

CoHere

Webinar

Boosting WASPLab® with
PhenoMATRIX®

Microbiology labs experience with A.I.

11 November 2021
16.00 to 17.00 CET

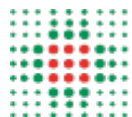


**PhenoMATRIX:
real-life
experience
of a high volume
bacteriology lab**

Simone Ambretti

Unit of Clinical Microbiology

**S.Orsola
University Hospital,
Bologna, Italy**



**SERVIZIO SANITARIO REGIONALE
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IRCCS Istituto di Ricovero e Cura a Carattere Scientifico

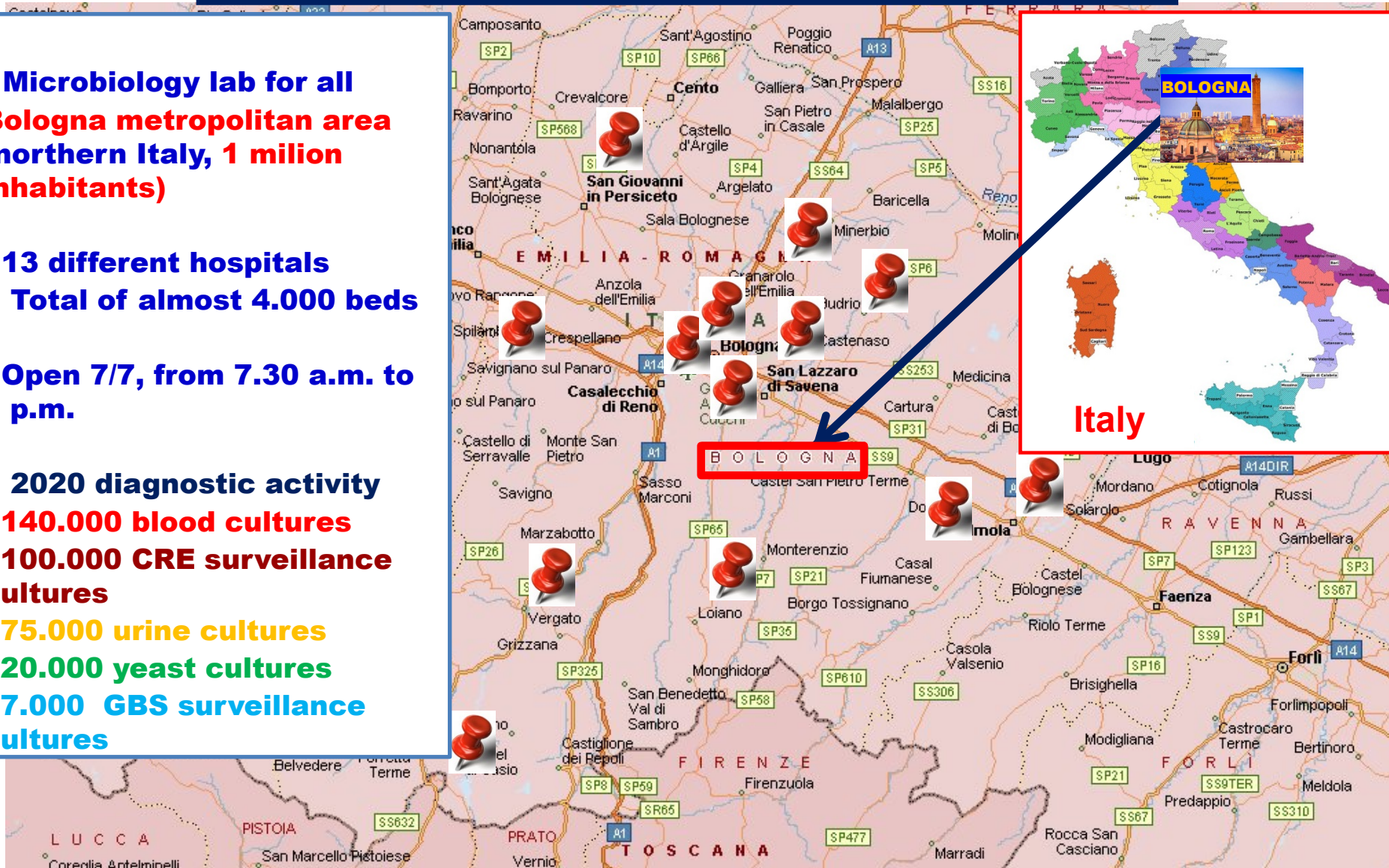
POLICLINICO DI **SANT'ORSOLA**



PHENOMATRIX : REAL LIFE EXPERIENCE OF A HIGH-VOLUME BACTERIOLOGY LAB

Unit of Clinical Microbiology S.Orsola University Hospital, Bologna, Italy

- ✓ **Microbiology lab for all Bologna metropolitan area (northern Italy, 1 million inhabitants)**
- ✓ **13 different hospitals**
- ✓ **Total of almost 4.000 beds**
- ✓ **Open 7/7, from 7.30 a.m. to 8 p.m.**
- ✓ **2020 diagnostic activity**
 - **140.000 blood cultures**
 - **100.000 CRE surveillance cultures**
 - **75.000 urine cultures**
 - **20.000 yeast cultures**
 - **7.000 GBS surveillance cultures**



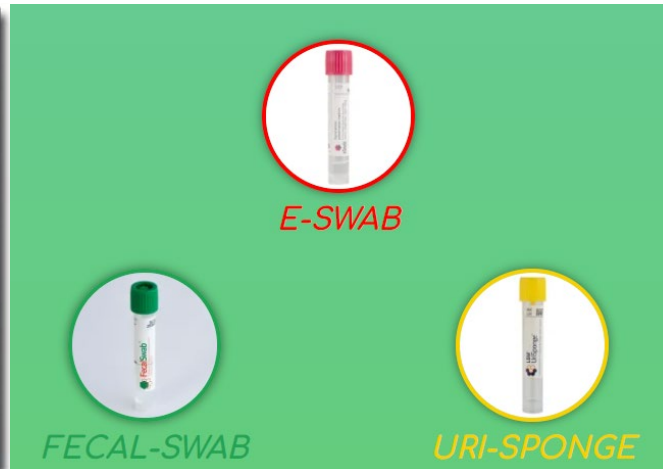
Unit of Clinical Microbiology, S.Orsola University Hospital, Bologna, Italy

OUR JOURNEY TO AUTOMATION IN BACTERIOLOGY

1° step: AUTOMATION OF STREAKING

➤ 2009 **WASP** (Walk-Away Specimen Processor)

from manual to automated streaking of liquid based samples (urine, stool, swabs) + enrichment broth inoculation + slide preparation



Liquid Based Microbiology

WASP

OUR JOURNEY TO AUTOMATION IN BACTERIOLOGY

2° step: AUTOMATION OF INCUBATION AND PLATE READING

➤ **2016 WASPLAB**



OUR JOURNEY TO AUTOMATION IN BACTERIOLOGY

3° step: AUTOMATION OF PLATE INTERPRETATION

Digital
microbiology



- 2017 Implementation of **segregation protocols** (NEGATIVE vs POSITIVE) for CRE surveillance rectal swabs and urine cultures



Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar



Rectal screening for carbapenemase-producing Enterobacteriaceae: a proposed workflow

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WASPLab

Rectal screening

ABSTRACT

Objectives: Active screening is a crucial element for the prevention of carbapenemase-producing Enterobacteriaceae (CPE) transmission in healthcare settings. Here we propose a culture-based protocol for rectal swab CPE screening that combines CPE detection with identification of the carbapenemase type.

Methods: The workflow integrates an automatic digital analysis of selective chromogenic media (WASPLab[®]; Copan), with subsequent rapid tests for the confirmation of carbapenemase production [i.e. detection of *Klebsiella pneumoniae* carbapenemase (KPC)-specific peak by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) or a multiplex immunochromatographic assay identifying the five commonest carbapenemase types]. To evaluate the performance of this protocol in depth, data for 21 162 rectal swabs submitted for CPE screening to the Microbiology Unit of S. Orsola-Malpighi Hospital (Bologna, Italy) were analysed.

Results: Considering its ability to correctly segregate plates with/without Enterobacteriaceae, WASPLab Image Analysis Software showed globally a sensitivity and specificity of 100% and 79.4%, respectively. Of the plates with bacterial growth ($n = 901$), 693 (76.9%) were found to be positive for CPE by MALDI-TOF/MS (KPC-specific peak for *K. pneumoniae*) or by immunochromatographic assay. Only 2.8% (16/570) of KPC-positive *K. pneumoniae* strains were missed by the specific MALDI-TOF/MS algorithm, being detected by the immunochromatographic assay. The mean turnaround time needed from sample arrival to the final report ranged between 18 and 24 h, representing a significant time saving compared with manual reading.

Conclusion: This workflow proved to be fast and reliable, being particularly suitable for areas endemic for KPC-producing *K. pneumoniae* and for high-throughput laboratories.

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PhenoMATRIX: real-life experience of a high volume bacteriology lab

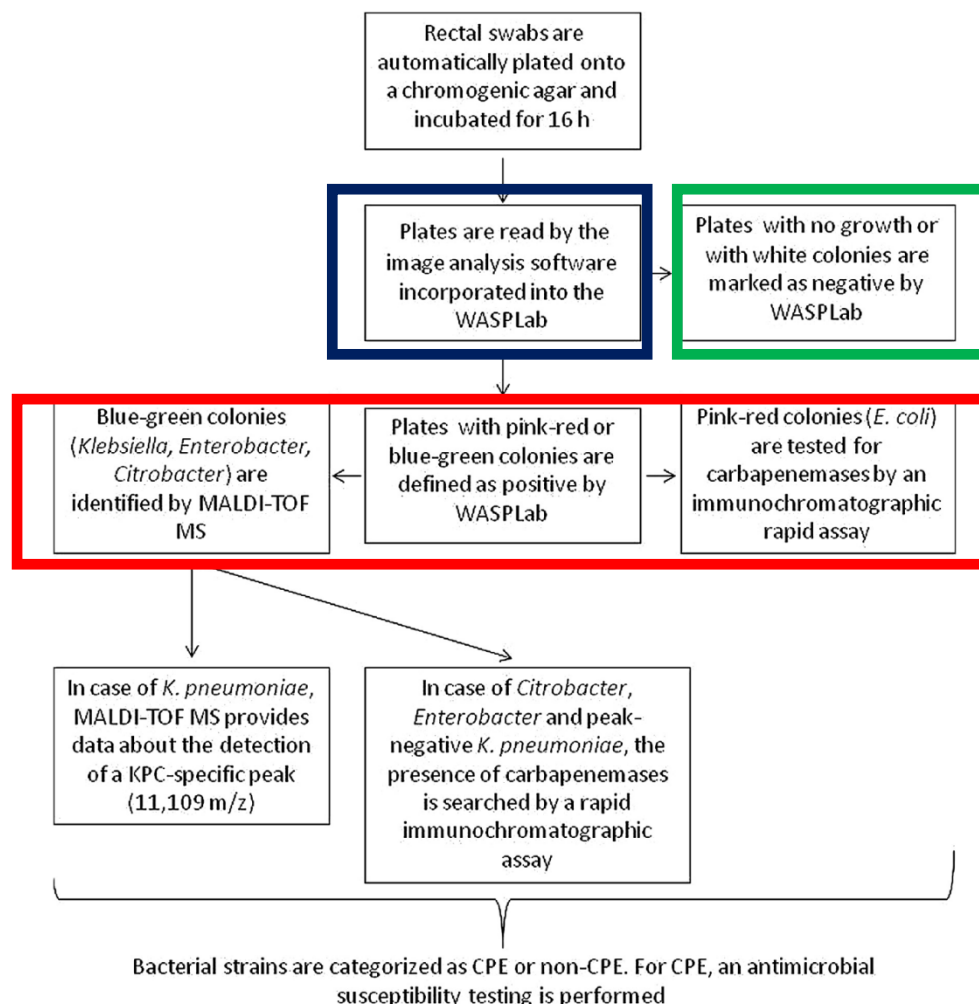


Fig. 1. Workflow for rectal carbapenemase-producing Enterobacteriaceae (CPE) screening.

PHENOMATRIX : REAL LIFE EXPERIENCE OF A HIGH-VOLUME BACTERIOLOGY LAB

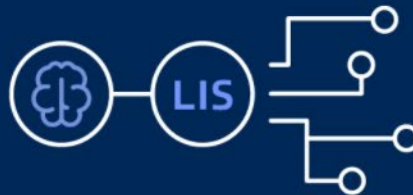
- 2020 introduction of **PhenoMatrix**: development of different protocols of automated plate-reading (CRE surveillance, GBS surveillance, yeast cultures, urine cultures)



**ARTIFICIAL
INTELLIGENCE
INTERPRETATION**



Algorithms



Lis Integration



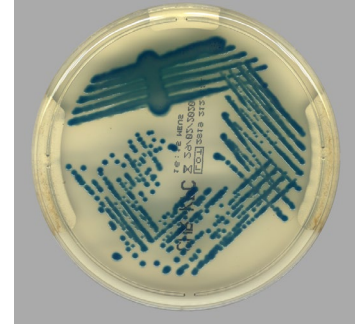
Applied Artificial Intelligence

AUTOMATIC INTERPRETATION (PRESUMPTIVE RESULT)

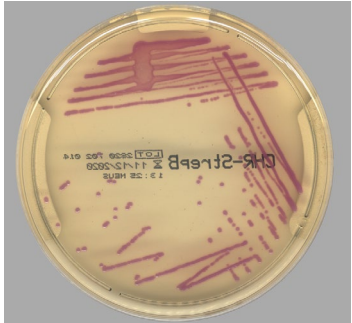
PHENOMATRIX : REAL LIFE EXPERIENCE OF A HIGH-VOLUME BACTERIOLOGY LAB

PHENOMATRIX PROTOCOLS

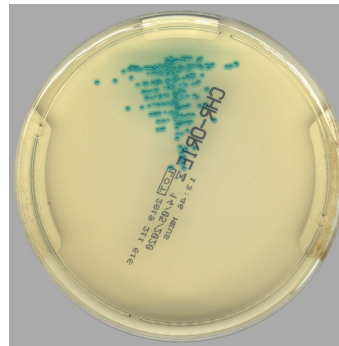
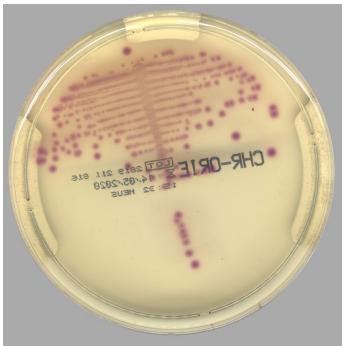
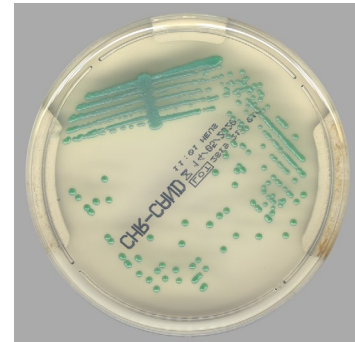
✓ CRE surveillance rectal swabs



✓ GBS surveillance vagino-rectal swabs



✓ Yeast cultures
(genital and respiratory samples)



✓ Urine cultures

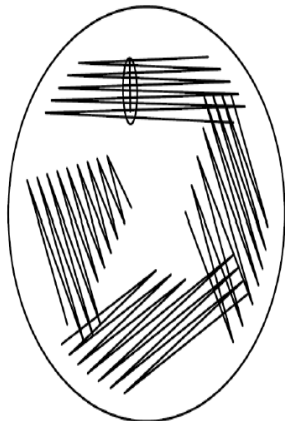
CRE surveillance rectal swabs



2020: 100.000 samples
Daily mean: > 330 swabs / day
Peaks: >800 swabs /day
Positivity rate: <5%

CULTURE PROTOCOL

Material = Rectal swab
Analysis = CPE screening



VACUTEST KIMA®, Chromagar KPC

DISEGNO DI SEMINA: 4 Quadrants Type 3

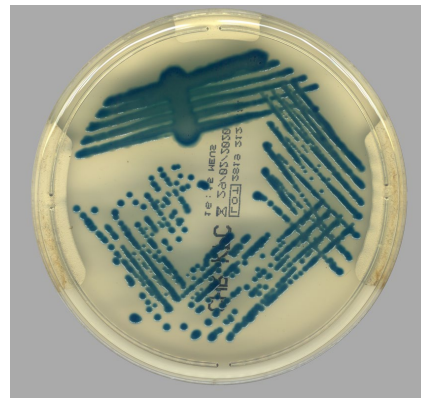
ANSA: 10 µl

INCUBATORE: O₂

TEMPO DI ACQUISIZIONE: 14 Ore

IMPOSTAZIONI ILLUMINAZIONE: B2024; W21

IMAGE ANALYSIS ALGORITHM

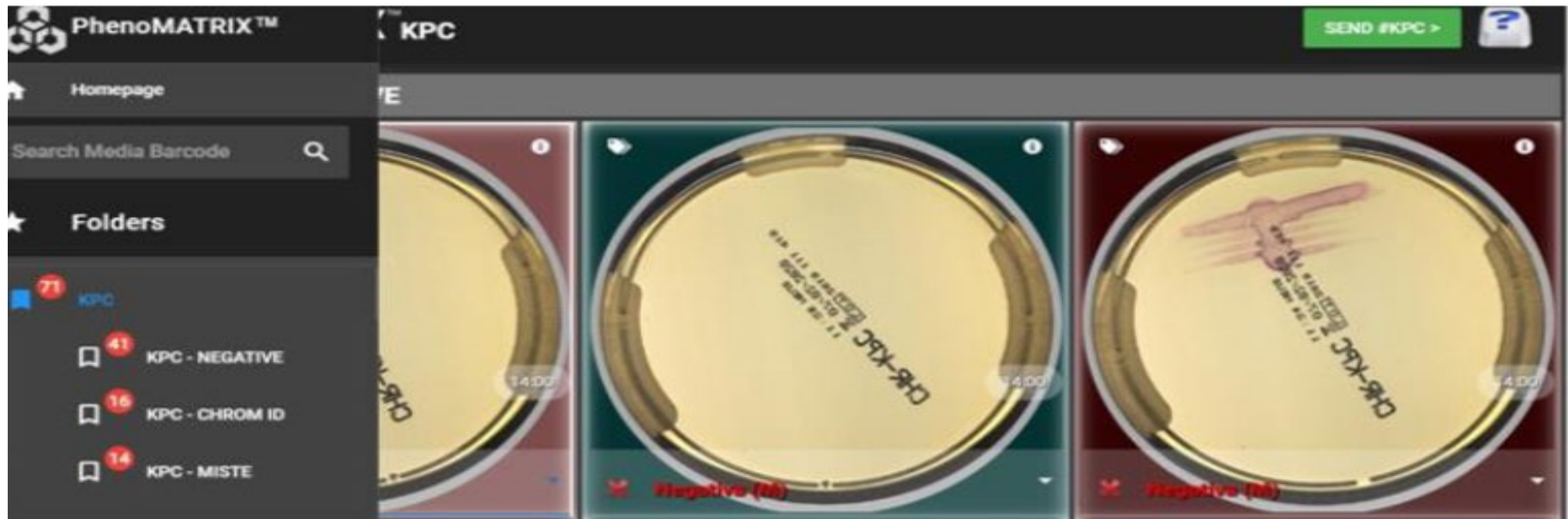


- ✓ Qualitative evaluation
- ✓ Colony detection based on size and colour

- Pink/red (*Escherichia coli*)
- Blue/green (KESC)
- Transparent/white (suspected for *Acinetobacter baumannii*) **BUT ONLY FOR ICU PATIENTS**

PHENOMATRIX : REAL LIFE EXPERIENCE OF A HIGH-VOLUME BACTERIOLOGY LAB

Samples segregated into 3 different folders: **NEGATIVE**, **CHROM-ID**, **MIXED**



KPC - NEGATIVE

Numero di isolati: 0

Negativa



Numero di isolati: 1

Tipo di isolato: Colonie trasparenti


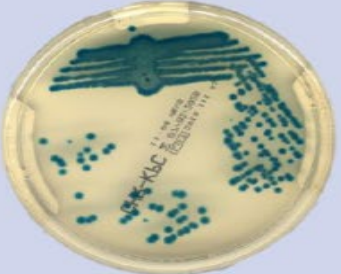

Dati LIS: Reparto non critico

Negativa



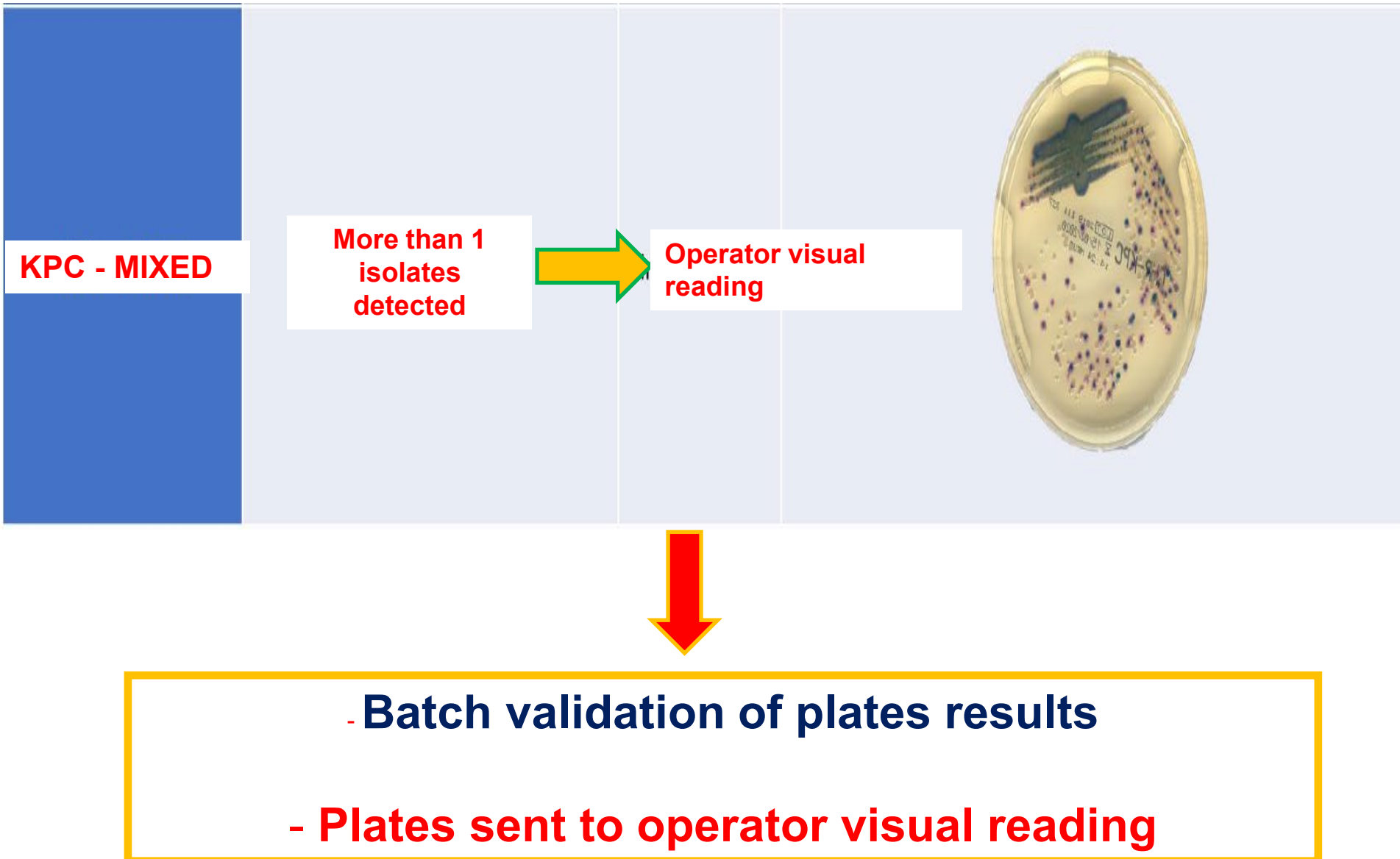
- Batch validation of plates results
- Negative results sent to LIS
- Plates sent to waste

PHENOMATRIX : REAL LIFE EXPERIENCE OF A HIGH-VOLUME BACTERIOLOGY LAB

| | | | |
|---------------|---|-------------|--|
| KPC - CHROMID | Numero di isolati: 1 Tipo di isolato: E. Coli | ECOLI |  |
| | Numero di isolati: 1 Tipo di isolato: KESC | BLU |  |
| | CUSTOM RULE BASED ON CLINICAL DATA Numero di isolati: 1 Tipo di isolato: Colonie trasparenti Dati LIS: Reparto critico | TRASPARENTI |  |

- Batch validation of plates results
- **positive result and presumptive ID sent to LIS**
- plates sorted to follow-up activities column

PHENOMATRIX : REAL LIFE EXPERIENCE OF A HIGH-VOLUME BACTERIOLOGY LAB



PROTOCOL VALIDATION PROCESS

Le prestazioni e le statistiche riportate nel presente documento sono relative alla raccolta di dati basati su campioni di routine.

Raccolta dei dati: dal 16 Dicembre 2019 al 04 Gennaio 2020

Quantità di dati: 5719 campioni

4.3 PRESTAZIONI GLOBALI

Nel grafico sottostante si riportano le percentuali globali rispetto alla Classificazione di PhenoMATRIX.

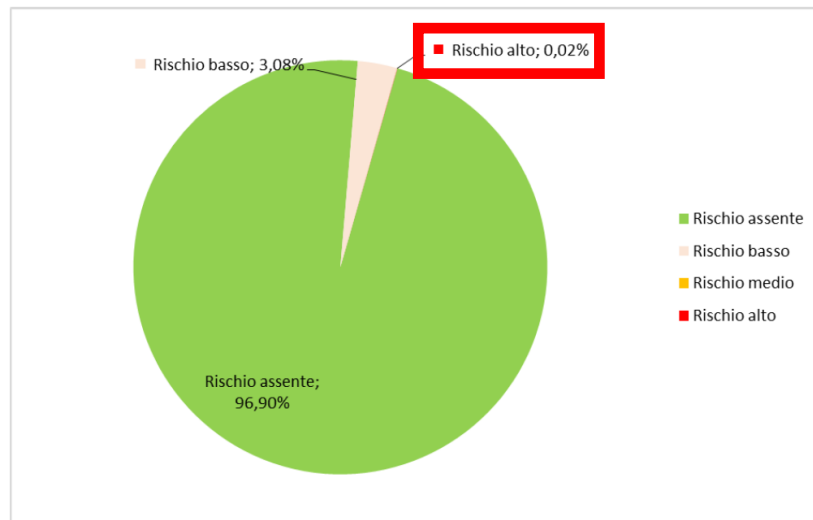


Figura 3 Statistiche dei Risultati PhenoMATRIX

I risultati PhenoMATRIX che necessitano una revisione in Screening o Lettura sono colorati di arancio.

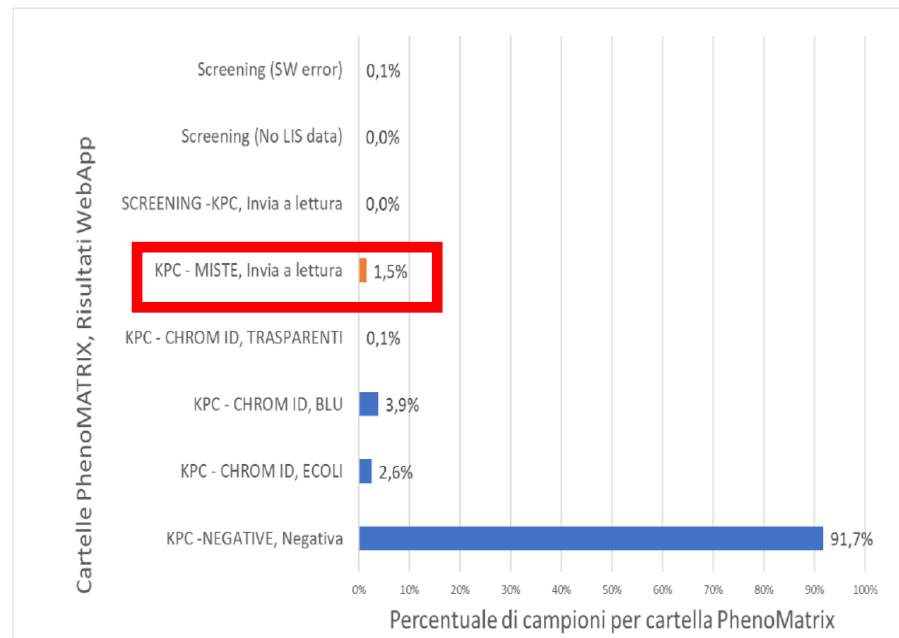


Figura 4 Piastre considerate per la Valutazione delle Prestazioni

PHENOMATRIX : REAL LIFE EXPERIENCE OF A HIGH-VOLUME BACTERIOLOGY LAB

GBS surveillance recto-vaginal swabs

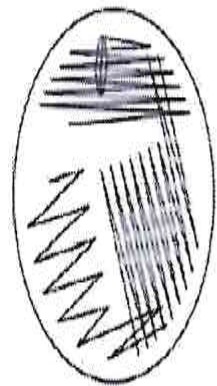


2020: 7.000 samples
Daily mean: 25 swabs / day
Positivity rate: 16-18%

CULTURE PROTOCOL

Material = Vagino-Rectal swab

Analysis = GBS screening



CHROMagar™ StrepB, VACUTEST KIMA

DISEGNO DI SEMINA: 3 quadranti tipo 3

ANSA: 1 microlitro

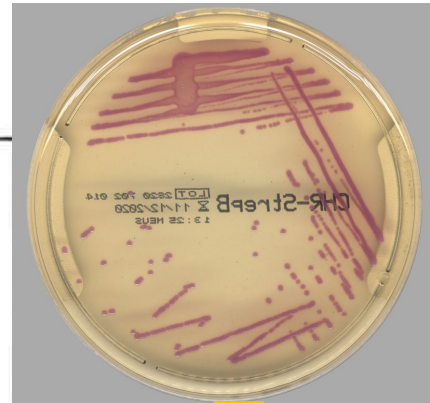
INCUBATORE: ossigeno

TEMPO ACQUISIZIONE: 16 ore

IMPOSTAZIONI ILLUMINAZIONE: W23

IMAGE ANALYSIS ALGORITHM

- ✓ Qualitative evaluation
- ✓ Colony detection based on size and colour



Purple colonies =
suspected for *Streptococcus agalactiae*)



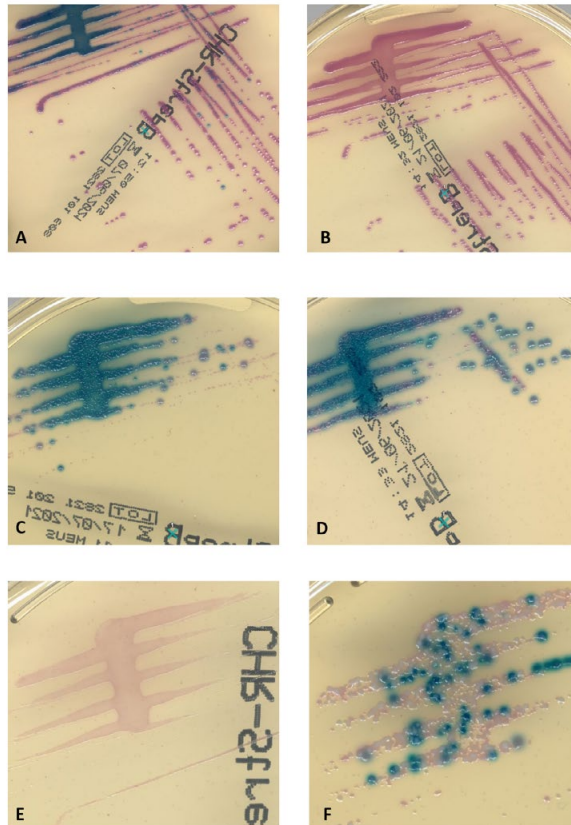
Performance of PhenoMatrix for the detection of Group B *Streptococcus* from recto-vaginal swabs



Claudio Foschi^{a,b,*}, Gabriele Turello^a, Tiziana Lazzarotto^{a,b}, Simone Ambretti^b

^a Microbiology, DIMES, University of Bologna, Bologna, Italy

^b Microbiology Unit, IRCCS S.Orsola-Malpighi Hospital, Bologna, Italy



Digital images of plates were automatically screened by Pheno-Matrix.

This software analyses the plates to identify differences in growth and colony colour.

A specific protocol was developed evaluating the medium type and the incubation time used by the laboratory software automatically sorted the cultures into the categories 'negative for GBS' and 'potential-positive for GBS'.

In our protocol, plates with no bacterial growth or with blue/colourless colonies are marked as GBS-negative, whereas plates with pink/light red/purple colonies are defined as presumptive GBS-positive.

PHENOMATRIX : REAL LIFE EXPERIENCE OF A HIGH-VOLUME BACTERIOLOGY LAB

2

C. Foschi et al. / Diagnostic Microbiology and Infectious Disease 101 (2021) 115427

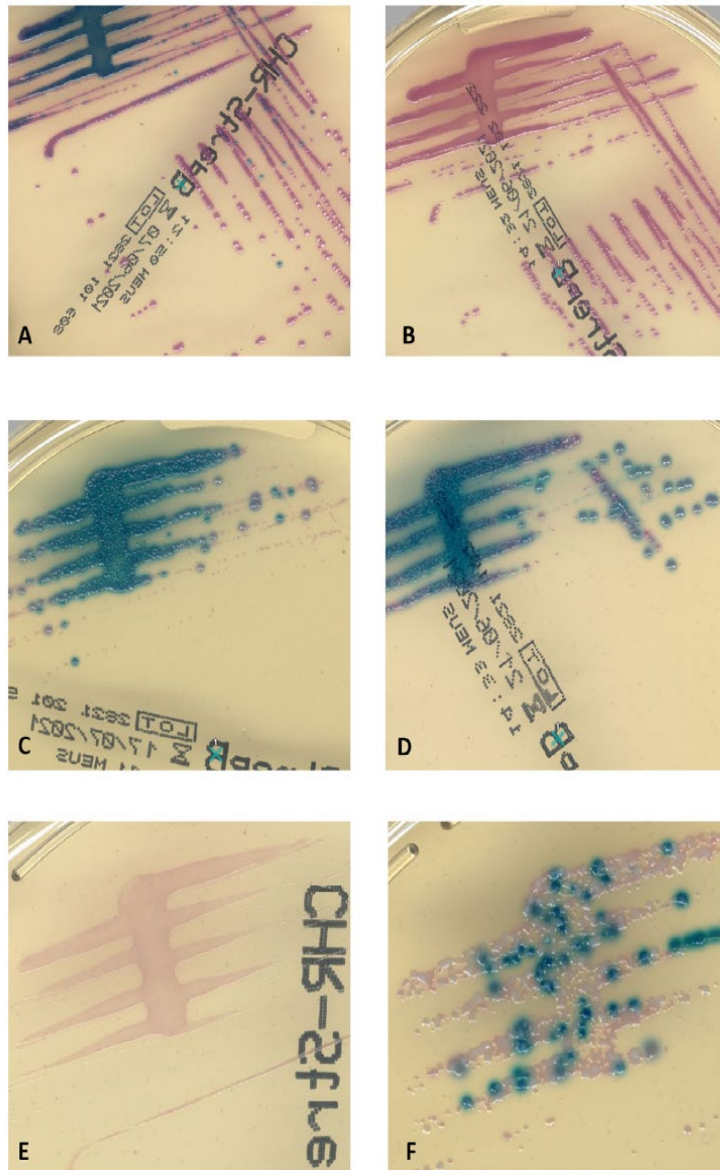


Fig. 1. . Images of representative cultures on CHROMagar StrepB medium. (A-B) Streptococcus agalactiae - GBS; (C) Streptococcus anginosus; (D) Enterococcus faecalis; (E) Streptococcus oralis; (F) Staphylococcus epidermidis.

Cultures were also reviewed manually by a technologist to check software analyses and scored for the presence or absence of colonies resembling GBS.

Colonies consistent with GBS were confirmed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) identification.

A specimen was considered a true-positive if a colony was confirmed to be GBS by MALDI-TOF.

A total of 587 plates (55%) were correctly segregated as GBS-negative by PhenoMatrix, with **no false-negative results**. The automatic reading system marked 481 plates (45%) as potentially positive; 158 of them were confirmed as GBS by MALDI-TOF.

The remaining cases (323; 30.2% of the total) sorted positive by PhenoMatrix were characterized by the absence of pink colonies at visual inspection (212 cases) or by a MALDI-TOF identification different from GBS (111 cases).

YEAST CULTURES: swabs, genital and respiratory samples

2020: 20.000 samples
Daily mean: 65 swabs / day

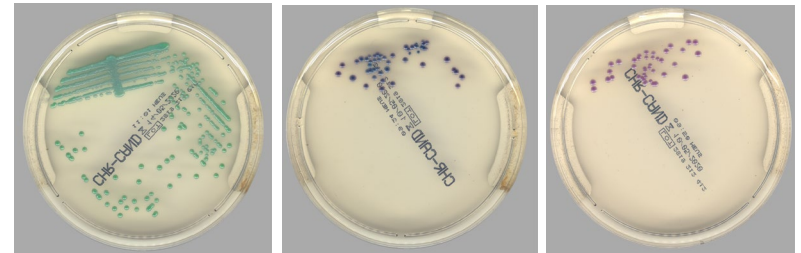
CULTURE PROTOCOL

Material = swabs, genital and
respiratory samples

Analysis = Yeast culture

IMAGE ANALYSIS ALGORITHM

✓ Quantitative evaluation

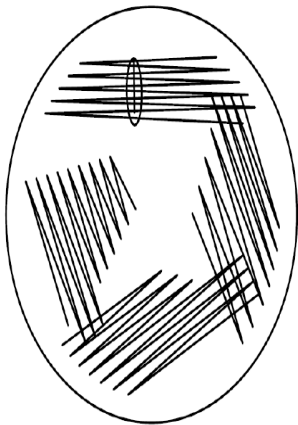


Colony detection based on colour:

- Green = *Candida albicans*
- blue = *Candida tropicalis*
- white/pink/purple = Other *Candida* species

Colonies counting:

- Different cut-off values for different types of samples



VACUTEST KIMA®, Chromagar Candida

DISEGNO DI SEMINA: 4 Quadrants Type 3



ANSA: 10 µl

INCUBATORE: O₂

TEMPO DI ACQUISIZIONE: 38 Ore

IMPOSTAZIONI ILLUMINAZIONE: B2021; W21

PHENOMATRIX : REAL LIFE EXPERIENCE OF A HIGH-VOLUME BACTERIOLOGY LAB



| | | | |
|-----------------|---|----------|---|
| CAND - NEGATIVE | Tipo di isolato: Non Candida O Crescita totale: 0 colonie | Negativa |  |
| | Campioni Respiratori Tipo 1 Numero di isolati: 1 Tipo di isolato: Candida Numero di colonie < 10 | Negativa |  |

-NO GROWTH OR
-*Candida* colonies count lower than
sample type- specific cut-off



- Batch validation of plates results
- negative result sent to LIS
- plates sent to waste

Introduction of Phenomatrix protocols for plate-reading automation of CRE and GBS Screening Specimens, Urine Cultures and Yeast Cultures on chromogenic media: real life experience of an high volume bacteriology lab

| Sottocartella PhenoMATRIX | Regole | Risultati WebApp | Esempio |
|------------------------------|---|------------------|---|
| CAND – CHROM ID | Regole Generali: Campioni Respiratori Tipo 3 | | |
| | Numero di isolati: 1 Tipo di isolato: C. albicans Numero di colonie: 5 - 70 | C.ALBICANS_CB4 |  |
| | Numero di isolati: 1 Tipo di isolato: C. albicans Numero di colonie: 71 - 149 | C.ALBICANS_CB5 |  |

| Sottocartella PhenoMATRIX | Regole | Risultati WebApp | Esempio |
|------------------------------|---|------------------|---|
| CAND – ID | Regole Generali: Campioni Genitali o Liquidi Seminali o Tamponi | | |
| | Numero di isolati: 1 Tipo di isolato: C. species Numero di colonie : 71 - 200 | C. SPECIES_MLT |  |
| | Numero di isolati: 1 Tipo di isolato: C. species Numero di colonie: >200 | C. SPECIES_MM |  |

GROWTH OF *Candida albicans* or *Candida tropicalis*
with colonies count higher than sample type- specific cut-off



- Batch validation of plates results
- positive result, colonies count, and species ID sent to LIS
- plates sorted to back-up activities column

GROWTH OF *Candida sp.* different from *Candida albicans* or *Candida tropicalis*
with colonies count higher than sample type- specific cut-off



- Batch validation of plates results
- positive result, colonies count, and presumptive ID (*Candida sp.*) sent to LIS
- plates sorted to MALDI-TOF ID column



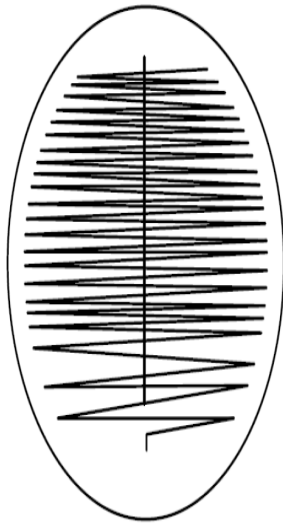
URINE CULTURES

2020: 75.000 samples
Daily mean: 250 swabs / day

CULTURE PROTOCOL

Material = urine

Analysis = urine culture



VACUTEST KIMA®, Chromagar Orientation

DISEGNO DI SEMINA: Single Streak Type 6

ANSA: 1 µl

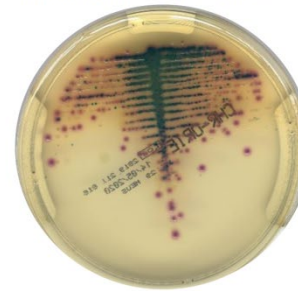
INCUBATORE: O₂

TEMPO DI ACQUISIZIONE: 16 Ore

IMPOSTAZIONI ILLUMINAZIONE: B2022; W21

IMAGE ANALYSIS ALGORITHM

- ✓ **Quantitative evaluation**
- ✓ **High level of complexity**



- o *Escherichia coli*
- o *Enterococcus*
- o KESC
- o PMP
- o *Staphylococcus saprophyticus*
- o *Streptococcus agalactiae*
- o *Pseudomonas*
- o colonie bianche
- o colonie trasparenti
- o microcolonie
- o sconosciuto

Result evaluation based on:

- **Global colonies counting**
- **Number of different types of isolates**
- **Count, colour and size evaluation of single type of colonies**
- **Sex and age of patients (for GBS)**

NOTE: LIS limitation did not allow to use other types of parameters (leucocytes count)

URINE CULTURES

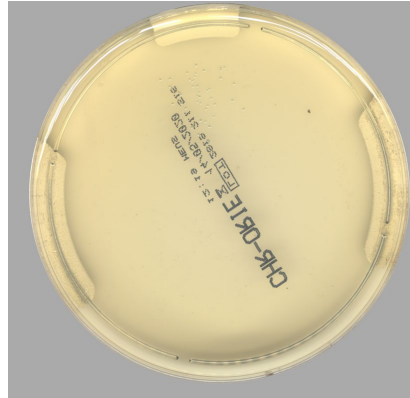
RESULT EVALUATION BASED ON COLONIES PREVALENCE RULES

Si definiscono le **Regole di Predominanza Globali** per la definizione del numero di isolati:
se un isolato è presente in quantità minore di 10 volte rispetto all'isolato più numeroso,
allora questo non viene considerato



URINE CULTURES

RESULT EVALUATION BASED ON PATIENTS DATA



EVALUATE AS POSITIVE FOR GBS

With lower colonies count (10^4 CFU/ml)

ONLY IF

Sex: Female

Age: >14, <50 YEARS

PHENOMATRIX : REAL LIFE EXPERIENCE OF A HIGH-VOLUME BACTERIOLOGY LAB

URINE CULTURES

| PhenoMATRIX Sottocartella | Regole | Risultati WebApp | Esempi |
|---------------------------|---|---------------------------|---|
| ECOLI | Regole generali: Tempo di Recording: 16h | | |
| | E. coli > 39 | ECOLI-CBMAS ECOLI-CBCM |  |
| BLU-BACNEG | Regole generali: Tempo di Recording: 16h | | |
| | KESC > 39 BAC NEG > 39 | BLU-CBMAS BLU-CBCM |  |

| PhenoMATRIX Sottocartella | Regole | Risultati WebApp | Esempi |
|---------------------------|---|--|---|
| ENTERO-STAFILO | Regole generali: Tempo di Recording: 16h | | |
| | Enterococco > 39 Stafilococco > 39 | ENTERO-CBMAS ENTERO-CBCM STAPH-CBMAS STAPH-CBCM |  |

GROWTH OF MONOMICROBIAL
Escherichia coli or other gram-negatives
with colonies count higher than sample type- specific cut-off



- Batch validation of plates results
- positive result, colonies count, and species ID or presumptive ID sent to LIS
- plates sorted to follow-up activities column (AST or MALDI-TOF ID plus AST)

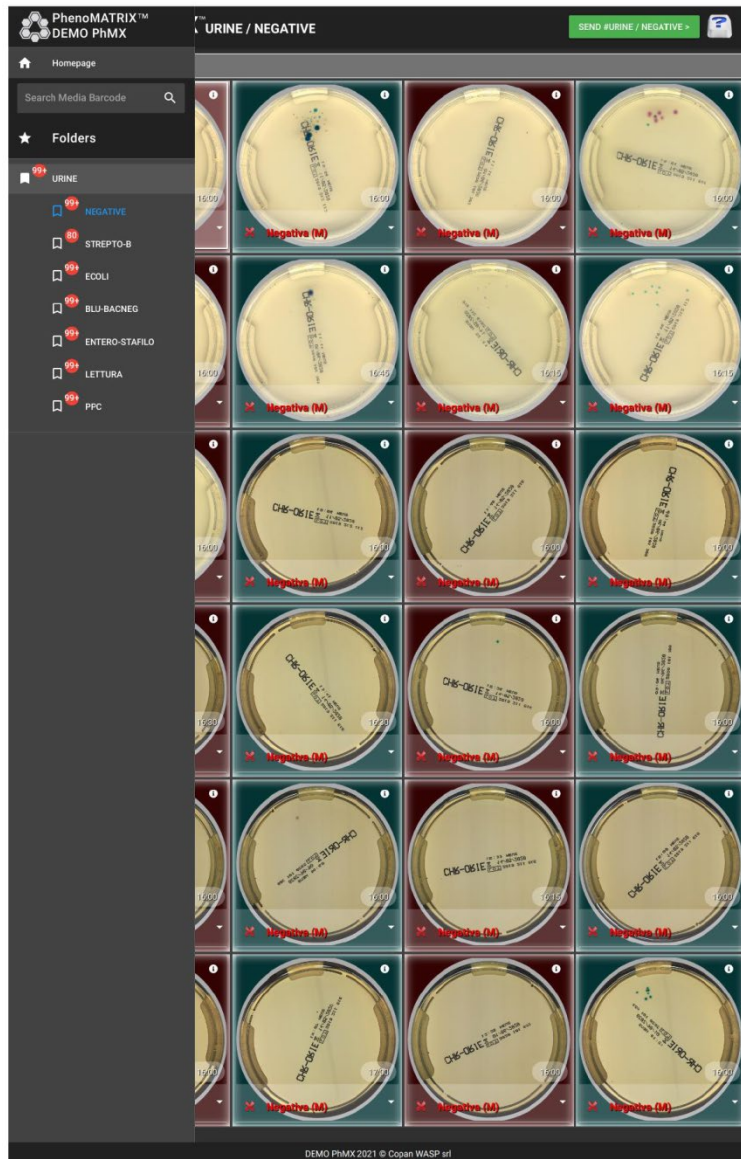
GROWTH OF MONOMICROBIAL
Enterococci or Staphylococci (*saprophyticus* or *aureus*)
with colonies count higher than sample type- specific cut-off



- Batch validation of plates results
- positive result, colonies count, and presumptive ID sent to LIS
- plates sorted to follow-up activities column (MALDI-TOF ID plus AST)

PHENOMATRIX : REAL LIFE EXPERIENCE OF A HIGH-VOLUME BACTERIOLOGY LAB

URINE CULTURES



| PhenoMATRIX Sottocartella | Regole | Risultati WebApp | Esempi |
|------------------------------|---|------------------|--------|
| PPC | Regole generali: Tempo di Recording: 16h | | |
| | 3+ isolati (no Strepto B) > 39 3+ isolati (con Strepto B e condizioni critiche) > 9 | PPC | |

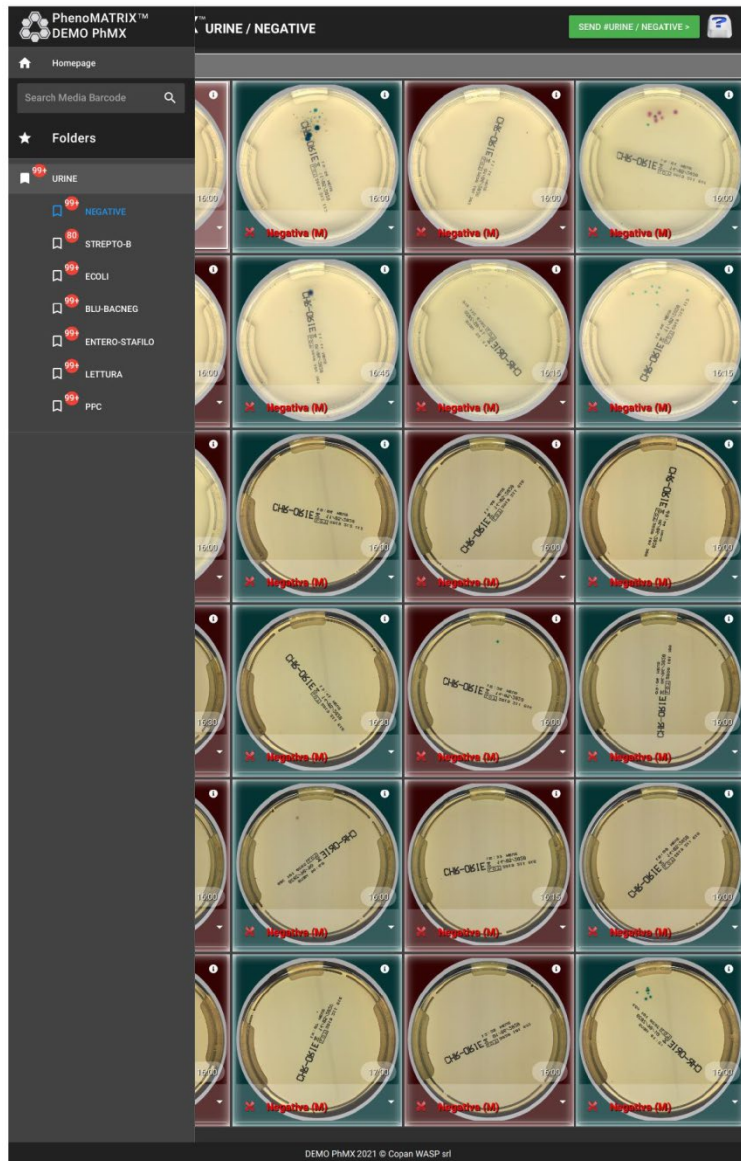
**POLYMICROBIAL GROWTH WITH THREE OR MORE
DIFFERENT TYPE OF COLONIES**



- Batch validation of plates results
- **CONTAMINATION** result sent to LIS
- plates sorted to back-up activities column

PHENOMATRIX : REAL LIFE EXPERIENCE OF A HIGH-VOLUME BACTERIOLOGY LAB

URINE CULTURES



LETTURA

Regole generali:

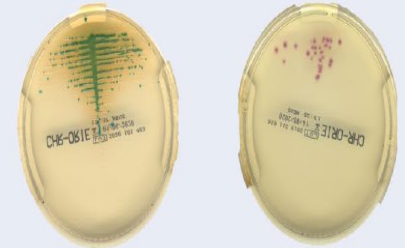
Tempo di Recording: 16h

1 isolato,
stafilo/entero/coli/bacneg
10-39

2 isolati > 39 (totale)

2 isolati (no Strepto B) 10-39

Invia In Lettura



**MONOMICROBIAL GROWTH OF POSSIBLE
PATHOGENS WITH INTERMEDIATE TOTAL COUNT**

OR

**POLYMICROBIAL GROWTH WITH TWO DIFFERENT
TYPES OF COLONIES**



- Batch validation of plates results

- Plates sent to operator visual reading

URINE CULTURES

4.4 URINE

Nel seguente grafico vengono illustrate le percentuali di comparsa dei risultati PhenoMATRIX per il protocollo URINE.

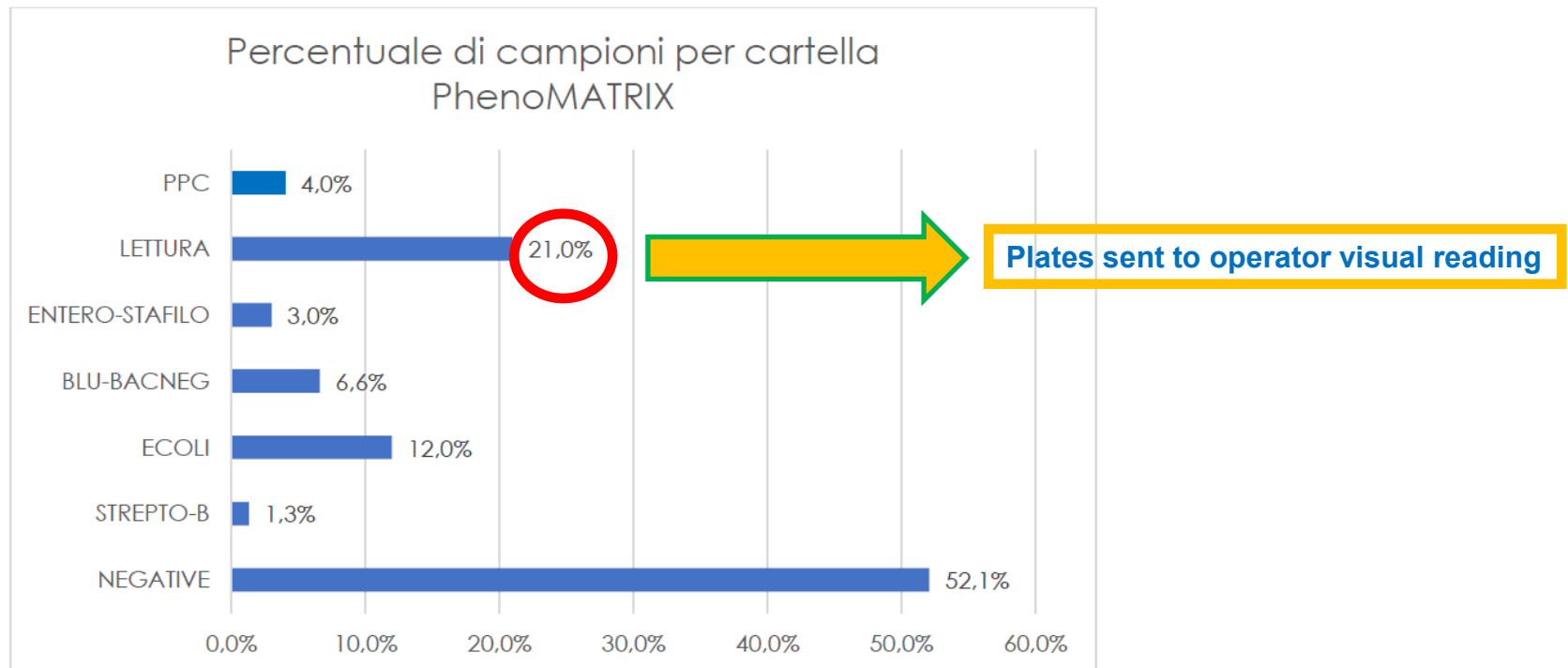
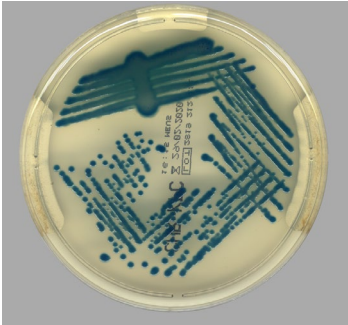


Figura 4 Piastre considerate per la Valutazione delle Prestazioni

PHENOMATRIX PROTOCOLS



✓ **CRE surveillance (330)**

+

✓ **GBS surveillance (25)**

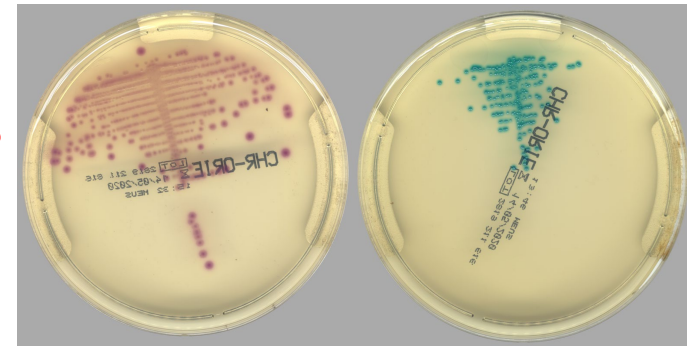
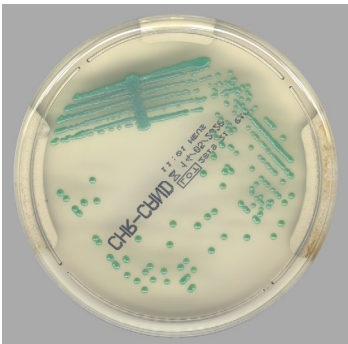
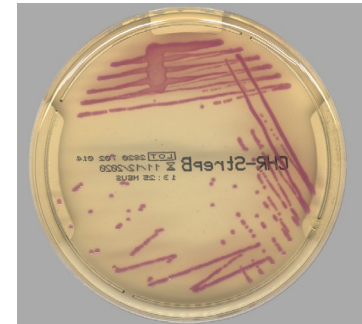
+

✓ **Yeast cultures (60)**

+

✓ **Urine cultures (250)**

= (665)



What about other samples???

PhenoMATRIX: real-life experience of a high volume bacteriology lab

Home BO
WL 46

Risultati
pendenti

Dashboard

714
PhenoMATRIX

770
Screening

Assegnamento
Lettura

Lettura

Stato Incubatore

| Incubatore | Percentuale di Riempimento |
|---|---------------------------------|
| 1 - Incubator 1 Temperatura: 37° C - Tipologia: CO2 | 13% (112 / 850) <div></div> |
| 2 - Incubator 2 Temperatura: 37° C - Tipologia: AIR | 38% (646 / 1700) <div></div> |
| 3 - Incubator 3 Temperatura: 37° C - Tipologia: AIR | 38% (648 / 1700) <div></div> |

Piastre Incubate

Cerca:

| | Barcode Medium | Barcode Campioni | Incubatore | Posizione | Stato | Azione |
|--|---|------------------|------------|-----------|-----------|--------------------------|
| | 420588030102 Tutte le fotografie di questo medium sono state viste | 4205880301 | 1 | L-H7 | INCUBATED | <input type="checkbox"/> |
| | 420587990102 Tutte le fotografie di questo medium sono state viste | 4205879901 | 1 | L-G7 | INCUBATED | <input type="checkbox"/> |
| | 420588050102 Tutte le fotografie di questo medium sono state viste | 4205880501 | 1 | L-F7 | INCUBATED | <input type="checkbox"/> |
| | 420587960102 Tutte le fotografie di questo medium sono state viste | 4205879601 | 1 | L-E7 | INCUBATED | <input type="checkbox"/> |

Visualizza 10 elementi

More 80% of WASPLAB samples are currently evaluated using a Phenomatrix plate-reading automated protocol

OUR JOURNEY TO AUTOMATION IN BACTERIOLOGY

➤ Urine:
WASP+WASPLAB+PHENOMATRIX

➤ Rectal swabs:
WASP+WASPLAB+PHENOMATRIX

➤ Yeast and SGB cultures:
WASP+WASPLAB+PHENOMATRIX

➤ Respiratory samples: WASP (streaking +
slide preparation) + WASPLAB

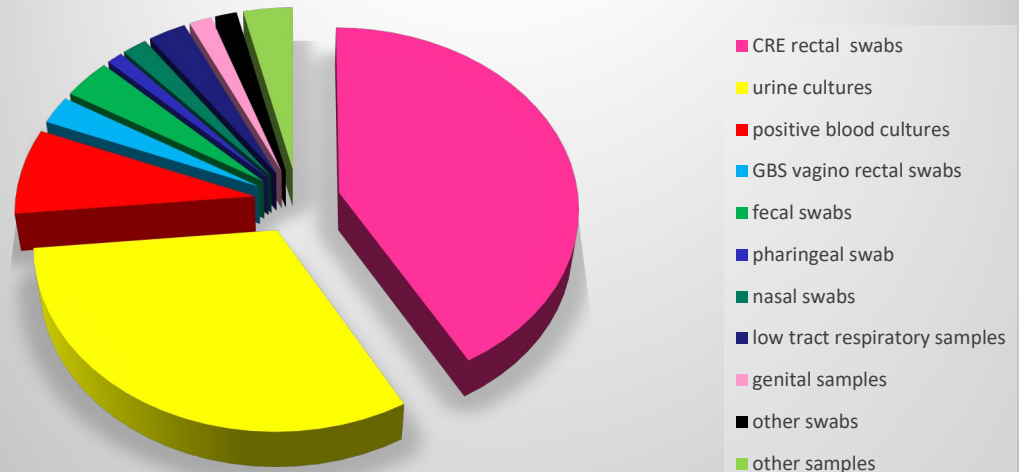
➤ Other swabs and genital samples:
WASP+WASPLAB

➤ Positive blood cultures: **WASP** (streaking
+ slide preparation)

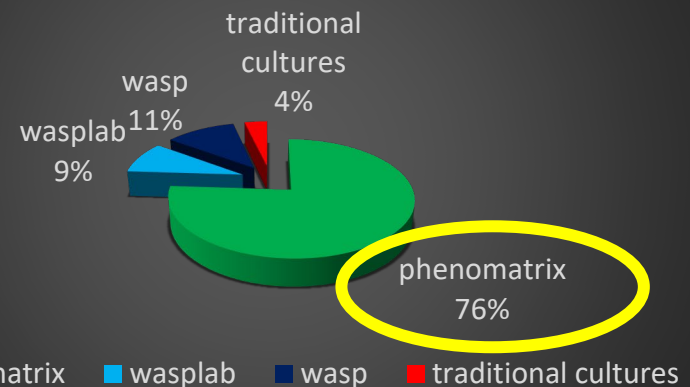
➤ Stool cultures: **WASP** (streaking and
broth inoculation)

➤ Other samples: manual streaking and
visual reading (traditional)

Sample distribution (2020 data)



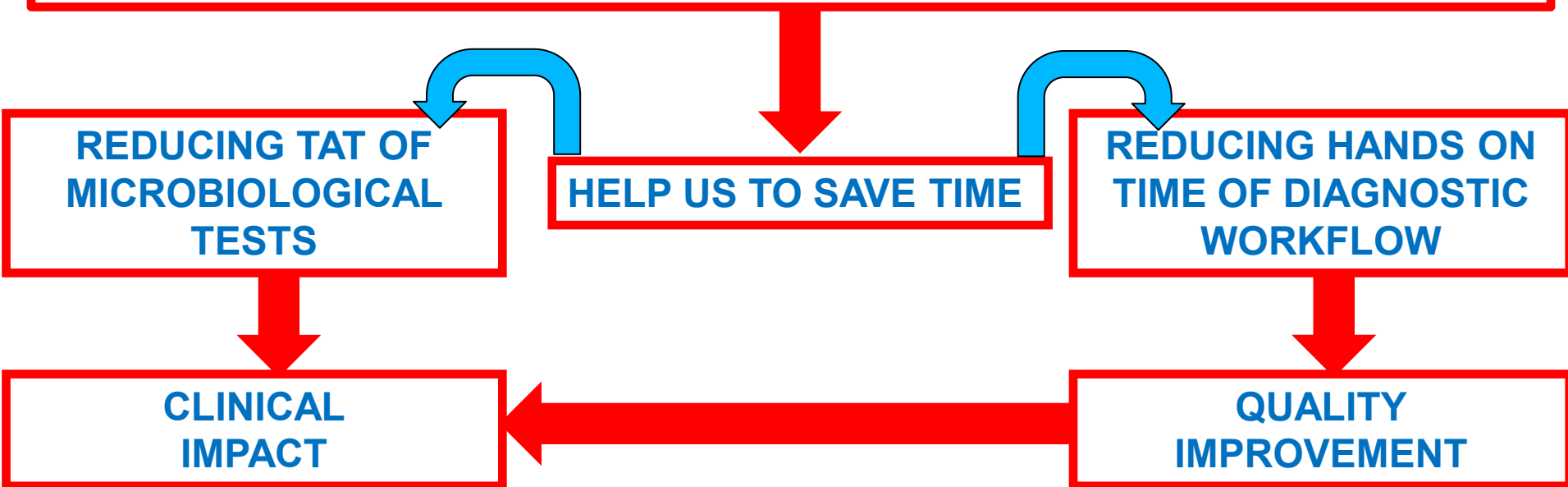
Level of automation



WHERE WE WERE, WHERE WE ARE, WHERE WE ARE GOING?

- Over the last decade we have implemented **FULL LAB AUTOMATION** in our bacteriology lab
- Starting from LBM, we introduced automated streaking (**WASP**), automated incubation and plate reading (**WASPLAB**) and automated sample interpretation (**PHENOMATRIX**)
- Currently almost 80% of bacteriology samples are routinely evaluated by AI protocols
- Is our journey into automation over? No!!!! Next steps:
 - Improvement of existing protocols
 - Development of new protocols (multiple plates samples)

AUTOMATION = STANDARDIZATION, TRACEABILITY, EFFICIENCY



CoHere

Webinar

Boosting WASPLab® with
PhenoMATRIX®

Microbiology labs experience with A.I.

**THANK
YOU FOR
YOUR
ATTENTION**

BOLOGNA

