FLUORESCENT ANTIBODY TECHNIQUE FOR IDENTIFICATION OF PRESUMPTIVELY POSITIVE GONOCOCCAL CULTURES

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LABORATORY confirmation of *Neisseria gonorrhoeae* by culture is sometimes complicated by inability to obtain a viable subculture. This inability usually results from failure to recognize gonococcal colonies until after they have been subjected to the oxidase reagent (dimethyl-p-phenylenediamine hydrochloride). This reagent rapidly kills the gonococci, usually by the time the colony has turned black—a matter of 5–6 minutes. Such an occurrence makes it impossible to obtain the subculture needed for the purification and fermentation procedures used in identification.

Ordinarily, when large numbers of colonies are present, no difficulty is encountered, since only a small amount of oxidase reagent is applied to colonies on part of the plate. When only two or three colonies are present on the plate, however, they may be overlooked and not be observed until the oxidase reagent has killed them. This situation is sometimes encountered in tests of cure after antibiotic therapy, in chronic gonorrhea of the female, and when the asymptomatic carrier state exists.

In our study, immunofluorescent procedures were used successfully to identify the nonviable oxidase-positive colonies as *N. gonorrhoeae*.

Materials and Methods

We used fluorescein-labeled antigonococcal rabbit serums absorbed with *Neisseria meningitidis* serogroup B (hereafter referred to as GC conjugate) to identify by four methods 18- to 48-hour cultures grown on Thayer-Martin selective medium (*1*). Sixty-four specimens were examined by methods A, B, and D, but only 48 by method C. Sixty percent of the specimens from each group were isolated from male patients. The cultures represented no more than four transfers from the patients.

Under method A, suspected colonies were smeared on microscope slides, heat fixed, gram stained, and read. The smears then were cleaned by removing the immersion oil with xylene, rinsed thoroughly with tapwater, and blotted dry. After being treated with GC conjugate, the smears were incubated for 30 minutes in a moist chamber at 35° C. The prepared slides were read with a fluorescence microscope. Under method B, cultures were treated with oxidase reagent for 6–10 minutes. The oxidase-positive colonies were smeared on a microscope slide and treated in the same manner with GC conjugate.

Under method C, the cultures were treated with oxidase reagent, and smears of positive colonies were gram stained and read. Smears were cleaned as in method A and treated with GC conjugate. Method D served as a control, since suspected colonies from the same culture were treated only with GC conjugate. All cultures were confirmed by sugar fermentation. Eight cultures of *Staphylococcus aureus* grown on conventional media were treated with (*a*) crystal violet, iodine, alcohol, and safranin, (*b*)

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crystal violet, (*c*) iodine, (*d*) alcohol, (*e*) safranin, (*f*) crystal violet-iodine, and (*g*) crystal violet-iodine-alcohol. The smears of the eight cultures of staphylococci so treated were subsequently also treated with GC conjugate.

N. meningitidis serogroups A, B, C, and D, grown on Thayer-Martin medium, and *Neisseria catarrhalis*, *Neisseria sicca*, *Neisseria flava*, *Neisseria subflava*, *Neisseria perflava*, *Neisseria flavescens*, *S. aureus*, the streptococci groups A, B, C, D, F, and G, *Mima polymorpha*, and *M. polymorpha* var. *oxidans*, all cultivated on conventional media, were treated with oxidase and fluorescent antibody (FA) (method B).

Results

Under method B, all of the cultures presumptively positive by oxidase fluoresced strongly 3 to 4+ by the FA technique, as did the control group D (see table). Smears from group D were subsequently gram stained. Examinations revealed typical gram-negative diplococci. In group A, in which the cultures had been pretreated with gram stain, only 56.2 percent fluoresced strongly 3 to 4+, while 32.8 percent gave a 2+ reaction and 10.9 percent stained only 1+. In group C, in which the cultures had been pretreated with oxidase reagent and gram stain, only 45.9 percent fluoresced strongly 3 to 4+, while 43.8 percent gave a 2+ reaction and 10.4 percent stained 1+ or less. Because of the reduced staining, we examined only 48 specimens by method C.

Staphylococcal cultures treated with the gram stain or a combination of the reagents used in the gram stain would fluoresce 1+ if treated with GC conjugate only when the complete gram stain was performed before the FA staining and safranin was not used. If, however, the alcohol step preceded the FA procedure, the FA technique did yield a ± reaction.

Bacteria (see Materials and Methods) other than *N. gonorrhoeae* did not fluoresce after treatment with oxidase, nor subsequently, with GC conjugate.

Discussion

In 1959, Deacon and others (*2*) described the application of the FA technique to the specific identification of *N. gonorrhoeae*. A subsequent study demonstrated that direct FA smears from suspected female carriers were not as efficient in detecting *N. gonorrhoeae* as either the culture or the delayed FA enrichment procedure (*3*). While these two procedures were equal in sensitivity in the primary diagnosis of untreated subjects, a comparison of the culture and delayed FA methods as a test of cure on treated patients was discouraging (*4*). It became evident in our study that FA-positive reactions were frequent when the delayed FA method was used, although cultures remained negative when both procedures were used as a test of cure. There are at least two reasons for this result. First, dead gonococci, carried over to the delayed culture slant and subsequently to the smear by the implanted swab, may fluoresce in the FA procedure as do other bacteria (*5*, *6*). Second, in vitro studies that Peacock conducted in 1967 demonstrated that other bacteria, notably streptococci, would fluoresce in the FA procedure following a treatment with lethal concentrations of penicillin.

In 1964, Thayer and Martin (*1*) described the selective medium for isolation of pathogenic *Neisseria*. Published reports in this country and abroad indicate that the selective culture method has been widely accepted (*7–13*). These studies show that the difficulties associated with

Fluorescent antibody results by four methods

Method	Number of patients	Degree of staining [1]									
		4+	Percent	3+	Percent	2+	Percent	1+	Percent	±	Percent
A	64	18	28.1	18	28.1	21	32.8	7	10.9	0	0
B	64	56	87.5	8	12.5	0	0	0	0	0	0
C	48	9	18.8	13	27.1	21	43.8	4	8.3	1	2.1
D	64	55	85.9	9	14.1	0	0	0	0	0	0

[1] None of the cultures showed negative results.

the culture method, such as overgrowth by contamination and the added time needed to isolate the gonococci for fermentation procedures, have apparently been eliminated. The presumptive cultural diagnosis was further strengthened by the demonstration that saprophytic *N. catarrhalis*, *N. sicca*, and so forth would not grow on the selective medium. Streptococci and staphylococci were also inhibited. From the foregoing data, cultures that are presumptively positive by oxidase can be confirmed by the FA technique as gonococci. In large general surveys in which cultures are used to determine the incidence of gonorrhea, the FA procedure can be used to rapidly confirm the culture diagnosis without use of sugar fermentation reactions.

Summary

Sixty-four cultures identified as *Neisseria gonorrhoeae* by standard methods were treated with oxidase reagent and gram stained by three experimental methods. The cultures were subsequently treated with *N. gonorrhoeae* fluorescein-labeled antiserums. Results showed that colonies of gonococci killed by action of the oxidase reagent (dimethyl-p-phenylenediamine hydrochloride) can be specifically identified by fluorescent antibody techniques. The gram stain, however, interferes with fluorescence of the gonococcal cell.

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Education Notes

Fellowships for a Master's Degree in Public Health. Fellowships for 1- and 2-year programs of study which prepare students for administrative positions at the policymaking level in public and private medical care programs, health insurance plans, and other agencies concerned with providing and financing personal health services are available from the University of Michigan.

The 1-year program is intended for students with an advanced degree or appropriate related work experience. The 2-year program is designed for those with only a bachelor's degree.

Both programs lead to the degree of master of public health and are designed to provide students with an understanding of the principles of the social science disciplines as they relate to the functioning of the medical care system; a working knowledge of community organization, administrative practice, and organizational behavior; and a thorough knowledge of the organization of medical care in the United States.

Fellowship stipends are $3,000–$3,600 (depending on the applicant's educational background), plus an additional $500 for each dependent, and full tuition.

For an application or further information write to Eugene Feingold, Department of Medical Care Organization, Room 3547 C, School of Public Health, University of Michigan, Ann Arbor 48104.

Environmental Health Fellowships. Under an interdepartmental program to give students broad training for careers in research, teaching, and practice in environmental health, the Consolidated University of North Carolina (Chapel Hill and Raleigh campuses) is offering environmental health fellowships for graduate study during 1968–69.

The program is sponsored jointly by the departments of environmental sciences and engineering, biostatistics, and epidemiology of the School of Public Health; the departments of city and regional planning, zoology, botany, chemistry, and geology of the School of Arts and Sciences; and the department of food science at North Carolina State University at Raleigh.

Students usually will enroll in the department of their basic specialty and then select courses in other departments to obtain a broad understanding of the problems of the environment and the application of their specialty to the solution of those problems.

The fellowships provide tuition, fees, and a stipend. The amount of the stipend will be in accordance with current Public Health Service regulations and university policy.

Additional information is available from the head of any of the departments mentioned, located at Chapel Hill 27514, except for the department of food science at Raleigh 27607.

Health Administrators Development Program. A 4-week course of lectures, readings, and discussions dealing with problems and developments in the organization of medical care services will be held at Cornell University, June 16 to July 12, 1968.

Thirty-five $500 scholarships are available to experienced health service administrators and planners. Executives from community hospitals, university medical centers, psychiatric hospitals, planning councils, State and Federal health agencies, and other health organizations will be considered for enrollment.

Scholarships cover tuition and other educational costs. The total charge to each participant is $400, which covers room and board.

For further information write to the Sloan Institute of Hospital Administration, Graduate School of Business and Public Administration, 315 Malott Hall, Cornell University, Ithaca, N.Y. 14850.

Oral Medicine Research Training Program. A program for qualified persons interested in oral medicine and clinical investigation at advanced levels of study has been established at the University of Pennsylvania School of Dental Medicine and the Philadelphia General Hospital.

Supported by the National Institute of Dental Research, Public Health Service, the program involves simultaneous instruction and research in oral medicine for candidates who will participate in departmental research under supervision and direction preparatory to selecting problems for independent investigation.

The aim is to recruit and train investigators who will provide leadership and direction for research projects. Some candidates will wish to return to clinical or basic scientific investigations, others to teaching.

Another objective is to provide postgraduate experience in oral medicine and research analogous to doctoral programs in the medical specialties. This ex-

perience will entail a new and different type of teaching by which a few participants will receive advanced instruction, most of it tutorial.

Consideration will be given to candidates having demonstrable competence in basic science and holding a medical or dental degree and who have also served a 1-year hospital internship. If there are gaps in a candidate's background, study in the basic sciences at the Graduate School of Arts and Sciences will be included.

Needs will vary with the interests, aptitudes, and aspirations of the participant. In general, research programs will be long term in concept and involve a variety of scientific backgrounds.

Persons who wish to be considered should make known their qualifications and research interests to one of the following: Dr. Vernon J. Brightman, assistant professor of oral medicine, Dr. Malcolm A. Lynch, associate in oral medicine, or Dr. Irwin I. Ship, professor of oral medicine, Oral Medicine Training Program, Philadelphia General Hospital, 34th Street and Curie Avenue, Philadelphia, Pa. 19104.

Pesticides and Public Health. A training course in pesticides and public health will be held at the National Communicable Disease Center in Atlanta, Ga., May 13–16, 1968.

Designed to present an overview of the health aspects of pesticides, the course will be directed to personnel from State and local health departments, Federal services, arthropod control districts, conservation groups, and other appropriate agencies. Biologists, sanitarians, engineers, chemists, physicians, nurses, veterinarians, other members of the health team, and persons whose employment includes responsibility for applying or dispersing pesticides or supervising their use are invited to apply for this course.

Additional information is available from the chief, State Services Section, Pesticides Program, National Communicable Disease Center, Atlanta, Ga. 30333.

Control of Mosquitoes and Mosquito-Borne Diseases. A training course in control of mosquitoes and mosquito-borne diseases is being sponsored by the Training and Consultation Section of the Aedes Aegypti Eradication Program of the Public Health Service. The course has been scheduled at the National Communicable Disease Center in Atlanta, Ga., May 20–24, 1968.

Directed to entomologists, sanitarians, engineers, physicians, laboratory workers, environmental health personnel, and other members of the public health team, the course content is designed to provide public health personnel with a basic understanding of mosquitoes and mosquito-borne diseases affecting the health and well-being of man. Primary emphasis is placed on epidemiology of mosquito-borne diseases, identification and biology of mosquitoes, and survey and control procedures.

Tuition and books are free. The only costs to applicants involve housing and subsistence. However, trainees should provide themselves with clothing suitable for fieldwork.

Additional information and applications are available from the National Communicable Disease Center, Atlanta, Ga. 30333, Attention: Training and Consultation Section, Aedes Aegypti Eradication Program.

Postprofessional Training in Community Mental Health. Advanced training in community mental health is being offered by the department of psychiatry of the University of North Carolina School of Medicine.

The curriculum and related field experiences focus on increasing professional competence for leadership and administration of community mental health programs. Emphasis is placed on didactic and practicum teaching of program planning, staff development, consultation, supervision, community organization, and analysis of existing institutional and community programs.

Completion of 11 months of training provides eligibility for a certificate in mental health program administration, and completion of 21 months of training with a major in the School of Public Health provides eligibility for a master's degree.

The training program is open to psychiatrists, health officers, psychologists, nurses with a bachelor of science degree, social workers, administrators, and social scientists.

Stipends from the National Institute of Mental Health, Public Health Service, are available at the postgraduate level according to the discipline and seniority of the trainee.

Further information and applications may be obtained from Dr. William G. Hollister, Department of Psychiatry, School of Medicine, University of North Carolina, Chapel Hill 27514.

phr synopses

COE, RODNEY M. (Washington University, St. Louis, Mo.), FRIEDMANN, EUGENE A., SIGLER, JACK, MARSHALL, DOUGLAS, and BREHM, HENRY P.: *The response to Medicare. Public Health Reports, Vol. 83, April 1968, pp. 271–276.*

Data from household interviews with older people in five Midwestern communities are presented in terms of respondents' awareness and knowledge about Medicare and their attitudes toward it. The results indicated that nearly all respondents were aware of the program, but that knowledge about specific aspects of the program varied by age, social class position, and perceived health status of the respondent. Similarly, about three-fourths of the respondents held favorable attitudes toward Medicare although these responses varied by age, sex, and social class. Despite these variations, the general conclusion was that the overall response to Medicare as an innovation in methods of payment for health and medical care services was positive.

WESTOFF, CHARLES F. (Office of Population Research, Princeton University), and RYDER, NORMAN B.: *Duration of use of oral contraception in the United States, 1960–65. Public Health Reports, Vol. 83, April 1968, pp. 277–287.*

An analysis was made of data on discontinuation of oral contraception collected in the 1965 National Fertility Study, an interview survey of a probability sample of married women throughout the United States. Based on these data, admittedly inadequate for diagnostic purposes, there does not appear to be any evidence of serious health problems associated with the use of the birth control pill.

About one-third of all women who had used the pill at any time since 1960 had discontinued use, either permanently or temporarily, by the time of interview in autumn 1965. The majority of these women stopped because of what they perceived as unpleasant side effects of the drug, most of which related to either undesirable reactions commonly associated with pregnancy or problems connected with the menstrual cycle. Typically, the women who discontinued oral contraception had experienced nausea or menstrual breakthroughs and stopped using the pill after one or two cycles.

The probability of discontinuation appears to have declined between 1960–62 and later years. Excluding reasons extraneous to the pill itself, such as stopping to have a child, the most recent dropout rate was approximately 3 to 4 percent per month over the first 3 months and 1 to 2 percent in subsequent months. The proportion continuing for at least 1 year was approximately 80 percent.

PEACOCK, WILLIAM L., Jr. (Public Health Service), WELCH, BOBBY G. MARTIN, JOHN E., Jr., and THAYER, JAMES D.: *Fluorescent antibody technique for identification of presumptively positive gonococcal cultures. Public Health Reports, Vol. 83, April 1968, pp. 337–339.*

Sixty-four cultures identified as *Neisseria gonorrhoeae* by standard methods were treated with oxidase reagent and gram stained by three experimental methods. These cultures were subsequently treated with *N. gonorrhoeae* fluorescein-labeled antiserums. Results showed that colonies of gonococci killed by action of the oxidase reagent (dimethyl-p-phenylenediamine hydrochloride) can be specifically identified by fluorescent antibody techniques. The gram stain, however, interferes with fluorescence of the gonococcal cell.

phr synopses

KENDRICK, MILDRED A. (Public Health Service), DISCHER, DAVID P., and HOLADAY, DUNCAN A.: ***Industrial hygiene survey of Metropolitan Denver. Public Health Reports, Vol. 83, April 1968, pp. 317–322.***

Limitations on staff and budget have forced the State and local occupational health units operating in the Metropolitan Denver area to restrict their activities to investigating a potpourri of high-risk situations and to responding to specific requests for assistance in studying occupational health problems. To define the occupational health problems in the area and provide a basis for determining the priorities to be followed in solving them, a survey of selected plants was undertaken. Five hundred plants were chosen from selected industrial categories and employee-size groups.

Exposures to hazardous agents or materials averaged about 30 per plant; industrial hygiene controls were absent or inadequate for about one-third of these exposures. The survey showed that approximately 30 percent of the study population (some 43,000 workers) were employed in plants which the surveyors judged to be in the high-risk category. About three-fourths of the employees in the high-priority establishments worked in manufacturing or trade.

The staff of the governmental occupational health units in the Metropolitan Denver area should make about 2,500 plant visits a year to give minimum occupational health services to the 6,700 plants represented in the study.

CONSTANTINE, DENNY G. (Naval Biological Laboratory), TIERKEL, ERNEST S., KLECKNER, MARLIN D., and HAWKINS, DOUGLAS M.: ***Rabies in New Mexico cavern bats. Public Health Reports, Vol. 83, April 1968, pp. 303–316.***

An epizootic of deaths of Mexican free-tailed bats at Carlsbad Cavern, N. Mex., in August 1955 at first appeared to be caused by rabies, stimulating epidemiologic studies to define the extent of the disease, its origin, and its public health significance.

Excessive bat mortality occurred in 1956 and in certain subsequent years. The deaths of the bats were associated with migration during inclement weather and unfavorably cool temperatures in the cavern due to the presence of relatively few bats. Rabies virus was detected in 3.9 percent of the fallen bats, a rate similar to that subsequently observed in clinically normal migrating bats. During nonepizootic periods, reletively few moribund or dead rabies-virus infected bats were found.

Migratory and other bat population movements made rates of rabies infection and the presence of rabies antibody in bat serums difficult to interpret. In monthly samples, rabies virus was detected in less than 1 percent of the bats collected in flight, and serum rabies-neutralizing antibody usually was found in 15 to 30 percent. Evidently, prenatal transfer of antibody occurs in the free-tailed bat; a decrease of antibody rate in young bats was followed by clinical rabies in bats of similar ages.

Naturally infected bats were not observed to develop furious rabies, and there were no unprovoked attacks by bats, but moribund bats bit when handled. Certain native Carnivora developed rabies after intramuscular or subcutaneous inoculations of the bat rabies virus. Rabies-virus transmission to Carnivora by bat bite was attempted and failed, but it has since been achieved.

Bats banded at the cavern migrated into Mexico, where in winter they shared caves with vampire bats. In an experiment, captive vampires got blood from free-tailed bat cagemates. Rabies virus might be transmitted to free-tailed bats in this manner in nature. Rabies infected suckling free-tailed bats, however, were found in New Mexico, which indicates an intraspecies source of infection.

Mexican free-tailed bats appear to be a potential source of rabies infection for man and Carnivora, and direct contact with bats should be avoided. Ventilation in the cavern appears adequate to prevent airborne rabies virus transmission, but a remote hazard of infection from this source may exist in corridors crowded with great numbers of flying bats entering or departing from the cavern.