NOTE

Samplers for Dip Testing or Swab Testing in Breweries

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ABSTRACT

Experiments with Millipore Yeast and Mold and Total Count™ samplers have demonstrated that recovery of typical brewery bacteria and yeasts from suspensions is poorer when samplers are used than when traditional membrane filtration is used. Based on these data, such samplers, at least in their present mode, cannot be recommended for use in the brewery.

Key words: *Bacteria, Dip testing, Membrane filtration, Swab testing, Yeast*

A Millipore (Millipore Corporation, Bedford, MA 01730) sampler used for swab (or dip) testing is basically a plastic support on which a 0.45-μm gridded membrane filter is bonded to an absorbent pad containing a dehydrated nutrient medium. When the sampler is immersed in liquid, the absorbent pad rapidly draws 1 ± 0.05 ml of liquid through the membrane, and the medium hydrates. Microbes are retained on the filter surface and are fed by the specified medium below. These samplers, although relatively expensive, are a remarkable innovation designed to allow small plants with limited expertise in microbiology to routinely monitor microbes in liquids or effluents, or on equipment, with minimal labor (2–7). At the present time, Coli-Count™, Total Count™, and Yeast and Mold samplers are available; other water testers and swab testers will soon be marketed.

These samplers have been found to be equivalent to other conventional assay procedures (6,7) and extremely useful for the assessment of microbial contamination of water and dialysis fluid (7), the recovery of *Pseudomonas* in soft drinks (by special *Pseudomonas* F dip testers [6]), and the determination of total count in water used for processing electron and microelectronic devices (1).

Although recommended for liquid products (3,5) and equipment (4), such samplers have not been specifically advocated or assessed for brewing. Our investigations evaluated Total Count and Yeast and Mold samplers for this purpose. Previous investigations[1] had indicated poorer recovery of organisms from brewery equipment with the swab testers than with conventional methods.

Experiments were conducted on yeasts grown in Sabouraud's dextrose broth (DIFCO) and bacteria grown in tomato juice broth (DIFCO) in side-arm Erlenmeyer flasks. Growth was monitored and roughly quantified as described earlier (8,9). The yeasts were obtained from the National Collection of Yeast Cultures (Nutfield, Surrey) or from the collection of Molson Breweries of Canada Ltd. (Montreal). All except *Saccharomyces carlsbergensis* were isolated as wild yeasts of brewery origin. Bacteria were also brewery contaminants and were supplied by reputable laboratories (10). Cultures in logarithmic growth were diluted in 0.1% sterile peptone water to obtain 30–35 colony-forming units per milliliter.

In the conventional procedure, 10 ml of sterile 0.1% peptone water was placed on the 0.45-μm 47-mm S-pak Millipore filter to aid in dispersal of organisms over the filter surface. A 1.0-ml quantity of diluted culture was added and then filtered through the membrane. Filters were then rinsed with 10 ml of the peptone water.

[1] Unpublished data.

The same diluted cultures of yeast and bacteria were immediately subjected to the dip-test, using either the Yeast and Mold or the Total Count sampler. Samplers were purchased and were used immediately upon receipt from the supplier. The media used on these samplers are Schaufus and Pottinger M-green yeast and mold broth,[2] which is recommended for detection of fungi in routine analyses of beverages, and a standard methods-based broth for bacteria.[3]

Six replicate analyses were made with both the traditional membrane filtration technique and the samplers. Care was taken to

[2] A medium of yeast extract, cerelose, peptone, $MgSO_4$, potassium phosphate, diastaste, and thiamine, supplemented with brom cresol green (BBL11286, Baltimore Biological Laboratories, Cockeysville, MD 21030).

[3] Personal communication.

TABLE I.
Recovery of Brewery Yeasts Using Yeast and Mold Samplers

Organism	Total CFU[a] Yeast/Mold Sampler[b]	Total CFU[a] Membrane[b]	Comparative Recovery[c] (%)
Saccharomyces carlsbergensis, MBCL L-5	2.5 (1.8)	23.7 (3.8)	10.5
Candida mycoderma, NCYC 327	28.5 (16.4)	135 (3.4)	21.1
Pichia membranaefaciens, NCYC 326	18.1 (5.4)	34.5 (2.4)	52.5
Hansenula anomala, NCYC 682	7.5 (4.6)	22.5 (4.0)	33.3
Brettanomyces anomalus, NCYC 449	38.7 (15.7)	73.8 (2.9)	52.4
Saccharomyces willianus, NCYC 114	13.8 (9.2)	31.5 (2.4)	43.8
Torulopsis colliculosa, NCYC 608	16 (7.9)	60.3 (2.7)	26.5
Kloeckera apiculata, NCYC 328	6.3 (4.3)	30.3 (2.2)	20.8

[a] Colony forming units per milliliter of prepared suspension.
[b] Mean of six replicas; standard deviation in parentheses.
[c] Assuming that membrane recovery is 100%.

TABLE II
Recovery of Brewery Bacteria Using Total Count™ Samplers

Organism	Total CFU[a] Total Count Sampler[b]	Total CFU[a] Membrane[b]	Comparative Recovery[c] (%)
Flavobacterium proteus, B125	68.5 (12.4)	72.2 (3.3)	94.9
Enterobacter agglomerans, #127	3.7 (3.4)	24.0 (2.1)	15.3
Lactobacillus brevis, BSO 31	25.3 (2.3)	106 (6.0)	23.9
Klebsiella oxytoca, #52	23.8 (20.0)	78.8 (4.0)	30.2
Gluconobacter oxydans subs *oxydans,* NCIB 9013	30.5 (16.4)	50.2 (4.3)	60.8
Citrobacter freundii, ATCC 8090	8.5 (3.3)	15.0 (2.5)	56.7
Actobacter sp., BSO 7	51.2 (7.2)	60.3 (2.6)	84.9

[a] Colony forming units per milliliter of prepared suspension.
[b] Mean of six replicas; standard deviation in parentheses.
[c] Assuming membrane recovery is 100%.

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follow exactly the manufacturer's protocol for samplers (3,5). Yeast cultures on the membranes from the traditional filtrations were cultivated on DIFCO Sabouraud's dextrose agar and bacteria cultures on DIFCO plate count agar. All incubations were conducted for 2–3 days (depending on the organism) at 27° C. Reincubation showed that no further growth occurred with prolonged incubation. Growth on membranes and samplers was counted, and means and standard deviations were calculated. Results are tabulated in Tables I and II. For comparative recovery calculations, we assumed that all organisms from logarithmically growing cultures were viable and recoverable by using S-pak membrane filtration and plating on the described media (9).

The recovery of yeasts and bacteria on the two types of samplers was poor. Although this study has the bias of comparing recovery of organisms on two separate media (ie, for yeast), it nevertheless evaluates a quality imperative in a good testing method—the ability to recover all microbes significant to the industry with precision and speed. We conclude that such demonstrated recovery is unacceptable, with the possible exception of *Flavobacterium* and *Acetobacter* spp. Not only was recovery often less than 30% of the recovery found by a traditional membrane filtration method, but the standard deviations for replicas of six samplers were often unaccountably high. The reason for such poor recovery is unknown but may relate to lack of adherence of the microbe to the filter, unequal water absorption by the pad in the samplers (and therefore unequal organism impaction on the filter), or poor growth of brewing organisms on the media chosen by the Millipore Corporation. Based on these findings, we conclude that dip-test samplers (or swab testers, which work on the same principle) may give substantially lower counts than does the described conventional filtration method for quantitation of brewery organisms in samples, in effluents, or on equipment.

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