The History of *Aspergillus* PCR

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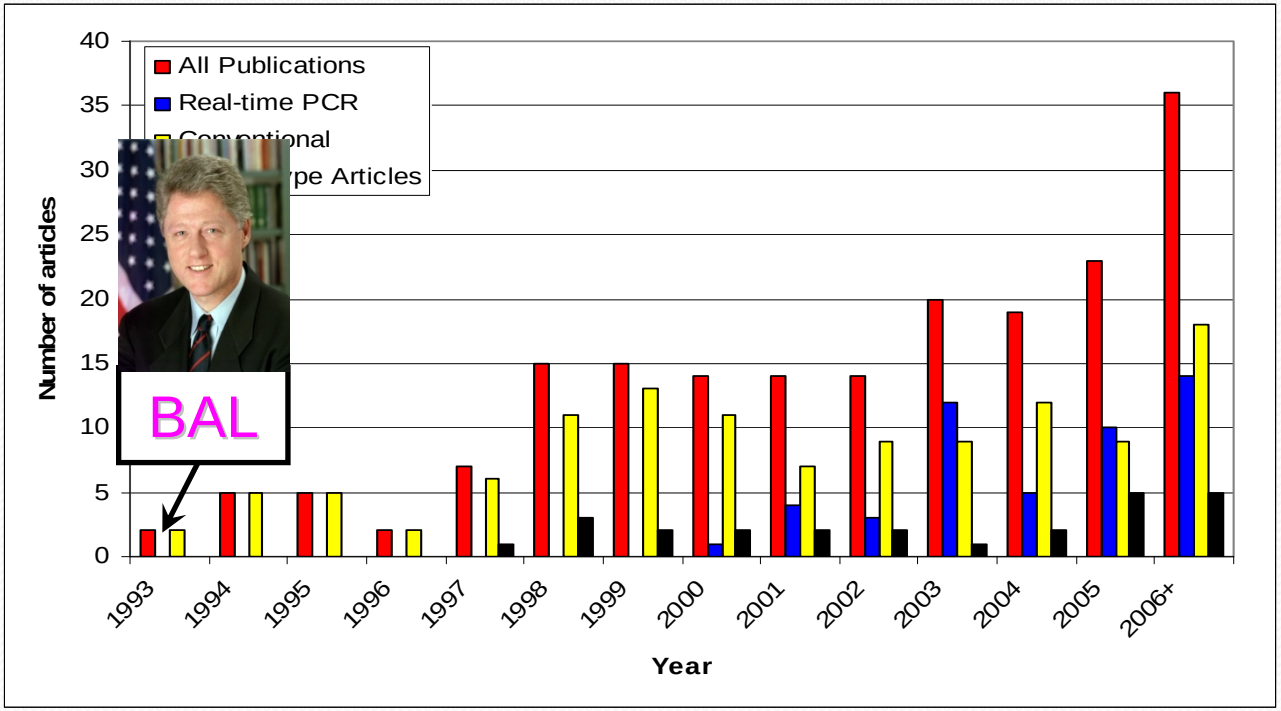
Aspergillus PCR through the ages!!



White and Barnes, 2008 Chapter 29 In *A.fumigatus* and Aspergillosis

PCR using respiratory specimens

- Permits the earliest opportunity for detection
- Positivity does not allows infer disease
  - Lung colonisation
  - Contamination
- Conidia/Hyphae
  - Targeted DNA extraction techniques
- Invasive specimen
  - Haemoptysis
  - Thrombocytopenia
- Pre-emptive approach?
  - Positivity = Early initiation of therapy
- Screening approach?
  - Negativity = Withhold therapy

Mean Sensitivity: 79%*

Mean Specificity: 94%*

*Tuon 2007 Rev Iberoam Micol

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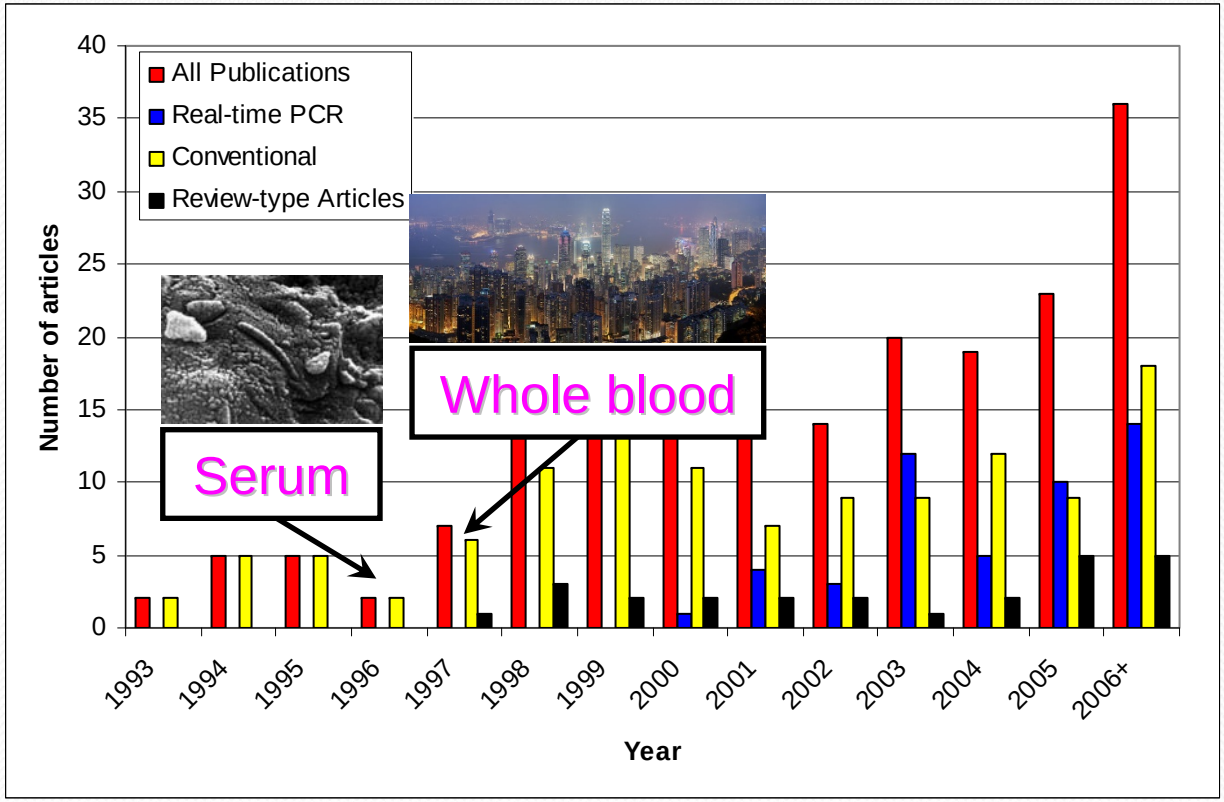
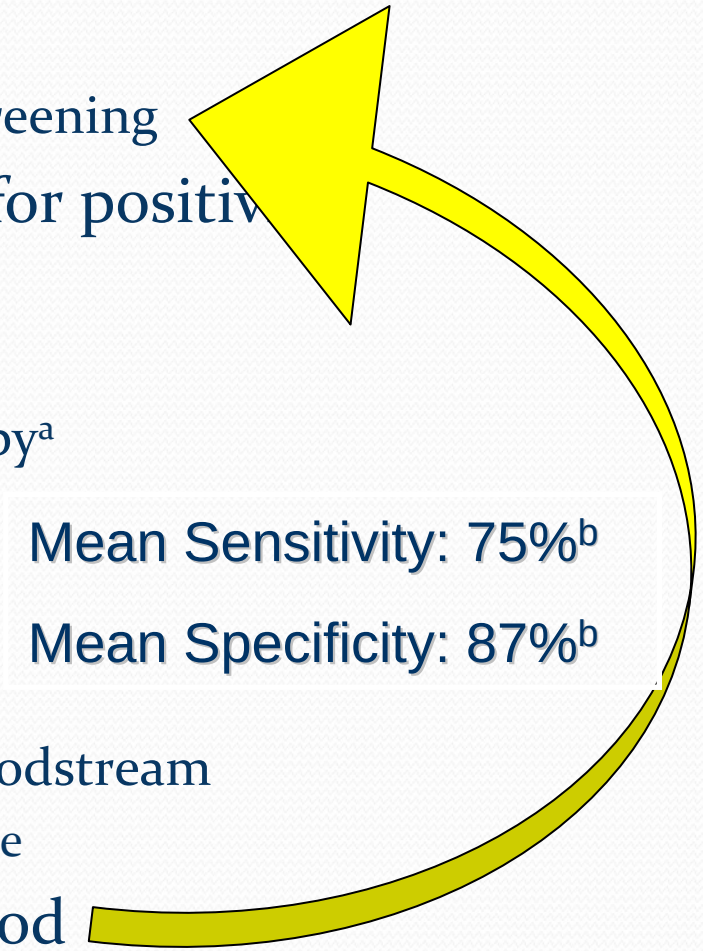


White and Barnes, 2008 Chapter 29 In *A.fumigatus* and Aspergillosis

PCR using blood specimens

- Non-invasive
  - High frequency large volume screening
- Invasive Disease progression for positiv
  - Requires angioinvasion
  - Pre-emptive approach?
  - PCR directed vs empirical therapy[a]
- Fungal target?
  - Blood cultures rarely positive
    - Non viable/free DNA source
  - Disease disseminates via the bloodstream
    - Viable/cell associated DNA source
- Fungal target is limited in blood



Mean Sensitivity: 75%[b]

Mean Specificity: 87%[b]

[a]Einsele *et al*, 2004 ASH

[b]Mengoli *et al*, 2009 Lancet Infectious Disease

PCR using serum or plasma

- Free circulating DNA only (DNAemia)
  - Hyphae lost during processing
- Simple DNA extraction process
- Direct comparison with GM ELISA
- Beneficial for patients on antifungal therapy
- Stability of free DNA
  - DNAses

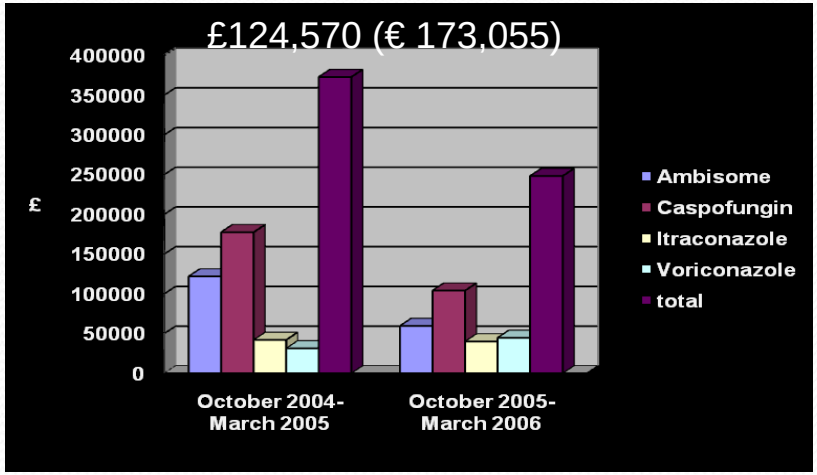
Mean Sensitivity: 72%*

Mean Specificity: 96.5%*

*White and Barnes, 2008 Chapter 29 In *A.fumigatus* and Aspergillosis

PCR using Whole Blood

- Targets both free and cell associated DNA?
  - In practice free DNA is destroyed or decanted
- Labour intensive
- Cost effective
- Larger volumes
  - Increases opportunity for detection
  - Increases possibility for contamination/inhibition
  - Internal control PCR
- Improved sensitivity?



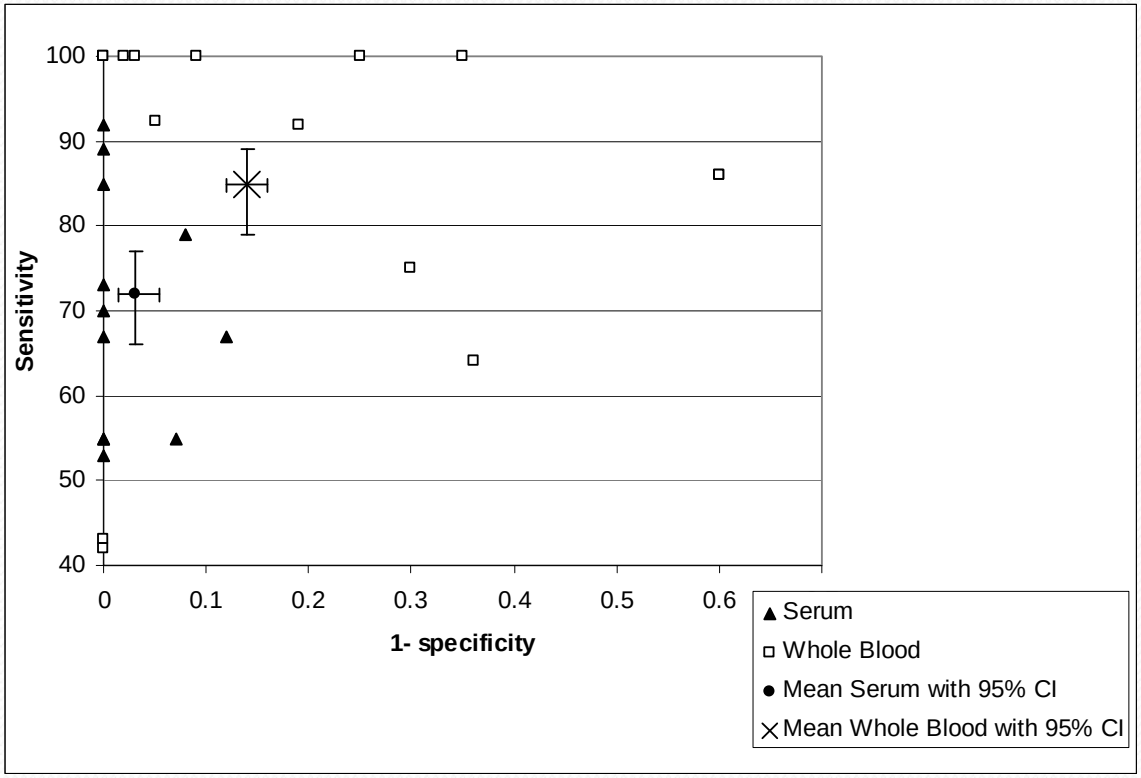
Mean Sensitivity: 85%[b]

Mean Specificity: 86%[b]

[a]Barnes *et al.* 2009 Journal of Clinical Pathology

[b]White and Barnes, 2008 Chapter 29 In *A.fumigatus* and Aspergillosis

Whole Blood or Serum/Plasma?



*White and Barnes, 2008 Chapter 29 In *A.fumigatus* and Aspergillosis

Combined Serum/Whole Blood approach

- Test both individually
  - Evidence for optimal specimen
- Primary screen: whole blood
  - Improved sensitivity
- Confirmation: serum
  - Improved specificity
- Combined extraction*
  - Target both free and cell associated DNA
  - Ultimate sensitivity?

*Morrisey *et al*. 2008 Abstract M1721 ICAAC

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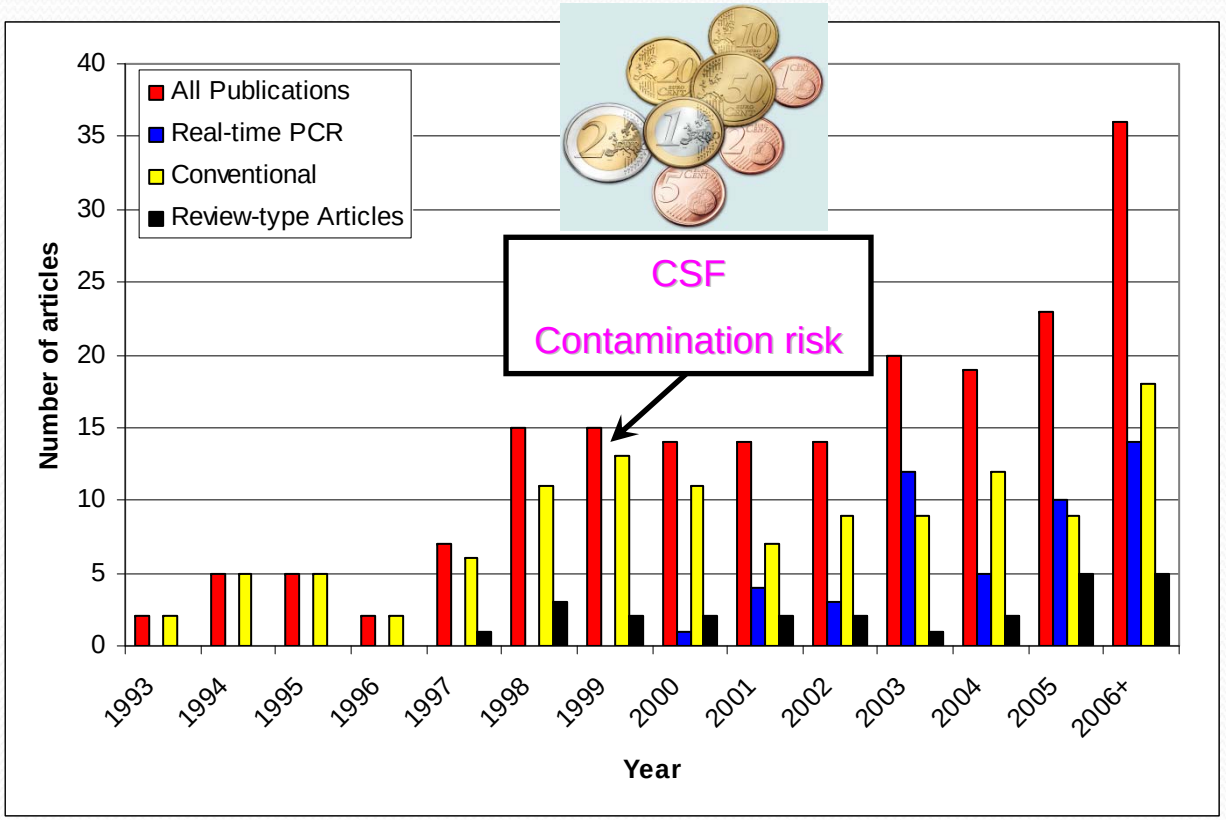

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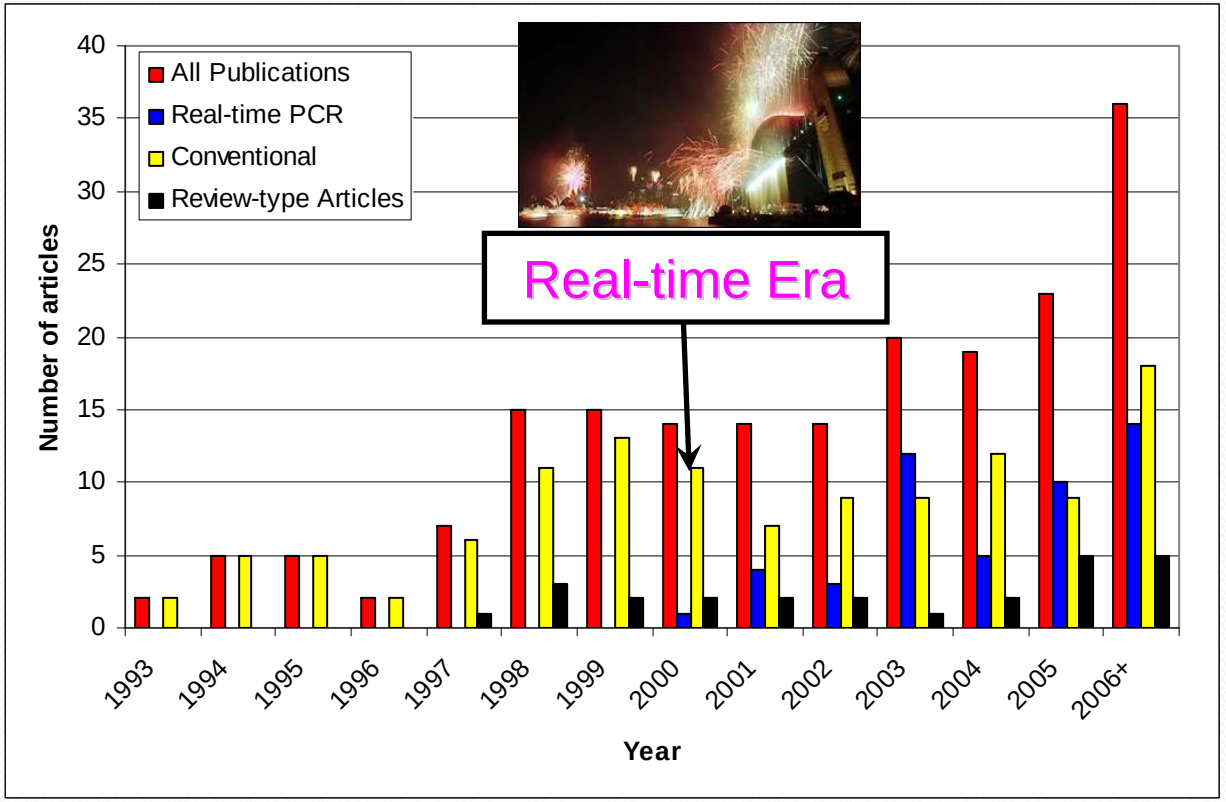
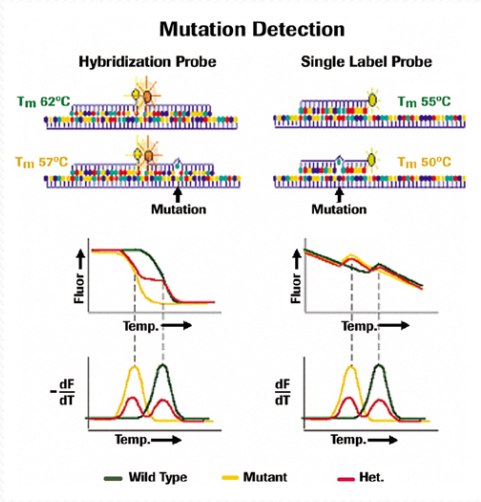
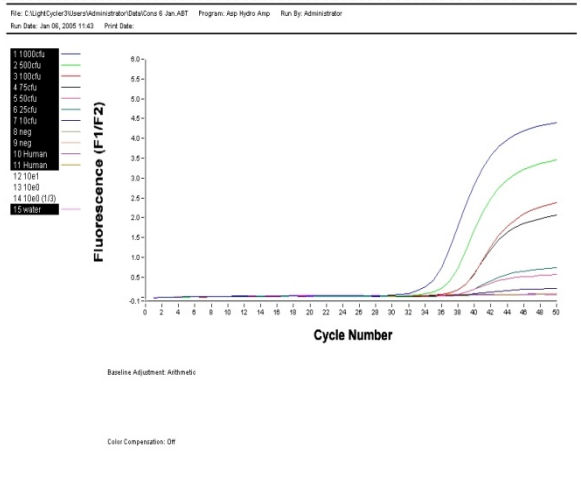


White and Barnes, 2008 Chapter 29 In *A.fumigatus* and Aspergillosis

Benefits of real-time PCR[1]

- Minimal contamination risk
  - No post amplification handling
  - UDG treatment
- Fast turn-around time
- High Sensitivity and Specificity
  - Using fluorescently labelled probes
  - Species/Genus level identification
- Multiplexing
- SNP detection
  - Antifungal resistance
- Quantification
  - Monitor fungal burden
  - Therapeutic response





[1] White PCR – Platforms, strengths and weaknesses, AAA 2006

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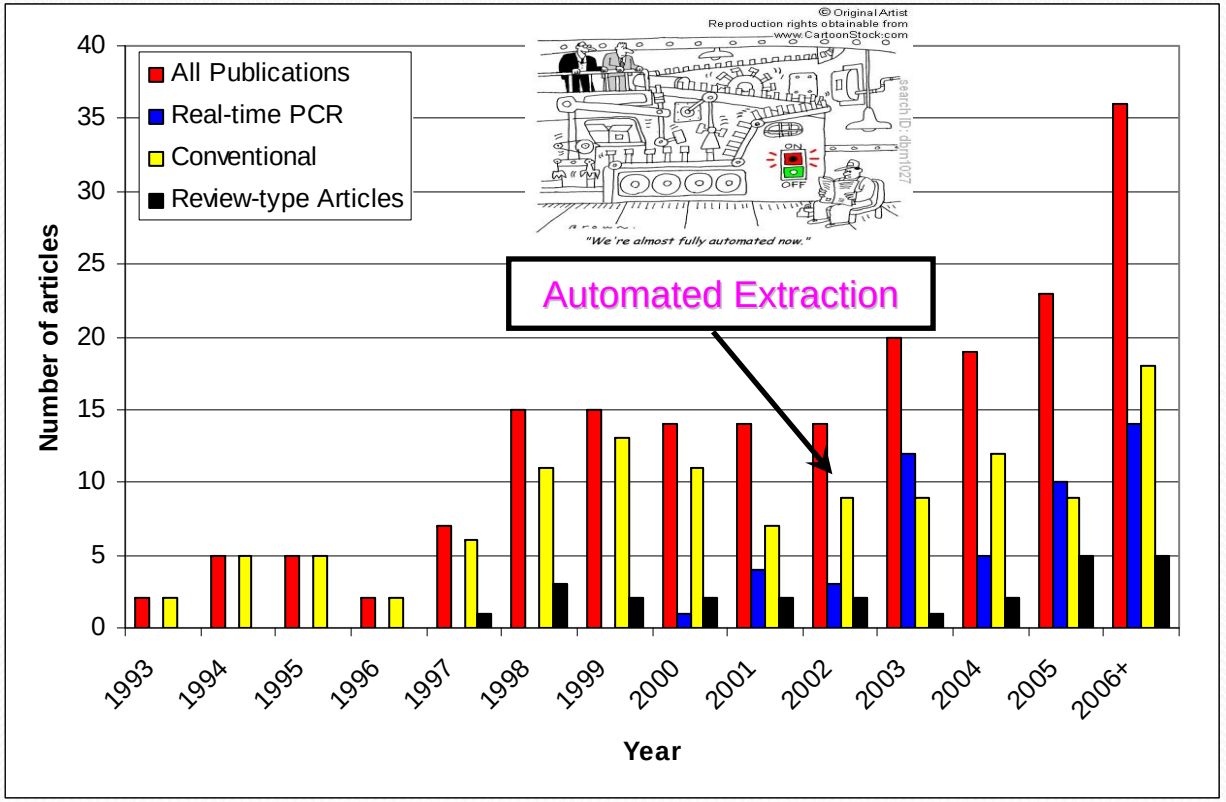

White and Barnes, 2008 Chapter 29 In *A.fumigatus* and Aspergillosis

DNA Extraction

- Rate limiting step
  - Providing extraction efficiency = PCR threshold
  - Removing inhibitory compounds
  - Avoid introducing contamination
- Methodology vary:
  - Specimen type = DNA target
  - Volume
- Complex methodology
  - Efficiency affected by user
- Automated extraction

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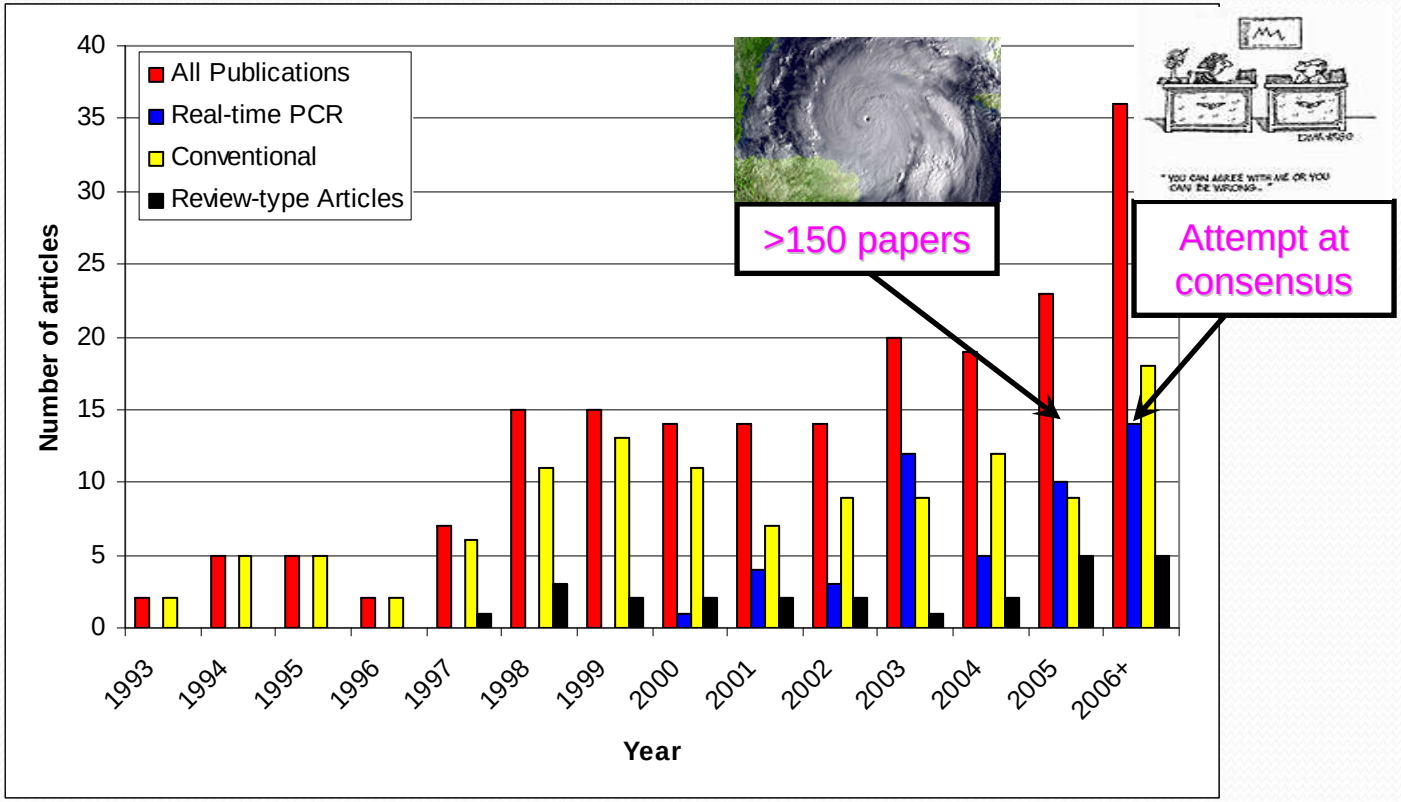

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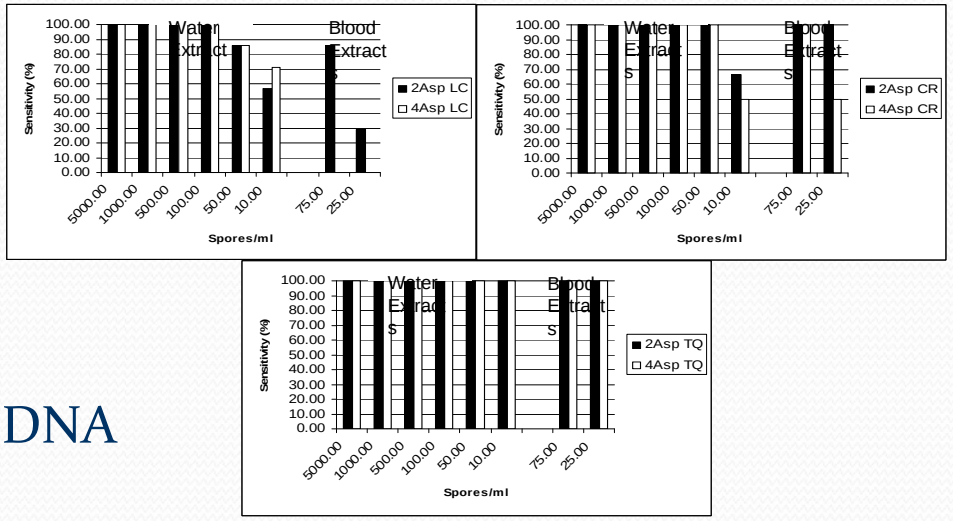
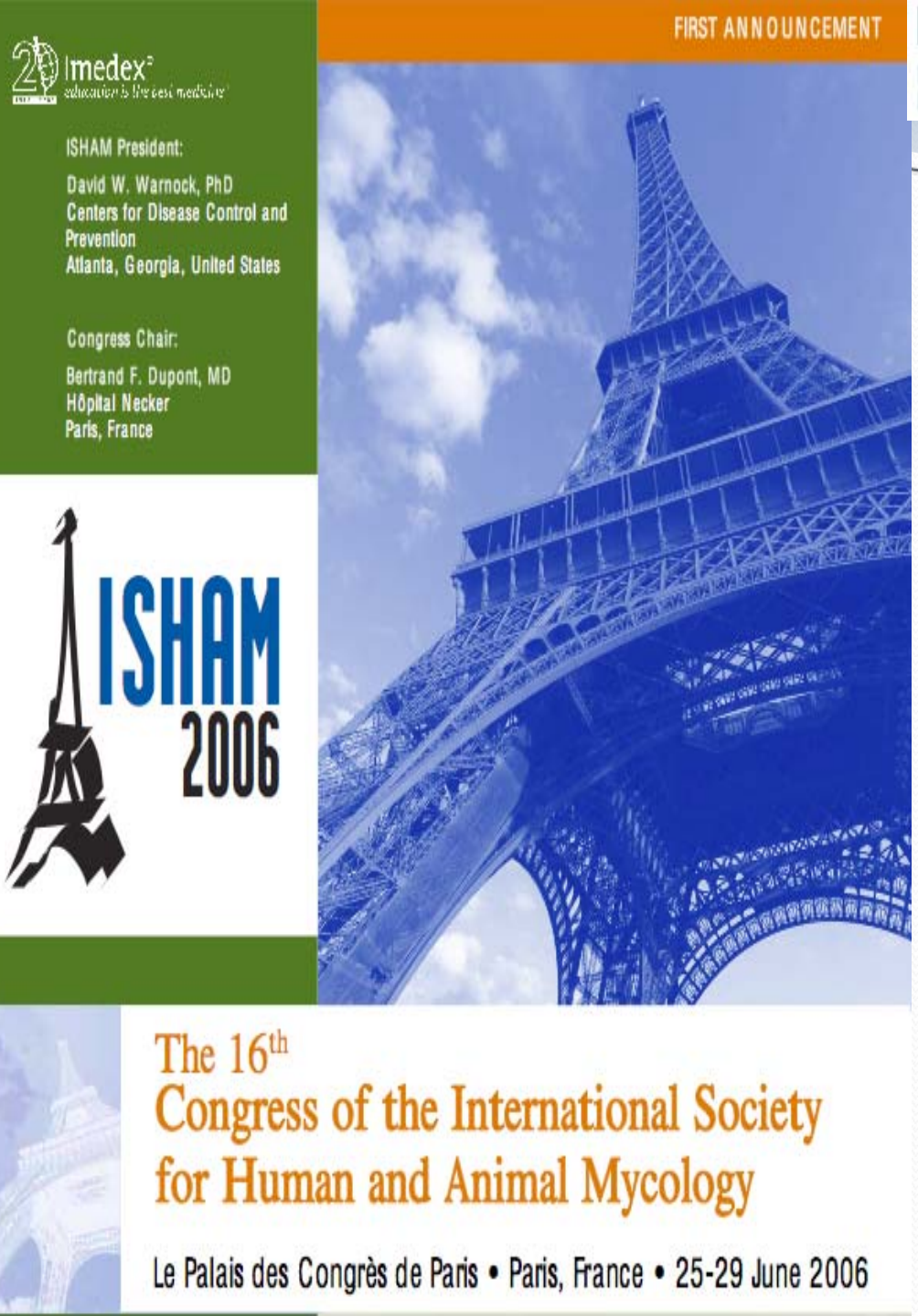
UK Standardisation of *Aspergillus* PCR[a]

- 2006 – First with multi-centre comparison of methods
- Distribution of QCMD panels
- Extraction based variation
  - Bead-beating in combination with Automated extraction
- Two optimal PCR methods – multi-centre testing
  - One for TaqMan
  - One for Light Cycler
- Sample type effect
  - Platform
- Amplification of human DNA



[a]White *et al.* 2006 J Mol Diag



ISHAM
INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

WORKING GROUP
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Sunday afternoon 25th June

Contact

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