CALIFORNIA STATE UNIVERSITY SAN MARCOS

PROJECT SIGNATURE PAGE

PROJECT SUBMITTED IN PARTIAL FULLFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

PROJECT TITLE: Microbial Survey of Ajinomoto Althea’s 2011/2012 Environmental Isolates

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THE PROJECT HAS BEEN ACCEPTED BY THE PROJECT COMMITTEE IN
PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF
SCIENCE IN BIOTECHNOLOGY.

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Aseptically manufacturing pharmaceutical grade sterile drug products requires that final drug products are sterile and free of contamination. In order to ensure the facilities in which the drug products are manufactured are clean themselves, pharmaceutical manufacturing facilities must have a sound cleaning and monitoring system. This is laid out in detail by the US Pharmacopeia USP <1116> and the Food and Drug Administration (FDA) in the code of federal regulations 21 CFR 211.113 which cover proper current Good Manufacturing Practices (cGMP) regarding control of microbial growth and contamination. Monitoring or specifically environmental monitoring not just routinely, but also during aseptic processing can give important information over time showing that a facility has proper cleaning procedures implemented for aseptic processing. Whenever microbial contaminants are found above Althea’s set action limits these organisms are sent out for identification, trended, and then investigated in an action report. Trending is done to ensure the facility is still under microbial control and that no in process or drug products are affected by the isolates found. In order to determine the risk an organism poses to a final drug product a microbial survey is first performed to better assess what kind of environmental isolates are expected to be found at Ajinomoto Althea. While the FDA does not specify objectionable organisms for sterile drug products, Althea recognizes that there are specific organisms that should be tested for as outlined in the U.S. Pharmacopeia USP <1111> Acceptance Criteria for Non-Sterile Drug Products. The FDA is headed toward a risk based approach in regulating cGMP facilities. Thus a formal risk analysis on microbial contamination may be required in the near future. Before generating a risk analysis a microbial survey of all the known identified microorganisms isolated within Ajinomoto Althea cleanrooms was done showing that Althea’s microbial isolates are “normal” human flora and environmental isolates found in cleanrooms. The microbial survey report lastly serves to provide a microbial analysis based on the 3 risk criteria: microbial abundance, occurrence, and objectionability.
Microbial Survey of Ajinomoto Althea’s 2011/2012 Environmental Isolates

By: Gabriel Rodriguez
May 2013
Semester in Residence Project April 2013
Faculty Advisors
Project Chair: Dr. Betsy Reid
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Committee Member: QC Micro. Supervisor Tara Byerly
Overview

• Introduction
  • Why Microbial Survey
  • What are Objectionable Microorganisms
  • USP<1116> and 21 CFR 211
  • Contamination Concerns

• Materials/Methods
  • Equipment and Sampling
  • Identifications
  • Measurement Criteria

• Results and Discussion
• Conclusion
• What’s Next?
• Literature Cited
• Questions
Introduction: Althea Technologies

- cGMP Biologics Manufacturing facility
- Process Development (PD)
- Cell Banking
- Fermentation
- Purification
- Final Drug Product Formulation/Fill-Finish (FF)
- Quality Testing
Why Microbial Survey?

• Determine what kind of microflora are the “norm” for Althea Technologies cleanrooms.
• Important in order to assess future shifts or changes in microbial trends.
• “Norms” can be established for types and kinds of microorganisms generally isolated at Althea.
• **USP <1116>** addresses establishing normal microbial flora and identifications to assess cleanroom sanitization programs and microbial contamination trends.
Contamination Areas of Concern

- BB and Final Product
- Buffers
- API
- In Process EM
- Personnel
- Raw Materials
# Althea’s Sampling Plan and Limits

<table>
<thead>
<tr>
<th>ISO Class/ Other Samples</th>
<th>Sampling Frequency (NLT= Not Less Than)</th>
<th>Action Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>NLT once/ week</td>
<td>Air: &gt;50CFU/m³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface: &gt;25 CFU/25cm²</td>
</tr>
<tr>
<td>7</td>
<td>NLT twice/week</td>
<td>Air: &gt;10CFU/m³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface: &gt;5 CFU/25cm²</td>
</tr>
<tr>
<td>6</td>
<td>NLT twice/week</td>
<td>Air: &gt;3CFU/m³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface: &gt;3 CFU/25cm²</td>
</tr>
<tr>
<td>5</td>
<td>NLT twice/week</td>
<td>Air: ≥1 CFU/m³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface: ≥1 CFU/m³</td>
</tr>
<tr>
<td>Water: PW, WFI, CS</td>
<td>NLT once/week</td>
<td>PW: ≥100 CFU/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CS/WFI: ≥20 CFU/200mL</td>
</tr>
<tr>
<td>CCG, DI water, EM(SDA)</td>
<td>NLT once/quarter</td>
<td>Depends on ISO class sample was taken from</td>
</tr>
<tr>
<td>Product Bioburden</td>
<td>Depends on Client</td>
<td>Depends on Client</td>
</tr>
</tbody>
</table>
Materials

• Tryptic Soy Agar (TSA)
• Sabouraud Dextrose Agar (SDA)
• TSA contact plates
• R2A
• SAS-180/R2S slit sampler
• Incubators
  • 20-25°C
  • 30-35°C
• Cryovials
3 Measurement Criteria

• Abundance
  • 10,905 representative microbial CFU’s for 2011/2012.
  • Measured by number of microbial isolates per identification.
  • 1 identification is representative of 1-150 (TNTC) CFU’s.

• Occurrence
  • 2300 microbial isolates for 2011/2012.
  • Measured by how many times a specific microorganism was sent out for identification.
    • i.e. Staphylococcus sp. was sent out for ID 818 times.

• Objectionability
  • Low Risk
    • Human Flora and Cleanroom Environmental microflora
  • Medium Risk
    • Fungi, Gram Positive sporulating bacteria
  • High Risk
    • Althea’s Highly Objectionable Organisms
    • Known Pathogens
Results and Discussion
Majority of Isolates localized to ISO 8-7

Objectionable microorganisms in the Product/BB section are *Enterobacteriaceae*. They are in higher amounts as most of these microorganisms include E.Coli and other bacteria used for fermentation production and cell banking identifications.

**Iso 8-7** have the greatest distribution of Identification representations.

**Iso 6** shows a much smaller range of CFU counts.

**Iso 5** rooms are nearly all 1 CFU/identification.

**Iso 8 and 7** have highest # of CFU counts due to action level excursions in BMG.

**ISO 8 Action** = >50 CFU/m³ for Air Viables and >25CFU/25cm² for Surface Viables.

**ISO 7 Action** = >10 CFU/m³ for Air Viables and > 5 CFU/25cm² for Surface Viables.

**ISO 6 Action** = >3 CFU/m³ and >3CFU/cm² for Surface and Air Viables.

**ISO 5 Action** = ≥1 CFU/m³ and ≥1 CFU/cm² for Surface and Air Viables.
Majority of Isolates have relatively low re-occurrences and abundances

- Majority of Occurrences are rare or “isolated incidents”.
- A review of 2011/2012 trend data shows that a majority of routine environmental action identifications are one time isolated incidents.
- Table 1 shows the 4 highest # of occurrences as being part of the normal human flora. In order: Staphylococcus (818), Bacillus (276), Micrococcus (259), and Corneybacterium (200).
- High Risk occurrences are reflective of Penicillium mold counts in Building 3 and Enterobacteriaceae isolates from E.Coli fermentation production runs.
Most common Cleanroom Bacteria are Gram-positive Bacteria

### Human Flora
- **Gram Positive Cocci (GPC)**
  - *Staphylococcus sp.*
  - *S. epidermidis*
  - *S. Aureus*
  - *Micrococcus sp.*
  - *Kocuria sp.*
  - *Streptococcus sp.*

### Environmental Isolates
- **Gram Positive Rods (GPR)**
  - *Bacillus sp.*
    - *B. cereus*
  - *Paenibacillus*
  - Cornyebacterium sp.
  - Gram Negative Rods (GNR)
  - *Pseudomonas sp.*
  - *E. coli*
  - *Methylobacteria sp.*

- **Fungi**
  - *Trichocomaceae*
  - *Aspergillus sp.*
  - *Penicillium sp.*
Top 10 Cleanroom microflora

- 2,263 Total ID’s sent out
  - Representing 10,905 isolated bacteria, mold, yeast
- 24,697 CFU’s enumerated in total (about 44% identified)
- Annual Trend Reports show 196,695 EM samples taken
- Top 10 represent 68% of all Identifications.
- 127 different species

<table>
<thead>
<tr>
<th>Rank</th>
<th>Species</th>
<th># of CFU’s</th>
<th>% of total CFU’s Identified</th>
<th>% of overall samples taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus sp.</em></td>
<td>2368</td>
<td>22%</td>
<td>1.2%</td>
</tr>
<tr>
<td>2</td>
<td><em>Bacillus sp.</em></td>
<td>1134</td>
<td>10%</td>
<td>.6%</td>
</tr>
<tr>
<td>3</td>
<td><em>Aspergillus sp.</em></td>
<td>871</td>
<td>8%</td>
<td>.4%</td>
</tr>
<tr>
<td>4</td>
<td><em>Micrococcus sp.</em></td>
<td>721</td>
<td>7%</td>
<td>.4%</td>
</tr>
<tr>
<td>5</td>
<td><em>Enterobacteriaceae</em></td>
<td>518</td>
<td>5%</td>
<td>.2%</td>
</tr>
<tr>
<td>6</td>
<td><em>Penicillium sp.</em></td>
<td>504</td>
<td>5%</td>
<td>.2%</td>
</tr>
<tr>
<td>7</td>
<td>Family: Trichocomaceae</td>
<td>485</td>
<td>4%</td>
<td>.2%</td>
</tr>
<tr>
<td>8</td>
<td><em>Corynebacterium</em></td>
<td>372</td>
<td>3%</td>
<td>.2%</td>
</tr>
<tr>
<td>9</td>
<td><em>Paenibacillus sp.</em></td>
<td>229</td>
<td>2%</td>
<td>.1%</td>
</tr>
<tr>
<td>10</td>
<td><em>Kocuria sp.</em></td>
<td>195</td>
<td>2%</td>
<td>.1%</td>
</tr>
</tbody>
</table>
Althea Technologies EM data, trend reports, and this microbial survey study follow current literature trends.
• Majority of Cleanroom isolates are Gram Positive Rods (GPR) and Cocci (GPC).
• Staphylococcus, Bacillus, Micrococcus, and Corneybacterium species are most abundant microorganisms found.
  • GPC, part of normal human flora
  • GPR, part of cleanroom microflora
• Most common fungi: Penicillium, Aspergillus, Trychopyhton.
• Therefore Objectionability for most of Althea’s Identifications are low risk bacteria or medium risk molds.
• Most Abundance and Occurrence levels are low indicating most identifications are one to few time “isolated incidents”.
• High Abundance and Occurrence levels are attributed to “normal” cleanroom microflora.

Conclusion

• Althea Technologies EM data, trend reports, and this microbial survey study follow current literature trends.
  • Majority of Cleanroom isolates are Gram Positive Rods (GPR) and Cocci (GPC).
  • Staphylococcus, Bacillus, Micrococcus, and Corneybacterium species are most abundant microorganisms found.
    • GPC, part of normal human flora
    • GPR, part of cleanroom microflora
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• High Abundance and Occurrence levels are attributed to “normal” cleanroom microflora.
## Risk Analysis Parameter Suggestion

### Location and Process
(Where in the facility and process or product are contamination found)

<table>
<thead>
<tr>
<th>Location</th>
<th>Risk Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISO 8 and ISO 7</td>
<td>Low (1)</td>
</tr>
<tr>
<td>ISO 6/ Personnel</td>
<td>Medium (2)</td>
</tr>
<tr>
<td>ISO 5/Product BB</td>
<td>High (4)</td>
</tr>
</tbody>
</table>

### Abundance and Occurrence
(Total amount of CFUs recovered in a process)

<table>
<thead>
<tr>
<th>Abundance and Occurrence</th>
<th>Risk Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under Alert Level</td>
<td>Low (1)</td>
</tr>
<tr>
<td>Alert Level</td>
<td>Medium (3)</td>
</tr>
<tr>
<td>Action Level</td>
<td>High (5)</td>
</tr>
</tbody>
</table>

### Objectionability
(Risk levels to minimize contamination)

<table>
<thead>
<tr>
<th>Objectionability</th>
<th>Risk Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental Isolates/Human Flora</td>
<td>Low (1)</td>
</tr>
<tr>
<td>Mold/GNC</td>
<td>Medium (3)</td>
</tr>
<tr>
<td>Recognized/Possible Pathogens</td>
<td>High (5)</td>
</tr>
</tbody>
</table>

### Risk Classification
(Location/Process + Abundance/Occurrence + Objectionability)

<table>
<thead>
<tr>
<th>Risk Classification</th>
<th>Risk Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk score ≤ 3</td>
<td>Low</td>
</tr>
<tr>
<td>Risk score 4 – 7</td>
<td>Medium</td>
</tr>
<tr>
<td>Risk score &gt; 7</td>
<td>High</td>
</tr>
</tbody>
</table>
What Next?

• Microbial Risk Analysis based on Trend Reports and Microbial Survey
  • Then a full Sterility Risk Analysis using a Microbial Risk Analysis + Aseptic Process Risk Analysis (Already Completed).

• Further Study for more significant findings with Parameters:
  • Location, identification/pathogenicity, abundance/occurrence.

• USP <1116> defines Risk Assessment Analysis:
  • “Analysis of the identification of contamination potentials in controlled environments that establish priorities in terms of severity and frequency and that will develop methods and procedures that will eliminate, reduce, minimize, or mitigate their potential for microbial contamination of the product/container/closure system.”
Literature Cited

- Abraham, A et. al. Food and Drug Administration, Bad bug book: Foodborne pathogenic, microorganisms, and natural toxins handbook
- Environmental Protection Agency (EPA), USDA Food and Inspection Service. (2012). Microbial risk assessment guideline: Pathogenic microorganisms with focus on food and water (EPA/100/J-12/001)
Questions???