© 2020 | International Journal of Scientific Research in Science and Technology
IJSRST | Volume 7 | Issue 3 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
DOI : https://doi.org/10.32628/IJSRST

Water Quality Testing (H_2S and MPN) Modalities in The Samples from India Mark-2 Hand Pumps in Bareilly and Rohilkhand Regions

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Introduction- India Mark-II hand pumps (IM-2) are installed to meet the clean potable water needs in public places. The main interventions are sanitary sealing with the well-constructed platform, foot apron, brick on edge, and soakage pit connected with the drain. However, there are significant problems concerning water quality contamination. Sanitary improvement is the intervention to minimize the risk of bacterial contamination. The present investigation has been accomplished under the guidance of the Divisional Commissioner of the Moradabad division with technical support from UNICEF. The mapping of India Mark-II hand pumps with poor quality conditions based on bacteriological contamination was done in 10 locations of Bareilly, and Rohilkhand regions, and risky hand pumps were identified and suggested for sanitary improvement to ensure quality assurance.

Water samples were aseptically brought to the laboratory and bacterial contamination was tested by the Most Probable Number count method and through H_2S vials.

Materials and methods

Water Sample collection from distinct locations: The water samples from 10 distinct locations of different villages of Rohilkhand regions were brought to the laboratory in autoclaved bottles aseptically in ice boxes and tested for coliform bacteria to ensure the faecal contamination in drinking water from hand pumps of poor conditions due to several sanitary problems. All the samples were tested on the same day through H_2S vials and by thorough testing through three steps of the MPN count method, viz., Presumptive test, confirmed test, and complete test, and investigated from the MPN table.

Presumptive test: for coliforms

Preparation of the culture medium:

The medium used in the **presumptive test** was **Lactose fermentation broth 1x and 2x** which contained 5g of peptone, 5g of lactose, and 3g of beef extract in 1000ml distilled water. For 2x broth, the quantity of all ingredients is doubled, except the distilled water.

H_2S vial Test:

The water samples to be screened for faecal pollution were placed in the H_2S vial, up to the pre-calibrated mark (20ml), and allowed to stand at ambient temperature (30-37^{0}C). Faecal pollution is indicated if the bottle's contents turn black within 12-18 hours; in this case, the water was graded as unfit for consumption.

Confirmed coliforms test:

Preparation of the culture medium: The medium used in the test was **Brilliant green lactose bile broth** medium (pH 7.4) which contained 10g of peptone, 10g of lactose, 20g of ox bile (purified and dehydrated), 13g of Brilliant green, 0.1% (w/v) in water, and 1000ml distilled Water.

The performance of this test was compared with the **presumptive coliform test**. Drinking water samples received for routine MPN assay were tested simultaneously with the confirmed test. Water samples with a coliform count of more than 4 per 100ml, as assessed by the MPN method, and those turning black in the H_2S vials were graded as unsatisfactory.

Complete test: Identification & culturing of coliforms:

The water samples positive in the **confirmed coliforms test** were cultured on **EMB agar and Endo agar**.

Eosin-Methylene Blue agar (EMB agar) is a selective and differential culture medium, ideally used for isolating faecal coliforms. It is a selective medium due to the inhibition of gram-positive bacteria by methylene blue. EMB agar's differential property comes from the eosin and methylene blue complex formation under acidic pH. EMB agar aids in distinguishing coliforms and faecal coliforms that indicate possible pathogenic microorganism contamination in water samples. Hence, it is used in water quality tests (the presence of *E. coli* in the water sample suggests the possibility of faecal contamination of water. On streaking the EMB Agar plates with inoculum from positive water samples, the colonies of ***E. coli*** give a **green metallic sheen** as distinguished colonies of lactose fermenting gram-negative bacteria from noncoliforms.

EMB Agar medium (pH 7.2) contains 10g of peptone, 5g of lactose, 2g of di-potassium hydrogen phosphate, 0.4g of eosin y, 0.0065g of methylene blue, 15g of agar, and 1000ml of distilled water. **Endo Agar (pH 7.5)** contains 10g of peptone, 10g of lactose, 3.5g of di-potassium hydrogen phosphate, 2.5g of sodium sulphite, 0.4g of basic fuchsin, 15g of agar, and 1000ml of distilled water.

RESULTS AND DISCUSSION

Table 1: Enumeration of water samples for the presence of coliform bacteria in different locations (phase 1)

S.No	Location of water sample from hand pumps	No. of Samples	P-Form	C-form	Complete form	MPN Mean	H_2S Vials	
							+ve	-ve
01.	'Himmatpur'	05	01	00	00	0.4	00	04
02.	'Mohanpur'	06	02	01	01	1.834	04	02
03.	'Akha'	07	03	01	01	1.142	02	05
04.	'Padarathpur'	05	00	00	00	0.00	00	05
05.	'Sirohi'	03	00	00	00	0.00	00	03
06.	'Bhuda'	07	03	01	00	0.571	00	07
07.	'Bichhuria' &'Himmatpur'	07	02	01	01	0.571	01	06
08.	'Nagaria Khurd'	07	00	00	00	1.286	00	07
09.	'Gren' & 'Hardua kajitalla'	07	02	02	02	2.571	02	05
10.	'Barai'	06	02	02	02	0.667	02	04
Total	**10 Locations**	**60**	15	08	07	9.042	11	49

Table 2: Enumeration of water samples for the presence of coliform bacteria in different locations (phase 2)

S.No	Location of sample	No. of Samples	P-Form	C-form	Complete form	MPN Mean	H_2S Vials	
							+ve	-ve
01.	'Himmatpur'	05	05	05	04	372.2	5	00
02.	'Mohanpur'	08	06	04	04	53.375	6	02
03.	'Akha'	09	07	04	03	14	7	02
04.	'Padarathpur'	09	05	05	05	72.667	5	04
05.	'Sirohi'	10	03	03	03	5.2	3	07
06.	'Bhuda'	10	07	06	05	27.9	5	05
07.	'Bichhuria'&Himmatpur	10	07	05	05	5.6	3	0
08.	'Nagaria Khurd'	10	07	07	05	31	05	0
09.	'Gren'&'Hardua kajitalla'	13	07	05	03	6.154	03	1
10.	'Barai'	10	06	05	05	180.3	01	0
Total	**10 Locations**	**94**	60	49	42	768.396	43	5

The change in colour due to the production of acid and gas bubble in the concavity of durham tube indicates a positive presumptive test for coliform bacteria. Production of gas in brilliant green bile broth tubes indicates a positive confirmed test for coliform bacteria in water sample. The number of tubes showing positive result are confirmed and MPN value is calculated from the table 3. H_2S vials also showed a direct positive correlation with the results of MPN testing. The mapping of India Mark-II hand pumps was systematically done and risky pumps were marked with cross marks, painted red with public participation. Finally, all the hand pumps indicating poor quality water due to contamination by faecal material were condemned till field-level implementations were laid down and sanitary improvements were made to ensure quality water free of contamination.

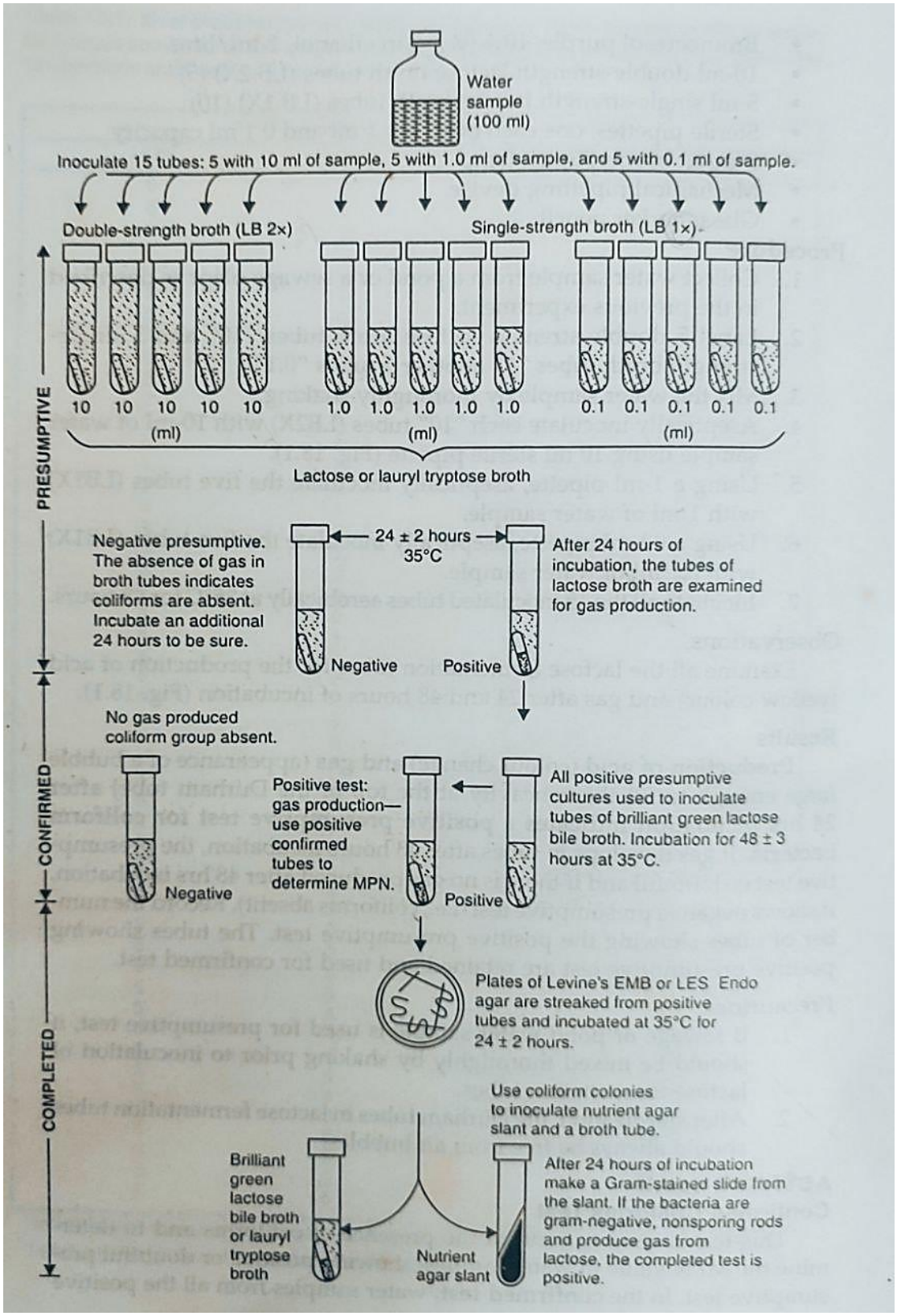
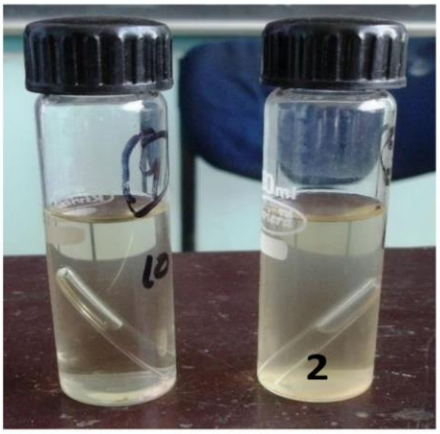
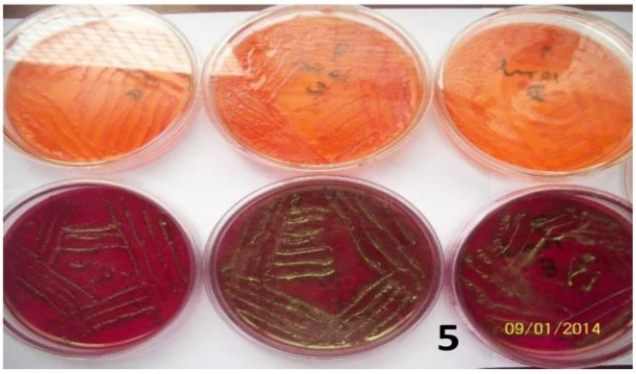
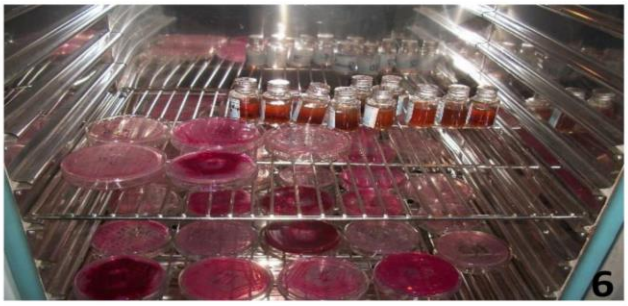
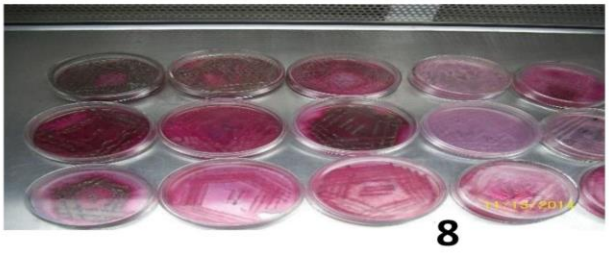


Fig. Schematic representation of water sample analysis by MPN method (courtesy: Experiments in Microbiology, Plant Pathology and Biotechnology by K. R. Aneja)









1. Samples load 2. Gas bubble in Durham tube in Lactose broth 3. H2S Vials 4. and 7. E. coli streaking 5. Colonies of E. coli on Endo Agar & EMB agar media 6. in BOD incubator 8. Pour plated on EMB agar

Table 3. Standard reference table for assessment of MPN

Most Probable Number Table					
Number of tubes giving a positive reaction for a 5-tube set			MPN (per 100 ml)	95% Confidence Limits	
10 ml	*1 ml*	*0.1 ml*		*Low*	*High*
0	0	0	<2	<1	7
0	1	0	2	<1	7
0	2	0	4	<1	11
1	0	0	2	<1	7
1	0	1	4	<1	11
1	1	0	4	<1	11
1	1	1	6	<1	15
2	0	0	5	<1	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	0	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	78
4	3	0	27	9	80
4	3	1	33	11	93
4	4	0	34	12	93
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	110
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	150
5	2	0	49	17	130
5	2	1	70	23	170
5	2	2	94	28	220
5	3	0	79	25	190
5	3	1	110	31	250
5	3	2	140	37	340
5	3	3	180	44	500

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