DIPARTIMENTO DI SANITA' PUBBLICA E MALATTIE INFETTIVE (DSPMI) UNIVERSITÀ DI ROMA LA SAPIENZA

TRAINING AND TRANSFER OF NOVEL METHODOLOGIES TO ENTERPRISES INVOLVED IN THE BACTERIAL, VIRAL, MYCOTIC AND PARASITIC INFECTIONS IN NATIONAL AND INTERNATIONAL COUNTRIES

SUPPORTED BY LAZIO REGION

PRINCIPAL INVESTIGATORS

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Aims of the Project

-Innovative methodologies to detect microorganisms and viruses in humans, medical devices and environment: present and future

ALESSANDRA della TORRE
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-Innovative methodologies to detect parasites and insects: present and future

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AIMS

I° YEAR (2016):

- recruitment of researchers;
- set-up novel methodologies for pathogen diagnosis and epidemiological surveillance;
- setting up and activation of the web-site;
- meetings on project results

II° YEAR (2017):

-finalization of the novel microbiological methods and protocols for epidemiological surveillancee;
-statistical analysis between novel and classical methods ;

-statistical analysis of epidemiological data ;
-evaluation of the results and socio-economic impact of the project;

-publication of the results and demonstrative movies in the web-site;
-setting up demonstrative laboratories in DSPMI;
-stage at Lazio region and national Enterprises or Institutions

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NOVEL METHODOLOGIES FOR PATHOGEN DIAGNOSIS

BACTERIA AND MYCETES

VIRUSES

PARASITES ----Prof.ssa ALESSANDRA della TORRE

NOVEL METHODOLOGIES FOR EPIDEMIOLOGICAL SURVEILLANCE OF THE RISK BY PATHOGENS AND CONTAMINANTS

BACTERIA AND MYCETES

VIRUSES

PARASITES AND INSECTS ----Prof.ssa ALESSANDRA della TORRE

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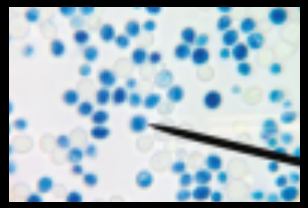
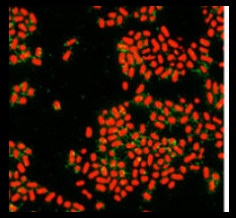
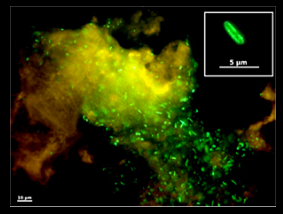
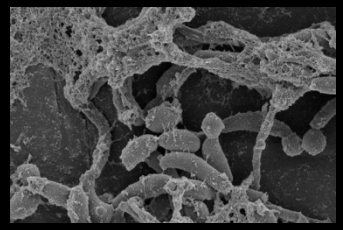
BACTERIA AND MYCETES
can acquire different morphologies corresponding to different lifestyles

	BACTERIA	MYCETES
PLANKTONIC FORM		
AGGREGATE FORM		
BIOFILM FORM		

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THE SUCCESS OF AN INFECTION IS STREACTLY RELATED TO

- MICROBIAL COLONIZATION OF THE HOST ----- MICROBIAL NUMBER
- PLANKTONIC OR BIOFILM LIFESTYLE
- FAILURE OF ANTIBIOTIC THERAPY

EVEN IF MICROBIOLOGICAL TESTS SHOW SEVERAL LIMITS AND DO NOT MIMIC THE IN VIVO INFECTIONS

CLINIC MICROBIOLOGY NEEDS TO DIAGNOSE THE ETIOLOGICAL AGENT TO PREVENT THE PATHOGENESIS AND THE SPREADING OF THE INFECTION , WHILE NEGLECT TO QUANTIFY THE NUMBER OF MICROORGANISMS COLONIZING THE INFECTION SITES

TO DATE ONLY ONE METHOD, FDA VALIDATED, IS UNIVERSALLY APPLIED TO COUNT MICROORGANISMS:
COLONY FORMING UNIT (CFU) COUNT

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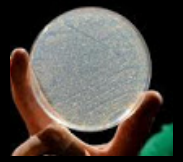
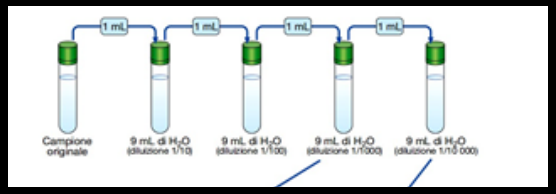
CFU COUNT

IS A METHOD RELIABLE TO COUNT VIABLE MICROORGANISMS PRESENT IN A SAMPLE

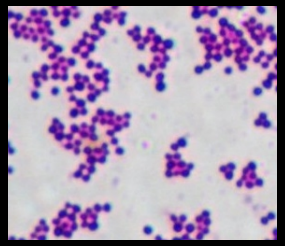


- THE SAMPLE IS INOCULATED ON SOLID MEDIUM
- IF THE NUMBER OF THE COLONIES IS > 300/ML, THE DILUTION OF THE SAMPLE IS REQUIRED

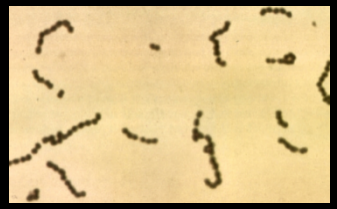


BUT

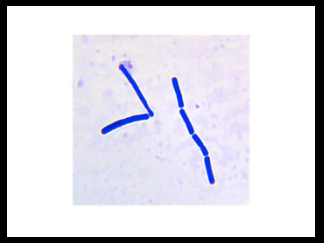
EACH COLONY CAN DERIVE FROM A SINGLE BACTERIUM OR SEVERAL AGGREGATE BACTERIA ACCORDING TO THE MORPHOLOGY OF THE GENUS



Staphylococcus



Streptococcus



Bacillus

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ADVANTAGES AND DISADVANTAGES OF CFU COUNT

ADVANTAGES

- VALIDATION FDA
- SIMPLE METHOD
- COUNT OF VIABLE PLANKTONIC BACTERIA

DISADVANTAGES

- ONE COLONY DERIVES FROM **X** BACTERIA
- IS NOT RELIABLE TO QUANTIFY BACTERIA IN BIOFILM

BIOFILM PLAYS A CRUCIAL ROLE IN THE INFECTIONS OF CATHETERS, MEDICAL DEVICES AND PROSTHESIS

BACTERIA IN BIOFILM ARE MORE RESISTANT TO ANTIBIOTICS COMPARED WITH PLANKTONIC FORM

A fundamental prerequisite in studying, controlling and/or counteracting biofilm formation and development is the possibility of quantifying the actual number of bacteria involved.

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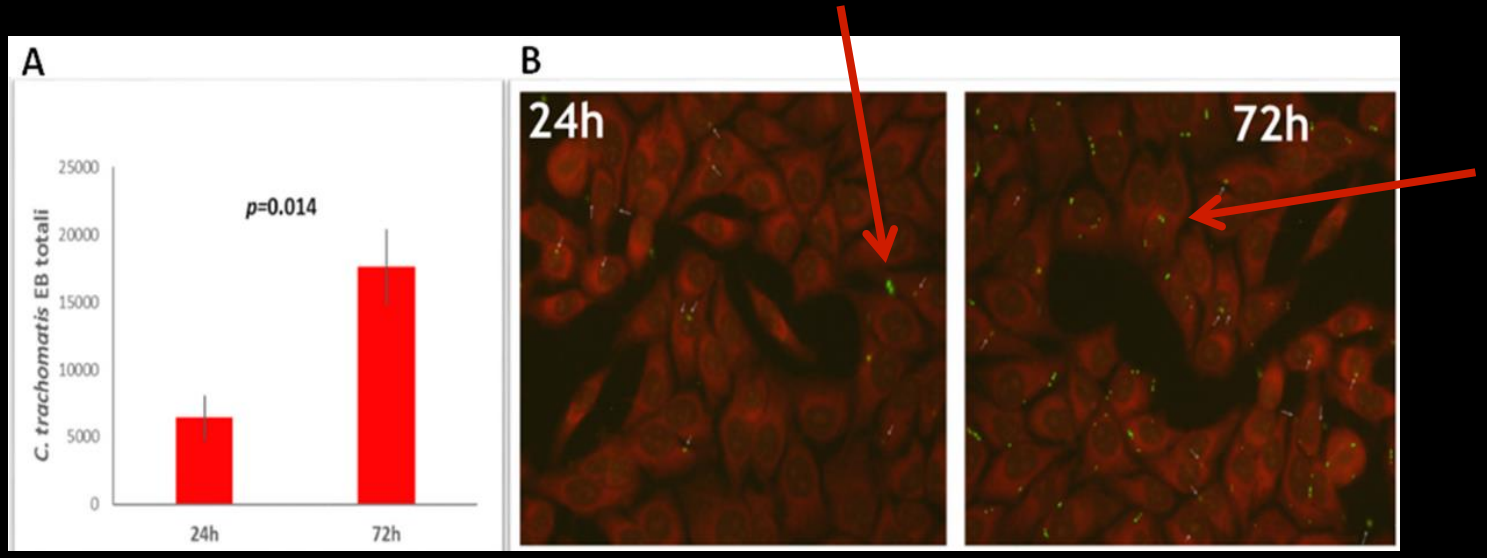
INFLUENCE OF BIOFILM IN *CHLAMYDIA TRACHOMATIS*, ETIOLOGICAL AGENT OF STERILITY AND PATHOLOGICAL PREGNANCIES

ASYMPTOMATIC INFECTION DIFFICULT TO DIAGNOSE AND ERADICATE

NOVEL DIAGNOSIS IN CERVICAL-VAGINAL SECRETION CHLAMYDIA TRACHOMATIS ADHERENT TO CANDIDA BIOFILM

(rosa.sessa@uniroma1.it)



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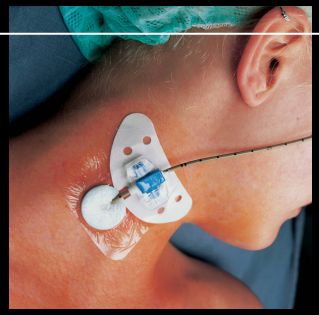
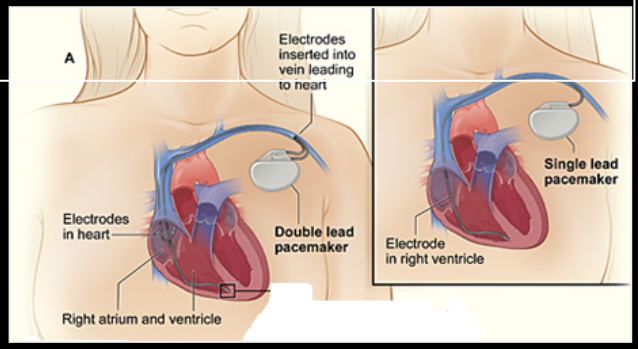
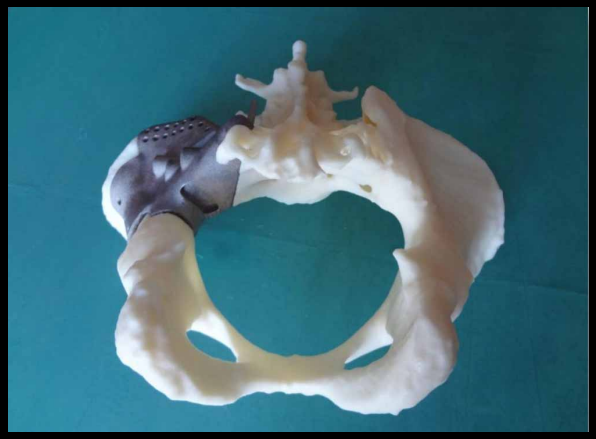
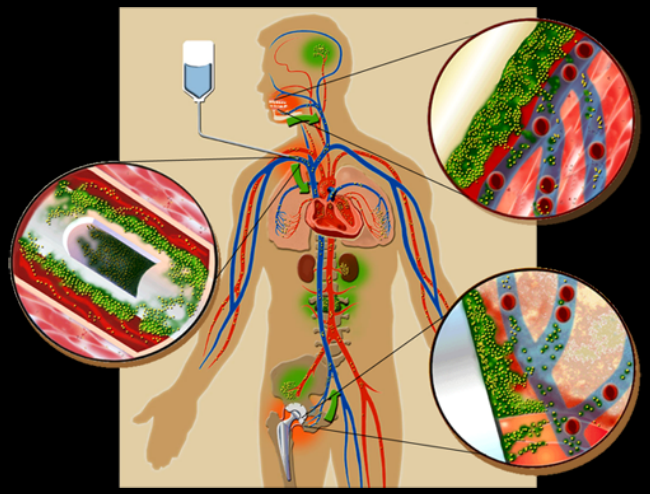
The implantation of catheters, medical devices and prostheses can frequently induce severe infections by bacteria adherent in biofilms is









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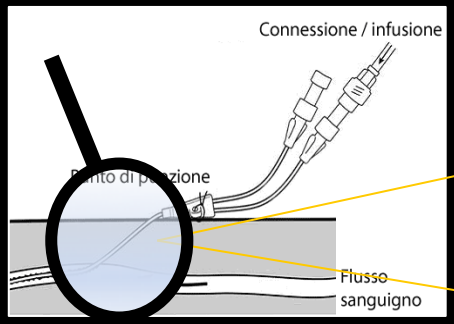
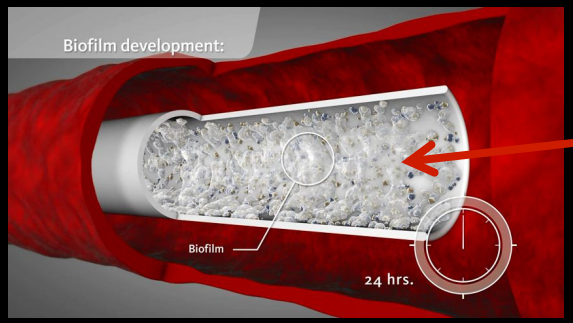


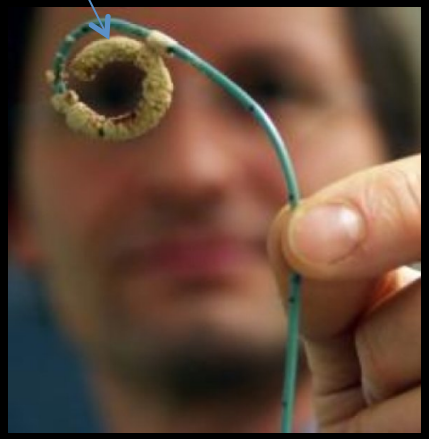
Biofilm difficult to eradicate

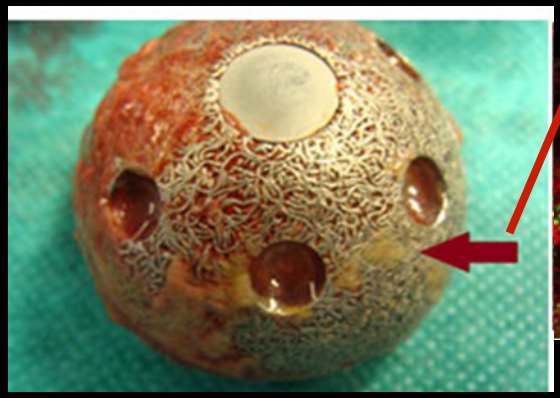
Resistant to antibiotics



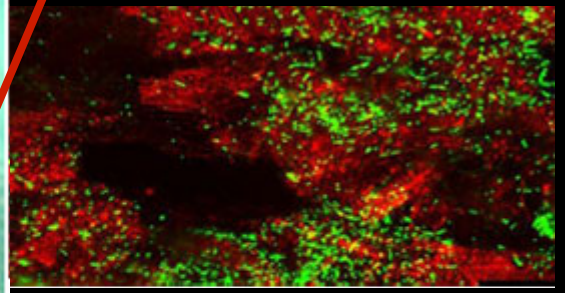
Colonization of the catheter's lumen

Stent colonization





Prosthesis colonization



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The implantations of 500.000 catheters / year induce

25.000 infections

12%- 25% mortality

Increase of hospitalization costs from 300 € to 5.616 € per patient

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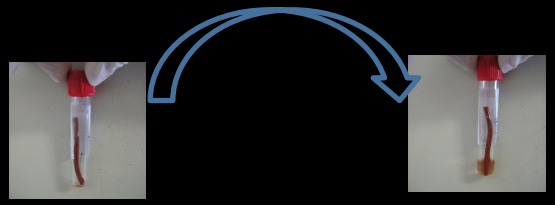
Policlinico Umberto I
Università di Roma La Sapienza

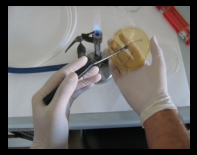
Cleri method

1) The catheter is immersed in physiological solution



2) Treatment with vortex and sonicator
to remove adherent bacteria

3) After the treatment, the microbial suspension is inoculated in selective and complete media incubated at 37° C for 24/48 h



4) After incubation, the CFU count is carried out from complete media while the microbial identification from selective media

CLERI METHOD IS VALIDATED

EVEN IF SHOWS SOME LIMITS

1. **DOUBT ON THE COMPLETE REMOVAL OF ADHERENT BACTERIA**

2. **THE NUMBER OF BACTERIA $\geq 10^3$ CFU/ml INDICATES THE INFECTION OF CATHETER, WHILE THE VALUE $< 10^3$ CFU/ml INDICATES THE UNINFECTED CATHETER**

3. **THE ANTIBIOGRAM IS PERFORMED ON THE PLANKTONIC FORM INSTEAD OF BIOFILM ONE**

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BIOTIMER ASSAY

- ENUMERATES BACTERIA ADHERENT, AGGREGATED AND IN BIOFILM THROUGH THE DETECTION OF A EXTRACELLULAR METABOLIC MICROBIAL PRODUCT
- DETECTS THE NUMBER OF MICROORGANISMS IN *SITU* WITHOUT ANY MANIPULATION OF THE SAMPLE
- UTILISES AN ORIGINAL REAGENT CONTAINING INDICATORS THAT SWITCH THEIR COLOR ACCORDING TO MICROBIAL METABOLIC PRODUCTS
- THE TIME REQUIRES FOR COLOR SWITCH OF THE INDICATOR IS RELATED TO THE NUMBER OF MICROORGANISMS PRESENT AT TIME 0 THROUGH SPECIFIC CORRELATION LINES

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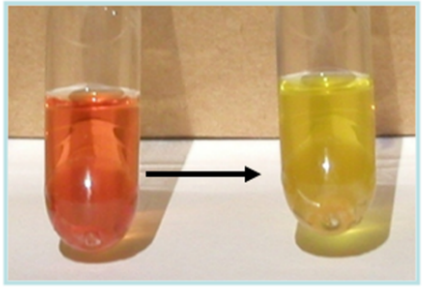
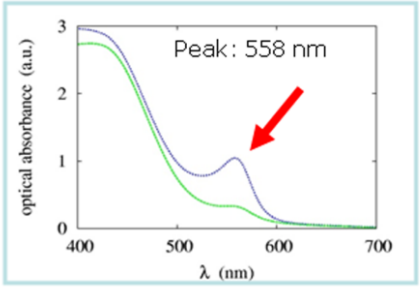
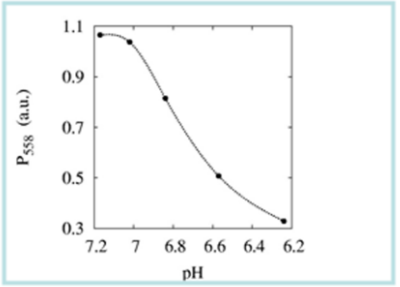
REAGENT TO DETECT FERMENTING MICROORGANISMS (BTA)



Reagent with phenol red:
initial color (left, red) and after switching (right, yellow).

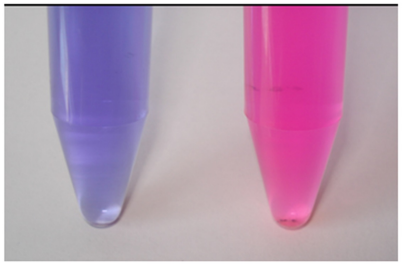


Spectrophotometric detection:
initial and switched reagent (blue and green line, respectively)

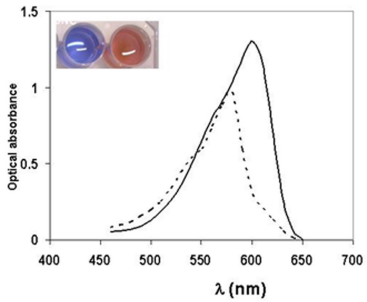


Reagent switch as function of pH (λ 558)

REAGENT TO DETECT NON- FERMENTING MICROORGANISMS (BTR)



Reagent with resazurin:
initial color (left, blue) and after switching (right, pink).



Spectrophotometric detection:
initial and switched reagent (solid and dotted line, respectively)

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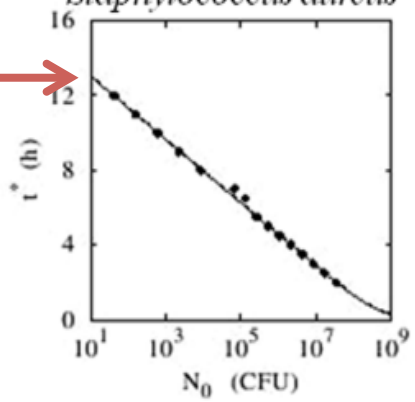
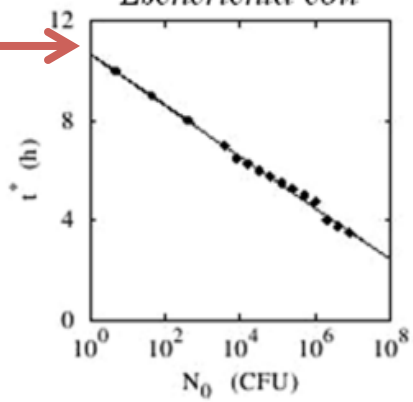
CORRELATION LINES

THE TIME OF COLOR SWITCH IS INVERSELY RELATED TO THE NUMBER OF MICROORGANISMS

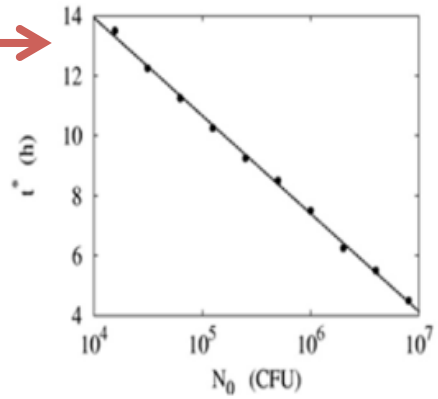
Staphylococcus aureus



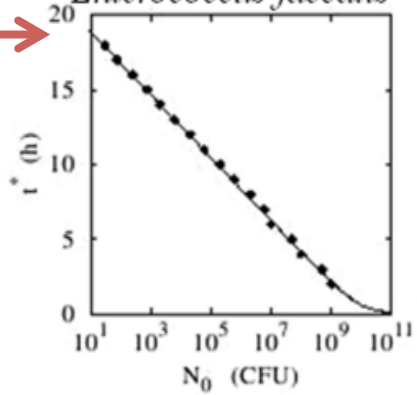
Escherichia coli



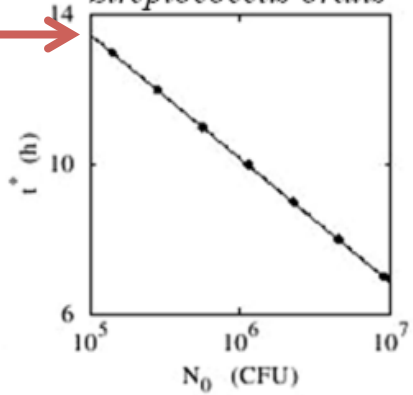
Streptococcus sobrinus



Enterococcus faecalis



Streptococcus oralis



Species	k^{-1} (h)	t_2 (min)
S. oralis	1.44 ± 0.0006	60
S. aureus	0.74 ± 0.02	31
E. coli	0.45 ± 0.008	19
E. faecalis	0.91 ± 0.008	38

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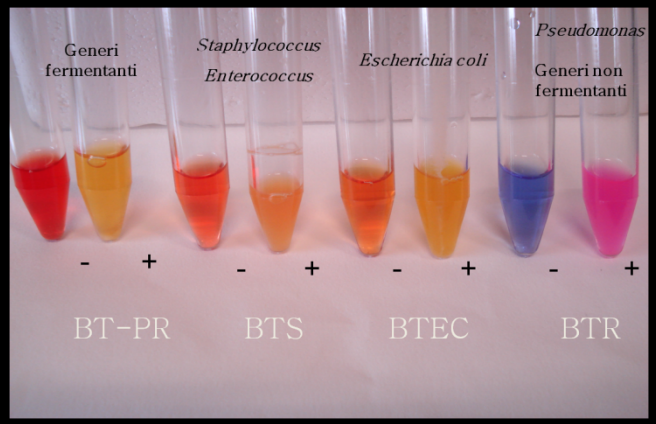
CATHETER 0R FRAGMENTS

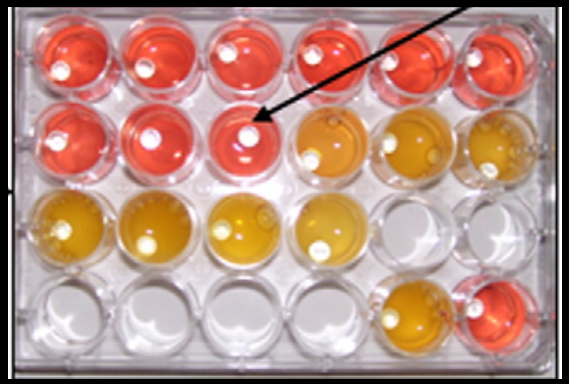
MICROBIAL IDENTIFICATION

MICROBIAL COUNT



TIME OF THE COLOR SWITCH CORRELATED TO MICROBIAL NUMBER THROUGH CORRELATION COURVES

ANTIBIOGRAM DIRECTLY ON CATHETER (BIOFILM)



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	84 CATHETERS ANALYZED WITH CLERI AND BIOTIMER ASSAYS			
METHOD	CLERI		BIOTIMER	
RESULTSI	9 STERILE	75 INFECTED	0 STERILE	84 INFETED
BACTERIA/ML	$<10^3$	$>10^3$	$10\text{-}10^2$	$>10^2$
ANTIBIOGRAM	NO	SI	SI	SI

	OXACILLINA	VANCOMICINA	TEICOPLANINA	LINCOMICINA	RIFAMPICINA
1	R	S	S	S	R
2	R	S	S	S	S
3	R	S	I	R	S
4	S	S	S	S	S
5	R	R	R	R	R
6	S	S	S	S	S
7	R	R	R	R	R
8	R	R	I	S	S
9	S	S	S	S	S

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COMPARISON BETWEEN CLERI AND BIOTIMER

THE MOST RELEVANT LIMITATION OF CLERI IS THE CUT-OFF CONCENTRATION OF >10^3 MICROORGAMISMS/ML, WHILE BIOTIMER ASSAY CLERLY DEMONSTRATES THAT THE PRESENCE OF < 10^3 MICROORGAMISMS/ML CANNNOT BE NEGLECTED BECAUSE INDICATES AN INFECTION BY MICROORGANISMS RESISTENT TO ANTIBIOTICS AS SHOWED IN THE PREVIOUS SLIDE

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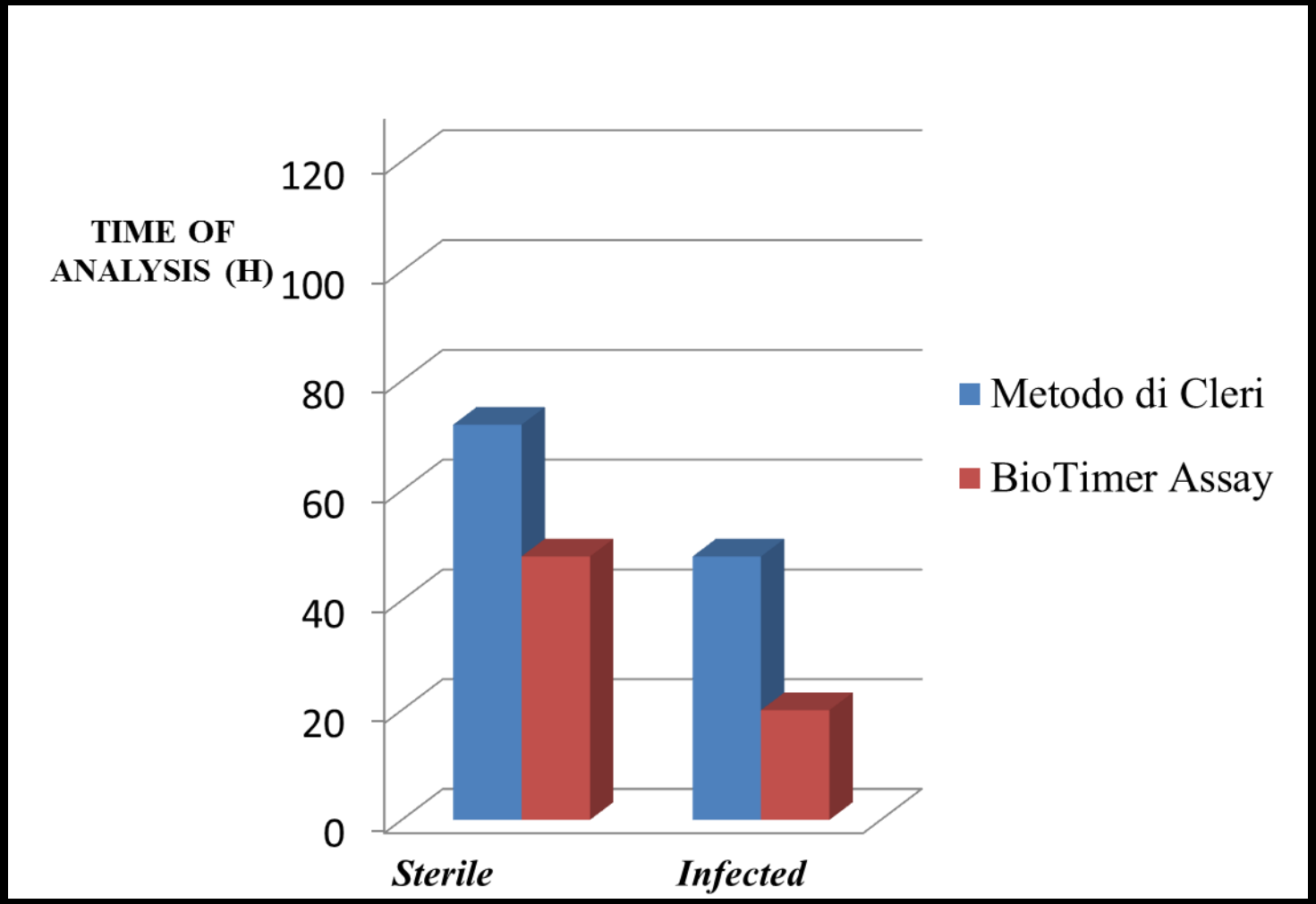
BIOTIMER ANALYSIS REQUIRE SHORTER TIME COMPARED WITH CLERI METHOD



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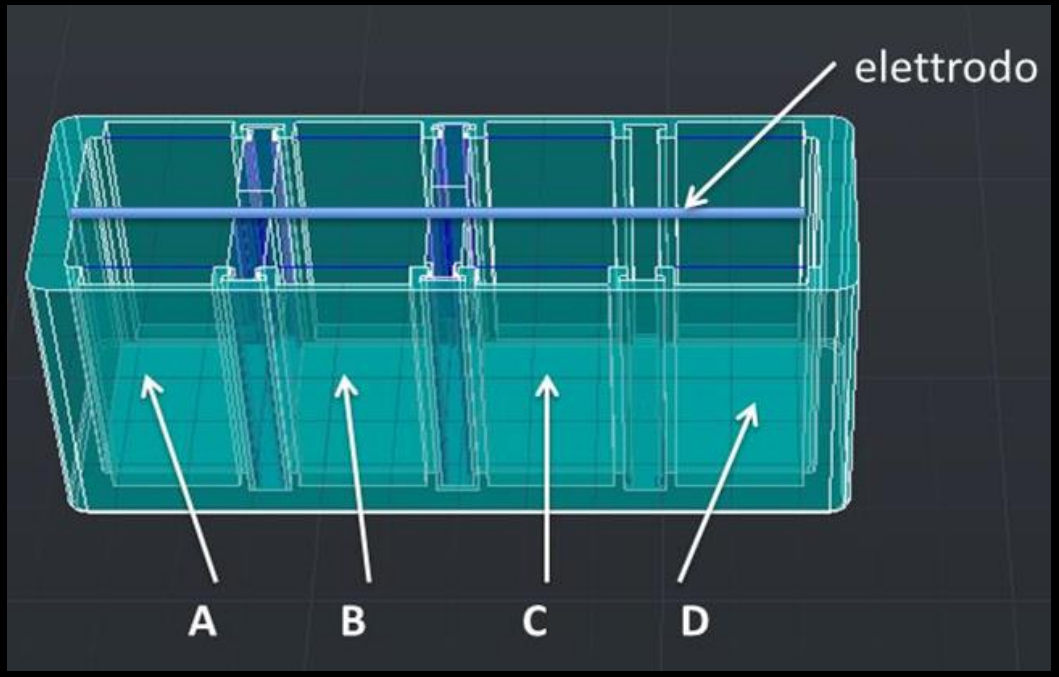
NOVEL ANALYTICAL APPARATUS TO ENUMERATE BACTERIA ADHERENT IN BIOFILM TO ATRIAL AND VENTRICULAR ELECTRODES



A,B,C,D DIFFERENT BIOTIME REAGENTS

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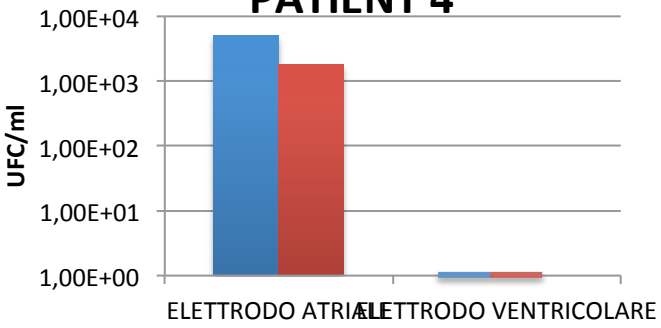
REGIONE LAZIO

RESULTS

LEGENDA:
- BIOTIMER
- CLERI

PATIENT 4



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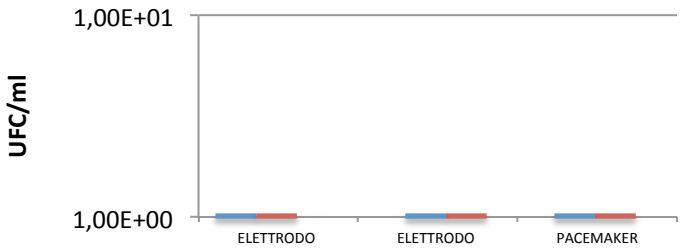

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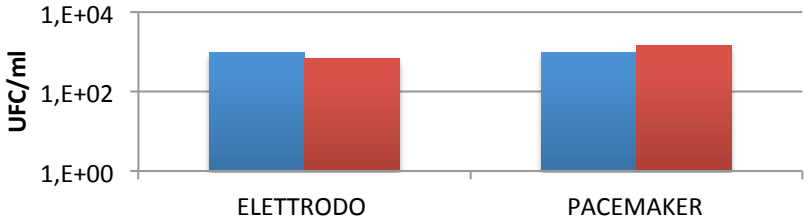

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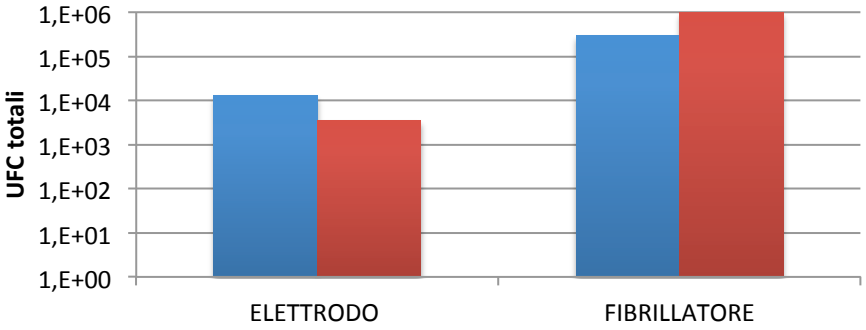
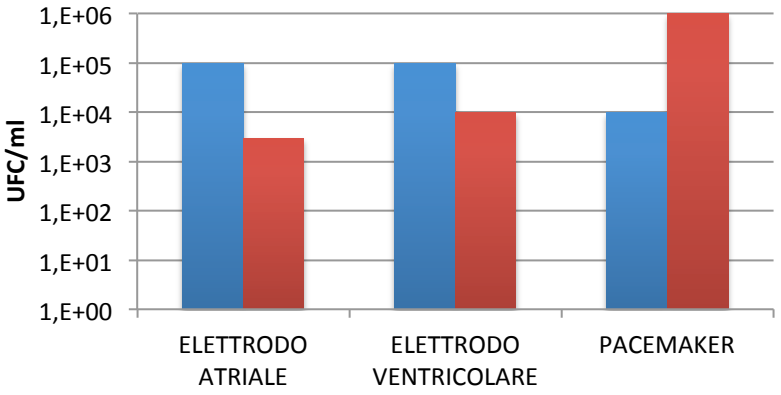


PATIENTE 2



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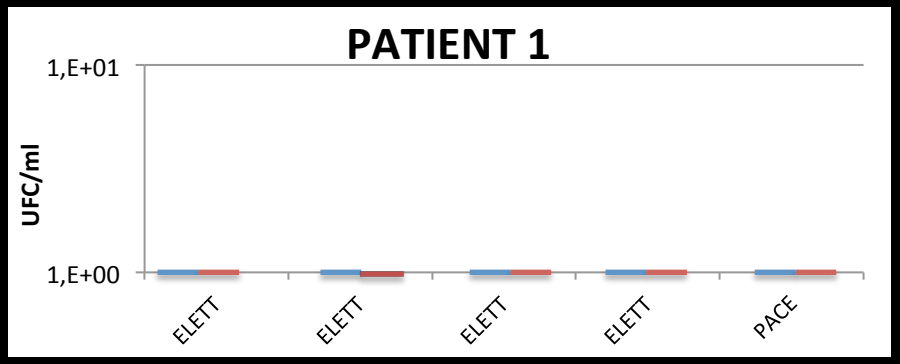
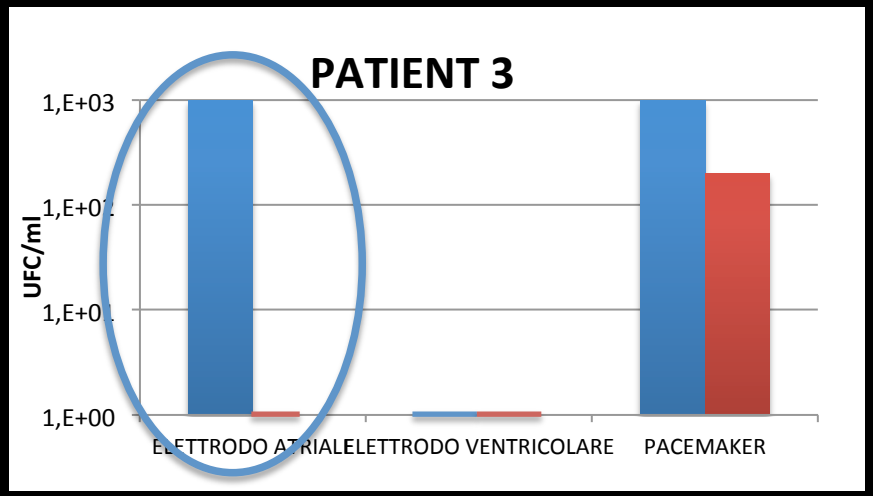
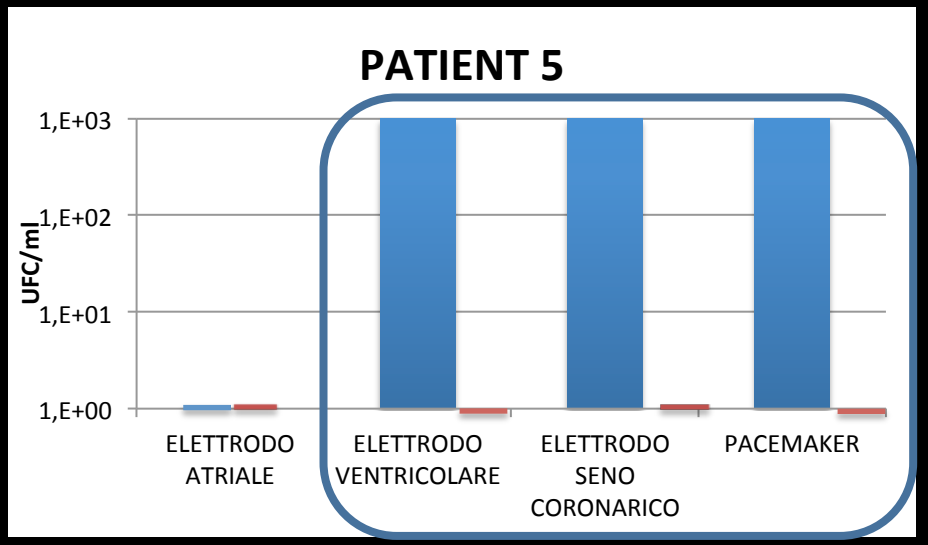
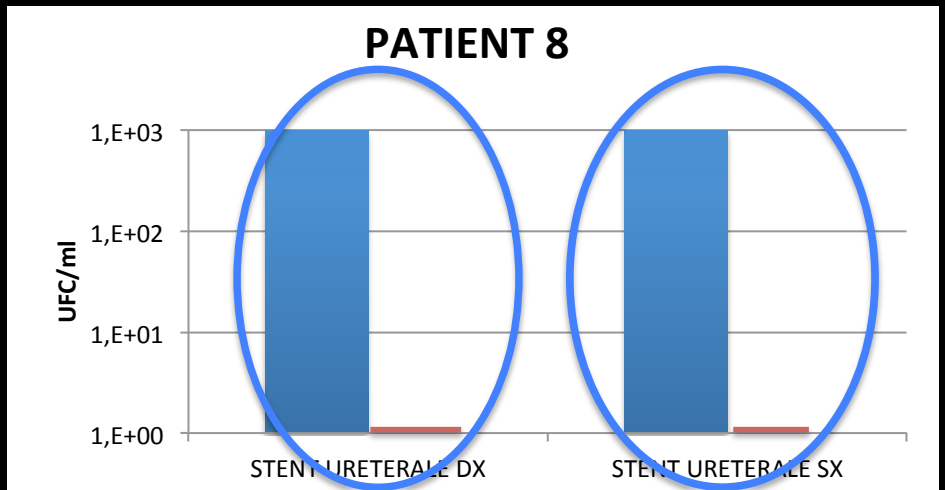
REGIONE LAZIO

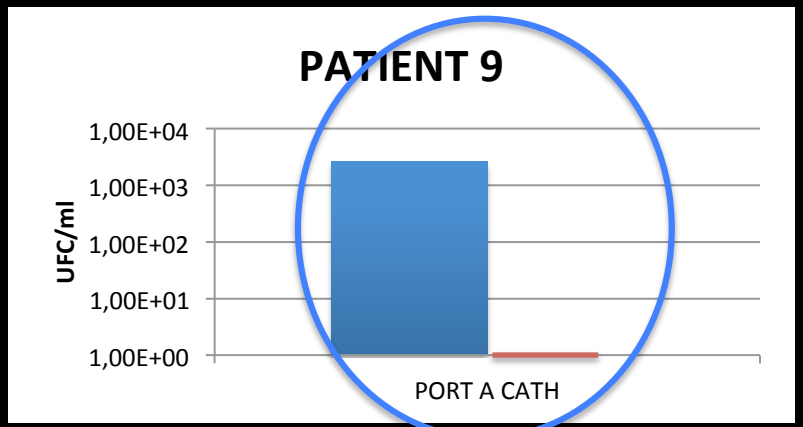
- BIOTIMER
- CLERI











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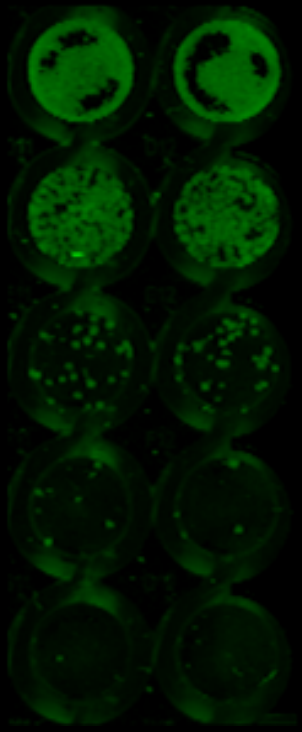
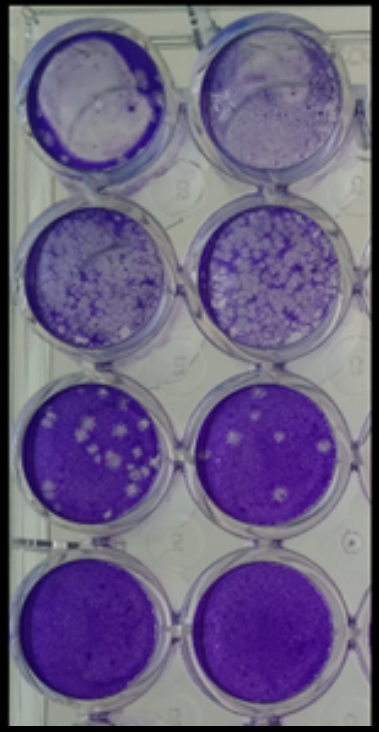
VIRUS

USE OF ODYSSEY FOR DNA OR RNA VIRUS TITRATION





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INNOVATION IN EVALUATING THE RISK OF VIRAL INFETIONS

➢ ISOLATION AND CHARACTERIZATION OF HUMAN POLYOMAVIRUS

Etiological agent of Multifocal Progressive Leukoencephalopathy

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➢ SCREENING OF CONGENITAL INFECTION BY CITOMEGALOVIRUS IN NEONATS

The 10-15% of neonates with congenital infection by Citomegalovirus shows a clear symptomatology while the 85-90% do not show any clinical sign.

However, the 5-15% of these neonates can show within the 2 years of life neurological pathologies as deafness, impairment of motor development, mental retardation, chorioretinitis. **Among 361 neonates , 67 were positive for congenital infection by Citomegalovirus.**

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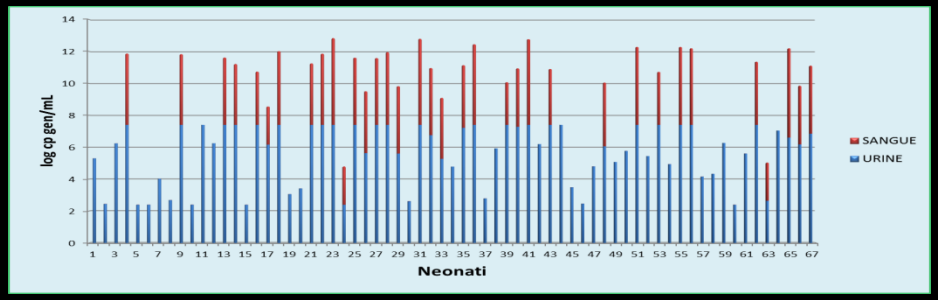

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EPIDEMIOLOGICAL SURVEILLANCE OF PATHOGENS IN THE HOSPITALS

THE HIGHEST MICROBIAL CONTAMINATION HAS BEEN DETECTED NEAR OF THE PATIENT

THE PERSISTENCE OF ALERT MICROORGANISMS

INEFFICACY OF SANIFICATION

FROM THESE RESULTS, THE SPECIFIC SURFACES SEEM TO PLAY AN IMPORTANT ROLE IN CROSS-CONTAMINATION WITHIN HOSPITAL WARD

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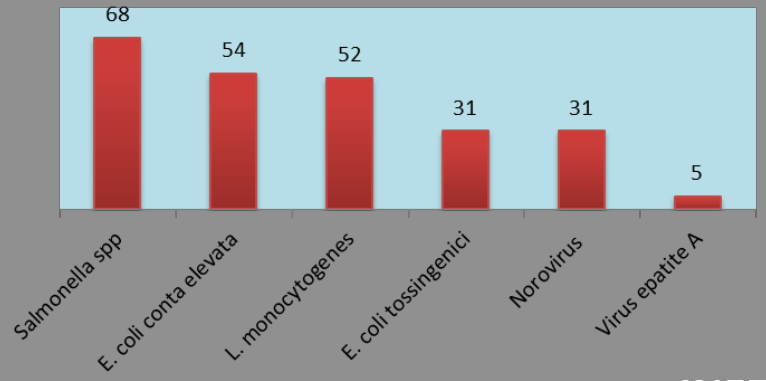
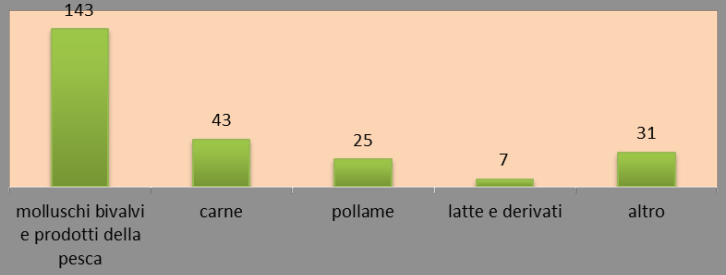
EPIDEMIOLOGICAL SURVEILLANCE OF PATHOGENS IN THE FOODS

FREQUENCY OF INFECTIONS ASSOCIATED TO DIFFERENT BACTERIAL GENERA



INFECTIONS RELATED TO DIFFERENT FOODS



maria.degiusti@uniroma1.it

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CONCLUSIONS

NOVEL MICROBIOLOGICAL METHODS TO

- ENUMERATE BACTERIA IN BIOFILM WITHOUT ANY MANIPULATION OF THE SAMPLE
- ANTIBIOGRAM DIRECTLY ON BACTERIA BIOFILM
- TITRATION OF DNA AND RNA VIRUS

INNOVATION IN EVALUATING THE RISK OF

- INFECTIONS BY POLYOMAVIRUS AND CITOMEGALOVIRUS

EPIDEMIOLOGICAL SURVEILLANCE OF PATHOGENS

- IN THE HOSPITALS
- IN THE FOODS

Contact : piera.valenti@uniroma1.it