Cleaning Validation
What do you need to consider to ensure a successful outcome?

White Paper

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Abstract
The data used to determine the success of a cleaning validation is built upon both the effective evaluation of the manufacturing plant and the robustness of the validated analytical method. In order to ensure the safety of the consumer, there must be a high degree of confidence in the analytical results in order to verify the absence of residues at the prescribed limits on the various equipment surfaces and to control microbiological contamination. This white paper discusses the over-arching strategy for performing a successful cleaning validation, with detail on some of the key factors to consider at both the manufacturing, microbiological and analytical stages, highlighting many common pitfalls to avoid.

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Introduction

Cross contamination must be avoided in the Pharmaceutical industry at all costs and successful cleaning validation ensures that patients are not put at risk due to cross contamination. The process can be divided into a number of sections each of which must be fully understood and areas of concern addressed to ensure a successful outcome across the entire process. This spans both the manufacturing and subsequent analytical and microbiological support.

The data used to confirm a positive/successful cleaning validation is underpinned by the results of validated analytical methods. It is essential that these results are truly representative as patient safety is based upon the absence of equipment residues.

So what are those areas of concern, what affects your ability to get a successful outcome and what do you need to consider when carrying out a Cleaning Validation exercise?

Figure 1. An overview of the areas to be considered to ensure success.
A detailed study of all the equipment to be cleaned must be carried out by a qualified team to ensure the chosen cleaning method will be successful. This is colloquially known as “Walking the plant”. It is important to understand what can affect your cleaning results and to either engineer out those areas of concern or include them in your cleaning procedure.

Areas of concern
All product contact pipe work should be thoroughly evaluated. The geometry of the pipe work can play a vital role in a successful outcome. Multiple bends, flanges, angles of pipework and dead ends, can all result in product retention and possible cross contamination. There are recorded cases in the industry of minor changes to pipework geometry resulting in cross contamination issues.

Remember – If you change the shape of a piece of pipework it may no longer clean as well.

Specific areas to be mindful of are:

1. **Service lines** – These are small bore lines and very difficult to clean. There have been a number of cases over the years where investigations into cross contamination issues have concluded that the service lines were at fault. Ensure that you have adequate safeguards in place (e.g. non return valves and filters) to ensure these lines do not get contaminated.

2. **Gaskets** – The microscopic nature of materials used to make gaskets result in product retention and possible swab failure. It is recognised that gaskets are necessary but a study should be carried out to ensure that there are not gaskets in the system that are no longer required and that all gaskets in the plant are in good condition to reduce the risks.

3. **Filter meshes** – These are designed to retain product and therefore can also result in cross contamination if not carefully cleaned.

4. **Valves** – Material can be drawn into valve housings and returned to the product stream at a later date resulting in cross contamination. All valves in the product stream should be investigated, a cross section dismantled and tested during validation to ensure that they do not present a risk to the outcome.

5. **Pumps and samplers** – These pieces of equipment are very difficult to clean successfully. It should be determined if they are really necessary. Can the geometry of the plant be altered to avoid their usage? If not they must be included in your cleaning exercise.

6. **Process investigations** – It is important that an understanding of what can go wrong in a process is obtained. Sometimes a process error at an earlier date can result in product being deposited in an area of the plant where it normally would not reach. This can then result in swab failures during cleaning or more critically Product contamination at a later date. E.g. Over-heating of a vessel can result in product being deposited in condenser return lines.

7. **Packaging lines** – It should be determined that there are no areas in the line where tablets could bounce and get stuck e.g. on the top of sensors etc. These may then fall off later into a different product and cause cross-contamination, patient injury or even death. Screens should be erected at these areas to ensure this cannot ever happen.

Remember – A good plant walk around, if performed thoroughly, with the right team, will not only reduce errors, but will also write most of your cleaning validation protocol. Swabbing points and plant break in lists can be decided during this investigation.
Design and control of cleaning methods

Good cleaning procedures are fundamental. A procedure that has been used for years may not necessarily be the best. A review of the methods should be carried out at validation to determine their suitability and effectiveness. Procedures should include factors such as:

1. Operation of valves during cleaning
2. Filling pipework with solvent and allowing dissolution time
3. Refluxing solvent around system and through condenser return lines

Manual cleaning is very subjective. Two different operators may not necessarily clean to the same degree. In order to achieve reproducibility it is critical that all operators carry out the procedure in the same way. Very detailed procedures and training are required to ensure the successful outcome of a manual cleaning exercise. It is sometimes easier to alter plant layout so that Clean in Place (CIP) can be performed in place of manual cleaning, e.g. moving a pan filter from beside the vessel to the floor below can result in a double bonus. The pan filter can then be washed using a CIP procedure and the move also results in a loss of a pump and extra pipework. This sort of rearrangement should be considered as it reduces risks of error.

Ensure that:

• Written procedures will deal with the outcome of a process failure based on knowledge of what can go wrong in your process. Often what can go wrong in a process is more critical to the cleaning than what can go right.
• All process operators are fully trained and their records are up to date. Validation exercises have failed in the past, during audits, due to failure to ensure that operators’ training records were complete and up to date.

Issues with sampling and testing

A considerable proportion of the testing failures experienced in the industry are not related to the ability to clean the equipment or perform the analysis. They are solely related to the skills of the person taking the swabs. It is easy to write and train a procedure on how to carry out a swab test but it is a different thing being able to perform a test correctly and reproducibly. So what can be done to reduce this risk and help secure a successful validation outcome?

• A number of issues can be identified related to the swabbing procedure and these must all be addressed if success is to be guaranteed.
• Control of swab equipment – It is essential that all the equipment used to carry out a swab test is reliably controlled. It is extremely easy to contaminate a swab and put your whole cleaning validation exercise at risk of failure. The swab material, the solvent used, any disposable gloves etc. must be very carefully controlled to reduce the risk of possible false results.

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Alongside the product manufacture, the analytical method is of critical importance when assessing the cleanliness of pharmaceutical manufacturing equipment. In order to satisfy this requirement, it is key to ensure a high level of confidence in the generation of results derived through the use of the method. This, in turn, is underpinned by the development of a suitable, fit-for-purpose analytical method, designed not only to be specific to the analyte, but also able to ensure its ability to quantify at the prescribed residue limits.

On the subject of residue limits, this is very much a subject in its own right. The regulatory authorities do not set limits for specific products. The key factors to bear in mind when approaching this with respect to a given pharmaceutical product:

- Defined limits should be logical
- They should be practical and achievable (in terms of method sensitivity)
- Verifiable

An approach for limit setting should be identified early on since this will have a direct impact on the analytical test method and its ability to detect the analyte(s) at the required level.

Determining cleanliness can prove to be a very challenging task, with the aim being to measure trace residues (target analytes) on the surfaces. The residue must initially be extracted from a surface, recovered from the extraction matrix and then suitably quantified. Note that sampling is usually performed via direct methods (swabbing), however indirect strategies (such as rinse samples) may also be used, though the latter is not a preferred approach.

Very often, the starting point in the development of a cleaning method would be based upon a previously developed assay method. The main aspects to be borne in mind are (1) attainment of suitable sensitivity to quantify at the limits, (2) specificity (between the target analyte and other matrix components) and (3) a curtailed run time to allow the requisite high sample throughput that is generally needed when analysing swab samples.

In many respects, the requirements for validating cleaning methods are closely aligned with a standard assay, evaluating factors such as specificity, accuracy (recovery), linearity, sensitivity, precision, robustness and solution stability. However, one area that does require some time is the swabbing technique itself. A large part of the development procedure itself is focused upon deriving a swabbing procedure that allows acceptable recovery of API residues from the required surface types. Furthermore, the choice of swab surface used will be dictated by conducting a plant walk-around and subsequent risk assessment of the manufacturing plant and determination of the key ‘risk’ areas where residues are most likely to remain following manufacture.

It should be noted that there is currently an increasing shift towards the use of Total Organic Carbon (TOC) Analysis to determine residues following cleaning of the manufacturing plant. The increasing view is that apart from looking at specific active traces, a ‘crude’ evaluation of contamination should be considered. The advantages of TOC over more traditional HPLC methods are (1) the ability to evaluate the overall contamination, and (2), reduced time and cost for the development, validation and subsequent analysis. This can potentially have very real positives from a manufacturing perspective as it will enable much more rapid turn-around time for the analytics, thereby reducing down-time for the plant.

Whilst TOC may be suitable in certain cases, these would be limited to manufacturing lines that have proven control of the cleaning process and hence where TOC results (i.e. non-specific, gross contamination) are determined to be well below the specified residue limits. If this is not the case, then it would be necessary to develop specific methods (such as HPLC) to determine the levels of a given active(s).
Swabbing

Often, insufficient time is invested in the actual swabbing regime, which can ultimately lead to a less than robust final method. Each of the surface types identified (discussed previously) should be spiked with the API (at the calculated residue limit) and experiments performed to demonstrate that acceptable recovery is repeatedly obtained. This can involve the evaluation of a number of different swabbing solvents and swab types together with optimisation of the actual swab technique. This, combined with the need to be able to repeatedly detect residues at very low levels, can contribute significantly towards the time required for successful method development and validation.

Recovery experiments essentially underpin all other validation parameters since the residue must be:
1. Successfully extracted from a surface
2. Recovered from the extraction matrix
3. Appropriately quantified

If the method is shown to have sufficient accuracy, then all of these parameters can be evaluated as a single entity. If inadequate results are obtained then each of the steps would need to be investigated individually to identify which of the component steps is responsible for the poor recovery, and allow screening of other solvents or swab material that may improve recovery. It should provide assurance that an analyte can be analysed in a matrix, which, for a finished product, would include excipients, impurities or perhaps solvents used in the manufacture of the actual API. If a cleaning agent is used, its composition should also be considered. An evaluation of specificity should first be conducted to ensure the absence of any interference between matrix components and the target analyte.

Another key factor to be considered throughout the development process is the stability of the analytical samples; that is, the ability of the active substance to degrade once in the extraction solvent. If appropriate expiry dates are not set, significant degradation could occur, leading to apparent low swab levels, which could actually be attributable to the instability of the active and not representative of the levels actually swabbed from the equipment surfaces – potentially giving a result that “passed” which should actually fail.

There is such an array of validation parameters that could be discussed, however this article cannot possibly do each of these justice. However, in terms of analytical method validation, there should be no unexpected surprises. All of the critical parameters to be assessed should be identified and evaluated prior to commencement of the formal validation. The method development should therefore be used to obtain the initial information to set the limits stipulated within a validation protocol and hence build in Quality by Design.
Microbiological Contamination

Aside from the manufacturing and analytical aspects, it is also paramount to consider cleaning validation from a microbiology point of view. This focuses on contamination control in pharmaceutical and healthcare facilities.

Pharmaceutical manufacturing process can be subjected to microbial contamination. Therefore a good cleaning and disinfection process is necessary to maintain a good hygiene in facilities.

Disinfectants are used to prevent microbial contamination of pharmaceutical products.

Where chemical disinfectants are used as part of contamination control, it is important to evaluate the efficacy of the disinfectants to ensure they are fit for purpose.

So why do you have to evaluate the efficacy of your disinfectant?

Apart from being a regulatory requirement, you need to establish whether your disinfectants are effective under the conditions they are used. Factors such as the surfaces they are used on, contact time, concentration and so on will alter the effectiveness of disinfectants. The test performed by the disinfectant manufacturer before release does not mimic the way you use them, and therefore does not establish they are fit for purpose.

Choosing a disinfectant

Disinfectants are usually chosen based on their chemical properties and expected effectiveness against microorganisms.

Before evaluating the efficacy of your disinfectant, it is important to consider a few points and ask do I have the right disinfectants in place?

- **Bactericidal and fungicidal agents** – It is important to use disinfectants that are effective against bacteria and fungi. This will ensure that bacteria and fungi on surfaces are killed.

- **Sporicidal agents** – It is important to have disinfectant that are effective against bacteria and fungi but some disinfectants are only effective against vegetative microorganisms. Not all disinfectants are effective against spores, therefore a sporicide should be added to the disinfectant rotation.

- **Sterile disinfectant** – If disinfectants are used in a cleanroom (Grade A and B) environment, they should be sterile prior to use. Where disinfectants are transferred into spray bottles before use, then the bottles should also be sterilised.

- **Available data** – Disinfectant manufacturers that supply to the pharmaceutical and healthcare industries perform various tests that evaluates the efficacy of their disinfectants. Ask the manufacturer for the available data to help you establish if the selected disinfectants are good enough.

Validating your disinfectant

The effectiveness of a disinfectant would depend on the biocidal properties of the disinfectant. However, a number of factors can affect the effectiveness depending on the way it is used.

The following factors most be considered in the validation process to help establish if your disinfectants are fit for purpose.

- **Contact time** – Apply contact times that are practical and are actually followed in the disinfection process. Shorter contact times can be validated to cover a worst case scenario. Consider a practical contact time when testing alcohol as it evaporates within a short period of time. Some sporicidal agents might need an extended contact time depending on prior knowledge provided by the manufacturer.

- **Surface** – Make sure the disinfectants are validated against the surfaces they are used on. In some cases, not all surfaces can be included in the cleaning validation, therefore a risk assessment approach must be adopted to determine the most appropriate surfaces to use.

Use the exact surface present in your facility as a slight modification might affect the efficacy of the disinfectant. For example if you have a coated vinyl floor within your facilities, then the exact vinyl surface should be used.

- **Condition** – The validation process should apply interfering substances to create a clean or a dirty condition. Substances like Bovine Serum Albumin can be added to mimic the amount of soiling present on surfaces. Select the most appropriate condition that mimics where disinfectants are used.

- **Temperature** – Validation should be performed under the same temperature the disinfectants are used.

- **Organisms** – Ensure the organisms used covers a variety so that the bactericidal, fungicidal and sporicidal ability of the disinfectants can be determined.

- **Environmental isolates** – Environmental isolates should be added to the validation process to help prove that the disinfectants are effective against organisms they would encounter on your facilities.

- **In use state** – Disinfectants should be validated at their in use state. If they are transferred to a spray bottle, then it should be validated at that state.

They should also be validated at the end of their hold time to cover a worst case scenario. The concentration at which the disinfectant are used must also be applied in the validation process.
Microbiological practical test

The efficacy of a disinfectant can be demonstrated with a suspension test or a surface test. Suspension test can be used to determine if the concentration at which a disinfectant is used is effective. Since the disinfectant is tested in a suspension, it does not mimic the way it is used in practice.

A surface test is designed to mimic a possible contamination on a surface which can be found in a pharmaceutical or healthcare facility in the presence of an interfering substance.

The Quantitative Surface Test EN 13697 can be used to validate the efficacy of a chemical disinfectant.

**Test process** – A known level of test organisms are dried on the surfaces then the disinfectant is added to the surface and left for the standard contact time used during disinfection.

The effect of the disinfectant is neutralised after the contact time and the recovery of surviving organisms will be used to evaluate the efficacy of the disinfectant. Numerous controls are used to ensure that the result is due to the effect of the disinfectant. See figure 5 below for an overview of the test process on stainless steel.

![Figure 6: Overview of the surface test process on a stainless steel surface](image)

Validate environmental monitoring media

To have confidence that your selected disinfectants are not influencing the recovery of organisms on media used to perform routine environmental monitoring, it is important to verify the process.

This validation will enable pharmaceutical and healthcare facilities to implement the use of adequate contact plates and swabs containing the appropriate neutralisers in the environmental monitoring process.

Conclusion

The over-arching principle when embarking on pharmaceutical cleaning validation is to ensure that the manufacturing and analytical aspects are conducted synergistically, with good communication between consultancy, manufacturing, analytical and microbiological teams. This will build solid foundations to ensure that the final analytical method is able to fully encompass the requirements of the cleaning process, and can reliably and consistently quantify the required residues at the calculated limits, and establish the efficacy of the disinfectants used on site. Hence ensuring the pharmaceutical product is safe for the consumer.
RSSL is an established expert in this field and our coordinated approach to cleaning validation with analysis, consultancy and training services ensures that clients in pharmaceutical and bio-pharmaceutical production satisfy their regulatory requirements. These services will provide you with a thorough grounding in process design and pitfalls to avoid, along with understanding of fundamental issues in this area. To find out more about our cleaning validation services please contact us on: +44 (0)118 918 4076, email enquiries@rssl.com, or visit www.rssl.com

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Steve has sixteen years’ industry experience, having graduated in 2000 from Kingston University with a Bachelor’s Degree in Pharmaceutical Science. He has spent his entire career to date working in the pharmaceutical sector and has gained all of his experience within a CRO environment, gaining exposure to a wide range of analytical techniques and product matrices. Steve’s main area of expertise is Early Phase Development, Validation and Stability Testing of New Chemical Entities and Formulations mainly in Pre-clinical Development or Phases I and II.

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