CRITICAL REAGENTS IN BIOASSAY

THEIR IMPORTANCE, CHARACTERIZATION AND STORAGE

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What are Critical reagents and why are they important

- Reagents that can curtail or alter the performance of assays
- Difficult to make, replace, acquire or substitute
- They can be any reagent identified by the user as critical to the life cycle of the assay.
- Critical reagents geared towards ligand Binding Assays
- Eg: Abs, special reagents like FBS, special buffers etc
- Generation of lots and materials that are also stable over the life cycle of the assay.

Critical reagents generation.
Points to consider

- L4 Global Harmonization Team
- US FDA and the EMA
- Critical reagents documentation
- In-house produced critical regent versus commercial source reagents
- Changing critical reagents
- Stability and storage of the critical reagents

Critical reagents documentation

- SOPs, documented analytical procedures, work instructions
- Reproducibility and consistency
- Types of critical information needed for documentation
- Characterization and qualification
- Handling and storage
- Side by side comparison or other appropriate documented procell to switch to another lot
- Pre-defined acceptance criteria for switch to another lot

In-house produced critical reagents versus commercially sourced reagents.

- Availability of reagents throughout the life cycle of the assay.
- Sufficient quantities
- Cost determines direction

Inhouse produced reagents summarized[1]

	Pros	Cons
Availability and cost-effectiveness	• Custom in house reagents can be tailored to suit the assay design. • Independence from commercial sources	• Needs special planning and infrastructure for in-house reagent production • In-house production of reagents (e.g., recombinant proteins/antibodies) requires significant investment in personnel (expertise) and capital • Cost-effectiveness and availability of in-house resources could be a major challenge
Characterization	• Reagents can be extensively characterized for the intended use, and certificate of analysis can be customized. • Lot-to-lot variability (especially in protein labeling) can be monitored and controlled. • Stability data can be generated in-house.	• Reagent characterization can be time-consuming and expensive. Time is of essence in most developmental projects. • In-house expertise may be limited.
Ease of use	• Better control on life cycle of reagents. • Availability of a sufficient amount of the reagents can be exerted by better	

Commercially produced reagents summarized[1]

	Pros	Cons
Availability and cost-effectiveness	Readily available off-the-shelf reagents from a variety of vendors	• Availability of a particular reagent may be discontinued without prior notice • Changes to quality attributes without upfront notification might impact assay performance
	Custom reagent production is not limited to production of critical reagents (e.g., recombinant proteins, antibody, and antibody conjugates) in-house. These reagents can be produced by commercial companies according to customer needs	Lot-to-lot variability. Lot changes may meet vendor release specifications but may be a significant risk to consumer
	Specific reagents (e.g., recombinant proteins, antibody, and antibody conjugates) can be custom prepared	Lot change may reflect both conjugation (replace by “final product”?) and starting materials
	Relatively inexpensive	Custom reagents are generally

Commercial produced reagents cont.[1]

	Pros	Cons
Characterization	Generally characterized for intended use, and certificate of analysis is provided	Certificate of analysis may not have all the characteristics that one would like to see Potential lot to lot variability requires monitoring
	Specific reagents can be characterized as per customer specification	Stability data are generally missing. It should be done in-house
Ease of use	A wide variety of conjugated proteins can be obtained and tested for superior performance in the assay	May require considerable efforts to select a reliable reagent and a reliable vendor
	Calibrators available in preweighed quantities	Concentration assignments for preweighed calibrators vary widely varied, especially if the concentration is expressed in international units
	Premade buffers and assay diluents tested to work with critical	Proprietary composition of specialty buffers (e.g., wash buffer)

Changing critical reagents

- When and why.
- FDA guidelines for changing critical reagents
- Major or Minor changes.
- Requalification, optimization of assays
- Side by side studies
- 3 plate studies in parallel or over a period (you will have to explain that bullet point)

Stability and storage of critical reagents

- Conditions guaranteeing the stability of the reagents
- Various conditions provide varying degrees of stability
- Room temp. vs refrigerated
- -20 °C vs – 80 °C
- Arrhenius equation; $k = Ae^{\frac{-E_a}{RT}}$
- K is the rate constant, A is the pre-exponential factor, Ea is the activation energy, R is the universal gas constant and T is the absolute temperature measured in Kelvin. (you will have to explain that bullet point)

Stability and storage of critical reagents

- Expiry date versus Retest date
- Scientific documentation over a period of time
- Systematic evaluation during the life cycle of the reagent
- Freeze thaw cycles, storage times and temperatures
- Something as simple as aliquotting
- Extensive controls and oversight on their storage.
- Useful tools: DoE, control charts

References

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