

NATIONAL MICROBIAL MONITORING PROGRAMME

2004

ASSESSMENT OF METHODS FOR COLIFORM AND *E. COLI* ENUMERATION

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Purpose of Presentation

TO DISCUSS

- THE ENUMERATION OF COLIFORMS AND *E.COLI*
 - THE COMPARISON OF METHODS ACCORDING TO THE ISO 17994 PROTOCOL
 - THE RESULTS OF A COMPARISON OF COLILERT AND MEMBRANE FILTRATION METHODS
 - A SUGGESTION
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Enumeration of Coliforms and *E. coli*

- **DRINKING WATER SHOULD BE FREE OF PATHOGENS**
 - **DO NOT TEST DIRECTLY FOR PATHOGENS – USE INDICATOR ORGANISMS**
 - **MOST COMMONLY USED INDICATORS ARE COLIFORMS AND *E. COLI***
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Enumeration of Coliforms and *E. coli*

- **FIRST USE OF BACTERIA AS INDICATORS – VON FRITSCH - 1880**
 - **ROUTINE EXAMINATION OF DRINKING WATER – LONDON – 1885**
 - **ESCHERICH DESCRIBED *BACILLUS COLI* FROM THE FAECES OF BREAST-FED INFANTS -1885**
 - **FIRST ENUMERATIONS BY MULTIPLE TUBE OR MPN –TEST DURATION 48 HOURS**
 - **LACTOSE BASED MEDIA**
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Enumeration of Coliforms and *E. coli*


MEMBRANE FILTRATION METHOD – MUELLER – 1943:

- FILTER WATER THROUGH 0.45 MICRON MEMBRANE RETAINING THE BACTERIA
 - PLACE MEMBRANE ON SELECTIVE MEDIUM CONTAINING LACTOSE AND INHIBITORS
 - INCUBATE AT 37 AND 44 DEGREES CELCIUS FOR 24 HOURS
 - COUNT PRESUMPTIVE BACTERIA
 - CONFIRM
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Enumeration of Coliforms and *E. coli*


CONFIRMATION STEP:

- TEST FOR ACID AND GAS FROM LACTOSE AT 37 AND 44 DEGREES CELSIUS
 - TEST FOR INDOLE FROM TRYPTOPHANE AT 44 DEGREES CELSIUS
 - TOTAL COLIFORMS PRODUCE ACID AND GAS AT 37 DEGREES CELSIUS
 - E. COLI PRODUCE ACID AND GAS AT BOTH TEMPERATURES AND INDOLE AT 44 DEGREES CELSIUS
 - TEST DURATION – 48 HOURS
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Enumeration of Coliforms and *E. coli*




ENZYME-BASED METHODS – 1988 ...

- DETERMINE COLIFORMS AND *E. COLI* CONCURRENTLY – AT 37 DEGREES CELCIUS
 - ADD WATER TO POWDERED SUBSTRATE
 - INCUBATE AT 37 DEGREES CELCIUS
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Enumeration of Coliforms and *E. coli*


ENZYME-BASED METHODS – 1988 ...

- COLIFORMS – ENZYME BETA GALACTOSIDASE – HYDROLYSIS OF CHROMOGENIC SUBSTRATE
 - *E. COLI* - FLUOROGENIC SUBSTRATE (MUG)- ENZYME BETA GLUCORONIDASE
 - TEST DURATION – 24 HOURS
 - IMPROVED RECOVERIES OF TARGET ORGANISMS
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Enumeration of Coliforms and *E. coli*

ENZYMЕ-BASED METHODS - COMMERCIAL PRODUCTS:

- COLILERT, COLISURE, m-COLIBLUE, COLI-COMplete, MICROSCAN
 - DISADVANTAGE: MOST ARE PRESENCE/ABSENCE TESTS
 - COLILERT QUANTITRAY – MPN
 - COLISURE AND COLILERT - USEPA APPROVED
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Enumeration of Coliforms and *E. coli*

ENZYME-BASED METHODS – MEMBRANE FILTRATION COMMERCIAL PRODUCTS:

- MERCK CHROMOCULT (USEPA APPROVED)
- OXOID MLGA MEDIUM (DWI APPROVED)
- ADVANTAGE: COUNTS
- TEST FOR COLIFORMS AND E. COLI ON ONE MEMBRANE AT 37 DEGREES CELCIUS
- NO CONFIRMATIONS
- RESULTS AFTER 24 HOURS




Comparison of Methods

- **ISO STANDARD 17994**
 - **PROTOCOL TO PROVIDE CRITERIA AND PROCEDURES FOR ASSESSING THE AVERAGE QUANTITATIVE EQUIVALENCE OF RESULTS OBTAINED BY TWO MICROBIOLOGICAL ANALYTICAL METHODS**
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Comparison of Methods

- ONE METHOD MAY, BUT NEED NOT BE, A STANDARD METHOD
 - METHODS CONSIDERED MAY BE BASED ON COUNTS OF COLONIES OR MPN-METHODS
 - SAME TARGET GROUP OF MICRO-ORGANISMS
 - SOUND STATISTICAL BASIS FOR EVALUATION
 - PAIRS OF CONFIRMED COUNTS
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Comparison of Methods

RANGE OF SAMPLES:

- **OVER A WIDE GEOGRAPHICAL AREA**
 - **APPROPRIATE FOR TESTS NORMALLY CONDUCTED**
 - **DRINKING WATER - CHLORINE STRESSED ORGANISMS**
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Comparison of Methods

- ENOUGH BACTERIA FOR LOW LIKELIHOOD OF ZERO COUNTS (TARGET 10-30 COLONIES PER PLATE)
 - TENTATIVELY SUGGESTS MIN OF 6 LABS
 - SUFFICIENT SAMPLES WITH COUNTS TO DETECT AN AVERAGE RELATIVE DIFFERENCE OF 10% - 256 SAMPLES
 - CONFIRM ALL PRESUMPTIVE TARGET COLONIES
 - EXPERT PANEL INCLUDING STATSTICIAN
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Results of a SA Trial

- **SEVEN LABORATORIES**
 - **EACH ANALYSED APPROX 50 SAMPLES**
 - **COLILERT QUANTITRAY VS. MEMBRANE FILTRATION**
 - **PROTOCOL USED STILL UNDER CONSIDERATION BY THE ISO WORKING COMMITTEE**
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Results of a SA Trial

- **MEMBRANE FILTRATION - EACH LAB USED ITS REGULAR SELECTIVE MEDIUM**
 - **FOUR USED SABS M-ENDO FOR TC AND M-FC FOR *E. COLI***
 - **TWO USED UK-MLSD MEDIUM**
 - **ONE USED CHROMOCULT ADDITINALLY**
 - **ONE USED M-ENDO FOR TC AND M-TECH FOR *E. COLI***
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Results of a SA Trial

RESULTS:

- COLILERT RECOVERED SIGNIFICANTLY MORE TCs THAN M-ENDO, CHROMOCULT AND MLSD
 - COLILERT RECOVERED MORE *E. COLI* THAN M-FC
 - INSUFFICIENT DATA TO SHOW OTHER SIGNIFICANCIES
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A Suggestion

- **COST OF COLILERT A FACTOR TO CONSIDER**
 - **MLGA (UK BASED INFO) AND CHROMOCULT CLAIMED TO BE LESS COSTLY**
 - **SOME LABORATORIES ALREADY GEARED TO DO MEMBRANE FILTRATION**
 - **COMPARE M-ENDO AND M-FC WITH CHROMOCULT AND MLGA**
 - **USE THE APPROVED ISO 17994 PROTOCOL**
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