International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 07 Issue: 06 | June 2020 www.irjet.net p-ISSN: 2395-0072

Motiloscope- A Novel Method and Device to Detect Bacterial Motility using Disk Type Sample Holder

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Abstract - *For the detection of bacterial movement a novel device designed which is useful to observe bacterial motility. In this device have a disc type of sample holder in which the bacterial suspension is added and coated with viscous oil i.e. paraffin oil. Then this sample holder is observed in present device between slit, reflecting mirror, and directly scans by objective lens with eyepiece lens. It has shutter arrangement to kept sample in device. The principal use of this apparatus in field of bacteriology is to observe bacterial motility, identification, evaluation and classification of different species of bacteria.*

Key Words: Motiloscope, paraffin oil and bacterial suspension.

1. INTRODUCTION

The ability of organism to move by it self called motility. Motility is closely link with chemotaxis, the ability to orientate along certain chemical gradient. Eukaryotic cell can move by one of the following locomotors organelles such as cilia, flagella or pseudopodia. Prokaryotes move by means of un-usual, propeller-like flagella unique to bacteria or by special fibrils that produce a gliding form of motility. Almost all spiral bacteria and about half of the bacilli are motile, whereas essentially none of the cocci is motile. Motility by means of flagella is of important for the identification of microorganisms, for instance Bacilli are motile except for the anthrax, which is instead capsular, therefore flagella could be produced to enable the organisms to run away from danger and to move from less favorable to more favorable environment. The same is observed with Clostridia, all are motile except Clostridium perfringens, which has a capsule. It has also been suggested that motility is an essential feature in the colonization hence pathogenicity of Helicobacter pylori. Flagella are long, slender, and helical and generally, several time the length of the cell. Individual flagella of a single species are uniform in diameter, but the flagella of different bacterial species vary from about 12 nm to 30 nm in diameter. Each flagellum has a very rigid, helical structure and actual motility results from the rotation of the flagellum in a manner similar to that of a boat propeller. Bacteria have shown that when the flagellum is rotating counterclockwise, the bacterium travels in a more or less straight line; but if the direction of flagellar movement is reversed, the organism will tumble aimlessly. [1]

When anticlockwise rotation is resumed, the cell moves off in a new direction. This ability is important, since it allows bacteria to change direction. Bacteria can sense nutrient molecules such as sugars or amino acids and move towards them - a process is known as chemotaxis. Additionally, they can also move away from harmful substances such as waste products and in response to temperature, light, gravity, etc. [2, 3]

If identification of a bacterium requires detection of the actual number and placement of flagella, special stains or electron microscope preparations are required, as flagella are too minute to be seen in un-stained live preparations with an ordinary light microscope. Often it is sufficient to know simply whether a bacterial species is motile, which helps differentiation between genera and species. The focus of this work is to bring the detection of motility concerning flagellar mode together with some basic novel apparatus is bacterial motility detector and method of their sample preparation. [4] The disadvantage of cavity slide method is fall of drop of bacterial suspension or drop of young fluid culture when they are inverted, wax coating required, short period of observation due to fall of drop of suspension. For these experimentations, more skill required and due to fall of drop of suspension or drop of young fluid culture, the bacterial motility cannot observe. This novel device and their designs overcome these problems.

1.1 Development of novel device and their designs

This device comprises different slit row arrangement with lenses, sources as white color LED (light emitting diode), light focusing device as to cut focus light diameter. It passes pin-point or very small diameter of light which, fall on Plano-convex lens of slit and this light is forwarded to another slit. In addition, it comprises slit for sample holder, which is involved with movable track. The two lenses system for observation of bacterial motility comprises objective and eyepiece lenses with knob for sample focusing to objective lens for observation of bacterial motility. The light source comprises LED bulb that connected by insulating wire with on/off switch and variable resistor for controlling intensity of light. The variable resistor comprises carbon composition potentiometer by means of voltage regulator. The power input supply given by adapter of an external AC to 3V DC. An apparatus and its whole parts are made of metal and it has shutter arrangement to keep sample into apparatus.[5] The apparatus for bacterial movement detector shown in following figure 1.

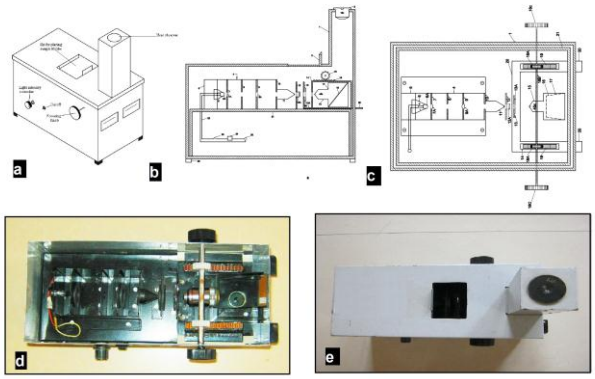

Fig -1: (a) Perspective drawing of device. (b) Side View drawing of device. (c) Top View drawing of device. (d&e) Actual construction of device.

1.2 Development of method of preparation and there design of sample holder

The disc type of sample holder comprises thick metal disk that has 3 to 4 mm diameter aperture. Below the metal disk a transparent thin glass plate are join by each other and handle are join with metal disk for handling sample holder. The disk type sample holder shown in following figure 2.

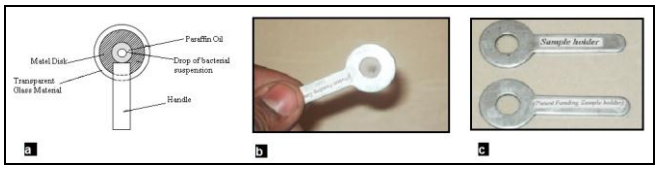

Fig -2: (a) Perspective drawing of disk types of sample holder. (b&c) Actual construction of disk types of sample holder.

A small drop of bacterial suspension or young fluid culture is add at the center of disk and coat with viscous oil that is, paraffin oil. The choice of viscous oil in preparation sample is to cover drop of a young fluid culture or bacterial suspension for keeping drop at center of disc. The drop and oil cannot mix with each other, so concentration of bacterial cell is within drop only. Then these sample holders observe in present apparatus between slit and reflecting mirror with eyepiece lens. Therefore this method and apparatus not required any wax coating for sample, not much skill require, the time period of observation increases. The disk type sample holder shown in following Figure 3.

Fig -3: (a) Application of a small drop of bacterial suspension or young fluid culture is at the center of disk. (b) Coating with viscous paraffin oil (c) Application of disk type sample holder in device.

1.3 Preparation of suspension of test bacterial sample for testing bacterial motility

The 24 hr old bacterial suspension of different species was prepared in sterile normal dextrose saline solution. By using these test bacteria, the sample holder is prepared.

2. SCREENING, IDENTIFICATION AND TESTING EFFICIENCY OF DIFFERENT MOTILE SPECIES OF BACTERIA BY USING THIS DEVICE

During this work, the different species of motile and non-motile bacteria selected. There are different 8 species of bacteria are Screen by this device. The Screening of these different species of bacteria is for identification of their motility as true motility and Brownian movement given in Figure 4. In the Table 1., we screened different species of bacteria by using this device.

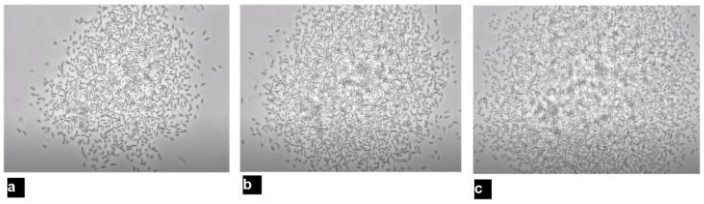

Fig -4: (a) First real-Time laps image of bacterial motility within 1 min. (b) Second real-Time laps image of bacterial motility next 5 min. (c) Third real-Time laps image of bacterial motility next 15 min.

The main objectives of this device is to observe bacterial motility with sophisticated for identification, evaluation, and classification of different species of bacteria like E. coli, fecal coliform and any other different species of bacteria, to confirm Brownian motion of bacteria. The Brownian motion of bacteria is due to oscillation of bacteria about mean position caused by bombardment of water molecules. In addition, to observer detail true motility, size and shape of different species of bacteria at longer period.

Table -1: Screening of different species of bacteria by novel device.

Sr. No	Different species of bacteria	Motile	Non-motile	Time period of observation
1	*E.coli*	+	-	Increases
2	*Bacillus cereus*	+	-	Increases
3	*Salmonella typhi*	+	-	Increases
4	*Diplococcus pneumoniae*	-	+	Increases
5	*Vibrio cholerae*	+	-	Increases
6	*Pseudomonas aeruginosa*	+	-	Increases
7	*Clostridium*	-	+	Increases

	sporogenes			
8	*Yersinia psedotuberculosis*	-	+	Increases

3. CONCLUSION

Apparently, this presenting apparatus will helpful for both young researchers and teaching assistant for laboratory classes especially for investigators from other disciplines dealing with bacteriology. By the use of this apparatus, the bacterial motility, true size and shape are easily observed. The time of period of observation increases for observation of different species of bacteria. It is sophisticated for identification, classification of different species of bacteria and effective methods for complete motility understanding.

4. FUTURE SCOPE OF RESEARCH

The next stage of research is to study how to detect bacterial motility by using this device with integrating with Microsoft Windows operating system will execute the processes to reduce the time required to bacterial motility observation, classification and diagnosis.

Further, after execution, the study of how this device manages to change contexts of processes with digital image processing for observation of bacterial motility will also be interesting.

ACKNOWLEDGEMENT

The authors thank R & D team of SAGLO Industry for development of this device.

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