Evaluation of Bacterial vaccines

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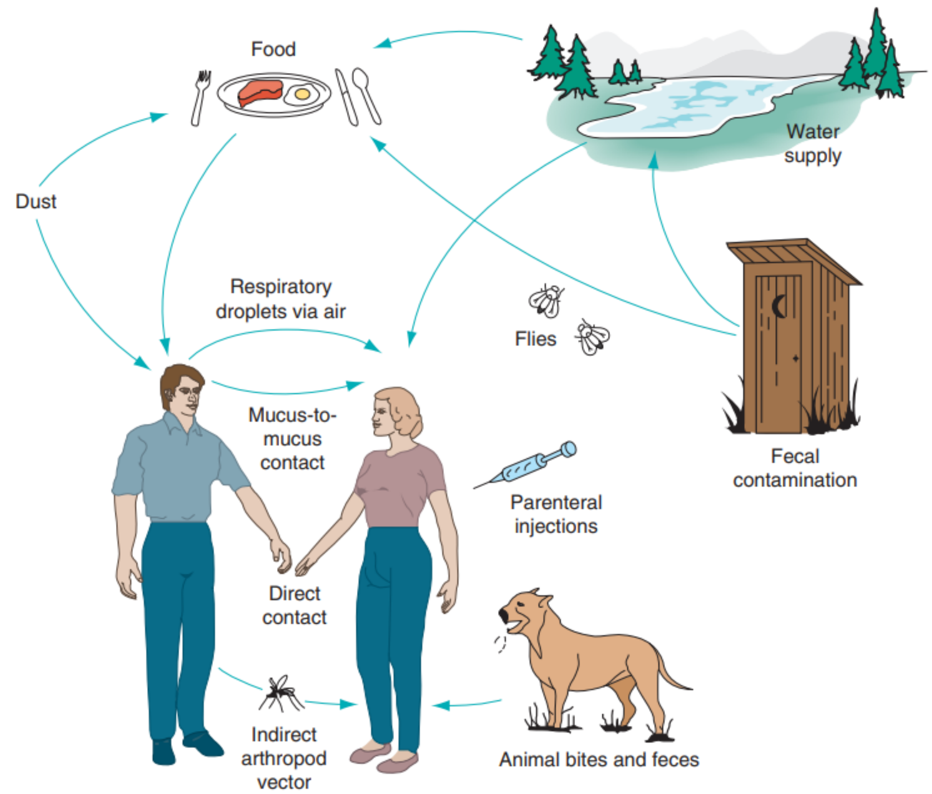
Overview

- Bacteria are ubiquitous,
- Only a small percentage cause disease,
- Bacterial infections have a large impact on public health,
- Generally, bacterial infections are easier to be treated than viral infections



Burton's Microbiology for the Health Sciences, 8th edn., ch. 11. Baltimore: Lippincott Williams and Wilkins

The role of enviroment and mode of transmissions

Reservoirs	*Disease examples*
Human	Typhoid fever, syphilis
Animal	Anthrax (cows), *Salmonella* (turtles), tularemia (rabbits), Lyme disease (white-footed mice)
Arthropods	Rocky Mountain spotted fever (ticks), endemic typhus (fleas), scrub typhus (mites)
Air	Tuberculosis
Soil	Tetanus, botulism, gas gangrene
Food	Vibrio, *E. coli* 0157:H7
Water	Shigella, Legionella

Mode of transmission	*Disease examples*
Contact	Streptococcal impetigo (skin-to-skin), gonorrhea (mucus membrane-to-mucus membrane), *Salmonella* (fecal–oral), syphilis (transfusion)
Airborne	Tuberculosis, Q fever, legionella
Droplet	Pertussis, meningococcus, *Haemophilus influenzae*
Vectors	Lyme disease (tick), Shigella (fly) epidemic typhus (lice), bubonic plague (fleas)
Vehicular	*Campylobacter* (food), trachoma (fomites)

Doron S and Gorbach SL., Elsevier, 2008

- Bacterial can be trasmitted by several mechanisms and they have been adapted to survive in water, soil, food and elsewhere.
- Some bacteria are endemic in certain geographic regions and nonexistent in others,
- Some bacteria infect vectors such animals or insects before being trasmitted to humans,
- Impact of **overflow,** a phenomen relevant for zoonotic diseases such as Lyme Disease (caused by Borrelia sp.)

Prevention of bacterial infections

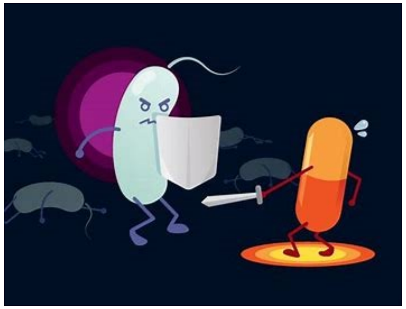
Prevention is important in this age of increasing antibiotic resistance

3 main strategies for bacterial infections control:
Which measure is most effective often depends on the reservoir of infection

- → Eliminate the sources of infection
- → Interrupt the chain of transmission
- → Protect the host against infection or disease

3 main types of prevention:

- → Tertiary prevention (treatment of infected people to prevent transmission to other humans)
- → Secondary prevention (treatment of infected people to prevent symptomatic infections)
- → Primary prevention (Vaccination). Prophylactic vaccines are urgently needed



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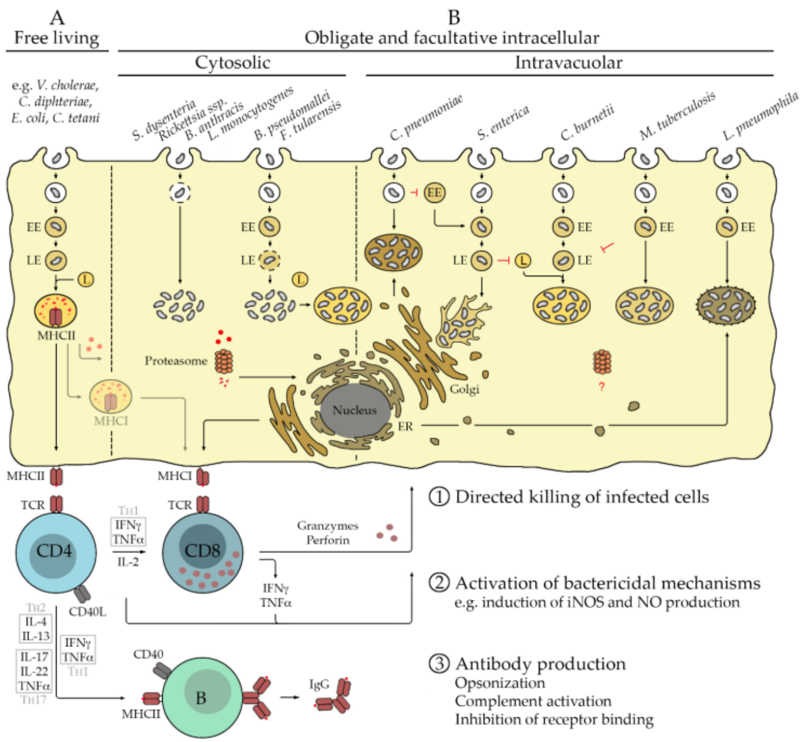
The complexity of immunological responses



Osterloh, Vaccines 2022, 10, 751

- Bacteria are complex organisms and are more difficult targets,
- They possess several antigens whose immunogenic potential is often unknown,
- Unclear if antigens can elicit long-lasting immunity
- Intracellular vs extracellular bacteria
- non-living whole cell antigens or subunit vaccines are not able to elicit T-cell mediated responses.

Current vaccine formulations development

	Preclinical	Phase I	Phase II	Phase III
Protein vaccine	• iNTS • *Shigella* species • *Neisseria gonorrhoeae*	• ExPEC	• *Pseudomonas aeruginosa* • GAS • *Staphylococcus aureus* • *Mycobacterium tuberculosis*	• *Clostridioides difficile*
Glycoconjugate	• *Klebsiella pneumoniae* • iNTS • GAS	None	None	• *Salmonella* species
Synthetic conjugate	• *K pneumoniae*	• *Shigella* species	None	None
Bioconjugate	• *K pneumoniae* • *S. aureus*	None	• ExPEC • *Shigella* species	None
OMV	• *N. gonorrhoeae* • *K pneumoniae* • *M. tuberculosis* • ExPEC • iNTS	None	• *Shigella* species	None
Live attenuated vaccine	• *C. difficile* • iNTS	None	• *Salmonella* species • *Shigella* species	• *M. tuberculosis*

Micoli et al. Nature Reviews, Microbiology, May 2021

- **Reverse vaccinology** (its first application for Neisseria meningitidis group B, enables the selection of potential vaccines on the basis of the genomic information of a bacterial strains)
- **Structural vaccinology** (Structural information combined with immunological and functional characterization of microbial antigen can be used to structurally design new candidate vaccine antigens. E.g., Monoclonal Ab selection)
- **OMV (**outer membrane vesicles) and **Generalised modules for membrane antigens** (GMMA) (Naïve outer membrane vesicles contain natural bacterial surface-exposed proteins in the correct conformation)
- **Bioconjugation** (covalent linking of a bacterial polysaccaharide to a carrier protein e.g. *Haem Influenza,*
- *Men C,A and ACWY), Penumococcus Serotypes 7, 10, 3 and Salmonella Ent.)*

Critical aspects to be considered for clinical development:

- Selection of suitable target population for efficacy trials
- Biomarkers for identifying correlates of protection
- Lack of a known correlate of protection is a major limitation in the ability to identify a protective vaccine
- Measure of functional immunity is critical for vaccine evaluation and it is required by regulators for new vaccine licensing

For the three main bacterial pathogens that cause bacteremic disease—Haemophilus influenzae type b (Hib), pneumococci, and meningococci—the correlates are opsonophagocytic or bactericidal antibodies, although binding antibodies are useful as surrogates.

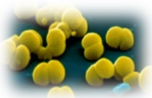
TABLE 2. Quantitative correlates and surrogates of protection after vaccination

Vaccine	Test	Level required	Reference(s)[a]
Anthrax	Toxin neutralization	1,000 IU/ml	87, 136, 149, 170, 191
Diphtheria	Toxin neutralization	0.01–0.1 IU/ml	14, 92
Hepatitis A	ELISA	10 mIU/ml	45, 110
Hepatitis B	ELISA	10 mIU/ml	66
Hib polysaccharides	ELISA	1 μg/ml	74
Hib conjugate	ELISA	0.15 μg/ml	73
Human papillomavirus	ELISA	ND[b]	140
Influenza	HAI	1/40 dilution	50, 171
Japanese encephalitis	Neutralization	1/10 dilution	63
Lyme disease	ELISA	1,100 EIA U/ml	128
Measles	Microneutralization	120 mIU/ml	24, 120, 158
Meningococcal	Bactericidal	1/4 (human complement)	96
Mumps	Neutralization?	ND	189
Pertussis	ELISA (toxin)	5 units	25, 173, 180.
Pneumococcus	ELISA; opsonophagocytosis	0.20–0.35 μg/ml (for children); 1/8 dilution	68, 81, 167
Polio	Neutralization	1/4–1/8 dilution	41, 95, 139
Rabies	Neutralization	0.5 IU/ml	196,
Rotavirus	Serum IgA	ND	49, 67, 104, 199, 200
Rubella	Immunoprecipitation	10–15 mIU/ml	2, 27, 53, 99, 141, 169
Tetanus	Toxin neutralization	0.1 IU/ml	13, 37,
Smallpox	Neutralization	1/20	89, 93, 139, 160
Tick-borne encephalitis	ELISA	125 IU/ml	77
Tuberculosis	Interferon	ND	46
Varicella	FAMA gp ELISA	≥1/64 dilution; ≥5 IU/ml	195
Yellow fever	Neutralization	1/5	79, 97
Zoster	$CD4^+$ cell; lymphoproliferation	ND	190

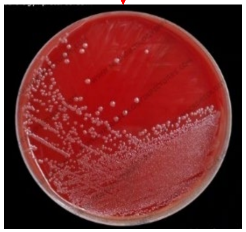
Plotkins, Clinical and Vaccine Immunology July 2010

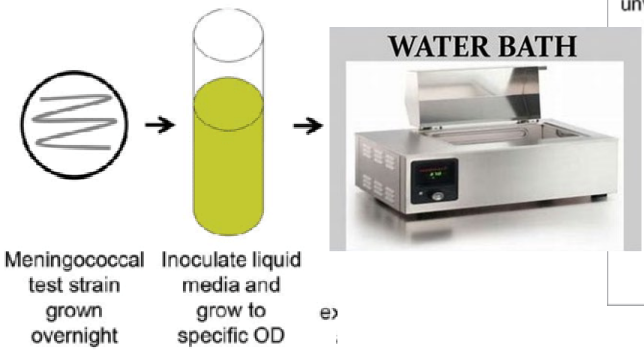
Manual-based Serum Bactericidal Assay (SBA)

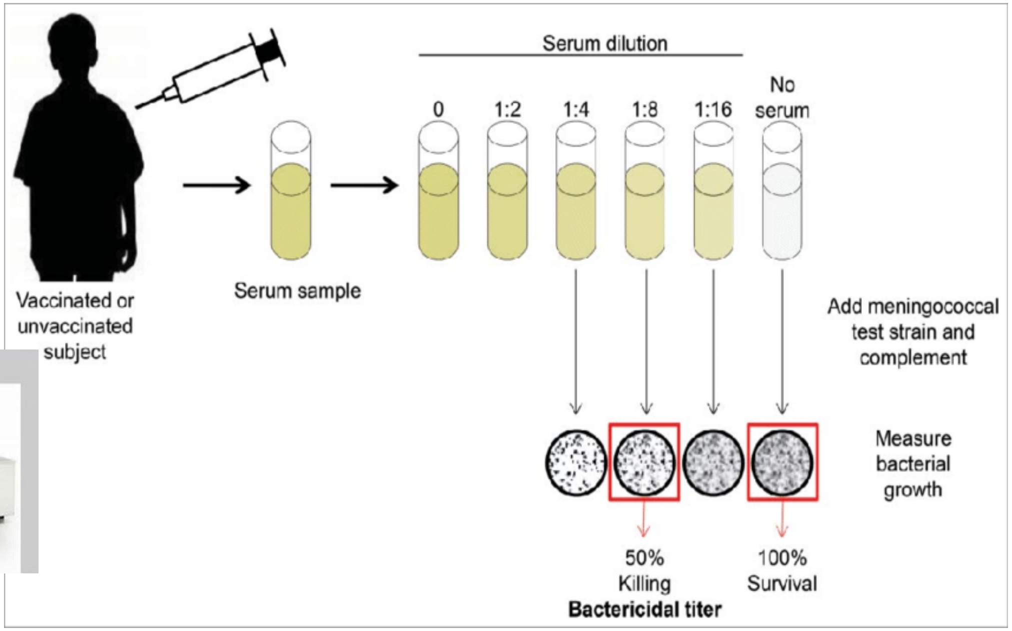
Based on the ability of antibodies present to kill the bacteria of interest. The killing is complement-mediated.



Incubation of bacteria O/N at 37°C







The serum killing activity is evaluated by plating the SBA reaction mix and counting the survival bacterial colony forming Units (CFUs) at each serum dilution. Bactericidal titers are calculated as the reciprocal of serum dilution that kills 50% of bacteria

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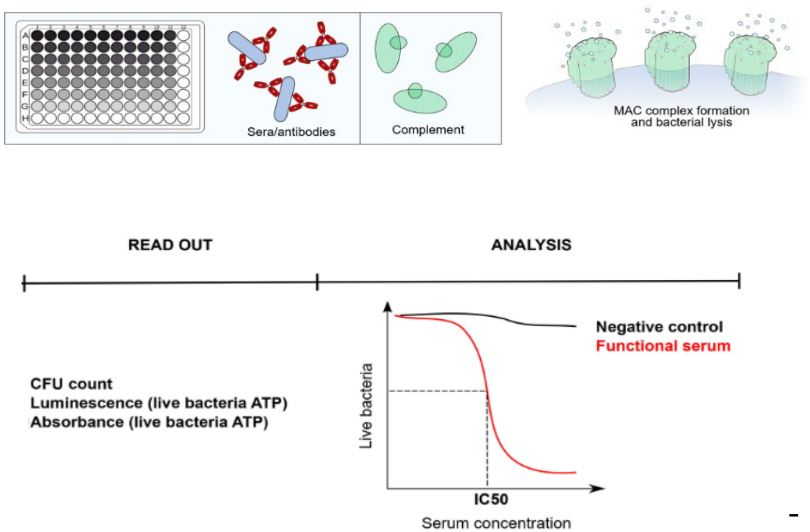
Luminescence-based Serum Bactericidal Assay (L-SBA)



https://www.frontiersin.org/files/Articles/1171213/fcimb-13-1171213-HTML/image_m/fcimb-13-1171213-g001.jpg

Table 6. Comparison between L-SBA and traditional CFU-based assay in terms of procedure advantages.

	L-SBA	traditional SBA with manual counts
Total time of execution	6 hours[1]	1.5 working day[2]
Data acquisition	2 minutes/SBA plate	2–3 hours/SBA plate[2]
Reproducibility	higher operator independency	lower operator independency
Assay throughput	1 operator/day: 24 individual sera in triplicate (6 SBA plates total)	1 operator/1.5 day: 12 individual sera in single (1 SBA plate[2])

[1]to execute 1 set of 6 SBA plates

[2]to execute 1 SBA plate, plating each reaction well in 1 full agar plate: 1 SBA plate corresponds to have 96 agar plates

doi:10.1371/journal.pone.0172163.t006

- Bacteria surviving the complement-mediated ab dependent killing are detected by measuring their metabolic ATP via the use of a reagent called Bac titer (Promega) .
- SBA reaction is not plated on the agar plate
- The signal obtained is proportional to the ATP present in SBA reaction which is directly proportional to the number of bacteria not killed by SBA.
- Bactericidal titer can be calculated by using a luminometer

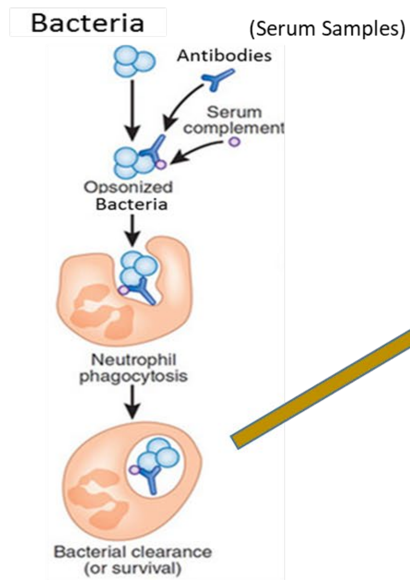
Opsonophagocytosis (OPA)

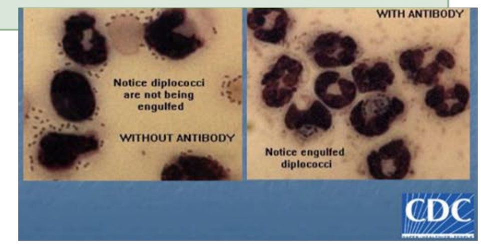
It is based on the bacteria complement mediated opsonizzation and killing

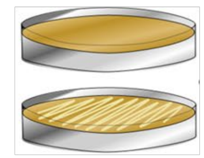
This assay use baby rabbit serum as the complement source and human promyelocytic cell line HL-60 cells as the phagocytic cells.

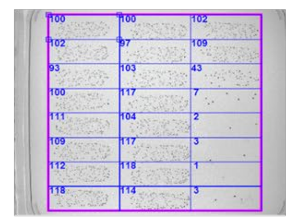
OPA measures the biological activity of serum antibodies, therefore it is believed to be highly relevant in assessing vaccine efficacy.
Its experimental set up involves four critical components (bacteria, serum antibodies, complement, and phagocytic cells).

Standard-, Flow Cytometric based-, Fluorescent- and Multiplex- OPA exist.









<u>Vismederi started the process of set-up to use the standard OPA for anti-S. *pneumoniae* antibodies detection</u>

The opsonophagocytic titer is calculated as the reciprocal of the serum dilution killing the 50% of colony (respect to the control)

Table 1. Advantages and disadvantages of currently available functional assays.

Assays	Advantages	Disadvantages
Traditional Killing OPA/MOPA pneumococcal-specific	• Standardised gold-standard assay	• Labour intensive • Time consuming • Can have high repeat rate ^
Fluorescent OPA/MOPA pneumococcal-specific	• Single-day assay • Eliminates colony-counting • Semi-automation	• Non-standardised output • Requires specialised equipment (i.e., flow cytometer or fluorometer) • Variable results for some serotypes
Serum Bactericidal Assay Hib and meningococcal	• Does not require phagocytic cell line	• Non-standardised reagents • Does not measure opsonophagocytic activity • Time consuming
Antibody Avidity Assay pneumococcal, Hib and meningococcal	• Easy to perform • Does not require live bacteria	• Non-biological assay • Non-standardised method (dilution vs. elution)

Vaccines 2021, 9, 677

Table 2. Correlates of protection for pneumococcal, Hib and meningococcal vaccines.

Vaccines	Correlates of Protection
PCV	ELISA >0.35 µg/mL OPA ≥8 titre
Hib	ELISA Long term: ≥1.0 µg/mL Short term: >0.15 µg/mL SBA ≥4 titre
Meningococcal *	SBA rSBA (≥8 titre) or hSBA (≥4 titre)

[Senza titolo]

Need of assay standardisation for functional assays

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