Innovative Technologies
Emerging technologies keep us one step ahead of emerging infectious diseases

Pathogens, diseases, and people move across borders. Infectious diseases constantly evolve. Protecting Americans and people around the world demands that we develop better tools to address diverse threats—from familiar yet pernicious diseases like rabies to emerging and rapidly spreading viruses like chikungunya. We use laboratory testing, disease tracking, and epidemiologic investigations to keep infectious diseases at bay. This work requires continually stoking the pipeline with cutting-edge diagnostics and laboratory assays. We also stay on the hunt for innovative solutions, like finding a way for scientists on different sides of the globe to share the same microscope so they can identify a particularly elusive pathogen.

To advance our mission of reducing illness and death from infectious disease, the National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), in collaboration with partners, has developed the following new tests and tools to better protect people from these wide-ranging, ever-changing threats.
Labs in the time of Ebola
Epidemic spurs laboratory innovation

PROBLEM
The first case of the Ebola epidemic in West Africa was reported in Guinea in March 2014. In the following months, the disease spread through the neighboring countries of Sierra Leone and Liberia. As of September 10, 2015, there have been about 28,000 total cases (suspected, probable, and confirmed) and more than 11,000 deaths. The rapidly spreading disease overwhelmed the limited capacity of laboratory processing and analysis in the three countries. Curbing the epidemic required developing ways to quickly and accurately diagnose and isolate Ebola-infected patients.

INNOVATION
From the beginning of the outbreak, NCEZID scientists worked to build and expand the capacity of labs in West Africa. They also worked with biotechnology and pharmaceutical companies to develop laboratory innovations for use in West Africa that will help prevent future outbreaks.

• Rapid diagnostic tests
  NCEZID scientists worked with several private companies to field test a rapid Ebola diagnostic test that looks for antigens in blood. Antigens are proteins produced by the Ebola virus that stimulate an immune response in the body. The test uses fingerstick or whole blood samples and can provide results in as few as 4 minutes.

• Molecular detection tests
  NCEZID laboratorians developed a real-time PCR (polymerase chain reaction) test, which detects genetic material from the virus in just a few hours. The US Food and Drug Administration has approved that this test can be used in emergencies (a process called Emergency Use Authorization). Using this approach, the laboratorians worked with several pharmaceutical companies to develop and distribute molecular detection tests.

• Hand-held device
  NCEZID is assisting in the development of a hand-held device that can be used in remote rural locations. This device will be able to detect several viral hemorrhagic pathogens, including Ebola, Marburg, and Lassa fever.

• Field laboratory
  NCEZID established a diagnostic field laboratory in Sierra Leone in August 2014. The CDC lab was able to process more than 2,000 samples in a 3-week period. As of July 2015, the lab had tested over 20,000 specimens. These results helped guide the care of patients in Ebola Treatment Units in Sierra Leone.

NCEZID’s work in helping to develop these laboratory tools is enhancing the disease detection capacity that is necessary for controlling the spread of Ebola in West Africa. These technologies and the expanded field lab capacities will help prevent future outbreaks of Ebola or other viral hemorrhagic fevers.
NCEZID microbiologist James Graziano and virologist Johanna Salazar test blood samples for Ebola at CDC’s lab in Bo, Sierra Leone. (David Snyder/CDC Foundation)
New vaccines to reduce an age-old scourge
Developing new vaccines for people and animals

PROBLEM
Rabies annually causes more than 59,000 deaths worldwide. Most of these cases of rabies result from rabid dog bites; however, the disease is also spread by other animals. Domestically, more than 90% of all animal rabies cases reported each year to CDC occur in wildlife, with wild carnivores (like raccoons and skunks) and bats as the principal hosts. Human rabies, which is rare in the United States, is almost always fatal. Despite the elimination of canine rabies in this country, the disease remains a threat to people and requires the annual investment of hundreds of millions of dollars in rabies disease diagnostics, prevention, and control.

INNOVATION
The good news is that rabies is a vaccine-preventable disease. Scientists at CDC are developing new rabies vaccines for both people and animals that will help improve rabies control throughout the world. Using innovations like advanced reverse genetics to recover historical rabies virus strains, NCEZID’s rabies experts are developing a series of safer and more effective vaccine candidates for use in people. Similarly, a rabies strain that is common for animal vaccinations has also been further weakened (attenuated) for improved safety and immunity.

Several of these experimental vaccines have been issued patents and licensure agreements in the United States, Europe, South Africa, and other countries.

A young girl in Haiti waits in line to get her family dog vaccinated. In Haiti, rabies vaccines for dogs are only available once a year through government campaigns. Haiti has the highest rate of canine rabies in the Western Hemisphere.
Many developing countries lack the resources to vaccinate enough dogs to eliminate rabies. NCEZID works in many countries to train people in the technical aspects of effective rabies vaccination programs.
A faster, easier screening test for diagnosing yellow fever
Results that used to take 2 days to obtain now take only 4 hours

PROBLEM

Yellow fever is widespread in tropical and subtropical regions of the world. In Africa, approximately 180,000 new cases occur every year with more than 25,000 deaths. Mosquitoes spread the virus to people through bites. Common symptoms include fever, chills, headache, and muscle aches. About 15% of people develop serious illness that can lead to bleeding, shock, organ failure, and sometimes death. Although an effective vaccine is available, outbreaks still occur around the world.

Yellow fever outbreaks can take time to detect. During this time, the disease can spread rapidly within a region. Quick, accurate identification of yellow fever is essential to controlling outbreaks. Currently, the most common, front-line diagnostic test takes 2 days to complete and requires highly trained laboratory staff. International reference laboratories provide local laboratories with specialized test components (reagents) needed to do the testing. After receiving reagents, laboratory staff must independently calibrate or determine the exact amount of each reagent needed to accurately perform a test. When done properly, this test calibration process reduces the number of inaccurate test results and wasted materials. However, many remote locations or resource-poor tropical countries may not have staff trained to calibrate reagents or freezers for storing some reagents.

INNOVATION

To address these challenges, NCEZID developed a ready-to-use laboratory test kit. Now a diagnostic test can be completed in less than 4 hours. The kit contains premade, premeasured components that can be stored for at least 6 months without the need for freezing. The new kit yields results that are as accurate as the 2-day test. Field trials in countries with yellow fever outbreaks will help determine if the test kit or instructions require any changes. Improving the turnaround time, quality, and consistency of regional yellow fever testing will lead to faster outbreak identification and mobilization of a public health response.
NCEZID microbiologist Jane Basile shows how to use the new yellow fever test kit that in less than 4 hours yields results that are as accurate as the 2-day test.
Taking the bite out of mosquito-borne viruses
A new and more accurate diagnostic test for dengue virus

PROBLEM
The bite of an infected Aedes aegypti or Aedes albopictus mosquito can spread viruses like those that cause dengue and chikungunya. More than one-third of the world’s population lives in areas at risk for infection with dengue virus, a leading cause of illness and death in the tropics and subtropics. As many as 400 million people are infected with dengue virus each year. Chikungunya virus has caused outbreaks worldwide, but had not been detected in the Western Hemisphere until 2013. Since then, it has spread in epidemic proportions to 45 countries and territories in the region, affecting more than 1.5 million people.

Surveillance and timely diagnosis of dengue and chikungunya diseases depend on accurate and reliable laboratory tests. Because dengue and chikungunya disease cause similar symptoms, clinicians must use laboratory testing to confirm a diagnosis or to identify an outbreak. Over time, viruses mutate, causing their genetic structure to change. These mutations can make a diagnostic test less accurate. For example, a 2010 dengue outbreak in Micronesia nearly went unrecognized because the virus had changed and the existing test could no longer detect it.

INNOVATION
NCEZID has collaborated with other institutes and used advanced molecular detection (AMD) methods (such as whole genome sequencing and next-generation sequencing) to sequence thousands of dengue virus samples collected over the past 20 years. NCEZID scientists used AMD methods to create a new and more accurate diagnostic test for dengue virus, which is now being used around the world. AMD technology also can be used to help develop highly specific tests for other related viruses, such as Zika virus, which has recently been reported for the first time in the Western Hemisphere.

Zika is a virus related to dengue viruses. Current diagnostic tests for Zika virus cross-react with dengue virus tests. Therefore, a more specific test is needed so that sick people get the correct diagnosis. By routinely monitoring pathogens using AMD technology, the course of epidemics can be tracked, effectiveness of diagnostic tests can be more easily evaluated, and outbreak response can be improved.

The bite of an Aedes aegypti mosquito can spread dengue virus.
NCEZID scientists used advanced molecular detection methods to create a new and more accurate diagnostic test for dengue virus, which is now being used around the world.
A novel solution for preserving test samples
Waterborne disease prevention experts develop a new universal liquid buffer

PROBLEM
Molecular testing is an important tool for detecting and identifying germs in samples collected from the environment (like water) and from patients (like blood or stool). Samples collected for molecular testing typically need to be stored or transported at refrigerated temperatures until they can be tested in the lab. This can be challenging when working in the field or in resource-poor settings, because refrigeration is often not available or reliable, and access to laboratory testing facilities may be limited. When storing and transporting samples, it is important to have a way to preserve them for molecular testing without destroying the genetic material.

INNOVATION
NCEZID’s waterborne disease prevention experts developed a universal liquid buffer, which preserves water and other clinical samples and keeps them stable and undamaged for up to 416 days depending on the temperature (ranging from 40°F to 95°F). Using this new technology, samples that are collected in the field and need to be transported to a laboratory facility for molecular testing can be stored either directly in the liquid buffer or on filter paper discs soaked in the buffer, called UNEX cards.

Like the liquid buffer, the UNEX cards preserve the genetic material in the sample needed for molecular testing and are very easy to transport. The UNEX cards and liquid buffer aid in safe handling of samples by inactivating the sample so it is no longer able to infect. This new technology provides an inexpensive way for the safe and stable storage of water and other samples collected in the field for transport to laboratories.

Recently, the UNEX cards were used to test 400 water samples that were sent to the NCEZID laboratory from Ghana and Ethiopia to confirm the presence of E. coli. The lab was able to process the 400 samples very quickly and economically—it cost only $100, or 25 cents a card. This technology opens the door for performing molecular testing in a variety of challenging environments, like large outbreaks in remote locations, where clinical and environmental samples can be safely collected, stabilized, and transported.
NCEZID scientist Jothikumar Narayanan, co-inventor of universal liquid buffer, holds a filter paper disc that was soaked in buffer.
Microscopes across the universe
Electronic platform connects NCEZID pathologists with scientists around the world

PROBLEM
Imagine that halfway across the world in a remote and resource-limited village, three family members have recently died with swelling of the brain. The local doctors who treated the villagers have theories as to what caused the deaths but don’t have access to advanced laboratory technologies, powerful microscopes, and laboratory experts to confirm their hunches. They have extracted brain tissue, but even shipping the specimens to a pathology lab, often the first step in diagnosing and treating patients, can be a challenge.

INNOVATION
NCEZID has launched a new electronic platform called ePathology, which allows physicians and scientists from anywhere in the world to electronically submit images and scanned pathology slides to CDC pathologists for evaluation. All they need is an internet connection. In this hypothetical case, the village doctors and pathologists at NCEZID view the slides of the brain tissue together through a video conference. NCEZID pathologists’ examination reveals characteristic structures in cells where viruses are multiplying, which confirms that this is a viral infection. They strongly suspect that rabies virus was the cause of death. With this information, local officials investigate the area for rabid animals, seek people who may have been bitten by a dog or animal, administer vaccines to those bitten, and educate the community about animal vaccination and steps to prevent rabies infection. In the coming months, we anticipate many real-life examples of ePathology in action, identifying and preventing infections and saving lives.

Looking further ahead, NCEZID plans to expand ePathology to offer users real-time telepathology imaging. This development will mean a physician or scientist from any point on the globe and NCEZID pathology experts in Atlanta will look through the same microscope at the same time, as if they were working side by side in the lab. In addition to sharing views, ePathology will also allow reference tools to be shared with users, including an image library and reviews of cases, which will be particularly useful in the diagnosis of rare or unknown illnesses.
Using real-time telepathology imaging, a scientist from thousands of miles away can share his microscope with NCEZID pathology experts in Atlanta.
A one-stop shop for diagnosis
Tweaking NCEZID’s online virtual reference laboratory

PROBLEM
In 2012, the average stay in a US hospital was 4.5 days and cost $10,354. Every minute counts for a hospitalized patient awaiting a diagnosis, and every minute of a hospital stay costs dollars—hundreds and even thousands when an illness is difficult to diagnose.

Although new advanced molecular detection technologies, such as whole genome and next-generation sequencing, can produce vast amounts of data to aid in the diagnosis, analyzing voluminous data can be time consuming. Part of the problem has been that the data are fragmented over multiple databases, requiring the time-consuming task of searching many databases to identify the source of the problem.

INNOVATION
A few years ago, NCEZID launched MicrobeNet, an online virtual reference laboratory designed to aid in faster and more accurate disease identifications. MicrobeNet is a multiplatform search tool that is curated by NCEZID microbiologists who are available to consult on the pathogens referenced in the MicrobeNet system. A researcher can find contact information for the NCEZID subject matter expert on each species information page. MicrobeNet currently has DNA sequence, phenotypic, and biochemical search capabilities for thousands of bacterial pathogens.

Recently, NCEZID has joined in partnership with two companies that will help MicrobeNet more completely realize its potential. ThermoFisher developed MicrobeBridge to make it easier for researchers to access MicrobeNet’s information and enables them to perform DNA sequence searches directly from sequencer instruments.

Another partnership, with Bruker Corporation, has created a cost-effective platform that allows hospitals, state public health labs, and others to rapidly search NCEZID’s databases as well as access the wealth of other information that MicrobeNet has to offer. MALDI-ToF instruments (Matrix-Assisted Laser Desorption Ionized Time of Flight) have the potential to significantly reduce turnaround times, speed up appropriate treatment and care, save money, and assist in infection control and outbreak surveillance. Both of the innovations will equip scientists and researchers to more easily and quickly access MicrobeNet data to identify pathogens that are causing illness and disease outbreaks.
NCEZID microbiologists are available to consult on pathogens referenced in the MicrobeNet system.
Far from the contagious crowd
Simulating how flu spreads

PROBLEM
A number of factors can influence the spread of influenza viruses at mass gatherings, including the number of flu cases within the community, the susceptibility of people attending an event, and the intensity and length of their interactions (also called social mixing). Researchers have worked to learn more about these factors, but have been limited by low participation rates and underreporting of short-duration social interactions.

INNOVATION
To address these limitations, NCEZID, in collaboration with the Oak Ridge National Laboratory (ORNL), assessed the use of innovative video analysis technology to capture the social mixing patterns among the approximately 400 attendees at GameFest, an event in Troy, New York in April 2013. Overhead video cameras were used to record attendees as they moved through the event and interacted with one another. A computer program was then developed to abstract the number and duration (in seconds) of social contacts from the video recordings when any two attendees were within about 6 feet of each other, the maximum distance that flu virus can typically spread from a cough or a sneeze. The team built a simulation model using the contact results obtained from the video analysis to more accurately describe how flu could spread at the GameFest event.

This was the first time that video analysis technology has been used for this purpose. Although more work is still needed, the NCEZID-ORNL approach could greatly increase opportunities for researchers to better describe social mixing patterns and improve modeling of disease spread in different settings.
A number of factors can influence the spread of influenza viruses at a mass gathering.
Decoding *Listeria*
A deadly—and elusive—foodborne germ

**PROBLEM**
Many germs can be spread through food. Some, like *Listeria*, can be deadly. Pregnant women, fetuses, newborns, older adults, and people with weakened immune systems are particularly vulnerable to *Listeria* infection. *Listeria* can contaminate many foods that we don’t usually cook, like deli meats, cheeses, and fruits and vegetables.

After someone eats food contaminated with *Listeria*, they may not get sick until weeks later, making it difficult to identify which food was the source. This means that more people could become sick before officials can determine the food source, issue a recall, or alert the public to a contaminated food.

**INNOVATION**
In 2013, NCEZID scientists, in collaboration with the US Food and Drug Administration, US Department of Agriculture, National Center for Biotechnology Information, and state and local health departments, began applying advanced molecular detection (AMD) methods through the *Listeria* Whole Genome Sequencing (WGS) Project. Combining whole genome sequencing of *Listeria* isolates and data on sick patients’ food histories has helped to speed detection and investigation of *Listeria* outbreaks.

In the first year of this project, these methods have detected more clusters of *Listeria* infections, solved outbreaks of *Listeria* infection faster by linking them to likely food sources, and identified *Listeria* in new foods. For example, one person’s *Listeria* infection was linked to contaminated prepackaged lettuce, which was then recalled by regulatory agencies.

In another example, an outbreak was linked to whole apples used in commercially produced prepackaged caramel apples. AMD methods showed that the apples were contaminated with the two *Listeria* strains involved in the outbreak. Because of these investigations, caramel apples and prepackaged lettuce were identified as sources of *Listeria* infection for the first time.

Combining AMD methods with data on sick patients’ food histories helped NCEZID investigators rapidly determine the foods responsible for outbreaks and take public health actions, including recalls, sooner than would have been possible using traditional methods.

In the future, investigators can apply lessons learned from the *Listeria* WGS project to outbreaks caused by other foodborne germs like *E. coli* O157 and *Salmonella*, which will save even more lives.
Advanced molecular detection methods showed that two *Listeria* strains were involved in the outbreak in prepackaged caramel apples. This was the first time *Listeria* had been found in caramel apples.
Antibiotic-resistant bacterial infections are a widely recognized public health threat. Less is known about the effects of antifungal resistance and drug-resistant fungal infections, which no longer respond to the antifungal medications that are designed to cure them. This emerging phenomenon of antifungal resistance is especially a concern for invasive infections like the fungus *Candida*.

**Invasive candidiasis**, a serious infection, occurs when *Candida* yeasts enter the bloodstream and spread throughout the body. It is the most common cause of healthcare-associated bloodstream infections in the United States. One *Candida* bloodstream infection can result in 3–13 additional days of hospitalization and $6,000–$29,000 in additional healthcare costs. Some types of invasive candidiasis are becoming increasingly resistant to the first- and second-line antifungal medications fluconazole and echinocandins. Compared with the more costly method of DNA sequencing, this new test uses fluorescent probes to detect resistance genes and provides a rapid and cost-effective way to screen for echinocandin resistance. Clinicians can use this important information to determine the appropriate treatment plan for patients, an important first step to improved health outcomes.
The emerging phenomenon of antifungal resistance is especially a concern for invasive candidiasis, a serious infection involving the fungus *Candida* pictured here.
Measuring immune response to the new HPV vaccine
New test provides much-needed answers

PROBLEM
Annually, there are approximately 528,000 cases of cervical cancer worldwide and 266,000 deaths, most of which are associated with human papillomavirus (HPV). Although most HPV infections clear on their own, HPV may cause cervical and other ano-genital cancers and oropharyngeal cancers.

The first HPV vaccine was introduced in the United States in 2006. This vaccine and a second vaccine approved in 2009 target the two types of HPV infections that are linked to about 70% of cervical cancers worldwide. These vaccines already have resulted in a 56% decrease in these two types of HPV infections among girls aged 14–19.

A new HPV vaccine (9vHPV) targets five additional cancer-causing HPV types and can offer protection from more than 80% of cervical cancers. To ensure that this vaccine is used most effectively, we need results of HPV serologic testing, which is the laboratory examination of blood to measure the body's immune response to HPV. Examining HPV serology, both before and after receiving the vaccine, helps us understand how people respond to different dosing schedules of the vaccine. It also provides insights into the immune responses in special populations (such as those with HIV or transplanted organs) and will help us track those who have received the vaccine.

INNOVATION
NCEZID’s HPV lab has developed a test to measure the immune system response to the nine types of HPV in the new HPV vaccine. The test (a high-throughput multiplex serology assay) uses one sample to provide much-needed answers to questions about how many doses of vaccine are necessary, when they need to be given, and whether giving other vaccines at the same visit will affect the immune response to the HPV vaccine. The assay is also needed to help us understand how those with impaired immune function make antibodies after vaccination and whether new vaccines being prepared in low-resource countries are comparable to approved vaccines. This feedback is critical for evaluating the success of the new vaccine and will inform the Advisory Committee on Immunization Practices, a group of medical and public health experts that develops recommendations on how to use vaccines.
Preteens should receive all three doses of the HPV vaccine series.
New tools and drugs to slow antibiotic resistance
Providing raw material to develop new antibiotics and diagnostic tests

**PROBLEM**

“Antibiotic resistance may be the single-most important infectious disease threat of our time,” CDC Director Tom Frieden said recently. Antibiotic-resistant germs—bacteria that no longer respond to the drugs designed to kill them—cause more than 2 million illnesses and 23,000 deaths each year. Because antibiotic resistance occurs as part of a natural process in which bacteria evolve, it can be slowed but not stopped. Therefore, we will always need new technologies—new diagnostic tests as well as drug-resistant antibiotics—to keep pace with resistant bacteria and improve how we track resistance and prescribe antibiotics.

**INNOVATION**

The Antimicrobial Resistance Isolate Bank is a joint project between CDC and the US Food and Drug Administration (FDA) to give groups invested in slowing antibiotic resistance, like pharmaceutical companies and diagnostic manufacturers, the “raw material” necessary to develop new antibiotics and diagnostic tests. The raw material is actually collections of bacterial isolates—called panels—that CDC and FDA routinely collect and analyze as part of public health surveillance and tracking activities. Once the panels are shared, researchers at pharmaceutical and diagnostic manufacturers can use the isolates to identify and characterize new strains of resistant bacteria, develop the next generation of diagnostics, and accelerate research for development of new antibiotics. The panels are accompanied by comprehensive data that can support studies to advance development of earlier diagnoses and more effective treatment options that slow the threat of resistance.

Microbiologist Brian Yoo prepares a panel of isolates for processing.
A panel of isolates from a freezer in one of CDC’s labs. The Antimicrobial Resistance Isolate Bank provides a diverse collection of bacteria to researchers to support development of more accurate diagnostic tests and more effective antibiotic drugs—all to help slow antibiotic resistance.
Looking ahead
Antibiotic resistance—Where are we going?

Antibiotics can fight infections and save lives when used at the right place, at the right time, and for the right duration. However, the overuse and misuse of antibiotics in our country is contributing to antibiotic resistance—when bacteria stop responding to the drugs designed to kill them.

New diagnostics have the potential to transform when and how antibiotics are used by helping health care providers confidently identify infection types and make treatment recommendations. Diagnostics can also be an essential tool for preventing transmission of infectious disease in healthcare and community settings. With fewer infections, there is less need to use antibiotics.

CDC’s Fiscal Year 2016 Antibiotic Resistance Solutions Initiative—which would support CDC activities in the National Action Plan for Combating Antibiotic-Resistant Bacteria—is a comprehensive approach that includes helping develop and implement transformative diagnostics in healthcare settings where they can have the biggest impact.

Our goal is to

- Make it easier to test the accuracy of new diagnostic tests and devices by providing the most current and important resistant bacteria to researchers and developers.
- Evaluate the impact diagnostics have on antibiotic use.
- Improve tracking and outbreak detection with surveillance diagnostics.
- Improve antibiotic use and reduce the spread of disease by making recommendations that include well-established diagnostic solutions.