EVALUATION OF PERFORMANCE OF FILMARRAY AND MICROSCAN TECHNOLOGIES IN IDENTIFICATION OF BLOOD STREAM INFECTION PATHOGENS AND THEIR ANTIBIOTIC RESISTANCE

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Evaluation of Performance of Filmarray and Microscan Technologies in Identification of Blood stream infection pathogens and their Antibiotic Resistance

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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This thesis has been submitted for examination with our approval as the university supervisors

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DEDICATION

I dedicate this work to my wife Jackline Mumbi who has been my encouragement to achieve my professional goal, and to my children Brian Kibet, Blessings Chebet and Ivy Cherotich whose unending love and support make everything I do possible.

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ABBREVIATIONS AND ACRONYMS

API	Analytical profile index
AST	Antimicrobial susceptibility testing
BAP	Blood agar plate
BCID	Bacteria culture identification
BSC	Biosafety cabinet
BSI	Bloodstream infection
CAI	Community acquired infection
CBA	Chocolate blood agar
CI	Confidence interval
CLSI	Clinical and laboratory standards institute
DNA	Deoxyribonucleic acid
ESBL	Extended spectrum beta lactamase
FA	Film Array
FDA	Food and Drug Administration
H_2S	Hydrogen sulfide
HAI	Hospital acquired infection
HEA	Hektoen enteric agar
HIV	Human immunodeficiency virus
ICU	Intensive care unit

IND	Indole
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
KPC	*Klebsiella Pneumoniae* Carbapenemase
MAC	MacConkey agar
MALDI- TOF	Matrix Assisted Laser Desorption Ionization Time of Flight
MHK	Microbiology hub Kericho
MIC	Minimum inhibitory concentration
MRSA	Methicillin resistant staphylococcus aureus
MS	MicroScan
MSSA	Methicillin sensitive *Staphylococcal aureus*
PBP	Penicillin-binding protein
PCR	Polymerase Chain Reaction
PH	Potential of Hydrogen
QC	Quality control
SCC	Staphylococcal cassette chromosome
SDA	Sabouraud dextrose agar
SF	SeptiFast
TAT	Turnaround time

TDA	Tryptophane deaminase
TSI	Triple sugar iron
UKNEQAS	United Kingdom National External Quality Assessment Service
USA	United States of America
USAMRD-A/K	United States Army Medical Research Directorate-Africa/Kenya
UTI	Urinary tract infection
VP	Voges proskauer
WHO	World Health Organization

ABSTRACT

Bloodstream infection (BSI) is a major cause of mortality with rates of 53% in sub-Saharan Africa. BSI is marked by the presence of bacterial and/or fungal microorganisms in the blood, which elicit an immunological response. The major pathogens responsible for BSI in both children and adults worldwide are bacteria, and fungi. These infections require quick, reliable and accurate diagnosis to aid in a more precise antimicrobial therapy to the patient. This study was carried out to identify primary BSI pathogens using FilmArray® (FA®) and MicroScan WalkAway 40 Plus® (MS®) technologies. The performance of these technologies was compared with culture based plus Analytical profile index (API) technique used as the gold standard using blood samples from patients attending Kisii and Homa Bay county referral hospitals. FA® technology identified the following BSI pathogens; *Enterococcus faecium, Staphylococcus aureus, Streptococcus pneumoniae, Enterobacter cloacae, Acinetobacter baumanii, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Candida albicans, Candida krusei,* and *Candida parapsilosis*. MS identified the following BSI pathogens to species level *Enterococcus faecium, Staphylococcus aureus, Staphylococcus capitis, Staphylococcus mutants, Staphylococcus epidermidis, Staphylococcus auricularis, Staphylococcus saprophyticus, Staphylococcus hominis, Staphylococcus lugudensis, Staphylococcus sciuri, Staphylococcus intermedius, Streptococcus pneumoniae, Streptococcus anginosus, Streptococcus bovis, Enterobacter cloacae, Acinetobacter baumanii, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Candida albicans, Candida krusei*, and *Candida parapsilosis.* The culture and API identified the following BSI microorganisms; *Enterococcus faecium, Staphylococcus aureus, Staphylococcus capitis, Staphylococcus simulans, Staphylococcus epidermidis, Staphylococcus auricularis, Staphylococcus saprophyticus, Staphylococcus hominis, Staphylococcus lugudensis, Staphylococcus sciuri, Staphylococcus intermedius, Streptococcus pneumoniae, Enterobacter cloacae, Acinetobacter baumanii, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Candida albicans, Candida krusei,* and *Candida parapsilosis.* The sensitivity and specificity of the three technologies were determined. The FA technology was able to identify *Enterobacter cloacae* with higher sensitivity of (100%, 4/4) than the MS technology and API which identified (50%, 2/4), this shows FA had higher sensitivity in identification of *Enterobacter cloacae*. The overall specificity of FA, MS and API was 99.04% (95% CI: 96.59-99.88%), sensitivity of 98.68% (95% CI: 95.33-99.84%). The MS had overall specificity of 98.56% (95% CI: 95.86-99.70%), sensitivity of 98.68% (95% CI: 95.30-99.84%). The FA technology had limitation in identifying *Staphylococcus capitis, Staphylococcus simulans, Staphylococcus epidermidis, Staphylococcus auricularis, Staphylococcus saprophyticus, Staphylococcus hominis, Staphylococcus lugudensis, Staphylococcus sciuri, Staphylococcus intermedius, Streptococcus anginosus*, and *Streptococcus bovis*, these organisms are not in the FA data base and they are majorly skin contaminants, also FA had limitation in identifying gram positive rods these are also not in its data base. MS and API had limitation in identifying some *Enterobacteria cloacae* However, FA had advantage of identifying resistant genes, these are mecA (methicillin), vanA/B (vancomycin) and KPC (carbapenems). FA also identifies coinfection of more than one pathogen. MS and API had advantage of expanded database allowing it to identify more BSI pathogens than FA. In this study there were no resistance

genes detected from the identified BSI pathogens, However, these were demonstrated using control isolates across the technologies under test; *Enterococcus faecium, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus haemolyticus, Salmonella typhi* and *Escherichia coli*. The FA and MS technology have not been used in public hospitals and have not been evaluated locally, further evaluation using a larger number of samples is recommended. FA and MS can be a good tool in rapid identification of blood stream infection which culminates in reduction of hospital stay and cost with better management of patients and reduction of misuse of drugs.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Bacteremia accounts for a large number of hospital admissions as a source of high morbidity and mortality throughout the world (Dagnew et al., 2013; Peker et al., 2018). In sub-Saharan Africa, Kenya included, there is limited information on bloodstream infection (BSI) leaving a vast gap in knowledge (Akoua-Koffi et al., 2015; Altun, Almuhayawi, Ullberg, & Ozenci, 2013; Kariuki et al., 2010). To close this gap newer diagnostic equipment will help in rapid diagnosis of BSI. Due to the urgent need to diagnose BSI, blood is one of the most critical specimens received in the microbiology laboratory for identification and antimicrobial sensitivity testing (AST) (Berkley et al., 2005; Prakash, Arora, & Geethanjali, 2011).

Blood culture has been the gold standard method for diagnosis of bacteremia in most hospitals throughout the world including Kenya (Kariuki et al., 2010; Peker et al., 2018; Prakash et al., 2011). The conventional culture and API was chosen as a gold standard for this study because this is the current standard identification method for blood stream infection pathogens in public hospitals in Kenya. Analytical Profile Index is a developed test kit for identification of Gram positive and Gram-negative bacteria and yeast. It contains 20 microtubes biochemical tests containing dehydrated substrates to detect the enzymatic activity or the fermentation of sugars by the inoculated organisms. During incubation, metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. When the carbohydrates are fermented, the pH within the cupule changes and is shown by a colour change.

Analytical Profile Index was invented in the 1970s in the United States by Pierre Janin of Analytab Products, Inc. currently the API test system is manufactured by BioMerieux. In 1977 It has been modified to include identification of non-fermentative gram-negative bacteria (Maina, Okinda, Mulwa, & Revathi, 2014).

However, the use of conventional diagnostic techniques to identify bacteria in blood culture down to species level has been a challenge, especially since conventional techniques are less sensitive as compared to PCR based methods and often require experienced laboratory technologists (Altun et al., 2013; Berkley et al., 2005; Kang et al., 2020). The automated technologies such as FA and MS provide rapid, sensitive identification, and minimize chance of misdiagnosis; these techniques are approved for use in the United States of America by the United States Food and Drug Administration (US-FDA) (Altun et al., 2013; Blaschke et al., 2013; Kang et al., 2020).

FilmArray is a sophisticated closed automated system multiplex polymerase chain reaction (PCR) that can identify BSI causative agents and antimicrobial markers within 1 hour of positive blood culture (Altun et al., 2013). The FA system software automatically controls lysis and extraction of DNA from the positive blood culture sample. With the DNA, the FA system can amplify it in order to perform analysis for identifying the pathogen. FA can identify 24 causative agents of BSI, 8 Gram positives, 11 Gram negatives, 5 yeast species and 3 antimicrobial resistance genes mecA, vanA/B and KPC from positive blood culture (Blaschke et al., 2013; Ray, Drew, Hardiman, Pizer, & Riordan, 2016).

MicroScan system has also been used for identification of blood-borne bacterial pathogens. MicroScan plus system was introduced into diagnostic microbiology laboratories by Siemens Medical solutions Diagnostics in 2007. The MS system offers simultaneous automation of overnight, rapid and specialty panels that test for both Gram negative and Gram-positive bacteria. MicroScan is able to identify microorganisms within 16 to 20 hours (Ombelet et al., 2021). The MS system also does this by combining conventional methods of biochemicals for microorganism identification**.** Identification of yeast by MS takes four(Ombelet et al., 2021). In comparison, FA takes one hour in identification of yeast and bacterial etiologies (Fhooblall, Nkwanyana, & Mlisana, 2016).

The following pathogens *Staphylococcus aureus, Candida albicans, Streptococcus pneumoniae, Enterococcus faecium* and *Salmonella typhi* are the

common causes of BSI and they mostly affect vulnerable group including the elderly and immunocompromised individuals. Newborns are also amongst the affected due to their lower immunity thus they often acquire BSI during birth or after birth due to lack of proper sanitation (Köten et al., 2012 ; WHO, 2011).

Diagnosis of BSI pathogens is very important in determining the course of treatment for the patient. The isolation of very important causative agents of BSI aid in determination in bacterial resistant patterns and infection trends in different localities (Kang et al., 2020; Swain & Otta, 2012). In management and diagnosis of these blood infections conventional culture media technique has been the gold standard method for diagnosis of bacteremia in most hospitals in Kenya (Dagnew et al., 2013; Kariuki et al., 2010; Maina et al., 2016).

The use of conventional diagnostic techniques to identify bacteria in blood culture down to species level has been a challenge, especially since conventional culture techniques are less sensitive in identification of BSI organisms down to genus and species level and this often requires experienced laboratory technologists and subjective interpretation (Altun et al., 2013; Kang et al., 2020).The FA and MS technologies have not been used in diagnosis of blood infections in Kenya. Their evaluation in this study was important in order to confirm whether they address the challenges encountered when using the gold standard.

1.1.1 Transmission of BSI

Most BSIs are contracted in hospitals (Markwart et al., 2020 ; WHO, 2011). BSI associated with catheter related urine tract infection (UTI) are the most common forms of hospital acquired infection (HAI), which are especially serious for patients in intensive care units (ICU) (Viscoli, 2016). Majority of these infections are caused by nosocomial infections mainly gram positives, gram negatives bacteria and yeasts (Dagnew et al 2013). These infections have been associated with long duration of stay in hospitals, and may vary according to section of hospitalization; that is, those in the general wards and ICUs are particularly susceptible to BSI (Kang et al., 2020).

Microorganisms causing HAI are mostly resistant to antimicrobials and are associated with high cost of treatment management, high treatment failure rates, increased circulation of antibiotic resistant strains, long hospital stay and negative impact to economies, particularly in the developing countries (Balkhy et al., 2006; Maze et al., 2018). Prompt and specific antibiotic response and understanding of the resistance patterns of the local pathogens therefore help to reduce mortality rates and control resistance to antimicrobials (Vlieghe et al., 2013).

1.1.2 Community acquired infection

These are infections diagnosed during hospital admissions in the outpatient section or infections acquired from the community, it could be due to an emerging infection in the community, majority of these patients present to outpatient clinics and hospitals with febrile illnesses (Myat et al., 2020) (Balkhy et al., 2006). Many of these patients require admission and treatment is normally based on clinical presentation alone, and so there is need for laboratory diagnosis in order to diagnose the real cause of febrile illness (Pradhan *et al.*, 2012). Methicillin resistant *Staphylococcus aureus* (MRSA) in particular has been associated with CAI (Williamson et al., 2013).

1.2 Statement of the Problem

Presently, Kisii Level Five and Homa Bay County referral Hospitals use conventional diagnostic techniques such as culture media in BSI identification from blood samples. However, these diagnostic methods are insensitive as compared to PCR methodologies and do not allow for accurate identification of BSI pathogens down to the species level, which compromises the efficacy of treatment regimens. Additionally, these conventional methods require considerably long turnaround time (TAT) from 12-72 hours. In potentially life-threatening cases of BSI, the microbial cause of infection must be identified as quickly as possible to ensure proper treatment and management of the disease. This automated methods FA and MS have never been used before in Kenya public hospitals and resistance genes have not been tested in these county referral hospitals.

1.3 Justification

Compared to the labor intensive and time-consuming techniques associated with the conventional culture media, new automated technologies such as FA and MS have fast TATs and offer increased specificity and sensitivity. This study was carried out using blood culture samples from patients attending Kisii Level Five and Homa Bay County Hospitals. These as county referral hospitals receive large number of patients with febrile illnesses yet they are ill-equipped to identify all the causative agents of bloodstream infections down to species level. These county referral hospitals use culture media for identification of BSI pathogens. This study therefore was able to identify the causative infectious pathogens of febrile illnesses using the automated technologies FA and MS. This study will aid the administrators of Kisii Level Five and Homa Bay county hospital and also hospitals in other counties of Kenya in choosing rapid diagnostic equipment for their microbiology laboratories hence assisting in management of the patients.

1.4 Hypothesis

1.4.1 Null Hypothesis

The use of FilmArray and MicroScan technologies does not improve identification of primary BSI pathogens in samples.

There are no resistance genes in BSI pathogens from Kisii and Homa Bay county hospitals.

1.5 Objectives

1.5.1 General objective

To identify primary BSI pathogens and antibiotic resistance markers in blood from patients attending Kisii and Homa Bay County hospitals using FilmArray®, MicroScan 40 plus®, and conventional culture and API biochemical technique.

1.5.2 Specific objectives

i. To identify primary BSI pathogens in samples collected from patients attending Kisii and Homa Bay county hospitals using BioFire FilmArray® and MicroScan 40 plus® and API technologies.
ii. To evaluate the sensitivity and specificity of BioFire FilmArray®, MicroScan 40 plus® and API in identification of BSI pathogens.
iii. To determine markers for methicillin, vancomycin and carbapenems resistance genes in selected primary BSI pathogens using FilmArray®.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bloodstream Infections

Bacteremia and candidemia are major causes of disease and death in most low income countries (Swain & Otta, 2012; Viscoli, 2016), with reported annual death rates of 20-50% worldwide (Dagnew et al., 2013). These infections require quick identification and antimicrobial susceptibility testing results to aid in a more precise antimicrobial therapy to the patient. Precise antibiotic treatment would reduce resistance of microorganisms to antibiotics and lower the high mortality rates associated with ineffective or nonspecific treatment approaches (Southern et al., 2015)

Blood culture and improved technologies such as FA and MS are essential in diagnosis and management of BSI (Arora et al., 2011,). Additionally they shorten TAT thus reducing on the number of deaths and costs associated with prolonged hospitalizations (Fhooblall et al., 2016).

2.1.1 Patient vulnerability

Newborns are among the most vulnerable to BSI due to their lower immunity thus they often acquire BSI during birth or after birth due to lack of proper sanitation (Viscoli, 2016; WHO, 2011). Consequently, about 10 million children aged below 5 years die worldwide each year (Macharashvili et al., 2009). Unfortunately, the young children have underdeveloped immunity which makes diagnosis of BSI difficult because there is no enough serological response to aid in diagnosis. Therefore, most clinicians resort to prescribing broad spectrum antibiotics that may result in antimicrobial resistance (Ballot, Nana, Sriruttan, & Cooper, 2012; Maina et al., 2016).

Similarly, the elderly persons are also more prone to BSI because of the decreased immune response and the clinical manifestation is not clear complicating the assessment and diagnosis (Wester et al., 2013). Patients with underlying diseases

and chronic conditions like cancerous tumors, leukemia, acquired immunodeficiency syndromes, renal failure, diabetes mellitus and those that use immunosuppressive drugs like steroids, irradiation, and malnutrition are also at an increased risk of BSI because these conditions lowers the immune response making them vulnerable to infections (Viscoli, 2016)

2.1.2 Environmental factors associated with BSI

Health care settings where sick persons intermingle freely with healthy subjects including health care workers pose a significant threat of transmitting pathogenic organisms (Kang et al., 2020). Poor infection control measures, such as concentrating patients with different ailments in one section coupled with frequently transferring patients from ward to ward can be a source of infection (O'Neill, Park, & Rosinia, 2018). Moreover, waterborne diseases, viruses and microbial flora which contaminate objects, examination devices like stet scope act as source of infection especially when they are used on patients without decontaminating it first or if patients come in contact with these objects (Ducel et al., 2002).

2.1.3 Microbial factors associated with antimicrobial resistance

The likelihood of productive infection with blood borne pathogens depends on the virulence of the pathogen, enzymatic inactivation of antibiotics by bacteria, alteration of the antibiotic target site, acquisition of resistance through mutations in chromosomal gene or through plasmids; this could also depend on host's immune status and the microbe's infectious dose (Christopher et al., 2013). In addition the normal flora of the patient or contaminated surfaces by other patients can be a source of further transmission of antimicrobial resistant strains (Reygaert, 2018).

Furthermore, overuse of antimicrobial agents promotes the selection and exchange of resistance genes. This is particularly dangerous since antibiotic treatments suppress the normal flora, allowing resistant strains to thrive and contribute to further circulation of resistant strains in the community (Morrison & Zembower, 2020). This increased selective presence for resistant microbes has been a great

challenge to treatment of life-threatening BSI infections (Cecchini, Langer, & Slawomirski, 2015; Reygaert, 2018).

2.2 FilmArray® versus other rapid techniques

FilmArray technology was first developed by Idaho technology and later changed to BioFire Diagnostics. It was cleared for use by FDA in 2011, in 2013 the blood culture identification panel received approval from FDA. In 2014 BioFire merged with BioMerieux. FilmArray extracts and purifies nucleic acids from blood culture sample and performs a nested multiplex PCR, the internal control has to pass for the PCR process to continue. During the first-stage PCR, the FilmArray performs a single, large volume, massively multiplexed reaction. Last, individual singleplex second-stage PCR reactions detect the products from the first stage PCR, all PCR procedures are performed by the machine automatically in an enclosed system. The resulted PCR products are evaluated using DNA-melting analysis. FilmArray software automatically generates results for each target and the identified organism displayed on the computer monitor connected to the machine.

Many rapid techniques have been used in identification of BSI pathogens in place of conventional techniques among them is Septi*Fast*® (SF) M^{GRADE} test (Roche Diagnostics GmbH, Mannheim, Germany), this technique is based on multiplex real-time PCR technology and it takes six hours to BSI pathogen identification in contrast to FA which takes one hour to BSI pathogen identification. SF has high running cost and it is labor intensive, FA has low running cost compared to SF and it is less labor intensive, in addition FA is capable of identifying resistant gene while Septi*Fast*® techniques cannot (Burdino et al., 2014; Kothari, Morgan, & Haake, 2014).

Other rapid techniques which has been used in identification of BSI pathogen include HemoFISH® Gram positive and HemoFISH® Gram negative (miacom diagnostics GmbH Dusseldorf, Germany) which is a beacon-based fluorescent in situ hybridization based on fluorescently labeled oligonucleotide probes complementary to specific targets, this technique takes 45 minutes to BSI pathogen identification the difference between this technique and FA is that it uses different

kits in identification of pathogen, it has different kit for gram negative and different kit for gram positives unlike FA which uses one BCID panel for all BSI pathogen identification and this is an added advantage to FA as compared to this technique (Sakarikou *et al*., 2014).

MALD-TOF® MS, Matrix Assisted Laser Desorption Time of Flight, (Bruker Daltonik GmBh, Leipzig, Germany), in particular has been shown to have limitation in identification of yeast and other bacteria as compared with FA which is able to identify most of the *candida species*. The difference between these techniques is cost of running the test which is a higher for FA and time of running many samples which for FA a sample is run at a time (Paolucci et al., 2014). FA has the ability to identify antimicrobial resistant genes which MALD-TOF® cannot do; this goes a long way in assisting clinicians in deciding which line of treatment to follow (Altun et al., 2013). As opposed to MALD-TOF which has to be based in the laboratory for research work and diagnosis, FA on the other hand can be used in small setup hospitals and clinics or field assignments and diagnosis with limited labour resource (Inglis et al., 2016). From previous studies, MALDI-TOF has a challenge in identification of most gram positive organisms like *Streptococcus species* and *Staphylococcus species* which are notable causes of BSI (Inglis et al., 2016). Using MALDI-TOF For fungal identification, preparation of sample is different from bacterial preparation, and takes time with different steps unlike FA which uses only one BCID panel which has same procedure has for fungal (Altun et al., 2013).

Verigene system (NanoSphere, Northbrook, IL) is a qualitative genotyping assay PCR based rapid technique which integrates amplicon hybridization to gold-labeled nanoparticle probes on a microarray, followed by silver signal amplification and detection by measuring relative intensity of scattered light by a photosensor. It uses different cartridges in detection of Gram positive and Gram negative microorganisms in contrast to FA which uses single BCID panel in identification of different microorganisms and it is not dependent on gram reactivity of microorganisms unlike Verigene system which relies on gram stain in selection of appropriate cartridge to use, also Verigene system is less sensitive in

identification of important BSI pathogens like *Salmonella species* unlike FA which has accurate target for *Enterobactericeae*. The Verigene system identification time for BSI pathogen is two and half hours and FA identification time is one hour which is shorter than Verigene system making it more preferable rapid technique for BSI pathogen diagnosis (Bhatti, Boonlayangoor, Beavis, & Tesic, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Kisii Teaching and Referral Hospital is a public hospital located in Kitutu Chache Constituency, Kisii County. It is located in Kisii town Central Business, District Hospital Road, and serves a population of over 1 million. Homa Bay County Referral Hospital is a government health center located in Homa Bay Township Sub-location, Homa-Bay Location, Asego Division, and Rangwe Constituency in Homa Bay County. It has a population of over 1 million. The common diseases in these areas are malaria, upper respiratory tract infections, typhoid, pneumonia, tuberculosis and HIV (County Government of Homa Bay, 2017).

Figure 3.1: Homa Bay County Hospital location

(Source: Google Maps 2021)

Figure 3.2: Kisii Level 5 Hospital Location

(Source: Google Maps 2021)

Blood culture samples received from Kisii Teaching and Referral Hospital and Homa Bay County Referral hospital were analyzed at Microbiology Hub Kericho, a United States Army Medical Research Directorate-Africa/Kenya (USAMRD-A/K) facility working in collaboration with Kenya Medical Research Institute (KEMRI). These collection sites were selected because they do not have the capacity to identify BSI pathogens to the species level. They use culture media for identification of BSI pathogens.

3.1.1 Study design

Blood culture samples from both in and out-patient groups presenting with febrile illnesses to Kisii Level Five and Homa Bay County Referral hospitals with temperatures above 38˚C were collected by trained laboratory technologist. A total of 168 blood culture samples which met the consenting criteria were collected. A total of 152 positive blood culture samples were analyzed for BSI using FA, MS technologies and Conventional culture and API biochemical technique was used as a gold standard. Each blood culture sample was tested across the techniques. Gram positive rods and contaminant organisms were not analyzed across the techniques because FA and MS have no identification panels to identify these microorganisms**.** This was initially determined using gram stain.

3.2 Collection and transportation of blood culture samples

Collection of blood culture samples for identification of BSI pathogens was done before administration of antibiotics, these samples were collected by trained laboratory technologist. Blood culture bottle was labeled with patient identification and date prior to inoculating the BacTec bottle (peads plus, aerobic and anaerobic bottles). The cap was flipped- off from the vial top, inspected for any cracks then wiped with alcohol swab. Puncture site was cleaned with alcohol swab and/or tincture of iodine and allowed to air dry for 30 seconds. The needle was then inserted bevel-upwards into the vein and required volume of blood was withdrawn. Five to 10 ml of blood for infants and 10-20 ml for adults. The tourniquet was loosened and cotton wool ball was placed over the puncture site. The needle was removed gently while gently pressing the cotton wool ball. The needle used to draw blood was replaced with a new one and blood aseptically transferred into the blood culture bottles.

Prior to transportation of blood culture samples to the processing lab MHK, identification of patient was checked on the request form against the labeled blood culture bottles. Blood culture samples were then wrapped with absorbent material. Each vial was put in a ziplock bag, packaged in cool boxes with Styrofoam in an upright position. Blood culture samples were transported at room temperature overnight using local courier services. Samples were received at MHK within 48 hours of collection. Upon arrival, samples condition was checked and patient identification verified using request form against labeled blood culture bottles then entered into laboratory logbook.

3.3 Sample size determination

Formula $n = Z^2 \times P(1-P)/d^2$, n = Sample size, Prevalence (p) = 11%

95% confidence interval (z) = 1.96

Precision $d^2 = 0.05$

$n = 1.96^2 \times 0.11(1-0.11)/0.05^2$

Calculated sample size (n) = 150 isolates.

The prevalence was taken from the study by Maze et al., 2018, "The epidemiology of febrile illness in sub-Saharan Africa: implications for diagnosis and management". The prevalence rate is based on East Africa data which Kenya is part of. The prevalence is 11% in Kenya (Maze et al., 2018)

3.4 Inclusion criteria and Exclusion Criteria

All blood culture samples that showed growth of microorganism after incubation at 35ºC incubator (BacTec 9050® instrument) were analyzed. All BSI pathogens identified by API were used to evaluate performance of FA and MS. This showed that they could be used to take part in the study.

3.4.1 Exclusion Criteria

The blood culture samples also which did not meet the acceptance criteria of blood volume, missing identifiers and same identifiers belonging to different samples were rejected. Blood culture samples which did not show growth were not processed.

3.5 Sample processing for identification of BSI pathogens

Blood samples were collected into BACTEC Plus Aerobic/F, Peds Plus Aerobic/F, Anaerobic/F and Lytic/10 Anaerobic/F vials (BD, United States). Blood culture samples bottle were incubated into BacTec 9050 instrument (BD, United States) for 5 days to account for slow-growing pathogens. A positive signal was indicated by an increased fluorescence caused by the carbon dioxide released by an organism reacting with the vial dye. Positive blood culture samples were removed and processed to identify the organism. First the blood culture sample were drawn using 2ml syringe and a drop placed in a glass slide for gram staining. The gram reactivity determined the subculture media to be used. For gram positive microorganisms blood agar plate was used. For gram negative microorganisms MacConkey agar (MAC) and Hektoen enteric agar (HEA) were used. For yeast-like Sabouraud Dextrose agar (SDA) was used. Samples were processed directly for FA procedure without need for prior subculture or gram stain. The identification across the techniques was based on BSI pathogens identified by API to evaluate performance of FA and MS.

Figure 3.3: Blood culture samples and Bactec equipment

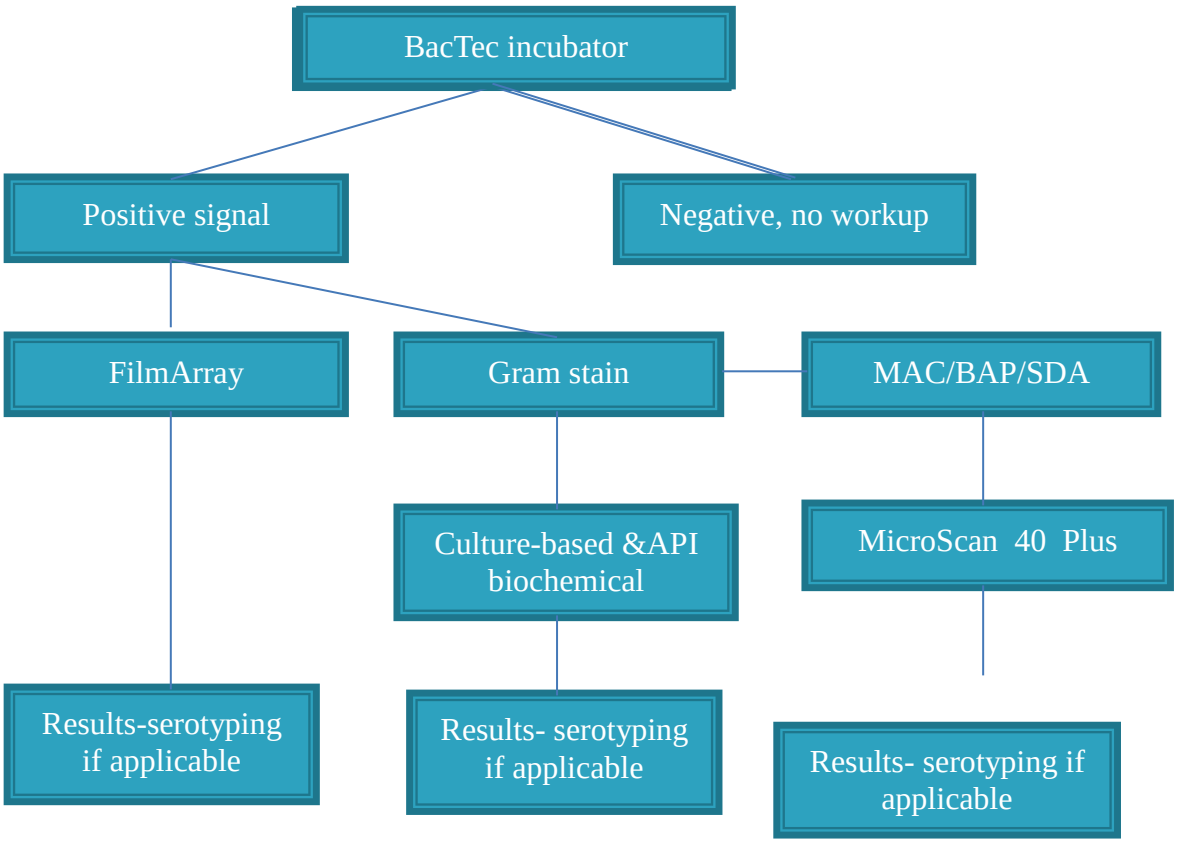

Figure 3.4: Flow chart showing the identification process of FilmArray, MicroScan and Culture and API

3.5.1 Gram stain procedure for identification of BSI pathogens

The procedure was done as per (Smith & Hussey, 2005). Gram stain slides prepared from positive blood culture samples were fixed a alongside one QC slide on the staining rack. The slides were then flooded with crystal violet stain (primary stain) for 1 minute. Then rinsed gently using tap water and excess water tipped off. Slides were again flooded with grams iodine (Mordant) for 1 minute and rinsed gently using tap water in a wash bottle and excess water tipped off. The slides were decolorized with acetone (Gram decolorizer solution) for 10-30 seconds. Then were gently rinsed using tap water in a wash bottle. Finally, slides were flooded with Grams Safranin for 1 minute, then gently rinsed using tap water and excess water was tipped off. Back side of the slides were wiped with 70% alcohol and air dried by

standing it on the slide drying rack. Slides were examined under oil immersion (100X objective). Interpretation, reporting and recording of results; Gram positive bacteria/yeasts: purple/ blue colour and Gram-negative bacteria: pink/ red colour. Gram stain results were reported as Gram positive or negative cocci, bacilli/rods.

3.5.2 Identification of BSI pathogens using culture and API method

Blood culture samples were incubated at 35ºC for 5 days in BACTEC 9050®. Blood culture bottle which signaled positive were gram-stained to determine gram reactivity that is gram positive or gram negative. Biochemical analysis method, the API strips (BioMerieux, United States) and media (MacConkey agar, blood agar plate, Hektoen enteric agar, triple sugar irons and Sabouraud dextrose agar plates) were brought to room temperature before use. The sample from positive blood culture was sub-cultured on suitable culture media and incubated for 24-48hrs. Five mL of deionized water was added to the tray along with the strips. The API ampules or equivalent suspension medium was inoculated with a single colony.

The inoculation of wells for gram negative using API 20E, gram positive using API 20 STAPH and API 20 STREP and yeast organisms using API 20C AUX was done as per manufacturer's instruction. Briefly, the bacterial suspension was inoculated into media microtubes and incubated at 35-37℃ for 18-24 hours. After the 24 hours incubation period, the following tests were spontaneous and were read immediately: Ortho nitro phenylβ-D-galactopyranosidase; Arginine dihydrolase; Lysine decarboxylase; Ornithine decarboxylase; Citrate; Hydrogen Sulfite; Urea; Gelatin; Glucose; Mannitol; Inositol; Sorbitol; Rhamnnose; Saccharose; Melibiose; Amylase and Arabinose.

For API 20E the following reagents were added, Tryptophane deaminase (TDA), Indole (IND) and Voges proskauer (VP) tests. For TDA test: A drop of TDA reagent was added and immediately checked for a reddish brown colour. ii) IND test: A drop of JAMES reagent was added and observed for a pink colour in the whole cupule. iii) VP test: A drop of VP 1 and VP 2 reagents were added incubated for 10 minutes to observe a pink or red color.

The Interpretation of API reactions were read in sets of three. For the positive tests they were awarded number of points as follows; In the first set a score of 1 was given followed by score of 2 and lastly a score of 4. If the reaction was determined negative a score of zero was given.; The 7-digit numerical profile was used to query the API database using the APIWEB identification software. Scores of ≥80% was considered high confidence and it was used for species identification. Microorganism identified only to genus level and needed further serotyped to get species level, this were sub-cultured on nutrient agar. (Appendix 2). The gram-positive rods were not worked up further.

Figure 3.5: API strip with a positive reaction

3.5.3 Identification of BSI pathogens using MicroScan 40 plus® procedure

Pure culture colonies were sub-cultured on MAC, BAP and SDA, 1 to 3 discrete colonies were picked using polyester tipped swab; this swab does not interfere with growth of bacteria. Then the swab with the colonies was suspended in 3 ml of inoculum water. This was mixed and checked for turbidity using turbidity meter which was equivalent to 0.5 McFarland (McFarland standards are used as turbidity standards in the preparation of suspensions of microorganisms). A volume of 100 µl of this solution was transferred and mixed with 25 ml of water with pluronic as recommended then poured into dry tray (D set tray). By use of inoculator Renok (multichannel pipette), 140 µl of suspended solution was transferred into either gram-negative combo panel or gram-positive combo panel, this was dependent on the panel to be used. The inoculated panels were then covered with the tray lid, bar-coded with patient name and loaded into the machine. The instrument then performed biochemical testing to determine microorganism identification. Microorganisms identified to family and genus level were further identified to species level using respective antisera typing reagent (Becton Dickson and

Company, Sparks, MD, USA). For *Salmonella species* serotyping, briefly, a drop of normal saline was placed into a clean glass slide, pure culture colonies were emulsified and checked for auto-agglutination. A drop of salmonella antiserum (35µl) reagent was added, mixed thoroughly and the slide was rotated for one minute while observing for agglutination which was indicative of positive results. The identified microorganisms were entered into laboratory book and microorganism stored in tryptic soy broth mixed with 50% glycerol at -80°C freezer for future reference.

A **B**

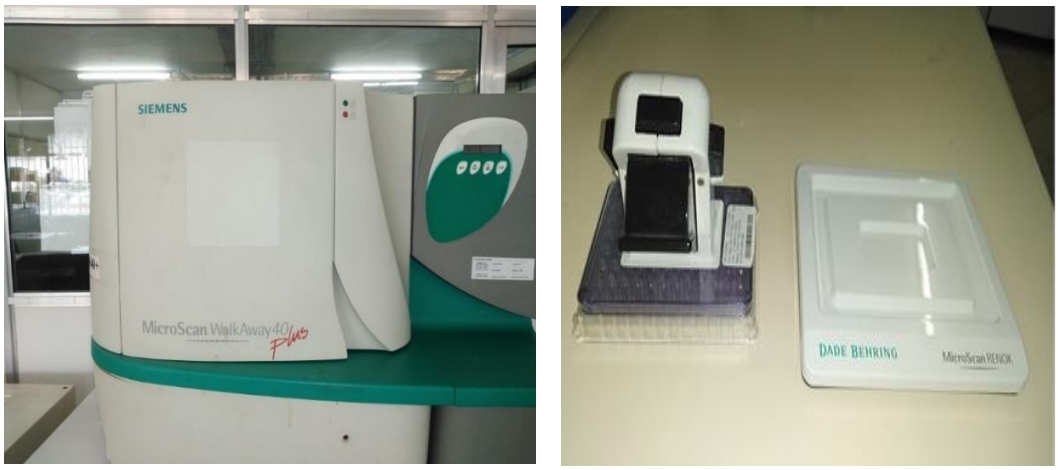

Figure 3.6: MicroScan Walkaway 40 plus technology and Renok pipette and BioFire FilmArray® procedure

3.5.4 Identification of BSI pathogens using FA procedure

A volume of 100 µl of broth was taken from the positive culture bottle and diluted in 500 µl sample dilution buffer. Then 300 µl of this sample solution was drawn and injected into the FA pouch for analysis. This reagent pouch stores all the necessary reagents for sample preparation in a freeze-dried format, plus the PCR primers, probes, enzymes, buffers and the internal control (*Saccharomyces pombe*) that are needed to isolate, amplify, and detect nucleic acid from blood culture sample. The loaded pouch was bar-coded and inserted into the instrument; bar-code was scanned

using a hand-held barcode reader to identify the sample identification in the pouch to the machine.

Microorganisms identified to family and genus level specifically as Enterobactericeae were further identified to species level using respective antisera typing reagent (Becton Dickson and Company, Sparks, MD, USA). For *Salmonella species* serotyping, briefly, a drop of normal saline was placed into a clean glass slide. Pure culture colonies were emulsified and checked for auto-agglutination. A drop of antiserum (35µl) reagent was added, mixed thoroughly and the slide was rotated for one minute while observing for agglutination which was indicative of positive results.

Figure 3.7: FilmArray BioFire technology

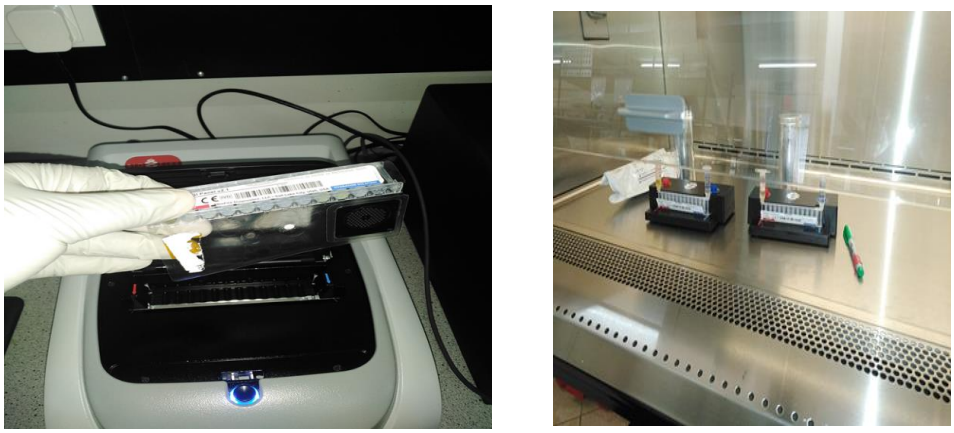

Figure 3.8: Blood culture identification panel

3.6 Identification of BSI pathogens Resistant genes

The genotypic markers for methicillin (mecA), vancomycin (vanA/B) and carbapenems (KPC) resistance genes in BSI pathogens were determined using FA technique, because it is a molecular technique. MicroScan and API are not molecular techniques and therefore were not used in determination of resistant genes. Known isolates from United Kingdom National External Quality Assessment Service (UKNEQAS) and seeded isolates previously determined to be resistant to vancomycin, carbapenem and methicillin antibiotics using molecular techniques were used to determine gene resistance. However, in this study the tested blood culture samples did not have the targeted resistant genes (vancomycin, carbapenem and methicillin).

3.7 Safety precaution

All blood culture samples were treated as potentially infectious and were processed under Biosafety level II cabinet (BSC II) and universal precaution was observed; donning on the right personal protective equipment while processing the samples.

3.8 Sensitivity and specificity of FA and MS technologies in identification of BSI pathogens

Sensitivity: Sensitivity was calculated using the following formula: sensitivity = number of samples with true-positive results/number of samples with true-positive results + number of samples with false-negative results × 100%.(Trevethan, 2017).

$$\textbf{Sensitivity} = \frac{\text{True positives}}{\text{True positives} + \text{False negatives}}$$

Specificity: The Specificity was calculated as the number of samples with true-negative results/ number of samples with true-negative results + number of samples with false-positive results x 100%. (Trevethan, 2017). Further statistical data analysis was calculated using Prism GraphPad version 8.0 (Tatsuno et al., 2019).

3.9 Data analysis

The sample size was based on the prevalence rate at the study carried out in East Africa, Kenya included which is 11%, the sample size was also dictated by the availability of resources to carry out the study. This test was done in order to obtain the accuracy of the FA and MS test which was going to be conducted in Kisii and Homabay County.

Identifications made using FilmArray®, MicroScan® and culture-based and API biochemical techniques were tabulated against each isolate tested and compared for diagnostic sensitivity and specificity. Sensitivity values and the 95% confidence intervals (CI) for both of these metrics were analyzed using the GraphPad Prism 8.0 software (Tatsuno et al., 2019). Presentation of identified BSI pathogens was determined and expressed using tables, pie charts and percentages.

CHAPTER FOUR

RESULTS

4.1 Primary BSI Pathogens

Out of 168 blood culture samples which met inclusion criteria were accessioned and processed for BSI pathogens identification. A total of 152 blood culture samples were positive for BSI and 16 were negative for BSI and were not processed further. One negative blood culture sample came from Kisii Level 5 Hospital and 15 negative blood culture samples came from Homa bay County Referral Hospital. The rejected samples were not accessioned and hence not processed for BSI identification. Out of 168 blood culture samples, 100 blood culture samples were collected from Kisii and 68 blood culture samples were collected from Homa bay.

From Kisii the following BSI pathogens were identified; *Enterococcus faecium* 6/99, *Staphylococcus aureus* 7/99, *Streptococcus pneumoniae* 6/99, *Enterobacter cloacae* 4/99, *Acinetobacter baumanii* 5/99, *Pseudomonas aeruginosa* 4/99, *Salmonella typhi* 26/99, *Escherichia coli* 5/99, *Klebsiella oxytoca* 2/99, *Klebsiella pneumoniae* 9/99, *Candida albicans* 6/99, *Candida krusei* 1/99, and *Candida parapsilosis* 0/99, *Staphylococcus capitis* 1/99, *Staphylococcus mutants* 1/99, *Staphylococcus epidermidis* 2/99, *Staphylococcus auricularis* 3/99, *Staphylococcus saprophyticus* 1/99, *Staphylococcus hominis* 1/99, *Staphylococcus lugudensis* 2/99, *Staphylococcus sciuri* 1/99, and *Staphylococcus intermedius* 1/99 *Streptococcus bovis* 0/99 and *Streptococcus anginosus* 0/99. (Table 4.1).

Table 4.3 shows how FA, MS and API identified each BSI organism. From Homa bay the following BSI pathogens were identified; *Enterococcus faecium* 2/53, *Streptococcus pneumoniae* 1/53, *Acinetobacter baumanii* 1/53, *Pseudomonas aeruginosa* 5/53, *Salmonella typhi* 31/53, *Escherichia coli* 3/53, *Klebsiella pneumoniae* 2/53, *Candida albicans* 3/53, and *Candida parapsilosis* 1/53, *Staphylococcus epidermidis* 1/53, *Staphylococcus auricularis* 1/53, *Streptococcus bovis* 1/53 and *Streptococcus anginosus* 1/53. Table 4.2 shows how FA and MS identified each BSI pathogen. On individual BSI pathogen identification, FA was

able to identify 4/4 of *Enterobacter cloacae* microorganisms. MicroScan and culture-based and API biochemical technique identified 2/4 of *Enterobacter cloacae* microorganisms. FilmArray and MS identified *Staphylococcus aureus* down to the species level this agreed with API the gold standard. The following staphylococcus were not identified to species level by FA as compared to MS and API gold standard; *Staphylococcus capitis, Staphylococcus mutants, Staphylococcus epidermidis, Staphylococcus auricularis, Staphylococcus saprophyticus, Staphylococcus hominis, Staphylococcus lugudensis, Staphylococcus sciuri, Staphylococcus intermedius.* These organisms were identified by MS and API technology down to species level and therefore they were not included as part of analysis across the tested technologies. FilmArray could not also identify *Streptococcus anginosus* and *Streptococcus bovis,* these organisms were identified by MS down to species level. These organisms are not in FA data base. *Salmonella typhi* grew on MAC and HE media without fermenting lactose and sucrose. The colonies appeared clear with black centers due to hydrogen Sulfide (H_2S) production. On TSI it appeared yellow on the butt due to acid production and pink on the slant due to alkaline production with blackening due to H_2S production.

The sample size was determined based on BSI 11% prevalence rate in East Africa which Kenya is part of and also to determine the limited number of samples based on the available resources to carry out the study. Hektoen enteric agar was used to culture gram negative suspected BSI pathogen to target isolation of *Salmonella* species. Blood agar plate was used to culture gram positive cocci bacteria to target isolation of *Staphylococcus* species and *Streptococcus* species.

Table 4.1: Identification of BSI from Kisii

ORGANISMS	FA	MS	API
Staphylococcus aureus	7	7	7
Staphylococcus auricularis	0	3	3
Staphylococcus epidermidis	0	2	2
Staphylococcus capitis	0	1	1
Staphylococcus simulans	0	1	1
Staphylococcus lugudensis	0	2	2
Staphylococcus saprophyticus	0	1	1
Staphylococcus intermedius	0	1	1
Staphylococcus sciuri	0	1	1
Staphylococcus hominis	0	1	1
Streptococcus pneumoniae	6	6	6
Streptococcus bovis	0	0	0
Streptococcus anginosus	0	0	0
Streptococcus species	5	5	5
Enterococcus faecium	6	6	6
Escherichia coli	5	5	5
Enterobacter cloacae	4	4	4
Klebsiella pneumoniae	9	9	9
Acinetobacter baumanii	5	5	5
Pseudomonas aeruginosa	4	4	4
Salmonella typhi	26	26	26
Klebsiella oxytoca	2	2	2
Candida albicans	6	6	6
Candida krusei	1	1	1
Candida parapsilosis	0	0	0

FA-FilmArray, MS-MicroScan, API-Analytical profile index Staphylococcus denoted “0” were not identified by FA to species level, FA identified these BSI

pathogens to genus level. However, MS and API identified these BSI pathogens to species level using Gram negatives were identified using API 20E, Gram positives API 20 STAPH and 20 STREP and yeast using 20C AUX. FA identified 4 *Enterobacter cloacae* while MS and API identified 2 *Enterobacter cloacae*. *Streptococcus bovis*, *Streptococcus anginosus* and *Candida parapsilosis* were not detected in BSI samples from Kisii.

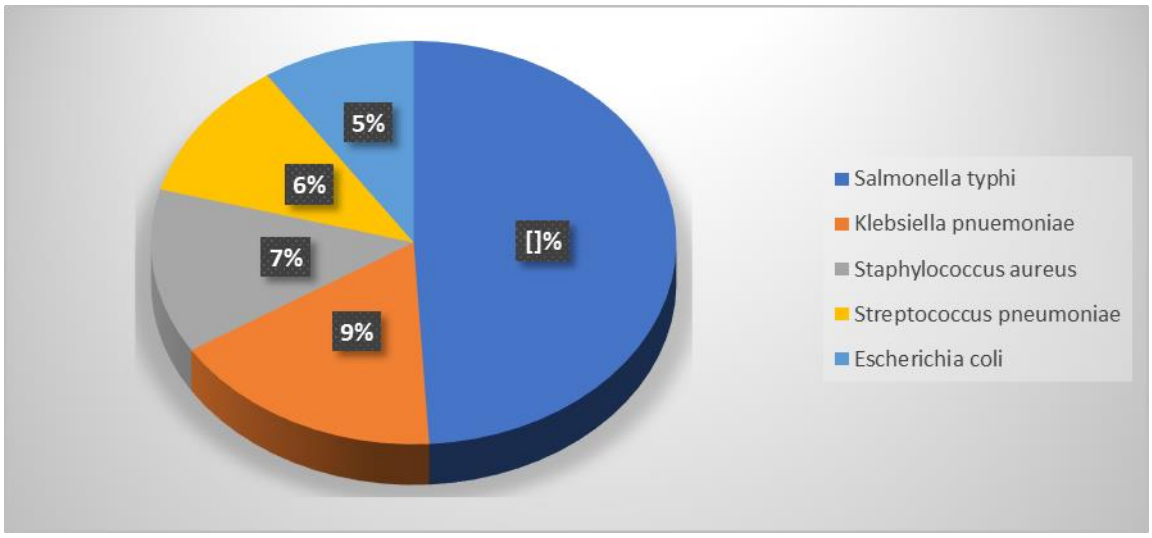

Figure 4.1: Most prevalent BSI from Kisii

Salmonella typhi (26%) was the most identified BSI from Kisii, also seen from the pie chart is, *Klebsiella pneumoniae* (9%), *Staphylococcus aureus* (7%), *Streptococcus pneumoniae* (6%) *followed by Escherichia coli* (5%).

Table 4.2: Identification of BSI from Homa Bay

ORGANISMS	FA	MS	API
Staphylococcus aureus	0	0	0
Staphylococcus auricularis	0	1	1
Staphylococcus epidermidis	0	1	1
Staphylococcus capitis	0	0	0
Staphylococcus simulans	0	0	0
Staphylococcus lugudensis	0	0	0
Staphylococcus saprophyticus	0	0	0
Staphylococcus intermedius	0	0	0
Staphylococcus sciuri	0	0	0
Staphylococcus hominis	0	0	0
Streptococcus pneumoniae	1	1	1
Streptococcus bovis	0	1	0
Streptococcus anginosus	0	1	1
Streptococcus species	0	0	0
Enterococcus faecium	2	2	2
Escherichia coli	3	3	3
Enterobacter cloacae	0	0	0
Klebsiella pneumoniae	2	2	2
Acinetobacter baumanii	2	1	2
Pseudomonas aeruginosa	5	5	5
Salmonella typhi	31	31	31
Klebsiella oxytoca	0	0	0
Candida albicans	3	3	3
Candida krusei	0	0	0
Candida parapsilosis	1	1	1

FA-FilmArray**, MS**-MicroScan, **API**-Analytical profile index

FA did not identify Staphylococcus to species level "0", however it identified them to genus level. MS and API on the other hand identified them to species level using

Gram negatives API 20E, Gram positives 20 STAPH and 20 STREP and for yeast API 20C AUX. Apart from *Staphylococcus auricularis* and *Staphylococcus epidermidis* in *Staphylococcus* family, other *Staphylococcus species*, including *Enterobacter cloacae, Klebsiella oxytoca* and *Candida krusei* were not detected in BSI samples from Homa Bay. FA did not identify *Streptococcus bovis* and *Streptococcus anginosus* because they are not in its data base, however they were identified by MS. "0" were BSI pathogens not identified in Homa bay.

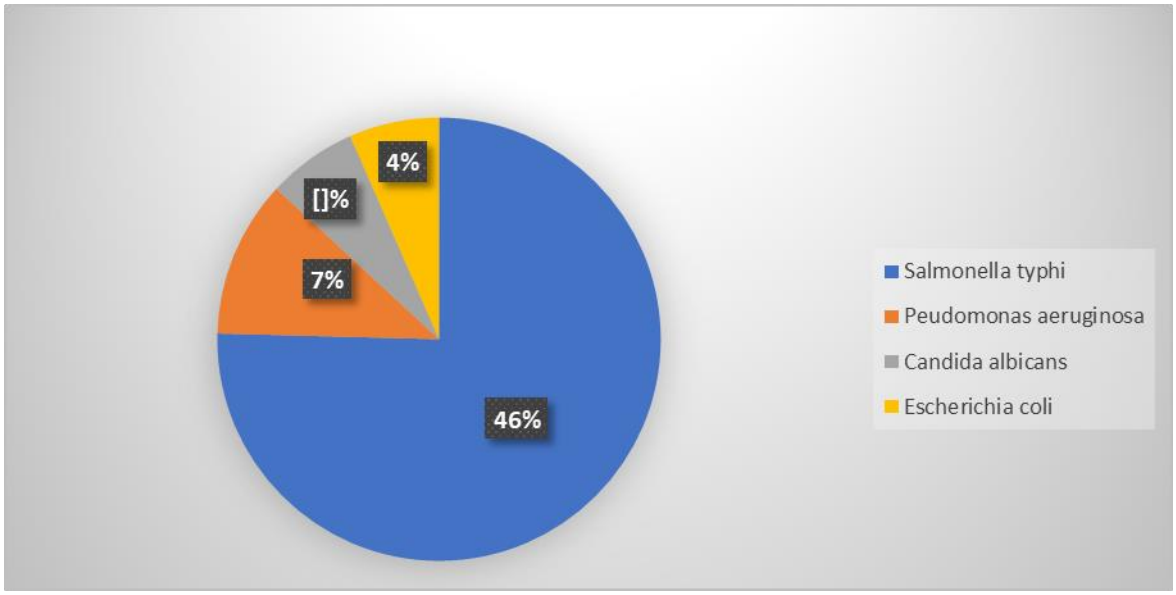

Figure 4.2: Most prevalent BSI from Homa bay

Salmonella typhi (46%) was the most identified BSI from Homa bay, also seen from the pie chart is *Pseudomonas aeruginosa* (7%), *Candida albicans* (4%) and *Escherichia coli* (4%).

Table 4.3: BSI identified by the three methods FA, MS and API biochemical technique

ORGANISMS	API (gold std)	FA	MS
Staphylococcus aureus	7	7	7
Streptococcus pneumoniae	7	7	7
Enterococcus faecium	8	8	8
Escherichia coli	8	8	8
Enterobacter cloacae	2	4	2
Klebsiella pneumoniae	11	11	11
Acinetobacter baumanii	7	7	6
Pseudomonas aeruginosa	9	9	9
Salmonella typhi	57	57	57
Klebsiella oxytoca	2	2	2
Candida albicans	9	9	9
Candida krusei	1	1	1
Candida parapsilosis	1	1	1

FA-FilmArray, **MS**-MicroScan and **API**-Analytical profile index.

The table shows different BSI pathogens identified using FA, MS and API (API 20E for Gram negatives, API 20 STAPH and 20 STREP and 20C AUX for yeast) as the gold standard. Different pathogens were identified from 152 blood culture samples to species level across the technologies. The selected BSI pathogen for analysis were based on API technique as the gold standard to allow for comparison purposes.

4.2 Sensitivity and Specificity

The table shows different specificities using the 3 technologies, FA, MS and API (gold standard).

Table 4.4: Specificity of FA compared to MS technology and API

ORGANISMS	FA	% FA specificity	MicroScan	% MS specificity	Culture and API	% Culture and API Specificity
Staphylococcus aureus	7	100	7	100	7	100
Streptococcus pneumoniae	7	100	7	100	7	100
Enterococcus faecium	8	100	8	100	8	100
Escherichia coli	8	100	8	100	8	100
Enterobacter cloacae	4	100	2	100	2	50
Klebsiella pneumoniae	11	100	11	100	11	100
Acinetobacter baumanii	7	100	6	85.71	7	100
Pseudomonas aeruginosa	9	100	9	100	9	100
Salmonella typhi	57	100	57	100	57	100
Klebsiella oxytoca	2	100	2	100	2	100
Candida albicans	9	100	9	100	9	100
Candida krusei	1	100	1	100	1	100
Candida parapsilosis	1	100	1	100	1	100

FA-FilmArray, **MS**-MicroScan, **API**-Analytical profile index

FA identified 4 *Enterobacter cloacae* while MS identified 2 *Enterobacter cloacae*. MS did not identify one *Acinetobacter baumanii* present in BSI sample which shows MS was less sensitive to identification of *Acinetobacter baumanii*. However, FA identified the 7 *Acinetobacter baumanii* which were present in BSI samples.

Table 4.5: Comparison of sensitivity and specificity of FilmArray and MicroScan technologies

	FilmArray (n=152)	MicroScan (n=152)
True positives	150	149
False positives	0	1
True negative	57	57
False negatives	2	2
Sensitivity	98.68% (95%CL: 95.30% to 99.84%)	98.68% (95%CL: 95.30% to 99.84%)
Specificity	99.04% (95% CI, 96.59-99.88%)	98.56% (95% CI: 96.59% to 99.88%)

4.2.1 Sensitivity of FA technology

Out of the 152 positive blood culture samples for BSI, FA technology was able to identify 150 bacterial isolates. This therefore implied that the sensitivity of FA was 98.68% (95%CL: 95.30% to 99.84%) as compared to the API gold standard they had similar sensitivity of 98.68% as shown in table 4.5.

4.2.2 Specificity of FA technology

Using FA technology was able to identify most of BSI bacterial isolates that were identified using culture and API techniques and specificity was 99.04% (95% CI, 96.59-99.88%) Positive predictive value was 100% (95%CL: n/a) and negative predictive value was 97.94% (95%CL: 92.30% to 99.47%) respectively.

4.2.3 Sensitivity of MicroScan technology

Out of the 152 positive blood culture samples for BSI, MicroScan technology identified 149 bacterial pathogens. Comparing the number identified using MS to the number established using culture and API method; it was found out that sensitivity of MS was 98.68% (95%CL: 95.30% to 99.84%) as shown in table 4.5.

4.2.4 Specificity of MicroScan technology

The overall specificity of MS was 98.56% (95% CI: 96.59-99.88%) MS Positive and negative predictive value was 99.33% (95%CL: 95.52% to 99.90%) and 96.61% (95%CL: 87.79% to 99.12%) respectively.

Table 4.6: Identification of resistance genes (mecA, vanA/B and KPC)

ORGANISMS	mecA	vanA/B	KPC
Staphylococcus aureus	0	na	na
Enterococcus faecium	na	0	0
Escherichia coli	na	na	0
Klebsiella pneumoniae	na	na	0
Salmonella typhi	na	na	0
Klebsiella oxytoca	0	na	0

na-Not applicable, mecA-Methicillin genes, vanA/B- Vancomycin resistant genes, KPC- carbapenemase resistant genes.

The table shows BSI pathogens and the resistance genes. The “0” indicates that no resistant gene was detected and na indicates that the gene is not applicable to the identified BSI pathogen

CHAPTER FIVE

DISCUSSION

5.1 Primary BSI Pathogens

Identification of 152 BSI pathogens in both Kisii county referral hospital and Homa bay county hospital, *Salmonella typhi* was the most prevalent and isolated bloodstream infection bacteria. *Salmonella typhi* for the longest time in resource limited countries has been associated with poor hygiene (Blaschke et al., 2013; Mbae et al., 2020; Shahunja et al., 2015). Other microorganism which have showed high bacteremia prevalence are *Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecium, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Candida albicans*. These organisms have shown resistance to mostly used antimicrobials due to misuse of these antimicrobials been dispensed over the counter though it was not detected in this study. Misdiagnosis as a result of use of empirical diagnosis as opposed to laboratory diagnosis is a concern towards antimicrobial resistant (Aamodt et al., 2015; Buys, Muloiwa, Bamford, & Eley, 2016; Daniel Maina et al., 2016; Rautanen et al., 2016; Tan et al., 2010).

Though not a metric evaluated in this study, the FA requires considerably less training and skill compared to the other methods. It has a less turn-around time of one hour to sample identification which help to balance its throughput limitations. MS and API on the other hand has high throughput but long turnaround time which makes it a limiting factor for BSI rapid results (Kang et al., 2020).

Of note, the FA is able to identify resistant genes such as mecA common with *Staphylococcus aureus,* vanA/B common with *Enterococcus and* KPC common with *Klebsiella pneumoniae* and other *Enterobacteriaceae*. While the MS has no capability to identify antimicrobial resistant genes commonly associated with BSI, it is able to perform phenotypic drug sensitivity by minimum inhibitory concentration. In fact, the MS has a wider range of antimicrobial testing capabilities with regularly updated software database in line with CLSI guidelines.

FilmArray is also able to identify co-infection microorganisms using one panel in a single run. However, in this study there was no co-infection observed, this was demonstrated using control samples. MS uses more than one panel in the event of co-infection which requires pure culture of every isolate. FA also has a limitation of identifying some Gram-positive organisms this is because FA BCID panel does not have the targets for all gram positives in its panel and database while MS has a special panel for gram positive identification. Identification of *Candida species* by FA was done using one panel same as identification of other BSI microorganism, MS uses different special panel specifically for yeast only (rapid yeast panel).

5.2 Sensitivity and specificity of BioFire FilmArray®, MicroScan 40 plus®

Bacteremia as a result of these microorganism have shown to thrive in immunocompromised individuals especially in areas where the infection rate of HIV (human immuno-deficiency virus) and malaria are high because the presentation and the symptoms are similar and this has been a challenge for diagnosis due to lack of capacity to identify and differentiate this organism (Isendahl et al., 2014).

Overall, the sensitivity of FA (98.68%), MS (98.68%) and API (98.68%) were identical, with an overall specificity of 99.04%. Moreover, the sensitivity of FA demonstrated in this study was similar to the sensitivity observed in a previous study carried out in Kazulu-Natal (Fhooblall et al., 2016) The differences in sensitivity came in *inability* of FA and MS *to agree in terms of genus and species* individual identification of BSI pathogens.

The higher specificity by FA in individual identification of BSI pathogens could be because it is a molecular-based platform. The FA identified *Enterobacter cloacae* with a higher accuracy than the other two methods, which could not identify the two isolates of *Enterobacter cloacae*. This is probably because the FA is a molecular technology and it has high sensitivity in identifying *Enterobacter cloacae*. However, the sensitivity of FA was limited when identifying *Streptococcus* the organisms in question *Streptococcus anginosus* and *Streptococcus bovis* are not available in the FA database and are not common causes of BSI (Altun et al., 2013) .The other Streptococcus species which were not identified to species level by either FA, MS or

API, were ruled as discordant results. I believe that these organisms were actually skin contaminants. Still, the overall specificity demonstrated by the FA is in line with previous paper evaluating the diagnostic capabilities of the system (Salimnia et al., 2016).

The MS was able to accurately identify the presence of BSI bacteria with similar specificity to the API method, MS had a specificity of 85.71% in detecting *Acinetobacter baumanii*. The MS surpassed the API strip method in the identification of *Streptococcus anginosus* and *Streptococcus bovis,* where the specificity was (100%) compared to API method (85.71%). These issues with MS in identifying *Staphylococcus* organisms and *Acinetobacter baumanii* is in line with previous studies, where the MS misidentified *Acinetobacter baumanii* (Jin et al., 2011)- (Patteet, Goossens, & Ieven, 2012).

In the past studies, the API strip analysis had a lower accuracy identifying *Citrobacter species*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter* species than automated platforms (Opota, Jaton, & Greub, 2015). Interestingly, this did not occur with this study, and could partly be because of background experience in performing the assay with extensive clinical microbiology experience and also the assays were performed alongside the controls. Microbiology labs typically address this lower accuracy by adding biochemical tests such as oxidase and catalase to increase accuracy. However, I did not incorporate these assays into the API strip analysis. In comparison to the FA and MS, the API method was more labor intensive. Furthermore, fastidious bacteria might not be identified if they fail to grow on culture media but can be identified directly from blood culture using FA. FilmArray® could not detect all genera and species in *Enterobactericeae* family because some of these organisms are not in the database. This required confirmation using biochemical testing and serotyping.

In resource-limited settings, the use of conventional methods in diagnosis of bacteremia has been a challenge to most public health facilities leading to misclassification of the diagnosis of BSI (Blaschke et al., 2013; Pradhan et al., 2012). The automated methods FA and MS proved to be more efficient, reliable and faster

in the identification of a wide range of BSI pathogens. These positive factors outweigh the use of API strips for microbial identification, which is considered the conventional standard in Kenya for diagnosis of BSI in public hospitals.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Although only 152 positive bacterial isolates were tested, the data generated in this study will go a long way in helping the clinicians in counties of Kisii and Homa bay who always use empirical treatment in management of BSI. In this study all the technologies FA, MS and API showed similar sensitivity (98.68%) and specificity (99.04 %) in identification of BSI pathogen, *Salmonella typhi* was the most identified pathogen in both Kisii and Homa bay counties. The difference came in identification of *Enterobacter cloacae* where by FA identified more *Enterobacter cloacae.*

FilmArray had a sensitivity of 98.68% (95%CL: 95.30% to 99.84%) and specificity of 99.04% (95% CI, 96.59-99.88%) and Positive predictive value was 100% (95%CL: n/a) and negative predictive value was 97.94% (95%CL: 92.30% to 99.47%). FA had a good detection sensitivity in identification of targeted BSI pathogens. FA had a challenge in identifying *Streptococcus bovis* and *Streptococcus anginosus* though these organisms are not in its data base and are normally skin contaminants but they can also cause endocarditis taking advantage of immunocompromised immunity. FA also had a challenge in identifying *Staphylococcus* species other than *Staphylococcus aureus* to genus level and *Streptococcus species* apart from *Streptococcus pneumonia* to genus level.

MicroScan had a sensitivity of 98.68% (95%CL: 95.30% to 99.84%) and specificity of 98.56% (95% CI: 96.59-99.88%). MS Positive and negative predictive value was 99.33% (95%CL: 95.52% to 99.90%) and 96.61% (95%CL: 87.79% to 99.12%), similar to FA, MS had a good sensitivity and specificity detection in identifying BSI pathogens. MS does not identify resistant genes because it is a phenotypic technique and not a molecular technique, it determines antimicrobial susceptibility testing by minimum inhibitory concentration (MIC) which is phenotypic, though this metric was not part of the analysis.

MicroScan was more sensitive in identification of *Streptococcus bovis* and *Streptococcus anginosus* than FA. Though FA is a rapid molecular technique which is a very important factor in identification of BSI in emergency cases. In terms of sampling processing API and MS was more involving in manipulation of samples. It took time for incubation, interpretation and culture reading before processing for identification. FA was direct in sample processing from the time of positive signal by Bactec incubator. Blood culture samples were processed directly for identification without need for gram stain first, this factor assist in release of results faster which assist in patient management.

FilmArray has a limited target BSI pathogen data base. In this study there were no resistant genes identified, however FA is able to identify resistant genes (mecA, vanA/B and KPC, Vancomycin and Carbapenem) which helps in reduction of drug resistant reducing on long hospital stay.

The study of the FilmArray and MicroScan 40 plus technologies on positive blood cultures demonstrated reasonable accuracy and practical benefits. The results agreed with the reference method used in this study in the majority of positive blood cultures tested. These technologies, if utilized for rapid communication of results to the clinicians, would dictate the choice of initial antimicrobial to patients and enhance patient management outcome.

6.2 Recommendations

I. Though the three technologies FA, MS and API had similar high sensitivity and specificity in identification of BSI pathogens. The culture and API followed by MS was more involving and laborious as compared to FA. It took long time to BSI pathogen identification and it requires experienced laboratory technologist to interpret the gram stains, culture media and API color interpretation. This calls for training and continuous education, despite been used as the gold standard method in most public hospitals.
II. The conventional method cannot be used in rapid identification of BSI pathogens as this will affect the outcome of the patient management.

III. The FA on the other hand has not been evaluated at Kenya public hospitals, but it has proved to be faster than culture and API and MS in identification of BSI pathogens and important BSI resistant genes which is crucial for patient management.

IV. Further evaluation of this automated method using a larger sample size is recommended.

V. The preliminary results from this study clearly suggest that both the FA and MS platforms are valuable tools in rapid identification of BSI. Each technology has its advantages and disadvantages, which must be considered.

VI. Implementation of either of these platforms could result in reduction of hospital stays, lower cost, better patient management and more appropriate use of antibiotics by clinicians and so the cost of the FA panels should not be considered a major drawback since early detection of BSI reduces economic burden on the patient.

REFERENCES

Aamodt, H., Mohn, S. C., Maselle, S., Manji, K. P., Willems, R., Jureen, R., … & Blomberg, B. (2015). Genetic relatedness and risk factor analysis of ampicillin-resistant and high-level gentamicin-resistant enterococci causing bloodstream infections in Tanzanian children. *BMC Infectious Diseases*, *15*(1), 1–9.

Akoua-Koffi, C., Tia, H., Plo, J. K., Monemo, P., Cissé, A., Yao, C., … & Becker, S. L. (2015). Epidemiology of community-onset bloodstream infections in Bouaké, central Côte d'Ivoire. *New Microbes and New Infections*, *7*(9), 100–104.

Altun, O., Almuhayawi, M., Ullberg, M., & Ozenci, V. (2013). Clinical evaluation of the Filmarray blood culture identification panel in identification of bacteria and yeasts from positive blood culture bottles. *Journal of Clinical Microbiology*, *51*(12), 4130–4136.

Balkhy, H. H., Cunningham, G., Chew, F. K., Francis, C., Al Nakhli, D. J., Almuneef, M. A., & Memish, Z. A. (2006). Hospital- and community-acquired infections: a point prevalence and risk factors survey in a tertiary care center in Saudi Arabia. *International Journal of Infectious Diseases*, *10*(4), 326–333.

Ballot, D. E., Nana, T., Sriruttan, C., & Cooper, P. A. (2012). Bacterial Bloodstream Infections in Neonates in a Developing Country. *ISRN Pediatrics*, *2012*, 1–6.

Berkley, J. A., Lowe, B. S., Mwangi, I., Williams, T., Bauni, E., Mwarumba, S., … & Scott, J. A. G. (2005). Bacteremia among Children Admitted to a Rural Hospital in Kenya. *New England Journal of Medicine*, *352*(1), 39–47.

Bhatti, M. M., Boonlayangoor, S., Beavis, K. G., & Tesic, V. (2014). Evaluation of

filmarray and verigene systems for rapid identification of positive blood cultures. *Journal of Clinical Microbiology*, *52*(9), 3433–3436.

Blaschke, A. J., Heyrend, C., Byington, C. L., Mark, A., Barker, E., Garrone, N. F., … & Poritz, M. A. (2013). *Rapid Identification of Pathogens from Positive Blood Cultures by Multiplex PCR using the FilmArray System*. *74*(4), 349–355.

Burdino, E., Ruggiero, T., Allice, T., Grazia, M., Gregori, G., Milano, R., … & Ghisetti, V. (2014). Combination of conventional blood cultures and the Septi Fast molecular test in patients with suspected sepsis for the identi fi cation of bloodstream pathogens ☆. *Diagnostic Microbiology and Infectious Disease*, *79*(3), 287–292.

Buys, H., Muloiwa, R., Bamford, C., & Eley, B. (2016). Klebsiella pneumoniae bloodstream infections at a South African children's hospital 2006-2011, a cross-sectional study. *BMC Infectious Diseases*, *16*(1), 1–10.

Cecchini, M., Langer, J., & Slawomirski, L. (2015). Antimicrobial resistance in G7 countries and beyond: economic issues, policies and options for action. In *Paris: Organization for Economic Co-operation and Development*.

Christopher, A., Mshana, S. E., Kidenya, B. R., Hokororo, A., & Morona, D. (2013). Bacteremia and resistant gram-negative pathogens among under-fives in Tanzania. *Italian Journal of Pediatrics*, *39*(1), 2015.

Cleven, B. E. E., Palka-Santini, M., Gielen, J., Meembor, S., Krönke, M., & Krut, O. (2006). Identification and characterization of bacterial pathogens causing bloodstream infections by DNA microarray. *Journal of Clinical Microbiology*, *44*(7), 2389–2397.

County Government of Homa Bay. (2017). *Homa Bay County Integrated Development Plan 2013-2017*.

Dagnew, M., Yismaw, G., Gizachew, M., Gadisa, A., Abebe, T., Tadesse, T., … &

Mathewos, B. (2013). Bacterial profile and antimicrobial susceptibility pattern in septicemia suspected patients attending Gondar University Hospital, Northwest Ethiopia. *BMC Research Notes*, *6*(1), 1.

Fhooblall, M., Nkwanyana, F., & Mlisana, K. P. (2016). Evaluation of the BioFire® FilmArray® blood culture identification panel on positive blood cultures in a regional hospital laboratory in KwaZulu-Natal. *African Journal of Laboratory Medicine*, *5*(1), 1–8.

Inglis, T. J. J., Bzdyl, N., Ly Joanna Chua, I., Urosevic, N. M., Leung, M. J., & Geelhoed, E. (2016). Improved blood culture identification by FilmArray in cultures from regional hospitals compared with teaching hospital cultures. *Journal of Medical Microbiology*, *65*(1), 56–61.

Isendahl, J., Manjuba, C., Rodrigues, A., Xu, W., Henriques-Normark, B., Giske, C. G., & Nauclér, P. (2014). Prevalence of community-acquired bacteraemia in guinea-bissau: An observational study. *BMC Infectious Diseases*, *14*(1), 1–9.

Jin, W. Y., Jang, S. J., Lee, M. J., Park, G., Kim, M. J., Kook, J. K., … & Park, Y. J. (2011). Evaluation of VITEK 2, MicroScan, and Phoenix for identification of clinical isolates and reference strains. *Diagnostic Microbiology and Infectious Disease*, *70*(4), 442–447.

Kang, C. M., Chen, X. J., Chih, C. C., Hsu, C. C., Chen, P. H., Lee, T. fen, …& Hsueh, P. R. (2020). Rapid identification of bloodstream bacterial and fungal pathogens and their antibiotic resistance determinants from positively flagged blood cultures using the BioFire FilmArray blood culture identification panel. *Journal of Microbiology, Immunology and Infection*, *53*(6), 882–891.

Kariuki, S., Revathi, G., Kiiru, J., Mengo, D. M., Mwituria, J., Muyodi, J., … & Dougan, G. (2010). Typhoid in Kenya is associated with a dominant multidrug-resistant salmonella enterica serovar typhi haplotype that is

also widespread in Southeast Asia. *Journal of Clinical Microbiology*, *48*(6), 2171–2176.

Köten, B., Becker, K., Podschun, R., Von Eiff, C., Meyer-Hoffert, U., Harder, J., & Gläser, R. (2012). Susceptibility of Staphylococcus aureus bacteremia strains to different skin-derived antimicrobial proteins. *Archives of Dermatological Research*, *304*(8), 633–637.

Kothari, A., Morgan, M., & Haake, D. A. (2014). Emerging technologies for rapid identification of bloodstream pathogens. *Clinical Infectious Diseases*, *59*(2), 272–278.

Macharashvili, N., Kourbatova, E., Butsashvili, M., Tsertsvadze, T., McNutt, L. A., & Leonard, M. K. (2009). Etiology of neonatal blood stream infections in Tbilisi, Republic of Georgia. *International Journal of Infectious Diseases*, *13*(4), 499–505.

Maina, D., Okinda, N., Mulwa, E., & Revathi, G. (2014). A five year review of api20e bacteria identification System's performance at a teaching hospital. *East African Medical Journal*, *91*(3), 73–76.

Maina, Daniel, Omuse, G., Revathi, G., & Adam, R. D. (2016). Spectrum of microbial diseases and resistance patterns at a private teaching hospital in Kenya: Implications for clinical practice. *PLoS One*, *11*(1), 1–12.

Markwart, R., Saito, H., Harder, T., Tomczyk, S., Cassini, A., Fleischmann-Struzek, C., … & Allegranzi, B. (2020). Epidemiology and burden of sepsis acquired in hospitals and intensive care units: a systematic review and meta-analysis. *Intensive Care Medicine*, *46*(8), 1536–1551.

Maze, M. J., Bassat, Q., Feasey, N. A., Mandomando, I., Musicha, P., & Crump, J. A. (2018). The epidemiology of febrile illness in sub-Saharan Africa: implications for diagnosis and management. *Clinical Microbiology and Infection*, *24*(8), 808–814.

Mbae, C., Mwangi, M., Gitau, N., Irungu, T., Muendo, F., Wakio, Z., … & Kariuki, S. (2020). Factors associated with occurrence of salmonellosis among children living in Mukuru slum, an urban informal settlement in Kenya. *BMC Infectious Diseases*, *20*(1), 1–12.

Morrison, L., & Zembower, T. R. (2020). Antimicrobial Resistance. *Gastrointestinal Endoscopy Clinics of North America*, *30*(4), 619–635.

Myat, T. O., Oo, K. M., Mone, H. K., Htike, W. W., Biswas, A., Hannaway, R. F., … & Crump, J. A. (2020). A prospective study of bloodstream infections among febrile adolescents and adults attending Yangon general hospital, Yangon, Myanmar. *PLoS Neglected Tropical Diseases*, *14*(4), 1–22.

O'Neill, L., Park, S. H., & Rosinia, F. (2018). The role of the built environment and private rooms for reducing central line-associated bloodstream infections. *PLoS ONE*, *13*(7), 1–12.

Ombelet, S., Natale, A., Ronat, J. B., Vandenberg, O., Hardy, L., & Jacobs, J. (2021). Evaluation of microscan bacterial identification panels for low-resource settings. *Diagnostics*, *11*(2).

Opota, O., Jaton, K., & Greub, G. (2015). Microbial diagnosis of bloodstream infection: Towards molecular diagnosis directly from blood. *Clinical Microbiology and Infection*, *21*(4), 323–331.

Organization, W. H. (2011). Report on the Burden of Endemic Health Care-Associated Infection Worldwide Clean Care is Safer Care. In *World Health Organization* (Vol. 3). Retrieved from www.who.int

Paolucci, M., Foschi, C., Tamburini, M. V, Ambretti, S., Lazzarotto, T., & Landini, M. P. (2014). Comparison between MALDI-TOF MS and FilmArray Blood Culture Identi fi cation panel for rapid identi fi cation of yeast from positive blood culture. *Journal of Microbiological Methods*, *104*, 92–93.

Patteet, L., Goossens, H., & Ieven, M. (2012). Validation of the MicroScan-96 for the species identification and methicillin susceptibility testing of clinical significant coagulase-negative staphylococci. *European Journal of Clinical Microbiology and Infectious Diseases*, *31*(5), 747–751.

Peker, N., Couto, N., Sinha, B., & Rossen, J. W. (2018). Diagnosis of bloodstream infections from positive blood cultures and directly from blood samples: recent developments in molecular approaches. *Clinical Microbiology and Infection*, *24*(9), 944–955.

Pradhan, R., Shrestha, U., Gautam, S. C., Thorson, S., Shrestha, K., Yadav, B. K., … & Murdoch, D. R. (2012). Bloodstream Infection among Children Presenting to a General Hospital Outpatient Clinic in Urban Nepal. *PLoS ONE*, *7*(10), 1–7.

Prakash, K. P., Arora, V., & Geethanjali, P. P. (2011). Bloodstream bacterial pathogens and their antibiotic resistance pattern in Dhahira region, Oman. *Oman Medical Journal*, *26*(4), 240–247.

Rautanen, A., Pirinen, M., Mills, T. C., Rockett, K. A., Strange, A., Ndungu, A. W., … & Spencer, C. C. A. (2016). Polymorphism in a lincRNA Associates with a Doubled Risk of Pneumococcal Bacteremia in Kenyan Children. *American Journal of Human Genetics*, *98*(6), 1092–1100.

Ray, S. T. J., Drew, R. J., Hardiman, F., Pizer, B., & Riordan, A. (2016). Rapid Identification of Microorganisms by FilmArray Blood Culture Identification Panel Improves Clinical Management in Children. *Pediatric Infectious Disease Journal*, *35*(5), e134–e138.

Reygaert, W. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, *4*(3), 482–501.

Sakarikou, C., Parisato, M., Cascio, G. Lo, & Fontana, C. (2014). Beacon-based (bbFISH ®) technology for rapid pathogens identification in blood cultures. *BMC Microbiology*, *14*(1), 1–8.

Salimnia, H., Fairfax, M. R., Lephart, P. R., Schreckenberger, P., DesJarlais, S. M., Johnson, J. K., … & Kanack, K. J. (2016). Evaluation of the FilmArray Blood Culture Identification Panel: Results of a Multicenter Controlled Trial. *Journal of Clinical Microbiology*, *54*(3), 687–698.

Shahunja, K. M., Leung, D. T., Ahmed, T., Bardhan, P. K., Ahmed, D., Qadri, F., … & Chisti, M. J. (2015). Factors Associated with Non-typhoidal Salmonella Bacteremia versus Typhoidal Salmonella Bacteremia in Patients Presenting for Care in an Urban Diarrheal Disease Hospital in Bangladesh. *PLoS Neglected Tropical Diseases*, *9*(9), 1–12.

Smith, A. C., & Hussey, M. A. (2005). Gram stain protocols. *American Society for Microbiology*, *1*(September 2005), 14.

Southern, T. R., Vanschooneveld, T. C., Bannister, D. L., Brown, T. L., Crismon, A. S., Buss, S. N., … & Fey, P. D. (2015). Implementation and performance of the BioFire FilmArray ® Blood Culture Identi fi cation panel with antimicrobial treatment recommendations for bloodstream infections at a midwestern academic tertiary hospital. *Diagnostic Microbiology and Infectious Disease*, *81*(2), 96–101.

Swain, B., & Otta, S. (2012). Blood stream infection in a teaching hospital. *Annals of Biological Research*, *3*(4), 1923–1928.

Tan, C. K., Lai, C. C., Wang, J. Y., Lin, S. H., Liao, C. H., Huang, Y. T., … & Hsueh, P. R. (2010). Bacteremia caused by non-faecalis and non-faecium enterococcus species at a Medical center in Taiwan, 2000 to 2008. *Journal of Infection*, *61*(1), 34–43.

Tatsuno, K., Ikeda, M., Wakabayashi, Y., Yanagimoto, S., Okugawa, S., & Moriya, K. (2019). Clinical Features of Bloodstream Infections Associated with Peripheral Versus Central Venous Catheters. *Infectious Diseases and Therapy*, *8*(3), 343–352.

Trevethan, R. (2017). Sensitivity, Specificity, and Predictive Values: Foundations,

Pliabilities, and Pitfalls in Research and Practice. *Frontiers in Public Health*, *5*(November), 1–7.

Viscoli, C. (2016). Bloodstream Infections: The peak of the iceberg. *Virulence*, *7*(3), 248–251.

Vlieghe, E. R., Phe, T., De Smet, B., Chhun Veng, H., Kham, C., Lim, K., …& Jacobs, J. A. (2013). Bloodstream Infection among Adults in Phnom Penh, Cambodia: Key Pathogens and Resistance Patterns. *PLoS ONE*, *8*(3), 59775.

Wester, A. L., Dunlop, O., Melby, K. K., Dahle, U. R., & Wyller, T. B. (2013). Age-related differences in symptoms, diagnosis and prognosis of bacteremia. *BMC Infectious Diseases*, *13*(1), 1.

Williamson, D., Lim, A., Thomas, M., Baker, M., Roberts, S., Fraser, J., & Ritchie, S. (2013). Incidence, trends and demographics of Staphylococcus aureus infections in Auckland, New Zealand, 2001-2011. *BMC Infectious Diseases*, *13*(1), 569.

World Health Organization. (2002). *Prevention of hospital-acquired infections World Health Organization*. https://doi.org/WHO/CDS/CSR/EPH/2002.12

APPENDICES

Appendix I: Ethical approval and consent to participate

Ethical clearance for this work was obtained from Kenya Medical Research Institute Scientific and ethical review unit (SERU-SSC) and WRAIR institutional review boards (IRBs) (SSC KEMRI #3686, WRAIR #2513). This work was also part of the larger Non-Typhoidal Salmonellosis protocol that had been cleared by the Kenya Medical Research Institute (KEMRI) ethics board.

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16th September, 2014

Mr. Ronald Kimutai Kirera
C/o Biochemistry Department
JKUAT

Dear Mr. Kirera

RE: APPROVAL OF RESEARCH PROPOSAL AND SUPERVISORS

Kindly note that your M.Sc. research proposal entitled: **"Identification of selected primary bloodstream infection pathogens in patients attending Kisii level 5 and Homa Bay County Hospitals"** has been approved. The following are your approved supervisors:-

1. Dr. Daniel Kariuki
2. Dr. Joseph Ng'ang'a
3. Cpt. Christine Hulseberg

Yours sincerely

PROF. MATHEW KINYANJUI
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

Copy to: Dean, SoBS COD, Biochemistry

/pw

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KEMRI/RES/7/3/1 **November 03, 2016**

TO: **RONALD KIRERA,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. LEAH KIRUMBI,**
ACTING DIRECTOR, CCR,
NAIROBI.

forwarded 11/11/2016.

Dear Sir,

RE: **KEMRI/SERU/CCR/045/3351 *(RESUBMISSION 1 OF INITIAL)*: IDENTIFICATION OF BLOODSTREAM INFECTION PATHOGENS FROM PATIENTS ATTENDING KISII LEVEL FIVE AND HOMA BAY COUNTY HOSPITALS USING FILMARRAY® AND MICROSCAN 40 PLUS® TECHNOLOGIES. (*VERSION 1.2 DATED 19 OCTOBER 2016*)**

Reference is made to your letter dated 19th October 2016. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledge receipt of the revised study documents on 26th October, 2016.

This is to inform you that the issues raised during the 255th meeting of the KEMRI/Ethics Review Committee (ERC) held on 20th September, 2016 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **03rd November, 2016** for a period of one year. Please note that authorization to conduct this study will automatically expire on **2nd November, 2017.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **22nd September, 2017.**

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

FOR: DR. EVANS AMUKOYE,
ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

RESEARCH ARTICLE

Identification of selected primary bloodstream infection pathogens in patients attending Kisii level five and Homa Bay county hospitals [version 1; peer review: 1 approved with reservations, 1 not approved]

Ronald Kirera[1], Erick Kipkirui[1], Margaret Koech[1], Abigael Ombogo[1], Janet Ndonye[1], Mary Kirui[1], Cliff Odhiambo Philip[1], Nancy Kipkemoi[1], Elizabeth Odundo[1], Alexander F. Flynn[1], Christine Hulseberg[1], Judd Walson[2,3], Joseph Nganga[4], Daniel Kariuki[4]

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Abstract

Background: Bloodstream infection (BSI) contributes to a substantial proportion of mortality in sub-Saharan Africa and is marked by the presence of bacterial and/or fungal microorganisms in the blood. Because BSI can be life threatening, it requires a timely, reliable and accurate diagnosis. This study retrospectively analyzed data of identified BSI pathogens and compared the performance of the different diagnostic technologies used in terms of accuracy, sensitivity, turnaround time (TAT) and cost.
Methods: Currently, culture followed by analytical profile index biochemical strips (API), (BioMerieux) are used as the conventional standard diagnostics in Kenyan public hospitals and labs. We compared the results of this standard to that of the BioFire FilmArray (FA) (BioFire Diagnostics) and MicroScan WalkAway-40 plus System (MS) (Beckman Coulter) used in diagnosis of BSI. The FA technology was able to identify 150/152 bacterial and yeast isolates with an overall accuracy of 99.04% (95% CI: 96.59-99.88%), sensitivity of 98.68% (95% CI: 95.33-99.84%), mean TAT of 8 hours 40 minutes per eight samples and running cost per sample of USD 140.11. The MS identified 150/152 isolates with an overall accuracy of 98.56% (95% CI: 95.86-99.70%), sensitivity of 98.68% (95% CI: 95.30-99.84%), mean TAT per sample was 42 hours and running cost per sample of USD 28.05. API detected 150/152 isolates, with an overall accuracy of 99.04% (95%

Open Peer Review

Approval Status ? ✗

	1	2
version 1 12 Oct 2020	?	✗

1. **Ciira Kiiyukia**, Mount Kenya University (MKU), Thika, Kenya

2. **Josette Raymond**, Bicêtre Hospital, Kremlin Bicetre, France

Any reports and responses or comments on the article can be found at the end of the article.

Appendix II: Raw data for FA, MS and culture-based/API

Sample ID	FA ID	MS ID	FA (resistant)	API
MHK001	Escherichia coli	Escherichia coli		Escherichia coli
MHK002	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK003	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK004	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK005	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK006	Staphylococcus	Staphylococcus auricularis		Staphylococcus auricularis
MHK007	Staphylococcus	Staphylococcus epidermidis		Staphylococcus epidermidis
MHK008	Pseudomonas aeruginosa	Pseudomonas aeruginosa		Pseudomonas aeruginosa
MHK009	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK010	Enterococcus faecium	Enterococcus faecium		Enterococcus faecium
MHK011	Staphylococcus aureus	Staphylococcus aureus		Staphylococcus aureus
MHK012	Pseudomonas aeruginosa	Pseudomonas aeruginosa		Pseudomonas aeruginosa
MHK013	Candida albicans	Candida albicans		Candida albicans
MHK014	Streptococcus pneumoniae	Streptococcus pneumoniae		Streptococcus pneumoniae
MHK015	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK016	Enterobacter cloacae	Enterobacter cloacae		Enterobacter cloacae
MHK017	Klebsiella pneumoniae	Klebsiella pneumoniae		Klebsiella pneumoniae
MHK018	Escherichia coli	Escherichia coli		Escherichia coli
MHK019	Staphylococcus	Staphylococcus capitis		Staphylococcus capitis
MHK020	Escherichia coli	Escherichia coli		Escherichia coli
MHK021	Enterobacter cloacae complex	Enterobacter cloacae		Enterobacter cloacae
MHK022	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK023	Pseudomonas aeruginosa	Pseudomonas aeruginosa		Pseudomonas aeruginosa
MHK024	Staphylococcus aureus	Staphylococcus aureus		Staphylococcus aureus
MHK025	Candida albicans	Candida albicans		Candida albicans
MHK026	Staphylococcus aureus	Staphylococcus aureus		Staphylococcus aureus
MHK027	Klebsiella pneumoniae	Klebsiella pneumoniae		Klebsiella pneumoniae
MHK028	Candida parapsilosis	Candida parapsilosis		Candida parapsilosis
MHK029	Klebsiella pneumoniae	Klebsiella pneumoniae		Klebsiella pneumoniae

MHK030	Pseudomonas aeruginosa	Pseudomonas aeruginosa		Pseudomonas aeruginosa
MHK031	Escherichia coli	Escherichia coli		Escherichia coli
MHK032	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK033	Escherichia coli	Escherichia coli		Escherichia coli
MHK034	Klebsiella pneumoniae	Klebsiella pneumoniae		Klebsiella pneumoniae
MHK035	Staphylococcus aureus	Staphylococcus aureus		Staphylococcus aureus
MHK036	Escherichia coli	Escherichia coli		Escherichia coli
MHK037	Klebsiella pneumoniae	Klebsiella pneumoniae		Klebsiella pneumoniae
MHK038	Pseudomonas aeruginosa	Pseudomonas aeruginosa		Pseudomonas aeruginosa
MHK039	Staphylococcus aureus	Staphylococcus aureus		Staphylococcus aureus
MHK040	Candida albicans	Candida albicans		Candida albicans
MHK041	Enterobactericeae	Salmonella typhi		Salmonella typhi
MHK042	Escherichia coli	Escherichia coli		Escherichia coli
MHK043	Salmonella typhi	Salmonella typhi		Salmonella spp.
MHK044	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK045	Escherichia coli	Escherichia coli		Escherichia coli
MHK046	Acinetobacter baumanii	Acinetobacter baumanii		Acinetobacter baumanii
MHK047	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK048	Klebsiella pneumoniae	Klebsiella pneumoniae		Klebsiella pneumoniae
MHK049	Candida albicans	Candida albicans		Candida albicans
MHK050	Acinetobacter baumanii	Acinetobacter baumanii		Acinetobacter baumanii
MHK051	Candida krusei	Candida krusei		Candida krusei
MHK052	Acinetobacter baumanii	Not detected		Acinetobacter baumanii
MHK053	Klebsiella pneumoniae	Klebsiella pneumoniae		Klebsiella pneumoniae
MHK054	Staphylococcus aureus	Staphylococcus aureus		Staphylococcus aureus
MHK055	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK056	Staphylococcus aureus	Staphylococcus aureus		Staphylococcus aureus
MHK057	Klebsiella pneumoniae	Klebsiella pneumoniae		Klebsiella pneumoniae
MHK058	Candida albicans	Candida albicans		Candida albicans
MHK059	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK060	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK061	Enterobacter cloacae complex	Not detected		Not detected
MHK062	Pseudomonas aeruginosa	Pseudomonas aeruginosa		Pseudomonas aeruginosa

MHK063	Acinetobacter baumanii	Acinetobacter baumanii		Acinetobacter baumanii
MHK064	Enterobacter cloacae complex	Not detected		Not detected
MHK065	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK066	Candida albicans	Candida albicans		Candida albicans
MHK067	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK068	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK069	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK070	Klebsiella pneumoniae	Klebsiella pneumoniae		Klebsiella pneumoniae
MHK071	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK072	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK073	Acinetobacter baumanii	Acinetobacter baumanii		Acinetobacter baumanii
MHK074	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK075	Klebsiella pneumoniae	Klebsiella pneumoniae		Klebsiella pneumoniae
MHK076	Klebsiella pneumoniae	Klebsiella pneumoniae		Klebsiella pneumoniae
MHK077	Acinetobacter baumanii	Acinetobacter baumanii		Acinetobacter baumanii
MHK078	Enterococcus faecium	Enterococcus faecium		Enterococcus faecium
MHK079	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK080	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK081	Enterococcus faecium	Enterococcus faecium		Enterococcus faecium
MHK082	Streptococcus species	Streptococcus species		Streptococcus species
MHK083	Streptococcus species	Streptococcus species		Streptococcus species
MHK084	Acinetobacter baumanii	Acinetobacter baumanii		Acinetobacter baumanii
MHK085	Enterococcus faecium	Enterococcus faecium		Enterococcus faecium
MHK086	Enterococcus faecium	Enterococcus faecium		Enterococcus faecium
MHK087	Streptococcus species	Streptococcus species		Streptococcus species
MHK088	Streptococcus species	Streptococcus species		Streptococcus species
MHK089	Streptococcus species	Streptococcus species		Streptococcus species
MHK090	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK091	Enterococcus faecium	Enterococcus faecium		Enterococcus faecium
MHK092	Pseudomonas aeruginosa	Pseudomonas aeruginosa		Pseudomonas aeruginosa
MHK093	Klebsiella oxytoca	Klebsiella oxytoca		Klebsiella oxytoca
MHK094	Enterococcus faecium	Enterococcus faecium		Enterococcus faecium
MHK095	Pseudomonas aeruginosa	Pseudomonas aeruginosa		Pseudomonas aeruginosa

MHK096	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK097	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK098	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK099	Not detected	Streptococcus anginosus		Streptococcus spp
MHK100	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK101	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK102	Enterococcus faecium	Enterococcus faecium		Enterococcus faecium
MHK103	Streptococcus pneumoniae	Streptococcus pneumoniae		Streptococcus pneumoniae
MHK104	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK105	Candida albicans	Candida albicans		Candida albicans
MHK106	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK107	Staphylococcus	Staphylococcus auricularis		Staphylococcus auricularis
MHK108	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK109	Candida albicans	Candida albicans		Candida albicans
MHK110	Staphylococcus	Staphylococcus lugudensis		Staphylococcus lugudensis
MHK111	Staphylococcus	Staphylococcus epidermidis		Staphylococcus epidermidis
MHK112	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK113	Staphylococcus	Not detected		Staphylococcus spp
MHK114	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK115	Staphylococcus	Staphylococcus mutants		Staphylococcus simulans
MHK116	Salmonella typhi	Salmonella typhi		Salmonella spp.
MHK117	Staphylococcus	Staphylococcus saprophyticus		Staphylococcus saprophyticus
MHK118	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK119	Streptococcus pneumoniae	Streptococcus pneumoniae		Streptococcus pneumoniae
MHK120	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK121	Not detected	Streptococcus bovis		Not detected
MHK122	Staphylococcus	Staphylococcus auricularis		Staphylococcus auricularis
MHK123	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK124	Staphylococcus	Staphylococcus intermidius		Staphylococcus intermidius

MHK125	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK126	Staphylococcus	Staphylococcus epidermidis		Staphylococcus epidermidis
MHK127	Candida albicans	Candida albicans		Candida albicans
MHK128	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK129	Staphylococcus	Staphylococcus sciuri		Staphylococcus sciuri
MHK130	Salmonella typhi	Salmonella spp.		Salmonella typhi
MHK131	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK132	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK133	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK134	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK135	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK136	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK137	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK138	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK139	Streptococcus pneumoniae	Streptococcus pneumoniae		Streptococcus pneumoniae
MHK140	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK141	Streptococcus pneumoniae	Streptococcus pneumoniae		Streptococcus pneumoniae
MHK142	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK143	Streptococcus pneumoniae	Streptococcus pneumoniae		Streptococcus pneumoniae
MHK144	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK145	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK146	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK147	Klebsiella oxytoca	Klebsiella oxytoca		Klebsiella oxytoca
MHK148	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK149	Staphylococcus	Staphylococcus lugudensis		Staphylococcus lugudensis
MHK150	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK151	Staphylococcus	Staphylococcus hominis		Staphylococcus hominis
MHK152	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK153	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK154	Staphylococcus	Staphylococcus auricularis		Staphylococcus auricularis
MHK155	Not detected	ND-Gram positive		ND-Gram positive

		rods		rods
MHK156	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK157	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK158	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK159	Pseudomonas aeruginosa	Pseudomonas aeruginosa		Pseudomonas aeruginosa
MHK160	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK161	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK162	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK163	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK164	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK165	Salmonella typhi	Salmonella typhi		Salmonella typhi
MHK166	Streptococcus pneumoniae	Streptococcus pneumoniae		Streptococcus pneumoniae
MHK167	Not detected	ND-Gram positive rods		ND-Gram positive rods
MHK168	Salmonella typhi	Salmonella typhi		Salmonella typhi
FA Control 1	Staphylococcus aureus	Staphylococcus aureus (MRSA)	Methicillin	Staphylococcus aureus (MRSA)
FA Control 2	Enterococcus faecium	Enterococcus faecium	Vancomycin (A/B)	Enterococcus faecium
FA Control 3	Pseudomonas aeruginosa	Pseudomonas aeruginosa	Imipenem, meropenem,	Pseudomonas aeruginosa
FA Control 4	Staphylococcus	Staphylococcus haemolyticus	Methicillin	Staphylococcus haemolyticus
FA Control 5	Staphylococcus aureus	Staphylococcus aureus (MRSA)	Methicillin	Staphylococcus aureus (MRSA)
FA Control 6	Staphylococcus aureus	Staphylococcus aureus (MRSA)	Methicillin	Staphylococcus aureus (MRSA)
FA Control 7	Staphylococcus aureus	Staphylococcus aureus (MRSA)	Methicillin	Staphylococcus aureus (MRSA)
FA Control 8	Staphylococcus aureus	Staphylococcus aureus (MRSA)	Methicillin	Staphylococcus aureus (MRSA)
FA Control 9	Staphylococcus aureus	Staphylococcus aureus (MRSA)	Methicillin	Staphylococcus aureus (MRSA)
FA Control 10	Staphylococcus aureus	Staphylococcus aureus (MRSA)	Methicillin	Staphylococcus aureus (MRSA)
FA Control 11	Staphylococcus aureus	Staphylococcus aureus (MRSA)	Methicillin	Staphylococcus aureus (MRSA)
FA Control	Enterococcus faecium	Enterococcus faecium	Vancomycin	Enterococcus faecium

12				(A/B)	
FA 13	Control	Enterococcus faecium	Enterococcus faecium	Vancomycin (A/B)	Enterococcus faecium
FA 14	Control	Enterococcus faecium	Enterococcus faecium	Vancomycin (A/B)	Enterococcus faecium
FA 15	Control	Salmonella typhi /Escherichia coli	Escherichia coli		Escherichia coli

Appendix III: Microscan assay procedure SOP

SOP Title: **MICRO SCAN ASSAY PROCEDURE SOP**	SOP No: **MHK-BAC 018**
	Version: **6**
Effective Date: 14 March 2018	Pages: 17

1. PURPOSE/APPLICABILITY:

1.1. Purpose

1.1.1. This SOP ensures proper use of the Micro scan 40plus in the identification of microorganisms.

1.1.2. This SOP elaborates the use of the conventional, Rapid and Synergy panels used for processing the patient samples and Quality control.

1.1.3. This SOP explains on phenotypic confirmation of ESBL presence requires confirmation testing, using the Micro Scan ESBL plus ESBL Confirmation Panel or Dried Gram Negative Panels with Streamlined ESBL dilutions.

1.2. Applicability

1.2.1. This standard applies to all designated quality assurance quality control (QAQC) personnel, Laboratory personnel, visiting students working within the MHK laboratory and USAMRU-K officer.

2. RESPONSIBILITIES:

2.1. It is the responsibility of all staff to follow the SOPs that impact the research and clinical activities performed.

2.2. Technical staff is responsible for the preparation, review and updating all SOPs relative to their daily operations.

2.3. The lab director/designee and QA/QC personnel are responsible for ensuring that all SOPs are updated annually to meet the standards outlined within this SOP.

2.4. Lab director is responsible for reviewing, signing, dating and approving all procedural standards and other policies.

3. MATERIALS AND EQUIPMENT:

3.1. Micro scan

3.2. Printer

3.3. Micro scan Bar code printer

3.4. Lab pro software (Micro scan software)

3.5. Monitor

3.6. Micro Scan Turbidity Meter

3.7. Rehydrating Inoculating system
3.8. Micro Scan Combo Panels and synergy
3.9. Isolate/ culture organism
3.10. Prompt inoculators
3.11. Inoculum water 3ml/6ml
3.12. 25ml innoculum water with pluronic
3.13. 10% bleach
3.14. Pipette and pipette tips
3.15. D and R inoculating trays
3.16. Tray lids
3.17. Yeast turbidity standard
3.18. Media plate for purity plate.

3.19. **Safety precautions**

4.1.1 Treat all isolates/ cultures are potentially infectious.

4.1.2 Always don PPE while processing/ preparing panels

4.1.3 Discard all used panels and materials as biohazard waste**.**

3.20. Dried Overnight and Rapid Chromogenic panels (gram negative aerobic bacilli and gram positive aerobic bacteria for 16-18 hrs), the colorimetric system guides light from an interference filter through optical fiber channels and then through the 96 wells of each panel. Light sensitive photodiodes detect the amount of light passed through each well and generate a corresponding electronic signal for each well. The resident computer in the WalkAway instrument compares these signals to stored control values and sends the data to LabPro for calculation and analysis.

Synergies plus panels (gram negative aerobic bacilli and gram positive aerobic bacteria for $2^1/_2$hrs) combine fluorometric identification and colorimetric MIC testing to provide rapid test results.

To identify minimum inhibitory concentrations (MICs) for a microorganism, the panel wells contain specific concentrations of antimicrobics. The turbidity or fluorescence will be less or non-existent in wells in which the antimicrobic has inhibited growth. The Walk Away instrument compares each test well reading with a threshold value. This value is a fixed number representing a certain percentage of relative absorbency or fluorescence that corresponds to clinically significant growth.

3.21. MAKING ORDER ENTRY

3.21.1. Patient Order Entry

3.21.1.1. Click patient order entry on command center

3.21.1.2. Select interface download option.

3.21.1.3. Click specimen entry and enter volunteer MHK number press enter

3.21.1.4. Click patient ID to enter volunteer study number

3.21.1.5. Enter other volunteer detail information e.g. source, age, gender, etc.

3.21.1.6. Click isolate to choose type of isolate e.g. Gram Negative Bacilli (GNB), Gram positive (GP).

3.21.1.7. Click order to enter type of panel to be used.

3.21.1.8. Click accept isolate and Save.

4.2.1 Changing Patient Orders

4.2.1.1 This procedure describes how to edit an existing patient order; delete a specimen, specimen test, isolate, or test group from an order; and finalize a specimen, specimen test, or isolate.

4.2.1.2 Editing a Patient Order

4.2.1.3 Type a specimen number and press **Enter** or click the **Specimen** Lookup button and double-click a specimen in the Specimens table. On the Specimens table, to view only active specimens, click **Active Specimens Only** on the **Data** menu.

4.2.1.4 To recall a specimen test, on the specimen tests tab, double-click a specimen test.

4.2.1.5 Click **Save** to save the specimen.

4.2.1.6 **To Delete a Specimen, Specimen Test, Isolate, or Test Group**

4.2.1.6.1 On the Patient Order Entry window, recall a specimen.

4.2.1.6.2 Click **Delete Specimen**. When the confirmation message appears, click **OK** to delete the specimen.

4.2.1.6.3 To Delete an isolate or test group, On the **Isolate Tests** tab, right-click an isolate and then click **Delete.**

4.2.1.7 **Editing a Patient ID**

4.2.1.7.1 On the Patient Review & Edit menu click on patient demographics then click ok on Warning window.

4.2.1.7.2 On the **Data** menu, click **Change Patient ID**. The **Change Patient ID** dialog box

4.2.1.7.3 Appears On the **Data** menu, click **Change Patient ID**. The **Change Patient ID** dialog box appears.

4.2.1.7.4 In the **Change To** box, type a unique patient ID and click OK.

4.2.1.7.5 When finished updating the patient order, click Save.

4.2.1.8 **To change the patient associated with a specimen**

4.2.1.8.1 On the Patient Order Entry window, click Clear on the toolbar, and then click Clear Patient.

4.2.1.8.2 Type a new ID in the Patient ID box and press Enter—or click the Patient ID Lookup button and then double-click a patient in the Patients table.

4.2.1.8.3 If the patient ID exists in LabPro, all available information displays.

4.2.1.8.4 If the ID is for a new patient, add optional patient information, if any.

4.2.1.8.5 Click **Save** to store the changes

4.2.1.9 Click **Save** to save the information.

4.2.2 **QC order Entry**

4.2.2.1 Click QC order Entry On the Command center

4.2.2.2 Type the panel lot number box and press enter.

4.2.2.3 If the panel lot number does exists, the pointer positions in the panel Type box.

4.2.2.4 If one matching panel the lot number is found, the information for that lot appears. Make sure the panel lot number is entered correctly. If not, click Clear on the toolbar, type the lot number, and press Enter. The Pointer positions in the Panel Type box.

4.2.2.5 If the lot number is correct, and you want to add a new QC order with the same lot number but a different panel type or received date, on the Data menu, click New Lot/Shipment. LabPro clears all data except the lot number and positions the pointer in the Panel Type box.

4.2.2.6 If more than one QC order with the same lot number is found, the Duplicate Lot Numbers dialog box appears. Select the desired lot number or click New Lot/Shipment to add a new QC order with the same lot number.

4.2.2.7 In the panel Type box, type the panel code and press Tab-or click the panel type Lookup button, double-click a panel in the Test Group table.

4.2.2.8 In the Received Date box, type the date the panel lot was received or click the Calendar button and then double-click the date.

4.2.2.9 **Note**: Although received date is not a required entry, it is highly recommended. The consistent entry of a received date each time

you add a new QC order makes it easier to locate and recall the order after it is saved.

4.2.2.10 The recommended ATCC Strains for QC are Pseudomonas aeruginosa ATCC 27853 and E. coli ATCC 25922 for gram negative panels.

4.2.3 **Edit or Deleting a QC Order**:

4.2.3.1 On the QC Order Entry window, in the Lot # box, type the panel lot number and press Enter to recall the order or, click the Lot # Lookup button and double-click an item on the Panel Lots list.

4.2.3.2 To edit; A lot number - Position the pointer in the Lot # box and type the new number, received date, Position the pointer in the Received Date box and type a new date, click the Calendar button and double click a day on the calendar, Delete an isolate order- Right click the isolate in the QC/Isolate Order list, and then click Delete Isolate on the shortcut menu, on the confirmation message, click OK, Delete the entire QC Order, Click Delete on the toolbar and then click Delete Lot, on the confirmation message, click OK.

4.2.3.3 Click save to store changes.

4.3 **Panel processing**

4.3.1 The panel is ordered directly during patients order entry

4.3.2 Print bar code labels

4.3.3 Press **feed** in the bar code printer and tear off the portion with the labels.

4.3.4 Place bar code labels to panels by holding the panel with the Micro Scan logo on the right side nearest you then affix the bar code label securely to the long side of the panel nearest to you and the Micro Scan logo. Make sure that the label is upright. Each panel must have individual barcode.

4.4 **Procedure for use of turbidity meter**

4.4.1 Principle: the meter uses colorimetric method by measuring the optical density of the solution's turbidity and giving a reading equivalent to 0.5 McFarland standard.

4.4.2 Place a solution in the blank slot of the turbidity meter (the same solution that one will use for preparation of the McFarland standard).

4.4.3 Prepare the suspension per specific requirements (see 4.4.4) and place in the next slot.

4.4.4 Check if the reading is within range (bacterial between 0.06 to 0.12 and yeast 0.8 to 1.2).

4.4.5 If the reading is above, dilute further using the same solution i.e. 3ml inoculums water if that is what was used and take readings again.

4.4.6 If within range, continue with the panel processing.

4.5 **Procedure for use of the RENOK Inoculator**

4.5.1 Hold the inoculators by pressing the ears

4.5.2 Fit on the inoculating tray (D or R) and release to hold the cover.

4.5.3 Aspirate/draw the suspension by holding the black button to the front of the inoculators and pull upwards.

4.5.4 Fit the lid on the panel to be inoculated and press the center button to dispense the inoculums.

4.5.5 Replace the lid on the inoculating tray and holding the ears, release the tray lid and return the RENOK to its holder.

4.6 Inoculate depending on the panel type with microorganisms from a culture plate or pallet from blood culture as follows:

4.6.1 Inoculating **Conventional panels**

4.6.1.1 **Using Turbidity Method (PC20/NBPC30/NBPC34/NMIC32)**

- Using a Swab, touch 3-4 isolated colonies with similar morphology (patients Sample or QC organisms) and make a suspension equivalent to 0.5 McFarland standards (0.06-0.12 on Dade microscan turbidity meter) in 3 ml inoculum water Or pallets from **blood culture bottles prepare as follows:**
  - Perform gram stain on the positive blood culture to verify a single isolate
  - Using 10ml syringe, transfer 10ml of the blood from the blood culture bottle to a sterile universal container using aseptic condition.
  - Centrifuge the bottle at 1400Xg for 10min
  - Transfer the supernatant to a new sterile universal bottle using a sterile plastic Pasteur pipette.
  - Centrifuge the new bottle at 1400Xg for 10 min
  - Remove and discard the supernatant

- The pallet containing the bacteria is used to standardize the 0.4% Saline with Pluronic, 6.5 ml or equivalent to give a turbidity equivalent to 0.5 Mc Farland.
- Transfer 100 ul into 25 ml Inoculums' water with pluronic. Mix and transferring into inoculators' D set (tap for 20seconds to clear the bubbles).
- Using the Renok, inoculate the suspension into the panel.
- Cover the panel with a lid and load into the Micro Scan with barcode label facing inside.
- Each panel will be automatically read after loading for 20 minutes to 3 hour (depending on the panel type).

4.6.1.2 **Conventional panels using prompt Inoculation method gram negative and gram positive panels.**

- Hold the prompt inoculation wand perpendicular to the surface of the agar and touch 3-4 isolated colonies as large as or larger than the wand tip.
- Remove the collar
- Place the wand in the prompt bottle after you have snapped off the bottle
- Shake the bottle vigorously to create a suspension of the bacteria in the 30 ml stabilized aqueous Pluronic-D.
- Pour the suspension into a seed tray (inoculators D sets)
- Position RENOK Rehydrating Inoculator on the transfer lid and transfer the suspension into the panel
- Cover the panel with a lid and load into the Micro Scan

4.6.1.3 **Microstrept plus Type 1 Panel (MSTRP1)**

- Make suspension equivalent to 0.5 McFarland standard (0.06-0.12 on turbidity meter) in 3 ml inoculums saline.
- Transfer 100ul into 25ml MH broth with 3% LBH. Mix.
- Using the Renok, inoculate MH broth suspension into the panel. (Twice draw &dispense before transferring into the panel).
- Place the panel cover &load in the microscan. Microscan Reads Results at 20-24 Hours.
- **Note**: MSTRP+1=MicroStrept plus panel Type 1(Special panel for broth dilution MIC for all the *Streptococcus spp* including *S. pneumoniae*)

4.6.1.4 **Rapid ID and MIC combination panel type**

- **NMIC panel**
- Prepare a suspension equivalent to 0.5 McFarland standard (0.06-0.10 on turbidity meter) in 6.5 ml 0.4% inoculums saline

- Transfer 100 ul into 25ml Inoculum water with pluronic. Mix and transfer into inoculators D sets and inoculate the panel using RENOK Rehydrating inoculator.

4.6.1.5 **Rapid gram negative ID**

- Prepare suspension equivalent to 0.5 McFarland standard (0.06-0.10 on turbidity meter) in 6.5 ml 0.4% inoculums saline.
- Use the inoculators R, it has two separate compartments.
- Transfer the remaining 6.5% inoculums saline into small compartment (ID part).Pour 25ml of un-inoculated inoculums water with pluronic-D into the large compartment.
- Inoculate the rapid Negative ID 3 panel using the Renok.
- Place the panel cover for gram negative MIC panel, Rapid ID Panel and Rapid gram negative ID and load in the Microscan.

4.6.1.6 **Synergy plus Gram negative Combo Panel Preparation**

- Using a sterile loop, emulsify colonies (from Sheep blood agar/ non selective media) or pallet from blood culture in 6.5ml inoculums saline.The final suspension should be equivalent to 0.5 McFarland standard. Use vortex to have a homogeneous suspension.
- Pipette 100ul of the standardized suspension into 25ml of Inoculum water with pluronic.
- Use the inoculators R for Renok inoculation, it has two separate compartments.
- Transfer the remaining 6.5% inoculums saline into small compartment (ID part).Pour 25ml of uninoculated inoculums water with pluronic-D into the large compartment.
- Place the panels in the Microscan, ID results will be ready in about 2.5Hours, sensitivity will be ready in about 5-10Hours and all will be complete in 18 Hours

4.6.1.7 **RYID(Rapid yeast ID)**

- Make suspension equivalent to yeast turbidity standard (0.8 on turbidity meter) in 3 ml inoculums water.
- Transfer 50ul into each labeled well.
- Place the panel cover & load in the Microscan. Results will be ready in about 4 Hours.

4.6.1.8 **ANO2 Panel: RAID (Rapid Anaerobic ID)**

- Prepare a suspension equivalent to Yeast turbidity standard (0.8 on turbidity meter) in 3 ml HNID broth.
- Transfer 50ul into each labeled well.
- Place the panel cover and load in the Microscan.

- For manual reading add 1-2 drops of mineral oil in urea and Indole (IND) wells Results are ready in 4 hours.

4.6.1.9 **HNID (Haemophilus and Neisseria ID)**

- Prepare a suspension equivalent to Yeast turbidity standard (0.8 on turbidity meter) in 3 ml inoculums water.
- Transfer 50ul into each labeled well.
- Place the panel cover and load in the Micro scan. Results are ready in 4 hours.

4.6.2 **Procedural note**:

4.6.2.1 **Always subculture from the suspension for purity check of the innoculum.**

4.6.2.2 Load the panels into the WalkAway instrument with the barcode label facing inside

4.6.2.3 The panels will be incubated in Micro Scan at 35 ºC for 2 to 42 hrs, depending on the panel type.

4.6.2.4 The WalkAway instrument then processes panels (refer to SOP on Use and maintenance).

4.6.3 **Results review and interpretation(Conventional and some rapid panels)**

4.6.3.1 On the Walk Away Monitor, the WalkAway Status, Load Status or Exception Status tab, double click a panel, or on the QC Order Entry window, read a panel in Micro scan instrument, the QC results summary and edit dialog box displays

4.6.3.2 Review the panel results; **Ø** symbol indicates an out-of-control MIC**,** the tested value for an antimicrobic in a Hold state is N/R and the out-of-control value is blank, the tested value and the out-of-control value for a biochemical in a Hold state is a 42 Hour

4.6.3.3 Check on the alert and/or most probable organism as the percentages are displayed.

Note: The percentage should be greater than 85% for the organism identified. if less than 85% or if there is an alert, trouble shoot as in 4.3 below**. Note: The results will be cross referenced to the colonial morphology of the initial culture plate and the purity plate.**

4.7 **ESBL Screening Procedures:**

4.7.1 **Principle:** Phenotypic confirmation of ESBL presence requires confirmation testing, (e.g. the MicroScan ESBL plus ESBL Confirmation Panel or Dried Gram Negative Panels) with Streamlined ESBL dilutions. These panels contain doubling dilution of ceftazidime with Ceftazidime/clavulanate; and cefotaxime with cefotaxime /clavulanate as per current CLSI recommendations for confirmation of

ESBL producing isolates. LabPro is customized to display this message if an isolate qualifies as a suspected ESBL Producer.

4.7.2 **ESBL Screen**

4.7.2.1 Process Dried Overnight Gram-Negative Panels, NBPC 30/34 as above.

4.7.2.2 Check panel processing alerts and exception message as Suspected ESBL and resolve the exception by, leaving the panel on the exception status tab then setting up the ESBL confirmation panel or test.

4.7.2.3 Leaving the results on the results summary and edit dialog box. Save the results (results are reported as final) but an isolate comment can be added indicating that further test on the isolate is pending. Set up the ESBL confirmation panel/test.

4.7.2.4 Save the results as suspected and report as final on the results summary and edit dialogue box.

4.7.3 **ESBL Confirmation**

- Set up the MIC combo 32 panel (refer to 4.2 on panel preparation).
- Check the results status and type in the organism name on the box for isolate.
- Save the results.

4.7.4 **ESBL Result and interpretation**.

4.7.4.1 **ESBL** indicates a confirmed ESBL when, the antibiotic(s) within the screening set that meet the conditions have the MIC reported and the interpretation **ESBL**.

4.7.4.2 The other screening antibiotics that do not meet the conditions will have the MIC reported and the interpretation **R***.

4.7.4.3 All other cephalosporins and penicillins will have the MIC reported and the interpretation **R***.

4.7.4.4 The **EBL? ESBL**, and **R*** footnotes will appear on the patient report form.

4.7.4.5 The carbapenem (imipenem and meropenem), beta-lactam/beta-lactamase inhibitor combinations, and all other antimicrobics will have normal MICs and **SIR** interpretations. The LabPro interface will transmit the interpretations **ESBL** and **R***

4.8 **Resolving Panel processing Alerts and Exception Messages.**

4.8.1 **Resolving Bar Code Errors**

4.8.1.1 A bar code read error may occur after the panel is loaded into a Walk Away Instrument, the instrument detects the bar code label on the panel, but it cannot read the data encoded in the bar code. For example, the bar code label is smudged, the print is light, or the reader malfunctions or is out of alignment.

4.8.1.2 To identify the tower slot location of any panel with a bar code read error, note the position of the bar code read error symbol on the **WalkAway Status** grid.

- Request access to the WalkAway instrument with the bar code read error. When access is granted, position the corresponding tower behind the panel access door.

4.8.1.3 After the instrument doors unlock, open the panel access door and unload the panel from the instrument.

4.8.1.4 Find the specimen or lot number; isolate number, and panel type on the bar code label for the unloaded panel.

4.8.1.5 Find the problem and correct it, request access and reload back the panels into the corresponding tower and lock the door.

Note: Ensure that the bar code faces toward the inside of the instrument.

4.8.2 **Resolving Unread Panels**

4.8.2.1 The panel was not loaded into a Walk Away instrument, check the towers and resolve the problem.

4.8.2.2 The bar code reader cannot read the bar code label on the panel (bar code read error), resolve the bar code error

4.8.2.3 The panel was loaded into the instrument, but the bar code reader cannot detect the bar code, resolve the bar code error

4.8.2.4 The panel was loaded without the lid, counter check and place the lid on the panel

4.8.3 **Resolving insufficient growth.**

4.8.3.1 Occurs at the final read when the Microscan does not detect significant growth well.

4.8.3.2 For Dried overnight and synergies plus panels.

- The ID may be valid but the MIC is reported as N/R. Visually check the panel.
- If you feel there is sufficient growth in the Growth well, you can record the MIC values on the Results (or QC Results) Summary and Edit dialog box. Click process test group data and then save.
- If the Growth well is not acceptable, reorder or delete the test group.

4.8.3.3 Rapid Fluorogenic Panels

- The ID portion is valid. For rapid Combo and MIC only panels, the MICs are reported as *N/R* considers testing the isolate on Dried Overnight MIC panel.
- **Note:** Delete the Rapid Combo panel test group before ordering Dried overnight MICs panels on the same isolate. Keep in mind the organism identification from the Rapid Combo panel and enter it

when ordering the new panel.

4.8.4 **Oxidase Required Exception**

4.8.4.1 Occurs when Dried Overnight Gram –Negative ID or Combo panel was ordered, the oxidase reaction defaulted to No Test. LabPro cannot differentiate between several potential organisms without the oxidase reaction.

4.8.4.2 Perform an oxidase test.

4.8.4.3 On the Results Summary and Edit dialog box, enter the oxidase result, and click Process test group data.

4.8.4.4 Review the results, and then click save.

4.8.5 **Resolving Low probability ID (First choice organism on the probable organism list has a probability less than 85%)**

4.8.5.1 Confirming the organism ID on the Results Summary and Edit dialog box, do one of the following :

- If the first choice on the probable Organism List is acceptable, save the panel data.
- If the first choice is not acceptable, select an alternate organism from the probable Organism List or Organism table, and then click Save or delete or reorder panel test group.

4.8.5.2 **Synergy plus panels only.** If processing a Synergy plus Gram negative panel, you must enter a valid organism before the walkaway instrument reads the first MIC to obtain rapid results .Failure to do this default processing continues, and MICs for antimicrobics with rapid limitation are held for 16/18 hour read. Once default processing is set, you must enter an organism before MIC results can be stored.

4.8.5.3 For Synergy plus Gram negative panel, Lab pro must have a valid organism ID to calculate the MICs. To obtain rapid results, you must enter a valid organism before there is enough growth in the panel Growth well for the Walkaway instrument to read the first MIC (generally at 41/2hours.If the above was not done, you can still enter an organism before the panel is complete to obtain 16/18 hour MIC results. If an organism is not entered before the panel is complete, LabPro reports all MICs as N/R.

4.8.6 **Resolving Very Rare Biotype**

4.8.6.1 Occurs when the organism biotype number is not in the standard LabPro database.

4.8.6.2 On Results summary and Edit dialog box, enter an organism code (or select an organism from the Organism table) and then save the panel data or delete and reorder the panel test group.

4.8.6.3 If processing Synergy plus Gram negative panel. Lab pro must enter a valid organism before the Walkaway instrument reads the

first MIC to obtain rapid results. Failure to do this defaulting processing continues, and MICs for antimicrobics with rapid limitations are held for a 16/18 hour read. Once default processing is set, you must enter an organism before MIC results can be stored.

4.8.6.4 If you are processing a Synergy plus positive negative panel. Lab pro must have a valid organism ID to calculate the MICs. To obtain rapid results, you must enter a valid organism before there is enough growth in the panel Growth well for the Walkaway instrument to read the first MIC (generally at $4^1/_2$hours.If the above was not done, you can still enter an organism before the panel is complete to obtain 16/18 hour MIC results. If an organism is not entered before the panel is complete, LabPro reports all MICs as N/R.

4.8.6.5 Take the following steps as needed to resolve this exceptions:

- Check purity plate.
- Compare the actual panel results to the results on the Results summary and Edit dialog box. If necessary edit and reprocess the panel results.
- Confirm that the correct oxidase result was entered (Dried Overnight Gram –Negative ID and Combo panels).
- Confirm the right family was selected .You may be able to change the family for Dried Overnight Gram– positive ID on the Results Summary and Edit dialog box.
- If processing in a Walkaway instrument, confirm that the instrument added the correct reagents to the panel. (Other biochemical tests assays such as API or single biochemical test can be done manually to verify the biochemical that should be positive for the organism.
- If necessary, use the biotype look up tool. On the Utilities Window, double click System and then Biotype Lookup.
- Repeat test may be performed for the same isolate.

4.8.6.6 Refer to MHK-BAC 023 Appendix 7.2 Processing options/ responses/ Corrective action.

NOTE:

1. Isolates resistant to routinely tested antimicrobial agents will be referred to an outside laboratory for testing supplemental agents.

   This will be addressed to as below or according to protocol specific requirements:

   WRAIR,
   Division of Bacterial and Rickettsial Diseases,
   503 Grant Ave.,
   Silver Spring, MD 20910.

2. Taxonomic changes may occur in the genus and or species of microorganisms leading to changes in antibiotics for antimicrobial susceptibility testing and interpretation. This information can be obtained from but not limited to manufacturer's bulletins', CLSI guidelines, CAP/ UKNEQAS participants' summaries and peer reviewed scientific journals. These changes will be implemented after approval.

Appendix IV: Operation and maintenance of Film Array machine

SOP Title: **OPERATION AND MAINTENANCE OF FilmArray machine**	SOP No: **MHK-EQP**
	Version **. 1.**
Effective Date: August 2013	Page 1 of 11

1. PURPOSE/ APPLICABILITY:

1.1. **Purpose:**

1.1.1. The FilmArray Blood Culture ID Panel (BCID) tests for a comprehensive panel of pathogens which cause blood infections. The FilmArray integrates sample preparation, amplification, detection, and analysis into one simple system that requires 3 minutes of hands-on time and has a total run time of about 1 hour.

1.1 **Applicability:**

1.2.1 This standard applies to all designated Quality Assurance Quality Control (QAQC) personnel, Laboratory personnel, visiting students working within the MHK laboratory and USAMRU-K officer.

1.2.2 All personnel who use the Idaho FilmArray Instrument are responsible for the maintenance.

2. RESPONSIBILITIES:

2.1 It is the responsibility of all staff to follow the SOPs that impact the research and clinical activities performed.

2.2 Technical staff is responsible for the preparation, review and updating all SOPs relative to their daily operations.

2.3 The lab director/designee and QA/QC personnel are responsible for ensuring that all SOPs are updated annually to meet the standards outlined within this SOP.

2.4 Lab director/designee is responsible for reviewing, signing, dating and approving all procedural standards and policies as well as any changes that are made.

3. EQUIPMENT AND MATERIALS:

3.1 FilmArray Instrument

3.2 Loading station

3.3 Hydration solution

3.4 Syringes and needles

3.5 Pasteur pipette

3.6 BCID Pouches

3.7 Turbidity meter

3.8 Sample buffer

3.9 Sample

4. PROCEDURES

4.1 Principle

The FilmArray reagent pouch stores all the necessary reagents for sample preparation, PCR, and detection in a freeze-dried format. Prior to a run, Hydration Solution is injected and positive aerobic blood culture sample combined with Sample Buffer into the pouch. The FilmArray instrument does the rest.

First, the FilmArray extracts and purifies all nucleic acids from the sample. Next, the FilmArray performs a nested multiplex PCR. During the first-stage PCR, the FilmArray performs a single, large volume, massively multiplexed reaction. Last, individual singleplex second-stage PCR reactions detect the products from the first stage PCR.

4.2 Operation

4.2.1. Pouch preparation.

4.2.1.1. Load the pouch into the loading station

4.2.1.2 The freeze-dried reagents in the pouch are resuspended with hydration solution using a 3 ml syringe fitted with a blunt metal cannula.

4.2.1.3. The cannula is inserted into the hydration port where it breaks a septum in the port. The vacuum in the fitment (pouch) draws liquid to fill wells 2 through 11 (~80 µl each). (Do not press the plunger)

4.2.1.4. Sample to be tested is mixed with two volumes of a denaturing sample buffer and injected into the pouch through the sample injection port, Well 1 draws in 300 µl of this mixture. (Do not press the plunger)

4.2.1.5. The loaded pouch is then inserted into the FilmArray instrument, and the pouch and sample are identified to the instrument by the operator using a hand-held bar code reader.

4.2.1.6. After the run is started all further steps are performed by the instrument.

4.3. **Safety**

4.3.1. Sample injection into the pouch is performed in a biosafety cabinet following the appropriate biohazard guidelines for working with potentially infectious samples.

4.3.2. Syringes with needles are thrown into the sharp container; used sample buffers and hydration liquid are thrown into biohazard bag.

4.3 Maintenance

4.3.1 **Cleaning**

4.3.1.1. the instrument, loading station and the barcode reader are wiped with 10% bleach 3 times followed by distilled water 2 times at least once a week

4.4 **Quality control**

4.4.1 Avoid touching the tip of the needle

Appendix V: Budget

Materials	Cost per sample (USD)		
	API	**MS**	**FA**
API BioMerieux	4.72	N/A	N/A
MacConkey agar	0.16	0.16	N/A
Hektoen enteric agar	0.32	N/A	N/A
Triple sugar iron agar	0.14	N/A	N/A
Blood agar base	0.14	0.14	N/A
Dish Petri CS-100 15X100m	1.05	N/A	N/A
Gram stain kit	0.22	0.22	N/A
Polyester tipped swabs	0.25	0.25	N/A
Sheep blood agar	0.24	0.24	N/A
3ml inoculum water	N/A	2.66	N/A
25ml inoculum water with pluronic	N/A	2.92	N/A
Inoculator D sets	N/A	1.88	N/A
Panel lid	N/A	0.71	N/A
Microscan Panel (GPC/NBPC)	N/A	7.64	N/A
Kovacs reagent	0.71	0.71	N/A
0.8% Sulfanilic acid (Nit 1)	0.49	0.49	N/A
0.5% N-N-Dimethyl-Alpha-Naphthylamine (Nit 2)	0.63	0.63	N/A
40% Potassium hydroxide (Vp1)	0.82	0.82	N/A
Alpha naphthol (Vp2)	0.54	0.54	N/A
10% Ferric chloride (TDA)	0.59	0.59	N/A
Peptidase reagent	0.49	0.49	N/A
NaOH	0.53	0.53	N/A
Oxidase reagent	0.52	0.52	N/A
Blood culture bottle	6.98	6.98	6.98

Blood culture identification pouch	N/A	N/A	124.58
Inoculating needle	0.12	0.12	N/A
Needles and syringes	0.3	0.3	0.3
Biohazard bag	0.2	0.2	0.2
Microscope slides	0.32	0.32	N/A
Sharps containers small	3.95	3.95	3.95
Lens cleaning paper	0.08	0.08	N/A
Bleach	1.44	1.44	1.44
Mineral oil	0.24	0.24	N/A
Oil Immersion	0.24	0.24	N/A
Absolute Ethanol	2.17	2.17	2.17
Pipette tips	0.08	0.08	N/A
Gloves	0.49	0.49	0.49
Total	**29.17**	**38.75**	**140.11**

Appendix VI: Salmonella spp plate

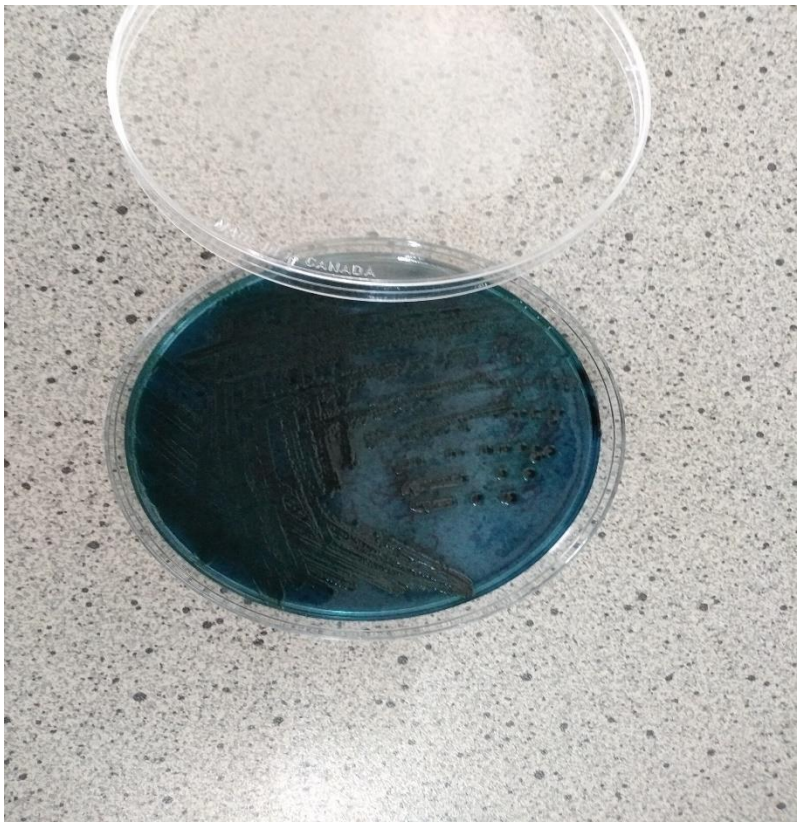


Figure 1: Salmonella spp plate

Appendix VII: Culture media plates

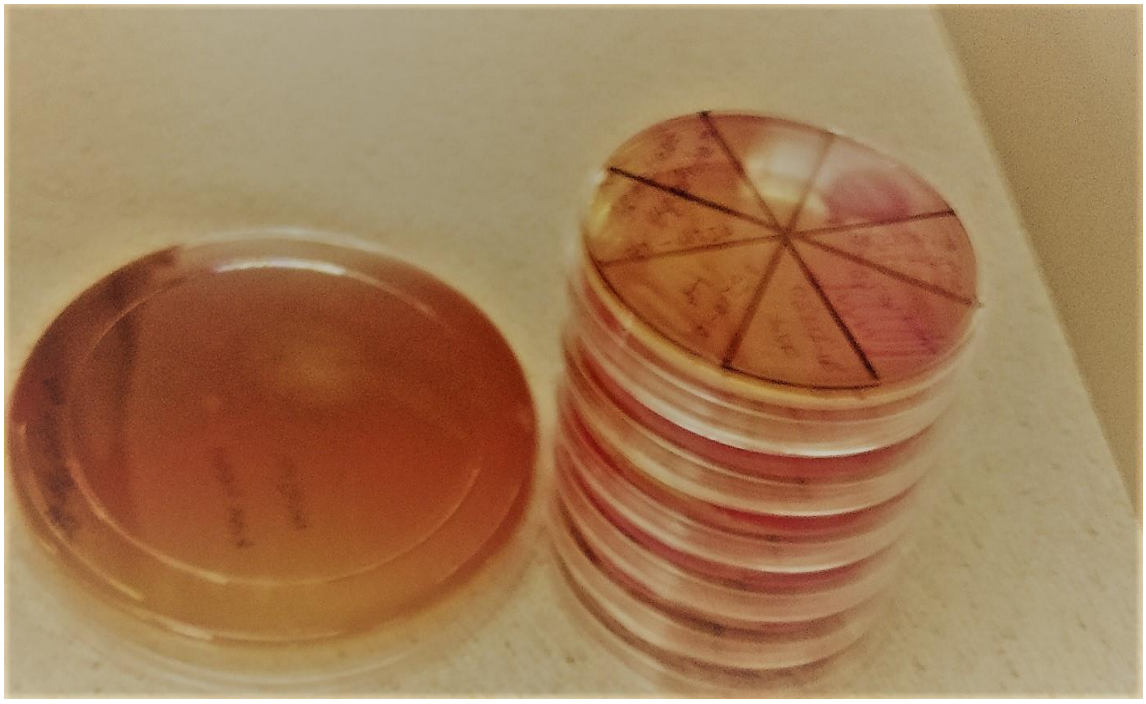


Figure 2: culture media plates

Appendix VIII: Yeast Identification

REF 20 210 07628**H** - en - 2010/02

®20 C AUX **IVD**

Yeast identification system

SUMMARY AND EXPLANATION

API 20 C AUX is a system for the precise identification of the most frequently encountered yeasts. The complete list of those species that it is possible to identify with this system is given in the Identification Table at the end of this package insert.

PRINCIPLE

The API 20 C AUX strip consists of 20 cupules containing dehydrated substrates which enable the performance of 19 assimilation tests. The cupules are inoculated with a semi-solid minimal medium. The yeasts will only grow if they are capable of utilizing each substrate as the sole carbon source.

The reactions are read by comparing them to growth controls. Identification is obtained by referring to the Analytical Profile Index or using the identification software.

CONTENT OF THE KIT (Kit for 25 tests)

- 25 API 20 C AUX strips
- 25 incubation boxes
- 25 ampules of API C Medium
- 25 result sheets
- 1 package insert

COMPOSITION

Strip

The composition of the API 20 C AUX strip is given below in the list of tests:

TESTS	ACTIVE INGREDIENTS	QTY (mg/cup.)
0	None	-
GLU	D-GLUcose	1.2
GLY	GLYcerol	1.2
2KG	calcium 2-Keto-Gluconate	1.2
ARA	L-ARAbinose	1.2
XYL	D-XYLose	1.2
ADO	ADOnitol	1.2
XLT	XyLiTol	1.2
GAL	D-GALactose	1.9
INO	INOsitol	2.36
SOR	D-SORbitol	1.2
MDG	Methyl-αD-Glucopyranoside	1.2
NAG	N-Acetyl-Glucosamine	1.2
CEL	D-CELlobiose	1.2
LAC	D-LACtose (bovine origin)	1.2
MAL	D-MALtose	1.2
SAC	D-SACcharose (sucrose)	1.2
TRE	D-TREhalose	1.2
MLZ	D-MeLeZitose	1.2
RAF	D-RAFfinose	1.9

Medium

API C Medium 7 ml	Ammonium sulfate	5 g
	Monopotassium phosphate	0.31 g
	Dipotassium phosphate	0.45 g
	Disodium phosphate	0.92 g
	Sodium chloride	0.1 g
	Calcium chloride	0.05 g
	Magnesium sulfate	0.2 g
	L-Histidine	0.005 g
	L-Tryptophan	0.02 g
	L-Methionine	0.02 g
	Gelling agent	0.5 g
	Vitamin solution	1 ml
	Trace elements	10 ml
	Demineralized water	to make 1000 ml
	final pH : 6.4-6.8 (at 20-25°C)	

Although API C Medium contains gelling agent, **it requires no prior heating** and may be as easily pipetted as a liquid medium. It is preferable to warm it at room temperature a few hours before use. **Do not shake**.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagents / Instrumentation

- API Suspension Medium, 2 ml (Ref. 70 700) or API NaCl 0.85 % Medium, 2 ml (Ref. 20 070)
- Sabouraud Medium (Ref. 42 026 or 43 171 or equivalent)
- McFarland Standard (Ref. 70 900) No. 2
- API 20 C AUX Analytical Profile Index (Ref. 20 290), **api*web*** ™ identification software (Ref. 40 011), ATB ™ instrument or ***mini API*** ® (consult bioMérieux)
- RAT Medium [Rice Agar Tween]

Material

- Pipettes or PSIpettes
- Ampule protector
- Ampule rack
- General microbiology laboratory equipment

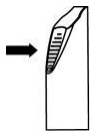
WARNINGS AND PRECAUTIONS

• **For *in vitro* diagnostic use and microbiological control.**

- **For professional use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).

- All specimens, yeast cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling yeasts should be observed throughout this procedure. Refer to "CLSI® M29-A, *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline* - Current revision". For additional handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories- CDC/NIH - Latest edition", or to the regulations currently in use in each country.

- Do not use reagents past the expiry date.
- Before use, check that the packaging and components are intact.
- Do not use strips which have been damaged : cupules deformed, etc.
- Open ampules carefully as follows:



  - Place the ampule in the ampule protector.
  - Hold the protected ampule in one hand in a vertical position (white plastic cap upper-most).
  - Press the cap down as far as possible.
  - Position the thumb tip on the striated part of the cap and press forward to snap off the top of the ampule.
  - Take the ampule out of the ampule protector and put the protector aside for subsequent use.
  - Carefully remove the cap.

- The performance data presented were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.
- Interpretation of the test results should be made taking into consideration the patient history, the source of the specimen, colonial and microscopic morphology of the strain and, if necessary, the results of any other tests performed, particularly the antimicrobial susceptibility patterns.

STORAGE CONDITIONS

The strips and media should be stored at 2-8°C until the expiry date indicated on the packaging.

SPECIMENS (COLLECTION AND PREPARATION)

API 20 C AUX is not for use directly with clinical or other specimens.

The microorganisms to be identified must first be isolated on a suitable culture medium according to standard microbiological techniques.

INSTRUCTIONS FOR USE

Preparation of the strip

- Prepare the incubation box (tray and lid) and distribute about 5 ml of distilled water or demineralized water [or any water without additives or chemicals which may release gases (e.g. Cl2, CO2, etc.)] into the honey-combed wells of the tray to create a humid atmosphere.
- Record the strain reference on the elongated flap of the tray. (Do not record the reference on the lid as it may be misplaced during the procedure).
- Remove the strip from its individual packaging and place it in the incubation tray.

Preparation of the inoculum

- Open an ampule of API Suspension Medium (2 ml) or an ampule of API NaCl 0.85 % Medium (2 ml) as indicated in the paragraph "Warnings and Precautions" of the package insert for these products, or use any tube containing 2 ml of the same solution without additives.
- Using a pipette, pick up a portion of a yeast colony either by suction or by successive touches. It is recommended to use young cultures (18-24 hours old).
- Prepare a suspension with a turbidity equal to 2 McFarland. This suspension must be used immediately after preparation.
-
- Open an ampule of API C Medium as indicated in the paragraph "Warnings and Precautions" and transfer approximately 100 µl of the previous suspension into it. Gently homogenize with the pipette, avoiding the formation of bubbles.

Inoculation of the strip

- Fill the cupules with the suspension obtained in the ampule of API C Medium. Avoid the formation of bubbles by placing the tip of the pipette against the side of the cupule. Care should be taken not to overfill or underfill the cupules (the surface should be flat or slightly convex, but never concave), otherwise incorrect results may be obtained.
- Place the lid on the tray and incubate at 29°C ± 2°C for 48-72 hours (± 6°hours).

READING AND INTERPRETATION Reading the strip

After 48 hours of incubation, or 72 hours (if the tests, in particular glucose, are not clearcut after 48 hours), compare growth in each cupule to the 0 cupule, which is used as a negative control. A cupule **more turbid than the control** indicates a **positive** reaction to be recorded on the result sheet.

In order to minimize the risks of contamination when reincubation is necessary, remove the lid only when reading the strip and replace immediately.

Morphology test

Determine the presence of hyphae (mycelium) or pseudohyphae (pseudomycelium) using RAT Medium [Rice Agar Tween].

Dispense 1 drop of the suspension obtained in the ampule of API Suspension Medium or API NaCl 0.85 % Medium onto RAT Medium or follow the manufacturer's recommendations. This test constitutes the 21st test of the strip. It is considered positive if hyphae or pseudohyphae are detected.

Interpretation

Identification is obtained with the **numerical profile**.

• Determination of the numerical profile:

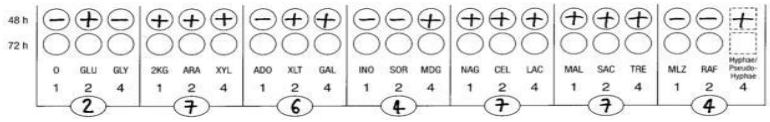
On the result sheet, the tests are separated into groups of 3 and a number 1, 2 or 4 is indicated for each. By adding the numbers corresponding to positive reactions within each group, a 7-digit number is obtained which constitutes the numerical profile.

• Identification:

This is performed using the database (V4.0)

* with the Analytical Profile Index :
  -Look up the numerical profile in the list of profiles.

* with the ATB ™ instrument, ***mini API*** ®, or **api*web*** ™ identification software :
  - Enter the 7-digit numerical profile manually via the keyboard.



2 764 774 *Trichosporon asahii*

api® 20 C AUX ……. 7628**H** - en - 2010/02

QUALITY CONTROL

The strips and media are systematically quality controlled at various stages of their manufacture.

Streamlined quality control may be used to confirm acceptable performance of the API 20 C AUX system after shipping/storage. This methodology may be performed by following the instructions above for testing and meeting the criteria stated in CLSI® M50-A Quality Control for Commercial Microbial Identification Systems.

As there are no substrates that are consistently sensitive to degradation during shipping conditions, streamlined quality control may be conducted by testing two strains: ***Cryptococcus laurentii*** **ATCC® 18803** that is mostly positive and ***Candida glabrata*** **ATCC 15126**, which is mostly negative for reactions on the API 20 C AUX system.

For those users who are required to perform **comprehensive quality control** testing with the strip, the following three strains should be tested to demonstrate positive and negative reactivity for the most of the API 20 C AUX tests.

1.	*Cryptococcus laurentii*	ATCC 18803	3. *Candida guilliermondii*	ATCC 6260
2.	*Candida glabrata*	ATCC 15126		

ATCC : American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA.

	0	GLU	GLY	2KG	ARA	XLT	GAL	INO	SOR	MDG	NAG	CEL	MAL	SAC	TRE	MLZ	
1.	–	+	–	+	+	+	+	+	+	+	+	+	+		+	+	–
2.	–	+	V	–	–	–	–	–	–	–	–	–	–		–	–	+
3.	–	+	+	+	+	+	+	–	+	+	+	+	–		+	+	–

Profiles obtained after 48 hours of incubation after culture on Sabouraud agar.

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

- The API 20 C AUX system is intended uniquely for the identification of yeasts included in the database (see Identification Table at the end of this package insert). It cannot be used to identify any other microorganisms or to exclude their presence.

- Only pure cultures of a single organism should be used.

RANGE OF EXPECTED RESULTS

Consult the Identification Table at the end of this package insert for the range of expected results for the various biochemical reactions.

PERFORMANCES

5156 collection strains and strains of various origins belonging to species included in the database were tested :

- 89.7 % of the strains were correctly identified (with or without supplementary tests).
- 6.1 % of the strains were not identified.
- 4.2 % of the strains were misidentified.

WASTE DISPOSAL

Unused ampules of API C Medium may be considered as non hazardous waste and disposed of accordingly. Dispose of all used or unused reagents (other than ampules of API C Medium) as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their type and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

WARRANTY

bioMérieux disclaims all warranties, express or implied, including any implied warranties of MERCHANTABILITY AND FITNESS FOR A PARTICULAR USE. bioMérieux shall not be liable for any incidental or consequential damages. IN NO EVENT SHALL BIOMERIEUX'S LIABLITY TO CUSTOMER UNDER ANY CLAIM EXCEED A REFUND OF THE AMOUNT PAID TO BIOMERIEUX FOR THE PRODUCT OR SERVICE WHICH IS THE SUBJECT OF THE CLAIM.

PROCEDURE	p. I
IDENTIFICATION TABLE	p. II
LITERATURE REFERENCES	p. III
INDEX OF SYMBOLS	p. IV

REF **20 210** 07628**H** - en - 2010/02

20 C AUX **IVD**

Yeast identification system

SUMMARY AND EXPLANATION

API 20 C AUX is a system for the precise identification of the most frequently encountered yeasts. The complete list of those species that it is possible to identify with this system is given in the Identification Table at the end of this package insert.

PRINCIPLE

The API 20 C AUX strip consists of 20 cupules containing dehydrated substrates which enable the performance of 19 assimilation tests. The cupules are inoculated with a semi-solid minimal medium. The yeasts will only grow if they are capable of utilizing each substrate as the sole carbon source.

The reactions are read by comparing them to growth controls. Identification is obtained by referring to the Analytical Profile Index or using the identification software.

CONTENT OF THE KIT (Kit for 25 tests)

- 25 API 20 C AUX strips
- 25 incubation boxes
- 25 ampules of API C Medium
- 25 result sheets
- 1 package insert

COMPOSITION

Strip

The composition of the API 20 C AUX strip is given below in the list of tests:

TESTS	ACTIVE INGREDIENTS	QTY (mg/cup.)
0	None	-
GLU	D-GLUcose	1.2
GLY	GLYcerol	1.2
2KG	calcium 2-Keto-Gluconate	1.2
ARA	L-ARAbinose	1.2
XYL	D-XYLose	1.2
ADO	ADOnitol	1.2
XLT	XyLiTol	1.2
GAL	D-GALactose	1.9
INO	INOsitol	2.36
SOR	D-SORbitol	1.2
MDG	Methyl-αD-Glucopyranoside	1.2
NAG	N-Acetyl-Glucosamine	1.2
CEL	D-CELlobiose	1.2
LAC	D-LACtose (bovine origin)	1.2
MAL	D-MALtose	1.2
SAC	D-SACcharose (sucrose)	1.2
TRE	D-TREhalose	1.2
MLZ	D-MeLeZitose	1.2
RAF	D-RAFfinose	1.9

Medium

API C Medium 7 ml	Ammonium sulfate	5 g
	Monopotassium phosphate	0.31 g
	Dipotassium phosphate	0.45 g
	Disodium phosphate	0.92 g
	Sodium chloride	0.1 g
	Calcium chloride	0.05 g
	Magnesium sulfate	0.2 g
	L-Histidine	0.005 g
	L-Tryptophan	0.02 g
	L-Methionine	0.02 g
	Gelling agent	0.5 g
	Vitamin solution	1 ml
	Trace elements	10 ml
	Demineralized water	to make 1000 ml
	final pH : 6.4-6.8 (at 20-25°C)	

Although API C Medium contains gelling agent, **it requires no prior heating** and may be as easily pipetted as a liquid medium. It is preferable to warm it at room temperature a few hours before use. **Do not shake**.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagents / Instrumentation

- API Suspension Medium, 2 ml (Ref. 70 700) or API NaCl 0.85 % Medium, 2 ml (Ref. 20 070)
- Sabouraud Medium (Ref. 42 026 or 43 171 or equivalent)
- McFarland Standard (Ref. 70 900) No. 2
- API 20 C AUX Analytical Profile Index (Ref. 20 290), **api*web*** **™** identification software (Ref. 40 011), ATB **™** instrument or ***mini API*** ® (consult bioMérieux)
- RAT Medium [Rice Agar Tween]

Material

- Pipettes or PSIpettes
- Ampule protector
- Ampule rack
- General microbiology laboratory equipment

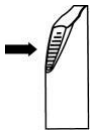
WARNINGS AND PRECAUTIONS

• **For *in vitro* diagnostic use and microbiological control.**

- **For professional use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- All specimens, yeast cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling yeasts should be observed throughout this procedure. Refer to "CLSI® M29-A, *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline* - Current revision". For additional handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories- CDC/NIH - Latest edition", or to the regulations currently in use in each country.

• Do not use reagents past the expiry date.

• Before use, check that the packaging and components are intact.
• Do not use strips which have been damaged : cupules deformed, etc.
• Open ampules carefully as follows:



- Place the ampule in the ampule protector.
- Hold the protected ampule in one hand in a vertical position (white plastic cap upper-most).
- Press the cap down as far as possible.
- Position the thumb tip on the striated part of the cap and press forward to snap off the top of the ampule.
- Take the ampule out of the ampule protector and put the protector aside for subsequent use.
- Carefully remove the cap.

• The performance data presented were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.
• Interpretation of the test results should be made taking into consideration the patient history, the source of the specimen, colonial and microscopic morphology of the strain and, if necessary, the results of any other tests performed, particularly the antimicrobial susceptibility patterns.

STORAGE CONDITIONS

The strips and media should be stored at 2-8°C until the expiry date indicated on the packaging.

SPECIMENS (COLLECTION AND PREPARATION)

API 20 C AUX is not for use directly with clinical or other specimens.

The microorganisms to be identified must first be isolated on a suitable culture medium according to standard microbiological techniques.

INSTRUCTIONS FOR USE

Preparation of the strip

• Prepare the incubation box (tray and lid) and distribute about 5 ml of distilled water or demineralized water [or any water without additives or chemicals which may release gases (e.g. Cl2, CO2, etc.)] into the honey-combed wells of the tray to create a humid atmosphere.

- Record the strain reference on the elongated flap of the tray. (Do not record the reference on the lid as it may be misplaced during the procedure).
- Remove the strip from its individual packaging and place it in the incubation tray.

Preparation of the inoculum

- Open an ampule of API Suspension Medium (2 ml) or an ampule of API NaCl 0.85 % Medium (2 ml) as indicated in the paragraph "Warnings and Precautions" of the package insert for these products, or use any tube containing 2 ml of the same solution without additives.
- Using a pipette, pick up a portion of a yeast colony either by suction or by successive touches. It is recommended to use young cultures (18-24 hours old).
- Prepare a suspension with a turbidity equal to 2 McFarland. This suspension must be used immediately after preparation.
- Open an ampule of API C Medium as indicated in the paragraph "Warnings and Precautions" and transfer approximately 100 µl of the previous suspension into it. Gently homogenize with the pipette, avoiding the formation of bubbles.

Inoculation of the strip

- Fill the cupules with the suspension obtained in the ampule of API C Medium. Avoid the formation of bubbles by placing the tip of the pipette against the side of the cupule. Care should be taken not to overfill or underfill the cupules (the surface should be flat or slightly convex, but never concave), otherwise incorrect results may be obtained.
- Place the lid on the tray and incubate at 29°C ± 2°C for 48-72 hours (± 6°hours).

READING AND INTERPRETATION Reading the strip

After 48 hours of incubation, or 72 hours (if the tests, in particular glucose, are not clearcut after 48 hours), compare growth in each cupule to the 0 cupule, which is used as a negative control. A cupule **more turbid than the control** indicates a **positive** reaction to be recorded on the result sheet.

In order to minimize the risks of contamination when reincubation is necessary, remove the lid only when reading the strip and replace immediately.

Morphology test

Determine the presence of hyphae (mycelium) or pseudohyphae (pseudomycelium) using RAT Medium [Rice Agar Tween].

Dispense 1 drop of the suspension obtained in the ampule of API Suspension Medium or API NaCl 0.85 % Medium onto RAT Medium or follow the manufacturer's recommendations. This test constitutes the 21st test of the strip. It is considered positive if hyphae or pseudohyphae are detected.

Interpretation

Identification is obtained with the **numerical profile**.

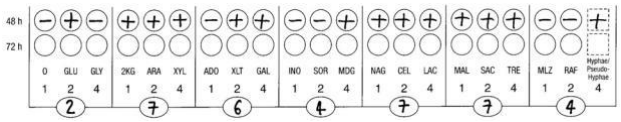
• Determination of the numerical profile:

On the result sheet, the tests are separated into groups of 3 and a number 1, 2 or 4 is indicated for each. By adding the numbers corresponding to positive reactions within each group, a 7-digit number is obtained which constitutes the numerical profile.

• Identification:

This is performed using the database (V4.0)

* with the Analytical Profile Index :
  -Look up the numerical profile in the list of profiles.
* with the ATB ™ instrument, ***mini API*** ®, or **api*web*** ™ identification software :
  - Enter the 7-digit numerical profile manually via the keyboard.



1 **764 774 *Trichosporon asahii***

api® 20 C AUX 07628**H** - en - 2010/02

QUALITY CONTROL

The strips and media are systematically quality controlled at various stages of their manufacture.

Streamlined quality control may be used to confirm acceptable performance of the API 20 C AUX system after shipping/storage. This methodology may be performed by following the instructions above for testing and meeting the criteria stated in CLSI® M50-A Quality Control for Commercial Microbial Identification Systems.

As there are no substrates that are consistently sensitive to degradation during shipping conditions, streamlined quality control may be conducted by testing two strains: ***Cryptococcus laurentii*** **ATCC® 18803** that is mostly positive and ***Candida glabrata*** **ATCC 15126**, which is mostly negative for reactions on the API 20 C AUX system.

For those users who are required to perform **comprehensive quality control** testing with the strip, the following three strains should be tested to demonstrate positive and negative reactivity for the most of the API 20 C AUX tests.

1.	*Cryptococcus laurentii*	ATCC 18803	3.	*Candida guilliermondii*	ATCC 6260
2.	*Candida glabrata*	ATCC 15126			

ATCC : American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA.

	0	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF
1.	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+
2.	–	+	V	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
3.	–	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	–	+	+

Profiles obtained after 48 hours of incubation after culture on Sabouraud agar.

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

- The API 20 C AUX system is intended uniquely for the identification of yeasts included in the database (see Identification Table at the end of this package insert). It cannot be used to identify any other microorganisms or to exclude their presence.

- Only pure cultures of a single organism should be used.

RANGE OF EXPECTED RESULTS

Consult the Identification Table at the end of this package insert for the range of expected results for the various biochemical reactions.

PERFORMANCES

5156 collection strains and strains of various origins belonging to species included in the database were tested :

- 89.7 % of the strains were correctly identified (with or without supplementary tests).
- 6.1 % of the strains were not identified.
- 4.2 % of the strains were misidentified.

WASTE DISPOSAL

Unused ampules of API C Medium may be considered as non hazardous waste and disposed of accordingly. Dispose of all used or unused reagents (other than ampules of API C Medium) as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their type and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

WARRANTY

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