INTRODUCTION

Interest in automation of Molecular workflow is increasing in clinical diagnostic laboratories, as it saves time in routine procedures, reduces tedious manual pipetting steps, and improves throughput and traceability.

BioMérieux solution targets the integration of the main steps of molecular diagnostics from sample to result (Extraction, PCR setup, and PCR reaction). The organization by modules (e.g. easyMAG®, easySTREAM™, amplification/detection platforms) enables to maintain and develop the required flexibility, both in terms of configuration and of compatible applications. The current NucliSens® V2 is able to connect the easyMAG® extraction platform, the easySTREAM™ and different PCR platforms to transfer relevant data from samples and test requests to the PCR run including the plate layout. In the clinical domain, this solution facilitates and supports the ARGENE product range by using dedicated assay protocols, but it is also compatible for Lab Developed Test (LDT), also called ‘Homemade’. In the marketplace, the presented solution aims to answer to the needs of Molecular Laboratories with medium/high throughputs which need to improve and develop their connectivity and automation.

The objective of this study was to verify the performances of the bioMérieux molecular automated solution, compared to manual process. The easySTREAM™ volumetric results, carry-over experiment, CMV R- gene® and EBV R-gene® on clinical samples quantification and Influenza A/B r-gene® on QCMD (Quality Control for Molecular Diagnostics) panel results are presented.

MATERIAL AND METHODS

Negative and positive clinical samples (EBV, CMV, FluA and FluB) were collected from different hospital centers or from QCMD panel. Samples were extracted on NucliSens® easyMAG® (bioMérieux) using the recommended protocol for these type of matrices (whole blood and reconstituted matrix). New improved whole blood extraction protocol (Specific B, 200/50) for whole blood and classical Specific B, 200/50 for the other matrices. 200µl of each sample (and 10µl of internal control when it applies) were placed in the easyMAP® vessel and the vessel was loaded onto the extractor. After the run, the easyMAP® vessels containing the eluates were loaded directly on a specific holder into easySTREAM™ to prepare the PCR reactions. PCR reactions were also manually prepared with the same eluates to compare performance. For both methods, PCR set up was performed as follows: 10 µl of purified nucleic acids were added to 15 µl of ready-to-use amplification/detection premix. The amplifications were performed on BioRad Dx Real Time System using the recommended protocols by bioMérieux.

All the data (samples data, PCR test requests and plate layouts) were transferred through the various elements of the automated platform using NucliSens® V2 software. An estimation of the time from samples to results has been done in parallel (data shown on the figure).

easySTREAM™ volumetric performance

<table>
<thead>
<tr>
<th>Type of volume</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>Median</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-dispense</td>
<td>5µl</td>
<td>3.3µl</td>
<td>3.09µl</td>
<td>3.10µl</td>
<td>5.80%</td>
</tr>
<tr>
<td>(Full size, dry vessel transfer) from PCR instrument</td>
<td>10µl</td>
<td>9.91µl</td>
<td>9.81µl</td>
<td>9.91µl</td>
<td>2.98%</td>
</tr>
<tr>
<td>Single-dispense</td>
<td>10µl</td>
<td>9.86µl</td>
<td>9.81µl</td>
<td>9.81µl</td>
<td>2.98%</td>
</tr>
<tr>
<td>(Full size, dry vessel transfer) from PCR instrument</td>
<td>15µl</td>
<td>14.99µl</td>
<td>14.94µl</td>
<td>14.94µl</td>
<td>2.98%</td>
</tr>
<tr>
<td>(Full size, dry vessel transfer) from PCR instrument</td>
<td>18 µl</td>
<td>17.83µl</td>
<td>17.81µl</td>
<td>17.81µl</td>
<td>2.98%</td>
</tr>
<tr>
<td>Multi-dispense</td>
<td>15µl</td>
<td>14.90µl</td>
<td>14.86µl</td>
<td>14.86µl</td>
<td>2.98%</td>
</tr>
<tr>
<td>(Full size, dry vessel transfer) from PCR instrument</td>
<td>20µl</td>
<td>19.74µl</td>
<td>19.73µl</td>
<td>19.73µl</td>
<td>2.98%</td>
</tr>
<tr>
<td>Multi-dispense</td>
<td>25µl</td>
<td>24.60µl</td>
<td>24.49µl</td>
<td>24.49µl</td>
<td>2.98%</td>
</tr>
<tr>
<td>Multi-dispense</td>
<td>50µl</td>
<td>49.20µl</td>
<td>49.00µl</td>
<td>49.00µl</td>
<td>2.98%</td>
</tr>
</tbody>
</table>

As expected, the volumetric performance were in the required specifications:
- For volume of 5µl: 10% accuracy and 10% CV
- For volumes between 5µl and 180µl: 5% accuracy and 5% CV

easySTREAM™ Carry-over (contamination test)

Mean Ct: 24.2 ± 0.11

No cross contamination observed

COMPARISON OF BIOMERIEUX WORKFLOW VS MANUAL RESULTS FOR INFLUENZA A/B QCMD PANEL

Comparison of qualitative results

- No impact on qualitative results has been identified using the easySTREAM™ instrument compared to manual PCR set up. Nevertheless, for some samples, a slight impact on Ct values or amplification profiles was observed in function of the time spent in the easySTREAM™ instrument: e.g. ≤3 h51 min for a full run of 96 wells, with no impact on qualitative clinical results.

Comparison of amplification profiles

CONCLUSIONS

The presented data demonstrate the key features necessary to provides to clinicians high-level capabilities of the “samples to results” automated bioMérieux solution:
- Automatic transfer of information between steps of the sample workflow,
- Facilitate transfer of disposables from one system to another,
- The versatile and compact easySTREAM™ Liquid Handling System, combined with the dedicated NucliSens® V2, allows a complete sample workflow from the extraction using easyMAG® instrument through automation of PCR set up using easySTREAM™ to different PCR platforms. This solution improves throughput, traceability, reproducibility and reliability and ensures the expect biological performance. It can easily be integrated in any laboratory workflow to link test requests, extraction and amplification steps, even for customers using home-brew PCR tests.

The BioMérieux automated molecular workflow provides: workflow, hand-on-time, risk of human error and time to results.

Benefits of the bioMérieux automated molecular workflow

- Sample ID transmission
- Automated matrix and assay protocol assignment
- Automated tests request transmission
- User-friendly sample generation
- Traceability
- Plate layout generation
- Vessels content transmission
- Full molecular workflow
- Support to workflow, connectivity, automation

Comparison of quantitative results

- Mean difference = 0.93 logcps/ml; SD=0.16% dispersion (mean ±2 SD)= 0.93 ± 0.32 logcps/ml
- Pearson coefficient correlation: R=0.966

The samples (in red) found negative with automated workflow but positive with manual have a viral load less than 2 log for EBV (cps/ml).

- Mean difference = 0.34 logcps/ml; SD=0.22% dispersion (mean ±2 SD)= 0.34 ± 0.68 logcps/ml
- Pearson coefficient correlation: R=0.871