Flow cytometry for detecting resistant strains *Pseudomonas aeruginosa*

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Summary

Antibacterial drugs are the most consumed group of drugs in the modern hospitals. Standard methods of antibiotic sensitivity are labour and time-consuming, taking up to 24 hours after the pure culture is isolated (the analysis typically lasts up to 72 hours). Working out express diagnostic methods is of importance, and studies are made in various directions [March-Rossello. 2015; Golus, 2016; Smith, 2015:]. Flow cytometry is a relatively new, but popular technology, and is used in various clinical spheres, such as Immunology, Oncology, Transplantation, Microbiology, Sea Biology and Industrial Biotechnology. The potential of the analysis of various cells' parameters and that of their morphology constantly expands. So do the functional potential of the analysis automation and acceleration [Wang, 2010; Jarzembowski, 2011; Shrestha, 2011; Faria-Ramos, 2013:].

Materials and methods

Clinical strains from patients were used after their identification and check for antibiotic sensitivity with commonly used methods. The absence and presence of significant antibiotic resistance were taken in consideration. 21 *P.aeruginosa* strains were used: 11 productive MBL and 10 nonproductive MBL. The isolation of pure cultures from clinical material was performed according to the commonly accepted scheme. Strain reidentification was done with MALDI-TOF mass spectrometry method. Antibiotic sensitivity was determined using
disc-diffusing method and delusion test [by EUCAST], the latter taken as gold standard test and the comparison method.

Partec CyFlow cytometer was used. Flow cytometry fluorescent dyes were used to stain viable and dead cells. Propidium iodide (water PI solution with the final concentration of 1 μ/ml, based on the proportion of 5 mcl to 100 μl of culture, 5-minute incubation in dark) and SYTO® 9 working solution (with the final concentration of 1 μM/ml 5 mcl per 100mcl of culture, 10-minute incubation) were used.

The bacterial culture with culture concentration of 0.5 MF was placed into the Muller-Hinton broth where the target antibiotic was present: for P. aerogenosa MBL+ and MBL- differentiation 0.1 mg/ml Meropenem and 0.1 mg/ml Polymixin were used. Culture in absence of antibiotic was used for negative control, and for positive control, the similar culture treated with 70% ethanol was used. After two-hour incubation, a 100 mcl sample was dyed, added 1 ml filtered saline solution and analyzed on flow cytometer.

For method validation, relative accuracy (AC), relative sensitivity (SE) and relative specificity (SP) in comparison with the delusion test were determined. Cohen’s kappa test, which determines the measure of agreement changing from 0 to 1, at the same time accepting or rejecting H-null, and its limit of confidence, was used to evaluate the methods’ agreement.

Results and discussions

P. aerogenosa strains were represented by MBL+ and MBL- strains. In MBL+ strain group, the average minimal inhibiting Meropenem concentration was 31.7±16.9 mg/l and that of Polyxemin equaled 0.55±0.8 mg/l. Generally, MBL- population was susceptible to Meropenem (average MIC being 0.8±0.21mg/l) and to Polymixin (1.0±0.8). Cytometry test showed that under the action of Meropenem, average PI+ value equaled 17.6%±4.2 for MBL+, and in case of Polyxixin, the value was 59.2%±4.2. Susceptible MBL- strains showed the average PI+ as 68.6%±16.7 when treated with Meropenem and 59.2%±4.2 in the presence of Polymixin.

Figure 1 shows P. aerogenosa overall blank result without antibiotic treatment.

![Figure 1](image1.png)

Fig. 1. Syto PI-stained P. aerogenosa under no antibacterial impact.

Figure 2 presents the resistant strain with PI+ events comprising 16%.

![Figure 2](image2.png)

Fig. 2. Syto PI- dyed resistant P. aerogenosa strain under the treatment of antibacterial drugs.

Figure 3 shows a diagram of a highly sensitive strain with 87% of population revealing signs of damage and 43% revealed dead.
Table 1 presents pseudo positive and pseudo negative results of cytometry method.

**Table 1 – Method validation based on** *P. aerogenosa* **model.**

<table>
<thead>
<tr>
<th>Cytometry test result</th>
<th>Standard methods revealed sensitivity (S)</th>
<th>Standard methods revealed resistance (R)</th>
</tr>
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<tbody>
<tr>
<td>Antibiotic sensitivity (S) revealed</td>
<td>29</td>
<td>1</td>
</tr>
<tr>
<td>Antibiotic resistance (R) revealed</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

Relative accuracy (AC), relative specificity (SP), relative sensitivity (SE), Cohen’s kappa and their limits of confidence were calculated. The measure of agreement value of 0.720 was taken as good.

**Table 2** – Accuracy, sensitivity, specificity and measure of agreement of Cytometry method based on *P. aerogenosa* model as compared with classical antibiotic sensitivity determination methods.

<table>
<thead>
<tr>
<th></th>
<th>AC</th>
<th>SP</th>
<th>SE</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudo positive</td>
<td>88.6%</td>
<td>90.9%</td>
<td>87.8</td>
<td>0.720</td>
</tr>
<tr>
<td>Pseudo negative</td>
<td>83.81-93.39</td>
<td>82.3-99.5</td>
<td>82.2-93.4</td>
<td>0.497-0.947</td>
</tr>
</tbody>
</table>

**Conclusion**

Clinical strains of *P. aerogenosa* with both antibiotic resistant and non-resistant characteristics determined with standard methods were tested. Cohen’s test showed good agreement between standard bacteriological tests and Cytometry method. Pseudo positive results were more frequent than pseudo negative ones. The agreement equaled 88.6%, specificity comprised 90.9%, while susceptibility was 87.8%.

In general, the method under development has shown good agreement with the commonly accepted Gold standard method, delusion test. There was insignificant difference in accuracy, specificity and test sensitivity revealed, depending on the culture tested. As to the negative aspects, although the tube-test is less time and labour-consuming compared with delusion test, it is still not advantageous in comparison with disc-diffusing method. However, the reduction of time provided by the method – 2.5 hours from pure culture isolation – is an advantage. Culture plate technique (inaccessible with the Cytometer modification available in our laboratory) would reduce labour and time-consumption and can be automated. Another problem is that the reagents and filter units to minimize possible contamination are too costly. Moreover, some important reagents are not registered in Kazakhstan as those of medical purpose. All these aspects present some hindrance for the development of the method. The economic evaluation will be presented by the end of the Project.
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References


