INSTITUTO SUPERIOR DE CIÊNCIAS DA SAÚDE EGAS MONIZ

MESTRADO INTEGRADO EM CIÊNCIAS FARMACÊUTICAS

DESIGN/OPTIMIZATION/INTRODUCTION OF DISPOSABLES FOR BIOTECH DRUG PRODUCT PROCESSES

Trabalho submetido por
Joana Demmich Barbosa Agostinho
para a obtenção do grau de Mestre em Ciências Farmacêuticas

Novembro de 2014

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Doutor André Mang
Prof. Doutora Carla Ascenso

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To my parents, who have always guided me and been by my side.

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Abstract

The Quality-Time-Cost Iron triangle is in the middle of a pharmaceutical commercial production process. However, nowadays pharmaceutical companies try to optimize the security level of sterility assurance, available capacity and costs, looking for innovative technologies in order to improve their processes.

Within this master thesis, the possibility to transfer active pharmaceutical ingredient solutions with a peristaltic pump and with overpressure in combination with a combined thawing and transfer disposable tube was tested. Moreover, the transfer speeds of three combined thawing and transfer disposable tubes in combination with the two transfer methods were measured in order to decide which combined disposable tube and which method is more suitable for the transfer of active pharmaceutical ingredient solutions and therefore, see if it is advantageous to replace the current single thawing and single transfer disposable tubes, taking also into account other additional factors.

It is possible to transfer active pharmaceutical ingredient with a peristaltic pump and overpressure. However, the use of an optimized combined thawing and transfer disposable tube in combination with the overpressure transfer method would be an innovative process and would bring quality and efficiency to a pharmaceutical sterile drug product manufacturing process.

Key words: disposable technologies combined thawing and transfer disposable tube, peristaltic pump, overpressure

Resumo

O triângulo de ferro qualidade-tempo-custo está no seio do processo farmacêutico de produção comercial. No entanto, nos nossos dias, as empresas farmacêuticas tentam otimizar o nível de segurança de garantia da esterilização, a capacidade disponível e os custos, procurando melhorar os seus processos produtivos por recurso a tecnologias inovadoras.

No âmbito desta tese, foi testada a possibilidade de transferência de soluções de ingredientes farmacêuticos ativos, por recurso a dois métodos de transferência de fluidos, bomba peristáltica e sobrepressão, em combinação com um tubo descartável de descongelamento e transferência. Além disso, foram medidas as velocidades de transferência através dos dois métodos utilizando três alternativas de tubos descartáveis combinados de descongelamento e transferência, de forma a decidir qual o método e o tubo descartável combinado mais adequados para a transferência de soluções de ingredientes farmacêuticos ativos e, por conseguinte, verificar se é vantajoso proceder à substituição dos atuais tubos descartáveis, um de descongelamento e outro de transferência, tendo igualmente em consideração outros fatores adicionais necessários à tomada de decisão.

É possível transferir ingredientes farmacêuticos ativos através de bomba peristáltica e através de sobrepressão. Contudo, a utilização otimizada de um tubo descartável combinado de descongelamento e transferência, empregando o método de transferência de sobrepressão, seria um processo inovador que traria qualidade e eficiência ao processo de produção de produtos farmacêuticos estéreis.

Palavras-chave: tecnologias descartáveis, tubos descartáveis combinados de descongelamento e transferência, bomba peristáltica, sobrepressão

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Abbreviations

ADC - Antibody drug conjugate

ANOVA - Analysis of variance

API - Active Pharmaceutical Ingredient

ASTM - American Society for Testing and Materials

Cfu – Colony forming units

cGMP – Current Good Manufacturing Practices

CI – Confidence interval

CIP – Clean- in- place

FDA – Food and Drug Administration

HPAPI – Highly potent active pharmaceutical ingredients

ID – Inner diameter

ISO – International Organization for Standardization

Kg – Kilograms

kGy - Kilogray

LAL - Limulus amebocyte lysate

Min - Minutes

OD – Outer diameter

OEB – Occupational exposure bands

OEL – Occupational exposure limits

QC – Quality Control

SIP – Sterilization- in- place

SOP – Standard operating procedures

StDev – Standard deviation

USP – United States Pharmacopeia

WFI – Water for injection

Glossary

Active Pharmaceutical Ingredient - any substance or combination of substances used in a finished pharmaceutical product, intended to furnish pharmacological activity or to otherwise have direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to have direct effect in restoring, correcting or modifying physiological functions in human beings.

Aseptic - free of pathogenic microorganisms.

Batch - a quantity of any drug produced during a given cycle of manufacture.

Basic clean rooms – clean rooms that have control of airflow, air filtration, air velocity, air changes, air pressure, airborne particulates, temperature, humidity and microorganisms.

Bioburden - population of viable microorganisms on or in raw materials, products, and labeling/packaging materials determined before sterilization.

Clean in place - cleaning of equipment in its assembled condition and at its location. This cleaning may be an automatic process or manual. Whatever the method, it must comply with the stringent hygiene regulations of the pharmaceutical industries.

Clean room area - an area (or room or zone) with defined environmental control of particulate and microbial contamination, constructed and used in such a way as to reduce the introduction, generation and retention of contaminants within the area.

Containment - a process or device to contain product, dust or contaminants in one zone, preventing it from escaping to another zone.

Contamination - The undesired introduction of impurities of a chemical or microbiological nature, or of foreign matter, into or on to a starting material, intermediate or pharmaceutical product during handling, production, sampling, packaging or repackaging, storage or transportation.

Class C – a clean area for carrying out less critical stages in the manufacture of sterile products. In this clean area personal must take hair cover, beard cover, face mask, a general protective suit, gathered at the wrists and with high neck, shoe covers and gloves. This area has a maximum permitted number of particles per m^3 at rest and in operation and has recommended limits for microbial contamination. The maximum permitted number of particles per m^3 equal to or greater than 0,5 μm is at rest 352000 and is in operation 3520000. The maximum permitted number of particles per m^3 equal to or greater than 5 μm is at rest 2900 and is in operation 29000. The recommended limits for microbial contamination in contact plates (Ø 55mm) are 25 cfu/plate.

Cross contamination - contamination of a starting material, intermediate product or finished product with another starting material or product during production or due to microorganisms.

Current Good Manufacturing Practices – is the aspect of quality assurance that ensures that medicinal products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the product specification.

Class D - a clean area for carrying out less critical stages in the manufacture of sterile products. In this clean area personal must take hair cover, beard cover, a general protective suit and shoe covers. This area has a maximum permitted number of particles per m^3 at rest and in operation and has recommended limits for microbial contamination. The maximum permitted number of particles per m^3 equal to or greater than 0,5 μm is at rest 3520000 and is not defined in operation. The maximum permitted number of particles per m^3 equal to or greater than 5 μm is at rest 29000 and is not defined in operation. The recommended limits for microbial contamination in contact plates (Ø 55mm) are 50 cfu/plate.

Endotoxin - lipopolysaccharide contained within the outer membrane of Gram-negative bacteria that may lead to pyrogenic reactions and other biological activities in humans.

Gamma irradiation – the operation of exposing a material to gamma rays in order to sterilize.

Gray space – non-defined clean room area.

LAL test - used for the detection and quantification of bacterial endotoxins.

Process qualification - confirming that the manufacturing process as designed is capable of reproducible commercial manufacturing.

Process validation - documented evidence which provides a high degree of assurance that a specific process will consistently result in a product that meets its predetermined specifications and quality characteristics. Process validation is a requirement of current

Good Manufacturing Practices. Process validation involves a series of activities taking place over the lifecycle of the product and process.

Qualification - action of proving and documenting that equipment or ancillary systems are properly installed, work correctly, and actually lead to the expected results. Qualification is part of validation, but the individual qualification steps alone do not constitute process validation.

Sanitization - process of decontamination that reduces viable microorganisms to a defined acceptance level.

Shelf life - The period of time during which a pharmaceutical product, if stored correctly, is expected to comply with the specification as determined by stability studies on a number of batches of the product. The shelf-life is used to establish the expiry date of each batch.

Spalling – is the removal of small particulate matter from the inner wall of flexible tubing when subjected to repeated deformation and flexing.

Standard operating procedures – an authorized, written procedure giving instructions for performing operations not necessarily specific to a given product but of a more general nature (e.g. equipment operation, maintenance and cleaning, validation, cleaning of premises and environmental control, sampling and inspection).

Sterile – complete absence of microorganisms.

Sterilization - complete destruction or removal of all microorganisms including spore-forming and non-spore-forming bacteria, viruses, fungi, and protozoa.

Sterilization in place – sterilization of equipment in its assembled condition and at its location.

Validation - action of proving and documenting that any process, procedure or method actually and consistently leads to the expected results. It includes the qualification of systems and equipment. In general, an entire process is validated.

1. Introduction

1.1. Disposable technologies

It has been about a decade since disposable products were introduced to the biomanufacturing industry. While disposables have been examined for years in less regulated environments, disposable manufacturers had to demonstrate their benefits and qualities to convince biomanufacturers to try them (Mintz, C. 2009; Whitford, W. G., 2010).

Pharmaceutical drug products should be produced with efficiency (reduced costs and less production time) and with high quality according to current Good Manufacturing Practices (cGMP) as shown in the iron triangle (see figure 1) (Valle, C., 2009; Sandle, T., & Saghee, M. R., 2011). Quality-Time-Cost is in the middle of the development process. At each verification point, choices have to be made, based on Quality-Time-Cost. There must be a balance between the three pillars of the triangle. For example too little time could lead to poor quality, which can prove very expensive later on. Therefore, ways have to be found to improve quality and still cut on time and costs (Legrand, B., 2004).

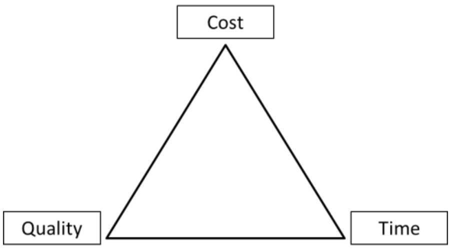


Figure 1. The Iron Triangle. Adapted from Atkinson, R. (1999).

However, the presence of microbiological contamination can affect the quality and the efficiency leading to batch rejection. Therefore, an emphasis on risk reduction should be an essential component of sterile product manufacturing. A focus on high quality operating clean rooms, trained staff, the use of sterilized clean room items and recent technologies are examples of what can be done to support this goal (Sandle, T., & Saghee, M. R., 2011).

The development from basic clean rooms to novel clean room technologies over the last years has resulted in a reduction on the risk of product contamination and in a simplification of the process operation. These novel technologies include the following: barrier isolated systems and RABS (restricted access barrier systems) to protect the aseptic filling; methods to protect the product from personnel contamination such as particle reducing air showers; sanitization and sterilization methods to decontaminate clean rooms and sterile disposable technologies. The use of disposable technologies allowed pharmaceutical companies to substitute equipment that needs to be sterilised by disposable items (Sandle, T., & Saghee, M. R., 2011).

Nowadays pharmaceutical companies are under pressure to increase productivity and profitability. In addition, there is a concern about costs, available capacity and sterility assurance, forcing them to look for available technologies in order to optimize their processes (Sinclair, A., & Monge, M., 2002; Aranha, H., 2004). As a consequence, some pharmaceutical companies have incorporated disposables technologies in the biopharmaceutical and biotechnology sectors (Sandle, T., & Saghee, M. R., 2011).

Single-use technologies, which are generally sterile, plastic items, have been adopted in order to replace traditional pharmaceutical processing items, like stainless steel that require cleaning, recycling and in-house sterilization (Sandle, T., & Saghee, M. R., 2011).

Disposables are available in different formats and can be rigid or flexible. They are usually manufactured from plastic polymers approved by the Food and Drug Administration (FDA) (Eibl, D., Peuker, T., & Eibl, R., 2011; Sandle, T., & Saghee, M. R., 2011). The plastic items are especially suitable to pre-sterilization with gamma irradiation, between 25 and 50 kGy, because of the relative transparency of plastics to ionizing radiation, which allow a disposable to be sterilized without the risk of spreading the sterilizing agent throughout the fluid path. The sterilization cycles must achieve a Sterility Assurance Level of at least 10^{-6}. This method of sterilization protects the disposable material, plastic, from degradation and eliminates the need for subsequent sterilization of the equipment. When gamma irradiation is used for sterilization it is important to take in consideration the appropriate irradiation dose and the inspection of the sterilized item for signs of degradation (Eibl, D., Peuker, T., & Eibl, R., 2011; Sandle, T., & Saghee, M. R., 2011; Repetto, R., et al., 2014). Other sterilization methods are acceptable to sterilize disposables. Examples of other sterilization methods are: autoclaving that is often used to sterilize components assembled by end-users and gas sterilization using ethylene oxide. However, gas sterilization is not widely used since by-products can be formed and some ethylene oxide can be retained. Each method is appropriate for different applications and should be evaluated for suitability (Repetto, R., et al., 2014).

1.1.1. Examples of disposable technologies

Table 1. Common types of disposable technologies used in the biopharmaceutical industry. Adapted from Sandle, T., & Saghee, M. R. (2011).T

Disposable technologies available to the biopharmaceutical industry
Tubing
Capsule filters
Single-use ion exchange
Single-use mixers
Bioreactors
Single-dose filtration systems
Product containing sterile bags
Aseptic connection devices
Biocontainer sampling bags

When using a disposable unit, there are a number of factors that should be considered, such as the number of manufacturing campaigns per year, the size of the production unit (volume and capacity requirements), the operating conditions (pressure, temperature and operation period) and the compatibility of the product with the disposable unit (Aranha, H., 2004).

1.1.2. Disadvantages

The main issue of using disposables that drug manufacturers and FDA are more concerned about, is the release of leachables and extractables (Kauffman, J. S., 2006; Mintz, C., 2009). Leachables are chemical components that migrate naturally over time, under normal conditions, from the disposable to the drug product formulation, when there is contact between both (Kauffman, J. S., 2006; Eibl, D., Peuker, T., & Eibl, R., 2011). The level and variability of the gamma irradiation that is used for the sterilisation of disposables can affect the level of leachables that migrate to the drug product formulation (Rao, G., Moreira, A., & Brorson, K., 2009). Extractables are chemical components that are extracted from the disposable that enters in contact with the drug product formulation when exposed to extreme conditions such as harsh solvents or high temperatures (Kauffman, J. S., 2006; Eibl, D., Peuker, T., & Eibl, R., 2011). The presence of leachables and/or extractables can modify the safety, quality, identity, strength or purity of the drug products. Therefore, the FDA offers guidance for protection against leachables and extractables (Kauffman, J. S., 2006).

1.1.3. Advantages

The adoption of disposables has been increasing due to a variety of advantages over traditional stainless steel systems, which include: decreased risk of cross-contamination, increased assurance of sterility, safety considerations, process efficiencies, cost saving and others (Aranha, H., 2004, Mintz, C. 2009; Sandle, T., & Saghee, M. R., 2011).

Cross-contamination

Cross contamination might result from microbiological contamination that can be transferred from one batch to another, and from product adulteration, which means that chemical residues can also be transferred from one batch to another. Disposables decrease the risk of cross-contamination because they are single used and consequently not used for further operations (Sandle, T., & Saghee, M. R., 2011).

Sterility assurance

The assurance of sterility is achieved due to a reduction of cross-transference of microorganisms that diminishes the risk of environmental microbial contamination and also due to a reduction of inadequate sterilisation cycles. The use of disposables avoid cross contamination since the need to clean components disappears, thus reducing manual handling. In addition, it allows the pharmaceutical companies to distance themselves from equipment that needs to be sterilized or offer a risk with their transfer to clean rooms. A reduction of microbial contamination is also achieved since structural integrity tests (testing for leaks) of disposable technologies are also made by the manufacturers and by the pharmaceutical company (Sandle, T., & Saghee, M. R., 2011; Jenness, E., & Gupta, V., 2011; Repetto, R., et al., 2014).

Safety considerations

The use of disposables is safe for the product and for the operator. On one hand, it is safe for the product because it reduces cross contamination as it is single used. In addition, the potential for microbial contamination is decreased because the intervention of the operator is less dependent since there is a reduction in assembly and handling of the system. On the other hand, it is safe for the operator to use single use systems when he manipulates cytotoxic drugs, so called highly potent drugs or radiolabeled chemicals. The handling of the operator with hazardous active pharmaceutical ingredients (API) is diminished because single use systems don't need clean in place (CIP) and sterilization in place (SIP) operations. Thus, disposable systems protect the operator from materials that can compromise his health and are in the best interest of manufacturing personnel (Aranha, H., 2004; Sandle, T., & Saghee, M. R., 2011).

Process efficiencies

The use of disposables has advantages with regard to process efficiencies in comparison with reusable stainless steel systems. Reusable stainless steel systems lead to equipment support and routine inspections that need labour, time, effort and money. With the use of disposables labour and time can be saved by decreasing operator dependence so that operators and other resources can be used for other tasks. Disposables also reduce process downtime and eliminate CIP and SIP, which save time, energy and costs. Time is also saved because qualification and validation are facilitated (Aranha, H., 2004; Eibl, D., Peuker, T., & Eibl, R., 2011). Moreover they save waste disposal and quantities of detergents and other cleaning chemicals that are used. The most important advantage is faster turnaround times. There is a reduction of the time taken to obtain a new batch ready for processing (Aranha, H., 2004; Mintz, C. 2009; Sandle, T., & Saghee, M. R., 2011).

Costs savings

One of the reasons that explain cost savings is the elimination of contamination events that lead to batch rejection and process downtime (Eibl, D., Peuker, T., & Eibl, R., 2011). Additionally, costs are reduced given that smaller batch sizes are possible to produce. Disposable technologies are also capable to operate outside the clean rooms, which are expensive (Valle, C., 2009). The costs are also reduced since the use of expansive cleaning procedures (corrosive chemicals, water for injection and sterilization procedures) are no longer required as disposables are pre-sterilized and discarded (Eibl, D., Peuker, T., & Eibl, R., 2011). With the use of disposables there is also a reduction of costs in installation, in process qualification and validation and in maintenance requirements (Wong, R., 2004; Eibl, D., Peuker, T., & Eibl, R., 2011; Sandle, T., & Saghee, M. R., 2011).

Therefore, the use of disposable technologies not only increases quality since these technologies decrease cross contamination, assure sterility and are safer but also reduces costs and time.

1.1.4. Qualification and Validation

GMP regulations that are involved with the production of drugs and active pharmaceutical ingredients (API´s) are supported by the FDA regulations 21 CFR Parts 210 and 211 and internationally they are supported by the European Union directive 356 (Day, N., 2004).

Disposable technologies are manufactured according to cGMP, and must be qualified and validated to guarantee that the equipment is not reactive, additive or absorptive, which is important to assure that the safety, identity, strength, quality or purity of the API is not affected (CFR, 2014).

Disposable technologies are manufactured with pharmaceutical grade polymeric materials that are tested to meet the requirements of USP<87>, USP<88>, USP<661> ISO 10993 and EP <3.1.9> (Creasey, J. & Thibion, V., 2013; Mueller, D., 2014). The qualification and validation methods have to demonstrate that the disposable is reliable, robust and safe (Belongia, B. M., & Allay, J. A., 2006).

Single use systems are sterilized with gamma irradiation, which can decrease the shelf life of disposables and can accelerate the degradation of the polymeric substances. Qualification tests must be done to determine the effects of irradiation and the stability of the polymer used. Some qualification tests are (Aranha, H., 2004; Jornitz, M. W., Cappia, J.-M. & Meltzer, T. H., 2009; Jornitz, M. W., Szarafinski, D., & Peuker, T., 2012; Mueller, D., 2014):

- Biocompatibility testing:
    - *USP* ‹87› biological reactivity tests, *in vitro,*
    - *USP* ‹88› biological reactivity tests, *in vivo.*
- Mechanical properties:
    - tensile strength,
    - elongation at break,
    - seal strength,
    - air leak test.
- Gas transmission properties:
    - ASTM D3985: oxygen,

  - ASTM F1249: water vapour.
- *USP* ‹661› test for plastics.
- European Pharmacopeia (EP) <3.1.7.>: Ethylene vinyl acetate (EVA) for containers and tubing.
- European Pharmacopeia (EP) <3.1.9.>: Silicone elastomers for closures and tubing.
- European Pharmacopeia (EP) <5.2.8.>: Minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products.
- Total organic carbon (TOC) analysis.
- pH and conductivity.
- Extractable and leachable tests with standard solutions.
- Chemical compatibility testing.
- Protein adsorption studies.
- Endotoxin testing.
- Gamma irradiation sterilization validation.
- Bacterial ingress test.

These tests are performed by the disposable technologies manufacturer under standard setting and solutions, so they only serve as guidance for the pharmaceutical company that buys the disposable technologies. Thus, before implementing the disposable technologies in the manufacturing process, the system users must validate them under specific requirements and process conditions (Aranha, H., 2004; Jornitz, M. W., Cappia, J.-M. & Meltzer, T. H., 2009; Jornitz, M. W., Szarafinski, D., & Peuker, T., 2012).

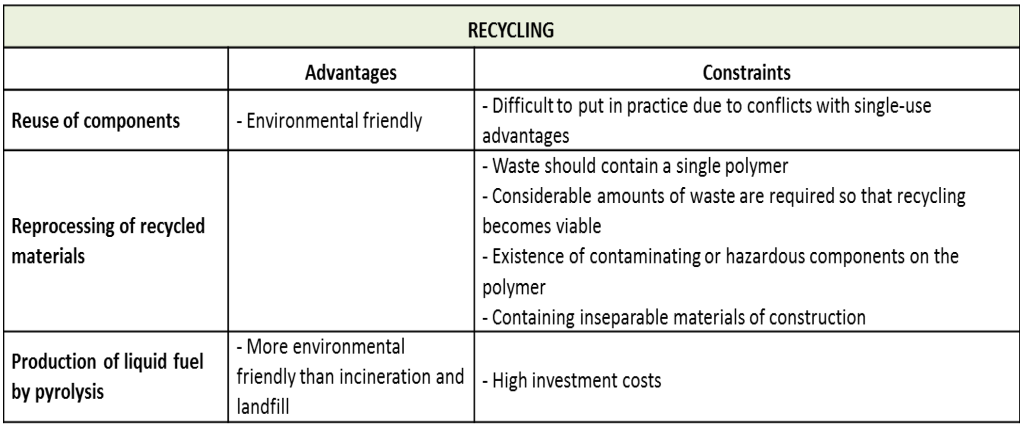
1.1.5. Environmental impact

The balance of ecosystems can be significantly affected by human activities and therefore have health, aesthetic and economic consequences to us. Consequently, sustainability is an important issue and companies must respect laws and regulations that are related to their business activities (Whitford, W. G., 2014). Companies should provide information on the environmental impact and sustainability of their manufacturing systems (Rawlings, B., & Pora, H., 2009). The environmental evaluation can be enabled through guidelines from industrial organization (ex: Bio Process Systems Alliance (BPSA)), regulatory guidelines and directives, data from suppliers and manufacturers and published articles, reviews, and case studies (Rawlings, B., & Pora, H., 2009).

The environmental impact of disposable technologies has become a concern in recent years (Rawlings, B., & Pora, H., 2009; Pora, H., & Rawlings, B., 2009). Thus, manufacturers should choose recyclable materials of construction for disposable systems instead of using materials made from non-renewable feedstocks (Rao, G., Moreira, A., & Brorson, K., 2009). Disposable systems should be designed and manufactured to ensure a minimum environmental impact and a maximum recyclability of waste (Rao, G., Moreira, A., & Brorson, K., 2009; Pora, H., & Rawlings, B., 2009).

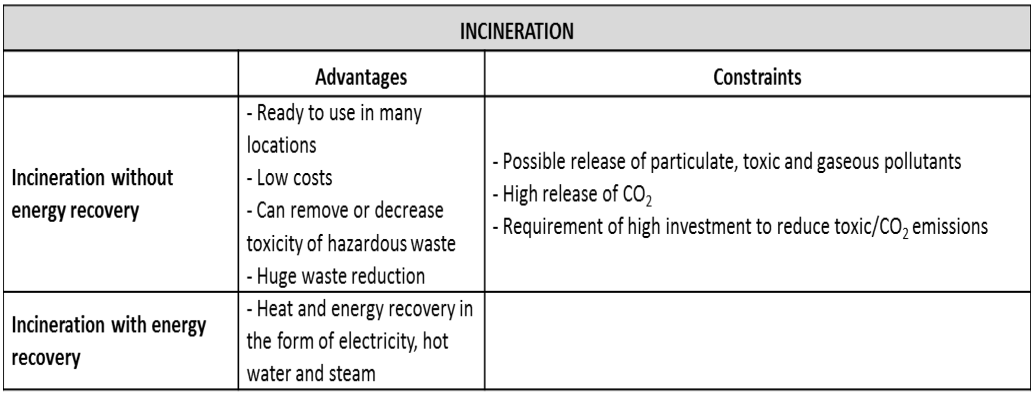
Another concern is the environmental impact of currently used disposal methods, which should consider the legislation in force to protect the environment and guarantee industrial sustainability. There are three major solid-waste disposal methods for disposable systems: 1) recycling options, which include the reuse of components, the reprocessing of recycled material and the production of liquid fuel; 2) incineration, with or without energy recovery; 3) landfills (non-hazardous waste, hazardous waste). The following tables summarize the advantages and constraints of the methods above mentioned (Pora, H., & Rawlings, B., 2009):

Table 2. Recycling advantages and constraints. Adapted from Pora, H., & Rawlings, B. (2009).



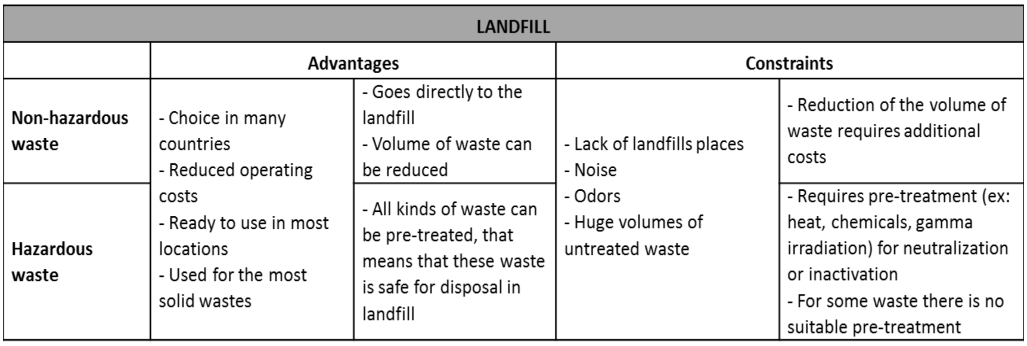
RECYCLING		
	Advantages	**Constraints**
Reuse of components	- Environmental friendly	- Difficult to put in practice due to conflicts with single-use advantages
Reprocessing of recycled materials		- Waste should contain a single polymer - Considerable amounts of waste are required so that recycling becomes viable - Existence of contaminating or hazardous components on the polymer - Containing inseparable materials of construction
Production of liquid fuel by pyrolysis	- More environmental friendly than incineration and landfill	- High investment costs

Table 3. Incineration advantages and constraints. Adapted from Pora, H., & Rawlings, B. (2009).



INCINERATION		
	Advantages	**Constraints**
Incineration without energy recovery	- Ready to use in many locations - Low costs - Can remove or decrease toxicity of hazardous waste - Huge waste reduction	- Possible release of particulate, toxic and gaseous pollutants - High release of CO_2 - Requirement of high investment to reduce toxic/CO_2 emissions
Incineration with energy recovery	- Heat and energy recovery in the form of electricity, hot water and steam	

Table 4. Landfill advantages and constraints. Adapted from Pora, H., & Rawlings, B. (2009).



LANDFILL				
	Advantages		**Constraints**	
Non-hazardous waste	- Choice in many countries - Reduced operating costs - Ready to use in most locations - Used for the most solid wastes	- Goes directly to the landfill - Volume of waste can be reduced	- Lack of landfills places - Noise - Odors - Huge volumes of untreated waste	- Reduction of the volume of waste requires additional costs
Hazardous waste		- All kinds of waste can be pre-treated, that means that these waste is safe for disposal in landfill		- Requires pre-treatment (ex: heat, chemicals, gamma irradiation) for neutralization or inactivation - For some waste there is no suitable pre-treatment

Taking into account the three disposal methods presented, the optimal choice would be the one with the lowest environmental impact. In order to help making this choice, a decision tree can be used, as shown below (Pora, H., & Rawlings, B., 2009):

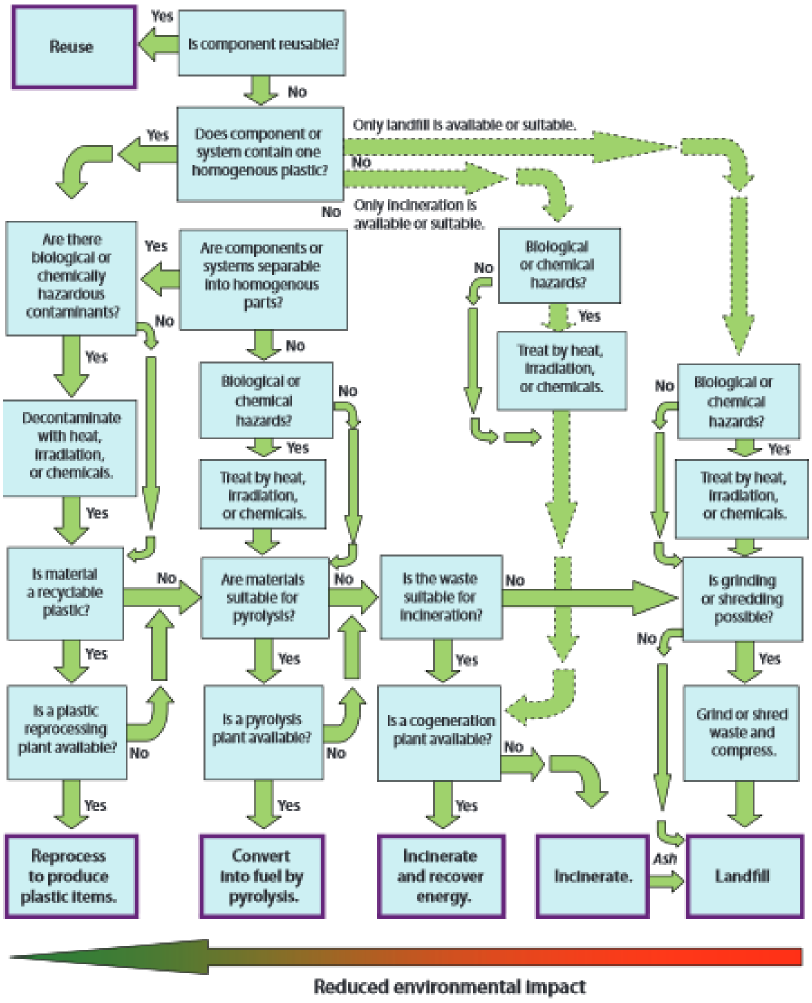


Figure 2. Decision tree for management of solid waste (Pora, H., & Rawlings, B., 2009).

1.2. Highly potent drugs

In the recent years, the pharmaceutical industry has developed new and more potent API due to advances in clinical pharmacology and oncology research (Mari, G., Moccaldi, A., & Ludovisi, G., 2005; Karmarkar, A. B., 2012). Highly potent active pharmaceutical ingredients (HPAPI) are becoming more selective in their interaction with biological targets and are particularly effective at small doses (Harris, R., 2012; Bowman, M., 2013). Examples of HPAPIs are hormones, cytostatics, narcotics and retinoids (Bowman, M., 2013; Denk, R., 2014). In addition, there is a new generation of highly potent drugs called antibody drug conjugates (ADCs) which are a monoclonal antibody conjugated with a HPAPI like a cytotoxic. The ADCs are selective in their interaction with the target and differentiate the healthy tissue from the diseased tissue, since the monoclonal antibody is joined to the HPAPI by a short linker molecule, which is designed to break down at the active site and release the cytotoxic (Bormett, D., 2008; Lonza, 2012; Wooge, C., 2014)

Every API must be evaluated and classified to assess potential toxicity, potency and potential hazards so that the level of containment that is required can be determined (Bormett, D., 2008). The drug substances can be classified according to their potency based on the use of occupational exposure limits (OEL) or occupational exposure bands (OEB) (Calkins, T., 2010; Wollowitz, S., 2010; Harris, R., 2012). The OEL is given in microgram (µg) per cubic meter (m^3) or milligram (mg) per cubic meter (m^3) and as the most likely and toxic route is by inhalation it is defined as the air concentration of the compound that can be inhaled by a worker over an 8h working day, 40 hours a week without adverse effects (Wollowitz, S., 2010; Denk, R., 2014). A lower value of OEL means a more potent compound which requires a higher level of containment (Van Arnum, P., 2009). The OEB group together substances by an approximate hazard level and this dictate the handling containment required to work with the substances (Wollowitz, S., 2010; Bowman, M., 2013). In the last years, a number of systems have been proposed to classify APIs (Harris, R., 2012). The industry uses different category systems ranging from three to six - category systems (Farris, J. P., Ader, A. W., & Ku, R. H., 2006; Calkins, T., 2010). There are also companies that developed their own category system, according to their equipment and facilities (Bowman, M., 2013). There are different category systems because companies have different products, facilities, equipment and processes (Farris, J. P., Ader, A. W., & Ku, R. H., 2006). The system that is more frequently used is the four category system, the SafeBridge System and its evaluation and categorisation are described in table 5 (Farris, J. P., Ader, A. W., & Ku, R. H., 2006; Harris, R., 2012; Bowman, M., 2013).

Table 5. High potency API evaluation and categorization. Adapted from Farris, J. P., Ader, A. W., & Ku, R. H. (2006), Harris, R. (2012), Bowman, M. (2013).

Criteria	Category 1	Category 2	Category 3	Category 4
Dosage/Day	>100 mg/day	10-100 mg/day	0,01-10 mg/day	< 0,01 mg/day
OEL	> 0,5 mg/m^3	10 µg/m^3– 0,5 mg/m^3	30 ng/m^3 – 10 µg/m^3	< 30 ng/m^3
Potency	Low potency	Moderate potency	Potent	High potent
Toxicity	None or slight	Low - moderate	Moderate	High toxicity

Active pharmaceutical ingredients can be potent when (Bormett, D., 2008; Bowman, M., 2013):

1. The active pharmaceutical ingredient has an OEL of $\leq 10\ \mu g/m^3$ of air as an 8 hour time weighted average.
2. The active pharmaceutical ingredient has a therapeutic dose of ≤ 10 mg/day.
3. The dose of 1 mg/kg/day of active pharmaceutical ingredient produce serious toxicity in laboratory animals.
4. The active pharmaceutical ingredient has high selectivity and/or has the potential to cause cancer, mutations, developmental effects or reproductive toxicity at low doses.
5. It is a novel compound of unknown toxicity.

The handling with highly potent active pharmaceutical ingredients requires a substantial investment in specialized containment to guarantee that workers and the working environment are protected from exposure (Bormett, D., 2008). A safe handling with highly potent active pharmaceutical ingredients can be achieved by using standard operating procedures (SOPs), trained workers, containment equipments, personal protective equipment, process isolation, facility design and cGMP activities (Bormett, D., 2008; Karmarkar, A. B., 2012).

1.2.1. Facility design

Handling with highly potent active pharmaceutical ingredients requires specialized equipment to achieve containment and attention to safety. A facility should be designed with equipment such as isolators with laminar flow hoods and appropriate ventilation for potent compound handling to protect workers and to maintain the product sterile. The room pressure should be negative to its surrounding rooms to guarantee that the potent products are retained in the working environment. The air should also be filtrated so that escaping product is captured before its release to the external environment. To prevent cross contamination or concentration of materials, the airflow in the facility must be single pass. Process isolation and containment equipment are very important to ensure that an entire manufacturing process is carried out in closed systems (from raw materials to product packaging) so that the chances of operators exposure can be minimized (Van Arnum, P., 2009; Calkins, T., 2010; Wooge, C., 2014).

The facility design for ADCs has to take in consideration that the HPAPI must be contained under negative pressure, but the antibody components and the final drug product should be handled under positive pressure to prevent microbial and particle contamination. Therefore, the handling steps involving HPAPI should take place in an isolator in a separate room, where the air is under negative pressure. HPAPIs can only leave this room if they are in solution, because this removes the risk of airborne contamination and the chances of operators or environment contamination are much lower. The conjugation to an antibody occurs in a second room which is under positive pressure and therefore protects the product from external contamination. The conjugation reaction must still take place in a closed system that enables full containment of hazardous materials, because despite being in solution HPAPIs are still hazardous (Calkins, T., 2010; Wooge, C., 2014).

Disposable technologies are a suitable option for handling with HPAPIs as they can improve containment. They protect and reduce the exposure of the operators from the compounds, they eliminate the need for extensive cleaning that potent drugs require and decrease cross contamination. The adoption of disposable technologies is of extreme importance in order to avoid the present risk of contamination of HPAPI in multiproduct facilities (Greb, E., 2010; Challener, C., 2014).

1.3. Cryo-vessel

Cryo-vessels are large-scale cryo-preservation systems made of stainless steel containers from 50-300 L. They are divided in two compartments. The inner compartment contains the API solution, whereas the outer compartment, named double coat is for the circulation of silicon oil. They are systematically frozen and thawed with a well-controlled heat or cold transfer fluid (silicon oil) that circulates in the outer compartment and goes in the internal compartment through an inner tube snake with fins, which efficiently transfer heat or cold into the solution (Arnitz, T., & Liebig, C., 2011; Kantor, A., MacMillan, S., Ho, K., Tchessalov, S., & Warne, N., 2011; Kolhe, P., & Badkar, A., 2013).

Cryo-vessels are used for freezing, storage, transport and thawing of API. The temperature storage can range from liquid storage at 2 – 8 °C to frozen storage down to -50 °C. The liquid storage from 2 - 8 °C can affect the quality and lead to degradation of proteins. Therefore, frozen storage is indicated to guarantee a higher stability and longer shelf life of proteins, decrease microbial growth and eliminate the foam formation during transport (Webb, S. D., Webb, J. N., Hughes, T. G., Sesin, D. F., & Kincaid, A. C., 2002; Arnitz, T., & Liebig, C., 2011; Kantor, A., Tchessalov, S., & Warne, N., 2011; Desu, H. R., & Narishetty, S. T., 2013).

As proteins lack stability when in solution, it was considered convenient since a long time, to freeze them because of the need to store proteins for a large period of time. New approaches to the freezing and storage of proteins have been developed in which the freeze-thaw process is better controlled due to the emergence of large scale production of proteins (Kantor, A., Tchessalov, S., & Warne, N., 2011).

Protein based API can be stored under a variety of conditions such as refrigerated liquids and spray dried powders. However, a considerable number of clinical and commercial API are stored in a frozen state (Kantor, A., Tchessalov, S., & Warne, N., 2011).

There are three types of container closure system normally used for frozen API: plastic or stainless steel bottles between 1-5 L, plastic bags between 1-16 L and stainless steel cryo-vessels between 50-300 L. Although there has been a development of the systems, there is still a need for all these three systems depending on the nature of the drug substance, its volume and storage temperature (Kantor, A., Tchessalov, S., & Warne, N., 2011; Kolhe, P., & Badkar, A., 2013).

1.4. Tubings and Connectors

Nowadays the economic situation is an obstacle for the development of new drugs. As a challenge, the pharmaceutical companies have to review their manufacturing systems and find ways to make them more reliable, flexible and cost-effective. Therefore traditional stainless steel equipment has been replaced by pre-sterilized disposable technologies (Boehm, J., 2010).

1.4.1. Tubings

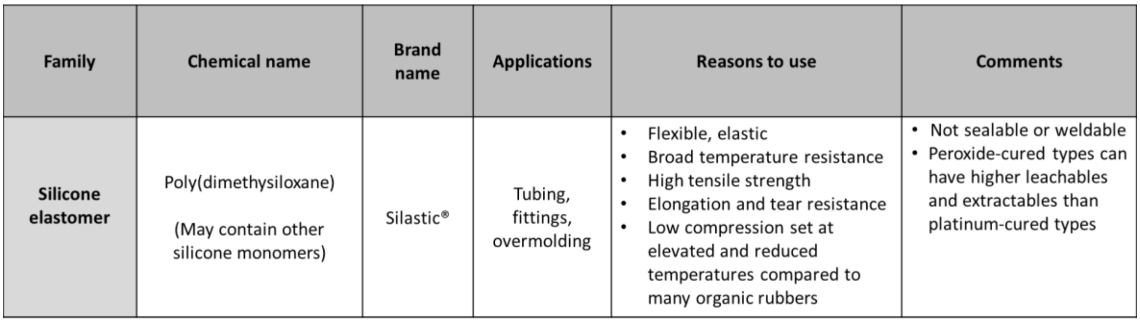
Flexible tubings have gained more acceptance in recent years due to its low costs associated with validation, CIP and SIP (Spontak, R. J., & Patel, N. P., 2000; Colas, A., Malczewski, R., & Ulman, K., 2004).

Tubings are involved in the manufacturing of pharmaceuticals and are currently used for fluid transfer, gas transfer, peristaltic pumping and filling operations. Tubings are made from various polymeric materials that may vary according to polymer class, molecular weight and additives. This variability confers a wide range of performance characteristics on the plastic materials. Examples of plastics that are commonly used are silicone and thermoplastic elastomer (Colas, A., 2001; Aranha, H., 2004).

Silicone can have diverse applications: as excipients in topical formulation, as excipients in controlled devices and in pharmaceutical manufacturing for siliconisation, as antifoam and for tubing. Silicone tubings are used in pharmaceutical manufacuturing operations for transfer processes. Two types of silicone tubings may be used: platinum-cured silicone tubings or peroxide-cured silicone tubings. It is preferable to use platinum-cured silicone tubings since it is naturally purer and it offer fewer leachables and extractables; they have smoother internal surfaces and it minimizes protein loss due to adhesion. Moreover peroxide cured tubings contain peroxide by-products that can be released and contaminate the transferred product (Colas, A., 2001, Aranha, H., 2004; AdvantaPure, 2013). The platinum-cured silicone tubings can have different applications such as in ultra-pure fluid transfer or in peristaltic pumps, where durability is required or in high/low pressure applications (Dow Corning).

Tubings that can be heat sealed and welded are often needed at some point in the fluid path. Instead of using multiple materials in the different sections of the fluid path since not all materials have the required characteristics (for example: heat sealed and welded capacity), a material that meets the needed requirements of the customer can be selected. Tubing materials such as thermoplastic elastomer (combine the material properties of rubbers and plastics) meet the conditions that are often required.This kind of tubing is flexible, has a high purity, is less permeable and is a peristaltic pump tubing that may be welded and sealed, thus eliminating the use of multiple materials in a fluid path (AdvantaPure, 2013). Thermoplastic elastomer can be divided in two groups: multiblock copolymers which consist of soft elastomers and hard thermoplastic blocks such as thermoplastic polyurethane and blends (Shanks, R., & Kong, I., 2012).

Table 6. Example of plastic used for tubing (Silicone). Adapted from Repetto, R., et al. (2014).



Family	Chemical name	Brand name	Applications	Reasons to use	Comments
Silicone elastomer	Poly(dimethysiloxane) (May contain other silicone monomers)	Silastic®	Tubing, fittings, overmolding	• Flexible, elastic • Broad temperature resistance • High tensile strength • Elongation and tear resistance • Low compression set at elevated and reduced temperatures compared to many organic rubbers	• Not sealable or weldable • Peroxide-cured types can have higher leachables and extractables than platinum-cured types

Table 7. Example of plastics used for tubing (Thermoplastic elastomer). Adapted from Repetto, R., et al. (2014).

Family	Chemical name	Brand name	Applications	Reasons to use	Comments
Thermoplastic elastomer	Thermoplastic polyurethane (TPU)	Ellastolan® Irogran®	Tubing	• Flexible – can be stretched to moderate elongations and return to close to original shape • Absence of significant creep • Autoclavable	• TPUs have a large variety of chemical structures • Critical to specify manufacturer and grade of TPU resins
Thermoplastic elastomer blends	Styrene-ethylene-butyl-styrene + polypropylene (SEBS-PP)	C-Flex® Kraton®	Tubing, fittings, overmolding	• Flexible – can be stretched to moderate elongations and return to close to original shape • Absence of significant creep • Weldable	• Autoclave conditions may cause tubing to deform

Other plastics that can be used for tubing:

Table 8. Example of plastic used for tubing (Copolymer). Adapted from Repetto, R., et al. (2014).

Family	Chemical name	Brand name	Applications	Reasons to use	Comments
Copolymer	Etylene vinyl acetate (EVA)	Evatane® Elvax®	Bags, tubing	• Soft and flexible • Good clarity and gloss • Good barrier properties • Excellent cold temperature properties	• Gamma irradiated EVA can lower pH of contents • Exctrables may be undesirable • Poor heat resistance • Attacked by polar solvents, hidrocarbons, oxidants and strong acids

Table 9. Examples of plastics used for tubing (Fluoropolymers). Adapted from Repetto, R., et al. (2014).

Family	Chemical name	Brand name	Applications	Reasons to use	Comments
Fluoropolymers	Poly(tetrafluoroethylene) (PTFE)	Teflon® Fluon®	Filters, tubing	• Biologically inert • Chemically resistant • Extreme cold or hot temperature resistance • Low exctrables and leachables	• Cannot be sterilized using gamma irradiation • Limited mechanical properties
	Poly(vinylidenefluoride) (PVDF) Poly(vinylidene fluoride-co-hexafluoropropylene	Kynar® Kynar Flex®	Filters, fittings, tubing, bags	• Biologically inert • Greater strength and wear resistance than PTFE • Broad temperature and chemical compatibility • Low extractables and leachables • Easily worked, molded and sealed	• Sterilizable by irradiation, steam or chemical • Some strong solvents can solubilize the material

In the cGMP environment flexible tubing has to be qualified and validated. So, the tubing that are considered for single use application as well as for multiple use application need to meet FDA, ISO, USP, EP and ASTM standards. In case of an inspection in the US it is sufficient that the tubing meet the US standards and in case of an inspection in the Europe it is sufficient that the tubing meet the Euopean standards. Therefore, according to the country where it is manufactured and according to the delivery country the following standards should be fulfilled (Colas, A., Malczewski, R., & Ulman, K., 2004; Hunt, D. G., 2013; Mueller, D., 2014):

US standards:

- FDA G95-1 Memorandum: Required Biocompatibility Training and Toxicology Profiles for Evaluation of Medical Devices.
- FDA 21 CFR 177.2600: Rubber articles intended for repeated use.
- USP <85>: Bacterial Endotoxins Test.
- USP <87>: Biological Reactivity Tests, In Vitro.
- USP <88>: Biological Reactivity Tests, In Vivo, Classification of Plastics: Class V and VI.
- USP <1663>: Extractables.
- USP <1664>: Leachables.
- ASTM F748-98: Standard Practice for Selecting Generic Biological Test Methods for Materials and Devices.

International standards:

- ISO 10993: Biological Evaluation of Medical Devices, Part 1: Evaluation and Testing.
- ISO 10993: Biological Evaluation of Medical Devices, Part 5: Tests for in vitro cytotoxicity.
- ISO 10993: Biological Evaluation of Medical Devices, Part 11: Tests for systemic toxicity.

European standards:

- EP <3.1.9>: Silicone elastomers for closures and tubing.
- EP <3.2.2>: Plastic containers and closures for pharmaceutical use.

- ASTM F748-98: Standard Practice for Selecting Generic Biological Test Methods for Materials and Devices.

1.4.2. Connectors

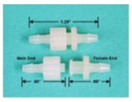
Many of the advantages of disposable technologies would be lost if manufactures could not safely connect systems to create an aseptic process, once a connector can be the determining factor in keeping a process strictly aseptic (Boehm, J., 2010).

The risk of contamination requires special attention in biopharmaceutical processes since almost every biopharmaceutical process implicates making sterile connections between fluid pathways. Traditionally, reusable fittings have been used, particularly those made of stainless steel (e.g. a stainless steel tri-clamp), but the tendency is towards the use of disposable connectors (Mach, C. J., & Riedman, D., 2008).

A wide variety of connectors are commercially available and the right choice depends on the needs and preferences of a given facility and customer (Boehm, J., 2010).

The different types of connectors are summarized in the table below (Rothe, S. & Eibl D., 2011; Niazi, S. K., 2012; Shukla, A., Mostafa, S., Wilson, M., & Lange, D., 2012):

Table 10. Different types of connectors. Adapted from Rothe, S. & Eibl D. (2011), Niazi, S. K. (2012), Shukla, A., Mostafa, S., Wilson, M., & Lange, D. (2012).

Luer Lock connectors	• Male and female are connected • Suitable for small volume flow rates	
Sanitary/Tri-clamps connectors	• A clamp connects two flat parts with a gasket between them • A connection with stainless steel equipment is also possible	
Quick (dis)connect fittings	• Male and female parts are connected via a click mechanism • To separate the connection a button on the female part must be pressed	
Aseptic connectors	• Allow aseptic connection of two sterile systems in a non aseptic environment • The joints can´t be disconnected	

There are different systems for aseptic connection:

1. Connection under laminar flow hood or in a clean room area class A:

Luer-lock connectors, tri-clamps and quick (dis)connect connectors provide fast and easy connections and should be used together with laminar flow hood. Careful handling with this type of connectors is needed to prevent contamination. Examples of this kind of connectors are: tri-clamps and MPC$^{®}$ Series from Colder Company (Boehm, J., 2010; Rothe, S. & Eibl D., 2011).

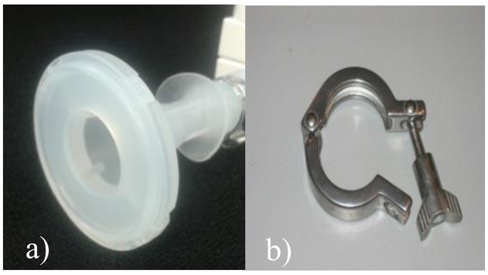


Figure 3. Triclamp disposable (a) and a stainless steel clamp (b)

Figure 4. Quick-connectors: MPC® Series from Colder Company

2. Sterilisation in place connectors:

These types of connectors create sterile connections between a range of disposable systems and stainless steel equipment. They can be sterilized at the point of connection. Examples of this kind of connectors are: Steam-Thru System$^{®}$ from Colder Company and Lynx ST$^{®}$ from Millipore Company (Boehm, J., 2010; Rothe, S. & Eibl D., 2011).

Figure 5. Steam-Thru System® from Colder Company

Figure 6. Lynx ST® from Millipore Company

3. Aseptic connectors

Aseptic connectors allow aseptic connection without laminar flow hood like conventional connectors. These connectors are secure, the connections can be achieved rapidly without any further support or additional equipment and permit a sterile fluid pathway in a non-aseptic environment (gray space). The aseptic parts on the connector side are sealed with sterile membrane filters or caps. After coupling, the sterile membrane filters or caps must be removed and both parts have to be clamped or fixed. The connection is then ready for use. It is not possible to disconnect connectors that are once connected. Examples of this kind of connectors are: Kleenpak KPC® by Pall, Lynx S2S® by Millipore, Opta SFT-1® by Sartorius Stedim Biotech, ReadyMate DAC® by GE and the Pure-Fit SC® by Saint-Gobain (Strahlendorf, K. A., & Harper, K., 2009; Boehm, J., 2010; Rothe, S. & Eibl D., 2011).

Figure 7. Kleenpak KPC® by Pall

Figure 8. Lynx S2S® by Millipore

Figure 9. Opta SFT-1® by Sartorius Stedim Biotech

Figure 10. ReadyMate DAC® by GE

Figure 11. Pure-Fit SC® by Saint-Gobain

The variety of plastics and connectors enables the development of innovative equipment and provides customization.

1.5. Fluid transfer

In the manufacturing process the fluid can be transferred either by pump systems or by pressurizing (Kubischik, J. & Schaupp, M., 2011).

1.5.1. Pump systems

Pumps can be classified in two types: positive displacement pumps and rotodynamic pumps. Both types are designed to transfer fluids but the way this is accomplished differs (Srinivasan, K. M., 2008).

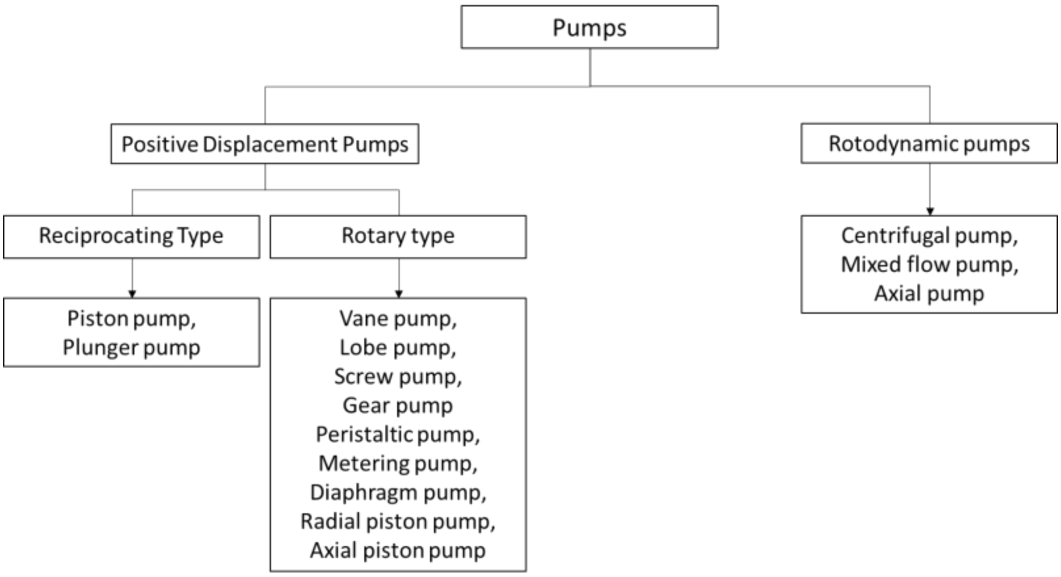


Figure 12. Classification of pumps. Adapted from Srinivasan, K. M. (2008).

A positive displacement pump moves the same amount of fluid for each rotating cycle of the system. It uses mechanisms that seals fluid in a chamber and forces it out by reducing the volume of the chamber. This action increases the fluid pressure. The motion can be rotary or reciprocating (Srinivasan, K. M., 2008; Gupta, A. K. & Arora, S. K., 2013).

In a rotodynamic pump there is a free passage of fluid between the inlet and outlet of the machine without sealing. Rotodynamic pumps have a rotating part called impeller, which rotates the fluid continuously and transfers energy from the rotor to the fluid (Garde, R. J., 1997; Gupta, A. K. & Arora, S. K., 2013).

Piston pumps have been a traditional technology in pharmaceutical technology. However due to stricter validation requirements and design innovations peristaltic pumps started to be considered instead of piston pumps (Lambert, P., (2008).

Piston pump

A piston pump is a positive displacement pump. It is a mechanical device that cycles through a suction phase and a pressure phase to move fluid. Internal parts of a piston pump (gaskets, seals, valves and internal surfaces) are in direct contact with the fluid. Piston pumps are known for their reliability and accuracy. However, they require regular maintenance and disassembly for cleaning and sterilization and cross contamination between batches is a concern. In addition, they also apply high pressure and high shear forces, that are inappropriate for biological drugs which are shear sensitive (Lambert, P., 2008).

Peristaltic pump

A peristaltic pump is also a positive displacement pump. In this pump type the fluid is moved forward by progressively squeezing and releasing the flexible tubing (Avis, K. E. (Ed.) 1995; Lipták, B. G. (Ed), 2003; Volk, M., 2014). The product only comes in contact with the flexible tubing. For this reason peristaltic pumps can be used for single aseptic processes (Lambert, P., 2008).

Peristaltic pumps using disposable tubes eliminate the possibility of cross contamination and the cleaning of the pump isn't required since the tubing is the only part that comes into contact with the fluid (Lipták, B. G. (Ed), 2003; Niazi, S. K., 2012). It also can be used with some biological drugs that are shear sensitive, since they apply low pressure and provide a gentle handling (Niazi, S. K., 2012). However, studies have shown that some tubing materials release particles (spalling) during the use with peristaltic pumps, due to their poor abrasion resistance and consequently, contaminating the solution (Bahal, S. M., & Romansky, J. M., 2002; Colas, A., Malczewski, R., & Ulman, K., 2004).

1.5.2. Pressurizing

Fluids can be transferred by using pressurized gas or vacuum, when pumps are not available, when their use might cause contamination or degradation due to mechanical shear or when handling abrasive or corrosive materials, thus avoiding pumping the materials. Pressurized systems use nitrogen, air or other gas under moderate to high pressure to force the transfer of the fluid (CCPS, 1993; Kerry, F. G., 2007).

1.6. Influence of Pump systems and Pressurizing in protein solutions

In some cases, during manufacturing, proteins undergo a variety of stresses leading to physical and chemical instability that can compromise the quality, safety and efficiency of the proteins, such as monoclonal antibodies and the yield of the final drug product. Physical instability causes changes in the higher order structure of proteins (secondary, tertiary or quaternary structure), which include denaturation, aggregation, precipitation or adsorption to surfaces. Chemical instability is a covalent modification of the protein via bond formation or cleavage (Banga, A. K., 2005; Bausch, U. J., 2008; Vázquez-Rey, M., & Lang, D. A., 2011; Ma, J. K., & Hadzija, B., 2013).

Protein aggregation can be induced by various processing steps during manufacture such as pumping and pressurization (Vázquez-Rey, M., & Lang, D. A., 2011).

Pumping processes expose proteins to mechanical shear forces that can cause protein aggregation and formation of particles. However, the extent of impact depends on the intensity and on the duration of exposure to such stress. Shearing creates a hydrophobic air/water interface which results in the alignment of protein molecules at the interface. The alignment of the protein molecules at the interface occurs since proteins denature exposing the hydrophobic residues to the air, forming unfolded intermediates that induce protein aggregation (Wang, W., 1999; Vázquez-Rey, M., & Lang, D. A., 2011; Ma, J. K., & Hadzija, B., 2013). To reduce shearing-induced protein aggregation, surfactants can be used as they compete with the protein molecules for the hydrophobic interfaces by binding directly to proteins or by increasing the viscosity of a protein solution limiting the movement of proteins (Wang, W., Nema, S., & Teagarden, D., 2010). Certain pumps like piston pumps and peristaltic pumps can form protein particles (for example white tornadoes) due to shear. However, peristaltic pumps generate less protein particles as piston pumps (Bausch, U. J., 2008; Roche Internal Report, 2008; Nayak, A., Colandene, J., Bradford, V., & Perkins, M., 2011).

Despite peristaltic pumps being advertised to be used with biological drugs that are shear sensitive, it was shown that they also can form protein aggregation and protein particles due to shear forces (Maggio, E. T., 2008). Some authors correlate the formation of protein aggregation with the material of the tubing (Thomas, C. R., & Geer, D., 2010, Vázquez-Rey, M., & Lang, D. A., 2011).

The formation of aggregates due to pumping can also be minimized using pressure for the transfer of fluids (Vázquez-Rey, M., & Lang, D. A., 2011).

Low to moderate pressures exert no significant effect on the aggregation of proteins. However, high pressure can cause protein denaturation and facilitate protein aggregation due to increased hydrophobic interactions. The pressure-induced protein aggregation has not been a major problem in the manufacturing of proteins therapeutics since the pressure that is required to unfold proteins must be greater than 100 MPa, which is beyond the range of routine exposure (Chang, B. S., & Yeung, B., 2010; Wang, W., Nema, S., & Teagarden, D., 2010; Meersman, F., Daniel, I., Bartlett, D. H., Winter, R., Hazael, R., & McMillain, P. F., 2013).

2. Target

The purpose of this master thesis is to test the possibility to transfer API solutions with peristaltic pump and overpressure in combination with a combined thawing and transfer disposable tube and to verify if it is advantageous to replace the current thawing and transfer disposable tubes used in the API thawing and drug product compounding process by a new combined thawing and transfer disposable tube in order to diminish the quality risks of manual handling, reduce assembling time and improve safety of ADCs.

With that purpose in mind, a series of practical experiments will be performed, which consist of testing three different combined thawing and transfer disposable tubes with two methods for moving fluid: peristaltic pump and overpressure. Testing a new combined disposable with overpressure and additionally, using a peristaltic pump for the transfer of API solution would be innovative in the methods used in Roche Diagnostics GmbH, Mannheim drug product compounding processes, since until today the only moving fluid method used for transfer has been overpressure with a specific tube with pressure capabilities. In addition, the transfer speeds will be calculated, the length and diameter of the tubes will be measured and the whole handling process evaluated in order to compare their benefits and disadvantages from the quality and efficiency perspective.

3. Types of Cryo-vessels

Roche Diagnostics GmbH, Mannheim operates with three different types of cryo-vessels, namely US (United States Cryo-vessel), Basel and Penzberger (European Cryo-vessels).

3.1. US Cryo-vessel Type

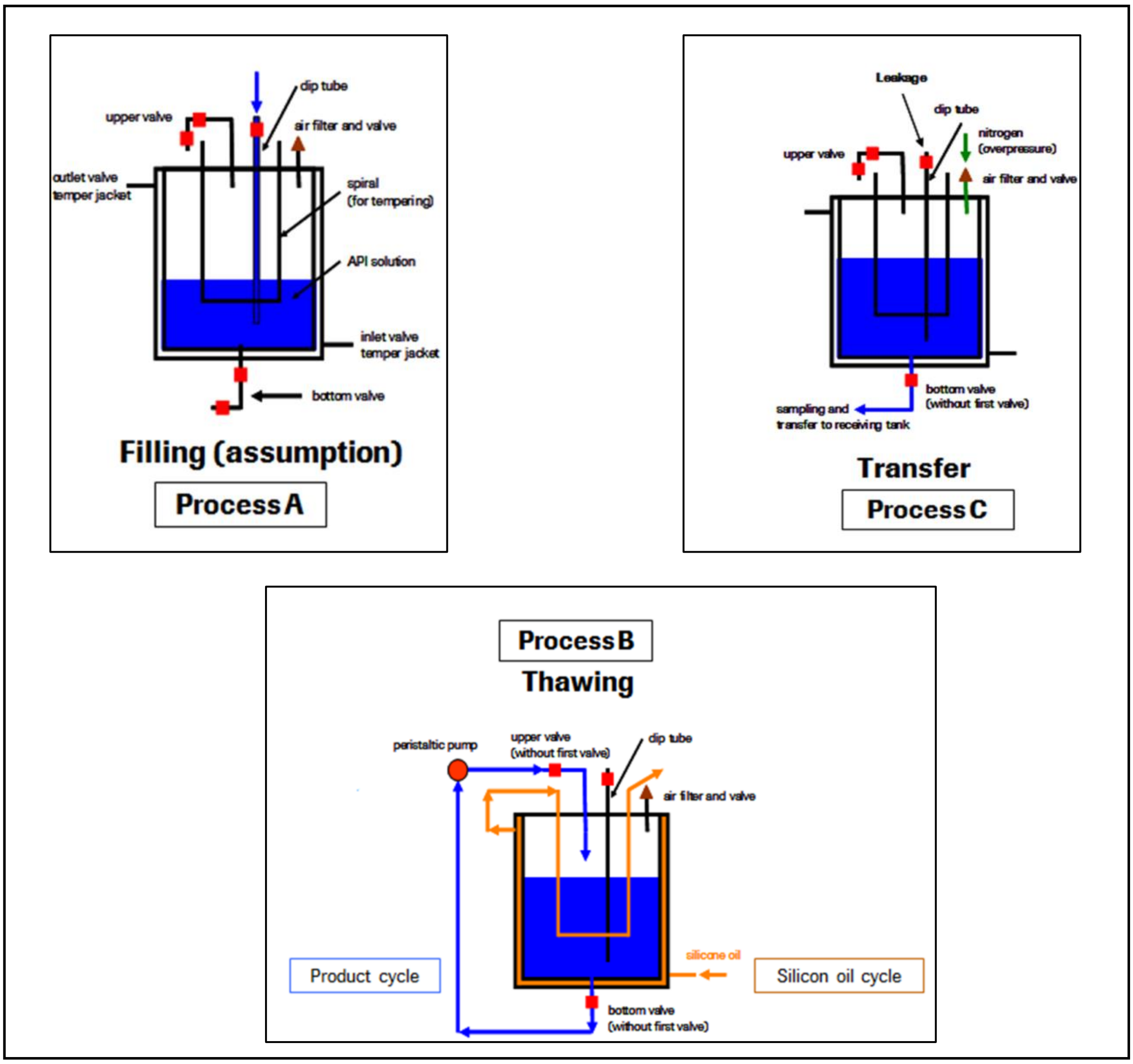


Figure 13. US Cryo-vessel type: Filling, Thawing and Transfer processes

Two sizes of US Cryo-vessel are used: 120 L (small vessel) and 300 L (big vessel).

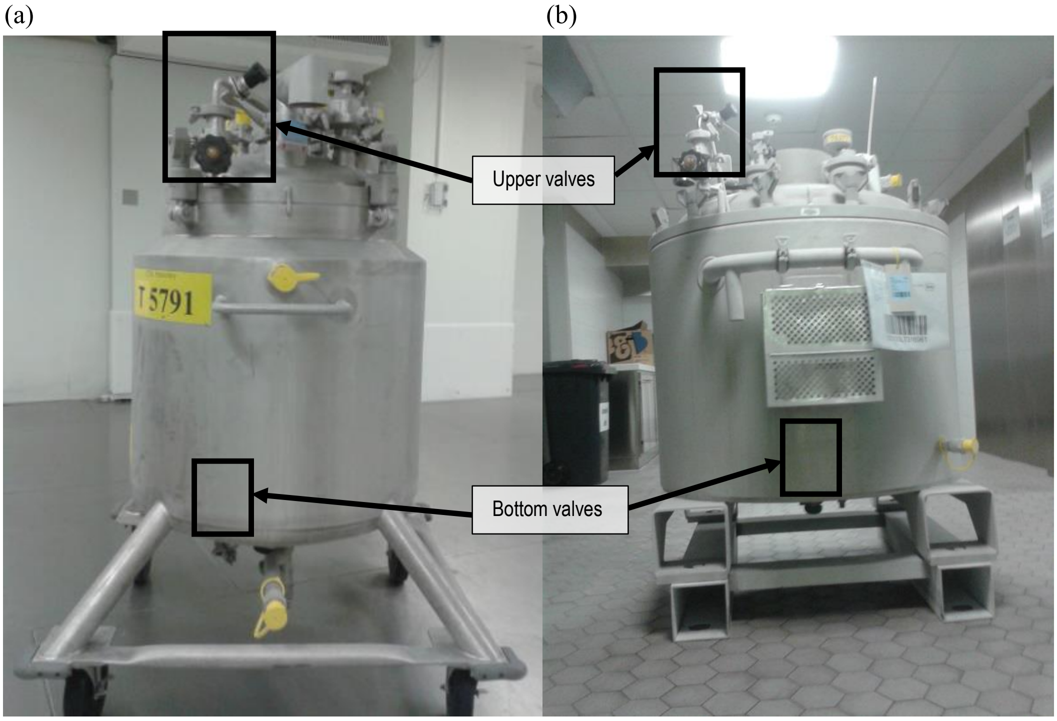


Figure 14. US Cryo-vessel type of (a) 120 L and (b) 300 L, with upper and bottom valves highlighted.

The product filling process (Process A) is the same regardless of what type of cryo-vessel is utilized. The filling of API solution is done by a dip tube, which extends from the top of the cryo-vessel to the bottom, allowing the filling of the inner compartment. After the filling process is completed, the cryo-vessel can be transported and stored.

The thawing process is divided in two cycles: silicon oil and product cycle (Process B).

Silicon oil cycle: the silicon oil circulates in the outer compartment of the cryo-vessel, named double coat and in the inner tube snake. The circulation of the silicon oil in the cryo-vessel warms the API solution allowing its thawing.

Product cycle: it is used a thawing disposable tube, that is connected on the bottom valve and on the upper valve and passes through a peristaltic pump, that pumps the API solution for its homogenization and for heat distribution during thawing. The API circulation starts always from the bottom valve to the upper valve, where it enters in the inner compartment.

The transfer of the API solution (Process C) is made through a transfer disposable tube that is connected to the bottom valve and to the receiving tank in the compounding room. The transfer process is done through overpressure with nitrogen, where the nitrogen has to pass through a 0,22 µm filter in the cryo-vessel to ensure sterility (figure 13, Process C).

3.2. Basel and Penzberger Cryo-vessel Types

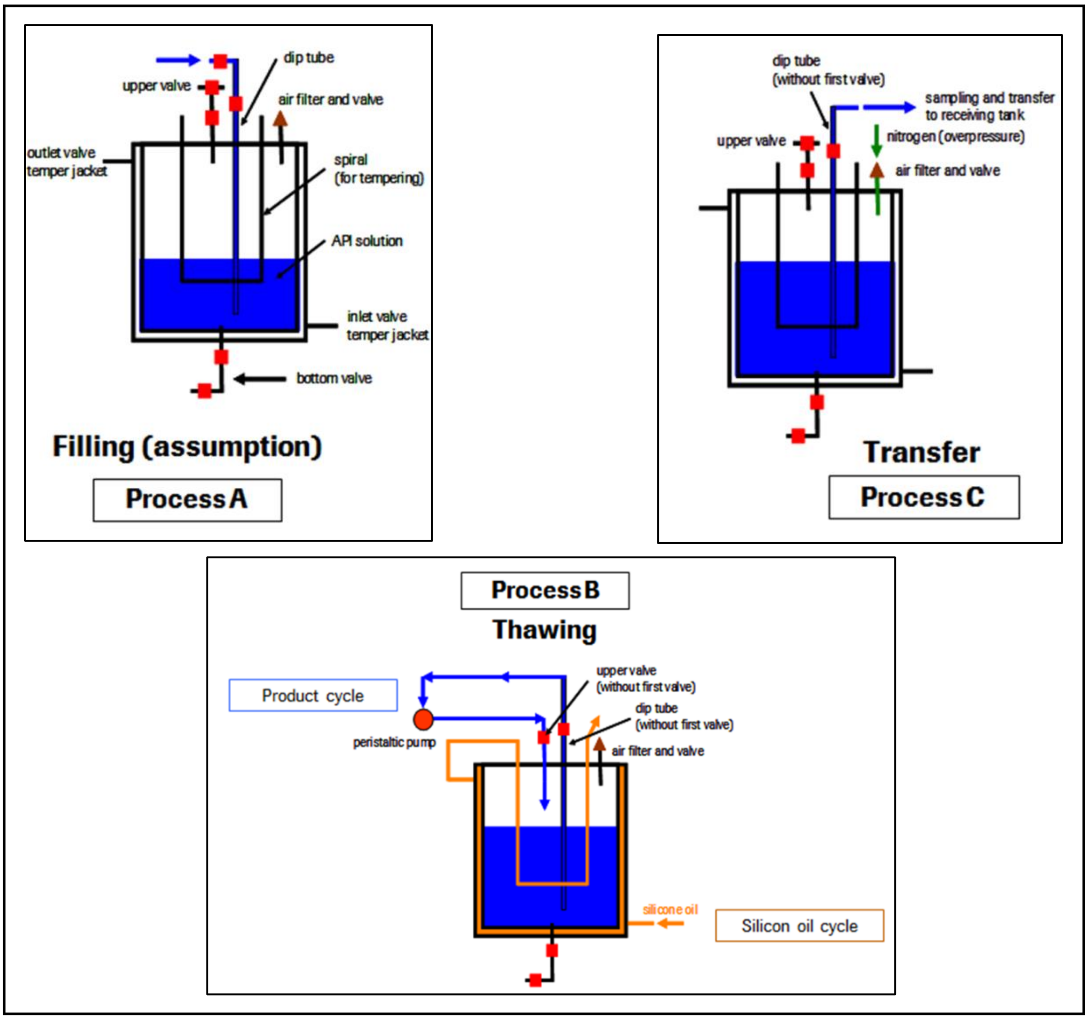


Figure 15. Basel and Penzberger Cryo-vessel: Filling, Thawing and Transfer processes

The Basel and Penzberger Cryo-vessel types only exist with the capacity of 300 L. They differ from each other in the localization of the upper valve and the dip tube.

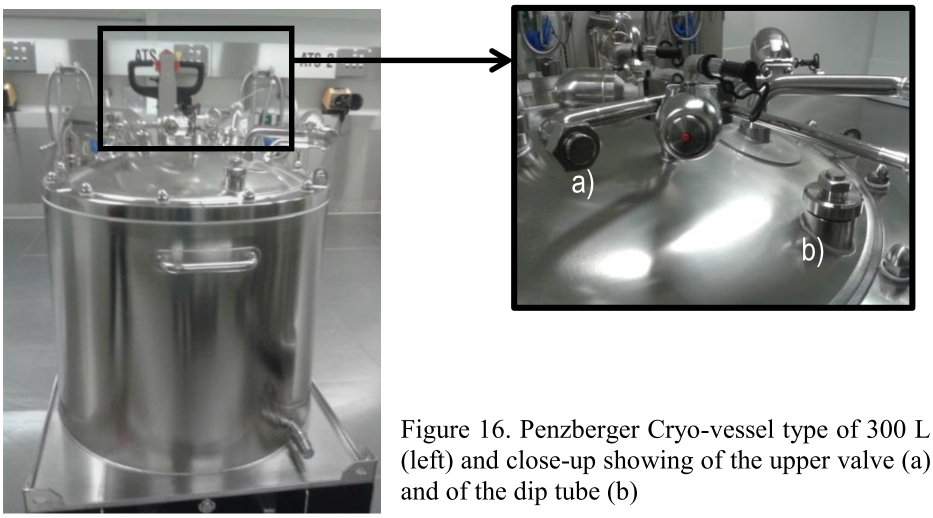


Figure 16. Penzberger Cryo-vessel type of 300 L (left) and close-up showing of the upper valve (a) and of the dip tube (b)

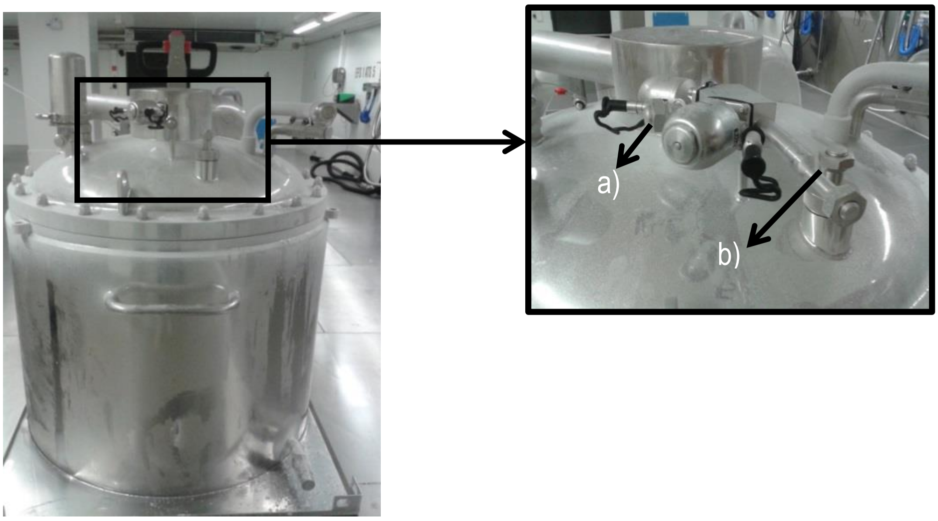


Figure 17. Basel Cryo-vessel type of 300 L (left) and close-up showing of the upper valve (a) and of the dip tube (b)

The differences between US Cryo-vessel type and Basel and Penzberger Cryo-vessel types are in the thawing process (Process B), in the product cycle and in the transfer process (Process C).

Product cycle (Process B): the thawing disposable tube is connected on the dip tube from where the API solution leaves, passes through a peristaltic pump, and on the upper valve where the API solution enters to the inner compartment.

The transfer of the API solution (Process C) is made through a transfer disposable tube that is connected to the dip tube and to the receiving tank in the compounding room. With the Basel and Penzberger Cryo-vessel types the transfer process is also done through overpressure with nitrogen where the nitrogen has to pass through a 0,22 µm filter in the cryo-vessel to ensure sterility (see figure 15 – Process C).

4. Today's Cryo-vessel process cycle (API), Roche Mannheim

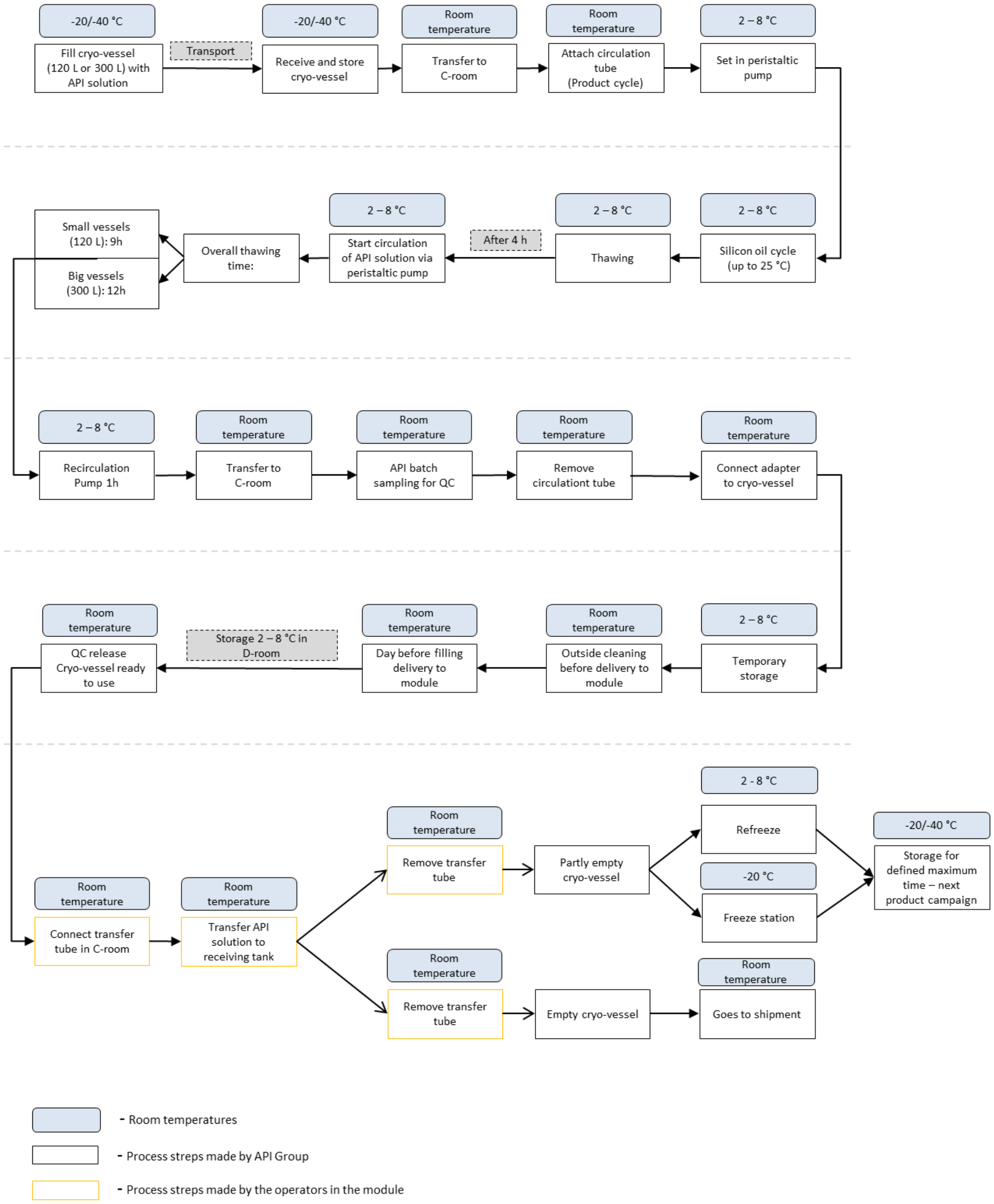


Figure 18. Today's Cryo-vessel process cycle in Roche Diagnostics GmbH, Mannheim

5. Today’s API thawing and transfer process

Before starting the thawing process the thawing disposable tube must be attached to the cryo-vessel in a class C room. The thawing disposable tube length is approximately 4 meters (m) and must be cut with a sterilized scissor in two parts, one with the length of approximately 3 m and the other with the length of approximately 1 m. Linked to the thawing tube is a Nova Septum® bag for bioburden sampling and a syringe used for endotoxins (LAL test) sampling. The polyethylene Nova Septum® bag was validated for bioburden and the polycarbonate syringe was validated for endotoxins (Roche Internal Document, 2010).

In a 2-8 °C room the thawing tube is connected to a peristaltic pump, which will allow the thawing process, product cycle.

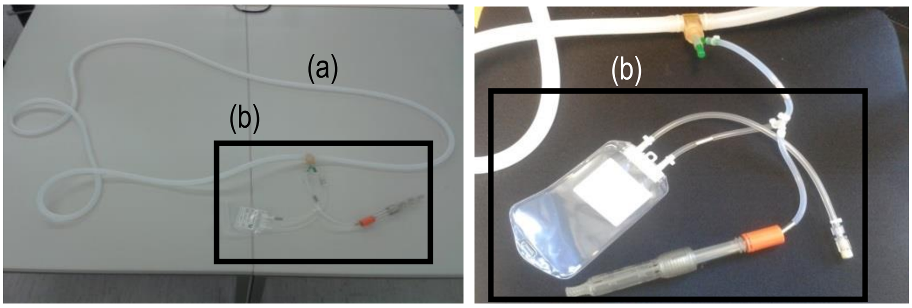


Figure 19. Today’s thawing tube (a) attached with a Nova Septum® and a syringe for sampling (b)

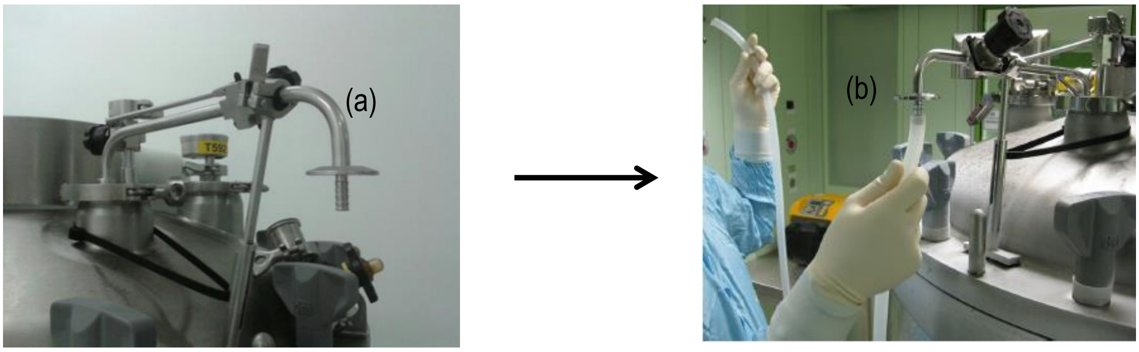


Figure 20. Upper valve of US Cryo-vessel (a) and connection of the thawing disposable tube with the upper valve before the thawing process (b)

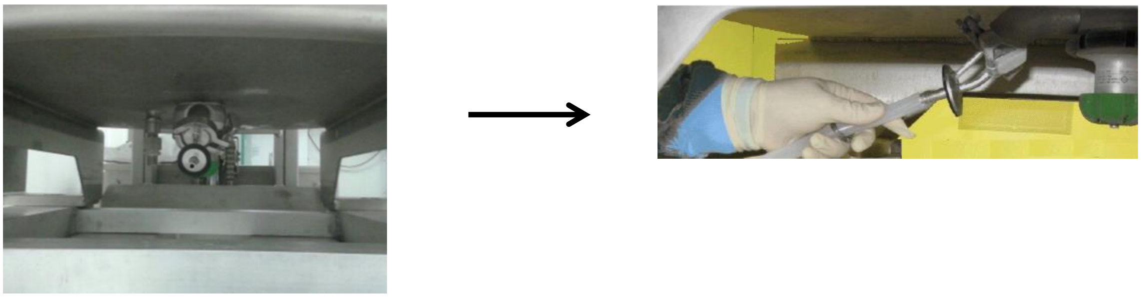


Figure 21. Bottom valve of US Cryo-vessel (a) and connection of the thawing disposable tube with the bottom valve before the thawing process (b)

Table 11. Today's Tube features

Tube features			
Tube	**Material**	**Diameter (mm)**	**Length transfer way (m)**
Today´s tubes	Thawing tube: Platinum-cured Silicone	Thawing tube: ID: 9 mm OD: 15 mm	
	Transfer tube: Silicone with Polyester yarn braiding	Transfer tube: ID: 9,5 mm OD: 12,7 mm	≈ 2

Table 12. Features of the today's transfer tube material. Adapted from AdvantaPure (2012).

Material	**Silicone with Polyester yarn braiding**
Features	• Platinum-cured silicone tubing • Polyester yarn braiding inside the wall enhances pressure capabilities • Meets all USP Class VI specifications • Meets European Pharmacopeia 3.1.9 specifications • Meets FDA 21 CFR 177.2600 specifications • Meets ISO 10993-5specifications

Subsequently, begins the silicon oil cycle. The silicon oil circulates in the outer compartment of the cryo-vessel, named double coat and in the inner tube snake, heating the API solution.

Figure 22. The silicon oil cycle. (a) Entry of silicon oil in the cryo-vessel; (b) Circulation of silicon oil in the double coat and in the inner tube snake in the cryo-vessel; (c) Exit of silicon oil out of the cryo-vessel

After four hours of heating starts the product circulation via peristaltic pump. The thawing process takes 9 hours in case of a small cryo-vessel (120 L) and 12 hours in case of a big cryo-vessel (300 L).

Figure 23. Peristaltic pump for product circulation and the today´s thawing disposable tube in the peristaltic pump for product circulation

During the thawing process the operators and a machine document the thawing parameters (GMP-batch record). After the thawing process the cryo-vessel is cleaned outside and transported to a C-room. There, the operators take samples for microbiological Quality Control (QC) analysis of bioburden (Nova Septum® bag) and endotoxins, due to a LAL test (syringe) and take a sample for chemical QC analysis of identity of the API solution. The batch record, the analysis of bioburden and endotoxins and the analysis of identity will be reviewed by the QC. After that, the QC makes the decision to release, reject or quarantine the cryo-vessel in accordance with the quality.

Subsequently they remove the thawing disposable tube and connect an adapter on the bottom valve in case of a US cryo-vessel. Before the implementation of an adapter, the operators in the compounding room had to lie down on the floor to connect the transfer tube with the compounding tank which increased the contamination risks. Therefore, the implementation of an adapter on the bottom valve aims to avoid having to lie down on the floor to connect the transfer tube from the cryo-vessel to the compounding tank. Consequently the adapter enables a safer connection between the cryo-vessel and the compounding tank and prevents operators from lying down on the floor minimizing the contamination risks.

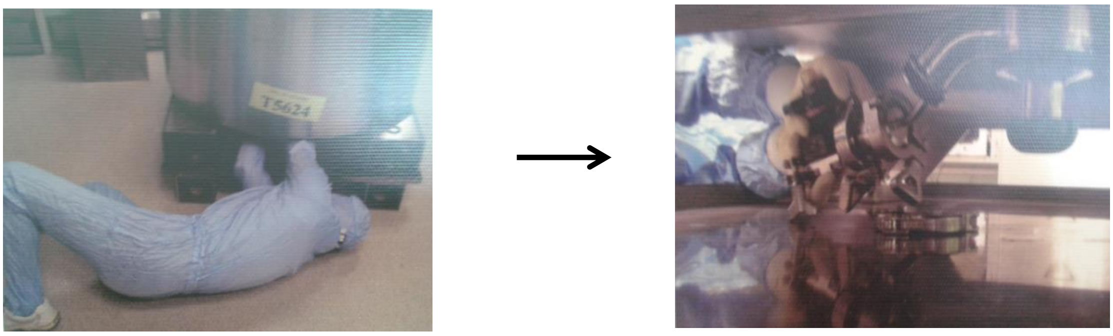


Figure 24. Operators lying on the floor to connect the transfer tube before implementation of the adapter

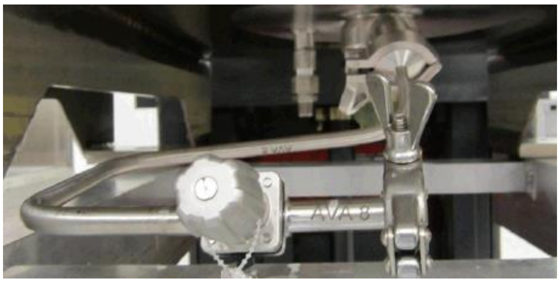


Figure 25. Adapter used for the today's connection of the transfer tube with the US Cryo-vessel

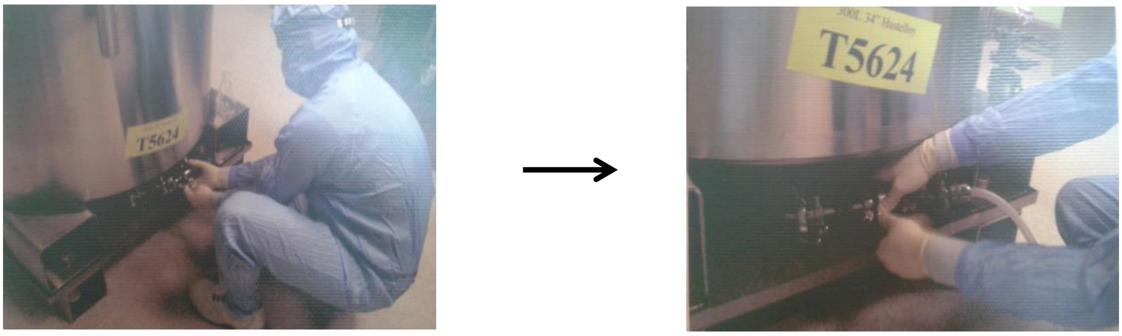


Figure 26. Operators doing the connection of the transfer tube after implementation of the adapter

When the QC decides that the microbiological and chemical samples and that the batch record meet the specifications, the cryo-vessel can be released.

In the compounding room, before connecting the transfer disposable tube from the cryo-vessel to the compounding tank the operators must to do a quick LAL test (Diagnostic Kit) for some products to check the microbiological quality after holding time (approximately two weeks). Sometimes it is necessary to use more than one cryo-vessel for the compounding. Therefore, the LAL test is made to prevent financial risks due to the possibility of the mixture of one API solution with suboptimal quality from one cryo-vessel with one with optimal quality from another cryo-vessel. If the result of the quick LAL test is negative they can connect the transfer disposable tube to the adapter in the cryo-vessel to and to the compounding tank and start the transfer process. The API solution is transferred trough overpressure to the compounding tank.

Figure 27. Today's transfer disposable tube

With this master work a first data base will be created for the decision if Roche Diagnostics GmbH, Mannheim can adopt a combined thawing and transfer disposable tube to reduce the operators manipulation and the assembly time. Moreover, with the implementation of a combined thawing and transfer disposable tube the operators will no longer need to connect an adapter on the bottom valve. Additionally it can be used with highly potent drugs (ADCs).

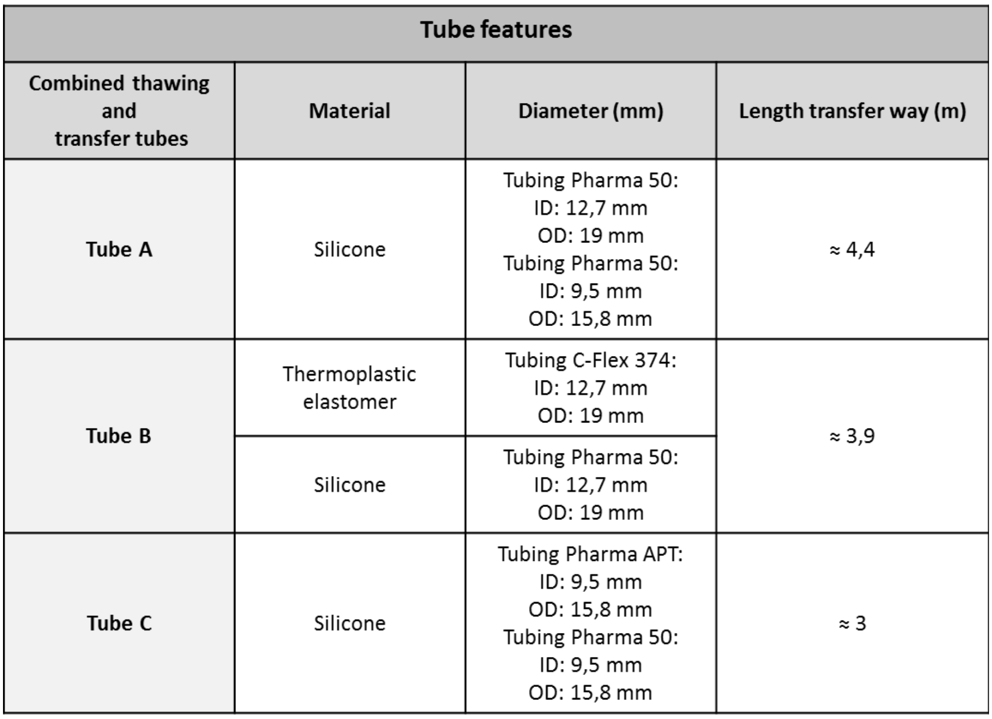
6. Materials and Methods

The possibility of transferring product with a peristaltic pump and with overpressure in combination with a combined thawing and transfer disposable tube was tested and the most suitable method verified. Additionally, the transfer speed (kg/min) of three different combined thawing and transfer disposable tubes (Tube A, B and C) was measured using both transfer fluid methods, peristaltic pump and overpressure, and compared with the transfer speed of today´s transfer with overpressure of Product A, taking also into account other additional factors as presented in the tables 11 and 13.

Tube A is the only combined thawing and transfer disposable tube that is already qualified according to the cGMP.

Tube B and C were specially designed for Roche Diagnostics GmbH, Mannheim.

Table 13. Combined thawing and transfer Tubes A, B and C features.



Tube features			
Combined thawing and transfer tubes	**Material**	**Diameter (mm)**	**Length transfer way (m)**
Tube A	Silicone	Tubing Pharma 50: ID: 12,7 mm OD: 19 mm Tubing Pharma 50: ID: 9,5 mm OD: 15,8 mm	≈ 4,4
Tube B	Thermoplastic elastomer	Tubing C-Flex 374: ID: 12,7 mm OD: 19 mm	≈ 3,9
	Silicone	Tubing Pharma 50: ID: 12,7 mm OD: 19 mm	
Tube C	Silicone	Tubing Pharma APT: ID: 9,5 mm OD: 15,8 mm Tubing Pharma 50: ID: 9,5 mm OD: 15,8 mm	≈ 3

Table 14. Features of the tube materials used in the experiment. Adapted from Dow Corning and Saint-Gobain (2014).

Material	Silicone		Thermoplastic elastomer
	Pharma 50	Pharma APT	C-Flex
Features	• Platinum-cured silicone tubing • For standard tubing transfer and filling operations • Minimal extractables • Burst pressure: 4,1 bar • Meets all USP Class VI specifications • Meets FDA 21 CFR 177.2600 specifications • Meets European Pharmacopeia 3.1.9 specifications	• Platinum-cured silicone tubing • Ideal for pumping applications • For use in peristaltic pumps • Has up to four times the pump life of standard platinum cured silicone tubing • Low extractables • Burts pressure: 3,4 bar • Meets all USP Class VI specifications • Meets FDA 21 CFR 177.2600 specifications • Meets European Pharmacopeia 3.1.9 specifications	• Significantly less permeable than silicone • For weldable applications as well as fluid transfer • Complies with USP Class VI specifications • Meets FDA 21 CFR 177.2600 specifications

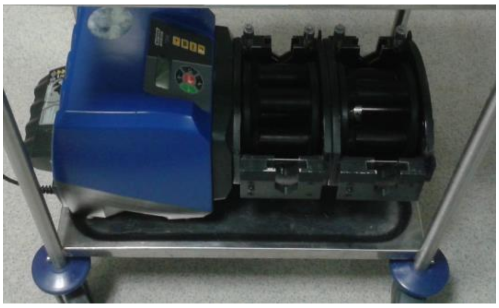


Figure 28. Peristaltic pump (up) and manometer and overpressure tube (right) used in the experiment

The experiments were performed on the pharmaceutical GMP equipment of Roche Diagnostics GmbH, Mannheim in the compounding room of Module 1 (pharmaceutical sterile drug product manufacturing process). The experiments were not performed under GMP conditions; for that reason the combined thawing and transfer disposable tubes were connected in a class D room.

Since Roche Diagnostics GmbH, Mannheim only works in a GMP environment the use of all types of cryo-vessels that were explained was not possible. In order to use a cryo-vessel for experiments, cleaning validation is needed due to possible API residues. The only cryo-vessel that was available to make the experiments was a US Cryo-vessel type (300 L), as it is a GMP training test vessel.

Figure 29. US Cryo-vessel (300 L) used in the experiment

The solution that was used for testing was water for injection (WFI). For each designed experiment with the exception of the experiment with Tube C (pressure) the cryo-vessel was filled four times with WFI to obtain a final amount of 1031 kg of transfer solution. For the experiment with Tube C with pressure the cryo-vessel was filled three times with WFI to obtain a final amount of 1031 kg of transfer solution.

Figure 30. Filling the US Cryo-vessel with WFI

Amount of transfer product

The maximum volume that can be transferred to the compounding tank is approximately 1050 L. The compounding tank measures the transfer product in kg. So when the transfer product is WFI and 1000 L of WFI should be transferred, the final amount in the compounding tank is 1000 kg since the density of water is approximately 1g/cm^3.

$$Density\ (g/cm^3) = \frac{Amount\ (g)}{Volume\ (cm^3)} = \frac{Amount\ (kg)}{Volume\ (dm^3)} = \frac{Amount\ (kg)}{Volume\ (L)} \Leftrightarrow$$

$$\Leftrightarrow\ Amount\ (kg) = Density\ (g/cm^3)\ \text{x}\ Volume\ (L)\ \Leftrightarrow$$

$$\Leftrightarrow\qquad Amount\ = 1\ \text{x}\ 1000 = 1000\ kg$$

However Product A, which is used for the comparing of the transfer process, has a density of approximately 1,031g/cm^3 which means that the final amount of transferred product in the compounding tank is 1031 kg.

$$Amount\ (kg) = Density\ (g/cm^3)\ \text{x}\ Volume\ (L)\ \Leftrightarrow$$

$$\Leftrightarrow \qquad Amount\ = 1{,}031\ \text{x}\ 1000\ =\ 1031\ kg$$

Since the final amount of the transferred product of Product A is 1031 kg it was transferred 1031 kg of WFI so that a comparison of the transfer speeds is possible.

6.1. Experiment

6.1.1. Tube A.

The first combined thawing and transfer disposable tube that was tested was Tube A. This tube is only suitable for the use of non-highly potent drugs and consists of three parts. Part A and B are intended to connect to the cryo-vessel for the thawing process and Part C combined with a Lynx S2S® connector is intended to connect to the compounding tank for the transfer process. Coupled to Part A are Nova Septum® bags that serve for batch sampling (Part D) (see figure 31).

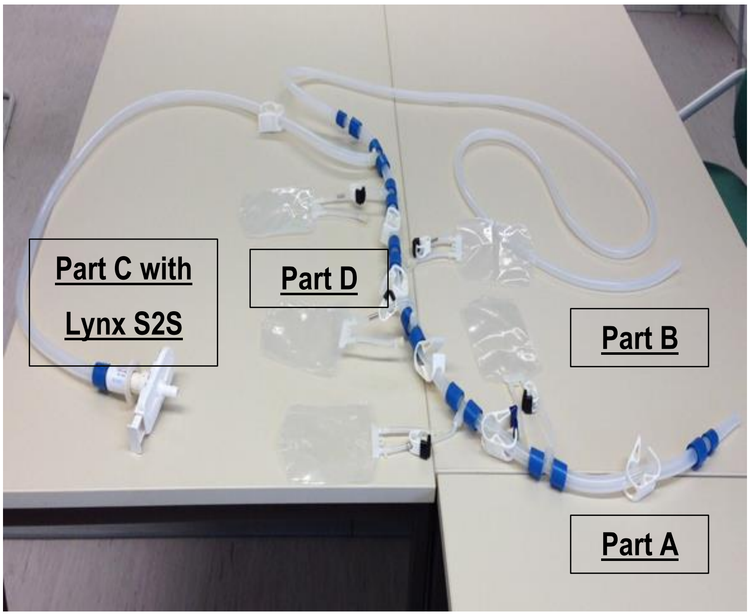


Figure 31. First disposable tube that was tested - Tube A. Part A is intended to connect with the upper valve, part B is intended to connect with the bottom valve, part C is intended to connect with the compounding tank and part D is intended to take samples

Tube A was connected to the cryo-vessel in a class D room and then transported to the compounding room, class C room. Part A was connected to the upper valve of the cryo-vessel with a metal clamp and part B was connected to the bottom valve of the cryo-vessel also with a metal clamp.

Figure 32. US Cryo-vessel with Tube A in class D room

Two methods were used as moving force for the transfer of the solution to the compounding tank: peristaltic pump and overpressure.

Experiment with peristaltic pump:

Although Part C has a Lynx S2S® connector, the experiment wasn´t performed with it as it was designed for another plant and didn´t fit for Module 1 processes. Therefore, the operators cut the connector and connected a short tube with a tri-clamp connection to the existing tube. In the compounding room the transfer tube (Part C) was connected to the compounding tank passing through a peristaltic pump with a tube connection piece. For the transfer process approximately 200 rpm were used.

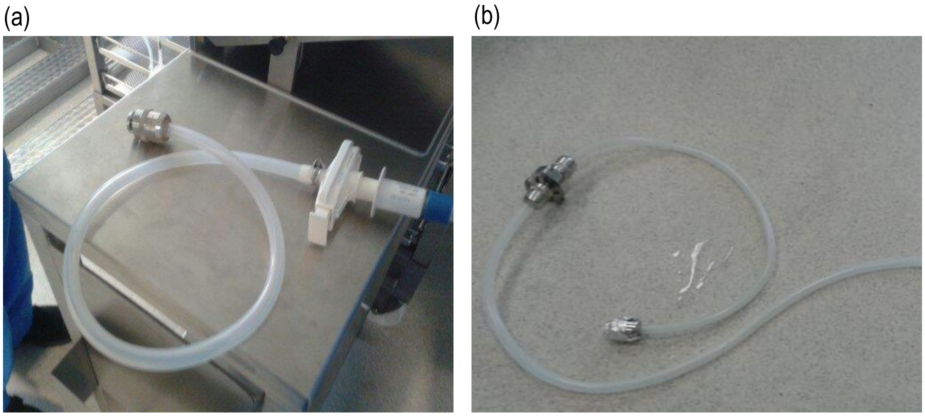


Figure 33. Part C of tube A with Lynx S2S® connector and with a tube connection piece (a) and Part C of Tube A after removing the Lynx S2S® connector and with a tube connection piece (b)

Figure 34. Part C of Tube A passing through the peristaltic pump

Experiment with overpressure:

In the compounding room the transfer tube without Lynx S2S® connector (Part C) was connected to the compounding tank with a tube connection piece. Afterwards, the overpressure tube (nitrogen) was connected to the cryo-vessel. For the transfer process approximately 0,5 bar was used. Before nitrogen enters into the cryo-vessel it has to pass a 0,22 µm filter to ensure sterility.

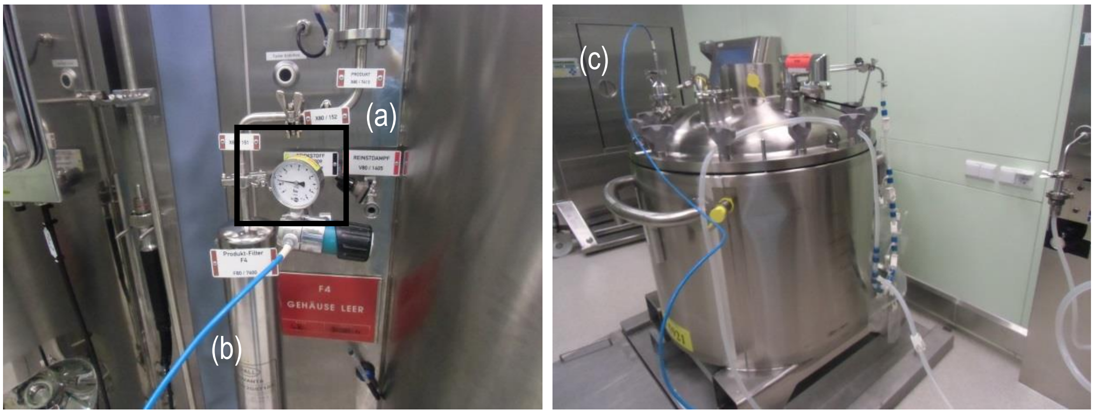


Figure 35. Manometer (a), overpressure tube (blue tube) (b) and overpressure tube connected to the US Cryo-vessel in class C-room (c)

Experiment design of Tube A with peristaltic pump and overpressure:

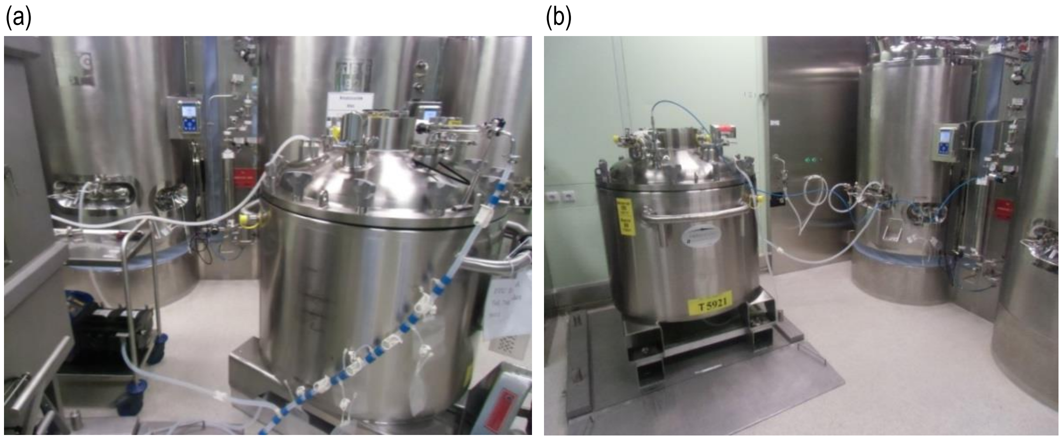


Figure 36. Experiment design of Tube A (a) with peristaltic pump and (b) with overpressure

6.1.2. Tube B.

The second tube that was tested was Tube B. This tube is a delivery tube for the connection of different cryo-vessels (y-connector). It can connect up to four different cryo-vessels (Part B, C, D and E) and can be used for the handling of API solutions and for highly potent drugs, ADCs. Part A is intended for the connection with the compounding tank and Part F is intended for flushing in case of highly potent drugs, ADCs manipulation allowing the cleaning of the tube. However, Tube B was tested as a combined thawing and transfer disposable tube to test the silicon tube material (see figure 37).

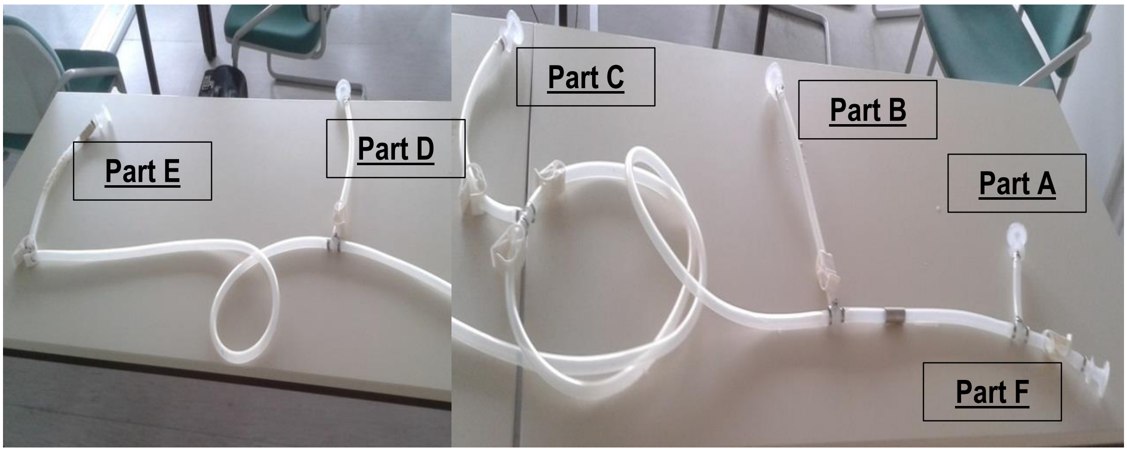


Figure 37. Second disposable tube that was tested - Tube B. Part A is intended to connect with the compounding tank, Part B, C, D, E are intended to connect with different cryo-vessels and part F is intended for flushing

Tube B was connected to the cryo-vessel in a class D room and then transported to the compounding room, class C room. Part A was connected to the upper valve with a tri-clamp connection and part C was connected to the bottom valve of the cryo-vessel also with a tri-clamp connection.

Figure 38. US Cryo-vessel with Tube B in class D room

Two methods were used as moving force for the transfer of the solution to the compounding tank: peristaltic pump and overpressure.

Experiment with peristaltic pump:

In the compounding room the transfer tube (Part E) was connected to the compounding tank with a tri-clamp connection passing through a peristaltic pump. For the transfer process approximately 200 rpm was used.

Figure 39. Part E of Tube B passing through the peristaltic pump

Experiment with overpressure:

In the compounding room the transfer tube (Part E) was connected to the compounding tank with a tri-clamp connection. Afterwards, the overpressure tube (nitrogen) was connected to the cryo-vessel. For the transfer process approximately 0,5 bar was used. Before nitrogen enters into the cryo-vessel it has to pass a 0,22 µm filter to assure sterility.

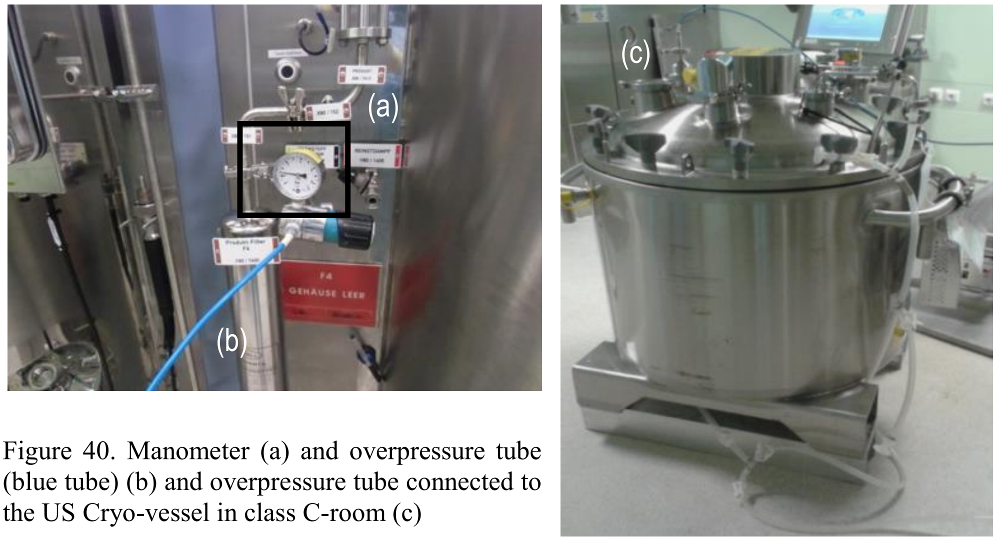


Figure 40. Manometer (a) and overpressure tube (blue tube) (b) and overpressure tube connected to the US Cryo-vessel in class C-room (c)

Experiment design of Tube B with peristaltic pump and overpressure:

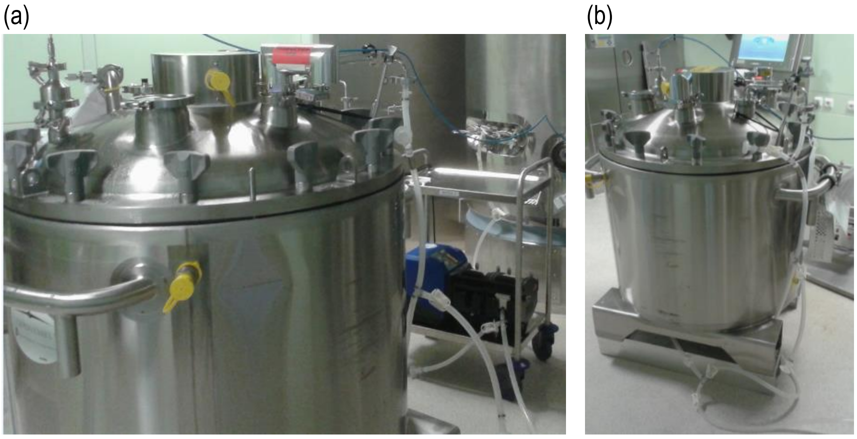


Figure 41. Experiment design of Tube B (a) with peristaltic pump and (b) with overpressure

6.1.3. Tube C.

The third combined thawing and transfer tube that was tested was Tube C. This tube is designed for using with API solutions and ADCs. Part A and B are intended to connect with the cryo-vessel with a tri-clamp connection for the thawing process. After the thawing process it is also possible to take batch samples with Part E. Part E is composed of a Nova Septum® bag for bioburden sampling and a syringe for endotoxin sampling. Connected to part A and B is Part D. Part D is designed for flushing when handling with highly potent drugs, ADCs, allowing the cleaning of the tube. Part C is used for the transfer process (see figure 42).

This combined disposable tube was designed to allow the connection of Part C with the y-connector (Tube B) and this one with the compounding tank. This concept was intended for highly potent drugs, ADCs.

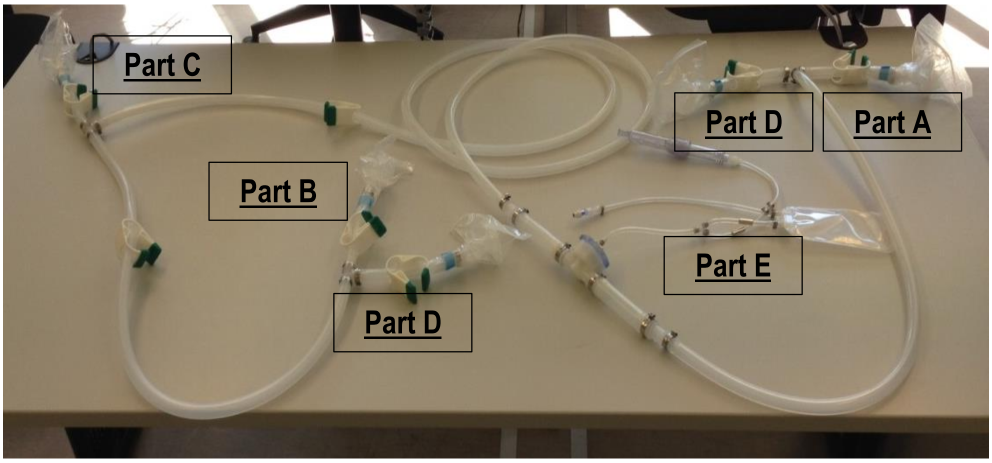


Figure 42. Third disposable tube that was tested - Tube C. Part A is intended to connect with the upper valve, part B is intended to connect with the bottom valve, part C is intended to connect with the compounding tank and part D is intended for flushing.

Tube C was connected to the cryo-vessel in a class D room and then transported to the compounding room, class C room. Part A was connected to the upper valve of the cryo-vessel with a tri-clamp connection and part B was connected to the bottom valve of the cryo-vessel also with a tri-clamp connection.

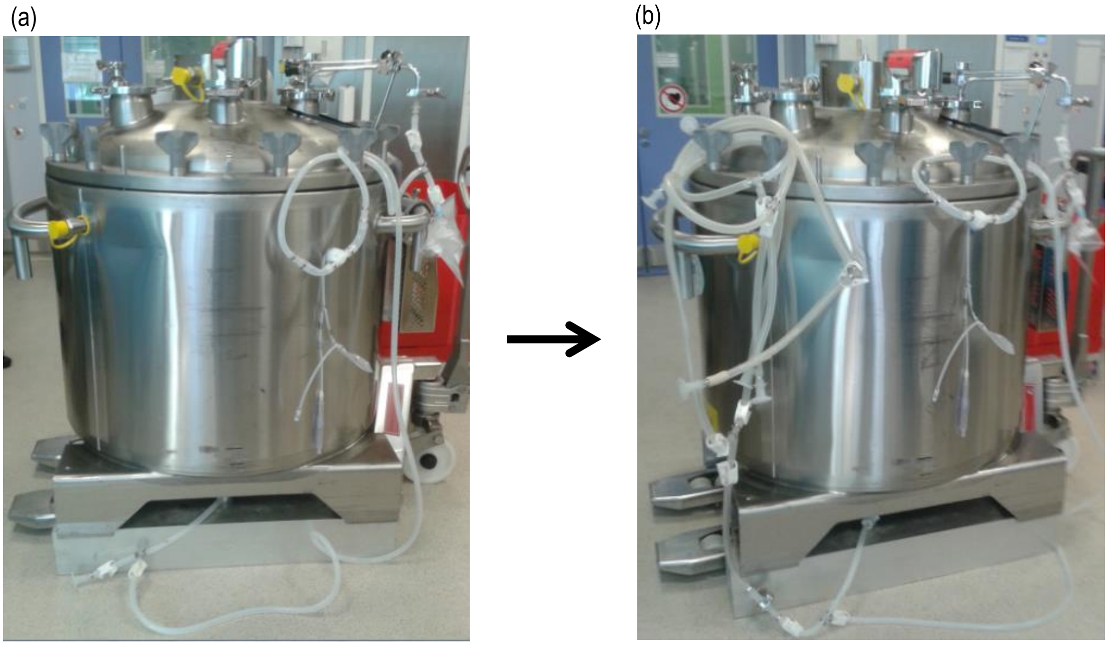


Figure 43. US Cryo-vessel with Tube C in class D room (a) and US Cryo-vessel with Tube C connected to the y-connector in class D room (b)

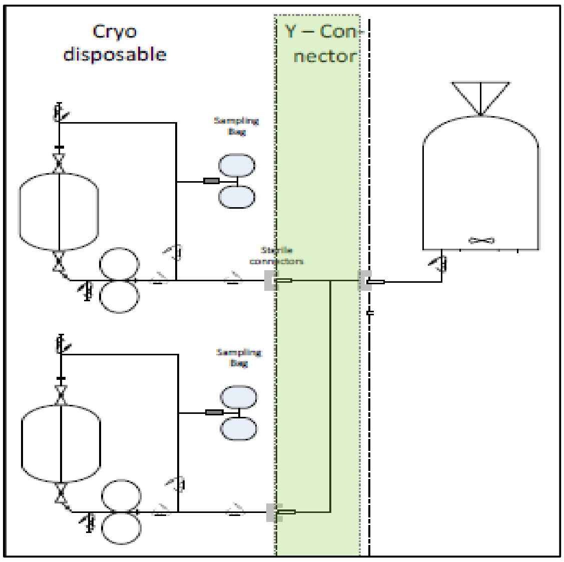


Figure 44. Transfer concept for ADCs: connection of Tube C with y-connector and connection of y-connector with the compounding tank

Two methods were used as moving force for the transfer of the solution to the compounding tank: peristaltic pump and overpressure.

Experiment with peristaltic pump:

In the compounding room the transfer tube (Part C) was connected to the y-connector with a tri-clamp connection passing through a peristaltic pump. The y-connector was connected to the compounding tank with a tri-clamp connection. For the transfer process approximately 200 rpm was used.

Figure 45. Part D of y connector (connected to Tube C) passing through the peristaltic pump

Experiment with overpressure:

In the compounding room the transfer tube (Part C) was connected to the y-connector with a tri-clamp connection. Then the y-connector was connected to the compounding tank as well with a tri-clamp connection. Subsequently, the pressure tube (nitrogen) was connected to the cryo-vessel. For the transfer process approximately 0,5 bar was used. Before nitrogen enters into the cryo-vessel it has to pass a 0,22 µm filter to assure sterility.

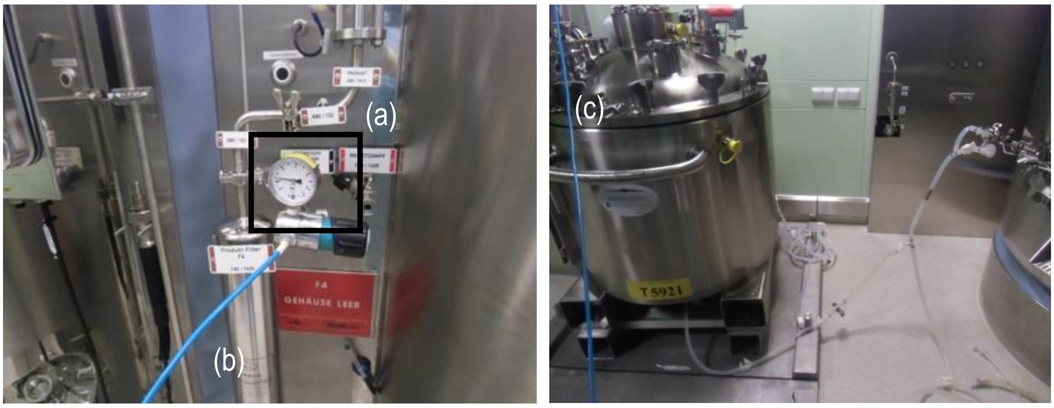


Figure 46. Manometer (a), overpressure tube (blue tube) (b) and overpressure tube connected to the Cryo-vessel in class C-room (c)

Experiment design of Tube C with peristaltic pump and overpressure:

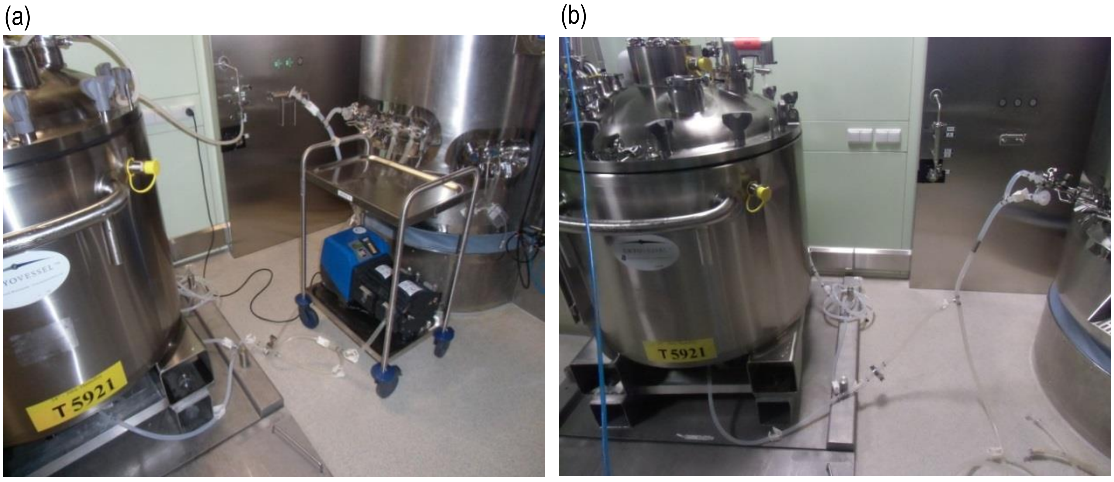

(a) (b)

Figure 47. Experiment design of Tube C with (a) peristaltic pump and (b) overpressure

7. Results

The possibility to transfer product with a peristaltic pump and with overpressure in combination with a combined thawing and transfer disposable tube as well as the most suitable transfer method were tested. Additionally, the transfer speeds of three combined thawing and transfer disposable tubes (Tube A, B and C) in combination with the two transfer methods were measured to see if it is advantageous to replace the current thawing and transfer disposable tubes by a new combined thawing and transfer disposable tube from the point of view of speed/time efficiency. The results are summarized on the table below, which also presents data regarding to the transfer speed from product A with the today´s transfer disposable tube. It is important to mention that the today´s disposable tube only has results for overpressure, since nowadays this is the only method used for the transfer of the product to the compounding tank in Module 1, Mannheim.

Table 15. Transfer speeds of Today´s Tube and Tube A, B and C

Transfer speed									
Methods	**Cryovessel**	**Tubes**							
		Today's Tube		Tube A		Tube B		Tube C	
		Product A		WFI		WFI		WFI	
		Transferred amount (kg)	Transfer time (min)	Transferred amount (kg)	Transfer time (min)	Transferred amount (kg)	Transfer time (min)	Transferred amount (kg)	Transfer time (min)
Peristaltic pump (≈ 200 rpm)	Refill 1			280	42	290	31	310	46
	Refill 2			640	95	560	63	610	91
	Refill 3			914	139	850	94	910	135
	Refill 4			1031	166	1031	113	1031	151
Overpressure N_2 (≈ 0,5 bar)	Refill 1	310	31	310	33	310	25	360	35
	Refill 2	610	59	610	65	640	53	710	75
	Refill 3	910	88	910	100	910	75	———	———
	Refill 4	1031	102	1031	115	1031	85	1031	114

The table shows that it is possible to transfer product with both methods: peristaltic pump and overpressure.

Moreover, according to the table it can also be seen that the today´s transfer tube needs 102 minutes (min) to transfer an amount of 1031 kg with overpressure.

Tube A could transfer an amount of 1031 kg through the peristaltic pump in 166 min and an amount of 1031 kg with overpressure in 115 min.

Tube B could transfer an amount of 1031 kg through the peristaltic pump in 113 min and an amount of 1031 kg with overpressure in 85 min.

At last, one can also observe that Tube C could transfer an amount of 1031 kg through the peristaltic pump in 151 min and an amount of 1031 kg with overpressure in 114 min.

Consequently one can say that the transfer method with overpressure is faster than the transfer method with peristaltic pump.

To summarize, Tube B reveals a better transfer speed with peristaltic pump and with overpressure.

Based on the above results it is possible to design three graphs. The first one compares the transfer speeds between the tubes with peristaltic pump, the second one compares the transfer speeds with overpressure and the third one compares the transfer speeds between the tubes with peristaltic pump and overpressure.

On the y axis the amounts of transferred product are expressed in kilograms, while on the x axis the time of transfer is represented in minutes. The transfer speeds are represented by plotted lines that combine the accumulated time (in minutes) and the transferred amounts to accumulate 1031 kg of product.

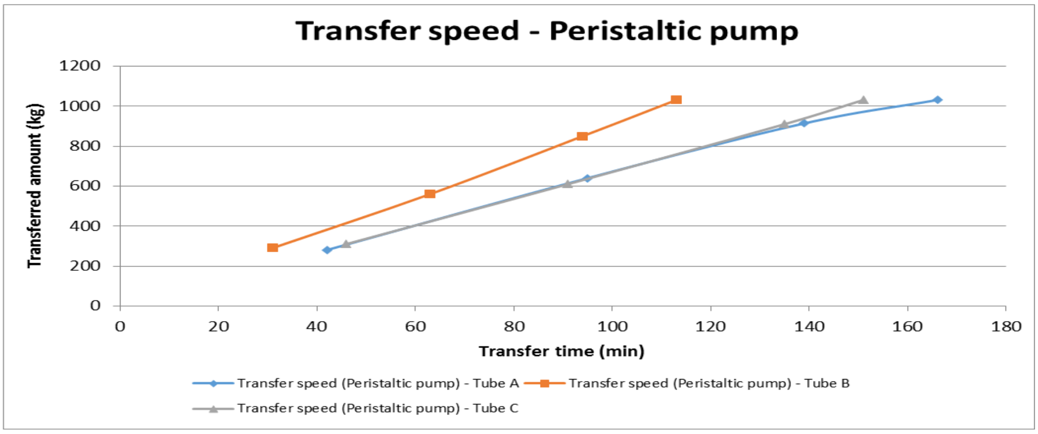


Figure 48. Transfer speed of the tubes with peristaltic pump

Tube B reveals a better transfer speed with peristaltic pump.

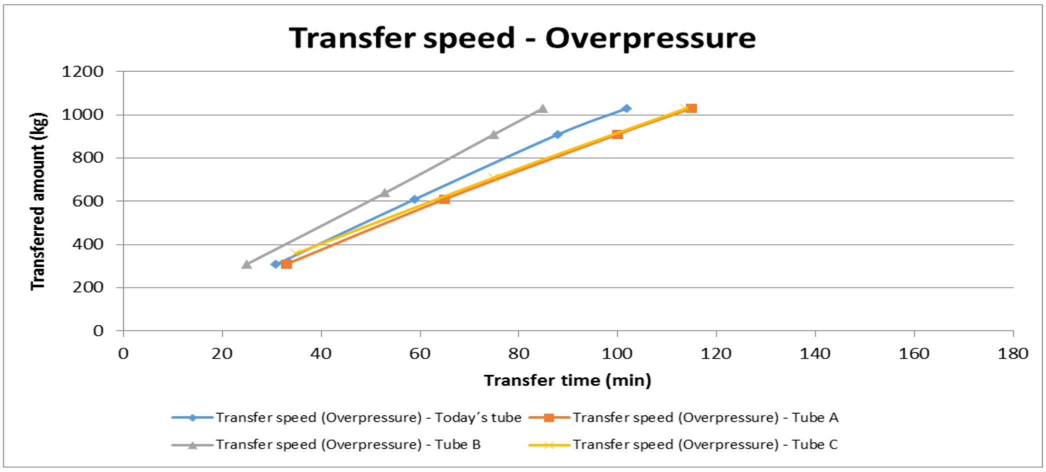


Figure 49. Transfer speed of the tubes with overpressure

Tube B also reveals a better transfer speed with overpressure.

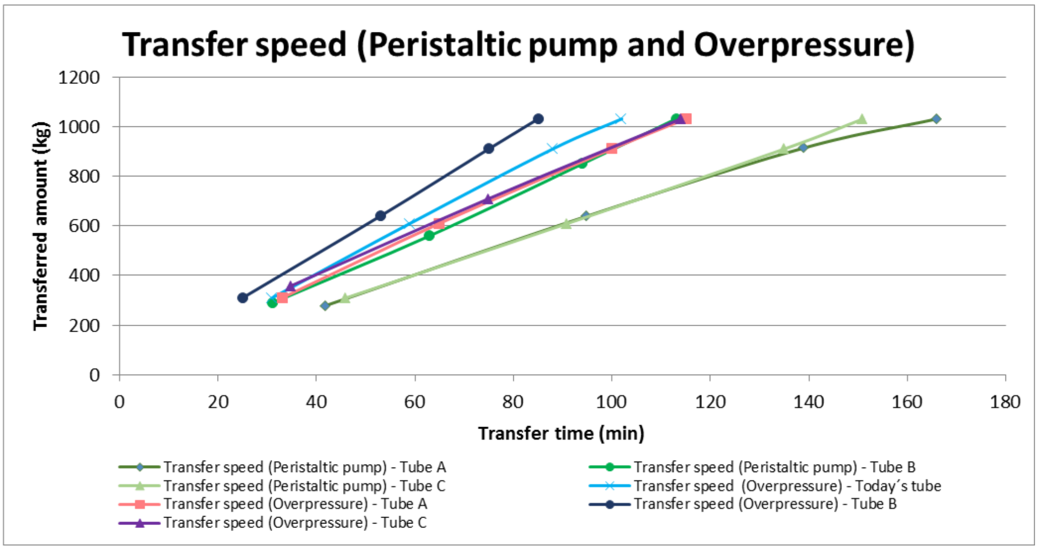


Figure 50. Transfer speed of the tubes with peristaltic pump and overpressure

The transfer speed of the tubes using the overpressure method is in each single subgroup higher than the one using the peristaltic pump method.

To see any possible statistical significance some statistical methods were used with Microsoft Excel 2010 and Minitab® Software Version 16. Some statistical measures and analysis are presented for a better understanding of the results. Aiming to enhance the presentation of the results the following scheme will be considered (Brook, Q., 2010):

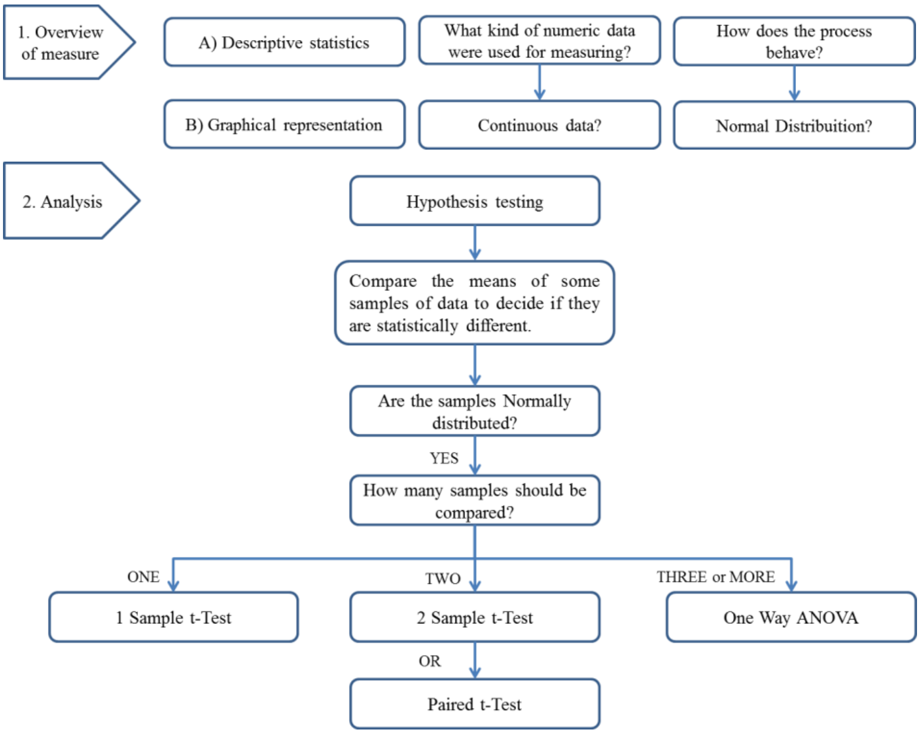


Figure 51. Overview of some statistical analysis. Adapted from Brook, Q. (2010).

The same results are presented with an additional column in the following table showing the transferred amount/time in each refill for each tube according to the two transfer methods (peristaltic pump and overpressure).

$$Tranferred\ amount/time = \frac{Transferred\ amount\ (kg)}{Transfer\ time\ (min)}$$

Table 16. Transferred amount/time of Today´s tube, Tube A, B and C

Tubes	Pump			Pressure		
	Transferred amount (kg)	Transfer time (min)	Transferred amount/time (Kg/min)	Transferred amount (kg)	Transfer time (min)	Transferred amount/time (Kg/min)
Tube 1 (Today´s tube)				310	31	10
				610	59	10,34
				910	88	10,34
				1031	102	10,11
Tube 2 (Tube A)	280	42	6,67	310	33	9,39
	640	95	6,74	610	65	9,38
	914	139	6,58	910	100	9,10
	1031	166	6,21	1031	115	8,97
Tube 3 (Tube B)	290	31	9,35	310	25	12,40
	560	63	8,89	640	53	12,08
	850	94	9,04	910	75	12,13
	1031	113	9,12	1031	85	12,13
Tube 4 (Tube C)	310	46	6,74	360	35	10,29
	610	91	6,70	710	75	9,47
	910	135	6,74	—	—	—
	1031	151	6,83	1031	114	9,04

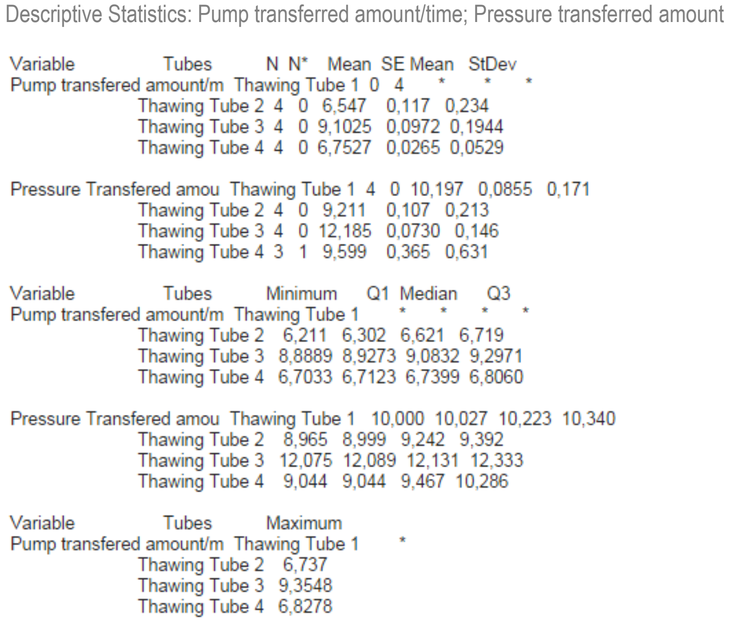
Given the reduced number of measured data points the statistical results should be analysed with caution. However, to better understand the data the following tools were used.

Table 17. Correspondence of the tubes for the statistical methods

Correspondence of the Tubes	
Thawing tube 1	Today´s tube
Thawing tube 2	Tube A
Thawing tube 3	Tube B
Thawing tube 4	Tube C

Overview of measures

Descriptive statistics



```
Descriptive Statistics: Pump transferred amount/time; Pressure transferred amount

Variable              Tubes          N  N*   Mean  SE Mean  StDev
Pump transfered amount/m  Thawing Tube 1  0   4      *       *      *
                      Thawing Tube 2  4   0  6,547   0,117  0,234
                      Thawing Tube 3  4   0 9,1025  0,0972 0,1944
                      Thawing Tube 4  4   0 6,7527  0,0265 0,0529

Pressure Transfered amou  Thawing Tube 1  4   0  10,197  0,0855  0,171
                      Thawing Tube 2  4   0  9,211   0,107  0,213
                      Thawing Tube 3  4   0 12,185  0,0730  0,146
                      Thawing Tube 4  3   1  9,599   0,365  0,631

Variable              Tubes          Minimum     Q1  Median     Q3
Pump transfered amount/m  Thawing Tube 1        *      *      *      *
                      Thawing Tube 2    6,211  6,302  6,621  6,719
                      Thawing Tube 3   8,8889 8,9273 9,0832 9,2971
                      Thawing Tube 4   6,7033 6,7123 6,7399 6,8060

Pressure Transfered amou  Thawing Tube 1   10,000  10,027  10,223  10,340
                      Thawing Tube 2    8,965   8,999   9,242   9,392
                      Thawing Tube 3   12,075  12,089  12,131  12,333
                      Thawing Tube 4    9,044   9,044   9,467  10,286

Variable              Tubes          Maximum
Pump transfered amount/m  Thawing Tube 1        *
                      Thawing Tube 2    6,737
                      Thawing Tube 3   9,3548
                      Thawing Tube 4   6,8278
```

Figure 52. Descriptive Statistics: Transferred amount/time with peristaltic pump and transferred amount /time with overpressure

The statistical results reveal a transferred amount/time near to the mean value of the different refills, thus verifying low standard deviations of the results in all of the tested tubes with the two transfer methods.

The maximum and minimum values and the median for the results of the different tests were calculated for a better understanding of the data characteristics.

Graphical representation

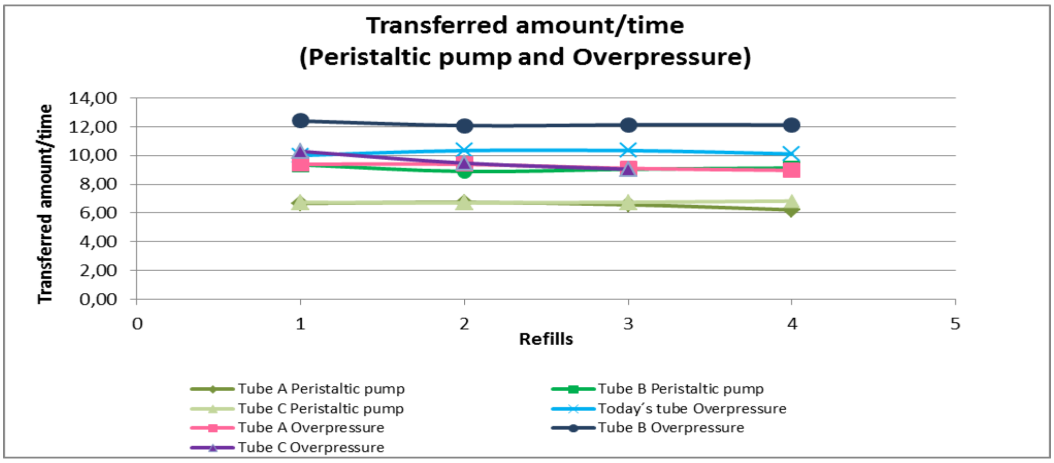


Figure 53. Transferred amount/time of the tubes with peristaltic pump and overpressure

The graph represents the transferred amount/time in the different refills for the tested tubes according to the two transfer methods.

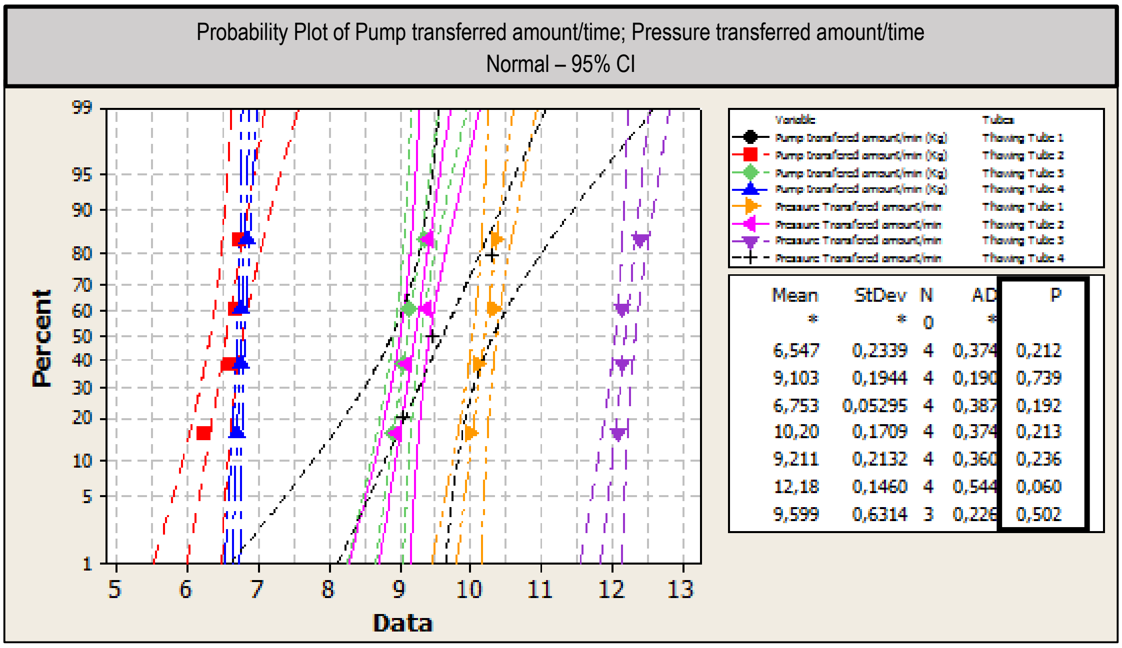
Numeric data used for measuring

The data obtained for the transfer amount/min are continuous data since they assume values over the entire measured period and are clearly not limited to whole numbers. The continuous data sometimes follow a normal distribution (Pedroso, A. C. & Gama, S. M. A., 2004; Brook, Q., 2010).

Process behaviour

To evaluate the process behaviour of the data, a probability plot was constructed for the today´s tube and the tested tubes (Tube A, B and C) with the two transfer methods (peristaltic pump and overpressure).

The probability plot will help to answer if the data follow a normal distribution. If it is the case they will fall in a straight line. However the line will never be perfectly straight and that is the reason for a 95% CI (confidence interval) limits on the diagram. Then if all points fall within the lines the data are normally distributed (Brook, Q., 2010).



Mean	StDev	N	AD	P
*	*	0	*	
6,547	0,2339	4	0,374	0,212
9,103	0,1944	4	0,190	0,739
6,753	0,05295	4	0,387	0,192
10,20	0,1709	4	0,374	0,213
9,211	0,2132	4	0,360	0,236
12,18	0,1460	4	0,544	0,060
9,599	0,6314	3	0,226	0,502

Figure 54. Probability plot of the transferred amount/time with peristaltic pump and overpressure

As shown in the probability plot all data fall within the lines and moreover all p-values are greater than 0,05. Consequently the data within each subgroup follow a normal distribution.

Analysis One Way ANOVA

Since the data follow a normal distribution and the number of samples is more than three a One Way ANOVA was performed to compare the means of the data samples and decide if they are statistically different.

For the One Way ANOVA technique there are two hypothesis: the null hypothesis and the alternative hypothesis. The null hypothesis tests that there is no difference in the means of the transferred amount/time values across the four different tubes-subgroups with peristaltic pump and overpressure. The alternative hypothesis tests that there is a difference.

Two Interval plots were calculated to compare the transferred amount/time of the different tubes with the two transfer methods. An interval plot is a graphical summary of the distribution of the samples that shows the samples central tendency and variability. Each dot represents a sample mean from a subgroup and each interval is a 95% CI for that sample mean (Pedroso, A. C. & Gama, S. M. A., 2004; Brook, Q., 2010).

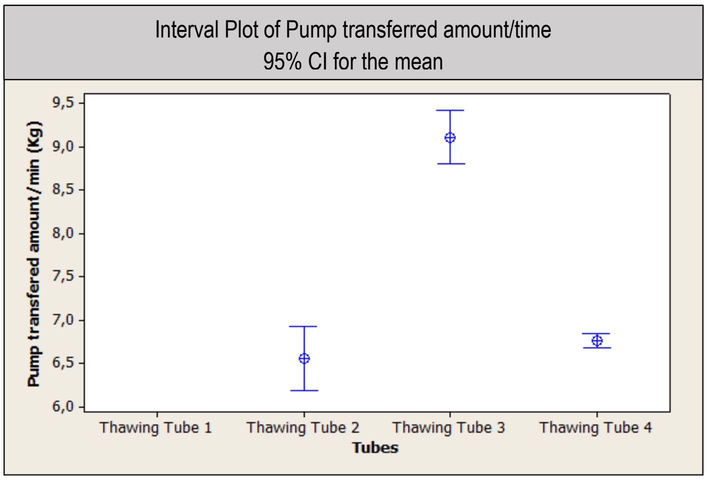


Figure 55. Interval plot for the transferred amount/time of the tubes with peristaltic pump

This plot compares the transferred amount/time of Tube A, B and C with peristaltic pump. The means seem to be different in the three tubes-subgroups but the difference between Tube A (thawing tube 2) and C (thawing tube 4) is probably not significant because the interval bars easily overlap. However, the difference between the means of Tube A (thawing tube 2) and C (thawing tube 4) with Tube B (thawing tube 3) is probably significant because the interval bars do not overlap.

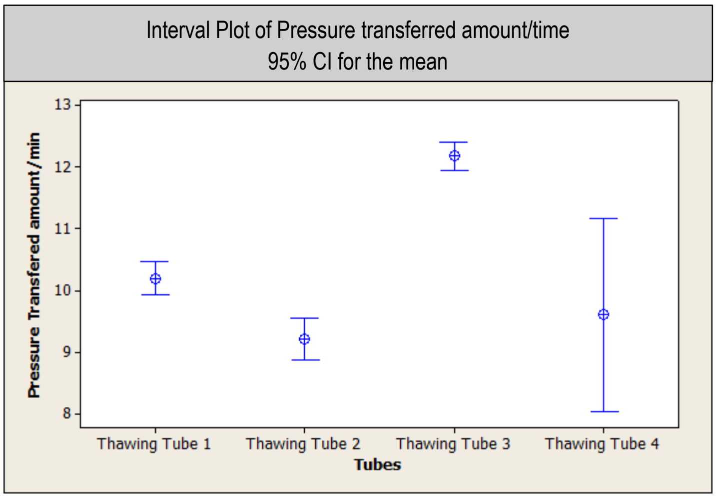
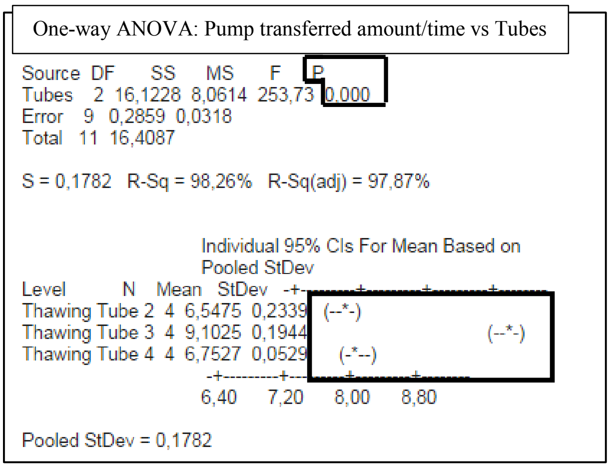


Figure 56. Interval plot for the transferred amount/time of the tubes with overpressure

This plot compares the transferred amount/time of Today´s tube, Tube A, B and C with overpressure. The means seem to be different in the four tubes-subgroups but the difference between Today´s tube (thawing tube 1) and Tube C (thawing tube 4) and the difference between Tube A (thawing tube 2) and Tube C (thawing tube 4) are probably not significant because the interval bars easily overlap. However, in this plot it can also be seen that the difference between the means of Today´s tube (thawing tube 1), Tube A (thawing tube2) and C (thawing tube 4) with Tube B (thawing tube 3) and the difference between Today´s tube (thawing tube 1) with Tube A (thawing tube 2) are probably significant because the interval bars do not overlap.

Since the p-value is greater than 0,05 for every sample (see figure 54) the data are all normally distributed. As represented in table 16 the number of samples is more than three so a One Way ANOVA test will be performed to compare the averages of the data samples to decide if they are statistically different.

The results of the ANOVA test can be presented in the ANOVA tables output (see figure 57 and 58).



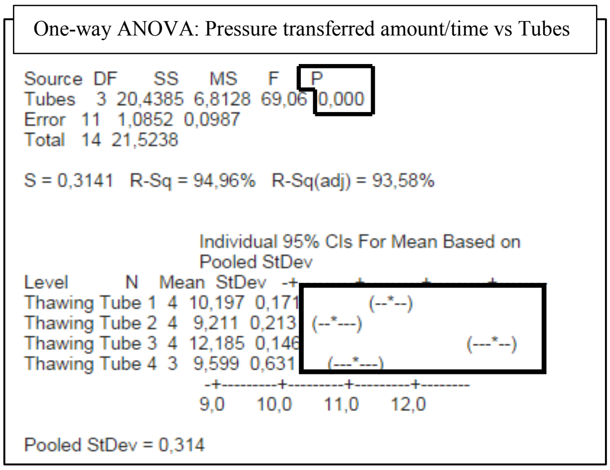
One-way ANOVA: Pump transferred amount/time vs Tubes

```
Source  DF      SS      MS       F      P
Tubes    2  16,1228  8,0614  253,73  0,000
Error    9   0,2859  0,0318
Total   11  16,4087

S = 0,1782   R-Sq = 98,26%   R-Sq(adj) = 97,87%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level           N   Mean   StDev  -+---------+---------+---------+--------
Thawing Tube 2  4  6,5475  0,2339  (--*-)
Thawing Tube 3  4  9,1025  0,1944                               (--*-)
Thawing Tube 4  4  6,7527  0,0529    (-*--)
                                  -+---------+---------+---------+--------
                                 6,40      7,20      8,00      8,80

Pooled StDev = 0,1782
```

Figure 57. One Way ANOVA table output for the transferred amount/time using peristaltic pump

The p-value on the ANOVA table output is the result for the hypothesis test. The p-value is 0, 000 which demonstrates that there is a difference in the means of the transferred amount/time values between the tubes-subgroups tested with the peristaltic pump method.

In the ANOVA table output a diagram of the samples means and their 95% CI is also presented. Tube B (thawing tube 3) is the sample with the average value that is most different from the others, so maybe the results of this tube have caused the p-value of the whole test to be 0,000. Consequently the null hypothesis should be rejected.



One-way ANOVA: Pressure transferred amount/time vs Tubes

```
Source  DF      SS      MS      F      P
Tubes    3  20,4385  6,8128  69,06  0,000
Error   11   1,0852  0,0987
Total   14  21,5238

S = 0,3141   R-Sq = 94,96%   R-Sq(adj) = 93,58%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level           N   Mean  StDev  -+---------+---------+---------+---------
Thawing Tube 1  4  10,197  0,171           (--*--)
Thawing Tube 2  4   9,211  0,213  (--*---)
Thawing Tube 3  4  12,185  0,146                             (---*--)
Thawing Tube 4  3   9,599  0,631     (---*---)
                            -+---------+---------+---------+--------
                            9,0      10,0      11,0      12,0

Pooled StDev = 0,314
```

Figure 58. One Way ANOVA table output for the transferred amount/time using overpressure

In this case the p-value is also 0, 000, which demonstrates that there is a difference in the means of the transferred amount/time values between the tubes tested with the overpressure method.

In the ANOVA table output it can be seen that Tube B (thawing tube 3) is again the sample with the average value most different from the others, so maybe the results of this tube have caused the p-value of the whole test to be 0,000. Consequently the null hypothesis should be rejected.

8. Discussion

In the present master thesis the possibility to transfer API solutions with a peristaltic pump and with overpressure in combination with a combined thawing and transfer disposable tube were tested, with the goal of finding the most suitable method. Moreover, the transfer speed of three combined thawing and transfer disposable tubes was measured to decide if it is advantageous to replace the current single thawing and single transfer disposable tubes, taking also into account other additional factors.

The results demonstrate that is possible to transfer a solution with a peristaltic pump and overpressure. On the one hand this was expected since the tubes that were tested are made of silicone which is suitable for pumps. On the other hand the tubes and connectors didn´t bust when overpressure was applied which means that the transfer process can also be done with this kind of tubes and connectors applying an overpressure of around 0,5 bar.

The three tested combined thawing and transfer tubes reveal different results for the transfer speed depending on their diameter and on the length of the transfer way. The results demonstrate that tube B (thawing tube 3) has a faster transfer speed in both methods used. Additionally the ANOVA tests showed a statistical difference in relation to the other tubes. Considering that the flow of what was transferred corresponds to a laminar flow then it is possible to use the Poiseuille’s equation (it was not calculated if the flow was laminar or turbulent as this is not one of the targets of this master thesis):

$$Q = \frac{\Delta P \cdot \pi \cdot R^4}{8 \cdot \eta \cdot L}$$

where

Q is the volumetric flow;

ΔP is the pressure difference between the two ends;

R is the radius;

η is the viscosity and

L is the length of the tube.

According to the Poiseuille’s equation, one can say that if the pressure difference, the viscosity and the length of the tube are constant, then the transfer speed is proportional to the diameter. Additionally if the pressure difference, the viscosity and the diameter are constant, then the transfer speed is inversely proportional to the length of the tube. Since the radius is the fourth potency in relation to the length one can say that the radius has more influence on the transfer speed as the length. Although tube B does not have the shortest length, it was the tube with the fastest transfer speed since it has a wider diameter than the other tubes and it is also why the ANOVA test showed a statistical difference of tube B in relation to the other tubes.

8.1. Advantages of a combined thawing and transfer tube

The advantages of replacing the current single thawing and single transfer disposable tubes by a combined thawing and transfer disposable tube are: there is no need for the use of two different disposable tubes, the single thawing and single transfer disposable tubes are combined in one disposable tube. Furthermore, there is a reduction on the quality risks since the manual handling of the adapter is no longer required and therefore the process is from the microbiological point of view more secure. Another advantage is the reduced assembling time as the removal of the transfer tube, the assembly of the adapter and of the transfer disposable tube are no longer needed (see figure 59). Moreover, if a combined thawing and transfer disposable tube with the possibility for flushing is chosen, the safety of ADCs will be improved.

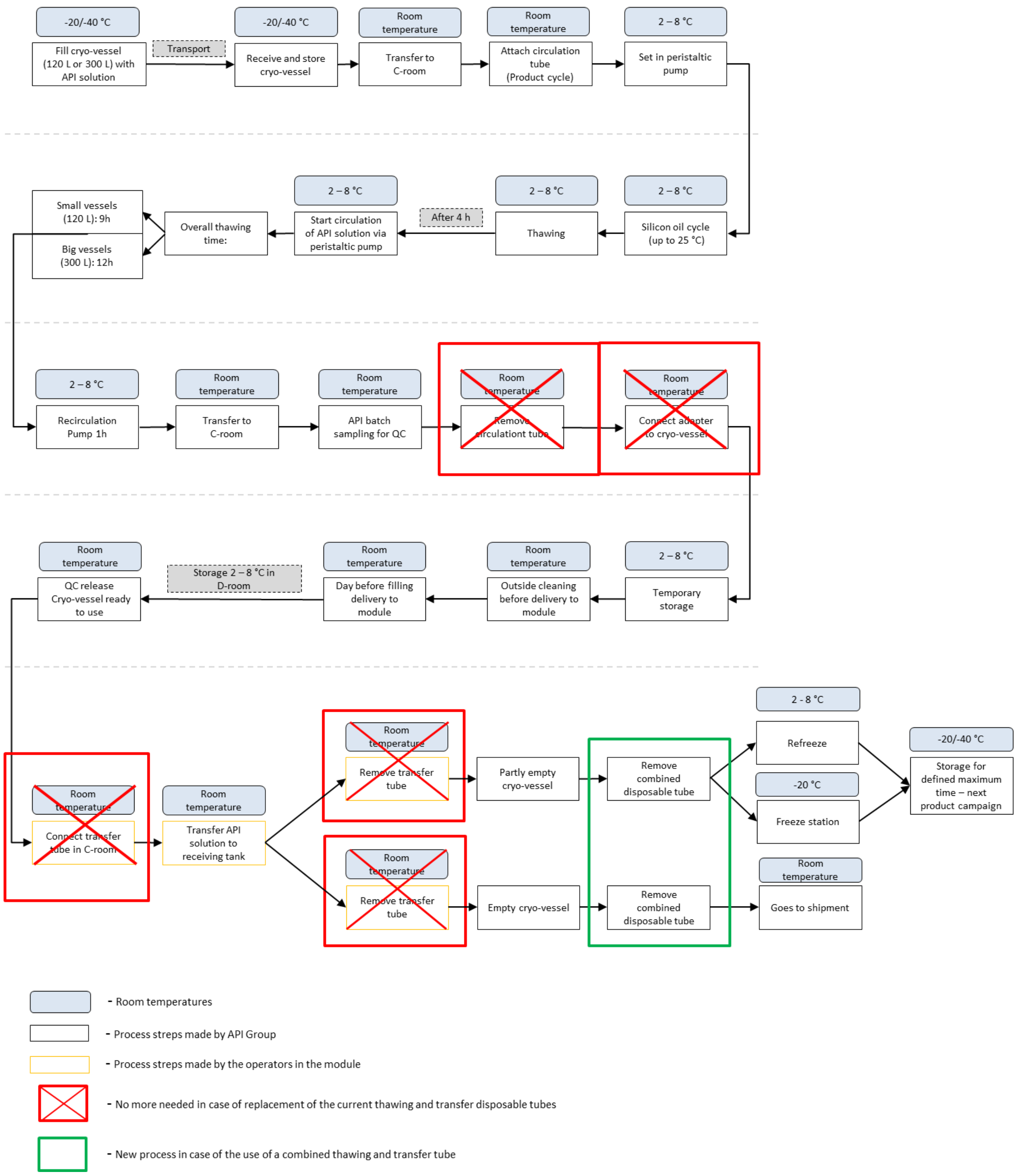


Figure 59. Cryo-vessel process cycle in Roche Diagnostics GmbH, Mannheim with the application of a combined thawing and transfer disposable tube

8.2. Disadvantages of a combined thawing and transfer tube

One of the disadvantages of a combined thawing and transfer disposable tube is that the more complex the disposable is, the more expensive it probably becomes. Moreover, it needs probably also more qualifications and it is more difficult to find a second supplier. Additionally, due to the complexity of the disposable the shelf life can be diminished.

To evaluate if a disposable technology is feasible the following steps can be followed:

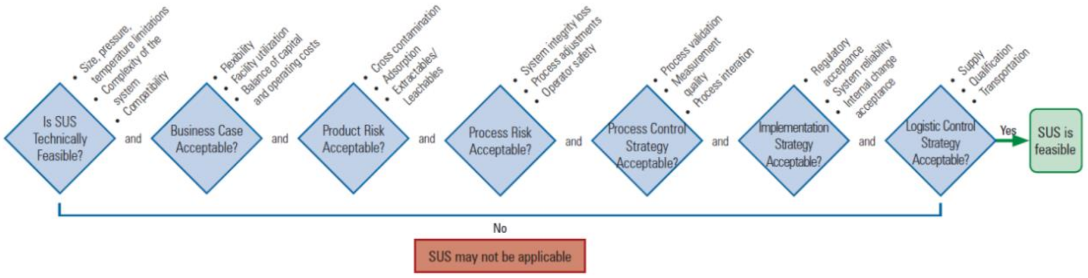


Figure 60. Steps that can be followed to evaluate if a disposable technology is feasible from the business point of view in GMP-environment (Repetto, R., et al., 2014).

8.3. Selection of an adequate combined thawing and transfer disposable tube

The literature review and the results showed that a combined thawing and transfer disposable tube can be designed in accordance with the customer's wishes allowing the possibility of choosing the tubing material, the diameter of the tubes, the length of the tubes, the connectors and other requirements. Thus, a combined thawing and transfer disposable tube can be adapted and optimized to the customer's needs.

To decide what kind of combined thawing and transfer tube should be designed a balance between Quality-Time-Cost should be taken into account and therefore, some factors have to be considered.

It is necessary to know what kind of product will be manufactured so that the disposable ensures the safety of the operators and of the environment.

Additionally, it must be considered what kind of method should be used for the transfer process, a pump or overpressure, since the tubing material has to be chosen.

The selection of the method for the transfer process has to take into account the quality and efficiency (time and costs). Therefore, the method to be chosen is the one that is faster and that results in less shear forces to prevent protein aggregation and the formation of particles and that guarantees a product with at least the quality that existed before using disposables.

There are a variety of polymeric materials available for tubing with different performance characteristics. The choice depends on what the customer wants. For example if the chosen method for transfer is a pump then it has to be taken in consideration if the tube has been tested and meets the needed standards and if it has an extended pump life. If the chosen method for transfer is overpressure then it is important to choose a tube that has been tested and meets the required standards and that can withstand the applied pressure. According to the tested tubes a combination of different materials was also used with the aim to achieve different performances.

However, the selected tubing materials should always be tested and qualified for leachables and extractables in order to prevent product contamination and guarantee the safety and quality of API solutions. Besides choosing the tubing materials, the diameter and length have also to be determined. The tubing has to have an appropriate diameter and length since it affects the transfer speed and therefore the efficiency. Moreover, the diameter has to be chosen so that the tube fits in the peristaltic pump of the thawing station and if the chosen method for the transfer process is a pump it has also to fit in the pump of the compounding process. Furthermore, the tubing should have a suitable length so that it can be connected to the thawing station and also from the cryo-vessel to the compounding tank. Additionally, the length should also be suitable to prevent yield loss.

The connectors that are attached to the tubing in order to permit the different connections, such as the connections to the cryo-vessel or to the compounding tank have to be chosen in accordance with the facility and the level of sterility that must be assured. They should protect the fluid path from microbiological contamination ensuring an aseptic process and additionally ensure that there are no leaks that can lead to product loss. Moreover an efficient connection should protect the operators and the surrounded environment. Another aspect that has to be considered is that the connectors can burst with overpressure compromising the fluid path. Therefore the choice of the connector and the applied pressure has to be considered.

8.4. Recommendation of a transfer method

According to the literature some protein solutions that are shear-sensitive can be used with peristaltic pumps (Niazi, S. K., 2012). However, because of peristaltic pumps some tubing materials can release particles due to their poor abrasion resistance (Bahal, S. M., & Romansky, J. M., 2002; Colas, A., Malczewski, R., & Ulman, K., 2004). Moreover, pumps like piston pumps and peristaltic pumps can cause protein aggregation and the formation of protein particles due to shear forces (Wang, W., 1999; Bausch, U. J., 2008; Maggio, E. T., 2008; Roche Internal Report, 2008; Nayak, A., Colandene, J., Bradford, V., & Perkins, M., 2011; Vázquez-Rey, M., & Lang, D. A., 2011; Ma, J. K., & Hadzija, B., 2013).

Regarding the results of this master thesis it is better to use overpressure for the transfer of product as it is faster (efficiency), as proved in the experiments and according to the literature it minimizes the possibility of protein aggregation (quality) since only low to moderate pressure is used (Chang, B. S., & Yeung, B., 2010; Wang, W., Nema, S., & Teagarden, D., 2010; Meersman, F., Daniel, I., Bartlett, D. H., Winter, R., Hazael, R., & McMillain, P. F., 2013**)**. Furthermore, there are no changes in the transfer method, once nowadays in Roche Mannheim, the transfer method that is used is overpressure. Therefore, fewer mistakes would probably be made by the operators ensuring quality. Nevertheless, obtaining stability data for each API solution that should be transferred is always required to test this hypothesis. Consequently, using overpressure is considered to be the best option for the transfer of product from the cryo-vessel to the compounding tank.

8.5. Recommendation of a combined thawing and transfer disposable tube

To decide what kind of combined thawing and transfer disposable tube is most suitable to replace the current single thawing and single transfer tubes in Roche Diagnostic GmbH, Mannheim some advantages and disadvantages of the tested tubes are presented in the table below (see table 18).

Table 18. Advantages and disadvantages of the tested tubes

Tested combined thawing and transfer disposable tubes	**Advantages**	**Disadvantages**
Tube A	• Qualification is already done	• Validation is required • Can´t be used with highly potent drugs, ADCs
Tube B	• Can be used in combination with a combined thawing and transfer disposable tube • Delivery tube for four different cryo-vessels • Was designed for Roche Diagnostics GmbH, Mannheim facilities, for ADC´s	• It can´t be used alone as it isn´t a combined thawing and transfer disposable tube
Tube C	• Can be used with highly potent drugs, ADCs • Was designed for Roche Diagnostics GmbH, Mannheim facilities, for ADC´s	• Validation and qualification are required

According to the advantages and disadvantages presented in the table 18, although Tube B showed the best results in terms of transfer speed it can´t be used alone as a combined thawing and transfer disposable tube once it is a delivery tube for the connection of different cryo-vessels. It can only be used in combination with a combined thawing and transfer disposable tube. Therefore, Tube A is adequate to replace the current single thawing and single transfer disposable tubes if it is used for non-hazardous API, since it is not suitable for highly potent drugs. In addition it is already qualified. On the other hand, tube C is adequate to replace the current single thawing and single transfer disposable tubes as it has been specially designed for highly potent drugs and for Roche Diagnostics GmbH, Mannheim. Regardless of the decision both tubes have to be optimized in respect to the diameter and lengths.

8.6. Future research

Taking into consideration the time constraints and the master thesis deadline, only a number of experiments were performed. Therefore, some additional tests and parameters were not part of the evaluation process.

First, it was not possible to evaluate if a combined thawing and transfer disposable tube is appropriate to all described types of cryo-vessels. Moreover, there is the need to see if this concept can be used in all Modules of Roche Diagnostic GmbH, Mannheim. Additionally, it must be tested if the combined thawing and transfer disposable tube is adequate for the thawing process and how it will be transported in the cryo-vessel from the API-group to the different Modules. Furthermore, only tube A is already qualified, therefore, if the choice is to use tube C qualification and validation have to be done from the start point of cGMP. At last, there are still GMP issues that have to be evaluated and the need of a cost-benefit analysis.

9. Conclusion

In the pharmaceutical industry, concerns about quality, time and costs, make it necessary for companies to analyse their processes and look for available technologies which allow process optimization. There are different disposable technologies available in which companies are investing due to their benefits.

In recent years, the pharmaceutical industry has developed new and more potent active pharmaceutical ingredients. The development of innovative active pharmaceutical ingredients, like antibody drug conjugates requires containment and appropriate equipment and methods to ensure quality, efficiency and safety. With disposable technologies it is possible to customize and therefore optimize the processes, handling steps and yields.

As shown, the use of a combined thawing and transfer disposable tube makes it possible to diminish the quality risks of manual handling, to reduce assembling time and improve safety of antibody drug conjugates. Moreover, the transfer of active pharmaceutical ingredient solutions is possible through the use of the two methods tested, peristaltic pump and overpressure, whereas the overpressure transfer method proved to be faster and has according to the literature research probably fewer effects on the stability of proteins. Moreover, this is a transfer method that is already used in the described pharmaceutical production area.

The decision of which combined thawing and transfer disposable tube should replace the current single thawing and single transfer disposable tubes must be made by the pharmaceutical company taking their needs into account. To conclude, the use of an optimized combined thawing and transfer disposable tube in combination with the overpressure transfer method would be an innovative process and would bring quality and efficiency to a pharmaceutical sterile drug product manufacturing process.

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