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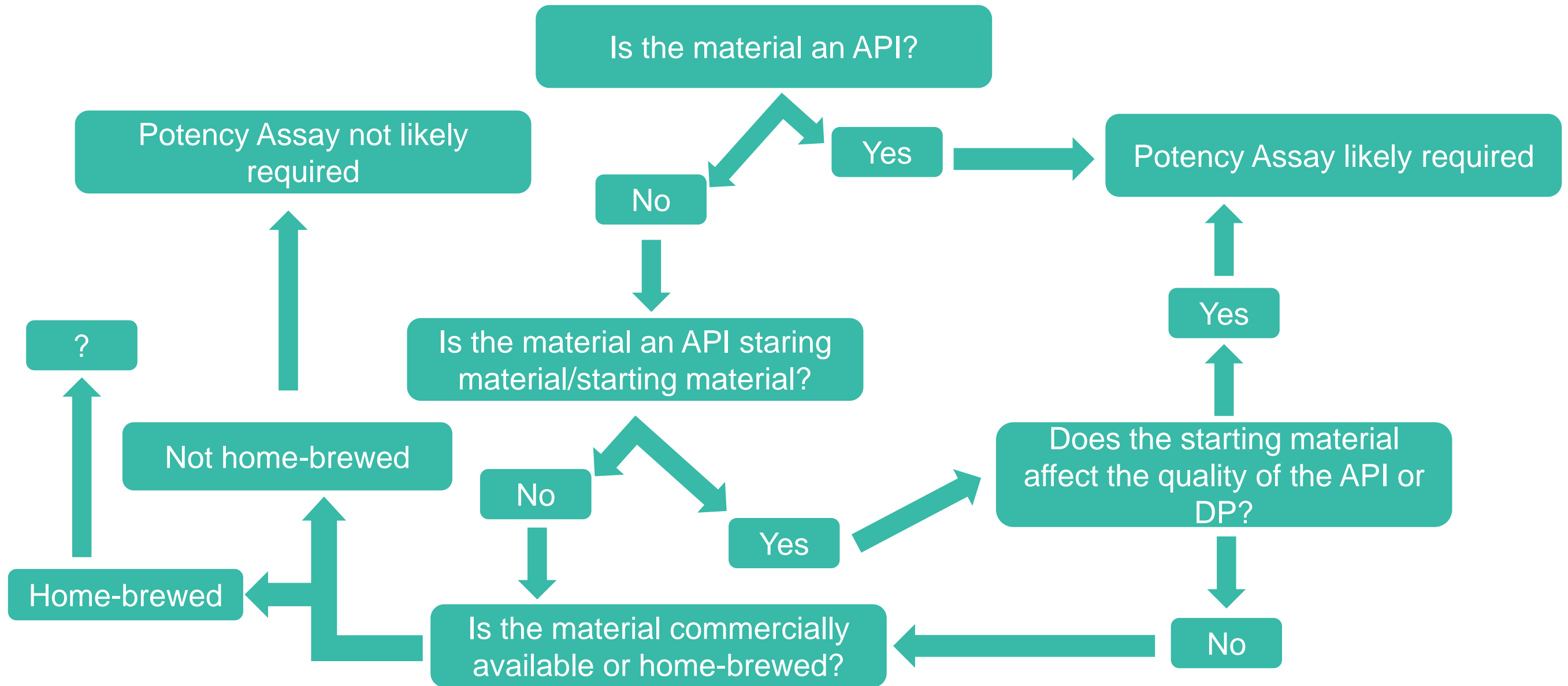
Terminology: How are critical materials defined?

▸ 21CFR: Component (Critical raw material) is any ingredient intended for use in the manufacture of a drug product, including those that may not appear in such drug product.

Definition	Terminology	
	US	EU
Materials used in the manufacturing process that will **not** remain in the active substance	Ancillary materials	Critical raw materials
Materials used in the manufacturing process that will remain in active substance	API starting material/starting material	Starting materials
All materials used in the manufacture of drug product	Critical raw materials/components	
Substance when used in the production of a drug, becomes an active ingredient in the drug product	Active Pharmaceutical Ingredient	Active Pharmaceutical Ingredient

▸ How to apply this terminology is not always clear in C&GT space
  – The same material can be classified as API or starting material depending on the therapy
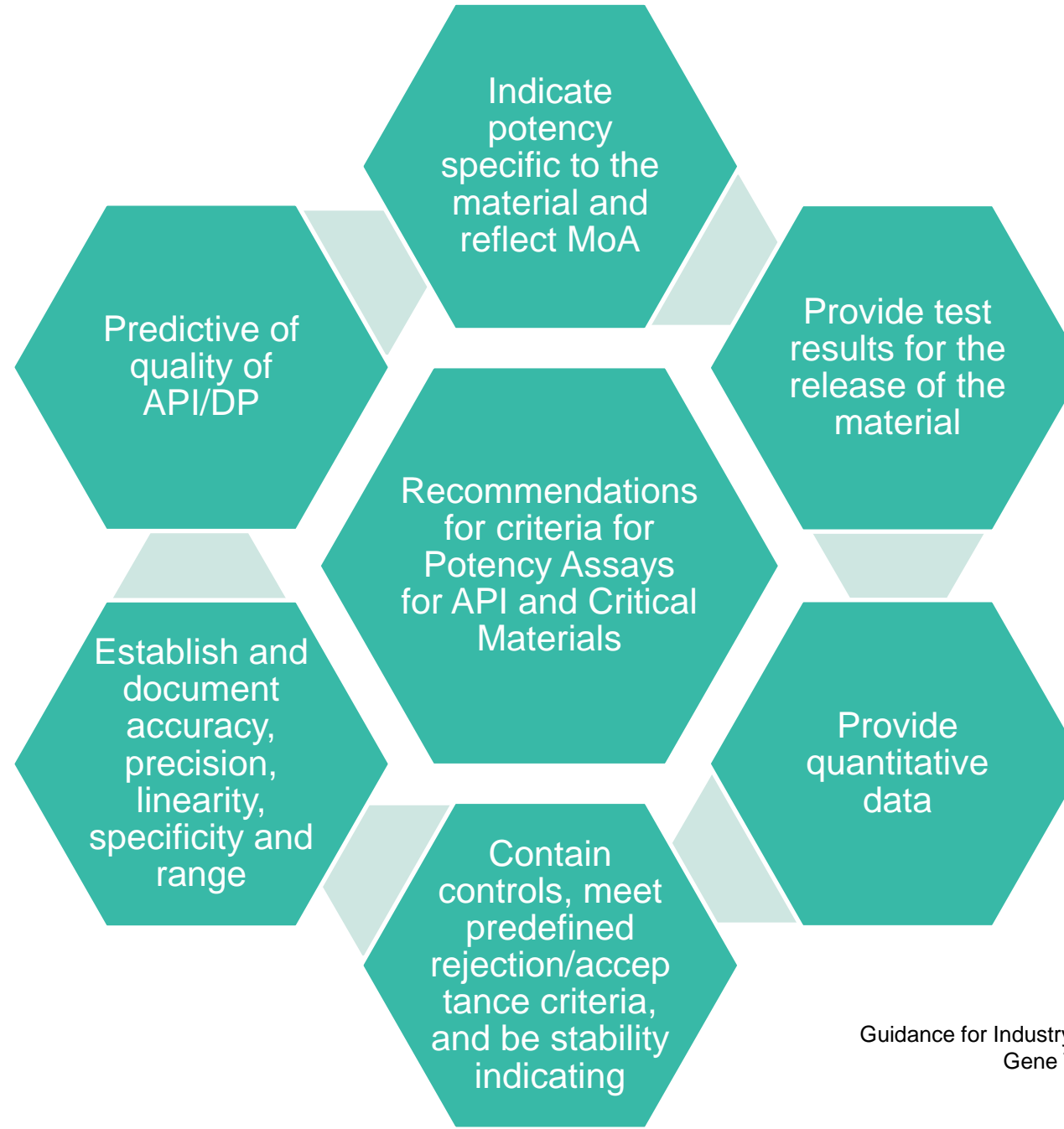▸ Different levels of compliance are required depending on how materials are categorized

Is a potency assay required for critical materials?

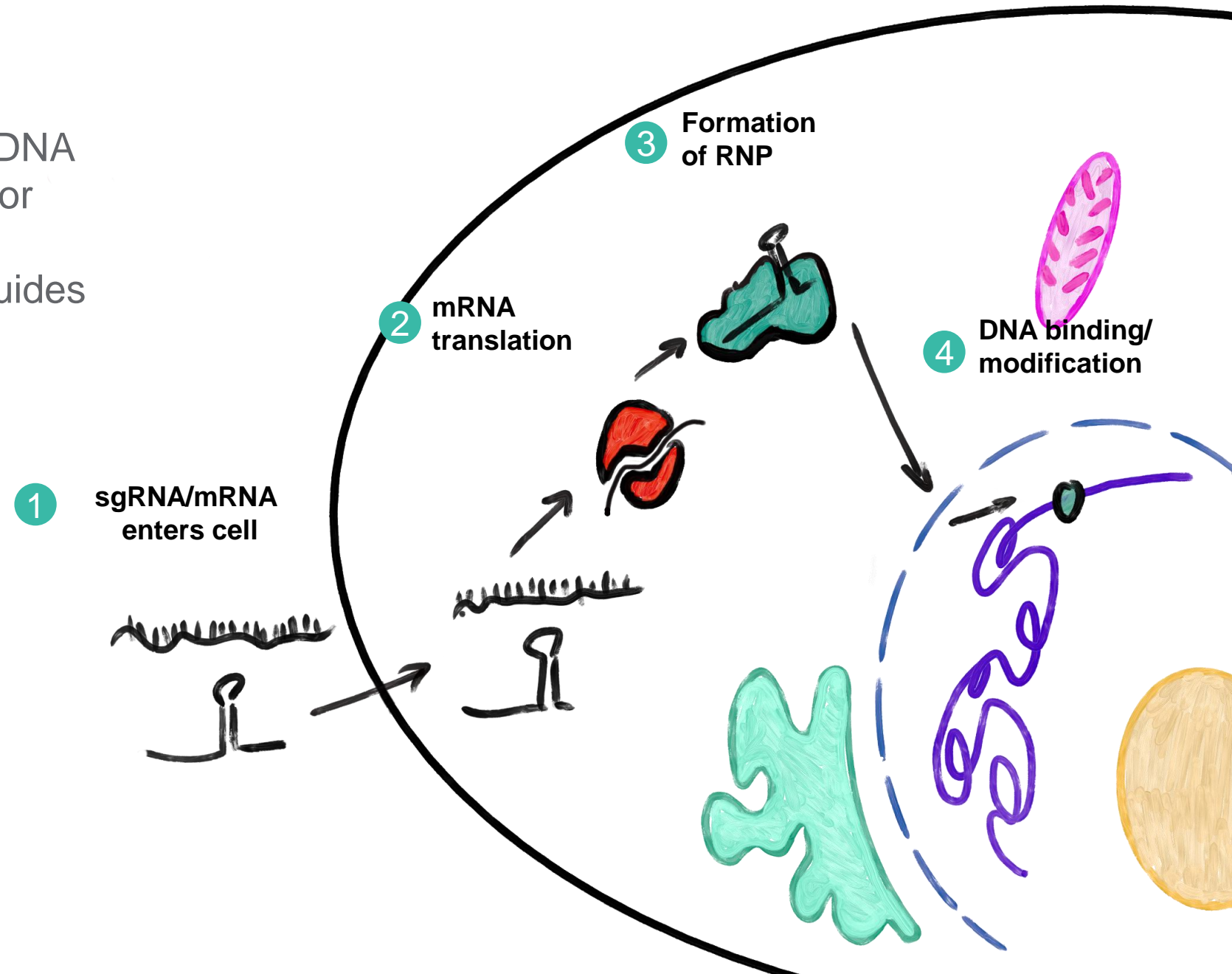# Language around potency and how we apply it to critical materials?

▸ By definition, much of the language around potency is linked to the DP

▸ Potency is defined as "the specific ability of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result (21CFR 600.3)

▸ How do we apply the definition of potency to API or materials?
  – Propose applying similar definition to these materials as we do to DP
  – "the specific ability of the ~~product~~ **material**, as indicated by appropriate laboratory tests obtained through the administration of the ~~product~~ **material** in the manner intended, to effect a given result
  – A quantitative measurement of biological activity

▸ Similar guidelines for potency assay development as with DP assays (with some caveats)

Suggestions for Developing/Optimizing Analytical Cell-Based Potency Assays for C&GT Products

- Indicate potency specific to the material and reflect MoA
- Predictive of quality of API/DP
- Provide test results for the release of the material
- Recommendations for criteria for Potency Assays for API and Critical Materials
- Establish and document accuracy, precision, linearity, specificity and range
- Provide quantitative data
- Contain controls, meet predefined rejection/acceptance criteria, and be stability indicating

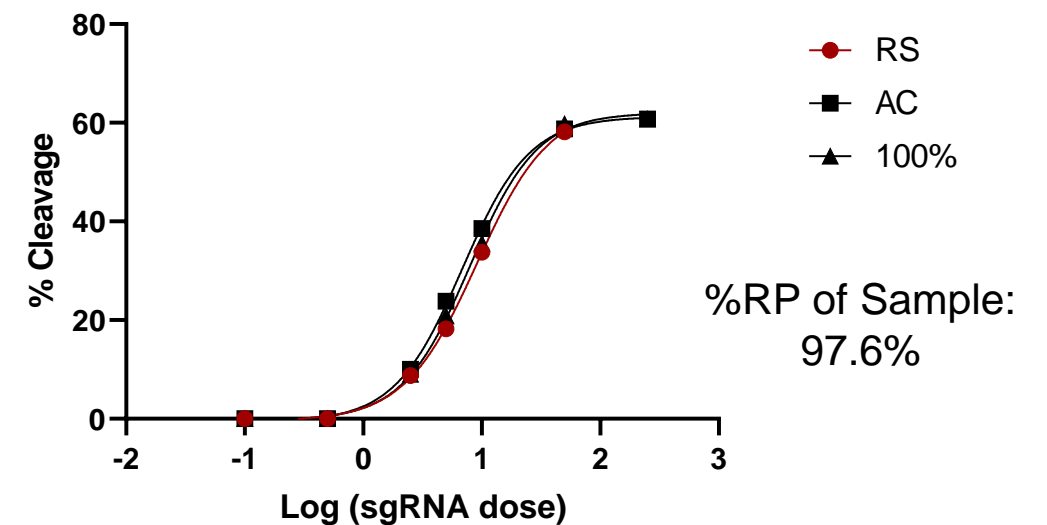Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products
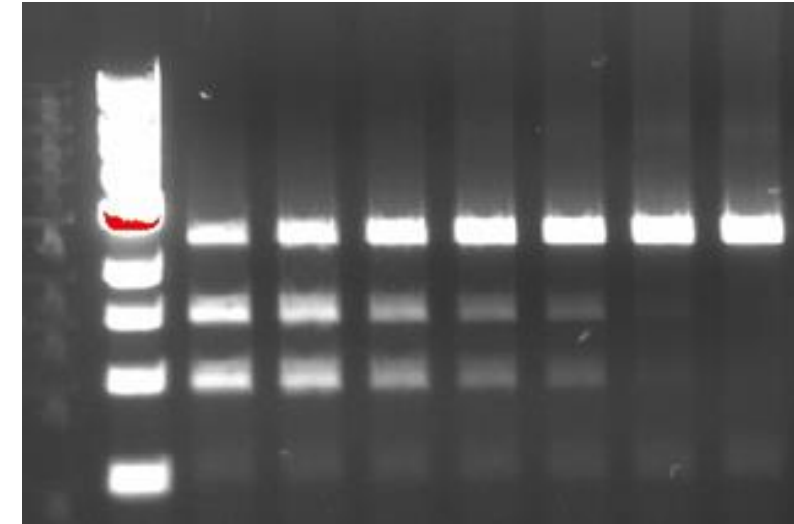
Example of a Potency Assay for gRNA Critical Material

- gRNA is an RNA sequence that recognizes a DNA target region and directs RNP complex there for editing
- Binds to form an RNP complex with BE and guides RNP to correct location within the genome
- It can be considered an API or an API starting material depending on therapeutic
- Therefore, a potency assay is needed for this material

3 Formation of RNP

2 mRNA translation

4 DNA binding/ modification
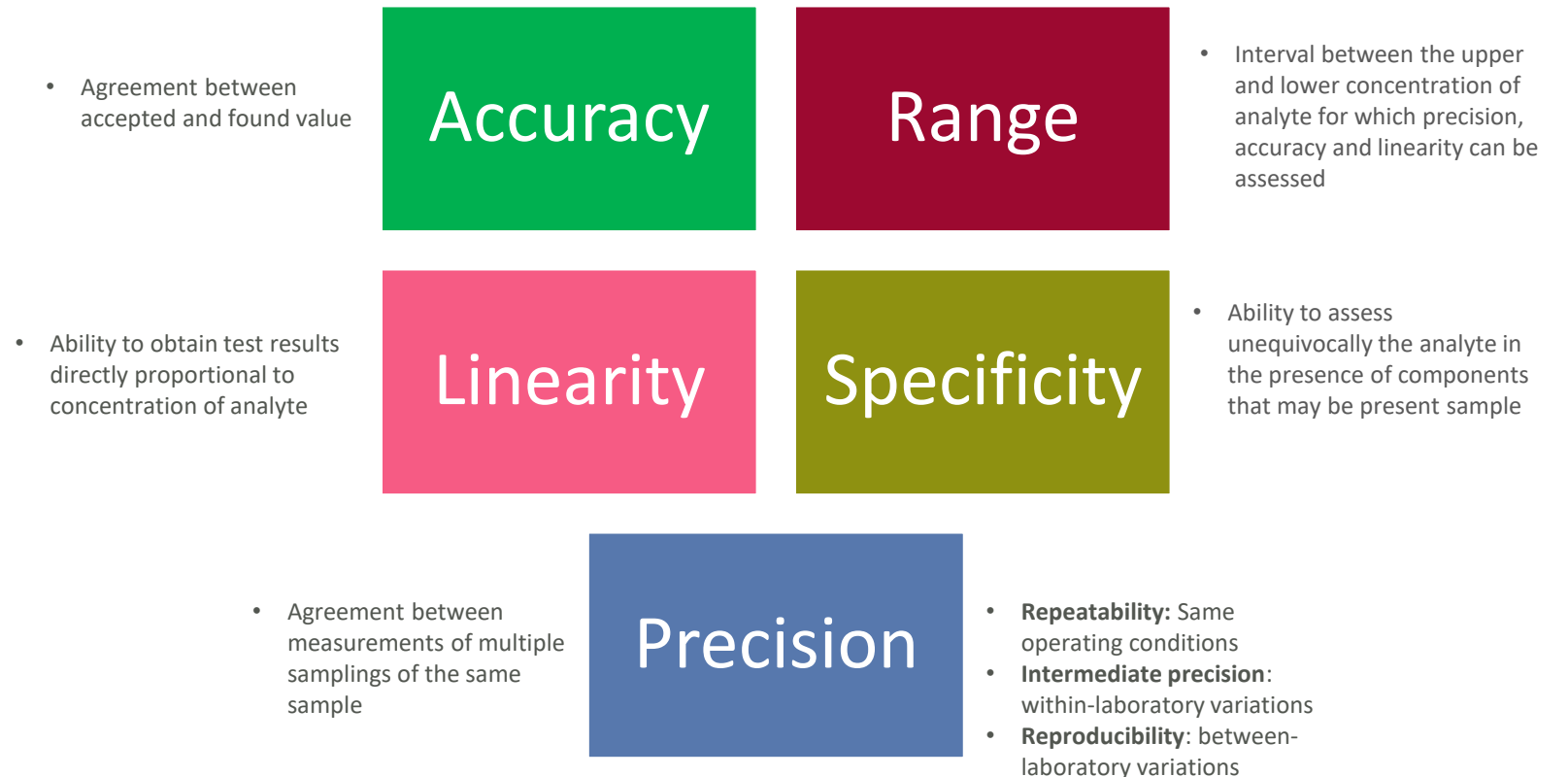
1 sgRNA/mRNA enters cell

Example of a Potency Assay for gRNA Critical Material

- Assay tests gRNAs ability to form an RNP complex and guide that RNP to DNA with a sequence of interest
- gRNA is added at various doses in the presence of Cas9 and its DNA target sequence
- Sample is run through a gel to determine the % cleavage from the full-length DNA target strand
- If gRNA is active, the DNA sequence is cleaved

- **Readout:**
- Potency assay must be quantitative
  - In the case of this assay, EC_{50} values were generated
- Readout is expressed as % Relative potency
  - De-risk assay runs by measuring potency relative to a reference standard
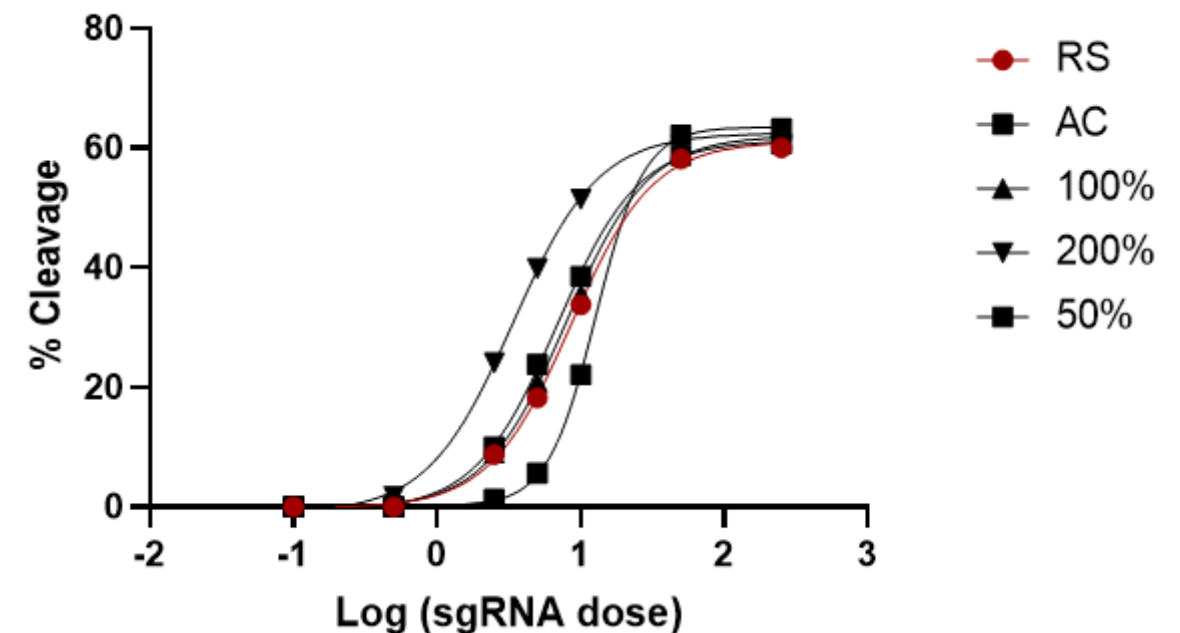 - Reference standard is well characterized, representative material

%RP of Sample: 97.6%

Qualification of a Potency Assay ICH *Q2(R1)* Criteria for Validation of Analytical Methods

- Qualification, also called phase-appropriate validation, is used for early phase (I/II) clinical studies
- Typically done for release assays
  - Valuable to do this for characterization assays as well
- Demonstrates an assay is "fit for purpose"
- Not defined anywhere officially, no specific guidance on analytical assay qualification, parameters around assay qualification are typically determined by the company
  - Conservative/less risky approaches to qualifications treat them like validations (i.e. Use guidance equivalent to what would be used for validation)
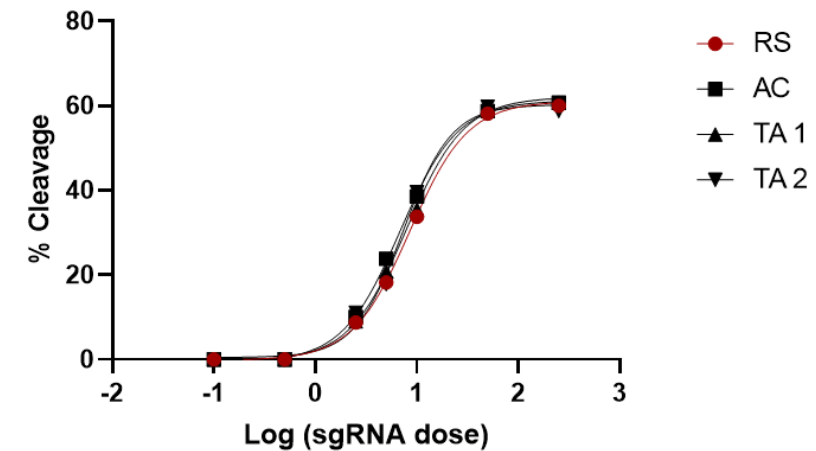
- Agreement between accepted and found value

Accuracy

Range

- Interval between the upper and lower concentration of analyte for which precision, accuracy and linearity can be assessed

- Ability to obtain test results directly proportional to concentration of analyte

Linearity

Specificity

- Ability to assess unequivocally the analyte in the presence of components that may be present sample

- Agreement between measurements of multiple samplings of the same sample

Precision

- **Repeatability:** Same operating conditions
- **Intermediate precision:** within-laboratory variations
- **Reproducibility:** between-laboratory variations

Accuracy

- Expresses the closeness of agreement between the value which is accepted as true and the value found *(Q2(R1))*

- Was assessed by calculating the % relative bias between the measured potency relative to the reference standard and the theoretical simulated potency level

  - gRNA was titrated at starting doses that would generate theoretical simulated potency values of 200%, 150%, 75%, and 50% potency

  - based off of the top point of the dose response curve at 100%

- Both within run accuracy and accuracy across all runs was assessed

- %Relative Bias < 20%

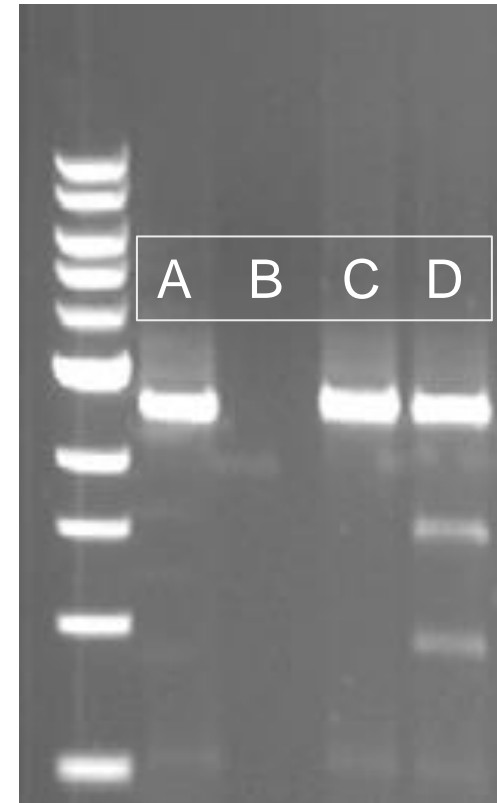- No directional (positive or negative bias)

Precision

- Expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions *(Q2(R1))*

- Precision may be considered at three levels:

  - Repeatability

  - Intermediate precision

  - Reproducibility

  - Was assessed by calculating the %CV of the reportable result (relative potency)
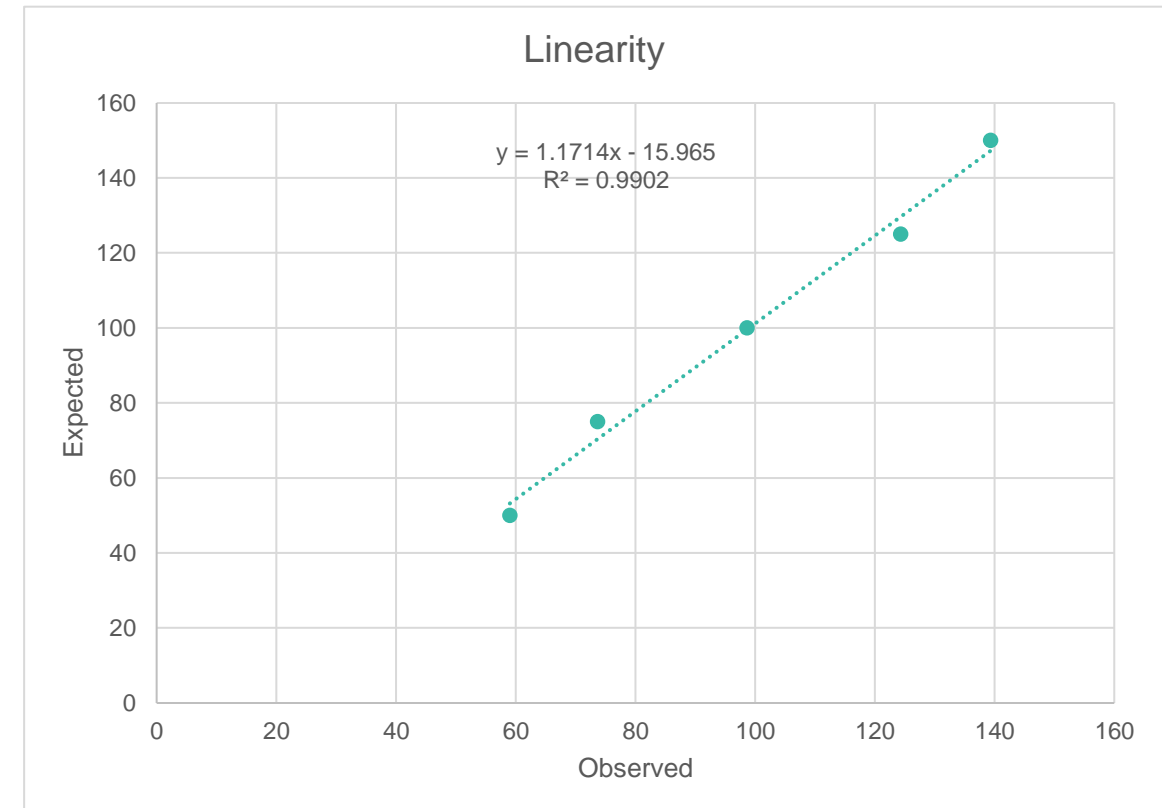
- %CV < 20%

Specificity

- Ability to assess unequivocally the analyte in the presence of components which may be expected to be present (Q2(R1))

- Provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample (Q2(R1))

- Samples were stained as per SOP

  - RS (D) shows signal

  - Specificity controls

    - No gRNA (A) shows no % cleavage

    - Blank (B) shows no bands

    - No Cas9 (C) shows no % cleavage

Linearity

- Ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample *(Q2(R1))*

- Assessed measured (% relative potency) vs. theoretical values (%values for which gRNA was theoretically dosed) across all runs

  - Linear Regression

- R^2=0.9902 (amount of linear association between two variables)

  - Theoretical vs. Measured

- Slope=1.17 (directly proportional)

Linearity

$y = 1.1714x - 15.965$
$R^2 = 0.9902$

Summary

- ▸ Understanding the potency of critical materials can provide meaningful insight into quality of API/DP

- ▸ Categorizing critical materials is not always straight forward in C&GT space
  - – Different levels of compliance are required depending on how materials are categorized

- ▸ Potency assays may be required for critical materials if potency is predictive of quality of the DP/API

- ▸ Similar guidelines for developing potency assays can be used for critical materials as are used for DP

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Thank you.

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