

# srRNA: Potent Compound or Biosafety Program Management

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# Problem Statement

- A company is developing a self-replicating RNA therapy. They need a vendor who can sterile fill this therapy.
- You have been following the NIH rDNA guidelines, classifying the drug product as BSL-2.
- Sterile dose vendors expect worker safety classification using the OEB banding system. This is standard practice for chemical-based active pharmaceuticals.
- What can you do?

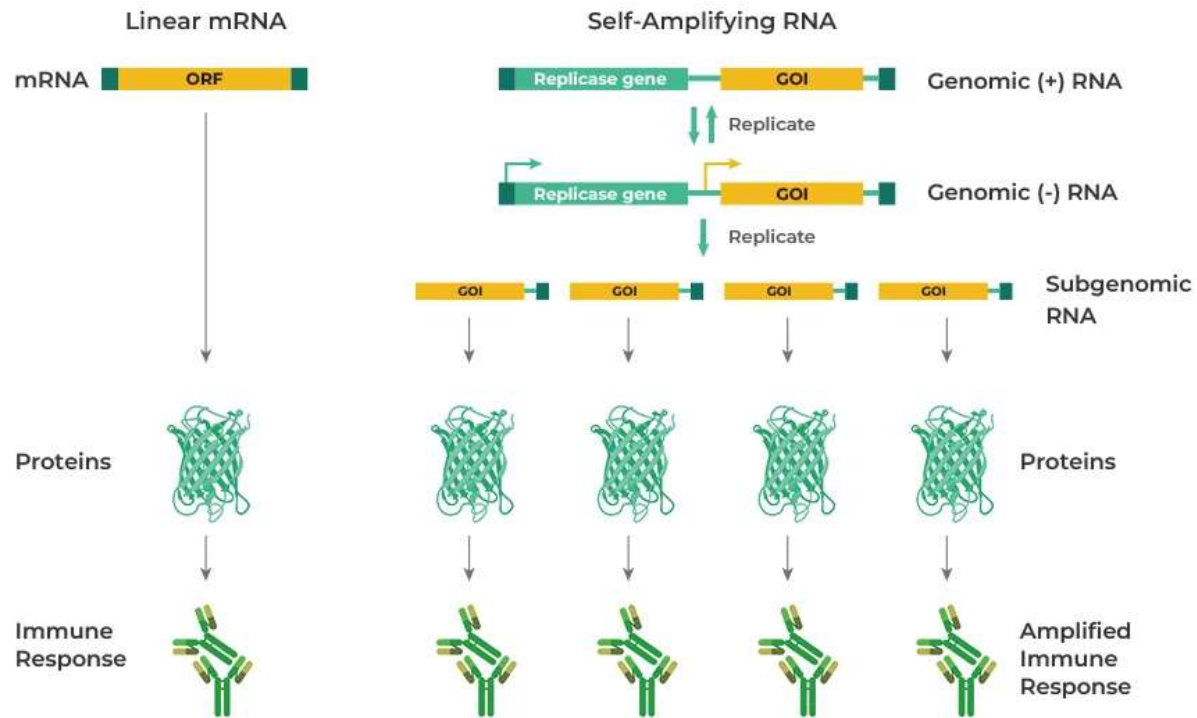
# Agenda

- What is self replicating RNA?
- How is it produced?
- Is it covered by the NIH rDNA Guidelines?
- Hybrid Biosafety-Industrial Hygiene Model
- Comparison of IH and Biosafety Protective Measures
- Review of Containment for Product Filling

# srRNA Composition

- srRNA
  - Alpha virus non-structural proteins (replicase)
  - Transgene
- Liposome
  - Sphere-shaped microscopic vesicles with the hydrophilic portion completely enclosed by one or more phospholipid bilayers

# mRNA versus Self-Replicating RNA





## How Long are srRNA systems active?



PROTEIN EXPRESSION-  
EXTENDED PERIOD.

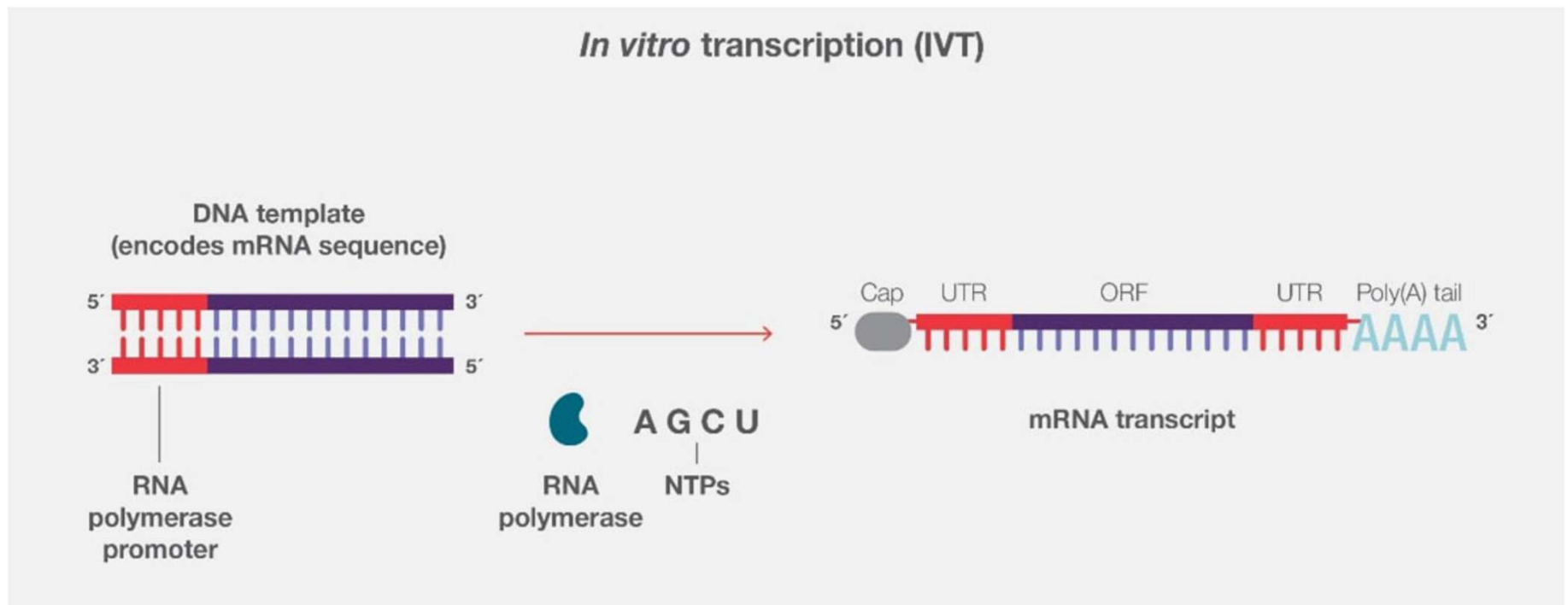


REPLICATION  
MACHINERY  
EXPRESSION- SHORTER  
EXPRESSION TIME, ~6  
HRS., THEN DEGRADED  
PER NORMAL HALF-  
LIFE.

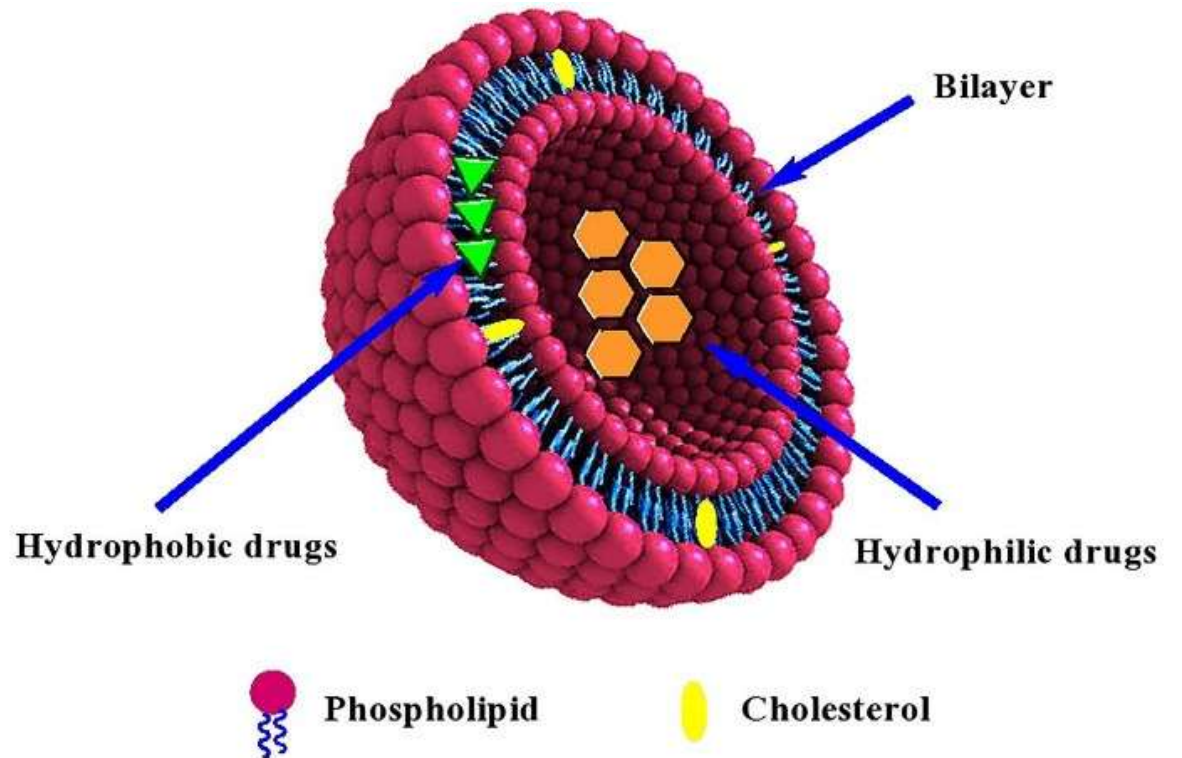


ANTIGEN  
PRODUCTION-  
USUALLY LASTS 5-7  
DAYS

# *In vitro* Transcription Manufacturing

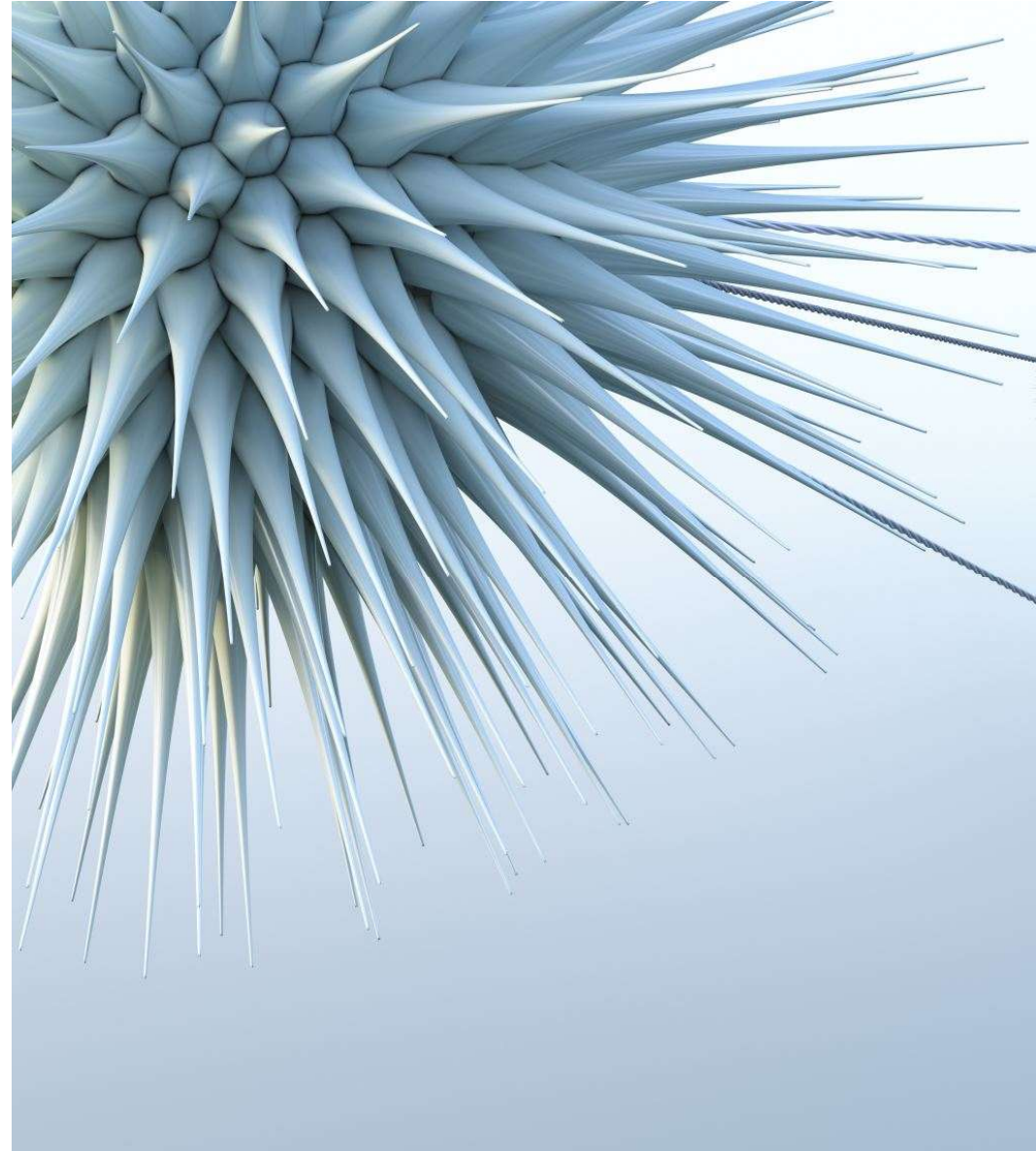


# Liposome- srRNA membrane



# Do srRNA systems infect cells?

- NO!
- Cellular entry is modulated by liposomes.
- Once srRNA enters a cell, antigen or protein production is limited to that cell.
- Affected cells do not release srRNA which can infect adjacent cells. srRNA does not act as a virus



# NIH rDNA Guidelines- Synthetic Nucleic Acid Molecules

- **Section III-E-1. Experiments Involving the Formation of Recombinant or Synthetic Nucleic Acid Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus**
- **Section III-C-1. Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants**
  - **Human gene transfer is the deliberate transfer into human research participants of either:**
    - **Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or**
    - **Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:**
      - **Contain more than 100 nucleotides; or**
      - **Possess biological properties that enable introduction of stable genetic modifications into the genome (e.g., cis elements involved in integration, gene editing); or**
      - **Have the potential to replicate in a cell; or**
      - **Can be translated or transcribed.**

# Hybrid Biosafety-IH Program

Material	Biological Safety/ Biorisk		Industrial Hygiene/ Toxicology	
	Program Management	Containment Level (e.g. BSL)	Program Management	Containment Level (OEB/ OEL)
Biological Agents & Materials	X	X		
Biologically Derived Molecules			X	X
Isolated Nucleic Acids (e.g., shRNA, miRNA or cDNA) delivered biologically (e.g. by a bacterial or viral vector system or by a plasmid)	X	X (Note: viral vector/plasmid containing the nucleic acid sequence & host cell will be assigned a containment level)		X (Note: the nucleic acid sequence will be assigned an OEB/OEL.
Isolated Nucleic Acids (e.g. siRNA, shRNA, miRNA)	X	Synthetic nucleotides fall under the NIH rDNA Guidelines	X	X
Biological Toxins <b>except</b> Regulated Toxins			X	X
Regulated Biological Toxins (e.g. Select Agents Toxins)	X	X		X

# Roles of Biosafety and Toxicology

## **Biosafety**

- Maintain compliance with NIH rDNA Guidelines
- Evaluate Risk Group and Biosafety Level

## **Toxicology**

- Evaluate chemical components (liposome, PEG, cholesterol)
- Evaluate transgene protein
- Assign OEB band for worker protection

# IH Controls

## CONTAINMENT CONTROLS

Exposure limits determine manufacturing safeguards

OCCUPATIONAL EXPOSURE LIMIT	BAND	PRODUCTION REQUIREMENTS
>1-10 mg/m <sup>3</sup>	1	Good manufacturing practices
>0.1-1 mg/m <sup>3</sup>	2	Good manufacturing practices (with local exhaust ventilation)
>0.01-0.1 mg/m <sup>3</sup>	3	Essentially no open handling (ventilated enclosures required)
>0.001-0.01 mg/m <sup>3</sup>	3+	Virtually no open handling (containment systems required)
≤0.001 mg/m <sup>3</sup>	4	No open handling (closed systems required)
≤0.001 mg/m <sup>3</sup>	5	No manual operations/human intervention (robotics or remote operations required)

SOURCE: Merck & Co.

# Biosafety vs. IH Controls

Biosafety Level 2 Controls and PPE	OEL 3 Controls and PPE
<p><b>Biological Safety Cabinet (BSC) Class II A2</b></p>	<p><b>Ventilated Balance Safety Enclosure</b></p>
<p>Open bench permitted if aerosol generation controlled. BSC Class 2 B2 if solvents are used.</p>	<p>Open bench permitted. Containment hood if solvents are used or aerosolization may occur</p>
<p><b>Decontamination procedure required</b></p>	<p><b>Decontamination procedure required</b></p>
<p>The laboratory supervisor ensures laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and appropriate records are maintained.</p>	<p>The laboratory supervisor ensures laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of chemicals, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and appropriate records are maintained.</p>
<p>Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials are housed or manipulated.</p>	<p>Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where chemicals are housed or manipulated.</p>

# Biosafety vs. IH Controls (cont.)

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<p>Sample transfer using tightly sealed container or zip-lock plastic bags</p>	<p>Sample transfer using tightly sealed container or zip-lock plastic bags</p>
<p>A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.</p>	<p>A sign is posted at the laboratory entrance indicating chemical risks. Posted information includes: chemical name, laboratory's OEL class, supervisor or other responsible personnel's name and telephone number, PPE requirements, and required procedures for entering and exiting the laboratory.</p>
<p>Persons wash their hands after working with potentially hazardous materials and before leaving the laboratory.</p>	<p>Persons wash their hands after working with potentially hazardous materials and before leaving the laboratory.</p>
<p>The risk assessment considers whether respiratory protection is needed for the work with hazardous materials. If needed, relevant staff are enrolled in a properly constituted respiratory protection program.</p>	<p>The risk assessment considers whether respiratory protection is needed for the work with hazardous materials. If needed, relevant staff are enrolled in a properly constituted respiratory protection program.</p>

# What OEB Band Should We Chose?

- OEB 3 best matches BSL-2
  - Aerosol control
  - Reduced open handling
  - Signage
  - PPE
- Containment for liposome component should be similar

# What OEB Band Should We Chose?

- Are there differences using the OEB system?
  - Yes
  - Evaluation of control measures uses wipe testing.
  - Without specific analytical methods non-toxic surrogates (mannitol, lactose) are used to determine if control measures are sufficient.
  - Similar testing methods are not normal practice in Biosafety

Sterile  
Isolator  
Filling Line



# Isolator Sterile Filling System

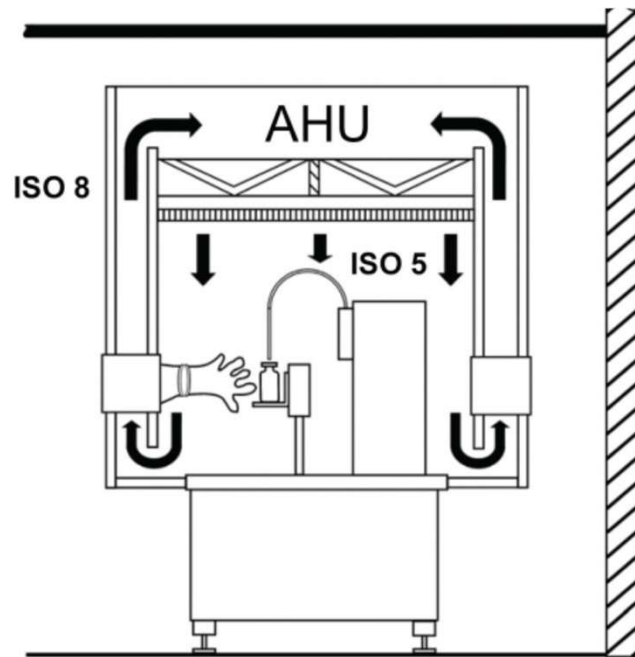
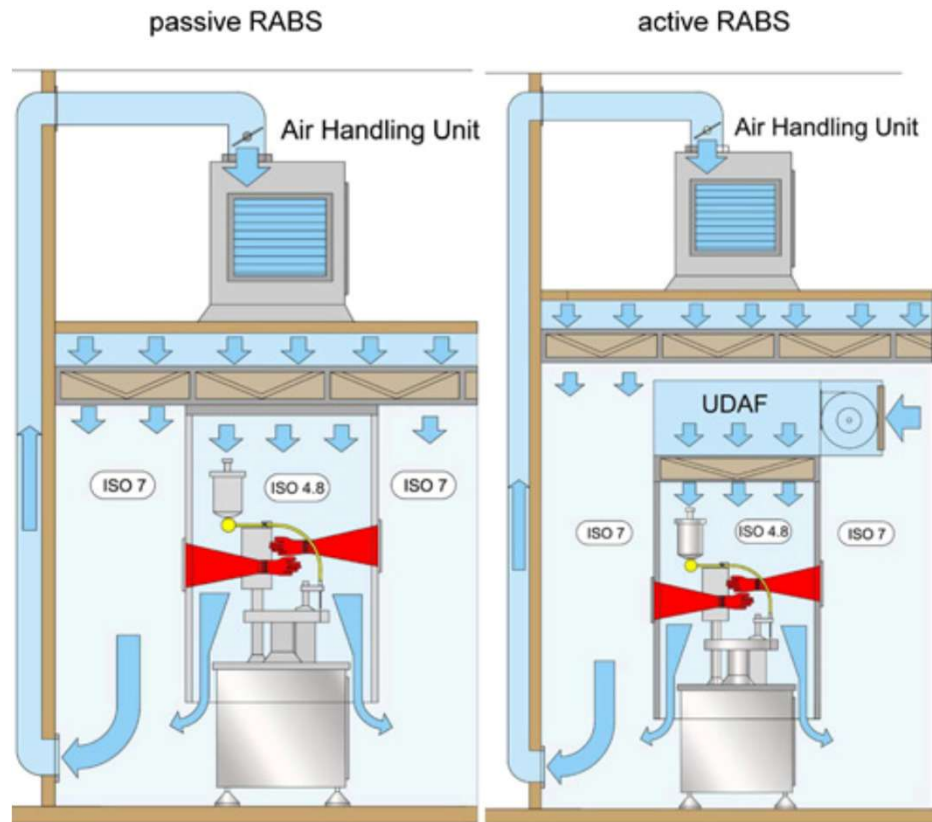


FIGURE 12.11

# Restricted Access Barrier System (RABS)



# Restricted Access Barrier System (RABS)

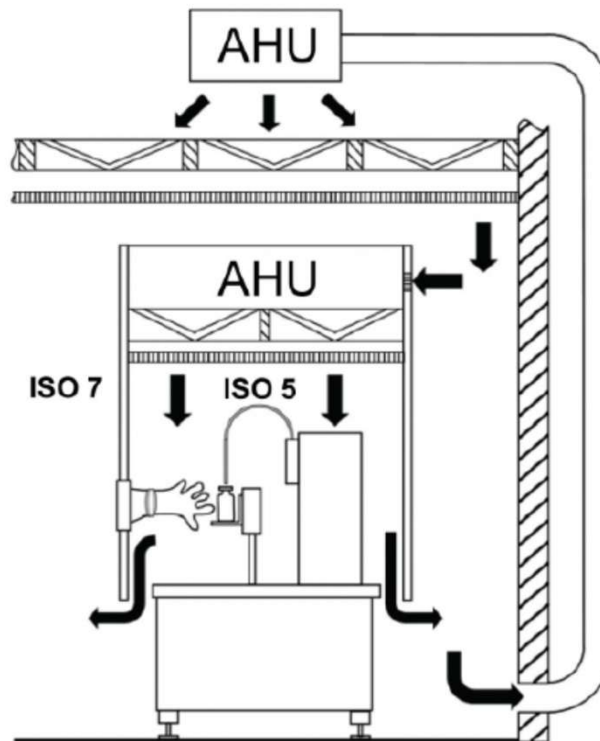


Figure 1. Basic RABS airflow diagram

# Comparison of RABS to Isolator

ISOLATORS	RABS
Closed system (product not exposed to external environment)	Open or closed
ISO 5 inside (unidirectional or turbulent flow)	Grade A; ISO 5 unidirectional airflow inside the critical area
Minimum background environment ISO 8 (Grade D)	Minimum background environment ISO 7 in operation (grade B) and ISO 5 (grade A in some cases)
Integrated decontamination system	Decontamination system into the room
Biodecontamination procedure using biologicals and chemical indicators (H <sub>2</sub> O <sub>2</sub> as bench mark process)	Validation procedure according to cleanroom standards
Entry to or exit from using closed transfer devices	Other transfer devices are possible; e.g. mobile LAF carts, open airlock, etc.
Prepared for handling highly potent or toxic products	Not for highly potent or toxic products
Leak tightness according to international standards ISO 14644-7/ ISO 10648-2. Pressure decay test before each cycle/batch	Airtightness typically not required; Unidirectional airflow as a dynamic barrier
Working at positive pressure or negative pressure or double design pressure (for example, cytotoxic product)	Working at a defined pressure (typically positive pressure)

# Sterile filling systems concerns

- Liquid filling system generate aerosols at high throughput speeds
- Isolators operate at positive pressure to maintain sterility.
- Isolators have low leakage rate (~1.5%).
- RABS exhaust sterile downflow air at waist level; exhaust captured by floor level returns