Avoiding Common Errors during Contamination Investigations

Kenneth H. Muhvich, Ph.D.
Principal Consultant
Micro-Reliance LLC

Sterility Assurance Failure Investigations – Importance?

- Prevent ‘bad’ product from going out the door and entering the marketplace
- Regulatory Authorities [FDA, EMA, other A’s] use the Quality of Failure Investigations performed as one METRIC by which they judge the capability of a facility to manufacture sterile products
**Important Deficiency**

*Inadequate Investigations* are consistently observed and cited during Regulatory Authority Inspections.

**Sterility Assurance Failures**

- Sterility Test Failure [obvious] – this test is very insensitive; if you have a growth-positive test that should really grab your attention!
- Media Fill contamination event
- Multiple Environmental Monitoring [EM] Alert or Action Limit Excursions
Investigation Approaches

- Need to have a ‘Common Sense’ approach, which is not very common
- Need to have a ‘Patient-focused’ approach. The great majority of sterile products are given to patients who are already very ill. “Don’t add insult to injury”
- Need to perform an aggressive, effective investigation; no easy task

Investigations – How to begin? / What to do first?

- Most people aren’t very experienced in performing failure investigations
- Facilities are for the most part under an acceptable state of contamination control
- So, many people haven’t seen a lot of Environmental Monitoring Excursions, Sterility Test or Media Fill Failures
Contamination Investigations
- So, what’s the plan?

- In many pharmaceutical companies there is no formal plan other than to follow a Chemistry test based OOS SOP and to form a cross-functional team to conduct the investigation
- There may be a checklist
- That’s OK to help begin the investigation, but the list shouldn’t be viewed as all inclusive

Check Lists - just a start

- Should be open ended so that one can think ‘outside the box’ and explore areas that have not already been identified or even thought about
- Some might include a decision tree to help get the investigation off on solid footing
Goals for the Investigation

- Determine the Source(s) of the microbial contamination
- Determine the **Root Cause** for the Contamination Event – how did the ‘bug’ [pathogenic microorganism not insect] get into the product or the highly controlled environment?

Goals for the Investigation

- One may **NOT** find the definitive Root Cause = Smoking Gun
- In my experience that **is** the typical case
- If you can find the definitive Root Cause 15-20% of the time you are doing good
Goals for the Investigation

- A ‘Probable Cause’ is the most common outcome of a contamination investigation
- One should be able to find this the majority of the time >60%
- An investigation result of ‘Possible Causes’ means that you haven’t worked hard or long enough yet

Goals for the Investigation

- Possible root causes result when a firm doesn’t look hard enough for the source of the microbial contamination
- One must locate the source of the microbial contamination to have any real chance of finding the root cause
What is the best approach?

One needs to employ a methodical approach in sterility assurance failure investigations such as that taken by police detectives, e.g., Columbo.

“WRONG Thinking”

- Definition – thought patterns that will prevent finding the source(s) of the particular microbial isolates and the root cause(s) for the contamination.
- Most common example of wrong thinking is making assumptions based upon a little bit of information or knowledge.
“WRONG Thinking”

- For example, information about the isolate from the contaminated media fill test unit is obtained ‘on line’ or from a Microbiology text book.
- The common source(s) listed are automatically assumed to be the same for the isolate from the pharmaceutical manufacturing facility.

That may not be the case at all.

- For example, just because a bacterium is ‘human borne’ doesn’t mean that was the source in any particular case.
- When the scope of the investigation is too narrow, it gives new meaning to the term ‘small minded’.
- Regulatory Authority inspectors make the same mistake and require unnecessary tasks [CAPA’s] to be performed, without any proof that they contributed to the failure event.
“WRONG Thinking”

- If you’ve seen a particular microorganism in your facility before, you think that you already ‘know’ where it came from
  - That may be true
  - But, it isn’t necessarily the case
  - The microbe may have remained undetected in a totally different area

“WRONG Thinking”

- Focusing the investigation on a really tight time frame around the date of the product or media fill that was contaminated
  - The particular microbe may have been in the facility at low levels for a long time [years], but not detected because only microbes from Alert & Action level excursions are identified
  - One may have to look at a years worth of data to figure out what is going on
“WRONG Thinking”

- Failure to conduct a thorough review of EM data for all classified areas, i.e., focusing only on the filling room in question
- Many facilities don’t have a true ‘Aseptic Core’, so microbes may be closer to the filling line than is desirable to prevent product contamination

“WRONG Thinking”

- Assuming that the filling line and the clean room surrounding it are in an adequate state of control
- “The FDA were happy with our data and said that we had a model filling operation” – this actual statement represents a failure of management to admit that the manufacturing area could be responsible for the contamination seen in the sterility test
“WRONG Thinking”

- Failure to consider the possibility that the filling line was contaminated
- “It must be the Micro Lab’s fault!”
- Assuming that the EM data used to make decisions during the investigation came from ‘worst case’ sample sites – that may **not** have been the case at all

“WRONG Thinking”

- Coming/jumping to conclusions about the contamination event without substantial information and data to support that
- Example, Propionibacterium acnes in Sterility Test failure
- “Must have come from the operators”
“The BIG Trap” - a Similar Circumstance

- You’ve seen it before at another facility with the ‘same’ microorganism
- So you assume that the source and/or root cause for the current contamination event is the same as the one that you saw before - **Wrong!**
- Easy trap to fall into
- I’ve been guilty of this myself

---

“The BIG Trap” - a Similar Circumstance

- So there is a failure to perform a thorough investigation, i.e., no depth and/or breadth
- CAPA’s are performed based upon the previous knowledge instead of the results of a thorough investigation into current events
- Leads to additional contamination events, because the source of the microbe and/or the root cause were not identified and properly mitigated
Extraordinary [investigative] Environmental Monitoring

- Extra EM performed using swabs in ‘nooks and crannies’ to look for the source of the contaminating microbe
- “You’ll never find anything!”
- “The facility has already been cleaned and sanitized 14 times since the product batch was filled”

Extraordinary [investigative] Environmental Monitoring

- Increased sampling frequency
- Sampling at sites that are hard to reach and may not be properly cleaned or sanitized [non-routine sample sites]
- Hundreds of samples may need to be taken; typically twenty (20) samples is viewed by regulatory authorities as just ‘going through the motions’
### Extraordinary/Investigative Environmental Monitoring

- “It is a waste of time, effort and resources!”
- Assumes that cleaning and sanitization was performed properly
  - Inside – Out [filling line]
  - Up and down
  - Cleanest areas to least clean

---

### Extraordinary/Investigative Environmental Monitoring

- Assumes that disinfectants used were sterile
- Assumes that disinfectants were used at the proper dilution [concentration]
- Assumes that disinfectants have been properly qualified for the current use dilution [that a meaningful disinfectant efficacy study has been performed]
Extraordinary Environmental Monitoring

- Assumes that the contact time qualified in the microbiology laboratory is actually achievable in the clean room as well
- Disinfectants have to be ‘wet’ to be effective
- Air flow in clean rooms can be substantial and the disinfectant can ‘dry off’ before the necessary contact time is achieved

Extraordinary Environmental Monitoring

- So, it may take more than one application to kill certain spore-forming microorganisms
- For example, a Paenibacillus species isolated from a sterility test required two applications of SporKlenz® to destroy its spores in a clean room setting
Observation can be KEY to Solving a Contamination Event

- A Safety Shower and Eye Wash Station
- Dead Legs!!
- Source of Gram-negative bacteria
- Can be a problem if near aseptic manufacturing process

Wrong Thinking - other examples

- Assumes that the sterility test samples were not contaminated externally during selection, handling and transport to the micro lab; so little or no decontamination required
- Assumes that triple bags of gamma-irradiated RODAC plates were properly decontaminated when transferred into the Aseptic Processing Area
Wrong Thinking - other examples

- Mold was transferred from a laboratory refrigerator to the aseptic core because of failure to properly decontaminate the outside of the bag containing RODAC plates.
- Lab technician was trying to be efficient and labeled plates in the lab on the bench top, contaminated them with mold which was then transferred to the manufacturing areas - one would assume that there in ‘no chance’ of this type of thing happening.

Wrong Assumptions

- Assume that gamma-irradiated ‘bunny suits’ were properly decontaminated when transferred into the Grade B gowning room.
- The bags containing the gowning materials could have a HUGE bioload which may have not been eliminated altogether.
Wrong Assumptions

- Assume that the 70% Isopropyl Alcohol [IPA] was suitable for material transfer decontamination at the interface of controlled and classified areas
- However, 70% IPA is not sporicidal
- Materials were not properly decontaminated – molds or Bacillus survived

Wrong Assumptions

- Assumption that all materials used in the Sterility Test were in fact sterile
- Case example – DMSO was used in a sterility test and thought to be sterile
- Used to dissolve the product so that it could be filtered through a membrane
Wrong Assumptions

- DMSO is bactericidal and readily kills vegetative cells
- But, it may **not** destroy bacterial spores
- Bacillus species was isolated from the sterility test canister
- Represents a false-positive sterility test

Wrong Assumptions

- In this particular case the manufacturing area was assumed to be at fault for the contamination
- Four (4) rooms of equipment [sterile ‘closed system’] were dismantled and sampled for viable microbes
- >1,000 samples were taken!
Wrong Assumptions

- **NO** Bacillus species were found at all
- When the sterility testing methodology was thoroughly examined it was discovered that the DMSO had *never* been sterilized, let alone by a qualified cycle

Wrong Assumptions

- Assumption that the ‘Qualified VHP Cycle’ will decontaminate 100% of the microbes existing on the outside of sterility test samples and testing materials
- This assumption ignores points of contact or mated surfaces
Wrong Assumptions

- Assumption that VHP was able to penetrate the Sterility Test isolator load
- Assumption that the loading pattern was the same as that originally qualified
- Or, that as long as you ‘can see space’ between the items in the load that the VHP will penetrate/flow everywhere it needs to go

Wrong Assumptions

- Assumption that *wiping* sterility test samples with a sporicide will be effective
- However, the necessary contact time may *not* be achieved to destroy all of the bacterial or fungal spores present on the exterior surfaces of sterility test samples
Wrong Assumptions

- Assumption that interviews with aseptic fill line operators ≥ two (2) weeks after a batch was filled will fail to yield any meaningful information.
- In my experience operators often remember ‘Oh Dear’ events that could have contributed to the microbial contamination seen, but were not recorded in the batch record.

Wrong Assumptions

- If you ask the operators, they are just as likely to remember something important that could have caused a batch contamination as not.
- I’ve seen operator interviews glean extremely important information that lead to discovery of the root cause for a microbial contamination event.
Wrong Assumptions

- Assume that the disinfectant efficacy results for compendial and ‘in-house’ isolates can be extrapolated to any adventitious ‘bugs’ that are brought into the manufacturing facility
- Increased frequency of isolation of any particular microorganism may be indicative of inadequate contact time or use of the wrong type of disinfectant

Conclusions

- Keep an open mind when conducting microbial contamination event investigations
- What you find will probably surprise or amaze you
- If you make assumptions you can go down the ‘wrong rabbit hole’ and fail to have any chance of finding the root cause for the Microbial Contamination Event observed
Conclusions

- Extraordinary/Investigative sampling and an Open Mind are the two most valuable tools that you have at your disposal when you are faced with performing an investigation for a sterility assurance failure event.