IDEXX Summary 5BD

Topic:	Zimbabwe Approval of Colilert for all waters
Title:	SAZS 629: Part 3 2004
Source:	Technical Committee H3: Effluent Waste Water under general direction of the Health, Safety and Environmental Council
Date:	2004

Report Highlights:

- Colilert is approved in its P/A and MPN tube format for the testing of all waters for coliforms, thermo-tolerant coliforms and presumptive *E. coli*.

CONTENTS Page

PREFACE

This Zimbabwe Standard Test Method, SAZS 629:2004: Water Quality – Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia Coli*: Part 3: Defined substrate technology (Colilert) method, is based on AOAC Official Method 991.15 published in 2000.

This standard method was prepared by Technical Committee H 3: Effluent Waste Water, under the general direction of the Health, Safety and Environment Council.

The following interests were represented on the technical committee entrusted with the preparation of this test method.

Tobacco Research Board	Mr J Mlilo (Chairman)
City of Harare	Mr L Chipfunde Mr D Makaza
Ministry of Health and Child Welfare,	
Blair Research Institute	Mrs G Rukure
Government Analyst Laboratory	Mr C Benhura
Standards Association of Zimbabwe	Mrs P Muzunzandare
Zimbabwe Alloys Ltd.	Mrs O Dzawo
Zimbabwe National Water Authority	Mr T Z Nherera
Standards Association of Zimbabwe	Mr W Karuwo (Technical Secretary)

ZIMBABWE STANDARD TEST METHOD

FOR

WATER QUALITY – DETECTION AND ENUMERATION OF COLIFORM ORGANISMS, THERMOTOLERANT COLIFORM ORGANISMS AND PRESUMPTIVE *ESCHERICHIA COLI*

PART 3: DEFINED SUBSTRATE TECHNOLOGY (COLILERT) METHOD

1. SCOPE

This part of SAZS 629 specifies a method for the detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia coli* (*E.coli*) by the defined substrate technology.

This method can be applied to all types of water, including those containing an appreciable amount of suspended matter. The method identifies *E.coli* specifically and can detect single viable coliforms or *E.coli* per sample.

2. DEFINITIONS

For the purpose of this standard the following definitions shall apply:

2.1 Coliform Organisms. Organisms capable of aerobic growth at either 35 ± 0,5 °C or 37 ± 0,5 °C in a liquid lactose culture medium with the production of acid and gas within 48 h.

2.2 *Escherichia Coli (E.Coli)*. Coliform organisms as described in 2.1 which also produce indole from tryptophan within 24 h, at either 44 ± 0,25 °C or 44,5 ± 0,25 °C.

3. PRINCIPLE

Defined substrate technology (DST) reagent system simultaneously enumerates total coliforms and *E.coli* directly and separately from a water test sample. Reagent contains o-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG). After inoculation of DST test, a clear solution results. Only total coliforms can hydrolyze ONPG to produce yellow chromogen. Same test tube or vessel contains MUG, which is hydrolyzed and fluoresences when *E.coli* grow. β-Glucuronidase has been found to be specific to the genus *Escherichia (Escherichia* and *Shigella)* and *Salmonella.* Practically, from water samples, only *E.coli* yields a positive result. Metabolism

of ONPG by β-D-galactosidase system of enteric bacteria is specific for total coliform group. Composition of inorganic salts in DST reagent does not support growth of nonenteric bacteria. Assay may be performed in most probable number (MPN) format or as presence-absence (P-A) test.

4. APPARATUS

4.1 Tubes. Glass, 12 mℓ. Sterile, free of microbial inhibitors (e.g residual detergent), and nonfluorescent at 366 nm.

4.2 Vessels. Glass, 120 mℓ. Sterile, free of microbial inhibitors (e.g residual detergent), and nonfluorescent at 366 nm.

4.3 Longwave Ultraviolet Light Source. 366 nm, 4 watt, hand-held lamp or equivalent.

5. REAGENT

For each 1 000 mℓ test sample, completely mix the following: 5 g ammonium sulfate, 50 mg manganese sulfate, 50 mg zinc sulfate, 100 mg magnesium sulfate, 10 g sodium chloride, 50 mg calcium chloride, 900 mg potassium dihydrogen phosphate, 6,2 g disodium hydrogen phosphate, 40 mg sodium sulfate, 1 mg ampotericin B, 500 mg ONPG, 75 mg MUG, and 500 mg solanium.

6. ENUMERATION

For MPN format, use sufficient reagent mixture (see Clause 5) in each tube to accept 10 mℓ test portion. For P-A format, use 10 times that amount in each vessel. If laboratory-prepared reagent is used, add powder to labelled tube, (4.1) or vessel, (4.2), containing test portion or, add well mixed water test portion to labelled tube or vessel containing predispensed reagent. Combine test portion and reagent aseptically, cap container tightly, and mix vigorously to dissolve reagent. Resulting solution is colourless. Incubate samples for 24 h at 35 ± 1,0 °C. Yellow colour in MPN tube or P-A vessel after incubation denotes presence of total coliforms. Expose positive total coliform tubes or vessel to hand-held 366nm lamp, (4.3). Fluorescence denotes presence of *E.coli*.

Use the standard MPN table below to determine MPN values. Report results as total coliform MPN/100 mℓ test sample or *E.coli* MPN/100 mℓ test sample.

TABLE – MOST PROBABLE NUMBERS PER 100 MℓOF SAMPLE, PLANTING 5 PORTIONS IN EACH 3 DILUTIONS IN GEOMETRIC SERIES

Number of positive tubes				Number of positive tubes				Number of positive tubes				Number of positive tubes				Number of positive tubes				Number of positive tubes			
10 mℓ	1 mℓ	0,1 mℓ	MPN	10 mℓ	1 mℓ	0,1 mℓ	MPN	10 mℓ	1 mℓ	0,1 mℓ	MPN	10 mℓ	1 mℓ	0,1 mℓ	MPN	10 mℓ	1 mℓ	0,1 mℓ	MPN	10 mℓ	1 mℓ	0,1 mℓ	MPN
0	0	0		1	0	0	2,0	2	0	0	4,5	3	0	0	7,8	4	0	0	13	5	0	0	23
0	0	1	1,8	1	0	1	4,0	2	0	1	6,8	3	0	1	11	4	0	1	17	5	0	1	31
0	0	2	3,6	1	0	2	6,0	2	0	2	9,1	3	0	2	13	4	0	2	21	5	0	2	43
0	0	3	5,4	1	0	3	8,0	2	0	3	12	3	0	3	16	4	0	3	25	5	0	3	58
0	0	4	7,2	1	0	4	10	2	0	4	14	3	0	4	20	4	0	4	30	5	0	4	76
0	0	5	9,0	1	0	5	12	2	0	5	16	3	0	5	23	4	0	5	36	5	0	5	95
0	1	0	1,8	1	1	0	4,0	2	1	0	6,8	3	1	0	11	4	1	0	17	5	1	0	33
0	1	1	3,6	1	1	1	6,1	2	1	1	9,2	3	1	1	14	4	1	1	21	5	1	1	46
0	1	2	5,5	1	1	2	8,1	2	1	2	12	3	1	2	17	4	1	2	26	5	1	2	64
0	1	3	7,3	1	1	3	10	2	1	3	14	3	1	3	20	4	1	3	31	5	1	3	84
0	1	4	9,1	1	1	4	12	2	1	4	17	3	1	4	23	4	1	4	36	5	1	4	110
0	1	5	11	1	1	5	14	2	1	5	19	3	1	5	27	4	1	5	42	5	1	5	130
0	2	0	3,7	1	2	0	6,1	2	2	0	9,3	3	2	0	14	4	2	0	22	5	2	0	49
0	2	1	5,5	1	2	1	8,2	2	2	1	12	3	2	1	17	4	2	1	26	5	2	1	70
0	2	2	7,4	1	2	2	10	2	2	2	14	3	2	2	20	4	2	2	32	5	2	2	95
0	2	3	9,2	1	2	3	12	2	2	3	17	3	2	3	24	4	2	3	38	5	2	3	120
0	2	4	11	1	2	4	15	2	2	4	19	3	2	4	27	4	2	4	44	5	2	4	150
0	2	5	13	1	2	5	17	2	2	5	22	3	2	5	31	4	2	5	50	5	2	5	180
0	3	0	5,6	1	3	0	8,3	2	3	0	12	3	3	0	17	4	3	0	27	5	3	0	79
0	3	1	7,4	1	3	1	10	2	3	1	14	3	3	1	21	4	3	1	33	5	3	1	110
0	3	2	9,3	1	3	2	13	2	3	2	17	3	3	2	24	4	3	2	39	5	3	2	140
0	3	3	11	1	3	3	15	2	3	3	20	3	3	3	28	4	3	3	45	5	3	3	180
0	3	4	13	1	3	4	17	2	3	4	22	3	3	4	31	4	3	4	52	5	3	4	210
0	3	5	15	1	3	5	19	2	3	5	25	3	3	5	35	4	3	5	59	5	3	5	250
0	4	0	7,5	1	4	0	11	2	4	0	15	3	4	0	21	4	4	0	34	5	4	0	130
0	4	1	9,4	1	4	1	13	2	4	1	17	3	4	1	24	4	4	1	40	5	4	1	170
0	4	2	11	1	4	2	15	2	4	2	20	3	4	2	28	4	4	2	47	5	4	2	220
0	4	3	13	1	4	3	17	2	4	3	23	3	4	3	32	4	4	3	54	5	4	3	280
0	4	4	15	1	4	4	19	2	4	4	25	3	4	4	36	4	4	4	62	5	4	4	350
0	4	5	17	1	4	5	22	2	4	5	28	3	4	5	40	4	4	5	69	5	4	5	430
0	5	0	9,4	1	5	0	13	2	5	0	17	3	5	0	25	4	5	0	41	5	5	0	240
0	5	1	11	1	5	1	15	2	5	1	20	3	5	1	29	4	5	1	48	5	5	1	350
0	5	2	13	1	5	2	17	2	5	2	23	3	5	2	32	4	5	2	56	5	5	2	540
0	5	3	15	1	5	3	19	2	5	3	26	3	5	3	37	4	5	3	64	5	5	3	920
0	5	4	17	1	5	4	22	2	5	4	29	3	5	4	41	4	5	4	72	5	5	4	1 600
0	5	5	19	1	5	5	24	2	5	5	32	3	5	5	45	4	5	5	81				

7. QUALITY CONTROL

Perform quality control as follows:

7.1 Reconstitute reagent in each of 3 tubes or vessels with appropriate volume of sterile, distilled water and mix thoroughly to aid dissolution.

7.2 Label tubes "*Escherichia coli*", "*Klebsiella pneumoniae*" and "*Pseudomonas aeruginosa*".

7.3 Touch sterile inoculating loop or needle to an 18 to 25 h pure culture slant of each of the 3 bacteria.

7.4 Transfer each bacterial inoculum to appropriately labelled tube or vessel.

7.5 Incubate inoculated tube or vessel for 24 h at 35 $\pm$ 1,0 °C.

8. EXPRESSION OF RESULTS

After the test confirm the results as:

a) *E.coli*: yellow and fluorescent;

b) *K.pneumoniae*: yellow only; and

c) *P.aeruginosa*: no colour, no fluorescence.

9. TEST REPORT

The test report shall contain the following information:

a) a reference to this part of SAZS 629;

b) all details necessary for complete identification of the sample;

c) the confirmatory media and tests used;

d) the time, temperature and conditions of incubation;

e) the results expressed in accordance with Clause 8; and

f) any other information relevant to the method.

