



WHITE PAPER

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Nitrogen Dioxide Biodecontamination:

A New, Effective and Cost-saving Option for Biodecontaminating Syringe Tubs Prior to the Filling Line

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INTRODUCTION

The prefilled syringe market continues to grow. In the past, products that are not candidates for oral administration (like large molecule compounds) would be delivered in a multi-dose vial container. Today, many of these products are administered in prefilled syringes to reduce the risk of injury to health care workers administering the drug, to reduce the container overfilling, and to improve dosing accuracy (Daedal Research, 2013).

To meet the growing demand, companies install small, medium and high-speed filling lines, depending on production needs. The majority of filling lines installed each year are small to medium speed lines, filling 100 to 200 syringes per minute, or about 1 to 2 syringe tubs per minute (Lysfjord, et al., 2010). These filling lines are used for commercial product, clinical trial or pre-clinical products. High-speed filling lines are typically reserved for large volume products, where the filling lines can fill syringes at a rate of six tubs per minute.

These filling lines rely on sterile, clean and ready-to-fill syringes that are packaged in tubs. Glass or polymer syringes are manufactured and placed in these (polystyrene) tubs for shipping. Typically, there are 100 syringes in a tub. These tubs are sealed with a Tyvek lid, forming a sterile barrier. Once the lid is applied, the tub is placed in an outer bag for additional protection, then placed in a carton. The syringes packaged in cartons are sterilized via ethylene oxide or gamma radiation and shipped to the site for filling.

Automated methods of introducing the syringes into the isolated filling line include a biodecontamination process after the tubs are removed from the outer bags. For smaller

filling lines, the usual process for introducing the syringes into the filling line includes the following steps: biodecontaminating the outside of the sterile barrier bag (or tub depending on the process), debagging the syringe tub, and de-lidding (removing the Tyvek lid from the tub). The de-lidding and aseptic filling will occur in a Grade A (ISO 5, Class 100) isolator. However, prior to the biodecontamination, the tubs of sterile syringes (either within bags or removed from bags) occurs in a less-controlled area, such as a Grade B or Grade C (ISO 7 or 8, respectively) area. Therefore, the risk of microbial contamination of the outer surface exists, which necessitates a biodecontamination step prior to lid removal and aseptic filling (Mouldenhauer, 2013).

In this document, the methods of syringe tub biodecontamination are reviewed before describing nitrogen dioxide (NO₂) as a beneficial option for the biodecontamination of syringe tubs prior to introducing the tubs into the filling line.

CURRENT METHODS

There are various methods used for biodecontamination of the syringe tub prior to entering the filling line. The three most common are: alcohol spray and wiping; vapor hydrogen peroxide biodecontamination; and, electron beam (e-beam) radiation. These methods are briefly described below.

Manual Alcohol Spray and Wipe

The tubs arrive in the sterile barrier bag. The outer surface of the bag is biodecontaminated with a spraying and wiping process. The bagged tub is placed in the debagging area and the bag is removed, either automatically or manually. The tub is then placed in the aseptic de-lidding area before moving to the filling area. There are deficiencies and risks associated with the spraying and wiping method (Vogt, 2010; Spolyar, 2010).

Pros

- Low capital equipment cost
- No known by-products on the syringes

Cons

- Manual, slow process
- Inconsistent process and lethality
- Difficulty validating a manual and highly variable process

Vapor Hydrogen Peroxide (VHP) Surface Biodecontamination

The syringe tubs are loaded into a biodecontamination airlock chamber (either in the outer bags or removed from the outer bags) (Vogt, 2010; Spolyar, 2010). Removing the outer bag in the isolator is less convenient than removing the outer bag outside the chamber, and the seams and folds of the outer bag are a greater challenge to the VHP biodecontamination process. After the VHP biodecontamination process, the tubs enter

the isolated volume of the filling line. Then, the Tyvek lid is removed from the tub and the syringes are presented for filling.

Pros

- Validation process is defined
- Same technology as used in many filling lines

Cons

- Capital cost of chamber
- Longer process duration
- VHP inability to reach complex geometries (in the folds of the bag or under the flap on the Tyvek lid)

Electron Beam Radiation Surface Sterilization

With the e-beam system, the tubs are continuously loaded into the tunnel – typically at a rate of six tubs a minute (Spoylar, 2010). As the tubs traverse the tunnel, three emitters are positioned in a way that all surfaces of the tub are irradiated and biodecontaminated. The tubs then enter the next section, where the Tyvek lid is removed, and after which the syringes are presented for filling.

Pros

- Continuous, high speed process
- Validation and dose requirements well defined

Cons

- Large capital cost and replacement cost of emitters
- Additional space in manufacturing area
- Radiation protection required

As a summary of the current methods, the three options are described below:

- Hand wiping – not reproducible, not appropriate for high volume applications
- Vapor hydrogen peroxide – not reproducible, not suited for high volume applications
- Electron beam radiation – reproducible, best suited for high-volume filling lines

After reviewing these biodecontamination methods, it is clear that a reproducible biodecontamination method is needed for small and medium speed filling lines.

Nitrogen dioxide gas is proven to be a better sterilant for this application, in the low and medium speed filling line market. The data below demonstrates that nitrogen dioxide gas provides a fast process (15 minutes), and consistent lethality, especially under the Tyvek flap on the tub lid.

NITROGEN DIOXIDE (NO₂) vs. HYDROGEN PEROXIDE (H₂O₂) STUDY

Noxilizer conducted a head-to-head study of nitrogen dioxide versus hydrogen peroxide biodecontamination processes to compare the lethality and cycle time of each process. The comparison testing used a biodecontamination airlock that is configured for both hydrogen peroxide vapor and nitrogen dioxide gas. An accurate comparison of the two sterilants was facilitated by using the same airflow rates, materials of construction, loading configuration and test environment. The syringe tubs were removed from the outer bag prior to being placed in the biodecontamination airlock. Preparing the microbiological challenge for both NO₂ and VHP processes is described below:

- Biological Indicators (BIs) were made from segments of tub lids consisting of a portion of the Tyvek and tub material, which were welded together in the Tyvek sealing process.
- The Tyvek flap on this portion of tub was lifted and inoculated in the seam with a spore suspension and allowed to dry.
- 10⁴ spores per centimeter of linear length of tub lid segments (this equals an inoculation rate of 10⁶ per tub as tubs have a circumference of about 100 cm).
- In addition, stainless steel BIs (10⁶) in Tyvek pouches were used throughout the chamber.

There is a small flap of Tyvek, on the tub lid, that is not sealed to the polymer tub surface, and has been used as a challenge location in previous testing (Spoylar, 2010). The BIs used for these tests were formed by cutting small sections of the Tyvek flap and syringe tub lip and inoculating the seam area. The area under this Tyvek flap is susceptible to contamination during handling and after debagging in the Grade B or Grade C area. To represent this challenge, spores were uniformly distributed on a section of tub rim that has a length that is 1% of the total tub rim circumference. Therefore, the rate of inoculation represents a challenge of 10⁶ spores per tub.

The load consisted of 10 tubs placed in the 200 L transfer airlock, 5 tubs on the top shelf and 5 tubs on the bottom shelf. A tub BI was placed on the lid of each of the 5 tubs that were on the bottom shelf (See Figure 1).

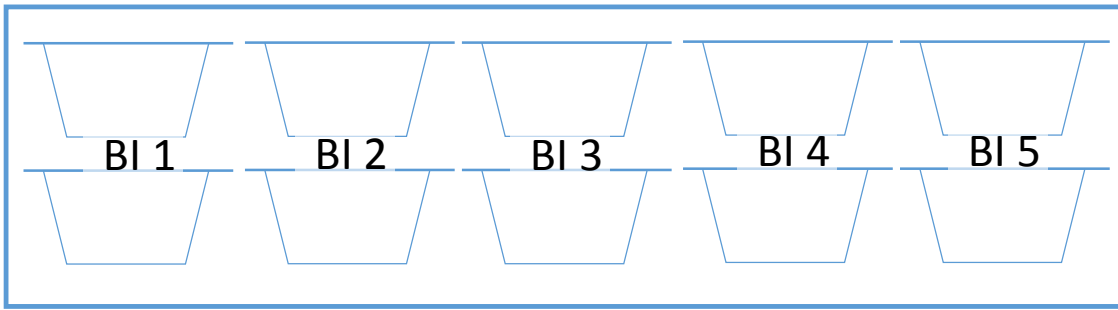


Figure 1. Diagram showing the arrangement of the tubs and the placement of the tub BIs. The top row of tubs are on a shelf, above the bottom row of tubs. The tub BIs are placed on top of the tubs on the bottom row.

In addition, stainless steel disk BIs with 10^6 spores in Tyvek pouches were placed throughout the chamber. This represents the standard method for developing and validating a biodecontamination cycle for hydrogen peroxide today. The tub BIs mentioned above present a more significant, yet realistic challenge, for biodecontaminating syringe tubs based on how the tubs are actually handled and the risk of contamination, especially in the Tyvek lid and tub lip area.

The exposure cycles used for both VHP and NO_2 processes were ones that completely inactivated biological indicators with 10^6 spores. The parameters for these cycles are shown in Table 1. The sterilant concentration and humidity with the biodecontamination process for a typical NO_2 cycle is shown in Figure 2. The microbiological results for the VHP and NO_2 cycles completed are shown in Table 2 and Table 3, respectively.

Table 1. Cycle parameters used to test tub biodecontamination.

NO_2 Process	Time (Minutes)	H_2O_2 Process	Time (Minutes)
Humidification to 60% RH	1	Dehumidification to 20% RH	10
Dosing (NO_2 added, 10 mg/L)	1	Dosing (Injection at 4.5 g/min)	1
Dwell (NO_2 circulated)	7	Dwell (Stabilization at 1.5 g/min.)	7
Aeration to 1 ppm	6	Aeration to 1 ppm	25
Total Process Time	15 Minutes	Total Process Time	43 Minutes

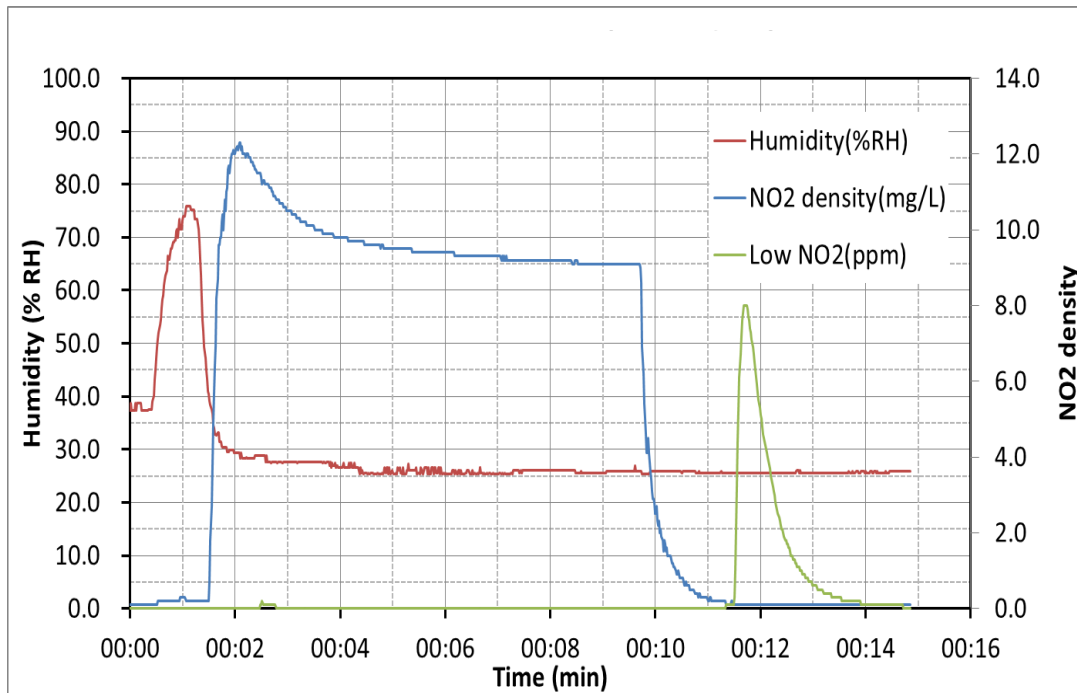


Figure 2. Typical nitrogen dioxide sterilization cycle for syringe tubs. In this graph, the red and blue lines represent FTIR readings. The green curve indicates the measurement from the EC cells in the chamber. EC cells were included in order to measure NO₂ at the lower levels, 1-10 ppm. Because of the sensitivity of the EC cells, they were not exposed to the chamber air until the FTIR results indicated that the NO₂ concentration had fallen to 10 ppm or less.

Table 2. BI results for the VHP cycles. N indicated 'negative', or sterile BI. P indicates 'positive', or that the BI had viable surviving spores.

Time	BI 1	BI 2	BI 3	BI 4	BI 5
8 min.	N	P	P	N	P
6 min.	N	P	N	N	P
4 min.	N	P	N	P	N

Table 3: BI results for the NO₂ cycles. N indicated 'negative', or sterile BI. P indicates 'positive', or that the BI had viable surviving spores.

Time	BI 1	BI 2	BI 3	BI 4	BI 5
6 min.	N	N	N	N	N
4 min.	N	N	N	N	N
2 min.	N	N	N	N	N

DISCUSSION

The nitrogen dioxide cycle parameters of 10mg/L achieved lethality on all BIs at as little as a 2 minute exposure – even in the most challenging location, the lip of the Tyvek lid and tub area. This demonstrates that a 15-minute cycle will provide consistent biodecontamination, including a reasonable safety margin.

The VHP microbiological results demonstrates that the biological indicators were not consistently biodecontaminated. As a result, the VHP biodecontamination process would require a higher dose of H₂O₂, or longer exposure times. Hydrogen peroxide failed to achieve consistent lethality results at the tub lid. While there was evidence that lethality could be achieved at 8 minute cycles with the lesser challenge – stainless steel disk BIs in Tyvek pouches. One would suspect the spore inactivation rate (inversely proportional to the D-value) on the polystyrene tub material (tub BIs) would be larger than the D-value on stainless steel, as has been reported in the literature (Sigwarth, et al.).

These results demonstrate that nitrogen dioxide provides a solution for the low and medium speed filling lines. The cost of an NO₂ biodecontamination airlock will be much less than an e-beam system. Also, the NO₂ process provides consistent lethality throughout the chamber and with an efficient cycle time.

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