

Ultra-rapid antibiotic susceptibility testing workflow for neonatal sepsis: from blood sample to AST result in 9 hours

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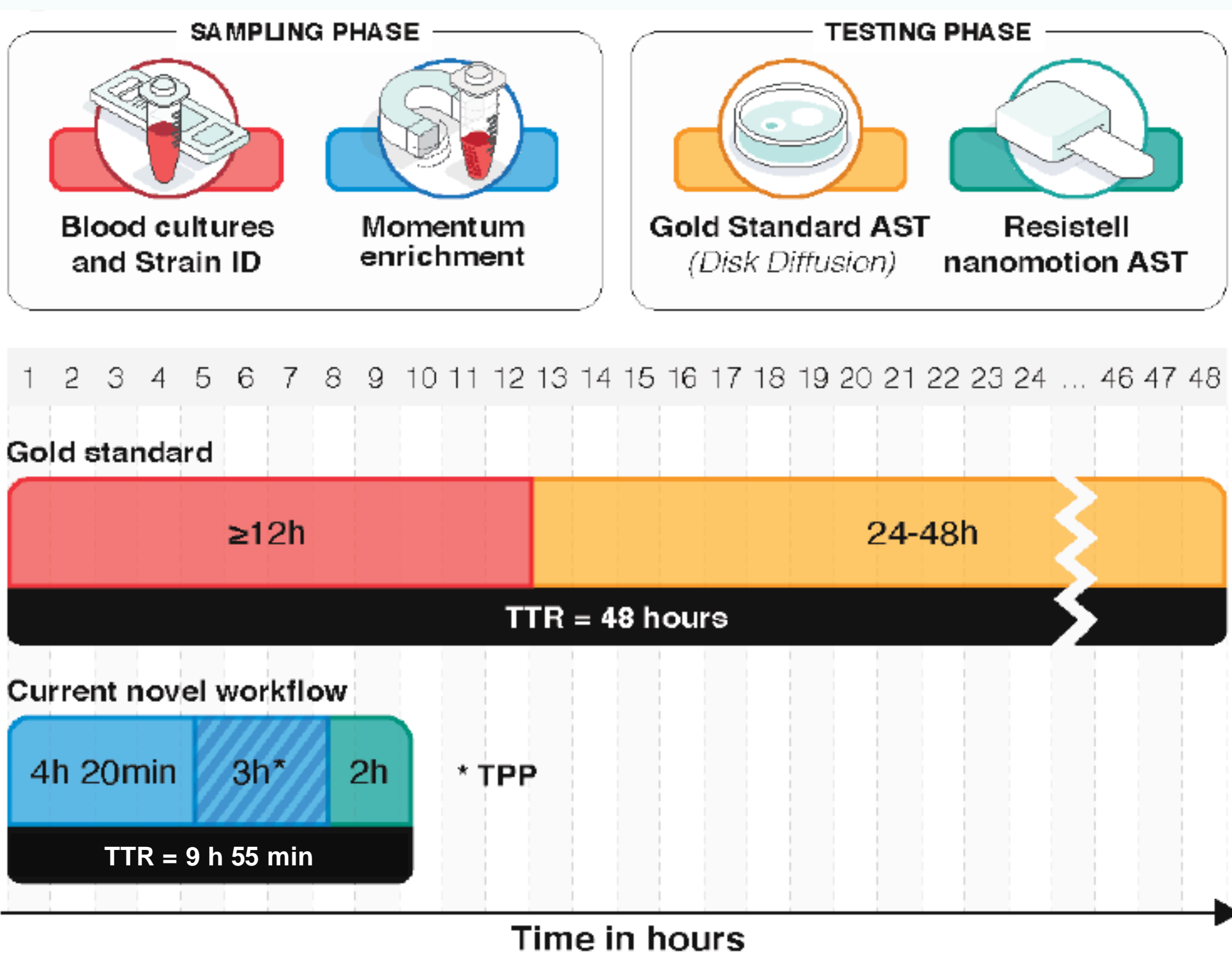
Neonatal sepsis

- Critical cause of morbidity and mortality globally due to bacteraemia.
- Diagnosis is made harder by the **small volume of blood** available for testing (up to 1 mL).
- Rapid detection and accurate identification of pathogens and their antibiotic susceptibility is essential for timely and targeted treatment.

This study

- Evaluates an ultra-rapid diagnostic platform for delivering Antibiotic Susceptibility Test (AST) results within hours using the **Momentum-Resistell platform**.
- SepsisSTAT[®] sample enrichment: Automates direct-from-blood microbial extraction (via magnetic beads) and enrichment, with qPCR-based growth monitoring and Gram identification.
- Phenotech[™] AST: A nanomotion-based phenotypic AST providing growth-independent susceptibility results in just 2 hours.

Time to Result impact of the Momentum-Resistell platform

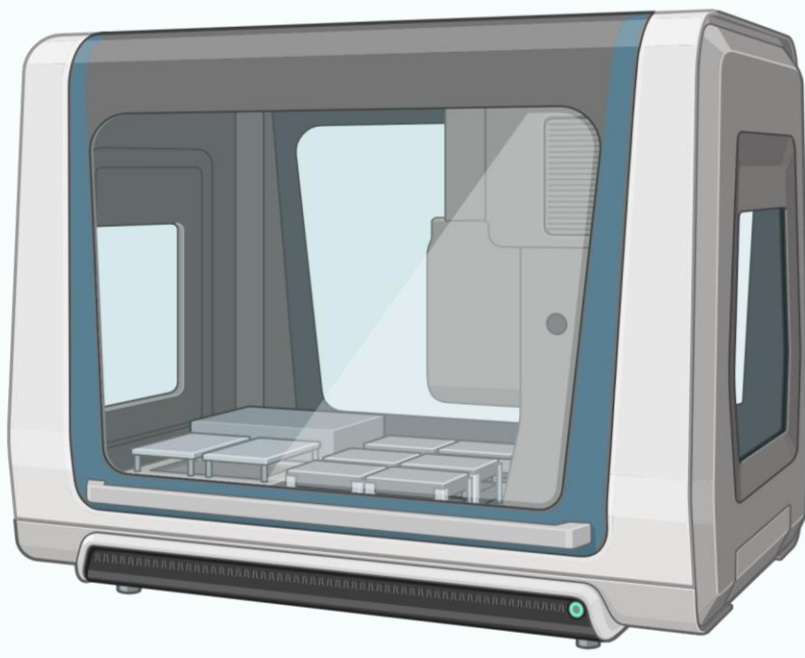


Bacteraemia diagnostic workflow timelines comparison between:

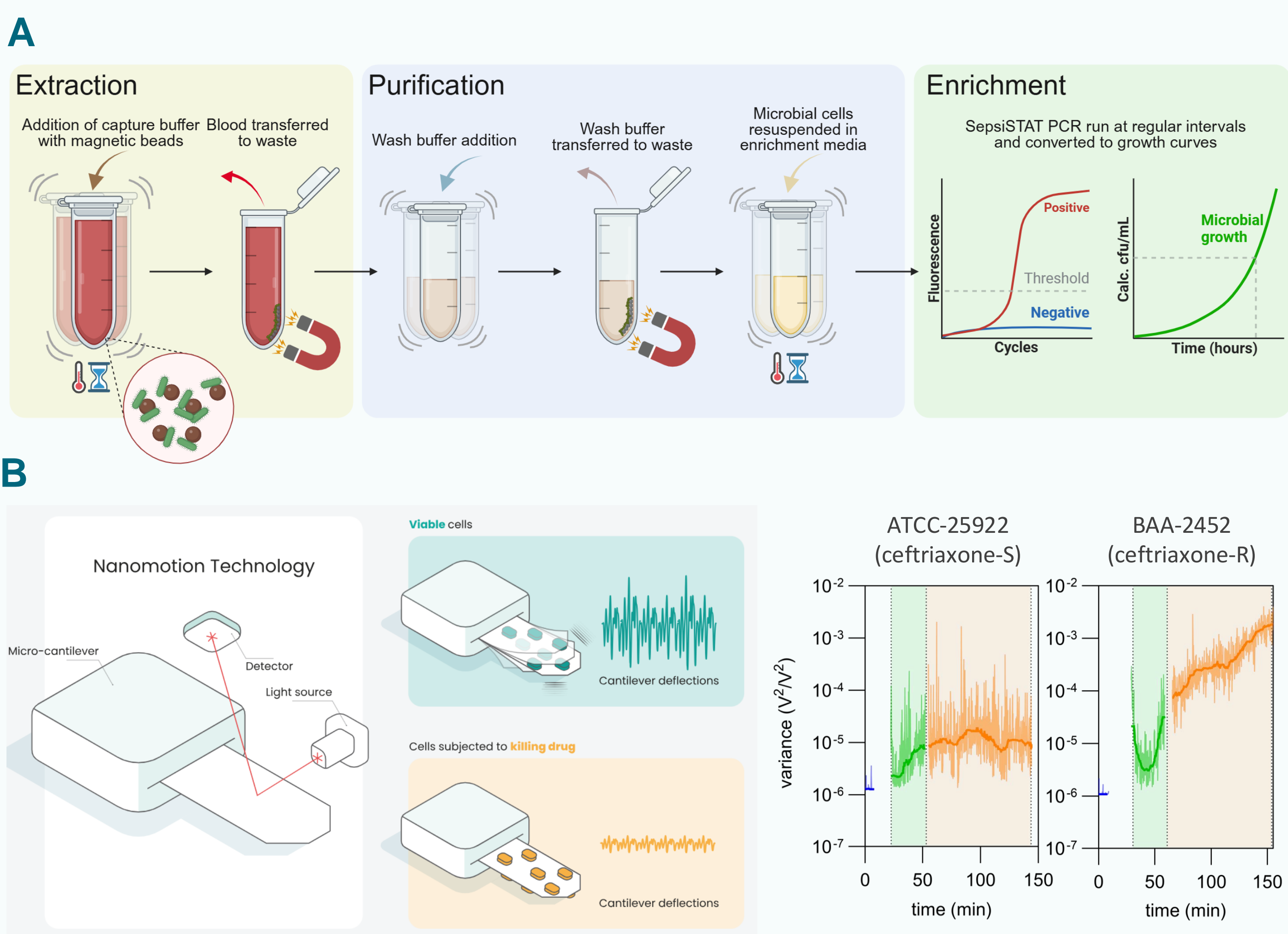
- **Gold Standard process** (blood culture and disk diffusion assay). Often take **≥48 hours** as they rely on blood culture for microbial detection and enrichment prior to antibiotic susceptibility testing (AST)
- This study's **novel Momentum-Resistell workflow** (SepsisSTAT[®] and Phenotech[™] AST, data presented here) **Time to result 9 h 55 min** (SD = 49 min)

Momentum SepsisSTAT + Resistell Phenotech combined workflow - Methods

MOMENTUM
BIOSCIENCE
SepsisSTAT[®]



Resistell
Phenotech[™]

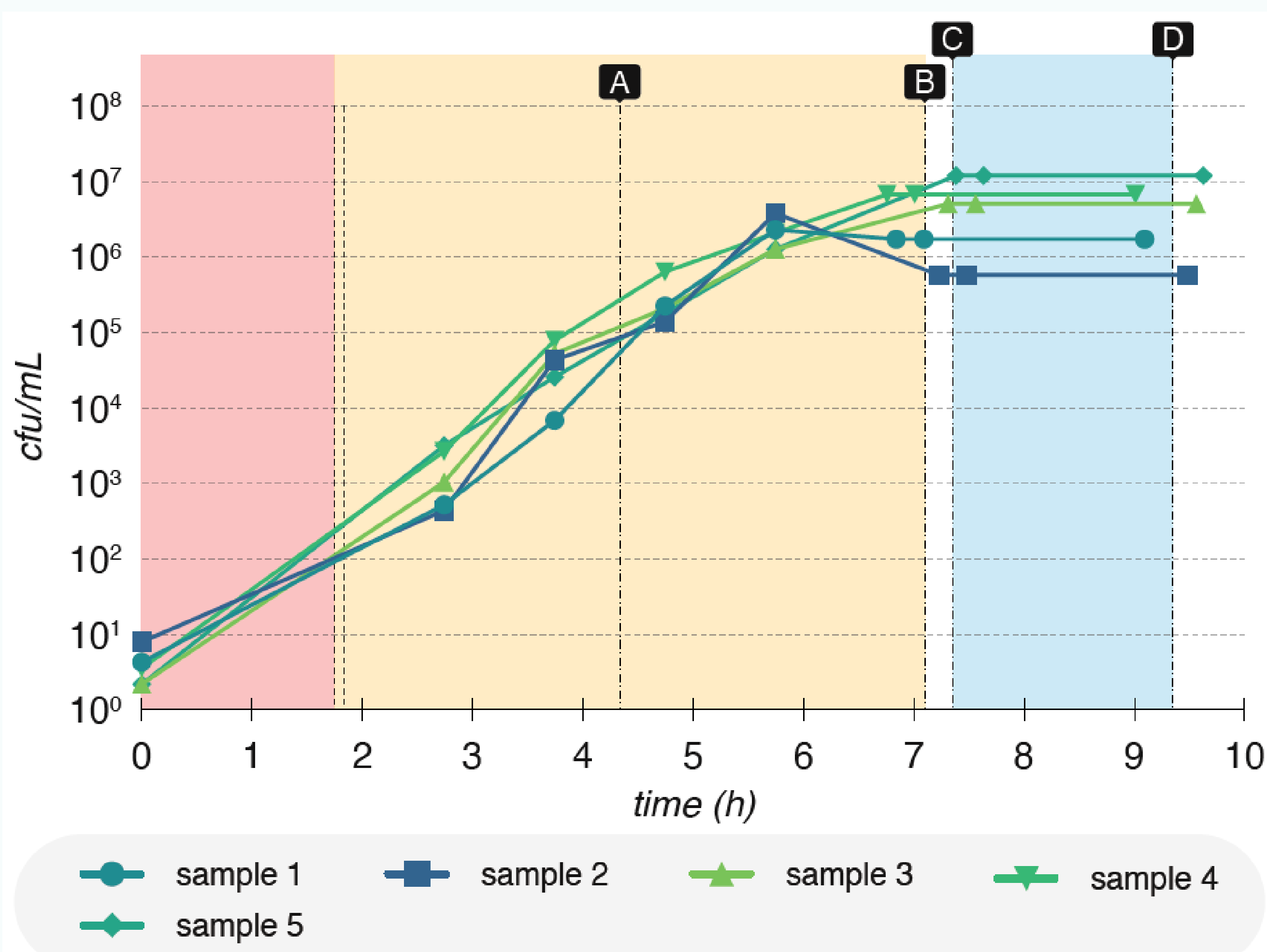


- Samples:**
- 1 mL blood spiked with ≤5 CFU/mL of clinically relevant *Escherichia coli* strains: ATCC-25922 (ceftriaxone-susceptible) and BAA-2452 (ceftriaxone-resistant)
- SepsisSTAT[®] processing (A):**
- Automated bacterial extraction and purification.
- Microbial enrichment monitored hourly via qPCR until reaching 5×10^6 CFU/mL.
 - CFU/mL estimates were derived using mathematical algorithms converting PCR Ct values.
 - Output: Bacterial suspensions at $\sim 5 \times 10^6$ CFU/mL.
- Sample preparation for Resistell Phenotech AST:**
- Samples centrifuged and pellets mixed with agarose for bacterial attachment to the cantilever.
- Nanomotion-based AST (Phenotech AST) (B):**
- Measurement of cantilever oscillations induced by viable bacterial cells.
- **Blank Phase (blue):** Baseline of cantilever vibrations in medium.
 - **Medium Phase (green):** Vibrations of attached bacteria.
 - **Drug Phase (orange):** Vibrations after ceftriaxone (2 µg/mL) exposure.
- Performance evaluation on machine learning classification algorithm:**
- Previously trained on 611 recordings with ~200 Enterobacteriales strains and evaluated by testing 48 blood cultures achieving 98% accuracy (100% sensitivity, 97% specificity).

Momentum SepsisSTAT + Resistell Phenotech combined workflow - Results

Figure 1: Momentum-Resistell combined workflow as CFU/mL over time.

- 5 biological replicates, 1 mL blood samples spiked with *E. coli* ATCC-25922 in different shapes of green and blue.



- **Red area:** Bacterial extraction and purification from spiked sample.
- **Yellow area:** Bacterial enrichment with qPCR CFU/mL hourly monitoring.
- **A:** Sample positivity detection and Gram identification.
- **B:** Enrichment completed for the Resistell AST.
- 10 min sample preparation and bacterial attachment to cantilever (**B** to **C**).
- **Blue area (C to D):** Nanomotion-based AST and sample phenotype classification.

Table 1: Detailed results for both *E. coli* strains tested with ceftriaxone

	Figure index	ATCC-25922 susceptible MIC = 0.047 µg/mL	BAA-2452 resistant MIC = 48 µg/mL
Biological replicates (N)	-	5	3
Inoculum (CFU/mL)	-	4.1 (SD = 2.4)	4.7 (SD = 1.2)
Time to positivity (SepsisSTAT)	A	4 h 20 min (SD = 0 min)	5 h 47 min (SD = 37.53 min)
Time to reach the targeted 5 x 10 ⁵ CFU/mL (estimated by SepsisSTAT)	B	7 h 06 min (SD = 16.92 min)	8 h 23 min (SD = 41.56 min)
Bacterial suspension for attachment to cantilever (CFU/mL)	C	5.3x10 ⁶ (SD = 4.6x10 ⁶)	3.1x10 ⁷ (SD = 1.2x10 ⁷)
Phenotech AST	C - D	2 h 5 min	2 h 5 min
Phenotech AST performance	-	100%	100%
Time to result (Momentum-Resistell platform)	D	9 h 26 min (SD = 16.92 min)	10 h 43 min (SD = 41.56 min)

Conclusions

- **Momentum SepsisSTAT[®]** delivers rapid bacterial detection (time to positivity) and Gram identification in 4 h 52 min (SD = 49 min).
- **Resistell Phenotech[™] AST** delivers AST results in 2 hours 5 min with 100% accuracy.
- This Momentum-Resistell platform combined diagnostic workflow for neonatal sepsis and time to accurate AST results in 9 h 55 min (SD = 49 min) significantly reducing the standard workflow of > 48 hours.