Single-cell phenotypic antimicrobial susceptibility testing of neonatal positive blood cultures in under two hours: a prospective, multicentre diagnostic accuracy study

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January 2026

Abstract

Background: Antimicrobial susceptibility testing (AST) for neonatal bloodstream infection takes about two days after a blood culture flags positive, and most rapid systems test Gram-negative organisms only. We assessed the accuracy and speed of Halo AI, a single-cell imaging AST system, used directly on positive neonatal blood cultures. **Methods:** In this prospective study at six NHS microbiology laboratories in England, we tested residual positive blood cultures from neonatal units between 15 May and 30 November 2025 with a laboratory prototype of Halo AI, against ISO 20776-1 broth microdilution interpreted with EUCAST breakpoints. **Findings:** We screened 1409 cultures, of which 1252 were eligible and 1214 were analysed (4842 organism–antibiotic pairs; 2908 Gram-positive, 1934 Gram-negative). Categorical agreement was 96.8% (95% CI 96.3–97.3). The very major error rate was 0.7% (8/1116; 95% CI 0.4–1.4) and the major error rate was 1.6% (60/3726; 1.3–2.1). Agreement was 96.8% for Gram-positive and 96.7% for Gram-negative pairs. Median time to result was 88 min (IQR 63–104) from cartridge loading, and 93.4% of cultures were reported within 2 h. Halo AI gave an invalid result for 11 of 1252 eligible cultures (0.9%). **Interpretation:** Single-cell imaging AST met accepted

accuracy thresholds for both Gram-positive and Gram-negative neonatal pathogens and reported a median of 88 min after loading, against 46.2 h after culture positivity for routine methods.

1 Introduction

Neonatal sepsis affects an estimated 3 million newborn babies each year and causes up to 570 000 deaths [1]. Antimicrobial resistance accounts for a growing share of these deaths. In many low-income and middle-income countries Gram-negative organisms are now the predominant cause, and they are often resistant to the empirical regimens that WHO recommends [2]. In a global cohort of infants with sepsis, 18% of those whose blood culture grew a pathogen died [3].

In England, NICE recommends benzylpenicillin with gentamicin for suspected early-onset infection and a narrow-spectrum combination such as flucloxacillin with gentamicin for late-onset infection, with any escalation guided by culture results [4]. Following that guidance requires an AST result. Conventional AST needs an overnight subculture and then an overnight test, so the result usually arrives about two days after the culture flags positive [5]. At the same time, antibiotic-resistant bloodstream infections in England continue to rise [6].

Rapid AST systems have shortened the wait for adults with Gram-negative bacteraemia [7, 5]. Neonatal sepsis, however, is frequently caused by Gram-positive organisms: group B streptococcus in early-onset disease and staphylococci in late-onset disease. Single-cell imaging follows individual bacteria and can show their response to an antibiotic within minutes, before growth of the population as a whole can be measured [8]. A point-of-care system based on this approach is already in use for urinary tract infection [9]. Halo AI uses the same approach, with a deep-learning model trained to track cells of differing morphology, including the chains and clusters formed by Gram-positive cocci.

We aimed to estimate the diagnostic accuracy and time to result of Halo AI, used directly on positive neonatal blood cultures in routine NHS laboratories, with broth microdilution as the reference standard.

2 Methods

2.1 Study design and setting

We did a prospective, multicentre diagnostic accuracy study, which we report in line with STARD 2015 [10]. Six NHS microbiology laboratories in England took part (sites A–F), each serving a level 2 or level 3 neonatal unit. A research ethics committee approved the study (REC reference 25/SC/0142). We used only residual material from cultures taken for clinical care, and Halo AI results were not reported to clinicians, so individual consent was not required.

2.2 Samples

We screened consecutive blood culture bottles from infants aged 0–90 days that flagged positive between 15 May and 30 November 2025. A culture was eligible if the Gram stain showed bacteria, if it was loaded onto Halo AI within 8 h of flagging positive, and if the organism identified by the site's routine method was within the validated scope of the system (coagulase-negative staphylococci, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp.). Cultures later found to be polymicrobial were excluded from the primary analysis.

2.3 Index test

Halo AI (Halo AI Ltd, Oxford, UK) is a single-cell phase-contrast imaging system. For this study it was run as a laboratory prototype, with one prototype at each site. Each consisted of a motorised inverted phase-contrast microscope with a heated stage and a camera, connected to a GPU workstation running the Halo AI software. The integrated benchtop instrument had not been built when the study was done.

The operator loads 50 μL of positive broth into a single-use cartridge, which dilutes the sample, removes most blood cells and distributes the bacteria into approximately 500 microchannels. The channels are arranged in nine sets. Eight sets contain a dried antibiotic at the EUCAST breakpoint concentration (benzylpenicillin, amoxicillin, flucloxacillin, gentamicin, cefotaxime, vancomycin, meropenem and amikacin) and the ninth is an antibiotic-free growth control. The prototype images the channels every 2 min at 37 °C. A convolutional neural network segments and tracks individual cells, and a second model classifies the response in each channel as

continued division or arrest. Once the organism identification has been entered, Halo AI reports each organism–antibiotic combination that has an EUCAST breakpoint as susceptible, susceptible increased exposure, or resistant. Operators did not know the reference results. The model version (2.3.1) was locked before enrolment began.

2.4 Reference standard

Isolates were subcultured and tested centrally by broth microdilution according to ISO 20776-1 [11], by staff who did not know the Halo AI results. Minimum inhibitory concentrations were interpreted with the EUCAST clinical breakpoint tables that were current when the study started.

2.5 Outcomes

The primary outcomes were categorical agreement (CA) and the rates of very major error (VME), major error (ME) and minor error (mE). A very major error was a result that was resistant by the reference method and susceptible by Halo AI, and the rate is given as a proportion of resistant reference results. A major error was a result that was susceptible by the reference method and resistant by Halo AI, given as a proportion of susceptible reference results. A minor error was any discordance involving the susceptible increased exposure category. We used acceptance thresholds of CA $\geq$ 90%, VME $\leq$ 1.5% and ME $\leq$ 3% [12]. Secondary outcomes were time to result from cartridge loading, time from culture positivity to a Halo AI result compared with routine AST, and the rate of invalid results.

2.6 Statistical analysis

Proportions are given with Wilson score 95% confidence intervals [13]. We set a target of 1200 cultures (about 4800 organism–antibiotic pairs), which would allow an overall CA of 95% to be estimated with a 95% CI half-width of less than 0.7 percentage points. Analyses by organism group were prespecified. Discordant results were repeated in duplicate by both methods, and the repeat reference result was taken as final. We used R version 4.4 for all analyses.

3 Results

We screened 1409 positive cultures, of which 157 were ineligible and 1252 were tested. A further 38 were excluded after testing (21 were polymicrobial, 11 had an invalid Halo AI result and 6 had a failed reference test), which left 1214 cultures and 4842 organism–antibiotic pairs for analysis (Figure 1). Of the cultures analysed, 342 (28.2%) came from early-onset episodes and 872 (71.8%) from late-onset episodes (Table 1).

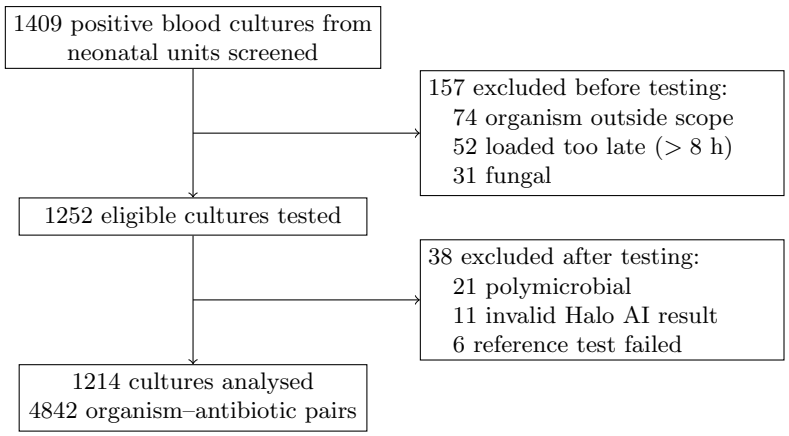


Figure 1: Study flow diagram (STARD).

Table 1: Organisms and onset of infection in analysed cultures.

Organism	Cultures	Early onset	Late onset	Pairs
Coagulase-negative staph. (CoNS)	412	18	394	1648
Staphylococcus aureus	168	22	146	672
S. agalactiae (GBS)	196	161	35	588
Escherichia coli	254	128	126	1270
Klebsiella spp.	112	9	103	448
Enterobacter spp.	72	4	68	216
Total	1214	342	872	4842

Overall CA was 96.8% (4687/4842; 95% CI 96.3–97.3). There were 8 very major errors (0.7% of 1116 resistant results; 0.4–1.4), 60 major errors (1.6% of 3726 susceptible results; 1.3–2.1) and 87 minor errors (1.8%; 1.5–2.2). Results were similar for Gram-positive pairs (CA 96.8%; VME 0.7%, 6/873; ME 1.6%, 33/2035) and Gram-negative pairs (CA 96.7%; VME 0.8%, 2/243; ME 1.6%, 27/1691). CA was above 95% for every organism (Table 2). Few

Klebsiella spp. and *Enterobacter* spp. isolates were resistant, so the VME estimates for these organisms are imprecise.

Table 2: Agreement with reference broth microdilution, by organism. CA, categorical agreement; VME, very major error; ME, major error; mE, minor error; CoNS, coagulase-negative staphylococci; GBS, group B streptococcus. No GBS isolate was resistant to any tested antibiotic.

Organism	Pairs	CA % (95% CI)	VME n/N (%)	ME n/N (%)	mE n (%)
CoNS	1648	96.2 (95.2–97.1)	4/721 (0.6)	22/927 (2.4)	36 (2.2)
S. aureus	672	97.3 (95.8–98.3)	2/152 (1.3)	7/520 (1.3)	9 (1.3)
GBS	588	98.0 (96.5–98.8)	0/0 (–)	4/588 (0.7)	8 (1.4)
E. coli	1270	97.3 (96.3–98.1)	2/200 (1.0)	14/1070 (1.3)	18 (1.4)
Klebsiella spp.	448	95.8 (93.5–97.3)	0/35 (0.0)	8/413 (1.9)	11 (2.5)
Enterobacter spp.	216	95.4 (91.7–97.5)	0/8 (0.0)	5/208 (2.4)	5 (2.3)
All	4842	96.8 (96.3–97.3)	8/1116 (0.7)	60/3726 (1.6)	87 (1.8)

Median time to result from cartridge loading was 88 min (IQR 63–104), and 1134 of 1214 cultures (93.4%) were reported within 2 h (Table 3). Gram-negative organisms were read fastest (median 52 min for *E. coli*) and coagulase-negative staphylococci slowest (median 102 min). The fastest result was reported 38 min after loading, for an *E. coli* culture. Sites identified the organism directly from the positive bottle while the cartridge was running, by MALDI-TOF mass spectrometry at four sites (median 35 min) and by a multiplex PCR panel at two (median 65 min). Routine AST at the same sites took a median of 46.2 h (IQR 40.1–52.8) from culture positivity.

Table 3: Time to result from cartridge loading.

Organism	Median, min (IQR)	Reported within 2 h (%)
CoNS	102 (89–118)	83.3
S. aureus	74 (65–86)	98.2
GBS	86 (76–97)	95.9
E. coli	52 (46–61)	100.0
Klebsiella spp.	58 (51–67)	100.0
Enterobacter spp.	63 (55–72)	100.0
All	88 (63–104)	93.4

4 Discussion

In 1214 positive neonatal blood cultures from six NHS laboratories, Halo AI agreed with reference broth microdilution for 96.8% of organism–antibiotic

pairs. The very major and major error rates were within accepted thresholds [12], and the median time to result was 88 min. Gram-positive organisms cause most neonatal bloodstream infections in high-income settings and are not tested by most rapid systems [7, 5]. In our study, accuracy for these organisms was as good as for Gram-negative organisms.

Halo AI is fast because it reads single cells. A system that measures growth in bulk needs several doublings before a susceptible population can be told apart from a resistant one, whereas arrest or continued division of individual cells can be seen within one or two generations [8]. Results for coagulase-negative staphylococci were slower than for the other organisms. We attribute this to their longer doubling time and to the tight clusters they form, in which individual cells are harder to follow.

Our study has several limitations. First, we tested residual samples and did not report results to clinicians, so we cannot yet show an effect on time to effective therapy or on outcomes. Second, coagulase-negative staphylococci are frequent contaminants, and some of the cultures we included will not have represented true infection. This does not affect analytical accuracy, but it limits clinical interpretation. Third, few *Klebsiella* and *Enterobacter* isolates were resistant, so the VME estimates for these organisms are imprecise, and no GBS isolate was resistant. Fourth, all sites were in England, where resistance is less common than in many of the settings where mortality from neonatal sepsis is highest [2, 3]. Fifth, the system was tested as a laboratory prototype, operated alongside routine work. The integrated benchtop instrument has yet to be built, and its performance will need to be confirmed against these results. Finally, several of the authors are involved in developing the system.

5 Conclusion

Single-cell imaging AST performed directly on positive neonatal blood cultures was accurate and gave results within 2 h of loading, where routine methods took a median of 46.2 h from culture positivity. The next steps are a randomised evaluation of its effect on time to effective therapy, and studies in settings where resistance is common.

Declarations

Contributors. EGR conceived the system, developed the model and drafted the manuscript. DKM ran the reference testing. PR coordinated

the laboratory sites. AF provided clinical input. AO supervised the study. All authors approved the final manuscript.

Declaration of interests. EGR is the founder and a shareholder of Halo AI. AO is an unpaid scientific adviser to Halo AI. The other authors declare no competing interests.

Funding. University of Oxford Impact Accelerator Award; Neonatal Infection Research Trust.

Data sharing. De-identified organism-level data are available from the corresponding author on reasonable request.

Acknowledgments. We thank the biomedical scientists at sites A–F, who ran the study alongside their routine work.

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