Team A2 :
Tiered Approaches to Method Validation

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on behalf of GBC A2

Why are Tiered Approaches Deemed Necessary?

Bioanalysis has evolved into

"Bioequivalence Bioanalysis":

Why are Tiered Approaches Deemed Necessary?

- In the interest of avoiding any regulatory critique the bioanalytical community has deferred to the gold standard : methods supporting Bioequivalence studies …. for everything that will be/could be submitted to a regulatory agency
- Pathological fear of the FDA form 483
- Existing BMV Guidance is all we have to “harmonize” around
- Impact on CROs has been particularly severe – which in turn has influenced whole community
- Leads to many circumstances (e.g. early drug development) where there is poor return on investment in the development, validation and use of a bioanalytical method

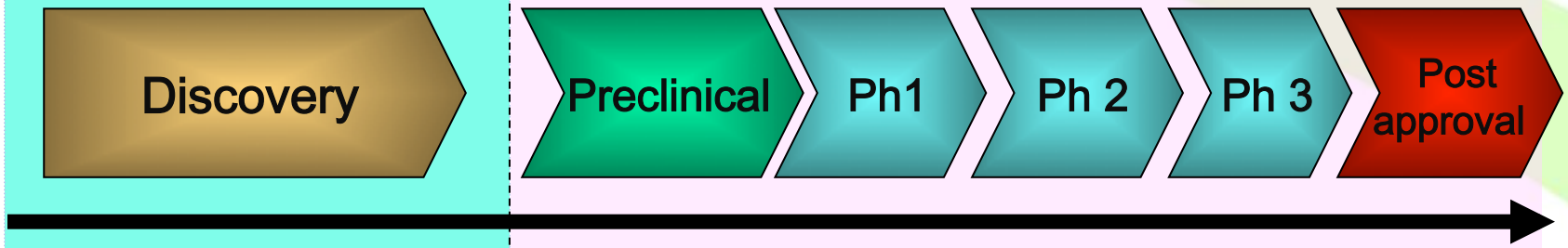
Why are Tiered Approaches Deemed Necessary?

- To provide a framework that will help rationalize level of bioanalytical method characterization
- To accommodate the practicalities now encountered in a modern bioanalytical laboratory – take the insights of 'tiered approach' beyond the metabolites discussion (CC-III):
  - Tissue analysis
  - Biomolecule LC/MS
  - Microsampling techniques
  - in vitro bioanalytical challenges
  - Novel technologies
  - Etc..
- To deliver data that returns value while still meeting/exceeding the required standards of quality appropriate for the intended use

Slides adapted from EBF presentation at AAPS Nov. 2009, courtesy of EBF

Non GLP

Clinical, GLP and non GLP



Screening methods

or

Qualified Methods

Validated Methods

Rodent and non Rodent plasma (unchanged drug)
Other matrices as appropriate
Metabolites as appropriate?

Human plasma
Other matrices as appropriate
Metabolites as appropriate?

Additional preclinical species
Other matrices as appropriate
Metabolites as appropriate?

Qualified Methods

- Samples from:
  - *In vitro* (e.g. PPB),
  - some mechanistic PK, PK/PD
  - most non standard matrices (e.g. tissues)
- Quantification of metabolites

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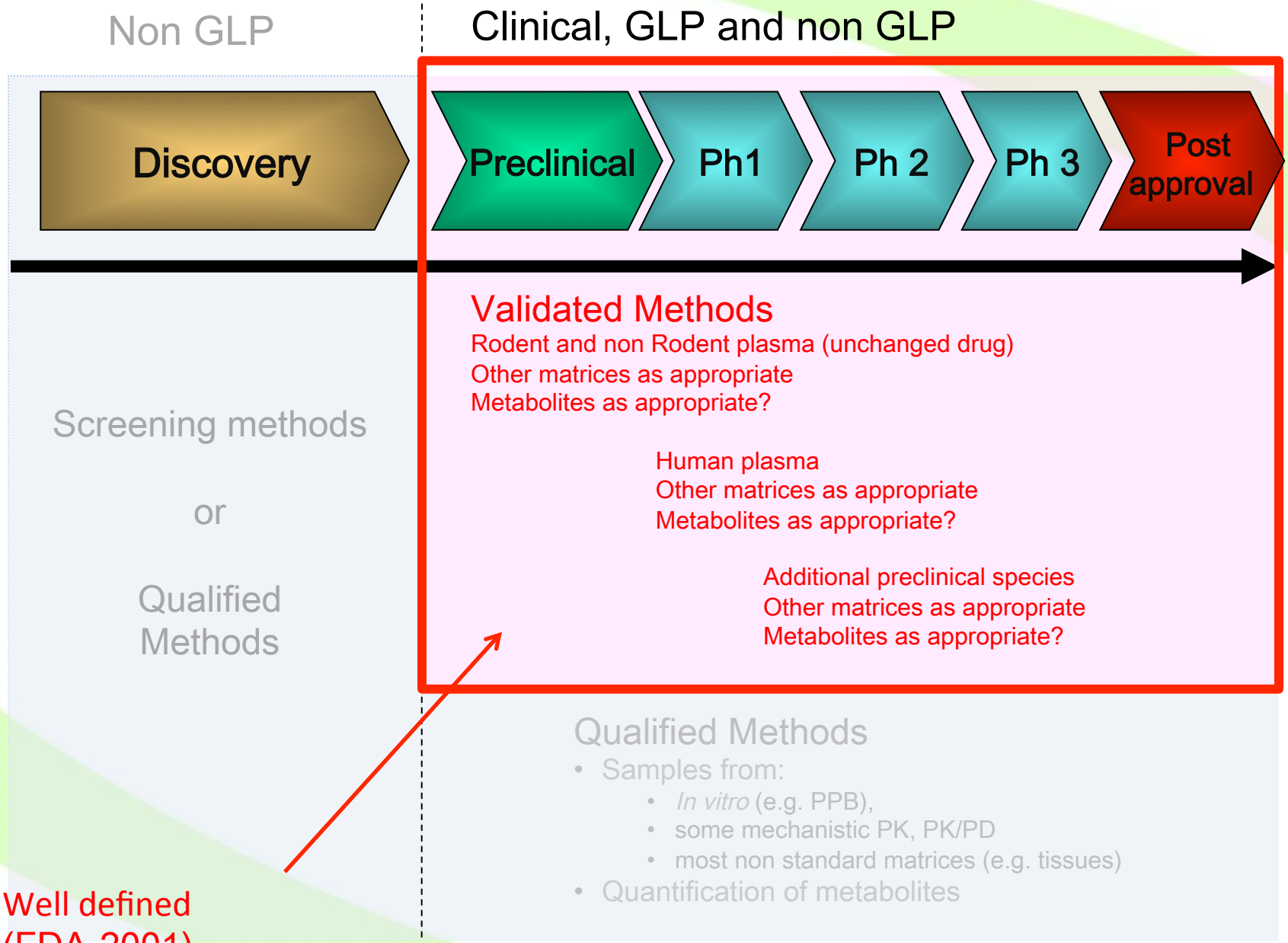

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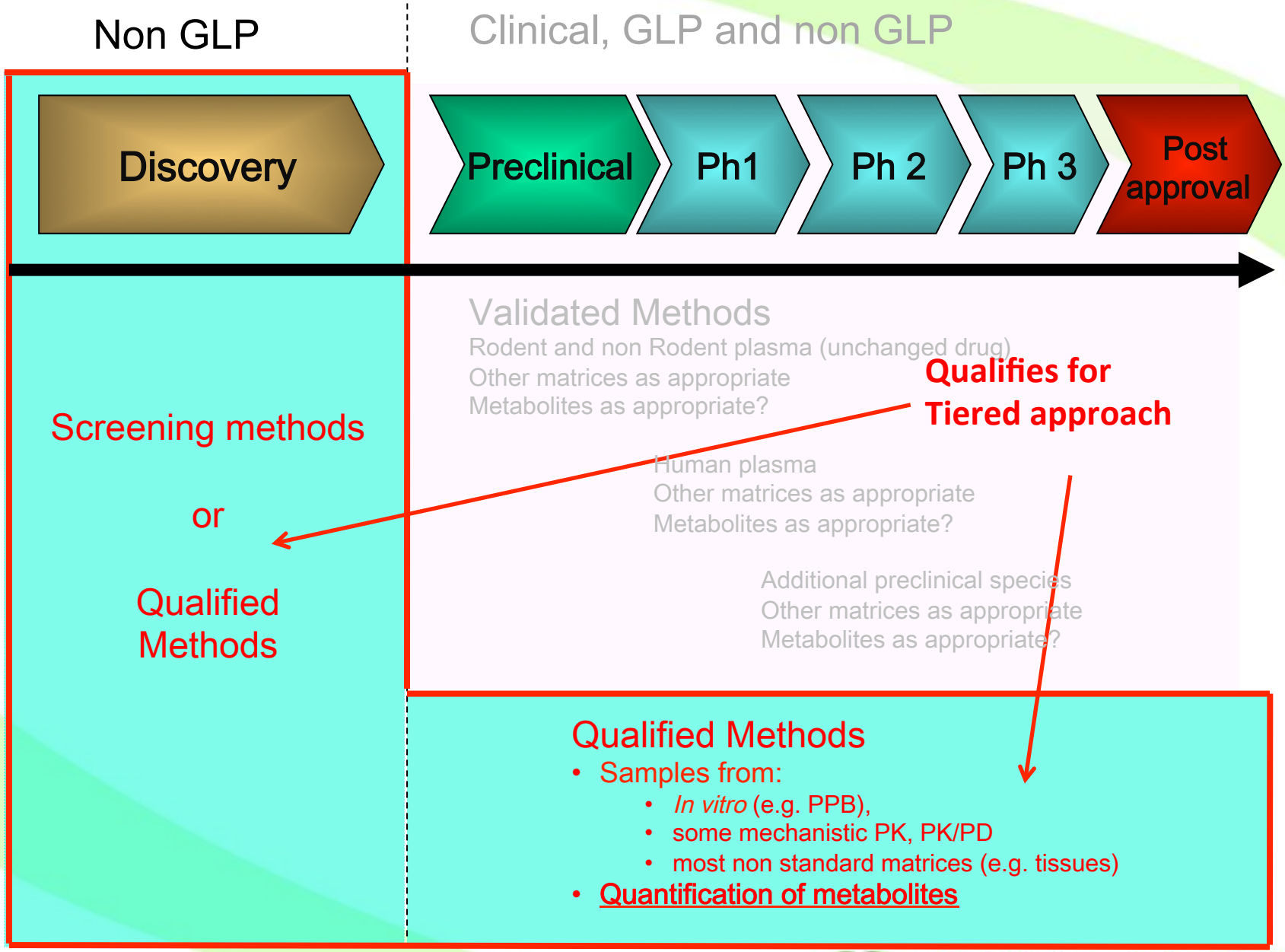

Glossary of Terms Used

Tiered Method Characterization:

- Different levels of method characterization depending on the intended objective of a preclinical or clinical study for which a method is to be used
- Embraces a fit-for-purpose (FFP) principle and envisions adding more tiers to the currently accepted tier of validated methods
- All tiers are developed from the beginning and each undergo limited characterization
- All proposed tiers should be an integral part of regulated bioanalysis, as appropriate.

Glossary of Terms Used

Validated method (Full/Partial/Cross):
Undergoes characterization as defined in regulatory guidances and provides absolute analyte concentration.

Proposed additional tiers:

Qualified method: Undergoes limited characterization with calibration standards and QC samples prepared using an authentic reference standard. The method provides absolute analyte concentration. The process of method characterization is called **method qualification** (not partial validation, or limited validation).

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Glossary of Terms Used

Research method:

Undergoes limited characterization with calibration standards prepared using a comparator reference standard, such as an in-situ (in-solution) standard with concentration estimated by radioactivity, NMR, or UV. The method provides estimated analyte concentration (for internal use).

Screening method:

Undergoes limited characterization based on relative instrument analyte response, where a reference standard is not available. The method provides relative analyte measurement.

When Use The Appropriate Tier

- Objective is to generate a road map that helps guide the decision making process of which tier to use and when.
- Guiding questions are:
  - How much risk is acceptable?
  - What limitations are you bound by?
  - What makes scientific sense under the drug development phase being addressed?
  - Understanding the established Tiers, do the principles of fit-for-purpose hold for the needed assay?

To this end the following decision diagrams are under construction and review:

Tiered Method Performance Parameters

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

Pre-Study Method Performance Assessment: Establishing performance of a new method, re-establishing an existing method, qualifying a new analyst, etc prior to study sample analysis

COA: A reference standard document that provides the source, identity, purity, storage conditions, expiration date and batch number of the reference standard.

FFP = fit for purpose

Tiered Method Performance Parameters

Parameters	Screening	Research	Qualified	Validated
Inter and intra assay accuracy & precision (CV)	Intra assay precision	Intra assay accuracy/precision (one-run)		Both, as per regulatory guidance and SOP
Selectivity from the biological matrix	Yes, using one matrix batch blank (depending on the use of the method)	Yes; obtained from one matrix batch blank by in-study run		Yes, as per regulatory guidance and SOP
Extraction recovery	Not applicable	No		Yes, as per regulatory guidance and SOP
Internal standard (IS)	Not applicable	Preferable		Yes, as per regulatory guidance and SOP
Carry-over	Run samples in the order of sample collection time points. Include in each run a blank following a high conc sample			Yes, as per regulatory guidance and SOP
Matrix-effect	Demonstrate absence of differential matrix effect between species of interest	If IS is used, check IS response across runs		Yes, as per regulatory guidance and SOP
Incurred sample bench-top evaluation	Reanalyze in the same run a couple of study samples (incurred samples) after ambient bench-top storage	Conduct incurred sample ambient bench-top evaluation In addition to spiked sample bench-top stability		Yes, as per regulatory guidance and SOP
F/T stability in the biological matrix	No, except for structural alert or historic data from similar cmpds.			Yes, as per regulatory guidance and SOP
Long term stability in the biological matrix	Not applicable	Not required if bench-top stability is proven	Not required if bench-top stability in matrix is proven unless long storage is intended	Yes, as per regulatory guidance and SOP

ISS = Incurred Sample Stability

Tiered Method Performance Parameters

Parameters	Screening	Research	Qualified	Validated
Processed sample stability	Not required except for structural alert	Not required except for structural alert; rely on bracketing calibration stds in each run		Yes, as per regulatory guidance and SOP
Stability in blood	Not applicable	Not required unless unstable in plasma or structural alert		As per regulatory guidance and SOP
Stock/working solution stability	Not applicable	FFP assessment		As per regulatory guidance and SOP
Accurate recording of raw data	Yes			As per regulatory guidance and SOP
QC checking raw data prior to reporting	Yes			As per regulatory guidance and SOP
Method description/report	Description in lab notebook or report			As per regulatory guidance and SOP
Method performance description or report	Description in lab notebook or report			As per regulatory guidance and SOP
Bioanalytical study sample analysis report	Description in lab notebook or report			As per regulatory guidance and SOP

FFP = fit for purpose

How to read this grid?

The grid can be read in different ways.

GBC is not proposing to become prescriptive.

In our discussions, we approached grid from 2 direction :

1. Prior to method establishment, you can define the tier needed to run the samples from your study, and establish the method accordingly
   - Can be applicable for 'standard studies' not needing validation but currently often analyzed using an inappropriate (i.e. too high) level of validation (e.g. metabolites assays in early development, urine analysis,…).
   - By predefining the tier needed, GBC intends to provide guidance to the bioanalytical community on choosing the right tier.

How to read this grid?

2. Nevertheless, often we might not be able to define the tier needed for our samples upfront. But, after method establishment, we can still assess (and if needed report back on) the tier to which our established method (at least) complies.

Some initial reflections

In both scenarios, science is driving our decisions:

- In scenario 1, the scientific needs were assessed as part of the type of study and the stage of development by previous investigator in other development programs, or by consensus by the bioanalytical community. The scientific needs were translated in the tier the community wants to propose for this kind of assays

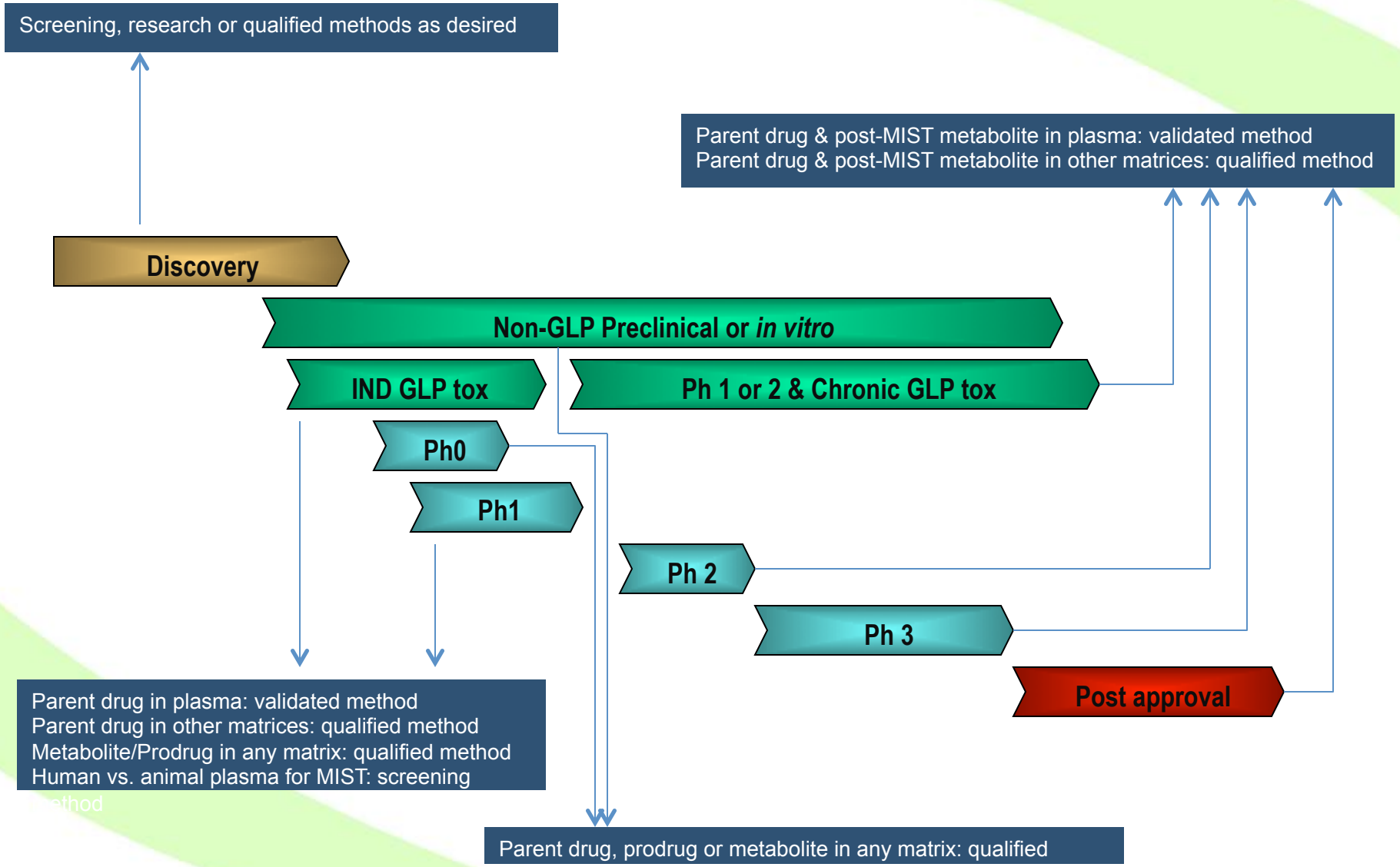
Some initial reflections

- In scenario 2, the scientist was unbiased towards the tier needed, but focussed on the science needed as part of his/her initial method establishment process. However, after his method establishment he/she can still assess the tier that the method complies to. This may be needed for internal communication or external acknowledgement of the work performed

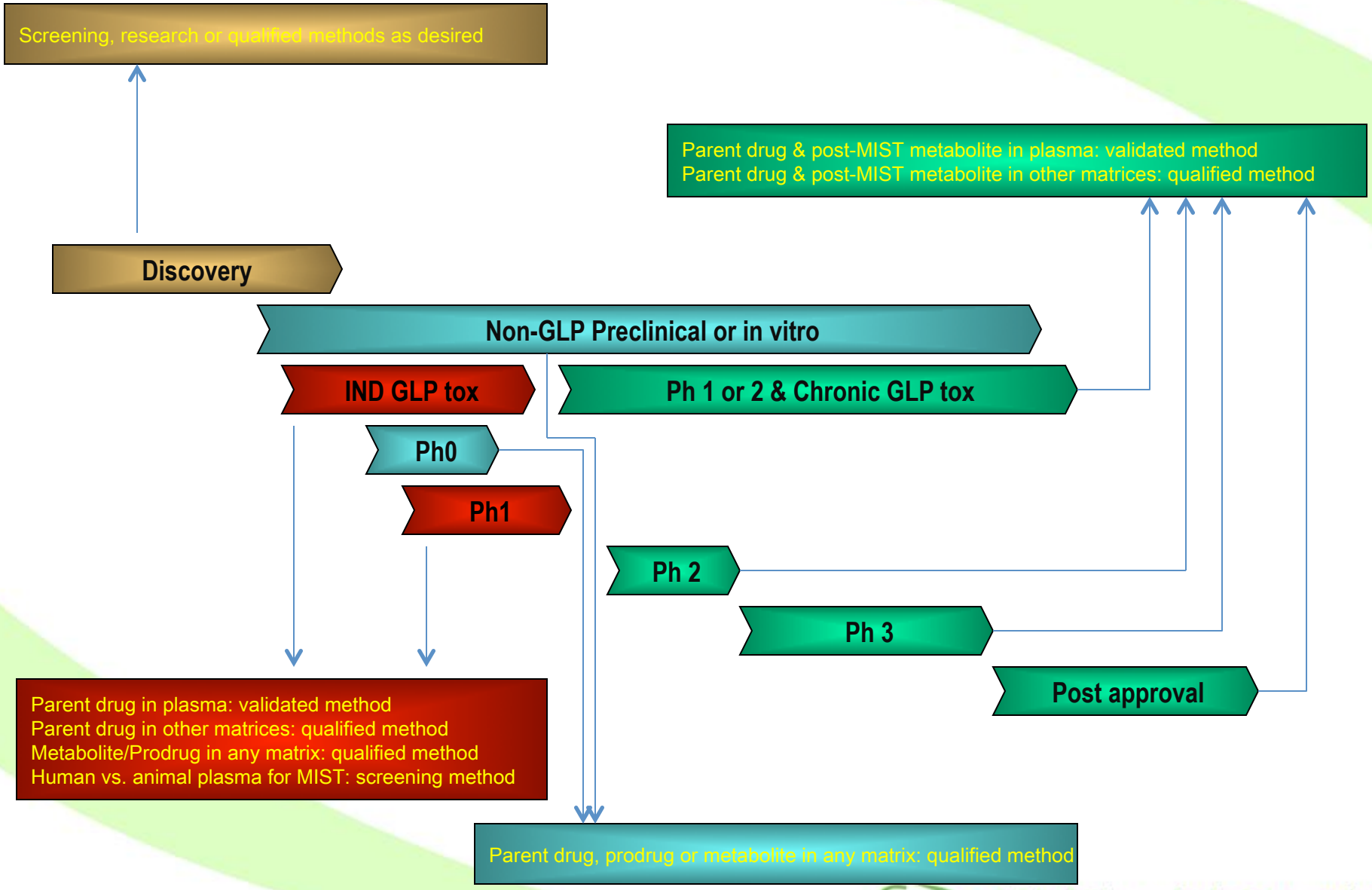
Also, the grid is not proposing a tick box mentality or trying to regulate what doesn't need to be regulated. We propose to read the different tiers as follow:

- If a method **at least** complies with the specifications of a certain tier, the method can claim that label. In order to be able to claim the label of the next tier, again, the assay has to comply with all the specifications from that tier.
- That approach allows the shades of grey needed in our day to day work and at the same time enables us to communicate about (or as needed to report back on) your method.

When to Use A Given Tier (pictorial presentation) – per Drug Development Phase



When to Use A Given Tier (pictorial presentation) – per Quality Association



Conclusions

- Still ‘work in progress’
- Team is comfortable presenting a stance on why tiered approaches are required
- We are confident in defining the four tiers of Screening, Research, Qualified and Validated
- Need further focus on fine-tuning the definitions of the parameters and characteristics of a bioanalytical method and fitting these into the tier categories
- Team is reaching a consensus on correlating the tiers to the bioanalytical need (i.e. when to use which tier)

References

1. P. Timmerman, M.A. Kall, B. Gordon, S Laakso, A. Freisleben, R. Hucker, *Best practices in a tiered approach to metabolite quantification: views and recommendations of the European Bioanalysis Forum*, Bioanalysis 2(7), 1185-1194 (2010)

2. B. Booth, *When do you need a validated assay?,* Bioanalysis 3(24), 2729-2730 (2011

3. Others to add

Acknowledgments

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