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# Surrogate CSF for the Bioanalysis of Oligonucleotide Therapeutics Through LBA

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## INTRODUCTION

- Bioanalytical ligand binding assays (LBA) support pharmacokinetic (PK) drug and pharmacodynamic (PD) biomarker profiling for preclinical and clinical studies.
- Per the ICH M10 guidance, LBA for PK bioanalyses requires calibration standards prepared in the same biological matrix as the study samples.
- Additionally, per the ICH M10 guidance, the biological matrices are the recommended diluents for study samples.
- Therefore, each biological matrix lot must be qualified for specificity and selectivity against the analyte, prior to use.
- These requirements pose unique challenges for rare matrices such as cerebrospinal fluid:
  - Long lead times for procurement
  - Expensive
  - Limited volumes per collection lot
  - Performance variance between lots
  - Requiring robust criteria for qualification, making it further challenging.

## SURROGATE CSF

- Compositional analyses indicate a 1000-fold lower protein content in CSF vs. blood-based matrices.
- **Table 1** lists the identified composition from a review article.
- *[1]Fogh JR et al. Anal Bioanal Chem 2020;412:1653-61*

**To identify a suitable surrogate CSF:**

- Dual-probe method for an ASO
- QC samples in native CSF
- Calibration standards in
  - 1% serum in PBS
  - 1% BSA in PBS
- Serum supplementation better mimics performance in native CSF

| | CSF | Plasma |
|---|---|---|
| Proteins (µg/mL) | 300–700 | 60,000–70,000 |
| -Albumin | 192 | 42,150 |
| -Globulins | 50 | 22,700 |
| -Transferrin | 14 | 2600 |
| -Fibrinogen | 0.6 | 2800 |
| Lipids (µg/mL) | 10–20 | 50,000 |
| $Na^+$ (mmol/L) | 140–145 | 135–147 |
| $Cl^-$ (mmol/L) | 115–120 | 95–105 |
| Glucose (µg/mL) | 600 | 900 |
| pH | 7.31 | 7.41 |

**Table 1.** Typical composition of CSF and plasma [1]

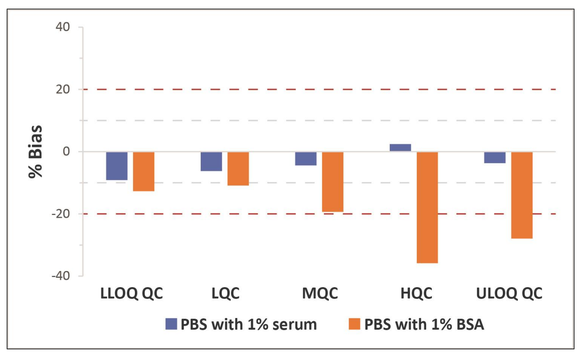


**Figure 1.** Serum vs. BSA-supplemented PBS as potential surrogate CSF candidates

## CLINICAL APPLICATION

- Original bioanalytical method for an ASO validated using native human CSF and successfully used to support the Phase I clinical study.
- The above-mentioned challenges were observed during a longer-duration Phase II study, primarily through variance in lot-to-lot performance of native CSF.
- Therefore, the feasibility of a surrogate CSF method was explored through preliminary testing as depicted in **Figure 1**.
- Performance of 1% serum-supplemented PBS demonstrated in precision and accuracy evaluations with native human CSF (**Table 2**).

**Table 2.** Calibration standards in surrogate CSF. QC sample sets in surrogate and native CSF.

| Precision and Accuracy | Prepared in surrogate CSF (1% serum supplemented PBS) | | | | | Prepared in native human CSF | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LLOQ QC | LQC | MQC | HQC | ULOQ QC | LLOQ QC_CSF | LQC_CSF | MQC_CSF | HQC_CSF | ULOQ QC_CSF |
| | 0.1500 ng/mL | 0.4000 ng/mL | 4.000 ng/mL | 75.00 ng/mL | 100.0 ng/mL | 0.1500 ng/mL | 0.4000 ng/mL | 4.000 ng/mL | 75.00 ng/mL | 100.0 ng/mL |
| Replicate – 1 | 0.1782 | 0.3815 | 4.060 | 77.74 | 87.64 | 0.1542 | 0.4170 | 4.033 | 75.43 | 89.83 |
| Replicate – 2 | 0.1723 | 0.3997 | 3.847 | 72.34 | 93.74 | 0.1628 | 0.4187 | 4.030 | 78.26 | 84.68 |
| Replicate – 3 | 0.1733 | 0.3655 | 3.833 | 74.87 | 87.78 | 0.1560 | 0.4032 | 3.627 | 71.98 | 81.95 |
| Intra-run Mean | 0.1746 | 0.3822 | 3.913 | 74.98 | 89.72 | 0.1577 | 0.4130 | 3.897 | 75.22 | 85.49 |
| Intra-run SD | 0.003158 | 0.01711 | 0.1272 | 2.702 | 3.482 | 0.004536 | 0.008501 | 0.2335 | 3.145 | 4.001 |
| Intra-run %CV | **1.8** | **4.5** | **3.3** | **3.6** | **3.9** | **2.9** | **2.1** | **6.0** | **4.2** | **4.7** |
| Intra-run %Bias | **16.4** | **-4.4** | **-2.2** | **0.0** | **-10.3** | **5.1** | **3.2** | **-2.6** | **0.3** | **-14.5** |
| Intra-run %Total Error | 18.2 | 8.9 | 5.4 | 3.6 | 14.2 | 8.0 | 5.3 | 8.6 | 4.5 | 19.2 |
| n | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

## PRECLINICAL APPLICATION

- 1% plasma—PBS tested as surrogate CSF, for *de novo* development.
- Performance demonstrated in precision and accuracy evaluations with native monkey CSF (**Table 3**).
- Dilutional linearity established with surrogate CSF as diluent for reliable quantitation of preclinical study samples at Cmax (**Table 4**).

**Table 3.** Calibration standards in surrogate CSF. QC samples sets in surrogate and native CSF.

| Precision and Accuracy | Prepared in surrogate CSF (1% plasma supplemented PBS) | | | | | Prepared in native monkey CSF | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LLOQ QC | LQC | MQC | HQC | ULOQ QC | LLOQ QC_CSF | LQC_CSF | MQC_CSF | HQC_CSF | ULOQ QC_CSF |
| | 40 pg/mL | 100 pg/mL | 2000 pg/mL | 150000 pg/mL | 200000 pg/mL | 40 pg/mL | 100 pg/mL | 2000 pg/mL | 150000 pg/mL | 200000 pg/mL |
| Replicate – 1 | 41.1 | 112 | 1900 | 164000 | 200000 | 34.4 | 102 | 2070 | 163000 | 200000 |
| Replicate – 2 | 36.6 | 109 | 2090 | 175000 | 193000 | 34.7 | 99.1 | 1990 | 162000 | 192000 |
| Replicate – 3 | 37.3 | 109 | 1960 | 165000 | 205000 | 38.7 | 106 | 2220 | 156000 | 203000 |
| Intra-run Mean | 38.3 | 110 | 1980 | 168000 | 199000 | 35.9 | 102 | 2090 | 160000 | 198000 |
| Intra-run SD | 2.42 | 1.73 | 97.1 | 6080 | 6030 | 2.40 | 3.46 | 117 | 3790 | 5690 |
| Intra-run %CV | **6.3** | **1.6** | **4.9** | **3.6** | **3.0** | **6.7** | **3.4** | **5.6** | **2.4** | **2.9** |
| Intra-run %Bias | **-4.2** | **10.0** | **-1.0** | **12.0** | **-0.5** | **-10.2** | **2.4** | **4.5** | **6.7** | **-1.0** |
| Intra-run %Total Error | 10.5 | 11.6 | 5.9 | 15.6 | 3.5 | 16.8 | 5.8 | 10.1 | 9.0 | 3.9 |
| n | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

**Table 4.** Dilutional linearity of QC sample (Cmax) in native CSF using surrogate CSF as diluent.

| Dilution Linearity | Nominal concentration – 5,000,000,000 pg/mL | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1X | 10X | 100X | 1,000X | 10,000X | 100,000X | 1,000,000X |
| | Measured concentration (pg/mL) | | | | | | |
| Replicate – 1 | >ULOQ | >ULOQ | >ULOQ | >ULOQ | >ULOQ | 5150000000 | 4320000000 |
| Replicate – 2 | >ULOQ | >ULOQ | >ULOQ | >ULOQ | >ULOQ | 5260000000 | 4450000000 |
| Replicate – 3 | >ULOQ | >ULOQ | >ULOQ | >ULOQ | >ULOQ | 4640000000 | 4810000000 |
| Mean | NA | NA | NA | NA | NA | 5020000000 | 4530000000 |
| S.D. | NA | NA | NA | NA | NA | 331000000 | 254000000 |
| n | 1 | 3 | 3 | 3 | 3 | 3 | 3 |
| % CV | NA | NA | NA | NA | NA | **6.6** | **5.6** |
| % Bias | NA | NA | NA | NA | NA | **0.4** | **-9.4** |

**Cross-validation of native CSF and surrogate CSF methods:**

- Specific workflow used for method cross-validation:

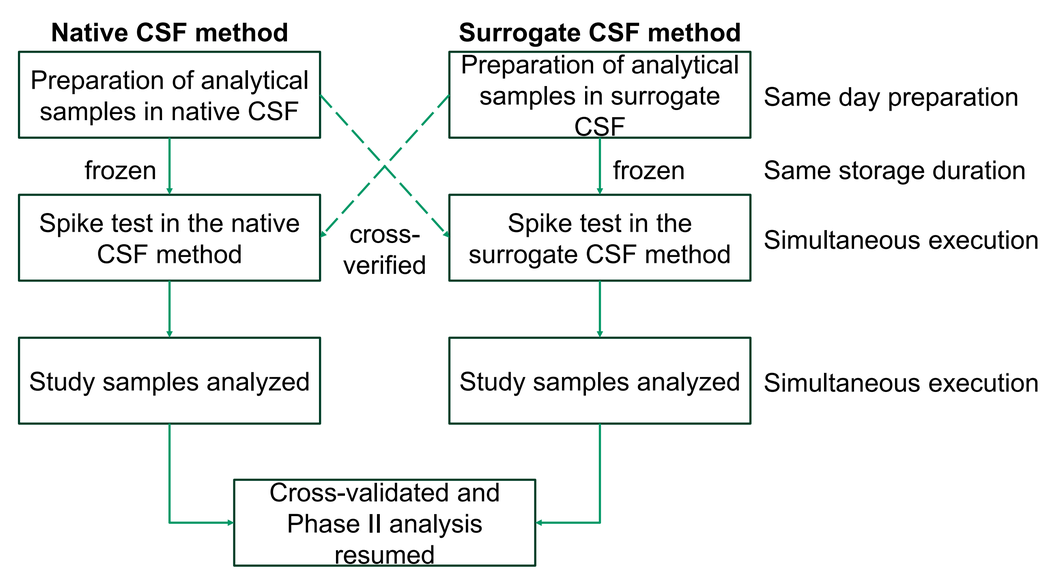


**Figure 2.** Cross-validation work-flow.

- Cross-validation first performed using QC samples.
- Cross-validation next performed using n≥30 study samples.
- All QC and study samples met acceptance criteria (100%).

## CONCLUSIONS

- Serum or plasma (at 1% v/v) supplemented PBS mimics native CSF performance in bioanalytical methods for oligonucleotide therapeutics.
- This similarity in performance extends outside the analytical range, as demonstrated with dilution linearity up to 1,000,000-fold.
- The presented clinical study demonstrates the ease of transferring validated native CSF methods to surrogate CSF methods without extensive changes in the methodology.
- Surrogate CSF preparations perform consistently across different lots, enhancing method robustness and reliability.