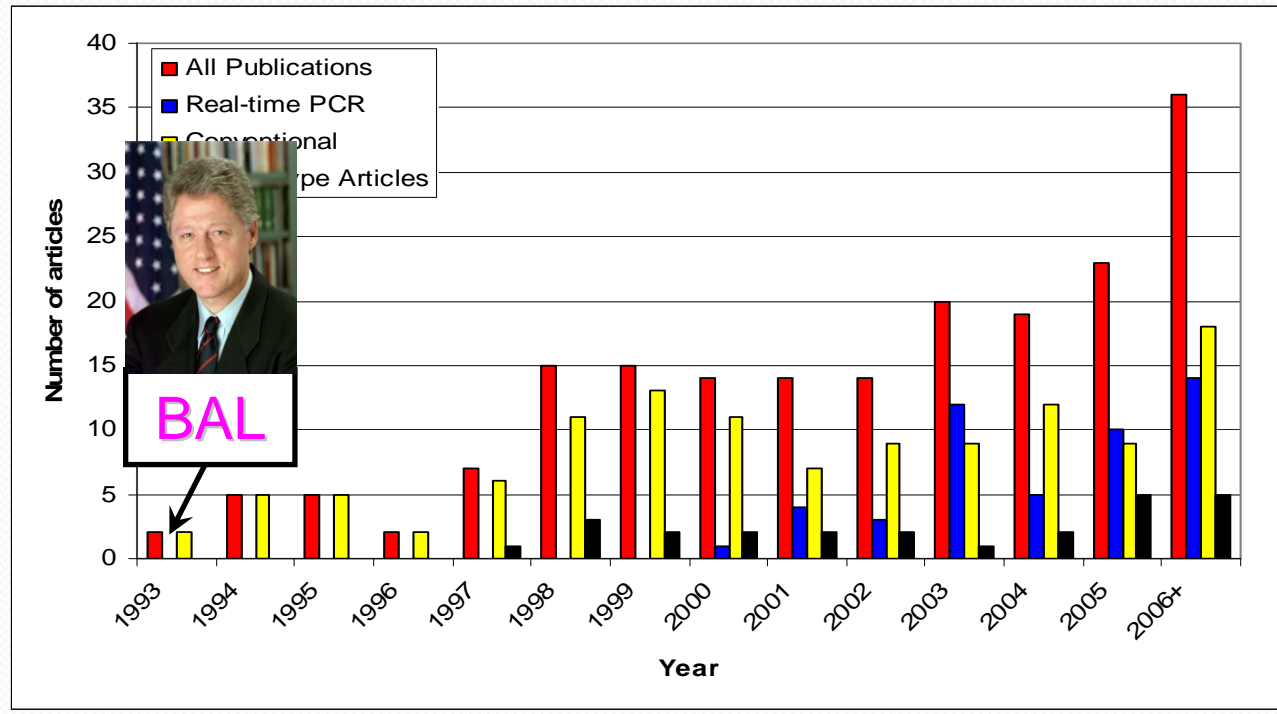




# The History of *Aspergillus* PCR

Dr P. Lewis White  
NPHS Microbiology Cardiff

# Aspergillus PCR through the ages!!



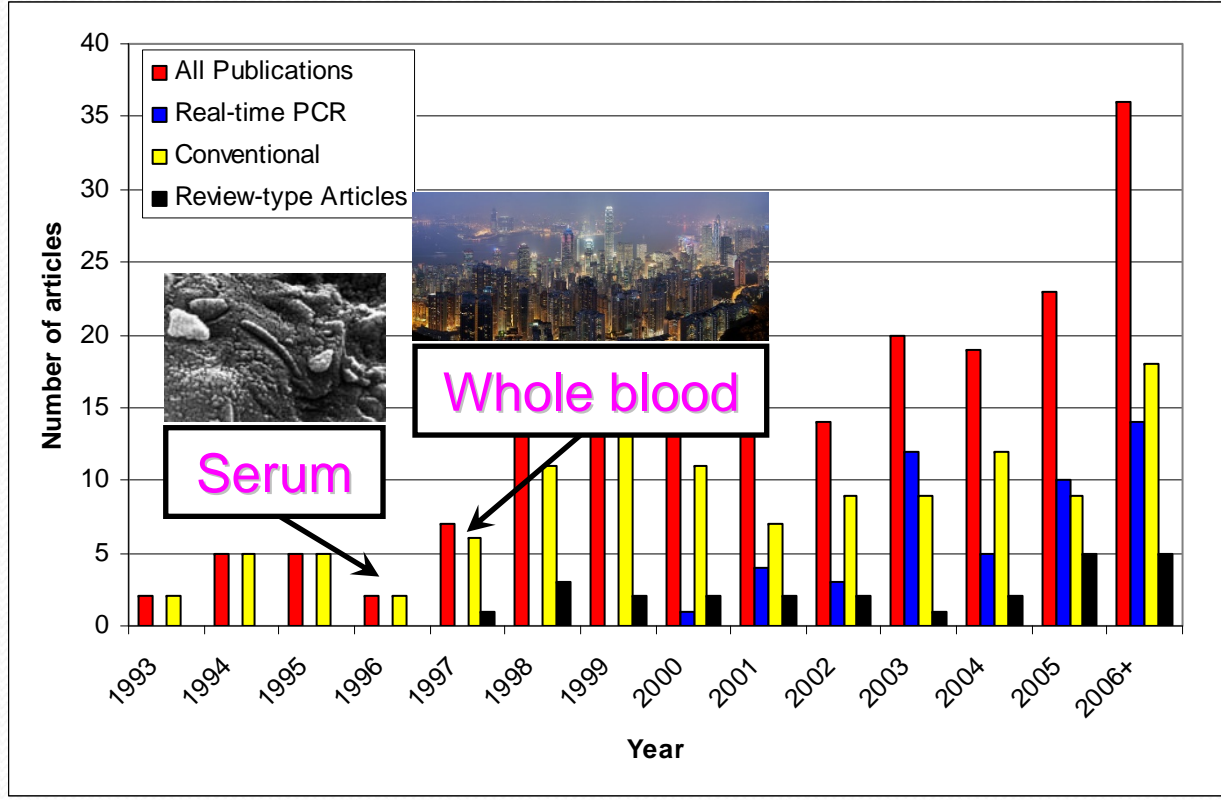
# PCR using respiratory specimens

- Permits the earliest opportunity for detection
- Positivity does not allow to 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%\*

# Aspergillus PCR through the ages!!



# PCR using blood specimens

- Non-invasive
  - High frequency large volume screening
- Invasive Disease progression for positive
  - Requires angioinvasion
  - Pre-emptive approach?
  - PCR directed vs empirical therapy<sup>a</sup>
- 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%<sup>b</sup>

Mean Specificity: 87%<sup>b</sup>

<sup>a</sup>Einsele *et al*, 2004 ASH

<sup>b</sup>Mengoli *et al*, 2009 Lancet Infectious Disease

## PCR using serum or plasma

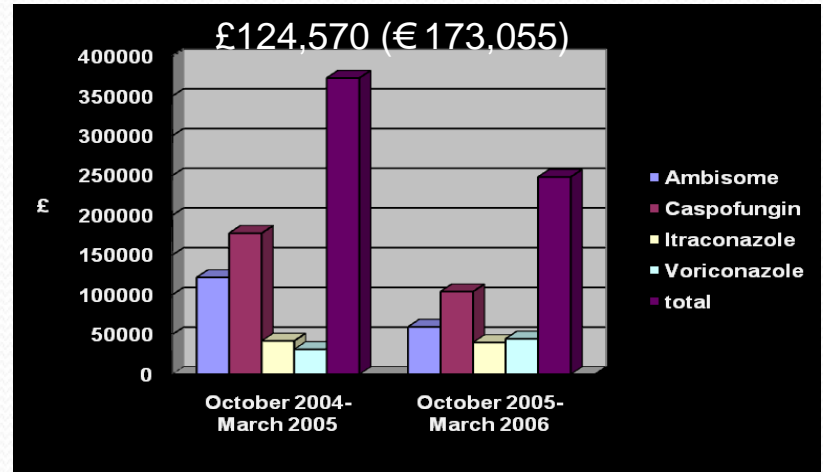
- Free circulating DNA only (DNAemia)
  - Hyphae lost during processing
- Simple DNA extraction process
- Direct comparison with GM ELISA
- Beneficial for patients on antifungal therapy
- Stability of free DNA
  - DNAses

Mean Sensitivity: 72%\*

Mean Specificity: 96.5%\*

# 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?



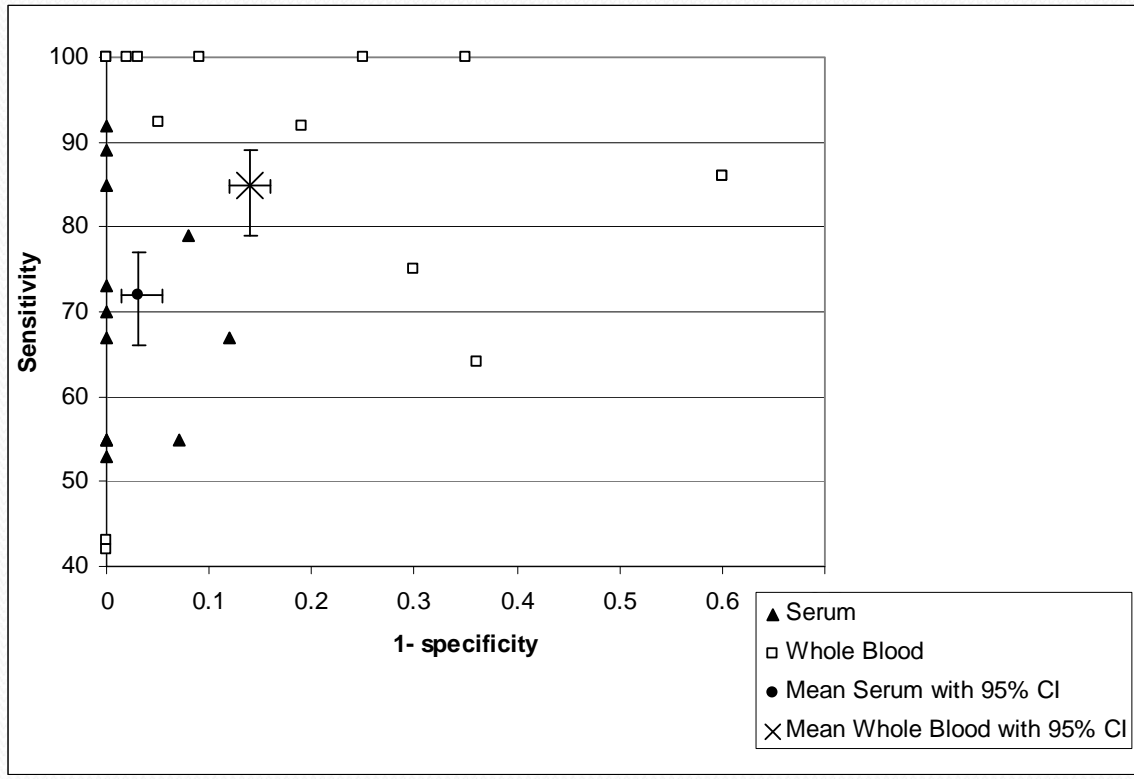
Mean Sensitivity: 85%<sup>b</sup>

Mean Specificity: 86%<sup>b</sup>

<sup>a</sup>Barnes *et al.* 2009 Journal of Clinical Pathology

<sup>b</sup>White and Barnes, 2008 Chapter 29 In *A.fumigatus* and Aspergillosis

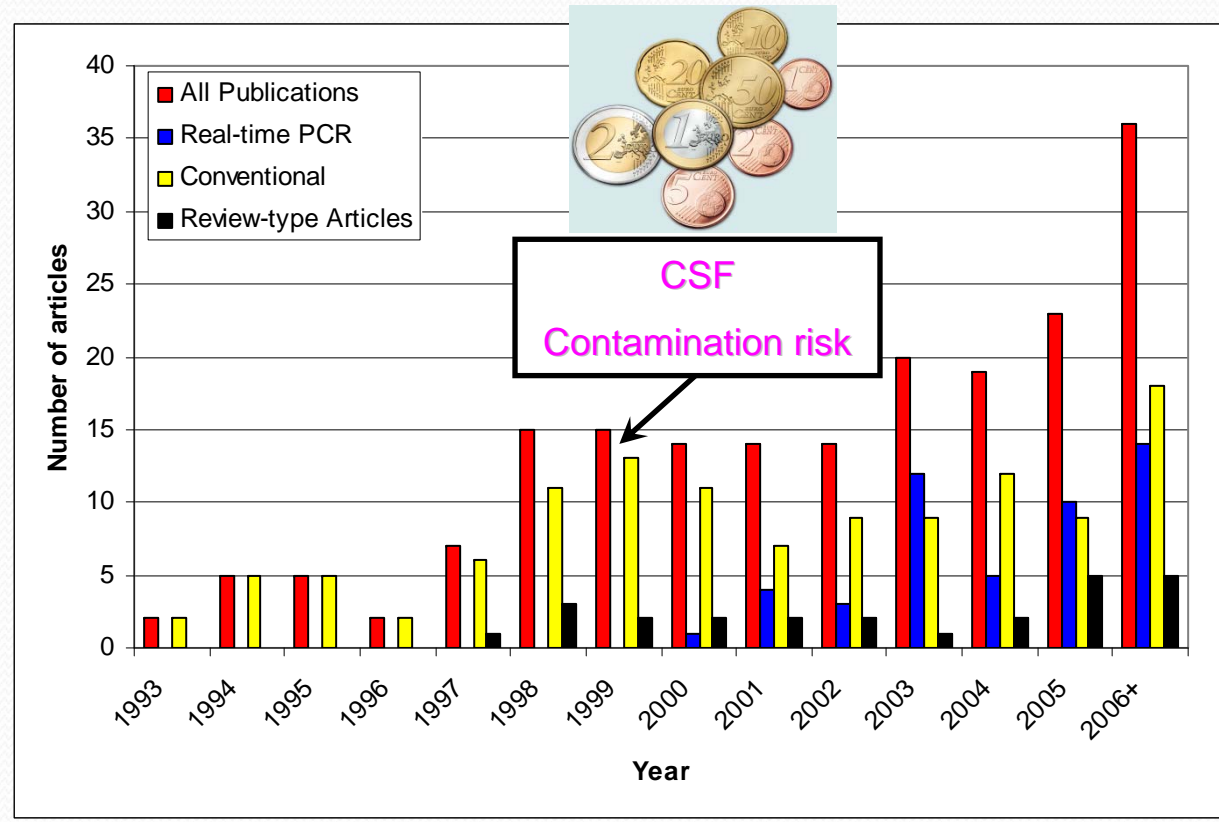
# Whole Blood or Serum/Plasma?



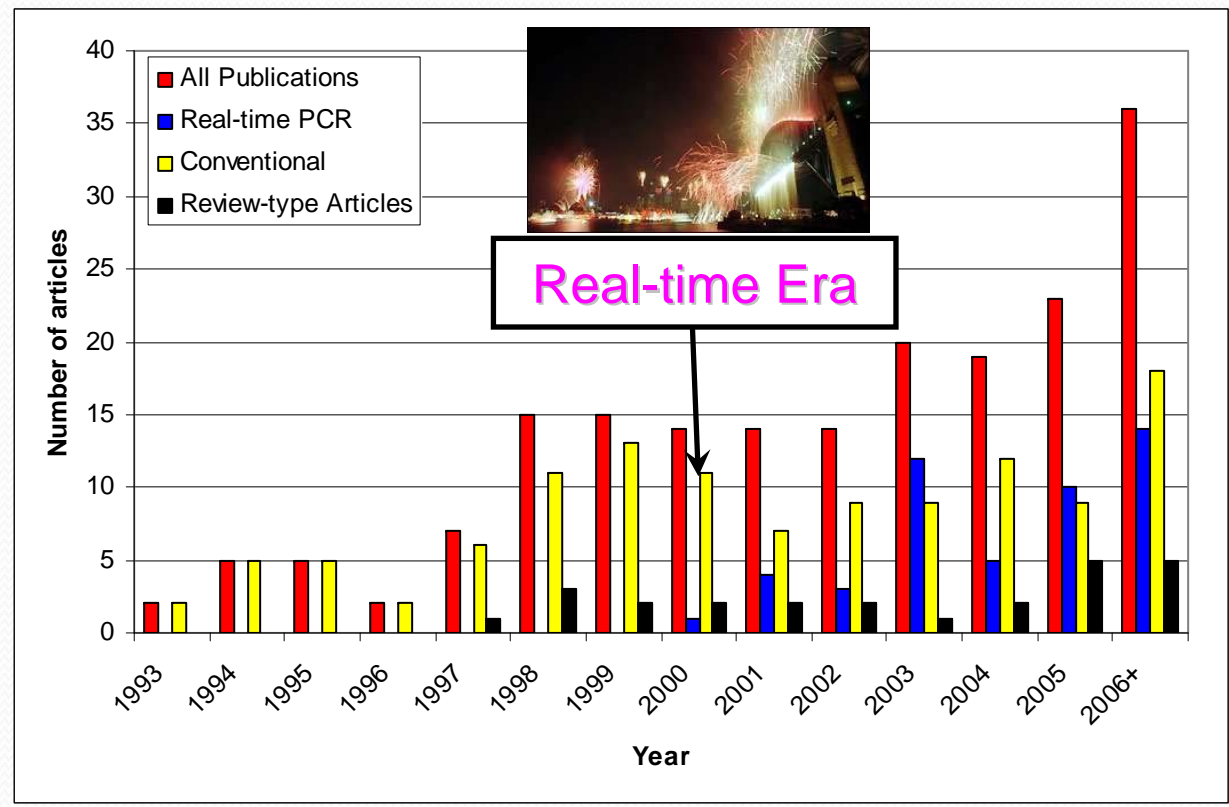
# Combined Serum/Whole Blood approach

- 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?

# Aspergillus PCR through the ages!!

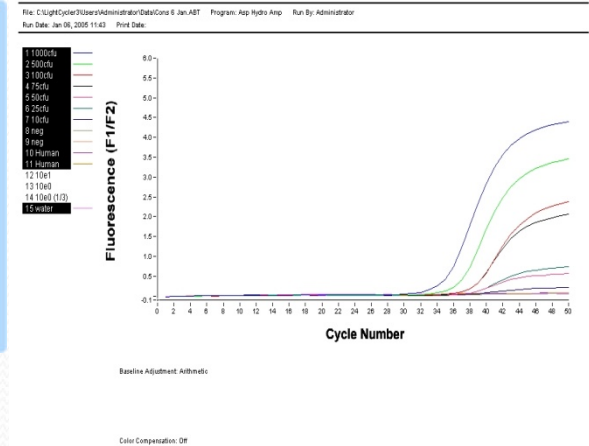
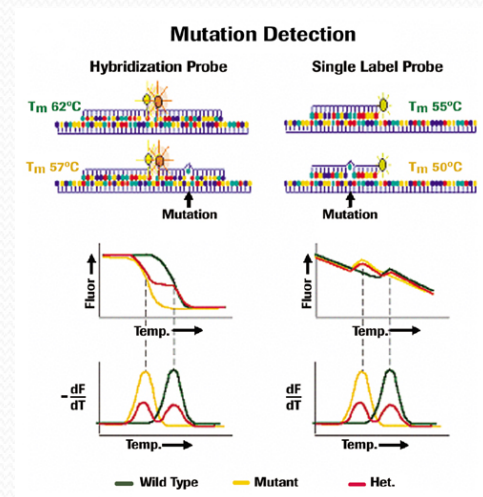


# Aspergillus PCR through the ages!!



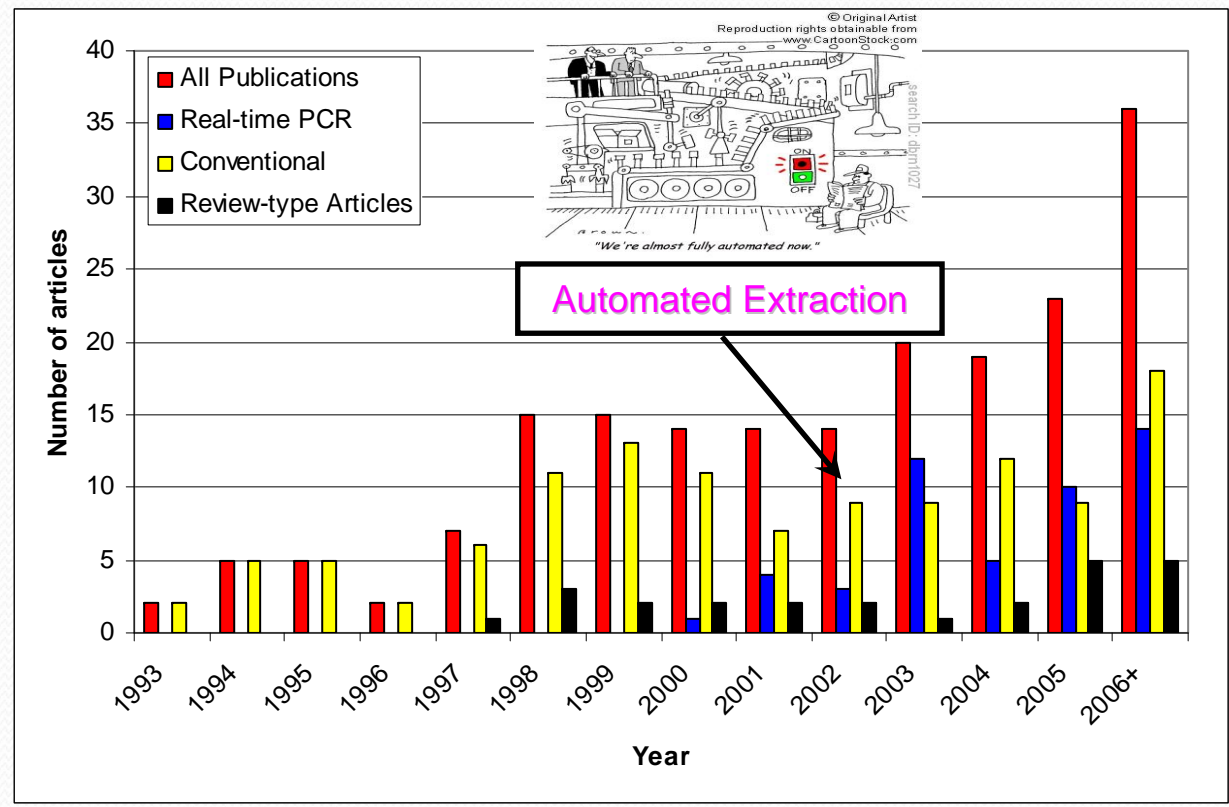
# Benefits of real-time PCR<sup>1</sup>

- 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



<sup>1</sup> White PCR – Platforms, strengths and weaknesses, AAA 2006

# Aspergillus PCR through the ages!!

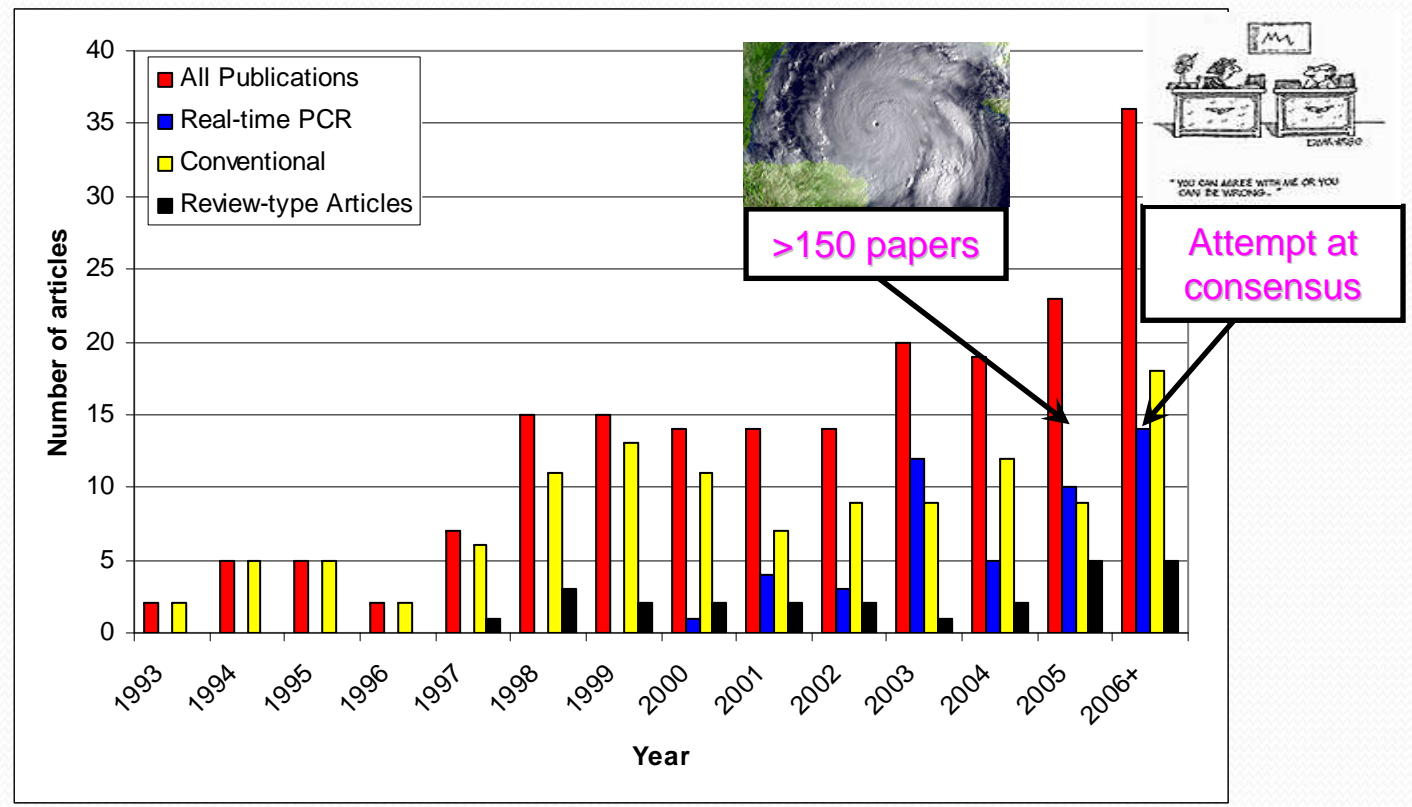


# DNA Extraction

- 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

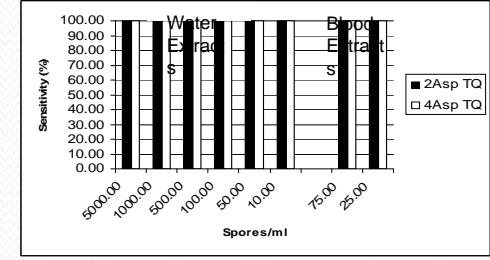
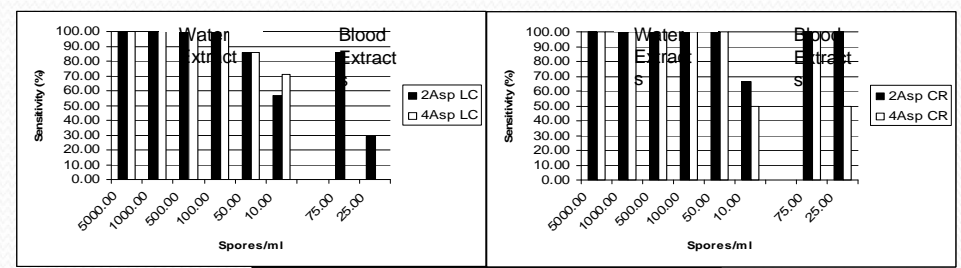


# Aspergillus PCR through the ages!!



# UK Standardisation of *Aspergillus* PCR<sup>a</sup>

- 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



<sup>a</sup>White et al. 2006 J Mol Diag

FIRST ANNOUNCEMENT

**ISHAM**  
INTERNATIONAL SOCIETY FOR  
HUMAN AND ANIMAL MYCOLOGY



WORKING GROUP  
EUROPEAN ASPERGILLUS PCR INITIATIVE  
**EAPCRI**

ISHAM President:

David W. Warnock, PhD  
Centers for Disease Control and  
Prevention  
Atlanta, Georgia, United States

Congress Chair:

Bertrand F. Dupont, MD  
Hôpital Necker  
Paris, France



**Sunday  
afternoon  
25th June**

**Contact**

[p.donnelly@usa.net](mailto:p.donnelly@usa.net)

The 16<sup>th</sup>  
Congress of the International Society  
for Human and Animal Mycology

Le Palais des Congrès de Paris • Paris, France • 25-29 June 2006