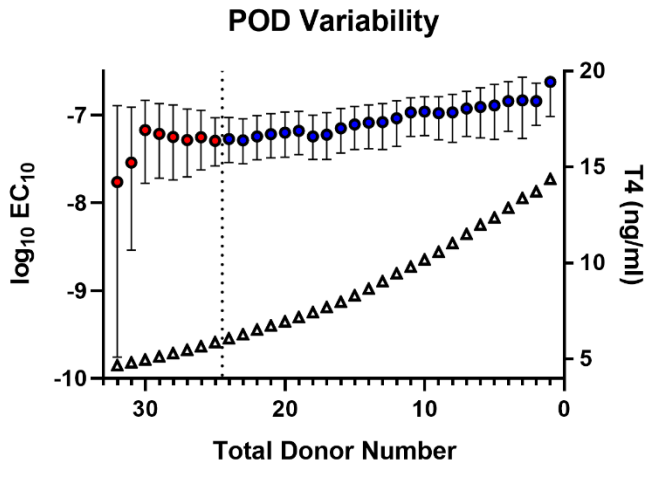


Donor Cohort	N	R2	IC10 nM (95% CI)
Total	32	0.51	17 (0.2-127)
Qualified	24	0.89	53 (29-94)

B



C



Donor qualification improves data modeling and decreases uncertainty in chemical potency determination.

Reference Chemical Benchmarks for Proficiency Testing

	Proficiency testing benchmarks		
Reference chemical	IC_{10} (95% CI)	IC_{50} (95% CI)	Units
Methimazole	53 (29–94)	234 (190–277)	nM
6-Propyl-2-thiouracil	76 (46–115)	363 (311–422)	nM
Sodium perchlorate	4 (2–6)	18 (12–29)	μM
Methomyl	NA	NA	NA

Reference chemical IC10 and IC50 potency values with 95% confidence intervals (CI) derived from the concentration-response modeling of the qualified donor cohort (*N*=24).

United States Environmental Protection Agency

Key Points

Advances AOP-based Thyroid Testing - The human thyroid microtissue assay fills an important 'key event' gap in the context of the thyroid adverse outcome pathway network by enabling functional testing of potential thyroid toxicants on hormone synthesis.

Standardizes Primary Human Thyrocyte Technology - The establishment of guidelines for donor procurement and primary thyrocyte qualification move the technology toward standardization in a manner that directly addresses cell quality as a key vulnerability with the use of organotypic model systems.

Sets Minimum Performance Guidelines - Establishing minimum performance parameters introduces flexibility into the assay to enable evaluation of a range of human responses.

Readiness for Method Transfer - The benchmark reference chemical potency ranges establish quantitative parameters for evaluating proficiency of method transfer and reproducibility.

Inter-laboratory Prevalidation of the Human Thyroid Microtissue Assay

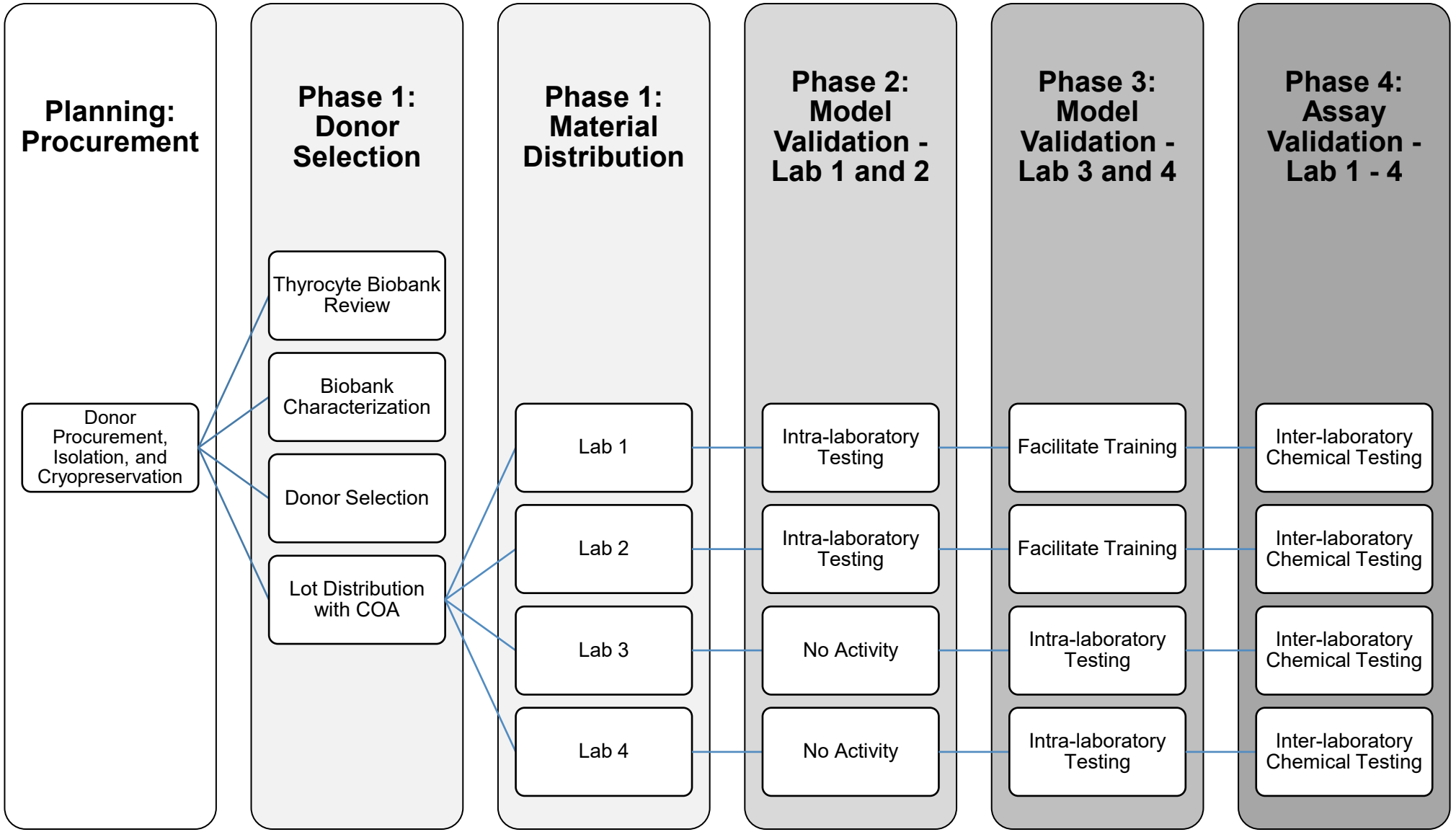
Goal: To structure and support a preliminary assessment of the test method reliability and relevance.

Independent Validation Management Team

Objectives

1. Test method standardization.
2. Test method transfer, training and intra-laboratory model performance evaluation.
3. Limited inter-laboratory reference chemical testing and assay performance evaluation.

Study Plan



Progress

Phase 2/3: Method Transfer and Model Validation

Objectives

1. Demonstrate method transferability using standardized operating procedures.
2. Evaluate biological model performance with quantitative metrics (dynamic range, precision, screening quality).
3. Demonstrate intra-laboratory reproducibility with a single donor lot of cells and standard reagents.

Note: No references chemicals are evaluated at this phase. The emphasis is on reproducibility of thyroid hormone synthesis to support model adoption.

Phase 2/3: Experimental Design

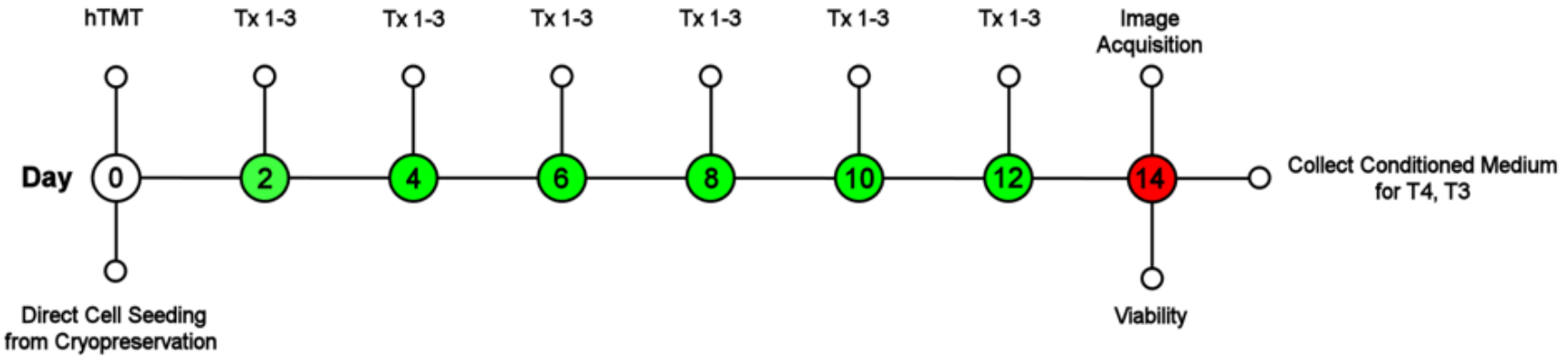


	Plate Map											
	1	2	3	4	5	6	7	8	9	10	11	12
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E	Tx - 1	Tx - 2	Tx - 3	Tx - 1	Tx - 2	Tx - 3	Tx - 1	Tx - 2	Tx - 3	Tx - 1	Tx - 2	Tx - 3
F	Tx - 1	Tx - 2	Tx - 3	Tx - 1	Tx - 2	Tx - 3	Tx - 1	Tx - 2	Tx - 3	Tx - 1	Tx - 2	Tx - 3
G	Tx - 1	Tx - 2	Tx - 3	Tx - 1	Tx - 2	Tx - 3	Tx - 1	Tx - 2	Tx - 3	Tx - 1	Tx - 2	Tx - 3
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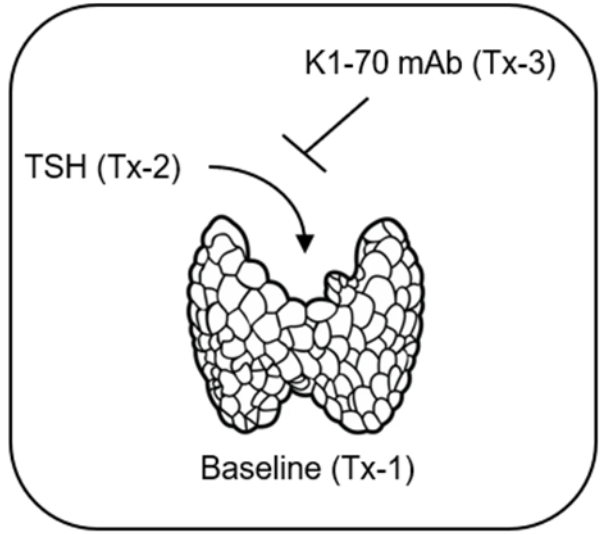
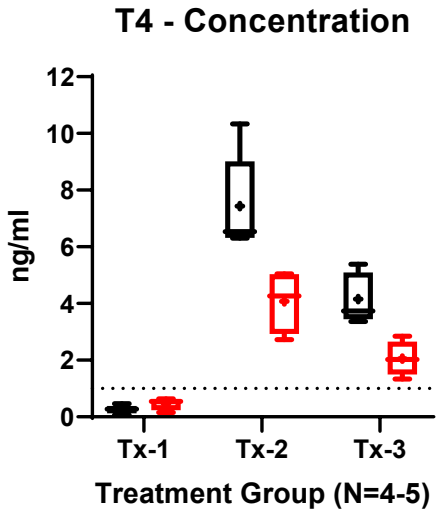
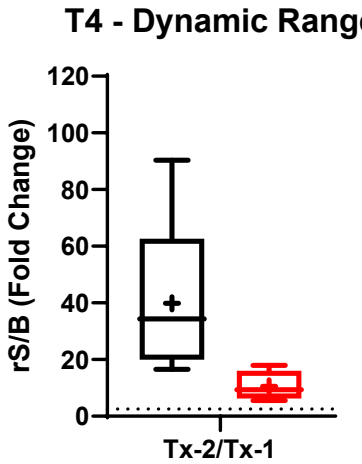
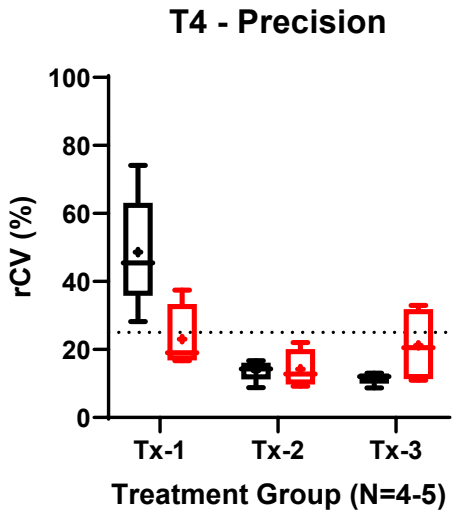


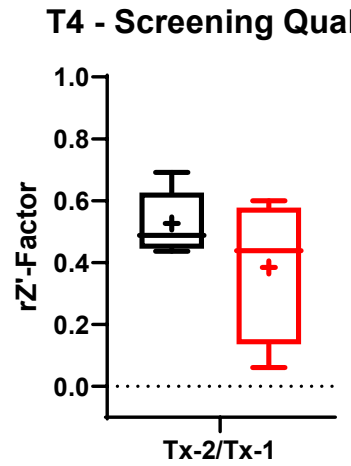
Treatment (Tx) groups include controls for: baseline activity (Tx-1), TSHR agonism (Tx-2), and TSHR antagonism (Tx-3).

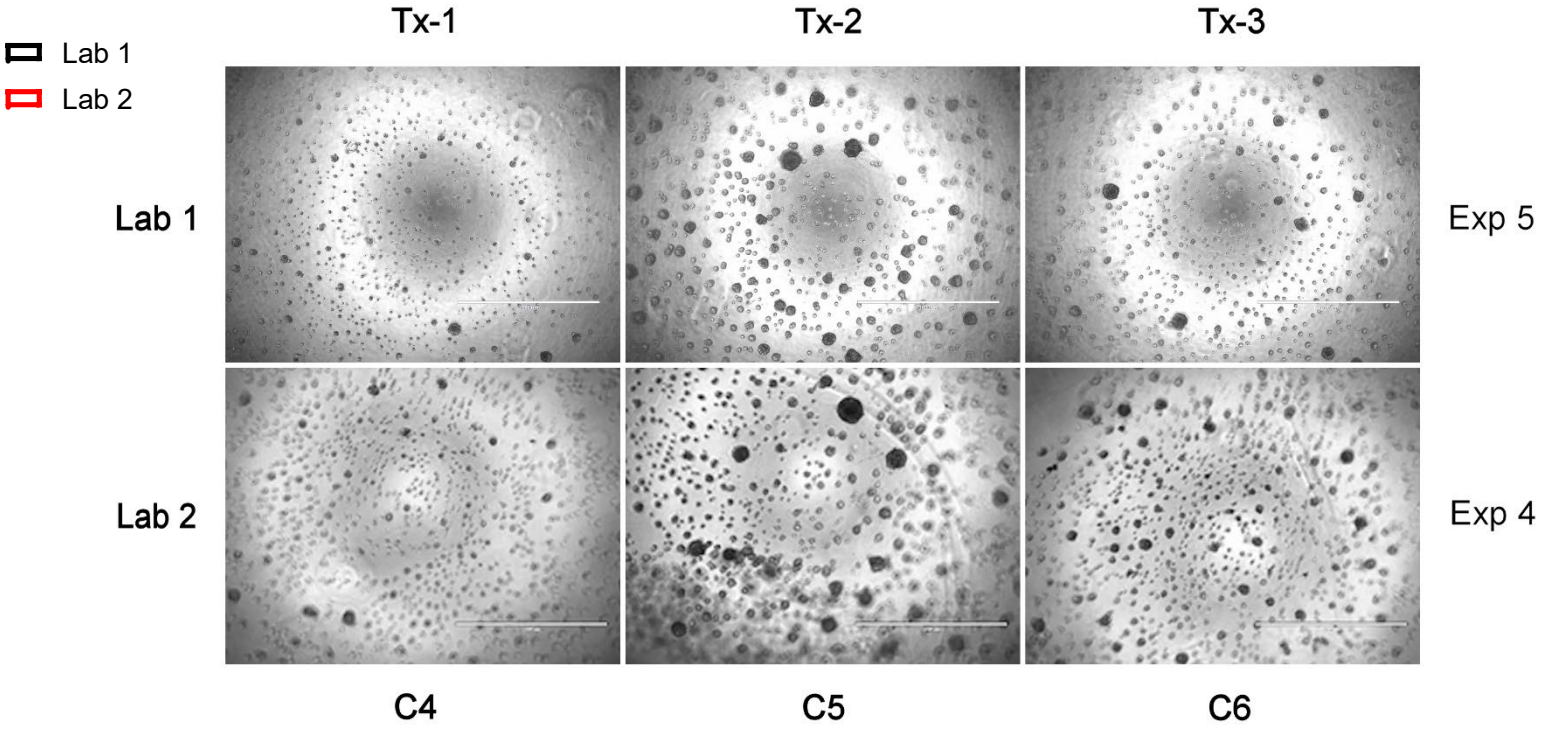
Phase 2 Results – Labs 1 and 2











- Minimum standards exceeded for quantitative performance metrics.
- Intra-laboratory reproducibility is good.
- Inter-laboratory reproducibility is fair.

Acknowledgements

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Agnes Karmaus
Dave Allen
Michaela Blaylock
Emily Reinke

Helen Tinwell
Julia Kuehnlenz
Frederic Schorsch
Olivier Blanck

Jessica LaRocca
Enrica Bianchi
Mercedes Biven
Wei Chen

Open Postdoc Position!
EPA Endocrine Disruptor Screening Program Fellowship

Reference Code:
EPA-OCSPP-2024-01

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