CROWD: Clean Rivers Of West Dorset

MEASURING BACTERIAL POPULATIONS IN CROWD RIVERS

John and Howard have been considering bacterial measurements with the aim of achieving a robust and readily achievable detection. This document has been prepared for discussion on 25th January 2024.

1. **Dipslides:** the simplest approach. Prepared slides are dipped into the sample water and incubated at 30°C for 24-48 hours. Weaknesses: only semiquantitative and lack sensitivity at below 10,000 cfu/100mL. They would have detected only the highest contamination levels found in 2023 on the Winniford. They would not detect colony counts in the BlueFlag range of "good" or "satisfactory" (200-500 cfu/100ml).
   Cost for initial evaluation £40: for 10 Dipslides. An incubator is available.

2. **Bacterial Filters:** a demountable bacterial filter would gather bacteria from a larger volume of water (most likely 10ml but possibly not 100ml). The extracted filter could then be wiped across a Dipslide (this may – but not certainly – detect 200-500 cfu/100ml) or be applied to an agar surface (see below).
   Cost for initial evaluation £60: prefilters (£10) two demountable filter holders (£20), 10ml syringes (£10), 50 x 0.45µm bacterial filters (£10) and "soft" tweezers (£10).

3. **Prepared agar plates:** (£30/10 plates) [or Petrifilms (3M, £25/50 plates) function like agar plates and are cheaper]. The bacterial filter could be laid on their surface for c20min or possibly throughout incubation at 30°C for 24 to 48 hours. This may result in a more complete transfer of viable bacteria than possible with surface wiping with Dipslides.
   Cost for initial evaluation £80: requirements as in 2 above plus £20 for a butane Bunsen-like flame if we use agar plates. It may not be needed with Petrifilms.

4. **Detecting both *E.coli* and coliform bacteria:** Dipslides, agar plates and Petriflms are available with selective media in which the colour developed by *E. coli* differs from that produced by coliform bacteria. So both can be counted on the same plate (or opposite sides of a Dipslide). Petrifilms may les good if all Enterobacteriaceae are to be counted. The developed bacterial colonies could be photographed to provide a permanent record. Free software is also available for counting of images.

5. **Technical difficulty:** Dipslides do not require any experience of sterile techniques. Agar plates or Petrifilms do require some technical skill, but this could easily be acquired by demonstration. Petrifilms require less diligence with sterile techniques. Disposable gloves would be use when handling the samples. All samples would be soaked in 5% bleach before safe disposal**.**

6. **Recommendations:**
   a. **Evaluate Dipslides (with bacterial filters if needed)**. Results should be calibrated with concurrent WW evaluation for the same samples and possibly later with BlueFlag measurements at Charmouth, to establish that our results cannot be fairly challenged by others.
   b. **Evaluate Selective Petrifilms.** Needed if Dipslides are too insensitive. Low cost. Require less sterile techniques but require a second Petrifilm if all enterobacteria are to be evaluated**.**

   Total cost of initial evaluation: £150.

   c. **Obtain funding for the initial <u>and</u> subsequent evaluations.** This would ensure that adopted approach can be continued through the 2024 bathing season. We would need extra Dipslides or Petrifilm/agar plates plus further filters. **Needs an extra budget for 2024 of £50 for Petrifilms or £150 for Dipslides or agar plates.** There is little point in an initial evaluation if funding is not available beyond that phase.

HJA, 12/01/2024.