Istituto Nazionale Biostrutture Biosistemi
I.N.B.B.
Consorzio Interuniversitario

# Utilizzo del metodo MBS per la diagnosi rapida delle infezioni delle vie urinarie

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# Urinary Tract Infection (UTI)

**"Clinically detectable condition associated with invasion by disease causing microorganism of some part of the urinary tract"**

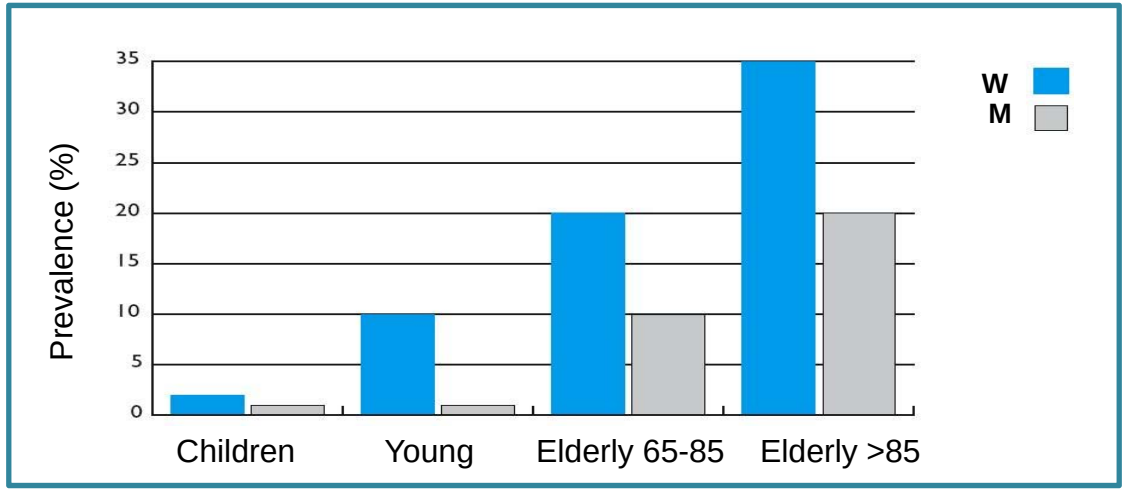
(Wang A et al.,Prim Care. 2013 Sep;40(3):687-706).

**Significant bacteriuria ≥ $10^5$ CFU/ml**

(M. S. Najar et al., Indian J Nephrol. Oct 2009)

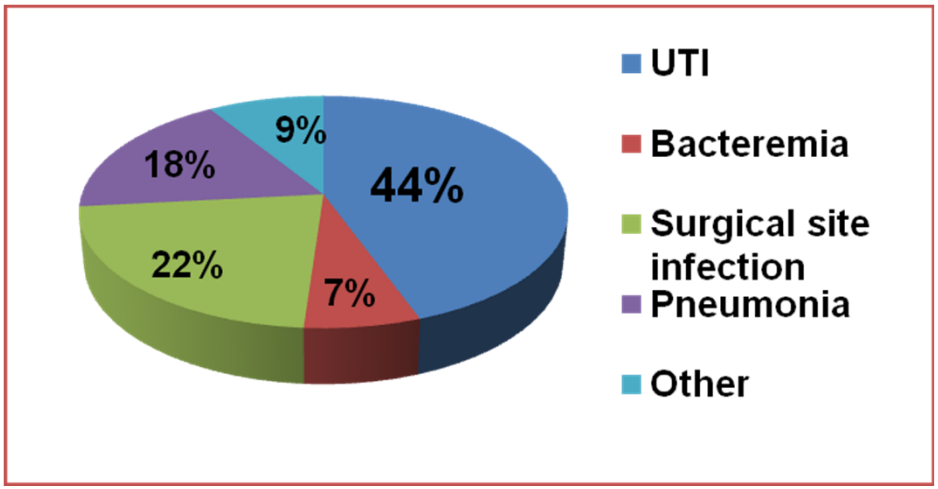
Center for Disease Control and Prevention *(CDC 1988)*

# Epidemiology of UTI




**The incidence of UTI varies according to sex and age**

**Mostly observed in women**




**UTI is the most common <u>nosocomial infection</u> and the second most frequent infections of all Emergency admissions**

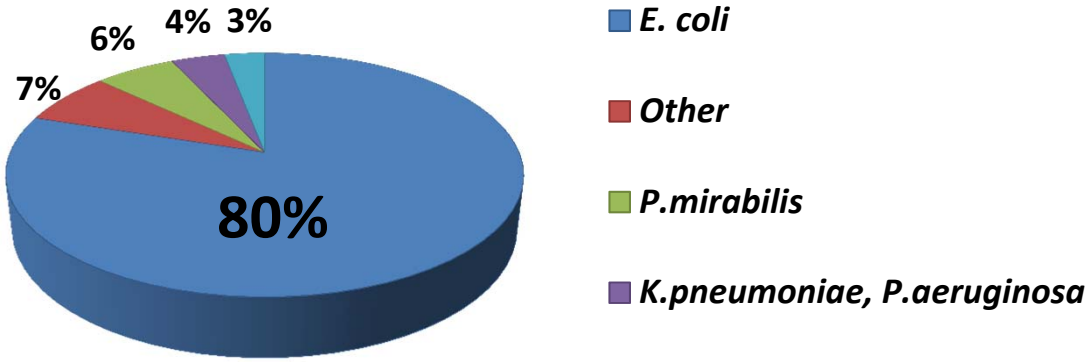
**80% of infections are associated with the use of an indwelling bladder catheter**

**Prevention of hospital-acquired infections, WHO/CDS/CSR/EPH/2002.12**

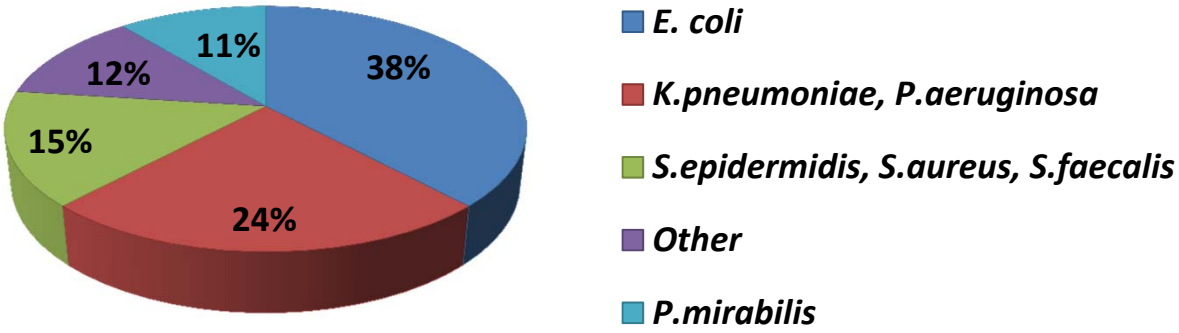
National Nosocomial Infections Surveillance (NNIS)

# Eziology of UTI

**Community-acquired UTI**




**Hospital-acquired UTI**



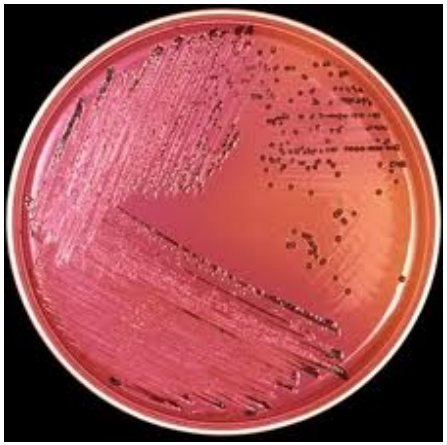

Wang et al. , Prim Care. 2013 Sep;40(3):687-706.

# LABORATORY DIAGNOSIS of UTI

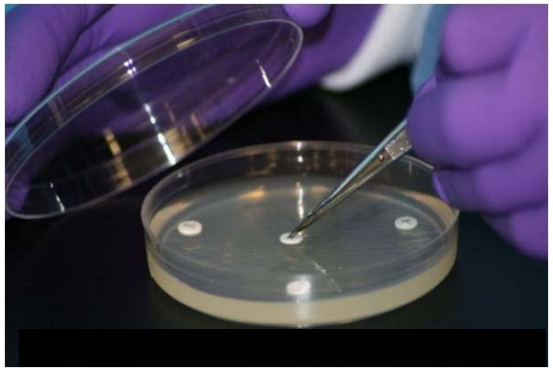
**(traditional)**

**1-2 days for bacterial count (diagnosis)**

**+ other 1-2 days for antibiogram (therapy)**



Colony count on solid medium



Antimicrobial susceptibility tests

The **Micro Biological Survey (MBS)** method is a colorimetric system developed for the selective detection and quantification of microrganisms present in food, water or on surfaces.

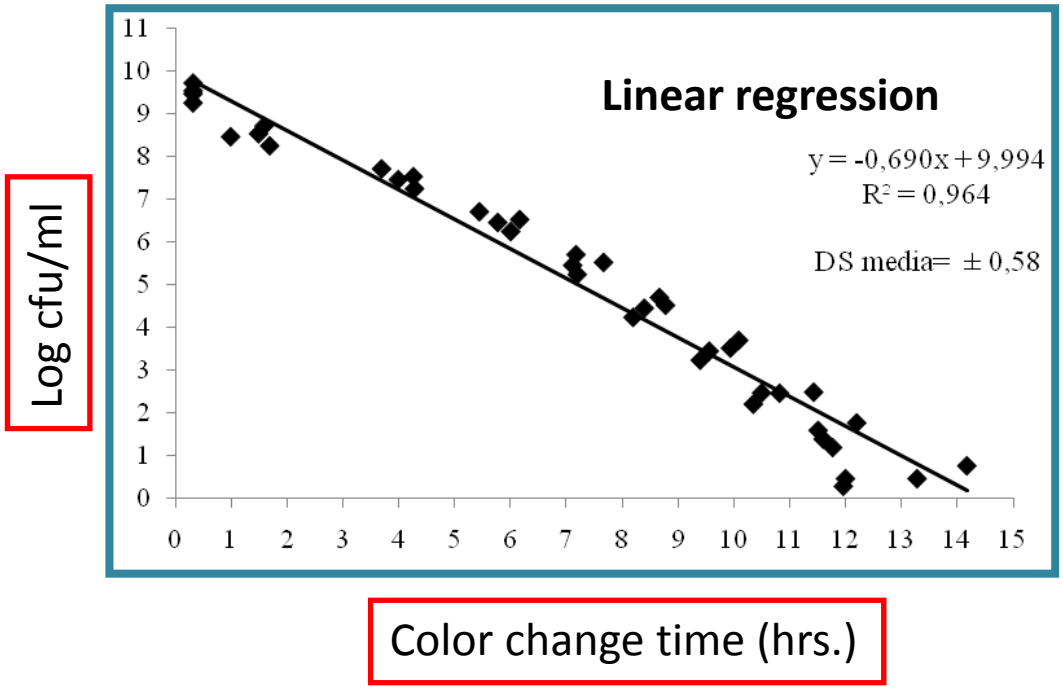
# Disposable vials

- **Ready to Use**
- **Pre-filled**
- **No sample pretreatment**
- **No Lab required**
- **Bacterial reaction causes colour change**

# MBS multireader

- **automatic color changes detection**
- **automatic bacteria counts**
- **automatic supply of an analysis report**

The analytical principle of MBS method is based on **measuring metabolism of bacteria** using redox indicators (dyes).




**Greater the number of living bacteria, faster the colour change**

# MBS METHOD COMPARISON TRADITIONAL PLATE COUNT ANALYSIS

I step: inoculum (about 1 ml)

II step: incubation

**Analysis Report** 2012/02/03 16:44:36

Company: Hygeia Laboratories - Berlin

Customer: Iceland - Amburg

Sample Number: 1 | Receiving Date: 2012/01/18 | Starting Time: 2012/02/03 10:28:10 | Ending Time: 2012/02/03 16:43:18

| | |
|---|---|
| Product Class: | Frozen |
| Product Type: | Cod |
| Sample Quantity: | 1g |
| Sample Back: | No |
| Sampling Procedure: | standard |
| Product: | Uncooked food |

This Report refers only to the given sample

| Date | Analysis - Analytical Method | CFU/g or CFU/ml | Limit | Note |
|---|---|---|---|---|
| 2012/02/03 16:43:18 | CBT-A01 Total Viable Count 30°C - MBS par | 1,253E05 | | - |

Operator: Edward Smith

Supervisor: _____

III step: PDF file reading

PDF file gives you directly the number of bacteria in the sample, without any calculation

I step: serial dilutions

II step: inoculum

III step: incubation

IV step: manual count

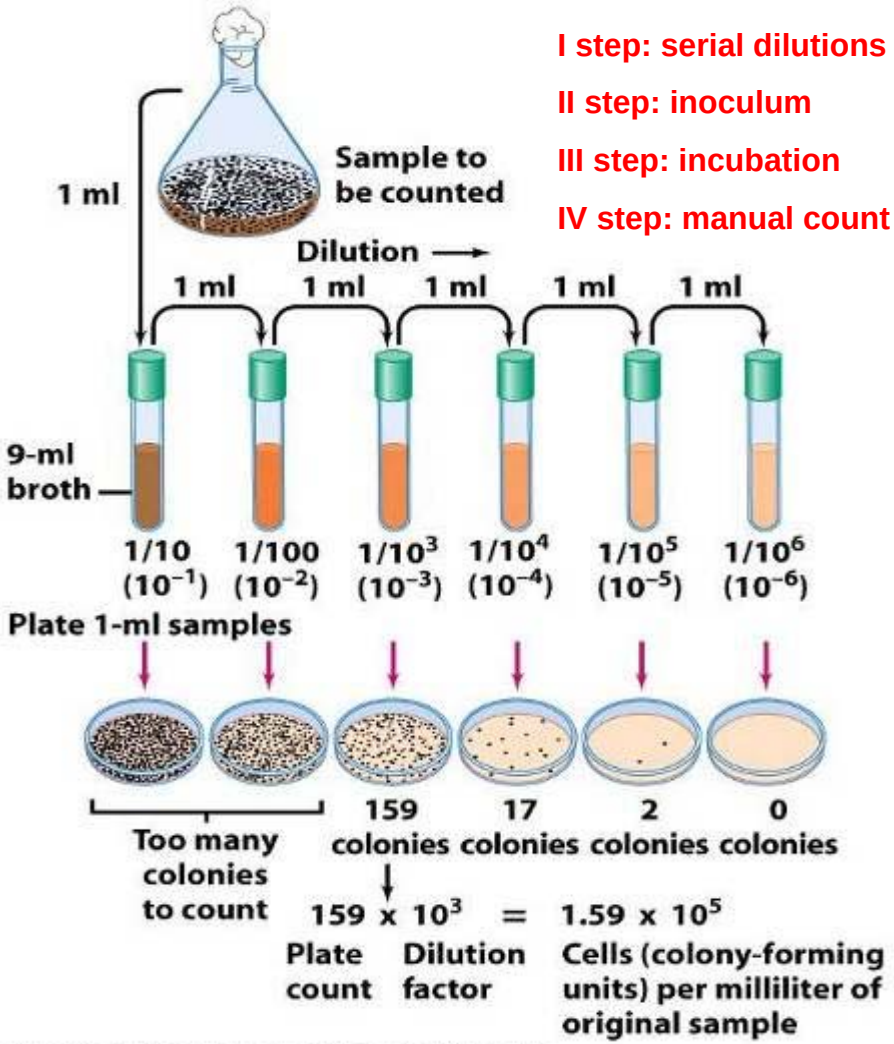



Figure 6-11 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

**3.5-5 HOURS**

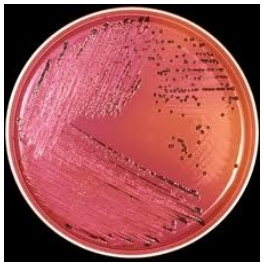
**24-48 HOURS**

# Aim of the study

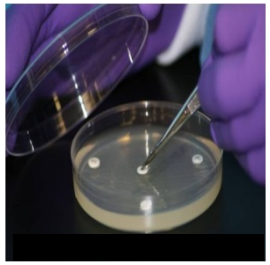
To confirm the reliability of POCT MBS method, as microbiological POCT for the detection of urinary tract infection and to test bacterial susceptibility to antibiotics, comparing this procedure with the traditional bacterial count + antibiogram (REFERENCE METHOD)

**SLOW (days)**

Traditional analysis in the central laboratory



Urine culture

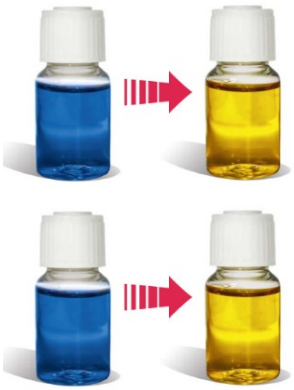


Antibiogram

**VS**

**FAST (hours)**

POCT MBS



Bacteria load

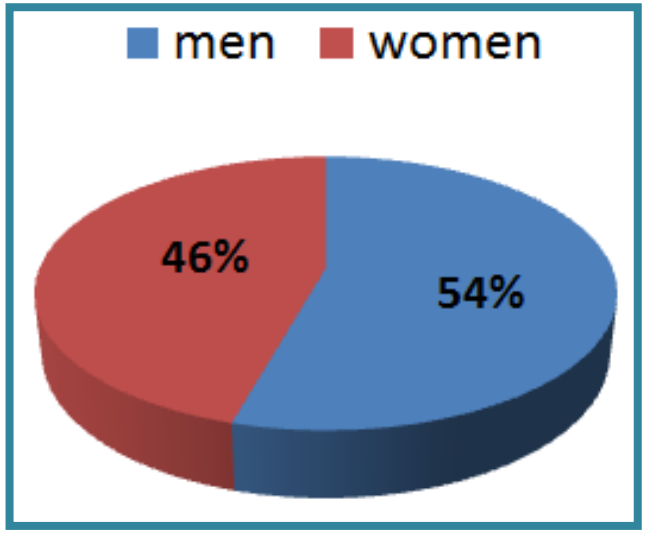
Bacterial susceptibility test

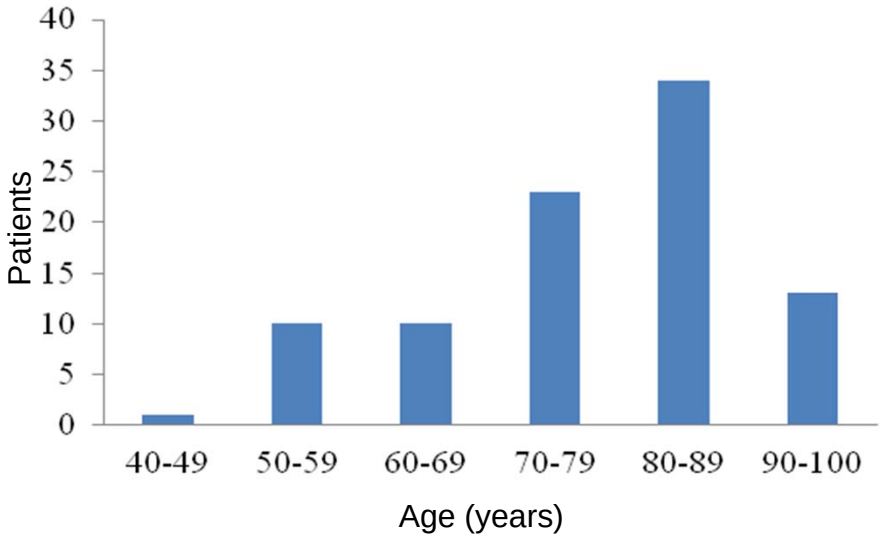
**sequencial analysis** **vs** **parallel analysis**

# Use of POCT MBS to evaluate the bacterial load in urine

# Clinical study

**122 subjects have been enrolled between November 2013 and July 2014 in Emergency Room with clinically suspected Urinary tract infections**




**89% with urinary catheter**

# ROC analysis

**Receiver Operating Characteristic (ROC) curve analysis used to compare the diagnostic performance of two or more laboratory or diagnostic tests**

**(Griner et al., Annals of Internal Medicine 94:555-600, 1981).**

| | | Reference method | | |
|---|---|---|---|---|
| | | **POSITIVE** | **NEGATIVE** | **TOTAL** |
| Alternative method | **POSITIVE** | **CP** (positive agreement) | **DP** (positive deviation: **false positives**) | (total positives for alternative method) |
| | **NEGATIVE** | **DN** (negative deviation: **false negatives**) | **CN** (negative agreement) | (total negatives for alternative method) |
| | **TOTAL** | **N+** (total positives for reference method) | **N-** (total negatives for reference method) | **N** (total number of test) |

**ACCURACY is the percentage of agreement between the two methods (positive agreement + negative agreement)/total number of tests**

**AC = Relative accuracy :** The relative accuracy is the degree of correspondence between the response obtained by the alternative method and the Reference methods.

**SE = Relative sensitivity :** The relative sensitivity is the ability of the alternative method to detect the analyte compared to the Reference methods.

**SP** = **Relative Specificity :** The relative specificity is the ability of the alternative method not to detect the target microorganism when it is not detected by the Reference methods.

# Urine culture vs POCT MBS

| | | URINE culture | | |
|---|---|---|---|---|
| | | POSITIVE | NEGATIVE | TOTAL |
| POCT MBS Method (at 5 hours) | POSITIVE | 31 | 9 | 40 |
| | NEGATIVE | 3 | 79 | 82 |
| | TOTAL | 34 | 88 | 122 |

**3 false negatives**

***S.aureus* infections: POCT MBS is slow responding towards S.aureus**

**9 false positives**

**HYPOTHESIS: Previous administration of antibiotics hinders replication of bacteria but not their redox metabolism**

**Receiver Operating Characteristic (ROC) curve analysis is widely used to compare the diagnostic performance of two or more laboratory or diagnostic tests**

| ROC ANALYSIS | urine culture vs MBS |
|---|---|
| Accuracy | 90.2% |
| Relative sensitivity | 91.2 % |
| Relative specificy | 90.0 % |

# urine colture vs urine sticks

| | URINE COLTURE | | | |
|---|---|---|---|---|
| Urine sticks (positive for both leukocytes and Nitrite) | | POSITIVE | NEGATIVE | TOTAL |
| | POSITIVE | 10 | 6 | 16 |
| | NEGATIVE | 16 | 40 | 56 |
| | TOTAL | 26 | 46 | 72 |

**16 false negatives** **6 false positives**

**Urinary Dipstick (Leukocytes+ nitrite)**

| ROC ANALYSIS | urine culture vs urine sticks |
|---|---|
| Accuracy | 69.4 % |
| Relative sensitivity | 38.5 % |
| Relative specificy | 87.0 % |

# urine colture vs PCT

| | | URINE COLTURE | | |
|---|---|---|---|---|
| | | POSITIVE | NEGATIVE | TOTAL |
| PCT (≥0.05 ng/ml) | POSITIVE | 31 | 73 | 104 |
| | NEGATIVE | 0 | 1 | 1 |
| | TOTAL | 31 | 74 | 105 |

**73 false positives** **All this patients possibly had a bacterial infection but NOT a UTI !**

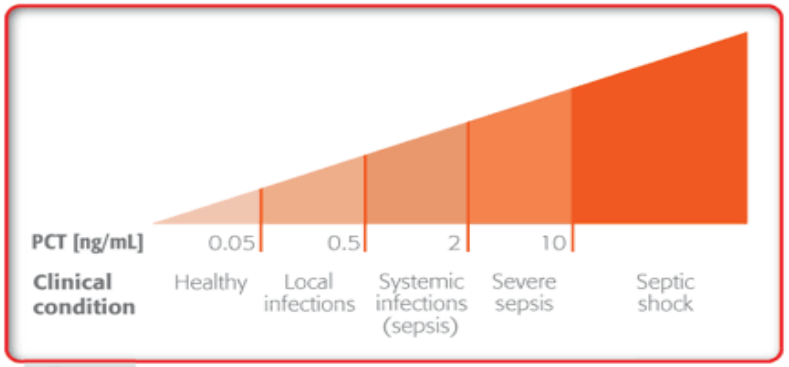
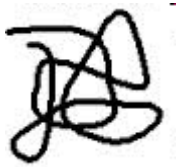



*Figure 1* PCT increase reflects the continuous development from a healthy condition to the most severe states of disease (severe sepsis and septic shock).

**Serum procalcitonin (PCT) ≥ 0.05 ng/ml**



| ROC ANALYSIS | urine culture vs PCT |
|---|---|
| Accuracy | 30.5 % |
| Relative sensitivity | 100 % |
| Relative specificy | 1.35 % |

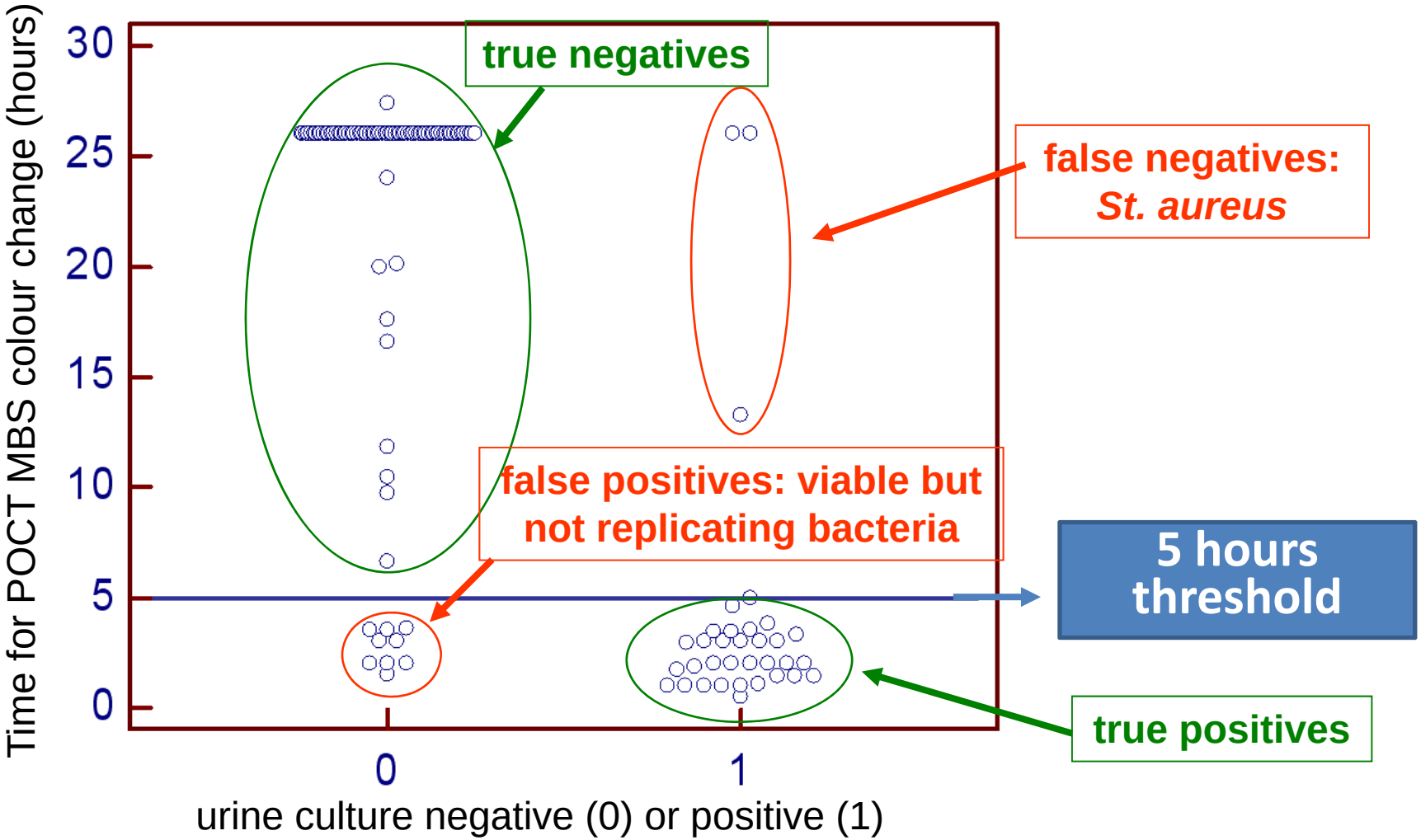
# Detailed ROC analysis of POCT MBS Experimental data

The Urine culture positivity (1) or negativity (0) and the time (hours) required for POCT MBS colour change have been recorded for each patient.

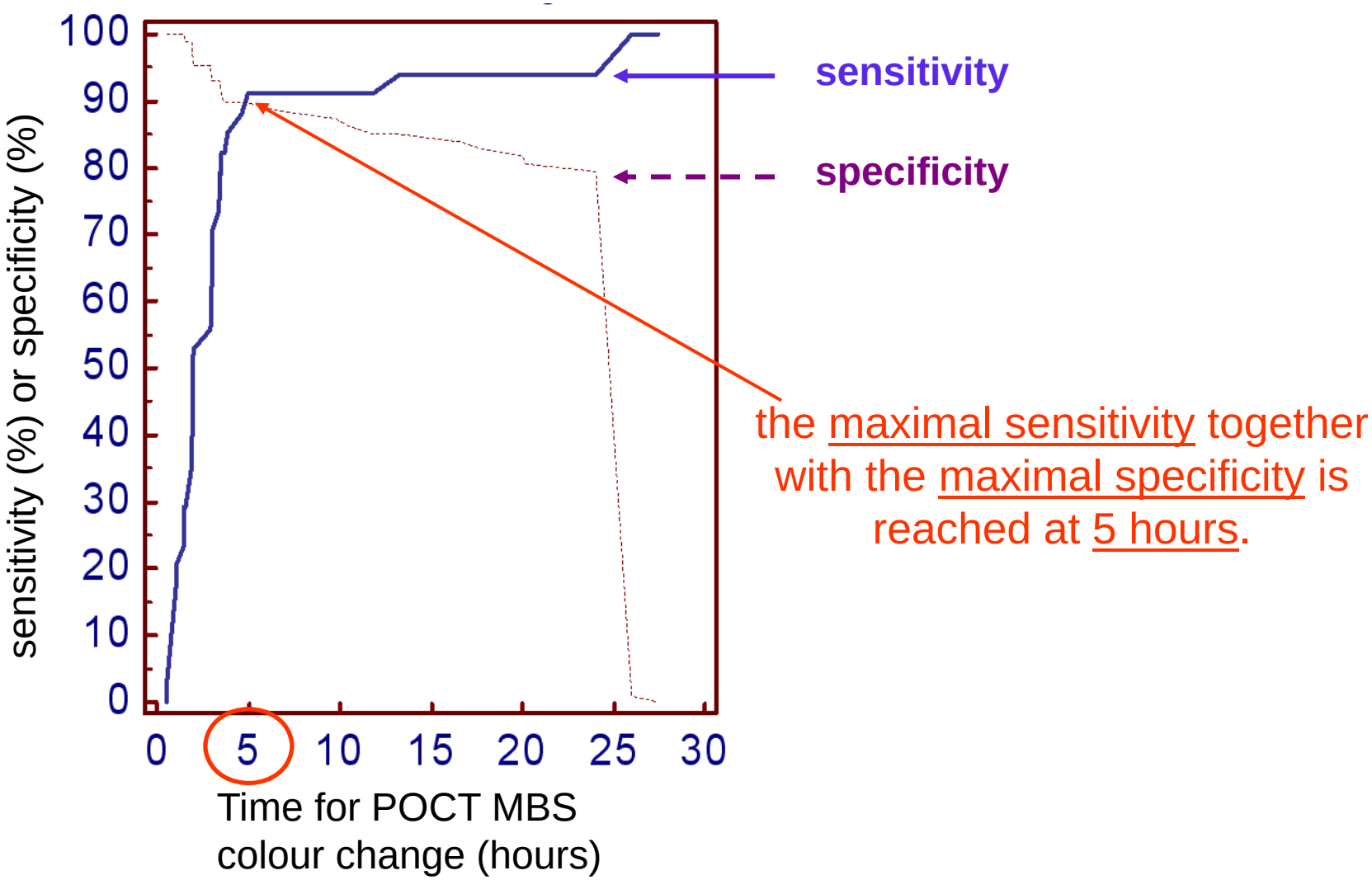
| Patient number | Urine culture positive (1) or negative (0) | POCT MBS colour change (hours) | EXAMPLES |
|---|---|---|---|
| 1 | 0 | 26 | ← true negative |
| 2 | 1 | 2 | ← true positive |
| ... | ... | ... | |
| 6 | 0 | 2 | ← false positive |
| ... | ... | ... | |
| 17 | 1 | 13.25 | ← false negative |
| ... | ... | ... | |
| 123 | ... | ... | |

# Detailed ROC analysis for POCT MBS dotplot

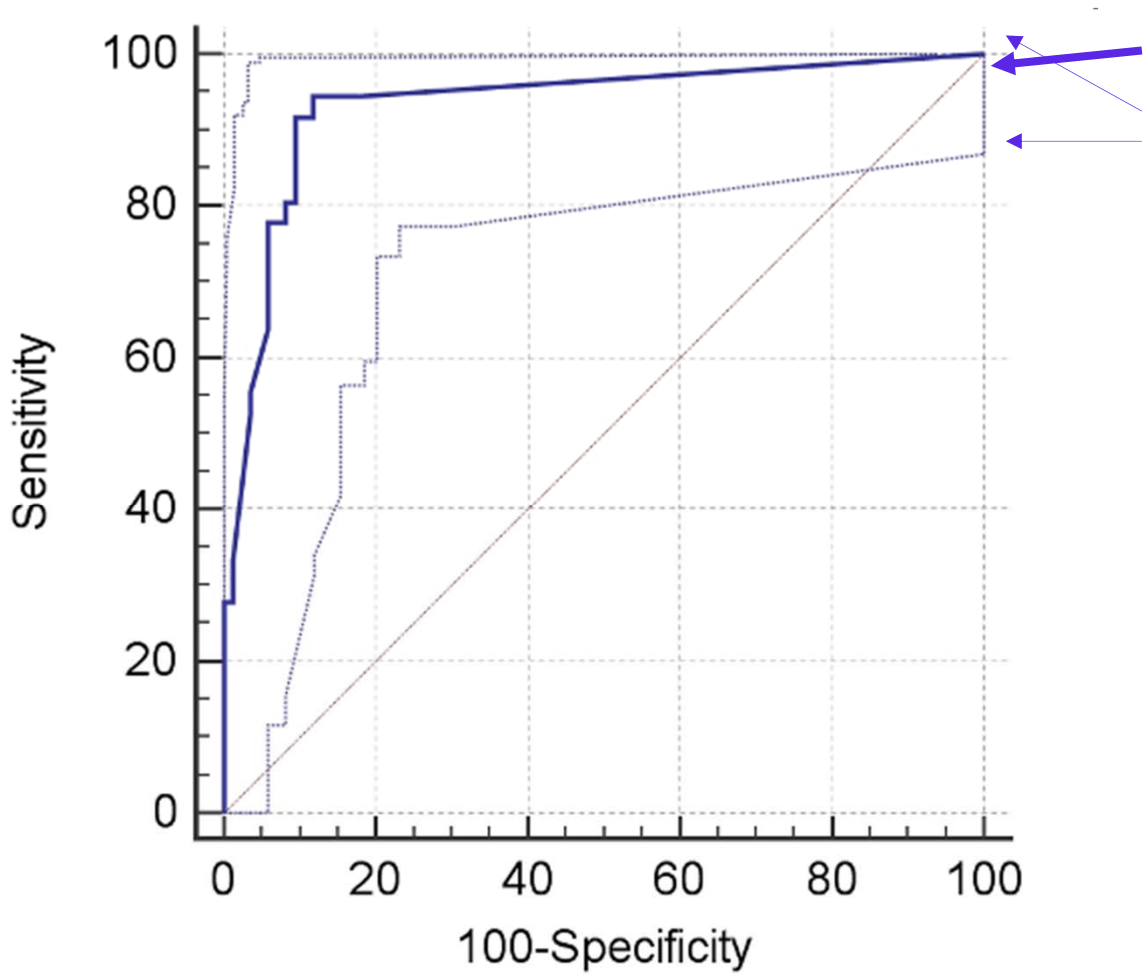
Each dot represents a patient



# Detailed ROC analysis of POCT MBS sensitivity vs specificity



# Detailed ROC analysis of POCT MBS Area Under the Curve (AUC)




POCT MBS **AUC: 0.927**
95% confidence interval:
0.966 to 0.865

# ROC analysis conclusions

| Method | Accuracy (%) (agreement with the reference methods) | Sensitivity (%) | Specificity (%) |
|---|---|---|---|
| POCT MBS (5 hours) | 90.2 | 91.2 | 90.0 |
| POCT MBS (3.5 hours) | 88.5 | 82.4 | 91.0 |

# ROC analysis conclusions

| Method | Accuracy (%) (agreement with the reference methods) | Sensitivity (%) | Specificity (%) |
|---|---|---|---|
| POCT MBS (5 hours) | 90.2 | 91.2 | 90.0 |
| POCT MBS (3.5 hours) | 88.5 | 82.4 | 91.0 |

**For POCT MBS the best compromise between fast response time and Accuracy is most likely reached after 3.5 hours (88.5 % Accuracy)**

# Use of POCT MBS to evaluate the resistance/susceptibility to antibiotics of bacteria causing UTI

# POCT MBS method plus ANTIBIOTICS

**Patients' urine was added to**

**Vials ± antibiotics**

- NO Antibiotic
- Amoxicillin-Clavulanate
- Ciprofloxacin
- Co-trimoxazole

# 2.1 antibiogram vs MBS (ciprofloxacin)

| | | URINE culture | | |
|---|---|---|---|---|
| | | RESISTANT | SUSCEPTIBLE | TOTAL |
| POCT MBS Method | RESISTANT | 25 | 3 | 28 |
| | SUSCEPTIBLE | 3 | 3 | 6 |
| | TOTAL | 28 | 6 | 34 |

Urine culture: R= MIC ≥ 1 mg/l

POCT MBS: R = colour change ≤ 5 hours

| ROC ANALYSIS | Ciprofloxacin |
|---|---|
| Accuracy | 82.4 |
| Relative sensitivity | 89.3 |
| Relative specificy | 50.0 |

# 2.2 antibiogram vs MBS (co-amoxiclav)

| | | URINE culture | | |
|---|---|---|---|---|
| | | RESISTANT | SUSCEPTIBLE | TOTAL |
| POCT MBS Method | RESISTANT | 11 | 14 | 25 |
| | SUSCEPTIBLE | 2 | 7 | 9 |
| | TOTAL | 13 | 21 | 34 |

Urine culture: R= MIC ≥ 8 mg/l

POCT MBS: R = colour change ≤ 5 hours

| ROC ANALYSIS | Co-amoxiclav |
|---|---|
| Accuracy | 52.9 |
| Relative sensitivity | 84.6 |
| Relative specificy | 33.3 |

# 2.3 antibiogram vs MBS (co-trimoxazole)

| | URINE culture | | | |
|---|---|---|---|---|
| POCT MBS Method | | RESISTANT | SUSCEPTIBLE | TOTAL |
| | RESISTANT | 19 | 9 | 28 |
| | SUSCEPTIBLE | 4 | 2 | 6 |
| | TOTAL | 23 | 11 | 34 |

Urine culture: R= MIC ≥ 4 mg/l

POCT MBS: R = colour change ≤ 5 hours

| ROC ANALYSIS | Co-trimoxazole |
|---|---|
| Accuracy | 62.0 |
| Relative sensitivity | 82.6 |
| Relative specificy | 27.3 |

# POCT MBS method plus ANTIBIOTICS

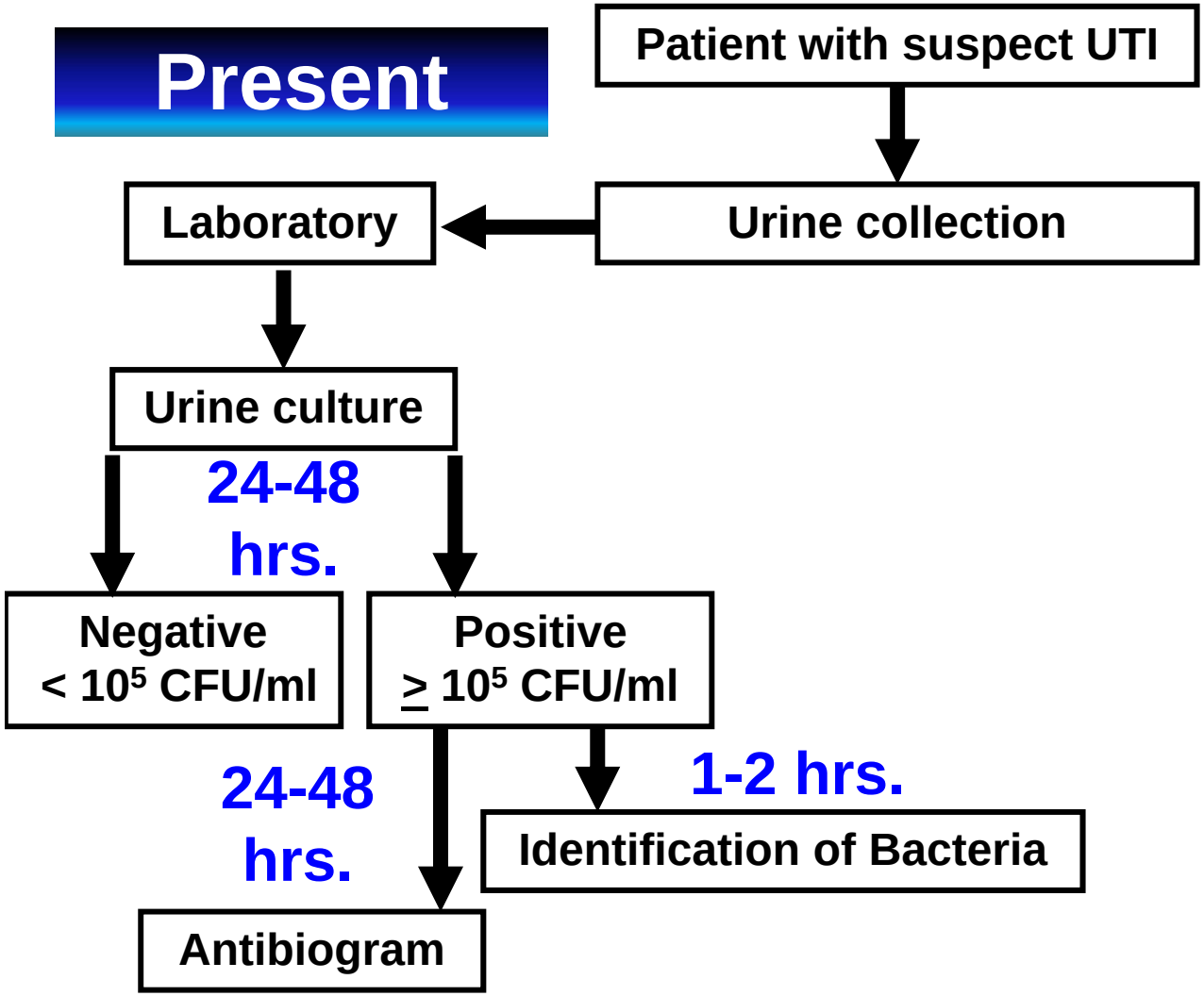
| ANTIBIOTIC (n=34) | **Accuracy %** (agreement) |
|---|---|
| Ciprofloxacin | **82.4** |
| Co-amoxiclav | **52.9** |
| Co-trimoxazole | **62.0** |

**The results are encouraging although there are too many false resistants that decrease observed Accuracy. It is possible that these false resistants are bacteria not able to replicate but still having metabolic activities.**

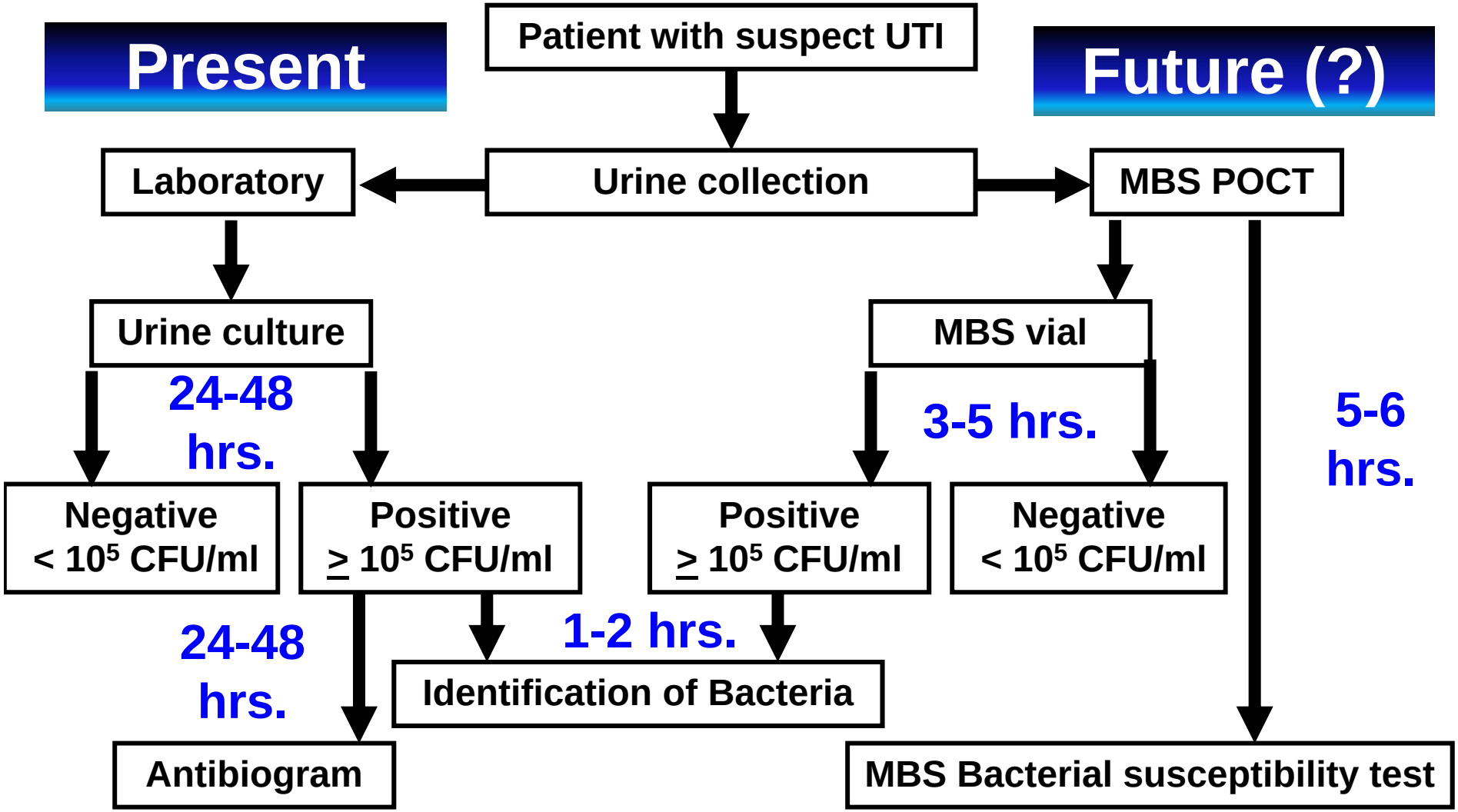
**It is thus possible that POCT MBS changed colour before the antibiotic could have an effect on bacteria replication.**

# Conclusions

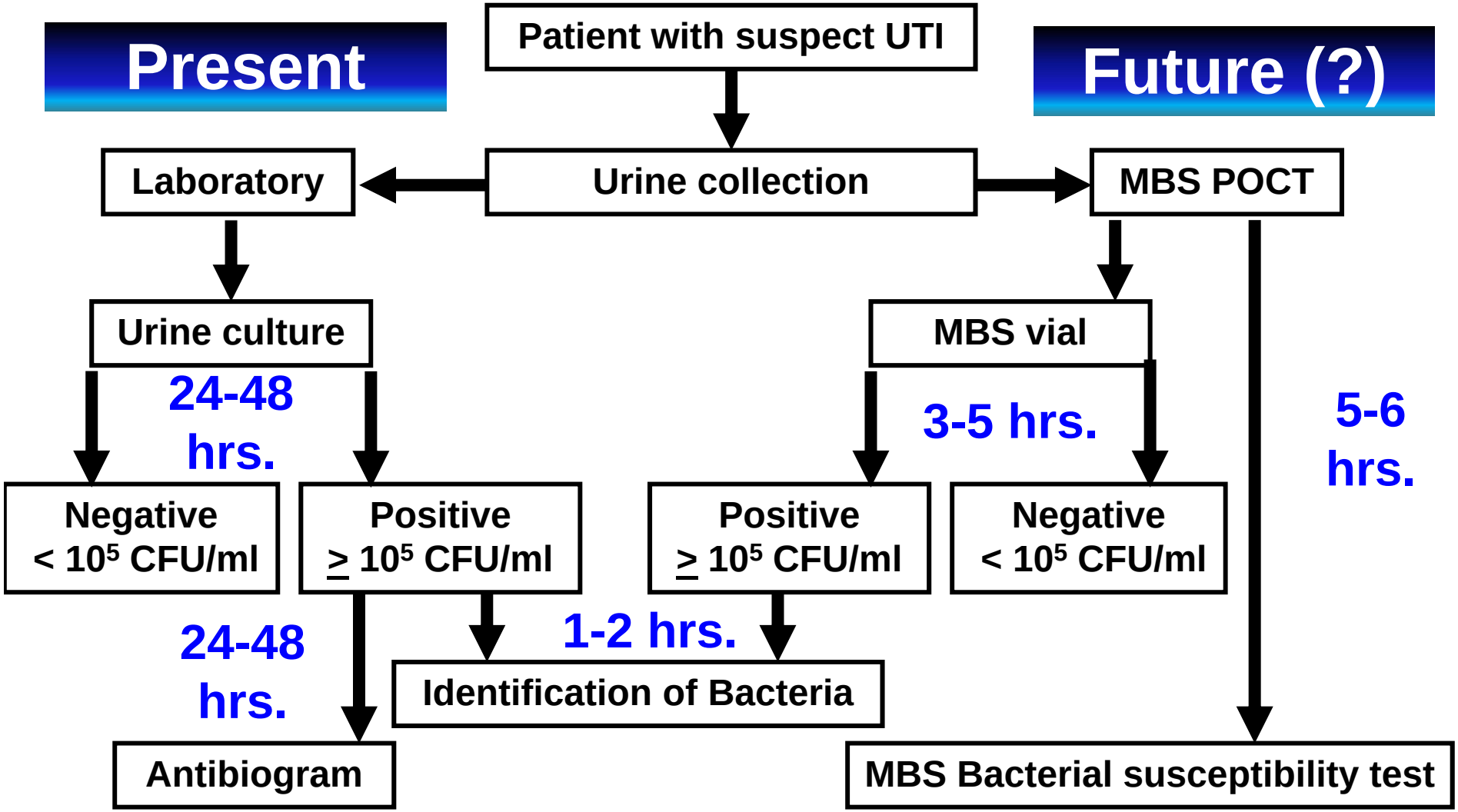
## Present



# Conclusions



# Conclusions




**Total analytical time**

**48-96 hrs. vs 3-6 hrs.**

# Collaborations

Prof. Giovanni Antonini, Prof. Paolo Visca,
dr.ssa Alyexandra Arienzo,
dr.ssa Valentina Cellitti, dr.ssa Valeria Ferrante

Prof. Salvatore Di Somma,
dr.ssa Flavia Cristofano

Prof. Roberto Messi

Ing. Alberto Mari, dr.ssa Giorgia Bottini,
dr.ssa Francesca Losito, dr.ssa Francesca R. Priolisi

Ing. Filippo Ugolini, ing. Tommaso Buranelli

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