Phenotypic methods for investigating the impact of variation on HBsAg antigenicity

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Impact of HBsAg mutants

- Use of both vaccine and antivirals selects for mutations which impact HBsAg antigenicity
- Raise concern over consequence of HBsAg mutants emerging in an era of increased levels of immunisation and use of antivirals
- What is the impact of HBsAg mutants on current public health and control policies
  - How common are these mutations in HBV infected populations?
  - Damaging in the antenatal setting?
  - Are these viruses being transmitted?

Prevalence of HBsAg mutants

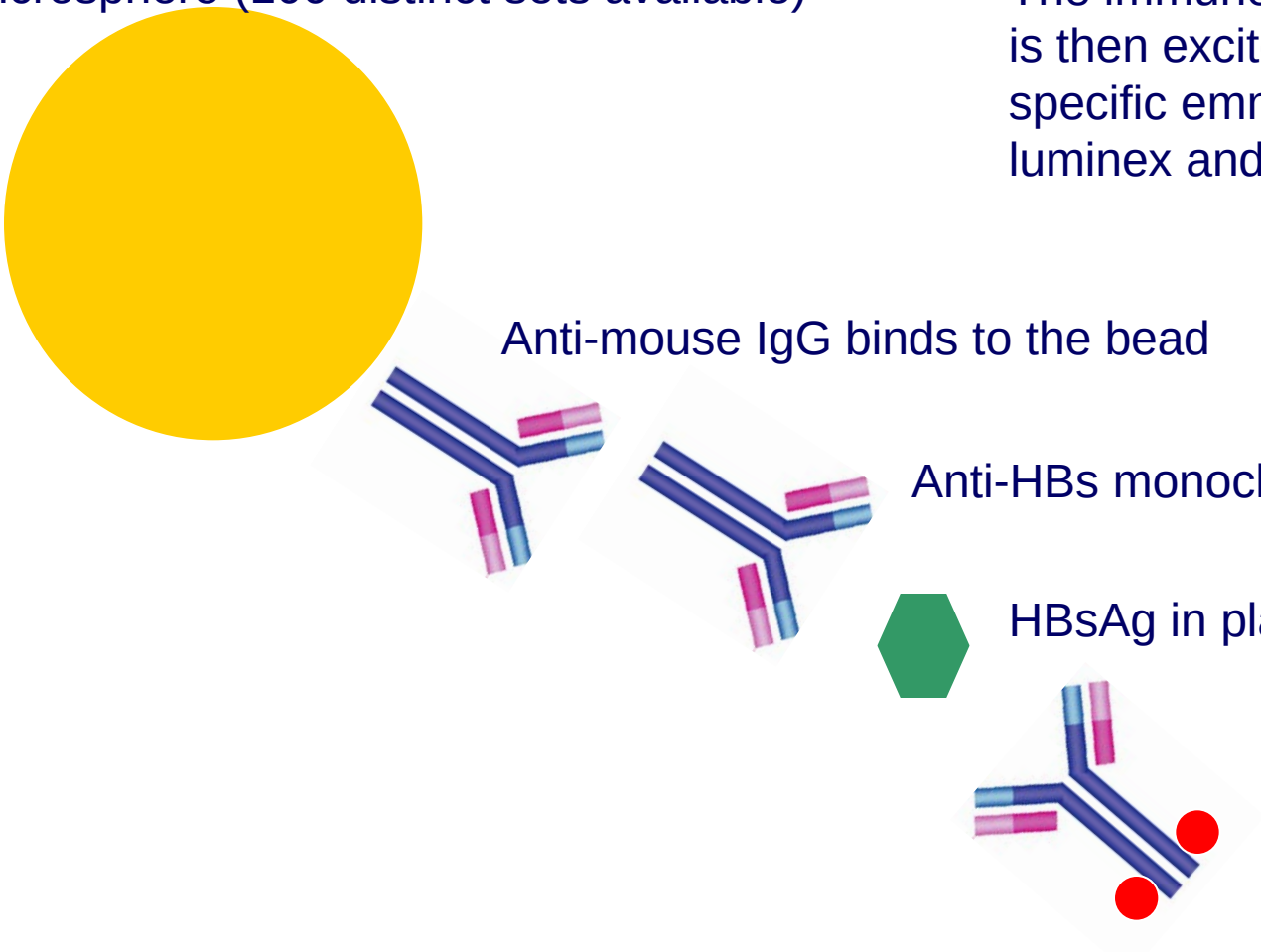
- Studies limited by viral sequencing which is both laborious and expensive
- Studies indicate that it was not always possible to infer accurately changes in antigenicty from direct sequencing
- Backbone of the virus played an important role in determining the impact of amino acid changes

Luminex assay

- *Ex-vivo* phenotyping directly from patients' sera
- HBsAg epitope mapping using monoclonal antibodies on the Luminex platform
- The advantage of Luminex technology lies in its sensitivity, high throughput and efficiency

Microsphere (100 distinct sets available)

The immune-complex/microsphere is then excited by the laser. The bead specific emmission is quantified by luminex and the bead identified



Anti-mouse IgG binds to the bead

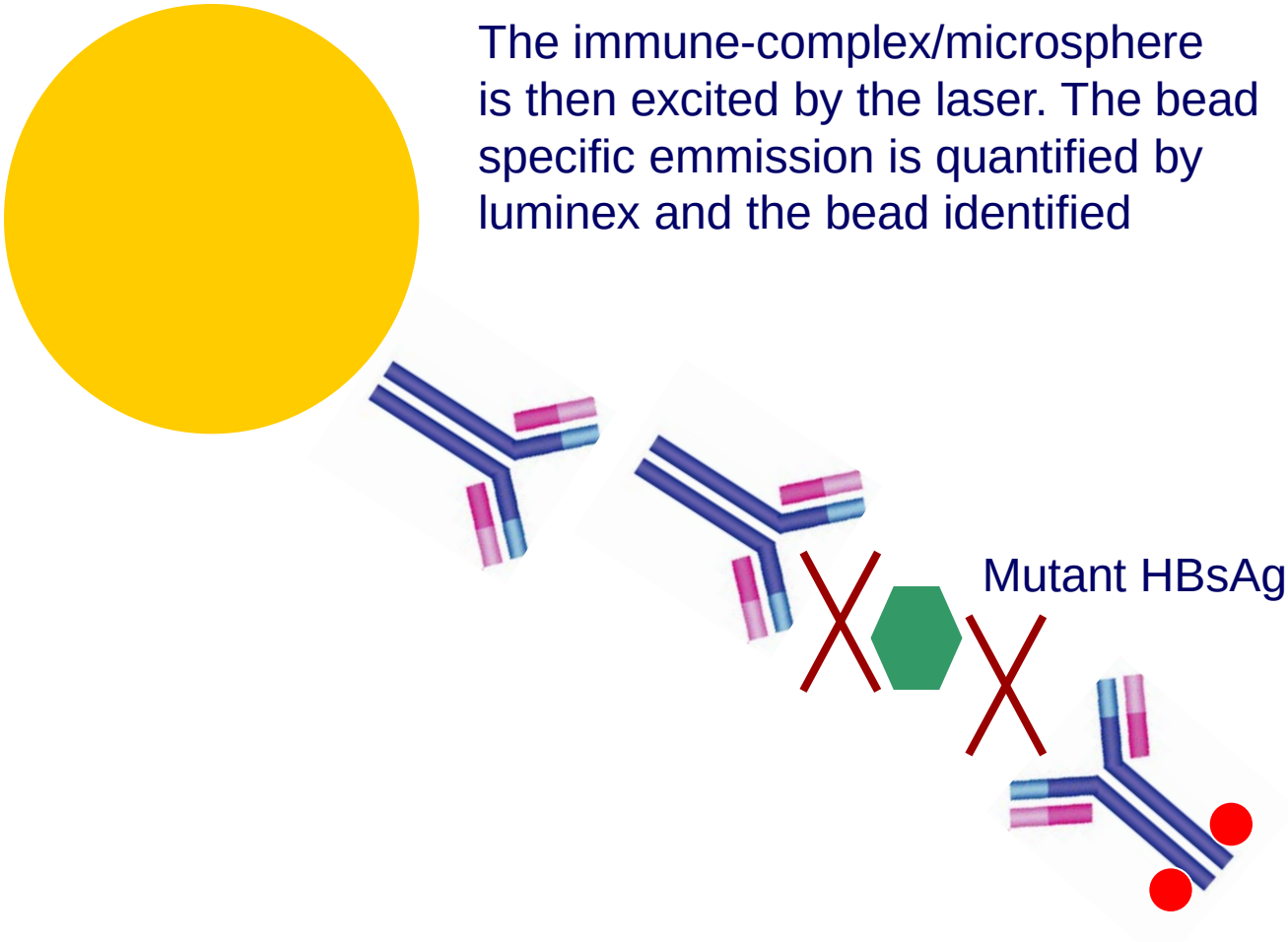
Anti-HBs monoclonal antibody

HBsAg in plasma

Polyclonal anti-HBs labelled with RPE

Phycoerythrin is excited by laser and emits fluorescence which is quantified by Luminex

The immune-complex/microsphere is then excited by the laser. The bead specific emmission is quantified by luminex and the bead identified



Mutant HBsAg in plasma

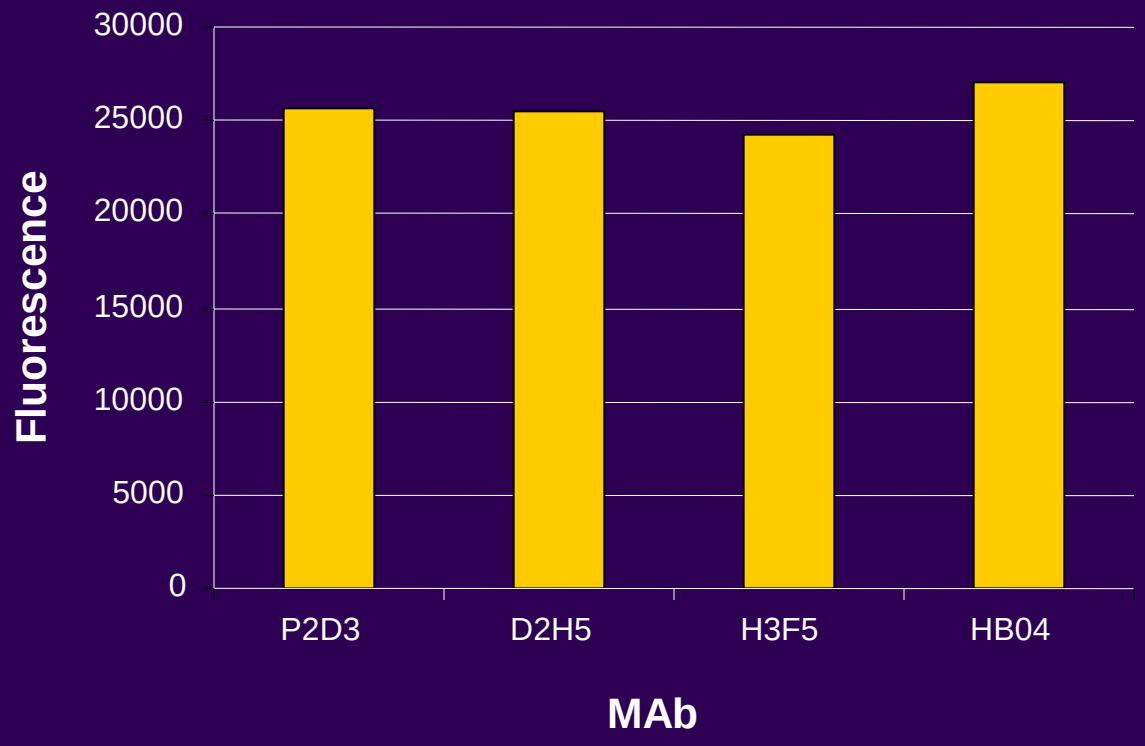
Polyclonal anti-HBs labelled with RPE

Decrease in fluorescence emitted by the Phycoerythrin

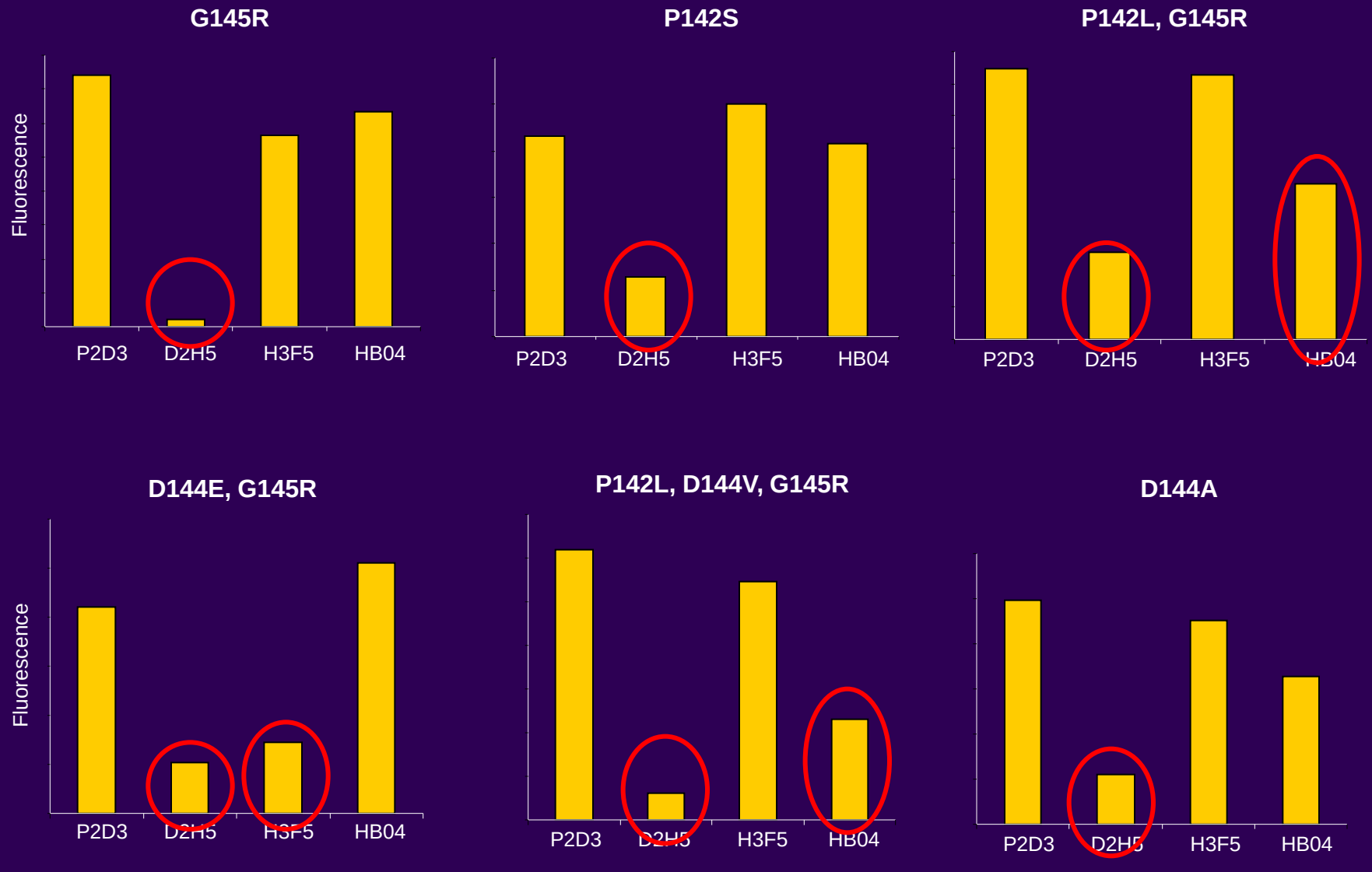
Luminex assay

- Microspheres beads – each has a unique internal dye (100 sets)
- Each different bead type can be conjugated to an individual MAb
  - Several epitopes of the HBsAg can be investigated
- Panel of MAbs recognising discrete and overlapping epitopes on the HBV envelope
  - HBsAg mini loop
  - HBsAg first loop
  - HBsAg second loop
  - Pre S1
  - Pre S2

Epitope mapping – WT sample



Epitope mapping



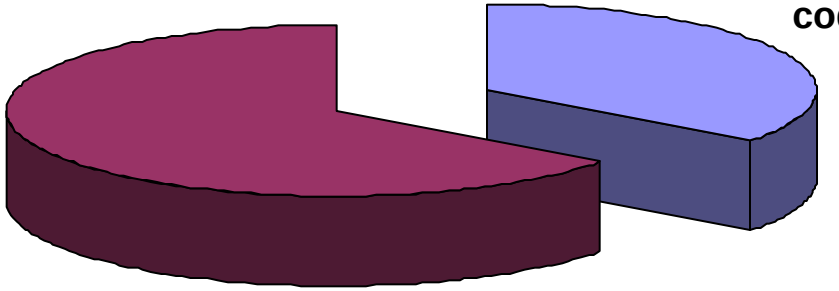
Epitope mapping

Application of epitope mapping

Mother to Baby study

- UK has a selective HBV immunisation policy
- Only babies born to HBV-infected mums receive prophylaxis
  - HBeAg pos mums – vaccine and HBIg
  - Anti-HBe pos mum – vaccine only
  - Anti-HBe pos mum with a VL >1.0e+06IU/ml – vaccine and HBIg
- HPA follows up babies at 1 year (received vaccine and HBIg)
- Data shows that policy is effective in preventing transmission
- A small number of babies are HBV infected at 1 year

N=52 babies



21 aa changes between codons 120-150 on sequencing

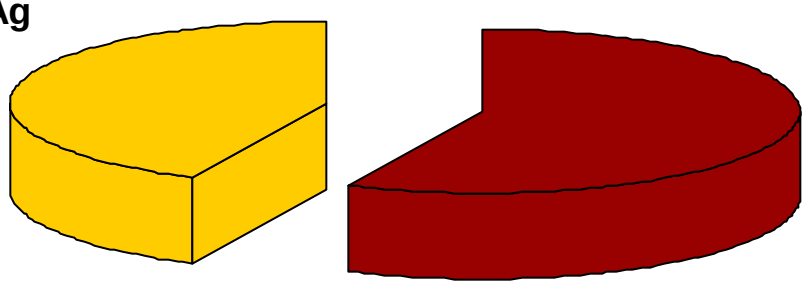
31 WT HBsAg on sequencing

Epitope mapping

29/31 WT phenotype

Epitope mapping

8 had WT HBsAg phenotype



13 had altered HBsAg antigenicity

Additional analysis

- Testing of additional HBV makers in babies
- Determine HBV VL in mums
- Looking at records to check immunisation and also response to vaccine
- Aim to follow up babies and determine outcome of infection

Drug-driven HBsAg mutants

- Drug driven changes impacting on the overlapping HBsAg resulted in the alteration of HBsAg antigencity

  Torresi J., Virology 2002, 293:305-313

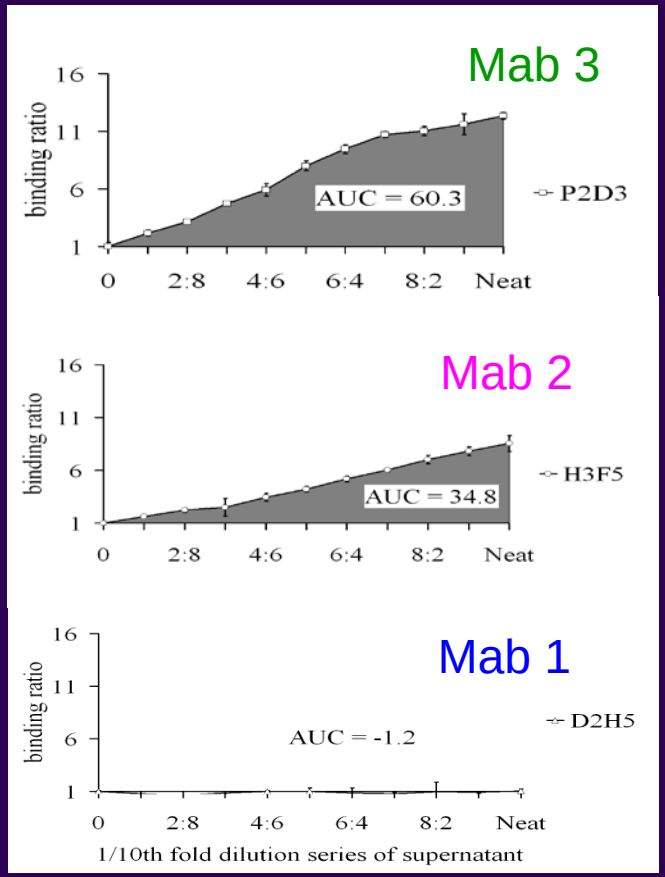
  - SDM to introduce drug driven changes (codons 164, 195 & 196)
  - Expressed proteins found to have reduced reactivity to vaccine induced antibody

- Drug-driven HBsAg mutant viruses result in successful infection in immunised chimps

  Kamili S., Hepatology 2009, 49:1483-91

Evidence of drug-driven HBsAg escape mutants

Expressed recombinant HBsAg bearing various combinations of amino acid substitutions associated with drug driven escape mutants



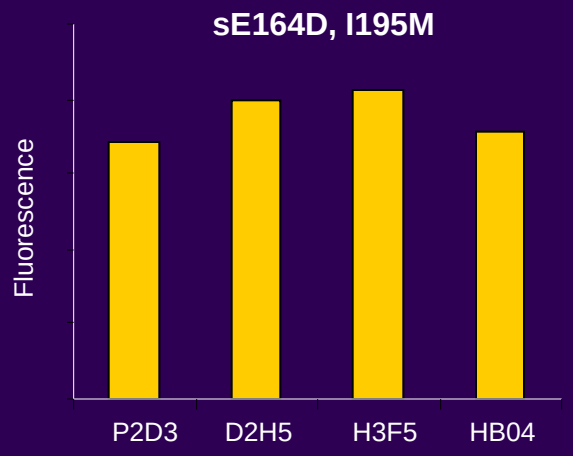
rtV173L/sE164D + rtM204V/sI195M

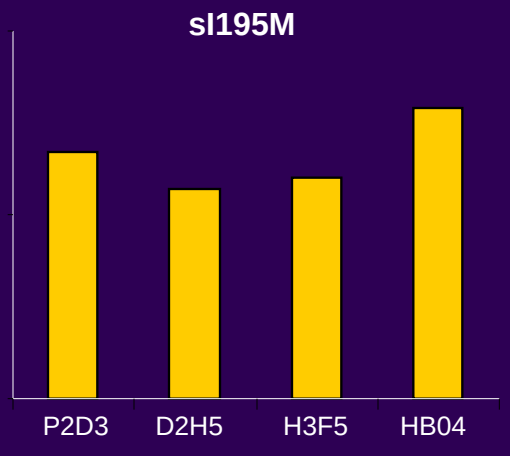
Total loss of one epitope induced by two mutations neither of which alone has a detectable effect

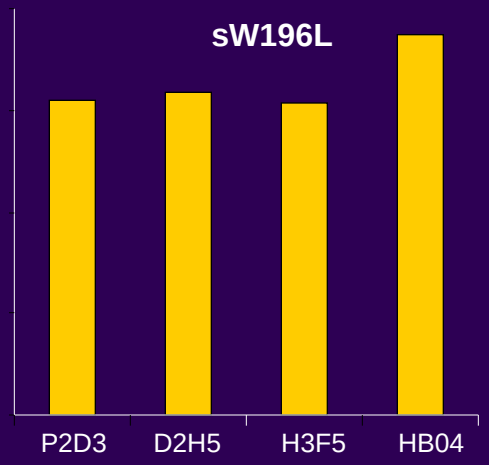
DRUG INDUCED MUTANTS BEHAVE LIKE VACCINE ESCAPE MUTANTS

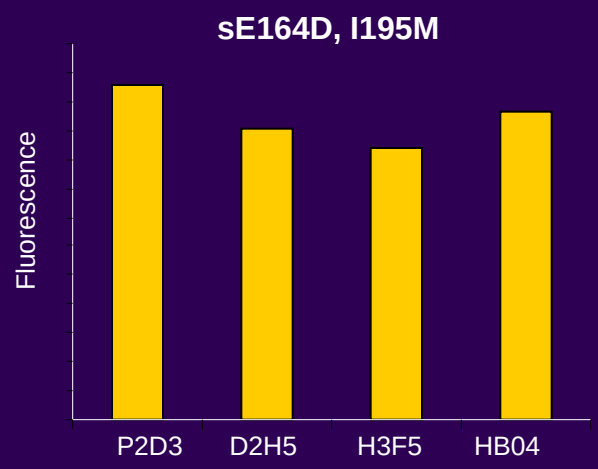
Sloan et al., Antiviral Therapy 2008, 13:439-447

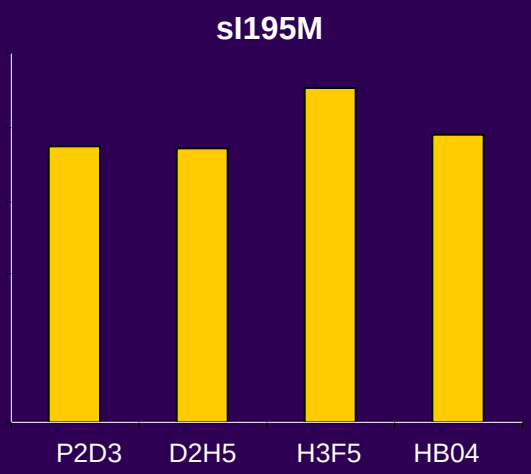
Epitope mapping – drug-driven HBsAg mutants

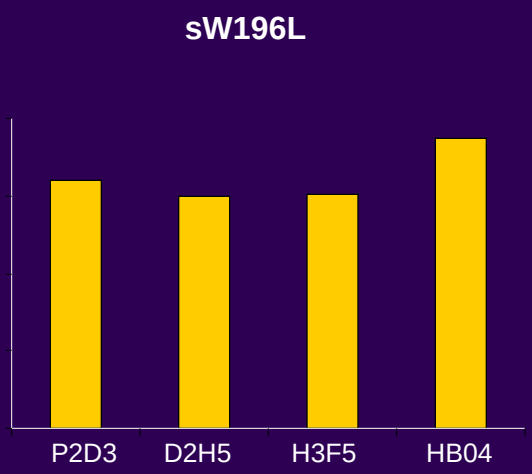












Epitope mapping – drug-driven HBsAg mutants

- 2008 longitudinal study undertaken in patients undergoing antiviral therapy

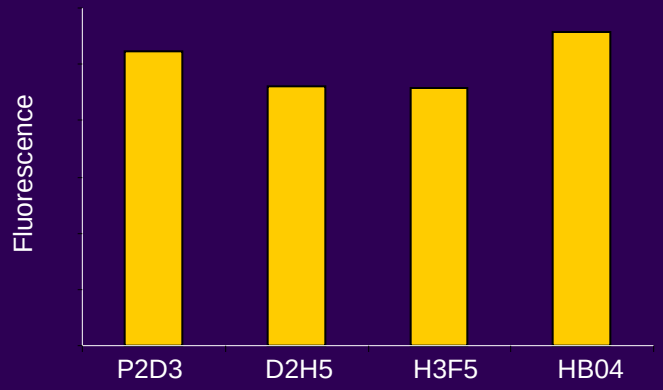
  Ijaz et al., J Med Virol 2008, 80:1160-1170

- Investigating dynamics of antiviral resistance mutations whilst on therapy
  - Lamivudine monotherapy
  - Treatment change to lamivudine & adefovir combination therapy or adefovir alone

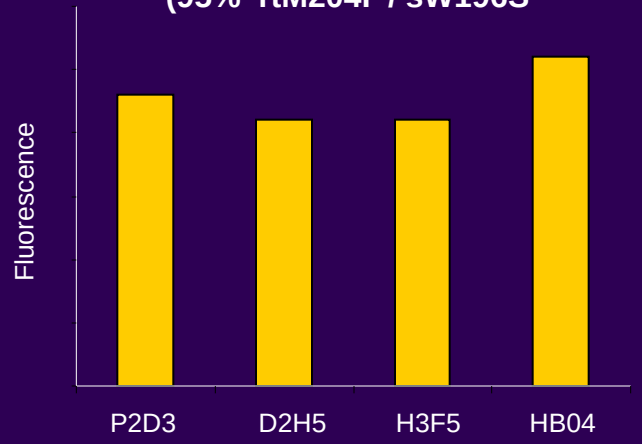
- Developed method based on pyrosequencing to quantify specific mutations (polymerase at codons 180, 181, 204)

Patient 1 (genotype B)

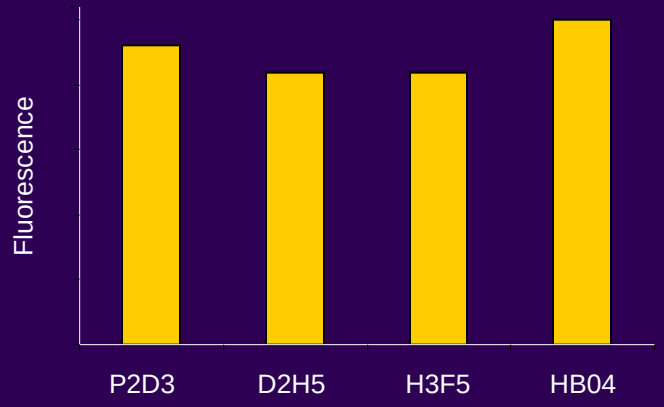
Baseline
(100% WT rt204 / WT in surface)



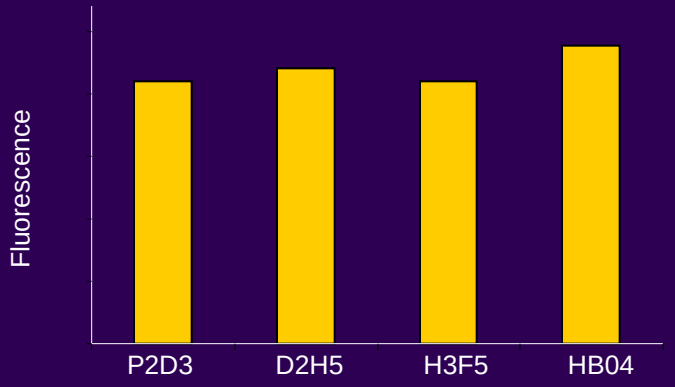
(95% rtM204I / sW196S



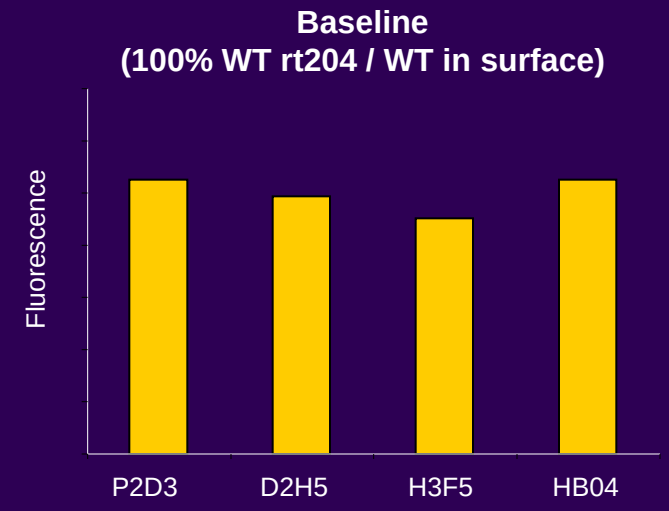
(94% rtM204I / sW196S)

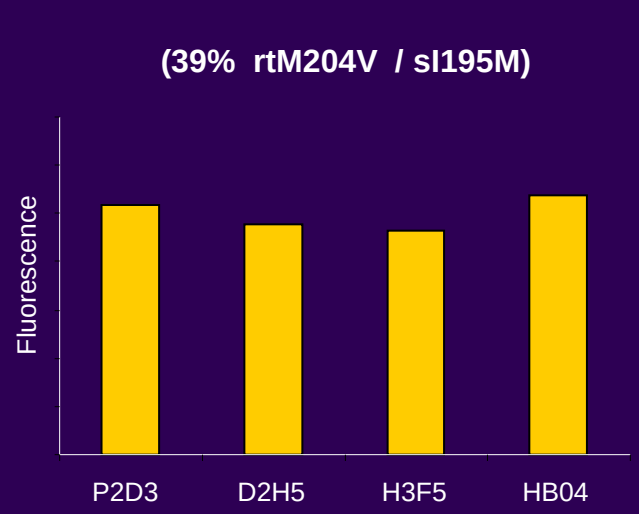


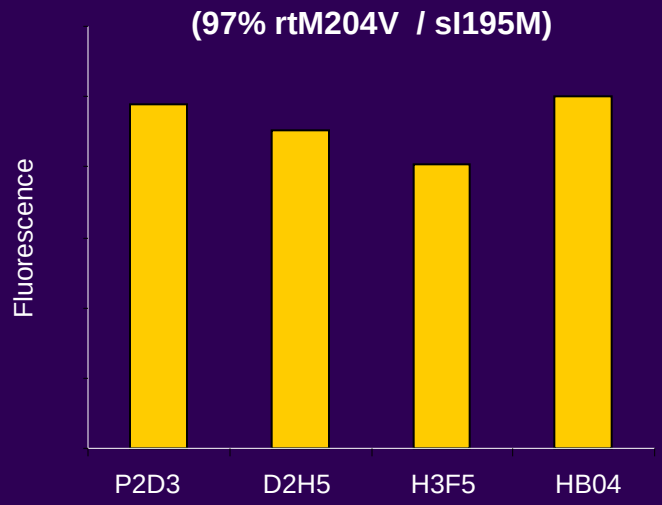
(100% rtM204I / sW196S)

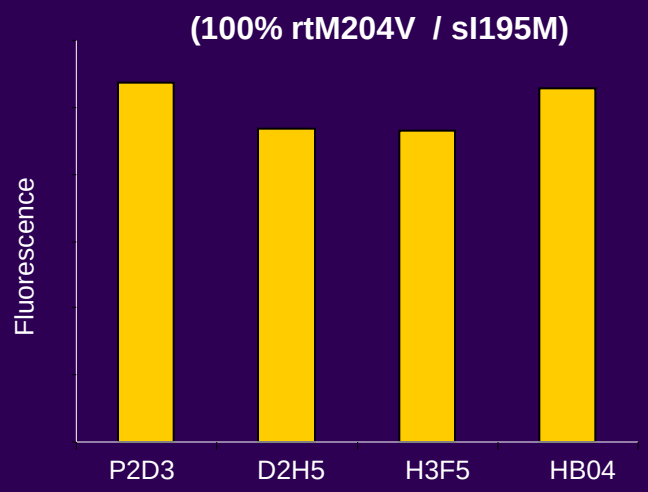


Patient 2 (genotype E)



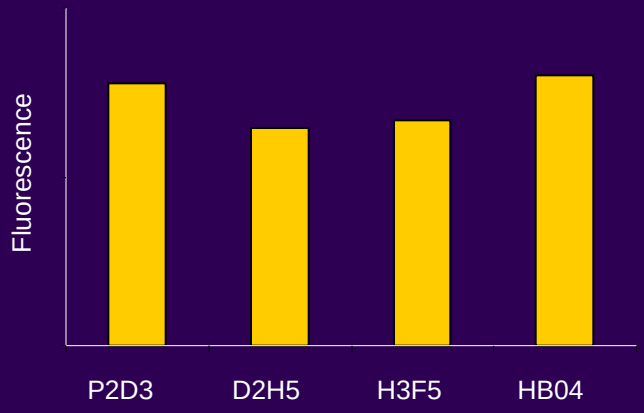




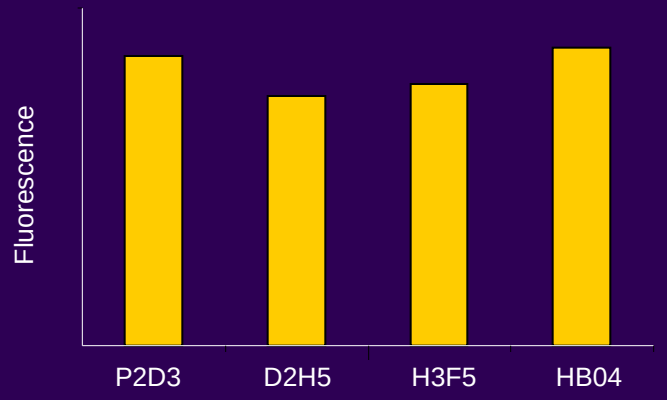


Patient 3 (genotype A)

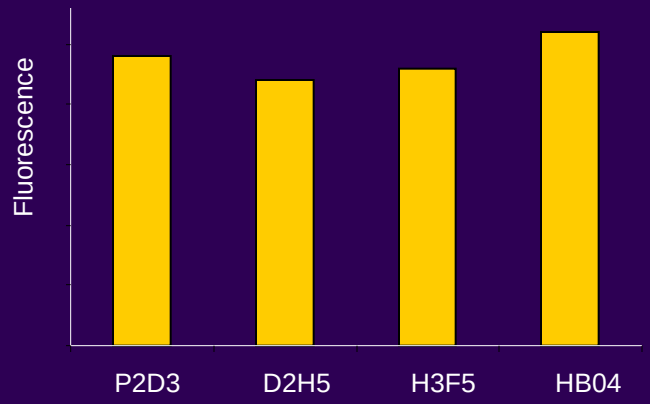
Baseline
(100% WT rt204 / WT in surface)



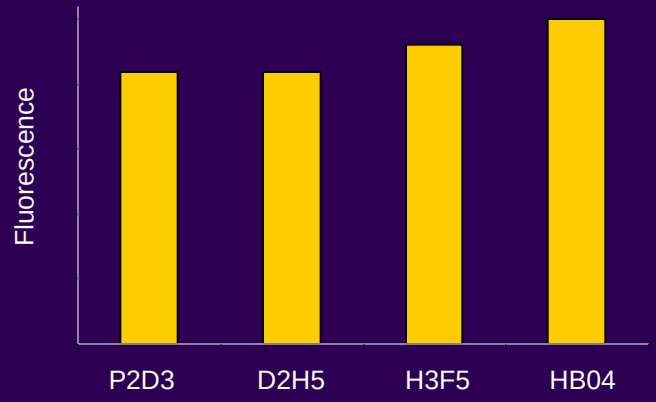
(58% rtV173L&M204V / sE164D&I195M)



(95% rtV173L&M204V / sE164D&I195M)



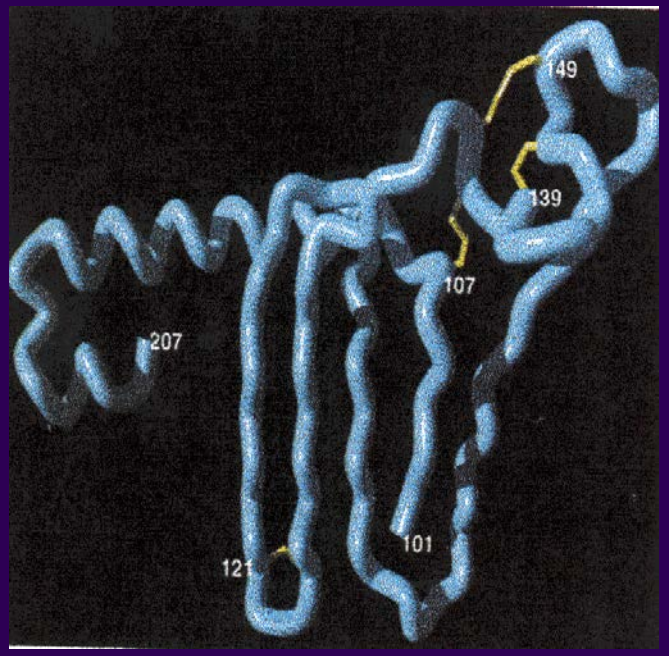
(97% rtV173&/M204V / sE164E/D&I195I/M)

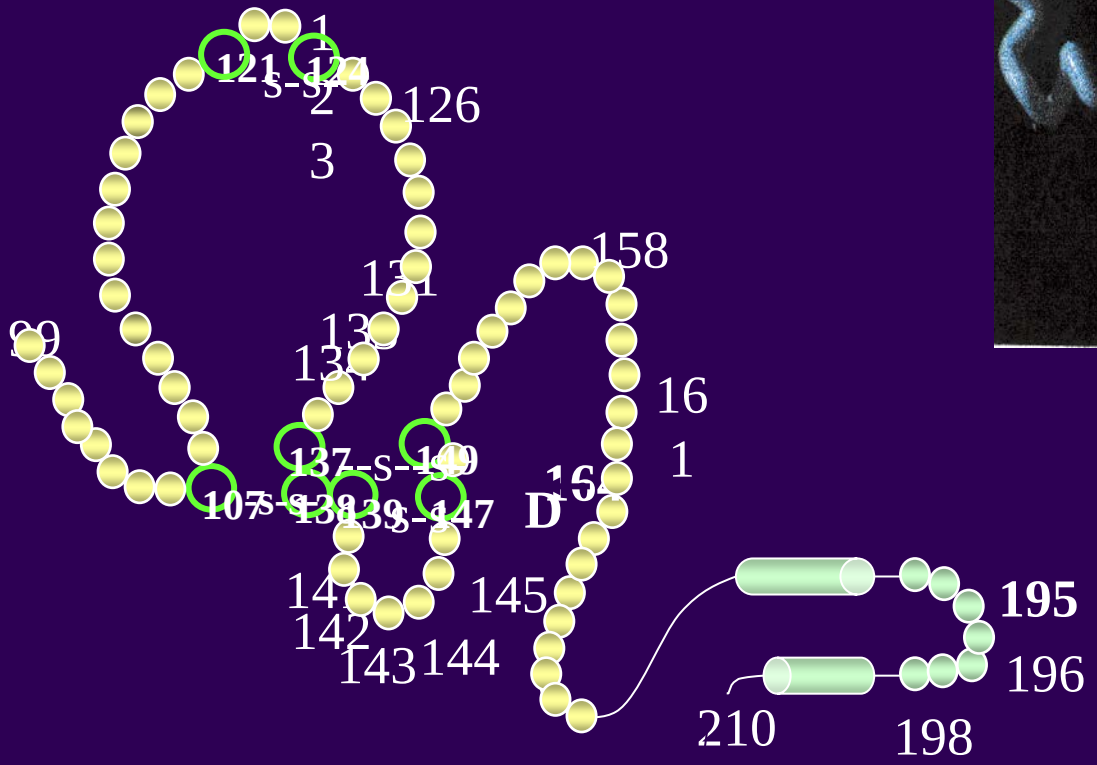


Epitope mapping – drug-driven HBsAg mutants

- No indication of epitope loss
- Panel of MAbs may not be appropriate for such analysis
  - P2D3, H3F5 and D2H5 have been used in other studies where epitope loss has been seen
- s/pol studies describing epitope loss have been undertaken using recombinant HBsAg
  - May not truly reflect native HBsAg conformation
  - Investigation into these viruses need to be in their ‘natural backbone’

HBsAg is a complex protein





Summary

- Epitope mapping using the luminex provides a rapid system for phenotyping HBsAg
- Allows for large population based studies to be undertaken which will improve our understanding of the prevalence of these mutants
- Data suggests that drug-driven HBsAg mutants do not impact on HBsAg antigenicity
- Data shows the importance of studying viruses in their natural backbone

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