Nebraska Public Health Laboratory Newsletter

A publication of the Nebraska Public Health Laboratory (NPHL) at the University of Nebraska Medical Center
Winter Issue 1997/1998

Introduction

Laboratory professionals are well aware of the many changes that are taking place in health care today and these changes are not just appearing in the private sector. Many of the testing services previously performed at the State Health Laboratory were transferred to the University of Nebraska Medical Center this past summer. Affected tests include those utilized or administered by State Health programs for HIV surveillance, sexually transmitted diseases, and child health. Testing that relates to water quality, environmental testing or agricultural applications has remained in Lincoln. The only significant change for most clinical laboratories is the address to which bacterial isolates should be sent for referral to the CDC. By consolidating testing services with those already available at the UNMC, considerable cost savings are possible due to reduced reagent expenditure and volume efficiencies. Nebraska has taken a leadership position in this effort and several states are considering similar moves. Some states such as Iowa and Wisconsin have operated the public health laboratories under the auspices of their state universities for years, whereas only recently the state of Nevada reorganized it's public health laboratory within the university system. While these efforts at economizing are worthy, much concern has been raised about whether our public health system is prepared to deal effectively with the next major epidemic. One potential epidemic that could have a significant impact is the Asian influenza A virus strain associated with birds (see accompanying story on page 4).

The purpose of this newsletter is to communicate timely information regarding laboratory testing to hospital and clinic laboratorians. We also want to convey our willingness to discuss or consult on current issues as they arise in the community .

Dr. Steven H. Hinrichs

Photograph of little brown myotis taken by J. Scott Altenbach. Brown bats are frequently encountered in houses throughout Nebraska. In July of 1997 alone, over 270 bats were removed from homes in the Omaha area. Only one bat was found to carry rabies in Nebraska during 1997. (Used by permission)

b-lactam resistance in the *Enterobacteriaceae*

by Paul D. Fey, Ph.D.

The development of new β-lactam antibacterial agents within the pharmaceutical industry has shifted into high gear once again due to a rapid development of resistance to older β-lactam compounds within the *Enterobacteriaceae*. Just as antibacterial agents such as cefotaxime, ceftriaxone, and other 3rd-generation cephalosporins (cephs) were created to counteract strains resistant to ampicillin and 1st-generation cephs, 4th-generation cephs (e.g. cefepime) were created to treat infections refractory to therapy with 3rd-generation cephs and aztreonam. This short review discusses β-lactam antimicrobial resistance mechanisms as well as the experience of the NPHL in the detection of strains

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Laboratory Diagnosis: *Chlamydia trachomatis/ Neisseria gonorrhoeae* Infections

by Peter C. Iwen, MS

Numerous culture and non-culture techniques are available to test for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in genital specimens. Culture was historically considered the "gold standard" for testing both pathogens and is still the recommended method for medical-legal cases where false-positive results are not acceptable. Culture methods however, in comparison to molecular tests, have been shown to generally be less than 90% sensitive for both species. One reason for the reduced sensitivity of culture is decreased viability of organisms when transporting specimens

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Health Risk of Common Bats

by Steven H. Hinrichs, M.D.

Several stories in the news this past summer caused attention to be focused on the health risk to humans following contacts with bats. Pathologists and technologists should be prepared to provide timely information to the public and local physicians regarding transmission to humans by bats. Since 1980, there have been 28 cases of human rabies acquired in the United States, and over half were caused by virus strains associated with bats. Ten of the fifteen cases caused by bat rabies viruses were of the virus strain found in the silver-haired bat. In 1995, two children died of rabies and although bats were present in the houses, no contact with bats had been recognized prior to the onset of rabies. In fact, the majority of the 15 patients who died since 1980 with rabies

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b -Lactam resistance

producing extended-spectrum β-lactamases.

β-lactam antibiotics act on the bacterial cell by binding to, and thus inactivating, enzymes which are involved in the construction of the cell wall. These enzymes are called penicillin-binding proteins or PBPs. Inhibition of cell wall construction ultimately leads to cell lysis and death. One way bacteria have overcome this challenge is through the production of β-lactamases, which cleave the amide bond in the β-lactam ring, rendering the antibiotic unable to bind PBPs.

The most common β-lactamases responsible for β-lactam resistance within species such as *Escherichia coli* and *Klebsiella pneumoniae* are two closely related enzymes called TEM-1 and SHV-1. These β-lactamases are plasmid mediated and therefore can be transferred from species to species by a process called conjugation. They confer resistance to β-lactam drugs such as ampicillin, piperacillin, ticarcillin, cephalothin, cefazolin and cefamandole. Infections with strains containing a TEM-1 or SHV-1 β-lactamase can be treated effectively with 3rd- and 4th-generation cephs, cephamycins (cefoxitin and cefotetan) as well as aztreonam, penicillin/β-lactamase inhibitor combinations and the carbapenems.

Unfortunately, through selective pressure in the hospital environment, bacterial strains have been isolated which carry mutant TEM-1 or SHV-1 β-lactamases that are not only able to hydrolyze 1st-generation cephs but are also able to hydrolyze 3rd-generation cephs (see chart) as well as aztreonam. These mutant enzymes are called **extended-spectrumb -lactamases** or **ESBLs**. To date, there have been over 100 ESBLs described throughout the world. Initially, most ESBLs were found in *E. coli* and *K. pneumoniae,* but since the enzymes are encoded on transferable plasmids, they have also been found in other members of the *Enterobacteriaceae* such as *Serratia marcescens* and *K. oxytoca.* Strains harboring ESBLs remain susceptible *in vitro* to the cephamycins, penicillin/β-lactamase inhibitor combinations, carbapenems and cefepime. However, it is not clear at this time whether treatment with any β-lactam besides a carbapenem, and in most cases cefepime, is efficacious in a serious infection.

The TEM-1 and SHV-1 β-lactamases and their derivatives (ESBLs) are distinct from the **cephalosporinases**, another group of β-lactamases that are encoded on the chromosomes of virtually all gram-negative organisms. These β-lactamases are encoded by a gene called *ampC* and hydrolyze cephalosporins more efficiently than penicillins. Even though an *ampC*-like gene is found in all species of the *Enterobacteriaceae,* certain species such as *E. coli* produce the cephalosporinase in such low amounts that it does not affect an isolate ' s susceptibility to β-lactam antibiotics. Other species such as *Enterobacter cloacae* and *Citrobacter freundii* may be induced to generate cephalosporinase at high levels when certain cephs or penicillins (e.g. cefazolin or ampicillin) are present. 3rd-generation cephs do not induce the cephalosporinase and isolates in most cases can initially be treated effectively with a 3rd-generation ceph. Unfortunately, all species of *Enterobacteriaceae* (including *E. coli*) may potentially develop mutations which are selected during therapy with third-generation cephs (called an ***ampC* mutant**). These mutant bacteria become resistant to all cephs (excluding cefepime), aztreonam, penicillins and penicillin/β-lactamase inhibitor combinations. Cefepime and the carbapenems retain excellent activity against *ampC* mutants.[1,2]

The laboratory challenge: -detection of ESBLs

Failure to detect resistance to antimicrobial agents may have dire consequences for the patient. Therefore, clinical microbiology laboratories are constantly monitoring and improving their susceptibility testing methods so accurate information will be conveyed to the physician. Fortunately, *in vitro* susceptibility testing accurately reveals an *ampC* mutants' resistance to 2nd- and 3rd-generation cephs and no additional testing is warranted. However, ESBLs are difficult to detect in the laboratory using standard *in vitro* assays and isolates that contain ESBLs may appear susceptible to 3rd-generation cephs or aztreonam. The difficulty arises from the fact that breakpoint panels from commercial automated susceptibility systems currently use an MIC breakpoint for ceftazidime, ceftriaxone, cefotaxime, and aztreonam that is 8 μg/ml. Some strains that express ESBLs have MICs to the 3rd-generation cephs and aztreonam as low as 2 μg/ml. Therefore, a patient may be initially treated with a 3rd-generation ceph based on laboratory results that suggest the organism is susceptible. Two different investigators, using genetically defined and well characterized ESBL isolates, recently found that using cefpodoxime at an MIC breakpoint of 2 μg/ml, accurately detected all strains expressing ESBLs. Commercial automated susceptibility systems already have, or will have in the near future, cefpodoxime on their panels/ cards to help identify strains that contain ESBLs.

Our laboratory is currently defining an ESBL producing isolate as any *Enterobacteriaceae* that is resistant or intermediate to either ceftazidime or ceftriaxone or cefotaxime or aztreonam, yet susceptible to cefoxitin and cefotetan. We confirm each case using a double-disk diffusion test[3], which tests the enzymes susceptibility to β-lactamase inhibitors such as clavulanate. In contrast, *ampC* mutants are resistant to both cefoxitin and cefotetan and are resistant to the inhibitory action of β-lactamase inhibitors. Our protocols for monitoring and testing for ESBLs are available to clinical laboratories. The NPHL is interested in monitoring the frequency of ESBLs throughout Nebraska and isolates may be referred for confirmatory testing or molecular typing. Contact Dr. Paul Fey for more information.

References

1. **Fekete, T., H. Tumah, J. Woodwell, V. Satishchandran, A. Truant, and P. Axelrod.** 1996. Comparative susceptibilities of *Klebsiella* species, *Enterobacter* species, and *Pseudomonas aeruginosa* to 11 antimicrobial agents in a tertiary-care university hospital. Am. J. Med. **100(suppl 6A)**:20S-25S.

2. **Sanders, W.E., H.H. Tenney, and R.E. Kessler.** 1996. Efficacy of cefepime in the treatment of infections due to multiply resistant *Enterobacter* species. Clin. Infect. Dis. **23:**454-

461.
3. **Thomson, K.S., and C.C. Sanders,** 1992. Detection of extended-spectrum β-lactamases in members of the family *Enterobacteriaceae:* comparison of the double-disk and three-dimensional tests. Antimicrob. Agents Chemother. **36:**1877-1881.

Other reviews of interest:
Medeiros, A.A. 1997. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. Clin. Infect. Dis. **24:** (Suppll): 519-545.

Sanders, W.E., and C.C. Sanders,1997. *Enterobacter* spp.: pathogens poised to flourish at the turn of the century. Clin. Micro. Rev. **10**:220-241.

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to or from off-

PACE 2C

site facilities (see Iwen, et al., Arch. Pathol. Lab. Med. 1996; 120: 1019-1022). To overcome the issue of viability, a number of non-culture test methods have been evaluated, including tests to detect antigen or genetic material. Two such molecular tests are the Gen-Probe PACE 2 assays (Gen-Probe, San Diego, CA), one for *C. trachomatis* (PACE 2CT System) and one for *N. gonorrhoeae* (PACE 2NG System). These tests use nucleic acid hybridization techniques for the detection of ribosomal RNA (rRNA) and they are widely utilized in public health laboratories throughout the United States because of reliable performance characteristics and ease of use. Recently, Gen-Probe made available another nucleic acid probe test that simultaneously detects both *C. trachomatis* and *N. gonorrhoeae* from a single patient sample (PACE 2C System). Since reported co-infection rates of 15% to 40% are not uncommon, a probe test to detect both organisms from each specimen is a desirable alternative for testing. The NPHL uses this Gen-Probe PACE 2C System to screen genital specimens simultaneously for both *C. trachomatis* and *N. gonorrhoeae*. The PACE 2C System uses chemiluminescent-labeled,single stranded DNA probes that are complementary to the rRNA of both target organisms. After the rRNA is released from the target organisms, the labeled probes combine with this rRNA to form stable DNA:RNA hybrids. The labeled DNA:RNA hybrids are separated from the non-hybridized probes and the DNA:RNA hybrids, if present, emit a light reaction which is detected using a luminometer. The results of testing are reported quantitatively in relative light units (RLUs). A positive screen indicates the presence of *C. trachomatis, N. gonorrhoeae,* or both subsequently requiring additional testing for verification and to identify specific species.

Specimen Collection

Endocervical and male urethral specimens are collected using the appropriate Gen-Probe PACE Specimen Collection Kit (Gen-Probe Urethral, Cat. No. 3275 and Gen-Probe Cervical, Cat. No. 3300). These two body sites are the only sites currently approved for testing with the combined assay. The proper swab should be used and placed back into the collection tube containing transport media after collection. The tubes are transported to the laboratory and held at room temperature until assayed. Samples not processed within 7 days are stored frozen at -20 to -70 ^{O} Celcius.

Specimen Processing

Upon receipt in the laboratory, swabs are processed according to manufacturer's recommendations. The processing involves steps in

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b-lactam Antibiotics

Penicillins

Gram-positive Activity		*Gram-negative Activity*	
-penicillin	-methicillin	-ampicllin	-amoxicillin
-oxacillin	-cloxacillin	-piperacillin	-ticarcillin
-dicloxacillin	-nafcillin	-mezlocillin	-azlocillin
		-carbenicillin	

Penicillin/b -lactamase inhibitor combinations

-ampicillin/sulbactam	-piperacillin/tazobactam
-amoxicillin/clavulanate	-ticarcillin/clavulanate

Cephalosporins/Cephamycins*

Generation Classification			
First	***Second***	***Third***	***Fourth***
-cephalothin	-cefamandole	-cefotaxime	-cefepime
-cefazolin	-cefuroxime	-ceftizoxime	
-cephapirin	-cefonicid	-ceftriaxone	
-cephalexin	-cefixime	-cefoperazone	
-cephradine	-cefpodoxime	-ceftazidime	
-cefadroxil	-cefprozil		
-cefaclor	-ceftibuten		
-ceforanide	-cefoxitin		
	-cefotetan		
	-cefmetazole		

Monobactams -aztreonam

Carbacephems -loracarbef

Carbapenems -imipenem
-meropenem

***Separation of first, second, third and fourth generation cephalosporins based on activity alone and not on date of introduction by the company.**

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preparation, hybridization, separation, and detection. Both negative and positive controls are

PACE 2C

included with each sample run. Upon reading each processed sample with a luminometer, a chemiluminescence reaction is detected which is converted into a RLU reading.

Interpretation

A positive screen result is a RLU reading of greater than 300 plus the mean of the Negative Reference (approximately 350 RLUs total) which indicates the possibility of *C. trachomatis* and/or *N. gonorrhoeae* rRNA present in the specimen. A negative screen result is considered when a RLU reading of less than 300 plus the mean of the Negative References is identified. All specimens with RLU readings of 300 or more are subjected to a second level of testing. Specimens with RLU readings of 300 to 1000 are considered "grey zone" and tested with Gen-Probe's Probe Competition Assays (PCAs) for both *C. trachomatis* (CT-PCA) and *N. gonorrhoeae* (NG-PCA). The PCAs are supplemental DNA probe tests that use the technique of competitive nucleic acid hybridization. They are used to detect a nonspecific signal in specimens that test high-negative or low-positive in the PACE 2 Systems. For specimens with RLU readings of greater than 1000, clinical studies have shown then to be true-positive and not requiring supplemental PCA testing for verification. These true-positive specimens in our laboratory are follow-up tested with individual *C. trachomatis* (PACE 2CT) and *N. gonorrhoeae* (PACE 2NG) assays to identify specific species. In rare instances, where follow-up testing with the individual assays have RLU readings between 300 and 1000, the result is retested using the PCA method for verification. If not enough sample is available for retesting, the result is reported as "inconclusive" and another sample requested. When identified as true positive by the confirmatory method, the specific etiological agent is reported.

Performance Evaluation

The PACE 2C assay has a reported sensitivity of 89.9% to 98.9% for endocervical and 93.3% to 97.1 % for male urethral specimens, with specificities greater than 95% for both sites. An evaluation by our laboratory comparing the PACE 2C assay with culture using a nucleic acid amplification test for discrepancy analysis, showed the sensitivity of culture for *N. gonorrhoeae* and *C. trachomatis* to be 88.9% and 89.2%, respectively, with a PACE 2C assay sensitivity of 96.3% and specificity of 98.8% (Iwen, et al., J. Clin. Microbiol. 1995; 33:2587-2591). It is important to note there are some limitations to consider when using this test methodology. Specimen adequacy cannot be assessed microscopically, so it is imperative that proper specimen collection techniques be used. Additionally, grossly bloody specimens (greater than 80 µL whole blood in 1 ml transport medium) may interfere with the performance of the probe test, thus requiring PCA testing. Finally, as is true with all laboratory tests, when positive and negative results contradict other clinical or patient information, verification tests, such as culture, should be considered.

Conclusion

Nucleic acid amplification tests are available for testing both pathogens. The high sensitivity and specificity of these tests, along with the ability to test urine, shows promise. However, studies comparing amplification tests with PACE 2 and PCA assays are limited and have had conflicting results. This, along with the higher cost for amplification testing, has limited their usefulness for large scale screening. NPHL currently process about 3000 genital samples per month. Cultures for both chlamydia and gonorrhea are offered for medical-legal cases and to test specimens such as conjunctiva, rectum, and from infants, nasopharynx and throat. The PACE 2C System is not FDA-approved for these sites, although the PACE 2CT for *C. trachomatis* has been approved to test

Screen for Chlamydia/Gonorrhea by DNA Probe

Availability:	Monday - Friday
Turnaround Time:	24 hours
Specimen:	Swab of endocervix or male urethra Swab of conjunctiva (for chlamydia only)
Container:	Gen-Probe transport container Send at room temperature
Causes for rejection:	Other sites and specimens submitted on wooden swabs are unacceptable
Reference range:	Negative for C.trachomatis and N.gonorrhoeae
Additional information:	Culture for both chlamydia and gonorrhea are available. Contact the Nebraska Public Health Laboratory for proper collection and transport information.

Asian Flu is for the Birds

by Steven H. Hinrichs, M.D.

The CDC is requesting increased surveillance for influenza this year in recognition of the potential threat following an outbreak of influenza A in Hong Kong. The influenza virus causing concern is an H5N1 serotype which previously was thought to only be capable of infecting chickens and other fowl, but not humans. The Public Health Laboratory at UNMC is part of a national and international surveillance program coordinated by the World Health Organization and the CDC. The goal of this program is to monitor the frequency of specific influenza serotypes and rapidly identify any significant change. This information is then used in determining which influenza strains are incorporated into the vaccine preparation each year. In addition to making a determination

Asian Flu

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Bats and Health

caused by a bat virus did not document or report a bat bite. While this information has raised concern regarding the possibility of transmission by aerosolized virus, it is believed that transmission was due to an unrecognized bat bite or scratch.

It is recommended that in situations where a bat is found in sleeping quarters or bedrooms and the possibility of bite exposure cannot be excluded, post-exposure prophylaxis should be given unless the bat can be captured and tested for presence of rabies[1]. The first case of rabies caused by virus from a brown bat was documented in 1997. To our knowledge, no brown bat has been found to transmit rabies to humans in Nebraska. However, a migratory bat, called the hoary bat can be found in Nebraska in the spring and fall and in some surveys up to 50% of these bats carry the rabies virus. Epidemiologic surveys are planned in the coming year to document the frequency of rabies in specific bat species in Nebraska.

This information does not call for a full-scale extermination of bats, especially in light of the valuable role they play in reducing mosquito and other flying insect populations. The risk of rabies to humans from bats in Nebraska appears to be very low. However, appropriate public awareness is needed and if an incidental bat exposure occurs the following steps should be taken:

1) Notify the Rabies Control Officer at the Nebraska Department of Health; Ph. #(402) 471-2937, and obtain approval for submission of bat (or other animal) to NPHL;

2) Do not damage the head when killing the animal. Keep refrigerated if not able to transport immediately.

3) Transport bat to NPHL. Proper packaging includes placing the animal in double containment, usually plastic bags and labeling as hazardous medical samples. Species can be transported in the U.S. mail with cold packs. A rabies form for documentation of essential information should accompany the specimen which can be obtained by calling (402) 559-2440.

To some degree, these stories detract from the equally important risk to humans and domestic animal from skunks and raccoons that carry rabies. We should make every effort to encourage vaccination of dogs and cats in our communities since this is one of the most effective ways to practice public health.

1) Centers for Disease Control and Prevention. Human Rabies—Conneticut, *MMWR*; **45** 207-209, 1996.

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about whether the dominant virus in any one year is of an A or B type, the subtype or serotype information is equally important. However, current techniques require an isolate to be available, one that can grow and replicate in cell culture. Therefore, physicians throughout the state are requested to communicate with the state epidemiologist, Dr. Tom Safranek, the development of any new outbreaks of influenza so that efforts can be made to collect samples. For epidemiological purposes, it is not necessary to test every sample associated with a particular outbreak and usually only 2 or 3 are necessary , particularly if the presence of influenza A has been documented in most individuals by the antigen assay.

Expectations of practicing physicians has rapidly changed from the past when very few specimens were collected for virus testing. With the advent of rapid antigen assays, such as the Bectin-Dickinson Influenza A assay, which are excellent when used on appropriate specimens, timely treatment can be instituted and spread of virus can be limited. In addition to rapid antigen assays, the development of rapid screening tests and their assembly into kits by companies such as Bartels or Chemicon has also made the detection of respiratory viruses possible and convenient for many hospital laboratories.

Currently it is possible to screen for the presence of parainfluenza virus types 1, 2 and 3, influenza virus A and B, adenovirus and respiratory syncytial virus. The Public Health Laboratory at UNMC is interested in helping other hospitals in establishing assays for viruses and can provide training for technologists as well as reference samples to establish initial proficiency. The virus laboratory has also provided mini-fellowship training experience for pathologists who are interested in expanding their capabilities. For more information on these educational opportunities please contact Dr. Steven Hinrichs at (402) 559-4116.

Burkholderia cepacia Confirmation

Although *Pseudomonas aeruginosa* remains the most common organism recovered from patients with cystic fibrosis, the isolation of another bacterial pathogen, *Burkholderia cepacia* has important clinical implications as well. *Burkholderia cepacia*, formerly known as *Pseudomonas cepacia*, is intrinsically resistant to a wide range of antimicrobial agents and may be especially virulent in patients with CF. Because of phenotypic similarities to other bacteria, it is frequently difficult to identify. The Cystic Fibrosis Foundation supports a clinical research laboratory at Allegheny University of the Health Sciences in Philadelphia, PA under the direction of Dr. John LiPuma that we have found helpful in characterizing these difficult to identify non-fermenting gram-negative rods. The testing is performed without charge and the only requirement for submission is that a brief summary, similar to the CDC form, be completed and mailed with the isolate. For more information or to obtain a copy of the submission form by FAX, please call Peter Iwen at (402) 559-7774.

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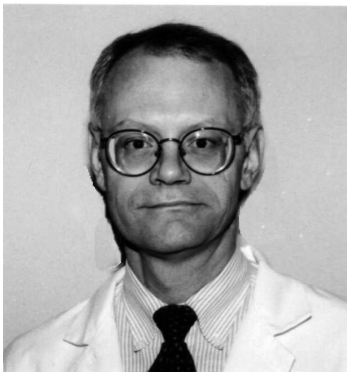
Coming in the spring issue : ***E. coli* O157:H7**

An issue of great relevance to the citizens of Nebraska and the Midwest is that of ***E. coli*** capable of causing hemorrhagic diarrhea and hemolytic uremic syndrome.



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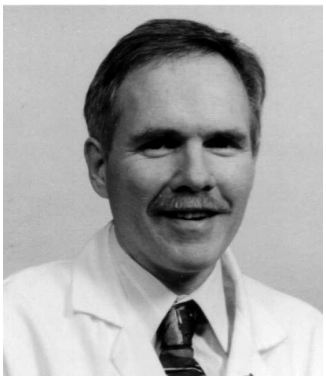

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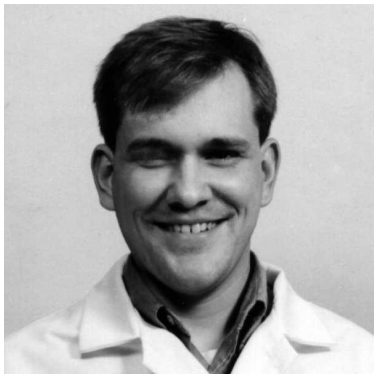

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