Concordance and repeatability tests to evaluate lab performance

Introduction
The purpose of the concordance validation was to evaluate if the same high quality of data could be obtained at Olink™-trained labs as at Olink Analysis Service labs, to ensure they can provide stable and accurate results that fulfill the Olink QC acceptance criteria.

Study design
The same set of samples was run on Olink™ Target 48, Olink™ Target 96 and Olink™ Explore panels at Sequanta Technologies in Shanghai, China, and at Olink Analysis Service in Boston, US. Operator 1 from Sequanta ran both the Target and Explore panels, while Operator 2 ran the Target panels only, and is therefore noted as N/A in the Explore entries in the results tables.

The detectability, CV and correlation were compared between the two sites and the evaluation was performed by the Olink Data Science team.

Samples
The sample set consisted of samples from patients with various diseases, as well as healthy donors. See Figure 1 for more information about samples types.

Table 1. The sample distribution for the different Olink panels.

<table>
<thead>
<tr>
<th>Olink™ panel</th>
<th>Number of samples</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target 48 Cytokine</td>
<td>16 samples</td>
<td>12 patients</td>
</tr>
<tr>
<td></td>
<td>(14 plasma and 2 serum)</td>
<td>4 healthy donors</td>
</tr>
<tr>
<td>Target 96 Neuro Exploratory</td>
<td>16 samples</td>
<td>12 patients</td>
</tr>
<tr>
<td></td>
<td>(14 plasma and 2 serum)</td>
<td>4 healthy donors</td>
</tr>
<tr>
<td>Target 96 Oncology II</td>
<td>16 samples</td>
<td>12 patients</td>
</tr>
<tr>
<td></td>
<td>(14 plasma and 2 serum)</td>
<td>4 healthy donors</td>
</tr>
<tr>
<td>Explore 384 Cardiometabolic</td>
<td>40 samples</td>
<td>30 patients</td>
</tr>
<tr>
<td></td>
<td>(36 plasma and 4 serum)</td>
<td>10 healthy donors</td>
</tr>
</tbody>
</table>

Results
All runs passed the Olink QC criteria. Refer to the software user manuals for Olink NPX Signature and Olink MyData Cloud for detailed information about the different QC criteria.

Detectability
Detectability of each assay was calculated as the percentage of samples with results above the estimated limit of detection (LOD), or, in the case of Olink Target 48, above the lowest quantifiable level (LQL).

Table 2. The average detectability of each panel by lab and operator

<table>
<thead>
<tr>
<th>Olink™ panel</th>
<th>Olink Analysis Service lab</th>
<th>Sequanta Operator 1</th>
<th>Sequanta Operator 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target 48 Cytokine</td>
<td>86%</td>
<td>87%</td>
<td>86%</td>
</tr>
<tr>
<td>Target 96 Neuro Exploratory</td>
<td>84%</td>
<td>80%</td>
<td>81%</td>
</tr>
<tr>
<td>Target 96 Oncology II</td>
<td>97%</td>
<td>99%</td>
<td>99%</td>
</tr>
<tr>
<td>Explore 384 Cardiometabolic</td>
<td>94%</td>
<td>97%</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Intra CV comparison
The coefficient of variance (CV) was calculated on a per assay basis, using the control samples that were included in each run (see Table 3 and 4). For this calculation, data below LOD or LQL was excluded.

Table 3. The average CV of each panel by lab and operator.

<table>
<thead>
<tr>
<th>Olink™ panel</th>
<th>Olink Analysis Service lab</th>
<th>Sequanta Operator 1</th>
<th>Sequanta Operator 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target 48 Cytokine</td>
<td>5.1%</td>
<td>3.2%</td>
<td>3.7%</td>
</tr>
<tr>
<td>Target 96 Neuro Exploratory</td>
<td>5.0%</td>
<td>6.6%</td>
<td>7.1%</td>
</tr>
<tr>
<td>Target 96 Oncology II</td>
<td>3.2%</td>
<td>3.1%</td>
<td>4.1%</td>
</tr>
<tr>
<td>Explore 384 Cardiometabolic</td>
<td>13.6%</td>
<td>7.5%</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Correlation

Correlations of NPX values between Analysis Service and Sequanta were calculated on a per assay basis for Olink Target 96 and Olink Explore. For Olink Target 48, the correlation of absolute concentrations in pg/mL was calculated. Each operator’s correlations were calculated separately. For this calculation, data below LOD or LQL was excluded.

Table 5. The average correlation values ($r$, Pearson correlation) of each panel by lab and operator.

<table>
<thead>
<tr>
<th>Olink™ panel</th>
<th>Olink Analysis Service &amp; Sequanta Operator 1</th>
<th>Olink Analysis Service &amp; Sequanta Operator 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target 48 Cytokine</td>
<td>0.96</td>
<td>0.97</td>
</tr>
<tr>
<td>Target 96 Neuro Exploratory</td>
<td>0.85</td>
<td>0.91</td>
</tr>
<tr>
<td>Target 96 Oncology II</td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>Explore 384 Cardiometabolic</td>
<td>0.94</td>
<td>N/A</td>
</tr>
</tbody>
</table>

PCA plots

Scatter plots along the two principal components were generated to compare global data performance. Figure 1 displays PCA plots from both labs combined to evaluate sample clustering across labs. When looking at the PCA of results from both labs combined, the same samples tend to cluster with themselves suggesting that the labs are performing similarly.

Conclusion

The results showed that Sequanta could produce the same high quality data as the Olink Analysis Service lab. The small variances seen can be explained by sample shipment and storage conditions as well as differences between kit lots.

Table 4. Quantified values and CVs of IL18 for each sample run on Olink™ Target 48 in both labs. CVs are presented for all three values from both labs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Olink Analysis Service (pg/mL)</th>
<th>Sequanta Operator 1 (pg/mL)</th>
<th>Sequanta Operator 2 (pg/mL)</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy serum</td>
<td>242</td>
<td>247</td>
<td>251</td>
<td>1.7%</td>
</tr>
<tr>
<td>ALS</td>
<td>380</td>
<td>415</td>
<td>392</td>
<td>4.6%</td>
</tr>
<tr>
<td>Healthy serum</td>
<td>492</td>
<td>442</td>
<td>441</td>
<td>6.4%</td>
</tr>
<tr>
<td>Solid tumor cancer</td>
<td>183</td>
<td>207</td>
<td>198</td>
<td>6.2%</td>
</tr>
<tr>
<td>Healthy plasma</td>
<td>309</td>
<td>308</td>
<td>290</td>
<td>3.5%</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>597</td>
<td>594</td>
<td>566</td>
<td>2.9%</td>
</tr>
<tr>
<td>Healthy plasma</td>
<td>153</td>
<td>147</td>
<td>134</td>
<td>6.6%</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>631</td>
<td>711</td>
<td>615</td>
<td>7.8%</td>
</tr>
<tr>
<td>CAD</td>
<td>209</td>
<td>225</td>
<td>206</td>
<td>4.8%</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>427</td>
<td>553</td>
<td>538</td>
<td>13.6%</td>
</tr>
<tr>
<td>Migraines, Hypertension</td>
<td>869</td>
<td>931</td>
<td>875</td>
<td>3.8%</td>
</tr>
<tr>
<td>Type II Diabetic donor</td>
<td>372</td>
<td>371</td>
<td>354</td>
<td>2.9%</td>
</tr>
<tr>
<td>Hypertension, Atrial fibrillation</td>
<td>299</td>
<td>294</td>
<td>280</td>
<td>3.4%</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>377</td>
<td>390</td>
<td>373</td>
<td>2.4%</td>
</tr>
<tr>
<td>Alzheimers disease</td>
<td>344</td>
<td>419</td>
<td>351</td>
<td>11.2%</td>
</tr>
<tr>
<td>Gout</td>
<td>623</td>
<td>737</td>
<td>704</td>
<td>8.5%</td>
</tr>
</tbody>
</table>
**Olink™ Target 48**

**Cytokine**

- ALS
- Alzheimer's Disease
- Breast Cancer
- CAD
- Follicular Lymphoma
- Gastric

**Sample Type**

- Healthy_Serum
- Healthy_Plasma
- CAD
- Breast
- Cancer
- Atopic
- ALS

**Description**

- Rheumatoid Arthritis
- Hypertension
- Migraines,
- Cancer
- Lung
- Atrial Fibrillation

**Olink™ Explore**

**Cardiometabolic**

- ALS
- Alzheimer's Disease
- Hepatitis C
- CAD
- IL-10
- Healthy_Serum
- Healthy_Plasma
- CAD
- Healthy_Serum
- healthy
- Healthy_Plasma
- Multiple Sclerosis
- Rheumatoid Arthritis
- Hypertension
- Migraines,

**Olink™ Target 96**

**Oncology II**

- ALS
- Alzheimer's Disease
- Breast Cancer
- CAD
- Follicular Lymphoma
- Gastric

**Sample Type**

- Healthy_Serum
- Healthy_Plasma
- CAD
- Breast
- Cancer
- Atopic
- ALS

**Description**

- Rheumatoid Arthritis
- Hypertension
- Migraines,
- Cancer
- Lung
- Atrial Fibrillation

**Neuro Exploratory**

- ALS
- Alzheimer's Disease
- Hepatitis C
- CAD
- IL-10
- Healthy_Serum
- Healthy_Plasma
- CAD
- Healthy_Serum
- healthy
- Healthy_Plasma
- Multiple Sclerosis
- Rheumatoid Arthritis
- Hypertension
- Migraines,

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**Figure 1.** PCA plots combining the Olink™ Analysis Service and Sequanta lab results to evaluate sample clustering across labs for several Olink panels, colored by sample description and shape representing lab and operator. Lines connect the same sample replicates.

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