

Microbiological Analysis of Water: Addressing The Challenges

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INTRODUCTION

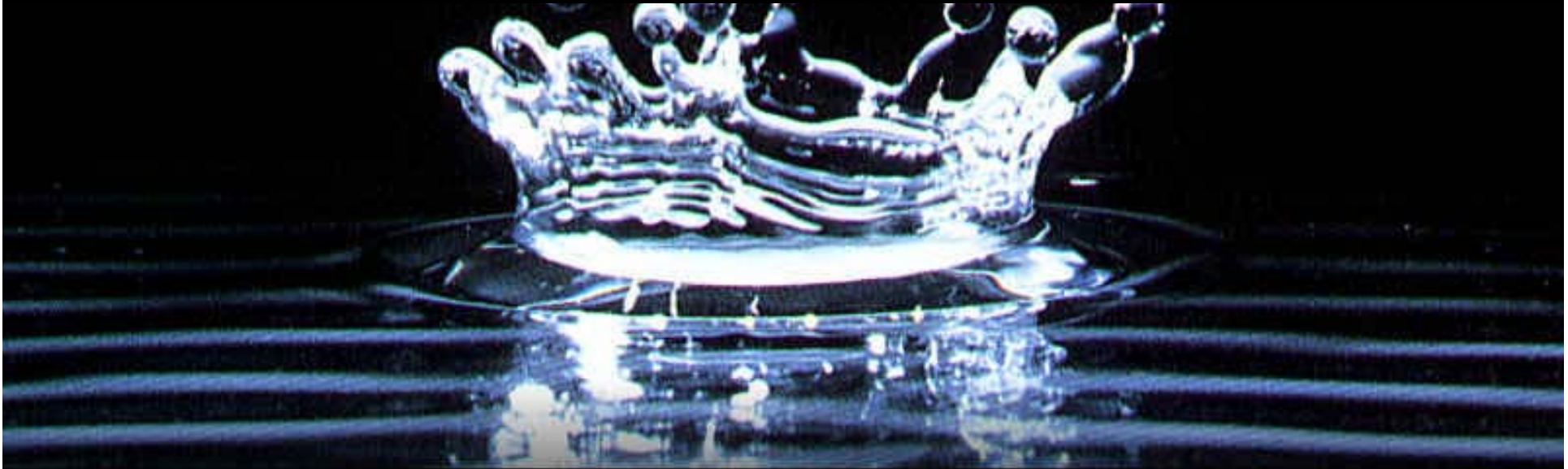
Microbes are ubiquitous, some are pathogens and transmitted by water

Sources of contamination:

- Fecal material
- Various types of water flowing together
- Wildlife

Fecal contamination plagued humans throughout history

Progress due to improved water treatment techniques



INTRODUCTION

Water reuse and generated wastewater are principal targets for pollution prevention strategies - **pathogens**



INTRODUCTION

Today 1 000 people world-wide die every hour due to microbial waterborne disease

Problems associated with microbial pathogens in drinking water are expected to worsen:

- Changes in human demographics
- Microbial adaptation and environmental changes
- Breakdown of public health measures



Human Demographics and Behaviour



Economic conditions encourage movement to cities:

- Rural urbanization
- Urban population growth

Increasing risk groups:

- Low socio-economic groups
- Very young
- Elderly
- Immunocompromised persons

Microbial Threats



Natural environment:

- Evolution of existing microbes
- Development of antimicrobial resistance
- Development of biofilms



Breakdown of Public Health Measures

Pathogens can remain in reservoir hosts or the environment

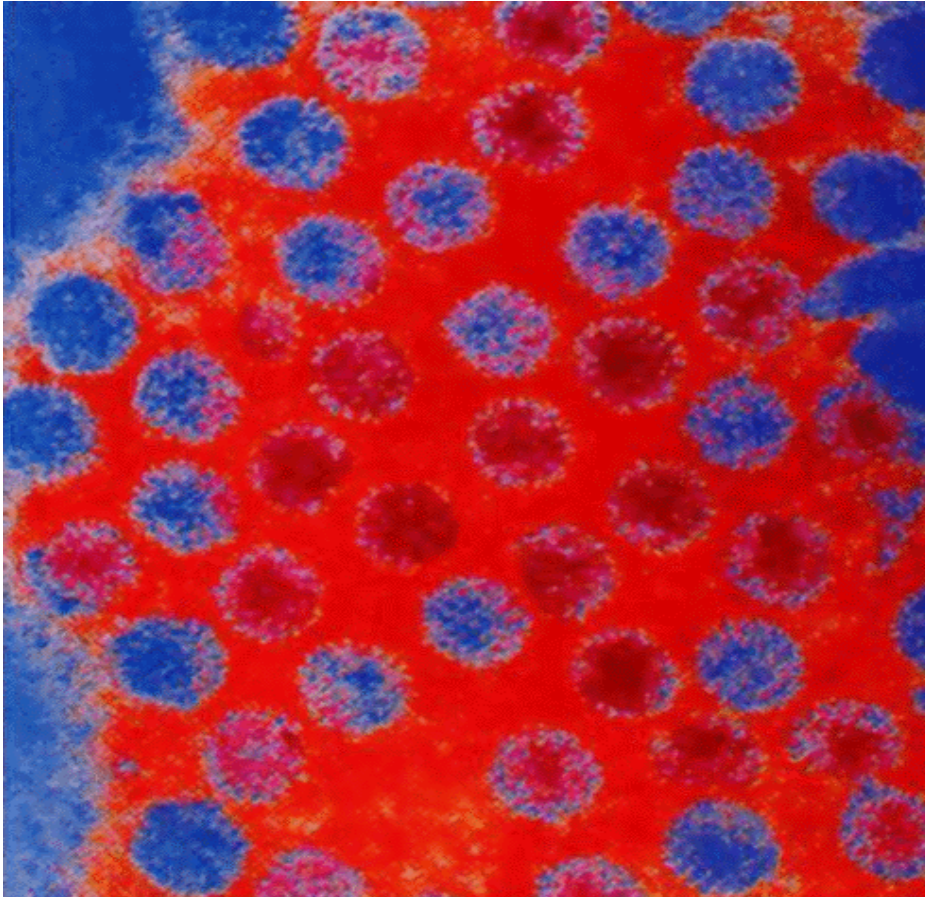
Breakdown of preventative measures

- *Cholera* spread aided by reduced chlorine levels
- *Cryptosporidium* infection due to nonfunctioning water filtration plant

Limitations in surveillance and the availability of diagnostic tests

AGENTS OF CONCERN

- More than 200 diseases transmitted through water
- Pathogens
 - Bacteria
 - Parasites
 - Viruses
 - Toxins
 - Metals
 - Prions
- Symptoms
 - Mild gastroenteritis
 - Life-threatening neurologic-hepatic and renal syndromes



Rotaviruses

Highly infectious

Infants, young children

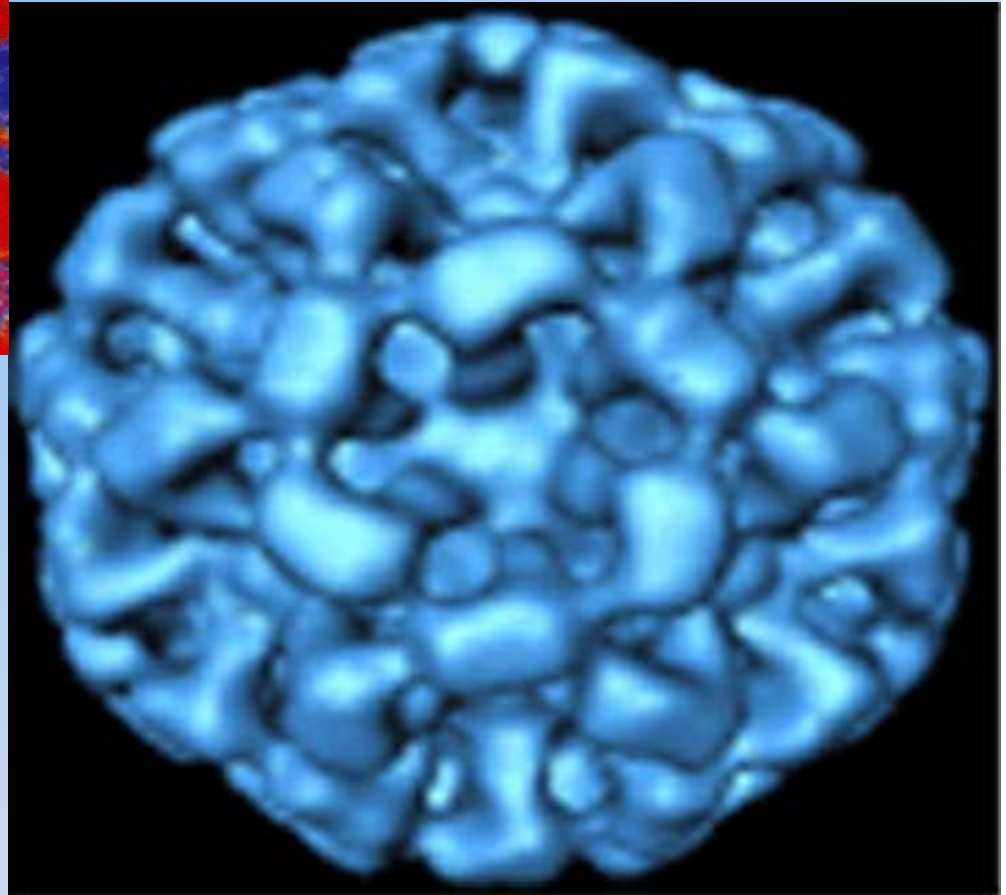
4 million cases per year

Norwalk virus

Various outbreaks

Older children and adults

Responsible for a 1/3 of gastroenteritis outbreaks



Illness and Deaths - Viral

Disease	Estimated total cases	% Foodborne transmission	Deaths
Norwalk-like virus	23 000 000	40	124
Rotavirus	3 900 000	1	0
Hepatitis A	8 3391	5	4



Giardia lamblia

Two forms: trophozoites and cyst

Campers at high risk

Common cause of disease in humans

Cryptosporidium

Inhabits many different animals

More prevalent in AIDS patients

Milwaukee outbreak:
400 000 cases, 100 deaths



Illness and Deaths-Parasitic

Disease	Estimated total cases	% Foodborne transmission	Deaths
<i>Cryptosporidium parvum</i>	300 000	10	7
<i>Giardia lamblia</i>	2 000 000	10	1



Salmonella

Most human pathogens belong to *S. enterica*

Multidrug resistant strains

Carriers are animals used in food production

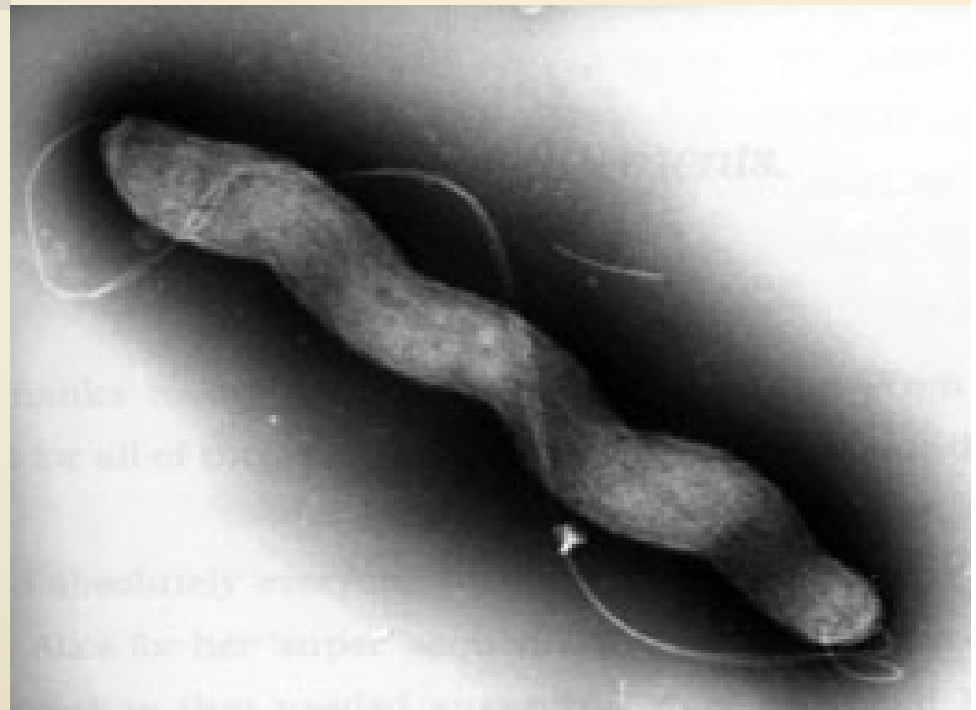
800 000 to 4 million cases

Campylobacter

C. jejuni / *coli* common in developed countries

Sources include: chicken, unpasteurized milk and untreated water

170 000 to 2.5 million cases





Shigella

Prevalent among children

Causes bacillary (asylum) dysentery

Highly communicable

500 000 deaths per year

Vibrio cholerae

Two serogroups cause epidemics (O1 and O139)

Caused 7 pandemics

Introduced in western hemisphere in 1992

Mortality rate is over 60%



Illness and Deaths - Bacterial

Disease	Estimated total cases	% Foodborne transmission	Deaths
<i>Campylobacter jejuni</i>	2 453 926	80	99
<i>E. coli</i> O157:H7	73 480	85	52
<i>Listeria monocytogenes</i>	2 518	99	499
<i>Salmonella</i> , non typhoid	1 412 498	95	553
<i>Shigella</i> spp	448 240	20	14
<i>Vibrio cholera</i> (toxigenic)	54	90	0
<i>Yesinia enterocolitica</i>	96 368	90	2

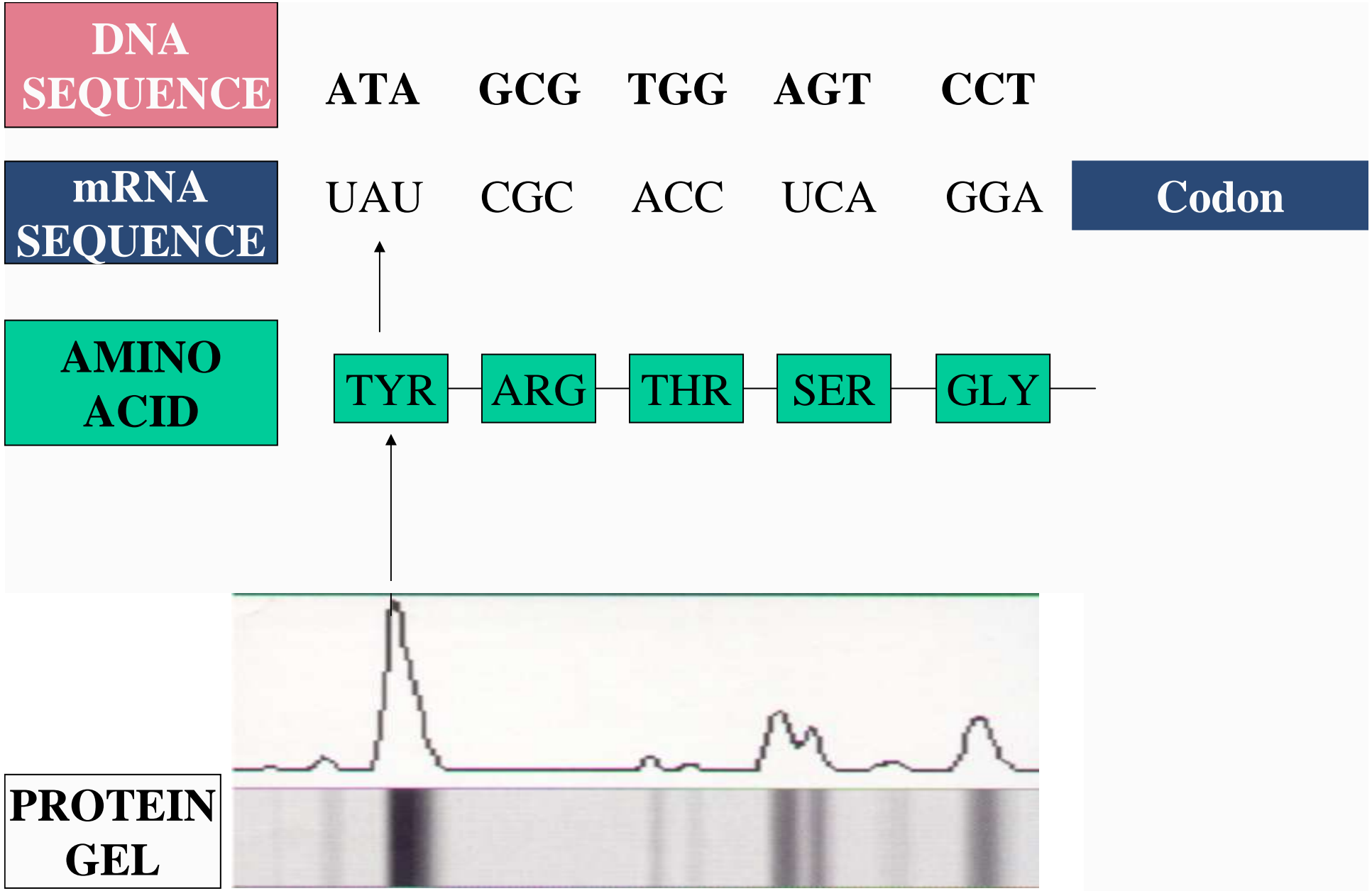
Detection Methods for Pathogens



- **What pathogens do you look for ?**
- **Do you wait for people to get sick ?**
 - **Retrospective epidemiology studies**
- **What approach do you use ?**
 - **Microscopy**
 - **Culture**
 - **Molecular detection**

GENOTYPIC CHARACTERISTICS





TESTING OF VIRUSES AND PROTOZOA

Pathogenic viruses and protozoa present in low numbers

Require large volumes of water to filtered which may be impractical

Cell cultures involves infecting growing cells with a water sample and visualizing any effects

Labour-intensive and time-consuming

Cultural tests developed for only 60% of viruses

Testing for protozoa done by microscopic analysis

Requires highly trained technicians

TESTING OF BACTERIA

Presence of indicator bacteria

Unreliable in indicating presence of several pathogens

Use of growth media and biochemical, physiological and serological tests

Some pathogens are fastidious in their growth requirements

Cells present in nonculturable state

Microbes are stressed in the environment

Time-consuming





MOLECULAR-BASED TESTS

To be effective, such tests must be:

- Specific for the target organism
- Sensitive enough to detect low numbers of the target microbe
- Simple in order to perform tests on a routine basis

Two common approaches:

- DNA hybridization
- Polymerase chain reaction



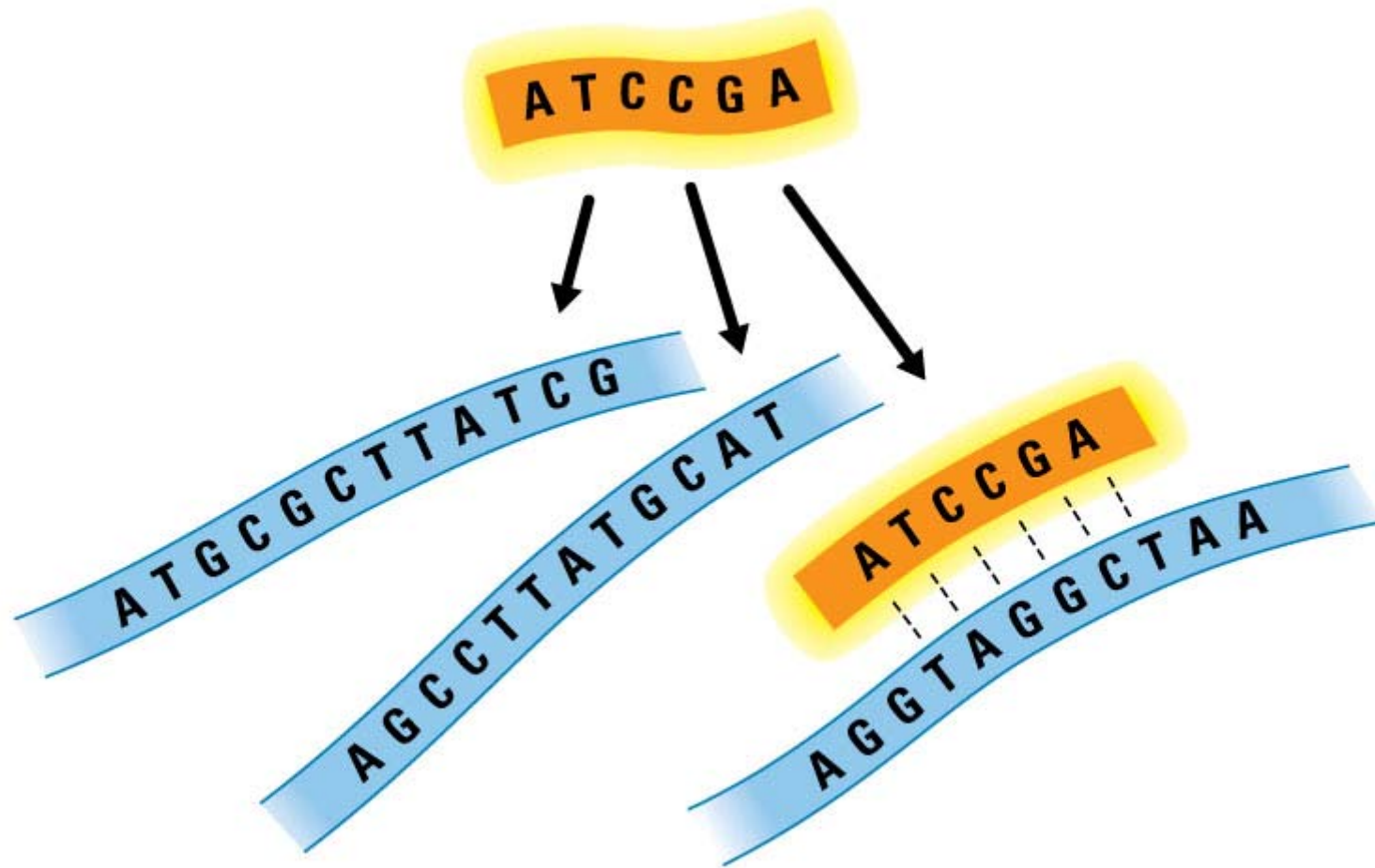
HYDRIDIZATION ASSAYS

Components:

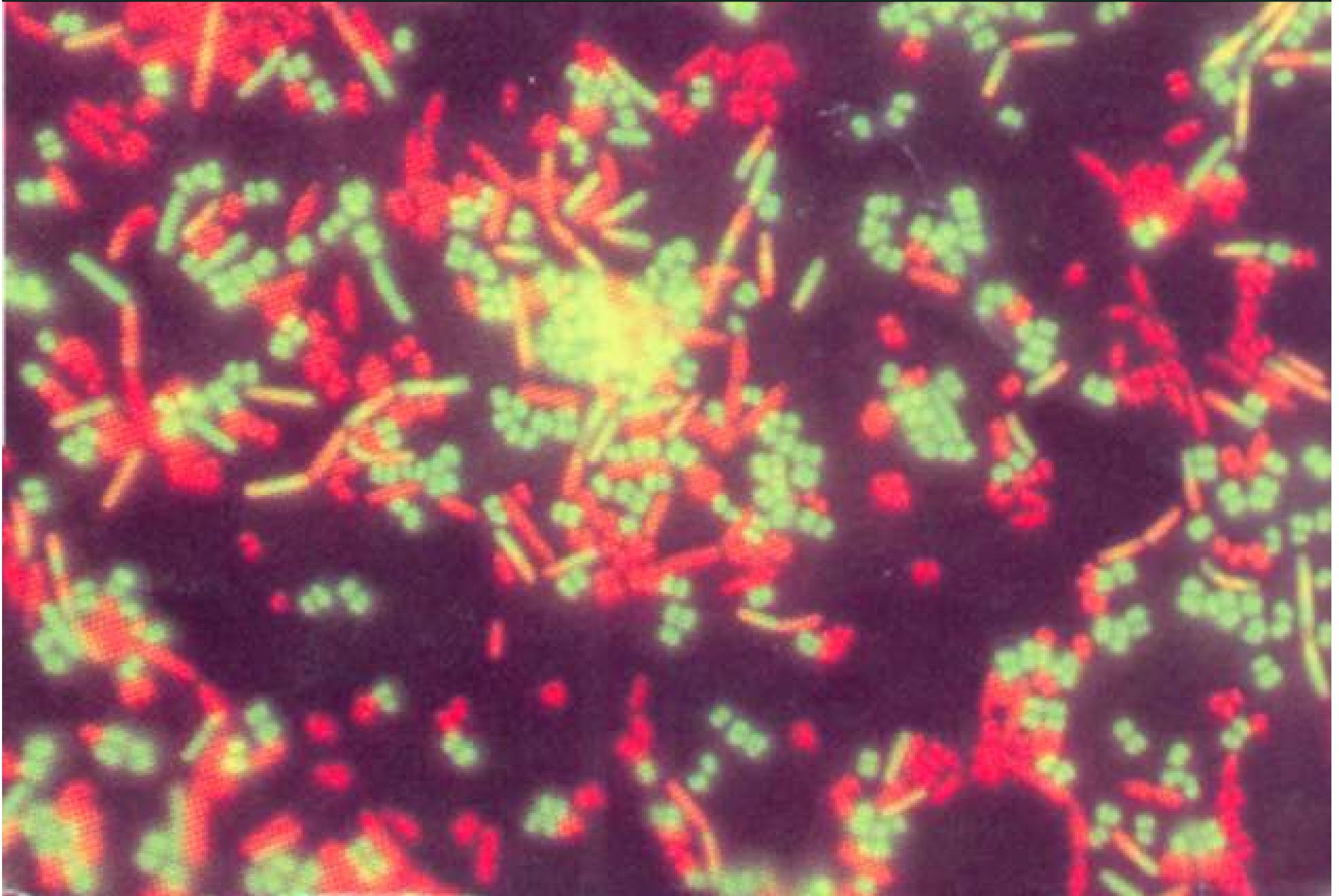
- Labeled probe molecule
- Target molecule (in sample)

Methodology:

- Immobilisation of target nucleic acid on solid support
- Incubation of the blot with denatured probe
- Detection of reporter molecule



SEROLOGICAL DIFFERENCES





POLYMERASE CHAIN REACTION

Components:

- DNA template
- Two synthetic oligonucleotides
- Enzyme (polymerase), dNTPs

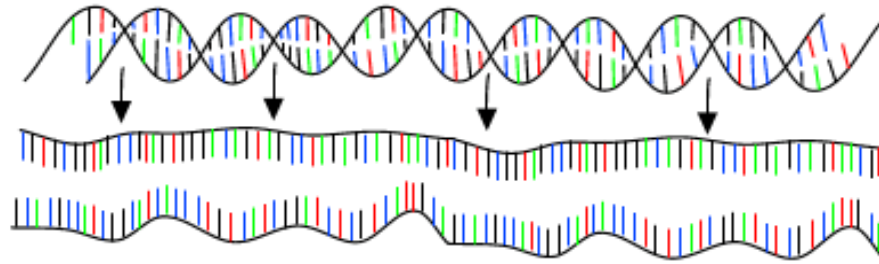
Reaction:

- Denaturation
- Annealing
- Extension

Automated thermal cycler

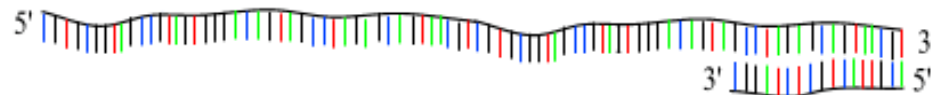
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



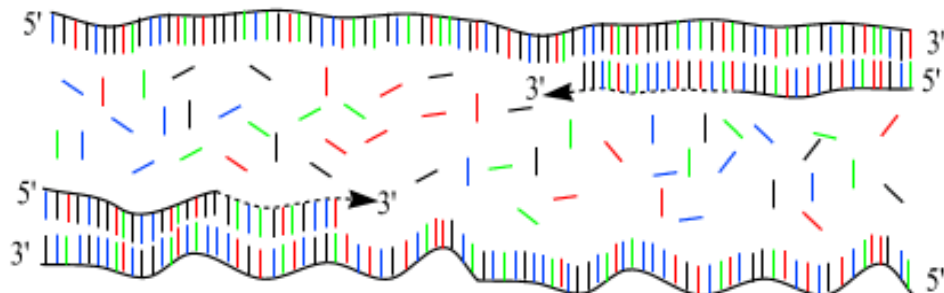
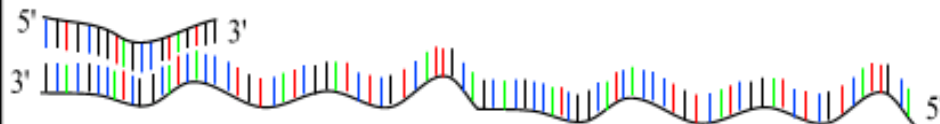
Step 1 : denaturation

94 °C



Step 2 : annealing

54 °C



Step 3 : extension

72 °C

CONCLUDING THOUGHTS

- Molecular techniques are the key to the future for microbiological analysis of water

