Implementation of a software for automatic reading of agar dilution plates at a *Neisseria gonorrhoeae* antimicrobial resistance surveillance laboratory

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BACKGROUND

The increasing prevalence of gonorrhea infections is one of the most challenging public health problems worldwide. *Neisseria gonorrhoeae* (GC), the bacterial pathogen responsible for this sexually transmitted disease, has developed resistance mechanisms to different antibiotics classes over time. The emergence of strains resistant to all currently recommended treatments has also been documented[1]. In the United States, GC antibiotic resistance (AR) surveillance is overseen by the CDC through several programs: the Gonococcal Isolate Surveillance Project (GISP), the "Enhanced GISP" (eGISP) and the "Strengthening the U.S. Response to Resistant Gonorrhea" (SURRG)[2]. All these AR surveillance programs entail phenotypic testing via agar dilution (AD), the gold standard method for determining minimal inhibitory concentrations (MIC) values in GC[3]. AD is a very laborious procedure, involving manual evaluation of growth on agar plates and data entry into the laboratory information system (LIS) (Fig. 1). Here we describe the evaluation of the BIOMIC V3 imaging system (Giles Scientific Inc) and its AD-specific software module with the goal of improving the efficiency of the AD procedure[4].

METHODS

AD for GC is routinely performed in our lab by processing batches of up to 32 isolates against 7 different antibiotics (ciprofloxacin, penicillin, cefixime, ceftriaxone, tetracycline, azithromycin, gentamicin) at 9-16 varying concentrations generated by completing 1:2 serial dilutions (Fig. 2). 0.5 McFarland suspensions of individual isolates in Mueller Hinton broth are then stamped onto agar plates (GC medium base containing IsoVitaleX supplement) containing different antibiotic concentrations using a Steer`s replicator. Three quality control strains (F18, WHO L, WHO U) are tested alongside the isolates. The inoculated plates are incubated at 36°C with 5% CO2 overnight. For this study 30 isolates including WHO strains and isolates previously tested through GISP, eGISP and SURRG were evaluated. MIC values from 83 antibiotic plates read both manually (Fig. 3 and Fig. 4) and with the BIOMIC V3 (Fig. 5 and Fig. 6) were entered into the LIS (Fig. 4 and Fig. 7) and processing time relative to these workflows was measured.

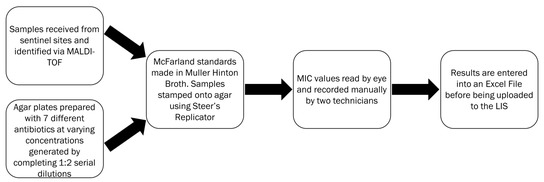


Figure 1. Current workflow. Schematic of the current procedure for testing of *Neisseria gonorrhoeae* for antibiotic resistance surveillance through the GISP, eGISP, and SURRG programs using manual reading.

Agar Dilution Testing

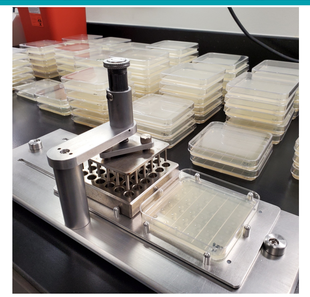


Figure 2. Isolate testing via agar dilution. Up to 32 samples in Muller Hinton Broth may be stamped onto 6x6 square agar plates using the Steers Replicator. Plates are incubated for 20-24hrs at 36°C with 5% CO_2.

Agar Dilution Manual Reading

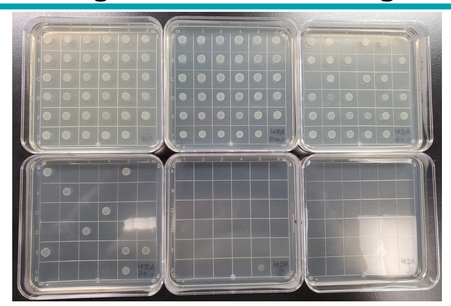


Figure 3. Manual reading. Representation of plates undergoing manual reading to determine the MIC of isolates to the antibiotics tested. This process is conducted by two technologists and takes approximately 2 hours.

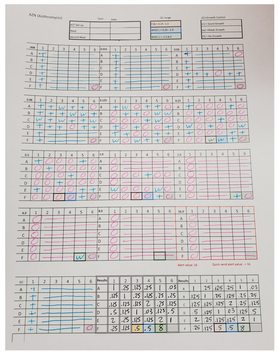


Figure 4. Manual recording of MICs. Growth of isolates are documented during visual inspection of plates. MICs are recorded as the first dilution with no visible growth. A second technician reviews the interpretations and recorded MICs and enters the results into an Excel file before uploading results into the LIS.

Agar Dilution Automated Reading and Data Entry

Figure 5. BIOMIC V3 automated agar dilution plate reader. Plates are placed in the imaging drawer with a custom 6x6 square plate holder and read with the BIOMIC V3 software. Image from Giles Scientific Inc.

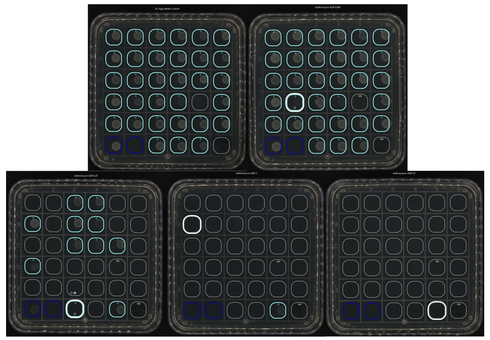
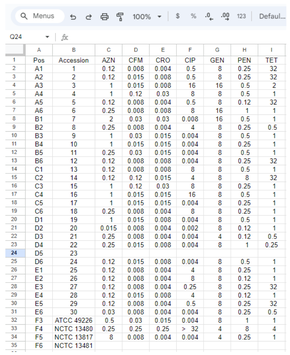


Figure 6. Automated reading. Images taken by the BIOMIC V3 system during automated reading of agar dilution plates. The color bordering an isolate indicate growth (cyan), no growth (grey), indeterminate growth (yellow), or isolates not being evaluated (blue).



	A	B	C	D	E	F	G	H	I
1	Pos	Accession	AZN	CFM	CRO	CIP	GEN	PEN	TET
2	A1	1	0.12	0.008	0.004	0.5	8	0.25	32
3	A2	2	0.12	0.015	0.008	0.5	8	0.25	32
4	A3	3	1	0.015	0.008	16	16	0.5	2
5	A4	4	1	0.12	0.03	8	8	0.5	1
6	A5	5	0.12	0.008	0.004	0.5	8	0.12	32
7	A6	6	0.25	0.008	0.008	8	16	1	1
8	B1	7	2	0.03	0.03	0.008	16	0.5	1
9	B2	8	0.25	0.008	0.004	4	8	0.25	0.12
10	B3	9	1	0.03	0.015	0.004	8	0.5	1
11	B4	10	1	0.015	0.015	0.004	8	0.25	1
12	B5	11	0.25	0.03	0.015	0.004	8	0.5	1
13	B6	12	0.12	0.008	0.008	0.004	8	0.25	32
14	C1	13	0.12	0.008	0.008	8	16	0.5	1
15	C2	14	0.12	0.12	0.015	4	8	8	32
16	C3	15	1	0.12	0.03	8	8	0.25	32
17	C4	16	1	0.015	0.015	16	8	0.5	1
18	C5	17	1	0.015	0.015	0.004	8	0.25	1
19	C6	18	0.25	0.008	0.004	8	8	0.25	1
20	D1	19	1	0.015	0.008	0.004	8	0.5	1
21	D2	20	0.015	0.008	0.004	0.002	8	0.12	1
22	D3	21	0.25	0.008	0.004	0.004	4	0.12	0.5
23	D4	22	0.25	0.015	0.008	0.004	8	1	0.25
24	D5	23							
25	D6	24	0.12	0.015	0.008	0.004	8	0.5	1
26	E1	25	0.12	0.008	0.004	4	8	0.25	1
27	E2	26	0.12	0.008	0.004	8	8	0.12	1
28	E3	27	0.12	0.008	0.004	0.25	8	0.25	32
29	E4	28	0.12	0.015	0.008	4	8	0.12	1
30	E5	29	0.12	0.008	0.004	0.5	8	0.25	32
31	E6	30	0.03	0.008	0.004	0.004	8	0.25	0.5
32	F3	ATCC 49226	0.5	0.03	0.015	0.004	8	1	1
33	F4	NCTC 13400	0.25	0.25	0.25	> 32	4	8	4
34	F5	NCTC 13817	8	0.008	0.004	0.004	4	0.25	1
35	F6	NCTC 13481							

Figure 7. Automated Data Export. The BIOMIC V3 system records the MIC results of the automated reading. When all the plates have been read the software generates a report that can be exported to an Excel file, as shown in this image. This file can then be used to upload results into the LIS.

RESULTS

- Automated reading greatly reduced the time dedicated to reading agar dilution plates
  - Manual reading takes 2hrs for two technologists
  - Automated reading without manual review takes 25 minutes and only one technologist
- Automated reading without manual review had 85.2-96.3% accuracy, when compared to manual reading.
- Automated reading with manual review had 99.9% accuracy, when compared to manual reading. It took 1hr to complete and required only one technologist.
- Manual reading required manual recording of data, manual entry into Excel and upload into LIS (Fig. 4)
- Automated reading allows export of MIC data to Excel for upload into LIS (Fig. 7)

LIMITATIONS

- Automatic image density scanning is less sensitive than the human eye for the detection of growth (Fig. 8A)
- AD-specific software was not able to generate an MIC in about 10-20% of the isolates
- Occasionally the software interprets bubbles or starch flakes in the agar as growth (Fig. 8B). This specificity issue occurred while scanning 4% of the total plates imaged
- Performance limitations were bypassed by including a manual review of the automatically generated MICs. This review took under 30 minutes to complete and increased accuracy to 99.9%

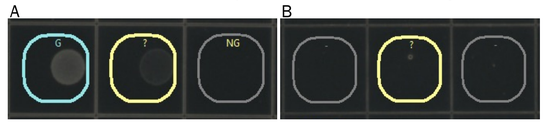


Figure 8. Limitations of software. The software is less sensitive than the human eye and requires manual review for A. Visible growth marked as indeterminate. B. A bubble in the well marked as indeterminate growth.

CONCLUSIONS

Automated reading for AD testing coupled with manual review displays an acceptable performance. Adoption of this method improves efficiency in the lab by reducing bench time, number of staff needed, ergonomic stress. Increased data accuracy in the post analytical phase (less transcription errors during data import in LIMS) is an additional potential benefit. However, this has not been measured in this study.

- Future directions: We will be working toward further validating this method and incorporating it into the GISP, eGISP, and SURRG testing activities.
- Summary: This pilot project has led to the development of an AD-specific BIOMIC software and of a workflow that can improve efficiency in labs performing AD.

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Sources

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