Drug metabolizing enzymes and transporter induction can result in clinically meaningful drug interactions. It is therefore important to identify potential drug interactions early in the drug development process. Both p450 gene and enzyme induction studies are helpful in identifying p450 inducing compounds. Measuring gene expression has traditionally relied upon RNA extraction from treated hepatocytes followed by RT-qPCR. An alternative, potentially more efficient, method for measuring gene induction in this setting is the automated multiplex HTG Edge system and HTG Edge chemistry, quantitative nucleic acid protection assay (qNPA).

We evaluated HTG Edge system’s multiplex p450 enzyme gene expression assay performance by assessing repeatability across multiple days and reproducibility across multiple instruments.

## Methods

### Materials

Primary human hepatocytes (donor list DQB) were sourced from BioreclamationIVT.

### Induction

Prototypical inducers and control were used to treat hepatocytes:
- Vehicle Control (DMSO): 0.1% DMSO
- Omeprazole (OME): 50 µM
- Phenobarbital (PB): 1000 µM
- Rifampicin (RIF): 50 µM

Media removed and cells lysed in HTG Lysis Buffer after 48 hours.

### HTG Edge system Repeatability and Reproducibility Studies

Table 1: Correlations Between All Pair-Wise Replicates By Processor

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>0.96</td>
<td>0.96</td>
<td>0.93</td>
</tr>
<tr>
<td>OME</td>
<td>0.95</td>
<td>0.98</td>
<td>0.99</td>
</tr>
<tr>
<td>PB</td>
<td>0.91</td>
<td>0.95</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Overall expression across conditions: Distributions of all probe expression were similar across 3 different processors and 3 different days as demonstrated by the boxplots below.

##### Distribution of all probe expression by processor

Mean and 95% confidence intervals for fold-changes between treatment and vehicle control.

### Correlation across conditions

Pearson correlation coefficients from pairwise comparisons between processors and days were greater than or equal to 0.90 for all treatments. The majority of correlation coefficients exceeded 0.95.

### Fold change comparison across conditions

A comparison of means and 95% confidence for fold change across conditions demonstrates a high degree of precision within treatments of each p450 enzyme.

## Results

HTG Edge system & chemistry

### HTG Edge system & chemistry

Results/Reports

### Overall expression across conditions

Distributions of all probe expression were similar across 3 different processors and 3 different days, as demonstrated by the boxplots below.

### Distribution of all probe expression by day

### Transfer samples/run on HTG Edge reader

**Table 2. Correlations Between All Pair-Wise Replicates By Day**

<table>
<thead>
<tr>
<th></th>
<th>Day 1 vs 2</th>
<th>Day 1 vs 3</th>
<th>Day 2 vs 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>0.98</td>
<td>0.96</td>
<td>0.97</td>
</tr>
<tr>
<td>OME</td>
<td>0.98</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>PB</td>
<td>0.99</td>
<td>0.98</td>
<td>0.97</td>
</tr>
<tr>
<td>RIF</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
</tr>
</tbody>
</table>

### Correlation

These analyses demonstrate the reproducibility and repeatability of HTG Edge system’s multiplex p450 enzyme gene expression assay for three separate induction treatments, across 3 different processors and across 3 different days.

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