



# ***ZONE OF INHIBITION TESTING***



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## WHAT IS A ZONE OF INHIBITION TEST?

The effectiveness of an antibacterial treatment on a surface or product can be quickly assessed using a Zone of Inhibition (ZOI) test. The procedure, formerly known as the Kirby-Bauer test, was developed as a result of pharmaceutical industry research on antibiotics, and was later used to evaluate the antibacterial capabilities of polymers and textiles.

## HOW IS A ZONE OF INHIBITION TEST PERFORMED?

A petri dish filled with nutrient agar and the requisite bacteria culture are streaked in a conventional Zone of Inhibition test. An inch-by-inch portion of a product sample that has been treated with an antimicrobial agent is cut out and placed onto the nutrient agar. The petri dish is then kept at 36°C for 18 to 24 hours, which is the ideal temperature for bacterial growth.

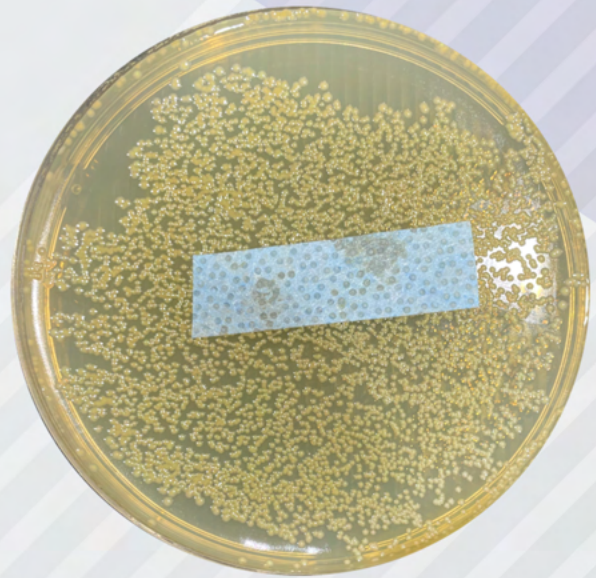
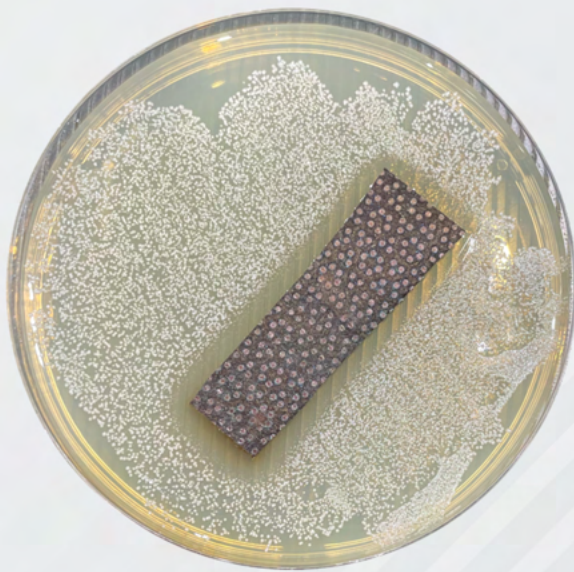
The bacteria on the nutrient agar should be well-established and apparent as a dense yellow lawn after the incubation period. A clearing zone might be discernible around test pieces where the antibiotic can travel into the nutritional media surrounding the sample.

The extent of this zone reflects the potency of the particular antimicrobial agent present in the treated sample.

## WHAT ARE THE LIMITATIONS OF THE ZONE OF INHIBITION TEST?

While the Zone of Inhibition test shows visibly compelling results, it has several limitations relating to the size of the zone.

- **A broad zone of inhibition is not always indicative of a product that is well-protected.** It could also be an indication that an antimicrobial agent is inadvertently leaching from a treated product and spreading into the environment. This might be partially caused by blatant incompatibility between the antimicrobial agent and the product, which would result in a sharp decline in antimicrobial performance. Additionally, leaking antimicrobials may unnecessarily expose the environment and the user to a biocide.
- The zone of inhibition test requires the antimicrobial agent to travel into the nutrient agar, therefore the absence of a visible zone does not necessarily indicate that the antimicrobial agent is ineffective. The antibiotic will not travel to form a visible zone of inhibition if the nutrient agar is incompatible with it. A lack of a visual zone in accordance with the Zone of Inhibition test guidelines may deceive the untrained evaluator into believing that the antimicrobial is worthless, even if it may be quite successful at protecting the product itself. Silver-based antimicrobials and zinc organometallic antimicrobials are examples of effective antimicrobials that do not have a propensity for the nutrient agar utilized in a Zone of Inhibition test.



An incubated test piece is shown in the images above (left and right). The clean zone that surrounds the Silver Oxide coated sample in the left image, however modest and well-established, serves as a sufficient indicator of antibacterial effectiveness. In contrast, the right image, the control shows no zone surrounding the sample.

## HOW DOES CMDC LABS USE THE ZONE OF INHIBITION TEST?

The Zone of Inhibition test is used by CMDC Labs to swiftly evaluate the efficacy of particular antimicrobial medicines or materials. A very broad zone of inhibition may indicate the product's instability and propensity to migrate rather than providing superior antibacterial product protection. Typically, a small area of clear and robust clearance is desired surrounding the product as a sign of antibacterial efficacy, durability, and stability.

The validity of the Zone of Inhibition test has mainly been contested, despite the fact that labs all over the world continue to frequently use it. The test method is frequently replaced today by more precise quantitative techniques like ISO 22196 or JIS Z2801.

# HOW CAN CMDC LABS TESTING SERVICES HELP YOU?

A wide range of testing and technical support services are provided by CMDC Labs. Numerous qualitative and quantitative tests are carried out at our microbiological labs in accordance with industry and international standards as AATCC, ASTM, EPA, ISO, JIS, and FZ/T.

Antimicrobial substances are tested against both Gram-positive and Gram-negative bacteria in our bacterial test lab to assess their range of performance. Traditional tests including Zone of Inhibition, environmental monitoring (such aerobic plate count), Minimum Inhibitory Concentration (MIC) studies, and neutralization studies are all part of our testing portfolio (ASTM E1054).

Every year, CMDC Labs can conduct over 30,000 bacterial and fungal tests utilizing industry-recognized test procedures created for a variety of product substrates and antimicrobial technologies.

Contact a team member immediately for additional details on our test capabilities and services.



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