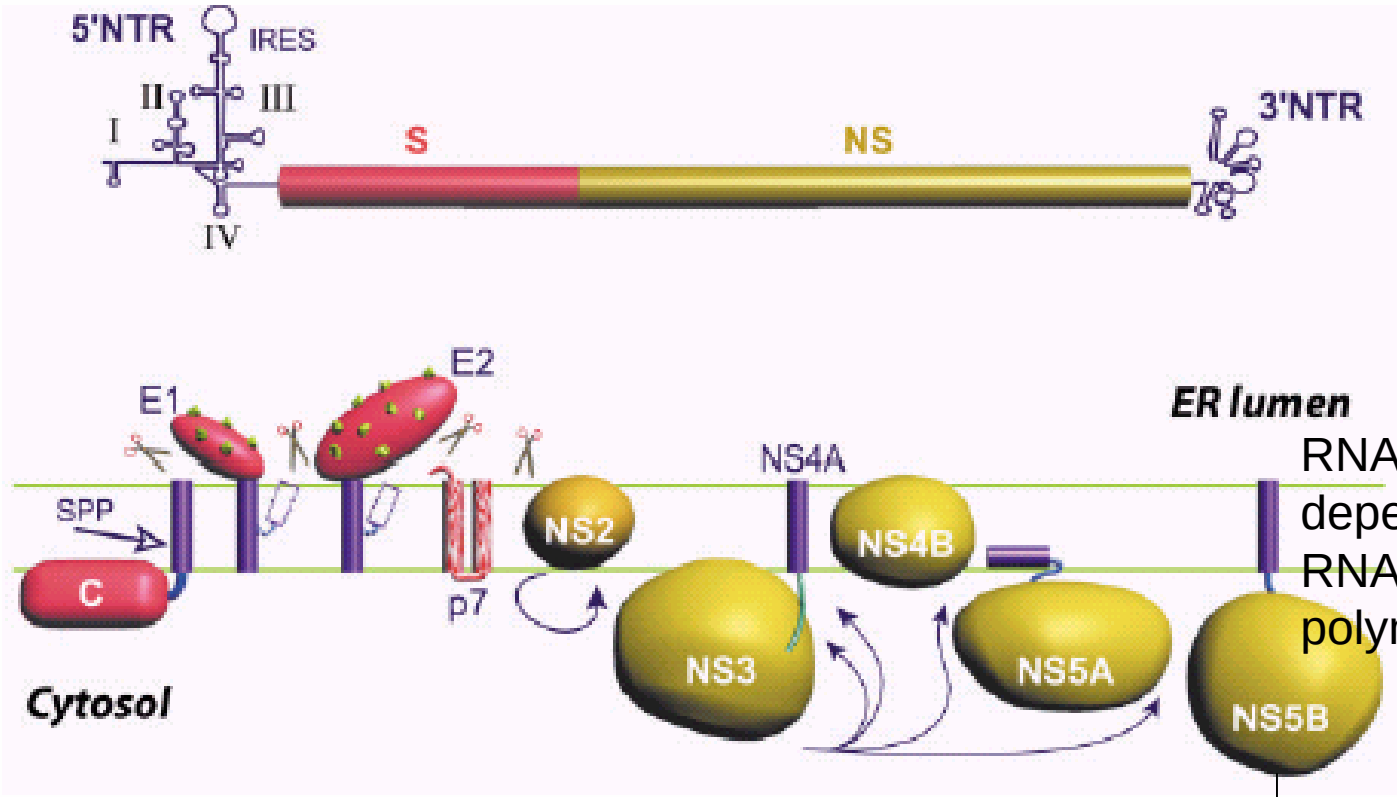
MOLECULAR EPIDEMIOLOGY OF HCV

Vall d'Hebron
Hospital



LACK OF PROOFREADING MECHANISMS

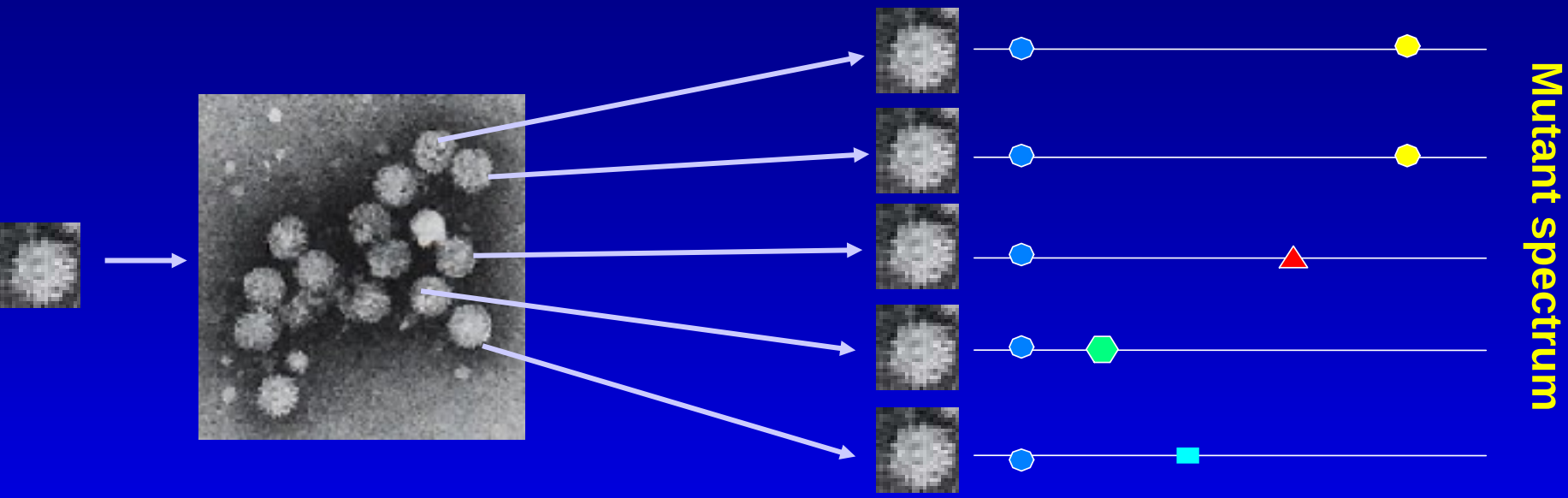
Mutation rates (mutations / nucleotide / cicle of replication)

10^{-3} - 10^{-5}

F.Penin et al Hepatol 2004;39:5-19

Exemple: 10^4 nts and mut.rate of 10^{-4} = 1 mutation/new genoma

QUASISPECIES NATURE OF HCV



Master sequence

Consensus sequence

JOURNAL OF VIROLOGY, May 1992, p. 3225–3229
0022-538X/92/053225-05$02.00/0

Vol. 66, No. 5

Hepatitis C Virus (HCV) Circulates as a Population of Different but Closely Related Genomes: Quasispecies Nature of HCV Genome Distribution

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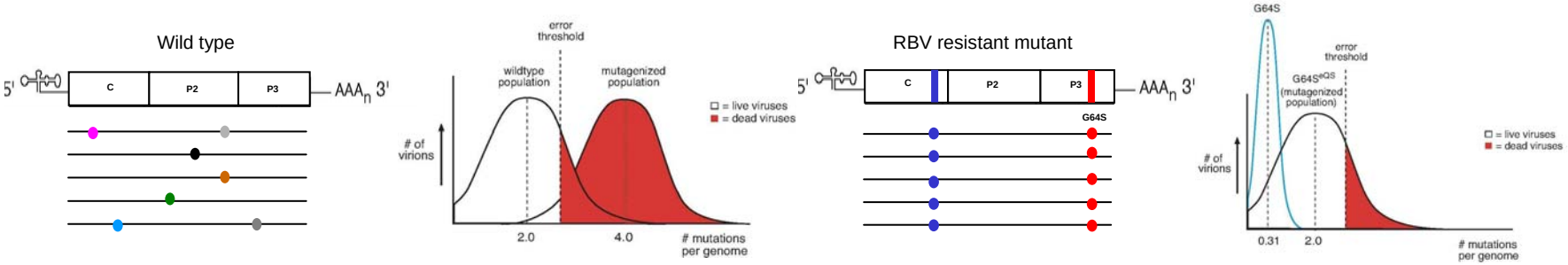
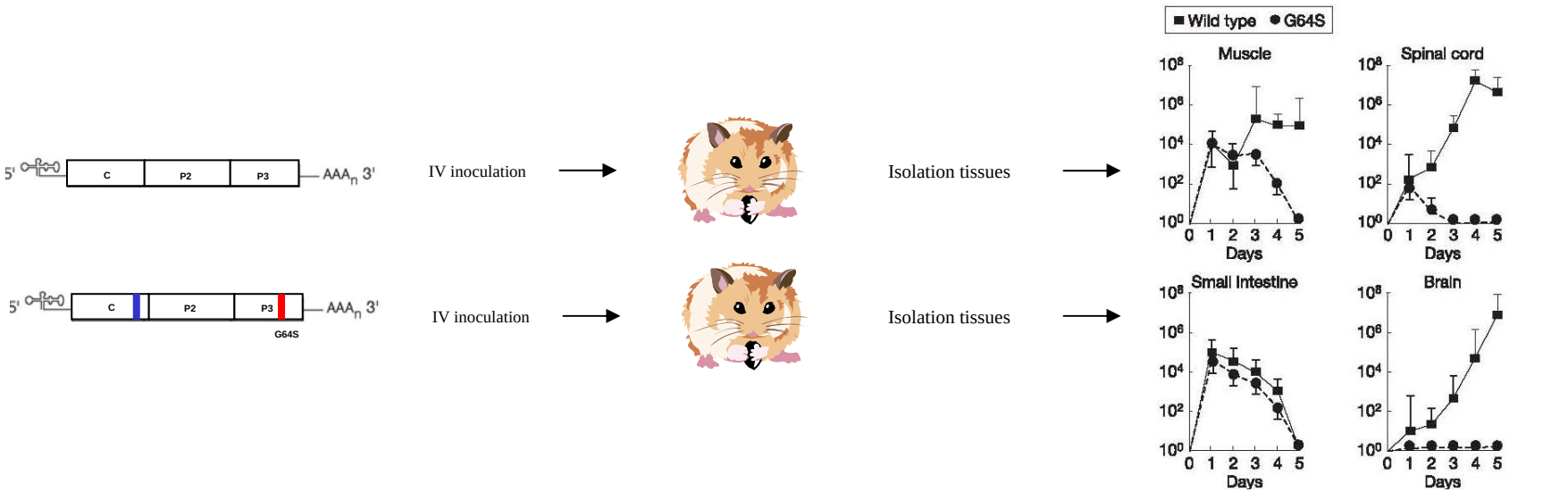
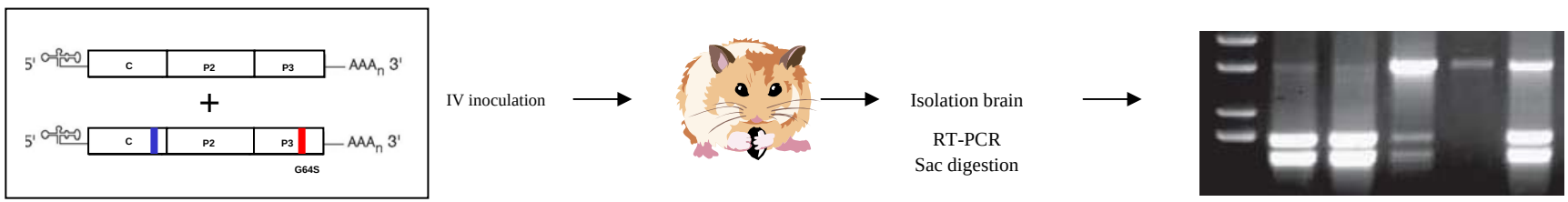
Quasispecies diversity, pathogenesis and cooperative interactions

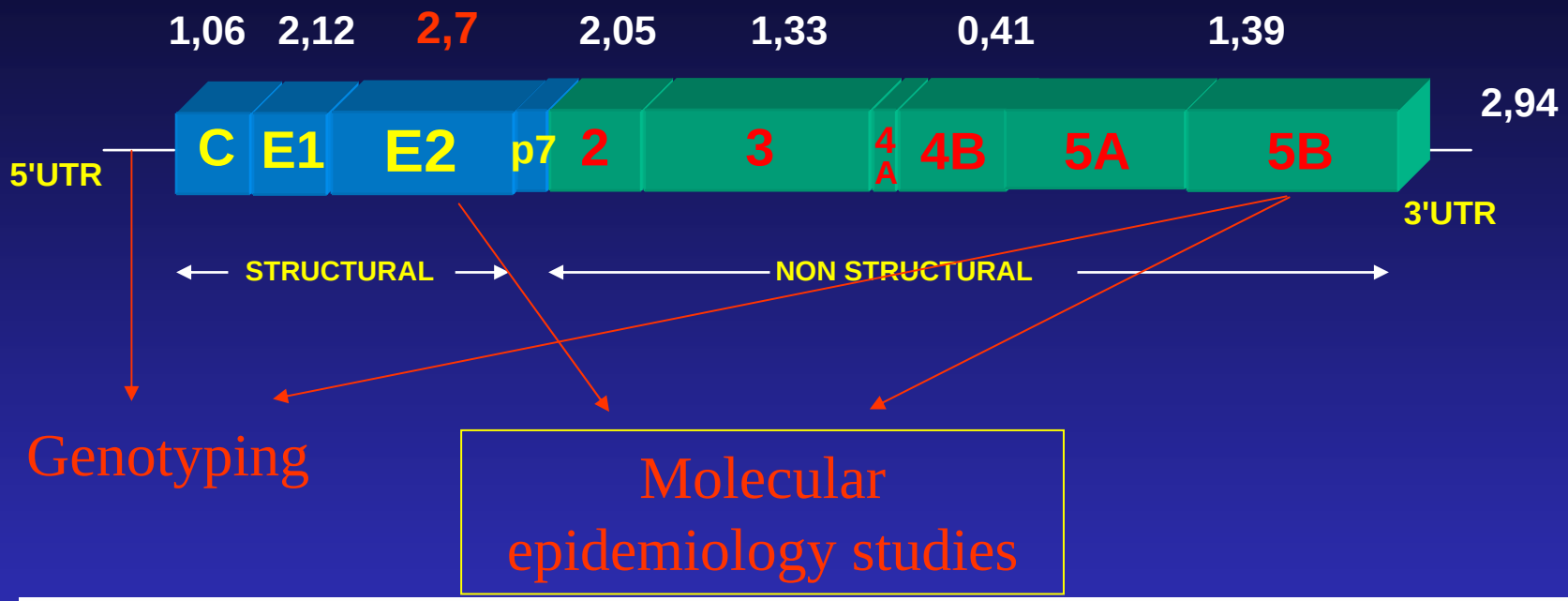


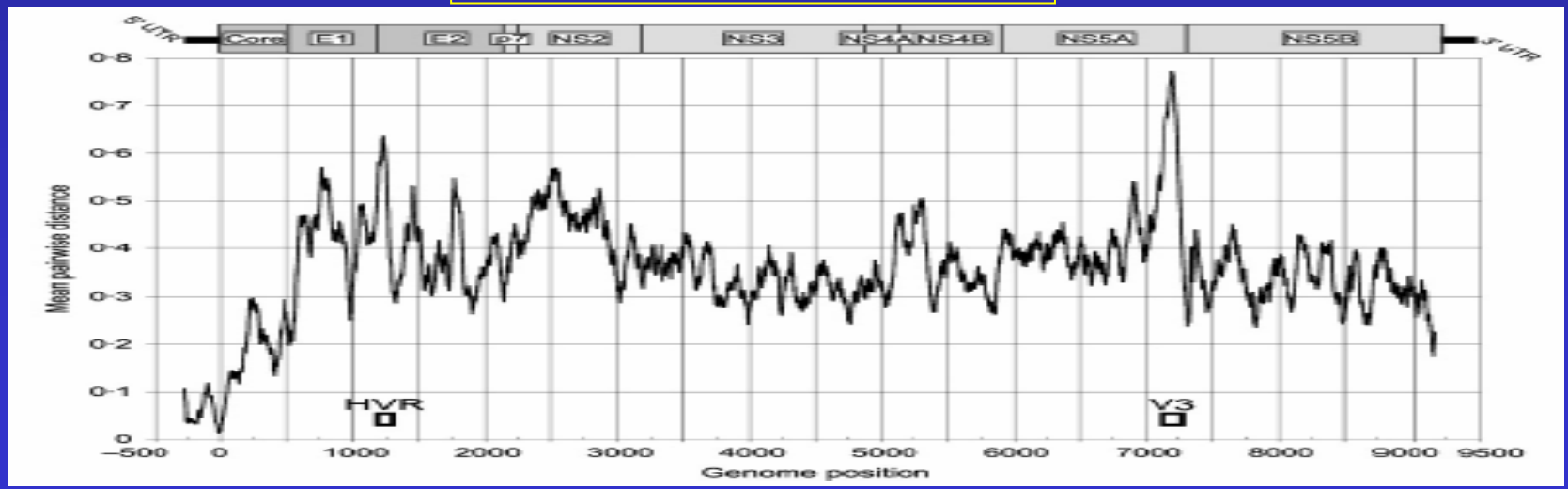




Vignuzzi M, et al. Nature 2005

RATE OF FIXATION OF MUTATIONS (mutations/site/year) ($x10^{-3}$)





COMPARATIVE GENETIC DISTANCE

	Nucleotides	Amino acids
Genotypes (Consensus. All genome):	31-34%	30%
Subtypes (Consensus. All genome):	20-23%	

Pawlotsky JM, Trends Microbiol 2004; 12:96-102

Quasispecies (Cloning. E1/E2) 10%

With Source of infection(Consensus. E2-PePHD) 1%

TRANSMISSION MECHANISMS OF HCV

A. PARENTERAL:

1. **Percutaneous:**
   - *Transfusion*
   - *Plasma product recipients (haemophiliacs, recipients of platelet concentrates, IVIG)*
   - *Organ transplantation*

2. **Nosocomial (Patient-to-patient, Haemodialysis patients...)**

3. **IVDU**

B. NON-APPARENT PARENTERAL:

1. **Health-care workers**

2. **Tattooing, Acupuncture.**

C. NON-PARENTERAL:

1. **Mother-to-infant**

2. **Sexual and household transmission**

MOLECULAR EPIDEMIOLOGY STUDY

A. Epidemiologic investigation:

A1- Confirmation of HCV infection and genotyping.

A2- On-site review of risk factors, diagnostic procedures, surgical interventions, medications and other treatments, interview with the health-care workers. Serological testing of patients undergoing the procedure before and after the patient

B. Molecular analysis of viral isolates (source-recipient):

1- Samples from patients and potential viremic sources

2.-RNA extraction

3.-RT-Nested-PCR

4.-Sequencing

5.-Phylogenetic analysis

PHYLOGENETIC ANALYSIS

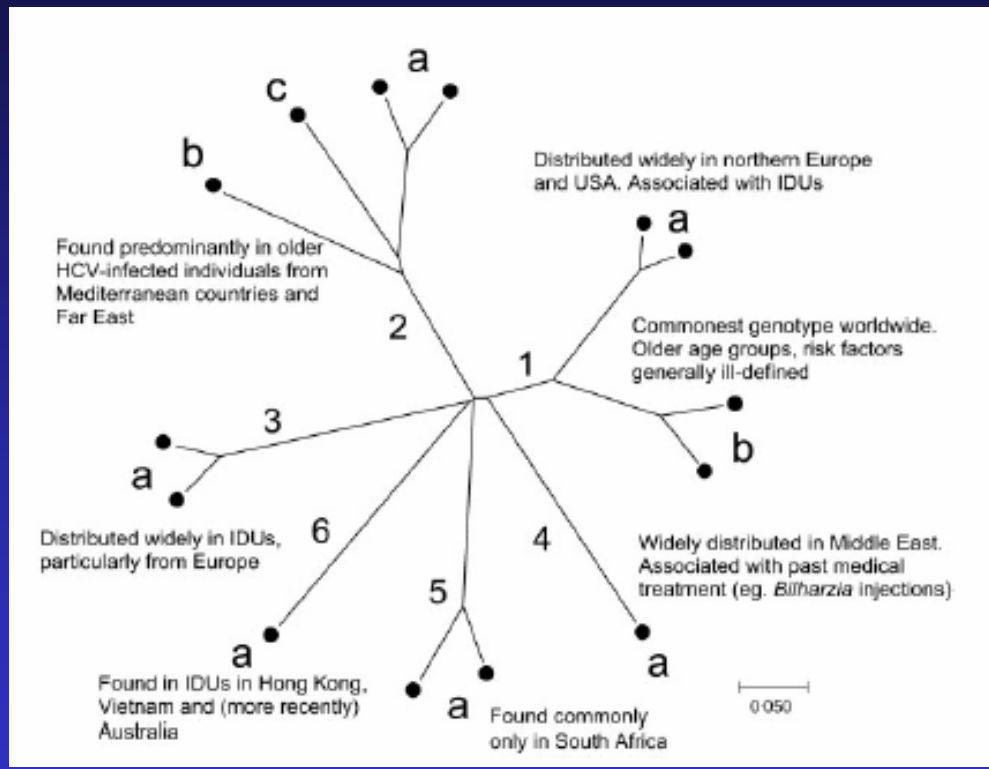
GENETIC DISTANCE:

Percentage differences between the same genomic is a measure of "genetic distance" between these nucleotide sequences.

PHYLOGENETIC TREES

Graphic representation of homology (or distance) among various species. It has a form of cladogram.

Each node with descendants represents the most common ancestor of the descendants, and the edge lengths correspond to time estimates.



http://evolution.genetics.washington.edu/phylip/software.html

TRANSMISSION OF HEPATITIS C VIRUS BY A CARDIAC SURGEON

JUAN I. ESTEBAN, M.D., JORDI GÓMEZ, PH.D., MARÍA MARTELL, PH.D., BEATRIZ CABOT, PH.D., JOSEP QUER, PH.D., JOAN CAMPS, M.D., ANTONIO GONZÁLEZ, M.D., TERESA OTERO, M.T., ANDRÉS MOYA, PH.D., RAFAEL ESTEBAN, M.D., AND JAIME GUARDIA, M.D.

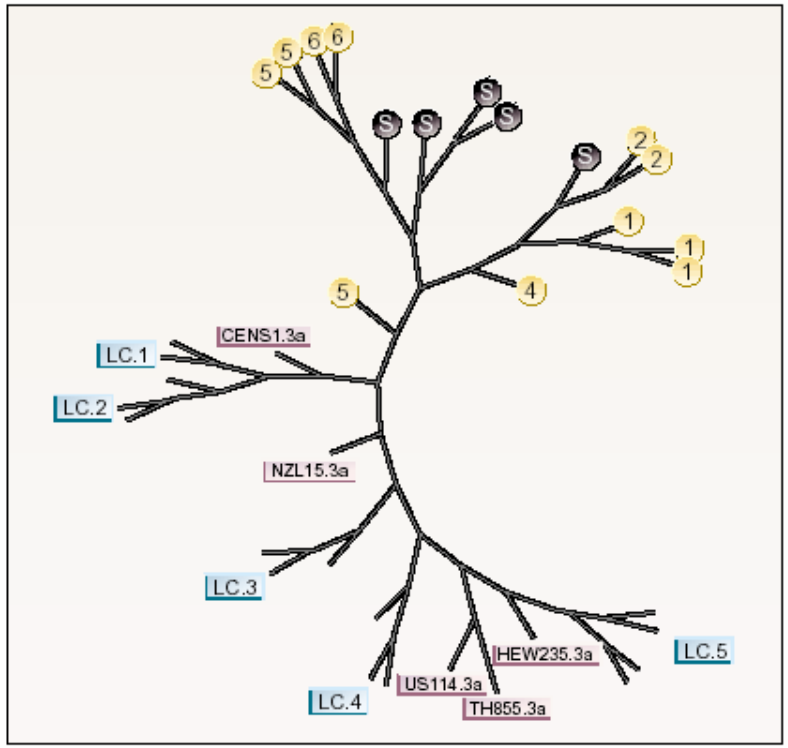
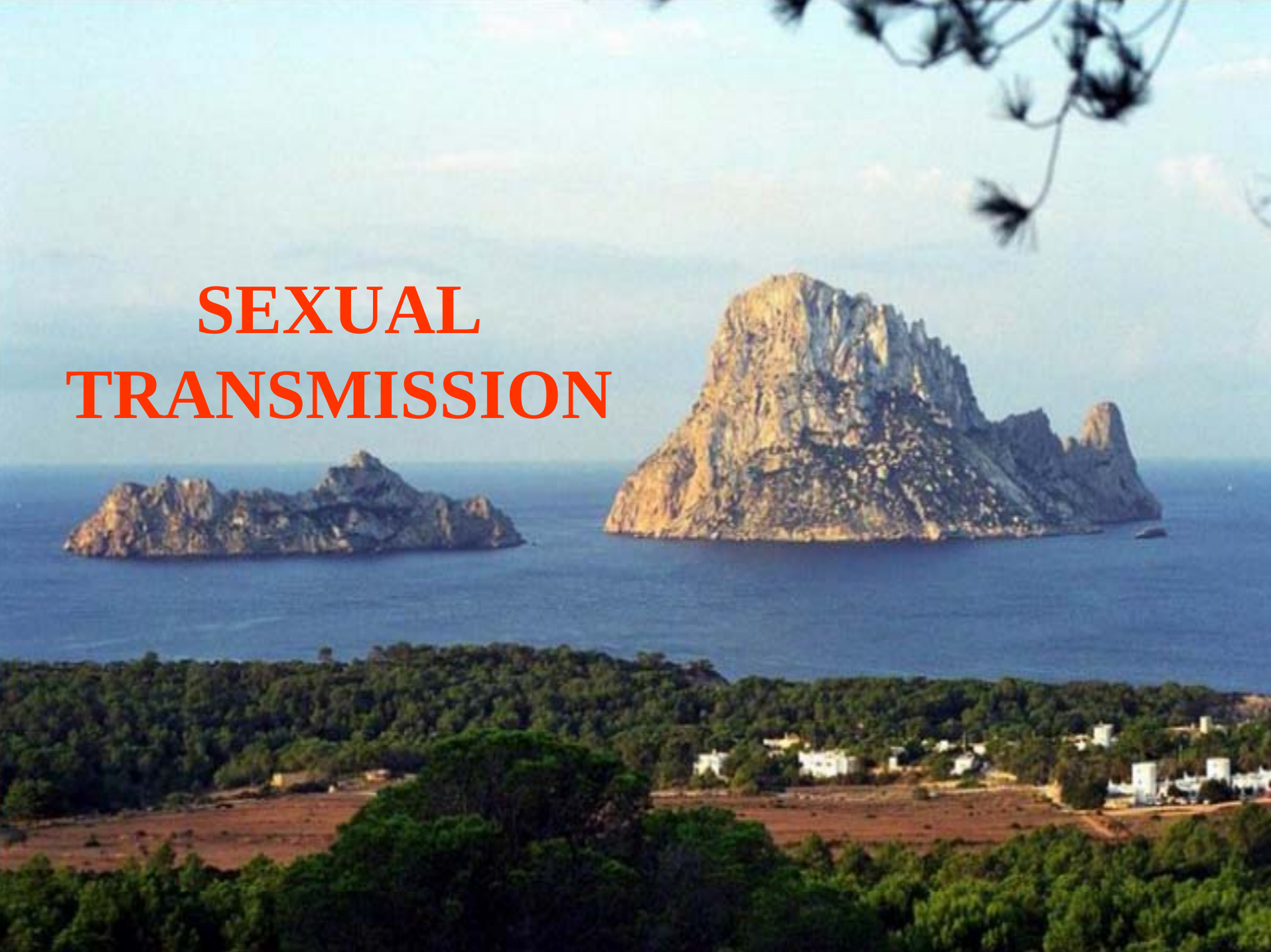


Figure 2. Neighbor-Joining Phylogenetic-Tree Analysis Comparing Coding Sequences in HCV Hypervariable Region 1 in Viral Isolates from the Surgeon (S), Five Patients (1, 2, 4, 5, and 6), Local Controls (LC.1 through LC.5), and Controls from the EMB Data Bank (CENS1.3a, NZL15.3a, US114.3a, TH855.3a, and HEW235.3a).

All sequences from the five patients and the surgeon clustered together in a monophyletic nest. The clustering of sequences from the patients and the surgeon was similar in 448 of 500 bootstrap analyses (89.6 percent). Each branch of the tree corresponds to a different cloned sequence (e.g., there are five branches for the surgeon because five of his cloned sequences were analyzed), except for Patient 4, whose single branch corresponds to three clones with identical nucleotide sequences.

N Engl J Med 1996;334:555-60.


SEXUAL
TRANSMISSION

SEXUAL TRANSMISSION

PATIENT August 1998. Acute infected patient. JAUNDICE.

DIAGNOSIS **HCV + G1a**

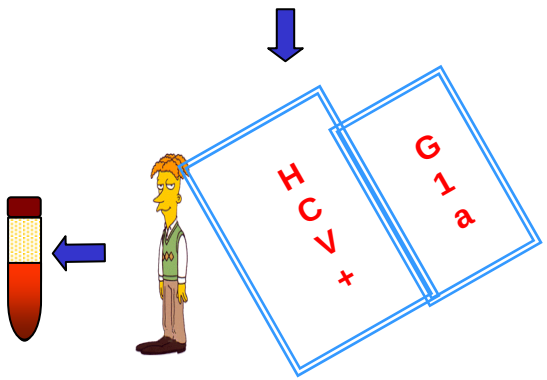
SOURCE OF INFECTION?

HISTORY She was a blood donor. HCV negative in December 1997

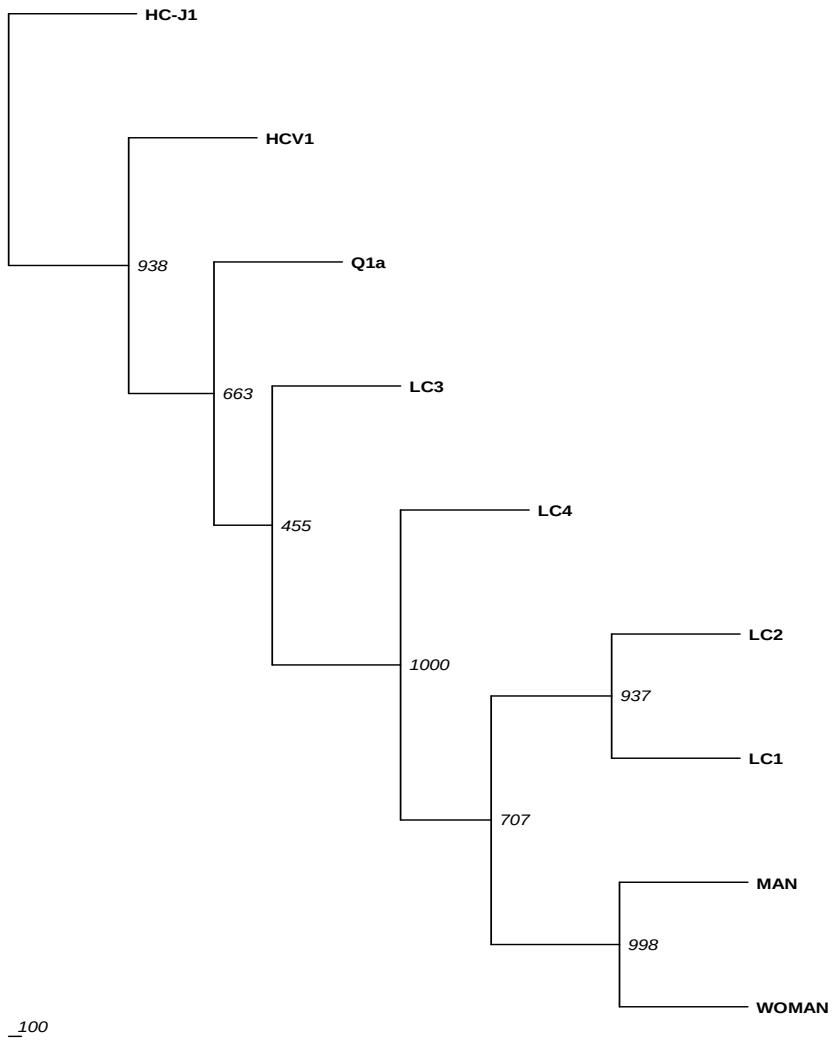
MEDICAL RECORD On-site review of risk factors, diagnostic procedures, surgical interventions, medications and other treatments

CONCLUSION

No risk other than being sexual partner of a chronic infected patient.

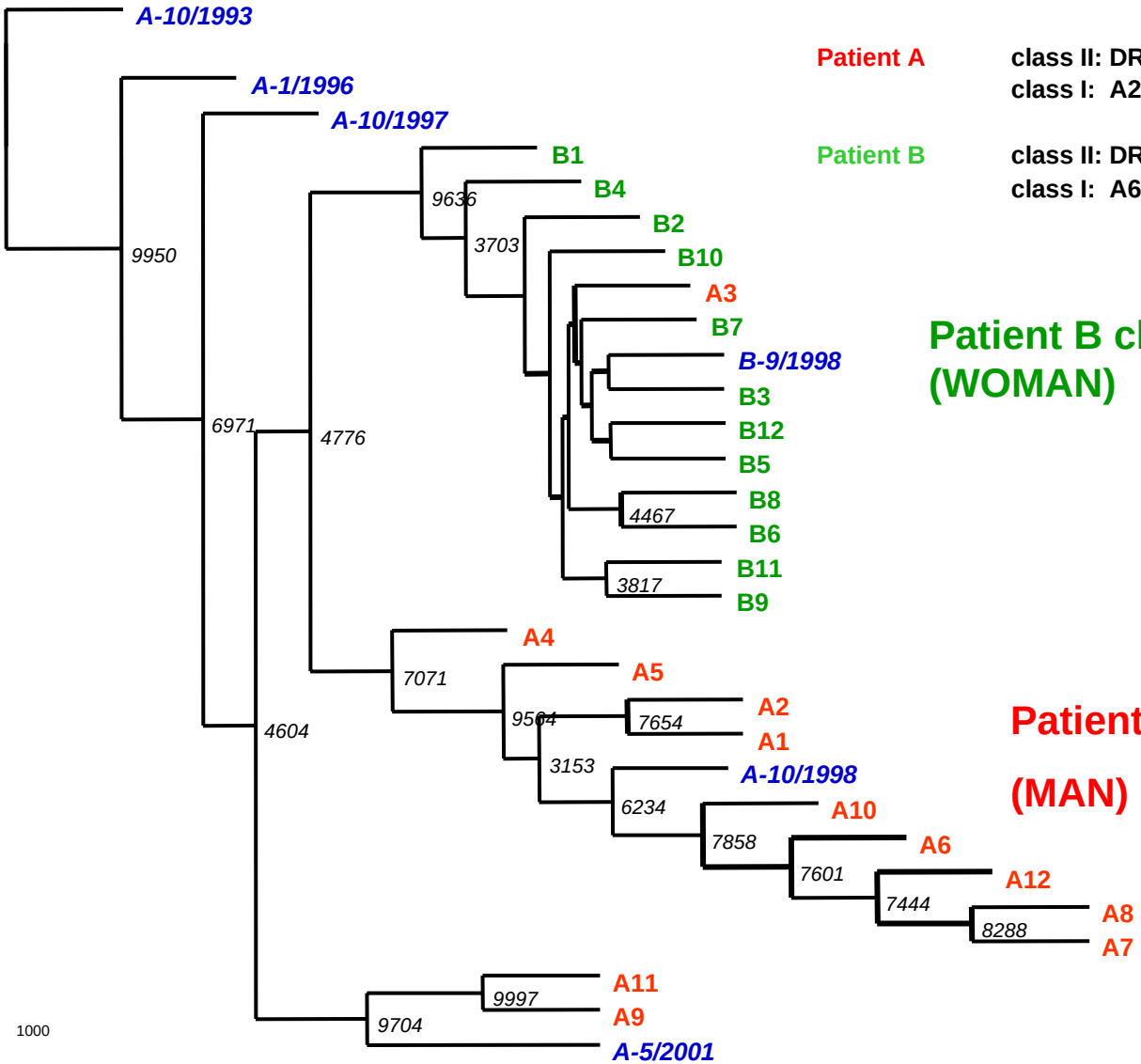


PHYLOGENETIC TREE E2-PePHD. SEXUAL TRANSMISSION (Consensus sequence analysis).



Quer et al. Sex.Transm.Dis. 2003; 30:470-471

PHYLOGENETIC ANALYSIS. Consensus and clonal sequences from patient A (chronic) and B (acute and self-recovering)



Patient A — class II: DR1, DR15; DR51; DQ5, DQ6; class I: A2, A28; B7, B14; Cw7, Cw8

Patient B — class II: DR4, DR11; DR52; DR53; DQ3; class I: A66, A69; B41, B55; Cw1

Patient B clones (WOMAN)

Patient A clones (MAN)

Quer J, et al. J Virol 2005; 79:15131-41

Similar viral yield

When BOTTLENECKING? / Evidences.

Genetic BOTTLENECK might operate in circumstances in which HCV transmission occurs through exposure to very small inoculum:

-Sexual contact:

- Detected **intermittently** in semen
- **Low viral load:** 85% of cases <50UI/mL, 15% average 200 UI/mL
- Sexual lesions can help direct contact with blood vessels.

-Accidental needlestick exposure, tattooing, acupuncture, mother-to-infant.

Reduction in viral diversity in the virus transmitted may compromise the capacity of a viral population to overcome immune-mediated clearance and to persist.

Health-care workers → Risk HCV after needlestick injury: **(0.013-10%)** **2%**

Mother-to-infant → Risk mother anti-VHC+: **2%**; and if RNA+ at delivery: **4-7%**

Sexual → Prevalence monogamous **2-3%**, multiple sex **4-6%**

Transfusion → Chronicity >80%


NOSSOCOMIAL
patient-to-patient
TRANSMISSION

PATIENT CTA

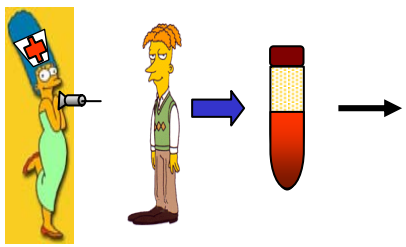
June/July 2004, patient CTA underwent clinical procedures

HCV negative at that time.

August 2004. Acute hepatitis patient with nausea, fatigue and jaundice**.**

Hepatitis?

A1- VIRUS, GENOTYPE



ANALYSIS

HCV +

G1b

A2- SOURCE OF INFECTION?

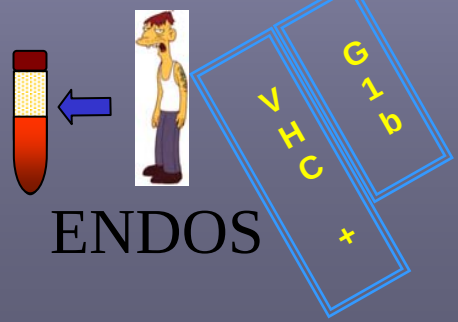
Department of Preventive Medicine and Epidemiology

Epidemiological Service. Public Health Agency of Barcelona.

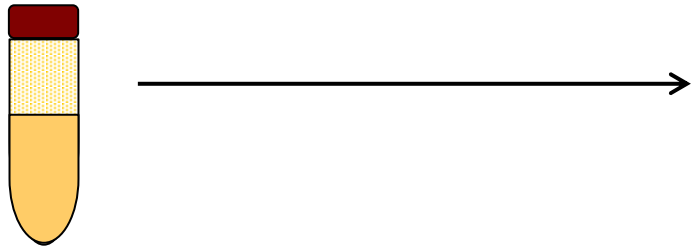
Department of Health Generalitat de Catalunya

MEDICAL RECORD OF THE
PATIENT WAS REVIEWED

He underwent an **ENDOSCOPY** with BIOPSY, 7-8 weeks before. The previous endoscopied patient was chronic HCV+.



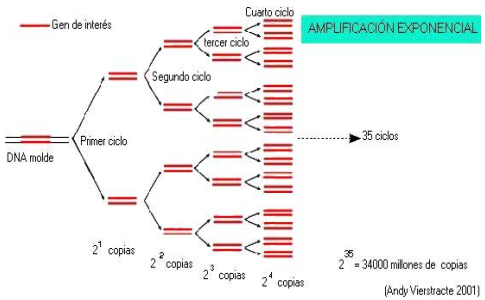
1. RNA EXTRACTION



SERUM

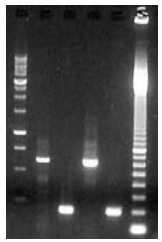
VIRAL RNA

2. RT-NESTED-PCR



PCR

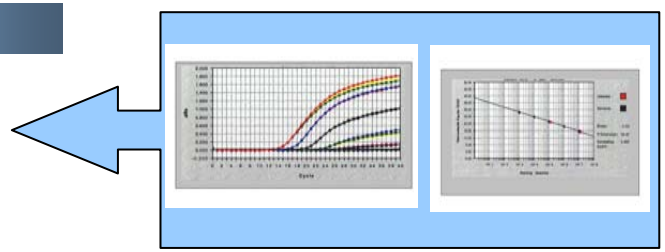
Agarose gel



Fragment 650 pb

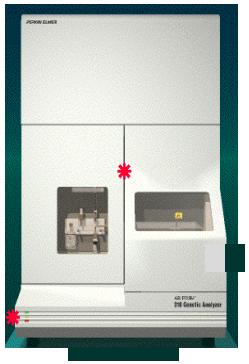
Fragment 200 pb

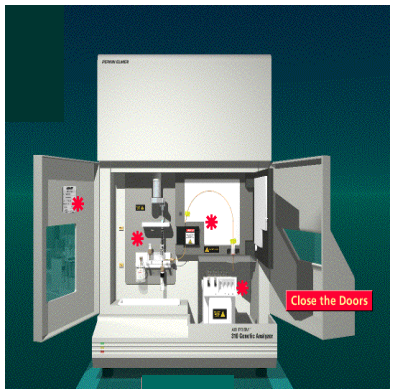
Real-time PCR

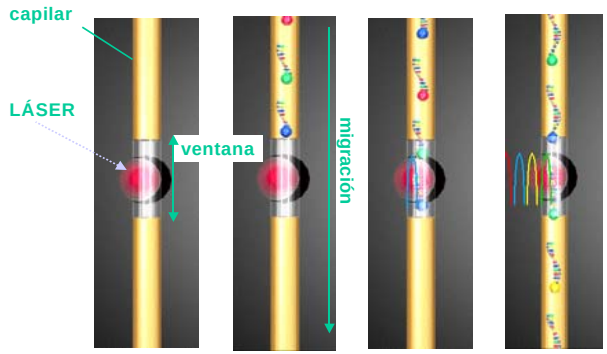


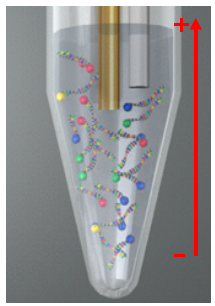
3. SEQUENCING

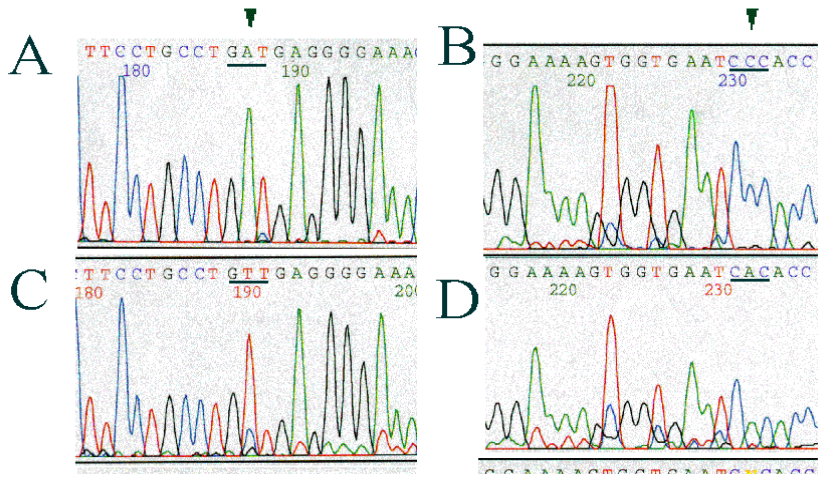
310 Genetic Analyzer **24/24 hours , 7/7 days**



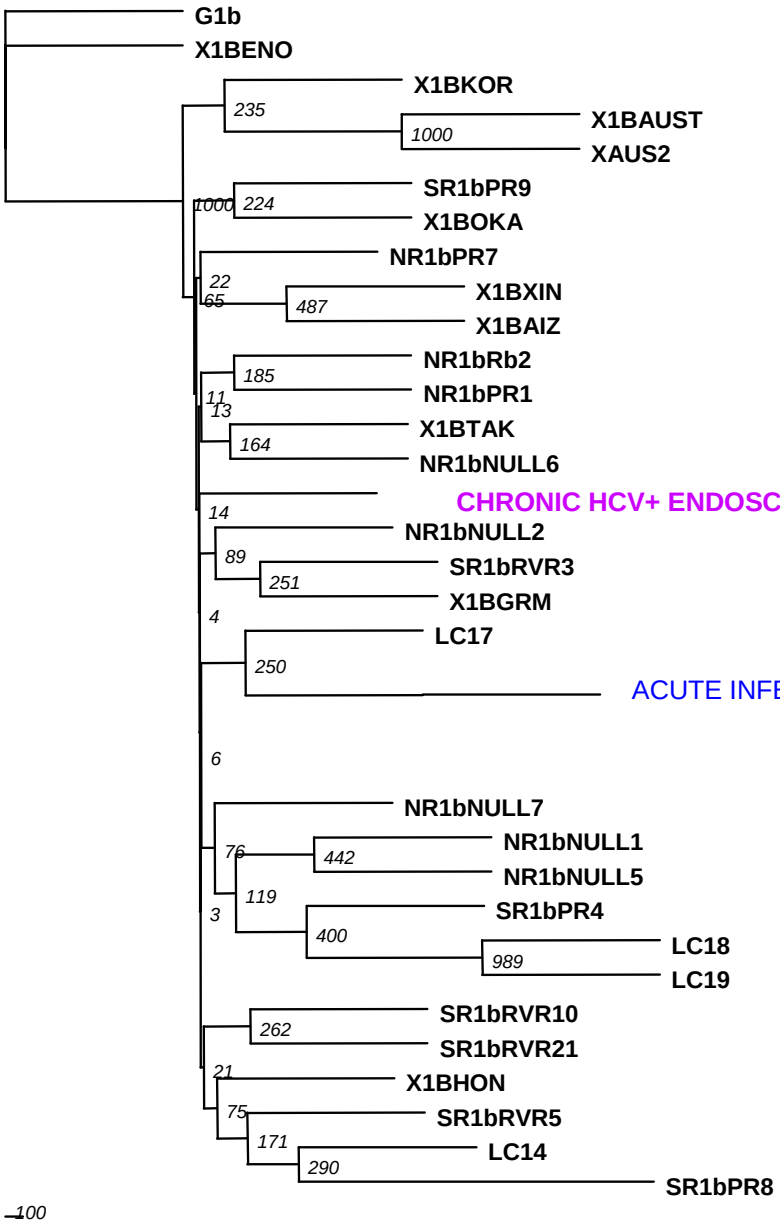








ENDOSCOPY?



ENDOS

CTA1

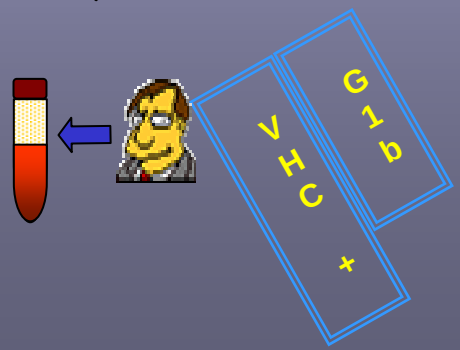
GENETIC DISTANCE:

CTA1-LC / CTA1-Genbank= 7.8-14.7%

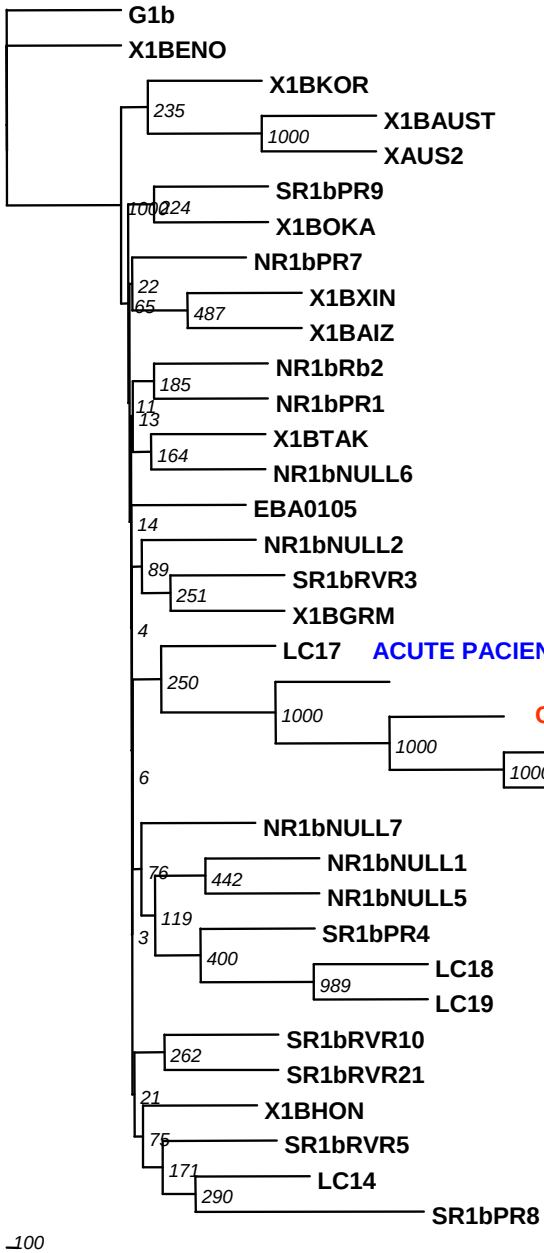
CTA- ENDOS = 12.4%

CTA patient, also underwent a **Contrast-Enhanced Computed Tomography scan** 7 weeks before jaundice.

The previous scanned patient was HCV+.



Contrast-enhanced CT?



CTA1

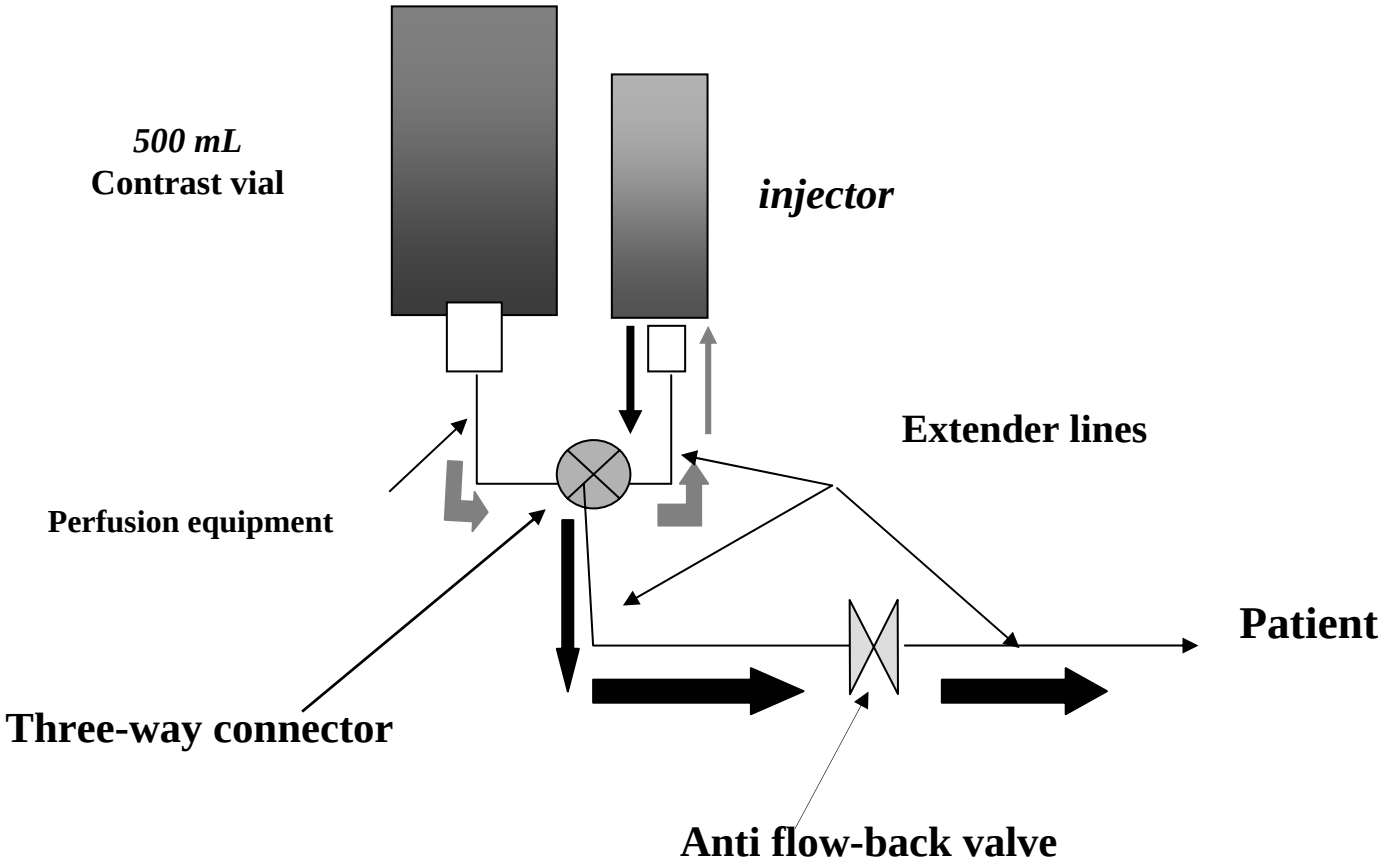
TAC

GENETIC DISTANCE:

CTA1-LC / CTA1-Genbank = 7.8-14.7%

CTA1- TAC = 0.6%

MULTI-DOSE CONTRAST MEDIUM EQUIPMENT



MOLECULAR EPIDEMIOLOGY. Conclusions

- To identify a source of infection
- To establish transmission mechanisms
- To modify procedures to prevent additional infections through nosocomial transmission:

  Patient-to-patient specially by using multidose vials

  During haemodialysis

  Infection to health-care-workers

  Health-care provider-to-patient transmission

It has legal, economical and medical practice implications:

- liability of health-care providers
- economical compensation to patients infected in the health-care setting
- potential restrictions to HCV-infected medical practitioners involved in invasive procedures.