**Evaluation of Bio-Rad® discs for antimicrobial susceptibility test by disc diffusion and the ADAGIO™ system for the automatic reading and interpretation of results**

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**Abstract**

Disc diffusion test method is still used worldwide for antimicrobial susceptibility testing. In this study, the performance of both, Bio-Rad® antibiotic discs (as compared to OXOID® discs) and the ADAGIO™ automated system for the reading of disc diffusion test results were evaluated with ATCC QC and wild strains of bacteria. Inhibition zones of both disc brands were read manually and using ADAGIO™. Category interpretation of results for each strain and antibiotic combination was summarized for each strain/antibiotic combination according to Clinical Laboratory Standards Institute MS-100 (2017 update) for manual and ADAGIOTM readings. This study included eight ATCC QC strains and 120 different wild strains, giving a total of 1226 antibiotic/bacteria combinations and 2486 manual readings. There were only one major error and four minor errors with Bio-Rad as compared to Oxoid (0.08% and 0.34% respectively) by manual readings. For the same number of antibiotic/bacteria combinations, there were only five minor and one major error (0.42% and 0.08 respectively) with Bio-Rad discs read by ADAGIO™. In addition, the number of times that the automatic reading needed manual edition with Bio-Rad discs was statistically lower than with Oxoid (3.7% vs 5.7%, p<0.05). This study findings support the hypothesis that Bio-Rad discs are not inferior to Oxoid and the performance of ADAGIO**™** are comparable to manual readings with both disc brands.

**Introduction**

Disc diffusion test method is still used worldwide for antimicrobial susceptibility testing (AST) [1,2,3]. This method is simple, flexible and inexpensive. In most laboratories the results reading is manually done, with significantly high hands-on time. The main disadvantages of this manual non-automated method are lack of standardization and documentation of the readings, human reading and transcription errors, lack of reagent traceability, the latter being mandatory in the era of certification and accreditation of clinical laboratories. In the last few years, full automation of the process has been suggested as a fair solution to the mentioned pitfalls [4]. Meanwhile, for those laboratories where full automation is still not an option, a camera-based system that could automatically read and analyze disc diffusion test may be a useful tool for standardization and documentation of the result readings and batch numbers of the plates and antibiotics.

ADAGIO™ is an automated system built around data management software and an imaging device that measures the inhibition zone size around antibiotic discs. It provides speed, accuracy and reproducibility, and means less hands-on time, no transcription errors and standardized antimicrobials susceptibility test readings. Antibiotic disks and media batch numbers can be recorded for their full traceability in every single test. The system automatically recognizes Bio-Rad antibiotic discs (Bio-Rad, Marnes-la-Coquette, France) regardless the position on the agar plate, leading to a significant improve in reading process and result interpretation security. While other systems in the market do not automatically recognize the discs content, therefore disc position template on the agar plate should be fixed in order to avoid results mismatch [5,6,7,8,9,10,11].

ADAGIO™ offers built-in expert system, which based on readings can detect potential errors and suggest results editing based on phenotypes of known antibiotic resistance mechanisms. ADAGIO™ software also includes a powerful tool for the monitoring of resistance trends and nosocomial infections.

Due to the fact that the use of ADAGIOTM with Bio-Rad discs improves reading process and result interpretation security as compared to other manufacturers, in the first phase of this study the performance of Bio-Rad discs was compared to the Oxoid discs (Oxoid Ltd, Basingstoke, Hans, UK), which has been recognized as the best by former studies [12].

In a previous study the performance of discs from nine manufacturers (including the Bio-Rad and Oxoid) was compared in 2014 and 2017 using EUCAST criteria [13]. This study showed that although there was a significant improving between 2017 and 2017 in the performance of Bio-Rad discs, in 2017 it was still slightly inferior to that of Oxoid discs.

The aim of the first phase of the present study was to evaluate Biorad discs by means of testing a larger number of strains as compared to Oxoid discs using Clinical Laboratory Standards Institute (CLSI) criteria. Oxoid discs and CLSI criteria are both the routine disc diffusion method in Israel, therefore were chosen as gold standard for the present study.

# In the second phase of the study, the performance of ADAGIO™ was evaluated with ATCC QC and wild strains against Bio-Rad and Oxoid discs, as compared to manual reading. To the best of our knowledge, there was just one study that evaluated the performance of ADAGIO™ to manual readingjust for fastidious bacteria. In this study we substantially enlarged the number of bacterial strains used [14].

**Material and methods**

Bio-Rad and Oxoid discs were tested against ATCC QC strains: *Escherichia coli* 25922, *Klebsiella pneumoniae* 700603, *Pseudomonas aeruginosa* 27853, *Staphylococcus aureus* 25923, *Streptococcus pneumonia*e 49619, *Haemophilusinfluenzae* 49247 and *Haemophilusinfluenzae*49766.

All tests were performed according to CLSI disc diffusion methodology [15].Discs were tested on Mueller-Hinton agar (MHA) or MHA supplemented with 5% defibrinated horse blood (both from Hy Laboratories, Rehovot, Israel) or HTM (MHA supplemented with hemin and β-NAD from Novamed Ltd, Jerusalem, Israel) depending on the organism tested.Each combination of agent and QC strain was tested in triplicates. Discs used for the triplicate tests were always from the same lot and the same vial. Alltriplicates tests were performed on the same day using three individually prepared inoculum suspensions. For each agent, one disc from Oxoidand Bio-Rad manufacturer was placed on the same 90 mm circular agar plate to minimize variationsdue to differences in inoculum size, media and incubation conditions. Zone diameters were measured to the nearestmillimeter with a caliper.The mean of readings was calculated and for each strain and antibioticcombination it was reported if the mean reading fell within the range or not.

Following that, the performance of Bio-Rad discs against wild strains of *Enterobacteriaceae, P. aeruginosa, Acinetobacter spp,Shigellaspp/Salmonella enterica, Haemophilusspp, Moraxella catarrhalis, Staphylococcus spp, Streptococcus spp*and *Enterococcus spp*as compared to Oxoiddiscs was evaluated. Zone diameters were measuredtwice by two techniciansand the mean of readings was calculated for each strain and antibioticcombination.Category interpretation of results (as susceptible, intermediate or resistant)was summarized for each strain/antibioticcombination according to CLSI MS-100 (2017 update). According to ISO 20776–2 guideline, category agreement was established according to the following: very major errorfor false susceptible interpretation, major error for false resistant interpretation and minor errors for false categorization involving intermediate result.

In the second phase of the study, the ADAGIO™ system for the automatic reading of disc diffusion results was evaluated as compared to manual reading, using both Bio-Rad and Oxoid discs against four ATCC strains (*E. coli* 25922, *S. aureus* 25923, *S. pneumoniae* 49619, and*H.influenzae* 49247) and wild strains ofEnterobacteriaceae, *P. aeruginosa*, *Acinetobacter spp,Shigellaspp, S. enterica,Haemophilusspp,M.catarrhalis,Staphylococcus spp,Streptococcus spp,*and*Enterococcus spp*). For each strain,two 90-mm circular agar plates were inoculated with the same bacterial inoculum at the same timeand Bio-Rad orOxoid discs of relevant antibiotics were placed on each plate. Plates were incubated together at the same conditions. Following automatic reading, diameters were manually edited at the ADAGIOTM screen when needed. The number of manual corrections was recorded (Table 5). ADAGIOTM readings were categorized to susceptible, intermediate or resistant and compared against manual reading of Oxoid disks. Error category was defined for all readings as described before.Statistical significance of differences was calculated using Fisher and Chi-Square method when appropriate.

**Results**

In the first part of the study, eight ATCC QC strains were tested in triplicates, giving a total of 34 strain/antibiotic combinations and 102 individual readings. As shown in Table 1,only the same two out of from 34 combinations (5.9%) were not within expected range with bothOxoid and Bio-Rad discs.

Table 2 shows the performance of Bio-Rad Discs as compared to Oxoid Discs forwild strains.From a total of 1192antibiotic/bacteria combinations (2384 duplicate readings) with 120 different wild strains, there were only one major error and four minor errors with Bio-Rad discs as compared to the Oxoid discs (0.08% and 0.34%respectively,Table 5).

In the second part of the study, among 32 strain/antibiotic combinations (64 readings) of four ATCC strains read by ADAGIOTM, only the one strain (3.1%) wasout ofexpected range (by one millimeter) with Bio-Rad discs. The same diameter was read manually, suggesting that the ADAGIOTM reading was correct (Table 3).

Table 4 shows the performance of ADAGIOTMreading with Bio-Rad and Oxoiddiscs as compared to manual reading with Oxoiddiscs forwild strains. From a total of 1192strain/antibioticcombinations with 120 different wild strains read by ADAGIOTM, there were five minor and one majorerror with Bio-Rad discs and one minor error and no major errors with Oxoid discs (0.42%, 0.08%, 0.08% and 0% respectively, Table 5).

Table 5 also shows the number of times that the automatic reading was manually edited for both Bio-Rad and Oxoid discs (3.7% versus 5.7% respectively). The number of times that the automatic reading was manually edited with Bio-Rad discs was significantly lower than with Oxoid discs (p<0.05).

**Discussion**

The first part of this study presents an evaluation of Bio-Rad discs for antimicrobial susceptibility test by disk diffusion test. As seen in table 1, no significant difference was observed between the performance of Bio-Rad and Oxoid discs against ATCC QC strains. Only the same two strain/antibiotic combinations (*S. pneumoniae* 49619 with meropenem and *H. influenzae* 49766 with ertapenem) were out of range with both disc brands. The fact that all triplicate readings for both brands in the two cases showed similar slightly out-of-range results may suggest that the problem was related to other causes (e.g. isolate, culture media, incubation conditions) and not the quality of discs. In any case, the performance of both brands was the same. The previously mentioned study performed by EUCAST[13] checked the performance of antibiotic discs from different brands and showed that average readings of Bio-Rad and Oxoid discs against ATCC strains were all within expected range. Our study confirms these findings.

A very good performance of Bio-Rad discs was also observed with wild strains: the percentage of minor and major errors wasvery low, 0.34% and 0.08% respectively,with no very major errors. The number of minor and major errors with Bio-Rad discs was statistically not higher than with Oxoid discs (p= 0.20,p=0.99 respectively) (Table 5).In contrast to the mentioned EUCAST study were Oxoid discs were find to be significantly superior to Bio-Rad, ourstudy checked a larger number of QC and wild strains andresults suggest that Bio-Rad discs are not inferior and can be used also without compromising patient safety.

In the second part of the study, the performance of ADAGIOTM was evaluated, first with ATCC QC strains and then with a large number of wild strains.

Only one of the QC strain/antibiotic combinations showed out-of-range results (*H. influenzae* against trimethoprim/sulfamethoxazole) by only one millimeter, and again the result was the same for both disc brands.

The performance of ADAGIOTM with wild strains showed also very good results, with only five minor and one major error with Bio-Rad discs (0.42% and 0.08%) out of 1192 strain/antibiotic combinations. No very major errors were observed with both brands. With Oxoid discs there was only one minor error (0.08%), and the difference between both brands was not statistically significant (Tables 4 and 5). From these results, it can be assumed that both disc brands are equivalent and can be used with the ADAGIOTM system indifferently.

As already stated, the advantage of using Bio-Rad discs with ADAGIOTMis the fact that the system automatically recognizes the disc content regardless the position on the agar plate, with no need of keeping a fix pattern, this leading to a significant improve in the reading processand result interpretation security, and also reducing overall hands-on-time.In addition, as shown in Table 5, manual editing of automatic reading was needed in a significant lower number of times with Bio-Rad discs than Oxoid(3.7% and 5.7% respectively, p= 0.02).

To summarize, this study findings support the hypothesis that Bio-Rad discs are not inferior to Oxoidfor antimicrobial susceptibility testing by disk diffusion test. It is the first study that includes a wide range of bacteria and shows an excellent performance of ADAGIO™ system with Bio-Rad discs for the automatic reading of disc diffusion results, this potentially leading toa significant improve in hand-on-time, accuracy, reagents traceability and patient safety. Additional conclusions from the evaluation were that the system is user friendly, requires a very short personnel training and a minimal maintenance.

Although the time needed for reading was not thoroughly evaluated in this study, obviously it was shorter with the ADAGIO™ system than with manual measurement. This study also demonstrated the ability of ADAGIO™ system to read different kinds of agar plates, even those supplemented with blood.

The only disadvantage we found in the system is the fact that it reads only open plates without lids, this being a potential safety issue that should be considered.

In conclusion, according to the results of this study, the ADAGIO™ system in combination with Bio-Rad antibiotic discs showed disc diffusion results not inferior to manual reading of Oxoid discs. This combination may be an excellent alternative to current manual techniques in order to improve standardization, traceability and patient safety in clinical microbiology laboratories.

**Table 1: Bio-Rad and Oxoiddiscs susceptibility results for ATCC strains**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **ATCC #** | **Expected inhibition zone** | | **Oxoid** | | | | | | **Bio-Rad** | | | | | |
| **Antibiotic** | **Range (mm)** | **1st read** | **2nd read** | **3rd read** | **Mean** | **σ** | **Within range** | **1st read** | **2nd read** | **3rd read** | **Mean** | **σ** | **Within range** |
| *E. coli* | 25922 | Amoxi/clav | 18-24 | 21 | 20 | 20 | 20.3 | 0.47 | yes | 22 | 23 | 22 | 22.3 | 0.47 | yes |
| Cefoxitin | 23-29 | 25 | 25 | 26 | 25.3 | 0.47 | yes | 25 | 24 | 25 | 24.7 | 0.47 | yes |
| Meropenem | 28-35 | 32 | 33 | 33 | 32.7 | 0.47 | yes | 35 | 34 | 33 | 34.0 | 0.82 | yes |
| Tobramycin | 18-26 | 22 | 22 | 22 | 22.0 | 0.00 | yes | 21 | 21 | 22 | 21.3 | 0.47 | yes |
| Ciprofloxacin | 30-40 | 34 | 35 | 35 | 34.7 | 0.47 | yes | 34 | 34 | 35 | 34.3 | 0.47 | yes |
| Piperacillin | 24-30 | 26 | 26 | 27 | 26.3 | 0.47 | yes | 26 | 26 | 26 | 26.0 | 0.00 | yes |
| Ertapenem | 29-36 | 35 | 35 | 36 | 35.3 | 0.47 | yes | 35 | 36 | 36 | 35.7 | 0.47 | yes |
| Cefotaxime | 29-35 | 31 | 31 | 33 | 31.7 | 0.94 | yes | 31 | 33 | 32 | 32.0 | 0.82 | yes |
| *E. coli* | 35218 | Amoxi/clav | 17-22 | 20 | 20 | 21 | 20.3 | 0.47 | yes | 22 | 22 | 22 | 22.0 | 0 | yes |
| Piperacillin | 12-18 | 17 | 16 | 16 | 16.3 | 0.47 | yes | 15 | 16 | 16 | 15.7 | 0.47 | yes |
| *K. pneumoniae* | 700603 | Cefotaxime | 17-25 | 20 | 21 | 21 | 20.7 | 0.47 | yes | 21 | 22 | 23 | 22.0 | 0.82 | yes |
| *P. aeruginosa* | 27853 | Tobramycin | 20-26 | 26 | 26 | 26 | 26.0 | 0.00 | yes | 26 | 25 | 25 | 25.3 | 0.47 | yes |
| Ciprofloxacin | 25-33 | 28 | 27 | 29 | 28.0 | 0.82 | yes | 28 | 27 | 28 | 27.7 | 0.47 | yes |
| Piperacillin | 25-33 | 30 | 32 | 31 | 31.0 | 0.82 | yes | 30 | 31 | 31 | 30.7 | 0.47 | yes |
| Ertapenem | 13-21 | 18 | 18 | 20 | 18.7 | 0.94 | yes | 20 | 20 | 20 | 20.0 | 0.00 | yes |
| Meropenem | 27-33 | 31 | 30 | 32 | 31.0 | 0.82 | yes | 31 | 31 | 31 | 31.0 | 0.00 | yes |
| Cefotaxime | 18-22 | 21 | 21 | 20 | 20.7 | 0.47 | yes | 21 | 21 | 21 | 21.0 | 0.00 | yes |
| *S. aureus* |  | Cefoxitin | 23-29 | 27 | 27 | 25 | 26.3 | 0.94 | yes | 26 | 26 | 25 | 25.7 | 0.47 | yes |
| Meropenem | 29-37 | 31 | 30 | 30 | 30.3 | 0.47 | yes | 32 | 31 | 32 | 31.7 | 0.47 | yes |
| Tobramycin | 19-29 | 24 | 24 | 22 | 23.3 | 0.94 | yes | 23 | 22 | 23 | 22.7 | 0.47 | yes |
| Ciprofloxacin | 22-30 | 24 | 23 | 24 | 23.7 | 0.47 | yes | 24 | 24 | 24 | 24.0 | 0.00 | yes |
| Penicillin | 26-37 | 31 | 32 | 33 | 32.0 | 0.82 | yes | 30 | 33 | 33 | 32.0 | 1.41 | yes |
| Ertapenem | 24-31 | 27 | 28 | 29 | 28.0 | 0.82 | yes | 27 | 28 | 28 | 27.7 | 0.47 | yes |
| Cefotaxime | 25-31 | 28 | 28 | 27 | 27.7 | 0.47 | yes | 27 | 28 | 28 | 27.7 | 0.47 | yes |
| *S. pneumoniae* | 49619 | Meropenem | 28-35 | 37 | 38 | 38 | 37.7 | 0.94 | no | 37 | 38 | 40 | 38.3 | 1.25 | no |
| Penicillin | 24-30 | 27 | 28 | 26 | 27.0 | 0.82 | yes | 27 | 27 | 27 | 27.0 | 0.00 | yes |
| Ertapenem | 28-35 | 34 | 35 | 35 | 34.7 | 0.47 | yes | 34 | 35 | 35 | 34.7 | 0.47 | yes |
| Cefotaxime | 31-39 | 34 | 32 | 34 | 33.3 | 0.94 | yes | 35 | 33 | 34 | 34.0 | 0.82 | yes |
| *H. influenzae* | 49247 | Amoxi-clav | 15-23 | 20 | 20 | 21 | 20.3 | 0.47 | yes | 20 | 21 | 21 | 20.7 | 0.47 | yes |
| Meropenem | 20-28 | 25 | 26 | 27 | 26.0 | 0.82 | yes | 26 | 25 | 27 | 26.0 | 0.82 | yes |
| Ciprofloxacin | 34-42 | 36 | 35 | 37 | 36.0 | 0.82 | yes | 36 | 35 | 36 | 35.7 | 0.47 | yes |
| Ertapenem | 20-28 | 25 | 26 | 25 | 25.3 | 0.47 | yes | 26 | 25 | 25 | 25.3 | 0.47 | yes |
| *H. influenzae* | 49766 | Cefotaxime | 31-39 | 34 | 35 | 35 | 34.7 | 0.47 | yes | 35 | 36 | 36 | 35.7 | 0.47 | yes |
| Ertapenem | 27-33 | 34 | 34 | 35 | 34.3 | 0.47 | no | 34 | 35 | 35 | 34.7 | 0.47 | no |

**Table 2: Performance of Bio-Rad discs as compared to Oxoiddisks forwild strains**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Organism** | **Total tested** | **No of isolate/antibiotic combinations** | **Antibiotic** | **Oxoid results** | | | **Bio-Rad errors (n)** | | |
| **S** | **I** | **R** | **Minor** | **Major** | **Very major** |
| *Enterobacteriaceae* | 30 | 510 | Amikacin | 27 | 0 | 3 | 0 | 0 | 0 |
| Amoxi/clav | 10 | 0 | 20 | 0 | 0 | 0 |
| Cefazoline | 6 | 1 | 23 | 0 | 0 | 0 |
| Cefotaxime | 17 | 0 | 13 | 0 | 0 | 0 |
| Ceftazidime | 17 | 0 | 13 | 0 | 0 | 0 |
| Ceftriaxone | 17 | 0 | 13 | 0 | 0 | 0 |
| Cefuroxime | 7 | 0 | 23 | 0 | 0 | 0 |
| Chloramphenicol | 22 | 0 | 8 | 0 | 0 | 0 |
| Ciprofloxacin | 19 | 1 | 10 | 0 | 0 | 0 |
| Ertapenam | 28 | 1 | 1 | 1 | 0 | 0 |
| Gentamicin | 21 | 1 | 8 | 0 | 0 | 0 |
| Imipenem | 29 | 1 | 0 | 0 | 0 | 0 |
| Meropenem | 19 | 1 | 0 | 0 | 0 | 0 |
| Ofloxacin | 19 | 1 | 10 | 0 | 0 | 0 |
| Piperazillin/Tazo | 26 | 2 | 2 | 0 | 0 | 0 |
| Piperacillin | 16 | 1 | 13 | 0 | 0 | 0 |
| Thrimet/Sulpha | 19 | 0 | 11 | 0 | 0 | 0 |
| *P. aeruginosa* | 11 | 88 | Amikacin | 10 | 1 | 0 | 0 | 0 | 0 |
| Ceftazidime | 9 | 0 | 2 | 0 | 0 | 0 |
| Ciprofloxacin | 3 | 1 | 7 | 0 | 0 | 0 |
| Gentamicin | 8 | 1 | 2 | 0 | 0 | 0 |
| Imipenem | 7 | 1 | 3 | 0 | 0 | 0 |
| Meropenem | 8 | 0 | 3 | 0 | 0 | 0 |
| Ofloxacin | 3 | 1 | 7 | 0 | 0 | 0 |
| Piperacillin | 8 | 0 | 3 | 1 | 0 | 0 |
| *Acinetobacter spp* | 11 | 187 | Amikacin | 9 | 0 | 2 | 0 | 0 | 0 |
| Amoxi/clav | 2 | 2 | 7 | 0 | 0 | 0 |
| Ampicillin/Sulbac | 9 | 0 | 2 | 0 | 0 | 0 |
| Cefazoline | 0 | 0 | 11 | 0 | 0 | 0 |
| Cefotaxime | 1 | 4 | 6 | 1 | 0 | 0 |
| Ceftazidime | 6 | 0 | 5 | 0 | 0 | 0 |
| Ceftriaxone | 1 | 4 | 6 | 0 | 0 | 0 |
| Cefuroxime | 1 | 1 | 9 | 0 | 0 | 0 |
| Chloramphenicol | 1 | 0 | 10 | 0 | 0 | 0 |
| Ciprofloxacin | 7 | 0 | 4 | 0 | 0 | 0 |
| Gentamicin | 9 | 0 | 2 | 0 | 0 | 0 |
| Imipenem | 9 | 0 | 2 | 0 | 0 | 0 |
| Meropenem | 9 | 0 | 2 | 0 | 0 | 0 |
| Ofloxacin | 7 | 0 | 4 | 0 | 0 | 0 |
| Pip/Tazo | 7 | 0 | 4 | 0 | 0 | 0 |
| Piperacillin | 4 | 3 | 4 | 0 | 0 | 0 |
| Trimet/Sulpha | 7 | 0 | 4 | 0 | 0 | 0 |
| *Shigellaspp / S. enterica* | 9 | 54 | Amoxi/clav | 8 | 0 | 1 | 1 | 0 | 0 |
| Ampicillin | 7 | 0 | 2 | 0 | 0 | 0 |
| Ceftriaxone | 9 | 0 | 0 | 0 | 0 | 0 |
| Ciprofloxacin | 8 | 0 | 1 | 0 | 0 | 0 |
| Tetracycline | 8 | 0 | 1 | 0 | 0 | 0 |
| Trimet/Sulpha | 9 | 0 | 0 | 0 | 0 | 0 |
| *Haemophilusspp* | 5 | 29 | Amoxi/clav | 5 | 0 | 0 | 0 | 0 | 0 |
| Ampicillin | 4 | 0 | 1 | 0 | 0 | 0 |
| Ceftriaxone | 1 | 0 | 0 | 0 | 0 | 0 |
| Cefuroxime | 5 | 0 | 0 | 0 | 0 | 0 |
| Ciprofloxacin | 4 | 0 | 0 | 0 | 0 | 0 |
| Gentamicin | 4 | 0 | 0 | 0 | 0 | 0 |
| Trimet/Sulpha | 5 | 0 | 0 | 0 | 0 | 0 |
| *M. catharrhalis* | 2 | 2 | Erythromycin | 2 | 0 | 0 | 0 | 0 | 0 |
| *Staphylococcus spp* | 21 | 170 | Cefoxitin | 16 | 0 | 5 | 0 | 0 | 0 |
| Clindamycin | 9 | 0 | 12 | 0 | 0 | 0 |
| Erythromycin | 7 | 0 | 14 | 0 | 0 | 0 |
| Fusidic acid | 19 | 0 | 2 | 0 | 0 | 0 |
| Gentamicin | 16 | 0 | 5 | 0 | 0 | 0 |
| Mupirocin | 2 | 0 | 0 | 0 | 0 | 0 |
| Ofloxacin | 15 | 0 | 6 | 0 | 0 | 0 |
| Rifampicin | 21 | 0 | 0 | 0 | 0 | 0 |
| Trimet/Sulpha | 20 | 0 | 1 | 0 | 0 | 0 |
| *Streptococcus spp* | 11 | 68 | Ampicillin | 3 | 0 | 0 | 0 | 0 | 0 |
| Ceftriaxone | 7 | 0 | 0 | 0 | 0 | 0 |
| Chloramphenicol | 8 | 0 | 0 | 0 | 0 | 0 |
| Clindamycin | 9 | 0 | 2 | 0 | 0 | 0 |
| Erythromycin | 9 | 1 | 1 | 0 | 0 | 0 |
| Levofloxacin | 6 | 0 | 1 | 0 | 0 | 0 |
| Oxacillin | 1 | 0 | 0 | 0 | 0 | 0 |
| Penicillin | 5 | 0 | 0 | 0 | 0 | 0 |
| Tetracycline | 4 | 0 | 3 | 0 | 0 | 0 |
| Vancomycin | 8 | 0 | 0 | 0 | 0 | 0 |
| *Enterococcus spp* | 20 | 84 | Ampicillin | 20 | 0 | 0 | 0 | 0 | 0 |
| Penicillin | 1 | 0 | 7 | 0 | 1 | 0 |
| Ciprofloxacin | 6 | 0 | 6 | 0 | 0 | 0 |
| Fosfomycin | 12 | 0 | 0 | 0 | 0 | 0 |
| Nitrofurantoin | 12 | 0 | 0 | 0 | 0 | 0 |
| Vancomycin | 20 | 0 | 0 | 0 | 0 | 0 |
| **Total** | **120** | **1192** |  | **799** | **30** | **353** | **4** | **1** | **0** |

**Table 3: ADAGIOTMreadingsagainst manual reading for ATCC Strains**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **ATCC #** | **Antibiotic** | **Expected value (mm)** | **ADAGIO® reading** | | | | **Manual reading** | | | |
| **Oxoid disks** | **Within range** | **Bio-Rad disks** | **Within range** | **Oxoid disks** | **Within range** | **Bio-Rad disks** | **Within range** |
| *E. coli* | 25922 | Imipenem | 26-32 | 32 | yes | 30 | yes | 32 | yes | 32 | yes |
| Amikacin | 19-26 | 22 | yes | 25 | yes | 25 | yes | 26 | yes |
| Ampicillin | 16-22 | 16 | yes | 16 | yes | 18 | yes | 18 | yes |
| Ampicillin+sulbactam | 19-24 | 22 | yes | 20 | yes | 24 | yes | 21 | yes |
| Augmentin | 18-24 | 20 | yes | 21 | yes | 21 | yes | 22 | yes |
| Ceftriaxone | 29-35 | 30 | yes | 30 | yes | 35 | yes | 34 | yes |
| Ciprofloxacin | 30-40 | 36 | yes | 36 | yes | 35 | yes | 37 | yes |
| Meropenem | 28-35 | 35 | yes | 34 | yes | 34 | yes | 34 | yes |
| Pepiracillin | 24-30 | 25 | yes | 26 | yes | 30 | yes | 27 | yes |
| Tetracycline | 18-25 | 24 | yes | 25 | yes | 24 | yes | 23 | yes |
| Trimeto/Sulfa | 23-29 | 28 | yes | 27 | yes | 27 | yes | 28 | yes |
| *S. aureus* | 25923 | Cefoxitin | 23-29 | 28 | yes | 26 | yes | 26 | yes | 25 | yes |
| Gentamicin | 19-27 | 25 | yes | 24 | yes | 25 | yes | 24 | yes |
| Clindamycin | 24-30 | 30 | yes | 30 | yes | 24 | yes | 27 | yes |
| Erythromycin | 22-30 | 27 | yes | 28 | yes | 24 | yes | 25 | yes |
| Rifampicin | 26-34 | 30 | yes | 31 | yes | 30 | yes | 30 | yes |
| Ofloxacin | 24-28 | 25 | yes | 24 | yes | 24 | yes | 25 | yes |
| Trimeto/Sulfa | 24-32 | 29 | yes | 31 | yes | 30 | yes | 30 | yes |
| *S. pneumoniae* | 49619 | Tetracycline | 27-31 | 31 | yes | 31 | yes | 30 | yes | 31 | yes |
| Chloramphenicol | 23-27 | 27 | yes | 27 | yes | 27 | yes | 27 | yes |
| Ampicillin | 30-36 | 32 | yes | 32 | yes | 35 | yes | 36 | yes |
| Levofloxacin | 20-25 | 23 | yes | 22 | yes | 22 | yes | 20 | yes |
| Vancomycin | 20-27 | 24 | yes | 27 | yes | 24 | yes | 25 | yes |
| Erythromicin | 25-30 | 30 | yes | 30 | yes | 28 | yes | 30 | yes |
| Clindamycin | 19-25 | 25 | yes | 25 | yes | 24 | yes | 24 | yes |
| Penicillin | 24-30 | 28 | yes | 27 | yes | 30 | yes | 26 | yes |
| Ceftriaxone | 30-35 | 31 | yes | 33 | yes | 32 | yes | 34 | yes |
| *H. influenzae* | 49247 | Ampicillin | 13-21 | 20 | yes | 21 | yes | 20 | yes | 21 | yes |
| Augmentin | 15-23 | 20 | yes | 23 | yes | 19 | yes | 21 | yes |
| Ceftriaxone | 31-39 | 38 | yes | 35 | yes | 35 | yes | 35 | yes |
| Trimeto/Sulfa | 24-32 | 32 | yes | 33 | no | 30 | yes | 33 | no |
| Ciprofloxacin | 34-42 | 35 | yes | 39 | yes | 40 | yes | 39 | yes |

**Table 4: Performance of AdagioTMreading with Bio-Rad and Oxoiddiscs as compared to manual reading with Oxoiddiscs for wild strains**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Organism** | **Total**  **tested** | **No of isolate /**  **antibiotic**  **combinations** | **Antibiotic** | **Oxoid discs results**  **(n)** | | | **Adagio Reading Errors (n) with Bio-Rad disks** | | | **Adagio Reading Errors (n) with Oxoid disks** | | |
| **S** | **I** | **R** | **Minor** | **Major** | **Very major** | **Minor** | **Major** | **Very major** |
| *Enterobacteriaceae* | 30 | 510 | Amikacin | 27 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Amoxi/clav | 10 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cefazoline | 6 | 1 | 23 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cefotaxime | 17 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceftazidime | 17 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceftriaxone | 17 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cefuroxime | 7 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chloramphenicol | 22 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ciprofloxacin | 19 | 1 | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ertapenem | 28 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| Gentamicin | 21 | 1 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| Imipenem | 29 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Meropenem | 19 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ofloxacin | 19 | 1 | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pip/Tazo | 26 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Piperacillin | 16 | 1 | 13 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trimet/Sulpha | 19 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. aeruginosa* | 11 | 88 | Amikacin | 10 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceftazidime | 9 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ciprofloxacin | 3 | 1 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gentamicin | 8 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Imipenem | 7 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Meropenem | 8 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ofloxacin | 3 | 1 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| Piperacillin | 8 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Acinetobacter spp* | 11 | 187 | Amikacin | 9 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Amoxi/clav | 2 | 2 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ampicillin/Sulbac | 9 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cefazoline | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cefotaxime | 1 | 4 | 6 | 1 | 0 | 0 | 1 | 0 | 0 |
| Ceftazidime | 6 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceftriaxone | 1 | 4 | 6 | 1 | 0 | 0 | 0 | 0 | 0 |
| Cefuroxime | 1 | 1 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chloramphenicol | 1 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ciprofloxacin | 7 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gentamicin | 9 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Imipenem | 9 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Meropenem | 9 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ofloxacin | 7 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pip/Tazo | 7 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Piperacillin | 4 | 3 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trimet/Sulpha | 7 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Shigellaspp / Salmonella enterica* | 9 | 54 | Amoxi/clav | 8 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| Ampicillin | 7 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceftriaxone | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ciprofloxacin | 8 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tetracycline | 8 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trimet/Sulpha | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Haemophilusspp* | 5 | 29 | Amoxi/clav | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ampicillin | 4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceftriaxone | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cefuroxime | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ciprofloxacin | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gentamicin | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trimet/Sulpha | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *M. catharrhalis* | 2 | 2 | Erythromycin | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Staphylococcus spp* | 21 | 170 | Cefoxitin | 16 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Clindamycin | 9 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 |
| Erythromycin | 7 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fusidic acid | 19 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gentamicin | 16 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mupirocin | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ofloxacin | 15 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rifampicin | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trimet/Sulpha | 20 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Streptococcus spp* | 11 | 68 | Ampicillin | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceftriaxone | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chloramphenicol | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Clindamycin | 9 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Erythromycin | 9 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Levofloxacin | 6 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oxacillin | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Penicillin | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tetracycline | 4 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Vancomycin | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Enterococcus spp* | 20 | 84 | Ampicillin | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Penicillin | 1 | 0 | 7 | 0 | 1 | 0 | 0 | 0 | 0 |
| Ciprofloxacin | 6 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fosfomycin | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nitrofurantoin | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Vancomycin | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | **120** | **1192** |  | **799** | **30** | **353** | **5** | **1** | **0** | **1** | **0** | **0** |

**Table 5: Summary: Performance of Bio-Rad discs and AdagioTMfor wild strains as compared to manual reading with Oxoiddiscs**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Isolate / antibiotic**  **Combinations (n)** | **Errors (n%)** | | | **Manual Editing of ADAGIOTM Readings (%)** |
| **Minor** | **Major** | **Very Major** |
| Bio-Rad disks | 1192 | 0.34 | 0.08 | 0 |  |
| Adagio reading with Bio-Rad disks | 1192 | 0.42 a | 0.08c | 0 | 44 (3.7) b |
| Adagio reading with Oxoid disks | 1192 | 0.08 a | 0c | 0 | 68 (5.7) b |

a p = 0.20 (n.s.)

b p = 0.02

c p = 0.99 (n.s.)

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