Using automation to support validation at ECVAM

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Outline

• Motivation.
• HTS/HCA facility at ECVAM.
• Factors to consider when automating assays.
• Case studies: 3T3/NRU and LUMI-CELL.
• Conclusions and outlook.
Motivation

Exploiting assay automation to support validation:

• Efficiency – generate data faster.
• Coverage – test broader chemical domain.
• Precision – minimise technical variance.
• Application – make ready for industrial use.
• Necessity – validation of HTS-specific assays.

HTS/HCA facility

Compound management

Advanced detection

Cell culturing

Compound repository
Automation platform

1 to 300μl volume handling

Analytical chemistry - NMR

- Verify chemical identity by checking NMR spectrum against structure.
- Determine absolute conc. in solvent.
- Determine absorbed water content.
- Assess purity and stability of chemicals.
- Detect chemical-reagent interactions.
- Routine QC-screening of libraries.
- Non-destructive thus sample is available for further analysis (MS).

Avoid spurious results, pointless retesting, and endless discussion!
Information content

Information

In vitro testing

Low content

Medium content

High content

Toxicity Screening → Toxicity Profiling

High Content Analysis

Automated quantitative imaging

Electrophysiology using MEA
Advanced MS and NMR

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Technical aspects

• Automation
  - liquid handling; plate manipulation, cell seeding, detection, timing

• Experimental design
  - plate format typically 96, 384, 1536 wells
  - number and position of chemicals/controls on one plate
  - conc-resp within or across plates within a run
  - degree of replication within and between runs
  - pipetting volumes and dilution protocols

• Preparation
  - chemicals prepared as DMSO stock solutions beforehand
  - volume of cells typically to fill 10 to 20 plates for a run

• Data
  - annotation, storage, retrieval, visualisation
  - normalisation, scaling model fitting

Challenges

• High setup and maintenance costs.
• High density plate formats (384 & 1536 wells).
• Handling liquids with variable phys-chem properties.
• Availability and quality of large chemical libraries.
• Scale and reproducibility of cell culturing.
• Effort and cost per experimental run.
• Operational and logistical complexity.
• Heavy price for small mistakes.
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3T3/NRU Assay

• 3T3 mouse fibroblasts
• 48 hour treatment duration
• EP - cellular uptake of Neutral Red dye
• 72 reference chemicals
• Stringent acceptance criteria and PS
  → Commissioning study (faithful implementation)
  → ECVAM validation study (56 chemicals)
  → Scale-up (quantitative HTS format)


3T3/NRU protocol

Plate layout

Original protocol

Cell treatment

NRU detection

Performance Assessment

Historic mean IC\textsubscript{50} of PC (SLS) over 6 months

Comparison of IC\textsubscript{50} of 12 reference chemicals
Results from 44 of 56 chemicals that could be tested and exhibited toxicity

**Quality assessment**

**Z' factor** - a performance indicator for bioassays. Evaluation of assay quality

\[
Z' = 1 - \frac{3\sigma_{C8} + 3\sigma_{C1}}{\mu_{C8} - \mu_{C1}}
\]

C1 highest and C8 lowest conc.
Quantitative HTS (qHTS)

Pharmaceutical industry

HTS

1536/3456 WP

PLATE 1

CONC. 1

PLATE 2

CONC. 2

Throughput

> 10^3 compounds/plate

• Single concentration for all compounds

• No replicates

• Fast and efficient for large compound libraries

• No dose-response curve, high uncertainty

• Many compounds/plate

• Large (fixed) conc. range

• Conc. varies over plates

• Many concentration points

• On-plate replicates and controls

Physical industry

HTS

96/384 WP

24/96 WP

Quality of data

Modelling variance in HTS experiments to estimate precision of various intra and inter plate designs (traditional versus qHTS)

\[
X_{jks} = \mu + \xi_j + n_{jk} + \epsilon_{jks}
\]
\[
Y_{ijk} = \mu + \eta_i + \xi_{ij} + n_{ijk} + \epsilon_{ijk}
\]

\[
\tau_X = \text{Var}(X_{-}) = \frac{\epsilon^2}{n_j} + \frac{\xi^2}{n_j n_K} + \frac{n_{jk}}{n_j n_K n_S} \approx 0.70
\]

\[
\tau_Y = \text{Var}(Y_{-}) = \frac{\epsilon^2}{n_j} + \frac{\xi^2}{n_j n_K} + \frac{n_{jk}}{n_j n_K n_S} + \frac{n_{ijk}}{n_j n_K n_S} \approx 0.89
\]


ICCVAM/NICEATM Coordinated Study

- Reporter gene assay - ER binding.
- Agonist and antagonist formats.
- 78 chemicals in test set.
- 3 test labs (manual) inc. ECVAM.
- Peer review March 2011.

Automation at IHCP

- qHTS format with 96-well plates.
- 16 plates/concentrations, DL of 2.
- 44 chemicals per plate, 2 replicates.
- 3 biological repeats.
- pos/neg control, fixed conc, 4 replicates.
- Acceptance and normalisation criteria needed adapting.
LUMI-CELL HTS Results

Genistein

LUMI-CELL HTS Results

o,p' - DDT
**Biphasic Model**

\[
\begin{align*}
\alpha - \omega + \frac{\text{Max} - \alpha}{1 + (\epsilon_{\text{up}}/x)^{\beta_{\text{up}}}} \\
y = \omega + \frac{1}{1 + (\epsilon_{\text{dn}}/x)^{\beta_{\text{dn}}}}
\end{align*}
\]

*FIGURE 2. Illustration of the meanings of the parameters of the general biphasic model for hill-shaped curves (eq 8).*

*Don’t throw the baby out with the bathwater!*

**LUMI-CELL Automation**

**NIH Chemical Genomics Center**

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Validation requirements

- Purpose of the validation study needs to be clear.
- Availability of ‘reliable’ reference chemicals.
- Assay protocols have to be optimised and well defined.
- Appropriate and unambiguous acceptance criteria.
- Right data treatment and statistical analysis.
- Importance of transferability and reproducibility.
- Level of formality and independent peer-review.

One size doesn’t fit all!

Conclusions and outlook

- Automation is a powerful tool that can support and expedite the validation process in many ways.
- Nobody will be out of a job - some roles may change.
- Proactive education and communication on HTS methods are needed to reap the full benefit.
- You don’t have to have a HTS facility to contribute.
- Data is just data. Knowledge enables us to convert that data into useful information for safety assessment.
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http://ihcp.jrc.ec.europa.eu/

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