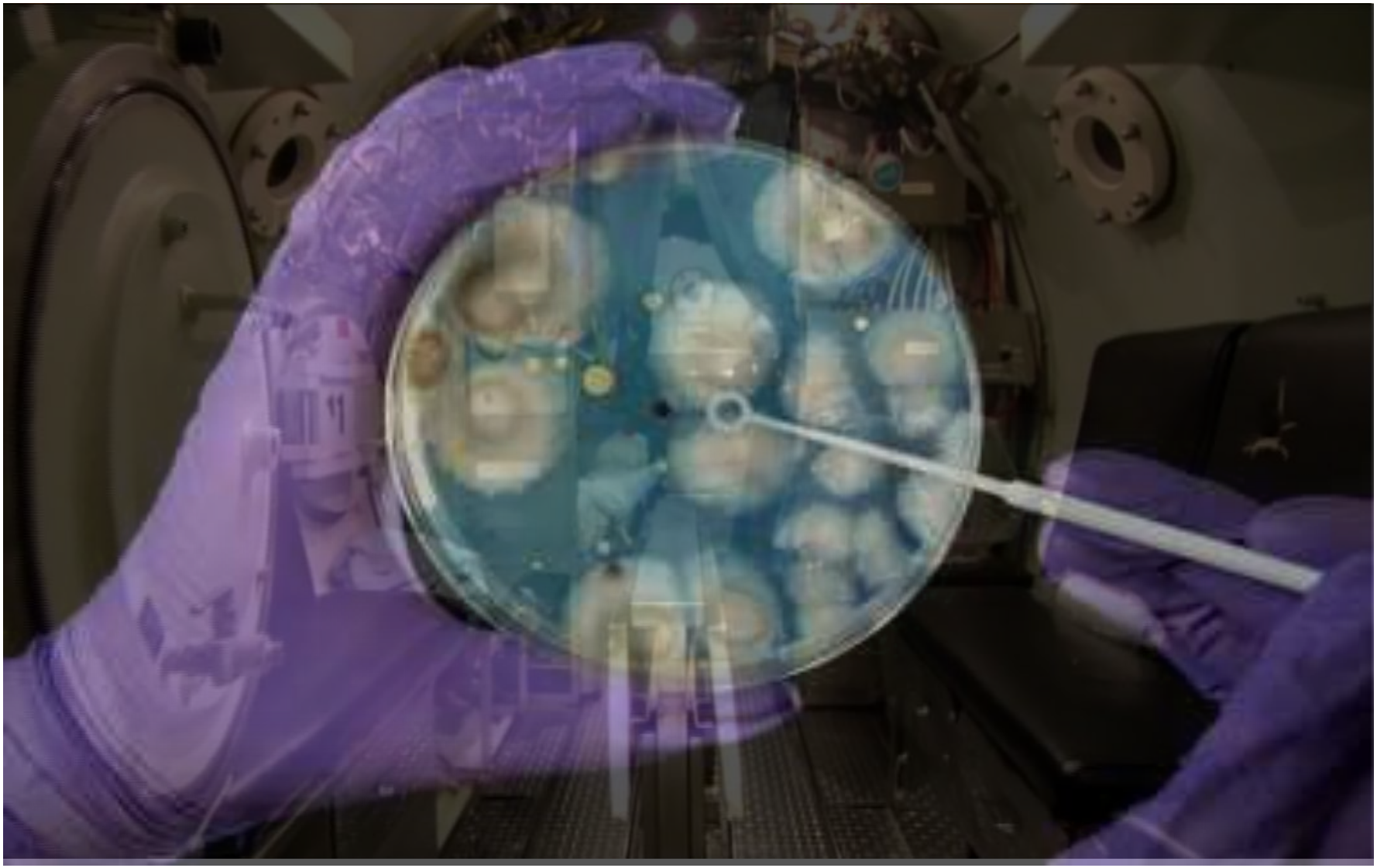
CONTROL OF PSEUDOMONAS AND OTHER PATHOGENS IN SATURATION CHAMBERS



PSEUDOMONAS AERUGINUSA (“PYO”)

Metabolic products

- Urine
- Faeces
- Sweat
- Dust (dead skin)

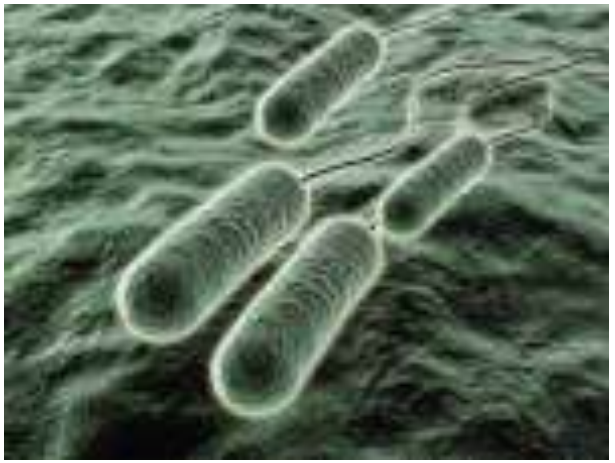
- “Introduced” products
- Foodstuff
- Books, paper etc
- Fresh water (drinking & sanitary)
- Seawater
- Mud etc

Chamber environment

- High PPO_2
- High temperature
- High humidity
- Recycled gas
- Close personal contact

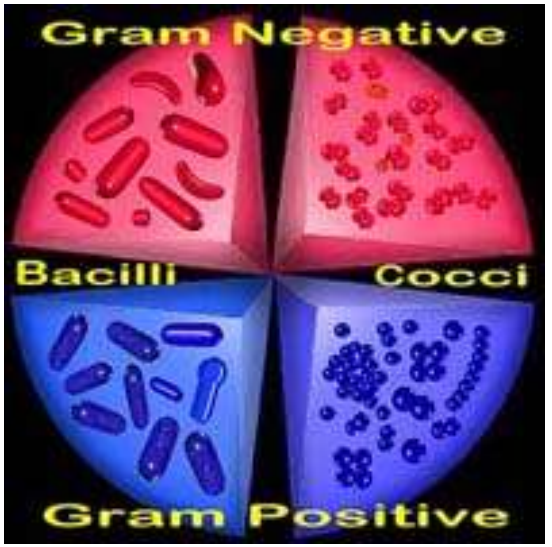
PSEUDOMONAS AEROGINUSA (“PYO”)

- ***PSEUDOMONAS* SPECIES > 190**
- **AEROBIC**
- **VERY MOBILE**
- **UBIQUITOUS**
- **OPPORTUNISTIC**
- **THRIVES WHERE CARBON SOURCE IS AVAILABLE**
- **GRAM NEGATIVE**



GRAM NEGATIVE / POSITIVE

- TWO **MAIN CATEGORIES** OF BACTERIA
- DEPENDS ON REACTION TO CRYSTAL VIOLET STAIN
- STAINING INVOLVES APPLICATION OF CRYSTAL VIOLET AND THEN DECOLOURING WITH A COUNTER-STAIN E.G SAFRANIN
- GRAM-POSITIVE BACTERIA WILL RETAIN THE CRYSTAL VIOLET



- WHILE

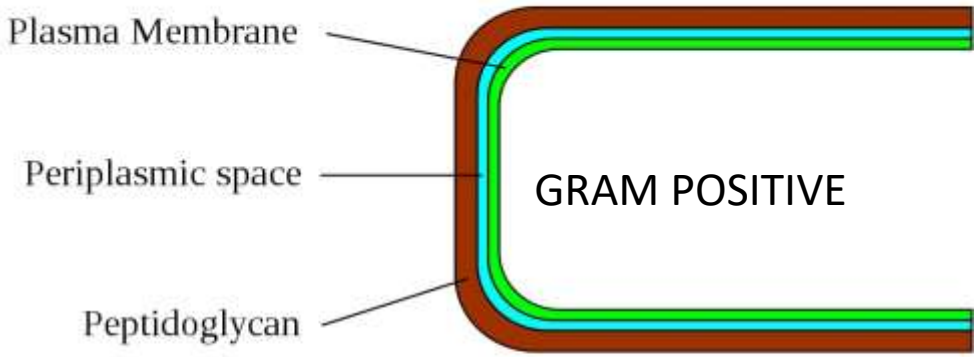
- GRAM-NEGATIVE BACTERIA WILL LOSE VIOLET DYE COLOUR AND TAKE UP THE COUNTER STAIN COLOUR (RED/PINK)

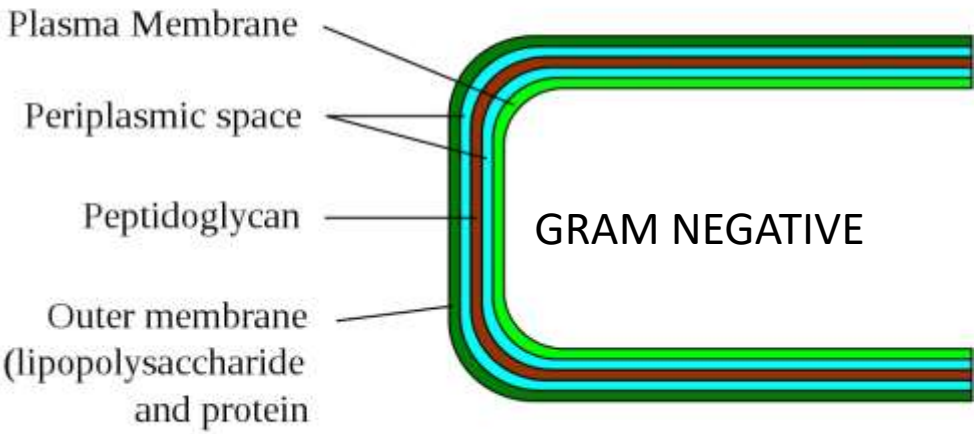
GRAM NEGATIVE / POSITIVE

STRUCTURE OF BACTERIAL CELL WALL DICTATES REACTION TO STAINING

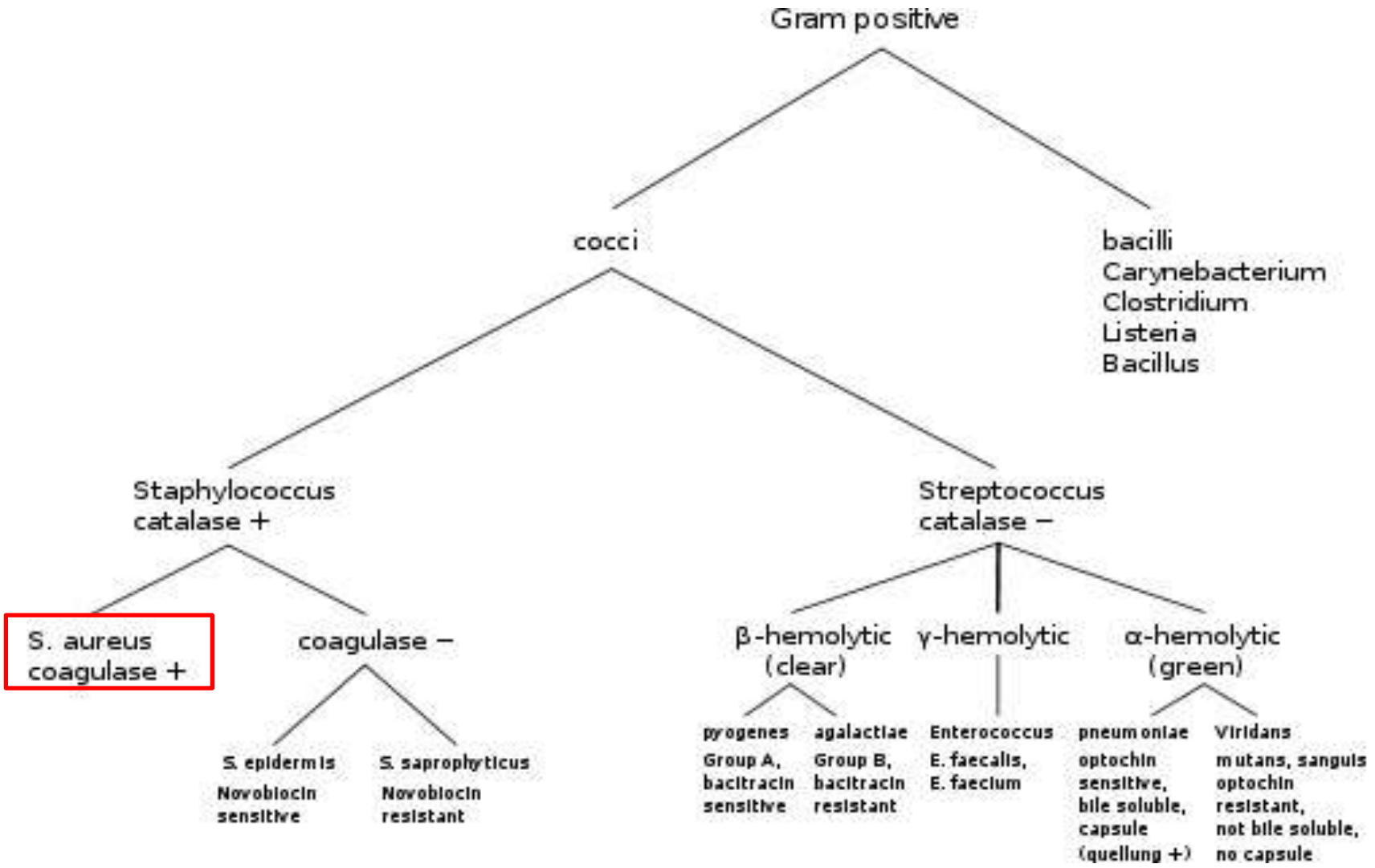
GRAM-NEGATIVE HAVE AN ADDITIONAL LAYER OF LIPOPOLYSACCARIDE WHICH PREVENTS STAIN TAKE-UP

THIS LAYER CAN INCREASE RESISTANCE TO ANTIBIOTICS

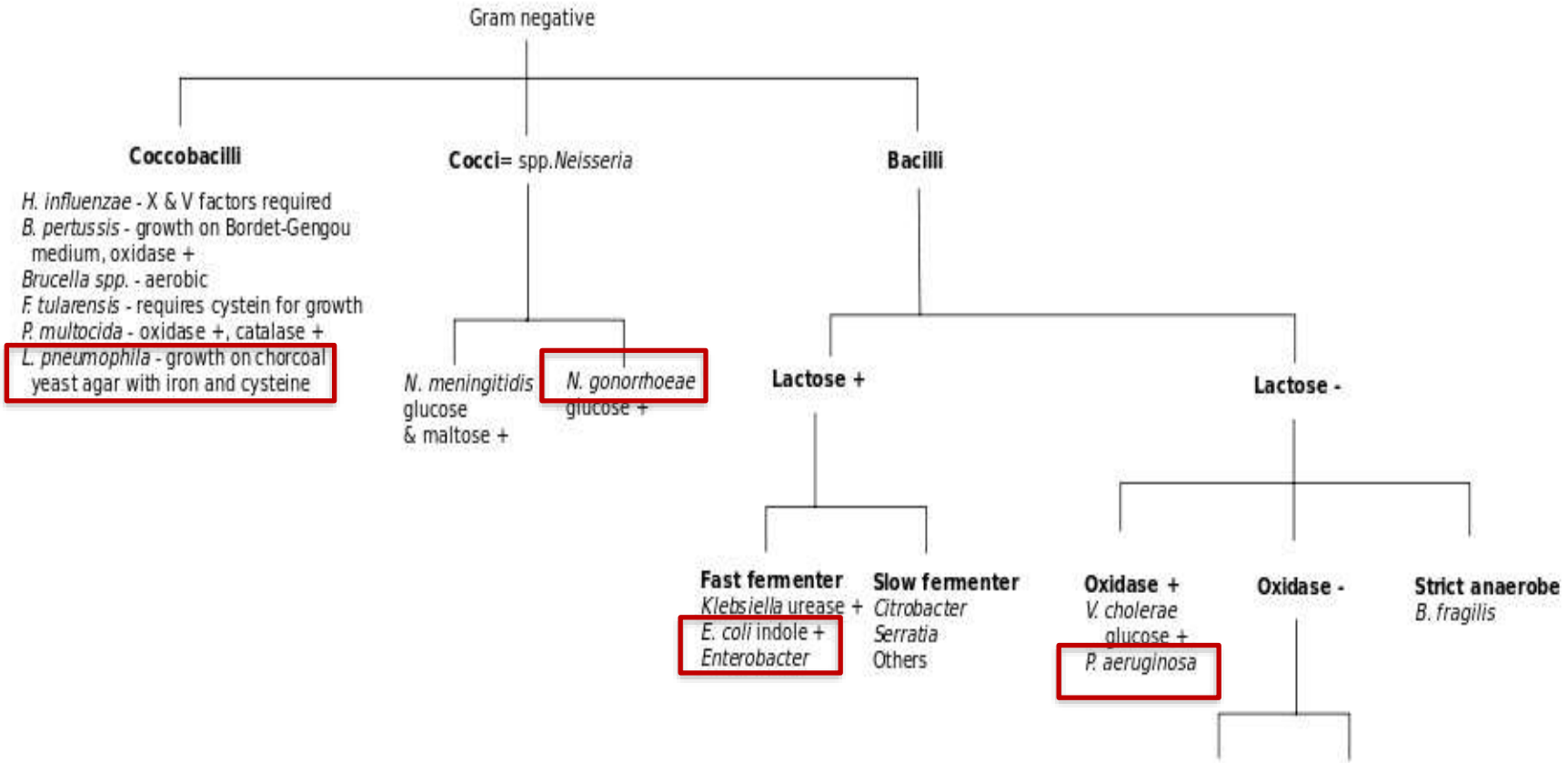




GRAM-POSITIVE BACTERIA



GRAM-NEGATIVE BACTERIA



Additional layer in cell wall protects against certain antibiotics (eg. Penicillin) and detergents/disinfectants

However, antibiotics have been developed which will attack gram-negatives (e.g. Ampicillin, Chloramphenicol, Streptomycin)

SOURCES

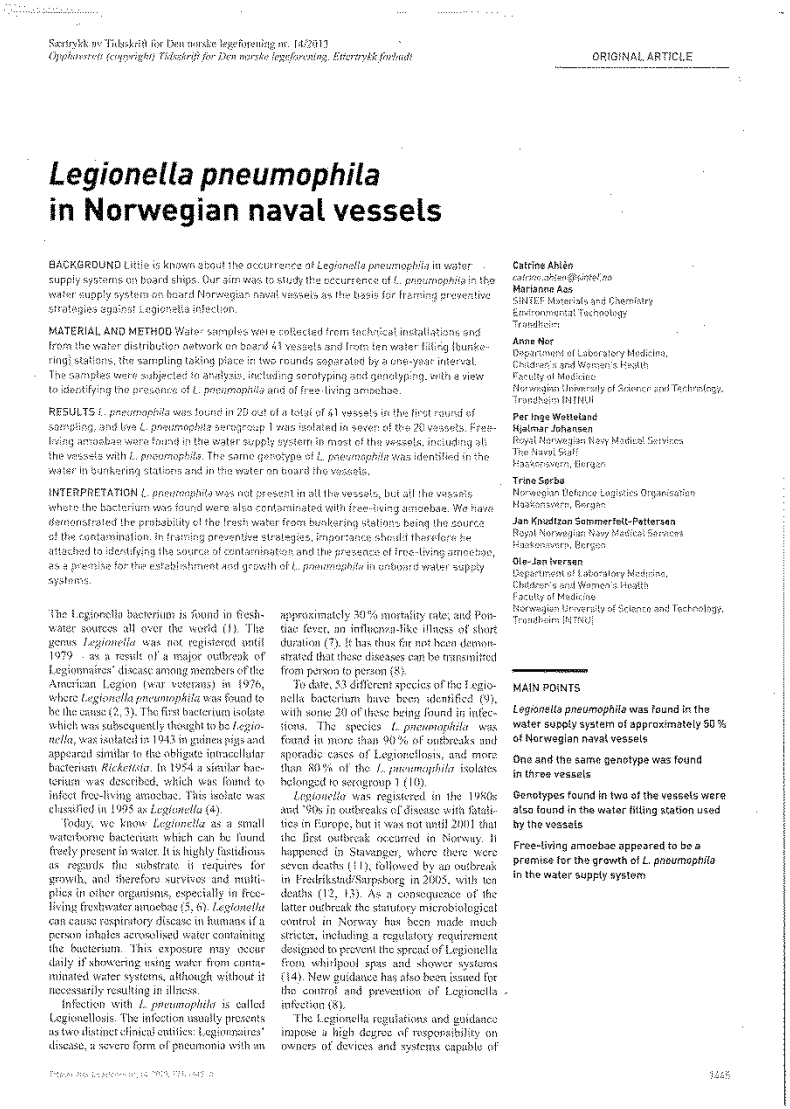
“INTRODUCED” PRODUCTS

- **FOODSTUFF**
- **BOOKS, PAPER ETC**
- **FRESH WATER**
- **SEAWATER**
- **MUD ETC**

~~**DIVERS!**~~

FRESH (SANITARY) WATER

BIOFILMS IN WATER SUPPLY SYSTEMS…….



Særtrykk av Tidsskrift for Den norske legeforening nr. 14/2013
Opphavsrett (copyright) Tidsskrift for Den norske legeforening. Ettertrykk forbudt

ORIGINAL ARTICLE

Legionella pneumophila in Norwegian naval vessels

BACKGROUND Little is known about the occurrence of *Legionella pneumophila* in water supply systems on board ships. Our aim was to study the occurrence of *L. pneumophila* in the water supply system on board Norwegian naval vessels as the basis for framing preventive strategies against Legionella infection.

MATERIAL AND METHOD Water samples were collected from technical installations and from the water distribution network on board 41 vessels and from ten water filling (bunkering) stations, the sampling taking place in two rounds separated by a one-year interval. The samples were subjected to analysis, including serotyping and genotyping, with a view to identifying the presence of *L. pneumophila* and of free-living amoebae.

RESULTS *L. pneumophila* was found in 20 out of a total of 41 vessels in the first round of sampling, and the *L. pneumophila* serogroup 1 was isolated in seven of the 20 vessels. Free-living amoebae were found in the water supply system in most of the vessels, including all the vessels with *L. pneumophila*. The same genotype of *L. pneumophila* was identified in the water in bunkering stations and in the water on board the vessels.

INTERPRETATION *L. pneumophila* was not present in all the vessels, but all the vessels where the bacterium was found were also contaminated with free-living amoebae. We have demonstrated the probability of the fresh water from bunkering stations being the source of the contamination. In framing preventive strategies, importance should therefore be attached to identifying the source of contamination and the presence of free-living amoebae, as a premise for the establishment and growth of *L. pneumophila* in onboard water supply systems.

Catrine Ahlèn
catrine.ahlen@sintef.no
Marianne Aas
SINTEF Materials and Chemistry
Environmental Technology
Trondheim

Anne Nor
Department of Laboratory Medicine,
Children's and Women's Health
Faculty of Medicine
Norwegian University of Science and Technology,
Trondheim (NTNU)

Per Inge Wetteland
Hjalmar Johansen
Royal Norwegian Navy Medical Services
The Naval Staff
Haakonsvern, Bergen

Trine Serba
Norwegian Defence Logistics Organisation
Haakonsvern, Bergen

Jan Knudtzon Sommerfelt-Pettersen
Royal Norwegian Navy Medical Services
Haakonsvern, Bergen

Ole-Jan Iversen
Department of Laboratory Medicine,
Children's and Women's Health
Faculty of Medicine
Norwegian University of Science and Technology,
Trondheim (NTNU)

MAIN POINTS

Legionella pneumophila was found in the water supply system of approximately 50 % of Norwegian naval vessels

One and the same genotype was found in three vessels

Genotypes found in two of the vessels were also found in the water filling station used by the vessels

Free-living amoebae appeared to be a premise for the growth of *L. pneumophila* in the water supply system

The Legionella bacterium is found in freshwater sources all over the world (1). The genus *Legionella* was not registered until 1979 – as a result of a major outbreak of Legionnaires' disease among members of the American Legion (war veterans) in 1976, where *Legionella pneumophila* was found to be the cause (2, 3). The first bacterium isolate which was subsequently thought to be *Legionella*, was isolated in 1943 in guinea pigs and appeared similar to the obligate intracellular bacterium *Rickettsia*. In 1954 a similar bacterium was described, which was found to infect free-living amoebae. This isolate was classified in 1995 as *Legionella* (4).

Today, we know *Legionella* as a small waterborne bacterium which can be found freely present in water. It is highly fastidious as regards the substrate it requires for growth, and therefore survives and multiplies in other organisms, especially in free-living freshwater amoebae (5, 6). *Legionella* can cause respiratory disease in humans if a person inhales aerosolised water containing the bacterium. This exposure may occur daily if showering using water from contaminated water systems, although without it necessarily resulting in illness.

Infection with *L. pneumophila* is called Legionellosis. The infection usually presents as two distinct clinical entities: Legionnaires' disease, a severe form of pneumonia with an approximately 30 % mortality rate; and Pontiac fever, an influenza-like illness of short duration (7). It has thus far not been demonstrated that these diseases can be transmitted from person to person (8).

To date, 53 different species of the Legionella bacterium have been identified (9), with some 20 of these being found in infections. The species *L. pneumophila* was found in more than 90 % of outbreaks and sporadic cases of Legionellosis, and more than 80 % of the *L. pneumophila* isolates belonged to serogroup 1 (10).

Legionella was registered in the 1980s and '90s in outbreaks of disease with fatalities in Europe, but it was not until 2001 that the first outbreak occurred in Norway. It happened in Stavanger, where there were seven deaths (11), followed by an outbreak in Fredrikstad/Sarpsborg in 2005, with ten deaths (12, 13). As a consequence of the latter outbreak the statutory microbiological control in Norway has been made much stricter, including a regulatory requirement designed to prevent the spread of Legionella from whirlpool spas and shower systems (14). New guidance has also been issued for the control and prevention of Legionella infection (8).

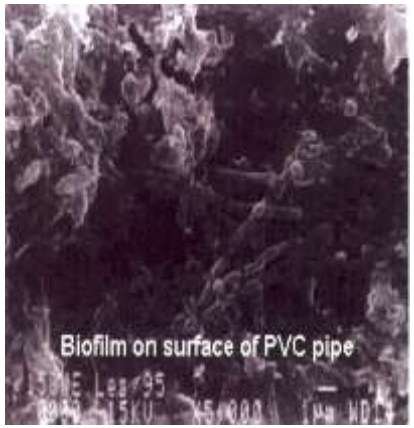
The Legionella regulations and guidance impose a high degree of responsibility on owners of devices and systems capable of

BACTERIAL FORMS IN WATER SUPPLIES

- FREELIVING (PLANKTONIC)

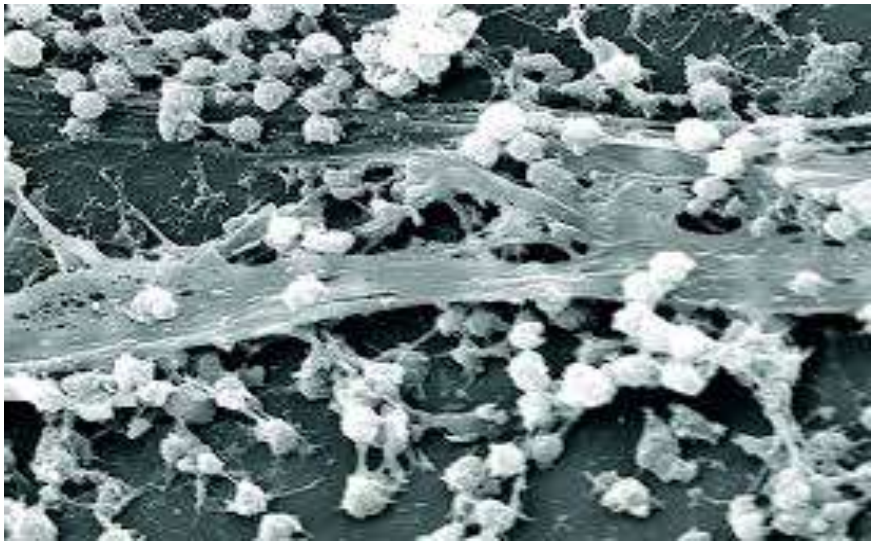
- INTRACELLULAR

- BIOFILM



BIOFILM

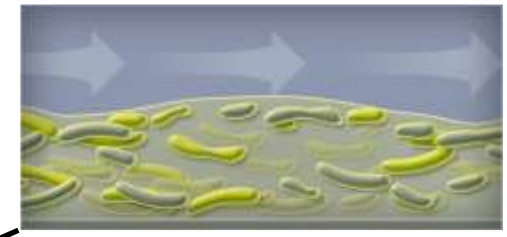
THE TERM “BIOFILM” DESCRIBES ANY ACCUMULATION OF MICROORGANISMS ADHERING TO INTERFACES (FREQUENTLY: SOLID/LIQUID). THE ORGANISMS IN A BIOFILM ARE EMBEDDED IN A MATRIX OF EXTRACELLULAR POLYMERIC SUBSTANCES (EPS).



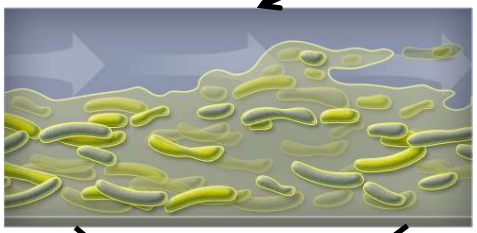
BIOFILM

Bacteria colonise inner surfaces of the drinking water installation and build up biofilms

Produce “glue”, Extracellular Polymeric Substance - EPS



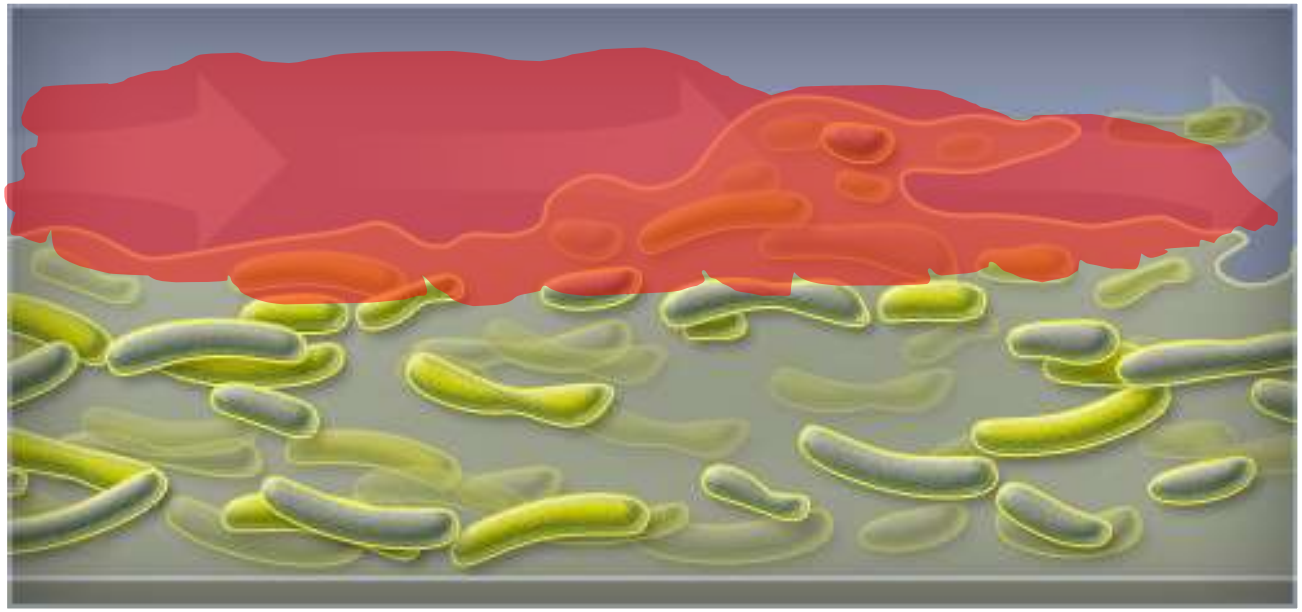
Quorum sensing.
Higher levels of homoserine lactones – HSL
Communication…..



Genetic changes and increased resilience

Biofilm particles can shear off and contaminate water for drinking & washing

DISINFECTION – HEAT TREATMENT



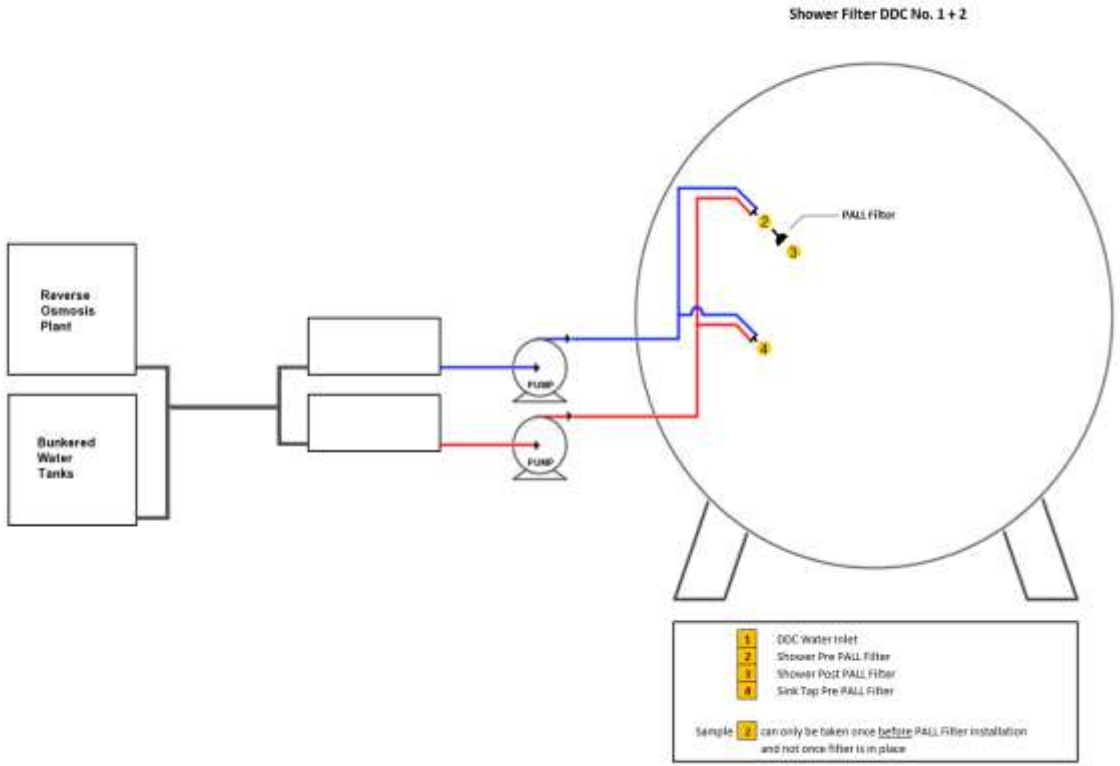
TOP LAYER MAY GET KILLED OR SLOUGHED OFF DEPENDING ON RESILIENCE

LOWER LEVELS CONTINUE TO THRIVE OR PROTECT THEMSELVES BY GOING INTO A DORMANT STATE OR FORM HARD COATING (CYST) OR VIABLE BUT NOT CULTURABLE PHASE (VBNC)

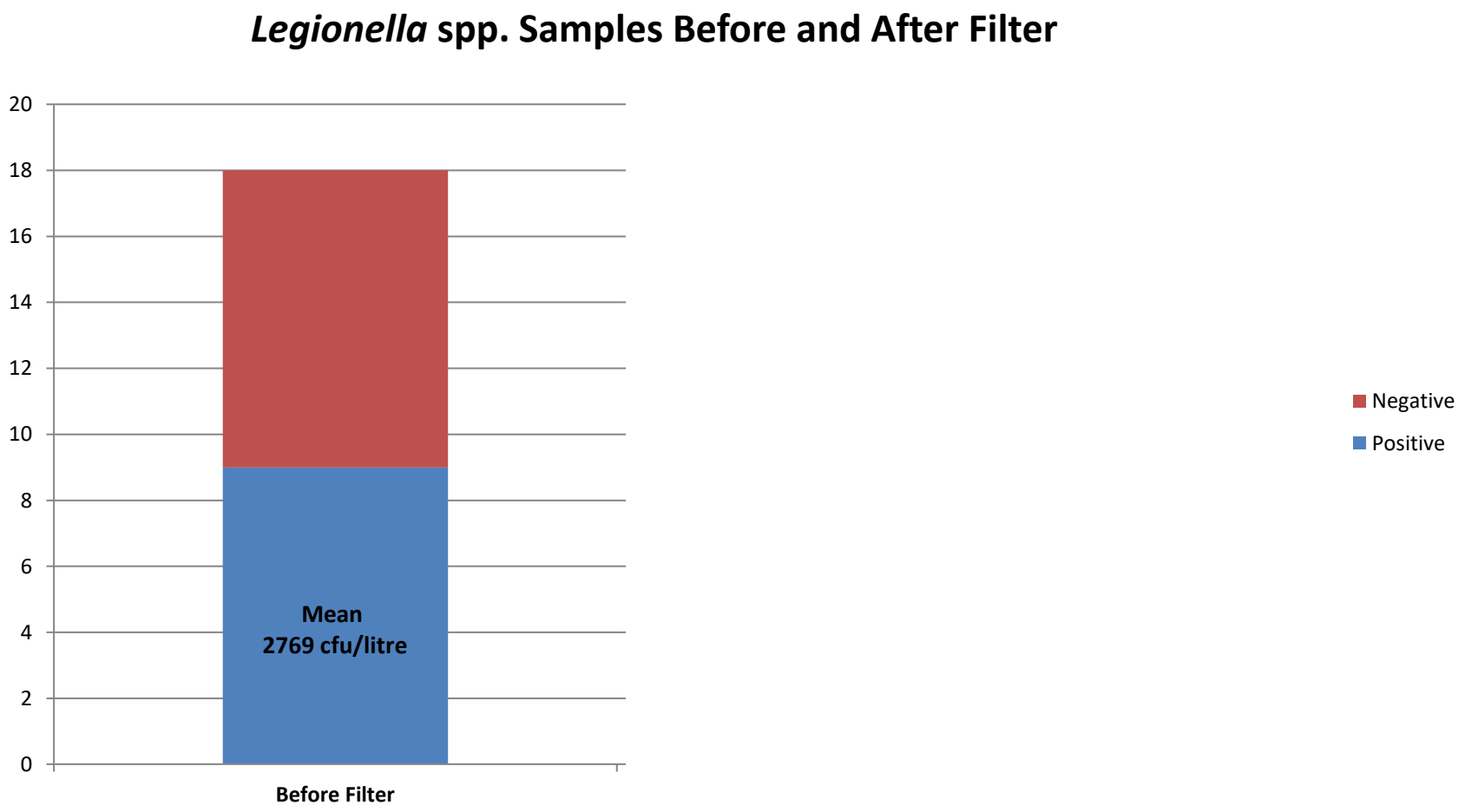
“REACTIVATE” WHEN CONDITIONS RETURN TO NORMAL

TRIALS

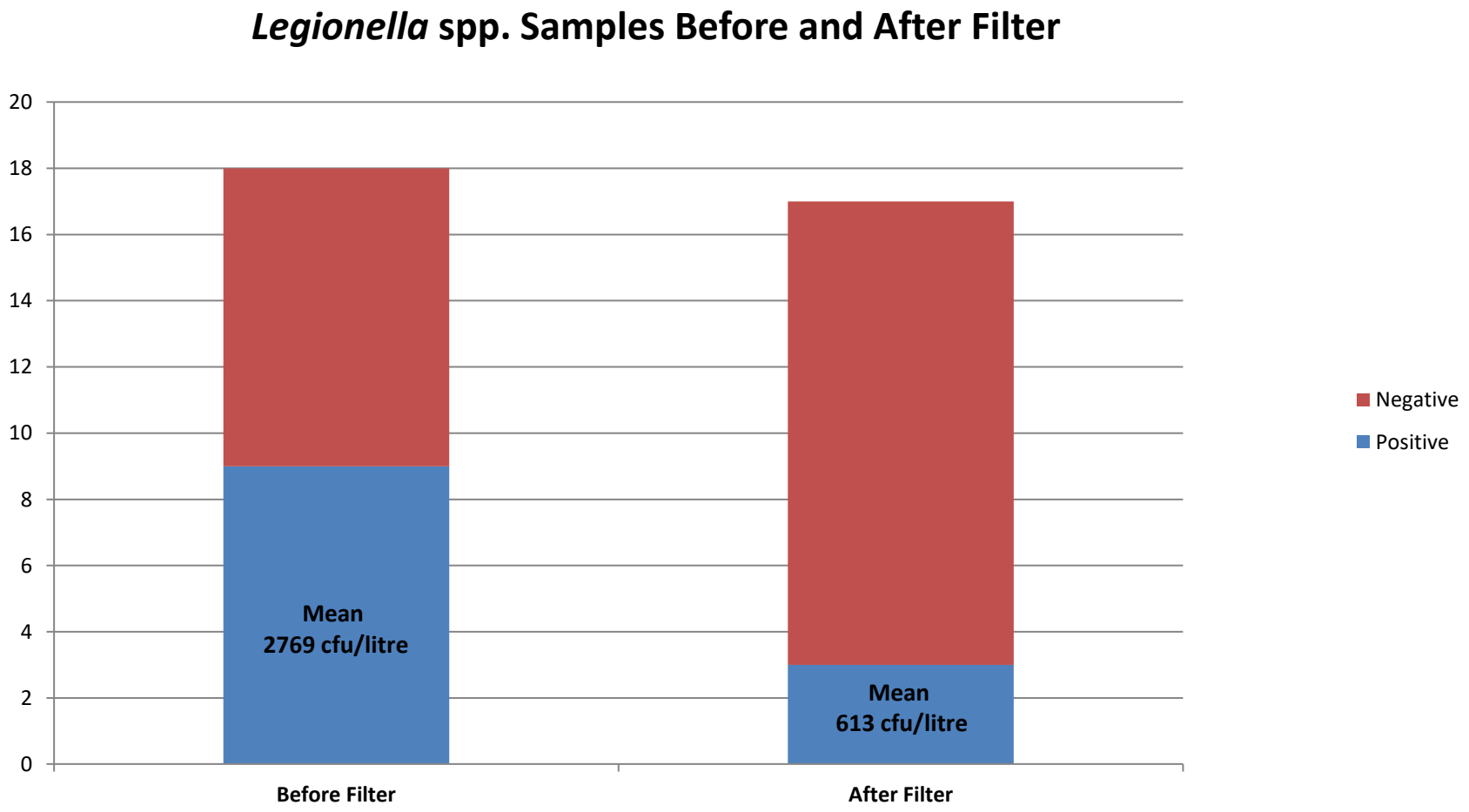
- DECIDED TO PERFORM SOME TRIALS ON 2 SHIPS



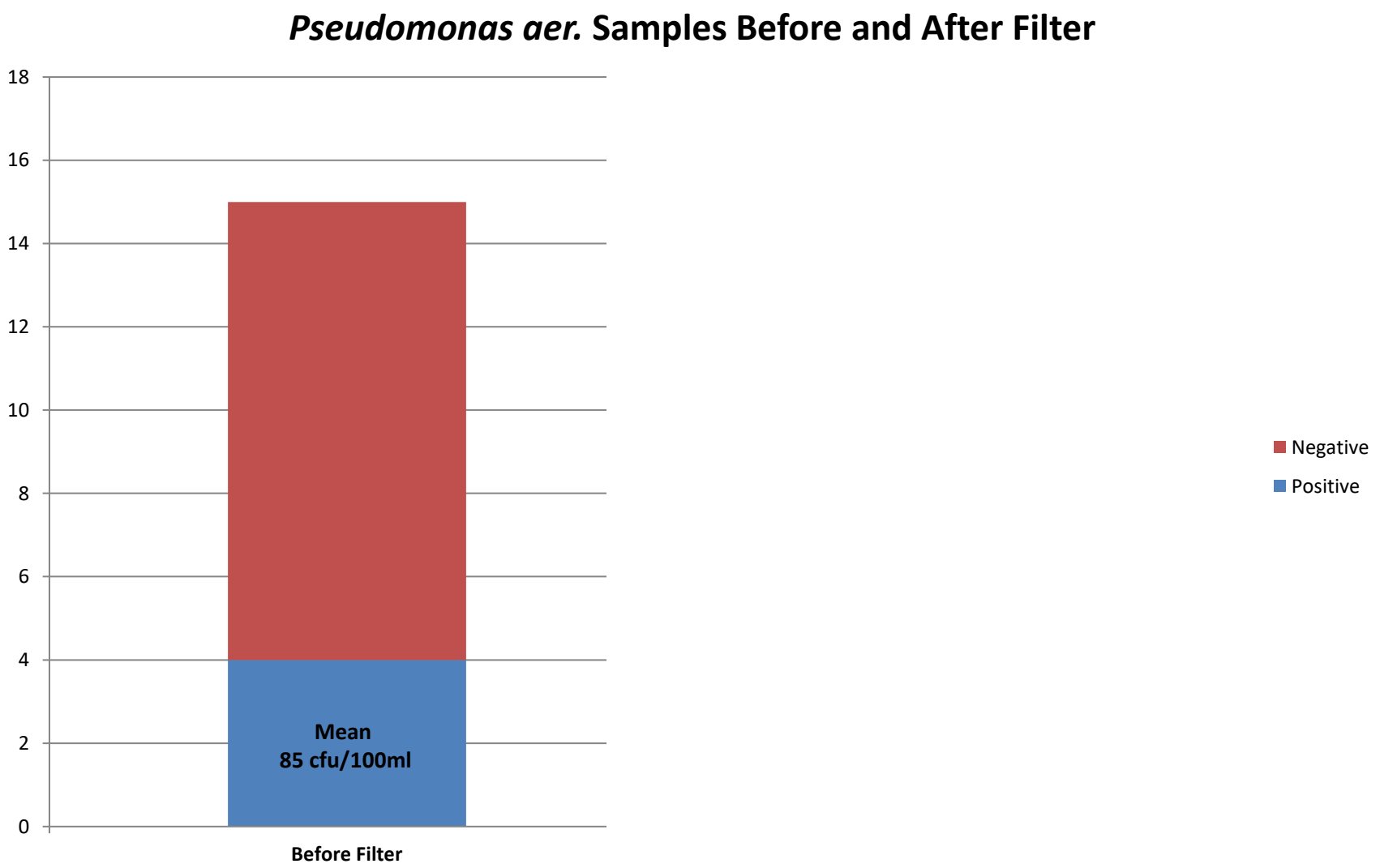
TRIALS VESSEL 1



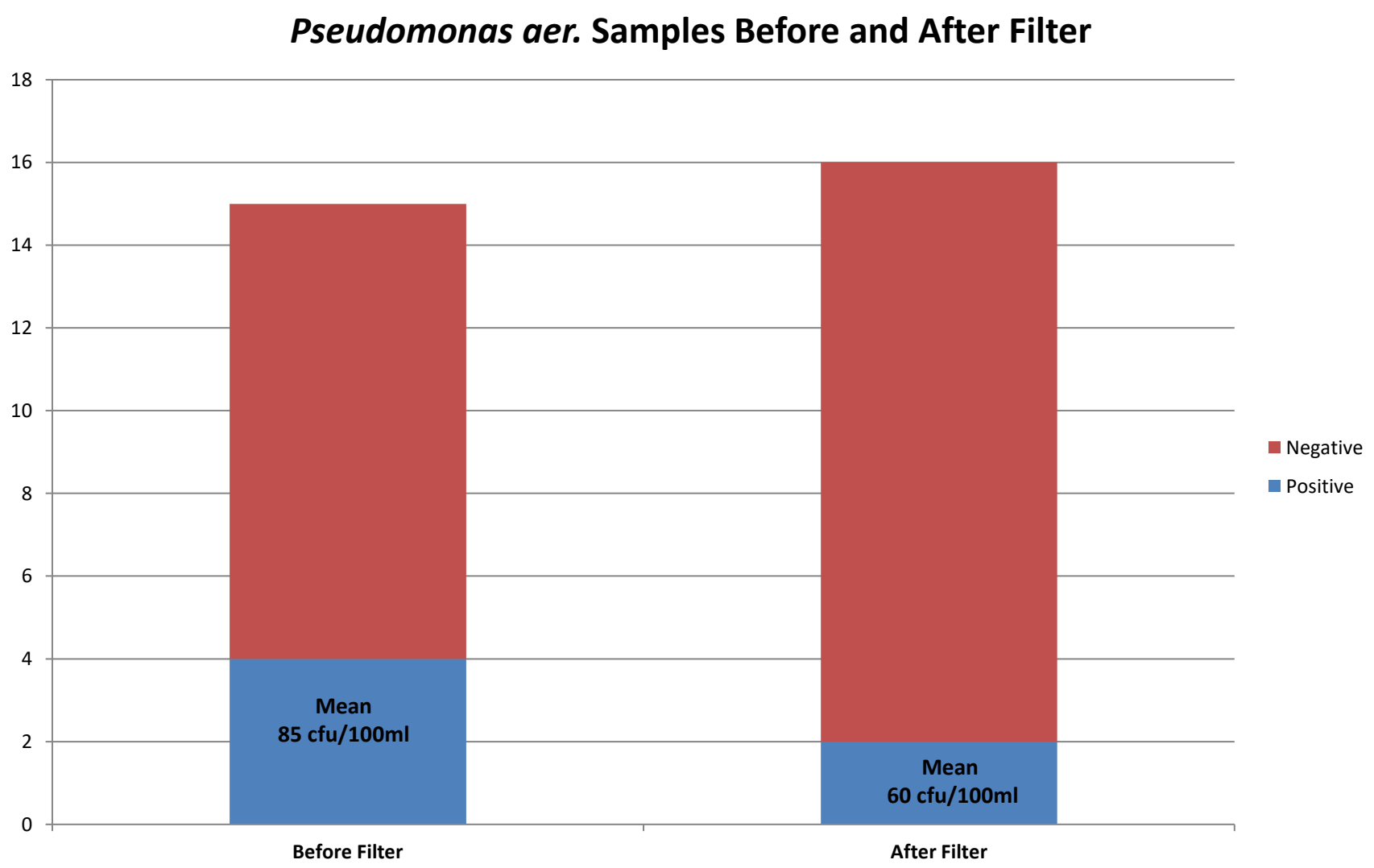
TRIALS VESSEL 1



TRIALS VESSEL 1



TRIALS VESSEL 1

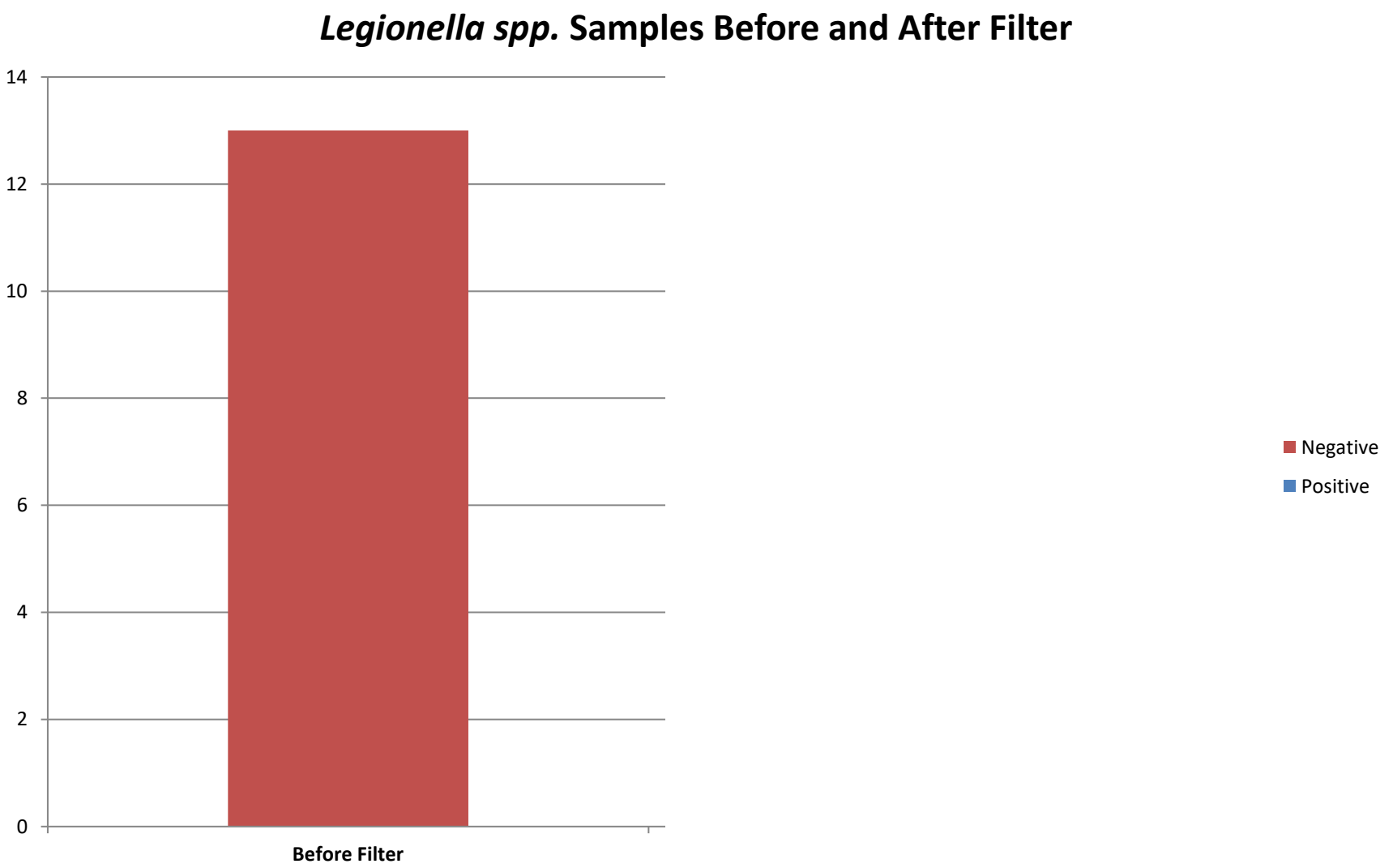


SAMPLING TECHNIQUES

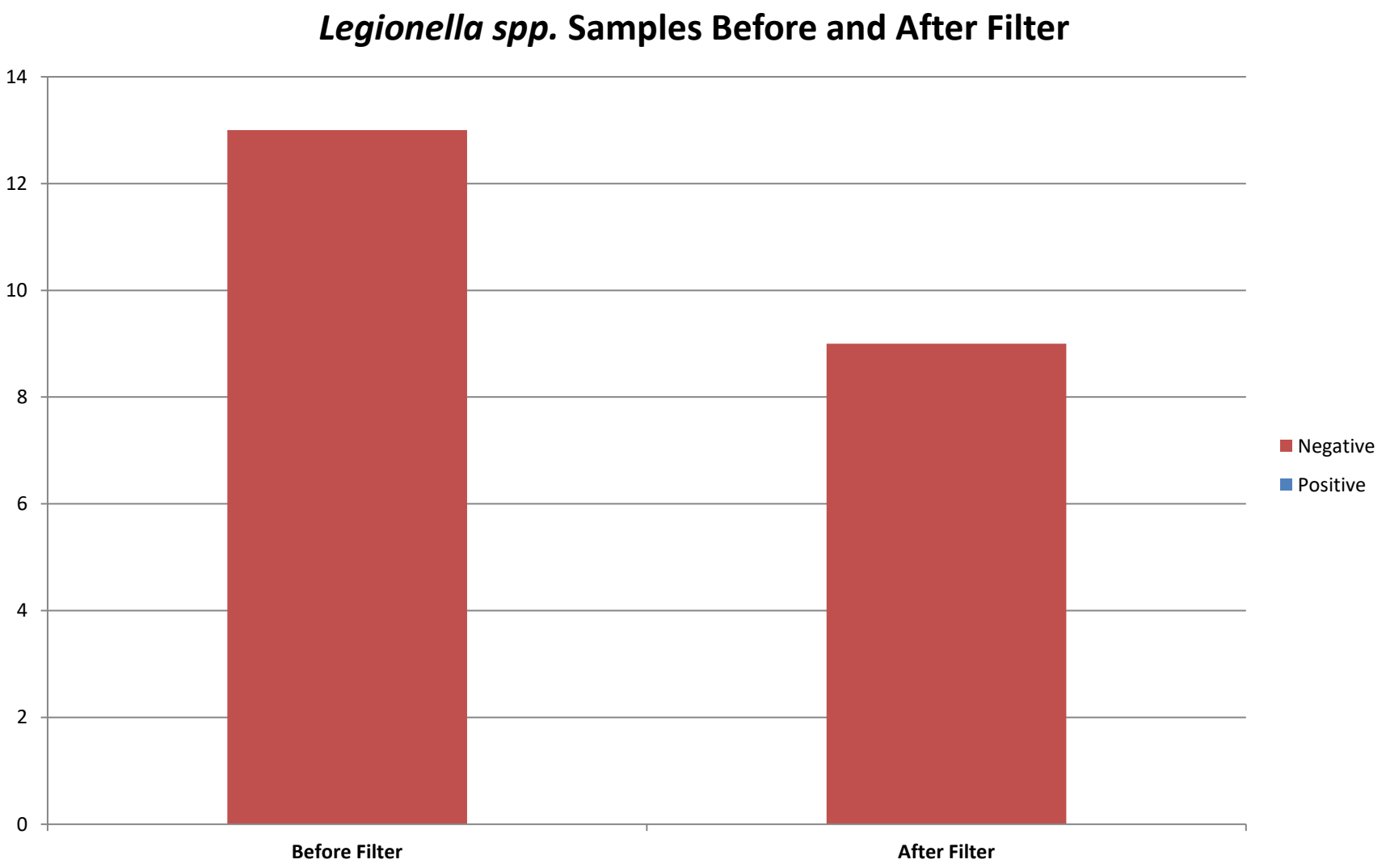
- SAMPLING TECHNIQUES EXTREMELY IMPORTANT, E.G. ENTRAINMENT, CONTACT WITH SAMPLE BOTTLE ETC

  - FALSE POSITIVES

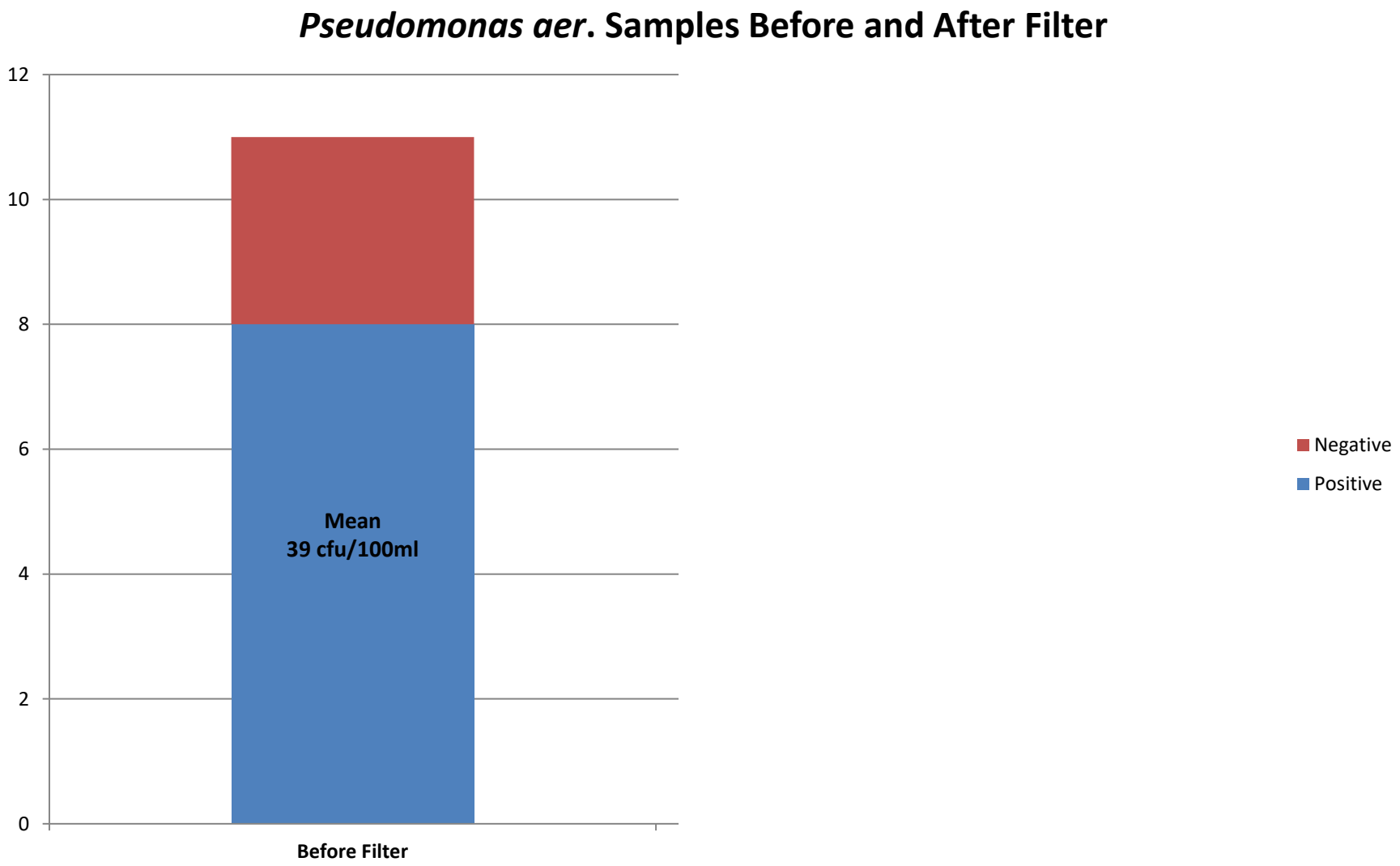
TRIALS VESSEL 2 - IMPROVED SAMPLING



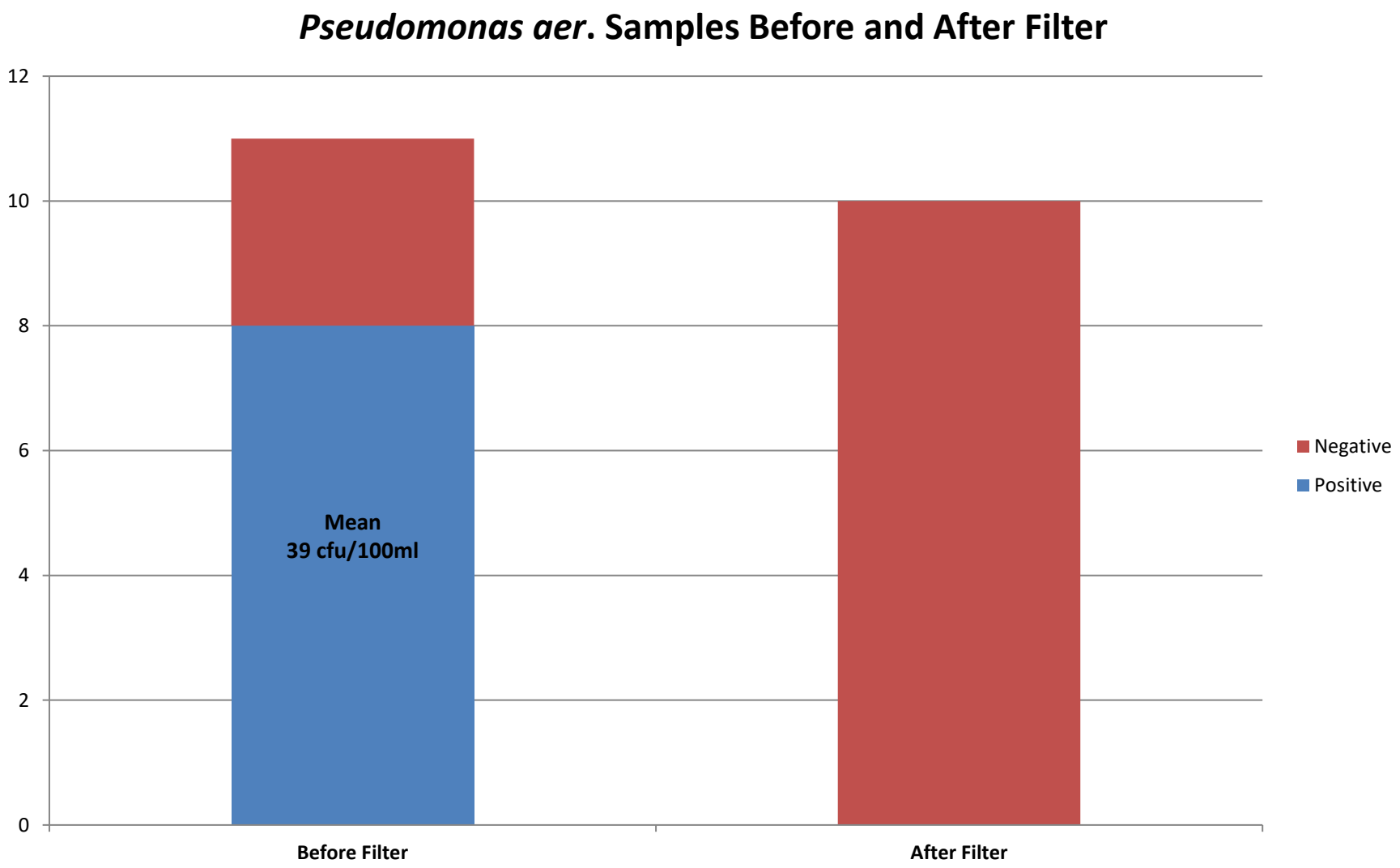
TRIALS VESSEL 2 - IMPROVED SAMPLING



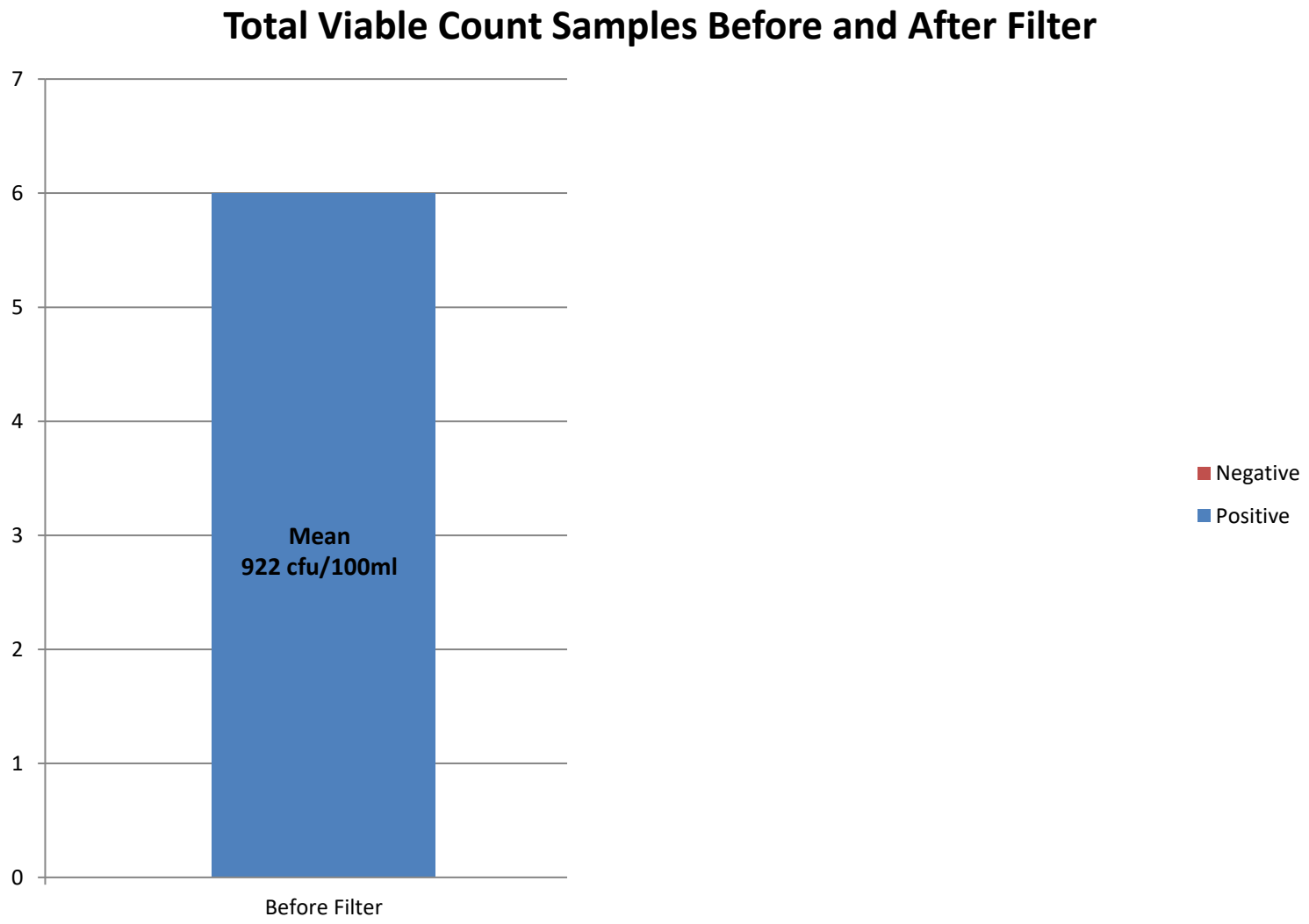
TRIALS VESSEL 2 - IMPROVED SAMPLING



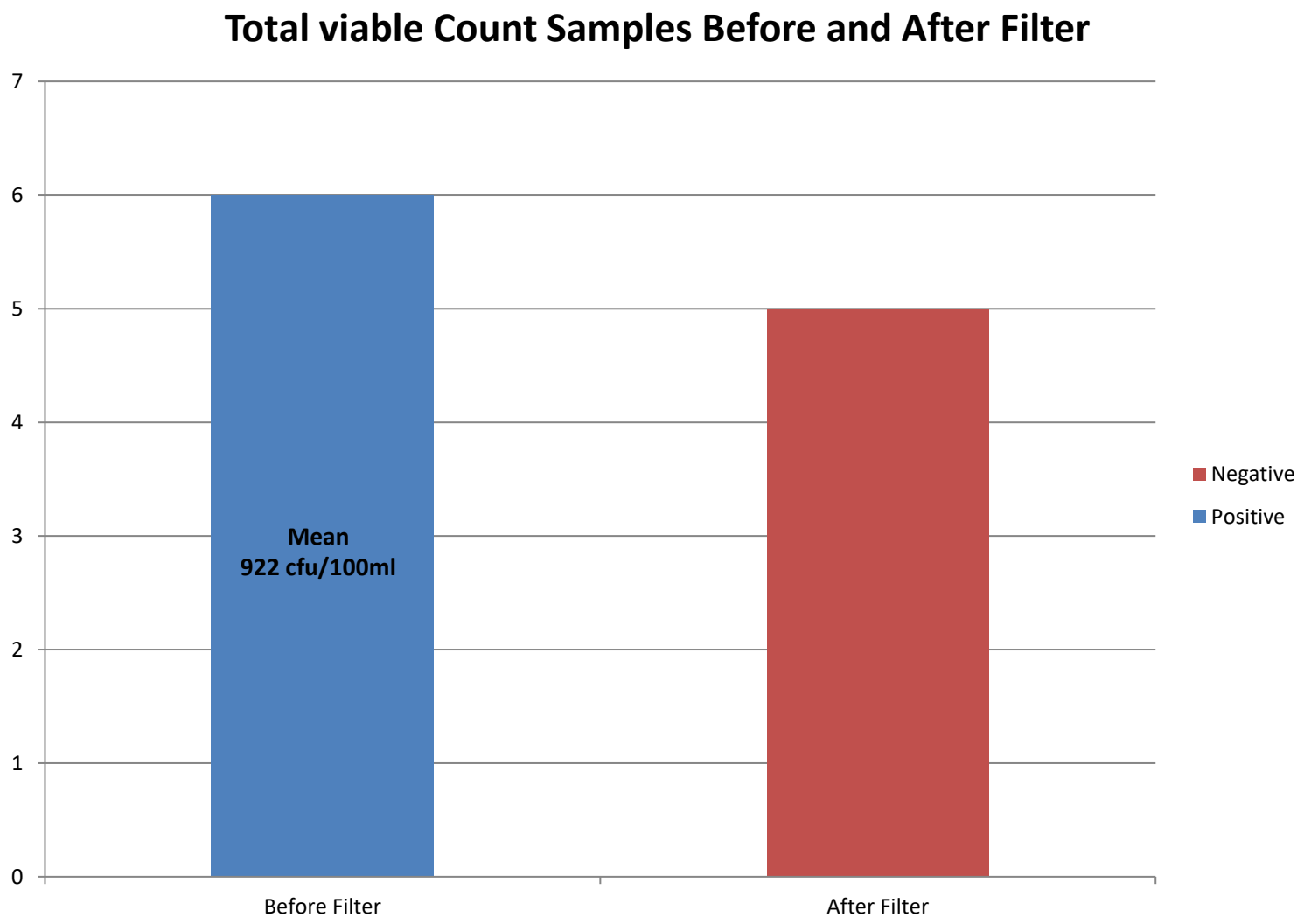
TRIALS VESSEL 2 - IMPROVED SAMPLING



TRIALS VESSEL 2 - IMPROVED SAMPLING



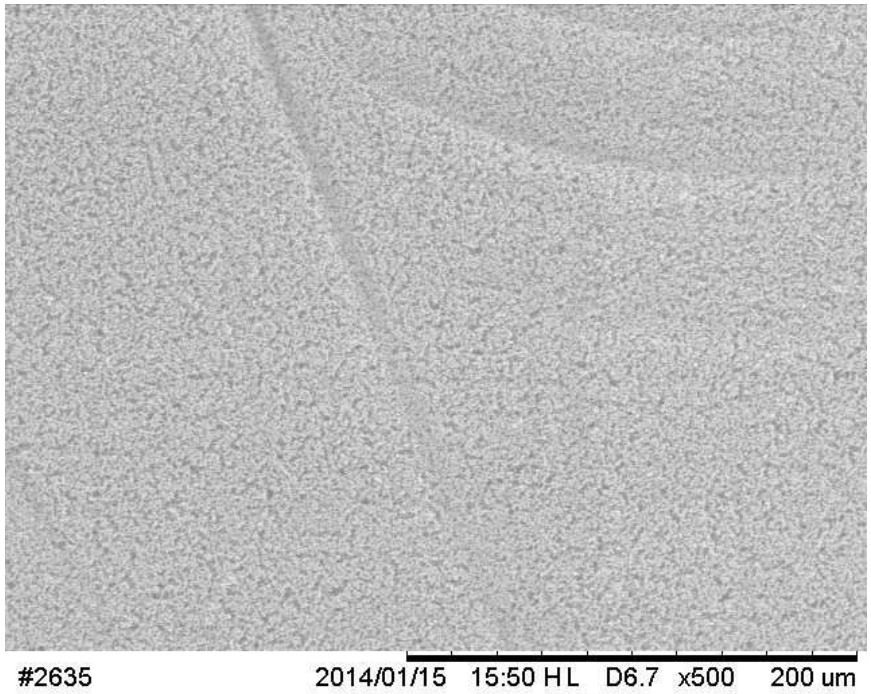
TRIALS VESSEL 2 - IMPROVED SAMPLING



POINT OF USE FILTERS

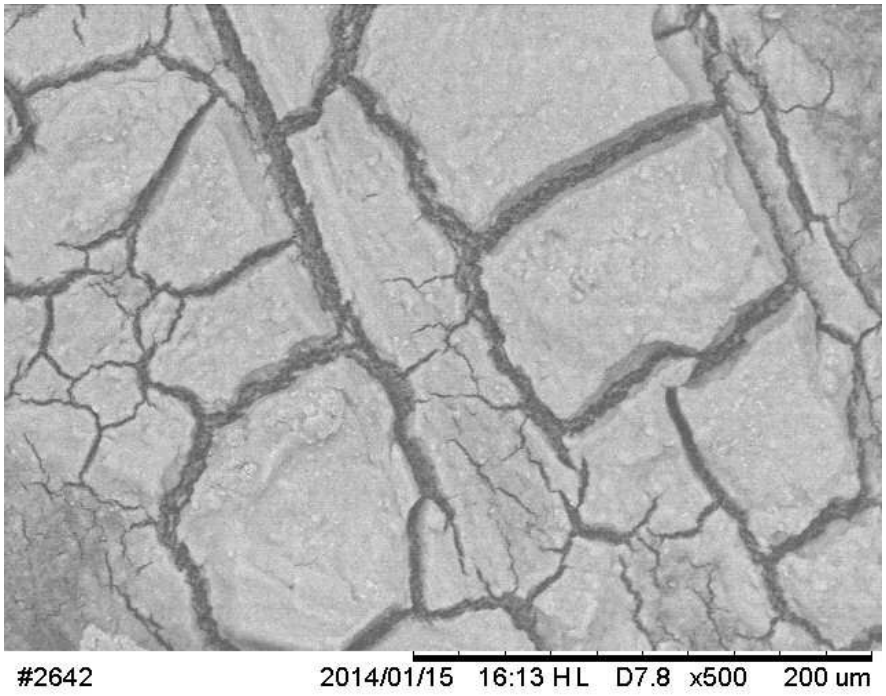
CHALLENGES FOR FILTERS

ANALYSIS OF FILTER CONDITION

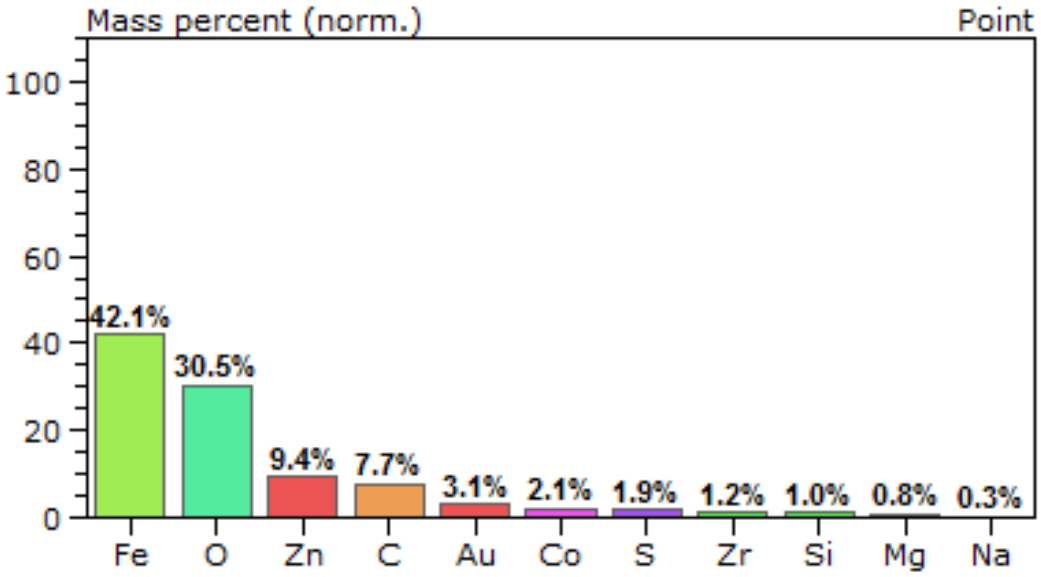


Control

After 3 weeks
In DDC



ANALYSIS OF FILTER CONDITION



Spectrum: Point

Element	AN	Series	norm. C [wt.%]	Atom. C [at.%]
Iron	26	K-series	42.07	20.65
Oxygen	8	K-series	30.47	52.21
Zinc	30	K-series	9.38	3.93
Carbon	6	K-series	7.70	17.57
Gold	79	M-series	3.09	0.43
Cobalt	27	K-series	2.08	0.97
Sulfur	16	K-series	1.94	1.66
Zirconium	40	L-series	1.16	0.35
Silicon	14	K-series	1.04	1.02
Magnesium	12	K-series	0.77	0.87
Sodium	11	K-series	0.29	0.35
		Total:	100.00	100.00

ROUTINE PREVENTATIVE MEASURES

STRICT PERSONAL HYGIENE- “WASH TOP – DOWN”!

GOOD CHAMBER HOUSE-KEEPING

FREQUENT BEDDING CHANGES

NO SHARED HEADPHONES

NO COTTON BUDS!

OWN HAT LINER

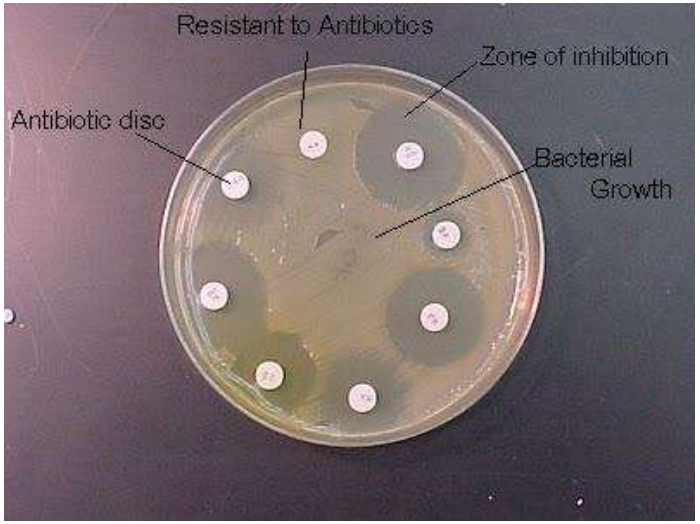
PROPHYLACTIC (PREVENTATIVE) EAR DROPS. ONE BOTTLES FOR EACH EAR PER DIVER (LABELLED). ALUMINIUM ACETATE IN ACETIC ACID

SHOWER AND TAP FILTERS

SWABBING

- SWAB AND YOU WILL FIND!
- ROUTINE AND WIDESPREAD SWABBING IS OF LITTLE USE

- SWABBING OF TOILETS, SINK DRAINS, BILGES ETC. WILL GIVE POSITIVE RESULTS (SAME AS IT WOULD AT HOME!)
- "JUDICIAL" SWABBING CAN BE VERY USEFUL



- SHOWER HEADS
- TAPS
- SUSPECTED INFECTION (BEFORE TREATMENT DROPS)

SUMMARY

- ALL SURFACES IN A POTABLE WATER SYSTEM, IN CONTACT WITH WATER WILL BE COLONISED BY BIOFILM.

- PLUMBING MATERIALS CAN BE NUTRIENTS FOR THE BACTERIA IN THE BIOFILM.

- CULTURE OF SAMPLES MAY NOT REVEAL THE EXTENT OF CONTAMINATION

  - RESULTS OF SAMPLES FROM SAME SOURCE CAN DIFFER WIDELY
    E.G. SAMPLES FROM SAME TAP ONE HOUR APART
    <1 cfu/100ML
    > 100 cfu/100ML

  - AMOUNT OF BACTERIA GROWN IN CULTURE MAY BEAR VERY LITTLE RELATIONSHIP TO ACTUAL LEVELS AS MANY BACTERIA ARE "VIABLE BUT NOT CULTURABLE" – VBNC.

- HEAT TREATMENT AND DISINFECTION HAVE TEMPORARY EFFECTS, STRESSED CELLS MAY GO IN TO VBNC STATE AND THEN "REACTIVATE" WHEN STRESS CONDITIONS HAVE GONE, ANY DEAD CELLS PROVIDE NUTRIENT FOR THE SURVIVORS AND NEWCOMERS

SUMMARY

- BIOFILM POPULATIONS ADAPT AND CAN INCREASE THEIR RESISTANCE TO DISINFECTANTS AND ANTIBIOTICS

- IT IS VIRTUALLY INEVITABLE THAT THE CHAMBER WATER SUPPLY SYSTEMS WILL BE POPULATED BY *PSEUDOMONAS* AND OTHER BACTERIA
- POINT OF USE FILTRATION (POU) IS RECOGNISED BY REGULATORS AS THE MOST EFFECTIVE DEFENCE IN WATER SUPPLY SYSTEM
- HENCE WIDELY USED IN HOSPITAL ICU/HDU, PAEDIATRIC UNITS, CANCER TREATMENT CENTRES AS WELL AS WASHING FACILITIES IN HEALTH CLUBS, GYMS ETC.
- LIFESPAN OF FILTERS REDUCED DUE TO POOR WATER QUALITY ON INLET SIDE
- CAN BE AN IMPORTANT TOOL IN REDUCING INFECTION RISK IN DIVERS.