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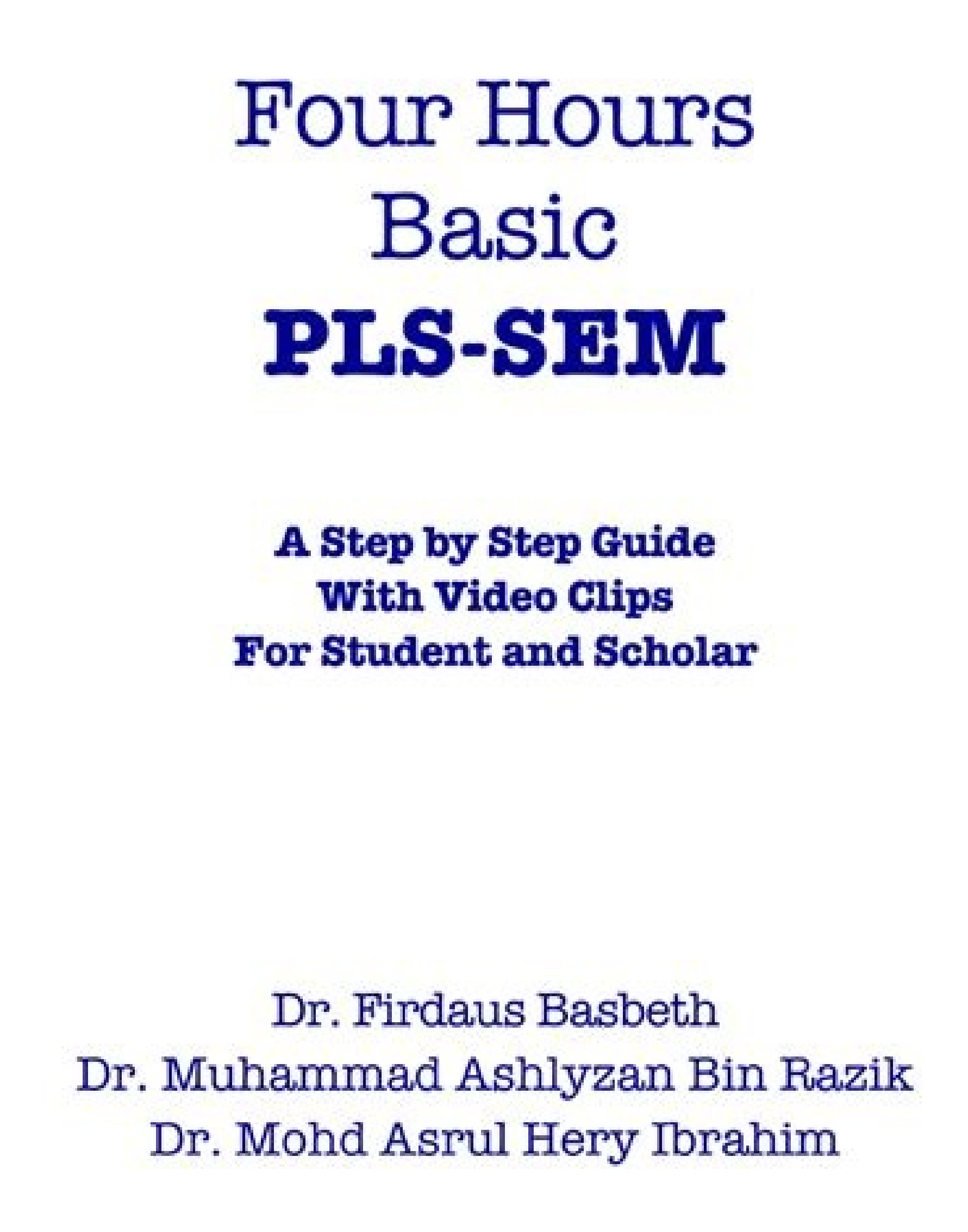

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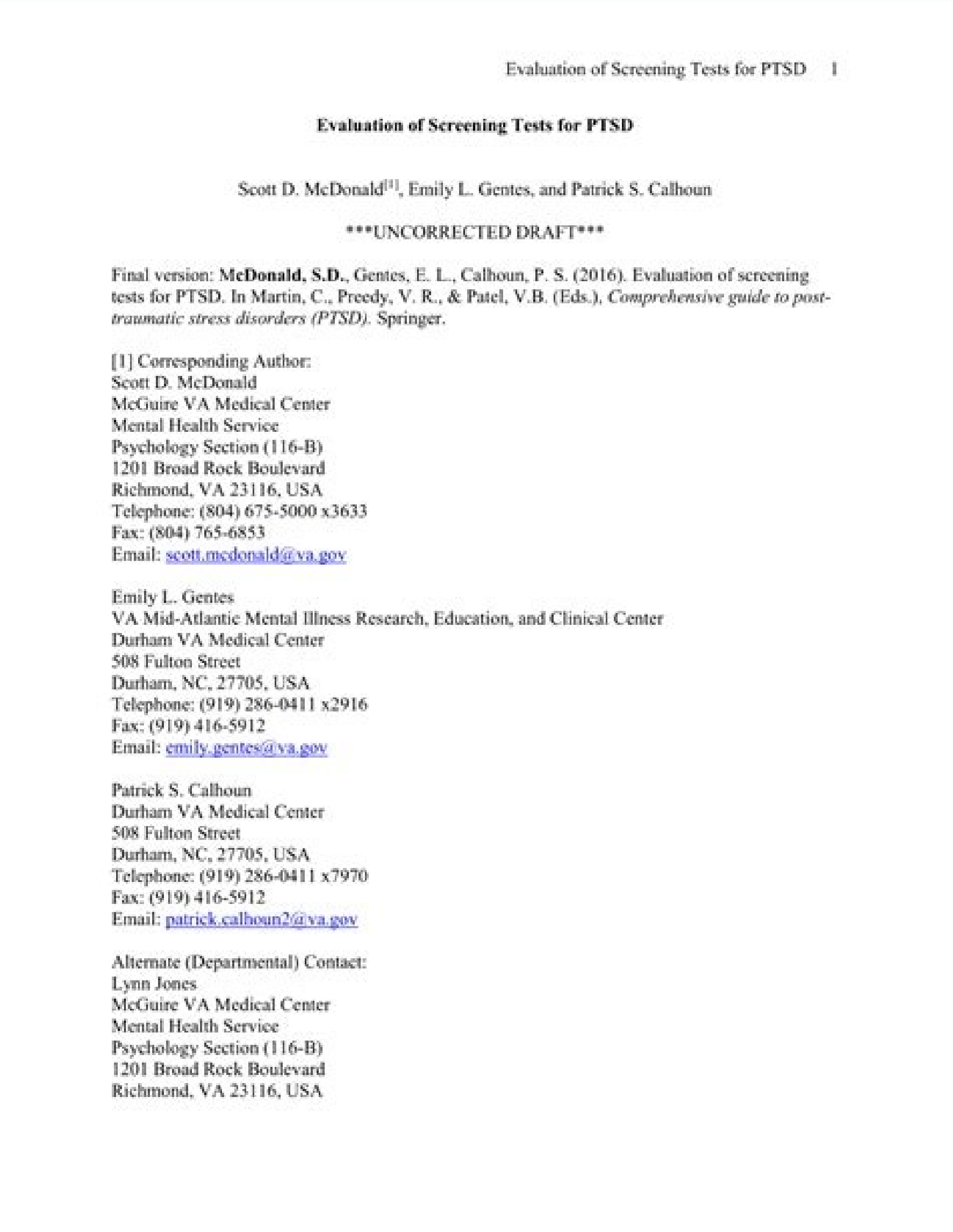

Evaluation of Screening Tests for PTSD

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UNCORRECTED DRAFT

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Polymer Rigidity Control for Endoscopic Shaft-Guide 'Plastolock' — A Feasibility Study

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Flexible endoscopes are used for diagnostic and therapeutic interventions in the human body for their ability to be advanced through tortuous trajectories. However, this very same property causes difficulties as well. For example, during surgery, a rigid shaft would be more beneficial since it provides more stability and it allows for better surgical accuracy. In order to keep the flexibility and to obtain the rigidity when needed, a shaft-guide with controllable rigidity could be used. In this article, we introduce the plastolock shaft-guide concept, which uses thermoplastics that are reversibly switched from rigid to compliant by changing their temperatures from 5°C to 45°C. These materials are used to make a shaft that can be rendered flexible to follow the flexible endoscope and rigid to guide it. To find polymers that are suitable for the plastolock concept, an extensive database and internet search was performed. The results suggest that many suitable materials are available or can be custom synthesized to meet the requirements. The thermoplastic polymer Purasorb® PLC 7015 was obtained and a dynamic mechanical analysis showed that it is suitable for the plastolock concept. A simple production test indicated that this material is suitable for prototyping by molding. Overall, the results in this article show that the plastolock concept can offer simple, scalable solutions for medical situations that desire stiffness at one instance and flexibility at another.
[DOI: 10.1115/1.4002494]

1 Introduction

For the investigation and treatment of areas in and around the digestive tract, flexible endoscopes [1] are used for many decennia to negotiate bends in organs and to approach hard to reach areas in the human body. In natural orifice translumenal endoscopic surgery (NOTES) and colonoscopy, for example, the indispensable flexibility of these instruments causes several difficulties [2–10]. An example of such difficulties during NOTES is shown in Fig. 1: A flexible endoscope is advanced through the esophagus, into the abdomen through an incision in the stomach wall, toward an organ for surgery. A grasper is introduced through a channel in the inserted endoscope to manipulate tissue. When the grasper is used to pull tissue, the flexible endoscope bends, failing to provide the stability required for the intervention because the endoscope shaft is too compliant to provide solid stability.

This situation can occur in all interventions that use flexible instruments in the human body, and it contains a conflict: There is a necessity to have a flexible endoscope shaft that enables insertion through tortuous body cavities, and a desire for a rigid shaft that allows greater surgical accuracy. This could be solved if the endoscope shaft had widely controllable rigidity or if it had a second shaft with controllable rigidity guiding it. The mechanism providing such functionality should retain the shaft curvature when changing rigidity. This would enable altering the shaft (guide) for each phase of the intervention to be rigid or compliant in any suitable shape.

If surgery through flexible endoscopes is to become a good alternative for current operating techniques, stable instrument support and spacing between instruments are indispensable [8]. To obtain instrument spacing without extremely reducing instrument sizes or using multiple endoscopes, the rigidity control mechanism should occupy as little space as possible. Meanwhile, it should still support scopes ranging from pediatric endoscopes to standard colonoscopes with flexural rigidities ranging from 67 N cm^2 to 330 N cm^2 [11,12].

In 2005, Rex et al. [13] and Swanstrom et al. [14] demonstrated a shaft guiding overtube with a rigidity control based on friction. Its shaft is a train of nested segments with tension wires running through the segments [15]. This smart and simple mechanism offers beneficial rigidity in its current form. However, its rigidity highly depends on the high tension forces applied to the small segments. Therefore, the segments cannot be made very thin, leaving little space for instruments. Other solutions for the same problem use vast amounts of controlled segments, using much space and increasing the complexity of the devices [16–19].

Size reduction and simplification of rigidity control mechanisms enable the application of rigidity control in smaller endoscopes and increase the space available for instruments and working channels in flexible endoscopes. It is expected that rigidity control mechanisms could be greatly simplified and downscaled if they were not dependent on applying forces or moving mechanical parts. One way to loose this dependency is to use an amorphous (or semicrystalline) thermoplastic polymer and heat or cool it around its glass-transition temperature (T_g) [20]. Amorphous and semicrystalline polymers will further be addressed to as "(partly) amorphous." The goal of this article is to demonstrate the feasibility of a concept using such a rigidity control mechanism within temperatures that are safe for the human body. Further presented are material requirements for this concept and a search for suitable polymers.

2 Plastolock Shaft Concept

2.1 Basic Concept. At temperatures below T_g, (partly) amorphous thermoplastic polymers are rigid with strong bonds between macromolecules. When heated, they become compliant, with weakened bonds between macromolecules. The transition around T_g is fast and reversible.

This rigidity control concept has been suggested in patents for applications ranging from medical catheters to inflatable spacecraft structures [21–23] but has, to our knowledge, never been demonstrated in literature for use inside the human body. The concept we investigated is called the "plastolock" shaft. It is an elongated shaft (Fig. 2) made of (partly) amorphous thermoplastic polymer. To change the shaft stiffness from rigid to compliant repeatedly and at will, fast heating and cooling of the material must be achieved. In the current concept, this is achieved using a heat carrying fluid flowing through channels in or around the plastolock shaft.

By passing warm fluid through the channels, the temperature rises and the polymer becomes compliant, enabling the plastolock shaft to be put to any shape desired and to be advanced along

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Annual Research Review: The transdiagnostic revolution in neurodevelopmental disorders

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Practitioners frequently use diagnostic criteria to identify children with neurodevelopmental disorders and to guide intervention decisions. These criteria also provide the organising framework for much of the research focussing on these disorders. Study design, recruitment, analysis and theory are largely built on the assumption that diagnostic criteria reflect an underlying reality. However, there is growing concern that this assumption may not be a valid and that an alternative transdiagnostic approach may better serve our understanding of this large heterogeneous population of young people. This review draws on important developments over the past decade that have set the stage for much-needed breakthroughs in understanding neurodevelopmental disorders. We evaluate contemporary approaches to study design and recruitment, review the use of data-driven methods to characterise cognition, behaviour and neurobiology, and consider what alternative transdiagnostic models could mean for children and families. This review concludes that an overreliance on ill-fitting diagnostic criteria is impeding progress towards identifying the barriers that children encounter, understanding underpinning mechanisms and finding the best route to supporting them. **Keywords:** Neurodevelopmental disorders; learning difficulties; working memory; ADHD; Autism; Developmental Language Disorder.

Introduction

Neurodevelopmental disorders (NDDs) affect children's cognition, academic attainment, behaviour, social interactions and lived experience. Up to 10% of children are identified as having one or more NDDs (e.g. NICE, 2019), and many more require support in or beyond school in these key areas of functioning (Department of Education, 2020). In this article, we consider how well current classifications of NDDs serve our understanding of this large and heterogeneous population of young people and review the conceptual, methodological and empirical developments that have set the stage for a radical rethink in both research and practice.

International diagnostic systems are widely used by clinicians to categorise difficulties, establish who receives additional support and inform intervention decisions. These systems provide the foundations for research too, framing conceptual thinking and guiding recruitment and analytic strategies. The most influential framework – the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association, 2013) – includes specific disorders of learning and communication, autism spectrum disorder (hereafter autism), attention-deficit hyperactivity disorder (ADHD) and a range of other neurodevelopmental conditions.

This taxonomy has evolved in several respects over recent decades. Its most recent instantiation, the DSM-5, has relaxed some of the boundaries between disorders, diminished the emphasis on fixed inclusionary and exclusionary criteria, and increased acknowledgement of the variability within disorders. This is most notable in the case of autism, where a single broad category of NDD comprised of two variable domains of potential difficulty has replaced the three domains of difficulty – in social interaction, social use of language and symbolic play – that were previously required for a diagnosis (e.g. Volkmar & McPartland, 2014). The addition of new diagnostic options such as autism *with* intellectual disability or autism *with* language delay demonstrates the limits to which a categorical model can tolerate heterogeneity. Other diagnostic categories such as social (pragmatic) communication disorder have also been incorporated. In essence, the DSM-5 is a system used for assigning individuals to discrete diagnostic categories that appears to be straining at its limits.

Categorical diagnoses have long been adopted as 'ground truth' by many researchers. Study design, sampling approaches, analytic methods and theory are built on the premise that disorder categories reflect an underlying reality, shaping every aspect of the scientific architecture of NDDs. This status quo faces three fundamental problems. First, current taxonomies are not particularly effective in capturing the needs of the full population of children requiring additional support in the broad areas of learning, behaviour or social functioning. The application of arbitrary thresholds leads to the failure to identify individuals with milder difficulties that nonetheless impact significantly on their lived experience. This diagnostic problem is amplified by inequalities in access to both diagnoses and therapeutic support

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Conflict of interest statement: No conflicts declared.

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GENETIC MATERIAL 3. ANAEROBE – bacteria that grow, live, and survive in the absence of oxygen STRICT/OBLIGATE ANAEROBE – absolutely do not require oxygen to grow, live, and survive Many Clostridum spp. Clostridum tetani – tetanus – spasmodic contraction of the muscle because of tetanospasmin 4. Distinct, discrete colonies within an obvious zone of inhibition should not be considered swarming. A properly made bacterial smear has the following characteristics: The smear occupies around 2/3 of a properly labeled slide The bacterial cells are evenly distributed The bacterial smear is not washed off the slide during staining Bacterial form is preserved and not distorted Figure 15 Bacterial Smear Preparation Page 25 of 131 Study Guide in Diagnostic Bacteriology Page 26 of 131 STEP 1: Label the glass slide on the frosted edge or on the far left corner. Best collected during febrile episodes. holartica – TYPE B – least severe; all forms of tularemia Francisella tularensis subsp. Membrane Phospholipid Bilayer usually WITHOUT carbohydrates and sterols **Except: Mycoplasma & Ureaplasma – with sterols ABSENT With Cell Wall Plants: Cellulose & lignin Algae: Glycan Fungi: complex carbohydrates (polymers) such as chitin, mannan, glucan, chitosan) Without Cell Wall: Animals & Protozoans SOMATIC/BODY CELLS – Mitosis NUCLEUS SEX CELLS/GAMETES – Meiosis Fluid Phospholipid Bilayer usually WITH carbohydrates and sterols. Once all disks are in place, replace the lid, invert the plates, and place them in a 35°C air incubator for 16 to 18 hours. NALC is digesting and NaOH is antibacterial Trisodium Phosphate and Benzalkonium Chloride (Zephiran) – recommended when Pseudomonas is present in sample Dithiothreitol and 2% NaOH – similar to example # 1 4% NaOH – strong alkali solution acts both as a mucolytic agent and antibacterial agent. pH – it is also important to consider the pH of the material to be treated and he agent itself. Dry Heat – oxidation of bacterial components Page 46 of 131 Study Guide in Diagnostic Bacteriology Page 47 of 131 a. In 1677, he observed bacteria in a water sample which he called "little animals" Figure 3: A drawing of the microscope used by Robert Hooke which he used to observe the blue inset image which has round structures which is believed to be fungal spores of Mucor He's often called the Father of Microbiology because in 1684, he described the different shapes of microscopic organisms from a tooth scraping which was evidenced by his letters to the Royal Society of London (Brock, 1989) (Figure 4). Arcobacter spp. GN Broth (Gram Negative Broth) – used to selectively favor the growth of Salmonella and Shigella while inhibiting all other normal intestinal flora. Selenite F Broth - used to selectively favor the growth of Salmonella while inhibiting all other normal intestinal flora. INCINERATION – burns materials into ashes; used in the disposable of biological wastes effective indication: 870-980oC for 2 seconds 3. Pale yellow pigment which is enhanced with additional incubation at room temp. meningitidis + + Weakly +; mostly - Glucose & Maltose N. Incubation with light PHOTOCHROMOGEN No pigment production (Non-photochromogen) Incubation without light Page 43 of 131 SCOTOCHROMOGEN Study Guide in Diagnostic Bacteriology Page 44 of 131 SELECTIVE MEDIUM FOR Neisseria spp. Table 4-4 summarizes the modified Kinyoun method of acid fast staining, the corresponding reagent/s in each step, and key reactions of acid fast and non acid fast organisms. Organisms such as Proteus mirabilis, which swarm, must be measured differently than nonswarming organisms. cholerasuis Most common cause of enterocolitis/gastroenteritis – S. METHODS OF ANTIMICROBIAL SUSCEPTIBILITY TESTING DIFFUSION METHOD – In 1960, Drs. Mycobacterium kansasii Also known as YELLOW BACILLUS – produces a yellow pigment when exposed to light due to the presence of beta-carotene pigment The pigment darkens to RED when prolonged incubation and exposure to light Causes Chronic Pulmonary disease similar to Tuberculosis that rarely disseminates KEY LABORATORY IDENTIFICATION: o Grows well at 37oC evident in 14-28 days o Grows slowly at 24OC and DOES NOT GROW at 42OC o RAPID Tween 80 Hydrolysis (in 3 days) o STRONG Reduction of NO_3 to NO_2 o RAPID Catalase activity o Pyrazinamidase (-) 5. Temperature – Generally, disinfectants are usually used at room temperature (200C to 220C). TRIPLE SUGAR IRON AGAR Protein Source: Peptone CHO: Lactose (1%) Sucrose (1%) Glucose(0.1%) pH Indicator: Phenol Red H_2S indicator: ferrous sulfate Sulfur Source: Sodium thisulfate ** Important TSI Reactions: 1. Example, Type IV pili enables bacterium to crawl or "twitch" across smooth surfaces. Borrelia burgdorferi Agent of LYME DISEASE which was first epidemiologically investigated during an increased incidence of rheumatoid arthritis in children in Lyme and Old Lyme, Connecticut, USA in 1975 Although the signs and symptoms, specifically the rash, were first observed in 1909 from Arvid Afzelius of Sweden who discovered a red papule which spreads into a larger lesion. Neufeld Quellung Reaction Page 76 of 131 Study Guide in Diagnostic Bacteriology Page 77 of 131 17. Schultz-Chalton Test NUTRITIONALLY VARIANT STREPTOCOCCI (NVS) Abiotrophia spp. STEP 2: INOCULATION OF THE MUELLER-HINTON PLATE 1. CLASSIFICATION OF CULTURE MEDIA ACCORDING TO FUNCTION/USE GENERAL PURPOSE/PRIMARY/BASIC/BASAL/SUPPORTIVE/GENERAL ISOLATION CULTURE MEDIA – contains basic nutritional requirements to support the growth of non-fastidious microorganisms. GENUS LEPTOSPIRA Tightly twisted organisms with one or both ends bent into a hook Aerobic and motile by periplasmic flagella which occur singly at each end of the cell Important Members: o Leptospira biflexa – non-pathogenic. X FACTOR (HEMIN/HEMATIN V FACTOR (Nicotinamide Adenine Dinucleotide or NAD) ENVIRONMENTAL GASEOUS REQUIREMENT AEROBE - bacteria that grow, live, and survive in the presence of oxygen STRICT/OBLIGATE AEROBE – absolutely requires oxygen to grow, live, and survive Micrococcus spp., Mycobacterium spp., Pseudomonas spp., Neisseria spp., Brucella spp., Francisella spp., Bordetella spp., Leptospira spp. Example, Alcohol and betadine has to be in contact for about 1-2 minutes to work properly. GENUS: Capnocytophaga Common species: Capnocytophaga ochraceae Key Characteristics: o Normal Flora of the Oral Cavity o Opportunistic Infection in the oral cavity o Rare cause of human infection o Yellow pigmented, thin, gram negative bacilli o Non-motile (no flagellum); exhibits GLIDING MOTILITY GENUS: Eikenella Common species: Eikenella corrodens Key Characteristics: o Corroding bacterium – PITS or CORRODE the surface of the agar o Normal Flora of the human mouth and upper respiratory tract o Important cause of HUMAN BITE INFECTON o Grows well in CAP and requires X factor (hemin) o Characteristic BLEACH LIKE ODOR o Nonsaccharolytic, oxidase (+), catalase (-), NON-MOTILE ∞ SECTION 20 ∞ FAMILY PASTEURELLACEAE GENUS: Pasteurella Common species: Pasteurella multocida KEY CHARACTERISTICS: Gram negative short bacilli/coccobacilli Facultative anaerobe Non-motile Fermentative Encapsulated Grows in BAP/CAP but NOT CAPNOPHILIC. Clostridium perfringens SEROTYPE A Gas gangrene/clostridial myonecrosis/Necrotizing Fascitiis Type 3 SEROTYPE B Pig Bell – ingestion of contaminated meat 2. gordonae (+) and M. szulgai (@ 35-37OC) M. Until his new colleague, Sahachiro Hata tested all compounds against the newly discovered microbe causing syphilis. Therefore, the following general considerations should be kept in mind when specimens are submitted to the clinical microbiology section of the laboratory: Specimen is collected and transported in a sterile, leakproof, and keeps the bacterial pathogen alive. Filtration – based on membrane gradient by differences in particle size; used for the sterilization of heat sensitive materials a. He theorized that either the microbes in the air repopulated the broth after Page 5 of 131 Study Guide in Diagnostic Bacteriology Page 6 of 131 boiling or the air enhanced the growth of microbes after boiling. Earliest Recorded Cases The first recorded case of infectious disease may have happened around 3180 B.C. which speaks of a great pestilence or deadly disease outbreak during the reign Emperor Shemsu of the First Dynasty in Egypt (Beck, 2000). MOT: Ingestion of contaminated meat/milk/dairy products Cutaneous – 2 stages VESICULAR STAGE – appearance of a small pimple surrounded by a fluid-filled vesicles at the point of entry ESCHAR STAGE – lesion ulcerates, dries, & BLACKENS EMETIC FOOD POISONING – ingestion of food contaminated with the emetic toxin results to headache, nausea, vomiting DIARRHEAL ingestion of food contaminated with the emetic toxin results to watery diarrhea NON PATHOGENIC – commom lab contaminant & used as biological indicator LECITHINA SE ASCOLI Precipitation Test Penicillin Susceptibil ity (10 ug) Growth in PLET Medium + + + + + B-hemolytic Lavender/p urple colonies + - - - + Alpha or Bhemolytic; ground glass appearance - - - - Motility Growth in BAP Non hemolytic - Colonies has a beaten egg white consistency ; medusa head appearance ; tenacious GENUS CLOSTRIDIUM KEY CHARACTERISTICS: Gram positive large SPORE FORMING BACILLI o oval subterminally located spores o round terminally located – C. He concluded that he had discovered a vaccine that differed from that of Jenner's by incorporating microorganisms into the vaccine (Brock, 1989). Smith, F.I. Kilbourne Martinus Beijerinck Walter Reed Paul Erlich Alexander Fleming J. Christie Atkins Munch Petersen (CAMP) Test Page 74 of 131 Study Guide in Diagnostic Bacteriology Page 75 of 131 10. Formaldehyde Vapor/Vapor Phase H_2O_2 - for HEPA filters and large spaces 3. OXIDASE (-) CATALASE (-) and NON-MOTILE Capnophilic and colonies are small and β-hemolytic on rabbit or human blood containing media such as Human Blood Bilayer Tween (HBT) Medium after 48 hours incubation Thought to be the cause of Bacterial Vaginosis but it is only associated with this disease. GRAM STAIN Page 83 of 131 Study Guide in Diagnostic Bacteriology Page 84 of 131 2. Grows in BAP but no hemolysis. However, the usual credit is given to Hans Jansen for his invention which contains two lenses in three sliding tubes with a 3X magnification when closed and 9X when fully opened (Jones, 1995) Robert Hooke A person with variety of interests such as physics, astronomy, chemistry, geology, biology, etc. o COMPOSITION: made up of protein material known as PILIN o TYPES/KINDS – there are seven (7) types that have been described depending on their size and the antigen that they carry (Type I, II, III....., Type VII). urealyticum + o SEROLOGICAL TEST o 50% of M. Table 7-2 provides an example of the components of this type of media. Mueller Hinton with Chocolate Agar Base or Haemophilus Test Medium (HTM) Agar Mycobacterium spp. He subjected his theory using an experimental set up (Figure 5) which entails the use of heated broth in swan-neck flasks, one allowed to cool and did not show any signs of microbial growth and the other was tipped which contaminated the heated broth as evidenced by turbidity. pertussis antibody o The stained smear is viewed using fluorescent microscope Page 104 of 131 Study Guide in Diagnostic Bacteriology Page 105 of 131 GENUS: Francisella KEY CHARACTERISTICS: Gram negative short bacilli/coccobacilli Obligate aerobe Intracellular parasite Requires medium containing cysteine, cystine, sulfhydryl, and a source of iron for enhanced growth CATALASE and OXIDASE (+) Fermenter of Glucose, Maltose, Mannose Agent of animal and human TULAREMIA – zoonotic infection/zoonoses Common reservoir is the cottontail rabbit Class A Agent of Bioterorrism – only approved laboratories should work up identification Common species: Francisella tularensis subsp. One of the sites of antigenic determinant in the bacterial cell Components may contribute to the pathogenicity of bacteria o Protein A – Staphylococcus aureus o M Protein – Streptococcus pyogenes o Mycolic Acid – Mycobacterium tuberculosis Responsible for the staining property and characteristics of bacterial cell Page 14 of 131 Study Guide in Diagnostic Bacteriology Page 15 of 131 The cell wall structure is different from one bacterium to another. They were thought to be viruses because they cannot produce ATP or survive outside a host cell It was also regarded as TRIC (Trachoma Inclusion Conjunctivitis) – infection associated with this organism Obligate intracellular organism hence would not grow in artificially prepared culture media Has gram-negative like cell wall, contains DNA and RNA, and are susceptible to variety of antibiotics, hence it was considered as a bacteria LIFE CYCLE OF CHLAMYDIA Page 127 of 131 Study Guide Diagnostic Bacteriology 128 IMPORTANT MEMBERS o Chlamydia psittaci (Chlamydophila now) Agent of PSITTACOSIS – disease of psittacine birds such as parrots, parakeets, cockatoos Agent of ORNITHOSIS – can also be carried by other birds like turkeys, pigeons, and chickens MOT: inhalation of contaminated aerosols or fomites or through person to person transmission 1-2 weeks incubation then chills, fever, malaise appear o Chlamydia pneumoniae (Chlamydophila now) Associated with mild respiratory infection It is believed that infection is spread from human to human without an animal reservoir Also known as TWAR (attributed to the first two strain isolated TW183 – isolated in 1965 from the eye of a control child in trachoma vaccine trial in Taiwan AR-39 – recovered the same year from the throat of a student with pharyngitis at the University of Washington o Chlamydia trachomatis Serotype A, B, Ba, C Causes trachoma – world's leading cause of blindness MOT: Close contact with infected individuals Trachoma begins with conjunctivitis which persists for months to years. Figure 4: A depiction of how Antoni Van Leeuwenhoek holding a miniature hand-held device towards a light source to visualize microorganisms which he documented by drawing their figures as seen on the figure. baumannii and A. through d. The results of the Kirby-Bauer disk diffusion susceptibility test are reported only as susceptible, intermediate, or resistant. haemophilum M. rettgeri Nosocomial Infections of skin (burn patients) and urinary tract (predisposed patients) P. Ignore the thin veil of swarming and measure the outer margin in an otherwise obvious zone of inhibition. INCLUSION BODIES – serves as depot or storage deposits under certain circumstances such as limited or excess of a particular nutrient. To avoid confusion, the first two letters of the first syllable are used when two or more genera begin with the same first letter (e.g. Staph and Strept. indologones o Most frequent human isolate o Dark yellow colonies on BAP o INDOLE (+) by xylene extraction o Oxidizes glucose but not mannitol in OF medium F. lwoffi A. Medically important bacteria are part of the Kingdom Monera which consists of prokaryotic organisms that do not contain a nucleus or a limiting membrane around their nuclear material. It changes its color fro BLUE to WHITE if ANAEROBIOSIS (complete anaerobic environment) is achieved Example is GasPak System (BD) A generator envelope is added is opened and activated by adding water This activates the generation of hydrogen and CO_2 o EVACUATION REPLACEMENT SYSTEM An air tight container such as Brewer Jar or GasPak Jar (BD) The air in the jar is removed and replaced with 85% N_2 10% H_2 5% CO_2 Methylene Blue Strips are used as indicator of effectiveness Already replaced by more modern techniques o ANAEROBIC GLOVE BOX METHOD Self contained anaerobic environment of 85% N_2 10% H_2 5% CO_2 is provded through external gas sources Other materials and specimens are passed into the chamber by an entry lock system Culture can be incubated in an incubator inside the chamber The system is incorporated with most techniques for culture and identification of an anaerobic organism o DISPOSABLE ANAEROBIC BAGS Convenient anaerobic incubation system Example is BioBag System (Type A) bye (BD) Consists of a plastic bag with H_2CO_2 gas generator that is released upon adding water PALLADIUM acts as catalyst RESAZURIN acts as indicator Bag is sealed after activation of gases and can hold two to three Petri dishes ANAEROBIC CULTURES are incubated at 35oC to 37OC for at least 48 hours o After examination, should be re-incubated for 2 to 4 days more to ensure that some of the slower growing bacteria, such as Actinomyces are not missed o Broth cultures should be held for 7 days before reporting negative or no growth o Page 131 of 131 STEP 3: Allow to air dry by putting the slide flat on the table surface or in a slide warmer. polysaccharea Predominant Normal Flora species: N. Another type (Type VII), also called as "F" or "sex" pilus is used to transfer DNA from one bacterial cell to another (BACTERIAL CONJUGATION). ACID FAST STAIN – another routinely used differential stain in the laboratory which is utilized to differentiate acid fast organism such as Mycobaterium tuberculosis which stains RED from non acid fast organisms which stains BLUE or GREEN depending on the counterstain used in the process using the Ziehl-Neelsen or Kinyoun Staining Methods. (+) from Streptococci spp. SPIRALS These are helical or twisted bacteria. PIGMENT PRODUCTION – due to carotenoids; observed using LJ medium which exhibits CHROMOGENICTY Property of Mycobacterium species 2. meningosepticum o Most often found causing human infection o Isolated from several water sources such as ice machines, water fountains, incubators, water baths o Nosocomial and extremely opportunistic causing: Page 99 of 131 Study Guide in Diagnostic Bacteriology o Page 100 of 131 Meningitis in neonates (especially in pre-mature infants) Pneumonia in immunosuppressed adults Key Lab Reactions: Delayed oxidation of glucose and mannitol in OF medium INDOLE (+) by xylene extraction Esculin Hydrolysis (+) ONPG (+) DNAse (+) UREASE (-) Pinpoint, glistening colonies. gordonae GROUP IV: RAPID GROWERS – pigmented yellow to orange in dark; pigment intensifies to orange or red when exposed to light source for 2 weeks M. It may also indicate presence of resistance mechanism in the organism. Neisseria gonorrhoeaeCommon Name: GONOCOCCUS/GONOCOCCI a. SUMMARY OF STANDARDIZED FACTORS IN SUSCEPTIBILITY TESTING Standardized Factors Inoculum equal to 0.5 McFarland Effect of Changes Too heavy = possible false resistance Too ight = possible false susceptibility Less than 15 min delay to > 15 mins delay = possible false resistance inoculation of testing medium Less than 15 min delay to > 15 mins delay = possible false resistance application of antibiotic discs Depth of the Culture Media (4mm) Too shallow = possible false susceptibility Too deep = possible false resistance Incubation Time (16-18 hrs) 10mm zone of inhibition Page 73 of 131 Study Guide in Diagnostic Bacteriology Page 74 of 131 7. He discovered an arsenic compound called atoxyl worked well with trypanosome parasites but is poisonous. Aids in the identification of Streptococcus agalactiae, Campylobacter jejuni, and Listeria spp. pallidum fixed on the slide o Treponemes staining with the fluorescent dye is a POSITIVE REACTION MICROHEMAGGLUTINATION TEST for T. And most of all, turn off your phone and avoid using the internet for unrelated tasks to maintain your focus.Review Your Notes and TextbookWhen you're getting started with your studying, take a look at the notes you have from class. aeruginosa P. Compatibility of Disinfectants – Some disinfectants may inactivate the action of another hence it is also important to consider the compatibility of the disinfectants. These ideas were expanded and popularized by Carolus Linnaeus and are documented in his primer, entitled Systema Nature, with the 10th edition published in 1758. Type-Specific Antibodies 2. Transparency of Fluid specimens (clear, hazy, turbid) Evidence of gas, foul smell, or sulfur granules – essential for recovery anaerobic organisms MICROSCOPIC EXAMINATION A direct microscopic examination of clinical sample is best achieved through bacterial smear preparation and subsequent staining. sonnei ALL Yersinia except enterocolitica Proteus Providencia Morganella Edwardsiella DARK VIOLET LLF: 36-72 hrs freundii koseri/diversus Characteristic growth of Rapid Lactose Fermenters in EMB 1. For sample from solid media, place a drop of sterile distilled water or NSS in a glass slide. It is important to remember and understand that NOT ALL selective culture media are differential but ALL differential culture media are selective. It was in 1799 that Lazaro Spallanzani demonstrated that some microbes are sensitive to heating than others while some required over an hour heating before being killed. morganii is the only known species in this genus Infections include nosocomial Infections of UTI and Wound Proteus Providencia Morganella All are MOTILE METHYL RED (MR) Positive DEAMINASE Positive LIA – R/A (red/yellow) Citrobacter Resembles Salmonella but differs because it is: o ONPG positive o LDC negative o Slow UreaseProducer (SUP) Important human pathogen which are commonly associated with UTIs & respiratory tract infections Table 14-6 Differentiation of Citrobacter species SPECIES C. Found in soil and water o Leptospira interrogans – causes of human and animal LEPTOSPIROSIS, a zoonosis Primarily parasitic on vertebrates other than humans such as rodents, cattle, dogs, cats, raccoons, and bats – sheds the organism in the URINE Modes of acquisition of the infection Direct contact with the urine of animals carrying the organism Indirect contact occurs when humans come in close contact with contaminated soil and water HIGH RISK: slaughterhouse workers, farmers, veterinarians, pet owners, sewage workers, campers, and those living in rodent infested areas LEPTOSPIROSIS – involved the kidney, liver, or CNS. scrofulaceum, M. Table 10: Spiral Morphologies Prominent Arrangement Illustration Spiral with two or more curves, quite rigid E.g. Spirillum minor/minus - causative agent of SODOKU (a rat bite fever infection) Loosely twisted spiral resembling a stretched spiral E.g. Borrelia spp. (Figure 1) TYPES OF PATHOGENS: True Pathogen: refers to an organism that will cause disease in a healthy host. Clostridum botulinum Food Botulism – ingestion of improperly canned goods Wound Botulism – severe wound infection Infant Botulism – ingestion of contaminated home-made honeys 3. SEMI-SOLID – a culture medium that contains 0.5%-1% agar. The four categories of staining frequently used in clinical microbiology lab are as follows: DIRECT/SIMPLE STAIN – usually contains one specific active chromogen in the stain which enhances the appreciation of bacterial size, shape and arrangement. Coughing subsides together with the other signs and symptoms c. Table 10 summarizes that important spirals of medical importance. MESOPHILIC – bacteria that can optimally grow between 20-40OC PSYCHROPHILIC/CRYOPHILIC – bacteria that can optimally grow between 0-20OC THERMOPHILIC – bacteria that can optimally grow between 50-60OC HYPERTHERMOPHILIC/EXTREMELY THERMOPHILIC – bacteria that can optimally grow between 80110OC such as the spore forming bacteria EURITHERMOPHILIC – bacteria that can grow at a wide range of temperature STERNOTHERMOPHILIC - bacteria that can grow at a narrow range of temperature pH REQUIREMENT – most pathogenic bacteria can grow in a neutral or slightly alkaline environment (pH 7.0 – 7.5), hence most culture media used in routine isolation of pathogens is adjusted to this pH range. packets of eight (8) E.g. Micrococcus luteus Coccus is divided by two (2) or more COCCI IN CLUSTERS planes of division in an irregular pattern Page 20 of 131 Study Guide in Diagnostic Bacteriology Page 21 of 131 E.g. Staphylococcus spp. Mouse Virulence Test 13. Patient appears recovered 6 days after the fever episodes only to relapse few days or weeks after RELAPSE attributed to the ability of the organism to alter its antigenicity. coli (EAEC) o Causes acute and chronic diarrhea. SYNONYMS: Indigenous Flora, Resident Flora, Normal Microbiota. These colonies are either mutant organisms that are more resistant to the drug being tested, or the culture was not pure and they are a different organism. ∞ SECTION 8 ∞ BACTERIAL CULTIVATION BACTERIAL GROWTH refers to the increase in the number of bacteria rather than in size. fortuitum – POSITIVE (+) M. Hence, understanding of the various methods of sterilization and disinfection is a must for a medical technologist to prevent unwanted spread of infection in the workplace. typhi – has Vi (virulent) Antigen which is equivalent to K Antigen 3. It d4ifferentiates gram positive bacteria which stain PURPLE or VIOLET from gram negative which stains RED or PINK. paratyphi A Causes enteric/typhoid fever – S. Na+ Hippurate Hydrolysis Test 11. PATHOGENICITY – refers to the ability of an organism to cause disease in a host organism. NaOH is usally used. haemolyticus H. ENDOTOXIN Page 79 of 131 Study Guide in Diagnostic Bacteriology Page 80 of 131 Table 13-2 Differentiation of Neisseria and Branhamella/Moraxella species SUPEROXOL TEST CARBOHYDRATE UTILIZATION TEST **Cystine Trypticase Agar (CTA) CATALASE OXIDASE + + + Glucose N. Antisepsis In the middle of 1800s, physicians were identified as sources of infectious agents from one ward to another. His classification scheme is very well adapted, accepted, and used until the present time which we regard as binomial nomenclature which basically classify organism using a general name (genus, genera, plural) followed by a one-word species name (species). Cardiobacterium hominis NORMAL FLORA of the upper respiratory tract. ducreyi H. TCBS Fermentation RED Reaction TEST Phenomenon V. Medically important bacteria that exhibit this form of gycocalyx is Staphylococcus epidermidis. It is also recommended for the transport of specimens for the recovery of Bacteroides ureolyticus. Place the appropriate antimicrobial-impregnated disks on the surface of the agar, using either forceps to dispense each antimicrobial disk one at a time, or a multidisk dispenser to dispense multiple disks at one time. LEPROMIN SKIN TEST Hypersensitivity test for LEPROSY Tuberculoid Type = (+) Lepromatous Type (-) Methods: o Fernandez Reaction – 24-48 hours o Mitsuda Reaction – 3-4 weeks PRIMARY PATHOGENS: 1. This is also the most widely and routinely used primary isolation media for most bacterial pathogens from clinical specimens. Continue to place one disk at a time onto the agar surface until all disks have been placed as directed in steps f. AGAR – solidifying agent of choice in the preparation of culture media because of the following reasons: Page 36 of 131 Study Guide in Diagnostic Bacteriology Page 37 of 131 It is not easily degraded by bacteria It can be melted at boiling point, resolidify upon cooling off starting at 42 0C, and can be melted again upon boiling EXAMPLES: Nutrient Broth (NB), Trypticase Soy Broth (TSB), Brain Heart Infusion (BHI), or Milk. which led him to invent telescopes and microscopes Page 4 of 131 Study Guide in Diagnostic Bacteriology Page 5 of 131 His invention led him to publish his best-seller Micrographia in 1665 which contains specific instructions for making single-lens, hand-held miniature microscope. NORMAL FLORA – bacteria that are in or on different sites of the body that usually do not harm the host unless the host defense is compromised. Test K. Page 113 of 131 Study Guide in Diagnostic Bacteriology Page 114 of 131 Slow growing organisms that produce buff colored colonies which are thin and transparent or smooth and asteroid margins may be present Found in the environment and have been isolated in water and soil samples Grows well 35oC to 37oC and 42oC BUT NOT at 24oC Identification is made based on its failure to yield positive reaction to most biochemical tests with the exception of HEAT STABLE CATALASE and Pyrazinamidase IMPORTANT: Has been noted with HIGH FREQUENCY in AIDS PATIENTS – usually involves gastrointestinal tract Mycobacterium avium o Agent of TB in chickens and rarely causes TB in chicken farmers o Has been complexed with Mycobacterium intracellulare (considered to be non-pathogenic) due to similar morphological and biochemical characteristics 4. FLAGELLAR ARRANGEMENT – not all bacteria have flagella, if they don't, they are usually called as ATRICHOUS while flagellated bacteria express their flagella in different parts of the bacterium usually in an organized manner and specific locations. NUCLEAR BODY 4. Leptospires grows 1-3 cm below the agar surface and may form linear disk of growth o Growth may be examined using DARKFIELD MICROSPCOPY o Can be isolated from CSF, blood, tissues during early bacteremic phase (first 10 days) o Can be isolated from the urine immune phase (2nd week up to 30 days) which requires alkalinization if cannot be cultured immediately Direct detection of leptospiral antigens o From Clinical Specimens – ELISA, Radioimmunoassay, Immunomagnetic capture o From infected tissues samples – imnnunofluorescence and immunohistochemistry Serological methods – agglutinating antibodies may appear during the 1st week of illness, during peak of the illness at around 3-4 weeks, and may persist for years o Microscopic/Macroscopic Agglutination test – established reference method but in a research setting o Hemagglutination tests o Enzyme Immuno Assay (EIA) ∞ SECTION 25 ∞ ATYPICAL BACTERIA GENUS MYCOPLASMA Classified as Mollicutes and are smallest free living organism known and are found in animals and plants They lack cell wall – easily change shape – PLEOMORPHIC RESISTANT to Antibiotics that inhibit cell wall synthesis such as Beta Lactam Drugs (PENICILLIN) Originally known as "Pleuro Pneumonia-Like Organisms" (PPLO) – first discovered causing pleuropneumonia in cattle Important Members: o Mycoplasma pneumoniae Previously known as EATON agent Frequent cause of community acquired pneumonia and tracheobronchitis in children and young adults. He described this procedure in a paper in 1867 which drastically reduced number of deaths after surgery. Used for the presumptive identification of catalase-negative, gram positive cocci DESCRIPTION Bacteria hydrolyzes the substrate using the enzyme L-pyrroglutamylaminopeptidase Bacteria hydrolyzes esculin to esculitin (black) Inhibitory Profiles – ability of the bacteria to grow in media supplemented with certain inhibitory substances INHIBITORY SUBSTANCE DESCRIPTION Vancomycin – inhibits gram positive Colisitin/Nalidixic Acid – inhibits gram negative Antibiotics Trimethoprim – inhibits Proteus spp. The average time is 3 weeks but may range from 3-60 days RUNYON'S CLASSIFICATION OF MYCOBACTERIA OTHER THAN TUBERCLE BACILLI (MOTT) GROUP I: PHOTOCHROMOGENS – develop yellow pigment when exposed to constant light source; nonpigmented in dark M. denitrificans K. Glutaraldehyde – for medical instruments (e.g. bronchoscopes, etc.) 4. TYPICAL SIZE 2. Coughing becomes more severe b. There are incubators that are equipped with humidity regulator. sicca, N. Mycobacterium leprae Agent of LEPROSY or HANSEN'S Disease – chronic infection of the skin, mucous membranes, and nerve tissues CAN'T GROW in artificially prepared media. penneri Most frequently isolated human pathogen; assoc. ∞ SECTION 5 ∞ BACTERIAL MORPHOLOGY Most medically significant clinical bacterial isolate usually ranges in size from 0.25μm to 1μm in width and 1 to 3μm in length which requires microscopy for visualization. aprophilus H. –anaerobic bacteria which chiefly found as a normal flora in the gastrointestinal tract (GIT) Slim, slender, threadlike bacillus, sometimes arranged in serpentine cord (crawling snake) pattern in stained smears E.g. Mycobacterium causative agent TUBERCULOSIS tuberculosis – of human Branching or filamentous bacilli E.g. Actinomyces spp. Also has glycolipids & glycoproteins PRESENT Free Ribosomes Ribosomes in the Endoplasmic reticulum Cytoplasmic/Plasma Membrane Mitochondria 70S (consists of 50S & 30S subunits) 80S (consists of 60S & 40S subunits) ∞ SECTION 4 ∞ BACTERIAL CYTOLOGY This section includes basics of bacterial cell structure and the function of these structures, as well as a brief discussion on the means by which bacteria metabolizes and their means of transferring genetic material from one bacterial cell to another which plays a pivotal role in mutation of these bacteria which is primarily important in the development of resistance to antimicrobial agents. lactamica, N. vulgaris INDOLE TEST ORNITHINE DECARBOXYLATI ON (ODC) CHLORAMPENIC OL SUSCEPTIBILITY - + S + - - R R S Proteus spp. He took a photo of this plate (Figure 8) and was alter able to isolate penicillin as the active inhibitor from the mould Penicillium notatum, later renamed to Penicillium chrysogenum. In 1881, Koch shifted his work to the agent of tuberculosis, Mycobacterium tuberculosis, with enhanced bacteriological techniques by recognizing that he would need a solid surface to grow microorganisms in pure culture. except Vibrio cholerae & Vibiro mimicus Bacillus spp. odoratum o The only non saccharolytic species of this genus o Characterized by fruity odor o INDOLE (-) o Yellow to green colonies on BAP F. GUIDELINES IN SELECTING THE IDEAL ANTIBIOTIC Selective Toxicity – this refers to the ability of the antimicrobial agent, such as antibiotic to exert lethal or detrimental effects on the causative agent but not on the host cells. ARYLSULFATASE TEST Key reaction for M. Sometimes resembles X, V, Y, Z or Chinese character E.g. Corynebacterium diphtheriae causative agent of DIPHTHERIA – Curved or comma shaped bacilli E.g. Vibrio spp. gastri, M. o A flourescein-labeled ANTI-HUMAN GLOBULIN REAGENT shall be added which will bind to the o Page 124 of 131 Study Guide Diagnostic Bacteriology 125 antibodies attached to the T. Freshly plowed field Haemophilus spp. Evenly spread the mixture about 2/3 area of the slide. Following incubation, measure the zone sizes to the nearest millimeter using a ruler or caliper; include the diameter of the disk in the measurement 2. Mycobacterium ulcerans INERT BACILLUS – yields negative reaction to most biochemical tests except HEAT STABLE CATALASE Found in stagnant tropical waters; also harbored in an aquatic insect's salivary glands Causes African BURULI ULCER and Australian BAIRNSDALE ULCER Page 114 of 131 Study Guide in Diagnostic Bacteriology Page 115 of 131 9. The first letter of the family name (similar to a human "clan") is capitalized and has a suffix –aceae (e.g. Enterobacteriaceae). Rim the plate with the swab to pick up any excess liquid. kansasii = POSITIVE (+) as quickly as 6 hours Important test to differentiate M. E.g. Streptococcus spp. The first microscope which is already available in the early 1600s was invented by an unknown individual. Consistency of fecal sample (formed, soft, watery) Presence of blood, mucus, or puss Volume of the specimen especially for bodily fluids such as blood, urine, synovial fluid, CSF, etc. lwoffi DIFFERENTATION A. A graphical representation of the phases of bacterial growth is called as BACTERIAL GROWTH CURVE which is illustrated by Figure 7-1. The said compound was then sold worldwide as Salvarsan in 1910. FACULTATIVE ANAEROBE – bacteria that have the ability to grow, line, & survive in small concentration of oxygen environment Staphylococcus spp., Streptococcus spp., Family Enterobacteriaceae MICROAEROPHILIC – bacteria that prefers small concentration of oxygen environment approximately 2%-10% Campylobacter spp., Helicobacter spp., Arcobater spp., and some Streptococcus spp. NITRATE REDUCTION – test to detect nitrosoreductase enzyme production which reduces Nitrate to Nitrite FILTER PAPER impregnated with $NaNO_3$ = BLUE COLOR (+) Broth (incolulated with the organism then added with HCl, Sulfanilamide, and N-1-naphthylethylenediamine = PINK to RED COLOR (+) MTB = POSITIVE (+) Niacin Test (M. Actinomycetes spp. Characterized by chronic and recurring fever with weight loss and anorexia o Undulant fever o Malta fever o Gibraltar fever o Mediterranean fever o Bang's Disease GROWTH IN MEDIA CONTAINING Common Species Brucella abortus Brucella mellitensis Brucella suis Brucella canis Animal Reservoir Cattle Goat Pig Dog UREASE Test (positive in) + + + + H_2S + - Thionine (20μg) + + + Basic Fuchsin (20μg) + + - Thionin Blue (2mg/mL) + + - IDEAL SPECIMEN for ISOLATION: 1st two weeks of fever – blood culture o Ideal Culture Media for isolation Castaneda Bottle (obsolete). 3. CYTOPLASMIC/PLASMA MEMBRANE 5. The two major divisions or domains within this kingdom are the true bacteria (EUBACTERIA) and archaea (ARCHAEBACTERIA). xylosoxidans Gram – short bacilli/coccobacilli Motile Aerobic Non-fermentative Oxidase (+) Non-lactose fermenter ONPG (-) Gram – short bacilli/coccobacilli Motile Aerobic Non-fermentative Oxidase (+) Page 97 of 131 Non-lactose fermenter ONPG (-) Please see separate table for differentiation Study Guide in Diagnostic Bacteriology Page 98 of 131 GENUS PSEUDOMONAS in FOCUS! Pseudomonas aeruginosa Important opportunistic pathogen and an important cause of nosocomial infection Causes the following infections: o Severe wound infections in burn patients o UTI & Nosocomial Pneumonia o Septicemia in immunosuppresed patients and infants o Chronic Lung Infections in Cystic Fibrosis patients o Septic arthritis in intravenous drug abusers o Destructive eye infection (keratitis & corneal ulcers) in contact lens wearers o Swimmer's ear – especially in athletes o Jacuzzi/Hot Tub Syndrome – SHANGHAI FEVER Pathogenic Determinants: o Pili/Fimbriae o Alginate o Elastase o Collagenase o Protease o Exotoxin A o Hemolysin o Endotoxin Important Laboratory Tests o Large, mucoid, spreading colonies o CETRIMIDE Agar – transparent selective culture media that aids in pigment production visualization o OXIDASE + to differentiate it from Family Enterobacteriaceae & other enterics o Oxidative Dextrose (Glucose) but NOT Maltose o Fermentative TSI Reaction: K/K o Growth @ 420C – to differentiate it from the rest of the pseudomonads o Acetamide Utilization + (blue slant) after 7 days of standard incubation DIFFERENTIATION OF PSEUDOMONAS SPECIES P. Mycobacterium bovis Causes TB in cattle. Until now, it is called KirbyBauer Method. INFECTION – refers to the entry, invasion and multiplication of pathogens in or on to the host body system which results to subsequent tissue injury and progress to overt disease TYPES OF INFECTION: Based on Source of Pathogen Endogenous Infection – infection arising from colonizing flora Exogenous Infection – infection arising from invading pathogen from the external environment Based on Clinical Onset of Signs and Symptoms Acute Infection – rapid/sudden onset of signs and symptoms which are usually severe to fatal that may lead to death Chronic Infection - gradual onset of signs and symptoms that are usually mild to moderate that may progress to long standing infection Based on Etiologic/Causative Agent Nosocomial Infection – infection acquired during hospitalization Zoonotic Infection (Zoonosis) – is an animal disease which can spread to humans; animal acquired infection Based on Clinical Manifestation Subclinical/Asymptomatic/Nonapparent – no obvious appearance of signs and symptoms and the person is unaware of the infection Clinical/Symptomatic/Apparent – associated with presence of overt signs and symptoms of the disease DISEASE – an altered health state in an infected host. If there is a lot of content to wade through, consider reviewing your notes before and after class, to keep the information fresh in your mind.Also, if you're studying for a test, make sure to have any notes in front of you about what subjects and topics are expected to be on the test. The amount of CO_2 generated, if exceeds the threshold set, will be detected as a positive growth. Table 9 summarizes the important cocci arrangements and examples of medically important bacteria that exhibit the morphology. Phenol/Phenolic Compounds/Bisphenols MOA: plasma membrane destruction and enzyme denaturation ∞ SECTION 10 ∞ ANTIBIOTIC SUCEPTIBILITY TESTING Although some organisms exhibit predictable susceptibility patterns to antimicrobial agents, it is impossible to predict, with any certainty, the effectiveness of a drug in treating infections caused by different microbes. diversus DISEASES Septicemia, wound infection, gastroenteritis Meningitis INDOLE TEST H_2S production ORNITHINE DECARBOXYLATION (ODC) - + V + - + Edwardsiella IMVC + + - Isolated from environment, many cold & warm blooded animals including reptiles, freshwater & aquarium fish, frogs, & turtles E. For each drug, indicate on the recording sheet whether the zone size is susceptible (S), intermediate (I), or resistant (R) based on the interpretation chart. scrofulaceum M. Neisseria meningitidis Common Name: MENINGOCOCCUS/MENINGOCOCCI 1. pseudotuberculosis 220C + + + MOTILITY 250C 370C + + - DISEASE/S ASSOCIATED Plague Enterocolitis Lymphadenitis FERMENTATION OF SUCROSE RHAMNOSE SORBITOL + - + + - ∞ SECTION 16 ∞ FAMILY VIBRIONACEAE GENUS: Vibrio KEY CHARACTERISTICS o gram negative (-) comma shaped, curved, straight bacilli o facultative anaerobe o oxidase positive (+) except V. Overlaying the broth with mineral oil after inoculation will enhance the recovery of anaerobes. Commonly, the genus is abbreviated with the first letter (capitalized) of the genus followed by a period and the species (e.g. S. o Positive Reaction – REACTIVE o Negative Reaction – NON-REACTIVE VENERAL DEISEASE RESEARCH LABORATORIES (VDRL) o Uses cardiolipin-lecithin-cholesterol antigen in a flocculation procedure o More sensitive than RPR, hence recommended for diagnosis and follow up of neurosyphilis o Procedure is standardized and antigen must be titrated Although NON-TREPONEMAL TESTS are useful for screening because relatively inexpensive and simple to perform, biological false positives may occur because cardiolipin is present in human tissue. TUBERCULIN SKIN TEST hypersensitivity test for TB uses Purified Protein Derivative (PPD) – heat killed, filtered, ammonium sulfate precipitated organism PPD injected intradermally; observed for 48 hours REDNESS/ERYHTHEMA/INDURATION – POSITIVE REACTION (2-10mm or greater) METHODS: Page 110 of 131 Study Guide in Diagnostic Bacteriology o o o o Page 111 of 131 Mantoux Volmer-Patch Von-Pirquet Tuberculin Tine Test Page 111 of 131 Study Guide in Diagnostic Bacteriology Page 112 of 131 12. Bacterial suspension is inoculated in the conjunctiva of the test animal. fortuitum-chelonae Complex Determines if the organism CAN GROW in 20% ferric citrate M. Streptococcus adjacens Streptococcus defective ** Will not grow in regular Blood Agar Plate (BAP), they need additional growth requirements ∞ SECTION 15 ∞ FAMILY NEISSERIACEAE Table 13-1 Differentiation of Family Neisseriaceae NEISSERIA MORAXELLA M/B. ADDITIONAL/SPECIAL GROWTH REQUIREMENTS – there bacteria that are very difficult to grow which we refer to as FASTIDIOUS which requires special or additional requirements to grow in culture media. Patient may recover 2-3 weeks after. Figure 12 illustrates the structure of a gram positive and gram negative bacterial cell wall. It's known to cause extraintestinal infections in humans who are immunocompromised. Antoni Van Leeuwenhoek An amateur microscope builder who considered microbiology as a hobby. PARTS EXTERNAL TO THE CELL WALL PILI (plural) or PILUS (singular) – protein projections that are thinner and shorter than flagella and are most usually found in gram negative bacteria. Page 33 of 131 Study Guide in Diagnostic Bacteriology Page 34 of 131 AEROTOLERANT ANAEROBE – bacteria that do not require oxygen but may tolerate or withstand limited exposure to oxygen Some Clostridium spp. In this section, various clinical specimens, their indications for examination, important considerations in terms of handling, processing, transport, and preserving for storage will be discussed. coli (EHEC) is negative. This type also serves as receptors for viruses that infect bacteria often referred to as BACTERIOPHAGE. This lead him to develop pure cultures of microorganisms. Colonies are usually: o After 24 hours incubation: Pinpoint o After 48 hours incubation: Rough, sticky, adherent, surrounded by greenish tinge If viewed under OIO, mature colonies exhibits four to six star-like configuration in the center of the colony resembling CROSSED CIGARS. He described the first microbe, a fungus or "hairy mould" which is believed to be Mucor (Figure 3). Kitasato W. CELL WALL 4. Patients exhibiting the characteristic "WHOOP" c. Two individuals provided solid evidence that preventing microbial contamination shall significantly reduce death rate. vulgaris – source of OX2 / OX19 o P. cholerae NON-01 o V. It is therefore important to follow, the correct preparation and dilution as prescribed by the manufacturer. simiae, M. coli but are lactose negative and non-motile Center for Disease Control (CDC) described four (4) subgroups and species based on biochemical and antigenic characteristics Table 14-2 Subgrouping of Shigella species SUBGROUP A B C D SPECIES S. This is a manual blood culture system which requires daily inversion or at least twice weekly to bathe the slide paddle with the broth culture medium, thereby allowing infrequent subcultures, hence less contamination. and Plesiomonas shigelloides Table 14-7 Differentiation of Yersinia species SPECIES Y. Hence in the 1990 Dr. Woese proposed a new phylogenic tree split into three (3) domains: the Archaea (archaebacteria), the Bacteria (eubacteria), and Eukarya (eukaryotes) (Figure 10). SEROVARITIES: Leptospira interrogans SEROVAR icterohaemorrhagiae – Weil's syndrome Leptospira interrogans SEROVAR canicola – Infectious jaundice Page 125 of 131 Study Guide Diagnostic Bacteriology 126 Leptospira interrogans SEROVAR autumnalis – Fort Bragg/Pretibial Fever Leptospira interrogans SEROVAR hebdomadis – Seven Day Fever Leptospira interrogans SEROVAR grippotyphosa – Marsch Fever Leptospira interrogans SEROVAR mitis/Pomona – Swine Herd Disease Important Laboratory tests: Culture Media of Choice o Fletcher's/Stuart's Medium or Ellinghausen-McCullough-Johnson-Harris (EMJH) Medium o Incubated in the dark for 4-6 weeks at 25-30OC. Decongested/Digested – to dissolve the thick mucus/mucin that might be trapping the bacteria in the sample. Key Terminologies Antibiotic – substance produced by bacteria and fungi that inhibits the growth of microorganisms Bacteriostatic – agents that have inhibitory effect on bacterial growth but do not necessarily kill the organisms Bactericidal – aganets that are able to exert lethal effects on microorganisms. Inhibitors: Brilliant Green, Bile salts, Citrate Differential: INDICATORS: pH indicator: Neutral Red H_2S Indicator: Ferric ammonium citrate Sulfur Source: Sodium thiosulfate fermentation CHO Incorporated: Lactose ACID pH RLF: 18-24 hrs Lactose fermenter LLF: 36-72 hrs LACTOSE DARK PINK H_2S + with blackening H_2S – w/o blackening COLORLE SS Non-Lactose fermenter H_2S + with blackening H_2S - w/o blackening Page 41 of 131 Study Guide in Diagnostic Bacteriology Page 42 of 131 HEKTOEN ENTERIC (HEA) AGAR Original Color – dark green Selective: For: Gram negative enteric bacilli Inhibitors: bile salts, citrate Differential: INDICATORS: pH indicator Bromthymol Blue (BTB) H_2S Indicator: ferric ammonium citrate Sulfur Source: sodium thiosulfate fermentation CHO Incorporated: Lactose ACID pH H_2S + with RLF: 18-24 hrs ORANGE Lactose fermenter LLF: 36-72 hrs LACTOSE BLUE/GRE EN Non-Lactose fermenter blackening H_2S – w/o blackening H_2S + with blackening H_2S - w/o blackening BISMUTH SULFITE (BSA) AGAR Selective: For: Salmonella spp. Intermediate – represents an organism that may require a higher dose of antibiotic for a longer period of time to be inhibited. Page 48 of 131 Study Guide in Diagnostic Bacteriology Page 49 of 131 Minimum Inhibitory Concentration (MIC) – the lowest concentration of an antimicrobial agent that visibly inhibits the growth of microorganisms. have a gram positive cell wall structure however because 60% of the cell wall is made of hydrophobic lipids mainly mycolic acid, it affects its permeability this makes it difficult to gram stain. Some species are capable of developing resistance to commonly used therapeutic agents. through g. Immunofluorescent Assays 3. al., 2001). arizonae ALL Shigella except S. Usually associated with destructive periodontitis often following dental manipulations. CITRATE UTILIZATION TEST 9. sicca + + + + + + - N. All measurements are made with the unaided eye while viewing the back of the petri dish. hydrophilia – most common human isolate Page 95 of 131 Study Guide in Diagnostic Bacteriology Page 96 of 131 Often associated with gastroenteritis Produces two (2) types of enterotoxin Heat labile enterotoxin Heat stable cytotoxic enterotoxin Also produces protease, lipase, nuclease – pathogenic determinants ∞ SECTION 17 ∞ FAMILY CAMPYLOBACTERIACEAE GENUS: Campylobacter Common species: Campylobacter jejuni Causes gastroenteritis and diarrhea OPTIMUM SELECTIVE CULTURE MEDIA: Modified Skirrow's Medium – Columbia Blood Agar base + Antibiotics (vancomycin, trimethoprim, & Polymyxin B) Key Characteristics: Gram negative comma, curved, S-shaped, seagull wing shaped bacilli Microaerophilic (5% O_2) Capnophilic Motile (one polar flagellum) Oxidase (+) Non-fermentative Na Hippurate Hydrolysis (+) Urease (-) OPTIMUM TEMPERATURE FOR GROWTH 15OC 25OC 42OC + ** Campylobacter fetus subspecies fetus – agent of infective abortion in cattle and sheep. (Salm. Monoclonal Antibodies EXAMINATION OF MOTILITY 1. myxofaciens Rare human isolate P. Amie's Charcoal Transport Medium – this is an improved semi-solid transport medium which contains charcoal and 0.3% w/v NaCl for the optimum recovery of Neisseria gonorrhoeae. o OTHER STAINS FOR ACID FAST ORGANISMS PAPPENHEIM STAIN Mycobacterium tuberculosis – RED Mycobacterium lacticola (smegmatis) – BLUE BAUMGARTEN STAIN – uses ROSOLIC A CID as a decolorizer Mycobacterium tuberculosis – BLUE Mycobacterium leprae – RED Page 26 of 131 Study Guide in Diagnostic Bacteriology Page 27 of 131 FLUOROCHROME STAIN – uses fluorescent dyes such as AURAMINE or RHODAMINE or combination of both. chelonae – NEGATIVE (-) 9. enterocolitica. His theory provided framework for Louis Pasteur's experiments in disproving the theory on spontaneous generation (Brock, 1989). It also grows in MAC & EMB FAMILY AEROMONADACEAE GENUS: Aeromonas KEY CHARACTERISTICS: o Gram negative (-) straight bacilli o Naturally found in fresh and sea water and are known to cause disease in cold blooded animals o May also be found in drains, sink traps, distilled & tap water o Most common human infection includes cellulitis, wound infections, and gastroenteritis o Facultative anaerobe o Fermentative o Indole positive (+) o Oxidase positive (+) o Motile o RESISTANT to vibriostatic agent O/129 (2,4 diamino-6,7-diisopropylpteridine) o NON-INOSITOL FERMENTER o A. The staining results aid in the selection of appropriate culture media to allow the growth of the pathogen. LYMPHOCYTOSIS 2. mucosa N. Spirillum minor/minus Gram negative, helical, and strictly aerobic organism MOT: Rat Bite – SODOKU 3. Ulcers are sterile or contain few spirochetes, if any. lactamica + + - N. Note that there are different charts for different organisms. whenever Staphylococcus and Streptococcus are discussed). Gilbert, F. GROSS/MACROSCOPIC EXAMINATION OF CLINICAL SPECIMENS After ensuring that general considerations mentioned above are considered, clinical specimens have to be observed based on its gross appearance which may provide useful information for the suitability of the specimen for the optimum recovery of the bacterial pathogen. Page 6 of 131 Study Guide in Diagnostic Bacteriology Page 7 of 131 Fannie Hesse, the wife of one of Koch's laboratory assistants, suggested the use of agar, a gelatinous material from seaweeds, as a solidifying agent for culture media. His later publication Microscopium in 1678 detailed his microscopic techniques. indologenes Gram – kidney or coffee bean shaped diplococci EXCEPT: Obligate Aerobe Gram – kidney or coffee bean shaped diplococci Obligate Aerobe Capnophilic Capnophilic Non motile Pyogenic CATALASE + OXIDASE + Non motile Not Pyogenic CATALASE + OXIDASE + **DNAse Test Gram – short bacilli (coccobacilli) Gram – short bacilli (coccobacilli) Gram – bacilli Obligate Aerobe Not Necessarily Capnophilic Non motile Pyogenic CATALASE + OXIDASE + Obligate Aerobe Obligate Aerobe Capnophilic Capnophilic Non motile Pyogenic CATALASE + OXIDASE - Non motile Pyogenic CATALAS E OXIDASE + **Butyrate esterase Test ** Non Fermenter of CHO Page 77 of 131 Indole Test Study Guide in Diagnostic Bacteriology Page 78 of 131 DISEASES CAUSED BY THE PATHOGENIC SPECIES OF NEISSERIA 1. for individual disk placement with forceps. INHIBITORS in CULTURE MEDIA INHIBITORS for GRAM POSITIVE BACTERIA DYES – crystal violet, eosin, methylene blue, brilliant green, etc. Example is bleach (sodium hypochlorite) that is easily inactivated by organic material. May last for 4 to 8 weeks b. as well as Legionella micdadei and coccidian parasites such as Cryptosporidium parvum, Cysclospora spp., and Isospora belli. DECLINE/DEATH PHASE Due to unfavorable environment for growth, bacterial cell division decreases while cell death becomes more accelerated. Theory of Spontaneous Generation For 200 years, scientist strongly believed that microorganisms spontaneously arose from non-living material which was due to their observation that a broth left in an open container becomes cloudy after 24 hours. TRANSPORT CULTURE MEDIA – a primary isolation culture media which maintains the viability of bacteria without allowing rapid multiplication if there is an anticipated delay in bringing the specimen collected bedside or remotely to the laboratory. SCHICK'S TEST – susceptibility test for Corynebacterium diphtheriae ARM (PORTION) + DIPHTHERIA TOXIN (+) redness/erythema GENUS LACTOBACILLUS Common species: Lactobacillus acidophilus Normal flora of the mouth, GIT, vaginal canal Usually nonpathogenic and has little clinical significance (rare cause of bacteremia and pneumonia Maintains normal HOMEOSTATIC ENVIRONMENT by producing large amount of lactic acid High acidic environment (pH 3 to 4) can't be tolerated by most bacteria Used as a PROBIOTIC AGENT in making YOGURTS Important Laboratory Tests: o GRAM STAIN – Gram (+) pleomorphic bacillus o Non-Motile o CATALASE (-) o H_2S (+) o Hydrolyzes esculin GENUS LISTERIA Gram positive(+) non spore forming bacilli Found in the environment such as soil, water, sewage, and decaying vegetation, and in the feces of humans, swine, and poultry Primarily an animal pathogen but humans may acquire the infection by direct contact with infected animals or animal products Common species Listeria monocytogenes o Most significant human isolate o Major source of infection is contaminated cabbage, raw fruit, pasteurized and pasteurized dairy products Page 120 of 131 Study Guide Diagnostic Bacteriology o o o o o o 121

Causes LISTERIOSIS – outbreak noted in 1988 thru ingestion of contaminated MEXICAN CHEESE Highest risk includes pregnant women, their fetuses, and newborns (may cause spontaneous abortion or still birth Most common manifestation of the disease is MENINGITIS Classic intracellular organism which can resist phagocytosis Culture Media of Choice: McBRIDE Medium IMPORTANT LAB TESTS: BIOCHEMICAL TESTS: CATALASE (+) H2S Production (-) MOTILE Narrow band of β-hemolysis in BAP Demonstration of Motility Characteristics: On wet prep/hanging drop technique: TUMBLING On Semi-Solid Medium (SIM): UMBRELLA LIKE GROWTH 2-5 mm below the agar surface; sometimes described as INVERTED CHRISTMAS TREE (best observed at 25oC) OCULAR TEST OF ANTON GENUS ERYSIPELOTHRIX Gram positive(+) non spore forming bacilli Commom species: Erysipelothrix (red skin, thread) rhusiopathiae (RED disease) o Veterinary infection and an occupational hazard for those handling meat, poultry, fish, and rabbits o Carried asymptomatically or causes infection in fish, cattle, horses, turkeys, and pigs o Infection occurs as cutaneous inflammation of the hands or fingers – ERYSIPELOID o BIOCHEMICAL TESTS: CATALASE (-) H2S Production (+) NON-MOTILE α/γ-hemolysis in BAP TEST TUBE BRUSH LIKE GROWTH in Gelatin Medium (12% GELATIN) but none in SIM GENUS KURTHIA Gram positive(+) non spore forming bacilli Found in soil and is an opportunistic pathogen Common species: Kurthia bessonnii MOTILE OBLIGATE AEROBE CATALASE (+) and OXIDASE (-) GENUS ROTHIA Gram positive(+) non spore forming bacilli Normal flora of the human mouth Rare cause of abscesses and endocarditis Page 121 of 131 Study Guide Diagnostic Bacteriology 122 ∞ SECTION 24 ∞ SPIROCHETES helically coiled and motile through PERIPLASMIC FLAGELLA cam move even in viscous liquid using locomotion, rotation, and flexion although has the characteristics of a gram negative (-) cell wall, they do not Gram stain very well staining is not useful for identification Includes Genus BORRELIA, TREPONEMA, and LEPTOSPIRA GENUS BORRELIA Loosely twisted resembling a stretched spiral MOT: tick/lice bite Microaerophilic Stains well with Giemsa or Wright stain = BLUE in color using either stain IMPORTANT MEMBERS: AGENTS OF RELAPSING FEVER 1. BACTEC ® - first automated growth detection system for blood cultures which detects radiolabeled carbon (14C). Ultra High Temperature (UHT): 72OC – 110OC – 72oC for 5 seconds 3. Gonorrhoeae Adult Male Adult Female Young Female (Children) – usually indicative of sexual abuse Newborns – Opthalmia neonatorum b. COLI INACTIVE Does not produce gas during fermentation Lactose negative and non-motile Previously known Alkalescens dispar Klebsiella IMVC - - + + Short, non-motile bacilli Slow urease producer INDOLE: Negative except K. S or C shaped bacilli, resembles seagull wing sometimes E.g. Campylobacter spp. If it’s a swab, is it already dry? flavescens B/M. This staining technique also includes a decolorization step which is the most critical step in the process. – cell wall is high in lipid which enables them to become resistant to most environmental stress such as desiccation Biofilm forming bacteria – certain bacteria can aggregate into communities of bacteria which makes then resistant to chemical and physical means of destruction Prions – naked pieces of proteins, similar to viruses but without the nucleic acid, that is most resistant to the action of heat, radiation, and chemicals. Creating a study schedule with the exam date in mind helps you pace your studying, and gives you time to ask questions before the test.Start or Join a Study GroupIf you work well in a group setting, consider starting or joining a study group. Also causes pneumonia & septicemia Same as above but UTI is usually nosocomial UTI/Wound Infection P. SPIRALS are very difficult to stain using gram staining however stainable spirals are usually gram negative Mycobacterium and Nocardia spp. Key Terminologies Sterilization – complete destruction of all microbial life, including bacterial spores Disinfection – selective elimination of a defined scope of microorganisms, including some spores Disinfectant – chemical agents applied to inanimate objects Antiseptic – substance applied to the skin to eliminate or reduce the number of bacteria present in a particular area. subflava ACINETOBACTER : 17 genospecies based on DNA hybridization Most prevalent human pathogen: A. sonnei # OF SUBTYPES/ SEROTYPES SYNONYM 1-12 1-8 1-18 1 Shiga’s Bacillus Strong’s Bacillus New Castle Manchester Bacillus Duval’s Bacillus Causes Shigellosis/Bacillary Dysentery o Early Stage Incubation period for 1-7 days Fever, abdominal cramping and pain, diarrhea o Diarrheic Stage Watery diarrhea for 3 days Page 91 of 131 Study Guide in Diagnostic Bacteriology Page 92 of 131 Dysenteric Phase Frequent stools with blood, puss, and mucus Bacteria had invaded the lining of the GIT Among the four (4) species, the most seriously pathogenic is S. This differentiates them from the K-E-S-H group. Hold the plate a few inches above a black, nonreflecting surface illuminated with reflected light 4. Patient exhibits sunken eyes, washerwoman’s hands, and pallor Page 94 of 131 Study Guide in Diagnostic Bacteriology Page 95 of 131 V. K or Envelope Antigen Associated with the capsular polysaccharide surrounding the cell wall Covers the “O” Antigen; hence inhibiting the type specific O antisera HEAT LABILE: After K Antigen typing, boil the sample for 30 mins and then retest for O antigen Klebsiella, Salmonella, and E. alginolyticus SF - yellow V. mirabilis – source of OXK (Kingsbury Strain) Providencia IMVC + + - + Lactose negative, DEAMINASE positive, H2S negative, motile but no swarming, ferments mannose and citrate positive LDC, ODC, & ADH negative (-) Page 92 of 131 Study Guide in Diagnostic Bacteriology Page 93 of 131 Table 14-5 Differentiation of Providencia species SPECIES FERMENTATION OF: ADONITOL TREHALOSE UREASE TEST DISEASES P. ANTIBIOTICS – Vancomycin INHIBITORS for GRAM NEGATIVE BACTERIA DYES – basic fuchsin and thionine for Brucella abortus CHEMICALS – potassium tellurite, sodium azide, phenylethy alcohol ANTIBIOTICS – Colistin, Nalidixic Acid, Trimethoprim (Proteus) INHIBITORS for FUNGI ANTIBIOTICS – Nystatin, Anisomycin, Ampothericin B. o MAIN COMPOSITION: Calcium Dipicolinate or Calcium-Dipicolinic Acid Complex Page 17 of 131 Study Guide in Diagnostic Bacteriology o o Page 18 of 131 MEDICALLY IMPORTANT SPORE-FORMING BACTERIA BACILLUS – forms spores or sporulates aerobically CLOSTRIDIUM – form spores or sporulates anaerobically Bacterial spores or endospores are usually round or oval, centrally or subterminally located except Clostridium tetani which has a TERMINALLY LOCATED SPORES which give it a characteristic appearance on stained smears being lollipop, ping-pong, tennis racquet, tack-head, and drumstick. Schandinn, Erich Hoffman Alice Evans Joseph McDade, Charles Shepard Stanley Prusiner Table 2: Key Events in the Development of Medical Microbiology. AUTOCLAVE – operates based on the principle of steam under pressure effective indication: STERILIZATION: 121oC for 15lbs/in2 for 15 minutes DECONTAMINATION: 135oC for 30lbs/in2 for 30 minutes biological indicator: Bacillus stearothermophilus b. flavescens M. This technique is still widely and routinely used in most microbiology laboratories which we call as Gram stain (Brock, 1989). Stains used in the microbiology section of the laboratory contain acidic and basic components that adhere to specific cellular components. SYMPTOMS – refers to any subjective evidence of disease. aegyptius – pink eye conjunctivitis H. The genus followed by the word species (e.g. Enterococcus species) may be used to denote the entire genus as a whole. Infection spreads to the cornea. Susceptible or Sensitive – is an interpretative category that indicates an organism that is inhbited by the recommended dose or achievable level of an antimicrobial agent Resistant - is an interpretative category that indicates an organism that is NOT inhbited by the recommended dose or achievable level of an antimicrobial agent. bacteria) contains 16s ribosomal RNA that are more closely related to eukaryotes than prokaryotes. Growth up to the edge of the disk can be reported as a zone of 0 mm. Vibrio spp. Quaternary Ammonium Compounds (QUATS) MOA: enzyme inhibition, protein denaturation, and disruption of plasma membrane a. Peracetic Acid – for surgical instruments 2. POLYMERASE CHAIN REACTION (PCR) ANIMAL INOCULATION TECHNIQUE ANTON’S OCULAR TEST – pathogenicity or toxigenicity test for Listeria monocytogenes strains which uses rabbits as test animal. Page 50 of 131 Study Guide in Diagnostic Bacteriology Page 51 of 131 6. Helicobacter spp. Unwashed stockings Rancid potato Serratia odorifera Corn tortilla/Fruity Pseudomonas aeruginosa Acinetobacter spp. Heavy Metals MOA: denaturation of enzymes and other essential bacterial proteins Mercury (Hg): active ingredient or merthiolate but this is already banned in the market due to its known toxicity Copper (Cu): CuSO4 crystals are used as algaecide in swimming pools and aquarium Silver (Ag): 1% AgNO3 – used as prophylactic agent in Crede’s Prophylaxis in suspected cases of Ophthalmia neonatorum 4. LYMPHOCYTOSIS 3. Use of Semi-Solid Culture Medium *** NOTE Listeria monocytogenes is known for its characteristic INVERTED XMAS TREE or OPEN UMBRELLA pattern in SULFIDE INDOLE MOTILITY (SIM) Medium 3. The swab should not be dripping wet. malmoense Page 107 of 131 M. Additionally, Vibrio cholerae, Salmonella and Shigella, and Yersinia pestis can also be optimally recovered using this medium. MAXIMUM STATIONARY/ KEY NOTES Bacteria are still adjusting to the new environment hence there is no cell division that occurs in this phase but they are activity synthesizing DNA & proteins that are necessary for cell division Phase where there is a sudden increase of bacteria because of rapid generation or doubling time. Flash: 72OC for 15 seconds c. xylosoxidans + + + - ∞ SECTION 19 ∞ FAMILY CAPNOCYTOPHAGACEAE GENUS: Flavobacterium Key Characteristics: DO NOT GROW in MAC Oxidase (+) Non-motile Most species produce yellow colored colonies in BAP Nitrate Reduction (-) Common species: F. His technique did not only achieve this, but he was able to differentiate bacteria by using alcohol as a decolorizing agent. pallidum antigens are mixed with patient’s serum o Agglutination is the positive reaction if the patient’s serum has antibodies to T. Low grade fever d. coli (EPEC) o Non invasive and non-toxigenic; associated with limited number of serogroups o Causes syndrome that is nosocomial and seen in infants and newborns o Causative agent of diarrhea outbreak in hospital nurseries or INFANTILE DIARRHEA o STOOL: watery with mucus but without blood EnteroINVASIVE E. To differentiate from C. (4) E.g. Micrococcus tetragena Coccus is divided by three (3) planes of SARCINA/E – coccus in cubical division in a regular pattern. tetani drumstick, tack head, lollipop, tennis racket bacillus o No spores – box or car shaped OBLIGATE/STRICT ANAEROBE/CATALASE Page 116 of 131 Study Guide in Diagnostic Bacteriology Common species Clostridium perfringens Clostridium botulinum Clostridium tetani Clostridium difficile Page 117 of 131 MOTILITY Growth in BAP Glucose Fermentati on Lactose Fermentation LECITHINASE Production LIPASE Production Reverse CAMP Test Stormy Fermentation of Milk - β– hemolysis (double zone) + + + - + + + β– hemolysis + - - + - - + β– hemolysis - - - - - - + β– hemolysis + - - - - - DISEASES/PATHOLOGY IN FOCUS: 1. Disseminated Gonococcal Infection (DGI) Endocarditis Gonococcal Arthritis c. Page 2 of 131 Study Guide in Diagnostic Bacteriology Page 3 of 131 Figure 1: Normal Flora of a Healthy Human Person Figure 2: Type of Pathogenic Biological Entities Page 3 of 131 Study Guide in Diagnostic Bacteriology Page 4 of 131 ∞SECTION 2∞ DEVELOPMENTAL NOTES IN MEDICAL MICROBIOLOGY INFECTIOUS DISEASES IN HISTORY During the ancient times before the birth of Christ, it is the belief of humans that disease is caused by evil spirits, invisible animals, or punishment sent by God. Page 7 of 131 Study Guide in Diagnostic Bacteriology Page 8 of 131 Hans Christian Gram in 1884 developed a simple differential staining technique because of his want to visualize bacteria in tissues (Figure 7). GRAM STAINING 2. bovis (POSITIV E +) from M. COMMOM/SOMATIC/ORDINARY PILI – usually shorter, numerous, sticky hair-like appendages that are primarily used for adherence to one another, host cells, and environment surfaces. Dr. Woese found that many species of prokaryotes (incl. GROWTH ON PRIMARY ISOLATION MEDIA: 3. Hence the field of medical microbiology continues present challenges which are perceived by modern medical technologists with sounds diagnostic knowledge and impeccable laboratory techniques and methods which have also evolved with sophistication. – kidney or coffee bean shaped diplococci except: Neisseria weaveri and Neisseria elongata Streptococcus pneumoniae flame/lancet shaped diplococcic – Coccus is divided by one (1) plane of COCCI IN CHAINS division but continuously dividing it. faecalis Oxidase + Glucose/Xylose Oxidation Nitrate Reduction Nitrite Reduction to N2 gas + A. Based on these findings, an arthropod vector (tick) was suspected Because of its very minute size, none of the patients could remember of having bitten by a tick prior to developing the rash until a tick, Ixodes dammini, was isolated from a patient’s rash. kingae K. Methyl Red (MR) – used for the detection of bacterial pathogen that metabolize glucose using the MIXED ACID PATHWAY Voges-Proskauer (VP) used for the detection of bacterial pathogen that metabolizes glucose using the BUTYLENE GLYCOL PATHWAY Simmon Citrate Agar (SCA) – used for the detection of bacterial pathogen that can utilize citrate as a sole source of carbon Triple Sugar Iron Agar (TSI) – used for the determination of bacterial pathogen’s ability to ferment glucose, sucrose, or lactose. Figure 4-3 illustrates flagellar arrangements in bacteria. Klebsiella – large sized mucoid dark violet colonies with or without dark center Page 40 of 131 Study Guide in Diagnostic Bacteriology Page 41 of 131 MC CONKEY (MAC) AGAR Original Color – light pink Selective: For: Gram negative enteric bacilli Inhibitors: Crystal Violet, bile salts, citrate Differential: INDICATORS: Neutral Red CHO Incorporated: Lactose Lactose fermenter LACTOSE Non-Lactose fermenter fermentation ACID pH RLF: 18-24 hrs DARK PINK LLF: 36-72 hrs COLORLESS SALMONELLA-SHIGELLA (SSA) AGAR Original Color – light orange Selective: For: Salmonella and Shiella spp. scrofulaceum (-) TWEEN 80 LIPASE TWEEN 80 (polyoxoethylene sorbitan mono-oleate) OLEIC ACID = Red /Pink (+) 7. He really did not bother to know the origin of these microorganisms and their link to human disease. Water Soluble – it must be water soluble to be readily absorbed into the blood. Gardnerella vaginalis (formerly called Haemophilus vaginalis & Corynebacterium vaginale) Gram variable bacilli or coccobacilli Normal flora of the anorectal area of both sexes and also of children, but definitely a normal vaginal flora of women of reproductive age. Some studies linking it to peptic or duodenal carcinoma. Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location, because some of the drug begins to diffuse immediately upon contact with the agar. fortuitum-chelonei complex M. Although he developed many vaccines such as the ones for anthrax and cholera, he’s most famous success is his development of rabies vaccine which led the French Government to establish the Pasteur Institute in Paris in 1888. Natural inhabitant of mucus secreting cells of the stomach OPTIMUM SELECTIVE CULTURE MEDIA: Campy-CVA – Brucella Agar base + Antibiotics (cefoperazone, vancomycin, & Amphotericin B) Key Characteristics: Gram negative comma, curved, S-shaped, seagull wing shaped bacilli Microaerophilic (5% O2) Capnophilic Motile (four to six polar flagella) Oxidase (+) Non-fermentative Na Hippurate Hydrolysis (-) Urease (+) – very strong urease activity Page 96 of 131 Study Guide in Diagnostic Bacteriology Page 97 of 131 OPTIMUM TEMPERATURE FOR GROWTH 15OC 25OC 37OC + GENUS: Arcobacter Common species: Arcobacter butzleri Causes gastroenteritis OPTIMUM SELECTIVE CULTURE MEDIA: Campy-CVA – Brucella Agar base + Antibiotics (cefoperazone, vancomycin, & Amphotericin B) Key Characteristics: Gram negative comma, curved, S-shaped, seagull wing shaped bacilli Microaerophilic (5% O2) Capnophilic Motile Oxidase (+) Non-fermentative Na Hippurate Hydrolysis (-) Urease (-) OPTIMUM TEMPERATURE FOR GROWTH 15OC 25OC 37 or 42OC + + - ∞ SECTION 18 ∞ FAMILY PSEUDOMONADACEAE PSEUDOMONAS P. Example is endoscopic instruments which can’t be autoclaved. ALL are: - Voges Proskauer (VP) positive (+) - produce large amount of gas in the TSI butt portion Pantoea IMVC - - + + Representative organism is Pantoea agglomerans (formerly Enterobacter agglomerans) Nitrate Reduction negative (-) Yellow pigment producer LDC, ODC, ADH negative (-). Usually seen in individuals who are in close contact such as camps, schools, or dormitories Also associated with pharyngitis, rhinitis , and ear infections Page 126 of 131 Study Guide Diagnostic Bacteriology 127 Complications: hemolytic anemia, skin rash, meningitis, and a temporary arthritis Mycoplasma hominis/Ureaplasma urealyticum A genital mycoplasmas Can colonize adults asymptomatically and are also cause of non-gonococcal urethritis in males Mycoplasma hominis also found as an agent of salpingitis and postpartal fever in females IMPORTANT LABORATORY TESTS o Culture Media of Choice – incubated either anaerobically or capnophilic cultivation E Agar Mycoplasma pneumoniae colonies have a FRIED EGG Shepard’s Medium appearance – dense center with transluscent A7B Medium periphery Edward Hayflick Agar o BIOCHEMICAL TESTS: Glucose Common species Arginine Urease Fermentation M. Ignaz P. diphtheria biotype intermedius C. The defeat of the Athenian Empire in 430 B.C. was believed to be due to a terrible plague (Plague of Athens) which wiped out one-third of the city (Cartwright & Biddiss, 1972). (NHACEK Group) and Streptococcus pneumoniae TEMPERATURE REQUIREMENT – most pathogenic bacteria would grow at temperature between 35-370C, hence incubator in the laboratory is usually set and maintained within this temperature range for routine isolation of pathogens. Page 38 of 131 Study Guide in Diagnostic Bacteriology Page 39 of 131 Lim Broth (Todd-Hewitt Broth with Colistin & Nalidixic Acid) – used to favorably isolate Streptococcus agalactiae (Group B Streptococci) especially from vaginal swab with dominating normal flora. LAG PHASE 2. cholerae 01 o Classical o El Tor V. Record the zone size on the recording sheet. PILI – most important pathogenic determinant T1 T2 – colony type of strains that are ABLE to retain their pili once grown in culture media T3 T4 T5 - colony type of strains that are NOT ABLE to retain their pili once grown in culture media 2. putida P. K Antigen – associated with the capsule 4. Pasteur’s work paved the way for aseptic technique to prevent contamination and industries benefited from his work by rendering food and food products sterile which lead to the concept of pasteurization in beers and wines. NIACIN TEST – All Mycobacterium species produce NIACIN and most posses another enzyme to convert free niacin to niacin ribonucleotide Important test for identifying MTB because it lacks the enzyme NIACIN + Cyanogen bromide (toxic reagent) = yellow colored solution MTB = POSITIVE (+) Niacin Test 4. Germany, his home nation, became the world leader in drug production. stutzeri Gram – short bacilli/coccobacilli Motile Aerobic Non-fermentative Oxidase (+) Non-lactose fermenter ONPG (-) XANTHOMONAS (NEW) STENOTROPHOMAS S. The number of generation per hour is called growth rate constant. The two major types of bacterial cell wall that he was able to identify were gram positive and gram negative. tarda – most important human pathogen which is the source of GI infection in tropics & subtropics Resembles E. mediasiatica - severe MODES of TRANSMISSION to humans: Tick bite Direct cutaneous inoculation from an infected animal Conjunctival inoculation Inhalation of infectious aerosols Ingestion of undercooked infected animal meat or contaminated water DIFFERENT TYPES OF TULAREMIA TYPES OF TULAREMIA INFECTION Ulceroglandular Glandular Oculoglandular Oropharyngeal Systemic (typhoidal) tularemia Pneumonic tularemia CLINICAL MANIFESTATIONS Common; ulcer & lymphadenopathy; rarely fatal Common; lymphadenopathy; rarely fatal Conjunctivitis and lymphadenopathy Ulceration in the oropharynx Acute illness with septicemia; no ulcer or lymphadenopathy Pneumonia; most serious form of tularemia IDEAL MEDIA and METHODS of IDENTIFICATION: Culture Media of Choice: o Blood-Cystine-Glucose Agar with thiamine o Modified/Buffered Charcoal Yeast Extract Agar (BCYE) o Chocolate Agar with ISOVITALEX Direct Fluorescent Antibody (DFA) Stain Antibody titers as low as 1:40 in the absence of previous disease is diagnostic and may rise up to 1:640 or greater within the 1st 3 weeks FORSHAY TEST – susceptibility test for tularemia Page 105 of 131 Study Guide in Diagnostic Bacteriology Page 106 of 131 ∞ SECTION 22 ∞ OTHER MISCELLANEOUS BACTERIA 1. pneumoniae). TWEEN 80 HYDROLYSIS TEST Key reaction for M. It is also recommended for the cultivation of beta-hemolytic streptococci, pneumococci, and other fastidious organisms. Vi Antigen – specific capsular antigen of Salmonella typhi IMMUNOLOGICAL 1. But most importantly, it inhibits or evades phagocytosis which contributes to the virulence of the bacteria. Growth in 6.5% NaCl (Salt Broth) – Halophilic Growth 9. Figure 5: The experimental set-up of Louis Pasteur which ultimately disproved the theory on spontaneous generation LINKING MICROBES TO HUMAN DISEASE The Germ Theory of Disease and Koch’s Postulates A German physician who paved the way in the better linking of microorganisms as an infectious cause of human disease. metschnikovii o nitrate reduction positive (+) o motile – “shooting star motility” o halophilic except V. Keeping a tidy desk means that you’ll have enough space to lay out all the papers and books you need, without dealing with distractions or clutter from other classes or assignments. Dick’s Test 18. This lead the way to the modern practice of antiseptic surgery, thus he was coined as the Father of Antiseptic Surgery. Adjust the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy. Some advantages and disadvantages are also emphasized. Inclusion bodies may be in the form of glycogen (carbohydrate reserves, polyphosphates (ATP reserves), and poly-β-hydroxybutyric acid (lipid reserves) o MUCH GRANULES – contains lipids (Mycobacterium tuberculosis) o VOLUTIN/BABES-ERNST BODIES/METACHROMATIC GRANULES – contains polyphosphates or inorganic phosphates (Corynebacterium diphtheriae) o BIPOLAR BODIES – prominent staining of each end of the bacilli Yersinia pestis using Methylene Blue or WAYSON stain giving it a “safety pin appearance” BACTERIAL SPORES/ENDOSPORES – complex multilayered highly refractile structure that can be found within the cytoplasm of the vegetative cell of the bacteria or in the environment when the bacterial cell has been disintegrated. Page 10 of 131 Study Guide in Diagnostic Bacteriology Page 11 of 131 ∞ SECTION 3 ∞ TAXONOMY AND CLASSIFICATION The first recorded attempts at classification indicate that Aristotle and others engaged in the practice as far back as 400 B.C. The classifications were morphological or phenotypical descriptions of plants and animals. Figure 8: Photo of the culture plate noted with inhibited growth around a mould growth by Alexander Fleming Table 1: Selected Significant Infectious Agents and Dates of Discovery PERIOD 1876 1879 1880 1882 1883 1892 MICROORGANISM Bacillus anthracis Neisseria gonorrhoeae Staphylococcus, Streptococcus, Pneumococcus Mycobacterium tuberculosis Corynebacterium diphtheria Clostridium perfringens Page 9 of 131 DISCOVERER Robert Koch Albert Neisser Louis Pasteur Robert Koch Edward Klebs, Fredrick Loeffler William Welch, George Nuttal Study Guide in Diagnostic Bacteriology 1894 1900 1903 1905 1918 1977 1982 Page 10 of 131 Yersinia pestis Coccidioides immitis Leishmania donovani Treponema pallidum Brucella abortus Legionella pneumophila Prions Emile John Yersin, S. Vaccination Edward Jenner Edward Jenner was coined as the father of immunology by validating a procedure that was undertaken by Benjamin Jesty in 1774 when he scratched himself with purulent material from a cow udder lesion into his arm and his family members. With these dyes, AFB fluoresce, absorbing UV light and emitting visible light perceived by the human eye as YELLOW, RED, or ORANGE lit bacilli depending on the dye or combination of these dyes used. oxytoca CITRATE: Positive LDC positive (+) Klebsiella pneumoniae o 90% of clinical isolate belong to this species o SYNONYM: Friedlander’s bacillus o Capsulated: mucoid colonies or tend to “string” o Normal Flora of the URTI and GI tracts of 5% of healthy individuals o Causes severe pneumonia, lung abscess, neonatal meningitis, gastroenteritis, UTI, wound infection o Most infections are nosocomial o Pneumonia is very necrotic and hemorrhagic causing currant jelly like sputum o Has been isolated in contaminated medications, IV solutions, & respiratory care equipment Klebsiella oxytoca o The only INDOLE POSITIVE Klebsiella species o Has been isolated in stool and blood cultures Klebsiella ozaenae o Associated with purulent sinus infection Klebsiella rhinoscleromatis o Causes granuloma of the nose & the oropharynx Enterobacter IMVC - - + + Motile bacilli Slow urease producer except E. Often described as “STARSHAPED” colonies in agar media CANNOT grow in MAC Oxidase (+) Catalase (+) Indole (-) Urease (-) Opportunistic pathogens, very low virulence. Transport Culture Media Cary-Blair Amie’s Charcoal Transport Medium Stuart’s Transport Medium SOLID – a culture medium which contains 1.5-3% (2-3%) agar. In the bottle, there is a 14C-labelled substrate which is then utilized or degraded by bacteria which liberates CO2 which is then measured in an ionization chamber. UREASE TEST Important test in differentiating M. Immunoglobulin A (IgA) Protease 3. Water/Liquid solutions/Antibiotics/Vaccines – usually uses a thin membrane filter of cellulose acetate with different pore size depending on the intended purpose: - 0.45-0.80µm – most bacteria, yeasts, and molds are retained but may allow passage of Pseudomonas- like organisms - 0.22 µm – used to filter Pseudomonas- like organisms; used for critical sterilization of parenteral solutions -0.01 µm – able to retain small viruses b. Specimen is properly identified with the patient with his/her information (e.g name, collection time & date, etc.) and collected in sufficient amount or volume for optimum recovery of the pathogen Specimen is collected before symptoms subsides and before any treatment is given Specimen is collected aseptically using proper disinfection technique on the site of infection to avoid contamination by normal flora Specimen is sent to the laboratory without delay under optimum conditions (e.g. optimum temperature & humidity) Accompanied by a request that matches the information on the specimen submitted. influenzae – serotype b capsulated – important cause of meningitis in children; URTI, epiglotitis H. Lastly, when bacteria are referred to as a group, their names are neither capitalized nor underlined (e.g. enterococci). Alcohol Page 47 of 131 Study Guide in Diagnostic Bacteriology Page 48 of 131 MOA: dehydration, lipid dissolution, and protein denaturation 4 70% Alcohol not 90%: Minimum Contact Time; 1-2 minutes or until completely evaporated 2. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. coli (VTEC) o Produces verotoxin an which lyses Vero Cell Line o STOOL: watery with excessive blood o Causes the following complications: Hemorrhagic colitis – abdominal cramps, watery diarrhea with blood; complications include acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia Hemolytic Uremic Syndrome – a manifestation of hemorrhagic colitis seen in infants and small children which starts with bloody diarrhea o Most common serotype is O157:H7 o The only E. It can also detect sulfide production and gas production. o Disseminated to various organs such as liver, eyes, bones, CNS – THE GREAT IMMITATOR/GREAT POX LATENT SYSPHILIS – ABSENCE of clinical symptoms with a POSITIVE serological test TERTIARY (LATE) SYPHILIS o Can occur 5 to 30 years after primary syphilis o May include CNS involvement and neurological abnormalities Paralysis, delusions, blindness, deafness, cardiovascular abnormalities and appearance of granulomatous lesions known as GUMMATA/GUMMAS Page 123 of 131 Study Guide Diagnostic Bacteriology 124 GUMMATA/GUMMAS – painful ulcers that enlarge and erupt. This interpretative category also allows a buffer zone between susceptible and resistant. flavescens) M. gergoviae (RUP) INDOLE: Negative CITRATE: Positive LDC negative (-) except E. Mycobacterium gordonae Also known as TAP WATER BACILLUS – found in variety of water sources Previously known as Mycobacterium aquae Considered as NON-PATHOGENIC but OPPORTUNISTIC to immunocompromised host KEY LABORATORY IDENTIFICATION: o Grows well at 37oC evident in 7 days o Yellow to Orange Colonies o TWEEN 80 Hydrolysis and HEAT STABLE CATALASE (+) 6. When testing Staphylococcus against oxacillin or vancomycin, or Enterococcus against vancomycin, incubate for a full 24 hours before reading. Due to the intense cellular immune response against the spirochete. (-) Aids in the identification of Vibrio, Pseudomonas, Neisseria, Campylobacter, Aermonas, etc. o f. Potassium permanganate is used to quench non-specific fluorescence and provides a dark background for better contrast. stuartii Rare human pathogen; UTI + + - - ** - + **Some strains may be urease negative Morganella IMVC + + - Motile but no swarming Lactose, citrate, H2S, and LDC negative (-) Urease & DEAMINASE, ODC positive (+) M. Compound Number 606 cured all test animals infected with syphilis. Moeller’s Broth – used to detect lysine decarboxylation, ornithine decarboxylation, and arginine dihydrolysis Stuart’s Urea Broth or Christensen Urea Agar – used to detect bacterial pathogen that hydrolyze urea substrate Page 44 of 131 Study Guide in Diagnostic Bacteriology Page 45 of 131 PART 3 CONTROL OF MICROORGANISMS ∞ SECTION 9 ∞ STERILIZATION AND DISINFECTION Laboratory safety especially in the microbiology section is crucial in the prevention of the spread of infectious agents from clinical specimens. BACTERIAL VAGINOSIS – characterized by foul smelling, grayish vaginal discharge, excessive vaginal discharge, and vaginal pH of greater than 4.5 LABORATORY DIAGNOSIS: Examination of vaginal secretion for the following parameters o pH of greater than 4.5 o presence of CLUE CELLS – epithelial cells covered with bacteria on the cell margins o Absence of Lactobacilli (gram positive bacilli) in gram stained smear o Positive WHIFF TEST – fishy amine odor upon the addition of 10% KOH to the secretions 5. 2. pallidum. Page 49 of 131 Study Guide in Diagnostic Bacteriology Page 50 of 131 4. boydii S. kansasii, M. Petri Dmitri Iosifovich Ivanovski T. INSPISSATION – thickening through evaporation effective indication: 75-80oC for 2 hours instrument: Inspissator 2. bovis = NEGATIVE (-) (M. FARCY – disseminated form of glander’s disease Burkholderia pseudomallei Cream to tan wrinkled colonies in BAP Motile via polar tuft flagella ADH (+) Highly oxidative – glucose, maltose, lactose, mannitol Causes MELIOIDOSIS – glanderslike disease which has a long latent period. He then started to chemically manipulate atoxyl and tested 900 different compounds but none of them worked. CELL DIVISION/MEANS OF REPRODUCTION 4. Decontaminated – to eliminate normal flora that contaminates the sample. sonnei ALL Yersinia except enterocolitica Proteus Providencia Morganella Edwardsiella Some produces H2S gas (blackening of the colony or medium) o In Triple Sugar Iron Agar (TSI) In Lysine Iron Agar (LIA) S – Salmonella S - Salmonella P – Proteus A – Arizona A – Arizona C – Citrobacter freundii Page 81 of 131 Study Guide in Diagnostic Bacteriology Page 82 of 131 C – Citrobacter freundii E – Edwardsiella E – Edwardsiella Some produces UREASE enzyme: o Rapid Urease Producers: P - Proteus P - Providencia M - Morganella o Slow Urease Producers: C – Citrobacter K – Klebsiella E – Enterobacter except E. fortuitum, M. Bile Esculin Hydrolysis Test 8. This might require you to organize them, review all of the important points and re-read portions of the textbook. May be stained sing aniline dyes (Wright’s or Giemsa) AGENTS OF LYME DISEASE 1. JEMBEC (John E. PATHOGENIC DETERMINANTS/VIRULENCE FACTORS - refers to any genetic, biochemical, or structural features that enable a pathogen to cause disease in a host organism. All seem to be involved in adherence or binding to host cell. Lesions are filled with treponemes, hence highly infectious. Therefore, ACID FAST STAIN is usually used instead. Vortex the saline tube to create a smooth suspension. However, even with the use of microscopes, bacteria appear to be colorless, transparent, and have a refractive index similar to the surrounding fluid making visualization very difficult, hence the use of stains are quite apparent. Bile Solubility Test Page 75 of 131 Study Guide in Diagnostic Bacteriology Page 76 of 131 15. Rare cause of endocarditis NON-MOTILE, OXIDASE (+), CATALASE (-), INDOLE (+) CAPNOPHILIC and grows on BAP Fermenter of glucose and maltose BUT NOT LACTOSE Page 106 of 131 Study Guide in Diagnostic Bacteriology Page 107 of 131 ∞ SECTION 23 ∞ GRAM POSITIVE BACILLI GENUS MYCOBATERIUM KEY CHARACTERISTICS: Gram positive (expected) but gram ghost or neutral on actual staining Slender, thin acid fast bacilli due to mycolic acid INCUBATION o Rapid growers – less than 5 day o Slow growers – more than 5 days o * Mycobacterium tuberculosis – 6-8 weeks incubation Obligate Aerobe CAPNOPHILIC – growth is enhanced by CO2 Requires High Protein Media to grow – fastidious Cell Wall Component – high in LIPID o Cord factor wax D o Mycolic Acid Agent of TUBERCULOSIS – chronic infectious disease o MOT: inhalation of contaminated droplets or aerosols DEVELOPMENTAL NOTES: First Isolated by ROBERT KOCH as the cause of tuberculosis 1886 – named as Bacterium tuberculosis – by Zoppf Later named as Mycobacterium tuberculo sis by Neumann because of the organisms “fungus-like” characteristics (myco) Mycobacterium tuberculosis – identified as cause of tuberculosis (TB) in man Mycobacterium bovis – identify v ed as cause of tuberculosis (TB) in cattle Mycobacterium avium – identified as cause of tuberculosis (TB) in chickens 1959 – RUNYON classified Mycobacteria other than tuberculosis (MOTT) bacilli based on pigment production and growth characteristics – arising to RUNYON’S CLASSIFICATION IMPORTANT LABORATORY TESTS FOR MYCOBACTERIUM 1. niger c. This is the first notation of the acid fastness or acid resistance property of tubercle bacilli. Number of Organisms – this factor basically refers to the amount of organisms present in the object to be treated referred to as microbial load (bioburden). Non-ionizing Radiation – uses low energy long wavelength ultraviolet rays to disinfect heat sensitive materials as well as large spaces II. burgdorferi Fluorescent immunoassay Indirect immunofluorescence Enzyme linked immunoassay (ELISA) Western Blot – may be considered as the GOLD STANDARD in the diagnosis of Borelliosis because of its high sensitivity and specificity GENUS TREPONEMA Tightly and helically twisted organisms which resembles a corkscrew appearance There are thirteen (13) species mostly are anaerobic but human pathogens are microaerophilic Had never been cultured in artificial media IMPORTANT PATHOGENIC MEMBERS – morphologically and serologically related: o Treponema pallidum subspecies pallidum Also known as GREAT POX/EVIL POX/French Disease/Italian Disease/Great Imitator/Social Disease/LUES Agent of VENERAL SYPHILIS (STD) MOT: direct sexual contact, blood transfusion, transplacental route. Examples: Spore forming bacteria – spores are coated with proteins, lipids, and carbohydrates as well as dipicolinic acid and calcium Mycobacterium spp. can be optimally recovered using this medium by adding 1% w/v sodium pyruvate (10g/L) or by reducing the agar content from 5g to 1.6 g/L. This medium is suitable for collection and shipment of specimens for epidemiological studies of Vibrio parahaemolyticus. Urease Hydrolyzes urea substrate which produces ammonia; the alakalinity changes the color of phenol red indicator to RED Catalase Breakdown H2O2 to oxygen and water resulting to rapid bubbling Cytochrome-c Oxidase Hippuricase Blue compound is formed when the indicator tetramethyl-paraphenylenediamine reacts with cytochrome c Hydrolyzes hippurate substrate visualized by addition of ninhydrin β-glucuronidase Hydrolyzes substrate that results to MUG which fluoresces under long-wave UV light Leucine Aminopeptidase Hydrolyzes substrate to Leucine and α-naphthylamine which reacts with DMAC to form a red color Hydrolysis of Substrate SUBSTRATE PYR (L-pyrrolidonyl-βnaphthylamide) Bile Esculin Detects rapid urease producing members of Family Enterobacteriaceae such as Proteus, Providencia, Morganella, and slow urease producing members such as Citrobacter, Klebsiella, Enterobacter, Yersinia, and Serratia Differentiates Staphylococci spp. aegyptius Pfeiffer bacillus Koch-Week bacillus Ducrey bacillus H. He concluded that the difference lies in the fact that the other clinic washes their hands with a chloride of lime sin between patients which dramatically reduced the cases of puerperal fever in birthing mothers. PHYSICAL METHODS: 1. CULTURE MEDIA TERMINOLOGIES: FOOD SOIL NOUN: growth of microorganisms CULTURE VERB: to grow/cultivate microorganisms INOCULATE/PLANT/CULTIVATE – introduction of microorganisms in a culture media TRANSPLANT/SUBCULTURE – the transfer of microorganisms from one culture media to another culture media Page 35 of 131 Study Guide in Diagnostic Bacteriology Page 36 of 131 CLASSIFICATION OF CULTURE MEDIA CLASSIFICATION OF CULTURE MEDIA ACCORDING TO COMPOSITION: Synthetic/Chemically Defined – composed of known and exact amounts of pure chemical substances which are commonly used in culturing autotrophic microorganisms such as algae or non-fastidious heterotrophs. Pathogenic for humans and animals. Suspend the organism in 2 ml of sterile saline. In essence, the bacterial cell transforms to a dormant state from a highly metabolic and growing state. Nomenclature refers to naming assignment of living organisms which includes all medically important bacteria that is discussed in this study guide. Too high or low temperature may inactivate disinfectants and sterilants. Mycobacterium tuberculosis (MTB) Known as KOCH’s bacillus Captain of ALL MEN of DEATH Causes TB in Man Infects lower/middle lungs lymphatics blood other organs (bones, meninges, spinal cord, heart, genitourinary tract, etc.) Specimen submitted for MTB Culture: o Sputum – deep cough collection, early morning for 3 consecutive days o Urine – collected early morning, 3 consecutive days and centrifuged for concentration o CSF – centrifuged and sediment should be inoculated to suitable media May be refrigerated – pellicle/clot formation – presumptive of MTB o Synovial, Peritoneal, Pericardial – should be centrifuged as well and sediments should be inoculated to suitable media Prior to processing SPUTUM sample: o DECONTAMINATION – kill or inhibit normal flora or other contaminating bacteria o DIGESTION – splitting of disulfide bonds in MUCIN/MUCUS which traps the MTB o Examples of Decontaminating/Digesting Agents: N-Acetyl-L-cysteine (NALC) and 2% NaOH – most common. cholerae 01 BIOTYPE DIFFERENTIATION BAP VogesPolymyxin B Chicken RBC BIOTYPES Key Note Hemolytic Proskauer (50u) Agglutination Pattern (VP) Test Susceptibility Classical Pandemics NonS of the past hemolytic El TOR Recent B-hemolytic + + R pandemics V. parahaemolyticus NSF – blue green Variable V. Clostridium difficile - antibiotic associated pseudomembranous colitis GENUS CORYNEBACTERIUM Also known as Kleb Loeffler’s Bacillus Aerobe Gram positive (+) non spore forming IRREGULAR BACILLI o Swollen, barbed, clubbed shaped ends due to the presence of METACHROMATIC GRANULES/BABES ERNST BODIES/VOLUTIN o X or V or Y arrangement or palisade or fence stick or cigar packets arrangement o Chinese Character Arrangement Most common species Corynebacterium diphtheriae o Pathogenic strain – causes two types of diphtheria ORAL/RESPIRATORY FORM MOT: Contaminated aerosol, nasopharyngeal route, nasal/oral discharges EXOTOXIN production leads to PSEUDOMEMBRANE formation in the respiratory tract (OROPHARYNX) “Bull’s Neck Appearance” CUTANEOUS DIPHTHERIA Page 117 of 131 Study Guide in Diagnostic Bacteriology Page 118 of 131 Characterized by lesions on the skin o Non-Pathogenic strain – does not cause disease to humans NORMAL FLORA MEMBERS – morphologically resembles Corynebacterium diphtheriae hence they are called DIPHTHEROIDS Page 118 of 131 Study Guide in Diagnostic Bacteriology o o o o Page 119 of 131 Corynebacterium xerosis Corynebacterium ulcerans Corynebacterium pseudotuberculosis Corynebacterium pseudodiphthericum IMPORTANT LABORATORY TESTS Page 119 of 131 Study Guide Diagnostic Bacteriology 120 1. Hence, flagellated bacteria are said to be moving of motile. SIGNS – refers to readily observable evidence of disease. CAPSULE – most important pathogenic determinant 2. SPECIFIC STAINS for METACHROMATIC GRANULES/BABES ERNST BODIES/VOLUTIN LAMB – most commonly used BURKE’S ALBERT 3. The following macroscopic observation should be noted prior to processing of the specimen: Is the sample swab or aspirate? To use a multidisk dispenser, place the inoculated MH agar plate on a flat surface and remove the lid o b. Joseph Lister A British surgeon who noted that o[en wound infections in patients were due to the germs in the air around the patient. Jenner tested his observation in milkmaids infected with mild variety of pox called cowpox. They also share similarities with eukaryotic organisms in terms of DNA (genes). jejuni: Will not grow in 42oC – thermophilic growth Susceptible to Cepalothin (C. gergoviae Y – Yersinia S – Serratia Some are DEAMINASE producing: P - Proteus P - Providencia M – Morganella Can be differentiated based on Lysine Decarboxylation (LDC) o LDC positive (+) K – Klebsiella E – Escherichia E – Edwardsiella S – Serratia S - almonella except S. There are three (3) common types: BUTT/DEEP SLANT BUTT-SLANT Page 37 of 131 Study Guide in Diagnostic Bacteriology Page 38 of 131 BOTTLED – culture media contained in a glass bottle that is usually used for blood culture CASTANEDA – a biphasic medium that is obsolete already that was used for blood culture to isolate Brucella abortus SEPTI CHEK ® - a broth slide system which is an innovation of the Castaneda bottle which consists of slide paddles containing Chocolate Agar, MacConkey, and Malt Extract Agar (selective for yeasts and fungi) attached to the top of a standard broth bottle. freundii C. simiae M. putida OXIDASE MOTILITY Glucose Oxidation Maltose Oxidation Arginine Dihydrolase (ADH) Growth at 42oC Nitrate Reduction P. Burgdorferi isolated a unicellular, loosely coiled spirochete from the gut of the Ixodes tick, hence the organism is named after him. The ideal antibiotic is devoid of allergenic properties such as allergic skin rashes, gastrointestinal upset, and anaphylaxis. Also, it’s advisable to prepare basic snacks and water so you can stay nourished while you study. Streptobacillus moniliformis Gram negative coccobacilli in chains Known as “NECKLACE BACILLUS” – string of beads appearance Causes: RAT BITE FEVER – MOT: Rat bite o HAVERHILL FEVER – MOT: Ingestion of contaminated meat/milk/dairy products 2. typhimurium DIAGNOSIS of TYPHOID FEVER: o 1ST WEEK (Early Typhoid Fever) Weakness, stomach pains, frontal headache, loss of appetite constipation Scybala Specimen of Choice: BLOOD o 2nd WEEK (Colonization Stage) Organism from the Peyer’s patches of the intestine travels to the lymphatic system and to the blood. STEP 4: MEASUREMENT OF ZONES OF INHIBITION 1. Mycobacterium avium-intracellulare Complex (MAIC) Also known as BATTEY BACILLUS due to an outbreak in Battey State Hospital in Rome, Georgia. May be overgrown by normal fecal flora, hence use a selective media: Cefsoludin Contains: Mannitol, peptones, yeast, neutral red indicator, Page 93 of 131 Study Guide in Diagnostic Bacteriology Irgasan Novobiocin Page 94 of 131 crystal violet Bull’s eye Colonies of Y. This broth has to be subcultured after 12 to 18 hours of incubation (Becton Dickinson, 1988) Thioglycolate Broth (THIO) – an enrichment broth for anaerobic bacteria but should be used solely in the isolation of anaerobic bacteria since it can also grow aerobes and facultative anaerobes. This also cause TB in dogs, cats, swine, and rabbits Virtually eliminated as a cause of human TB by the advent of cow’s milk pasteurization in the 1940’s Source of TB Vaccine for humans – Bacillus of Calmette & Guerin (BCG Vaccines) – share similar antigenic determinants as MTB – this is based on the principle of CROSS-REACTIVITY in IMMUNOLOGY. NUCLEIC ACID (DNA/RNA) PROBES 2. MINERAL ELEMENTS – needed as co-factors in various metabolic process of the bacteria (e.g. Ca2+, Mg2+, Fe2+, Sulfates, Phosphates) SALT – bacteria can tolerate salt concentration below 6%, however there are certain bacteria that can survive high salt concentration environment hence they’re called as HALOPHILIC bacteria or HALOPHILES (salt-loving) Staphylococcus spp. Some of which are capable of producing methane gas, however, they are not implicated as a cause of human disease hence they will be discussed further in this material. The first letter of the genus is capitalized followed by the species in lowercase; both the genus and species should be italicized in print or should be underlined when written in script (e.g. Streptococcus pyogenes or Streptococcus pyogenes). mirabilis DISEASES P. List of diseases and their treatments are even scribed in Egyptian Medical Papyrus dated to around 1500 B.C. (Hunderfund, 1987). Alexander Fleming In 1928, a Scottish Physician’s untidiness accidentally led him to discover penicillin when he noticed from an old pile of old culture a mould which inhibited the growth of Staphylococcus. BACILLUS (BACILLI) These are rod shaped, cylindrical, or elongated bacteria but it’s interesting to know that this is not always true to all bacilli since some of them also varies in morphologies. Near the end of the Trojan War (1190 B.C.), the Greek Army was largely wiped out by an epidemic which is now believed as bubonic plague. dysenteriae S. LOG/LOGARITHMIC / EXPONENTIAL PHASE 3. This growth is affected by various factors such as optimum growth requirements, dynamics of growth, including the use of a medium that can be artificially prepared in the laboratory. SEX/FERTILITY/F PILUS – usually longer and singular, long and hollow protein tubes that is primarily used for bacterial conjugation. gallinarum, S. fluorescens P. Contact Time – it is critical to observe proper contact time of the agent and the object to be disinfected or sterilized. Manifestation of symptoms takes years hence the synonym Vietnam Time Bomb DIFFERENTIATION OF ALCALIGENES SPECIES A. Except for Salmonella typhi Inhibitors: brilliant green CHO Incorporated: lactose Salmonella spp. He named the rash as ERYTHEMA CHRONICUM MIGRANS (Bull’s Eye Skin Lesion) Most of the cases of Lyme disease were seen during the summer months. It was eventually massed produced and was useful by American and British troops fighting in the World War II to prevent battlefield infections (Beck, 2000). Louis Pasteur A French chemist which first believed that microorganisms occurs in dust particles in the air which when fell on an uncovered broth will reproduce rapidly which makes the broth to appear cloudy after some time. Table 4: Comparison of a Gram positive and Gram negative Bacterial Cell Wall CELL WALL COMPONENT Peptidoglycan Periplasmic Space/Area Outer Membrane Teichoic Acid Lipoteichoic Acid Lipoproteins Lipopolysaccharide Layer GRAM POSITIVE Thick Layer Absent Absent Present Present Absent Absent GRAM NEGATIVE Thin Layer Present Present Absent Absent Present Present Table 5 summarizes gram staining technique with the corresponding reagent/s utilized in each step and the key color exhibited by a gram positive and gram negative cell wall. Livy (Titus Livius), a roman historian, mentioned of widespread prevalence of high death rate diseases in ancient Rome in 790, 710, 640 B.C. (Beck, 2000). o Bartonella bacilliformis Agent of Carrion’s disease MOT: bite of a sandfly (Genus Lutzomyia) CARRIERS: Asymptomatic humans After a 3 week incubation: EARLY STAGE: OROYA FEVER o Gradually: anorexia, headache, malaise, low grade fever o Abruptly: chills high grade fever, headache, and mental status changes ERUPTIVE STAGE: VERRUGA PERUANA (Peruvian Lesion) o Appearance of red to purple, non tender nodules in crops over 1-2 months and persist for months or years ∞ SECTION 26 ∞ ANAEROBIC BACTERIA ANAEROBES – organisms that require a reduced oxygen tension and fail to grow on solid media in the presence of oxygen They are generally normal flora of skin and mucous membranes o Bacteroides fragilis – significant and abundant normal flora of the gastrointestinal tract o Fusobacterium – significant normal flora of the upper respiratory tract including the mouth, nasopharynx, oropharynx, and nasal passages o Actinomyces species – normally found in the upper respiratory tract and GIT o Propionibacterium acnes – important anaerobic normal flora of the skin o Anaerobic Lactobacillus –normal flora of the female genital tract Usually involved in POLYMICROBIC INFECTION – causes infection together with aerobic and facultative anaerobic organisms Infections are usually ENDOGENOUS (from the inside of the body) but some are EXOGENOUS (outside source) SPECIMEN COLLECTION, TRANSPORT, CULTURE MEDIA, AND INCUBATION Specimens preferred for anaerobic culture o Needle aspiration – most preferred o Blood Culture system – allows growth of strict aerobe, facultative anaerobes, and anaerobic organisms Transport Media o Pre-reduced AND Anaerobically Sterilized (PRAS) system by REMEL – semisolid agar and an anaerobic atmosphere suitable for aspirates o Gassed-Out Collection Tubes Non supportive medium with CYSTINE (reducing agent) and RESAZURIN (redox indicator) Tubes are flushed with O2-free CO2 then the specimen is injected through a rubber stopper in the tubes ANAEROBIC CULTURE MEDIA o Generally contains supplements such as hemin, blood, Vitamin K, as well as Sodium Bicarbonate (NaHCO3) which provides the source of CO2 Page 130 of 131 Study Guide Diagnostic Bacteriology 131 Reducing agents, such as THIOGLYCOLIC ACID, Sodium Thioglycollate, and Cystine may be added to absorb oxygen o PRIMARY ISOLATION requires three (3) sets of culture media Non-selective blood agar plate Anaerobic selective media Enriched Broth – THIOGLYCOLLATE BROTH o Although anaerobic culture media do not need to be stored anaerobically, they need to be held in reduced form for 8-16 hours prior to inoculation ANAEROBIC INCUBATION SYSTEMS o ANAEROBIC JAR METHOD Hydrogen is added to the jar that will form a reaction inside that will remove the oxygen PALLADIUM – catalysts inside the jar which needs to be replaced and reactivated every time the jar is being used, since it is inactivated by the metabolic products produced by the bacteria Reactivation of PALLADIUM is achieved by heating the pellets in a Dry/Hot Air Oven for 2 hours at 160oC to 170OC. STEP 2: For sample coming from liquid medium, gently tap the broth culture and aseptically transfer 1-2 loopful of the sample at the center of the slide and evenly spread to about 2/3 area f the slide. Escherichia coli – medium sized dark violet colonies with greenish metallic sheen. Table 7-3 COMPLEX (Undefined) Culture Media A COMPLEX or UNDEFINED CULTURE MEDIUM COMPONENT QUANTITY Dipotassium phosphate, K2HPO4 2.5 g Casein peptone 17 g Soybean peptone 3g Sodium chloride, NaCl 5g 8Glucose 2.5 g Distilled water 1 litre TISSUE CULTURE MEDIA - live cells harvested from organs of humans and animals that supports the growth of obligate intracellular organisms that cannot grow in artificially prepared culture media. diphtheria biotype mitis 5. catarrhalis ONPG Test Neufeld Quellung Reaction - + + + + - + + - Glucose; Maltose; Lactose Glucose; Maltose; Sucrose & fructose Non-fermenter Non-fermenter Other Notable Neisseria species N. dysenteriae because it uniquely produces the following pathogenic determinants: o Aside from ENTEROTOXIN & ENDOTOXIN which the other three (3) species also have, it produces NEUROTOXIN – causes paralysis and death o Table 14-3 Differentiation of Shigella species SPECIES S. cholerae NON-01 o No enterotoxin and does not agglutinate 01 anti-sera o Causes very mild diarrhea and extraintestinal infection (skin/wound infection) DIFFERENTIATION OF PATHOGENIC VIBRIO SPECIES Growth in Lactose CHOLERA STRING KANAGAWA PATHOGENIC SPP. coli (EHEC) o Also known as Verotoxigenic E. Therefore, any POSITIVE non-treponemal test must be confirmed with a TREPONEMAL TEST. fortuitum-chelonae Complex which is POSITIVE in 3 days Tripotassium Phenolphthalein ARYLSULFATASE FREE PHENOPHTHALEIN = Pink (+) 8. coli that is Non-Sorbitol Fermenter (NSF); colorless colonies in Sorbitol MacConkey Agar (SMAC) ANAEROGENIC E. IRON UPTAKE TEST Key reaction to differentiate M. cholerae SF - yellow + + V. gergoviae S – Shigella SEROLOGICAL CHARACTERISTICS THREE (3) TYPES OF ANTIGENIC DETERMINANTS Useful in the identification of the following species & epidemiological investigation: o Escherichia coli o Klebsiella o Shigella o Salmonella 1. Spectrum of Activity – the compound must be useful in treating diseases caused by a number of different types of microorganisms not only gram positive and gram negative, but also mycobacterial spp., fungal spp., parasites, and viruses. Table 8 summarizes the Page 19 of 131 Study Guide in Diagnostic Bacteriology Page 20 of 131 important cocci arrangements and examples of medically important bacteria that exhibit the morphology. Optimum pH can be checked and monitored using a pH meter (sterile) after preparing the culture media usually before dispensing into tubes or culture media plates (petri dish) ACIDOPHILIC – acid loving bacteria (e.g. Lactobacillus acidophilus) ALKALIPHILIC – alkali loving bacteria (e.g. Gardnerella vaginalis) HIGH OSMOTIC PRESSURE – OSMOPHILIC Bacteria (Archaebacteria spp.) BACTERIAL GROWTH PHASES refers to the stages of bacterial growth. pneumoniae + M. Zephiran: Benzalkonium chloride b. Sulfamethoxazole-Trimethoprim (SXT) Susceptibility 12. Infection may be mild or severe accompanied by myalgia, nausea, vomiting, fever, headache, and chills in ACUTE PHASE. Isolated from several sources in nature & environment Has been isolated from contaminated IV fluids in a nationwide septicemia outbreak in the 1970s Salmonella IMVC - + - Very complex genus More than 2200 serotypes using Kauffman White Classification o They have been grouped as follows on the basis of Somatic O Antigen: Serotypes 1, 2, 3 o They have been grouped as follows on the basis of Flagellar H Antigen: Groups A1, A2, B1, B2, etc. Subculture to BAP helps in identification because it fails to grow in BAP Antibody titer fourfold rise to at least 1:128 or greater is diagnostic GENUS: Brucella KEY CHARACTERISTICS: Gram negative bacilli Strict/Obligate aerobe Intracellular organism Normal flora of the urinary and gastrointestinal tracts of goats, pigs, & cows Agent of brucellosis in animals. Hans Christian Gram used this difference in categorizing or grouping bacteria on the basis of their different cell structure. Not until Paul Ehrlich discovered the cure for syphilis. If the placement of the disk or the size of the zone does not allow you to read the diameter of the zone, measure from the center of the disk to a point on the circumference of the zone where a distinct edge is present (the radius) and multiply the measurement by 2 to determine the diameter 7. o TREPONEMAL TEST – uses specific treponemal antigens in the test systems Three (3) TYPES Treponema pallidum IMMOBILIZATION (TPI) o Uses LIVE TREPONEMES o After incubation with patient’s serum, a positive test is indicated by IMMOBILIZATION of the spirochetes due to the attachment of specific antibodies to the organisms FLOURESCENT TREPONEMAL ANTIBODY – ABSORBED (FTA-Absorbed) o Uses a sorbent (non pathogenic treponemal strain) to remove cross reacting antibodies o After mixing the sorbent with the patient’s serum which removes the cross reacting antibodies, it is incubated in a slide with nonviable T. It’s a way to collaborate with others, ask questions and help others with things they may be struggling with. STAINING Staining imparts an artificial coloration not only to bacteria but for other material found on clinical specimen smear that allows them to be visualized better using the magnification of microscope. Teaching the material to classmates can be an effective tool to gaining thorough understanding of the topics.Take BreaksWhile studying is important, so is taking breaks. Borrelia recurrentis agent of LOUSE borne RELAPSING FEVER characterized by fever muscle and bone pain, and confusion. Optochin (TAXO P) Susceptibility 16. To differentiate: o Late lactose fermenter o Citrate Negative (-) o To differentiate from Serratia: o DNAse negative Page 89 of 131 Study Guide in Diagnostic Bacteriology Page 90 of 131 Lipase negative Gelatinase negative o o Klebsiella Enterobacter Serratia Hafnia Biochemically similar. CONVALESCENT STAGE a. DRY/HOT AIR OVEN – used in the sterilization of heat resistant materials effective indication: 160-180oC for 1.5 to 2 hours biological indicator: Bacillus subtilis var. which is helical but rigid while the Spirochetes which are helical as well but more flexible in movement. However, these diseases and the one’s causing them were better understood through time. Chromobacterium violaceum Motile, facultative anaerobe, OXIDASE (+) Rare cause of human infection. Figure 9: Scheme of classifying living organisms by Dr. Whitaker. – shooting star motility Page 31 of 131 Study Guide in Diagnostic Bacteriology Page 32 of 131 Campylobacter spp. If you’re a student, regardless of your age, solid studying habits can help you succeed. flexneri S. paratyphi A H – Hafnia o LDC negative (-) P - Proteus P - Providencia M – Morganella C – Citrobacter Y – Yersinia E – Enterobacter except E. (swarming) Nystatin/Anisomycin/Ampothericin B – inhibits fungal growth Page 30 of 131 Study Guide in Diagnostic Bacteriology Page 31 of 131 Phenyl Ethyl Alcohol – inhibits gram negative Bismuth sulfite/desoxycholate – inhibits most normal fecal flora except Salmonella & Shigella Basic Fuchsin/Thionine – inhibits Brucella abortus Crystal Violet/Eosin/Methylene Blue/Brilliant Green – inhibits gram positive Chemicals Dyes Carbohydrate Utilization UTILIZATION TYPE Respiration/Oxidation Fermentation DESCRIPTION Ability of the bacteria to breakdown carbohydrate with O2 as the final electron acceptor which yields high amounts of ATP (energy) hence a more effective way of carbohydrate utilization Ability of the bacteria to breakdown carbohydrate anaerobically with an organic product as the final electron acceptor which yields low amounts of ATP (energy) hence a less effective way of carbohydrate utilization Organic Compound Utilization – ability of the bacteria to utilize certain organic compounds as a sole source of carbon leading to the production of an alkaline or basic product which changes the color of the indicator present in the culture media. Growth on PAI’s Medium or LSS – enhance the production of metachromatic granules 4. Rare cause of human infection acquired by direct contact, trauma, or inhalation. Opportunistic Pathogens: refers to organisms that will cause disease in an immunocompromised host. MORE FROM QUESTIONSANSWERED.NET Study Guide in Diagnostic Bacteriology Page 1 of 131 PART 1 Introduction to Diagnostic Bacteriology ∞ SECTION 1 ∞ GENERAL DEFINITIONS FOCUS OF DIAGNOSTIC BACTERIOLOGY FOR MEDTECH Page 1 of 131 Study Guide in Diagnostic Bacteriology Page 2 of 131 Definition of Terms Related to Infectious Process and Disease Transmission PATHOGENS – disease causing microorganisms such as bacteria, fungi, protozoans, and viruses. pallidum (MHATP) o RBC sensitized with T. This helps you focus on the areas that you need to review the most.Create a Study ScheduleIf you’re studying for a big test or exam, don’t plan on doing all of the studying last-minute. tuberculosis and M. Dip a sterile swab into the inoculum tube. Pasteurization – used for the preservation of alcoholic beverages such as beers, wines, and also dairy products such as milks and yogurt a. Basically the bacteria decrease its cytosol and increase the strength and thickness of its cell envelope. Bacteria/Structure (Capsule) – UNSTAINED Background – COLORED/STAINED o INDIA INK or NIGROSIN – background is BLACK o CONGO RED - background is RED o ANTHONY - background is PURPLE ∞ SECTION 7 ∞ METHODS OF STUDYING BACTERIA After staining clinical specimens, appropriate culture media must be chosen for the favorable growth of the pathogen of interest. and g. o h. Because of his work on the etiology of anthrax and tuberculosis, he was able to outlined postulates or the steps needed to prove that specific organism caused an infectious disease (Ochoa & Corey, 1997) (Figure 6). catarrhalis M. CHEMICALS – bismuth sulfite, bile salts (sodium desoxycholate), thiosulfate, citrate, etc. Uses cardiolipin-lecithin antigen to detect reagin TWO (2) TYPES RAPID PLASMA REAGIN (RPR) Test – antigen is coated with carbon and reaction is observed against white card to aid visibility of black flocculation. KEY LABORATORY IDENTIFICATION: o Grows well between 35oC to 37oC in 3 weeks Middlebrook media and 6-8 weeks in LJ Medium o DO NOT grow at 24oC and 42oC o Colonies are buff and small and may be rough or smooth. For the purpose of organization, we will study the structure of a bacterial cell based on the following categories in order by which they are discussed in this section (1) Cell Wall; (2) Parts internal to the cell wall; and (3) Parts external to the cell wall. Ophuls, H.C. Moffett William Leishman Fritz R. BACTERIAL GROWTH REQUIREMENTS Nutritional Environmental NUTRITIONAL CARBON – needed for the synthesis of cellular components Carbon Dioxide from the air – autotroph Organic Compounds in the Culture Media (Glucose) - heterotroph ENERGY SOURCE Page 32 of 131 Study Guide in Diagnostic Bacteriology Page 33 of 131 Light – phototroph Chemical energy – chemotroph ELECTRON SOURCE Inorganic molecule (Fe2+) – lithotroph Organic molecule – organotroph NADH reduced from NAD FADH2 reduced from FAD NITROGEN – needed for the synthesis of proteins Free nitrogen from the air Nitrogenous compounds in the Culture Media (e.g. peptone, yeast, beef extract) WATER/MOISTURE/HUMIDITY – bacterial cell is 70% water; it’s like a sealed plate of moisture hence the humidity level should be maintained at 70% which can be achieved by placing a container of water inside the incubator which should be monitored regularly so that it doesn’t dry out or become contaminated with mould. Positive reaction is the appearance of redness or conjunctivitis in the inoculated eye. o c. parainfluenzae H. Animal sources includes: o Poultry (chicken, ducks, turkeys o Pets (dogs, cat, hamsters, turtles) o Others (pigs, sheep, cows, donkeys, snakes, parrots) Human infection is through the ingestion of contaminated food or food products, or contaminated hands Page 90 of 131 Study Guide in Diagnostic Bacteriology Page 91 of 131 To differentiate from Shigella o Salmonella is motile except S. PERIOD 1665 1667 1796 1850 1861 1862 1867 1876 1881 1882 1884 1885 1887 1892 1893 1899 1900 1910 1928 1953 1977 1983 1995 DEVELOPMENTAL NOTES Publication of the first description of microbes Observation of “little animals” Smallpox Vaccination – first scientific validation Advocating Handwashing in the prevention of the spread of disease Spontaneous Generation disproved Publication of the paper supporting the germ theory of disease Practice of Antiseptic Surgery Discovery of Bacillus anthracis which became the first proof of germ theory Utilization of solid culture media for bacterial growth Outlined Koch’s Postulate Development of Acid-fast stain Gram Stain developed First Rabies Vaccination Invention of the Petri Dish Discovery of Viruses Zoonosis – first described Viral dependence on living host cells for reproduction recognized Proof the mosquitoes carry the agent of yellow fever Discovered the cure for syphilis Discovery of Penicillin Proposed and built the DNA Model Development of the DNA sequencing method Invention of the Polymerase Chain Reaction (PCR) Publication of the first microbial genomic sequence KEY SCIENTIST/S Robert Hooke Anton van Leeuwenhoek Edward Jenner Ignaz Semmelweis Louis Pasteur Louis Pasteur Joseph Lister Robert Koch Robert Koch Robert Koch Paul Erlich Hans Christian Gram Louis Pasteur Richard J. pullorum o Salmonella is H2S producing o Salmonella is gas producing o Samonella is LDC positive (+) except S. urethralis M. He then injected the same test animals with virulent strains and was surprised to know that some test animals did not die. Formation of nodules on the elbows, knees, toes, or fingers which may ulcerate Fisherman and lifeguards – increased rate of infection 7. This should be done before replacing the petri dish lid as static electricity may cause the disks to relocate themselves on the agar surface or adhere to the lid. A burst of exercise can help you clear your head and get motivated for another round of studying. 4. Middlebrook 7H10 or 7H11 Medium CULTURE MEDIA FOR MOTILITY – Sulfide Indole Motility Medium (SIM) ANAEROBIC CULTURE MEDIA Reducing Agents: Thioglycollc Acid, Sodium Thioglycolate, or Cystine Examples: Bacteroides Bile Esculin Medium Kanamycin-Vancomycin Laked Blood Agar CHARACTERISTIC/BIOCHEMICAL CULTURE MEDIA Sulfide Indole Motility Medium (SIM) - used for observation of hydrogen sulfide gas production, indole production, and motility. coli) IMVC + + - Synonym: Colon bacillus/Golden bacillus Most common cause of UTI (90% of UTI cases) Most important and common indicator of fecal contamination in water Normal flora of the gastrointestinal tract (70% of GIT NF) Important cause of nosocomial infection: o UTI o Meningitis o Pneumonia o Septicemia DIARRHOGENIC STRAINS of E.COLI EnteroTOXIGENIC E.coli (ETEC) o Related with enterotoxin production which mediates secretion of water and electrolytes in the GIT causing watery stool o Produces two types of toxin; heat labile toxin (LT) and heat stable toxin (ST) o Incubation period is 1 to 2 days and actual illness lasts for 5 to 10 days o Only grows in BAP o Causes transient diarrhea related to travelling. O Antigen – associated with the cell wall 2. It serves as a resting or hibernating stage for bacteria when they are exposed to unfavorable conditions. O or Somatic Antigen HEAT STABLE; associated with the cell wall lipopolysaccharide layer which includes the ENDOTOXIN portion E. Using the published CLSI guidelines, determine the susceptibility or resistance of the organism to each drug tested. In principle, contact time may be affected by all previous factors already mentioned as well as temperature. ORGANIC COMPOUNDS Citrate Acetate Malonate Acetamide Amino Acid Degradation – certain bacteria produce enzyme which degrades amino acid in media leading to the production of product which changes the color of the indicator present in the culture media AMINO ACID ENZYME REACTION PRODUCT Decarboxylase Cadaverine Lysine Ornithine Decarboxylase Putrescine Arginine Dihydrolase Citrulline Tryptophan Tryptophanase Indole Phenylalanine Deaminase Phenylpyruvic Acid ANTIGENIC DETERMINATION BY SEROLOGICAL TYPING 1. Figure 14 illustrates the three (3) primary shapes of bacteria as mentioned including common arrangement patterns of cocci. influenza H. STEP 1: INOCULUM PREPARATION AND STANDARDIZATION OF INOCULUM 1. It is associated with K Antigen (KAPSULE Antigen) and a slight change in the capsular Page 18 of 131 Study Guide in Diagnostic Bacteriology o Page 19 of 131 composition change the antigenicity of the bacteria which is the main reason why Streptococcus pneumoniae has approximately 80 capsular serotypes. o g. INDICATORS in CULTURE MEDIA Dyes and Chemical Substances such as pH indicators Page 39 of 131 Study Guide in Diagnostic Bacteriology Page 40 of 131 MOST COMMONLY AND ROUTINELY USED SELECTIVE & DIFFERENTIAL CULTURE MEDIA IN THE LABORATORY EOSIN METHYLENE BLUE (EMB) AGAR Original Color – dark violet Selective For: gram negative enteric bacilli Inhibitors: eosin and methylene blue Differential INDICATORS: eosin and methylene blue CHO Incorporated: LACTOSE LACTOSE fermentation Lactose fermenter Non-Lactose fermenter ACID pH RLF: 18-24 hrs COLORLESS *** Differentiation of Family Enterobacteriaceae based on Lactose Fermentation RLF Escherichia, Enterobacter, Klebsiella LLF Hafnia, Serratia, Citrobacter Salmonella arizonae Shigella sonnei Yersinia enterocolitica NLF ALL Salmonella except S. VOGES PROSKAUER (VP) TEST 8. aerogenes ODC: Positive Page 88 of 131 Study Guide in Diagnostic Bacteriology Page 89 of 131 Enterobacter cloacae o Predominant clinical isolate o Associated with bacteremia, respiratory tract infections, UTI, and wound infection in burn patients o Also contaminates IV fluids and other hospital instrumentation Enterobacter taylorae o Unique member because it is lactose negative but ONPG positive Enterobacter sakazakii o Biochemically similar to Enterobacter cloacae o Differentiated by its yellow pigment production intensifies at 25oC o Has been associated with neonatal sepsis and meningitis Enterobacter gergoviae o Resembles Enterobacter aerogenes o Rapid Urease Producer (RUP) o Causes infection of the urinary tract, and blood Serratia IMVC - V + + Opportunistic, unique due to the production of DNAse, LIPASE, GELATINASE Late lactose fermenter, ONPG positive Slow Urease Producer LDC positive (+) ODC positive (+) Serratia marscesens o Associated with pneumonia and septicemia in immunocompromised patients (undergoing chemotherapy) o Free living in soil and water and can be present in the hands of health care personnel o Causes nosocomial infection to patient undergoing cathetherization & other types of medical instrumentation o Also causes neonatal meningitis & found to be an agent of “community acquired endocarditis in IV drug users o Destructive and invasive due to the proteolytic enzymes that it produces and resistance to antibiotics including cepalothin and colistin o PRODIGIOSIN: red pigment at RT incubation; 5% of isolate exhibits this pigment production at 370C Serratia liquefaciens o Very similar to S. There are two types: SMALL – size of a standard saucer plate (100mm in diameter) LARGE/BIG – size of a standard dish plate (150 mm diameter) Usually used in Antibiotic Susceptibility Testing TUBED – usually container in glass tubes such as Wassermann tubes with different volume capacity (3mL, 5mL, 10mL) or in a tube with a flat bottom and a screw cap. Agar replaced gelatin because it remained solid at higher temperatures. He developed a DIFFERENTIAL TYPE of staining technique based on the staining characteristics of the bacterial cell wall which we popularly known as GRAM STAIN which has become a routine and basic technique in rapidly grouping or differentiating bacteria in the clinical laboratory. Paul Ehrlich in 1882 evaluated the work of Koch and was able to note that tubercle bacilli is difficult to stain so he used a different dye and an acid decolorizer which lessens the staining time to around 45-60 minutes from a 24hour process. typhi, S. This finding provided the first important evidence which form bases of the germ theory of disease. However, some doctor did not believe this enhance refused to embrace the practice. This is most commonly known as Direct Fluorescent Antibody (DFA) Stain o The smear is flooded with fluorescein-isothiocyanate-conjugated B. Thus, there is no absolute or official classification scheme for bacteria. LYSINE IRON AGAR (LIA) Protein Source: Peptone CHO: Glucose (0.1%) pH Indicator: Brom Cresol Purple (BCP) H_2S indicator: ferric ammonium citrate Sulfur Source: Sodium thiosulfate Amino Acid: Lysine ** Important LIA Reactions: 1. Watson, F. 6. Bacteria multiply and becomes concentrated in the ciliated epithelial cells and mucous membranes of the respiratory tract e. Sitting at a desk for hours can be draining, so make sure to space out time for yourself to get up, stretch or even take a short walk. Cepacol: Cetylpyridium chloride 5. Inhibitor Gram + inhibitor Gram - inhibitor Fungal Inhibitor Vancomycin Colistin Nystatin Vancomycin Colistin Nystatin Trimethoprim Vancomycin Vancomycin Decreased concentration of Vancomycin; added with Lincomycin Colistin Colistin Anisomycin Amphotericin B Trimethoprim Trimethoprim Colistin Amphotericin B Trimethoprim CULTURE MEDIA FOR ANTIBIOTIC SUSCEPTIBILITY/SENSITIVITY TESTING (AST) Most bacteria: Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) Haemophilus spp. Similarly, the body of Pharaoh Ramses V manifested pustules in his face, neck, and shoulders which is believed as the earliest evidence of smallpox (Kiple, 1997). Touch the colony of interest using an inoculation loop or needle or applicator stick. It is now known that Borrelia burgdorferi is found in tissues of several mammals including field mice and deers. o Known to produce adherent biofilm. pneumoniae infection develops a nonspecific cold-reacting antibodies known as COLD AGGLUTININ o COLD AGGLUTININS – IgM Antibodies that agglutinate human group O RBCs at 4oC but not at 37OC o These antibodies can be detected in the patient's serum shortly after the onset of disease and peak during the convalescent period. CHEMICAL METHODS: 1. One is the Spirillum spp. Coccus is divided by two (2) planes of TETRAD/S – coccus in packets of four division. The modern idea of taxonomic classification was introduced by several scientists in the 1600s, including John Ray, an English naturalist a general scheme in describing plants and animals, and Gaspard Bauhin in 1623 who first used a two-word (binomial) name to classify plants. sonnei CATALASE TEST ONPG TEST MANNITOL FERMENTATION ORNITHINE DECARBOXYLATION (ODC) + + + + + + + + Proteus IMVC Found in soil, water, focally contaminated materials UNIQUE CHARACTERISTICS: Rapid Urease Producers (RUP- RXN within 4 hours); typical swarming motility (growth in waves or swarms); H_2S producing in TSI but not in LIA Table 14-4: Differentiation of Proteus species SPECIES P. marinum (POSITIVE +) from M. He began to spray carbolic acid in the air around his patients prior to surgery (Barba, 1999). Nature of Surface to be Disinfected - Some instrument that we use in the laboratory sometimes are made up of biomaterial which exempts them to disinfection or sterilization due to possible damage. Human may acquire the infection by: o Ingestion of contaminated meat/milk products o Direct animal contact Causes BRUCELLOSIS – in man. The species are abbreviated as "sp." (singular) or "spp." (plural) when the species is not specified. Inflamed gall bladder, lungs, periosteum & other organs. There are two (2) types: LIQUEFIABLE – this is the type of solid culture media that we prepare in the laboratory that can be boiled and melted. xenopi M. Drug Clearance – the antibiotic must not readily secreted by the kidney and metabolized by the liver to allow sufficient plasma levels. While your studying strategies may evolve as you progress in your educational career, here are basic tips and advice to help you get the most out of your study sessions.Set Up Your Study SpaceOne of the effective ways to make the most out of your study sessions is to have it set up properly. Figure 15: A Plane of Division splitting a coccus Table 8: Cocci Morphologies Plane of Division Resulting Arrangement Illustration Coccus is divided by one (1) plane of DIPLOCOCCUS/DIPLOCOCCI division All diplococcic appears to be two cocci adjacent to each other except the following: Neisseria spp. CONGENITAL SYPHILIS Syphilis may be passed from infected mother to the fetus during primary, secondary, or latent syphilis which may result to miscarriage or still birth NEWBORNS with congenital syphilis may be seen with bone malformation, widespread skin rashes, meningitis or hepatosplenomegaly Transplacental transfer usually happens after the 4th MONTH of PREGNANCY IMPORTANT LABORATORY TESTS: Demonstration of spirochetes using DARK FIELD MICROSCOPY using sample from skin lesions during primary and secondary syphilis ONLY Serological Tests available – antibodies detected which can be categorized as o NON-TREPONEMAL TESTS – detects REAGIN or WASSERMANN antibodies (non-specific) antibodies produced in response to the infection. smegmatis (lacticola) 3. They are as follows: o MONOTRICHOUS – single polar flagellum (Figure 13A) AMPHITRICHOUS – single or clusters of flagella at both poles (Figure 13B) LOPHOTRICHOUS – single tuft of cluster of flagella at a pole (Figure 13C) BILOPHOTRICHOUS – two tufts or clusters of flagella at both poles (Figure 13D) PERITRICHOUS – single flagellum randomly distributed around the bacteria (Figure 13E) Figure 13 Types of Flagella Arrangements GLYCOCALYX – exterior high molecular weight appendage or structure usually made up of polysaccharide polymers or sometime polypeptides which are produced be certain bacteria depending on environmental and growth conditions surrounding the bacterial cell. pestis Y. with wound infection & UTI. enterocolitica – LLF, ONPG positive, weakly fermentative, requires cold enrichment. Page 45 of 131 Study Guide in Diagnostic Bacteriology Page 46 of 131 Concentration of Disinfecting Agent – The amount needed to destroy microorganisms varies based on the agent to be used. Bacteremia can also result from a primary bite infection o Inhalation of the bacteria Can lead to pneumonia, bronchitis, sinusitis Risk are found in animal handlers/raisers with predisposing respiratory infection GENUS: Actinobacillus (now part of the Genus Aggregatibacter) Common species: Actinobacillus (Aggregatibacter) actinomycetemcomitans KEY CHARACTERISTICS: Gram negative short bacilli/coccobacilli Facultative anaerobe Non-motile Fermentative Grows in BAP/CAP but CAPNOPHILIC (requires 5-10% CO_2). thermoresistable (can grow @ 52oC) M. Organism enters the body through the mucous membranes or breaks in the skin Incubation period varies from 10-90 days (average of 14-21 days) STAGES OF VENEREAL SYPHILIS PRIMARY SYPHILIS – appearance of a painless, firm, smooth CHANCRE (HARD CHANCRE) with regional lymphadenopathy and early invasion of the blood SECONDARY SYPHILIS o Onset is 6 weeks to 6 months after the appearance of the chancre o Chancre heals leaving little or no scarring o Widespread of skin and mucous membrane lesions (CONDYLOMATA LATA) typically found on the palms of the hands and soles of the feet. Campylobacter spp. VIRULENCE – refers to the degree of pathogenicity; the power by which a pathogen can cause severe disease. Although preventing contamination through antisepsis is a significant step in the practice of modern day medicine, protection of antiseptic agents in only limited since scientists have observed that person who have had the disease catches the same disease eventually. Enterobacter - medium sized dark violet colonies with or without dark center. – usually composed of chocolate agar base with antibiotics Culture Media Thayer Martin Agar (TM) Modified Thayer Martin Agar (MTM) Martin-Lewis Agar (MLA) New York City Agar (NYC) GC-LECT Proteus spp. o Increased Antibody Titer to O Ag ≥ 1:160 – ACTIVE INFECTION o Increased Antibody Titer to H Ag ≥ 1:160 – PAST INFECTION/IMMUNE STATE o Increased Antibody Titer to Vi Ag – CARRIER STATE Shigella IMVC + + - Inert bacilli (yields negative reaction to most tests) Resembles E. typhi has distinct appearance) Inhibitors: Bismuth sulfite CHO Incorporated: Glucose ****Salmonella typhi colonies appear as black colonies with silver metallic sheen BRILLIANT GREEN (BGA) AGAR Selective: For: Salmonella spp. Thus, method which uses the body's natural defenses were developed to prevent infection. Boiling – destroys vegetative cells of bacteria but not their spores Effective indication: 100oC for 15-30 minutes 2. Colonies are usually small, smooth, gray or translucent, and non-hemolytic CANNOT grow in EMB/MAC Produces MUSTY ODOR – resembling the smell of mushrooms Page 100 of 131 Study Guide in Diagnostic Bacteriology Page 101 of 131 Oxidase (+) Catalase (+) Indole (+) Urease (-) Harbored in the oral cavity, respiratory and gastrointestinal tracts of healthy domesticated animals which are the common reservoirs. cholerasuis, S. Selective also for Aeromonas spp. UREASE TEST Page 86 of 131 Study Guide in Diagnostic Bacteriology Page 87 of 131 10. Buboes may occur FREI TEST – delayed hypersensitivity test to LGV that uses purified LGV antigen o False negative results also occur hence test is not useful anymore o IMPORTANT LABORATORY TESTS Cytological examinations using stains Giemsa – chlamydial inclusion bodies appear purple Iodine solution - chlamydial inclusion bodies appear light to dark brown CHLAMYDIAL INCLUSION BODIES Chlamydia trachomatis Halberstaedler-Prowazek bodies WITH Glycogen Chlamydophila psittaci Levinthal cole Lillie bodies WITHOUT Glycogen Culture in Cell Lines McCoy Cells – heterploid murine cells pretreated with cycloheximide Others – HeLa-229 cells, L-929 cells, and BHK-21 cells Page 128 of 131 Study Guide Diagnostic Bacteriology 129 Serological methods – limited usefulness since high prevalence of C. He also tried to infect a boy with cowpox by inserting cowpox into an incision that he made in the boy's arm, like the milkmaids, the boy did not get sick of smallpox. These stains contain electrochemically active molecules called CHROMOGENS. The pellets must be stored in a desiccator until next use METHYLENE BLUE STRIPS are used as indicator in the system. lwoffi Table 13-3 Differentiation of A. Place the disk on the plate over one of the dark spots on the template and gently press the disk with the forceps to ensure complete contact with the agar surface. The archaebacteria are unique microorganisms in terms of cell wall components and membranes. Bacteria are under external stresses to undergo mutations (Lobry & Sueoka, 2002). Batch: 62.5oC for 30 minutes b. Basically bacteria grow in number and do not grow in size. CHOCOLATE AGAR PLATE (CAP) – used as a primary isolation media highly fastidious bacteria such as the NHACEK group and Streptococcus pneumoniae which requires supplementation. CATARRHAL STAGE a. Inulin Fermentation 14. Study Guide in Diagnostic Bacteriology Page 85 of 131 4. This innovation was called Petri dish (Brock, 1989). The rash typically appeasr after a bite of a tick. Adverse Side Effects – the compound must not, as much as possible, develop life threatening side effects to the host that will negate the purpose of treating the patient. Methods of Sterilization I. Minimum Bactericidal Concentration (MBC) – the lowest concentration of an antimicrobial agent that results in the death (greater than or equal to 99.9%) of the test organism. This makes it necessary to conduct susceptibility tests, the results of which must be communicated to the clinician. This provided a method that gave a reproducicble and reliable results. Page 22 of 131 Study Guide in Diagnostic Bacteriology Page 23 of 131 Irregular bacilli – club shaped or barb shaped arranged in palisade, fence stick, cigarette packet. above. diphtheria biotype belfanti C. COLI or E. One of the most routinely used methods in the laboratory to identify and characterize bacteria in clinical specimens is the observation of cell size, shape, and distinct or unique arrangement which is collectively referred to as bacterial morphology. for the use of the multi-disk dispenser or steps e. Found in soil and water Unique because of the violet pigment that it produces – VIOLACEIN 6. Using the forceps carefully remove one disk from the cartridge. Figure11 illustrates the typical bacterial cell structure. Moist Heat – coagulation of bacterial proteins including bacterial enzymes a. NON-LIQUEFIABLE – these are solid culture media but they cannot be boiled and melted like the culture media containing agar EXAMPLES: rice grains, potato slices, cooked meat CLASSIFICATION OF CULTURE MEDIA ACCORDING TO MANNER OF DISPENSING/FORMATION PLATED – usually contained in a container that can be made of glass (pyrex) or disposable plastic. This section discusses important key terminologies related to control of microorganisms in the laboratory as well as their intended purpose and mechanism of actions. (-) as well as Bacillus spp. Chest pain due to lung infiltration of neutrophils and alveolar macrophages. Partially remove the lid of the petri dish. asiaticum M. Waterhouse Friderichsen Syndrome – hemorrhagic disease of the adrenal gland because of Neisseria meningitidis 3. Since majority of specimens are being collected by patients and non-laboratory health care personnel who may be unfamiliar with the consequences of poorly collected and handled specimens, it is the prime responsibility of the laboratory to provide clear and concise instructions on this pre-analytical aspect of testing. coli but differs because it is: o H_2S positive o Lactose negative LDC &ODC positive but ADH negative Yersinia Small coccobacilli that produce small pinpoint colonies on MAC Y. TOXIGENICITY TEST Best pathogenicity test for Corynebacterium diphtheria 2 TYPES: o IN VIVO - animal inoculation using GUINEA PIG o IN VITRO – Modified ELEK's TEST 6. Figure 7: Steps in Gram Staining EARLY EFFORTS OF MICROBIAL CONTROL Along with the rapid discovery of microorganisms as cause of infectious diseases, it is also imperative for scientist to work on how to control them, hence preventing the development of disease. It is not necessary to administer the drug numerous times or at frequent intervals. EARLY CLUES OF MICROBIAL EXISTENCE Early Microscopes The invention of the microscope is probably the single most important catalyst in the development of microbiology and the continuous innovation in this instrument over time immensely contributed to our understanding of microbial existence and their role in human disease. gordonae and MAIC (NEGATIVE -) UREA UREASE AMMONIA (changes the PHENOL RED INDICATOR to RED (+) 11. Manufacturers usually optimize this factor to achieve maximum activity. Table 7-2 Chemically Defined Culture Media A CHEMICALLY DEFINED CULTURE MEDIUM COMPONENT QUANTITY Dipotassium phosphate, K_2HPO_4 7g Potassium phosphate, monobasic, 2g KH_2PO_4 Hydrated magnesium sulfate, $MgSO_4$0.2 g $7H_2O$ Ammonium sulfate, $(NH_4)_2SO_4$ 1g Glucose 5g Distilled water 1 litre Non-synthetic/Non-Chemically Defined/COMPLEX – composed of complex materials that are rich in vitamins and nutrients that are not usually represented by a chemical formula such as peptones, beef or yeast extract, plant extracts, etc. Paul Ehrlich In 1897, he shifted his attention to chemotherapy. Page 84 of 131 4. IMMUNE PHASE follows after acute phase and is associated with antibody production and elimination of the organism. This particular tick is abundant in the wooded areas of Lyme and Old Lyme Sooner later, Dr. W. SELECTIVE and DIFFERENTIAL CULTURE MEDIA – selective culture media favors the growth of the organism of interest using inhibitors added in the culture media while differential culture media contains indicatos which changes in color as a result of a product produced be a chemical reaction in the components of the media such as glucose. Cramming might seem appealing, but it can be stressful and not as effective as a thought-out schedule. 8. This staining technique is very useful in the identification of medically important capsulated bacteria as well as capsulated strains of Cryptococcus spp. Lymphadenopathy may also be present o STAGE TWO: dissemination occurs through the blood, and may affected several organs which may includes the bones (arthritis), CNS (meningitis, paralysis, dizziness, etc.) heart (palpitation), liver o STAGE THREE: also known as CHRONIC STAGE, includes chronic neurological abnormalities, arthritis, and skin lesions IMPORTANT LABORATORY TESTS: o Modified Kelly's Medium (BSK-II) – but yield of growth is very low; not preferred o Immunoserological tests – demonstrate presence of antibodies to B. ducreyi – soft chancre or chancroid (STD) ∞ SECTION 21 ∞ OTHER MISCELLANEOUS GRAM NEGATIVE BACILLI GENUS: Legionella KEY CHARACTERISTICS: Gram negative thin or narrow bacilli Aerobe Motile – one or more polar or subpolar flagella Faintly staining in GRAM STAIN (replace Safranin O with Giemsa or basic fuchsin) WEAKLY CATALASE POSITIVE; GELATINASE & MOTILITY POSITIVE Isolated from water sources: o Natural water sources – ponds, creeks, streams, wet soil o Artificial Water sources – water cooling towers, air-conditioning systems, heating systems, shower heads, plumbing systems Causes LEGIONELLOSIS – manifests as pneumonia characterized by flulike symptoms, myalgia, fever. cholerae strains: V. FUNCTIONS: it serves as an outside barrier for sudden osmolarity changes (high/low salt concentrations). COMPOSITION: made up of protein material known as FLAGELLIN Associated with H Antigen (Hauch Antigen) which is very useful is serologically typing and identifying species of Salmonella. 9. Apparently, the healthy test animals developed the disease and he concluded that only this microorganism is capable of causing this disease. Figure 14: General Bacterial Shapes and Common Cocci Arrangements Cocci (Coccus) These are round or spherical shaped bacteria. stutzeri + + + - + + + V + + + V + + + + + + + + - + + - - V + BURKHOLDERIA SPECIES (formerly Pseudomonas) Burkholderia cepacia yellow serrated colonies in BAP that are weakly oxidase (+) ADH (-) and LDC (+) Causes onion bulb rot in plants and foot rot in humans Page 98 of 131 Study Guide in Diagnostic Bacteriology Page 99 of 131 Burkholderia mallei Smooth and cream to white colonies in BAP and weakly oxidase (+) The only non-motile among all pseudomonads CAN'T GROW in 42oC Causes GLANDER's DISEASE – infectious disease of horses, goats, sheep, & donkey. paratyphi, S. Due to the complexity and ability of these microbes to adapt and mutate their genomic information, the challenge still continues in isolating, identifying, and characterizing these microorganisms in the interest of not only knowing the cause of the disease of humans but ultimately to find cure or treatment to these diseases. This, probably is the first evidences of vaccination which were all published in his book entitled "An Inquiry into the Causes and Effects of the Variolae Vaccinae" in 1798 (Brock, 1989). Additional substances may be added to enrich the media for growth of microorganisms that are very difficult to grow (FASTIDIOUS). Example is bleach and quaternary ammonium compounds which may negate each other. Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry or immersing the forceps in alcohol then igniting. Alkaline Peptone Water (APW) - used to selectively favor the growth of Vibrio while inhibiting all other normal intestinal flora due its high pH. Table 7 Summary of Modified Kinyoun Method for Partially/weakly Acid-Fast Organisms C KEY STEPS (MODIFIED KINYOUN METHOD) Primary/Initial Staining T Mordanting A Decolorization/Differentiation M Counterstaining CODE REAGENT/S CARBOL FUCHSIN CHEMICAL: TERGITOL ACID ALCOHOL (1% H_2SO_4 in 70% Ethanol) METHYLENE BLUE DURATION/TIME 5 MINUTES (rinse) Continuous drop until washing is colorless (rinse) 30 seconds (rinse) RED NON-ACID FAST RED RED RED RED COLORLESS RED BLUE ACID FAST PARTS INTERNAL TO THE CELL WALL CYTOPLASMIC/PLASMA MEMBRANE – a phospholipid bilayer embedded with proteins that envelops the cytoplasm but does not contain sterols (except Mycoplasma/Ureaplasma) o FUNCTIONS Separates the intracellular components of the bacterial cell from the extracellular environment Acts as an osmotic barrier between the inside and outside of the bacterial cell by allowing selective permeability of the membrane to macromolecules Site of electron chain transport necessary for energy production, hence maintaining the viability of the bacterial cell Houses enzymes involved in outer membrane and cell wall synthesis, and the assembly and secretion of extracytoplasmic and extracellular substances MESOSOMES – folds or invagination along the length of the cytoplasmic/plasma membrane which serves as a point of attachment for chromosomes FREE RIBOSOMES – sites of protein synthesis in bacterial cells which has a size of 70S comprised of two subunits being 50S and 30S. (Figure 1) COLONIZATION: refers to the establishment of substantial number of microorganisms usually in the skin or mucosa but there's no penetration of tissues. marscesens but differentiated to S. Disease often referred to as PRIMARY ATYPICAL PNEUMONIA because if it accompanied by dry cough instead of typical exudates associated with bacterial pneumonia Another common name is WALKING PNEUMONIA because patients appear normal or moderately ill even if the areas of the lungs are heavily infiltrated by the bacteria Disease is usually common in the autumn and winter months. Halogens MOA: inhibits protein function and acts as strong oxidizing agents Chloride (Cl) in NaOCl: used as disinfecting agents in many laboratory and hospitals spaces, surfaces, and also in treating water for potability Iodine (I_2) in Betadine used as a household antiseptics and surgical antiseptics 3. This medium is prepared in a plastic container with a snap-top lid with modified Thayer Martin Agar. terrae-triviale Study Guide in Diagnostic Bacteriology Page 108 of 131 GROUP II: SCOTOCHROMOGENS – pigmented yellow to orange in dark; pigment intensifies to orange or red when exposed to light source for 2 weeks M. baumannii KINGELLA NO_3 Reduc. INFECTIOUS DISEASE – is an illness caused by a pathogen which invades body tissues and causes damage COMMUNICABLE DISEASE – is an infectious disease that is capable of spreading from person to person. globijii Methods of Disinfection I. COLONIAL/CULTURAL CHARACTERISTICS SIZE – relative size of the bacterial colony COLONY SIZE DESCRIPTION Pinpoint colonies less than 1mm Small about the same size of a pinhead Medium Slightly larger than a pin head Large Usually 6-8mm in diameter MARGIN – appearance of the edge of the colony Edge of the Colony DESCRIPTION Smooth or entire Circular without interruption Undulate Wavy edge Rough or rhizoid Crenated edge Lobate Lobulated edge Fringed or filamentous Branchlike edge Fingerlike Uneven rounded projections Irregular Uneven length of projection with no definite shape Page 28 of 131 Study Guide in Diagnostic Bacteriology ELEVATION – height of the colony Height of the Colony Flat Raised Convex Umbilicate (innie) Umbonate (outie) TEXTURE or CONSISTENCY Texture of the Colony Brittle or splinters Creamy or butyrous Dry & Waxy Rough & Warty Mucoid Page 29 of 131 DESCRIPTION No visible elevation or height Slight elevation Dome shaped Depressed or concaved center Raised or bulging center DESCRIPTION Crumbling colony (e.g. Nocardia spp.) Butterlike (e.g. Staphylococcus spp.) Sticky colony (e.g. Diptheroids) Cauliflower appearance (e.g. Mycobacterium spp.) Wet & sticky colony (e.g. Streptococcus pneumonia HEMOLYTIC PATTERN – exhibits the bacteria's ability to lyse RBCs in the culture media Hemolysis type DESCRIPTION Clear zone around the colony; complete hemolysis Beta Greenish or brownish zone around the colony; Alpha incomplete/partial hemolysis Gamma No hemolysis around the colony Inner alpha hemolysis surrounded by an outer beta Alpha prime hemolysis PIGMENTATION – ability of the bacteria to produce unique coloration their colony Colony Color Bacteria producing the pigment Micrococcus luteus Lime yellow Golden yellow Staphylococcus aureus Blue Green Pseudomonas aeruginosa Serratia marscesens Red Staphylococcus albus Porcelain white Chromobacterium violaceum Violet ODOR – certain bacteria produce characteristic odor in culture media Colony Odor Bacteria producing the odor Staphylococcus spp. Table 5 Summary of Gram Staining Technique: Reagent/s and Reactions CODE KEY STEPS V Primary/Initial Staining I Mordanting 1 MINUTE (rinse) GRAM POSITIVE Violet/Purple GRAM NEGATIVE Violet/Purple 1 MINUTE (rinse) Violet/Purple Violet/Purple REAGENT/S DURATION/TIME CRYSTAL VIOLET IODINE Page 15 of 131 Study Guide in Diagnostic Bacteriology A Decolorization/Differentiation S Counterstaining Page 16 of 131 ACETONE ALCOHOL Quick ON & Rinse Violet/Purple COLORLESS SAFRANIN RED OR "O" 30 seconds (rinse) Violet/Purple RED/PINK GRAM VARIABILITY (a characteristic exhibited by gram positive bacteria) Natural gram variability: Mobiluncus spp., and Gardnerella vaginalis, Acquired gram variability (for gram positive bacteria) o Contributing factors Use of old culture pH of staining reagents bacterial autolysis staining reaction time GUIDING RULES IN THE GRAM STAIN REACTION OF MEDICALLY IMPORTANT BACTERIA All COCCI are Gram POSITIVE except Neisseria, Branhamella/Moraxella, Veillonella All BACILLI are Gram NEGATIVE except Mycobaterium, Bacillus, Clostridium, Corynebacterium, Lactobacillus, Listeria, Erysipelothrix, Aerobic Actinomyces, Rothia, Kurthia (MBCCLLEARK) Mycoplasma & Ureaplasma usually have a gram negative reaction NOT because it has a gram negative cell wall but because they DO NOT HAVE a CELL WALL. Table 7-1 Bacterial Growth Phases GROWTH PHASE 1. marinum, M. o EXPECTED Gram Stain Reaction: Gram positive – violet/purple o ACTUAL Gram Stain Reaction: Gram ghost or neutral – colorless or sometimes faint blue ACID FAST ORGANISMS Certain bacteria have increased lipid content in their cell wall containing waxy layer of glycolipids and fatty acids mainly MYCOLIC ACID which is a strong hydrophobic molecule which makes the cell wall of these bacteria difficult to penetrate however once stained, this exact cell wall component makes it difficult to decolorize even if strong acids are used hence the term "acid fast" for organisms exhibiting this property. Unfortunately, his colleagues despised and hated him for it appears that he's suggesting that they are unclean. (See steps a. gastri, MAIC) 5. HEAT STABLE CATALASE TEST – MOST Mycobacterium species are CATALASE (+) Slide is warmed at 68oC for 20 mins + 30% H_2O_2 = Bubbling or Effervescence (+) M. Cost Effective – minimal pricing to allow patients to afford it financially. These are usually physical manifestation of the disease such as rashes, bleeding, etc. Backbone structures: N-acetylglucosamine (NAG) N-acetylmuramic acid (NAM) **Except: Mycoplasma, Ureaplasma, Spiroplasma, & Anaeroplasma Asexual: BINARY FISSION – spontaneous splitting/division of bacterial cells given the optimum growth requirements EXCEPT: Rickettsiae – reproduce by binary transverse fission **DOUBLING/GENERATION TIME – time/period required for a bacterial cell to divide into two. MEDICALLY IMPORTANT CAPSULATED BACTERIA o Neisseria meningitidis Capsule is primarily made of: o Haemophilus influenzae serotype b POLYSACCHARIDE o Streptococcus pneumoniae POLYMERS o Klebsiella pneumoniae o Bacillus anthracis - capsule is made up of D-GLUTAMIC ACID SLIME LAYER – loose or diffused, thick, viscous unorganized material that appears to be detached from the bacterial or not firmly attached to the cell wall of the bacteria. This is also the phase where bacteria are most metabolically active hence most susceptible to the action of antimicrobial agents After essential nutrients are depleted, toxic products accumulate, or oxygen becomes limiting, the rate of Page 34 of 131 CELL DIVISION INCREASE IN NUMBER NO NO YES YES YES NO Study Guide in Diagnostic Bacteriology Page 35 of 131 PLATEAU PHASE cell division equates the rate of cell death 4. Here's a summary of colonial or cultural characteristics that are examined in plate reading that certainly helps in the identification of bacterial pathogens. enterocolitica Y. Place the dispenser over the agar plate and firmly press the plunger once to dispense the disks onto the surface of the plate. Discard the swab into an appropriate container. such as Clostridium noyvi, most Bacteroides spp., Fusobacterium spp., Peptostreptococcus spp., and Porphyromonas spp. This section deals with the most commonly used antimicrobial agents, their spectrum of activity, side effects, mode of action or how they affect organisms, mechanism of resistance, and methods used in susceptibility testing. coli – 164 serotypes o Serotype O111 – infantile diarrhea o Serotype O157 – verotoxin production Shigella – serotypes A, B, C, D Salmonella – 60 different serotypes Page 82 of 131 Study Guide in Diagnostic Bacteriology Page 83 of 131 2. LYMPHOCYTOSIS IDEAL MEDIA and METHODS of IDENTIFICATION: Bordet Gengou (B-G) Agar contains potato, blood, glycerol (methicillin or cephalexin preferred to be added) Regan Lowe (R-L) Agar – horse blood, charcoal agar, cephalexin and amphotericin B Methods of Specimen collection: o Cough plate Method – not done anymore o Nasopharyngeal swab or washings – most preferred and commonly done Alternatively, NASOPHARYNGEAL SWAB can be smeared and fixed on a sterile slide. H or Flagellar Antigen Associated with the flagella; hence only motile members have this antigen HEAT LABILE used to serotype within species of Salmonella & other bacteria: o Salmonella has two (2) different types of H Antigen Phase 1 Phase 2 KAUFFMAN-WHITE Classification o Classification scheme of Samonella spp. It is highly resistant to desiccation, heat, chemical agents. Martin Microbiological Environmental Chamber) – used for the optimum recovery of Neisseria gonorrhoeae. lacunata ACINETOBACTER A. Before 1983, three (3) Salmonella spp. pullorum Usually isolated from cold blooded animals and not from human or other warm blooded animals. existed: S. STEP 4: Allow the slide with smear to pass through a Bunsen burner or alcohol lamp flame about three times to heat fix the smear on the slide. Currently, Lyme disease is the most common arthropod borne disease in the US KNOWN VECTORS: o Ixodes dammini – northeastern, eastern and north central US Page 122 of 131 Study Guide Diagnostic Bacteriology 123 o Ixodes pacificus – northwestern area of the US o Ixodes ricinus – found in Europe LYME DISEASE – characterized by headache, low grade fever, fatigue, abdominal pain which may be mistaken for several other diseases such as rheumatoid arthritis, systemic lupus erythematosus, and viral meningitis STAGES OF LYME DISEASE: o STAGE ONE: appearance of the lesion erythema chronicum migrans after a tick bite in some but not all patients. especially in cerebrospinal fluid sample in cases of meningitis. This container is commonly known as PETRI DISH after its proponent Richard Julius Petri. Member of the HACEK group which are known cause of slowly progressive (subacute) bacterial endocarditis GENUS: Haemophilus KEY CHARACTERISTICS: Gram negative short bacilli/coccobacilli Non-spore forming Non-motile Facultative anaerobes Fermentative Capnophilic (5-7% CO_2) Page 101 of 131 Study Guide in Diagnostic Bacteriology Page 102 of 131 Common species Other Name H. kansasii M. Sulfide Indole Motility (SIM) Medium – used for observation of hydrogen sulfide gas production, indole production, and motility. SEPTI-CHEK (BD) Brucella Agar – contains the following additive: 5% horse or rabbit serum Pancreatic digest of casein Peptic digest of animal tissues Yeasts Sodium bisulfite rd 3 weeks onwards – serological tests – gold standard in the diagnosis of Brucella infection o Identification of febrile agglutinins – detects antibodies in patient's serum that react with antigen of killed Brucella o A fourfold rise in the antibody titer between the acute and convalescent sample is considered significant REQUIRES BIOHAZARD LEVEL III in handling specimens suspected with Brucella Page 103 of 131 Study Guide in Diagnostic Bacteriology Page 104 of 131 GENUS: Bordetella KEY CHARACTERISTICS: Gram negative short bacilli/coccobacilli Obligate aerobe Intracellular parasite Common species Bordetella pertussis Bordetella parapertussis Bordetella bronchiseptica Colonial Morphology Mercury droplets Brown colonies No specific color UREASE test + + Nitrate Motility Reduction Tests + + + + + + Oxidase Test + + Associated Diseases: Bordetella pertussis Whooping Cough or Pertussis Cough Bordetella parapertussis Milder form of whooping cough Bordetella bronchispetica Kennel's Cough PATHOGENIC DETERMINANTS OF Bordetella pertussis Pertussis toxin Filamentous hemagglutinin Cytotoxin Capsule Endotoxin STAGES OF PERTUSSIS INFECTION: 1. It does not kill bacterial spores and cannot be used as disinfectants Sporicidal – a disinfectant that acts upon bacterial spores Tuberculocidal - a disinfectant that acts upon Mycobacterium spp. YES NO Figure 7-1 Bacterial Growth Curve CULTURE MEDIA is an artificial preparation in the laboratory which contains basic foundation of nutrients and a solidifying agent (if needed) to support the growth of microorganisms. Mycobacterium terrae Also known as RADDISH BACILLUS May be complexed with Mycobacterium triviale forming Mycobacterium terrae-triviale Complex which grows slowly at both 25oC and 37OC 8. MEMBRANE BOUND ORGANELLES SITE OF PROTEIN SYNTHESIS SITE OF ENERGY PRODUCTION RIBOSOMAL SIZE Page 13 of 131 PROKARYOTE EUKARYOTE 0.2 - 2 µm in diameter 0.5 – 5 µm in length CIRCULAR; complexed with RNA **Except: Streptomyces spp. Cary-Blair Transport Medium – is a low nutrient medium and contains phosphate buffer instead of glycerophosphate which prevents bacterial overgrowth by Escherichia coli, Citrobacter freundii, Klebsiella aerogenes. Ethylene Oxide (ETO) gas – for large spaces and spaceships and other heat sensitive materials biological indicator: Bacillus subtilis var. Nocardia spp. hominis + U. Crick W. Calymmatobacterium granulomatis (Klebsiella granulomatis now) Causes a sexually transmitted disease (STD) – DONOVANOSIS – manifested by ulcerogranulomatous lesions of the skin and mucosa of the genital and inguinal areas. aerogenes and E. mimicus o NON-INOSITOL FERMENTER o SUSCEPTIBLE to vibriostatic agent O/129 (2,4 diamino-6,7-diisopropylpteridine) o STRING TEST positive (+) o Medically important species: Vibrio cholerae V. baumannii OLD NAME Acinetobacter calcoaceticus var anitratus KEY CHARACTERISTICS Obligate aerobe Non motile Oxidase negative DISTINCT Saccharolytic; acidifies most carbohydrates CHARACTERISTICS including glucose and xylose GROWTH ON BAP Large gummy, translucent, gray to white, convex, entire margin GROWTH ON MAC Bluish or peach to pink tint GROWTH ON EMB Cornflower blue colonies A lwoffi Acinetobacter calcoaceticus var lwoffi Obligate aerobe Non motile Oxidase negative Asaccharolytic Ammonia like odor DISEASES CAUSED BY Acinetobacter spp. The mere attachment causes diarrhea o STOOL: watery with mucus EnteroHEMORRHAGIC E. Zone sizes are not reported to physicians. Fever rises to a high plateau Rose spots (pale skin rashes on the chest & abdomen) may appear Specimen of Choice: URINE/STOOL o 3RD WEEK (Convalescence Stage) With proper antibiotic treatment, immune system would have developed antibodies against the bacteria Serologic tests are best done during this stage of the disease (Widal or Thypidot Test) Specimen of Choice: SERUM WIDAL Test – tube agglutination test using serial dilution to determine patient's antibody titer to antigenic determinant of the bacteria. DIRECT FLAME – direct application of flame in aseptic technique b. In 1969, Dr. Robert Whitaker formalized the classification of living organisms into five (5) kingdoms namely (1) Animalia (animals); (2) Plantae (plants); (3) Mycetae (Fungi); (4) Protista (mostly algae and protozoans); and (5) Monera (bacteria) (Figure 9). Presence of Organic Material - Blood, puss, and mucus are examples of organic materials that may prevent the full contact of the agent to the organisms, hence limiting its action. Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5 minutes, but no more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step STEP 3: PLACEMENT OF ANTIBIOTIC DISKS 1. METHYL RED (MR) TEST 7. Because of its slime layer, it can adhere to silicone implants such as prosthetic heart valves causing post open heart surgical endocarditis. When measuring zone diameters, always round up to the next millimeter. Conjunctival scarring and corneal vascularization leading to blindness Serotype D, E, F, G, H, I, J, and K Associated with diseases through venereal routes Infections include: o Non-gonococcal urethritis, epydidymitis, proctitis o Mucopurulent cervicitis, endometriosis, salpingitis o Neonatal pneumonitis and inclusion conjunctivitis Serotype L1, L2, L3 Associated with LYMPHO GRANULOMA VENEREUM (LGV) – disease transmitted through venereal routes Initial lesion is small, painless genital ulcer that may be unnoticed and spontaneously heal Disease progresses to lymphadenopathy of the inguinal lymph nodes. Biofilms – certain bacteria have to ability to form communities of layers of bacteria with protective shield which is called as biofim. cholerae 01 – agglutinate 01 anti-sera and produces a very potent enterotoxin CHOLERAGEN – responsible for the massive amount of water and electrolyte loss Characterized by "Rice Water Stool" – watery stool with gray mucin Has three (3) serotypes: o Inaba – Philippines o Ogawa – India o Hikojima – Japan Dehydration is usually the cause of death. Inhibitors: Thiosulfate, citrate, bile salts Differential: INDICATORS: Bromthymol Blue (BTB) Page 42 of 131 Study Guide in Diagnostic Bacteriology CHO Incorporated: Sucrose SUCROSE Page 43 of 131 fermentation ACID pH YELLOW Sucrose fermenter Non-sucrose fermenter BLUE-GREEN MANNITOL SALT (MSA) AGAR Original Color – light/salmon pink Selective: For: Staphyloccocus spp. testosteroni S. putrefaciens A. The two most commonly used differential stain in clinical microbiology laboratory are: GRAM STAIN – most routinely used differential stain in the microbiology laboratory. Table 6 summarizes the two common methods of acid fast staining, the corresponding reagent/s in each step, and key reactions of acid fast and non acid fast organisms. UTI Meningitis Respiratory Tract Infection Wound Infections associated with water & soil Bacteremia contamination Important cause of nosocomial and opportunistic infection because they can contaminate: Endotracheal tubes Dialysis Machine Respiratory care equipment Catheters Page 80 of 131 Study Guide in Diagnostic Bacteriology Page 81 of 131 ∞ SECTION 16 ∞ FAMILY ENTEROBACTERIACEAE Very large family hence classification and taxonomy has greatly changed over past recent years Currently, there are 27 recognized genera and more than 100 species and subspecies Found in soil and water, vegetables Also found as normal flora of the gastrointestinal tract of humans and animals Significant human pathogen and common clinical isolate Causes wide array of infections in humans, most common are UTI, gastroenteritis and septicemia Key Characteristics: Gram negative (-) non-spore forming bacilli and coccobacilli Facultative Anaerobe Some possess pili or fimbriae which they use for host cell attachment ALL are GLUCOSE FERMENTERS ALL reduces Nitrate (NO_3) to Nitrite (NO_2) except Pantoea agglomerans ALL are CATALASE (+) positive except Shigella dysenteriae All are motile because of their peritrichous flagella except Shigella and Klebsiella and @ 370C Yersinia All are cytochrome oxidase (-) negative Generally aerogenic except Shigella, the following may or may not be aerogenic: o S – Salmonella o S – Serratia o P – Proteus o P – Providencia o K – Klebsiella Can be differentiated based on Lactose Fermentation: o RLF Escherichia, Enterobacter, Klebsiella freundii o o LLF Hafnia, Serratia, Citrobacter Salmonella arizonae Shigella sonnei Yersinia enterocolitica koseri/diversus NLF ALL Salmonella except S. Figure 6: The Koch's postulate which was used to prove the cause and effect of infectious diseases. Development of Additional Microbiological Tools Richard Julius Petri developed in 1887 a double dish with the upper dish slightly larger than the bottom dish. avium-intracellulare M. Diagnosis is done through TISSUE BIOPSY o DONOVAN BODIES – small, straight or curved, pleomorphic bacilli with rounded ends with characteristic polar granules, giving a safety pin appearance within mononuclear cells 4. H Antigen – associated with the flagella 3. In principle, the higher the number of organisms, the longer the exposure time needed to eliminate 99.9% of the microorganisms. Causes: Turista Montezuma's Revenge Tokyo Two-step Delhi Belly EnteroPATHOGENIC E. Chemotherapy For so long, doctors tried to treat infection using chemicals such as mercury and arsenic for syphilis and bark tree quinine for malaria but they were also toxic to the host which eventually caused patients' deaths. shigelloides - only medically important species Carried by most cold blooded animals such as amphibians Found in soil and water Human infection is acquired ingestion of contaminated or unwashed food. Common species: o Legionella pneumophila Legionnaire's Disease/PONTIAC FEVER o Legionella micdadei – Pittsburg Pneumonia/Broad St. Pneumonia o Legionella bozemanii – Wiga Agent of Pneumonia Page 102 of 131 Study Guide in Diagnostic Bacteriology Page 103 of 131 IDEAL MEDIA and METHODS for IDENTIFICATION: Buffered Charcoal Yeast Extract Agar (BCYE) – contains L-cysteine, activated charcoal, alpha-ketoglutarate Growth in BCYE is circular, glistening, and convex with an entire margin. View the plate using a direct, vertical line of sight to avoid any parallax that may result in misreading. PYRAZINAMIDASE TEST Important test in differentiating M. tularensis – TYPE A – most severe; all forms of tularemia Francisella tularensis subsp. STEP 5: INTERPRETATION AND REPORTING OF RESULTS 1. Flulike symptoms such as runny nose & infrequent coughing c. typhi (most common cause of typhoid fever) S. Inoculate the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculum. FUNCTIONS: primarily it also serves as a form of protection from phagocytosis, or in some instances, it helps the bacteria to adhere to host tissues or synthetic implants such as prosthetic heart valves. Borrelia burgdorferi NUCLEOID 7 – 100 µm in diameter > 10 µm in length LINEAR; complexed with basic histones & other proteins Made up of PEPTIDOGLYCAN (aka murein or mucopeptide layer) in most bacteria. To add disks one at a time to the agar plate using forceps, place the MH plate on the template. Spore forms may need more contact time than its vegetative counterpart. Enterococcus spp. After 2-3 weeks incubation, the disease begins with a mild respiratory infection that later be associated with fever, headache, malaise, and a persistent, dry, nonproductive cough. such as Clostridium perfringens, Bacteroides fragilis, most strains of Proprionibacterium and Lactobacillus CAPNOPHILIC – bacteria that requires 5%-10% CO_2 to grow Neisseria spp., Haemophilus spp., Aggregatibacter spp., Cardiobacterium spp., Eikenella spp., and Kingella spp. Also known as Woolsorter OR Ragpicker's Disease Intestinal/Gastrointest inal – most serious form. Inhibitors: High concentration of salts (7.5%) Differential: INDICATORS: Phenol Red CHO Incorporated: Mannitol fermentation ACID pH Mannitol fermenter YELLOW Non-Mannitol fermenter DARK PINK MANNITOL LOWENSTEIN JENSEN (LJ) MEDIUM Original Color – light green Selective: For: Mycobacterium spp. The recent discovery of his writings referred to as the "Hooke Folio" dated November 1677, confirmed Antoni Van Leeuwenhoek's findings (Gest, 2007). The commonly used simple stains are Crystal Violet, Gentian Violet, Methylene Blue, Malachite Green DIFFERENTIAL STAIN – contains two or more chromogens which further differentiate specific component within the bacterial cell which aids in the differentiation or grouping of bacteria. Page 24 of 131 Study Guide in Diagnostic Bacteriology Page 25 of 131 FUNDAMENTALS OF SPECIMEN COLLECTION Failure to recover the bacterial pathogen from clinical specimens may result from faulty and improper specimen collection and processing. There are two (2) forms: o CAPSULE – uniform and condensed organized material that is firmly attached to the cell wall of the bacteria. INDOLE TEST HsS Indicator: Ferrous ammonium sulfate Amino Acid: tryptophan Page 85 of 131 Study Guide in Diagnostic Bacteriology Page 86 of 131 6. In 1876, he initially worked on Bacillus anthracis, causative agent of anthrax, by inoculating healthy mice with the bacillus and its spores. tuberculosis complex are ALL HEAT STABLE CATALASE (-) which includes: Mycobaterium tuberculosis Mycobacterium bovis Mycobacterium africanum Mycobacterium microti Mycobacterium canett Page 108 of 131 Study Guide in Diagnostic Bacteriology Page 109 of 131 Page 109 of 131 Study Guide in Diagnostic Bacteriology Page 110 of 131 6. STAIN FOR CELL WALL o Victoria Blue Dye – cell wall stains blue STAINS FOR CAPSULE o HISS - capsule stains PALE BROWN o TYLER - capsule stains LIGHT VIOLET o MUIR - capsule stains LIGHT BLUE o GIN – capsule is unstained bt the bacteria will be stained with its margins delineated by the ink o WADSWORTH – capsule stains PINKISH and bacteria stains BLUE o WELCH – capsules stains PALE VIOLET STAINS FOR METACHROMATIC GRANULES/BABES ERNST BODIES/VOLUTIN o Loeffler's Alkaline Methylene Blue (LAMB) – granules stain RED o Albert – granules appear BLUE BLACK o Neisser – granules appear DARK BLUE o Lindegran - granules appear REDDISH BROWN o Burke's Technique – a modified gram's staining technique o Ljubinsky – granules stain DARK VIOLET STAINS FOR BACTERIAL SPORES/ENDOSPRES o Fulton-Schaeffer – spores are GREEN o Dorner – spores are RED o Wirtz-Conklin - spores are GREEN STAINS FOR FLAGELLA o Leifson o Gray o Silver o Fisher-Conn TANNIC ACID – important component in flagellar stain which coats, swells, precipitates the flagella enhancing its visualization STAINS FOR RICKETTSIA o Castaneda – stains BLUE o Machiavelo – stains RED o Giemsa – stains BLUE STAINS FOR CHLAMYDIA o Gimenez – elementary bodies stains RED o Machiavelo – stains RED o Giemsa – stains PURPLE o o STAINS FOR SPIROCHETES Fontana-Tribondeau – spirochetes stains DARK BROWN or BLACK Levaditi Silver Impregnation - spirochetes stains BLACK Page 27 of 131 Study Guide in Diagnostic Bacteriology o Page 28 of 131 India Ink Negative Stain – spirochetes are UNSTAINED; background is BLACK STAINS FOR MYCOPLASMA o Dienes – stains BLUE STAIN FOR BIPOLAR BODIES (Yersinia pestis) o Wayson – bipolar bodies stain RED INDIRECT/NEGATIVE/RELIEF STAINING – a type of staining which actually provides coloration to the background of the smear while rendering the bacteria and covering structure such as capsule unstained. This is also used a base medium in the preparation of other culture media EXAMPLES: Nutrient Broth (NB), Nutrient Agar (NA), Trypticase Soy Broth (TSB), Trypticase Soy Agar (TSA), and Brain Heart Infusion (BHI) ENRICHED CULTURE MEDIA – contains the basic nutritional requirements to support the growth of nonfastidious microorganisms with additives, enriching substances, or supplements to support the growth of fastidious microorganisms. If it is determined by repeat testing that the phenomenon repeats itself, the organism must be considered resistant to that drug. However, Kinyoun method is more commonly used today which uses higher concentration of phenol in the primary stain solution which aids in the better penetration of the primary stain (Forbes et.al., 2007) Table 6 Summary of Acid Fast Staining Technique: Reagent/s and Reactions C KEY STEPS (ZIEHL-NEELSEN METHOD) Primary/Initial Staining H Mordanting A Decolorization/Differentiation M C Counterstaining KEY STEPS (KINYOUN METHOD) Primary/Initial Staining T Mordanting A Decolorization/Differentiation M Counterstaining CODE CODE REAGENT/S CARBOL FUCHSIN PHYSICAL: HEAT ACID ALCOHOL (3% HCl in 95% Ethanol) METHYLENE BLUE REAGENT/S CARBOL FUCHSIN CHEMICAL: TERGITOL ACID ALCOHOL (3% H_2SO_4 in 95% Ethanol) METHYLENE BLUE Page 16 of 131 RED NON-ACID FAST RED RED RED 2 MINUTES (rinse) RED COLORLESS 1 MINUTE (rinse) RED DURATION/TIME ACID FAST RED BLUE NON-ACID FAST RED RED RED 2 MINUTES (rinse) RED COLORLESS 1-3 MINUTEs (rinse) RED BLUE DURATION/TIME 4-5 MINUTES (rinse) 5 MINUTES (rinse) ACID FAST Study Guide in Diagnostic Bacteriology Page 17 of 131 OTHER ACID FAST ORGANISMS A modification of the acid fast staining procedure may be done for partially or weakly acid fast bacteria such as Nocardia spp., Rhodococcus spp. Exposure should be limited to 15 minutes as it will kill Mycobacteria as well CULTURE MEDIA o PRIMARY ISOLATION MEDIA (egg based media) Lowenstein-Jensen – coagulated whole eggs and malachite green as inhibitor (0.025 g/dL) Petragnani - coagulated whole eggs and malachite green as inhibitor (0.052 g/dL), MORE inhibitory than the other, used for HEAVILY CONTAMINATED specimens American Thoracic Society - coagulated whole eggs and malachite green as inhibitor (0.02 g/dL) - LESS inhibitory than the other, used for STERILE specimens o MEDIA for ANTIBIOTIC SUSCEPTIBILITY TESTING (transparent media – agar based) Middlebrook 7H10 – salts, vitamins, cofactors, oleic acid, albumin, catalase, biotin, glycerol, GLUCOSE, malachite green as inhibitor (0.0025 g/dL) Middlebrook 7H11 – salts, vitamins, cofactors, oleic acid, albumin, catalase, biotin, glycerol, CASEIN HYDROLYSATE, malachite green as inhibitor (0.0025 g/dL) o MTB Cultures are maintained for 8 weeks (2 months) before reporting as negative or no growth o MTB Colonies – tan to buff (non-pigmented), dry, rough, warty, granular with cauliflower appearance STAINING METHODS o ACID FAST STAIN Ziehl Neelsen Technique (HOT METHOD) Kinyoun Technique (COLD METHOD) o FLUORESCENT STAIN – more sensitive but more difficult and expensive to perform Auramine O – primary stain Page 112 of 131 Study Guide in Diagnostic Bacteriology Page 113 of 131 Rhodamine – Counterstain MTB – yellow green bacilli SEMIQUANTITATIVE METHOD OF REPORTING FOR ACID FAST BACILLI (AFB) o Center for Disease Control (CDC) Method – by oil immersion field (OIF) NUMBER OF AFB SEEN REPORT 0 NEGATIVE (-) + / - (repeat using new 1-2 AFB/300 OIF specimen) 1-9 AFB / 100 OIF 1+ 1-9 AFB / 10 OIF 2+ 1-9 AFB / OIF 3+ MORE THAN 9 AFB / OIF 4+ o National Tuberculosis Association Method – by slide NUMBER OF AFB SEEN REPORT 0 NEGATIVE (-) Report #, repeat using new 1-2 AFB/slide specimen 3-9 AFB/slide Rare or 1 + >9 AFB/slide Few or 2 + ≥ 1 AFB / OIF Numerous or 3 + o American Lung Association(ALA) Method – by smear/slide NUMBER OF AFB SEEN REPORT 0 NEGATIVE (-) doubtful, repeat using new 1-2 AFB/smear specimen 3-9 AFB/smear Rare or 1 + >10 AFB/smear Few or 2 + ≥ 1 AFB / OIF Numerous or 3 + KEY LABORATORY IDENTIFICATION: o Grows well between 35oC to 37oC in 14-21 days in LJ and Middlebrook media o DO NOT grow at 25oC and 42oC to 45OC o NIACIN TEST (+), NO_3 Reduction (+), Pyrazinamidase (+), BUT HEAT STABLE CATALASE (-) o MTB Colonies – tan to buff (non-pigmented), dry, rough, warty, granular with cauliflower appearance 2. pallidum o Treponema pallidum subspecies pertenue Agent of YAWS – chronic, non-venereal disease of the skin and bones MOT: direct contact with open skin lesions INITIAL LESIONS appear 3-4 weeks after exposure, heals spontaneously, and reappears as secondary lesion months later SECONDARY LESIONS ulcerate, heals, and re-appear in crops for several years TERTIARY LESIONS can occur in the skins and bones leading t disfiguration of the face o Treponema pallidum subspecies endemicum Agent of BEJEL – endemic, non-venereal syphilis MOT: poor sanitation or poor personal hygiene, such as through sharing of eating or drinking utensils PRIMARY LESIONS – occur in the oral cavity SECONDARY LESIONS – occur in the oral mucosa TERTIARY LESIONS – widespread and may occur in the skin, bones, nasopharynx o Treponema pallidum subspecies carateum Agent of PINTA – ulcerative skin disease MOT: direct contact with infected lesions SKIN LESIONS – flat red and become depigmented but do not ulcerate Lesions remain confined in the skin unlike Bejel and do not disseminate to the bones ANTIBIOTIC of CHOICE: PENICILLIN o JARISCH-HERXHEIMER SYNDROME – an adverse reaction with penicillin treatment exhibited by fever, body pain/malaise, vomiting, headache, etc. ONPG TEST MEDICALLY IMPORTANT ENTEROBACTERICEAE SPECIES: Escherichia coli (E. Air: High Efficiency Particulate Air Filter (HEPA) – has a pore size of 0.3 µm; usually used in Biological Safety Cabinet (BSC) and rooms of immunocompromised patients II. marinum GROUP III: NONPHOTOCHROMOGENS – white to tan in color; CANNOT develop pigment on exposure to light M. For the purpose of guidance and uniformity, the following guidelines were employed in this material. o a. A bacterial smear is a dried preparation of bacterial cells in a clean glass slide. paraprophilus H. Semmelweis In 1850, Dr. Semmelweis shared his very simple observation to his colleagues during a lecture that a maternity clinic which he used for his obstetrics instruction has four times higher death rate compared to the other clinic that he supervises. Growth on selective & differential medium: TINSDALE Agar - Corynebacterium diphtheriae grows black colonies due to tellurite reduction CYSTINE TELLURITE & POTASSIUM TELLURITE, & MAC LEOD MEDIUM - grows gray-black colonies with due to tellurite reduction 4 BIOTYPES or SUBPECIES based on COLONIAL VARIETIES C. 5. Page 8 of 131 Study Guide in Diagnostic Bacteriology Page 9 of 131 Louis Pasteur While working with diseases anthrax and fowl cholera, he discovered that bacterial culture can be rendered "nonvirulent." He coined the term attenuated to describe weakened strains of microorganisms which did not cause disease when injected in healthy test animals. bovis and M. Fitz Hugh Curtis Syndrome – perihepatic infection of the liver by Neisseria gonorrhoeae Page 78 of 131 Study Guide in Diagnostic Bacteriology Page 79 of 131 2. o FLAGELLA (plural) or FLAGELLUM (singular) – exterior protein filaments or whip-like projections which is embedded in the cell envelope with a motor attached in a basal body responsible for its propeller-like rotation of the flagella which makes bacteria move. Bauer, Kirby, Sherris, and Truck standardized an anitbiotic susceptibility method known as disk diffusion method. based on O & H Antigens o Based on this scheme, there are 2200 serovar (serotypes) of Salmonella o PROTOCOL for this scheme: Salmonella isolates should be tested with polyvalent O antisera If positive (+), specific monovalent test should be done If negative (-), boil the suspension for 15 mins and retest Serogroup determined Heating destroys the capsular antigen which may block the reactivity of O Antigen Serotype should be found usually by a reference laboratory IMPORTANT LABORATORY TESTS IN THE DIAGNOSIS OF ENTEROBACTERIACEAE INFECTION 1. Simply, concentrated agents do not necessarily mean that it would work better. Inhibitors: Malachite Green SPUTUM SAMPLE needs to be: 1. - Darting Motility Listeria monocytogenes – Tumbling Motility 2. entiritidis In 1983,it was determined that only one (1) species existed, S. Used for the presumptive identification of Escherichia coli and Streptococcus anginosus group; Enterohemorrhagic E. Sterile – free from microorganisms Factors that Affect the Degree of Killing of Microorganisms Types of Organisms – different organisms have varying ability in withstanding and chemical and physical treatment due to the different biochemical composition of these organisms and various mechanisms that they use to protect themselves. vulnificus + - - + - GENUS: Plesiomonas KEY CHARACTERISTICS: o gram negative (-) pleomorphic bacilli that may be seen in singly, in pairs, or in chains or even in long filamentous forms o facultative anaerobe o oxidase positive (+) o motile o STRING TEST negative (-) o INOSITOL FERMENTER o P. The rules of bacterial nomenclature are governed by the International Code for Bacterial Nomenclature, with changes and additions published in the International Journal of Systematic Bacteriology. szulgai, M. o GENUS CHLAMYDIA Formerly known as BEDSONIA which means large virus. coli (+) for K Antigen S. In general, bacterial shape exists in three (3) major forms namely (1) spherical or rounded bacteria which is called as COCCI (plural) or COCCUS (singular); (2) rod or cylindrical shaped called as BACILLI (plural) or BACILLUS (singular); and (3) helical or twisted in shape called as SPIRALS. Table 7-4 presents examples of tissue culture media used in microbiology laboratory Table 7-4 Tissue Culture Media Used in Microbiology Laboratory TISSUE CULTURE MEDIA Vero Cell Line Mc Coy Cell Line Chicken Embryo A549 Cells HELA Cell Line Hep-2 Cell Line SOURCE Kidney cells of an African Green Monkey Mouse Cell line Fertilized chicken egg Human lung carcinoma Human cervical carcinoma Human epithelial cells of larynx carcinoma CLASSIFICATION OF CULTURE MEDIA ACCORDING TO PHYSICAL STATE/CONSISTENCY LIQUID – a culture medium that doesn't contain a solidifying agent. The broth has to be subcultured every 6 to 8 hours of incubation for it to work effectively (Gilligan, Janda, Karmali, & Miller, 1991) Todd-Hewitt Broth – a liquid enrichment recommended for the production of Streptococcal haemolysin and the cultivation of streptococci prior to serological grouping. Meningococcemia – presence of Neisseria meningitides in the blood with or without meningitis Acute Chronic PATHOGENIC DETERMINANTS OF Neisseria gonorrhoeae 1. PAROXYSMAL STAGE a. gergoviae and E. are the source of antigen for the Weil-Felix Reaction (serologic test for the diagnosis of Rickettsial diseases) because it shares common polysaccharide o P. Borrelia hermisii and Borrelia parkerii Agent of TICK borne RELAPSING FEVER VECTOR: Ornithodoros hermisii and Ornithodoros parkerii IMPORTANT LABORATORY TESTS: Grows well in modified Kelly's Medium – Barbour Stoenner-Kelly Medium (BSK-II) in 7-14 days between 30-35oC THICK BLOOD FILMS – preferred due to low number # of organisms in the blood. These molecules interact with cells and non cellular materials through ionic bonds which results to the cells or specific material being dyed or stained with the color of the chromogen. Ionizing Radiation- works by alkylation of nucleic acid of bacteria using high energy short wavelength deep penetrating gamma rays; used for heat sensitive materials biological indicator: Bacillus pumilis 4. It is important to consider that biofilm formation may require longer contact time or increase in the concentration of the agent. These dyes remain in the cell wall of acid fast organism even after decolorization. chelonae, M. Flagellar Stains Leifson Gray Silver Fisher-Conn TANNIC ACID – important component in flagellar stain which coats, swells, precipitates the flagella enhancing its visualization GENOTYPICAL CHARACTERIZATION - means of identifying bacteria using its genetic material (DNA/RNA) using more advance and sophisticated technologies which are accurate and specific however, these methods are quite costly and would require special knowledge and skills to be performed. TRANSGROW – also used for the optimum recovery of Neisseria gonorrhoeae. Penetrability - the antibiotic must penetrate well into the tissues and even cross the blood-brain barrier which allows the treatment of abscesses and central nervous system infections. trachomatis antibody exist in general population Complement fixation – useful in the diagnosis of Chlamydophila psittaci ELISA Direct Fluorescent Antibody (DFA) RICKETTSIAE Includes the genus Rickettsia, Ehrlichia, Coxiella, and Rochalimea (now part the Genus Bartonella) Gram negative obligate intracellular bacteria Important Point to remember: o All are transmitted by VECTOR ONLY except Coxiella which can also be spread through AEROSOL o All CANNOT SURVIVE outside a living animal host or vector EXCEPT Coxiella o All requires tissue culture media EXCEPT Rochalimea (Bartonella) quintana CLINICALLY SIGNIFICANT RICKETTSIAE GROUP SPECIES Rickettsia rickettsi Spotted fever Typhus Scrub typhus Q Fever Ehrlichiosis Rochalimea (Bartonella) INFECTION Rocky Mountain Spotted Fever (RMSF) Rickettsia akari Rickettsial POX Rickettsia australis Australian Tick Typhus Rickettsia conorii Boutonneuse Fever Rickettsia prowazekii Epidemic typhus Sporadic Typhus Brill-Zinsser Disease (Recrudescent typhus) Rickettsia typhi Murine typhus Rickettsia (Orientia) Scrub typhus tsutsugamushi Coxiella burnetii Q Fever Ehrlichia canis Ehrlichiosis Ehrlichia chaffeensis Ehrlichia equi Ehrlichia sennetsu Rochalimea Quintana Trench fever TRANSMISSION Ticks Mites Ticks Ticks Lice Flying squirrels Reactivation of latent infection Fleas Chigger bites (mite) Ticks, aerosols Ticks Lice IMPORTANT LABORATORY TEST o Culture in Tissue Culture Media Chicken embryo "BALUT" HeLa or Vero Cell Line o Manifestation of characteristic Fox Like Lesions called "ESCHAR" o Serological test – presumptive only, lacks specificity WEIL-FELIX REACTION – detects antibodies to rickettsial infection that agglutinates antigens from Proteus species: Proteus vulgaris – OX-2 and OX-19 Proteus mirabilis – OX-K o Indirect Immunofluorescent Antibody (IFA) Assay – considered as gold standard in the diagnosis of Rickettsioses GENUS BARTONELLA formerly Rochalimea Gram negative (-) short bacilli Fastidious and grows well in CAP but not in MAC Grows slowly; average of 12-14 days but may take as long as 45 days OXIDASE (-) Page 129 of 131 Study Guide Diagnostic Bacteriology 130 Important members o Bartonella quintana – louse borne disease – agent of TRENCH FEVER o Bartonella henselae – recognized as the primary cause of Cat-scratch disease (CSD) Afipia felis – another suspected agent of CSD but currently doubtful due to inability of infected patients to mount an immune response against the organism and lack of established procedures and methods for isolation. The terms FIMBRIAE (Latin, fringe) and PILI (Latin, hairs) are commonly used synonymously (Brinton, 1965; Duguid & Anderson, 1967). paratyphi A/B Most common cause of bacteremia - S. Figure 10: Scheme of classifying microorganisms by Dr. Carl Woese. faecalis A. However, their activity may increase at a certain degree by a corresponding increase in temperature or may decrease when temperature is decreased. A modified Thayer-Martin agar is prepared in the inner flat surface of the bottle under CO_2. cholerae and V. His family survived a smallpox epidemic (Beck, 2000). Mousy/Mouse Nest DENSITY – optical property to pass light through the bacterial colony COLONY DENSITY DESCRIPTION Light won't shine through the colony Opaque Page 29 of 131 Study Guide in Diagnostic Bacteriology Transluscent Transparent Page 30 of 131 Light will shine through the colony Light shines through the colony BIOCHEMICAL CHARACTERISTICS Enzyme Production BACTERIAL ENZYME DETECTED MECHANISM OF DETECTION APPLICATIONS Coagulase Forms CLOT in rabbit's plasma Differentiates pathogenic from non pathogenic staphylococci β-galactosidase Hydrolyzes orthonitro-phenylgalactopyranoside substrate producing a YELLOW colored product orthonitro phenol Differentiates late lactose fermenting members of Family Enterobacteriaceae from non lactose fermenting members. coli (EIEC) o Invades or colonize the intestinal epithelium o produces Shiga-like toxin hence causing SHIGELLA-LIKE DIARRHEA o STOOL: watery with blood, puss, and mucus EnteroAGGREGATIVE E. They did not get smallpox. Colonies may appear as "water droplets" in Middlebrook media o Can be differentiated from MTB because it is NIACIN TEST (-), NO_3 Reduction (-), Pyrazinamidase (-) 3. – causative agent of RELAPSING FEVER and LYME DISEASE Page 23 of 131 Study Guide in Diagnostic Bacteriology Page 24 of 131 Tightly twisted spiral resembling a cork screw E.g. Treponema pallidum - causative agent of VENEREAL SYPHILIS Tightly twisted spiral with one or both ends bent into a hook, sometimes even resembling an interrogative symbol E.g. Leptospira interrogans - causative agent of zoonotic infection LEPTOSPIROSIS PART 2 GENERAL AND BASIC PRINCIPLES IN BACTERIAL IDENTIFICATION ∞ SECTION 6 ∞ ROUTINE SPECIMEN PROCESSING The accuracy of the test results reported by the clinical microbiology laboratory puts heavy emphasis on the integrity of the processing of the clinical specimen which is the primary source of the pathogen. Page 87 of 131 Study Guide in Diagnostic Bacteriology Page 88 of 131 o Uses PILI to attach and colonize the intestinal epithelium. gonorrhoeae N. EXAMPLES: Stuart's Transport Medium – a non-nutritional semi-solid substrate for the preservation of Neisseria species and other fastidious organisms such as Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes, and Corynebacterium diphtheriae. Sanger Kary Mulis The Institute for genomic Research (TIGR) Modern Microbiology: The Quest Continues Today, microbiology laboratory is a fusion of traditional techniques developed in the late 1800s and the newer molecular methods. The specimen is inoculated directly into the medium, and the moisture inside dissolves the bicarbonate tablet to create the CO_2 atmosphere. Grows well in Nine-Banded Armadillo or Mouse Footpad MOT: UNKNOWN but believed to be: o Person to person spread VIA aerosolization o Direct skin contact or via penetrating wounds, such as thorns or the bite of an arthropod o Breast milk from lepromatous mother o Transplacental transmission also possible TREATMENT: SULFONE & DAPSONE TWO TYPES of LEPROSY o LEPROMATOUS/NODULAR TYPE Widespread anergic form of the disease Progressive or disseminated type In person who lacks the specific cell mediated immunity to these antigens, results to spread of the infection all over the body Characterized by cutaneous lesions ranging from diffuse generalized skin involvement to widespread symmetrically distributed nodules called LEPROMAS filled with the bacilli Lesions usually seen in COOLER parts of the body such as anterior third of the eyes, nasal mucosa, superficial peripheral nerve trunks MANY/PLENTY organisms found in AFB stain of the skin lesions LEPROMIN TEST (-) o TUBERCULOID/ANESTHETIC TYPE Localized form of the disease Non-progressive or localized type Usually seen in person with effective cell mediated immunity leading to resistance of the infection Characterized by few, well-circumscribed anesthetic macules or plaques, accompanied by enlargement of the peripheral nerves near the skin lesions FEW/SCARCE found in AFB stain of the skin lesions LEPROMIN TEST (+) Page 115 of 131 Study Guide in Diagnostic Bacteriology Page 116 of 131 GENUS BACILLUS KEY CHARACTERISTICS: Gram positive large SPORE FORMING BACILLI Aerobe/ Facultative Anaerobe CATALASE + Arranged in CHAINS in smear resembling a bamboo fishing pole arrangement Common species Bacillus anthracis Biohazard Level III Bioterorrism Agent Bacillus cereus Agent of Fried Rice Poisoning Bacillus subtilis Associated Disease/s ANTHRAX (Greek word: Coal) – zoonotic infection 3 FORMS Pulmonary/Inhalationa l – MOT: Inhalation of spores while sorting wool. kansasii (NEGATIVE -) PYRAZINAMIDE PYRAZINAMIDASE Pyrazinoic acid 10. ENDOTOXIN PATHOGENIC DETERMINANTS OF Neisseria meningitidis 1. Table 9: Bacilli Morphologies Prominent Arrangement Illustration Single Bacillus - rod shaped bacillus Diplobacillus – bacillus in pair Streptobacillus – bacillus in chains Coccobacillus – bacillus that are small, short, stout/plump Page 21 of 131 Study Guide in Diagnostic Bacteriology Page 22 of 131 Small and short bacillus arranged in school of fish, rail road track, or fingerprint pattern in stained smear E.g. Haemophilus ducreyi – causative agent of SOFT CHANGRE/CHANCROID Large, square cut-ends, spore forming bacillus, arranged in chains E.g. Bacillus anthracis – causative agent of ANTHRAX Large, rounded ends, non spore forming bacillus, arranged in chains E.g. Fusobacterium spp. Development of Resistance – the antibiotic must not induce development of resistance which may result due to mutation of the organism such as production of drug inactivating enzymes, alteration of drug targets, and alterartions in the mechanism of drug uptake. (+) and Clostridium spp. Table 3 tabulates the general characteristics of prokaryotic organism vis-à-vis eukaryotic organism. Replace the lid to minimize exposure of the agar surface to room air. In 1977, an American Microbiologist, Dr. Carl Richard Woese defined a new domain, or kingdom of life, the Archaea, based on his work sequencing the 16s ribosomal RNA. jejuni is RESISTANT) GENUS: Helicobater Common species: Helicobacter pylori (formerly known as Campylobacter pylori) Causes Type B gastritis, peptic and duodenal ulcers. Plasma Drug Concentration Toxicity – the agent must be administered in the plasma of the blood even at high concetration necessary to eliminate the organisms with no adverse effects to the host. It was not after 200 years that the origin and the capability of these microorganisms to cause human disease are described in detail. These are usually perception of the patient having the disease such as headache, dizziness, etc. phlei M. He developed a solid media using gelatin but this can't be incubated at temperatures higher than 300C. It is discussed in detail in Table 7-1. These may accumulate, precipitate out, and form an inclusion body which is not bounded by a membrane freely floating in the cytoplasm of the bacterial cell. ROUTES OF INFECTION: o Scratch, bite, lick of an infected animal, usually a cat causing: PASTEURELLOSIS – wound infection Characterized by pain, redness, swelling, cellulitis, and a purulent discharge Rapid onset of inflammation Fever developing 3-5 days after infection May complicate to osteomyelitis, meningitis, and joint infection. It was suggested that they should wash their hands before handling patients. Lift the dispenser off the plate and using forceps sterilized by either cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure complete contact with the agar surface. o d. Figure 12: Gram Positive and Gram Negative Bacterial Cell Wall

Structure Table 4 highlights the unique or different structure or features in a gram positive and gram negative cell wall for easy reference. Using a sterile inoculating loop or needle, touch four or five isolated colonies of the organism to be tested. Page 13 of 131 Study Guide in Diagnostic Bacteriology Page 14 of 131 Figure 11: Typical Bacterial Cell Structure BACTERIAL CELL WALL Functions of Bacterial Cell Wall Provides shape and rigidity to the bacterial cell. None of the various substances found in the plasma or the gastrointestinal system inactivate the drug. cholerasuis which wasc then subdivided into seven (7) subspecies Arizonae, once considered as separate genus is now a Salmonella subspecies Table 14-1 Salmonella Nomenclature Summary Salmonella Subgroup 1 Former Genus Salmonella Subspecies KEY NOTES Cholerasuis   2 3a 3b 4 5 6 Salmonella Arizona Arizona Salmonella Salmonella Salmonella Salamae Arizonae Diarizonae Houtenae Bongar Indica Usually isolated from humans Highly pathogenic to humans Almost all clinical isolates of Samonella are from this group Includes S. Between the two methods, ZIEHL-NEELSEN method is preferred over the KINYOUN method because of its higher sensitivity in detecting acid fast bacilli (Somoskovi et. The first step towards ensuring that these results are reliable and accurate begins with proper collection of the clinical specimen, proper handling and timely transport to the laboratory. BLOOD AGAR PLATE (BAP) – used to support the growth of fastidious organisms and to exhibit hemolytic properties of bacterial pathogens. Addition of polymyxin to this medium favors the isolation of Neisseria gonorrhoeae. 1. He initially used potato slices but not all microorganisms grew in them. Lysine Iron Agar (LIA) - used for the determination of bacterial pathogen's ability to decarboxylate or deaminate lysine. Cell Wall Proteins CW Protein I CW Protein II CW Protein III 4. diphtheria biotype gravis C. gallinarum and S. o A fourfold rise in antibody titer between the acute phase and convalescent phase specimens is diagnostic for CURRENT INFECTION o Also, an antibody titer of greater than 1:128 is diagnostic of a current infection as well. maltophilia Gram – short bacilli/coccobacilli Motile Aerobic Fermentative Oxidase (-) Non- Lactose fermenter ONPG (-) COMAMONAS SHEWANELLA ALCALIGENES C. Ammonia like Nocardia spp. Table 7-3 provides an example of the components of this type of media. Use this suspension within 15 minutes of preparation. It can also detect glucose fermentation, sulfide production and gas production. N-acetyl-L-cysteine (NALC) is usually used. colonies appear as white colonies resembling a snowflake surrounded by brilliant red medium THIOSULFATE CITRATE BILE SALTS SUCROSE (TCBS) AGAR Original Color – light green/olive green Selective: For: Vibrio spp. cinerea N. Table 3: Comparative Characteristics of Prokaryotes and Eukaryotes Page 12 of 131 Study Guide in Diagnostic Bacteriology CRITERIA OF DIFFERENTIATION 1. Meningitis Serotype A – meningitis belt in Africa; epidemic meningitis during hot & dry seasons Serotype B – common in US & Europe Serotype C - common in US & Europe Serotype Y - common in US & Europe Serotype W 135 – invasive strain; epidemics worldwide 2. After the standard incubation of 18-24 hours, inoculated plates are retrieve from the incubator and the colonial or cultural characteristics of the bacterial colonies that grew in each culture media for each specimen is examined, this is referred to as PLATE READING. Mycobacterium marinum Literally means "OF THE SEA" Associated with skin infections occurring as red to blue lesions Infection acquired by contact to poorly chlorinated or unchlorinated fresh or salt water May be harbored in swimming pools, fish aquariums, and water cooling towers Causes SWIMMING POOL GRANULOMA – more serious form of infection. However, the enormous use of penicillin as an antimicrobial agent was not realized until 1940 where it was purified by Ernst Chain and subjected to clinical trials by Howard Florey. These microorganisms are found in extreme condition environments such as high temperature, high osmotic pressure, high acidity, and high salt concentration. ENRICHMENT BROTH – a primary media used to support or favor the selective growth of pathogens in a specimen, such as stool or sputum, where the number of normal flora outnumber the pathogens. parahaemolyticus X Factor Normal Flora URT V Factor BAP (Sheep) + + + + + - + + - + + + + + + + - BAP/CAP Porphyrin (other sources Test (D-ALA – horse blood) Test) + + + + + + Associated diseases: H. Agar content is increased because charcoal interferes with the gelling properties of the medium. SELECTIVE/SPECIAL – stains that specifically highlight or emphasize certain bacterial cell structures or components which aids in the presumptive identification of the bacteria. VECTOR: human louse – Pediculus humanus subspecies humanus 2. o e. However, the scheme proposed in Bergey's Manual of Systematic Bacteriology is the most widely accepted system (Boone & Castenholtz, 2001). Incubation period ranges from 7-10 days b. Domestic cats and dogs are common reservoir but also harbored by rodents, rabbits, birds. Page 11 of 131 Study Guide in Diagnostic Bacteriology Page 12 of 131 The rules governing taxonomy are fairly rigid, and therefore debate is inevitable. Hanging Drop/Wet Mount Preparation *** NOTE Vibrio spp. marscesens by its ability to ferment arabinose Serratia rubidaea o also produces red pigment – prodigiosin Serratia odorifera o produces rancid, potato like odor **NOTE: Serratia rubidaea and Serratia odorifera have never been associated with human infection Hafnia   IMVC - V + motile and late lactose fermenter ONPG positive (+) LDC positive (+) ODC positive (+) Hafnia alvei o Representative organism of this genus; formerly Enterobacter alvei o Although it has been recovered from stool and wound specimens, its clinical significance is still questionable o Resembles Enterobacter. The resulting arrangement of cocci depends on the PLANE of DIVISION (Figure 15). TIME REQUIRED TO GROW – Mycobacterium species exhibits wide range of growth times. TYNDALLIZATION – fractional discontinuous sterilization effective indication: 100oC for 30-60 minutes instrument: Arnold's Sterilizer b.

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