HELSINKI UNIVERSITY OF TECHNOLOGY
Department of Electrical and Communications Engineering
Master's Programme in Electrical Engineering

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HYGIENE MONITORING BIOSENSING SYSTEMS IN HOSPITAL ENVIRONMENTS

Master's Thesis
Laboratory of Computational Engineering

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ABSTRACT OF THE MASTER'S THESIS

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This thesis concerns the monitoring of biogenic impurities and hygiene in hospital environments by means of biosensing systems. The objective is to find biosensing systems applicable in hospital environments and able to identify pathogens that cause nosocomial infections, on surfaces, in the air and water.

In the first part of the thesis, nosocomial infections were investigated, along with their prevalence, mortality, financial aspects and the most common microorganisms causing them. Furthermore, existing national and international nosocomial infection surveillance programmes, as well as organisations and journals associated with this field, were pinned down. Collaboration with the Meilahti Helsinki University Central Hospital enabled examination of their nosocomial infection status and familiarisation with their current prevention practices. The most important is good hand hygiene, trailed by, among others, pressurisation and filtration of air; limited use of tap water; and good overall cleanliness. Products that monitor or test the hygiene level are not in routine use. Three areas, where biosensing systems could be of use in a hospital, were identified. These are the ventilation system, the water distribution system and cleaning.

The second part of the thesis discusses biosensing systems. Various methods were mapped in an overview of this field, comprising electrochemical, optical, microelectromechanical, nucleic acid- and cell-based systems. Based on this, the most promising methods – laser- and light-emitting diode-induced fluorescence and biocavity lasing – were explored further. These have the potential to detect microbes eminently fast, even in real time. Use of the fluorescence method to verify the functioning of filters in the ventilation system has been evaluated, as well as the biocavity laser's potential to validate the purity of water and the cleanliness of surfaces in conjunction with cleaning. Rapid biosensing systems based on the aforementioned novel methods are also applicable to, among others, flow cytometry devices used for gene sequencing.

Keywords: biosensing systems, hygiene monitoring, biosensor, nosocomial infection, hospital, microbe, detection
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Detta diplomarbete behandlar övervakningen av biogena orenheter och hygienen i sjukhusmiljöer med hjälp av biosensorsystem. Målet är att finna biosensorsystem, som är lämpliga i sjukhusmiljöer och har förmågan att identifiera patogena organismer, som orsakar sjukhusinfektioner, på ytor, i luften eller i vatten.

I första delen av arbetet undersöktes sjukhusinfektionernas förekomst, dödlighet och ekonomiska aspekter, samt de vanligaste mikroorganismerna som förorsakar sjukhusinfektioner. Dessutom klarlades vad det finns för nationella och internationella övervakningsprogram av sjukhusinfektioner, samt organisationer och tidskrifter som berör detta ämnesområde. Samarbete med Helsingfors Universitets Centralsjukhus i Mejlans möjliggjorde undersökning av sjukhusinfektionernas situation på sjukhuset och de nuvarande förhindrande åtgärderna. Den viktigaste åtgärden är god handhygien, efterföljt av bl.a. filtrering av luft och tryckreglering; begränsad användning av kranvatten; och allmän renlighet. Produkter som övervakar eller testar hygiennivån används inte rutinmässigt. Biosensorsystem kunde användas i tre områden i ett sjukhus. Dessa är ventilationssystemet, vattenledningsnätet och städningen.

Arbetets andra del behandlar biosensorsystem. En kartläggning av områdets olika metoder gjordes. Den överblickar elektrokemiska, optiska, mikroelektromekaniska, nukleinsyre- och cellbaserade system. På basis av detta utforskades de mest lovande metoderna vidare, nämligen laser- och lysdiod-inducerad fluorescens samt en biokavitetlaser. Dessa har potential att detektera mikrober ytterst snabbt, t.o.m. i realtid. Fluorescensmetodens tillämpning för att verifiera funktionsdugligheten hos ventilationssystemets filter har utvärderats, likaså biokavitetlaserns potential att bekräfta renheten av vatten och ytor i samband med städning. Snabba biosensorsystem som baserar sig på de förutnämnda nya metoderna kan också tillämpas i bl.a. flödescytometriska apparater som används till gensekvensering.

Nyckelord: biosensorsystem, hygienövervakning, biosensor sjukhusinfektion, sjukhus, mikrob, detektion
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Acronyms and Abbreviations

AJIC	American Journal of Infection Control
APIC	Association for Professionals in Infection Control and Epidemiology, Inc.
CDC	Centers for Disease Control and Prevention
EARSS	European Antimicrobial Resistance Surveillance System
ECCMID	European Congress of Clinical Microbiology and Infectious Diseases
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
EU	European Union
FDA	Food and Drug Administration
HELICS	Hospital in Europe Link for Infection Control through Surveillance
HICPAC	Healthcare Infection Control Practices Advisory Committee
HIS	The Hospital Infection Society
HUCH	Helsinki University Central Hospital
HUS	Helsingin ja Uudenmaan sairaanhoitopiiri
IDSA	Infectious Diseases Society of America
MIT	Massachusetts Institute of Technology
MMWR	Morbidity and Mortality Weekly Report
NASA	National Aeronautics and Space Administration
NHS	National Health Service
NNii	National Network of Immunization Information
NNIS	National Nosocomial Infections Surveillance
SaHTi	Sairaalahygienian Tiedotuslehti
SHEA	The Society for Healthcare Epidemiology of America
SIRO	Sairaalainfektio-ohjelma
SSHY	Suomen Sairaalahygieniayhdistys ry
U.K.	United Kingdom
U.S.	United States
WTEC	World Technology Evaluation Center, Inc.

AC	alternating current
ACH	air changes/hour
AIDS	Acquired Immune Deficiency Syndrome
AMP	adenosine monophosphate
AMV	Avian Myeloblastosis Virus
ATP	adenosine triphosphate
BAWS	biological agent warning sensor
BioMEMS	biological microelectromechanical systems
BSI	bloodstream infection
cDNA	complementary DNA
CoNS	coagulase-negative staphylococci
CW	continuous wave
DC	direct current
DdRp	DNA dependent RNA polymerase
DPA	dipicolinic acid
cfu	colony-forming unit
DNA	deoxyribonucleic acid
ECL	electrochemiluminescence
EM	electron micrograph
ESBL	extended spectrum beta-lactamase
FLAPS	fluorescent aerodynamic particle sizer
FTIR	Fourier transform infrared
HEPA	high efficiency particulate air
HIV	Human Immunodeficiency Virus
HPV	hydrogen peroxide vapour
ICU	intensive care unit
IR	infrared
ISE	ion-selective electrode
LD_{50}	lethal dose sufficient to kill 50% of persons exposed
LED	light-emitting diode
LIF	laser-induced fluorescence
LOD	level of detection
M	molarity (mol/l)
MEF	metal-enhanced fluorescence
MEMS	microelectromechanical systems
MRSA	methicillin-resistant *Staphylococcus aureus*
NADH	reduced nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NASBA	nucleic acid sequence-based amplification

PCR	polymerase chain reaction
PMT	photomultiplier tube
PP_i	the anion of pyrophosphoric acid; $P_2O_7^{4-}$
ppm	parts per million
QCM	quartz crystal microbalance
QD	quantum dot
RNA	ribonucleic acid
RSV	Respiratory Syncytial Virus
RT	reverse transcriptase
RT-PCR	reverse transcription polymerase chain reaction
SAW	surface acoustic wave
SERS	surface-enhanced Raman scattering
SPFA	single particle fluorescence analyzer
spp	species
SPR	surface plasmon resonance
TIR	total internal reflection
TM	transverse magnetic
TRPA	tobramycin-resistant *Pseudomonas aeruginosa*
TVO	total viable organisms
UTI	urinary tract infection
UV	ultraviolet
UV-APS	ultraviolet aerodynamic particle sizer
UVDL	ultraviolet diode laser
VAT	value added tax
VCSEL	vertical-cavity surface-emitting laser
VISA	vancomycin-intermediate *Staphylococcus aureus*
VRE	vancomycin-resistant *Enterococcus*
VRSA	vancomycin-resistant *Staphylococcus aureus*

Chapter 1

Introduction

> ***Jeffrey Goines:*** *You know what crazy is? Crazy is majority rules. Take germs for example.*
> ***James Cole:*** *Germs?*
> ***Jeffrey Goines:*** *Uh-huh. Eighteenth century, no such thing, nada, nothing. No one ever imagined such a thing. No sane person. Along comes this doctor, uh, Semmelweis, Semmelweis. Semmelweis comes along. He's trying to convince people, other doctors mainly, that there's these teeny tiny invisible bad things called germs that get into your body and make you sick. He's trying to get doctors to wash their hands. What is this guy? Crazy? Teeny, tiny, invisible? What do they call it? Uh-uh, germs? Huh? What? Now, up to the 20th century, last week, as a matter of fact, before I got dragged into this hellhole. I go in to order a burger at this fast food joint, and the guy drops it on the floor. James, he picks it up, he wipes it off, he hands it to me like it's all OK. "What about the germs?" I say. He says, "I don't believe in germs. Germs is a plot made up so they could sell disinfectants and soaps." Now he's crazy, right?*
>
> \- *From the movie* Twelve Monkeys

Medical science has come a long way since the 17th century, when "the Father of Microbiology", the dutch scientist Anton van Leeuwenhoek, discovered bacteria swimming inside a raindrop. At the time, the connection between these newly unearthed microorganisms and disease remained unknown. The common belief was, that disease was spontaneously generated. In the mid-19th century the Hungarian-Austrian physician Ignaz Semmelweis, while working at the Vienna General Hospital, concluded that childbed fever was caused by unknown "cadaveric material", carried from the autopsy room to examined patients on the hands of physicians and nurses. As a consequence, he introduced a hand-washing policy; hospital workers had to wash their hands with a solution of chlorinated lime between autopsy work and patient examination. This lead to a drop in the childbed fever mortality rate from 12.24% to 2.38%. Semmelweis' findings were, however, rejected by the medical community since he lacked scientific evidence to support his theory. It was not until a

few decades later that the germ theory of disease was developed by, among others, the french microbiologist and chemist Louis Pasteur. According to the theory, which is the foundation of modern medicine, microorganisms are the cause of some or all disease. [16]

This steered the combat against disease on a more scientific path, which in 1928 lead to the discovery of the first antibiotic, penicillin, by Alexander Flemming. Unfortunately, it did not take long for the first bacteria to develop antibiotic resistance. Only four years after the breakthrough of Flemming, the first case of penicillin resistant bacteria was reported.

During the following decades, research in new antibiotics was huge. Mortality due to bacterial infection decreased significantly compared to the pre-penicillin era. It was thought that antibiotics had provided the final solution for infectious diseases, as indicated by the U.S. Surgeon General, William H. Stewart's, alleged comment in 1967 and 1969: *"... that we had essentially defeated infectious diseases and could close the book on them [infectious diseases] ... "* [54, 135]. Research and development in novel antibiotics was subsequently de-emphasized. From 1983 to 2001 only 47 new antibiotics were approved by the U.S. Food and Drug Administration (FDA) and the Canada Health Ministry. Between 1998 and early 2005 only nine have been approved [114].

This decrease in novel antibiotics hitting the markets in the last 30 years, and the emergence of multidrug-resistant bacteria, have caused a new health threat. The situation is especially serious in hospitals, where the abundant use of antibiotics creates a selective pressure on the microbes in the environment, causing resistant strains to thrive. These form a serious threat to already weakened patients. In July 2004 the Infectious Diseases Society of America (IDSA) reported that about 2 million people get bacterial infections annually in U.S. hospitals. Of these cases, 90,000 are fatal and approximately 70% involve bacteria resistant to at least one drug [17].

This thesis concerns hygiene monitoring in hospital environments by means of biosensing systems. The objective is to identify how and which biosensing systems can be incorporated into the hospital environment in order to assist in the prevention of nosocomial infections.

Chapter 2 gives the essential background in nosocomial infections and biosensing systems. The prevalence of these infections in Finland and abroad, their financial aspects, the most common microbial culprits and national, as well as international organisations, regulations and journals concerned with the subject are discussed. Furthermore, the chapter will give a general introduction to what biosensing systems are.

In Chapter 3 the current nosocomial infection situation at the Helsinki University Central Hospital (HUCH) in Meilahti is described, as well as the present means of prevention of these infections. Different hospital areas where biosensing systems could be used are identified.

Chapter 4 contains an overview of current biosensing systems. What is presently feasible and which promising technologies are under development are covered in this chapter, all the while keeping the focus on the basic underlying principles.

Various approaches to biosensing systems, which could be employed in the identified hospital areas in Chapter 3, are discussed in Chapter 5.

Finally, concluding remarks are given in Chapter 6.

Chapter 2

Background

2.1 Nosocomial infections

The term *nosocomial infections* (from the Greek word *nosokomeion*, meaning hospital) refers to infections that have neither been confirmed clinically nor through laboratory tests as the patient was admitted to the hospital. This is the definition by the American governmental organisation Centers for Disease Control and Prevention (CDC), and it is likewise the most common definition used in Finland [92, 72]. The synonym *hospital-acquired infection* is also used quite often, however, the more general term *health-care-associated infection* is used more and more frequently, since there is a risk of getting infected after admission to any health-care facility. In this thesis, the terms *nosocomial infection* and *hospital-acquired infection* are used interchangeably.

The CDC's definition is not the only one. It is quite common to establish some time frames around the time of hospital admission to separate hospital- and community-acquired infections. Inweregbu *et al* define nosocomial infections as those occuring within 48 hours of hospital entry, three days of discharge or 30 days after an operation [78]. To further complicate things, different physicians tend to define these infections in fairly differing ways. This makes it difficult to estimate their true prevalence.

2.1.1 Prevalence

Various studies, both national and international, have been conducted to determine nosocomial infection rates.

In a recent prevalence study (February 2005) of nosocomial infections in adult acute wards in Finland, an average of 8.5% (703/8234) of the examined patients had at least one

nosocomial infection. Of these, 10% occured in intensive care units (ICUs). Nosocomial infections occured more frequently among the elderly, patients who had undergone surgery and patients with more serious primary diseases. A total of 30 hospitals participated in the study: five university hospitals, 15 central hospitals and 10 other acute care hospitals [100].

Similar studies in the United Kingdom also indicate that approximately 9% of patients in hospitals suffer nosocomial infections at any one time. In England and Wales at least 100,000 nosocomial infections occur each year [73]. This estimate was considered to be, in all likelihood, an underestimate, because it excluded, among others, teaching hospitals and ICUs, and did not account for infections breaking out after the patients had left the hospital.

In April 1999 a one-week-period prevalence study of nosocomial infections was conducted in 18 acute care hospitals in Switzerland [131]. Besides unravelling the current prevalence, the study also investigated how much hospital size affects the nosocomial infection rates. All adult patients in medical, surgical and intensive care units were included, except patients in dermatology[1], ophthalmology[2], ear-nose-throat, gynecology-obstretics, bone marrow transplant, and burn units, and long-term care wards. Of 4252 patients, 429 suffered from one or more nosocomial infections, i.e. 10.1%. Of all nosocomial infections (470), 29.7% occured in ICUs, 9.3% in medical, 9.2% in surgical and 14.1% in mixed wards. The conditions of patients in larger hospitals were more often fatal than in smaller hospitals, and in addition, they were more frequently afflicted by multiple diseases. The nosocomial infection prevalence rate was highest (10.9%) in large hospitals, followed by 10.0% and 6.1% in intermediate and small hospitals, respectively. The study concluded that smaller hospitals treat the less ill, but not with a reduced nosocomial infection risk compared to intermediate and large hospitals.

In 2003 Erbay *et al* [49] reported on a two-year survey of nosocomial infections in a Turkish university hospital. An adult ICU served as the setting for the study, and a total of 434 patients were included (with the condition that the patient stayed in the ICU more than 48 hours). One hundred and thirteen of these patients were inflicted by hospital-acquired infections, i.e. 26%.

A similar, one-year U.S. retrospective cohort study of patients admitted to the medical and surgical ICU at the University of Maryland Medical Center (an urban teaching hospital), between January 1997 and 1998, was reported on in 2002 [110]. During the 12-month period a total of 1261 patients stayed in either ICU, but only those staying more than 48 hours were included in the study. Thirteen percent of these were afflicted by nosocomial infections (109/851).

[1] The branch of medicine concerned with skin, hair and nail diseases.
[2] The branch of medicine concerned with eye diseases.

Table 2.1 summarizes the results of prevalence studies conducted in various European countries between 1984 and 2003 [55]. Although a rigorous comparison between nations' nosocomial infection prevalences is not advisable due to methodological differences in the data acquisition, some general observations can be made. The table confirms that the prevalence of nosocomial infections in Finland (based on the recent study [100]) is of the same order of magnitude as in the other European countries, where similar surveys have been made. Furthermore, it indicates that nosocomial infections are roughly equally common in most European countries.

Table 2.1: Results of national prevalence studies on nosocomial infection rates in European countries. [55]

Country	Year	Prevalence (%)	Patients	Hospitals
Belgium	1984	9.3	8723	106
Czechoslovakia	1984	6.1	12260	23
Great Britain	1993–1994	9.0	37111	157
Germany	1994	3.5	14966	72
France	1996	6.7	236334	830
Spain	1997	6.9	51674	214
Greece	1999	9.3	3925	14
Italy	2000	4.9	18667	88
Slovenia	2001	4.6	6695	19
Norway	2003	5.1	12257	60
Finland	2005	8.5	8234	30

2.1.2 Predisposing factors

Predisposition to nosocomial infections essentially comes down to the following two causes [150]:

- An increased risk of bacterial colonisation.
- An impaired host defence.

These are due to a myriad of factors, which can be categorized into four main groups: factors related to the patient's underlying health status, factors related to an acute disease process, factors related to invasive procedures and finally, other factors related to the patient's treatment [150]. Table 2.2 further expands these groups into the most important risk factors.

High age, chronic lung disease and malnutrition, for example, all involve a greater risk to nosocomial infections, because of the host's impaired immune status. Trauma also affects the patient's immune system and thus makes it easier to get infected. Several treatments, like antibiotic therapy, blood transfusions and sedative drugs have been uncovered to have the same effect. Severe burns result in a reduced ability of the skin to prevent bacteria

Table 2.2: Risk factors to nosocomial infections. [78]

Factors that predispose to nosocomial infection
Related to underlying health status
Advanced age
Malnutrition
Alcoholism
Heavy smoking
Chronic lung diease
Diabetes
Related to acute disease process
Surgery
Trauma
Burns
Related to invasive procedures
Endotracheal or nasal intubation[1]
Central venous catheterization[2]
Extracorporeal renal support
Surgical drains
Nasogastric tube[3]
Tracheostomy[4]
Urinary catheter
Related to treatment
Blood transfusion
Recent antimicrobial therapy
Immunosuppressive treatments
Stress-ulcer prophylaxis[5]
Recumbent position
Parenteral nutrition[6]
Length of stay

[1]The placement of a tube into the windpipe through the mouth or nose.
[2]The placement of a catheter into a large vein in the neck, chest or groin.
[3]A small flexible tube inserted through the nose into the stomach.
[4]Surgery to create a small opening through the neck into the windpipe.
[5]Stress ulceration is a mucosal injury in the stomach, related to critical injury.
[6]Nutrition is given to the patient intravenously.

from penetrating into the body, consequently increasing the risk of infection. Furthermore, all kinds of invasive procedures where foreign objects, e.g. catheters, are inserted into the body, put the patient at a higher risk of bacterial colonisation. This becomes evident from the results of a U.S. investigation of 205 medical-surgical ICUs participating in the National Nosocomial Infections Surveillance (NNIS) System [125]. Surveillance data collected between 1992 and 1998 on 498,998 patients revealed that mechanical ventilation was part in 83% of all nosocomial pneumonia cases, 97% of all urinary tract infections (UTIs) involved urinary catheterization and 87% of all direct bloodstream infections (BSIs) involved central venous catheterization.

2.1.3 Mortality

Unfortunately, nosocomial infections are more than a serious inconvenience. These diseases may have fatal outcomes, either by being the direct cause of death, or simply by being the last straw. It is estimated that nosocomial infections play a hand in the deaths of around 90,000 people in the United States every year [17, 154]. In the United Kingdom, about 5,000 annual deaths are estimated to be the direct cause of nosocomial infections [73, 108]. In Finland, nosocomial infections are thought to be an accomplice in 2,000–5,000 patients' deaths each year [92], based on international studies in countries with equal public health service standards. Twenty percent of these patients are judged not to have a mortal primary disease.

In 1994 Dinkel *et al* [48] reported on a survey which had been conducted in acute care hospitals in Germany from 1978 to 1989. The purpose of the study was to find out the impact of nosocomial infections on mortality rates. The presence of a hospital-acquired infection was determined to increase the mortality rate threefold without correction for age and gender. With correction, the increase was twofold. The increase in the risk of death was largest among 20–40-year-old patients. Perhaps surprising was the fact that trauma increased the hospital mortality rate due to nosocomial infections by a factor of three, even after correcting for age. These findings seem to be roughly consistent with the mortality rates in an adult ICU in a Turkish university hospital: 60.9% for patients with nosocomial infections, versus 22.1% for non-infected [49].

2.1.4 Financial aspects

Hospital-acquired infections have major financial implications. This is mainly due to two factors: patients are hospitalized longer and the treatment costs are higher. The former is a natural consequence of prolonged disease, whereas the latter arises from the fact that nosocomial infections are often hard to treat and require expensive medication.

How much longer infected patients stay in hospital depends on the type of infection. Historical cohort studies (original papers [58, 64, 107, 118, 147, 156], data from [155]) have shown that nosocomial bloodstream infections extend the hospitalization the longest, requiring an extra stay of about 10–14 days. The same figures for nosocomial pneumonia, operative wound infections and urinary tract infections are nine, five and two days, respectively. Other nosocomial infections are estimated to require one extra day of stay.

More recent estimates say that nosocomial urinary tract infections add 1–4 days to the hospital stay, whereas nosocomial pneumonias add as much as 7–30 days [110]. The median stay in an adult ICU in a Turkish university hospital was 13 days for infected patients and two days for non-infected [49]. On average, it is considered that nosocomial infections

approximately double the length of hospitalization, which usually means one extra week [78, 92].

The costs of excess stay alone in hospitals in the United States due to hospital-acquired bloodstream infections are $3.5 billion per year according to one source [39]. This figure does not include costs from other types of nosocomial infections, and treatment costs like antibiotics. Another source, [154], declares that in 1995 the costs of nosocomial infections were approximately $4.5 billion. These numbers are, of course, suggestive estimates. On a less grand scale, DiGiovine *et al* [47] performed a study at the University of Michigan Hospital in the United States, to among other things, evaluate the additional costs that nosocomial bloodstream infections cause per incident. Sixty-eight cases with infection during a three-year period from the beginning of 1994 to the end of 1996 were compared to 68 closely matched noninfected controls. The infected group had, on average, 10 days of excess stay, resulting in average additional costs amounting to $34,500 per case. In a similar Spanish case-control study of 49 cases of intravenous catheter-related infection in the bloodstream, the hospital stay was prolonged by approximately 20 days, with additional costs of approximately 3,100 euros per incident [124]. The study was performed retrospectively, of data collected between January 1992 and December 1998 in the ICUs in Hospital de Sabadell in Barcelona, Spain.

Surgical-site infections required patients to stay an average of 6.5 extra days in hospital in a U.S. cohort study performed in the late 1990s [82]. The corresponding costs were about $3,000, but if the infection did not appear before discharge (which is a common scenario) and the patient had to be hospitalized again, the costs increased to just over $5,000. The setting was a community hospital and 255 pairs of matched patients with and without surgical-site infections were included. Perhaps the costliest of surgical-site nosocomial infections are those associated with open heart surgery. According to a fairly recent American report [38] the estimated additional costs for a deep sternal wound infection were as much as $81,000, and even the costs for a superficial sternal wound infection were significant: about $10,000. A British report in 2001 evaluated, that patients with hospital-acquired infections were, on average, 2.9 times more expensive than equivalent noninfected patients. This translated into additional costs of £3,154 per patient [119]. The study included 3980 patients admitted to selected wards[3] of a district general hospital between April 1994 and May 1995. Of these, 7.8% (309) developed various nosocomial infections. Based on the results of the study, the total annual economic burden on the National Health Service (NHS) attributable to these infections from the selected wards was derived. The conclusion was, that an estimated 320,994 patients are infected each year, costing £930.62 million to the NHS.

The burden on the national economy due to hospital-acquired infections has not been

[3]Medical; surgical; orthopaedic; urology; gynaecology; ear, nose and throat; elderly care; and obstretic patients (who had had a Caesarean section).

studied extensively in Finland. Most estimates are based on international results applied to the scales of Finnish hospitals. Table 2.3 reproduces the estimates for additional hospitalization, cost per infection and total annual cost attributed to different types of hospital-acquired infections in Finland, as described in [92]. These should, in all probability, be considered as minimum expenses, since the costs per infection are rather small compared to the figures outlined in the above studies. Thus it would seem, that these infections cost at least 65 million euros each year to the Finnish society.

Table 2.3: Estimates of key economic figures of nosocomial infections in Finland, based on international studies. [92]

Infection type	Additional hospital days/infection	Euros/ infection	Million euros/year
Wound infection	7	2,300	20
Pneumonia	6	2,000	10
Bloodstream infection	7	2,300	13
Urinary tract infection	1	330	6
Other infections	5	1,700	16
	mean 4	mean 1,320	sum 65

The direct costs due to prolonged hospitalization are, however, not the only costs arising from nosocomial infections. Possible rehabilitation, auxiliary devices and disability to work also have financial implications. Another cost-increasing factor is the possible antibiotic resistance of microorganisms. This can increase the treatment expenses dramatically, as conventional antibiotics have to be replaced by second or third line antibiotics. Some of these require serum monitoring because of toxicity, which further increases costs. Table 2.4 shows comparative costs of treatments to two infections, in cases when the causative bacteria are sensitive, and resistant in various degrees to antibiotics. The partial data has been reproduced from a recent article by G.L. French [52], but originally it has been extracted from *Guy's, St. Thomas' and Lewisham Hospitals. Formulary: A Guide to Prescribing*, 5th Edition, 2002. These two examples demonstrate exemplarily the significant financial burden generated by even modestly resistant microbes. In the case of *Pneumococcal meningitis*, intermediate resistance to penicillin increases the costs by almost a factor of five. The treatment of a serious *Staphylococcus aureus* infection gets only 2.4 times more expensive if the bacterium is resistant to penicillin[4], but in the worst case scenario (Linezolid MRSA), the necessary drugs are 53 times more expensive than ordinary penicillin (Table 2.4).

An outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) occured in three hospitals in the U.K. between April 1991 and December 1992. A total of 400 patients were infected or colonised. Cox *et al* reported on this incident in 1995, and concluded that the total cost of the outbreak was no less than £403,600 [40]. The dominant cost was the

[4] In practice, *S. aureus* infections are never treated with penicillin, since virtually all strains are resistant to this drug. This treatment example is just for price comparison.

Table 2.4: The treatment costs of two bacterial infections are compared in cases where the causative bacteria have various degrees of antibiotic resistance. [52]

Infection	Treatment example	Cost per week	Increase in cost compared to sensitive strain
Pneumococcal meningitis			
Penicillin-sensitive	High dose IV benzyl penicillin	£30.00	
Penicillin intermediate-resistant	Ceftriaxone	£139.65	4.7×
Penicillin high-level-resistant	Ceftriaxone+vancomycin*	£261.74	8.7×
Serious S. aureus infection			
Penicillin-sensitive	IV benzyl penicillin	£11.76	
Penicillin-resistant	IV flucloxacillin	£28.56	2.4×
Methicillin-resistant	IV vancomycin*	£187.74	16×
Methicillin-resistant, vancomycin ineffective	IV linezolid	£623.00	53×

These are basic U.K. NHS costs excluding VAT (2002 prices). Purchasers often get significant discounts on these prices, and they are given for comparative purposes only.

*Requires serum monitoring, which adds to the cost.

isolation wards (£303,600), but microbiology, drugs and cleaning were also major contributors to the overall expenses. Kim *et al* [81] investigated the costs of MRSA in a Canadian hospital between April 1996 and March 1998, and found that 20 patients were infected during the time period, corresponding to 2.9 MRSA cases per 1,000 hospital admissions. On average, these patients spent 14 additional days in hospital, and caused excess total costs amounting to $287,200, i.e. $14,360 per patient. If the MRSA infection rate is 10–20%, they estimated that every year, MRSA infections cost Canadian hospitals $42–$59 million (at 1997 prices).

In light of these notable financial figures, it becomes prudent to ask how much of nosocomial infections are preventable, and is their prevention cost-effective? Harbarth *et al* give an answer to the first question in a recent review [65]. Thirty reports published between January 1990 and October 2002 were assessed to conclude that nosocomial infection rates can be decreased by 10–70% depending on the setting, type of nosocomial infection, prevention method and previous infection rate. These findings led Harbarth *et al* to estimate that at least 20% of all nosocomial infections can be prevented in all probability. The second question is not as easy to give a straightforward answer to. Cost-benefit analyses are always more complicated, as their results depend heavily on the initial assumptions. In the mid-1990s R.P. Wenzel [155] calculated in his analysis that one life-year can be saved at a lower cost by a nosocomial infection control programme than other various medical treatments, e.g. mammography and cholesterol reduction. In 2002 Stone *et al* reported on a systematic audit of articles published between 1990 and 2000 containing cost analyses of nosocomial infection control interventions [139]. Based on these reports Stone *et al* summarized, that the attributable costs of most infections were larger than

their intervention costs, although standard deviations were frequently large, because of the small number of results and their inconsistencies. Stone *et al* also highlighted that most papers only included a simple cost analysis without a comparison group.

To summarize the financial aspects of nosocomial infections, it can be said that they are expensive, and while definite figures of the cost-effectiveness of intervention programmes cannot be given in general, it is most often economically beneficial to prevent them. One cannot, however, just blindly stare at the dollars, euros and pounds, but one also has to keep in mind the humane aspects of preventing these infections; rescuing the patient from suffering, sparing relatives and friends from mental hardship and contributing to the quality of life.

2.2 The usual suspects of nosocomial infections

2.2.1 Bacteria

The majority of hospital-acquired infections are caused by bacteria. Bacteria are prokaryotic, mostly unicellular organisms, i.e. they lack a cell nucleus. In bloodstream infections they stand for over 90% of all episodes [101, 160]. Studies have shown, that it is generally the same bacteria causing these infections both in Europe and the U.S., although there are discrepancies in the precise percentages between countries, wards and sites of infection [18, 138]. This can be seen, for example, in the data in Table 2.5.

Table 2.5: Comparison of American and Finnish results for the most common pathogens associated with nosocomial bloodstream infections. [101, 160]

	% of BSIs (rank)	
Pathogen	USA	Finland
CoNS	31.3 (1)	30.9 (1)
Staphylococcus aureus	20.2 (2)	10.5 (3)
Enterococcus species	9.4 (3)	6.2 (4)
Candida species	9.0 (4)	3.8 (8)
Escherichia coli	5.6 (5)	11.2 (2)
Klebsiella species	4.8 (6)	5.1 (5)
Pseudomonas aeruginosa	4.3 (7)	5.0 (7)
Enterobacter species	3.9 (8)	3.3 (9)
Serratia species	1.7 (9)	–
Acinetobacter baumannii	1.3 (10)	–
Viridans streptococci	–	5.1 (6)
Group B streptococci	–	2.8 (10)
Isolates, n =	20,978	1,621

NOTE. BSI, bloodstream infection. CoNS, coagulase-negative staphylococci. The figures of the U.S. study do not include polymicrobial episodes, unlike the Finnish. The *Candida* species are fungi.

The table compares the most common pathogens associated with nosocomial bloodstream infections in Finland and the U.S. according to two recent surveys [101, 160]. It should be noted that the *Candida* species are fungi. With a few exceptions, the results are amazingly similar. Among the bacterial species, the only larger differences are in the predominances of *Staphylococcus aureus* (9.7 percentage units less in Finland) and *Escherichia coli* (5.6 percentage units more in Finland). These figures should, however, not be compared in detail, since the U.S. study does not include polymicrobial infections, unlike the Finnish study. Coagulase-negative staphylococci (CoNS) still cause most (almost one-third) of the nosocomial bloodstream infections in both countries.

Which bacteria predominate hospital-acquired infections naturally depends on the site of infection. The leading culprit of bloodstream infections will not, for example, be the same as in the case of urinary tract infections. This becomes evident from the data in Table 2.6, which shows the predominances of the five most common pathogens in ICUs by type of infection. The data is from U.S. hospitals participating in the NNIS System between January 1992 and May 1999 [18]. In agreement with later Finnish and American studies [101, 160], CoNS reign supreme as the begetters of bloodstream infections, whereas *S. aureus* and *E. coli* cause the majority of pneumonias and urinary tract infections, respectively.

Table 2.6: Distribution of the five most common pathogens associated with bloodstream infections, pneumonia and urinary tract infections in ICUs, according to NNIS data from January 1990 to May 1999. [18]

Pathogen	BSIs	Pneumonia	UTIs
Enterobacter spp	4.9%	11.2%	–
Escherichia coli	–	–	17.5%
Klebsiella pneumoniae	–	7.2%	6.2%
Pseudomonas aeruginosa	–	17.0%	11.0%
Staphylococcus aureus	12.6%	18.1%	–
CoNS	37.3%	–	–
Enterococcus spp	13.5%	–	13.8%
Candida albicans	5.0%	4.7%	15.8%
Isolates, n =	21,943	39,810	30,701

NOTE. BSI, bloodstream infection. UTI, urinary tract infection. CoNS, coagulase-negative staphylococci. *Candida albicans* is a fungus.

When the overall predominance of hospital-acquired bacteria is considered, most of the infections are due to Gram-positive[5] bacteria [78]. In the 2005 prevalence study in Finland [100], the most common bacteria causing nosocomial infections were *E. coli* (13%), *S. au-*

[5]Gram-staining differentiates bacteria into two large classes, depending on the properties of their outer cell walls. Gram-positive bacteria appear violet in microscope studies after the Gram-staining procedure. They have outer cell walls consisting of up to 90% peptidoglycan, which can be stacked in no fewer than 20 layers. Gram-negative bacteria have outer cell walls consisting of only a few layers of peptidoglycan, and a secondary membrane consisting mostly of lipopolysaccharide. Gram-staining is a fast first step on the road to identify bacteria. Gram-negative bacteria are, in general, more dangerous than Gram-positive, but there are exceptions. [16]

reus (10%), *Enterococcus faecalis* (9%), CoNS (8%) and *Pseudomonas aeruginosa* (6%). In a one day point prevalence study in 1992 in 17 European countries, the distribution of the most common nosocomial bacteria in ICUs was the following: *S. aureus* (30%), *P. aeruginosa* (29%), CoNS (19%), *E. coli* (13%) and enterococci (12%). Thus, approximately the same bacteria occupy the highest ranks, although the percentage distribution is not the same everywhere in the Western World. These main bacterial begetters will be discussed in more detail below. The *Legionella* species have also been included, since they are present in the water distribution systems of many hospitals [67].

Enterobacteriaceae

The *Enterobacteriaceae* family is a large group of Gram-negative bacteria. These facultative anaerobic organisms[6] are rod-shaped and usually 1–5 μm long. Many members of this family are part of the normal gut flora of both humans and animals, and generally do not cause infections, except in favourable conditions. The most important of these, which cause nosocomial infections, are *E. coli*, *Klebsiella* spp, *Enterobacter* spp, *Proteus* spp, *Serratia marcescens*, *Citrobacter freundii*, *Morganella morganii* and *Providencia stuartii*. The first three are the most common. An electron micrograph (EM) of *E. coli* bacteria is depicted in Fig. 2.1.A.

Enterobacteriaceae spread through patient contact in the wards and may cause epidemics. The *Enterobacter*, *Klebsiella* and *Serratia* species can also spread from moist locations in the environment. They are the main culprits of urinary tract infections, but are associated with many types of hospital-acquired infections and often cause secondary bloodstream infections. Of the Gram-negative rod-shaped bacteria, *E. coli* and the *Klebsiella* species stand for most of the bloodstream infections. This is due to *E. coli* and *Klebsiella* strains capable of producing extended spectrum beta-lactamase (ESBL), which is becoming more and more ordinary. Beta-lactamases are enzymes capable of inactivating antibiotics based on beta-lactam, a four-atom ring (three carbons and one nitrogen), which prevents the formation of bacterial cell walls. Thus ESBL producing bacteria are resistant to many antibiotics, like penicillin and even third generation cephalosporins. One of the only potent option left, is to use carbapenems, but as resistance to these antibiotics increases, as it surely will in time, the arsenal of modern medicine starts to deplete.

In a U.S. study [160] of nosocomial bloodstream infections, 2% of *E. coli* and 12% of *Klebsiella* spp isolates were resistant to cefotaxime, a third generation cephalosporin. Thirty-four percent of the *Enterobacter* spp isolates were resistant to the drug, thanks to the species' ability to produce chromosomal AmpC beta-lactamase, which inhibits third generation cephalosporins. In an equivalent Finnish study [101] the cefotaxime resistance

[6]Facultative anaerobic organisms can produce adenosine triphosphate (ATP) both in aerobic conditions (oxygen is present) and anaerobic conditions (oxygen is not present).

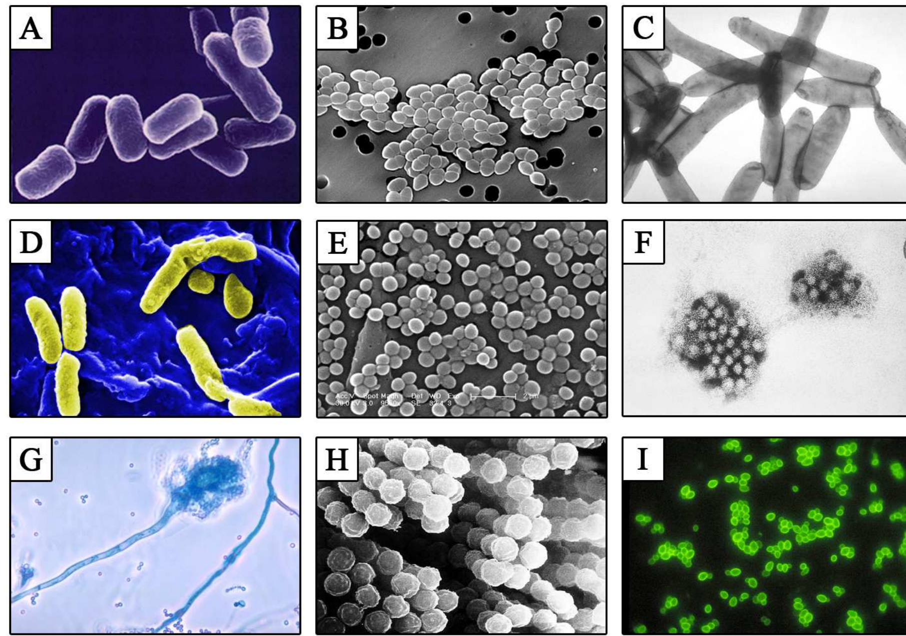


Figure 2.1: Micrographs of some pathogens causing nosocomial infections. Figures A–E depict bacteria, figure F a virus and figures G–I fungi. A) Electron micrograph (EM) of *Escherichia coli*. B) EM of vancomycin-resistant enterococci. C) EM of *Legionella pneumophila*. D) EM of *Pseudomonas aeruginosa*. E) EM of methicillin-resistant *Staphylococcus aureus*. F) EM of Noroviruses. G) Photomicrograph of a specialized *Aspergillus fumigatus* filament were a spore is developing. H) EM of *Aspergillus* spp spores. I) Photomicrograph with fluorescent antibody staining of oval budding *Candida albicans* cells. Sources: A) NASA, B–I) Public Health Image Library (CDC); B and D by Janice Carr, E by Janice Carr/Jeff Hageman, G by Dr. Libero Ajello, H by Robert Simmons/Janice Carr, I by Maxine Jalbert/Dr. Leo Kaufman.

rates were 2% for *E. coli*, 1% for *Klebsiella* spp and 39% for *Enterobacter* spp. Bloodstream infections caused by members of the *Enterobacteriaceae* family are more often fatal than those caused by other microbes. [16, 85]

Enterococcus spp

The enterococci are Gram-positive bacteria. Like *Enterobacteriaceae* they are facultative anaerobes and part of the normal gut flora of humans, although they can be found in the soil, food and animals as well. Physically enterococcus species are so alike staphylococci, that distinction is not possible based on this characteristic alone. The two most common species to cause infections are *Enterococcus faecalis* and *Enterococcus faecium*, the former standing for 90% of all enterococci infections. The bacteria are mostly transmitted from patient to patient on the hands of the medical staff, but sometimes also through the environment. Enterococci can become a part of the natural gamut of skin bacteria in the severely ill, further increasing the risk of peer contamination. They cause nosocomial urinary tract, bloodstream and wound infections. The major problem with enterococci infections is, that the species are intrinsically resistant to many antibiotics, *E. faecium*

more so than *E. faecalis*. Many species are resistant to beta-lactam antibiotics, reducing the treatment options in worst case scenarios to drugs like vancomycin and linezolid.

A growing concern is the increase in *Enterococcus* species resistant to vancomycin. These strains are called vancomycin-resistant *Enterococcus*, abbreviated VRE. Bacteria from such a strain are depicted in Fig. 2.1.B. The portion of VRE strains have been increasing in the U.S. since the 1990's. In 2000, 26% of isolates in ICUs were resistant to vancomycin [36]. In the case of bloodstream infections, vancomycin resistance in *E. faecium* isolates has increased from about 47% in 1995–1997 to almost 70% in 2000-2002 [160]. So far, VRE episodes in Finland have not been abundant. Of enterococci causing nosocomial bloodstream infections during 1999–2000, VRE stood for only 1%. Episodes of VRE remain fairly rare in most of Europe, at least when the more dominating species *E. faecalis* is concerned, if not for *E. faecium*, which shows much greater resistance levels in Central and Southern Europe (Fig. 2.2).

VRE is a very hardy organism. It can survive on dry surfaces, like doorknobs, bedclothes, bedposts, headboards and medical instruments for long times. Thus, the most effective prevention method against it is appropriate hand hygiene. The scary aspect of VRE is that the genes responsible for the resistance are usually located on a transferable plasmid[7], which can be transmitted to other, more pathogenic organisms, e.g. staphylococci [153]. [16, 87, 126]

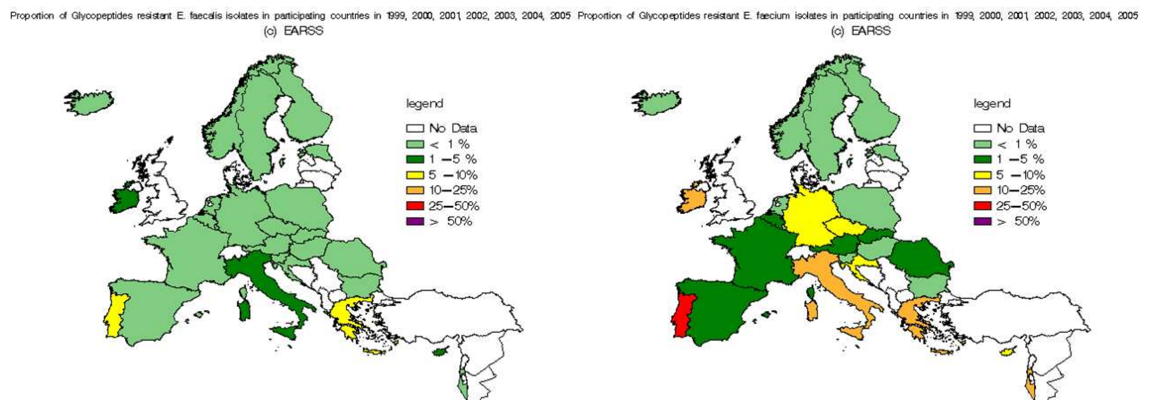


Figure 2.2: *Left:* Proportion of *E. faecalis* isolates resistant to glycopeptide antibiotics (e.g. vancomycin) in European countries between 1999 and 2005. *Right:* Same as on the left, but for the more resistant *E. faecium*. [3]

[7]Plasmids are circular double-stranded DNA molecules outside the chromosomal DNA. They are common in bacteria.

Legionella spp

Legionella species are present in small quantities in all freshwater environments in nature. There they live as parasites inside protozoa[8], and can proliferate inside mammalian cells by similar means. The bacteria are Gram-negative, and approximately 0.3–0.9 × 2–20 μm in size. They are aerobic, non-fermentative and rod-shaped, which can be seen in Fig. 2.1.C, depicting *Legionella pneumophila*.

There are 48 known species, *L. pneumophila* being the most important from a nosocomial infection perspective, trailed by *Legionella micdadei*, *Legionella bozemanii*, *Legionella dumoffii* and *Legionella longbeachae*. *L. pneumophila* and *L. micdadei* do not autofluoresce (see Subsection 4.2.2), contrary to *L. bozemanii* and *L. dumoffii* which fluoresce bluish white light when illuminated with UV light [19]. Legionella pathogens were not a problem before the second half of the 20th century, however, modern plumbing systems represent viable environments for legionellae to multiply in. They can easily colonise water distribution and air conditioning systems in virtually any building; hospitals and hotels included. In fact, a Finnish study revealed that 30% of hot water distribution systems in Finland are havens of *L. pneumophila*. The study included 67 buildings from different parts of the country, most of them apartment buildings [161]. In water systems lacking the presence of protozoa, legionellae survive wrapped in biofilms[9]. Whether they are able to multiply extracellularly within biofilms, or not, is currently unknown. Legionellae are always transmitted through the environment, but do not survive in dry environments. Usually, people get infected by breathing water aerosols containing *Legionella* bacteria. Another path of infection is direct contact with, for example, a wound. The infection can manifest itself in a couple of ways. Legionnaires' disease is a severe pneumonia, with a mortality rate of approximately 15% despite appropriate treatment. Pontiac fever resembles a common cold, and passes on its own in about a week. Sometimes predisposed people are completely asymptomatic.

None of the infections are known to be contagious, but they most often appear in clusters or epidemics. In 1999 there were two major *Legionella* epidemics in Europe, both originating from flower and whirlpool bath exhibitions. Of the combined 413 infected people, 33 died. Approximately 10–20 cases of *Legionella* infections occur in Finland annually and the bacteria are known to inhabit the water distribution systems of many hospitals. This knowledge makes it possible to battle these opportunistic microbes, although they can hardly be completely eradicated. Still, bacterial counts can be reduced by cleaning and disinfecting critical parts like shower heads and taps. *L. pneumophila* proliferates at temperatures from 25 to 42 °C, 37 °C being the optimal growth temperature. Thus, one

[8]Protozoa are single-celled, eukaryotic microorganisms larger than bacteria. Amoeba, for example, are protozoa.

[9]Biofilms are slime layers which some bacteria can produce around themselves to stay attached to inanimate objects made of stone, metal or wood in aquatic environments.

of the most effective disinfection methods is the "superheat and flush" -method, i.e. the temperature of the circulating water in the system is raised to 60–77 °C for several days, and all taps are left running for half an hour each day. This is, of course, less effective in old buildings where a lot of renovations tend to leave closed pipes where the water does not circulate. The increased water temperature may also cause patient and staff burns, which is why this method is typically used only when an epidemic is suspected. Another feasible disinfection method is to use ultraviolet (UV) light, which kills the bacteria [90]. Chlorinating the water system is not effective, since the species can withstand chlorine fairly well. [16, 51, 67]

Pseudomonas aeruginosa

Pseudomonas aeruginosa is an opportunistic pathogen capable of surviving in very modest environments. Essentially any carbon source is enough for the bacteria to flourish. *Pseudomonas* species have been found in soap residue, bottled mineral water, antiseptics based on ammonium and even diesel and jet fuel. They are widely found in the environment; food, water and moist areas. The species are Gram-negative, aerobic and rod-shaped (Fig. 2.1.D). *P. aeruginosa* is seldom harmful to healthy persons, but the species often colonises mucous membranes of immunocompromised patients and patients undergoing antibiotic treatment. Like other species of the genus, *P. aeruginosa* secretes a compound of two siderophores[10], pyochelin and pyoverdine, under iron-depleted conditions. This substance is fluorescent. The bacterium spreads by human contact, or from contact with moist areas in the environment. *P. aeruginosa* causes nosocomial infections in the urinary tract, respiratory tract, burns, wounds and the bloodstream. The species has an inherent resistance to most penicillins and cephalosporins, and is quick to develop new resistances. A genetic mutation can increase the imperviousness of the bacterium's outer cell wall, which prevents some antibiotics, like carbapenems, from penetrating. The cell wall also contains efflux pumps which can rapidly pump out selected antibiotics before they start killing the bacterium. Selection pressure can refine these pumps, further enlarging the repertoire of ineffective antibiotics. In addition, *P. aeruginosa* is able to form protective biofilms on inanimate objects, e.g. catheters. *Pseudomonas* bacteria possessing all these abilities can be resistant to all existing antibiotics. However, such infections mostly occur only in long-term intensive care patients with multiple chronic diseases. [16, 66]

Staphylococcus spp

The *Staphylococcus* species can be divided into two classes: the coagulase-positive and the coagulase-negative. Coagulase-positive species can produce plasma-coagulating enzymes,

[10] Siderophores are small molecular substances which have a high affinity to iron. Microorganisms use them to take up iron from the environment.

whereas coagulase-negative cannot. *Staphylococcus aureus* is the only coagulase-positive *Staphylococcus*, the other 31 species being coagulase-negative. Besides, *S. aureus* is catalase positive, i.e. able to convert hydrogen peroxide (H_2O_2) to water and oxygen. The staphylococci are Gram-positive, facultative anaerobes and spherical in shape, with a diameter of approximately 1 μm. In electron micrographs the bacteria look like clusters of grapes (Fig. 2.1.E). *S. aureus* is a major player in nosocomial infections, as are many coagulase-negative staphylococci (CoNS), of which *Staphylococcus epidermis* and *Staphylococcus saprophyticus* are the most significant species. *S. epidermis* is a part of the normal human skin flora. The more virulent *S. aureus* is present in the nose or nasopharynx of most children and many adults. Approximately every third adult has a permanent nasal colony of the bacteria. CoNS are the most common causes of bloodstream infections (Table 2.5). This is probably due to the increased use of central venous catheters, which are one of the most notable risk factors for bloodstream infections. *S. aureus* is the second most common cause of nosocomial bloodstream infections in the U.S., where the pathogen is twice as common as in Finland (Table 2.5). Both CoNS and *S. aureus* cause a lot of surgical-site infections. In these cases the bacteria are usually transmitted from the patient's own skin or nose. They also have a great ability to attach to inanimate objects, where *S. epidermis* forms biofilms. Furthermore, *S. aureus*, which can survive on dry surfaces, is one of the main pathogens causing nosocomial pneumonias.

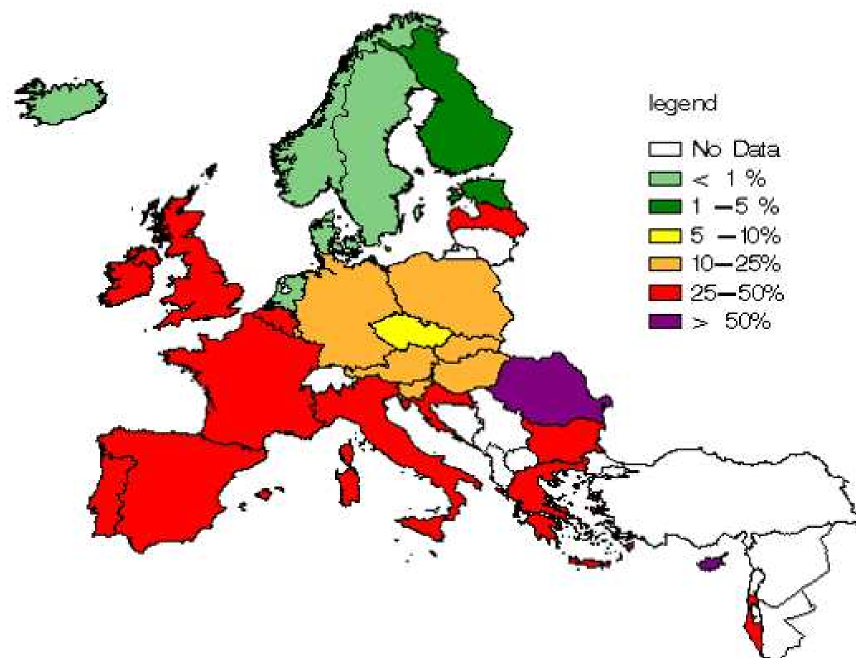


Figure 2.3: Proportion of MRSA isolates in European countries between 1999 and 2005. [3]

Resistance to antibiotics is usual among staphylococci. A genetic mutation can change the bacteria's penicillin-binding protein, consequently rendering them resistant to all beta-lactam antibiotics. Since methicillin is used to test this resistance in laboratories, these strains are called methicillin-resistant. Most isolates of *S. epidermis* in Finnish hospitals possess this resistance. Methicillin-resistant *Staphylococcus aureus* -strains, abbreviated MRSA, account for 30–50% of *S. aureus* isolates in the U.S. and many countries in Europe, except in the Netherlands and the Scandinavian countries, where MRSA rates are more or less below 1% (Fig. 2.3). Strains susceptible to methicillin are thought to acquire resistance from their resistant CoNS brethren. MRSA spreads through contact, primarily between patient and hospital personnel. Thus, MRSA-patients are single-room isolated and personnel are required to use gloves and protective clothing during inspection. Appropriate hand hygiene is essential in preventing further transmission (see Section 3.2.1). Susceptible strains of MRSA are treated with glycopeptide antibiotics, principally vancomycin. But not even this drug provides a final solution, because in recent years "vancomycin-intermediate" *S. aureus* (VISA) strains have been found in patients in Japan, Europe and North America. Many of these strains, which show tolerance to vancomycin, are results of long-term vancomycin treatment. They are usually associated with infections where it is not possible to determine if the treatment failure was due to the severity of the infection, or the decreased susceptibility to the drug. Unfortunately, a vancomycin-resistant *S. aureus* (VRSA) strain with high level resistance was found in two unrelated patients in the U.S. in 2002. The gene which is responsible for the resistance had been passed on by a VRE strain. Thus *S. aureus* has, in comic-style, but still accurately, been called a "Superbug". [16, 52, 86]

2.2.2 Viruses

Viruses are intracellular parasites of eukaryotic cells. They cannot reproduce by themselves, but need to invade and control a host cell for this. Where the sizes of bacteria are measured in the length scales of micrometres, viruses are measured in tens to hundreds of nanometres. Their structure is very simple compared to their host cells. A protective shell made of proteins, lipids and glucoproteins[11], called a capsid, holds a nucleic acid genome. The nucleic acid can be either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), and it can be single- or double-stranded, linear or circular [16, 152]. Because viral infections are much harder to study than bacterial infections, the true proportion of nosocomial infections caused by viruses is uncertain, although when monitoring has been performed the rate has been approximately 5%. In pediatric wards viruses accounted for as much as around 23% of nosocomial infections [21]. The following groups of viruses have been identified as the main viral agents for nosocomial transmission [22]:

[11] A glucoprotein is a protein with an attached carbohydrate.

- Respiratory route viruses.
  - Transmitted via large (median diameter over 5 μm) or small droplets. Small droplets are produced from sneezing, talking or coughing and can travel long distances. Large droplets are transmitted from person to person upon contact.
  - Transmission is seasonal, parallelling the disease activity in the community. The peak incidence occurs during the winter months.
  - Examples:
    - Respiratory Syncytial Virus (RSV). A major causative agent of respiratory disease among infants and young children.
    - Influenza Viruses A and B. Highly infectious.
    - Rhino- and Coronaviruses. Responsible for most episodes of the common cold.
- Fecal-oral route viruses.
  - Viruses which can replicate in the intestines and may cause gastroenteritises.
  - Examples:
    - Rotavirus. Especially significant among infants, children under five and the elderly.
    - Small Round-Structured Viruses (noro- and astroviruses). Noroviruses (Fig. 2.1.F), which belong to the caliciviruses, are the principal causative agents of stomach diseases. Noroviruses cause epidemics in e.g. hotels, hospitals, schools and on cruise ships. They typically cause heavy vomiting and spread exceptionally well. Conventional disinfectants are ineffective against them and they mutate rapidly. The viruses spread on contact, through drinking water and foodstuff. They infect patients, as well as personnel, without prejudice and cause absences from work.
- Herpesviruses.
  - Seldom a cause of nosocomial infections. Usually transmitted from person to person upon contact.
  - Examples:
    - Herpes Simplex Viruses.
    - Varicella-Zoster Virus. The most infectious herpesvirus. Causes chickenpox and shingles. The only herpesvirus which can be transmitted to humans by aerosols.
- Blood-borne viruses.
  - Transmission is only possible through transfer of body fluids or tissue from an infected to an uninfected person. Transmission in health-care settings is

basically limited to needle stick injuries and incidents where infection control procedures have not been abided by.

- Examples:
  - Hepatitis B and C Viruses.
  - Human Immunodeficiency Virus (HIV) Type 1.

- Exotic viruses.
  - Viral Hemorrhagic Fevers. Severe life-threatening diseases caused by a variety of viruses. Among the ones forming the greatest risk of nosocomial transmission are the Ebola and Marburg viruses, as well as the Lassa and the Congo Crimean hemorrhagic fever viruses, with recorded mortality rates ranging from 50–90%, 23–70%, 10–40% and 15–20%, respectively [136].

2.2.3 Fungi

Fungi are eukaryotic organisms, meaning that unlike bacteria, they have a nucleus where the DNA is situated. Fungi are less frequently the causative agents of nosocomial infections, however, their role is becoming more important. From a nosocomial infection standpoint the most significant fungi are the yeast and filamentous fungi, specifically the *Candida* species from the former and the *Aspergillus* species from the latter. [152]

Aspergillus spp

The *Aspergillus* species (Fig. 2.1.G) are mould fungi, which form filaments (long chains of cells) called hypha. The species are ubiquitous in the environment, especially where oxygen is plentiful, since the microbes are aerobic. Some *Aspergillus* species are able to survive in extremely nutrient-poor environments. In nature, *Aspergillus* spp are particularly found in water, hay and compost. Outdoors, *Aspergillus* spores[12] ride in the air dependless of the season, the peak concentration occuring during the late summer. Indoors, the spores are encountered in buildings with damage due to damp, and if the air ventilation system or water distribution system has been contaminated. Fungal spores are inevitably present in construction concrete as well, from where they can be released during renovations. The spores are very small and stay airborne for extended periods of time, travelling long distances. They pose a serious threat in hospital environments, where construction and renovation is common.

[12]The term spore has several different meanings in biology. In this context a spore is the reproductive cell of a fungus, analogous to the seed of a plant. In the case of bacteria, some of them can change into dormant forms, called spores, in times of stress or when there is a lack of nutrients. Bacterial spores can survive in harsh environments where vegetative bacteria would surely perish.

The most common *Aspergillus* spp causing invasive disease are *Aspergillus fumigatus* and *Aspergillus flavus*. They are both opportunistic pathogens, rarely causing disease in healthy persons, but rather in patients with compromised immune systems. The infection sites are usually in the lower respiratory tract or wounds. The group of diseases caused by *Aspergillus* spp is called aspergillosis. Ninety percent of aspergillosis cases are caused by *A. fumigatus*, which is the most pathogenic of the species. *A. fumigatus* spores (Fig. 2.1.H) are about 3–5 μm in size and protected from the human body's defence mechanisms by means of a surface protein which makes the spores hydrophobic. In addition, *A. fumigatus* can grow in 37 °C, contrary to its nonpathogenic counterparts. Aspergillosis among immunocompromised patients is associated with a very high mortality rate, approaching 90% at the highest. Currently these patients are kept in rooms with positive air pressure and high efficiency particulate air (HEPA) filters[13] to reduce their chance of exposure to *Aspergillus* spores. The medical treatment of a serious *Aspergillus* infection is extremely expensive, with costs capable of approaching 1,000 euros per day, for a period of one month, at the Helsinki University Central Hospital [26]. [16, 128]

Candida spp

The *Candida* species are yeast fungi which cause infections in humans opportunistically. Most of the fungal nosocomial infections are caused by them. As *Candida* yeasts are quite common on the skin and the genital area, in the respiratory tract and the gastrointestinal tract of perfectly healthy persons, and thus a part of our normal flora, severe *Candida* infections are ordinarily endogenous. This does not rule out cross-infection, as the fungi have been found to spread from patient to patient with the hospital personnel as mediators, and can be present on inanimate surfaces in the hospital environment. In other words, the species are spread by contact, stressing the importance of appropriate hand hygiene. The most prominent *Candida* spp causing nosocomial infections is *Candida albicans* (Fig. 2.1.I), followed by *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, *Candida kefyr* and *Candida lusitaniae*. While *C. albicans* is the genus' main begetter of nosocomial infections (standing for approximately 60% of all *Candida* bloodstream infections in a U.S. NNIS study between 1989 to 1999 [148]), the other species are being reported more and more frequently, above all in the U.S. One explaning factor might be these species' resistance to some antifungal drugs. As with *Aspergillus* infections, *Candida* spp are mainly a threat to patients with an impaired immune defence, and thus already severely ill. Consequently, the attributable mortality is ambiguous. Ordinary infection sites are the urinary tract, the bloodstream and surgical sites, gastrointestinal in particular. Nosocomial bloodstream infections are more frequent in the U.S. than in Finland (Table 2.5). In addition, *C. albicans* infections are associated with nosocomial

[13]HEPA filters are designed to prevent 99.97% of all particles above 0.3 μm in diameter from penetrating.

pneumonia (Table 2.6). [53, 129]

2.3 The nosocomial infection community

2.3.1 Surveillance programmes

National Nosocomial Infections Surveillance

In the U.S., a system called the National Nosocomial Infections Surveillance (NNIS) was initiated in the early 1970s. The system is coordinated by the CDC's Division of Healthcare Quality Promotion, and during the past 30 years the number of participating hospitals has increased from about 60 to approximately 300. The objectives of the system are to monitor hospital-acquired infections; their prevalence, causative pathogens, antimicrobial resistances and risk factors. Furthermore, prevention strategies are developed and assessed, and information of nosocomial infections is provided to all parties concerned. The NNIS frequently releases public reports on the collected nosocomial infection data and publishes prevention guidelines, which are available on the Internet. The NNIS is the only hospital-acquired infection monitoring system in the U.S., and is voluntary to hospitals. [8]

Sairaalainfektio-ohjelma

Sairaalainfektio-ohjelma (SIRO) is the Finnish equivalent of NNIS. It is a national hospital-acquired infection programme, begun in 1998 and funded by the National Public Health Institute (*Kansanterveyslaitos* in Finnish) and the Ministry of Social Affairs and Health (*Sosiaali- ja terveysministeriö* in Finnish). The objectives of the programme are to prevent hospital-acquired infections, to improve and develop their surveillance, to establish common prevention guidelines and recommendations, to educate and to do research, and to investigate hospital epidemics. Currently, bloodstream infections and surgical site infections are recorded, including the causative pathogens and their drug susceptibilities. Of the surgical site infections only those related to coronary surgery, hip and knee endoprostheses, and upper thigh bone fractures are recorded. Participation in the programme is voluntary. Between 1999 and 2004 there were 10 participating hospitals. Guidelines and reports of the programme are freely available on the Internet. [10]

Hospital in Europe Link for Infection Control through Surveillance

In the European Union (EU), surveillance of nosocomial infections on an international level is carried out by the Hospital in Europe Link for Infection Control through Surveil-

lance (HELICS) programme. HELICS functions as a "Network of Networks", utilising the already existing surveillance systems in some European countries. The general goals of the programme are to create an EU covering surveillance system of nosocomial infections and to create a European database of these infections. This is achieved by harmonising data acquisition methods in participating countries by introducing agreed methodologies. Therefore, the quality and comparability of the data is improved, which enables identification of effective prevention measures. The programme is meant to help countries without surveillance systems to set up their own networks by providing expertise and support. Further aims are to allow countries to more easily share surveillance experiences and to collaborate in and guide the development of enhanced surveillance schemes and prevention practices, in order to reduce nosocomial infection rates. The nosocomial infection data is acquired by the HELICS-associated networks, which collect the data using standardised protocols, and send it to the HELICS Management Team, which performs the necessary analyzations and enters the data into the database. Each HELICS-associated network has a coordinator, who participates in the decisions of how the data acquisition protocols are standardised and how the data is managed. Sixteen countries or regions contributed to the pilot data collection between 2000 and 2003, with either ICU or surgical site infection data, or in some cases with both. A further seven countries are expected to participate as of 2004, and by March 2005 an additional eight countries were in the process of establishing data contribution. [6]

European Antimicrobial Resistance Surveillance System

Another EU supported programme is the European Antimicrobial Resistance Surveillance System (EARSS) initiated in January 1999. The principles of operation are the same as in the HELICS programme. EARSS is a network of national networks, collecting data on antimicrobial resistance in Europe. Its objective is to monitor the prevalence and spread of the principal bacteria possessing clinically meaningful resistances to antimicrobials. Presently 31 countries participate in the effort, providing data from approximately 800 public health laboratories throughout the continent. The nationally assembled data from antimicrobial susceptibility tests is transfered to the EARSS Management Team located at the Netherlands Institute for Public Health. This team validates the consistency of the data and publishes the results on the EARSS website, which features an interactive database where the user can choose how and which results are presented. Fig. 2.3 represents an example of this, depicting the proportion of MRSA isolates in all participating countries during 1999–2005. Resistance data is collected and reported on the following seven bacteria: *Streptococcus pneumoniae*, *S. aureus*, *E. faecalis*, *E. faecium*, *E. coli*, *Klebsiella pneumoniae* and *P. aeruginosa.* Trends in the antimicrobial resistance of these pathogens are analysed and publicly made available on the EARSS website. Besides these activities, EARSS provides guidelines and protocols on testing methods in order to

improve the quality and value of the received data. [3]

2.3.2 Organisations, conferences and journals

Association for Professionals in Infection Control and Epidemiology, Inc.

The Association for Professionals in Infection Control (APIC) was founded in 1972 in the U.S. The organisation was the fruit of a realisation that an organised, systematic approach to the control of hospital-acquired infections was needed. In 1994 its name was changed to the Association for Professionals in Infection Control and Epidemiology, Inc. (APIC). This was intended to reflect the evolution of the organisation to also encompass other health care systems than hospitals in particular. Today APIC, whose headquarters are in Washington, D.C., is an international multidisciplinary voluntary organisation with more than 10,000 members. The association aims to support, affect and improve the quality of health care. The tools for this are infection control and epidemiology. APIC members receive training and education in these disciplines, as well as issues of the APIC News magazine and *The American Journal of Infection Control* (AJIC). AJIC is APICs official publication, covering topics on, among others, infection control, epidemiology, infectious diseases and disease prevention. Additionally, infection control guidelines by the CDC and APIC are published in AJIC. *APICS's Annual Educational Conference & International Meeting* is the organisations foremost educational event, arranged once a year. [1]

Centers for Disease Control and Prevention

The Centers for Disease Control and Prevention (CDC) is an organisation under the U.S. Department of Health and Human Services. The CDC was originally founded in 1946 (then named the Communicable Disease Center) to help control malaria, but has since then evolved into one of the foremost contributors to public health through its efforts in the prevention of, among others, infectious and chronic diseases, injuries and environmental health threats. The CDC's mission is "to promote health and quality of life by preventing and controlling disease, injury, and disability". Among the many activities to accomplish this are to do research, investigate and identify health problems, provide education and leadership, study and implement prevention strategies, exercise publishing and to give recommendations and guidelines on topics related to public health. *Emerging Infectious Diseases* is a monthly published journal aiming to recognise infectious diseases with an increased incidence during the past 20 years or that might become more prevalent in the short-term future, and how to prevent these diseases. The journal communicates findings of both the CDC and international researchers. *Morbidity and Mortality Weekly Report* (MMWR) is another of the CDC's publications. It contains preliminary data reported to

CDC by state health departments each week. The *MMWR Recommendations and Reports* contains policy statements on all areas the CDC are dealing with. Some of the guidelines and recommendations offered by the CDC are presented in Table 2.7. [2]

Table 2.7: Some of the guidelines on infection control available at the CDC website. Source: `http://www.cdc.gov/ncidod/dhqp/guidelines.html` (Accessed 27 Feb, 2006)

Guideline or recommendation	Year
Preventing Healthcare-Associated Pneumonia	2003
Environmental Infection Control in Healthcare Facilities	2003
Hand Hygiene in Healthcare Settings	2002
Prevention of Intravascular Device-Related Infections	2002
Prevention of Surgical Site Infecitons	1999
Infection Control in Healthcare Personnel	1998
Prevention of Catheter Associated Urinary Tract Infections	1981

European Society of Clinical Microbiology and Infectious Diseases

The European Society of Clinical Microbiology and Infectious Diseases (ESCMID), which was founded in 1983, aspires to improve the diagnosis, treatment and prevention of infectious diseases. The non-profit organisation encourages and endorses research and education in all disciplines related to infectious diseases. The ESCMID organises the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) each year, as well as a number of educational meetings. Since 2002 a one-week Summer School has been held for clinical microbiologists and infectious disease specialists. Awards, grants and fellowships are bestowed on young researchers and scholars to support the fields of clinical microbiology and infectious diseases. A special Task Force has been assembled to collaborate with the EU in research programmes and the organisation of conferences, in order to get more involved in the various related EU activities. Today the society has more than 2,750 members from 93 countries. The administrative headquarters are situated in Basel, Switzerland, but the ESCMID has additional offices in Munich, Germany, where the organisation is registered. *Clinical Microbiology and Infection* is the society's official journal, dealing with topics discussing everything related to infectious diseases, e.g. clinical and basic microbiology, nosocomial infections, molecular pathogenesis of infectious diseases, and immune mechanisms. In addition, the society has an official newsletter, the ESCMID *News*, primarily targeted at the members of the society. Medical guidelines on clinical microbiology and infectious diseases are available for free at the ESCMID website, categorised into guidelines supported by the ESCMID, and those by other organisations. [4]

The Hospital Infection Society

The roots of the Hospital Infection Society (HIS) can be traced back to the early 1980s, but it was not until 2002 that the society moved into permanent premises in London, U.K. The objectives of the HIS are to further the knowledge, and to distribute information of the prevention and control of hospital and health care associated infections. The society organises, in collaboration with the London School of Hygiene and Tropical Medicine and the Public Health Laboratory Service, a multidisciplinary course (The Diploma in Hospital Infection Control) for both national and international infection control personnel and students. The course provides training in a broad spectrum of scientific areas related to the control of hospital infections. Other activites of the HIS are to award one major and some minor research grants annually, and to feature the *Journal of Hospital Infection*, the society's official journal. The journal covers all kinds of topics related to nosocomial infections, e.g. surveillance, methods of prevention, outbreaks, and design of hospitals. Besides the meetings which the HIS arranges annually, a major international conference is organised every three or four years. The upcoming HIS 2006 Conference in October is the sixth and takes place in Amsterdam, the Netherlands. [14]

Infectious Diseases Society of America

As the name already tells, the Infectious Diseases Society of America (IDSA) is an association of physicians, scientists and medical personnel involved in infectious diseases and their prevention. IDSA aims to improve the health of individuals as well as communities by encouraging research, education and prevention methods associated with infectious diseases. Advancement in research related to infectious diseases is supported through awards, and conferences are offered throughout the year for educational and networking purposes. IDSA has two scholarly journals, *The Journal of Infectious Diseases* and *Clinical Infectious Diseases*. The former was initially published in 1904, and contains original research articles on all matters related to infectious diseases; the origins of the diseases, the causative pathogens, diagnosis and treatments. The latter contains articles with similar topics, the emphasis is, however, on clinical practice. Moreover, each issue includes a special section discussing salient themes like antimicrobial resistance, HIV/AIDS and biological weapons. IDSA is an influential advocate of quality microbiology, HIV care, bioterrorism defence and food safety. The society runs the "Emerging Infectious Network", which is a sentinel network consisting of infectious diseases consultants who report on unusual episodes they encounter. Furthermore, IDSA is a cofounder of the National Network of Immunization Information (NNii), which provides information on vaccines and immunisation. [7]

The Society for Healthcare Epidemiology of America

The Society for Healthcare Epidemiology of America (SHEA) was founded in 1980 to advance the field of health care epidemiology, by which all kinds of activities to study or improve the outcomes of patient cares in health care settings is meant. The objective of SHEA is thus to raise the quality of all patient care and the safety of all health care personnel. Previously a large portion of SHEA's activities have been focused on nosocomial infections and their prevention and control, which will continue in the future as well. Furthermore, SHEA influences public policy in regards to standard setting, prevention and control of infectious diseases, promotes and provides support for research and education in health care epidemiology and fosters both national and international collaboration in this field of science. SHEA organises courses in collaboration with the CDC and runs an annual meeting for health care epidemiologists. The society's official journal, *Infection Control and Hospital Epidemiology*, appears monthly and is directed at anyone concerned with infection control or epidemiology in a health care setting. [15]

The Finnish Society for Hospital Infection Control

The Finnish Society for Hospital Infection Control, or Suomen Sairaalahygieniayhdistys ry (SSHY) in Finnish, was founded as early as 1975. The society's purpose is not unlike its international counterparts, as its mission is to improve patient and staff safety in health care facilities by promoting hygiene and infection prevention strategies. SSHY organises meetings and seminars for its 1,300 members, awards grants to purposes in agreement with the society's mission, and publishes a newsletter, *Suomen Sairaalahygienialehti*, six times a year. The newsletter has been in print since 1983, although under a different name, *SaHTi*, until 1993. The major event arranged by SSHY is the "National Hospital Hygiene Days", occuring simultaneously with the society's annual general meeting in February/March. [12]

2.3.3 Regulations of the public authorities

The role of hospital-acquired infections in the Finnish legislation took a quantum leap in 2004, when the existing law on infectious diseases (23.7.1986/583) was modified. In section 3, subsection 8 of the law, a hospital-acquired infection is defined (for the first time in Finnish legislation) as an infection, which has developed or emerged during treatment in a health care functional unit. The law specified surveillance and prevention responsibilities of hospital-acquired infections, which had not been included previously. The purpose of the inclusion was to support regional organisations involved in the prevention of hospital-acquired infections, as well as to authorise regional registers of infections

caused by antimicrobial resistant bacteria. The sixth section of the law enforces the hospital districts to prevent, monitor and detect hospital-acquired infections in their area of responsibility. The National Public Health Institute (Kansanterveyslaitos) serves as the national consultant in the prevention of all infectious diseases. [5, 127]

2.4 What are biosensing systems?

In this work, the same definition for *biosensing systems* is used as in a 2004 report on *International Research and Development in Biosensing* by the World Technology Evaluation Center (WTEC), Inc [132]. *Biosensing systems* are broadly considered as all approaches to the detection of biological substances. This differs from *biosensors*, which specifically utilise biological recognition elements in sensing. Thus biosensors are merely a branch in the "biosensing systems–tree", which is constituted of many diverse means. Among these are electrical, electronic and optical devices; cell-, enzyme-, and nucleic acid-based methods and instruments performing chemical analyses. The purpose of these is to produce detectable signals indicating ongoing biological phenomena.

Chapter 3

Helsinki University Central Hospital

On a national level, Finland is divided into 20 hospital districts. There are five university hospital districts, the largest being *The Hospital District of Helsinki and Uusimaa* (Helsingin ja Uudenmaan sairaanhoitopiiri; HUS). The 31 municipalities constituting HUS are subdivided into five health care regions, of which one is the Helsinki University Central Hospital (HUCH) -region. It comprises the metropolitan area and its immediate environs. The HUCH is nationally liable for treating rare and serious diseases, requiring special expertise and technology. The largest hospital belonging to the HUCH is the Meilahti Hospital, situated just three kilometres from the Helsinki city centre.

Planning on the hospital area in Meilahti begun as early as in 1898, but the first clinic was not finished until 1934. In 1939 the state and the City of Helsinki agreed to build a general hospital in Meilahti. The work was to be done in stages. The first to be constructed was the Children's Hospital, opened in 1945, followed by the Eye and Ear Hospital in 1951. The Meilahti Hospital was inaugurated on 20 November 1965, with the first patients arriving on 1 December, the same year. Nowadays, the largest hospital in the country employs about 1,400 nurses, 400 physicians and several hundred other health care professionals. The hospital has 31 wards, 10 outpatient clinics and 538 beds. Annually, approximately 30,000 patients are treated in the wards, and 150,000 patients visit the outpatient clinics. Moreover, fully 8,000 operations are performed every year, almost half of them constituting fast emergency operations. The entire hospital campus is undergoing a major building project, in course of which the Meilahti Hospital will be extensively renovated. [13]

The collaboration with the HUCH Meilahti started with a brief meeting in the summer of 2005. Our liaison at the hospital is M.D. Veli-Jukka Anttila, a specialist in infectious diseases.

3.1 Nosocomial infections at the Meilahti Hospital

An all-embracing rate on nosocomial infections at the Meilahti Hospital is not available. The reason is, that such a figure contains no information that could help in the prevention struggle. Hence, no efforts have been made to define it. Instead, the rates of certain types of nosocomial infections are thoroughly monitored, e.g. bloodstream infections and surgical infections related to hip endoprostheses. Nevertheless, the nosocomial infection situation at the Meilahti Hospital is not thought to diverge from other national or international hospitals, although a vigorous comparison is not justified, since many delicate and severely ill patients, like transplantation patients, from all over Finland are centralised there [26].

The occurrence of antimicrobial resistance among pathogens is kept under close watch. Six small MRSA epidemics occured at the Meilahti Hospital in 2005. There were 85 new episodes of MRSA. During the past few years the number of MRSA episodes has turned down, partly because of the increase in hand disinfectant consumption (Subsection 3.2.1 and Fig. 3.3). The VRE situation is very good at the hospital and no succumbed cases were encountered in 2005, whereas the previous increase in ESBL episodes seems to be steadying. *A. fumigatus*, on the contrary, was isolated from 23 patients, compared to 16 in 2004. There has also been some problems with tobramycin-resistant *P. aeruginosa* (TRPA) -bacteria at one of the hematological wards, and despite assiduous efforts, the source of the bacteria has not been found. [144]

3.1.1 Bloodstream infections

Data on the predominance of nosocomial BSIs is useful to hospital hygiene personnel, as it contains information which aids to prevent them, e.g. how the infections arise (central venous catheterization is a common cause). The gratifying aspect of BSIs is that it is not very hard to determine whether the infections are hospital-acquired or community-acquired. In 2005 the overall number of BSIs at the Meilahti Hospital was 614, slightly more than during the previous year (599). Of these, 43% were hospital-acquired, compared to 45% in 2004. Only one MRSA strain was isolated from the blood samples, and the antimicrobial resistance of *E. coli* (ESBL) isolates stayed more or less the same as in 2004. [144]

3.1.2 Septicaemia-associated mortality

The mortality associated with hospital-acquired septicaemia varies depending on the causative pathogens. Some are more virulent than others. *P. aeruginosa* is one of the

bacteria showing the highest mortality among the patients at the Meilahti Hospital. During the past 10 years the mortality has ranged between approximately 20 and 70%. The steady increase during the previous two years was fortunately turned down in 2005. A significant portion of the mortality is due to TRPA. Another bacterium with high mortality rates is *S. aureus*. The mortality has swayed between 20 and 25% during the previous three years, but was periodically very low earlier. MRSA has played a very small role in these episodes. *E. coli* mortality, on the other hand, has decreased in the past two years and remains low. Hospital-acquired *Pneumococcus* infections occur seldom, but are quite lethal when they do. It should be noted that the above percentages are derived from often extremely small populations, so one additional case or one case less could sway them radically. Furthermore, the underlying health status of deceased patients is often pessimistic. [144]

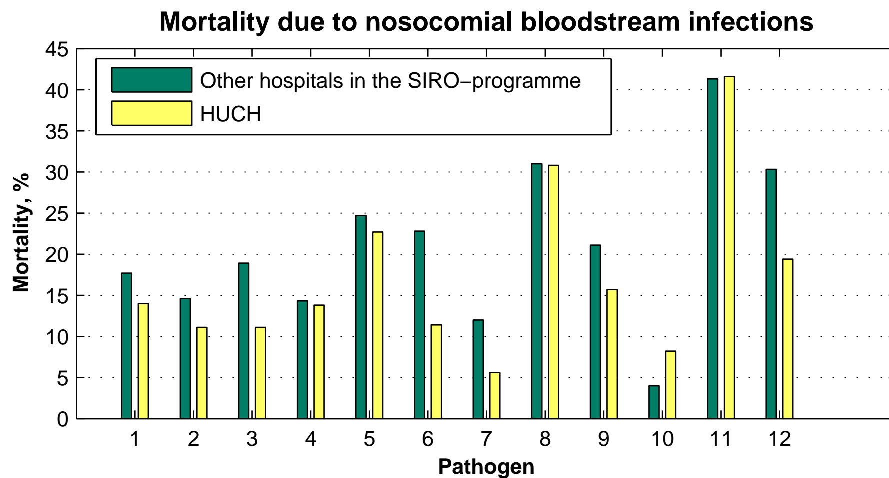


Figure 3.1: Comparison of 28-day mortality rates due to nosocomial bloodstream infections between the HUCH and all other hospitals in the SIRO-programme from 1998 to 2005. The pathogens are as follows: 1) All reported episodes, not just those reported here in detail 2) coagulase-negative staphylococci 3) *S. aureus* 4) *E. coli* 5) *Enterococcus* spp 6) *Klebsiella* spp 7) *Streptococcus viridans*-group 8) *P. aeruginosa* 9) *Enterobacter* spp 10) *Streptococcus agalactiae* 11) *C. albicans* 12) Other yeasts.
Source: SIRO – The Finnish national nosocomial infection programme, [26].

When comparing the 28-day mortality rates due to nosocomial bloodstream infections between the HUCH and other hospitals in the SIRO-programme from 1998 to 2005 (Fig. 3.1), an overall trend seems to be that the rates are somewhat lower at the HUCH. For some pathogens, e.g. the *Klebsiella* spp and the *Streptococcus viridans*-group, the mortality rates are even close to half as low at the HUCH. During this period of eight years, a total of 5,701 nosocomial bloodstream infections were diagnosed in the hospitals participating in the SIRO-programme. The HUCH stood for 3,084 of these. [26]

3.2 Current nosocomial infection prevention practices

The nosocomial infection prevention practices are related to the different means by which pathogens spread. From the viewpoint of nosocomial infections, the most important ones are *contact*, *droplet* and *airborne infections*. Infection by *contact* is the most common of these three. Pathogens are usually transmitted on the hands of health care workers from one patient to another when the treatment involves personal contact. The pathogens can originate from patient secretion, which often contains large amounts of bacteria, or from contaminated areas in the inanimate environment. The pathogens can survive for long periods of time on the hands, and are easily transmitted further. *Droplet* infection means that the pathogens are spread through large droplets, produced, for example, when sneezing, coughing or even talking. The droplets are heavy enough to be pulled down by gravity against airflows, and rarely fly farther than a metre. Accordingly, infection requires close proximity to the source. *Airborne* infection is spread through pathogen-containing aerosols, e.g. scurf, tiny droplets and dust particles. These are less than 5 μm in size, and are suspended in the air, consequently able to travel long distances with the aid of airflows. In the following subsections the discussion will be concentrated on prevention practices affecting these three pathways. Other vectors, like blood and food, are more seldom the mediators of nosocomial infections and hence omitted. [152]

3.2.1 Hand Hygiene

Good hand hygiene is paramount in the prevention of hospital-acquired infections. It sets up an effective road block on the contact infection pathway. Since this is the major highway for nosocomial pathogens, it is estimated that hospital-acquired infections can be reduced by roughly 50% by appropriate hand hygiene, as indicated in a study by Pittet *et al* [117]. But what does good and appropriate hand hygiene mean? The answer comprises the following topics: *handwashing*, *hand disinfection*, *surgical hand disinfection*, *tending the skin of the hands*, and *the use of protective gloves*.

Handwashing is nowadays used just to remove visible dirt from the hands. Previously handwashing was recommended in order to reduce the transient bacterial flora of the hands, but already Ignaz Semmelweis observed that simple handwashing with soap and water was not effective in the reduction of childbed fever episodes in the 1840s. Nonetheless, handwashing with soap was still recommended by the CDC in 1985 and hand disinfection with alcohol was not advised as a regular action. It took 10 more years before alcohol disinfection became the primary recommended alternative in the U.S. [112].

Finland, on the contrary, has been one of the pioneers in the use of alcohol-based disinfec-

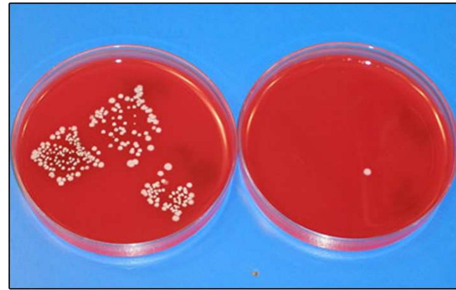


Figure 3.2: Comparison of the growth of bacterial colonies on blood agar plates before (left) and after (right) hand disinfection with an alcoholic disinfectant. Source: the Hand Hygiene Resource Center; `http://www.handhygiene.org/` (Accessed 8 Mar, 2006)

tants instead of soap as a means to remove the transient bacterial skin flora. Alcohol-based disinfectants have been shown to be much more effective, and by adding 1-2% of glycerol to the emulsion, the hands will not dry as after a traditional soap wash. The emulsions which are used in Finland contain 70 mass % ethanol and 3.0 mass % tert-butanol. They rapidly destroy bacteria and most viruses, including HIV, RSV, the rotavirus and hepatitis A and B viruses. The excellent thing about alcohol as a disinfectant is, that it instantly kills bacteria and they do not develop resistance towards it, although there are a few pathogens (e.g. *Clostridium difficile*) which alcohol disinfectants are ineffective against. For the alcohol emulsion to have proper effect, enough of it (usually 2–4 ml) has to be applied so that the hands remain wet for 20–30 seconds, and the correct technique has to be used when rubbing it into the hands. This means that all parts of the hands have to be worked through, and special care be given to the fingertips and the thumb. Alcohol disinfection is performed before and after *each patient contact*. Despite the clear evidence of the usefulness of alcohol disinfection (Fig. 3.2), the above guidelines are not always complied with. The reasons for this are not indifference and disbelief, but rather forgetfulness during hectic moments and an assumption that the alcohol disinfectant is used more than what is actually true [146].

The use of alcohol disinfectants is usually monitored by measuring how much of the emulsion is consumed. This serves partly as a motivating force, since the personnel on each ward can see how much has been used, and compare it to previous months and years. During the past few years, the consumption has been steadily increasing in the three biggest university hospitals in Finland (Fig. 3.3). The hospital-wide consumption is typically presented as litres per 1,000 patient-days, which is not very concrete. However, the numbers can be transformed into something useful quite easily. In 2005 the consumption at the Meilahti Hospital was 56 litres/1,000 patient-days (Fig. 3.3). This equals to 56 ml per patient per day. If the dose is 3 ml, the alcohol disinfectant is used, on average, about 19 times per patient each day at the hospital. Naturally, the consumption is not the same on all wards. The consumption is dependent on the number of patient contacts the health care workers perform, meaning that more is consumed in ICUs than, for example,

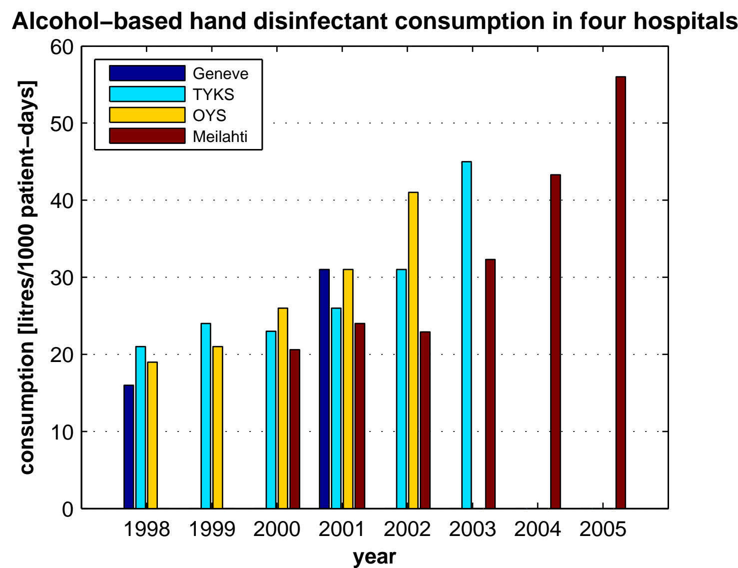


Figure 3.3: Comparison between the consumption of alcohol-based hand disinfectants during 1998–2005 in four hospitals: Geneva University Hospital (Geneve), Turku University Hospital (TYKS), Oulu University Hospital (OYS) and Meilahti HUCH (Meilahti). Source: The Hospital Hygiene Committee at the Meilahti Hospital.

on surgical or medical wards (Fig. 3.4).

Surgical hand disinfection is a more profound disinfection procedure. The aim is to remove the transient bacterial skin flora, as well as to reduce the resident flora, eventhough 20–25% of the skin's bacteria reside so deep that it is impossible to remove them without causing permanent skin damage. When performing a surgical hand disinfection, the disinfectant has to be applied all over the hands and even up to the elbow, with continuous replenishments so that the hands are wet for 2–3 minutes. Protective gloves are used to protect both the patient and the medical employee. Gloves are used when touching areas which are heavily contaminated with microbes (e.g. blood, secretion and wounds) and for example mucous membranes and catheters. The gloves are disposable, patient-specific and procedure-specific. They greatly reduce the amount of microbes attaching to the hands, although they do not provide a complete protection. Therefore, the hands need to be disinfected after use. The use of protective gloves should not lead to slackened hand disinfection habits with alcoholic emulsions. [142]

3.2.2 Pressurisation and filtration of air

There are basically two types of patients who stand out when pressurisation and filtration of air is considered in order to prevent nosocomial infections. These are 1) the patients who are prone to infections (e.g. transplantation and leukaemia patients), and 2) the patients who easily transmit diseases (e.g. pulmonary tuberculosis patients) [26]. They

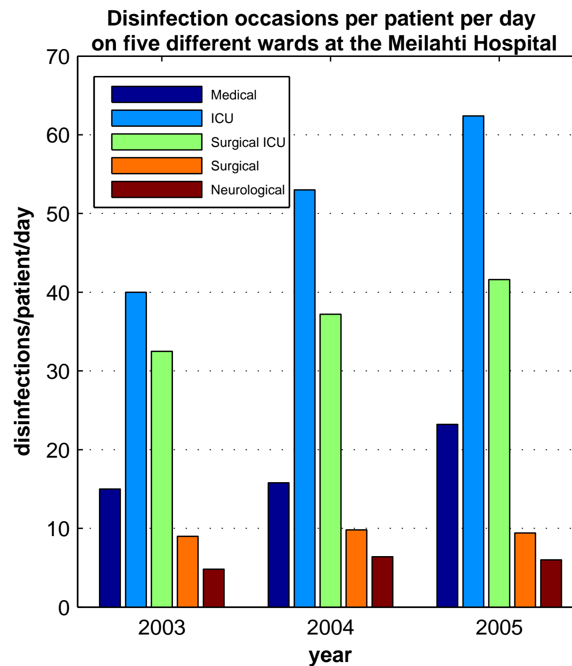


Figure 3.4: Comparison between the number of disinfection occasions on five wards at the Meilahti Hospital during 2003–2005. The figures have been calculated assuming that on one occasion 3 ml of disinfectant is consumed. Source: The Hospital Hygiene Committee at the Meilahti Hospital.

place different requirements on the pressurisation and filtration of air in the rooms where they are treated.

The infection risk of patients in group 1 has to be minimised. Thus, their rooms are held at a positive pressure compared to the ward corridor. This ensures that no microbes enter from outside the room. Moreover, the intake ventilation ducts are equipped with HEPA filters, preventing environmental airborne microbes, like *Aspergillus* spores, from entering. At the Meilahti Hospital, all new operating theatres, the hematological wards and the isolation rooms in the cardiosurgery ICUs are HEPA filtered and positively pressurised. The pressurisation of some operating theatres is adjustable. For instance, in case an infectious tuberculosis patient is undergoing surgery, the air pressure has to be negative, i.e. the air flows from outside into the operating theatre [26].

The reverse applies to patients in group 2. Their ability to *spread* infections has to be minimised. Hence, these patients are isolated and placed in rooms with a negative air pressure compared to the ward corridor. The negative air pressure ensures that airborne microbes in the room are confined to the room, and only removed via the exhaust outlets.

The number of microbes in the air depends on the number of people in the area, their movements, and the efficiency of the ventilation. Most airborne microbes originate from the skin of humans, but less than 1% are pathogenic (*S. aureus* dominates this fraction)

[141]. The potency of the ventilation is expressed in air changes/hour (ACH), which affects the time required to remove airborne contaminants to a certain degree (Fig. 3.5).

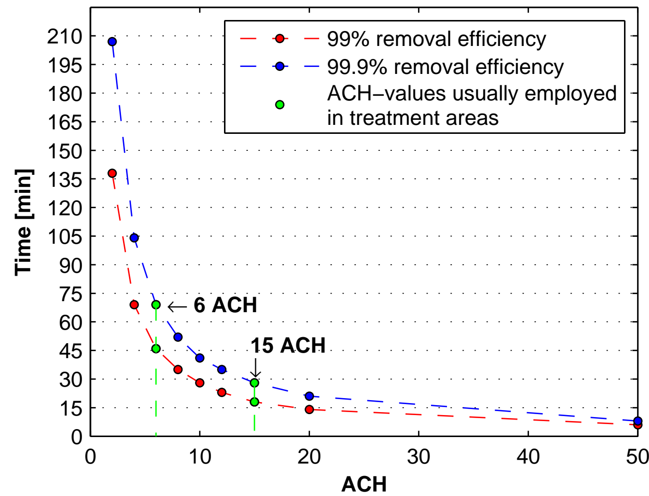


Figure 3.5: The time required to remove 99% and 99.9% of all airborne contaminants in a room with various degrees of air changes/hour (ACH). These values apply to an empty room where no aerosol-generating sources (e.g. persons) are present. Perfect air mixing in the room is likewise assumed when calculating the times. This seldom occurs in real life. [123]

The *Guidelines for Environmental Infection Control in Health-Care Facilities* by the CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC) specifies that protected environment rooms, where immunocompromised patients are kept, and airborne infection isolation rooms should have a positive/negative air pressure of ≥2.5 Pa in relation to the corridor, and ≥12 ACH in new rooms and ≥6 ACH in old rooms [123]. The same guidelines also dictate the ventilation requirements for operating theatres. A positive pressure in relation to corridors and adjacent areas has to be maintained. The ventilation system should provide ≥15 ACH, of which ≥3 ACH should be fresh air, i.e. not recirculated filtered air. In addition, all air, both recirculated and fresh, should be filtered with 90% efficiency. As already mentioned, all new operating theatres at the Meilahti Hospital are equipped with HEPA filters, which provide a much more efficient filtration than the current recommendation.

3.2.3 Control of waterborne microbes

In order to control the spread and proliferation of waterborne microbes like *Legionella* and *Pseudomonas* species in the water distribution systems, the warm and cold water temperatures are subject to the following recommendations. Warm water should be ≥51 °C and cold water <20 °C [123]. The hot water should be in constant circulation and the hot and cold water should not be mixed before the tap. Other general guidelines include

to keep seals and nozzles of showers and taps clean of sediment; to clean and disinfect cooling towers no less than twice a year with monthly checks; to clean heat exchangers and cold water basins and disinfect them with chlorine once a year; to clean and disinfect water filters once every 1–3 months; and to avoid sections in the piping where the water does not flow at all times [79].

At the Meilahti Hospital tap water is never used to clean medical instruments, because it is known that there are waterborne microorganisms in it. Instead, sterile water is utilised, for example, when rinsing equipment used for respiratory passage suctioning in ICUs. Shower heads and water conduits are temperature disinfected once a week. The shower heads are special made for hospital use by Oras Ltd to withstand high temperature washing. An unconventional method employed at the hospital is to leave the taps running for a while each morning, to flush out the microbes which have gathered in the stagnant water during the night. Care is also given to make sure that tap water is not aerosolised anywhere in the hospital, as this could produce airborne bacteria capable of causing respiratory nosocomial infections. [26]

3.2.4 Cleaning

The purpose of routine cleaning in a hospital is to reduce the amount of pathogens on surfaces in the inanimate environment below an infectious level. Faintly alkaline or neutral detergents are used. During cleaning, dust, organic and inorganic matter is removed, thus annihilating the breeding ground of bacteria. This prevents pathogens from thriving, and since many microorganisms cannot survive on dry surfaces for long, no disinfection is needed. Routine disinfection of surfaces is *de facto* not recommended in hospitals, because it is not worthwhile. Cleaning and drying with clean tools is sufficient on low-risk surfaces such as walls, floors, ceilings, beds and sanitary installations [122]. The floors of wards, for example, are heavily contaminated with bacteria, but are little affected by cleaning and subsequently soon retain the same level of contamination. However, this does not matter, seeing that the pathogens are mostly confined to the floor and air flows and person traffic do not contribute to their abundance [141]. The focus of cleaning is on surfaces which are frequently touched by patients and the personnel, e.g. doorknobs, water tap handles, light switches and bed rails. Another important cleaning procedure is the disinfection of secretion spills (blood and other body substances). By way of exception, disinfectants are used to clean up spills of secretion. Chlorine is generally used. The dilution should consist of 5,000 ppm available chlorine if it is directly applied on the spill, or 500 ppm available chlorine if the spill is initially dried up. The disinfection of secretion spills is the responsibility of all health care workers, and it has to be performed immediately after the incident [113]. The guidelines at the Meilahti Hospital dictate that all visible smears have to be removed prior to disinfection [26]. Further recommendations on and descriptions of

the cleaning practices in hospitals are available in [113, 123].

3.2.5 Other prevention practices

Besides the prevention practices briefly described above, there are a plethora of other routine measures which in their own small ways contribute to the prevention of hospital-acquired infections. There are preventative regulations on the cleaning and disinfection of treatment and other instruments; washing the laundry and the linen; isolation procedures; the allowance of flowers and plants in patient-care areas; the use of scrubs and protective clothing; and intubation and catheterization, just to mention a few. Hospital architecture and the space between beds in multipatient rooms are also examples of affecting factors. Even the constantly ongoing renovations at the Meilahti Hospital have to be incorporated into the prevention schemes. The doctors and nurses responsible for hospital hygiene have to be informed of the construction work, in order to minimise its effects on patient safety. Dust and spores are not easily confined to the construction area alone, and spores can cause serious nosocomial infections in immunocompromised patients.

The construction work does not even have to take place inside the hospital to be of consequence. In mid-October 2005 construction and excavation work was initiated outside the Meilahti Hospital's 13-bed stem cell transplantation ward, in the immediate vicinity of intake ventilation openings. Measures had to be taken to ensure the safety of the stem cell transplantation patients, since construction work inside or next to a hospital is known to increase the risk of mould infections [130, 145]. A protective barrier was erected between the construction site and the ventilation ducts. Among the other protective actions was to measure the air particle counts in the patient rooms five times a week until the end of the construction work at the end of the year. Particles above 0.3 μm were counted with a handheld particle counter. It proved the HEPA filters to be operative and able to keep the patient rooms safe from fungal spores in this case [26].

The details of the prevention practices associated with the above scenarios are not within the scope of this thesis, and are therefore dismissed. Many of the recommendations concerning these topics can be found in [123].

3.3 Possible uses of biosensing systems

Based on the previous sections of this chapter, and Chapter 2, three attractive areas where biosensing systems could be utilised to help in the battle against nosocomial infections stand out. These are:

- The ventilation system.

- The water distribution system.
- Cleaning.

In the case of the ventilation system, the objective of a biosensing system would be to verify the continuous effectiveness of the air filters, especially in premises with HEPA filters. By monitoring the bacterial burden of the water distribution system a biosensing system could warn about excessive levels of pathogens in the water. Since purer water is usually obtained by allowing taps to run a while, a biosensing system could work as an informer of when it is safe to use the water. Cleaning could likewise benefit from a rapid method to assess the effectiveness of the cleaning effort.

Detection is, in theory, possible on three levels: 1) qualitative (are microbes present or absent), 2) quantitative (are microbe levels within acceptable limits or not) and 3) discriminative (identification and separation of species). A quantitative detection level is a minimum requirement for biosensing systems employed in all three areas. It would suffice for biosensing systems concerned with the ventilation and water distribution system, whereas cleaning might benefit from discrimination of microbes, although it is not an absolute necessity.

Possible concepts of biosensing systems applicable in these areas are discussed in Section 5.3.

Chapter 4

Overview of Biosensing Systems

In the following sections different approaches to detect biological substances are discussed. All available or emerging means may not be included, but the chapter will try to give a glimpse of the overall status of biosensing technologies.

4.1 Electrochemical systems

The electrochemical biosensors were one of the first types of biosensing devices developed, and still play an essential role in biological detection. Electrochemical biosensors (Fig. 4.1) rely on a chemical reaction, which can be determined and converted into an electrical signal. The reaction takes place on a biocatalyst (A) where enzymes, antibodies or cells are usually immobilized. The reaction is determined by the transducer (B) and transformed into a signal. The signal is amplified (C), processed (D) and finally displayed (E). The working life time of electrochemical biosensors is application dependent and varies from single use to a few days or months.

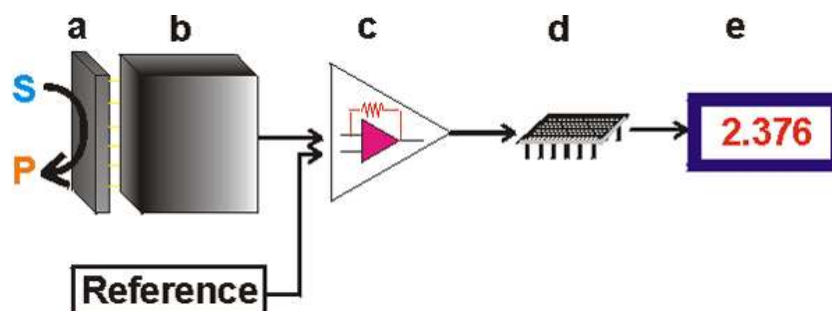


Figure 4.1: A schematic diagram of the principles of an electrochemical biosensor. A) A substrate (S) is turned into a product (P) on the biocatalyst. B) The reaction is detected and transformed into an electrical signal by the transducer. C) The signal is amplified, D) processed and E) displayed. [35]

4.1.1 Potentiometric biosensors

A potentiometric biosensor utilises an ion-selective electrode (ISE) to transform a chemical reaction on the biocatalyst into a detectable signal. Such a device requires two electrodes. One is the working electrode and the other the reference electrode. The working electrode is immersed in the sample and the reference electrode is kept in a solution containing the ion of interest at a fixed concentration. Hence the potential of the reference electrode is constant. The potential difference between the two electrodes depends on the concentration of the ion at the working electrode. This difference can be measured and consequently the concentration of the ion at the working electrode is determined. The most common potentiometric instrument is the pH electrode, which relies on a reaction where H^+ ions are either absorbed or produced. Many other ions, e.g. NH_4^+, I^- and CN^-, can be utilised as well, although it is the choice of membrane material on the working electrode which enables the fabrication of electrodes for the detection of a wide variety of agents. This is because with the right enzymes, the substrate of interest can be catalysed into a product and an ion that is detectable by an ISE. [35, 140]

4.1.2 Amperometric biosensors

Amperometric biosensors are based on the reduction or oxidation of an agent on the working electrode. As in the case of the potentiometric biosensor, a reference electrode is needed. The amperometric biosensor works by applying a potential between the two electrodes. This produces a current, which is proportional to the redox reaction at the working electrode. By measuring this current, it is possible to determine the concentration of the analyte at the working electrode. Basically it is feasible to detect any substance which can be oxidised or reduced with an amperometric biosensor. [35, 140]

Recently Llaudet *et al* reported on an amperometric biosensor for the real time measurement of adenosine triphosphate (ATP) in biological tissue [98]. ATP is the cell's special carrier of energy and plays an important part in extracellular signalling. Llaudet *et al* coated Pt electrodes with a porous silicate[1] biolayer, onto which two enzymes, glycerol kinase and glycerol-3-phosphate oxidase, were entrapped. In a two-step reaction with these enzymes, involving glycerol and oxygen, hydrogen peroxide (H_2O_2) is produced when ATP is present at the electrode. When the Pt electrode is operated at +500 mV relative to the reference electrode, the hydrogen peroxide breaks down into oxygen, protons and electrons. The subsequent current is the evidence of ATP in the sample. Clear ATP signals were obtained at concentrations as low as 100 nM (molarity M = mol/l).

[1] A silicate is a molecule comprised of a silicon oxide (Si_xO_y), some metals and occasionally hydrogen.

4.1.3 Conductometric biosensors

Conductometric biosensors rely on the measurement of the conductivity of a film or a bulk material, to detect the binding of an analyte to the sensor. Usually the resistance is measured using direct current (DC), but it is not unusual to use alternating current (AC), since it allows changes in the capacitive impedance to be detected. Thin films have been used as gas sensors, since their conductivity changes due to surface chemisorption[2]. In essence, conductometric biosensors are non-selective, but selectivity can be achieved by using modified surfaces. [140]

An example of this is the device developed by Cui *et al.* They have constructed silicon nanowires coated with molecular receptors [41]. As the target analytes bind to the receptors on the nanowire's surface, this results in a depletion or accumulation of carriers in the nanowire structure. This can be detected by a direct change in the nanowire's conductivity. In a test setup the binding of the protein streptavidin to biotin[3] molecules (also known as vitamin B_7 or vitamin H) entrapped on the nanowire was detected down to a concentration of 10 pM.

4.2 Optical systems

4.2.1 Quantum dot fluorescent labelling

Fluorescence labelling is a widely used method to observe structural compartments and molecules in cells, which are virtually transparent under visible light. Traditionally, this has been accomplished by the use of organic molecules with fluorescent properties. These fluorophores can bind either directly to the target molecules or indirectly via antibodies. In 1998 however, Alivisatos *et al*, as well as Nie *et al*, reported on the first use of quantum dot (QD) nanocrystals as biological labels [31, 34].

Quantum dots are small groupings of semiconductor atoms (~1,000 to as many as 100,000 atoms in one QD). Their sizes range from a few nanometres to tens of nanometres. Quantum dots have extraordinary electronic and optical properties, arising from the confinement of the electrons' motions by potential barriers in all three dimensions [9, 24, 116]. Compared to organic fluorescent molecules, quantum dots possess several advantages. Organic fluorophores can only be effectively excited with light, whose wavelength is in a very narrow window of the spectrum. This is followed by the emission of light at a longer wavelength. Both the excitation and emission wavelengths are unique for different fluorophores.

[2]Chemisorption is the process by which a molecule sticks to a surface by forming a chemical bond.

[3]Molecular formula of biotin: $C_{10}H_{16}N_2O_3S$

Quantum dots, on the other hand, can be excited with light from a broad spectrum. Thus, just a single light source is needed to excite QD labels of different colours, whereas each organic fluorophore demands its own excitation source. The emission spectra of QDs are also narrower and lack long tails, which makes it possible to discern the signals of QDs of many different colours simultaneously. Furthermore, QDs have longer fluorescent lifetimes and are brighter emitters of fluorescent light.

Sadly, there are a few drawbacks to QDs. Like their organic counterparts they have been reported to "blink", i.e. drift between emission modes of "on" and "off" [89]. Nevertheless, there is some controversy about the matter, as another report stated that the blinking might be due to the colloidal QDs' close proximity to surfaces, which is the case in most experiments. No blinking was observed from QDs in solution with no close surfaces (which corresponds to *in vivo* experiments) [94]. Greater disadvantages are, however, the inherent toxicity of many QDs and their insolubility in water. This renders them unusable for *in vivo* experiments as such, and therefore they have to be made biocompatible. The usual approach is to encapsulate the hydrophobic QDs with a layer of amphiphilic molecules[4]. The hydrophobic parts of these molecules are bound to the surface of the QD, leaving the hydrophilic head groups in contact with the aqueous medium. These coatings are unfortunately prone to disintegration. An approach to produce more stable shells is surface silanization. In this process a shell of hydrophilic silica (silicon dioxide, SiO_2) is grown around the QDs [57]. A hydrophilic shell is also needed since some QDs, like those made of cadmium and selenium, are toxic to many cells. From the *in vivo* perspective the stability of the shell becomes paramount, as most QD labels would stay in the tissue for months or even years [116].

4.2.2 Autofluorescence

Autofluorescence, i.e. the intrinsic fluorescence of substances within a microorganism, is seldom a desired property of samples studied under microscopes. There autofluorescence can be a real problem, but it is a phenomenon which can be exploited in biosensing schemes. The intrinsic fluorescence is due to certain molecules which fluoresce when irradiated with light of specific wavelengths. Especially the autofluorescence of bacterial spores has been utilised to separate them from non-biologic matter. Ho *et al* have constructed a fluorescent aerodynamic particle sizer (FLAPS) for the detection of bioaerosols [70, 71]. They used an ultraviolet (UV) laser at wavelengths from 340 to 360 nm to get an autofluorescence response from *Bacillus globigii* spores, a harmless *Bacillus anthracis*[5] simulant. The fluorescent emission can be detected on wavelengths between 400 and 550 nm. It is most likely the biological molecule reduced nicotinamide adenine dinucleotide

[4] Amphiphilic (amphipathic) molecules have both hydrophilic ("water loving") and hydrophobic ("water fearing") properties.

[5] *B. anthracis* is the bacterium causing the deadly disease anthrax.

(NADH) which emits fluorescent light in this case. NADH plays an essential role in the energy production of cells and is present in all cells, but to different extents.

Recently, Davitt *et al* have reported on an instrument with the same objective, however, making use of arrays of UV light-emitting diodes (LED) instead of UV lasers. These UV LED arrays, emitting light at 290 nm and 340 nm, target aerosolised particles containing the biological molecules tryptophan (an amino acid) and NADH. Tryptophan was detectable at concentrations below 125 μM and NADH at 140 μM [44, 43]. For a more detailed description see Subsection 5.3.1.

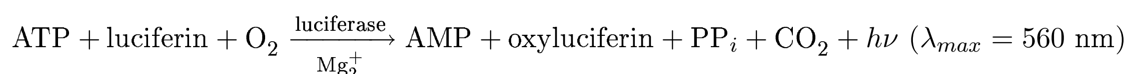
At least one study has been conducted to assess whether autofluorescence can be used as a viability marker for bacterial spores. Laflamme *et al* discovered that the autofluorescent spores were 1.7 times more culturable when compared to the corresponding population of non-autofluorescent spores [91].

4.2.3 Chemi- and bioluminescence

Chemiluminescence is the process where a chemical reaction produces light. In its simplest form, chemiluminescence arises when two reactants, A and B, react in the presence of a suitable catalyst and produce some products and light: $\mathrm{A} + \mathrm{B} \xrightarrow{\text{catalyst}} \text{products} + \text{light}$. If the chemiluminescence takes place inside a living organism, it is called bioluminescence. Usually, the emitted light is born when an O−O bond of an organic peroxide compound is cleaved in the reaction. This is the case in the most common chemiluminescence reaction, where hydrogen peroxide and luminol (5-amino-2,3-dihydro-1,4-phtalazinedione) react in alkaline conditions, catalysed by the enzyme horseradish peroxidase. Herein $h\nu$ is the energy of the emitted photon, where h is Planck's constant and ν the frequency. [16, 29]

$$2\,\mathrm{H_2O_2} + \text{luminol} + \mathrm{OH^-} \xrightarrow{\text{peroxidase}} 3\text{-aminophthalate} + \mathrm{N_2} + 3\,\mathrm{H_2O} + h\nu \ (\lambda_{max} = 430 \text{ nm})$$

Bioluminescence involves an enzyme-catalysed reaction, where a luciferin[6] is oxidised by a luciferase[7]. This process enables the detection of ATP when luciferase from the firefly *Photinus pyralis* is used. The light-producing biochemical reaction happens in multiple stages, but in a simplified form ATP, luciferin and oxygen molecules are catalysed by luciferase and magnesium ions, producing oxyluciferin, by-products and light.



$$\text{ATP} + \text{luciferin} + \mathrm{O_2} \xrightarrow[\mathrm{Mg_2^+}]{\text{luciferase}} \text{AMP} + \text{oxyluciferin} + \mathrm{PP}_i + \mathrm{CO_2} + h\nu \ (\lambda_{max} = 560 \text{ nm})$$

[6]Luciferin is a common name for pigments found in living organisms capable of bioluminescence, for instance, fireflies, deep-sea fish and microbes.

[7]Luciferase is the name of a group of enzymes used as catalysts in bioluminescence reactions.

Here PP_i is the anion of pyrophosphoric acid[8], whereas AMP stands for adenosine monophosphate, which is the twice hydrolysed form of ATP. The emitted light has a peak wavelength of 560 nm (yellow-green). The above reaction is the most energy efficient chemiluminescent reaction known, boasting a quantum efficiency of 88%. This means that nearly all of the input energy is converted into light.

Another important biological substance which can be detected by bioluminescence is NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate (NADP), an important coenzyme in cells. In this case, the reaction is also multistaged, but different reactants are necessary and the luciferase of the marine bacteria *Vibrio fischeri* or *Vibrio harveyi* is used to catalyse the reaction. The emitted light has a peak wavelength of 490 nm (blue-green).

Electrochemiluminescence (ECL) is a third type of luminescence. Basically, it is a matter of a chemical reaction which is electrically stimulated to produce light.

4.2.4 Fourier transform infrared spectroscopy

Molecules can be characterised by the wavelengths of the electromagnetic spectrum which are absorbed when light is passed through them. By recording the intensity of the penetrated light at different wavelengths, a unique fingerprint is obtained for the sample, enabling its identification. Which frequencies are absorbed depends on the resonant frequencies of the chemical bonds in the molecules. These, in turn, are dependent on the bond type, the length of the bond and the masses of the atoms connected by the bond.

In Fourier transform infrared (FTIR) spectroscopy, coherent infrared (IR) light is guided through an interferometer before passed through the sample. In its most simple form, the interferometer consists of two mirrors; one stationary and one moving, and a beamsplitter. The IR light is split into two perpendicular arms by the beamsplitter. The mirrors are positioned at the ends of these arms and the reflected beams are reunited by the beamsplitter. By moving the other mirror, a phase shift is introduced between the recombined beams and an interference pattern is formed. The phase shifted beams are passed through the sample. A detector measures the signal, which is the interferogram, and a mathematical Fourier transformation is performed on it. The spectrum which is thus produced is identical to the one obtained from dispersive IR spectroscopy by recording absorbance, but the frequency of the monochromatic IR source does not have to be adjusted. This technique can not just be used to probe the composition of the sample, but also to discriminate and identify microorganisms, since they exhibit unique IR spectra as well [102, 103].

[8] The structure of PP_i is $P_2O_7^{4-}$.

4.2.5 Holographic sensors

Holographic sensors are unique in the sense that the same sensing element functions as both the interrogator of the sample and the optically reporting transducer [99]. In other words, there is no need for further electronic processing. The principles behind a simple reflection hologram as a biological and chemical sensor are rather elemental.

Conventional "white-light reflection holograms" consist of glass or plastic substrates covered with a gelatin-silver halide[9] photographic emulsion. This holographic plate is exposed to light from a single laser, whose beam is split in two. The interference of the reference beam, which is passed via a mirror straight from the laser, and the object beam, which is reflected from the target object, and the subsequent development and post-processing give rise to fringes of the silver halides within the gelatin. The fringes lie in planes parallel to the gelatin surface. The planes lie approximately half a wavelength from each other. Upon illumination with white light, the diffraction gratings work as wavelength filters and reproduce the original object as a monochromatic three-dimensional image. The partial reflections from the fringes interfere constructively, and produce a spectral peak whose wavelength is described by the Bragg equation ($\lambda_{max} = 2nd\cos\theta$), where n is the average refractive index, d the distance between the fringe planes and θ the angle of incident light. Now, the basis for a holographic sensor is the spectral peak wavelength's dependence on n and d. If either of these is modified by any means, it will have an impact on the wavelength and intensity, i.e. the colour and the brightness, of the hologram. [16, 99]

The group of Christopher R. Lowe at the University of Cambridge, U.K., have developed holographic sensors for the monitoring of pH [105] and glucose [95]. The pH monitoring sensors were fabricated using a thin, polymeric, hydrogel[10] film, containing acidic or basic monomers, instead of the gelatin. Ionisation of these monomers causes the hydrogel to swell, and hence, the fringe spacing is changed, which increases the diffraction wavelength of the hologram and red shifts the colour. A contraction of the hydrogel results in the opposite, and the colour blue-shifts. Thus, the pH of the medium, which is in contact with the hologram, is sensed by the sensor. Lowe *et al* have also turned this holographic sensor into a biosensor able to detect urea and penicillin, by immobilising catalysing enzymes on the hologram surface [106].

The glucose-sensitive holographic sensor works much in the same way, except that a glucose binding ligand is used in the hydrogel film. The advantages of holographic sensors are their inexpensiveness, readiness to be mass-produced, longevity and ability to be reused.

[9] A silver halide is a compound of silver and a halogen. The most common silver halides are silver bromide (AgBr), silver chloride (AgCl) and silver iodide (AgI).

[10] Hydrogels are networks of polymer chains which can absorb vast amounts of water.

4.2.6 Surface plasmon resonance

A surface plasmon is a transverse magnetic (TM) electromagnetic surface wave, which exists at the interface between one medium with a positive dielectric constant (insulator) and another medium with a negative dielectric constant (metal) [151]. Surface plasmons arise when p-polarised monochromatic light of the right wavelength propagates through a dielectric prism coated with a thin layer of a noble metal (usually gold) under the condition of total internal reflection (TIR). Under the right conditions the incident photons interact with the free electrons of the metal surface and are absorbed and converted into surface plasmons [104]. This leaves a gap in the reflected light intensity profile (Fig. 4.2). This phenomenon is called surface plasmon resonance (SPR).

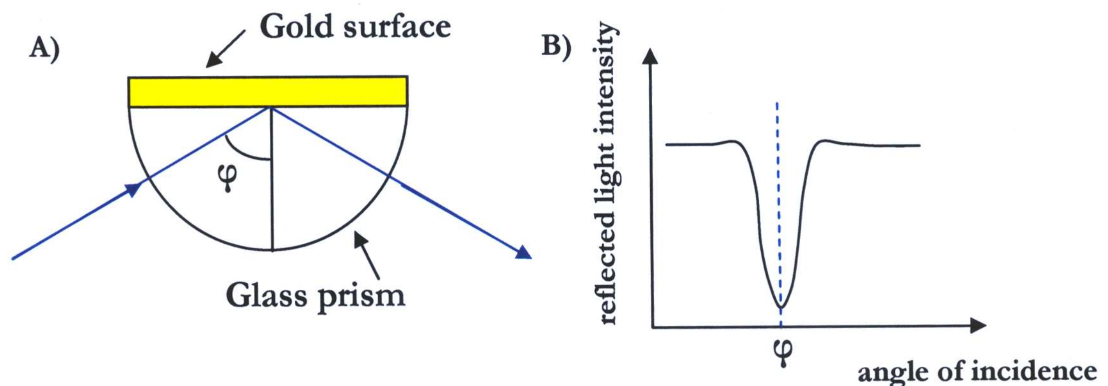


Figure 4.2: A) Monochromatic light is totally reflected in the interface between a glass prism and a thin gold surface. B) Surface plasmons emerge as the wavelength and angle of incidence of the photons are just right. This results in a dramatic drop in the intensity of the reflected light.

As photons are reflected under TIR conditions they create an electrical field on the other side of the interface. Surface plasmons create a similar field on either side of the interface along which they propagate. This electrical field is called an evanescent wave, since it decays exponentially as a function of the distance from the interface. The velocity of the evanescent wave is naturally dependent on the refractive index of the medium it travels in. Consequently, it is very sensitive to matter bound to the far side of the metal surface. The velocity of the evanescent wave directly affects the velocity of the plasmon, which in turn affects the angle at which resonance occurs.

So the angle at which resonance occurs is dependent on the matter bound to the surface of the metal coating. This property makes the phenomenon suitable for use as a selective detector. One part, the ligand, of an interaction pair of molecules, is immobilised onto the metal/sensor surface. The other part, the analyte, is present in an aqueous buffer solution. The solution is run past the sensor surface, which forms one of the walls in a flow channel. As the analyte molecules are bound to the ligands on the sensor surface, the plasmon resonance angle changes (Fig. 4.3). This can be measured very precisely. The

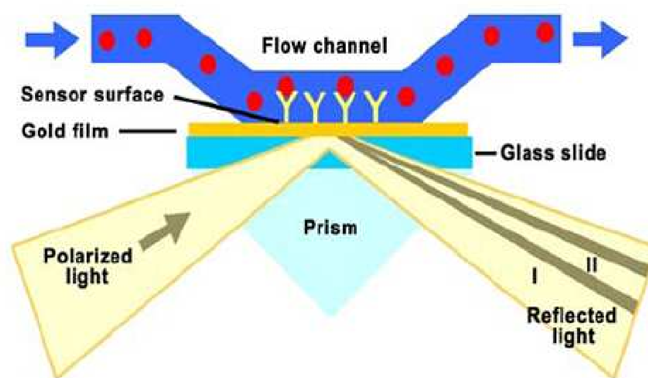


Figure 4.3: The basic components of a SPR biosensing device. The red dots in the flow channel are the analytes and the yellow 'Y's are the ligands immobilised onto the sensor surface. [32]

changes in reflectivity are proportional to the amount of analyte bound near the surface. SPR devices are extremely sensitive, but also expensive. [32]

4.2.7 Surface-enhanced Raman scattering

Before going into what surface-enhanced Raman scattering (SERS) is, the principles of Raman scattering are briefly explained to begin with.

As photons hit molecules they can scatter either elastically or inelastically. The former is called Rayleigh scattering and means that the scattered photons have the same energy, i.e. same frequency and wavelength as the incident photons. The latter is called Raman scattering and involves scattered photons whose energy is different compared to the incident photons. If the energy of a scattered photon is lower than the incident photon's, i.e. the wavelength is longer, the process is called Stokes scattering. The opposite process is called anti-Stokes scattering (the energy of the scattered photon is higher). But what gives birth to the two different Raman scattering processes?

Raman scattering is entwined with the vibrational state of the interacting molecule. In Stokes scattering the incident photons lose energy due to excitation of the molecule's vibrational ground state to a higher vibrational state (Fig. 4.4). In anti-Stokes scattering, in contrast, the incident photons gain energy since they interact with a molecule in an excited vibrational state. So in essence, the Raman effect probes the vibrational states of a molecule, and subsequently the scattered light is a fingerprint of the molecule's structure, since the vibrational levels depend on the atoms and bonds of the molecule [83].

A major disadvantage of conventional Raman spectroscopy is its insensitivity. In the mid-1970s, however, the group of Fleischmann experienced greatly enhanced Raman scattering from roughened silver electrodes onto which pyridine, an aromatic organic molecule, had

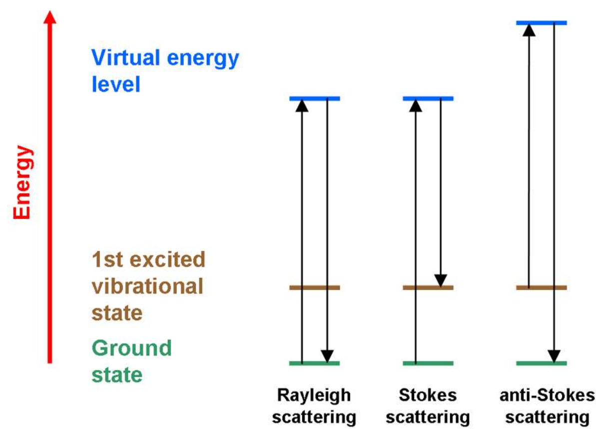


Figure 4.4: Comparison between the Rayleigh and the two types of Raman (Stokes and anti-Stokes) scattering processes. Rayleigh scattering is an elastic process since the incident and scattered photons have equal energy. Raman scattering is inelastic, as the scattered photons have either a lower (Stokes) or higher (anti-Stokes) energy than the incident photons. [16].

been adsorbed. This effect was later named surface-enhanced Raman scattering. SERS is observed from molecules which are in the vicinity of metallic "nanostructures". These can be electrode surfaces, often roughened, but not necessarily, evaporated island films or colloids. The strongest enhancements are obtained from clusters of colloidal particles, 10–100 nm in size. These can be either in a colloidal solution or deposited on a surface. The most commonly used substrates are the coinage metals; gold, silver and copper, due to their superior enhancing powers [33]. Colloidal clusters or island films of gold or silver can provide enhancement factors over 10^{12} [83]. The entire mechanisms behind the SERS process are not understood, but experiments indicate that two effects contribute to the enhancement. The first effect is an electromagnetic field enhancement due to the excitation of surface plasmons on the metal surface in the molecule's proximity. The second effect is a chemical enhancement, which is weaker than the electromagnetic, but since these effects are independent their effects are multiplicative if simultaneously present. The chemical enhancement does not occur for all molecules, for example, the SERS intensities of CO and N_2 differ by a factor of 200 [33]. Details of these enhancement effects can be read in the two references already mentioned in this subsection.

SERS has many applications, ranging from single-molecule detection and identification of microorganisms to immunoassays and the probing of DNA without labelling.

4.3 Microsystems for biosensing applications

Microelectromechanical systems (MEMS) are manufactured with techniques adapted from the fabrication of electronic semiconductor chips and range from micrometres to millimeters in size [132]. MEMS differ from conventional electronic components in the sense that

mechanical parts, which can move or can be mechanically perturbed, are also incorporated into the design. If the MEMS device is "[...] used for processing, delivery, manipulation, analysis, or construction of biological and chemical entities" [28] it is called a biological microelectromechanical systems (BioMEMS) device. The discussion in the following two subsections will focus around BioMEMS technologies harnessing mass sensing and microfluidic systems, albeit the MEMS field is broad and this merely scratches its surface.

4.3.1 Mass sensing

BioMEMS devices provide an excellent platform for recognition of biological substances through mass sensing. A great advantage of mass sensing is the redundancy of biological labels and markers, as the mass itself generates the detector response. Unfortunately, the target molecules have to be selectively identified and bound to a surface, which leaves open the possibility of nonspecific adsorption and subsequent false positives [132]. These problems are, however, being addressed by, for instance, employing reference devices or suitable surface chemistries, but are well and truly not unique to this class of sensors. Micro- and nanodevices capable of mass sensing can be split into *piezoelectric crystal-based* and *silicon MEMS-based* devices.

Piezoelectric crystal-based devices create standing or travelling acoustic waves which propagate across the sensor surface. The acoustic waves are generated by temporally or spatially varying electrical signals acting on a film of piezoelectric material (e.g. quartz or zinc oxide). The propagation of the acoustic waves depends on the matter bound to the sensor surface, thus providing a means to recognise the presence of the target analyte. Piezoelectric devices using surface acoustic waves (SAWs) and quartz crystal microbalances (QCMs) are the most well-known, although they may experience unacceptable damping in aqueous environments. [132]

Silicon MEMS-based devices consist of a micro- or nano-scale cantilever (or an array of them) made of a non-piezoelectric material, often silicon. These diving board type structures, which are 0.2–1 μm thich, 20–100 μm wide and 100–500 μm long [133], can operate in two modes: surface stress change detection and mass change detection. In the former, the biochemical reaction of the target species binding to one side of the cantilever changes the surface free energy. This changes the surface stress and subsequently results in the cantilever bending (Fig. 4.5). In mathematical terms, the deflection of the tip of the cantilever can be expressed as

$$\Delta z = 4\left(\frac{L^2}{t}\right)\frac{(1-\nu)}{E}\left(\Delta\sigma_1 - \Delta\sigma_2\right) \tag{4.1}$$

where L is the length and t the thickness of the cantilever, E Young's modulus[11], ν Poisson's ratio[12] and $\Delta\sigma_1$ the change in the surface stress on the top surface and $\Delta\sigma_2$ on the bottom surface of the cantilever [28]. The bending can be read either optically or electrically. Optical detection utilises a laser beam targeted at the tip of the cantilever. As the cantilever bends, the angle of the reflected beam will change, which can be measured very precisely. Electrical detection can be, for example, capacitive, where a plate-to-plate gap varies due to the bending of the cantilever. The sensitivity of this sensor is increased by reducing the spring constant, but the total area of the cantilever should also be considered, as it dictates how many target analytes need to bind to the surface in order to produce a detectable bending.

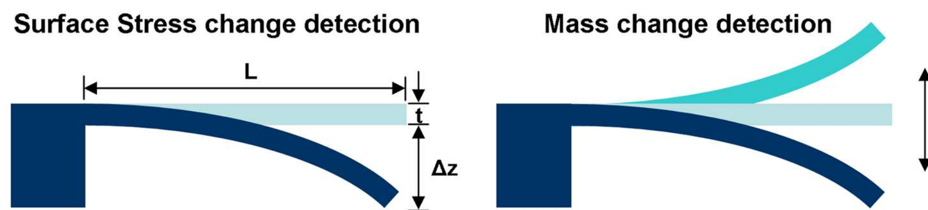


Figure 4.5: Principles of surface stress change and mass change detection using a microcantilever. Adapted from [28].

Mass change detection, on the other hand, involves a vibrating cantilever (Fig. 4.5). When the target species binds to the cantilever, its resonant frequency shifts (the cantilever's spring constant is assumed to remain unchanged), which can be measured either electrically or optically. By comparing the resonant frequency with and without the analyte bound to the sensor, the change in mass can be calculated with

$$\Delta m = \frac{k}{4\pi^2}\left(\frac{1}{f_1^2} - \frac{1}{f_0^2}\right) \tag{4.2}$$

where k is the spring constant, f_0 is the unloaded resonant frequency and f_1 is the loaded resonant frequency [28]. The sensitivity of the mass change detection mode is increased by reducing the mass of the cantilever and decreasing the lower limit of detectable resonant frequency shifts. Like the SAW and QCM devices the vibrating cantilever experiences damping in fluidic mediums, making the stress sensing mode the preferred choice in these cases. Nevertheless, if the target species is a whole organism, e.g. a cell or a bacterium, this mode is, in all probability, ineffective as a detection technique, since the organism might not produce the necessary uniform change in stress over the cantilever surface. The vibrating mode, in turn, has been used to detect microorganisms, e.g. *E. coli*, by attaching

[11] Young's modulus is a measure of the stiffness of a material. It is the ratio between tensile stress and strain. [16]

[12] Poisson's ratio is associated with the stretching and contraction of a body when a force is applied to it in one direction. It is the ratio between the contraction strain perpendicular to the force and the extension strain in the direction of the force. [16]

microbe-specific antibodies on the cantilever surface [76].

Microcantilever devices have a response time in the milliseconds and commonly feature nanogram (10^{-9}), picolitre (10^{-12}), femtojoule (10^{-15}) and attomolar (10^{-18}) sensitivities [143]. Microcantilever-based sensors resonating at lower frequencies than SAW-based sensors (20–200 kHz vs. 5–500 MHz) can be more sensitive, and indeed, it is estimated that a nanoscale cantilever, whose resonant frequency reaches the megahertz mark, could be capable of mass sensing approaching the zeptogram (10^{-21}) level, i.e. single molecules [133].

4.3.2 Microfluidic systems

Microfluidic systems are fabricated using many of the same methods familiar from the production of MEMS. Unlike classical MEMS, however, they contain fluidic microcomponents (e.g. channels, valves, pumps, reservoirs, fluid interconnects, filters) in addition to the sensors and detectors [132]. Originally, the field of microfluidic systems had two approaches. One was to combine microsensors with these fluidic microcomponents, creating whole systems. The other was to miniaturise the biochemical laboratory or analytical chemical methods onto a single chip [25]. The latter has received far more emphasis, especially genetic analysis. The PCR procedure (see Subsection 4.4.1), for instance, has been implemented in a microfluidic device [84].

Since the size of a cell is of the same magnitude as common microfluidic devices (10–100 μm) microfluidic systems are perfectly suitable for cell analysis. Some further advantages of these devices are their fine ability to manipulate single objects of cellular dimensions, the fast mass and heat transfers and the possibility to generate strong electrical fields with small voltages in these systems [25]. A microfluidic device for thorough cell analysis is required to be able to perform 1) sampling, 2) trapping and sorting, 3) treatment, and 4) analysis of cells. In the following, a few miniaturised concepts of cell trapping and sorting will be unfolded. For methods concerning the other procedures the reader is referred to the already mentioned review by Andersson *et al* [25].

Mechanical trapping of cells is possible by, for instance, microfabricated filters. These filters consist of parallel, rectangular channels of such width and height that particles of larger dimensions are prevented from entering. Because electric fields are quite easy to generate and structure on microchips, electrokinetic separation and transportation of molecules and cells is perhaps a more attractive alternative. Transportation of cells (pumping) is possible through electrophoresis (the movement of a charged substance due to a uniform electric field), as almost all biological cells have more or less the same electrophoretic mobilities. [25]

Separation and trapping by electric fields can be accomplished with dielectrophoresis. In dielectrophoresis a non-uniform electric field exerts a force on a dielectric particle, i.e. a charged particle is not required. As all particles have dielectrophoretic properties, depending on their chemical make-up, their physical attributes and the frequency of the AC electric field, it is possible to manipulate them by tuning the field. This has enabled separation of cells and viruses of different kinds in microfluidic devices [74, 111]. Advantages of dielectrophoresis are its non-invasiveness and easy incorporation into microfluidic systems thanks to similar manufacturing technologies [75].

Finally, cell sorting is feasible with flow cytometry. In flow cytometry a stream of fluid, which contains suspended particles, is targeted by a laser beam. Several detectors are focused on the intersection and both scattered and fluorescent light are measured. Different particles in the stream produce different scattering and fluorescent signals, and by analysing these, discrimination between different populations becomes possible. Particles of one population can then be charged after passing the laser beam and later extracted from the stream into a separate flow [16]. Flow cytometry of, for instance, *E. coli* has been demonstrated by McClain *et al* [109].

4.4 Nucleic acid-based systems

Nucleic acid-based systems utilise the ever-present, organism-specific nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) to detect microorganisms. These systems are typically extremely sensitive, but fairly slow and often arduous.

4.4.1 Polymerase chain reaction

Polymerase chain reaction (PCR) is a technique to amplify a specific region of a DNA sequence *in vitro*. The DNA is enzymatically replicated and increases in an exponential manner. The amount of DNA from the region can be amplified a billionfold, facilitating analysis as more DNA is available. PCR has many applications and is frequently used in medical and biological research laboratories. Besides its use in paternity testing, detection of hereditary diseases, genetic fingerprinting, cloning of genes, and diagnosis of infectious diseases, PCR is also applicable to biosensing systems. The traditional PCR technique will be briefly described below, but there exists a legion of modified versions, like nested, inverse, reverse transcription (used to amplify data contained in RNA), asymmetric, touchdown, colony, quantitative, and quantitative real time PCR, which will be omitted, except for quantitative PCR which will be just shortly mentioned. The reader is referred to [16] for descriptions of these.

PCR is able to copy very short DNA fragments, commonly up to 10 kb (kilo base pairs), although some variations of the technique are able to extend this to 40 kb. Nonetheless, these fragments are infinitesimal compared to the whole genome of an eukaryotic cell[13]. The DNA fragment which is to be amplified has not only to be short, but also well-defined. The PCR procedure needs the following basic ingredients: a *DNA template* which contains the fragment which is to be copied, two *primers* which mark the beginning and end of the fragment, *DNA polymerase* which is the enzyme doing the copying, *nucleotides* which are the building blocks of DNA, and a *buffer* which the polymerase is compatible with.

The DNA amplification is carried out in three steps in a thermal cycler, which adjusts the temperature of the mixture to the appropriate level for each step. These three steps have to be repeated in several (20 to 35) cycles. Let us walk through these three steps and see what happens in each of them.

1. *Denaturing.* The mixture is heated to 94–96 °C for 1–5 minutes in order to separate the strands of the DNA double helix. The heat breaks the hydrogen bonds between the strands and thus produces single-stranded DNA molecules. Because of the necessary high temperature of this step, DNA-polymerase enzymes from hyperthermophilic[14] bacteria or archaea have to be used (e.g. *Thermus aquaticus* or *Pyrococcus furiosus*). The DNA-polymerase of these organisms stays stable even at high temperatures.

2. *Annealing.* Once the double-stranded DNA has been denatured, the temperature is lowered to allow the primers to bind to the single DNA strands. The primers are short oligonucleotides[15] (usually 18–25 base pairs, but never more than 50), which have been chosen to flank the interesting DNA fragment to be copied. One primer binds to the beginning of the fragment on one strand and the other to the end on the other strand. Consequently, there is only one primer attached to each single-stranded DNA molecule. The temperature of this step depends on, among other things, the primers' lengths, but is usually around 45–70 °C. This step takes 1–2 minutes.

3. *Extension.* During this step the DNA-polymerase copies the DNA starting from the primers. The temperature and time of this step depends on the DNA-polymerase, but the time is dependent on the length of the fragment as well.

After each cycle the newly synthesized DNA fragments also serve as templates, thus doubling the amount of DNA fragments in the mixture. After a few cycles most of the DNA

[13] The human genome consists of approximately three billion base pairs.

[14] Hyperthermophilic organisms live in excruciatingly hot environments, with optimal temperatures around 80–110 °C.

[15] An oligonucleotide is a short molecule of nucleic acid monomers, forming a single-stranded DNA chain. They are commonly used as probes and primers.

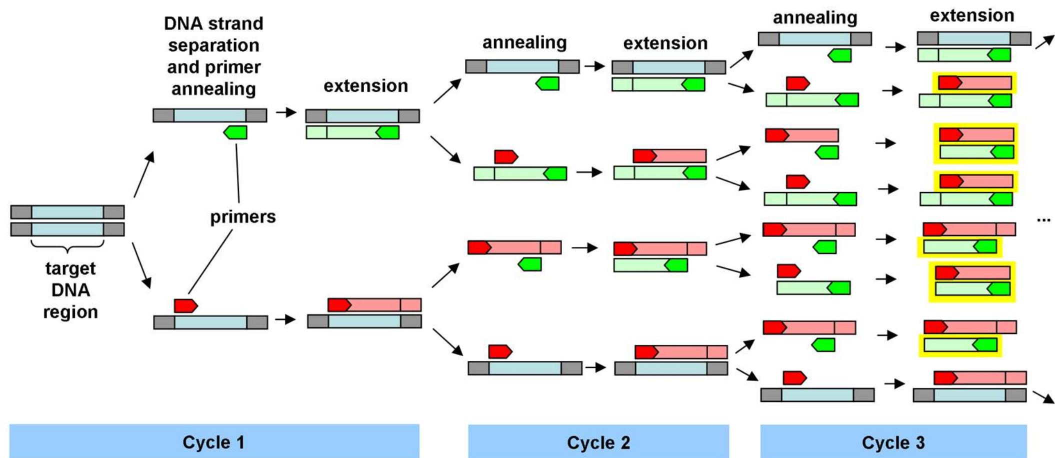


Figure 4.6: A schematic diagram of the polymerase chain reaction procedure. After three cycles 8 out of 16 DNA strands are duplicates of either strand from the target region (enveloped in yellow). Adapted from [23].

strands are of a single kind and correspond to the DNA region between the original primers. Starting from a single double-stranded DNA template, three cycles into the process 8 out of 16 DNA chains are exact copies of either strand from the target region (Fig. 4.6). It only takes four more cycles to increase this ratio to 240 out of 256, and when the procedure is finished the great majority of DNA chains are from the amplified region.

The PCR method is exceptionally sensitive, as it is possible (at least in theory) to start with a single DNA template and amplify it to detectable levels. Quantitative PCR can be used to quickly measure the amount of PCR product. The three main methods are agarose gel electrophoresis, SYBR green dyeing and the use of fluorescent reporter probes. The latter two enable quantitative real time PCR, as they rely on fluorescence and the ongoing reaction can be monitored as it unfolds. [16, 23]

4.4.2 Nucleic acid sequence-based amplification

Nucleic acid sequence-based amplification (NASBA) is a method which resembles PCR in many ways. However, the target of NASBA is primarily RNA, which is amplified very sensitively *in vitro*, just like in reverse transcription PCR (RT-PCR), but here the similarities between the procedures end. Whereas PCR consists of three repeating steps, NASBA comprises only one *initiation phase* and a single self-sustained *amplification phase*. Furthermore, the amplification step is isothermal, eliminating the need for thermal cycling equipment. The biggest difference between NASBA and RT-PCR is the fact, that the single-stranded RNA amplicons produced by NASBA are easily detectable by molecular probes. A further advantage of NASBA is its capability to amplify the target single-stranded RNA molecules even in the presence of double-stranded DNA with the same

sequence. Only when the target RNA is abscent or target DNA is present over 1,000-fold compared to the target RNA, is it possible that the procedure amplifies DNA as well, although less efficiently. RNA sequences which can be efficiently amplified by NASBA are about 100–250 nucleotides in length. [46]

A modified NASBA procedure has also been presented for the specific amplification of DNA [46], but this text will focus on the RNA NASBA method.

The RNA NASBA method proceeds as follows (Fig. 4.7). During the initiation phase the mixture of isolated RNA and specific "forward" and "reverse" primers, which mark the beginning and end of the target sequence on the RNA, are incubated at 65 °C. This allows the forward primer to hybridise to the target RNA, because it possesses a sequence which is complementary to the beginning of the target sequence. The temperature is subsequently lowered to 41 °C and three enzymes are added: avian myeloblastosis virus (AMV) reverse transcriptase[16] (RT), RNase H and T7 DNA-dependent RNA polymerase[17] (DdRp). The AMV RT will now extend the forward primer which has bound to the RNA, thus producing a cDNA-RNA hybrid. At the same time, however, RNase H degrades the RNA of the hybrid molecule. In essence, this produces a cDNA strand, whose beginning consists of the forward primer, followed by the target sequence, the reverse primer's complementary sequence and finally a possible sequence of the rest of the original RNA molecule. Consequently, the reverse primer hybridises to its complementary sequence on the cDNA strand, and the AMV RT extends it to the end of the cDNA strand containing the forward primer. Hence, a double-stranded DNA molecule is produced of the target sequence on the RNA. The forward primer, which is now incorporated into this molecule, contains a promoter of T7 DdRp, and since the promoter is now double-stranded, the T7 DdRp can attach itself to it and synthesize many new RNA strands (called amplicons) which are complementary to the target RNA sequence.

Moving forward, NASBA proceeds to the amplification phase, where the target RNA is copied exponentially. The reverse primer hybridises to the new RNA molecules, and are subsequently extended by AMV RT. Simultaneously, RNase H degrades once again the RNA from the cDNA-RNA hybrid molecule, followed by the forward primer's hybridisation to the cDNA strand. Using this as a template the AMV RT completes the cDNA strand and a partial double-stranded DNA molecule is formed, containing the double-stranded T7 promoter sequence. T7 DdRp, in turn, uses it to synthesize new RNA molecules complementary to the original target sequence. This cyclic phase repeats itself over and over again, for one to two hours, thereby multiplying the amount of RNA amplicons in the mixture. [46]

[16]Reverse transcriptase is an enzyme which synthesizes a complementary DNA (cDNA) strand from an RNA strand. This is performed by the enzyme's RNA-dependent DNA polymerase. The enzyme's DNA-dependent DNA polymerase then synthesizes a complementary strand from the cDNA so the double-stranded DNA is formed.

[17]DNA-dependent RNA polymerases polymerise RNA from a DNA template.

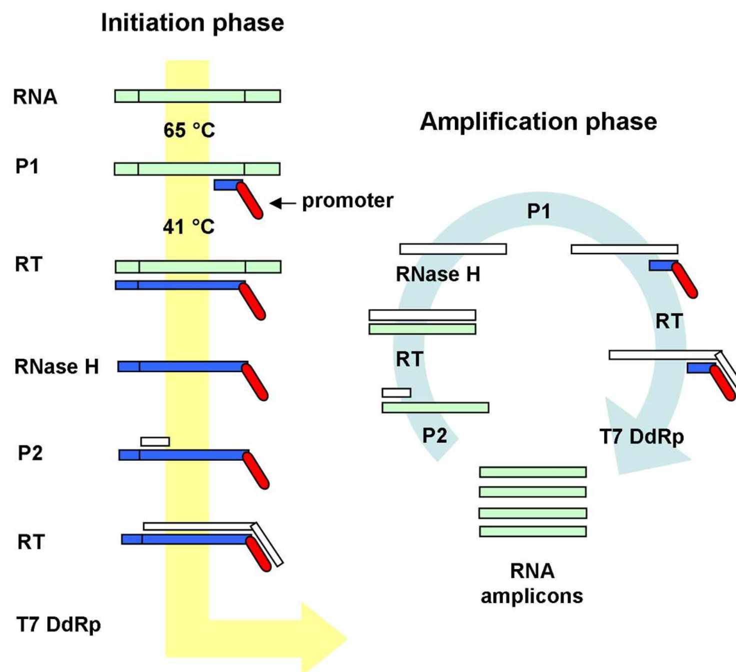


Figure 4.7: A schematic diagram of the nucleic acid sequence-based amplification procedure. P1 is the forward primer and P2 the reverse primer. The target RNA sequence is located between the vertical lines on the RNA bars in the initiation phase. Adapted from [46].

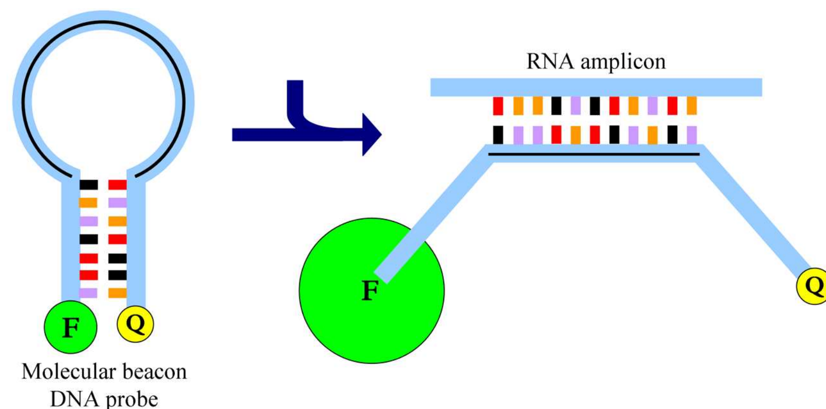


Figure 4.8: Principles of a molecular beacon. *Left.* When not hybridised to a complementary amplicon, the fluorophore (F) and the quencher (Q) of the molecular beacon are close to each other and the fluorescence signal is doused. *Right.* Hybridisation to a complementary RNA strand opens the hairpin structure of the beacon, thus separating the quencher from the fluorophore, which can now emit fluorescent light. Adapted from [46].

The RNA amplicons are perfectly suited for detection by sequence-specific probes. The probes are oligonucleotides with a nucleotide sequence complementary to a part of the amplicon's sequence, thus able to hybridise with the amplicon. Presently, the most commonly utilised detection method involves ECL from ruthenium-labelled probes, but fluorescent probes, for instance, have also been used. These detection systems are not initiated before the amplification phase. An emerging detection method, which would clear the way for

real time detection during the cyclic phase, uses molecular beacons (Fig. 4.8). A molecular beacon is an oligonucleotide with a fluorophore attached to one end and a quencher to the other. A part of the nucleotide sequence between these molecules is complementary to a part of the amplicon's sequence. When the molecular beacon is not hybridised to a RNA amplicon, the nucleotide chain forms a hairpin structure, bringing the fluorophore and the quencher in close proximity to each other. The quencher will now douse the fluorophore's fluorescent signal. But when the molecular beacon hybridises to an amplicon, the hairpin structure is opened, the quencher and fluorophore are far apart, and the fluorophore shines like a beacon.

NASBA has been used to detect the RNA of many bacteria, fungi and viruses. Further details can be found in [46]. The NASBA procedure has even been combined with a membrane-strip-based biosensor for successful rapid detection of viable *B. anthracis* spores [27].

4.5 Cell-based sensors

Traditionally, the bulk of sensors and detection methods use biomolecules like antibodies, protein ligands and nucleic acids as recognition elements. In stark contrast to these are the newly emerging types of sensors utilising *live* cells and tissues. These cell-based sensors are still in their infancies, but research has been spurred especially by their application to the drug discovery process. [132]

In cell-based sensors living cells and tissues are combined with conventional synthetic materials. Receptors which recognise the extracellular agents of interest are embedded in the cell membrane and nucleus (Fig. 4.9). This triggers a response from the cell, e.g. release of Ca^{2+} stores, gene expression, membrane potential change, contraction, etc. These cellular outputs naturally need to be transduced into solid-state signals. The two most common transduction schemes harness electrical and optical recognition methods. Cells can be engineered to express elements that report cellular events by optical or luminescent means. Examples of such elements are beta lactamases and green fluorescent proteins. Cells of neural origin, or other electrically active cells, can be combined with microelectrodes to produce hybrid sensors consisting of both living and inanimate matter. The microelectrodes can detect impedance changes in the excitable cells, thus identifying ongoing cellular recognition events.

Among the advantages of cell-based sensors are their ability to detect a wide variety of substances, like other organisms (e.g. viruses, bacteria), mechanical forces, toxins and biomolecules; as well as their ability to detect unanticipated threats (e.g. mutated pathogens or novel chemicals), since these will have a functional impact on mammalian

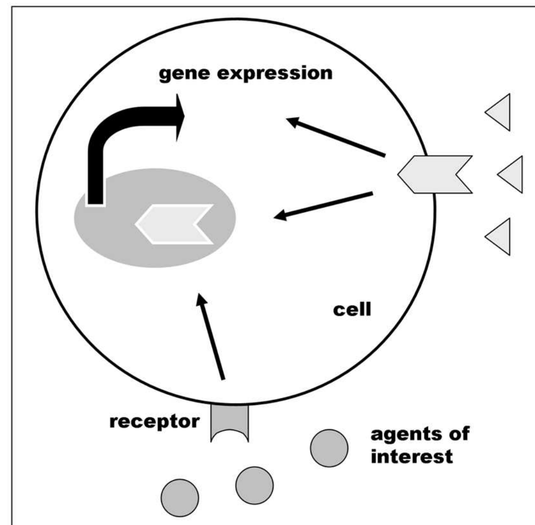


Figure 4.9: A schematic diagram of cell-based sensing. The target extracellular agents are sensed by membrane-bound or nuclear receptors. Multiple (circle, triangle) inputs of both known and unknown origin can be detected by outputs like gene expression. [132]

cells, which are the eventual targets. In addition, enzymes, receptors and antibodies are more stable in these biological systems, which are their natural environments; and from a manufacturing standpoint they are unique in the sense that as living systems they are self-replicating as long as a suitable energy source is available. [132]

Despite the many advantages, cell-based sensors pose a lot of problems to be solved as well. They are, for instance, environmentally sensitive and must not only be kept alive, but also sterile, i.e. free from bacteria and fungi, and in a liquiform environment. Furthermore, the hybrid interface between living and non-living matter is difficult to engineer at best, and the transduction of cellular signals to solid-state signals is tricky. [132]

More details of cell- and tissue-based sensors can be found in a special issue (2001, **16**(7–8)) of the journal *Biosensors and Bioelectronics*.

Chapter 5

Biosensing in Hospital Environments

5.1 Existing hospital hygiene products

A quick search was conducted on the Internet to get an idea of what hospital hygiene products are currently available. The search strings *hospital hygiene monitoring products* and *hospital hygiene ventilation monitoring* were fed to the Google search engine (`http://www.google.com/`) and the results browsed for relevant hits. The principal aim was to investigate the existance of commercially available hygiene monitoring devices for hospital use, hence the word *monitoring* in the search strings. This search found none capable of continuous monitoring, which is why other hygiene products were also included in this brief investigation. The site `http://www.rapidmicrobiology.com/` (Accessed 10 May, 2006) turned out to be an adequate portal to the essential companies, by providing lists of manufacturers according to sector and product area. Most of the manufacturers and their products discussed below were found by selecting the ”clinical” sector and ”environmental monitoring” or ”rapid test” product areas. It should be noted that an exhaustive survey of the market for hospital hygiene products was not the objective, and hence the number and gamut of presented products is limited.

Advanced Analytical Technologies Inc

U.S.-based Advanced Analytical offers a device with accompanying test kits for the automated enumeration of microorganisms. Samples are prepared with one of two test kits, *FasTest* Total Viable Organisms (TVO) Kit or *FasTest* Biomass Test Kit. In both kits, a buffer reagent is added to the bacterial sample, followed by the addition of Nucleic Acid Dye. The dye permeates the cell membranes of live bacteria, consequently staining both live and dead biomass. In the former kit, another solution, BRAG3, is added after the

Nucleic Acid Dye staining. The BRAG3 solution works as a counter-stain to the Nucleic Acid Dye, leaving just live cells fluorescent. The thus labelled bacterial cells can be enumerated with the RBD 3000 device developed by the company. The RBD 3000 uses flow cytometry (see Section 4.3.2) to quantify labelled bacteria down to a detection limit of 100 cfu/ml. Sample processing is fully automated and results are obtained in barely 20 minutes.
Website: `http://www.aati-us.com/` (Accessed 10 May, 2006).

BioControl Systems Inc

BioControl is a supplier of industrial microbiology testing products. The company offers two hygiene monitoring products, FLASH® and LIGHTNING MVP™. FLASH® is a single use instant test stick capable of detecting protein residues. Since protein residues are one of the cardinal nutrient sources for microbes, they offer a measure of cleanliness. The test surface is swabbed with the test stick and the result can be read as a colour change within five seconds. The level of detection is only qualitative (yellow means clean and blue/green dirty). The detection is based upon a protein-binding dye which is immobilised on the swab. The LIGHTNING MVP™ instrument is a hand held device which is used along with the company's sampling devices. There are surface and liquid sampling devices (swab-resembling sticks) and pH, temperature, and conductivity probes. The disposable surface and liquid sampling devices are used to obtain ATP samples. Detection and quantification is based on bioluminescence (see Subsection 4.2.3) measured with the LIGHTNING MVP™ instrument.
Website: `http://www.biocontrolsys.com/` (Accessed 11 May, 2006).

BIOQUELL UK Ltd

BIOQUELL is a supplier of bio-decontamination solutions and air filtration equipment. The company has developed a hydrogen peroxide (H_2O_2) vapour (HPV) generator called Clarus®. Bio-decontamination of a room or a ward with a Clarus® unit requires the space to be sealed and no persons may reside in the area during the process. The computer controlled Clarus® unit will distribute HPV evenly into the air, until saturation conditions for the air/hydrogen peroxide mixture are reached. This leads to the microcondensation of H_2O_2 onto all exposed surfaces. Approximately 1 μm of hydrogen peroxide is laid down, and if there are microbes present on the exposed surfaces the H_2O_2 will kill them. After a predetermined decontamination time the Clarus® unit recirculates the air, dehumidifying it and catalytically converting the HPV into water and oxygen in the process. Complete neutralisation of the hydrogen peroxide is obtained by using aeration units or the building's ventilation system. No toxic residues are left in the decontaminated area. The process has

no adverse effects on materials or electronics. A room is decontaminated in four hours, whereas a complete ward requires 12 hours.
Website: `http://www.bioquell.com/` (Accessed 11 May, 2006).

Biotrace International Plc

U.K.-based Biotrace is a provider of fast quantitative hygiene monitoring systems. The company's product range comprises several reagents and complementary instruments. The CLEAN-*TRACE*® and AQUA-*TRACE*® hygiene tests are "pen-like" devices which are swabbed to collect the sample. The former is used on surfaces and the latter on water. Both are based on the bioluminescence of ATP (see Subsection 4.2.3), so total microbial counts can be assessed with them. The tests are read by the UNI-*LITE*® hand held luminometers, which produce the results within 30 seconds. Biotrace has also several colour change tests which measure protein residues on the swabbed surface. These require no instruments.
Website: `http://www.biotrace.co.uk/` (Accessed 10 May, 2006).

Lifa Air Ltd

Lifa Air is a Finnish company offering solutions for the indoor air quality. The Lifa Ventilation hygiene concept is a take-no-prisoners system consisting of several air duct cleaning units. The DuctControl Maxi can be used to visually inspect the cleanliness of air ducts before and after cleaning. It comprises a video camera/TV system with an incorporated remote controlled mini-tractor capable of, for instance, temperature and airflow measurements and dust sampling. The DuctControl Mini is a similar, but smaller and hand controlled version intended for vertical shafts and small horizontal ducts. The Lifa Special Cleaner 20 is a electrically driven cleaning machine intended for small ducts. By the choice of brush and other accessories, the unit can clean both circular and rectangular ducts. A counterpart for large ducts is embodied in the Lifa Hydmaster, which is hydraulically driven and can be equipped with a video camera to assess the cleaning result in real time. Finally, the HepaClean 4000 is a low-pressure vacuuming unit which can be attached to the maintenance opening of the ventilation duct. The exhaust air is either directed outdoors or filtered through a HEPA filter.
Website: `http://www.lifa.net/` (Accessed 11 May, 2006).

Orion Diagnostica Oy

Finland-based Orion Diagnostica has several tests available for microbiological hygiene monitoring. The company's Drycult® TPC dipstrip is intended for assessing the hygiene status of water samples, whereas the Hygicult® dipstrips are for surface hygiene monitoring. The Hygicult® dipstrips consist of plastic plates coated with culture medium. Samples can be obtained from both surfaces and liquids by pressing, brushing or embedding the dipstrip. After sampling, the dipstrip has to be incubated from one to three days depending on the test and incubation temperature. The result is interpreted by comparing the colour of the dipstrip to an appended colour map. There are dipstrips for the total bacterial and fungal counts (Hygicult® TPC), enterobacteria (Hygicult® E), yeasts and moulds (Hygicult® Y&F), β-glucuronidase-positive organisms (Hygicult® E/β-GUR) and coliform bacteria (Hygicult® CF).
Website: `http://www.oriondiagnostica.com/` (Accessed 10 May, 2006).

Pall Europe Ltd

Pall is a developer of filtration and separation products for, among others, the health care sector. Disposable water filters can be found among its filtration products. The Pall-Aquasafe™ range contains water filter products for shower heads, taps and in-line processing. There are filters for all microorganisms and filters for removal of just *Legionella* spp. All filters are disposable, but intended for up to 7, 14 or 31 days use, depending on the type of filter. Filtration is based on a double layer 0.2 μm membrane. The filter housing contains a bacteriostatic additive to prevent the filtered bacteria from proliferating.
Website: `http://www.pall.com/healthcarewater` (Accessed 11 May, 2006).

Sartorius AG

Among the many products offered by Sartorius, the MD8 airscan® air sampler and the gelatine filter units can be found. The disposable gelatine membrane filters are used to collect the samples. A filter is attached to the nozzle of the air sampler, which functions much like a reversed vacuum cleaner and traps airborne microbes in the gelatine membrane. After sampling, the gelatine filters can be directly placed on agar plates, which are incubated according to normal microbiological procedures.
Website: `http://www.sartorius.com/` (Accessed 11 May, 2006).

Smiths Detection

Although not a supplier of hygiene products, Smiths Detection offers products for the detection of biological agents. In their Trace Detection product selection there are detection systems targeted at the biological warfare defence market. Two of these are the Bio-Seeq^{TM} and the Bio-Detector. Both are capable of detecting bacterial and viral pathogens in approximately 30 minutes or less. Bio-Seeq^{TM} is a portable, hand held thermocycler, and utilises thus the PCR (see Section 4.4.1) procedure to detect agents of mass destruction. The accompanying sample preparation cartridge contains all necessary tools and reagents to ensure that the sample is correctly prepared before inserted into the thermocycler. The instrument is built for field use, can process six different samples simultaneously, and able to detect 1 cfu (colony-forming unit) in about half an hour. Bio-Detector, on the other hand, is a portable, automated detector capable of detecting up to eight agents concurrently. Bio-Detector relies on fluorescently labelled antibodies and a light-addressable potentiometric sensor, which finally measures the change in pH indicating the presence of biological warfare pathogens in the sample.
Website: `http://www.smithsdetection.com/` (Accessed 17 May, 2006).

5.2 Why not microbiological monitoring?

There are three basic disadvantages to traditional microbiological monitoring. These are: 1) (lengthy) transportation of the sample from the test site to a laboratory, 2) processing of the sample usually takes between 18 and 24 hours due to necessary incubation, and 3) results are obtained visually as a subjective interpretation of a human being [30]. The so called "fast" microbiological tests, like for example Orion Diagnostica's Hygicult®, mostly aim to remedy the first issue by offering an on-site, portable platform. However, this does not make the detection of microbes rapid. Novel methods and systems with rapid performance usually address the second and third issue. A real time, continuous monitoring system would have to tackle all three [30].

5.3 Advanced biosensing systems with hygiene monitoring potential

5.3.1 Detection of bioaerosols using semiconductor UV light sources

The intrinsic fluorescence of bacteria was introduced as a viable detection method in Subsection 4.2.2. This subsection, in turn, will dig deeper into the autofluorescence of airborne microbes and the application of UV LEDs as the excitation source. There is nothing novel about UV laser-induced fluorescence (LIF) as a bioaerosol detection method. A number of UV LIF bioaerosol sensors have been developed, among others the already mentioned FLAPS by the Canadian Ministry of Defence [70, 71], the Ultraviolet Aerodynamic Particle Sizer® (UV-APS) by TSI, Inc. (`http://www.tsi.com` Accessed 22 May, 2006) [20], the Biological Agent Warning Sensor (BAWS) by MIT Lincoln Laboratory [121] and the Single Particle Fluorescence Analyzer (SPFA) by the Naval Research Laboratory [50]. The UV-APS is essentially an upgraded version of the FLAPS, and it is the only one of these commercially available.

Traditionally, the UV light source employed in these systems has consisted of a pulsed solid-state laser, such as a Nd:YLF[1] or Nd:YAG[2] laser. However, these lasers are expensive and bulky. Relief to this plight comes in the form of ultraviolet diode lasers (UVDLs) and UV LEDs. UVDLs and UV LEDs in the mid-deep UV region have not been previously commercially available and are only now emerging on the markets. Germany-based PicoQuant GmbH (`http://www.picoquant.com/` Accessed 22 May, 2006), for example, now offers a UV LED with a 280 nm center wavelength and a pulse width in the subnanoseconds. The company is also expected to release a 265 nm version very soon. Advantages of these semiconductor light sources are their compactness, inexpensiveness and easy accomodation into a multiple-wavelength source, enabling concurrent excitation of different biofluorophores [158].

The principal biofluorophores important to UV LIF detection (Table 5.1) are the aromatic amino acids and some biogenic molecules involved in the metabolism of cells [137]. The three aromatic amino acids tyrosine, tryptophan and phenylalanine are present in nearly all proteins, and can therefore be considered as universal markers for matter with biological origin. These show considerable absorption only below 300 nm, with optimal excitation in the deep UV band ∼260–290 nm [80]. They emit fluorescent light in the 300–400 nm band, however, tryptophan is a much stronger fluorophore than the other two [137]. NADH[3]

[1] Neodymium-doped yttrium lithium fluoride (YLF), $LiY_{1\text{-}x}Nd_xF_4$.

[2] Neodymium-doped yttrium aluminium garnet (YAG, $Y_3Al_5O_{12}$). YAG is a synthetic crystalline material.

[3] Molecular formula of NADH: $C_{21}H_{29}N_7O_{14}P_2$.

and riboflavin[4] are the two main biofluorophores involved in cell metabolism. Excitation of these is performed at wavelengths around 350 nm, followed by emission peaks at 450 nm for NADH and 550 nm for riboflavin. In the literature, this emission is attributed to NADH and riboflavin even in the case of bacterial spores, even though these are in a dormant state with reduced metabolism. Thus, there are only trace amounts of NADH and riboflavin in the spores, and such clear emissions at these wavelengths should not be observed. It is still unclear what causes this emission [137].

Table 5.1: Summary of the principal biofluorophores for UV LIF detection and their excitation (λ_{ex}) and emission wavelengths (λ_{em}).

Biofluorophore	λ_{ex} **(nm)**	λ_{em} **(nm)**	**References**
Tryptophan	260–290	300–400	[80, 137]
NADH	340–360	400–550	[71]
Riboflavin	340–360	550 (peak)	[137]
Tb^{3+}-DPA compound	275 (peak)	543 (peak)	[68]

Of all living organisms on Earth, bacterial spores are the unique possessors of dipicolinic acid (DPA; 2,6-pyridinedicarboxylic acid; $C_7H_5NO_4$) [68]. Not even vegetative bacteria contain the acid, as it is one of the keys to the survival of the spores in inhospitable environments. DPA is present in high concentrations (up to 1 M) in bacterial spores and can constitute 5–15% of the spore mass, corresponding to approximately 10^{15} molecules [97]. DPA binds with great affinity to the lanthanide metals and exhibits strong fluorescence with some of them, e.g. terbium, europium and dysprosium. The lanthanide metals show only weak fluorescence on their own, and DPA none at all. However, upon complexation, the fluorescent signal of the bound lanthanide metal is enhanced over a 1,000-fold. The strongest enhancement (over 20,000×) is observed from Tb^{3+} ions bound to DPA [68]. The fluorescence life time of terbium is likewise increased to the order of 1 ms [97]. The Tb^{3+}-DPA compounds can be excited with light in the deep ultraviolet (275 nm peak wavelength) and the fluorescent emission can be observed at wavelengths of green visible light (543 nm peak wavelength). Ponce *et al*, for instance, have developed a biological warfare agent early warning system which relies on the extraction of DPA from airborne bacterial spores into a $TbCl_3$ glycerol solution, followed by UV light-induced fluorescence [96, 97]. A pulsed Xenon lamp was used to irradiate the sample with UV light.

UV LIF bioaerosol detection is hampered by both non-biological and indigenous biological aerosols constantly present in, at least, the outdoor air. Industrial chemicals and engine exhausts compounded of aromatic hydrocarbons are examples of the former, whereas pollen, fungi and dander are of the latter [137]. Many of these interferents are excited by wavelengths in the deep UV, which target tryptophan. Longer wavelengths targeted at NADH and riboflavin do not encounter this dilemma in the same extent. The trade-off comes in the form of weaker fluorescence compared to tryptophan [159].

[4] Vitamin B_2. Molecular formula: $C_{17}H_{20}N_4O_6$.

UV LIF sensors based on UVDLs and UV LEDs are being developed in many research laboratories, mainly in the U.S. The sensors are intended for use as early warning and trigger devices, continuously screening the air for elevated bacterial spore counts indicating an ongoing biological warfare attack. Discrimination between different species of bacteria is not the objective, indeed it is thought unlikely that the fluorescence spectra could provide this kind of identification [37], but rather rapid quantitation of bioaerosols. Species-level identification is left to other, even more advanced, methods and instruments once a possible biowarfare agent has been detected.

In late 2004 Wilson *et al* reported on the BioLert^{TM}2×16C5+1 LIF bio-particle sensor being developed by Hach Homeland Security Technologies in the U.S [158, 159]. The instrument uses two UVDLs at 375 and 405 nm wavelengths to excite particles. The use of several excitation wavelengths yields more information about the interrogated particles and increases specificity. Before the particles enter the fluorescence detection assembly, large (>10 μm diameter) and small (<0.5 μm diameter) particles are removed from the air flow, thus leaving particles in the size range of bacteria and bacterial spores respirable by humans. An integrated air sampling component with an inlet rate of ~35 litres per minute continuously samples the surrounding air. The fluorescence signals from particles are so small, that it is common practice to use photomultiplier tubes (PMTs) in LIF detection systems. The BioLert^{TM} is no exception. It uses a 16 pixel PMT with 15 nm bands in the 360–600 nm range to detect the fluorescent emission of bioaerosols.

More recently, Davitt *et al* have reported on the development of a sensor incorporating a linear array of UV LEDs [43, 44]. The array comprises 32 individual UV LEDs which are 200 μm wide and 50 μm high. The elements are separated by 50 μm from each other, resulting in a total height of ~3.2 mm for the entire array. The dimensions of the array are designed to be compatible with the properties of the jet stream carrying the aerosols which are to be detected. As with all bioaerosol detection devices, aerosols are gathered from the surrounding air and injected through a nozzle into an optical cell where the fluorescence detection takes place. The nozzle used by Davitt *et al* produces a 200 μm wide stream of air, which remains non-turbulent for 1 cm, i.e. well past the length of the array. The LED elements of the array have a common n-electrode, but each one can be controlled individually. The AlGaN and AlGaInN LEDs fabricated by the group of Davitt have peak wavelengths at either 290 nm or 340 nm. Continuous wave (CW) output powers of 1 mW per array element have been reached with the 340 nm LEDs, whereas the 290 nm LED arrays are capable of 100 μW per element. Unfortunately, both LED arrays have considerable tails at longer wavelengths, especially the 290 nm array. Thirty percent of its output power is at wavelengths longer than 310 nm, which necessitates the use of excitation filters, since the longer wavelengths can interfere the with the fluorescence emission. The 290 nm excitation wavelength is targeted at tryptophan, contrary to the 340 nm wavelength which is targeted at NADH.

Incoming particles are detected by elastically scattered light from a 635 nm diode laser targeted at the air stream just after the nozzle. This serves as the identifier of particles in the system, and triggers the UV LED array. The array is covered with a hyperhemispherical sapphire lens in order to triple the transfer efficiency of light from the array to the particle trajectory. The 32 LED elements of the array are fired in rapid succession, thus tracing the particle's path of flight for 2.5 ms. One PMT captures the scattered excitation light from the particles and a 32-anode PMT captures the fluorescence emission (Fig. 5.1). An LED scatter filter blocks scattered excitation light and a fluorescence emission filter blocks the 635 nm diode laser light. In addition, the fluorescent emission is dispersed onto the 32-anode PMT by a UV transmission grating. This produces 32-point spectral data of each particle.

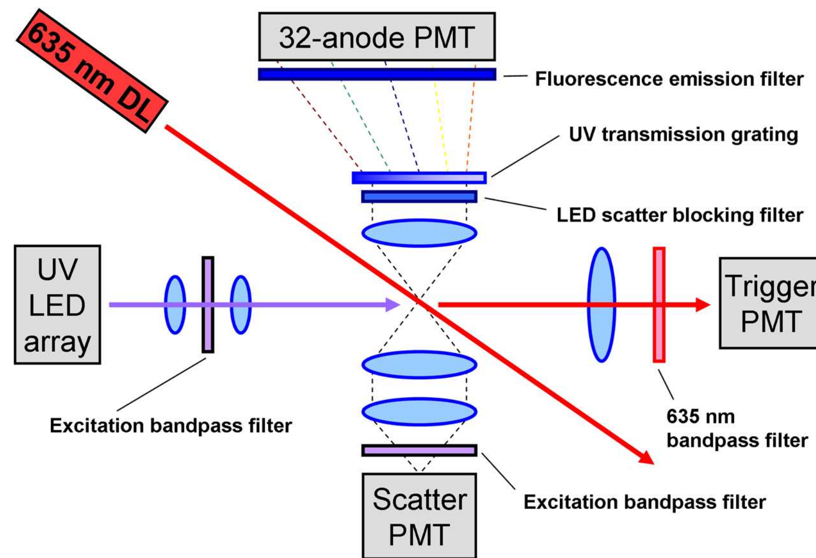


Figure 5.1: A schematic diagram of the apparatus for UV LED-induced fluorescence detection of airborne microbes by Davitt *et al.* Adapted from [44].

The UV LED array device has been tested with aerosolised water droplets containing tryptophan and NADH, with excitation at 290 nm and 340 nm, respectively. The system described above, with aerosols arriving at random intervals, was able to detect NADH at a concentration of 350 μM. Detection levels of tryptophan using the 290 nm LED array was not specified in [44], although experiments had been performed with 0.5% tryptophan-doped water droplets. Davitt *et al* had also performed some initial tests with dry 6 μm particles of tryptophan, yielding promising results, but still in need of further study. Employing a simpler detection scheme where particles did not arrive randomly and were not detected by a triggering diode laser, yielded tryptophan and NADH detection levels below 125 μM and 140 μM, respectively. In this purely experimental setup, the UV LED array and fluorescence PMT were triggered by the aerosol-generating apparatus. This, of course, does not correspond to the real life situation. Hence, the diode laser and trigger PMT have to be used to detect incoming particles and trigger the LED array, as already

described above.

In 2004 Dasgupta *et al* reported on another deep UV LED for biological sensing, being developed at the Sandia National Laboratory in the U.S. [42]. The AlGaN LED had a peak wavelength at 291 nm and an output power of 0.5 mW when operated at 100 mA. Continuous operation characteristics were discouraging because of life time issues, but the LED was very stable in pulsed mode. A gated fluorescence detection system with the LED as light source was built to measure the fluorescence of terbium bound DPA. The system was able to detect 0.4 nM DPA, equalling approximately 500 bacterial spores (based on the amount of DPA in *Bacillus Subtilis* spores, 0.365 fmol [68], a close relative of *B. anthracis*). This kind of level of detection (LOD) is already relevant in practice, since estimates of LD_{50} (lethal dose sufficient to kill 50% of persons exposed to it) for inhalational anthrax vary between 2,500 and 55,000 spores [45, 77]. If the average breathing volume of a normal person is 7.5 l/min, then the equivalent air concentration is ~330–7,330 spores/l over one minute. With a LOD of 500 spores and an efficient air sampler, this system could detect the airborne spores before the LD_{50} is inhaled by people.

The systems described above have all more or less been inclined towards the detection of biowarfare agents, i.e. *bacterial* spores. However, the situation is somewhat different in a hospital environment. There the threat from airborne pathogens comes mainly in the form of fungal spores carried inside the facility by the ventilation system, or vegetative bacteria aerosolised by, for instance, a coughing pneumonia tuberculosis patient. As far as quick searches on the Internet can tell, no LIF system for the specific detection of fungal spores exists. Instead, flow cytometry and PCR, for instance, have been used to detect airborne fungi [120, 157]. Vegetative bacteria on the other hand have been included in many of the papers cited in this subsection [20, 80, 137, 158], and show comparative levels of intrinsic fluorescence to bacterial spores, albeit the tested species have been distinct from those encountered in hospital environments (see Subsection 2.2.1). The intrinsic fluorescence of one fungus (*Sporisorium Cruentum*) was mentioned in [137], but listed as an interferent. Notwithstanding the fact that fungal spores do not contain DPA, the other biofluorophores present in them should make it possible to detect them using UV LIF. The autofluorescence properties of common hospital-acquired bacteria and fungal spores like *A. fumigatus* should be investigated thoroughly, but UV LIF systems might indeed offer a means to monitor relevant bioaerosols in hospitals in real time, at least quantitatively.

5.3.2 Biocavity laser

A novel optical biosensing system has for some time been under development at Sandia National Laboratories in the U.S. [59, 60, 61, 62, 63]. The portable device, called a biological microcavity laser, or just 'biocavity laser', is a kind of flow cytometer, where cells flow in single file in a channel having dimensions slightly larger than the cells' (micrometers

or tens of micrometers). The laser of the instrument is based on vertical-cavity surface-emitting laser (VCSEL) technology, meaning that the laser beam is emitted perpendicular to the gain medium's surface. The laser consists of the following parts (Fig. 5.2). A lower Bragg reflector (a semiconductor mirror) is situated on an n-type substrate, followed by the laser's gain medium, the GaAs/AlGaAs quantum well. Between the quantum well region and the upper Bragg reflector (a dielectric mirror) there is a gap – a microcavity – which is in fact a section of the flow channel.

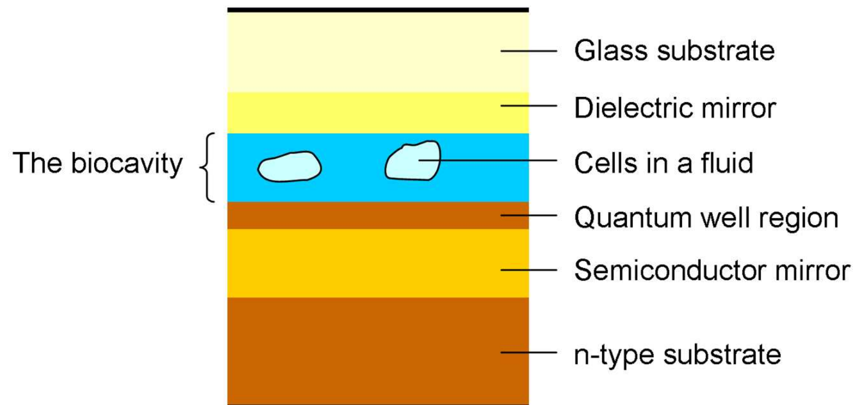


Figure 5.2: A schematic diagram of the structure of the biocavity laser. Adapted from [61].

Hence, the microcavity is an intrinsic part of the laser, as is everything flowing through it. The sample can be blood, cells from culture, or tissue.

The thickness of the quantum wells in the GaAs/AlGaAs semiconductor and the cavity modes have been tuned so the emitted light's wavelength is near 850 nm. The population inversion is achieved either by photon pumping or by carrier injection. Due to the reflective mirrors the emitted light is bounced back and forth inside the structure hundreds of times, amplifying the light. Only a small fraction is allowed to leak through the topmost dielectric mirror. Since the microcavity lies between the two reflective mirrors, the optical properties of anything within it will affect the genesis of stimulated emission. When the microcavity contains fluid only, one laser mode is observed. However, when the fluid contains cells, the spectrum of the resonated light has several spectral peaks and it is also red-shifted. This is due to the fact that cells have higher refractive indices than the fluid and will act as tiny lenses as the light passes through them. This gives rise to a coherent state.

The choice of laser wavelength, near 850 nm, is because between 600 and 1200 nm human cells are highly transmissive and exhibit low absorption. In other words, the cells are almost transparent. Complex molecules like proteins, RNA and DNA, in which carbon-carbon and carbon-nitrogen double bonds are abundant, are the main contributors to the refractive index of mammalian cells at these wavelengths. Simple molecules like water and

sugars play a less significant role. Consequently, the light emission contains information about the cells in the microcavity, specifically information related to the concentration of these complex biomolecules, as well as cell size and shape. The laser beam can be detected and captured with a high-resolution spectrometer. The spectrum and spatial image of the transverse modes can then be analysed to identify the sample, since variations in intensity and spacing of the spectral peaks are specific to each sample.

Gourley *et al* who have been working on the biocavity laser at Sandia, have found several applications for the device. Among the first clinical applications reported was its use to measure intracellular haemoglobin in red blood cells [60, 61]. This is motivated by the fact that 20% of the world's population is estimated to suffer from anaemia. Iron deficiency anaemia is the most common type, and results in decreased levels of haemoglobin in red blood cells [16]. Using the biocavity laser, Gourley *et al* were able to determine the haemoglobin distribution of normal and anaemic red blood cells from just 10 pl of whole blood. The device was clearly able to distinguish between the anaemic and normal blood. The haemoglobin concentration can be determined from the spectrum if the effective cavity length is known, and by using refractive indices of haemoglobin solutions from the literature. Thus, shifts in the resonant peaks of the spectrum can be calibrated to equal changes in the refractive index of the solution in the biocavity. Unknown concentrations can be determined via the relationship $n = n_0 + \alpha C$, where n is the observed refractive index, n_0 is the index of the surrounding fluid, α is the increment to the index introduced by the entity in the fluid and C is the concentration (g/100 ml). Histograms of the haemoglobin concentration of normal and anaemic blood revealed a higher mean concentration and a lower standard deviation for normal blood than anaemic blood.

Furthermore, the biocavity laser could be used to analyse cells to find rare events [61]. For instance, physicians use counts of $CD4^+$ cells to assess how severe the situation of a HIV patient is, since their numbers diminish as AIDS progresses. The techniques of today require a venipuncture, but the biocavity laser could do with the sample volume obtained from a finger puncture. Other rare events include finding stem cells in the bloodstream and malignant cancer cells in a sample.

Recent reports have mainly concentrated on this latter application, i.e. detecting cancer in cells with the biocavity laser [60, 61, 63]. Cancer, being one of the leading causes of death in the developed world, still has no definite cure, although means by which to slow down the disease exist. A key constituent in the fight against cancer is its early detection. The biocavity laser has been assessed for this task.

Gourley *et al* discovered that changes in the biomolecular mass of a cell flowing in the biocavity affected the emission spectrum. This can be used to determine if a population of cells have the rapidly growing characteristic of cancer cells. The cell cycle, which is the sequence of events during which a cell duplicates and divides into two, consists of four

main phases. During the long G_1 (gap) phase, the cell prepares for DNA synthesis and duplicates much of its biomolecular mass. Once certain checkpoints have been passed, the cell enters S phase, during which the DNA strands are replicated. This is followed by the second gap phase, G_2 – a very short resting stage when the cell prepares for division. The final proteins and other biomolecules necessary for division are also produced in this phase. Consequently, the mass of a cell in G_2 is larger than a cell in G_1. G_2 is succeeded by mitosis – M phase – during which the cell duplicates into two daughter cells. Normal cells would spend very little time in G_2 phase, which means that only a few cells in a population would be observed in this phase. Rapidly growing cancer cells, on the contrary, would show a different distribution of cells in the G_1 and G_2 phases.

The group at Sandia has observed this with the biocavity laser using cancerous brain cells [60, 61]. Since the biomass of cells in G_2 is larger than cells in G_1, they possess a higher refractive index. Thus, the wavelength of light which has interacted with such cells is shifted to a longer wavelength compared to that produced from cells in G_1. A histogram of spectral shifts for normal and cancerous cells showed that, in the case of normal cells, a main peak – standing for 98% of the cells in the tested population – corresponded to cells in G_1, whereas a tiny peak at a longer wavelength shift – standing for the remaining 2% of the cells – corresponded to cells in G_2. When a population of tumour cells were tested, the distribution was much broader, and more cells comprised the second peak corresponding to G_2. This indicated that the phases of the cells were more interspersed throughout the cell cycle, characterising rapidly growing tumour cells.

A significant advantage of the biocavity laser over traditional methods, besides the smaller required sample volume, is its speed. Samples are analysed almost instantly – in microseconds – and in the above case, only a few hundred cells were necessary to detect cancer. This corresponds to an unsubstantial tissue sample of 1 nl. Moreover, cancer cells can be detected without invasive chemical labelling, in other words, in the same state they appear in the body.

Attempts have also been made to detect cancer in single cells with the biocavity laser [60, 63]. This was accomplished by increasing the sensitivity of the lasing spectra by imaging the far field profile of the lasing mode and the spectra concurrently. A two-dimensional array detector is used to obtain spectral information in one dimension and spatial information in the other dimension. This imaging is performed in the far field, meaning that the image plane is situated at a distance $z > 2D^2/\lambda$ from the source (the cell), where λ is the wavelength of light and D is the largest dimension of the source aperture. This method was tested with normal and cancerous liver cells [63]. A three-dimensional cluster plot of the lasing wavelength shift, lasing linewidth and peak lasing intensity revealed distinct clusters of normal and diseased cells, confirming that it actually is possible to detect cancer in single cells using biocavity laser spectroscopy.

Furthermore, the biocavity laser has been used to study mitochondria [63]. Mitochondria are present in huge quantities (up to 25% of a cell's cytoplasm) in well-nigh all eukaryotic cells [16]. They are the cell's 'power plants', converting organic matter into ATP. In recent years, the health status of mitochondria has been found to play a role in a number of diseases, for instance, Alzheimer's and Parkinson's disease [69, 149], and Huntington's disease [115]. Mitochondria have submicrometer diameters – normal human mitochondria, for example, are usually 700–800 nm in diameter, i.e. close to the wavelength of light. Hence, they are hard to study with standard light microscopes. The biocavity laser, on the other hand, with a cavity size of a few micrometers, was able to distinguish the swelling of mitochondria induced by calcium. This swelling is observed in the aforementioned diseases and is an indication of a diseased mitochondrion. Samples of both normal and diseased mitochondria were analysed as well, producing distinguishable lasing spectra [63].

Besides the many medical applications, the biocavity laser could be used to detect and identify dangerous biological warfare agents among non-pathogenic agents in only minutes. Some time ago, the device was shown to be able to distinguish *Bacillus subtilus* and *Bacillus pumilus* spores, which are inseparable under the microscope and are simulants of the deadly *B. anthracis* spores [59]. However, this application has apparently received no further attention in the literature, so far.

Nevertheless, based on this, there is reason to believe that the biocavity laser could be used not only to interrogate eukaryotic cells, but also prokaryotic cells, i.e. bacteria. Since the device needs a sample in liquid form, the quality of tap water could probably be assessed without complications, rapidly determining its bacterial burden. Possibly, with a lasing spectra sensitive enough, discrimination of pathogens could be a reality. A sample taken from a surface into a liquid could likewise be analysed, thus providing data on the cleanliness of surfaces. These scenarios seem feasible at a glance and should be be studied further in detail.

Sandia is not the only place where microcavity laser spectroscopy is studied. Lear *et al* at the Colorado State University in the U.S. have taken example from Gourley *et al* and created a microfluidic cavity surface emitting laser of their own [88]. A more recent endeavour by the Colorado group explores a simplified setup which does not utilise VCSEL technology, but rather a Fabry-Perot microcavity built on glass substrates [134]. Unlike the biocavity laser, the light source of this instrument is an LED. The instrument was used to analyse blood and was able to distinguish red blood cells and other blood cells. Red blood cells, however, could not be differentiated from yeast cells by the present method. The method is still a promising alternative to the biocavity laser and follows many of the same principles.

5.4 Gene sequencing and other overlapping fields

Although the focus in this thesis has been on hygiene monitoring in hospital environments, the methods outlined in the previous section and Chapter 4 are applicable in a variety of fields. When it comes to hygiene monitoring, hospitals are not the only places where it is important that microbes are kept in line. The pharmaceutical industry, for example, has extremely high requirements on cleanliness, not to talk about the food industry, where continuous monitoring of the food processing environment is crucial to ensure food safety. There are also other fields – some of which have already been mentioned in connection with the detection methods – that utilise or benefit from the same kind of detection systems. These are, for instance, the detection of biological warfare agents, cell sorting, cell imaging and medical diagnostics. Optoelectronic devices utilising UV LEDs and diode lasers also have applications in the disinfection of water and surfaces [11].

Another field where advanced biosensing methods are making their way is gene sequencing. Today gene sequencing, which is the process by which the nucleotides of a DNA strand are determined, is usually performed by a method called ”chain terminator sequencing”, or ”Sanger sequencing” named after its developer Frederick Sanger [16]. One drawback of this method is the necessity to use radioactive or fluorescent labels. This may come to change in the future by the development of sophisticated, rapid biosensing systems similar to those described in this thesis, but aimed at gene sequencing.

Such devices are envisaged to use, for instance, mechanisms of surface plasmon resonance or metal-enhanced fluorescence (MEF) [93]. One such concept is a flow cytometer, where a single DNA strand is initially bound to a bead. The nucleotides are progressively cleaved from the other end of the strand by an exonuclease[5] and subsequently transported into a flow chamber. The walls of the flow chamber are coated with metal particles, which enhance the intrinsic fluorescence of the nucleotides. Upon excitation with light of two different wavelengths, the emission spectra of different nucleotides could be distinct enough to enable identification of the nucleotide in the chamber (Fig 5.3) [56, 93]. Thus, DNA sequencing could be performed without any chemical labelling at all.

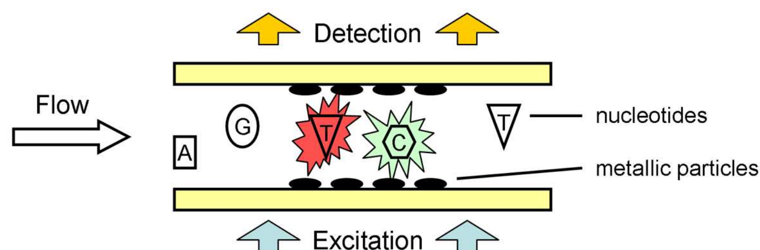


Figure 5.3: Metal-enhanced fluorescence DNA sequencing. Adapted from [93].

[5]Enzymes which releave nucleotides from the terminal ends of nucleotide chains one at a time.

Chapter 6

Summary and Conclusions

Nosocomial infections are a real problem in health care facilities. Roughly every tenth patient is afflicted by such an infection at any one time. Patients with an increased risk of bacterial colonisation or an impaired immune defence are predisposed to nosocomial infections, which also increase the risk of death. Their financial implications are huge; the additional costs vary from thousands to tens of thousands of dollars or euros per episode, depending on the severity and site of the infection. The majority of hospital-acquired infections are caused by bacteria, however, fungi are playing an ever increasing role. The most common bacterial species to blame are bacteria in the *Enterobacteriacea* family, *Enterococcus* spp, *Legionella* spp, *P. aeruginosa* and *Staphylococcus* spp, in no particular order. *Aspergillus* spp and *Candida* spp are the cardinal fungal culprits. The role of viruses is less significant. They are thought to stand for only a small amount of all nosocomial infections.

The largest hospital in the Helsinki University Central Hospital -region is the Meilahti Hospital. Many severely ill patients, among others transplantation patients with an increased risk for nosocomial infections, are treated at the hospital. The rates of certain types of nosocomial infections are closely monitored at the hospital, for instance, bloodstream infections. The overall rate is not thought to diverge from other national or international hospitals. So far, antimicrobial resistance has been fairly low and septicaemia-associated mortality rates have been lower than in other hospitals participating in the SIRO-programme. Among the current nosocomial infection prevention practices, good hand hygiene is the most important one. Other practices include the pressurisation and filtration of air, limited use of tap water, temperature disinfection of shower heads and water conduits, and keeping a good level of overall cleanliness to prevent microbes in the environment from thriving.

Products that monitor or test the hygiene level are not in routine use, but three areas where such monitoring could be of benefit were identified. These are the ventilation system, the

water distribution system and cleaning. Rapid biosensing systems could be used to detect microbes in these areas. A biosensing system could verify the continuous effectiveness of the air filters in the ventilation system, make sure that tap water does not contain harmful pathogens, and validate the cleanliness of surfaces after the cleaning effort.

The term 'biosensing systems' comprises a vast amount of different approaches to detect biological substances. These can be roughly divided into electrochemical, optical, microfabricated, nucleic acid- and cell-based systems. These contain methods from potentiometric biosensors to quantum dot labelling, and surface plasmon resonance to nucleic acid sequence-based amplification. The optical systems are perhaps the most versatile, and thus it is no surprise that two promising methods suitable for the three aforementioned areas were found from this category.

One is based on laser- and light-emitting diode-induced fluorescence, and the other is a biocavity laser. The former has the potential to detect microbes in the air, whereas the latter could analyse samples of water or samples taken from surfaces. Both have the ability to perform analyses in well-nigh real time – a requirement for an online monitoring system. These systems should still be extensively studied, but it is the conclusion of this work, that they possess the greatest potential to fulfill the requirements of these applications.

The biosensing systems described in this thesis are applicable to many fields beyond the one under focus herein, namely hygiene monitoring. The detection of biological warfare agents, medical diagnostics and gene sequencing are but some of the others.

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