Viability Assay Validation Protocol Telecon, January 23, 2012 - Ducord

BLA 125407 Duke University CCBB T-con minutes 1-23-2012
RE: Draft -----(b)(4)------ Viability Assay Validation Protocol
Duke University: Amanda Parrish, Bruce Burnett.
FDA: Denise Gavin, Mark Davidson.
Background: ----(b)(4)---- assay is used to test CBU viability after processing but prior to adding cryoperservant and cryopreservation. ----------(b)(4)---------- % viability is calculated.
Because viability is a critical product quality attribute, you should make every attempt to build control into this assay wherever possible. We recommend that you perform some basic robustness studies to consider how error may be introduced (e.g. sample preparation, operator fatigue, instrument quality, reagent quality, etc) and limit them in your assay.
Some basic suggestions were recommended to reduce assay variability:

- Making validation protocol and SOPs as detailed as possible so that operators follow precise instructions.
  - e.g. step is to mix…describe how to mix, i.e. pipette sample up and down 10 (x) times…not 5-10 times… that you have determined is adequate to achieve suitable homogeneity of sample.
  - Make sure quantitative measurements are used, i.e. “very low cell viability” is not quantitative and could introduce bias. See below.
- Make sure sample preparation is very well controlled. Because samples chosen for validation studies are not representative of sample that will normally be assessed by assay, you should qualify sample as suitable:
  - Sample preparation considerations will be an issue for all assays you plan to validate. Please provide rationale with validation report.
  - Make sure details are included related to how samples are prepared, frozen, stored and thawed prior to assay.
  - Determine affect freeze/thaw has on assay variability?
  - You should performed preliminary studies to demonstrate that DMSO does not affect assay results?
- Replicates should be analyzed in triplicate at a min to get more statistical power.
- Your list of equipment: you should look at affect of the ---(b)(4)--- on readout of this assay. We recommend evaluating affect of different (b)(4), quality of --------(b)(4)--------
  - ***DUKE: It is possible there is only one (b)(4) in lab.***
  - ***FDA: If this is the case you should discuss instrument controls, i.e. instrument cleaning, ---(b)(4)---, etc***

DUKE: We will put in more descriptive language into protocol with more precise measurements and recommend that all replicates be run in triplicate.
Sponsor was informed that the above questions should be addressed in validation report and that they do not need to be re-submitted for review.

Discussion continued with recommendations/comments related to specific validation parameters evaluated in validation protocol:

Dr. Burnett state that they are not looking at accuracy since they ----------(b)(4)----------.

FDA: We understand this difficulty, and will allow you to infer accuracy from precision, linearity and other parameters being examined in the validation protocol. But you will need to demonstrate good control. You may also consider comparison to another validated assay for viability (i.e.-(b)(4)-), this comparison may support accuracy of the method.

1. Repeatability will be assessed using one operator looking at one sample prepared at ------(b)(4)--------- in triplicate with (b)(4) CV. This is different from initial validation plan submitted for 1-6-12 t-con, where you described using (b)(4) cord blood unit as the samples. Using (b)(4) cells can introduce variability, so you should control this aspect of protocol. As discussed above want to provide detailed discussion of sample prep and qualification as discussed above.
    a. ***DUKE: Viability goes down over time so wanted to have sufficient homogeneous sample to perform validation studies for repeatability as well as intermediate precision.***

- As mentioned previously, how sample will be prepared should be described in detail. Please clarify in protocol.
- Various cell concentrations will be generated by ----------(b)(4)----------
  - Q: is this -----(b)(4)----? should be a set amount…not a range.
- Q: protocol states this gives ‘very low viability” upon thawing.
- As discussed above language should be quantitative and precise and should be modified to reflect quantitative number of live/dead cells.

DUKE: The amount will be specified after preliminary studies.

2. Intermediate precision: you should include additional assay matrix variables in your table in the validation report showing relationship between variables.
3. Specificity: controls: ----(b)(4)---- cells listed under linearity.

- Sample prep details should be provided
  - Will 1 unit or multiple units units be used?
  - We recommend you mix in a known amount of (b)(4) since not clear from current protocol.

DUKE: We hope to use -(b)(4)- to make aliquots for later testing as described. We understand, we will revise to make sure precise amounts are used.

FDA: Because specificity here is related to potency, expect that CV will be low, if you can do (b)(4) for repeatability why not for specificity?

4. Limit of Quantitation:

  - How did you determine that (b)(4) cells is the quantitative limit? Have you performed dilution studies to determine limit of quantitation? Evaluated too concentrated and too diluted samples…?
  - This should be done in a ranging study….instead of leaving up to operator discretion.
    - Nothing should be up to operator discretion in a validated assay.
    - Decisions should be based on scientific knowledge of how assay performs. Thus, need to know upper limit of number of cells.

5. Linearity:

- Again, sample prep details and replicates in triplicate.
- You should add column to analysis table that includes your acceptance criteria (result within (b)(4) variability of target value).

6. Range: not planned …this is discussed above under LOQ. Recommended doing a ranging study.

DUKE: We will recommend a ranging study.