International Interlaboratory Proficiency Testing Program for Measurement of Azole Antifungal Plasma Concentrations: a Five-Years Data Analysis


1st International Workshop on Clinical Pharmacology of Antifungal Drugs and Fungal Diseases

Berlin, 26th of April 2013
International Interlaboratory Proficiency Testing Program

Wide application of analytical methods to measure azole antifungal plasma concentrations

Validation tool when analysing antifungal plasma concentrations and enhance methods’ accuracy, precision and specificity.

- Proficiency testing program started in 2007: first initiative of an international interlaboratory QC program for the measurement of azole antifungal agents
- Rounds organized by the Dutch Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology (KKGT).
Methods overview (1 round)

**Obtain plasma**

**Establish High/Low conc.**

**Spike**

**Spiked Human Plasma**

**Perform confirmative check with ‘own’ HPLC (<5% target conc.)**

**Calculate deviation (-20% to +20% considered to be correct)**

**Send high&low spiked plasma to participating lab**

**Analysis (< 6 weeks) + details on analysis**

**Report results anonymously & give feedback on performance**

Fluconazole, Itraconazole, OH-itraconazole, Voriconazole, Posaconazole, Flucytosine (since 2010)

**Repeat round (2/year)**
Worldwide participation of laboratories

Number of laboratories:

<table>
<thead>
<tr>
<th>Country</th>
<th>2008</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>USA</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Switzerland</td>
<td>4</td>
<td>6</td>
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<tr>
<td>Spain</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Italy</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Belgium</td>
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<td>3</td>
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<tr>
<td>Germany</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>France</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Canada</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Sweden</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Korea, South</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Australia</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
| **Total**        | 35   | 57   (+38.6%)
<table>
<thead>
<tr>
<th>Method</th>
<th>Absolute</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>1238</td>
<td>55,0</td>
</tr>
<tr>
<td>LC-MS</td>
<td>977</td>
<td>43,4</td>
</tr>
<tr>
<td>GC-MS</td>
<td>5</td>
<td>0,22</td>
</tr>
<tr>
<td>UPLC</td>
<td>31</td>
<td>1,38</td>
</tr>
<tr>
<td>Total</td>
<td>2251</td>
<td>100%</td>
</tr>
</tbody>
</table>
HPLC / LC-MS analyses per drug

- HPLC overall preferred method of analysis (except for posaconazole)
- Flucytosine: Almost no labs use LC-MS (5.5%)
- Voriconazole analyzed the most (29%), flucytosine the least (4.9%)
Analysis per drug/year

- Increase in number of analyses per year for each drug
- More participations over time
- Development of new analytical methods
1 out of five analyses outside 80-120% limit

Analyses within 80-120% 2008-2012

- 80.8% of analyses within 80-120% (7.7% >120% and 11.5% <80%)

- (Very weak) negative correlation between relative inaccuracy and year of the program (r = -0.054, n=2251, p=0.012).
Hydroxy-itraconazole is most difficult to determine adequately

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>Mean ab. Inacc. (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLZ</td>
<td>12.9</td>
<td>287</td>
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<tr>
<td>ITZ</td>
<td>29.6</td>
<td>451</td>
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<tr>
<td>OH-ITZ</td>
<td>34.4</td>
<td>348</td>
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<tr>
<td>PCZ</td>
<td>30.0</td>
<td>402</td>
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<tr>
<td>VCZ</td>
<td>12.1</td>
<td>652</td>
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<tr>
<td>FLUC</td>
<td>13.0</td>
<td>111</td>
</tr>
</tbody>
</table>

- Significant effect of antifungal drug on absolute inaccuracy was found. Laboratory, method of analysis or concentration did not influence results [4-way ANOVA, $F=3.58$; $P=0.003$]

- LOW concentration (ITZ, hITZ, PCZ) yield a worse than average performance.

- HIGH concentrations (FLZ, FLUC), have a better performance.
HPLC more frequently out of bounds (absolute)

- LC-MS significantly better in total analyses within 80-120% ($\chi^2 (1, N = 2215) = 11.035$, $p < .005$).

- Mean absolute inaccuracy of 22.3% for HPLC and 19.3% for LC-MS NOT significantly different ($n = 2215$, $p=0.379$, unpaired $t$-test)
High concentrations can be determined more accurately.

- Analyses at low concentrations significantly less accurate than for the higher concentrations (75.5% versus 86.6% correct analyses, respectively; p<0.001, four-way ANOVA).
- Methods used have LLOQ higher than ‘low’ concentration for specific antifungals.
- Error forms based on CLSI guidelines: 23 respondants
- Sources of error: Clerical (C), Methodological (M), Technological (T), Equipment (E), Organizational (O) or ‘other’
Conclusions

- After 5 years of analysis by other laboratories:
  - From 33 to 57 laboratories, still growing
  - HPLC and LC-MS mostly used as method of detection (55.0% and 43.4%, respectively)
  - Increase in number of analyses/year within 80-120% margin over 5 years
  - LC-MS significantly more analyses within 80-120%
  - High concentrations measured significantly more accurate
  - Explanation for inaccuracies: concentration below LLOQ/ close to LOD
Future prospects

• Program always open for new participants (costs €250/year, 2-rounds)

• Recruit more laboratories

• Include more antifungal drugs (alphabetical order)
  – Echinocandines
    • Anidulafungin
    • Caspofungin
    • Micafungin
  – Newer azoles?
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