Assessing contamination control of pre-sterilised container tub transfers into an aseptic manufacturing filling isolator via a de-bagging/no-touch-transfer process step

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Experimental contamination transfer challenge studies were designed to assess whether contamination control and sterility are maintained when using a no-touch-transfer (NTT) de-bagging tub transfer method to introduce pre-sterilised containers into a FlexPro50 aseptic manufacturing isolator/restricted-access barrier system filling line system. Importantly, the sterile tubs of product containers are enclosed in double steri-bags, and remain sterile through the supply chain to point of filling. Use of NTT means that any of the current automated bio-decontamination steps usually employed prior to material transfer into Grade A areas are rendered unnecessary; since the bag contents remain sterile and protected and can be transferred without exposing the Grade A aseptic processing zone to the outer bag. To support this rationale, two key contamination control studies were undertaken during processing of tub transfers with the NTT/de-bagging technology; 1) surface-to-surface transfer of human commensal microorganisms representing the challenge of ‘worst case’ operator handling, and 2) a structured approach evaluating the risk of airborne contamination as tubs move through the NTT process steps into the Grade A isolator environment by a particle challenge method (limitation of risks method). In these studies, the barrier–isolator environment remained robust to adverse particle movement, without microbial contamination transfer. In addition, the sterile tub outer surfaces were confirmed as maintaining sterility during the NTT process step. These results provide proof of concept of NTT technology.

Key words: Pre-sterilised containers, no-touch-transfer (NTT), de-bagging, aseptic manufacturing filling isolator, L-R method.

Introduction
There is a significant trend in the market towards biological products with more targeted delivery systems and improved efficacy which can be given in smaller doses and manufactured on a smaller scale1. Personalised medicines, advanced therapeutic medicinal products and investigational medicinal products have to be filled in controlled environments, but in much smaller batches at clinical trial phases and potentially in final production batches. Consequently, new product types are supported by new technology developments including increasing use of pre-sterilised single-use disposable systems for sterile product holding, and fluid pathways that transfer product to point of fill. Pre-sterilised product containers in different formats (syringes, vials and cartridges) are particularly useful for sterile medicinal products manufactured in small batches2. This increased use of pre-sterilised product containers has challenged established container tub transfer methods for entry into filling line isolators/restricted access barrier systems (RABS).

The industry response has been developments in process/scale compatible manufacturing technologies, and here we examine an important development in pre-sterilised container processing. Transferring tubs of pre-sterilised containers into the Grade A/ISO 5 filling zone traditionally necessitates bio-decontamination of the outside of the tub of containers at entry into the filling zone. Transfer of material must not compromise the stringent requirements for good manufacturing practice (GMP) EU controlled environment classification – Grade A/ISO 53–5.

Traditionally, pre-sterilised container manufacturers provided no assurance that the tub outer surfaces were sterile at manufacture and through the supply chain, so by default an
automated decontamination step at entry to filling lines was required; tub surface bio-decontamination steps were either eBeam, cold/low temperature plasma or vaporised hydrogen peroxide.

Recent developments presented by pre-sterilised container manufacturers have provided the necessary assurance that outer tub surfaces are sterile at manufacture with assurance this sterility is maintained through the supply chain via use of tamper proof carton closing. Such assurance of sterility is a prerequisite to implementation of no-touch-transfer (NTT) process steps that further maintain the tub sterility in transfer to point of de-lidding (removal of tub Tyvek® cover) and filling/container closing.

Currently, if specified, the outer tub surface bio-decontamination step, e.g. eBeam, requires the tub to be unpackaged from the protective steri-bag with consequent exposure to contamination in the surrounding environment or lower classification environments, so even if the tub outer surfaces were sterile, due to the in-process contamination risks, a tub outer surface decontamination step was required at entry to Grade A filling environments. If a tub surface bio-decontamination step is specified, it is acceptable to process pre-sterilised containers starting with a single steri-bag and without assurance the outer tub surfaces are sterile.

Considering alternative methodology, with the assurance that outer tub surfaces of pre-sterilised container tubs are also sterile, NTT technology can be applied that does not expose the tub to a lower environmental classification than Grade A through process steps of outer packaging removal and tub transfer into Grade A processing zones. The NTT de-bagging method requires double steri-bag packaging and removes the need for a post-delivery automated bio-decontamination process step by ensuring the sterile tubs and contents are transferred to the aseptic environment under controlled environmental conditions, including operator to process separation via RABS in the final steri-bag NTT/de-bagging step.

Carefully controlled NTT techniques are used for double bags to remove the initial outer-bag at entry to either a Grade B cleanroom with a RABS-filling barrier system or at entry to a RABS-NTT system in a Grade C cleanroom where filling is completed in an isolator filling system. The RABS–NTT has a controlled environment to Grade B with a Grade A air supply at the transfer point of the sterile tub. It is recommended that a manual disinfection wipe step is used to reduce bioburden entering the Grade C cleanroom on the double-bagged tubs. At the final entry into the Grade A filling zones, the application of semi-automated or fully automated de-bagging/NTT technology, depending on the line filling speed, with sterile tub transfer under Grade A air supply conditions is applied, and thus the tub pre-sterilised containers are protected throughout a continuum from primary manufacture to filling (see Figure 1).

Every new technology requires experimental evidence to prove the concept and validate its use in a process operating with GMP. Although a small number of US Food and Drug Administration/EU-approved facilities are using de-bagging technology, supportive data in the public

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**Figure 1.** Pre-sterilised containers manufacturing, supply chain distribution and NTT process steps for transfer into Grade A processing zones for filling.
domain is required that sterile tubs (inside and out) which have the necessary assurance of sterility can be transferred together with sterile containers into Grade A/ISO 5 zones via the NTT process whilst sterility and environmental control is maintained.

The study objective was to verify that tubs of sterile containers which are protected by a steri-bag can enter the filling zone without contaminating the sterile tub outer surface or the filling zone when using a de-bagging NTT mechanism that does not employ an automated tub surface bio-decontamination step. This study simulates a worst case contamination scenario to fully interrogate the robustness of the NTT process. Human commensals representing the most likely contamination challenge through packaged tub handling, were deliberately inoculated onto the steri-bag outer and the bag cutting blade. To challenge the NTT–RABS environment, the tubs were singly wrapped in a steri-bag rather than doubly bagged so there was no NTT step at bag loading to the RABS that contained the final NTT mechanism at entry to the Grade A isolator. Furthermore, the surrounding cleanroom was Grade D, although Grade C is the recommendation for isolators filling sterile products.

It was considered that if the process was robust to a worst case scenario, it would operate at acceptable risk reduction to potential contamination in a manufacturing facility working under GMP and using good aseptic technique. The focus points for environmental monitoring of the contamination transfer studies are shown in Figure 2, which is a diagrammatic representation of the NTT process.

The NTT process has been described previously by Drinkwater et al., but in brief the process comprises of the following steps. The pre-sterilised containers for transfer in the NTT process, e.g. nested filling containers in tubs are placed in membrane steri-bags (double bags) made of Tyvek® filter material. A number of bagged tubs are then placed in a perforated carton – all layers allow ethylene oxide (ETO) to pass through into the product containers to render the contents sterile. After this classical certified sterilisation step, the carton of sterile tubs of containers are then secured by a tamper-proof label-tape to provide assurance of maintained sterility through the supply chain.

On receipt at a manufacturing facility, the carton tamper-proof closures are checked for integrity and if valid the bagged tubs are removed and transferred within the facility to point of use following documented procedures. The still-enclosed bag contents are moved to a tub loading preparation area for material transfer via NTT.

During the final de-bagging step, the steri-bag is cut open by a semi-automatic blade cutting mechanism whilst clamped so that particles do not enter inside the bag onto the tub. The operator (or machine) then completes the NTT process and pushes the sterile tub into the Grade A area under Grade A air supply protection without touching the sterile tub (Figure 2).

The NTT concept relies on the fact the tubs are ETO sterilised together with double steri-bags and the process of NTT is only under Grade A airflow protection and the transfer zones are protected from contamination via outer bag surfaces using the NTT technology. In principle, no
decontamination step is required other than initial manual
disinfection on tub packaging at transfer into a Grade C
cleanroom if isolator technology is used in the filling
process.

The experiments reported here were conducted in an
isolator engineering facility with an isolator filling system
housed in a Grade D cleanroom (Figure 3). Two potential
routes of contamination were identified: 1) surface-to-
surface microbiological contamination transfer, and 2)
airborne contamination transfer that may be in the form of
non-viable particulate or microbe carrying particles. To
reproduce the most likely microbiological challenge, the
microorganisms collected to inoculate surfaces were
human commensals from finger touch.

To risk assess the possibility of airborne
contamination during the process, the method for
limitation of risks (the L-R method) was applied with its
visualisation and challenge test. Airborne contamination
transfer through the NTT tub transfer was evaluated by
monitoring of particle concentration during the particle
(smoke) challenge test to study if there was compromise
to the controlled process environments. In these studies,
the barrier technology remained robust to adverse
particle movement during the NTT/de-bagging process.

Furthermore, zero colony forming units (CFU) were
recorded via environmental monitoring in the Grade A
filling area and on outer tub surfaces after the ‘worst case
scenario’ purposeful inoculation of the steri-bags and NTT
equipment. These results provide proof of concept of NTT
technology and it is up to individual sites to qualify NTT
for a given application.

Materials and methods

Contamination transfer challenge studies included
analysis of potential airborne and surface contamination
during the process of tub transfer via the de-bagging/NTT
principles into a FlexPro50 de-lidding/filling machine
(Groninger GmbH) inside a small batch isolator
connected to a RABS enclosing the NTT mechanism with
a surrounding Grade D cleanroom environment (F. Ziel
GmbH), as shown in Figures 3, 4(a) and 4(b).

All environmental zone classifications were verified by
classical environmental monitoring as EU Annex 1 GMP
compliant before the tub transfer studies. The transfer
tubs were pre-sterilised containers sealed with Tyvek
lids and were provided by an approved supplier, Nuovo Ompi
Stevenato Group, Italy.

Tub transfer studies were completed with a single outer
packaging bag as an experimental worst case scenario. In
manufacturing process operations, double bagged sterile
tubs are best practice. Single wrapped sterile tubs of
nested containers used in the study were not disinfected on
entry to the RABS Grade B NTT zone to provide an
additional contamination challenge. Furthermore operator
gowning in the Grade D Cleanroom was deliberately
basic – an overall, shoe covers and no hair cover to
contribute to the worst case challenge scenario.

Two key experiments were conducted: Experiment 1) a
structural approach to physical risk assessment using
particles from a smoke pencil as tracer and challenge and
an optical particle sensor for detection, and Experiment 2)
confirmation of absence of surface contamination transfer
with NTT technology via classical microbiological
environmental monitoring methods which comprised
contact plates and swabs.

Qualification of room and isolator environmental

conditions

Prequalification testing verified that the isolator interior
complies with EU-GMP Grade A (ISO Class 5, the
de-bagger unit (RABS) in front of isolator in-feed ‘mouse-
hole’ complies with EU-GMP Grade B – with Grade A air
supply at critical tub opening/transfer point.

Operator intervention into the de-bagging NTT zone is
only possible via RABS gloves. Tub initial entry from the
Grade D Cleanroom into a preparation area with unidirectional airflow (UDF) protection in front of a tub entry ‘mouse-hole’ performs to EU-GMP Grade C compliance. These classifications were determined in advance of the tub transfer/contamination challenge studies by environmental monitoring methods as referenced in EU GMP Annex 13–5,11.

**Experiment 1: L-R method**

Experiment 1 utilised the application of the L-R method with its visualisation of air movements and its challenge test of the process environment. The L-R method provides a reliable procedure for assessing potential ‘microbe carrying particles’ as microbiological risks of airborne contamination in clean zones in a systematic way. It relies upon sensitive measurements of potentially non-visible particle movements and not simple visualisation of smoke movement. Particle challenge testing and calculation of the risk factor presents an effective way for measuring different types of risk in medicinal product manufacture and environmental monitoring12.

It can be used for tracing the dispersion routes of airborne contamination, for identification of risk situations, for evaluating risks connected to single process steps, for immediate evaluation of changes, and for assessment of potential risks. A modification of the L-R method can be used for evaluating the response of sampling locations in clean zones.

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**Figure 4. NTT equipment: pre-sterilised container transfer into filling system.**
The illustrative technique of smoke studies provides a useful technique for visualising air movements and dispersal of contaminants. This technique requires that isothermal smoke is released continuously and almost momentum free using a diffuser. The smoke pattern can be recorded by means of still photography and video. Ljungqvist et al. demonstrated that smoke particles are typically so small that under normal turbulent conditions, they are dispersed in the same way as gases.

During the challenge test, the process simulation and operating conditions should preferably exaggerate the human interference and interventions in order to more rapidly identify potential risk situations. To assure the result, generally not less than three measurements of not less than 1 minute each should be performed for each intervention and at each representative location. The maximum concentration (number/ft³) value of each intervention and location, respectively, forms the base for risk factor calculations. The advantage with this approach is the uncomplicated, immediate registration of results using electronic discrete particle counters. The critical regions become contaminated only by non-viable particles, and this approach can be safely used in microbiological clean zones with no added risk of contamination.

In this study, the barrier technology was challenged with a smoke pencil during NTT to represent an airborne challenge (simulating total particulates into airborne microorganisms’ movements) and a particle counter was used to scan and map the particle movements.

The first step of risk assessment with the L-R method was a confirmation study that the de-bagger/NTT zone operates to Grade B with Grade A air supply and that the FlexPro 50 isolator modules meet Grade A (particles and microorganism levels) requirements. Figure 5 shows the testing environment and probe placement (P1–P8). The L-R method is performed in three steps:

1. Visualisation (by using the smoke technique) of the main air movements and identification of turbulent regions and critical vortices where contaminants can be accumulated or dispersed in an unpredictable way.
2. The particle challenge test, which identifies potential risk situations. It involves placing the probe of a particle counter in the critical area where during normal operations the product is exposed, and taking continuous total particle counts while generating particles in the surrounding air (e.g. by using air current test tubes) to a challenge level of more than 300,000 particles equal to and larger than 0.5 µm per cubic foot (~ 10⁷ particles per m³). These measurements were carried out during simulated tub transfer production activity.
3. The third step is to evaluate the risk situation by calculating the risk factor, which is defined as the ratio between measured particle concentration (number/ft³)
in the critical region and the challenge level in the surrounding air. Because of limited measurement accuracy at high concentrations, a value of 300,000/ft³ is used as a challenge level in all risk factor calculations.

**Experiment 2: Contamination transfer challenge studies**

The standard environmental monitoring technique selected to assess surface contamination transfer was contact plating. More extensive environmental monitoring was carried out as a control during set up to confirm the contamination control status of the testing environment. Due to the accuracy of Experiment 1 (L-R method) in visualising airborne contamination risk at ‘mouse-hole’ entry, settle plates were not sampled during NTT as it was considered there was no added value.

In this experiment, surfaces of the manufacturing set up were purposefully contaminated with human commensals as previously stated to be a ‘worst case’ scenario including the bag opening cutting knife and outer single steri-bag. A simple method of non-gloved hand inoculation was selected as the most likely ‘real world’ source of contamination over test isolates as a simulation for operator error. NTT was carried out and to check for contamination in Grade A areas via contact plating on the tub post-NTT, sampling of both the outer tub surfaces and inner Tyvek® cover was conducted to confirm maintained sterility (Figure 6).

The first step of the contamination transfer challenge studies was to confirm environmental control zones met EU GMP Annex 1/ISO 14644-1 environmental classifications. The zones included RABS (de-bagger) grade B zone, Grade A air supply at tub transfer point into Grade A filling isolator (de-lidding section), and de-lidding/filling Grade A isolator. The small batch isolator interior conformed to GMP Class A/ISO grade 5 conditions concerning particle limits and microbiological air quality. The de-bagger conformed to GMP Class B conditions concerning particle limits and microbiological air quality.

Furthermore, the UDF unit used for opening the first steri-bag (when used) conformed to Grade C and the cleanroom Grade D. Additionally, as a pre-qualification to the NTT studies, the tub outer surface was confirmed sterile inside a Grade A microbiology laboratory isolator independent of an NTT step. For this control test, the bag was manually surface disinfected then opened under Grade A conditions with good aseptic technique and contact plate samples of the tub surfaces taken to confirm they were sterile as per the manufacturers guarantee. Furthermore, active air sampling in the RABS–NTT at the critical material transfer location was carried out as a prequalification to ensure the RABS environment conformed to Grade B and critical tub transfer zone conformed to Grade A air supply (particulate and microbiological levels). Settle plating was also carried out in classification to prove the controlled environments met classification conditions. The following experiment was then carried out.

1. Inoculation of the challenge surfaces were carried out as shown in Figure 7. Initially, the tub outer bag was inoculated. The inoculum was human commensal from finger dabs. Inoculation of the outer bag was via human commensal skin contact onto each of the 10 tubes before the transfer process. The sample size selected was 10 tub transfers (single wrapped) from Grade C (preparation area) to Grade B (debagger, RABS) through the mouse-hole into a Grade A isolator. Bioburden studies were completed to confirm the human commensal challenge.
2. The study design for environmental monitoring sampling was via contact plates for each consecutive steri-bag before the NTT process step (outside the Grade A isolator) and directly on tub outer surfaces after the NTT process step and arrival into the Grade A filling isolator.

To reduce the amount of activity and materials in the Grade A isolator, tubs were recovered (in sterile bags) from the filling isolator after NTT and moved to a test isolator in an adjacent microbiological laboratory for inner Tyvek lid surface contact plating.

3. Completion of an actual (not simulated) tub transfer procedure with NTT de-bagging into de-lidding/filling isolator.

4. Following the initial five tub transfers at the midway point in the experiments, the cutting knife (which opens the bag) was also inoculated. As before, it was inoculated with human commensals via direct skin contact and then the remaining five tub transfers were completed.

5. The focus of the study microbiological sampling during the NTT process steps was via contact plates on outer surfaces of the tub after transfer into the Grade A process isolator to confirm maintained sterility. Surface contact plate sampling of the sterile tub outer surfaces after transfer was undertaken as shown in Figure 8 (a) and in additional steps in a laboratory isolator (Figure 8 (b) and (c)) of inside the Tyvek lid and on the top of the tub liner.

   Contact plating was completed on the outer single steri-bag before NTT as a bioburden confirmation.

   The first eight tubs had a single contact plate sample after the NTT process and the last two (worst case) tub transfers had duplicate contact plate samples to check tub top side and underside for sterility.

Figure 7. Inoculation procedure and example of recovered bioburden.

Figure 8. Contact plating of the tub inner and outer surfaces.
6. Incubation of contact plates for 36 hours then a further 7 days, which gives the best chance of detecting/visualising a range of microorganisms at formation growth states. The CFU were counted and recorded at these two timepoints3,11.

Results

L-R method

The challenge test (see step 2 in Experiment 1 method), was performed first with no activity in the preparation area or in the RABS de-bagger (Table 1), and then with a bag in the transfer opening (Table 2). After that, the challenge test was carried out during simulated activity (Table 3). From the measurements taken during these simulated situations, the third step – calculation of the risk factor – was performed. The results and calculated risk factors are shown in Tables 1 to 3.

When the risk factor is less than $10^{-4}$ (0.01%) during the challenge test, it is assumed there are no risks of airborne microbiological contamination during normal operational conditions according to experimental findings from more than 30 studied aseptic production lines16. The evaluation of NTT undertaken here with the L-R method demonstrated that the risk factors are low, thus there was no airborne microbial contamination during the tested activities.

Analysis of the L-R method highlighted that the following points should be considered. Firstly, in the tub loading preparation area, bags should not be stored along the inner wall of this zone and only on the pathway to ‘mouse-hole’ entry to the RABS–NTT zone as this was shown to disrupt airflow. Secondly, at entry to the RABS–NTT zone, a further NTT technique (using double bags) would provide a more controlled tub entry not only reducing risk of contamination transfer but also prevent possible adverse airflow movements at operator intervention process points.

Results of Experiment 2: Contamination transfer studies

Experiment 2 results are shown in Tables 4 and 5. A deviation from an expected result was that the initial bioburden at the cutting knife (before inoculation) was 1 CFU, but the plate growth was outside the streak line of the swab indicating a false-positive. However, false-positive or not, the 1 CFU was still below the 5 CFU not to exceed level of the de-bagging RABS–NTT zone so does not influence results. The results per sample type for contact plates are shown in Table 6.

Even with this worst case challenge, no contamination was detected in the Grade A area on tub surfaces.

The most probable root cause for the high counts at outer bag in bioburden studies (marked as red) is finger contact of contact plate edge due to operator error.

Table 1. L-R method – results from tests without activity and no bag in the transfer area.

<table>
<thead>
<tr>
<th>Challenge region</th>
<th>Probe position</th>
<th>Particles per ft³</th>
<th>Risk factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside preparation area (II)</td>
<td>P2 In de-bagger</td>
<td>52</td>
<td>$1.7 \times 10^{-4}$</td>
</tr>
<tr>
<td>Outside, in front of de-bagger (III)</td>
<td>P4 In de-bagger</td>
<td>19</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td>Inside de-bagger, on table</td>
<td>P4 In de-bagger</td>
<td>7,192</td>
<td>$2.4 \times 10^{-2}$</td>
</tr>
<tr>
<td>Inside de-bagger, on table</td>
<td>P6 In de-bagger</td>
<td>5</td>
<td>$&lt;10^{-4}$</td>
</tr>
</tbody>
</table>

Table 2. L-R method – results from tests without activity and with one bag in the transfer area.

<table>
<thead>
<tr>
<th>Challenge region</th>
<th>Probe position</th>
<th>Particles per ft³</th>
<th>Risk factor</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outside, in front of preparation area (I)</td>
<td>P1 In preparation area</td>
<td>11</td>
<td>$&lt;10^{-4}$</td>
<td>Bag in transfer area</td>
</tr>
<tr>
<td>Inside preparation area (II)</td>
<td>P2 In de-bagger</td>
<td>27</td>
<td>$10^{-4}$</td>
<td>Bag in transfer area</td>
</tr>
<tr>
<td>Outside, in front of de-bagger (III)</td>
<td>P4 In de-bagger</td>
<td>22</td>
<td>$10^{-4}$</td>
<td>Bag in transfer area</td>
</tr>
<tr>
<td>Outside, in front of de-bagger (III)</td>
<td>P6 In de-bagger</td>
<td>0</td>
<td>$&lt;10^{-4}$</td>
<td>Bag in transfer area</td>
</tr>
<tr>
<td>Inside de-bagger, on table</td>
<td>P4 In de-bagger</td>
<td>11,585</td>
<td>$3.9 \times 10^{-2}$</td>
<td>Bag in transfer area</td>
</tr>
<tr>
<td>Inside de-bagger, on table</td>
<td>P6 In de-bagger</td>
<td>0</td>
<td>$&lt;10^{-4}$</td>
<td>Bag in transfer area</td>
</tr>
</tbody>
</table>
Table 3. L-R method – results from tests with simulated activity, transfer and opening of bags.

<table>
<thead>
<tr>
<th>Challenge region</th>
<th>Probe position</th>
<th>Particles per ft³</th>
<th>Risk factor</th>
<th>Response to activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside preparation area (II)</td>
<td>P2 In de-bagger</td>
<td>53</td>
<td>$1.8 \times 10^4$</td>
<td>Low</td>
</tr>
<tr>
<td>Outside, in front of de-bagger (III)</td>
<td>P4 In de-bagger</td>
<td>8</td>
<td>$&lt; 10^4$</td>
<td>Low</td>
</tr>
<tr>
<td>Outside, in front of de-bagger (III)</td>
<td>P7 In de-bagger at working height</td>
<td>15</td>
<td>$&lt; 10^4$</td>
<td>Low</td>
</tr>
<tr>
<td>Inside de-bagger, in front of opening to isolator</td>
<td>P8 In isolator, close to opening to de-bagger</td>
<td>0</td>
<td>$&lt; 10^4$</td>
<td>Low</td>
</tr>
</tbody>
</table>

Table 4. Environmental monitoring results per sample type.

<table>
<thead>
<tr>
<th>Active air</th>
<th>Swab</th>
<th>Contact plates</th>
<th>Laboratory isolator settle plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only sampled during qualification for classification of controlled areas</td>
<td>Swab of the cutting knife before and after deliberate contamination to confirm the bioburden (see Table 5)</td>
<td>Contact plates were taken before and after NTT from tub surfaces. The steri-bag outer was sampled before and after deliberate inoculation to confirm bioburden. After the steri-bag is removed by NTT the tub outer surfaces were plated in the test isolator. The tub inner surfaces with the Tyvek® lid removed were recovered from the test equipment and in a laboratory isolator to confirm sterility was not compromised during NTT as an independent confirmation study (see Table 6 for results).</td>
<td>Only sampled during qualification for classification of controlled areas. Settle plate positioning is shown in Figure 6. All settle plates showed 0 CFU recovery in critical test areas.</td>
</tr>
<tr>
<td>Isolator: 0 CFU</td>
<td>RABS: 1 CFU</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Confirmation of bioburden after inoculation.

<table>
<thead>
<tr>
<th>Position</th>
<th>Plate 1 (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knife bioburden in Grade B before inoculation</td>
<td>1</td>
</tr>
<tr>
<td>Knife after inoculation (positive control)</td>
<td>59</td>
</tr>
</tbody>
</table>

Table 6. Results per sample type: contact plates.

<table>
<thead>
<tr>
<th>Position/tub sample number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer bag bioburden</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Outer bag after inoculation</td>
<td>9</td>
<td>27</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>15</td>
<td>9</td>
<td>83</td>
<td>34</td>
</tr>
<tr>
<td>Tyvek® outside, inside study isolator</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tyvek® inside laboratory isolator</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inner lid, laboratory isolator</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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Growth round the edge of the plate is a possible sampling error and this was evident when investigated. Fortunately, these errors did not occur on the critical Grade A samples, i.e., those testing if the process is successful. Also higher bioburden on the outer bag at starting may be due to use of the ‘worst case’ challenge single steri-bagged tub passing through the lower class cleanroom. Usually the process would use double steri-bagged tubs at this stage and bioburden would be extremely low.

- For the outer bag No. 6, the CFU count of 29 was higher than the positive control (10).
- For the outer bag No. 10, it was visible that someone had touched the agar surface.

Discussion and conclusion

Although the sample size is small, the high level of ‘worst case’ challenge conditions make for compelling data to support the concept of NTT. No surface or airborne contamination was found in the Grade A zone after NTT. Visualisation of air movements alone provides an understanding of the situation but does not assure or measure the degree of microbiological safety of aseptic processes. Taken together, both the risk assessment with the L-R method and contamination transfer study are a good test of the NTT method and concept.

L-R method demonstrates NTT is a robust process

This study had some limitations, one of which is the fact that the experimental set up was a simulation of a real life medicinal product aseptic manufacturing filling line with the preparation area for initial tub loading limited for the study as a loading table and not having a raised conveyor as the final design for actual processing. The airflow movement in the experimental RABS–NTT with airflow protection (high-efficiency particulate air filters) suggest that the RABS–NTT barrier could be improved at the preparation entry. As this was a mock-up, it was useful to understand the impact of having a less well engineered tub entry zone on airflow movement in the RABS–NTT entry point as turbulence was found that should be avoided in a real world scenario to maintain good aerodynamic protection.

Classical microbiological sampling methods provide further evidence for method

Despite the high chance of contamination, these NTT studies show that the NTT process for tub transfer via a debagger unit complies with the stringent regulatory requirements for maintained sterility. Even with worst case situations (single packed tubs, transfer of human commensals, sub-standard gowning of operators, Grade D environmental surround), no contamination transfer occurred. Although the study was limited in challenge sample size and study runs, the significant worst case and compromised contamination conditions increased the confidence in the results.

To provide a high level of assurance that tub sterility is not compromised in transfer into filling lines, it is recommended that sterile tubs are double bagged on delivery. For an isolator filling line, as used in studies, the first bag is typically removed under UDF together with an NTT technique on entry to the RABS–NTT de-bagging system. The second (final) steri-bag would typically remain sterile on the outside or only have very low bioburden before the final bag is removed with the semi-automated NTT de-bagging system at entry into the Grade A filling zone. In the case of the research study, only a single steri-bag was used and there was no disinfection step for the outer bag surfaces, so the outer bag was contaminated before it passed into the RABS–NTT de-bagging zone presenting a further contamination challenge.

Next steps to further interrogate NTT

These experiments indicate that NTT is a secure process but further studies would be optimal to fully test the method. Furthermore, it may be appropriate to do case-by-case qualifications on site. Further studies could include analysis of a greater array of microbiological isolates, but in principal the most likely challenge from human commensals (handling of tubs, i.e. by fingers) has been tested.

More studies are recommended to add assurance and reference data, including more extensive environmental monitoring sampling, and an increased number of tub transfers to allow statistical analysis.

As the contamination control element of the NTT process relies on Grade A air supply, from down-flow air and out-coming air via the mouse-hole to the Grade A isolator, it is recommended that this airflow is well characterised with smoke studies and monitored (down-flow velocities) in process operations. As part of operational qualification, it is also recommended to include tub surface monitoring (to verify sterility and zero CFU recovery) in conjunction with environmental monitoring sampling for the controlled zones (RABS and isolator).

Adoption of new technologies for a new market

Alternative technologies are now starting to be considered for many applications in GMP, and NTT offers a simplicity and cost saving with flexibility that is much needed as new product profiles are developed with varying batch sizes and bio-compatibility requirements. Effectively the NTT process uses no decontamination chemicals and has no process residuals that can cause bio-compatibility challenges to biological products so provides added advantage at a cost saving.

This alternative process needs no bio-contamination step as tubs are assured as sterile in manufacture, through the supply chain to point of use and evidence is provided here that the NTT process is robust to prevent compromise of the tub sterility of Grade A filling environments.

The cost and expense of the existing tub decontamination technologies used in product manufacture such as eBeam do not necessarily suit small batch processing, e.g. clinical trial batches or small batch biological products.
Conclusions and recommendations

These microbiological and airborne particulate contamination studies were limited in study runs and range of environmental monitoring sampling but the challenge was considered greater than the aseptic processing worst case providing confidence in the results.

This is a very positive indication that NTT with tubs that start with sterile outer surfaces is a secure process without contamination transfer to the containers and filling environment; and that it is a viable alternative to transfer processes that require a tub surface decontamination step, e.g. cBeam or VHP®/vH2O2.

This is a contribution to knowledge and understanding and the indications are that NTT is a robust process and proves in principle the concept, but to ensure GMP compliance individual sites should independently undertake contamination control qualification testing and process monitoring. These results should apply to any isolator processing pre-sterilised containers but each site should conduct their own qualification studies and validation of all process steps, including NTT.

Following further research, microbiological contamination challenge studies at user sites may not be required with reference taken from peer-reviewed studies. User sites could benefit, however, from applying the L-R method with its visualisation and particle challenge test in NTT process qualification together with environmental monitoring of the controlled environments.

References