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Aim: To Correlate Spatial Pattern of Colonies on Culture Plate with Antibiotic Sensitivity by MIC Method on BD Phoenix and BD Kystra Systems

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Abstract: ***The Study tries to correlate spatial patterns of colonies on culture plate as in Primary, Secondary and Tertiary Streak Lines; Symmetrical, Asymmetrical with antibiotic profile (Shown as resistant/ Sensitive/ Intermediate) in MicBd Phoenix and BD Kystra Systems. Virulence of Bacteria by PCR Testing of Virulence Genes, Toxin studies are put to use to confirm positive correlation. While Gram Staining, the spatial pattern seen on slide is a mirror image of tissue pathology. This is suggestive as friction coefficient of solid and liquid part of patient sample is proportional to three dimensional pathology of hollow organ as in radiology images (imprinting, single cell imaging, precision medicine***

Keywords: Single Cell Medicine, Precision Medicine, BD Kystra

1. Introduction

Fluid mechanics is involved in the growth, progression, metastasis, and therapy of cancer. Blood vessels transport oxygen and nutrients to cancerous tissues, provide a route for metastasizing cancer cells to distant organs, and deliver drugs to tumors. The irregular and leaky tumor vasculature is responsible for increased interstitial pressure in the tumor microenvironment, whereas multiscale flow-structure interaction processes control tumor growth, metastasis, and nanoparticle-mediated drug delivery. We outline these flow-mediated processes, along with related experimental and computational methods for the diagnosis, predictive modeling, and therapy of cancer. A standard teaching amongst infectious disease specialists is that pneumonia due to Candida species is a “very rare event”. These organisms regularly colonize the mouth, and their numbers can increase if antibiotics are given. When yeast forms are seen on Gram stain and Candida are grown in sputum cultures, they are generally dismissed as oropharyngeal contaminants from colonizing organisms. However, in severely immune compromised patients, Candida spp. is known to invade locally causing thrush or, occasionally, pneumonia.

Differentiation refers to the series of steps that a cell goes through to become mature. As a cell develops, it begins to show its own purpose and role in the human body, a process known as differentiation.

Cells may be immature because they are rapidly growing from a new start, such as in the development of a baby in the womb; however immature cells that lack differentiation also occur normally in adults for instance, in tissues and organs that constantly replace old cells with new ones, such as the bone marrow[1].

Fully differentiated cells are the ones we learn about in basic biology: red blood cells, brain cells or neurons or muscle cells, for instance. Differentiation is the process that shapes the immature cell’s destiny, determines the cell’s distinct role and results in specific characteristics tailored to the adult cell’s purpose[2]. A skin cell is unlike a blood cell, for example. A mature, well-differentiated cell usually has a very specific role to play, with characteristics typical of the organ or tissue where i

Differentiation in Cancer

In cancer, the process of differentiation may not occur normally. Cancer cells may be stuck in one phase of differentiation, may be less developed and may not function as well as the surrounding, healthy cells[3]. In fact, sometimes these cells are so poorly differentiated that, under a microscope, they don’t even look like the cells that they developed from.

Pathologists are doctors who are trained to analyze cells and tissues, such as those submitted in biopsy specimens, to make a determination about the disease[4]. It used to be that pathologists relied heavily on what’s called morphology how the cells looked under the microscope: the size, shape or richness of color when special dyes and stains were applied[5].

This is still done and yields important information about differentiation, but now there are other tests that are used as well. These tests can identify specific molecules on the outside of the cells that can sometimes be used to tell how well differentiated a cell is.

2. Material and Methods

Microscope, BD PHOENIX MACHINE AND MIC PANELS, BLOOD AGAR, MACCONKEY AGAR, MUELLER HINTON AGAR,CLED AGAR,BACTERIAL

ENDOTOXIN KIT, GRAM STAIN KIT, WIRE LOOP.

BUNSrine Culture Using the Calibrated Loop/Surface Streak Method

Urine culture is the gold standard for diagnosing UTI. Different versions of the calibrated loop/surface streak method have been used since the 1960s to semi-quantify, isolate and start a presumptive identification of the microorganism(s) present in a urine specimen[5]. It is important to iterate that all samples should first be subject to a dipstick testing and/or microscopic examination to look for the presence of nitrites, white blood cells, red blood cells or bacteria[6, 7].

Step-by-Step Procedure of the Calibrated Loop/Surface Streak Method

1) Tip over the container to re-mix the urine sample.
2) Remove the cap and dip the end of a sterile 1-µL inoculating loop (white) into the urine and remove it vertically making sure that there is no urine up the loop.
3) Tip and spread the inoculum over the surface of a standard nutrient agar plate (60 × 15 mm) prepared according to the instructions of the manufacturing company.
   - Make a single streak across the centre. Then, spread the inoculum evenly distributed in a cross-zigzag arrangement to the primary streak, as shown in **Figure 1**.
4) Re-dip the end of the same 1-µL loop into the urine and remove it vertically making sure that there is no urine up the loop.
5) Tip and spread the inoculum over the surface of a glucose-topped MacConkey agar plate (60 × 15 mm). Spread as described above. Prepare the glucose-topped

MacConkey Agar Plates as following

1) Disinfect the port of a bag of 5% glucose intravenous infusion solution (1000 mL) with 70% isopropyl-alcohol-impregnated cotton ball or pad and allow to dry.
2) Aspirate 2 mL of the 5% glucose solution using a sterile needle and syringe.
3) Drop the aspirated solution on the surface of a standard MacConkey agar plate (60 × 15 mm) prepared according to the instructions of the manufacturing company.
4) Spread it by tilting the plate in different directions.
5) Leave the plate on the bench at room temperature for at least 1 h in order to allow the solution to infuse and the surface to dry.
   - Re-dip the end of the same 1-µL loop into the urine and remove it vertically making sure that there is no urine up the loop.
   - Tip and spread the inoculum over the surface of a standard MacConkey agar plate (60 × 15 mm) prepared according to the instructions of the manufacturing company. Spread as described above.
   - Incubate the plates aerobically at 35–37 °C for at 18–24 h.
6) In the following day, count the number of colonies on the surface of each medium. Each colony growing on the agar plate represents one colony forming unit (cfu)/µL (according to the size of the loop), which is equal to 1000 cfu/mL. Remember that nutrient agar is the primary medium used for counting colonies.

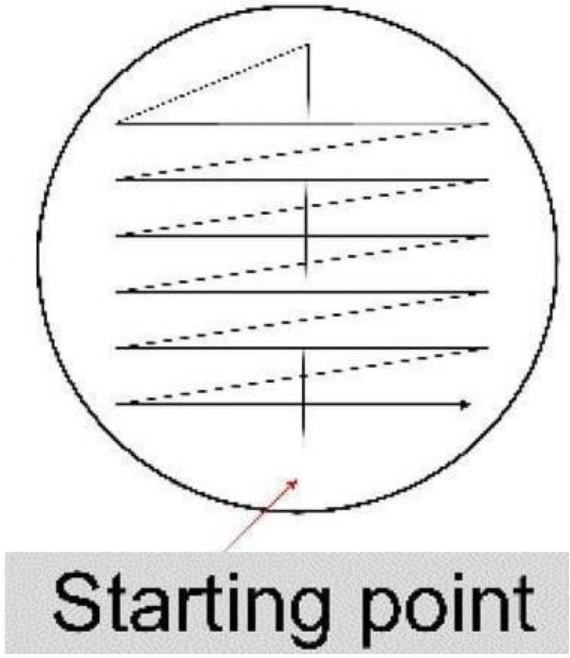

Figure 1: Urine culture using the calibrated loop/surface streak method.

General Purpose Media Are Sufficient for Urine Culture in Low-Resource Settings

According to traditional guidelines, blood agar (non-selective medium) and MacConkey agar (selective and differential for Gram-negative rods) are probably the most commonly recommended and used media for routine urine cultures8. As an alternative, cysteine lactose electrolyte-deficient (CLED) agar or chromogenic agar have been proposed as standard media for urine culture[6]. Sabouraud agar should be added, in addition to the usual bacterial media, to culture the urine of patients in particular care units or if yeasts have been seen by microscopic examination[6]. In this guideline, we propose using nutrient agar, MacConkey agar, and glucose-topped MacConkey agar for the routine urine culture. However, the choice of media for routine urine culture should be made locally based on available resources and the desired approach of identification. Blood agar is replaced by nutrient agar in order to keep the costs low and since Gram-negatives have frequently accounted for the majority of anticipated pathogens.

Only if needed, blood agar will be applied as part of the Level-2 advanced bacterial identification for Gram-positives. MacConkey agar is a commonly used medium for other bacterial cultures, such as pus and cerebrospinal fluid. MacConkey agar with glucose facilitates rapid differentiation between glucose-fermenters (mainly *Enterobacterales*), which will grow as pink colonies regardless of their ability or disability to ferment lactose, and the non-fermenters (such as *Pseudomonas* spp. and *Acinetobacter* spp.), which will always grow as colourless colonies. Importantly, our protocol does not aim to undervalue or discourage the use of CLED and Chromogenic Agar. It rather provides an alternative approach.

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Gram Staining Procedure

The gram staining procedure involves four major steps; staining with crystal violet, fixing the dye, applying a decolorizer, and counterstaining Flood air dried, heat fixed smear of cells for 1 minute with crystal violet staining reagent. Please note that the quality of the smear (too heavy or too light cell concentration) will affect the Gram Stain results.

- Wash slide in a gentle and indirect stream of tap water for 2 seconds.
- Flood slide with the mordant: Gram's iodine. Wait 1 minute.
- Wash slide in a gentle and indirect stream of tap water for 2 seconds.
- Flood slide with decolorizing agent (acetone-alcohol decolorizer). Wait 10-15 seconds or add drop by drop to slide until the decolorizing agent running from the slide is clear.
- Flood slide with a counterstain, safranin. Wait 30 seconds to 1 minute.
- Wash slide in a gentile and indirect stream of tap water until no color appears in the effluent.

Allow the slide to air dry by tilting it onto a paper towel or over a sink. Alternatively, gently dry the slide by blotting it using a lint-free bibulous paper. Please do not use a wiping motion, as it can remove the smear.

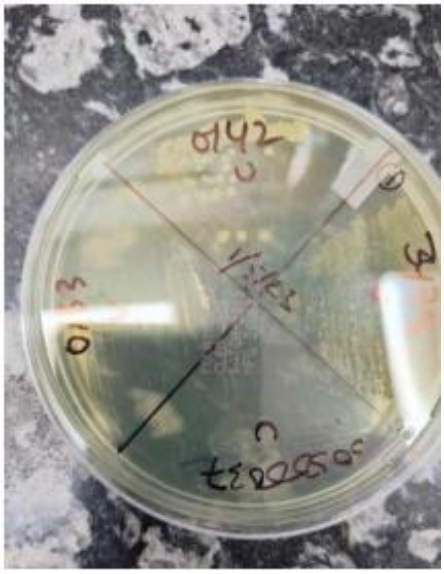
The slide is now ready to view under the microscope. First, focus on the image using the high dry objective lens marked 40x. Then, without removing the slide, switch to the high-power oil immersion objective lens marked 100x. Use immersion oil and observe the staining procedure's results under oil immersion (100x) using a bright field microscope. This will result in an overall magnification of 1,000x.

SAMPLE 245: NORMAL GROWTH COLONIES SEEN ON CULTURE PL1A4TE8

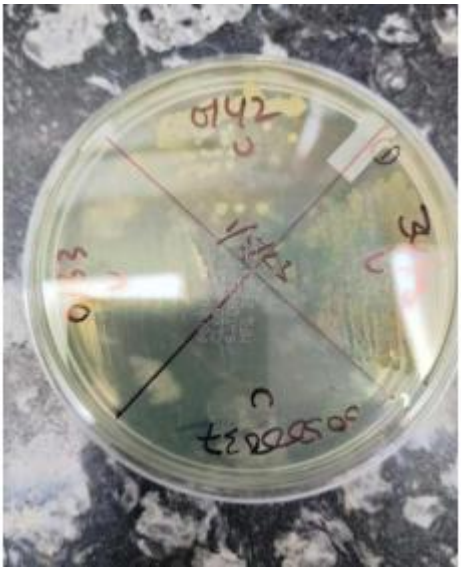
Gram stain were done on culture plate significant growth 100000

Sample Report: BD Phoenix Mic Report

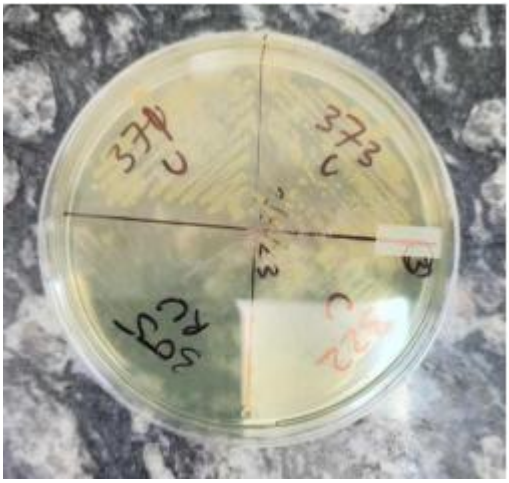
Mixed Growth Colonies Seen on Culture Plate



Sample 0142: Colonies Growing Placed sparsed pattern on Culture Plate

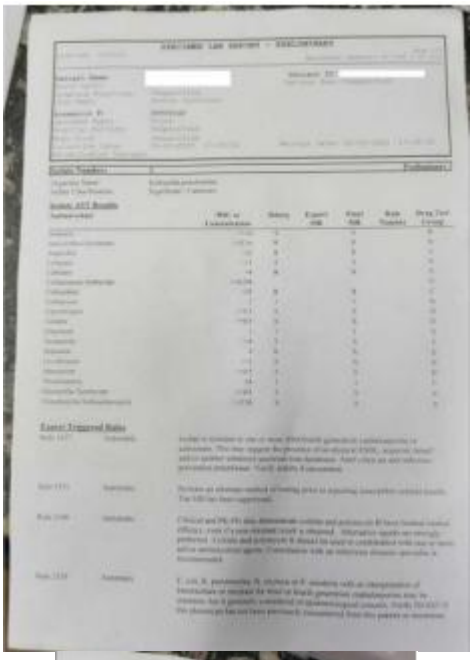


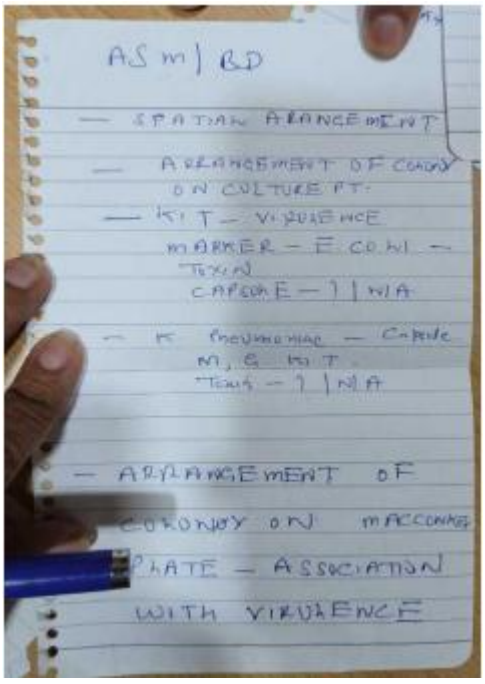
Sample 3475: Low Confluent Growth Seen On Culture Pla

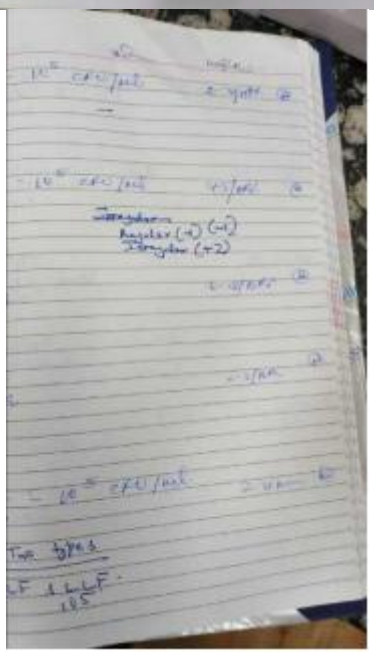


373 U: COLNIES PLACE GROWTH EVEN PATTERN REGULAR ON CULTURE PLATE

Antibiotic result showing maximum senstive in well sparsed colony on culture plate



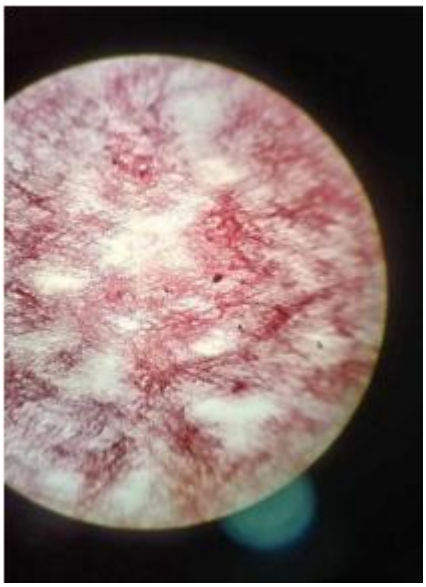




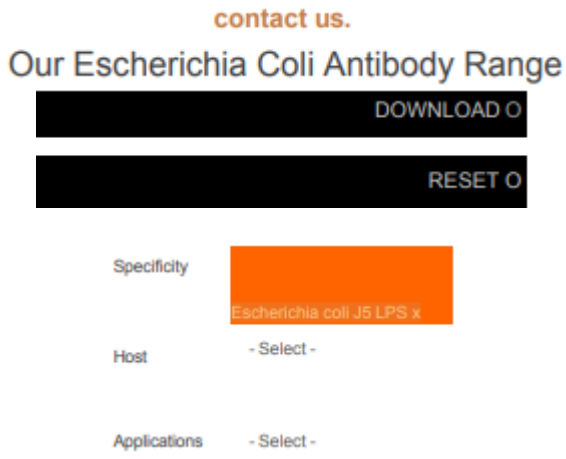
Antibiotic Profile Showing Odd/Even Sensitive/Resistant In Evenly Distributes Colony Pattern On Culture Plate

U5434: LESS DENSE GROWTH COLONIES SEEN ON CULTURE PLATE

U 5326: PATTERN OF GROWTH OF COLONIES: WELL SPARSED



Please use the filters to sort the attributes in the table below in order to find the antibody that fits your exact requirements. If you need any further assistance please do not hesitate to



Circular shape bacterial growth seen on cututre plate

allowing toxin elaboration at colonization sites (e.g., the presence ot £ tampon) suffice for initiation of clinical illness.

The *S. aureus* Genome

The entire genome has been sequenced for numerous strains of *S. aur* interesting revelations are (1) a high degree of nucleotide sequence simil different strains; (2) acquisition of a relatively large amount of genetic horizontal transfer from other bacterial species; and (3) the "pathogenicity" or "genomic" islands—mobile genetic elements g enterotoxin and exotoxin genes or antimicrobial resistance determine these islands are those carrying mccX, the gene responsible for methi Methicillin resistance-containing islands have been designated *staphyl cfjromosonje mecs {SCCmecs)* and range in size from -20 to 60 kb. To dat have been identified. Type 4 and type 5 SCC/necs have been associated A acquired MRSA strains.

A limited number of MRSA clones have been responsible for most commur associated infections worldwide. A comparison of these strains with thi outbreaks (e.g., the phage 80/81 strains from the 1950s) has revealed pre nucleotide sequence over time. This observation suggests that these determinants facilitating survival and spread.

Regulation of Virulence Gene Expression

In both toxin-mediated and non-toxin-mediated diseases due to 5. *aureus*, the virulence determinants associated with infection depends on a series of regul: accessory gene regulator and staphylococcal accessory regulator (sar)] control the expression of many virulence genes. The regulatory gene *agr* is p sensing signal transduction pathway that senses and responds to bacterial der3 Staphylococcal surface proteins are synthesized during the bacterial exponen phase in vitro. In contrast, many secreted proteins, such as toxin, the enteroto assorted enzymes, are released during the postexponential growth phase.

It has been hypothesized that these regulatory genes serve a similar function i Successful invasion requires the sequential expression of these different bact< Bacterial adhesins are needed to initiate colonization of host tissue surfaces. 'release of various enzymes enables the colony to obtain nutritional support ai bacteria to spread to adjacent tissues. Studies with mutant strains in which thi genes are inactivated show reduced virulence in several animal models of *S.*.

Diagnosis

Staphylococcal infections are readily diagnosed by Gram's stain (Fig. 129-1) examination of abscess contents or of infected tissue. Routine culture of : usually yields positive results, and blood cultures are sometimes post infections are localized to extravascular sites. Polymerase chain reaction (PC have been applied to the rapid diagnosis of 5, *aureus* infection and are ina clinical microbiology laboratories. To date, serologic assays have not prov

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	CONTROL STANDARD ENDOTOXIN 500NG/ VIAL Make: Wako	**1**
	Lysate Reagent water 125 ml Make: Wako	**1**
	Micropipette Tips 2-200 ul Sterile indivally wrapped	**00/P**

3. Discussion

Figure 1: Gram Stain Bal fluid (Pink Air Vacoules and Mucin correspond to mucous in lungs and air spaces)

The study tries to correlate spatial patterns of colonies on culture plate as in primary, secondary and tertiary streak lines; symmetrical, asymmetrical with antibiotic profile (shown as resistant/ sensitive/ intermediate) in Mic BD phoenix and BD Kystra systems.

Virulence of bacteria by PCR testing of virulence genes, toxin studies are put to use to confirm positive correlation.

While gram staining, the spatial pattern seen on slide is a mirror image of tissue pathology. This is suggestive as friction coefficient of solid and liquid part of patient sample is proportional to three dimensional pathology of hollow organ as in radiology images (imprinting, single cell imaging, precision medicine)

F**ollow up:** to be done on BD kiestra system with statistics correlation.

Competing Interests Statement:
I declare that I have no significant competing financial, Professional or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

Jayant Balani

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[3] Sample Reports BD PHOENIX MIB Reports Culture Plates And Bacterial Growths Register Entry: Types of Colonies
[4] LYMPH NODE : ARRANGEMENT OF POLYMORPH CELLS
[5] Escherichia Coli Antibody Range
[6] Geno-Sen's Staphylococcus aureus (Real Time PCR Kit Quantitative)