

Paper -V

SCIENCE AND TECHNOLOGY

VACCINES: IMMUNITY, FUNDAMENTAL CONCEPTS AND MODERN VACCINE PRODUCTION

Introduction to Immunity, Vaccination Principles, and Hepatitis B Vaccine Production with Indian Context

INTRODUCTION

Vaccines are biological preparations that stimulate the immune system to recognize and defend against specific pathogens without causing the disease itself. They represent one of the most successful public health interventions in history, preventing an estimated 2-3 million deaths annually worldwide.

PART 1: INTRODUCTION TO IMMUNITY

What is Immunity?

Immunity is the body's ability to resist infection and disease through the actions of specialized cells, proteins, and organs that recognize and eliminate foreign invaders (pathogens) like bacteria, viruses, fungi, and parasites.

Types of Immunity

1. Innate Immunity (Natural/Non-specific)

- Present at birth; first line of defense
- Does not target specific pathogens; responds similarly to all invaders
- Components:
 - Physical barriers: Skin, mucous membranes, stomach acid
 - Cellular defenses: Phagocytes (macrophages, neutrophils), natural killer (NK) cells
 - Chemical defenses: Complement proteins, interferons, inflammatory mediators
- Response is rapid (minutes to hours) but not long-lasting or memory-based

2. Adaptive Immunity (Acquired/Specific)

- Develops after exposure to specific pathogens or vaccines
- Highly specific to particular antigens (molecular signatures of pathogens)
- Components:
 - Humoral immunity: B-lymphocytes produce antibodies that neutralize pathogens in blood and body fluids
 - Cell-mediated immunity: T-lymphocytes directly kill infected cells or help coordinate immune responses
- Response is slower (days to weeks) initially but creates immunological memory for faster, stronger responses upon re-exposure.

	Innate Immunity	Acquired Immunity
Origin	Present at birth	Develops after exposure to antigen
Specificity	Non-specific; responds similarly to all pathogens	Highly specific to particular antigens
Memory	No immunological memory	Creates memory B and T cells for faster future responses
Response Time	Rapid (minutes to hours)	Slower initially (days to weeks), but faster upon re-exposure
Components	Skin, mucous membranes, phagocytes, complement proteins	B-lymphocytes (antibodies), T-lymphocytes, memory cells
Examples	Skin barrier against bacteria; fever during infection	Antibodies after measles infection; protection from Hepatitis B vaccine

Types of Adaptive Immunity

1. Active Immunity

- Body produces its own antibodies.
- Long-lasting protection.

Examples:

- Natural infection (e.g., chickenpox)
- Vaccination

2. Passive Immunity

- Antibodies are transferred from another source.
- Immediate but temporary protection.

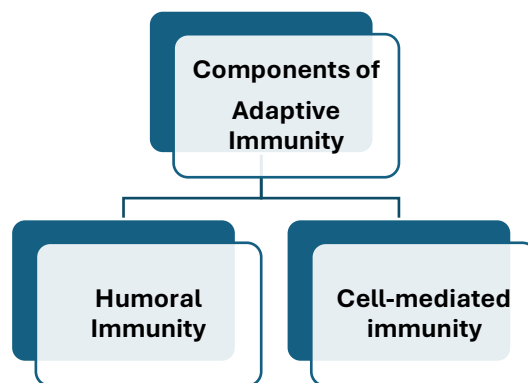
Examples:

- Maternal antibodies through placenta and breast milk
- Antisera and immunoglobulin injections .

Active vs Passive Immunity

Type	Source	Onset	Duration	Example
Active Immunity	Body produces its own antibodies after exposure to antigen	Slow (days-weeks)	Long-lasting (years-lifetime)	Natural infection; vaccination
Passive Immunity	Pre-formed antibodies transferred from another source	Immediate	Short-term (weeks-months)	Maternal antibodies via placenta/breast milk; antivenom; monoclonal antibody therapy

Components of Adaptive Immunity



Humoral Immunity

Humoral immunity is a type of **adaptive (acquired) immunity** mediated by **B-lymphocytes (B-cells)** and the **antibodies** they produce. It protects the body primarily against **extracellular pathogens** such as bacteria, viruses before cell entry, and toxins present in body fluids (blood and lymph).

Mechanism of Humoral Immunity

1. Entry of Antigen

A foreign substance (antigen) such as a bacterium, virus, or toxin enters the body.

Functions of Humoral Immunity

1. Neutralization of toxins and viruses.
2. Agglutination (clumping) of pathogens.
3. Opsonization (enhancing phagocytosis).
4. Activation of complement proteins.
5. Formation of immunological memory.

Examples

- Protection after **Hepatitis B vaccination**.
- Antibody production following **measles vaccination**.
- Natural immunity after recovering from diseases like chickenpox.

Cell-mediated immunity

Cell-mediated immunity is a type of **adaptive (acquired) immunity** mediated primarily by **T-lymphocytes (T-cells)** rather than antibodies. It is particularly effective against **intracellular pathogens** such as viruses, certain bacteria, fungi, parasites, and abnormal cells including cancer cells.

Mechanism of Cell-Mediated Immunity

1. Antigen Entry and Processing

- A pathogen infects body cells.
- Infected cells or antigen-presenting cells (APCs) process the antigen and display it on their surface using Major Histocompatibility Complex (MHC) molecules.

2. Activation of T-Cells

- Helper T-cells (CD4⁺) recognize the antigen-MHC complex.
- They release cytokines that activate other immune cells.

3. Clonal Expansion

- Activated T-cells multiply rapidly to produce a large number of identical cells.

4. Effector Functions

A. Cytotoxic T-Cells (CD8⁺)

- Directly destroy infected, cancerous, or foreign cells.
- Release perforins and granzymes that induce cell death.

B. Helper T-Cells (CD4⁺)

- Stimulate B-cells, macrophages, and cytotoxic T-cells.
- Coordinate the immune response.

C. Memory T-Cells

- Persist for long periods.
- Provide rapid response upon re-exposure to the same antigen.

Functions of Cell-Mediated Immunity

1. Destruction of virus-infected cells.
2. Elimination of intracellular bacteria (e.g., tuberculosis).
3. Rejection of transplanted organs.
4. Surveillance and destruction of cancer cells.
5. Regulation and coordination of immune responses.

Types of T-Lymphocytes

T-Cell Type	Function
Helper T-cells (CD4 ⁺)	Activate and regulate immune responses
Cytotoxic T-cells (CD8 ⁺)	Kill infected and abnormal cells
Regulatory T-cells	Suppress excessive immune reactions
Memory T-cells	Provide long-term immunity

Examples of Cell-Mediated Immunity

- Immune response against influenza virus-infected cells.
- Defense against tuberculosis.
- Rejection of transplanted organs.
- Protection induced by certain vaccines through T-cell memory.

Humoral Immunity vs Cell-Mediated Immunity

Feature	Humoral Immunity	Cell-Mediated Immunity
Main Cells	B-lymphocytes	T-lymphocytes
Effector Molecule	Antibodies	Cytotoxic T-cells
Target	Extracellular pathogens	Intracellular pathogens
Memory	Present	Present
Example	Protection by vaccines	Defense against virus-infected cells

Immunological Memory: The Basis of Vaccination

- After first exposure to a pathogen or vaccine, the immune system generates:
 - Effector cells: Fight the current infection
 - Memory B and T cells: Long-lived cells that "remember" the specific antigen
- Upon re-exposure, memory cells rapidly activate, producing a faster, stronger, and more effective response
- This is why vaccines can prevent disease without causing illness: they safely create memory without the risks of natural infection

Key Immune Cells and Molecules

- **B-lymphocytes (B cells):** Produce antibodies; differentiate into plasma cells (antibody factories) and memory B cells
- **T-lymphocytes (T cells):**
 - Helper T cells (CD4+): Coordinate immune responses by activating B cells and other T cells
 - Cytotoxic T cells (CD8+): Directly kill virus-infected or cancerous cells
- **Antibodies (Immunoglobulins):** Y-shaped proteins that bind specific antigens; neutralize pathogens, mark them for destruction, or activate complement
- **Antigen-Presenting Cells (APCs):** Dendritic cells, macrophages that process and display antigens to activate T cells
- **Cytokines:** Signaling molecules that regulate immune cell communication and activity

PART 2: FUNDAMENTAL CONCEPTS IN VACCINATION

What is a Vaccine and How Does It Work?

A vaccine is a biological preparation containing antigens (or instructions to make antigens) from a pathogen, administered to stimulate the immune system to develop adaptive immunity and immunological memory without causing the actual disease.

Key Terminology

- **Antigen:** Foreign substance that triggers immune response
- **Antibody:** Protein produced by B cells that specifically binds to antigens
- **Memory cells:** Long-lived B and T lymphocytes that enable rapid response upon re-exposure
- **Active immunization:** Body produces its own antibodies after vaccine exposure
- **Passive immunization:** Pre-formed antibodies transferred from another source (e.g., maternal antibodies, antivenom)

Edward Jenner and the Birth of Vaccination

- **1796:** Edward Jenner (father of vaccination) inoculated 9-year-old James Phipps with cowpox material from milkmaid Sarah Nelmes
- **Observation:** Boy did not develop smallpox upon subsequent exposure
- **Principle:** Exposure to a related, less virulent pathogen (cowpox) confers immunity to a more dangerous one (smallpox)
- **Legacy:** Term "vaccine" derived from Latin "vacca" (cow); foundation of modern immunology

MECHANISM OF VACCINATION

How Vaccines Work: Step-by-Step

1. Antigen Introduction

- Vaccine delivers harmless form of pathogen (attenuated, killed, subunit, mRNA, or DNA)
- Antigen-presenting cells (dendritic cells, macrophages) capture and process antigens

2. Immune Activation

- Processed antigens displayed on MHC molecules to activate helper T cells (CD4+)
- Helper T cells activate B cells to produce specific antibodies (humoral immunity)

- Cytotoxic T cells (CD8+) activated to kill infected cells (cell-mediated immunity)

3. Memory Formation

- Some activated B and T cells differentiate into long-lived memory cells
- Memory cells persist for years to decades, providing rapid response upon re-exposure

4. Protective Immunity

- Upon future pathogen encounter, memory cells quickly expand
- Antibodies neutralize pathogens; cytotoxic T cells eliminate infected cells
- Disease prevented or significantly attenuated

Types of Vaccines

CLASSIFICATION OF VACCINES

I. LIVE ATTENUATED VACCINES

Concept: Pathogens weakened (attenuated) to reduce virulence while retaining antigenicity and ability to replicate.

Methods of Attenuation:

- Serial passage in non-human cells or at suboptimal temperatures (e.g., rabies in chick embryo)
- Chemical treatment (formaldehyde) or UV irradiation to induce mutations
- Genetic engineering to delete virulence genes

Examples:

- Viral: MMR (measles, mumps, rubella), Oral Polio Vaccine (OPV/Sabin), Yellow fever, Rotavirus
- Bacterial: BCG (tuberculosis), Typhoid (Ty21a oral)

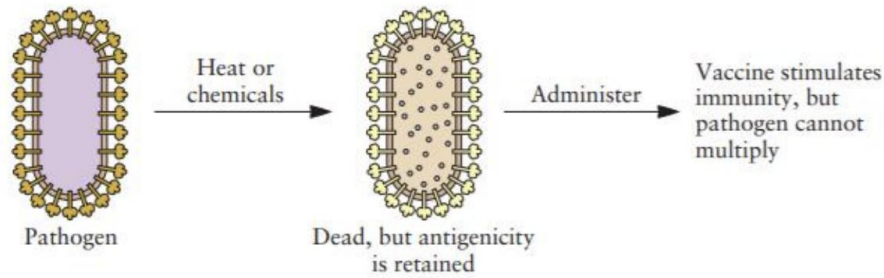
Advantages:

- Strong, long-lasting immunity (often single dose)
- Induces both humoral and cell-mediated immunity
- Mimics natural infection without disease risk (in immunocompetent individuals)
- No adjuvant typically required

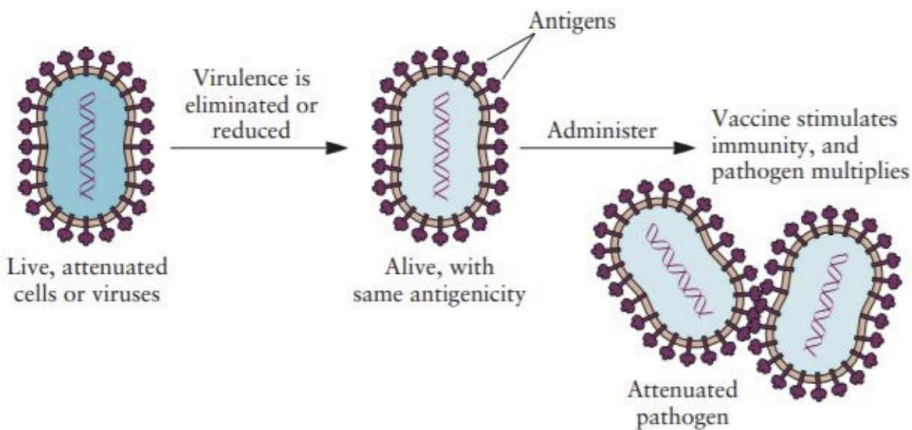
Disadvantages:

- Risk of reversion to virulence (rare but serious; e.g., vaccine-derived poliovirus)
- Contraindicated in immunocompromised individuals, pregnant women

A Heat-killed or formalin-inactivated vaccine



B Live attenuated vaccine



Difference Between Live Attenuated Vaccines and Killed (Inactivated) Vaccines

Feature	Live Attenuated Vaccines	Killed (Inactivated) Vaccines
Nature	Contain live but weakened (attenuated) microorganisms	Contain dead/inactivated microorganisms
Dose Administered	Usually single dose sufficient	Multiple doses and boosters required
Stability	Less stable	More stable
Immunity Induction	Induce both humoral and cell-mediated immunity	Mainly induce humoral (antibody-mediated) immunity
Cell-Mediated Immunity	Good	Poor
Duration of Immunity	Long-lasting (many years)	Relatively short-lived
Antibody Response	Mainly IgG and strong mucosal immunity (IgA in some cases)	Mainly IgG antibodies

Need of Adjuvant	Usually not required	Usually required
Reversion to Virulence	Possible (rare)	Not possible
Immune Response	Strong and resembles natural infection	Comparatively weaker
Production Method	Virulent organisms are attenuated by prolonged culture, passage through different hosts, or adverse growth conditions	Virulent organisms are killed/inactivated using heat, formaldehyde, alkylating agents, or radiation
Safety	Not suitable for immunocompromised individuals	Safer for immunocompromised individuals
Cost	Generally lower	Relatively higher due to repeated doses
Storage Requirements	Require careful cold-chain maintenance	Easier storage and transportation
Examples	BCG, Measles vaccine, MMR vaccine, Oral Polio Vaccine (Sabin)	Cholera vaccine, Pertussis vaccine, Anthrax vaccine, Inactivated Polio Vaccine (Salk), Rabies vaccine

III. SUBUNIT, RECOMBINANT, POLYSACCHARIDE, AND CONJUGATE VACCINES

Concept: Contain specific pathogen components (proteins, sugars) rather than whole organisms.

Types:

- **Protein subunit:** Purified antigenic proteins (e.g., Hepatitis B surface antigen – HBsAg)
- **Recombinant:** Antigen produced via rDNA technology in yeast, bacteria, or mammalian cells
- **Polysaccharide:** Capsular sugars from bacteria (e.g., Pneumococcal polysaccharide vaccine – PPSV23)
- **Conjugate:** Polysaccharide linked to protein carrier to enhance immune response in infants (e.g., Hib, PCV13)

Examples:

- Hepatitis B vaccine (recombinant HBsAg in yeast)
- HPV vaccine (virus-like particles of L1 protein)

- Acellular pertussis vaccine (purified pertussis toxins)
- Pneumococcal conjugate vaccine (PCV13)

Advantages:

- Highly safe; no risk of infection
- Targeted immune response; fewer side effects
- Suitable for immunocompromised individuals

Disadvantages:

- May require adjuvants and multiple doses
- Complex manufacturing; higher cost
- Primarily induce humoral immunity; weaker cell-mediated response

IV. TOXOID VACCINES

Concept: Inactivated bacterial toxins (toxoids) that induce immunity against toxin-mediated diseases.

Examples: Tetanus toxoid, Diphtheria toxoid (part of DPT/DTaP)

Advantages: Highly effective against diseases where pathology is toxin-mediated

Disadvantages: Do not prevent bacterial colonization; require boosters

V. MODERN VACCINE PLATFORMS

1. Viral Vector Vaccines

- Use harmless virus (adenovirus, VSV) to deliver pathogen antigen genes
- Examples: Covishield (Oxford-AstraZeneca COVID-19), Ebola vaccine (rVSV-ZEBOV)
- Advantages: Strong cellular/humoral immunity; rapid development
- Limitations: Pre-existing vector immunity may reduce efficacy; rare adverse events

2. mRNA Vaccines

- Contain mRNA encoding pathogen antigen; host cells produce antigen to trigger immunity
- Examples: Pfizer-BioNTech, Moderna COVID-19 vaccines; GEMCOVAC-OM (India)
- Advantages: Rapid design/production; no live virus; easily updated for variants
- Limitations: Ultra-cold chain requirements; limited long-term safety data

3. DNA Vaccines

- Contain plasmid DNA encoding antigen; host cells express antigen to stimulate immunity
- Example: ZyCoV-D (India; world's first DNA vaccine approved for human use)
- Advantages: Stable at higher temperatures; simple manufacturing; potential for multiplexing
- Limitations: Lower immunogenicity in humans; requires specialized delivery (needle-free injector)

4. Virus-Like Particle (VLP) Vaccines

- Self-assembling protein structures mimicking virus exterior but lacking genetic material
- Examples: HPV vaccine (Gardasil, Cervavac), Hepatitis B vaccine (also recombinant subunit)
- Advantages: Highly immunogenic; safe; stable
- Limitations: Complex manufacturing; may require adjuvants

PRODUCTION OF VACCINES: GENERAL STEPS

1. Strain Selection and Seed Lot Preparation

- Select pathogen strain with desired antigenic properties
- Create master and working seed lots under controlled conditions

2. Bulk Antigen Production

- Culture pathogen or host cells (yeast, bacteria, mammalian) in bioreactors
- Control pH, temperature, aeration, nutrients for optimal yield

3. Harvesting and Purification

- Separate antigen from culture medium via centrifugation, filtration, chromatography
- Remove contaminants: host cell proteins, DNA, endotoxins

4. Inactivation/Attenuation (if applicable)

- Kill pathogen (heat, formaldehyde) or attenuate via serial passage/genetic modification

5. Formulation

- Combine antigen with adjuvants (aluminum salts), stabilizers (sugars, amino acids), preservatives
- Adjust pH, osmolarity; ensure sterility

6. Fill-Finish

- Aseptically fill vials or pre-filled syringes under GMP conditions

- Label with batch number, expiry date, storage conditions

7. Quality Control

- Identity: Confirm antigen is correct (immunoassays, sequencing)
- Purity: <10 ppm host DNA, <1% host cell proteins, no endotoxins
- Potency: Animal studies to confirm antibody induction
- Sterility: Absence of bacteria, fungi, mycoplasma
- Safety: No residual reagents above limits

8. Distribution

- Cold chain maintenance (2-8°C for most; -70°C for some mRNA vaccines)
- Vaccine vial monitors (VVMs) track temperature exposure

Key Vaccination Concepts

Herd Immunity (Community Immunity)

- When a sufficiently high proportion of a population is immune (through vaccination or prior infection), transmission of the pathogen is interrupted, protecting even unvaccinated individuals
- Threshold varies by disease: Measles requires ~95% immunity; Polio ~80%; SARS-CoV-2 varies by variant
- Critical for protecting those who cannot be vaccinated (infants, immunocompromised, allergic individuals)

Vaccine Efficacy vs Effectiveness

- Efficacy: Performance under ideal, controlled conditions (clinical trials); e.g., "95% efficacy" means 95% reduction in disease among vaccinated vs unvaccinated in trial
- Effectiveness: Performance in real-world conditions; may be lower due to variants, storage issues, population differences
- Both are important for policy decisions and public communication

Primary Series and Booster Doses

- Primary series: Initial doses required to establish protective immunity (e.g., 2 doses for most COVID vaccines, 3 doses for Hepatitis B)
- Booster doses: Additional doses given later to restore waning immunity or broaden protection against variants

PART 3: PRODUCTION OF MODERN VACCINES – FOCUS ON HEPATITIS B VACCINE

Evolution of Vaccine Production

- First generation: Live attenuated or inactivated whole-pathogen vaccines (e.g., smallpox, polio)
- Second generation: Subunit, toxoid, and conjugate vaccines using purified components
- Third generation: **Recombinant DNA, viral vector, mRNA, and DNA vaccines** using genetic engineering

Recombinant DNA Technology in Vaccine Production

A **recombinant vaccine** is a vaccine produced using **recombinant DNA (rDNA) technology**, in which the gene encoding a pathogen's antigen is inserted into a host organism (such as yeast, bacteria, or mammalian cells). The host then produces the antigen, which is purified and used as a vaccine.

Unlike conventional vaccines, recombinant vaccines do **not contain the whole pathogen**.

Immune Response

After vaccination:

Humoral Immunity

- B-cells produce antibodies against the antigen.

Cell-Mediated Immunity

- T-cells recognize antigen-presenting cells and develop immune memory.

Immunological Memory

- Memory B and T cells provide long-term protection.

Examples of Recombinant Vaccines

Vaccine	Antigen Produced
Hepatitis B Vaccine	HBsAg
HPV Vaccine	L1 capsid protein
Recombinant Influenza Vaccine	Hemagglutinin protein
Some COVID-19 Protein Subunit Vaccines	Spike protein

Advantages of Recombinant Vaccines

Advantage	Explanation
High Safety	No live pathogen used
No Risk of Infection	Cannot cause disease
High Purity	Contains only specific antigen
Large-Scale Production	Easy industrial production
Consistent Quality	Uniform vaccine batches
Reduced Side Effects	Fewer contaminants
Suitable for Immunocompromised Patients	Safer than live vaccines

Disadvantages of Recombinant Vaccines

Disadvantage	Explanation
Requires Adjuvants	Antigen alone may be less immunogenic
Booster Doses Needed	Immunity may decline over time
Higher Production Cost	Requires advanced biotechnology facilities
Complex Manufacturing	Multiple purification and quality-control steps

Recombinant Vaccines vs Conventional Vaccines

Feature	Recombinant Vaccines	Conventional Vaccines
Pathogen Used	No whole pathogen	Whole pathogen often used
Safety	Very high	Moderate to high
Risk of Infection	Absent	Possible in live vaccines
Purity	High	Lower
Production Method	Genetic engineering	Culturing pathogens
Reversion to Virulence	Impossible	Possible in live vaccines

Production of Hepatitis B Vaccine

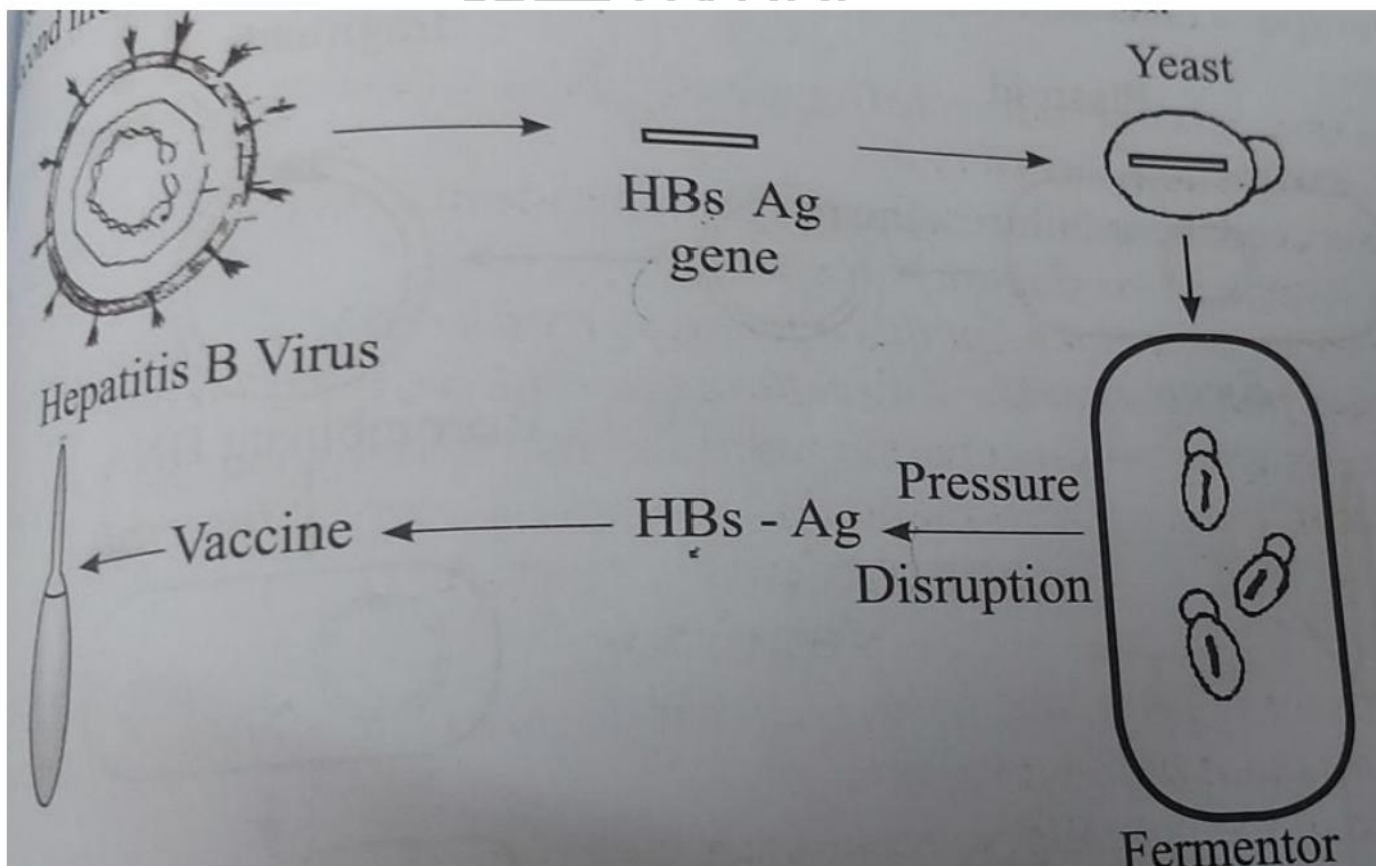
Background:

- [Hepatitis B vaccines](#) were the first recombinant Virus-like particles (VLPs) based vaccines and were introduced for human use in 1986.
- Hepatitis B virus (HBV) is a 42 nm double-shelled DNA virus of the [Hepadnaviridae](#) family that replicates in the liver and can cause [hepatic dysfunction](#), cirrhosis and cancer in chronically infected and symptomatic carriers.
- Modern recombinant HBV vaccines (since 1986) use yeast cells to produce HBsAg, eliminating safety concerns and enabling mass production.

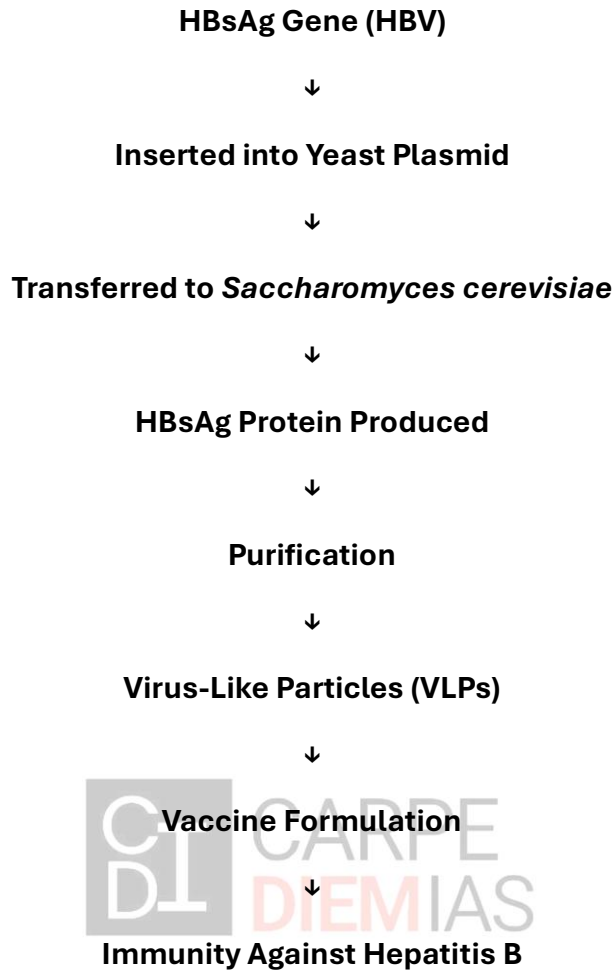
Two types of hepatitis B vaccine are commercially available:

- [Plasma-derived hepatitis B vaccine](#) and
- [Yeast recombinant hepatitis B virus vaccine](#).

The two vaccines are equally immunogenic, protective, and safe. However, in most countries the [recombinant vaccine](#) is considered the vaccine of choice.



Process



Recombinant Hepatitis B Vaccine Production

1. **Gene Isolation:** HBsAg gene isolated from HBV DNA or synthesized
2. **Vector Construction:** Gene inserted into yeast plasmid under strong promoter (alcohol dehydrogenase)
3. **Yeast Transformation:** Recombinant plasmid introduced into *Saccharomyces cerevisiae*
4. **Fermentation:** Transformed yeast grown in bioreactors; HBsAg produced and self-assembles into 22-nm virus-like particles (VLPs)
5. **Purification:** Chromatography, ultrafiltration to isolate HBsAg VLPs; remove yeast contaminants
6. **Formulation:** HBsAg + aluminum hydroxide adjuvant + stabilizers
7. **Quality Control:** Confirm identity, purity, potency, sterility, safety
8. **Distribution:** Cold chain (2-8°C) to vaccination centers

Advantages

- **Safety:** No live pathogen; zero infection risk from vaccine
- **Purity:** Only protective antigen; minimal contaminants
- **Scalability:** Fermentation easily scaled for mass production
- **Consistency:** Uniform, high-quality antigen batches
- **Adaptability:** Rapid redesign for new pathogens/variants

Indian Examples

- **Shanvac-B** (Serum Institute): Recombinant Hepatitis B vaccine; WHO-prequalified
- **Cervavac** (Serum Institute): Indigenous HPV vaccine (VLP); launched 2023; included in UIP
- **Corbevax** (Biological E): Recombinant RBD-based COVID-19 vaccine; yeast expression

PLASMA-DERIVED HEPATITIS B VACCINE

Concept, Production, Safety, Transition to Recombinant Vaccines,

Plasma-derived hepatitis B vaccine was the first-generation vaccine against Hepatitis B virus (HBV), developed in the early 1980s before recombinant DNA technology enabled safer, scalable production. It was prepared from the plasma of chronic HBsAg carriers, containing purified, inactivated 22-nm hepatitis B surface antigen (HBsAg) particles adsorbed onto an aluminum adjuvant. While historically significant and proven safe through extensive surveillance, it has been largely replaced by recombinant HBsAg vaccines produced in yeast since the mid-1980s. Understanding this transition is essential for appreciating advances in vaccine technology, biosafety, and India's role in global vaccine supply.

CONCEPT AND PRODUCTION PROCESS

Basic Principle

- Chronic HBV carriers have high levels of HBsAg in their blood (up to 500 µg/mL)
- Plasma from these donors is collected, pooled, and processed to purify HBsAg particles
- Purified HBsAg self-assembles into 22-nm virus-like particles (VLPs) that mimic native virus structure but lack viral DNA, making them non-infectious
- Particles are inactivated, formulated with aluminum adjuvant, and packaged as vaccine

Step-by-Step Production

Step 1: Donor Selection and Plasma Collection

Step 2: Viral Inactivation and Purification

Step 3: Formulation and Quality Control

Step 4: Fill-Finish and Distribution

Historical Use and Transition

- India initially imported plasma-derived HBV vaccines in the 1980s for high-risk groups (healthcare workers, dialysis patients)
- **1997:** India launched its **first indigenous recombinant HBV vaccine** (Shanvac-B by Shantha Biotechnics, later acquired by Sanofi)
 - Produced in yeast using recombinant DNA technology
 - One-third the cost of imported vaccines; WHO-prequalified for global procurement
- **Universal Immunization Programme (UIP):** Hepatitis B vaccine introduced nationwide in 2007-08 as part of pentavalent vaccine (DPT-HepB-Hib)
 - Uses recombinant HBsAg produced in yeast (multiple Indian manufacturers)
 - Coverage: >90% of infants receive three doses by 12 months of age

Why Replace Plasma-Derived Vaccine?

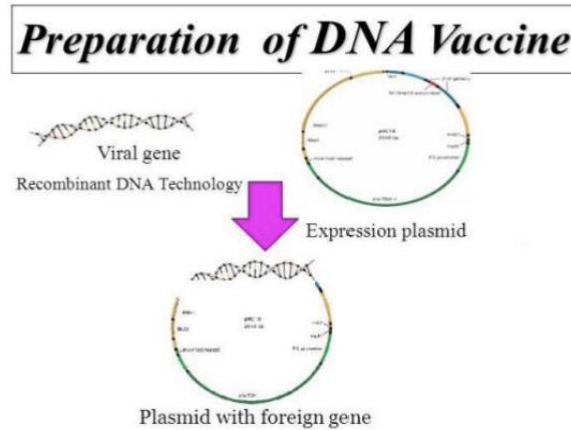
1. **Safety Perception:** Despite proven safety, public concern about using plasma from infected individuals persisted
2. **Scalability:** Limited supply of HBsAg-positive plasma; recombinant technology enables unlimited production
3. **Consistency:** Biological variability in plasma donors vs. controlled fermentation in yeast
4. **Cost:** Recombinant production became more economical at scale

Ethical Considerations: Avoid reliance on human plasma from potentially vulnerable donor populations

DNA VACCINES: CONCEPT AND PRODUCTION

DNA vaccination is a technique for protecting an organism against disease by injecting it with genetically engineered DNA to produce an immunological response.

These vaccines contain DNA that codes for specific proteins (antigens) from a pathogen. DNA vaccines have emerged as a safer alternative to standard live and inactivated vaccines for treating human and animal viral **diseases**.



Schematic representation of DNA vaccine preparation

Production Steps

1. **Plasmid Construction:** Antigen gene inserted into bacterial plasmid with promoter, poly-A signal, selectable marker
2. **Bacterial Transformation:** Plasmid introduced into *E. coli*; grown in fermenters to amplify plasmid copies
3. **Plasmid Purification:** Separate circular plasmid from bacterial DNA via alkaline lysis, chromatography, filtration
4. **Formulation:** Plasmid DNA + stabilizers (e.g., sucrose) + delivery enhancers (e.g., electroporation buffer)
5. **Quality Control:** Confirm plasmid identity, purity, endotoxin levels, sterility
6. **Delivery:** Intramuscular/intradermal injection; often with electroporation or gene gun to enhance uptake

Advantages

- Focused immune response on antigen of interest
- Cost-effective; large DNA quantities produced rapidly
- Stable at higher temperatures; easier transport/storage
- No risk of infection; no live pathogen handled
- Induces both MHC class I and II presentation (humoral + cell-mediated immunity)

Limitations

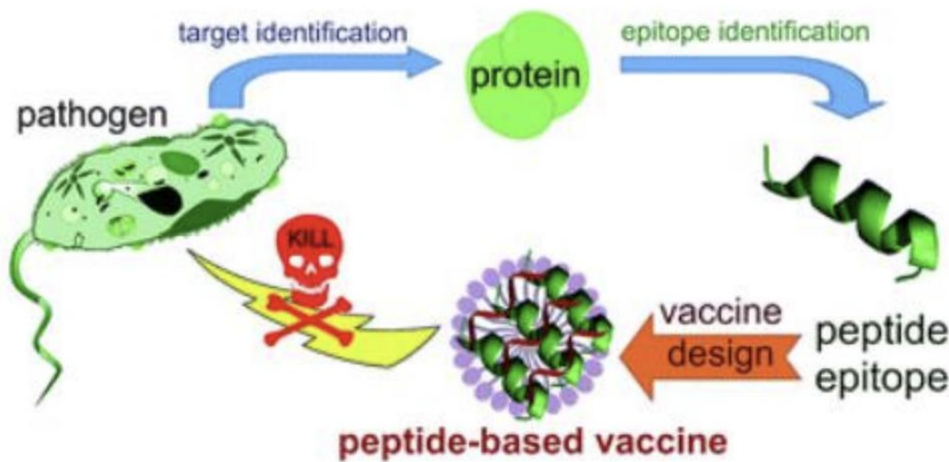
- Lower immunogenicity in humans vs. other platforms
- Risk of genomic integration (theoretical); requires careful design
- Potential for immune tolerance to antigen
- Limited to protein immunogens; not suitable for polysaccharide antigens

Indian Milestone: ZyCoV-D

- World's first DNA vaccine approved for human use (2021)
- Encodes SARS-CoV-2 spike protein; delivered via needle-free injector
- Stable at 2-8°C; three-dose primary series
- Demonstrates India's end-to-end capability in novel vaccine platforms

PEPTIDE VACCINES

Peptide-based synthetic vaccines contain peptides which mimic the epitopes of the antigen that triggers immune responses. Peptide vaccines are synthesized peptides of 20–30 amino acids, which are highly immunogenic and to trigger the desired immune response.



Production

- Solid-phase peptide synthesis (SPPS): Stepwise addition of amino acids on resin beads
- Characterization: NMR, HPLC to confirm sequence and purity
- Conjugation to carrier protein (e.g., KLH) if needed for T-cell help
- Formulation with adjuvant (aluminum salts) and stabilizers

PART 4: INDIAN CONTEXT IN VACCINE DEVELOPMENT AND PRODUCTION

Vaccine Manufacturing Capacity

- India produces over 60% of the world's vaccines by volume, earning the title "pharmacy of the world"
- Key manufacturers:
 - Serum Institute of India (Pune): World's largest vaccine manufacturer by volume; produces Covishield, pentavalent vaccine, PCV, rotavirus vaccine
 - Bharat Biotech (Hyderabad): Developed Covaxin (whole-virion inactivated COVID-19 vaccine), Typbar-TCV (typhoid conjugate vaccine), Rotavac
 - Biological E (Hyderabad): Produces Hepatitis B, DPT, and COVID-19 vaccine (Corbevax, protein subunit)
 - Zydus Cadila (Ahmedabad): Developed ZyCoV-D, the world's first DNA vaccine approved for human use
 - Panacea Biotec, Haffkine Institute, and others contribute to domestic supply

Indigenous Vaccine Development Milestones

- **Covaxin (BBV152):** Whole-virion inactivated SARS-CoV-2 vaccine developed by Bharat Biotech in collaboration with ICMR-NIV; received WHO Emergency Use Listing; administered over 200 million doses in India
- **ZyCoV-D:** Plasmid DNA vaccine encoding SARS-CoV-2 spike protein; developed by Zydus Cadila; first DNA vaccine approved globally; needle-free intradermal delivery
- **GEMCOVAC-OM:** India's first indigenous mRNA vaccine for Omicron variant; developed by Gennova Biopharmaceuticals; received emergency use authorization
- **Rotavac and Rotasiil:** Indigenous rotavirus vaccines developed with DBT support; included in UIP, reducing diarrheal mortality
- **Typbar-TCV:** Typhoid conjugate vaccine by Bharat Biotech; first WHO-prequalified typhoid vaccine; used in India and Africa

Immunization Programs in India

Universal Immunization Programme (UIP)

- Launched in 1985; one of the world's largest public health programs

PART 5: CHALLENGES AND WAY FORWARD

Persistent Challenges

- **Vaccine Hesitancy:** Misinformation, cultural beliefs, and distrust affect uptake; requires targeted communication and community engagement
- **Cold Chain Limitations:** Maintaining temperature integrity in remote, hot, or conflict-affected areas; power outages and equipment failures
- **Equity and Access:** Ensuring last-mile delivery to marginalized communities; addressing gender, caste, and geographic disparities
- **Emerging Pathogens and Variants:** Rapid evolution of viruses (e.g., SARS-CoV-2, influenza) requires agile vaccine development and updating
- **Manufacturing Scalability:** Balancing domestic needs with global supply commitments; technology transfer and intellectual property considerations
- **Adverse Event Monitoring:** Robust pharmacovigilance to detect rare side effects while maintaining public confidence

Strategic Recommendations

- **Strengthen Primary Health Care:** Integrate vaccination with other maternal and child health services for higher coverage and efficiency
- **Invest in Next-Generation Platforms:** Support indigenous development of mRNA, viral vector, and universal vaccine technologies for pandemic preparedness
- **Enhance Cold Chain Infrastructure:** Solar-powered refrigerators, drone delivery, and real-time monitoring via eVIN/U-WIN
- **Combat Misinformation:** Proactive science communication through trusted community leaders, social media, and school curricula
- **Promote Regional Collaboration:** South-South technology transfer, joint clinical trials, and pooled procurement for affordable vaccines
- **Align with BioE3 Policy:** Leverage biomanufacturing hubs, regulatory sandboxes, and AI-driven vaccine design for innovation and scale

Future Outlook

- **Universal Vaccines:** Research on vaccines targeting conserved viral regions (e.g., influenza hemagglutinin stalk, coronavirus RBD) to provide broad, long-lasting protection against variants

- **Thermostable Formulations:** Lyophilized mRNA, nanoparticle-encapsulated antigens, and dry powder vaccines to reduce cold chain dependence
- **Personalized Vaccinology:** Using genomics and immunoprofiling to tailor vaccine schedules and formulations for high-risk groups
- **One Health Approach:** Integrating human, animal, and environmental surveillance to anticipate and prevent zoonotic outbreaks requiring vaccines
- **Atmanirbhar Bharat in Vaccines:** Building end-to-end indigenous capacity from antigen design to fill-finish, reducing import dependence and enhancing strategic autonomy

QUICK REVISION POINTS

- Immunity: Innate (non-specific, rapid) vs Adaptive (specific, memory-based); Active (body produces antibodies) vs Passive (pre-formed antibodies received)
- Vaccine types: Live attenuated (MMR, OPV), Inactivated (IPV, HepA), Toxoid (tetanus, diphtheria), Subunit/Conjugate (HepB, PCV), Viral vector (Covishield), mRNA (Pfizer, GEMCOVAC-OM), DNA (ZyCoV-D), VLP (HPV)
- Hepatitis B vaccine production: Recombinant DNA technology; HBsAg gene inserted into yeast; fermentation; purification; formulation with aluminum adjuvant; quality control
- Indian vaccine landscape: Serum Institute, Bharat Biotech, Biological E, Zydus Cadila; Covaxin, ZyCoV-D, GEMCOVAC-OM as indigenous milestones
- UIP: 12 vaccines; Mission Indradhanush for coverage gaps; CoWIN, eVIN, U-WIN for digital tracking
- Regulatory bodies: CDSCO (approval), NTAGI (policy), ICMR (research), GEAC (for GM components)
- Challenges: Hesitancy, cold chain, equity, variants, scalability, pharmacovigilance
- Way forward: Next-gen platforms, thermostable vaccines, personalized vaccinology, One Health, Atmanirbhar Bharat