Searching for microbes
Part XII.
Virology – Part One

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To practical of VLLM0421c

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Content of this slideshow

Viruses

Hepatitis viruses

HIV

Diagnostics of hepatites and HIV infection

Practical diagnostic approaches

Story

- Buy the peach, Ma'am! cried a seller on an exotic marktet. Mrs. Green bought them and took them to the hotel.
- In the hotel room she wanted to wash them, but oops – water is not runing. What to do now? Mrs. Green was unsure, but the peaches were SO nice… So, she decided to eat several fruits.
- After coming back from abroad, Mrs. Green became yellow…

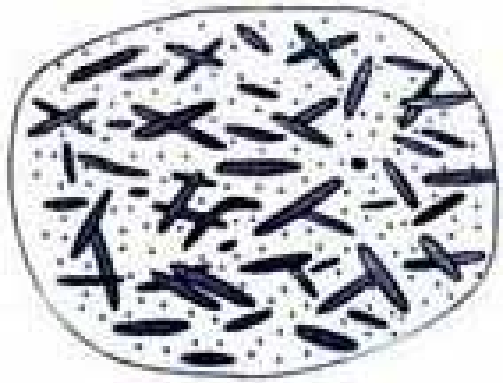
Who was the criminal?

- Hepatitis A virus. But it could be also hepatitis E virus – both of them are transmitted by dirty hands and unwashed nutrients.
- Besides these hepatites there are also other, transmitted by blood or sexually
- There are various hepatites, and the viruses causing them are different. But one problem is common: the diagnostic should respect the fact that they are viruses, not bacteria.

Viruses

How viruses look like

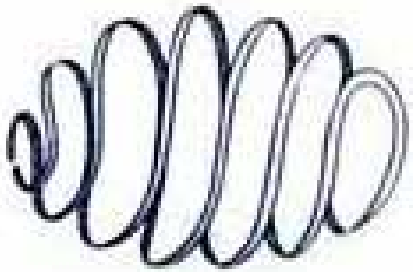
http://vietsciences.free.fr/khaocuu/nguyenlandung/virus01.htm



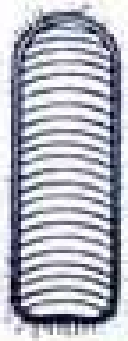
(a) Vaccinia virus

(b) Paramyxovirus (mumps)

(c) Herpesvirus



(d) Orf virus



(e) Rhabdovirus

(f) T-even coliphage

(g) Flexuous-tailed phage

(h) Adenovirus

(i) Influenza virus

(m) Tubulovirus

What are viruses?

- Viruses are acellullar particles, it is unsure, whether they are organisms at all
- On viral origin there are several teories, it is not sure that all of them have the same origin
- Like cellullar organisms, they atempt to „keep the gene", but they need an alien cell to it
- Besides human viruses we have animal viruses, plant and bacterial viruses (bacteriophages)
- Prions – „mistakes in protein" are not viruses, but they use to be described in virology.

Classifying viruses

- According to nucleic acid: DNA viruses and RNA viruses
- According to number of DNA/RNA strands: single stranded (ss) and double stranded (ds), in single stranded also according to "plus" or "minus" strand
- According to presence of viral envelope both DNA, and RNA are classified into enveloped and unenveloped
- Further they are clasified into families and genera, like bacteria or animals; species names are usually not used.

Viral particle – virion

- Virion is not a cell. Virions are smaller than majority of cells, including bacterial cells: mostly 20 – 300 nm
- Composition of a virion
  - nucleocapsid or nucleus + capsid
  - envelope (in enveloped viruses)
  - in some viruses atypical composition (VHB)

When a virus is just present in a host cell, its structure and arrangement is different from virus in outer environment

Nucleocapsid

- is present in all viruses
- is composed of nucleic acid (DNA, RNA) and protein capsid
- capsid may have helicoidal, cubic or other symetry.
- viruses with cubic symetry form so named pseudocrystals – regular formations

Lipoprotein envelope

- only enveloped viruses have it.
- it is a lipidic bilayer, coming from host cell (originally cytoplasmic membrane, nuclear membrane, etc.) with viral proteins inside
- sometimes the membrane is connected by a specific protein to the nucleocapsid.
- enveloped viruses are less resistant (they die at envelope dammage e. g. by drying)
- More about viruses: see Bonus info

Hepatitis viruses

Hepatitis viruses

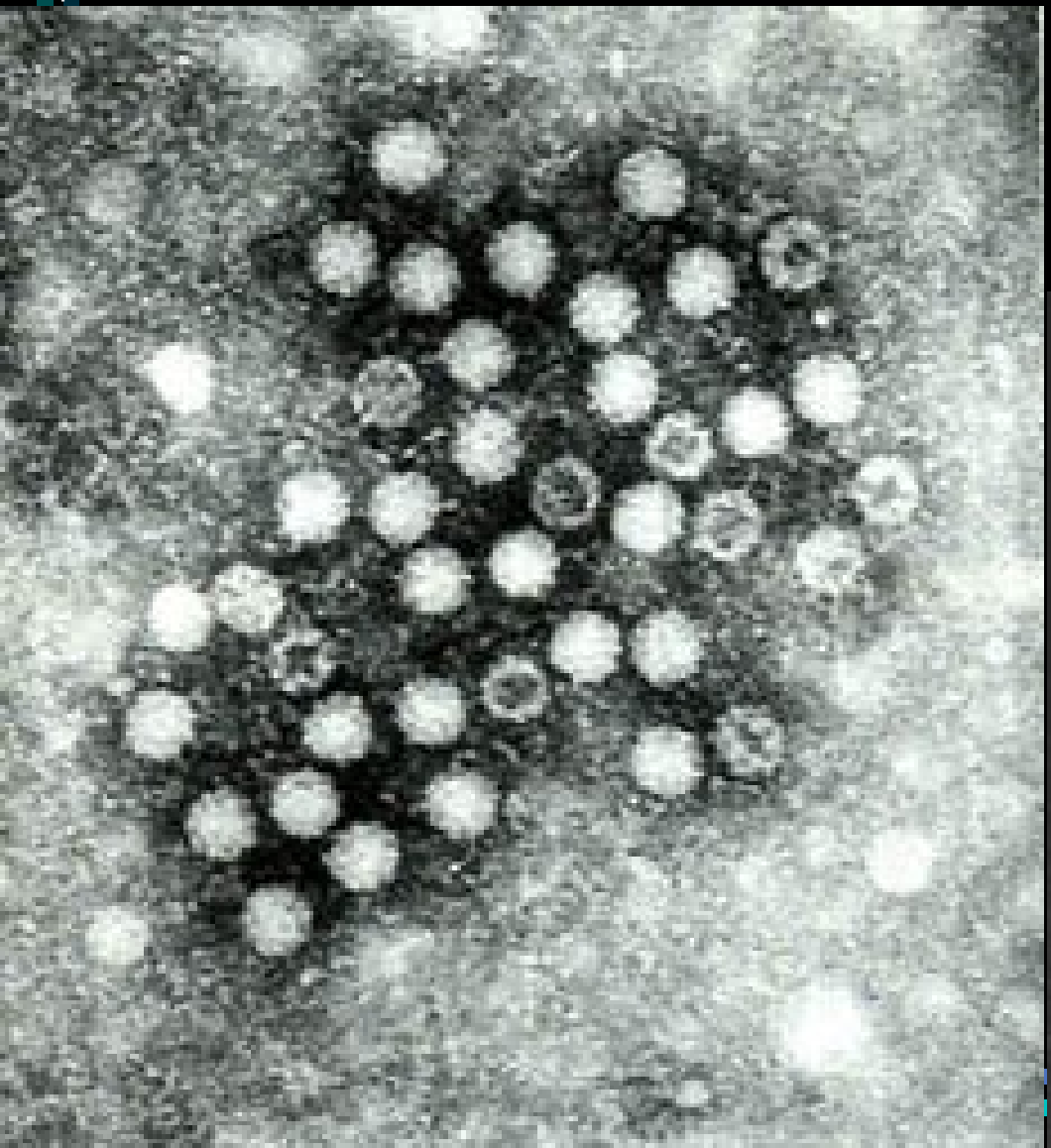
- There are five main types of hepatites VHA to VHE, caused by viruses HAV to HEV. Each belongs to another family, **majority are RNA viruses, but hepatitis B virus is a DNA virus**
- **VHA and VHE** (mnemotecnic help: vocals) are transmitted by **fecal oral route** (hands), **they do not become chronical**
- **VHB, VHC and VHD** (consonants ☺) – transmission by **blood, or sexual** (in VHC rather unimportant), **they may swith into chronicity**

Survey of hepatites

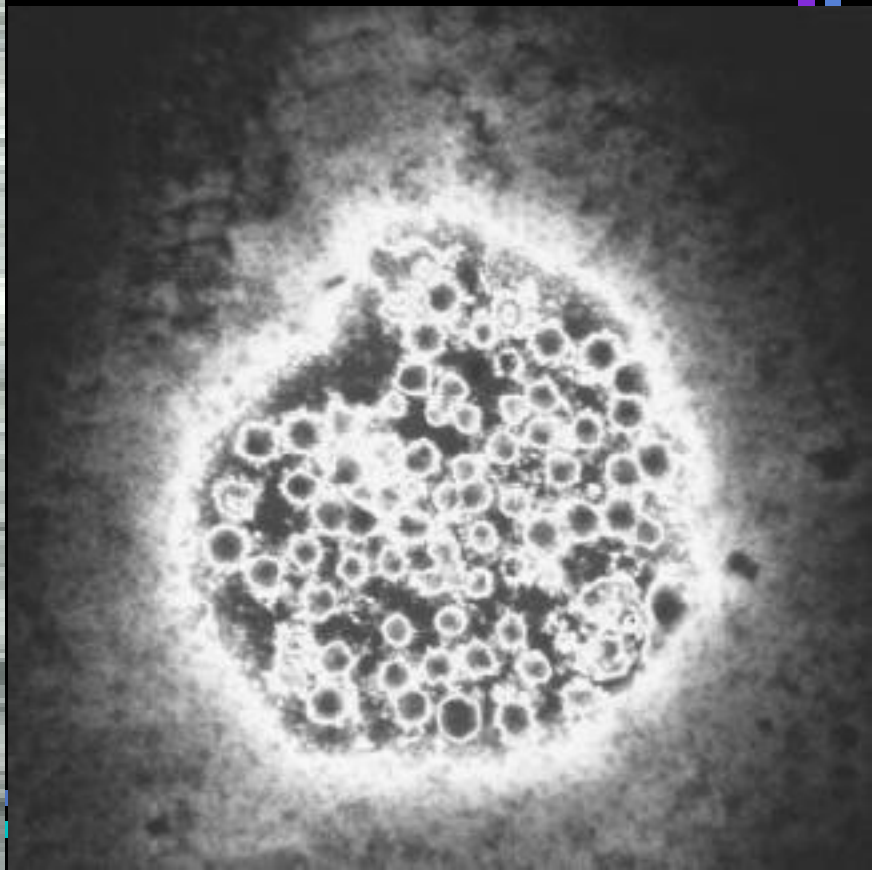
Hepatitis	Virus	Transmission
HAV	Picornavirus	faecal-oral
HBV	Special group of DNA viruses	sexual, blood
HCV (+ HGV)	Flavivirus	blood
HDV	Delta agens – viroid	sexual, blood
HEV	Related to caliciviruses	faecal-oral

Hepatitis A virus

http://www.epidemic.org/cgi-bin/hepcglossary.cgi?query=HepatitisA&caller=theFacts/viruses/viralReplication.html

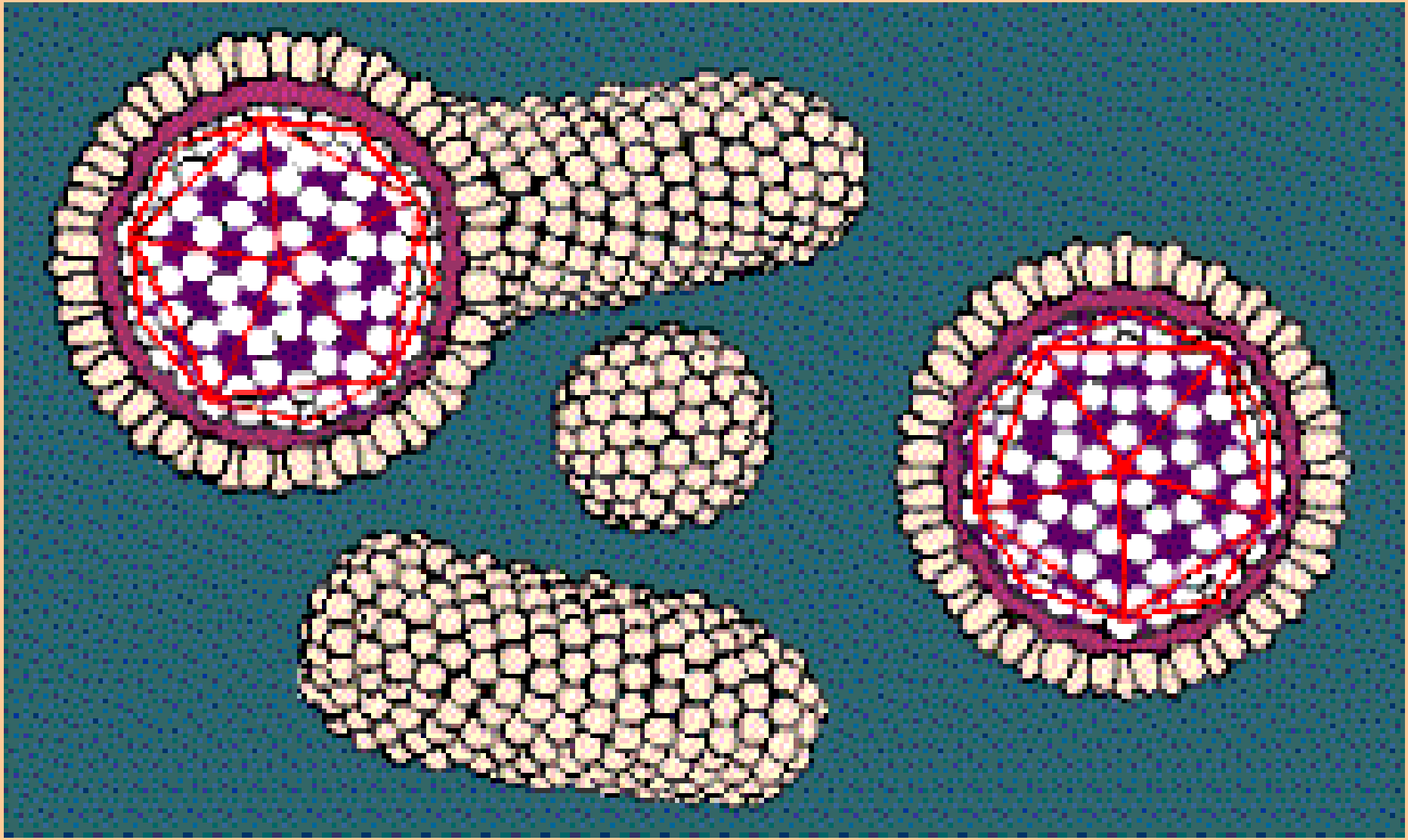


www.faqs.org/health/Sick-V2/Hepatitis.html.

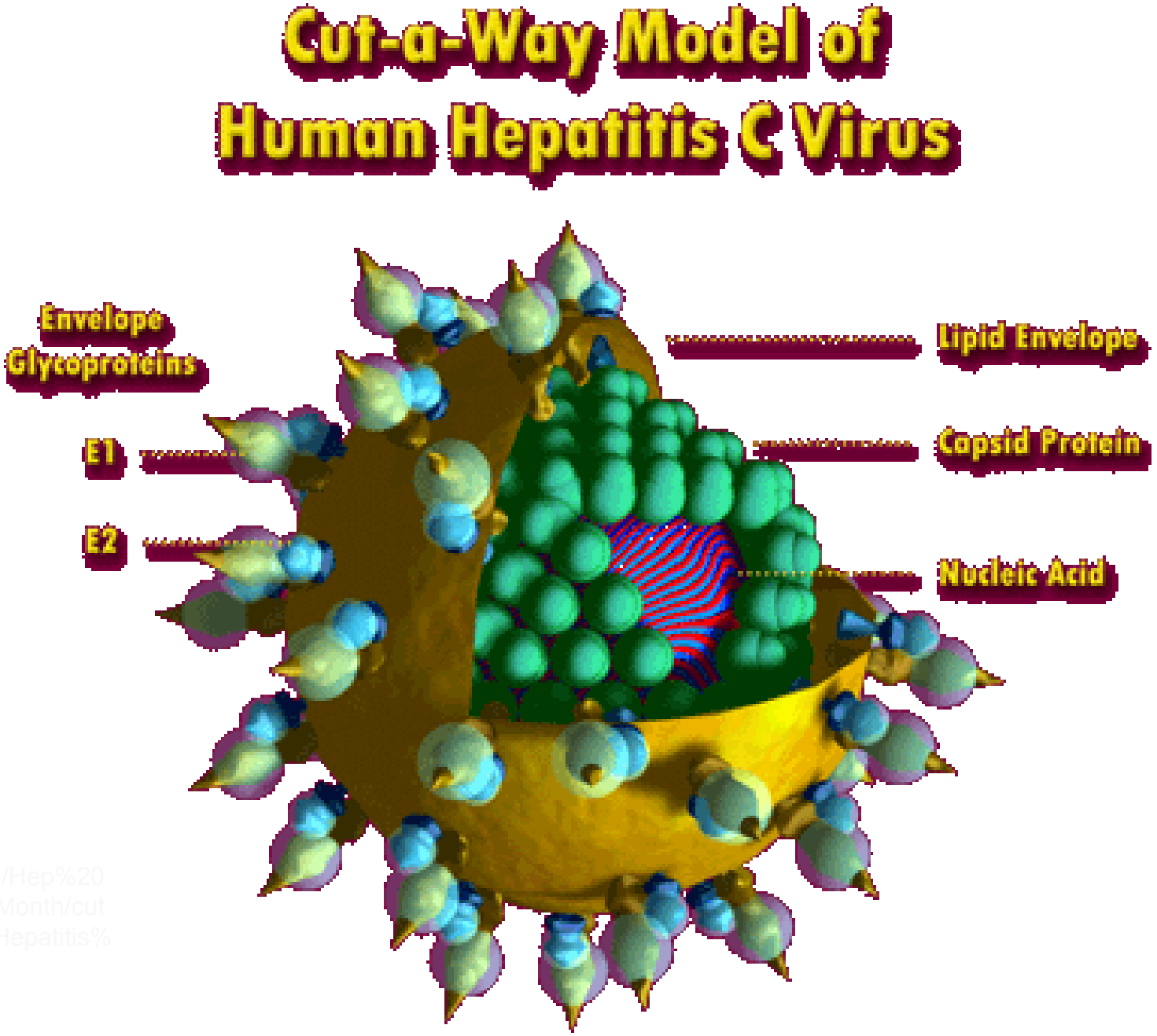


Hepatitis B virus

www.uct.ac.za/depts/mmi/stannard/hepb.html.

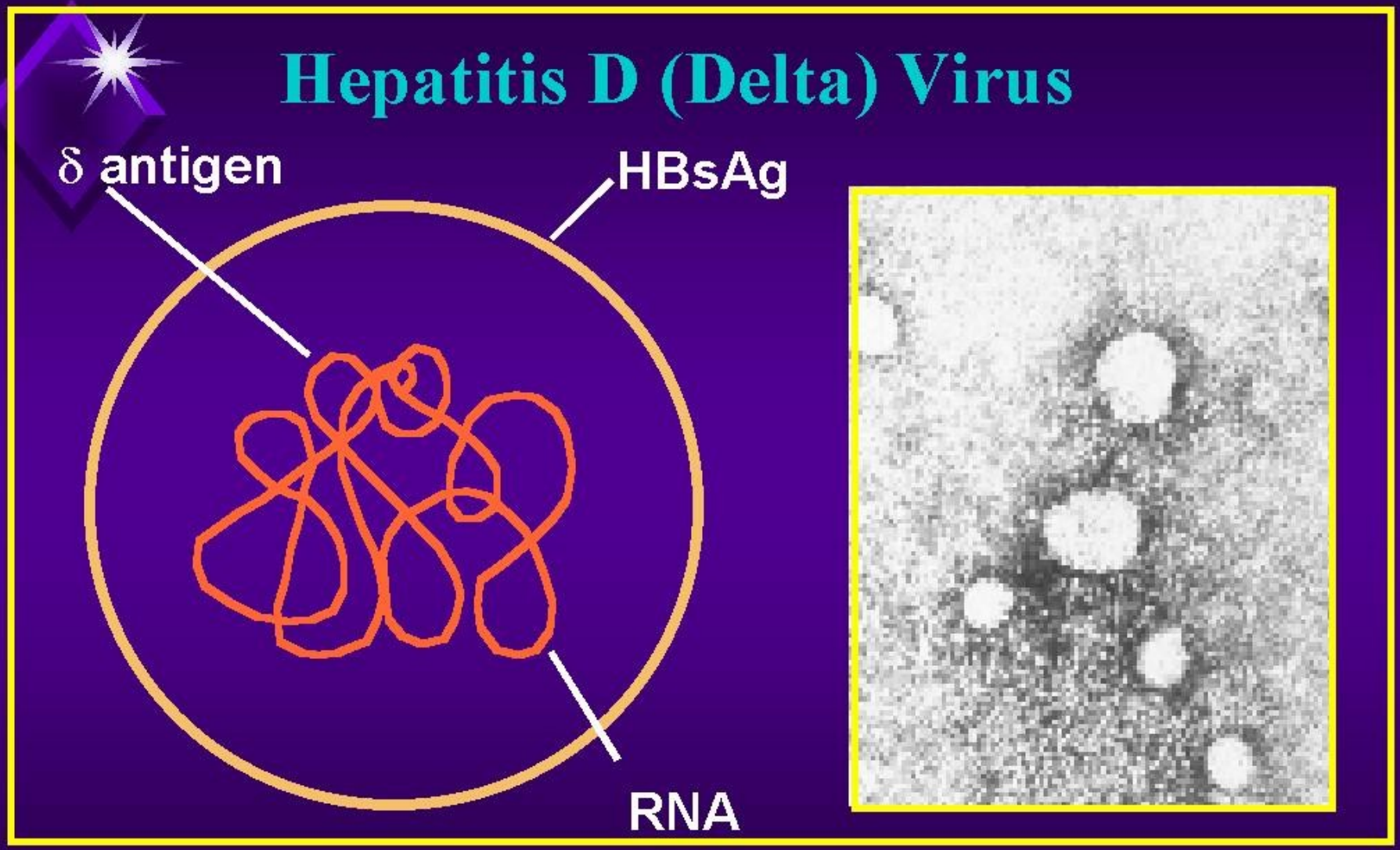


Hepatit virus



http://www.pcswn.com/Hep%20C%20Awareness%20Month/cut%20model%20of%20Hepatitis%20C%20virus.gif

Hepatitis D virus



virology-online.com/viruses/HepatitisD.htm

Hepatitis E virus

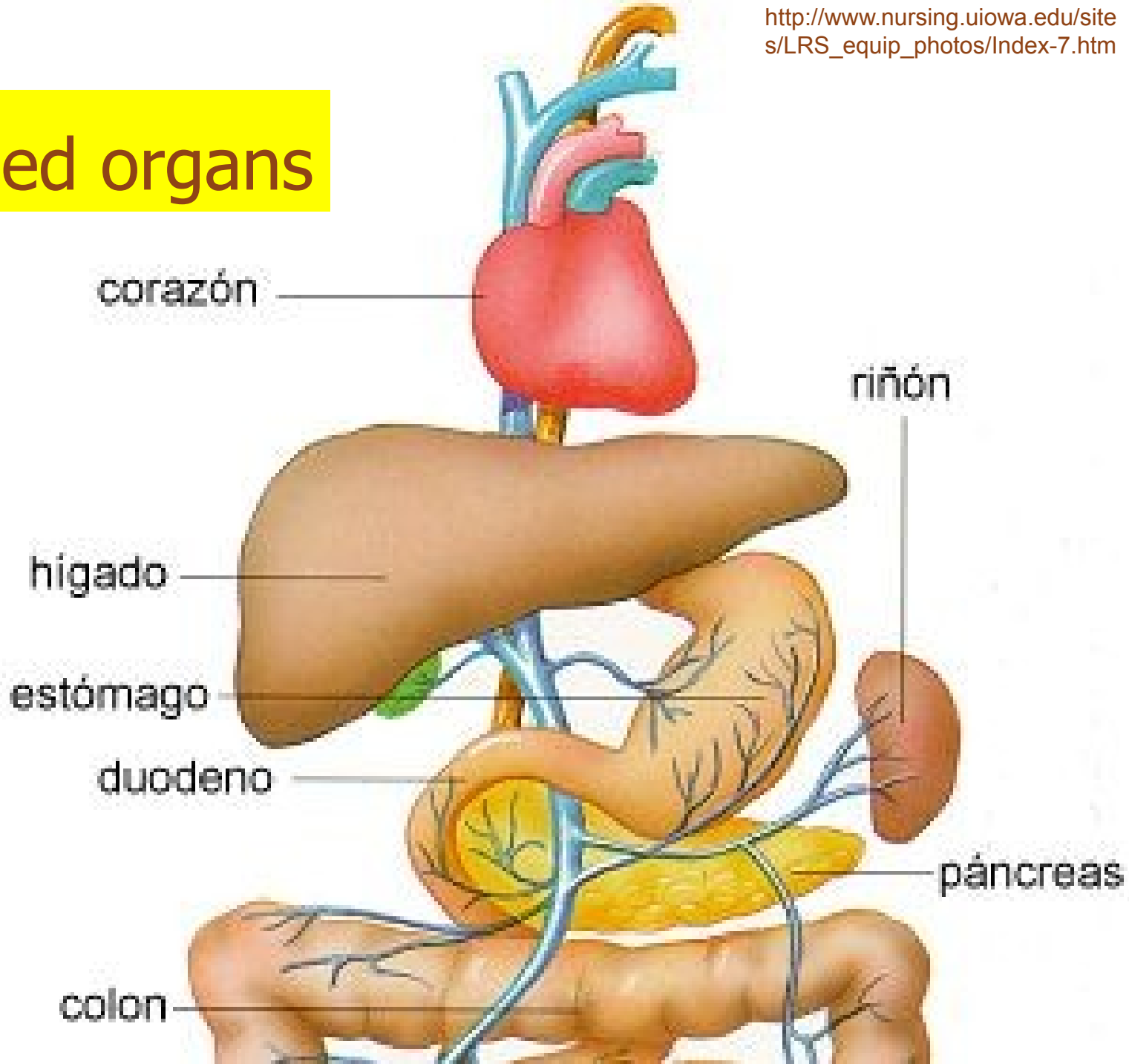
http://vietsciences.free.fr/khaocuu/nguyenlandung/virus01.htm

Hepatites

- They are **infectious liver inflamations**, with jaundice as a typical symptoma. Not all jaundices mean a hepatitis: in some inborn diseases, or in cholelitiasis (stones in bile ways) jaundice is present, too.
- **Patient** has fever, intestinal problems, yellow colour of sclera or skin, changed urine and stool colour etc. Hepatitis B, C and D may become chronical, and they may act as precanceroses.

Diseased organs

http://www.nursing.uiowa.edu/sites/LRS_equip_photos/Index-7.htm



Patients with jaundice

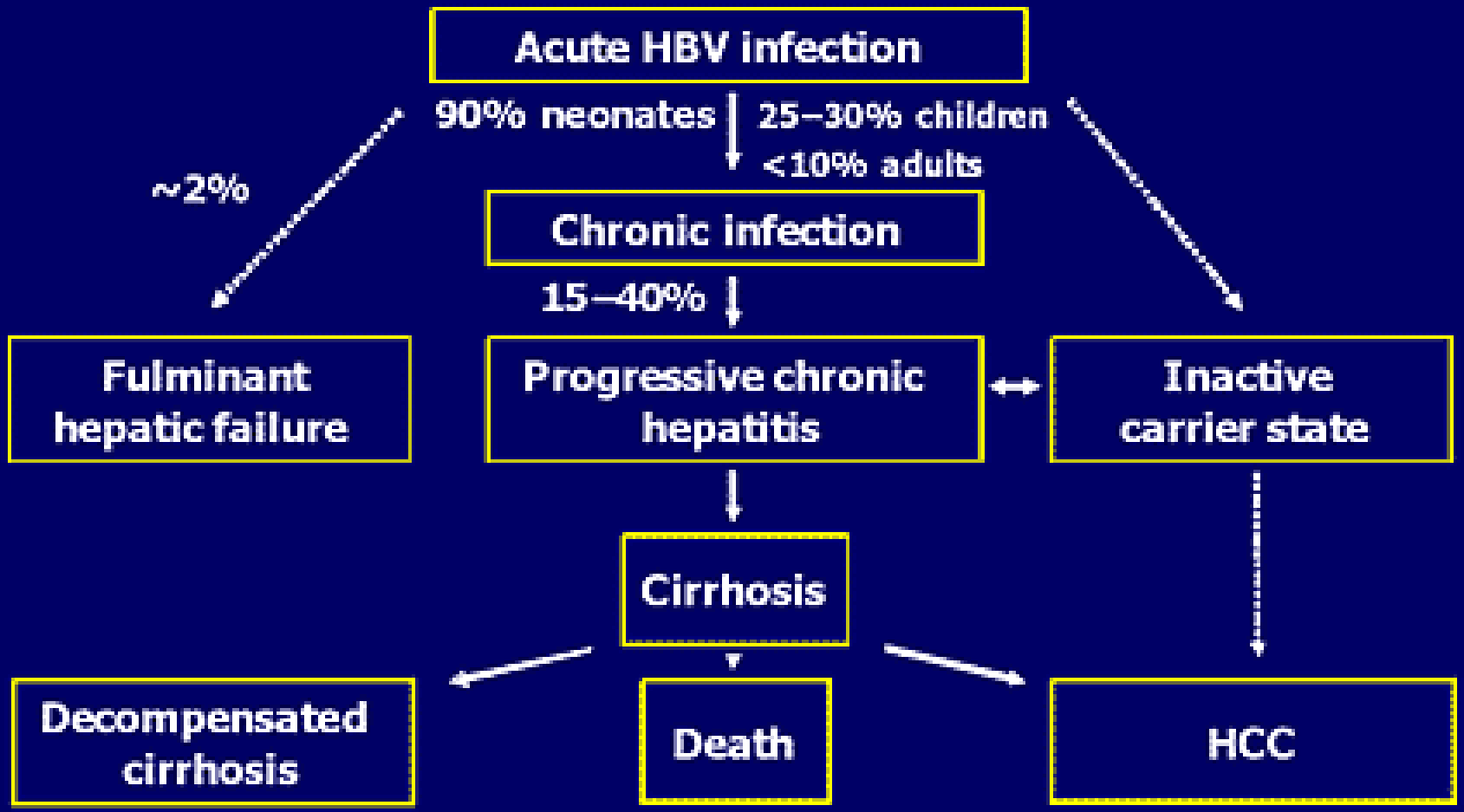
http://www.gihealth.com/images/imgJaundiceBig.jpg

medicine.ucsd.edu/Clinicalimg/skin-jaundice.html

Mostly hepatitis B is a classical health problem

- switch to chronicity, possibility of cirrhosis or hepatoma
- sooner transmission in hospitals, today only sexual transmission and i. v. drug abusers
- screening of hepatitis B common in many situations (before operations etc.)

Natural History of Hepatitis B



www.pegasys.com/hcp/efficacy-hepatitis-b.aspx

3. Lok ASF, McMahon BJ. *Hepatology.* 2004;39:857-861.

4. Lok ASF, McMahon BJ. AASLD Practice Guidelines. Available at: https://www.aasld.org/eweb/docs/chronichep_B.pdf.

HBV

A Global Health Problem

Country	HBsAg+ (%)
China	5.3–12[2]
S. Korea	2.6–5.1[2]
India	2.4–4.7[2]
Taiwan	10–13.8[2]
Viet Nam	5.7–10[2]
Japan	4.4–13[2]
Africa	5–19[2]
Russia	1.4–8[2]
Europe	0.3–12[2]

WHO. Hepatitis B. 2002. Available at: http://www.who.int/csr/disease/hepatitis/whocdscsrlyo20022/en/.

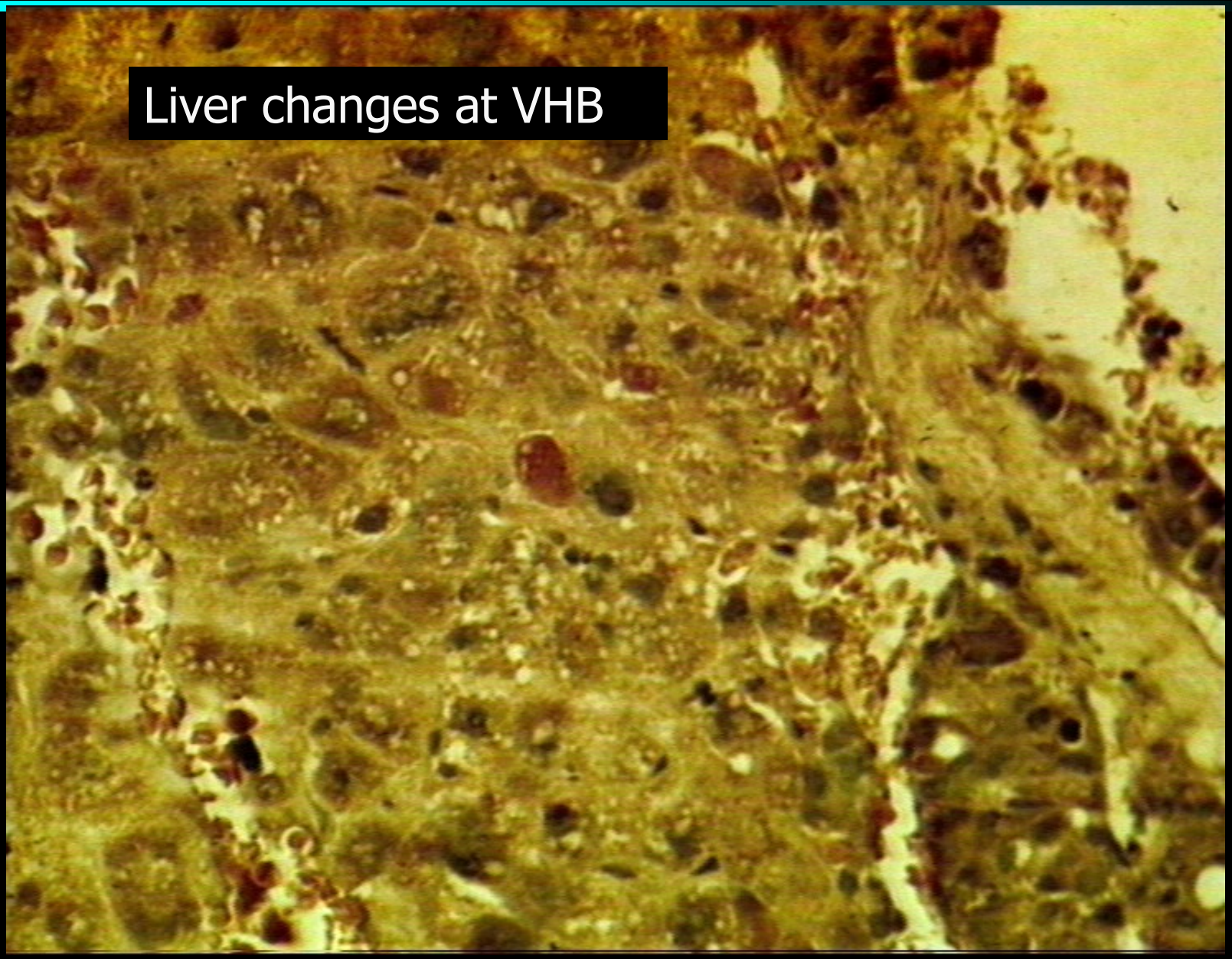
Custer B et al. *J Clin Gastroenterol.* 2004;38(10 suppl):S158-S168.

WHO. Seroprevalence of hepatitis B in WPRO. Available at: http://www.wpro.who.int/pdf/EPI/seroprevalence_hepatitisB_WPRO.pdf.

www.pegasys.com/hcp/efficacy-hepatitis-b.aspx

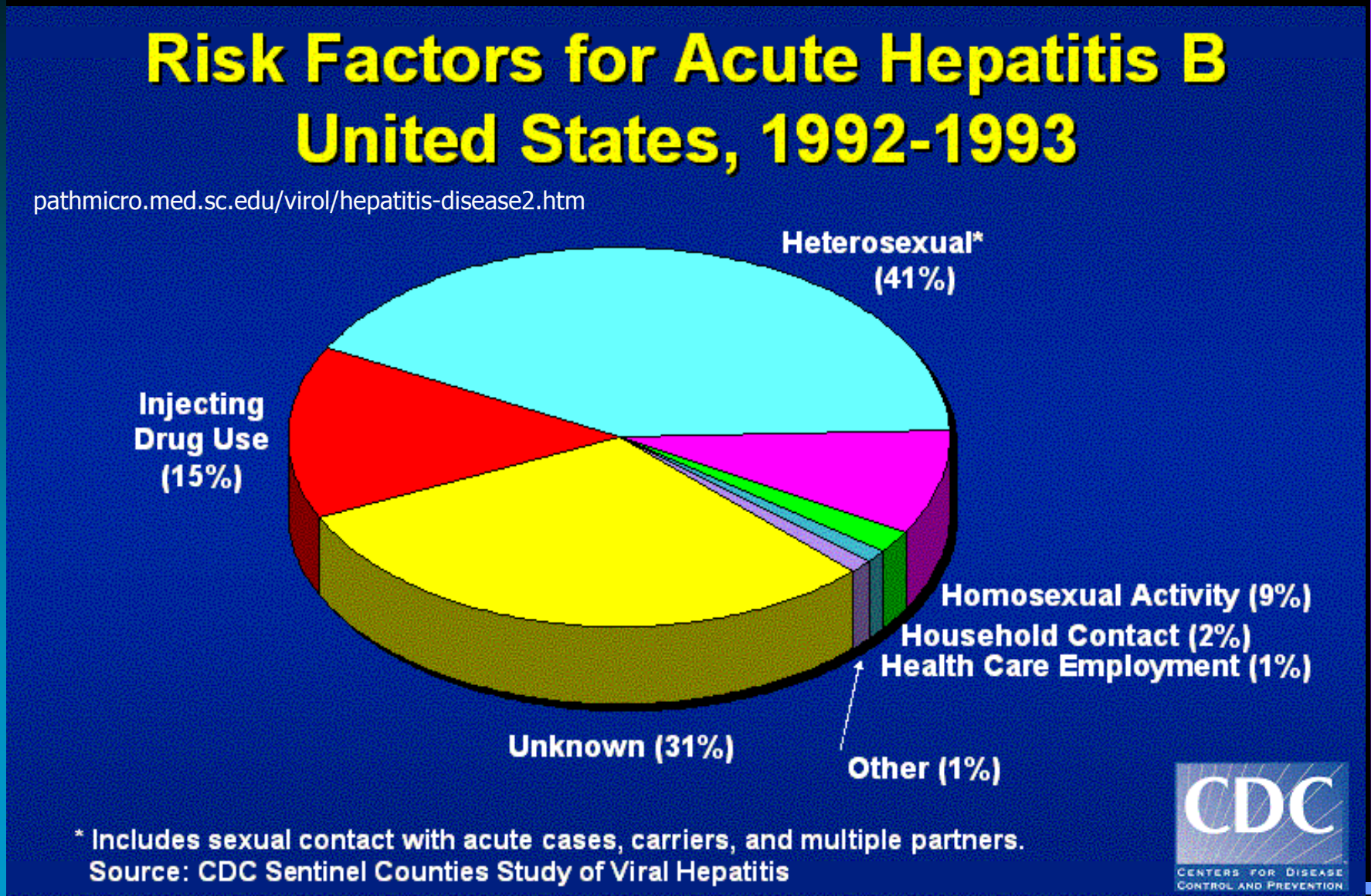
pathmicro.med.sc.edu/virol/hepatitis-disease2.htm

Up: pacient with jaundice at hepatitis B. Right: woman from Thai refugee camp, with hepatoma after hepatitis, later died because of her disease

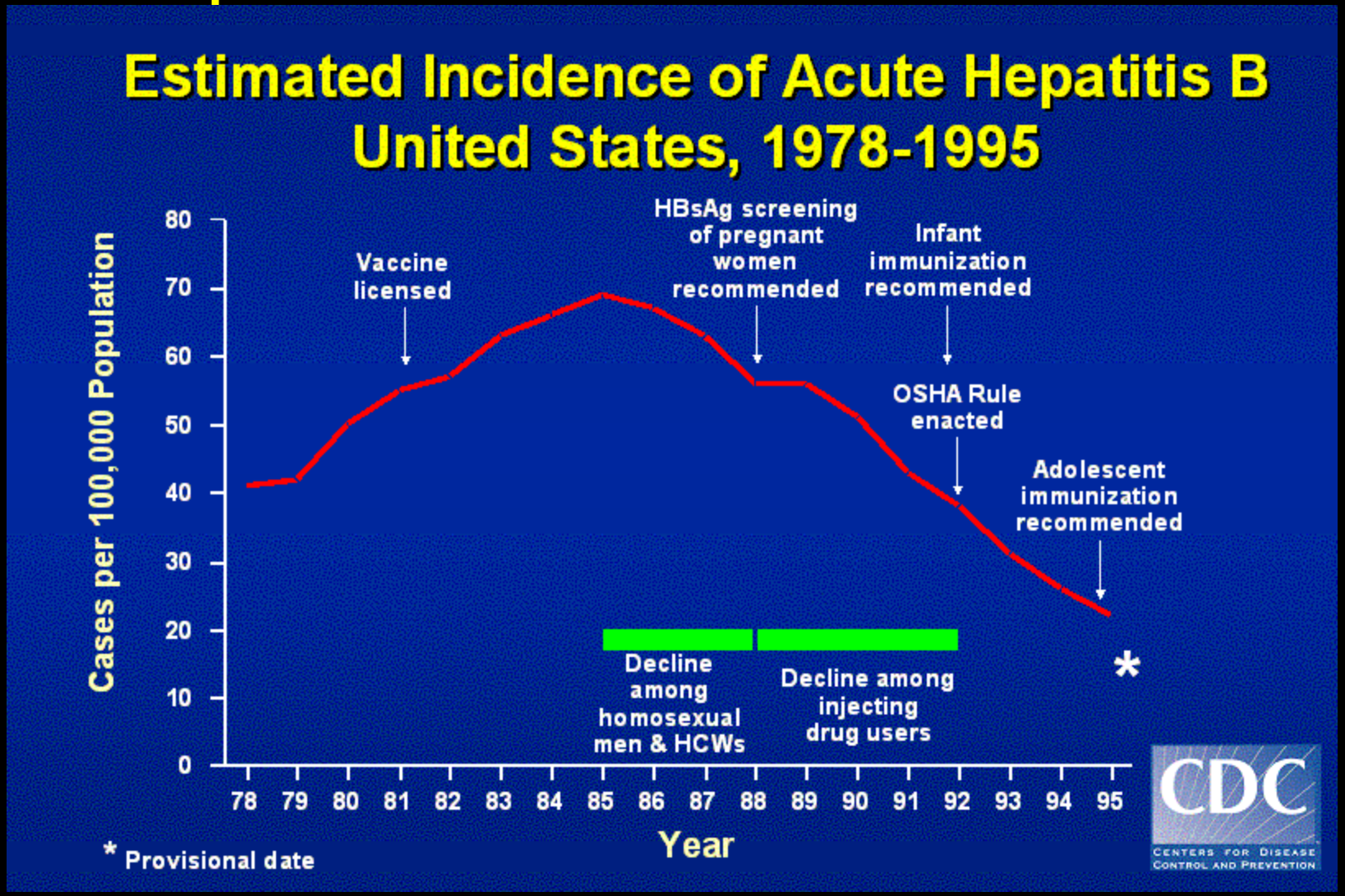


pathmicro.med.sc.edu/virol/hepatitis-disease2.htm

Risk factors at hepatitis B



Development of disease in the USA



pathmicro.med.sc.edu/virol/hepatitis-disease2.htm

Prevention and treatment of hepatites

- **Vaccination agains hepatitis B** is part of normal vaccination calendar in many countries
- **Vaccination agains hepatitis A** is available and recommendable in people travelling to various countries, including Mediterranean
- In some hepatites, treatment by **interferons** is used
- Otherwise **hepatoprotective drugs** are used, and other symptomatic therapy

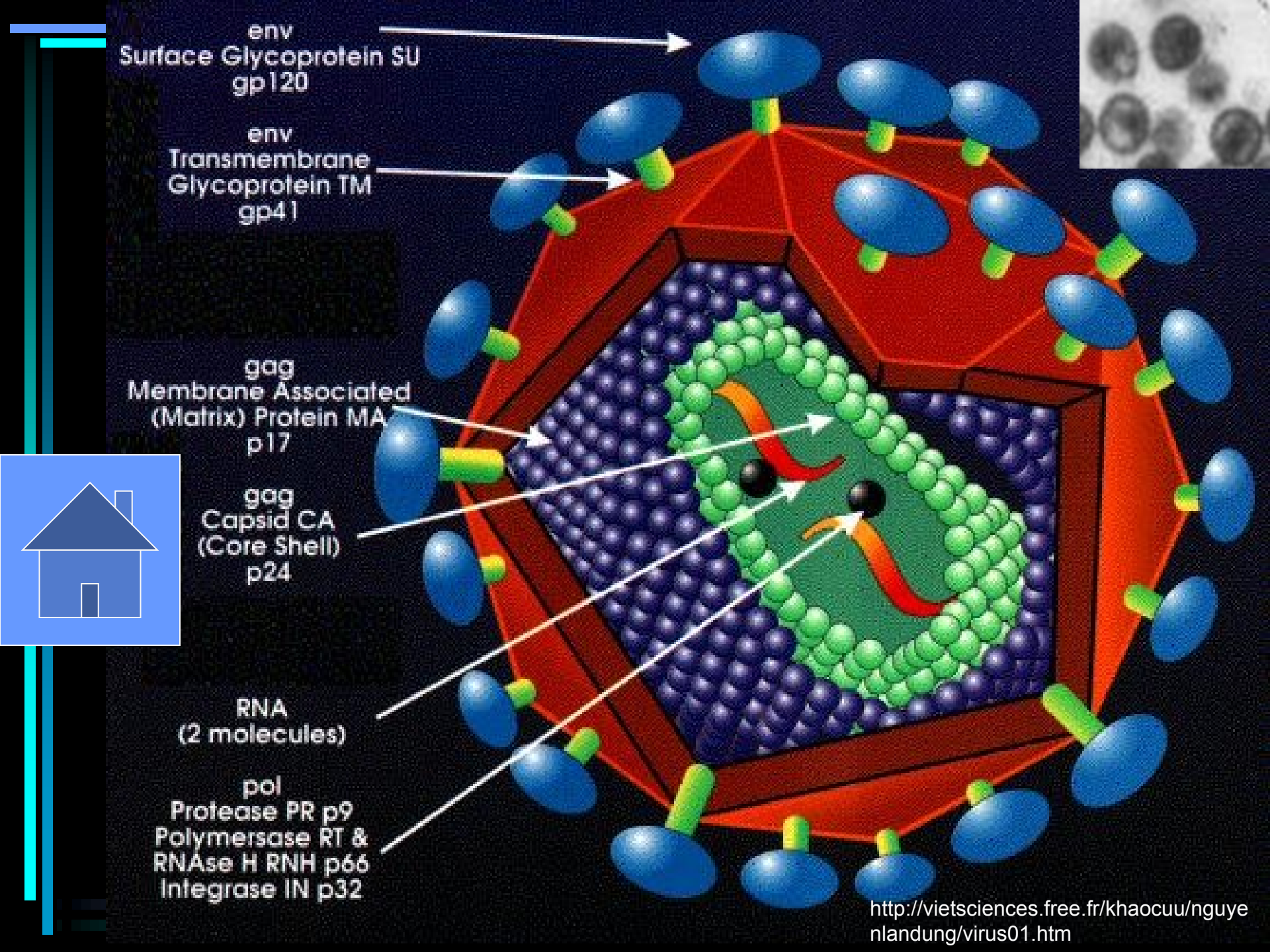
HIV

HIV – Human Immunodeficiency Virus

- **It is a retrovirus**, it has reverse transcriptase (an enzyme transcripting RNA into DNA)
- HIV virus exists in **two types**, majority of infections are caused by Type 1 virus
- Transmitted by **blood, sexually and moter-to-child transmission**
- There is a lot of **drugs agains HIV,** but ther effectivity is limited

Diseases caused by HIV

- Virus attacs mainly **cellullar immunity**
- After a non-specific **primary infection** there is a long latent period, where no symptomas
- Then generalized lymphadenopaty occur, step by step opportune infections are found, and the disease comes to complete **AIDS**
- AIDS has no own symptomas. The patients have symptomas of **opportune infections** (toxoplasmosis, pneumocystosis, various mycoses etc.) and **tumors**



http://vietsciences.free.fr/khaocuu/nguyenlandung/virus01.htm

Diagnostics of hepatites and HIV infection

And now diagnostics. Repeating first.

- Aim of microbiological methods: to detect a pathogen, eventually its antibiotic susceptibility.
- Direct methods are all methods of
  - detection of a whole microbe as morphological of fysiological unit
  - detection of its part (antigen,nucleic acid)
  - detection of its product (e. g. a toxin)
- Indirect methods: detection of antibodies against the microbe. Positivity = the microbe met the host IN HISTORY (weeks / months / years)

Methods of medical microbiology

Methods used in today's task are yellow and bold

- Direct methods *(work with **a sample** or **a strain**)*
  - Microscopy (wet mount, staining…)
  - Culture (liquid and solid media)
  - Biochemical and similar identification
  - **Antigen detection (using antibody)**
  - Animal experiment (isolation, toxins)
  - **Nucleic acid detection**
- Indirect methods
  - **Antibody detection (using antigen)**

Direct detection of viruses

- Culture → isolation (virus is often not multiplied, only kept living). Needs cells. More in next part.
- Microscopy: electronoptical, optical for detection of in vivo/in vitro effect of viruses (inclusions, cytopathic effects)
- Biochemical identification not here
- Animal experiment here = viral isolation
- DNA/RNA detection – viruses > bacteria
- AG detection in sample – common

Indirect detection of viruses

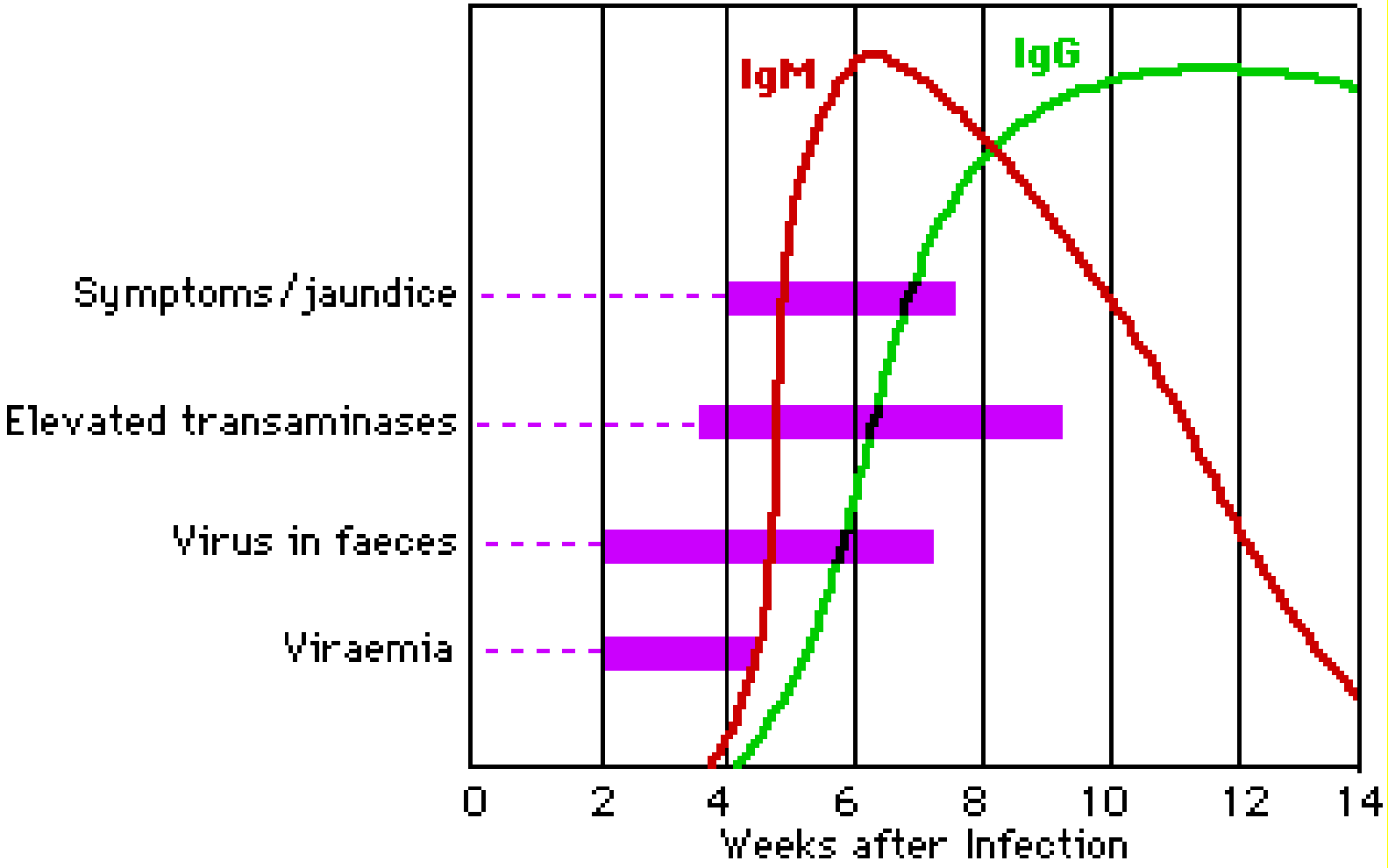
- Mostly used: CFT, various neutralisations (HIT, VNT) and in recent time mostly reactions with labelled components (mostly ELISA)
- Attention! Not all reaction where serum is used as specimen, are indirect detection methods! In systhemic viroses very often the virus itself or its antigen is present in serum and it is possible to find it here by a direct detection

Diagnostics of hepatitis A, C, D, E

- HAV. Using ELISA method, we determine anti-HAV IgM + IgG, or IgM and total Ig
- HCV. Again ELISA detection of IgM and IgG, and also PCR method.
- HDV. Determining delta antigen (HDAg), antibodies (anti-HD) or viral RNA PCR
- HEV. Again IgM and IgG ELISA detection, PCR is in developement

HAV ☺

Developement of Hepatitis A



www-micro.msb.le.ac.uk/3035/HAV.html.

Specfic diagnostics of HBV

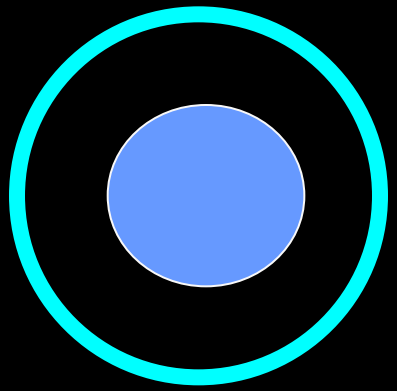
- In the middle of hepatitis B virion there is a **nucleocapsid**, where DNA is placed. Two core proteins, antigens **HBcAg** and **HBeAg** are present
- The **envelope** is formed by another antigen **HBsAg**
- HBsAg is overproduced, and so also **empty envelopes are present in serum**

In the empty HBsAg, delta agens may invade – hepatitis D causative agent

Delta agens

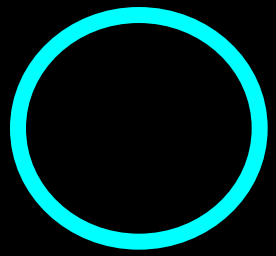
- Delta agens is a **viroid**, particle with uncertain virological classification
- Delta agens may infect humans together with HBV (**coinfection**), or following after such infection (**superinfection**)
- Presence of delta agens worsens prognosis of viral hepatitis

Virus of hepatitis B



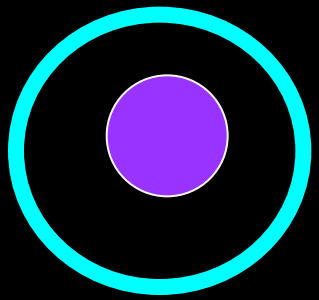
Complete virion (Dane particle)

42 nm



Only HBsAg

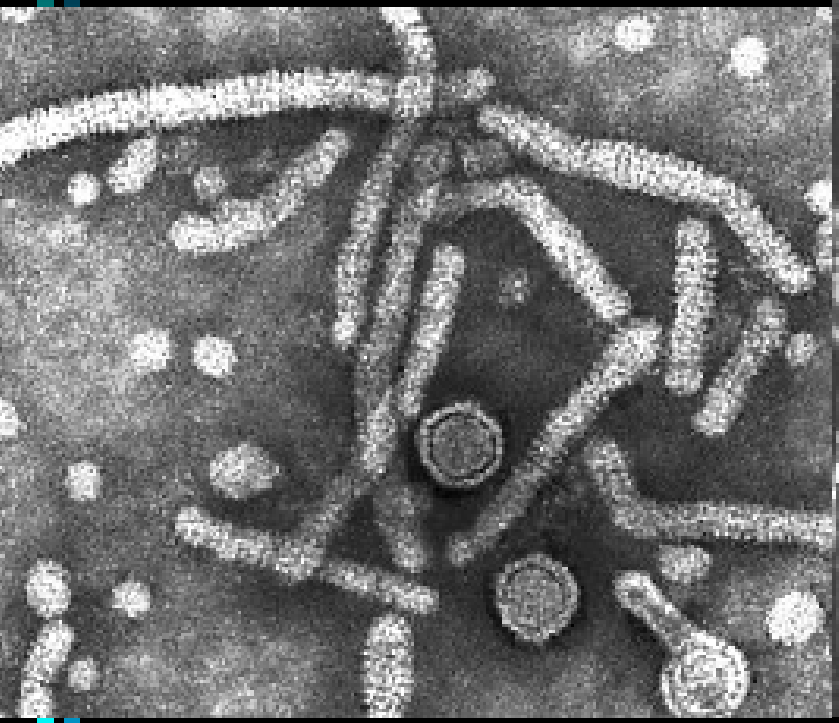
22 nm

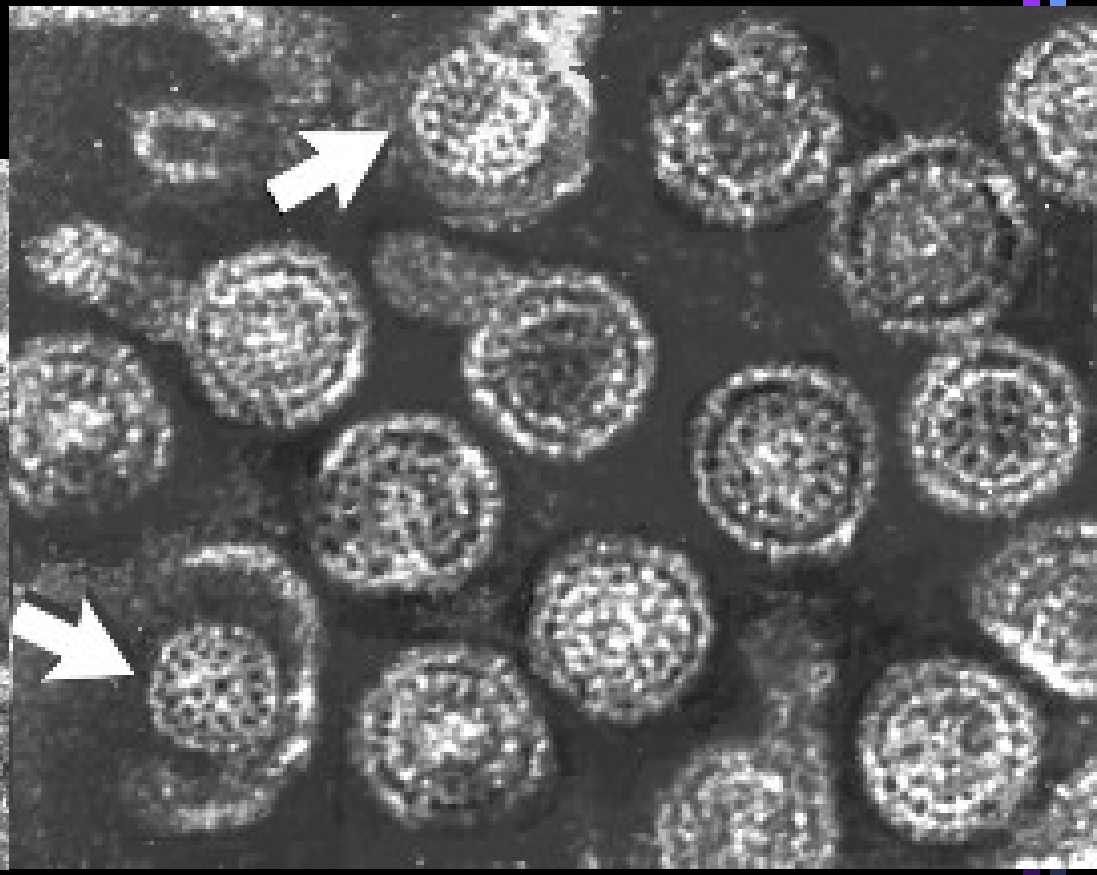


HBsAg, inside it delta agens (VHD)

35 nm

Complete virions and empty HBsAg



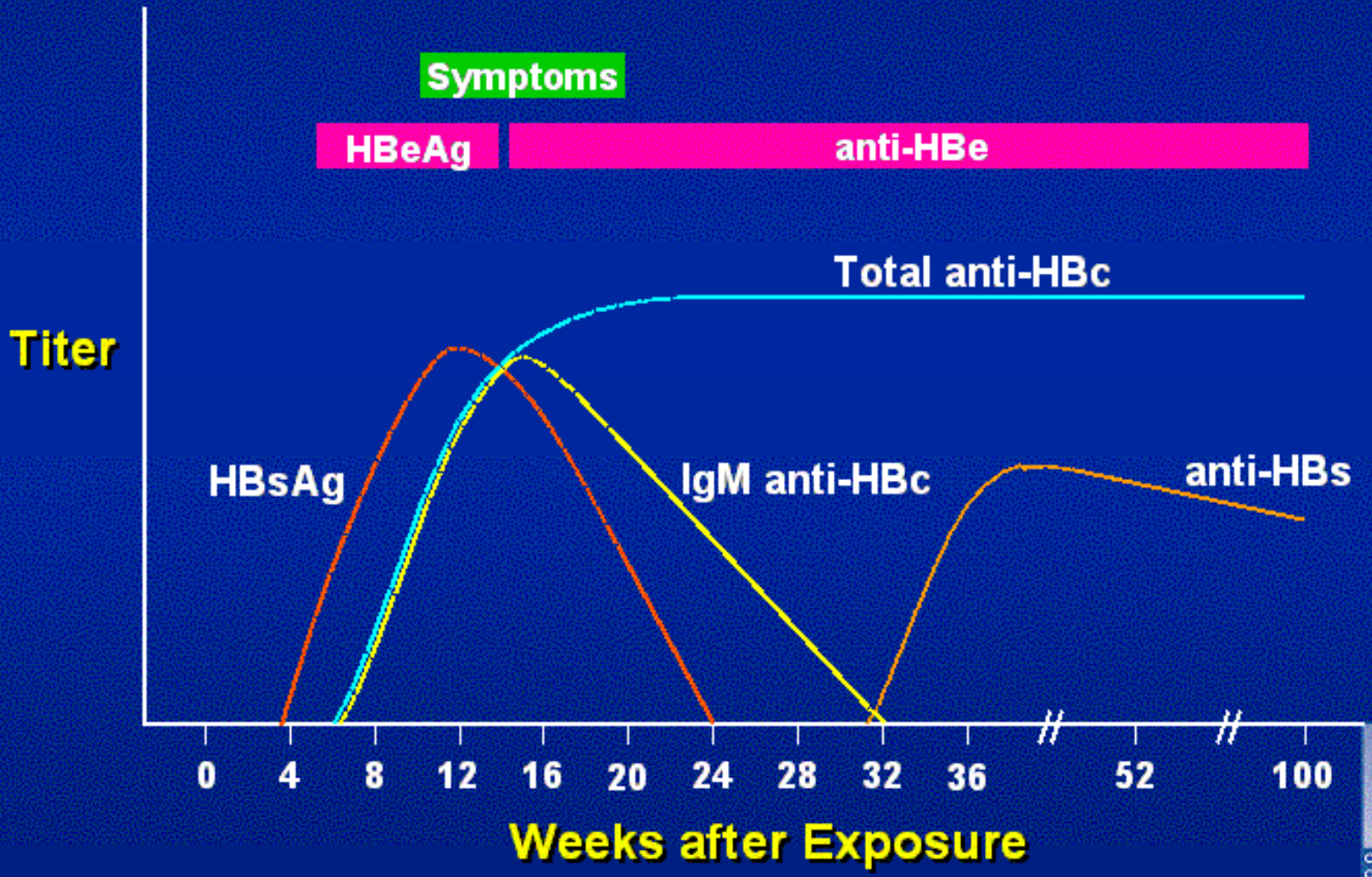


www.uct.ac.za/depts/mmi/stannard/hepb.html.

HBV diagnostics

- HBV has **three antigens important for diagnostics**. Only two of them can be found in patient's serum: **HBsAg** and **HBeAg**.
- **HBsAg is overproduced**, present in huge amounts in serum → suitable for screening
- Antibodies may be assessed against all three of them: **anti-HBs**, **anti-HBe** and **anti-HBc**.
- Also **PCR** is sometimes exploited, **hepatal enzymes** detection etc.
- Interpretation needs combination of results

Acute Hepatitis B Virus Infection with Recovery
Typical Serologic Course

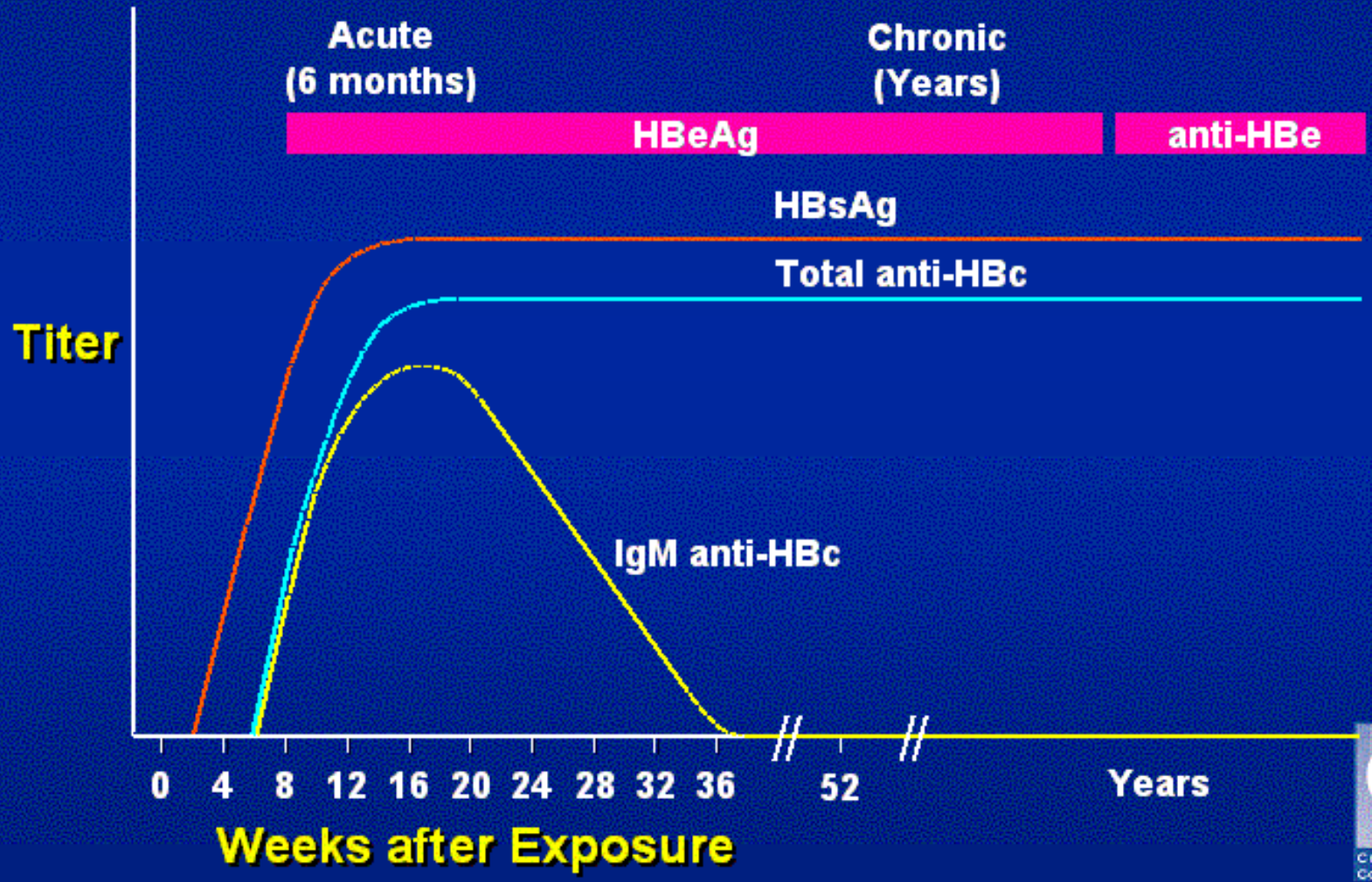


CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

pathmicro.med.sc.edu/virol/hepatitis-disease2.htm

Progression to Chronic Hepatitis B Virus Infection

Typical Serologic Course



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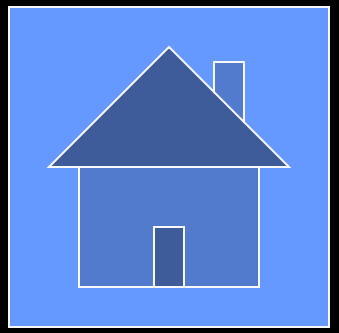
HIV diagnostics



- **Antibodies** against envelope glykoproteins using ELISA tests are assessed. When the result looks like positive, the serum specimen is sent to reference laboratory, that **confirms** the result using western blotting
- **Direct diagnostics** using PCR can be performed. Virus isolation is possible, but very difficult and not performed routinelly

Practical diagnostic approaches

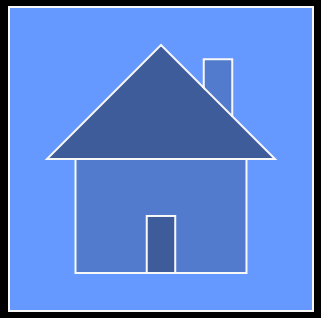
Practical diagnostics of hepatites and HIV infection: ELISA

- Although some ELISA reactions are to antigen detection and some for antibody detection, **practical approaches are simillar**.
- **Counting cut off:** average of cut off wells, or average of negative controls + constant
- Often **cut off 10 % = borderline values**
- In some cases, especially in VHA diagnostics, we do not examinate IgM and IgG antibodies, but **IgM and total antibodies**. It is clear that negative IgM and positive total antibodies mean practically presence of IgG antibodies.

Practical diagnostics of hepatites and HIV infection: PCR

- PCR is mostly used in **HCV diagnostics, eventually HIV diagnostics**
- The procedure is **identical to that described in J11 practical session**

The End



>>> Lambda phage >>>

http://www.ucm.es/info/genetica/grupod/Cromovibac/cromovibac.htm

More to viruses
Viral replication

- either in **nucleus** (mostly in DNA viruses), or in **cytoplasm** (mostly RNA viruses) of the host cell.
- before replication virus has to **get rid of its protein envelope**
- proper course of replication depends on the **viral type** (RNA/DNA, single stranded – ss/ double stranded – ds).

Individual ways of replication

- **In majority of DNA viruses** DNA-polymerase produces the complementary file
- **In hepadnaviruses** (VHB) DNA is transkribed to RNA, according to that reverse transcriptase makes DNA again
- **In RNA viruses** RNA polymerases are used
- **In retroviruses** (HIV) reverse transcriptase produces DNA along RNA. Cellullar RNA polymerase is then used for transcription.

Production of viral proteins

- is **necessary** to enable the virus to exit the cell and to spread further
- the proper process is again **related with the viral type**
- in any case, virus **uses partially the proteosynthetic apparate of the host cell**.

Individual possibilities of protein formation

- **+ss RNA:** direct translation possible
- **–ss RNA:** „+ file“ has to be completed
- **ds RNA:** after – file + file is completed
- **retroviruses:** reverse transkriptase formates DNA and then cellular polymerases work
- **ds DNA:** usually according to – file a + RNA file is formated
- **ss DNA:** second file is formated and then is is like in ds DNA

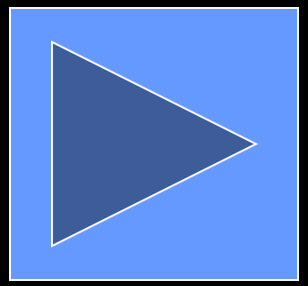
Viruses dependent to other viruses

Some strange viruses cannot live without presence of other viruses

- **Adenoasociated viruses (AAV)** belong to parvoviruses. Replication is possible only in presence of helping virus (adenovirus)
- **Hepatitis D virus** – delta agens – is a viroid. It is an incoplete particle, that is only able to survive in envelope of hepatitis B virus (mostly formed by HBsAg).

Viruses and outer environment, disinfection

- **some viruses are very sensitive** (e. g. HIV)
- **on the other hand, some are much more resistant** than bacteria (e. g. rhinoviruses)
- many disinfectants effective to bacteria have **no effect to viruses, or elevated conectrations are neccessary**; this concerns mostly **non-enveloped viruses**. Iodine preparations and peroxides use to be effective.
- **Prions** are very resistant towards high concentrations of disinfectants and high temperatures



Viruses as causative agents of diseases

A child infected by cytomegalovirus

Ways of transmission

- Ways of transmission are various; obviously, they are the same **as in bacteria**
- there are viruses tramsmitted by **droplet infection, fecal oral route, sexual transmission, vector** (tictk, mosquito) or **blood** (injection).
- in majority of viruses **„vertical" transmission** is also possible (mother to foetus)

Pathogenicity factors

- Unlike situation in bacteria, in viruses usually **individual pathogenic structures are not defined**, rather the whole virus is considered to be one pathogene particle
- This corresponds also with the fight with infection, where **antibodies neutralize the activity of the whole virus**.

This is also related with elevated importance of neutralisation methods in diagnostics

Course of viral infection

- in viruses inflamations are different from bacteria
- it is mostly related with **intracellullar parasitism of viruse**
- mostly **granulocytes are less important, lymfocytes more important, more important cellullar immunity**
- components of non-specific humoral immunity are different, too (mostly interferons).

Latent infection

- host cell **enables penetration of a virus into a cell**
- **its multiplication** and release from the cell is not possible
- on the other hand, virus **survives** in the cell, sometimes it is even integrated into the chromosome
- in some cases, later this **latent infection may be activated,** so the infection appears again
- it is typical in some **herpesviruses**.

Viruses and cancers

- Some viruses are are related with **some types of cancer**, mostly EB virus (causative agent of infectious mononucleose – takes part in formation of Burkitt lymphoma) and HHV8 together with HIV (Kaposi sarkoma), or human papilomavirus (HPV) – influence to cancer of cervix uteri
- The reason is that the **viral promotor** in such cases may **activate expresssion of human cellular oncogens**, that would be otherwise suppressed (it would not be present).

Fighting viruses I – antivirotics

- Used in **some viruses** only
- Usually only **limited influence in therapy**
- Enteric, oral or local (sometimes the same chemical, e. g. aciclovir – HERPESIN)
- ***In vitro* sensitivity testing still rather experimental**
- **Among the most common:** aciclovir, famciclovir, ganciclovir (herpesviruses), amantadin, rimantadin, oseltamivir, zanamivir (influenza), azidotymidin, PMPA (HIV virus)

Fighting viruses II – imunotherapy

- We use both **passive (antibodies) and active immunisation (vaccination)**
- Among **regullar vactination:**
  - Living attenuated viruses: all components of MMR (mumps, measles, rubella)
  - Non-living vaccines: viral hepatitis B, polyomyelitis (Salk)
- Among **other vaccines:** lyssa (prophylaxis, not prevention) tick born encephalitis, VHA etc.
- Among **passive immunisation** e. g. antibodies against varicella-zoster virus, HBV etc.