The European PCR standardisation process

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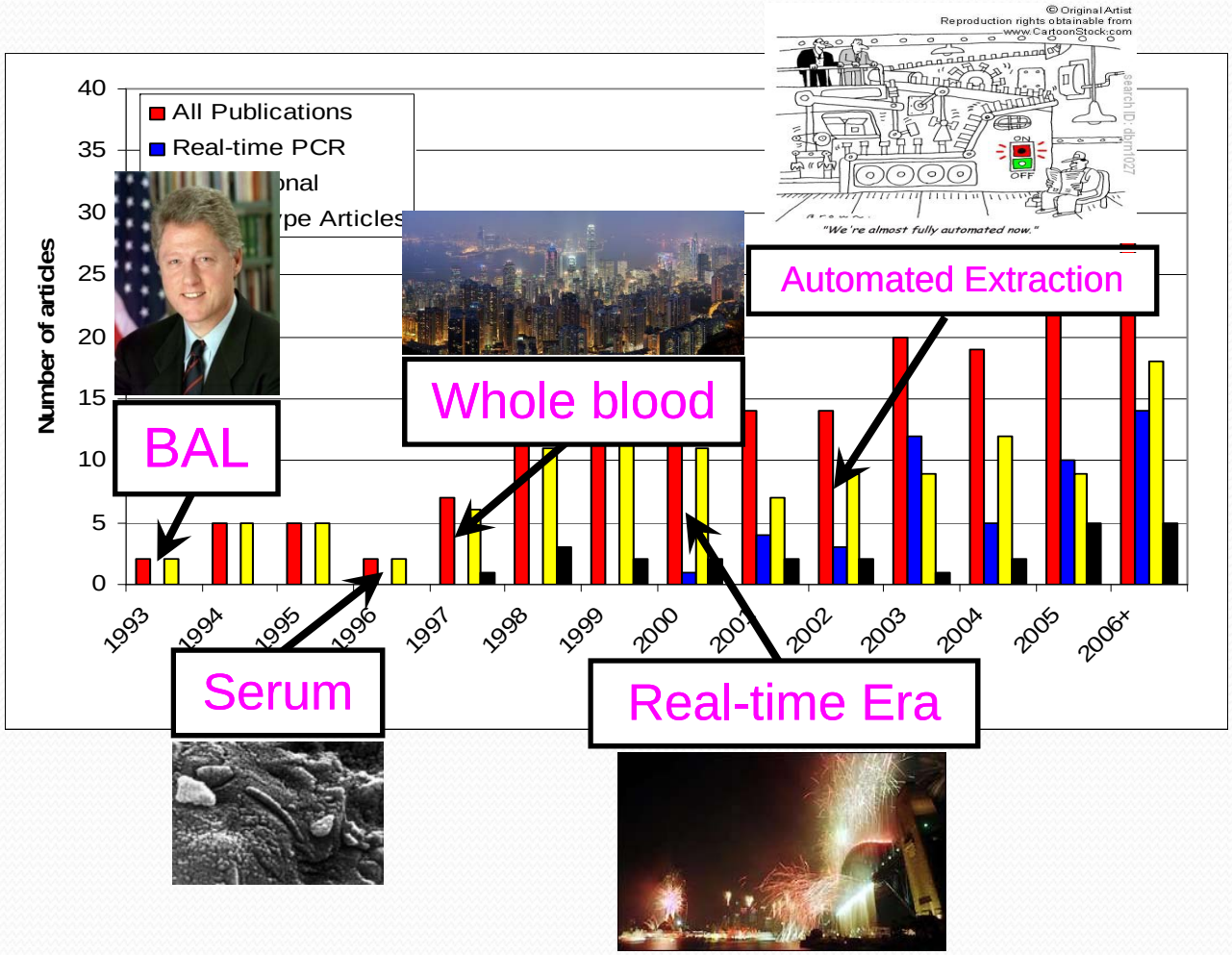
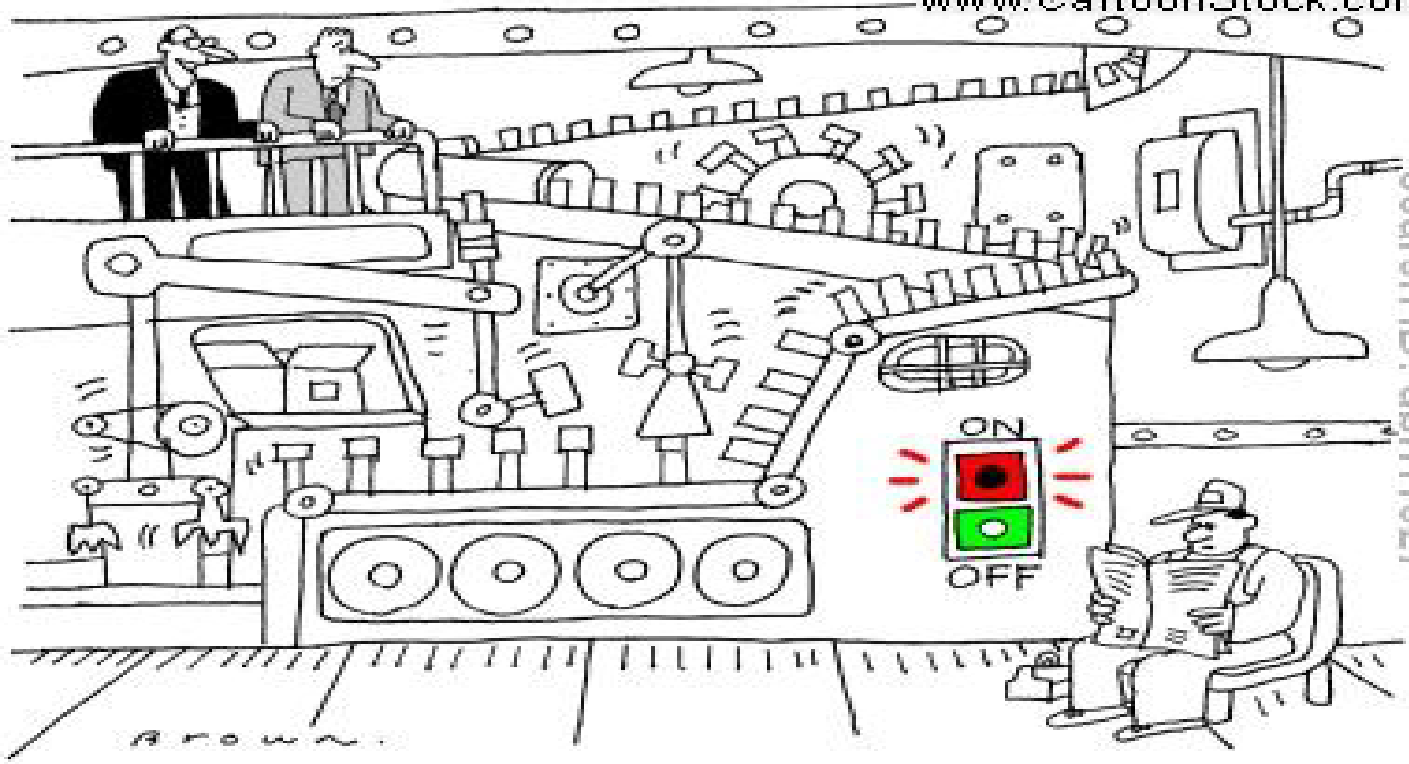
Aspergillus PCR through the ages!!

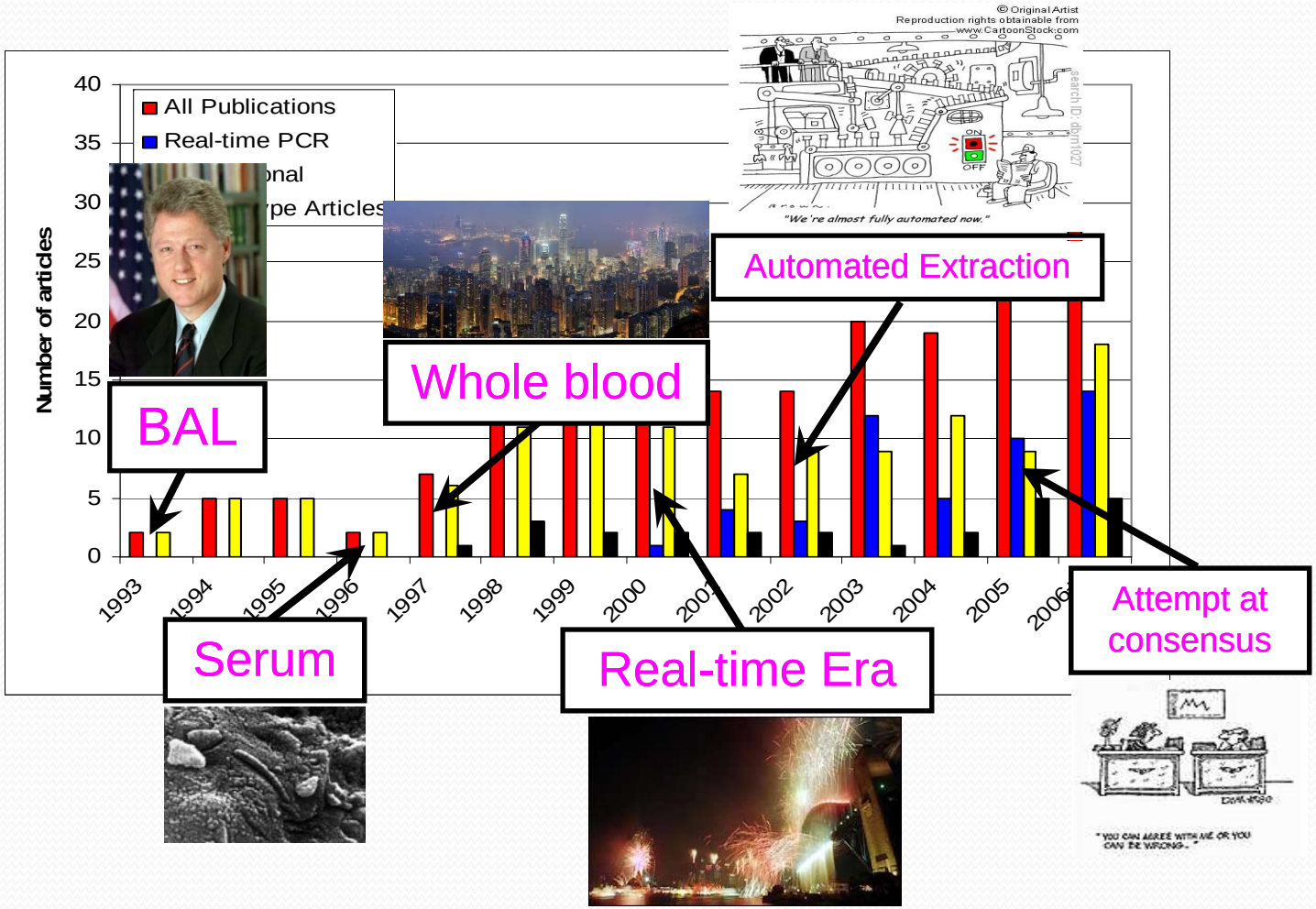


White and Barnes, 2009 Chapter 29 In *A.fumigatus* and Aspergillosis



"We're almost fully automated now."

Aspergillus PCR through the ages!!



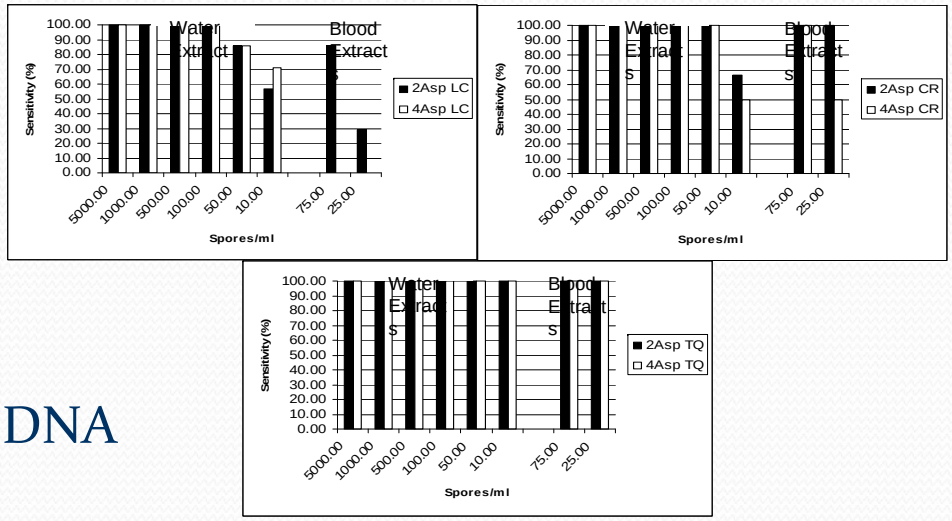
White and Barnes, 2009 Chapter 29 In *A.fumigatus* and Aspergillosis

Consensus?

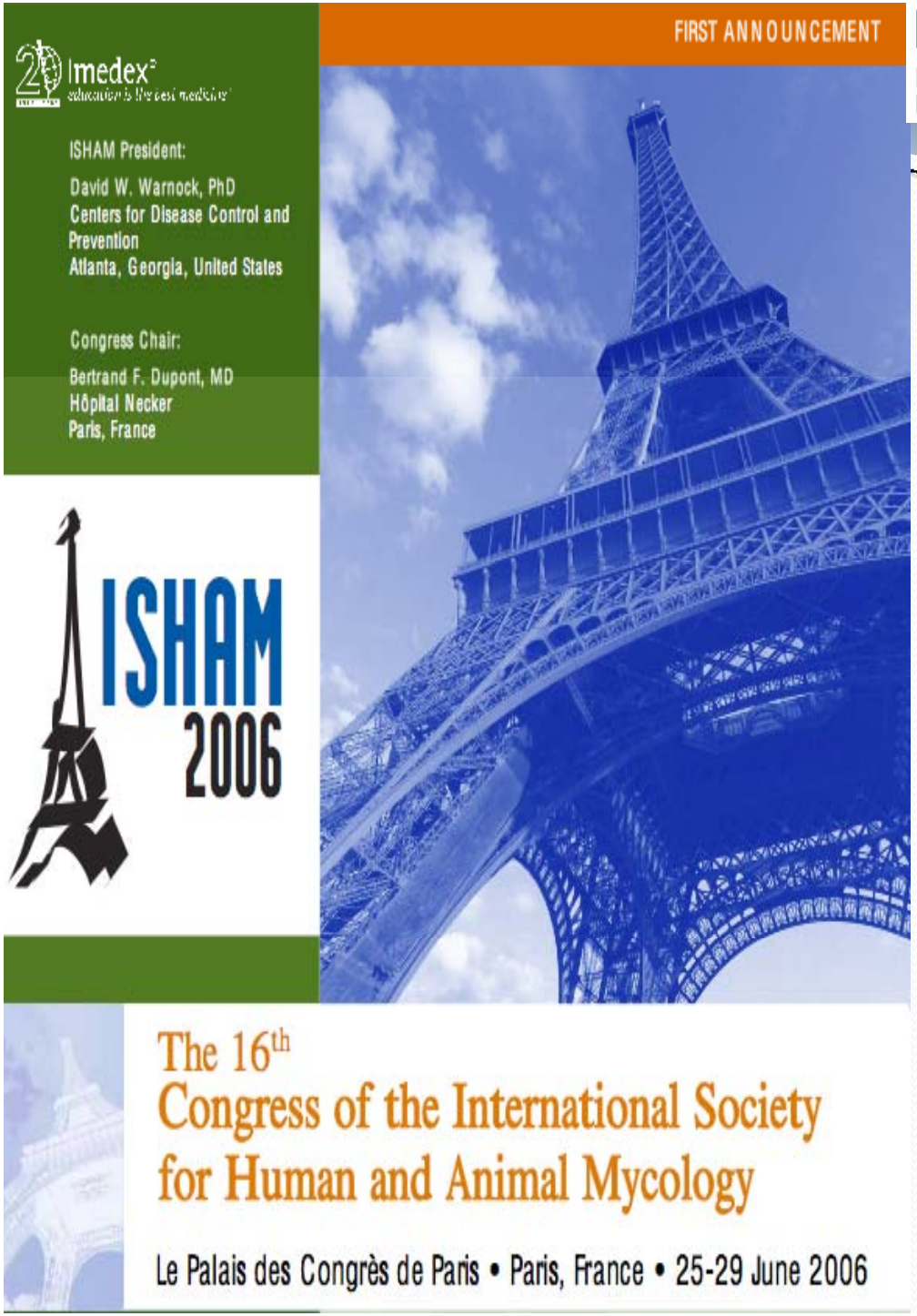
You can agree with me or you can be wrong

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
EUROPEAN ASPERGILLUS PCR INITIATIVE
EAPCRI

Sunday afternoon 25th June

Contact

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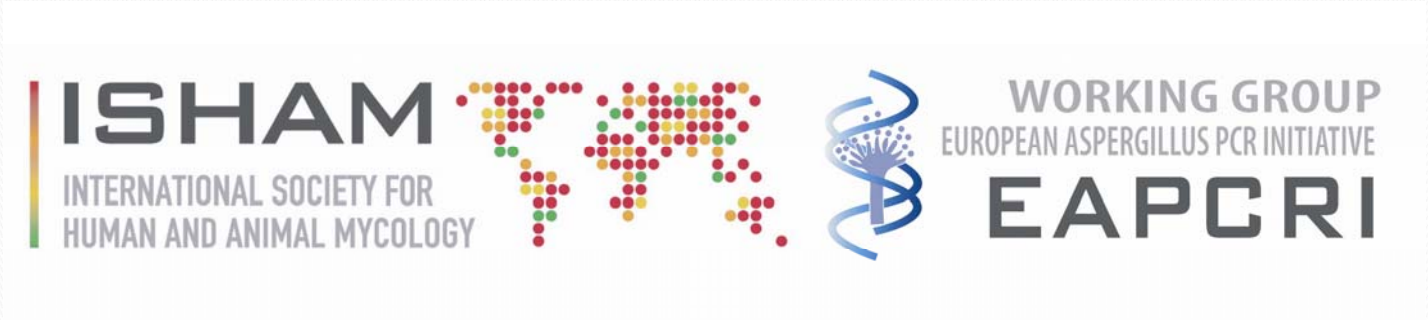
European Collaboration



PCR

Excellence in science
EBMT
European Group for Blood and Marrow Transplantation

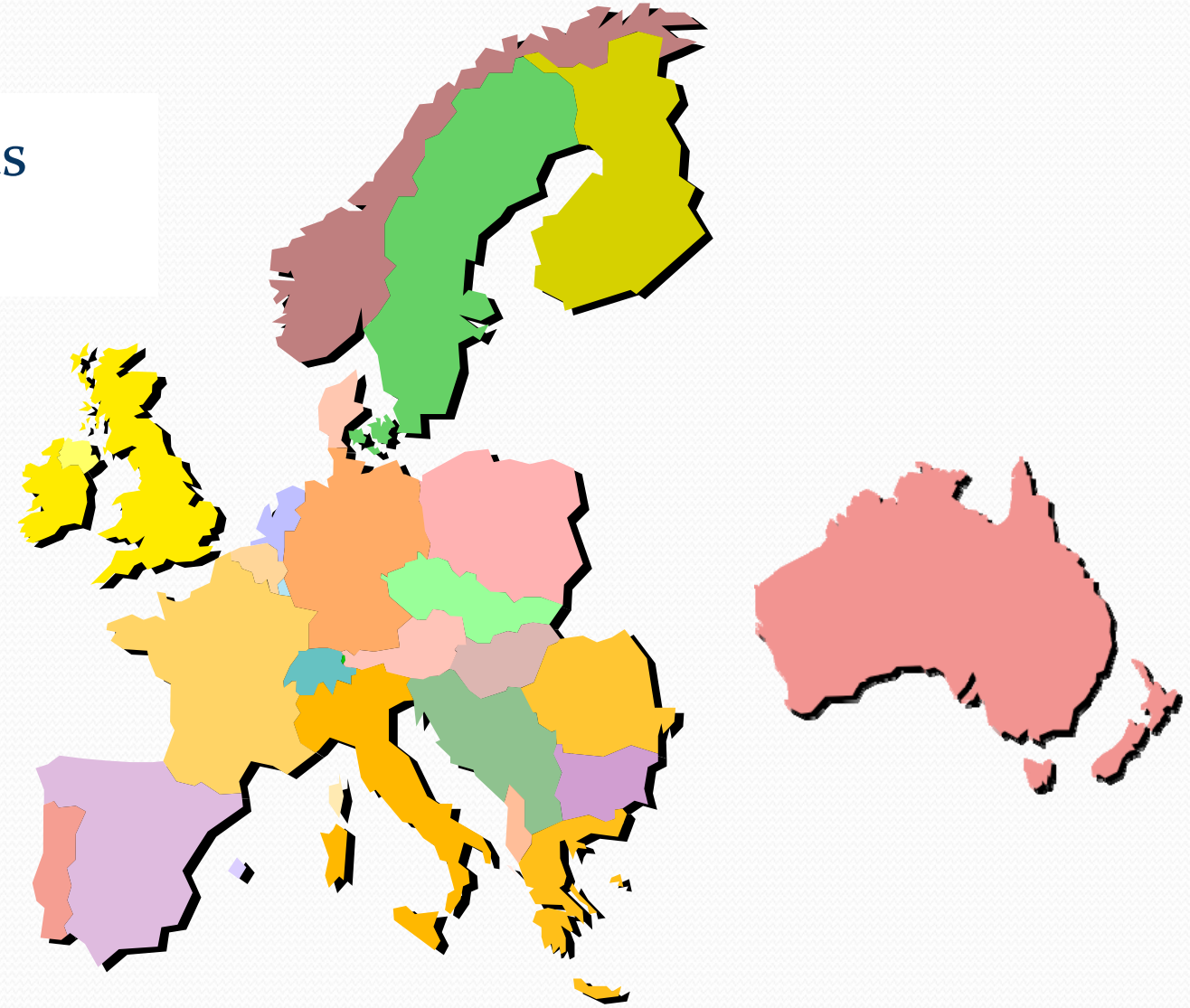
HSCT

EORTC
European Organisation for Research and Treatment of Cancer

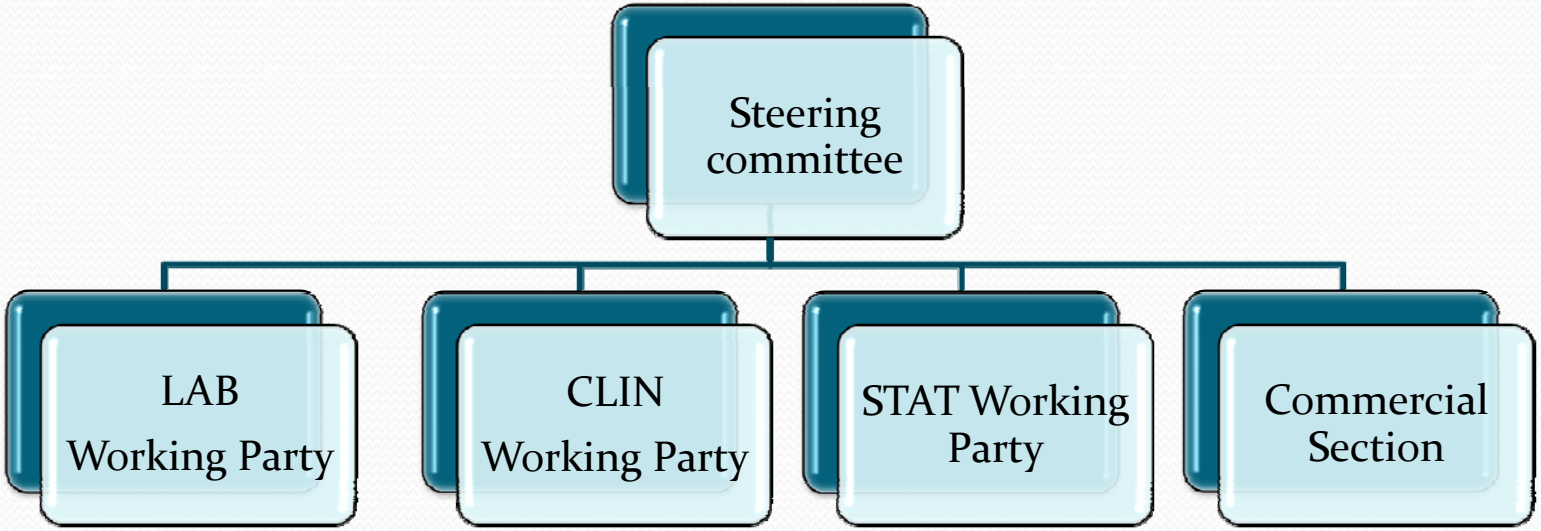
chemotherapy

Interested parties

- 86 participants
- 69 centres
- 24 countries



Structure of the EAPCRI

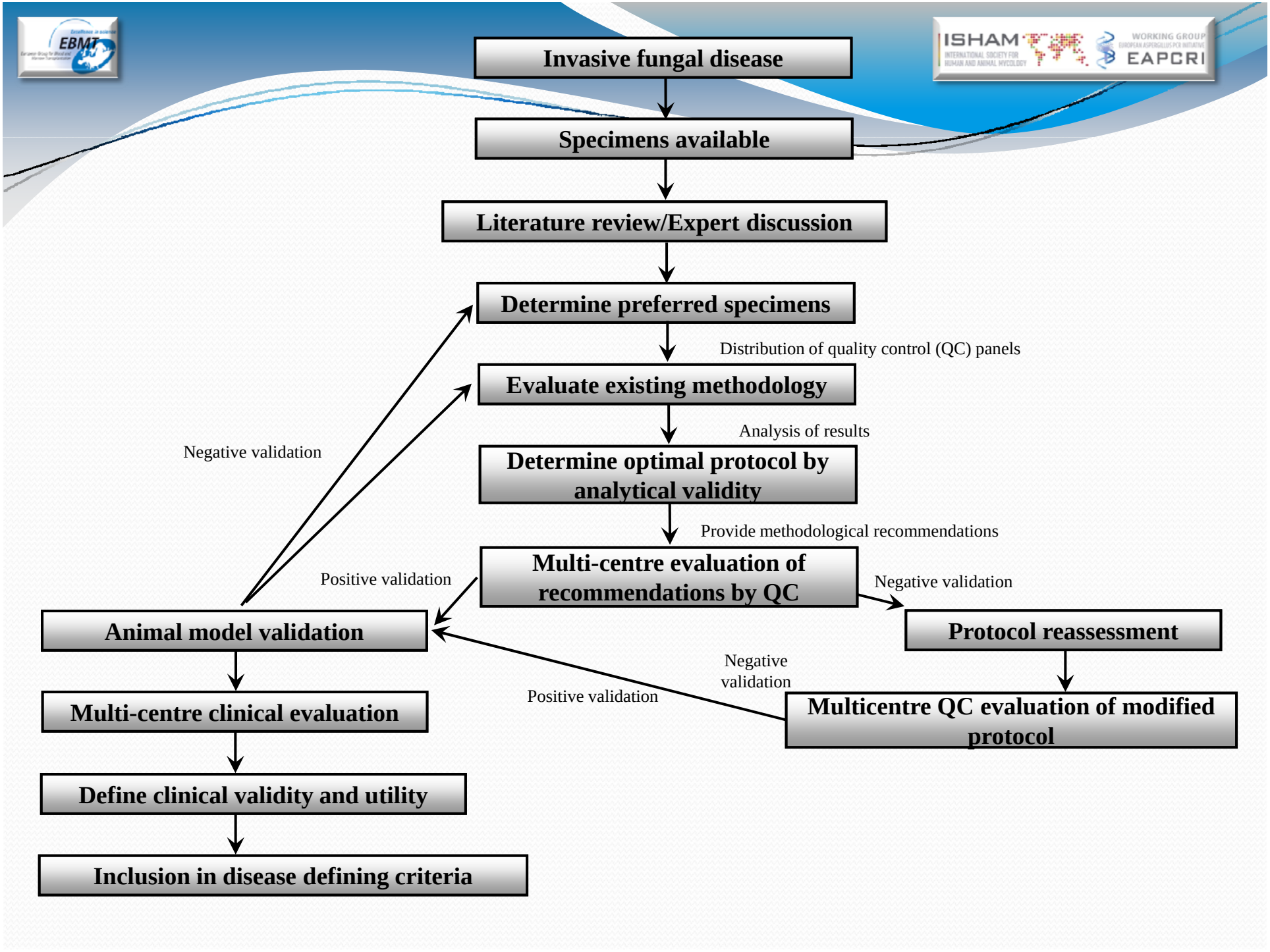


EAPCRI Objective

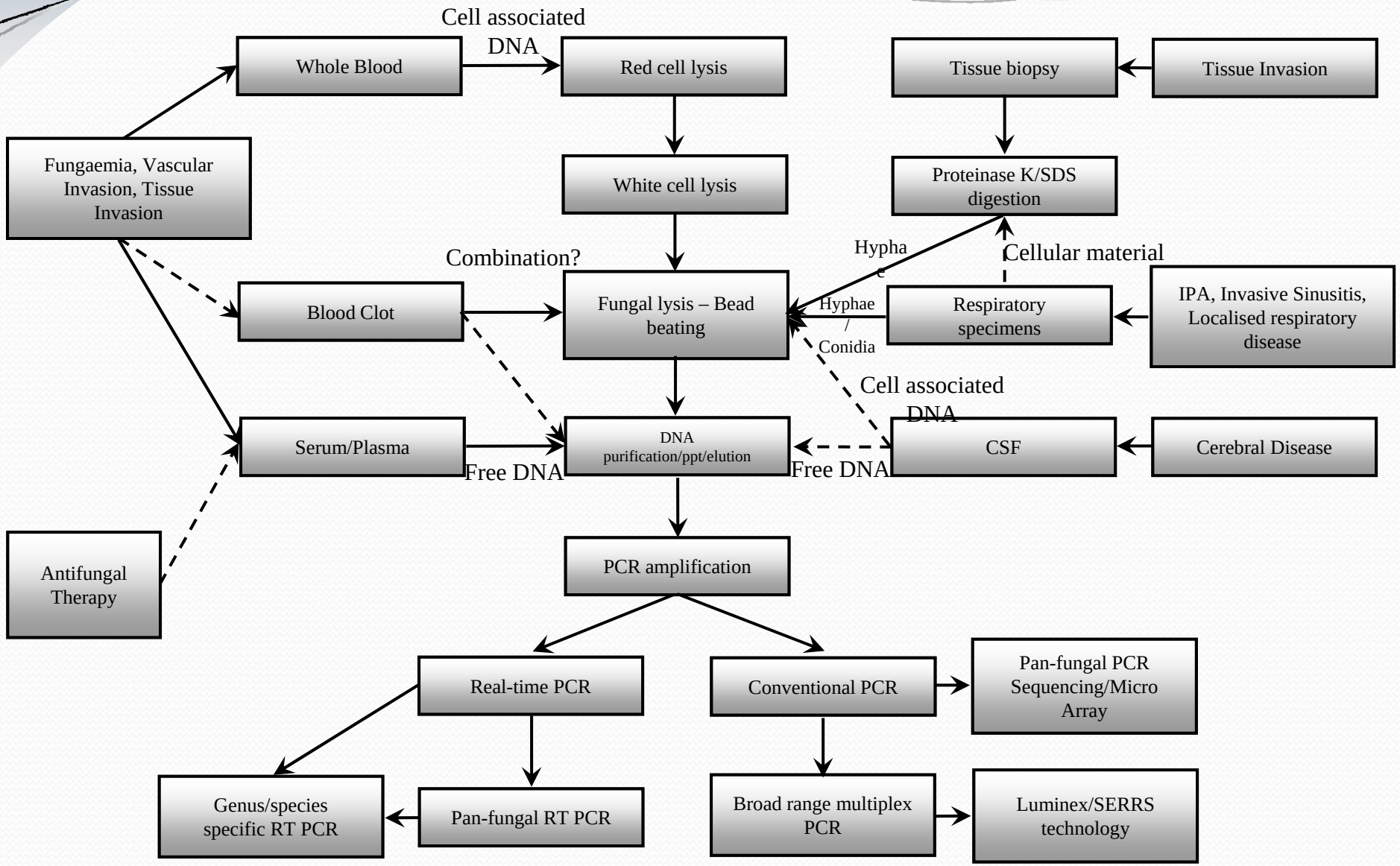
- Provide optimal methodology for inclusion in a multi-centre clinical trail to evaluate the performance and impact of PCR diagnosis
- Lead to inclusion in future consensus criteria for defining disease
- Improve the diagnosis of IA

Aim

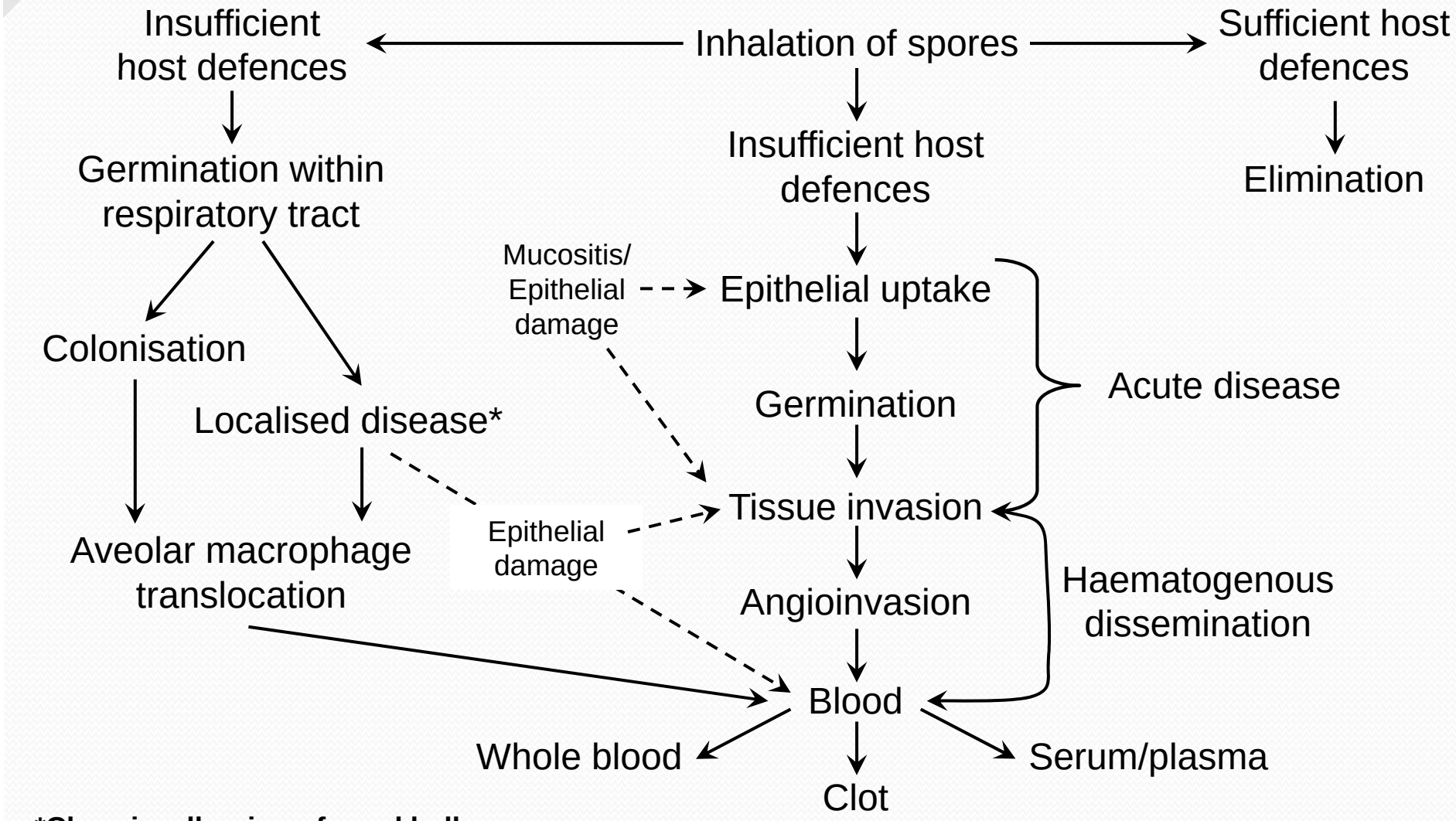
- To define a standard for - not a standard - PCR for Aspergillus in blood specimens


Invasive fungal disease
Specimens available
Literature review/Expert discussion
Determine preferred specimens
Distribution of quality control (QC) panels
Evaluate existing methodology
Analysis of results
Determine optimal protocol by analytical validity
Provide methodological recommendations
Multi-centre evaluation of recommendations by QC
Negative validation
Positive validation
Negative validation
Protocol reassessment
Animal model validation
Negative validation
Positive validation
Multicentre QC evaluation of modified protocol
Multi-centre clinical evaluation
Define clinical validity and utility
Inclusion in disease defining criteria

Options for *Aspergillus* PCR



Principles behind testing blood – inhalation



***Chronic, allergic or fungal ball**

Format for EAPCRI evaluation

- Distribution of QC panels
- Evaluate PCR amplification alone
- Evaluate DNA extraction in combination with PCR amplification
- Identify common parameters that enhance performance

Results from 1st distribution

- DNA panel
  - 85.7% of centres achieved required threshold (equivalent to DNA extracted from 50 *A. fumigatus* conidia
- Whole blood panel
  - Sensitivity: 64.5% (95% CI: 49.0-77.5)
  - Specificity: 89.6% (95% CI: 79.1 – 95.2)
  - DOR: 15.1 (95% CI: 4.4 – 52.2)
  - Only 50% of centres achieving PCR threshold maintained this cut-off when extracted DNA from WB
- Centres maintaining threshold used entire specimen and bead-beating

2nd Distribution – with recommendations

Protocol	sensitivity	95% CI	specificity	95% CI	DOR	95% CI
All	80.6%	68.2 – 88.9	86.3%	76.1 – 92.6	39.8	12.4 – 127.3
Compliant	88.7%	79.8 – 94.0	91.6%	79.1 – 96.9	119.9	44.9 – 319.9
Non-compliant	57.6%	37.9 – 75.2	77.2%	61.2 – 87.9	8.9	1.7 – 45.5

Bivariate meta-regression analysis between logit sensitivity and the additional covariates

	All centres		Centres with 100% specificity	
logit sensitivity	t	P	t	P
Optimal protocol	2.98	0.008	2.69	0.018
Blood volume used	-	NS	-	NS
RCLB	-	NS	-	NS
WCLB	-	NS	-	NS
NaOH	-	NS	-	NS
Beads	2.65	0.016	2.59	0.023
Purification	-	NS	-	NS
Manual Steps >9	-	NS	-	NS
ITS	-	NS	-	NS
18S	-3.56	0.002	-	NS
28S	-	NS	-	NS
Internal control	2.79	0.012	2.85	0.015
Elution volume	-	NS	-	NS

Multivariate meta-regression analysis between logit sensitivity and the additional covariates

	Centres with 100% specificity	
logit sensitivity	t	P
Optimal protocol	-	N.S
Blood volume used	-	NS
RCLB	-	NS
WCLB	2.95	0.018
NaOH	-	NS
Beads	2.57	0.033
Purification	-	NS
Manual Steps >9	-	NS
ITS	-	NS
18S	-	NS
28S	-	NS
Internal control	2.25	0.054
Elution volume	-2.53	0.035

Invasive fungal disease

Specimens available

Literature review/Expert discussion

Determine preferred specimens

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Evaluate existing methodology

Analysis of results

Determine optimal protocol by analytical validity

Provide methodological recommendations

Multi-centre evaluation of recommendations by QC

Animal model validation

Multi-centre clinical evaluation

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The current EAPCRI recommendations are:

All recommendations apply to EDTA whole blood.

1. A minimum of 3 ml of blood needs to be extracted
2. Bead-beating is required for lysis of fungal cells
3. A real time PCR platform using a multi-copy target and species / genus-specific hybridization probes
4. Analysis of all specimens in duplicate, if discrepancy occurs, repeat on identical DNA extract
5. An Internal control PCR is essential (preferably non-human)
6. The use of a negative control for DNA extraction and PCR assay is required
7. Elution volume <100µl
8. EDTA is the only anticoagulant to be used, sodium citrate and heparin should not be used
9. Some commercial products have been linked with fungal contamination. All batches of reagents should be screened for possible contamination prior to use

Acknowledgements

The EAPCRI LWP:

- Juergen Loeffler (Chair), Wuerzburg
- Stephane Bretagne, Paris
- Niklas Finnström, Cepheid, Toulouse
- Willem Melchers, Nijmegen
- Lewis White, Cardiff
- Lena Klingspor, Stockholm
- Elaine Mc Culloch, Glasgow
- Bettina Schulz, Berlin

- Website: www.eapcri.eu