Decision tree to simply select a test for faecal contamination of drinking water

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Introduction

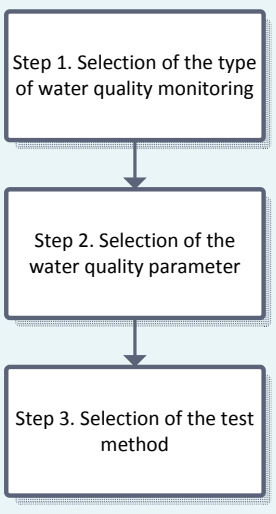
Many challenges exist in monitoring (drinking) water quality, particularly in resource-limited settings. Currently available microbial water tests often have a number of limitations within this context and require specific expertise. Tests are often cumbersome and while new technologies are emerging that promise to be cheaper, more user-friendly and faster, their efficacy and suitability for water quality testing needs to be reviewed.

Aim of the drinking water quality test decision tree

Various microbial tests are available, and choosing a suitable method for a particular application and setting is challenging. This decision tree provides guidance for the selection of appropriate monitoring parameters and choosing suitable tests for estimating these parameters.

How does the decision tree help you to select drinking water quality tests?

The decision tree supports the selection of microbial water quality tests for different purposes in safe drinking water production. The decision tree guides the user in three steps to a list of fit-for-purposes microbial water quality tests.The decision tree is meant for microbial water quality testing of water used for drinking or the production of drinking-water. To select a test for determining the microbial water quality three steps should be made:



Step 1. Selection of the type of water quality monitoring
It may be possible that within your organization or project, multiple water samples originating from different positions in the water supply chain must be tested, which are used for different types of monitoring. In contrast, the decision tree is meant to be used for a group of samples originating from a particular position in the water supply chain and which should be subjected to one specific type of monitoring. Hence, to address all questions in a project, it may be necessary to use the decision tree several times for the selection of different water quality tests for different monitoring purposes.

Step 2. Selection of the water quality parameter
It is not practical or possible to test water for all of the parameters that may cause health risks, therefore information based choices have to be made. This decision tree provides guidance for the selection of indicators of fecal contamination, the detection of pathogens is not within the scope of this decision tree. Selected parameters need to be both informative and economical. The selection of informative parameters depends on different circumstances, including the use of the water, the purpose of testing, water type (source) and hazards. Available resources and operator proficiency are likely to restrict the number of desired parameters as well as the frequency of testing.

Step 3. Selection of the test method
After selection of the parameters to be tested, a choice should be made for the methods used to measure these parameters. This decision tree only considers in detail methods for measuring indicators.

More detailed background information is described in the accompanying document.

Use of the colours

In the decision tree, different tekst box colours have a particular significance:

Grey: questions guiding you through the decision tree

Pale orange: examples (for clarification)

Blue: Additional Information regarding testing conditions

Yellow: Restrictions of testing options

Question to be answered with YES or NO

Green: selection – link to the next page [PDF: click with left mouse button; Visio: CRTL + click left mouse button]

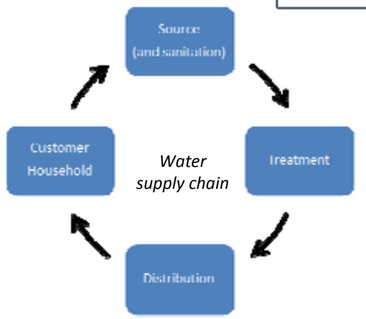
Orange: selection (optional), link to the next page[PDF: click with left mouse button; Visio: CRTL + click left mouse button]

Red: not required, no follow up

Yellow: selection to go back in the decision tree [PDF: click with left mouse button; Visio: CRTL + click left mouse button]

Start the decision tree (click here)

Drinking-water or water used for the production of drinking-water



Note:
It may be possible that within your organization or project, multiple water samples must be tested, originating from different positions in the water supply chain.
The decision tree is meant to be used for a group of samples originating from a particular position in the water supply chain. Hence, to address all questions in a project, it may be necessary to use the decision tree several times for the selection of different water quality tests.

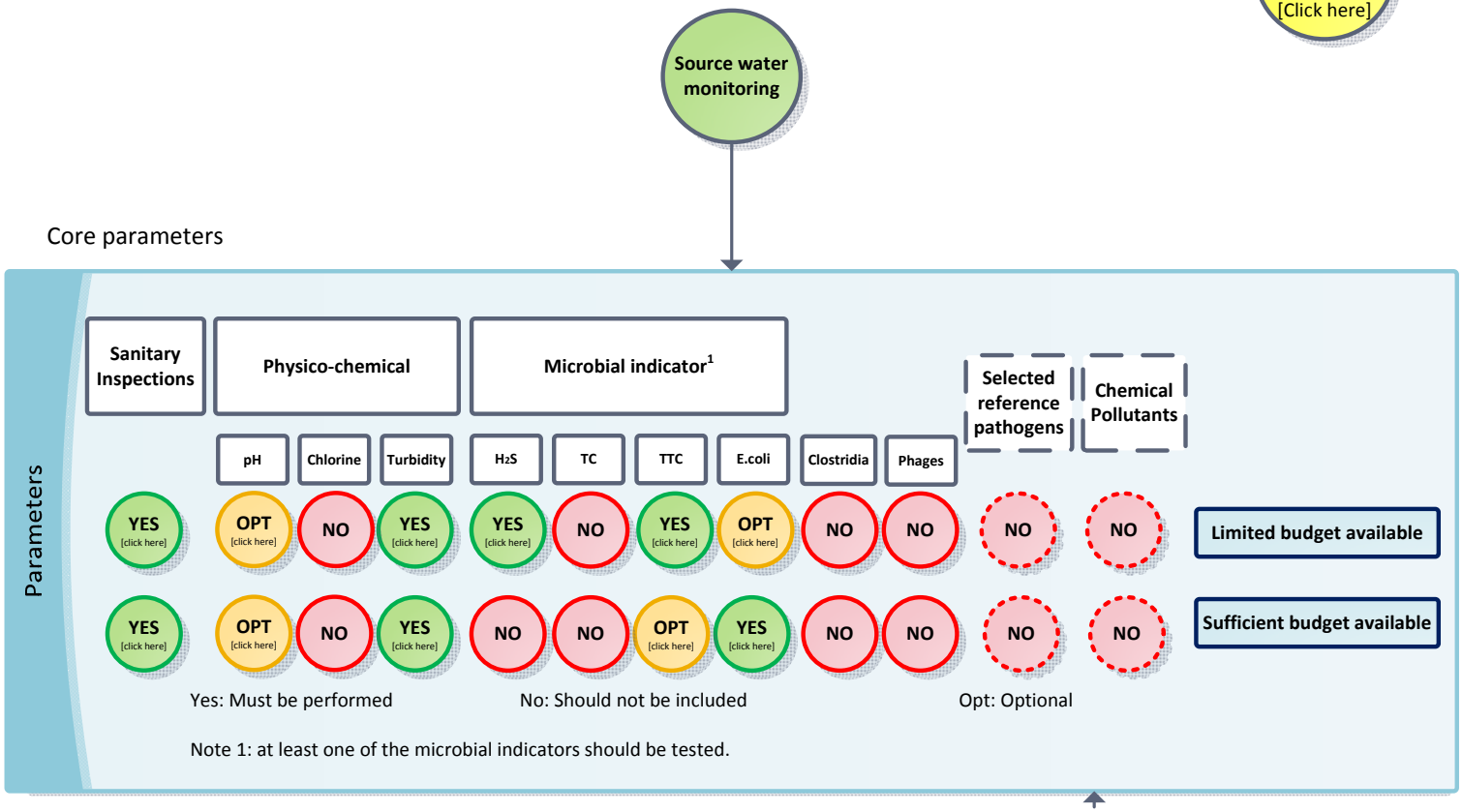
For instance, a survey of the quality of drinking water sources in a certain region may include different water types, such as dug wells and surface water. Also, the testing may be carried out by different agencies that do not all have the same laboratory equipment available.

Back to introduction [Click here]

Purpose of testing (drinking)water

Position of the sample in the water supply chain	Source (including sanitation)	Treatment			Distribution network, Consumer		Source-to-Tap	
Examples	• *Unprotected surface water* • *Poorly protected dug well* • *Rainwater storage*	• *Chlorination* • *(Sand)filtration* • *UV* • *Ozone*			• *Public tap* • *Pipelines* • *Reservoirs* • *Household*		• *Outbreak investigation* • *Awareness raising among consumers*	
Purpose of testing	Do you want to understand the characteristics and variations of source water quality?	Do you want to validate the treatment processes of your source water?	Do you want to check if the steps of the water supply system continue to work effectively?	Do you (as supplier) want to check if the water meets the regulatory standards after the treatment process?	Do you want to verify the performance of the drinking-water supply chain to supply safe drinking-water?	Do you (as independent agency) want to control the safety of drinking-water being consumed?	Do you want to investigate the source of an outbreak?	Do you want to raise awareness among consumers at the household level?
Examples	• *Investigation of fecal contamination of source waters, e.g. surface water, dug well* • *Compare different sources or collection points for selection of source water* • *Investigate effect of e.g. heavy rainfall on source water quality*	• *Confirm that control measures in the treatment, e.g. chlorination or sand filtration, are able to perform well given the source water quality*	• *Control chlorination by measuring chlorine residuals and pH, turbidity* • *Control filtration by measuring turbidity*	• *Compliance of the provided water to a national standard, e.g. absence of E. coli from 100 ml samples*	• *Assess possible recontamination during distribution to consumer* • *Assess whether the structure around a hand pump is complete and undamaged*	• *Compliance of the provided water to a national standard (on a national scale)* • *Assess waterborne health effects* • *Assess safety of drinking water in a household, community or area*	• *Investigate water samples to locate the source of a Cholera outbreak* • *Assess water borne health effects*	• *Inform consumers about the likeliness the water they consume is safe/ unsafe* • *Show consumers that water quality depends on household safe storage of drinking-water* • *Show the effect of point-of-use water treatment options*
Type of monitoring	**Source water monitoring** [click here]	**Validation** [click here]	**Operational monitoring** [click here]	**Compliance** [click here]		**Surveillance** [click here]	**Outbreak** [click here]	**Awareness raising** [click here]

Back to Selection of Monitoring [Click here]



Additional parameters

Not in the scope of this decision tree.
In addition to the core Indicators, it may be necessary to measure additional parameters which are selected based upon water type and hazards.
Note that some of these factors will also affect the frequency and location of momitoring for core indicators.

Water type.
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Unimproved sources: unprotected dug well; unprotected spring; cart with small tank or drum provided by water vendor; tanker truck provision of water; surface water (river, dam, lake, pond, stream, canal, irrigation channel); bottled water

Hazards.
Risks that may affect water quality may be related to local circumstances related to human activities and to naturally occurring micro-organisms.
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Peak events: heavy rainfall, sewage overflow, outbreaks
Maintainance: leaking pipelines, deadends, back flow, frequently interruptions

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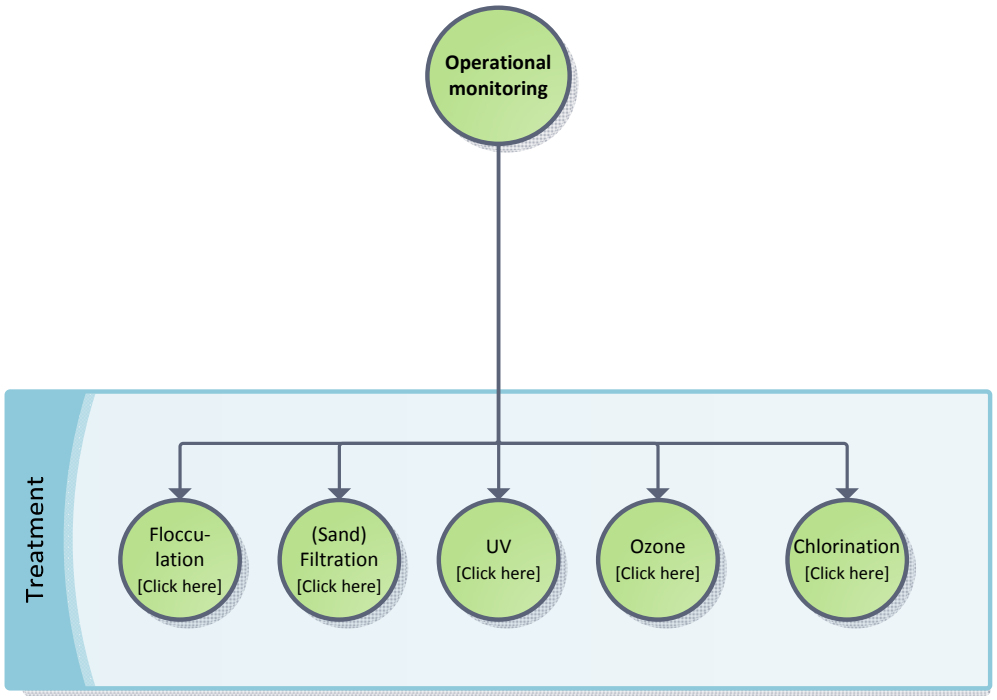

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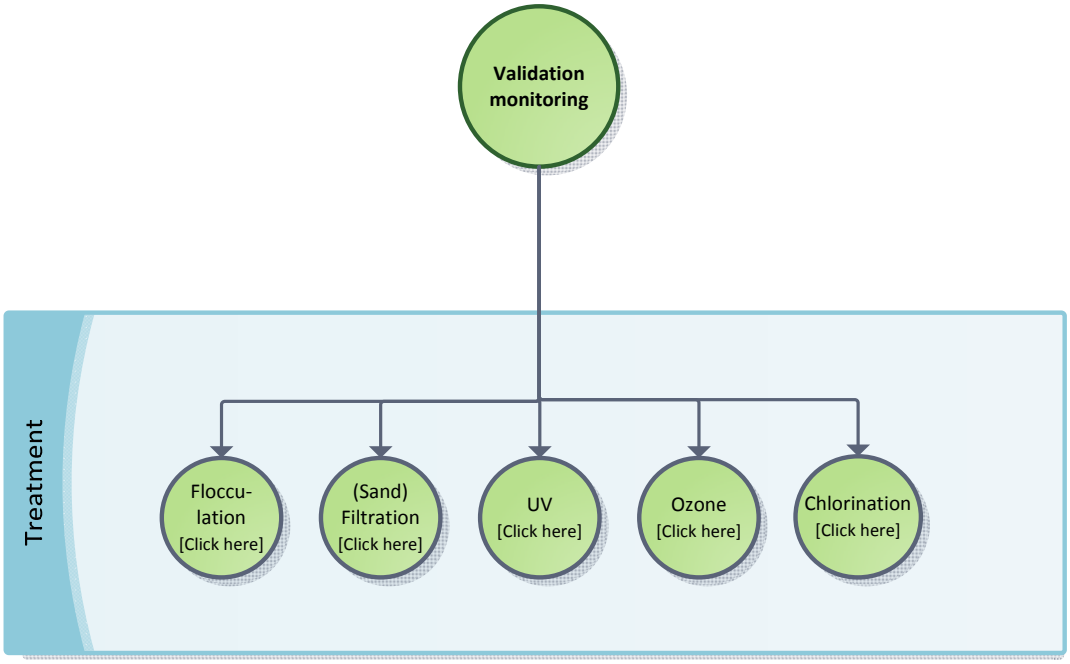

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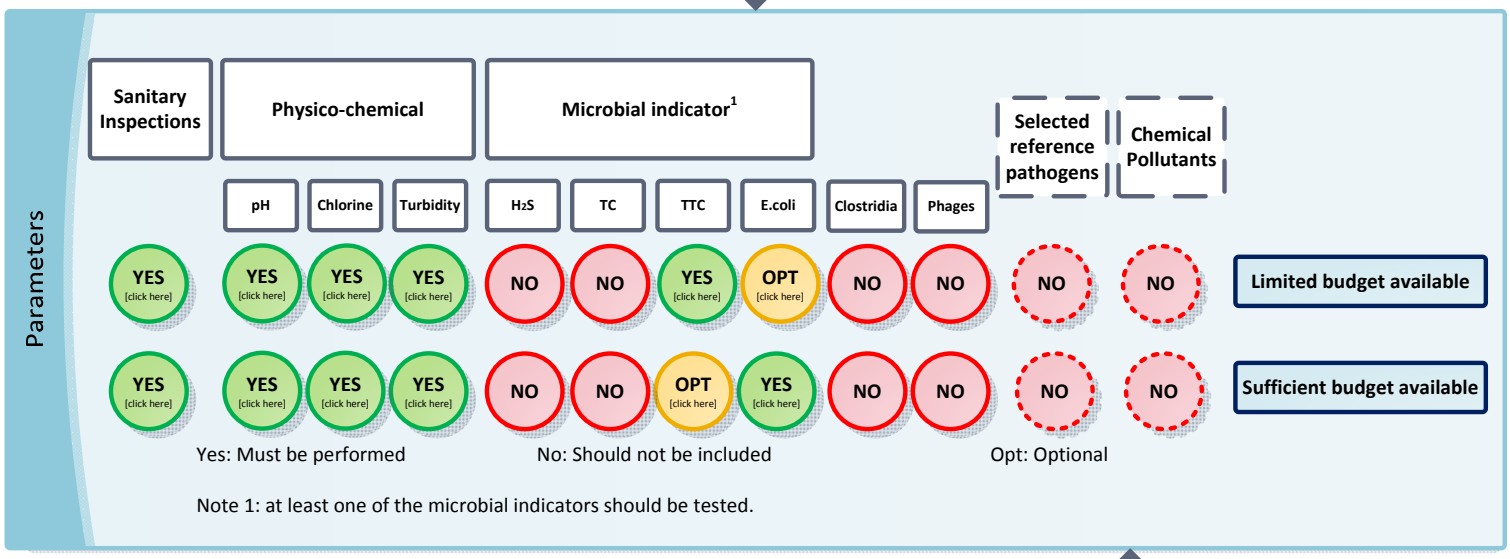
Compliance monitoring

Core parameters



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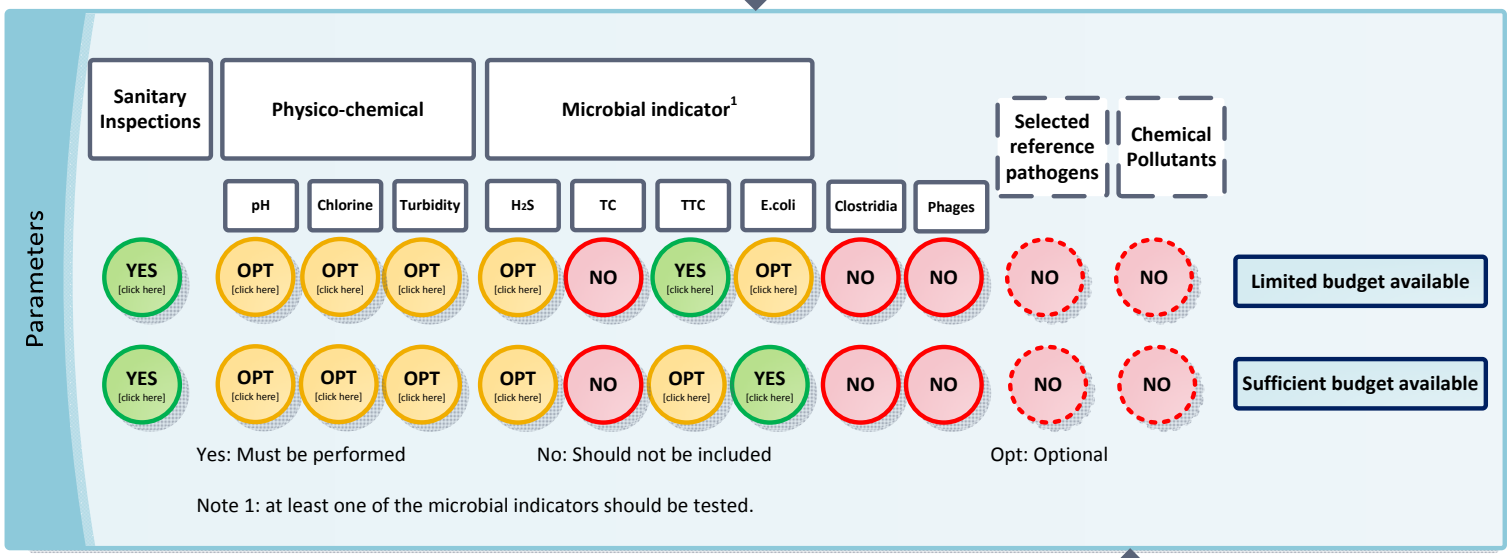
Surveillance

Core parameters



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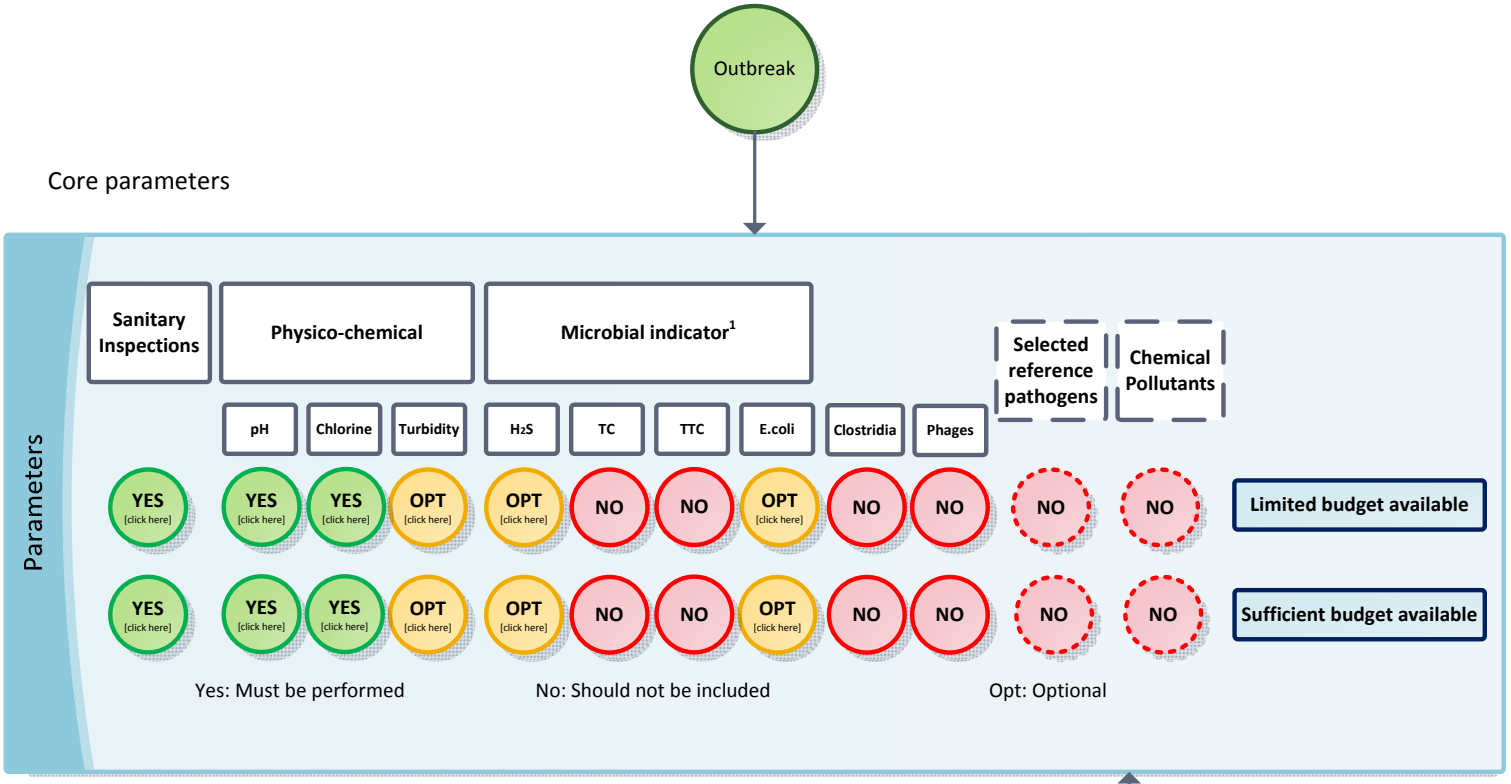

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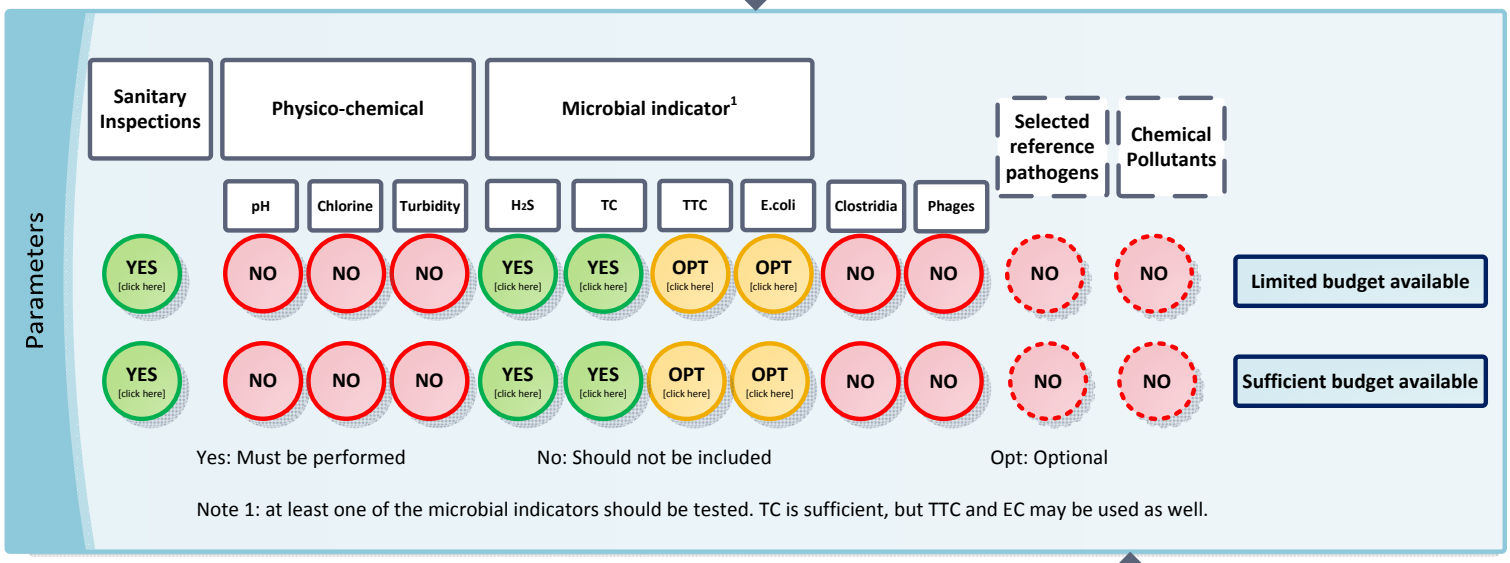
Awareness rising

Core parameters



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Back to Selection of Monitoring [Click here]

Validation Monitoring UV

Core parameters

Parameters

	Sanitary Inspections	Physico-chemical			Microbial indicator[1]						Selected reference pathogens	Chemical Pollutants	
		pH	Chlorine	Turbidity	H_2S	TC	TTC	E.coli	Clostridia	Phages			
	NO	NO	NO	YES [click here]	NO	YES [click here]	OPT [click here]	OPT [click here]	NO	NO	NO	NO	Limited budget available
	NO	NO	NO	YES [click here]	NO	YES [click here]	OPT [click here]	OPT [click here]	OPT	OPT	NO	NO	Sufficient budget available

Yes: Must be performed No: Should not be included Opt: Optional

Note 1: at least one of the microbial indicators should be tested. TC is sufficient, but TTC and EC may be used as well.

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Validation Monitoring Chlorination

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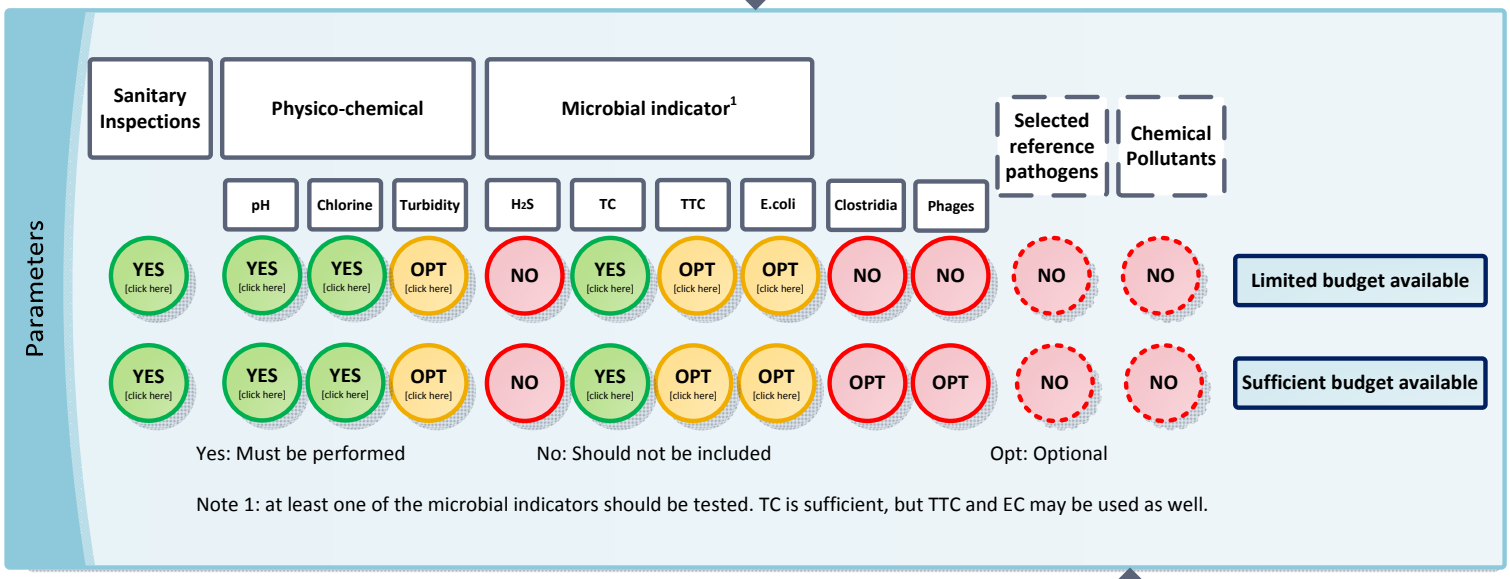
Core parameters



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Validation Monitoring Ozone

Core parameters

Parameters

Sanitary Inspections	Physico-chemical			Microbial indicator[1]					Selected reference pathogens	Chemical Pollutants	
	pH	Chlorine	Turbidity	H_2S	TC	TTC	E.coli	Clostridia	Phages			
NO	YES [click here]	YES [click here]	OPT [click here]	NO	YES [click here]	OPT [click here]	OPT [click here]	NO	NO	NO	NO	Limited budget available
NO	YES [click here]	YES [click here]	OPT [click here]	NO	YES [click here]	OPT [click here]	OPT [click here]	OPT	OPT	NO	NO	Sufficient budget available

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Back to Validation Monitoring [Click here]

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Validation Monitoring (Sand) Filtration

Core parameters

Parameters

Sanitary Inspections	Physico-chemical			Microbial indicator[1]						Selected reference pathogens	Chemical Pollutants	
	pH	Chlorine	Turbidity	H_2S	TC	TTC	E.coli	Clostridia	Phages			
NO	NO	NO	YES [click here]	NO	YES [click here]	OPT [click here]	OPT [click here]	NO	NO	NO	NO	Limited budget available
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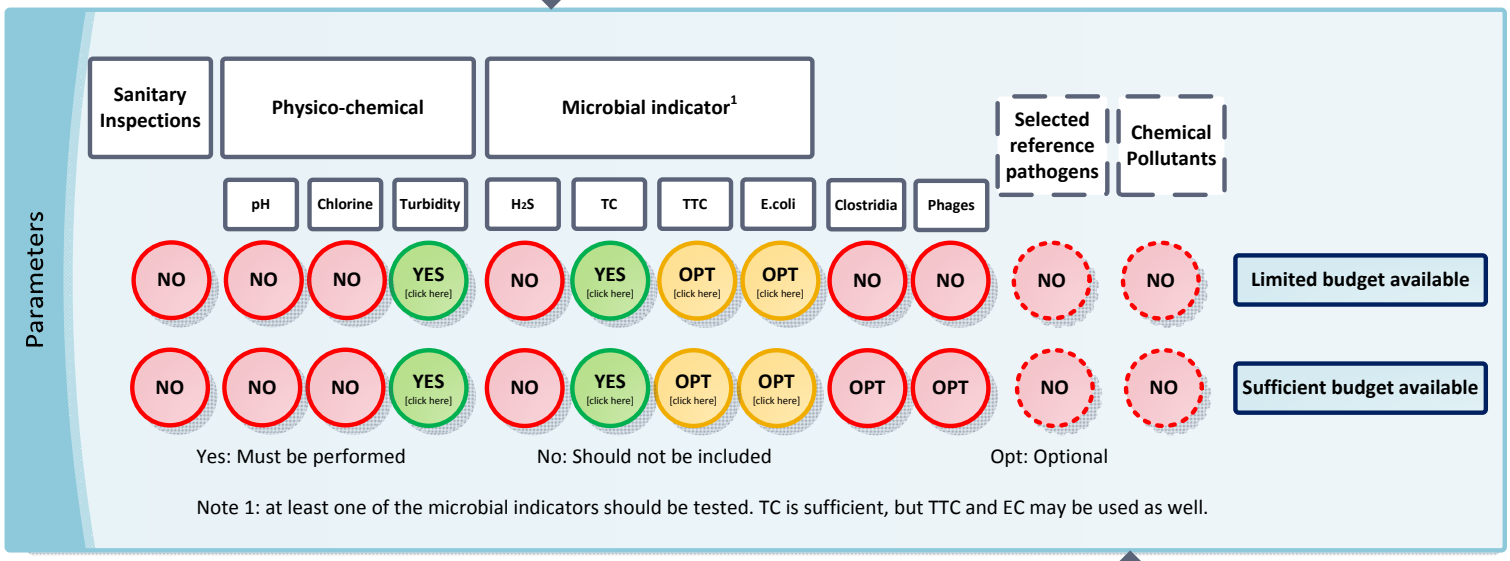
Back to Selection of Monitoring [Click here]

Validation Monitoring Flocculation

Core parameters



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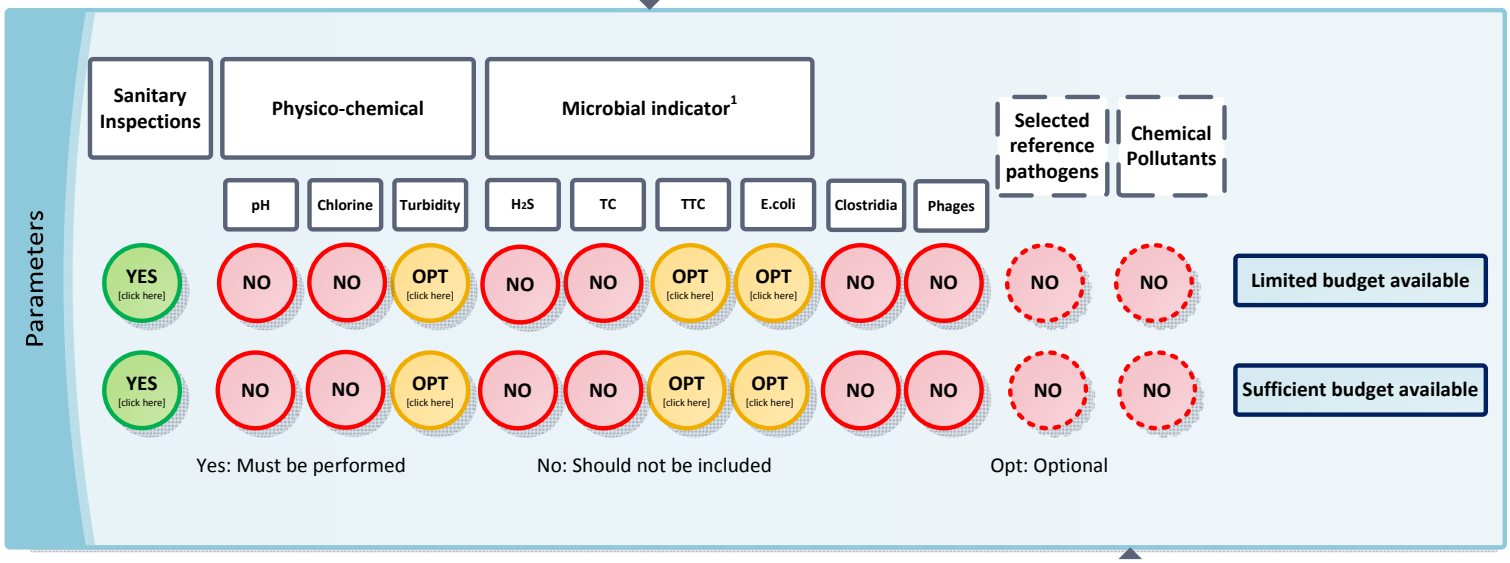
Operational Monitoring UV

Core parameters



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Back to Operational Monitoring [Click here]

Back to Selection of Monitoring [Click here]

Operational Monitoring Chlorination

Core parameters

Parameters

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	pH	Chlorine	Turbidity	H_2S	TC	TTC	E.coli	Clostridia	Phages			
YES [click here]	YES [click here]	YES [click here]	YES [click here]	NO	NO	OPT [click here]	OPT [click here]	NO	NO	NO	NO	Limited budget available
YES [click here]	YES [click here]	YES [click here]	YES [click here]	NO	NO	OPT [click here]	OPT [click here]	NO	NO	NO	NO	Sufficient budget available

Yes: Must be performed

No: Should not be included

Opt: Optional

Additional parameters

Not in the scope of this decision tree.
In addition to the core Indicators, it may be necessary to measure additional parameters which are selected based upon water type and hazards.
Note that some of these factors will also affect the frequency and location of momitoring for core indicators.

Water type.
The water type can be divided into improved and unimproved sources, where improved sources mostly are less susceptible to microbial contamination.
Improved sources: . Examples are piped water into dwelling, yard or plot; public tap or standpipe; tubewell or borehol; protected dug well; protected spring; rainwater collection
Unimproved sources: unprotected dug well; unprotected spring; cart with small tank or drum provided by water vendor; tanker truck provision of water; surface water (river, dam, lake, pond, stream, canal, irrigation channel); bottled water

Hazards.
Risks that may affect water quality may be related to local circumstances related to human activities and to naturally occurring micro-organisms.
Naturally occuring: chemicals (e.g. Fluoride and Arsenic) and microbial contaminants (e.g. *Vibrio cholera*, *Legionella* and *Cyanobacteria*)
Human derived: industry (e.g. slaughterhouses), recreation, agriculture (e.g. manure disposal) and sanitation (e.g. septic tanks and sewage treatment plants).
Peak events: heavy rainfall, sewage overflow, outbreaks
Maintainance: leaking pipelines, deadends, back flow, frequently interruptions

Back to Operational Monitoring [Click here]

Back to Selection of Monitoring [Click here]

Operational Monitoring Ozone

Core parameters

Parameters

Sanitary Inspections	Physico-chemical			Microbial indicator[1]						Selected reference pathogens	Chemical Pollutants	
	pH	Chlorine	Turbidity	H_2S	TC	TTC	E.coli	Clostridia	Phages			
NO	YES [click here]	NO	YES [click here]	NO	NO	OPT [click here]	OPT [click here]	NO	NO	NO	NO	Limited budget available
NO	YES [click here]	NO	YES [click here]	NO	NO	OPT [click here]	OPT [click here]	NO	NO	NO	NO	Sufficient budget available

Yes: Must be performed

No: Should not be included

Opt: Optional

Additional parameters

Not in the scope of this decision tree.
In addition to the core Indicators, it may be necessary to measure additional parameters which are selected based upon water type and hazards.
Note that some of these factors will also affect the frequency and location of momitoring for core indicators.

Water type.
The water type can be divided into improved and unimproved sources, where improved sources mostly are less susceptible to microbial contamination.
Improved sources: . Examples are piped water into dwelling, yard or plot; public tap or standpipe; tubewell or borehol; protected dug well; protected spring; rainwater collection
Unimproved sources: unprotected dug well; unprotected spring; cart with small tank or drum provided by water vendor; tanker truck provision of water; surface water (river, dam, lake, pond, stream, canal, irrigation channel); bottled water

Hazards.
Risks that may affect water quality may be related to local circumstances related to human activities and to naturally occurring micro-organisms.
Naturally occuring: chemicals (e.g. Fluoride and Arsenic) and microbial contaminants (e.g. *Vibrio cholera*, *Legionella* and *Cyanobacteria*)
Human derived: industry (e.g. slaughterhouses), recreation, agriculture (e.g. manure disposal) and sanitation (e.g. septic tanks and sewage treatment plants).
Peak events: heavy rainfall, sewage overflow, outbreaks
Maintainance: leaking pipelines, deadends, back flow, frequently interruptions

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Operational Monitoring (Sand) Filtration

Core parameters

Parameters

Sanitary Inspections	Physico-chemical			Microbial indicator[1]						Selected reference pathogens	Chemical Pollutants	
	pH	Chlorine	Turbidity	H_2S	TC	TTC	E.coli	Clostridia	Phages			
YES [click here]	NO	NO	YES [click here]	NO	NO	OPT [click here]	OPT [click here]	NO	NO	NO	NO	Limited budget available
YES [click here]	NO	NO	YES [click here]	NO	NO	OPT [click here]	OPT [click here]	NO	NO	NO	NO	Sufficient budget available

Yes: Must be performed

No: Should not be included

Opt: Optional

Additional parameters

Not in the scope of this decision tree.
In addition to the core Indicators, it may be necessary to measure additional parameters which are selected based upon water type and hazards.
Note that some of these factors will also affect the frequency and location of momitoring for core indicators.

Water type.
The water type can be divided into improved and unimproved sources, where improved sources mostly are less susceptible to microbial contamination.
Improved sources: . Examples are piped water into dwelling, yard or plot; public tap or standpipe; tubewell or borehol; protected dug well; protected spring; rainwater collection
Unimproved sources: unprotected dug well; unprotected spring; cart with small tank or drum provided by water vendor; tanker truck provision of water; surface water (river, dam, lake, pond, stream, canal, irrigation channel); bottled water

Hazards.
Risks that may affect water quality may be related to local circumstances related to human activities and to naturally occurring micro-organisms.
Naturally occuring: chemicals (e.g. Fluoride and Arsenic) and microbial contaminants (e.g. *Vibrio cholera*, *Legionella* and *Cyanobacteria*)
Human derived: industry (e.g. slaughterhouses), recreation, agriculture (e.g. manure disposal) and sanitation (e.g. septic tanks and sewage treatment plants).
Peak events: heavy rainfall, sewage overflow, outbreaks
Maintainance: leaking pipelines, deadends, back flow, frequently interruptions

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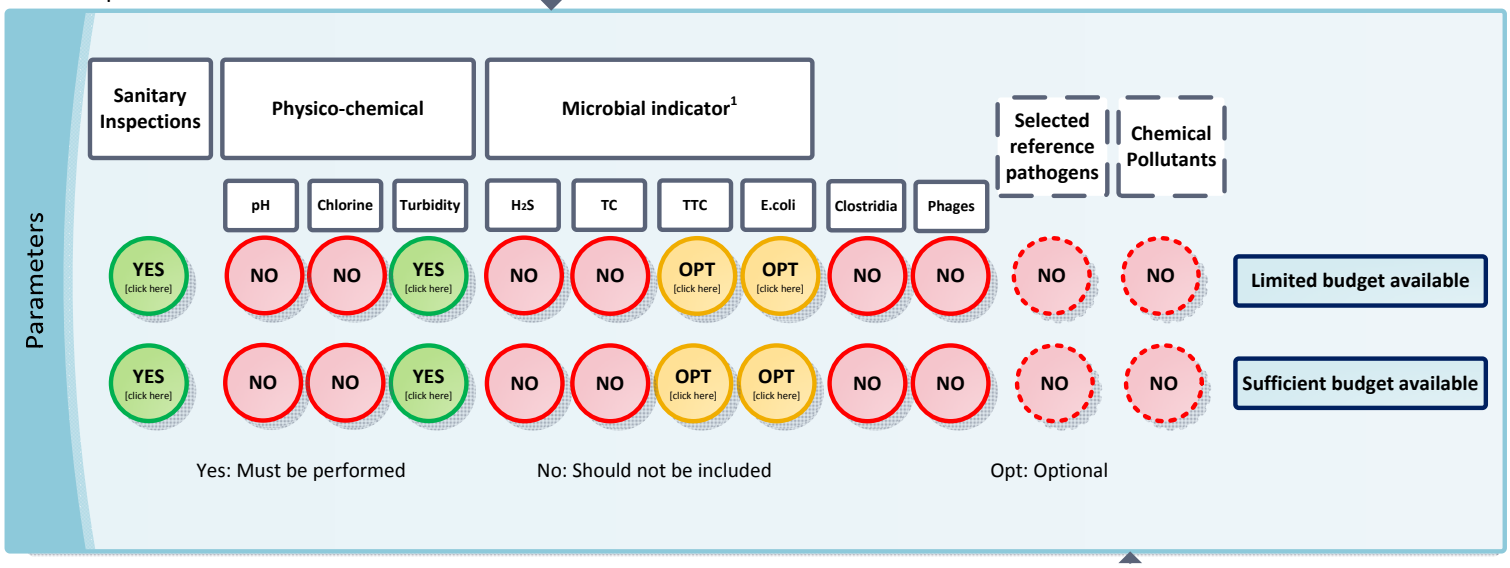
Operational Monitoring Flocculation

Core parameters



Additional parameters

Not in the scope of this decision tree.
In addition to the core Indicators, it may be necessary to measure additional parameters which are selected based upon water type and hazards.
Note that some of these factors will also affect the frequency and location of momitoring for core indicators.

Water type.
The water type can be divided into improved and unimproved sources, where improved sources mostly are less susceptible to microbial contamination.
Improved sources: . Examples are piped water into dwelling, yard or plot; public tap or standpipe; tubewell or borehol; protected dug well; protected spring; rainwater collection
Unimproved sources: unprotected dug well; unprotected spring; cart with small tank or drum provided by water vendor; tanker truck provision of water; surface water (river, dam, lake, pond, stream, canal, irrigation channel); bottled water

Hazards.
Risks that may affect water quality may be related to local circumstances related to human activities and to naturally occurring micro-organisms.
Naturally occuring: chemicals (e.g. Fluoride and Arsenic) and microbial contaminants (e.g. *Vibrio cholera*, *Legionella* and *Cyanobacteria*)
Human derived: industry (e.g. slaughterhouses), recreation, agriculture (e.g. manure disposal) and sanitation (e.g. septic tanks and sewage treatment plants).
Peak events: heavy rainfall, sewage overflow, outbreaks
Maintainance: leaking pipelines, deadends, back flow, frequently interruptions

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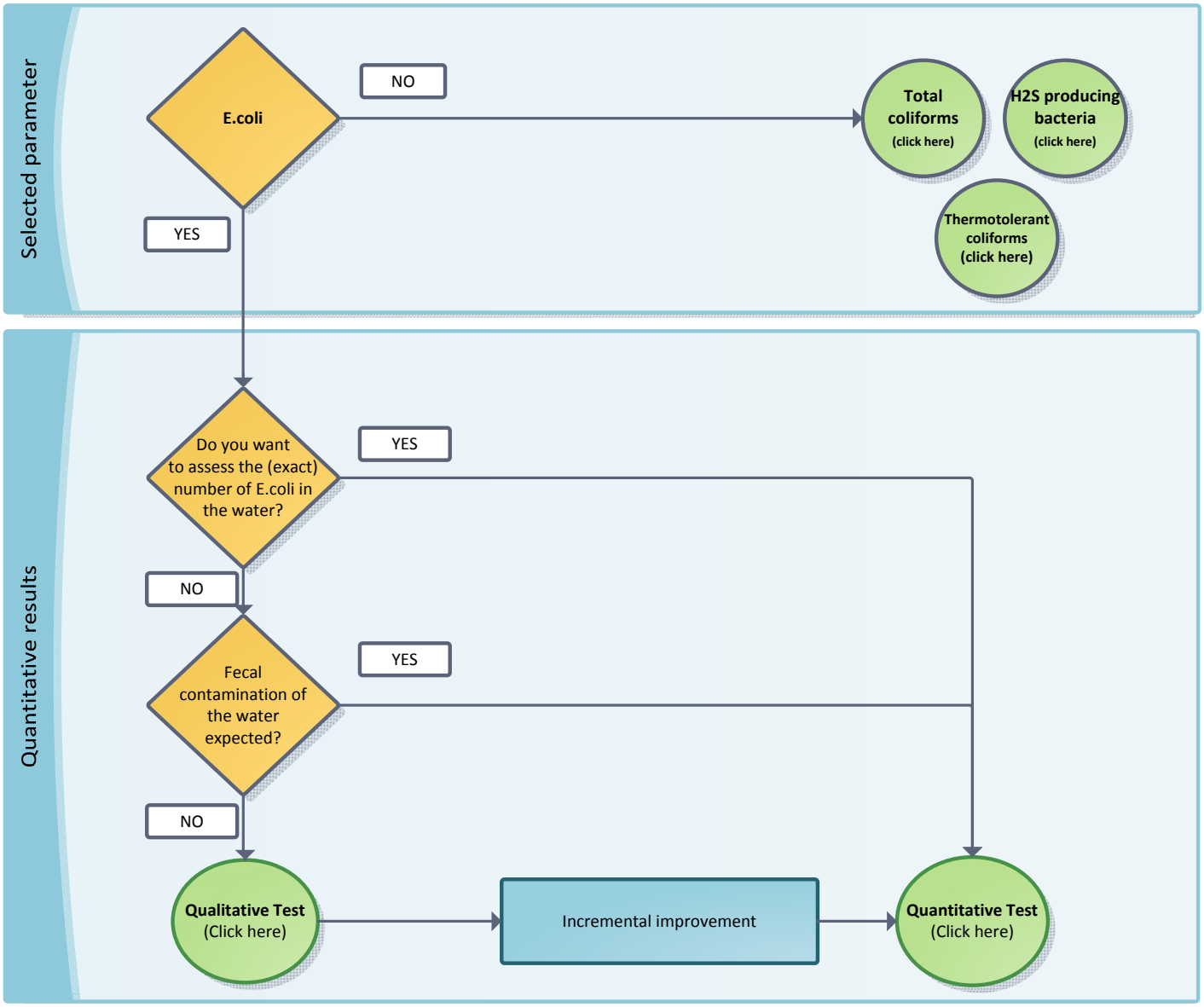

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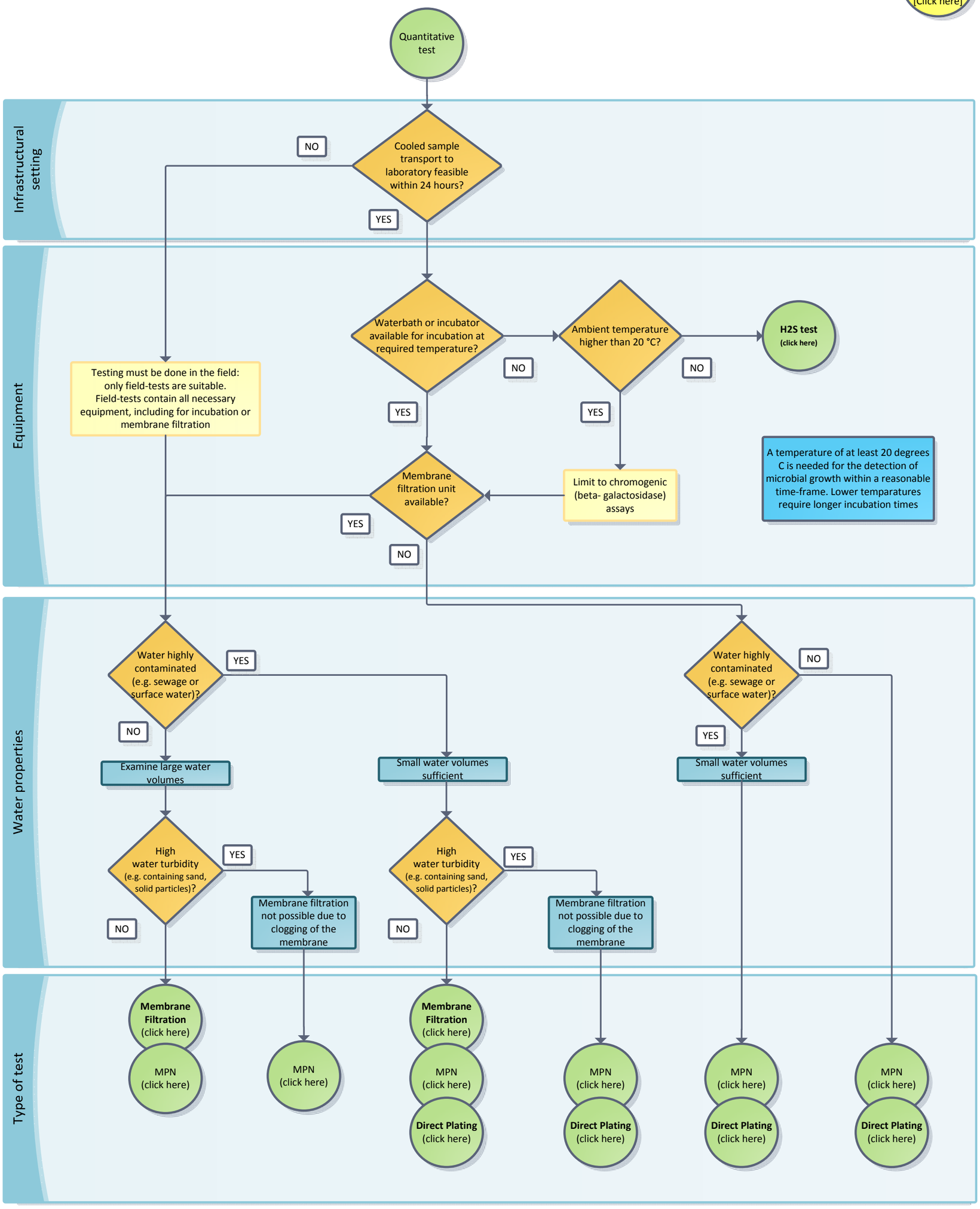

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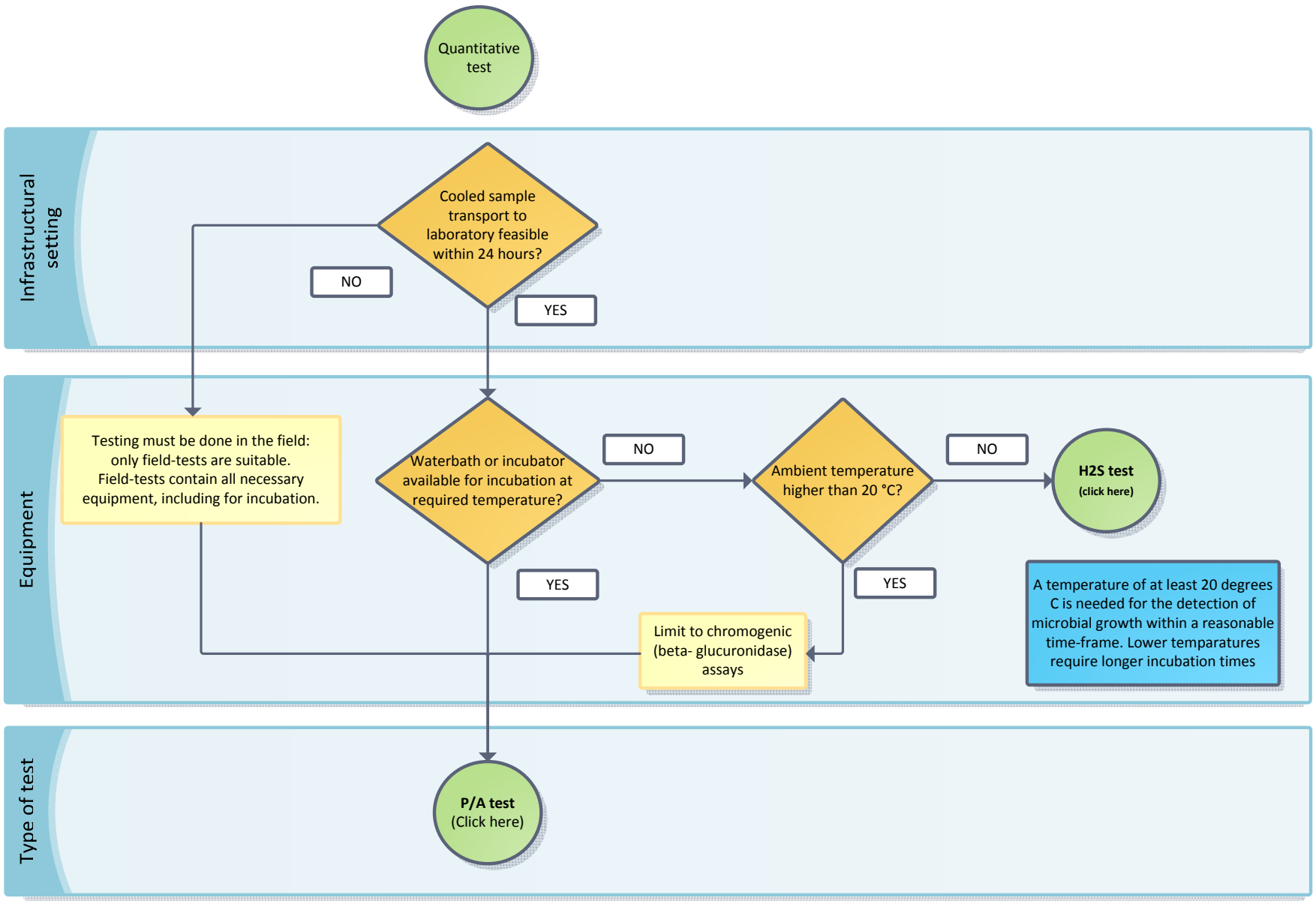

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P/A E.coli

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A water sample is inoculated into liquid medium that facilitates growth of *E.coli*. The only conclusion that can be drawn using this method is the **presence or absence** of *E.coli* in this test volume, but no information can be yielded about its concentration. P/A tests can be applied to verify that water quality meets regulatory standards (e.g. absence in 100ml).

Advantages presence/absence:
- Applicable to both clear and turbid samples.
- Allow growth of injured bacteria.
- Require minimal equipment (including incubator), time and effort, and use inexpensive media.

Disadvantages presence/absence:
- No quantitative data provided, only qualitative data.
- Only appropriate in circumstances where the micro-organisms of interest are rarely found.

Detection limit
Most available methods/kits are sensitive enough to detect a single colony forming unit (cfu) per 100 ml of water. The detection limit depends on the tested water volume and therefore the detection limit can vary.

Examples for detection based on chromogenic media (beta-glucuronidase)

Media	Shelf-life	Storage
HiSelective *E.coli*	1 year	RT
AquaCHROM	2 years	RT
Readycult	3 years	RT
E*Colite	1 year	RT
EC Blue	1 year	RT
Colilert/Quantitray	1 year	RT
Modified Colitag	2 years	RT
Watercheck	3 years	RT

RT = room temperature
For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Possibly suitability for application at ambient temperatures (needs to be validated if not explicitly indicated by the manufacturer).
- Limited training needed for use and interpretation of results.

Disadvantages:
- Higher cost than classical media.

Examples for detection using classical media

Media	Shelf-life	Storage
Lauryl tryptose broth*	3 years	RT
Lactose broth*	3 years	RT
EC-broth	3 years	RT

RT = room temperature
* Additional test (brilliantgreen lactose bile broth) needed.

For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Lower cost than GluA based detection.
- Cheaper when processing large numbers of samples.

Disadvantages:
- More training needed for use and interpretation of results.

Field testing kits

If the water quality testing must be done in the field, field testing kits are available. These kits contain all the equipment (e.g. incubator) and reagents needed for the analyses. Examples of field testing kits are:
- Compartmentalised bag test
- Aquatest
- Colifast field kit
- Hach: presence/absence test; MEL/m-ColiBlue Field Filtration Lab

If the equipment needed for the analyses in the field are available all media (both classical and based on glucuronic acid) can be used.
Also see UNICEF catalogue (Unicef, Water quality assessment and monitoring, Technical Bulletin No.6).

Relevant parameters for waste production are its volume and its biosafety consequences.
Waste, such as incubated petridishes, could be microbiologically contaminated and should be decontaminated before thrown away. Therefore, decontamination methods (e.g. chlorine addition or heat-sterilization) need to be considered.
Desinfection methods also deserve consideration when re-using materials as such materials need to be disinfected after use (in case more samples are tested).

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MPN E.coli

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The **most probable number** (MPN) approach uses replicated presence/absence tests of different volumes or dilutions to estimate the concentration of *E.coli* in a water sample based on which sample volumes grow *E.coli* (presence) and which do not (absence). The most probable number of *E.coli* present in the original sample can be calculated on the basis of the numbers of positive and negative test portions observed after incubation.

Advantages most probable number:
- MPN tests are essentially multiple P/A tests and therefore have comparable advantages
  - The method is applicable to clear and turbid samples.
  - Allow growth of injured bacteria.
- Quantitative data are provided.
- Require minimal equipment (including incubator), time and effort, use inexpensive media.

Disadvantages most probable number:
- More equipment, media, time and effort are required than with P/A testing.
- More reliable results require more replicas of the multiple tubes, which causes a significant increase in required time, materials and cost.
- Interpretation of the results requires trained personnel.

Detection limit
Most available methods/kits are sensitive enough to detect a single colony forming unit (cfu) per 100 ml of water. The detection limit depends on the tested water volume and therefore the detection limit can vary.

Examples for detection based on chromogenic media (beta-glucuronidase)

Media	Shelf-life	Storage
Compartmentalised bag test	<9 months	RT
Aquatest	2 years	RT
Colilert/Quantitray	1 year	RT
EC BlueQuant	1 year	RT
Colitag	2 years	RT
ColiPlate	3 years	RT

RT = room temperature
For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Possibly suitability for application at ambient temperatures (needs to be validated if not explicitly indicated by the manufacturer).
- Limited training needed for use and interpretation of results.

Disadvantages:
- Higher cost than classical media.

Examples for detection using classical media

Media	Shelf-life	Storage
Lauryl tryptose broth*	3 years	RT
Lactose broth*	3 years	RT

RT = room temperature
* Additional test (brilliantgreen lactose bile broth) needed.

For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Lower cost than GluA based detection.
- Cheaper when processing large numbers of samples.

Disadvantages:
- More training needed for use and interpretation of results.

Field testing kits

If the water quality testing must be done in the field, field testing kits are available. These kits contain all the equipment (e.g. incubator) and reagents needed for the analyses. Examples of field testing kits are:
- Compartmentalised bag test
- Aquatest

If the equipment needed for the analyses in the field are available all media (both classical and based on glucuronic acid) can be used.
Also see UNICEF catalogue (Unicef, Water quality assessment and monitoring, Technical Bulletin No.6).

Relevant parameters for waste production are its volume and its biosafety consequences.
Waste, such as incubated petridishes, could be microbiologically contaminated and should be decontaminated before thrown away. Therefore, decontamination methods (e.g. chlorine addition or heat-sterilization) need to be considered.
Desinfection methods also deserve consideration when re-using materials as such materials need to be disinfected after use (in case more samples are tested).

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Direct Plating E.coli

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E.coli can be enumerated by plating sample dilutions on plates containing a (semi-) solid medium, usually containing agar, and counting the colonies after incubation. The **direct method** can be carried out by spreading the sample onto a solid medium (spread plates) or by mixing the sample with a molten medium containing gel-forming substances (pour plates). After incubation, *E.coli* forms colonies in selective medium, which can be identified based on their morphology (which includes coloration due to color-producing additives in the medium)

Advantages direct method:
- Quantitative data are provided.
- Limited requirements for equipment (including incubator), media, time and effort.
- Applicable to both clear and turbid samples.

Disadvantages direct method:
- Not applicable for samples containing low numbers of the tested organisms.
- Use of aseptic techniques and interpretation of the results requires trained personnel.
- Possible underestimation of the micro-organisms of interest due to damage caused by the high temperature of molten media used to pour plates.

Detection limit
For direct plating a small volume of the water is tested, mostly varying from 0.1 – 1.0 ml. Therefore, the methods are able to detect a single colony forming unit (cfu) per 1 ml of water (100 CFU/100ml). The detection limit depends on the tested water volume and therefore the detection limit can vary.

Examples for detection based on chromogenic media (beta-glucuronidase)

Media	Shelf-life	Storage
Chromocult	5 years	RT
CHROMagar	3 years	RT
3M Petrifilm	1.5 year	cold
Compact dry EC	2 years	RT
MI agar	3 years	RT
Coliscan Easygel	1 year	cold
ColiGel	1 year	RT
m-Coliblue	1 year	cold
Rapid *E.coli*2	> 1 year	RT

RT = room temperature
For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Possibly suitability for application at ambient temperatures (needs to be validated if not explicitly indicated by the manufacturer).
- Limited training needed for use and interpretation of results.

Disadvantages:
- Higher cost than classical media.

Examples for detection using classical media

Media	Shelf-life	Storage
MacConkey agar	> 1 year	RT
Tergitol-TTC*	> 1 year	RT
Lactose-TTC*	> 1 year	RT

RT = room temperature
* Additional test needed.

For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Lower cost than GluA based detection.
- Cheaper when processing large numbers of samples.

Disadvantages:
- More training needed for use and interpretation of results.

Relevant parameters for waste production are its volume and its biosafety consequences.
Waste, such as incubated petridishes, could be microbiologically contaminated and should be decontaminated before thrown away. Therefore, decontamination methods (e.g. chlorine addition or heat-sterilization) need to be considered. Desinfection methods also deserve consideration when re-using materials as such materials need to be disinfected after use (in case more samples are tested).

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Membrane Filtration E.coli

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Membrane filtration is an established method for the concentration of *E.coli* in water. Microbes are retained on a membrane filter through which the water sample is filtered and the membrane is placed onto an appropriate medium and incubated. Colonies with typical appearances are counted.

Advantages membrane filtration:
- Quantitative data are provided, including for micro-organisms present at low concentrations.
- The tested volume of the water can be varied.
- The method achieves high precision.
- Requirements for equipment (including incubator), media, time and effort are modest.
- Membrane filtration provides less waste compared to methods that do not concentrate the water sample.

Disadvantages membrane filtration:
- The method can not be applied with turbid samples (due to clogging of te membrane).
- Additional costs due to the requirement of membrane filtration equipment for use in the laboratory or for portable kits.
- Additional costs of sterile membrane filters and effort due to cleaning the membrane filter unit.
- Staff needs to be trained for aspectic techniques and interpretation of the results.

Detection limit
Using membrane filtration the tested water volume can be increased up to several liters of water. Filtration of 100ml of water is commonly used, and the methods is able to detect a single colony forming unit (cfu) per 100 ml of water (1CFU/100ml), whereas the filtration of 1 liter has a detection limit of 0.1 CFU per 100mlThe detection limit depends on the tested water volume and therefore the detection limit can vary.

Examples for detection based on chromogenic media (beta-glucuronidase)

Media	Shelf-life	Storage
Chromocult	5 years	RT
CHROMagar	3 years	RT
3M Petrifilm	1.5 year	cold
Compact dry EC	2 years	RT
MI agar	3 years	RT
ColiGel	1 year	RT
Coliscan MF	1 year	cold
m-Coliblue	1 year	cold
Rapid *E.coli*2	> 1 year	RT

RT = room temperature
For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Possibly suitability for application at ambient temperatures (needs to be validated if not explicitly indicated by the manufacturer).
- Limited training needed for use and interpretation of results.

Disadvantages:
- Higher cost than classical media.

Examples for detection using classical media

Media	Shelf-life	Storage
MacConkey agar	> 1 year	RT
Tergitol-TTC	> 1 year	RT
Lactose-TTC	> 1 year	RT
mEndo medium	4 years	RT
mFC	4 years	RT

* Additional test needed; RT = room temperature

For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Lower cost than GluA based detection.
- Cheaper when processing large numbers of samples.

Disadvantages:
- More training needed for use and interpretation of results.

Field testing kits

If the water quality testing must be done in the field, field testing kits are available. These kits contain all the equipment (e.g. incubator and filtration unit) and reagents needed for the analyses. Examples of field testing kits are:

- DelAqua: bacteriological kit no.1; no.2. and no.3
- Millipore: water test kit
- WagTech: Potaflex; Potakit; Potalab and Potatest
- Hach: MEL/m-ColiBlue Field Filtration Lab

If the equipment needed for the analyses in the field are available all media (both classical and based on glucuronic acid) can be used. Also see UNICEF catalogue (Unicef, Water quality assessment and monitoring, Technical Bulletin No.6).

Relevant parameters for waste production are its volume and its biosafety consequences.
Waste from cultivation, such as incubated petridishes, could be microbiologically contaminated and should be decontaminated before It is thrown away. Therefore, decontamination methods (e.g. chlorine addition or heat-sterilization) need to be considered. Desinfection methods also deserve consideration when re-using materials as such materials need to be disinfected after use (in case more samples are tested).

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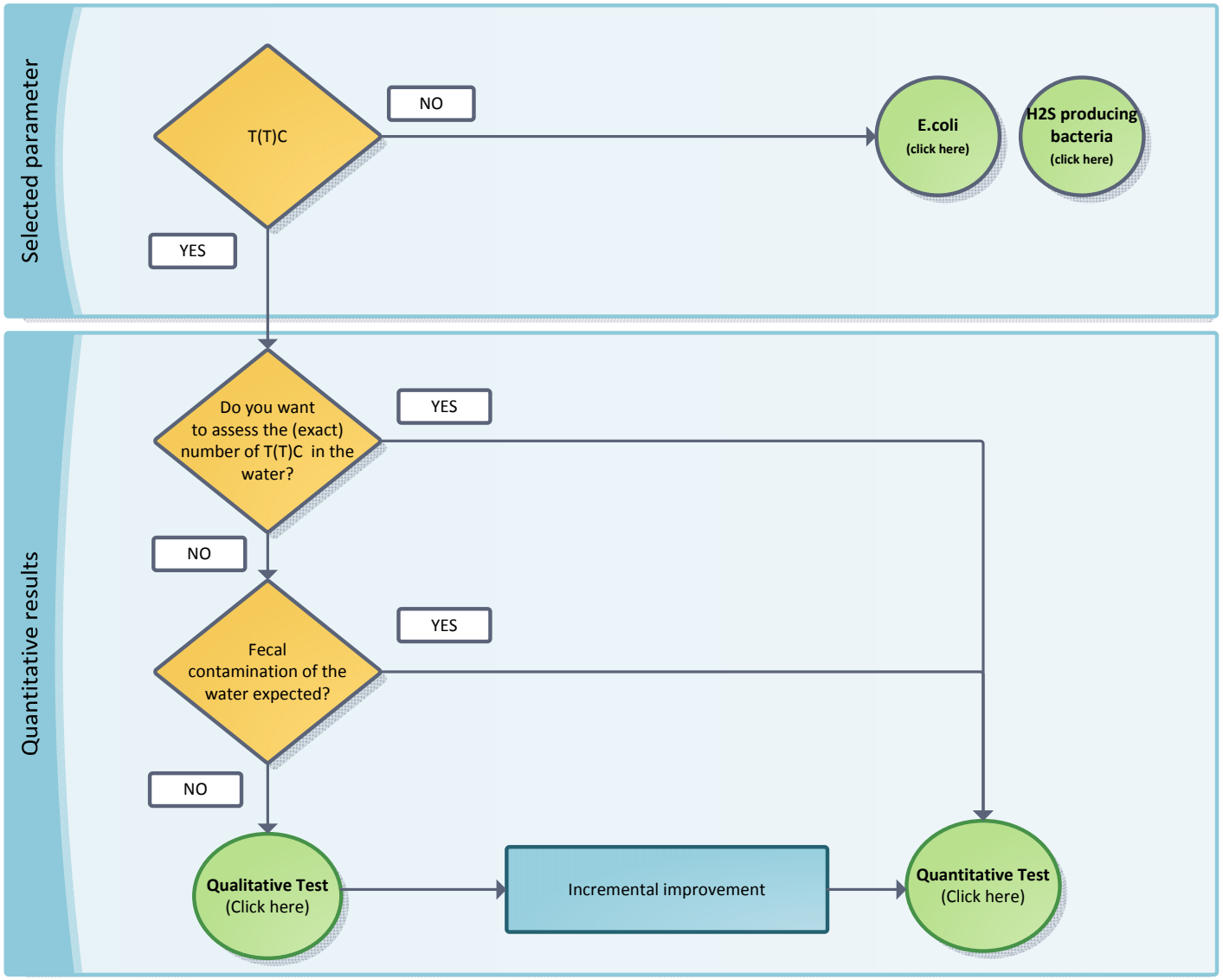

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Quantitative test

Infrastructural setting

Sample transport to microbiological laboratory in cold chain within 24 hours?

NO

YES

Equipment

Testing must be done in the field: only field-tests are suitable. Field-tests contain all necessary equipment, including for incubation or membrane filtration

Waterbath or incubator available for incubation at required temperature?

NO

YES

Ambient temperature higher than 20 °C?

NO

YES

H2S test (click here)

Limit to chromogenic (beta- galactosidase) assays

Membrane filtration unit available?

YES

NO

A temperature of at least 20 degrees C is needed for the detection of microbial growth within a reasonable time-frame. Lower temparatures require longer incubation times

Water properties

Water highly contaminated (e.g. sewage or surface water)?

NO

YES

Examine large water volumes

High water turbidity (e.g. containing sand, solid particles)?

NO

YES

Membrane filtration not possible due to clogging of the membrane

Small water volumes sufficient

High water turbidity (e.g. containing sand, solid particles)?

NO

YES

Membrane filtration not possible due to clogging of the membrane

Water highly contaminated (e.g. sewage or surface water)?

YES

NO

Small water volumes sufficient

Type of test

Membrane Filtration (click here)

MPN (click here)

MPN (click here)

Membrane Filtration (click here)

MPN (click here)

Direct Plating (click here)

MPN (click here)

Direct Plating (click here)

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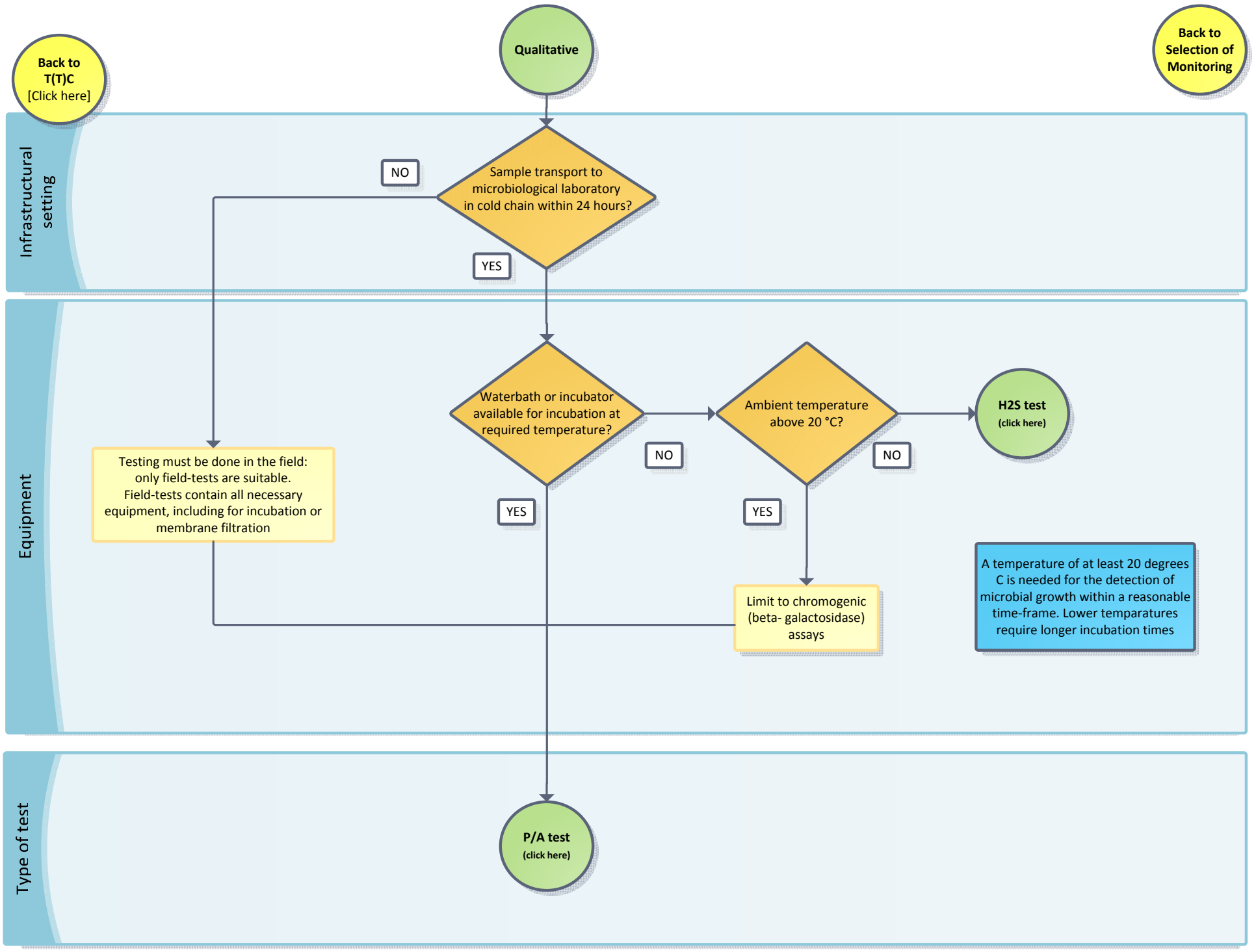
MPN (click here)

Direct Plating (click here)


Back to T(T)C [Click here]
Qualitative
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Infrastructural setting
NO
Sample transport to microbiological laboratory in cold chain within 24 hours?
YES
Equipment
Waterbath or incubator available for incubation at required temperature?
Ambient temperature above 20 °C?
H2S test (click here)
NO
NO
YES
YES
Testing must be done in the field: only field-tests are suitable. Field-tests contain all necessary equipment, including for incubation or membrane filtration
Limit to chromogenic (beta- galactosidase) assays
A temperature of at least 20 degrees C is needed for the detection of microbial growth within a reasonable time-frame. Lower temparatures require longer incubation times
Type of test
P/A test (click here)

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Presence / Absence: Total coliforms and thermotolerant coliforms

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A water sample is inoculated into liquid medium that facilitates growth of a particular micro-organism or a group of micro-organisms. The only conclusion that can be drawn using this method is the **presence or absence** of the micro-organism in this test volume, but no information can be yielded about its concentration. P/A tests can be applied to verify that water quality meets regulatory standards (e.g. absence in 100ml).

Advantages presence/absence:
- Applicable to both clear and turbid samples.
- Allow growth of injured bacteria.
- Require minimal equipment (including incubator), time and effort, and use inexpensive media.

Disadvantages presence/absence:
- No quantitative data provided, only qualitative data.
- Only appropriate in circumstances where the micro-organisms of interest are rarely found.

Detection limit
Most available methods/kits are sensitive enough to detect a single colony forming unit (cfu) per 100 ml of water. The detection limit depends on the tested water volume and therefore the detection limit can vary.

Examples for detection based on chromogenic media (beta-galactosidase)

Media	Shelf-life	Storage
HiSelective *E.coli*	1 year	RT
AquaCHROM	2 years	RT
Readycult	3 years	RT
E*Colite	1 year	RT
EC Blue	1 year	RT
Colilert/Quantitray	1 year	RT
Modified COlitag	2 years	RT
Watercheck	3 years	RT

RT = room temperature
For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Possibly suitability for application at ambient temperatures (needs to be validated if not explicitly indicated by the manufacturer).
- Limited training needed for use and interpretation of results.

Disadvantages:
- Higher cost than classical media.

Examples for detection using classical media

Media	Shelf-life	Storage
Lauryl tryptose broth*	3 years	RT
Lactose broth*	3 years	RT
EC-broth	3 years	RT

RT = room temperature
* Additional test (e.g. brilliantgreen lactose bile broth) needed.

For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Lower cost than GluA based detection.
- Cheaper when processing large numbers of samples.

Disadvantages:
- More training needed for use and interpretation of results.

Field testing kits

If the water quality testing must be done in the field, field testing kits are available. These kits contain all the equipment (e.g. incubator) and reagents needed for the analyses. Examples of field testing kits are:

- Compartmentalised bag test
- Aquatest
- Colifast field kit
- Hach: MEL/m-ColiBlue Field Filtration Lab

If the equipment needed for the analyses in the field are available all media (both classical and based on glucuronic acid) can be used.
Also see UNICEF catalogue (Unicef, Water quality assessment and monitoring, Technical Bulletin No.6).

Relevant parameters for waste production are its volume and its biosafety consequences.
Waste, such as incubated petridishes, could be microbiologically contaminated and should be decontaminated before thrown away. Therefore, decontamination methods (e.g. chlorine addition or heat-sterilization) need to be considered.
Desinfection methods also deserve consideration when re-using materials as such materials need to be disinfected after use (in case more samples are tested).

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Most Probable Number: Total coliforms and thermotolerant coliforms

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The **most probable number** (MPN) approach uses replicated presence/absence tests of different volumes or dilutions to estimate the concentration of microorganisms in a water sample based on which sample volumes grow the target microorganisms (presence) and which do not (absence). The most probable number of micro-organisms present in the original sample can be calculated on the basis of the numbers of positive and negative test portions observed after incubation.

Advantages most probable number:
- MPN tests are essentially multiple P/A tests and therefore have comparable advantages
  - The method is applicable to clear and turbid samples.
  - Allow growth of injured bacteria.
- Quantitative data are provided.
- Require minimal equipment (including incubator), time and effort, use inexpensive media.

Disadvantages most probable number:
- More equipment, media, time and effort are required than with P/A testing.
- More reliable results require more replicas of the multiple tubes, which causes a significant increase in required time, materials and cost.
- Interpretation of the results requires trained personnel.

Detection limit
Most available methods/kits are sensitive enough to detect a single colony forming unit (cfu) per 100 ml of water. The detection limit depends on the tested water volume and therefore the detection limit can vary.

Examples for detection based on chromogenic media (beta-galactosidase)

Media	Shelf-life	Storage
Colilert/Quantitray	1 year	RT
EC BlueQuant	1 year	RT
Colitag	2 years	RT
ColiPlate	3 years	RT

RT = room temperature

For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Possibly suitability for application at ambient temperatures (needs to be validated if not explicitly indicated by the manufacturer).
- Limited training needed for use and interpretation of results.

Disadvantages:
- Higher cost than classical media.

Examples for detection using classical media

Media	Shelf-life	Storage
Lauryl tryptose broth*	3 years	RT
Lactose broth*	3 years	RT

RT = room temperature
* Additional test (e.g. brilliantgreen lactose bile broth) needed.

For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Lower cost than GluA based detection.
- Cheaper when processing large numbers of samples.

Disadvantages:
- More training needed for use and interpretation of results.

Field testing kits

If the water quality testing must be done in the field, field testing kits are available. These kits contain all the equipment (e.g. incubator) and reagents needed for the analyses. Examples of field testing kits are:
- Compartmentalised bag test
- Aquatest

If the equipment needed for the analyses in the field are available all media (both classical and based on glucuronic acid) can be used.
Also see UNICEF catalogue (Unicef, Water quality assessment and monitoring, Technical Bulletin No.6).

Relevant parameters for waste production are its volume and its biosafety consequences.
Waste, such as incubated petridishes, could be microbiologically contaminated and should be decontaminated before thrown away. Therefore, decontamination methods (e.g. chlorine addition or heat-sterilization) need to be considered.
Desinfection methods also deserve consideration when re-using materials as such materials need to be disinfected after use (in case more samples are tested).

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Direct plating: Total coliforms and thermotolerant coliforms

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Coliforms can be enumerated by plating sample dilutions on plates containing a (semi-) solid medium, usually containing agar, and counting the colonies after incubation. The **direct method** can be carried out by spreading the sample onto a solid medium (spread plates) or by mixing the sample with a molten medium containing gel-forming substances (pour plates). After incubation, the micro-organisms form colonies in selective medium, which can be identified based on their morphology (which includes coloration due to color-producing additives in the medium)

Advantages direct method:
- Quantitative data are provided.
- Limited requirements for equipment (including incubator), media, time and effort.
- Applicable to both clear and turbid samples.

Disadvantages direct method:
- Not applicable for samples containing low numbers of the tested organisms.
- Use of aseptic techniques and interpretation of the results requires trained personnel.
- In case pour plates are used, underestimation of the true number of micro-organisms is possible due to damage caused by the high temperature of molten media.

Detection limit
For direct plating a small volume of the water is tested, mostly varying from 0.1 – 1.0 ml. Therefore, the methods are able to detect a single colony forming unit (cfu) per 1 ml of water (100 CFU/100ml). The detection limit depends on the tested water volume and therefore the detection limit can vary.

Examples for detection based on chromogenic media (beta-galactosidase)

Media	Shelf-life	Storage
Chromocult	5 years	RT
CHROMagar	3 years	RT
3M Petrifilm	1.5 year	cold
Compact dry EC	2 years	RT
MI agar	3 years	RT
Coliscan Easygel	1 year	cold
ColiGel	1 year	RT
m-Coliblue	1 year	cold
Rapid *E.coli*2	> 1 year	RT

RT = room temperature
For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Possibly suitability for application at ambient temperatures (needs to be validated if not explicitly indicated by the manufacturer).
- Limited training needed for use and interpretation of results.

Disadvantages:
- Higher cost than classical media.

Examples for detection using classical media

Media	Shelf-life	Storage
MacConkey agar	> 1 year	RT
Tergitol-TTC	> 1 year	RT
Lactose-TTC	> 1 year	RT
mEndo medium	4 years	RT
mFC	4 years	RT

RT = room temperature
* Additional test (e.g. brilliantgreen lactose bile broth) needed.

For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Lower cost than GluA based detection.
- Cheaper when processing large numbers of samples.

Disadvantages:
- More training needed for use and interpretation of results.

Relevant parameters for waste production are its volume and its biosafety consequences.
Waste, such as incubated petridishes, could be microbiologically contaminated and should be decontaminated before thrown away. Therefore, decontamination methods (e.g. chlorine addition or heat-sterilization) need to be considered.
Desinfection methods also deserve consideration when re-using materials as such materials need to be disinfected after use (in case more samples are tested).

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Membrane filtration: Total coliforms and thermotolerant coliforms

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Membrane filtration is an established method for the concentration of microorganisms in water. Microbes are retained on a membrane filter through which the water sample is filtered and the membrane is placed onto an appropriate medium and incubated. Colonies with typical appearances are counted.

Advantages membrane filtration:
- Quantitative data are provided, including for micro-organisms present at low concentrations.
- The tested volume of the water can be varied.
- The method achieves high precision.
- Requirements for equipment (including incubator), media, time and effort are modest.
- Membrane filtration provides less waste compared to methods that do not concentrate the water sample.

Disadvantages membrane filtration:
- The method can not be applied with turbid samples (due to clogging of the membrane).
- Additional costs due to the requirement of membrane filtration equipment for use in the laboratory or for portable kits.
- Additional costs of sterile membrane filters and effort due to cleaning the membrane filter unit.
- Staff needs to be trained for aspectic techniques and interpretation of the results.

Detection limit
Using membrane filtration the tested water volume can be increased up to several liters of water. Filtration of 100ml of water is commonly used, and the methods is able to detect a single colony forming unit (cfu) per 100 ml of water (1CFU/ 100ml), whereas the filtration of 1 liter has a detection limit of 0.1 CFU per 100mlThe detection limit depends on the tested water volume and therefore the detection limit can vary.

Examples for detection based on chromogenic media (beta-galactosidase)

Media	Shelf-life	Storage
Chromocult	5 years	RT
CHROMagar	3 years	RT
3M Petrifilm	1.5 year	cold
Compact dry EC	2 years	RT
MI agar	3 years	RT
ColiGel	1 year	RT
Coliscan MF	1 year	cold
m-Coliblue	1 year	cold
Rapid *E.coli*2	> 1 year	RT

RT = room temperature
For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Possibly suitable for application at ambient temperatures (needs to be validated if not explicitly indicated by the manufacturer).
- Limited training needed for use and interpretation of results.

Disadvantages:
- Higher cost than classical media.

Examples for detection using classical media

Media	Shelf-life	Storage
MacConkey agar	> 1 year	RT
Tergitol-TTC	> 1 year	RT
Lactose-TTC	> 1 year	RT
mEndo medium	4 years	RT
mFC	4 years	RT

* Additional test (e.g. brilliantgreen lactose bile broth) needed; RT = room temperature
For more information see Bain et al 2012 (*International Journal of Environmental Research and Public Health*, Vol.9, p.1609-1625).

Advantages:
- Lower cost than GluA based detection.
- Cheaper when processing large numbers of samples.

Disadvantages:
- More training needed for use and interpretation of results.

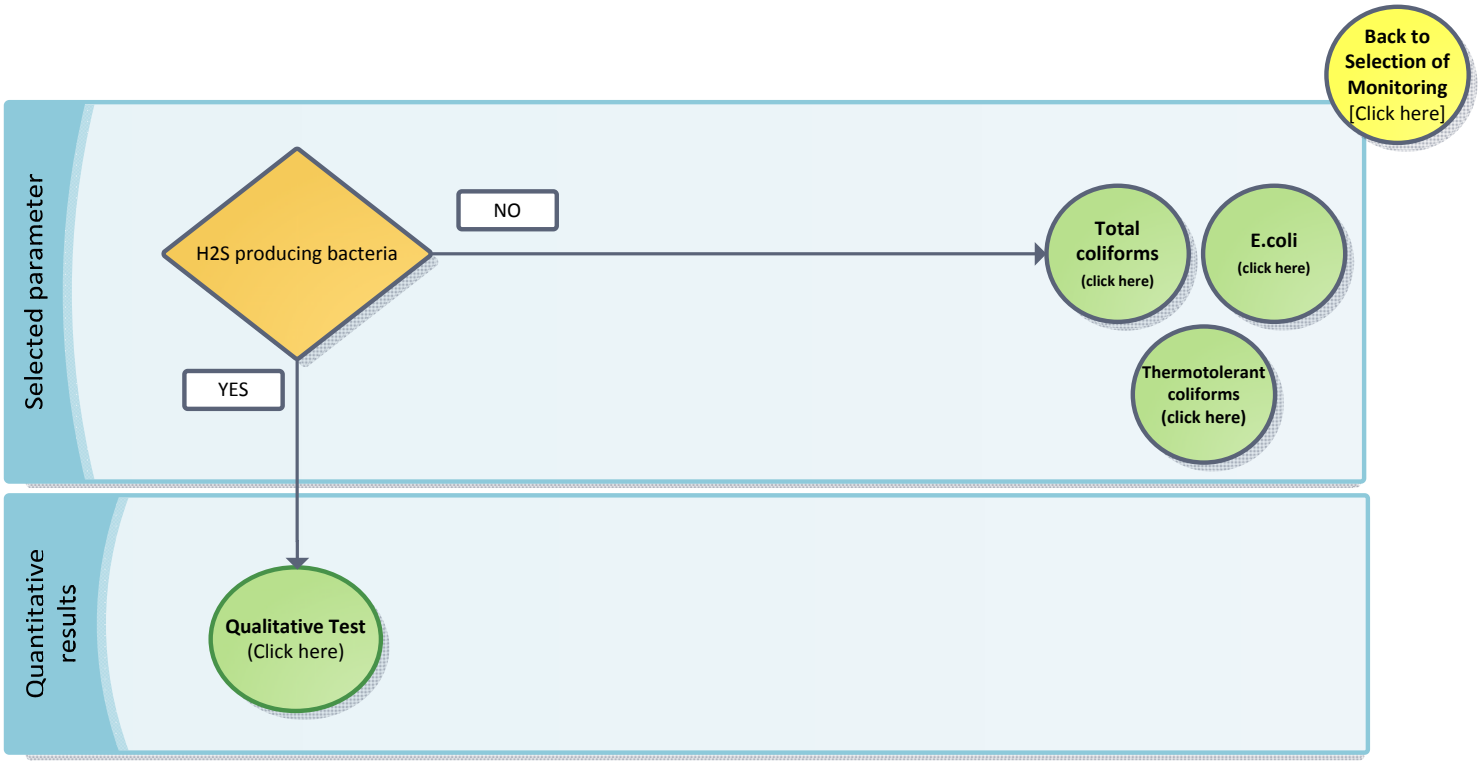
Field testing kits

If the water quality testing must be done in the field, field testing kits are available. These kits contain all the equipment (e.g. incubator and filtration unit) and reagents needed for the analyses. Examples of field testing kits are:

- DelAqua: bacteriological kit no.1; no.2 and no.3
- ELE International Ltd: Paqualab system 25 and Paqualab system 50
- Millipore: water test kit
- Potapak Limited: potapak
- WagTech: Potaflex; Potakit; Potalab and Potatest
- Hach: MEL/m-ColiBlue Field Filtration Lab

If the equipment needed for the analyses in the field are available all media (both classical and based on glucuronic acid) can be used.
Also see UNICEF catalogue (Unicef, Water quality assessment and monitoring, Technical Bulletin No.6).

Relevant parameters for waste production are its volume and its biosafety consequences.
Waste from cultivation, such as incubated petridishes, could be microbiologically contaminated and should be decontaminated before It is thrown away. Therefore, decontamination methods (e.g. chlorine addition or heat-sterilization) need to be considered. Desinfection methods also deserve consideration when re-using materials as such materials need to be disinfected after use (in case more samples are tested).


Back to Selection of Monitoring [Click here]
Selected parameter
H2S producing bacteria
NO
YES
Total coliforms (click here)
E.coli (click here)
Thermotolerant coliforms (click here)
Quantitative results
Qualitative Test (Click here)

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Presence / Absence: H_2S producing bacteria

Several types of bacteria, including some coliform bacteria, *Clostridium perfringens* and other sulfite reducing clostridia and some other enteric bacteria, produce hydrogen sulfide, which can be measured by using a cheap and rapid chemical test.

Advantages presence/absence:
- Applicable to both clear and turbid samples
- Substantially cheaper and faster compared to alternatives, these tests potentially offer the possibility to screen large numbers of rural water sources
- No need for expensive incubators or specialist microbiological expertise

Disadvantages presence/absence:
- No quantitative data provided, only qualitative data
- The specificity of the H_2S test is variable and optimal conditions for conducting the test remain unclear

A positive result of H2S test indicates the potential presence of fecal contamination. Negative results of H2S test suggest the absence of fecal contamination, but does not guarantee this.
H_2S testing is not recommended as a substitute for more specific and better established indicators such as *E. coli*.

Examples for H_2S detection

Media	Shelf-life	Storage
Pathoscreen	1 year	RT
LTEK H2S (20ml)	2 years	RT
LTEK H2S (100ml)	1 year	RT
HiWater	2 years	RT
Pathogel (including coliforms)	1 year	RT

RT = room temperature
For more information see Bain et al 2012.

Validation of H2S test against EC or TTC test is needed when the test is deployed in a new setting.
Be careful in situations where contaminated samples are very rare

Sanitary inspection

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Not in the scope of this decision tree

General information
A sanitary inspection is an on-site assessment of actual and potential contamination hazards affecting the water supply systems, that may be a risk to water systems.

Available methods
Sanitary inspections are usually carried out using standardized forms for observations and interview. The form consisting of a set of questions to be answered with “yes” or “no” will be filled in. The questions answered with “yes”, posing a risk of contamination, will be counted. The more questions answered with “yes”, the greater the risk of contamination.
Sanitary inspections consider fecal contamination risks posed to water sources.

Although in case no question is answered with yes, it does not mean that the water source is safe. Water analyses can check the quality of the water and therefore the safety of water.

For a complete a set of recommended checklists, see: *Water Safety Plans: Managing drinking-water quality from catchment to consumer, appendix C. WHO 2005*

pH

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Not in the scope of this decision tree

General information
pH usually has no direct impact on consumers, but it is a very important operational water quality parameter.

Optimal pH
The pH should preferably be less than 8 for effective disinfection with chlorine. For prevention of corrosion, the WHO recommends a pH of 6.5 or higher in drinking water. The pH of the water entering the distribution system must be controlled to minimize the corrosion of water mains and pipes in household water systems *(WHO, Drinking-water guidelines 4th edition)*.

Available methods
pH measurements can be carried out by using one of the following methods:
- pH indicator paper
- liquid colorimetric indicators
- electronic meters

(WHO, Water Quality Monitoring)

Chlorine

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Not in the scope of this decision tree

General information

For disinfection of drinking-water, chlorine is commonly used.
Some people are able to taste or smell chlorine in drinking-water at concentrations as low as 0.3 mg/l. Above a residual free chlorine concentration of between 0.6 and 1.0 mg/L there is an increasing likelihood of complaints from consumers. The taste threshold for chlorine is below the health-based guideline value of 5 mg/l (*Drinking-water guidelines, 4th edition, WHO 2011* and *Unicef Handbook on Water Quality, Unicef, 2008*).
Free chlorine may affect other analyses, such as bacteriological tests, and should be removed before testing by the addition of a small amount (usually one drop) of 0.1 mol/l sodium thiosulphate solution (*Water Quality Monitoring, A Practical Guide, UNEP/WHO 1996*).

Optimal free chlorine residual concentration

For effective disinfection with chlorine, the residual concentration of free chlorine should be 0.5 mg/l after at least 30 minutes contact time. The pH should be lower than 8.5. Disinfection with chlorine is very effective, provided it is used correctly with low-turbidity water. Throughout the distribution network, free chlorine residuals should be maintained, and at the point of delivery (consumers) the minimal concentration should be 0.2 mg/l.

Available methods

Most chlorine measurement use DPD (N,N diethyl-p-phenylene diamine) which causes a color change to pink in the presence of chlorine. Several suppliers for chlorine tests are available.

Turbidity

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Not in the scope of this decision tree

General information

Turbidity in water is caused by suspended particles or colloidal matter that obstructs light transmission through the water. It may be caused by inorganic or organic matter or a combination of the two. Microorganisms (bacteria, viruses and protozoa) are typically attached to particulates, and removal of turbidity by filtration will significantly reduce microbial contamination in treated water. Furthermore, turbidity can interfere with the effectiveness of disinfection methods.

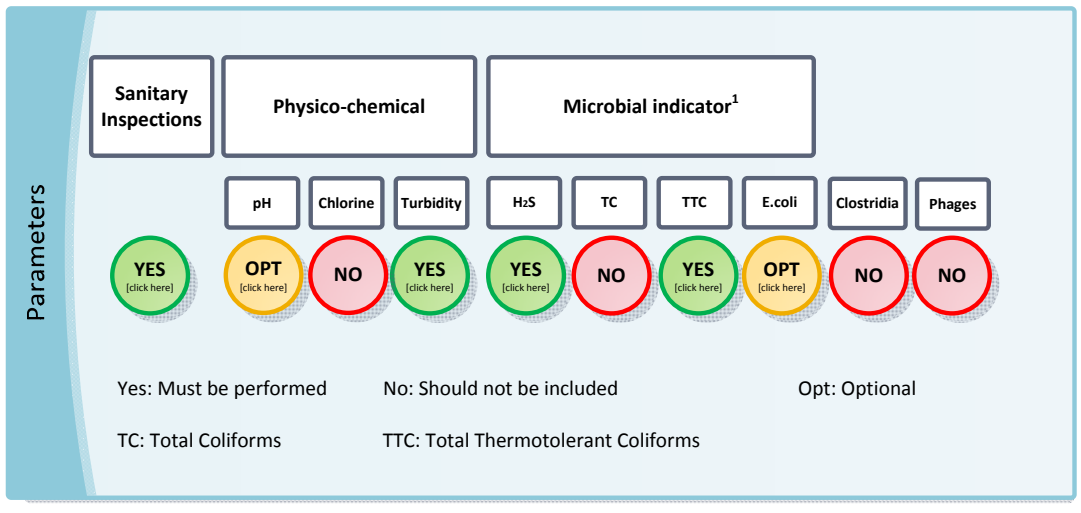
Turbidity is measured by nephelometric turbidity units (NTU) and can be initially noticed by the naked eye above approximately 4.0 NTU. Turbidity can also have a negative impact on consumer acceptability of water as a result of visible cloudiness.

Optimal turbidity

High levels of turbidity can shield pathogens from disinfectants. Therefore, effective disinfection, such as ozone, chlorination or UV, requires that the turbidity is less than 1 NTU, but preferable 0.2 NTU or less.

Available methods

Turbidity can be measured using turbidity tubes or electronic turbidity meters.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Sanitary Inspections
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Microbial indicator[1]
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Chlorine
Turbidity
H2S
TC
TTC
E.coli
Clostridia
Phages
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OPT [click here]
NO
YES [click here]
YES [click here]
NO
YES [click here]
OPT [click here]
NO
NO
Yes: Must be performed
No: Should not be included
Opt: Optional
TC: Total Coliforms
TTC: Total Thermotolerant Coliforms