

Assessing the limit of detection of *Candida auris* screening methods using direct-to-agar, broth-enriched-culture, direct-polymerase chain reaction, and broth-enriched-polymerase chain reaction

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INTRODUCTION

Candida auris is an emerging multidrug resistant yeast that causes severe invasive infections and nosocomial outbreaks¹, with mortality rates ranging from 30-72%². First isolated in 2009, individual cases or outbreaks have since been reported in over 20 countries across 5 continents¹. During the COVID-19 pandemic, *C. auris* outbreaks have been reported in healthcare facilities around the globe^{3,4,5,6}, including nosocomial outbreaks of pan-resistant *C. auris* resistant to all three classes of antifungal medications in the United States⁷. In Canada, 26 cases of *C. auris* have been reported as of January 2021⁸, with the first reported outbreak in a community healthcare facility in the spring of 2018⁹.

C. auris is considered to be a notable threat to global health because: a) multidrug resistance is common, limiting treatment options, b) its ability to colonize on skin and persist on surfaces allows it to spread between patients in healthcare settings, and c) it can be misidentified by commonly available biochemical methods and automated testing instruments¹, thereby delaying the implementation of infection prevention protocols and treatments. As such, a sensitive, timely, and reliable method of identifying *C. auris* is needed for its management worldwide.

The Centers for Disease Control and Prevention (CDC) offers several strategies for *C. auris* screening, including direct plating onto chromogenic or salt/dulcitol agar, using salt/dulcitol enrichment broth, and polymerase-chain reaction (PCR)⁹. However, the benefits of each method from a limit-of-detection perspective are currently unclear. Our group recently assessed different screening methods in terms of their ability to detect *C. auris* and their ease of implementation (see AMMI Canada – CACMID 2022 Poster P100) This current study aims to determine the limit of detection (LOD) differences of the optimal culture, broth, and PCR-based methods identified in our earlier study.

METHODS

- 11 *C. auris* isolates, representative of the East Asian, South Asian, and South American clades, were tested following the method described herein and summarized in Figure 1.

For each *C.auris* isolate:

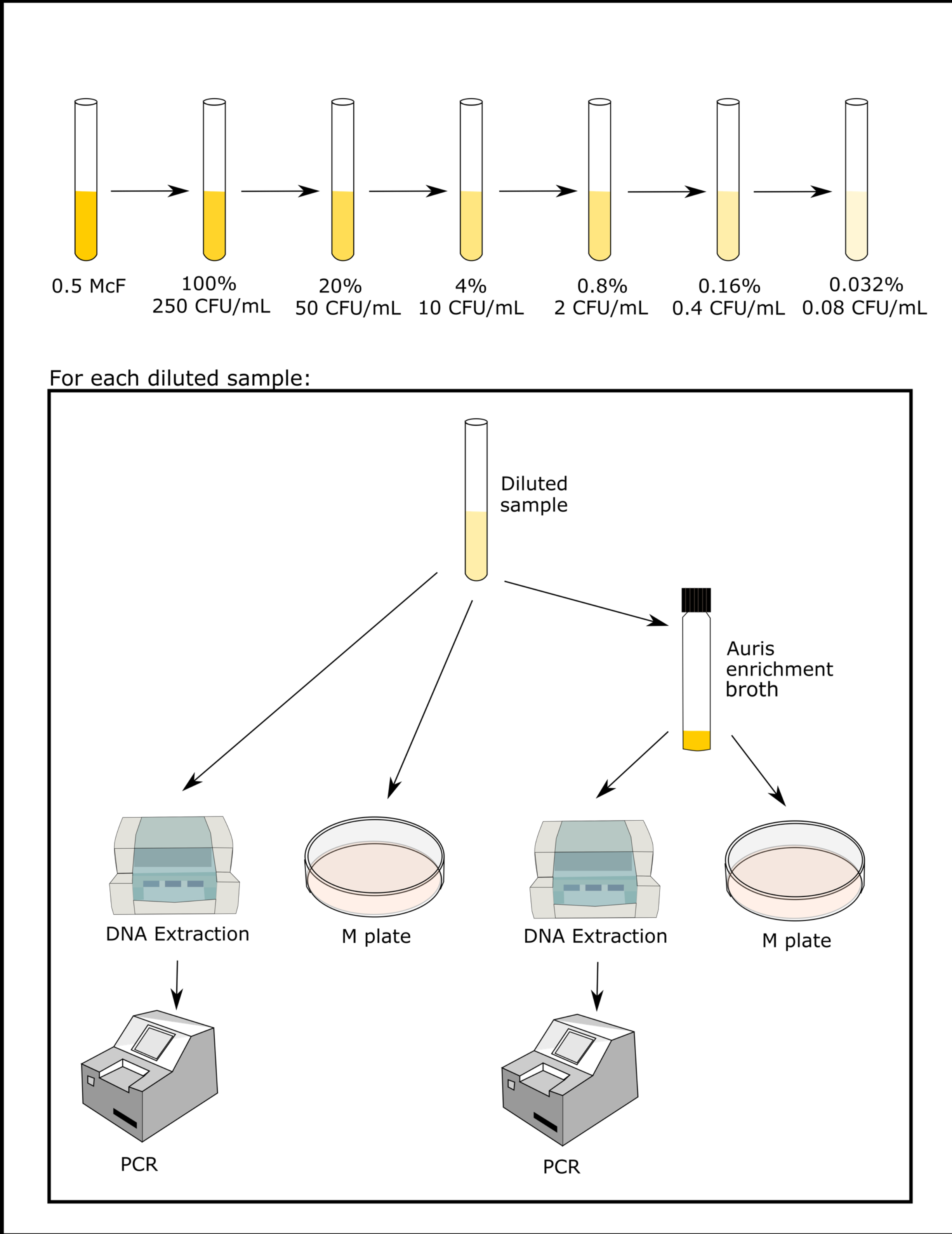


Figure 1: 5-fold dilutions of mock specimens, and study methods flow chart

METHODS (continued)

- A 0.5 McFarland in saline was prepared from each isolate, and inoculated into fresh residual nasal-axillary-groin-perineum-rectal swabs (Copan ESwab™ with liquid Amies), with a final concentration of 250 CFU/mL.
- Six 5-fold dilutions (from 250 CFU/mL to 0.08 CFU/mL) of each spiked sample were prepared.
- Each diluted sample was:
 - Directly inoculated onto Colorex Candida Plus (Micronostyx, Ottawa) (M) agar and directly tested for *C. auris* using PCR
 - First inoculated into Auris Enrichment Broth (Thermo Fisher Scientific, Massachusetts) (AEB) and incubated for 48 hrs, before inoculating onto M and before testing for *C. auris* using the BioGX *C. auris* research-use-only PCR using EasyMag (bioMérieux) extraction.
- The limit of detection was calculated using a Probit analysis (https://biostats.shinyapps.io/LOD_probit/) and the Analyse-it Method Validation Edition (Analyse-it Software, Ltd., UK), both at a probability of 95%.
- The range for absolute LOD values was assessed using back-calculated initial sample concentrations and a review of 0.5 McFarland concentrations in the literature.

RESULTS

- The calculated LODs are shown in Table 1 and Figure 1.
- AEB-enriched methods were the most sensitive methods
 - Culture-based, AEB-enriched methods were on average nearly 200 times more sensitive than culture-based, direct-to-agar methods but the halo effect from AEB-enriched cultures (see AMMI Canada – CACMID 2022 Poster P100) has similar sensitivity with culture-based, direct-to-agar methods.
 - PCR from AEB-enriched specimens was on average ~ 100 times more sensitive than direct PCR from specimen.
 - Notably culture-based AEB-enriched methods had the same sensitivity as PCR from AEB-enriched specimens.
- Direct PCR from specimen was the second most sensitive method
 - Direct PCR from specimen was nearly 2 times more sensitive than direct-to-agar methods.
- Culture-based, direct to agar methods had the lowest sensitivity

- Examples of the varying LOD of each testing procedure is show in Figure 2 using a representative spiked specimen sample and a summary of all data taking into account LOD, cost, turn-around-time, breakthrough of other species, and workload is shown in Table 2.

Detection Method	Probit			Analyse-it	Average Limit of Detection (CFU/mL)
	Limit of Detection (CFU/mL) (p=95)	Lower CI	Upper CI	Limit of Detection (CFU/mL) (p=95)	
Culture-Based, Direct-to-Agar	537	62	4677	431	484
Culture-Based, AEB-Enriched	3	1	10	2	3
Halo Effect	530	49	5728	420	475
PCR, Direct-from-Specimen	308	71	1337	241	275
PCR, AEB-Enriched	3	1	8	2	3

*Confidence intervals were not provided by Analyse-it

Table 1: Limit of detection values for each screening method, calculated using the Probit analysis tool and Analyse-it

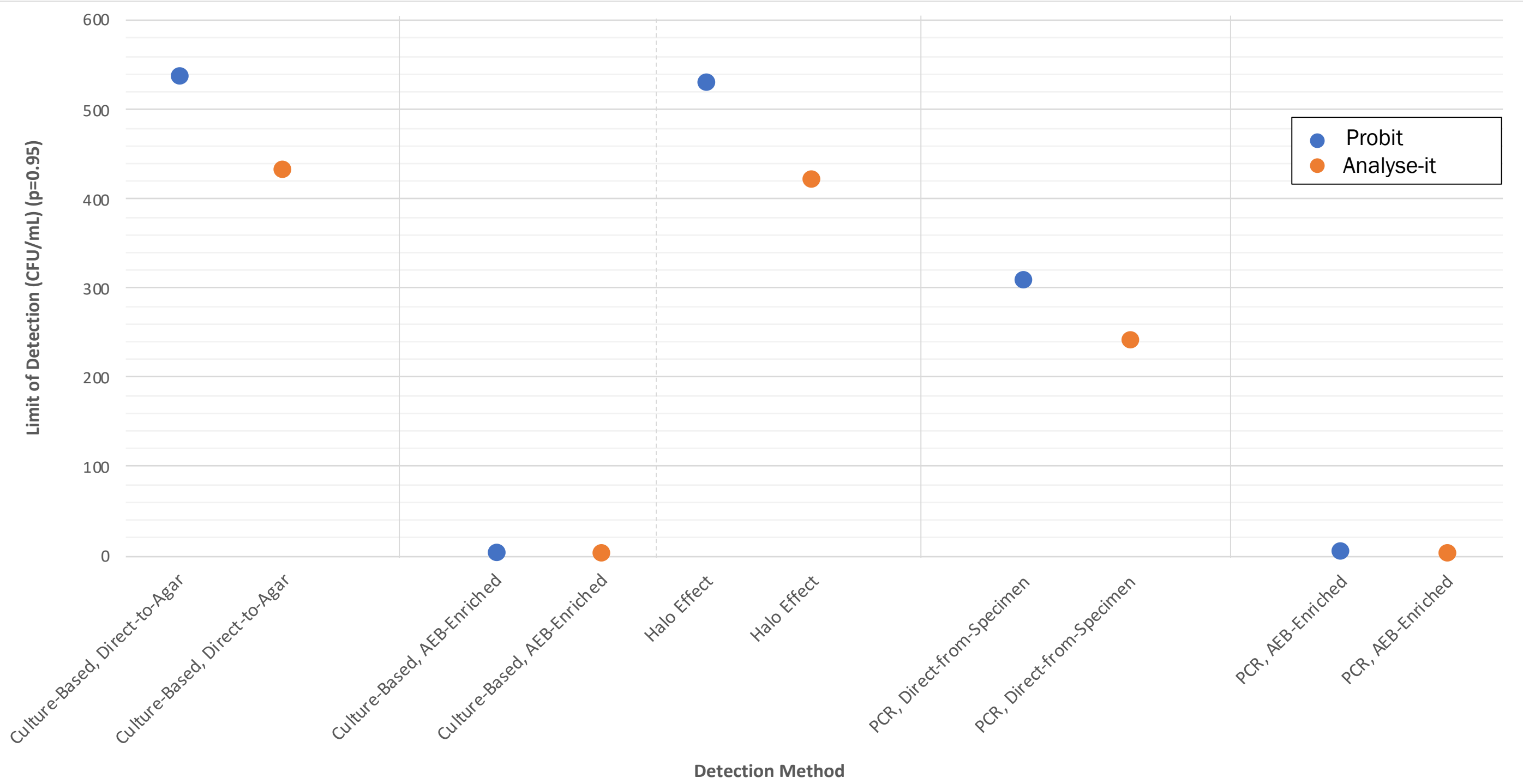


Figure 2: Limit of detection values for each screening method, calculated using the Probit analysis tool and Analyse-it

RESULTS (continued)

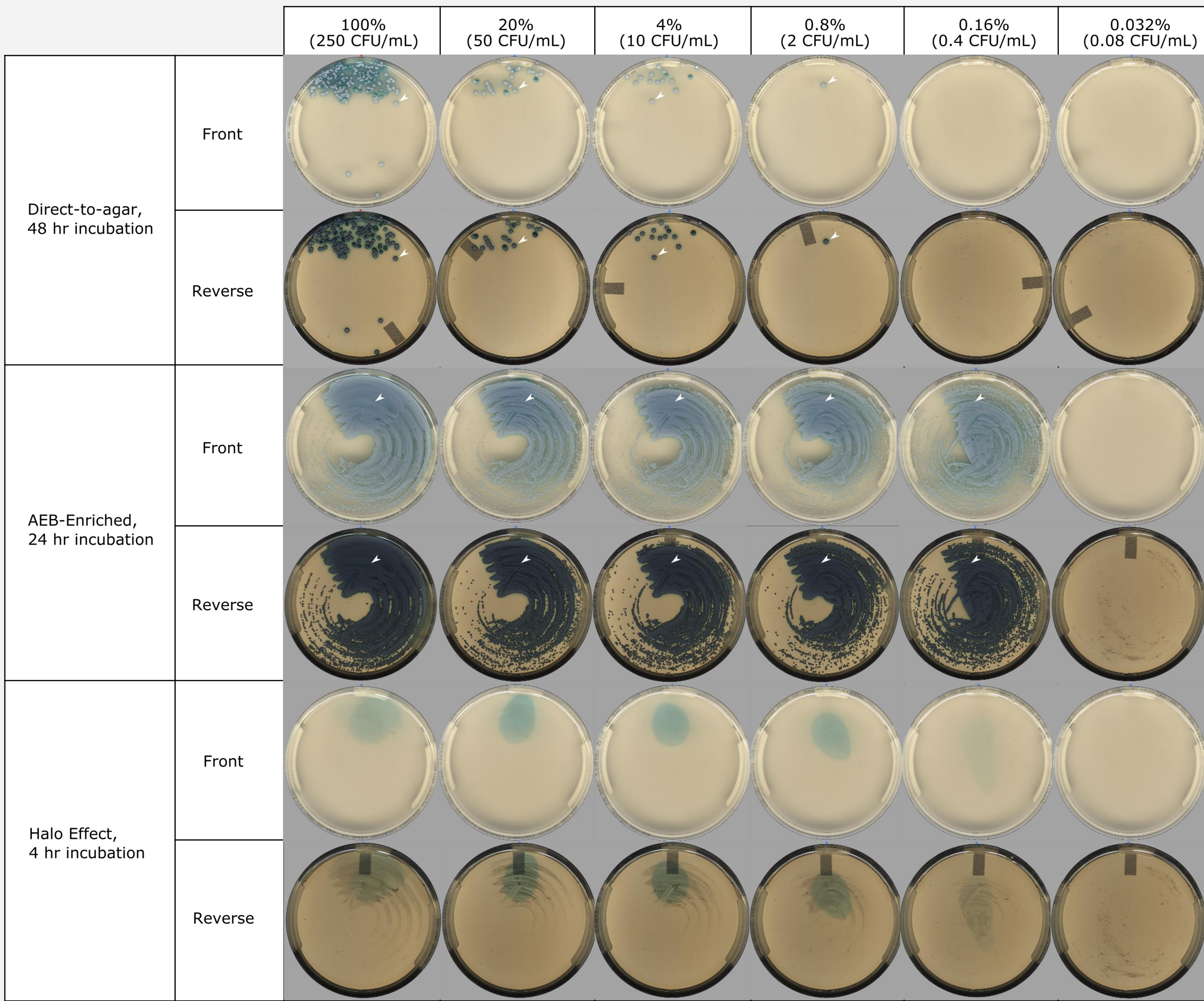


Figure 2: Growth of a spiked specimen sample at each dilution, for direct-to-agar procedure after 48-hr plate incubation at 37°C, and for AEB-enriched procedure after 4-hr (halo effect) and 24-hr plate incubation at 37°C. Front and reverse plate images shown. White arrows indicate *C. auris* growth. Images taken using Walk Away Specimen Processor (WASP)

C. auris screening procedure	LOD determined with Probit (CFU/mL)	LOD determined with Analyse-it (CFU/mL)	Cost/screen	Turnaround time	Breakthrough of other species	Workload in clinical lab
Culture-based, Direct-to-agar	537	431	\$	≤ 48 hrs	++	+
Culture-based, AEB-enriched	3	2	\$\$	≈ 72 hrs	+/-	++
Halo Effect	530	420	\$\$	≈ 52 hrs	+/-	++
Direct PCR from specimen	308	241	\$\$\$\$\$\$	≤ 24 hrs	N/A	+++
PCR from AEB-enriched specimen	3	2	\$\$\$\$\$\$	≈ 24-48 hrs	N/A	++++

Table 2: Summary table comparing *C. auris* screening methods

CONCLUSIONS

- AEB-enrichment of specimens increased the sensitivities of both culture-based and PCR screening procedures compared to direct-from-specimen procedures.
- Culture-based, direct-to-agar screening methods are the most cost-effective and easily implemented into the clinical lab workflow, but allow for greater breakthrough of other species.
- Culture-based, AEB-enriched screening methods are more sensitive, but require slightly more time, a higher cost, and extra broth-enrichment steps in the workflow.
- PCR screening methods have the shortest turnaround time, but highest cost workload without significantly improved sensitivity compared to culture-based methods.

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