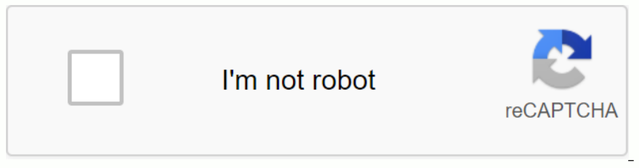

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Coliform bacteria test pdf

Bacteriological Analytical Manual (BAM) Main Page authors: Peter Feng (ret.), Stephen D. Weagant (ret.), Michael A. Grant (dec.), William Burkhardt Review History: October 2020 - Section I A.3 adapted to reflect that enrichment should take place at 35 ± 0.5ºC and not at 35 ± 1ºC. July 2017 - Chap. 4 Sec. For the completed phase of testing for E. coli, the incubation temperature of EC tubes is changed from 45.5 ± 0.2°C to 44.5 ± 0.2°C. The change is made in part as resulted in the poor ability of the control ten ATCC25922 to grow and ferment lactose to produce acid and gas at 45.5 ± 0.2 °C. Using 44.5 ± 0.2°C it will also be in accordance with used for fecal Coliform analysis in shellfish and shellfish meat (Sec. VI) as well as conditions used for E. coli testing by other International organizations. February 2013 – Shellfish analysis method revised in accordance with the APHA Investigation of seawater and shellfish, 4th ed. February 2013 – Membrane filter methods added to water analysis. Chapter Contents Escherichia coli, originally known as Bacteria coli commune, was identified in 1885 by the German paediatrician, Theodor Escherich (14, 29). E. coli is widely spread in the intestine of humans and hot-blooded animals and is the dominant faculty sanarbe in the bowel and part of the essential bowel flora that maintains the physiology of the healthy host (9, 29). E. coli is a member of the family Enterobacteriaceae (15), which includes many genera, including well-known pathogens such as Salmonella, Shigella and Yersinia. Although most strains of E. coli are not considered pathogens, they can be opportunistic pathogens that cause infections in immunocompromised hosts. There are also pathogenic strains of E. coli that when tumulated causes digestive disease in healthy people (see Chap. 4A). In 1892, Shardinger proposed the use of E. coli as an indicator of material infestation. It is based on the premise that E. coli is abundant in human and animal bowel movements and does not usually occur in other niches. Moreover, since E. coli can easily be detected by its ability to ferment glucose (later changed to lactose), it was easier to isolate than known gastrointestinal pathogens. From there, the presence of E. coli in food or water has accepted as an indication of recent faecal pollution and the possible presence of honest pathogens. Although the concept of using E. coli as an indirect indicator of health risk was sound, it is complicated in practice, due to the presence of other enteric bacteria such as Citrobacter, Klebsiella and Enterobacter which can also ferment lactose and are similar to E. coli in phenotypic properties, so that they are not easy to distinguish As a result, the term coliform was created to describe this group of enteric bacteria. Coliform is not a classification, but rather a working definition used to describe a group of Gram-negative, faculty's erobic bar-shaped bacteria that lactose acid and gas to produce within 48 h at 35°C. In 1914, the American Public Health Service adopted the summary of coliforms as a more convenient standard of sanitary significance. Although colyforms were easy to detect, their association with faecal infection was questionable because some coloids were found naturally in environmental samples (6). This led to the introduction of the faecal coloforms as an indicator of pollution. Fecal coliform, first defined based on the works of Eijkman (12) is a subset of total coliform that grows and ferments lactose at increased incubation temperature, from there also referred to as thermotolerant coliforms. Faecal coloform analyses are done at 45.5°C for food testing, except for water, shellfish and shellfish crop anales, which use 44.5°C (1, 3, 30). The fecal coliform group mostly consists of E. coli, but some other enterics such as Klebsiella can also ferment lactose at these temperatures and thus, are considered to be feeble coliforms. The inclusion of Klebsiella spp in the working definition of material cola forms reduces the correlation of this group with defective infestation. As a result, E. coli went to emerged as an indicator, partly facilitated by the introduction of newer methods that can quickly identify E. coli. Currently all 3 groups are used as indicators, but in different applications. Detection of cola forms is used as an indicator of sanitary quality of water or as a general indicator of sanitary condition in the food processing environment. Fecal coliforms remain the standard indicator of choice for shellfish and shellfish harvesting waters; and E. coli is used to indicate recent material contamination or inanitary processing. Almost all methods used to detect E. coli, total coliforms or defectal coliforms are summary methods based on lactose fermentation (4). The Most Likely Number (MPN) method is a statistical, multi-step test consisting of suspected, confirmed and completed phases. In the assay, series of thinings of a monster are vaccinated in sauce media. Analysts achieve the number of gas positive (fermentation of lactose) tubes, from which the other 2 phases of the assessment are performed, and then use the combinations of positive results to consult a statistical table (Appendix 2) to estimate the number of organisms present. Typically, only the first 2 phases in coliform and defectal coliform analysis are performed, while all 3 phases are done for E. coli. The 3-tube MPN test is used for testing most foods. Analysis of seawater using a multiple dilution range should not use less than 3 tubes per dilution (5 tubes are recommended); in certain cases, a single thunder range with no less than 12 tubes can also be acceptable. (For additional details, see: FDA. National Shell Fish Sanitation Program, Manual of Operations. 2009 DHHS/PHS/FDA, Washington DC). Similarly, analysis of bivalve molluscan shellfish should be performed using a multiple dilution MPN MPN whereoeing not less than 5 tubes per lunning should be used, see section IV. There is also a 10-tube MPN method used to test bottled water or samples that are not expected to test highly infected (3). Analysis of citrus juice for E. coli is performed as an absence/presence method, see section V. Also, there is a solid medium plates method for coliforms that use Violet Red Bile Agar, which contains neutral red pH indicator, so that lactose fermentation leads to the formation of pink colonies. There are also membrane filtration tests for coliform and defectal coliforms that measure aldehyde formation due to fermentation of lactose. This chapter also includes variations of the above tests that use fluorogenic substrates to detect E. coli (18), special tests for shell visalysis, a brief consideration of bottled water testing and a method for testing large volumes of citrus juice for presence of E. coli in conjunction with the Juice HACCP rule. I. Conventional method for coliforms, faecal coliforms and E. coli Equipment and material Coated water bath, with circulating system temperature of 44.5 ± 0.2°C. The temperature for water baths for the shellfish programme is 44.5°C ± 0.2 °C. Water level must be above the medium in immersed tubes. Immersion type thermometer, 1-55°C, about 55 cm long, with 0.1°C subdivisions, certified by the National Institute of Standards and Technology (NIST), or Equivalent Incubator, 35 ± 0.5°C. Balance with capacity of >2 kg and sensitivity of 0.1 g Blender and mixer jar (see Chapter 1) Sterile graduate pipes, 1.0 and 10.0 mL Sterile tools for sample handling (see Chapter 1) Dilution bottles made of borosilicate glass, with polyethy screw caps equipped with Teflon liners. Commercially prepared thining bottles with sterile Butterfield's phosphate buffer can also be used. Quebec colony counter, or equivalent, with the sized lens Longwave UV light [~365 nm], does not exceed 6 W. pH meter Media and Reagent Brilliant Green Lactose Gal (BGLB) sauce, 2% (M25) Lauryl tryptose (LST) loaves (M76) Lactose Broth (M74) EC sauce (M49) Levine's eosin-methylene blue (L-EMB) agar (M80) Tryptone (tryptophane) bro (M164) MR-VP sauce (M104) Cocker's citrate will be (M72) Sheet Count agar (PCA) (standard methods) (M124) Butterveld's phosphate-buffered water (R11) or equivalent dilution (Note: Same formulation is referred to as Buffered Dilution Water in the American Public Health Association. 1970. Recommended Procedures for the Investigation of Seawater and Shellfish, 4th ed. APHA, Washington, DC., p14-15) Kovacs' reagents (R38) Voges-Proskauer (VP) reagents (R89) Gram stain reagents (R32) Methyl red indicator (R44) Violet red gallery (VRBA) (M174) VRBA-MUG agar (M175) EC-MUG medium (M50) Lauryl tryptose MUG (LST-MUG) sauce (M77) Peptone Verdun, 0.5% (R97) MPN - Suspected test for coliforms, defective coliforms and E. Weighing 50 g of food in sterile high-speed blender jar (see Chapter 1 and current FDA FDA programs for instructions on sample size and compose) Frozen samples can be mipied by storage for <18 h at 2-5°c, but do not deaf. Add 450 ml of Butterfield's phosphate buffered water and mix for 2 min. If <50 g sample is available, portion equivalent to half the sample and add sufficient volume of sterile thining to make a 1:10 threw. The total volume in the mixer jar should completely cover the blades. Prepare decimal dilutions with sterile Butterfield's phosphate diluted or equivalent. Number of dilutions to be prepared depends on expected colosform density. Shake all suspensions 25 times in 30 cm arc or vortex mixture for 7 s. Using at least 3 consecutive thrones, inoculating 1 ml aliquots of each throne in 3 LST tubes for a 3 tube MPN analysis (other analysis may require the use of 5 tubes for each throne; See IV). Lactose sauce can also be used. For better accuracy, use a 1 ml or 5 ml pipe for inoculation. Do not use pipes to deliver<10% of their total volume; E.g. a 10 mL pipe to deliver 0.5 ml. Keep pipe in angle so that its bottom edge rests against the tube. No more than 15 min should pass from time to time the sample is mixed until all recovery is vaccinated in appropriate media. Incubate LST tubes at 35°C± 0.5°C. Examine tubes and record reactions to 24 ± 2 h for gas, i.e. displacement of medium in fermentation scale or slightly agitated when tubes gently agitate. Re incubate gas-negative tubes for an additional 24 h and examine and record again on 48 ± 3 h. Feed confirmed test on all suspected positive (gas) tubes. MPN - Confirm test for coliforms From each gassing LST or lactose sauce tube, wear a looping of suspension to a tube of BGLB sauce, avoid bunch if present. (a sterile wood applicator stick can also be used for these transfers). Incubate BGLB tubes at 35°C ± 0.5°C and examine for gas production at 48 ± 3 h. Calculate most likely number (MPN) (see Annex 2) of coliform based on ratio of confirmed gassing LST tubes for 3 consecutive dilutions. MPN - Confirmed testing for fecal coliforms and E. coli From each gassing LST or Lactose sauce tube of the Suspected test, transferring a looping of each suspension to a tube of EC sauce (a sterile wood applicator stick can also be used for these transfers). Incubate EEC tubes 24 ± 2 h at 44.5°C and examine for gas production. If negative, refined and investigated again on 48 ± 2 h. Use results of this test to calculate defective coliform MPN. To continue with E. coli analysis, proceed to Section F below. The EC sauce MPN method can be used for seawater and shellfish as it complies with recommended procedures (1). MPN - Completed test for E. coli. To perform the completed test for E. coli, stir in each gassing EC tube gently, remove a sauce and sauce for insulation on an L-EMB agar plate and incubate for 18-24 h at 35°C ± 0.5°C. Investigate plates for suspicious E. coli colonies, i.e. dark centered and and with or without metal sheep. Transfer to 5 suspicious colonies from each L-EMB plate to PCA slants, incubate them for 18-24 h at 35°C ± 0.5°C and use for further testing. NOTE: Identifying any 1 of the 5 colonies as E. coli is sufficient to consider that EC tube as positive; therefore, not all 5 isolates may be tested. Line Gram stain. All cultures that appear as Gram-negative, short sticks must be tested for the IMViC reactions below and also vaccinated back into LST gas production. Indole production. Inoculate tube of tryptone sauce and incubate 24 ± 2 h at 35°C ± 0.5°C. Test for invasion by adding 0.2-0.3 ml of kovacs' reagents. Appearance of clear red color in upper layer is positive test. Voges-Proskauer (VP) reactive compounds. Inoculate tube of MR-VP sauce and incubate 48 ± 2 h at 35°C± 0.5°C. Transfer 1 ml to 13 × 100 mm tube. Add 0.6 ml α-naphthol solution and 0.2 ml 40% KOH, and shake. Add some crystals of creatine. Shake and leave 2 h. Testing was developed positive as eosin pink color. Methyl red-reactive compounds. After VP testing, incubate MR-VP tube additional 48 ± 2 h at 35°C± 0.5°C. Add 5 droplets of methyl red solution to each tube. Clear red color is positive testing. Yellow is adverse reaction. Citrate. Slightly inoculating tube of Koser's cirate cutting; avoid observable turbidity. Incubate for 96 h at 35°C ± 0.5°C. Developing clear turbidity is positive response. Gas of lactose. Inoculate a tube of LST and incubate 48 ± 2 h at 35°C ± 0.5°C. Gas production (displacement of medium from inner scale) or effervescence after soft agitation is positive reaction. Interpretation: All cultures that (a) lactose with gas production within 48 h at 35°C yeast, (b) appear as Gram-negative nonsporeforming vines and (c) giving IMViC patterns of ++-- (biotype 1) or -+-- (biotype 2) are considered E. coli. Calculate MPN (see Annex 2) of E. coli based on ratio of EC tubes in 3 consecutive dilutions containing E. coli. NOTE: Alternatively, instead of performing the IMViC test, use API20E or the automatic VITEK biochemical test to identify the organism as E. coli. Use growth of the PCA slants and perform these assays as described by the manufacturer. Solid medium method – Coliforms Prepare violet red bile agar (VRBA) according to the manufacturer's instructions. Cool to 48°C before use. Prepare, homogenize, and decially thin sample as described in article I. C above so that insulated colonies will be obtained when plated. Transfer two 1 ml aliquots from each thinning to petri dishes, and use one of the following two casting plates methods depending on or injured or stressed cells are thought to be present (1). Pour 10 ml VRBA tempered to 48°C into plates, turn plates to mix, and allow to clot. To prevent surface growth and distribution of colonies, consult with 5 ml VRBA, and cause to solidifies. As resuscitation throw a basal layer of 8-10 ml of three-pttiese soy agar temper to 48 ° C. C. plates to mix, and incubate at room temperature for 2 ± 0.5 h. Then consult with 8-10 ml of melted, cooled VRBA and late to solidifise. Reverse set plates and incubate 18-24 h at 35 °C. Incubate dairy products at 32°C (2). Examine plates under enlargement glass lens and with relief. Count purple red colonies that are 0.5 mm or larger in dia dianare and are surrounded by zone of disgraced bile acids. Plates must have 25-250 colonies. To confirm that the colonies are colonies, choose at least 10 representative colonies and each transfer to a tube of BGLB sauce. Incubate tubes at 35°C. Investigate at 24 and 48 h for gas production. NOTE: If gas-positive BGLB tube shows a chutken, feed Grams stain to ensure that gas production was not due to Gram-positive, lactose-fermentation bacilli. Determine the number of coliforms per gram by multiplying the number of suspicious colonies per percent confirmed in BGLB by thining factor. Alternatively, E. coli colonies can be distinguished under the coliform colonies on VRBA by adding 100 µg of 4-methyl-umbelliferyl-β-D-glucuronide (MUG) per ml in the VRBA consultation. After incubation, observe for quenching fluorescence around colonies under long wave UV light. (see LST-MUG section II for theory and appropriateness.) Membrane Filtration (MF) Method - coliforms: see Section III. Bottled Water. Food homogenates will easily clog filters, from there MF is most suitable for analysing water samples; However, MF can be used in analysing liquid foods that do not contain high levels of particles such as bottled water (see Section III for the application of MF). II. LST-MUG Method for Detection E. Coli in Chilled or Frozen Food Exclusive Bivalve Molluscan Shellfish The LST-MUG assay is based on the enzymatic activity of β-glucuronidase (GUD), which clings to the substrate 4-methylumbelliferyl β-D-glucuronide (MUG) to release 4 methylumbelliferone (MU). When exposed to langwave (365 nm) UV light, MU displays a quenching fluorescence that is easily visualized in the medium or around the colonies. More than 95% of E. coli produces GUD, including anaerogenic (non-gas-producing) strains. One exception is enterohemorrhagic E. coli (EHEC) of serotype U157:H7, which is consistentLY GUD (11, 17). The lack of GUD phenotype in U157:H7 is often used to distinguish this serotype from other E. coli, although GUD positive variants of O157:H7 exist (24, 26). The production of GUD by other members of the family Enterobacteriaceae is rare, except for some shigellae (44 -58%) and salmonellae (20-29%) (18, 27). However, the unintentional detection of these pathogens by GUD-based essays is not regarded as a disadvantage from a public health perspective. Expression of GUD activity is affected by catabolite suppression (8) so on occasion, some E. coli is GUD negative, even if they carry the uidA gene (gusA) that codes for the enzyme (19). In Most analyses however, about 96% of E. coli isolates tested are GUD positive without the need for enzyme enzyme (27). CUP CAN be incorporated into almost any medium for use in the detection of E. coli. But some media such as EMB, which contain fluorescative components, are not suitable as they will mask the fluorescence of MU. When MUG is incorporated into LST medium, coliforms can be incorporated based on gas production of lactose and E. coli is thought to be identified by fluorescence in the medium under long wave UV light, so it is able to provide a suspected identification of E. coli within 24 h (18, 28). The LST-MUG method described below is adopted as Official Final Action by the AOAC for testing for E. coli in chilled or frozen foods, exclusive shellfish (28). See Sec. IV.4. D. for precautions in the use of MUG in the test of shellfish. For information on MUG assay contact, Dr Bill Burkhardt III (email William.Burkhardt@fda.hhs.gov), FDA, CFSAN, Dauphin Island, AL, 36528; 251-406-8125 CAREFUL: To observe for fluorescence, examine unioniculated LST-MUG tubes under langwave (365 nm) UV light in the dark. A 6-watt hand-hero UV lamp is sufficient and safe. When using a more powerful UV source, such as a 15-watt fluorescent lamp, wear protective glasses or glasses. Also, before use in MUG assays, examine all glass tubes for car fluorescence. Cerium oxide, which is sometimes added to glass as a quality control measure, will fluoresce under UV light and interfere with the MUG test (25). Using positive and negative control strains for MUG reaction is essential. Equipment and materials: see section I.A above and in addition, New, disposable borosilicate glass tubes (100 × 16 mm) New, disposable borosilicate glass Durham scales (50 × 9 mm) for gas collection Longwave UV lamp, Not to exceed 6-watt Media and reagening: see section I.B above Suspected LST-MUG test for E. Prepare food samples and perform the MPN Suspected test as described in section I.C. above, except use LST-MUG tubes instead of LST. Make sure to inoculate one tube of LST-MUG with a well-known GUD-positive E. coli isolates as positive control (ATCC 25922). In addition, inoculate another tube with a culture of Enterobacter aerogenes (ATCC 13048) culture of Enterobacter aerogenes (ATCC 13048) or a Klebsiella pneumonia as negative control, to facilitate differentiation of sample tubes that show only growth of those who are both growth and fluorescence Incubate tubes for 24 to 48 ±2 h at 35°C. Examine each tube for growth (turbidity, gas) and then examine tubes in the dark under langwave UV lamp (365 nm). A blusher fluorescence is a positive supposed test for E. coli. Studies by Moberg et al. (28) show that a 24 h fluorescence lecture is an accurate predictor of E. coli and can identify 83-95% of the E. coli-positive tubes. After 48 h of incubation, 96-100% of E. coli-positive tubes can be identified (28). Perform a confirmed test at all suspected buise out of the door 'a lusty shortening of each fluorescent tube after L-EMB agar to stripe and 24 ± 2 h by 35 ° C to inkubeer. inkubeer. protocols set out in I. F, above, for Completed Test for E. coli. Calculate MPN of E. coli based on combination of confirmed fluorescent tubes in 3 consecutive dilutions. III. Investigation of bottled water usage of bottled water is rapidly increasing globally. In the US alone, more than 3.6 billion litres of bottled water were consumed in 1998 (International Bottled Water Association, Alexandria, VA). Unlike potable water, which is regulated by the US EPA, bottled water is legally classified as food in the US and is regulated by the FDA (Federal Register. 1995. 21 CFR Part 103 et al. beverage: bottled water; final rule. 60(218) 57076-57130). FDA defines bottled water as water intended for human consumption and it is sealed in bottles or other containers with no additional ingredients, except that it may contain safe and suitable antimicrobial drugs and within restrictions, some added fluoride. Bottled water can be used as a beverage in itself or as an ingredient in other beverages. These regulations do not apply to soft drinks or similar beverages. In addition to bottled water or drinking water, in 21 CFR Part 103 FDA also defines different types of bottled water that meet certain criteria. These identities include artesian or artesian well water, groundwater, mineral water, purified or demineralised water, sparkling bottled water, springwater and well water. In addition, sterile water is defined as water that meets the requirements under the Sterility Test in the United States Pharmacoupeia. Colycyform organisms are not necessarily pathogens and are rarely found in bottled water, but they serve as an indication of madness or possible pollution. Surveys have shown that coliforms are useful indicators of bottled water quality, but some countries also monitor additional microbial populations as indicators of bottlewater quality (10, 33). Under the current bottled water quality standard, FDA has established a microbiological quality requirement based on coliform detection levels. These levels can be obtained through membrane filtration (MF) or by 10-tube MPN analysis of ten 10-ml analytical units. For information on bottled water methods contact Dr Bill Burkhardt III (email William.Burkhardt@fda.hhs.gov), FDA, CFSAN, Dauphin Island, AL, 36528; 251-406-8125 Equipment and Materials. Incubator at 35°± 0.5°C. Membrane filtration units (filter base and funnights): glass, plastic, or stainless steel; wrapped in foil or paper and dieilized. Ultraviolet sterilization room for sterilization filter base and t judges (optional). Filter manifold or vacuum jars to keep filter twists. Vacuum source (line vacuum, electric vacuum pump or water aspirator). Membrane filters; Sterile, white, roasted, 47 mm diath, 0.45 m pore size (or equivalent, as specified by the manufacturer) for summary of Petri dishes, sterile, plastic, 50 × 12 mm, with tight fitting lids. Forceps designed to transfer without damage. Culture media. Lauryl sulfate tryptose (LST) sauce (M-76). Brilliant green lactose gall salad (BGLB) (M-25). M-Endo Medium (BD#274930) (m-196). LES-Endo Agar (BD#273620) (m-197). Ten tube MPN coliform test - Suspected and confirmed procedures. For routine examination of bottled water, take 100 ml sample and inoculate 10 tubes of 2X LST (10 ml of medium) with 10 ml unintested sample each. Incubated tubes at 35°C. Investigate tubes at 24 ± 2 h for growth and gas formation as evidenced by displacement of medium in fermentation scale or slightly when tubes are agitated gently. If negative at 24 h, recumber tubes for an additional 24 h and examine for gas again. Perform a confirmed test on all suspected positive (gasification) tubes as follows: gently stir every positive LST tube and, using a 3.0 - 3.5 mm sterile loop, transfer one or more loopy suspension to a tube of BGLB sauce. Sterile wood applicator sticks can also be used for transfer by inserting it at least 2.5 cm in the sauce culture. Incubate BGLB tubes ± 48 kids 2 h at 35°C. Investigation for gas production and record. Calculate MPN with 10 tube MPN Table (9221.III), p. 9-52, Standard methods for examining water and waste water (3). NOTE: if a sample is found to contain coliforms (at any level) follow procedure set out in Sec. I. F. above to determine if it is E. coli. Bottled water is not allowed to contain E. coli. Membrane filters method for colay forms. Filter 100 mL test sample and transfer the filter to M-Endo medium (M-196) or LES Endo Agar (M-197) and incubate at 35 °C± 0.5°C for 22-24 h. Count colonies that are pink to dark red with a green metal surface. The sheep can vary from point to complete coverage of the colony. Use of low power, dissexual type microscope to examine filters is recommended. Fastening - If there are 5 to 10 sheen colonies on the filter, confirm everything by the inoculating growth of each sheep colony in tubes of LST and incubate at 35 °C± 0.5°C for 48 h. If the number of sheep colonies more than 10, randomly choose and confirm 10 colonies representing all sheep colonies. Any gas positive LST tubes should be subcultured to BGLB and incubate at 35°C± 0.5°C for 48 hours. Gas production in BGLB within 48 h is a confirmed colliform test. Report results as number of colicform colonies per 100 ml. NOTE: Standard Method, 1998, 20th ed, p. 9-60 (3), allows for simultaneous inoculation of LST and BGLB during verification. However, BGLB is somewhat inhibitor, so the method described above, where samples subculture from LST to BGLB are considered a more sensitive authentication test and therefore recommended. NOTE: if a sample is found to contain coliforms (at any level) follow procedure set out in Sec. I. F. above to determine if it is E. coli. Bottled water is not allowed to contain E. coli. IV. Investigation of Shellfish and Shellfish Meat The Official procedure for bacteriological analysis of and imported bivalve molluscan shellfish are fully and properly described in the APHA's Recommended Procedures for the investigation of Seawater and Shellfish, 4th ed. 1970 (1). The methods, including the conventional 5-tube MPN for coliform, fecal coliform and standard total plate count for bacteria (see Part III, APHA's Recommended Procedures the examination of Seawater and Shellfish, 4th ed. 1970 (1), are described below for examining shell stock, fresh-locked meat, fresh-closed frozen shellfish, and shellfish frozen at half These procedures do not apply to the investigation of scale analyses (crabs , lobster and shrimp) or processed shellfish meat such as bread, closed, pre-cooked and heat-processed products (see section I. C. this chapter). There are also many methods used for testing for shellfish harvesting and environmental water for faecal colosforms. One example, the mTEC agar (M-198) is a suitable membrane filter medium for summary of defective colain forms in marine and estuarine waters. In short, following the filtration of 100 ml of water, the filter rights must be washed down twice with approximately. The filter is then transferred to mTEC agar and incubates for 22-24 h at 44.5°C in Ethyfoam. All yellow-green or yellow-brown colonies are counted asfectal coliforms. Only plates with less than 80 colonies are counted. However, analyses of environmental water will not be covered here in detail, as environmental water analyses are done by the American EPA (3) and the quality of shellfish crop waters are primarily the responsibilities of each State's shellfish control authorities (20). Sample Preparation Using 10-12 shellfish, obtained 200 g shellfish beverages and meat. Mix 2 mins, with 200 mL of sterile phosphate buffered dilution water or 0.5% peptoon water (R97) to yield a 1:2 dilution of sample. Analysis of the soil sample

must commence within 2 min after mixing. Make series dilutions in 0.5% sterile peptoon water or sterile phosphate buffered dilution water. MPN – Suspected and confirmed test for Coliform Use Lactose Broth (M74) or Lauryl Tryptose Broth (M76), at ankle strength in 10 ml volumes. For 5-tube MPN analysis, inoculate the 5 tubes at each cycling as follows: To each of 5 tubes, add 2 ml of the mixed homogenate (equivalent to 1 g shellfish). Add 1 ml of 1:10 thining of homogenate (0.1 g shellfish). In each of 5 tubes, add 1 ml of 1:100 thining of homogenate (0.01 g shellfish). In each of 5 tubes, add 1 ml of 1:1000 thining of homogenate (0.001 g shellfish). Further dilutions may be necessary to avoid indetermined results. Incubate tubes at 35°C ± 0.5°C then follow instructions in section 1.C and enter Confirmed test as shown in 1.D above, under Conventional method for Coliforms, defectal coliforms and E. coli. Calculate MPN as described in Section 1.D above, except that shellfish analysis specifies that the coliform density as per 100 g sample is expressed as per g. MPN - Suspected and confirmed test for Fecal Coliforms in Shellfish Perform suspected test as described in section II above. To confirm positive tubes, carry one loop of gas positive LST tubes to EC sauce and incubate in a covered circulating water bath at 44.5°±0.2°C for 24 ± 2 hours. Gas production in EEC is a positive confirmation test for faecal coloforms. Calculate the MPN per 100 g forfectal coliform as described above for coliforms. MPN - EC-MUG Method for determining E. coli in Shellfish Meat The MUG test for β-glucuronidase (GUD) described above for detecting E. coli in chilled and frozen foods can also be used for testing for E. coli in shellfish meat; but with slight changes. This is due to the fact that food such as shellfish meat contains natural GUD activity (32). As a result, oyster homogenate can be vaccinated directly into LST-MUG tubes in the suspected phase of the MPN test can cause false positive fluorescence reactions. From there, in the analysis of E. coli in shellfish meat, the MUG reagent is added to the EC medium and used in the confirming phase of the asset. The EC-MUG tubes, incubate at 44.5°C + 0.2°C, can be used in the confirming phase of a conventional 5-tube MPN assay to determine faecal coliform levels in shellfish meat, then by examining tubes for fluorescence under long wave UV, an E. coli MPN can also be readibly obtained (32). See section 1.A and 1.B above for materials and reagenings needed. Use commercially prepared dehydrated EC-MUG, or prepare medium by adding MUG to EC sauce (0.05 g/L) (M50). Various sources of MUG connection are suitable: Marcor Development Corp., Carlstadt, NJ; Biosinth International, Itasca, IL; Sigma Chemical Co., St. Louis, MO and Hach Chemical, Loveland, CO. Dispense 5 ml in new dispersable borosilicate glass tubes (100 × 16 mm), new dispensable glass Durham vials (50 × 9 mm) for gas collection. Sterilize EC-MUG sauce tubes at 121°C for 15 min; storage up to 1 week at room temperature or up to a month under refreshment. Enter the 5-tube MPN Suspected and confirm test for fecal Coliforms in Shellfish as described above in Section 3 except use EC-MUG tubes instead of EC for the confirmed test. Determination of fluorescence in EC-MUG sauce requires the use of 3 control tubes, one used with E. coli as positive control; one with Enterobacter aerogeness (ATCC 13048) or K. pneumonia as negative control; and control an uninculated tube as EC-MUG medium batch. Inoculate the positive and negative controls at that time when Confirmed Test is performed and incubate all tubes at 44.5°C ± 0.2°C for 24 h. Read fluorescence as described above under LST-MUG assay. Note that some (<10%) >E. coli is anerogenic (gas negative), but should be MUG positive. Includes all fluorescence positive tubes in the E. coli MPN calculations. Determine E. coli MPN / of the tables in the BAM (Annex 2) using combination of fluorescence positive tubes at </10%) > </10%) > thinnning. NOTE: If analysis is to determine compliance with established E. coli limits, it will be necessary to confirm the presence of E. coli in MUG positive tubes. V. Analysis for E. coli in citrus juice Analysis for E. coli was implemented to identify potentially polluted juice or for verifying the effectiveness of HACCP during the processing of unpasteurized juice (21 CFR Part 120, Vol. 66, No. 13, January 19, 2001). The standard method commonly used for testing for E. coli is the MPN however, it does not seem sufficient for juice testing due to the acid (pH 3.6 to 4.3) of juice, which can interfere with the test, plus it only allows for testing 3.33 ml sample. Unlike most E. coli methods, which summary assays, the following method is a simple Presence/Absence test that can examine 10-ml volume of juice (34, 35). This assay, designated as modified ColiComplete (CC) Method, is a change of AOAC Official Method 992.30, which uses MUG for detecting E. coli (see Section on LST-MUG Method for Details). Equipment and materials Coated water bath, with circulating system temperature of 44.5 ± to maintain 0.2°C. Water level must be above the medium in immersed tubes. Incubator, 35 ± 0.5°C Longwave UV light [~365 pm], Not to exceed 6 W. Media and reagents: Universal Preenrichment Broth (UPEB) (M188) or can be purchased from BD (#223510) EC medium (M49) ColiComplete (CC) disks (#10800) - BioControl, Bellevue, WA Sample, Preparation, Preparation and Analysis Feed asays Aseptically, inoculates 10-ml portion of juice in 90 ml of UPEB and incubates at 35°C ± 0.5°C for 24 h. After enrichment, mix and carry 1 ml of each UPEB enrichment hose in 9 ml EC sauce with a CC disc. Incubate EC/CC sauce tubes at 44.5± 0.2°C in a circulating water bath for 24 ± 2 h. Includes a tube vaccinated with a MUG (+) E. coli strain as positive control and another with K. Pneumonia or Enterobacter aerogenesis (ATCC 13048) as negative control. Examine tubes in the dark and under long wave UV light. The presence of blue fluorescence in either tube is an indication that E. coli is present in the sample. Note: The CC discs also contain X-gal, which when clicked blue color on or around the disc through β galacttosdays. This reaction is analogous to measuring acid/gas production of fermentation of lactose from there, the presence of blue color is an indication of coliforms. VI. Other methods for summary of coliforms and E. coli There are many other methods for summary of coliforms and E. coli , including several that use fluorogenic reagents such as MUG or other chromogenic substrates for suspected detection and identification of coliform and E. coli in foods. Many of these tests, such as the Petrifilm dry rehydratable film, the hydrophobic grid membrane filter/MUG / MUG) method (13), ColiComplete disc (16), Colilert (AOAC 991.15), was evaluated by collaborative studies and adopted as official first or finals by the AOAC. There are also many changes from the membrane filtration assessments developed for testing for coliform, fecal coliform and E. coli and some of these can be useful in testing foods such as milk and beverages, but it is mostly used for water, environmental water, and shellfish harvest water analysis (5, 7, 20, 22, 23, 31). References to American Public Health Association. 1970. 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