Improved KCN Medium

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A semisolid tetrazolium salt agar medium developed for testing sensitivity to KCN is easier to interpret, has a longer storage life, and is less critical in the size of inoculum than is Moeller KCN broth medium.

The ability of an organism to grow in the presence of KCN is used in the differentiation of several groups of Enterobacteriaceae, in particular to distinguish between Salmonella (KCN sensitive) and Citrobacter (KCN resistant) (2, 4). The KCN broth of Moeller is the most widely accepted medium for performing the test. There are several problems with this broth: (i) it has a short storage life because of the evaporation of the cyanide as HCN; (ii) it is sometimes difficult to evaluate weak positive cultures; and (iii) a very small inoculum is required so that initial turbidity does not interfere with readings.

Gershmann (3) modified Moeller medium by adding 0.005% 2,3,5-triphenyltetrazolium chloride (TTC). In the presence of viable or growing cells, TTC is reduced to an insoluble red pigment. Buttiaux et al. (1) used a semisolid KCN medium for the identification of lactose-fermenting enterobacteriaceae. The medium is Moeller's basic formulation modified by the addition of 1 g of agar per liter and an increase in the KCN concentration from 0.0075 to 0.025%.

The medium proposed in this paper is a combination of the modification by Gershmann and Buttiaux et al. It uses TTC to indicate growth and agar to localize the growth pattern.

The organisms used in this study were obtained from the collections of the Food and Drug Administration and the National Center for Disease Control, Atlanta, Ga., or were isolated from clinical and food sources. Cultures were maintained on brain heart infusion agar slants overlayed with sterile mineral oil and held at 4 C. They were transferred to fresh brain heart infusion agar slants 24 h before testing. The organisms isolated from food and clinical specimens were identified by biochemical or serological reactions, or both, as described in Edwards and Ewing (2).

The semisolid KCN base was prepared by adding 1.2 g of agar to 1 liter of KCN broth base (BBL), rehydrated as directed. The medium was boiled and sterilized for 15 min at 121 C. After cooling to 45 C, 15 ml of a cold 0.5% KCN solution and 5.5 ml of a 1% filter-sterilized solution of TTC were added. Ten milliliters of the medium was placed in sterile screw-capped tubes (15 by 100 mm) containing 3 ml of sterile mineral oil. The time required for tubing the medium was kept to a minimum to avoid loss of HCN. The medium was stored at 4 C. Tubes of KCN broth were prepared as above except that agar and TTC were not incorporated.

The agar medium was inoculated from 24-h slants with a straight stab three-quarters down the length of the agar in the tubes. Sufficient inoculum was used so that visible traces remained along the stab line. The tubes were then incubated for 48 h at 37 C. A positive reaction was indicated by the reduction of the indicator producing a red coloration along the entire stab line.

Tubes of KCN broth were inoculated with a needle in a manner avoiding initial visible turbidity. The tubes were then incubated for 48 h at 37 C. A positive reaction was indicated by the presence of turbidity in the tube.

A total of 327 cultures, representing 12 different genera and at least 80 species, were inoculated simultaneously into the two media, and after 48 h at 37 C the growth responses were compared (Table 1). In all but two cases, both media were identical in the detection of KCN sensitivity.

The storage life of the agar medium was determined by testing four KCN-resistant and four KCN-sensitive organisms at weekly intervals for two months. The results of this testing indicated that the agar medium did not lose sensitivity during this time interval. The storage life of Moeller KCN broth is reported to be 2 weeks (2, 4, 5).

The size of inoculum is not as critical in the KCN-TTC agar as it is in the KCN broth. The KCN broth test is interpreted on the basis of turbidity. Too large an inoculum results in a slight turbidity or sediment on the bottom of
### TABLE 1. Comparison of growth response in KCN broth and KCN-TTC agar

| Organism           | No. of species | No. of serotypes or strains | Total No. of cultures | KCN broth | KCN-TTC | Correlation (%)<sup>a</sup> |
|--------------------|----------------|-----------------------------|-----------------------|-----------|---------|-----------------------------|
|                    |                |                             |                       | Pos       | Neg     | Pos             | Neg     |             |
| Escherichia coli   | 1              | 32                          | 100                   | 1<sup>a</sup> | 99<sup>a</sup> | 2<sup>a</sup> | 98<sup>a</sup> | 97.6    |
| Shigella           | 4              | 13                          | 14                    | 0         | 14<sup>a</sup> | 0               | 14<sup>a</sup> | 100     |
| Edwardsiella       | 1              | 1                           | 2                     | 0         | 2<sup>a</sup> | 0               | 2<sup>a</sup> | 100     |
| Salmonella<sup>b</sup> | 65           | 30                          | 108                   | 2         | 106<sup>a</sup> | 3<sup>a</sup> | 105<sup>a</sup> | 99.1    |
| Arizona            | 1              | 13                          | 17                    | 2<sup>a</sup> | 15        | 2               | 15        | 100     |
| Citrobacter        | 1              | 12                          | 17                    | 17        | 0         | 17              | 0         | 100     |
| Klebsiella<sup>c</sup> |             |                             |                       | 26        | 25<sup>a</sup> | 1<sup>a</sup> | 25<sup>a</sup> | 100     |
| Enterobacteriaceae |                |                             |                       | 22        | 21<sup>a</sup> | 1<sup>a</sup> | 21<sup>a</sup> | 100     |
| Serratia<sup>d</sup> |             |                             |                       | 2         | 0         | 2               | 0         | 100     |
| Proteus            | 4              |                             | 16                    | 16        | 0         | 16              | 0         | 100     |
| Providencia        | 2              |                             | 2                     | 2         | 0         | 2               | 0         | 100     |
| Aeromonas          | 1              |                             | 1                     | 0         | 1         | 0               | 1         | 100     |

<sup>a</sup> Based on results from three trials.
<sup>b</sup> Same strains positive or negative in both media.
<sup>c</sup> One of two positive for both media.
<sup>d</sup> Not all cultures were identified to species.
<sup>e</sup> Two of three positive for both media.
<sup>f</sup> Cultures identified to genus only.
<sup>g</sup> No serology done on species.

The data presented shows that the KCN-TTC semisolid medium is as effective as Moeller KCN broth in detecting KCN-resistant and -sensitive organisms. The localization of growth and color reaction due to addition of agar and TTC makes this medium considerably easier to interpret than the plain broth. The agar medium can be stored for a longer period of time and is less critical in the size of inoculum.

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**LITERATURE CITED**

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