

Development and Utilisation of the acCELLeratortm Cell Culture System within the Drug Discovery Pipeline

Gary Allenby

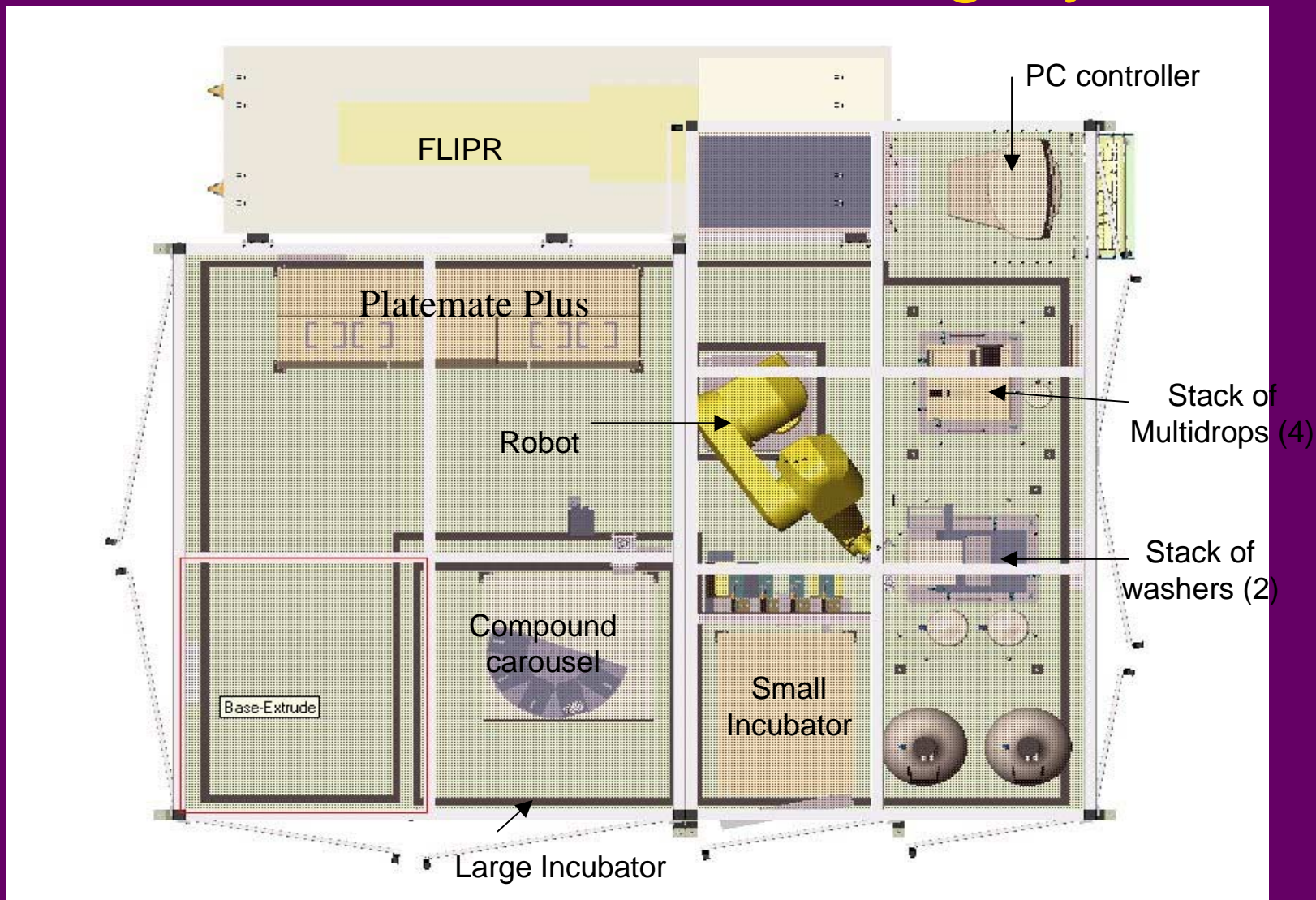
Lead Generation

AstraZeneca R&D Charnwood, U.K.

Introduction

- Increasingly, more cell-based HTS are being performed:
 - 60% 2002, 70% 2003 have been cell-based
 - CD's from functional assays with faster timelines
 - Why? Closer to 'real' biology, more relevant? More information earlier – less iterative cycles, make “right” med chem choices earlier
- AZ Charnwood HTS focused on cell-based HTS (750K wells, 1 compound/well)
 - FLIPR
 - FMAT
 - HCS
 - Ion channels

Automated FLIPR Screening System



Have we achieved our goal?

- Automated throughput
 - 160 x 384 wells/day for 4 days/week
205k per week, therefore 500K = 3 weeks
- FTE requirement (minimal)
 - Need 1FTE to set-up reagents and system
 - But:
 - Need at least 1FTE for tissue culture (160 x 384 well plates per day is a lot of flasks to prepared)
- New bottle-neck?
 - Automated cell culture needed to deal with this throughput

Why Automate?

- Scale of the problem
 - Automation of cell assays = increased throughput
 - 384 well x 2000 plates x 10,000 cells/well = 80 billion cells
 - Each day requires 50 + 10 + 2 x T225cm² flasks
 - Thank-less task = 1 person/screen/day performing cell culture
 - Skills base and working practice (tied to bench work)
 - More advanced automation = more cell based assays
 - Adherent cell lines require 24 hours incubation = 3 or 4 screening days/week or weekend work
 - 1 million compounds = 3,200 plates @
 - » 400/week = 8 weeks
 - » 500/week = 6 weeks

Why Automate?

- Data Quality and Fidelity
 - Cell-based HTS is inherently more variable than non-cell assays. Why?
 - Operator induced variability in technique (cell number)
 - ‘Batch’ variability dependent on varying harvesting procedures
 - Contamination issues (both cross and ‘bugs’)
 - Assay transfer difficulties between people in AD, HTS and HtL
 - Increasing number of ‘more complex cell-based assays entering arena’
 - Laser scanning con-focal microscopy on live cells in plates (INCell)
 - Whole cell binding assays (FMAT)
 - High throughput patch clamping (IonWorks)

Bottom Line for Cell-based HTS

- We are no longer looking at populations of cells in wells BUT individual cells as a statistically significant population:
 - FLIPR (384 well) = 10,000 cells
 - IN Cell Analyser = 100 cells
 - Ion Works HT = 1 cell
- **Homogeneity of cell populations is critical** cell to cell, plate to plate, day to day and week to week to 'succeed' in cell-based HTS – the production of quality 'hits'.

HOW? What did AZ want?

- Assume
 - Address adherent cell lines in flasks at present
 - 8 to 12 cell lines in Lead Generation at once
 - 2 to 4 cell lines in HTS at once
 - Up to 2 cell lines produced for HTS without operator intervention
 - Operator intervention on a daily basis but minimal
- Automated system that had to be capable of:
 - Harvest cells
 - Passage cells
 - Plate cells
- High capacity on the system
- Minimal operator intervention for both harvesting and plating
- Flexibility in equipment utilisation
- Simple operator intervention for processing with rapid turnaround time
- No contamination or cross contamination issues (operator or cells)
- Rapid harvesting and plating to minimise variability in batch processing
- Simple GUI – easy to operate

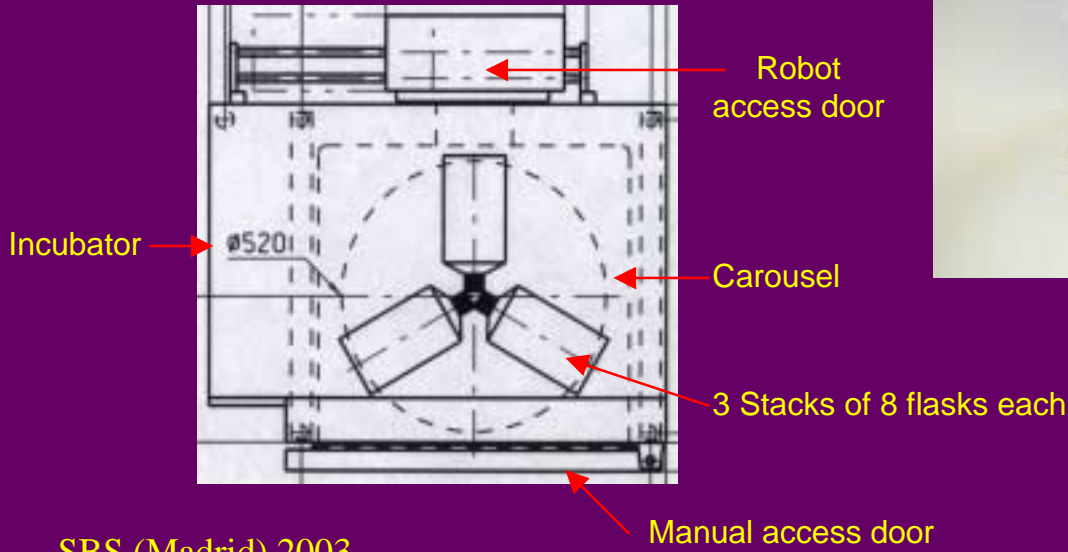
Approach to Automation

- Where possible identify tried and tested laboratory equipment and use an integration company with high quality engineering expertise (RTS Thurnall)
- Identify the risk factors (areas) in the process and minimise risk by designing around these:
 - Sterile liquid handling; how? (Essen instruments)
 - Cell-culture flask design; (Corning Incorporated)
 - No robotics incubator for flasks; (Kendro)
 - Need sterile conditions; how? (Bigneat)

Flask Incubator

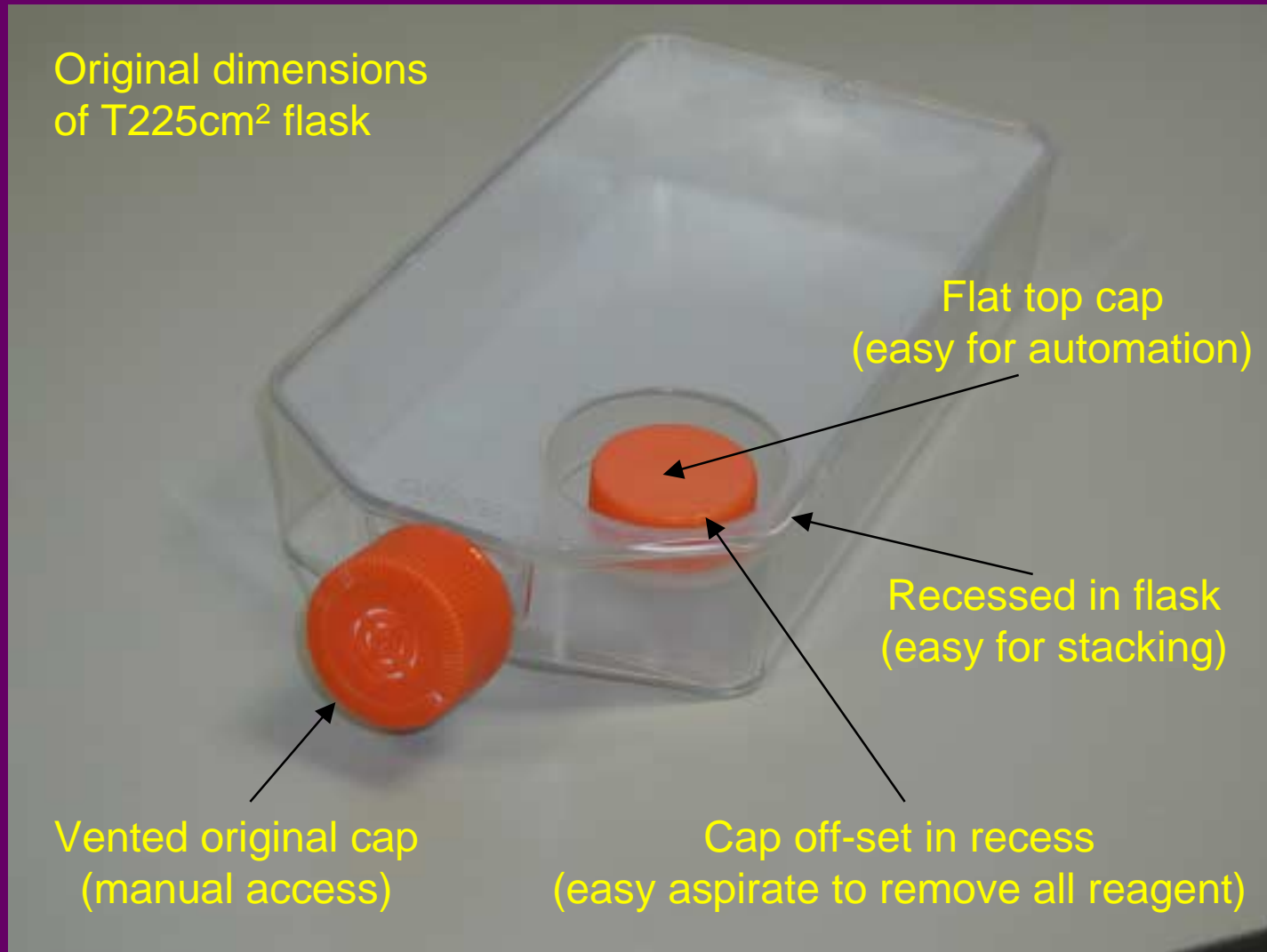


Demountable Stackers
(transfer to "regular" incubators)



SBS (Madrid) 2003

Automation 'Friendly' Flask



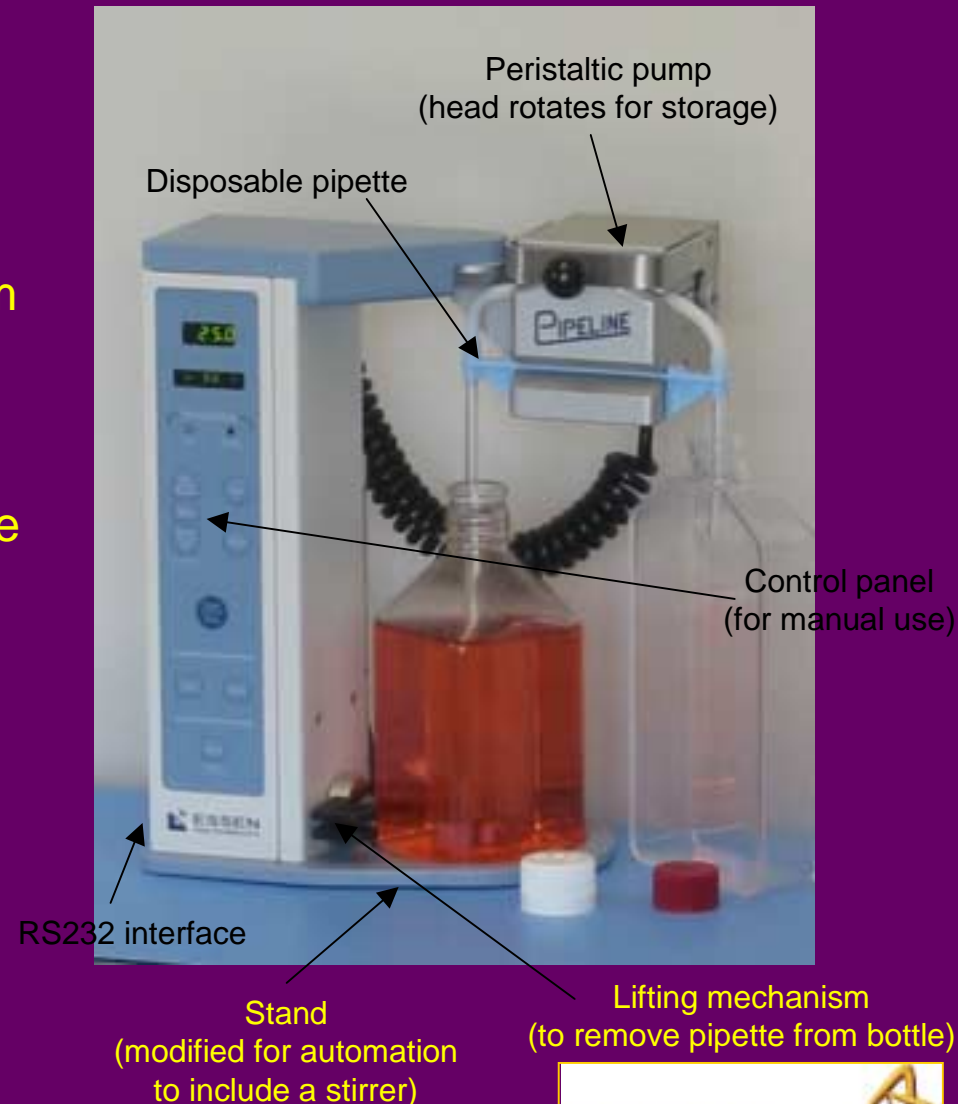
Sterile Reagent and Cell Dispensing

Automated dispensing operation of cells from a harvested cell batch to a flask

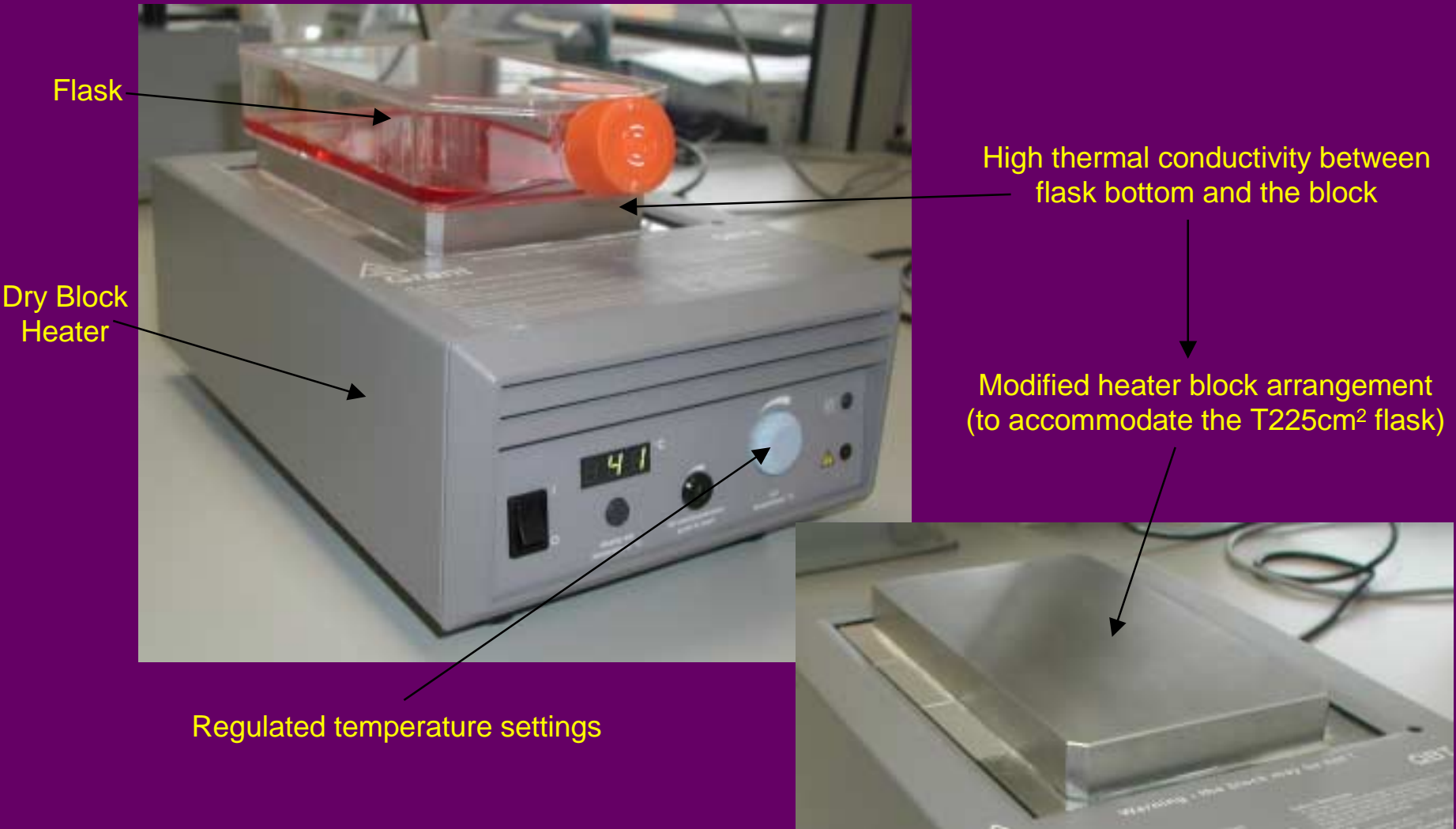
Automated mixing of cells harvested from a flask into the cell batch bottle with complete recovery

Automated recovery of the bottle from the dispenser

Rapid and simple changeover of the disposable pipette

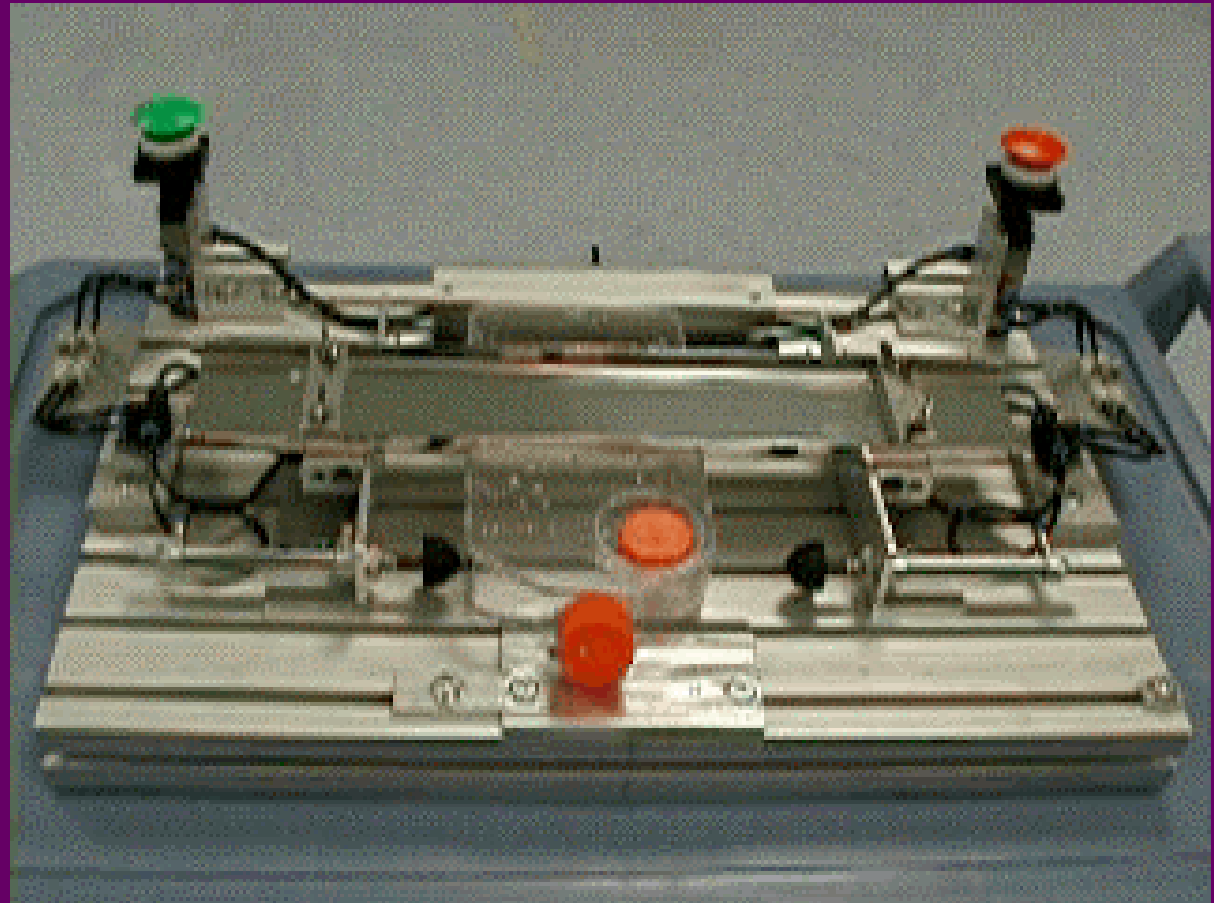


Dissociation of Cell from Flask



Dissociation of the Cells from the Flask

- Video to show
 - Action of the tapper



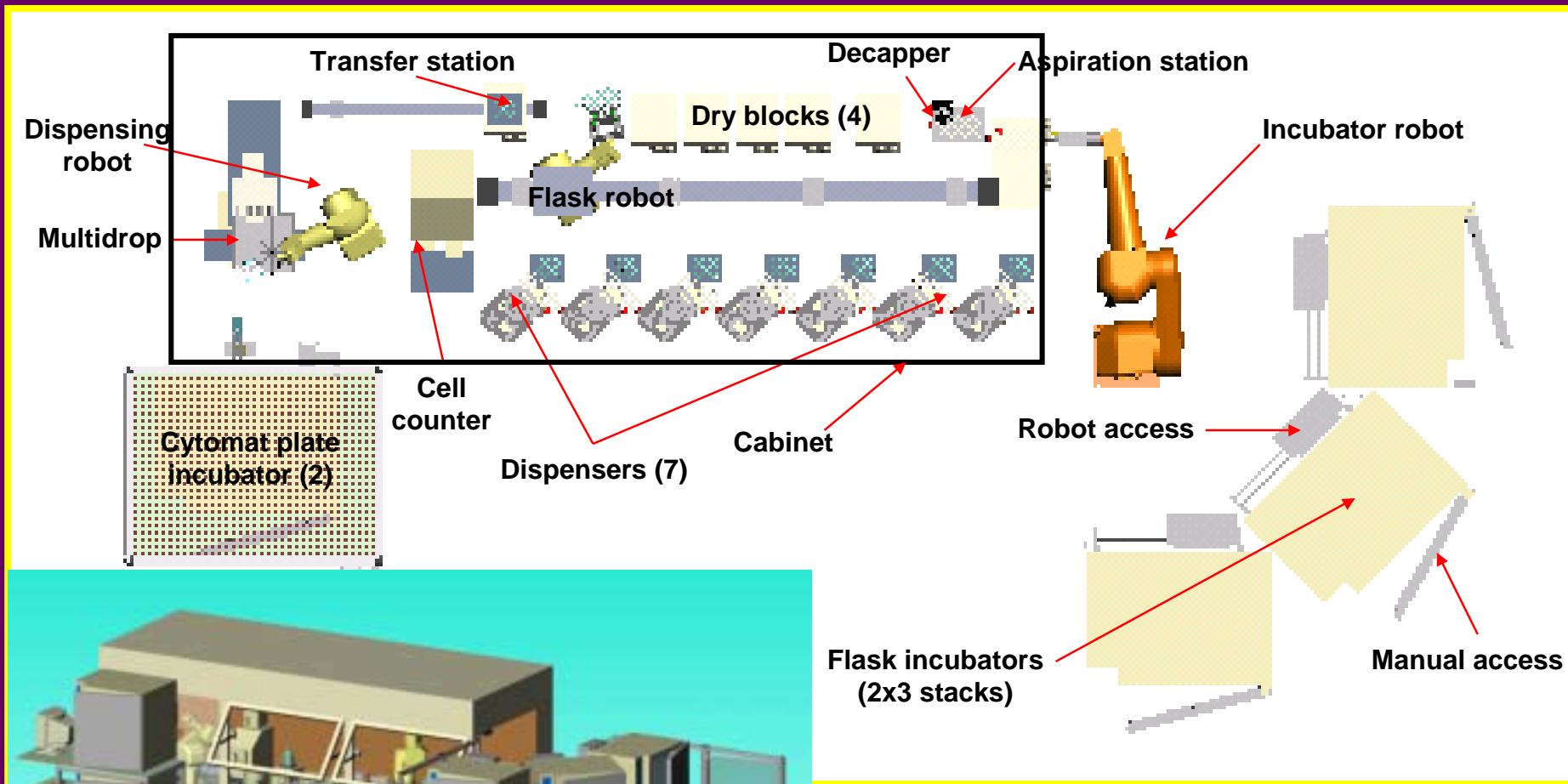
Comparison of 'Standard' versus 'Semi-Automated' Cell Culture Methodology

Method: Adherent CHO cells grown in T225cm² flasks, remove media, wash, add 10ml dissociation solution, incubate @ 37oC, tap flask, remove cells, and count/check viability (Cedex cell counter)

Methodology	Incubation And Tapping	Temperature (oC) after:		Cell number (x10 ⁵ /ml)	Viability (%) (trypan blue exclusion)
		5 mins	10 mins		
Standard	Heraeus BB6220 By hand Manual pipette	31	31	11.6	92
Semi-automation	Dry Block Heater Tapping Unit Pipeline dispenser	33.5	35.5	12.0	90

Take home message: Comparable data for cell isolation (number of cells and viability) between 'Standard' and 'Semi-automated' methodologies

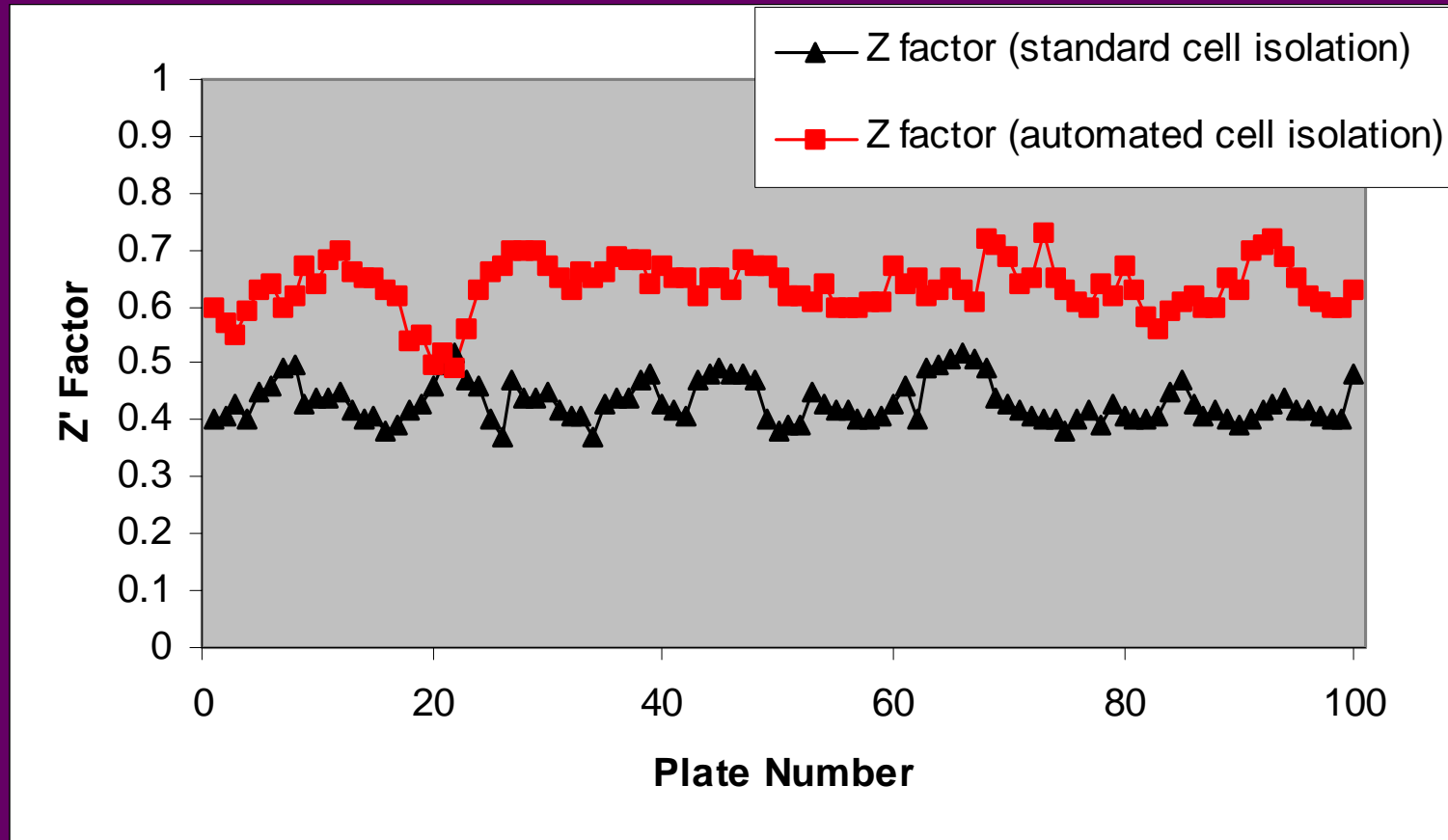
Overview of the System



Video of acCELLerator in action

Data Quality from acCELLerator

CHO cells stably expressing Chemokine receptor are screened in 384 well plates in Auto FLIPR over 16 hour period for 100 plates



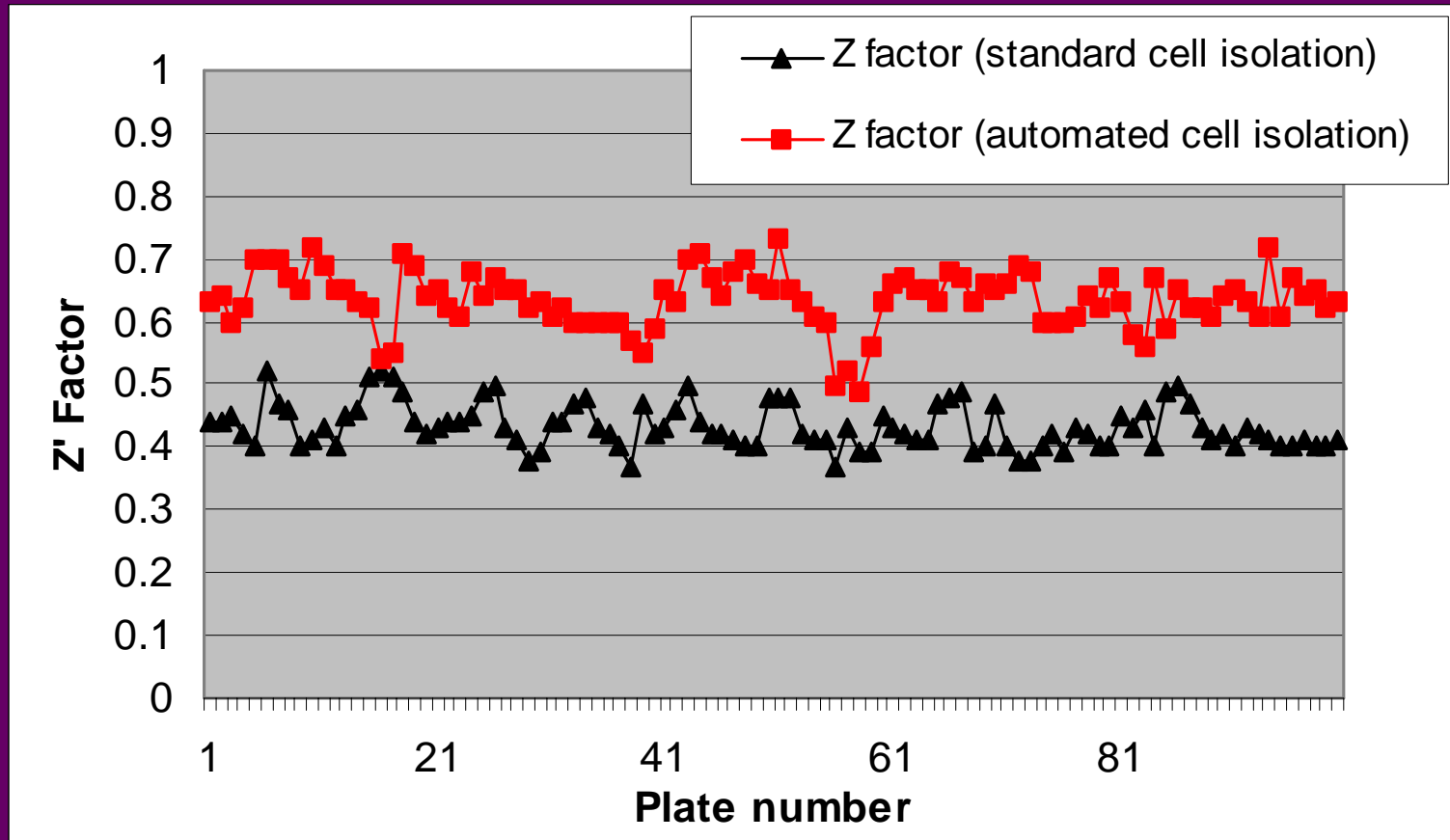
$$Z' = 1 - \frac{((3 \times \text{SD of max}) + (3 \times \text{SD of min}))}{(\text{Mean max} - \text{Mean min})}$$

(Mean max - Mean min)

32 replicates for positives and negatives

Data Quality from acCELLerator

HEK 293 cells stably expressing Chemokine receptor are screened in 384 well plates in Auto FLIPR over 16 hour period for 100 plates



$$Z' = 1 - \frac{((3 \times \text{SD of max}) + (3 \times \text{SD of min}))}{(\text{Mean max} - \text{Mean min})}$$

(Mean max - Mean min)

32 replicates for positives and negatives

Has the Specification been Addressed?

- **Functionality**
 - **Capacity**
 - 45 flasks can be processed to produce 180 384-well plates in 4 to 6 hours
 - capacity to supply current and future cell-based HTS
 - **Independent operation**
 - The harvesting and plating functions operate simultaneously giving greater flexibility and allowing parallel processing
 - **Membrane supply**
 - **Improved quality control**
 - database of all records

Has the Specification been Addressed?

- Advantages

- Consistency

- culture and harvest conditions same each day
 - ease transfer from AD to HTS

- Increased screening

- plates available for screening 5 days/week
 - 20% increase on current FLIPR screening

- Incubator stacks compatible with FLIPR automation

- Quick set-up and change-over time

- change media bottles, dispenser pipette, pre-filled stacks slot in/out of all incubators

- Less people per screen therefore cost effective

- Consumable costs remain approx. equivalent

acCELLerator™ Advantages

- System models the process performed manually – no re-validation
- Expandable flask and plate incubator capacity (customisable)
- Interchangeable stackers between automated and manual (cheaper) incubators
- Parallel processing of flasks and plates for harvesting and plating or passaging – improves speed
- Space for integration of other equipment
- Easily interchangeable equipment (Pipeline™, multidrop and dry block heater) for quick error recovery from breakdowns
- No cross contamination issues between cell lines

Operation within Lead Generation

- One person to operate the system for:
- HTS
 - Generate 2 screen days of plate (2 x 150) overnight without manual intervention
- Assay Development and Hits-to-Lead
 - Passage and plate as necessary with operator intervention to replace consumables if necessary between runs
- Looking at providing cells for other functions
 - DMPK – Caco-2

Thanks go to.....

- Elaine Anderson (DiscoverRx)
- Kathy Dodgson
- Collaborators:
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 - Essen Instruments
 - Corning
 - Kendro
- Dave Keeling and Elaine Sullivan
 - gary.allenby@astrazeneca.com