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Review Article

Operation, Qualification and Documentation of Tangential Flow Filtration System to produce Drug Substance of Vaccines in Good Manufacturing Practices Facility

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Abstract

Vaccines against infectious diseases including emerging etiological agent are being produced through various major steps in Good Manufacturing Practices approved world class facilities with the core divisions of Upstream & Downstream processes followed by quality attribute testings as per approved license from the regulatory authorities. The current review article will afford the reviewer to get hold of awareness on the operation and qualification of downstream equipment's especially Tangential Flow Filtration system and its validation as a purification tool for the manufacturing of Drug substance in vaccines against various pathological agents. The Scope of current article nourish and educate the industrial scientific personnel's and entrepreneur, who are working in biopharmaceutical industries like vaccines and Bio-therapeutics to acquire the scientific knowledge for operation, optimization, qualification and documentation of tangential flow filtration system to produce drug substance of vaccines in GMP facility. Even though the article summarizes and offers the methodological approach for the selection of TFF system as a purification tool for downstream process, The authors opinionated that the thorough familiarity given in this article regarding the pre-process concepts which starts from the industrial equipment selection & purchase procedure of the system and its operation, dual side qualification, validation, optimization of process variables with experimental execution for the successful production of vaccine drug substance would be definitely be unique and worthy. After review of this article, one can gain the complete comprehensive knowledge and assurance about the Selection, Purchase, Qualifications, Validation and Process utilization and Optimization of process variables with sustaining consistent quality of the product as output with benefit of expertise.

Keywords: tangential flow filtration (TFF) system; operation; qualification; documentation; Validation; bacterial vaccines; viral vaccines; GMP facility

Introduction

Tangential Flow filtration is unique, versatile and easiest filtration method for purification of various bio-molecules. These filtration methods can be used in wide range of biological industries such as immunology, virology, vaccinology, microbiology, biochemistry and molecular biology. In addition to this, the Tangential Flow Filtration (TFF) system can also be used from a wide range of research analysis to commercial large scale manufacturing units to deliver the purified quality product. This technique is so simple to use, easy to learn and less time consuming for various process applications. The virtue of TFF system is to remove all contaminants which may be present in the sample of interest. Being as a best purification method, the TFF system can be used in wide range of purification processes which involves smaller quantities of the sample with desired set of parameters. While there are many article about the optimizations and methodology development, the practical approach for qualification and application based information's not clearly delineated. The current article is devoted to give the reader clear information about the usage, selection and qualification, optimization requirement of TFF system in bacterial and viral vaccine process applications. Vaccines are being produced from a variety of sources including tissue extracts, bacterial cells, virus particles, recombinant mammalian, yeast and insect cell produced proteins and nucleic acids. The most common method of vaccine production is based on an initial fermentation process followed by purification (Downstream). Production of vaccines is a complex process involving many different steps and processes. Selection of the appropriate purification method is critical to achieving desired purity of the final product. Clarification of vaccines is a critical step that strongly impacts product recovery and subsequent downstream purification. There are several technologies that can be applied for vaccine clarification.

Selection of a harvesting method and equipment depends on the type of cells, product being harvested, and properties of the process fluids. These techniques include membrane filtration (microfiltration, tangential-flow filtration), centrifugation, and depth filtration (normal flow filtration). Historically vaccine harvest clarification was usually achieved by centrifugation followed by depth filtration. With the introduction of TFF system, the membrane-based technologies have gained prominence in vaccine clarification [1] The increasing use of single use technologies in upstream processes necessitated a shift in harvest strategies [1-2]. The current review offers best support for the selection of TFF system as a best tool to adopt the downstream procedures for bacterial and viral vaccine applications through the membrane-based approach.

About Tangential Flow filtration (TFF) system

The filtration methods have already been existed in the various biopharmaceuticals, to increase the ease of operation, faster, more flexible, and efficient and self-clean ability, the membrane-based filtration approach has been introduced in to work environments especially downstream processing areas. Until the development of TFF system method, the downstream operations took much more time for fluid filtrations and consumed more man efforts. Now days with the introduction of the TFF system from early 90s, the operations become so effortless in less time and manpower consumption at a remarkable yield in terms of product output [3]. The Perspective based advantages of the TFF system are summarized in the Table 1.

Perspective	Advantage
Easy to set up and use	Just make connections with required parameters and Plug and Play use
Fast and efficient	It can be used with in short span of time and efficient than centrifugation techniques
Multi use Method	Two steps can be done with the single system. Concentrations and Diafiltrations
Research and Commercial Purpose	One TFF system can be used for Research and Development and Pilot scale to commercial Applications
Economical	TFF system having membranes can be cleaned validated and reuse even in commercial scale also.
Reprocessing	Reprocessing also possible for any breakdown processes by using the same membrane
Easy optimization	Easy to optimize the Purification process of interest to deliver the desired substance
Space occupation and less labor cost	The TFF system itself doesn't occupy much space due to compact design and single work man ship operated

Table 1: *Perspective based advantages of TFF system.*

Principle of TFF system

As the name itself (Tangential Flow Filtration or Cross flow filtration) indicates the filtration principle based on the passage of feed which is parallel to the flow of membrane, where permeate will be collected as a filtrate and retentate is return back to the feed tank. The retentate is that part of the feed that does not pass through the membrane, while permeate is the part of the feed that does pass through the membrane. However based on the purification process the output might be contaminant free biological cells or purified biomolecules (Protein, Nucleic acids or carbohydrate etc).

This method is also known as Microfiltration system. Depending on the Pore size of the membrane which is being used in the TFF system, the techniques are classified as Microfiltration having the pore size ranges from 0.1µm -10 µm and the diafiltration use the pore sizes between 0.001 to 0.1 µm.

In contrast to the direct flow filtration (DFF) method, TFF system allows the passage of sample through the feed and sweeps away the aggregating molecules which cause the clogging of membrane, allows the smaller molecules which are lesser than the membrane pores size are moved out of the membrane. Where as in DFF method the molecule larger than the membrane pore size are accumulated at the membrane surface and forms a gel which block the flow of liquid through the membrane which subsequently decreases the flux rate as the inflow volume increases. Hence TFF can be so faster and efficient than DFF method [3].

Uses of TFF system

The TFF system can be used for the concentration, and diafiltration, and ultrafiltration of the given sample.

Concentration. The concentration of given sample depends on the type of sample that is being filtered. For protein purification, Molecular weight cutoff membrane to be chosen to filter the given sample. One must be taken care while choosing the MWCO membrane to get the maximum recovery rate of the product from the sample. General Thumb rule is to select a membrane with MWCO is that 3-6 times lower than the molecular weight of the protein/sample of interest. Concentration is a process where the membrane separates out the feed sample into the desired molecule and undesired impurities which will be flushed out to drain. During this process the desired molecule or product will be collected back to the sterilized tank and the undesired one will be eliminated through the waste.

Diafiltration. Diafiltration is a fractionation process that washes smaller molecules through the membrane and retain the larger molecules can be collected back in to the retentate without changing the concentration. There are two different methods in diafiltration process:

(i) Continuous diafiltration, where the diafiltration solution is added at the feed solution at the same rate of filtrate is generated.

(ii) Discontinuous diafiltration, where the solution is first gets diluted and concentrated back to the initial volume. By using diafiltration method the smaller molecules which are less than the membrane of interest and other salts will get eliminated to produce the purified product.

Ultra-filtration. Ultra-filtration membranes, with much smaller pore sizes between 0.001 and 0.1 µm, are used for concentrating and desalting dissolved molecules (proteins, peptides, nucleic acids, carbohydrates, and other biomolecules), exchanging buffers, and gross fractionation. Ultrafiltration membranes are typically classified by molecular weight cutoff (MWCO) rather than pore size [3].

The TFF application had been used significantly in the clarification of microbial and mammalian cell culture clarification processes where the protein can be purified from the harvest culture using microfiltration membranes. In some of these microfiltration TFF applications (*e.g.*,

mammalian cell culture clarification), the product (protein) freely passes through the microfiltration membrane and is recovered on the permeate side, while the contaminating unwanted impurities are retained on the feed side of the membrane. In certain other microfiltration TFF applications such as allantoic fluid clarification in egg-based flu process, the virus product may get concentrated on the feed side of the microfiltration membrane (similar to an ultra-filtration step), while the contaminating impurities like ovalbumin, etc. may get removed into the permeate side [4].

Selection criteria of TFF system

The selection of specific TFF system and its filtration membrane rely on the type of the molecule is being used for purification, concentration and filtration. However in general, for purification of bacterial and viral culture and biomolecules, following selection criteria to be adopted for proper choice of the preferred system to achieve desired results.

(i) Define the purpose of TFF system for application of interest.

(ii) Selection of Membrane for process application.

(iii) Selection of membrane flow channel configuration i.e., Screen channel, suspended screen channel, open channel. Mostly open channel membranes are being used for various cell-based products.

(iv) Selection of the Nominal Molecular Weight Cutoff (NMWC) for the membrane.

(v) Define the membrane area for application of interest.

(vi) Define the criteria for optimization of operational parameter.

Operation requirement of TFF system

The TFF system is acquainted with wide range application and makes itself a boost up for the biopharmaceutical downstream manufacturing process. TFF system is widely used in various biopharma, vaccines and biological manufacturing applications for clarification of fermentor cultures, cell cultures in case of monoclonal antibody productions and small molecule separations. The economic point of view makes the system so affordable even for Research & Development and commercial applications as compared to centrifugation techniques which are being used in high solid separations requires high capital investment, maintenance, and human hours. Recent recovery optimization research studies conducted for Neisseria meningitidis outer membrane vesicles had shown that the concentration and recovery of the biomass and separation of OMV for measuring the yield [5].

Operation

TFF step is limited by a maximum throughput or capacity obtainable under a given set of operating conditions, which may potentially limit or determine process sizing. The low TMP requirement, along with the potential throughput limitations, demands a special approach or methodology to develop a robust, optimized process condition for a microfiltration TFF process. Figure 1 is showing operation flow pattern of the TFF system in easy, systematic, and understandable manner. Nano porous sandwiched membranes could also been used to separate or isolate virus particles in recent research studies [4-5].

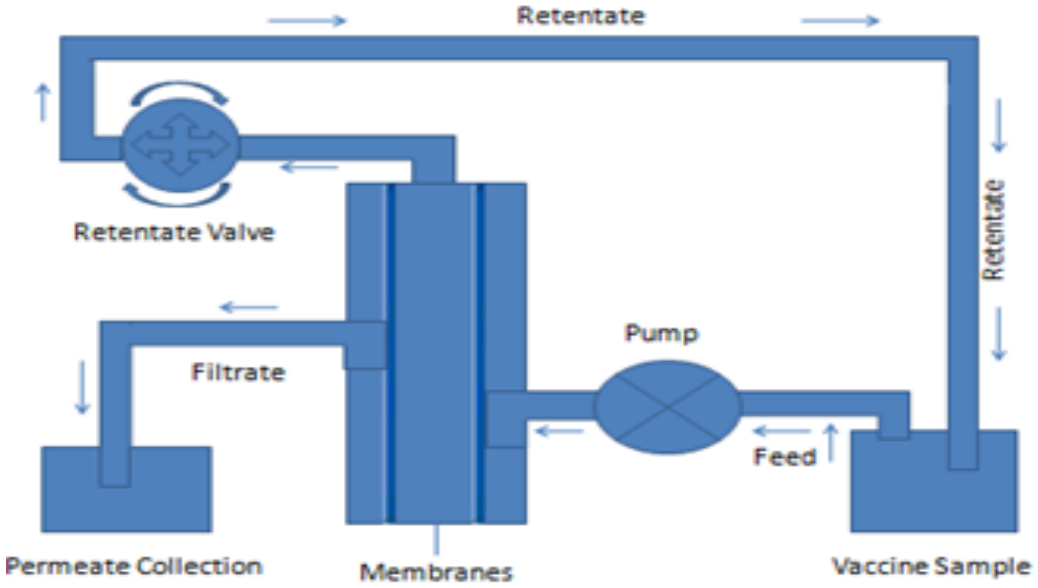

Figure 1: *Operation flow pattern of TFF system*

Customization of the TFF system could have been achieved depending on the type of purification process, the same was done for the separation and purification of HIV virus and its cell particles by fabrication of TFF system membrane and affinity separation [6].

Qualification and Validation Requirements

Prior to the validation of a TFF process, it is necessary to ensure that the process has been adequately defined during process development stage and accurately scaled up onto a well-designed and established system. If the criteria have been met, the task of executing the overall validation process which should include process characterization, process validation, and cleaning validation modules becomes much more straightforward with high odds of success.

Qualification & Validation of tangential flow filtration (TFF) is required to ensure the process delivers a product of consistent quality, safety, and efficacy and yield in terms of percentage of recovery. A meticulous and sound validation program not only satisfies regulatory requirements, but also provides a valuable source of information which facilitates the development of future processes procedures, training of production/operating personnel, and troubleshooting for the validated process. Validation of TFF shares many common fundamentals with validation of other traditional operations and equipment. Existing personnel and procedures should be readily adapted to execute the TFF

validation protocols. IQ's and OQ's will most likely follow well-known formats. In performance qualification, key areas needing attention include: assessment of compatibles, testing of parameters affecting membrane retention and selectivity, cleaning, sanitization, and membrane life span [7].

Cleaning Validation & Process validation. The validation of the TFF system is crucial and important stage for further use. Here it is describe in three simple steps and each step was discussed in sequential manner to understand easily for user [7].

The first step of cleaning validation demonstrates that the filtering membranes are returned to a state where the process will perform reproducibly each run which can be met and tracked by process performance as well as membrane data from run to run or batch to batch. The typical measures of process performance are process flux, process time, product yield, and product purity. While these measurements tie most directly to process success and are the most relevant, they require that product feedstock be committed to the cleaned membranes (without risk marker such as previous product traces) without knowing whether the membranes have yet reached their reuse limit. Therefore, in addition to process data, it is desirable to also track clean membrane data most typically membrane integrity and clean membrane permeability. Membrane integrity should be monitored before and after each use and it can be achieved by membrane integrity system, and the vendor's specification for membrane failure should be used as a criterion for replacing the membranes. Clean membrane permeability/ability can be measured with water flux divided by transmembrane pressure (TMP) which should be measured and trended from run to run. Although a drop in clean membrane permeability/flux may not be as directly related to process performance as the actual process flux, it is a simple measurement that can be an early indication of degradation of membrane performance to decide its lifespan. The reduction of clean membrane permeability to a certain percentage (probably less than 90%) of the initial value may be used as membrane change-out criteria or it depends on the user purpose. However, it is important to note that measured permeability values are often related to the system on which the measurement is taken, especially for high permeability membranes (ultrafiltration >30kDa and all microfiltration membranes) where measured TMPs are very low and often within the measurement error. Differences in the placement of pressure transmitters in relation to the membranes and differences in pressure loss in piping also add to variations in permeability measurements from system to system. For these reasons, a change-out criterion based on a permeability measurement should only be set when the data is collected on the same system on which it will be applied. In addition to this the product or the sample consistency which is being passed or filtered through the membrane is also a set mark for measurement of cleaning permeability.

The second step of cleaning validation is ensuring that contaminants (Traces of previous batch sometimes called as risk markers) are adequately removed to prevent run-to-run carryover. Risk marker carryover studies can either be performed between batches using placebo/real product, or by testing rinse solutions or extraction solutions. The placebo/real product, rinse solution, or extraction solution is analyzed for residual product for instance protein, other host cell proteins, DNA, excipients as risk markers, and residual cleaning and storage reagents. Care should be taken not to set the specification for any contaminant level as the limit of detection (LOD) of the assay, as LODs often decrease as assays adopt with new technology. Carryover studies must be performed out to the established limit of membrane reuses and to design the cleaning optimization process. Membrane re-use validation and carryover validation are often done concurrently at full-scale during production in order to minimize cost and effort without compromising the quality of product. This can be done by defining in place appropriate sampling, analysis, and quarantining of final product until cleaning rinse samples shown to be acceptable. In addition to run-to-run carryover, users of TFF membranes must also demonstrate that the preservative (shipping) solution is completely removed from the devices after a specified flushing protocol sometimes the same to be followed as per the vendor specified protocol prior to the first use with product. The assay showing clearance of the preservative or storage solution should be easy to use, and validation of an assay which can be run on the manufacturing floor will save processing time versus having to submit samples for QC testing and waiting for results before beginning a process run.

The third step of cleaning validation is a final step and it is to demonstrate that bio-burden and endotoxin levels are to be analyzed and kept under control using the specified cleaning protocol. This is of particular importance if the TFF unit operation is near the end of the purification process. Sterility requirements and maximum endotoxin levels should be specified for often not just for the final processed product pool but for incoming buffers and product as well which are in contact with the membrane. Bioburden and endotoxin elimination from the membranes and system using the specified cleaning reagents (concentration, temperature, and exposure time) must be documented. In addition, storage solutions should be evaluated for bacteriostatic/ virucidal capability over the intended storage time.

Special considerations surround the cleaning validation of a microfiltration system due to exposure to cells. Whether bacterial, yeast, or mammalian, the cleaning of the system must be adequate to show inactivation and removal of all cells and cell debris between runs and also necessary to make consistent. The biggest validation issue surrounding cell harvest is the containment of recombinant organisms and equipment decontamination. If a claim is made that the filtrate from the TFF system will be free of recombinant organisms, then protocols should specify testing of the filtrate for such similar organisms.

Succinct knowledge on IQ, OQ and PQ

Design Qualification (DQ) is the documented verification that the proposed design of the system/equipment is suitable for the intended purpose, meeting regulatory and process needs as per the predefined requirement of the user.

Installation Qualification (IQ) involves the selection of the system as per the users process requirement and its design preparation, suitability, installation at the users lab followed by the cross checking of the system as per the pre-determined user specifications.

Operational Qualification (OQ) refers that the installed system is being operated as per the predetermined specifications flawlessly.

Performance Qualification (PQ) is to establish confidence through appropriate testing that the finished product or process produced by a specified process meets all release requirements for functionality and safety and that procedure are effective and reproducible.

Validation of Trans Membrane Pressure and Flux

Objective. Validation of Trans membrane pressure (TMP) and flux for maximum yield of microbial antigen per vaccine batch.

Scope. Trans membrane pressure (TMP) and flux are critical parameters for TFF system to optimize maximum yield with consistency in batch-wise production for bacterial and viral vaccines [8].

Materials. Major materials are TFF system (TFF system with validated Flow meters, Pressure gauzes, Diaphragm valves for operation of Feed, Retentate, and Permeate lines), Slice cassette membrane (Specification of slice cassette membrane id 0.1 m^2 surface area, and 0.2µm pore size), Peristaltic pumps, and Sterile poly propylene bio-containers.

Process and results. The process parameters of using TFF system are extensively important to the process and the product as well to sustain the consistent quality. To optimize the filtration process using membrane-

based approach involves the operational parameters such as temperature, pressure and flow rate. In case of bacterial antigen for vaccine production, optimization of TMP with respect to pump frequency ranging from 5 to 25 Hz will be performed. Selection of TMP is always dependent on parameters; frequency of lobe pumps and pressures exerts at feed, retentate & permeate. As the TMP increases, the permeate flux also increased with reduced process time but the fouling of the membrane was observed due to ramp up the TMP with low flux. Hence this was considered as optimization point where the antigenic yield became constant above which unstable TMP and flux was obtained. The experimental details are compiled and described in table 2 (8). Finally, it is GMP requirement to check sterility of the retentate as product for presence and absence of bacterial & fungal contamination by culture method. In this study, sterility test of the retentate was performed in suitable & specific culture media and found sterile after 14 days incubation for bacterial & fungal contamination, individually.

The pressure is maintained across the membrane is defined as TMP (Transmembrane pressure) which is calculated as the average pressure at the feed and retentate of sample, which is to be subtracted with the permeate pressure denoted as below formula:

$$\text{Trans Membrane Pressure (TMP)} = \frac{(\text{Feed}+\text{Retentate})}{2} - (\text{Filtrate/Permeate})$$

Pump Frequency (Hz)	Pressure (bar)			TMP (bar)	Flux (LMH)
	Feed	Retentate	Permeate		
5	0.1	0.1	0.0	0.10	0.3
10	0.2	0.2	0.0	0.20	0.6
15	0.4	0.1	0.0	0.25	0.6
20	0.6	0.2	0.0	0.40	0.78
25	0.8	0.2	0.0	0.50	0.65

Table 2: *Optimum TMP & Flux details for the bacterial trial batch process*

After calculation of the TMP for TFF system, you can plot a graph on the basis of selected parameters against the obtained yield of bacterial antigen before execution of actual vaccine batches to observe the consistency in the yield. Consistency of the executed trial batches for maximum yield of bacterial antigen along with optimized TMP can be observed as shown in figure 2.

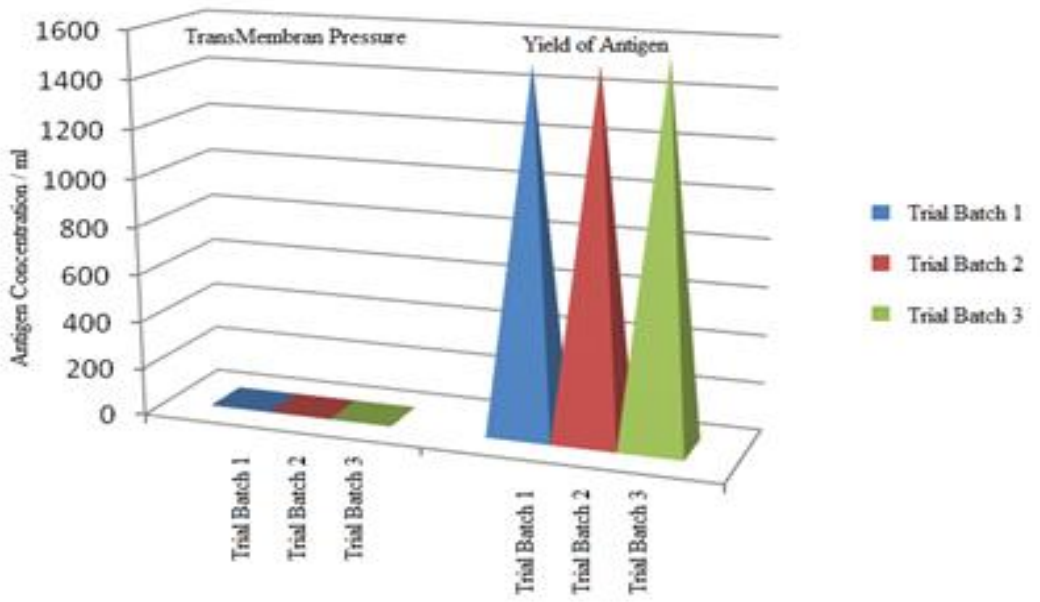

Figure 2: *The graph shows about Trans Membrane Pressure (TMP) optimization at the 0.25 bar at 15 Hz frequency and the obtained product output in terms of Antigen concentration (Viral and bacterial antigen for vaccine preparation) in trial batches with consistency of antigen yield.*

Data Compilation & Analysis. Based on the inference obtained from the plotted graph, it was observed that the bacterial antigen yield was consistent at the frequency of 15Hz with 0.25bar TMP at no product loss. All data will be collected and used to prepare in tabular form for its analysis purpose. The obtained data shows the result that with a TMP of 0.25 bar, the TFF system would be yielding maximum recovery of bacterial antigen.

Preservation of membranes. It is strictly advised to preserve the filtration membranes in the solutions as specified by vendor to maintain the life span of the membrane which would subsequently saves cost of the user.

Process Optimization. For any process optimization, one should perform product trail batch with data obtained from the process development stage. All parameters would be finalized before starting the trial batch to

ensure the batch is able to give the successful data to go ahead further or not. It is also necessary to monitor that the optimized parameters are providing consistent data or not.

Documentation

The following essential and adequate procedural and documentable activities to be performed as per the regulatory authority frame work of World Health Organization (WHO) and International Council for Harmonization (ICH). This current article has adopted the WHO and ICH and IP guidelines in preparation as well as execution of the experiment.

Preparation of URS. The User requirement specifications are the preliminary step before buying any equipment. This is documented evidence that the user will prepare specifications based on the process requirement which is going through thorough reviews among the team members and finally get approved. Once the URS is prepared, the user selects the suitable manufacturer by external survey in the market who offers best in terms of cost and requirement specifications.

Procurement process. The procurement if the downstream processing systems flow through the various internal and external procedural module such as Preparation of Indent with URS at the user side, Pre- qualification of the vendor, Design finalization as part of Design Qualification, Pumping & Instrumentation Diagram (P&ID), technical and financial evaluation, document and approval, Issue Purchase Order (PO) including with the requirement of FAT & SAT.

Factory Acceptance Test (FAT). This test to be carried out at the equipment manufacturer site to inspect the equipment is fabricated and operated as per the designed user requirement specification without any deviations. Further all the material compliance tests, operational steps and related documents shall be verified mutually before the approval.

Site Acceptance Test (SAT). This test is to be carried out at the user site/user industry, where the equipment solely shall be used to inspect the equipment which is being operated and deliver satisfactory results at the whole discretion of the user. All the necessary documents from both the user and the manufacturer side should be reviewed mutually and finalized followed by the mutual approvals.

Installation & Qualifications. This is to be carried out at the user site where availability of all utility services, along with suitable certified documents such as IQ, OQ, PQ, and their approvals.

Product trial. This is to ensure about performance of TFF system for manufacturing the vaccine after successful completion of qualification and validation with necessary approved documents.

Review, approved and archived the documents. Data integrity is the most essential task in any biopharmaceutical industry to review feedback and learn few or more new technical aspects or to modify the procedural steps and data retrieval for periodical supervision. The main approved documents which are considered in the current topic of the article includes Standard operating Procedure, Record sheets, Log books, Master Formulae Record, Batch Manufacturing record, Various Cleaning and Process validation Protocols and reports, Trend analysis reports, Risk analysis reports, Engineering documents, Troubleshooting documents etc. The key role of the documents would be known from the archival of the approved documents which can be accessed at later times whenever and wherever required either as hard copy or an electronic copy with the prior permission from the concerned authority.

Applications in Vaccine Industries

TFF system is acquainted with dynamic features and it is applying as downstream tool in biopharmaceutical industries especially in bacterial and viral vaccines production. On the basis of literature available, authors summarized combination of the technologies used for major bacterial and viral vaccines production with after the year 2000. Due to virtue of the TFF system, it was also used in various nano particle filtration technologies for the purification of biopharmaceutical products and chemicals with high percent of product recovery in less time which substantially proved its wide spread application in different fields of industries [9].

The application and usability of the TFF system in the field of marine industrial technology and aquatic microbial ecology is very appreciable and productive. Initial studies showed that the TFF system can be extensively applicable for the purification and separation of various bacterial and viral particles. But the recent studies also revealed that the type of the membrane could be used for the specific microbial particles for its separation and elimination with maximum recovery might help and opens the window for future research scope in various fields [10].

The TFF system is widely used in various vaccine bulk preparations in manufacturing industries approved by World Health Organization (WHO). In early 90s the vaccine clarification and concentration experiments which were done on optimization of TFF process variables in terms of product yield and recovery was successfully evident that the TFF system in vaccine and biological industries is so advantageous than the traditional centrifugation process which opened new era for futuristic expansions in process developments and manufacturing fields [11].

TFF system is widely acceptable and useful in purification of polysaccharide with protein conjugates especially polysaccharide released from the haemophilus influenza type b strain and streptococcus pneumonia of all serotypes during the production of conjugate vaccines. The outcome from the study of the PS-CRM_{197} conjugate showed that the low molecular weight polysaccharide could strongly bind to the carrier protein with high affinity and ultimate purification could lead to removal of impurity in the form of free polysaccharide [12].

The versatility of the TFF system enables its user to work on various purification principles involving huge number of biomolecules, cells and cultures. The advantages of TFF system are so flexible especially in vaccine industry for manufacturing and purification of bacterial and viral vaccine drug substance. The variety of bacterial and viral cultures and its purification with the help of TFF system and its role was extensively increased for various research and commercial purposes due to its ability of product high recovery and less time-consuming strategies in process.

The combination of technologies used for multiple numbers of bacterial vaccines [13-20] and viral vaccines [21-29], which were produced using primary & secondary specifications of the TFF system for clarification as a vital tool, are widely accepted in the market with continuous generation of revenue in the industrial sector. Different types of vaccines with production scale are summarized in table 3 for bacterial vaccines and table 4 for viral vaccines.

Vaccine	Production Technology	Vaccine type	Production scale	Reported year	Reference Number	Specification of TFF system for clarification	
						Primary	Secondary
Diphtheria toxin	Fermentation	Toxoid	Industrial	2002	13	0.45 µm Prostak™ device (TFF)	NA
A cellular pertussis	Fermentation	Sub-unit vaccine	Pilot	2009	14	0.45 µm Prostak™ device (TFF)	NA
Typhoid vaccine	Fermentation	Conjugated polysaccharide	Pilot	2010 & 2013	15 & 16	0.45 µm Hydrosart® cassette (TFF)	NA
pDNA vaccine	Fermentation	Plasmid DNA vaccine	Pilot	2010	17	Flocculation	Depth filtration (NFF)
Meningococcal vaccine	Fermentation	Conjugated polysaccharide	Pilot	2011	18	Extracellular Pilot 0.2 µm hollow fiber cartridges (TFF)	300 kDa cassettes (TFF)
Tetanus toxin	Fermentation	Toxoid	Industrial	2013	19	0.22 µm Prostak™ device (TFF)	NA
Pneumococcal vaccine	Fermentation	Conjugated polysaccharide	Pilot	2014	20	300 kDa Pellicon® 2 cassettes (TFF)	NA

Table 3: *Combination of technologies used for clarification of bacterial vaccines*

Vaccine	Substrate	Vaccine form	Production scale	Reported year	Reference Number	Specification of TFF system for clarification	
						Primary	Secondary
Rotavirus vaccine	Insect cell culture	Virus like particle	Pilot	2007	21	Centrifugation at 1000 ×g for 10 min at 4 °C	Ultracentrifugation of the supernatant at 100,000 ×g for 1 h at 4 °C
Influenza virus	MDCK cell culture	Inactivated virus	Pilot	2007	22	0.65 µm polypropylene depth filter (NFF)	NA
Rotavirus vaccine	Vero cell culture	Live viral vaccine	Pilot	2011	23	Centrifugation at 2831 ×g for 30 min and 4424 ×g for 10 min at 4 °C	0.45 µm hollow fiber (TFF)
Enterovirus 71	Vero cell culture	Killed viral vaccine	-	2011	24	0.65 µm filter (NFF)	NA
Canine adenovirus vector	MDCK-E1 cell culture	Live viral vector	Pilot	2013	25	Settling of cells with micro carrier	Sartobrand® P 0.45µm filter (NFF)
Polio virus vaccine	Vero cell culture	Inactivated virus	Production	2013a & 2013b	26 & 27	Diatomaceous earth deposit on a stainless steel mesh filter with 75 µm pore size, or Millistak + ® C0HC (Depth filter)	0.45 and 0.22 µm filtration (NFF)
Yellow fever vaccine	Vero cell culture	Inactivated virus	Pilot	2014	28	Sartopure® PP2 (8.0 µm),	NFF Sartoclean® CA (3.0 µm +0.8 µm), NFF
Hepatitis C	Insect cell culture	Virus like particle	Pilot	2015	29	Polypropylene filter, Polygard ® CN 5.0 µm (NFF)	Polypropylene filter, Polygard® CN 0.3 µm (NFF)

Table 4: *Combination of technologies used for clarification of viral vaccine*

Conclusion

The versatility of the TFF system enables the user so advantageous in various industrial applications especially downstream processing of number of biomolecules in biopharmaceutical industry to produce high yield quality product with minimum process loss. Due to its wide application scope, TFF system makes high priority requirement in purification of various biological mixtures. Current presentation is determined the importance of major process variables particularly TMP and Flux, which are interlinked in process application. Our validation study has shown that the yield of the final product is consistent when the TMP and flux are maintained in standardized fashion. Therefore, the wastage of the product output with cost-effectiveness has considerably reduced and negligible throughout the experiment. In current article, authors provided up-to-date knowledge about the TFF system including operation, qualification and documentation to produce the drug substance of vaccines in GMP facility and its dynamic applications in production of bacterial and viral vaccines.

Abbreviations:

TFF - Tangential Flow Filtration
NFF - Normal Flow Filtration
TMP - Trans membrane Pressure
Hz - Hertz
DQ - Design Qualification
IQ -Installation Qualification
OQ - Operational Qualification
PQ - Performance Qualification
P&ID - Pumping and Instrumentation Diagram
FAT - Factory Acceptance Test
SAT - Site Acceptance Test
WHO - World Health Organization
ICH - International Council for Harmonization
IP - Indian Pharmacopoeia
URS - User Requirement Specification
DFF - Direct Flow Filtration
LOD - Limit of Detection
QC - Quality Control
DNA - Deoxyribo Nucleic Acid
GMP - Good Manufacturing Practices
MWCO - Molecular weight Cutoff
NMWC - Normalized Molecular weight Cutoff
PO - Purchase Order

Conflict of interests

Authors declare that there is no conflict of interest.

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