Simple Method for Cross-indexing a Reference File

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In attempting to keep a reference file of current research one soons finds oneself in the perplexing situation of having several hundred cards on hand without an adequate cross-indexing system in operation. If, during the early stages of collecting reference material, one utilizes a multiple-card system, *i.e.* a separate card for each subject under which one particular reference can be filed, the number of cards needed soon makes space a limiting factor.

The ideal system, of course, is that in which only one card per reference is employed and which permits that reference to be selected from the file under any of several different subject headings. This ideal has been achieved in the punch-card system. However, to the average scientific worker this system presents two serious disadvantages: (1) Since special cards are used, the initial cost is fairly high; (2) having obtained these cards, transfer of the references from the original cards to the new ones necessitates an enormous amount of secretarial work.

The present communication describes a method for cross-indexing a file which is already in existence. The chief aims have been to obviate extra secretarial work and to achieve some advantages of the punch-card system.

In essence, the operation for cross-indexing involves notching the reference card along its top or side edges at positions corresponding to each of several different subject headings indicated on a master card.

Compilation of the master card is the most important task, since its design determines the ultimate efficiency of the system. Several features of the design deserve consideration: (1) One must decide at the outset which subject headings are likely to be most useful; the indexing system will do anything required of it, provided the subject headings are properly chosen. (2) The various headings are then divided into two classes, the *general* and the *specific*; *i.e.* for the general heading *Metabolism* there may be the specific headings *carbohydrate, fat, nitrogen, phosphate*, etc. (3) The importance of arranging these headings along the edges of the master card becomes apparent when one realizes that only the top edges of the cards are immediately visible in the file drawer. Since a reference is more commonly sought under a specific rather than a general subject heading, the former are made more readily available by arranging them along the top edge of the master card.

The master card itself is made from an ordinary blank card of the size used in the file, holes 2 mm. in diameter and about 2 mm. apart being punched along each of its top and two side edges. Subject headings are then listed opposite the holes.

In the author's file, general headings such as *Biochemistry, Metabolism, Enzymology, Pharmacodynamics*, are listed along the left-hand margin and specific headings along the top edge. Since it is also useful to know the tissue or organism used in a physiological study, the right-hand margin contains such specific headings as *amphibia, yeast, liver, brain*, etc.

To index a reference, the master card is superimposed over the card containing the reference, pencil marks being made on the latter through the holes in the master card opposite the appropriate headings. The reference card is then notched by punching semicircles opposite the marks. If the master card has been properly designed, each card should have at least one notch on each of its two side edges. (There may not be a notch along the top edge if the subject is of a general nature.)

The notched cards are finally filed alphabetically, by authors, under sections corresponding to the general headings listed on the left-hand margin of the master card.

The availability of data under this system is readily apparent. General information is obtainable at once, since the file is arranged in general sections. As each card in the general section has been punched in the same position along its left-hand margin, the section as a whole now exhibits a groove, along its side. Any card out of place shows up as a break in the groove.

To find a specific reference in a general section, the cards are first brought into alignment by moving them against one side of the drawer. The master card is then held in position in front of the section, or moved horizontally over the section if it is a long one, and the desired cards selected. Subjects listed along the right-hand margin are discovered by removing the section in whole or in part and bringing the cards into alignment both side and bottom; the master card is then used in the same manner to find a specific reference.

By this method it is possible to select one or two specific references out of several hundred cards in a matter of seconds. Furthermore, within a given section (e.g. *Metabolism*) all the references relating to one specific heading (e.g. *phosphate*) may be selected, or within the same section all the references pertaining to a specific tissue may be found with equal ease.

A Culture Medium for the Primary Isolation of Fungi

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Although Sabouraud's dextrose agar is widely employed for the cultivation of fungi, it has many shortcomings, particularly when used for the primary isolation of pathogenic fungi from material containing a mixed flora including bacteria, yeasts, actinomycetes, or saprophytic fungi. Gram-positive and gram-negative bacteria grow luxuriantly and rapidly on the medium, making it difficult to isolate pathogenic fungi from specimens such as feces, sputum, or exudates. Many clinical specimens must be treated with 70 per cent alcohol or other agents to destroy surface bacteria if dermatophytes are to be successfully isolated on Sabouraud agar. The acid reaction of the medium may prevent or retard the development of those pathogenic fungi for which the optimum pH is in the neutral or alkaline range. Furthermore, the utility of Sabouraud agar is seriously hindered by the rapid spreading growth of saprophytic fungi, which are invariably present in pathological specimens and in laboratory air. Since most pathogenic fungi grow more slowly than saprophytic species, they are isolated with difficulty in many cases or not at all in others. A new medium for the primary isolation of fungi was therefore thought to be very much needed. Studies were commenced along three separate lines of endeavor with that specific aim in mind.

The problem of restricting the spreading of fungi on agar media was first examined. After many different formulas had been tested, the following was found to support growth of 34 strains of pathogenic and saprophytic fungi in our collection as small, but well myceliated, discrete, *nonspreading* colonies at a rate comparable to that of Sabouraud agar: 1 per cent dextrose, 1 per cent granular peptone, 1.5 per cent dehydrated oxgall, and 2 per cent agar in water (no pH adjustment required).[1]

It was at first erroneously believed that oxgall in the concentration used above would inhibit gram-positive bacteria, particularly staphylococci. When this was proven not to be the case, other antibacterial agents known to be active against gram-positive bacteria were studied with a view to their incorporation into the medium. After a number of trials with different compounds, crystal violet[2] in final dilution of 1/100,000 was found to inhibit staphylococci and other gram-positive bacteria but not to hinder the growth of 34 known species of saprophytic and pathogenic molds, yeasts, and actinomycetes, as well as numerous strains of fungi isolated from laboratory air.

TABLE 1

FUNGI CAPABLE OF GROWING ON OXGALL-CRYSTAL VIOLET AGAR CONTAINING 30 UNITS OF STREPTOMYCIN/CC.

Pathogenic species	
*Blastomyces dermatitidis**	*Microsporum audouini*
" *brasiliensis*	" *canis*
Coccidioides immitis	" *gypseum*
Histoplasma capsulatum	*Trichophyton Schoenleinii*
Sporotrichum schenkii	" *violaceum*
Hormodendrum Pedrosoi	" *rubrum*
" *compactum*	" *mentagrophytes*
Phialophora verrucosa	" *sulfureum*
Cryptococcus neoformans	*Epidermophyton floccosum*
Candida albicans	*Monosporium apiospermum*
Torula (Elton)†	*Geotrichum* sp.
Nonpathogenic species	
Rhizopus nigricans (+)	*Candida candida*
" " (−)	*Fusarium*
Aspergillus herbariorum	*Alternaria*
Penicillium expansum	*Cladosporium*
" *notatum*	*Mucor mucedo*
Neurospora sitophila	*Scopulariopsis brevicaulis*

* Two strains.

† Isolated from a fatal case of meningitis.

Many chemical agents known to exert selective bacteriostatic activity versus gram-negative bacteria were investigated for addition to the oxgall-crystal violet agar; these included brilliant green, sodium azide and potassium tellurite. In no instance was it found possible to inhibit simultaneously all members of the Enterobacteriaceae family and the *Pseudomonas* genus without retarding or inhibiting some species of pathogenic fungi. Because of the wide range of activity of streptomycin against gram-negative bacteria and an observed high tolerance of one pathogenic fungus, *Blastomyces dermatitidis*, to streptomycin (*1*), this antibiotic was selected for trial. The hope that other species of fungi would show similar tolerance to streptomycin was fulfilled.

Oxgall-crystal violet agar was sterilized at 10–12 pounds pressure for 15 minutes[3] (115–117.7° C.) and then cooled to approximately 46°C. Sufficient streptomycin sulfate[4] in 5 cc. of sterile saline was then added and well mixed, immediately prior to pouring, to provide a final concentration of 30 units of the antibiotic/cc. of agar. All fungi in our collection were unaffected by the concentration of streptomycin used and grew unrestrictedly, with the exception of *Nocardia asteroides* (Table 1). Numerous laboratory aerial fungi, developing at approximately the same rate of growth in the streptomycin-containing oxgall-crystal violet agar as in Sabouraud agar, grew as small, well-isolated, *nonspreading* colonies (Fig. 1).

The degree of inactivation of streptomycin by brief exposure to 46°C. was not measured, but this was assumed to be negligible, since solutions of streptomycin can be heated to 100°C. for 10 minutes with loss of less than 50 per cent (*2*). In order to ascertain the stability of the antibiotic in the medium, streptomycin-containing oxgall-crystal violet agar was incubated at room temperature and 37°C. for varying periods and tested daily with pure cultures of *Escherichia coli* and fecal suspensions. Not only freshly prepared medium but also plates of medium incubated beforehand at both temperatures for a period of several weeks showed continued and undiminished, selective, inhibitory activity toward *E. coli* as well as most of the gram-negative bacteria present in normal and pathological feces. This attested to considerable stability of streptomycin in the poured medium.

A medium containing dextrose, peptone, oxgall, agar, and 30 units of streptomycin/cc. (excluding crystal violet) was streaked heavily with 10 different specimens of sputum. Since it was observed that a considerable number of gram-positive bacteria were able to form colonies on the medium, the presence of crystal violet as well as streptomycin was considered necessary in the formula.

The completed medium, containing, in distilled water, 1 per cent dextrose, 1 per cent peptone, 1.5 per cent oxgall, 2 per cent agar, 1/100,000 crystal violet, and 30 units of streptomycin/cc. of agar was tested in parallel with Sabouraud agar by heavy inoculation with specimens of feces and sputum obtained from the Clinical Diagnostic Laboratory, Charity Hospital, New Orleans. Approximately three times as many colonies of fungi and twice as many different fungal species could be recovered in pure culture from the new medium as from Sabouraud agar. Many specimens producing only a heavy bacterial overgrowth on Sabouraud agar developed 30–100 well-isolated mold and yeast colonies on the new medium (Fig. 2).

When used together in oxgall agar, crystal violet and streptomycin inhibited the growth of both gram-positive and gram-negative bacteria from heavy inocula of feces, sputum, and other grossly contaminated specimens. Molds and yeasts were permitted to grow as nonspreading, well-separated colonies which were easy to isolate in pure culture.

[1] All elements of this formula are Bacto grade, Difco Laboratories, Detroit, Michigan.

[2] Crystal violet (indicator): dye content, 91 per cent; National Aniline Division, 40 Rector Street, New York City. *Stock solution:* 1.25 grams dissolved in 25 cc. of 95 per cent ethyl alcohol and kept in a tightly stoppered bottle. *For use:* 0.2 cc. added/liter of medium.

[3] Heating in excess of this pressure and temperature may cause the formation of an insoluble precipitate on the surface of the agar due to heat instability of the oxgall.

[4] Two commercial brands found satisfactory.

Present studies indicate that the new medium holds considerable promise as a diagnostic tool for the primary isolation of fungi from specimens possessing a mixed bacterial and fungal flora. It might also be employed (1) for an easier estimation of the *normal* fungal flora of feces, sputum, and other human discharges; (2) for a more accurate evaluation of human disorders of the upper and lower respiratory and gastrointestinal tracts caused by fungi; (3) as a simpler method of single-cell isolation of fungi; (4) for an accurate quantitative estimation of viable saprophytic fungi in foodstuffs by plating techniques; (5) for an easier estimation of the fungal flora and content of the air; and (6) as a more rapid and proficient method for the examination of feces and sputum in incipient infections of the civil population with fungal agents of disease. These possible applications will, of course, require confirmation by experimentation.

Addendum: It is our practice to distribute the medium in 500-cc. quantities in 1-l. flasks, sterilize, and store in the refrigerator until needed, at which time the agar is remelted and cooled to approximately 46°C. Streptomycin in sterile saline is then added and mixed thoroughly, and the agar is poured, 27–30 cc./plate. Plates of agar are left at room temperature 6–8 hours and then stored in the refrigerator. The poured agar appears transparent and light blue. In inoculating the medium, a generous quantity of sputum or fecal suspension in saline is vigorously spread over the surface of the agar using a sterile swab. Streaking by means of a wire loop is not required. Skin and nail scrapings and infected hairs are planted directly on the surface of the agar without preliminary treatment. Plates are incubated at room temperature, preferably at 30°C., but not at 37°C.

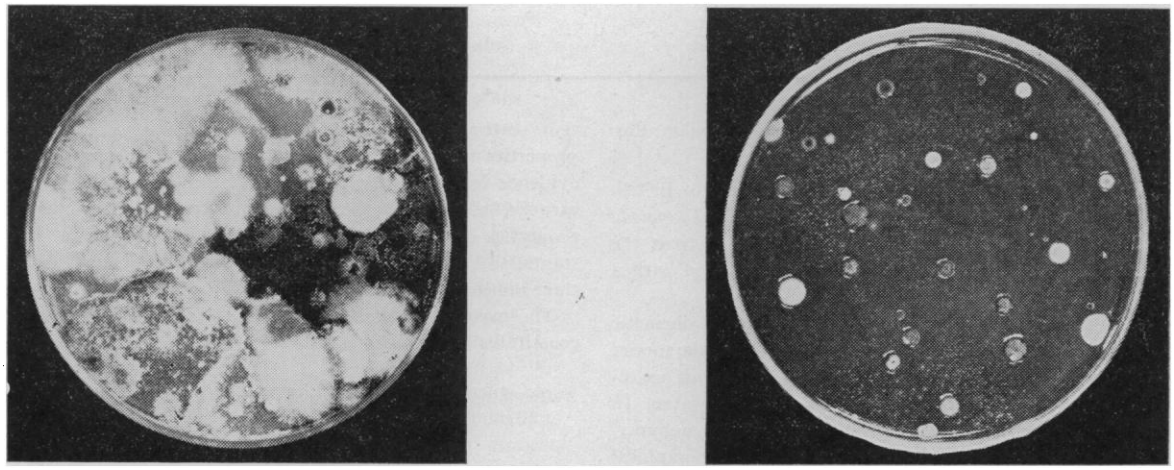

FIG. 1. Comparative appearance of media exposed to air for 4 hours, incubated 4 days at 30° C. *Left:* Sabouraud agar—marked spreading activity of aerial saprophytic fungi. *Right:* New medium—spreading activity reduced; note discrete, small colonies of fungi.

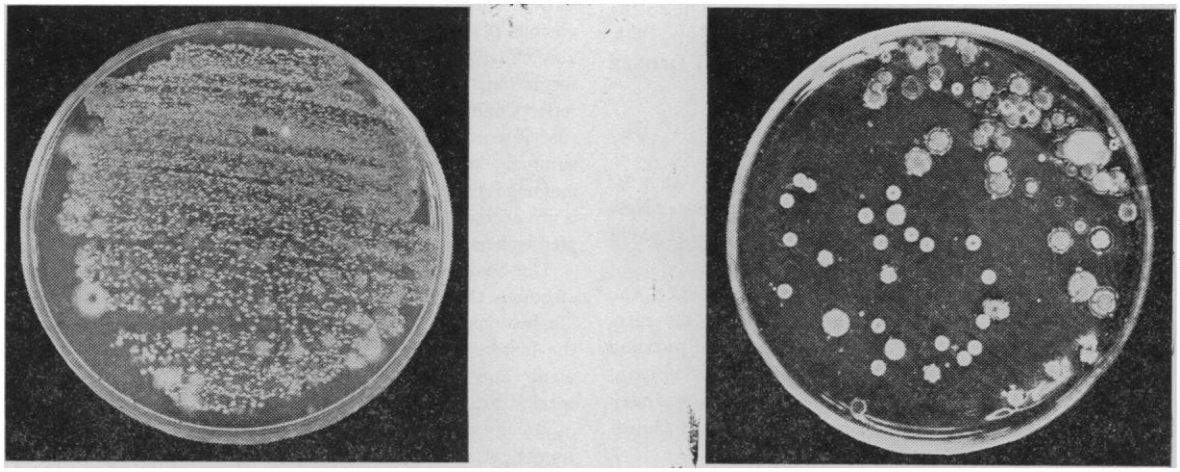

FIG. 2. Comparative appearance of media inoculated with heavy fecal suspension, incubated 4 days at 30° C. *Left:* Sabouraud agar—abundant bacterial growth which hindered fungal development. *Right:* New medium—minimal bacterial growth; note numerous, discrete, small colonies of fungi.

References

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