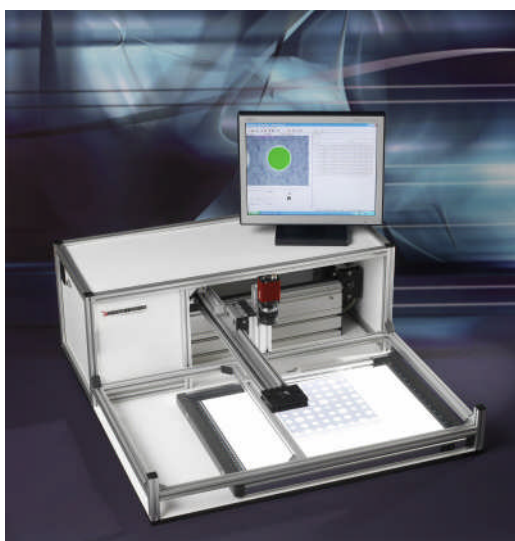


Measurement of reaction zones on large bioassay plates An automated solution using the AutoZONE system

Description of system

The AutoZONE consists of a gantry with a robotic arm, an A3 light pad which provides high frequency daylight quality light, and a PC with Protocol software installed.



The application – measurement of reaction zones on large bioassay plates

This is used to determine the potency of antibiotics, vaccines, and antimicrobial additives to foods.

Typically, these plates are up to 30 x 30cm in size, and will have wells cut into the medium, arranged in an array of 8 x 8.

Due to the large size of the plate, it is not practical to capture a single image of the whole plate. Also, the individual wells would appear very small relative to the whole image, making measurements difficult and inaccurate. Therefore, by using the AutoZONE system each well is imaged individually. This is done by moving the camera on the robotic arm, such that it moves to each well in turn and captures an image from each well. The diameter of the detected zone around each well is calculated and placed in the results table. Thus, each zone almost completely fills the field of view, making the measurements more accurate.

The method

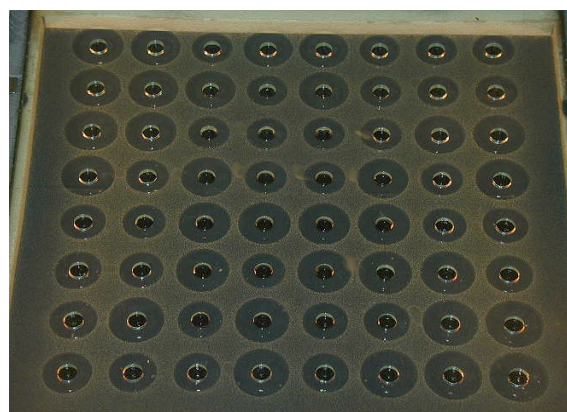
Several dilutions of test antibiotic/vaccine are placed in random well positions on the plate. Pre-known concentrations of a standard antibiotic/ vaccine are also placed in random well positions.

Usually a Latin square design is used to ensure that the operator does not know what wells contain test or standard sample.

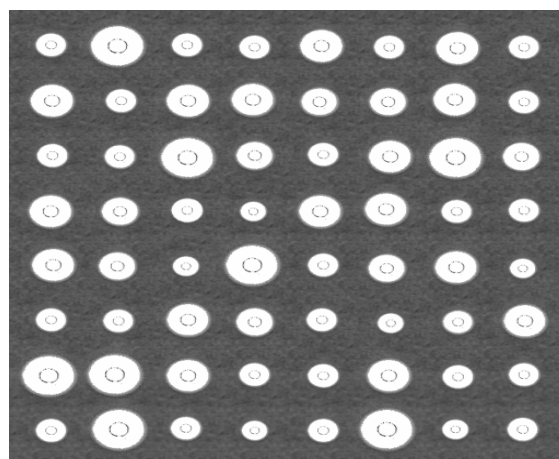
The plates are then incubated.

The antibiotic/vaccine diffuses out from each well. The antibiotic kills pre-coated bacteria on the plate, and creates a clear zone of inhibition around the well.

The vaccine interacts with the antigen on the plate, to create an antigen/antibody complex, which is counterstained to make it visible.



The result for both antibiotics and vaccines is a plate with clear zones of varying diameters around the wells.



The zone diameters now have to be measured.

The size of the zones relate to the potency of the antibiotic/vaccine. The smaller zones relate to lower potency antibiotic/vaccine, and vice versa.

Manual measurements

Most laboratories measure the zones manually. This is done by using mechanical callipers. Since zones are usually not perfectly circular, several diameter measurements are taken per well, and a mean is calculated. In order to see the zones clearly for measurement, the plate is usually placed on a light box with a large magnifying glass. After measurement the results are entered into a spreadsheet or statistics package for analysis.

The disadvantages of manual measurement are:

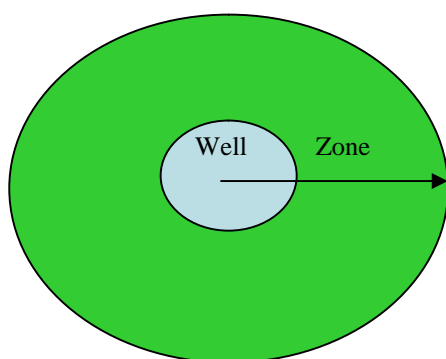
1. Time consuming - antibiotic plates can take about 45 minutes and vaccine plates about one hour. Often two people are required;
2. Error prone;
3. Highly subjective;
4. Low reproducibility;
5. Results require transcription;
6. No image archiving.

Automated measurement using AutoZONE

The robotic arm on the gantry system transports the camera to each well in turn. An image is captured of each well, and the area of the zone is detected using imaging technology. The detected *area* is then converted to a *diameter* by using the mathematical formula $\pi \times r$ squared.

This is the mathematical equivalent of taking an infinite number of diameter measurements, and then calculating the mean.

The AutoZONE performs this calculation whilst the arm is moving to the next well. Thus, the calculations are made “on-the-fly”, which greatly speeds up the process.



$$\text{Zone area} = \pi r^2$$

Benefits of automated measurement

1. Very fast - the AutoZONE can read a 64 well plate in ~70 seconds.
2. Very accurate - the diameter is calculated from the detected area.
3. An image of each well is archived - this enables a review of the sample at any later time.

4. Full results audit trail - essential for regulated environment.
5. Easy data handling - direct results exported to Excel or statistics package.
6. Huge time savings - increase laboratory efficiency.
7. No transcription errors - automatic reporting.

Analysis of results

There is a need for statistical analysis of results.

Typically, the Latin square method will have been used to ensure that operators do not know what sample or concentration is deposited in each well. At this stage, the Latin square code is broken in order that the results can be analysed.

Unistat v5.6 statistical package

A complete statistical package, called Unistat v5.6, with an add-in bioassay module is available.

The most common form of bioassay is where the potency of one or more *test* preparations is determined by comparison to a *standard* preparation.

The Unistat v5.6 bioassay module supports potency calculations employing parallel line, slope ratio and quantal response methods, complete with confidence intervals, validity tests and graphical representations.

A demo version of the Unistat 5.6 software is available for download from: www.unistat.com

All support is provided by Unistat.