

# Q-LINEA

Rapid MALDI sample prep from ASTar cartridge output:  
proof-of-concept

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ASTar is not CE-marked or FDA-cleared for MALDI sample prep. Not for clinical use.

# Background

## ➔ **AS<sup>T</sup>ar is an IVDR-marked and FDA-cleared rapid AST (rAST) system**

AS<sup>T</sup>ar is approved for use for rAST from monomicrobial Gram-negative positive blood cultures (PBCs).

## ➔ **Organism ID**

Use of AS<sup>T</sup>ar, and several other rAST systems on the market, requires organism identification from another source.

## ➔ **MALDI ID**

MALDI is a widely utilized low-cost identification method with extensive organism libraries but requires an isolate colony extending overall time.

## ➔ **MALDI directly from PBCs**

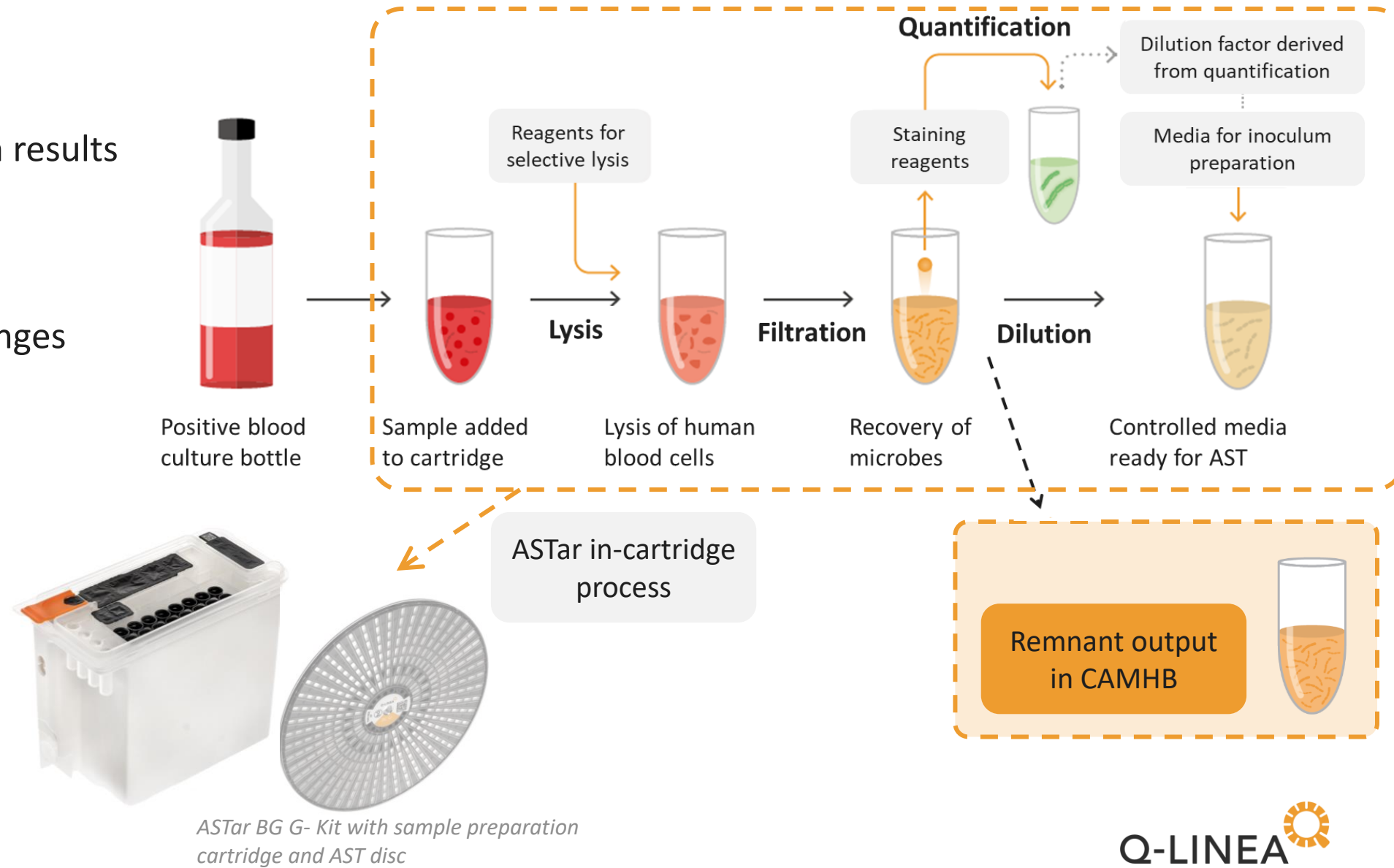
Enabling ID via MALDI directly from positive blood culture may make rAST workflows and costs attainable for more clinical microbiology labs. Current rapid MALDI workflows require either extensive hands-on time or additional equipment.



Examples of rapid AST and MALDI systems

# ASTar automated sample preparation from PBC

- Sample preparation results in purified bacterial suspension.
- Remnant output ranges from 1.8 – 2.8 mL.

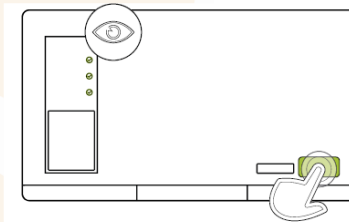
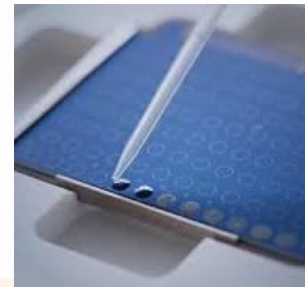
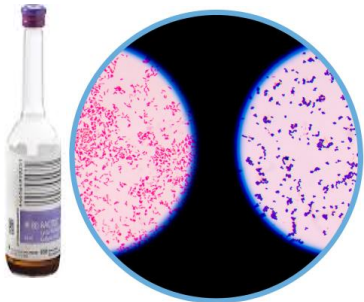
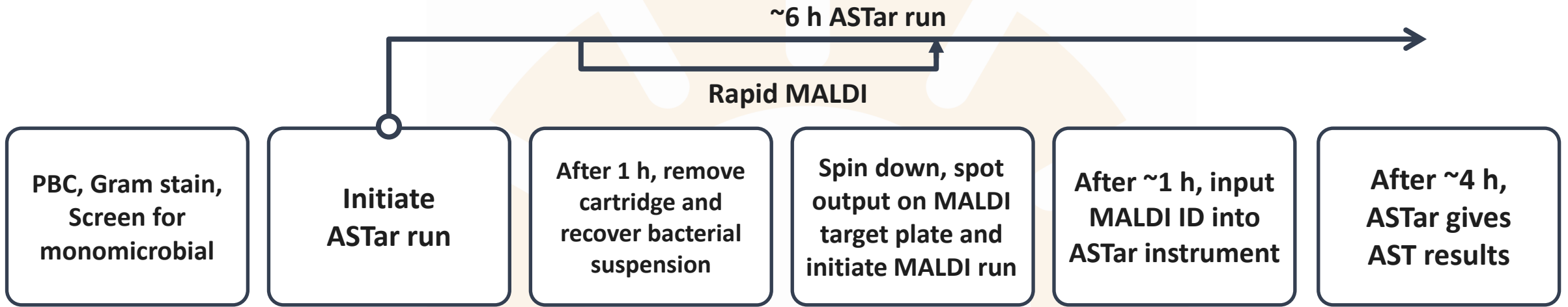


# MALDI prep protocol details: experimental design

- In this early proof-of-concept study, we explored the potential utility of the automated sample preparation output of ASTar, a purified bacterial suspension, for rapid MALDI ID.
- Contrived PBCs inoculated with Gram-negative isolates were processed within 2.5 – 14 h of positivity in BACT/ALERT VIRTUO using the ASTar BC G- Kit, and remnant suspension was retrieved from the cartridge within 40 minutes of sample prep and disc inoculation completion.
- Minimal sample prep:
  1. Centrifugation at 13,000 rpm for 3 minutes, removal of supernatant
  2. MALDI target plate single spotting of pellet
  3. Addition of MALDI matrix and ID by MALDI BiotyperAbout 1/3 of available remnant output was used, creating an opportunity for additional downstream uses.

# PBC end-to-end workflow

Experimental design. Not an approved use of the AStar System.



AST result report Q-LINEA

Sample ID: C635530906  
Pathogen ID: Escherichia coli (Pathogen added from AStar)

| Antibiotic                    | MIC (µg/ml) | Interpretation |
|-------------------------------|-------------|----------------|
| Ampicillin                    | >64         | R              |
| Amoxicillin-clavulanate       | >64         | R              |
| Meropenem-meropenem           | 0.125       | S              |
| Piperacillin-tazobactam       | 0.1         | S              |
| Ceftazidime                   | 0.1         | S              |
| Ceftazidime-avibactam         | 0.1         | S              |
| Colistin                      | 0.1         | S              |
| Meropenem                     | 0.125       | S              |
| Meropenem-meropenem           | 0.125       | S              |
| Tigecycline                   | 0.125       | S              |
| Linezolid                     | 0.125       | S              |
| Levofloxacin                  | 0.125       | S              |
| Trimethoprim-sulfamethoxazole | 0.0625      | S              |

**Additional Information**

Sample ID: C635530906    Lot number: 00123456789    Lot number: 00123456789  
 Date: 2024-06-03 10:11    Date: 2024-06-03 10:11    Date: 2024-06-03 10:11  
 Pathogen ID: Escherichia coli    Pathogen ID: Escherichia coli    Pathogen ID: Escherichia coli  
 Breakpoints (EU)    Breakpoints (EU)    Breakpoints (EU)

**Comments and Expert rules**

1. MIC above threshold concentration 1 µg/ml may indicate ERM production.  
 2. Resistance identified may be common for general conditions of epidemiological concern. Confirm ID and susceptibility if uncertainty in the identification. Check with infection prevention to determine appropriate actions.  
 3. Consideration should be given to other pathogens other than those listed. Consultation with an infectious disease specialist is recommended.

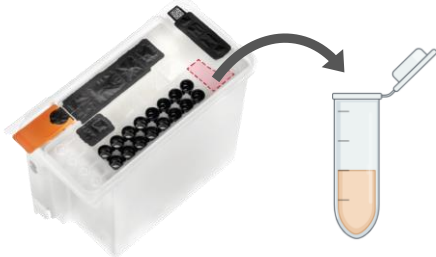
**Notes**

**Information**

AStar version: 0.1.0    Software version: 0.1.0    Software version: 0.1.0  
 AStar version: 0.1.0    Software version: 0.1.0    Software version: 0.1.0

# Sample prep from remnant output: workflow

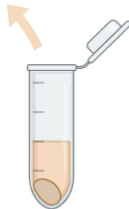
Pipette out sample remnant (600µl)



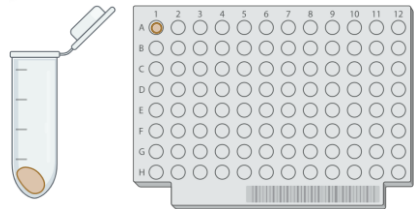
Centrifuge at 13,000 rpm for 3 min



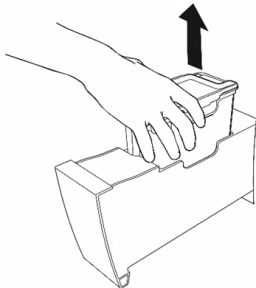
Remove supernatant



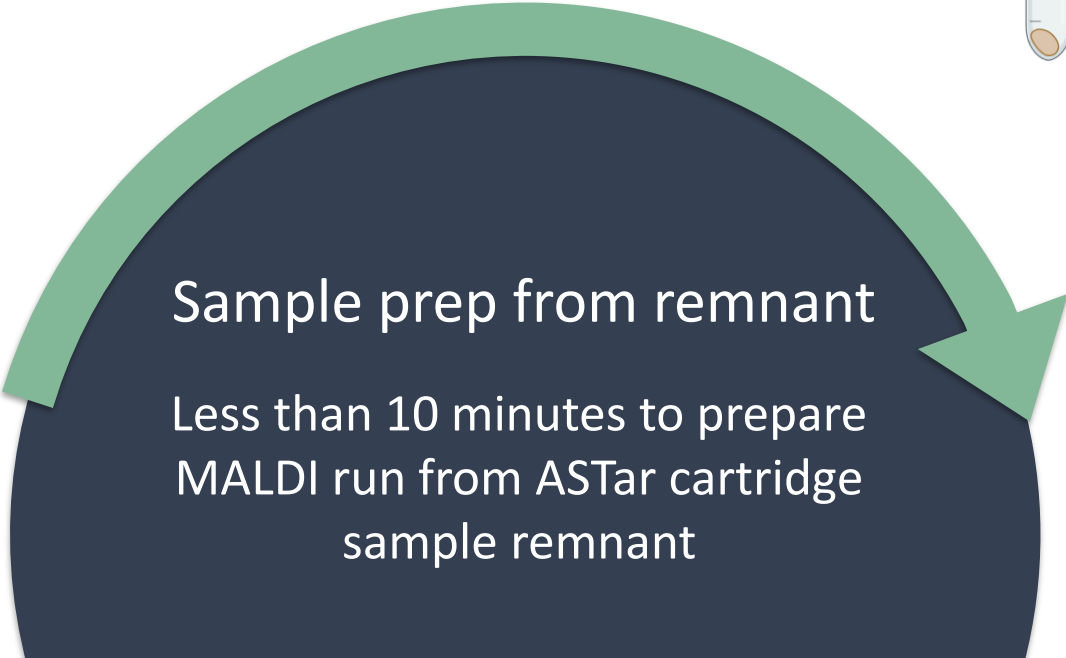
Single spotting of pellet onto MALDI target plate



Remove used cartridge from ASTar



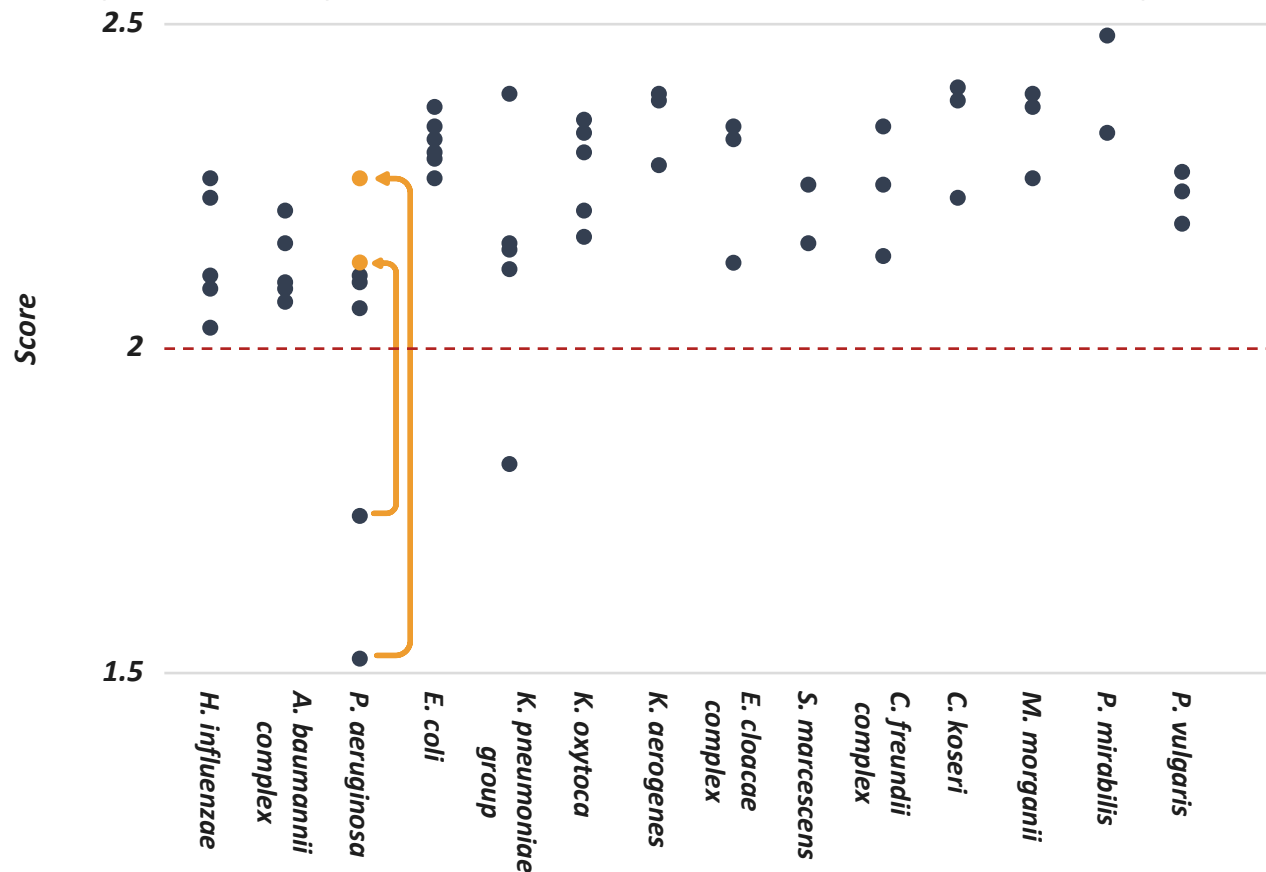
Addition of MALDI Matrix and ID



# Performance data

98% of samples (n=53) yielded high-confident ID scores (>2.0) using the Bruker Biotyper, matching classifications.

Two *P. aeruginosa* samples initially scored <1.8; re-test from the same aliquot scored >2.0 for both samples (orange)



# Limitations

- This early feasibility study consisted of a low n and limited organism taxa. More robust studies are needed to determine potential for routine usage.
- As this workflow does not represent an approved usage of AS*T*ar nor any MALDI instrument, regulatory considerations must be addressed before implementation into a clinical workflow.
- While AS*T*ar's claims include ~95%<sup>1</sup> of monomicrobial Gram-negative blood-culture-causing-organisms by prevalence, this workflow will result in some runs being initiated prior to awareness of an unclaimed organism ID, which will require an alternate AST method.

# Next steps

- In this data set, single spotting resulted in a high percentage of high-confidence ID scores. Future explorations may be warranted to determine whether dual spotting further improves this percentage.
- The presented procedure only uses about 1/3 of available bacterial suspension. Potential applications include:
  - Using the bacterial suspension output for lateral flow assays, such as NG CARBA 5 and CTX-M Multi, to provide some genetic resistance marker information in addition to the MALDI-generated ID. Future work is being planned to determine the feasibility of this approach.
  - The same bacterial suspension output may also enable rapid disk diffusion methods for novel or recently cleared antimicrobials, but future studies are needed to determine feasibility.

# Conclusions

- Use of the remnant bacterial suspension generated by the AStar cartridge following inoculation of the AStar disc resulted in accurate, high-confidence MALDI scores.
- The workflow demonstrated the feasibility of organism identification via MALDI within 2 hours from blood culture positivity, a saving of one day compared to MALDI ID from colony.
- The incremental cost of the experimental workflow was less than \$5 USD for a rapid MALDI ID, compared to an estimated \$100 price per kit for commonly used rapid molecular ID methods.
- This proposed workflow may enable broader utilization of rapid AST, which could lead to decreased time to optimal therapy for bacteremic patients.

# Acknowledgements

## Q-linea

- Stina Vincentsson
- Elin Ring
- Jan Grawé
- Jenny Göransson
- Michelle Myers
- Natalie Brown
- Oliver Mainwaring

## Clinical isolates

Clinical isolates from sites in Europe and US, and strain collections such as CDC & FDA Antibiotic Resistance Isolate Bank and Antibacterial Resistance Leadership Group (ARLG).

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