A mother in Puerto Rico holds her son, who has microcephaly, a condition potentially linked to infection with Zika virus in pregnant women.

CARLOS GIUSTI/AP/REX/SHUTTERSTOCK

SCREENING

In the blood

When threats emerge to the blood supply, public-health officials must make difficult decisions to reduce the risk of infections being transmitted by transfusions.

BY CASSANDRA WILLYARD

In March 2016, staff at Puerto Rico's blood banks packed up their gloves and gauze, needles and collection bags. Then they began to turn away potential donors. The link between Zika virus and the surge in Brazilian babies born with abnormally small heads was tenuous, but public-health officials thought that the mosquito-borne virus could be transmitted through blood — and didn't have a way to test for it.

In the continental United States, blood banks began to ask people who had travelled recently to regions where Zika virus was a problem to wait one month before donating. But in Puerto Rico, where mosquitoes already carried the virus, it was impossible to tell who had been infected and who had not — up to 80% of those who contract Zika virus never fall ill. So the US Food and Drug Administration (FDA), the federal agency charged with ensuring the safety of the US blood supply, recommended that the collection of blood be halted in regions where there had been local transmission of the virus.

At Banco de Sangre de Servicios Mutuos in San Juan, Puerto Rico's largest blood bank, staff had to dispose of 1,500 bags of red blood cells, plasma and other blood products that had already been tested for other pathogenic agents, processed and labelled. "It was a very challenging time," says Jose Alsina, the facility's chief operating officer. "We were trying to figure out how we were going to survive."

The blood supply in the developed world is remarkably safe, but that wasn't always the case. In the 1960s, the risk of contracting hepatitis B virus from a blood transfusion was around 30%. In the late 1970s and early 1980s, about half of all people with haemophilia in the United States became infected with HIV. Screening assays have since made the transmission of viruses through donated blood rare. But the system that health authorities use to keep the blood supply free from pathogens isn't perfect.

When threats such as Zika virus appear suddenly, public-health officials must scramble to work out how to best protect the blood supply in the absence of a screening test. And even when pathogens emerge more slowly, screening tests can take years to materialize. In some cases, the infectious agents seem to defy detection. Some technologies don't merely detect pathogens in the blood, but inactivate them as well. At the moment, however, such systems are only approved for use on certain components of the blood such as platelets and plasma.

These limitations mean that public-health officials must make tough choices. The public has a low tolerance for risk in the blood supply. Yet testing for particular pathogens may not always be practical or warranted.

EMERGING INFECTIONS

The Zika-virus crisis highlights perfectly how sticky these situations can get. Less than one month after Puerto Rico stopped collecting blood, the FDA announced the availability of a test that detects Zika-virus RNA in

samples of blood. The test allowed Servicios Mutuos and other blood banks affected by Zika virus to start receiving donations again.

Meanwhile, blood banks in unaffected parts of the United States continued to turn away donors who had travelled to areas in which Zika virus was being transmitted. But late in August 2016, the FDA issued fresh guidance: blood-donation centres would need to test each donation for the virus. According to Louis Katz, chief medical officer and acting chief executive officer at America's Blood Centers, the largest network of independent blood-collection centres in North America, US blood banks screened more than 10 million units of blood for Zika virus in the first year of testing, at an estimated cost of US$137 million.

Whether that was money well spent is a matter of debate. At the time, there was considerable uncertainty with regards to how far Zika virus would be able to spread in the United States, says Peter Marks, director of the FDA Center for Biologics Evaluation and Research in Silver Spring, Maryland. What's more, evidence had just surfaced that the virus could be transmitted sexually, and that men could harbour it in their semen for weeks. "It seemed most rational to test uniformly across the United States," Marks says.

But many in the blood-collection community saw the guidance as an overreaction. In a joint statement, the American Association of Blood Banks, the American Red Cross and America's Blood Centers called the move "wholly inappropriate". James AuBuchon, chief executive officer of Bloodworks Northwest, a blood bank in Seattle, Washington, points out that the *Aedes* species of mosquitoes that transmit Zika virus are not found in the Pacific Northwest region of the United States, making the risk of transmission there extremely small. "We would have to test for hundreds of years before interdicting a case that would have led to an unfortunate neurologic consequence for a fetus," he says.

"All screening assays will have a number of false positives."

Moreover, most of the diseases that are transmitted by mosquitoes don't pass easily from person to person through blood. "There are hundreds of thousands of mosquito-borne cases, and only a handful of transfusion transmissions," says Susan Stramer, vice-president of scientific affairs for the American Red Cross in Gaithersburg, Maryland. For Zika virus, in particular, only four cases have been reported of transmission through blood transfusion — and none of the recipients developed symptoms. "There was such great concern about the prospect of a microcephalic baby after a transfusion transmission that Zika testing was implemented without any formalized risk-based decision-making," Katz says.

The FDA will re-examine the issue in coming months, says Marks. "Had we known with hindsight what we know now, we likely would have done things differently."

Mosquitoes trapped in Dallas County, Texas, are sorted as part of the response to a case of Zika virus.

LM OTERO/AP/REX/SHUTTERSTOCK

TICK TOCK

Zika virus isn't the only emerging pathogen on the FDA's radar. A slower-moving threat to the blood supply is spreading through the northeast and upper-Midwest regions of the United States — the tick-borne parasite *Babesia microti*, which infects red blood cells and causes the malaria-like disease babesiosis. People with a history of babesiosis are prohibited from giving blood, but as with Zika virus, many who are infected develop only mild symptoms or none at all. Blood banks don't routinely screen for *B. microti*, and the pathogen has become the most commonly reported transfusion-transmitted parasite in the United States. Between 1979 and 2009, almost 200 people in the country were infected by *B. microti* from transfusions; at least 27 died. "*Babesia* is probably one of the single greatest concerns when it comes to transfusion safety," says Stramer. In fact, in 2009, Stramer, Katz and other members of a committee on transfusion-transmitted diseases identified *B. microti* as a high-priority pathogen. However, profit margins for blood screening are much lower than for pharmaceuticals, and only a handful of companies are working to develop screening tests for the parasite.

One company to show an interest in testing for *B. microti* is biotechnology company Imugen, based in Norwood, Massachusetts. In 2012, Imugen partnered with the American Red Cross to test two of its assays — one that detects antibodies raised against *B. microti* and another that detects the parasite itself. Over a period of two years, the American Red Cross screened[1] almost 90,000 samples of blood from donors in Connecticut, Massachusetts, Minnesota and Wisconsin, removing from the blood supply any donation that tested positive. Twenty-nine people who received unscreened blood contracted babesiosis. None of those who received screened blood developed the disease. Stramer and her colleagues have continued to test some of the blood collected in certain US states, and none of the 375,000 units screened by July 2017 have transmitted the parasite.

Last year, Oxford Immunotec, a diagnostics company with headquarters in the United States and the United Kingdom, acquired both Imugen and Immunetics, another Massachusetts-based company developing a *B. microti* screening test. In August 2017, the American Red Cross began using another assay, developed by Spanish pharmaceutical company Grifols, to screen for *B. microti*, as part of a clinical trial to test the assay's performance. Health-care company Roche, based in Basel, Switzerland, is also working on a screening test for the parasite.

At present, however, the FDA has yet to approve any screening test for *B. microti* or issue guidance on screening. So the Red Cross does not test every unit collected — even in states where *B. microti* is prevalent. The screening adds about $20 to the cost of a unit of blood. That's an increase of roughly 7–10%, and "not all hospitals want to pay", Stramer says.

In 2010, the FDA's Blood Products Advisory Committee advised the agency to adopt regional testing. But when the committee met again in 2015, they upped their recommendation to cover all 50 US states. Stramer expects that the FDA will require screening only in certain regions. But developing such guidance could prove even trickier. "How do you define a state with enough prevalence to justify donor screening?" asks Katz.

TRICKY TEST

Other potentially transmissible agents, such as proteins called prions that are thought to cause the fatal neurodegenerative disorder Creutzfeldt–Jakob disease (CJD), are much rarer and pose even more of a challenge to detect.

Prions exist in two forms, one of which is folded correctly and harmless. Misfolded prions can act as infectious agents, causing a chain reaction of misfolding in other previously normal prions. The abnormal proteins then clump together in the brain, with

worsening neurological effects. Classical CJD can be inherited or can occur spontaneously. But variant CJD (vCJD), first described by researchers in the United Kingdom in 1996, occurs in people who ate prion-contaminated meat from cows with the prion disease bovine spongiform encephalopathy, also known as mad cow disease.

Unlike classical CJD, vCJD seems to be transmissible by blood transfusion. In December 2003, researchers identified the first transfusion recipient to develop vCJD. Soon after, two more blood recipients developed the disease. Both had received blood from the same donor. In all, three recipients of transfusions developed the disease and a fourth had abnormal prions in his lymphoid tissue when he died.

Researchers have been working on a blood-screening test for vCJD for years. But because misfolded prions are scarce compared with normal proteins, their detection can be tricky. One of the first blood tests[2], built in 2011 by researchers at the UK Medical Research Council Prion Unit at University College London, relied on prions' affinity for binding to metal. The assay correctly identified 71% of infected samples. In 2016, two research groups published reports of even more sensitive assays. A team led by Claudio Soto, a neurologist at the University of Texas Health Science Center at Houston, devised a method for amplifying misfolded prions that mimics the chain reaction that happens in the body, making them easier to detect. The test accurately classified blood from 14 cases of vCJD and 153 controls[3]. And a group of researchers from France and the United Kingdom used a similar method[4]. But they went one step further — identifying vCJD in the blood of two patients more than a year before they developed clinical symptoms.

"Those assays look very promising," says Patricia Hewitt, a transfusion medicine specialist with the UK National Health Service Blood and Transplant authority. But it's not clear how they will perform in a large, healthy population that could include people infected with vCJD who aren't yet exhibiting symptoms of the disease. The French–British study managed to pick up vCJD in two such samples, but "that's not enough data", Hewitt says. And validating the assays could be difficult because there are precious few samples of blood from infected individuals — fewer than 250 people have developed vCJD worldwide.

Hewitt also points out the need for a confirmation assay. "All screening assays will have a number of false positives," she says. Confirmation is especially important when the assay is meant to detect a disease such as vCJD. Without confirmation, blood banks would have to inform healthy people that they might have a fatal neurodegenerative disease for which there is no cure — and that might not cause symptoms for years. "That's scary to contemplate," AuBuchon says.

And even if it were feasible to develop a screening test, it's unclear whether there would be the scientific rationale or political will to screen for vCJD. All of the individuals who had abnormal prions transmitted through transfusions received the blood in the 1990s, before the UK government started to require blood banks to eliminate white blood cells, which play a part in spreading the infectious prions. There have been no cases linked to transfusion since.

However, Soto thinks that at least some countries will still want the assay. Soto founded a Houston-based company called Amprion to help commercialize the technology behind his research group's test, and says that the French government has already shown interest.

CLEAN SLATE

Screening blood donations for pathogens using a battery of separate assays takes a considerable amount of effort and money. But there may be an easier way that tackles the underlying cause. At least two companies are working on pathogen-reduction systems, which eliminate most infectious agents from blood in one go. Such systems could, in theory, wipe out unknown as well as known pathogens. "The beauty of this," says Marks, "is that it helps protect you against what you don't know." That could be a boon when sudden outbreaks of viruses such as Zika or Ebola occur.

The FDA approved one such pathogen-reduction system, called INTERCEPT, for platelets and plasma in 2014. Developed by Cerus of Concord, California, the system relies on a molecule called amotosalen that binds to RNA and DNA. When exposed to ultraviolet light, it creates crosslinks between strands of nucleic acid, "effectively shutting down all transcription or translation or replication from the cells", says Cerus's chief medical officer, Richard Benjamin. Platelets, plasma and red blood cells do not possess DNA or RNA. INTERCEPT therefore leaves these products unharmed but inactivates pathogens. (There is no evidence, however, that INTERCEPT would have an effect on prions.)

Two weeks after blood banks in Puerto Rico halted blood collection over concerns about Zika virus, Alsina implemented INTERCEPT at Servicios Mutuos. The blood bank was still importing red blood cells and plasma, but the system allowed the centre to resume collecting platelets. That was a relief, Alsina says, because platelets' five-day shelf life makes importing them difficult.

Medical-device company Terumo BCT, based in Lakewood, Colorado, has developed its own pathogen-reduction system called Mirasol. The technology relies on the vitamin riboflavin and ultraviolet light to disable pathogens. Terumo BCT markets Mirasol in Europe for platelets, plasma and whole blood and, in 2017, launched its first US-based clinical trial of the system. Meanwhile, Cerus is also applying INTERCEPT to red blood cells. In May, the company collaborated with Servicios Mutuos to launch a phase III trial of the system in Puerto Rico.

Pathogen-reduction systems could save money by eliminating the need for some screening assays, and could also address a more-worrisome problem. Bags of platelets can become contaminated with bacteria during the collection process and those who receive bacteria-laden platelets can develop serious infections. To reduce this risk, most blood-collection centres in the United States culture samples from each batch of platelets. However, some bacteria escape detection, and at least 1 in 100,000 people who receive a platelet transfusion develop sepsis.

Both INTERCEPT and Mirasol have been approved in Europe, but their use varies from country to country. Katz points out that in the United States, only about half of all platelets collected can be processed using the current platform. The concentration of platelets, for example, can't be too low or the system won't work. And pathogen-reduced platelets cost about 10–20% more per unit. "Hospitals are not anxious to pay for it," he says. According to Cerus, only about 60 hospitals are now transfusing pathogen-reduced platelets.

The FDA is mulling how to handle the bacterial threat. In 2016, the agency issued draft guidance that outlined two possible strategies: treating platelets with pathogen-reduction technologies or implementing a rapid test in hospitals to detect bacteria before platelets are transfused. "I don't think there's any controversy that we need an intervention," Katz says. "The question is the scope of the intervention." Katz indicates a third option. In the United Kingdom, blood-collection centres have implemented a delay in culturing their platelets. Instead of taking samples 24 hours after collection, they wait 36–48 hours, when the growth of bacteria is more likely to be detected. And they allow hospitals to keep platelets for seven days instead of the conventional five. That strategy reduced the number of transfusion-related transmissions of bacteria by 90%.

The FDA has yet to issue its final guidance, and Katz is unsure about what the agency will decide. But he thinks that more people should be included in such decisions. "We do lots of things in transfusion medicine that are not cost-effective," he says. For Katz, everything boils down to just one issue: how safe is safe enough? That's a question that should be answered by society, he says, not just regulators and blood-donation centres. ■

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1. Moritz, E. D. *et al. N. Engl. J. Med.* **375,** 2236–2245 (2016).
2. Edgeworth, J. A. *et al. Lancet* **377,** 487–493 (2011).
3. Concha-Marambio, L. *et al. Sci. Transl. Med.* **8,** 370ra183 (2016).
4. Bougard, D. *et al. Sci. Transl. Med.* **8,** 370ra182 (2016).