Cerebrospinal fluid appearance as a diagnostic criterion for suspected bacterial meningitis in children less than five years in East Africa.

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DECLARATION

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

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DEDICATION

I dedicate this Thesis to my daughter, Natalie Watitu, my parents Dr. and Mrs.
Mate and all my family members for their love, support and encouragement
during this process.

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A wise man once said:

Our deepest fear is not that we are inadequate. Our deep fear is that we are powerful beyond measure. It is our light, not our darkness, that most frightens us. We ask ourselves, who am I to be brilliant, gorgeous, talented and fabulous. Actually, who are we not to be! As we let our own bright light shine, we unconsciously give others permission to do the same. As we are liberated from our fear, our presence automatically liberates others-

Williamson, A: Return to Love.

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ABBREVIATIONS AND ACRONYMS

CSF	- Cerebrospinal Fluid
CNS	- Central Nervous System
CI – 95%	- Confidence interval (95%)
DPT	- Diphtheria, Pertussis (whooping cough) and Tetanus
GAVI	- The Global Alliance for Vaccine and Immunization
H. influenzae (HIN)	- *Haemophilus influenzae*
***H. influenzae* b**	- *Haemophilus influenzae type b*
HepB	- Hepatitis B
ITROMID	- Institute of Tropical Medicine and Infectious Diseases
JKUAT	- Jomo Kenyatta University of Agriculture and Technology
KEMRI	- Kenya Medical Research Institute
LA	- Latex agglutination
LP	- Lumbar Puncture
N. meningitidis (NME)	- *Neisseria meningitides*
netSPEAR	- Network for the Surveillance of Pneumococcal disease in the East African Region
PPV	- Positive predictive value
PCR	- Polymerase chain reaction
ROC	- Receiver operating characteristic
SOP	- Standard operating procedures

S. pneumoniae (SPN)   - *Streptococcus pneumonia*

URTI   - Upper respiratory tract infections

WBC   - White blood cell

ABSTRACT

The study focused on the sensitivity and specificity of cerebrospinal fluid appearance and white blood cell (WBC) count as potential laboratory screening indicators compared to culture method. This is because Cerebrospinal fluid (CSF) culture facilities are expensive and difficult to maintain in resource poor laboratory settings yet this is the gold standard for diagnosis. Early signs of meningitis are often subtle and nonspecific resulting in unacceptably high mortality and morbidity rates in children, especially those from developing countries where rapid access to medical attention and resources is unavailable. Diagnosing acute bacterial meningitis in children is likely to be missed in a third of cases at district hospitals in sub-Saharan Africa where adequate and reliable laboratory resources are lacking. Most affected patients now survive due to antibiotic use, though many children still die or suffer permanent neurologic sequelae as a result of bacterial meningitis. Data was gathered from samples collected from children aged below five years admitted at the participating hospitals between the time periods 2001 to 2008. This was carried out retrospectively from the period 2001 to 2005 and prospectively from 2006 to 2008. A total of 32,152 samples were collected for the entire period. Of the 29,153 samples collected with reported appearance, 4.49% of them were positive for pathogenic organisms out of which three micro-organisms were of most interest to the surveillance; *Streptococcus pneumoniae, Haemophilus influenzae* and *Neisseria meningitidis* that accounted for 50.7% of the positive isolates. Turbid appearance had a sensitivity of 72% (95% CI 69 – 74) and specificity of 96%. Clear appearance had a sensitivity of 18% (95% CI 16.1 – 20.3) and a specificity of

17%. White blood cell count greater than 5 (WBC>5) per microlitre had a sensitivity of 81.5% (95% CI 77.2 - 85.3) and a specificity of 78.7%. White blood cell count greater than 10 (WBC>10) per microlitre had sensitivity of 80.1% (95% CI 75.7 – 84) and specificity of 91.3%. The 32,152 samples were also divided into Pre- and Post- Hib eras to account for the introduction of the *Haemophilus influenzae* type b vaccine that was introduced in the region. The pre-Hib, vaccine era accounted for 26.8% of the samples of which 5.0% were culture positive. The post-Hib data accounted for 73.2% of the total samples of which, 11.8% were culture positive. Although the number of positive isolates in the pre and post Hib eras varied significantly, there were insignificant differences in the sensitivities and specificities of turbidity and white cell counts. The recommendation is that, presence of a turbid sample is a good indicator of presence of an etiological agent. However, using (one evaluation method) appearance alone is not adequate. Additional use of white blood cell counts, as a screening criterion should be included to increase the test sensitivity to a point where it is useful for surveillance. The benefit of this study results is that this information can be used to advise laboratory personnel on what minimum criteria can be used to analyze CSF samples to reduce on missed cases and also maximize on the limited laboratory resources.

CHAPTER ONE

1.0   INTRODUCTION

1.1   Background

This work carried out is part of a larger multi-country study under the programme of netSPEAR, which is the Network for Surveillance of Pneumococcal disease in the East African Region. The main objective of the surveillance is to estimate the disease burden from bacterial infection in the region in light of the soon to be introduced pneumococcal conjugate vaccine. This surveillance sub-protocol; which forms my thesis work, revolved on hands-on isolation, identification and recording of the bacteria obtained from the collected cerebrospinal fluid samples obtained from children by clinicians.

Meningitis is the inflammation of the protective membranes that cover the brain and spinal cord. These membranes are commonly referred to as the meninges and are composed of the pia-mater which is the innermost membrane, the dura-mater that is the outermost membrane that lies below the bones of the skull and the arachnoid membrane that is found between the pia-mater and the dura-mater. The subarachnoid space separates the arachnoid and pia-mater membranes, and is filled with cerebrospinal fluid.

Cerebrospinal Fluid (CSF) is a dynamic metabolically active clear fluid surrounding and cushioning the brain and spinal cord. It occupies the ventricles, the

subarachnoid space and the central canal of the spinal cord. Normal CSF contains glucose, electrolytes, amino acids, but has very little protein and few cells that provide immunological protection (Dimitri, 2006).

Meningitis can be categorized according to the onset of symptoms into acute, sub-acute and chronic inflammation. The onset of sub-acute disease may take one to seven days; chronic cases more than 7 days while for acute meningitis the onset of signs takes less than 24hours. Meningitis can also be categorized according to aetiological agents involved; it may be due to bacterial, viral or fungal infection. Each of these aetiological causes is illustrated but for the purposes of this work, emphasis will be on the bacterial cause of meningitis in children under five years.

1.1.1 Causes of Meningitis

1.1.1.1 Viral Meningitis

This is an infection that is also referred to as aseptic meningitis. Although viral meningitis is the most common cause of meningitis, it is less severe and rarely fatal in people with normal immune systems than other causes of meningitis and clears up with no specific treatment (CDC E-brief, 2008). Viral meningitis is quite common and a relatively mild illness, when compared to most bacterial meningitis. Usually, the symptoms last from 7 to 10 days and the patient recovers completely (CDC E-brief, 2008). Although different viruses can lead to viral meningitis, the enteroviruses are the most common causes with other viruses like mumps,polio, herpes simplex viruses,measles and influenza also contributing to this infection.

Majority of people who get infected by viral meningitis do not exhibit symptoms or show low-grade fever, irritability in children, mouth sores or a cold. These symptoms however normally clear on their own and very few people go on to develop meningitis (Nigrovic *et al.*, 2007). Diagnosis of viral meningitis is based on clinical history compatible with meningitis, and a physical exam in which pain is elicited upon flexing the neck. A spinal tap that collects spinal fluid by use of a small needle inserted into the back will often show cells under the microscope compatible with a viral infection (Amir & Cordia, 2009).

The mortality rate of viral meningitis without factoring in the neonatal age is less than 1%; the morbidity rate is also low. The incidence of viral meningitis drops with age with children in their first year of life being at the highest risk of infection. This is however reduced by the vaccination of these children from mumps, polio, and measles viruses that greatly reduce the risk of viral causes of meningitis (Cherry & Feigin, 1998). Since viral meningitis normally does not have specific treatment regimes, doctors normally advice for the patent to have bed rest, plenty of fluids, and medicine to relieve fever and other symptoms. Antibiotics treatment is not advised since they are not useful in the treatment of viral infections. (Perez-Velez *et al.,* 2007).

1.1.1.2 Fungal Meningitis

Fungal meningitis is very rare in persons with normal immune systems, it is however a life threatening disease and may be caused by a variety of fungi, including most commonly *Cryptococcus neoformans* and *Candida albicans.*

Meningitis caused by a fungal infection is not common and is mostly observed in people with immune-compromised systems due to disease like HIV/AIDS or leukemia sufferers or even from immunosuppressant drug therapy. Cryptococcal meningitis is the most common cause of fungal meningitis that is observed (Safdieh *et al.,* 2008).

The organism is common in the environment and transmission is through the inhalation of air borne yeast cells. Majority of healthy people will not develop an infection from these inhaled fungi; however people with severely depressed immune systems may be unable to prevent the fungi from surviving and causing infection. (http://www.meningitis.org/sect5/subsect10). Symptoms are almost similar to those from other causes of meningitis and include headache, fever, stiff neck, lethargy, irritability and confusion. Left untreated, fungal meningitis can be fatal. It can also cause the following damage such as hearing loss, learning problems, difficulty talking, seizures, or paralysis (Slavoski & Tunkel*,* 1995).

Diagnosis is usually based on the analysis of blood and cerebrospinal fluid (CSF) samples. Repeated lumbar punctures may be required to culture the organism. Results show whether or not the cerebral spinal fluid stains show yeast, culture

grows cryptococcus, or it is positive for cryptococcus antigen. A blood test, also known as the serum cryptococcal antigen test, can be sensitive to HIV positive patients (Nigrovic *et al.*, 2007). Unlike some other meningitis infections like the meningococcal, there is no risk of someone with fungal meningitis spreading the infection to other people. There is no current vaccine for fungal meningitis and treatment is usually by the administration of antifungal medication like fluconazone.

1.1.1.3 Bacterial Meningitis

The most difficult meningitis to treat is caused by bacteria that in some cases can be fatal. The disease mostly affects children under the age of 5 years, with the highest rates in the first years of life (Arditi *et al.,* 1998; DiFabio *et al.,* 2001). The disease is less common in infants younger than 3 months because during the first few months of life, passively acquired maternal antibodies protect most infants.

Acute bacterial meningitis remains an important cause of death and neurologic sequelae in African children. Up to 35% of meningitis sufferers develop severe disabling complications like residual headaches, deafness or hearing problems, learning difficulties and seizures in about one in twelve survivors according to studies by Berkley *et al.,* 2004. Between 700,000 and 1 million children below five years old die due to invasive pneumococcal disease each year in developing countries around the world (Arditi *et al.,* 1998).

The clinical features of meningitis are often non-specific and may overlap with those of malaria in malaria endemic regions (PneumoADIP, 2008 & Wright *et al.,* 1993). Early diagnosis and appropriate antibiotic treatment are the most important steps in management, but published data suggest that fewer than half of the cases of childhood meningitis are identified at first assessment in hospitals in this region (Berkley *et al.*, 2004).

Acute bacterial meningitis in children is caused by a variety of organisms. The severity of disease depends on factors such as age, disease presentation, and causative organism. The main causes of bacterial meningitis in infants and children under 5 years are *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae* type b.

1.2 Statement of the Problem

Cerebrospinal fluid (CSF) culture facilities are expensive and difficult to maintain, and laboratory facilities are limited in most hospitals in sub-Saharan Africa. In the sites that do have these facilities, there may also be issues with quality control like having enough material to carry out the quality controls, on the growth media and the number of samples that may be reliably and affordably analyzed. Since meningitis is a life-threatening disease with severe complications, clinicians aim to minimize missed cases. Because signs may be subtle and overlap with other diseases (such as cerebral malaria) lumbar punctures should be done according to a

sensitive, evidence-based protocol (Berkley *et al.,* 2004). Such a protocol has been incorporated into national paediatric care guidelines in Kenya (Ministry of Health: Basic Paediatric Protocols, 2004). With such protocols, as many as 12 lumbar punctures may have to be done to identify each true case of meningitis (Berkley *et al.,* 2004). Consequently, the majority of cerebrospinal fluid samples reaching the laboratory will not be from children with meningitis. In practice in resource-poor settings, both clinicians and laboratory technologists use the appearance of cerebrospinal fluid (turbidity) as an initial screen for the presence of meningitis. Where culture facilities are limited, clear samples may not be cultured. This study aims to determine the risks and benefits of this practice.

1.3   Justification

The World Health Organization (WHO; 1997) has estimated that in the sub Saharan region, *Hemophilus influenza* causes over 100, 000 childhood deaths, *Streptococcus pneumoniae* causes over 250, 000 childhood deaths whereas *Neisseria meningitidis* is the cause of large epidemics in many African countries.

Acute bacterial meningitis is not limited to developing countries but is an important cause of childhood deaths and neurological disorders in the East African region due to limited resources, limited access to health, poor living conditions and in affordability of vaccines and medicine by the general public. It is for these reasons that understanding the efficacy of using cerebrospinal fluid appearance as a criterion to determine whether to perform culture will help hospitals know whether

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or not they are missing out on a significant percentage of cases and optimize use of scarce culture resources.

This will indirectly also influence laboratory personnel work load by helping to predict what samples will have positive etiological agents from those that are negative for disease causing bacterial organisms thus reducing the number of samples requiring culture analysis. This study was carried out under the framework of another study carried out to assess diagnosis criteria for acute bacterial meningitis by clinicians prior to the introduction of *Haemophilus influenzae* type b (Hib) conjugate vaccine (Berkley *et al.,* 2004).

1.4   Hypothesis

1.4.1   Null Hypothesis

There is no significant difference in the number of bacterial cases correctly diagnosed if cerebrospinal fluid appearance is used as compared to culturing all specimens for the diagnosis of bacterial meningitis.

1.4.2   Alternate Hypothesis

There is a significant difference in the number of bacterial cases correctly diagnosed if cerebrospinal fluid appearance is used as compared to culturing all specimens for the diagnosis of bacterial meningitis.

1.5   Objectives

1.5.1   General Objective

To determine the effectiveness of using cerebrospinal fluid (CSF) appearance, as a indictor of predicting bacterial meningitis in children aged less than five years in the East African region

1.5.2   Specific Objectives

The specific objectives of the study were to:

1.   To determine the sensitivity, specificity and positive predictive values of cerebrospinal fluid appearance and White blood cell count for culture positivity.

2.   To determine the accuracy of diagnosis of acute bacterial meningitis by use of cerebrospinal fluid appearance in the pre and post conjugate Hib vaccine era.

3.   To compare the effectiveness of using CSF total white blood count and CSF appearance to determine culture positivity

1.6   Research Questions

1.   What are the sensitivity, specificity and positive predictive values of cerebrospinal fluid appearance in determining culture positivity?

2.   Is there a significant difference in diagnosis of acute bacterial meningitis by use of cerebrospinal fluid appearance in the pre and post conjugate Hib vaccine era?

3.   Is screening for sample positivity by use of cerebrospinal fluid total white blood cell count be more effective than using the appearance of cerebrospinal fluid?

1.7   Scope of the Study

The study was conducted in multiple sites in East Africa as part of a larger surveillance system called netSPEAR (Network for the Surveillance of Pneumococcal disease in the East African Region).

The netSPEAR surveillance work is intergraded into the World Health Organization (WHO) pediatric bacterial meningitis surveillance activities that span global. Site selection was based on geographical representativeness of the hospital, the capability of the hospitals to carry out lumbar punctures and have a functioning laboratory.

The study focused on children under 5 years of age who underwent lumbar punctures for suspected meningitis. These samples were collected and analyzed for the diagnosis of bacterial meningitis.

Kenya has three (3) provincial general hospitals, two (2) referral hospitals and two (2) district hospitals. Uganda had four (4) hospitals sites in the surveillance of which, three (3) were referral hospitals while the fourth is the National Referral Hospital. Tanzania had two (2) sites included at the time of this data collection, one being the National Hospital and the other being a research-based hospital. Ethiopia had one hospital site in the surveillance network.

Selection of sites eligible for pneumococcal disease surveillance was by record evaluation and key stakeholders visiting the hospital. Examples of evaluation criteria were the ability of the hospital to carry out lumbar punctures and having a laboratory with the ability to analyze cerebrospinal fluid samples.

1.8   Study Limitation

The data was collected as part of routine surveillance and not as research. Clinical practices between hospital sites thus varied depending on the hospital practices though attempts were made to standardize the procedures by use of Standard Operating Procedures for all the surveillance sites. The main limitation to this study was that the netSPEAR database had unreliable white blood cell count data. This led to the use of only Kilifi data on white cell counts that covered this time period so as to enable comparative analysis. The retrospective data also has lots of missing variables that was beyond the researcher getting this data thus leading to different

sample numbers being analyzed for the various categories that were of interest in this study.

The usage of antibiotics prior to the collection of LP, improper handling and transportation of specimen and media and/or utilization of human blood, which inhibits growth of isolates, may have contributed to underestimation of true meningitis cases due to culture negative cases of meningitis.

Since the study covered the East African region, the researcher bore the cost of transport and accommodation and purchase of material while working in the hospital laboratories which was a limitation on the timeline and scope of work. The long processes of getting the needed paperwork and authorization hindered access to hospital records for collection of retrospective data. There was also the fear by some hospitals that the hospital was under audit, which was not the case.

CHAPTER TWO

2.0   LITERATURE REVIEW

2.1   Introduction

2.1.1   Pneumococcal meningitis

This is caused by the *Streptococcus pneumoniae* bacteria, also referred to as the "*Pneumococcus*". It is a leading cause of meningitis, pneumonia (inflammation in the lungs) and septicaemia (blood poisoning) (Collignon, 2000). The pneumococcus is also a common cause of middle ear infections (otitis media) and sinusitis.

Neonates and infants are at a greater risk of pneumococcal meningitis than any other age group due to deficiencies in their humoral and cellular immunity and phagocytic function. In humoral immunity, antibody production from plasma cells attaches to antigens and flags them for destruction. In cellular immunity, the macrophages, CD4 cells and helper T-cells provide protection against pathogens. The phagocytic function is when the white blood cells protect the body by ingesting harmful foreign particles and thus fight infections and provide subsequent immunity.   Their inefficient complement pathway compromises the immune system (Chaudhuri *et al*, 2008). People with weaker immune systems are at a greater risk of contracting bacterial meningitis.

2.1.2 *Haemophilus influenzae* type b (Hib) meningitis

Hib meningitis is caused by the *Haemophilus influenzae* type b bacterium. The organism is observed globally but is not easy to confirm due to difficulty in isolating if patients have received prior antibiotic treatment. It is however treatable by use of the Hib vaccine. Introduction of the Hib vaccine into the routine immunization programs of Kenya and Uganda has ensured reduction of *Haemophilus influenzae* type b meningitis cases within the region. In Uganda, the highly protective Hib-vaccine prevents almost 30,000 cases of severe disease (both meningitis and pneumonia) and more than 5,000 deaths among children younger than five years. This has also been shown in a Kenyan study (Scott *et al, 2006)* that showed that the vaccine prevented an estimated 3,370 hospitalizations in 2005 and an 88% reduction of laboratory confirmed cases of invasive Hib disease from pre-vaccine levels. *Haemophilus influenzae* type b infection is now rare in areas with routine conjugate Hib vaccination (CDC E-brief, 2008).

2.1.3 Meningococcal meningitis

This is bacterial meningitis caused by *Neiserria meningitidis* bacteria. It is a common cause of bacterial meningitis epidemic cases especially in the meningitis belt in sub-Saharan Africa that occurs periodically every 5-12 years (Teyssou *et al,* 2007). It is most prevalent during dry weather conditions. Meningococcal meningitis is characterized by petechial rash in patients and can also lead to septicaemia when the bacteria enter the blood stream.

2.1.4   The Meningitis Belt in Africa

This hyperendemic region on the African continent runs from Gambia and Mali in the west to Ethiopia and part of Eritrea, Kenya and Uganda to the east. (See figure 1). The region is characterized by dusty winds, cold nights and upper respiratory tract infections common in the dry season between December and June. These factors contribute to the damaging of the nasopharyngeal mucosa leading to an increase in the risk of acquiring meningococcal disease.

Other contributing factors include social habits like crowding in houses and person displacements due to pilgrimages also predispose these people to higher risks of infection. The cyclic characteristic of these epidemics is due to the herd immunity effect where transmission is blocked when a critical vaccinated mass of the population extends protection to the unvaccinated (Paul Chinnock, 2009).

The main serogroups in Africa are *N. meningitidis* A, C and W135. Prevention of this infection is by vaccinations that are used for epidemic control especially in the meningitis belt and prophylaxis for close contacts to an infected person. Routine immunization is limited by factors like low protection that lasts only for three to five years thus the person does not develop long lasting immunity. The vaccine also cannot be used in children under two years of age since they do not respond to conventional polysaccharide vaccines. (http://www.who.int/mediacentre/factsheets/fs141/en/).
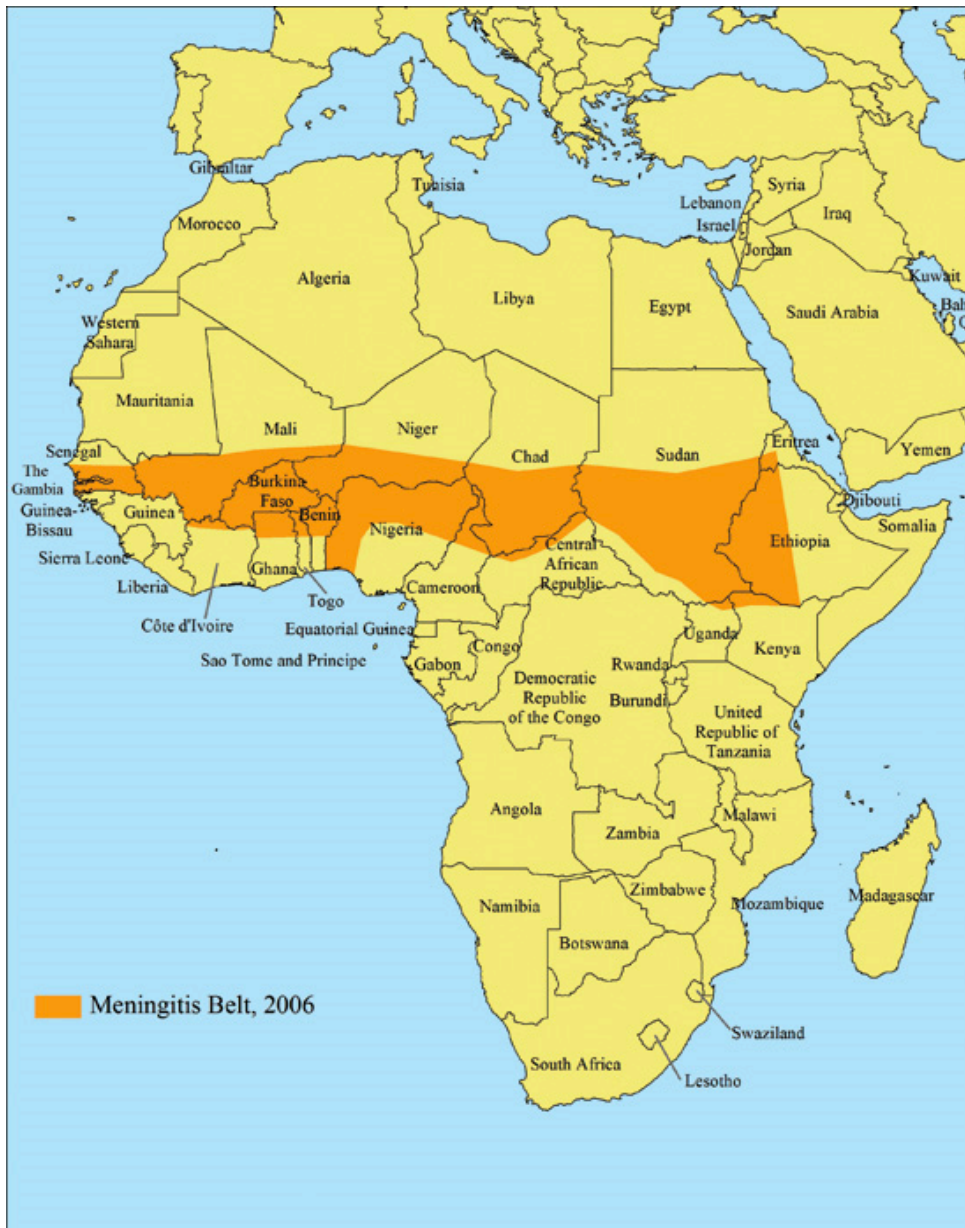
Figure 1: Meningococcal Meningitis Belt on the African continent.

Map courtesy of CDC

2.1.5 Pathophysiological mechanisms of acute bacterial meningitis.

Bacterial meningitis leads to tissue destruction due to inflammation of the meninges. Bacterial access to cerebrospinal fluid is normally through two main

routes; either through the bloodstream or by direct contact between the meninges and either the nasal cavity or the skin. This may result to brain oedema and hydrocephalus that leads to increased intracranial pressure and seizures.

The central nervous system interaction with bacterial meningitis leads to brain dysfunction that more often leads to death or severe neurological complications. Once bacteria have entered the bloodstream, they enter the subarachnoid space in places where the blood-brain barrier is vulnerable. Direct contamination of the cerebrospinal fluid may arise from recent trauma to the skull that makes the bacteria in the nasopharyngeal cavity enter the meningeal space. This can also happen in people with cerebral shunts or extraventicular drain.

Inflammation that occurs in the subarachnoid space is not a direct result of bacterial infection but is attributable to the immune system response of bacterial entry into the central nervous system (CNS). When the immune cells of the brain identify bacteria, they respond by releasing large amounts of cytokines that recruit other immune cells into an immune response. The blood-brain barrier becomes more permeable, leading to swelling of the brain due to fluid leakage from blood vessels. Large amounts of white blood cells enter the cerebrospinal fluid, causing inflammation of the meninges and swelling due to accumulation of fluid between the cells. The blood vessel walls also become inflamed and all these reactions lead to an increased intracranial pressure; lowered blood pressure, and brain cells are deprived of oxygen and undergo apoptosis (Saez-Llorens *et al,* 2003).

2.1.6 Transmission

Meningitis is not highly contagious but can be spread through direct contact with nose and throat secretions either through coughing or sneezing. The bacteria can also spread through kissing or sharing eating utensils, cigarettes or toothbrush. The bacterial transmission is normally by asymptomatic individuals. The bacteria are present in the nose and throat of these individuals but they do not get infected since their immune system produces enough antibodies to contain the bacteria. This prevents the bacteria from spreading to other parts of the body (Shapiro *et al,* 2008).

2.1.7 Risk factors of bacterial meningitis

The risk factors include age, where most cases of bacterial meningitis occur in children younger than five years especially in the developing countries where routine immunization with the vaccine is not common. Infectious diseases also tend to spread quickly in large groups of persons in congregations thus people living in community settings like dormitories, military bases, boarding schools and child care facilities are also at risk of infection. Factors that may compromise your immune system, including AIDS, diabetes and use of immunosuppressant drugs also make a person more susceptible to meningitis. Splenectomy, which is the surgical removal of the spleen, an important part the immune system and head injuries or brain surgery, may also put patients at risk for meningitis (Shapiro *et al*, 2008).

2.1.8 Prevention of bacterial meningitis

Prevention is normally by immunization using vaccines, also called immune-prophylaxis or by giving antibiotics to persons who are in close contact with infected patients "antimicrobial chemo-prophylaxis". Prevention of the meningococcal meningitis is by vaccination against common serotypes like A and W-135 prevention of Hib meningitis especially in the developing countries is by use of the penta-valent vaccine that vaccinates against diphtheria-pertussis-tetanus-hepatitis B and Hib.

2.2 Theoretical review

2.2.1 Diagnosis of bacterial meningitis

Ideally, the clinical suspicion of bacterial meningitis should be supported by cerebrospinal fluid (CSF) indices consistent with bacterial infection, and confirmed by the recovery of a bacterial pathogen (Bradley *et al,* 2001). This is obtained in children by carrying out a lumbar puncture (LP), which should be performed whenever the diagnosis of meningitis is suspected on the basis of clinical signs. However, early signs of meningitis are often subtle and nonspecific and, therefore, may be recognized only in retrospect (Stephen *et al*, 1986).

Cerebral spinal fluid analysis starts with the collection of the fluid though a lumbar puncture. This is done by positioning the patient, usually lying on the side, applying local anesthetic, and inserting a needle into the *dural sac* (a sac around the

spinal cord) to collect cerebrospinal fluid (Straus *et al,* 2006). The initial appearance of the fluid may provide an indication of the nature of the infection: cloudy CSF indicates higher levels of protein, white and red blood cells and/or bacteria, and therefore may suggest bacterial meningitis (Tunkel *et al*, 2004). Proper interpretation of tests on CSF is the key tool in the diagnosis of meningitis caused by bacterial infections (Dean *et al,* 2003).

Examination of the CSF of a patient with acute bacterial meningitis characteristically reveals a cloudy fluid, consisting of an increased white blood cell count and predominance of polymorphonuclear leucocytes, a low glucose concentration in relation to serum value, a raised concentration of protein, and positive Gram stained smear and culture for the causative microorganism (Stephen *et al,* 1986; Arditi *et al,* 1989).

This is in comparison to the normal parameters observed in a child who is not infected. Examination of this cerebral spinal fluid will reveal a clear fluid, consisting of white blood cells less than 5 per ml of sample, a glucose count of between 50-80mg/dl in relation to serum value, protein levels of between 20-45 mg/dl and a blank gram stain smear and no culture of an organism (Tunkel *et al,* 2000; Ballabh *et al,* 2004 ; Owens *et al*, 2008).

The probability of visualizing bacteria on a Gram stained preparation of cerebrospinal fluid is dependent on the number of organisms present. In microscopic examination of the fluid, the Gram stain is positive in 60 to 80 percent of untreated cases of bacterial meningitis and in 40 to 60 percent of partially treated

cases (Dean *et al*, 2003). The lower limit of detection is about 10 colony-forming units/ml in cerebrospinal fluid (Feldman, 1976).

The experience of laboratory personnel is also crucial since up to 10 percent of initial Gram stains are misread (Pruitt, 1998). About 87% of patients with bacterial meningitis will have a bacterial count higher than 1,000 per mm^3 while up to 99% will have more than 100 per cubed millimeter (Khurana, 1996).

Culture in the analysis of cerebrospinal fluid remains the gold standard for diagnosis of bacterial meningitis and the specimens should ideally always be cultured, even when the fluid appears to be crystal clear and is without WBCs. Although cerebrospinal fluid culture is the ideal method, it is rarely available in local hospitals throughout the East African region and antibiotic treatment prior to a lumbar puncture can decrease the sensitivity of culture (Berkley *et al*, 2001). The yield of positive cerebrospinal fluid cultures falls from 70–85% to below 50% in patients previously treated with antibiotics.

Additional tests such as latex agglutination (LA) allow rapid detection of bacterial antigens in cerebrospinal fluid but the specificity for LA is very low leading to the occurrence of false positives thus LA is not routinely used today (Dougherty *et al*, 1986). Polymerase Chain Reaction (PCR) has high sensitivity and specificity for many infections of the central nervous system, is fast, and can be done with small volumes of cerebrospinal fluid (Clnque *et al*, 1997). It is however expensive and

require specialized training which renders it unsuitable for use in our regional hospital settings.

The Ziehl-Neelsen stain is mostly utilized for the identification of cryptococcal meningitis that is an opportunistic fungal infection common in immune compromised persons. Its reliability as a diagnostic tool has also been shown to be poor with a smear positivity of only 4.6% (Nwokendi, 2007 & Thwaites *et al*, 2000). The India ink stain is utilized for the diagnosis of Cryptococcal meningitis in CSF; however, testing for cryptococcal antigen in blood or CSF is more sensitive, particularly in persons with AIDS (Bicanic *et al,* 2004; Sloan *et al.,*2008 and Saag *et al*, 2000).

The Ziehl-Neelsen stain and Indian ink were not utilized in this study since CSF analysis was according to a set standard operating procedure that was designed for the identification of bacterial causes of meningitis.

CHAPTER THREE

3.0   MATERIALS AND METHODS

3.1   Study design

The study was a retrospective analysis of routinely collected data on cerebrospinal fluid analysis from netSPEAR sites across East Africa. Ethical approval for this study was granted by the Kenya Medical Research institute (KEMRI) and the National Ethical Review Committee (ERC).

3.2   Study area

The study area covered 14 health facilities in the East African region spanning Kenya, Uganda, Tanzania and Ethiopia. These facilities have a common characteristic of being eligible for Vaccine Fund support by GAVI and by political, economic and language ties.

Site selection was based on geographical representativeness of the hospital; the capabilities of the hospitals to carry out lumbar punctures and have a functioning laboratory and commitment of the hospital on improving practices to facilitate surveillance. The netSPEAR surveillance work is intergraded into the WHO pediatric bacterial meningitis surveillance activities, which carries out the surveillance on a global scale.

Site included in this study are: -

Uganda

- Mbale Regional Referral Hospital

- St. Mary's Lacor Hospital

- Mbarara Regional referral Hospital

- Mulago National Referral Hospital

Kenya

- Moi Teaching and Referral Hospital

- Embu provincial General Hospital

- Nyeri Provincial General Hospital

- Nakuru Provincial General Hospital

- Machakos district Hospital

- Kenyatta National Hospital

- Kilifi District Hospital

Tanzania

- Muhimbili National Hospital

- Muheza /Teule Hospital

Ethiopia

- Black Lion (Tikur Anbessa) Hospital, Addis Ababa

The main aim of the surveillance was to maximize the data generated within the various government health systems by collaboration between clinicians, laboratory scientists and public health personnel. This data would then be used to advocate for strengthening and expanding immunization programmes within the region.

3.3 Study populations

3.3.1 Inclusion criteria

- Any child aged more than 2 months and below 5years who had a lumbar puncture (LP) performed where meningitis was suspected

- Had fever or a history of fever in the last 48 hours and exhibited one or more of the following features:

- Any reduction in the level of consciousness

- Confusion

- Stiff neck

- Bulging fontanelle

- Fits if age is less than 6 months

- Partial seizures – seizure affecting one part

These criteria were written up in a poster (appendix 1) that was available in the clinical wards as part of the SOP's used by clinicians. The Lumbar puncture (LP) was carried out unless a contraindication was present, usually during admission of

the child to hospital and before treatment was admitted. However, treatment was given if the LP could not be carried out immediately for logistical reasons.

3.3.2   Exclusion criteria

- Data from children less than 5 years who did not have an LP taken.
- Infection of the skin at the site of taking the LP
- If the child was gasping or needed immediate resuscitation
- Pupils respond sluggishly or asymmetrically to light
- In patients whom the positioning for taking an LP would compromise cardiopulmonary function
- Evidence of raised intracranial pressure (other than a bulging fontanelle) such as depressed consciousness level, focal neurological signs or hypertension.

3.4   Sample and Sampling Method

The study employed a simple random sampling technique, which was preferred because the respondents (children) and the subject of the study make the population homogeneous. According to Mugenda and Mugenda (2003), simple random sampling is a probabilistic sampling technique that ensures each subject, object or respondent have an equal chance of representation of which 10 percent of the accessible population is considered adequate for analysis. This is also in agreement to the Fisher formula (Fisher *et al*, 1978), which I used to calculate the minimum

number of positive *Streptococcus pneumoniae, Haemophilus influenzae* and *Neisseria meningitidis* isolates needed for analysis.

3.4.1   Calculation of sample size

Using the Fisher formula (Fisher *et al*, 1978), a minimum sample size number was determined by the formula shown below

$$n = \frac{Z^2_{1-\alpha/2} \, P \, (1-P)}{d^2}$$

Where

n= minimal positive isolate size

α = Level of significance (0.05)

$Z^2_{1-\alpha/2}$ = Standard normal deviate (1.96)

d^2 = Absolute precision (0.05)

P = It is the assumed that 10% of the admission children population will give a positive CSF culture result (Berkley *et al,* 2004)

$$n = \frac{(1.96)^2 \, (0.1)(0.9)}{(0.05)^2}$$

=138 children is the minimum sample size of children needed.

The number of children included in the given time frame from 2001 to 2008 satisfies the minimum samples required for this study.

3.4.2 Sampling method

All pediatric CSF sample data was collected in the participating hospital sites between the years 1999 to the year 2008 (which encompasses a pre and post Hib vaccine introduction). Kenya introduced the *Haemophilus influenzae* type B vaccine in 2001 while Uganda's was introduced in 2002. The years preceding these periods encompass the pre-vaccine era. Tanzania and Ethiopia also fall under the pre-vaccination group since they were yet to introduce the vaccine into their childhood immunization programs by the start of this study. All children with clinical presentation of any form of meningitis were enrolled into the study and samples were extracted from them.

3.5 Data Collection

3.5.1 Sample registration

All samples were recorded in an issued laboratory logbook and transferred to a data management program utilized by the netSPEAR programme. The program was used as the database to record patient demographic, clinical and laboratory details and provide a primary-key (PK) number to ensure patient confidentiality. Registration of specimens was done on receiving the specimen by verifying that the name, number and specimen date on the vials agreed with the details on the accompanying form. The laboratory logbook was periodically crosschecked for illegible or inconsistent data submitted electronically to netSPEAR.

3.5.2 Data collection

Data was collected according to variables in the microbiology request form (appendix2)

Table 1: Variables used to collect CSF data from Microbiology request forms

a)	Patient name	b)	Geographical location of patient
c)	Inpatient number	d)	Patient age (years/months)
e)	Gender	f)	Ward
g)	Consultant	h)	Specimen type
i)	Data of specimen collection	j)	Time of specimen collection
k)	Clinical diagnosis	l)	Final culture results
m)	Preliminary CSF results for:		
	Appearance, protein levels		
	Gram stain, Glucose levels		
	WBC counts, RBC counts		

3.5.3 Analysis of Cerebrospinal Fluid (CSF)

Sample information was collected using microbiology request forms. Clinical officers and pediatric resident physicians made the initial clinical assessment supervised by a consultant pediatrician. Analysis of the CSF was as per the Standard operating procedures (Appendix 3) that structured the use of Gram stain,

White blood cell counts, protein and glucose counts and use of culture for the isolation and identification of bacterial causes of meningitis. These SOP's were developed with the assistance of the KEMRI-Wellcome Trust Programme and rthe Ministry of Health. This was done in the context of routine clinical care and results made available to treating clinicians.

Cerebrospinal fluid appearance was scored by a Microbiology technologist without knowledge of the patient's clinical details and classified as either clear, turbid, xanthochromic or blood stained. Blood stained samples were categorized according to the levels of blood in the sample as described in the table two below.

Table 2: Stratification of bloodstain scores in CSF samples

Blood spec	Translation
Blood +	Slightly blood stained
Blood ++	Moderately blood stained
Blood +++	Deeply blood stained

The cerebrospinal fluid (CSF) leukocyte count was determined manually by the use of a modified Neubauer counting chamber. (Murex Diagnostics, Dartford, United Kingdom).

The cerebrospinal fluid was cultured in 5 percent sheep blood agar and enriched chocolate blood agar plates and positive organisms isolated using standard techniques. (Appendix 4 and 5) Meningitis was defined as definite if culture was

positive and as probable if there was a positive CSF culture or positive CSF latex agglutination test, or bacteria seen on Gram-stain. *Haemophilus influenzae* identification was through colony morphology and X and V factor analysis. *Streptococcus pneumoniae* was identified through colony morphology, α-hemolysis and Optochin susceptibility testing.

3.5.4 Provisions for data verification and validation in the field and laboratory

Any positive pneumococcal isolate that was obtained was sent to netSPEAR's coordinating laboratory in Kilifi for reconfirmation, serotyping and antimicrobial susceptibility testing E-test (AB Biodisk, Sweden).

3.5.5 Data Storage

Data was stored in an SQL database in a password-protected computer with CD backups. Regular data review and cleaning was carried out before being backed up on a second off-site machine that was also password protected. Access to the collected data was restricted to authorised persons only and only accessible with the consent of the principle investigator. Patient records were coded by use of a computer generated primary key.

3.6   Data Analysis and Presentation

Analysis was carried out using STATA (version 10) software after transferring the data from the netSPEAR program.

Analysis was carried out to compare the appearances of samples with respect to culture positivity and sensitivity. Culture of the sample was used as the gold standard to test the common assumption that clear appearance of cerebrospinal fluid is an indication of absence of bacterial pathogens. Sensitivity, specificity, proportion of missed cases, and negative and positive predictive values were calculated with 95% confidence intervals. The analysis was repeated on subgroups to examine differences between the post and pre-Hib era. The sensitivity, specificity, proportion of missed cases and negative and positive predictive values for CSF white blood cell count was then determined and compared. Sensitivity is the probability that a test result will be positive when the disease is present, (true positive). Specificity is the probability that a test result will be negative when the disease is not present, (true negative). Positive predictive value is the probability that the disease is present when the test is positive. Negative predictive value is the probability that the disease is absent when the test is negative.

CHAPTER FOUR

4.0   RESULTS

4.1   Study sample and isolate distribution

In the pre-Hib era covering the period 1999 to 2001, a total of 8,620 samples were generated of which 429 were culture positive. This data encompassed Kilifi before vaccine introduction that had 6,122 samples with 3.7% having positive isolates. Tanzania contributed 1,752 samples of which 6.7% were positive isolates and Ethiopia had 746 samples of which 10.7% were positive isolates.

The post-Hib data covering the period 2004 to 2008 comprised of Kenya and Uganda data with a total of 23,532 samples of which, 4.3% were culture positive. Kenya (inclusive of Kilifi from the year 2004) had 13,138 samples of which 3.9% were culture positive while Uganda had 10,394 samples of which 4.8% had positive isolates.

The gap between the 2001 and 2004 allows a time gap for the vaccine effects to be truly within the post-Hib by excluding the transitional phase of the vaccine introduction. Ugandan sites on the other hand; joined the surveillance in their post-Hib era. From this a total of 1,991 samples of which 4.5% had isolates were not included for analysis (see table 3).

Table 3:Contribution of data by different sites and before and after introduction of Hib conjugate vaccine

Country/Site	Pre-Hib n (culture positive)	Post-Hib* n (culture positive)	Total
Kenya, Kilifi	6122 (232)	6,879 (242)	13001 (474)
Kenya,other sites	_	6,259 (267)	6259 (267)
Uganda sites	_	10,394 (504)	10394 (504)
Tanzanian sites	1752 (117)	_	1752(117)
Ethiopian site	746 (80)	_	746 (80)
TOTAL	8620 (429)	23,532 (1013)	32152 (1442)

This excludes the 2001-2004 that allows for transition from pre to post vaccine.

Kenya and Uganda have contributed a large percentage of the number of cerebrospinal fluid samples reported with both countries accounting for 90% of the total number of samples. This is also reflected in the number of positive isolates with both countries contributing over 80% of the total number of positive isolates.

On the distribution of positive isolates, Kenya and Uganda contributed over 70% of the total number of positive isolates as illustrated by the figure 3. This is attributable to Kenya and Uganda both having more sites in their respective countries than Tanzania and Ethiopia. The two countries also joined the surveillance network and reported data earlier than Ethiopia and Tanzania.
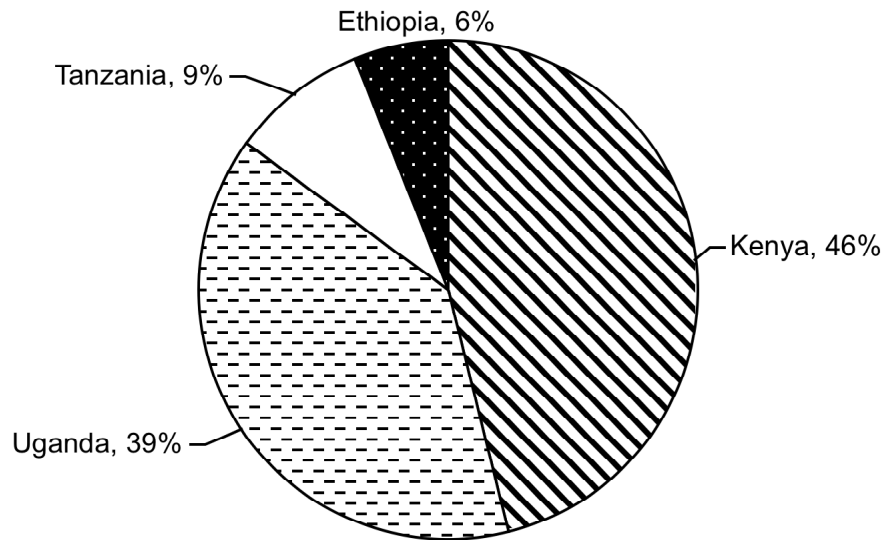
Figure 2: Distribution of positive organisms isolated by each country

Of the 29,153 CSF samples collected during the entire period of study (1999 to 2008) that had a reported appearance, 4.5% were positive for growth of organisms. Of these isolates, three microorganisms were of interest to the surveillance programs; these were *Streptococcus pneumoniae, Haemophilus influenzae* and *Neisseria meningitidis* that accounted for 50.7% of the 1,309 isolates.
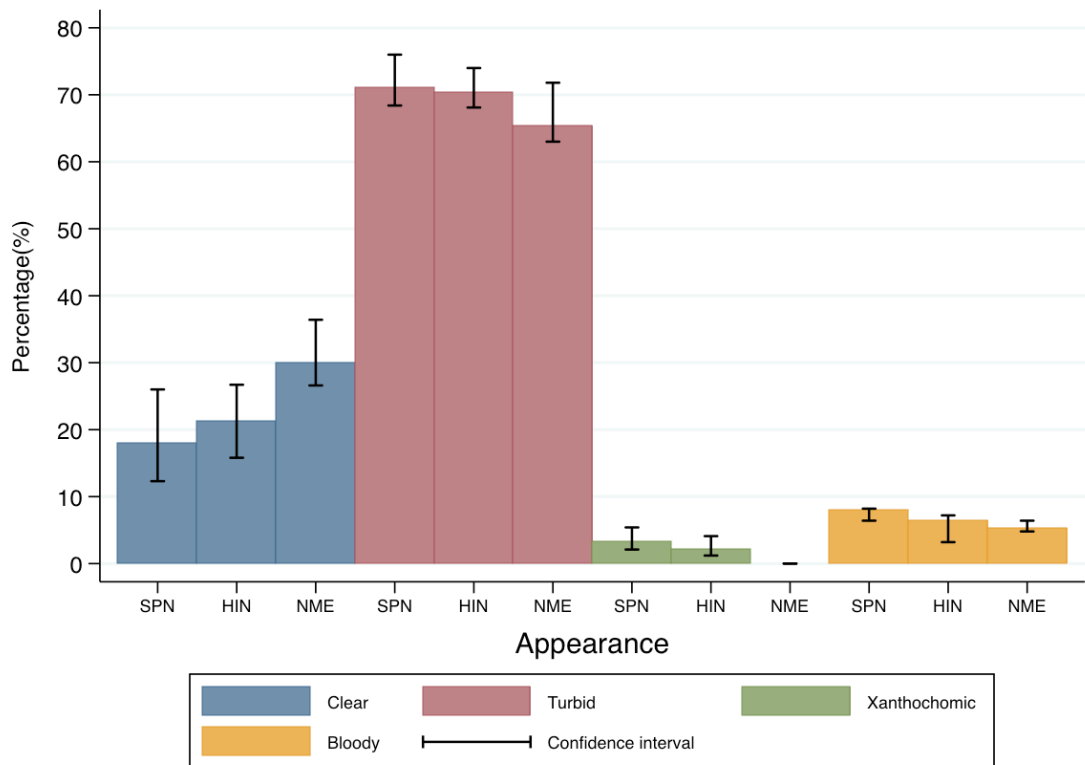
Figure 3: ***Distribution of S.pneumoniae*** **(SPN),** ***H. influenzae*** **(HIN)** ***and*** ***N.meningitidis*** **(NME)** ***according to cerebral spinal fluid appearance***

The distribution of the three main causal organisms of meningitis according to appearance shows that most of the organisms were identified from turbid cerebrospinal fluid samples with Xanthochromic appearance being the least indicator for presence of organism in CSF.

Appearance and White Blood cell count were utilized to calculate the sensitivities, specificities and predictive values of determining presence or absence of bacteria in the Cerebral Spinal Fluid. Sensitivity is the proportion that appearance was able to predict positive results. It was obtained by dividing the number of positive isolates

that had the testing appearance by the total number of positive isolates determined by culture. Specificity being the ability of a test to identify negative results was calculated by dividing the number of false positive isolates by the total number of positive isolates determined by culture. Positive Predictive Value is the proportion with a positive test that does have the bacteria. This was calculated by dividing the number of positive isolates that had the testing appearance by the total number of positive isolates determined by the test.

Table 4: Sensitivities, specificities, positive predictive values and proportion of missed cases according to appearance.

| Type of cases | Appearance | | | | | Proportion of missed cases |
	Sample size	Isolates	Sensitivity (95% CI)	Specificity (%)	PPV (%)	
Clear	23662	240	18 (16.1 - 20.3)	17	1	82
Turbid	1968	940	72 (69 - 74)	96	47.8	28
Xanthochromic	1113	88	6.8 (5.5 - 8.3)	96	7.9	93.2
Blood +	796	32	2.4 (1.7 - 3.4)	97	4	97.6
Blood ++	542	15	1.1 (0.6 - 1.8)	98	2.8	98.9
Blood +++	116	2	0.1 (0 - 0.5)	99.6	1.7	99.9
Xanthochromic /Turbid	2986	993	75 (72.6 - 77.3)	93	33.3	25
Turbid/Blood	3353	973	73.5 (71 - 75.9)	91.6	29	26.5
Xanthochromic /Blood	2508	131	9.9 (8.3 - 11.6)	91.6	5.2	90.1

Table 5: Sensitivities, specificities, positive predictive values and proportion of missed cases according to White Blood cell count

White Blood Cells	Sample size	Isolates	Sensitivity (95% CI)	Specificity (%)	PPV (%)	Proportion of missed cases
WBC >5	2558	304	81.5 (77.2 - 85.3)	78.7	12	18.5
WBC >5 and WBC missing	3827	360	96 (93.5 - 97.7)	67	9.4	4
WBC >10	1215	299	80.1 (75.7 - 84)	91.3	24.6	19.9
WBC >10 and WBC missing	2484	355	94.7 (91.9 - 96.8)	79.7	14.3	5.3
Turbid and WBC>10 or WBC missing	2987	355	95.2(92.5-97.1)	74.9	11.9	4.8

When the sensitivities of the appearances and the WBC counts was represented graphically, one notes that turbidity or a combination of turbidity and another appearance had relatively high sensitivities as seen in the figure 5 below. The WBC count sensitivities are also much higher than those of appearance.
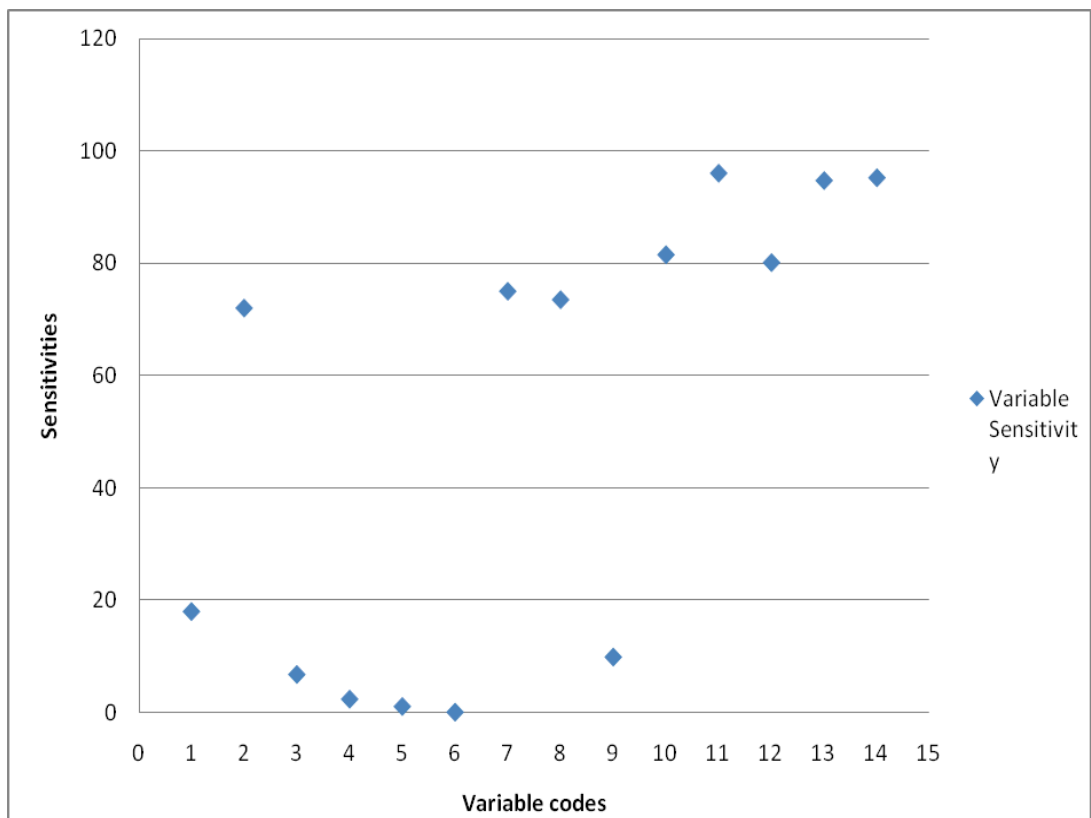
Figure 4: Graphical representation of sensitivities of appearances and White blood cell counts.

Key to variable codes in figure 4

	Variable	Variable code
Appearance	Clear	1
	Turbid	2
	Xanthochromic	3
	Blood +	4
	Blood ++	5
	Blood +++	6
	Xanthochromic/Turbid	7
	Turbid/Blood	8
	Xanthochromic/Blood	9
White Blood Cell	WBC >5	10
(WBC) table	WBC >5 and WBC missing	11
	WBC >10	12
	WBC >10 and WBC missing	13
	Turbid and WBC>10 and WBC missing	14

A comparison of the pre and post Hib-era sensitivities and specificities was also done. There were insignificant differences in their sensitivities and specificities although the number of positive isolates in the pre and post Hib eras varied significantly as seen in table 6. The two variables also showed trends that were not significantly different in the values obtained for the sensitivities and specificities of

each groups corresponding appearance category. Some differences in the two eras are however observed in the PPV values of blood+ and Xanthochromic/turbid groups.

Table 6: Sensitivities, specificities, positive predictive values and proportion of missed cases comparison of the pre-Hib era

Pre-Hib	Sample size	Isolates	Sensitivity (95% CI)	Specificity (%)	PPV (%)	Proportion of missed cases
Clear	6807	56	13.2 (10.1 – 16.8)	17.6	0.8	86.8
Turbid	586	334	79 (74.8 - 82.7)	96.9	57	21
Xanthochromic	410	34	8.1 (5.7 - 11.1	95.4	8.3	66
Blood +	433	20	4.7 (2.9 - 7.2)	94.9	46	99.3
Blood ++	272	6	1.4 (0.5 - 3.0)	96.8	2.2	98.6
Blood +++	11	0	0 (0 - 0.8)	99.9	0	100
Xanthochromic /Turbid	937	346	81.8 (77.8 -85.4)	92.8	0.37	18.2
Turbid/Blood	1264	349	82.5 (78.5 - 86)	88.8	27.6	17.5
Xanthochromic /Blood	1092	56	13.2 (10.1 - 16.8)	87.4	5.13	44

Table 7: Sensitivities, specificities, positive predictive values and proportion of missed cases comparison of the post-Hib era

| Post-Hib	Sample size	Isolates	Sensitivity (95% CI)	Specificity (%)	PPV (%)	Proportion of missed cases
Clear	18837	192	19.1 (16.8 - 21.7)	20.9	1	80.9
Turbid	1526	692	69 (66 - 71.8)	96.3	45.4	31
Xanthochromic	846	70	7.2 (5.6 - 9	96.5	8.3	92.8
Blood +	515	19	1.9 (1.2 - 3)	97.8	3.7	98.1
Blood ++	385	11	1.1 (0.6 - 2)	98.3	2.9	98.9
Blood +++	109	2	0.2 (0.02 - 0.7)	99.5	1.8	99.8
Xanthochromic /Turbid	2307	735	73.3 (70.4 - 76)	93	31.9	26.7
Turbid/Blood	2495	716	71.4 (68.5 - 74.2)	92.1	28.7	28.6
Xanthochromic /Blood	1821	100	9.9 (8.2 - 12)	92.4	5.5	90.1

Plotting sensitivity (true positive rate) against the false positive rate (1- true negative rate) in a Receiver Operating Characteristic (ROC) plot enables one to measure how well a parameter can distinguish between two diagnostic groups (diseased versus. normal).

The various appearance categories plotted in the ROC graph below shows that the points C, D, E, F, G, H and J are good screening indicators with relatively high sensitivities and low specificities. These categories give the best possible predictions of presence of an etiological agent in the given sample.
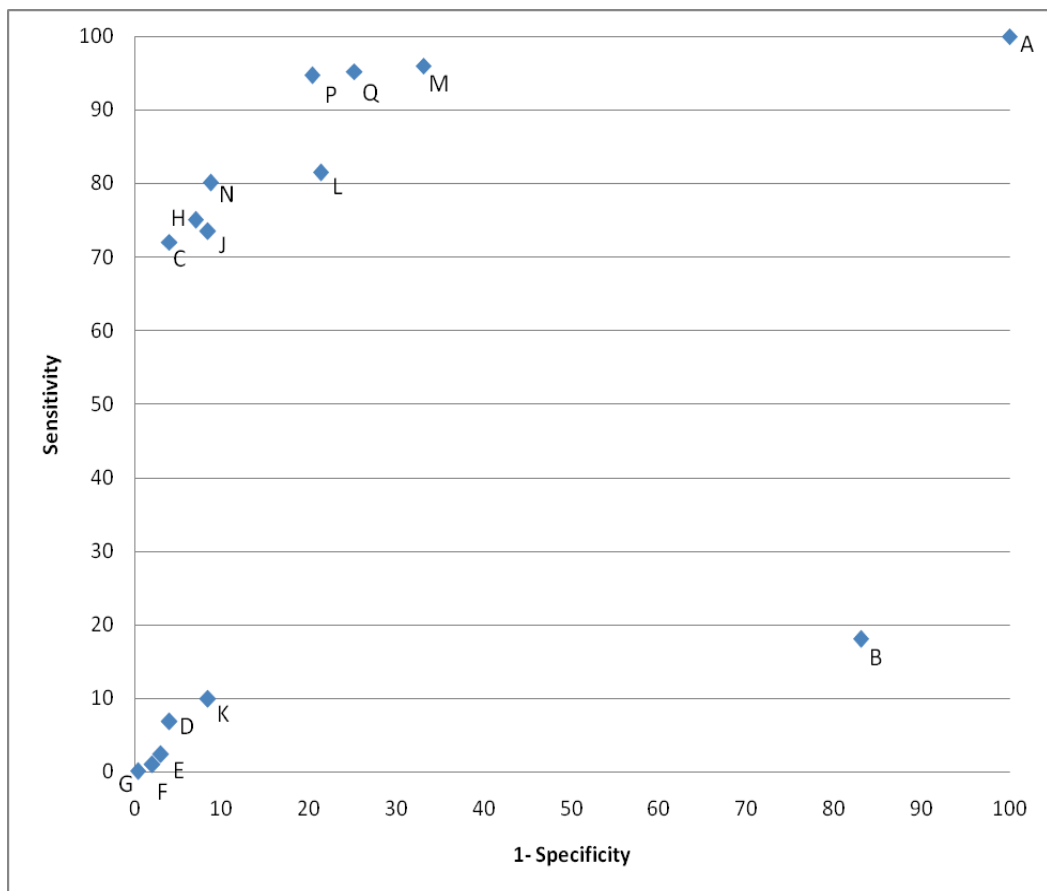
Figure 5: Receiver Operating Characteristic (ROC) plot of sensitivity against
(1- specificity)

The letter codes in the ROC graph above represents variables that coincide to an appearance variable and white blood cell count as illustrated in the coding:

Receiver Operating Characteristic (ROC) variables for figure 5

Code	Variable	Code	Variable
B	Clear	**J**	Turbid/Blood
C	Turbid	**K**	Xanthochromic/Blood
D	Xanthochromic	**L**	WBC >5
E	Blood +	**M**	WBC >5 or WBC missing
F	Blood ++	**N**	WBC >10
G	Blood +++	**P**	WBC >10 or WBC missing
H	Xanthochromic/Turbid	**Q**	Turbid or WBC>10 or WBC missing

CHAPTER FIVE

5.0   DISCUSSION

The main aim of carrying out this study was to establish how sensitive and/or specific appearance of a cerebrospinal fluid sample is at determining the presence or absence of meningitis since culture is an expensive venture in resource constrained laboratories in East Africa region. A common practice is to observe the appearance of the sample and from this, one decides on whether culture will be carried out or only the preliminary tests of Gram Stain and WBC count will be carried out. It is this practice that formed the basis of carrying out this research on how effective this method is in diagnosis of suspected Bacterial Meningitis. Lack of rapid accurate diagnosis and treatment of bacterial meningitis using appropriate antibiotic can swiftly lead to neurological disorders and at times even death.

In children over 2 months, the main bacteria that causes meningitis are *Streptococcus pneumonia (SPN)*, *Haemophilus influenzae (HIN)* and *Neisseria meningitis (NME)* are also the main bacterial causes of ear and other respiratory infections including pneumonia and sinusitis (Chaudhuri *et al,* 2008).

Distribution of the appearance according to reported appearances of the three organisms under study reveals that turbidity was the most common appearance in the positive samples. This is expected since cerebrospinal fluid is essentially a sterile body fluid that under normal circumstances should be clear. We however

notice that in a small fraction of samples that were deemed as clear, there was the isolation of *S.pneumoniae, H.influenzae and N.meningitidis*. It is this category of samples that makes one raise the question of whether using sample appearance is an effective criterion for determining whether to culture a sample of suspected bacterial meningitis in children aged under five years in the East African region.

The main findings were that out of a total of 29,153 samples. Samples with positive isolates accounted for 18%. Two hundred and ninety one (291), which represent 1% of the samples, had positive growth of an isolate compared to 48% among those with a turbid sample. The cerebrospinal fluid appearance that had the best predictive value for positive isolation of an isolate was turbid appearance with a PPV of 47.8%, sensitivity of 72% (95% CI 69 − 74) and a specificity of 96%. When different sets of appearances were examined they did not differ: the sensitivities of Xanthochromic/Turbid and Turbid/Blood were at 75 (95% CI 72.6 - 77.3) and 73.5 (95% CI 71 - 75.9) respectively. The increase in the sensitivity or specificity was however only in combination of appearances that included turbid appearance. A combination of Xanthochromic/Blood had a low sensitivity and a large proportion of missed cases.

Blood appearance had high specificity but was coupled with low sensitivity. Turbidity and Xanthochromic/Turbid showed a much better trade-off of the two variables that were both relatively high and with the lowest proportions of missed cases in their respective categories.

White blood cell (WBC) count analysis was used to compare if screening by total white blood cell count of the sample would be more effective than appearance. From the table 4 White blood cell count had higher sensitivities than the best of the appearance sensitivity of 75% (95% CI 72.6 - 77.3) in the Xanthochromic/Turbid group. WBC>5 had sensitivity 81.5% (95% CI 77.2 - 85.3) and a specificity of 78.7% while WBC>10 had a sensitivity of 80.1% (95% CI 75.7 − 84) and specificity of 91.3%.

Including any cerebrospinal fluid sample that had a missing WBC count into the analysis improved the sensitivities of both the WBC>5 and WBC>10 and reduced the proportion of missed cases but lowered their specificities. WBC>10 has the highest specificity with a relatively high sensitivity of 80% thus is a good predictor of absence or presence of disease. Adding samples with missing WBC count to this category increases the sensitivity but lowers the specificity.

From the results, we found that screening for WBC >10 had a high sensitivity of obtaining positive isolates. Combining the diagnostic methodologies, i.e. inclusion of turbidity into the screening criteria, increased the number of samples tested resulting to increased sensitivity but lowered specificity.

Analysis of the pre and post-Hib eras did not show any significant differences in the screening criteria depending on sample appearance. One however notes a decrease in the number of reported positive turbid sample from 5.6% pre-Hib to 4.5% post Hib, an increase in proportion of positive clear CSF sample to total from

0.8% pre-Hib to 1.0% post Hib. Positive Xanthochromic proportions across the two periods did not change.

Congested living conditions in regions with few health units coupled with the dry season are conditions that cause the increase in meningitis cases (Tunkel *et al,* 2004). Uganda also falls under the meningitis belt that stretches from Senegal in the west to Ethiopia in the east. The region, experiences meningitis cycles whenever the dry season sets in. according to W.H.O. (http://www.who.int/csr/don/2006_03_21/en/index.html)

The penta-valent vaccine consisting of Diphtheria, Pertussis (whooping cough) and Tetanus (DPT)*, Hepatitis* B (HepB) and *Haemophilus influenzae* type b (Hib) commonly known as DPT-HepB-Hib has been introduced in the Routine National Immunization Programmes in Uganda and Kenya. Tanzania is yet to introduce the Hib penta valent vaccine. The penta-valent vaccine has managed to decrease the prevalence of *Hemophilus influenzae* but unfortunately; it does not protect children against *Streptococcus pneumoniae and Neisseria meningitidis* bacteria (Scott *et al,* 2006). There is however a new 10-valent pneumococcal vaccine which is targeted for introduction in the region with Kenya and Rwanda having obtained approval to introduce the vaccine into their routine vaccination programs. This is targeted at reducing disease caused by the *Streptococcus pneumoniae* in children in accordance to the Millennium development Goal number four − reducing child mortality by two thirds by the year 2015 (http://www.un.org/millenniumgoals/childhealth.html).

According to study by Stephen *et al.* (1986) on meningitis outbreak in early 2006 mainly due to *Neisseria Meningitidis* serotype W135 as cause of suspected meningitis cases and mortality, there were two main epidemic loci, one in West Africa and the other in Eastern Africa. The East African locus affected Kenya, Sudan and Uganda with. Uganda experienced a total of 551 suspected meningitis cases with 59 deaths while Kenya had 74 suspected cases with 15 deaths due to this microorganism. It is for such reasons that fast and effective screening of the CSF samples that will in the future go a long way in decreasing the morbidity rates due to meningitis by increasing the effectiveness of screening for etiological agents using appearance and/ white blood cell count especially in the absence of expensive culture methods.

Pneumococcus is the leading cause of pediatric bacterial meningitis in the region and presence of a turbid sample is a good indicator of presence of an etiological agent. However, using appearance alone is not sensitive enough and the additional use of white blood cell count as a screening criterion increases the sensitivity of the test to >90%. The effect of the Hib vaccine introduction in the East African region has had immense effects of reducing purulent meningitis. In a Rwandan study by Muganga *et al* (2007), the Hib vaccination effectiveness against meningitis was 52%. There was a reduction of the proportion of cerebrospinal fluid with purulence from 26.0% during 2002, to 15.9% during 2003. Similar declines are hoped for in the introduction of pneumococcal conjugate vaccine.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study demonstrated there is a significant difference in the number of bacterial cases correctly diagnosed when cerebrospinal fluid appearance is used as a pre-screening criterion in the diagnosis of bacterial meningitis. However, the additional use of white blood cell count as a pre-screening criterion increases the sensitivity of diagnosis. The null hypothesis thus does not hold true and is therefore rejected.

6.2 Recommendations

Most regions in the developing world have limited capacity to carry out diagnosis of pneumococcal disease of which meningitis falls under that said, high quality surveillance is important and thus the need for ensuring that the use of multiple screening methods utilized are effective at diagnosis of etiological agents in samples.

The general public needs to be made aware of the symptoms to look out for. Clinicians also need to be aware of the clinical signs that lead to one diagnosing suspected meningitis in the given setting. These signs have been discussed in the paper by Berkley *et al (2004)*. Perhaps most importantly, this study underlines the importance of conducting LPs in suspected cases, since most children do have meningitis, and enhancing the use of CSF white cell counts to detect those that do have this disease.

REFERENCES

Amir Vokshoor and Cordia Wan. (2009). Staff Neurosurgeon, Department of Neurosurgery Spine Surgeon, Diagnostic and Interventional Spinal Care. *St John's Health Center Contributor Information and Disclosures*. **155:** 1087-97

Arditi M., Edward O., John S., Tina Q., William J., Gordon E., Ellen R., Laurence B., Kwang S., Ram Y. and Sheldon L. (1998) Three-year multicenter surveillance of pneumococcal meningitis in children: clinical characteristics and outcome related to penicillin susceptibility and dexamethasone use. *Pediatrics*. **102 (5):** 1087-97.

Arditi M., Ables L.and Yogev R. (1989). Cerebrospinal fluid endotoxin levels in children with *H.influenzae* meningitis before and after administration of intravenous ceftriaxone. *J Infect Disease*.**160:** 1005-11.

Ballabh P., Braun A. and Nedergaard M. (2004). The blood-brain barrier: an overview. Structure, regulation, and clinical implications. *PubMed Neurobiol Diseases*.**16:**1-13.

Berkley J., Anne C., Isaiah M., Brett S., Charles R. and Newton C. (2004). Indicators of Acute Bacterial Meningitis in Children at a Rural Kenyan District Hospital. *Pediatrics*.**114:**713-719.

Berkley J., Mwangi I., Ngetsa C., Mwarumba S., Lowe B., Marsh K. and Newton C. (2001). Diagnosis of acute bacterial meningitis in children at a district hospital in sub-Saharan Africa. *The Lancet*.**357 (Issue 9270):**1753-1757.

Bicanic T. and Harrison T.S. (2004). Cryptococcal meningitis. *British Medical Bulletin* **72:** 99–118.

Bradley J., John T. and Peyman S. (2001). Lumbar Puncture in Pediatric Bacterial Meningitis: Defining the Time Interval for Recovery of Cerebrospinal Fluid Pathogens after Parenteral Antibiotic Pretreatment. *Pediatrics*. **108(No. 5):**1169-1174.

Chaudhuri A., Martinez-Martin P. and Martin P.M. (2008). EFNS Guideline on the Management of Community-acquired Bacterial Meningitis: Report of an EFNS Task Force on Acute Bacterial Meningitis in Older Children and Adults. *European Journal of Neurolology* **15 (7):** 649–659.

Cherry J.D. and Feigin R.D. (1998). Aseptic meningitis and viral meningitis. *Textbook of Pediatric Infectious Diseases*. **4**[th] **ed**. Philadelphia: Saunders: 450-457.

Cinque P., Scarpellini P., Vago L., Linde A. and Lazzarin A. (1997). Diagnosis of central nervous system complications in HIV-infected patients: cerebrospinal fluid analysis by the polymerase chain reaction. *AIDS*. **11:**1-17

Collignon, P. and Turnbrige, J. (2000). Antibiotic resistance in Streptococcus Pneumoniae. *Journal of Paediatrics*. **35:**558-561.

Dean A., Mark M., Reeves. and Demitri A. (2003). Cerebrospinal Fluid Analysis. *American Family Physician,Tripler Army Medical Center*. Honolulu, Hawaii. 2003; 68(6).

Di Fabio J., Jose L., Elizabeth A., Clara I., Fernando H., Maria C., Teresa E., Gabriela B., Maria N., Ingrid H., Juan C., Maria C., Vera R., Mabel R., Raul C., Alejandra L., Marguerite T., James A. and The Paho Sireva-Vigia Study Group *et al*. (2001). Evolution of Streptococcus pneumoniae serotypes and penicillin susceptibility in Latin America, Sireva-Vigia Group, 1993 to 1999. *Pediatric Infectious Disease Journal*. **20(10):**959-967.

Dimitri P. (2006). Neuropathology: An interactive course for medical students and residents, Cerebrospinal fluid. Page.**254** Chapter 14.

Dougherty J. and Roth R. (1996). Cerebral spinal fluid. *Emerg Med Clin North America*. **4:**281-297.

Feldman W. (1986). Concentrations of bacteria in cerebrospinal fluid of patients with bacterial meningitis. *Journal Pediatrics*. **88:**549-552.

Khurana R. (1996). Intracranial hypotension. *Semin Neurol*. **16(1):**5-10.

Ministry of Health, Kenya. (2004). Basic Paediatric Protocols. Nairobi. Government Printers.

Muganga N., Uwimana J., Fidele N., Gahimbare L., Gessner D., Mueller J., Bekithemba R., Mhlanga B., Katsande R., Herbinger K. and Rugambwa C. (2007). *Haemophilus influenzae* type b conjugate vaccine impact against purulent meningitis in Rwanda. *Vaccine.* **l25:** issues 39-40: pp 7001-7005.

Nwokendi Evans E. (2007). Laboratory confirmation of clinically suspected cases of pulmonary TB in Kano, Nigeria. **6:**401-414

Network for Surveillance of Pneumococcal disease in the East African Region- A collaborative proposal from Kenya Medical Research Institute, Wellcome Trust and the WHO/AFRO PBM surveillance team. 2008. Unpublished manuscript.

Nigrovic K., Kuppermann N. and Macias K. (2007). Clinical Prediction Rule for Identifying Children With Cerebrospinal Fluid Pleocytosis at Very Low Risk of Bacterial Meningitis. *JAMA*. **297:**52-60.

Owens T., Bechman I. and Engelhardt B. (2008). Neurovascular Spaces and the Two Steps to Neuroinflammation. *J Neuropathol Exp Neurol.* **67:**1113-1121.

Paul Chinnock. (2009). Tropical Diseases Research to Foster Innovation & Knowledge Application: Major epidemic strikes Africa's meningitis belt. Neglected diseases in Africa workshop, Banjul,Gambia.

Pérez-Vélez C.M., Anderson M.S., Robinson C.C., McFarland E.J., Nix W.A. and Pallansch M.A. (2007). Outbreak of neurologic enterovirus type 71 disease: a diagnostic challenge. *Clin Infect Diseases.Medline citation.* **45(8):**950-957.

PneumoADIP N. (2008). *Preventing Disease and Reinforcing Need for Immediate Action to Prevent Pneumococcal Disease and Save Lives.* International Annual Symposium. Baltimore, USA.

Pruitt A. (1998). Infections of the nervous system. *Neurological Clinical journal.* *16:*419-447.

Saag M.S., Graybill R.J. and Larsen R.A. (2000). Practice guidelines for the management of cryptococcal disease. *Infectious Diseases Society of America.* **30(4):** 710–718.

Safdieh J.E., Mead P.A., Sepkowitz K.A., Kiehn T.E. and Abrey L.E. (2008). Bacterial and Fungal Meningitis in Patients with Cancer. *Neurology* **70 (12):**943–947.

Karen D., Moses N., Salome C., Amina I., Tatu K., Mike E., Newton C. and Scott *A*. (2006). Effectiveness of Haemophilus influenzae Type b (Hib) Conjugate Vaccine Introduction into Routine Childhood Immunization in Kenya. *JAMA: the Journal of the American Medical Association* **296(6):**671-678

Sez-Llorens X. and McCracken G.H. (2003). Bacterial meningitis in children. *The Lancet*. **361 (9375):** 2139-48.

Shapiro E., Aaron N., Wald E. and Chiponis D. (2008). Risk factors for development of bacterial meningitis among children with occult bacteremia. *The Journal of Pediatrics.* **109(1):**15-19

Slavoski L.A. and Tunkel A.R. (1995). Therapy of fungal meningitis. *Clinical Neuropharmacol.* **18 (2):**95–112.

Sloan D., Dlamini S., Paul N. and Dedicoat M. (2008). Treatment of acute cryptococcal meningitis in HIV infected adults, with an emphasis on resource-limited settings. *Cochrane Database System Review.* **1002(4):** 1465-1858.

Stephen J., Feigin R., Denis L. and McCracken G. (1986). W.H.O Report on the task force on diagnosis and management of meningitis. *Pediatrics*.**78:** 959-982.

Straus S.E., Thorpe K.E. and Holroyd-Leduc J.,(2006). How do I perform a lumbar puncture and analyze the results to diagnose bacterial meningitis? *JAMA: the Journal of the American Medical Association.* **296 (16):**2012–2022.

Teyssou R. and Muros-Le Rouzic E (2007). Meningitis epidemics in Africa: a brief overview. *Vaccine.* Sep 3; 25 Suppl 1:A3-7

Thwaites G., Chau T.T., Mai N.T., Drobniewski F., McAdam K. and Farrar J. (2000). Tuberculous meningitis. *Journal of Neurology, Neurosurgery, and Psychiatry* **68 (3):** 289–99.

Tunkel A.R., Hartman B.J. and Kaplan S.L. (2004). Practice guidelines for the management of bacterial meningitis. *Clinical Infectious Diseases.* **39 (9):** 1267–1284.

Wright P., Avery W., Ardill W. and McLarty J. (1993). Initial clinical assessment of the comatose patient: cerebral malaria vs. meningitis. *Pediatric Infectious Disease Journal.* **2:**37-41.

Internet references

http://www.who.int/csr/don/2006_03_21/en/index.html. Accessed on 20th October 2010.

http://www.cdc.gov/washington/EGlobalHealthEditions/eGlobalHealth0708.htm. CDC E-brief 2nd Quarter, 2008. Accessed on 20th October 2010.

http://www.meningitis.org/sect5/subsect10. Meningitis Research Foundation (2003). Accessed on 30 November 2010.

http://www.who.int/mediacentre/factsheets/fs141/en/ Fact sheet N°141 Revised 2003 WHO | Meningococcal meningitis Fact sheet. Accessed July 2010.

APPENDICES

Appendix 1: Clinical indications for Lumbar Puncture in children aged 0-59 months

Clinical Indications for Lumbar Puncture in Children aged 0-59 months.

LP for >2 months

Do an LP in <u>any</u> child aged more than 2 months with a history of fever and **one** or more of:

- ✓ Any reduction in the level of consciousness
- ✓ Confusion
- ✓ Stiff neck
- ✓ Bulging fontanelle
- ✓ Fits if age <6 months and older than 6 years
- ✓ Partial seizures – seizure affecting one part of the body only

Do an LP even if the malaria slide is positive.

Approximately 8 / 10 cases of meningitis are detected using these signs so if these signs are absent but you are suspicious of meningitis do an LP

LP in a child aged < 2 months

<u>As well as the above indications</u>

Do an LP if there is fever or hypothermia plus:

- ✓ Severe lethargy / Inability to feed
- ✓ A high pitched cry / obvious irritability

A Lumbar Puncture should not be done immediately if;
- x The child requires emergency resuscitation
- x The pupils respond poorly to light or there are other signs of cerebral oedema
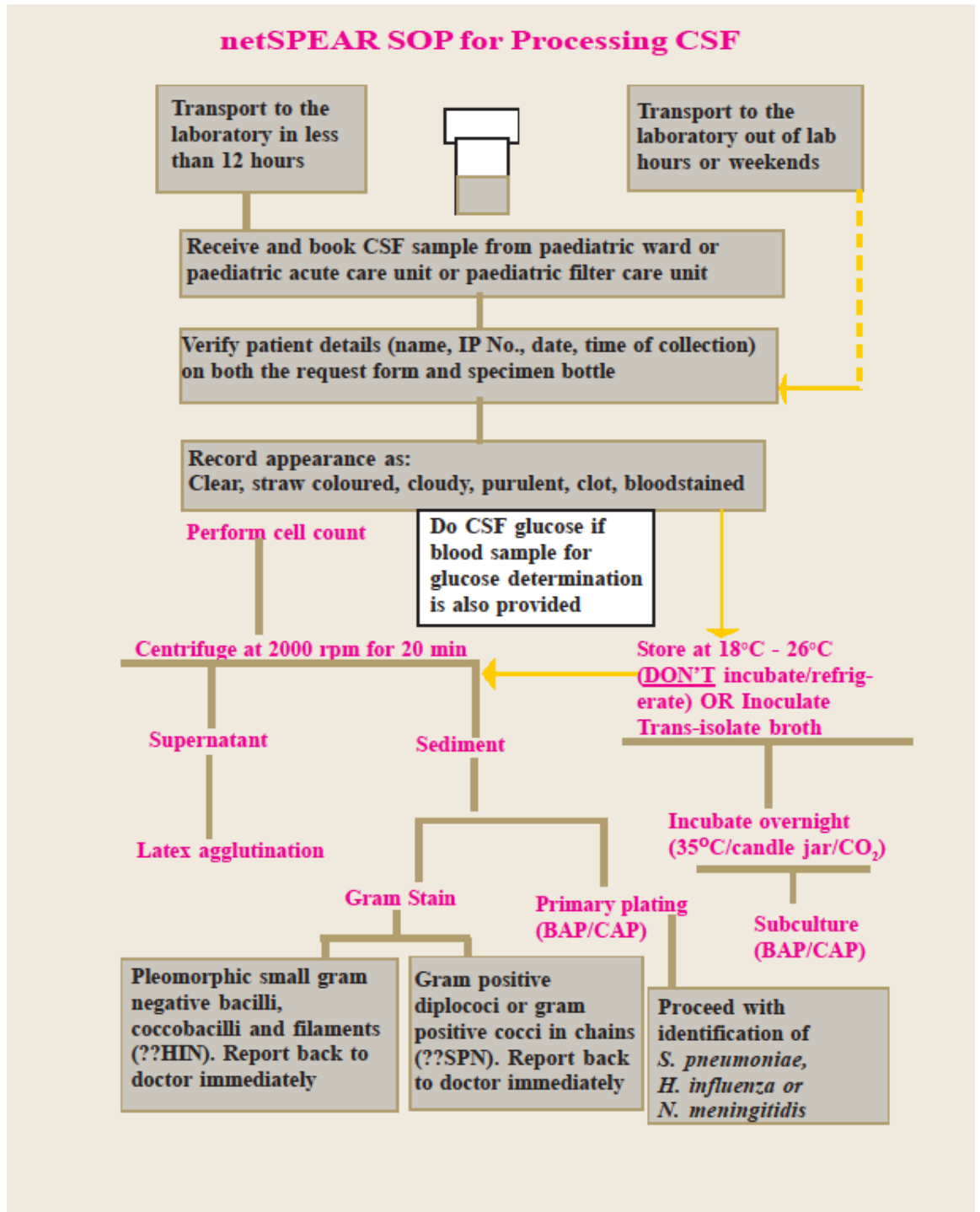- x There is skin infection at the site of LP

Appendix 2:   Microbiology lab request form

Microbiology Request Form.

Please tick or fill boxes as appropriate and write legibly.

Specimen type	CSF ☐	Blood ☐
	Aspirate ☐	Other ☐

IP Number ☐☐☐☐☐☐☐☐☐

Last Name _____

Other names _____

Sex ☐   Age (yrs) ☐   Age (mths) ☐

District of residence _____

Ward _____   Consultant _____

Specimen collected	Date	/ /
	Time	: am / pm

Diagnosis / Clinical – *please tick if 'Yes' in all appropriate boxes.*

Has patient received antibiotics before specimen collection? ☐

Neck Stiffness ☐	Meningitis ☐
V. Severe Pneumonia ☐	Severe Pneumonia ☐
Sepsis ☐	Other (write):

Results

CSF Interim Results	CSF appearance	
	Gram Stain	
	WCC	X10^6/L	Protein	
	RBC	X10^6/L	Glucose	

Please Check with Lab at 24-48 hours for final CSF result.

Blood Culture Result - Final	Organism cultured	

Appendix 3:   netSPEAR SOP for processing CSF

netSPEAR SOP for Processing CSF

Transport to the laboratory in less than 12 hours

Transport to the laboratory out of lab hours or weekends

Receive and book CSF sample from paediatric ward or paediatric acute care unit or paediatric filter care unit

Verify patient details (name, IP No., date, time of collection) on both the request form and specimen bottle

Record appearance as: Clear, straw coloured, cloudy, purulent, clot, bloodstained

Do CSF glucose if blood sample for glucose determination is also provided

Perform cell count

Centrifuge at 2000 rpm for 20 min

Store at 18°C - 26°C (DON'T incubate/refrigerate) OR Inoculate Trans-isolate broth

Supernatant

Sediment

Latex agglutination

Incubate overnight (35°C/candle jar/CO$_2$)

Gram Stain

Primary plating (BAP/CAP)

Subculture (BAP/CAP)

Pleomorphic small gram negative bacilli, coccobacilli and filaments (??HIN). Report back to doctor immediately

Gram positive diplococi or gram positive cocci in chains (??SPN). Report back to doctor immediately

Proceed with identification of *S. pneumoniae*, *H. influenza* or *N. meningitidis*

ON RECEIPT OF CSF SPECIMEN

Verify name, number and specimen date on the vials and request forms.

Check that there are three vials, two for CSF and one for blood. Accept if only one CSF vial is submitted. Record the specimen in the computer using the netSPEAR lab programme/laboratory register.

If it's a new specimen then register as new patient in the computer (if you are using the netSPEAR software).

Observe and record the macroscopic appearance of the CSF.

Do cell count on UNCENTRIFUGED sample

Centrifuge remaining sample at 2000rpm for 20 minutes. Gram stain and observe.

Inoculate 1 drop of CSF (or the sample from Trans-isolate broth) onto BAP and CAP plates and incubate in candle jar. Perform glucose estimation in the CSF and blood sample. Record all results in the request form and the computer. Inform the ward/clinician on the preliminary results within 1 hour. Store the remaining CSF in a freezing vial in the top compartment of the refrigerator.

DAY 2

After overnight incubation, remove plates from incubator and examine for growth.

Re-incubate if there is no growth. On BAP and CAP look for small grey colonies on fresh BAP and incorporate Optochin discs. Proceed with identification for *S. pneumoniae.* (See SPN SOP for details).

Further on CAP look for small grayish semi-opaque smooth colonies.

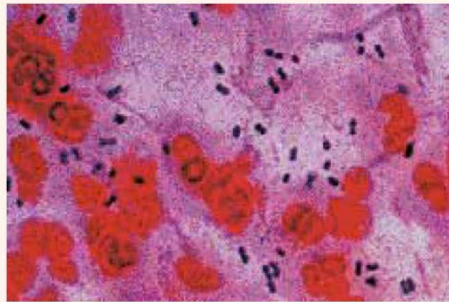Gram-stain and proceed with identification for *H. influenzae* (See main HIN SOP).

Appendix 4: netSPEAR flowchart for laboratory identification of Streptococcus

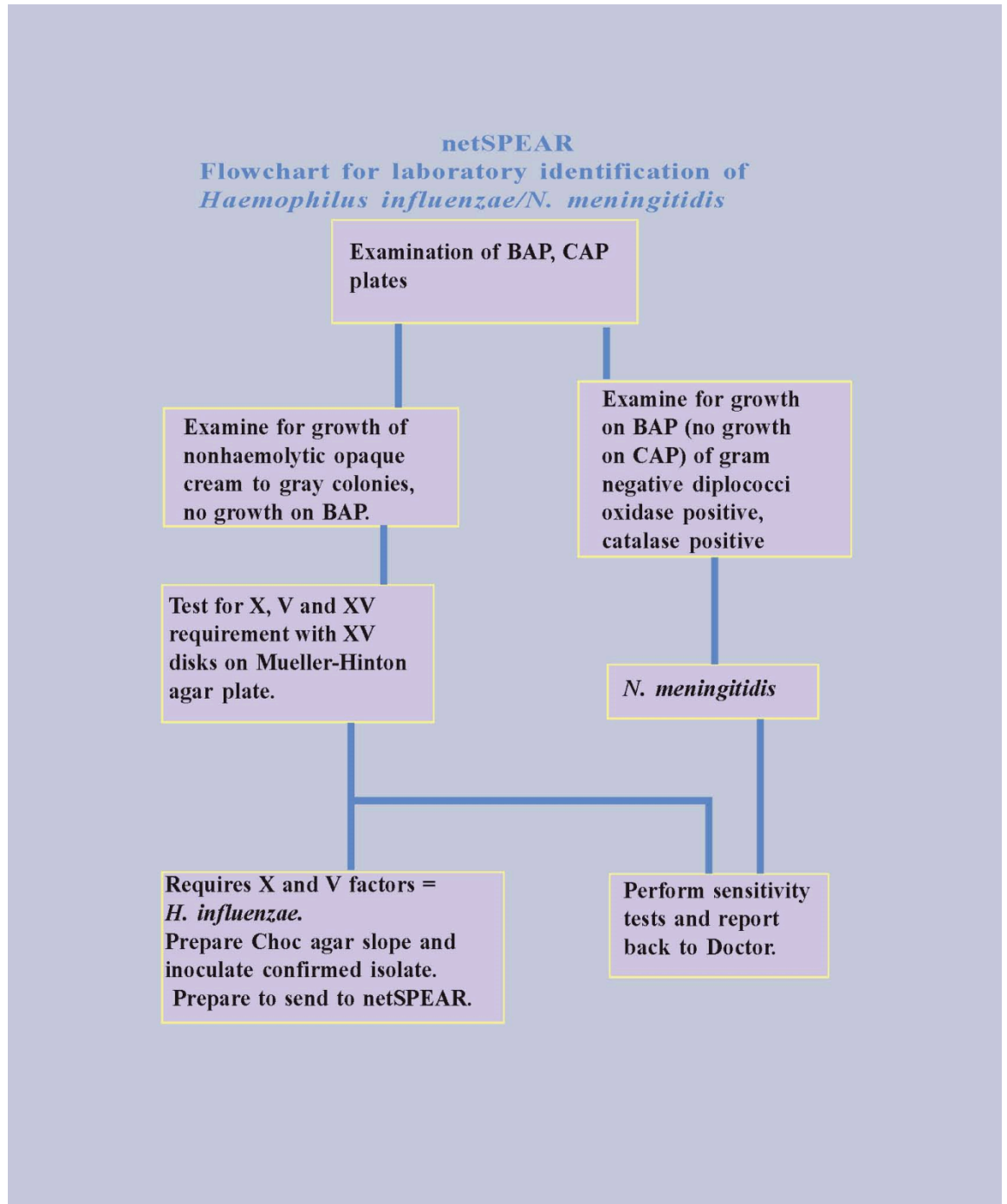*pneumoniae/ Neisseria meningitidis*
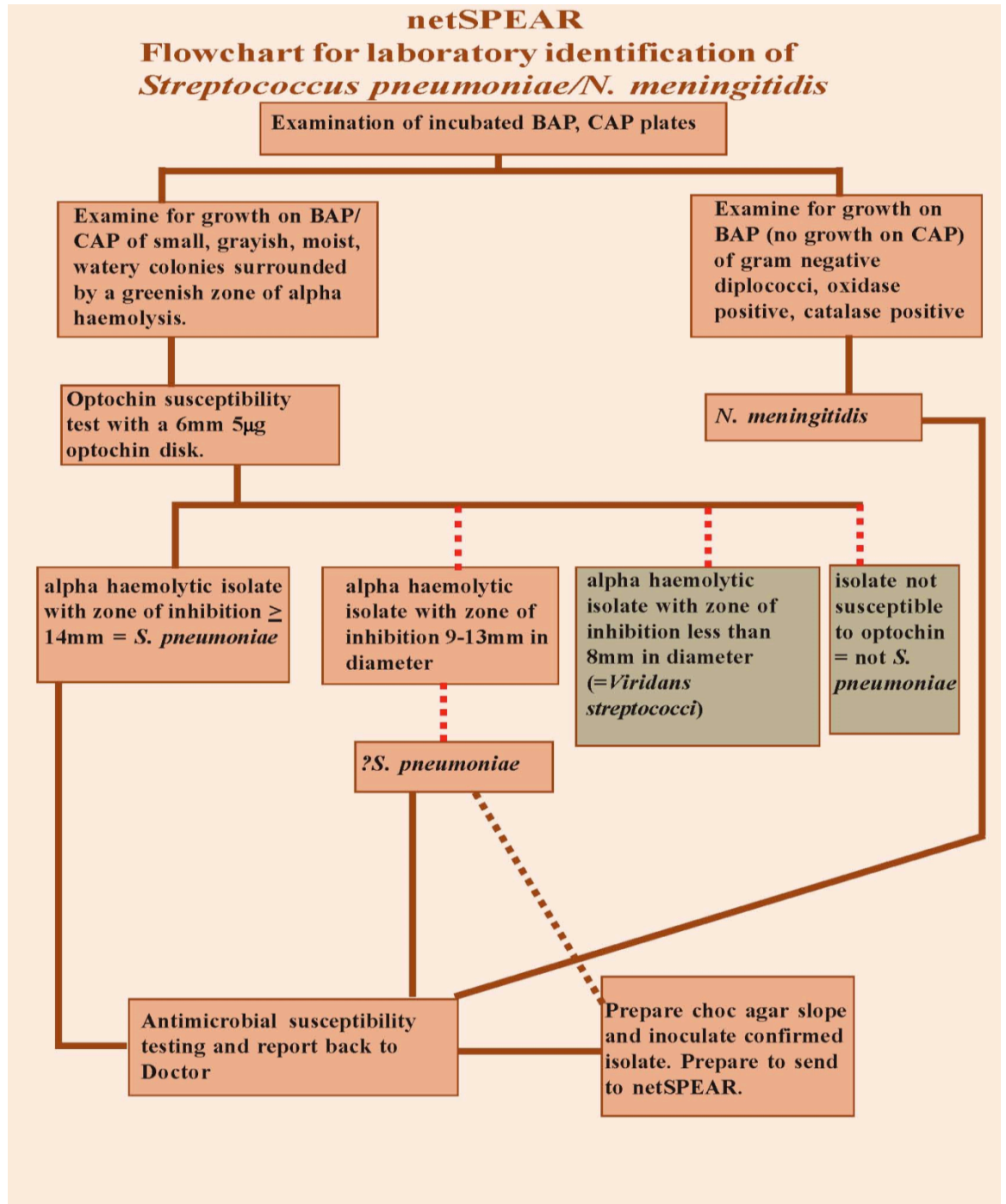
Streptococcus pneumoniae gram stain

Streptococcus pneumoniae on blood agar plate
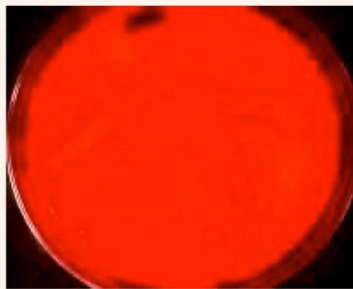
**Optochin test: S. pneumoniae (sensitive) S. sangius (resistant)**
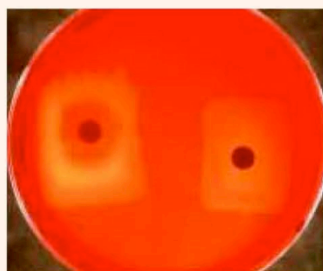
Appendix 5:   netSPEAR flowchart for laboratory identification of Haemophilus influenzae/ Neisseria meningitidis

Haemophilus influenza has certain specific growth requirements: it will not grow on a routine blood agar (TSB, trypticase sheep blood) plate, but will grow on a chocolate agar plate (where the blood is heat-denatured). It will, however, grow on a blood agar plate next to a streak of beta-hemolytic Staph (*Staphylococcus aureus*).

More specifically, it will grow on a plate (here, Mueller-Hinton agar) that has been fortified with both X-factor (found to be hemin) and V-factor (found to be NAD) but not either one alone. The plate below has been streaked with enough *Haemophilus influenza* to form a lawn on the plate, but there is only growth around the XV filter, which has been fortified with both X and V factor (Hemin and NAD).
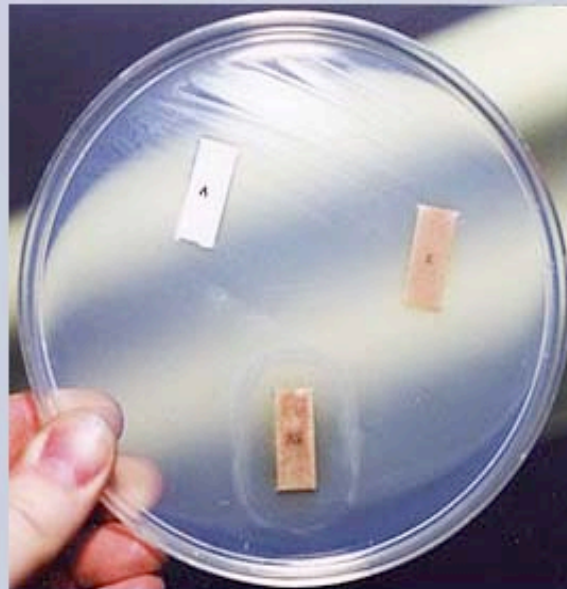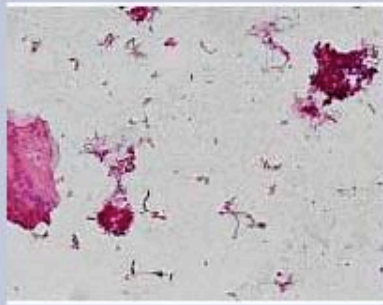
Haemophilus influenzae gram stain

Haemophilus influenzae growth on Chocolate agar.