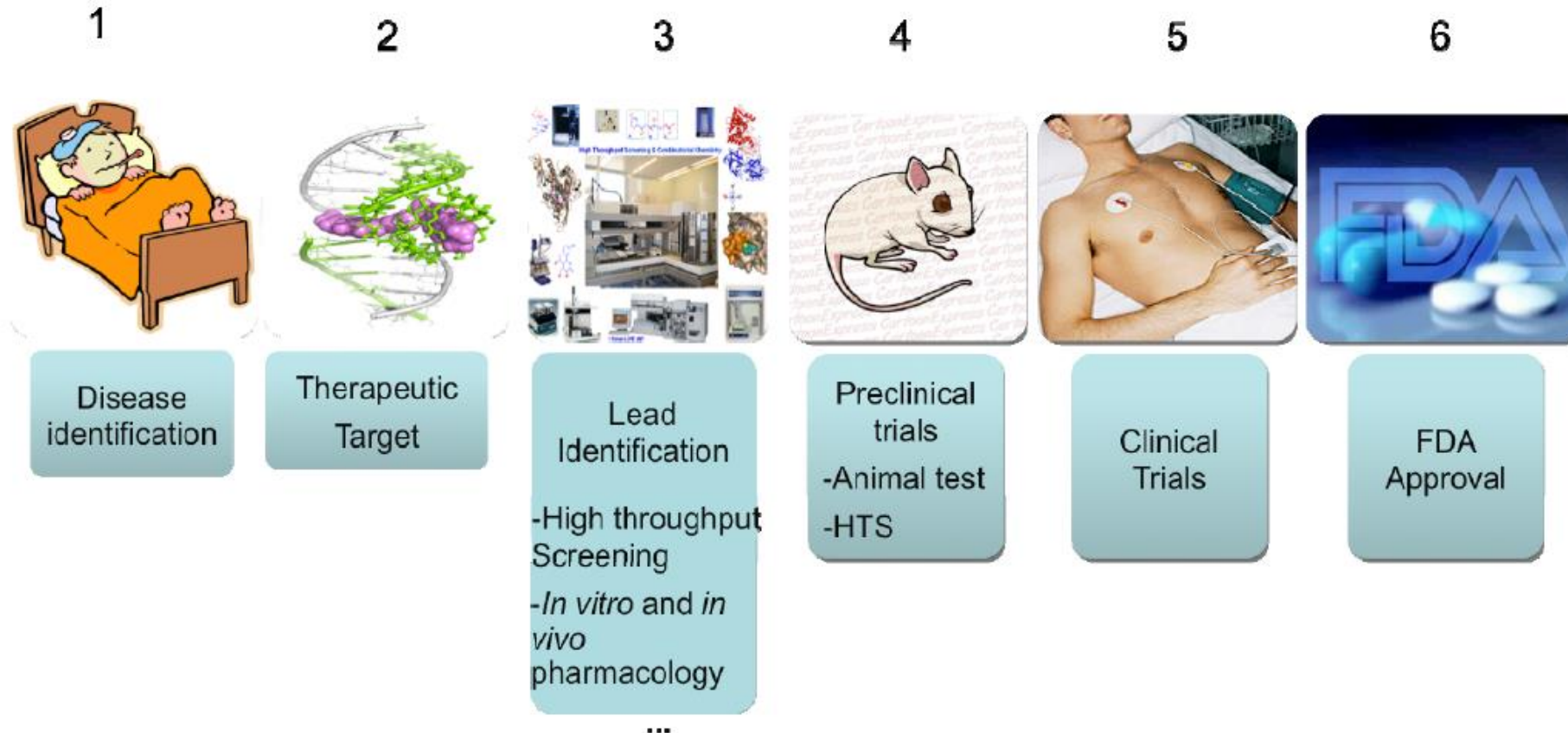


High Throughput Screening and Assay development for Drug Discovery

Prof M Kaur

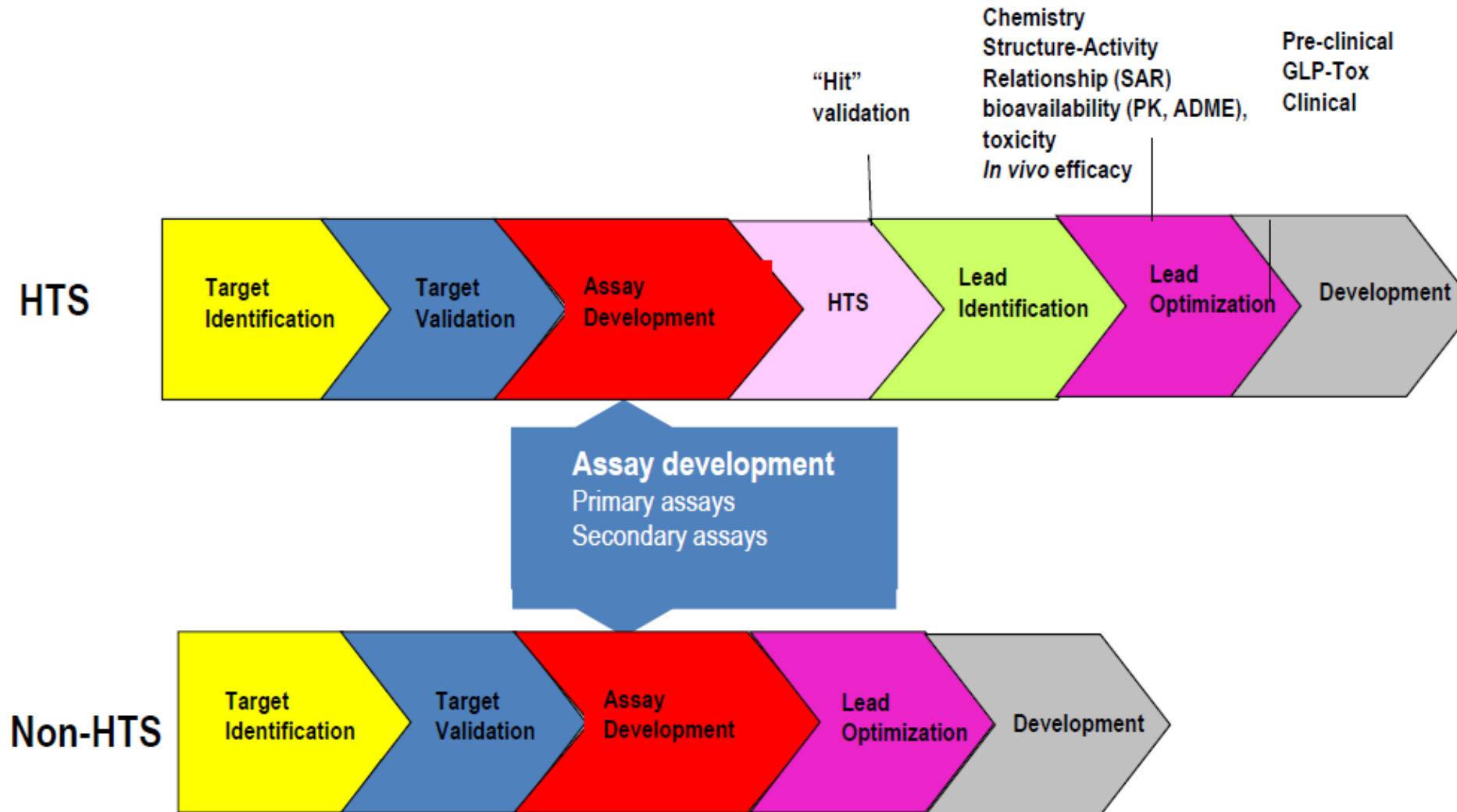
Stages of Drug Discovery



High-throughput screening (HTS):
Lead identification and Preclinical toxicology



The Drug Discovery Process: The Assay Development Stage



What is Bioassay?

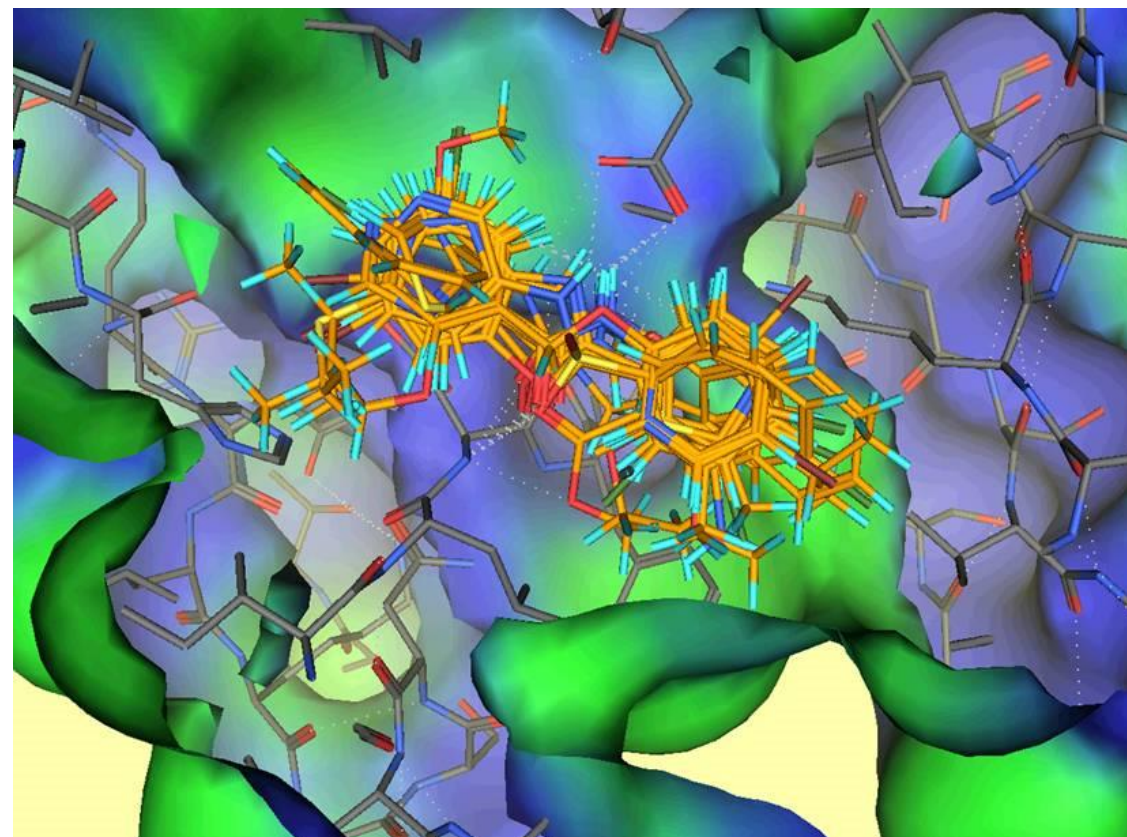
Bioassay or biological assay/screening is any qualitative or quantitative analysis of a substance that uses a living system, such as an intact cell, as a component.

Broad Categories

- *Virtual Screenings*
- *Primary Bioassays*
- *Secondary Bioassays*
- *Preclinical Trials*
- *Clinical Trials*

Virtual and *In Silico* Screenings

- Ligand based or Target based
- Target Selection
- Data Mining (Chemical space of over 10^{60} conceivable compounds)
- Screening of Libraries of Compounds Virtually
- Lead Optimization
- Prediction of Structure-Activity Relationships
- *It Save, Time, Money and Efforts*



Primary Bioassays

- Non- physiological Assays
- Biochemical or Mechanism-Based Assays
- Microorganism-based bioassays
- Cell-based Bioassays
- Tissue-based Bioassays
- Many other *In Vitro* bioassays/assays

A hit rate of 1% or less is generally considered reasonable

Secondary Bioassays

- Animal-based assays (*In Vivo*)
- Toxicological Assessments in whole animals
- ADME Studies
- Behavioral Studies
- Preclinical Studies

In Vitro Bioassays

In Vitro: In experimental situation outside the organisms. Biological or chemical work done in the test tube (in vitro is Latin for “in glass”) rather than in living systems

- *Toxicity Assays*
 - MTT assay
 - Cancer cell line assays

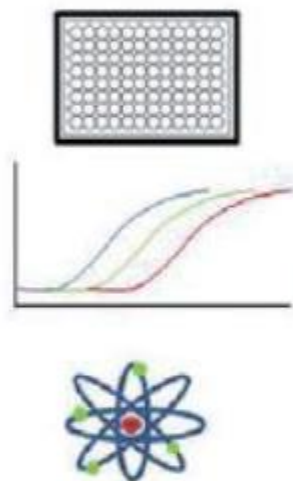
In Vivo Screenings

In Vivo: Test performed in a living system such as antidiabetic assays, CNS assays, antihypertensive assays, etc.

- *Animal Toxicity*
 - Acute toxicity
 - Chronic toxicity
- *Pre-Clinical Trials*
- *Clinical Trials*

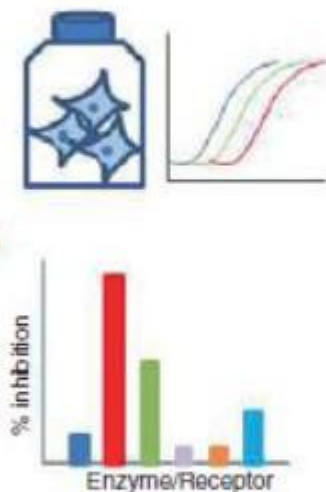
Assay development: A critical part of the “hit” discovery process

Primary assays



- HTS & selective library screens; structure based design
- Reiterative directed compound synthesis to improve compound properties

Secondary assays



- *in vitro* & *ex vivo* secondary assays (mechanistic)
- Selectivity & liability assays

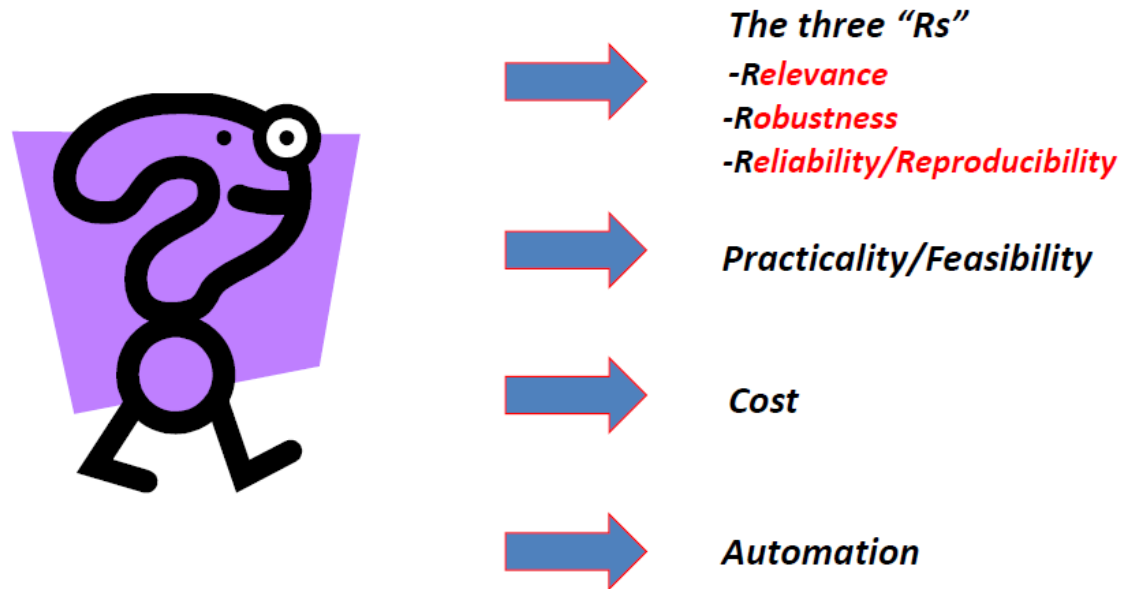
“HITS”

A “hit” is a compound which has the desired activity in a compound screen and whose activity is confirmed upon retesting

Assay Development for Drug Discovery



Key Considerations in Assay Development



“The quality of an assay determines the quality of the data: compromising on assay development can have substantial downstream consequences”

Assay development: From the bench to HTS

When you go

From This



To This



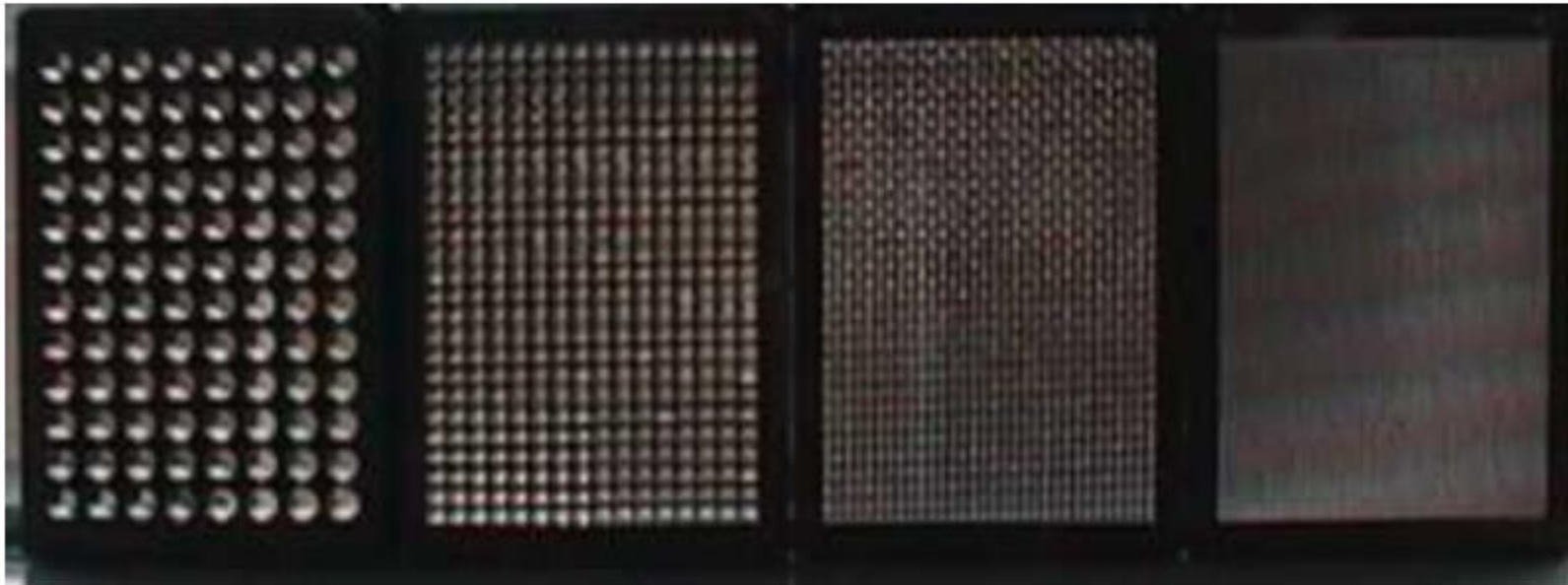
HTS Technology

- ❖ Robotics
- ❖ Miniaturization
- ❖ Sophisticated Assay Chemistry
- ❖ Sophisticated Software and Database



Everything is done in Microtiter Plates

96, 384, 1536, 3456



96-well
100-200 μ l

384-well
25-50 μ l

1536-well
4-10 μ l

3456-well
1-2 μ l



Microtiter plates

96-Well plate
(80 compounds/plate)

384-Well plate
(320 compounds/plate)

1536-Well plate
(1280 compounds/plate)

Miniaturization

10,000
Compounds/day

100,000
Compounds/day

125 Plates/day

1250 Plates/day

32 Plates/day

313 Plates/day

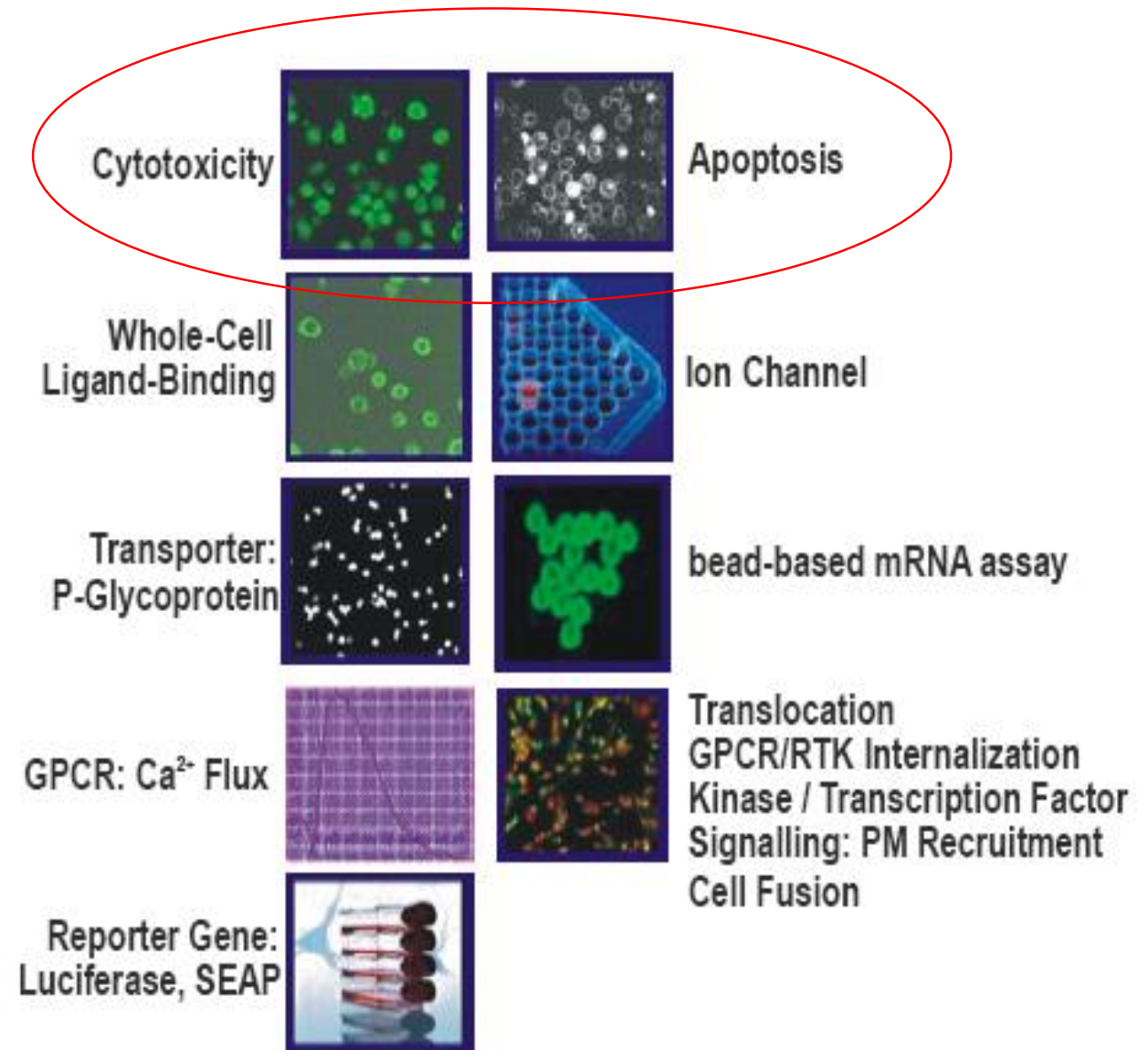
8 Plates/day

78 Plates/d



From a Workshop on High Throughput / High Content Screening Applications to Target-based Drug Discovery Research

Examples of Assays



Data Analysis and Management

Software packages for HTS (e.g. **Activitybase**, **Spotfire**) are available to carry out the principle tasks like

- a) Storage of raw data
- b) Quality control
- c) Transformation of data into information
- d) Documentation
- e) Reporting

Results from 1 plate in Enzyme Inhibition Screen

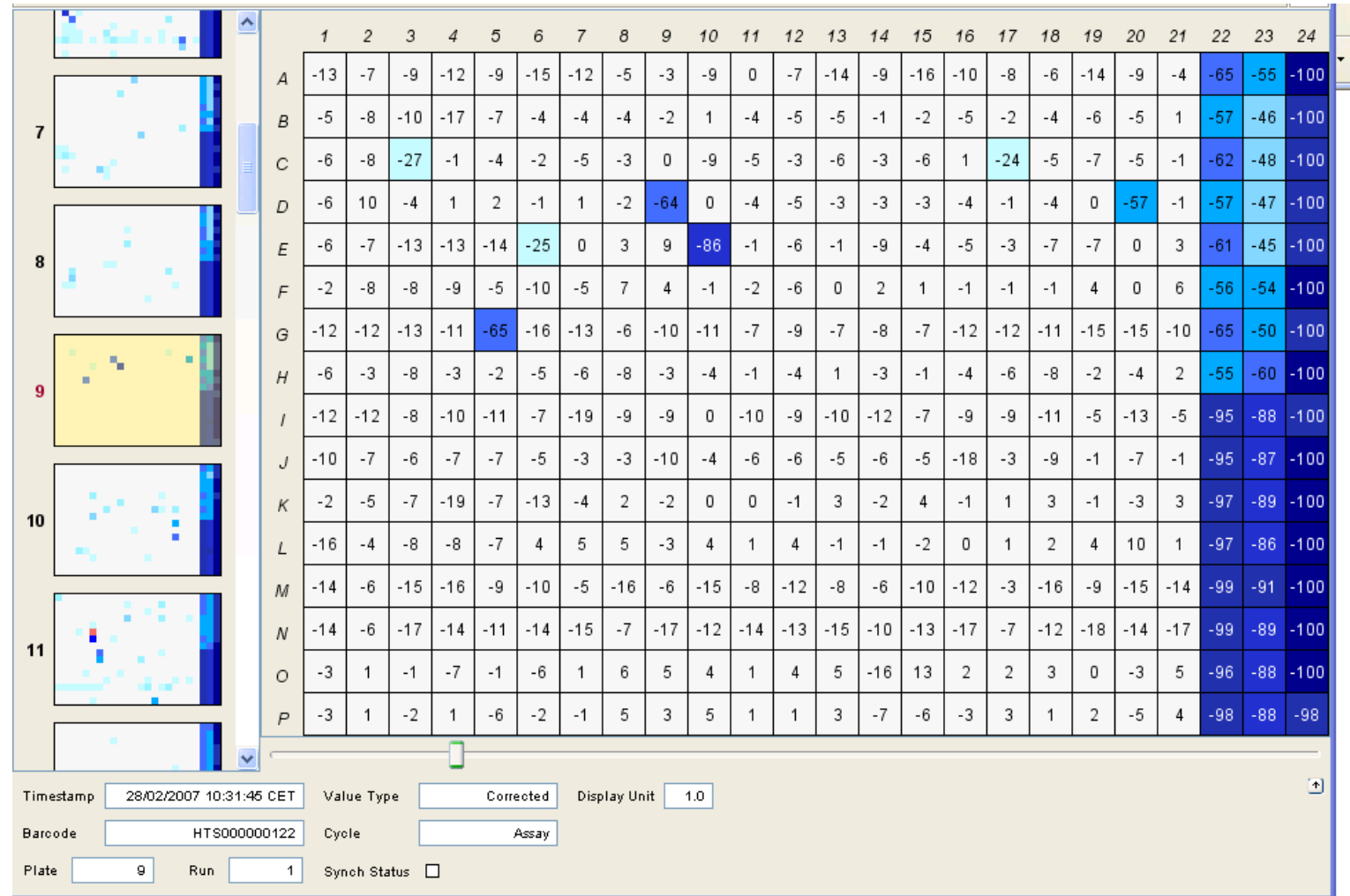


Plate Statistics									
	Compound	Neutral Control	Standard 1	Standard 3	Inhibitor Control	Standard 2	Standard 4		
Mean	-6.267	-1.730	-59.74	-50.75	-99.85	-97.11	-88.20	20.34	Sig. / Back.
Median	-5.373	0	-59.05	-49.32	-100	-97.11	-88.29	0.7718	Z' Factor
Std. Dev.	9.339	6.913	4.021	5.264	0.5502	1.530	1.362	0.8242	RZ' Factor
RStd. Dev.	5.936	5.711	4.659	5.711	0.1503	2.104	0.9017	0 & 0	Inhib. & Stim. Counts

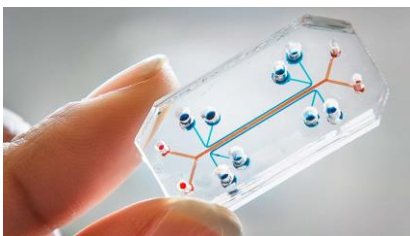
Considerations for HTS

- Outsourcing as cost of set-up is heavy
- 100,000 compounds library-over \$300,000

Recent Advances

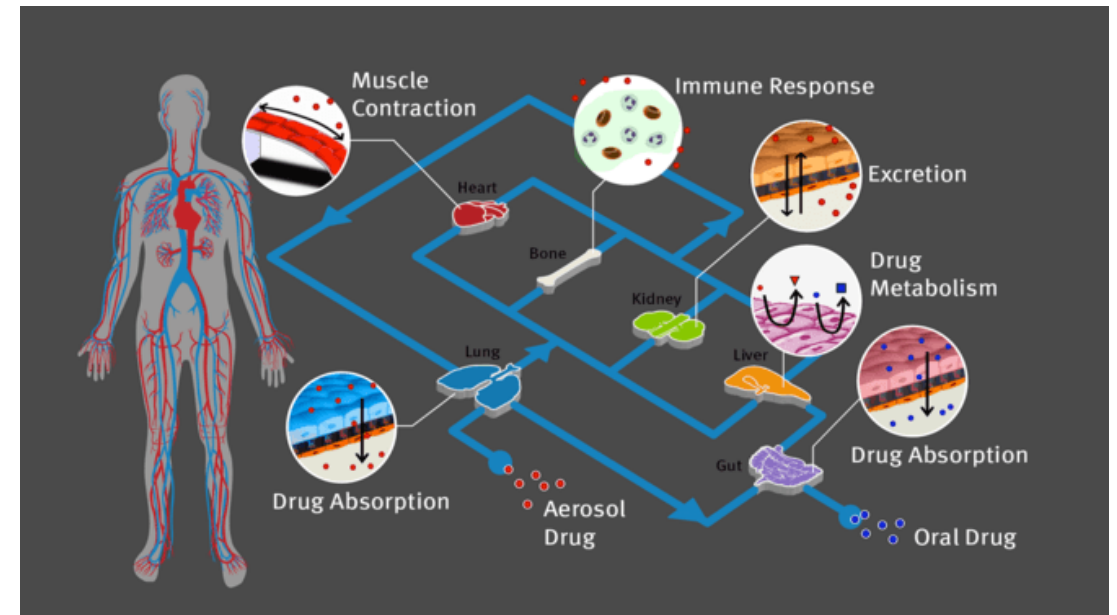
‘Organs-on-a chip’

Microchips lined by living human cells that could revolutionize drug development, disease modelling and personalized medicine



<i>Plate density (wells/plate)</i>	<i>Assay volume (μL)</i>	<i>Throughput (tests/d)</i>	<i>Reagent costs/well (\$)</i>
96	50–200	10,000	0.50
384	20–50	40,000	0.20
1536	2.5–10	60,000	0.05

<https://thenewstack.io/organs-on-chips-emulates-human-organs-for-better-biomedical-testing/>



See video here: <https://wyss.harvard.edu/technology/human-organs-on-chips/>

