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Considerations on Matrix Sources for Biomarker Assays

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16 September 2020

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Key messages Recent AAPS paper: Often matrix is neglected Decision for Data User

- Sample matrix selection matters and is even technology dependent
- Matched serum, hep plasma and EDTA plasma samples were analyzed for 32 soluble protein biomarkers with ELLA and ELISA
- Concentrations differed
- Consider using platelet free sources
- Carefully select the right anticoagulant: immune cell activation
- Heparin can change protein interactions
- Literature is not always correct
- Recommendation to use a systematic and data driven approach.
- **Understand the biology**

Reference: Considerations for Soluble Protein Biomarker Blood Sample Matrix Selection, Joel A. Mathews, Yan G. Ni, Connie Wang, Jon E. Peterson, Chad Ray, Xuemei Zhao, Daoyu Duan, Sara Hamon, John Allinson, Martha Hokom, and Greta Wegner,The AAPS Journal (2020) 22: 38;

https://link.springer.com/article/10.1208/s12248-020-0412-0

Thanks to Yan Ni for discussions

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The Source of Your Blank Matrix -

Questions for Assay Developers

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Common Practice in Many Laboratories for Biomarker Assays

- Method development
  - Start MD using matrix from the freezer stocks
  - Leftovers from other studies
  - Potentially commercially sourced diseased matrix
- Validation (or qualification)
  - Freshly purchased or from lab's stock
  - Same commercially available matrix as in MD
- Sample analysis: unexpected behavior can occur
  - Interferences
  - High background
  - Instability
  - Levels not as expected

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What do guidances request?

- FDA BMV (2018):
  - "The sponsor should prepare the calibration standards in the same biological matrix as the samples in the intended study. ……. When surrogate matrices are necessary, the sponsor should justify and validate the calibration curves."
  - "Sponsors should prepare QCs in the same matrix as the study samples to be assayed with the validated method"
  - "The sponsor should make up calibrators and QCs in lots of blank matrix that is free of interference or matrix effects"
  - "All stability determinations …. should use a set of samples prepared from a freshly made stock solution of the analyte in the appropriate analyte-free, interference-free biological matrix."
- So for most biomarker assays we need to be flexible with those expectations.

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What do guidances request?

- EMA BMV (2012) although BMs not in scope:
  - "Although the use of extracted matrix (e.g. charcoal, immuno-affinity) or alternative matrix (e.g. protein buffers, dialysed serum) is not recommended, the use of such matrices may be necessary when there is no other strategy to quantify the analyte of interest. The calibration standard curve may be prepared in these surrogate matrices. QC samples should be prepared in the actual sample matrix and the accuracy should be calculated to demonstrate the absence of matrix effect."
- ICH M10 draft has similar considerations.

- So this seems to be a pragmatic way forward for calibrators and QCs in biomarker assays.
- But, for stability, parallelism and other assessments true matrix samples are still needed.

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Questions to be asked

- Commercial sources: how representative are those (even for HV studies)?
  - Bags versus tubes
  - Plasma / serum preparation conditions?
  - What do we really know from the procedures or matrix donors?
  - Especially with rare matrices. What can we realistically expect?
    - Example: Aqueous humor.
- Diseased populations, what is relevant?

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Examples

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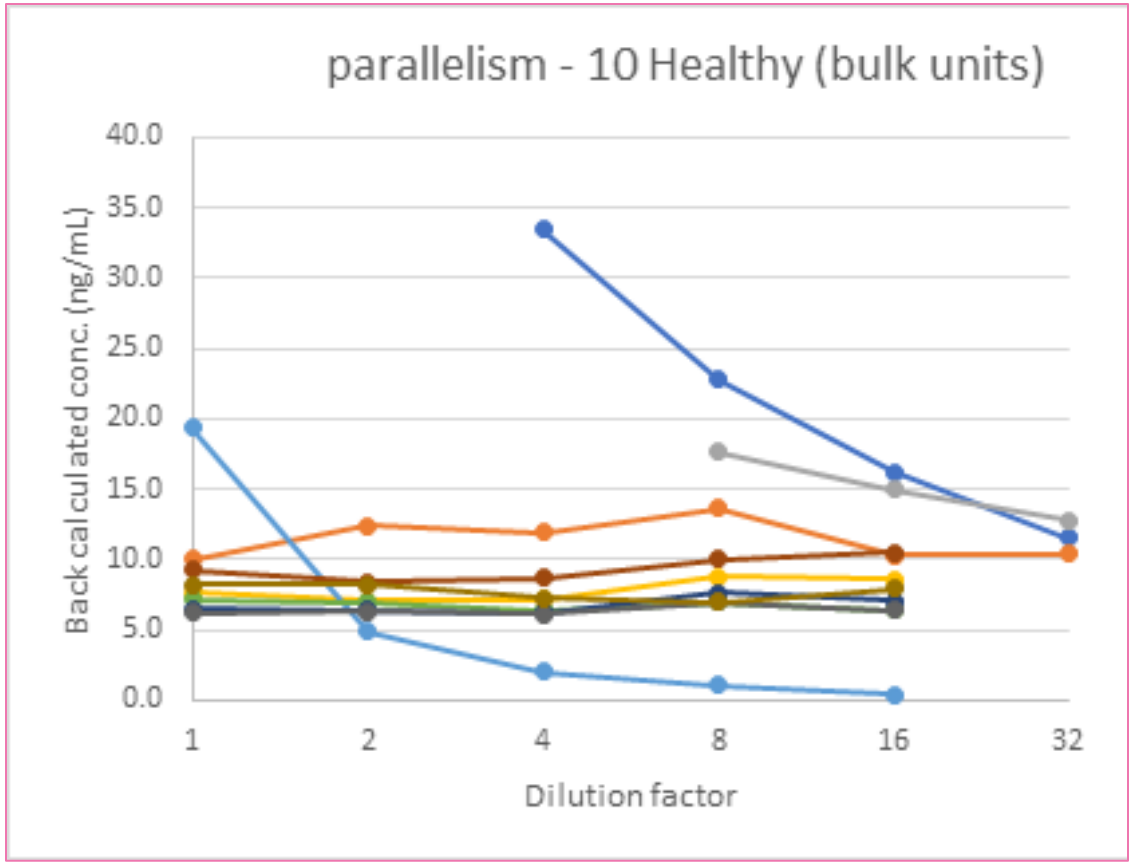
Matrix Source: Method Development vs. Validation Phase

- Method development “Biomarker X”, first experiment, commercial matrix source from HVs used. Months old.



- At least 2 out of 9 matrices do not behave “parallel”

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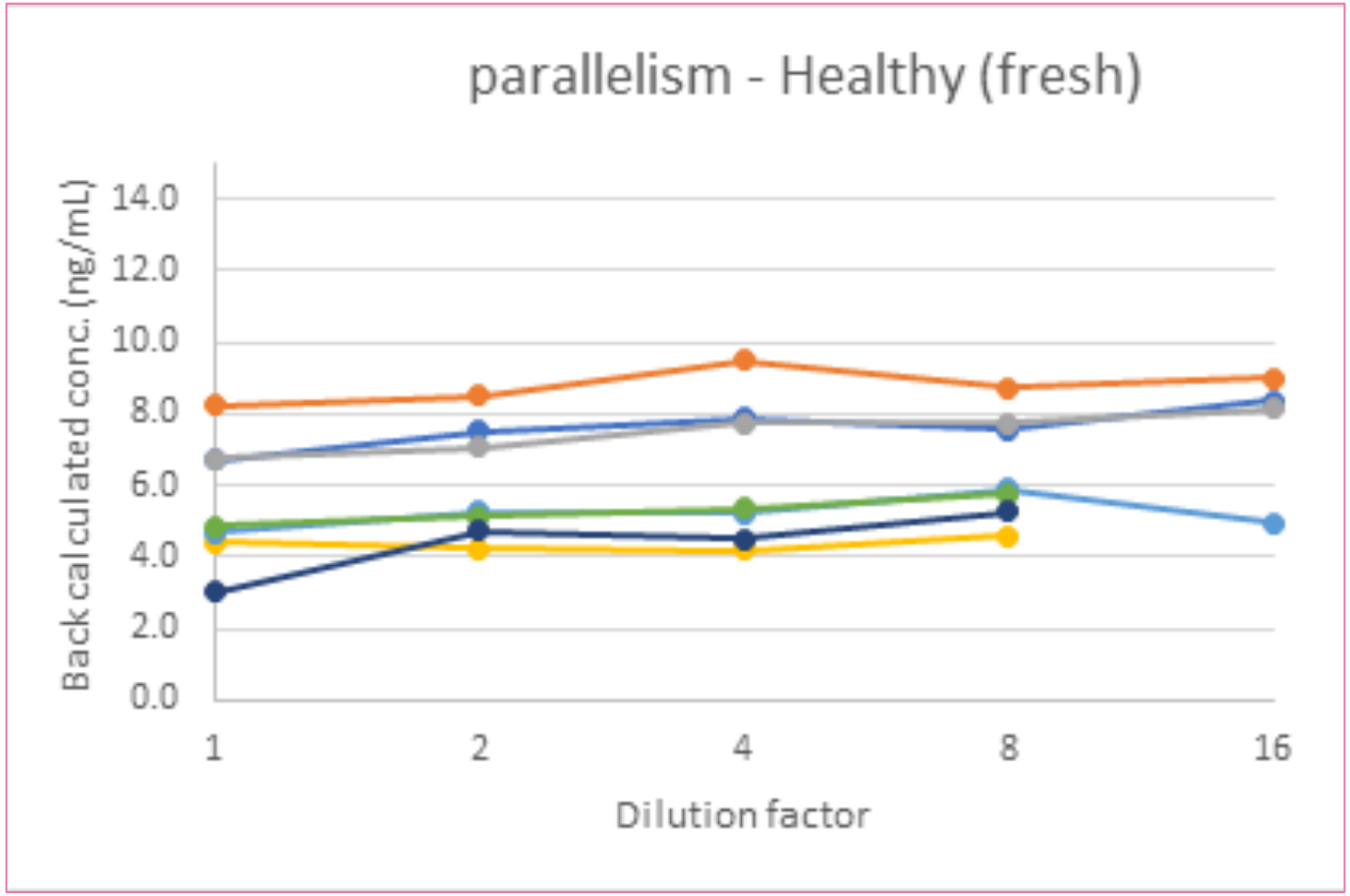
Matrix Source: Method Development vs. Validation Phase

- Method development, second experiment, fresh in-house sourced matrix used



- This seems much better (although limited number of lots used)

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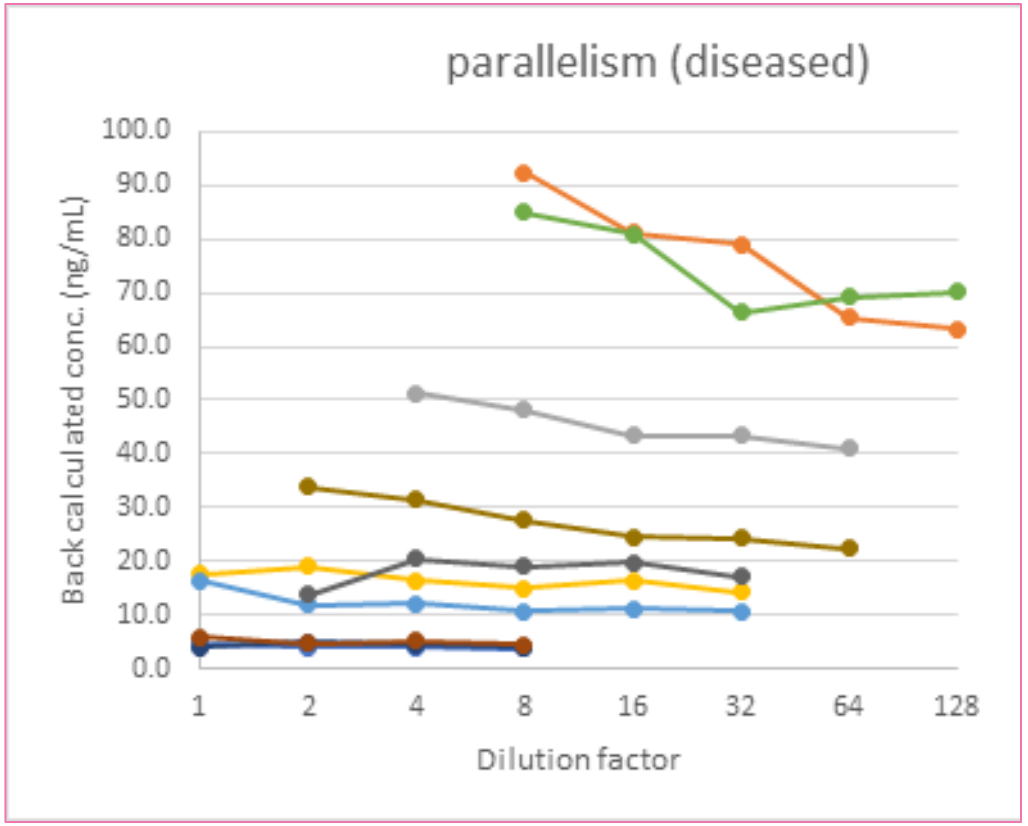
Matrix Source: Method Development vs. Validation Phase

- Validation, also diseased matrix from commercial origin used



- Some patient samples have higher levels and those seem to behave less parallel.
- Is this all acceptable? It is certainly not straightforward!

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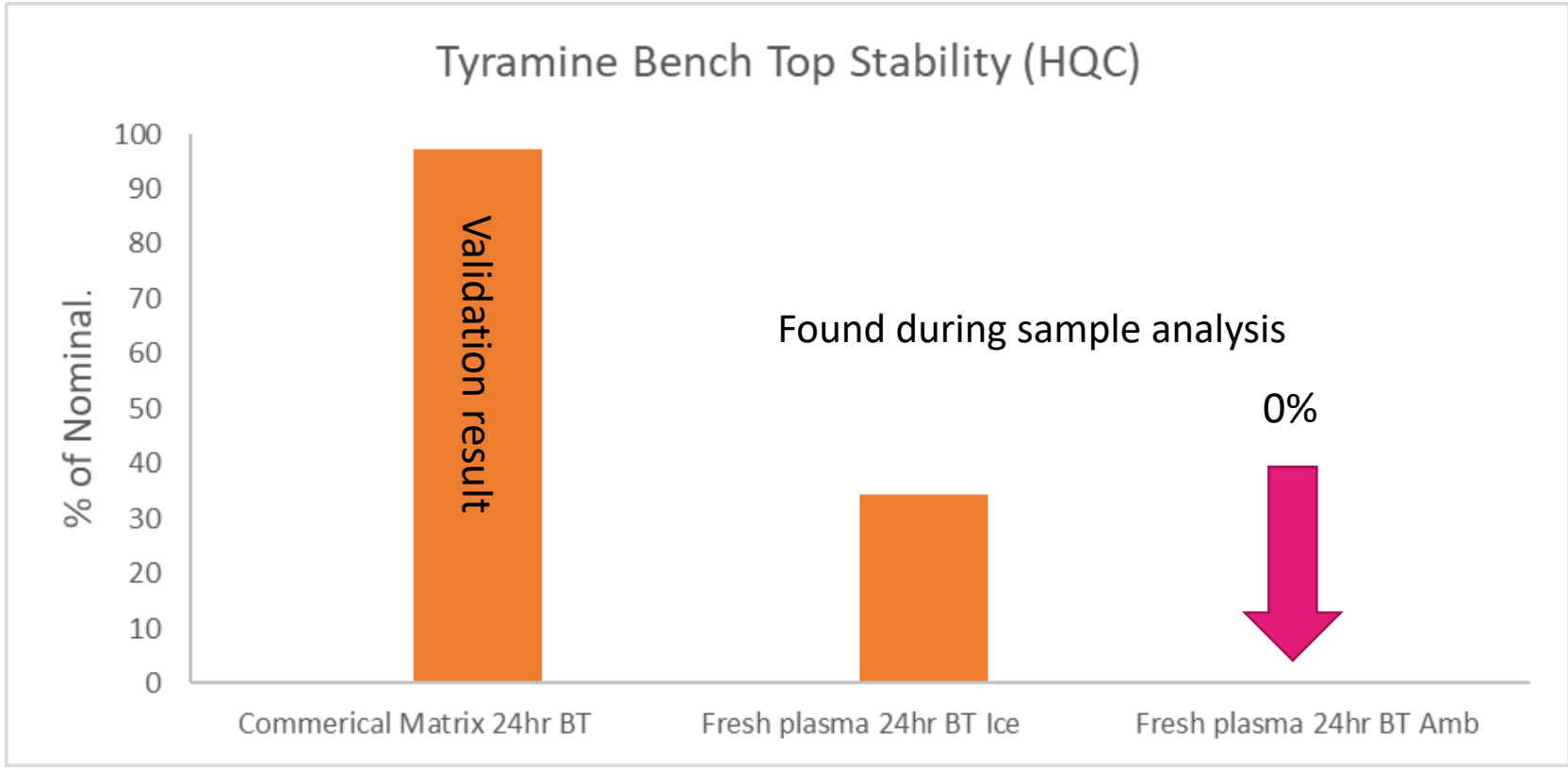
Freshness: Validation vs. Sample Analysis Phase

- MD in practice: how <u>fresh</u> are the matrices? F/T cycles?



- Conclusion: Antioxidants are needed after all

From: How Fresh is Fresh?- Overcoming stability errors encountered with “fresh” vs stored matrix
Corey Ohnmacht, PharmSci360, 05 November 2019

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Wrap-up

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Final Considerations Questions – Not Answers

- What can realistically be proven during MD / Val?
  - How homogeneous and well-known is the studies' patient population?
    - Diet?
    - Effect of different treatments
    - Patient's age
- Use of pre-dose samples for validation
  - Consider the ICF
  - Timing is not ideal
- Parallelism: Is the highest concentration always representative?
- How representative is the matrix lot with the lowest concentration (e.g. for preparing the calibration curve)?
- Precipitation in sample tubes: spin down?
- Minimum sample volume required and tube type

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Conclusion

- Understand the biology of the biomarker
- Consider what you are trying to prove during validation
- There is no simple way out
- Realize that the perfect situation does not exist, assay validation is always only a surrogate for the real analysis situation
- End user and assay developer need to communicate and agree on expectations!