

# The Waiting Game

A stack of several clear plastic petri dishes with red lids is the central focus, slightly out of focus. In the background, a stethoscope with a blue chest piece and a green tube is visible against a dark, textured surface. The overall lighting is dramatic, with highlights on the edges of the dishes and the stethoscope.

Richard Goldstein's patient

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unraveling of a genetic puzzle

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could save the lives of a million

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children a year | BY SUSAN SELIGSON

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GOLDSTEIN'S PRIDE: The world's largest collection of genetically indexed clinical specimens of some of the planet's most persistent bacterial pathogens.



## Goldstein's

### office

in the Medical Campus's pristine BioSquare complex appears to have fallen victim to

its own private earthquake. A listing tower of cardboard boxes threatens the last navigable floor space near the biologist's desk, which is buried under an avalanche of hemorrhaging manila folders.

"I'd clean it if I had the time," says Goldstein, a School of Medicine professor of pediatrics, who specializes in infectious diseases and whose expressive eyes, silver mane, and jowls bring to mind the actor Bert Lahr, of Cowardly Lion fame. The office flotsam is the result of the lab's move, Goldstein explains, as he lurches to extinguish the sounds of an atonal composition from a classical radio station. But the move, from the gothic quarters of the Maxwell Finland Laboratory on Albany Street, was made five years ago.

Any assumptions that Goldstein's dishevelment extends to his work area dissolve when the scientist opens a door to his lab, revealing proof of an ardently compulsive mind. Here, in a series of end-to-end industrial-size deep freezers, is Goldstein's pride, inspiration, and obsession: the world's largest collection of genetically indexed clinical specimens of some of the planet's most persistent bacterial pathogens. The biggest of these — nearly 1,000 isolates — is of *Streptococcus pneumoniae* (a.k.a. pneumococcus), a common microbe that each year causes deadly meningitis in millions of vulnerable people, mainly children. The startling adaptability and human toll of this bacterium, which lives harmlessly in the noses of most humans, have consumed Goldstein's forty-year career.

Responsible for the deaths of more than a million children each year and with the single vaccine against it only partly effective, pneumococcus is the white whale to Goldstein's inner Ahab. The current vaccine, Prevnar, based on the chemistry of the bacterium's capsule, or coating, works on just seven of ninety-one known pneumococcal strains. Relentlessly, and sometimes at his own expense, Goldstein has been blazing a scientifically novel path to a better vaccine, and his out-of-the-box approach may lead to vaccines

for other menacing bacteria as well. His strategy will have consumed years of genetic testing and retesting until — and Goldstein is on the cusp of this — he hones in on a vaccine that will stop all strains of *Streptococcus pneumoniae* in their tracks.

To do that, he needs to isolate the DNA that is both common to all strains and resistant to mutation. The common proteins expressed by these unvarying genes will, he hopes, trick the body into thinking that it is being attacked and tell the immune system to create antibodies that will go to war with every strain of the bacterium.

Goldstein expects that search to take three years or more. When it's done, however, he'll be on the home stretch and can turn to well-established protocols for vaccine development, including cloning and animal testing.

#### CHARLES DARWIN WAS HERE

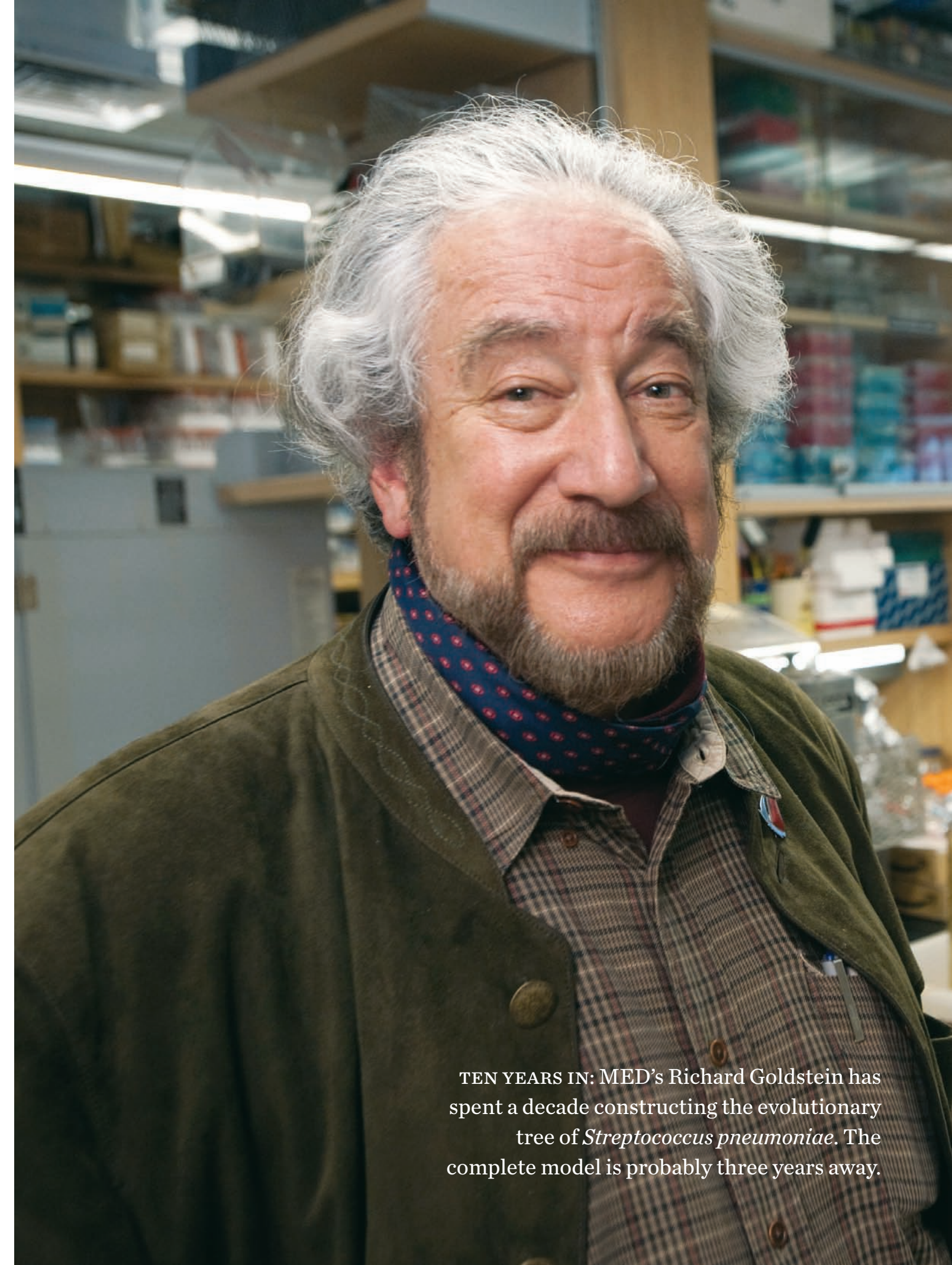
"The healthy high school football star who suddenly dies of meningitis — that's most likely *Streptococcus pneumoniae*," says Goldstein, who was recently awarded a three-year, \$300,000 research grant from the Hartwell Foundation, which selects ten "exceptional individuals" annually and funds "innovative, cutting-edge biomedical applied research" that advances children's health.

If grants for innovation had existed in the nineteenth century, one would likely have been awarded to Goldstein's idol, Charles Darwin. Among the charts and graphs in his presentation at a recent conference of molecular biologists in Tel Aviv, Goldstein included a portrait of his prolific muse. "Everything I'm saying now was said by Darwin in 1859," he says. He owns thirteen first editions of Darwin's books — his "pride and joy." Darwin penned forty volumes, and "they didn't all come from going to Galapagos," Goldstein says. "They came from him looking out the window of his study. He wrote a whole book on domestic ants." Just as these seemingly prosaic creatures riveted the father of the theory of evolution, Goldstein finds provocation in the familiar.

According to the federal Centers for Disease Control and Prevention, most people have been pneumococcus carriers at some point in their lives, although the bacterium is most common in young children. Mostly harmless, it's an exclusively human pathogen that can viably ride a sneeze six feet from one nose to another. Occasionally it spreads to other body parts, causing ear infections, sinusitis, and

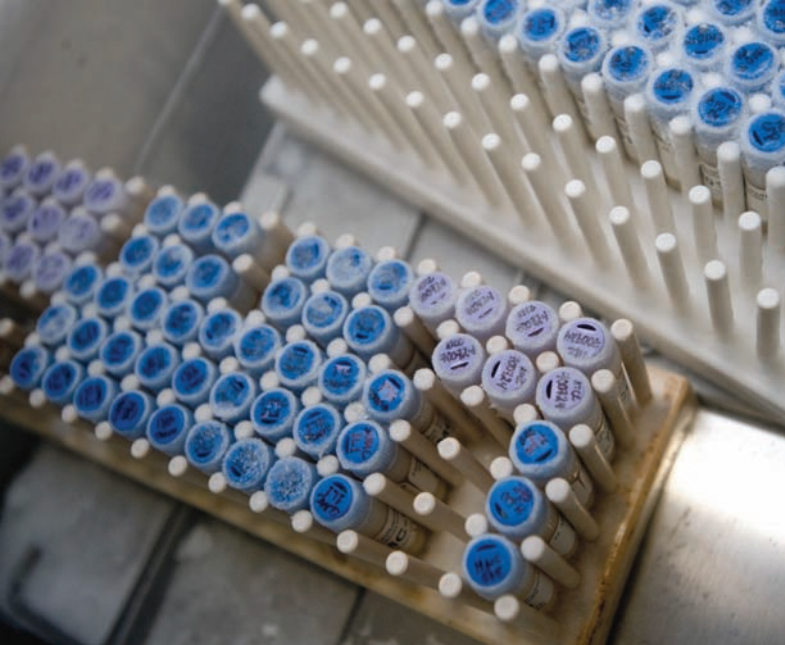
**WEB EXTRA**  
MED's Richard Goldstein talks about his idol, Charles Darwin, at [bu.edu/bostonia](http://bu.edu/bostonia).





TEN YEARS IN: MED's Richard Goldstein has spent a decade constructing the evolutionary tree of *Streptococcus pneumoniae*. The complete model is probably three years away.





### **DANGEROUS STRAINS**

Goldstein believes the bacterium's extraordinary virulence stems from its vast evolutionary diversity.

lung infections. Worse, it can get into places in the body that are normally free of bacteria, including the blood, or infect the lining of the brain and the spinal cord, causing meningitis. Pneumococcus is the most common cause of bacterial meningitis, contagious pneumoniae, and ear infections worldwide.

While pneumococcus is not always deadly, it is always a stubborn adversary. The first penicillin-resistant pneumococcal bacteria were discovered in the 1960s. Since then, strains have emerged that are resistant to other, more costly antibiotics. A highly effective vaccine — a preparation that resembles the bacterium closely enough to rally the body's defenses, or antibodies, but not actually infect it — makes more scientific and economic sense than playing catch-up with improved antibiotics.

Question one for Goldstein is why pneumococcus makes some people sick and not others. He believes the bacterium's feisty virulence is a result of its vast evolutionary diversity. How then to fashion a vaccine that keeps pace with pneumococcus's mutation rate so as not to require the development of new, modified — and perhaps prohibitively costly — vaccines? The answer, he believes, is to make one vaccine that fits all pneumococcus mutations, past, present, and future.

"My vaccine will be much less expensive because it won't be based on the capsule," says Goldstein, who has published papers in the *New England Journal of Medicine*, *Nature*, and many other leading journals. "They'll never make a ninety-one-variant vaccine because it's expensive and time-consuming. So, how to get around it? That's my Hartwell proposal."

Derived from the complex sugars that form the bacteria's capsule, the existing vaccine was developed in the 1990s by Goldstein's longtime mentor and friend Porter Anderson, then a professor at Harvard Medical School. Modest and soft-spoken, Anderson is a hero to many in the infectious disease world for his development, in the late 1960s, of the now routinely administered Hib, or *Haemophilus influenzae* type b vaccine, which has nearly obliterated the disease in the United States.

"I've known Richard since 1974 or 1975, when he

was an assistant professor at Harvard Medical School and so was I," recalls Anderson, who is officially retired but volunteers much of his time at Children's Hospital Boston. "He is bright and industrious and energetic. The work he does requires a great deal of data generation and analysis, and he goes at it tooth and nail. He has painted a pretty complete picture of the different pneumococcus strains using sophisticated techniques that I never try to do, and appreciate."

"It all goes back to the tree," Goldstein says, gesturing toward a chart that looks nothing like a tree, but more like a vertical bar code. In Goldstein's world, the foundation for any antibacteria vaccine is the microbe's evolutionary tree, which uses DNA-mapping technology to display concentrations of mutant genes, and the "cold spots" — appearing as sparse areas on the otherwise dense bar code — where genes appear to resist alteration.

"The simplest and undeniable conclusion from my perspective as an evolutionary biologist, as well as a microbial population biologist," Goldstein says, is that for a vaccine to be absolutely effective against *Streptococcus pneumoniae*, it will have to be based not on the bacterium's capsule, which is constructed of complex sugars, but on a common protein that is conserved across the complete evolutionary cast of pneumococcus. "This," he says, "is the reason for my seemingly unending efforts to acquire isolates, phylogenetically characterize each, and build the ever-growing tree until it reflects the actual diversity of the species."

Goldstein has successfully built trees for several pathogenic bacteria, including the strain of *E. coli* that causes urinary tract infections, but the pneumococcus tree, which tracks the evolution of every infected lung and spinal fluid sample in his collection, has so far taken ten years.

### **HALL OF PNEUMOCOCCI**

There appear to be no natural boundaries to Goldstein's workday. His battered leather bag thrown over a shoulder, he marches in his signature clogs from meetings to the lab to the think tank that doubles as his Cambridge home. One might say he's infected, if only intellectually, with *Streptococcus pneumoniae*. Some who know him think he's obsessed. Anderson marvels at his patience.

"I never took a sabbatical," says Goldstein, who unwinds by driving his convertible with the top down in all types of weather. He describes the vehicle as "an ancient Saab, number-one in crash test safety ratings, socially responsibly made by citizens of an admirable, progressive society, and bit of embarrassment re its decrepit state."

While they look to the uninitiated like a nondescript mother lode of tiny test tubes, the contents of Gold-

# The *Cepacia* Solution

## How to stop an equal-opportunity rotter

The tale of the bacterium called *Pseudomonas cepacia* illustrates how a microbe can be, in the mind of a rigorous biologist, a thing of beauty, evolutionarily speaking — even if it causes something as repugnant as onion rot.

*Burkholderia cepacia* was identified during World War II when Walter Burkholder, a Cornell professor, noticed that unpicked onions were rotting inside from “slippery skin disease.” As it turned out, the ubiquitous and

versatile *B. cepacia* is an equal-opportunity rotter — it rots organic matter of any kind, says MED’s Richard Goldstein. *Cepacia*, in the form of foot rot caused by tromping barefoot through rice paddies, caused more downtime among U.S. soldiers in Vietnam than any other ailment. So powerful is *cepacia*, the bacterium that most often survives hospital disinfectants, that only bleach kills it.

Packing the most DNA of nearly all known bacteria, *cepacia*, as Goldstein

puts it, loves the mucus lining of the lungs of children with cystic fibrosis. “*Cepacia* carries five to six times the amount of DNA as *Streptococcus*,” he says. “It’s ready for anything, anywhere.”

For safety, a visitor with CF would be turned away from Goldstein’s lab, where isolates of the bacteria are stored. But once *cepacia* picks its environment, its genome shuts down except for the business at hand. “That *cepacia* in the lungs of a kid with CF,” he says,

“will never rot an onion again.”

It was Goldstein and his colleagues who sounded a warning to the agricultural industry, in the form of a paper published in the journal *Emerging Infectious Diseases*, not to use *cepacia* to kill crop fungus, something it does quite efficiently. “Our paper said, ‘Stop!’” recalls Goldstein, noting a precedent similar to opening Pandora’s primordial box. “You don’t want to start something you can’t control.” SS

stein’s minus-eighty-degree freezers amount to a Hall of Pneumococci in a microscopic museum of natural history. How does one man secure such a staggering number of far-flung samples from the mucus and fluids of sick people? “I can’t describe what a monumental task it was to get these isolates from all over the world,” Goldstein says. He wheedles, prods, and in some cases barter lab services for the samples, many of which come from a Navajo reservation — Native Americans are more susceptible to pneumococcal infection than the population at large.

The quest for a vaccine for the bacterium picked up its pace fifteen years ago after Goldstein moved from Harvard Medical School to BU’s Maxwell Finland Laboratory for Infectious Diseases and the former Boston City Hospital (now Boston Medical Center), where he was far more likely to encounter the ravages of infectious diseases. There is poetic justice to Goldstein’s laboring within view of a plaque honoring Finland, who died in 1987 at age eighty-five. Head of Boston City Hospital’s department of medicine for half a century, the Russian-born Finland was among the first researchers to sound the alarm about overuse of antibiotics and about drug-resistant bacteria, and his work pioneering treatment of pneumonias is credited with cutting the disease’s mortality rate by a third.

These days, in slight overdrive after winning the Hartwell, Goldstein is looking toward the post-tree, and more mainstream, phases of his pneumococcus research. He will clone — the molecular biologist’s

word for genetic cutting and pasting — the crucial, recurring, nonmutating genes into self-replicating DNA carriers called plasmids. Like “firing a machine gun,” the selected genetic material will trigger the production of the protein Goldstein hopes will be the antigen, or antibody target, of the vaccine. “Then you purify it — you want only that protein,” he explains.

Once the protein is purified, the next step is “like shooting fish in a barrel,” he says. “I’ll call FedEx and send it to a mouse farm, a sterile facility where mice won’t have been exposed to pneumococcus, and six to eight weeks later I’ll get what’s called polyclonal antibodies — the mice’s antibodies to the isolated protein. Then I’ll mix it with *Streptococcus pneumoniae*, and if it binds — it will show a fluorescent reaction in a lab procedure — I’ll know it’s a target.”

The next, crucial question: is the antibody killing the bacteria? Not all antibodies do, Goldstein says. If the answer is yes, it’s back to the lab to see if the antibodies are homologous, killing just the strain that was the target protein’s source, or heterologous, meaning they kill every strain on the all-important tree.

If, and only if, these steps prove successful and the antibodies prove heterologous, Goldstein and his team will advance to vaccine development trials. “That’s the proof of the pudding,” says Goldstein. Will this new vaccine protect laboratory animals from not just some, but all strains of pneumococcus?

If it does, he says, “I’ll dance naked in the street.” ■