FACS TECHNOLOGY AND ANTIBODY DETECTION ASSAYS IN TODAY'S AND TOMORROW'S MICROBIOLOGY LABORATORY

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DECLARATION OF POTENTIAL CONFLICTS OF INTEREST

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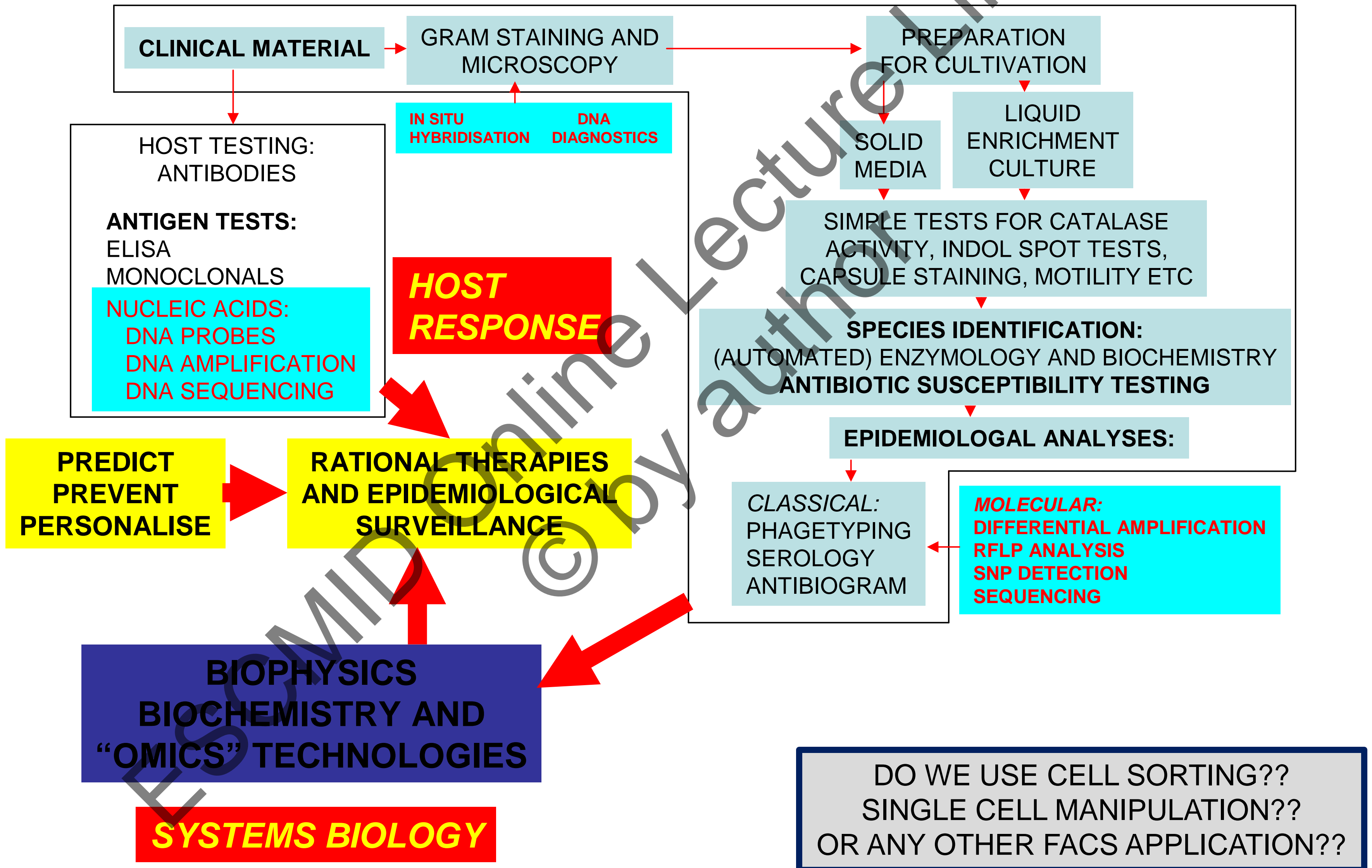
CLINICAL MICROBIOLOGY: THE OVERALL PICTURE



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FACS DEFINITION

Flow cytometry (abbreviated: **FCM**) is a technique for counting and examining microscopic particles, such as cells and chromosomes, by suspending them in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of up to thousands of particles per second. Flow cytometry is routinely used in the diagnosis of health disorders, especially blood cancers, but has many other applications in both research and clinical practice. A common variation is to physically sort particles based on their properties, so as to purify populations of interest.

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WikipediA

GOLD STANDARD MICROBIOLOGY TECHNOLOGY

GROWTH OF COLONIES ON A NUTRIENT MEDIUM
DURING A PERIOD OF INCUBATION

95% OF ALL CULTIVATED AND PUBLISHED SPECIES
BELONG TO 5 OF 53 RECOGNISED BACTERIAL PHYLA

CRYPTOBIOSIS, DORMANCY, MORIBUND, LATENT,
VIABLE BUT NON-CULTIVABLE

1% OR LESS OF ALL MICROSCOPICALLY OBSERVABLE ORGANISMS
ARE SCORED AS VIABLE BY PLATE COUNTING METHODS

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METHODS FOR DEFINING BACTERIAL VIABILITY

Method	Speed	No. of cells analyzed	Ease of use	Typical costs (excluding labor)
Plate counting	Preparation of dilutions and plating take minutes. Hundreds of plates can be prepared per day. Incubation of plates for 1 to 7 days typical before results are obtained.	Viable counts are typically based on plates with 30 to 300 cells.	Minimal training required in aseptic technique and safe handling of microbes.	Plastic consumables and media components. Incubation at growth temp.
Microscopy	Dilution (if necessary) and staining take minutes. Some stains may require incubation of, e.g., 10 to 30 min. Manual microscopic analysis may take several minutes per sample. A hundred samples could conceivably be processed in a day. Results are obtained immediately.	Typically 100 to 500 cells per sample are scored as viable or dead. Image analysis can be used to automate the process of identifying and scoring viable/dead cells.	Minimal training in safe handling of microbes and stains (some of which are carcinogenic).	Microscope slides and coverslips. Stains. Cost of purchasing and maintaining microscope or fluorescence microscope.
Flow cytometry	Dilution (if necessary) and staining take minutes. Some stains may require incubation of, e.g., 10 to 30 min. Manual sample presentation may take several minutes per sample. Automated samplers can be loaded with, e.g., a 96-well plate of samples. Hundreds of samples can be processed in a day. Results are obtained immediately, although postacquisition analysis of data is common.	Typically 10,000 to 100,000 cells per sample are analyzed. As stain uptake is quantified, intermediate results between live and dead are possible.	In addition to the above, training is needed in operation and quality control of flow cytometer. Experience required for protocol development and data analysis.	Sample tubes and stains. Costs of purchasing and maintaining flow cytometer.

MONOCLONAL ANTIBODIES, FLUORESCENT COMPOUNDS, SPECTRALLY DISTINCT PROBES

Hazel Davey, AEM Aug 2011

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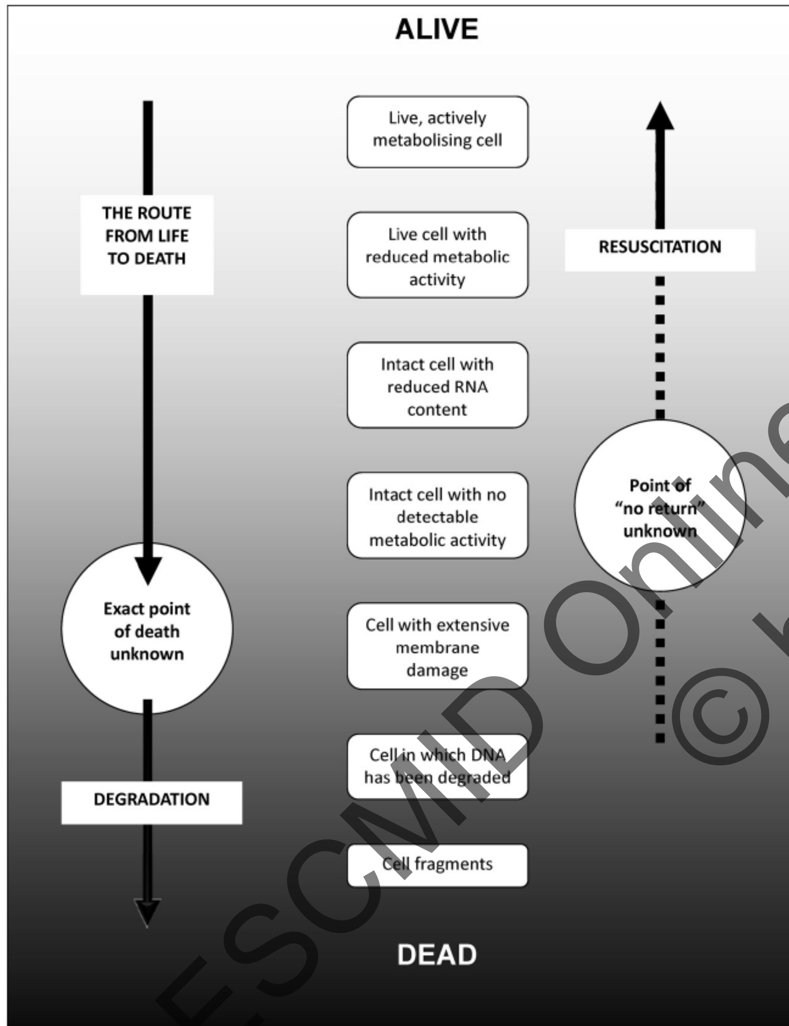
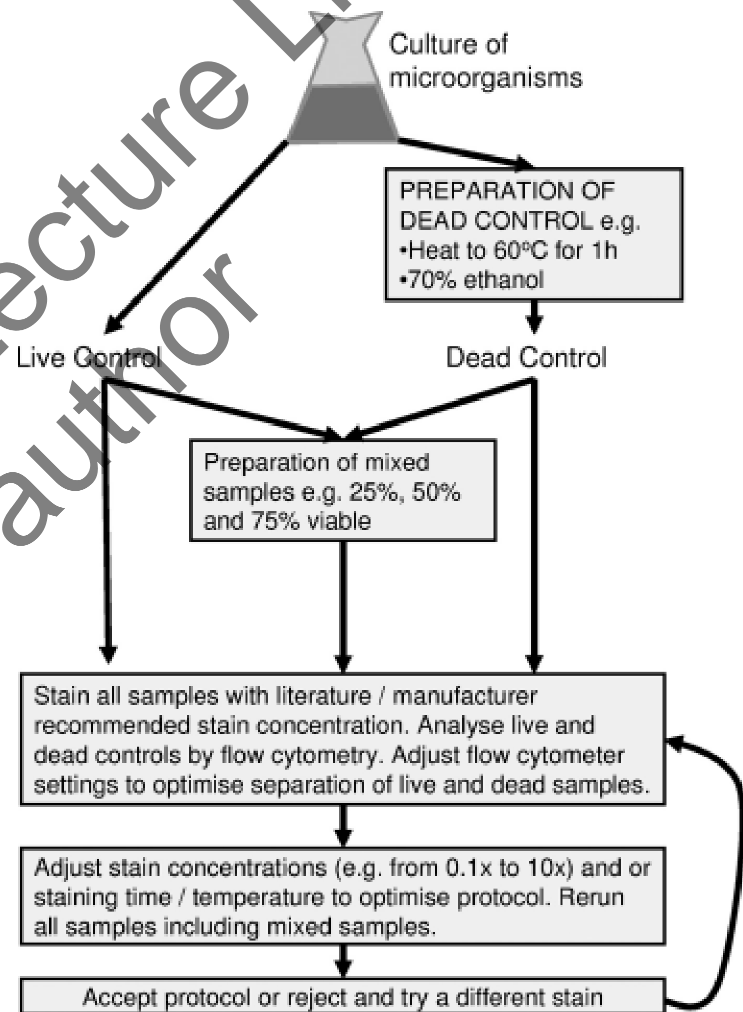
BACTERIAL VIABILITY TRAITS AND FACS MEDIATED ASSESSMENT





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Hazel Davey, AEM Aug 2011

Applied and Environmental Microbiology, Aug. 2005, p. 4679–4689 Vol. 71, No. 8
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.8.4679–4689.2005
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Genetic Diversity of Viable, Injured, and Dead Fecal Bacteria Assessed by Fluorescence-Activated Cell Sorting and 16S rRNA Gene Analysis

Kaouther Ben-Amor,[1,2]* Hans Heilig,[1] Hauke Smidt,[1] Elaine E. Vaughan,[1]
Tjakko Abee,[2,3] and Willem M. de Vos[1,3]

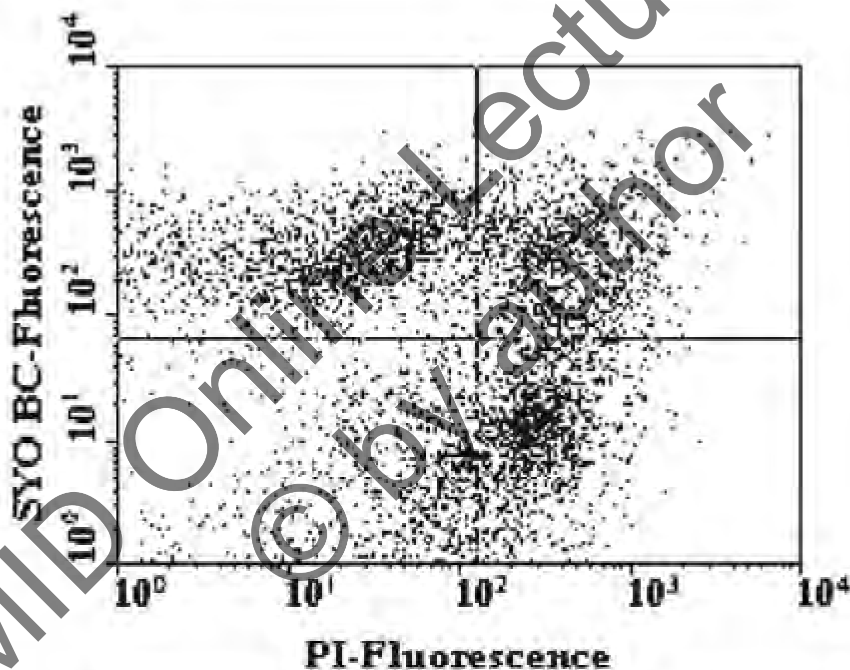

FIG. 1. FCM analysis of a fecal sample stained with SYTO BC and PI. The two-color dot plot discriminated between SYTO BC-stained viable cells (upper left quadrant), double-stained injured cells (upper right quadrant), PI-stained dead cells (lower right quadrant), and STYO BC- and PI-stained injured cells. Results were obtained with the FACSCalibur.

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MICROBIOLOGICAL FACS STATUS REPORT

"While flow cytometry holds many advantages and exciting opportunities for the microbiologist, it has not yet become as widely used as this potential deserves."

Hazel Davey, AEM Aug 2011

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CURRENT DEVELOPMENTS IN OLD FASHIONED STUFF: ELISA VERSUS LUMINEX TECHNOLOGY

Sulfo-NHS
EDC
MES (coupling buffer)

bead
COOH
HOOC
COOH
NH_2
protein

The fluorescently-colored beads are resuspended in NaH_2PO_4 (activation buffer)

Protein is added. Incubation for 2 h.

Protein binds to beads

After washing, beads are counted and stored in the dark at 4°C

protein
bead

Biomolecules are coupled to different fluorescently-colored beads and the beads are mixed in one well

Serum is added and antigen-specific antibodies will bind to the proteins

A secondary antibody, labeled with a fluorescent dye, is introduced

96-wells plate is ready for analysis

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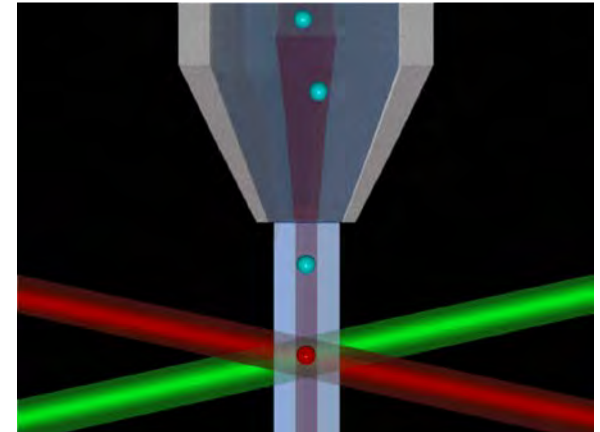
LUMINEX TECHNOLOGY

- Assays are read using a compact analyser:

  Luminex 100 with IS 2.3 software

- The analyser samples the well
- Beads pass rapidly through two lasers
- Lasers excite fluorescent dyes
  - Red laser - bead classification
  - Green laser - assay result
- Results are reported in Median Fluorescence Intensity (MFI)



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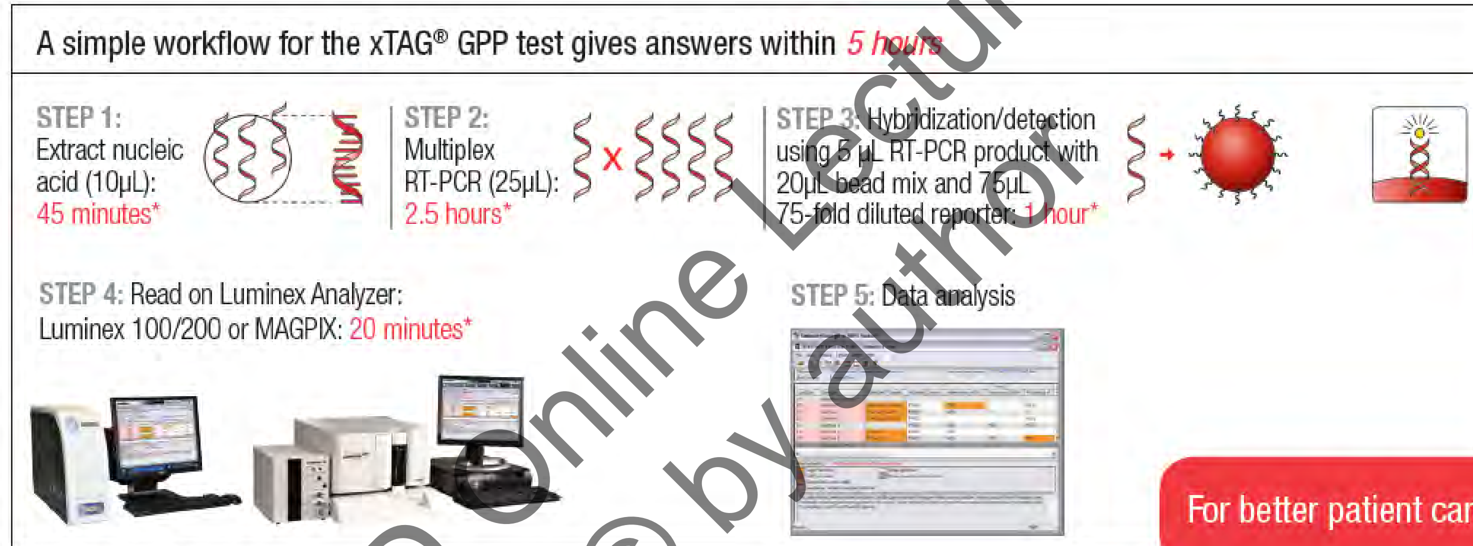
ALSO FOR NUCLEIC ACID APPLICATIONS



*Time estimates for 24 tests

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Highly multiplexed
Cost effective
Innovative

TEST DEVELOPMENT

Eur J Clin Microbiol Infect Dis (2011) 30:521–526
DOI 10.1007/s10096-010-1113-x

ARTICLE

Development of a multiplexed bead-based immunoassay for the simultaneous detection of antibodies to 17 pneumococcal proteins

S. Shoma · N. J. Verkaik · C. P. de Vogel · P. W. M. Hermans · S. van Selm · T. J. Mitchell · M. van Roosmalen · S. Hossain · M. Rahman · H. Ph. Endtz · W. J. B. van Wamel · A. van Belkum

The MFI values obtained for HPS with the multiplex assay were between 82% and 111% (median 94%) of those obtained with the singleplex assays. Therefore, it was considered to be legitimate to use the multiplex assay.

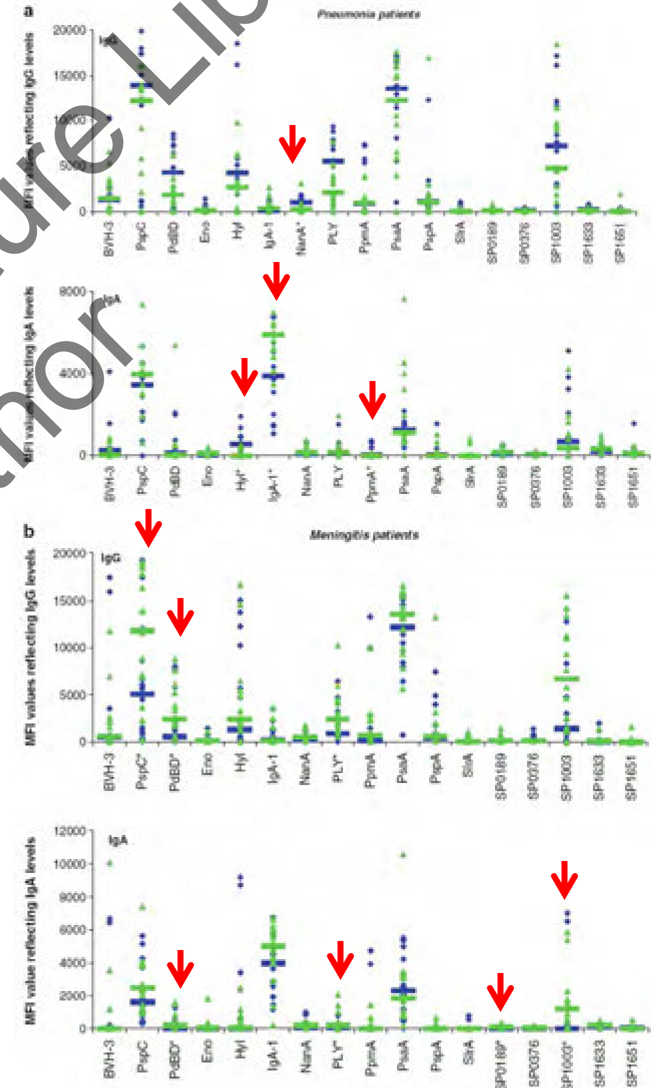

Fig. 1 Median fluorescence intensity (MFI) values reflecting levels of antigen-specific IgG and IgA for 17 *Streptococcus pneumoniae* antigens in the serum samples of pneumonia (**a**) and meningitis patients (**b**). The *blue diamonds* represent pneumonia/meningitis patients caused by *S. pneumoniae* and the *green triangles* represent pneumonia/meningitis patients that are positive for bacterial species other than *S. pneumoniae*. The *horizontal bars* indicate the median levels of anti-pneumococcal antibodies for these two groups. The *asterisks* indicate significant differences

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HYPOTHESES AND RESEARCH QUESTIONS RELATING TO *S. AUREUS* CARRIAGE

1. Is there a difference in antibody levels to different *S. aureus* antigens in serum of carriers and non-carriers?
2. Is there a correlation between antibody levels in serum and antibody levels in nasal secretion?
3. Are anti-staphylococcal antibody levels in serum stable over time?
4. Age related differences among humans?
5. What is the effect of infection on antibody levels and specificity?
6. Are there differences in the humoral responses between patients with different types of infections?
7. Are antibodies protective?
8. Etc etc etc etc …..

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SERUM SAMPLES, NASAL SECRETIONS AND NASAL SWABS

- Venous blood and at least three consecutive nasal swabs were collected from healthy volunteers or patients.
- A second blood sample was drawn from a certain number of volunteers after half a year and carrier state was re-determined.
- Nasal secretions were collected and processed from multiple volunteers.

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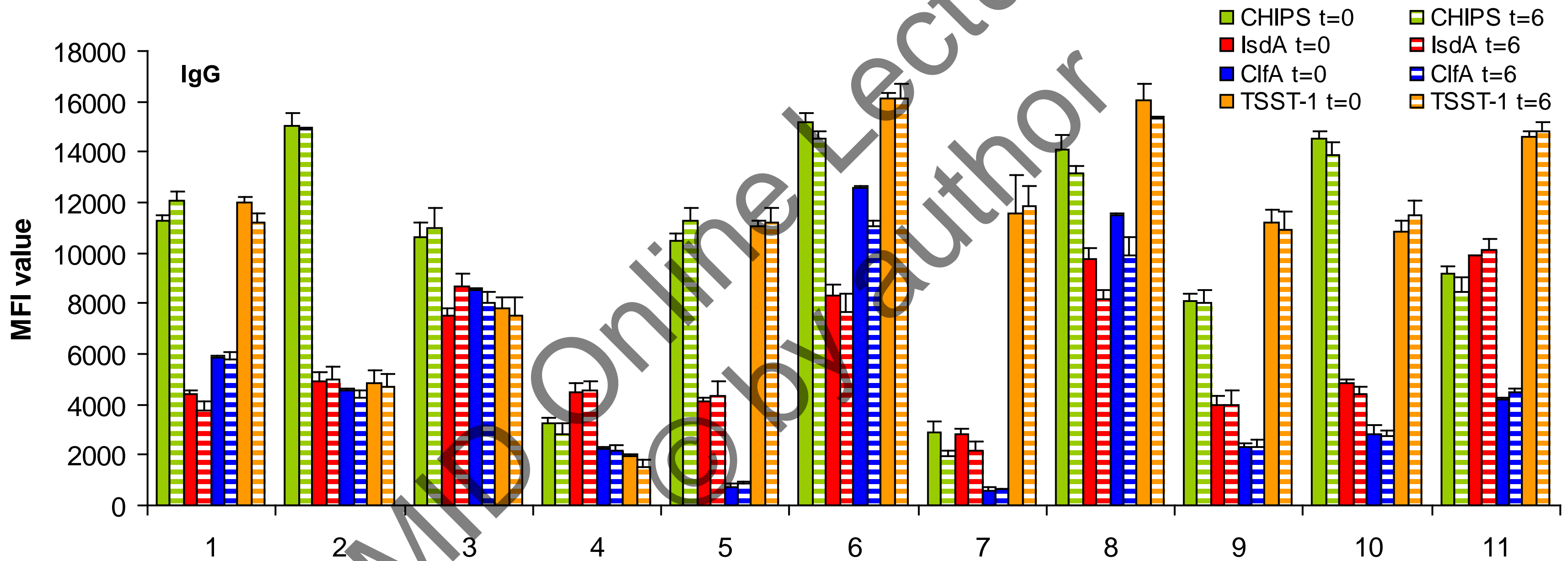
STABILITY OF ANTIBODY LEVELS OVER TIME



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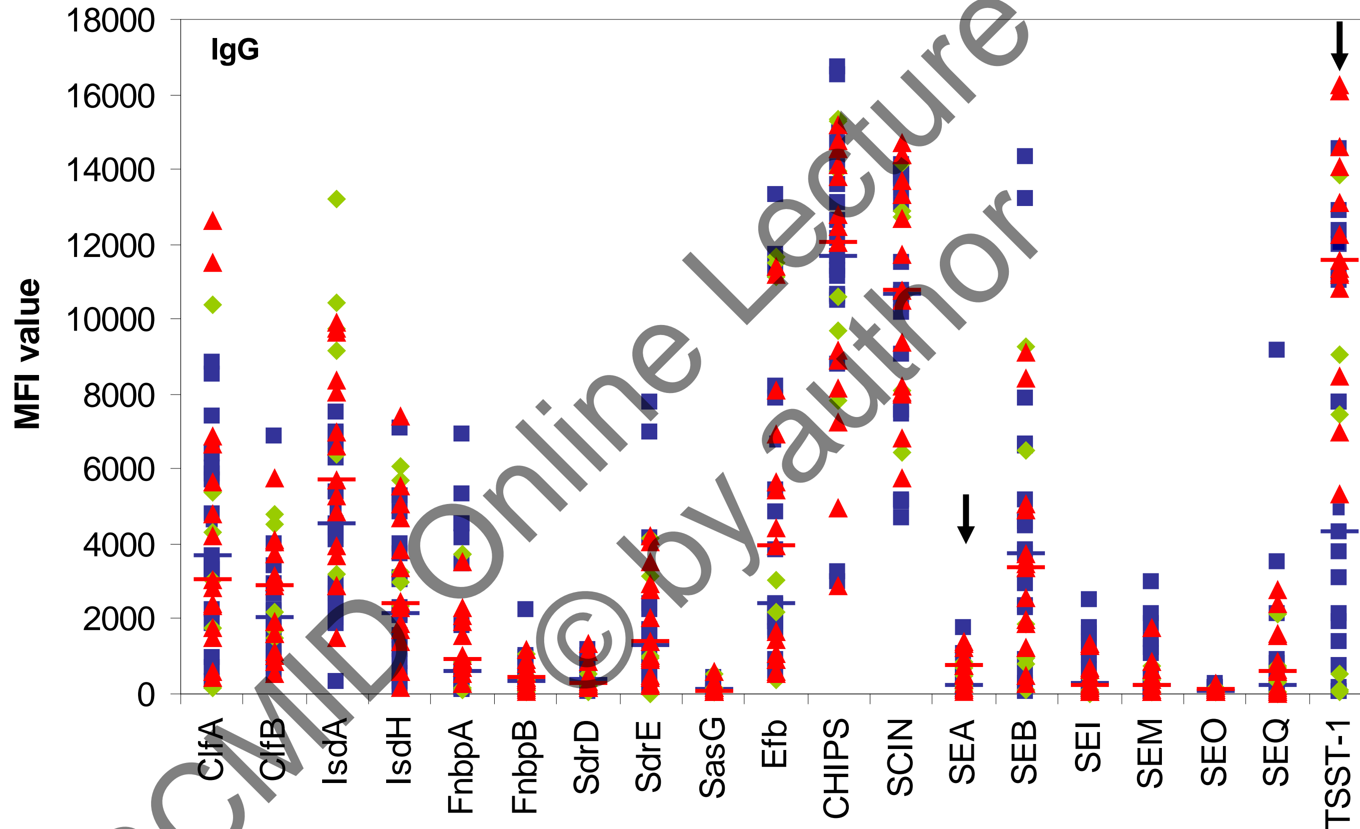
IgG LEVELS IN (NON-)CARRIERS



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Reclassification of *Staphylococcus aureus* Nasal Carriage Types

Alex van Belkum,[1] Nelianne J. Verkaik,[1] Corné P. de Vogel,[1] Hélène A. Boelens,[1] Jeroen Verveer,[1] Jan L. Nouwen,[1] Henri A. Verbrugh,[1] and Heiman F. L. Wertheim[1,2]

[1]Department of Medical Microbiology and Infectious Diseases, Erasmus MC, Rotterdam, The Netherlands; and [2]Oxford University Clinical Research Unit, National Institute for Infectious Tropical Diseases, Bach Mai Hospital, Hanoi, Vietnam

Background. Persistent nasal carriers have an increased risk of *Staphylococcus aureus* infection, whereas intermittent carriers and noncarriers share the same low risk. This study was performed to provide additional insight into staphylococcal carriage types.

Methods. Fifty-one volunteers who had been decolonized with mupirocin treatment and whose carriage state was known were colonized artificially with a mixture of *S. aureus* strains, and intranasal survival of *S. aureus* was compared between carriage groups. Antistaphylococcal antibody levels were also compared among 83 carriage-classified volunteers.

Results. Persistent carriers preferentially reselected their autologous strain from the inoculum mixture ($P = .02$). They could be distinguished from intermittent carriers and noncarriers on the basis of the duration of postinoculation carriage (154 vs. 14 and 4 days, respectively; $P = .017$, by log-rank test). Cultures of swab samples from persistent carriers contained significantly more colony-forming units per sample than did cultures of swab samples from intermittent carriers and noncarriers ($P = .004$). Analysis of serum samples showed that levels of immunoglobulin G and immunoglobulin A to 17 *S. aureus* antigens were equal in intermittent carriers and noncarriers but not in persistent carriers.

Conclusions. Along with the previously described low risk of infection, intermittent carriers and noncarriers share similar *S. aureus* nasal elimination kinetics and antistaphylococcal antibody profiles. This implies a paradigm shift; apparently, there are only 2 types of nasal carriers: persistent carriers and others. This knowledge may increase our understanding of susceptibility to *S. aureus* infection.

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FACS BASED ANTIBODY DETECTION

1. Easy to develop and validate.
2. Small volume of serum (or other samples) required.
3. Suited for bacterial, viral, fungal and parasitic antibody assessment.
4. (Semi-)Quantitative.
5. Highly multiplex.
6. Also suited for DNA typing.
7. Reproducible.

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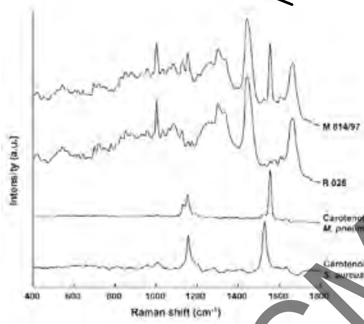
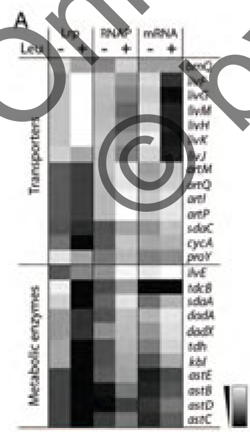
IMMEDIATE DIAGNOSTIC OBJECTIVES

ANTIMICROBIAL SUSCEPTIBILITY TESTING





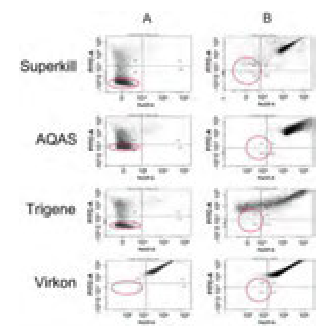


TABLE 1. Reproducibility of select NMR resonances for five bacterial genera

Strain no.	Resonance (ppm) at indicated location for:									
	E. coli		*S. aureus*		*K. pneumoniae*		*P. aeruginosa*		*E. faecalis*	
	A	B	A	B	A	B	A	B	A	B
1	0.96	1.48	3.01	3.27	1.32	3.26	2.36	3.27	1.48	2.36
2	0.96	1.48	3.04	3.27	1.32	3.26	2.35	3.27	1.48	2.34
3	0.96	1.47	3.03	3.27	1.32	3.26	2.36	3.27	1.47	2.36
4	0.97	1.48	3.03	3.27	1.32	3.26	2.38	3.27	1.47	2.36
5	0.97	1.48	3.03	3.27	1.32	3.26	2.36	3.27	1.47	2.36
6	0.96	1.47	3.03	3.27	1.32	3.26	2.36	3.27	1.48	2.36
7	0.96	1.47	3.03	3.27	1.32	3.26	2.36	3.27	1.48	2.34
8	0.97	1.48	3.03	3.27	1.32	3.26	2.36	3.27	1.47	2.35
9	0.96	1.47	3.03	3.27	1.32	3.26	2.36	3.27	1.47	2.36
10	0.96	1.47	3.03	3.27	1.32	3.26	2.34	3.27	1.48	2.36

And: PCR, MS, viability markers, stringent response, glycomics, lipomics, etc

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SOME FINAL CONSIDERATIONS

- Nucleic acid diagnostics is there to stay and is (slowly) getting its (much deserved) clinical recognition.
- Molecular markers for human infection susceptibility have been identified and appropriate use is under consideration.
- Mass spectrometry is now revolutionising microbial identification but needs optimisation in order to be compatible with clinical specimens.
- Biophysics also comprises Raman and other vibrational spectroscopies, single cell manipulation, metabolomics etc!!!!!!!!!!
- Detection of human antibodies and microbial antigens in highly multiplexed fashions is possible.
- Laboratory automation is just beginning.
- Data flow, data flow, data quantities, data quantities and …..
- WGS (Whatever Generation Sequencing).
- PHENOTYPE VERSUS GENOTYPE VERSUS SPECTRAL TYPE

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