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**RELATIONSHIP OF TIME LAPSE
TO BACTERIAL POPULATIONS
CREATED BY THE DAILY OBSERVATION OF SELECTED BART
TESTERS.**

Terms

Time lapse is defined as that time taken between the initiation of a BART test and the first observation of a positive reaction.

Bacterial population is defined by the mean number of predicted active cells per ml in the water sample being tested. This is based on the Time lapse to the first observation of a defined reaction within the test. The range is established by assuming the sample went positive one hour after the previous days determination of population (high) and also assuming that the test actually went positive one hour before the reading on the day after the critical day under consideration when the test would still have appeared negative (low). A range is therefore presented based upon the mean (assuming that the test actually went positive at that moment) with a high and low range set by the time span over which the positive detection could have occurred within that day.

Reaction is considered to be any of the defined changes that had occurred within the 15ml test sample that occurred during while the sample was being incubated.

Daily observation refers to the semi-quantitative methods that employ the observation of the tester on a daily basis for the occurrence of defined reactions.

Selected BART testers refer to those testers that can be applied for the detection of the activities of specific communities of bacteria that may be present within the sample.

| | | Time lapse (days) | | | | |
|----------|---------|-------------------|------------|---------|--------|----|
| | | 1 | 2 | 3 | 4 | 8 |
| SRB-BART | maximum | 50,000,000 | 15,800,000 | 539,000 | 36,600 | 68 |
| | mean | 23,200,000 | 731,000 | 46,600 | 5,200 | 31 |
| | minimum | 999,000 | 59,700 | 6,340 | 1,100 | 16 |

| | | Time lapse (days) | | | | |
|-----------|---------|-------------------|-----------|---------|--------|-------|
| | | 1 | 2 | 3 | 4 | 5 |
| SLYM-BART | maximum | 50,000,000 | 6,620,000 | 468,000 | 57,400 | 9,440 |
| | mean | 8,800,000 | 632,000 | 70,000 | 11,100 | 2,400 |
| | minimum | 805,000 | 85,700 | 13,200 | 2,760 | 749 |

| | | Time lapse (days) | | | | |
|----------|---------|-------------------|---------|---------|--------|---------|
| | | 1 | 2 | 3 | 4 | 6 |
| IRB-BART | maximum | 1,000,000 | 492,000 | 123,000 | 30,700 | 191,000 |
| | mean | 566,000 | 141,000 | 36,300 | 8,800 | 850 |
| | Minimum | 162,000 | 40,500 | 10,100 | 2,530 | 158 |

| | | Time lapse (days) | | | | |
|----------|------|-------------------|-----------|---------|--------|-------|
| | | 1 | 2 | 3 | 4 | 6 |
| HAB-BART | high | 40,000,000 | 5,200,000 | 355,000 | 39,100 | 6,290 |
| | mean | 6,900,000 | 454,000 | 47,800 | 7,400 | 1,590 |
| | low | 563,000 | 59,000 | 8,830 | 1,800 | 502 |

| | | Time lapse (days) | | | | |
|---------|---------|-------------------|---------|--------|-------|-----|
| | | 2 | 3 | 4 | 5 | 6 |
| DN-BART | maximum | 5,000,000 | 180,000 | 13,600 | 1,790 | 362 |
| | mean | 242,000 | 17,200 | 2,140 | 417 | 115 |
| | minimum | 21,800 | 2,580 | 183 | 129 | 45 |

| | | Time lapse (days) | | | | |
|----------|---------|-------------------|---------|--------|-------|-----|
| | | 1 | 2 | 3 | 4 | 6 |
| APB-BART | maximum | 5,000,000 | 623,000 | 54,500 | 7,440 | 396 |
| | mean | 817,000 | 68,000 | 8,920 | 1,690 | 143 |
| | minimum | 85,400 | 10,700 | 1,970 | 493 | 63 |

| | | Time lapse (days) | | | | |
|------------|----------------|-------------------|------------|-----------|---------|--------|
| | | 1 | 2 | 3 | 4 | 5 |
| BRB | maximum | 20,000,000 | 13,200,000 | 1,660,000 | 271,000 | 55,600 |
| | mean | 16,500,000 | 2,020,000 | 321,000 | 64,500 | 15,800 |
| | minimum | 2,460,000 | 382,000 | 75,000 | 18,100 | 5,230 |

| | | Time lapse (days) | | | | |
|------------------|----------------|-------------------|-----------|---------|--------|-------|
| | | 1 | 2 | 3 | 4 | 5 |
| POOL-BART | maximum | 10,000,000 | 3,450,000 | 196,000 | 19,300 | 2,950 |
| | mean | 4,760,000 | 255,000 | 23,800 | 3,500 | 740 |
| | minimum | 332,000 | 24,500 | 4,160 | 852 | 235 |

| | | Time lapse (days) | | | | |
|------------------|----------------|-------------------|---------|--------|-------|-----|
| | | 1 | 3 | 6 | 12 | 18 |
| ALGE-BART | maximum | 10,000,000 | 829,000 | 74,700 | 2,010 | 173 |
| | mean | 2,270,000 | 383,000 | 39,500 | 1,300 | 129 |
| | minimum | 991,000 | 185,000 | 31,700 | 870 | 98 |

| | | Time lapse (days) | | | | |
|------------------|----------------|-------------------|-----------|---------|--------|-----|
| | | 1 | 2 | 3 | 4 | 5 |
| FLOR-BART | maximum | 10,000,000 | 3,450,000 | 196,000 | 19,300 | 295 |
| | mean | 4,760,000 | 255,000 | 23,800 | 3,500 | 740 |
| | minimum | 332,000 | 29,500 | 4,160 | 852 | 235 |

Uncertainty in the data generated by the BART tester becomes restricted by making observations on a daily basis. This means that a reaction would only be observed when it is recorded at one of the daily observations. Such observations are self limiting in the generation of precision and, at best, only a semi-quantitative prediction of the population can be achieved. For a reaction to occur it must be assumed that the reaction went positive between the last observation and the next observation. This would mean that there is a potential for the number of active bacteria to have involved a Time lapse that could have occurred over a two day period between the previous observation and the observation two days later when the reaction would definitely have been observed. Thus the uncertainty extends from just after the previous observations that the tester was negative to just before the next observation would have been made after the observation where the reaction would have occurred before the next observation. The range takes into account these variables as the maximum possible population (where the reaction occurred just after the previous observation) and the minimum population (where the reaction occurred after the observation but before the next observation). The mean population calculated therefore reflects the most probable number for that sample with the possible errors reflected in the maximum and minimum populations values given as p.a.c. /ml.

Clearly if the observation did not detect a reaction on one day but did on the next day then the calculation of the population defaults to the next day but the minimum value does recognize the potential for late reactions to be generated. This method of daily observation is therefore semi-quantitative given approximate values. In the case of daily observations, there is an automatic calculation of the range of variability based upon the Time lapse minus 0.9 of a day for the maximum predicted population and Time lapse plus 0.9 of a day for the minimum predicted population. This forms the range of uncertainty in the test.

It is recommended that either BART-V-READ or a BART-READER be employed if quantitative precision is required. BART-V-READ detects reactions within 15 minutes of their appearance in the tester and BART-READER detects every second. In these cases then the precision improves dramatically towards fully quantitative with better precision obtained with the BART-READER due to the frequency of the readings (i.e. every second with data retention every ten seconds if there is no significant change in the values). Triplicate testing using either of these systems generally shows variation at less than 5% except when an outlier occurs. Outlier is defined as a result that lies more than two standard deviations away from the mean and, for the BART testers, generally occurs in one in eighteen tests and gives a radically different result. Generally such an outlier water sample would have contained either a much greater, or lesser, amount of biocolloidal or particulate material than the normal samples thus creating shifts in the Time lapse.

Water samples are subject to change in the level or bacterial activity and the position of the bacteria within the sample that can both affect the suitability of the 15ml sample gathered for a BART test. It is recommended that the BART testing should be conducted within four hours of the samples being taken with the water maintained at its ambient temperature if close to room temperature, or allowed to warm up to room temperature. Under these circumstances the water sample should be shaken gently just before the removal of water for BART testing to re-suspend any denser materials that may have been settling out. This would minimise the potential for outliers to be generated during the testing. The advantage of the BART testers is that they can be employed in the field minimising the effects that storage might have had on the bacteria in the sample. If the testers are filled in the field and then moved to a more secure place for daily observation at room temperatures, then the testers can be filled in the field and moved while being kept upright and keeping the tests at roughly room temperature. If the tests have to be transported in a vehicle then place the tests near to the floor or the vehicle roughly in the middle of the vehicle to avoid sudden shocks and vibrations. Field testers can be placed in 1½" square seedling trays or the lab testers can be placed in the BART-RACK trays as eight tests per tray.

If water samples are to be kept for longer than four hours before testing then the water can be placed in a cooler over ice. This will reduce the activity of the bacteria and may

cause a lengthening in the Time lapses (and a smaller predicted population). Where the BART tester is being observed daily, then these increases in Time lapse may not be significant to the semi-quantitative prediction of populations.

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