# Diagnostic tables for the common medical bacteria

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### INTRODUCTION

A device to help to sort the characters of micro-organisms, and so speed their identification, was described by Cowan & Steel (1960). By itself the device is useless and its value depends entirely on the tables constructed for it. In the original paper a simple table of IMViC reactions was given to illustrate the use of the device. The present paper describes a simplified form of the Determinator and gives tables suitable for use in the identification of the types of organisms in our Collection, namely, those bacteria of interest to medical and veterinary workers.

### CONSTRUCTION AND USE OF TABLES

The original Determinator, described by Cowan & Steel (1960), is shown in Pl. 1, figs. 1, 2. Diagnostic tables were made with up to twenty-eight characters on quarter-inch-squared foolscap paper. Names of characters were listed on the left side of the paper and the characters of the different organisms recorded in alternate columns by appropriate letters or signs (e.g. R for rod, S for sphere, $+$, $-$, etc.). The table was aligned with the top edge of the frame, and the characters of the unknown organisms were then written in the columns scribed on the Perspex sheet. When the characters of all the unknown strains had been recorded, one column at a time was isolated by the mask and the Perspex sheet moved from left to right until a column was found on the diagnostic table in which the characters corresponded closely or exactly to the characters of the unknown organism. A key indicated the genus, or in some cases species, of the organism on the diagnostic table.

A simplified form of the Determinator using the same diagnostic charts can be made from a strip of Perspex about $9 \times 2.5$ in. by scribing lines at quarter-inch intervals parallel to the short side (Pl. 1, fig. 3). The spaces between the lines are numbered, to align the strip with the characters on the tables. The characters of one unknown strain are written in grease pencil on the Perspex sheet, which is placed and aligned on the diagnostic table. The Perspex strip is moved across the table keeping the first line in alignment. The characters recorded on the strip are compared with those of the table and when a similar set of characters is found the unknown organism can be identified.
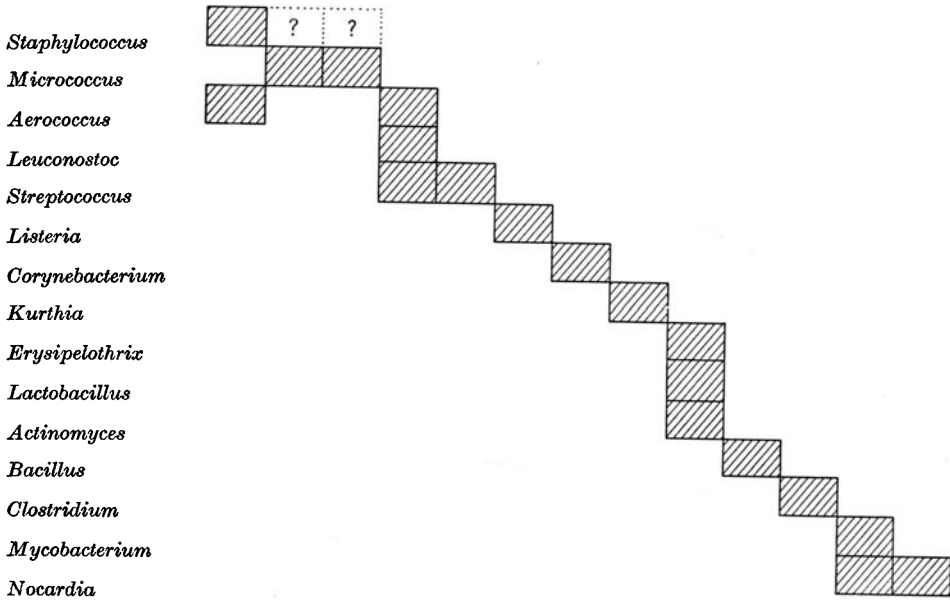
Diagnostic tables were made in three stages, and different tables were constructed for Gram-positive and Gram-negative organisms. Table 1 for Gram-positive bacteria and Table 2 for Gram-negative have been combined with figures; genera with the characters shown in each column are shaded.

*Gram-positive bacteria*

Table 1, which is included in Fig. 1, shows how a division of Gram-positive organisms may be made on a few characters such as shape (sphere or rod), acid-fastness, spore production, motility, catalase, oxidase, the production of acid from glucose and the method of attack of glucose, whether by fermentation or by oxidation (Hugh & Leifson, 1953). Not all possible combinations of the characters are shown in Table 1, which is limited to those combinations of characters that

Table-Fig. 1. *Stage 1 diagnostic table for Gram-positive organisms*

|  | a | | | b | | c | | | d | e | f | g | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | $a_1$ | $a_2$ | $a_3$ | $b_1$ | $b_2$ | $c_1$ | $c_2$ | $c_3$ |  |  |  | $g_1$ | $g_2$ |
| Shape | S | S | S | S | S | R | R | R | R | R | R | R | R |
| Acid-fastness | − | − | − | − | − | − | − | − | − | − | − | + | − |
| Spores | − | − | − | − | − | − | − | − | − | + | + | − | − |
| Motility | − | − | − | − | + | + | − | + | − | d | d | − | − |
| Catalase | + | + | + | − | − | + | + | + | − | + | − | d | + |
| Oxidase | − | − | − | − | − | − | − | − | − | d | − | − | − |
| Glucose (acid) | + | + | − | + | + | + | d | − | + | d | d | + | + |
| Glucose F or O | F | O | − | F | F | F | F | − | F | F or O | F | O | O |



Staphylococcus
Micrococcus
Aerococcus
Leuconostoc
Streptococcus
Listeria
Corynebacterium
Kurthia
Erysipelothrix
Lactobacillus
Actinomyces
Bacillus
Clostridium
Mycobacterium
Nocardia

+ = 100–80 % strains positive, d = 79–21 % strains positive, − = 20–0 % strains positive, S = sphere, R = rod, F = fermentation, O = oxidation.

are found to occur among the bacteria dealt with in this paper. The distribution of the different genera is shown diagramatically in Fig. 1 which is based on Table 1. Staphylococci and micrococci occur in the first three columns of the table. *Aerococcus viridans* is shown in the first ($a_1$) and fourth ($b_1$) columns, the reason being that the catalase reaction with this organism is not always easy to read. Workers who are used to observing the production of a large volume of gas when $H_2O_2$ is

added will record the reaction with *A. viridans* as negative, whereas those used to a normal negative catalase reaction (for example, with streptococci) will notice a small production of gas when $H_2O_2$ is added and will record this as positive. To

Table 1 *a*. *Stage 2 table for* Staphylococcus *spp. (including micrococci) and* Aerococcus viridans

|  | Staph. aureus | Staph. saprophyticus | Staph. lactis | Staph. roseus | Staph. afermentans | A. viridans |
|---|---|---|---|---|---|---|
| Catalase | + | + | + | + | + | ± |
| Coagulase | + | − | − | − | − | − |
| Nitrate reduction | + | d | + | d | d | − |
| Gelatin liquefaction | + | d | d | − | d | − |
| Urease | + | d | d | d | d | − |
| VP | + | + | − | − | − | − |
| Glucose (acid) | + | + | + | + | − | + |
| Glucose F or O | F | F | V | | − | F |
| Red pigment | − | − | − | + | − | − |

V = some strains F, others O.

Table 1 *b*. *Stage 2 table for* Streptococcus *groups and species*, Leuconostoc *spp.*, Corynebacterium pyogenes *and* Aerococcus viridans

|  | Str. pyogenes | Str. agalactiae | Str. dysgalactiae | Str. equisimilis | Str. equi | Str. bovis | Str. faecalis | Str. sanguis | Str. hominis | Str. lactis | Str. cremoris | Str. pneumoniae | Str. mitis | Str. uberis | C. pyogenes | L. mesenteroides | L. dextranicum | L. citrovorum | A. viridans |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antigen | A | B | C | C | C | D | D | H | K | N | N | − | − | − | H? | − | − | − | − |
| Haemolysis | β | d | α | β | β | d | d | α | − | α | α | α | α | α | β | | | | α |
| 6·5 % NaCl (growth) | − | − | − | − | − | − | + | − | − | − | − | − | − | − | | | | | + |
| pH 9·6 (growth) | − | − | − | − | − | − | + | − | − | − | − | − | − | − | | | | | + |
| Na hippurate (hydrolysis) | − | + | d | − | − | − | d | − | − | d | − | − | − | + | | | | | + |
| 40 % bile (growth) | − | + | − | + | d | + | + | + | + | + | + | − | − | + | | | − | − | + |
| 10 % bile (growth) | − | + | − | − | − | + | + | + | − | + | + | − | − | d | | | − | − | + |
| 60° C.–30′ (survival) | − | − | − | − | − | + | + | d | − | d | d | − | − | + | | | | | + |
| 45° C. (growth) | − | − | − | − | − | + | + | d | + | − | − | − | d | + | | | | − | d |
| Arginine hydrolysis | + | + | + | + | + | − | + | + | − | + | − | − | d | + | + | − | − | − | − |
| Ethylhydrocuprein | − | − | − | − | − | − | − | − | − | − | − | + | − | − | | | | | − |
| Litmus milk | A | AC | B | B | − | A | B | AC | RAC | RAC | RAC | AC | RAC | RAC | AC | A | AC | A | A |
| Mannitol (acid) | − | − | − | − | − | d | + | − | − | d | − | d | − | + | − | + | − | − | d |
| Sorbitol (acid) | − | − | d | − | − | d | d | − | − | − | − | − | − | + | − | • | − | − | d |
| Arabinose (acid) | − | − | − | − | − | d | d | − | − | d | − | − | − | + | + | + | − | − | d |
| Raffinose (acid) | − | − | − | − | − | + | − | − | + | − | − | + | − | − | + | − | + | − | d |
| Trehalose (acid) | + | + | + | + | − | d | + | + | + | d | d | + | − | + | | | | − | + |
| Glycerol (acid) | − | + | − | + | − | − | + | − | − | − | − | + | − | + | | − | − | − | + |
| Maltose (acid) | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | − | + |
| Salicin (acid) | + | d | d | d | + | + | + | + | + | d | d | d | + | + | − | + | − | − | + |
| Esculin | − | − | − | d | − | + | + | + | + | d | d | − | + | + | | + | − | − | d |
| Sucrose agar | | | | | | (D) | − | D | L | | | | | − | | D | D | − | − |

Milk: A = acid; R = reduction; C = clot; B = acid produced but clotting variable (see Table 1 *bb*).
Sucrose agar: D = dextran produced; L = levan produced; (D) = some strains produce dextran.

make provision for both these results *A. viridans* is shown under both columns $a_1$ and $b_1$. A detailed division of organisms shown in columns $a_1$, $a_2$ and $a_3$ is shown in Table 1*a*, the division of staphylococci being that used by Shaw, Stitt & Cowan (1951). In that paper *A. viridans* was recorded as the alpha group because most of the strains produced alpha haemolysis on blood agar.

The second set of tables for the columns $b_1$ and $b_2$ of Table 1 are shown in Table 1*b* where, by means of a larger number of different characters, the species of streptococci are shown and compared with those of related organisms such as *Leuconostoc* species, *Aerococcus viridans*, and *Corynebacterium pyogenes*. We have found an unexpectedly large proportion of *Streptococcus faecalis* strains are motile; hence the necessity for column $b_2$. In the streptococci, it is necessary to have a third stage to show the division of *Str. faecalis* into varieties (Table 1*bb*).

Table 1*bb*. *Stage* 3 *table for species and subspecies of group D streptococci*

| | *Str. bovis* | *Str. durans* | *Str. faecalis* var. *faecalis* | *Str. faecalis* var. *liquefaciens* | *Str. faecalis* var. *zymogenes* | *Str. faecalis* var. *faecium* |
|---|---|---|---|---|---|---|
| Growth at pH 9·6 | − | d | + | + | + | + |
| Haemolysis | − | β or − | − | − | β | − |
| Arginine (hydrolysis) | − | + | + | + | + | + |
| Litmus milk | A | A | RAC | RAC | RAC | A |
| Mannitol (acid) | d | + | + | + | + | + |
| Sorbitol (acid) | − | − | + | + | + | − |
| Arabinose (acid) | d | − | − | − | − | + |
| Raffinose (acid) | + | − | − | − | − | − |
| Gelatin liquefaction | − | − | − | + | d | − |
| Tellurite (growth) | − | − | + | + | + | − |

Table 1*c*. *Stage* 2 *table for* Listeria, Corynebacterium *and* Kurthia

| | *L. monocytogenes* | *C. diphtheriae* var. *gravis* | *C. diphtheriae* var. *mitis* | *C. diphtheriae* var. *intermedius* | *C. ulcerans* | *C. xerosis* | *C. hofmannii* | *C. murium* | *C. renale* | *C. ovis* | *C. pyogenes** | *C. bovis* | *C. equi* | *C. haemolyticum* | *K. zopfii* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Catalase | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + |
| Motility | + | − | − | − | − | − | − | − | − | − | − | − | − | − | + |
| Nitrate reduction | − | + | + | + | − | + | + | + | d | − | − | − | + | − | − |
| Glucose (acid) | + | + | + | + | + | + | − | + | + | + | + | − | − | + | − |
| Starch (acid) | d | + | − | − | + | − | − | + | d | + | + | − | − | d | − |
| Sucrose (acid) | d | − | − | − | − | + | − | + | − | d | d | − | − | − | − |
| Dextrin (acid) | + | + | + | + | + | d | − | d | d | + | + | − | − | + | − |
| Maltose (acid) | + | + | + | + | d | + | − | + | d | + | + | − | − | + | − |
| Lactose (acid) | d | − | − | − | − | − | − | − | d | d | + | − | − | + | − |
| Trehalose (acid) | + | − | − | − | + | − | − | + | − | − | − | − | − | − | − |
| Gelatin liquefaction | − | − | − | − | + | − | − | − | − | − | + | − | − | − | − |
| Urease | − | − | − | − | + | − | + | d | d | + | − | − | + | − | − |
| Arginine hydrolysis | | − | − | − | − | − | − | − | + | + | + | − | − | | |
| Haemolysis on blood agar | d | − | + | − | + | − | − | − | d | + | + | − | − | + | − |

* Included here because of morphological similarity to corynebacteria.

In the same way the second stage of columns $c_1$, $c_2$ and $c_3$ is shown in Table 1 $c$, and in this table is included *Corynebacterium pyogenes* because of its morphological similarity to corynebacteria. It is also shown in Table 1 $b$, since Cummins & Harris (1956) found that this organism had the characters of a streptococcus.

The expansion of column $d$ (*Erysipelothrix*, *Lactobacillus* and *Actinomyces*) is

Table 1 $d$. *Stage 2 table for* Lactobacillus, Erysipelothrix *and* Actinomyces *spp.*

|  | *Thermobacterium* (Lactobacillus) | *Streptobacterium* (Lactobacillus) | *Betabacterium* (Lactobacillus) | *E. insidiosa* | *A. bovis* | *A. israelii* | *A. naeslundii* |
|---|---|---|---|---|---|---|---|
| Growth at 15° | − | + | d | − |  |  |  |
| Galactose (acid) | d | + |  | + | + | + |  |
| Lactose (acid) | d | d | d | + | + | + | d |
| Maltose (acid) | d | + | + | d | + | + | d |
| Mannitol (acid) | d | + | − | − | − | d | − |
| Raffinose (acid) | d | d | d | − | − | d | + |
| Salicin (acid) | d | + | d | − | − | d | d |
| Sucrose (acid) | d | d | d | − | + | + | + |
| Xylose (acid) | − | − | d | d | − | d | − |
| Starch hydrolysis | − | − | − | − | + | − | − |
| Nitrate reduction | − | − | − | − | − | + | + |
| Tomato juice favours growth | + | + | + | − | − | − | − |

Thermobacterium includes *L. acidophilus*  
  *L. bulgaricus*  
  *L. helveticus*  
  *L. leichmannii*  
  *L. lactis*

Streptobacterium includes *L. plantarum*  
  *L. casei*

Betabacterium includes *L. brevis*  
  *L. fermenti*

Table 1 $e$. *Stage 2 table for some* Bacillus *species*

|  | *B. megaterium* | *B. cereus* | *B. anthracis* | *B. licheniformis* | *B. subtilis* | *B. pumilus* | *B. polymyxa* | *B. stearo-thermophilus* |
|---|---|---|---|---|---|---|---|---|
| Morphological group | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 |
| VP | − | + | + | + | + | + | + | − |
| Mannitol | + | − | − | + | + | + | +* | − |
| Gelatin liquefaction | + | + | + | + | + | + | + | + |
| Starch hydrolysis | + | + | + | + | + | − | + | + |
| Nitrate reduction | − | + | + | + | + | − | + | + |
| Urease | + | d | − | d | d | − | − |  |
| Growth at 65° C. | − | − | − | − | − | − | − | + |
| Anaerobic growth in glucose broth | − | + | + | + | − | − | + | + |
| Motility | + | + | − | + | + | + | + | + |
| Citrate | + | + | + | + | + | + | − | − |

1 = Sporangia not swollen. 2 = Sporangia swollen by oval spores. * = Gas produced.

shown in Table 1 d. Ideas on the classification of lactobacilli are changing and Rogosa & Sharpe (1959) follow Orla-Jensen (1919) by dividing the genus *Lactobacillus* into three subgenera, *Thermobacterium*, *Streptobacterium* and *Betabacterium*. The genus has only limited interest for the medical bacteriologist and in Table 1 d we have not shown a further subdivision into species. Langford & Hansen (1954) surveyed the genus *Erysipelothrix* and concluded that only one species, *E. insidiosa*, was justified; we have accepted their findings and therefore do not show *E. rhusiopathiae* and *E. muriseptica*. The genus *Actinomyces* is restricted to the microaerophilic species and much of the data shown came from papers by Howell, Murphy, Paul & Stephan (1959) and Pine, Howell & Watson (1960).

Table 1 f.  *Stage 2 table for clostridia*

| | *C. oedematiens* | *C. welchii* | *C. septicum* | *C. histolyticum* | *C. sordellii* | *C. bifermentans* | *C. tetani* | *C. tetano- morphum* | *C. sporogenes* | *C. botulinum* * | *C. chauvoei* | *C. tertium* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Motility | + | − | + | + | + | + | + | + | + | + | + | + |
| Spore (terminal) | − | − | − | − | − | − | + | + | − | − | − | + |
| Glucose (acid) | + | + | + | − | + | + | − | + | + | + | + | + |
| Lactose (acid) | − | + | + | − | − | − | − | − | − | d | + | + |
| Sucrose (acid) | − | + | − | − | − | − | − | − | − | − | + | + |
| Salicin (acid) | − | − | + | − | − | + | − | − | − | d | − | + |
| Indole | − | − | − | − | + | + | + | d | − | − | − | − |
| Gelatin | + | + | + | + | + | + | + | − | + | d | + | − |
| Serum (digestion) | − | − | − | + | + | + | d | − | + | d | − | − |
| Nitrate reduction | − | + | + | − | − | − | − | − | − | − | + | + |
| Meat (digestion) | − | − | − | + | + | + | − | − | + | d | − | − |
| Iron milk | GC | AGC | d | DB | CDB | CDB | d | − | DB | d | AGC | AC |

* = Subdivided by other tests.
A = acid; G = gas; C = clot; D = digestion; B = black.

Table 1 g.  *Stage 2 table for* Mycobacterium *and* Nocardia

| | Warm-blooded tubercle bacilli | Saprophytic acid-fast bacilli | Anonymous acid-fast bacilli | *Nocardia* |
|---|---|---|---|---|
| Growth in 3 days | − | + | − | + |
| Growth at 22° C | − | + | + | + |
| Pigmentation | − | + | d | d |
| Catalase | d | + | + | + |
| Pathogenicity for guinea-pig | + | − | d | − |

In Table 1 e is shown the second stage for *Bacillus* species; many of the details are from the monograph by Smith, Gordon & Clark (1952) but we have not shown all the species recognized by those authors, neither have we made provision for non-motile or asporogenous variants. Although gelatin is liquefied by all the species shown in Table 1 e, it is included there because it is not liquefied by certain species (*B. coagulans* and *B. lentus*) not shown but which might, on occasion, be isolated as a contaminant in a medical laboratory. Smith, Gordon & Clark regard *B. mycoides* as a variety of *B. cereus*; we have not shown *B. mycoides* for, in the tests detailed in Table 1 e, it does not differ from *B. cereus*. Examination of a colony, however, would clearly show the only significant difference between them.

Similarly the difference between *B. subtilis* and *B. licheniformis* is best seen in the colony form, and these examples show that the tables should be used in conjunction with characters best seen at the bench but which are difficult or impossible (as the odour of staphylococci) to describe.

Table 1*gg*. *Stage 3 table for saprophytic mycobacteria and* Nocardia *spp.*

| | *Myco. phlei* | *Myco. smegmatis* | *Myco. fortuitum* | *Myco. rhodochrous* | *N. asteroides* | *N. brasiliensis* | *N. madurae* |
|---|---|---|---|---|---|---|---|
| Casein (decomposition) | − | − | − | − | − | + | + |
| Tyrosine (decomposition) | − | − | − | d | − | + | + |
| Xanthine (decomposition) | | | − | − | − | − | − |
| Starch hydrolysis | + | + | + | + | d | d | d |
| Growth at 52° C. | + | − | − | − | − | − | − |
| Growth at 50° C. | + | d | − | − | d | − | − |
| Growth at 45° C. | + | + | − | d | d | − | d |
| Growth at 40° C. | + | + | d | d | + | d | + |
| Growth at 10° C. | − | d | d | + | − | d | − |
| Survive 60° C.–4 hr. | + | − | − | d | | | |
| Survive 50° C.–8 hr. | | | | | + | − | d |
| Arabinose (acid) | + | + | − | − | − | − | + |
| Dulcitol (acid) | − | + | − | − | − | − | − |
| Sorbitol (acid) | + | + | − | + | − | − | − |
| Maltose (acid) | − | − | − | d | − | − | d |
| Mannitol (acid) | + | + | d | + | − | + | + |
| Inositol (acid) | − | + | − | − | − | + | d |
| Benzoate (utilization) | − | + | − | + | − | − | − |
| Citrate (utilization) | + | + | + | + | d | + | + |
| Mucate (utilization) | − | + | − | − | − | − | − |
| Succinate (utilization) | + | + | + | + | + | + | d |
| Urease | + | + | + | d | + | + | d |
| MacConkey (indicator change) | − | d | + | − | − | − | − |

Anaerobic spore-formers are distinguished in Table 1*f* which is based on data from Reed & Orr (1941), Memorandum (1943), Smith (1955), and Willis (1960). We have followed Brooks & Epps (1959) in keeping separate *Clostridium bifermentans* and *C. sordellii*, but unlike Moussa (1959) we have not combined *C. chauvoei* with *C. septicum*. Finer subdivisions could be made by compiling third-stage tables of the different toxins produced by *C. welchii*, *C. oedematiens* and *C. botulinum*.

*Mycobacterium* and *Nocardia* are considered briefly in Table 1*g*. The tubercle and anonymous bacilli are not dealt with further, as the methods used to distinguish the tubercle bacilli are not susceptible to representation in our type of table, and the information on the anonymous mycobacteria is insufficient to justify naming definite species.

Further details of the saprophytic acid-fast bacilli and *Nocardia* species are shown in Table 1*gg*, compiled mainly from Gordon & Mihm (1957, 1959, 1961), and R. E. Gordon (personal communication). According to Dr R. E. Gordon only three species of *Nocardia* have been adequately described, but three other sorts may later be found worthy of specific rank.

*Gram-negative bacteria*

The first stage in the identification of Gram-negative bacteria is shown in Table 2 (Fig. 2). Column *a* covers the Gram-negative cocci, *Neisseria* and the newly defined genus *Gemella* (Berger, 1960*a*, 1961). Because of its morphological similarity on first isolation to *Neisseria*, 'Bacterium anitratum' is included in Table 2*a*. *Diplococcus mucosus* (von Lingelsheim, 1906; Cowan, 1938) would be

Table-Fig. 2. *Stage 1 diagnostic table for Gram-negative organisms*

|  | *a* | *b* | *c* | *d* | *e₁* | *e₂* | *f₁* | *f₂* | *f₃* | *g₁* | *g₂* | *g₃* | *h* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  | *e* | | *f* | | | *g* | | | |
| Shape | S | R | R | R | R | R | R | R | R | R | R | R | R |
| Motility | − | + | − | − | − | + | + | + | − | − | − | + | − |
| Catalase | d | + | + | − | + | + | + | + | + | + | + | + | NT |
| Oxidase | d | − | − | − | + | + | + | − | − | − | + | + | d |
| Glucose F or O | F O or − | F | F | F | F | F | O | O | O | − | − | − | NT |

Neisseriaceae
Enterobacteriaceae
*Klebsiella*
*Shigella*
*Actinobacillus*
*Pasteurella*
*Aeromonas*
*Vibrio*
*Pseudomonas*
*Chromobacterium*
*Loefflerella*
*Brucella*
*Moraxella*
*Bordetella*
*Alcaligenes*
*Lophomonas*
*Haemophilus*
*Achromobacter*

+ = 100–80 % strains positive, d = 79–21 % strains positive, − = 20–0 % strains positive, S = sphere, R = rod, F = fermentation, O = oxidation, NT = Not testable in ordinary media.

included if cultures were available, but all those sent to us as *D. mucosus* became bacillary after further subculture, failed to reduce nitrates, were of low pathogenicity for mice and were identified as 'Bact. anitratum' which is shown on the line for *Achromobacter* in Table 2. Newly described species of *Neisseria*, *N. caviae* Pelczar (1953) and *N. animalis* Berger (1960*b*), are included, taking data from the papers of the original authors. The species *N. sicca*, *N. flava* and *N. perflava*, listed

by Branham & Pelczar in *Bergey's Manual* (1957, p. 483–5), are combined as *N. pharyngis* Wilson & Smith (1928).

The motile Gram-negative rods that are catalase-positive, oxidase-negative and

Table 2*a*. *Stage 2 table for* Neisseria, Gemella *and* 'Bacterium anitratum'

| | N. gonorrhoeae | N. meningitidis | N. catarrhalis | N. flavescens | N. pharyngis | N. animalis | N. caviae | 'Bacterium anitratum' | G. haemolysans |
|---|---|---|---|---|---|---|---|---|---|
| Nutrient agar (growth) | − | − | + | + | + | + | + | + | + |
| Pigment | − | − | − | + | + | − | + | − | − |
| Growth at 22° C. | − | − | + | + | + | + | + | + | + |
| Glucose (acid) | + | + | − | − | + | + | − | + | + |
| Maltose (acid) | − | + | − | − | + | + | − | − | + |
| Sucrose (acid) | − | − | − | − | + | + | − | − | + |
| Fructose (acid) | − | − | − | − | + | + | − | − | + |
| Haemolysis | − | − | − | − | − | − | d | − | + |
| Catalase | + | + | + | + | + | + | + | + | − |
| Oxidase | + | + | + | + | + | + | + | − | − |

Table 2*b*. *Stage 2 table for motile Enterobacteriaceae and similar organisms*

| | Salmonella typhi | Salm. arizonae | Other Salmonella | Escherichia coli | Citrobacter freundii | Ballerup-Bethesda | Enterobacter cloacae | E. aerogenes | Hafnia alvei | Proteus vulgaris | P. mirabilis | P. morganii | P. retgeri | Providence | Serratia marcescens | P. pseudotuberculosis | C. violaceum | C. lividum* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| lcose (gas) | − | + | + | + | + | + | + | + | + | + | + | + | d | + | d | − | − | − |
| nnitol (acid) | + | + | + | + | + | + | + | + | + | − | − | − | + | − | + | + | − | − |
| lcitol (acid) | d | − | + | d | d | d | d | d | − | − | − | − | − | − | − | − | − | − |
| tose (acid) | − | (+) | − | + | + | d | + | + | − | − | − | − | − | − | d | − | − | − |
| rose (acid) | − | − | − | d | d | d | + | + | d | + | + | − | d | (+) | + | − | d | − |
| ole | − | − | − | + | − | − | − | − | − | + | − | + | + | + | − | − | − | − |
| atin liquefaction | − | (+) | − | − | − | − | + | d | − | + | + | − | − | − | + | − | + | − |
| s | + | + | + | − | + | + | + | + | + | + | + | + | − | − | − | − | − | − |
| | − | − | − | − | − | − | + | + | + | − | d | − | − | − | + | − | − | − |
| ase | − | − | − | − | d | d | − | − | − | + | + | + | + | − | − | + | − | − |
| ine decarboxylase | + | + | + | d | − | − | − | + | + | − | − | − | − | − | + | − | − | − |
| ginine decarb- ylase | − | + | + | d | + | + | + | − | − | − | − | − | − | − | − | − | + | |
| ithine decarb- ylase | − | + | + | d | d | d | + | + | + | − | + | + | − | − | + | − | − | − |
| tamic acid carboxylase | − | − | − | + | − | − | − | − | − | + | + | + | + | + | | | | |
| N | − | − | − | − | + | + | + | + | + | + | + | + | + | + | + | − | + | |
| ser's citrate | − | + | + | − | + | + | + | + | + | d | − | − | + | d | + | − | (+) | + |
| conate | − | − | − | − | − | − | + | + | + | − | d | − | − | − | + | − | − | d |
| lonate | − | + | − | − | − | − | d | + | + | − | − | − | − | − | d | − | d | − |
| nylalanine | − | − | − | − | − | − | − | − | − | + | + | + | + | + | − | − | − | − |
| ment | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | + | + |

' Included only for comparison. Its attack on carbohydrates is oxidative.

+ = positive within 48 hr. except for gelatin where + = positive within 7 days at 22° C., (+) = delayed
itive; d = some strains + or (+), others −.

ferment carbohydrates are some Enterobacteriaceae, *Pasteurella pseudotuberculosis* (motile at 22° C. but not at 37° C.) and *Chromobacterium violaceum*. This purple-pigmented organism may be confused with *C. lividum*, which oxidizes carbohydrates, and does not grow at 37° C. *C. lividum* properly belongs in column $f_2$ (see Table 2f) but it is shown in Table 2b for comparison with *C. violaceum*. Some

Table 2c. *Stage 2 table for* Shigella, Klebsiella, Pasteurella, Actinobacillus lignieresi, *and* Achromobacter equuli

| | S. dysenteriae* | S. flexneri and boydii | S. sonnei | A–D group | K. aerogenes | K. pneumoniae | K. edwardsii var. edwardsii | K. edwardsii var. atlantae | K. rhinoscleromatis | K. ozaenae | P. septica | P. pestis | P. pseudotuberculosis | Actino. lignieresi | Achromo. equuli |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Glucose (gas) | − | | | | + | + | − | + | − | d | | | | | − |
| Mannitol (acid) | − | + | + | + | + | + | + | + | + | + | d | + | + | + | + |
| Dulcitol (acid) | − | − | − | d | d | + | − | − | − | − | d | − | − | − | |
| Lactose (acid) | − | − | (+) | d | + | + | (+) | (+) | − | (+) | − | − | − | d | |
| Sucrose (acid) | − | − | (+) | d | + | + | + | + | + | d | (+) | − | − | + | |
| Indole | d | d | − | + | − | − | − | − | − | − | + | − | − | − | − |
| Gelatin liquefaction | − | − | − | − | − | − | − | − | − | − | − | − | − | − | |
| H₂S | − | − | − | − | − | − | − | − | − | − | d | d | − | + | − |
| MR | + | + | + | + | − | + | d | + | + | + | − | + | + | − | − |
| VP | − | − | − | − | + | − | + | d | + | − | − | − | − | d | − |
| Urease | − | − | − | − | + | + | + | + | − | d | − | − | + | d | |
| Lysine decarboxylase | − | − | − | d | + | + | + | + | − | d | − | − | − | − | |
| Arginine decarboxylase | − | − | d | d | − | − | − | − | − | − | − | − | − | − | |
| Ornithine decarboxylase | − | − | + | d | − | − | − | − | − | − | + | − | − | − | |
| Glutamic acid decarboxylase | − | − | + | + | − | | | | − | − | | | | | |
| KCN | − | − | − | − | + | − | + | + | + | + | + | − | − | − | |
| Gluconate | − | − | − | − | + | + | + | d | − | − | | | | | − |
| Malonate | − | − | − | − | + | + | d | − | + | − | | | | | − |
| Fimbriae | − | d | − | + | + | + | − | − | − | − | | | | | |
| MacConkey (growth) | + | + | + | + | + | + | + | + | + | + | − | + | + | + | d |
| Citrate | − | − | − | − | + | + | d | + | − | d | − | − | − | − | − |

\* Except serotype 1 (Shiga's bacillus).

Table 2d. *Stage 2 table for* Shigella dysenteriae *and* Actinobacillus lignieresi

| | S. dysenteriae serotype 1 | A. lignieresi | | S. dysenteriae serotype 1 | A. lignieresi |
|---|---|---|---|---|---|
| Glucose (gas) | − | − | Lysine decarboxylase | − | − |
| Mannitol (acid) | − | + | Arginine decarboxylase | − | − |
| Dulcitol (acid) | − | − | Ornithine decarboxylase | − | − |
| Lactose (acid) | − | d | Glutamic acid | − | |
| Sucrose (acid) | − | + |   decarboxylase | | |
| Indole | − | − | KCN | − | − |
| Gelatin liquefaction | − | − | Gluconate | − | − |
| H₂S | − | + | Malonate | − | − |
| MR | + | − | MacConkey (growth) | + | + |
| VP | − | d | Citrate | − | − |
| Urease | − | d | Nitrate reduction | + | + |

data for the Enterobacteriaceae are taken from Edwards & Ewing (1955); Ewing, Davis & Edwards (1960); Ewing, Davis & Reavis (1959) and Kauffmann (1954); for the chromobacteria from Sneath (1960).

Non-motile Enterobacteriaceae (*Klebsiella* and *Shigella* spp.) and *Pasteurella* spp. are shown with *Actinobacillus lignieresi* and *Achromobacter equuli* (*Actinobacillus equuli* in *Bergey's Manual*, 1957, p. 415) in Table 2c. 'Bacterium anitratum' is sometimes confused with these organisms and provisionally diagnosed as a shigella or a 'paracolon', but it is clearly distinguished by its inability to reduce nitrate and its oxidative attack on glucose. Decarboxylase reactions for *Shigella* were taken from Ewing *et al.* (1960); the classification and reactions of the genus *Klebsiella* are from Cowan, Steel, Shaw & Duguid (1960), with a minor change in the characters of *K. ozaenae*.

Table 2e. *Stage 2 table for* Aeromonas, Vibrio, Pasteurella, *and* Achromobacter

| | Aero. liquefaciens | Aero. hydrophila | Aero. salmonicida | V. cholerae | P. septica | P. haemolytica var. haemolytica | P. haemolytica var. ureae | Achromo. equuli |
|---|---|---|---|---|---|---|---|---|
| Motility | + | + | − | + | − | − | − | − |
| Glucose (gas) | + | + | + | − | − | − | − | − |
| Lactose (acid) | + | − | − | + | − | d | − | + |
| Sucrose (acid) | + | + | − | + | + | + | + | + |
| Xylose (acid) | − | + | d | − | d | + | − | + |
| Sorbitol (acid) | + | − | − | − | d | d | + | − |
| Glycerol (acid) | + | + | − | d | − | d | − | − |
| H₂S | + | + | − | + | d | d | − | − |
| VP | + | + | − | d | − | − | − | − |
| Growth at 4° C. | + | + | + | − | − | − | − | − |
| Lysine decarboxylase | − | − | − | + | − | | | − |
| Arginine decarboxylase | + | + | −* | − | − | | | − |
| Ornithine decarboxylase | −* | − | +* | + | + | | | − |
| Indole | d* | + | −* | + | + | − | − | − |
| Citrate | +* | d* | +* | − | − | − | − | − |
| Malonate | −* | d* | d* | − | − | − | − | − |
| Aesculin | −* | +* | +* | | − | | | |
| Haemolysis | + | + | + | d | − | + | + | − |
| Needs serum | − | − | − | − | − | + | + | − |
| MacConkey (growth) | + | + | + | + | − | d | − | d |
| Urease | − | − | − | − | − | − | + | + |
| Gluconate | + | + | − | − | − | − | − | − |

\* = results from Lysenko (1961).

Shiga's bacillus (*Shigella dysenteriae* serotype 1) differs from other *Shigella* spp. in being catalase-negative and appears in Table 2d. *Actinobacillus lignieresi* varies in its catalase production (Phillips, 1960) and is shown in Tables 2c and 2d.

Table 2e combines columns $e_1$ and $e_2$ of Table 2 and shows the distinguishing characters of *Aeromonas* spp., *Vibrio cholerae*, *Pasteurella septica*, *P. haemolytica* and, because some strains are weakly oxidase-positive, *Achromobacter equuli* (Steel, 1961). Our experience of *Aeromonas* spp. is limited and we have used Lysenko's (1961) data to supplement our observations. *Pasteurella septica* appears in

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Tables 2c and 2e because different strains produce different oxidase reactions. Generally the oxidase test divides bacteria at the generic level and it is possible that further investigation of *P. septica* (which is a gathering together of many so-called species) may suggest that division is desirable. *P. haemolytica* has been divided into two varieties (Henriksen & Jyssum, 1960), both of which are shown in Table 2e.

Table 2f. *Stage 2 table for* Pseudomonas, Loefflerella, Chromobacterium, Pasteurella pestis, Brucella neotomae *and* Achromobacter anitratus

| | *Pseudo. aeruginosa* | *Pseudo. fluorescens* | *L. pseudomallei* | *C. lividum* | *L. mallei* | *B. neotomae* | *Pasteurella pestis* | *A. anitratus* |
|---|---|---|---|---|---|---|---|---|
| Motility | + | + | + | + | − | − | − | − |
| Oxidase | + | + | + | − | − | − | − | − |
| Pigment | + | + | d | + | − | − | − | − |
| Diffusion of pigment | + | + | − | − | − | − | − | − |
| MacConkey (growth) | + | + | + | − | − | − | + | + |
| Growth at 37° C. | + | + | + | − | + | + | + | + |
| Nitrate reduction | + | d | + | + | + | + | + | − |
| Gelatin | + | d | + | − | − | − | − | d |
| Urease | + | d | d | − | + | + | − | + |
| H₂S | − | − | − | − | d | + | d | − |
| Citrate | + | + | + | + | + | − | − | + |
| Gluconate | + | + | − | d | − | − | − | − |
| Malonate | d | + | d | − | − | − | − | d |
| Starch hydrolysis | − | − | + | − | − | − | − | − |
| Mannitol (acid) | d | d | + | − | − | − | + | − |
| Salicin (acid) | − | − | d | − | − | − | + | − |
| Arabinose (acid) | +* | −* | d | | − | + | + | + |
| Xylose (acid) | +* | −* | + | | − | − | + | + |
| Glycerol (acid) | +* | −* | + | − | − | − | d | − |

* = results from Lysenko (1961).

Table 2g. *Stage 2 table for* Alcaligenes, Bordetella, Brucella *and* Moraxella

| | *A. faecalis* | *Lophomonas alcaligenes* | *Bordetella bronchiseptica* | *B. parapertussis* | *Moraxella lwoffi* | *Brucella* spp. | *Bordetella pertussis* | *Moraxella* spp. |
|---|---|---|---|---|---|---|---|---|
| Motility | + | + | + | − | − | − | − | − |
| Oxidase | + | + | + | − | − | d | + | + |
| Nitrate reduction | d | + | + | − | − | + | − | d |
| Urease | − | − | + | + | − | + | − | − |
| MacConkey (growth) | + | + | + | − | + | d | − | − |
| H₂S | d | + | d | − | − | d | − | − |
| Gelatin | d | − | − | − | − | − | − | d |
| Citrate | d | d | d | − | + | − | − | − |
| Serum agar (growth) | + | + | + | + | + | + | − | + |
| Polar flagella | − | + | − | − | − | | | |
| Glycerol oxidized | − | + | − | | | | | |
| KCN | + | − | + | − | − | | − | |
| Bordet–Gengou (growth) | + | | + | + | | | + | |

The oxidizing Gram-negative rods (columns $f_1$, $f_2$ and $f_3$ of Table 2) are combined in Table 2*f*. *Pseudomonas aeruginosa* (*P. pyocyanea*) and *P. fluorescens* are the only two species of *Pseudomonas* of medical significance; a wider survey of this genus is contained in the monograph by Lysenko (1961), from which some of our data are taken. Whitmore's bacillus (*Loefflerella pseudomallei*) has many characters of *Pseudomonas* (Brindle & Cowan, 1951) and is included in that genus by Haynes in *Bergey's Manual* (1957, p. 100) and by Lysenko (1961). In *Bergey's Manual* (p. 417) the glanders bacillus is included by Haupt in the genus *Actinobacillus*, but as *A. lignieresi* attacks glucose by fermentation and the glanders bacillus attacks it by oxidation, a fundamental distinction, we feel that this is a misguided move. *Pasteurella pestis* occupies a peculiar position in that the Hugh & Leifson (1953) test shows oxidation of glucose in the early stages of growth and fermentation in the later stages; we have therefore included it in both Tables 2*c* and 2*f*.

Table 2*h*. *Stage 2 table for* Bordetella pertussis *and* Haemophilus *spp.*

|  | *B. pertussis* | *H. influenzae* | *H. suis* | *H. canis* | *H. parainfluenzae* |
|---|---|---|---|---|---|
| Bordet–Gengou medium (growth) | + | − | − | − | − |
| Fildes digest agar (growth) | − | + | + | + | + |
| Requires X factor | − | + | + | + | − |
| Requires V factor | − | + | + | − | + |
| Indole | − | + | − | + | d |
| Nitrate reduction | − | + | + | + | + |

'Bacterium anitratum' appears in Table 2*f* (under the name *Achromobacter anitratus*) because it oxidizes glucose, and in Table 2*a* because, on first isolation, its morphology may suggest that it is a coccus. *Chromobacterium lividum*, which produces a purple pigment, is correctly placed in Table 2*f*; it appears in Table 2*b* purely for comparison with *C. violaceum*. *Brucella neotomae* Stoenner & Lackman (1957) is not a typical *Brucella*, as it oxidizes glucose sufficiently strongly to show acid production in serum water sugars. The other *Brucella* species are combined and shown in Table 2*g*; we have shown them as failing to produce acid from glucose and other carbohydrates but we are aware that by special methods acid production can be shown (Pickett & Nelson, 1955).

As Henriksen (1960) would exclude all oxidase-negative organisms from the genus, *Moraxella lwoffii* is shown separately from other *Moraxella* spp. *Alcaligenes faecalis* is not homogeneous and differs from *Bordetella bronchiseptica* in urease activity. *B. parapertussis* differs by the oxidase reaction from other *Bordetella* species (Lautrop, 1960). *Lophomonas* Galarneault & Leifson (1956) is the genus created for the polarly flagellated rods that fail to attack carbohydrates.

*Haemophilus* spp. have exacting nutritional requirements and do not grow on the media used in the biochemical tests shown in our tables; a special Table 2*h* has been made using features such as X and V requirements, indole production and nitrate reduction.

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## DISCUSSION

Intelligent use of the tables demands technical skill and sensitive but specific methods for the individual tests. As in all determinative bacteriology, a true identification can only be based on careful work; the tables will not help the man who is in too much of a hurry to carry out all the tests required. We have not stipulated particular methods for individual tests because our tables have been constructed from a variety of sources. We have relied mainly on our own results but, particularly where we have examined relatively few strains, we have not hesitated to include the results of others who have examined much larger series, e.g. we had only five strains of *Actinobacillus lignieresi* but Phillips (1960) examined 225 strains and we have drawn on his findings. Other sources on which we have drawn freely are *Topley and Wilson's Principles* (1955) and *Bergey's Manual of Determinative Bacteriology* (1957), and on various monographs by specialist workers. By doing so we hope we have increased the value of the tables, but in a few instances we have found that the experts do not agree; we have then used our own experience and judgement.

The generic and specific names are those we normally use in our work in the National Collection of Type Cultures and they are not always those found in standard texts or *Bergey's Manual*. In particular we differ in regarding *Achromobacter* as a convenient place in which to put Gram-negative rods of uncertain taxonomic position; we do not, therefore, attempt to define the genus or recognize a type species because a dump-heap cannot have a type and we hope that the species we include will soon be found more permanent homes in better established genera.

The main tables for Gram-positive and Gram-negative bacteria give, with the minimum of information, the chief distinguishing features of the bacteria commonly met in medical bacteriology. It will be seen that the genus of many bacteria can be determined by using one table, species by two tables, and that three stages are needed mainly for subspecific identification.

Attempts to cover all possible characters in a given genus have not been made. Thus, indole-producing and gelatin-liquefying strains of *Salmonella* are known but these characters have been recorded as negative in Table 2*b*. Kauffmann (1960) has reported fifty-two *Salmonella* serotypes as malonate-positive, but apart from the Arizona strains these are a minority of the total number of serotypes in the genus, which has therefore been tabulated as malonate-negative.

Characters chosen for differentiation are mainly those applicable in any routine bacteriological laboratory. Exceptions are fimbriation (Duguid, Smith, Dempster & Edmunds, 1955), which is of value in the genus *Klebsiella* (Cowan *et al.* 1960), and mode of flagellation, which aids the differentiation of *Alcaligenes* and *Lophomonas*.

Three points need to be emphasized about the diagnostic tables: (i) they cannot be considered in isolation, they must be taken in conjunction with other evidence, such as colony form, that cannot be included in the tables. (ii) The tables do not characterize an organism, all they do is to focus attention on the tests most

valuable for differentiation. (iii) The tables do not form part of any classification but they may draw attention to similarities and relations that are not otherwise apparent.

## SUMMARY

A simple device by which the characters of an unknown organism can be compared with those of named genera and species is described. The comparison is made by using 'diagnostic tables' of characters found to have differential value.

Separate tables for Gram-positive and Gram-negative bacteria were made; the first stage indicated the probable genus into which the unknown fell and a second (and occasionally a third) stage was needed to identify the species or subspecies.

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## EXPLANATION OF PLATE

Fig. 1. Determinator, showing Perspex sheet on which characters of unknowns are marked in grease pencil, and a simple diagnostic table of IMViC reactions.

Fig. 2. Determinator in use in which the characters of one unknown are isolated by a mask.

Fig. 3. Simple type of Determinator on which the characters of one unknown strain have been marked.
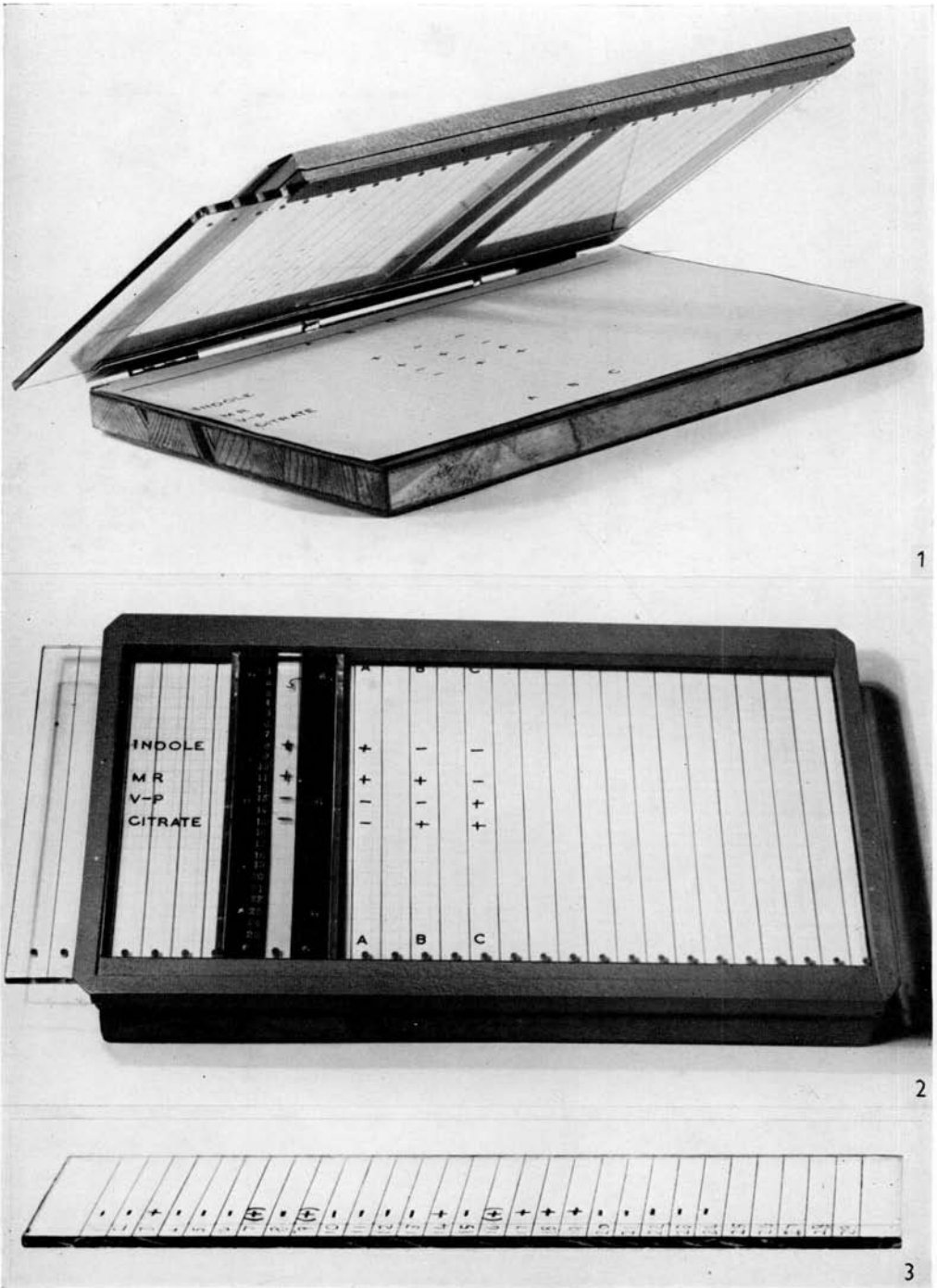
S. T. COWAN AND K. J. STEEL
*(Facing p. 372)*