High-Throughput Solubility

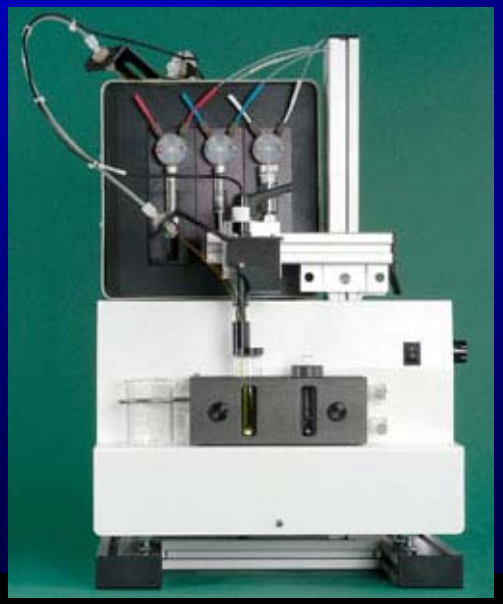
Alan Hill
Physico-Chemical Characterisation
Analytical Chemistry
MDR

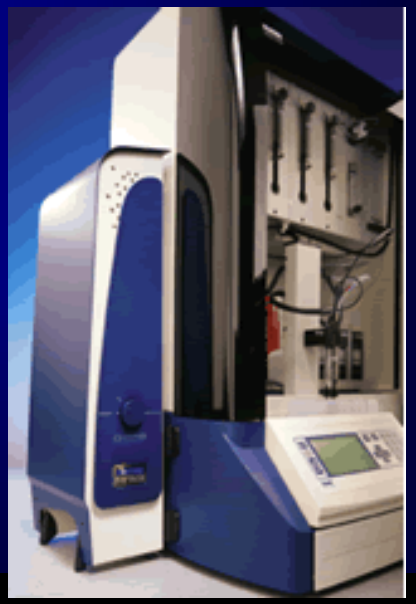
Solubility- Overview

- Solubility (along with Hydrophobicity) are probably the most important physico-chemical properties.
- It impacts on all aspects of drug design and heavily implicated in ADME issues
- Solubility is a complex consequence of several fundamental physico-chemical properties: This level of complexity renders calculation of little utility.
- Measurement is essential.

Solubility – From Solid 'The gold standard'

- Undoubtedly the most desired form of Solubility measurement.
- Several approaches available.
  - In-house
    - shake-flask' approach with variety of analytical endpoints, i.e. UV absorption, HPLC
  - Commercial
    - Pion – pSol
    - Sirius – CheqSol





GlaxoSmithKline

Solubility – From Solid ‘The gold standard’

- Although desirable there are issues!
- Low throughput techniques – insufficient capacity to meet demands of early stage chemistry.
- Requires ‘Solid’
  - Often not available until later in drug development process

GlaxoSmithKline

Solubility from DMSO stock solutions: Precipitative Solubility

- A pragmatic approach to no solid.
- Nearly all early stage drug discovery is carried out from DMSO stock solutions.
  - Ease of handling
    - Sample can be pipetted !
  - Compound efficient
    - Can easily manipulate compound amounts in the ug range.

GlaxoSmithKline

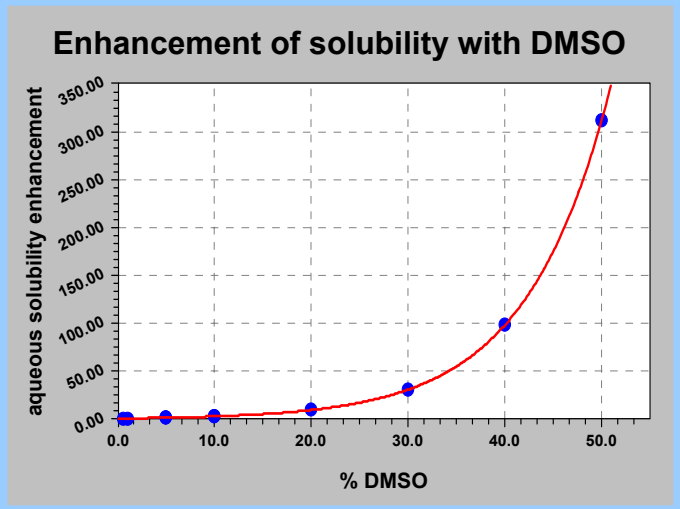
Precipitative Solubility….

- Approaches such as ‘shake-flask’ can be readily adapted to accommodate the use of DMSO stock solutions.
- However there are criticisms levelled at solubility from DMSO solutions.
  - Solubility enhancement due to presence of co-solvent
  - Lack of dissolution step

Aqueous Solubility Enhancement using DMSO as a co-solvent

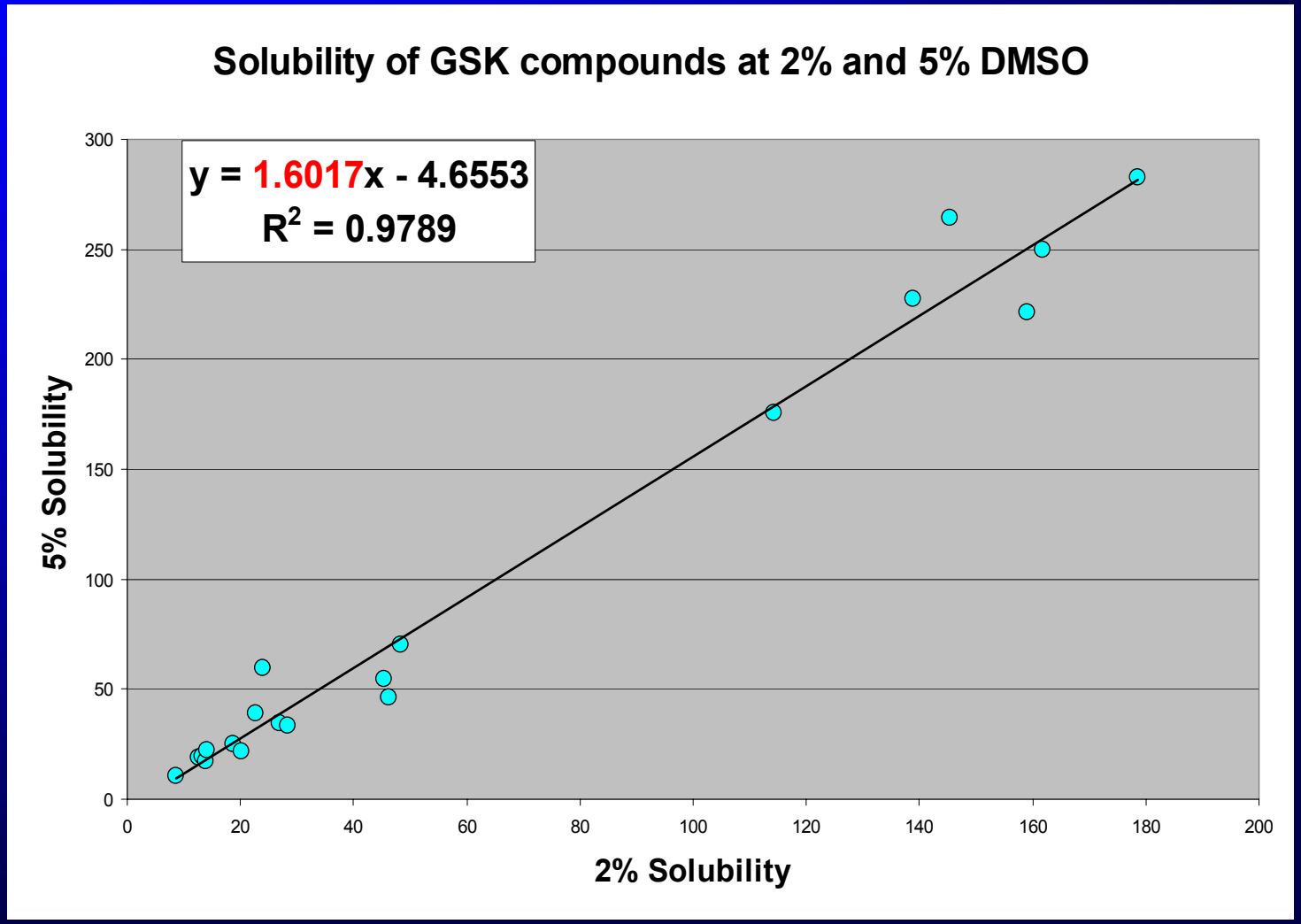
- GSK's compound collection is stored at 10mM in frozen DMSO solutions
- Compounds are screened from aqueous dilutions of these solutions
- Yalkowsky showed that Increasing the % DMSO from 2% to 5% in water increases a compound's solubility by 1.5 times

Aqueous Solubility enhancement
v
%DMSO



GlaxoSmithKline

This enhancement applies to GSK compounds



GlaxoSmithKline

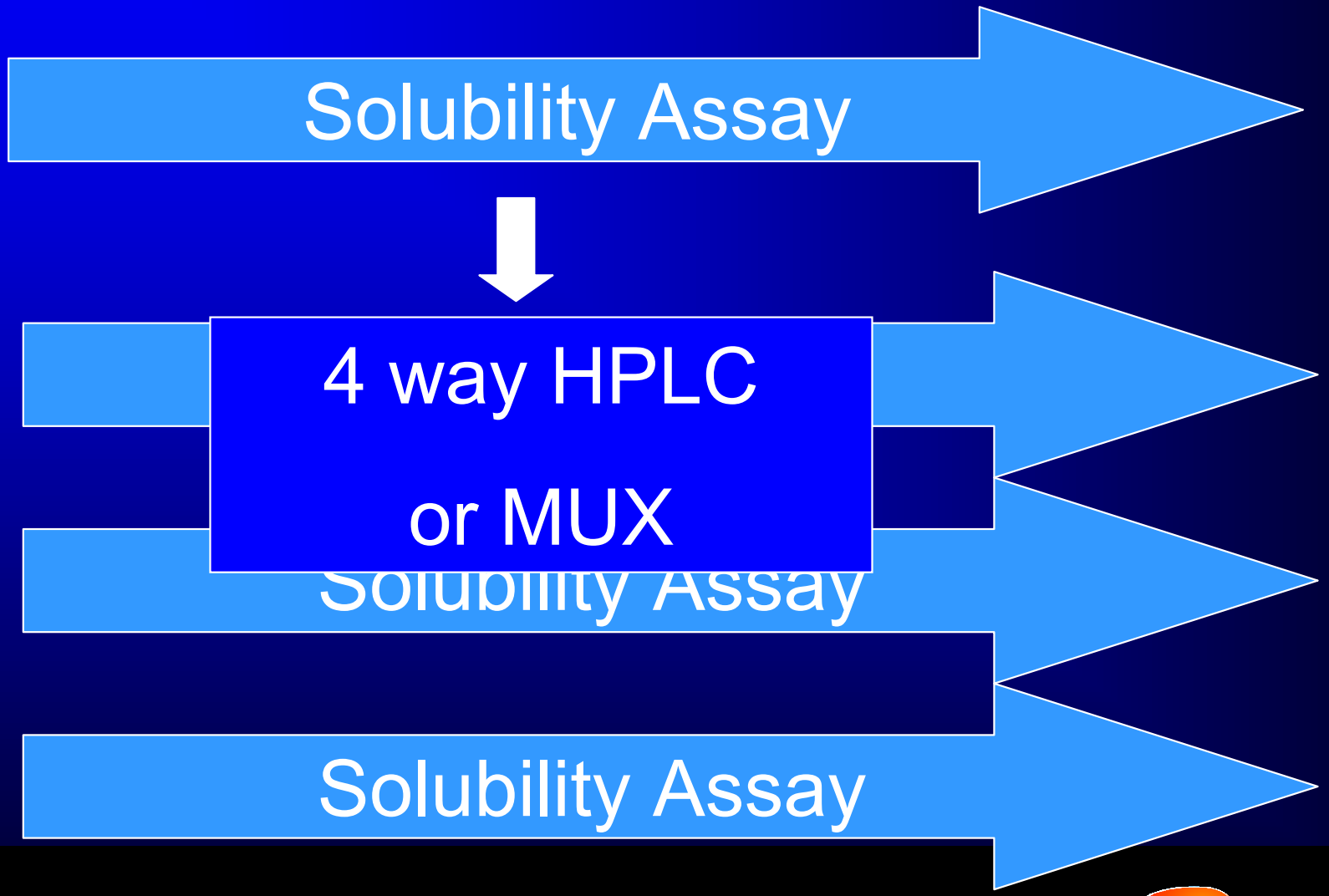
Precipitative Solubility….

- Solubility from DMSO is a best case scenario.
  - If a compound is insoluble from DMSO stock solution it is extremely unlikely to be soluble from solid.

- This is invaluable information to have at the early stage of the drug discovery process and allows compounds to be ‘killed’ quickly.

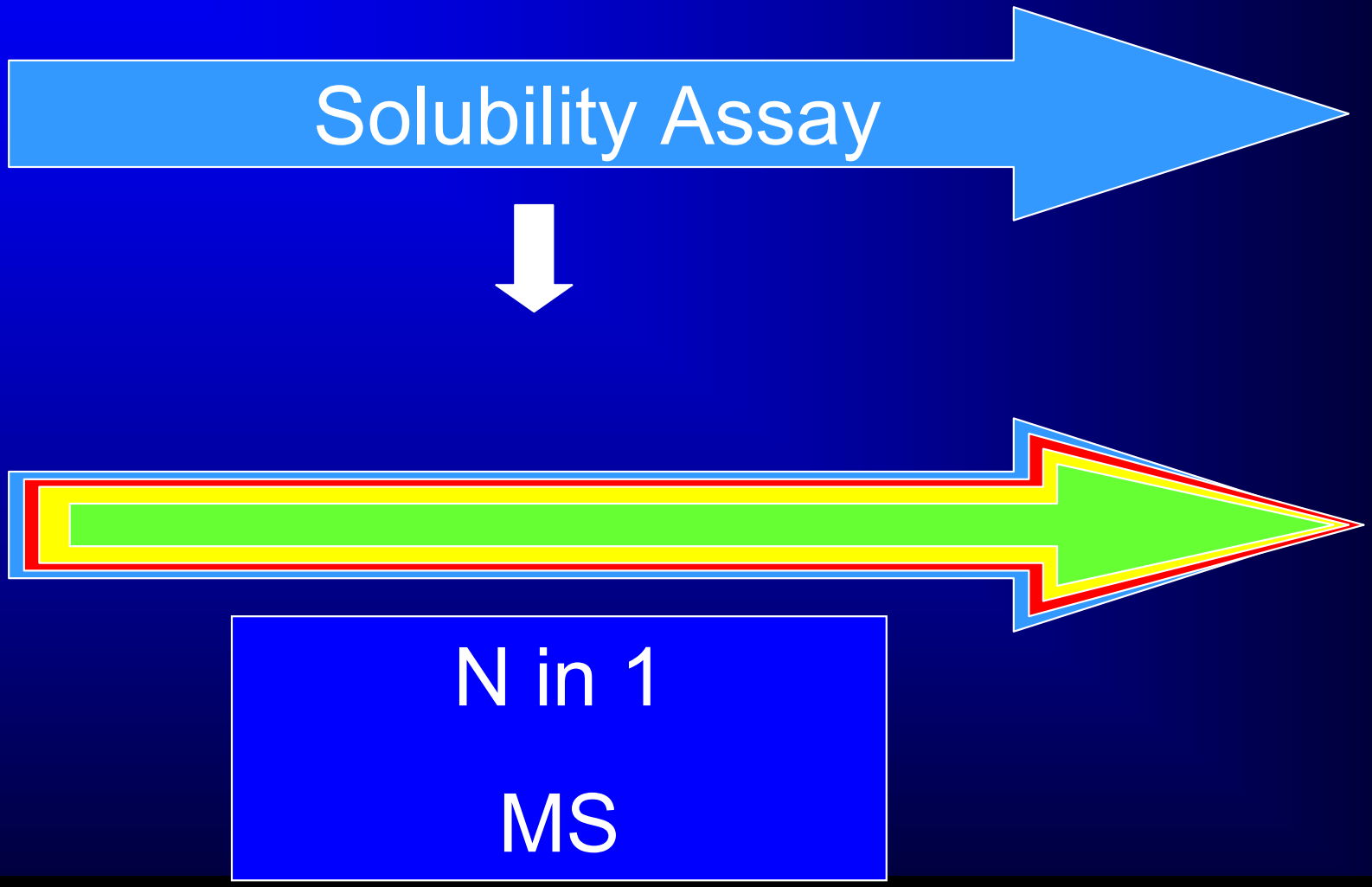
How do we make a method High-throughput?

- How do we measure more samples?



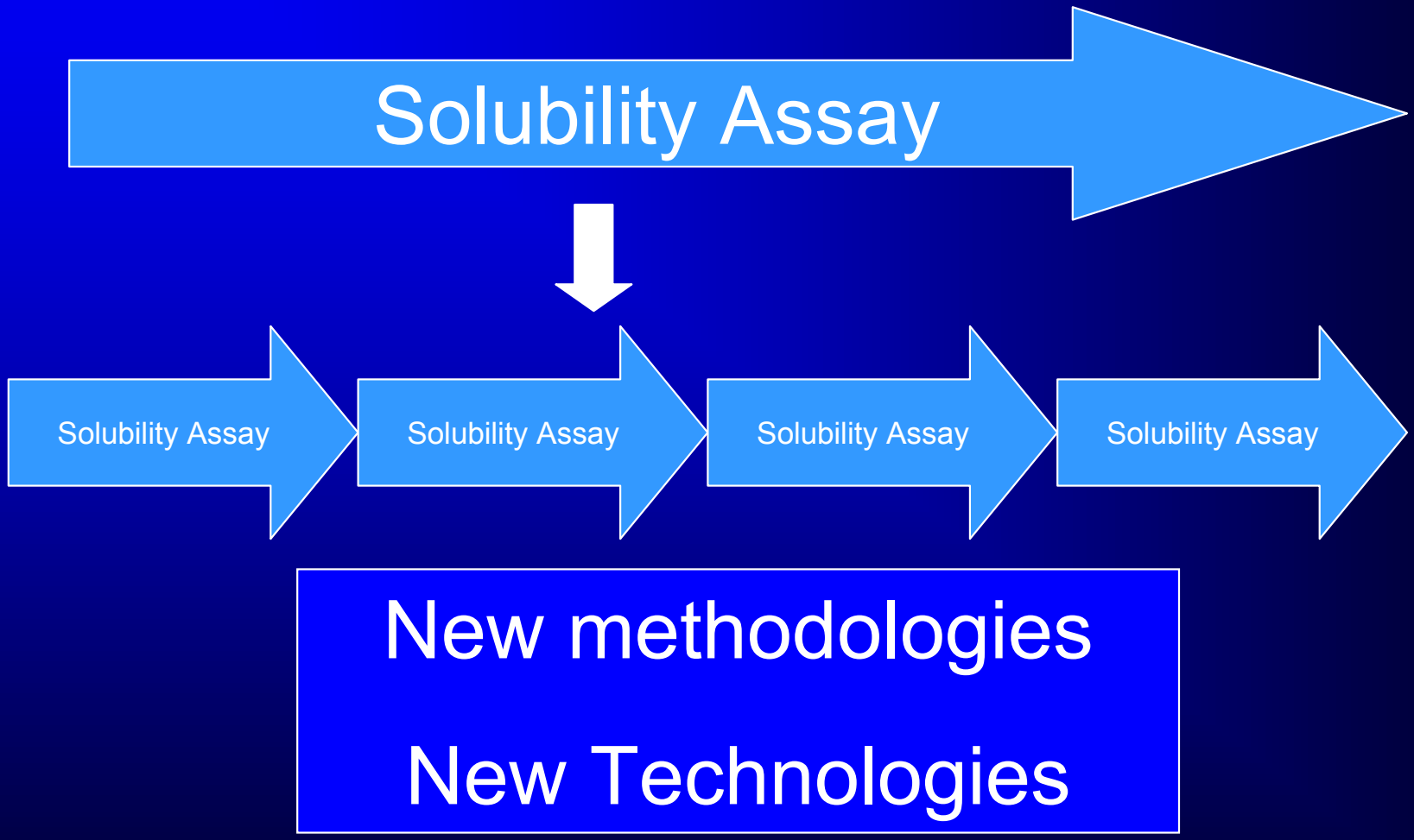
GlaxoSmithKline

How do we make a method High-throughput?



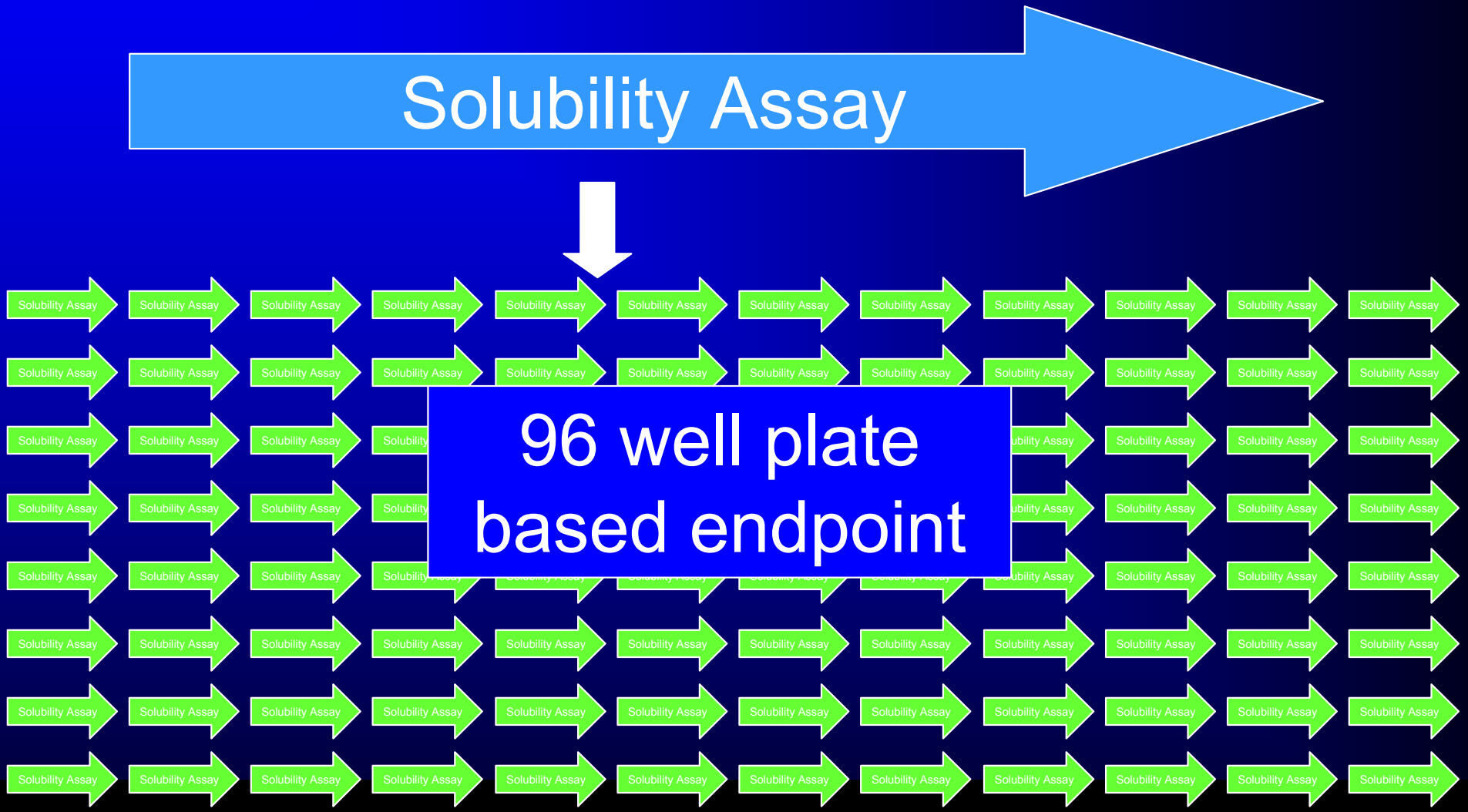
GlaxoSmithKline

How do we make a method High-throughput?

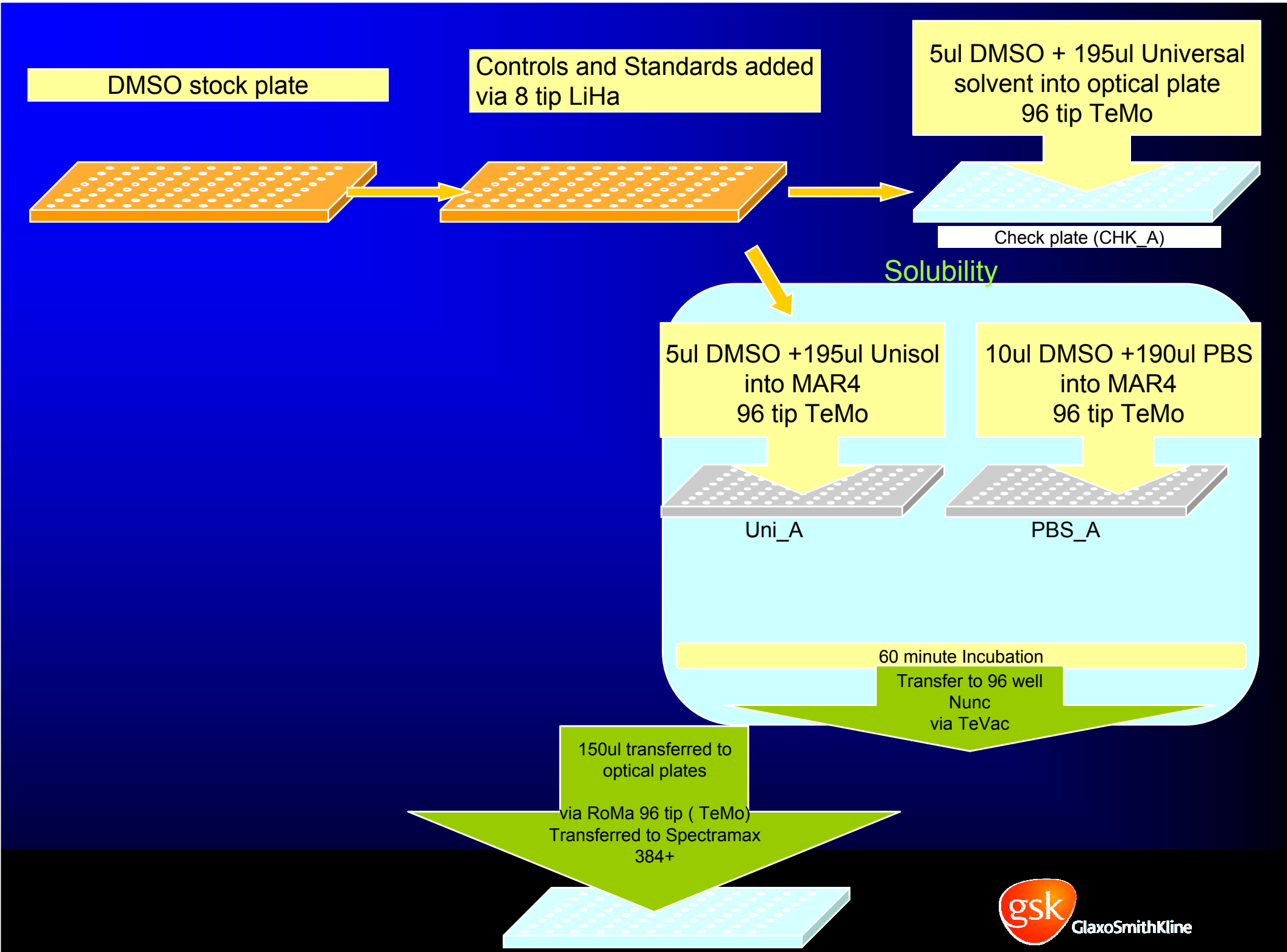


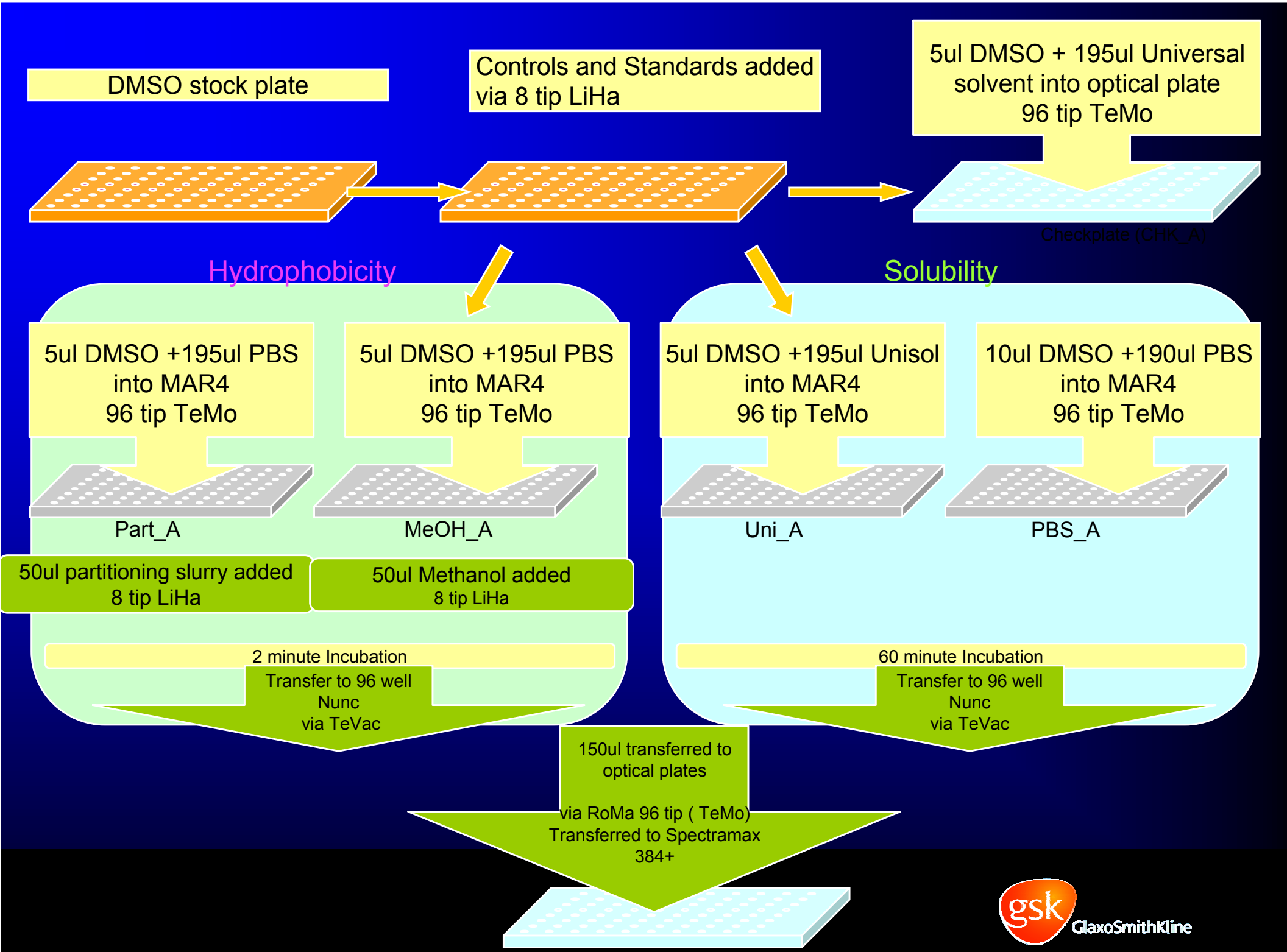
GlaxoSmithKline

How do we make a method High-throughput?



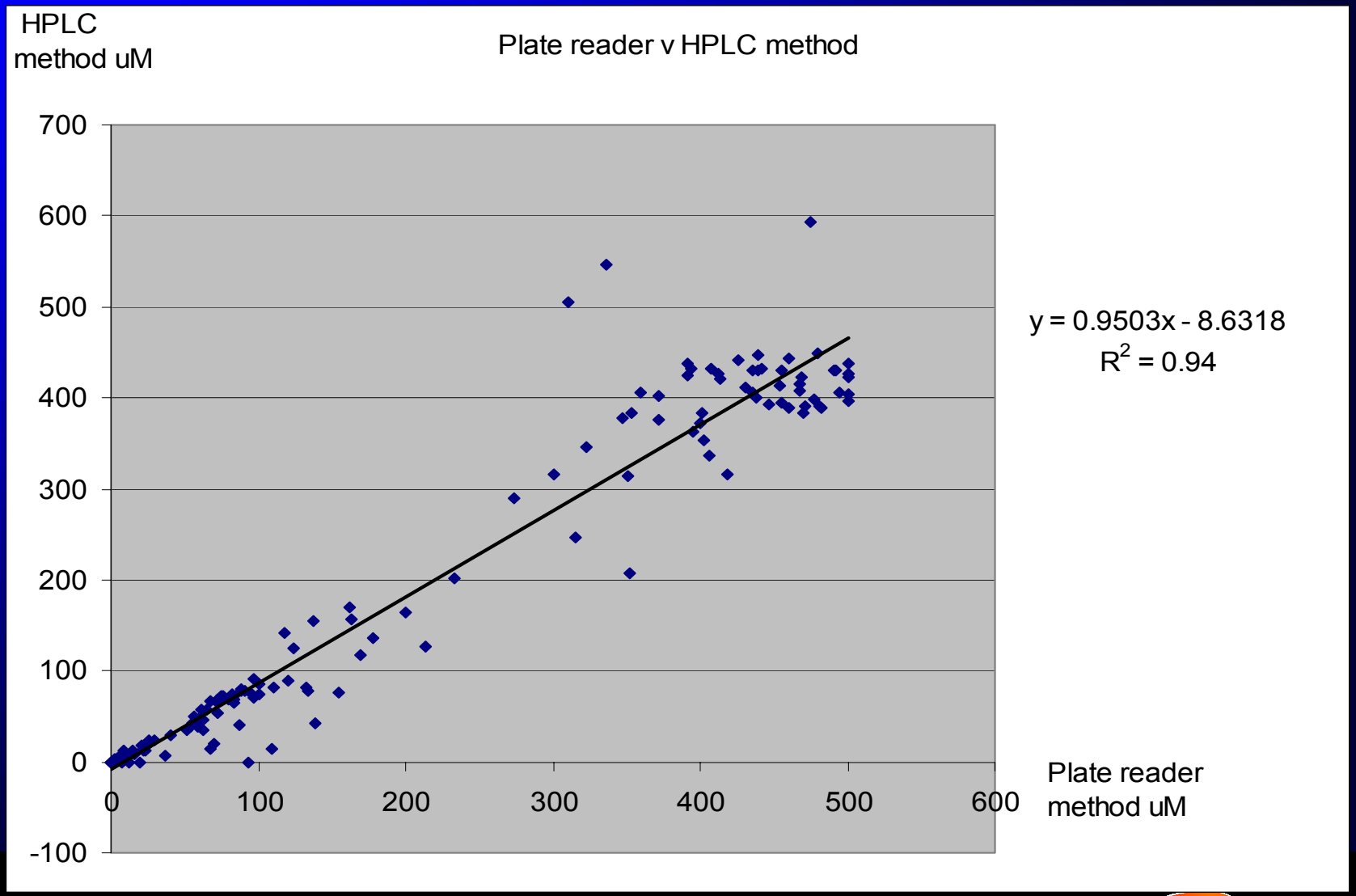
GlaxoSmithKline


DMSO stock plate
Controls and Standards added via 8 tip LiHa
5ul DMSO + 195ul Universal solvent into optical plate 96 tip TeMo
Check plate (CHK_A)
Solubility
5ul DMSO +195ul Unisol into MAR4 96 tip TeMo
10ul DMSO +190ul PBS into MAR4 96 tip TeMo
Uni_A
PBS_A
60 minute Incubation
Transfer to 96 well Nunc via TeVac
150ul transferred to optical plates
via RoMa 96 tip (TeMo)
Transferred to Spectramax 384+
gsk
GlaxoSmithKline


DMSO stock plate
Controls and Standards added via 8 tip LiHa
5ul DMSO + 195ul Universal solvent into optical plate 96 tip TeMo
Checkplate (CHK_A)
Hydrophobicity
Solubility
5ul DMSO +195ul PBS into MAR4 96 tip TeMo
5ul DMSO +195ul PBS into MAR4 96 tip TeMo
5ul DMSO +195ul Unisol into MAR4 96 tip TeMo
10ul DMSO +190ul PBS into MAR4 96 tip TeMo
Part_A
MeOH_A
Uni_A
PBS_A
50ul partitioning slurry added 8 tip LiHa
50ul Methanol added 8 tip LiHa
2 minute Incubation
60 minute Incubation
Transfer to 96 well Nunc via TeVac
Transfer to 96 well Nunc via TeVac
150ul transferred to optical plates
via RoMa 96 tip (TeMo)
Transferred to Spectramax 384+
GlaxoSmithKline

The DRAF-H Physchem robotic platform

Comparison of precipitative solubility by high and medium throughput methods



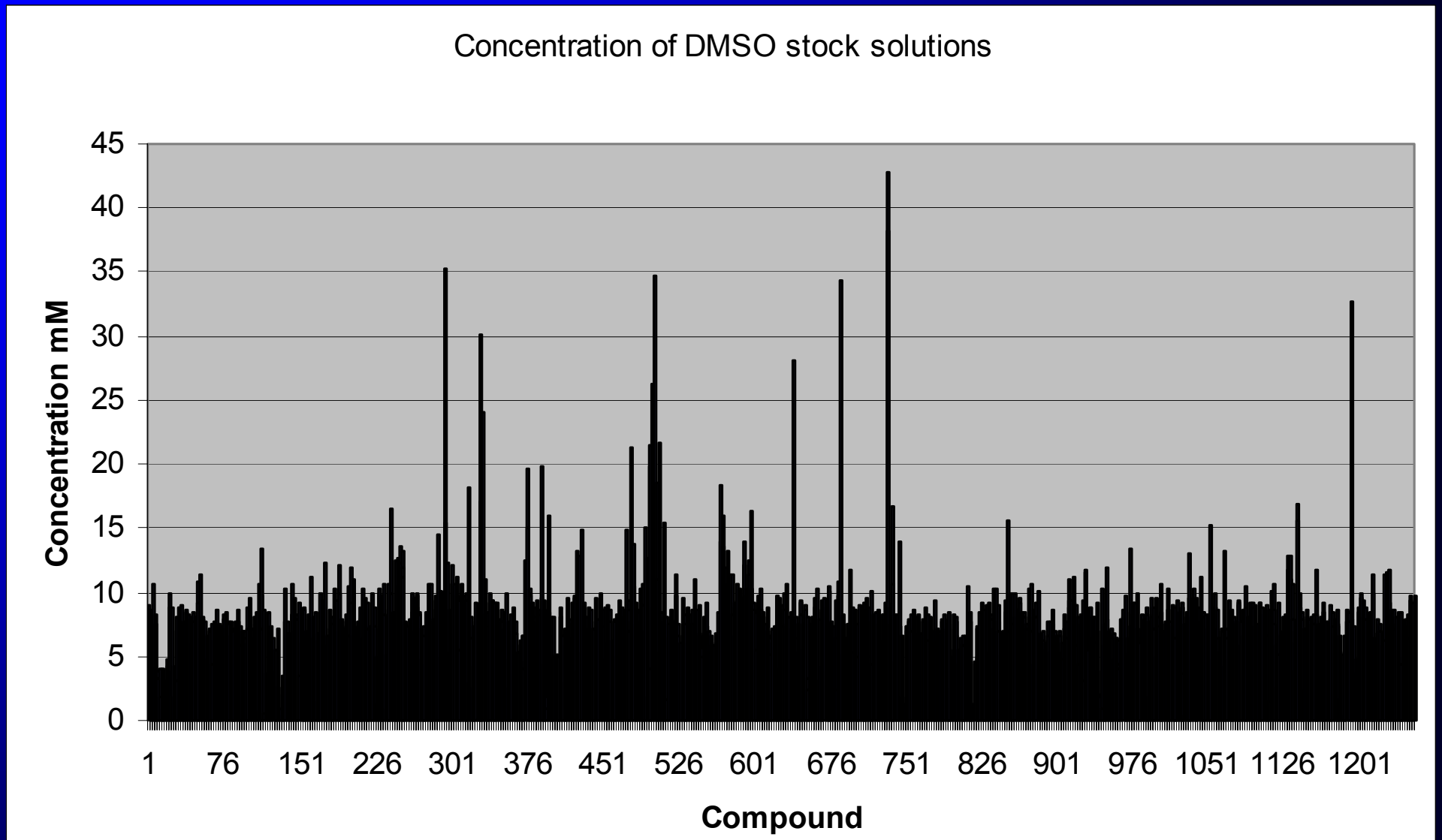
GlaxoSmithKline

Precipitative Solubility….

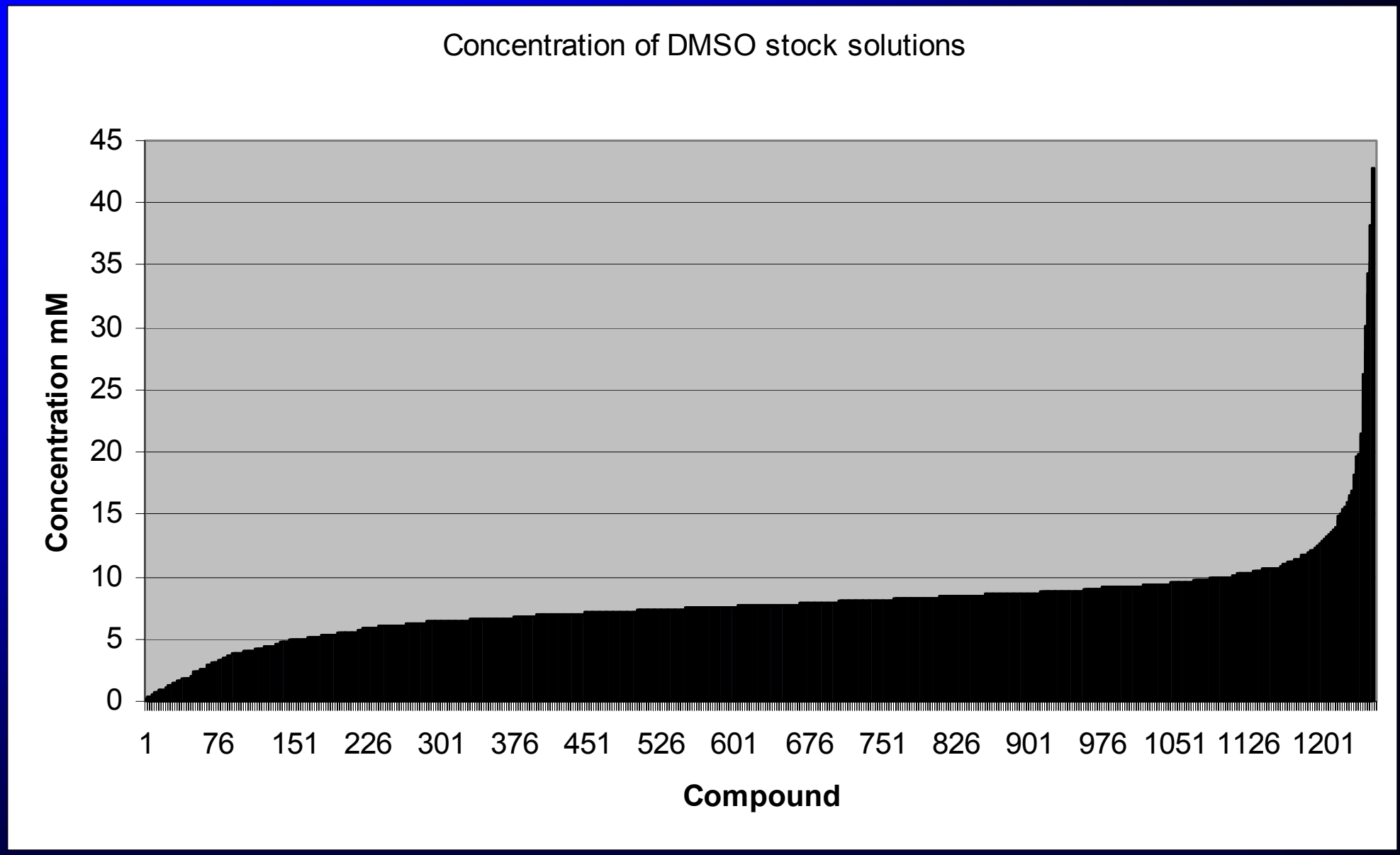
- All of our solubility assays use the stock solution provided as the calibration standard.
- This is assumed to be a 10mM solution

- However!

GlaxoSmithKline



GlaxoSmithKline



GlaxoSmithKline

Precipitative Solubility….

- How do we get around the problem of varying stock concentrations.

Universal standard

- Require an analytical technique that employs a universal standard.

CLND

Chemi-Luminescent Nitrogen Detection

GlaxoSmithKline

Why CLND?

Chemi-Luminescent Nitrogen Detection

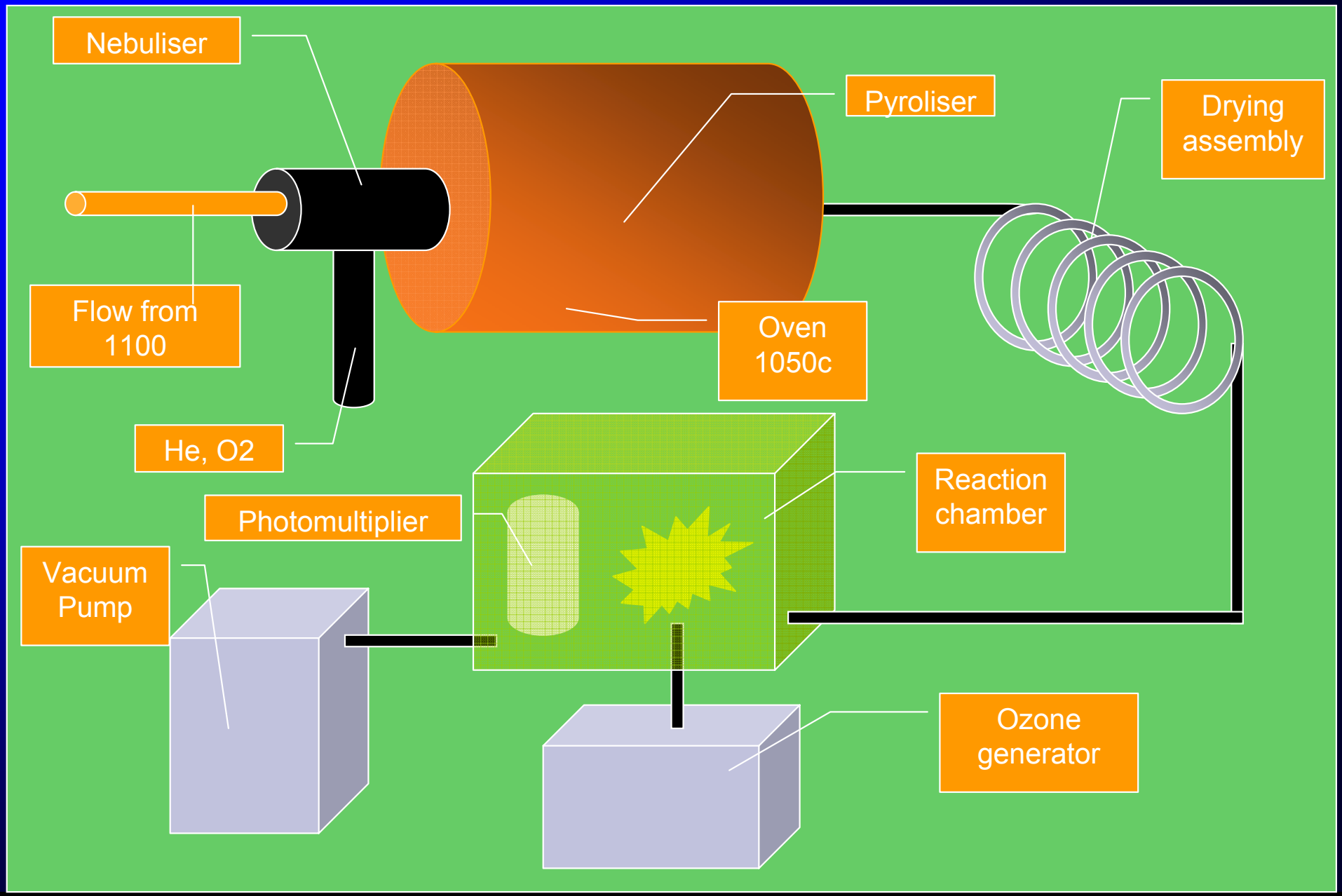
- Detector provides a linear response to molecular Nitrogen.
- Allows use of Universal not compound specific standards.
- Knowledge of N count of molecule of interest allows direct quantification.

GlaxoSmithKline

HP1100 with Antek 8060C CLND

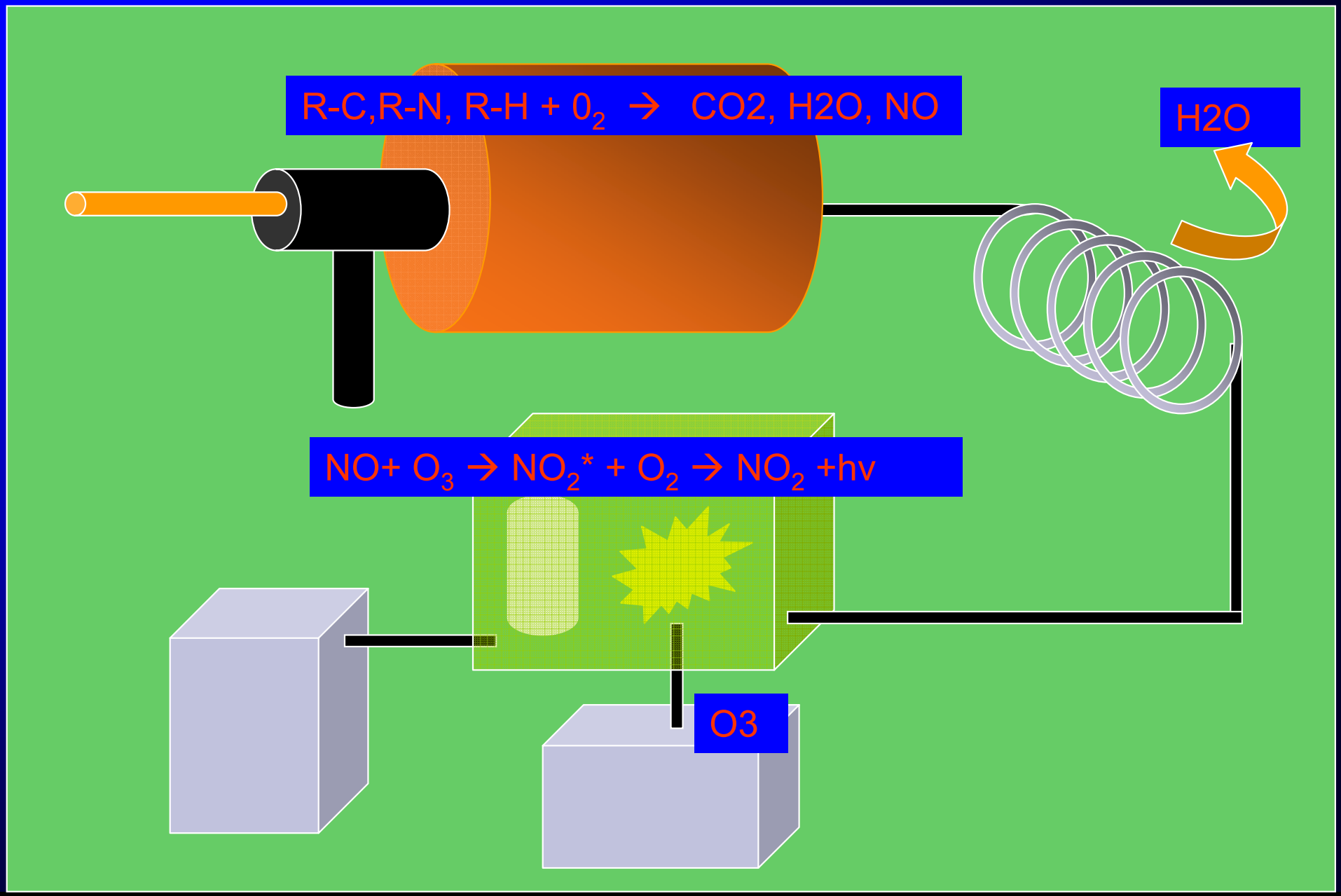
GlaxoSmithKline

CLND Chemi-luminescent Nitrogen detection - Architecture



GlaxoSmithKline

CLND Chemi-luminescent Nitrogen detection - Chemistry



GlaxoSmithKline

CLND - Good news and Bad news

- The Good news
  - The ANTEK 8060 CLND is a highly sensitive Nitrogen detector
- The Bad news
  - The ANTEK 8060 CLND is a highly sensitive Nitrogen detector

The key to the successful implementation of CLND technology

Good House-Keeping

Nitrogen is every where, keeping it's presence to a minimum is essential for success.

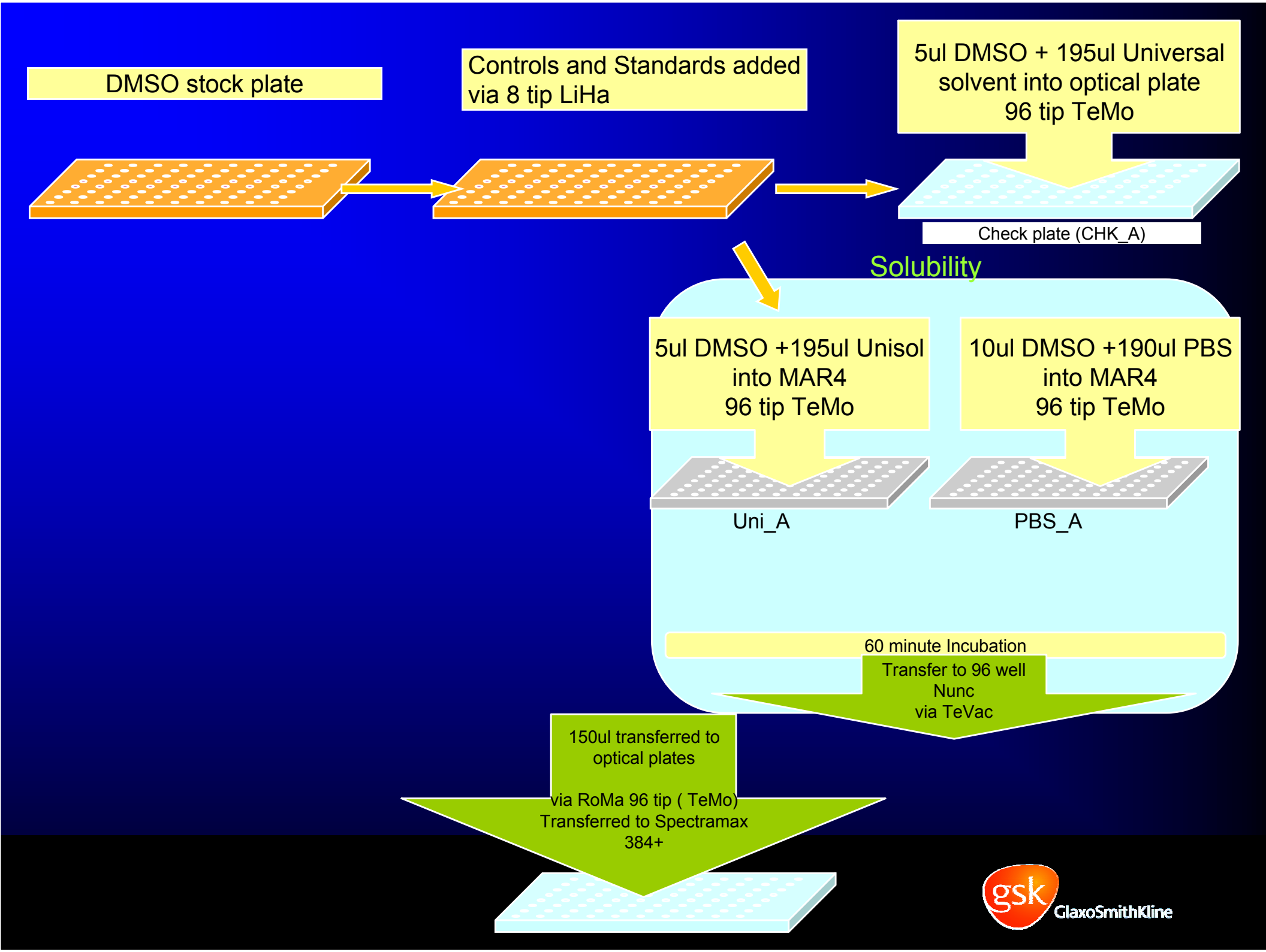
- Dedicated apparatus - Acetonitrile + Ammonium Acetate free HPLC equipment
- Dedicated labware - Local washing up
- Careful selection of reagents and labware - MTP and reagents

GlaxoSmithKline

Precipitative Solubility - Outline of method:

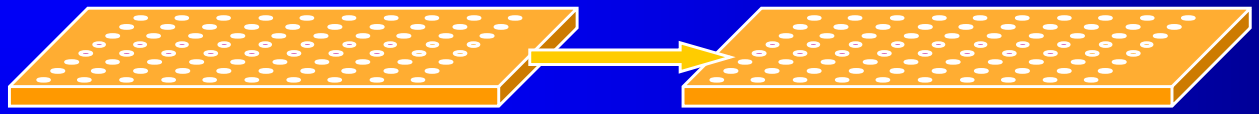
- Precipitative solubility from 5% 10mM DMSO stock solutions in pH7.4 PBS.
- Experiment performed in 96 well format.
- Quantification of DMSO stock solution and aqueous solution by flow-inject CLND calibrated against Caffeine.
- Molecular Nitrogen count calculated using FindNN (written by John Hollerton)

GlaxoSmithKline


DMSO stock plate
Controls and Standards added via 8 tip LiHa
5ul DMSO + 195ul Universal solvent into optical plate 96 tip TeMo
Check plate (CHK_A)
Solubility
5ul DMSO +195ul Unisol into MAR4 96 tip TeMo
10ul DMSO +190ul PBS into MAR4 96 tip TeMo
Uni_A
PBS_A
60 minute Incubation
Transfer to 96 well Nunc via TeVac
150ul transferred to optical plates
via RoMa 96 tip (TeMo)
Transferred to Spectramax 384+
gsk
GlaxoSmithKline

DMSO stock plate

Controls and Standards added via 8 tip LiHa



Solubility

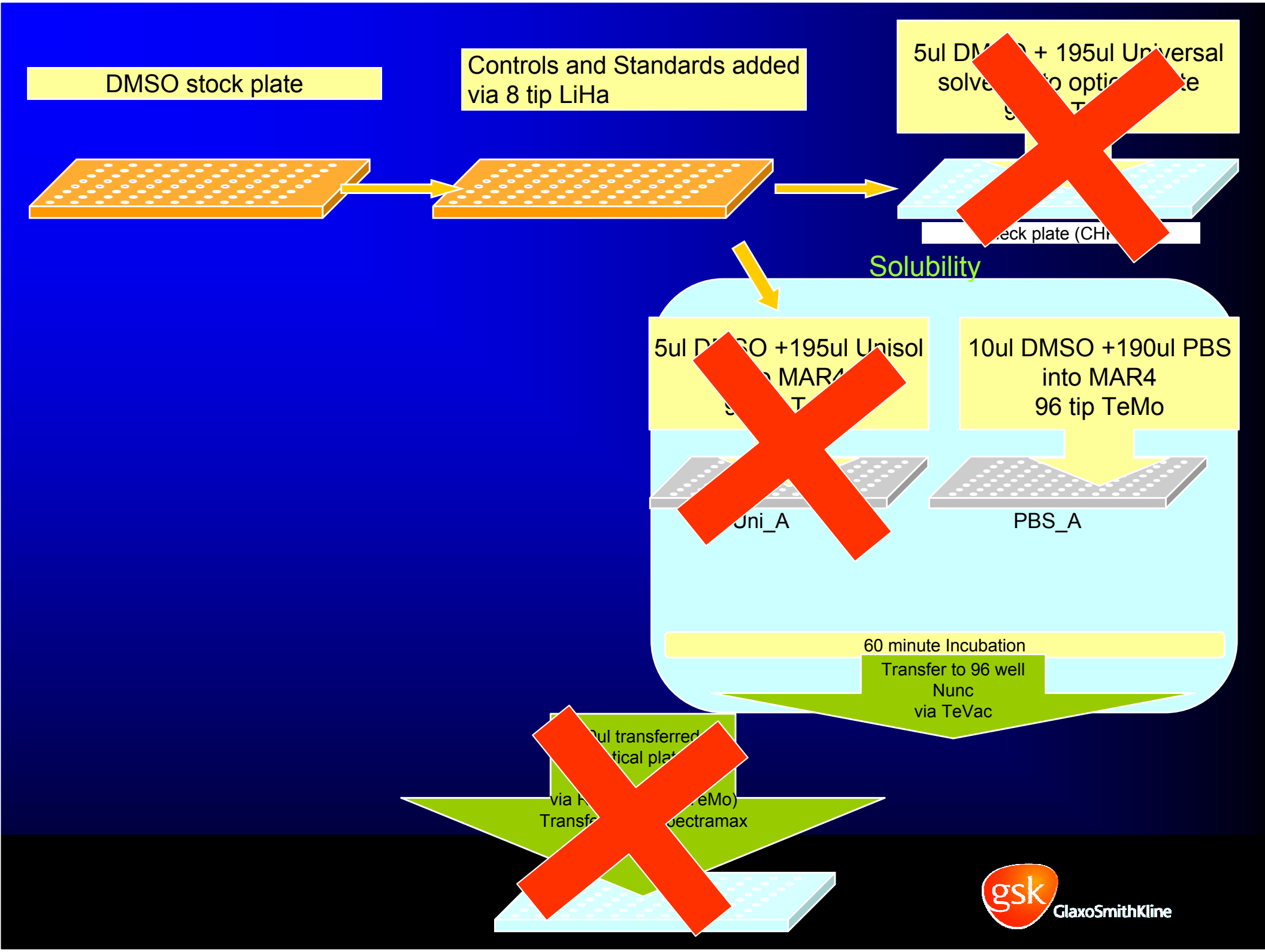
5ul DMSO +95ul PBS into Solvinert Solubility plate
96 tip TeMo

60 minute Incubation

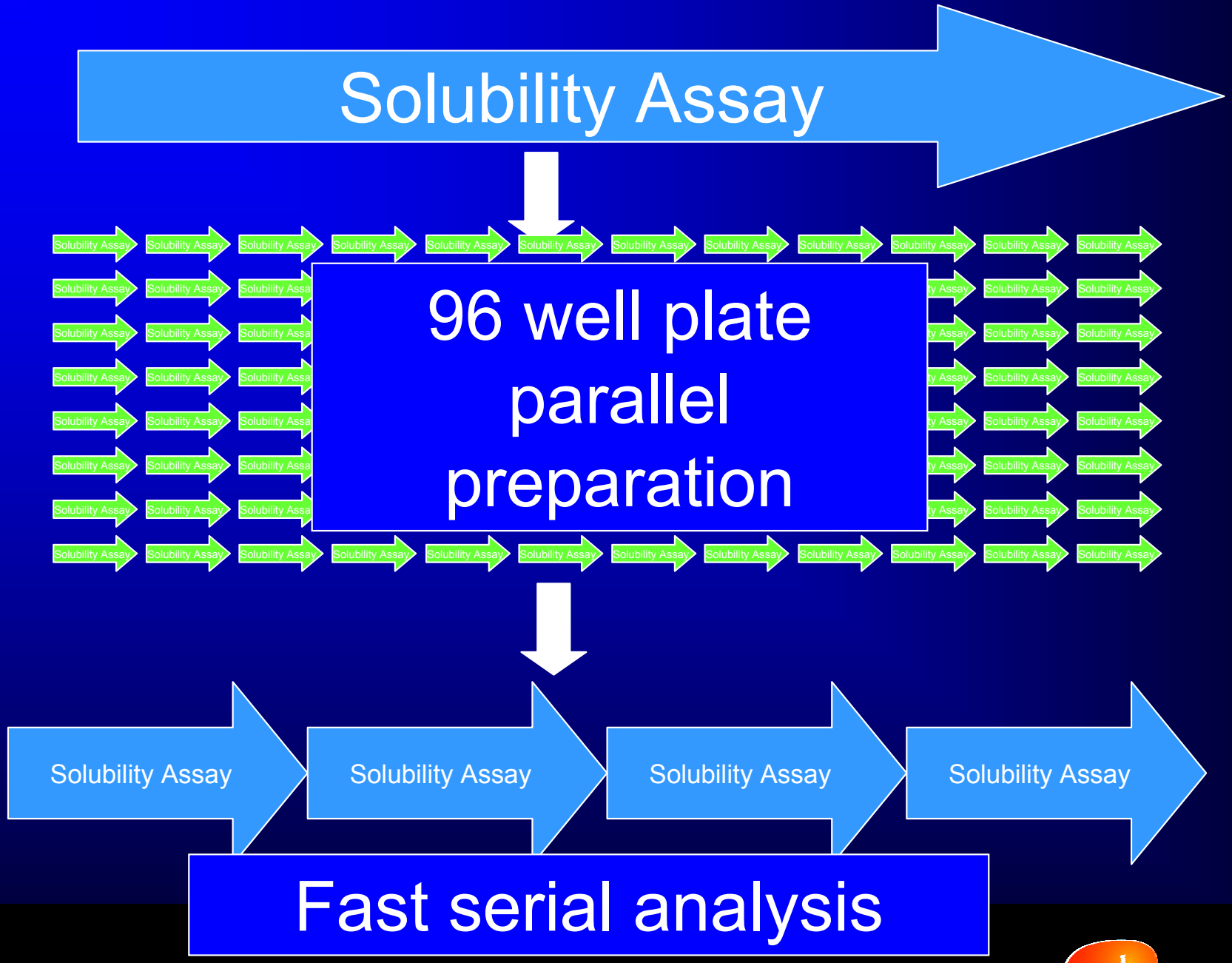
Transfer to 96 well Nunc via TeVac

DMSO stock plate and analytical plate transferred to HP1100 Flow inject CLND system

GlaxoSmithKline

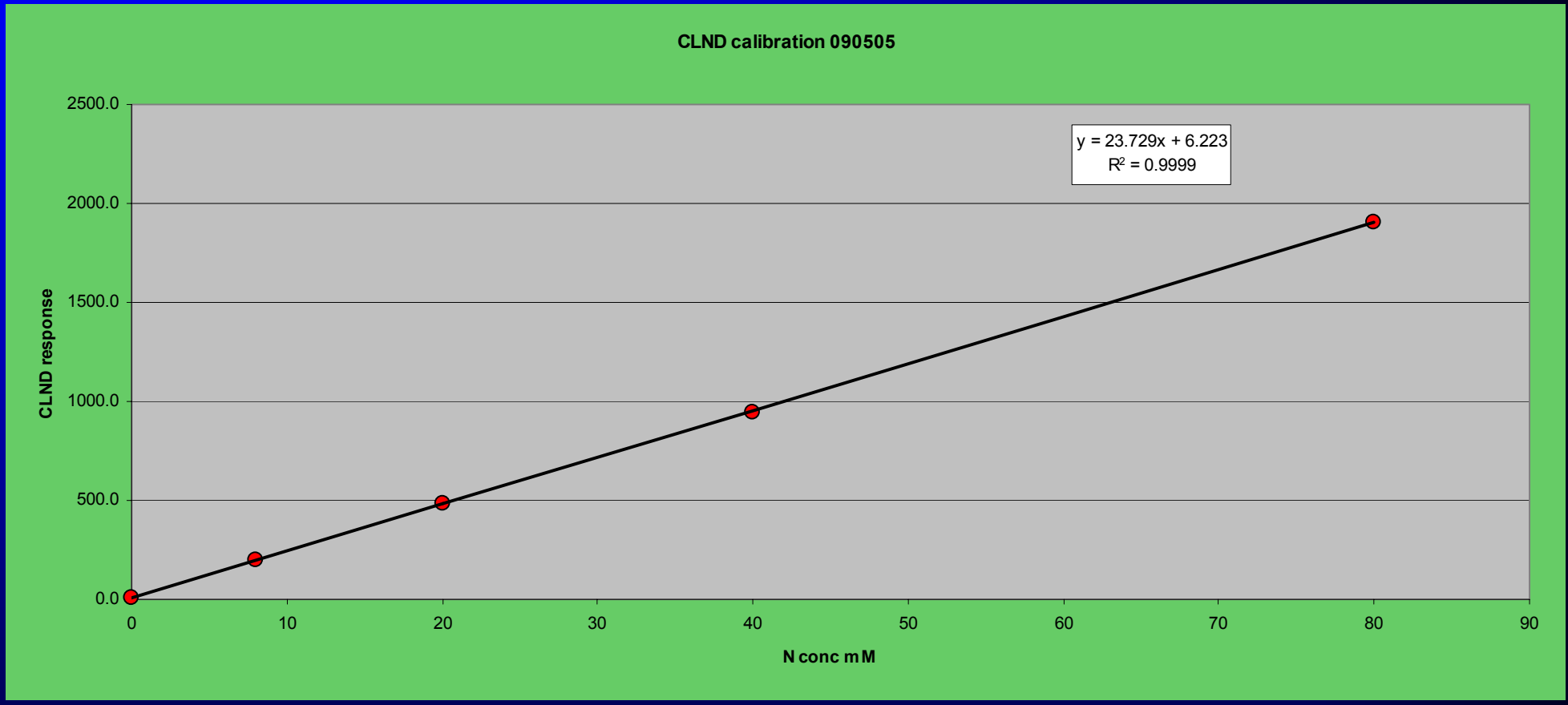

DMSO stock plate
Controls and Standards added via 8 tip LiHa
5ul DM + 195ul Universal solve to optic te
eck plate (CH
Solubility
5ul D SO +195ul Unisol MAR4
10ul DMSO +190ul PBS into MAR4 96 tip TeMo
Uni_A
PBS_A
60 minute Incubation
Transfer to 96 well Nunc via TeVac
ul transferred
tical plat
via H eMo)
Transf ectramax
gsk
GlaxoSmithKline

How do we make a method High-throughput?



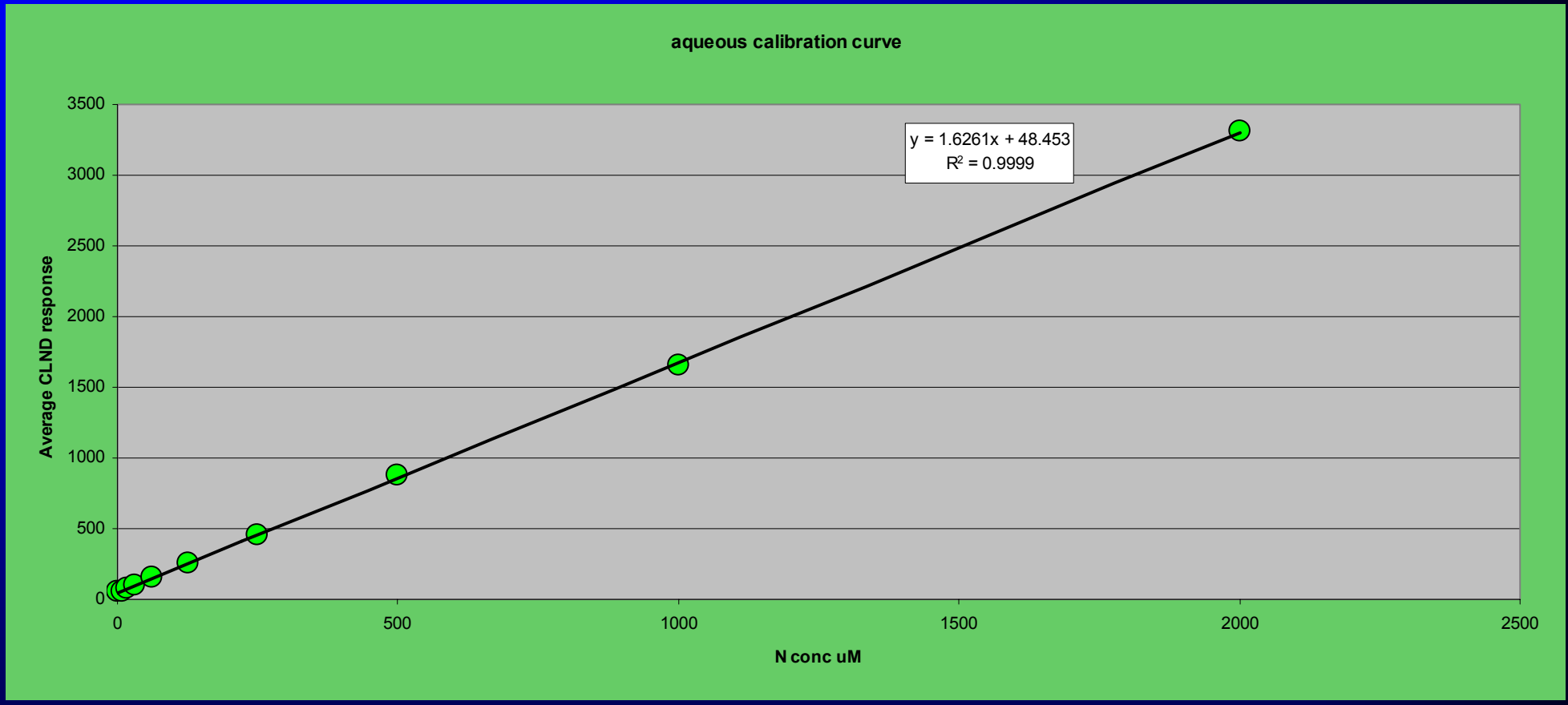
GlaxoSmithKline

Results: cont..



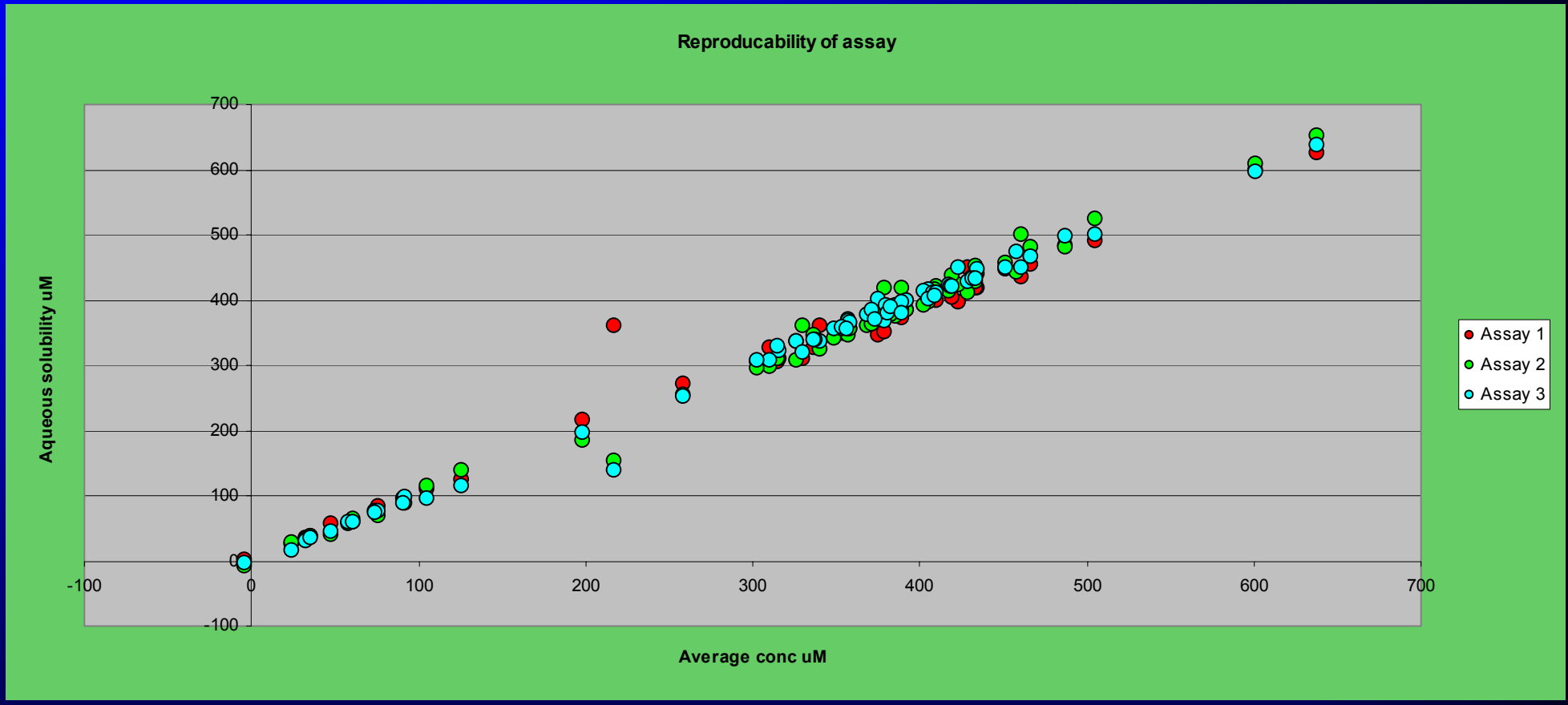
GlaxoSmithKline

Results: cont..



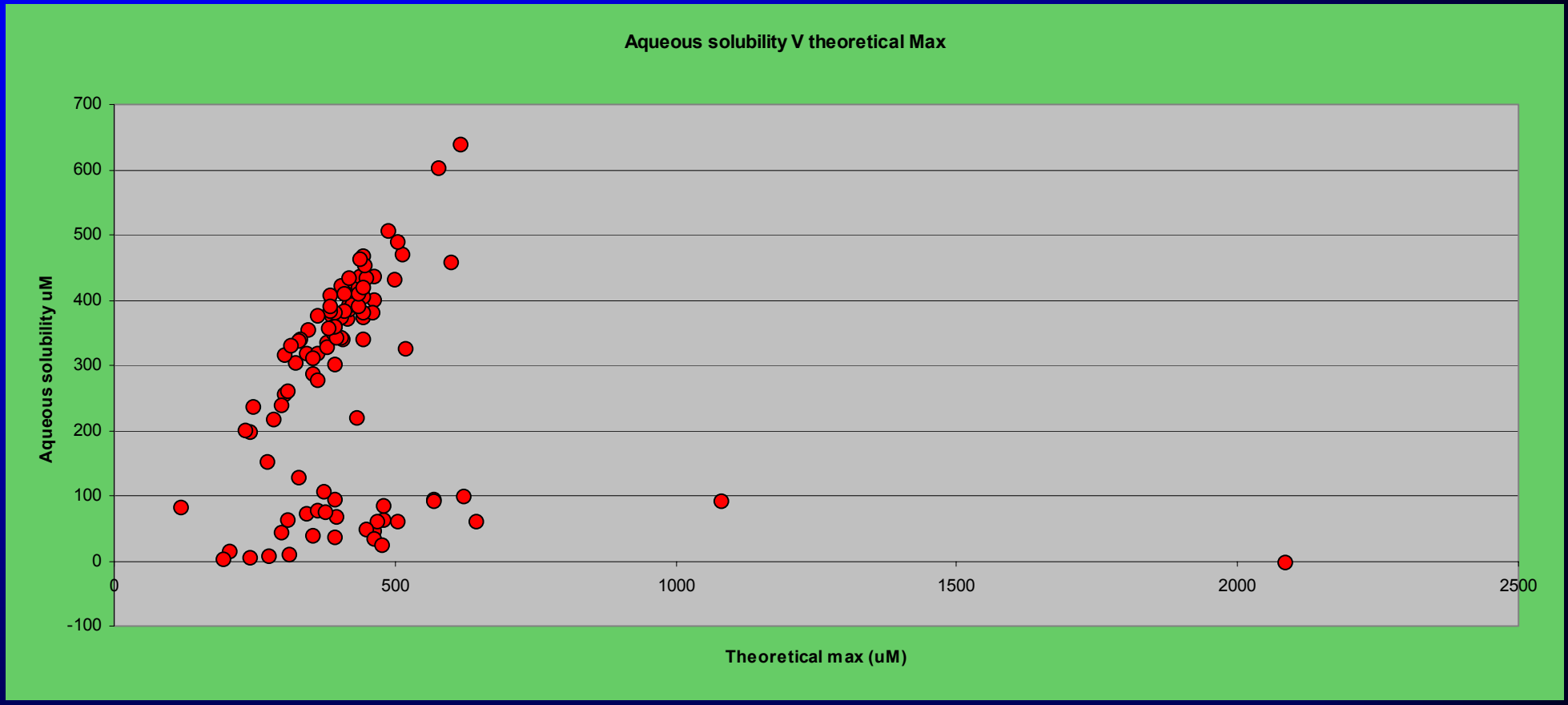
GlaxoSmithKline

Results: cont..



GlaxoSmithKline

Results: cont..



GlaxoSmithKline

Advantages of method:

- Fast – 160 samples readily accommodated in single experiment.
- Compound Efficient – only 6 ul of 10mM DMSO stock consumed in Assay.
- Large dynamic range – can measure from uM – 120mM
- Aqueous results not dependent upon stock concentration.
- Additionally yields DMSO stock concentration.
- Cheap – consumable cost 20p per sample

GlaxoSmithKline

Disadvantages:

- No separation step – subject to interference from impurities.
- Possible non linear response of various Nitrogen moieties.
- No Nitrogens present in Molecule.
- Too Many Nitrogens present in molecule
  (most encountered to-date 27)

Dual Injector twin channel CLND solubility work station – capacity 500 samples day-1

GlaxoSmithKline

Summary:

- Various options to automate and enhance capacity of assays are available.
- High throughput methods should ideally be designed to accommodate new technologies as they become available.
- Appropriate choice of technology allows solubility determination to be routinely run at a level to fully support the chemistry efforts of large pharma.

GlaxoSmithKline

Acknowledgements:

- Carol Carmody, Steve Besley, Nick Taylor, John Hollerton – GSK
- Steve Duffin – CSP
- The GSK WW PHYSCHEM group

GlaxoSmithKline