# New Method for Automated Disk Diffusion Test

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**Abstract**— Microbial resistence to antibiotics is a very important parameter in the selection of a proper therapy and in the control of resistence spreading. One of the most used methods for measuring microbial susceptibility is the disk diffusion test. The test is based on diffusion of concentrated antibiotics from paper disk into agar. Concentration of antibiotics in the agar is dependent on distance from the center of the disc. Near antibiotic disc is concentration much higher due difficult permeation of antibiotics in agar. The diffused antibiotics then inhibit growth of sensitive strains. The zones are usually measured manually or by electronic calipers. Several aproaches for automatic inhibition zone detection have been introduced in recent years. Nevertheless, most of the algorithms are based on a similar radial profile analysis. We have designed a novel image-processing algorithm for measuring the size of inhibition zones of antibiotics based on an analysis of corrected image and rated this image with multicriterial algorithms (based on an observation of the radius profile). The algorithm was tested on 100 clinical isolates, resulting in calculation accuracy of 89% (ratio of success computed radii). If we include alternative radii (to be selected manually), the precision of the calculation rises to 98% (tolerance deviations between manual and automatic measurements were 2 mm). The achieved accuracy was independent of the culture medium (e.g., Muller-Hinton, blood agar, chocolate agar). The main advantage of the algorithm is the invariantness to the tested bacterial strain and culture medium. The new algorithm offers an alternative way to determine inhibition zones and evaluate antimicrobial susceptibility.

## 1. INTRODUCTION

The disk diffusion test is one of the most commonly used methods for microbial susceptibility testing which determined the size of inhibition zones of sensitive and resistant strains.... The test is based on diffusion of concentrated antibiotics from paper disk into agar. Concentration of antibiotics in the agar is dependent on distance from the center of the disc. Near antibiotic disc is concentration much higher due difficult permeation of antibiotics in agar. The diffused antibiotics then inhibit growth of sensitive strains. The usual method for zone measurement is mostly based on manual reading of inhibition zones around the disk.

The presented algorithm eliminates effect of unwanted artifacts presented in scanned image (bacteria distributing residues, asymmetric inhibition zones etc.) and suggests the diffusion zones diameter (or alternatives) with comparable probability to any other presented system.

## 2. MATERIAL AND METHODS

Algorithms were developed and tested on clinical samples from St. Anne Faculty Hospital in Brno, Czech Republic. 100 clinical samples were used for measuring inhibition zones. Samples were cultivated on Muller-Hilton, blood or chocolate agar. Among analyzed samples was *Streptococcus spp., Staphylococcus aureus, S. epidermidis, Pseudomonas aeroginosa, Enterococcus spp., Escherichia coli* and some others unspecified clinical strains. All samples were cultivated 24 hours in 37°C. Standard petri dish with diameter 90 mm was used. Picture was taken by HP scanjet G3110 on 300DPI (bottom up).

Evaluation of the size of the diffusion zone consisted of the picture acquisition, image preprocessing, finding a dish and antibiotic disks, thresholding image, measurement of inhibition zones, confirmation or manual correction, processing output,and parameters extraction.

Image preprocessing consists of changing the image size ($500px \times 500px$) to increase the computation speed. It is necessary to take a picture in high resolution (minimum 300DPI), so the speed of calculation will increase 10 times.

Furthermore, the image is converted to grayscale. It is important to select the correct RGB conversion coefficients. Improperly chosen values can minimize the contrast between the bacterial coating and the bacterial cultivating medium (agar) or highlight the unwanted artifacts and degrade the measurement.

It is necessary to correct the image before applying the thresholding operator. Areas (background plates, antibiotic targets) and parts which do not belong to the investigated area were corrected. These areas were replaced by a homogeneous field. To avoid histogram changes, brightness of the replaced area was chosen equal to the median (or average) of the brightness of the image. Figure 1 shows the histograms of raw and modified image.

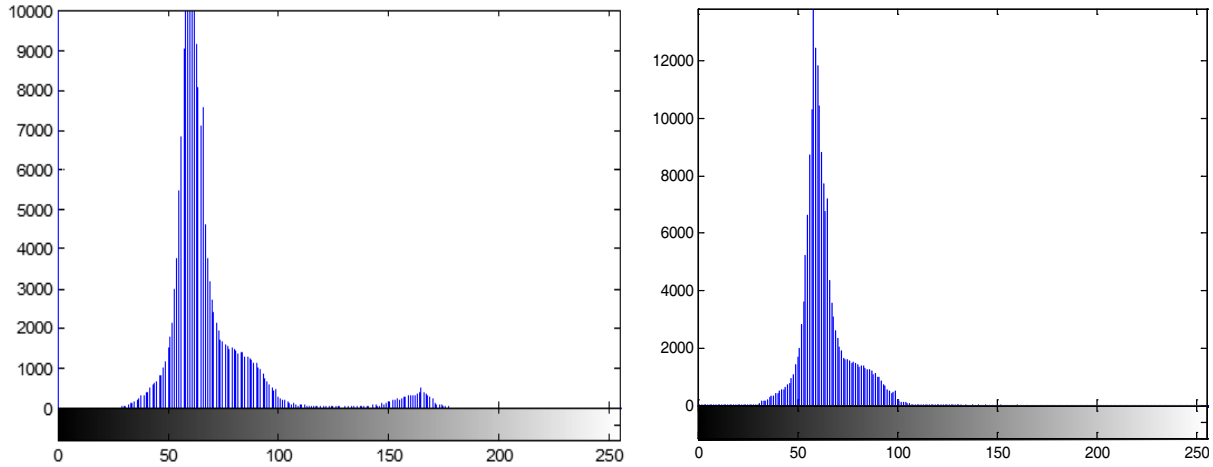


Figure 1: The histograms of raw and modified image.

Hough transform was applied to edge patterns. Result image was used to find a dish and antibiotic disks. First, the image was converted to grayscale, then Canny edge detector was applied (multi-step algorithm for finding the edges) and Hough transform was applied to this image. Hough transform is able to find a circle of known radii and calculate their coordinates — see [1].

Further, it is necessary to binarize the picture. Thresholding algorithm is applied to the modified image. The best results were achieved by using local thresholding (iterative thresholding, Otsu operator).

Otsu operator is described in [2]. To increase the efficiency, we have to equalize the image histogram. In the described procedure, both operators were applied (Otsu and Iterative). Combination of the results of both measurements is described below.

For the measurement of the size of these zones a unique algorithm is used which operates in the following steps:

- Initializing the radius to a value slightly greater than the radius of the disc. Do it for all investigated zones.
- Calculating the average brightness on the circle with the exclusion of conflict points.
- Conflicting points are those which belong to a different circle than the investigate.
- Circle points is evaluates by using Mid-point algorithm [3].
- If the calculated average brightness is greater than the specified threshold — end the circle calculation.
- Incrementing all radii of unfinished circles.
- For the unfinished circle — jump to point 2.

It is important to use an algorithm which reliably finds a circle whose circumference brightness is increased above a given brightness level. The algorithm is based on computing points, which do not belong to other circles. The problem is how to choose the optimum brightness level. Analytical deduction of the optimal luminance level is difficult and would require a complex description of the image. The described algorithm uses a different approach. Circles (the edges of the zone of inhibition) were detected using $N$ different thresholds. For each investigated zone, we obtained $N$

potential radii of inhibition zones. The calculated radius probabilities are given by their frequencies in the measured set of size $N$. The radius with the biggest probability (most often measured) is considered to be final — other radii can be proposed as an alternative. The frequency calculation is supplemented by tolerance — this means that the measured radii are divided into groups that have similar radius size. The resulting value is given by the arithmetic mean in grouped values.

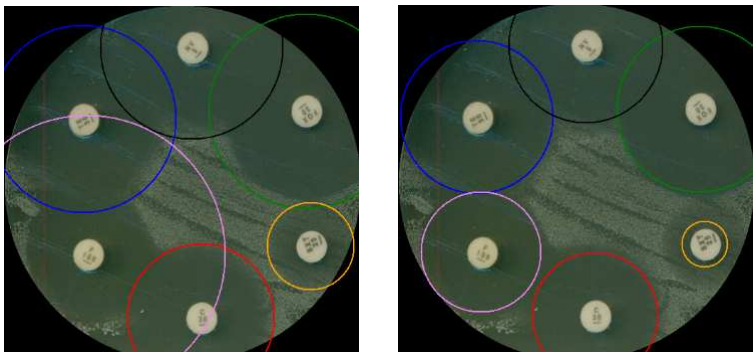

Figure 2: Incorrectly and correctly detected inhibition zones.

## 3. RESULTS AND CONCLUSION

In 98% of cases, the system measured zone correctly or the right size was in suggested alternative zones (user must choose the right zone — semi automate mode). Other testing would be necessary for chocolate agar and for yeasts.

The developed algorithm gives reliable and reproducible results even for damaged samples. If the border zone is not evident, the algorithm will calculate alternative zones. The user can then select the preferred one. The algorithm was tested on 100 dishes, resulting in calculation accuracy of 89%. If we include alternative radii (to be selected manually), the precision of the calculation rises to 98% (tolerance deviations between manual and automatic measurements were 2 mm). Figure 2 shows the output of the program. Abbes et al. [4] describe similar algorithm based on the analysis of the radial profile of the zone. This approach has achieved accuracy (the same tolerance of diversity) of 78%. The algorithm can be modified by time optimization and automatic labels reading.

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## REFERENCES

1. Cuenca-Estrella, M., A. Gomez-Lopez, M. O. Gutierrez, M. J. Buitrago, and J. L. Rodriguez-Tudela, "Reliability of the WIDERYST susceptibility testing system for detection of in vitro antifungal resistance in yeasts," *Antimicrobial Agents and Chemotherapy*, Vol. 52, 1062–1065, 2008.
2. Zabidi, M. A., N. M. Yusoff, and Z. S. A. Kader, "Preliminary comparative analysis of antibacterial effects of activated and non-activated of expired platelet concentrate by disc diffusion method," *Indian Journal of Pathology and Microbiology*, Vol. 55, 47–51, 2014.
3. Sood, S. and R. Gupta, "Antibiotic resistance pattern of community acquired uropathogens at a tertiary care hospital in jaipur, rajasthan," *Indian J. Community Med.*, 39–44, India, 2012.
4. Abbes, S., H. Trabelsi, I. Amouri, H. Sallemi, S. Nej, C. Fatma, F. Makni, and A. Ayadi, "Methods for studying the in vitro susceptibility of Candida spp. to antifungals," *Annales De Biologie Clinique*, Vol. 70, 635–642, 2012.
5. Canton, R., M. Perez-Vazquez, A. Oliver, B. S. Del Saz, M. O. Gutierrez, M. Martinez-Ferrer, and F. Baquero, "Evaluation of the wider system, a new computer-assisted image-processing device for bacterial identification and susceptibility testing," *Journal of Clinical Microbiology*, Vol. 38, 1339–1346, 2000.