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RESEARCH COMMUNICATION

The use of the GENETRAK[1] *Escherichia coli* probe kit for the detection of three atypical *E. coli* isolates

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ABSTRACT

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A commercially available *E. coli* probe kit was used to test 1 lactose negative *E. coli* isolate and 2 hydrogen sulphide-producing *E. coli* isolates. The isolates were confirmed as *E. coli* by means of the API system. The GENETRAK *E. coli* DNA probe kit reacted positively with the lactose negative isolate, but negatively with the hydrogen sulphide-producing isolate.

DNA probes are establishing themselves as a viable tool in diagnostic work. Their genetic specificity and ability to recognize a targeted DNA sequence in a heterogenous genetic mix favours their use over standard culture techniques (Parsons 1988).

The DNA sequence of *E. coli* has been well elucidated (Harel, Lapointe, Fallara, Lortie, Bigras-Poulin, Lariviere & Fairbrother 1991). Sequences encoding enterotoxins and virulent antigens have been determined and are used in probes to detect such virulent *E. coli* strains (Harel, *et al*. 1991). Since *E. coli* is a gut commensal, it also acts as an indicator of faecal contamination and in some cases it is therefore important to be able to detect the presence of any *E. coli*, whether virulent or not. The GENETRAK *E. coli* DNA probe has been designed to perform this function.

GENETRAK Systems successfully designed an *E. coli* probe using a database of DNA sequences collected from a wide variety of sources. A sequence characteristic of all available strains was selected from this database. The sequence had to be specific enough to exclude related genera, yet sufficiently sensitive to incorporate all strains.

Recently a lactose negative *E. coli* isolate from a chicken and 2 hydrogen sulphide-producing isolates, 1 from horse faeces (isolated by J. Carstens, Department of Infectious Diseases) and 1 from a chicken, were isolated at the Faculty of Veterinary Science at Onderstepoort. It was decided to test these atypical isolates using the GENETRAK *E. coli* probe kit.

Lactose fermentation and hydrogen sulphide production are atypical traits of *E. coli* (Krieg & Holt 1984). In tables presented by Ewing (1986), all iso-

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lates tested were negative for hydrogen sulphide production, although it is mentioned that an occasional strain may produce hydrogen sulfide. Isolates showing these characteristics were identified as *E. coli* by use of the API 20 E system. Each isolate was tested on the API 20 E on 3 different occasions. On all 3 occasions, identical results were obtained. A 98,8 % positive identification as *E. coli* for the hydrogen-sulphide-producing isolate and a 97,7 % positive identification as *E. coli* for the lactose negative isolate were recorded. The results of the API 20 E tests carried out on these isolates can be seen in Table 1. The GENETRAK *E. coli* probe test was also repeated 3 times for each isolate. In each instance the lactose negative isolate produced a positive result and the hydrogen sulphide-producing isolate, a negative result. The positive and negative controls supplied with the kit produced the correct results.

A lactose negative *E. coli* would pass undetected when grown by standard culture on MacKonkey Agar. The positive result obtained by the probe therefore indicates an improvement on the conventional methods. Lactose negative *E. coli* are regarded as being phenotypically intermediate to *E. coli* and *Shigella* spp. (Krieg & Holt 1984). The specificity of this GENETRAK *E. coli* probe was tested by using a variety of genetically closely related species (Chan, Wilson, Hsu, King, Halbert & Klinger 1989). They found that all 15 strains of *Shigella* tested by means of the probe produced a positive result. There is, however, a 95 % genetic homology between *E. coli* and *Shigella* (Chan, *et al.* 1989). There is no real taxonomic justification for regarding them as separate entities other than that of avoiding the confusion that would inevitably be caused by their reclassification (Krieg & Holt 1984).

Hydrogen sulphide-producing variants of *E. coli* were first reported by Lautrop, Orskov & Gaarslev (1971). They also demonstrated that hydrogen sulphide variants can transfer this capacity to ordinary *E. coli* strains, indicating that hydrogen sulphide production is plasmid-mediated. Layne, Hu, Balows & Davis (1971) also support this observation. If the plasmid is episomal, i.e. if it inserts within the bacterial genome, there is a slight possibility that it could insert in the target sequence, thereby defying recognition by the probe. There is a greater possibility of the plasmid either inserting elsewhere on the genome, or of its being extra-chromosomal. If this is the case, the *E. coli* isolates tested probably have a target sequence different from the one used in the probe. This presents the possibility that either the probe is incapable of detecting all strains of *E. coli* or the hydrogen sulphide-producing isolate is, in fact, not *E. coli*, but a closely related, yet genetically distinct species. This is strongly refuted by Lautrop *et al.* (1971) who states that hydrogen sulphide variants must be identified as E. coli as they are in

TABLE 1 Bicohemical results of three atypical *E. coli* isolates, obtained from the API 20 E

API 20 E test	H2S + Horse	H2S + Chicken	Lactose negative Chicken
ONPG	+	+	+
Arginine	–	–	–
Lysine	+	+	+
Ornithine	+	+	+
Simmons citrate	–	–	–
Hydrogen sulphide	+	+	–
Urease	–	–	–
TDA	–	–	–
Indole	+	+	+
Acetoin	–	–	–
Gelatin hydrolysis	–	–	–
Glucose	+	+	+
Mannitol	+	+	+
Inositol	–	–	–
Sorbitol	+	+	+
Rhamnose	+	+	+
Sucrose	+	+	+
Melibiose	+	+	+
Amygdaline	–	–	–
Arabinose	+	+	+

complete agreement with the pattern typical for *E. coli* and even contain recognized *E. coli* antigens. This then points to the need to improve the probe in such a way as to incorporate this variant strain. As data accumulates, improvement is inevitable and some fine-tuning of this probe would increase confidence in it as a very reliable screening technique for *E. coli*.

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