

BIOTRAK® REAL-TIME VIABLE PARTICLE COUNTER ROOT CAUSE INVESTIGATION GUIDANCE

APPLICATION NOTE CC-123 (US)

Introduction

This guidance provides recommendations for how the BioTrak® Real-Time Viable Particle Counter can be used to greatly improve the user's ability to identify a definitive root cause in the event of an airborne viable excursion. Investigations of airborne viable excursions in cleanrooms using traditional techniques notoriously fail to identify a definitive root cause. No matter how extensive the investigation, due to the limitations of the tools available, luck often plays a large role in actually identifying an elusive "smoking gun." As stated by the FDA in the *Guidance for Industry, Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice*:

"Environmental monitoring methods do not always recover microorganisms present in the sampled area. In particular, low-level contamination can be particularly difficult to detect."

One factor that plays a large role in this difficulty is the fact that active air samplers can only offer a snapshot of a very brief period of time, for a small area, making detection, especially at the low levels seen in very clean environments, a matter of being in the right place at the right time. In contrast, the BioTrak Real-Time Viable Particle Counter has two features that make it an ideal tool to use in root cause investigations:

Right Place	Right Time
The BioTrak Real-Time Viable Particle Counter has a scanning feature that can be used to scan equipment, utility penetrations, filters, etc., and provide rapid, real-time indication of a source of viable contamination.	The BioTrak Real-Time Viable Particle Counter can be set up to continuously monitor at a location to capture transient events of elevated viable contamination that can be directly correlated to the activities being performed in the area.



Integrating BioTrak Real-Time Viable Particle Counter into Root-Cause Investigations

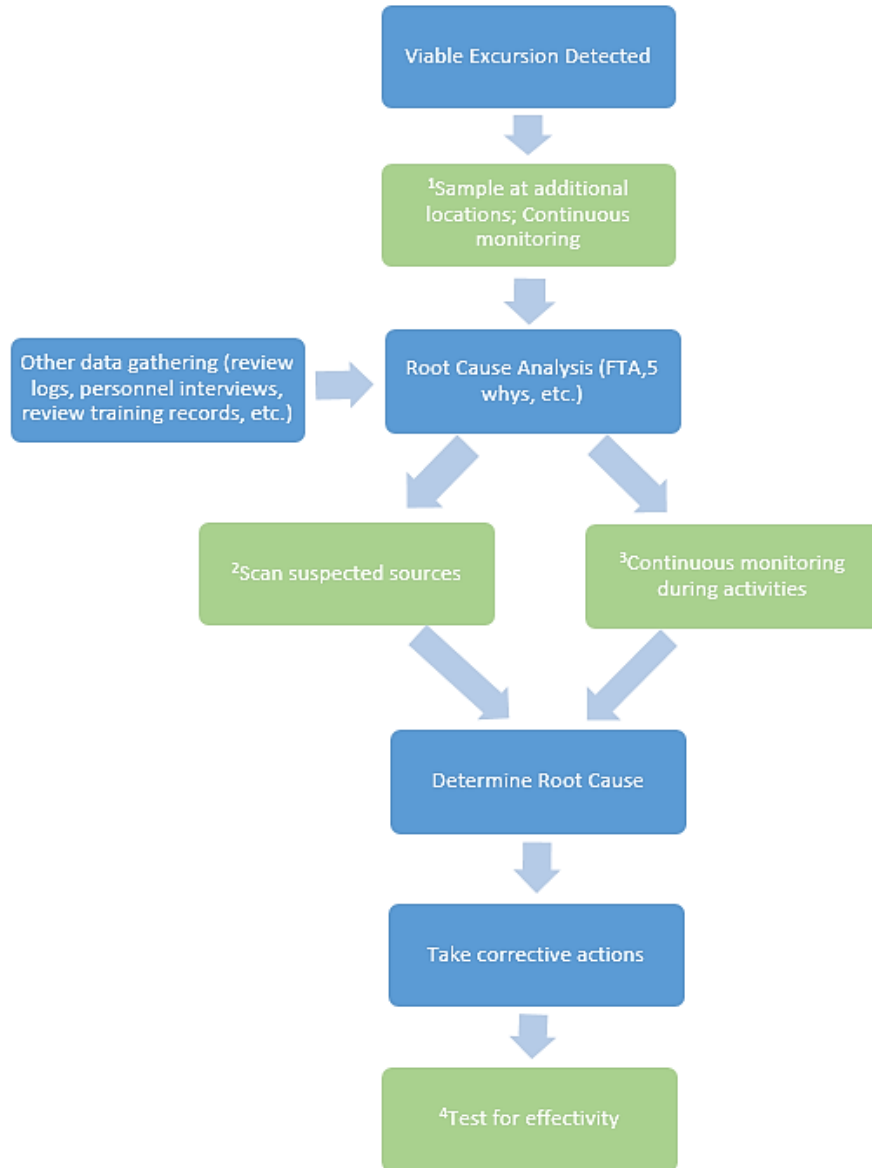


Figure 1: Root Cause Investigation Utilizing the BioTrak Real-Time Viable Particle Counter

Figure 1 is a flowchart of a typical root cause investigation process with the four green boxes indicating steps where integration of the BioTrak Real-Time Viable Particle Counter can improve the process. The following explains how the BioTrak Real-Time Viable Particle Counter would be used during these steps:

1. Sample Additional Locations; Continuous Monitoring

Routine monitoring locations may or may not have other locations in close proximity. Therefore, monitoring at additional locations around the site of the excursion is often performed to try to better pinpoint the location of the possible cause. Given the urgency usually associated with trying to resolve the issue, particle counts are often taken because, unlike active air sampling that requires plates to be incubated for multiple days, they provide real-time data. Unfortunately, total particle counts do not necessarily correlate with viable contamination and may direct the root cause analysis in the wrong direction. If viable samples are taken, either the investigation is delayed until

results are available, or there is a risk that work will need to be repeated because the initial assumptions were not correct. Sampling with the BioTrak Real-Time Viable Particle Counter resolves both of these issues by measuring the level of airborne viable contamination and doing so in real time.

The recommended procedure would be to sample the additional sites by taking 36 one-minute samples with the BioTrak Real-Time Viable Particle Counter at each location. Taking one-minute samples over that time will indicate if any viable contamination detected is continually present or if it is the result of discrete events. Sampling over a 36 minute period is also recommended because this is the amount of time required for the instrument to sample approximately one cubic meter of air. This will generate a result that is comparable to those that have historically been obtained using an active air sampler.

Box 1: What to Expect Before you Begin

The concentration of viable particles detected by the BioTrak Real-Time Viable Particle Counter will usually, if not always, be higher than the concentration of CFU detected by active air sampling except in aseptic areas where both techniques typically yield a count of zero. This is primarily due to the higher sensitivity of the detection method employed by the BioTrak Real-Time Viable Particle Counter. The BioTrak Real-Time Viable Particle Counter uses laser induced fluorescence (LIF) to detect all particles with fluorescent characteristics consistent with a viable microorganism, irrespective of its metabolic state or culturability.

Review the results obtained from sampling the additional sites to see if there is any indication that the contamination source is related to a particular area. Place the BioTrak Real-Time Viable Particle Counter at the indicated location to perform continuous monitoring. If the additional sampling did not indicate a particular location, place the BioTrak Real-Time Viable Particle Counter at the original site of the excursion. Set the sample time to one minute and the number of cycles to ∞ . Continuous monitoring will provide time related results that can be used to correlate increased contamination levels with such things as manufacturing activities, shift changes, cleaning, equipment operation, HVAC cycles, etc. Run the BioTrak Real-Time Viable Particle Counter for as long as is feasible, but, a minimum of 24 hours is recommended to try to capture as much data as possible to determine the effect of any cyclical events that occur on a daily basis. This data can then be reviewed, along with the other data compiled during the investigation, to perform a root cause analysis.

2. Scan Suspected Sources

If a possible contamination source is identified by the root cause analysis process, it can be very difficult to confirm using traditional testing methods—particle counting may not be an adequate surrogate and classical microbiology methods take days to get a result. These methods are limited in their ability to localize the contamination source as active air sampling can only give you a contamination level for a general area, while contact plates or swabs only sample a very limited area on surfaces only. In contrast, a filter scanning probe can be used with the BioTrak Real-Time Viable Particle Counter to scan a source and supply immediate feedback on airborne viable levels from a discrete location.

To perform a scan, a scanning probe needs to be attached to the BioTrak Real-Time Viable Particle Counter. If a recipe has not been created for scanning, one will need to be created. Select Beep for the Count Mode. Once started, Beep Mode will run continuously until it



is manually stopped, thus, any parameters entered on the timing tab will be ignored. Set to count in Cumulative Mode. Enable the 0.5 viable alarm and disable all other total particle and viable alarms. Hold the probe in an area away from the suspected sources and press start to determine the baseline viable particle levels for the area. Set the 0.5 alarm threshold to a level that is approximately half of the determined baseline. Press start and scan the suspected sources by moving the probe methodically over all areas of interest. Each second, the BioTrak Real-Time Viable Particle Counter will divide the actual number of viables detected by the entered threshold and emit a corresponding number of beeps. For example, if the threshold is set at 20 and 60 viable particles are detected; the BioTrak Real-Time Viable Particle Counter will beep at a rate of 3 beeps per second. Therefore, the level of viable contamination at a location can be determined by the frequency of the beeps emitted while scanning that location. Reset the alarm threshold as needed to aid in the detection process.

Box 2: Important Notes on "Beep Mode"

- I. Make sure **only one alarm** is enabled when using Beep Mode. If multiple alarms are configured, the BioTrak Real-Time Viable Particle Counter will search for which alarm to operate, starting with the smallest to largest Total Particulate Channel selected followed by the smallest to largest Viable Particulate Channel selected.
- II. Move slowly when using Beep Mode. Particles take time to traverse the tubing (up to several seconds depending on the tubing length) resulting in a delay in detection.
- III. No data is recorded while the BioTrak Real-Time Viable Particle Counter is operating in Beep Mode. If a record is desired, it is recommended to record a video while the scanning is performed. Alternatively, a quantitative result could also be obtained by running the BioTrak Real-Time Viable Particle Counter in Automatic Mode while holding the scanner at the contamination source identified through scanning. If employing this method, additional sites also need to be tested to provide context to the results.

3. Continuous Monitoring

If the root cause analysis has identified the possible root cause as being related to an event or an activity that was performed in the area, the traditional methods are once again limited in their ability to confirm this hypothesis. Besides the issues stated earlier, these methods do not provide a good way to time relate viable counts to an event that may have occurred or an activity that may have been performed during sampling. The BioTrak Real-Time Viable Particle Counter can solve these issues by sampling continuously over a long period with short sample times so that counts can be directly correlated to an event or activity in real time.

To perform continuous monitoring, set the BioTrak Real-Time Viable Particle Counter at the location of interest and set the sample time to one minute (shorter or longer sample times may also be used as needed to correlate to the activities being performed) and the number of cycles to ∞ . Perform the suspected activities or simulate the suspected event that resulted in the elevated counts. Any spikes in viable particles will provide immediate feedback to determine if an activity or event is the root cause.

4. Test for Effectivity

Once a root cause has been identified and actions have been taken to correct the issue, testing needs to be performed to confirm that those actions have been effective. Scanning and/or continuous monitoring should be repeated to verify that the issue has been resolved and the area can be immediately returned to use with confidence that the issue has truly been resolved.

Conclusion

Investigations into viable air excursions are very difficult to perform using traditional methods. The data that could be generated has been very limited, not very timely, or both. This often leads to high levels of frustration as the problem persists, with vast amounts of time and resources being consumed while the root cause remains elusive. The BioTrak Real-Time Viable Particle Counter is a powerful tool that can make this process more efficient with a much higher likelihood of being successful. By providing airborne viable counts in real time and having scanning abilities, the BioTrak Real-Time Viable Particle Counter puts you in the right place at the right time—to identify the root cause and return your room to a state of control as quickly as possible.



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