

INSTANT DIPSTICK TESTKIT

BENCH PROTOCOL

Principle and features

The INSTANT DIPSTICK TESTKIT is a simplified low cost variant of the "Bacterial Contamination Screening Kit". It is also a "field testkit" which allows for ultra-rapid gross screening of the contamination of water samples or solid surfaces samples by microbes and biological residues. Instant Dipstick tests assess the degree of contamination in an indirect way through measurement of the amount of ATP (Adenosine TriPhosphate) which is the energy source of all living organisms. Biological residues (such as dead biota or food residues) are biodegradable and their presence rapidly leads to bacterial development. Such residues are therefore also a signal for the contamination of water samples, or the degree of cleanliness of surfaces.

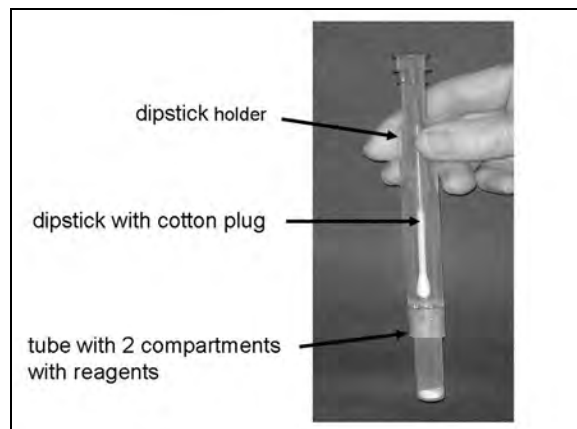
Instant Dipstick tests are applicable to any kind of freshwater or solid surface, but they do not give information on the presence or absence of pathogenic microbes.

The ATP is measured by chemical luminescence through a "luciferine/luciferase" reaction with a specific chemical reagent, in a small **luminometer**.

The tests can be performed at ambient temperature, with maximum luminescence output in the range 15°C to 25°C.

Dipsticks

The Instant Dipstick Testkit contains 2 sealed aluminium bags each with 10 Dipstick Units. Each Dipstick Unit allows to analyse one sample and is composed of 3 distinct parts (see *Figure*): a dipstick holder, a dipstick with cotton tip and a tube with 2 compartments containing a liquid ATP extractant and a solid ATP reagent respectively.



The dipsticks must be stored in the refrigerator (2-8°C) but should not be frozen, and have a shelf life of 6 months to 1 year.

The dipstick Units are used with the Luminometer and the materials contained in a small lightweight "Lumi-Box", and allow to perform two distinct types of analyses :

- a Direct Dipstick test which measures the "total amount of ATP" from living bacteria, biological residues and/or other small biota which contaminate waters or solid surfaces
- a "Prefilter" test which only measures the ATP from the living bacteria present in the water sample, after prior elimination of all "non-microbial" ATP.

Direct Dipstick Test on water samples

1. Take a water sample in a clean vessel.
2. Take the dipstick out of the holder, submerge the cotton tip in the water sample and swirl the dipstick gently to hydrate it completely (i.e. with 100 µl of water).
3. Take the dipstick out of the water, shake it slightly to eliminate all excess water and put the dipstick back into the holder.
4. Push the wet cotton tip through the cover of the tube with the reagents to immerse the tip completely in the ATP extractant . Swirl the dipstick gently to ensure a good contact of the extractant with the water (and the bacteria) inside the cotton tip.
5. After about 1 minute, push the cotton tip further down into the tube, through the separation between both compartments.
6. Grab the dipstick firmly with one hand and pull the tube slightly downwards in the holder with the other hand (without removing the dipstick totally from the holder).
7. Push the tube back into the holder in order to make the ATP extractant flow from the top compartment into the bottom compartment of the tube.
8. Repeat the former operation several times until all extractant solution has passed into the lower compartment of the tube.

N.B. : For a reliable measurement, it is imperative that the total volume of extractant has passed into the bottom compartment.

9. Swirl the Dipstick Unit gently to mix the extractant solution with the ATP reagent and to dissolve the latter completely.
10. Place the Dipstick Unit vertically in one of the holes of the foam insert of the Lumi-Box.
11. After about 2 minutes, take the luminometer , open the lid and insert the Dipstick Unit.
12. Close the luminometer and switch it on by pushing the "Power" button. Wait till the instrument has calibrated itself by a 10 seconds countdown visible on the display and followed by a beep signal.

N.B. : The luminometer must always be kept vertically when containing a Dipstick Unit !

13. Push the "Enter" button. The luminometer will calibrate again for 10 seconds after which the luminescence score will appear (in RLU) on the display.
14. Score the RLU figure on the Results Sheet.
15. Remove the Dipstick Unit from the luminometer and switch it off by pushing the "Power" button.

N.B. : When a dipstick is kept for more than 15 seconds in the luminometer after the scoring, the instrument will give beep signals indicating that the Dipstick Unit must be removed.

Evaluation of the contamination of water samples by bacteria and biological residues

A gross estimation of the degree of contamination of the water sample can be made as follows :

- < 20 RLU : extremely low contamination
- < 100 RLU : low contamination
- 100-1000 RLU : significant contamination
- > 1000 RLU : very high contamination

Direct dipstick test on solid surfaces (swab procedure)

1. Pull the dipstick out of the holder and hydrate the cotton tip by pouring a few drops of mineral water (from a freshly opened bottle) on the tip, till it is completely wet. Alternatively a small volume of water can be poured on the surface to be examined.
2. Swab the surface to be tested with the cotton tip.
3. Put the dipstick back into the holder and proceed further as of step 4 of the procedure for analysis of water samples.

Evaluation of the degree of cleanliness of solid surfaces

The same “rule of thumb” can be applied as for the evaluation of the degree of contamination of water samples. Very low RLU values (<20 RLU) indicate that the analysed surface is “hygienically” clean i.e. not contaminated by microbes, molds or food residues. RLU scores > 100 in turn are a clear signal for the presence of biological contaminants.

Prefilter test

The outcome of Direct Dipstick tests on water samples reflects the “overall” degree of contamination of the water not only by bacteria, but possibly also by biological residues and small biota which may have adsorbed on the cotton tip of the dipstick. Depending of the type of water, the amount of ATP originating from “non-bacterial” biota and/or residues can either be negligible or very substantial. The prefilter test procedure “separates” all “non-microbial” material from the bacteria and only measures the ATP from the bacteria present in the water sample.

1. Unscrew the 2 parts of the small filter holder and put them in the 2 holes in the foam insert of the Lumi-Box.
2. With the aid of the plastic pincette, take one prefilter from the small box with prefilters and put it in the hollow compartment of the filter holder.

N.B. : Don't touch the prefilter with the fingers during the operation to avoid bacterial contamination from the hand.

3. Reassemble the filter holder and connect it to the 5 ml syringe.
4. Take a water sample in a clean vessel and insert the free tip of the filter holder in the water.

5. Pull the plunger of the syringe upwards to fill the syringe with water.

N.B. : During this operation all “non-bacterial” material (i.e. all particles with a size > 15 µm) will be trapped on the outside of the prefilter and only the bacteria will pass the prefilter with the water sucked up into the syringe.

6. Disconnect the syringe from the filter holder.

7. Take one Instant Dipstick Unit, pull the dipstick out of the holder and slowly drop water from the syringe over the cotton tip of the dipstick, till the tip is completely wet.
8. Put the dipstick back into the holder and proceed further as of step 4 of the Direct Dipstick Test.

Evaluation of the microbial contamination of water samples

A gross estimation of the amount of bacteria present in the analysed water can tentatively be made as follows :

- | | |
|---|------|
| < 20 RLU : only a few bacteria | - |
| < 100 RLU : relatively low numbers of bacteria | + |
| > 100 RLU : a significant number of bacteria | ++ |
| > 500 RLU : high microbial contamination | +++ |
| > 1.000 RLU : very high microbial contamination | ++++ |

N.B. : The former figures are indicative and should not be interpreted strictly quantitatively, but preferably by – and + signs to express the importance of the microbial contamination.

Verification step

The degree of microbial contamination of a water detected in a few minutes by the former two screening methods, can subsequently be verified by a simple low cost “plate count” procedure on small discs containing a bacterial nutrient agar. The inoculation of a “compact Dry TC disc” with 1 ml water sample can be carried out “in situ” concurrently with the dipstick or prefilter screening assays, and shall be followed by incubation of the disc at room temperature for several days. The growth of the bacteria eventually leads to tiny red colonies which are clearly visible on the disc. Although not all types of bacteria will grow on the nutrient agar, the number of microbial colonies (Colony Forming Units = CFU) is nevertheless still grossly representative for the degree of bacterial contamination of the water.

Decontamination of the syringe and the filter holder subsequent to application of a prefilter test

In order to avoid cross-contamination from one water sample to the other, the syringe and the prefilter holder must be “decontaminated” after each prefilter test. This decontamination can be carried out in the field with the aid of the materials included in the “Decontamination Box”.

A decontamination solution is first prepared by dissolving a “decontamination tablet” in 25 ml mineral water (from a freshly opened bottle) The syringe connected to the prefilter is then filled with decontamination solution. After a contact time of a few minutes, the syringe is emptied and filled with “clean water” (from the bottle with mineral water), which is then also flushed out after a short contact time.

Depending of the magnitude of the contamination assessed in the previous prefilter test, the decontamination step with the specific chemical solution and the rinsing step with clean water shall be repeated a few times.