Hepatitis E – virus and vaccination

(virology, epidemiology, diagnostics and vaccines)

A/Prof David Anderson

Macfarlane Burnet Institute for Medical Research and Public Health

Melbourne, Australia

Hepatitis E virus

- **Similarities and contrasts with hepatitis A virus**
- **The (unmet) need for reliable diagnostics**
- **Diagnostics - Discovery and development**
  - Antigens and antibodies
- **Vaccines – Current status**

Hepatitis E virus and infection

- **Acute, generally self-limiting**
  - *25% mortality in 3rd trimester of pregnancy*
- **Most common in developing nations**
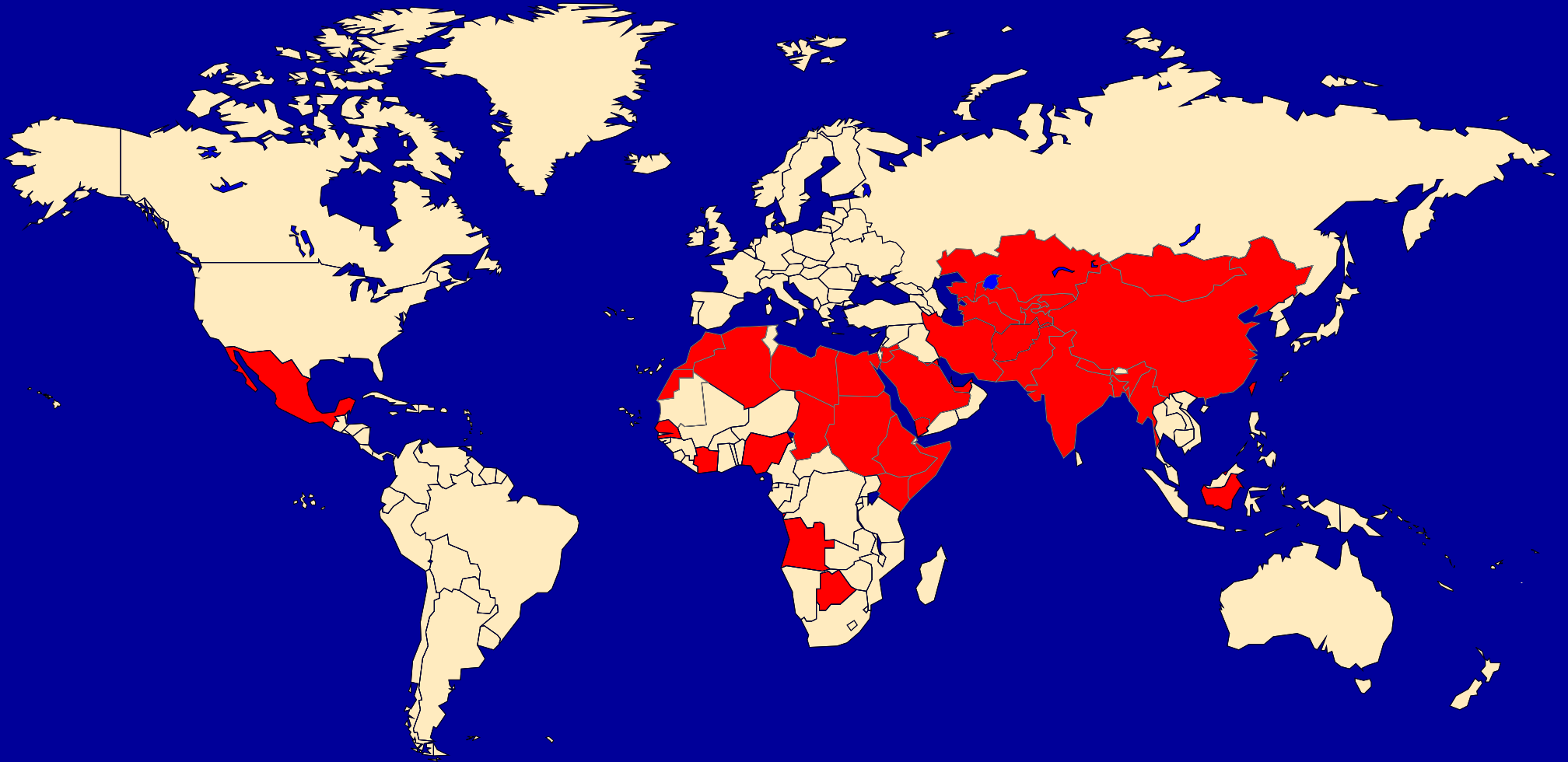- **Strain differences**

Physical characteristics

HAV

- Naked, 27-30 nm particles
- (+) RNA, 7.5 kb
- 1 polyprotein
- Three capsid proteins (VP1, VP2, VP3)
- Enteric transmission

HEV

- Naked, 32-34 nm particles
- (+) RNA, 7.2 kb
- 3 primary proteins
- One capsid protein (PORF2)
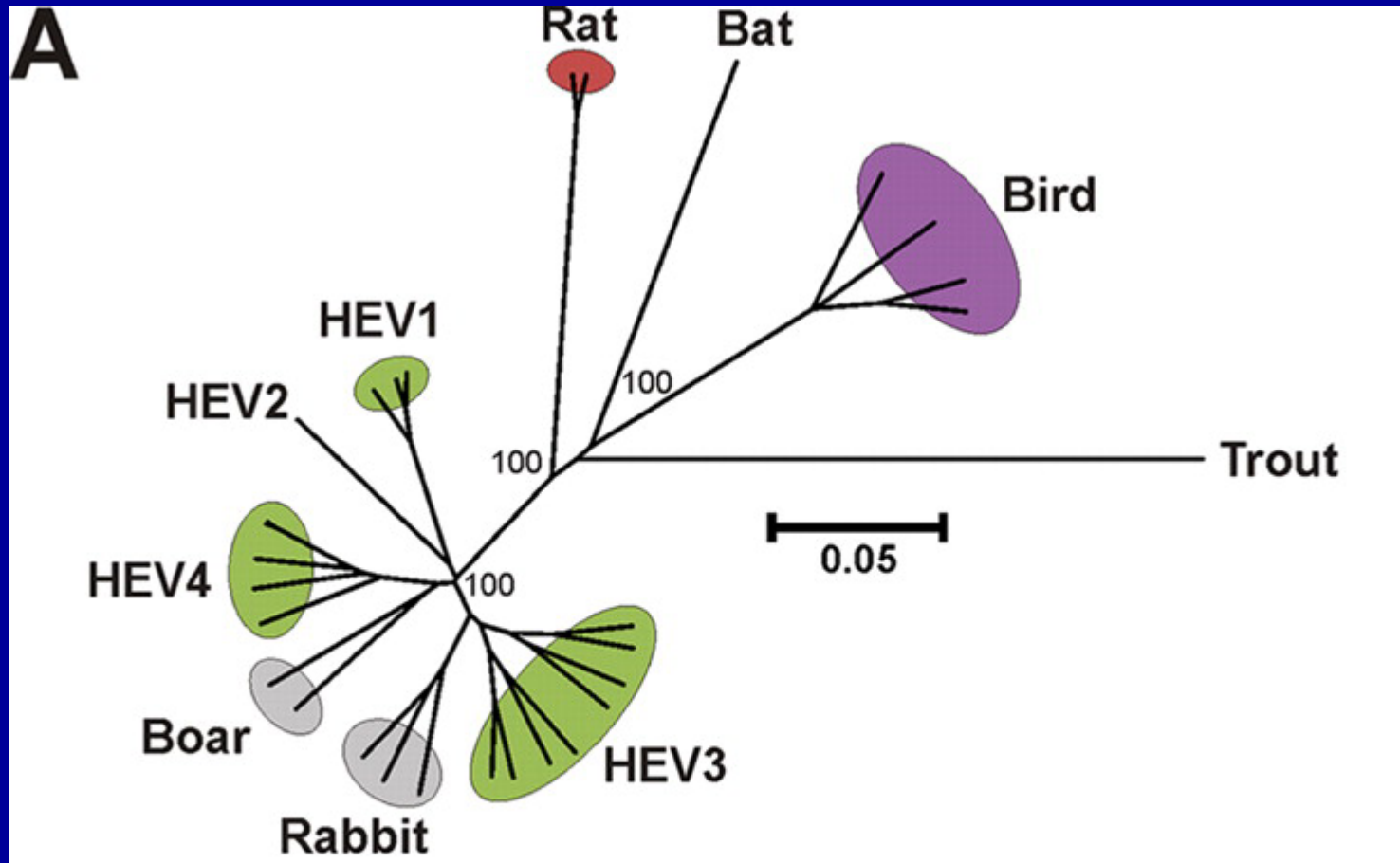- Enteric transmission

Geographic distribution of human HEV

(CDC data)

Swine HEV - Meng et al, 1997, NIH

- ≈100% of USA swine anti-HEV positive

- Unique HEV sequence

- Similar strain isolated from patients in USA (Schlauder et al, 1998)

Many other HEV-related viruses….

Disease burden

HAV

- Very common in developing world, but low morbidity
- Common in developed countries with wide socioeconomic gaps
- Foodborne outbreaks
  - International trade in fresh foods, eg strawberries, lettuces from Mexico-USA

HEV

- Common in much of developing world; high morbidity
- Rare in developed countries*
  - Travellers, zoonotic
- Foodborne and vectors in developed countries
 - Consumption of raw liver and meat in Japan, swine HEV

Control of HEV - the role of diagnostics

◆ **Effective vaccine should be available soon for developing countries (Hecolin; Xiamen Innovax Biotech Co Ltd)**
 – **GSK/Novavax vaccine passed Phase 3 in 2007, but not moving?**

◆ **Price and supply, duration of protection uncertain?**

◆ **Need to identify outbreaks/high incidence areas so that vaccine can be used, and water purification can be addressed**

◆ **Less concern about contact transmission (vs HAV)**

Ideal diagnostics for HEV

- Inexpensive and robust (field use and developing countries)
- Sensitive and specific, in areas where there is high prevalence
- High positive predictive value in all countries, including low prevalence
- Rapid, Point of care (RPOC) ideal for developing countries
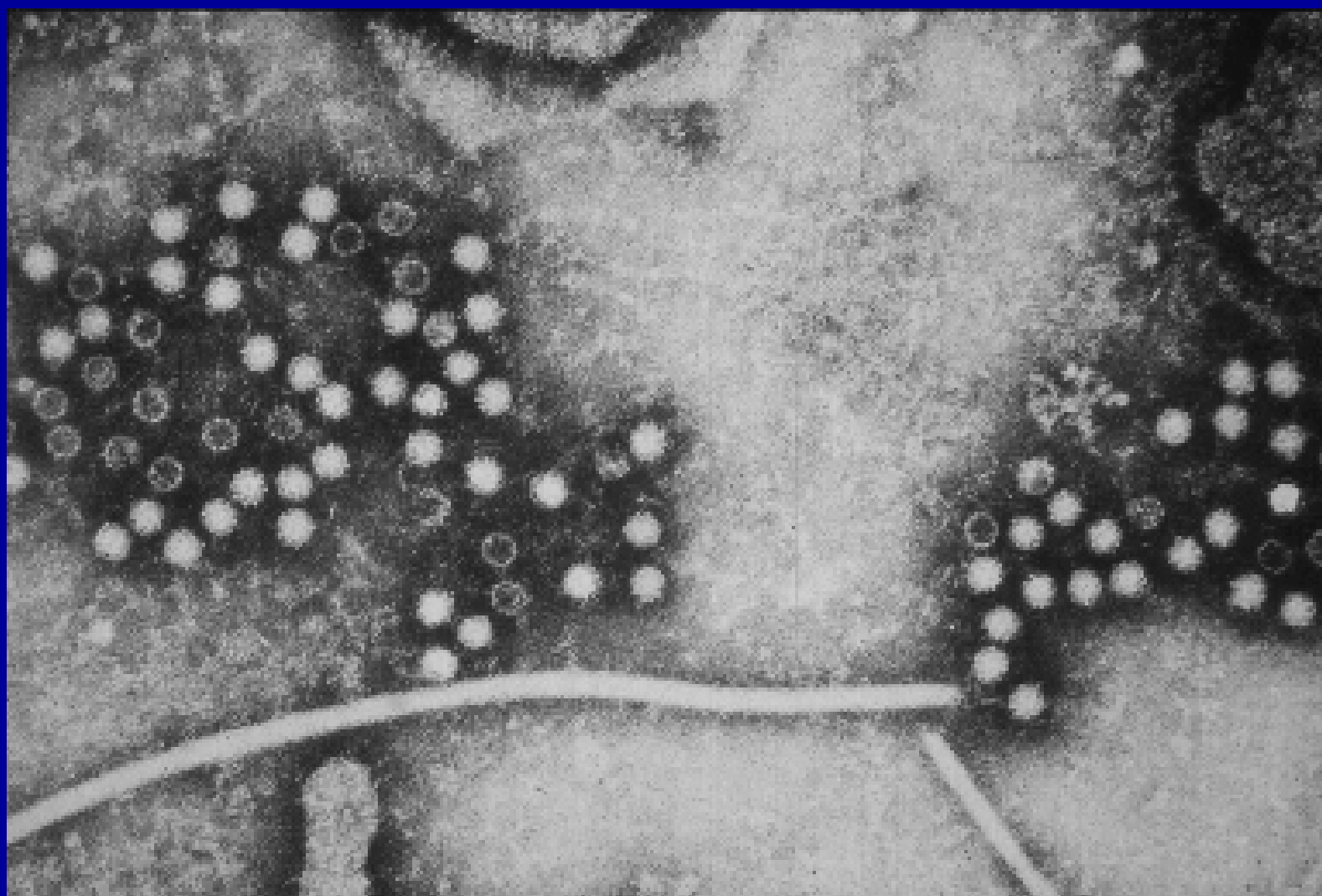
Serological responses to HAV and HEV

HAV IgG

HEV IgG

HAV IgM

HEV IgM

1   2   3   4   6   12   24

Months post infection
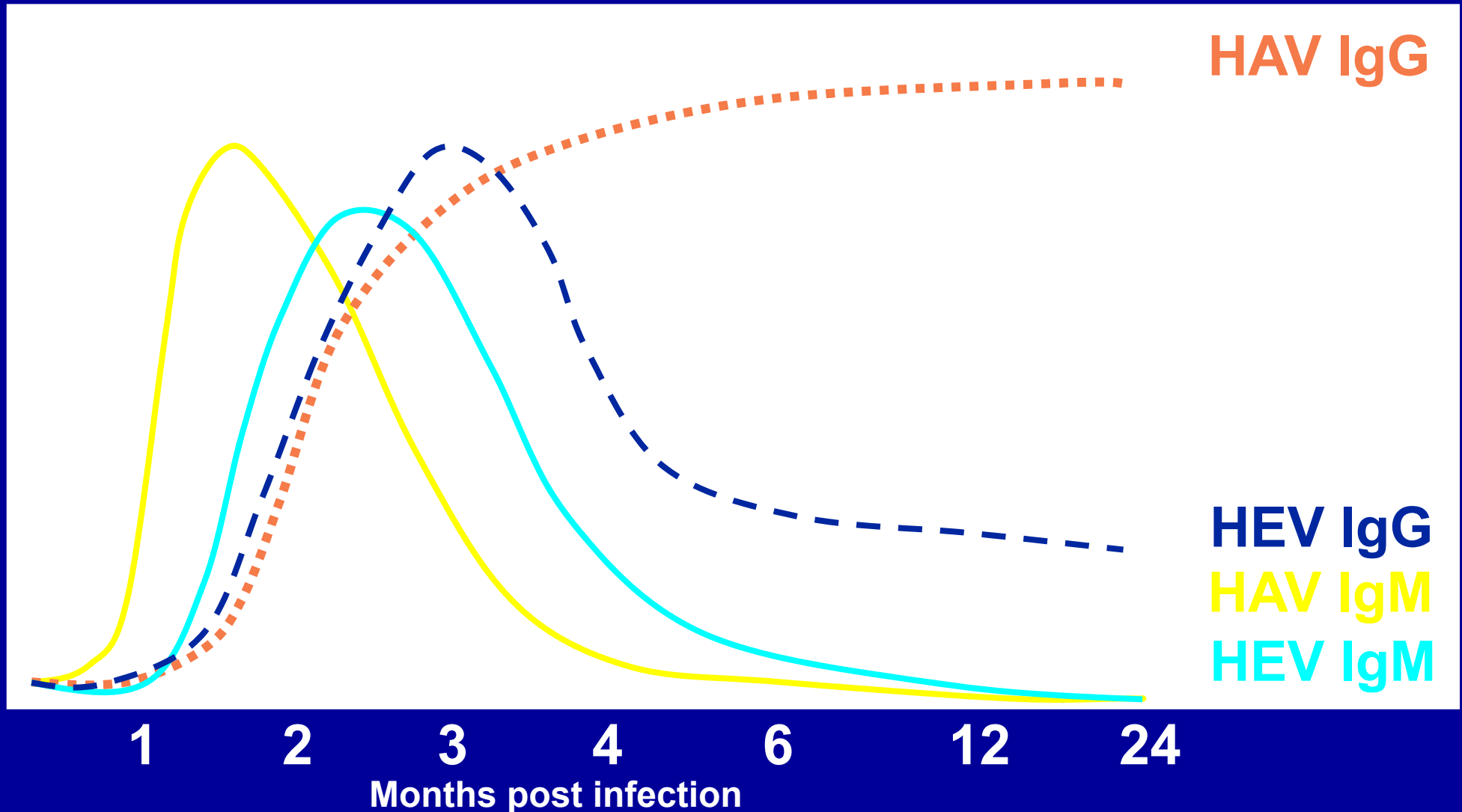
Diagnostic challenges

HAV

- Good antigen (virus), but expensive and difficult to make

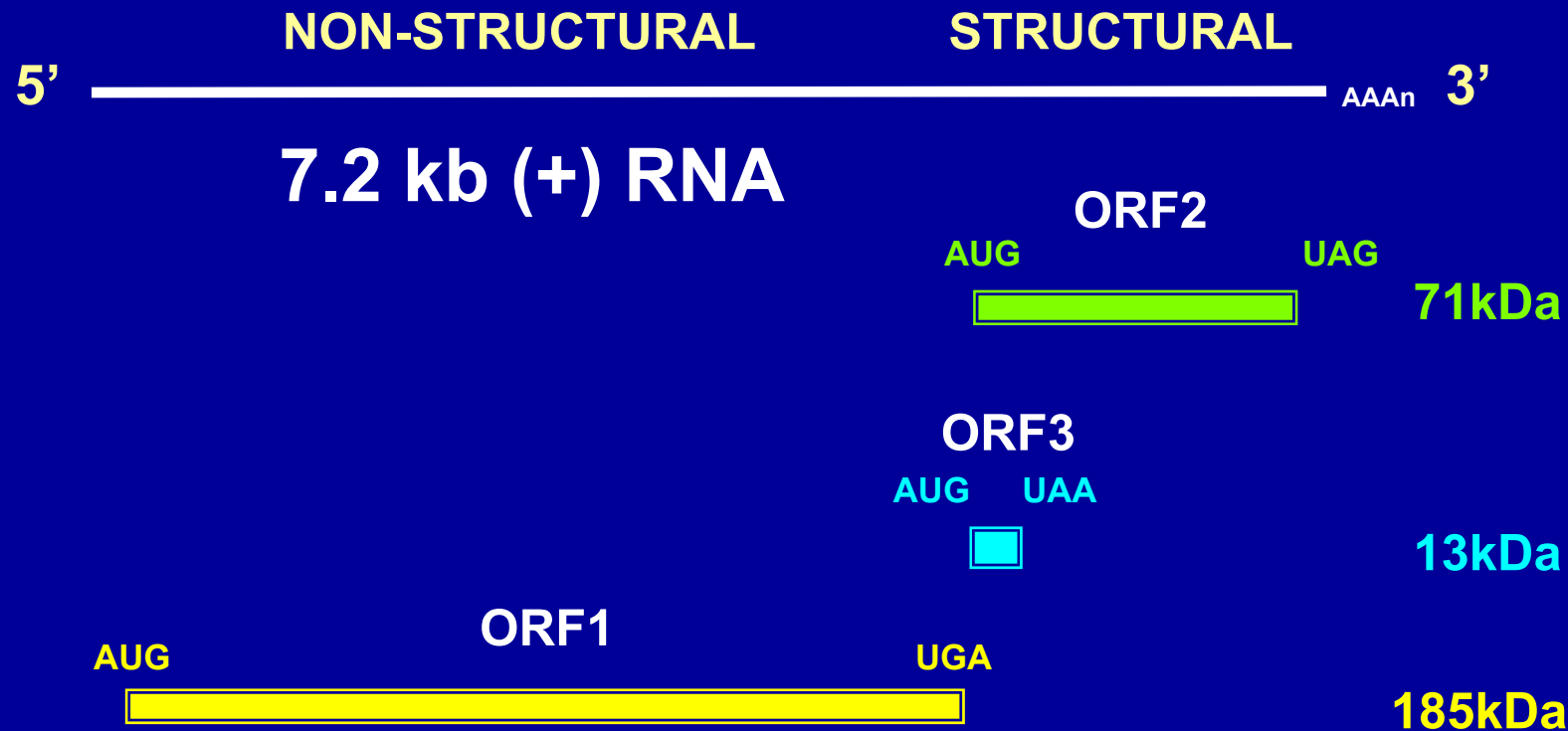- Good ELISAs, but expensive

- No rapid (RPOC) tests

HEV

- Variable antigens, easy to make

- Poor ELISAs (low sensitivity and/or specificity)

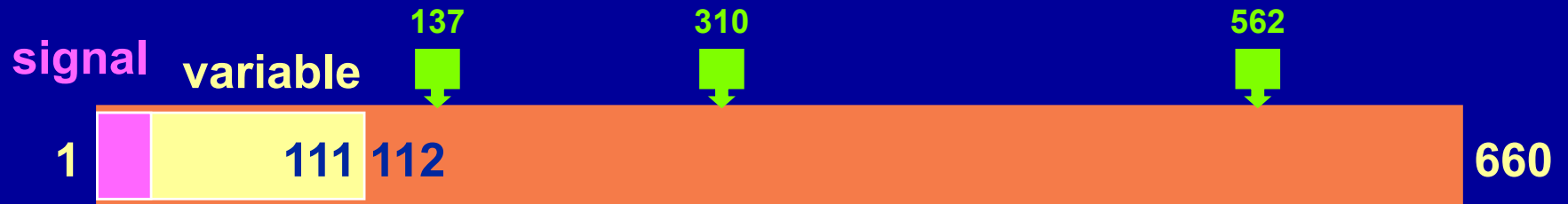- No rapid (RPOC) tests

The unmet need for HEV diagnostics

- Genelabs IgG, IgM and Abbott IgG
- Highly variable sensitivity (≈60%) and sensitivity (≈80%) – not related to virus strain differences
- All based on ORF2 and ORF3 proteins expressed in *E. coli*

HEV proteins - PORF2

◆ **PORF2: major capsid protein**

◆ **Protective antibody response** (Tsarev *et al*, 1994)

Antigenic structure of HEV

- Which antigens are appropriate for diagnosis and vaccine development?
- Which are the immunodominant epitopes?

- Are these epitopes associated with the protective immune response?

Recombinant HEV antigens (1)

Protein	aa	Reactivity	
		Acute	Conv
ORF3	124	++/-	+/-
ORF2	660		
C-2	435	++/-	+/-
SG3	327	++/-	+/-

Genelabs HEV IgM ELISA
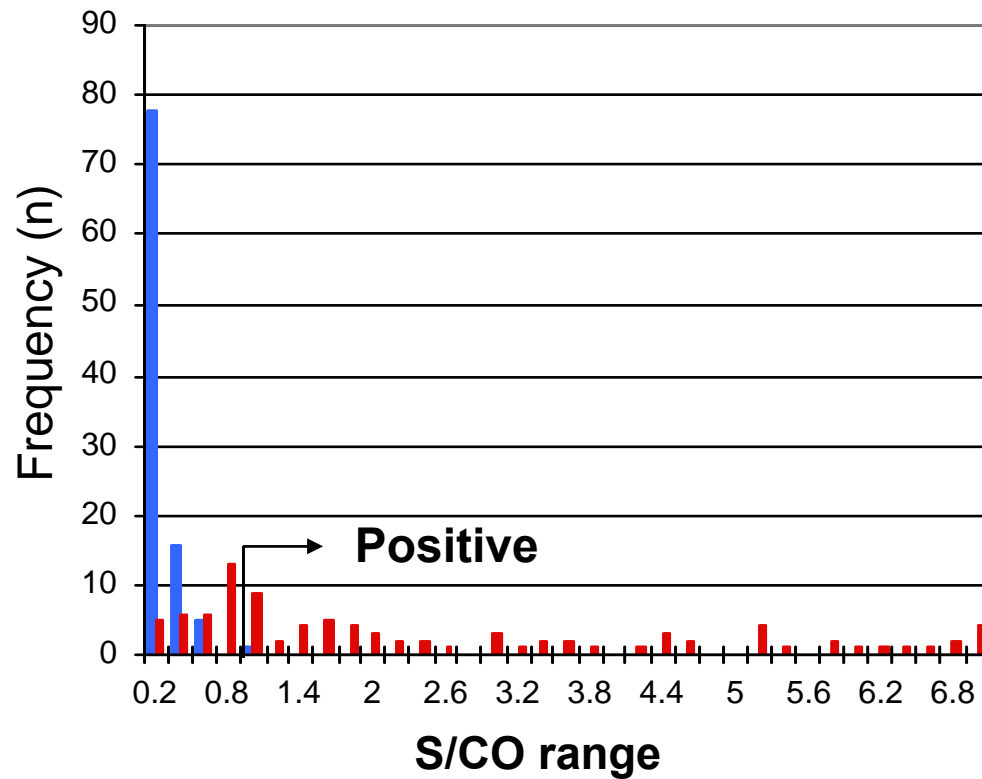
Recombinant HEV antigens (2)

	Protein	aa	Reactivity	
			Acute	Conv
	ORF3	124	++/-	+/-
	ORF2	660		
	C-2	435	++/-	+/-
	SG3	327	++/-	+/-
	ORF2 (baculovirus) VLPs	548	++++	++

Hepatitis E reactivity - VLP ELISAs

Population	anti-HAV	anti-HEV
Intravenous drug users	66.4	23.0
Homosexual men	32.3	15.9
Blood donors (Baltimore, Sacramento, New York)	16.0	22.9

Mast et al, 1997

Recombinant HEV antigens (3)

Protein	aa	Reactivity	
		Acute	Conv
ORF3	124	++/-	+/-
ORF2	660		
C-2	435	++/-	+/-
SG3	327	++/-	+/-
ORF2 (VLPs)	548	++++	+++
GST-ORF2.1	273	++++	+++

Western blot of patient sera, ORF2.1 deletion series
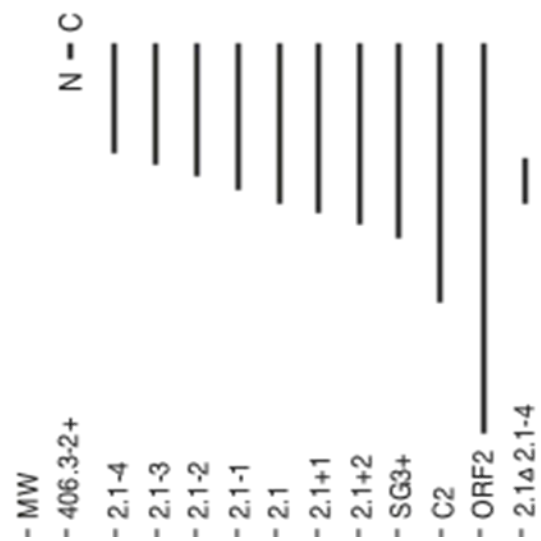
N – C

MW
406.3-2+
2.1-4
2.1-3
2.1-2
2.1-1
2.1
2.1+1
2.1+2
SG3+
C2
ORF2
2.1Δ2.1-4

Acute
patient

Convalescent
patient

Western blot of patient sera, ORF2.1 deletion series

Acute patient

Convalescent patient
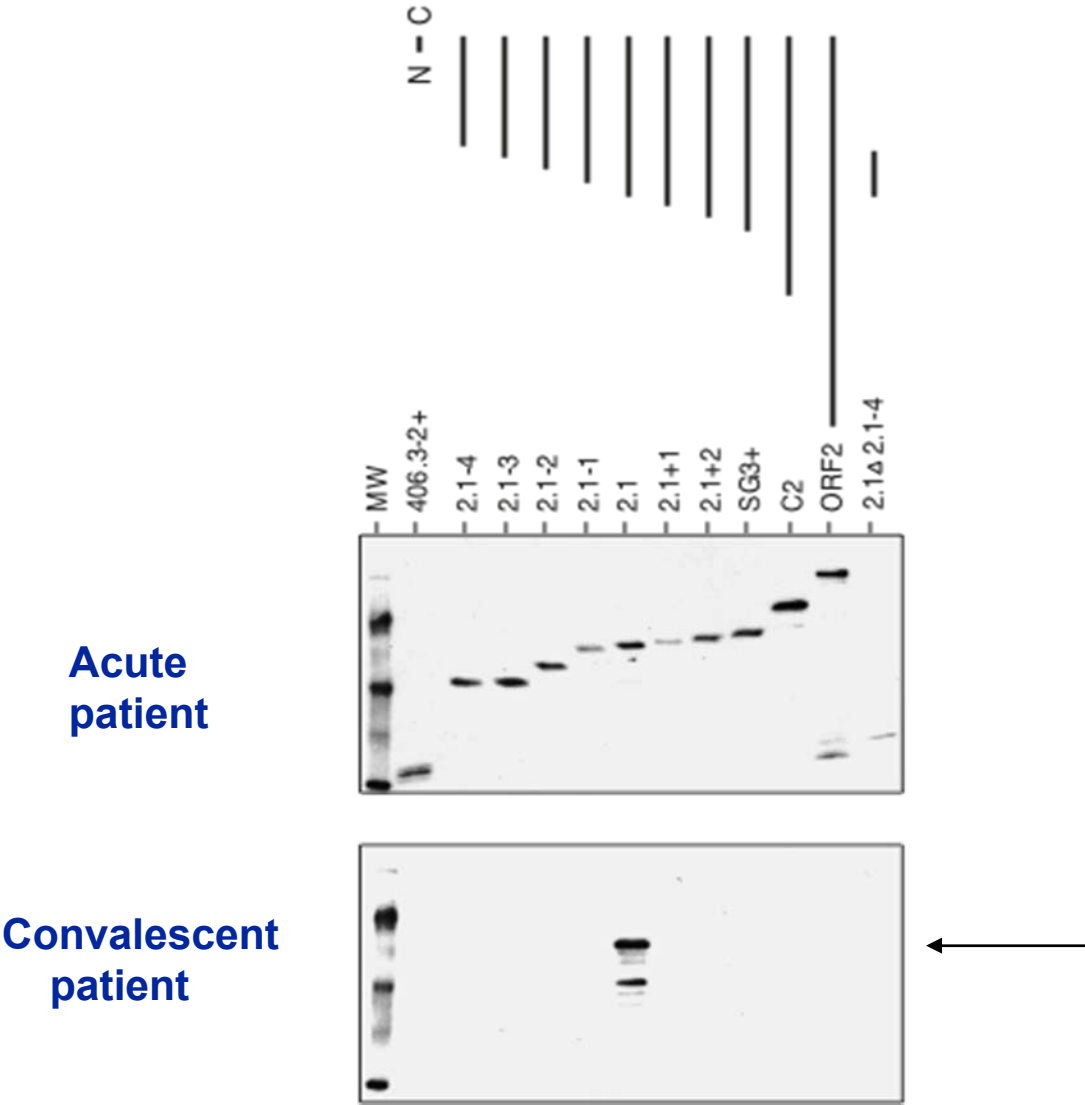
Western blot of MAbs against the deletion series
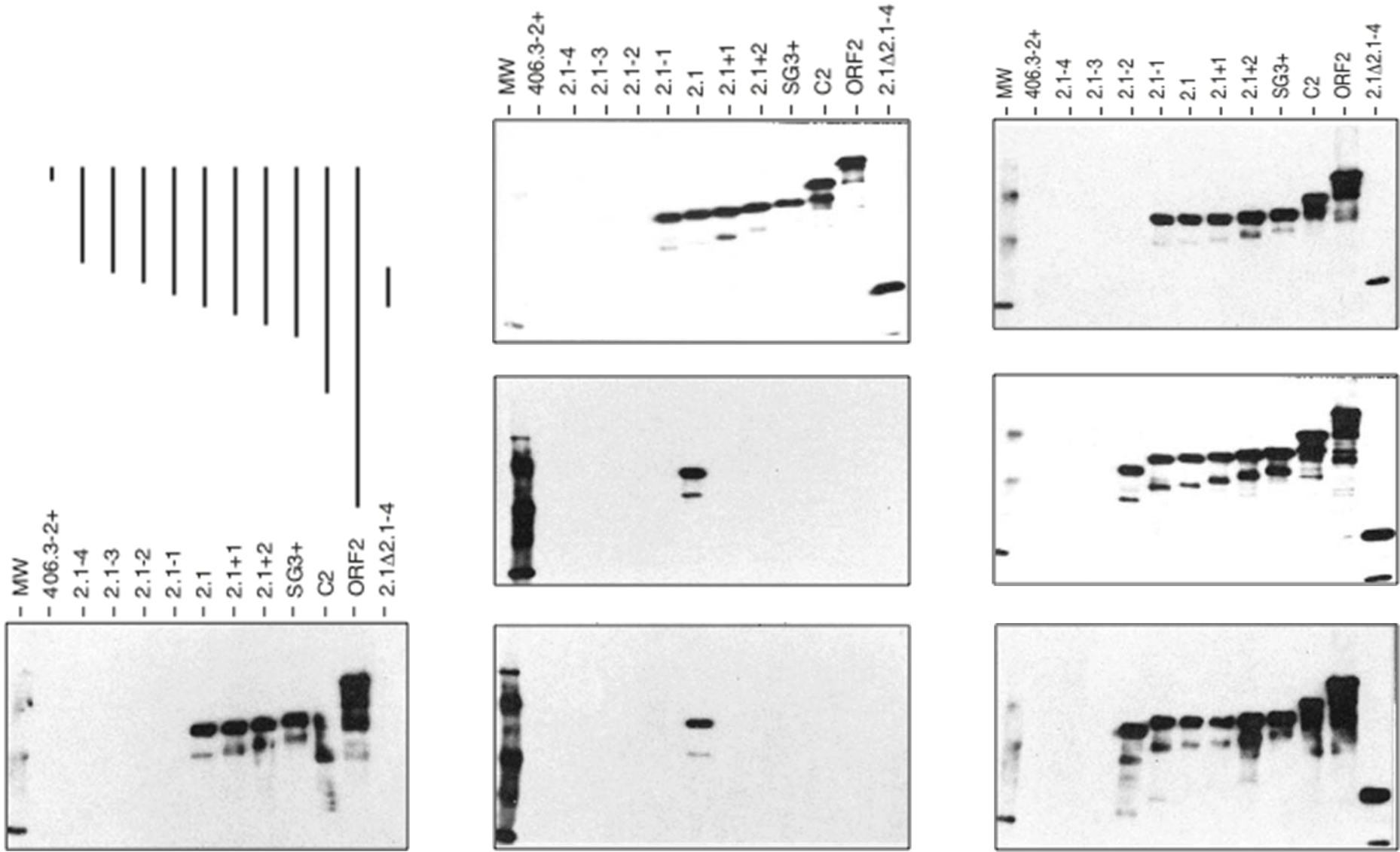
MAb	Isotype	Epitope[a]	PORF2 IF[b]	Blocking, %[c]
4B5	IgG$_1$	394-414, conformational	++++	0-4
3B2	IgG$_1$	414-434	++++	0
1E6	IgG$_{2b}$	434-457	++++	28-38
4B2	IgG$_1$	ORF2.1, conformational	+	52-59
2E2	IgG$_1$	ORF2.1, conformational	+/-	60-76

Genelabs HEV IgM ELISA
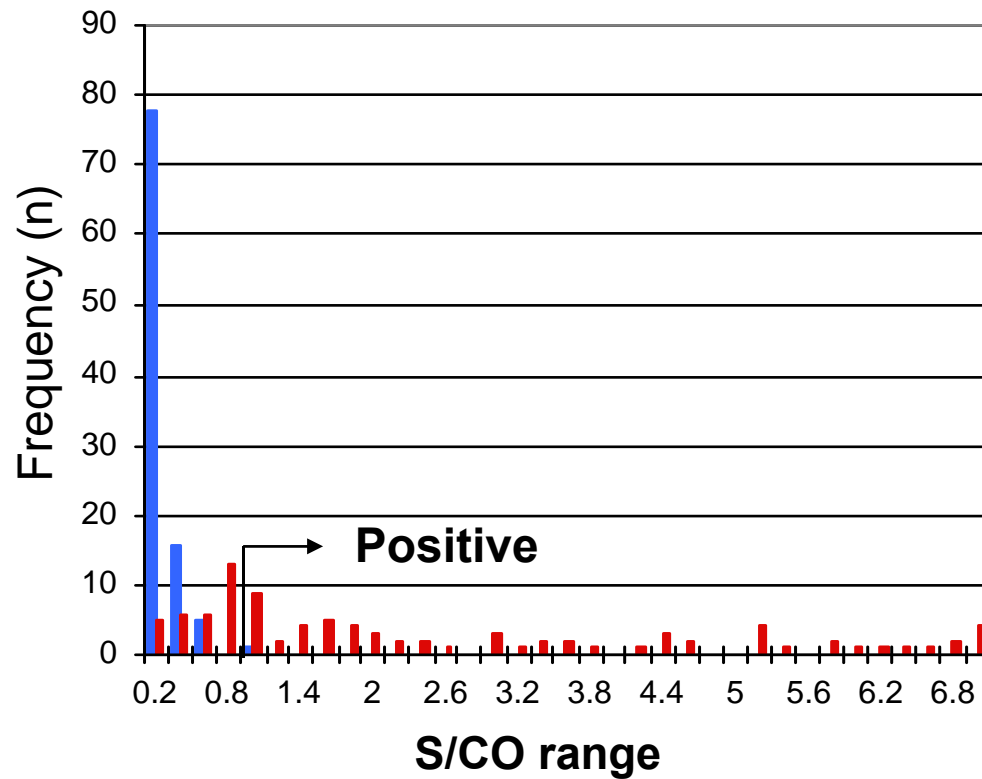
Burnet/Select HEV IgM ELISA
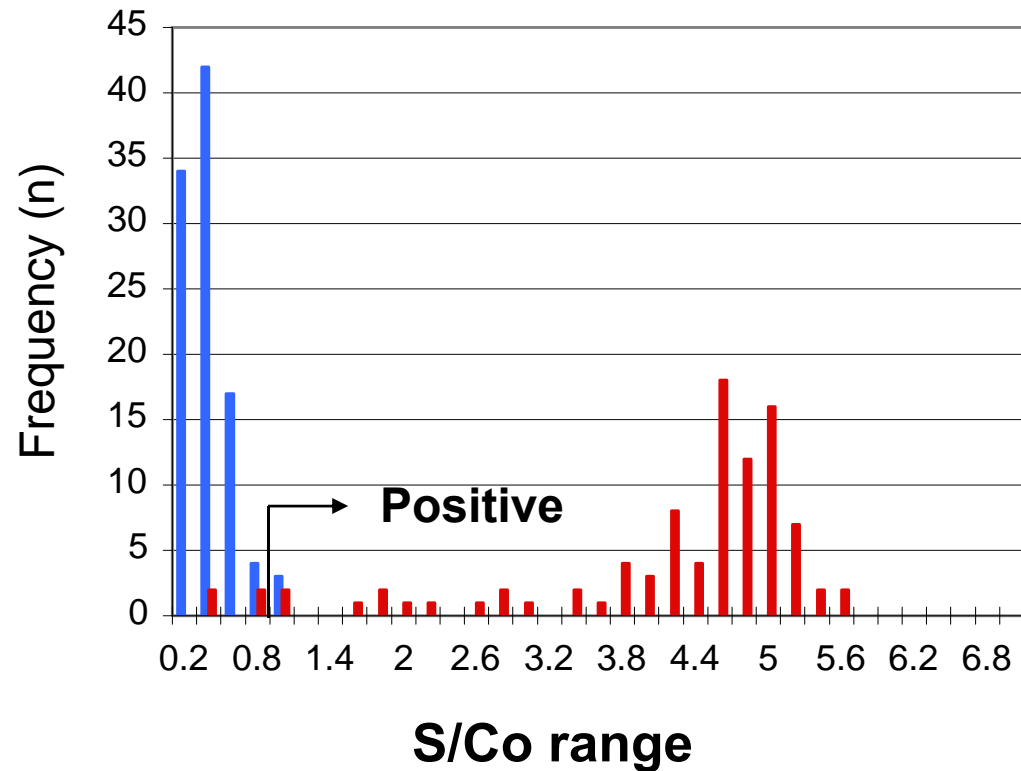
Burnet/Select HEV IgM ELISA

Population (n)	IgM reactive (%)
Australian blood donors (400)	1 (0.25)
Disease state sera (other hepatitis) (84)	1 (1.2)
Nepal, prevalence (1126)	16 (1.4)
Nepal epidemic (94)	92 (97.9)

IgM anti-HEV: Sporadic hep, Nepal

Dr Iswar Shrestha, Kathmandu
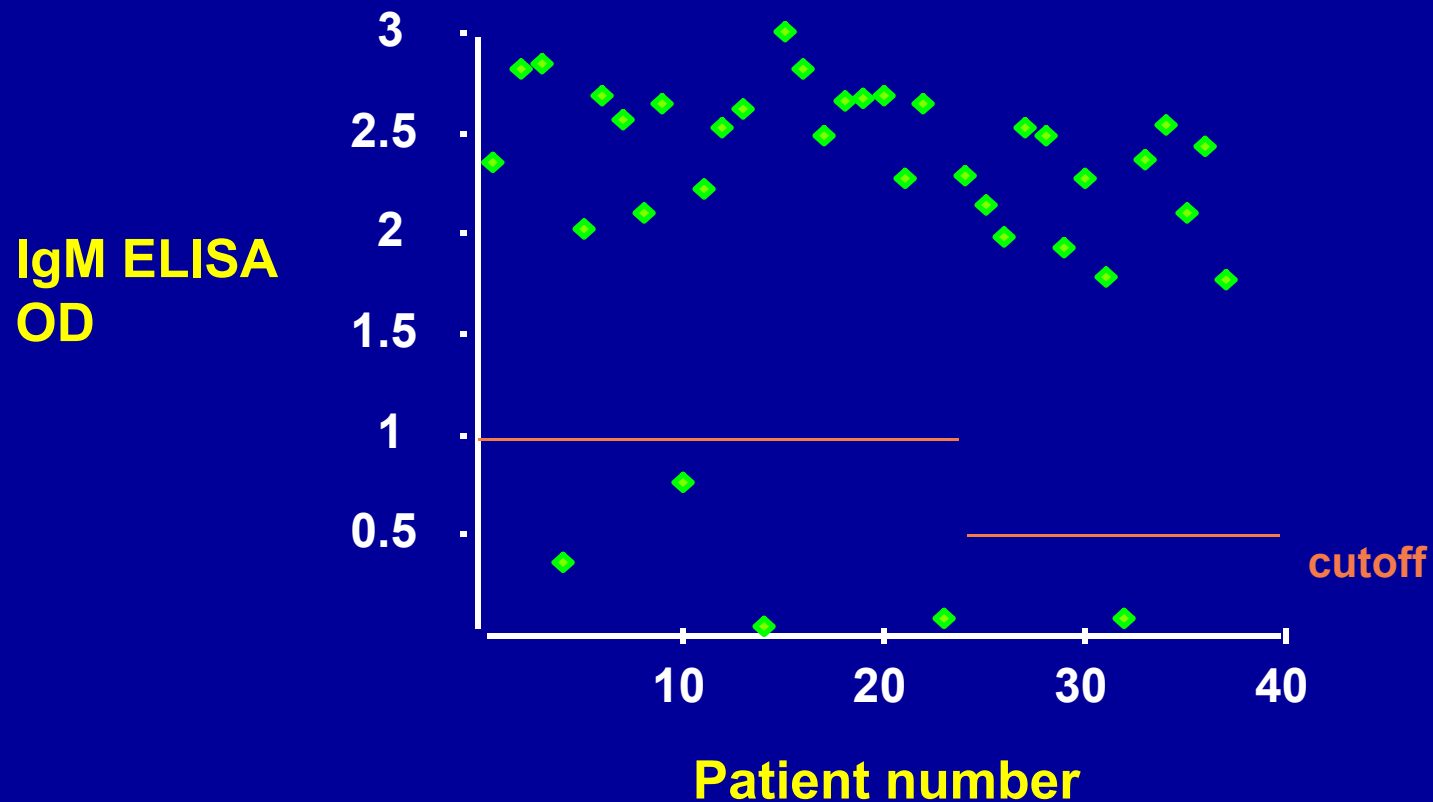
Current status of HEV diagnostics

◆ **HEV ELISAs based on ORF2.1 protein**
- **MP Biomedicals, Asia-Pacific**
- **IgM (diagnostics) ELISA 3.0**
- **IgG (seroprevalence)**
- **Antigen sandwich ELISA 4.0 (seroprevalence, zoonotic infections and seroprevalence)**

Genelabs HEV IgM ELISA (1 - old assay)
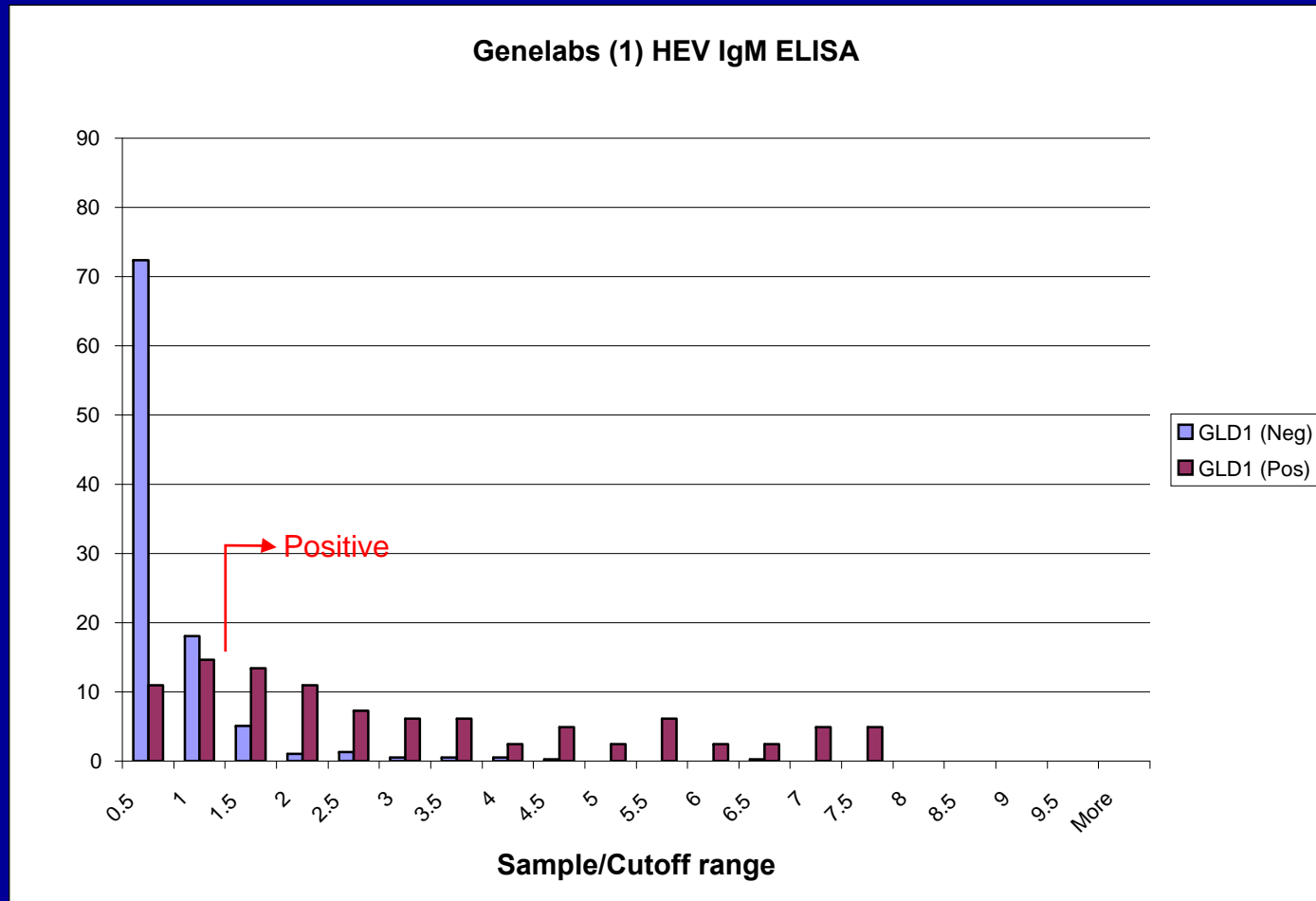
MP Bio HEV IgM ELISA 3.0

Genelabs (2) HEV IgM ELISA
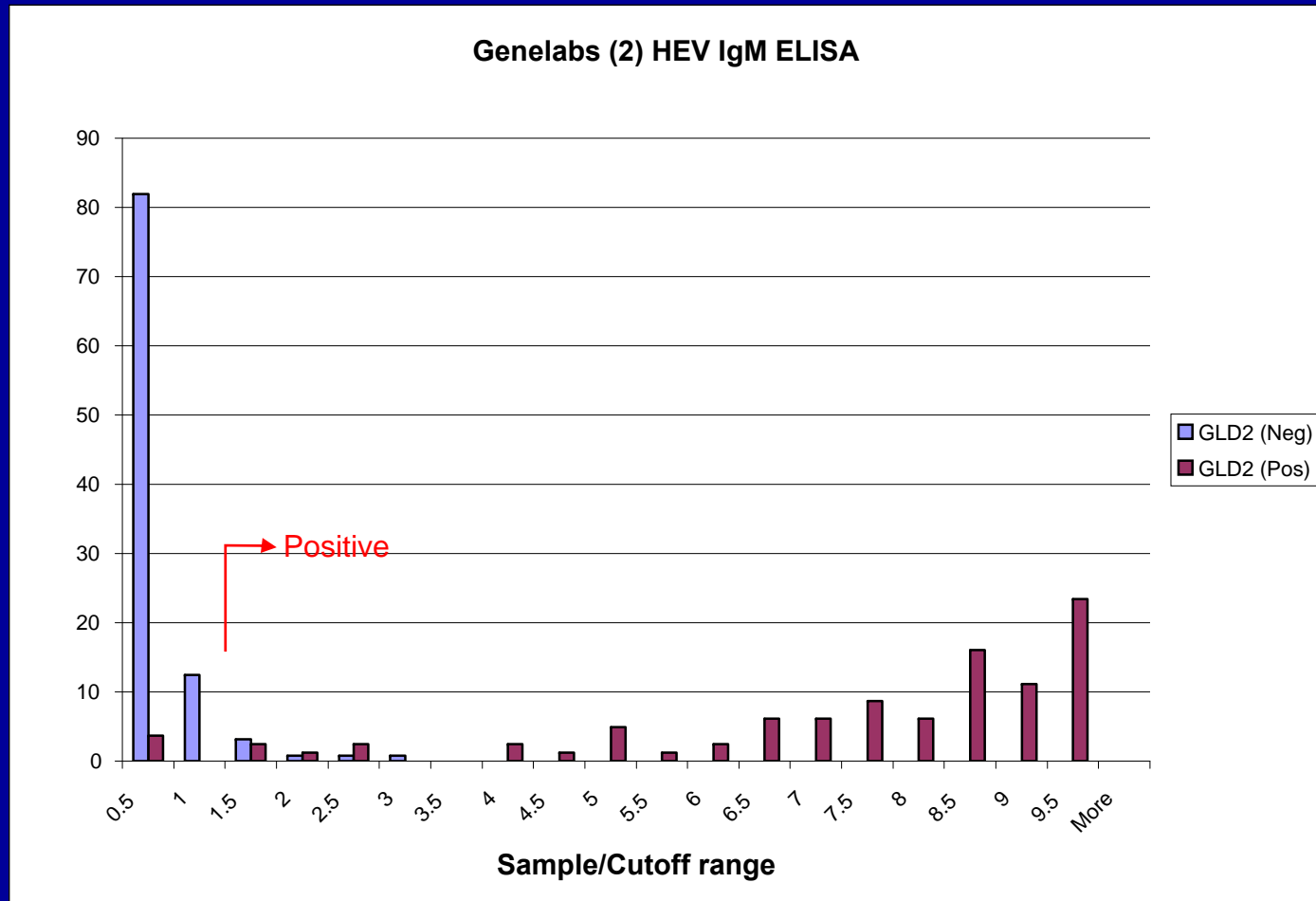
Genelabs new HEV IgM ELISA

	ELISA
Population (n)	IgM Pos (%)
Healthy donors (95)	1 (1.1)
Other hepatitis (88)	3 (3.4)
Other disease (25)	1 (4.0)
Control total (208)	5 (97.6% spec)
Acute HEV (151)	149 (98.7% sens)

H.Y.Chen et al, CDLI 2005

IgM anti-HEV: Sporadic hep, Nepal

"Patients would arrive at the clinic in the morning, and wait until I had time to run the test so they could get the result" Dr Iswar Shrestha, Siddhi Polyclinic, Nepal
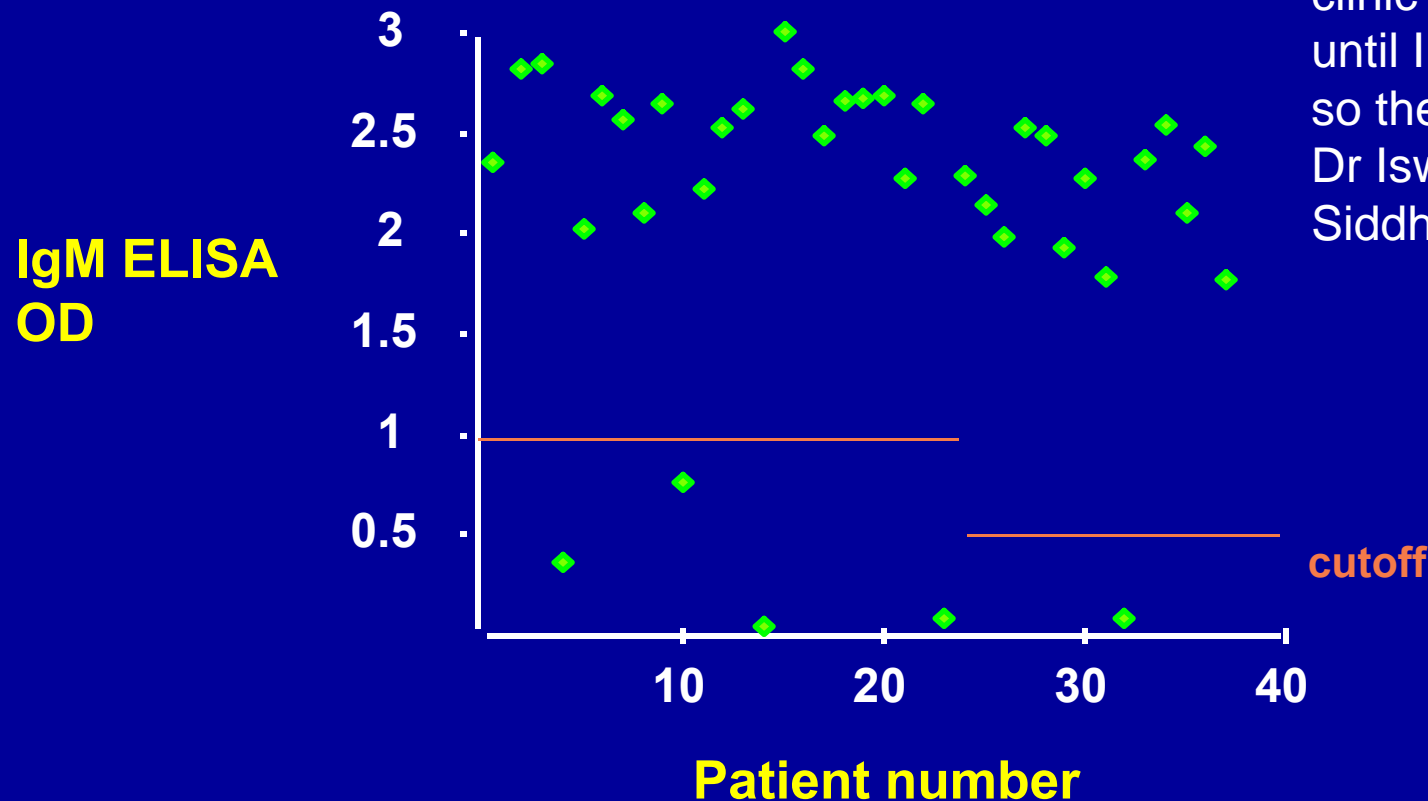
Dr Iswar Shrestha, Kathmandu

Rapid Immunochromatographic Tests

Gold Conjugate

(1) Gold conjugate (gold-MAb-antigen), rehydrated with 4 drops Rehydrating Reagent

Result Window

Sucker Pad

T – Test Line
C – Control Line

Limit line

(3)Serum reaches limit line, card secured closed reversing flow

Sample Pad

(2)25μl undiluted serum (or blood) added to sample pad
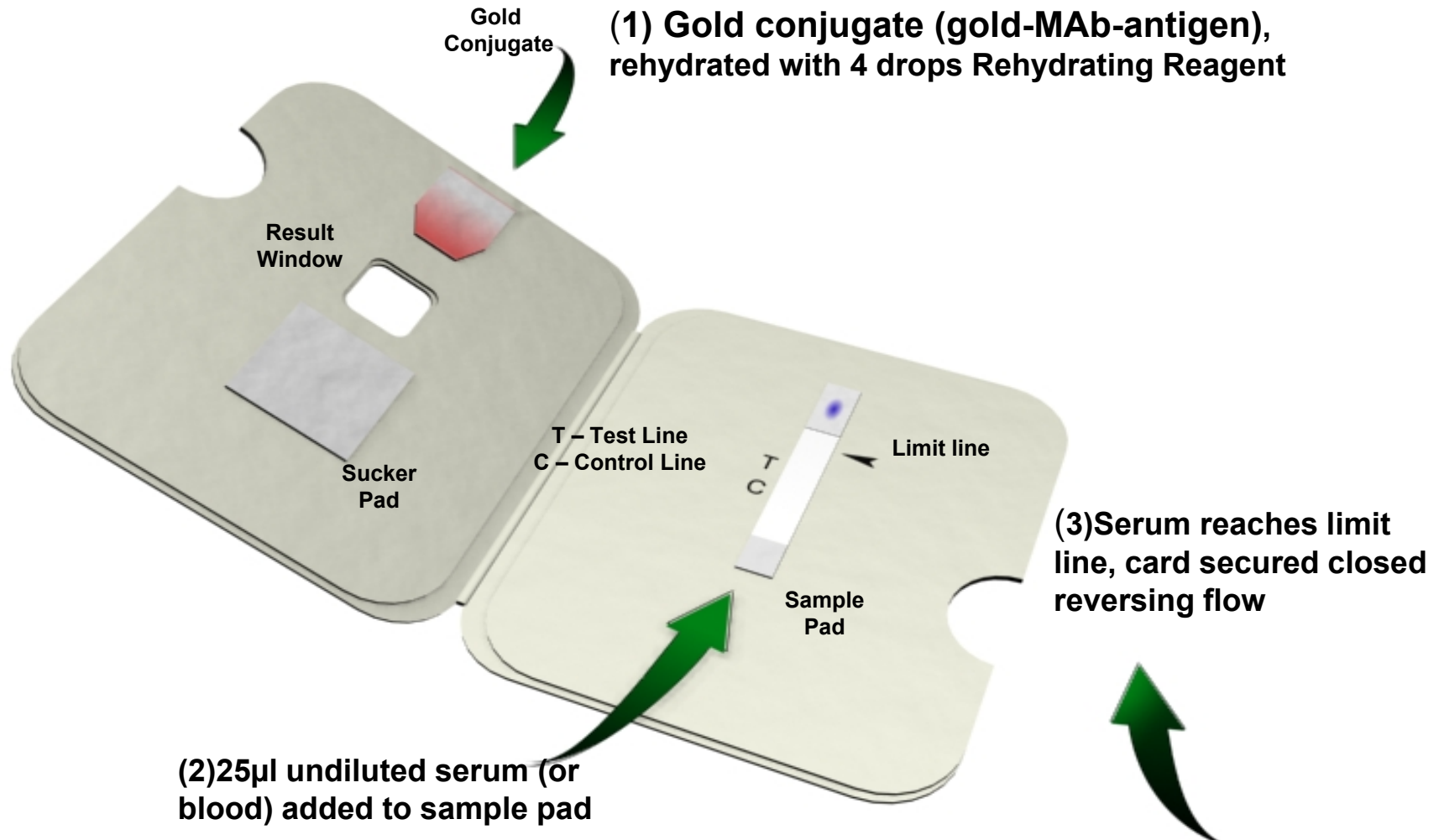
HEV IgM Rapid Test: Reverse Flow Technology

See red colour on <u>Test Line</u>

Always see red colour on <u>Control Line</u>

GOLD

Anti-HEV Mab
HEVAg

GOLD

Mab
HEVAg

conjugate buffer

flow

Anti-Hu IgM

Anti-mu

PROTOTYPE ASSAY
FOR RESEARCH PURPOSES ONLY

HEPATITIS E IgM
RAPID ASSAY

SelectVaccines
LIMITED

CONJUGATE PAD:

•Gold conj. to anti-HEV Mab, bound to HEV antigen

•reverses flow
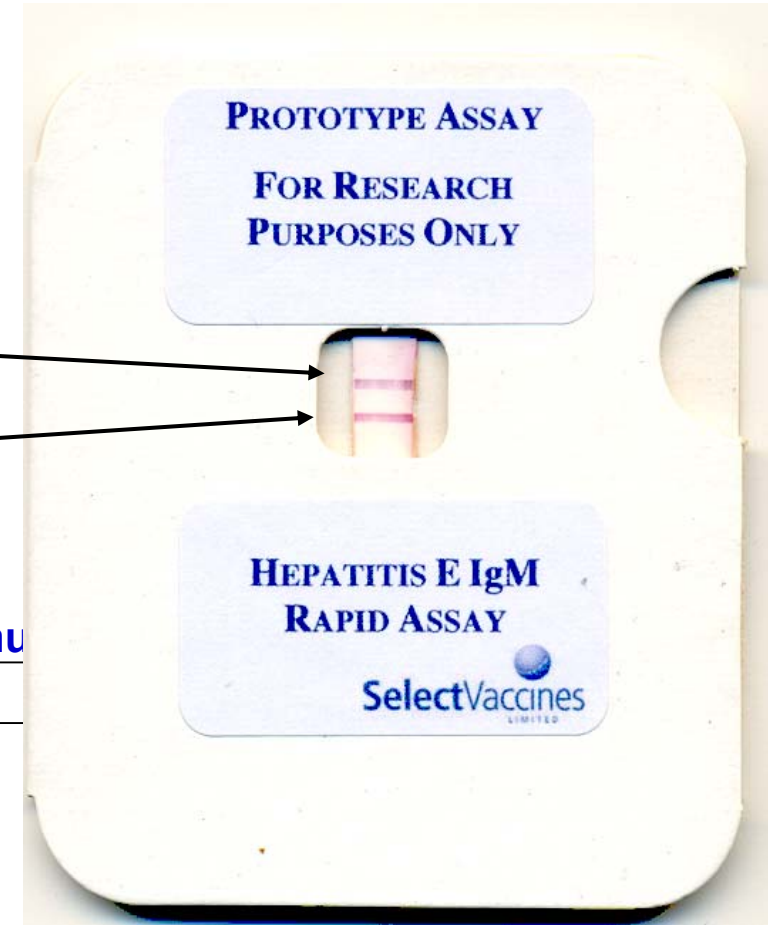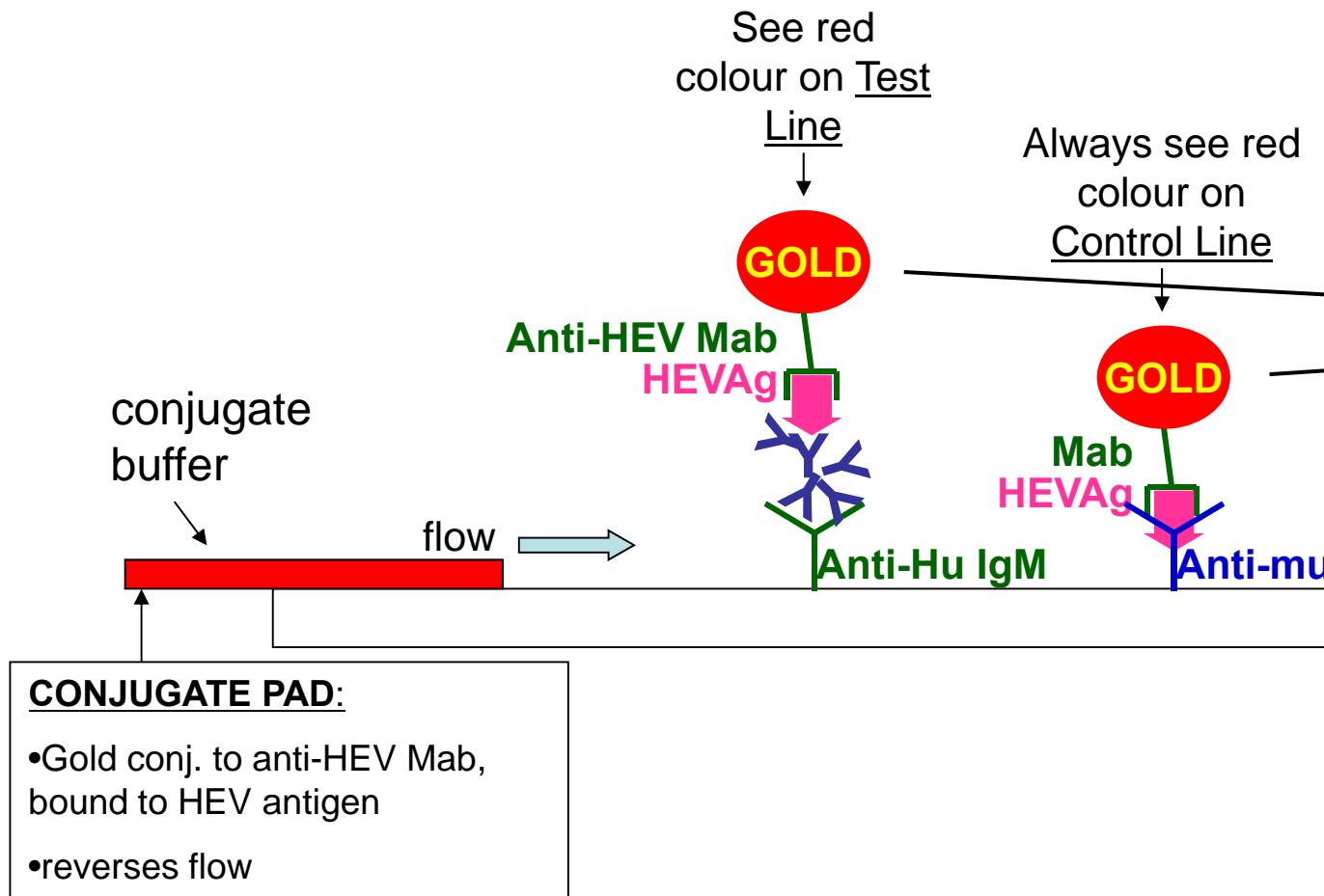
--------------------**NITROCELLULOSE MEMBRANE**--------------------

Hepatitis E IgM rapid test

Genelabs Diagnostics (MP Biomedical) Assure™
- licensed from Select Vaccines Ltd

HEV patients
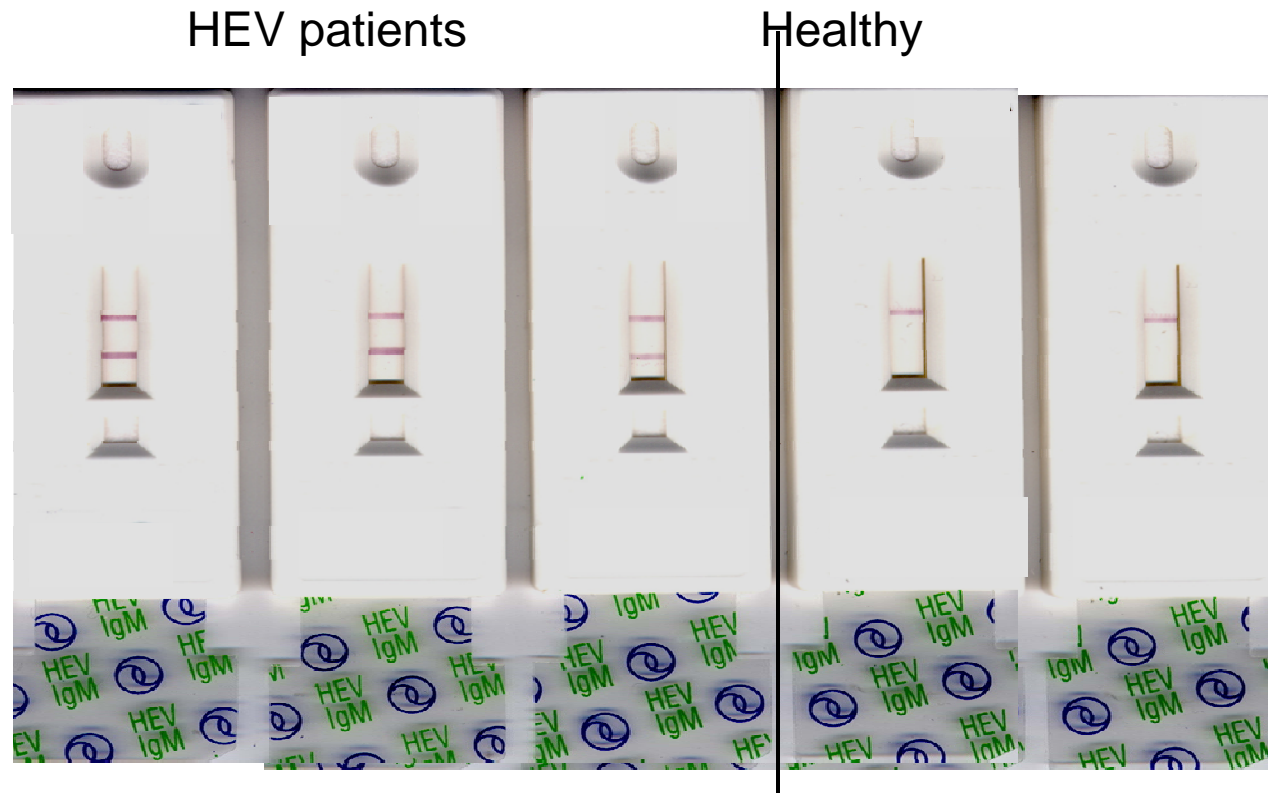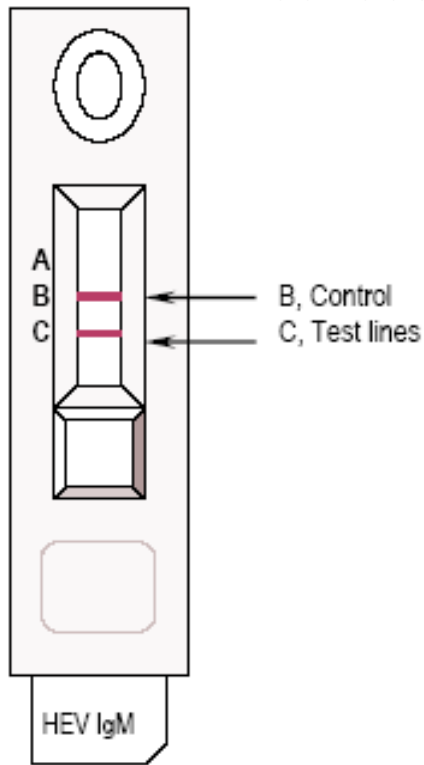
Healthy

Genelabs Assure™ HEV IgM rapid

	ELISA	Assure rapid
Population (n)	IgM Pos (%)	IgM Pos (%)
Healthy donors (95)	1 (1.1)	2 (2.1)
Other hepatitis (88)	3 (3.4)	0 (0)
Other disease (25)	1 (4.0)	1 (4.0) *RF
Control total (208)	5 (97.6% spec)	3 (98.6% spec)
Acute HEV (151)	149 (98.7% sens)	146 (96.7% sens)

Genelabs Assure™ - AFRIMS

K Myint et al, AJTMH 2005

Genelabs Assure™ - AFRIMS

Population (n)	Assure rapid IgM Pos (%)
Healthy donors (100)	0
Other hepatitis (175)	0
RF positive (26)	0
Acute HEV (200)	186 (93%)

K Myint et al, AJTMH 2005

Hepatitis E Rapid Assay in use in refugee camp in Chad

Photo courtesy Dr Greg Armstrong, CDC, Atlanta

MP Bio Assure™ HEV IgM rapid

Study	Sensitivity (n)	Specificity (n)
Nepal, PR China	96.7% (n=151)	98.6% (n=208)
Nepal, PRC, Indonesia	93.0% (n=200)	100% (n=275)
PR China*	97.0% (n=502)	96.5% (n=683)
Overall	96.0% (n=853)	97.7% (n=1166)

* Two site clinical trial in China

Alternative HEV Diagnostics

Assay	Sensitivity	Specificity
Baculo 62K IgM (NIH)	Very high	Very high
Quantitative IgM (AFRIMS)	Very high	High
Mosaic IgM (CDC)	High	Very high
RT-PCR	High – very high	"Gold standard"

RT-PCR has been invaluable in understanding transmission of indigenous/zoonotic HEV in presumed non-endemic countries, BUT it is not really suitable for routine diagnosis

Molecular diagnostics for HEV

◆ **Serology has specificity problems in low-incidence settings (eg zoonotic infections in W. Europe)**

◆ **Unsatisfactory PPV in low-incidence settings**

◆ **RT-PCR is "gold standard" for SPECIFICITY, but less sensitive than IgM serology in outbreak settings**
  – **Short duration and low titre of viral RNA in serum – very labile?**

◆ **Balanced approach needed – serology for returned travellers, RT-PCR for unexplained acute hepatitis?**

Vaccines for hepatitis E (and A) viruses

◆ **Hepatitis A**
  – **Inactivated virus from cell culture**
  – **Antibody is protective (and easily measured)**
  – **Many commercially available, including Twinrix**
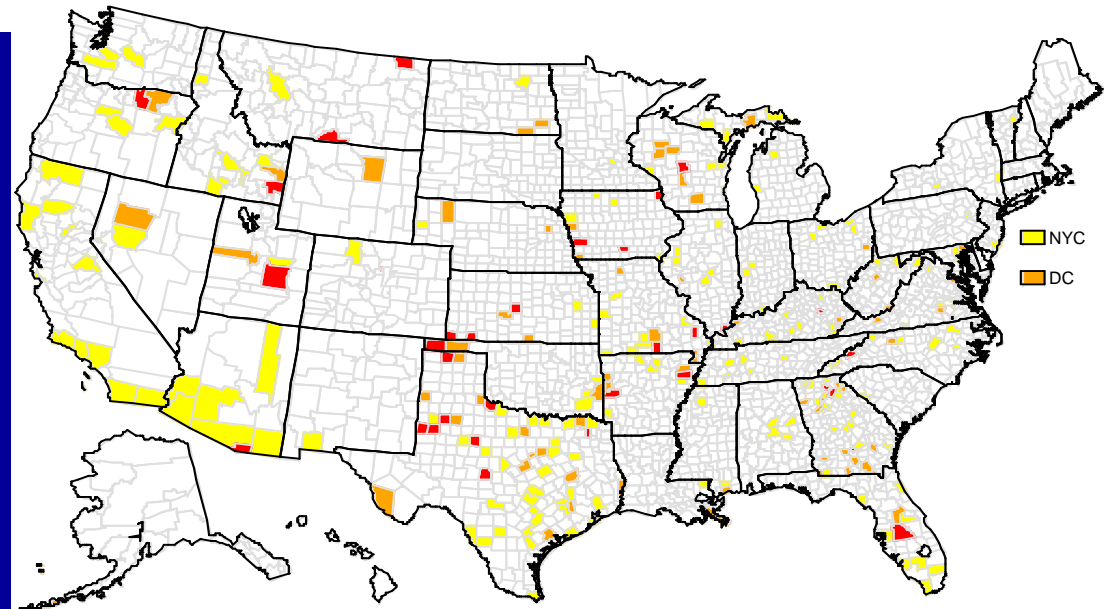 – **Widespread use has had dramatic public health impact**

◆ **Hepatitis E**
  – **Recombinant protein from insect cell culture or *E. coli***
  – **Very effective in Phase 3 clinical trials, Nepal and China**
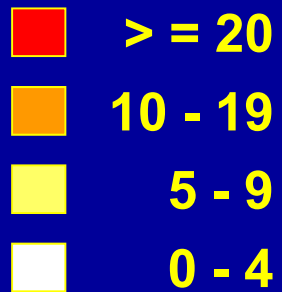 – **Have not been commercially released – China imminent**

1987-97 average incidence
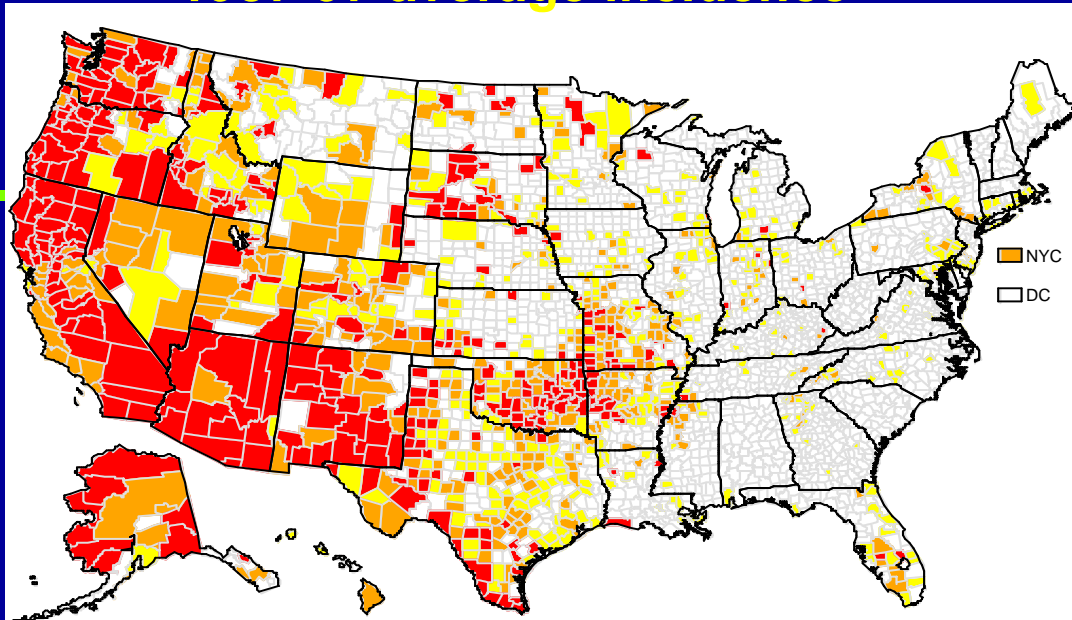
2002 incidence

Rate per 100,000

> = 20

10 - 19

5 - 9

0 - 4

HEV vaccines

- **GSK/Novavax – HEV VLPs from insect cell culture**
  - Successful Phase3 trials in Nepal 2005, published 2007

- **Xiamen Biotech, Jiangsu – Hecolin (HEV 239)**
  - Successful Phase 3 trials in Jiangsu, 2010
    - » Zhu FC et al, Lancet 376(9744):895-902, 2010
    - » Licensed SFDA in Jan 2012

  - 30 μg *E. coli*-derived protein + Alum at 0, 1, 6 mo

  - Placebo: 15 cases HEV in 48,663 recipients
  - Vaccine: 0 cases HEV in 48,693 recipients
    - » Efficacy 72-100%

Recombinant HEV antigens (4)

Protein	aa	Reactivity	
		Acute	Conv
ORF3	124	++/-	+/-
ORF2	660		
C-2	435	++/-	+/-
SG3	327	++/-	+/-
ORF2 (VLPs)	548	++++	+++
GST-ORF2.1 aa 394-660	273	++++	+++
HEV 239 aa 368-606 (13 nm particles - Hecolin)	239	++	++

BURNET INSTITUTE

Ian Potter Hepatitis Research Laboratory
Fan Li, Michaela Riddell, Joe Torresi
Abigail Clements, Teresa Howard, Anne Healy,
Katie Anders, Mary Garcia, Jocelyn Diaz,
Simone van der Waarsenburg, David Anderson

Centre for International Health
Nick Crofts, Mike Toole, Chris Morgan

Pathogenesis and Clinical Research programme
Suzanne Crowe, Robyn Lloyd, Helen Komodromou

Select Vaccines Ltd
Martin Soust, Fan Li

GENELABS DIAGNOSTICS

Hsiao Ying Chen, Yang Lu,
Priscilla Fong, Weiping Hu,
Chee Poh Chia, Ming Guan

ELSEWHERE

Khin S.A. Myint, AFRIMS, Thailand
Zhuang Hui, Beijing Medical University
Stephen Locarnini, VIDRL
Iswar Lal Shrestha,
    Siddhi Polyclinic Nepal
Gillian Mearns, AMRAD ICT
Alan Landay, Rush University