Using PamChip MLPA for the detection of European Bunyaviruses

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Study Design
Seven European Bunyaviruses, particularly (TOSV, SFSV, SFNV, TAHV, INKV, BATV and UUKV) are targeted. The viruses are prepared, hybridized and data analysis is performed, see each process outlined below:

Preparation of samples
1. Viral culture, virus isolates are grown in VeroB4 cells or VeroE6 cells.
2. RNA is extracted from viral culture.
4. MLPA probes designed to detect DNA signatures.
5. Analysis of MLPA amplicons using Gel Electrophoresis.
6. Step 3. and Step 5. are performed for quality control.

Hybridization
The samples are hybridized on a PamChip® MLPA under 5 different temperatures (50°C, 53°C, 56°C, 59°C, 62°C (i.e. temperature profile) in a PamStation®12 instrument. Images are taken during the process. (see Figure 1, white boxes highlight specific probes hybridizing to specific virus).

Data Analysis
Image analysis followed by melting point curve analysis are performed (presented in Ref1). Sensitivity and specificity of results are compared with other technologies (table 1).

Key Findings
The PamChip® MLPA application successfully detects the seven types of virus and shows good correlation in terms of sensitivity and specificity to monoplex real-RT-PCR (table 1). Detecting multiple pathogens with higher sensitivity then current techniques has been shown.

“Author Quote”
As far as I know, this is the first time there is a microarray based diagnostic kit for these types of viruses.

Background
While climate warming in Europe and global social changes have led to focusing attention to novel emerging infectious diseases, there are many hardly noticed European arboviral infections causing neurological disorders like encephalitis or meningoc-encephalitis and fevers. The aetiology of aseptic meningoc-encephalitis in 50% of the cases in Europe for example remains unclear. Developing better performing assays then the current assays is key to advancing the current knowledge of infections.

Conclusion
The PamChip® MLPA sufficiently sensitive and specific for the detection of European Bunyaviruses and this can be developed into a diagnostic tool for the rapid analysis of infections due to European Arboviruses.

References:

Table 1: Technology comparison table

<table>
<thead>
<tr>
<th>Virus/assay</th>
<th>RT-PCR</th>
<th>MLPA</th>
<th>MLPA-microarray</th>
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<tbody>
<tr>
<td>TOSV</td>
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<td>UUKV</td>
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Figure 1: Representative images of MLPA amplicon hybridisation on flow-through microarray for the detection of seven the European arboviruses, TOSV, SFSV, SFNV, TAHV, INKV, BATV and UUKV