Implementation of A Digital Imaging System for Reading and Interpretation of Broth Microdilution Antimicrobial Susceptibility Testing

J. Bodnar, E. Nazarian, C. Jacobsen, K. Wahl, K. Musser, M-C. Rowlinson
Wadsworth Center, New York State Department of Health, Albany, NY

Project Overview

Wadsworth Center (WC) evaluated three digital imaging systems for Antimicrobial Susceptibility Testing (AST) of gram-negative organisms by broth microdilution:

- Sensititre™ ARIS™ 2x (ARIS) – an automated plate reading system
- Sensititre™ Vizion™ System (VIZ)- a manual digital minimal inhibitory concentration (MIC) viewing system
- BIOMIC V3 System (BMV3)- a digital imaging system with automated plate-reading and CLSI guidelines for interpretation.

- All three systems were used to evaluate the Sensititre™ Gram Negative GN7F AST plate with:
  - 6 required quality control (QC) organisms
  - 60 organisms from CDC AR Isolate Bank (40 Enterobacterales, 10 *Pseudomonas aeruginosa*, and 10 *Acinetobacter baumannii*)

- Accuracy of results was determined by comparing each system's MICs with CDC AR Bank MICs. Essential and categorical agreement as well as functionality and ease of use were also considered.

GN7F Panel Layout

Figure 1:

THERMO SCIENTIFIC™ SENSITITRE™ GRAM NEGATIVE PLATE FORMAT

Plate Code: GN7F Plate Type: MIC

	1	2	3	4	5	6	7	8	9	10	11	12
A	AMI 8	TGC 1	FEP 2	DOR 0.5	ETP 0.25	IMI 1	MERO 0.5	FAZ 1	TAZ 1	AZT 1	LEVO 0.5	AXO 0.5
B	AMI 16	TGC 2	FEP 4	DOR 1	ETP 0.5	IMI 2	MERO 1	FAZ 2	TAZ 2	AZT 2	LEVO 1	AXO 1
C	AMI 32	TGC 4	FEP 8	DOR 2	ETP 1	IMI 4	MERO 2	FAZ 4	TAZ 4	AZT 4	LEVO 2	AXO 2
D	P/T4 8/4	TGC 8	FEP 16	DOR 4	ETP 2	IMI 8	MERO 4	FAZ 8	TAZ 8	AZT 8	LEVO 4	AXO 4
E	P/T4 16/4	C/T 2/4	CIP 0.25	MIN 1	ETP 4	CZA 2/4	MERO 8	FAZ 16	TAZ 16	AZT 16	LEVO 8	AXO 8
F	P/T4 32/4	C/T 4/4	CIP 0.5	MIN 2	ETP 8	CZA 4/4	GEN 2	TOB 2	A/S2 4/2	AMP 8	TET 4	AXO 16
G	P/T4 64/4	C/T 8/4	CIP 1	MIN 4	NIT 32	CZA 8/4	GEN 4	TOB 4	A/S2 8/4	AMP 16	TET 8	AXO 32
H	SXT 2/38	C/T 16/4	CIP 2	MIN 8	NIT 64	CZA 16/4	GEN 8	TOB 8	A/S2 16/8	POS	POS	POS

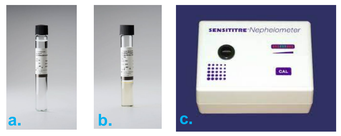
ANTIMICROBICS

AMI	Amikacin
AMP	Ampicillin
A/S2	Ampicillin / sulbactam 2:1 ratio
AZT	Aztreonam
FAZ	Cefazolin
FEP	Cefepime
TAZ	Ceftazidime
CZA	Ceftazidime/avibactam
C/T	Ceftolozane/tazobactam 4
AXO	Ceftriaxone
CIP	Ciprofloxacin
DOR	Doripenem
ETP	Ertapenem
GEN	Gentamicin
IMI	Imipenem
LEVO	Levofloxacin
MERO	Meropenem
MIN	Minocycline
NIT	Nitrofurantoin
P/T4	Piperacillin / tazobactam constant 4
TET	Tetracycline
TGC	Tgecycline
TOB	Tobramycin
SXT	Trimethoprim / sulfamethoxazole
POS	Positive Control

Sensititre™ ARIS™ 2x

Figure 2: Complete Sensititre™ system includes ARIS™ 2x, Vizion™, AIM™ (Automated Inoculation Delivery System), Optiread™ (Automated Fluorometric Plate Reading System), and computer with SWIN™ software.

Left to right:
a. Demineralized water
b. Mueller Hinton Broth with TES
c. Nephelometer

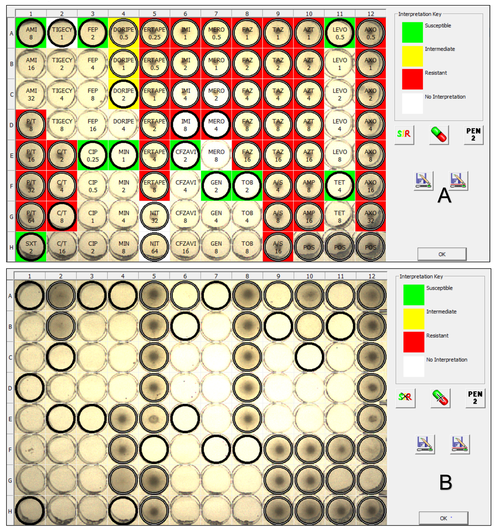


Sensititre™ ARIS™ 2x (ThermoFisher Scientific)

Advantages	• A closed system with onboard, temperature-controlled, timed incubation of Sensititre AST plates. • AST plates are automatically read at set times (18 or 24 hours) and results are automatically interpreted and held for review in the SWIN™ software. • System indirectly connects to Laboratory Information Management System (LIMS).
Limitations	• Connection to LIMS requires the use of an intermediate program. • System reads Sensititre plates only. • AutoRead feature occasionally misreads skipped wells and sporadically fails to detect visible growth leading to erroneous results.

Sensititre™ Vizion™ System

Figure 3: Image A. *Klebsiella aerogenes* growth on GN7F plate image with overlays (antibiotic names and interpretations).
Image B. *Pseudomonas aeruginosa* growth on GN7F plate image without Overlays.

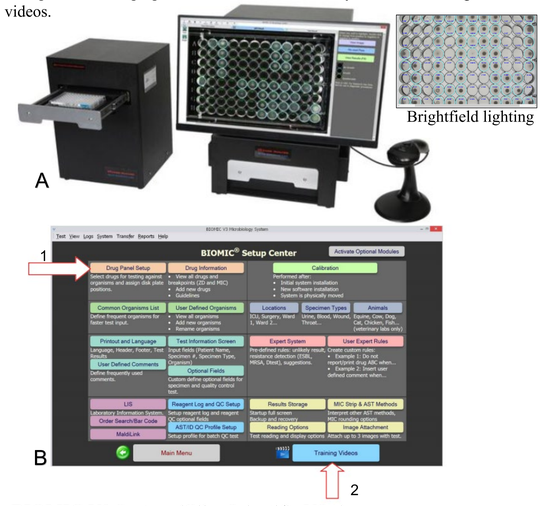


Sensititre™ Vizion™ System (ThermoFisher Scientific)

Advantages	• A digital MIC viewing system for reading Sensititre AST plates with automatic interpretation of results. • Brightfield and darkfield backgrounds with adjustable brightness allow for optimal reading. • Live and still Vizion images allow for plate to be removed during reading and the plate image can be observed simultaneously with a mirrored plate reader. • Plates are held for review in the SWIN™ software and can be used to compare auto and manual reads with different report options. • Plate images can be saved for manual review of skipped wells, trailing endpoints, and contamination. Interpretations, MICs and antibiotic names can be overlaid on the saved image (see Figure 4, Image A).
Limitations	• More hands-on time with a manual read.

BIOMIC V3 System

Figure 4: Image A. BIOMIC system with darkfield lighting on screen and brightfield option. Top-down camera included with system. Bottom-up camera reader separate.
Image B. Screenshot of system set up page. Arrow 1 indicates custom AST panels can be prepared and Arrow 2 shows easy access to training videos.



BIOMIC V3 System (Giles Scientific USA)

Advantages	• Open system using digital imaging to automatically read and interpret plates. • Brightfield and darkfield lighting options with fixed brightness allows for optimal reading of AST plates. • Manual review of autoread MICs and interpretations are available to verify results before accepting. Results are interpreted using current CLSI guidelines. • System directly or indirectly connects with the LIMS. • System can be used with a variety of AST plates and manufacturers including custom plates. Other test modules available such as disk diffusion, organism ID and colony counts.
Limitations	• May overcall wells at edge of plate due to camera angle. Top-down (mCIM) and bottom-up cameras (BMD) available for testing.

Results of System Comparison

Overall performance of the three AST plate reading systems:

- Reproducibility between users and comparison to expected results for all systems were acceptable.
- QC strains performed as expected for all systems. Very few random errors were observed across 9 days of testing. Any errors that were seen were corrected upon a single repeat.
- MICs for each system were compared to the expected result (CDC AR Bank). Essential Agreement (EA) and Categorical Agreement (CA) for the ARIS 2x was ≥90 except AZT (CA=89%).
- Total number of errors decreased or improved when the ARIS 2x was confirmed using the VIZ or the BMV3.
- Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* isolates performed as expected on all systems although some errors were observed.

*Note - possible deterioration of a glycerol stock was noted with one isolate due to lack of expected resistance with the carbapenems.

Notable errors:

- ARIS 2x (AutoRead) had ≥4 errors (minor and major) with Aztreonam (AZT), Cefepime (FEP), Doripenem (DOR), Ertapenem (ETP), Meropenem (MEM), Piperacillin/tazobactam (P/T) and Tetracycline (TET).
- Proteae tribe performed as expected for the ARIS 2x except *Proteus mirabilis.* Three of five isolates resulted in ≥5 errors (minor and major) on the ARIS 2x.
- DOR resulted in a very major error with *Proteus mirabilis*. The expected result was MIC=4 (R). The ARIS 2x result was >4 (R). The VIZ read the MIC=1 (S). Upon repeat, the VIZ MIC = 2 (I). The result was corrected to a minor error for the VIZ.
- The EA for all drugs on the VIZ was ≥94% and the CA ≥92%.
- MEM and TET resulted in ≥5 errors (minor and major) with all three systems. FEP resulted in 7 errors with the ARIS 2x, 4 errors with the Vizion (VIZ) (Manual Read) and 3 errors total with the BIOMIC.
- The total EA for all drugs for the BMV3 was ≥93% and the CA ≥92%. The overall performance of the BMV3 was comparable to the VIZ.

Table 1.

Drug	Agreement					
	% Essential Agreement			% Categorical Agreement		
	ARIS	VIZ	BMV3	ARIS	VIZ	BMV3
Amikacin	99%	96%	99%	100%	98%	98%
Ampicillin	94%	100%	100%	96%	98%	98%
Ampicillin/Sulbactam	98%	100%	100%	95%	97%	96%
Aztreonam	97%	97%	97%	89%	92%	94%
Cefazolin	100%	100%	100%	94%	92%	94%
Cefepime	90%	96%	93%	92%	94%	96%
Ceftazidime	100%	97%	100%	100%	96%	99%
Ceftazidime/Avibactam	98%	100%	100%	100%	100%	98%
Ceftolozane/Tazobactam	98%	100%	100%	98%	99%	99%
Ceftriaxone	100%	98%	98%	98%	100%	99%
Ciprofloxacin	100%	100%	100%	99%	94%	98%
Doripenem	94%	96%	97%	93%	93%	96%
Ertapenem	93%	98%	98%	94%	99%	99%
Gentamicin	100%	99%	99%	95%	95%	96%
Imipenem	100%	100%	100%	99%	99%	99%
Levofloxacin	100%	97%	97%	98%	95%	99%
Meropenem	94%	94%	100%	92%	92%	96%
Minocycline	100%	100%	95%	94%	94%	92%
Nitrofurantoin	100%	100%	100%	92%	94%	94%
Piperacillin/Tazobactam	94%	96%	96%	96%	97%	97%
Tetracycline	93%	100%	100%	90%	96%	95%
Tigecycline	97%	100%	100%	95%	97%	97%
Tobramycin	100%	100%	100%	94%	99%	98%
Trimethoprim/Sulfamethoxazole	98%	98%	98%	99%	98%	97%
All Drugs (>=)	90%	94%	93%	89%	92%	92%

Conclusion

The Sensititre™ ARIS™ 2x (ARIS), the Sensititre™ Vizion™ System (VIZ), and the BIOMIC V3 System (BMV3) all resulted in acceptable verifications of the GN7F panel using QC and CDC AR bank isolates.

Although the ARIS had the least hands-on time, it resulted in the greatest number of errors. The VIZ generated the least number of errors and enhanced the ARIS results by eliminating errors or correcting them to a less significant error by manual reading. Skipped wells, difficult to read endpoints, and contaminated wells can be resolved using a manual reading system. The SWIN™ software allowed the manual and auto-read results to appear side by side for reading. Plate images, reports and QC results can also be stored using this software.

The BMV3 performed similarly to the VIZ. However, a comparison of the VIZ and BMV3 cameras, gave a slight advantage to the VIZ due to its' adjustable brightness used for difficult to read MICs. Although there was more hands-on time with the VIZ it was easier to use and produced better quality images. An advantage of the BMV3 is its ability to connect directly to LIMS and to read and import other tests such as the Modified Carbapenem Inaction Method (mCIM) and custom AST plates for Expanded AST testing for aztreonam/avibactam. Additional modules are optional on the BMV3 creating a very diverse testing menu.

Based on these findings, WC will implement the GN7F panel testing with reading on the VIZ or BMV3 systems as part of the Antimicrobial Resistance Laboratory Network testing algorithm. This will enhance the laboratory's capability to detect emerging resistance, pan-resistance, and susceptibility patterns in an accurate and efficient manner.

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