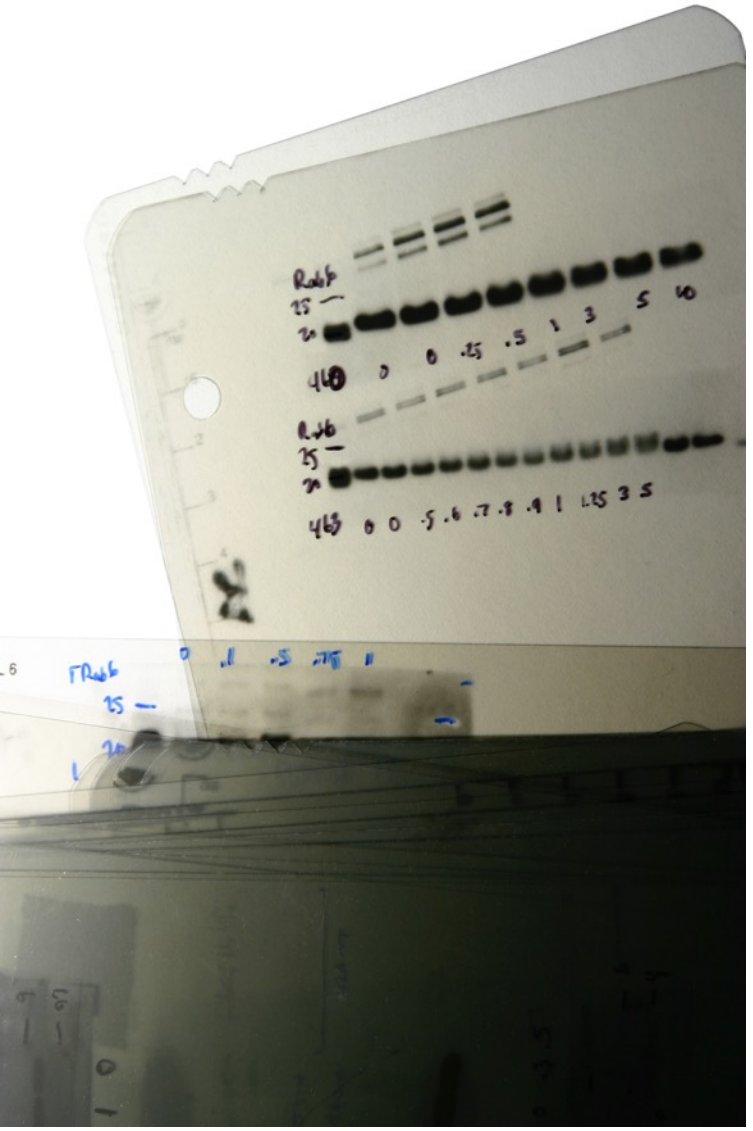
Canadian Centre for Alternatives to Animal Methods

Centre Canadien des méthodes de substitution à l'expérimentation animale

Recombinant antibodies for the life sciences: Time to move beyond animals, *eh*?

Dr. Charu Chandrasekera
Executive Director
CCAAM/CaCVAM
University of Windsor
Ontario, Canada

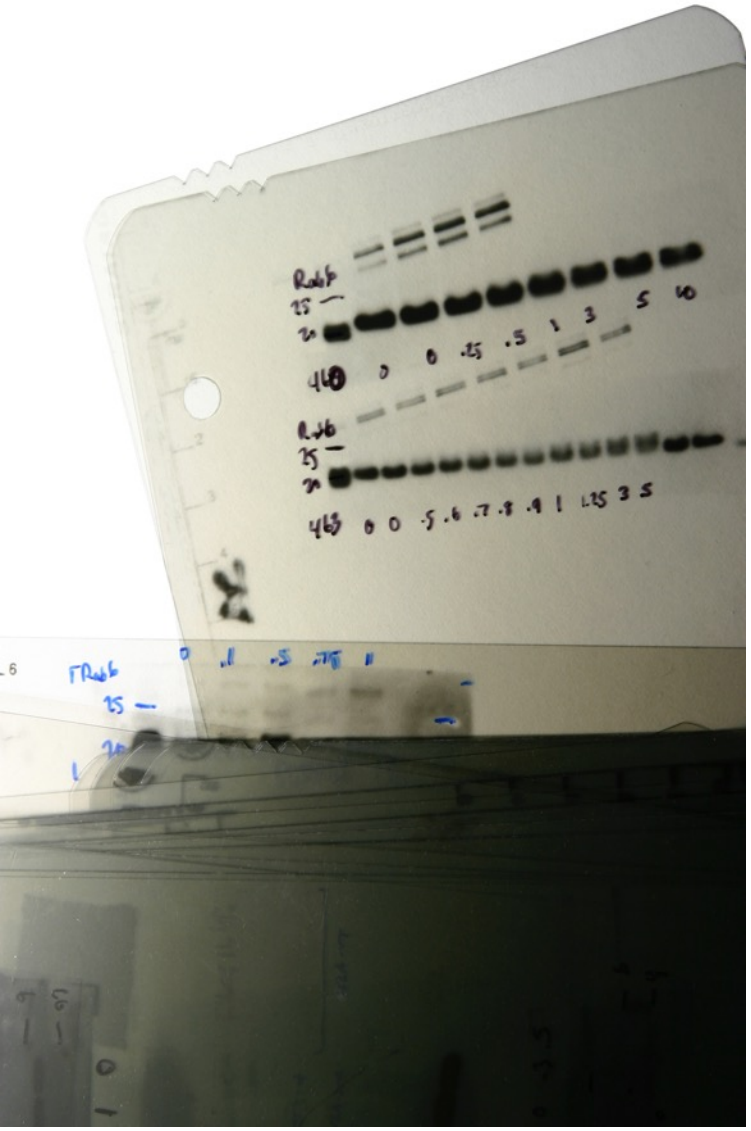
Research

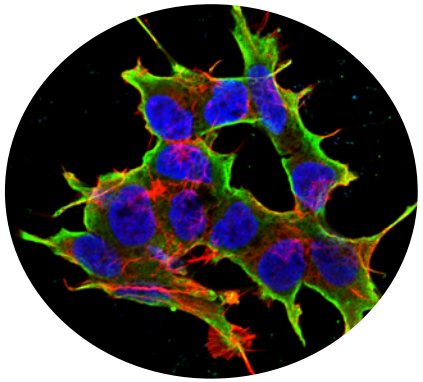


Diagnostic

Therapeutic

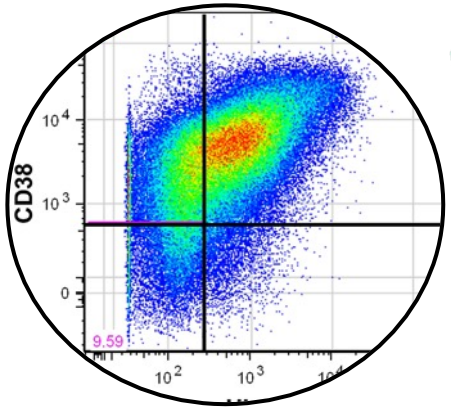
Research



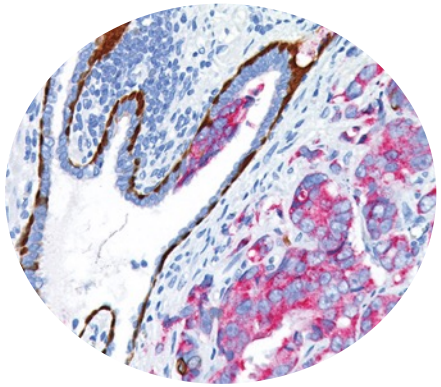


Immunocytochemistry

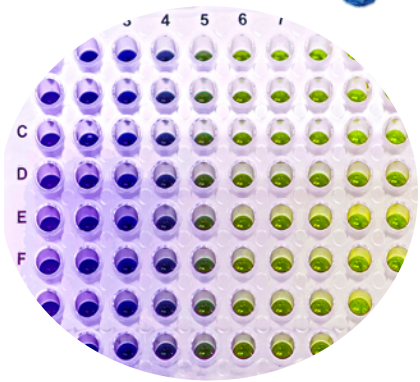
Immunoblotting &
Immunoprecipitation



Flow Cytometry

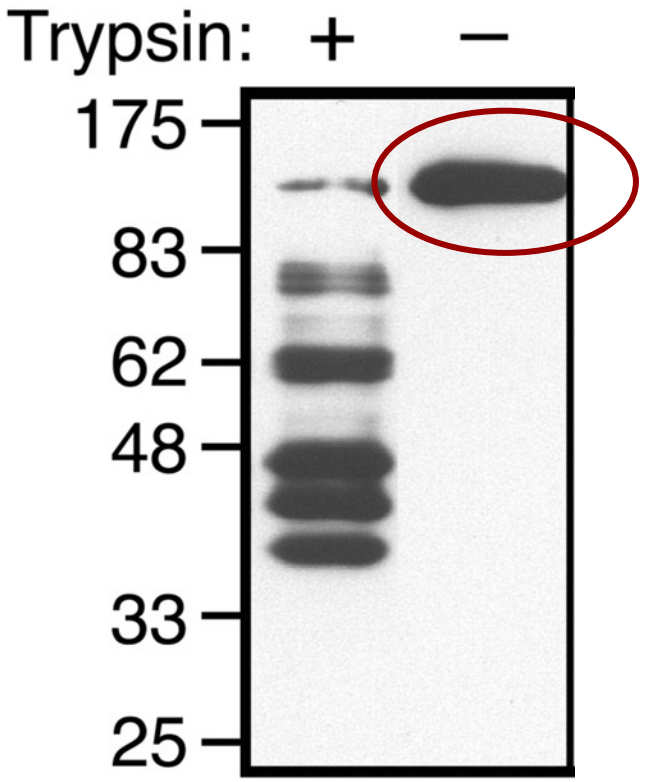


Immunohistochemistry

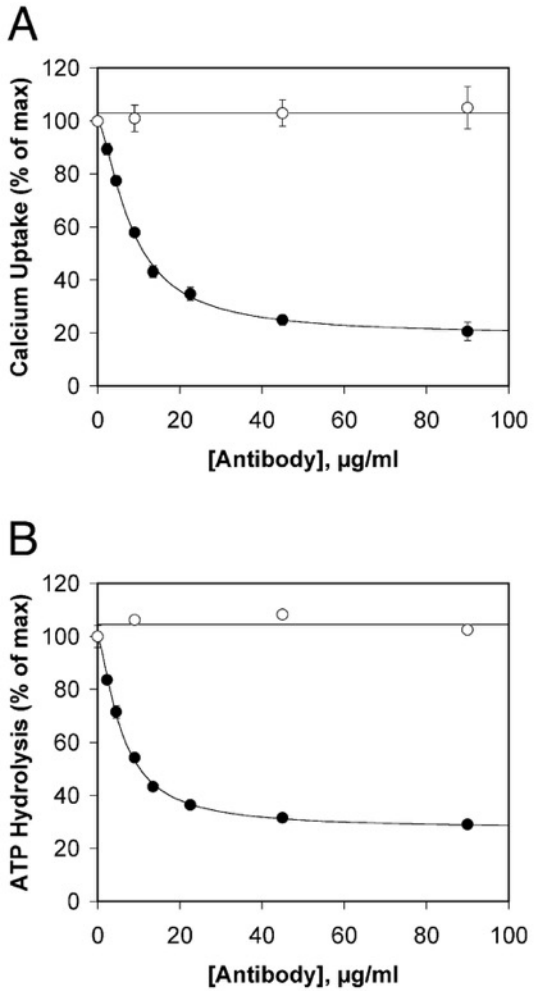


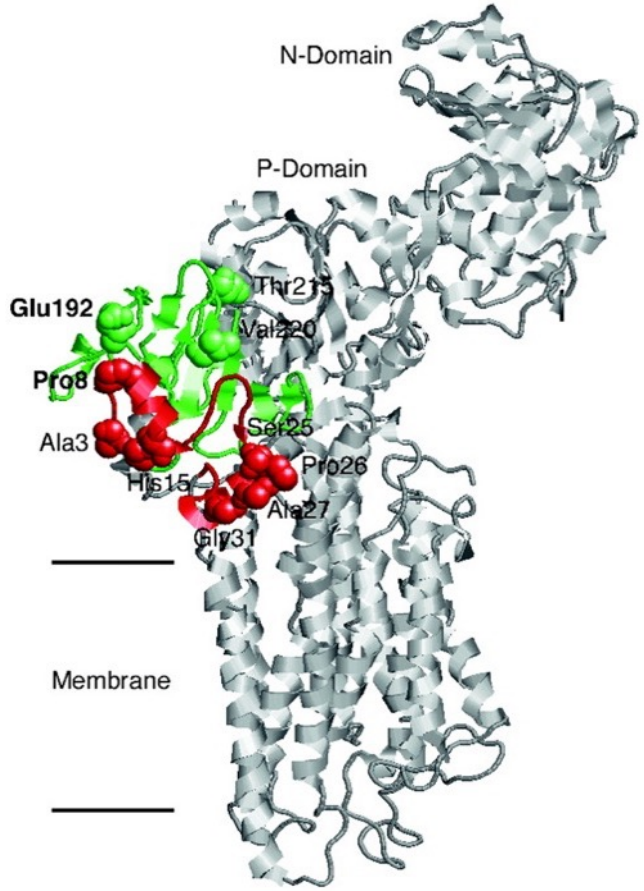
ELISA

Inhibition of human SERCA3 by PL/IM430: Molecular analysis of the interaction



PL/IM430
Superb Monoclonal Antibody





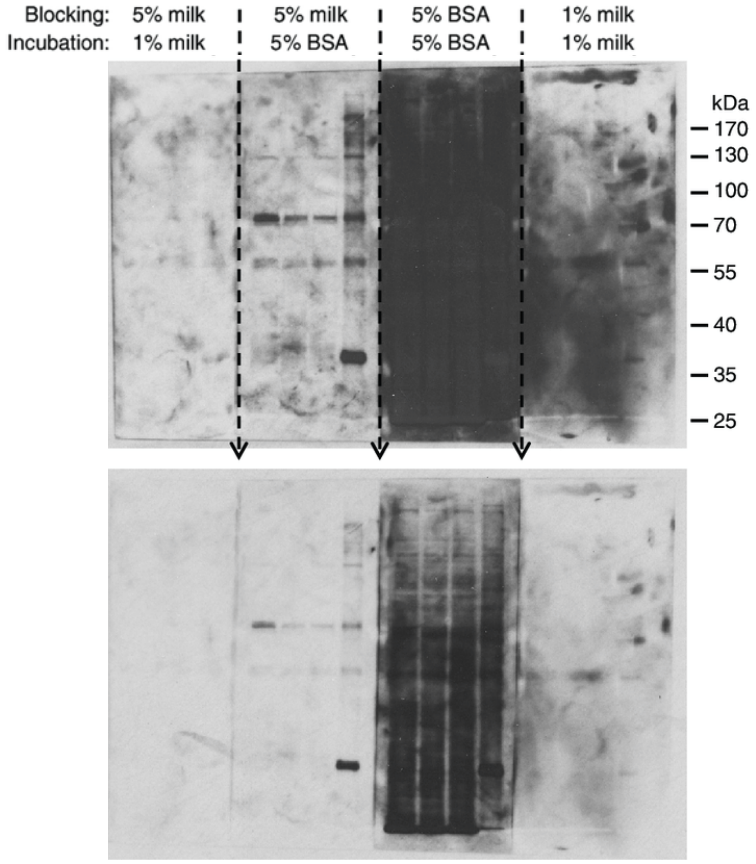
Chandrasekera CP, Lytton J. J. Biol. Chem. 2003;278:12482-12488

Buy one. Try it out. Optimize. Throw it out.

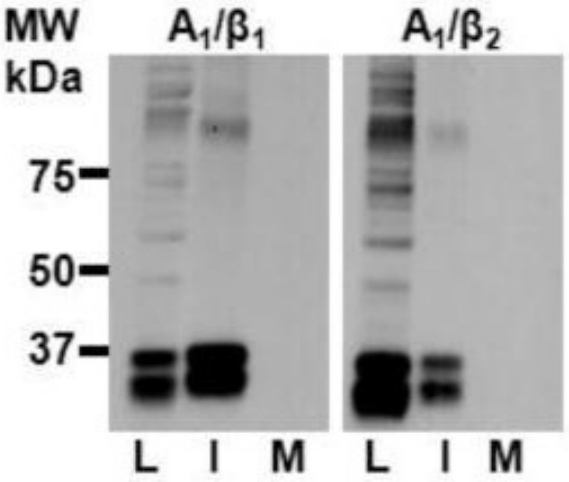
Repeat until successful.

G-Protein Coupled Receptor Antibodies:

Adenosine & β-adrenergic Receptors



Not my blot, but a very accurate representation of the mouse heart Westerns back then:
Only published gene expression data!



Human heart tissue

Third decade of the 21st century

Canadian Centre for Alternatives to Animal Methods

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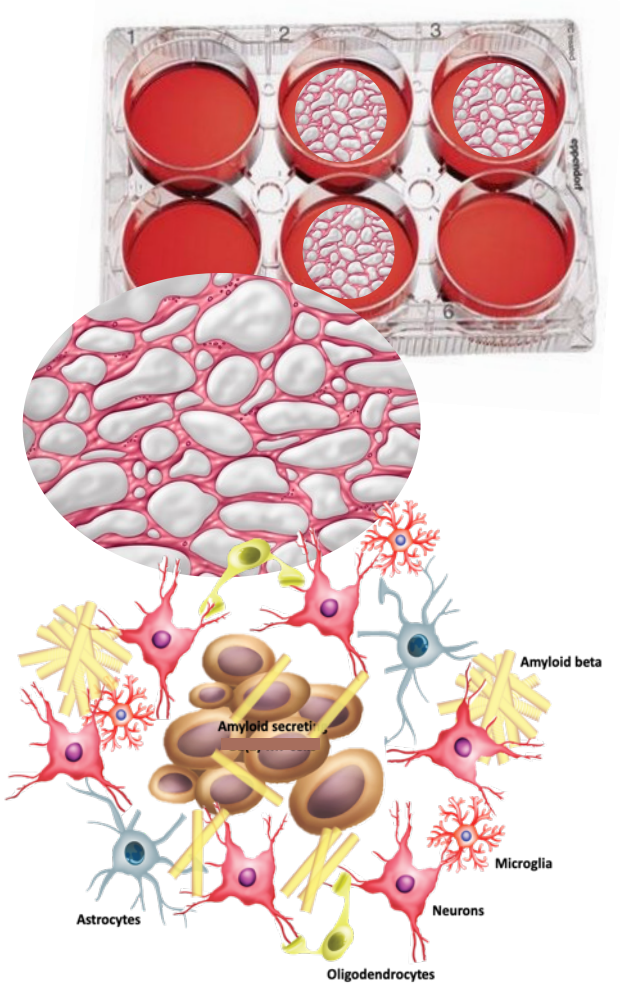
Promote the replacement of animals in Canadian biomedical research, education, and regulatory testing through 21st century science, innovation, and ethics.

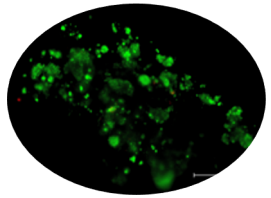


Unlike in the first or the second decade,
now I actually ***care*** about where my antibodies come from…

Disease-in-a-Dish

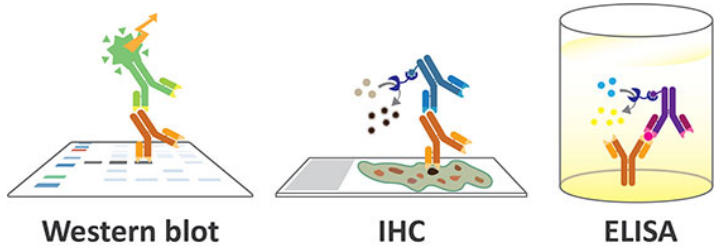


Dozens & dozens of antibodies for characterization

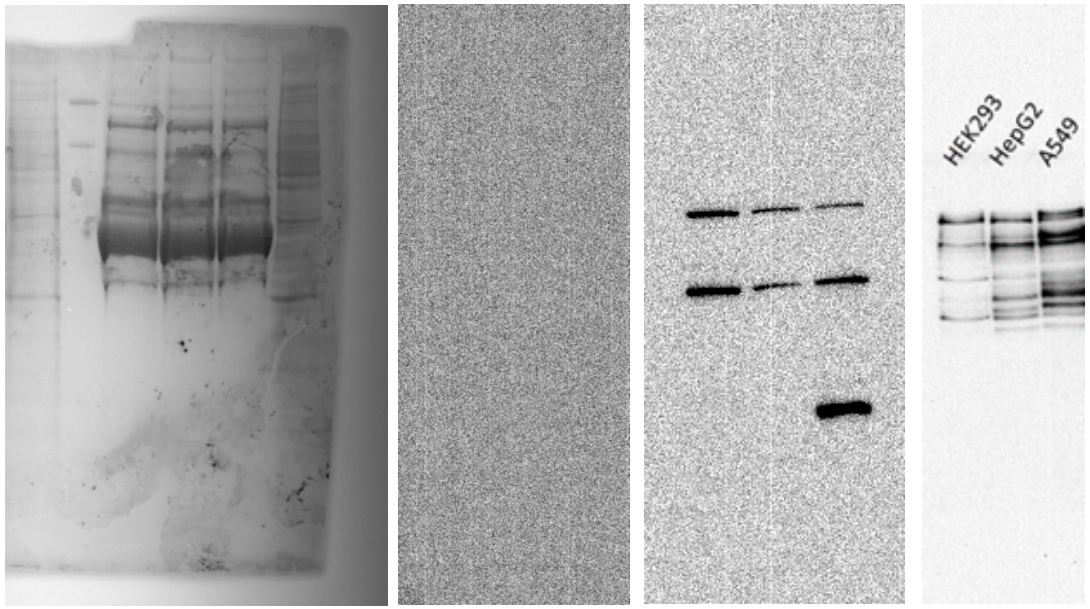


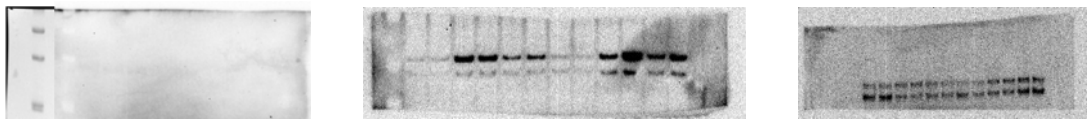
3D-Bioprinted Multicellular Liver-in-a-Dish Model

5 cell types; dozens of specific markers

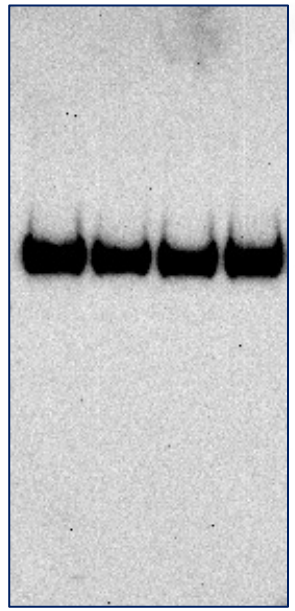


What's on the label doesn't always correspond to what's in the tube!





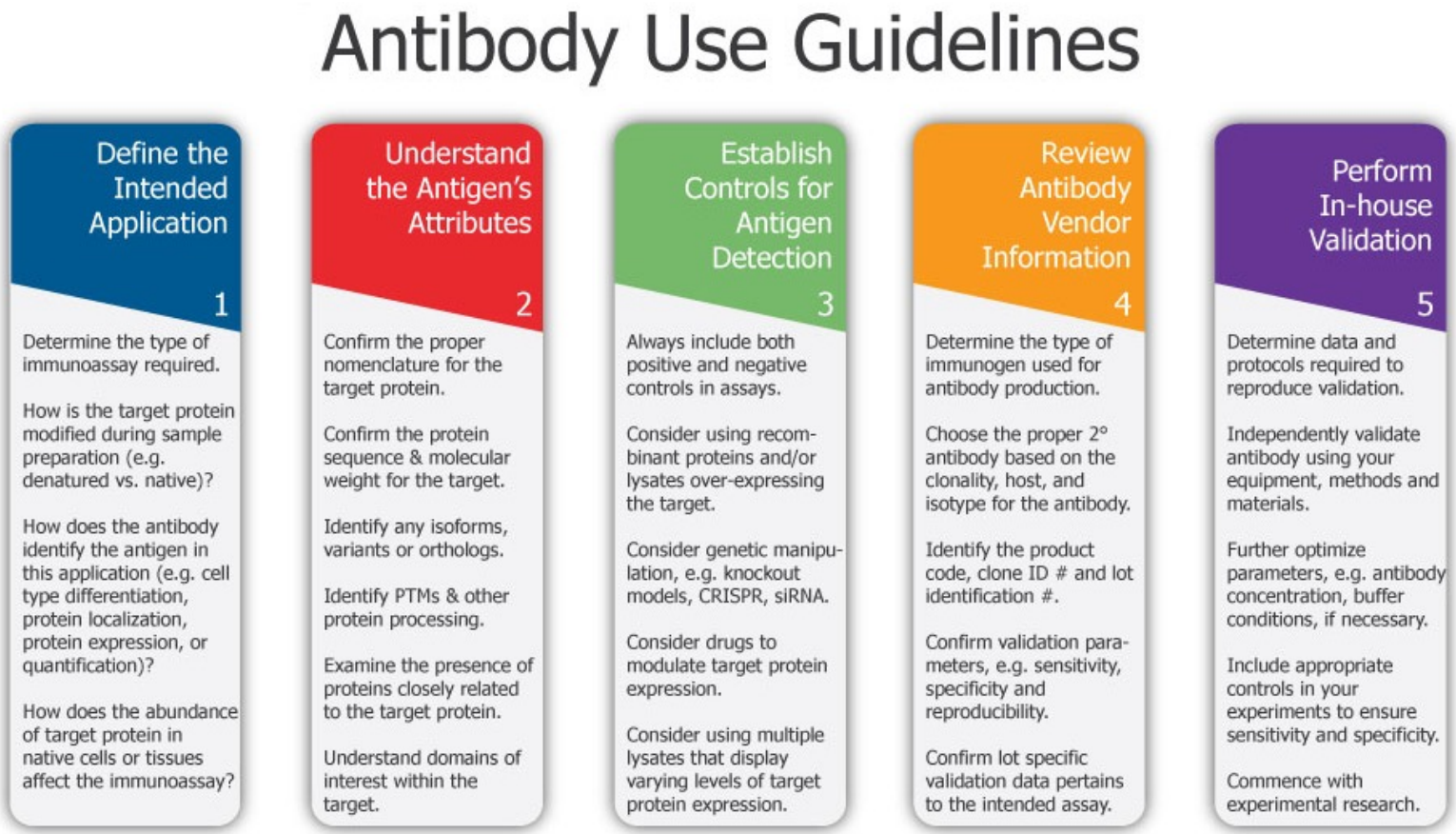
Human albumin
(not bovine)



- **Tried a dozen Abs** (mouse, rabbit, chicken, goat)
- **Lack of species specificity of target recognition**

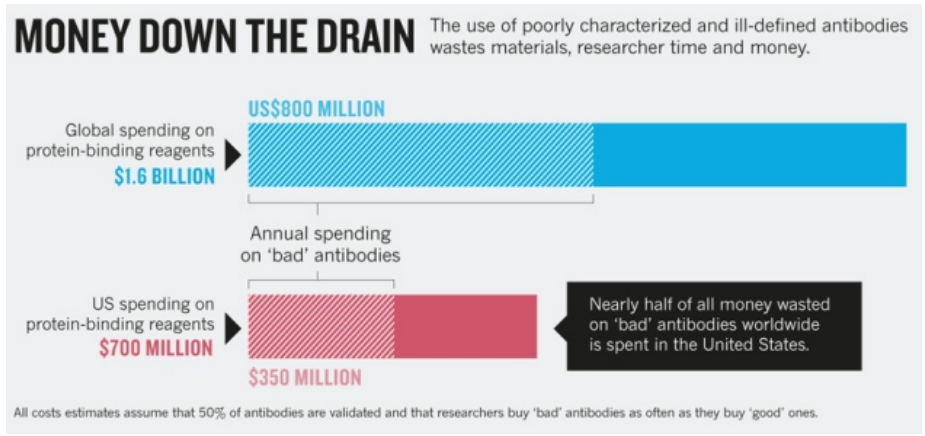
Specificity Validation:

Very tedious, trial-and-error process commonly left to end-users.



Disclaimer: Only using publicly available information from commercial suppliers to illustrate a point.

Reproducibility crisis



nature International weekly journal of science

NATURE | NEWS

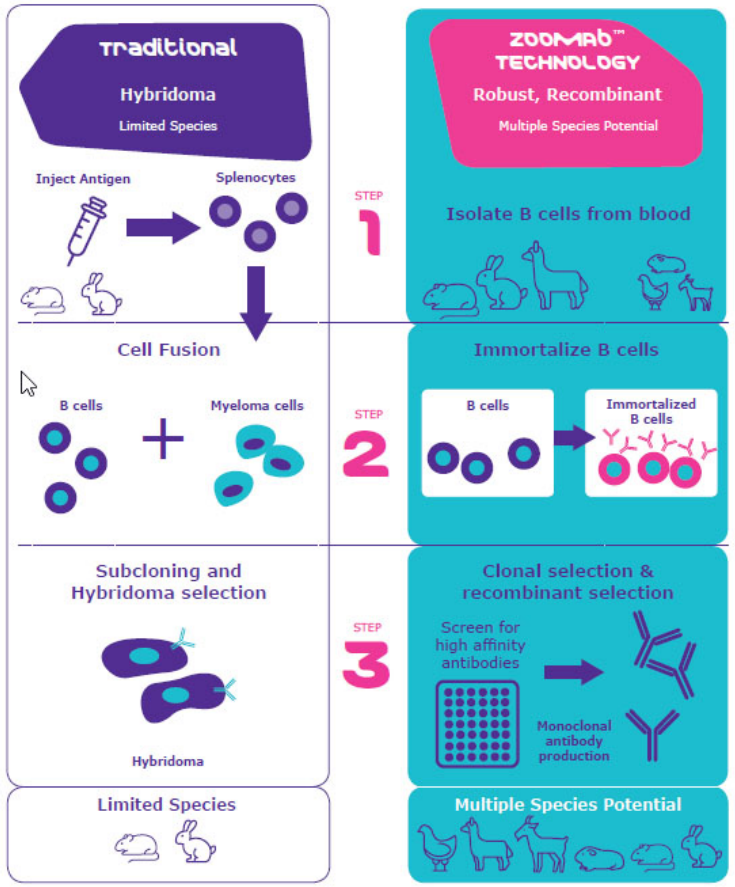
US government issues historic $3.5-million fine over animal welfare

Antibody provider Santa Cruz Biotechnology settles with government after complaints about treatment of goats.

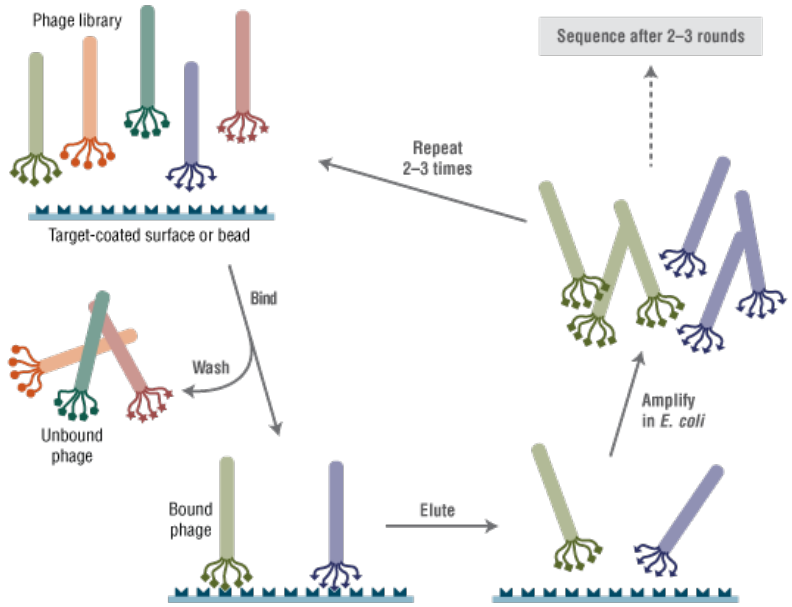
Sara Reardon

20 May 2016

Definition of Recombinant Antibody?



Pseudo-Animal-Free



Truly Animal-Free Recombinant

Disclaimer: Only using publicly available information from commercial suppliers to illustrate a point.

abcam

Recombinant antibodies: reproducible with tailored specificity

RELATED

- Knockout validation
- Carrier-free formulations for recombinant antibodies
- Directly conjugated antibodies
- Poster: automated CRISPR engineering for antibody validation

Recombinant antibodies give you the highest level of consistency between batches, peace of mind with an uninterrupted supply, and the ability to engineer sensitivity and specificity.

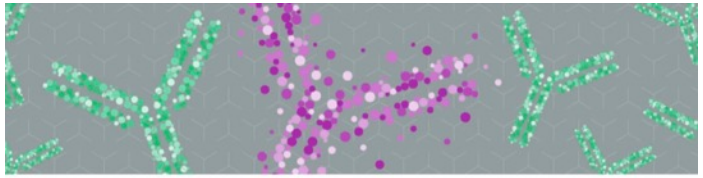
Monoclonal antibodies are typically made using B-cells from an immunized animal to form immortal hybridoma cells that secrete the desired antibody clone. This hybridoma technique produces highly consistent, specific and sensitive monoclonal antibodies in large quantities. However, over time, hybridoma cell lines can experience genetic drift, resulting in slight variations to the antibodies produced. There is also a growing demand for antibodies against difficult targets, ie toxins, nucleotides, and membrane-bound proteins, that can't always be made with this *in vivo* model.

Recombinant antibodies overcome many of the limitations of hybridoma-production of monoclonal antibodies.

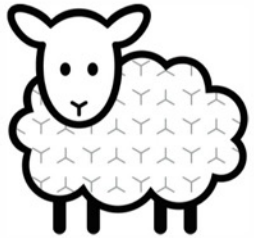
What are recombinant antibodies?

Recombinant antibodies are produced *in vitro* by cloning antibody genes for immune-specific heavy and light antibody chains into high-yield expression vectors. These vectors are then introduced into expression hosts (eg bacteria, yeast, or mammalian) to generate the recombinant monoclonal antibodies. Recombinant antibodies can be used wherever you would normally use a traditional monoclonal antibody.

Benefits of recombinant antibodies

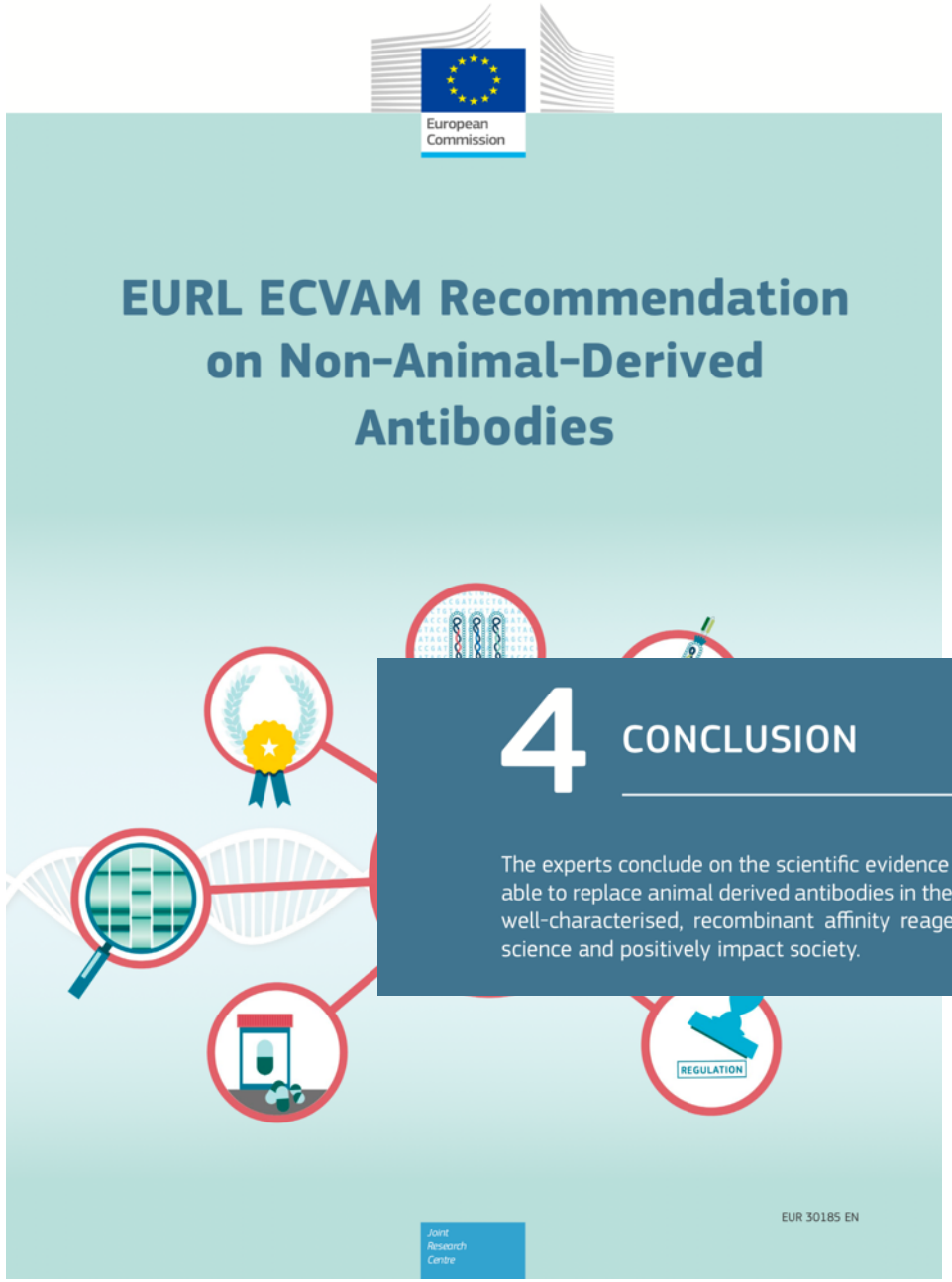


We have over 10,000 recombinant RabMab® antibodies!



RabMab® Rabbit Monoclonal Antibodies	Recombinant Antibodies
Low background	High consistency & Reproducibility
High Specificity	Improved Sensitivity
High affinity (10^{-12} K_D possible)	Improved Specificity
Diverse epitope recognition	Animal-free production

Disclaimer: Only using publicly available information from commercial suppliers to illustrate a point.



Correspondence | Published: 05 October 2020

Non-animal-derived monoclonal antibodies are not ready to substitute current hybridoma technology

África González-Fernández, Francisco J. Bermúdez Silva, Marcos López-Hoyos, César Cobaleda, Lluís Montoliu, Margarita Del Val & Kirk Leech

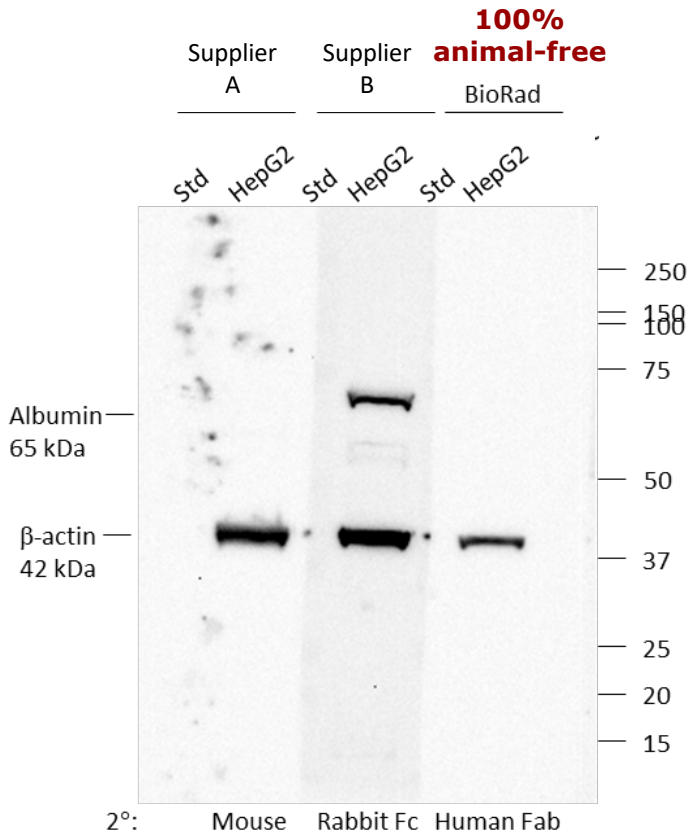
Nature Methods (2020) | Cite this article

To the Editor – We write on behalf of the COSCE (Confederation of Spanish Scientific Societies) Transparency Agreement on Animal Research, supported by the EARA (European Animal Research Association). In May 2020, the European Commission's Joint Research Centre (EC-JRC) released a recommendation on the development of non-animal-derived antibodies, urging government authorities, funding agencies and publishers to endorse the use of these antibodies to improve scientific reproducibility[1]. These recommendations were based on the work done by the Scientific Advisory Committee (ESAC) of the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM). Recent correspondence to *Nature*[2] and *Nature Methods*[3] claims that non-animal antibodies are ready to replace animal-derived ones in all known applications. In our view, however, both the EC-JRC document and the published correspondence contain distorted perceptions of the current possibilities for non-animal-derived antibodies. While we are all committed to replacing animal experimentation with alternative methods, these methods need further scientific validation to justify replacing the use of animals without affecting the desired outcome of the experiment.

Do animal-free antibodies work *as well*?

Commercially available
100% animal-Free

- **β-actin →**
- Calnexin
- β-catenin
- Vinculin
- HSP 5
- Cyclophilin B
- Vimentin
- GAPDH

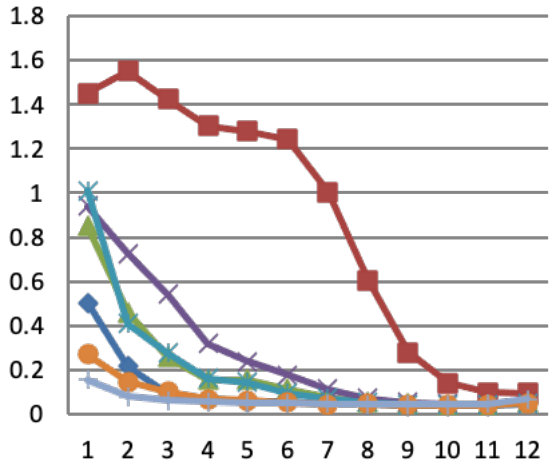


*Not a perfect side-by-side comparison;
2 antibody variable.
(Trying Protein A/G-HRP, but even then.)

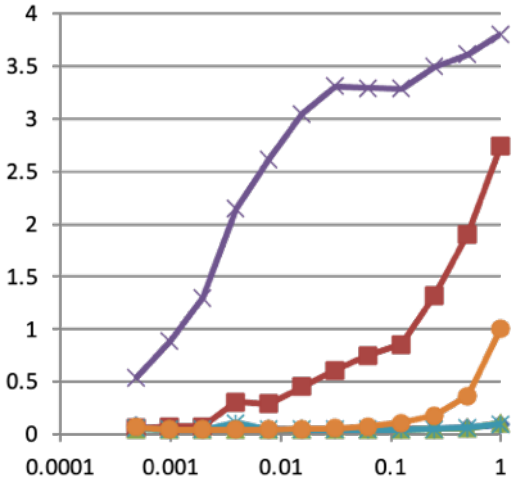
Planning to test other applications:
Immunoprecipitation, ELISA, and Immunofluorescence

Physicians Committee for Responsible Medicine

Peptide antigen

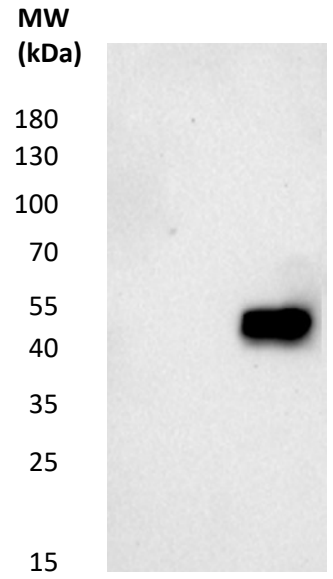


Protein antigen



Serial dilution from 1 ug/mL

Our 100% recombinant antibody



10 ng/lane antigen

Animal-derived counterpart – Mouse ascites antibody

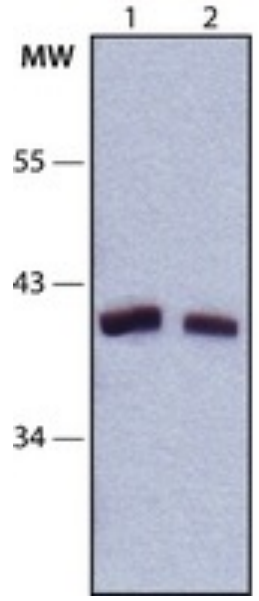
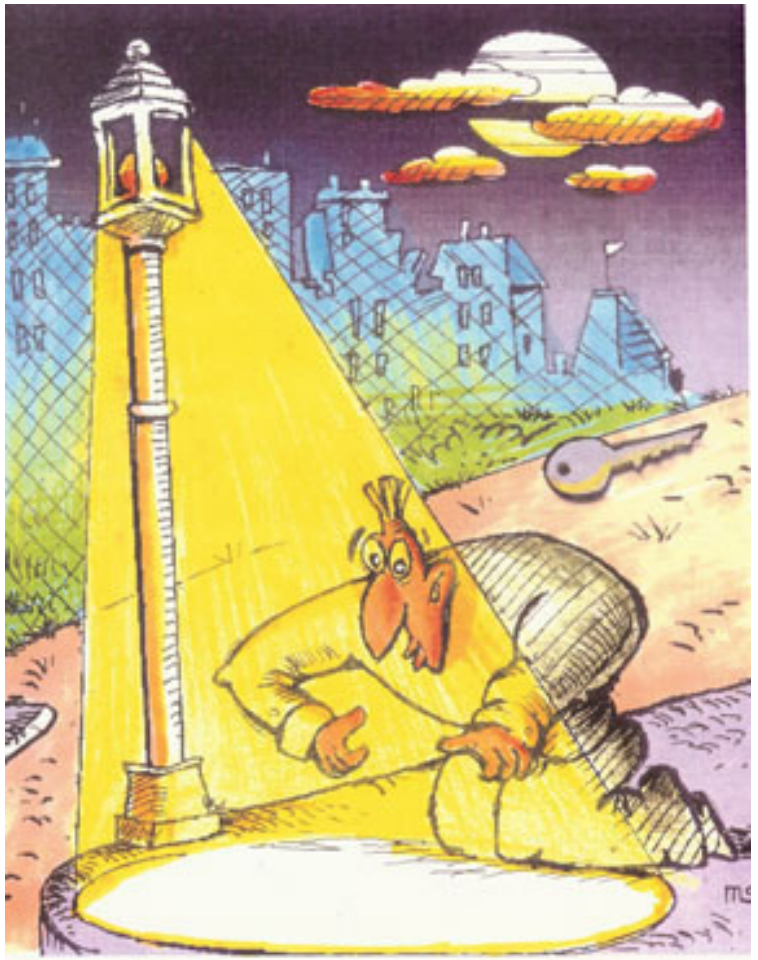
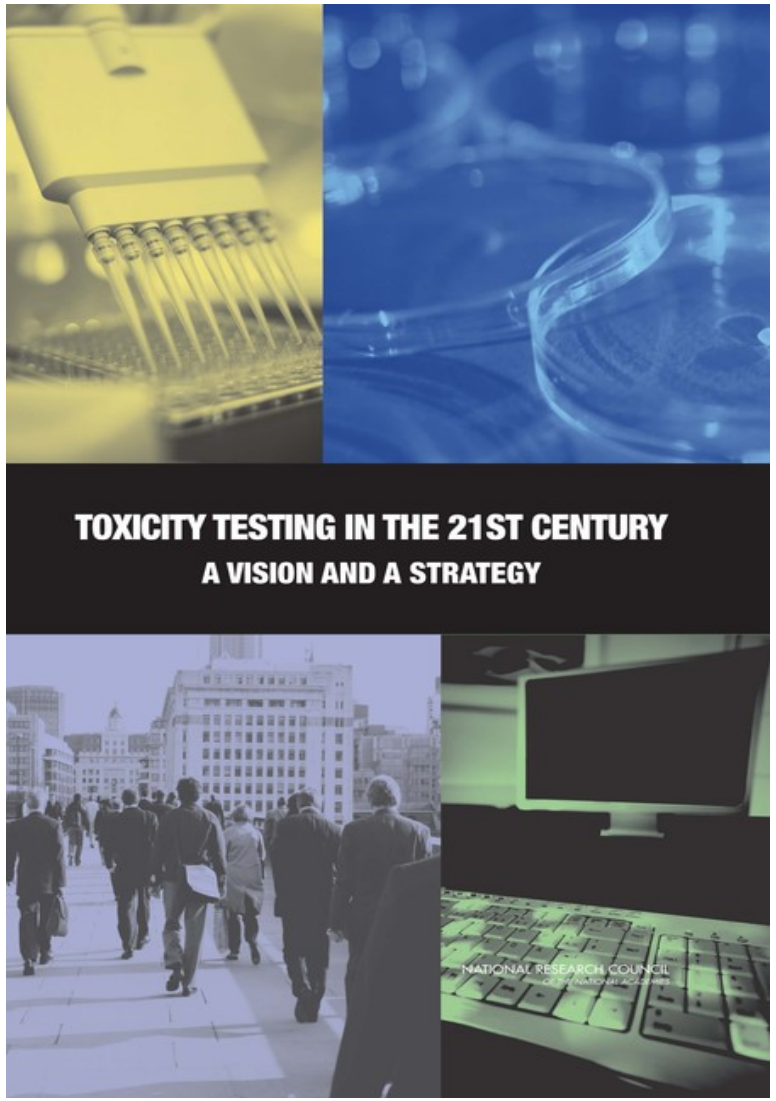


Image from supplier website

The evidence exists.



Stop looking under the lamp post.



EPA says it will eliminate animal testing by 2035

By Ali Zaslav, CNN

Posted at 1:07 PM ET, Tue September 10, 2019

Thank You!

www.uwindsor.ca/ccaam