# Bridging a biomarker kit

Hannah Vernon-Browne

Drug Development Solutions

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

# Contents

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

# Biomarker Kits

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

- Kits have advantages over self-developed assays
  - Reduced timelines
  - Purchase of large quantities
- Reliance on quality kit production
- Classic components:
  - Pre-coated plate / beads
  - Detection antibody
  - Standard + kit controls
  - Diluents
  - May have signal amplifiers

# Kits as Critical Reagents + Bridging Kits

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

- Critical reagents may impact the performance of an assay
  - Antibody binding affinities
- Changing kit lots
  - Long term stability assessments, short expiry dates, long clinical studies etc
- “Bridging” kit lots
  - Not currently defined → trying to create a standard

# Our Dynamic Approach

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

- Best practice → run 30 samples on both lots + compare
  - Design of experiment style
  - Issues with patient consent, logistics, resource
- Common practice → run old + new standards and QCs using the new kit reagents (below)
  - As many endogenous as possible, across analytical range
- Difficult to have “standard” approach → differing CoU, instruments, performance

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Old STD 1 | Old STD 1 | Old Blank | Old Blank | | | | | ULOQ | ULOQ | New STD 1 | New STD 1 |
| B | Old STD 2 | Old STD 2 | ULOQ | ULOQ | | | | | HQC | HQC | New STD 2 | New STD 2 |
| C | Old STD 3 | Old STD 3 | HQC | HQC | | | | | MQC | MQC | New STD 3 | New STD 3 |
| D | Old STD 4 | Old STD 4 | MQC | MQC | | | ULOQ | ULOQ | LQC | LQC | New STD 4 | New STD 4 |
| E | Old STD 5 | Old STD 5 | LQC | LQC | | | HQC | HQC | LLOQ | LLOQ | New STD 5 | New STD 5 |
| F | Old STD 6 | Old STD 6 | LLOQ | LLOQ | | | MQC | MQC | | | New STD 6 | New STD 6 |
| G | Old STD 7 | Old STD 7 | | | | | LQC | LQC | | | New STD 7 | New STD 7 |
| H | Old STD 8 | Old STD 8 | | | | | LLOQ | LLOQ | New Blank | New Blank | New STD 8 | New STD 8 |

# Our Dynamic Approach

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

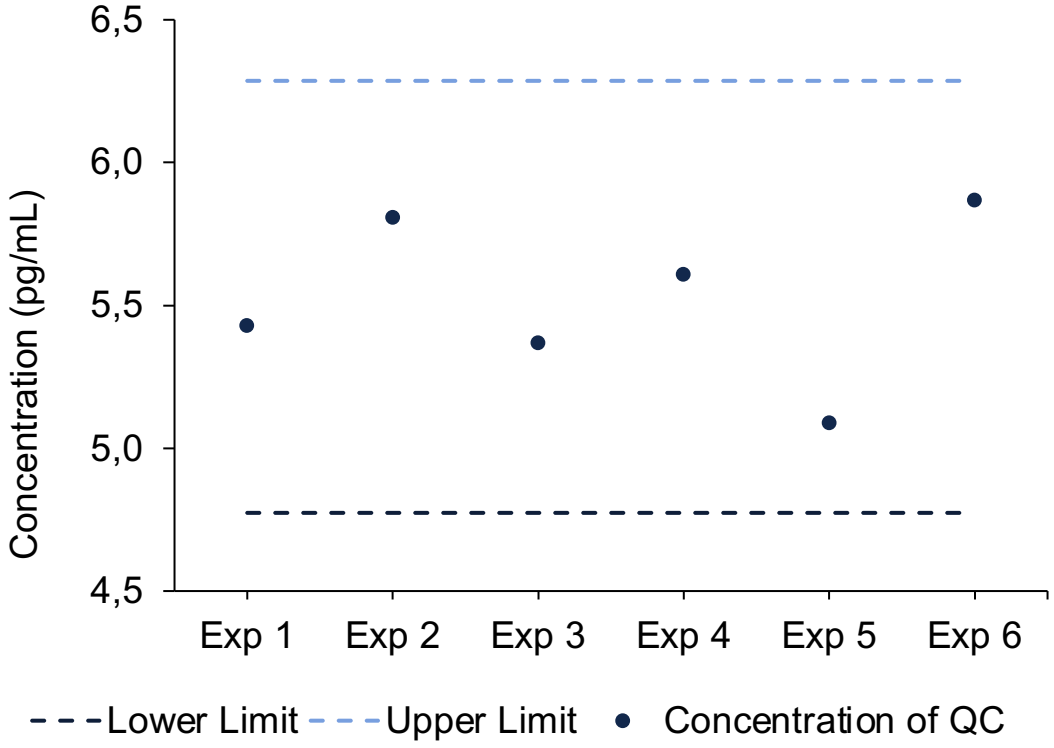
Develop the assay

Perform “precision runs” during validation

Statistical analysis determines prediction intervals + inter-assay precision

Acceptance criteria for bridging

Example QC data from precision runs, demonstrating the prediction intervals



# Case Study: NF-L & GFAP

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

- Neurofilament-light and glial fibrillary auxiliary protein
  - Neuromarkers in serum
- HD-X: Ultra-sensitive bead-based immunoassay platform

- Bridging for a LTS study
  - Beads (capture labelled)
  - Detection Antibody
  - Multiplex Standard

- Run old standard, new standard and QCs
  - Pre-made curve

# Bridging Kit Lot 1 + 2 – QCs + Kit Controls

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

| | NFL MQC | NFL LQC | NFL LLOQ |
|---|---|---|---|
| *Established Concentration (pg/mL)* | *17.1* | *4.84* | *0.877* |
| *Lower Limit (pg/mL)* | *11.3* | *3.54* | *0.542* |
| *Upper Limit (pg/mL)* | *22.9* | *6.14* | *1.21* |
| Mean Concentration from Old Curve (Kit Lot 1) | 21.6 | 4.56 | 1.14 |
| Mean Concentration From New Curve (Kit Lot 2) | 21.7 | 4.33 | 0.987 |
| *Established Inter-Assay Precision* | *7.3* | *6.7* | *9.5* |
| % Difference | 0.5 | -5.0 | -13.2 |
| *Mean Inter-Precision from Precision Runs* | *7.8* | | |
| Mean % Difference | **6.2** | | |

| | GFAP MQC | GFAP LQC | GFAP LLOQ |
|---|---|---|---|
| *Established Concentration (pg/mL)* | *184* | *43.1* | *9.28* |
| *Lower Limit (pg/mL)* | *70.3* | *27.0* | *5.31* |
| *Upper Limit (pg/mL)* | *298* | *59.2* | *13.3* |
| Concentration from Old Curve (Kit Lot 1) | 168 | 25.9 | 14.2 |
| Concentration From New Curve (Kit Lot 2) | 157 | 23.2 | 12.4 |
| *Established Inter-Assay Precision* | *10.6* | *9.3* | *10.6* |
| % Difference | -6.7 | -10.3 | -12.2 |
| *Mean Inter-Precision from Precision Runs* | *10.2* | | |
| Mean % Difference | **9.8** | | |

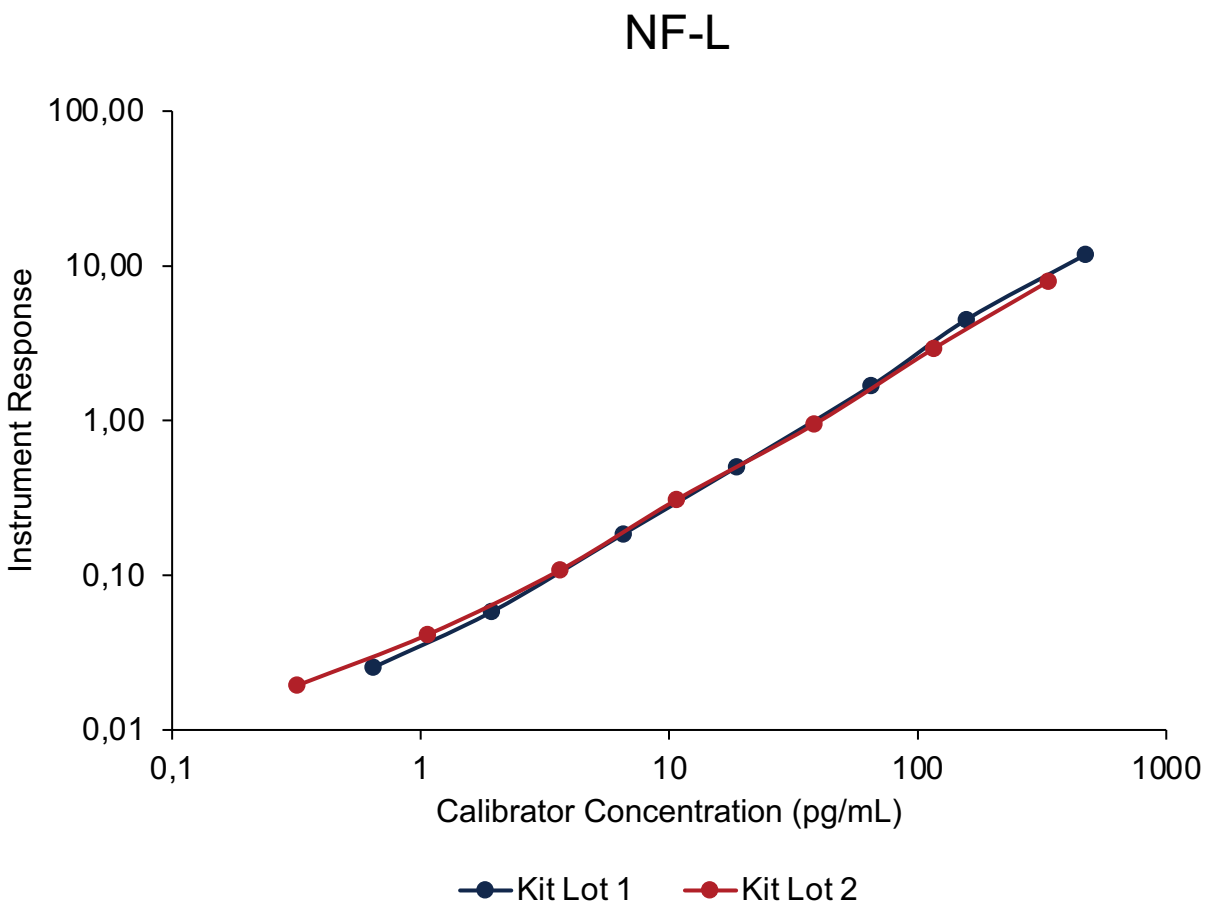
# Importance of Instrument response

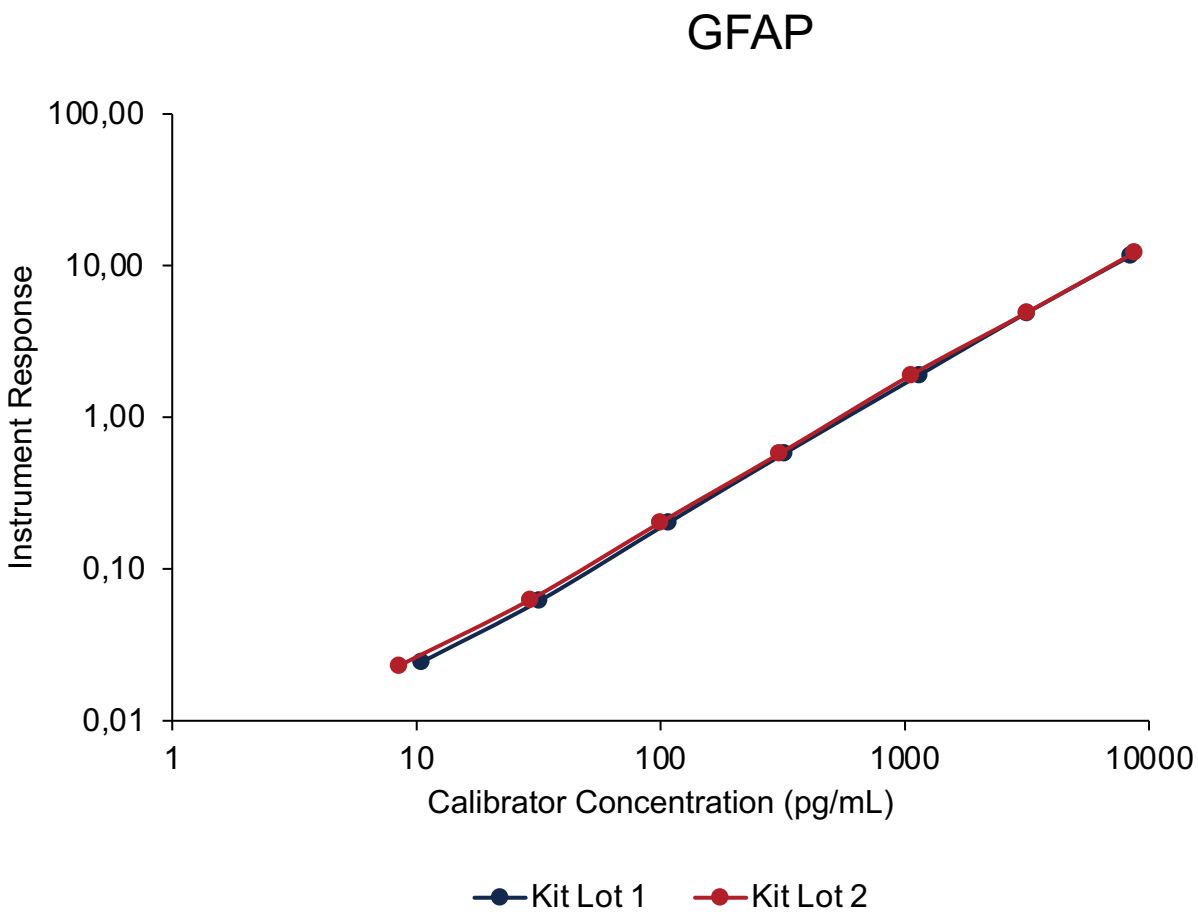
DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

- HD-X instrument response is Average Enzymes per Bead (AEB)
- Uses beads & fluorescence
- Top standard ~ 10 AEB
- Bottom standard ~ 0.03 AEB
- Blank ~ 0.01 AEB
- Signal needs to stay consistent

# Kit Lot 1 + 2 - Curve Comparisons

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.



# Kit Lot 2 + 3 – QCs + Kit Controls

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

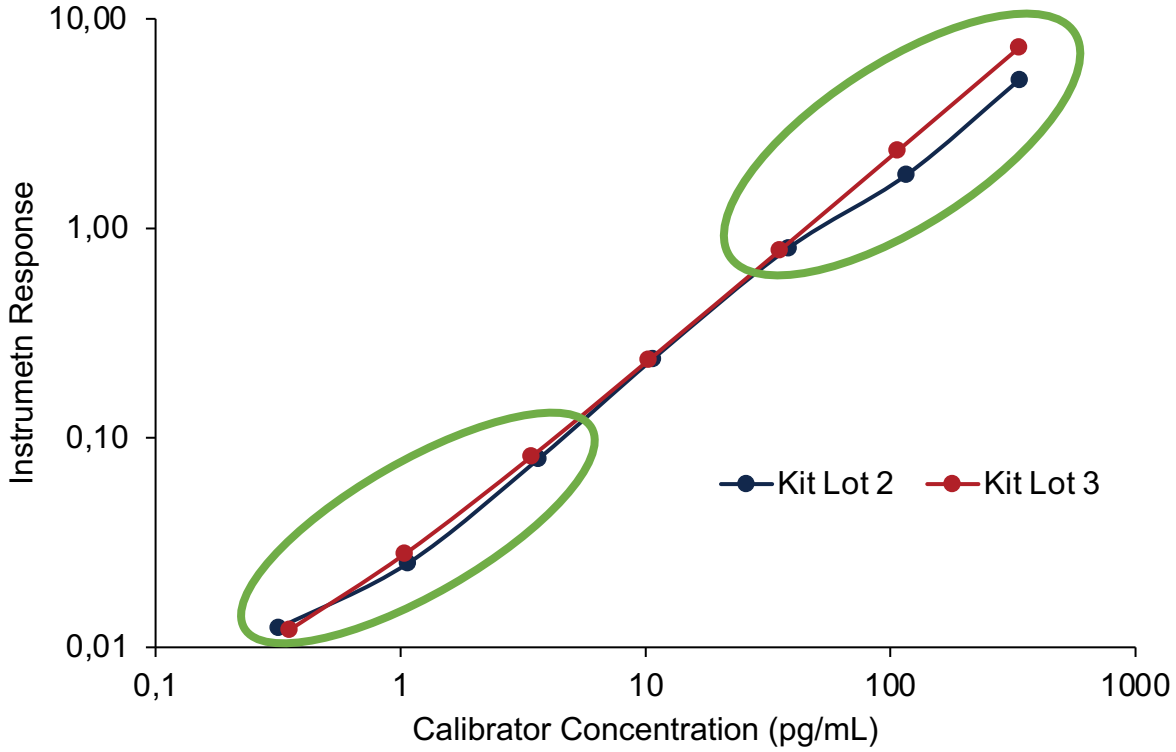
| | NFL MQC | NFL LQC | NFL LLOQ |
|---|---|---|---|
| *Established Concentration (pg/mL)* | *17.1* | *4.84* | *0.877* |
| *Lower Limit (pg/mL)* | *11.3* | *3.54* | *0.542* |
| *Upper Limit (pg/mL)* | *22.9* | *6.14* | *1.21* |
| Mean Concentration from Old Curve (Kit Lot 2) | 29.8 | 6.35 | 1.60 |
| Mean Concentration From New Curve (Kit Lot 3) | 25.8 | 5.68 | 1.50 |
| *Inter-Assay Precision* | *7.3* | *6.7* | *9.5* |
| % Difference | -13.4 | -10.6 | -6.7 |
| Mean Inter-Precision from Precision Runs | 7.8 | | |
| Mean % Difference | **10.2** | | |

| | GFAP MQC | GFAP LQC | GFAP LLOQ |
|---|---|---|---|
| *Established Concentration (pg/mL)* | *184* | *43.1* | *9.28* |
| *Lower Limit (pg/mL)* | *70.3* | *27.0* | *5.31* |
| *Upper Limit (pg/mL)* | *298* | *59.2* | *13.3* |
| Mean Concentration from Old Curve (Kit Lot 2) | 174 | 28.2 | 12.0 |
| Mean Concentration From New Curve (Kit Lot 3) | 185 | 29.3 | 12.3 |
| *Inter-Assay Precision* | *10.6* | *9.3* | *10.6* |
| % Difference | 6.1 | 4.1 | 3.1 |
| Mean Inter-Precision from Precision Runs | 10.2 | | |
| Mean % Difference | **4.4** | | |

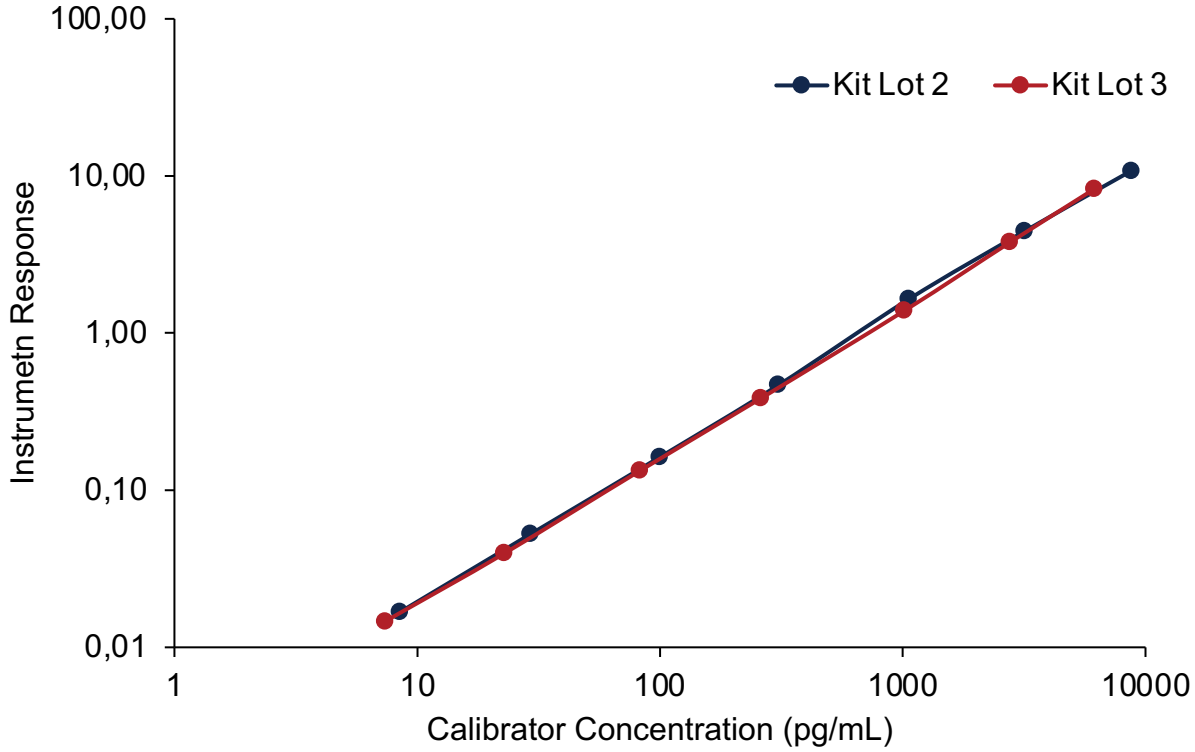
# Kit Lot 2 + 3 - Curve Comparisons

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

NF-L



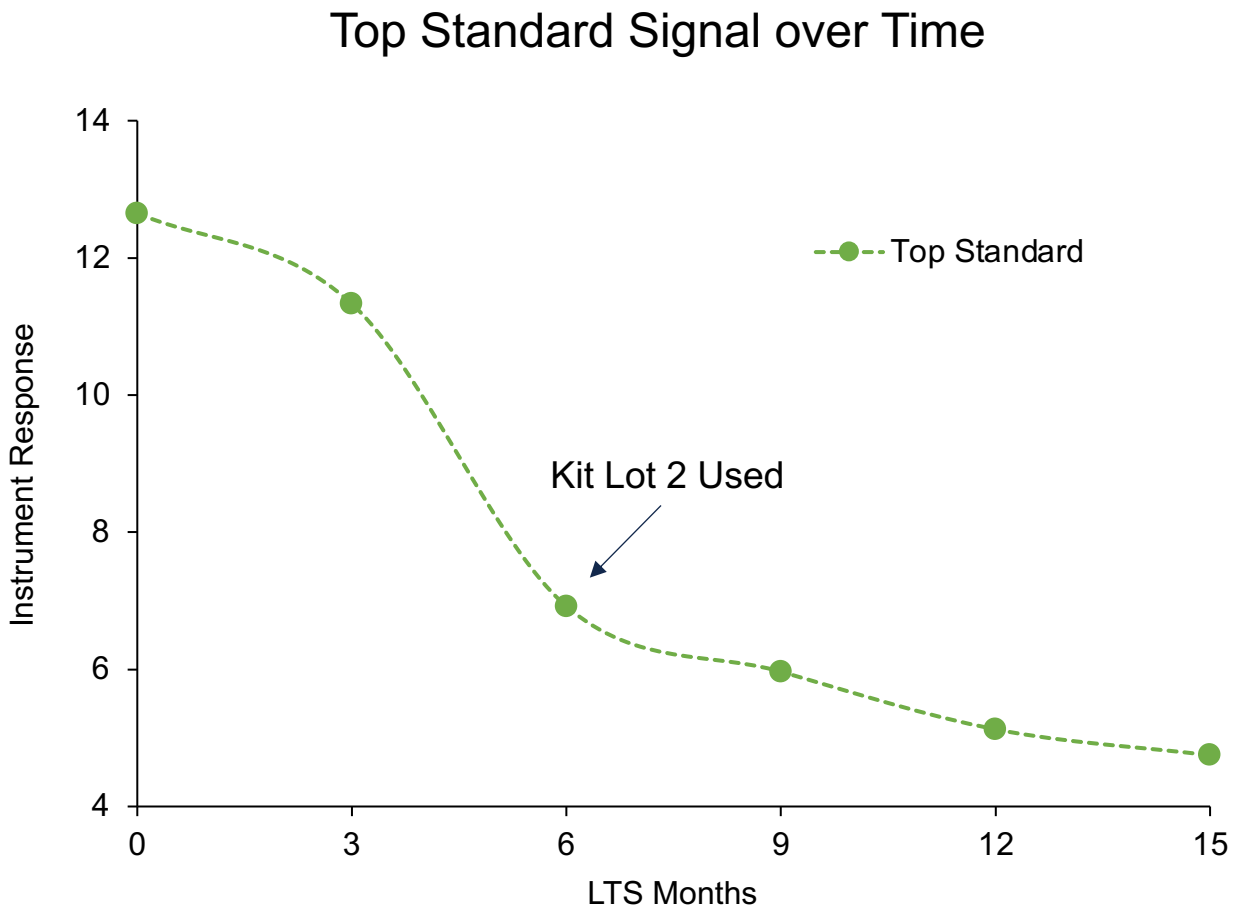

GFAP

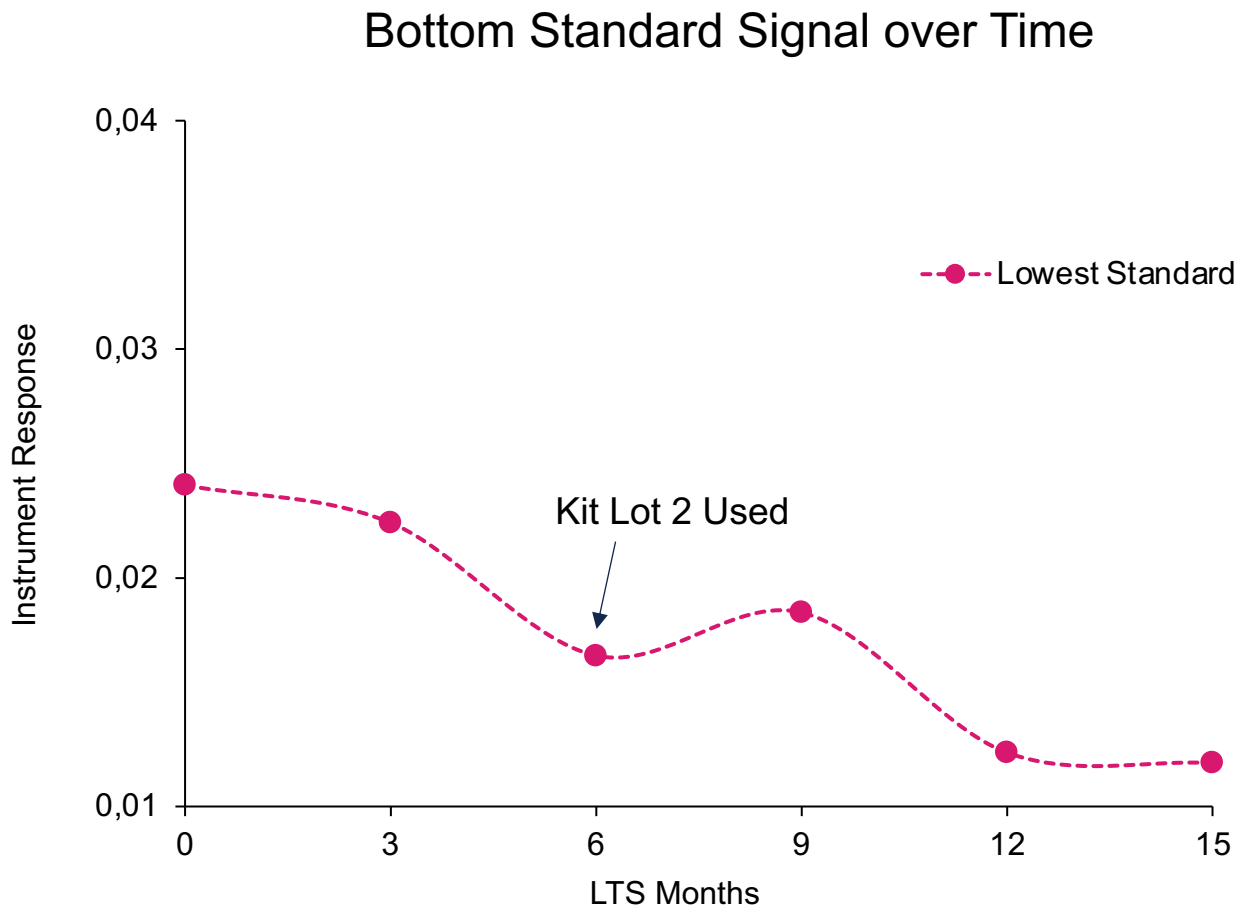



…Kit Lot 2 Standard???

# Trend of NF-L Standard Points Over Time

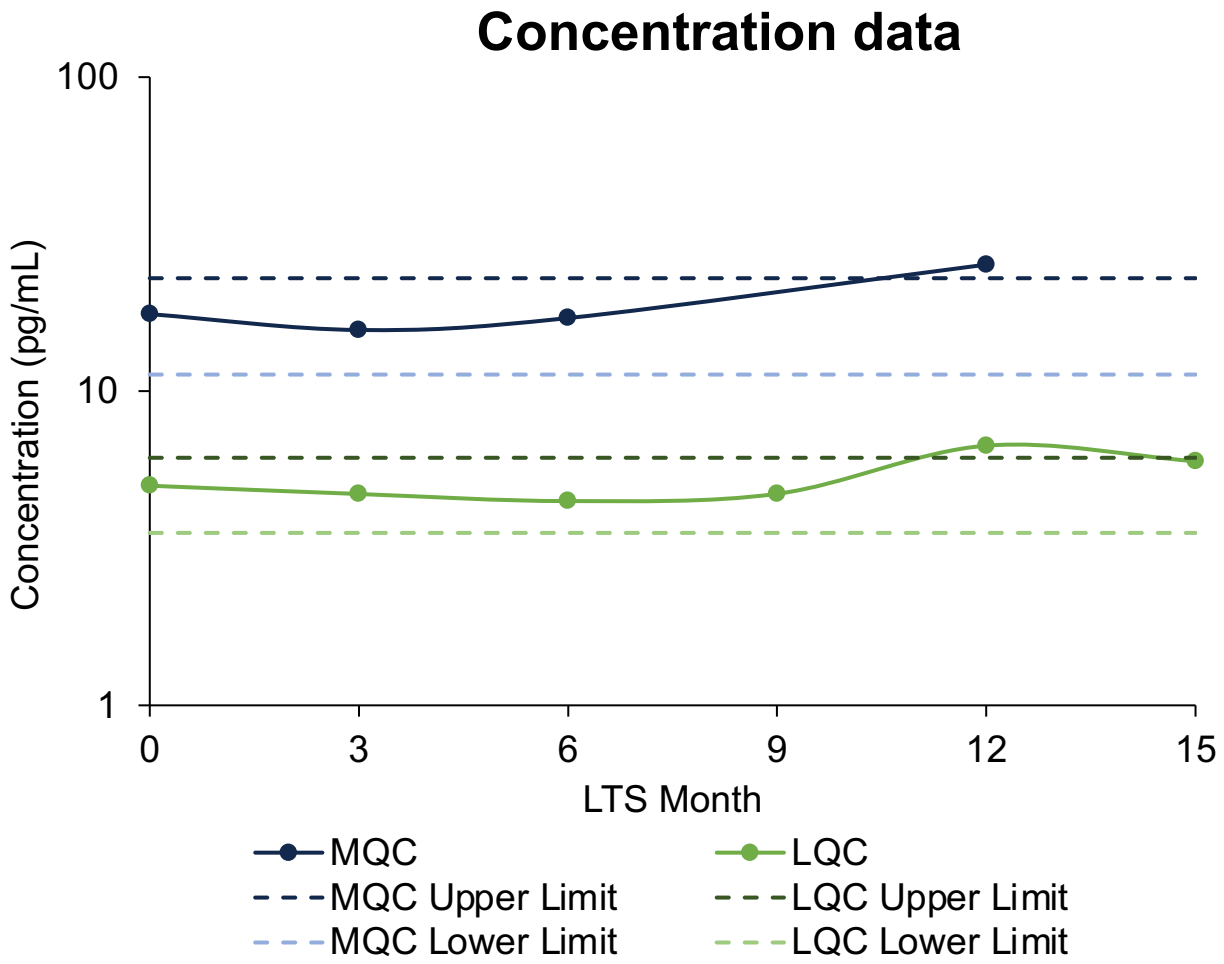
DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

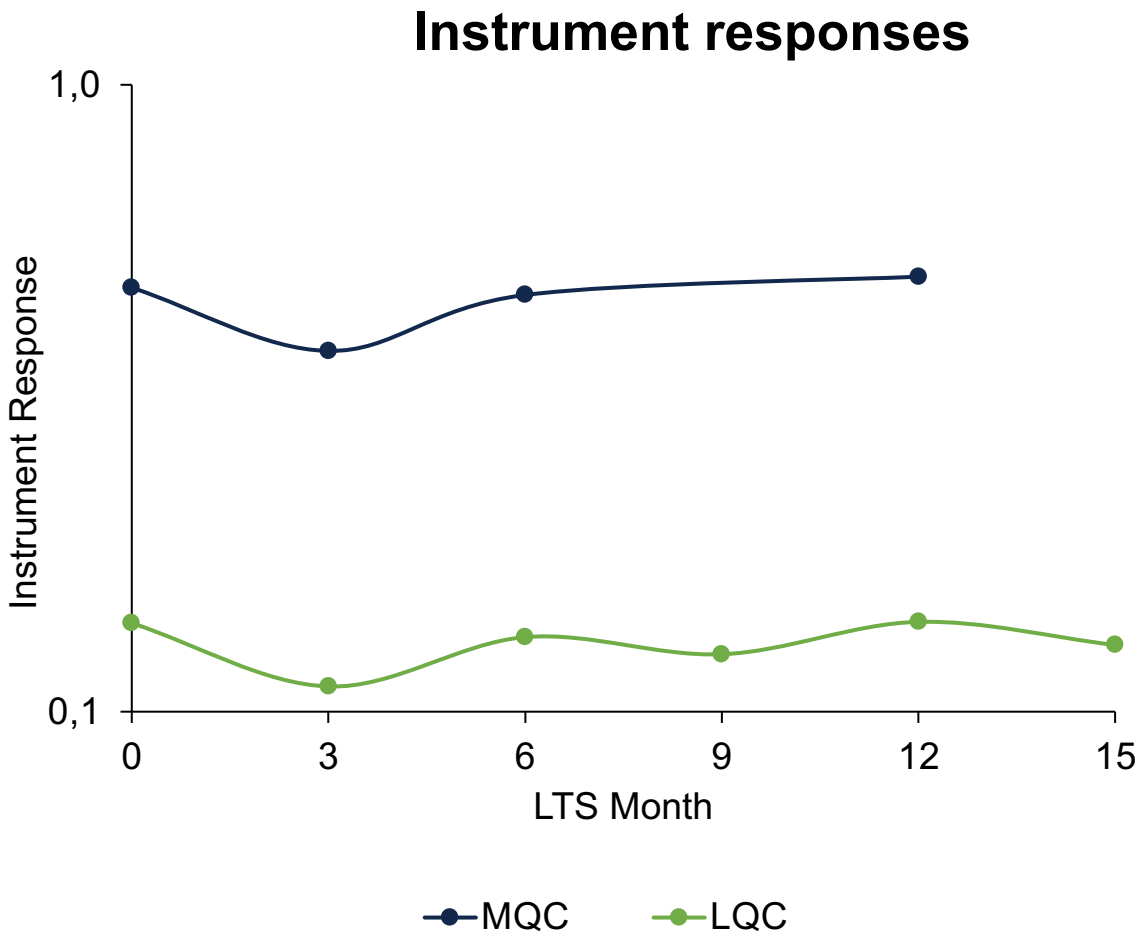


# NF-L Long Term Stability

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.



# NF-L Standard Stability – What Next?

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

- Ongoing troubleshooting…
  - Standard from kit lot 1
    - Trending data to see if performance still FFP
  - Alternative calibrator
  - Correction factors
- Does the C of U require this amount of work?
- Running samples in profiles

# Conclusion

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

- Kits lots may have differing performances
- Bridging is required to ensure the kit lot performance remain fit for purpose
  - Context of use dependent
- NF-L GFAP Case Study
  - Complexity of multiplex
  - Importance of instrument responses

DRUG DEVELOPMENT SOLUTIONS
Part of Alliance Pharma, Inc.

# Thank you for your attention

## Any questions?

drugdevelopmentsolutions.com

@DDSDrugDev @drugdevelopmentsolutions @drugdevelopmentsolutions