



HEALTH PROTECTION BRANCH

OTTAWA

ANALYSIS OF SPROUTS FOR COLIFORMS, *ESCHERICHIA COLI*,
AND *KLEBSIELLA PNEUMONIAE*.

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1. **APPLICATION**

This method is applicable to the detection of viable bacteria (*Coliforms*, *Escherichia coli*, and *Klebsiella pneumoniae*) in sprouts (alfalfa, bean, onion, radish and any other seed products germinated for consumption) to determine compliance with the requirements of Sections 4 and 7 of the Food and Drugs Act. This published version replaces previous drafts prior to September 1999.

2. **PRINCIPLE**

The presence of coliforms in a food usually indicates that it has been manufactured under unsanitary conditions. The presence of faecal coliforms and specifically *E. coli* usually indicates potential (post-processing) contamination of the product with faecal matter. *K. pneumoniae* is an opportunistic pathogen and can also be an indicator of faecal contamination. This test involves a multiple tube fermentation technique which estimates the "Most Probable Number" (MPN) of total coliforms, of faecal coliforms and of *E. coli* following the method described in MFHPB-19. Aliquots from serial dilutions are plated on *Klebsiella* Selective agar which is incubated at 44.5°C.

3. **DEFINITION OF TERMS**

See Appendix A of Volume 3.

4. **COLLECTION OF SAMPLES**

4.1 See Appendix B of Volume 3.

4.2 Each sample unit shall contain at least 100 g.

4.3 Keep the sample units refrigerated (0-4°C).

5. **MATERIALS AND SPECIAL EQUIPMENT**

The following media, to be prepared and sterilized according to the manufacturer's instructions or those given in MFHPB-19:

- 1) Peptone (0.1%) Water diluent (commercially available).
- 2) Lauryl Sulfate Tryptose (LST) broth (commercially available).
- 3) Brilliant Green Lactose 2% Bile (BGLB) broth (commercially available).
- 4) *Escherichia coli* (EC) broth (commercially available).
- 5) Levine's Eosin Methylene Blue (L-EMB) agar or Endo agar (commercially available).
- 6) Nutrient Agar (NA) (commercially available).
- 7) IMViC Media:
 - a. Tryptone broth (commercially available),
 - b. Buffered Glucose broth (commercially available),
 - c. Simmon's Citrate (SC) agar (commercially available),The choice of further identification schemes (7.5.17) may require alternate media.
- 8) *Klebsiella* Selective Agar.
- 9) Covered water bath, with circulating system to maintain temperature of 45.0°C. Water level should be above the medium in immersed tubes.
- 10) Thermometer, 1-100°C, with 0.1° subdivisions, calibrated and certified by the National Research Council (NRC), the National Bureau of Standards, or equivalent.
- 11) Incubator, 44.5°C.
- 12) Colworth stomacher 400, blender or equivalent.

NOTE: It is the responsibility of each laboratory to ensure that the temperature of the incubators or waterbaths are maintained at the recommended temperatures. Where 35°C is recommended in text of the method the incubator may be at 36 +/-1.0° C. Similarly, lower temperatures of 30 or 25 may be +/- 1.0°C. However, where higher temperatures are recommended, such as 43 or 45.5°C, it is imperative that the incubators or waterbaths be maintained within 0.5°C due to potential lethality of higher temperatures on the microorganism being isolated.

6. PROCEDURE

Each sample unit may be analyzed individually or the analytical units may be combined where requirements of the applicable sampling plan can be met. Carry out the test in accordance with the following instructions:

6.1 Handling of Samples Units

- 6.1.1 In the laboratory prior to analysis, except for shelf-stable foods, keep sample units refrigerated (0-4°C).
- 6.1.2 Analyze sample units as soon as possible after their receipt in the laboratory.

6.2 Preparation for Analysis

- 6.2.1 Have ready sterile peptone water, and other needed agars and broths (see MFHPB-19) and *Klebsiella* Selective Agar.
- 6.2.2 Clean the surface of the working area with a suitable disinfectant.

6.3 Preparation of Sample

- 6.3.1 Check pH of the food suspension. If the pH is outside the range of 5.5-7.6, adjust pH to 7.0 with sterile NaOH or HCl.

- 6.3.2 Prepare succeeding decimal dilutions as required using a separate sterile pipette for making each transfer.
- 6.3.3 Shake or vortex all dilutions (7.3.2) immediately prior to making transfers to ensure uniform distribution of the microorganisms present.

6.4 Analysis

- 6.4.1 Complete the analysis for Coliforms. Fecal Coliforms and *E. coli* following the MPN procedure described in MFHPB-19.
- 6.4.2 From each serial dilution, plate 0.1 mL onto *Klebsiella* Selective Agar and incubate at 44.5°C for 24 to 48 h..
- 6.4.3 Use typical strains of *E. coli* and *K. pneumoniae* (ATCC strains or equivalent) as controls.

6.5 Confirmation

- 6.5.1 Follow MFHPB-19 for confirmation of Coliforms, Fecal Coliforms and *E. coli*.
- 6.5.2 Typical colonies of *K. pneumoniae* (usually brilliant yellow orange and fairly large) can be screened following Table 1. It is recommended that urea slants and some other biochemicals be inoculated to screen for this bacteria. Confirmation can continue using rapid ID kits for *Enterobacteriaceae*.

7. References

- 7.1 R.M. Atlas. 1993. Handbook of Microbiological Media. (Ed. L.C. Parks) CRC Press. London.
- 7.2 R. Shinebaum and E.M. Cooke. 1985. Isolation and Identification of Microorganisms of Medical and Veterinary Importance. Society of Applied Bacteriology. Pp. 35-38.
- 7.3 A.D. Hitchins, P.A. Hartman and E.C.D. Todd. Coliforms - *Escherichia coli* and its Toxins. Compendium of Methods for the Microbiological Examination of Foods. 3rd Edition. p. 328.

8. PREPARATION OF MEDIA

Follow manufacturer's instructions for the agars and broths.

- 8.1 Prepare all media (agars, broths, and reagents) listed in MFHPB-19. Media used for screening (urea and citrate etc) can be found in MFHPB-20
- 8.2 *Klebsiella* Selective Agar

(composition per liter)

Agar	15 g
DL-Phenylalanine	10.0 g
L-Ornithine-HCl	10.0 g
Raffinose	7.0 g
Pancreatic digest of casein	2.5 g
Yeast extract	2.5 g

K ₂ HP0 ₄	2.0 g
Phenol red solution	10.0 ml
Carbenicillin solution	10.0 ml

pH 5.6± 0.2 at 25°C

Phenol Red Solution:

Add 0.5 g Phenol Red to 50% ethanol and bring volume to 10.0 ml. Mix thoroughly.

Carbenicillin Solution:

Add 0.05 g Carbenicillin to distilled water and bring up to 10.0 ml. Filter sterilize.

Preparation of media:

Add components, except carbenicillin solution, to 990 ml distilled water. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min. Cool to 45-50°C. Aseptically add 10.0 ml carbenicillin solution. Mix thoroughly. Adjust pH to 5.6 to 5.7 with 1 N HCl. Pour into sterile Petri dishes.

Table 1. Biochemical Reactions That Can be Used for Screening Presumptives.

Bacteria	Urea	Motility	Indole	Citrate	TSI	LIA	Growth at 44.5°C on KSA
<i>Klebsiella</i>	+	-	-	+	A/A gas	K/K	Yes
<i>Enterobacter</i>	-	+	-	+	A/A no gas	K/K	No
<i>E. coli</i>	-	+	+	-	K or A/A gas	K/K	No
<i>Citrobacter</i>	-	+	+/_	+	K/H ₂ S	K/A	No
<i>Klebsiella</i> species				Growth at 44.5°C			
<i>K. pneumoniae</i>				Yes			
<i>K. oxytoca</i>				No			
<i>K. terrigena</i>				No			
<i>K. planticola</i>				No			
<i>K. ozaenae</i>				to be determined			
Others				to be determined			