

Public Health Reports

Vol. 64 • FEBRUARY 18, 1949 • No. 7

A Transparent Dextrose Serum Tellurite Plating Medium

**Its Use as an Adjunct to Microscopic Examination of Smears
Made From Loeffler Slants in Routine Diphtheria Diagnosis**

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The purpose of this study was to develop a plating medium on which *C. diphtheriae* would exhibit differential colonial features. With such a medium, recognition of these characteristics could be used in the diagnostic laboratory to supplement the study of cellular morphology in smears made from Loeffler slants. Diagnostic reporting should thus become more objective and more reliable.

The usefulness of such a medium would depend on whether or not diphtheria colonies would be large enough to show their characteristics after 18–24 hours incubation, that is, at the time diagnostic smears are usually made. Furthermore this plating medium should be inhibitory for as many as possible of the commensal organisms found in nose and throat cultures.

Review of Literature

The classical and most widely used diagnostic medium for the cultivation of *C. diphtheriae* for more than 50 years has been Loeffler's coagulated blood serum. Advantages claimed for it have been that it was highly favorable for the growth of the diphtheria bacillus and that the typical morphology and arrangement of the organism was best brought out on this medium. Such may have seemed to be true to those who used it without making comparative studies, but in the hands of a critical worker it has clearly left much to be desired.

Commenting on the diagnostic utility of Loeffler, Cooper (1) and his associates wrote "those bacteriologists who continue to depend entirely on Loeffler medium are doing second-rate bacteriological work

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as far as diphtheria is concerned." And with reference to growth and cellular morphology when cultivated on this medium, Mueller and Miller (2) said "the normal, well-rounded diphtheria bacillus probably never has been observed from Loeffler medium because the latter provides only a starvation diet. The rapidity and selectivity of its supposed growth-promoting properties are illusory."

Other disadvantages to the use of Loeffler derive from the fact that being entirely nonrestrictive a great variety of commensal organisms grow on this medium when it is inoculated with nose and throat swabs and any small number of diphtheria bacilli present may be obscured by being overgrown. Loeffler may also be rendered quite useless due to its digestion by spore-forming or other proteolytic organisms in the inoculum, or it may not support growth of some strains of *C. diphtheriae*. Further, such variations occur in its composition from lot to lot, and the morphology of diphtheria bacilli is so affected by these differences, as shown by Goldsworthy and Wilson (3), that the organisms are at times quite unrecognizable. Finally, the identification of *C. diphtheriae* from Loeffler rests solely on the ability of the examiner to recognize accurately under the microscope a wide range of morphological variants.

Because of these drawbacks a variety of media—most of which contained one or another of the salts of tellurous acid—have more recently been proposed as substitutes for Loeffler or as complementary to it.

Without going into details of composition or the specific advantages claimed for the various formulae, the following summary indicates why they have been advocated:

1. They have been less inhibitory for all types of *C. diphtheriae*.
2. They yielded more positives than did other media.
3. They inhibited more commensal organisms.
4. They were not digested by proteolytic organisms.
5. They brought out differential colonial characteristics of diphtheria and dipthomorphic organisms.

The disadvantages of these media have been, mainly, that if they significantly restricted commensal organisms, especially diphtheroids, they also inhibited *C. diphtheriae*, or the cellular morphology of *C. diphtheriae* was so altered as to make microscopic recognition of the organism very difficult.

The Ideal Medium

Categorically stated, the ideal medium should possess the following characteristics for use in culturing naso-pharyngeal swabs for diag-

nostic purposes, for use as a plating medium, or for isolation of pure cultures of *C. diphtheriae*:

1. It should be noninhibitory for all types of *C. diphtheriae*.
2. It should have no material effect on the characteristic cellular morphology of the organisms.
3. It should give good growth on Petri plates of all strains of *C. diphtheriae* at 18–24 hours incubation.
4. Translucency of the medium is highly desirable if it is to be used for plating purposes, as this facilitates differentiation of colonies.
5. Colonial differentiation of all strains of *C. diphtheriae* from other organisms growing on the medium should be easy, or, alternatively, selectivity of the medium should be such as to inhibit growth of all organisms except *C. diphtheriae*.
6. It should inhibit commensal organisms as much as possible.
7. The ingredients should be easily obtainable and stable when stored for any length of time.
8. Preparation of the medium should be easy and quick—not calling for repeated filtrations or tedious adjustments of reaction.
9. On storage the prepared medium should be stable.
10. Large or small quantities of the medium should be prepared with equal ease.

Introduction of Tellurite Media

Following the introduction of tellurite salts as a component part of selective media for isolation of *C. diphtheriae*, many different formulae were proposed and some of them have been quite widely used. Initially, these media were considered as especially valuable for isolation and typing purposes as suggested by Douglas (4), Anderson, Happold, McLeod, and Thompson (5), and Horgan and Marshall (6), and many others. For routine diagnostic purposes, however, Loeffler slants continued to be used for the most part though the greater accuracy of tellurite media in the detection of *C. diphtheriae* was reported by Sutherland and Iredale (7), Wilson (8), Anderson (9), Knox (10), Frobisher (11), Perry and Petran (12), and Kellogg and Wende (13). Others, as for example Wright (14), employed tellurite as a means of eliminating false positive diagnoses due to the presence of diphtheroids and false negatives due to overgrowth of *C. diphtheriae* by commensal organisms; Young (15) used it because it was more productive; Cruickshank (16) found it to be not only more productive but to yield a considerably higher percentage of confirmed diagnostic cultures; Knox (10) reported it gave nearly twice as many positives as Loeffler in the examination of convalescents and contacts, and

Frobisher (11) detected three times as many carriers by plating negative and doubtful Loeffler cultures onto tellurite.

Reviewing the literature, then, on the use of tellurite media, it thus appears that they have been used by different workers in different ways and for different purposes.

Experimental Procedures

As a preliminary step comparative studies of many of the more recently suggested media were undertaken with particular attention being paid to the following points:

1. Was the medium inhibitory for any type of *C. diphtheriae*.
2. Was the medium inhibitory for any of the commensal organisms commonly encountered in nose and throat cultures.
3. Did the medium support sufficiently active growth of *C. diphtheriae* so that colonies coming up on the plates in 18–24 hours were large enough to show differential features.
4. Did the color of the medium allow easy recognition of differential colonial characters.
5. Were any special difficulties encountered in the preparation of the medium.
6. Was the prepared medium stable.

No attempt will be made to catalog the observations recorded on all media tested. Certain generalizations, however, may be made:

1. The media containing fresh blood or blood and cystine, such as those of Clauberg, and Frobisher, in general support good growth of diphtheria bacilli, although they may inhibit some strains of the small-colony variety.

The colonies, however, on these media after 18–24 hours incubation do not exhibit differential features and are therefore of no confirmatory value at the time diagnostic smear examinations from Loeffler are usually being made. Further disadvantages of these media are that while they may or may not inhibit commensal organisms they are not usable for any extended time after preparation.

2. The media containing heated blood, such as the chocolate-tellurite agar of Anderson, do permit differentiation of the *gravis* and *mitis* types but they also inhibit certain diphtheria strains. Of equal importance is the fact that colonies are too small at the critical period to be recognized with assurance.

More recently a heated hemoglobin tellurite agar has been proposed by Galbraith, Bramhall and Fraser which is said not to be inhibitory for any type of diphtheria. It is also claimed to bring out differential colonial characteristics. Our experience, however, has been that at 18–24 hours this medium was highly inhibitory for small-colony strains and did not bring out any differential colonial features whatever.

3. Those media such as that of Mueller, markedly restricting growth of commensal organisms, are also inhibitory for certain types of *C. diphtheriae*, give colonies having no differential features, and do not promote rapid enough growth to be helpful in 18–24 hours after inoculation.

Formula of Dextrose Serum Tellurite Agar

The ingredients needed for the preparation of this medium are available in any diagnostic laboratory.

Preparation of the medium from the basic components as it is needed each day is entirely possible, but it will be more convenient to keep poured plates on hand, ready for immediate use. In lieu of this, sterilized basic agar and sterile dextrose serum tellurite solution, ready to be added to the melted basic agar, may be kept in stock and the number of plates needed may be poured immediately prior to their use.

The directions which follow are for the preparation of 100 cc. of the completed medium. The amounts of the various components to be used in making up larger quantities may be determined by simple multiplication.

To prepare 100 cc. of the medium

Weigh 4.5 grams of Difco proteose No. 3 agar.

Weigh 0.5 gram of Difco Bacto-agar (granular).

Dissolve the above in the Arnold sterilizer in 100 cc. of buffered distilled water pH 7.1–7.2.*

Sterilize in autoclave at 121° C. for 15 minutes.

Cool to 50° C. in a water bath. This is important.

Aseptically add 8.0 cc. of stock dextrose serum tellurite solution.**

Mix thoroughly.

Pour plates aseptically and not less than 5 mm. thick.

Incubate at 37° C. to test sterility.

Store plates in cold room; they are good for at least a month.

**Preparation of Buffered Distilled Water*

- a. M/15 anhydrous disodium phosphate (Na_2HPO_4). Divide molecular weight given on the bottle by 15 and dissolve this amount of the salt in 1,000 cc. of distilled water.
- b. M/15 anhydrous sodium acid phosphate (NaH_2PO_4). Divide molecular weight given on the bottle by 15 and dissolve this amount of the salt in 1,000 cc. distilled water.
- c. To prepare 1,000 cc. buffered distilled water of pH 7.2 add 72.0 cc. of (a) above and 28.0 cc. of (b) above to 900 cc. of distilled water.

***Preparation of Stock Dextrose Serum Tellurite Solution*

The following sterile components are combined aseptically to give the working solution. Human serum is specified, as it is easily obtainable

from the serologic laboratory, but beef or other serum may be substituted although our results with these have not been as good as with human serum. Either crystalline potassium tellurite or the powdered salt is satisfactory and is used in 0.5 percent concentration.

Human serum.....	30.0 cc.
Dextrose solution (20%).....	12.0 cc.
Potassium tellurite solution (0.5%).....	6.0 cc.

Note: For preparation of the above.

1. Human (Wassermann) serum is pooled and sterilized by Seitz filtration.
2. Dextrose solution (20% in distilled water) is sterilized by Seitz filtration.
3. Potassium tellurite solution (0.5%) is prepared as follows: Grind 0.5 gm. of C. P. dry crystalline potassium tellurite very fine in a small dry mortar. Add 10.0 cc. buffered distilled water gradually after grinding. Stir well and allow to settle. Remove clear supernatant by pipette to a 100 cc. graduate. Add more buffered water and stir. Allow to settle and remove supernatant to the graduate. Repeat until all the tellurite appears to be dissolved. Finally, add a few drops of 10% KOH to the mortar. Rinse the sides of the mortar with 10.0 cc buffered distilled water and add this to the graduate. Make up the volume to 100.0 cc. Seitz filter. The final pH will be about 9.6.

Caution: Keep the crystalline potassium tellurite in a dessicator.

Colonial Morphology

Since the beginning of this study it has been observed that colonial size and appearance on different media vary much more than had been realized. However, because of uniformity of composition of the medium the colonial characteristics of *C. diphtheriae* on dextrose serum tellurite agar are remarkably constant although there are inherent differences between the various types, i. e., intermedius, mitis, gravis, and small-colony variety.

To facilitate recognition and differentiation of colonies of *C. diphtheriae* and other organisms coming up on the plates, the following descriptions may be found helpful. In a diagnostic laboratory the most significant characteristics are those observed at the end of 18–24 hours incubation because it is at that time that parallel smear preparations from Loeffler are generally examined.

In all instances colony examination is best made using a hand lens of about 8x magnification but a colony microscope is an advantage in the study of colonial detail.

Intermedius colony type

24 hours—very small gray colony, 0.25–0.5 mm (av. 0.3 mm); very slightly raised, but sometimes flat with tiny black dot, or dark gray center. Edge is serrated or entire, and surface somewhat matt-like in some strains: uniform in size.

Mitis-like colony type

24 hours—variation in colony size common, 0.3–1.25 mm (av. 1.0 mm); gray, sometimes a dull gray with darker gray center, smooth, round, convex, and glistening. Entire edge; butyrous consistency.

Gravis-like colony type

24 hours—size 1–1.50 mm (av. 1.25 mm), gray, with dark grayish-black center, translucent periphery, raised and round. In some strains edge is crenated to a variable extent; is brittle and tends to fracture radially when touched with a needle.

Small-colony type

24 hours—colonies minute (0.25 mm and less); grayish with slight darkening in some colonies, variation in size, round, convex, entire edge, somewhat rough.

Colonial morphology of other organisms appearing on the plates

1. *Staphylococci*: in 24 hours colony varies in size in different strains, somewhat flat with elevated center, glistening, round, black, or bluish black and with very thin translucent entire periphery.
2. *Diphtheroids*: in 24 hours, very small dead white and pin-point in size, but a few strains have brown tint and are larger.
3. *Yeasts*: in 24 hours, white, moist to dry, round and varying in size according to species.
4. *Spore formers*: in 24 hours, usually light brown, usually mucoid, good size but not spreading unless the medium is quite moist. Generally entirely suppressed.
5. *Micrococci*: in 24 hours, round, raised, grayish, glistening, and resembles the mitis diphtheria type colony somewhat. Often shows some blackening in center of colony which helps to differentiate it, but this is not always seen.
6. *Streptococci*: in 24 hours, vary in size, texture, and elevation; majority of strains small, flat, round, with glistening black centers, periphery is thin, translucent; some strains are light brown in color while others are black. These may have to be picked to be certain of their identification.
7. *Pneumococci*: in 24 hours, round, flat, dull, dark greenish colony with lighter greenish edge; matt-like texture.

Cellular Morphology

As stated above, accurate diagnosis, based on examination of stained smears, depends on the ability of the microscopist to recognize a range of morphological variants of the diphtheria bacillus. This cellular variation—which is most pronounced when the organism is grown on a medium of inconstant composition such as Loeffler slants—is generally thought of as quite characteristic and easily recognizable. On the other hand it is commonly believed that diphtheria bacilli when grown on tellurite media are shorter, thicker, and stain more uniformly, and are thus more difficult to recognize. For this reason it has been suggested by some authors that both media should be used, the colonies being picked from the tellurite medium and the morphology being studied from the Loeffler. Such a procedure may be satisfactory if the objective is to isolate pure cultures, but it certainly has nothing to recommend it in the routine diagnostic laboratory where a report to the physician must be made at the earliest possible moment.

It is of interest, therefore, to call attention to the fact that the cellular morphology of diphtheria bacilli on dextrose serum tellurite agar is not so different from that seen from Loeffler as to present a serious problem in recognition, and therefore smears may be made directly from the plates. It is true that many of the cells will be thicker and more heavily stained—probably because the medium offers a more

nutritive base than Loeffler—but there is a remarkable uniformity of cellular morphology as related to colonial type.

This correlation is indicated by the following description:

Intermedius colony type

Pleomorphic, with club-shaped forms occasionally seen. Few or no metachromatic granules. Many solid staining forms. Barred cells are deeply stained with pale blue areas between the bars.

Mitis-like colony type

Pleomorphic, with well developed metachromatic granules in most strains. Barred forms infrequent. Typical, rather slender rods as compared to intermedius colony type above.

Gravis-like colony type

Uniform, short, stout, heavily staining. Occasional tear, club and wedge-shaped forms seen. Some barred and shadow forms may be present. Most organisms resemble diphtheroids.

Small colony type

Slender, branching, filamentous bacilli. Fungus-like arrangement.

Comparative Results Obtained Using Loeffler Slants and Dextrose Serum Tellurite Plates

As a means of determining the relative utility of Loeffler slants and dextrose serum tellurite plates in routine diagnostic work, nose and throat swabs received from physicians were first streaked on Loeffler slants and then on the plates. After 18–24 hours incubation smears were made from the Loeffler slants, the plates were scanned for typical diphtheria colonies and when these were seen, smears were made from them for microscopic study. Examination of the stained smears in each instance was made by an experienced person.

Results obtained in parallel examinations made from Loeffler slants and dextrose serum tellurite plates in 259 positive specimens are shown below. Excluded are the specimens in which the Loeffler and plate were both negative.

	*L+	L+	L-	L unsatisfactory
	**T+	T-	T+	T+
Total (259) -----	222	6	29	2

*L=Loeffler **T=Tellurite

Analysis of these data indicates agreement in 222 of the specimens. In six instances a positive diagnosis was made from the Loeffler slant when the tellurite plate was negative, but in two of these cases it would seem probable that the organism seen in the Loeffler smear and called diphtheria was really a diphtheroid as this was the only thing which grew on the plates. On the other hand there were 29 cases in which the smears from Loeffler slants were negative and the plates showed the presence of diphtheria bacilli. Finally, there were two

instances in which no examination could be made from the Loeffler slants, due to digestion of the medium by a proteolytic organism, but typical colonies of diphtheria appeared on the plates.

In summary, then, it appears the slant and plate were in agreement in 85 percent of the cases, in 2 percent of the cases the slant was positive and the plate negative, and in 11 percent of the cases the slant was negative and the plate positive.

As a result of this comparative testing it has become evident that on the plates *C. diphtheriae* produces easily recognized colonies and that at the critical time—after 18–24 hours incubation—diphtheroids are usually completely inhibited, or, if they do come up can be readily distinguished. Simple scanning of the plates, then, serves to confirm as true diphtheria bacilli the tentative conclusion of the microscopist as to the identity of cells seen in smears made from Loeffler slants. Negative reports may also be made with assurance in instances where diphthomorphic organisms are seen in smears made from Loeffler but where there are no characteristic colonies on the plates.

Résumé

Ever since its introduction Loeffler's coagulated blood serum has been the medium most widely used in diagnostic laboratories for growth of diphtheria bacilli from nose and throat swabs. The advantages of this medium have been presumed to be that: (1) it grew diphtheria rapidly; (2) it grew all types of diphtheria organisms; (3) it was conducive to the development of the most characteristic cellular morphology and arrangement of the organism thereby making recognition easy in stained smears made from the medium.

In recent years Loeffler has been criticized because it has been shown that: (1) its "supposed growth-promoting properties are illusory"; (2) its failure to restrict commensal organisms often resulted in overgrowth when only a few diphtheria bacilli were present; (3) it was often rendered useless because of digestion by proteolytic organisms; (4) its composition was extremely variable; (5) the cellular morphology of diphtheria bacilli is much modified by differences in composition of the medium; (6) it fails to grow some strains of diphtheria bacilli.

To overcome these drawbacks a variety of media, all containing one or another of the salts of tellurous acid, have been developed and given more-or-less wide trial. Advantages claimed for these media have been stated to be that: (1) they gave more positives than did other media; (2) they were not digested by proteolytic organisms; (3) they inhibited many commensal organisms; (4) if they were used as plating media they brought out differential colonial characteristics of diphtheria colonies.

The disadvantages of these media have been: (1) that if sufficiently restrictive to inhibit commensal organisms they have also inhibited some strains of diphtheria; (2) the cellular morphology of diphtheria bacilli was so altered as to make microscopic recognition of the organism difficult; (3) colonies of *C. diphtheriae* have been too small after 18–24 hours' incubation to exhibit differential features.

This investigation was undertaken to develop a plating medium on which: (1) no known strain of diphtheria bacilli would be inhibited (2) colonies of diphtheria would be large enough perhaps to show differential features after 18–24 hours growth; (3) cellular morphology of diphtheria bacilli would not be so altered as to be difficult of recognition; (4) commensal organisms from the nose and throat might be inhibited to a large extent, and (5) pure cultures of diphtheria might be picked after 18–24 hours incubation, thus shortening the time needed for the performance of virulence tests.

After the trial of many formulae and an experience extending over a number of years the described dextrose serum tellurite medium was evolved. In our hands it has met the stated needs, and the comparative results using it in parallel with Loeffler have been set forth in the table. In this summary the time of comparison covered was only about a year, but actually the comparison has been in progress for the past several years.

The points of most significance in connection with dextrose serum tellurite agar are: (1) it is *noninhibitory* for any strain of diphtheria within our experience; (2) it is inhibitory for many of the commensal organisms found in nose and throat cultures; (3) it is uniform in composition; (4) the morphology of diphtheria bacilli is practically unaffected when the organisms are grown on different lots of the medium; (5) the different colonial types of diphtheria are distinguishable; (6) at 18–24 hours of age the diphtheria colonies are differential, and confirmatory evidence as to their identity may be obtained from microscopic examination of smears made from them.

Conclusions

1. Reliance on microscopic examination of stained smears from Loeffler slants for diagnosis of diphtheria may result in error because:

- (a) Not all strains of *C. diphtheriae* will grow on this medium.
- (b) The cellular morphology may be so altered as to be unrecognizable because of variations in the composition of the medium.
- (c) Small numbers of diphtheria bacilli may be masked due to overgrowth of commensal organisms.
- (d) The medium may be digested by proteolytic organisms present in the inoculum

2. The laboratory diagnosis of diphtheria would become more objective and reliable if the specimen from the patient could be planted simultaneously on Loeffler and a plating medium on which *C. diphtheriae* would appear in differential colonies and from which microscopic examination could be made directly.

3. The formula for such a medium has been presented.

4. The advantages of this medium are:

(a) It grows all types of *C. diphtheriae*.

(b) It yields more positives than does Loeffler.

(c) The growth of diphtheroids is usually surpassed up to 24 hours and when they do come up it is in easily differentiated colonies.

(d) The characteristic cellular morphology of *C. diphtheriae* growing on the medium is not greatly altered.

(e) The colonial morphology of *C. diphtheriae* is distinct and recognition of typical colonies serves to confirm tentative conclusions based on smear examinations.

(f) The colonies are large enough to be easily identified after 18–24 hours incubation, i. e., at the time when diagnostic smears are being made from Loeffler.

(g) If isolation of pure cultures is desired this may be accomplished at 18–24 hours after inoculation of the medium.

(h) It is easy to prepare and of uniform composition.

(i) It does not become toxic on storage.

(j) It is not digested by proteolytic organisms.

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A Flocculation Test as a Possible Method for Differentiating Immunologic Types of the Poliomyelitis Virus¹

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All early attempts to develop an *in vitro* serological test for poliomyelitis were unsuccessful, as pointed out by Raffel and Schultz (1). More recently, complement fixation reactions with the Lansing strain of poliomyelitis virus (2) have been obtained with highly concentrated antigens prepared by several cycles of ultracentrifugation. However, the amount of material and work required for the preparation of this antigen made this method impractical for use as a routine procedure.

Attempts to develop an *in vitro* test were begun in 1940 with the development of the bacterial agglutination (B. A.) test (3, 4). Results obtained with this test suggested that a possible approach might lie in the adsorption of the virus by other particles. Inasmuch as the adsorption of the virus particle on the bacterial cell is influenced by factors many of which are not understood and therefore cannot be controlled, it was considered that the substitution of another particle for the bacterial cell might present an advantage. Since the virus particle bears a negative charge it was thought that a positively charged protein (protamine) might overcome some difficulties of adsorption.

In the test to be described (the flocculation reaction) it is presumed that the positively charged protamine molecule adsorbs a few negatively charged virus particles as well as other molecules in the suspension. Thus these "built up" particles may possess sufficient

¹ Aided by a grant from the National Foundation for Infantile Paralysis.

² From the Neurotropic Virus Unit, The George Williams Hooper Foundation, University of California, San Francisco.

mass and other properties necessary to give a visible reaction in the presence of specific antibody.

Early in the work it became evident that central nervous system tissue contains a substance or substances which interfere with the flocculation reaction. These substances could be removed occasionally by the rapid and repeated freezing and thawing process outlined by Casals (5). Freezing and thawing at certain specific pH levels aided in the removal of the interfering substance, yet only 10 percent of the antigens thus prepared proved active. After the purification of influenza virus by methanol precipitation in the cold (6) was achieved, this procedure was applied to the virus of poliomyelitis. However, it was found that the undesirable normal component was precipitated with the virus. Nevertheless, this led eventually to the now successful method of removing this inhibiting substance. The method used at the present for the preparation of the flocculating antigen is a combination of freezing and thawing, pH adjustment, and methanol precipitation similar to that outlined by Gollan (7). The final preparation represents a 10-fold concentrate of the original 10 percent suspension.

Before performing the flocculation test, the correct amount of protamine to be added to the virus suspension must be determined. Various quantities of protamine sulfate solution are added to constant portions of partially purified virus suspension and incubated first at room temperature and then at 43° C. to fix the combination. These antigens are added to serial 2-fold dilutions of inactivated monkey convalescent serum. The tubes are then incubated at 43° C. for 3 hours. The precipitate is composed of thin, grey, transparent flakes. The amount of protamine that gives the best reaction is then added to the virus suspension, making the antigen used in setting up the flocculation test with unknown sera. This is conducted in the same manner as the preliminary titration.

A detailed description of the procedure is being prepared for publication elsewhere (8).

It is encouraging to find that antigens can be stored at -15° C. for at least one month without loss of activity. This activity appears to be related to the virus since Lansing antigen possessing an infectivity titer of 10^{-5+} gave reactions of 1:160 with monkey convalescent serum while an antigen with a titer of 10^{-4} gave a reaction of only 1:20 with the same serum. The flocculation test is simple and all reagents can be titrated and standardized. At present the antigen is not titrated since we are still on the threshold of the reacting dose. Improvement in preparation of the antigen should result in preparations sufficiently active to be titrated.

Antigens prepared from mouse brains and spinal cords infected with Lansing poliomyelitis virus gave reactions with pooled adult human

serum and hyperimmune MEF₁ (a strain known to be antigenically similar to Lansing and adaptable to cotton rats and mice) monkey serum (9). Normal monkey sera failed to react. Also antigens prepared from normal mouse brains as well as those infected with the Jungeblut murine-adapted SK virus failed to react with the above sera. The specificity of the reaction was further indicated when monkey serum taken before immunization failed to elicit a reaction with the Lansing antigen while strong reactions were obtained with serum taken after immunization with the MEF₁ virus. Serum from monkeys immunized with an unrelated strain of monkey-adapted poliomyelitis virus failed to react. In addition, partially purified suspensions of Lansing virus first added to the serum inhibited the flocculation reaction while similarly prepared normal mouse brain and Jungeblut SK virus suspensions failed to do so.

After the specificity of the test had been determined it was applied to antigens of the following monkey adapted strains: the MEF₁ strain from Dr. P. K. Olitsky, the Kosh and Campbell strains which were isolated in our laboratory, the BK and McKay supplied by Dr. John Kessel, and the Brunhilde and Frederick strains supplied by Dr. H. Howe.

The antigens prepared from infected tissue were tested with monkey sera. The Lansing, MEF₁, Kosh, Campbell, and McKay sera were from convalescent monkeys. The serum was drawn 16 days after paralysis developed. Convalescent monkey serum to the BK virus was obtained from Dr. John Kessel. Hyperimmune monkey sera to the Brunhilde and Frederick viruses were obtained from Dr. H. Howe.

Typical reactions obtained with two antigens (MEF₁ and Kosh) tested against several sera are presented in tables 1 and 2.

Table 1. Reactions with MEF₁ antigen

Sera	Reciprocal of serum dilution						Antigen control
	10	20	40	80	160	320	
MEF ₁		+	+	++	+++	+	0
Frederick.....	0	0	0	0	0	0	0
Brunhilde.....	0	0	0	0	0	0	0
Kosh.....	0	0	0	0	0	0	0

Table 2. Reactions with Kosh antigen

Sera	Reciprocal of serum dilution						Antigen control
	10	20	40	80	160	320	
Kosh.....		0	+	+	+	+	0
Brunhilde.....	0	0	0	0	0	0	0
Frederick.....	0	0	0	0	0	0	0
Campbell.....	0	+	+	+	0	0	0

Antigens prepared from the eight strains of poliomyelitis virus have been tested with some or all of the eight sera and in most cases by reciprocal titrations. When it became evident that the Lansing and MEF₁ viruses were closely related, the Lansing virus and serum were excluded from later titrations.

From these reactions the relationship of the eight viruses, as determined by the flocculation reaction, is indicated in table 3.

Table 3. *Relationship of poliomyelitis viruses by flocculation reaction*

Sera	Antigens							
	Lans	MEF ₁	Kosh	Camp	BK	Fred	McKay	Brun
Lansing.....	+	+	0	-----	0	0	-----	-----
MEF ₁	+	+	0	-----	0	0	-----	-----
Kosh.....	0	0	+	+	+	+	+	+
Campbell.....	0	0	+	+	+	0	-----	-----
BK.....	0	0	+	+	+	0	-----	-----
Brunhilde.....	0	0	0	0	0	0	0	+
Frederick.....	0	0	0	0	0	+	+	-----
McKay.....	-----	-----	+	+	+	0	+	-----

Of the eight strains of poliomyelitis virus tested there appear to be two distinct groups. The Lansing and MEF₁ viruses are closely related as has been shown by neutralization tests. They are distinct from five of the other six viruses (not compared with McKay strain). No close antigenic relationship between the Lansing and BK strains is indicated by challenge of BK convalescent monkeys (10). The second group includes Kosh, Campbell, BK, McKay, Brunhilde and Frederick viruses. Whether the failure of the Brunhilde and Frederick sera to react with Kosh, Campbell and BK antigens indicates possible sub-groups or results from the difference in the preparation of the sera (hyperimmune rather than convalescent) must be determined by further study. In either case these viruses are included within the group by the reaction of their antigens with the Kosh serum and by the reaction of the Frederick serum with the McKay antigen.

These results agree in general with those obtained in monkey neutralization and immunity experiments. In our laboratory the Campbell serum has neutralized the Kosh virus. Kessel (10) has demonstrated BK convalescent monkeys to be resistant to challenge with the McKay and Frederick viruses.

These results suggest that the flocculation test might offer a method for the grouping of the poliomyelitis viruses. The test offers a relatively inexpensive method since the cords of two monkeys supply sufficient antigen to test eight or ten sera.

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INCIDENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED JANUARY 29, 1949

A total of 15,266 cases of measles was reported for the current week, as compared with 13,392 last week and a 5-year (1944-48) median of 6,712. Increases were recorded in all geographic divisions (the largest in the East South Central and South Atlantic) except the New England and West North Central. Of the current total an aggregate of 10,522 cases occurred in 12 States, as follows (last week's figures in parentheses): Massachusetts 1,171 (1,373), New York 993 (839), Pennsylvania 1,145 (1,060), Michigan 559 (391), Wisconsin 583 (511), Maryland 774 (724), Virginia 815 (455), Kentucky 511 (97), Alabama 521 (250), Texas 2,086 (2,005), Oregon 474 (550), California 890 (685). The totals for the year to date and since the average seasonal low week in September are, respectively, 51,608 and 104,001 cases, as compared with last year's figures, the highest of the past 4 years, of 33,414 and 68,360. The respective corresponding 5-year medians are 20,285 and 46,409.

The current influenza incidence is 4,534 cases, as compared with 4,585 last week and a 5-year median of 14,253. An aggregate of 3,959 cases was reported in 6 States, as follows (last week's figures in parentheses): Virginia 464 (440), South Carolina 625 (601), Alabama 101 (66), Arkansas 290 (221), Texas 2,327 (2,558), Arizona 152 (147).

Of 95 cases of poliomyelitis (last week 113, 5-year median 36), California reported 30, Texas 14, North Carolina 9, and Arizona 6.

Of 26 cases of tularemia (last week 29, 5-year median 17), 8 occurred in Georgia, 5 in North Carolina, 3 in Illinois, and 10 in 6 other States. The total for the year to date is 145, same period last year 101, 5-year median 104.

During the week 2 cases of psittacosis were reported in Virginia. Pennsylvania and Kansas each reported 1 case of anthrax, and Kentucky and Tennessee each 1 case of Rocky Mountain spotted fever.

Deaths recorded during the week in 94 large cities in the United States totaled 9,518, as compared with 9,910 last week, 10,478 and 9,654, respectively, for the corresponding weeks of 1948 and 1947, and a 3-year median of 10,156. The total to date is 40,114, same period last year 42,360. Infant deaths totaled 635, last week 685, 3-year median 677. The cumulative figure is 2,745, same period last year 2,913.

Telegraphic case reports from State health officers for week ending January 29, 1949

(Leaders indicate that no cases were reported)

Division and State	Diphtheria	Encephalitis, infectious	Influenza	Measles	Meningitis, meningococcal	Pneumonia	Polio-myelitis	Rocky Mountain spotted fever	Scarlet fever	Small-pox	Tularemia	Typhoid and paratyphoid fever	Whooping cough	Rabies in animals
NEW ENGLAND														
Maine.....				374	1	9			9				10	
New Hampshire.....				10									5	
Vermont.....	1			276	2				10				10	
Massachusetts.....	9			1,171					313				67	
Rhode Island.....				1,195		9			5				4	
Connecticut.....	2		11	207	2	71			42				14	
MIDDLE ATLANTIC														
New York.....	3		42	993	5	289	3		264			2	154	5
New Jersey.....	1			338	3	64	2		127				64	5
Pennsylvania.....	7	1		1,145	4		1		261			6	74	
EAST NORTH CENTRAL														
Ohio.....	12		6	62	1	50	1		334		1	1	41	23
Indiana.....	13	2	60	50	1	26	1		58			1	30	17
Illinois.....	1	1	3	64	6	106			293		8	1	41	2
Michigan.....	2			569	1	31	3		367				57	
Wisconsin.....			23	583	7	17			83				32	1
WEST NORTH CENTRAL														
Minnesota.....	5			22	1	3	2		92				3	
Iowa.....				17	3		3		65				4	5
Missouri.....	3		11	350	7	25			26		2	1	5	
North Dakota.....				17					19				4	
South Dakota.....			3	33	1	4			4				4	
Nebraska.....				10			2		10					
Kansas.....	1		3	286	2	28	1		35				2	1
SOUTH ATLANTIC														
Delaware.....				12					5				1	
Maryland.....	4			774	2	41	1		31			1	4	
Dist. of Col.....				49	1				11			1	5	
Virginia.....	1			815		106	2		17				20	2
West Virginia.....	1		464	144		19			34				6	
North Carolina.....	11		44	232			9		37			2	25	
South Carolina.....	10			695	1	173			6		5	2	36	3
Georgia.....	1		27	84	2	51	1		21		2	3	15	1
Florida.....	4	1	22	112		14	1		9		8		12	

EAST SOUTH CENTRAL										
Kentucky	3	36	511	4	41	1	85	4	154	11
Tennessee	2	62	84	5	48	1	48	1	3	3
Alabama	14	101	621	1	57	2	12	2	62	2
Mississippi*	5	31	13	1	38		6	1	8	
WEST SOUTH CENTRAL										
Arkansas	5	290	279		89		4	3	1	2
Louisiana	2	43	43	1	25		7		4	
Oklahoma	6	47	907		25		22	1	1	5
Texas	36	2,327	2,086	11	478	14	27	5	85	34
MOUNTAIN										
Montana		9	50		12		7			
Idaho	3	53	50			1	• 22		2	
Wyoming			22	1	7					
Colorado	1	74	182		32		23		6	
New Mexico	1	2	302		16		14	1	1	
Arizona	3	81	152		37	6	3		20	
Utah*	4	76	76		12		4	2	20	
Nevada										
PACIFIC										
Washington		14	344	3	1	5	63		25	
Oregon	1	26	474		30	2	9		20	
California	8	28	880	7	53	30	116	4	52	2
Total	190	6	4,534	86	2,182	95	2,982	26	38	1,207
Median, 1944-48	294	6	14,253	216		36	3,123	4	46	2,117
Year to date 4 weeks	775	29	17,341	350	8,488	494	10,235	145	148	4,208
Median, 1944-48	1,277	26	46,635	909	20,285	160	10,939	104	169	8,985
Seasonal low week ends	July 10		(30th)	(37th)	(11th)	Mar. 20	(32nd)	(35th)	(11th)	(39th)
Since seasonal low week	5,889	53,611	104,001	1,194	27,821	13,547	32,933	3,267	14,241	3,267
Median, 1943-48	8,843	90,193	46,409	2,413			49,510	4,420		33,837

* Period ended earlier than Saturday.

^a New York City and Philadelphia only, respectively.

^b Including cases reported as streptococcal infection and septic sore throat.

^c Including paratyphoid fever, currently reported separately, as follows: New York 1; Georgia 1; salmonella infections, not included, were reported as follows: New York 3; Maryland 1.

^d *Arizhar*: Pennsylvania 1; Kansas 1.

^e *Pittococci*: Virginia 2 cases, 1 each in Henrico and Louisa Counties.

^f *Alaska*: Measles 3; pneumonia 1; streptococcal throat 17.

^g Territory of Hawaii: Influenza 18; measles 410; whooping cough 4.

TERRITORIES AND POSSESSIONS

Virgin Islands of the United States

Notifiable diseases—October–December 1948.—During the months of October, November, and December 1948, cases of certain notifiable diseases were reported in the Virgin Islands of the United States as follows:

Disease	October	November	December
Cancer.....	1		5
Chickenpox.....	1		
Fever, undetermined.....			1
Filariasis.....	2		
Gonorrhoea.....	9	18	15
Hookworm disease.....			4
Measles.....	11	13	
Pneumonia, broncho.....	1		
Syphilis.....	26	24	13
Tuberculosis, pulmonary.....		3	

FOREIGN REPORTS

CANADA

Provinces—Communicable diseases—Week ended January 8, 1949.—During the week ended January 8, 1949, cases of certain communicable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chicken pox.....		63	2	225	1,045	57	102	161	321	1,976
Diphtheria.....		1		30	5	1		3		40
Dysentery, bacillary.....				1						1
German measles.....		3		23	14		2	9	4	55
Influenza.....		29			7					36
Measles.....		125		110	221	106	79	195	84	920
Meningitis, meningococcal.....		1	1	1					1	4
Mumps.....		17		50	329	41	37	16	211	701
Poliomyelitis.....									1	1
Scarlet fever.....		6		153	89	7	4	9		277
Tuberculosis (all forms).....		7	17	69	20	6	4	18	43	184
Typhoid and paratyphoid fever.....				3	1					4
Undulant fever.....					2	1			1	4
Venereal diseases:										
Gonorrhoea.....	2	24	10	132	72	22	20	37	40	359
Syphilis.....	4	5	10	72	40	14	3	7	8	163
Other forms.....									2	2
Whooping cough.....		3		72	40	8	14			137

Diphtheria

Alberta.—Information from Calgary, Canada, dated January 27, 1949, states that six cases of diphtheria with one death have occurred at Nobleford, Alberta, just north of Lethbridge.

CUBA

Habana—Communicable diseases—5 weeks ended December 31, 1948.—During the 5 weeks ended December 31, 1948, certain communicable diseases were reported in Habana, Cuba, as follows:

Disease	Cases	Deaths
Chickenpox.....	3
Diphtheria.....	15
Measles.....	2
Tuberculosis.....	7
Typhoid fever.....	11	1

Provinces—Notifiable diseases—5 weeks ended December 31, 1948.—During the 5 weeks ended December 31, 1948, cases of certain notifiable diseases were reported in the provinces of Cuba, as follows:

Disease	Pinar del Rio	Habana ¹	Matanzas	Santa Clara	Camaguey	Oriente	Total
Cancer.....	5	6	12	24	3	27	77
Chickenpox.....	3	4
Diphtheria.....	1	18	2	2	1	4	28
Hookworm disease.....	7	7
Leprosy.....	1	1	1	1	4
Malaria.....	1	5	1	11	10	402	430
Measles.....	4	1	5
Poliomyelitis.....	1	1	2
Scarlet fever.....	1	1
Tuberculosis.....	3	12	8	15	16	19	73
Typhoid fever.....	8	19	15	6	38	86
Undulant fever.....	1	1
Whooping cough.....	104	104

¹ Includes the city of Habana.

JAPAN

Notifiable diseases—4 weeks ended December 25, 1948, and accumulated totals for the year to date.—For the 4 weeks ended December 25, 1948, and for the year to date, certain notifiable diseases were reported in Japan as follows:

Disease	4 weeks ended December 25, 1948		Total reported for the year to date	
	Cases	Deaths	Cases	Deaths
Diphtheria.....	1,636	215	16,170	1,498
Dysentery, unspecified.....	182	64	14,638	4,044
Encephalitis, Japanese "B" ¹	12	9	17,666	2,950
Gonorrhoea.....	13,041	217,918
Influenza.....	142	2,809
Malaria.....	102	2	4,933	42
Measles.....	3,884	54,660
Meningitis, epidemic.....	83	21	2,038	512
Paratyphoid fever.....	173	5	2,890	148
Pneumonia.....	7,512	110,593
Scarlet fever.....	390	6	2,918	44
Smallpox.....	1	29	1
Syphilis.....	14,805	214,535
Tuberculosis.....	27,204	378,523
Typhoid fever.....	661	74	9,414	1,123
Typhus fever.....	37	1	488	33
Whooping cough.....	3,843	52,789

¹ Includes suspected cases.

Note.—The above figures have been adjusted to include delayed and corrected reports.

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

Note.—The following reports include only items of unusual incidence or of special interest and the occurrence of these diseases, except yellow fever, in localities which had not recently reported cases. All reports of yellow fever are published currently.

A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

Cholera

India—Madras Province.—Cholera has been reported in Madras Province, India, as follows: Week ended December 18, 1948, 1,115 cases with 594 deaths; week ended December 25, 1948, 1,207 cases with 642 deaths.

Pakistan—Chittagong.—During the period January 1–15, 1949, 24 cases of cholera with 17 deaths were reported in Chittagong, Pakistan.

Plague

Belgian Congo—Stanleyville Province.—During the week ended January 22, 1949, 2 fatal cases of plague were reported northeast of Blukwa in Stanleyville Province, Belgian Congo.

Burma.—Plague has been reported in Burma as follows: Week ended November 27, 1948, 39 cases, 35 deaths; period November 28–December 31, 1948, 151 cases, 111 deaths; week ended January 8, 1949, 52 cases, 38 deaths.

Portugal—Azores.—Bubonic plague has been reported in the Azores Islands as follows: During the week ended November 13, 1948, 1 case at Ponta del Gada, Arrifes District; week ended November 27, 1948, 1 case Ribeira Grand, Matriz District.

Smallpox

Bahrein Islands.—During the week ended January 15, 1949, 19 cases of smallpox with 2 deaths were reported in the Bahrein Islands.

India—Ahmedabad.—Smallpox has been reported in Ahmedabad, India, as follows: For the week ended January 1, 1949, 32 cases, 16 deaths; week ended January 8, 1949, 80 cases, 32 deaths.

Iraq.—For the week ended January 22, 1949, 48 cases of smallpox with 9 deaths were reported in Iraq, including 15 cases, 4 deaths in Baghdad City.

Syria—Aleppo.—During the week ended December 25, 1948, 29 cases of smallpox were reported in Aleppo, Syria.

Transjordan—Amman.—Smallpox has been reported in Amman, Transjordan, as follows: For the week ended December 18, 1948, 7 cases; week ended January 8, 1949, 2 cases; week ended January 15, 1949, 11 cases.

Yellow Fever

Panama.—Information dated January 31, 1949, states that the outbreak of jungle yellow fever reported recently at Pacora, Panama, is under control. Of the confirmed deaths reported, the last one is stated to have occurred on December 30, 1948.

DEATHS DURING WEEK ENDED JAN. 22, 1949

[From the Weekly Mortality Index, issued by the National Office of Vital Statistics]

	Week ended Jan. 22, 1949	Correspond- ing week, 1948
Data for 94 large cities of the United States:		
Total deaths.....	9,910	10,305
Median for 3 prior years.....	10,181	-----
Total deaths, first 3 weeks of year.....	30,596	31,882
Deaths under 1 year of age.....	685	728
Median for 3 prior years.....	722	-----
Deaths under 1 year of age, first 3 weeks of year.....	2,110	2,233
Data from industrial insurance companies:		
Policies in force.....	70,650,802	66,909,483
Number of death claims.....	13,338	14,692
Death claims per 1,000 policies in force, annual rate.....	9.8	11.5
Death claims per 1,000 policies, first 3 weeks of year, annual rate.....	9.4	11.3

Notifiable Diseases, Third Quarter, 1948¹

The figures in the following table are the totals of the monthly morbidity reports received from State health authorities for July, August, and September, 1948. These reports are preliminary and the figures are more or less incomplete and subject to correction by final reports. The figures may be assumed to represent the civilian population only, although in some instances a few cases in the military population may be included. The comparisons made are with similar preliminary reports; but, owing to population shifts in many States since the 1940 census, the figures for some States may not be comparable with those for prior years, especially for certain diseases. Each State health officer has been requested to include in the monthly report for his State all diseases that are required by law or regulation to be reported in the State, although some do not do so. The list of diseases required to be reported is not the same for each State. Only 11 of the common communicable diseases are notifiable in all the States. In some instances cases are reported, in some States, of diseases that are not required by law or regulation to be reported and the figures are included although manifestly incomplete. There are also variations among the States in the degree of, and checks on, the completeness of reporting of cases of the notifiable diseases; therefore comparisons as between States may not be justified for certain diseases. As compared with the deaths, incomplete case reports are obvious for such diseases as malaria, pellagra, pneumonia, and tuberculosis, while in many States other diseases, such as puerperal septicemia, rheumatic fever, and Vincent's infection, are not reportable.

In spite of these and other deficiencies inherent in morbidity reporting, these monthly reports, which are published quarterly and annually in consolidated form, have proved of value in presenting early information regarding the reported incidence of a large group of diseases and in indicating trends by providing a comparison with similar preliminary figures for prior years. The table gives a general picture of the geographic distribution of certain diseases, as the States are arranged by geographic areas.

Leaders are used in the table to indicate that no case of the disease was reported.

Consolidated monthly State morbidity reports for July, August, and September 1948

Division and State	An-thrax	Chick-enpox	Con-junc-tivitis ²	Diph-theria*	Dysen-tery, amebic	Dysen-tery, bacil-lary	Dysen-tery, unde-fined	En-cepha-litis, infec-tious	Ger-man mea-sles	Hook-worm disease	Influ-enza	Ma-laria ³	Mea-sles*	Men-ingitis, menin-gococ-cus*	Mumps	Oph-thal-mia	Fella-gra	Pneu-monia, all forms
NEW ENGLAND																		
Maine.....		279		5					20		6		535	9	85			113
New Hampshire.....		32							9		17		200	2	57			9
Vermont.....		171							37				204	1	50			6
Massachusetts.....		917		61	2	30		5	166			6	3,260	16	1,566	497		476
Rhode Island.....		37		2	2	4		1	51		2	2	44	2	67			35
Connecticut.....		350		4	1	5		2	51		7	4	528	9	516	1		285
MIDDLE ATLANTIC																		
New York.....	1	1,809		56	109	77		7	167	32	13	19	6,074	44	619	17		1,520
New Jersey.....		157		22	12	2			205		15	12	3,742	21	2,281	42		467
Pennsylvania.....	3	777		48	3	1	1	4			18		2,841	36	1,246	5		618
EAST NORTH CENTRAL																		
Ohio.....		758		65	10	19	2		49		13	5	986	18	362	119		343
Indiana.....		72		16	2	8	5	9	22		21	7	264	5	117		1	60
Illinois.....		799		42	84	42		26	60		25	17	614	45	1,036	26		920
Michigan.....		880		15	219	22		5	100	15	3	10	2,693	19	800			311
Wisconsin.....		1,221		4	2			2	129		33	1	2,732	9	1,453			48

Consolidated monthly State morbidity reports for July, August, and September, 1948—Continued

Division and State	Pollomyelitis*	Rabies in man	Rheumatic fever	Rocky Mountain spotted fever	Scarlet fever*	Septic sore throat	Smallpox*	Tetanus	Trachoma	Trichinosis	Tuberculosis, all forms*	Tuberculosis, respiratory	Tularemia	Typhoid fever*	Paratyphoid fever	Typhus fever, endemic	Undulant fever*	Vincent's infection	Whooping cough*
NEW ENGLAND																			
Maine.....	29		2		58	4				5	131	123		7	13 5			2	85
New Hampshire.....	21				11	19		1			37							4	24
Vermont.....	10				20	1												4	92
Massachusetts.....	133				450	7		2		13	816	767		11	16		2	11	688
Rhode Island.....	6		45		36	14				2	166	152		1	2		2	1	25
Connecticut.....	84				67	61		3		1	447	424		4	13 3		25		94
MIDDLE ATLANTIC																			
New York.....	891			19	14 513			7		52	3,510	3,270		25	13 24	5	58		1,429
New Jersey.....	540			3	115	18		5		10	755			11	2	1	12		713
Pennsylvania.....	475		224	6	398			1		7	1,254		1	69	13 7		27		830
EAST NORTH CENTRAL																			
Ohio.....	863		26	5	566	1	1	10		4			3	44	7		44	2	562
Indiana.....	258		4	7	123	12		15	3		793	747		25	2		15	5	144
Illinois.....	745		22	11	294	18		12	4	7	2,044	1,908	7	29	6		147	29	580
Michigan.....	488		106		328	44		19		2	1,802		1	15	13 87		55		402
Wisconsin.....	386			1	132	5					685			3			85		495
WEST NORTH CENTRAL																			
Minnesota.....	840		16	1	119	20		2			738		1	11	13 63		53		105
Iowa.....	883			3	63	8				1	214		4	3	1		186		88
Missouri.....	195		2	8	62	3		10	348		557		26	33	1		17	1	89
North Dakota.....	83				29		1				89	86		3	2		3	12	45
South Dakota.....	200			2	10				5		47						23		12
Nebraska.....	494		5		81		1			1	148			2			28		78
Kansas.....	222		1	3	73	1		3			263	285	2	7	1		42	9	202
SOUTH ATLANTIC																			
Delaware.....	114			1	5	1					131	131		3	13 2	1	13	9	7
Maryland.....	107		14	28	66	13		5			1,178	1,146		9	2				206
District of Columbia.....	118			4	61									9					61
Virginia.....	399		1	35	77	567		3			1,087	1,066	16	39	9	1	22		469
West Virginia.....	122			8	85	9					559		2	24	2		3		125
North Carolina.....	1,906			56	132	7		1			813	775	6	23	3		6		494
South Carolina.....	280		80	3	29	1,670		19			90		4	41	1		1		682
Georgia.....	161		17	20	101	48		19			852	842	13	57	21		39	15	124
Florida.....	150				32	19		18	35		769		4	22	13 29	42	13	22	130

EAST SOUTH CENTRAL																		
Kentucky.....	138	1	10	11	144	7	2	15	29	548	538	1	74	4	8	10	7	161
Tennessee.....	256	2	6	29	195	128		14		1,862		10	56	2	6	23	79	289
Alabama.....	138			11	89		2	3	1	827	966	3	24		76	36		183
Mississippi.....	119	2	10		46					991		4	16	3	13	25		23
WEST SOUTH CENTRAL																		
Arkansas.....	106	1	2	1	60	372		5	34	727	715	63	55	2	3	11		180
Louisiana.....	80	1	11	2	17	6		15		904	870	1	47	6	20	12		38
Oklahoma.....	279			14	63	28	1	2	60	632	616	22	27	13	1	26		127
Texas.....	876	1		1	119	1,155		47	47	2,800		17	125	11	118	186		1,443
MOUNTAIN																		
Montana.....	32			2	41	11			18	208	206	5	2	1		1	2	64
Idaho.....	47		10	1	19	40				74		1	4	8		9	9	45
Wyoming.....	62		2	1	8			1		15	3	16				2		33
Colorado.....	85		32	4	58	238				485		3	17	11		70	11	210
New Mexico.....	54		11	1	26	5		1	7	845	843		7			2		82
Arizona.....	103		14	1	11	12			155	539	521		12			1		101
Utah.....	51		19	2	12	13		1	3	833	832	8	23			8	17	8153
Nevada.....	9		1	1	1					10						1	5	11
PACIFIC																		
Washington.....	165		76		129	6				360		2	4	13		12	28	92
Oregon.....	91		18	2	63	37				194	179	1	5			6		275
California.....	3,074		112	1	424	55		23	4	2,286	2,143	4	48	43	7	41		684
Total.....	16,678	8	899	308	5,659	4,673	9	206	744	33,484	19,511	267	1,054	341	493	1,423	254	13,114
Third quarter 1947.....	6,235	9	842	324	6,311	4,198	9	167	263	35,183	18,076	332	1,210	13,375	612	1,873	459	45,081
Median 1943-47.....	5,766	9	15,841	266	11,912	1,754	38	151	372	29,998	17,688	208	1,809	353	1,770	1,501	544	34,371
Alaska.....	1		2		5					44	44		2				1	36
Hawaii Territory.....			8		2	15		3		190	175		1	1	5			84
Panama Canal Zone ¹⁰								1		1114			6	1	4			118

See footnotes on page 228.

Footnotes for Table on Pages 224 to 227

Diseases marked with an asterisk () are reportable by law or regulation in all the States, including the District of Columbia. Typhoid fever is reportable in all the States; paratyphoid fever in all except 6 States. Syphilis is reportable in all the States and the District of Columbia but is not included in the table. Some States have increased and some have reduced the list of reportable diseases since the latest published compilation of reportable diseases (PUBLIC HEALTH REPORT 59:317-340) (Mar. 10, 1944. Reprint No. 2544).

- 1 For reports for first and second quarters of 1948 see pages 650 and 1,424 of the Public Health Reports for July 16 and Oct. 29, 1948, respectively.
- 2 Includes cases of terato- and suppurative conjunctivitis and of pink eye.
- 3 In some instances the infection was acquired outside of the United States.
- 4 Reported as ophthalmia neonatorum.
- 5 Iolux preparation only.
- 6 New York City only.
- 7 Exclusive of 6 cases officially induced.
- 8 Includes nonresident cases.
- 9 Contracted outside of State.
- 10 Includes the titles of Colon and Panama.
- 11 In the Canal Zone only.
- 12 Includes cases with heart involvement.
- 13 Includes cases reported as salmonella infection.
- 14 Includes septic sore throat.
- 15 3-year median (1948-47).

The following list includes certain rare conditions, diseases of restricted geographical distribution, and those reportable in or reported by only a few States; last year's figures in parentheses (where no figures are given, no cases were reported last year):

- Actinomycosis:** Massachusetts 2, New York 2, Minnesota 2 (7), Kentucky 1, Idaho 2.
- Botulism:** Tennessee 1 (2), Alaska 2.
- Cancer:** North Dakota 231, Kansas 981, South Carolina 369, Georgia 56, Florida 451, Kentucky 2, Tennessee 643, Alabama 1,013, Mississippi 416, Arkansas 184, Louisiana 648, Montana 287, Idaho 202, New Mexico 184, Utah 65 (includes nonresidents).
- Coccidioidomycosis:** Arizona 5, California 19 (10).
- Colorado tick fever:** Colorado 15 (4).
- Dengue:** South Carolina 2 (4), Florida 1, Texas 10 (5), Panama Canal Zone 1.
- Dermatitis:** New Hampshire 12 (7), Missouri 7 (20), Kentucky 41 (mycotic dermatitis), Arkansas 1 (3).
- Diarrhea:** Rhode Island 4, Connecticut 10, New York 71 (82), Pennsylvania 68 (77), includes enteritis, Ohio 738 (832) includes enteritis, Indiana 2 (4), Illinois 16 (18), Michigan 24 (11), Maryland 10 (8), South Carolina 4,206 (3,267), Florida 36 (16), Oklahoma 1, Idaho 13, gastroenteritis, Colorado 4, Nevada 1 enteritis, New Mexico 103 (32), Washington 6, California 8 (25), Alaska 6, includes enteritis.
- Dog bite:** Massachusetts 3,723, Illinois 5,221 (4,504) all animals, Michigan 3,432 (3,129), Arkansas 177 (161), all animals.
- Encephalitis (other forms):** Ohio 1, Michigan 24, Maryland 5, Florida 1, Idaho 3, Colorado 4, New Mexico 2, Panama Canal Zone 1.
- Erysipelas:** New Hampshire 3, Vermont 1, Connecticut 4, Ohio 10, Indiana 2, Illinois 17, Michigan 12, Wisconsin 6, North Dakota 1, South Dakota 2, Kansas 2, Maryland 4, Florida 17, Kentucky 1, Tennessee 11, Arkansas 5, Louisiana 3, Montana 3, Idaho 7, Colorado 11, New Mexico 1, Washington 2, Oregon 9, Alaska 1, Hawaii Territory 4.
- Favus:** Florida 1.
- Food poisoning:** New York 161, Indiana 6 (14), Illinois 6 (6), Minnesota 78 (70), Kansas 4, Idaho 6, Colorado 1 (4), New Mexico 8 (4), Washington 12 (35), Oregon 5 (2), California 110 (665), Hawaii Territory 56.
- Granuloma inguinale:** West Virginia 1, Florida 252 (71), Tennessee 14 (27), Mississippi 18 (65), Louisiana 40 (47), Idaho 1, Utah 1, California 5.
- Impetigo contagiosa:** New York 45, Ohio 40 (2), Indiana 27 (27), Illinois 13 (11), Michigan 186 (278), Missouri 10 (30), North Dakota 17, Kansas 11 (28), Maryland 2 (7), Kentucky 13, Montana 4 (6), Idaho 4 (20), Wyoming 3 (2), Colorado 5 (2), Nevada 41 (26), Washington 113 (99), Alaska 9, Hawaii Territory 9.
- Jaundice (including hepatitis and Weil's disease):** Maine 2 (6), Rhode Island 1 (1), Connecticut 1, New York 35 (44), Pennsylvania 16 (16), Illinois 3 (4), Michigan 4 (1), Minnesota 3 (1), Maryland 2 (2), Florida 5 (6), Kentucky 13 (1), Tennessee 7 (7), Montana 2, Idaho 2 (6), Nevada 1, Washington 3 (9), Oregon 5 (17), California 25 (47), Alaska 36, Hawaii Territory 10, Panama Canal Zone 6.
- Lead poisoning:** Kansas 1.
- Leprosy:** New York 1 (1), Florida 1, Louisiana 3 (2), Texas 4 (4), California 2 (2), Hawaii Territory 4.
- Lymphocytic choriomeningitis:** Maine 1, Massachusetts 16 (1), Rhode Island 3, Minnesota 1, Tennessee 5 (2).
- Lymphogranuloma venereum:** Missouri 1 (5), Florida 73 (35), Kentucky 1, Tennessee 19 (27), Mississippi 17, Louisiana 36 (30), Arizona 2 lymphogranuloma undefined, California 11.
- Mononucleosis:** Connecticut 27, Ohio 2, Michigan 11, Minnesota 67, Maryland 12, Kentucky 1, Tennessee 4, Idaho 3.
- Pittacosis:** Michigan 1, California 1.
- Prurperal septicaemia:** New York City 4, Florida 1 (1), Tennessee 1 (1), Mississippi 1, Arkansas 3, Louisiana 1.
- "Q" Fever:** Nebraska 1.
- Rabies in animals:** New York 114 (154), Pennsylvania 32, Ohio 120 (160), Indiana 173, Illinois 22 (48), Michigan 100 (73), Wisconsin 2, Minnesota 2, Iowa 8, Kansas 5 (19), Virginia 35, South Carolina 46 (43), Georgia 65, Florida 26 (115), Kentucky 79, Alabama 82 (91), Arkansas 15 (23), Louisiana 8 (2), Oklahoma 35, Texas 245 (247), Arizona 5, California 44 (42).
- Relapsing fever:** Texas 35 (34), Nevada 1 (1), California 4 (17), Panama Canal Zone 5.
- Rickettsialpox:** New York 52.
- Ringworm (including ringworm of the scalp):** Connecticut 8, Pennsylvania 39 (85), Ohio 22 (5), Indiana 9, Illinois 79 (75), Michigan 162 (163), Minnesota 12 (9), Missouri 12 (4), Kansas 8, Maryland 1, Kentucky 17 (5), Arkansas 1, Idaho 11 (14), Utah 4 (16), Washington 7 (134), Oregon 1.
- Scabies:** Pennsylvania 114 (56), Ohio 20, Indiana 10, Michigan 125 (128), Missouri 7 (12), North Dakota 4, Kentucky 5 (8), Montana 15 (2), Idaho 9 (31), Wyoming 5, Nevada 2 (4).
- Schistosomiasis:** New York City 1.
- Silicosis:** New Hampshire 1, Kansas 2, Arkansas 1 (3), Colorado 2, New Mexico 7 (2), includes anthracosis.
- Yaws:** Panama Canal Zone 3.