APPROACHES AND LESSONS LEARNED IN ESTABLISHING HT-ADME ASSAYS

Solvo Meet-the-Experts Transporter Conference

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05-Sept-2019

EVERY STEP OF THE WAY

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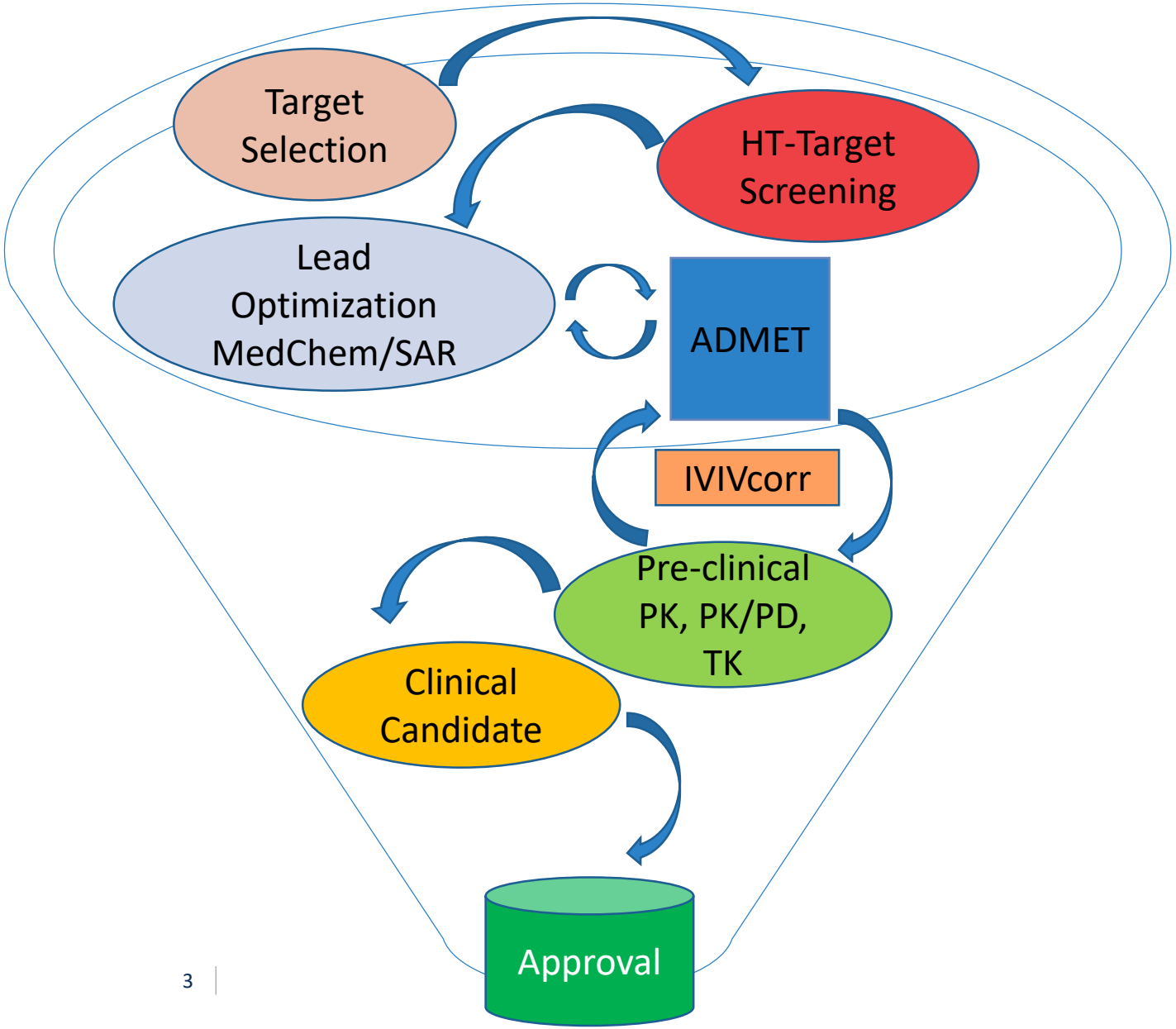
PRESENTATION OUTLINE

- WHY **ADME** AND WHY **HT**?
- APPROACH FOR SETTING UP HT-ADME PLATFORM
- QUALIFICATION ASSAY DATA / STATISTICAL ANALYSES TO DEMONSTRATE PERFORMANCE
- LESSONS LEARNED / SUMMARY

IMPORTANCE OF ADME FOR DRUG DISCOVERY & DEVELOPMENT



- *In vitro* ADME data are important for triaging compounds in early discovery to enable *in vivo* studies: PK, PK/PD, efficacy and TK studies, before IND selection
- Critical to optimize DMPK properties to enable successful outcomes in preclinical and clinical studies
- Saves time and money to establish an IV-IV-correlation during the discovery phase

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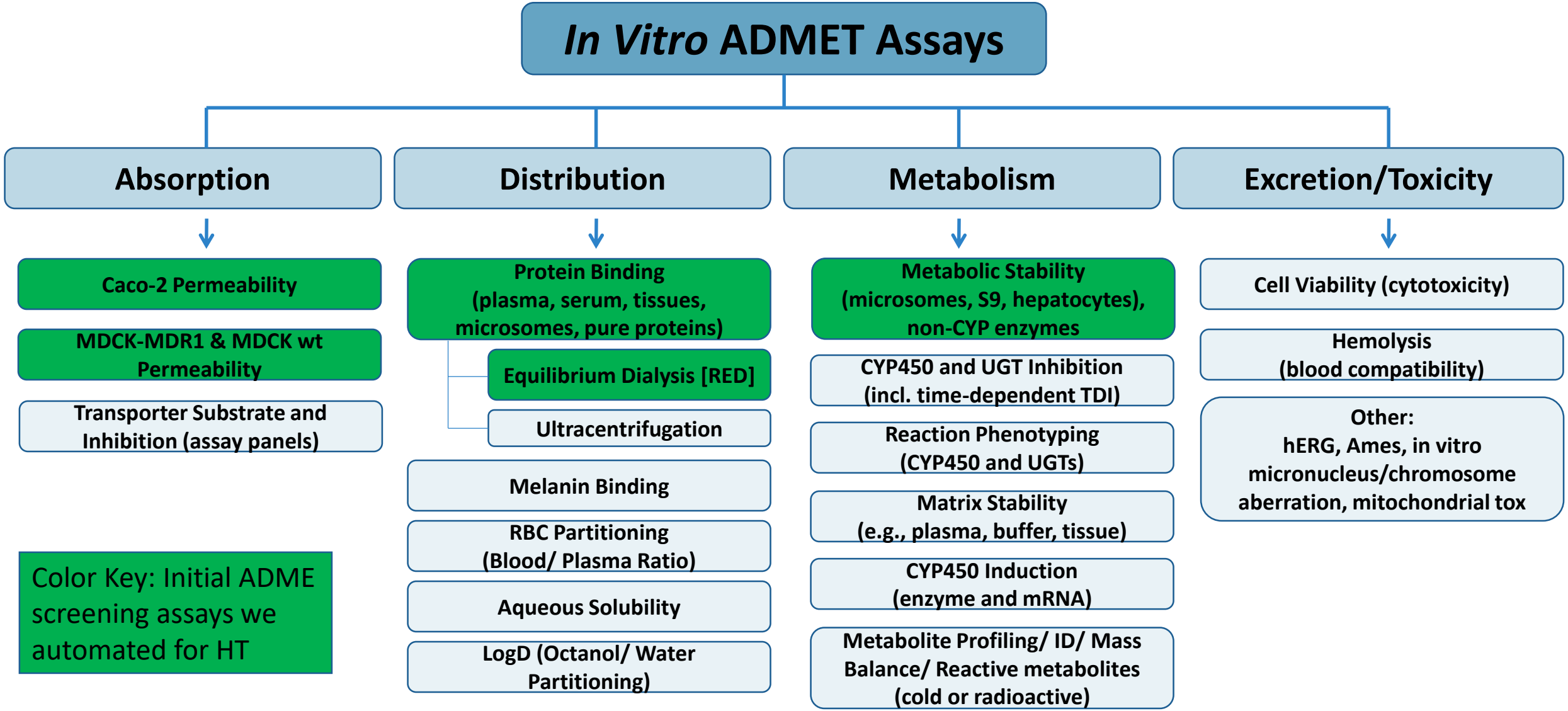
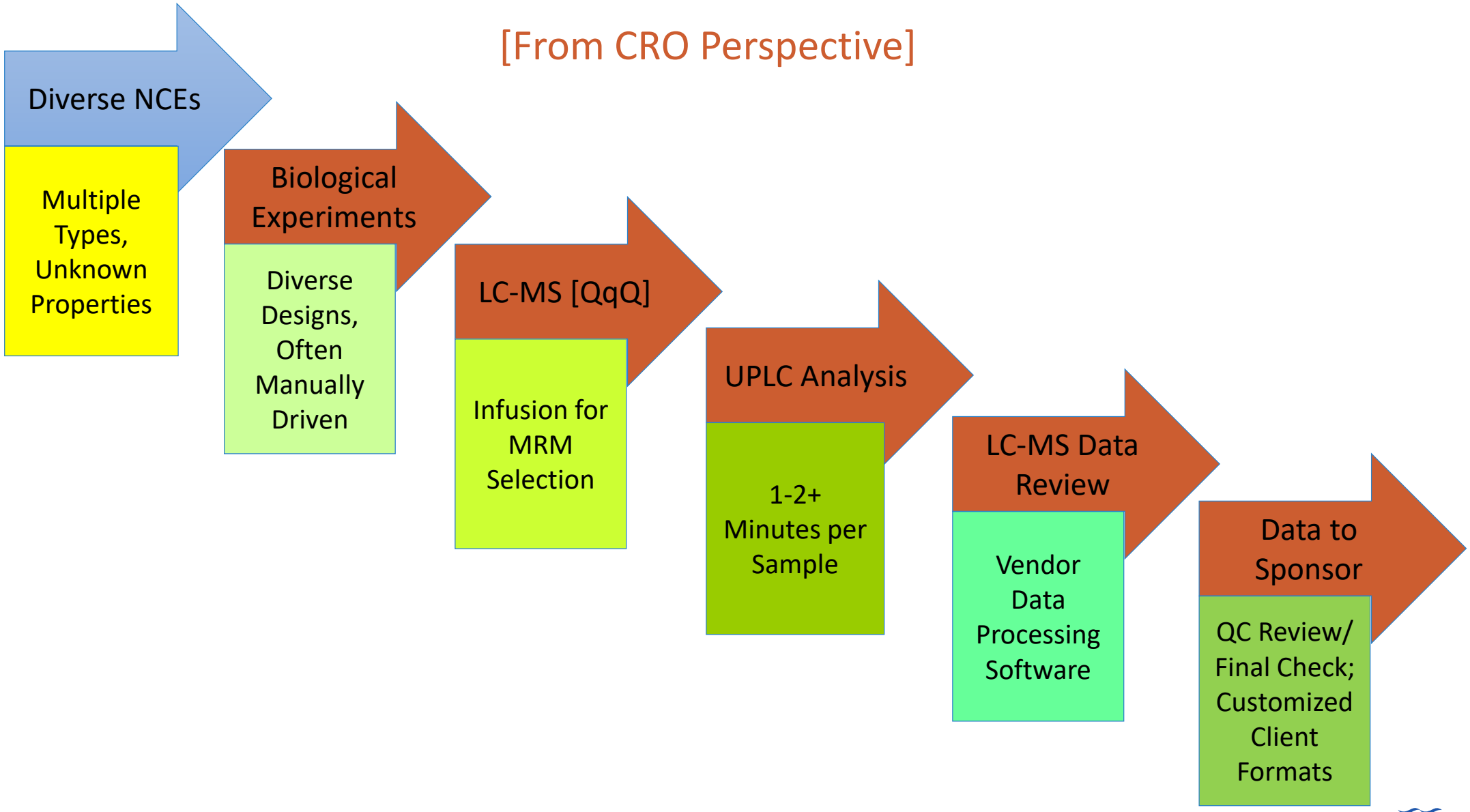
VARIETY OF IN VITRO ADME ASSAYS



BOTTLE-NECKS WITH HIGH-VOLUME ADME NEEDS

[From CRO Perspective]



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HT-ADME PLAN TO ADDRESS BOTTLE-NECKS

- Life science industry needs capacity and rapid TAT to drive drug discovery programs
- Target objectives (based on clients' requests):
  - Focus on first tier assays:
    - Liver microsomal metabolic stability
    - Plasma protein binding (RED; Rapid Equilibrium Dialysis)
    - Permeability (Caco-2, MDCK-MDR1)
  - Fast data TAT: ≤5 Days from compound receipt to delivery of results
  - Capacity: ~500 compounds/week for primary screening assays
  - Reformatting: ability to cherry-pick compounds and to handle a variety of inputs (a challenge in CRO environment)
- Implement assay automation and HT-LC-MS/MS workflow (within ~8 month period)
- LC-MS/MS optimization and data
  - Facile MRM development and optimization (DQ)
  - Efficient data processing and review (leverage Gubbs GMSU)
- ADME LIMS
  - Efficient data storage, flexible calculation and reporting

RESOURCE INVESTMENTS TO ENABLE HT-ADME

- Investments initiated in 2017 to enable HT-ADME capability to support both LT- and HT-ADME demands (capacity and flexible experimental designs)
- Liquid handling automation:
  - Evaluated 3 vendors in detail (hardware and software)
  - Purchased 2 Hamilton Vantage (2m; 8- and 96-channel; temp-control, shaking)
- HT-LC-MS/MS
  - Evaluated LC systems and visited labs with ADDA's; considered QE accurate mass
  - Purchased 2 Apricot ADDA with Agilent 1260s pumps and Sciex 5500 MS/MS
- 2 systems provide redundancy along with capacity (to minimize downtime)
- A small dedicated team with internal and external expertise to enable HT set-up and qualification/validation
- LIMS: Evaluated 3 vendors in detail, selected Edge BioRails/Morphit (UK)

HT AUTOMATION (TO GENERATE AND ANALYZE SAMPLES)

Hamilton Vantage robot [2m deck; N=2]
- Compound cherry-picking
- Heating, cooling, shaking, timed incubations
- Barcoding

ADDA LC-MS/MS [N=2]
- 96/384-well plates
- Automated MRM method development
- T/E and gradient modes
- Flexible LC column and mobile phase selections

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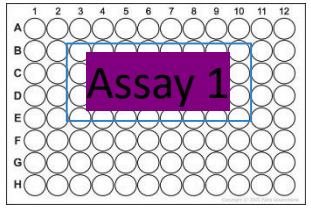
HT-ADME WORKFLOW: SOURCE→ ASSAY → BIOANALYSIS

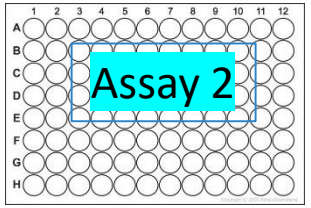
Client compounds provided in plates

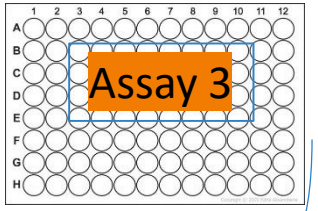
Store ≤-80°C for back-up

Imprint/aliquot

Cherry-pick/ make assay-ready plates







Used for all *in vitro* assays

Assay

Perform MetCL, PPB, Permeability Assays

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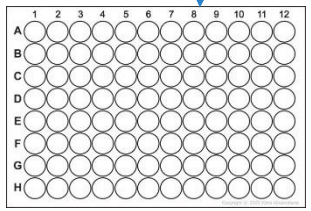
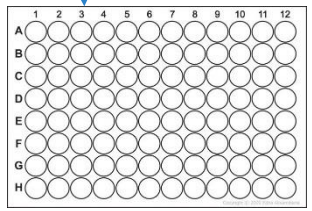
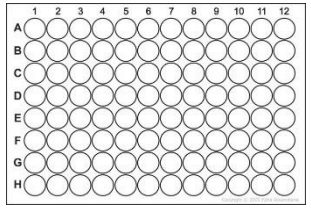
MRM Optimization using DQ

Bioanalysis







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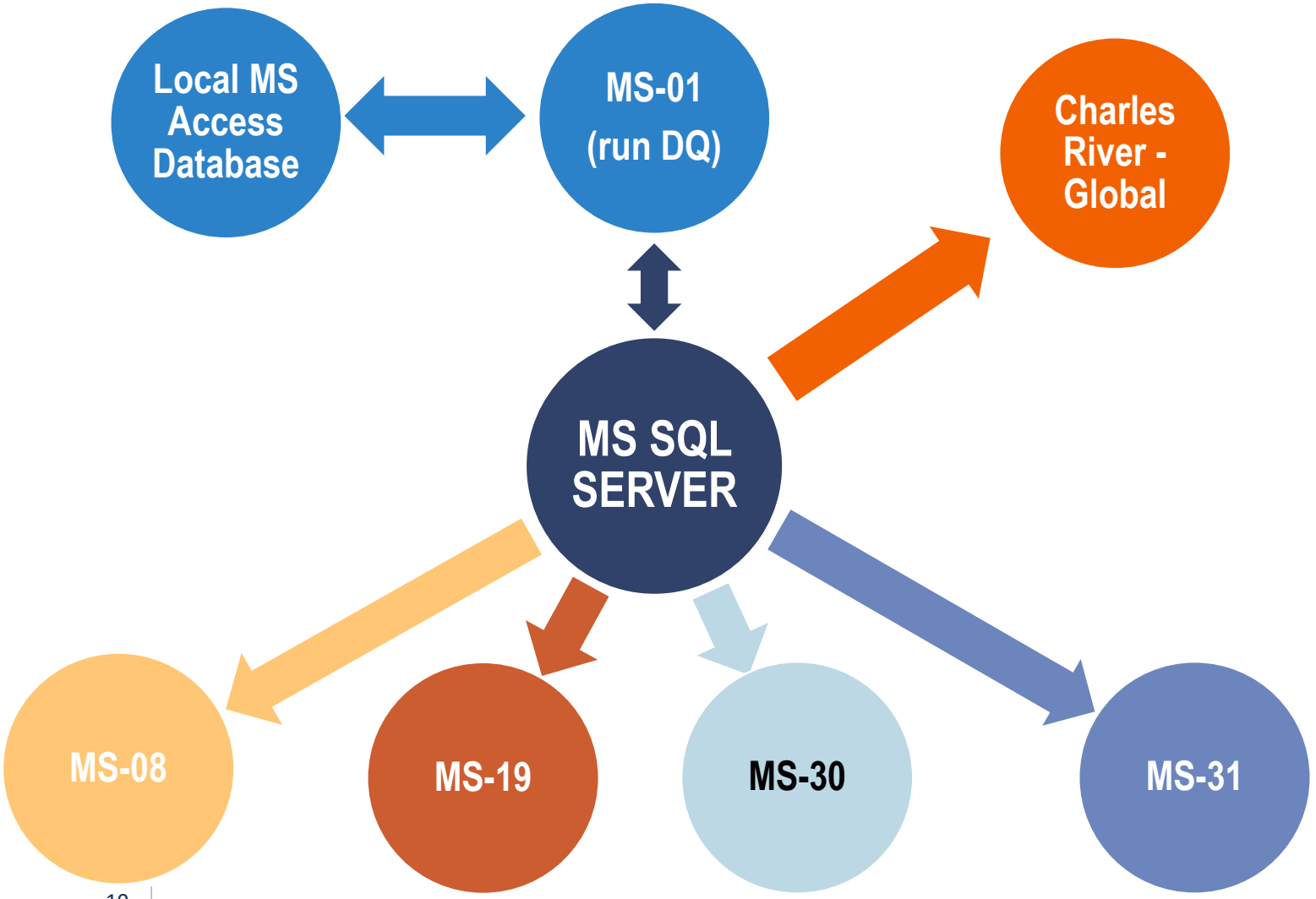
DISCOVERYQUANT®: MRM OPTIMIZATION



- Single database which serves as central repository of all compounds assayed within the *in vitro* ADME group
- Optimize once on a single MS (4000)
  - Run compound plates overnight
  - ~1 min/compound (fast tuning)
  - ~2 min/compound (fine tuning)
- Utilize the central database without having to repeat manual tuning and eliminate redundancies
- Global MS SQL server
  - Share MRM conditions
  - Review spectra

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ADDA HT-LC/MS/MS SYSTEMS

- Dual-arm, high-speed multiplexing autosampler
  - ~10 seconds/injection
- Two modes of operation:
  - Trap and elute
    - TIS for “clean” samples
    - APCI for plasma samples
  - Gradient
- Software: Sound Analytics
  - DiscoveryQuant (DQ) for automated MRM optimization
  - LeadScape for acquisition

APRICOT DESIGNS ADDA

+

UFLC Pumps

ABI 5500 MS

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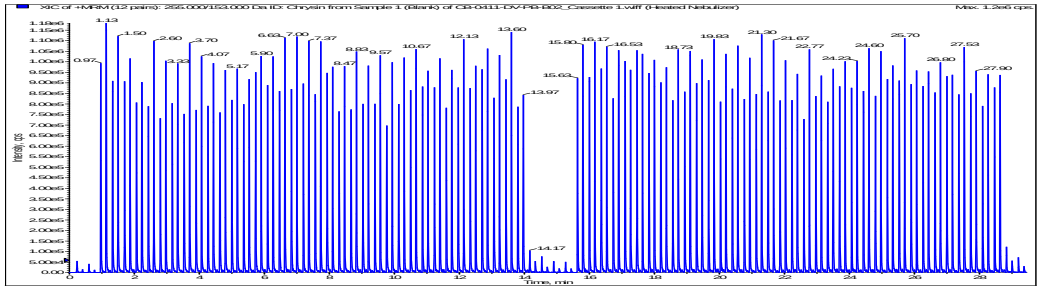
ADDA HT-LC-MS/MS PLATFORM

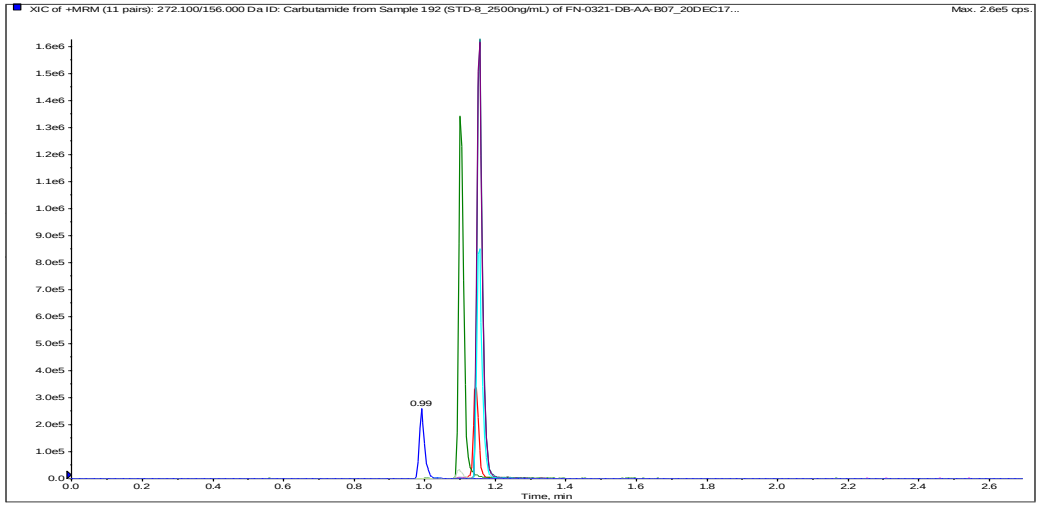
- Performs T/E and gradient modes on the fly
  - 384-well, 96-well, etc.
  - High-quality peak shape
- Diverse column chemistries [T/E]
  - RP C_{18}: 10 mm to 30 mm, 3 µM
  - HILIC for polar molecules
- ADDA proven to be very robust and reliable
- More flexible than RapidFire
- Need data processing software to be able to integrate multiple peaks and analytes in a single file

T/E



HT-Permeability Assay
[160 Samples Analyzed in 30 minutes]

Gradient



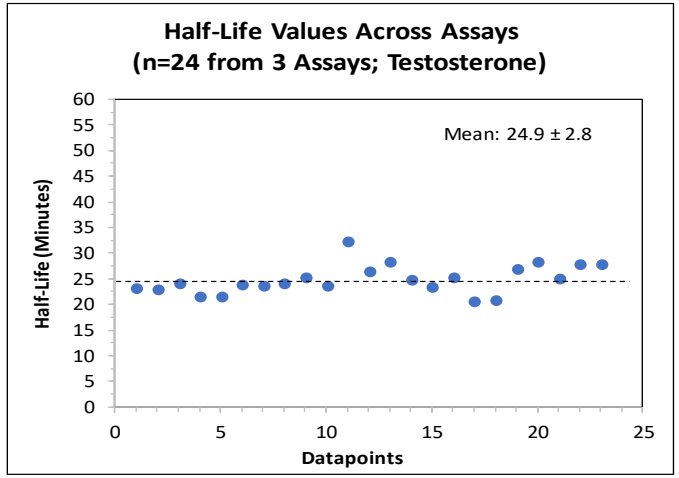
STEPS FOR PROCESS QUALIFICATION

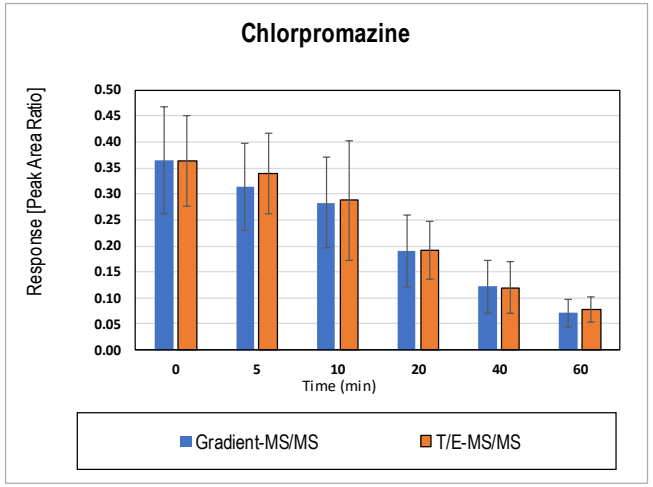
- Define final assay procedure
- Optimize pipetting (liquid classes, liquid into dry plate or solution, lab temp/humidity)
- Optimize workflow efficiency (deck layout, minimize back&forth movements)
- Run through procedure in simulation mode, then blank reagents, then real assay
- Demonstrate results agree with expected values (literature and internal historical)
- Assess data consistency across runs, time
- Demonstrate agreement between manual vs. robotic, and gradient vs. T/E
- Assess performance using known drug compounds, plus test “real world” discovery compounds

QUALIFICATION:
METABOLIC STABILITY ASSAY

HT-ADME ASSAY VALIDATION RESULTS FOR METABOLIC STABILITY ASSAY

- Demonstrated consistent robot and HT-LC/MS performance:



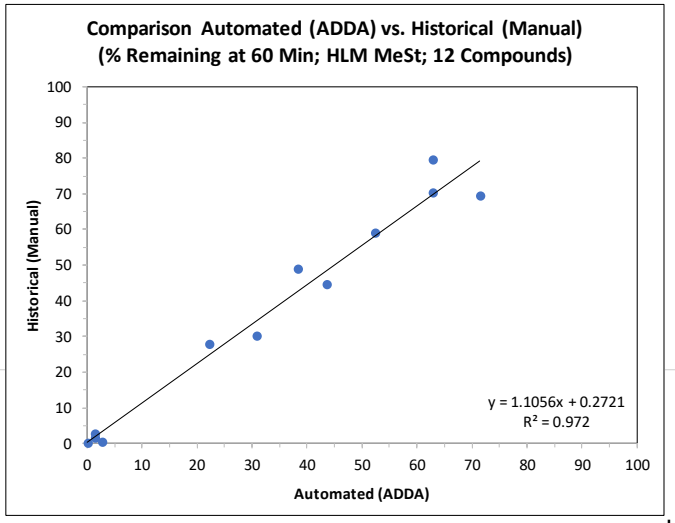


T/E vs. Gradient:
1 µM compound
0.5 mg/mL HLM
37°C
Time course

- Demonstrated equivalent results between automated assay and manual (historical) MeSt assay:

	Mean % Remaining at T_{60min}		Mean T 1/2 (min)		Mean CL_{int} (mL/min/kg)	
Compound ID	**Automated**	**Manual**	**Automated**	**Manual**	**Automated**	**Manual**
7-EC	2.8 ± 2.5	1.8 ± 0.2	9.7 ± 1.4	9.1 ± 0.3	131 ± 15	137 ± 4
Testosterone	22.3 ± 6.3	24.0 ± 4.6	24.9 ± 2.8	29.0 ± 2.7	50.7 ± 5.5	43.3 ± 3.9
Imipramine	52.4 ± 4.2	57.8 ± 6.6	66.7 ± 10.9	79.0 ± 17.0	19.2 ± 3.0	16.4 ± 3.2
Terfenadine	1.5 ± 0.8	1.5 ± 1.1	11.3 ± 1.7	10.4 ± 1.8	113 ± 17	123 ± 20
Quinidine	62.9 ± 5.9	70.3 ± 5.7	103 ± 31	108 ± 25	13.3 ± 4.0	12.1 ± 3.1

[Equivalency also demonstrated with 40 sponsor discovery compounds]



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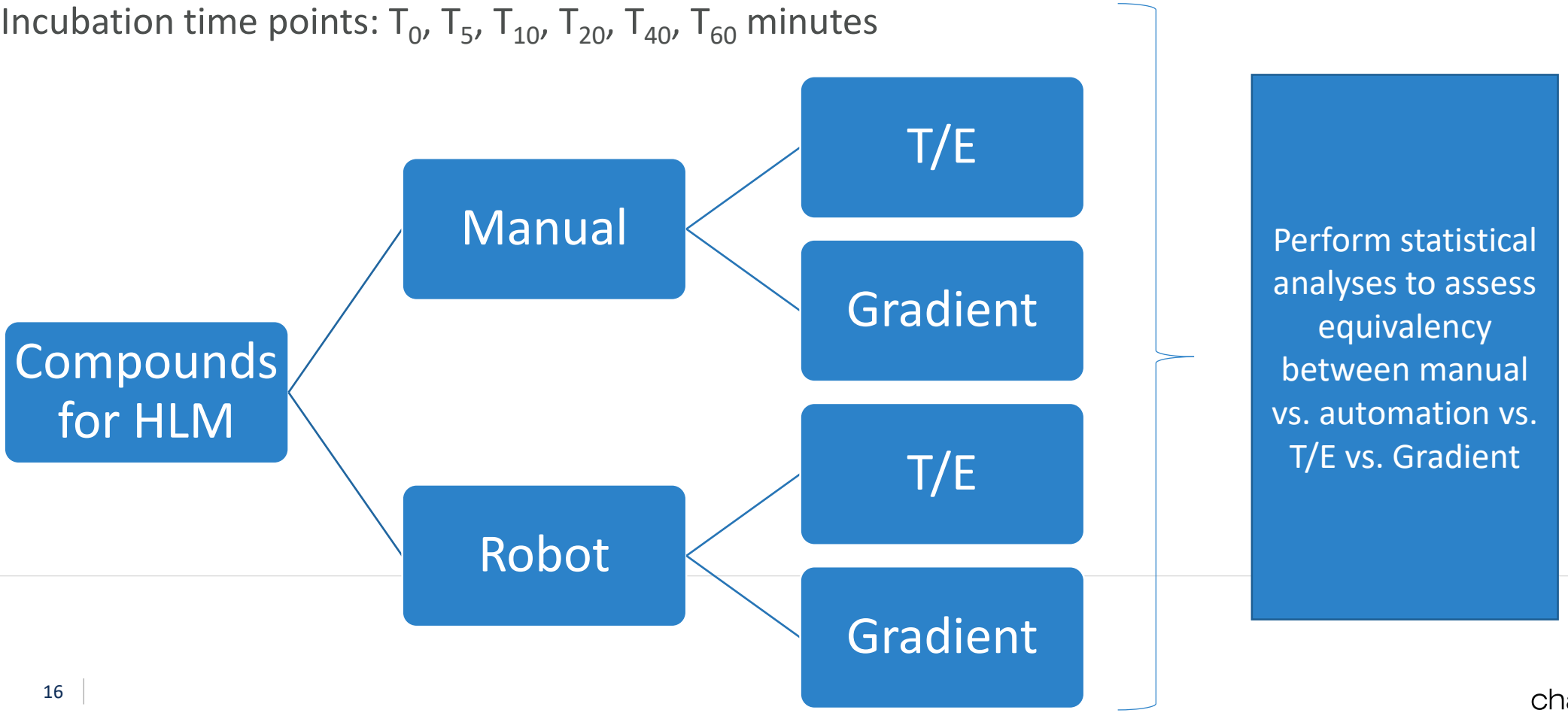
QUALIFICATION USING UNKNOWN NCE'S

- 40 discovery compounds submitted by Sponsor for HLM
- Concentration: 1 µM
- Incubation time points: T_0, T_5, T_{10}, T_{20}, T_{40}, T_{60} minutes



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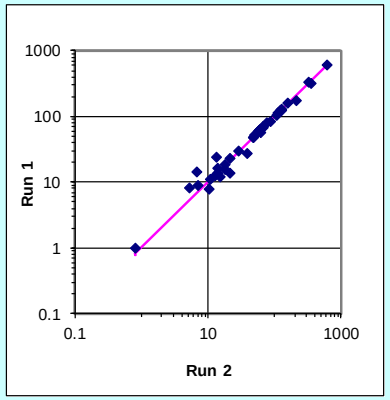
HLM: MANUAL VS AUTOMATION FOR NCEs [N=40] T/E & GRADIENT [CL_{INT}]

T/E vs. GRADIENT with 45-degree Line

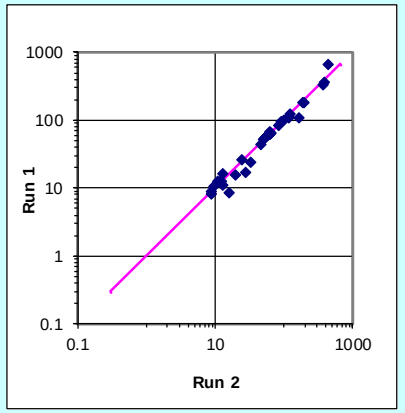


Correlation Coefficient of **LOG-LOG** Plot = 0.988

15 points below the 45-degree line
23 points above the 45-degree line

Manual Experiment

T/E vs. GRADIENT with 45-degree Line

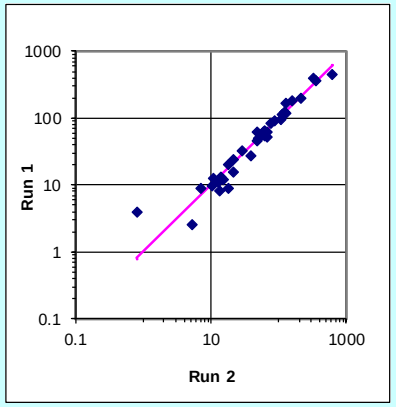


Correlation Coefficient of **LOG-LOG** Plot = 0.987

16 points below the 45-degree line
19 points above the 45-degree line

Automation Experiment

Robot vs. Manual with 45-degree Line

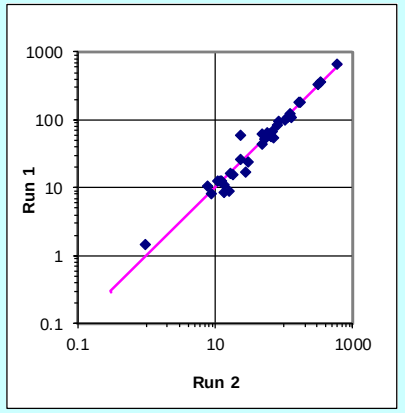


Correlation Coefficient of **LOG-LOG** Plot = 0.962

19 points below the 45-degree line
19 points above the 45-degree line

Gradient Analysis

Robot vs. Manual with 45-degree Line



Correlation Coefficient of **LOG-LOG** Plot = 0.980

17 points below the 45-degree line
20 points above the 45-degree line

T/E Analysis

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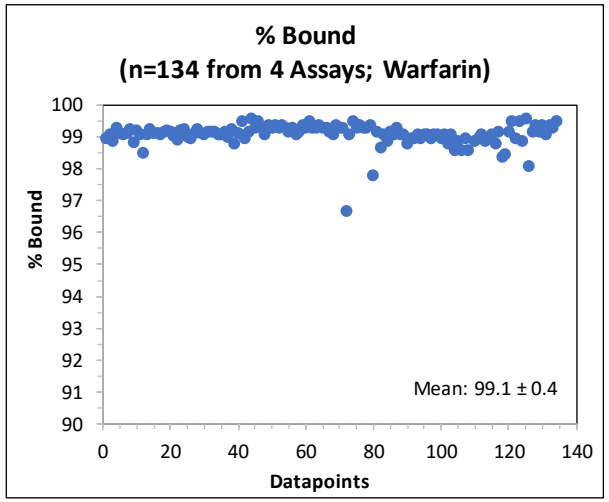
QUALIFICATION:
PROTEIN BINDING ASSAY (RED)

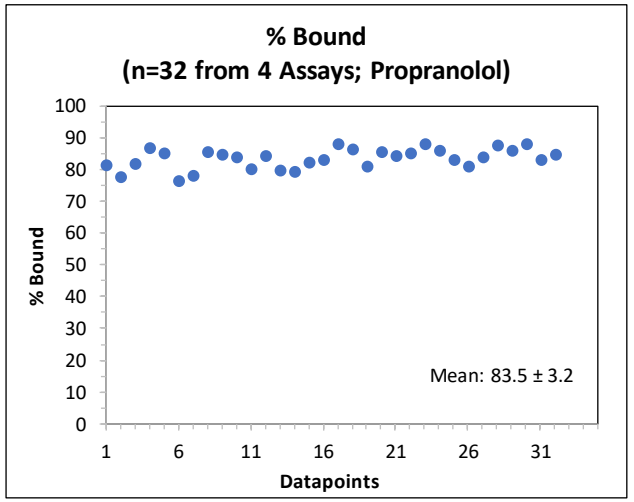
HT-RED PLASMA PROTEIN BINDING

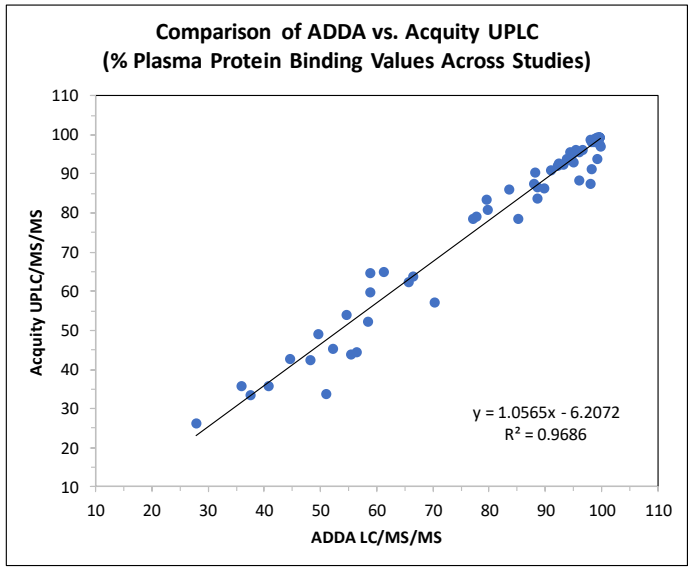
- Typically test 1-2 µM compound (from 1000X DMSO stock)
- Replicates: N=1 to 3
- Incubation time: 6 hrs with gentle mixing in 5% CO_2 at 37°C (to provide longer time to reach equilibrium, and to maintain pH)
- Matrices: Mouse, rat, dog, monkey, human plasma
  - Human lots are pre-screened (warfarin % binding, common control compounds, since we have noticed more unacceptable lots in recent years)
  - May be valuable to pre-screen with AGP binders too (plasticizers etc. can affect)
- Often include a concurrent matrix stability and recovery control (T_0 vs T_6)

HT-ADME ASSAY VALIDATION RESULTS: RED PPB ASSAY

➢Demonstrated consistent robot performance:



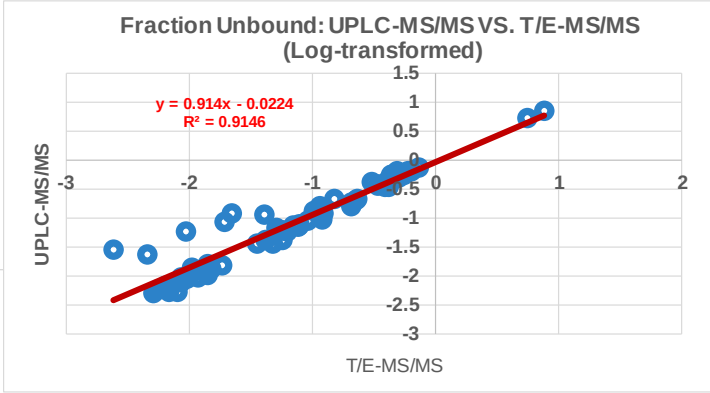




➢Demonstrated equivalent results between automated assay and manual (historical) assay:

	Mean % Bound	
Compound ID	**Automated**	**Manual**
Chlorpromazine	97.3 ± 1.4	95.8 ± 5.1
Digoxin	47.7 ± 18.3	45.6 ± 12.2
Propranolol	83.5 ± 3.2	83.8 ± 0.6
Verapamil	87.4 ± 0.8	87.7 ± 3.6
Warfarin	99.1 ± 0.4	99.1 ± 0.3

Equivalency also demonstrated with 68 sponsor discovery compounds:



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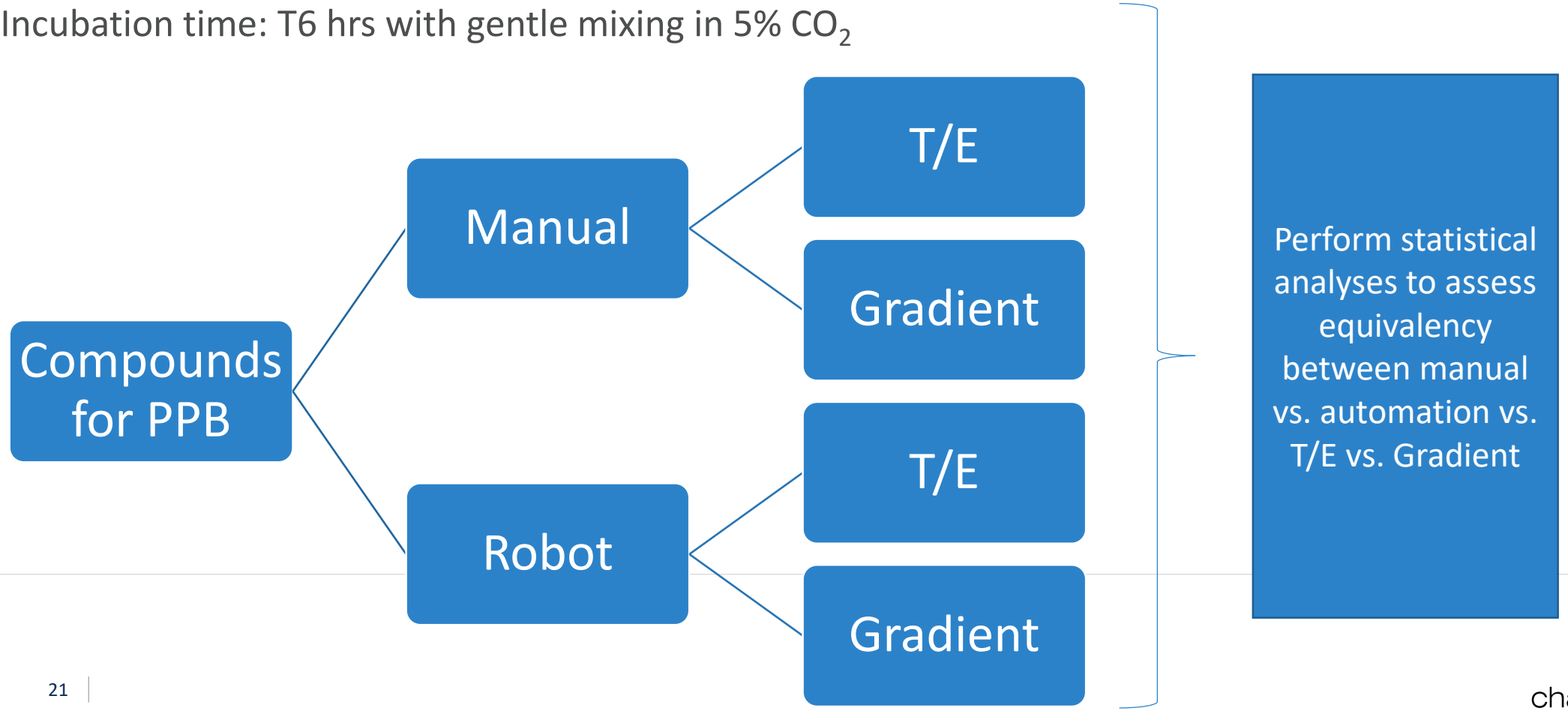
PPB [RED] VALIDATION USING UNKNOWN NCES

- 40 discovery compounds submitted by Sponsor for PPB
- Concentration: 1 µM
- Incubation time: T6 hrs with gentle mixing in 5% CO_2



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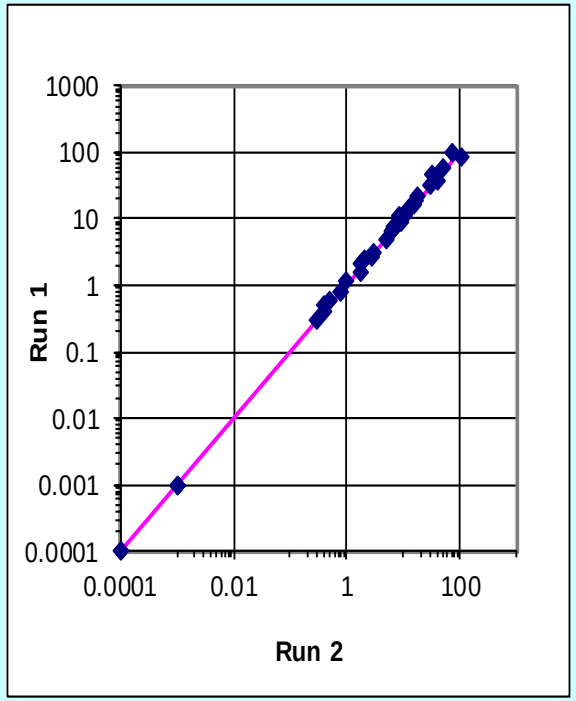
PPB [RED] USING AUTOMATION: T/E AND GRADIENT ANALYSIS

Fraction Unbound

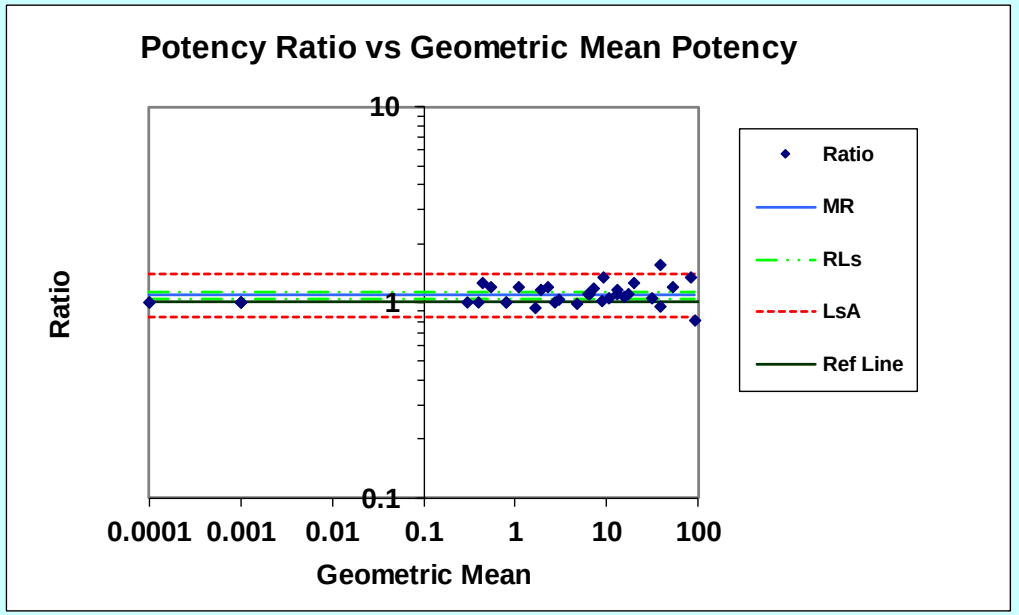
T/E vs. Gradient with 45-degree Line



Correlation Coefficient of **LOG-LOG** Plot = 1.000

4 points below the 45-degree line
21 points above the 45-degree line

Automation



	Statistical Analysis of "Bland-Altman" Plot						
	Equivalence Test Results				**Reproducibility Test Results**		
	N	35					
	MR	1.09			MSR (Within-Run)	1.28	
	RLs	1.04			LsA	0.85	
		1.13				1.39	
Sig Diff Between					SD	0.0380	
Runs Test, p =	0.0004						

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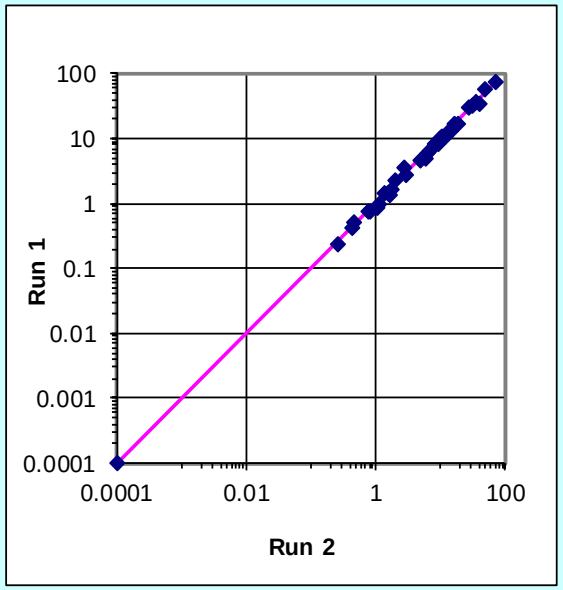
PPB [RED] USING MANUAL: T/E AND GRADIENT ANALYSIS

Fraction Unbound

T/E vs. Gradient with 45-degree Line

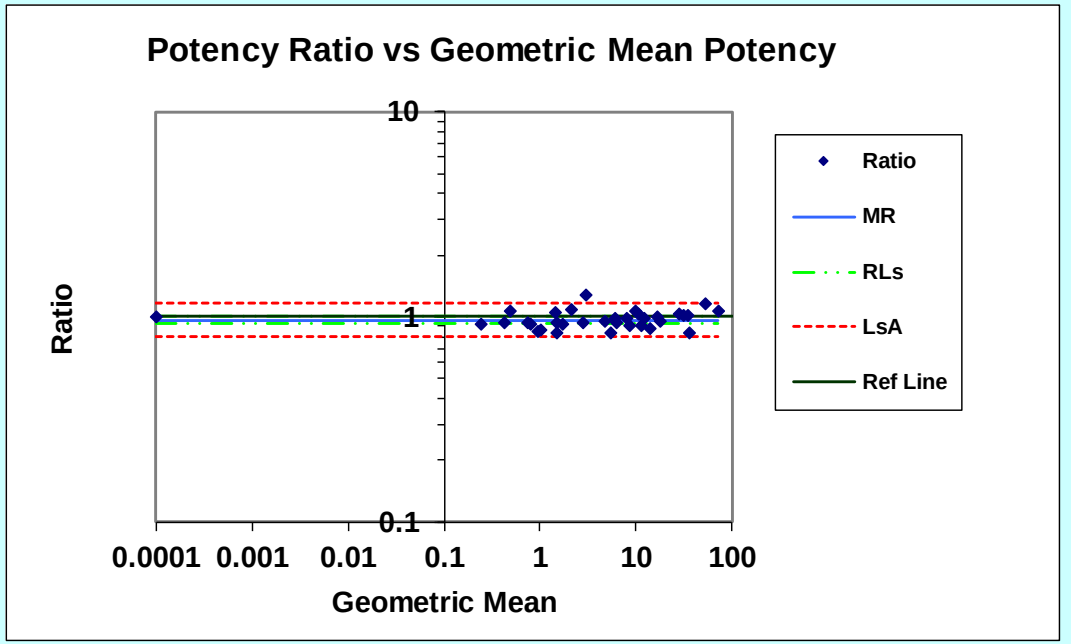


Correlation Coefficient of **LOG-LOG** Plot = 0.999

21 points below the 45-degree line
12 points above the 45-degree line

Manual

PPB [RED] MANUAL Assay T/E & Gradient



	Statistical Analysis of "Bland-Altman" Plot							
	Equivalence Test Results				Reproducibility Test Results			
	N	34						
	MR	0.97			MSR (Within-Run)		1.21	
	RLs	0.94				LsA	0.80	
		1.00					1.17	
	Sig Diff Between					SD	0.0294	
	Runs Test, p =	0.0487						

T/E and Gradient Analysis Demonstrated Equivalent Performance By Deploying ApCI for T/E analysis

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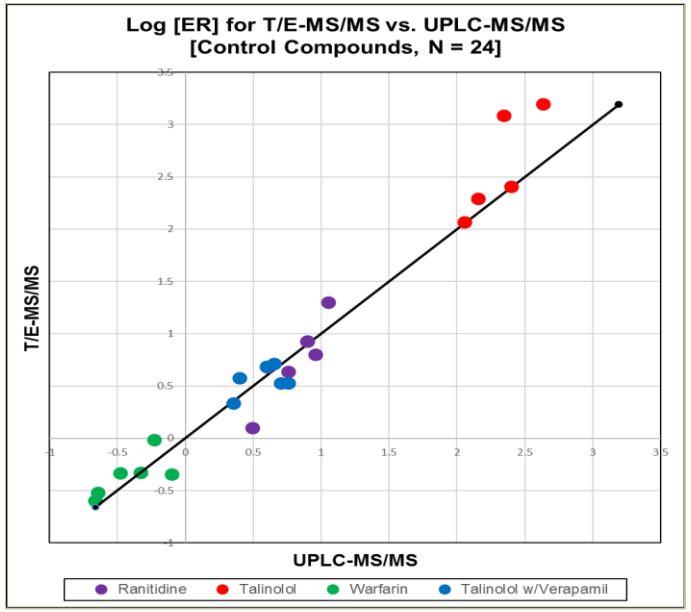
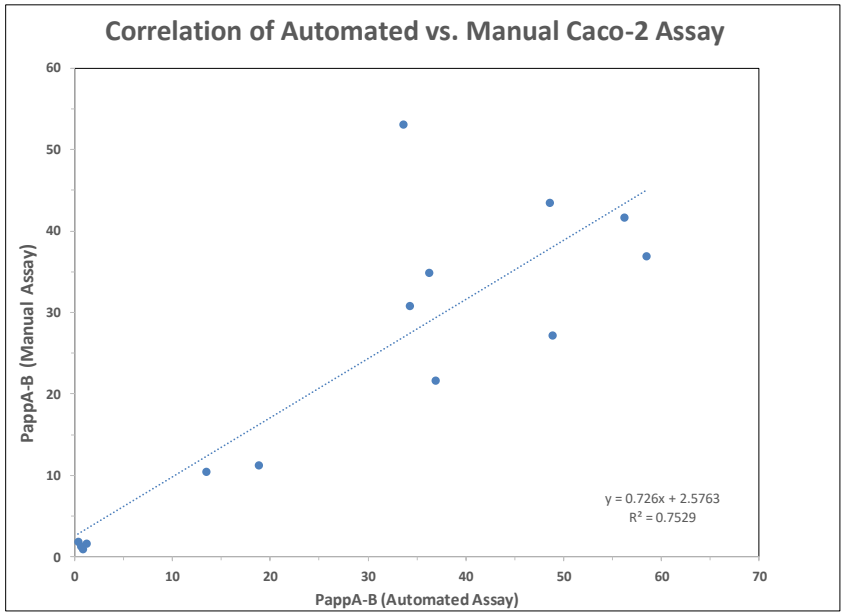
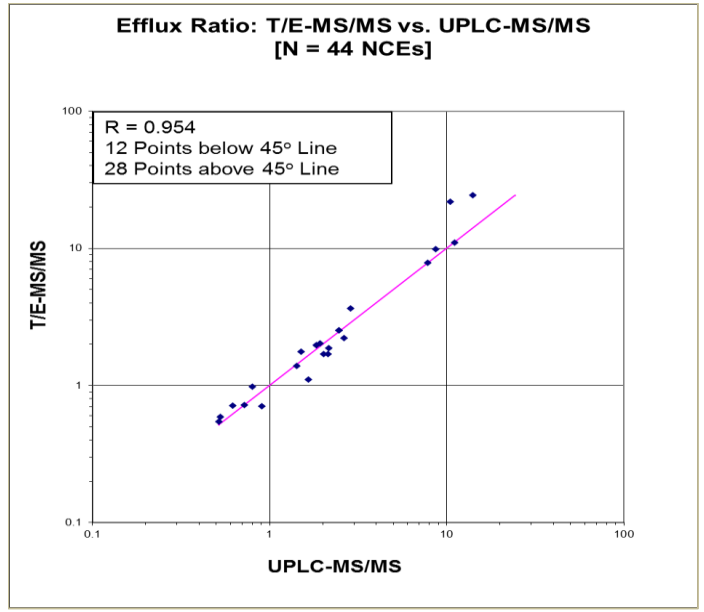
QUALIFICATION: PERMEABILITY ASSAY

HT-ADME ASSAY VALIDATION RESULTS FOR PERMEABILITY ASSAY

- Typically 10 µM, n=3, 2 hours incubation
- Demonstrated consistent performance (gradient vs T/E):







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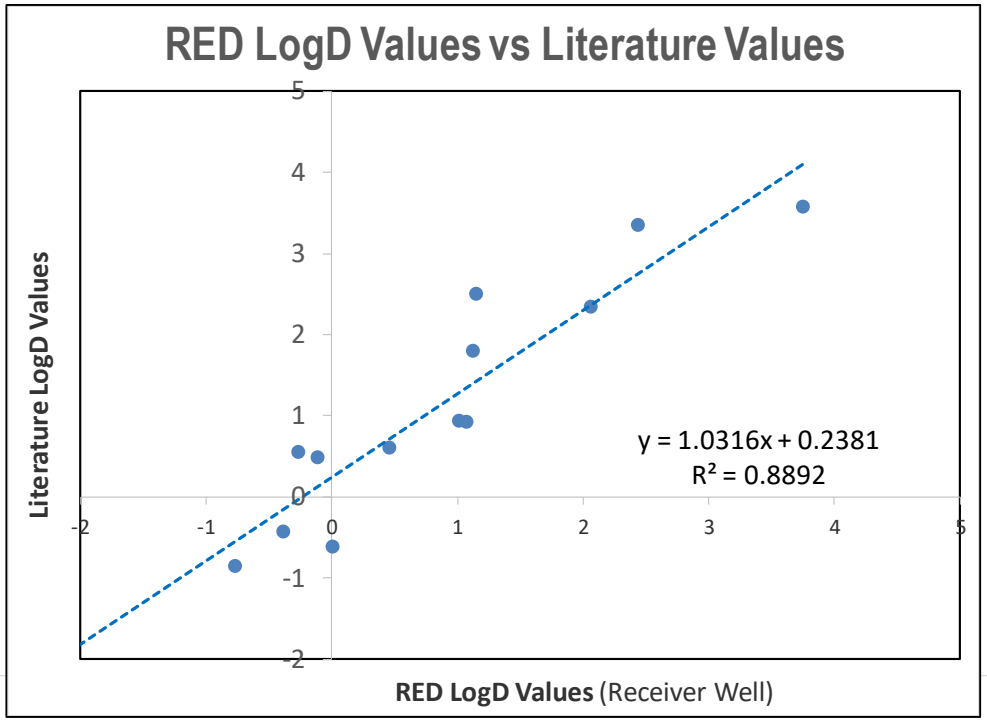
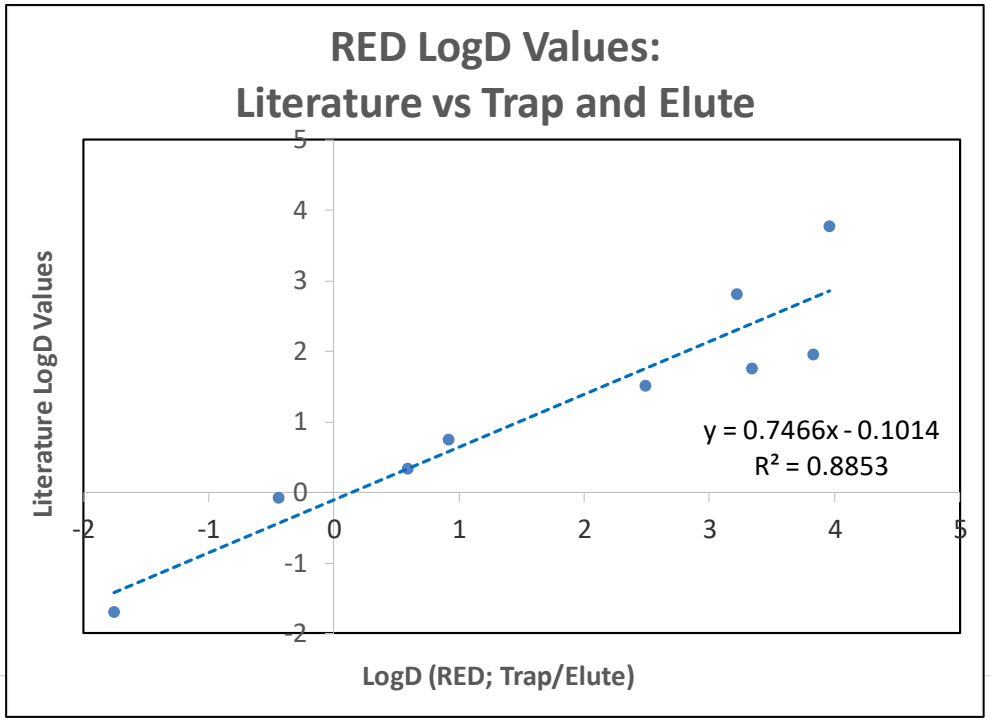
OTHER HT-ADME ASSAY PROJECTS

- Have added additional assay capabilities (CYP inhibition, aqueous solubility, etc.)
- Working on logD (octanol/water) assay using RED units (spike PBS at 100 µM, dialyze vs. octanol, sample PBS after 18 hours); format easier to automate than shake-flask.





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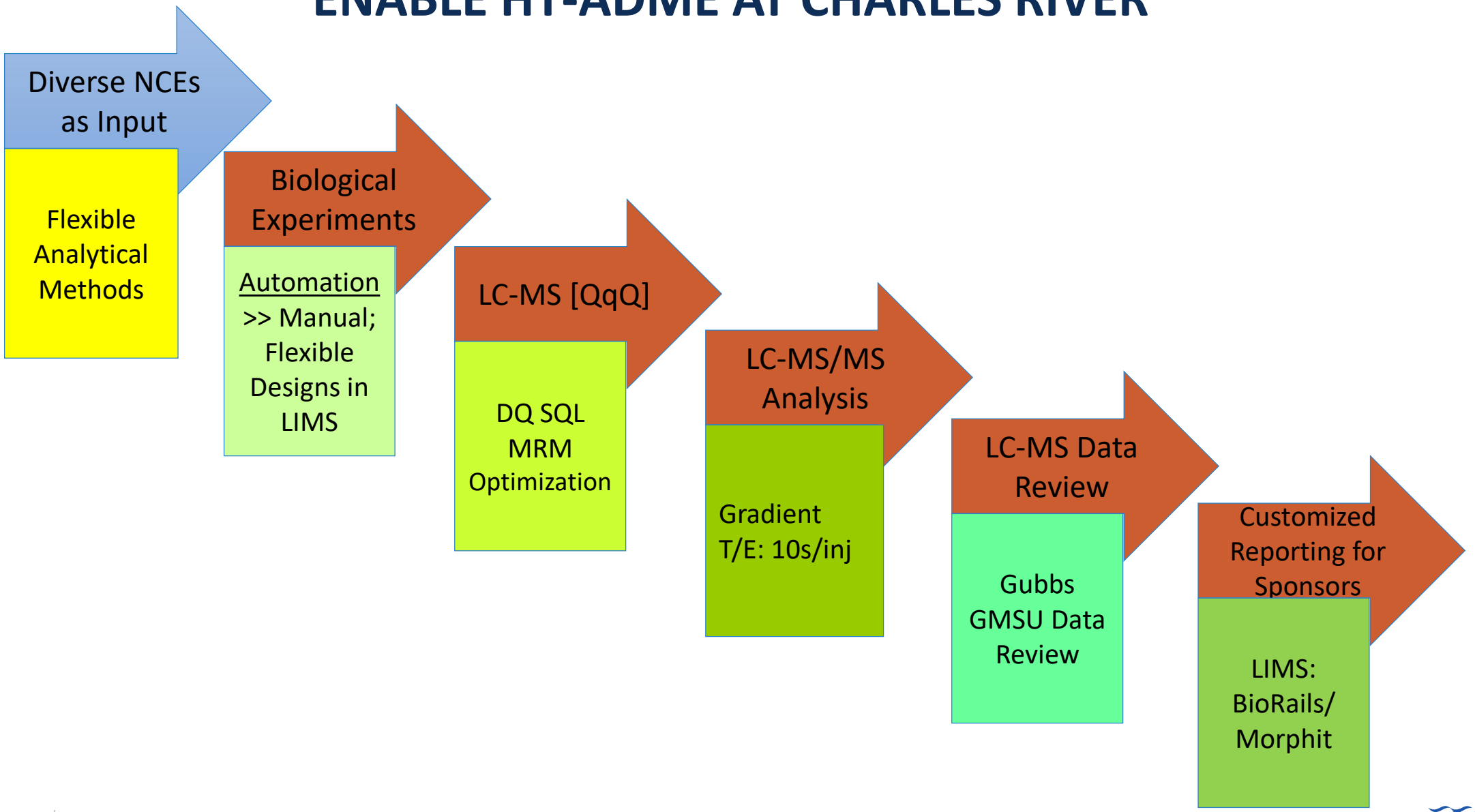
SUMMARY: BOTTLE NECKS ADDRESSED TO ENABLE HT-ADME AT CHARLES RIVER



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LESSONS LEARNED

Liquid Handling Automation

1) Hamilton hardware is solid, dependable (following some tweaks after installation)
2) Should have looked into variety and details of available hardware options in more detail (some accessories were more useful than others)
3) Hamilton Venus (VoV) software is very powerful but was more complex and longer learning curve than expected (initial vendor assistance was very important; need internal expert; found different programmers have different styles)
4) Pipetting accuracy is very dependent on parameters (e.g., liquid class, speed, height above well or cells, etc.); use real reagents vs. water for testing; in hindsight would have been helpful to define optimal pipetting parameters and plasticware earlier in process

HT-LC-MS/MS; Data Management

1) ADDA hardware observed to be solid, reliable
2) Slight stream differences observed, but is mitigated by running a complete set of samples for each compound within a single stream only
3) Fast data processing is key to reduce bottle-neck
4) Evaluated MultiQuant, LeadScape and GMSU, etc. (often using GMSU)
5) Evaluated diverse ADME LIMS options; critical to be track compounds in/data out; decide how much flexibility is required (decided to implement BioRails/Morphit; very happy with choice)

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- SoundAnalytics
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- Edge/BioRails (Andrew Lemon)

Any further questions:

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