



LA NOSTRA
ESPERIENZA,
LA VOSTRA
SICUREZZA.

Stato dell'arte dei metodi alternativi: dallo sviluppo al percorso di validazione

*Benessere degli animali da laboratorio e metodi alternativi alla
sperimentazione animale, Roma, 15 Ottobre 2019*



OUTLINE





Sperimentazione *in vivo*



La sperimentazione *in vivo* utilizza gli animali come modelli sperimentali.



Il modello animale è definito come:



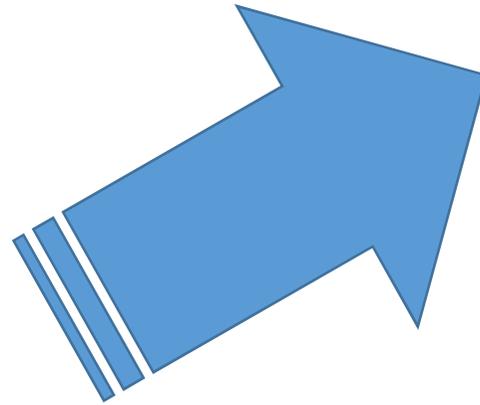
- ✓ Una condizione che permette di studiare processi biologici e comportamentali fondamentali
- ✓ Una possibilità di indurre processi patologici che riproducano, almeno per certi aspetti, lo stesso fenomeno patologico osservato negli umani, o in altre specie animali



La sperimentazione animale tra passato e futuro



Ieri...



Oggi...



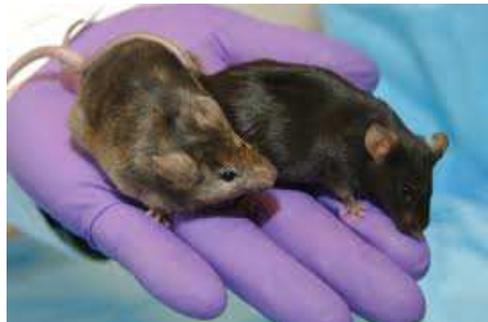


Cosa è un metodo alternativo



METODI ALTERNATIVI ALLA SPERIMENTAZIONE ANIMALE

Con il termine "metodi alternativi" sono indicate tutte le procedure adottate allo scopo di **ridurre l'uso di animali, di sostituirli completamente nella sperimentazione, ma anche di limitarne o eliminarne le sofferenze**. Secondo l'articolo 37 del D.lgs. 4 marzo 2014, n. 26, il Ministero della salute è chiamato a promuovere lo sviluppo e la ricerca di approcci alternativi, che non prevedono l'uso di animali o utilizzano un minor numero di animali o che comportano procedure meno dolorose, nonché la formazione e aggiornamento per gli operatori degli stabilimenti autorizzati.





Sperimentazione *in vitro*



Sperimentazione *in vivo*:

Studi sviluppati mediante la valutazione degli effetti di differenti sostanze biologiche su di un organismo vivente, che sia esso appartenente al regno animale, uomo incluso, o vegetale.

Sperimentazione *in vitro*:

Studi sviluppati con l'utilizzo di microrganismi, cellule e/o molecole di natura biologica al di fuori del loro normale contesto biologico.

Sperimentazione *ex vivo*:

Studi sviluppati su di un tessuto mantenuto vivente all'esterno del suo organismo originale.

Sperimentazione *in silico*:

Studi sviluppati mediante l'ausilio di supporti computerizzati oppure attraverso processi di simulazione al computer.

Differences between *in vitro*, *in vivo*, and *in silico* studies

There are three broad categories of experiments: *in vitro* studies, *in vivo* studies, and *in silico* studies. Each study type has conveniences and liabilities. Understanding the liabilities of study types offers insight into the validity of researchers' conclusions.

Curr Drug Discov Technol. 2015;12(4):218-24.

From *in vitro* Experiments to *in vivo* and Clinical Studies; Pros and Cons.

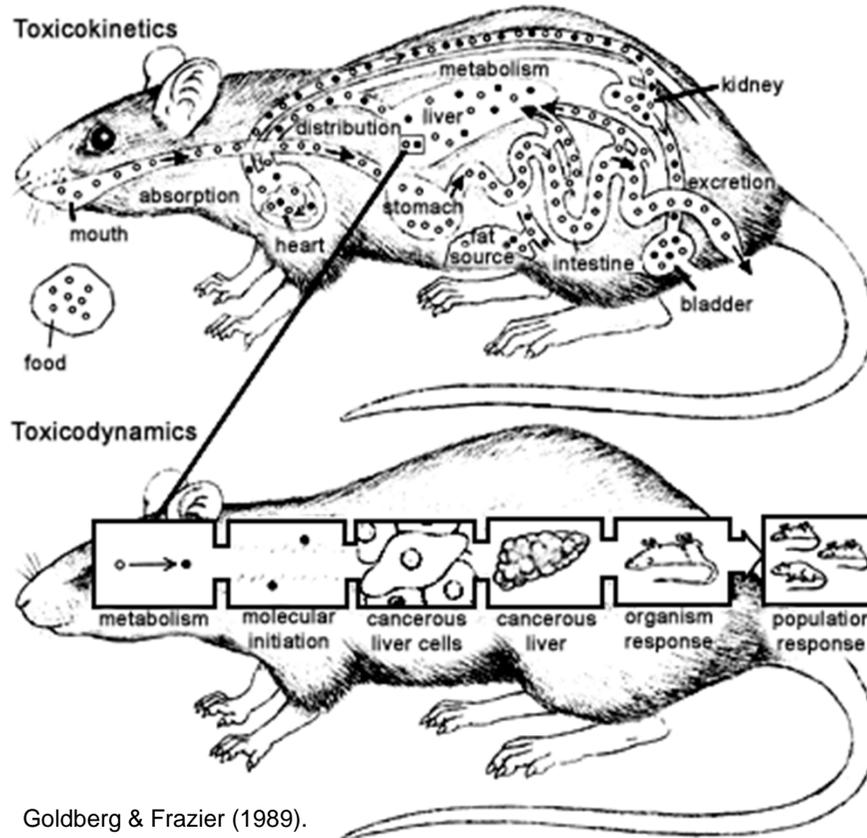
Saeidnia S, Manayi A, Abdollahi M¹.



Coerenza delle informazioni

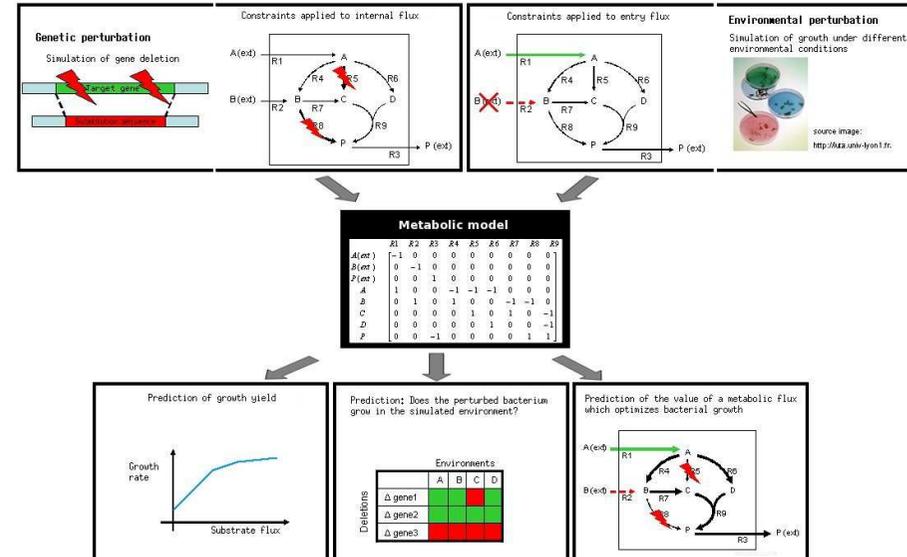


In vivo model

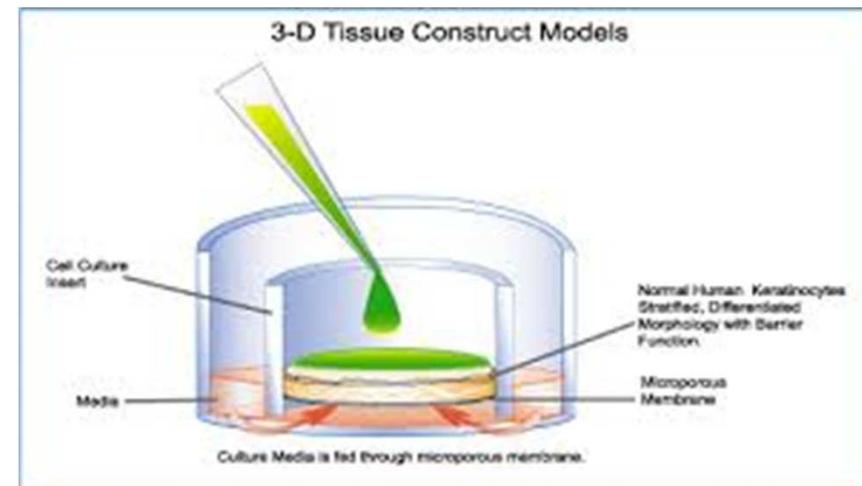


Toxicokinetics (absorption, distribution, metabolism, storage, and excretion of a chemical) and **toxicodynamics** (effects of the chemical and its metabolites on an organism)

In silico model



In vitro model





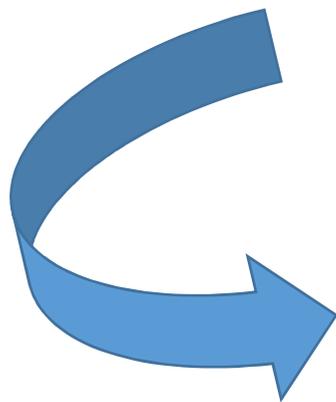
Quadro normativo



Gazzetta ufficiale dell'Unione europea

DIRETTIVE

DIRETTIVA 2010/63/UE DEL PARLAMENTO EUROPEO E DEL CONSIGLIO
del 22 settembre 2010
sulla protezione degli animali utilizzati a fini scientifici



DECRETO LEGISLATIVO 4 marzo 2014, n. 26

**Attuazione della direttiva 2010/63/UE sulla protezione degli animali
utilizzati a fini scientifici. (14G00036)**

(GU n.61 del 14-3-2014)





Quadro normativo



Il Centro di Referenza Nazionale per i metodi alternativi, benessere e cura degli animali da laboratorio ha integrato il Centro di Referenza Nazionale per i Substrati Cellulari con le attività da esso svolte.

Decreto Legislativo 04 marzo n. 26/2014

Capo V

Art. 37 Approcci Alternativi

Comma 2

Il Laboratorio Substrati Cellulari ed Immunologia Cellulare dell'IZSLER, è stato individuato come punto di contatto unico incaricato di fornire consulenza sulla pertinenza normativa e sull'idoneità degli approcci alternativi proposti per gli studi di convalida.



Cosa è la validazione?

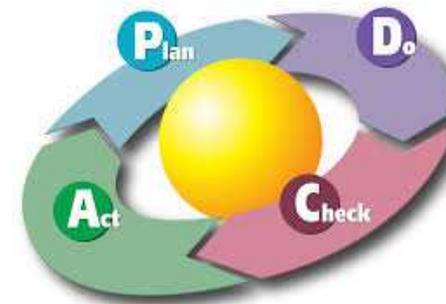


VALIDAZIONE:

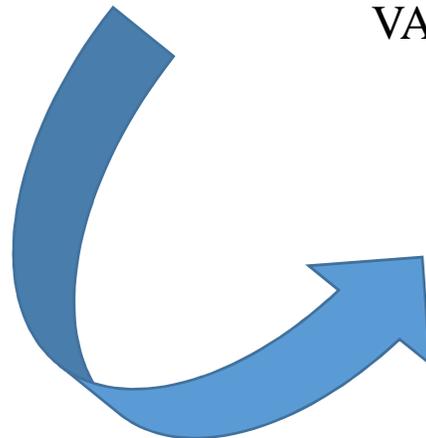
«conferma attraverso esame e l'apporto di evidenza oggettiva che i requisiti particolari per l'utilizzazione prevista siano soddisfatti»



VALIDAZIONE «SCIENTIFICA»



VALIDAZIONE «REGOLATORIO»





Percorsi di validazione



Unclassified

ENV/JM/MONO(2005)14

Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development

18-Aug-2005

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

OECD SERIES ON TESTING AND ASSESSMENT
Number 34

GUIDANCE DOCUMENT ON THE VALIDATION AND INTERNATIONAL ACCEPTANCE OF NEW
OR UPDATED TEST METHODS FOR HAZARD ASSESSMENT

ENV/JM/MONO(2005)14
Unclassified

Validazione PREDITTIVA

- Non esistono dati valutabili
- Nuovi dati sperimentali
- Vantaggi, limiti del test

Validazione RETROSPETTIVA

- Esistono già dati consultabili
- Validazione anche parziale di dati
- Validazione anche solo di pochi step



Percorsi di validazione



Un metodo rappresenta un «sistema sperimentale» che può fornire una serie di informazioni che spaziano dalle caratteristiche chimiche di una sostanza agli effetti avversi, etc.

Gli studi di validazione possono essere indirizzati verso diversi scopi

Individuazione nuovi
endpoints

Sostituzione di un test

Miglioramento di un
test già esistente

Sviluppo di un nuovo
metodo

Revisione di un
metodo già presente



Percorsi di validazione



Si possono individuare **5 categorie** di metodiche/strategie da utilizzare per un percorso di validazione

Metodo di screening

- Rapido
- Test preliminare
- Riduzione
- Non è sufficiente per un'analisi del rischio completa



Metodo definitivo

- Caratterizza un potenziale rischio definito
- Informazioni dose/risposta, effetti avversi
- Insieme ad altri test fornisce dati completi

Metodo di sostituzione

- Disegnato per sostituire in modo completo un test già in uso
 - Deve fornire vantaggi rispetto al test in uso
- Deve soddisfare criteri rigorosi per dimostrare le medesime informazioni o superiori rispetto al test da sostituire





Percorsi di validazione



Metodo accessorio

- Fornisce dati complementari
- E' utile nel pannello completo della valutazione di una sostanza



Pannello di test

- Test eseguiti nello stesso tempo o in sequenza ravvicinata
 - Sono tutti test complementari fra di loro
- L'insieme dei test viene trattato come un unico metodo



Percorsi di validazione



TABLE 1. PRINCIPLES AND CRITERIA FOR TEST METHOD VALIDATION

- a) **The rationale for the test method should be available.**
This should include a clear statement of the scientific basis, regulatory purpose and need for the test.
- b) **The relationship between the test method's endpoint(s) and the (biological) phenomenon of interest should be described.**
This should include a reference to scientific relevance of the effect(s) measured by the test method in terms of their mechanistic (biological) or empirical (correlative) relationship to the specific type of effect/toxicity of interest. Although the relationship may be mechanistic or correlative, test methods with biological relevance to the effect/toxicity being evaluated are preferred.
- c) **A detailed protocol for the test method should be available.**
The protocol should be sufficiently detailed and should include, *e.g.*, a description of the materials needed, such as specific cell types or construct or animal species that could be used for the test (if applicable), a description of what is measured and how it is measured, a description of how data will be analysed, decision criteria for evaluation of data and what are the criteria for acceptable test performance.
- d) **The intra-, and inter-laboratory reproducibility of the test method should be demonstrated.**
Data should be available revealing the level of reproducibility and variability within and among laboratories over time. The degree to which biological variability affects the test method reproducibility should be addressed.
- e) **Demonstration of the test method's performance should be based on the testing of reference chemicals representative of the types of substances for which the test method will be used.**
A sufficient number of the reference chemicals should have been tested under code to exclude bias (see paragraphs on "Coding and Distribution of Test Samples").
- f) **The performance of the test method should have been evaluated in relation to relevant information from the species of concern, and existing relevant toxicity testing data.**
In the case of a substitute test method adequate data should be available to permit a reliable analysis of the performance and comparability of the proposed substitute test method with that of the test it is designed to replace.
- g) **Ideally, all data supporting the validity of a test method should have been obtained in accordance with the principles of GLP.**
Aspects of data collection not performed according to GLP should be clearly identified and their potential impact on the validation status of the test method should be indicated.
- h) **All data supporting the assessment of the validity of the test method should be available for expert review.**
The detailed test method protocol should be readily available and in the public domain. The data supporting the validity of the test method should be organised and easily accessible to allow for independent review(s), as appropriate. The test method description should be sufficiently detailed to permit an independent laboratory to follow the procedures and generate equivalent data. Benchmarks should be available by which an independent laboratory can itself assess its proper adherence to the protocol.

Unclassified

ENV/JM/MONO(2005)14

Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development

18-Aug-2005

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY



Validazione metodi alternativi: ECVAM



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European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

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[EURL ECVAM Recommendations](#)

[EURL ECVAM Status Reports](#)

[Validation & regulatory acceptance](#)

[Test Method Submission](#)

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[EURL ECVAM's latest tweets](#)

About EURL ECVAM

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- [Activities & Services](#)
- [Customers](#)
- [Location](#)
- [Work opportunities](#)
- [FAQs 'Alternatives to animal testing'](#)



Vision & Mission

The aim of EURL ECVAM (the **European Union Reference Laboratory for Alternatives to Animal Testing**) is twofold:

- to promote the scientific and regulatory acceptance of non-animal tests which are of importance to biomedical sciences, through research, test development and validation and the establishment of a specialised database service
- to co-ordinate at the European level the independent evaluation of the relevance and reliability of tests for specific purposes, so that chemicals and products of various kinds, including medicines, vaccines, medical devices, cosmetics, household products and agricultural products, can be manufactured, transported and used more economically and more safely, whilst the current reliance on animal test procedures is progressively

Watch our video!

Scientists from EURL ECVAM show you what they are doing to advance safety assessment of chemicals without relying on animal testing



Transcript also available: [EN](#); [FR](#); [DE](#); [IT](#).

Version updated June 2014.

Related terms

- [European Union Reference Laboratory](#)
- [QSARS](#)
- [Alternative test methods](#)
- [in silico methods](#)
- [carcinogenicity](#)
- [biologics](#)
- [guidelines](#)
- [in vitro methods](#)
- [vaccine](#)
- [health](#)





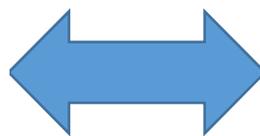
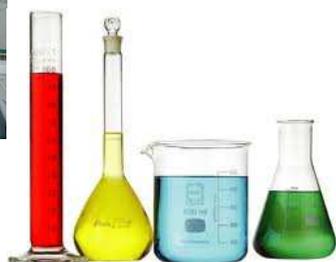
Validazione metodi alternativi



VALIDAZIONE

**Sviluppo/ottimizzazione
del test**

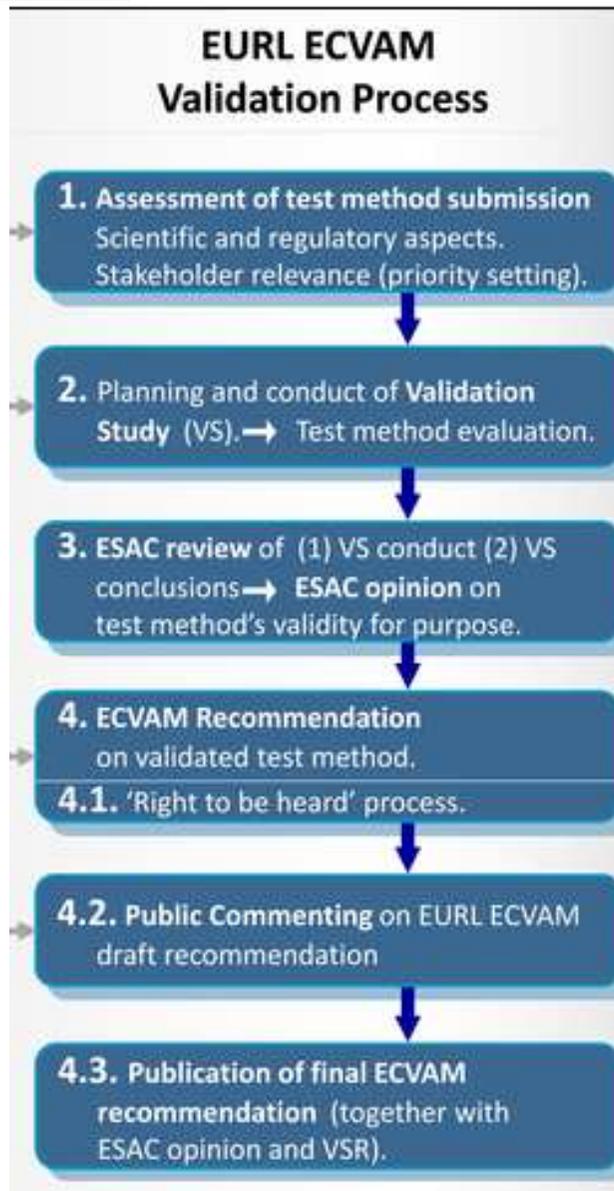
**Consenso
regolatorio/Riconoscimento
Internazionale**



EURL ECVAM



Percorso di validazione



- Interazione con le parti interessate (PARERE) e con il relativo *forum* (ESTAF)



- Collaborazione internazionale (ICATM) e network di laboratori di validazione (EU-NETVAL)





PARERE



PARERE is a network of national regulators that provides EURL ECVAM with upstream input and preliminary views on potential regulatory relevance of methods or approaches submitted to EURL ECVAM for validation and/or peer review.



- Verifica del regolatorio e delle potenzialità test proposti e strategie
- Facilitare il flusso di informazioni tra EURL ECVAM ed l'autorità regolatoria nell'ambito dello sviluppo e validazione dei metodi
- Identificazione di esperti del regolatorio per la partecipazione a determinati gruppi di lavoro/ricerca
- Fornire commenti alle raccomandazioni fornite da ECVAM
- Supportare e promuovere il ruolo di EU-NETVAL



ESTAF



The EURL ECVAM Stakeholder Forum (ESTAF) is our instrument for ensuring close dialogue is maintained with our non-governmental stakeholders. This includes parties from industry, academia and civil society organisations.

EURL
ECVAM
European Union Reference Laboratory
for Alternatives to Animal Testing

**EURL ECVAM Stakeholder Forum
(ESTAF)**
Open invitation to submit
Expressions of Interest

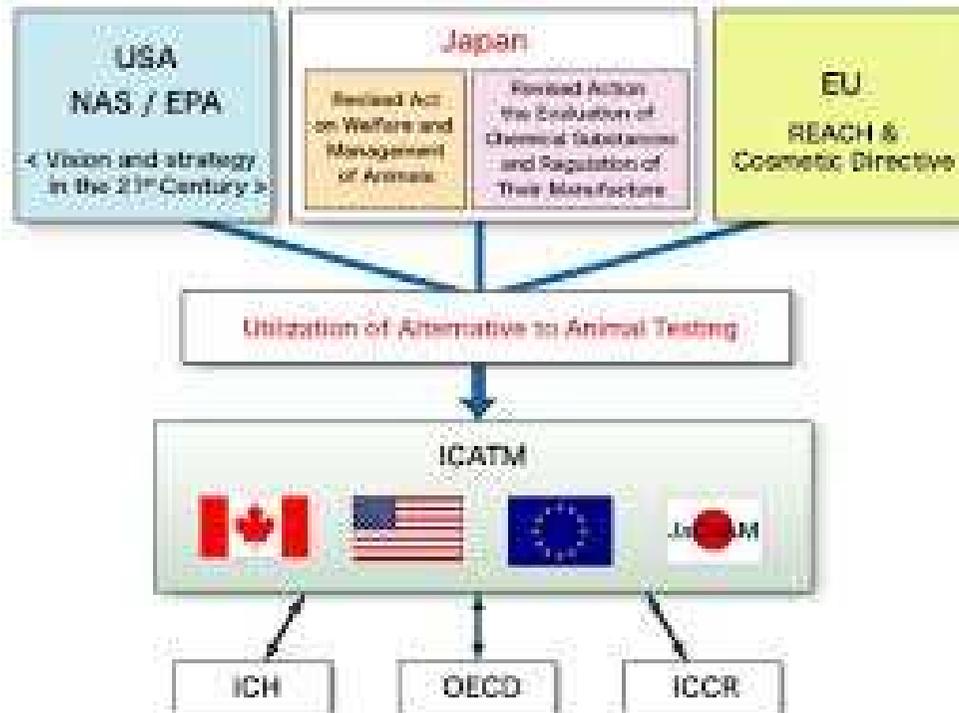
- Facilitare ed implementare il dialogo tra le parti interessate
- Fornire aggiornamenti relativi all'applicabilità di metodi dal punto di vista dell'utilizzatore
- Comunicare aggiornamenti scientifici
- Discutere gli aspetti teorico/pratici dello sviluppo /validazione e uso dei metodi
- Fornire una piattaforma di supporto e partecipare alle attività di ECVAM
- Fornire commenti sulle bozze delle raccomandazioni di ECVAM



ICATM



ICATM partners are working together to promote enhanced international cooperation and coordination on the scientific development, validation and regulatory use of alternative approaches.



- Stabilire cooperazioni internazionali per i punti critici di studi di validazione
- Armonizzare le raccomandazioni per implementare i metodi alternativi
- Verificare che i metodi alternativi approvati dal regolatorio garantiscano la stessa sicurezza per persone/animali ed ambiente rispetto ai metodi *in vivo*



EU-NETVAL



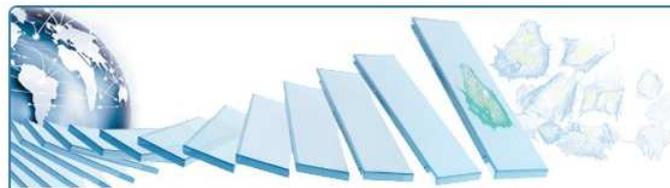
EU-NETVAL's mission is to provide support for EURL ECVAM validation studies to assess the reliability and relevance of alternative methods that have a potential to replace, reduce or refine the use of animals for scientific purposes.



- Implementazione della Direttiva Europea 63/2010
- Partecipazione a studi di validazione di metodi alternativi
- Partecipazione a meeting annuali per focalizzare l'attenzione sui nuovi punti di interesse
- Collaborazione internazionale nello sviluppo/applicazione di metodi alternativi



Submission to ECVAM



TRACKING SYSTEM FOR ALTERNATIVE METHODS TOWARDS REGULATORY ACCEPTANCE

TSAR tracks the progress of alternative, non-animal methods, for testing chemicals or biological agents such as vaccines towards acceptance as a recognised test method for use in various sectors



FEATURED TEST METHODS

Presubmission

Per una valutazione preliminare del metodo

Complete submission

Compilazione di un formato dettagliato.

Decisione finale per il percorso di validazione

KeratinoSens assay for the testing of skin sensitizers

Test Method Number:	TM2010-03 (EU)	Stage of Submission:	Assessment finalised
Short Name of TM:	KeratinoSens	Stage of Validation:	Finalised
Responsible Organisation:	EURL ECVAM - European Union	Stage of Peer-review:	Finalised
DB-ALM Protocol No.:	155	Stage of Recommendation:	Published
General Comments:	The validation was performed externally between 2009 and 2010	Stage of Regulatory acceptance/Standards:	Adopted



Monocyte Activation Test

Test Method Number:	TM2016-06 (BRA)	Stage of Validation:	Ongoing
Short Name of TM:	MAT	Stage of Regulatory acceptance/Standards:	Drafting of new regulatory standard/guideline
Responsible Organisation:	BraCVAM - Brazil		



Bioaccessibility testing (Bioelution) of metals, inorganic metals compounds and metals-containing materials: simulated gastric fluid

Test Method Number:	TM2016-02 (EU)	Stage of Submission:	Receipt of revised full submission
Short Name of TM:	Bioelution		
Responsible Organisation:	EURL ECVAM - European Union		



A new in vitro eye irritation test with a 3D-reconstructed human cornea epithelium, MCTT HCE™

Test Method Number:	TM2016-04 (KOR)	Stage of Submission:	Assessment finalised
Short Name of TM:	MCTT HCE™-EIT	Stage of Validation:	Ongoing
Responsible Organisation:	KoCVAM - Republic of Korea		





Validazione Metodi Alternativi



	Acute, Subchronic and Chronic Toxicity Tests Determine the effect of a chemical and mortality during various lengths of exposure
	Reproductive Toxicity Tests Assess the effect of a chemical on reproduction and fecundity
	Developmental Toxicity Tests Evaluate the capacity of a chemical to cause developmental abnormalities in an embryo, fetus or neonate
	Ocular- and Skin-Irritation Tests Measure the ability of a chemical to irritate the skin or eyes
	Hypersensitivity Tests Assess the tendency of a chemical to cause allergic reactions and other allergic responses
	Phototoxicity Tests Determine the extent to which a chemical is phototoxic by sunlight, thereby enhancing its toxicity
	Toxicokinetic Studies Explore the absorption, distribution, storage and excretion of a chemical in the body
	Behavioral Tests Monitor the effects of a chemical on behavior during development and in the adult

Acute toxicity

Aquatic toxicity

Aquatic bioconcentration and bioaccumulation

Biologicals

Carcinogenicity

Eye irritation/Serious eye damage

Genotoxicity

Phototoxicity

Repeated Dose Toxicity

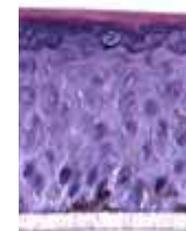
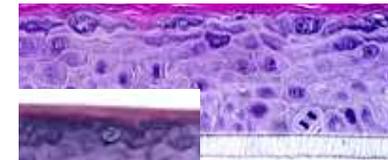
Skin Corrosion

Skin Irritation

Skin Sensitisation

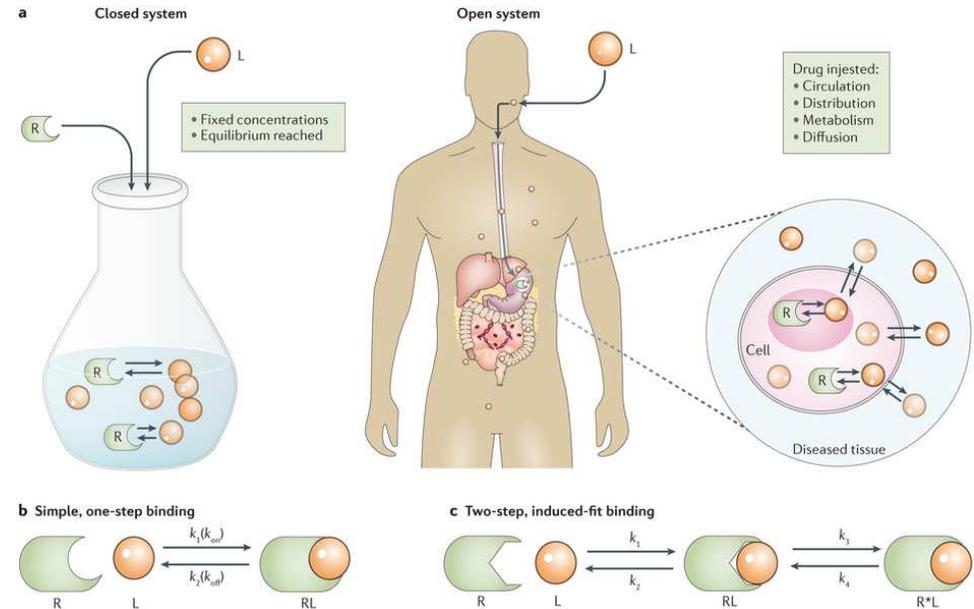
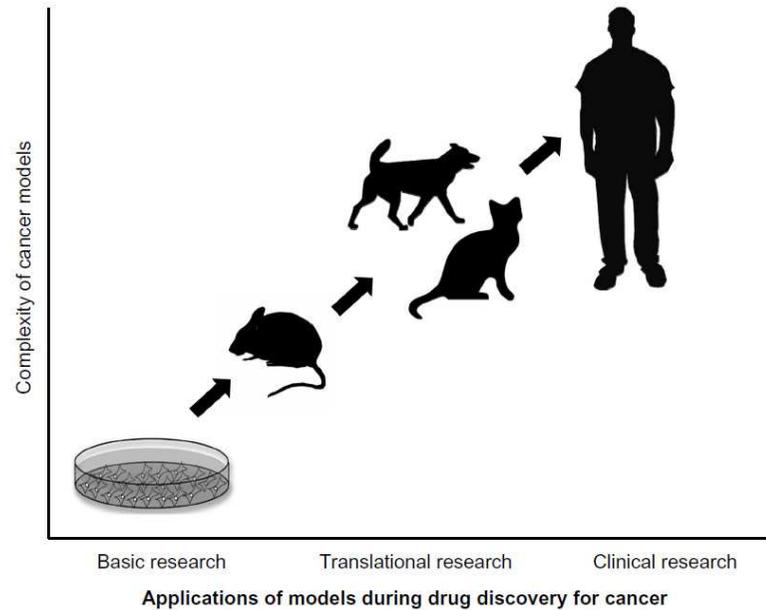
Toxicokinetics

Cornea *in vitro*





Dall' *in vivo* all' *in vitro*



Nature Reviews | Drug Discovery

Curr Eye Res. 2017 Jan 27:1-9. doi: 10.1080/02713683.2016.1262428. [Epub ahead of print]

Development and Assessment of a Novel Canine Ex Vivo Corneal Model.

Proietto LR¹, Whitley RD¹, Brooks DE¹, Schultz GE², Gibson DJ², Berkowski WM Jr¹, Salute ME¹, Plummer CE¹.



Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit



Review

Alternative approaches for identifying acute systemic toxicity: Moving from research to regulatory testing

Jon Hamm^{a,*}, Kristie Sullivan^b, Amy J. Clippinger^c, Judy Strickland^a, Shannon Bell^a, Barun Bhatarai^d, Bas Blaauwer^e, Warren Casey^f, David Dorman^g, Anna Forsby^h, Natàlia Garcia-Reyeroⁱ, Sean Gehen^j, Rabea Graepel^k, Jon Hotchkiss^d, Anna Lowit^l, Joanna Matheson^m, Elissa Reaves^l, Louis Scaranoⁿ, Catherine Sprankle^a, Jay Tunkel^o, Dan Wilson^a, Menghang Xia^p, Hao Zhu^q, David Allen^a

Ethical Use of Animal Models in Musculoskeletal Research

Matthew J. Allen,¹ Kurt D. Hankenson,² Laurie Goodrich,³ Gregory P. Boivin,^{4,5} Brigitte von Rechenberg⁶

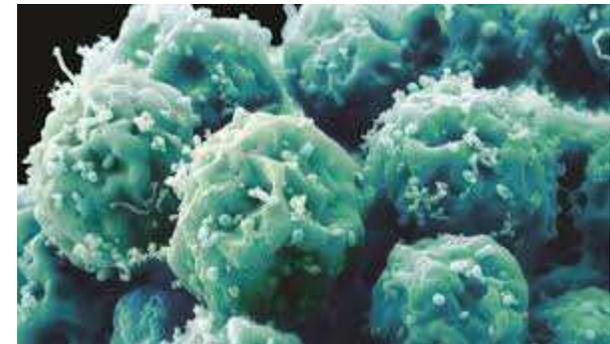
Research Article

In Vivo Pig-a Gene Mutation Assay: Guidance for 3Rs-Friendly Implementation

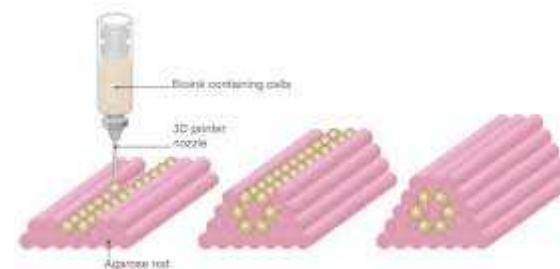
Marian Raschke,^{1*} Bernd-W. Igl,¹ Julia Kenny,² Joanne Collins,² Stephen D. Dertinger,³ Carson Labash,³ Javed A. Bhalli,⁴ Cameron C.M. Tebbe,⁴ Kylie M. McNeil,⁴ and Andreas Sutter¹



Colture cellulari tra passato e futuro



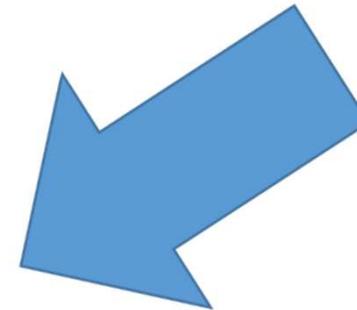
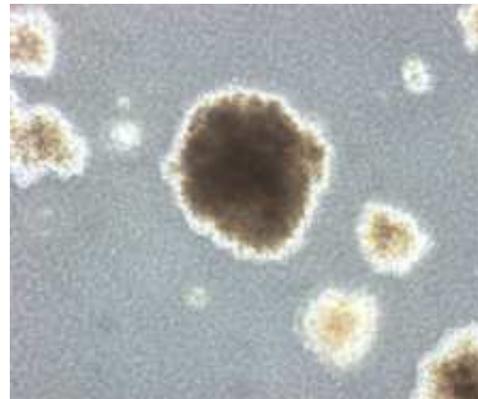
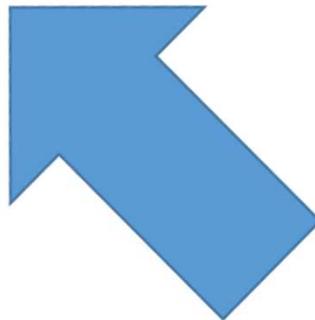
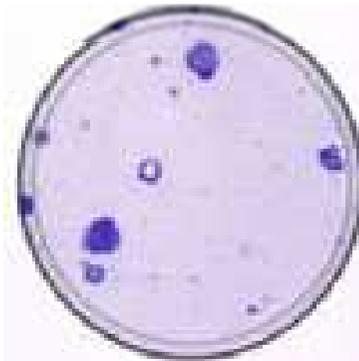
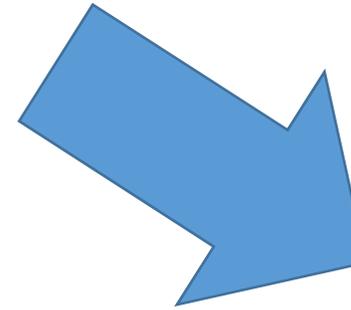
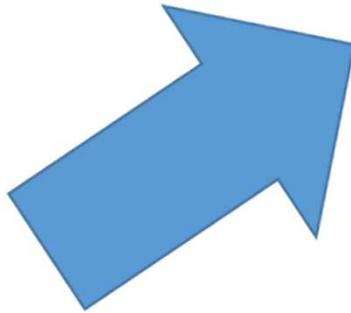
How bioprinting works



Source: Modern Meadow



Test di Tumorigenicità *in vitro*





Sistemi totalmente «Animal Free»



Utilizzo di supporti colturali chimici vs animali: SERUM-FREE E TRIPSINA ARTIFICIALE

Cytotechnology 23: 95–101, 1997.
© 1997 Kluwer Academic Publishers. Printed in the Netherlands.

Special Issue

Basal medium development for serum-free culture: a historical perspective

David Jayme, Toshio Watanabe & Toshiaki Shimada
Life Technologies, Inc., Grand Island, NY USA and Life Technologies Oriental, Tokyo, Japan

Aspetti pratici

- Adattamento graduale
- Standardizzazione dell'ambiente di crescita (no controlli microbiologici)
- Nessun contaminante di origine animale

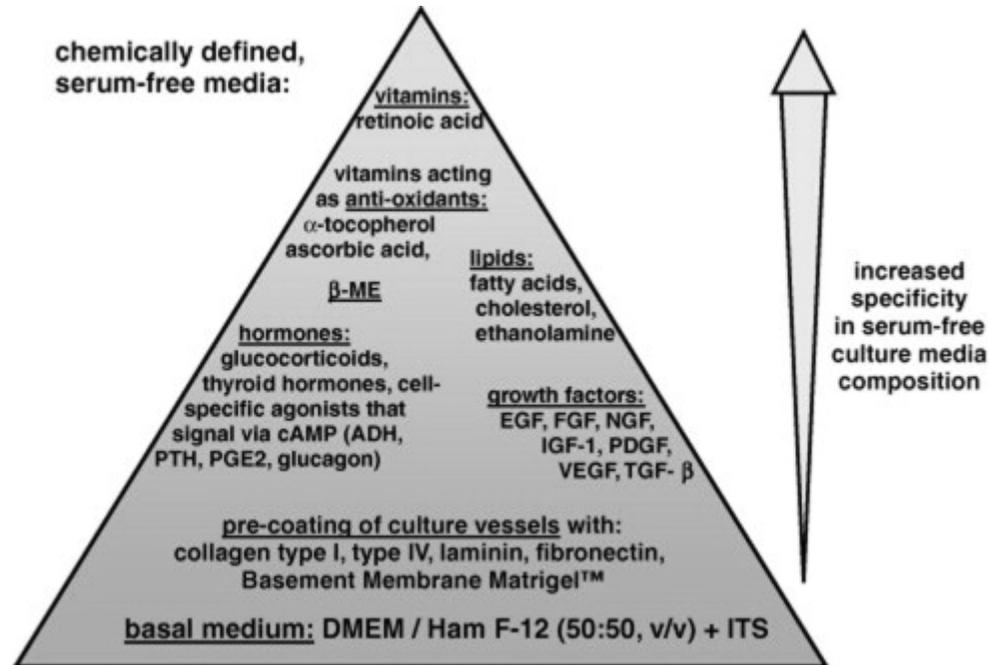
Aspetti Etici

- SFB ottenuto da feti
- Benessere animale
- Approccio alternativo





Sistemi totalmente «Animal Free»

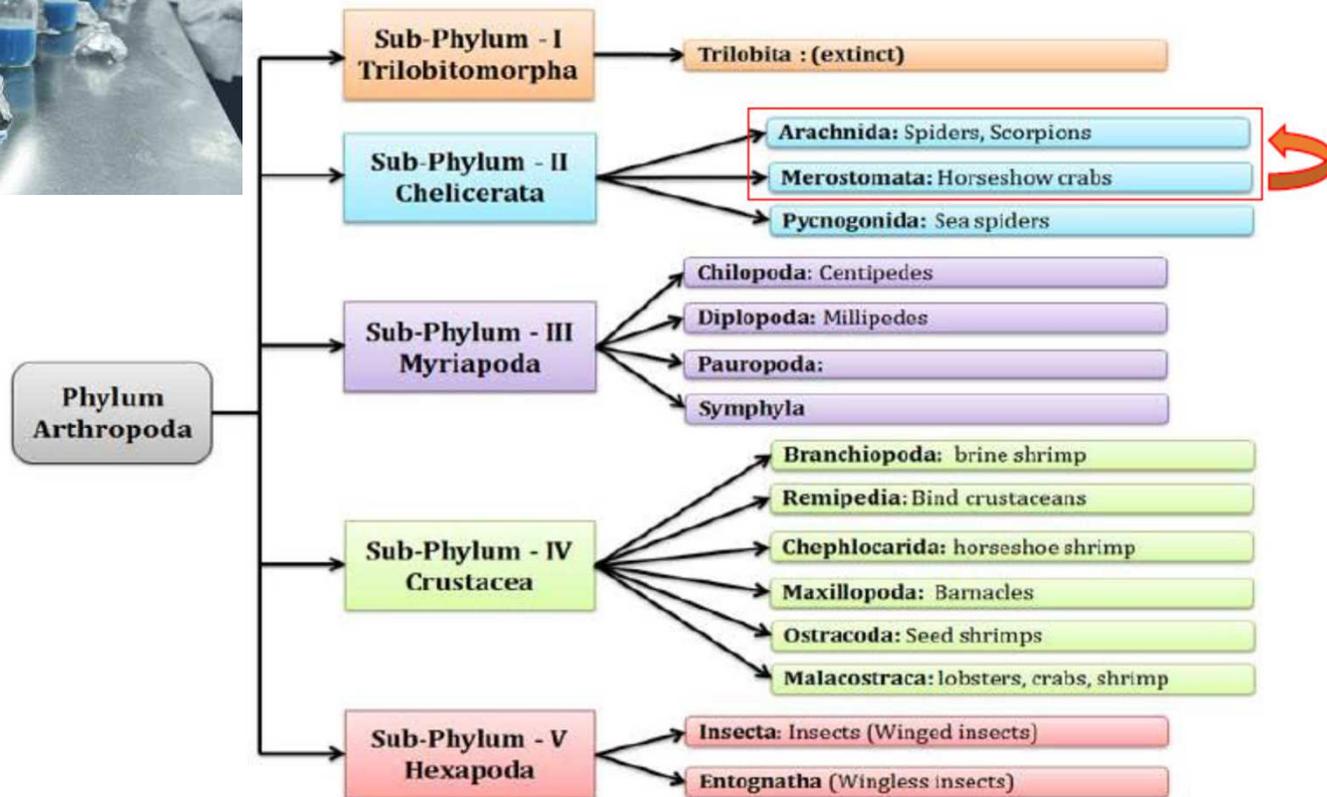


Sciencedirect.com



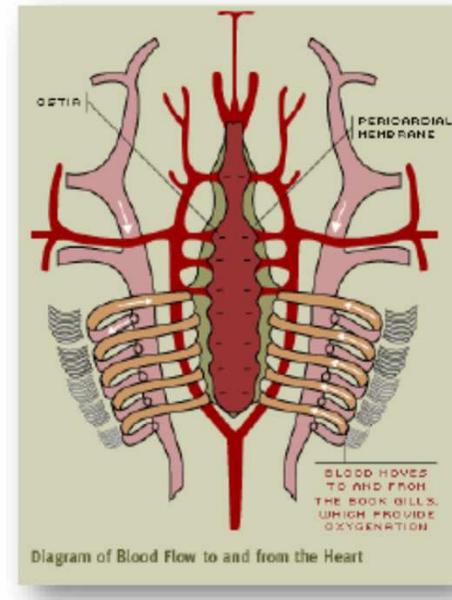
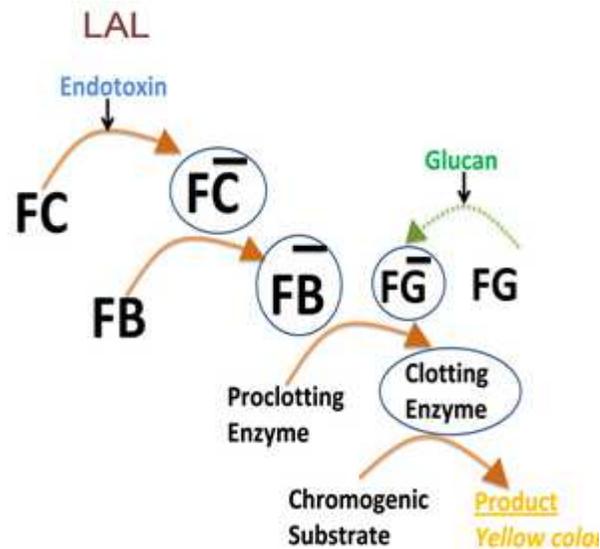


LAL TEST





PRINCIPIO DEL TEST



- Il limulo ha un sistema circolatorio aperto, con una pompa centrale che ha la funzione di spingere il sangue (emolinfa) direttamente a contatto dei tessuti. Questo poi rientra nel cuore grazie a dei forellini, che possiamo assimilare a valvole cardiache primitive.
- A differenza del sangue di vertebrati, che utilizza l'emoglobina per trasportare ossigeno, il sangue del limulus utilizza l'emocianina, contenente rame, che dona al sangue il colore blu. Gli «emociti» sono però cellule poco presenti nell'emolinfa.
- **L'emolinfa è invece ricca di amebociti**, cellule immunitarie primitive grazie alle quali è possibile il LAL test



OGGI



SOSTENIBILITÀ – INIZIATIVE 3R

Reduction & Refinement:

La tecnologia a cartucce permette di **ridurre del 95%** il lisato necessario/test

Attualmente vengono eseguiti 70.000.000 di test/anno (dato in crescita) – 500.000 limuli per il bleeding – ca. 140 test/limulo

Se tutti i test venissero eseguiti su cartuccia, sarebbero sufficienti 25.000 limuli/anno

Replacement:

Ricerca di nuove tecnologie alternative all'utilizzo del reagent LAL test.

Ha senso se si riesce ad ottenere una tecnologia che sia **eguale o superiore** all'attuale LAL.

FOCUS: sicurezza del paziente

Programmi di sostenibilità:

Programmi di sostenibilità e ripopolamento del Limulus da parte di alcune aziende biomediche





L'ALTERNATIVA AL METODO ALTERNATIVO....

METODI A CONFRONTO

	METODI DI FARMACOPEA				METODI NON DI FARMACOPEA
	Gel clot	Cinetico	Endpoint	PTS	rFC-based
Sensibilità massima	0,015 EU/ml	0,001 EU/ml*	0,015 EU/ml	0,005 EU/ml	Fino a 0,001 o 0,005 EU/ml
Accuratezza	50%-200%	50%-200%	50%-200%	50%-200%	Come LAL
Tempo di incubazione	60 minuti	dipende da curva e produttore	non meno di 1 ora	15 minuti circa	60-120 min + 10min pre-incubazione
Tempo preparazione test (escluso campione)	30 minuti circa	30 minuti circa	30 minuti circa	1 minuto	Variabili da 30 min a 1 ora + eventuale 18h pre-incubazione

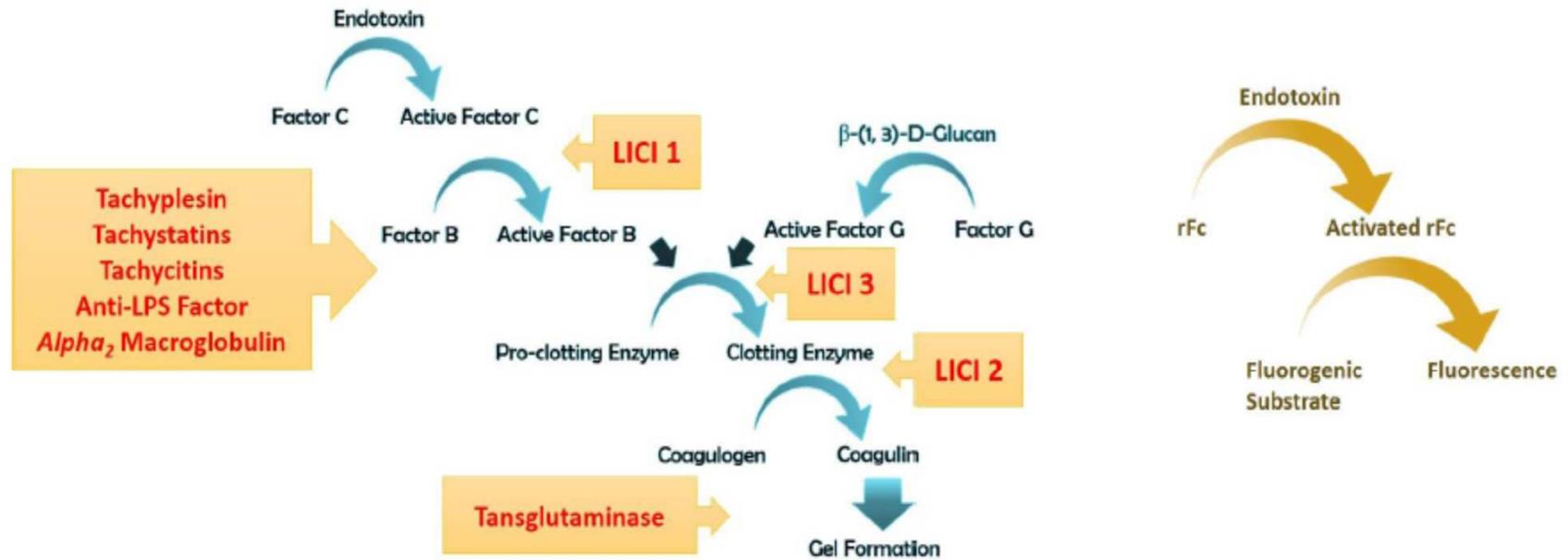
* 0,001 EU/ml: con lettori di provette e metodo turbidimetrico
con lettori di piastre e metodo cromogenico, esclusivamente con micropiastre e lisato CRL



LAL «SINTETICO»



CASCATA ENZIMATICA LAL VS. rFC





SPECIFICITÀ A CONFRONTO



STUDIO COMPARATIVO SULLA SPECIFICITÀ DEI METODI ALTERNATIVI

Table 2 Naturally occurring endotoxin (NOE) パネルによるライセート試薬と組換え試薬の評価

naturally occurring endotoxin		測定値(EU/mL) ^{*1}					
no.	由来	Endospey	ES-II	Kinetic-QCL	PyroSmart	PyroGene	EndoZyme
native endotoxin							
1	<i>Escherichia coli</i>	543	621	554	404	818	743
2	<i>Enterobacter cloacae</i>	897	1329	1176	298	1287	1098
3	<i>Pseudomonas aeruginosa</i>	2400	4141	2768	2340	3376	2456
4	<i>Ralstonia pickettii</i>	214	360	254	92	454	244
5	<i>Serratia marcescens</i>	400	504	447	108	459	312
水							
6	湖沼水	95.6	100.7	139.5	62.7	72.0	35.3
7	河川水 1	222.0	247.6	295.0	244.5	231.0	134.0
8	河川水 2	204.5	284.4	303.5	82.5	198.5	98.0
9	生活排水 (家庭排水用浄化槽)	111.0	160.3	164.0	86.0	138.0	77.9
10	市販ミネラルウォーター	0.114	0.116	0.140	0.088	0.034	0.030
11	水道水	8.105	10.964	14.820	10.285	4.830	1.295

*1 Measured value; Mean value of 2 institutes

Samples:

#6, Lake water;

#7, River water 1;

#8, River water 2;

#9, Domestic waste water;

#10, Commercially available mineral water;

#11, Tap water

*2 機関の平均値

Kikuchi et al, Collaborative Study on the Bacterial Endotoxins Test using Recombinant Factor C based Procedure for Detection of Lipopolysaccharides – Pharmaceutical and Medical Device Regulatory Science, Vol 48 No 4, May 2017



Percorso di validazione



Unclassified

ENV/JM/MONO(2014)23

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

11-Jul-2014

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

European drive to validate *in vitro* methods for the detection of thyroid disruptors

NEW SCOPING DOCUMENT ON IN VITRO AND EX VIVO ASSAYS FOR THE IDENTIFICATION OF MODULATORS OF THYROID HORMONE SIGNALLING

Series on Testing and Assessment

No. 207

JUL 04 2017 The JRC's EURL ECVAM has launched a validation study to assess *in vitro* methods for the detection of chemicals which disrupt thyroid function.



There is global concern about substances, natural and man-made, which have the potential to interfere with the endocrine system.

The thyroid's main role in the endocrine system is to regulate metabolism through the action of thyroid hormone, by extracting iodine from the blood and incorporating it into thyroid hormones.

Cells and human body systems depend on the thyroid to manage their metabolism and for regulating vital body functions, including breathing, heart rate, central and peripheral nervous systems, body weight, muscle strength, menstrual cycles, body temperature and cholesterol levels.

Some man-made chemicals have the potential to interfere with the functioning of the thyroid and related hormone signalling processes which can result in adverse health effects in humans and other organisms that have been sufficiently exposed.

A total of 17 *in vitro* methods have been identified by EURL ECVAM as candidates for this validation study which will be carried out in collaboration with the **European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL)**.

Those methods which perform well may be selected for further assessment with a view to their eventual use in a regulatory context, in support of EU initiatives to address the potential risks to human health and the environment posed by **endocrine disruptors**.

ENV/JM/MONO(2014)23
Unclassified



Fish Embryo Test



OECD/OCDE

236

Adopted:
26 July 2013

OECD GUIDELINES FOR THE TESTING OF CHEMICALS

Fish Embryo Acute Toxicity (FET) Test

INTRODUCTION

1. This Test Guideline (TG) 236 describes a Fish Embryo Acute Toxicity (FET) test with the zebrafish (*Danio rerio*). This test is designed to determine acute toxicity of chemicals on embryonic stages of fish. The FET-test is based on studies and validation activities performed on zebrafish (1)(2)(3)(4)(5)(6)(7)(8)(9)(10)(11)(12)(13)(14). The FET-test has been successfully applied to a wide range of substances exhibiting diverse modes of action, solubilities, volatilities, and hydrophobicities (reviewed in 15 and 16).

2. Definitions used in this Test Guideline are given in Annex 1.

PRINCIPLE OF THE TEST

3. Newly fertilised zebrafish eggs are exposed to the test chemical for a period of 96 hrs. Every 24 hrs, up to four apical observations are recorded as indicators of lethality (6): (i) coagulation of fertilised eggs, (ii) lack of somite formation, (iii) lack of detachment of the tail-bud from the yolk sac, and (iv) lack of heartbeat. At the end of the exposure period, acute toxicity is determined based on a positive outcome in any of the four apical observations recorded, and the LC_{50} is calculated.

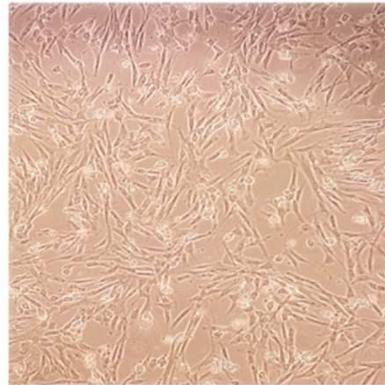




Valutazione Thyrotropin-releasing hormone (TRH)



Sistema che si basa su una linea cellulare modificata in grado di esprimere differenti recettori funzionali, in grado di evidenziare il comportamento dello ione calcio.



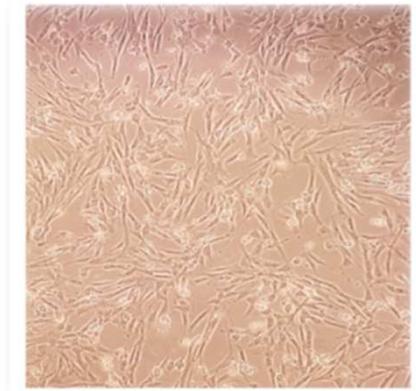
Questo sistema permette di valutare lo stato funzionale della tiroide, dopo aver stimolato le cellule con differenti composti chimici.



Conclusioni



- ✓ I metodi alternativi alla sperimentazione animale rappresentano una fondamentale fonte di innovazione
- ✓ Forniscono informazioni differenti rispetto al modello *in vivo*
- ✓ Difficoltà di esecuzione e di validazione
- ✓ Costi spesso molto elevati
- ✓ Laboratori dotati di tecnologia scientifica di alto livello
- ✓ Rappresentano il futuro della sperimentazione clinica
- ✓ Investimenti sia in ambito pubblico sia privato



GRAZIE A
TUTTI PER
L'ATTENZIONE



ISTITUTO ZOOPIROFIATTICO SPERIMENTALE
DELLA LOMBARDBIA E DELL'EMILIA ROMAGNA
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