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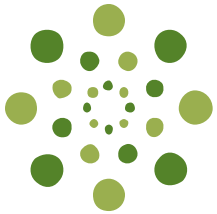


AIT Bioscience

Transforming the Process. Partnering for Results.

BIOANALYTICAL SOLUTIONS FOR BIOTHERAPEUTICS AND SMALL MOLECULE THERAPEUTICS

A TIERED APPROACH TO BIOANALYSIS SUPPORTING PRECLINICAL AND TOXICOLOGY STUDIES: BALANCING BUDGET, REGULATIONS AND NEEDS

JENNIFER VANCE

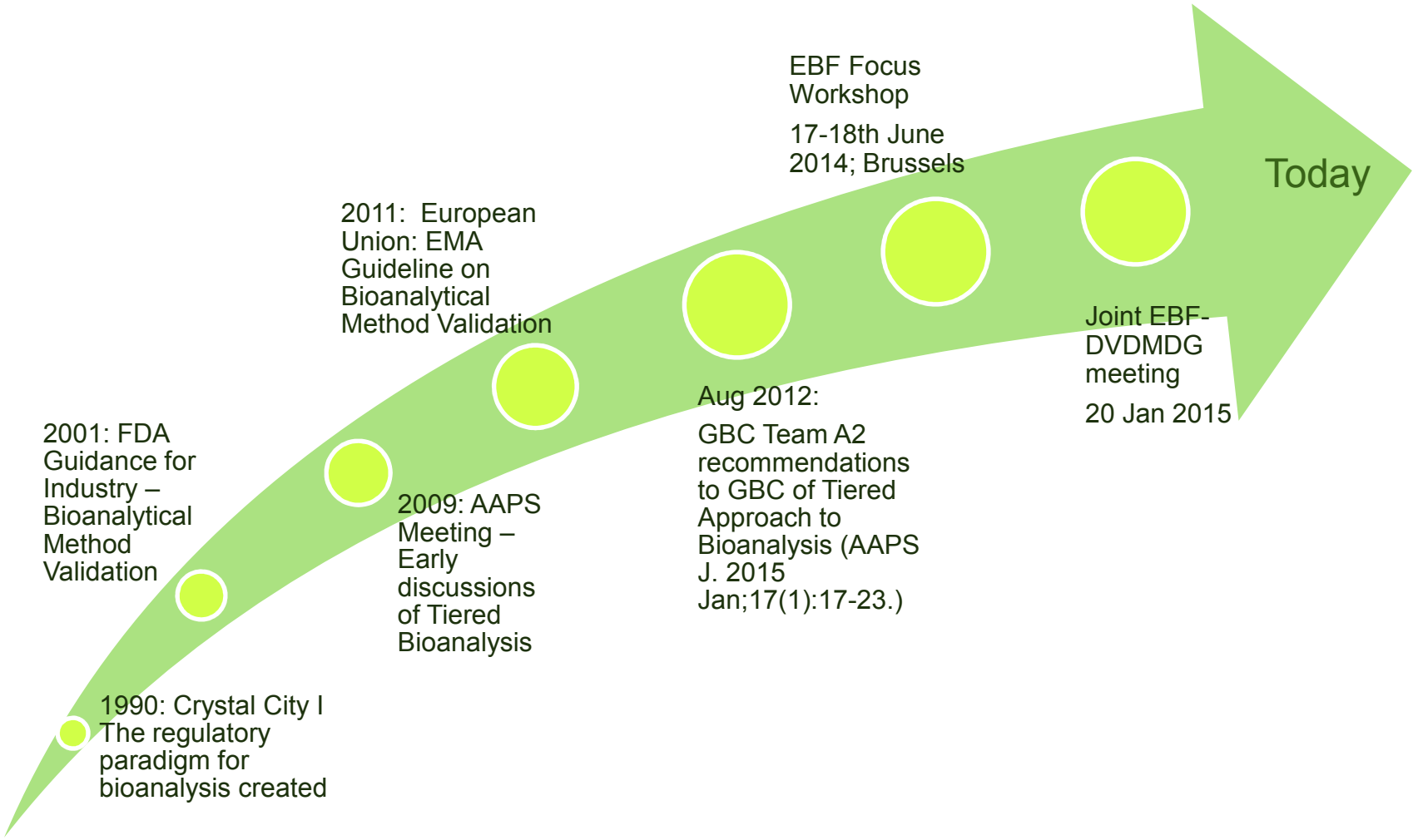
17 JAN 2018

PRESENTATION OUTLINE

- WHAT IS A TIERED APPROACH TO BIOANALYSIS?
  - Historical Discussion And Recommendations
- HOW AIT BIOSCIENCE HAS IMPLEMENTED A TIERED APPROACH
  - Execution In An All ELN Environment
  - Successes
    - CASE STUDY 1: A screening level approach to evaluate drug candidates
    - CASE STUDY 2: A research level surrogate matrix approach to understanding tissue PK
    - CASE STUDY 3: A qualified approach to secondary analyte analysis in a GLP-tox program
  - And Challenges

WHAT IS A TIERED APPROACH TO BIOANALYSIS?

HISTORICAL DISCUSSION AND RECOMMENDATIONS



WHAT IS A TIERED APPROACH TO BIOANALYSIS?

RECOMMENDATIONS FROM THE GBC A2 TEAM

- Differentiates four tiers of method characterization:
  - Validated method
  - Qualified method
  - Research method
  - Screening method
- Embraces a fit-for-purpose (FFP) principle and supplements the currently accepted single tier of a validated method
- All tiers are developed with the end objective in mind and each undergo limited characterization
- All tiers are proposed as an integral part of regulated bioanalysis, i.e. as appropriate for the intended use
- Appropriate to the phase of drug development

WHAT IS A TIERED APPROACH TO BIOANALYSIS?

RECOMMENDATIONS FROM THE GBC A2 TEAM

<table>
<tr><th>Requirements</th><th>Screening</th><th>Research</th><th>Qualified</th><th>Validated</th></tr>
<tr><td>Analyte Concentration obtained</td><td>Relative</td><td>Estimated</td><td colspan="2">Yes</td></tr>
<tr><td>Reference standard *</td><td>Not required</td><td>Comparator</td><td>Authenticated Reference Standard</td><td>Yes with COA,
as per regulatory guidance and SOP</td></tr>
<tr><td>Method development</td><td colspan="3">Yes, but limited</td><td>Yes</td></tr>
<tr><td>Pre-study method performance assessment **</td><td colspan="2">No - Rely on method development & in-study data</td><td>Preferred</td><td>Yes (pre-study validation)
as per regulatory guidance and SOP</td></tr>
<tr><td>Calibration curve for pre-study and in-study runs</td><td>Not applicable</td><td colspan="2">Yes, but fewer calibration standards allowed (> 3)</td><td rowspan="4">Yes, as per regulatory guidance and SOP</td></tr>
<tr><td>Matrix of cal stds and QCs identical to study samples</td><td>Not applicable</td><td colspan="2">Preferred</td></tr>
<tr><td>Independent QCs via second weighing</td><td colspan="2">Not applicable</td><td>Preferred</td></tr>
<tr><td>Acceptance criteria (AC) for calibration curves and QCs for pre-study and in-study runs</td><td>Not applicable</td><td colspan="2">Yes; AC can be broader than ±15/20%; required
FFP AC may be set a-posteriori instead a-priori</td></tr>
</table>

WHAT IS A TIERED APPROACH TO BIOANALYSIS?

RECOMMENDATIONS FROM THE EBF TEAM

ASSAY APPROPRIATE SCIENTIFIC VALIDATIONS

- Urine Analysis at all development stages
- Tissue Homogenate Analysis at all development stages
- Quantitation of Metabolites in plasma

STAGE APPRORITATE SCIENTIFIC VALIDATIONS

- Clinical: SAD/MAD Studies
  - Not conducting a full regulatory validation until key criteria such as basic PK behavior, metabolite profile and method range are established
- Preclinical: Dose range finding, Early Toleration Studies, even 28-day Tox
  - Inviting discussion on where the most appropriate application of the Guidance should begin

WHAT IS A TIERED APPROACH TO BIOANALYSIS?

RECOMMENDATIONS FROM THE EBF TEAM

REGULATORY VALIDATION

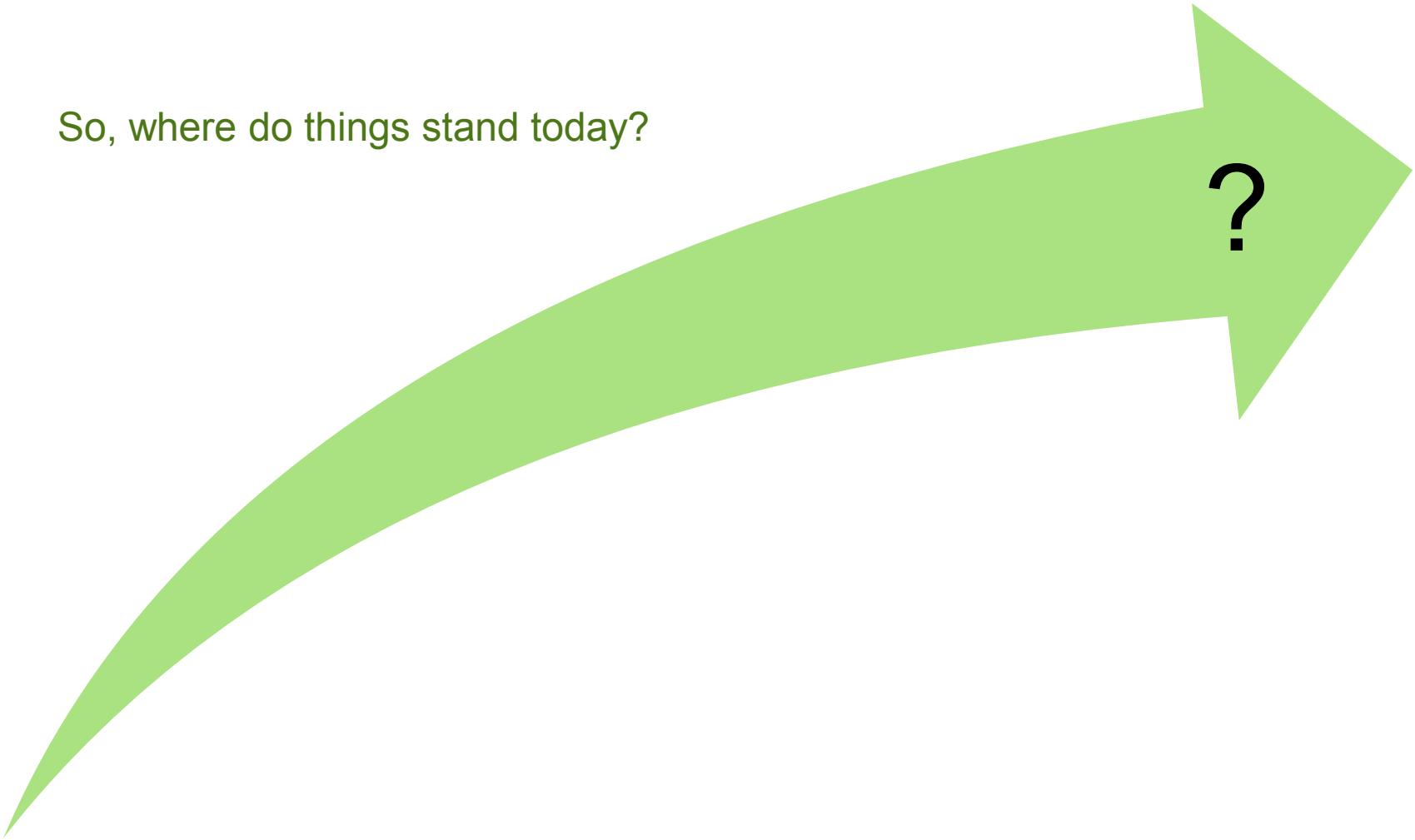
- pre-study validation
- Min. 3 P&A runs
- 15% P&A (20% at LLOQ)
- Matrix Effect, Recovery, Co-administered medications
- Dilution integrity
- Multi Lot Selectivity to include hemolyzed & lipemic
- Stability: LTS, Bench Top, Freeze-Thaw

SCIENTIFIC VALIDATION

- Single run pre-study validation when it makes sense to do so
- Consider in-study validation for certain elements (e.g. stability, dilution integrity)
- Combine stability experiments to simplify
- Wider P&A criteria may be appropriate for the end point decision (e.g. 20%/25%)

WHAT IS A TIERED APPROACH TO BIOANALYSIS?

So, where do things stand today?



AIT BIOSCIENCE APPROACH TO TIERED BIOANALYSIS

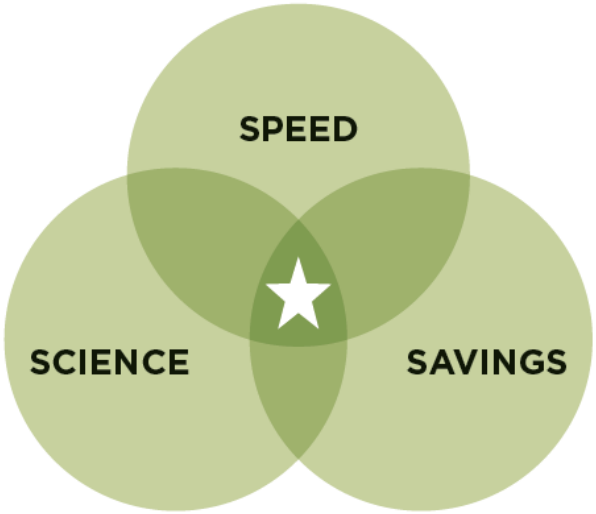
AIT BIOSCIENCE 4-LEVEL MODEL

	Price	Precision and Accuracy	Robustness Assessment
Screening	$	Good	None
Research	$$	Better	Minimal
Qualified	$$$	Best	Good
Validated	$$$$	Best	Best

AIT BIOSCIENCE APPROACH TO TIERED BIOANALYSIS

SCREENING LEVEL

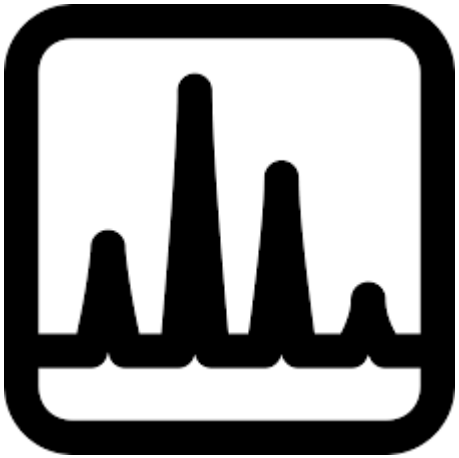
- **Speed *with* science for success**
  - **Speed**
    - **5 day turn around**
    - **3 days expedited**
    - **Quick answers:** To provide “yes/no” or “go/no-go” data for early discovery and exploratory tox
  - **Science**
    - 30% CV, 40% at LLOQ
    - Use of surrogate internal standard
  - **Savings**
    - Fit-for-purpose pricing balances budgets with intended use of data
    - 1/8th of the cost of a validated method



AIT BIOSCIENCE APPROACH TO TIERED BIOANALYSIS

RESEARCH LEVEL

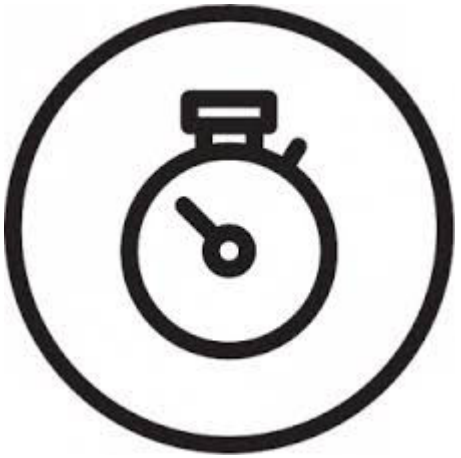
- **When you need to compare data from run to run**
  - **Uses**
    - Dose range finding
    - Formulation evaluation
    - Systemic exposure
    - Bioavailability
  - **Some robustness built in**
    - Typically 1-3 days, cursory chromatography and sample preparation method development
    - Targeted performance 20% CV/Bias (30% LLOQ)
    - Research characterization run
    - Calibrator and QC data provided in Excel spreadsheet
  - **Still a savings**
    - Method characterization ~ 25% of validation



AIT BIOSCIENCE APPROACH TO TIERED BIOANALYSIS

QUALIFIED LEVEL

- **Ready to validate when you are**
  - **What it is**
    - Meets FDA, EMEA, and CVM criteria for validation
      - Intra-run Precision and Accuracy +/-15%
        (20% at LLOQ) for small molecules
      - Intra-run Precision and Accuracy +/-20%
        (25% at LLOQ) for large molecules
      - Carry-over <20%
    - Stability samples stored but not evaluated
    - Completely documented with report
  - **What it isn't**
    - Regulated or Suitable for regulated work
    - Quick and Cheap
    - Complete
  - **When to use it**
    - For non-regulated studies when continuation to regulated studies is uncertain



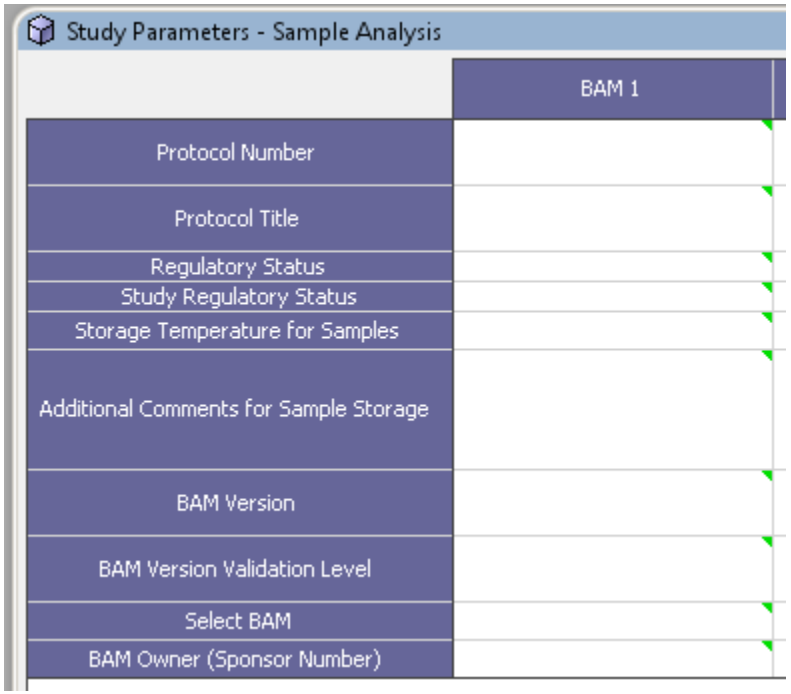
AIT BIOSCIENCE APPROACH TO TIERED BIOANALYSIS

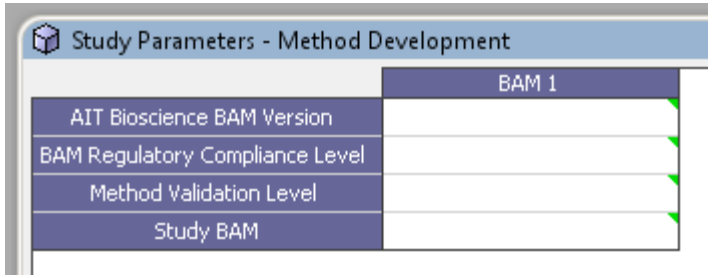
VALIDATED LEVEL

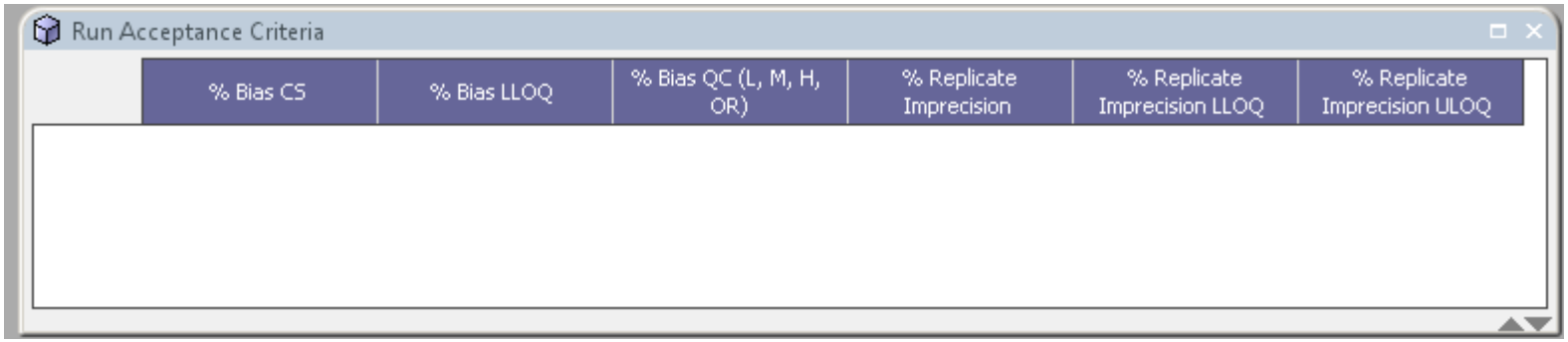
- **Meets all regulatory guidances and internal SOPs**
  - **What it is**
    - Accurate
      - At least 3 runs demonstrating acceptable precision and accuracy over at least 2 days
    - Rugged
      - Includes stability data to cover entire sample collection and analysis process
    - Complete
      - Fully documented with report
      - Audited by QA
  - **What it isn't**
    - Quick/ Cheap
    - Easy
  - **When to use it**
    - For every analyte and matrix for IND-supporting studies
    - Clinical studies

EXECUTING A TIERED APPROACH

USING THE ELN TO CONTROL A TIERED APPROACH – STUDY SET UP







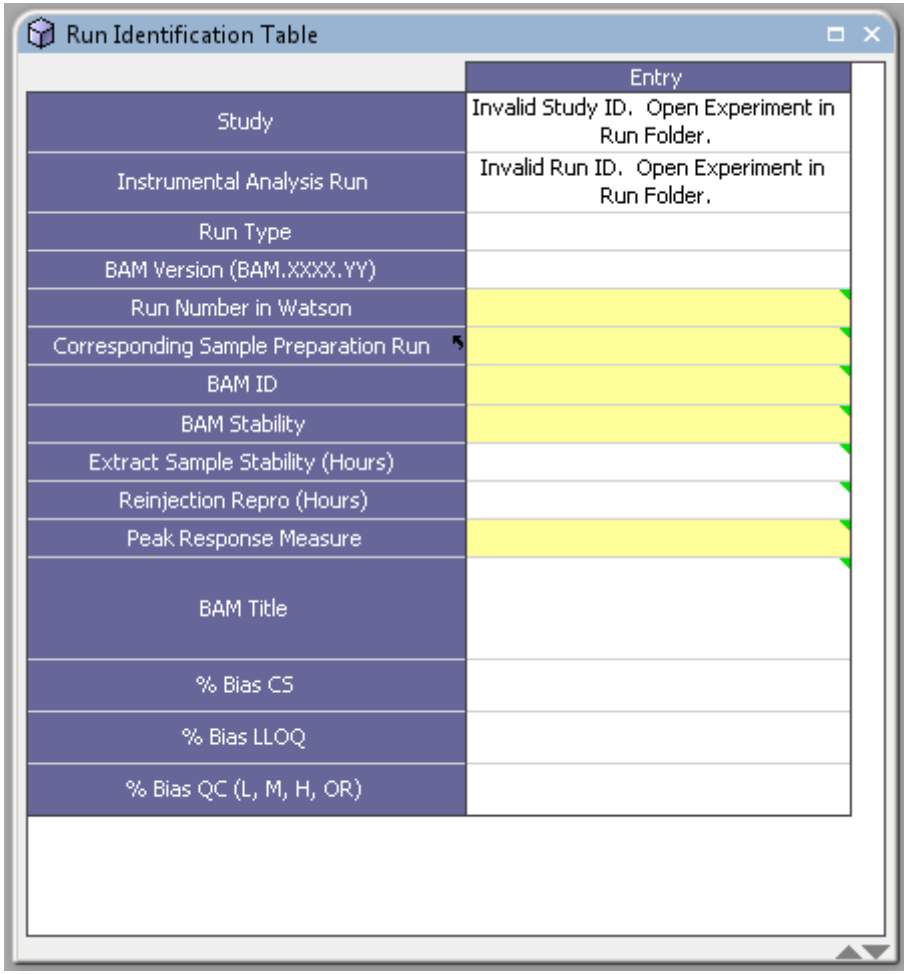
EXECUTING A TIERED APPROACH

USING THE ELN TO CONTROL A TIERED APPROACH – STUDY EXECUTION

ELN checks training status against user training records as well as regulatory status prior to any work being completed.

EXECUTING A TIERED APPROACH

USING THE ELN TO CONTROL A TIERED APPROACH – STUDY REVIEW

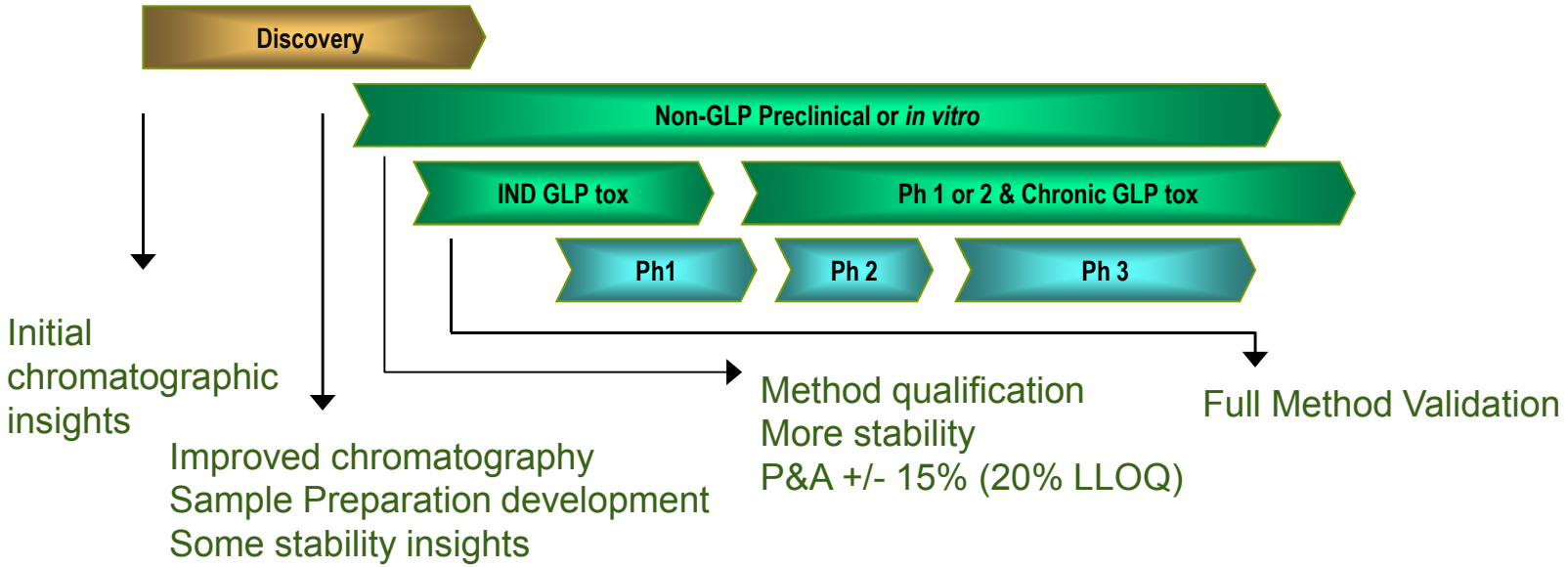


EXECUTING A TIERED APPROACH

SUCCESSES AND CHALLENGES

- Successes

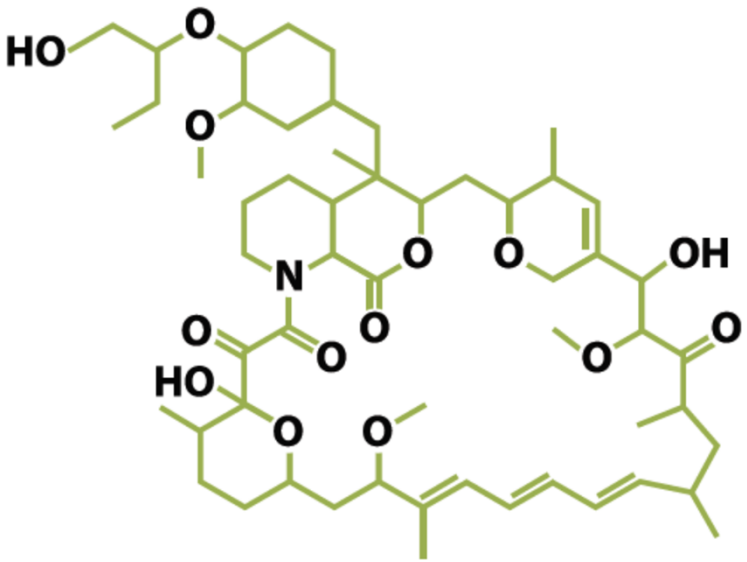
  When a sponsor is experienced and has a well planned and well-organized drug development program, AIT Bioscience travels through the process learning more about the molecule and progressively developing method understanding and robustness.



BALANCING BUDGETS AND NEEDS

CASE STUDY 1: A SCREENING LEVEL APPROACH TO EVALUATE DRUG CANDIDATES

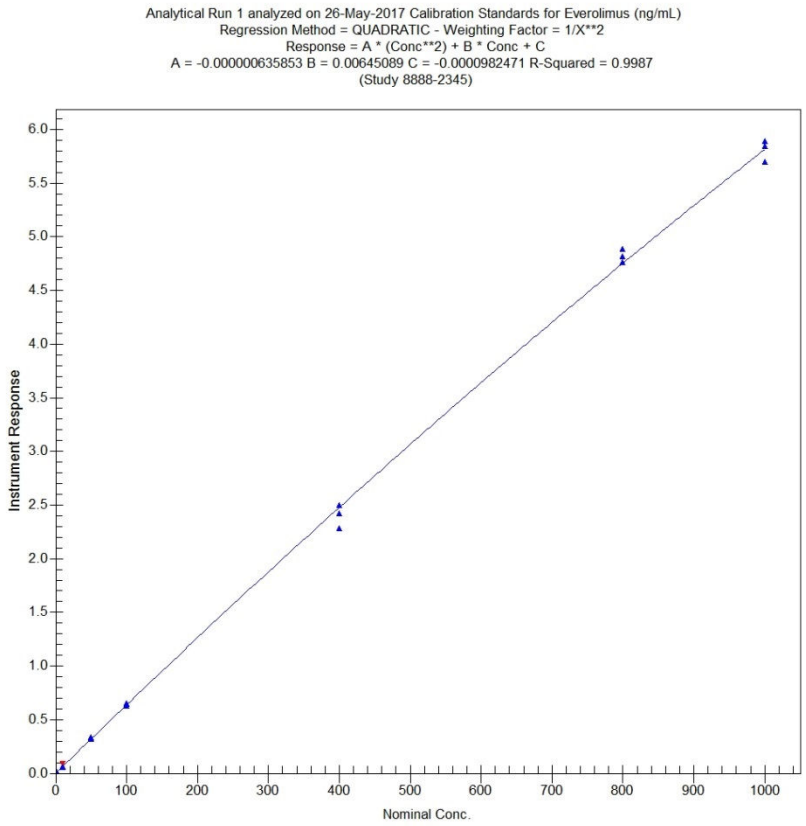
- Everolimus in human plasma



- Scientific Preview provided insights into internal standard selection and chromatography conditions
  - Column: C18
  - Mobile Phase A: Water with 0.1% Formic acid
  - Mobile Phase B: Methanol with 0.1% Formic acid
  - Internal Standard: Atorvastatin

BALANCING BUDGETS AND NEEDS

CASE STUDY 1: A SCREENING LEVEL APPROACH TO EVALUATE DRUG CANDIDATES



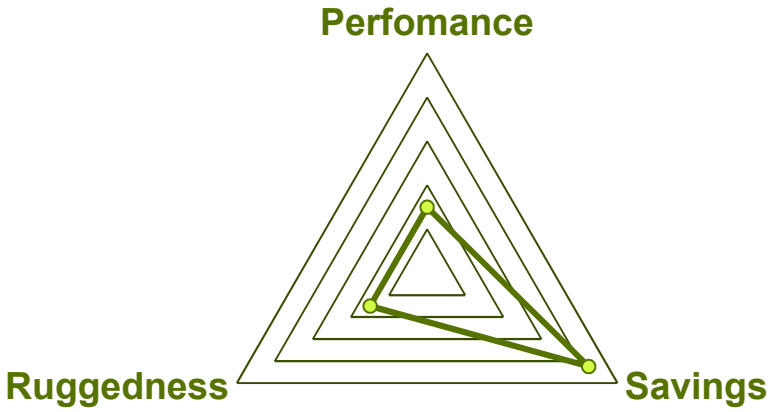
Analytical Performance of Everolimus Samples in Rat Plasma (Study 8888-2345)					
Run Date	**Curve Number**	**Sample @ 3.00 ng/mL**	**%Bias**	**Sample @ 750 ng/mL**	**%Bias**
26-May-17	1	3.16	5.3	749	-0.1
		3.07	2.3	717	-4.4
		3.1	3.3	733	-2.3
Mean		**3.11**		**733**	
S.D.		**0.0458**		**16**	
%CV		**1.5**		**2.2**	
%Theoretical		**103.7**		**97.7**	
%Bias		**3.7**		**-2.3**	
n		**3**		**3**	

BALANCING BUDGETS AND NEEDS

CASE STUDY 1: A SCREENING LEVEL APPROACH TO EVALUATE DRUG CANDIDATES

+ Ability to make an informed decision early and fast
  - Data within 5 days of sample receipt
  - Single run data with calibrators in triplicate, no QCs
+ Budget friendly
  - Single assay set-up charge (<15% the cost of validation)
  - Per sample rate for unlimited samples run in a single run

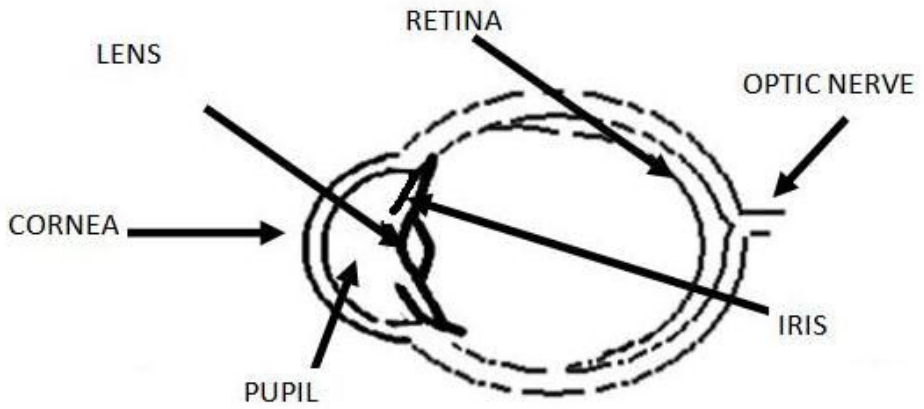
- Data is not designed to be compared between runs



BALANCING BUDGET AND NEEDS

CASE STUDY 2: A RESEARCH LEVEL SURROGATE MATRIX APPROACH TO UNDERSTANDING TISSUE PK

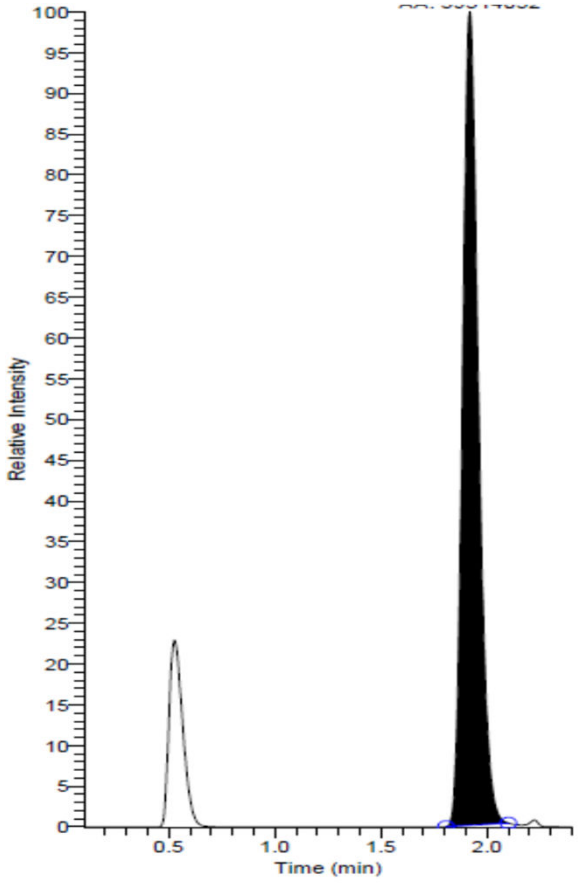
- Small molecule PK in rabbit ocular tissues



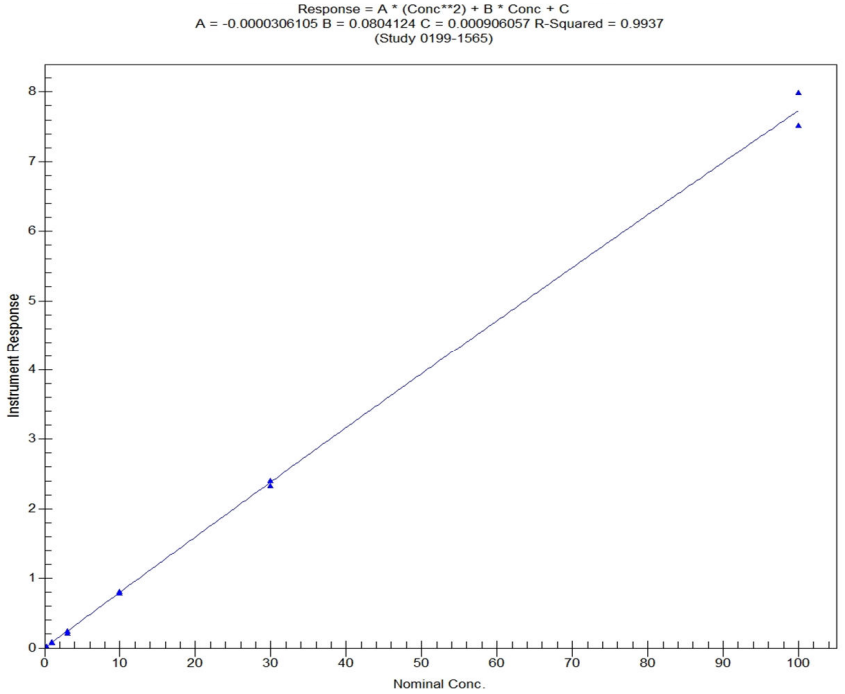
- Samples in plasma as well as ocular tissues:
  - Sclera
  - Lens
  - Cornea
  - Aqueous humor
  - Vitreous humor
  - Retina
  - Iris/ Choroid Body
  - RPE-choroid
- A single research method was developed in plasma and used as a surrogate matrix for all tissue analyses

BALANCING BUDGET AND NEEDS

CASE STUDY 2: A RESEARCH LEVEL SURROGATE MATRIX APPROACH TO UNDERSTANDING TISSUE PK



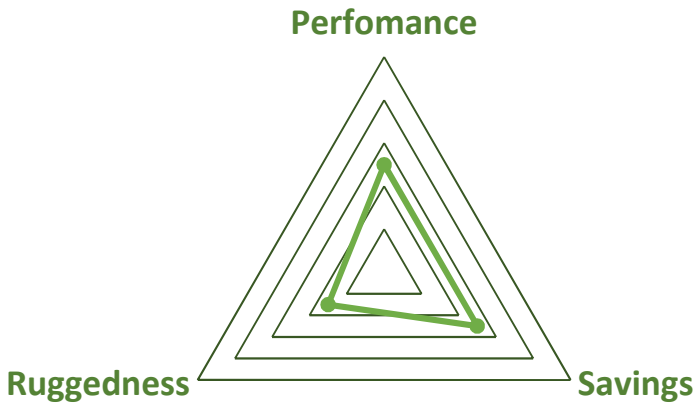
	0.300 ng/mL	3.00 ng/mL	30.0 ng/mL
Mean	0.31	3.04	30.6
% CV	9.6	4.1	4.7
% Bias	3.3	1.3	2



BALANCING BUDGET AND NEEDS

CASE STUDY 2: A RESEARCH LEVEL SURROGATE MATRIX APPROACH TO UNDERSTANDING TISSUE PK

- Performance targets of 20% (30% at LLOQ)
- Research level provided enough data to see distribution and initial PK
- 1 research method < 5% the cost of 8 validated methods



BALANCING BUDGET AND NEEDS

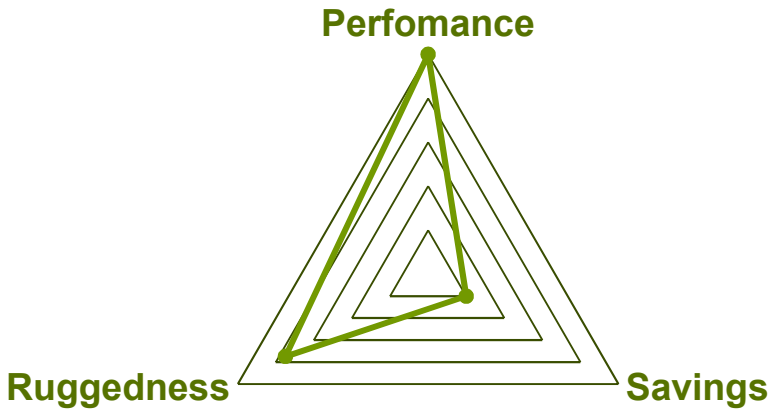
CASE STUDY 3: A QUALIFIED APPROACH TO SECONDARY MATRIX ANALYSIS IN A GLP-TOX PROGRAM

- Oral anti-cancer agent currently in Phase 2/3 clinical trials
- Initial Method Validation
  - Human K_2EDTA plasma
  - Full regulatory validation to support Phase 1 clinical trials
  - Completed in Early 2013
- May 2014 sponsor now requires additional PK data in human urine
  - Use a Fit-for-Purpose approach ("Qualified Method")
  - Meets precision and accuracy standards of a fully validated method
  - Does not include all of the additional experiments of a fully validated method (matrix effect, recovery, etc)

BALANCING BUDGET AND NEEDS

CASE STUDY 3: A QUALIFIED APPROACH TO SECONDARY MATRIX ANALYSIS IN A GLP-TOX PROGRAM

- Method that meets regulatory criteria
- More expensive than a research method, but less costly than full validation
- Also used for secondary and rare matrices
- Also used for metabolites being quantitated in a separate assay



EXECUTING A TIERED APPROACH

- Challenges
  - Subjective inclusion/exclusion criteria for some tests
  - Scope Creep
  - Redundant instead of Progressive
  - Overly cautious approaches
  - Robustness level of method not consistent with sample analysis needs at that level
  - Determining the level of formality and review that is appropriate at each level

A TIERED APPROACH TO BIOANALYSIS

THOUGHTS AND DISCUSSION

- What approaches are others using?
- Are you seeing similar challenges?
- Are you seeing successes?

THANK YOU

Acknowledgements:
- AIT Bioscience MD group
- AIT Bioscience Leadership Team
- GBC A2 Team
- EBF Team