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the University of New South Wales in Sydney, Australia. The good news is that hepatitis C can often be treated. And as improved medications with greater efficacy and shorter treatment times reach the market, there is a greater imperative to find and treat the people who are infected.

QUICK AND CHEAP

Several companies have rapid antibody tests for HCV in the pipeline. The first one to hit the market was developed by OraSure Technologies, a biotechnology firm in Bethlehem, Pennsylvania. The company's OraQuick HCV Rapid Antibody Test was approved for use in Europe at the end of 2009 and in the United States in mid-2010, and is gradually being rolled out in these regions.

In the United States, the OraQuick test can be carried out with blood from a finger prick. In Europe, the less invasive method of taking a mouth swab for saliva has also been approved. Either way, a health professional places the sample into a vial and inserts a plastic strip that looks like a home pregnancy test: two red-purple lines means that the patient has antibodies to HCV¹. The results are ready in 20 minutes.

"We believe it's going to dramatically expand testing opportunities," says Stephen Lee, OraSure's chief science officer. "Testing will be able to go on in a broad range of settings — doctors' offices, community outreach centres, needle-exchange clinics, even mobile testing vans." Similar rapid tests have greatly increased HIV screening and diagnosis over the last decade.

The next step in the diagnostic journey, testing for viral RNA, typically involves a method such as the polymerase chain reaction (PCR) to amplify the virus's genetic material to a detectable level. This step is time-consuming and expensive — much of the reason why antibody testing is usually carried out first. "One of the big issues is making RNA testing affordable," says John Ward, director of the Division of Viral Hepatitis at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. Faster RNA testing, he says, would also be "a fantastic development".

One approach to improving RNA testing comes from a team led by Hassan Azzazy, a chemist at the American University in Cairo. "We are going directly to detecting HCV RNA in a single reaction, without amplifying the RNA of the virus," he says.

Azzazy's test uses tiny particles of gold, each about 15 nanometres in diameter². Gold nanoparticles have an unusual optical property known as 'surface plasmon resonance': when the particles are distributed evenly throughout a liquid, they reflect light in a way that makes them appear red; however, when they clump together, they look blue.

To perform the test, Azzazy first takes serum from the patient and treats it to extract RNA from any virus present. To this treated serum he adds short pieces of DNA that are

DIAGNOSTICS

A testing journey

Results ready in minutes and more efficacious drugs will help find and treat the hundreds of millions of carriers.

BY SARAH DEWEERDT

Obtaining an accurate diagnosis of hepatitis C often means travelling a long road, with many stops along the way. First, you'll need a blood test to check for antibodies to the hepatitis C virus (HCV), an analysis that can take as long as two weeks. But even with antibodies, you might not be infected: 15–25% of exposed people clear the virus without needing any treatment.

The next step is to determine whether you have an active infection, and that means another blood draw to test for the presence of viral RNA — and another week or two of waiting for results. If that test comes back positive, you may need additional blood tests to determine your genotype (see 'Playing the odds') and the genotype of the virus, both of which affect how you are likely to respond to treatment.

This drawn-out journey is only part of the problem. HCV infection is often 'silent', with

symptoms not developing for twenty years or more. Consequently, many infected people are unaware they have hepatitis C and never seek medical help. Estimates are that fewer than half of the people who are chronically infected know they have HCV. For some, this will not be a problem as the disease will not progress; for others it could be fatal.

Scientists and public-health advocates are now turning their attention to developing tests that are faster, cheaper and less invasive. They are also considering how to design screening programmes to identify people who might, unknowingly, be harbouring the virus. "It doesn't matter how good your treatments are if the majority of your affected population is not diagnosed," says Gregory Dore, head of the Viral Hepatitis Clinical Research Program at

As many as 1 in 33 baby boomers might be infected.

complementary to HCV RNA. The gold nanoparticles are then added to this solution. In the absence of HCV RNA, the primers stick to the gold nanoparticles and separate them — and the solution appears red. If the virus is present, the primers pair with the viral RNA instead and the gold nanoparticles aggregate, turning the solution blue (see ‘Red light, blue light’). The reaction can be carried out in a test tube, and the whole process takes about 30 minutes. “We don’t need fancy infrastructure to run the test or to interpret the results,” Azzazy says. It should cost about one-seventh as much as the current HCV RNA tests, he says, and could make antibody testing unnecessary.

ONE-STOP TEST

Researchers at Brazil’s Carlos Chagas Institute, part of the Oswaldo Cruz Foundation in Rio de Janeiro, aim to streamline the diagnostic process even further. Marco Krieger and colleagues say that a single RNA test could suffice both to diagnose HCV infection and to identify which subtype of the virus is present, which will help guide treatment.

There are at least six genotypes of HCV, most of which have several subtypes. In Brazil, subtypes 1a, 1b, 2a, 2b, 2c and 3a account for nearly all HCV infections. Because different genotypes are present in different parts of the world, a diagnostic test designed for one country might not work in another, says Marco Krieger, who is leading the project.

His group’s test makes use of a technology called a liquid microarray, in which short pieces of DNA are attached to tiny plastic beads, or microspheres, floating in a sample³. It’s similar to a gene chip in that it can detect many nucleic acid sequences simultaneously, which makes the test both rapid and flexible.

Currently, the test is designed to identify both genetic sequences that are common to most viral subtypes, and variable sequences that distinguish between the subtypes. Unlike current RNA tests, the method can detect many different sequences in a single reaction, and Krieger says that it could easily be adapted to detect a different set of genotypes. “The assay flexibility also allows us to add new sequences in the future, for instance, sequences related to drug resistance if necessary,” he says.

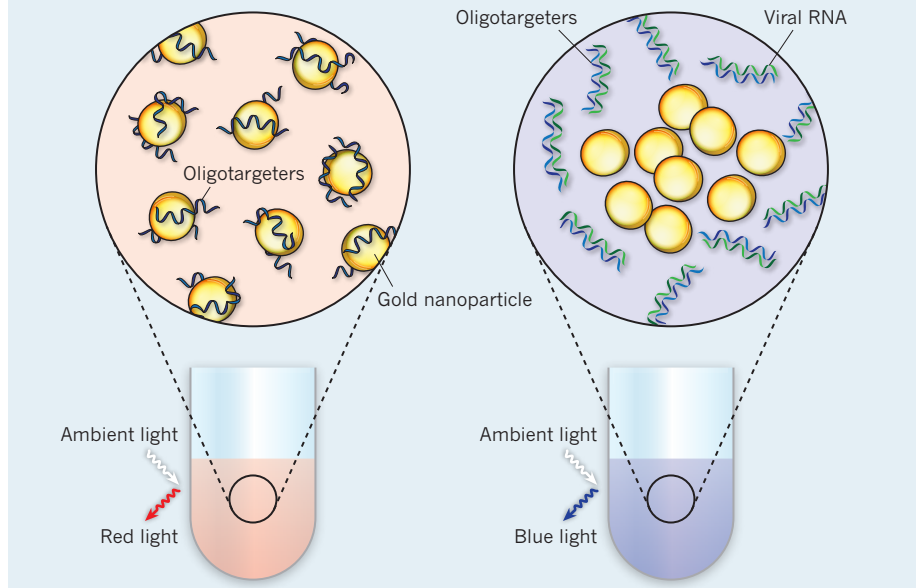
SCREENING SELECTION

As more diagnostic technologies are developed, other issues arise. Ward points out the need for standards to ensure that results of different tests can be compared. The CDC has begun discussions with the World Health Organization about collaborating to create such guidelines.

Another equally important issue is how to apply the tests. In the United States and many other developed countries, where the overall prevalence of HCV infection is low, mass screening isn’t cost effective. The CDC’s current HCV screening recommendations,

RED LIGHT, BLUE LIGHT

Gold nanoparticles are added to a test tube containing a patient’s serum and oligotargeters — short pieces of DNA designed to match genetic material from the hepatitis C virus (HCV). If there is no HCV, the oligotargeters stick to the nanoparticles and separate them and the solution appears red. If HCV is present, the oligotargeters pair with the viral RNA and the gold nanoparticles clump, turning the solution blue.



developed in the late 1990s, advise testing those known to have been at risk of exposure to the virus. But many people who contracted the virus several decades ago, perhaps through a blood transfusion or youthful experimentation with drugs, don’t realize they are at risk and do not come forward to be tested.

According to researchers at the Amsterdam Public Health Service, the Internet could be used to find these people and help them. A team led by Maria Prins designed and validated an online screening questionnaire about risk factors such as former injection drug use, blood transfusions before blood bank screening began and immigration from countries with a high prevalence of HCV infection⁴.

A mass media campaign including television and Internet advertisements brought almost 41,000 people to the website (www.heptest.nl), and 9,653 completed the questionnaire. Of the 1,480 people who were found to be at risk and eligible for a free blood test, 420 — nearly 30% — were tested. Prins was encouraged by this participation rate; in a similar effort aimed at syphilis, only 10% of those identified as at risk followed through and had a test, she says.

Study participants said they liked the convenience and anonymity offered by the online process. The biggest hurdle was encouraging people to take the questionnaire in the first place. “People don’t see hepatitis C as something very threatening, and they confuse it with hepatitis B,” says Prins. “They think they have already been vaccinated.” In the future, the team may combine screening efforts for the hepatitis B virus and HCV.

To avoid a looming crisis, however, public

health authorities will need to cast a wider net and not wait for people to seek out a test. In particular, it makes sense to target certain age groups. In developed countries, the peak of HCV transmission was between the 1960s and 1980s. Many of those infected during this period will soon begin to develop liver damage and possibly cancer: in the United States, as many as 1 in 33 baby boomers might be infected. According to a computer model designed by a team led by Lisa McGarry of i3 Innovus, part of the health-care information technology company Ingenix, in Medford, Massachusetts, screening everyone born between 1945 and 1970 would mean carrying out more than 80 million tests in the United States alone — but it could prevent 50,000 deaths. In fact, new CDC guidelines due to be released in 2012, might include a similar recommendation for age-based screening for HCV.

“A routine test in that age group is what you want,” says Eugene Schiff, director of the Center for Liver Diseases at the University of Miami in Florida. He argues that this greatly expanded screening is key to making the journey to diagnosis shorter — and the burden of hepatitis C lower. “You’re not going to get it done otherwise.” ■

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