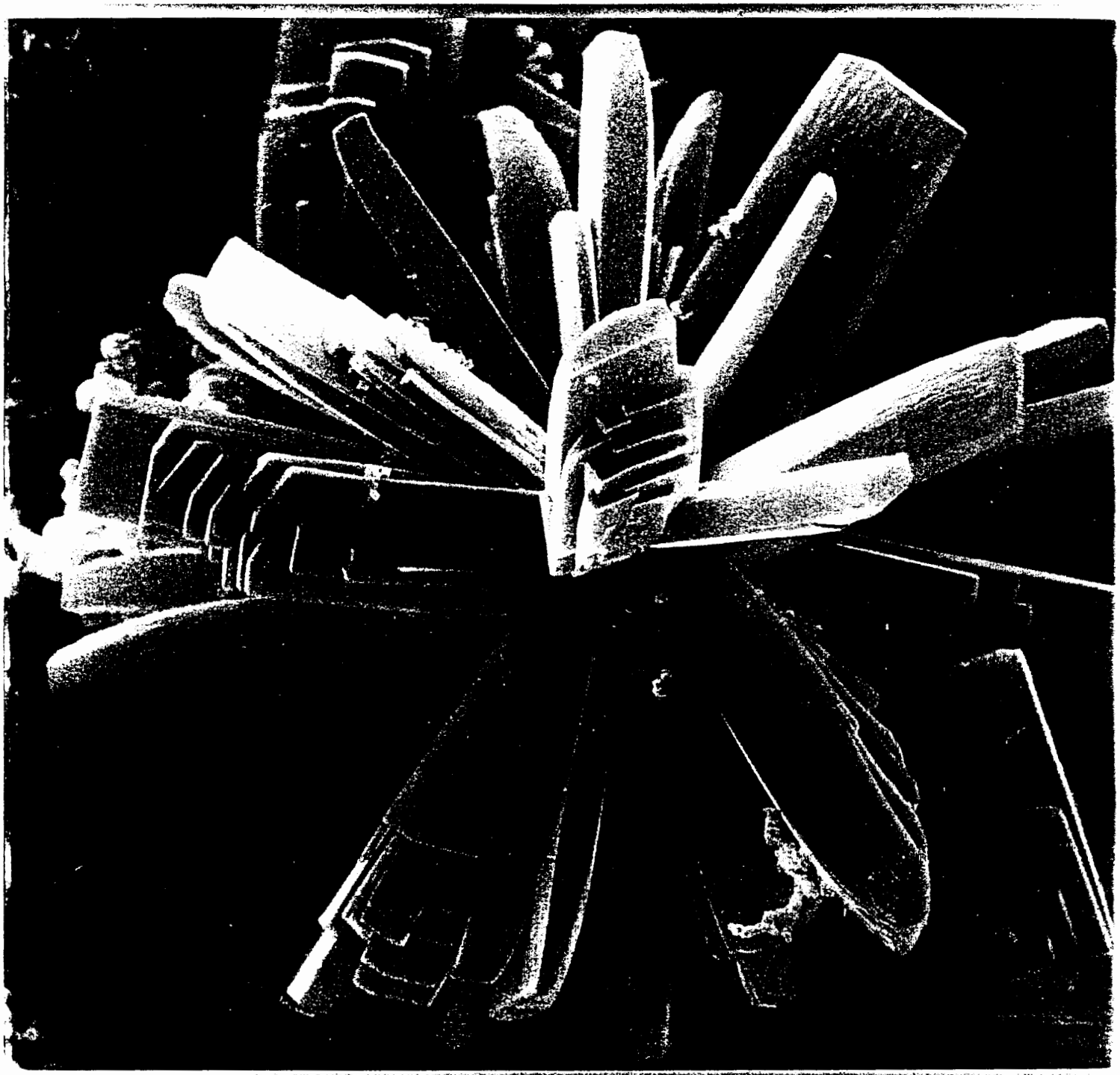
BRITISH JOURNAL OF

BIOMEDICAL SCIENCE



VOLUME 55 NUMBER 4 DECEMBER 1998 ISSN 0967–4845

British Journal of Biomedical Science 1998; 55: 238–241

Evaluation of CHROMagar Orientation medium for the identification of surveillance cultures: a novel method

J. L. RIGBY and T. J. NEAL

Department of Medical Microbiology, Royal Liverpool and Broadgreen University Hospital, 7th Floor Duncan Building, Daulby Street, Liverpool L69 3GA, England, UK

(Accepted 26 June 1998)

Abstract: Microbiological surveillance helps prevent the spread of potentially pathogenic organisms on high-risk units. This study describes a simple method for identifying aerobic Gram-negative bacilli isolated from a neonatal intensive care unit screening programme. The identification scheme combines CHROMagar with three commonly used laboratory tests and multipoint technology. The procedure produced identical results when compared with an established identification method (Mast ID) over a three-week period. Financial evaluation suggests that significant cost savings could be achieved with the new scheme.

Key words: Gram-negative bacteria. Infant, newborn. Infection control. Intensive care.

Introduction

Pre-term and unwell babies nursed on a neonatal intensive care unit (NICU) are highly susceptible to colonisation and infection with a variety of nosocomial organisms including yeasts, aerobic and facultatively anaerobic Gram-negative bacilli (GNB), and primary pathogens such as *Staphylococcus aureus* and β-haemolytic streptococci.[1] Colonised babies represent a potential cross-infection risk on the unit and the problem is increased if the isolate exhibits antibiotic resistance. Surveillance schemes for the detection of specific problem organisms can assist in controlling the spread of infection.[2]

The Liverpool Women's Hospital currently operates a weekly surveillance programme that examines throat and rectal swabs from each baby on the NICU. For several years, identification of GNB isolated from the NICU weekly screen was undertaken using Mast ID™ (Mast Diagnostics Limited, Merseyside, UK). This multipoint system uses 18 separate tests to identify isolates to species level. A six-disc antibiogram using Stokes' comparative method for sensitivity testing[3] was also performed. These methods are time-consuming and costly.

The aim of this study was to assess an in-house method for identifying GNB, with the intention of reducing workload while maintaining the quality and usefulness of the results. This method is based on CHROMagar™ Orientation medium (M-Tech Diagnostics Limited, Warrington, UK) and combines both multipoint and chromogenic technology.

Materials and methods

A retrospective computer analysis of surveillance samples from the NICU was performed to determine the pattern of GNB colonisation on the unit (Fig. 1). A method was devised to identify the GNB commonly isolated on the unit to genus level, at a min-

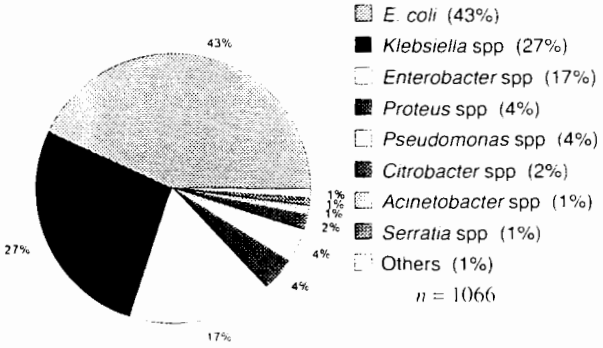


Fig. 1. Percentages of aerobic and facultatively anaerobic Gram-negative bacilli identified from NICU weekly screen swabs.

Table 1. Interpretation of reactions on the identification media

Medium	Appearance	Interpretation
CHROMagar Orientation	Coloured multipoint inoculum	Blue, pink or colourless (± brown halo)
Motility	Diffuse pink pigment throughout agar	Positive
	Pigment confined to point of inoculum	Negative
Amygdalin	Yellow pigmentation of agar surrounding inoculum	Positive
	No yellow pigment	Negative
DNase	Clear zone (no precipitation) around inoculum after addition of HCl (1 mol/L)	Positive
	Absence of clear zone around inoculum	Negative

imum. All babies on the NICU were routinely swabbed on admission and weekly thereafter for surveillance of potential pathogens. Throat and rectal swabs received from each baby were inoculated onto 5% horse-blood agar (37°C in 5% CO_2), MacConkey agar (37°C in air) and Sabouraud's agar (30°C in air). After overnight incubation, the plates were examined for the presence of GNB, yeasts and primary pathogens. All GNB isolated were inoculated into tryptone water (2 mL) and incubated at 37°C in air for 2–4 h. Each suspension was then dispensed into a well of a 19-pin multipoint inoculator block, and also subcultured onto MacConkey agar to ensure purity. A 10 μg gentamicin disc was placed on the MacConkey purity plate, prior to incubation, to provide an early indication of gentamicin resistance.

The identification scheme consisted of four principal tests: CHROMagar™ Orientation medium, motility agar, amygdalin agar and DNase agar, which were inoculated by multipoint and read after overnight incubation at 37°C in air (Table 1). The identification of both *Escherichia coli* and *Proteus* spp relies on the CHROMagar plate alone; *E. coli* produces a rose pink-coloured colony, and *Proteus* spp a colourless colony with a diffuse brown halo in the surrounding medium. Colourless colonies without a

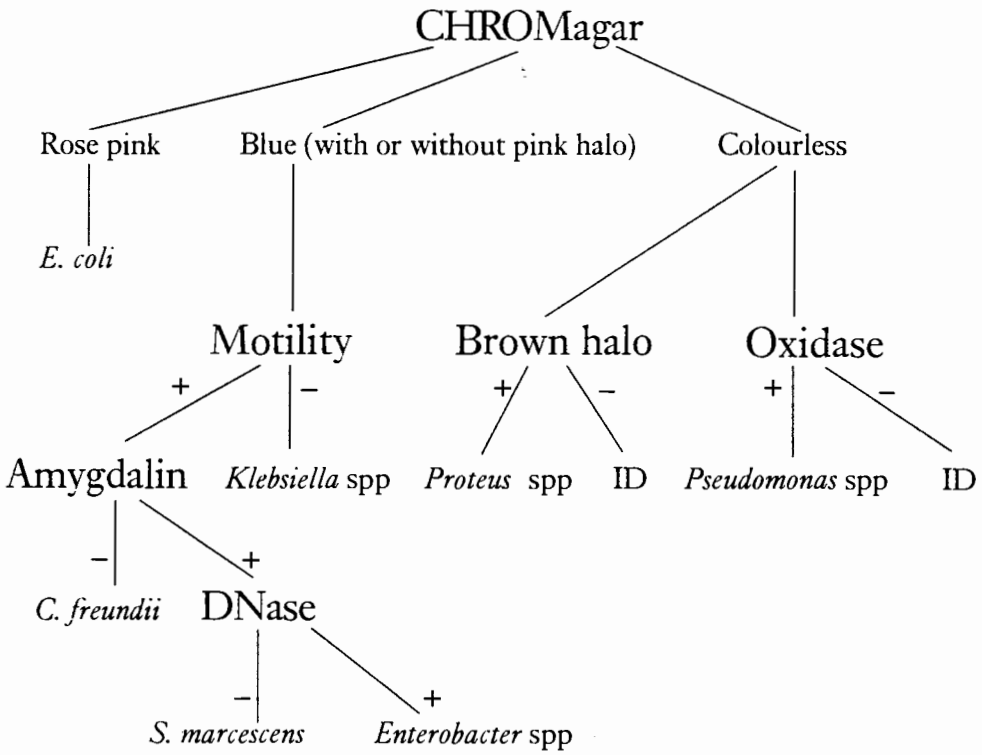


Fig. 2. Coliform identification flowchart.

Table 2. Coliform ID reference chart

CHROMagar			Motility	Amygdalin	DNase			
Pink	Blue	Colourless	(+/−)	(+/−)	(+/−)			
1	2	4	1	2	4	ID no.	Additional tests	Identification
+	−	−	−	−	−	10		*E. coli*
+	−	−	+	−	−	11		*E. coli*
−	+	−	+	−	−	21		*Citrobacter freundii*
−	+	−	−	+	−	22		*Klebsiella* spp
−	+	−	+	+	−	23		*Enterobacter* spp
−	+	−	+	+	+	27		*Serratia marcescens*
−	−	+	+	−	−	41	Oxidase+	*Pseudomonas* spp
−	−	+	+	−	−	41	Brown halo+	*Proteus* spp
−	−	+	+	+	−	43	Brown halo+	*Proteus* spp

brown halo were differentiated by the oxidase test. A positive oxidase test was indicative of *Pseudomonas* spp, while an oxidase-negative organism required further identification by API 32E (bioMérieux, Marcy l'Etoile, France). *Klebsiella, Enterobacter, Serratia* and *Citrobacter* species all appeared blue on CHROMagar medium (sometimes with a pink halo), and were difficult to differentiate. Further tests were required to distinguish these genera, and motility, amygdalin and DNase media were used for this purpose, as demonstrated in the coliform identification (ID) flowchart (Fig. 2).

Results were recorded on a coliform ID template and a two-digit ID number generated. The identification of the organism was determined by comparing the two-digit ID number with a coliform ID reference chart (Table 2). If the profile obtained was not found in the reference chart, the organism was identified fully using API 32E.

Over a three-week period, all Gram-negative isolates from the NICU screening programme were processed using the method described, in parallel with the Mast ID™ system. Seventeen wild strains, previously identified by API 32E, were included to control both methods.

Results

Seventy-six isolates were identified by both methods over the three-week period (Table 3). No isolates were identified incorrectly by the scheme described. On one occasion only was further identification necessary; the isolate was a *Salmonella* sp (control organism), and although the numerical profile obtained was the same as for *Proteus* spp and

Table 4. A summary of costs of the two ID schemes (in UK pounds)

	18-test Mast ID	Revised ID scheme
*Labour**		
Cost *per* week	31.91	15.42
Cost *per annum*	1659.32	801.84
Consumables		
Cost *per* week	17.17	7.37
Cost *per annum*	892.84	383.24
Total cost *per annum*	2552.16	1185.0
Saving *per annum*		1367.08

*Mid-point of the biomedical scientist (MLSO1) scale

Table 3. Comparison of 18-test Mast ID™ and a revised CHROMagar™-based scheme

18-test Mast ID		Revised ID scheme			
Identification	No. of isolates	Identification	No. of isolates	Profile	Extra tests
E. coli	35	*E. coli*	35	10	
Klebsiella pneumoniae	11	*Klebsiella* spp	20	22	
Klebsiella oxytoca	9				
Enterbacter cloacae	2	*Enterbacter* spp	5	23	
Enterobacter aerogenes	3				
Citrobacter freundii	2	*Citrobacter freundii*	2	21	
Serratia marcescens	5	*Serratia marcescens*	5	27	
Proteus mirabilis	3	*Proteus* spp	3	41	Brown halo+
Salmonella spp	1	Requires API32E	1	41	
Pseudomonas spp	1	*Pseudomonas* spp	1	41	Oxidase+

Pseudomonas spp, the oxidase text was negative and no brown halo was seen on the CHROMagar™ Orientation medium. On four occasions (results not shown) identification could not be achieved due to mixed cultures. Labour and consumable costs were estimated for both methods (Table 4).

Discussion

The Mast ID™ system used currently for the identification of GNB from surveillance samples is both labour-intensive and costly, and provides an excessive amount of information not always required for infection control. The intended application of CHROMagar™ Orientation medium was the isolation and differentiation of urinary tract pathogens such as *E. coli* and *Klebsiella*, *Proteus* and *Pseudomonas* species. However, its ability to discriminate between the majority of GNB facilitated its use as the primary identification medium in the scheme described. In line with the findings of Merlino *et al.*,[4] difficulty was encountered in differentiating the various blue colours produced by *Klebsiella*, *Enterobacter* and *Citrobacter* species. As these organisms represented 46% (Fig. 1) of the colonising isolates from our population, additional tests were required to discriminate between them. The three additional tests (motility, DNase and amygdalin) were derived from a study of the expected reactions of GNB.[5] In combination with the CHROMagar™ Orientation medium, motility, DNase and amygdalin agar provided identification to genus level, at a minimum, in almost 98% of GNB isolates from the NICU. The procedure was simple to perform and all tests were easy to interpret.

In a trial performed over a three-week period, no isolates were identified incorrectly when compared to the current Mast ID™ system. However, computer analysis of 1066 previous isolates revealed one *Citrobacter diversus* and three *Serratia liquifaciens* which would have been identified incorrectly as *Enterobacter* spp, 0.38% of the total number of isolates.

One of the major benefits of the identification scheme described is the estimated saving of over £1350 *per annum*. In addition, a large reduction in workload and time at both bench level and in media preparation would be realised without a reduction in the quality of service provided. We recommend this simple identification scheme to those laboratories processing large numbers of surveillance samples.

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