Design of

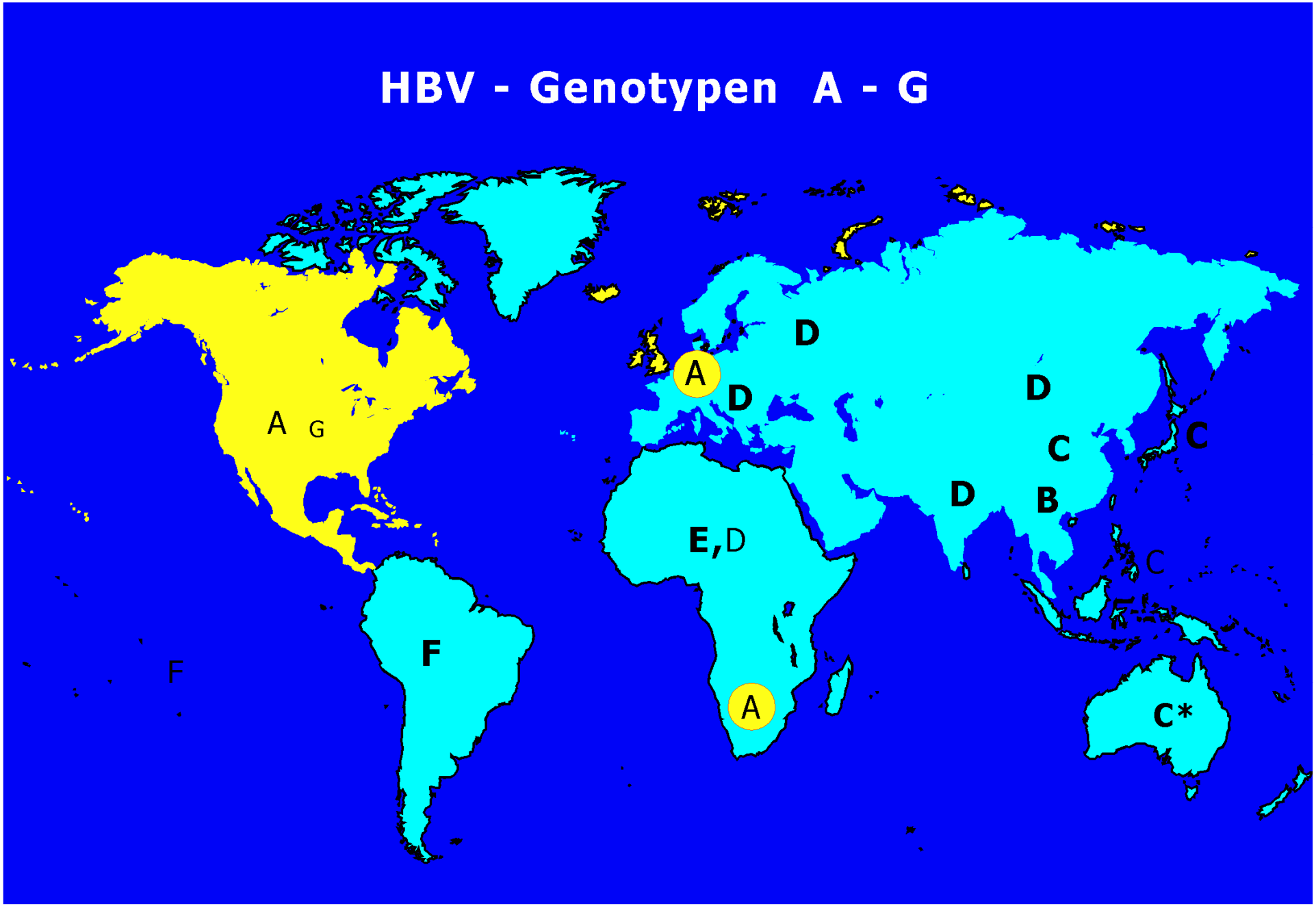
WHO Genotype Panels for HBsAg and HBV-DNA

and of

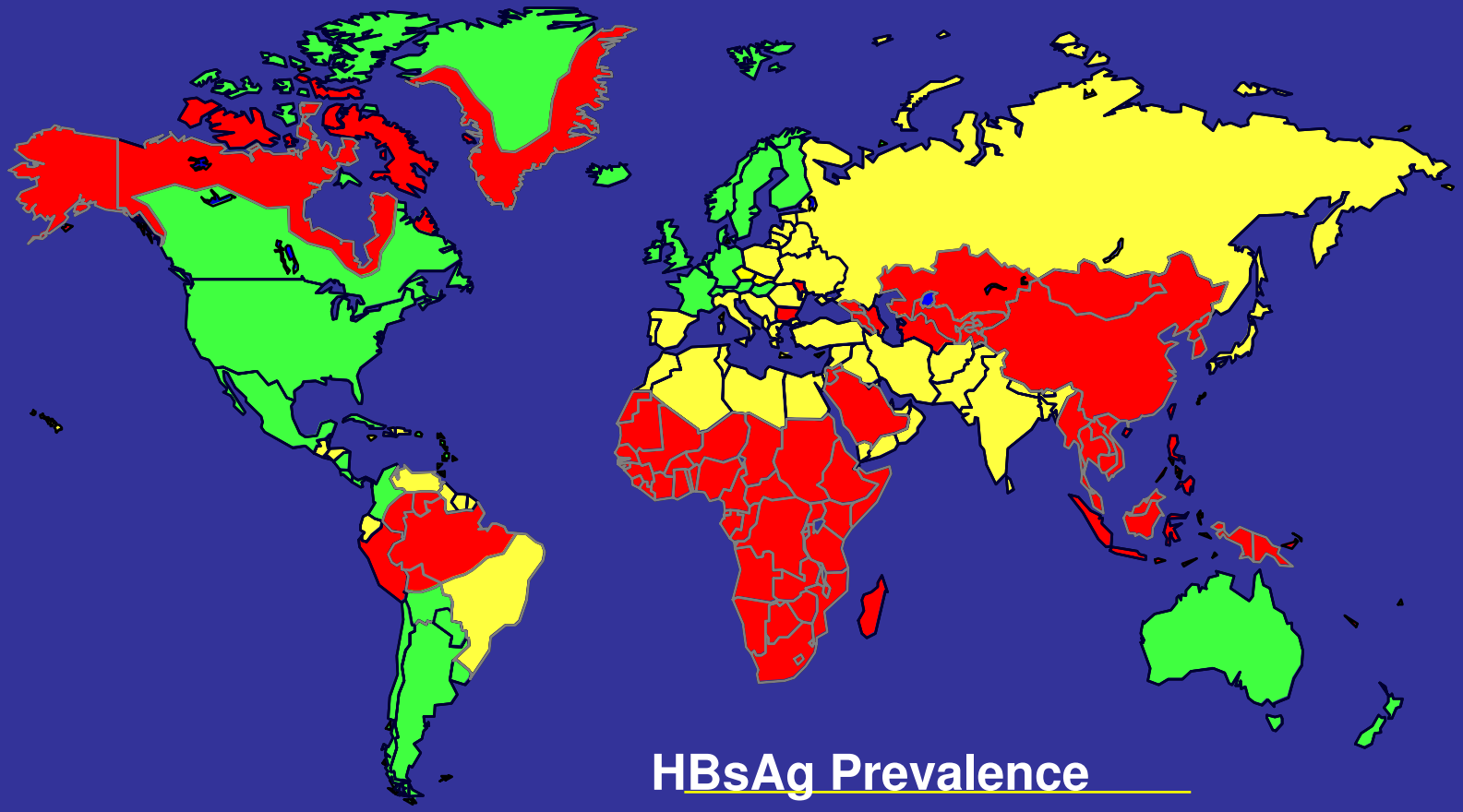
WHO anti-HBc Standard

WHO Genotype Panels for HBsAg and HBV-DNA

Worldwide distribution of HBV genotypes



Worldwide Frequency Distribution of Chronic HBV Infections



WHO International Standards

- **WHO IS HBsAg**
  genotype A, subtype A2

- **WHO IS HBV-DNA**
  genotype A

representing only 1% of HBV-infected population

HBV genotype panels

WHO Consultation on “Global Measurement Standards and their use in the in vitro Biological Diagnostic Field" (Geneva, June 7-8, 2004)

“….it was agreed that the contribution of other Hepatitis B virus genotypes on the sensitivity of test kits for HBsAg should be investigated further. It is recommended that Regulatory Authorities devise panels for kit evaluation that include HBsAg reactive specimen with subtypes and genotypes from their local regions."

HBV genotype Panels

Aim

- HBsAg panel
- HBV-DNA panel

from the same hi(+) units
reflecting all major HBV-genotypes / major genosubtypes

if lyophilisation: validation
no inactivation step

project introduced to and accepted by ECBS 2005

Cooperation: Prof W. Gerlich (Univ. Giessen)

HBV genotype panels
-current status-

- collection of plasma units worldwide
  - plasma from Russia, Germany, Japan, Brasil, South Africa arrived at PEI
  - represent genotypes **A, B, C, D, (E), (F)** and mixed genotypes
  - plasma from Brasil (genotype F?), Egypt, China and Iran has been announced

HBV-genosubtypes: worldwide distribution

Genotype	Genosubtype	HBsAg subtype	Frequency	Main Geographical Distribution
A	A1	*adw2, ayw1*	high	Africa
	A2	***adw2***	**high**	Europe, North America, Australia
	A3			Cameroon, Democratic Republic of Congo, Gabon
B	**B1**	***adw2***	**high**	Far East (Japan, China, Taiwan)
	B2	***adw2/adw3,***	**high/low**	Far East (China, Japan, Vietnam/Thailand)
	B3	*adw2*	high	Far East (Indonesia, Sumatra, Sulawesi)
	B4	*ayw1*	high	Vietnam
C	**C1**	***adr/ayr/adw2***	**high/high/low**	Far East (Japan, China)
	C2	***adr/ayr; ad***	**high/high**	Thailand, China/Vietnam
	C3	*adrq-/adw2*	high/low	Pacific (New Zealand to Polynesia), Micronesia/Far East
	C4	*ayw3*	low	Northeast Australia
D	D1	*ayw2*	high	Mediterranean, Middle East, India
	D2	***ayw3/ayw4***	**high/low**	worldwide/USA
	D3	***ayw2/ayw3***	**high/high**	South Africa, Alaska/Europe, Costa Rica
	D4	*ayw2,*	high	Oceania, Somalia,
	not identified	*adw3*	low	Eastern Europe Spain
E	-	*ayw4*	high	Africa
F	F1	*adw4q-/ayw4*	high/low	Cental America, Argentina, Spain, Alaska/Nicaragua
	F2	*adw4q-/ayw4*	high/low	South America, Polynesia, France/Venezuela
G	-	*adw2*	low	USA, Mexico, Europe
H	-	*adw4*	low	Central America (Nicaragua, Mexico), California
Recombinant Strains				
A/D		*adw2*		India
C/D		*ayw2*		Tibet
C/?		*adw2*		Vietnam

HBV genotype panels

Current work

- Characterization of candidate materials (HBsAg, HBV-DNA, Sequencing)

- Pilot experiments to separate HBsAg from infectivity (HBV-DNA) and to fully characterize candidate materials (Prof W Gerlich, Univ. Giessen)

HBV genotype panels

Prof Gerlich (Giessen)

- Pelleting of infectivity (99,9%) by sucrose ultracentrifugation

  dilution in plasma for HBV-DNA genotype panel

- Enrichment of 20 nm filaments by sucrose gradient centrifugation and flotation in CsCl
- HBsAg content by Laurell immune-electrophoresis
- residual HBV-DNA by qtNATs

  dilution in plasma for HBsAg genotype panel

WHO IS antiHBc

WHO IS antiHBc

antiHBc

- serological marker for past HBV-infection
- in rare cases HBV-DNA (low+)
- in rare cases HBV-transmissions reported from antiHBc-pos donors to recipients
- blood screening marker in several countries
  - previously as surrogate marker for NANBH
  - considerations to rely in future on antiHBc combined with ID HBV-NAT

WHO IS antiHBc

What is the ideal candidate material ?

- „heterogenous“ antiHBc-assays
  - competitive / non-competitive assays
  - reducing agents in specimen diluent for increase of specificity?
  - often same antigen source
  - „confirmation“ of unspecific results by „different“ assays

- no confirmation assay for antiHBc
- no common algorithm for „antiHBc-positive“

WHO IS antiHBc

What is the ideal candidate material ?

- no strict correlation between analytical (=dilutional) and diagnostic sensitivity of antiHBc-assays

- WHO antiHBc standard could be useful for
  - defining minimal diagnostic sensitivity of assays
  - quality control of batches

Anti-HBc screening of blood donors – Comparison of nine different Anti-HBc tests

M. Schmidt, C.M. Nübling, H. Scheiblauer, M. Chudy, L. Walch, E. Seifried, W.K. Roth, M.K. Hourfar (2006) *Vox Sanguinis* **91,** 237-243

- screening of 10.000 blood donors with 2 antiHBc-assays
- characterization of all antiHBc-reactive donations (207) using
  - 7 further antiHBc-assays
  - antiHBs-assays, antiHBe-assay
  - 3 HBV-NATs

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Characterization of 207 antiHBc-reactives

			antiHBc-reactivity in 9 different assays		
			9/9 assays	≥5/9 assays	<5/9 assays
	antiHBc only	**27** (13%) **HBV-DNA pos: 1**		**10**	**17**
one second marker pos	**antiHBc + antiHBs**	**67** (32%) >100 mIU antiHBs/ml: 49 <100 mIU antiHBs/ml: 18	**49**	**11**	**7**
	antiHBc + antiHBe	**7** (3%)	**7**		
both second markers pos	**antiHBc + antiHBs + antiHBe**	**106** (51%) >100 mIU antiHBs/ml: 98 <100 mIU antiHBs/ml: 8	**106**		
		207 173 (84%) antiHBs-pos	**162 (78%)**	**21 (10%)**	**24 (12%)**

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s/co-values antiHBc high low

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Serological Pattern of antiHBc-/HBV-DNA positives (in-house NAT, Frankfurt)

Characterization of 10 antiHBc-/HBV-DNA positives

s/co-values antiHBc: high / low

			antiHBc-reactivity in 11 different assays		
			11 / 11 assays	≥ 6 assays	≤ 5 assays
	antiHBc only	4	3	1	
one second marker pos	antiHBc + antiHBs	2	2		
	antiHBc + antiHBe	1	1		
both second markers pos	antiHBc + antiHBs + antiHBe	3	3		
		10			

HBV transmission by erythrocytes
- anti-HBc / HBV-DNA positive -

Assay name		Dec 01
Murex aHBc	CO/S	10,79
bioelisa anti-HBc	CO/S	1,51
bioelisa anti-HBc	CO/S	n.d.
Enzygnost Anti HBc monoclonal	CO/S	0,67
Enzygnost Anti HBc monoclonal	CO/S	0,99
Immulite 2000 anti-HBc	CO/S	1,66
Monolisa aHBc Plus	S/CO	3,22
Ortho aHBc	S/CO	5,83
Architect Anti-HBc	S/CO	2,29
AxSYM Core	CO/S	6,25
ADVIA Centaur HBcT	S/CO	0,26
Elecsys Anti-HBc	CO/S	43,48
PRISM HbCore	CO/S	2,38
HBV-DNA	qual.	positive
PRISM HBsAg	S/Co	0,20
Murex HBsAg version 3	S/Co	0,47
Immulite aHBc IgM	U/ml	<2,00
Murex aHBs	S/Co	0,31
Murex HBeAg/anti-HBe	S/Co	0,43
Murex HBeAg/anti-HBe	Co/S	0,44

WHO IS antiHBc
- candidate materials -

- **pooled plasma from transmission case**
  antiHBc weak pos, HBV-DNA pos
  >2.000 ampoules a 0.5 ml
  to be lyophilised
  for „diagnostic“ sensitivity

- **NIBSC 95/522**
  antiHBc strong pos, antiHBs pos
  2.700 ampoules a 1 ml
  lyophilised
  for „analytical“ sensitivity

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- D. Watts (South Africa)

HBV genotype panels

- M. Schmidt (Frankfurt)

WHO IS antiHBc

- H. Scheiblauer, S. Nick (PEI)
- M. Ferguson (NIBSC)