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GMP (Good Manufacturing Practice) and Validation in Biotechnology

In Special Consideration of Fermentation and Biotransfer Processes

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Process validation and registration in connection with GMP (Good Manufacturing Practice) and other related consumer protection and quality assurance measures are of importance for the biotechnological production of proteins and fine chemicals. GMP is increasingly applied to manufacturing steps upstream, such as fermentation. Thus, the aim of the conference is the identification and discussion of 'burning issues' with special consideration to validation and registration of manufacturing processes using fermentation technology for the production of proteins and fine chemicals.

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GMP and Biosafety Aspects in the Production of Recombinant IFN alpha-2a

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Introduction

Human Interferon α-A (hIFNα-A) is formed by leukocytes upon viral infections. It is one of the non-glycosylated cytokines of the human immune system. The size of hIFNα-A is 165 amino acids, 19300 D and contains 2 S–S bonds. These data show that this protein is predestinated to be produced in *E.coli*. *Roche*'s rIFNα-2a, trade name *ROFERON-A* has been introduced in the European and US market in 1986. There are many viral and cancer therapeutic areas, where rIFNα-2a is successfully applied, such as condyloma acuminata [1], hairy cell leucemia [2], hepatitis B and C, AIDS-related *Kaposi*'s sarcoma, renal cell cancer, *etc.*

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Production Process

rIFNα-2a has been produced in 1000-l working volume production scale, with two inoculum stages of 50 l and 0.75 l, respectively (*Fig.*). The production organism is *E. coli 294* (K12) and the expression plasmid is a derivative of pBR322 with a tetracycline resistance marker and tryptophane promotor control. This host-vector-system has been classified into biosafety containment level GILSP.

Biological Safety Aspects

It is important to note that worldwide no adverse effects have ever been observed due the application of recombinant organisms [4]. As a consequence it is difficult to supervise the health status of the personnel in a plant, because it is not possible to check for clearly defined symptomes. Despite this fact plant managers must guarantee the safety of the plant personnel [5]. Every production site has its own technical characteristics and features and hence its own, adapted safety instruments to guarantee biosafety requirements. These characteristics are the result of:

- international and national guidelines
- company organisation and culture
- history of the product and its development
- site, building, brand and size of plant
- personnel and its training and background.

Therefore, detailed biosafety measures, even for the same product, *cannot* be identical at different sites. In Switzerland each plant has to submit the 'Kurzbericht gemäss Störfallverordnung' to the authorities, where biological system, production plant and organisation have to be described [6]. In the fermentation process for rIFNα-2a *Roche* in Basel (*Fig.*), there are several safety measures in operation, the main goal

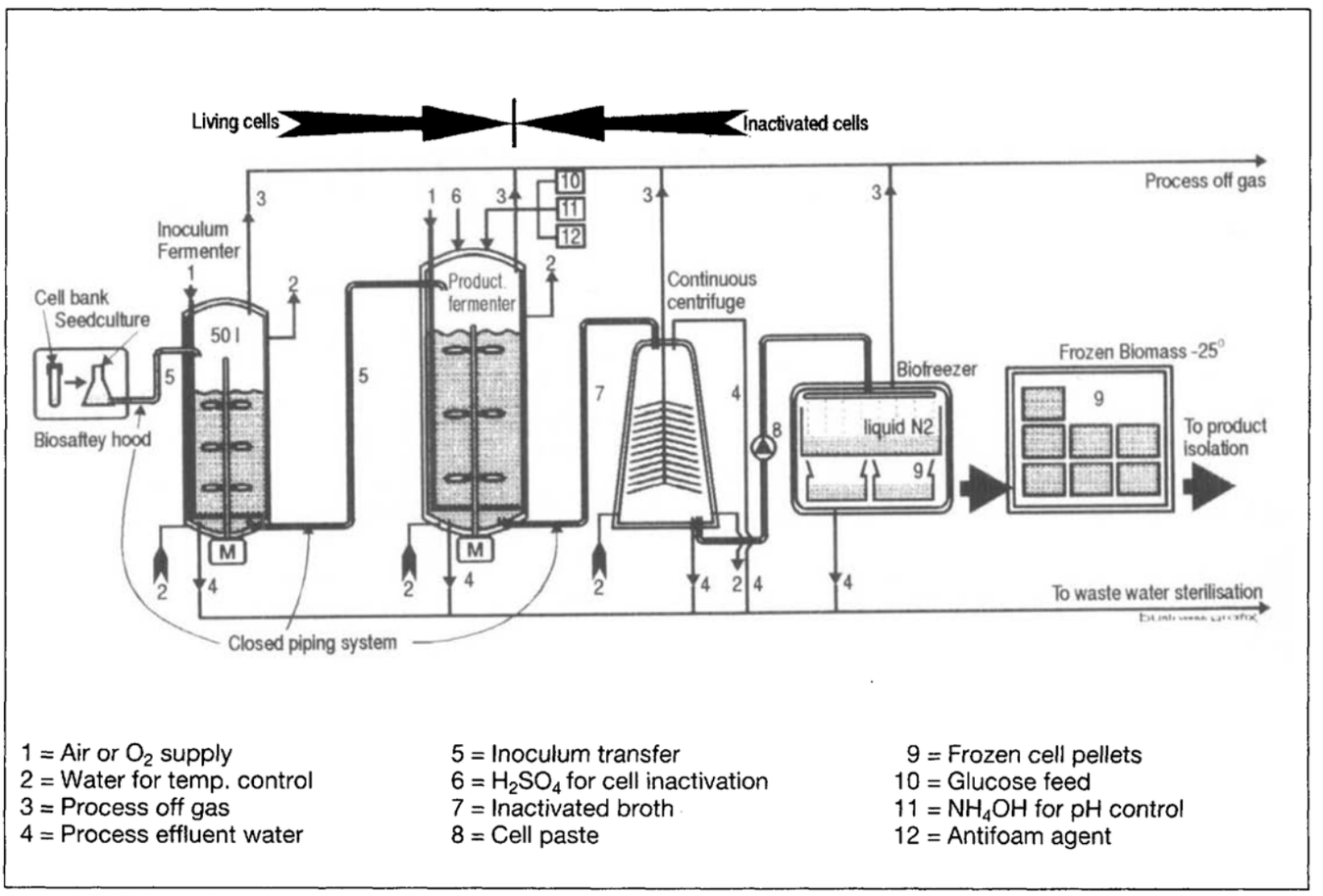

Figure. ROFERON-A *fermentation process and cell harvest scheme*

Table. *Biological and Product Safety in the rIFNα-2a Production*

	Biological Safety	Product Safety
Designation:	GILSP [3] (Good Industrial Large Scale Practice)	cGMP (current Good Manufacturing Practice)
Aim:	Protection of personnel and environment	Protection of product
Means:	*E. coli K12* pBR322 vector Safe and non toxic product Personnel training program	SOPs for process and analysis Hygiene and extended IPC Raw- and end-product controls Personnel training program

being to avoid exposition of personnel to *E. coli* and to prevent contamination of the product in a closed production system: Starting in a biohazard laminar flow hood (class 100), cell suspension of a working cell bank ampulle (stored in liquid N_2) is inoculated into a shake flask. The shake flask culture is run in a dedicated shaker apparatus, located adjacent to a 150 l (total volume) *inoculum* fermenter. At the end of the flask culture the cells are pumped with a peristaltic pump, while still on the shaker, through a presterilized tubing into the *inoculum* fermenter. From there, after incubation, 50 l are pressed by air-pressure through a steam-sterilized stainless steel pipe into the 1500 l production fermenter. At the end of the production culture, H_2SO_4 is used to inactivate the *E. coli* cells as well the rDNA. This inactivation was validated by PCR technique [5]. The inactivated cell suspension is then transfered by pressure into a continuous centrifuge. The supernatant is rejected and transfered into the waste-water sterilisation plant, while the biomass is directly transfered by gravity into a closed glass container. From here the biomass slurry is pumped by a peristaltic pump into the biofreezer apparatus [7], where it is frozen in liquid N_2 to small pellets, falling into alu-sacks (coated with *Teflon*) and sealed. The off-gas of the production fermenter is filtered by two 0.2-μ membrane fil-

ters in series, before it is released into the environment. Inlet and outlet filters are tested for integrity before every lot. All liquid wastes from the plant, *i.e.* condensates originating from sterilisation of fermenters, transfer pipes, autoclaves, centrifugation process and rinsing water from cleaning procedures are collected and heat sterilized batchwise for 30 min at 121° before release to the sewage plant.

Biosafety Measures Survey

Materials
- raw-material control (nutrients, cell-bank)
- biomass containing the product (to product extraction)
- liquid wastes – heat sterilisation – sewage plant
- solid wastes – heat sterilisation – combustion
- gaseous wastes – sterile filtration

Plant equipment
- maintenance plans for equipment and instruments
- validation and calibration plans for equipment and instruments
- computer and process controler validations
- cleaning validations of equipment

Personnel
- medical supervision at intervals
- training and information regularly
- hygiene assessments of plant, equipment and personnel
- written SOPs for all operations and analysis

All of the above mentioned points apply equally for cGMP production, thus indicating that product safety and biosafety do not exclude but support each other. There are FDA inspectors, who consider biosafety and product safety as one package.

Product Safety Aspects – cGMP Measures

Biotechnological products generally differ in size, structural elements and complexity from chemically sythetized drugs. As a consequence the end-product analysis of biological products alone are not as conclusive as for small compounds. In-process-control analysis, such as for rDNA, growth, product integrity and activity and for contaminations are, therefore, very important in order to guarantee safe and pure biotechnological products. In the fermentation process some points are often issues for discussions, because procedures and rationals are not clearly established and defined and it is sometimes very difficult to find appropriate analytical methods at all. Some points are listed below:

1. *Cell-bank validation* requirements. Here many points and even designations were rather confusing in the past. Therefore, newly revised recommendations have been elaborated by ICH [8].
2. Analytical assessment of *contaminations of the production culture* by foreign organisms are a sometimes questioned topic, because the normally used analytical methods are often not suited for such assessments and validations. In case of microbial cultures with high cell concentrations in the range of 10^8–10^{11}/ml and fast growing production populations, it is often difficult to find contaminant cells below 0.01%, amounting to 10^4–10^7/ml. This low percentage, however, still represents a rather high cell number and could be a thread to the product. Therefore, a case-by-case approach to detect contaminations has to be applied, based on experiences with contaminant cells occurring in the production environment. Contaminations generally occur from technical failures, such as broken valve membranes, O-rings, double mechanical seals, *etc.* In order to avoid such situations, a preincubation of the fermenter for some hours at fermentation conditions prior to inoculation might uncover weak points.
3. *Cross contaminations and change-over procedures* from one product or organism to another in multipurpose plants are other sensitive areas, because fermentors are defined as sterile vessels, which are heat sterilized, so that products and organisms are destroyed (DNA, proteins) and it becomes difficult to detect residual breakdown compounds of unknown structure. Specific case by case procedures have to be elaborated and validated.
4. *Cleaning procedures* of all equipment, such as fermenters, auxiliary tanks, transfer pipes, centrifuges, filters, *etc.* after each fermentation lot or after a production campain. These procedures again have to be designed on a case by case basis, considering specific ingredients, which can be detected on a low level.

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