Regulatory Perspectives In-use Storage of Biologic Products: Phase Appropriate Microbial Challenge Studies

CASSS Midwest Regional Virtual Forum: Clinical-in-use Stability Studies

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Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.

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Pharmaceutical Quality

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Drugs are no different.

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Patients expect safe and effective medicine with every dose they take.
Pharmaceutical quality is assuring *every* dose is safe and effective, free of contamination and defects.
It is what gives patients confidence in their next dose of medicine.
Outline

• Regulatory landscape
  – FDA regulations and guidance on in-use stability of products, including biological products.
    • IND Phase appropriate for clinical use
    • BLA requirements to support label for commercial use
• Design of microbial challenge studies to support expiry dating based on in-use stability data
• Common issues
• Case studies
• Conclusions
FDA REGULATIONS AND GUIDANCE
Labeling

- Labeling of licensed and approved products is a requirement of the Food, Drug and Cosmetic Act (FD&C Act) and the Public Health Service Act (PHS Act).
  - Labeling is reviewed as part of a BLA, NDA, and ANDA to ensure the information is correct and safe for use.

- 21 CFR 201.57 provide specific requirements on the content and format or labeling for human prescription drug and biological products.
  - Label must contain specific direction on dilution, preparation... and administration of the dosage form, if needed.....storage conditions for stability of the reconstituted drug, when important...
21 CFR 211.137
Expiration dating

(a) To assure that a drug product meets applicable standards of identity, strength, quality, and purity at the time of use, it shall bear an expiration date determined by appropriate stability testing described in §211.166.

(c) If the drug product is to be reconstituted at the time of dispensing, its labeling shall bear expiration information for both the reconstituted and unreconstituted drug products.
(g) New drug products for investigational use are exempt from the requirements of this section, provided that they meet appropriate standards or specifications as demonstrated by stability studies during .... clinical investigations.

– Labeling of IND products shall bear expiration information for the reconstituted drug product.
21 CFR 211.166
Stability testing

• (a) There shall be a written testing program designed to assess the stability characteristics of drug products......The written program shall be followed and shall include:
  – .....(5) Testing of drug products for reconstitution at the time of dispensing (as directed in the labeling) as well as after they are reconstituted.
Guidance: ICH Q8 (R2)
Pharmaceutical Development (2009)

• More specific guidance on how to comply with the regulations:
  – Where relevant, microbial challenge testing under testing conditions that, as far as possible, simulate patient use should be performed during development and documented in this section.
Guidance: ICH Q1A(R2)
Stability Testing of
New Drug Substances and Products

• Storage conditions:
  – Stability testing of the drug product after constitution or dilution, if applicable, should be conducted to provide information for the labeling on the preparation, storage condition, and in-use period of the constituted or diluted product.
Guidance: ICH Q1A(R2) Statements/Labeling

• A storage statement should be established for the labeling ..... 
  – ....specific instructions should be provided... 
  – There should be a direct link between the label storage statement and the demonstrated stability of the drug product.
Guidance: ICH Q5C
Stability Testing of Biotechnological/Biological Products

• Stability after Reconstitution of Freeze-Dried Product
  – The stability of freeze-dried products after their reconstitution should be demonstrated for the conditions and the maximum storage period specified on containers, packages, and/or package inserts.

• Labeling
  – .....precisely defined storage temperatures are recommended. Specific recommendation should be stated…”
FDA Expectations: IND Clinical Materials

• No specific FDA guidance specifying amount of data, design of study or other details.
• Good practice to mimic the clinical preparation and usage after discussing with clinicians.
• Formal in-use stability should be collected before filing IND and data should be provided in the IND filing.
  – Without supporting data, the in-use time should be limited to 4 hours.
• The type and amount of data are risk-driven.
  – The primary concern during IND development is patient safety.
FDA Expectations: BLA Approval

• Microbial Challenge Studies:
  – An applicant should provide microbiological challenge data in Section 3.2.P.2.6 of the BLA to support the label’s recommended storage conditions of the reconstituted/diluted drug product.
  – If no data are provided the product labeling should recommend that the reconstituted/diluted product storage period is not more than **4 hours** at either room temperature or 2-8°C.
FDA recommendations provided during IND CMC meetings

• Microbiological studies in support of the post-reconstitution and/or post-dilution storage conditions:
  – Describe the test methods and results that employ a minimum countable inoculum (10-100 CFU) to simulate potential microbial contamination that may occur during dilution.
  – The test should be run at the label’s recommended storage conditions, be conducted for twice the recommended storage period, bracket the drug product concentrations that would be administered to patients, and use the label-recommended solutions.
  – Periodic intermediate sample times are recommended. Challenge organisms may include strains described in USP <51> Antimicrobial Effectiveness Testing, plus typical skin flora or species associated with hospital-borne infections.
  – Not more that a 0.5 log10 increase in microbial growth should be the acceptance criteria and no observable upward trend in growth.
  – *In lieu* of these data, the product labeling should recommend a storage period of not more than 4 hours.
RATIONALE FOR MICROBIAL CHALLENGE STUDIES
Published References

• Rationale for in-use microbial challenge studies is provided in publications:
Contamination Reports

• One systematic review of contamination rates in Europe from 2000 to 2018 in the clinical and pharmacy environment by Larmené-Beld et al. reported:
  • 100-fold higher chances of contamination when reconstitution of medication prior to administration is performed in the clinical environment compared to the pharmacy setting.
  • The overall contamination rate of doses prepared in the hospital environment was 7.47% and 0.08% for doses prepared by pharmacy staff (Eur. J. Clinical Pharmacol. 2019, 75:609-617).

  — Significant safety concern.
In-use Microbial Challenge Studies

• Justification for studies:
  – A drug product is liable to contamination with adventitious agents when a container closure system is breached during reconstitution and/or dilution.
  – Storage conditions may allow for microbial proliferation prior to patient administration.
  – Many biological product solutions after reconstitution or dilution are growth promoting.
DESIGN OF MICROBIAL CHALLENGE STUDIES
Purpose of Microbial Challenge Studies

• To provide evidence that a reconstituted or diluted biological product will not exhibit growth of accidental contaminants during in-use storage. Evidence provided by data from studies.
  – Challenge reconstituted and/or diluted products with specific microorganisms and assess growth promotion properties under storage conditions relevant to the proposed label instructions.
  – No growth is defined as no increase greater than or equal to 0.5 log 10 than the initial CFU/mL value.
    • Application of a two fold safety factor
      – Storage time is ½ time tested showing growth
    • Determine start of exponential growth
    • Establish storage time
Microbiological Challenge Studies:
Study Design

• Study should be designed to simulate operations conducted to prepare and store the reconstituted and/or diluted drug product.
  – Inoculum:
    • A reconstituted/diluted preparation is inoculated with a low inoculum (< 100 CFU/mL).
    • The inoculum should be measurable and repeatable.
  – Storage conditions:
    • The inoculated reconstituted/diluted preparation is held using the storage conditions (time and temperature) specified in the product’s proposed labeling.
  – Drug product concentrations:
    • Should bracket the drug product concentrations that would be administered to patients.
• Use the label-recommended reconstitution solutions and diluents.

Microbiological Challenge Studies: Study Design (cont.)

– Sampling:
  • Sample and test the inoculum preparation for microbial growth.
  • Periodic intermediate sample times are recommended.
  • Include sampling timepoints that are 2 times of the maximum storage and administration periods proposed in product labeling.

– Challenge microorganisms:
  • Use challenge strains described in USP <51>, and typical skin flora and species associated with nosocomial infection.

– Microbial Growth:
  • An increase in a microbial population of more than 0.5 log10 (i.e., more than a three fold increase in CFU/mL) or an upward trend in microbial counts is indicative of growth promoting conditions.
  • Assess the start of exponential growth.
• Acceptance criteria:
  — Not more than 0.5 log10 increase in microbial growth or no observable upward trend in microbial counts for at least twice the maximum storage and administration periods proposed in product labeling
Common issues

• In-use microbial studies not conducted:
  – No supporting data.
  – No data to support the microbial stability of diluted DP during long infusions times (days).
  – No data to support room temperature (RT) equilibration times for DP and DP infusions for periods longer than 4 hours and/or at temperatures above RT (e.g., 37-40°C).
• Insufficient time points included in the studies.
  – Use of interim timepoints not assessed.
  – Data not supportive of storage times longer than 4 hours.
• Microbial growth not adequately assessed.
  – Inoculum too high or too low.
  – Incorrect assessment of significant growth: Log 10 not calculated from the initial timepoint but from preceding timepoint.
CASE STUDIES
Case studies

• Case studies are derived from microbiological challenge data from various IND and BLA submissions.

• Only a few examples will be presented to illustrate the complexity of the data and highlight the need for case by case supportive data for labeling instructions.

• Examples cover storage of reconstituted lyophilized products, diluted in various diluents, manual transfer and filling of DP from vials to syringes.
Case 1:
In-use studies from a BLA

• Lyophilized monoclonal antibody (mAb) product
  – Formulated with L-histidine, L-arginine, sucrose, polysorbate 80.
  – Reconstituted with sterile water for injection (SWFI).
  – Proposed storage at 20-25°C and at 2-8°C.

• Reconstituted drug product diluted with
  – Normal saline (sterile 0.9% sodium chloride injection) or,
  – Lactated Ringer’s solution.
  – Proposed storage at 20-25°C and at 2-8°C.

• Data provided in the BLA to support in-use storage times for the reconstituted and diluted product.
Case 1:
In-use studies from a BLA (cont.)

- Microbial challenge studies were performed using USP <51> microorganisms (E. coli, P. aeruginosa, A. brasiliensis, C. albicans, S. aureus) and environmental microorganisms (S. epidermidis and E. faecalis).

- The applicant initially defined an increase in growth as more than 0.5 log10 increase than the previous value and the acceptable hold time was selected based on the storage time when no growth was observed.
  - The FDA requested that the applicant use time 0 as a comparator for an increase in growth.
  - Additionally, raw microbial data in CFU/mL was requested during the BLA review and analyzed using time 0 to assess trends.
    - Applicant repeated the in-use studies during the BLA review.
Case 1:
Lyophilized DP reconstituted with SWFI -
Growth at 20-25°C and 2-8°C

<table>
<thead>
<tr>
<th>Storage Temp.</th>
<th>Challenge</th>
<th>0h</th>
<th>4h</th>
<th>8h</th>
<th>12h</th>
<th>24h</th>
<th>48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-25°C</td>
<td>E. coli</td>
<td>27</td>
<td>20</td>
<td>21</td>
<td>23</td>
<td>33</td>
<td>127</td>
</tr>
<tr>
<td>20-25°C</td>
<td>E. faecalis</td>
<td>18</td>
<td>61</td>
<td>TNTC</td>
<td>TNTC</td>
<td>TNTC</td>
<td>TNTC</td>
</tr>
<tr>
<td>20-25°C</td>
<td>P. aeruginosa</td>
<td>14</td>
<td>13</td>
<td>10</td>
<td>7</td>
<td>16</td>
<td>36</td>
</tr>
<tr>
<td>2-8°C</td>
<td>E. coli</td>
<td>27</td>
<td>22</td>
<td>22</td>
<td>20</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>2-8°C</td>
<td>E. faecalis</td>
<td>15</td>
<td>14</td>
<td>26</td>
<td>29</td>
<td>44</td>
<td>63</td>
</tr>
<tr>
<td>2-8°C</td>
<td>P. aeruginosa</td>
<td>15</td>
<td>14</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

- **E. faecalis:**
  - Significant growth after 4 hours at 20-25°C and after 24 hours at 2-8°C.
  - Growth trending up between 12 and 44 hours at 2-8°C.
- **E. coli:**
  - Significant growth after 48 hours at 20-25°C.
- **Storage time for reconstituted DP was limited to 8 hours at 2-8 °C.**
Case 1: 
DP reconstituted with SWFI – in use stability

• Growth promoting for *E. faecalis*:
  – at 20-25°C after 4 hours and at 2-8°C after 24 hours
    • trending up after 8-12 hours at 2-8 °C
• Storage instructions for the lyophilized DP reconstituted with SWFI
  • Use immediately after reconstitution, or
  • Store for 8 hours at 2-8°C.
Case 1: Reconstituted DP Diluted in Saline - Growth at 20-25°C and at 2-8°C

- **P. aeruginosa:**
  - Significant growth at 20-25°C after 48 hours and trending up after the 24 hour timepoint.
- **E. coli:**
  - Significant growth at 20-25°C after 48 hours.
Case 1: Reconstituted DP diluted in Lactated Ringer’s Solution - Growth at 2-8°C

*E. coli:*
- Significant growth observed at 2-8°C after 24 hours
- Upward trend noted starting after 12 hours at 2-8°C.
Case 1: 
Reconstituted DP diluted – in-use stability

• Saline diluent:
  – Significant growth of *P. aeruginosa* at 20-25°C after 48 hours.
  – Trending up after the 24 hour time point.
  – Label instructions when saline is the diluent:
    • Store 24 hours at 2-8°C.
    • Store 12 hours at 20-25 °C.

• Lactated Ringer’s diluent:
  – Significant growth of *E. coli* at 2-8°C after 24 hours.
  – Growth trending up after the 12 hours at 2-8°C.
  – Label instructions when Lactated Ringer’s is the diluent:
    • Use immediately after dilution.
    • Store 6 hours at 2-8°C.
Case 2: IND product

• Lyophilized therapeutic product
  – Formulated with citrate, L-Lysine, trehalose, polysorbate 80, pH 7.0.
  – Reconstituted and diluted in saline
  – Long infusion times were required.
  – Microbial challenge studies were recommended to support proposed infusion time using *E. coli*, *P. aeruginosa*, *E. cloacae*, *S. aureus*, *M. luteus* and *C. albicans*. 
Case 2: IND Product (cont.)

DP Diluted in Saline - Growth at 20-25°C

- Significant growth of Gram negatives in saline at 20-25°C.
- Applicant conducted further challenge studies with *E. cloacae* to better characterize the growth rate.
- Studies needed to support long infusion times.
Case 2: IND Product (cont.)
DP Diluted in Saline - Growth of *E. cloacae* at 20-25°C

- Significant growth was observed between 12 and 18 hours and the growth trended up from the 12 hour timepoint.
- The applicant required various dosing and infusion times over 24 and 48 hours.
- IV bag was fitted with an in-line filter with more frequent IV bag changeout.
- Storage and transport of IV bag was restricted to 2-8°C.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>CFU/mL</th>
<th>Log10 Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>67</td>
<td>0.08</td>
</tr>
<tr>
<td>12</td>
<td>152</td>
<td>0.43</td>
</tr>
<tr>
<td>18</td>
<td>860</td>
<td>1.08</td>
</tr>
<tr>
<td>24</td>
<td>5700</td>
<td>2.01</td>
</tr>
<tr>
<td>48</td>
<td>410000</td>
<td>3.86</td>
</tr>
</tbody>
</table>
Case 2: IND product (cont.)

Instructions for use

• Label contained specific instructions to prevent accidental contamination:
  – Preparation in a USP <797> compliant facility, in an ISO class 5 laminar flow hood with appropriate EM specifications and monitoring for the admixing area, personnel required to don protective clothing and gloves, by personnel trained to prepare oncology drugs. Gloves and surfaces to be disinfected.
  – Administration required various dosing and infusion times over 24 to 48 hours.
  – IV bag fitted with an in-line filter.
  – Storage and transport of IV bag: 2-8°C, if not used immediately.
  – Infusion times:
    • Product in saline: 24 - 48 hours
    • After BLA approval, the Sponsor added a preservative to extend the infusion time.
Case 3: IND Phase 1/2b Product

- Agency requested a “Beyond Use Date” study to evaluate the microbiological stability of the prepared DP in sterile normal saline IV bags to support the extended in-use hold times in the clinical settings described in the Investigator’s Brochure (IB).
  - The DP was intended for a sick neonatal pediatric patient population at high risk for nosocomial infections.
- The sponsor submitted data and concluded:
  - “After product preparation in IV bags, the prepared DP --- may be stored at 2°C to 8°C for up to 96 hours or at room temperature (maximum 27°C) for a maximum of 6 hours total including the infusion time.”
- FDA did not agree that the data provided by the sponsor was supportive of the proposed storage time and the sponsor reduced to the in-use storage time instructions in the product label.
Case 3: IND Phase 1/2b Product

• Challenge studies were conducted as follows:
  – The DP (mAb) formulated with citrate, sodium chloride, L-arginine at pH 5.8 was diluted in saline.
  – The diluted product was challenged with a low inoculum (10-100 CFU/mL) of five USP <51> microorganisms (S. aureus, E. coli, P. aeruginosa, A. brasiliensis and C. albicans).
  – Growth was assessed after 0, 6, 24, and 48 hours at 23-27°C and after 0, 24, 48, 72, and 96 hours at 2-8°C.
Case 3: IND Phase 1/2b Product
Growth Data Submitted (23-27°C)

Table 1. 23-27°C Test Sample Results. Log10 values for recovered Challenge Microorganism

<table>
<thead>
<tr>
<th>Organism</th>
<th>0</th>
<th>6</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus brasiliensis</td>
<td>1.51</td>
<td>1.72</td>
<td>TNTC</td>
<td>TNTC</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1.77</td>
<td>1.79</td>
<td>TNTC</td>
<td>TNTC</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1.58</td>
<td>1.81</td>
<td>TNTC</td>
<td>TNTC</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1.79</td>
<td>1.88</td>
<td>TNTC</td>
<td>TNTC</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1.51</td>
<td>1.97</td>
<td>TNTC</td>
<td>TNTC</td>
</tr>
</tbody>
</table>

TNTC = Too Numerous To Count (See Section 10)

- **E. coli:**
  - A 0.46 log10 increase was observed after 6 hours.
  - Intermediate timepoints between 6 and 24 hours were not tested
  - Storage time of not more than 4 hours (including infusion time) was recommended.
Case 3: IND Phase 1/2b Product Growth Data Submitted (2-8°C)

- No significant growth of test microorganisms was observed during the 96 hour test period at 2-8°C.
- The duration of the study did not extend beyond the proposed storage time of 96 hours.
Case 3: IND Phase 1/2b Product –
In-use hold times for IND studies

• Storage time of not more that 4 hours (including infusion time) was recommended at 23-27°C.
  • A 0.46 log10 increase was observed after 6 hours and intermediate timepoints between 6 and 24 hours were not tested.
• Storage time of not more than 48 hours at 2-8°C was recommended.
  • The duration of the study did not extend beyond the proposed storage time of 96 hours.
• Current regulatory expectation is that the microbial in-use study be performed for a period at least two times longer than the proposed storage time (Metcalfe, 2009).
Case 4: BLA in use stability – DP transfer from vial to a syringe

• A DP formulated with various excipients including L-Histidine, trehalose, sucrose, PS-80 L-Methionine, WFI, pH 5.5 was filled in single-use vials.
  – The DP solution is transferred to a syringe for SC injection.
  – The applicant conducted microbial challenge studies to determine syringe storage conditions prior to use.
Case 4: BLA in use stability –
DP transfer from vial to a syringe (cont.)

• Challenge studies were conducted in accordance with published literature (Ricci et al. 2015, Metcalfe 2009 and Lolas and Metcalfe 2011.)

• Storage conditions tested were 2-8°C for up to 14 days and 20-25°C for 8 hours.

• “No growth” was defined as not more than or equal to 0.5 log_{10} unit higher than the initial value.
Case 4: BLA in use stability – DP transfer from vial to a syringe (cont.)

- **2-8°C:**
  - No increase in growth of challenge microorganisms was observed over a 14 day storage time.
  - A 7 day in-use storage time was recommended.

- **20-25°C:**
  - Significant growth of *E. coli* was observed at 8 hours.
  - This result was reproducible as the challenge study with *E. coli* was repeated by the applicant and in both cases 0.5-0.6 log10 increase in growth was observed at the 8 timepoint.
  - A storage time no longer than 4 hours was recommended.
Case 4: BLA in use stability –
DP transfer from vial to a syringe (cont.)

• Case 4 in-use stability data showed that the syringe could be stored for 7 days at 2-8°C, but only for 4 hours at 20-25 °C (ambient temperature).
  – The data indicated that the initiation of exponential growth of *E.coli* occurred around the 8 hour time point at 20-25°C.
  – The proposed storage time at 20-25°C of 4 hours was based on half the time (8 hours).
  – However, the practice of transferring product into a syringe for storage was not recommended and was not implemented.
Case 5: BLA in-use stability

• Lyophilized therapeutic protein formulated with citrate/sucrose/PS80 (pH 6.5) and reconstituted with SWFI.

• In-use challenge:
  – USP <51> microorganisms plus S. epidermidis
  – Initial microbial challenge level ≤ 100 CFU/mL
  – Storage conditions:
    • 2-8°C for 0, 24, 36, 48, 72 hours
    • 20-25°C for 0, 4, 8, 12, 16, 24, 36, 48 hours
  – Acceptance criteria: Microbial recovery of not more than a 0.5 Log increase from the control inoculum.
Case 5:
BLA in-use stability - Results

20-25°C:
- No significant growth of challenge microorganisms up to the 16 hour timepoint.
- *E. coli*: significant growth between the 16 and 24 hours and growth trending up at the 12 hour timepoint.
- *P. aeruginosa*: significant growth between 24 and 36 hours.
- Data supports label instructions for reconstituted vial storage at 20-25°C for 8 hours.

2-8 °C:
- No growth of challenge microorganisms during the 72 hour storage time in the reconstituted vial.
- Data support label instructions for storage at 2-8°C for 24 hours.
Overall conclusions

• Due to the diversity of DP formulations and conditions of dose preparation and administration, microbial challenge studies are necessary to ensure patient safety.

• Microbial ingress of contaminants can occur once the primary container closure sterile barrier system is breached.
Overall conclusions (cont.)

• During IND development, patient safety is the primary concern:
  – In-use storage times or long infusion times may need to be limited unless supportive data is generated.
  – Additional safety concerns may indicate the need for supportive data (e.g., patient population [neonatal, pediatric], administration (long and complex infusion times), etc.
• BLAs should contain microbial in-use data to support labeling instructions for storage after the primary container is breached and after the DP is reconstituted and/or diluted and stored for more than 4 hours.
• A review of several BLA submissions indicates that microbial growth can occur at both 2-8°C and at 20-25°C.
  – In general, biological formulations tend to be more growth promoting for Gram negative microorganisms.
  – Certain diluents are more growth promoting (e.g., Lactated Ringer’s).
Salus populi suprema lex esto

(the health of the people is the highest law)
Ancient precept from “Epidemics and Society), Frank Snowden
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