Introduction

The DxFLEX flow cytometer provides quantitative and multi-parametric analysis quickly on suspended cells or other bio-particles at the cellular level. The DxFLEX flow cytometer is based on the successful CytoFLEX* research cytometer. The DxFLEX flow cytometer has been approved in China for clinical use since 2015. As a more advanced flow cytometry system, the DxFLEX flow cytometer boasts the added advantages of: (i) more fluorescent channels, (ii) high sensitivity, (iii) and a more user-friendly software over its predecessor - the FC500 flow cytometer. This clinical study evaluated the performance of the DxFLEX flow cytometer by comparing it to the FC500 flow cytometer (the predicate) using the DuraClone B27 reagent kit. The DxFLEX flow cytometer was evaluated in conjunction with DxFLEX Daily QC Fluorospheres. All validations were performed on the DxFLEX flow cytometer in its 3 lasers, 13 detectors configuration.

A product of the major histocompatibility complex, the human leukocyte antigen B27 (HLA-B27) is known to be associated with several disease states including ankylosing spondylitis, acute anterior uveitis, and Reiter syndrome (1). Flow cytometry testing has emerged as the most popular testing method used to screen for the HLA-B27 antigen (2) as it is reliable, inexpensive and relatively simple to perform (3,4). Accordingly, the use of more versatile and sensitive flow cytometry systems may prove to be of tremendous benefit to this diagnostic effort.

Materials and Methods

The DxFLEX flow cytometer system consists of Fluid Containers, a Cytometer (including CytExpert for DxFLEX software), and the optional accessory of an Autoloader (a standard 32-tube carousel). It is designed for clinical applications and offers performance, compact design, and streamlined installation and operation. Four configurations are available with up to 3 lasers and 13 detectors. The 3 lasers, 13 detectors configuration was used in this study, but only three channels (FITC, PE, and PC5.5) were validated using the DuraClone B27 application. DxFLEX Daily QC Fluorospheres (PN C39283) is a suspension of fluorescent microspheres, intended for verification of the DxFLEX flow cytometer's optical alignment and fluidics system.

* For Research Use Only. Not for use in diagnostic procedures
The DxFLEX flow cytometer Clinical Study was a method comparison study conducted by comparing the DxFLEX flow cytometer to the FC500 flow cytometer while using DuraClone B27 reagent kit for a qualitative performance evaluation. In brief, de-identified residual whole blood specimens collected in K₂EDTA were prepared using the DuraClone B27 reagent kit and samples were acquired once on the FC500, and once on DxFLEX flow cytometer. Final data were analyzed by the same operator on the site for both methods to qualitatively assess the testing result for each specimen as HLA-B27 Positive (+), HLA-B27 Negative (-), or HLA-B27 Indeterminant, which is in line with the DuraClone B27 IFU classification. Two sites were enrolled and overall a minimum of 150 HLA B27 positive (+) specimens and a minimum of 150 HLA B27 negative (-) specimens were targeted to be collected. The overall study workflow is depicted below in Figure 1.

The study endpoints were an assessment of the qualitative phenotypical agreement of the DuraClone B27 typing on the DxFLEX flow cytometer and FC500 flow cytometers including Positive Percent Agreement (PPA), Negative Percent Agreement (NPA) and Overall Percent Agreement (OPA). Two-sided 95% confidence intervals were estimated for the PPA, NPA and OPA. The target for the PPA, NPA, and OPA was 90% with the lower bound of the 95% confidence interval at 80%. The score method was used to calculate two-sided 95% confidence intervals.

DxFLEX Daily QC Fluorospheres were used to verify the optical alignment and fluidics system of the DxFLEX flow cytometer instruments. The laser delay, laser power, gain, MFI and rCV for all thirteen fluorescence channels were validated (successfully met QC acceptance criteria as per instrument IFU) on every testing day during this study.

Figure 1. Overall Study Workflow

**Statistical Analysis**

These analyses were performed to evaluate the study endpoints. The diagnostic performance of the DxFLEX flow cytometer compared to the FC500 for HLA-B27 typing was evaluated by calculating the: PPA (% of specimens HLA-B27 positive (+) by FC500 also identified as positive by DxFLEX flow cytometer, Formula 1), NPA (% of specimens HLA-B27 negative (-) by FC500 also identified as negative by DxFLEX flow cytometer, Formula 2) and the OPA (sum of the % of specimens identified HLA-B27 positive (+) and HLA-B27 negative (-) by FC500 also identified as positive or negative correspondingly by DxFLEX Flow cytometer, Formula 3). See Table 1.
Table 1. Agreement Matrix

<table>
<thead>
<tr>
<th>DxFLEX Flow cytometer</th>
<th>FC500</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ind</td>
<td>Ind</td>
</tr>
</tbody>
</table>

Formula 1: PPA = a/(a+b+c+i)
Formula 2: NPA = e/(d+e+f+h)
Formula 3: OPA = (a+e)/(a+b+c+i+d+e+f+h)

Results and Discussion

Table 2. Demographics of Enrolled and Included Patient Specimen Donors

<table>
<thead>
<tr>
<th>Gender</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>185</td>
</tr>
<tr>
<td>Female</td>
<td>192</td>
</tr>
<tr>
<td>Not Provided</td>
<td>0</td>
</tr>
<tr>
<td>Sum</td>
<td>377</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient (Donor Age)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Not provided</td>
<td>7</td>
</tr>
<tr>
<td>Infant (&gt;1 month to 2 years)</td>
<td>0</td>
</tr>
<tr>
<td>Child (&gt;2 to 12)</td>
<td>1</td>
</tr>
<tr>
<td>Adolescent (&gt;12 to 21)</td>
<td>15</td>
</tr>
<tr>
<td>Adult (&gt;21 to 65)</td>
<td>310</td>
</tr>
<tr>
<td>Geriatric (&gt;65 to 89)</td>
<td>44</td>
</tr>
<tr>
<td>Protected (&gt;89 and Older)</td>
<td>0</td>
</tr>
<tr>
<td>Sum</td>
<td>377</td>
</tr>
</tbody>
</table>

Altogether, 377 specimen data were analyzed to evaluate the performance agreement of the DxFLEX Flow cytometer and FC500 in this study. See patient specimen donor demographics in Table 2 above.

DxFLEX Daily QC Fluorospheres were used to verify the optical alignment and fluidics system of the DxFLEX Flow cytometer instruments in this study. The laser delay, laser power, gain, MFI and rCV for all thirteen fluorescence channels were validated on every testing day during this study. For the FC500, QC testing was performed using Flow-Check Fluorospheres. All QC criteria as per the instrument IFUs were met for both instruments on all testing days.

For the combined Sites, we observed that 153 specimens tested HLA-B27 positive for both instruments, and 209 tested HLA-B27 negative on both instruments (Table 3). Two (2) specimens were HLA-B27 indeterminate for both instruments. Additionally, there were 13 cases that were HLA-B27 indeterminate on the FC500, but HLA-B27 positive on the DxFLEX Flow cytometer instrument; these were classified as discordant specimens.
For the Site 1, a total of 221 specimens were enrolled, where 102 samples were HLA-B27 positive from both DxFLEX Flow cytometer and FC500 testing, and 108 samples were HLA-B27 negative for both (Table 4). A total of 11 specimens were considered as HLA-B27 indeterminant on FC500 testing, while they reported as positive on DxFLEX Flow cytometer.

For the Site 2, a total of 156 specimens were enrolled, 51 were HLA-B27 positive for both instruments, and 101 were negative for both instruments (Table 5). Two (2) specimens tested HLA-B27 indeterminant on the FC500, but tested HLA-B27 positive on the DxFLEX Flow cytometer. Another 2 specimens tested HLA-B27 indeterminant on both the FC500 and DxFLEX Flow cytometer.

After further investigation of the 11 discordant specimens from Site 1, it was determined that neither specimen age or sample age had any impact on this observed discordance. Interestingly, LDT results for these discordant specimens agreed with that of the DxFLEX Flow cytometer and were HLA-B27 positive for all 11 specimens. The 2 outstanding discordant specimens from the Site 2 study had no available LDT results.

Given these findings, we speculate that the observed discordance could be in part due to the difference in instrument sensitivity and resolution between the DxFLEX Flow cytometer and the FC500. The DxFLEX Flow cytometer being the more sensitive of the two, also has superior resolution (7 decades of resolution) compared to that of the FC500 (4 decades of resolution). Greater instrument sensitivity means that the instrument has an enhanced capability of differentiating between low fluorescence signals from populations and instrument background (5). The greater resolution of the DxFLEX Flow cytometer means that it is more effective at distinguishing dimly stained populations from unstained populations within a given sample.

Table 3. Combined Sites: Concordance of Qualitative Results

<table>
<thead>
<tr>
<th></th>
<th>HLA-B27 Positive</th>
<th>HLA-B27 Negative</th>
<th>HLA-B27 Indeterminant</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXFLEX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-B27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>153</td>
<td>0</td>
<td>13</td>
<td>166</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>209</td>
<td>0</td>
<td>209</td>
</tr>
<tr>
<td>Indeterminant</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sum</td>
<td>153</td>
<td>209</td>
<td>15</td>
<td>377</td>
</tr>
</tbody>
</table>

Table 4. Site 1: Concordance of Qualitative Results

<table>
<thead>
<tr>
<th></th>
<th>HLA-B27 Positive</th>
<th>HLA-B27 Negative</th>
<th>HLA-B27 Indeterminant</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXFLEX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-B27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>102</td>
<td>0</td>
<td>11</td>
<td>113</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>108</td>
<td>0</td>
<td>108</td>
</tr>
<tr>
<td>Indeterminant</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sum</td>
<td>102</td>
<td>108</td>
<td>11</td>
<td>221</td>
</tr>
</tbody>
</table>
Statistical analysis for specimens all together (Table 6) and for each individual site (Table 7 and Table 8) were summarized, which indicated the performance of the DxFLEX Flow cytometer met study acceptance criteria. PPA to classify the HLA positive (+) agreement between DxFLEX Flow cytometer and FC500 was 100.00% for all enrolled specimens. NPA to classify the HLA negative (-) agreement was 94.14% for all specimens combined. The overall OPA for all specimens was 96.53%. In addition, the statistical results from each individual site also indicated a comparable performance of DxFLEX while comparing to the FC500.

Table 5. Site 2: Concordance of Qualitative Results

<table>
<thead>
<tr>
<th></th>
<th>HLA-B27 Positive</th>
<th>HLA-B27 Negative</th>
<th>HLA-B27 Indeterminant</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>DxFLEX Flow cytometer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-B27 Positive (+)</td>
<td>51</td>
<td>0</td>
<td>2</td>
<td>53</td>
</tr>
<tr>
<td>HLA-B27 Negative (-)</td>
<td>0</td>
<td>101</td>
<td>0</td>
<td>101</td>
</tr>
<tr>
<td>HLA-B27 Indeterminant</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sum</td>
<td>51</td>
<td>101</td>
<td>4</td>
<td>156</td>
</tr>
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Table 6. Combined Site Performance Agreement

<table>
<thead>
<tr>
<th>Agreement</th>
<th>Estimate</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
<th>Acceptance Criteria</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA</td>
<td>100.00%</td>
<td>97.55%</td>
<td>100.00%</td>
<td>80%</td>
<td>Pass</td>
</tr>
<tr>
<td>NPA</td>
<td>94.14%</td>
<td>90.24%</td>
<td>96.55%</td>
<td>80%</td>
<td>Pass</td>
</tr>
<tr>
<td>OPA</td>
<td>96.53%</td>
<td>94.16%</td>
<td>97.96%</td>
<td>80%</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Table 7. Site 1: Performance Agreement

<table>
<thead>
<tr>
<th>Agreement</th>
<th>Estimate</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
<th>Acceptance Criteria</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA</td>
<td>100.00%</td>
<td>96.37%</td>
<td>100.00%</td>
<td>80%</td>
<td>Pass</td>
</tr>
<tr>
<td>NPA</td>
<td>90.76%</td>
<td>84.20%</td>
<td>94.76%</td>
<td>80%</td>
<td>Pass</td>
</tr>
<tr>
<td>OPA</td>
<td>95.02%</td>
<td>91.31%</td>
<td>97.20%</td>
<td>80%</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Table 8. Site 2: Performance Agreement

<table>
<thead>
<tr>
<th>Agreement</th>
<th>Estimate</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
<th>Acceptance Criteria</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA</td>
<td>100.00%</td>
<td>93.00%</td>
<td>100.00%</td>
<td>80%</td>
<td>Pass</td>
</tr>
<tr>
<td>NPA</td>
<td>98.06%</td>
<td>93.19%</td>
<td>99.47%</td>
<td>80%</td>
<td>Pass</td>
</tr>
<tr>
<td>OPA</td>
<td>98.70%</td>
<td>95.39%</td>
<td>99.64%</td>
<td>80%</td>
<td>Pass</td>
</tr>
</tbody>
</table>
Conclusion

The DxFLEX Flow cytometer demonstrated its overall improved performance by meeting the acceptance criteria based on its performance agreement with its predicate instrument. Furthermore, the DxFLEX Daily QC Fluorospheres were successfully used to verify the daily instrument performance which included laser delay, laser power, gain, MFI and rCV for all thirteen fluorescence channels.

References


