

Reproducibility of Fully Automated AST for Direct Near Patient Testing

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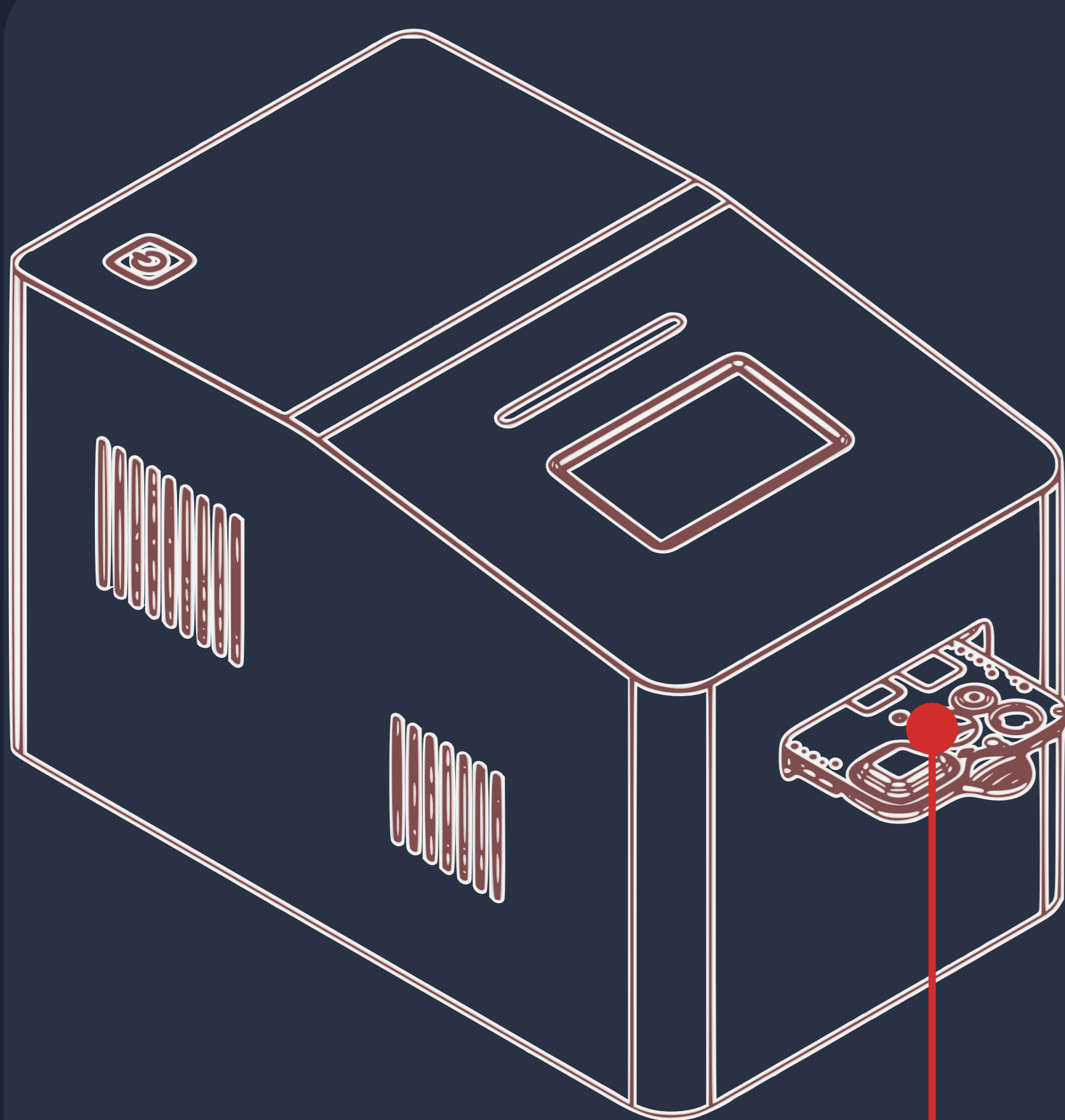
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Background

Determining the susceptibility of an infectious organism before antimicrobial selection can improve the outcome of antimicrobial chemotherapy and reduce the societal burden of worsening antimicrobial resistance (AMR) crisis. One barrier to such personalized approach to antimicrobial treatment is the long turnaround time (TAT) of the Antimicrobial Susceptibility Tests (AST). In addition to shortening of AST measurement itself, total TAT can be shortened by performing it directly on the patient sample. Astrego Diagnostics developed a fully automated rapid AST system for direct AST in near patient testing to address this unmet need. We have evaluated this system across different studies and presented the results in this poster.

Astrego's AST System

The system reports bacteriuria positive at concentrations above 5×10^4 CFU/mL within 5 to 15 minutes. For positive samples, qualitative AST is performed with 5 different antibiotics included in the panel within 30 min (Gram negatives) to 45 min (Gram positives).



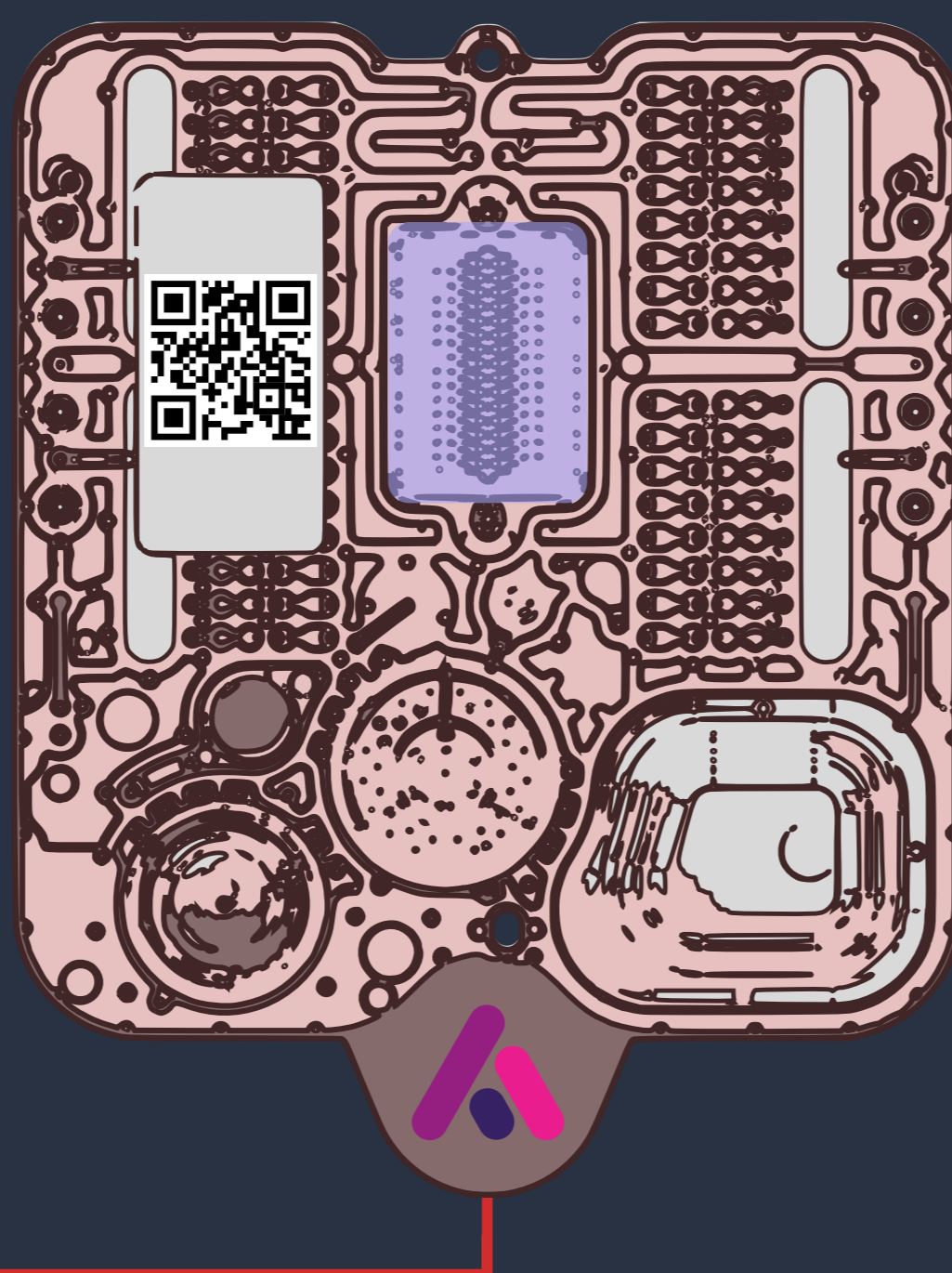
Analyzer

25x22x35 cm (9.1x8.7x13.8 inches)
Fully automated, stand-alone desktop analyzer

AST Panel

Single use cartridge with on-board reagents, fluidic control components and nanofluidic chip, which operates with 400 μ L freshly collected urine. Provides phenotypic AST result for the following 5 antibiotics:

- Amoxicillin-Clavulanic Acid
- Ciprofloxacin
- Fosfomycin
- Nitrofurantoin
- Trimethoprim



Comparative Performance Evaluation

The performance characteristics of the Astrego's system are established via comparative evaluations, where the qualitative AST results of Astrego's panel correlate with broth microdilution performed according to ISO 20776-1: 2019 and agar dilution.

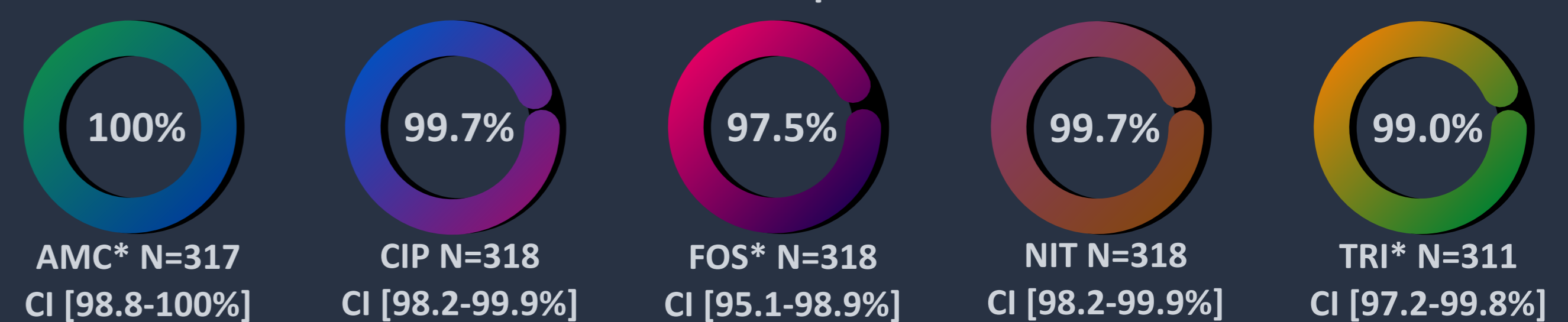
The AST performance study is conducted according to ISO 20776-2:2007, where applicable given that the standard is for AST from colonies on agar plates, while the Astrego's panel is indicated for freshly collected urine samples with actively growing bacteria.

Surrogate samples are prepared with fresh, recent, or stock isolates spiked into MHBII, grown overnight at 37°C and sub-diluted 1:2000 into MHBII to grow for 2 additional hours. The culture is then sub-diluted to target sample concentrations of 10^5 to 10^6 CFU/mL for positive and under 5×10^4 CFU/mL for negative samples.

AST Reproducibility

10 different bacterial isolates (from 5 target species) tested in technical triplicates, at 3 different study sites, over 3 days, by 6 different operators and on 13 different analyzers. In total 6 *E. coli* strains are included and 1 strain from each of the target species (*K. pneumoniae*, *P. mirabilis*, *E. faecalis* and *S. saprophyticus*).

CA for N tests with 10 unique isolates and 95% CI



The study is balanced in species by the prevalence. As the species ID is not reported by the system, the reproducibility results are evaluated for all species combined.

False negative results - 0.8%.

Technical errors - 2.38%.

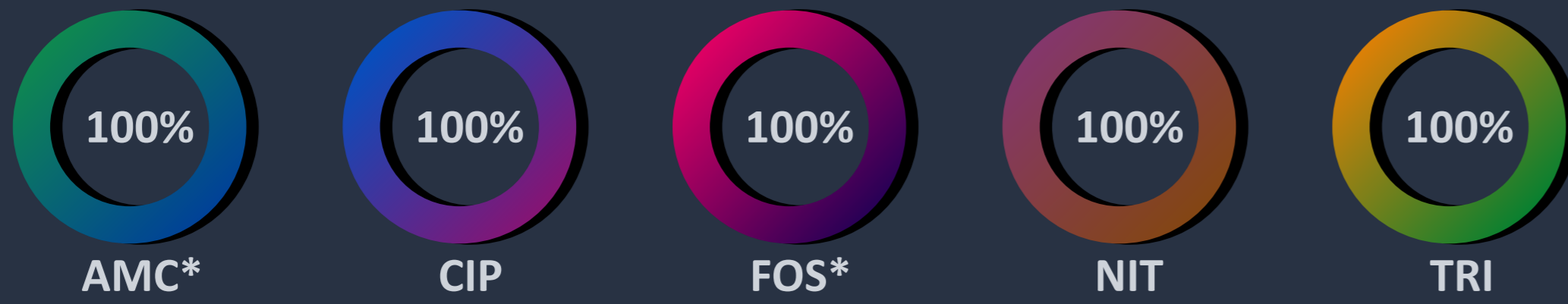
In-house AST Reproducibility

The data is collected from routine final quality control of Astrego's AST panel production. QC isolates are spiked into Mueller Hinton Broth II (MHBII), grown overnight at 37°C and sub-diluted 1:2000 into MHBII to grow for 2 additional hours. The culture is then sub-diluted to a target sample concentrations of 1×10^5 to 5×10^5 CFU/mL.

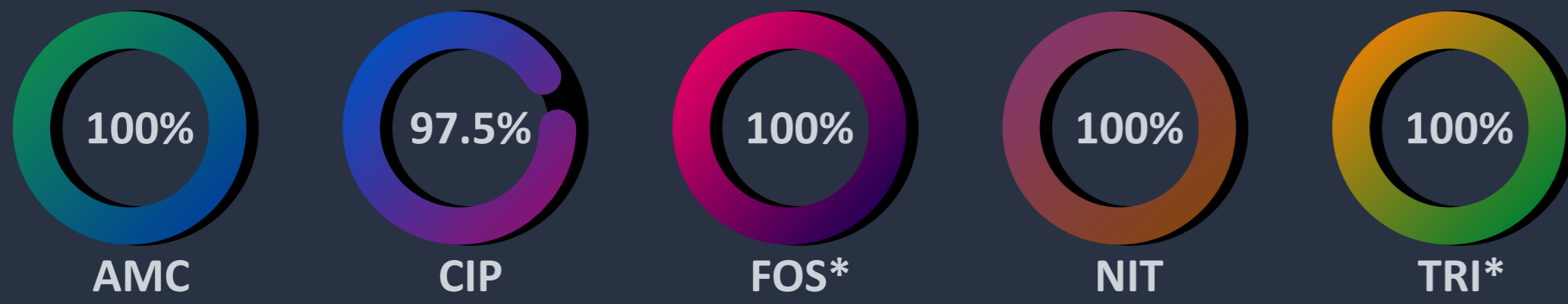
400 μ L of sample is transferred into the cartridge and the automated test is initiated.

Results are delivered within 30 min for Gram negatives, and 45 min for Gram positives.

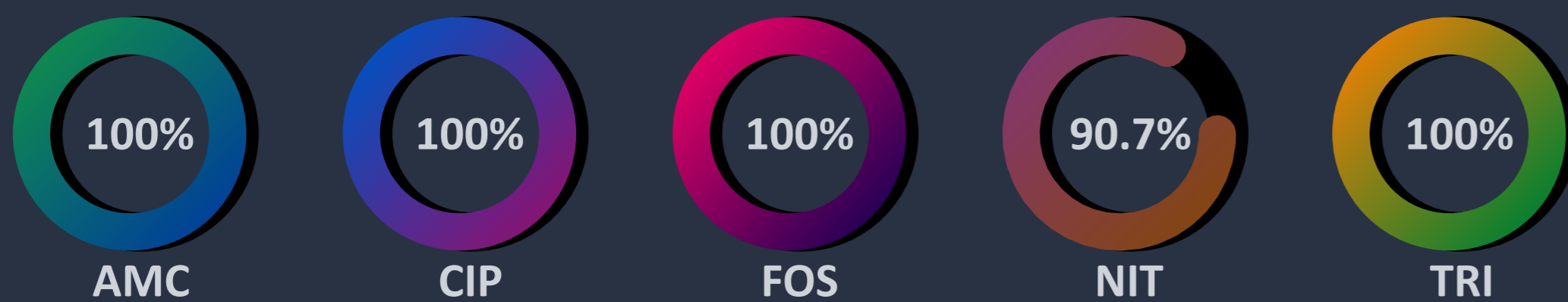
Staphylococcus saprophyticus
N=39 (40)



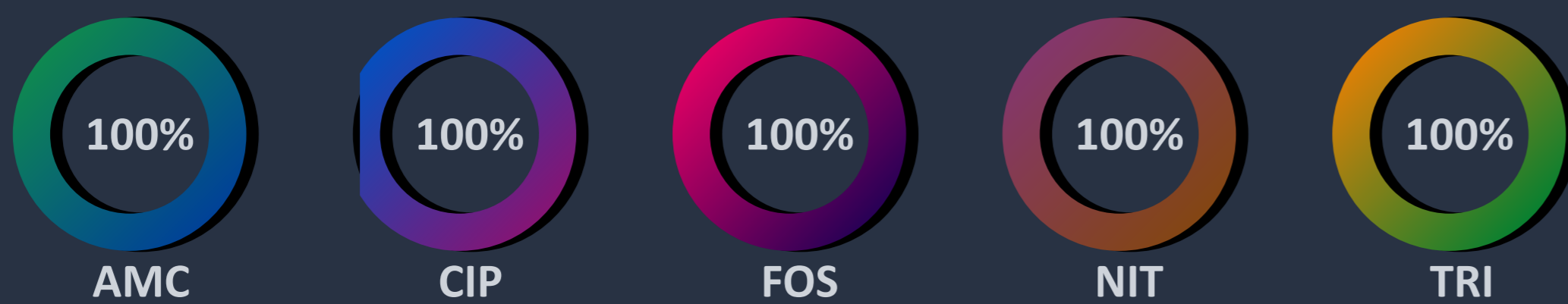
Enterococcus faecalis
N=40 (40)



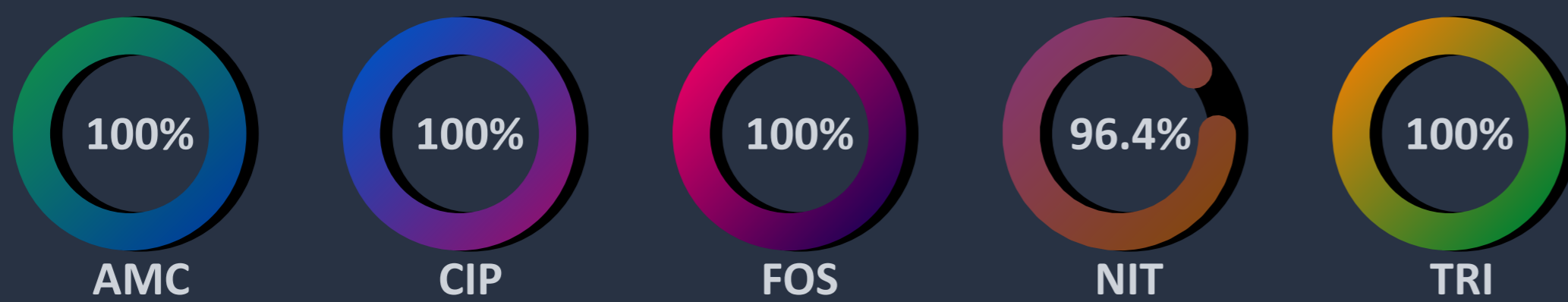
Klebsiella pneumoniae
N=54 (56)



Proteus mirabilis
N=56 (56)



Escherichia coli
N=63 (64)



- Bacteriuria performance: 100% true positive (technical errors are excluded).

- In the occurrence of a technical error, which could compromise the correct AST output, the system suppresses all or individual AST results. In the FQC dataset 4 tests (1.6% of all performed) resulted in technical errors (all AST results suppressed by the system).

* When a species/antibiotic combination that is not suitable for testing is detected, the system returns NA as a result. Under such conditions, NA as a result is considered accurate due to the Astrego system's ability to accurately identify the species and apply correct rules.

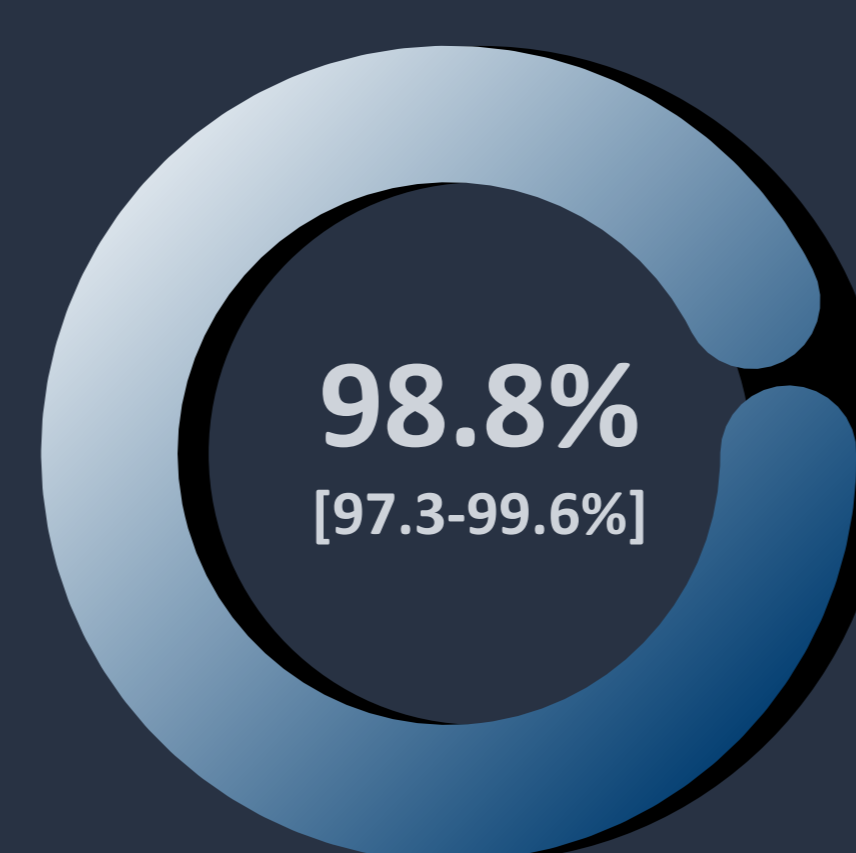
Bacteriuria Detection & AST Accuracy

Susceptible and resistant isolates from the five most common uropathogenic species are tested to evaluate the AST accuracy of the system. In total, 430 unique bacterial isolates are included. The species distribution for the selected bacterial isolates is shown in the table below.

187 negative samples divided in two groups: blank samples (N=54) and samples spiked below 5×10^4 CFU/mL (N=133), are also tested to evaluate the system's specificity.

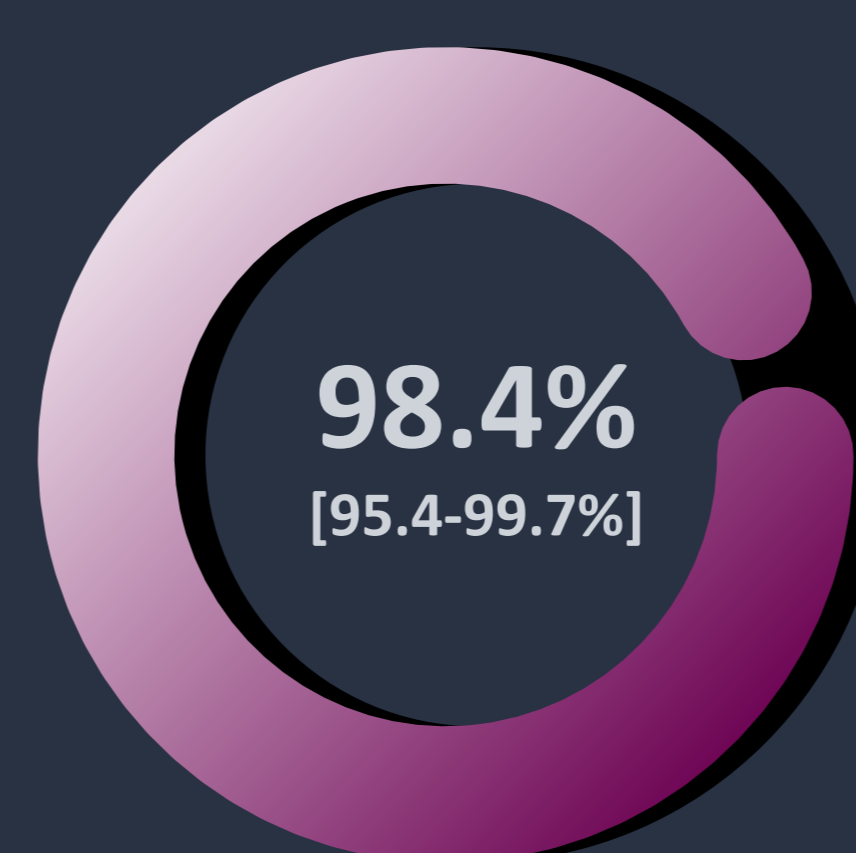
Sensitivity

N=430 positive samples
CI - 95%



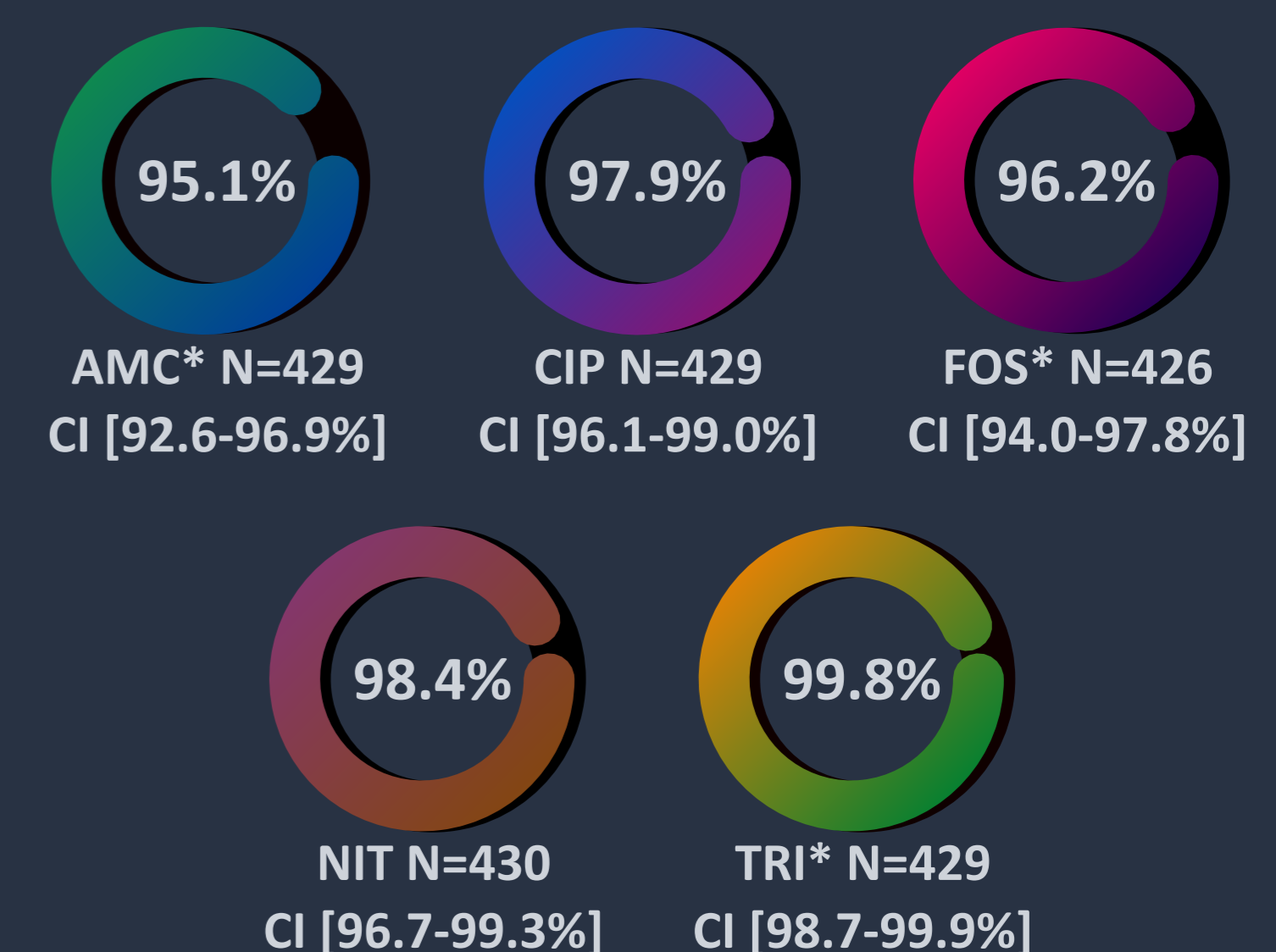
Specificity

N=187 negative samples
CI - 95%



AST Accuracy

CA for N unique isolates with 95% CI



Species	N total
<i>Escherichia coli</i>	313 (73%)
<i>Klebsiella pneumoniae</i>	62 (14%)
<i>Proteus mirabilis</i>	15 (3.5%)
<i>Enterococcus faecalis</i>	21 (4.9%)
<i>Staphylococcus</i>	19 (4.4%)

The absence of actively growing bacteria or the presence of such at concentrations below the cut-off results in NEGATIVE bacteriuria. Note that reported NEGATIVE bacteriuria does not equate to lack of bacteria.

Technical errors, Bacteriuria Detection: 3% all results suppressed; AST Accuracy: 2.5% all tests - all results suppressed, 4.9% true positive - AST result suppressed.

- To maximize the number of AST results in the study, bacterial isolates in assays with false negative result, and non-reported AST, are repeated.

* When a species/antibiotic combination that is not suitable for testing is detected, the system returns NA as a result. Under such conditions, NA as a result is considered accurate due to the Astrego system's ability to accurately identify the species and apply correct rules.

Conclusion

Astrego's AST system provides acceptable AST accuracy (95.1% to 99.8%) and reproducibility (97.5% to 100%) during comparative performance evaluations at 3 different sites. This performance with an unprecedented turnaround time of 30-45 min can revolutionize the antimicrobial therapy by providing accurate guidance to physicians prior to prescription - a radical advance in personalization of antimicrobial therapy.

Acknowledgements

We would like to express our gratitude and appreciation to all Astrego's employees, consultants and external partners, who made it possible to design, produce and evaluate Astrego's ultra rapid *in vitro* diagnostic system. In addition, we thank the clinical microbiology laboratory employees of Karolinska Sjukhuset in Huddinge and Akademiska Sjukhuset in Uppsala, Sweden who invested their valuable time to evaluate our system and provide crucial feedback. Finally, for their contribution and support, a special thanks to Dr. Volkan Özenci (professor and senior consultant in Clinical Microbiology at Karolinska University Hospital, Stockholm) and Dr. Sofia Persson (consultant physician in Clinical Microbiology at Akademiska Sjukhuset, Uppsala University Hospital).