

*VHPB MEETING*  
*PREVENTION AND CONTROL OF VIRAL HEPATITIS IN GREECE*  
*ATHENS, 15-16 MAY 2007*

# **SURVEILLANCE IN BLOOD BANK**

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# **B L O O D   S A F E T Y**

**The safety of blood supply remains a major concern of public health and requires continuous efforts to ideally reach zero-risk probability.**

**The public's fear and awareness of the potential of viral transmissions through blood transfusion has been a major driving force for improvement of blood safety.**

**The residual risk of transmitting blood-borne viruses through transfusion varies depending on the demographic and the socio-economic conditions in each country.**

**The risk of transmitting viral infection to recipients of blood components has been significantly reduced over the last years owing to a combination of measures.**



## SAFETY OF BLOOD TRANSFUSION

- 🔥 THOROUGH DONOR SELECTION AND QUESTIONNAIRE TO ELIMINATE HIGH-RISK DONORS
- 🔥 APPROPRIATE USE OF BLOOD AND BLOOD COMPONENTS.
- 🔥 TREATMENT OF BLOOD PRODUCTS (PATHOGEN INACTIVATION, LEUCOREDUCTION)
- 🔥 IMPLEMENTATION OF ADVANCED AND HIGHLY SENSITIVE SCREENING ASSAYS FOR

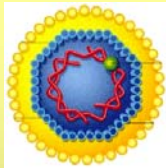
### TRANSFUSION TRANSMITTED VIRAL INFECTIONS

*Hepatitis B virus (HBV)*

*Hepatitis C virus (HCV)*

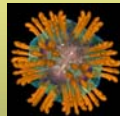
*Human Immunodeficiency Virus (HIV)*

#### HBV



6,2% of world population , ~ 350 million HBV carriers,  
1 million get infected every year in Europe

#### HCV



3% of world population  
~ 170 million chronic HCV  
3-4 million get infected every year

#### HIV

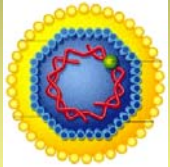


0,5% of world population  
~36,1 million have AIDS  
2.8 million die every year



## SEROLOGY TESTING OF BLOOD AND BLOOD COMPONENTS

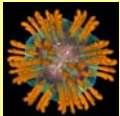
### HBV



Detection of HBV infection is based mainly on detection of HBsAg marker.  
3<sup>rd</sup> generation immunoassay or chemiluminescence method for detection of HBsAg (MEIA, ChLIA) (<0.1ng/ml)

Detection of anti-HBc in some countries/ Optional in Greece

### HCV



Detection of HCV infection is based on serology screening of antibodies against several HCV antigens

Mainly 3<sup>rd</sup> generation immunoassay (EIA, MEIA, ChLIA), and/or HCV core detection / (Confirmation Western Blot)

### HIV

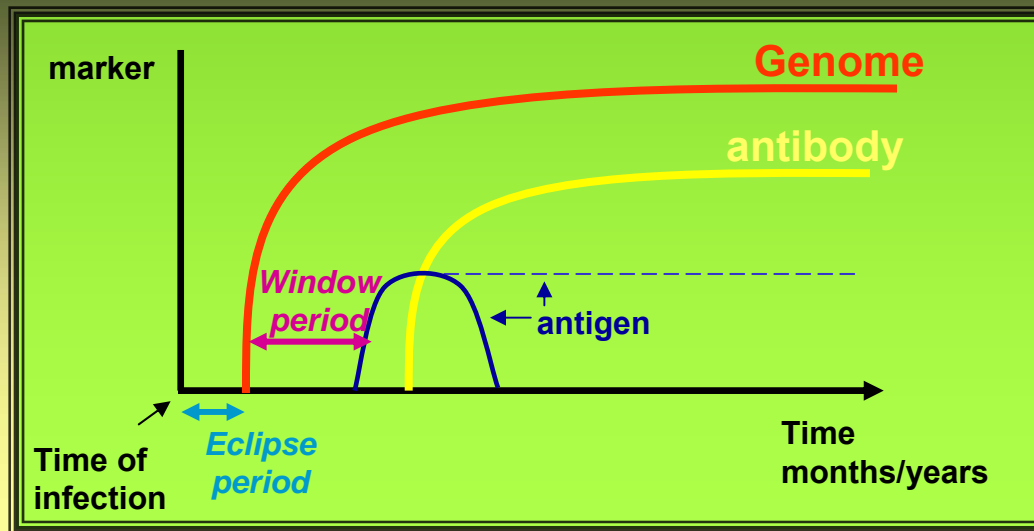


Detection of HIV infection is based on:

- screening of antibodies against several HIV antigens using 3<sup>rd</sup> generation immunoassays MEIA or ChLIA / (Western Blot confirmation testing)
- screening of p24 antigen



## GENERALISED COURSE OF INFECTION (TTIs)



**Window Phase period (WP):** serologically “silent” period that precedes the development of antibodies in the initial infection. An infected donor may harbor large amounts of viral particles in the absence of symptoms and be infectious even though he remains seronegative.

**Eclipse period:** Virus replication is restricted to tissue sites and there is no detectable viraemia.

NAT Yield Cases: the identification of a sample as NAT positive and antibody negative [ NAT (+) / antibody or antigen (-) ]

ID – NAT : Screening of NAT in individual donations

MP – NAT : Screening of NAT in mini pools.



## NUCLEIC ACID TESTING - NAT TECHNOLOGY

- ❖ Detects viral nucleic acids (RNA or DNA) prior to the appearance of viral antigens or antibodies in the circulation thus offering significant advantages in the diagnosis of the viral infection
- ❖ Allows for the detection of very low levels of viral RNA or DNA in the early stages of infections during which current serology screening assays are unable to detect infection. Offers high sensitivity and specificity for viral nucleic acids.
- ❖ Simultaneous detection of all three viruses HIV, HCV και HBV using Multiplex assays.

### Advantages

- Narrows Window Period considerably
- Detects viral infections earlier than other screening assays
- Enhances safety of the blood supply by reducing the residual risk of viral transmissions linked to window period.



## NUCLEIC ACID TESTING - NAT

- 🔥 **Mid 90s** Introduction of NAT by plasma manufacturers on a voluntarily basis in order to provide an extra safety margin on top of inactivation procedures. Testing was performed in mini-pool format for HCV-RNA, thus reducing the viral burden in the starting pools for fractionation.
- 🔥 **1999** Paul Ehrlich Institute (PEI) was the first regulatory body in Europe that has set a requirement for HCV NAT testing of cellular blood components (with a sensitivity of 5000 IU/ml (20000geq/ml) for HCV-RNA). In house developed test systems in mini-pool on 96 samples.
- 🔥 **1999** Japanese Red Cross initiates the implementation of NAT testing.
- 🔥 **1999** European Union required that all fractionated plasma products are HCV-RNA negative using NAT (with a sensitivity of 100 IU/ml of HCV-RNA in the final pool, ~230 viral particles/ml)
- 🔥 **Beginning of 2000** many countries in Europe introduce NAT technology for HCV and HIV using ready made diagnostic NAT kits in minipools of 48, 24 and/or 16 samples.



## **NUCLEIC ACID TESTING - NAT**

- 🔥 Mandatory NAT testing for HIV RNA in: France and Holland (2001), Belgium and Switzerland (2002), Poland (2003) and Germany (2004).**
- 🔥 Mandatory NAT testing for HCV RNA in: Austria, Germany, Holland and Switzerland (1999), Finland, Norway, Slovenia (2000), France and Poland (2001), Belgium and Italy (2002), Spain (2003).**
- 🔥 NAT testing has been optionally introduced in other EU countries (England, Ireland, Luxemburg, Portugal, Greece)**
- 🔥 HBV NAT testing has already been introduced in countries like Austria, Germany, Italy, Lithuania, Poland, Spain, Portugal and Greece.**





# NUCLEIC ACID TESTING - NAT TECHNOLOGY

Implementation of NAT technology in addition to the pre-existing antibody / antigen-based screening has dramatically reduced the **Residual Risk of Transmission**

## 1) Direct risk measurement (1985-1990)

(direct measurement of viral transmission risks from antibody screened transfusions)

## 2) Risk modeling (1991-present)

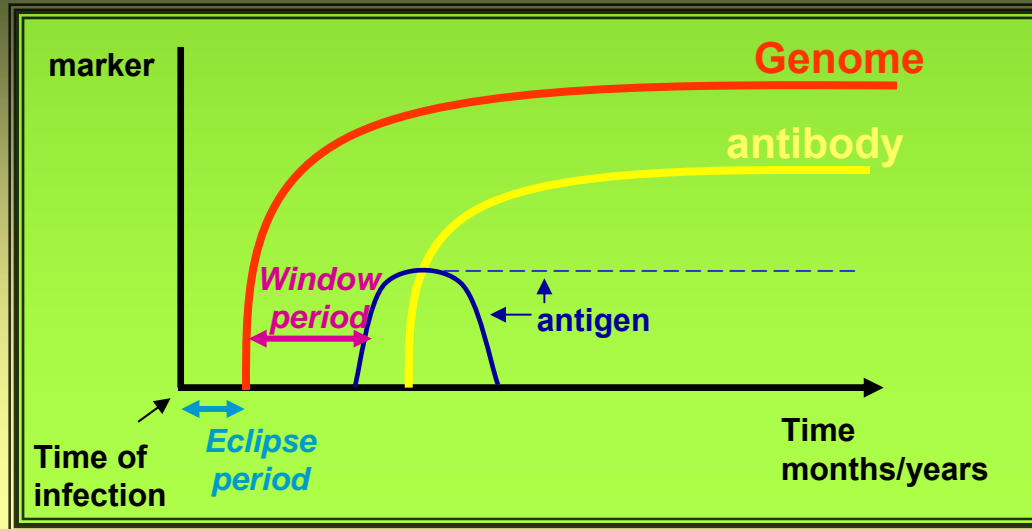
*i) Incidence Rate –Window Period model*

*ii) Viral dynamics and rates of detection of donors with incident infections (viraemia doubling time, viral load parameters etc.)*

- Schreiber GB et al, *N. Engl. J. Med.* 96, 334, 1685-90
- Busch M et al, *Transfusion*, 2005, 45: 254-264
- Weusten et al, *Transfusion* 2002,



## GENERALISED COURSE OF INFECTION (TTIs)



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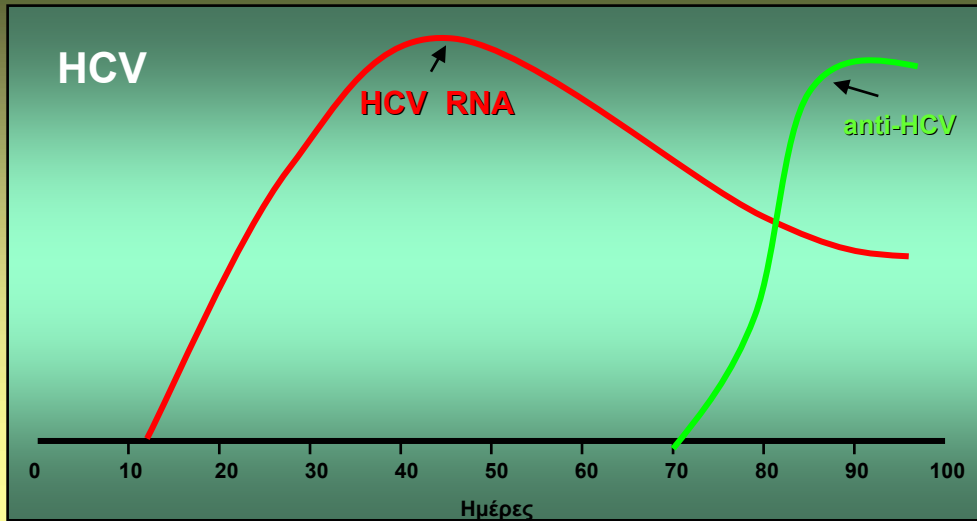
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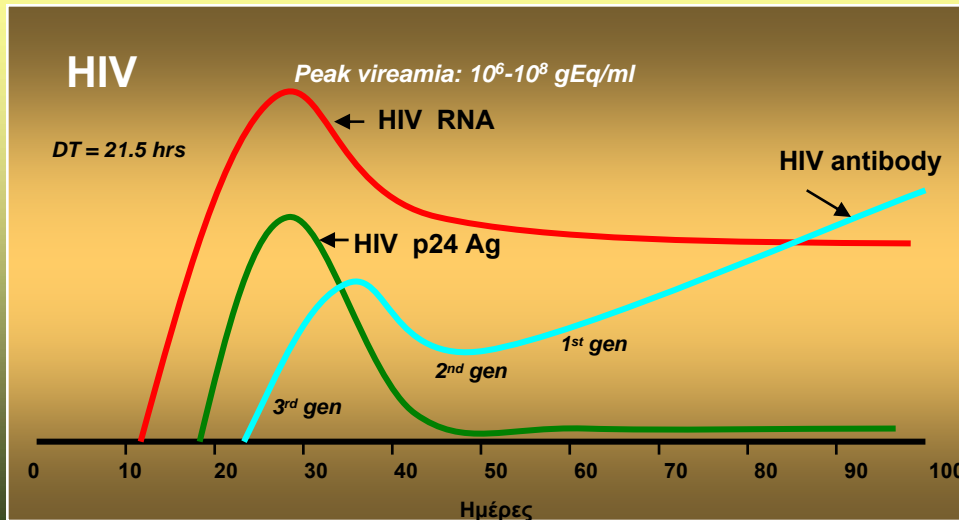
**MP – NAT :** NAT Screening in mini pools.

# HCV INFECTION PROFILE



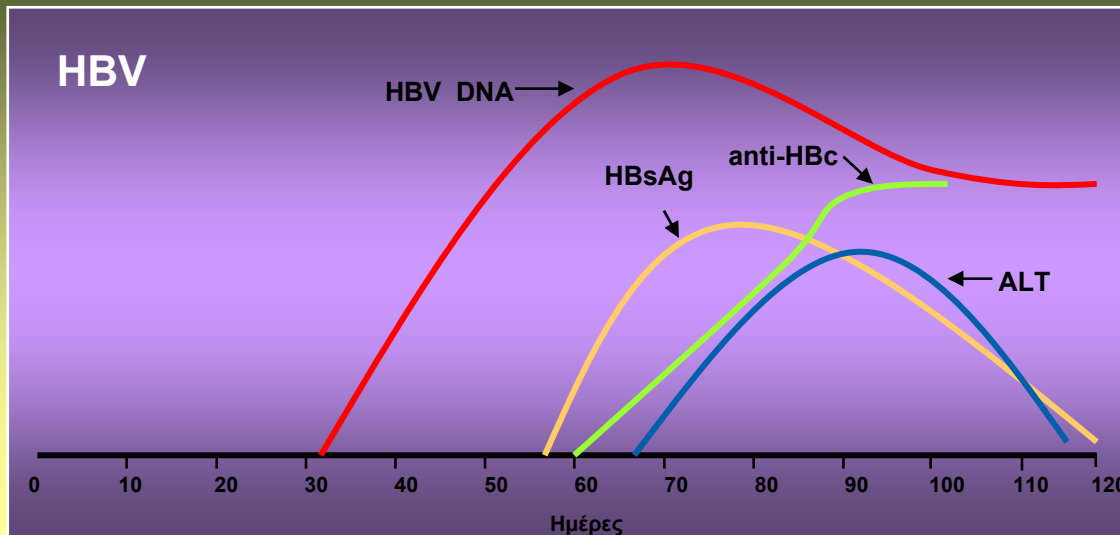
**Ab Detection** 70-80 days  
**RNA Detection** 12-22 days  
**Window reduction** 70%

# HIV INFECTION PROFILE



**Ab Detection** 22 days  
**p24 Ag Detection** 17 days  
**RNA Detection** 11 days  
**Window reduction** 50%

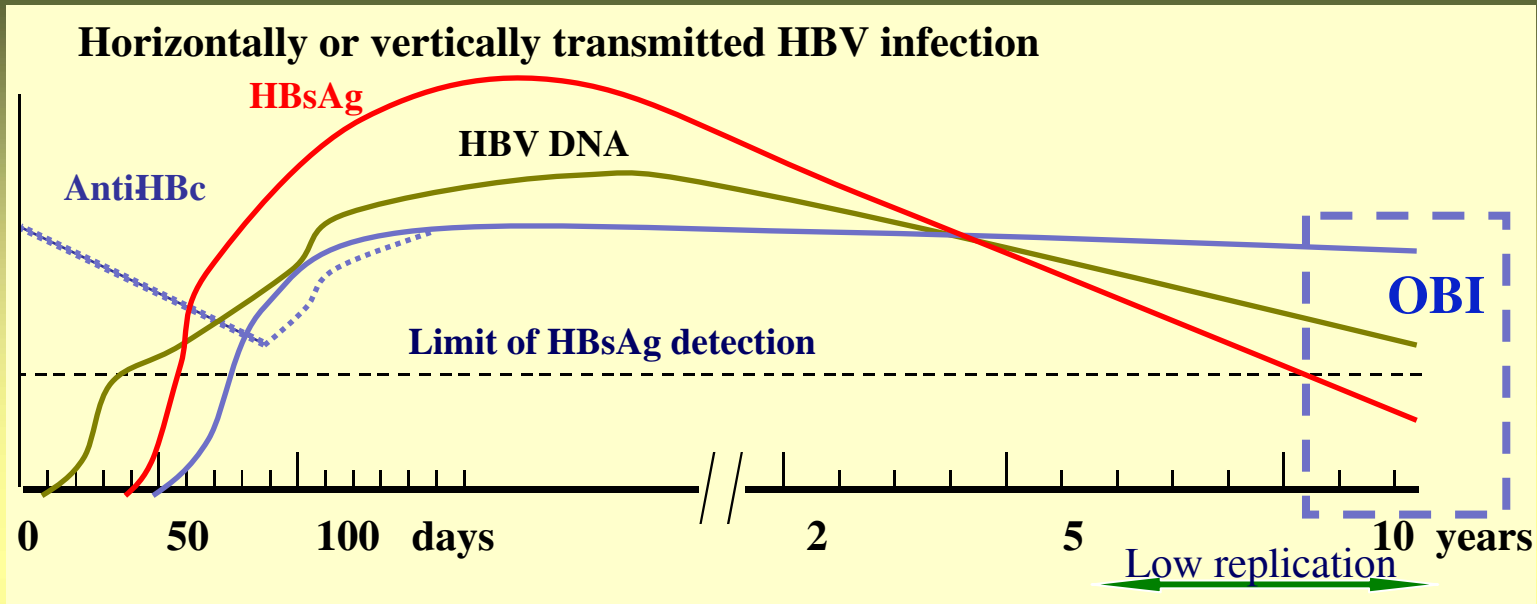
# HBV INFECTION PROFILE



- The window period is about 59 days / introduction of even more sensitive serology assays for HBsAg  $< 0.1\text{ng/ml}$   $\rightarrow$  38 days
- The doubling time for HBV virus is approximately 2.5 days.
- Detection of HBV DNA: 24 days
- The prevalence of HBV infection is higher (compared with that of HCV και HIV) therefore the residual risk is also higher.

*Koppleman MH et al, Transfusion 2005, 45, 1258-66*  
(Countries with low, medium or high endemicity)

# Physical history of HBV infection



Slide by  
JP ALLAIN

## Occult HBV infection (OBI)

❖ The presence of HBV DNA in the blood or tissues of patients, without detectable HBsAg, with or without antibodies to HBe antigen, outside the pre-seroconversion window period.

**With the introduction of NAT technology two different types of HBV DNA positive / HBsAg negative blood samples are detected**

**(Discrimination between WP NAT yield cases and occult NAT yield cases)**

## **Occult HBV infection (OBI)**



### **Various forms of occult HBV infection:**

- ❖ ***Recovery from infection defined by the presence of anti-HBs***
- ❖ ***Chronic hepatitis B where the infection is related to escape mutants that are poorly or not recognised by either polyclonal or monoclonal antibodies in assays***
- ❖ ***Chronic hepatitis B at the healthy carriage stage marked by the presence of anti-HBc with or without detectable anti-HBe***
- ❖ ***Chronic hepatitis or healthy carriage without any marker of HBV infection other than HBV DNA***

**The viral load in OBI varies from 10<sup>4</sup> IU/ml - 500 IU/ml, often <100 IU/ml and fluctuates.**

- ❖ **Transmission through blood or blood products infected with OBI is under investigation and possibly depends on the quantity of the infected product, the viral load and the immunocompetence of the recipient.**
- ❖ **Already 3 cases of transmission through OBI.**

**(Ullum H et al, Vox Sanguinis 2006, 91 suppl 3, p65**

**Gerlich WH et al, Journal of Clinical Virology 36 Suppl 2006, S18-S22)**



🔴 For the period 1996-2006 about 5.6 million blood units have been serologically screened.

### **Seroprevalence**

<b>HBsAg :</b>	<b>0.4%</b>
<b>HCV :</b>	<b>0.08%</b>
<b>HIV :</b>	<b>0.007%</b>

*Data from SKAE (Madrid, 2007)*

## **NUCLEIC ACID TESTING IN GREECE :**

🔴 Implementation of NAT was initially launched for HCV RNA/ HIV RNA in 2003 in a number of blood establishments.

🔴 Since 2005 HBV DNA testing was also included and until the end of 2006 the number of blood transfusion centers performing NAT screening has increased.

🔴 Preliminary data of NAT screening for the period 2003-2006 reveal the following:



# PRELIMINARY DATA OF SIMULTANEOUS NAT TESTING OF BLOOD SAMPLES FOR HCV RNA/ HIV RNA /HBV DNA

**TIME PERIOD : 2003 - February 2007**

TOTAL OF BLOOD SAMPLES : **348.721**  
HCV RNA / HIV RNA : **169.694 (49%)**  
HCV RNA / HIV RNA / HBV DNA : **179.027 (51%)**

<b>INFECTIOUS MARKER</b>	<b>NAT ID YIELD</b>	<b>Frequency/ blood units</b>	<b>Prevalence/ million units</b>
HIV	3	1 / 133.373	7.5
HCV	8	1 / 50.015	20
HBV	38 (occult)	1 / 6.080	164.5
<b>TOTAL</b>	<b>49</b>	<b>1/189.468</b>	<b>462</b>

*Data from SKAE (Madrid, 2007)*





## RISK PER UNIT OF TRANSMISSION OF MAJOR VIRUSES

	<b>pre- NAT</b>	<b>MP-NAT</b>	<b>ID-NAT</b>
<b>HIV</b>	1/1.300.000	1/1.900.000	1/3.000.000
<b>HCV</b>	1/230.000	1/ 1.600.00	1/2.300.000
<b>HBV</b>	1/180.000	1/210.000	1/410.000

*H.Klein, Santorini, 2007*



## OCCULT HBV CASES

<b>HBV</b>	<b>38 (occult)</b>	<b>1 / 6.080</b>
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### **INFECTIOUS**

#### **MARKER**

#### **negative**

#### **positive**

HBsAg	38 (100%)	0 (0%)
anti-HBc	0 (0%)	38 (100%)
anti-HBs	37 (97.4%)	1 (2.6%)
anti-HBe	33 (87%)	5 (13%)

HBV DNA consistently .....30 (80%)

HBV DNA inconsistently .....8 (20%)

Quatitative PCR in 12 (63%)

Viral load 24-152 copies / ml

Collaborative study for systematic molecular and biochemical characterisation of these infections

**Sequencing**

**Follow up samples**



## SUMMARY

- ❖ The implementation of NAT technology is particularly significant, since it reinforces the safety of blood supply. Introduction of NAT technology shortens the window period between exposure to infection and reactivity by serological assays. Consequently, the residual risk of transmission of HIV, HCV και HBV infection is reduced significantly.
- ❖ NAT technology cannot replace serology screening assays for antibodies and/or antigens yet. NAT and serology complement each other.
- ❖ Comparison of our results with those from other EU countries demonstrate an increased incidence of NAT yield cases for all three infections. **(1 HIV / 133.373, 1 HCV / 50.015 και 1 HBV/ 6.080 blood units)** supporting the importance of establishing NAT as a routine screening test in the entire blood supply in Greece.
- ❖ Screening in ID-NAT seems to be better compared to screening in MP-NAT, especially in cases with low viral load in combination with high incidence rates of infections.
- ❖ NAT yield is usually higher following testing of donations collected form 1st time donors than from repeat donors.



## SUMMARY

- ❖ Surveillance of transfusion transmitted infections (TTIs) has shown prevalence of OBI 1/6080 blood units. These data demonstrate that Greece is an area of medium endemicity for HBV infection especially for OBI.
- ❖ For HBV infection the benefit from MP-NAT implementation seems rather minimal compared to that when performing more sensitive serology HBsAg screening. It appears that performing ID-NAT, more window period (WP) or occult phase cases may be detected (occult NAT cases), especially in Greece where the last years the prevalence of HBV infection appear to rise.
- ❖ Due to the high number of donors with OBI missed by HBsAg screening, the probability of having a potentially infectious donation released in the blood supply is higher for HBV than for HCV or HIV.
- ❖ The benefit of HBV NAT regarding the number of potential infectious units intercepted before donation is clear. The benefit regarding avoided morbidity and mortality should be further evaluated.
- ❖ The clinical significance of OBI and the impact on donor management should be further studied.