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Abstract No.

# Bacterial Motility Assay in 384-well Format for HTS

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## Abstract

The emergence of antibiotic resistant strains of pathogenic bacteria drives the discovery of new antibiotics. Exploiting novel targets is considered the most promising way to develop new antibiotics that combat drug resistant strains. Motility is a virulence factor for many bacteria including *Vibrio cholerae* and is a promising target for drug discovery that has yet to be adapted to High Throughput Screening (HTS). Presented here is an HTS assay developed in 384-well format to specifically identify compounds that inhibit motility.

## Introduction

It is known that in many pathogenic bacteria motility, driven by chemotaxis, is an important virulence factor required for the pathogen to reach mucosal surfaces especially in areas with fast flow. A simple assay to measure bacterial motility is the swarm (soft) agar assay (1). In the swarm agar test bacterial motility is measured as the distance (halo) bacteria swarm away from the inoculation site and can be on the order of 5-10mm in 12 hours at 30C.

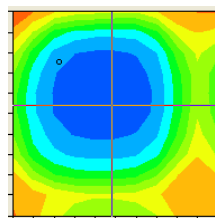


**Figure 1** Swarm agar test for bacterial motility in a 100mm Petri dish. Wild type and motility mutants were inoculated on LB plates containing 0.3 % agar.

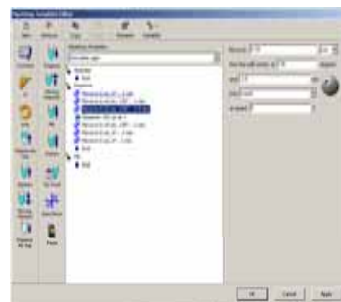
To illustrate bacterial motility, examples of wild type motile bacteria, a mutant expressing reduced motility, a flagellum-less (non-flagellated) mutant and a mutant that makes a paralyzed flagellum (flagellated non-motile) were inoculated in a 100mm Petri dish (Fig. 1). Differences in motility are obvious but have been difficult to adapt to the HTS format. The use of a semisoft agar motility assay has been reported for 96-well format (2) and has been replicated in our lab (Fig. 4A) using wild type motile and a non-motile mutant of *V. cholerae*. As far as proof of principle, this is an encouraging development but does not meet the needs of truly high throughput screening. We further miniaturized the assay to 384-well format to increase throughput (Fig. 4B).

## Methods

To adapt this assay to 384-well format, the inoculation site needed to be out of the field of view for the Abs read. This was accomplished by inoculating the plate in the top left corner of the well and reading the OD to the right and down from well center (Fig. 2). Even after 48 hours of incubation, the colonies of non-motile bacteria did not interfere with the absorbance read. A Biomek FX was used to inoculate the plate using standard p30 pipette tips and a custom pipetting template (Fig. 3). This template allowed the tips to be offset to the top left corner of the well and controlled the depth that the tip penetrated into the soft agar. The read location in the well was set using the plate dimension calibration on a Perkin Elmer Envision to be slightly off center to the bottom right to avoid interference from bacterial growth at the inoculation site (Fig 2). Following the absorbance read, alamarBlue was added to the plates for an additional viability read. Drug delivery was done with an Echo550 which transferred 50nl of test compound onto the surface of the soft agar. Compounds were allowed to diffuse into the agar for 2 hours before inoculation with bacteria.



**Figure 2** 384-well inoculation site (o) vs. read location (cross hair). By offsetting the read location from the inoculation site, non-motile bacterial colonies did not interfere with the absorbance read.



**Figure 3** FX pipetting template used to inoculate in the corner of the well. Note the offset of 0.18 cm at 135 degrees from center (diagonal toward top left corner) Tips then move to -2.5mm from the agar surface to inoculate.

## Results

Plates were inoculated with motile bacteria on the left half and non-motile on the right in both 96 and 384-well format (Fig 4A and B). Z values were 0.87 and 0.79 respectively indicating that this approach could distinguish motile from non-motile bacteria. To differentiate between non-motile and non-viable bacteria, alamarBlue was added to the plates following the absorbance read. Tetracycline, a known bactericidal compound, was used as a non-viability control. From the Abs 615 values it is impossible to differentiate between Motile + tet, Non-motile + tet and Non-Motile. Once alamarBlue is added, the difference between Non-motile and Non-motile + tet is obvious (Table 1). AlamarBlue conversion is relatively rapid at room temperature and is easily incorporated into the plate handling process following the absorbance read.



**Figure 4A** 96-well plate inoculated with *V. cholerae*. Columns 1-6 contain motile *V. cholerae* while columns 7-12 contain a non-motile mutant. Z value is 0.87.



**Figure 4B** 384-well plate inoculated with *V. cholerae*. Columns 1-12 motile and 13-24 the non-motile mutant. Z value is 0.79

	Motile	Motile+tet	Non-motile	Non-motile+tet
Abs615	1.25	0.25	0.29	0.24
Alamar Blue	8,082,494	272,548	9,869,575	255,445

**Table 1**-Comparison of Mobility and Viability. Absorbance for motile bacteria is high while non-viable (+tet) or non-motile are low. Viability determined by addition of alamarBlue clearly differentiates between non-viable (FI low) and viable (FI high) for both motile and non-motile strains.

## Conclusion

Motility is an important virulence factor for some bacteria. The soft agar Swarm assay has been used historically to evaluate motility, but has not been amenable to High Throughput Screening. By adapting this soft agar assay to 384-well format and evaluating Abs 615 negative wells for viable non-motile bacteria, a robust and specific motility assay has been developed for HTS.

## Acknowledgments

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## References

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