General Microbiology

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Lecture at The Scandinavian School of Cardiovascular Technology

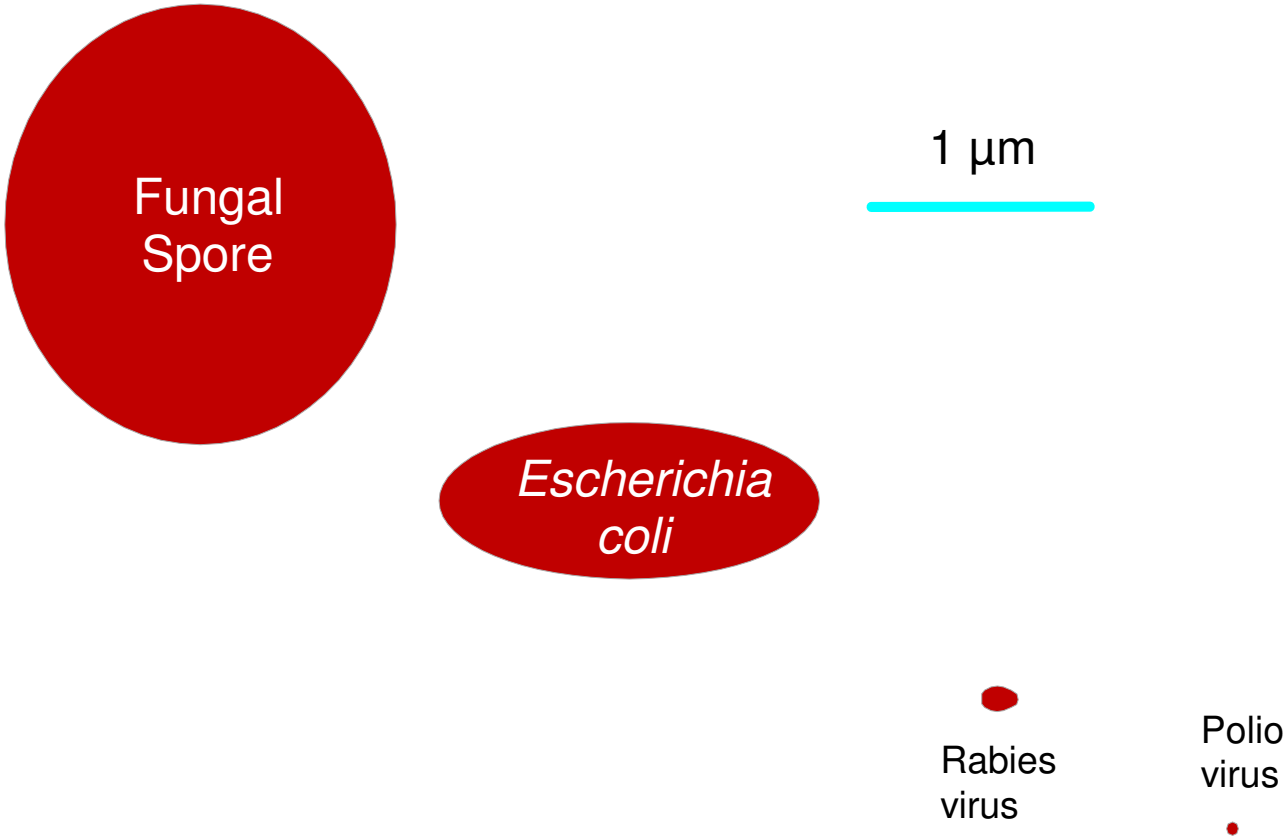
February 2013

Programme

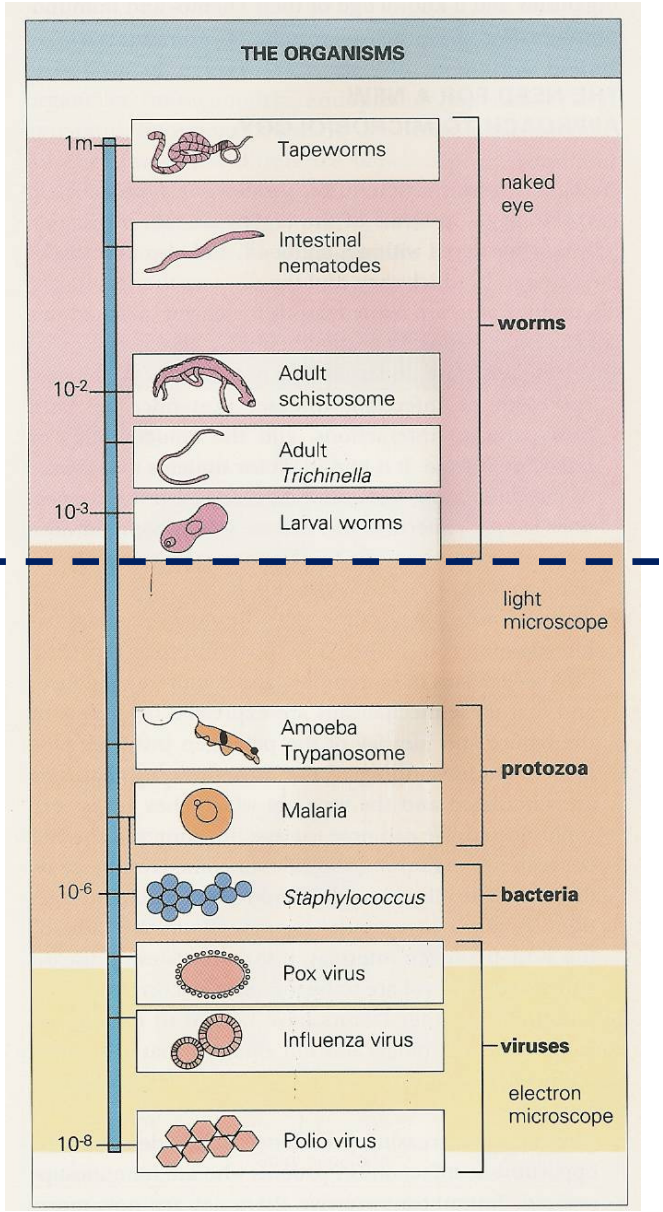
- Lecture 1: General Microbiology
- Lecture 2: Host-parasite interactions
- Lecture 3: Sterilization, Disinfection and Chemotherapy
- Lecture 4: Clinical Microbiology

What is a Micro-organism?

- Very small (you need a microscope to see them!)
- They are all over (most micro-organisms are **ubiquitous**)
- Relatively few species are human pathogens!



Relative sizes of the organisms causing infectious diseases



Micro-organisms

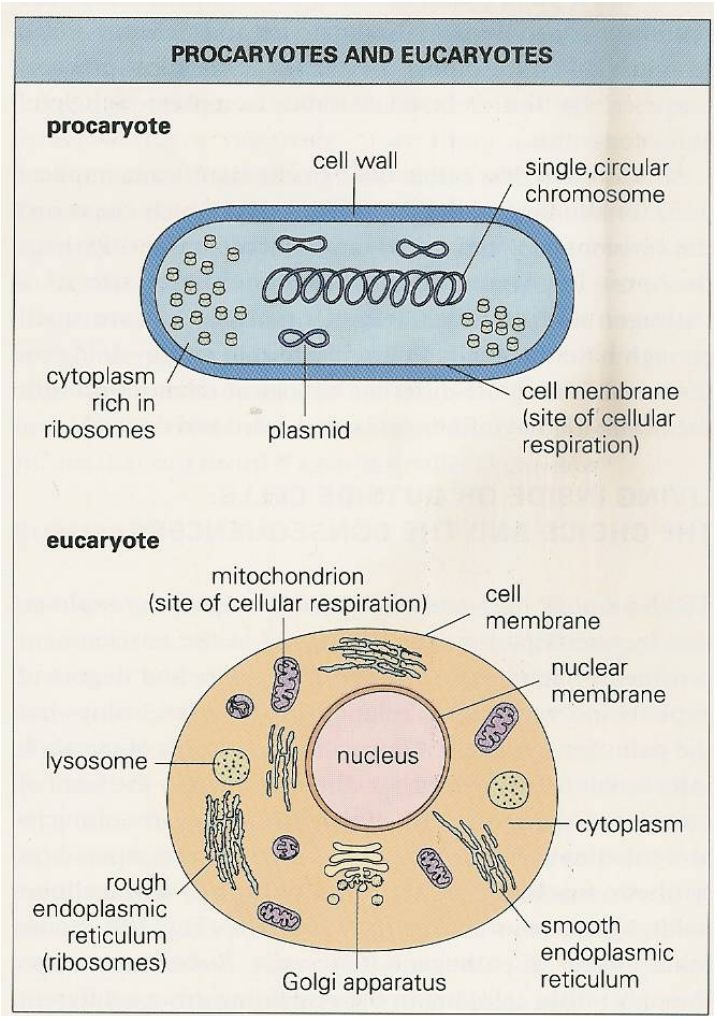
Characteristics of the major groups of organisms that causes infectious diseases

CLASSIFICATION OF MAJOR PATHOGENS					
	viruses	**bacteria**	**fungi**	**protozoa**	**worms**
nucleic acids	DNA or RNA	DNA and RNA	DNA and RNA	DNA and RNA	DNA and RNA
nuclear membrane	no	no	yes	yes	yes
external cell wall	no	yes (usually) rigid peptidoglycan	yes rigid chitin	no	no
antibiotic sensitivity	no	yes	no	some	no
replication/ reproduction	within host cells	within and outside host cells by binary fission	within and outside host cells by binary fission and sexually	within and outside host cells by binary fission and sexually	outside host cells, sexually

Micro-organisms

Prokaryotic cells *versus* **Eukaryotic** cells

Bacteria



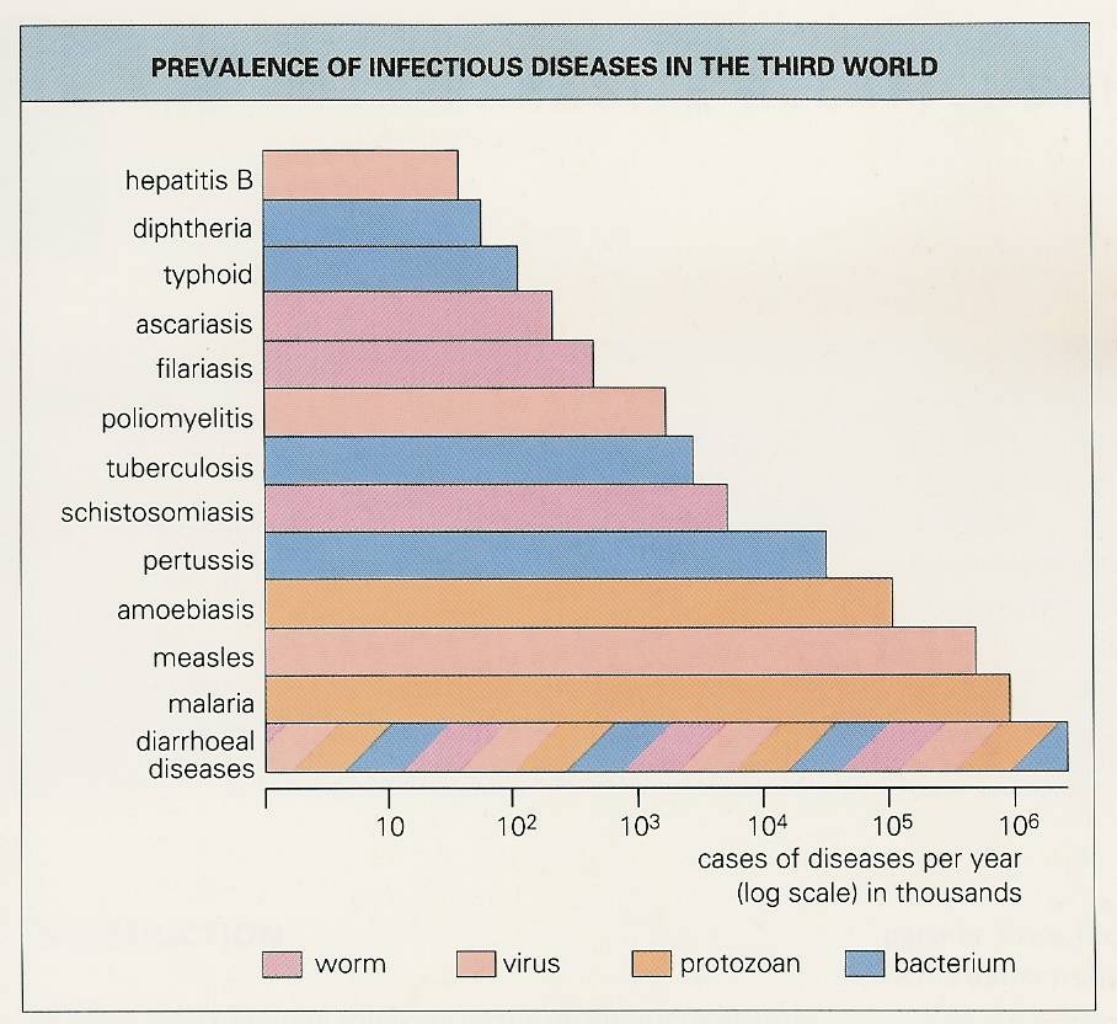
Prokaryotes: pro = before (rudimentary)
karyon = nucleus

Eukaryotes: eu = **true**
karyon = **nucleus**

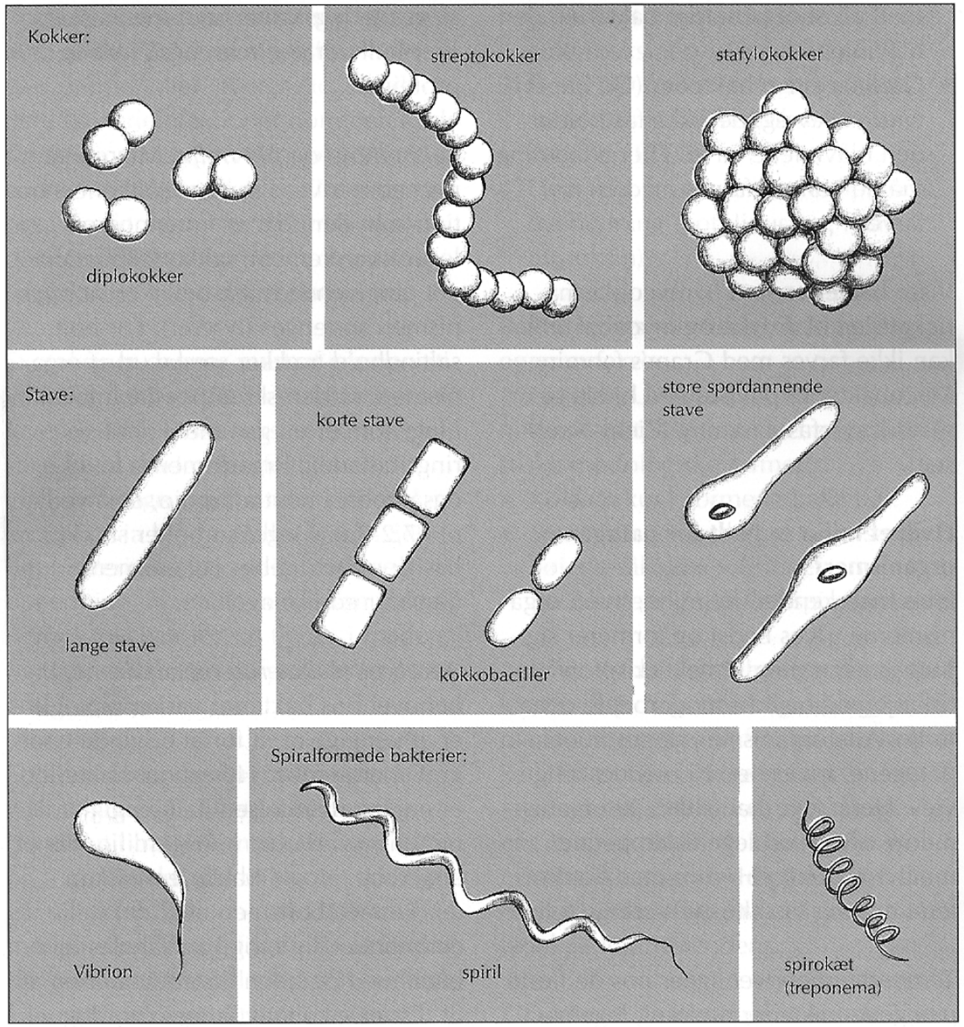
from Greek

Fungi

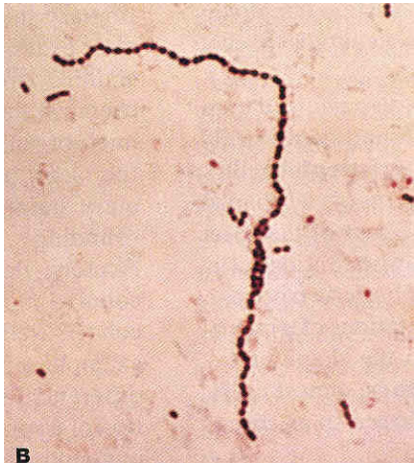
Cause of infectious diseases



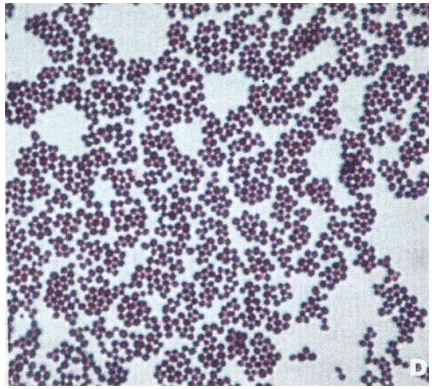
Morphology (Bacteria)



Cocci

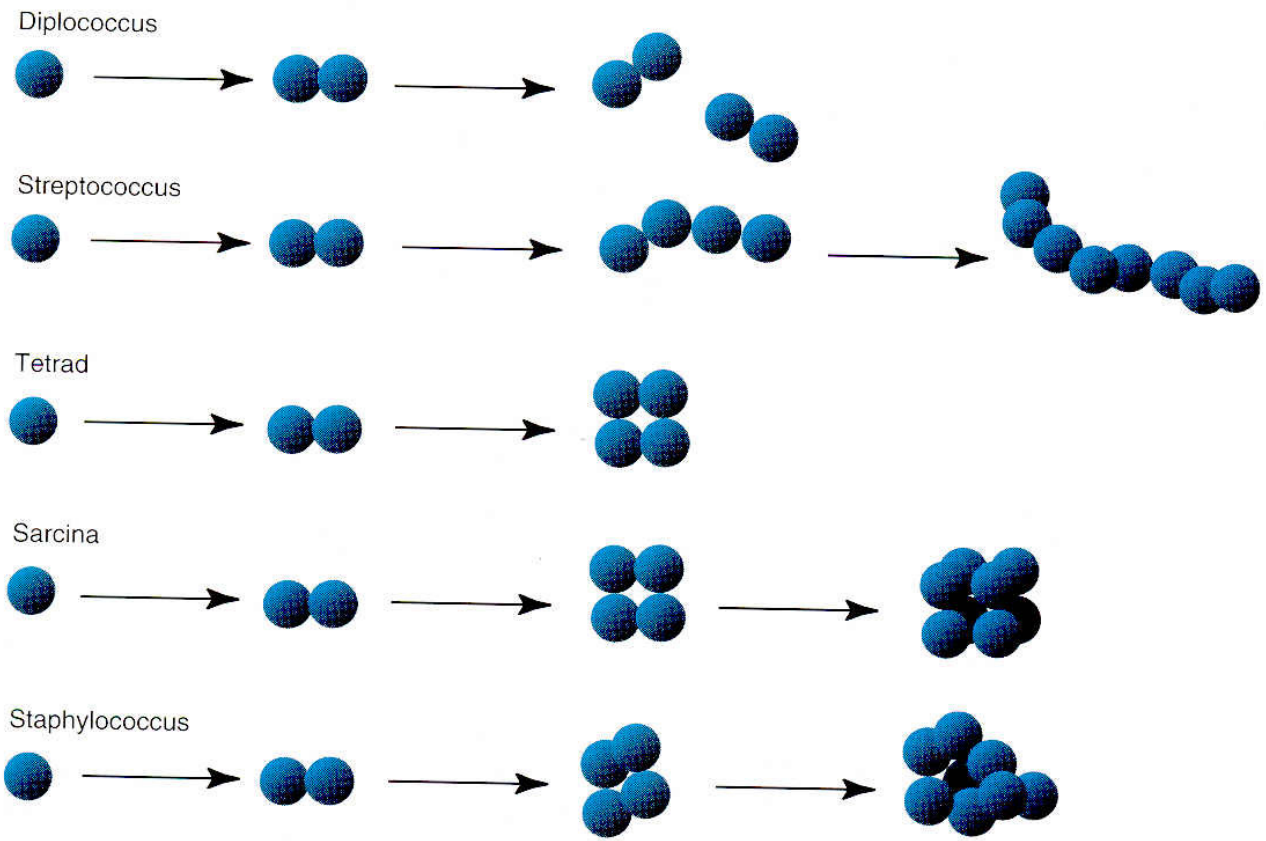


Rods



Spirochetes

Arrangements of bacteria determined by their pattern of binary fission



The Gram Stain

Bd. 2. 1884.

Fortschritte der Medicin.

Unter Mitwirkung hervorragender Fachmänner herausgegeben von

Dr. Carl Friedländer,
Privatdocent der pathol. Anatomie.

Verlag von Theodor Fischer's medicinischer Buchhandlung,
Berlin NW., Dorotheenstrasse 8.

No. 6. | Diese Zeitschrift erscheint am 1. und 15. jeden Monats. Abonnement für den Jahrg. von 24 Nummern M. 20. | 15. März.

Original-Mittheilung.

Ueber die isolirte Färbung der Schizomyceten in Schnitt- und Trockenpräparaten.

Von Dr. C. Gram aus Kopenhagen.

(Die Gelegenheit und den grössten Theil des Materials zu den folgenden Untersuchungen verdanke ich Hrn. Dr. Biess, Director des städt. allgem. Krankenhauses in Berlin.)

Wie bekannt giebt die Methode der isolirten Färbung der Tuberkelbacillen von Koch und Ehrlich mit oder ohne Doppelfärbung sehr schöne Bilder, weil die Bacillen durch die Contrastwirkung sehr deutlich hervortreten.

Es wäre ja sehr wünschenswerth, wenn man eine ähnliche Methode für die isolirte Färbung der übrigen Schizomyceten hätte, mit denen die Mikroscopiker Tag für Tag mehr und mehr sich beschäftigen.

Bei meinen Untersuchungen — als Mitarbeiter des Herrn Dr. Friedländer im Leichenhause des städtischen Krankenhauses in Berlin — hatte ich die Nachweisung der Kokken in Schnittpräparaten von den Lungen der Pneumoniker und der Versuchsthiere übernommen, und habe, wie es bereits von Friedländer in seiner Abhandlung über die Mikrokokken der Pneumonie [1]) kürzlich erwähnt worden ist, auf experimentellem Wege eine isolirte Färbung für die Pneumoniekokken gefunden; d. h. eine Färbung, durch welche die Kerne und andere Gewebeelemente ungefärbt bleiben, während die Kokken stark gefärbt hervortreten und daher viel leichter aufzufinden sind, während sie in den gewöhnlichen Präparaten besonders bei den Pneumonien, wo sie sehr häufig in den Exudatzellen liegen, gar nicht zu sehen sind.

Weitere Untersuchungen über die Verwendbarkeit dieser Methode auch für andere Schizomyceten haben nach und

[1]) Fortschritte der Medicin. No. 22, 1883.

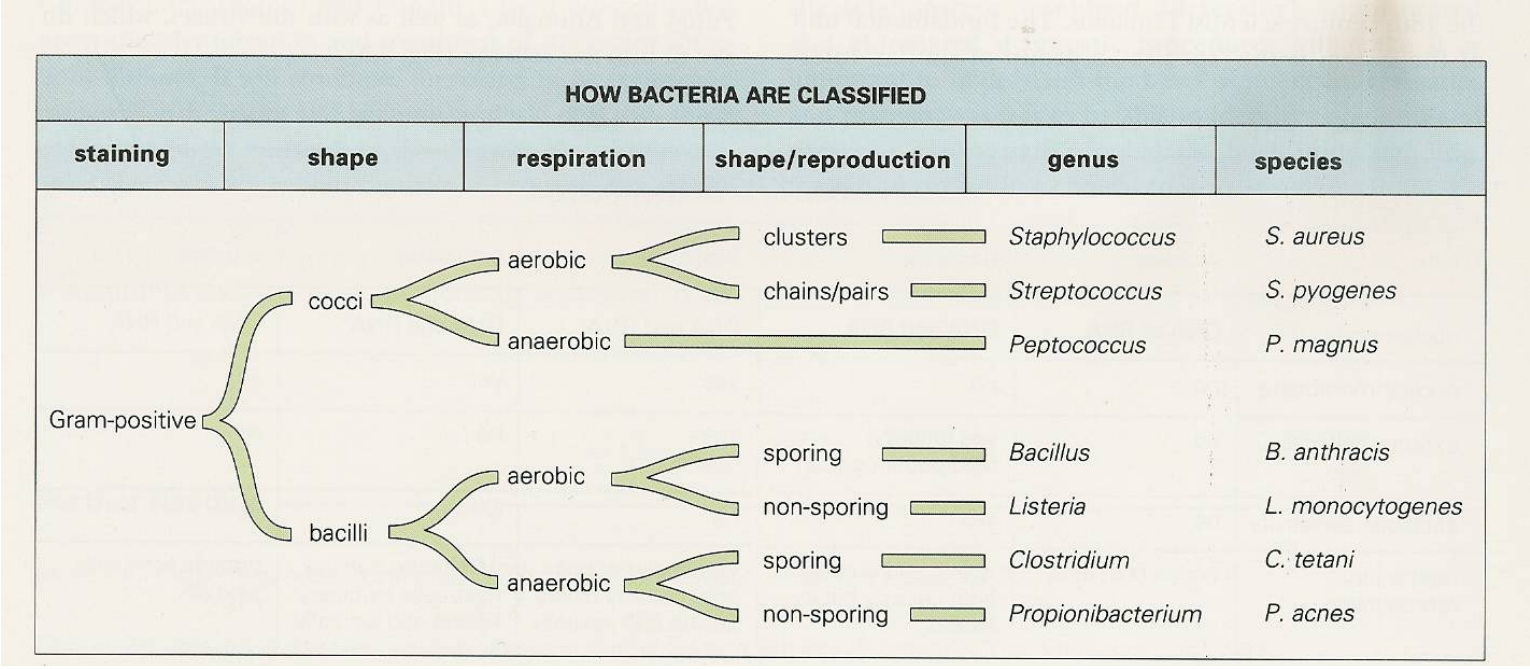
Hans Christian Gram

Gram negative bacteria
Gram positive bacteria

Characteristics used to classify bacteria

Example

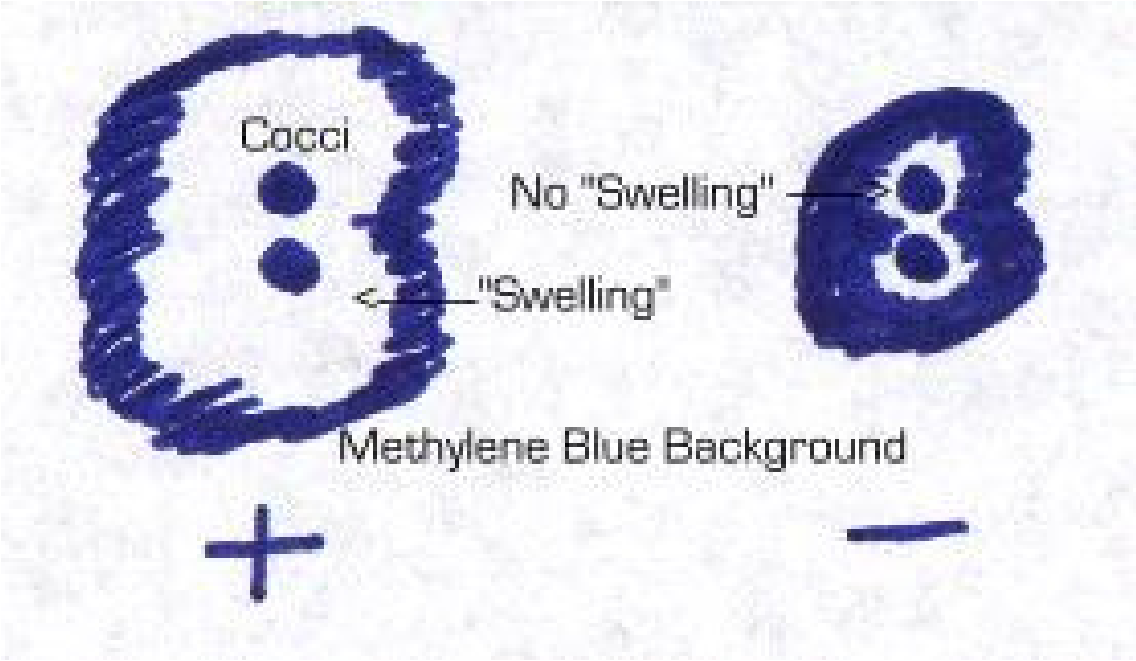
Gram-positives

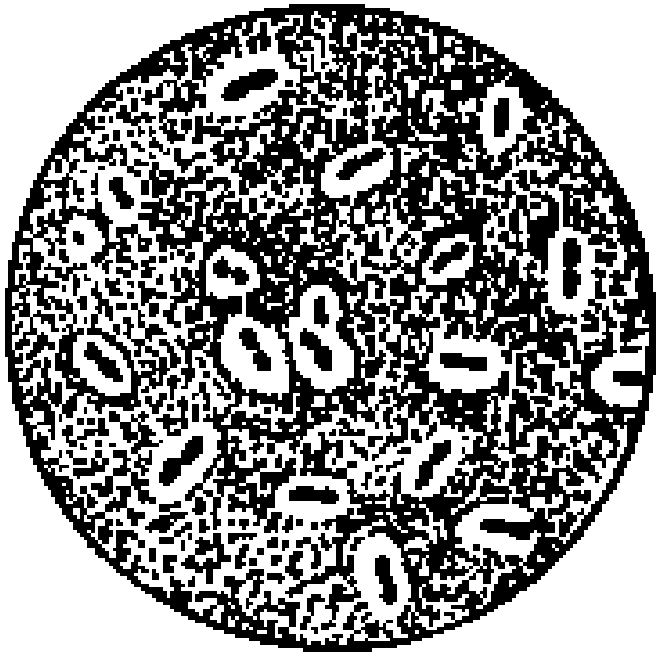


Simplified scheme

Pneumococci (*S. pneumoniae*)

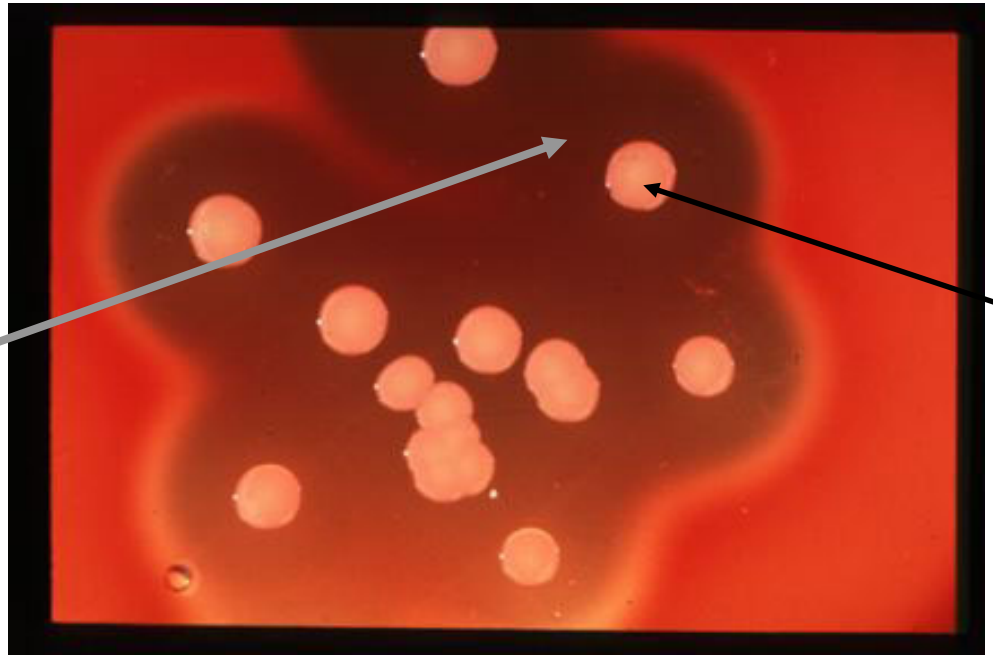
- α hemolytic, optochin sensitive
- diplococci with a polysaccharide capsule (>80 types)
- **capsule swelling**: polyvalent antiserum (omni serum) directed against the capsule is added to the sputum sample - if *S. pneumoniae* is present the capsule will swell and may be visualized by counterstaining or viewed directly in a wet smear under the microscope.



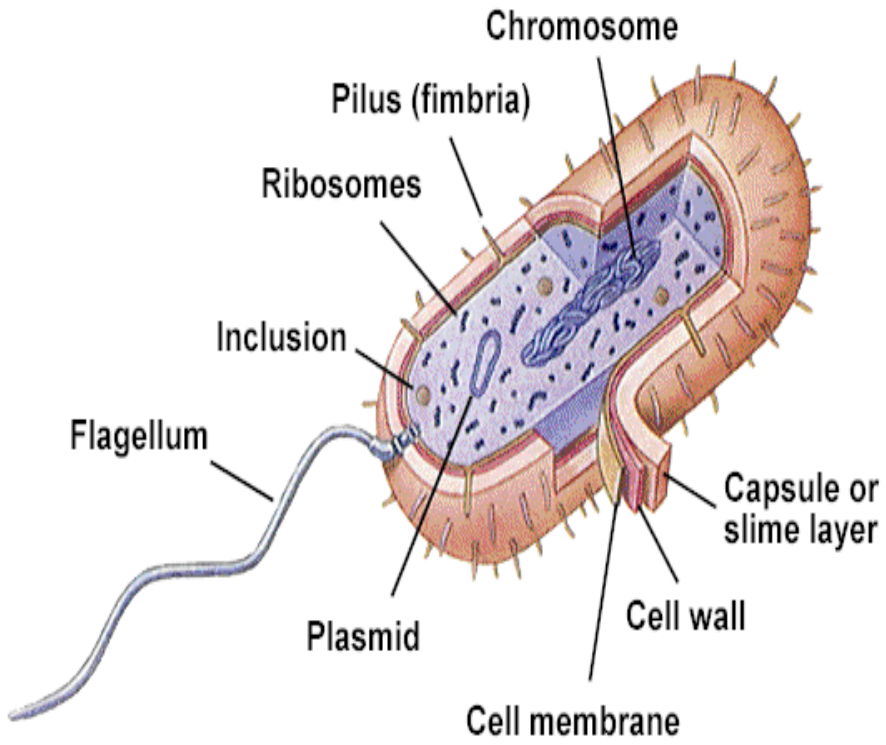


Morphology on blood agar

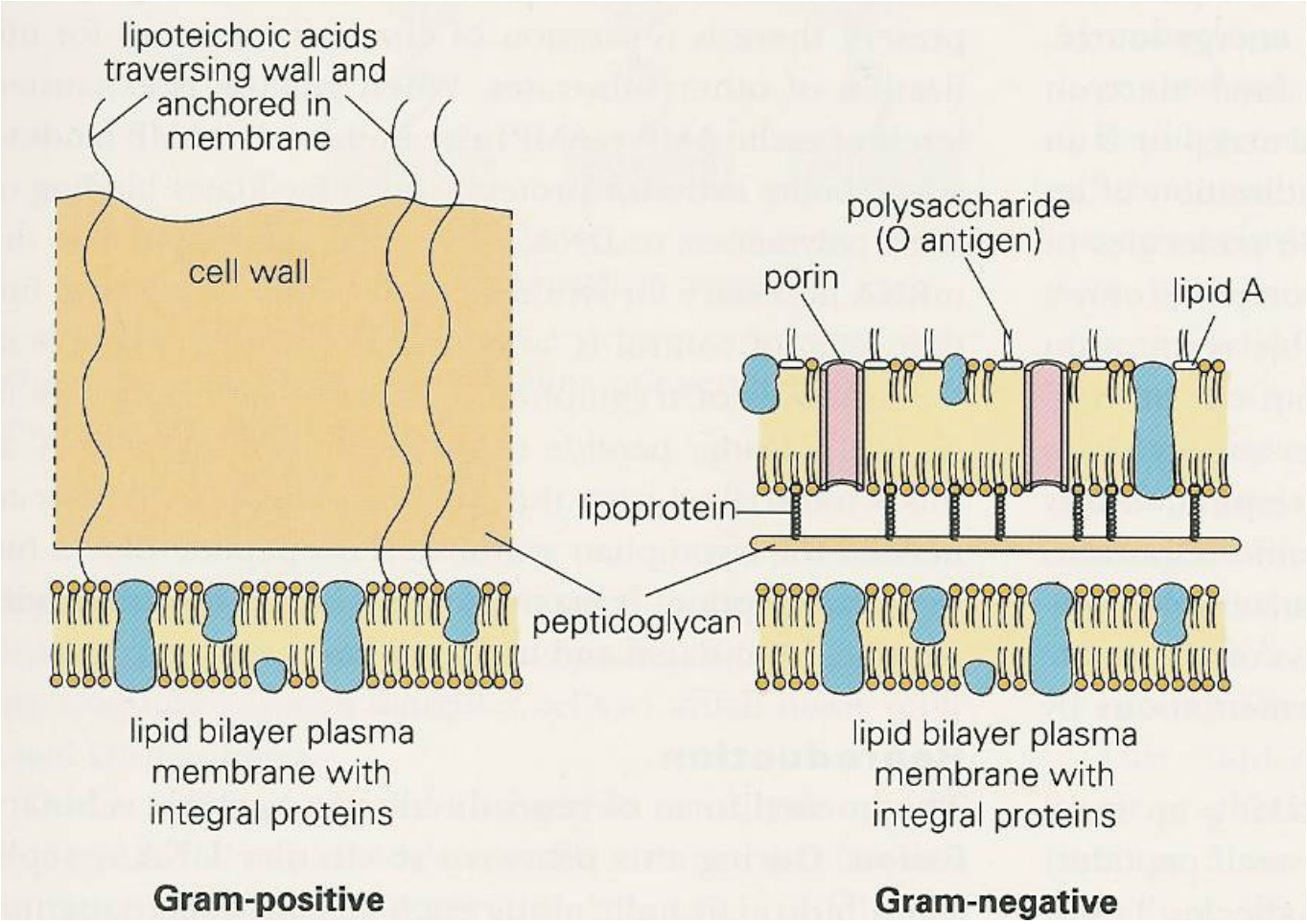
- *S. aureus* may show beta-haemolysis. Other *Staph. species* are alpha- or gamma-haemolytic.



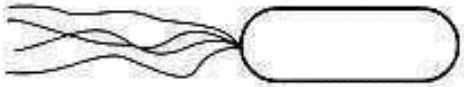
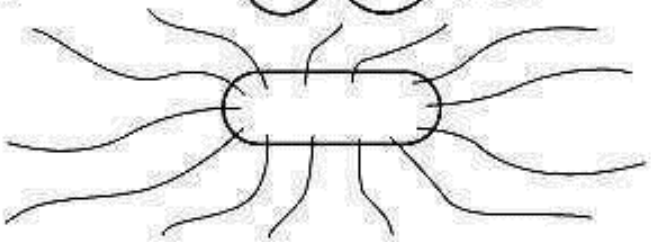
The bacterial cell



Bacterial cell walls



Specialized functions

Structure	Flagella Type	Example
	Monotrichous	*Vibrio cholerae*
	Lophotrichous	*Bartonella bacilliformis*
	Amphitrichous	*Spirillum serpens*
	Peritrichous	*Escherichia coli*

Flagella

Classification of *Staphylococcus aureus*

Example

Family	Micrococcaceae
Genus	Staphylococcus
Species	***Staphylococcus aureus***
Type	*S. aureus*, phage type 80
Strain	*S. aureus*, ATCC 29213
Clone	**e.g. *spa type* CC22 – t032**

Phenotypic examinations

Morphology
Biochemical reactions
Resistance pattern
Phage type

Genotypic examinations

Spa-type
Pulsed Field Gel Electrophoresis
Ribotyping
SCCmec typing

Single locus DNA-sequencing of the repeat region of the *Staphylococcus* protein A gene (**spa**) can be used for reliable, accurate and discriminatory typing of MRSA. Repeats are assigned a numerical code and the spa-type is deduced from the order of specific repeats.

Jens K. Møller

Medically important groups of bacteria

Gram positives

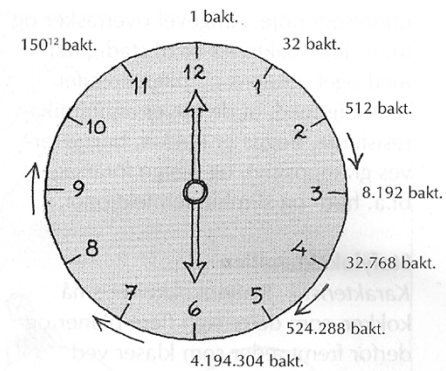
Gram negatives

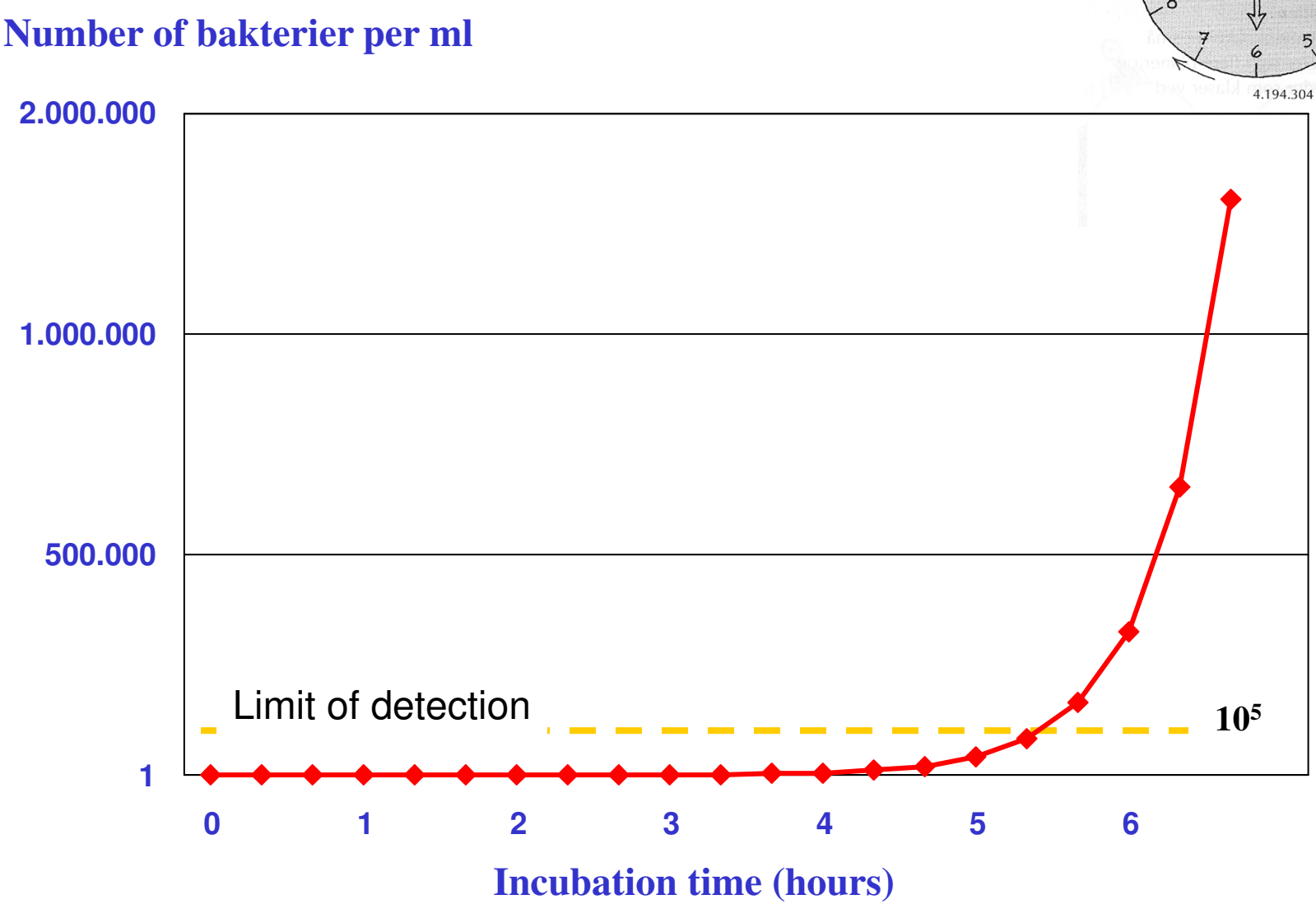
TABEL 3.1 Bakterierne inddelt efter gramfarvning, form og iltbehov

Grampositive kokker	
aerobe	*Staphylococcus aureus, S. epidermidis, S. saprophyticus, Streptococcus pyogenes, S. pneumoniae, Enterococcus,. faecalis*
anaerobe	Peptostreptococcus
Grampositive stave	
aerobe	*Bacillus anthracis, Bacillus saprophyticus, Bacillus cereus, Corynebacterium diphteriae, Listeria monocytogenes, Mycobacterium tuberculosis.*
anaerobe	*Clostridium tetani, Cl. perfringens, Cl. difficile, Cl. botulinum,* Lactobacillus, *Actinomyces Israeli*
Gramnegative kokker	
aerobe	*Neisseria gonorrhoeae, N. meningitidis, Moraxella catarrhalis*
Gramnegative stave	
fakultativt anaerobe	*Escherichia coli, Klebsiella pneumoniae,* Proteus, *Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi, Shigella dysenterie, Campylobacter jejuni, Helicobacter pylori, Bacteroides fragilis, Yersinia enterocolitica, Yersinia pestis, Vibrio cholerae, Haemophilus influenzae, Bordetella pertussis, Legionella pneumophila.*
Spirokæter	*Borrelia burgdorferi, Borrelia recurrentis, Treponema pallidum.*
Klamydia	*Klamydia trachomatis, Klamydia pneumonie, Klamydia psittaci*
Rickettsier	Rickettsier
Mycoplasma	*Mycoplasma pneumoniae*

Growth in a blood culture bottle

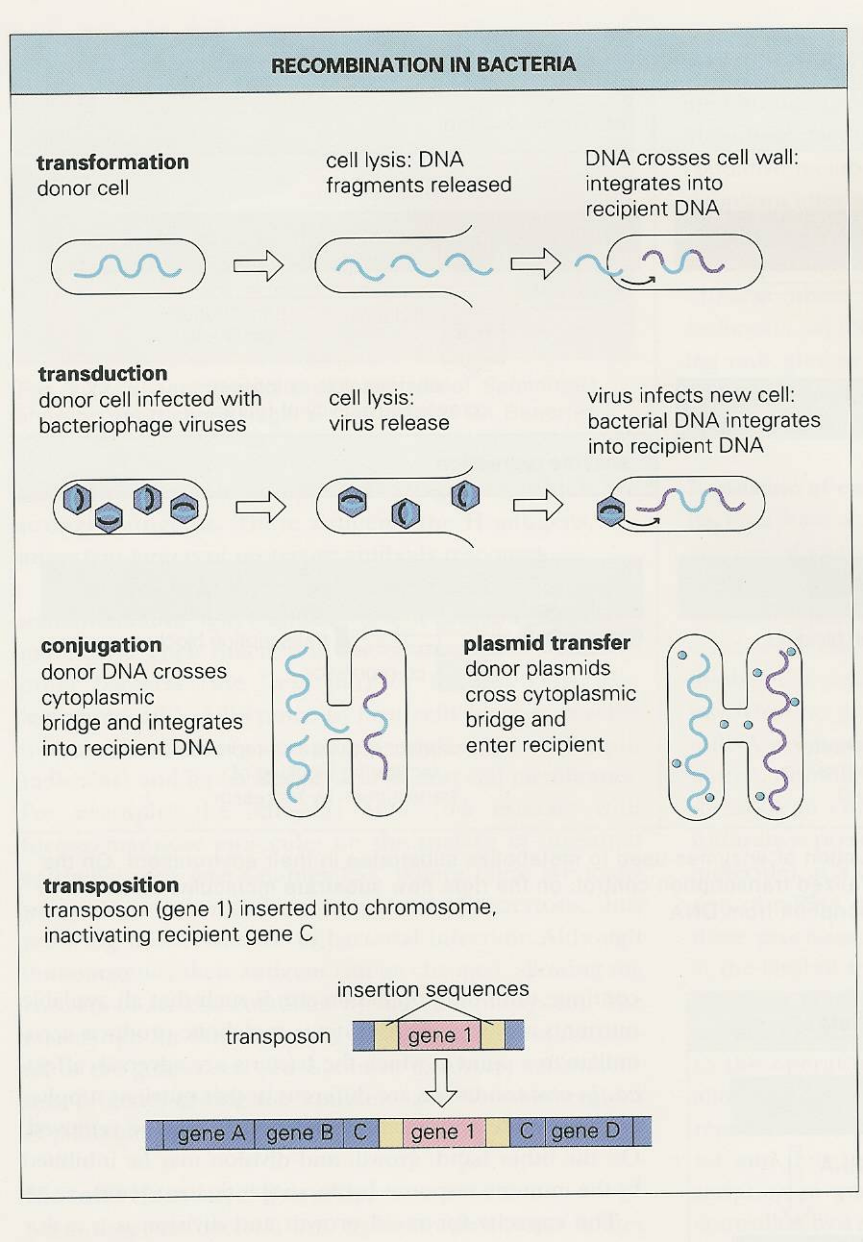
(under ideal growth conditions, the bacteria doubles in 20 minutes)





Jens K. Møller, 2004

Exchange of genetic material in Bacteria

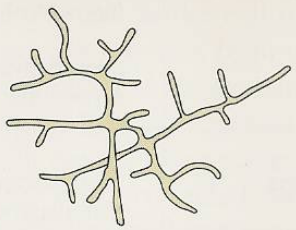


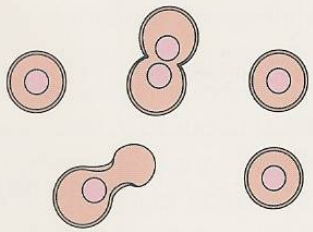
Fungi

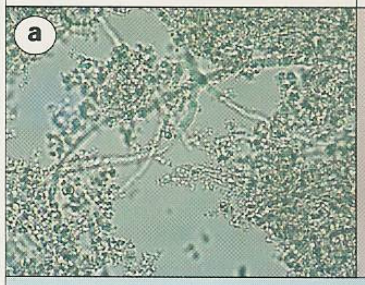
CLASSIFICATION OF FUNGI

by growth form

filamentous	**yeasts**
growing as multinucleate, branching hyphae, forming a mycelium	growing as ovoid or spherical single cells multiply by budding and division







by type of infection

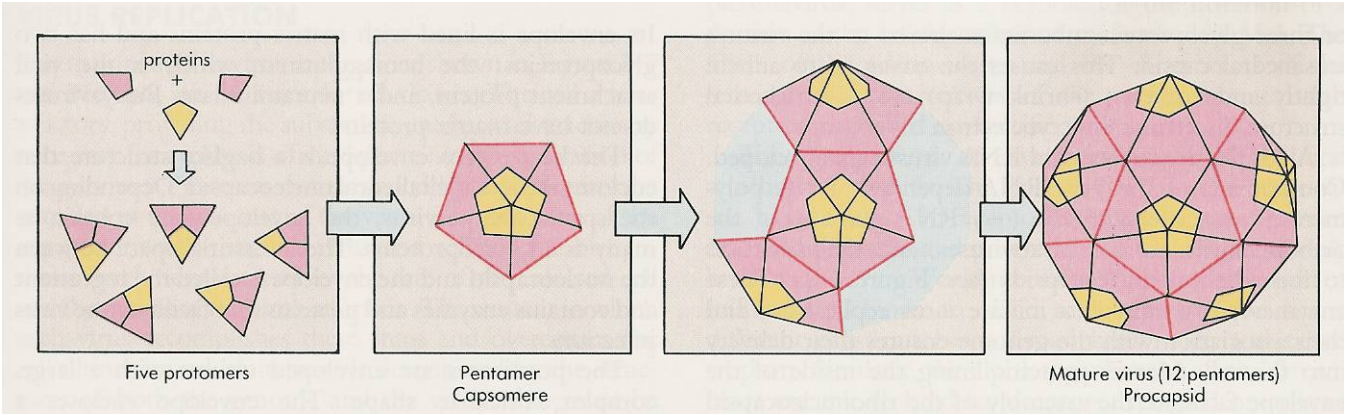
superficial mycoses	**deep mycoses**
Epidermophyton *Microsporum* *Trichophyton* *Sporothrix*	*Aspergillus* *Blastomyces* *Candida* *Coccidioides* *Cryptococcus* *Histoplasma* *Paracoccidiodes*

Dermatomycosis
Superficial and cutanous

Systemic or
subcutanous

Virus – the morphology (Capsid)

Example

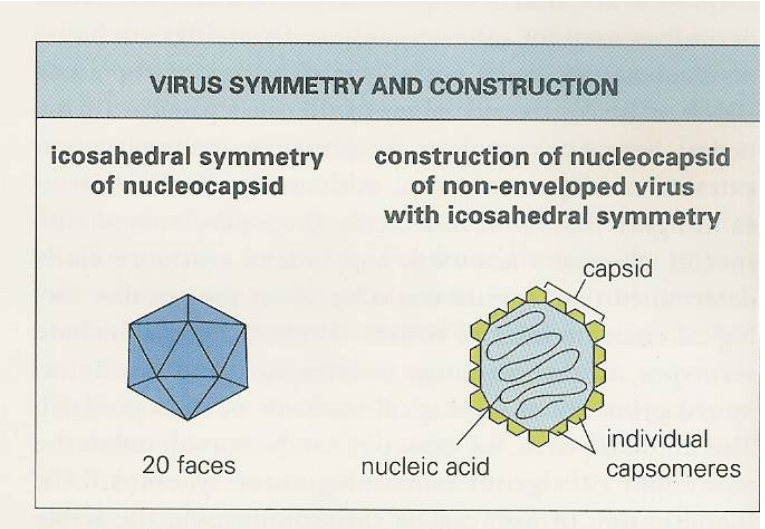


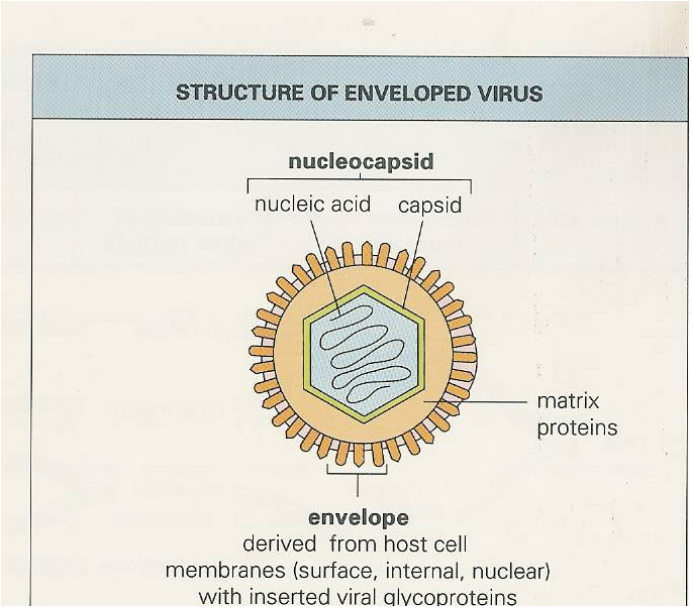
A virus is a very simple construction

(A capsomere is the basic subunit protein in the capsid of a virus)

Capsid is the shell or coating made up of proteins around a virus' genetic material

Virus





Virus are obligate intracellular organisms!

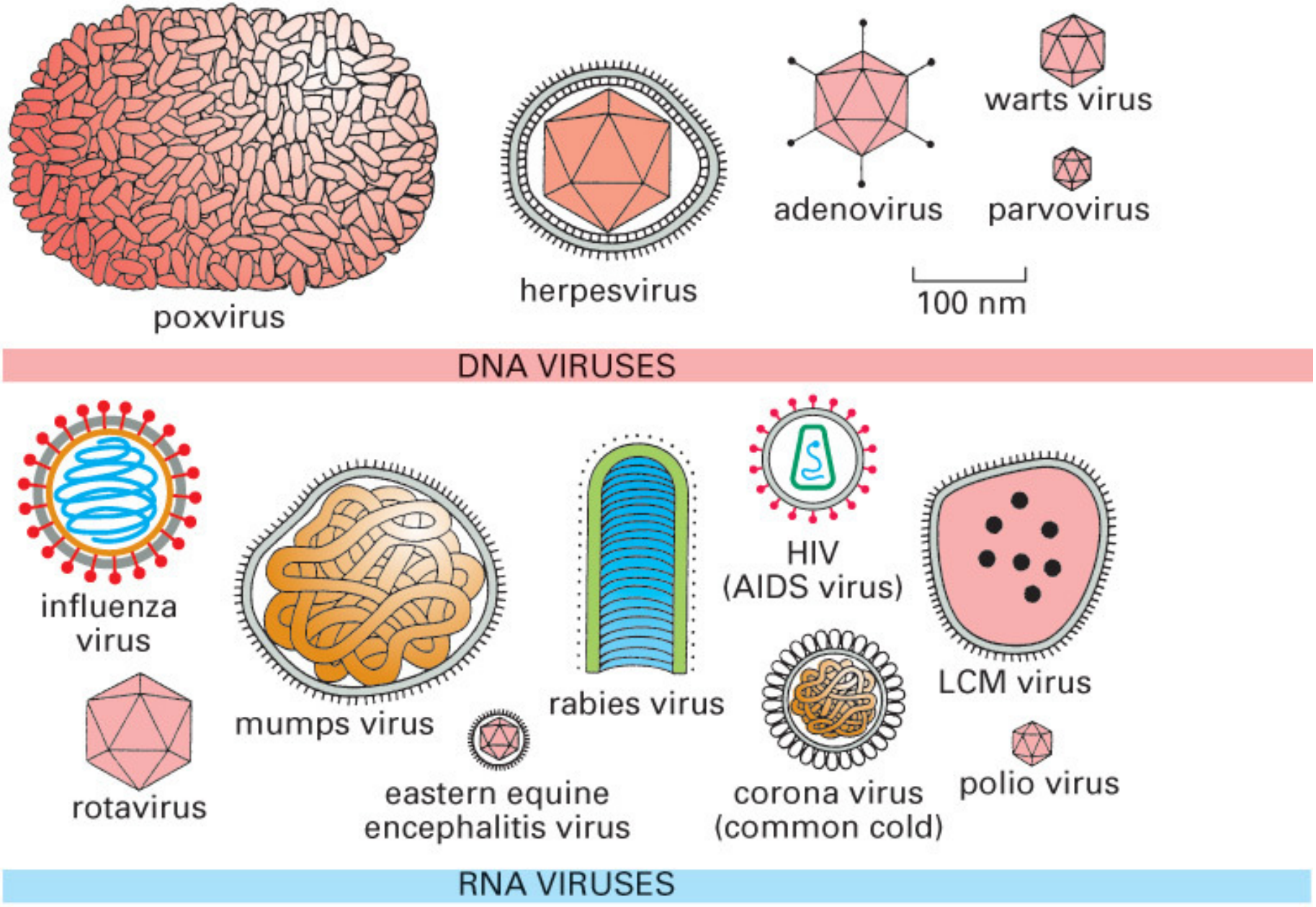
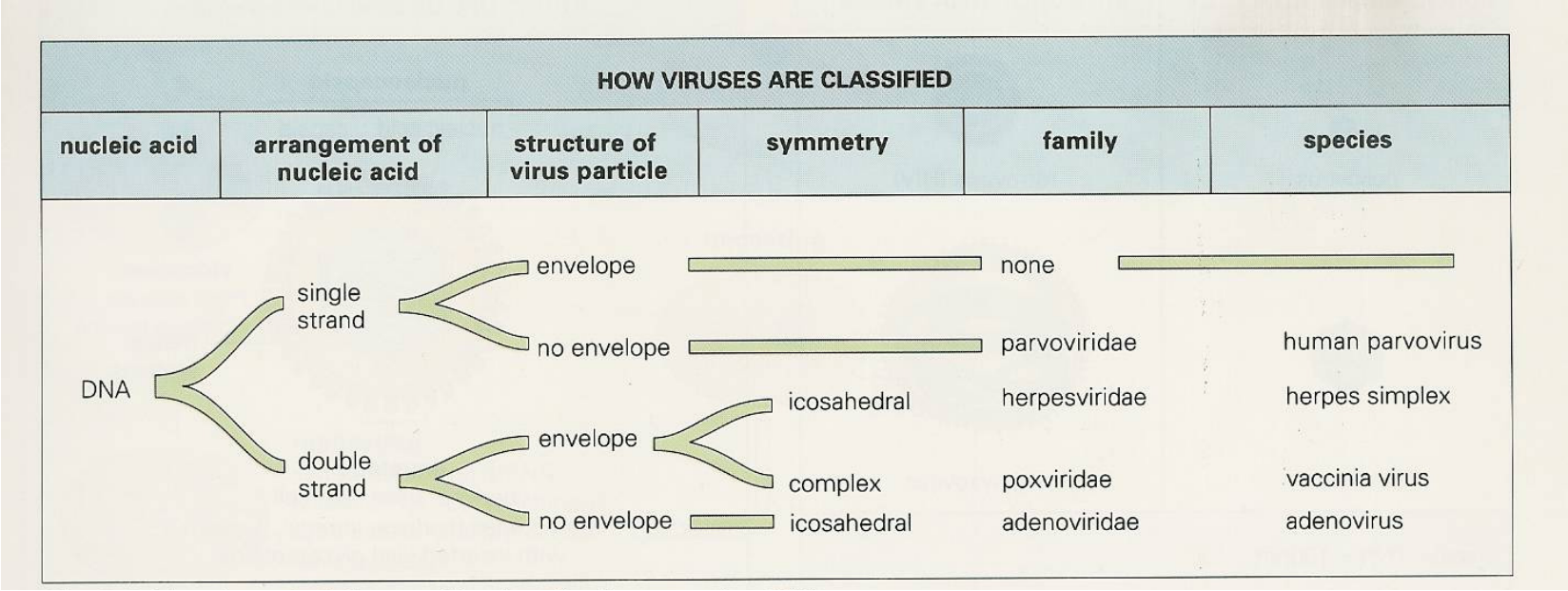


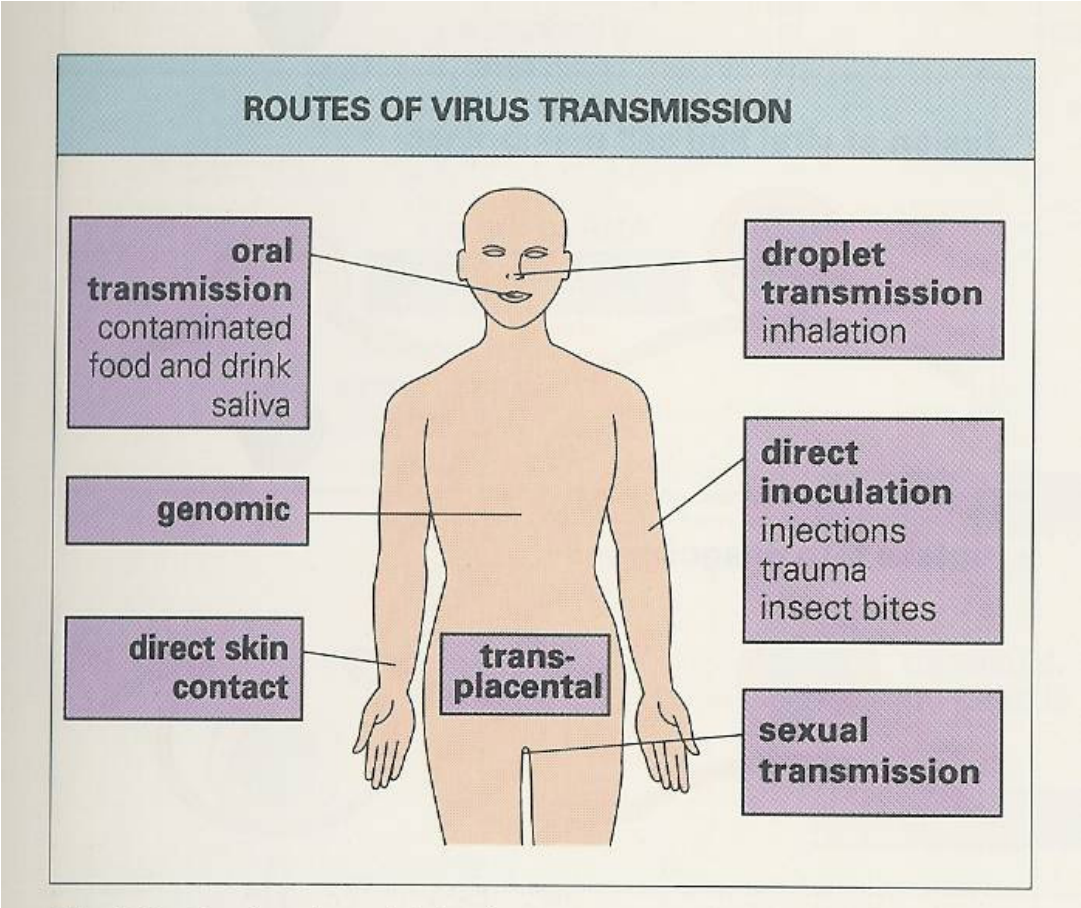
Figure 25–12. Molecular Biology of the Cell, 4th Edition.

The characteristics used to classify virus

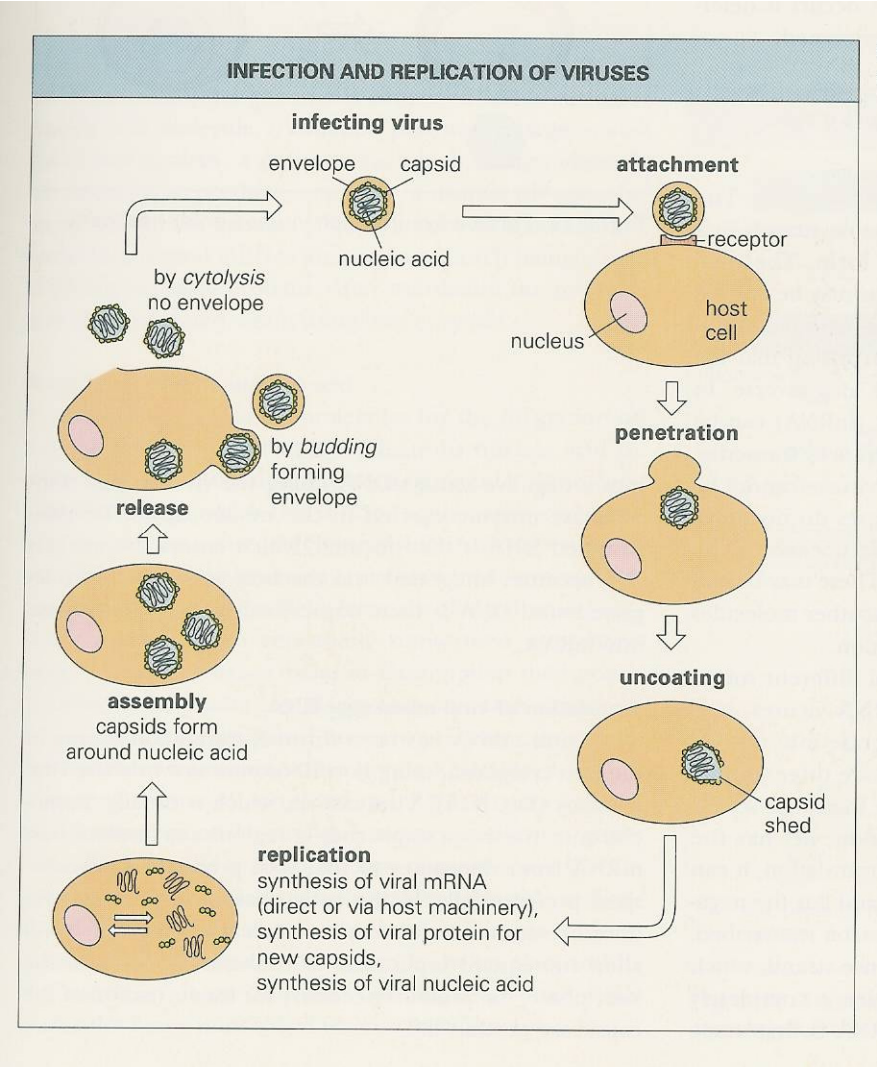
DNA virus



Routes by which a virus enters the body

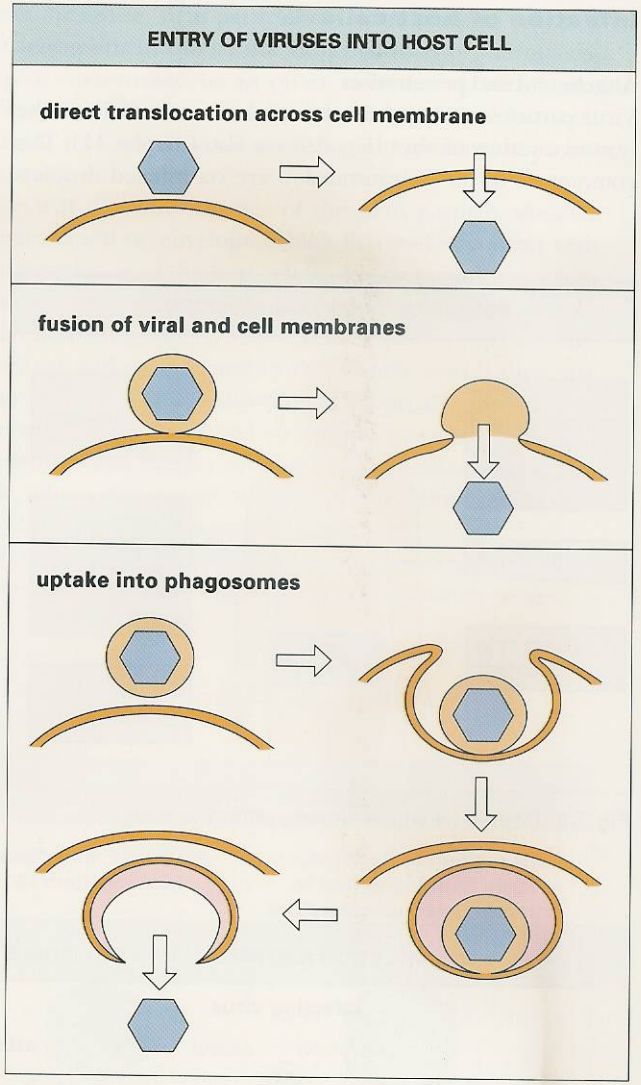


Replication of a virus

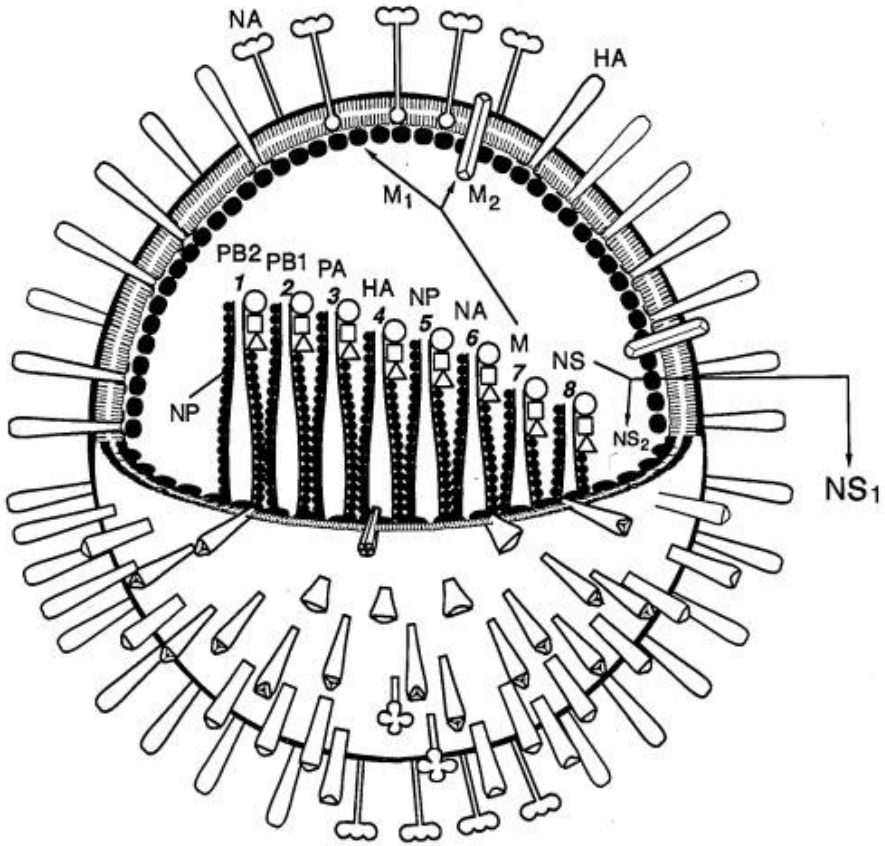


Receptors and virus entry into host cells

CELL MEMBRANE RECEPTORS FOR VIRUSES	
virus	**receptor molecule**
influenza	sialic acid on glycoproteins, including the glycophorin A molecule
rabies	acetylcholine receptor
HIV	CD4 molecule on T cells
Epstein–Barr	C3d receptor on B cells
vaccinia	epidermal growth factor receptor
reovirus type 3	β-adrenergic hormone receptor
encephalomyocarditis	glycophorin A molecule
rhinovirus	intercellular adhesion molecule-1 (ICAM-1)



Influenza virus structure



Structure of influenza virus. The diagram illustrates the main structural features of the virion. The surface of the particle contains three kinds of spike proteins: the hemagglutinin (HA), neuraminidase (NA), and matrix (M2) protein embedded in a lipid bilayer derived from the host cell and covers the matrix (M1) protein that surrounds the viral core. The ribonucleoprotein complex making up the core consists of at least one of each of the eight single-stranded RNA segments associated with the nucleoprotein (NP) and the three polymerase proteins (PB2, PB1, PA). RNA segments have base pairing between their 3´ and 5´ ends forming a panhandle. Their organization and the role of NS2 in the virion remain unresolved. (From Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Fig. 47-2)

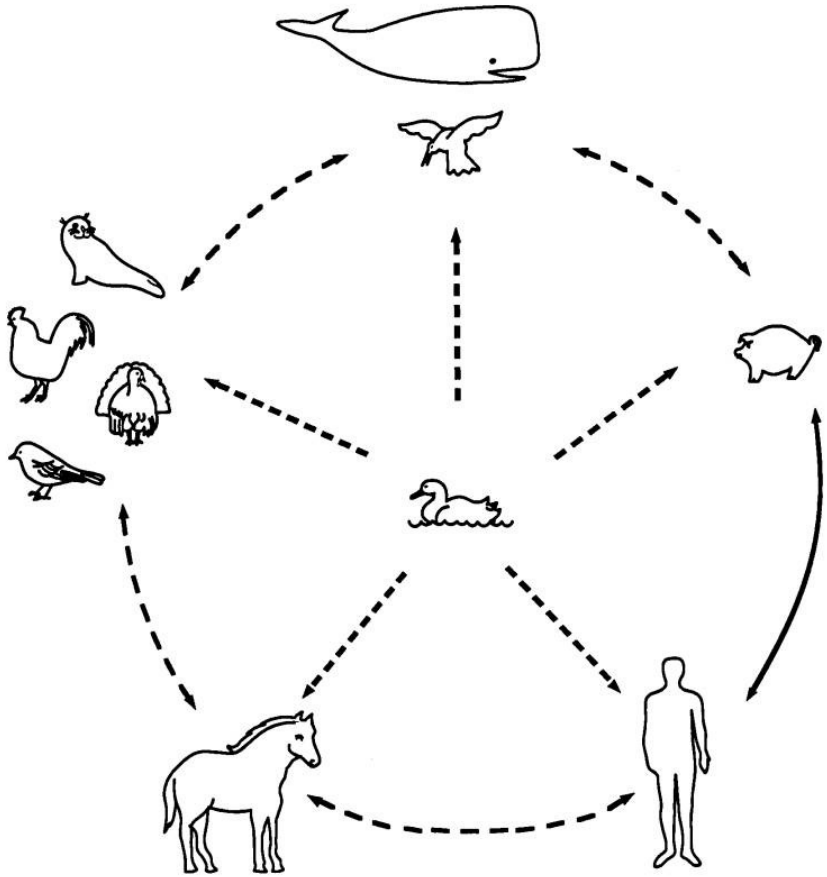
Influenza A hemagglutinin and neuraminidase subtypes

Subtypes	Species of origin[a]			
	Humans	Swine	Horses	Birds
Hemagglutinin				
H1[b]	PR/8/34	Sw/Ia/15/30	—	Dk/Alb/35/76
H2	Sing/1/57	—	—	Dk/Ger/1215/73
H3	HK/1/68	Sw/Taiwan/70	Eq/Miami/1/63	Dk/Ukr/1/63
H4	—	—	—	Dk/Cz/56
H5	—	—	—	Tern/S.A./61
H6	—	—	—	Ty/Mass/3740/65
H7	—	—	Eq/Prague/1/56	FPV/Dutch/27
H8	—	—	—	Ty/Ont/6118/68
H9	—	—	—	Ty/Wis/1/66
H10	—	—	—	Ck/Ger/N/49
H11	—	—	—	Dk/Eng/56
H12	—	—	—	Dk/Alb/60/76
H13	—	—	—	Gull/MD/704/77
H14	—	—	—	Dk/Gurjev/263/82
H15	—	—	—	Dk/Austral/341/83
Neuraminidase				
N1	PR/8/34	Sw/Ia/15/30	—	Ck/Scot/59
N2	Sing/1/57	Sw/Taiwan/70	—	Ty/Mass/3740/65
N3	—	—	—	Tern/S.A./61
N4	—	—	—	Ty/Ont/6118/68
N5	—	—	—	Sh/Austral/1/72
N6	—	—	—	Dk/Cz/56
N7	—	—	Eq/Prague/1/56	FPV/Dutch/27
N8	—	—	Eq/Miami/1/63	Dk/Ukr/1/63
N9	—	—	—	Dk/Mem/546/74

[a]The reference strains of influenza viruses, or the first isolates from that species, are presented.
[b]Current subtype designation.

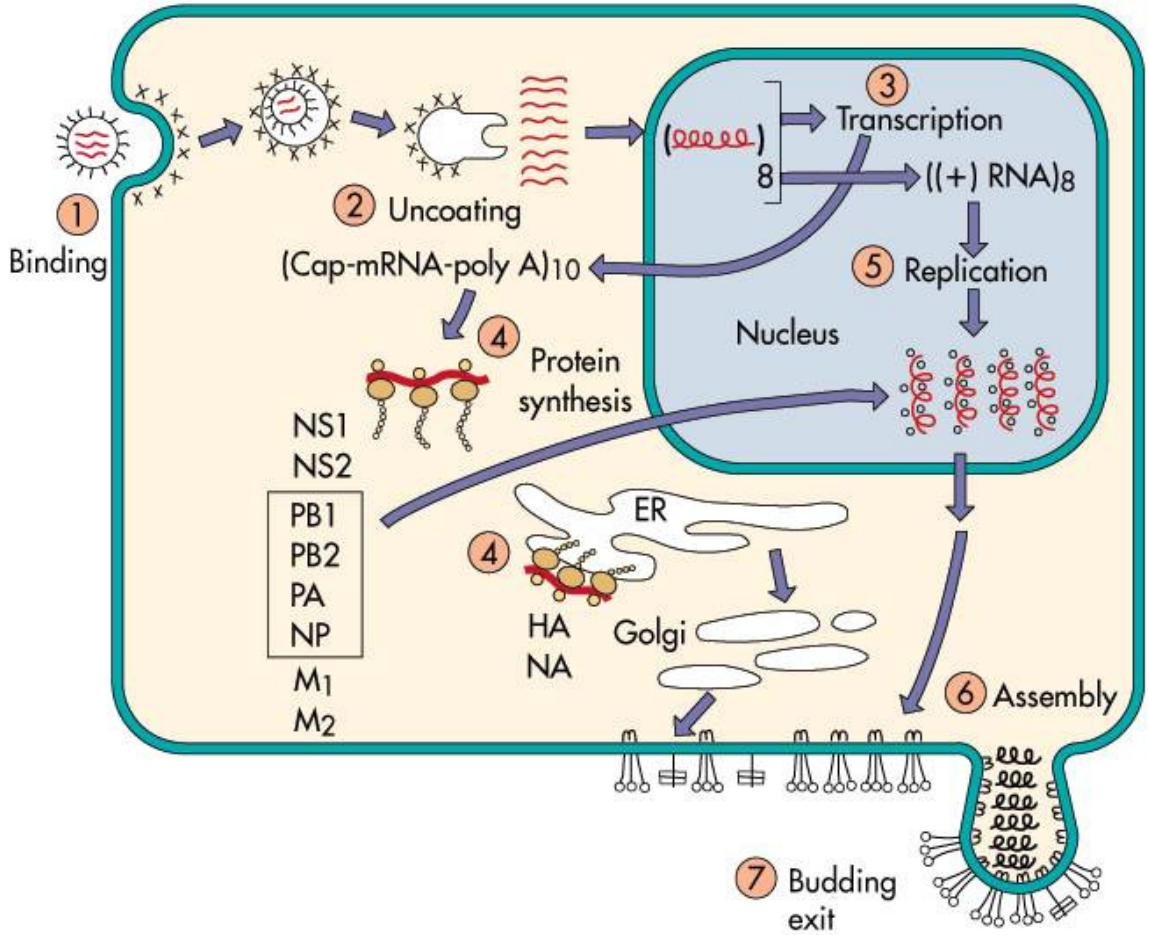
Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Table 47-1

Influenza A reservoir



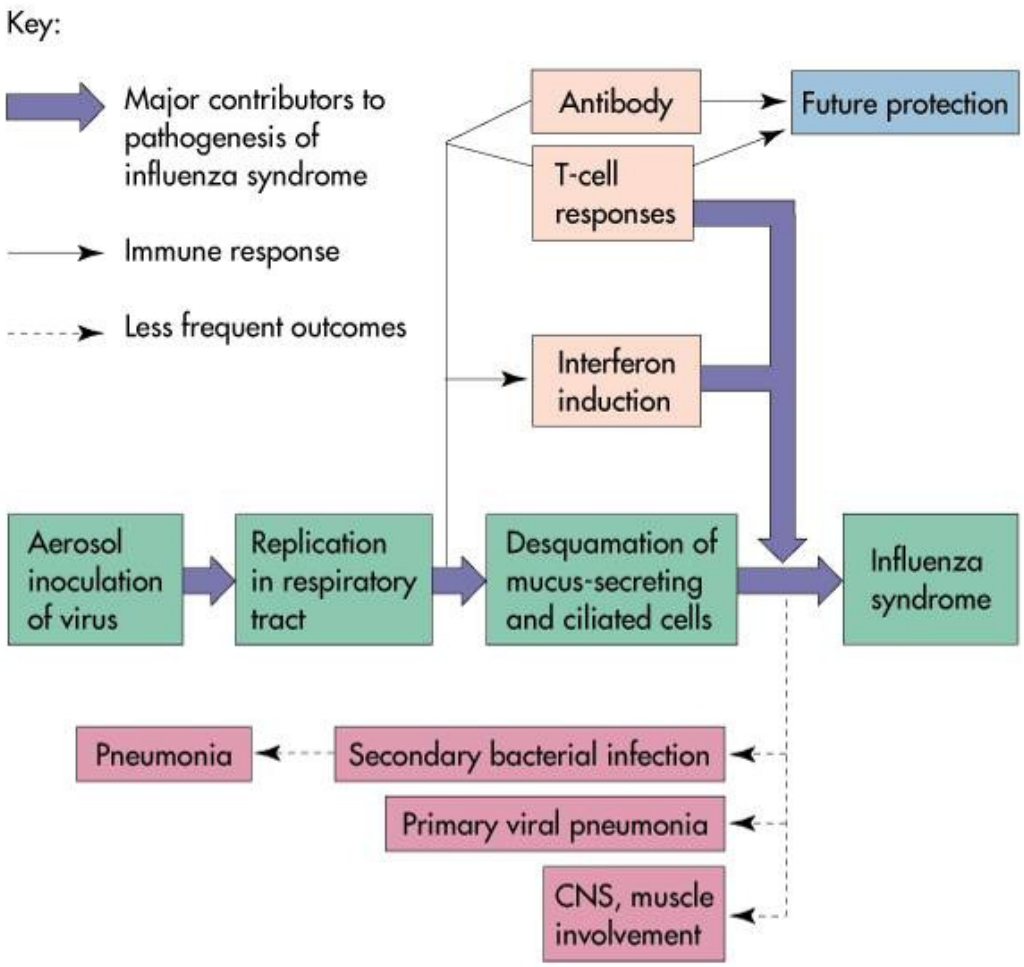
The reservoir of influenza A viruses. The working hypothesis is that wild aquatic birds are the primordial reservoir of all influenza viruses for avian and mammalian species. Transmission of influenza has been demonstrated between pigs and humans (solid lines). There is extensive evidence for transmission between wild ducks and other species, and the five different host groups are based on phylogenetic analysis of the nucleoproteins of a large number of different influenza viruses. (From Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Fig. 47-3.)

Influenza replication



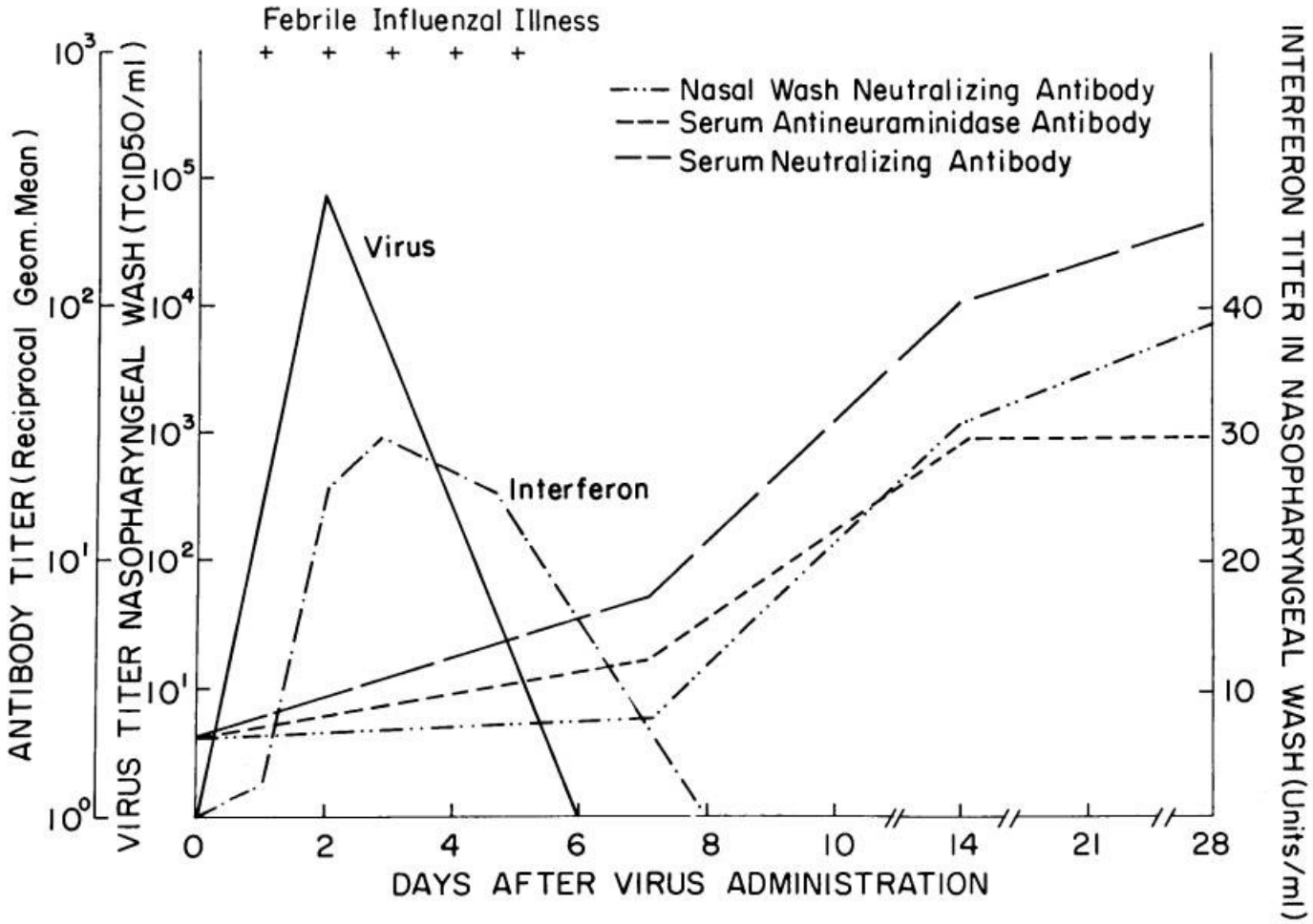
From Medical Microbiology, 5th ed., Murray, Rosenthal & Pfaller, Mosby Inc., 2005, Figure 60-2.

Influenza pathogenesis



From Medical Microbiology, 5th ed., Murray, Rosenthal & Pfaller, Mosby Inc., 2005, Figure 60-3.

Influenza pathogenesis in humans



Six seronegative volunteers received 104.0 TCID50 of wild-type A/Bethesda/1015/68 virus intranasally on day 0. (From Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Fig. 47-10.)