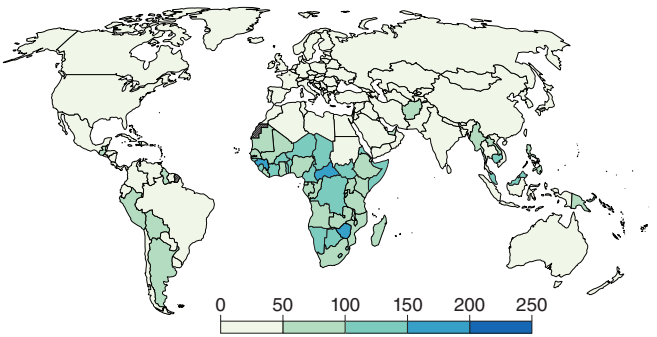
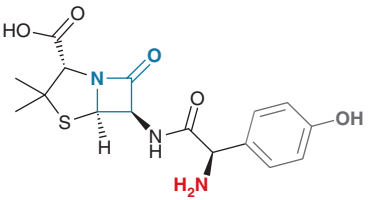
WorldLinks | Saving Lives with Azo Dyes

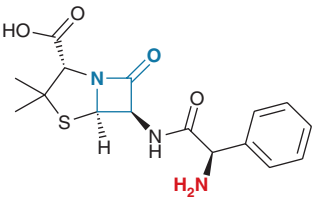
Pneumonia is one of the most common causes of death worldwide, and it kills more children than any other infectious disease. Every year, pneumonia takes the lives of over 650,000 children under the age of five. Antibiotics are a relatively cheap and effective treatment because most cases of pneumonia are caused by bacteria. In the United States, most children have access to healthcare and effective β-lactam antibiotics such as amoxicillin and ampicillin, so pneumonia is not a leading cause of death. Children living in impoverished parts of the world, however, are much more likely to die from pneumonia.



The estimated annual death rate from pneumonia and other lower respiratory tract infections per 100,000 people.



amoxicillin
1° amine
β-lactam
phenol

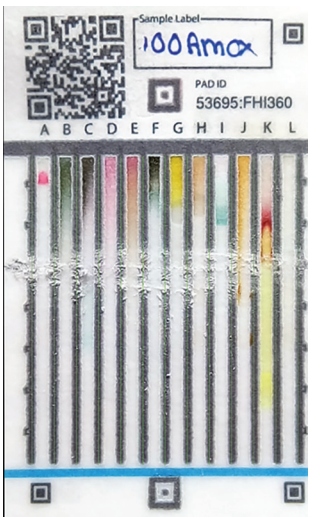


ampicillin
1° amine
β-lactam

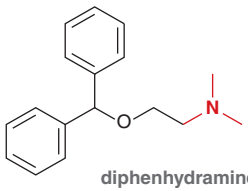
The reason low-income countries have higher death rates is in part due to a lack of resources—an inadequate healthcare infrastructure hampers the diagnosis and treatment of pneumonia, and poor families may be unable to afford treatment—but the reality behind the inequality is far more complex. Other contributing factors include a lack of vaccines, undernutrition, a lower prevalence of breastfeeding, environmental pollution, secondhand smoke, and overcrowded living spaces.

Another challenge in treating deadly diseases in developing countries is the presence of substandard or falsified drugs in the supply chain. In 2017, the World Health Organization reviewed over 100 papers about the quality of commercially available drugs in low- and middle-income countries. They found that 10% of the drugs tested were either substandard (did not meet required quality standards) or falsified (fraudulently misrepresented their identity, composition, or source). As a result, a child may be taking an antibiotic that is not the full prescribed dose, or perhaps it contains no antibiotic at all because the pills are made from 100% chalk! Severe pneumonia has a 6% death rate, so these inferior drugs cause thousands of excess deaths per year.

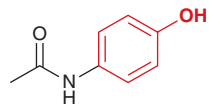
Low- and middle-income countries have neither the resources nor the facilities to increase regulatory oversight, so how can these substandard drugs be identified? One clever solution has been devised by Dr. Marya Lieberman, a chemistry professor at the University of Notre Dame. She developed the Paper Analytical Device (PAD), which can be used to test medications in countries that do not have capabilities for sophisticated analytical testing. They can also be used for low-cost, rapid testing of street drugs. Each lane on the card contains reagents that will test for a certain functional group. The lanes are created with a special printer that applies wax instead of ink, thereby creating a hydrophobic barrier to separate each analytical test. The solid to be analyzed is introduced to each lane by simply scraping it across the PAD, and the card is then left to stand in a shallow pool of water. The process is like developing a thin layer chromatography (TLC) plate in an organic solvent. In this case, as the water moves up the through the paper by capillary action, the reagents contained within each channel eventually reach the analyte at the swipe line. The aqueous solution dissolves the drug sample and mixes it with the reagents, effectively running several analytical tests simultaneously (in different channels). Within five minutes, the card can be assessed visually, by looking for color changes. If a smartphone is available, a mobile app can be used to read and analyze the PAD.



On the card shown, lane F uses a Cu(II) reagent to test for β-lactams. Lane B is a ninhydrin test for primary and secondary amines, as found in the antibiotic drugs ampicillin and amoxicillin. Lanes D and E test for tertiary amines, as found in cocaine and diphenhydramine (Benadryl, a common cutting agent in street drugs). Lane K utilizes an azo coupling reaction to test for phenols, as found in amoxicillin and the over-the-counter drug acetaminophen (Tylenol, an inexpensive painkiller used as a substitute for other active drug ingredients in some fake medicines).



diphenhydramine
(a 3° amine)



acetaminophen
(a phenol)

For the azo dye test, lane K is pre-loaded with *para*-nitroaniline, $NaNO_2$ and tosic acid (TsOH). These solids are stable until the card is placed in water, at which point the water moves up the lane by capillary action and dissolves each reagent in turn. The nitrite anion (NO_2^-) encounters the acid and is protonated to give nitrous acid (HONO), which converts the *para*-nitroaniline into a diazonium salt. Next, the mixture passes through the analyte before reaching the final reagent pre-loaded on lane K, a spot of NaOH above the "swipe line" that neutralizes the TsOH and deprotonates any phenol group in the analyte, enabling it to act as a nucleophile. As a result, an azo dye is produced, giving a yellow, orange, red, brown, green, or even black color as a positive test. The azo dye color, along with the results of the other lanes, show whether the positive azo dye test is amoxicillin or acetaminophen.

By putting a powerful analytical lab in the palm of your hand, a low-cost method for testing drugs such as the PAD can save lives. Public health officials around the world can now identify substandard or falsified drugs, thanks to some clever, colorful chemistry and the creative ingenuity of a chemistry professor.