Newly Harmonized USP Chapters <61>, <62> and <1111>
<61> Microbial Limits Tests

<1111> Microbiological Attributes of Nonsterile Pharmaceutical Products
<61> Microbiological Examination Of Nonsterile Products: Microbial Enumeration Tests

<62> Microbiological Examination Of Nonsterile Products: Tests for Specified Microorganisms

<1111> Microbiological Quality of Nonsterile Pharmaceutical Products
Microbiological Examination Of Nonsterile Products

- **<61> Microbial Enumeration Tests:**
  - Total aerobic microbial count (TAMC)
  - Total combined yeasts and molds count (TYMC)

- **<62> Tests for specified microorganisms:**
  - *Salmonella*
  - *Pseudomonas aeruginosa*
  - *Staphylococcus aureus*
  - *Escherichia coli*
  - *Clostridia*
  - Bile-Tolerant Gram-Negative Bacteria
  - *Candida albicans*
Notable Differences Between Current Chapter <61> and Harmonized Version

Validation – Organisms for TSA

- **USP old <61>:** *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella*

New version:
- *Staphylococcus aureus* ATCC 6538 (NCIMB 9518, CIP 4.83, NBRC 13276)

- *Pseudomonas aeruginosa* ATCC 9027 (NCIMB 8626, CIP 82.118, NBRC 13275)

- *Bacillus subtilis* ATCC 6633 (NCIMB 8054, CIP 5262, NBRC 3134)
Notable Differences Between Current Chapter <61> and Harmonized Version

Validation – Organisms for SDA

- **USP old <61>:** Not mentioned
- **New version:**
  - *Candida albicans* ATCC 10231 (NCPF 3179, IP 48.72, NBRC 1594)
  - *Aspergillus niger* ATCC 16404 (IMI 149007, IP 1431.83, NBRC 9455)
Media Growth Promotion – Methodology

- *USP old <61>*: Not detailed
- New version: Use *less than* 100 CFU per media. Counts must be within 50% of control.
- For inhibitory microorganisms in selective media use *more than* 100 CFU per media. No growth must be after maximal incubation period.
Notable Differences Between Current Chapter <61> and Harmonized Version

Methodology – Membrane Filtration

- **USP old <61>:** Not Listed

- New version:
  - Transfer validated amount to two filters. Wash each filter by validated method.
  - Total Aerobic Microbial Count (TAMC) filter is placed on TSA, incubated at 30-35°C for 3 to 5 days.
  - Total Yeast and Mold Count (TYMC) filter is placed on SDA, incubated at 20-25°C for **5 to 7** days.
When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:

- $10^1$ CFU: max. acceptable count = 20;
- $10^2$ CFU: max. acceptable count = 200;
- $10^3$ CFU: max. acceptable count = 2000, and so forth.
Interpretation of results and retests:

- **USP old <61>:** Must meet specs. Retest allowed using 25 gram sample.

- **New version:** Must be within two-fold of specification for product. No retest allowed.
Notable Differences Between Current Chapter <62> and Harmonized Version

Test for *Pseudomonas aeruginosa*

- *USP old <61>*: Bring specimen up to 100 mL with TSB. Incubate. If growth, streak on Cetrimide Agar Medium. Compare colonies for characteristics given – if absent, meets specification. If suspect colonies present, streak colonies onto Pseudomonas Agar Medium for the Detection of **Fluorescein and Pseudomonas Agar for the Detection of Pyocyanin.** Compare colonies for characteristics given on these additional agars– if absent, meets specification. Confirm suspect colonies with oxidase test. Must be oxidase negative to meet specifications.
Test for *Pseudomonas aeruginosa* (continued)

- New version:
  - Inoculate a suitable amount of TSB with 1 g of sample. Incubate at 30-35°C for 18 to 24 hours.
  - If growth, streak onto Cetrimide Agar and incubate at 30-35°C for 18-72 hours. Examine colonies for distinctive morphology. **Confirm identity of grown colonies.**
Test for *Salmonella* spp

- **USP old <61>:** Bring specimen up to 100 mL with Fluid Lactose Medium. Incubate. If growth, pipet 1 mL into 10 mL: Fluid Selenite Medium, Fluid Tetrathionate Medium. Incubate 12-24 hours. Streak growth of both Fluid Selenite and Fluid Tetrathionate onto: Brilliant Green Agar, Xylose-Lysine-Deoxycholate Agar, Bismuth Sulfite Agar. Incubate for growth – examine colonies for characteristic morphology. If colonies with characteristic morphology seen, gram stain and examine for gram-negative rods. Stab-Streak colonies with gram-negative rods into a butt-slant of Triple Sugar-Iron-Agar. Incubate the slants and examine for red slants with yellow butts. If seen, product fails specification.
Test for *Salmonella* spp (continued)

- New version: Inoculate a suitable amount of TSB with 1 g of sample. Incubate at 30-35°C for 18 to 24 hours. If growth, transfer 1 mL to 10 mL *Rappaport Vassiliadis Salmonella Enrichment Broth*. Incubate at 30-35°C for 18-24 hours. If growth, streak onto *Xylose-Lysine-Deoxycholate Agar*. Examine colonies for distinctive morphology. Confirm identity of grown colonies.
Test for Bile-tolerant Gram-negative Bacteria

- *USP old* <61>: None provided
- New version: Sample preparation, test for absence and quantitative test are provided
Test for Clostridia

- **USP old <61>**: None provided
- **New version**: Specific tests for the presence of Clostridia provided
Notable Differences Between Current Chapter <62> and Harmonized Version

Test for *Candida albicans*

- *USP old* <61>: None provided
- New version: Incubate TSB at 20-25°C for 5-7 days. If growth, streak onto SDA. Incubate at 20-25°C for 2 days. Examine colonies for distinctive morphology. Confirm identity of suspect colonies. If no growth, or if confirmatory tests show absence of *C. albicans*, product passes.
The new version also contains two tables:

- **Table 1: Criteria for microbiological quality of nonsterile dosage forms**
  - Entries for total aerobic microbial counts (TAMC)
  - Entries for total combined yeasts/molds count (TYMC)
  - Entries for specified microorganisms
Table 2: Criteria for microbiological quality of nonsterile substances for pharmaceutical use

- Entries for total aerobic microbial counts (TAMC)
- Entries for total combined yeasts/molds count (TYMC)
## Notable Differences Between Current Chapter `<1111>` and Harmonized Version

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>TAMC (CFU/g or CFU/ml)</th>
<th>TYMC (CFU/g or CFU/ml)</th>
<th>Specified microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-aqueous preparations for oral use</td>
<td>$10^3$</td>
<td>$10^2$</td>
<td>Absence of <em>Escherichia coli</em> (1 g or 1 ml)</td>
</tr>
<tr>
<td>Aqueous preparations for oral use</td>
<td>$10^2$</td>
<td>$10^1$</td>
<td>Absence of <em>Escherichia coli</em> (1 g or 1 ml)</td>
</tr>
<tr>
<td>Rectal use</td>
<td>$10^3$</td>
<td>$10^2$</td>
<td>-</td>
</tr>
</tbody>
</table>
| Oromucosal use | $10^2$ | $10^1$ | Absence of *Staphylococcus aureus* (1 g or 1 ml)  
Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) |
<table>
<thead>
<tr>
<th>Use</th>
<th>Limit 1</th>
<th>Limit 2</th>
<th>Notable Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal use</td>
<td>$10^2$</td>
<td>$10^1$</td>
<td>Absence of <em>Pseudomonas aeruginosa</em> (1 g or 1 ml)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Absence of <em>Staphylococcus aureus</em> (1 g or 1 ml)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Absence of <em>Candida albicans</em> (1 g or 1 ml)</td>
</tr>
<tr>
<td>Transdermal patches</td>
<td>$10^2$</td>
<td>$10^1$</td>
<td>Absence of <em>Staphylococcus aureus</em> (1 patch)</td>
</tr>
<tr>
<td>(limits for one patch</td>
<td></td>
<td></td>
<td>Absence of <em>Pseudomonas aeruginosa</em> (1 patch)</td>
</tr>
<tr>
<td>including adhesive layer</td>
<td></td>
<td></td>
<td>and backing)</td>
</tr>
<tr>
<td>Inhalation use (special</td>
<td>$10^2$</td>
<td>$10^1$</td>
<td>Absence of <em>Staphylococcus aureus</em> (1 g or 1 ml)</td>
</tr>
<tr>
<td>requirements apply to</td>
<td></td>
<td></td>
<td>Absence of <em>Pseudomonas aeruginosa</em> (1 g or 1 ml)</td>
</tr>
<tr>
<td>liquid preparations for</td>
<td></td>
<td></td>
<td>Absence of bile-tolerant gram-negative bacteria (1 g or 1 ml)</td>
</tr>
<tr>
<td>nebulisation)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In addition to the micro-organisms listed in Table 1, the significance of other micro-organisms recovered is evaluated in terms of:

- use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract);
- nature of the product: its ability to support growth, the presence of adequate antimicrobial preservation;
- method of application;
- intended recipient: risk may differ for neonates, infants, the debilitated;
- use of immunosuppressive agents, corticosteroids;
- presence of disease, wounds, organ damage.
Table 2. Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use

<table>
<thead>
<tr>
<th>Substances for pharmaceutical use</th>
<th>TAMC (CFU/g) or (CFU/mL)</th>
<th>TYMC (CFU/g) or (CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^3$</td>
<td>$10^2$</td>
</tr>
</tbody>
</table>
Changes to <61>
- Negative control every time that the product is tested

Changes to <62>
- Negative control every time that the product is tested
- Delete indicative property testing of XLD agar with E.coli
- Clarification to Clostridium Testing

Changes effective May 1, 2009
Incubators

Current Chapter
- 20 – 25 °C
- 30 – 35 °C
- 35 – 37 °C
- 41 – 43 °C
- 43 – 45 °C

Harmonized Version
- 20 – 25 °C
- 30 – 35 °C
- 42 – 44 °C
In the United States, the tests were originally scheduled to become effective Aug. 1, 2007, but the implementation date has been postponed to May 1, 2009 on the basis of comments received by USP.
The implementation schedules

In Europe, implementation has three different schedules depending upon the situation:

- 1. Substances covered by a monograph specification: use the methods of the European Pharmacopoeia (A) until the monograph is revised and implemented (projected date: January 2009).

- 2. Substances not covered by a monograph specification: use either the methods of the European Pharmacopoeia (A) or the harmonised methods (B) until January 2010. From January 2010: use harmonised methods (B).

- 3. Preparations: use either method of the European Pharmacopoeia (A) or the harmonised method (B) of chapter 5.1.4 until January 2010. For new preparations, use of harmonised method (B) is advisable. From January 2010: Use harmonised method (B) of chapter 5.1.4 (7).
What do we need?

- Development and validation group
- 3 lots of tested material for suitability test
- The list of methods should be checked for relevance
- The method approval by the RA must not be delayed
- RA agreement and signature for revalidation (?)
- Global or local policy for expanded specification in drug products
- R&D researcher must inform us about the intended recipients
- In case of discrepancy: which specification should be used: vendors or pharmacopoeial?
Thank you