MANAGEMENT OF NON-VIABLE PARTICLE EXCURSIONS IN THE CONTEXT OF THE CURRENT ISO14644-2

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1. INTRODUCTION

Non-viable monitoring aims at evaluating extrinsic contamination close to the most critical points, in order to detect a possible degradation of the system and not at evaluating intrinsic particles generation. The current annex 1 (1) states in the "Clean room and clean air device monitoring" chapter, that "*It is accepted that it may not always be possible to demonstrate conformity with particulate standard at the point of fill when filling is in progress, due to the generation of particles droplets from the product itself*". Similarly, the current annex 1 in the chapter 9 comes back to this point, specifically for particles bigger than 5 µm.
It is a matter of facts that products generate intrinsic particles that, by nature, do not have any impact on their quality. Moreover, most used particles counters are not specific. They cannot make the difference between extrinsic and intrinsic particles.
Intrinsic particles can contribute to significantly increase the signal and, so, suggest the system is deteriorating, even if it is not the case.
But then, how to limit intrinsic particles impact on the signal? How to increase the measure specificity? How to improve monitoring relevance?
The intention of this article is to take the opportunity of the recent release of the ISO Standard 14644-2 (2), of its new chapter on alternative monitoring approaches and of the upcoming release of the new annex 1 to come back to some basics in terms of non-viable particle monitoring and to propose 3 fields of discussion for a more relevant monitoring:

- Management of non-viable particule excursions;
- Tubing between the isokinetic probe and the particle counter;
- Positioning, orientation and height of the isokinetic probe.

2. MANAGEMENT OF NON-VIABLE PARTICULE EXCURSIONS

If the microbiological environmental monitoring does not live up to all its promises, can the monitoring of non-viable particles, whether in terms of particle number or size, help in decision making? Rarely yes, often no... But it will certainly create a lot of confusion!
It is rare for viable and non-viable particles to be simultaneously non-compliant. Both physical and biological monitoring tests suffer from limits that are both opposite and complementary: unlike cultures of bacteria, yeasts, or molds on agar culture media, particle counters are calibrated instruments that are highly sensitive but absolutely not specific.
In contrast, particulate monitoring has a major drawback that distinguishes it from Pasteurian microbiological methods: results are almost instantaneous!

Besides that, non-viable particulate monitoring therefore has the huge flaw of not being specific. The intentions of this kind of monitoring are very laudable in principle. However, in practice each batch requires an opening again and again of the Pandora's Box and, in the absence of specificity and because of a high sensitivity, they generate signals that are difficult to analyze: intrinsic particles, electric peaks... Following an environmental excursion and because investigations are not always conclusive, it is tempting to compensate for this lack of specificity by recalling the precautionary principle and rejecting doubtful bottles, with the questionable result of: "The more I discard, the more of what remains is good"...

Generally, the annex 1 (1) is well informed and comprehensive: "*It is accepted that it may not always be possible to demonstrate low levels of ≥ 5.0 µm particles at the point of fill when filling is in progress, due to the generation of particles or droplets from the product itself*" and adds "*The occasional indication of ≥ 5.0 µm particle counts may be false counts due to electronic noise, stray light, coincidence, etc.*" It is reminded that a low electronic noise is acceptable with up to 5 counts per hour. It is not said that the number of counts can be reduced by 5 but that the "electronic noise" is not just a legend, may have a significant impact on the official non-viable environment and may trigger some non-relevant and time-consuming investigation.

The manufactured/filled product itself can create a mist of intrinsic micro-particles, almost always invisible, and which by nature have no impact on its quality (See drawing #1). Extrinsic particles, for their part, are from product's environment. Extrinsic particles are thus the only potential contaminants of products. This is naturally not the case for intrinsic particles. Otherwise, we would have to consider that the product can contaminate itself... In addition to this evidence, the challenge consists of making the difference between intrinsic and extrinsic particles during non-viable particle monitoring.

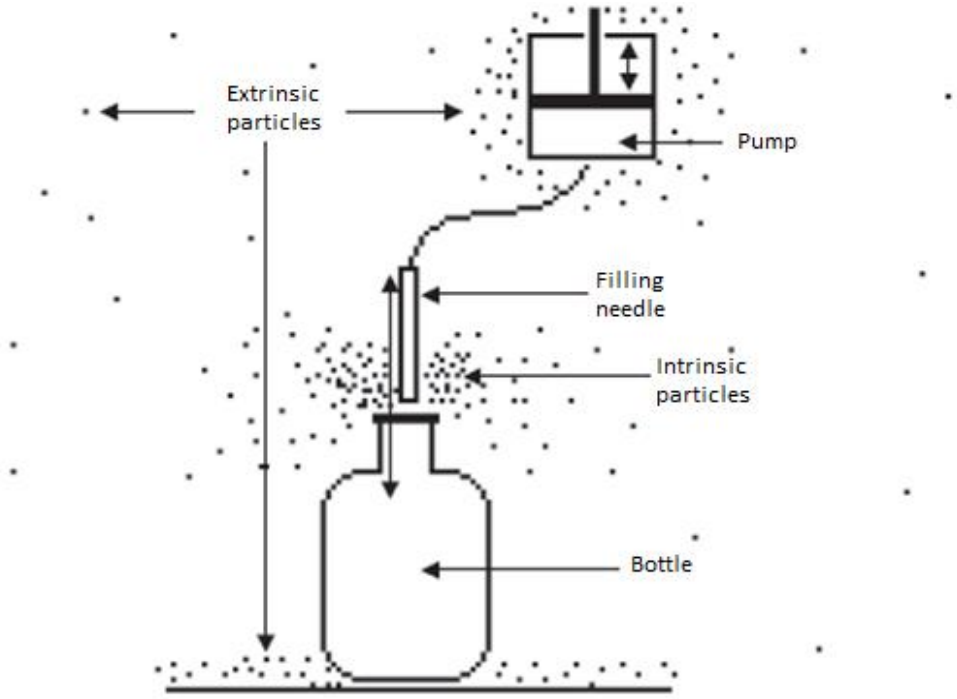

Figure1: Extrinsic versus intrinsic particles

What should we do when a peak is detected? Should we stop the aseptic activities? Should we discard all the vials potentially contaminated by some non-viable particles? While waiting for specific counters able to differentiate viable and non-viable particles, how can we avoid being blinded by "false positive" signals and separate the wheat from the chaff? The current annex 1 (1) states: "*Appropriate alert and action limits should be set for the results of particulate and microbiological monitoring*." Is this not an invitation for trend analyses? Moreover, this same annex 1 (1) also states: "...*consecutive or regular counting of low levels is an indicator of a possible contamination event and should be investigated*." This expectation is certainly of great significance:

- The reality is that an isolated peak, even a massive one (especially a massive one!), is probably much less disturbing to Qualified Persons than a succession of smaller peaks;
- Particulate contamination makes probably more sense in terms of frequency than peak size;

The presence of peaks, whether big or not, means that an investigation must be performed!

Where the current U.S.P. monograph <1116> (2) is very innovative in terms of microbiological contamination frequency, the European authorities are not less so in terms of particulate contamination frequency in the E.U. annex 1 (1) since 2009!

For all practical purposes, it is probably necessary to remember that the annex 1 (1) does not impose a strict particulate monitoring in terms of particle number. Assessing the frequency of peaks is appropriate and especially more relevant in

terms of impact assessment and decision-making for release (3). There is still an "unfortunate" assimilation between clean room classification and particulate monitoring, but the E.U. annex 1 (1) quickly dispels any confusion: "*Classification should be clearly differentiated from operational process environmental monitoring*." Moreover, the limits in the Chapter 4 of the E.U. annex 1 (1) are exclusively limited to clean room classification and never to particulate monitoring activity. If additional proof is needed (1): "*Appropriate alert and action limits should be set for the results of particulate and microbiological monitoring*." The "alternative methods" as described in the recent I.S.O. Document 14644-2 (4) goes in this direction.

Until now, this article extensively discusses particle monitoring on its quantitative aspect. Although not specific, this monitoring still presents a very small qualitative part.

Although until now the literature is not very rich in terms of relationship between particle size and origin, it is regularly heard that "large sizes," greater than 5 µm, would be of intrinsic origin, whereas particles of "small sizes" would be more of extrinsic origins.

However, the literature has for a long time reminded of the close link between the number and size of non-viable particles on one hand and the risk of microbiological contamination on the other.

In terms of number, many scientific studies have reported a correlation in ISO 5 areas between the number of non-viable and viable particles (5, 6, 7, 8 and 9) and the associated risk of product contamination (10 and 11).

As for size, Noble, Lidwell, and Kingston (12) already indicated that the larger the particle, the greater the probability that it carries a bacterium. They consider that bacteria are most often carried by particles whose equivalent diameter is usually between 4 and 20 µm. Greene, Vesley, Bond, and Michaelsen (13) have specifically studied the correlation between particle size and microbial contamination in operating blocks. They found that 75.6% of microbial contaminants are associated with particles of dimensions greater than 5 µm.

From there, it is regrettable to think that it is possible to tolerate, without question, 0.5 µm particles. An increase in peak frequency, whether involving particles of 0.5 µm or 5 µm or more, results *a priori* in environmental degradation or at least in a change in the production environment. Finally, particle size is ultimately of more interest for production people because it orients the investigation. But it is of little interest for Qualified Persons.

The advantage of non-viable particulate monitoring is certainly not to evaluate the generation of intrinsic particles or electric peaks, which are coincidences whatever the sizes of the particles or peaks. However, it is valuable to detect an extrinsic contamination close to the most critical points, to alert production people of an unusual situation, and to bring them to raise questions in order to detect a possible degradation of the system.

In the context of an investigation have you already observed unexpected particle counts with no H.V.A.C. (Heating Ventilating Air Conditioning) failure, no gas leak

and no intervention? It is difficult to investigate, isn't it? Are you sure they are due to true particles?

As explained previously, this article makes the choice not to strictly consider the number of particles but prefers to address non-viable excursions considering the number of peaks.

It is always difficult to manage a non-viable particle excursion and this chapter is a new attempt to modestly propose 2 decision trees as supportive tools to make appropriate decisions.

It is suggested to consider for both 0.5 and 5 µm particles 1 peak as an alert limit and 3 consecutive peaks as an action limit in agreement with the alternative approaches of the recent I.S.O. Standard 14644-2 (4). The 2 following decision trees are proposed to make decisions respectively in the context of alert (**Erreur ! Source du renvoi introuvable.**2) and action limits (**Erreur ! Source du renvoi introuvable.**3).

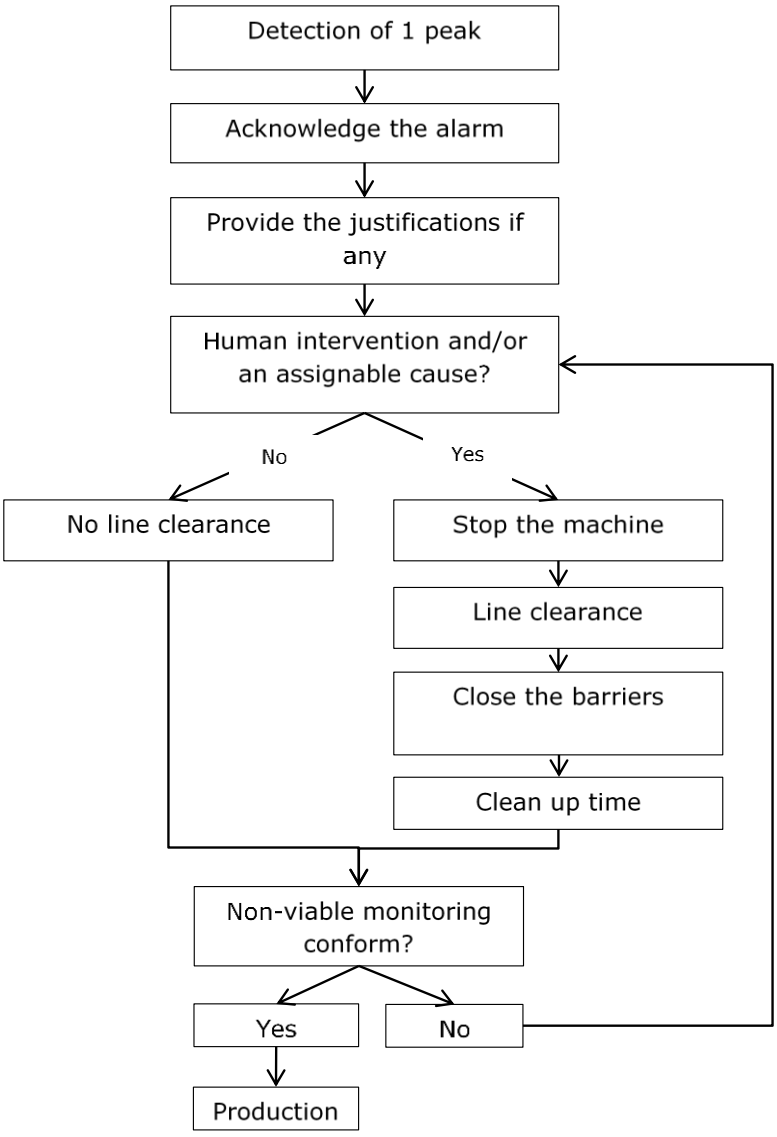

Figure 2: Decision tree, non-viable particles and alert limits

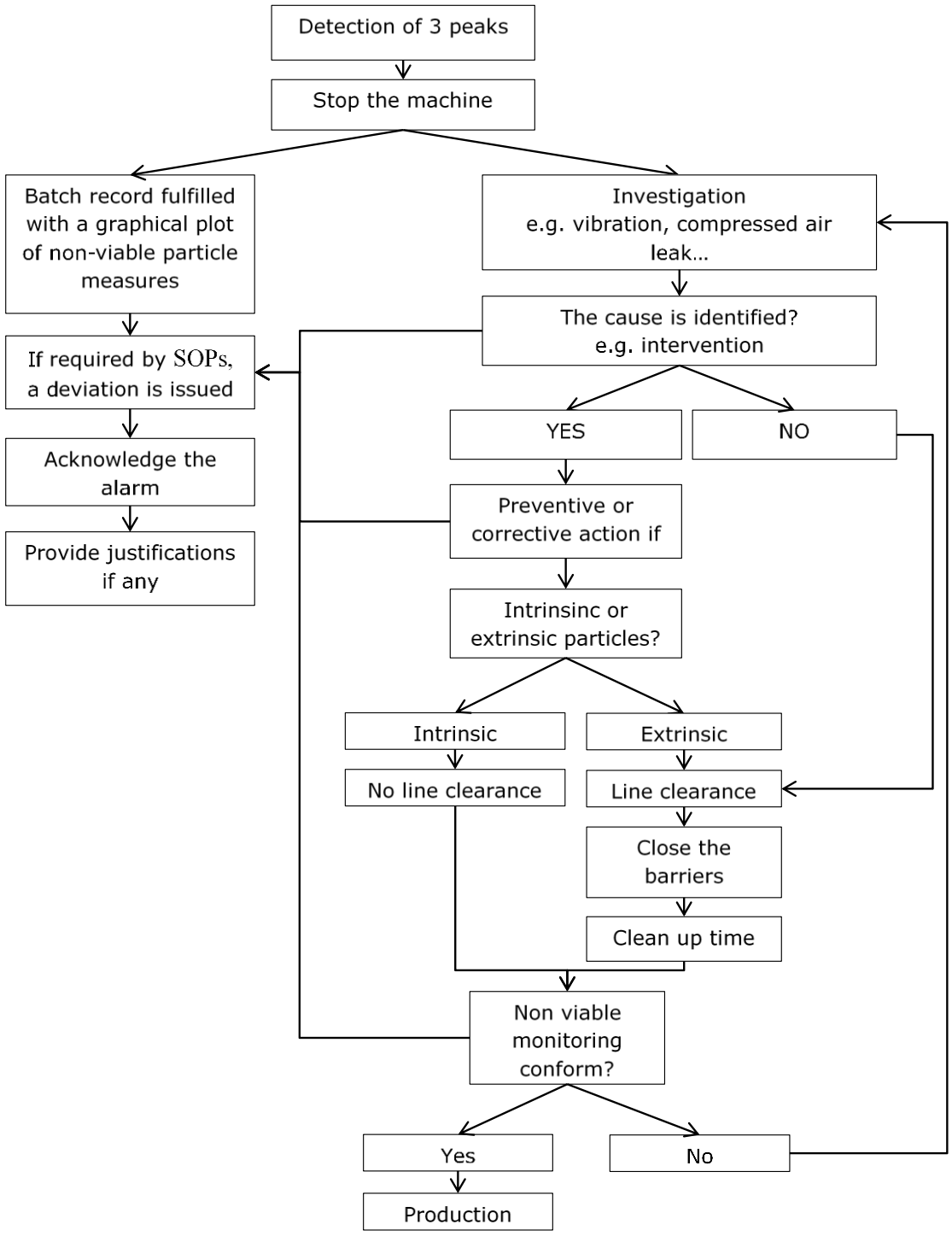

Figure 3: Decision tree, non-viable particles and action limits

3. TUBING BETWEEN THE ISOKINETIC PROBE AND THE PARTICLE COUNTER

By experience, we know that most of particle counts are actually false positives and it is absolutely not acceptable as in these conditions there is a risk to hide some less obvious true positives particle counts and of course a frightening risk not to detect a

potential source of contamination. It is even less acceptable when you understand that most of these false positives can be avoided or at least limited.
The annex 1 (1) states that *"...the length of tubing and the radii of any bends in the tubing must be considered..."* It is important to remember that the shorter and the straighter the tubing between the isokinetic probe and the particle counter, the better. Otherwise small particles can impact inside the bends and even a single small vibration of the tubing may release aggregates of particles to the counter, e.g. vibration of the product flexible due to pressure variations, opening and closure of some automatic valves, unidirectional air flow or simply the mechanical vibrations of the filling machine itself… There is no effective way to clean the inside of these tubing, therefore, it is also advised to change them on a periodic basis, e.g. once or twice a year depending of the processes. Where possible, it is also interesting to withdraw the tubing and to assemble the isokinetic probe directly to the particle counter.
In order to prevent some false positives and release some particles from the inside of the tubing between the isokinetic probe and the particle counter it is also advised in the case of mobile counters to precede any monitoring by a flush of the tubing for no less than 5 minutes.

4. PROBE POSITIONNING, ORIENTATION AND HEIGHT

The beginning of the article intended to manage excursion but it would be even better to avoid them. Most of all, priority should be given to a reduction of particles from the product generated throughout actual aseptic filling operations. Otherwise, extrinsic particles from products generating an important "fog" or "smoke" are completely diluted and represent only a small proportion of the whole signal. Monitoring would "mechanically" lose sensitivity.
The risk, then, would be that limits no longer allow detecting extrinsic particles variations and thus a degradation of the system, should it occur.
To mitigate this risk and decrease false positives, it is becoming necessary to thoroughly consider the probe positioning, and orientation, while ensuring that the possible turbulences, associated with its presence have no detrimental effects on the product.

4.1. Probe positioning

A former draft of the "sterile products produced by aseptic processing" guidance specified: "*Measurements to confirm air cleanliness in aseptic processing zones should be taken [...] at the sites where there is most potential risk to the exposed sterilized product and container-closures*."
It is obvious that in class A, the most critical zone is very close to sterile products A grade A topologic study seems to be a pre-requisite to the probe positioning, in order to look for a "worst case" position.

According to the U.S. Aseptic Process Guideline (14), the probe in grade A should be positioned at less than one foot (30 cm) from the critical zone (product, closure systems, containers).
As already mentioned, some operations can generate a high concentration of particles which, by nature, does not pose any product contamination problem.
The measure may not be feasible at less than one foot from the critical area and may not differentiate non contaminating particles from contaminating ones (14). In the latter case, the measure may be carried out at a distance sufficient to measure the true extrinsic particulate concentration representative of the product environment while getting rid of the intrinsic particulate non contaminating concentration. For example, aseptic addition of irradiated Penicillin generates a sterile dust:

- The isokinetic probe should be close enough to collect a representative sample of the air surrounding Penicillin;
- The isokinetic probe should be distant enough not to capture Penicillin particles.

4.2. Probe orientation

Documentary references are scarce. Nevertheless, the ISO Standard 14644-1, states: *"The sampling head must be placed in front of the flow. Should the direction of the flow to be sampled be uncontrolled or unpredictable (for example, a non-directional flow), the sampling head entry must be directed upwards."*
"Parallel to the flow" is expected and, such an orientation is acceptable without any justification. This orientation is intuitive as, actually, the particulate monitoring aims at detecting an extrinsic particulate contamination increase, which signs a system dysfunction. An orientation parallel to the flow, towards the flow origin, allows, this way, to capture extrinsic particles brought by the flow, while reducing, or even cancelling intrinsic particles bias.
Other orientations are possible, but they should be validated on a case by case basis, by demonstrating that particles are neither under sampled nor over sampled by the probe and the achieved results should undergo a mathematical correction.

4.3. Smoke test

Once determined the probe orientation and the distance between the probe and bottles, a smoke test cannot be considered a "bad science". Even an isokinetic probe can by its presence generate light turbulences and then concentrate very close to the product a cloud of particles picked up for example from the filling needles or from the pumps.
Smoke tests allow materializing the turbulences nearby the probe, to find the appropriate probe height and to assess the contamination risks:

- In figure 4. The probe is properly positioned:
    - Worst case between the bottle and a particles generator (a pump, for example);
    - Does not pick up the product intrinsic particles;

    - Its turbulences do not deviate the particles from the pump towards the bottle.

- In figure 5. The probe is badly positioned:
    - Worst-case between the bottle and the particles generator (the pump, for example);
    - Pick up the product intrinsic particles;
    - Its turbulences do not deviate the pump particles towards the bottle.
- Figure 6. The probe is badly positioned:
    - Worst-case between the bottle and a particles generator (the pump, for example);
    - Does not pick up the product intrinsic particles;
    - Its turbulences deviate the particles from the pump towards the bottle.

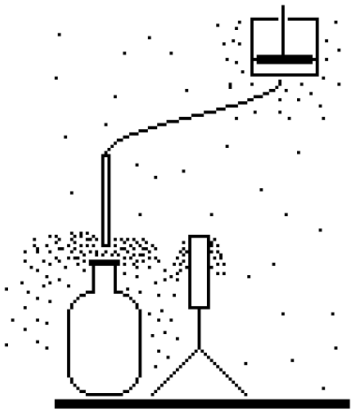

Figure 4: The probe is properly positioned

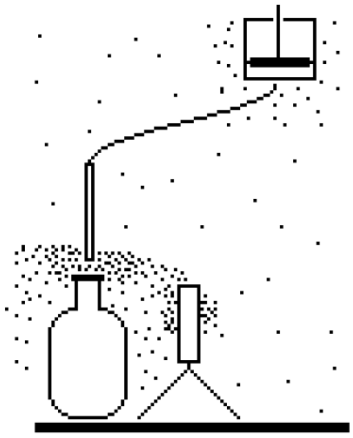

Figure 5: The probe is positioned too low

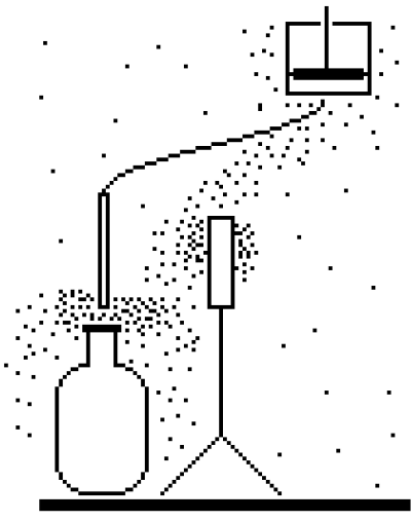

Figure 6: The probe is positioned too high

5. CONCLUSION

Monitoring a clean room, and more particularly a grade A area, is very common, but measurements representativeness, conclusions veracity and, of course, implemented actions pertinence depend on the probe positioning, orientation and height. With these parameters judiciously fixed, the particulate monitoring increases in power and becomes an incomparable tool to distinguish immediately and continuously a non-particulate event from a clean room detrimental contamination, to differentiate a simple intrinsic puff from an extrinsic avalanche and, so, reasonably decide the future of the sterile products produced by aseptic processing.

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