# THE APAC TIMES

Emerging Innovations In Cancer Immunotherapy


## SAFETY & QUALITY MEASURES FOR APCEDEN® PRODUCTION


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In recent years, Quality Assurance (QA) and Good Manufacturing Practices (GMP) have become increasingly significant in the biomedical industry. Biological therapeutics are distinguished from chemical pharmaceuticals drugs as they are derived from living organisms with a molecular composition too complex to be defined by physical or chemical means. Moreover, the potential for contamination of materials with agents coming from starting materials or the environment require special Quality Control (QC) and quality assurance mechanisms. Therefore, the manufacturer has the primary legal responsibility to ensure the safety, quality, and efficacy of the products they manufacture and sell.

Production of cancer therapy usually involves isolation and proliferation steps, including growing appropriate cells using substances of animal origin, which makes it susceptible to introduce any contaminant and to amplify low levels of contamination. Manufacturing is a complex activity with risks and often requires the handling of living organisms which are sometimes pathogenic for humans. The release of these agents, with the possibility of contamination/ cross-contamination, is regarded as a serious danger to the workforce and the environment. Thus, all the materials should be well protected.

A great attention is laid to all the parameters associated with handling, preparation, logistics, storage and isolation of the biological samples. At production site, the facility is systematically inspected, decontaminated and sterilized to ensure that the biological product is pure, safe, potent and efficacious. The laboratory procedures, data, tests and studies are planned, performed, monitored, recorded and reported, to ensure the quality and integrity of the data generated by a laboratory. Before entering a biological production area, personnel should either change their clothes for clean laboratory clothing or cover their clothes with appropriate laboratory garments. Hair covering, face masks, gowns and shoe covers should be used in production areas. Furthermore, the quality control manager inspects and certifies that the instruments are monitored, calibrated and maintained properly as per manufacturer's guidelines to ensure maximum efficiency

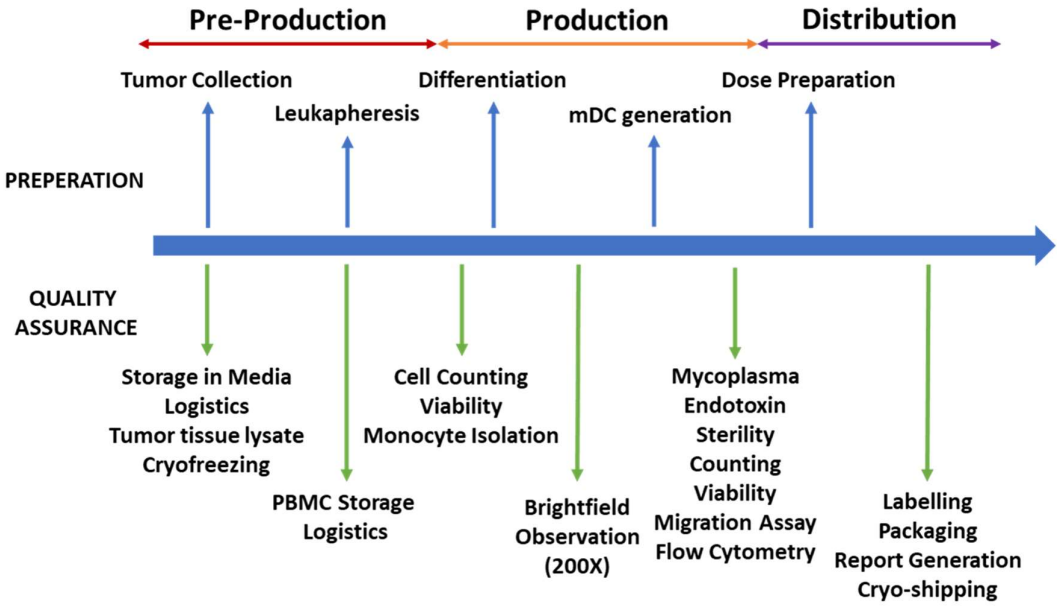


*Table 1:* List of QC steps involved in APCEDEN® generation.




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in the work flow. Before entering a biological production area, personnel should either change their clothes for clean laboratory clothing or cover their clothes with appropriate laboratory garments. Hair covering, face masks, gowns and shoe covers should be used in production areas. Furthermore, the quality control manager inspects and certifies that the instruments are monitored, calibrated and maintained properly as per manufacturer's guidelines to ensure maximum efficiency in the work flow.

APCEDEN®, a product of APAC Biotech Pvt Ltd is personalized Dendritic cells based immunotherapy approved by the Indian Government for use in advanced/refractory Prostate, Ovarian, Colorectal and Lung cancers. It is now available at major hospitals across India. We have divided our manufacturing step into 3 parts to simplify and elaborate the quality control checkpoints involved with each step.

## Pre-Production-

This step begins form the time patient is enrolled after being meticulously inspected by our fellow clinical teams. Once enrolled, the patient is requested to sign a medical agreement with APAC Biotech and all their previous medical reports are documented. Once enrolled, our team coordinates with their fellow clinician to approve a predefined date for the tumor tissue collection and the Leukapheresis.

Since our Dendritic Cell (DC) therapy is an autologous (patient derived), the process involves the collection of patient's derived tumor tissue and monocytes. The tumor collection kit is specially designed for the collection and shipment of the tumor tissue is dispatched from APAC Central Lab to the site of surgery. The kit contains one vial with fluid in it for storing tissue during surgery. Once excised, the tissue needs to be dipped fast in the provided fluid. The fluid ensures that the tumor tissue integrity (protein and RNA of the tissue remains intact and is not lost during the process of transport). The tissue should be cut into small pieces to ensure that it gets completely dipped in the given fluid. Once the tissue is enclosed, the vial is placed in dry ice and our logistics team ensures that the tissue reaches the APAC production facility safely within 24hrs. Shipping in dry ice induces cell cycle arrest, thus reducing cell lysis and mutation.

Once the tissue arrives at central facility, it is initially checked for the storage temperature (-40$^{o}$c) as a drop-in temperature might indicate mutation and loss of genetic and protein material by the cell into the media. The tissue is also visually inspected by our quality control manager for labelling and UPIN number issued for the patient sample. If it qualifies the checkpoint, the sample is taken to our facility where its carefully removed and weighed. On obtaining the sufficient amount of tissue (1-2 gram), the tissue is homogenized to induce complete cell lysis. The efficiency of lysis is analysed through 7AAD viability assay. If the cell viability is 99%, the lysate is sterile filtered to remove fungal, bacterial or large macromolecules that might be harmful. Once filtered, the lysate vial is collected, aliquoted, labelled and stored at -80$^{o}$C to avoid protein degradation.

Like tumor collection, a PBMC (Peripheral Blood Mononuclear Cells) kit is employed for collection and transportation of the PBMC from the hospital to the APAC central lab. Prior to transport, the nutriprep media is injected into the leukapheresis bag to ensure viability and safe transportation of PBMCs isolated from patients' blood. The bag is then transported at room temperature (upper limit 25°C) within 6 hrs of collection. In cases where transportation requires more than 12 hrs, the bag has to be transported at 4-8°C with ice packs.



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## Production-

Following processing and stringent quality control, once all parameters are OK, the quality control manager issues a certificate for the initiation of manufacturing of APCEDEN®. Any deviation from the set criteria leads to the rejection of the APCEDEN® preparation.

The viability of monocytes in PBMC is calculated through 7AAD staining and Trypan Blue. Once, a sufficient percentage viability is achieved, the total number of monocytes in PBMC are tallied with the hospital generated report by performing flow cytometry for Cell surface receptor characterization of Monocytes (CD14) in the PBMC (Figure 1). Once it fits all the criteria, the lab proceeds to monocyte isolation.

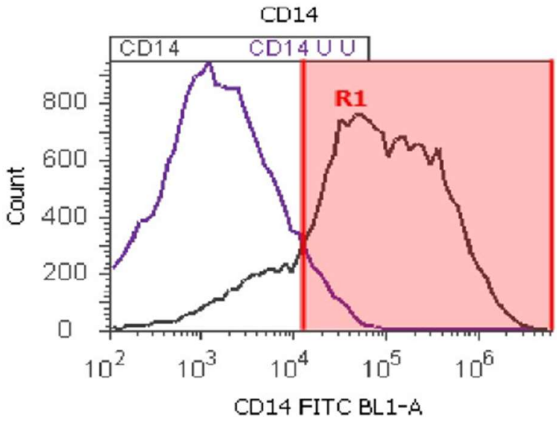


*Figure 1:* Flowcytometry data depicting number of monocytes in patient PBMC sample.

Following the isolation of monocytes, the cells are incubated with a cocktail of tumor tissue lysate and growth factors to prime immature dendritic cell differentiation. During the 8-day process, the cells are placed at 37°C $Co_2$ incubator and are regularly inspected under bright field magnification of 200X. On the 8th day, the immature Dendritic Cells are isolated and analysed for-

**Endotoxin**

The bacterial endotoxins can give rise to adverse reactions to our cell culture and can cause undesirable effects during the production process. Besides maintaining aseptic conditions, reagents such are serum and culture media are frequently analysed for the presence of bacterial endotoxin. Limulus amoebocyte lysate (LAL) assay is a calorimetric assay which is generally employed for quantification of endotoxin levels in lab reagents and cell culture media.

**Mycoplasma**

These microorganisms cannot be detected by microscope and affects the DC cell growth in media. They tend to compete with the nutrients present thus affect the timeline involved in DC proliferation. Commercially available MycoAlert kit is used for each sample to identify the presence of mycoplasma in the culture. This luminometric assay is performed as per manufactures guidelines and requires the use of culture supernatant. If present, the culture is decontaminated for 3 days by administration of antibiotics that specifically targets mycoplasma.

**Sterility**

Once the cells are isolated from the culture, they are sterile filtered by passing through microfilter to get rid of bacteria, fungi or any large macromolecules. Surface swabs from lab surface and equipment and air samples are collected from lab and cultured on agar plates monthly to identify the need for decontamination and sterilization.

**Viability (7AAD)**

Once thee cells are sterile filtered, the percentage viability of mature Dendritic Cell's (mDC) is recorded for each sample using 7AAD staining. An adequate viability of mDC's is essential for dose preparation and to safeguard an effective immune response against the cancer cells.



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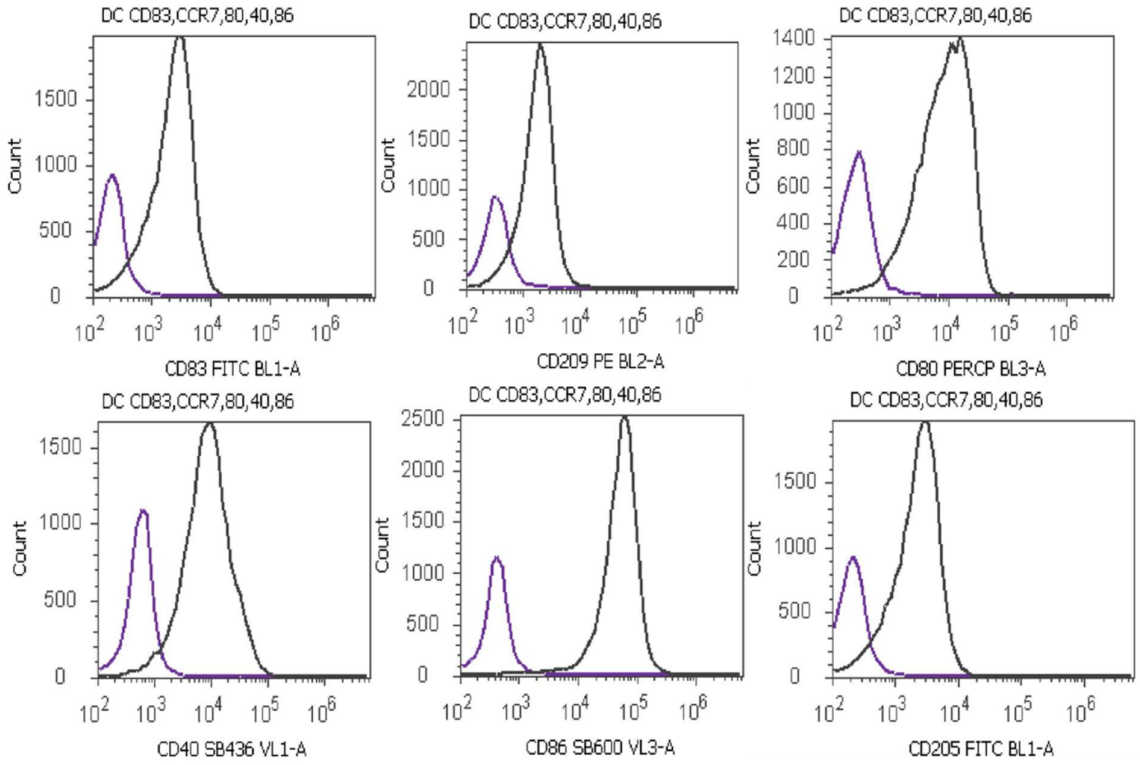


*Figure 2:* Analysis of cell surface markers expression on mDC following 8-day incubation using Flowcytometry.

**Surface Receptor / Phenotypic Studies: CD 40/80/83/86/205/209/HLA-DR**

Differentiation of monocytes to mDC's is analysed for each sample by quantification of cell phenotypic markers that are expressed only onto the surface of mDC's. Flow cytometry is employed for this procedure. Cells are stained with an optimised concentration of fluorescently labelled antibody that binds to the cell surface markers. The reports generated are saved, printed and delivered to the patient along with the dose (Figure 2).

**Migration Marker Studies: CCR7**

Cell migration is a central process in the development and maintenance of multicellular organisms. Vital process such as immune response requires the orchestrated movement of cells in particular directions to specific locations. Since cells migrate in response to stress signals such as chemical signals and mechanical signals, CCR7 is a key regulator that governs trafficking of DC under both inflammatory and steady-state conditions. Validation of expression of CCR7 is vital for cell maturation and generation of an effective dose.

**Intracellular marker: CD 208 (DC-LAMP)**

Besides phenotypic markers, intracellular markers are often quantified to ensure DC maturation and proliferation.

## Distribution-

Once all the production checkpoints are validated and the reports are approved by the product manager, the doses are carefully aliquoted into total of 12 ampoules (Figure 3) with each dose

*Figure 3:* A vial of APCEDEN® containing mDC's is ready for distribution and transfusion.

labelled with the patient's serial number. Each dose consists of 5-7 million DC's distributed in a set of 2 vials. 0.5 ml vial is for subcutaneous administration while 1.5ml vial is administered intravenously along with 100ml of saline. These doses are submerged in vapor phase liquid nitrogen and are shipped via cryo-tank to the delivery site along with the QC reports.

## Conclusion-

With diseases of great economic importance such as cancer, there is a need, for a mutual understanding between the therapy manufacturer and the control authority. In other words, FDA, ISO and QC departments of the government regularly guide our company so that all concerned bodies appreciate the consequences of a requirement for higher potency of treatment. It is essential to create therapy characteristics, safety profile and test parameters that link with the clinical quality, efficiency and efficacy of the product under strict and documented rigorous controls imposed by GMP. A great deal of attention is needed regarding the handling and organization of data of more novel and highly sensitive technologies along with patients' privacy and non-disclosure of personal information to any scientific body without their prior consent.



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