Public Health and Clinical Laboratories *in the* Diagnosis of Enteric Bacterial Infections

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Remarkable progress has been made in the prevention and treatment of many infectious diseases, but very few, if any, have been completely eradicated. The classical acute bacterial infections of the intestinal tract still occur with sufficient frequency to be of concern to the physician in practice and in public health work, and the syndrome of acute infectious diarrhea of the newborn from time to time complicates the management of hospital nurseries. Although in the past 20 years reported cases of typhoid and paratyphoid fever in this country have decreased from 23,000 to approximately 4,100 per year (*1*), the annual incidence of reported bacillary dysentery in the past 10 years has increased from 19,000 to approximately 28,000. These figures do not include the instances of diarrhea of unspecified cause nor those cases of specific infection which are not on record because they were not reported.

This discussion is concerned largely with the role of the laboratory in the diagnosis of the *Salmonella* and *Shigella* infections of man. The salmonellae include the true typhoid and paratyphoid organisms of human origin which give rise to the classical enteric fevers, as well as more than 200 different identifiable serologic types which may be pathogenic for man in sporadic cases or outbreaks of acute enteritis but the reservoir of which is in the lower animals. The dysentery bacilli—the shigellae—are now recognized by the International Shigella Commission as constituting four major groups of a total of 30 types. (These are the organisms of acute bacillary dysentery and are practically always of human origin.) The alkalescens-dispar organisms are now accepted as constituting a special group, more closely related to *Escherichia* than to *Shigella* but still of interest in enteric bacterial infections.

Dr. Tiffany, officer in charge of laboratory consultation services of the Communicable Disease Center, Public Health Service, presented this paper at the twenty-first annual meeting of the Southern Branch of the American Public Health Association, Baltimore, Md., April 17, 1952.

Why Identification?

Our concern with the laboratory diagnosis of these infections arises from three considerations:

1. The nature and cause of an enteric infection cannot be determined with certainty without the assistance of appropriate laboratory tests. Typhoid or paratyphoid fever, the "food poisoning" type of *Salmonella* infection, and amebic and bacillary dysentery may frequently be suspected with a fair degree of accuracy on the basis of history, epidemiology, and clinical aspects, but the borderline or atypical cases are frequent enough to make accurate diagnosis impossible without laboratory confirmation.

2. Identification of the causative organism

should prove of assistance in the rational therapy of enteric infections in view of the reported favorable results with certain of the broad-spectrum antibiotics, particularly in the treatment of the systemic *Salmonella* infections (*2–5*) and of bacillary dysentery (*6, 7*).

3. It is important to know the source of the infection and to judge the likelihood that the case may in turn infect others. Is the organism of human origin, as for example the typhoid bacillus, perhaps derived from a permanent carrier, or does it belong to a group, such as *Salmonella typhimurium* or *Salmonella anatum*, commonly derived from an animal reservoir or a temporary human carrier? In the United States many *Salmonella* infections in the human are traceable to poultry and swine and to food products derived from them (*8*). *Shigella* infections, on the other hand, as well as true typhoid fever, are always of human origin. The epidemiology of a case must be understood if the spread is to be limited and recurrence prevented. This knowledge is incomplete unless the causative organism is known. The control problem is one thing if the infecting organism is proved to be the typhoid bacillus with its tendency to cause prolonged illness, to spread from person to person, and to give rise to the permanent carrier state. The problem is quite different if the organism is *S. typhimurium* or some other *Salmonella* of animal origin, with the likelihood of single accidental exposure and less probability of person-to-person transmission.

The laboratory procedures essential to the final identification of the salmonellae and shigellae have been well defined and are well known to the bacteriologists engaged in this work. Final critical identification of an organism may, however, be time-consuming and require materials and skills not available in the majority of laboratories. The time which is often necessary to accomplish complete identification and the consequent delayed report, sometimes couched in terms of antigenic factors and details with which the physician is unfamiliar, have caused some dissatisfaction and have led to the opinion that laboratory diagnosis of these organisms is largely academic. Fortunately, much information of value to the physician and public health worker may be on hand within a few days. Studies are now under way which for some of these organisms may shorten the diagnostic interval to hours. Within the last several years a number of workers (*9–11*) have defined the simplified procedures which make possible within a relatively short time the classification of the majority of organisms encountered as to the *Salmonella* or *Shigella* genus, the major group within the genus, and in many instances the complete specific identification of the pathogens most commonly seen, leaving only the infrequent problem cases for the reference laboratory.

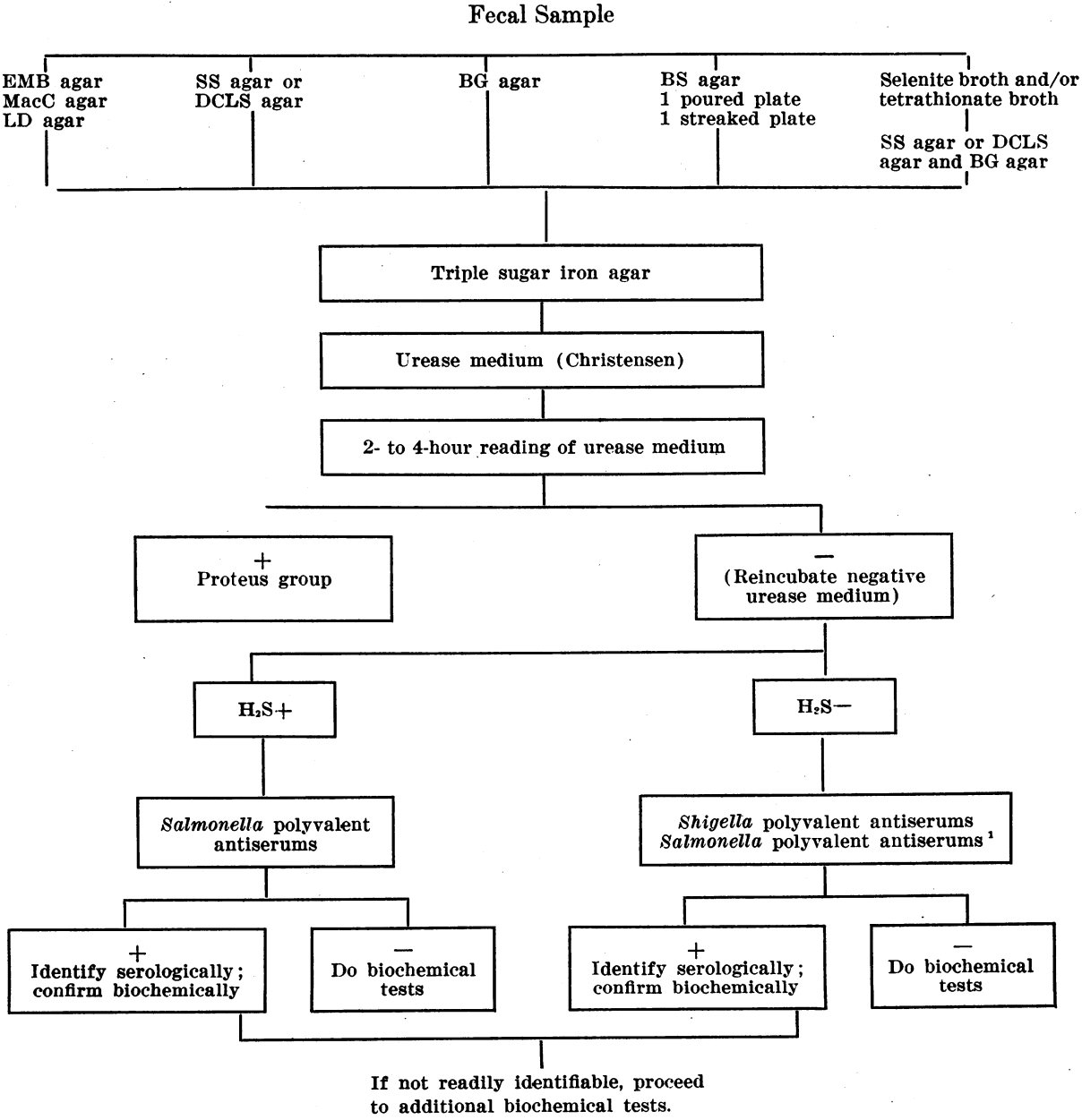
Degree of Identification Suggested

The degree of identification that may best be attempted by the various laboratories under consideration will depend mainly on need, facilities, experience, and type of laboratory. The several stages of procedure essential to complete identification of these enteric organisms are given in table 1, adapted from Edwards and Ewing (*11*). The salient features of procedure are further condensed in table 2.

The arrangement in table 2 suggests that the procedures themselves may logically fall into four stages of ascending complexity appropriate to different laboratories. It is not possible to be arbitrary about how much of the outlined procedure should be attempted by the various clinical and public health laboratories. The completeness of the service offered in any instance will depend on several factors such as demand, location and size of the laboratory, accessibility of possible reference laboratories, availability of reagents and, above all, upon the experience and interest of the laboratory staff. The interests of the two categories of laboratory are also somewhat different. The clinical laboratory will be expected to determine as early as possible whether the organism is a *Salmonella* or a *Shigella* in order that specific therapy may be more accurately focused. The public health laboratory will be concerned with more detailed information as to group and type of organism for the purpose of control and prevention of spread. However, both the practicing physician and the health officer will want, in the end, the same kind of information—full identification of the infecting organism.

The clinical or hospital laboratory which is equipped and staffed to do even a modest amount of cultural bacteriology could very well provide an effective screening service by carrying the procedure through the first four stages of enrichment, primary and secondary plating, isolation to TSI slants, and exclusion of the troublesome *Proteus* organisms by the routine use of urease medium. Organisms which on TSI slants give reactions consistent with salmonellae or shigellae and which produce no alkaline reaction on the urease medium are

Table 1. Outline of procedure for identification of Salmonella and Shigella cultures



[1] Occasional *Salmonella* cultures may fail to produce hydrogen sulfide in TSI agar. Also certain salmonellae and shigellae cross agglutinate. *Salmonella typhi* and *Salmonella gallinarum* are anaerogenic. Rarely, anaerogenic cultures of other types appear.

Table 2. Condensed summary of procedure for Salmonella and Shigella identification

1	1. The specimen: (a) Crude for immediate examination. (b) Preserved, buffered glycerol saline for transport. 2. Enrichment and primary plating media. 3. TSI slants (also give information on H_2S). 4. Urease media (allow detection and discard of *Proteus*).
2	5. Polyvalent *Salmonella* antiserum. Polyvalent *Shigella* antiserum. Preliminary biochemical tests (consistent with the genus): glucose, lactose, sucrose, mannitol, salicin, adonitol, citrate, MR, VP, indol, motility.
3	6. Group determination: *Salmonella* (6 group serums): A, B, C_1, C_2, D, E. Also Vi serum. *Shigella* (5 group serums): A, B, C, D (dysenteriae or Shiga, flexneri, boydii, sonnei, alkalescens-dispar,[1] respectively). 7. Simplified typing which will identify *S. typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Salmonella paratyphi C*, *S. choleraesuis*, and *S. typhimurium*. H factor serums a, b, c, d, i, and 1,2 and 1,5.
4	8. Complete critical typing, with complete biochemical study where necessary. Requires: (a) A complete set of O and H factor serums for salmonellae. (b) A complete set of group and type specific antiserums for the shigellae. (c) A staff experienced in the work.

[1] Alkalescens-dispar is included here although not now actually considered to belong to the *Shigella* group.

definitely open to suspicion as pathogens and merit further study. It is quite possible that this laboratory also might make its screening procedure more effective by performing the 11 simple biochemical tests indicated, which would further serve to exclude some organisms from consideration. Simple serology with commercially available antiserums might also be done. Unless the clinical laboratory in question happens to be one of the relatively few which are in a position to carry the examination further, the suspected isolate must at this point be referred to another laboratory—usually a public health laboratory.

The public health laboratory, whether at the city, county, or State level, as well as the large hospital laboratory, should be able to accept either the referred isolate described, or the primary specimen in transport solution, and carry it through procedures 6 and 7—group determination and even type recognition of the more commonly encountered forms. For the shigellae, this involves the biochemical and serologic procedures necessary to identify the organism as a dysentery bacillus and to place it in the dysenteriae, flexneri, boydii, sonnei, or alkalescens-dispar groups, and requires five serums. For the salmonellae, it is necessary to determine that the organism is indeed consistent with a member of the *Salmonella* group and to ascertain whether it is one of the species commonly of human origin, or whether it falls among the far greater number which are commonly derived from animals. Even these animal strains may often give rise to the temporary carrier state in man, whence they may for a time be the source of secondary cases.

Simplified Serologic Kits

This information in regard to the salmonellae can very largely be obtained by use of a simplified typing kit consisting of the six O factor serums A, B, C_1, C_2, D, and E; the five phase one H factors a, b, c, d, i, and the phase two H factors 1,2 and 1,5. The somatic Vi antiserum is, of course, also essential. Group A consists only of *Salmonella paratyphi A* and is rarely encountered in the United States. Most cases of *paratyphi A* infection seen here have usually originated in Mexico. In view of increased international travel today, however, it is perhaps well to include the serums necessary for the identification of this human pathogen.

Proper use of this simplified typing kit, plus the Kauffman-White schema, supplemented by appropriate use of a few biochemical tests, will allow the laboratory to identify *S. typhi*, *S. paratyphi A*, *Salmonella paratyphi B*, *Salmonella paratyphi C*, *Salmonella choleraesuis*, and *S. typhimurium*. In other words, proper use of such a kit will serve to identify, at least as to group, 98 percent of the *Salmonella* species pathogenic for man and likely to be encountered in the United States.

The biochemical tests and materials useful for the salmonellae are the same as those required for the shigellae. The four *Shigella* groupings serums and the alkalescens-dispar group serum suffice to give most of the information needed concerning a suspected dysentery bacillus.

For diagnostic work with the salmonellae and shigellae to be of value, the laboratory staff concerned must understand the properties of these organisms and their serologic relationships as set forth in the Kauffman-White schema and in the classification for shigellae proposed by the International Shigella Commission (*11*). Appropriate group and type specific factor serums must be available. Simplified serologic kits for the salmonellae and shigellae have been provided by the enteric bacteriology laboratory of the Communicable Disease Center to State health department laboratories desiring them. Appropriate serums for the simplified typing of the salmonellae and shigellae are now available commercially.

Certainly, every State public health laboratory should be able to provide the service so far outlined in identification of the salmonellae and shigellae. Private and public health city or county laboratories may also offer this degree of service. Excellent service in the laboratory diagnosis of enteric bacterial infections has been available for a long time in many hospital and city and county laboratories. The determining factors are demand, the availablity of the necessary diagnostic factor serums, and an experienced staff.

Work for the Reference Laboratory

In order to perform the final and complete critical typing of all *Salmonella* and *Shigella* strains which may be encountered, it is necessary to have on hand a much larger number of the O and H factor *Salmonella* serums as well as the necessary grouping and typing serums for the shigellae. When a new, unusual, or atypical organism is involved, final identification may be time-consuming and may require several weeks of attention from a staff thoroughly versed in all the vagaries of enteric bacteria. Studies requiring this degree of detail can only rarely be carried out routinely by the local laboratory and are the special province of the reference laboratory.

There are several well-known laboratories in this country which are equipped to undertake complete identification of the *Salmonella* organisms and which are associated with State or city health departments, a few hospitals, or the Public Health Service. Whatever their organizational position, these reference laboratories have certain features in common—they are staffed by individuals who have long experience with these organisms and who for the most part make their own serums.

The serologic relationships of the *Shigella* organisms have only recently been more clearly defined, and complete serologic analysis has not been as widely practiced on this genus. The good hospital or public health laboratory should be able, however, to isolate the organisms and identify them as to group. For complete typing, the shigellae may also be sent to the several appropriate reference laboratories in this country.

Anyone attempting to work in the laboratory with these enteric organisms will soon encounter the paracolon bacteria and will find them troublesome. The paracolon bacteria comprise a considerable spectrum of organisms falling into numerous subgroups with relationships ramifying among the salmonellae, the shigellae, and the colon bacilli. Although generally nonpathogenic and of nuisance value only in laboratory diagnosis, some strains may cause serious illness in man. They cannot therefore, always be disregarded. Much work remains to be done with the paracolon bacteria; there is at present no royal road to their recognition and exclusion. They are, in general, slow lactose fermenters and may give a delayed urease test only faintly positive after 48 hours of incubation. It seems inevitable that any system of screening enteric pathogens, as suggested here, will catch many paracolons in the net. The true identity of these organisms will have to await study by the reference laboratory whose staff can cope with the vagaries of the group.

Type identification of typhoid bacilli by means of bacteriophage is an important tool in epidemiology. But phage typing of the typhoid bacillus and of *S. paratyphi B* is a highly specialized procedure requiring care in the preparation and maintenance of the parent strains of bacteria and phages, and special training on the part of the staff. In view of these considerations and the low incidence today of typhoid fever, it has been considered preferable that specimens for this work be referred to one of the

14 special bacteriophage typing centers established in the United States (*12*).

Pathogenic Types of Escherichia coli

In the diagnosis of enteric bacterial infections a new field of considerable interest has been opened within the past few years. There is now evidence that at least certain identifiable strains of colon bacilli may be capable of causing primary enteritis.

Kauffman (*13*) in 1944 and in 1947 (*14*) published the results of his studies on the *Escherichia coli* group to which he had applied those techniques of antigenic analysis which have proved so valuable with the salmonellae. He also suggested "as a working hypothesis" that a number of the *E. coli* groups serologically identifiable by these techniques would prove of importance in certain of the infectious diseases of man. Approximately 125 O groups of colon bacilli have now been defined. Members of two of these groups have been isolated from cases of infectious diarrhea of infants, and also, on occasion, from enteritis in the adult, under circumstances which indicate a causal relationship. These two types are 055-B5 and 0111-B4, and may be identified by simple slide and tube agglutinations using appropriate serums. It would appear that at least every major public health laboratory should have on hand these serums and should be familiar with their proper diagnostic use in cases of enteritis from which the more common pathogens seem to be absent. Other serologically distinct coli types may be shown to be related to human disease.

Rogers and associates (*15*) in England in 1949 suggested the value of chloramphenicol on the basis of their experience with a small series of cases of enteritis in children apparently caused by a serologic variety of *E. coli*. Smith and his associates (*16*) in 1950 reported encouraging results with chloramphenicol in the treatment of cases of infection with coli type 055. Only this year a hospital outbreak of infantile diarrhea has been reported by Modica, Ferguson, and Ducey (*17*) in which *E. coli* 0111-B4 was isolated from 45 cases. Chloramphenicol, aureomycin, and terramycin appeared effective in treatment. If this experience with chloramphenicol and other antibiotics is borne out, it is possible that the larger hospital laboratories may likewise find use for these diagnostic serums.

Recommendations

1. Every State public health laboratory and the larger local public health laboratories should be equipped to isolate *Salmonella* and *Shigella* organisms from the primary specimen, to identify them as belonging in all probability to the *Salmonella* or to the *Shigella* genus, and to carry them through group identification.

Laboratories of this caliber should also be able to identify specifically the typhoid bacillus, *S. paratyphi B* and *C*, as well as the more common salmonellae of animal origin, such as *S. typhimurium*, *S. choleraesuis*, and a few others which may by experience have been found common and important in a given locality.

2. The local hospital, clinic, or smaller public health laboratory may either refer its specimens directly to the nearest laboratory equipped to handle them throughout, or may process the specimens to the point of detecting suspicious organisms and of determining that these are at least not *Proteus* or *Pseudomonas*.

These smaller laboratories might even find it practicable to apply the simpler biochemical tests indicating that the organism in question is consistent with a *Salmonella* or *Shigella*. The suspicious organism should then be referred to the appropriate laboratory.

3. Complete critical typing is the function of the larger and specialized reference laboratory which may, depending upon circumstances, be functioning at the local, State, or national level.

One last comment, somewhat in the nature of a plea, appears appropriate. The bacteriologists who examine and study these specimens in the laboratory will be eternally grateful to the physicians who submit the specimens if they will send in at the same time a brief summary of the outstanding facts pertaining to the case: animal or human origin; case or suspected carrier; age and occupation; acute or insidious onset; duration of illness; probable exposure; possible food source. Many of the bacteriolo-

gists engaged in this work are deeply interested in the clinical and epidemiological data enumerated above. Without this information their own horizon is narrowed and their usefulness in the matter of communicable disease control is impaired.

NOTE: The Communicable Disease Center does not wish to duplicate services which are available locally. The CDC laboratory branch, therefore, accepts specimens for study only when submitted through a State health department laboratory (*18*).

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State and Territorial Health Officers' Conference

The 1952 Annual Conference of the Surgeon General of the Public Health Service and Chief of the Children's Bureau with State and Territorial health officers, mental health authorities, and representatives of State hospital survey and construction agencies will be held from December 8 through December 11. Open sessions will be held in the auditorium of the Federal Security Building, Washington, D. C., on December 9 and December 11, beginning at 9:30 a. m. The remainder of the conference will be devoted to executive sessions and committee meetings.