

Microbiological parameters, concept and testing procedure

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Microbiological parameters

- **Indicator organisms**

- 1) *Escherichia coli* or *E. coli*.

- 2) Faecal coliform

- ***Vibrio cholerae***

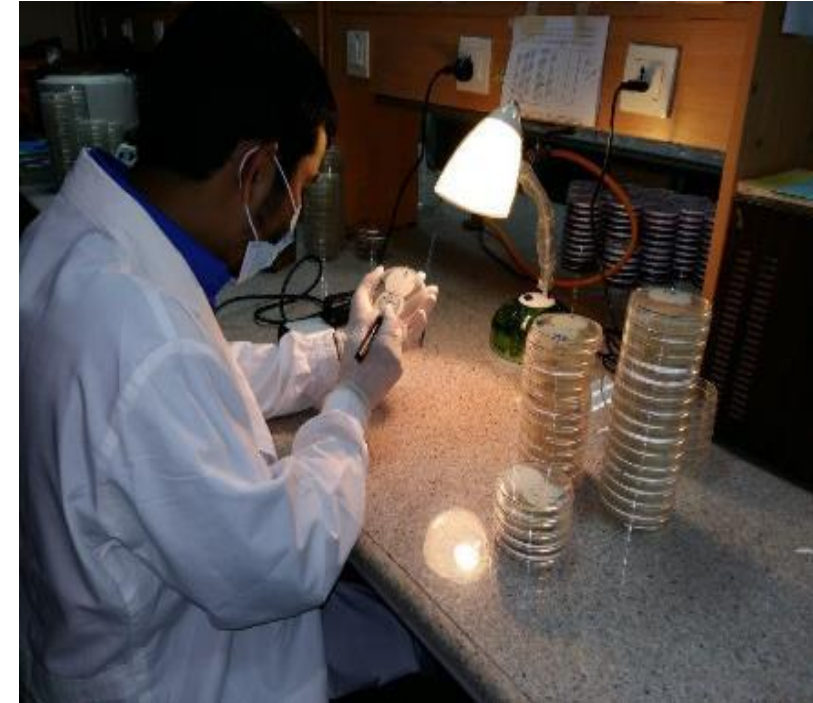
- ***Helminth eggs***

Escherichia coli

- ❑ *E. coli* normally colonizes an infant's gastrointestinal tract within 40 hours of birth, arriving with food or water or with the individuals handling the child.
- ❑ *E. coli* is a type of fecal coliform bacteria commonly found in the intestines of animals and humans.
- ❑ The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination. Sewage may contain many types of disease-causing organisms.
- ❑ Since it is always found in feces, it is a more direct indicator of fecal contamination and the possible presence of enteric pathogens.

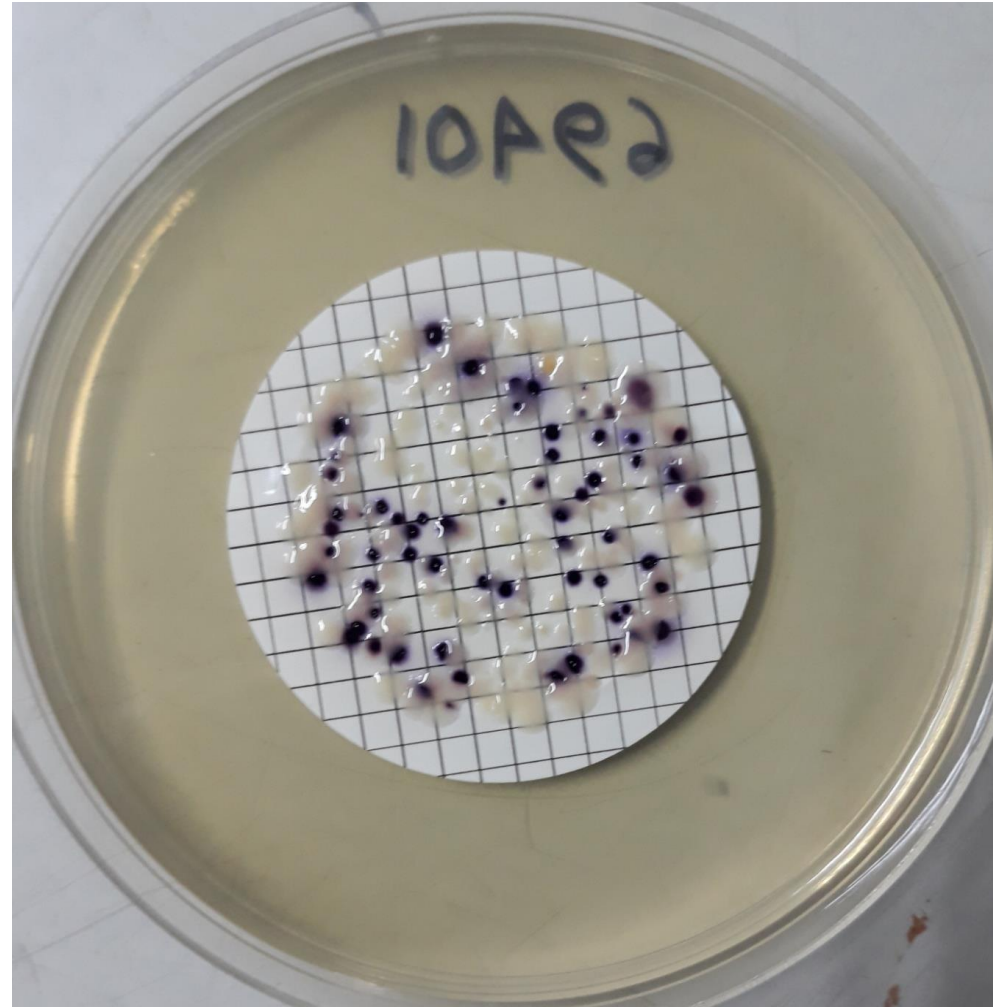
E. Coli detection method

- Sample collection and transportation to the lab maintaining the cold chain (4°-10°C)
- Then, samples are decimally diluted (e.g. 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4})
- Processing using mTEC agar:
 - Filtration
 - Drop-plate (alternatively used)
- Incubation at 37°C for 2 hours and further at 44°C for 18-24hrs.
- Red or magenta colonies are counted and result given in CFU/100 ml.



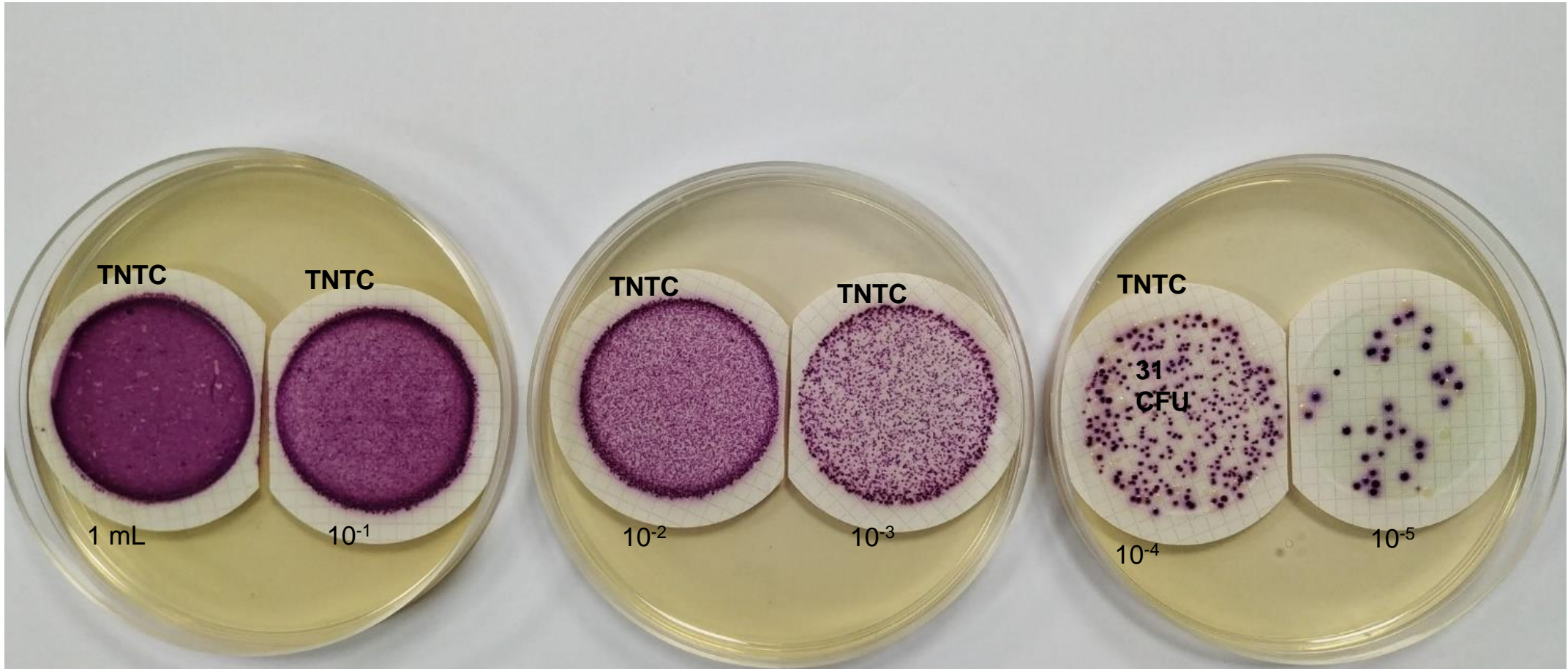
Analysis of Water Samples in the Laboratory

How *E. coli* look like in the chromogenic agar media



E. coli (Purple color colonies in modified mTEC agar plate)

Serial dilution of the Grab/FSTP samples to get countable bacterial colony



N.B. TNTC – Too Numerous to Count, CFU- Colony Forming Units

If 10⁻⁵ dilution contains 31 CFU, then 100 mL original sample will contain 31 x 10⁷ CFU

Evaluation of *E. coli* Petri plates and gold standard laboratory culture media



Two different batch of samples were tested for evaluation of the culture plates used to enumerate *E. coli*. In the first batch, two different volumes (1 ml and 5 ml) of household drinking water sample collected from Rohingya Camp were filtered and the filter papers were placed on **(1)** Petri plates and **(2)** Laboratory selective culture media (MI) for *E. coli* enumeration. Interpretation of the plates after overnight incubation are given below:

- (1) The characteristic blue colonies of *E. coli* are visible and can easily be enumerated.
- (2) The characteristic blue colonies of *E. coli* are highly visible and can easily be enumerated.



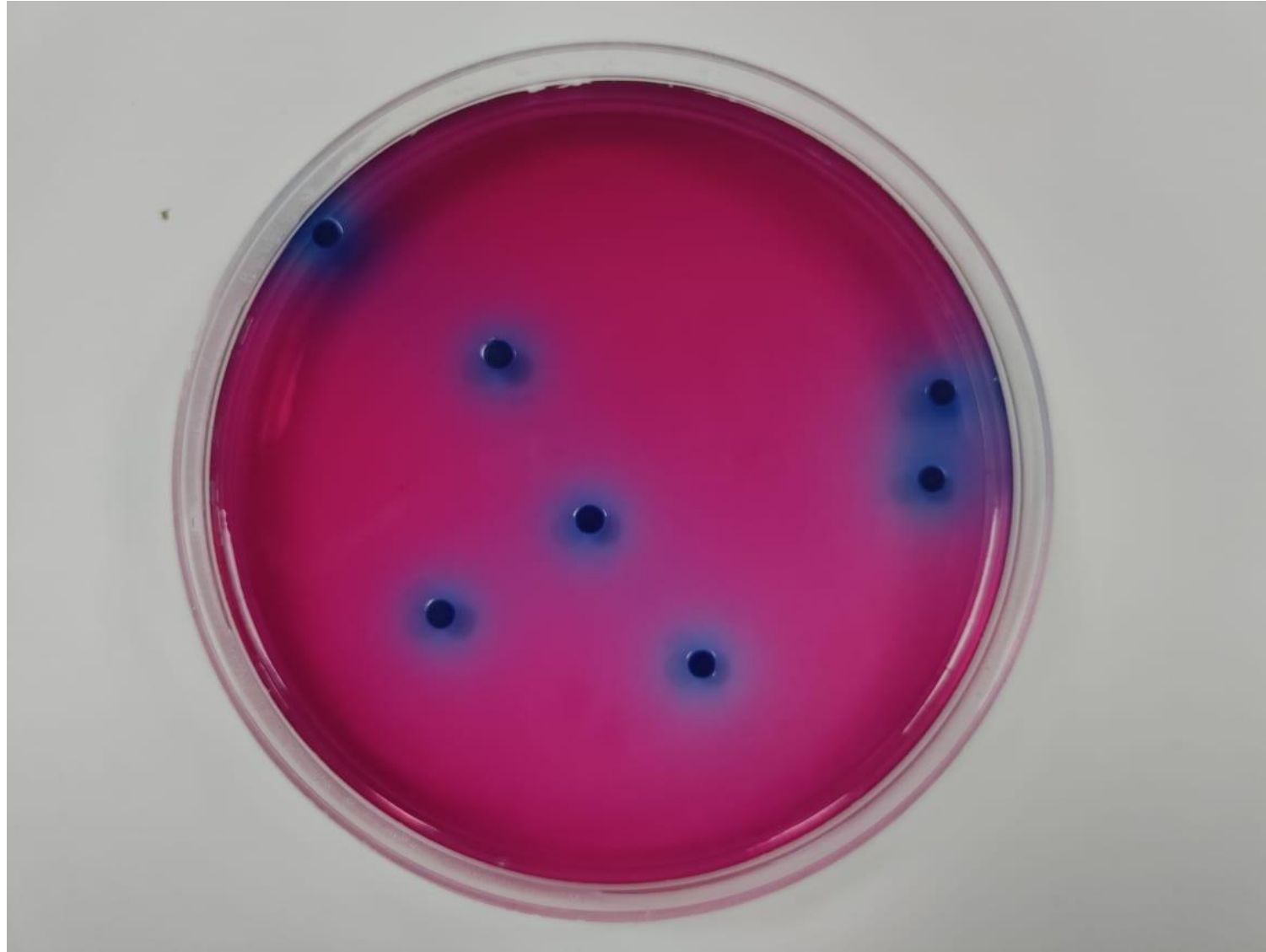
How *E. coli* look like under Microscope?

Faecal coliforms

- Indicates recent faecal contamination- greater risk that pathogens are present than if only total coliform bacteria is detected.
- *E. coli* is a sub-group of the faecal coliform group.

Faecal coliforms detection method

- Sample collection and transportation to the lab maintaining the cold chain (4°-10°C)
- Then, samples are decimally diluted (e.g. 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4})
- Processing using MFC agar:
 - Filtration
 - Drop-plate (alternatively used)
- Incubation at 44°C for 18-24hrs.
- Blue to dark blue colonies are counted and result given in CFU/100 ml.



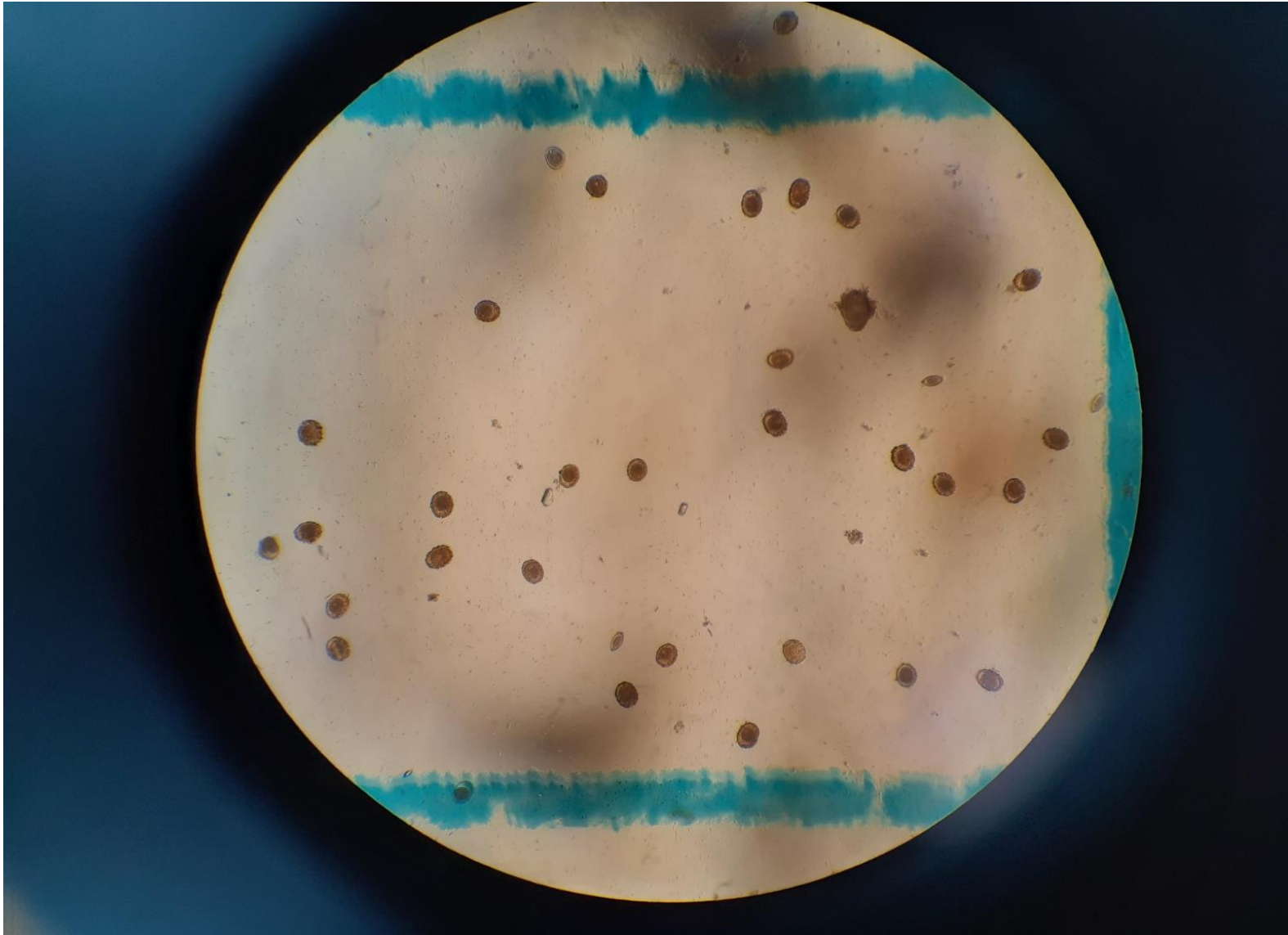
Faecal coliforms on mFC agar plate

Vibrio cholerae

- *Vibrio cholerae* is a "comma" shaped Gram-negative bacteria with a single, polar flagellum for movement.
- Numerous strains, some are pathogenic and some are not.
- Intestinal infection and increased mucous production causing diarrhea and vomiting leading to extreme dehydration and, if not treated, death.
- Transmitted through the feces of an infected person, often by way of unclean drinking water or contaminated food.

Helminth eggs

- **Helminth eggs are the infective agents for the types of worm diseases known globally as helminthiases and are contained in variable amounts in wastewater, sludge and excreta**
- **Soil transmitted helminths (CDC & WHO explanation) are considered an important public health parameter in sanitation.**
- **Monitoring treatment plant effluents for helminth eggs is considered highly important.**



Helminth eggs under microscope

Laboratory of Government Chemist (LGC)

Proficiency Testing Report from Laboratory of the Government Chemist (LGC)

- LGC is an international life sciences measurement and testing company.
- They provide proficiency testing schemes with localized support across a truly global network to over 13,000 laboratories in more than 160 countries.
- For the purposes of performance assessment z and z' scores are used based on assigned values and the laboratory results.

z/z' score	Interpretation	Color coding
$ z \leq 2.00$	Satisfactory result	Green
$2.00 < z $ and < 3.00	Questionable result	Amber
$ z \geq 3.00$	Unsatisfactory result	Red
No score given	See below	No colour coding

Here,

$$z = \frac{(x-X)}{SDPA}, \quad z' = \frac{(x-X)}{S\sqrt{SDPA^2 + (U_xAV)^2}}$$

X = Assigned value, $SDPA$ = Standard deviation for proficiency assessment, U_xAV = Uncertainty of the assigned value

Laboratory Performance Report (Physiochemical Parameters)

1H - Major Inorganic Components - Hard Water

Analyte	Analyst	Method	Result	Units	Assigned Value	Ux AV	SDPA	Z Score	Comment
Calcium	Lab Result	Titrimetry	73.80	mgCa/L	72.50	0.32	5.438	0.24	Satisfactory
Magnesium	Lab Result	Flame AAS	6.05	mgMg/L	6.20	0.04	0.465	-0.32	Satisfactory
Total Hardness	Lab Result	Titrimetry	83.2	mgCa/L	82.6	0.3	8.26	0.07	Satisfactory
Alkalinity	Lab Result	Titrimetry	207.4	mgHCO ₃ /L	201.1	0.8	20.11	0.32	Satisfactory
Chloride	Lab Result	Ion chromatography	45.60	mgCl/L	45.44	0.20	3.408	0.05	Satisfactory
Sulfate	Lab Result	Ion chromatography	30.29	mgSO ₄ /L	29.88	0.19	2.241	0.19	Satisfactory

2H - Nutrients and Others - Hard Water

Nitrite	Lab Result	Ion chromatography	0.315	mgNO ₂ /L	0.292	0.001	0.0219	1.05	Satisfactory
Nitrite	MSI	UV-VIS	0.304	mgNO ₂ /L	0.292	0.001	0.0219	0.55	Satisfactory
pH at 20-25°C	Lab Result	pH Meter	7.03	not specified	7.08	0.00	0.1	-0.50	Satisfactory
Conductivity (20°C)	Lab Result	Conductivity Meter	725	µS/cm (20°C)	720	2	54.0	0.09	Satisfactory
Nitrate	Lab Result	UV-VIS	4.88	mgNO ₃ /L	4.88	0.05	0.366	0.01	Satisfactory
Total dissolved solids	Lab Result	Calculated from conductivity	315.0	mg/L	303.0	4.1	30.3	0.40	Satisfactory

5A - Metals for Hydride Generation (Preserved in 0.5% Hydrochloric Acid)

Arsenic	Lab Result	Other	6.08	µg/L	5.88	0.02	0.588	0.34	Satisfactory
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Sample: 4G - Metals in Groundwater (in 0.5% Nitric Acid)

Iron	Lab Result	AAS	450	µg/L	434	4	32.6	0.49	Satisfactory
Manganese	Lab Result	Other	50	µg/L	52.4	0.5	3.93	-0.61	Satisfactory

* Please note, participant performance for this analyte has been assessed using a z' score, rather than a z score, in order to account for the measurement uncertainty of the assigned value which is not negligible when compared to the SDPA.

Laboratory Performance Report (Biological Parameters)

412 - Potable Water Indicator combination

Analyte	Analyst	Method	Result	Units	Log ₁₀	Assigned Value	Ux AV	SDPA	Z Score	Comment
Total aerobic count @ 37°C	Lab Result	Other	93	cfu/ml	1.97	85	0.01	0.35	0.11	Satisfactory
Escherichia coli	Lab Result	MEMF MI 35	52	cfu/100ml	1.72	63	0.01	0.35	-0.24	Satisfactory
Coliforms	Lab Result	Other	57	cfu/100ml	1.76	65	0.01	0.35	-0.16	Satisfactory
Enterococci (faecal streptococci)	Lab Result	MEMF SB 37	58	cfu/100ml	1.76	52	0.01	0.35	0.14	Satisfactory

Laboratory Performance Report (Physiochemical Parameters)

35 - BOD/COD at high concentration in waste water

Analyte	Analyst	Method	Result	Units	Assigned Value	Ux AV	SDPA	Z Score	Comment
COD	Lab Result	Closed reflux	427	mgO ₂ /L	421.5	1.5	21.1	0.26	Satisfactory
BOD	Lab Result	Other	203	mgO ₂ /L	190	7.3	19	0.64*	Satisfactory

* Please note, participant performance for this analyte has been assessed using a z' score, rather than a z score, in order to account for the measurement uncertainty of the assigned value which is not negligible when compared to the SDPA.

Laboratory Performance Report (Biological Parameters)

414 - Process Water

Analyte	Analyst	Method	Result	Units	Log ₁₀	Assigned Value	Ux AV	SDPA	Z Score	Comment
Total aerobic count	Lab Result	Other	10600	cfu/ml	4.03	7100	0.02	0.35	0.50	Satisfactory
Pseudomonas aeruginosa	ZHM	Other	3700	cfu/ml	3.57	3500	0.06	0.35	0.07	Satisfactory
Yeast	Lab Result	Other	2900	cfu/ml	3.46	1500	0.05	0.35	0.82	Satisfactory
Mould	ZHM	Other	2400	cfu/ml	3.38	2110	0.03	0.35	0.16	Satisfactory
Yeast & Mould	Lab Result	Other	5200	cfu/ml	3.72	3290	0.03	0.35	0.57	Satisfactory

419 - Surface/Waste/Bathing Water

Analyte	Result Field	Analyst	Method	Result	Assigned Value
Salmonella species	Result	Lab Result	Other	Detected	Detected