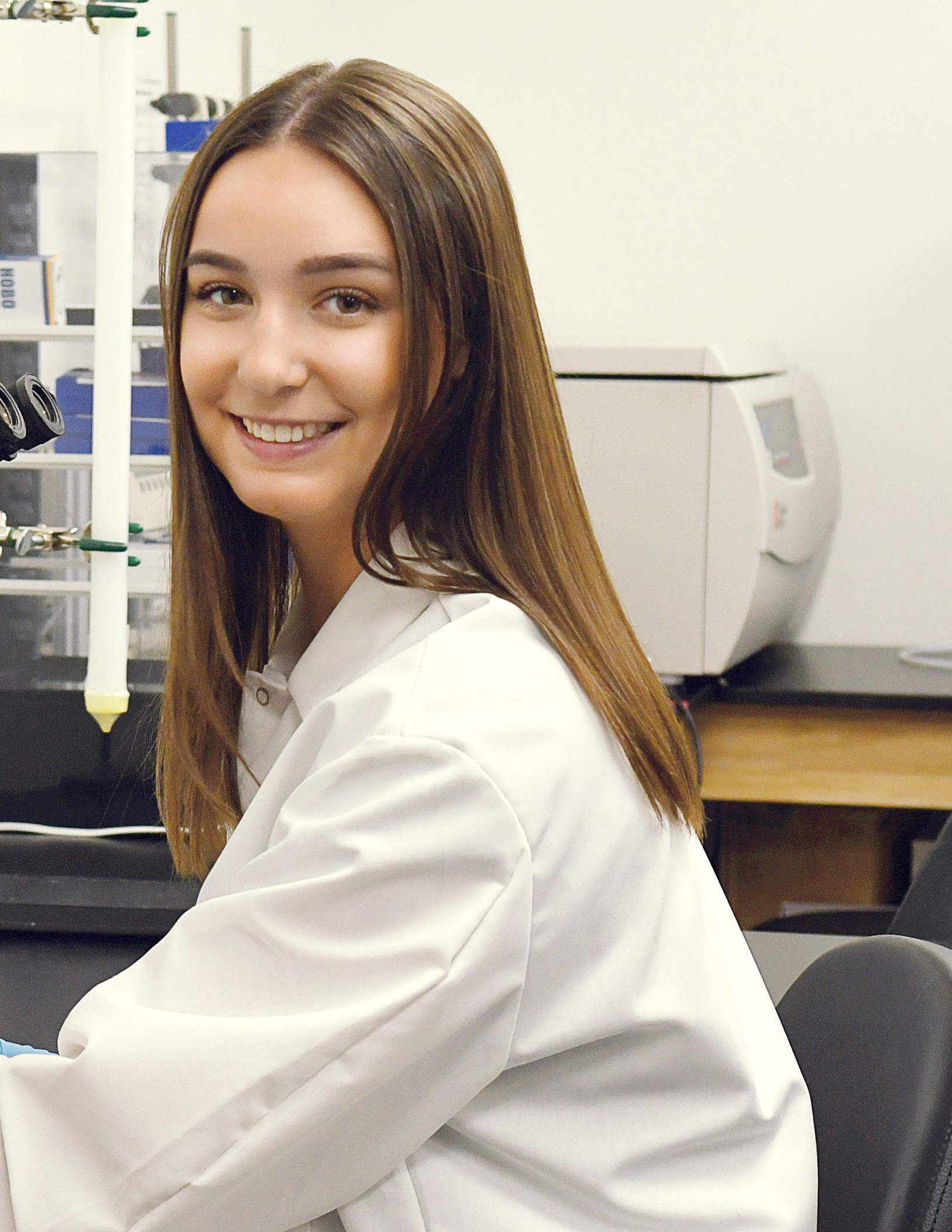
microBYTES

HARDY DIAGNOSTICS
A Culture of Service™

CLINICAL MICRO
VOL.1


Our heroes
wear lab coats.



CLINICAL MICRO
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Contents

Hardy Diagnostics may have had humble beginnings but since has grown to become one of the top producers of culture media in the country. Not only that, but Hardy Diagnostics has the unique distinction of being a 100% employee owned company. The Hardy Diagnostics ESOP was created in 2012, and in October 2015, Jay Hardy sold the remainder of his majority share in Hardy Diagnostics back to his employees. Hardy Diagnostics now operates as a 100% Employee Owned ESOP.

So how did we get here? Meet our founder and president, Jay Hardy.

"Many people have asked me how I got started in business. First of all, you will need to know that our company, currently consisting of 430 co-workers, manufactures culture media that microbiologists use in the laboratory. Culture media is what we call the "bug food" that bacteria and fungi feast upon in order for microbiologists to determine the identity of the pathogen and to help determine how to kill them in order to restore the health of the patient.

The year was 1980, and I had just finished a one year internship at a hospital in Santa Barbara to train as a Medical Technologist. These are people that are licensed to conduct laboratory tests in a clinical setting. The requirements are a bachelor's degree and a rigorous year of practical training in the hospital lab. After finishing and passing the California State Board exams, my dream had been realized and I had finally become a full-fledged Medical Technologist.

However, there were no jobs available at the time! Having come from the LA area, spending a year in the Central Coast of California was like paradise to me, so I very much wanted to stay in Santa Barbara. Disappointed and dejected about not being able to find work in my new profession, I was talking to my friend who had also completed the internship. We did not know which way to turn, but somehow came up with the idea of making culture media. My father was an entrepreneurial pharmacist who operated many drug stores during his career, so starting a new business seemed to be a somewhat natural path for me to follow.

My friend and I started our fledgling business on a shoestring budget. We rented two small rooms in what had once been a motel in Santa Barbara. After borrowing $10,000 from each of our dads, and rescuing some antiquated equipment from a trash heap, our little business was ready to be launched. We started with one customer, which was the hospital where we had trained. Over the years, we began to service more and more hospitals in Central California, and eventually grew to a company that now supplies over 10,000 laboratory customers worldwide with over 13,000 products that are used in the laboratory.

I often stop and wonder how different my life would have been if I had gotten my wish and had been offered a job back in 1980. I am constantly reminded of one of my favorite sayings when faced with adversity, which is the Marines' motto: 'Adapt, Improvise, Overcome.' We did just that, and I'm now enjoying the ride with no regrets!"

Jay Hardy, CLS, SM(NRCM),
Co-Founder and President

HardyCHROM™ SS NoPRO

HOSPITAL-ASSOCIATED INFECTIONS

A Public Health Priority

Antimicrobial resistance (AR) is one of the biggest public health challenges of our time. Each year in the U.S., at least 2.8 million people get an antimicrobial-resistant infection, and more than 35,000 people die. Fighting this threat is a public health priority that requires a collaborative global approach across sectors.

Late last year the Centers for Disease Control and Prevention (CDC) announced it invested $22 million to nearly 30 organizations around the world to combat antimicrobial resistance and other health care threats through the establishment of two new networks—the Global Action in Healthcare Network (GAIHN) and the Global AR Laboratory and Response Network (Global AR Lab & Response Network).

These two new networks, paired with additional short-term research projects, will span more than 50 countries worldwide and build programs that focus on preventing infections in health care through proven infection control; build laboratory capacity to detect antimicrobial-resistant organisms in health care, the community, and environment; and develop new and innovative ways to more rapidly detect and respond to threats like AR and COVID-19. This work builds on initial efforts to combat AR and is structured to complement ongoing global work underway by CDC and public health partners worldwide.

We know that AR is not going away and new threats will continue to emerge. We also know that evidence-based prevention in health care stops AR threats and other infectious diseases.

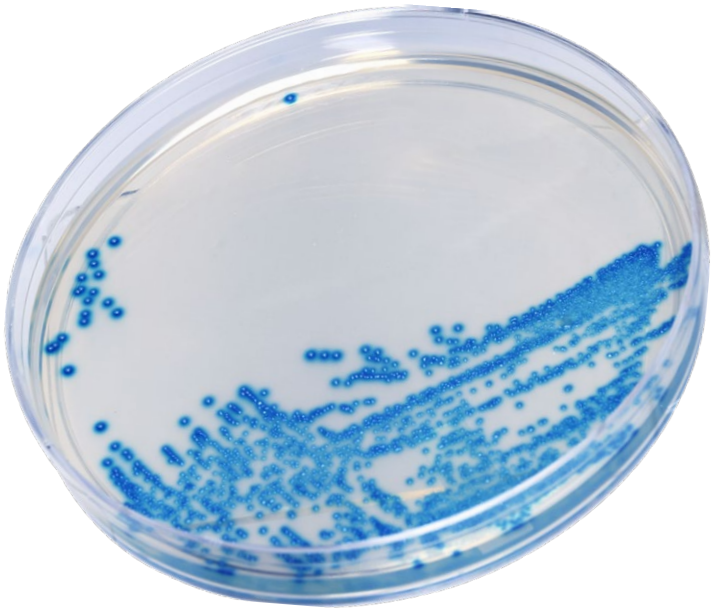
Pathogens are acquiring new methods of resistance and developing novel ways of surmounting antimicrobials at unprecedented rates. In some instances, bacteria, such as carbapenem-resistant *Enterobacterales*, are impenetrable to most available drugs, limiting treatment options drastically.

Transmission of healthcare-acquired pathogens (HAP) is chiefly related to the contamination of surfaces and equipment, including medical devices (e.g. catheters and ventilators).

Nosocomial, or hospital-associated infections (HAI), are a leading cause of morbidity and mortality in the United States; each year, about one in 25 U.S. hospital admissions are diagnosed with at least one infection because of complications in health care.

HAIs include central line-associated bloodstream infections (CLABSI), catheter-associated urinary tract infections (CAUTI), surgical site infections (SSI), hospital-acquired pneumonia (HAP), and ventilator-associated pneumonia (VAP).

While there are many diseases and organisms in health care settings, the CDC regards the emergence of carbapenem-resistant Enterobacterales (CRE) as an urgent threat, requiring immediate action. Mortality rates of up to 50% have been associated with hospitalized patients.



Urinary tract infections (UTI's) are the most common type of HAI. They affect the urinary tract (kidneys, bladder, urethra and ureters). About 75% of these UTI's are linked to the use of catheters, especially if the catheter is used for extended periods of time. HardyCHROM™ UTI (Cat. no. G313) is a differential culture medium that facilitates the isolation and differentiation of urinary tract pathogens, including Gram-negative and Gram-positive bacteria. The development of various colors, due to chromogenic substances in the media, allows for the easy differentiation of microorganisms from the primary set-up of a urine specimen.

Carbapenem resistance among *Enterobacterales* can result from several different resistance mechanisms; however, resistance through the production of carbapenemases is of utmost concern.

Carbapenems are generally administered as a last resort for treating drug-resistant Gram-negative infections. Approximately 30% of all CRE carries a mobile genetic element (MGE) that codes for carbapenemase production, which can facilitate the transfer of resistance.

While there are many variants of these enzymes, most carbapenemase producers harbor one or more of the 'big five' families: KPC, OXA-48-like, NDM, VIM, and IMP. Diagnostic laboratories should therefore implement assays that are capable of detecting at least four, or preferably, all five of these families.

Enzyme detection is critical to effective treatment, since it directs the course of antimicrobial therapy, and thus promotes good antimicrobial stewardship.

To help our customers fight back against antimicrobial resistance (AMR), Hardy Diagnostics offers tools to implement the most robust infection control measures. Patient specimens can first be easily screened for CRE using the chromogenic plate, **HardyCHROM™ CRE**. Then the major carbapenemase enzymes can be identified using the **NG-Test® CARBA 5** kit. This kit produces results in only 15 minutes and requires no equipment.

Hardy Diagnostics is expanding the diversity of antimicrobial resistance tests to include **NG-Test® MCR-1**, to detect the colistin resistance gene *mcr-1*, and **NG-Test® CTX-M MULTI**, to detect CTX-M enzymes when an ESBL is suspected, though both products are labeled for "Research Use Only" as of now. **NG-Test® MCR-1** is a rapid immunoassay designed to detect the colistin resistance gene *mcr-1* from bacterial colonies. The MCR (mobilized colistin resistance) gene family— *mcr-1*, *mcr-2*, and *mcr-3*—are an emerging AMR threat. These genes confer plasmid-mediated resistance to colistin (polymyxin E), known as one of the last-line antimicrobials for treating patients infected with multi-drug resistant Enterobacterales. **NG-Test® CTX-M MULTI** detects CTX-M variants in bacterial colonies when an ESBL is suspected. Currently, more than 170 CTX-M variants have been identified and are presently the most widespread resistance enzymes of clinical significance. All plasmid-mediated types of resistance are easily transferable within hospitals and in the community. Identifying CTX-M ESBLs early is crucial for preventing spread of resistance genes. Although ESBLs are less common in the U.S. compared to other regions in the world, **NG-Test® CTX-M MULTI** is useful in cases where hospitals have a higher CTX-M prevalence within the institution or in the immediate community, as it would provide a more rapid and cost-effective tool compared to molecular detection methods.

Chromogenic Media

HardyCHROM™ CRE (Carbapenem-Resistant Enterobacterales) Agar

HardyCHROM™ CRE is a selective and differential chromogenic agar medium intended for the qualitative and presumptive detection from stool specimens of *Escherichia coli* that are non-susceptible to carbapenems as pink colonies and KES (*Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Enterobacter cloacae* complex, and *Serratia marcescens*) that are non-susceptible to carbapenems as blue colonies.

HardyCHROM™ CRE

15x100mm plate, 10/pk	**G323**

Rapid Test

NG-Test® CARBA 5

Rapid detection and differentiation of the five most common carbapenemases produced by *Enterobacterales* and *Pseudomonas aeruginosa* resistance mechanisms.

NG-Test® CARBA 5

20/pk	**CARBA5**

Rapid Test

NG-Test® MCR-1

Rapid test for the detection of Colistin resistance via expression of *mcr-1* gene from a bacterial colony. Not for *in vitro* diagnostic use.
Research use only.

Rapid Test

NG-Test® CTX-M MULTI

Rapid test for the detection of CTX-M groups 1, 2, 8, 9, 25 extended-spectrum ß-lactamases bacterial colony. Not for *in vitro* diagnostic use.
Research use only.

NG-Test® MCR-1

20/pk **NGBMCRS23000**

NG-Test® CTX-M MULTI

20/pk **NGBCTMS23000**

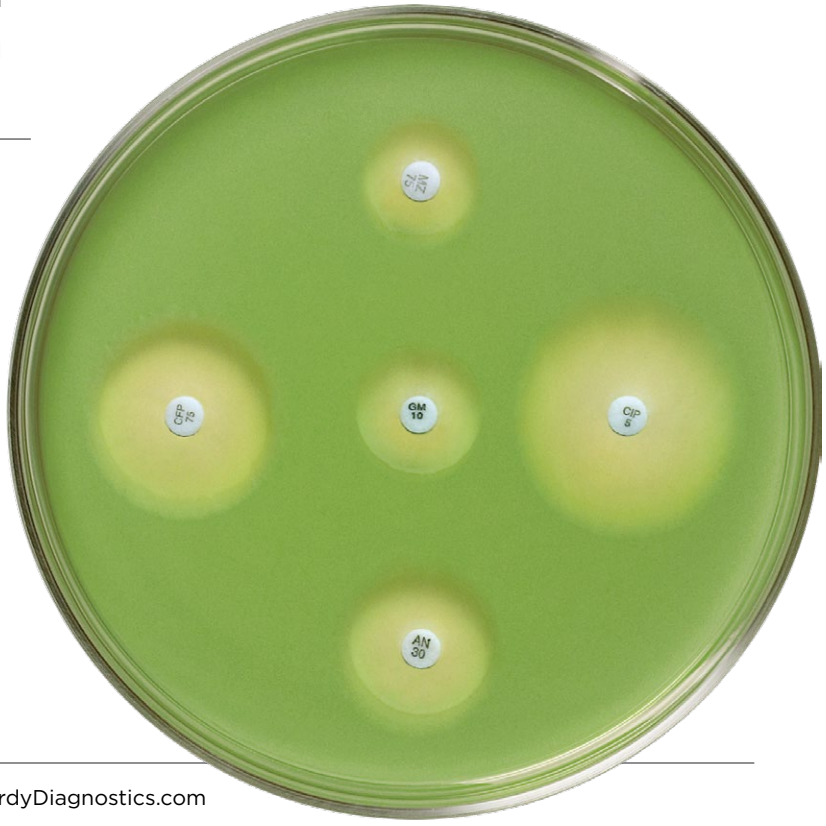
WHO WAS SIR ALEXANDER FLEMING

and how did he contribute to antimicrobial stewardship?

In 1928, Scottish scientist Sir Alexander Fleming made his monumental discovery of penicillin while experimenting with staphylococcal bacteria. Quite by accident, he found one of his uncovered agar plates had become contaminated with mold spores, and bacteria surrounding the mold growth were inhibited. He later identified the mold as a member of the *Penicillium* genus and began to perform further experiments to determine the inhibitory agent. The agent, now termed penicillin, was later found to fight against bacteria that cause diseases such as pneumonia, meningitis and scarlet fever. Fleming's findings did not catch on quickly, as it took more than a decade from the time of his publications for the scientific community to embrace penicillin. It was scientists Howard Florey and Ernst Chain who wanted to use penicillin as a treatment for soldiers during World War II. After mass production and successful use of penicillin throughout the war, Fleming was ultimately awarded the Nobel Prize for Physiology/Medicine in 1945. What we now refer to as antimicrobial resistance was first addressed by Fleming during a 1945 interview with the New York Times. While noting the importance of an antimicrobial, such as penicillin, he also warned about the abuse of such a critical discovery, *"In such cases, the thoughtless person playing with penicillin is morally responsible for the death of the man who finally succumbs to infection with the penicillin-resistant organism. I hope this evil can be averted."* As if on cue from Fleming's ominous prediction, just ten years into its widespread use, penicillin resistance began to emerge.

During the past few decades, many strains of bacteria have evolved resistance to antimicrobials. Infectious bacteria are much harder to control than their predecessors were ten or twenty years ago.

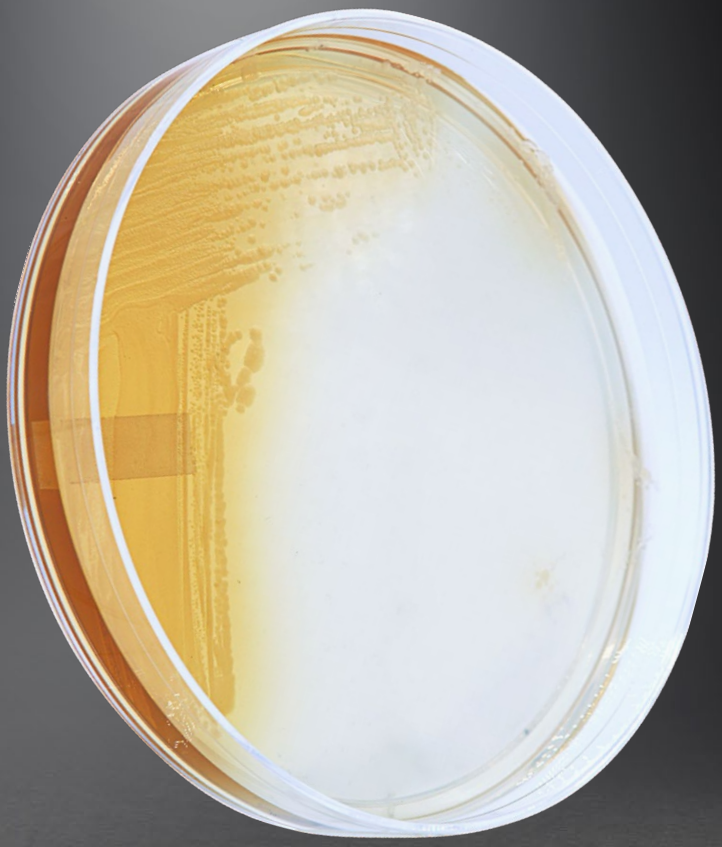
While the pace of antimicrobial discovery was stunted in the early 2000s, there has been an uptick of new broad spectrum antimicrobials approved by the FDA within the last ten years geared toward multi drug-resistant organisms (MDROs) such as Ceftolozane/Tazobactam, Ceftazidime/Avibactam, Meropenem/Vaborbactam, Delafloxacin, Plazomicin, Omadacycline, Eravacycline, Lefamulin, Imipenem/Relebactam, and Cefiderocol.



Chromogenic Media

HardyCHROM™ ESBL (Extended Spectrum Beta Lactamase) Agar

HardyCHROM™ ESBL is a selective and differential chromogenic medium which is intended for the qualitative and presumptive detection from stool specimens of: 1) Enterobacterales that are potentially non-susceptible to ceftazidime and cefpodoxime; and 2) Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli, Klebsiella pneumoniae* and *Klebsiella oxytoca*.

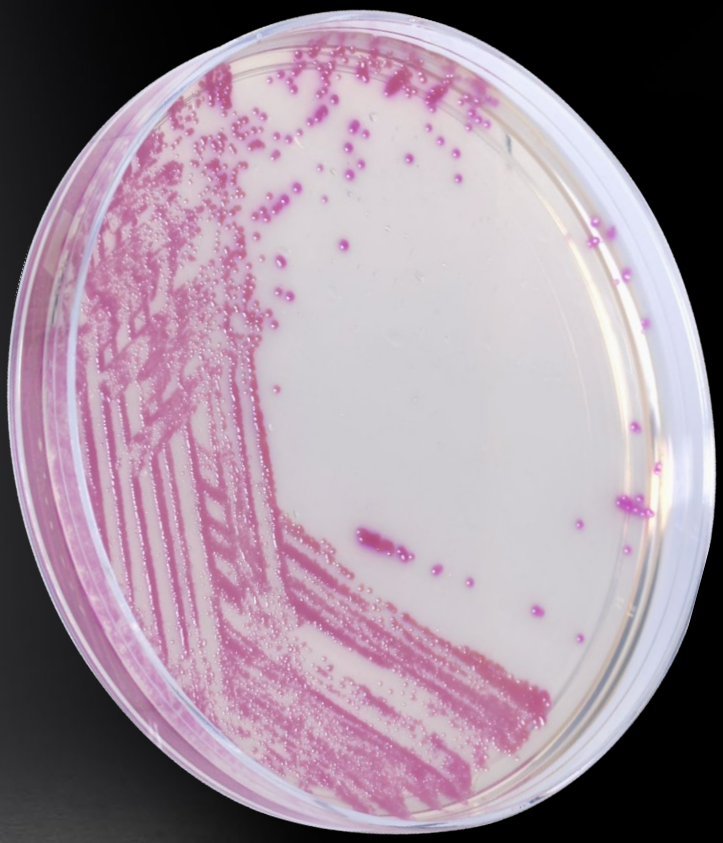


HardyCHROM™ ESBL

15x100mm plate, 10/pk **G321**

HardyCHROM™ MRSA (methicillin resistant *Staphylococcus aureus*) Agar

HardyCHROM™ MRSA is a selective and differential culture medium that facilitates the isolation and identification of methicillin resistant *Staphylococcus aureus* (MRSA) to aid in the prevention and control of MRSA infections in health care settings. The test is performed on anterior nares swabs from patients and health care workers to screen for MRSA colonization.



HardyCHROM™ MRSA

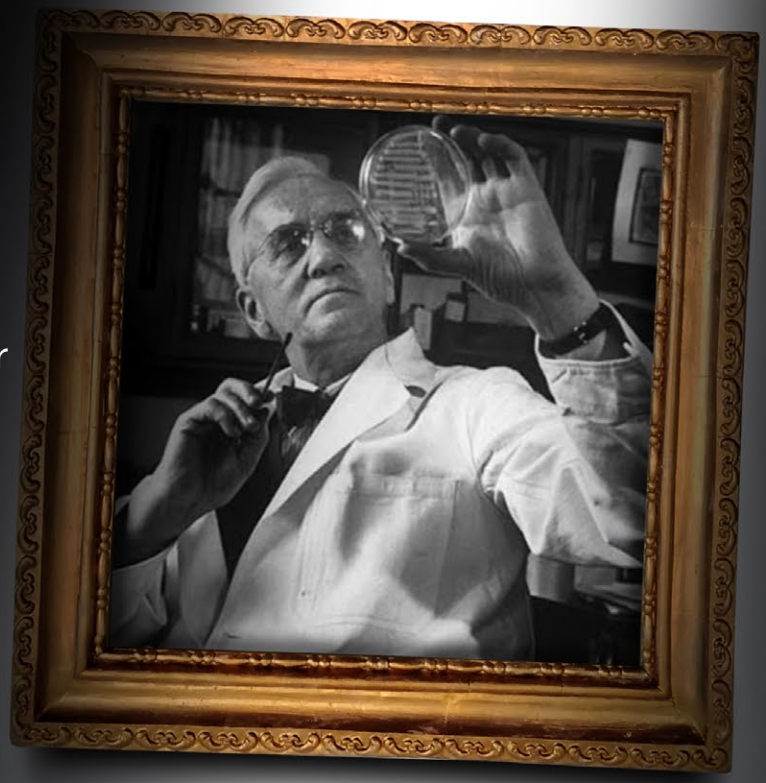
15x100mm plate, 10/pk **G307**

INCREASING RESISTANCE

Chromogenic Media

HardyCHROM™ Group A Strep Agar

HardyCHROM™ Group A Strep Agar is a chromogenic medium recommended for the selective cultivation and differential isolation of Group A Streptococcus (*S. pyogenes*). Colonies are identified based on color (red, red-brown, or red-orange colonies) among other non-GAS bacteria in the complex throat flora (blue, clear or white colonies) after 24 hours of incubation.

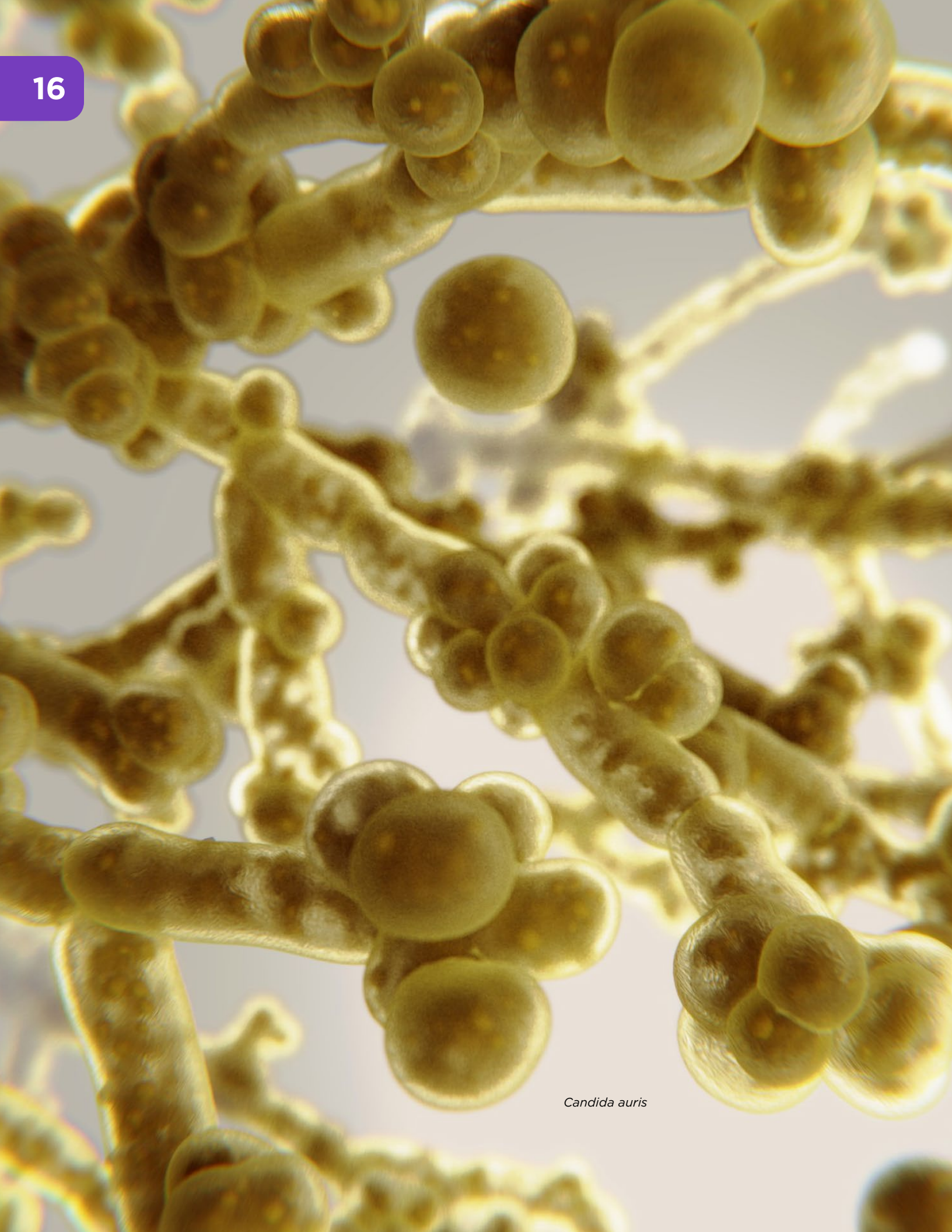


* In 2019, the CDC moved the threat level of Group A Streptococcus (GAS) to concerning, citing that GAS is increasing resistance to erythromycin and that clindamycin complicates the treatment of GAS infections. The percent of invasive GAS infections that are resistant to erythromycin has nearly tripled in eight years. ** Additionally, new research published in the Journal of Clinical Microbiology is showing strains of group A streptococcus that are leading to lower antibiotic susceptibility. In this research, investigators are warning public health officials to prepare for the possibility that group A Streptococcus (GAS) could soon become antibiotic-resistant.

**CDC 2019 Antibiotic Resistance Threats Report*
*** ContagionLive, Feb 4, 2020.*

HardyCHROM™ Group A Strep

15x100mm plate, 10/pk **G337**



Candida auris

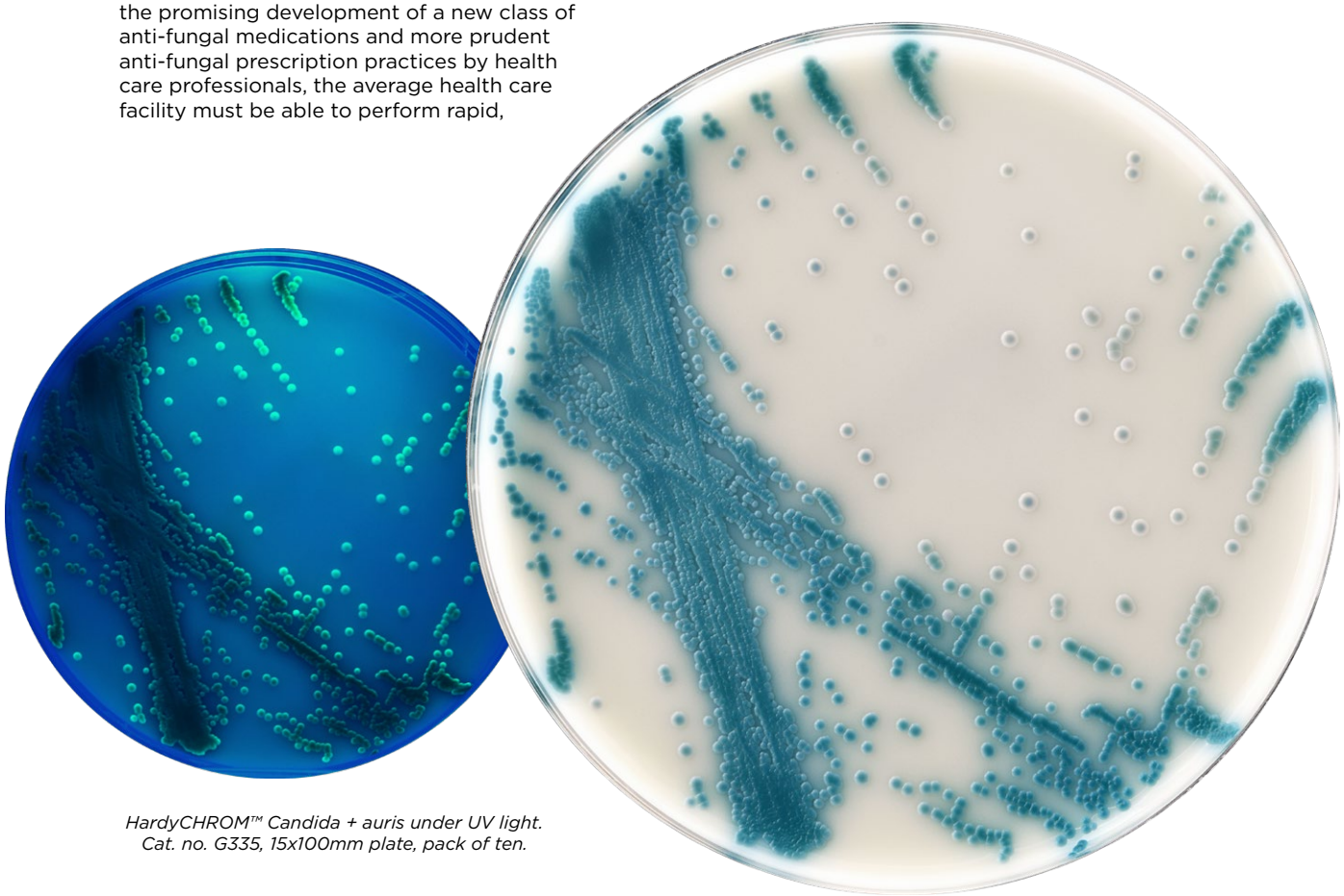
PAN-RESISTANT CANDIDA AURIS

An Urgent Threat

C. auris was first identified in 2009 in Japan. Retrospective review of *Candida* strain collections found that the earliest known strain of *C. auris* dates to 1996 in South Korea. *C. auris*, which quickly became a cause of severe infections around the world, is remarkable in that it is often drug resistant and difficult to identify without specific technologies that are not readily available in a clinical setting, namely PCR and MALDI-TOF mass spectrometry. Additionally it is concerning in that, with traditional testing methods, it is easily mistaken for other, more common *Candida* species, even on differential media. It can cause invasive candidiasis in at-risk populations, contribute to widespread *Candida* outbreaks, and progress to candidemia just as readily. However, as a potentially multi drug-resistant organism (MDRO), each of the above situations takes on an increased urgency.

The technology required for *C. auris* identification must be advanced. Even with the promising development of a new class of anti-fungal medications and more prudent anti-fungal prescription practices by health care professionals, the average health care facility must be able to perform rapid, accurate identification. Until this happens, our health care system will continue losing time, money, and lives to *C. auris* outbreaks that have caused up to 60% mortality rates in other health care facilities around the world.

In late 2021, Hardy Diagnostics introduced **HardyCHROM™ Candida + auris** (Cat. no. G343). **HardyCHROM™ Candida + auris** is recommended for the selective isolation and differential identification of *Candida* species. Colonies of *C. auris* will appear white with a characteristic teal to teal-green "bullseye" center and show a unique fluorogenic reaction under UV light. This medium will also identify *C. tropicalis*, *C. glabrata*, *C. albicans*, and *C. krusei*. The fluorescent reaction of *C. auris* on the **HardyCHROM™** plate increases the reliability of identification. Most typical yeast colonies tested for fluorescence at 48 hours using a UV lamp at 365nm will be negative, except for *C. auris* which will be positive at 48-72 hours.



HardyCHROM™ Candida + auris under UV light. Cat. no. G335, 15x100mm plate, pack of ten.

Salmonella spp.

NO PROTEUS, NO PROBLEM

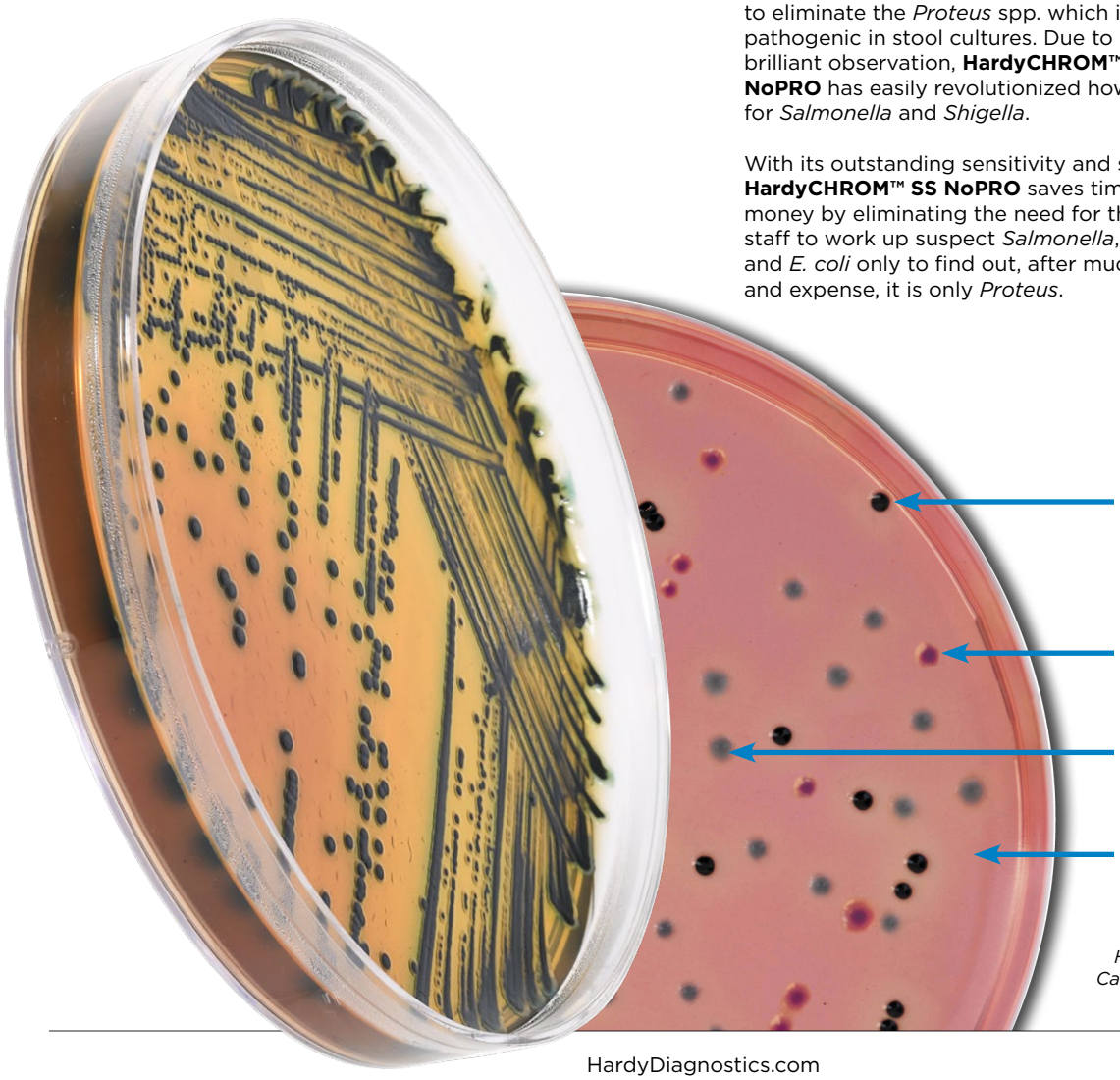
In 1968, Hektoen Enteric Agar was introduced by the Hektoen Institute of Chicago. HE agar is a selective and differential agar used for the culture of *Salmonella* and *Shigella* from patient stool samples. HE agar contains indicators of lactose fermentation and hydrogen sulfide production while possessing inhibitors to prevent the growth of Gram-positive bacteria.

Unfortunately, HE agar also allows for the growth of *Proteus* which can lead to an abundance of false positives which mimic *Salmonella*. It is because of this that *Proteus* has been given the name the "Great Impostor." *Proteus* spp. are Gram-negative, saprophytic Proteobacteria and are therefore abundant in stool cultures. Many microbiologists over the years found themselves frustrated working "through the noise" that *Proteus* presented to try and find *Salmonella* cultures on traditional HE plates.

In 2012, Hardy Diagnostics introduced HardyCHROM™ SS Agar which was a chromogenic selective and differential medium for the detection and cultivation of *Salmonella* and *Shigella*. The color differentiation on the plate was meant to help labs save time through the color identification of the different species of bacteria. H_2S producing *Salmonella* would appear as large clear colonies with black centers while non-H_2S producing *Salmonella* and *Shigella* would grow as teal colonies. However, the problem of *Proteus* still persisted.

Then four years later, Alani Vasquez, the Research and Development Manager at Hardy Diagnostics made a brilliant discovery. While working on the development of a new product, she discovered a new compound with activity specifically against *Proteus* spp. This compound was added to the **HardyCHROM™ SS NoPRO** (Cat. no. G327) to eliminate the *Proteus* spp. which is non-pathogenic in stool cultures. Due to her brilliant observation, **HardyCHROM™ SS NoPRO** has easily revolutionized how we test for *Salmonella* and *Shigella*.

With its outstanding sensitivity and specificity, **HardyCHROM™ SS NoPRO** saves time and money by eliminating the need for the lab staff to work up suspect *Salmonella*, *Shigella* and *E. coli* only to find out, after much time and expense, it is only *Proteus*.



Salmonella enterica
Colonies with large black centers and a clear perimeter.

Escherichia coli
Small pink colonies.

Shigella sonnei
Teal-colored colonies.

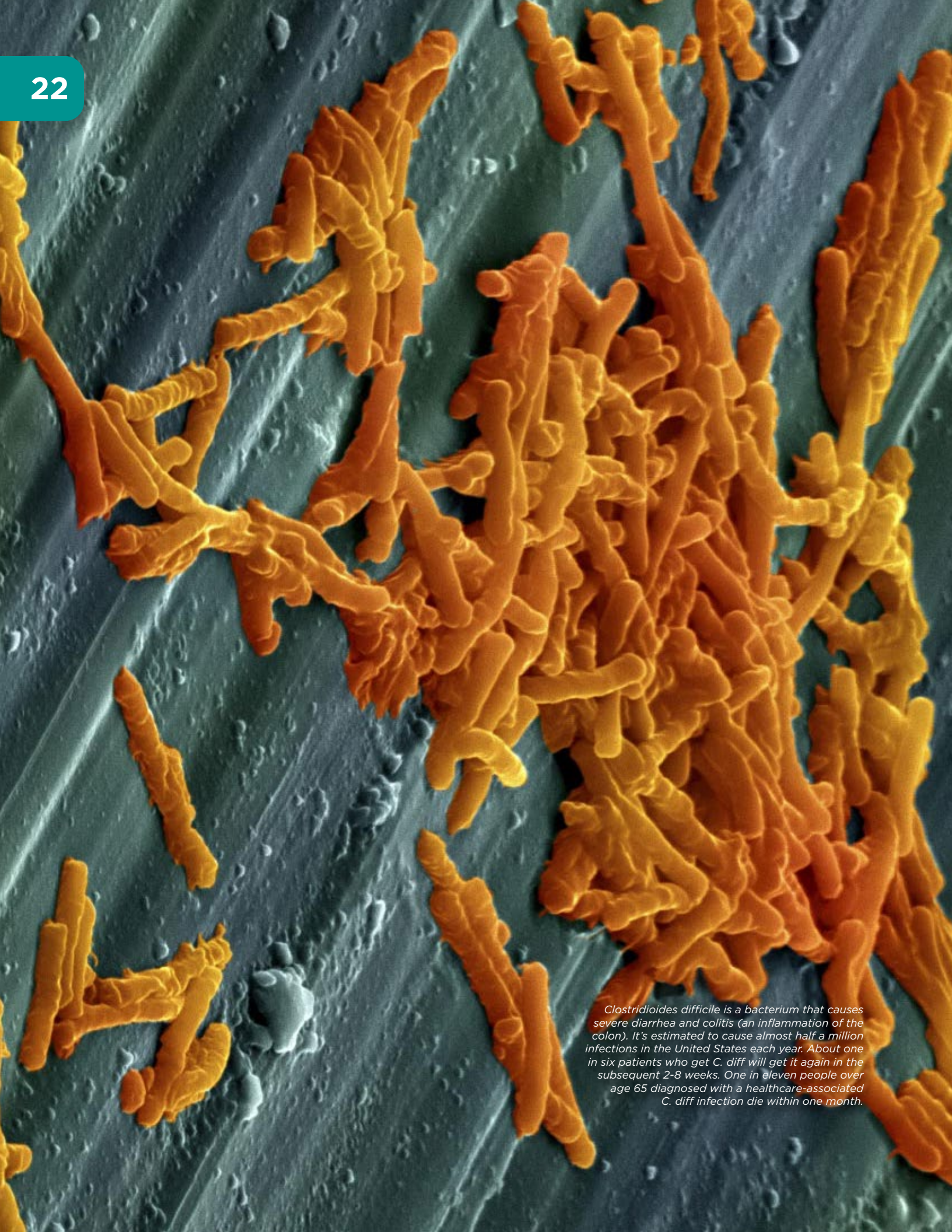
***Proteus* spp.**
You won't find it here!

HardyCHROM™ SS NoPRO. Cat. no. G327, 15x100mm plate, pack of ten.

Bottle filling at the Ohio manufacturing plant.

With bi-coastal manufacturing facilities and nine strategically placed fulfillment centers for expedited shipping nationwide, Hardy Diagnostics is here for you. For 42 years, our mission has been to diagnose and prevent disease by supplying our laboratory partners with necessary life-saving products.

HARDY DIAGNOSTICS
A Culture of Service™



Clostridioides difficile is a bacterium that causes severe diarrhea and colitis (an inflammation of the colon). It's estimated to cause almost half a million infections in the United States each year. About one in six patients who get C. diff will get it again in the subsequent 2-8 weeks. One in eleven people over age 65 diagnosed with a healthcare-associated C. diff infection die within one month.

THE DIFFICULTIES OF C. DIFFICILE

Clostridioides difficile (*C. diff*) is a major health threat with an estimated 223,900 cases in hospitalized patients in the United States alone and more than 30,000 deaths annually across the globe. It is especially threatening for the elderly and those with compromised immune systems. Of those 30,000 killed, around 90% were age 65 or older.

C. diff infections (CDI) can be found most often around hospitals and clinics, especially among patients who have previously been given antimicrobials. Once prescribed and implemented, antimicrobials can affect composition of gut bacteria by killing off normal flora (non-pathogenic bacteria). This is effective against most bacteria; *C. diff* however, is an exception. Because it is resistant to some antimicrobials, *C. diff* remains undisturbed during the patient's treatment. Because *C. diff* is no longer subdued by normal gut flora, it wastes no opportunity to multiply and flourish in this particularly favorable environment. At this point, *C. diff* often takes hold and causes extreme discomfort with multiple symptoms including diarrhea, severe cramps, irritable bowel syndrome, and colitis. Some patients even perish from this tragic illness.

C. diff is found in many healthy individuals. This does not mean these individuals suffer from the infection, merely that the bacteria are present in their gut flora. Normally, other bacteria present in a healthy patient would subdue *C. diff*. However, when a patient's gut flora is compromised by antimicrobials during a hospital stay, they are at a higher risk for CDI due to overgrowth of *C. diff*. Consequently, there is an increased need for strict sanitation procedures to reduce the transmission among patients and staff members. If a hospital cleaning crew does not adhere to proper cleaning protocols, this infection can spiral out of control and may result in many thousands of dollars in health care expenses and even patient death.

*Looking for flexibility? The **BioCode® MDx-3000** allows you to mask and unmask C. diff toxin gene results in the lab, giving you control of your reports.* *See more on page 32.*

SEROGROUPING OF STREP

The Legacy of Rebecca Lancefield

Some species of streptococci are unique in that they are classified or grouped according to the antigenic structure of their cell walls. Ever wondered how this was discovered? The genus *Streptococcus* contains many pathogens from numerous body sites. Over 50 species of these gram-positive cocci are currently recognized.

From a clinical perspective, *Streptococcus* species are separated into two major groups: alpha and beta-hemolytic for their characteristic growth on Blood Agar plates. A well-known example of alpha-hemolytic *Streptococcus* is *Streptococcus pneumoniae*, which causes inflammatory conditions such as otitis media, sinus infections, and even meningitis or sepsis. Other alpha-hemolytic *streptococci* include the viridans streptococci, which are normally commensal and rarely cause disease, with a notable exception being subacute bacterial endocarditis.

S. pneumoniae is easily distinguished from other streptococci by its alpha hemolysis, sensitivity to optochin, and solubility in bile. The other major group of streptococci, the beta-hemolytic streps, are rather difficult to differentiate based on biochemical tests alone. They all produce streptolysin, which is responsible for their ability to lyse red blood cells, and can typically be pathogenic to humans and other animals.

Originally termed *Streptococcus hemolyticus* as a group, it was apparent in the early 1900's that these organisms warranted further classification. It was this realization that led Rebecca Lancefield to perform her groundbreaking work on classifying these organisms into 18 categories that would later be known as "Lancefield Groupings." These groupings provide the basis for identification of these potentially dangerous organisms that we use today.

Rebecca Craighill Lancefield was born in Staten Island, New York in 1895 to a colonel in the U.S. Army Corps of Engineers. She spent her childhood traveling from city to city as many children of military families often do. She originally studied English at Wellesley College in Massachusetts.

Sparked by an interest in a zoology class, Lancefield changed her major and pursued graduate work at Columbia University in the field of bacteriology. Upon obtaining her master's degree at Columbia, Lancefield was offered a position at the Rockefeller Institute for Medical Research as a technical assistant in the lab of Dr. Alphonse Dochez, who discovered that the common cold was caused by a virus, and Dr. Oswald Avery, one of the fathers of immunochemistry, and whose team discovered that DNA is the molecule that encodes genetic information. Lancefield remained at the Rockefeller Institute (now the Rockefeller University) for 40 years until she retired in 1965.

Her early work focused on pneumococcus, but quickly shifted to research on the various *Streptococcus hemolyticus* outbreaks at military facilities during World War I. It was not known at the time whether the infections were due to a single virulent species, or the result of a group of distinct separate species previously categorized as one group. Lancefield's work was inspired by Dr. Avery's earlier work using serological methods to agglutinate and identify pneumococcus species.

Within a year, Rebecca Lancefield's diligent work initially identified four distinct serogroups of streptococci which composed 70% of 125 initial strains under study. As a co-author, she, Dr. Dochez and Dr. Avery published their landmark findings in the Journal of Experimental Medicine on June 1 of 1919. She concluded: "By the reaction of 'agglutination,' four distinct immunological types and a certain number of unclassifiable strains have been discovered among the 125 strains studied. Individuals of the same type are closely related to one another immunologically, and the different types can be sharply distinguished one from the other." The strains have been saved to this day and some became reference strains for Group A streptococci in the Lancefield collection.

*Rapid Strep Grouping Kits, such as **StrepPRO**, (Cat. no. PL030HD) are a result of Dr. Lancefield's diligent work in discovering the serogroups of beta-hemolytic strep.*

Lancefield's work involved preparing antigens by collecting bacteria centrifuged from broth cultures for 18 hours, and resuspending them in an HCl/NaCl mixture. The mixture was then heated, neutralized with NaOH and the precipitate discarded. The resulting supernatant would contain the antigen mixture Lancefield used for her precipitin tests. The antigen mixture could be concentrated by using consecutive overnight treatments of a sodium acetate/alcohol precipitation.

The antibodies Lancefield used were prepared by inoculating rabbits with heat-killed broth cultures, followed by inoculation with live cultures. Six to eight weeks later, the rabbit serum was collected and could be used for agglutination tests. Because non-specific precipitin reactions with antigens of heterologous strains mixed with the pure serum, an adsorption treatment was necessary. This involved mixing antigens of the heterologous strains with the inoculated rabbit serum and allowing the precipitin reaction to occur, thus cleaning out any non-specific antibodies as well as any other agglutinating factors in the serum. The result was a clean serum that was type-specific for the antigen of the organism that the rabbit was originally inoculated with. These sera could be used to characterize unknown streptococci into serogroups.

Lancefield later pursued her Ph.D at Columbia University on the study of viridans streptococci and their possible link to rheumatic fever. Lancefield's work contributed to discredit this link.

From 1924-1928, Lancefield discovered two different types of antigenic targets within *Streptococcus hemolyticus*. One was the M protein, associated with the 'matte' appearance of colonies when grown on agar and is related to virulence. *Streptococcus pyogenes* produces this protein, which is associated with cross-reactivity with heart muscle tissue, and is the true causative agent of rheumatic fever. The other antigenic target Lancefield discovered was the C carbohydrate. It quickly became apparent that the C carbohydrate could be used to distinguish between certain species of streptococci. For example, it was noticed that streptococci from throat infections shared a C carbohydrate that was serologically distinct from the C carbohydrate of streptococci isolated from bovine mastitis and both were distinct from strains isolated from equine strangles. It seemed that streptococcal disease and C carbohydrate type were correlated. Four years of Lancefield's work with the M protein and C carbohydrate culminated in 1928 with the rapid publication of seven papers in the Journal of Experimental Medicine. Initially, the human strains of streptococci that shared a C antigen were termed "Group A." Group B streptococci were initially isolated and classified from bovine sources. Group C streptococci were isolated from an epizootic in guinea pigs.

Starting in 1938, Dr. Lancefield also was able to classify the Group B Streptococci into "types" according to their capsular polysaccharides (similar to her work with pneumococcus). Later, Lancefield was also able to show that white blood cells were unable to engulf bacteria possessing M protein, thus initially evading the host's immune defense. Furthermore, when M protein protection was overcome by specific antibodies, the antigenic reaction was type-specific, meaning that an immune response of one serotype of *S. pyogenes* did not confer lasting protection to the more than 60 other known types of *S. pyogenes*.

By 1940, her work led to the serological grouping of beta-hemolytic streptococci known as Lancefield's groupings (A-H, L and M). Her contributions toward our understanding of pathogenic streptococci are innumerable and have shaped the way we identify and treat streptococcal infections. In addition, they provide the basis for the epidemiological study of streptococcal groups.

Through and beyond World War II, Dr. Lancefield's lab earned the name "The Scotland Yard of Streptococcal Mysteries," and was sent thousands of strains of streptococci for analysis and study. Dr. Lancefield's team diligently worked through these strains and her work is still kept in several volumes of notebooks, mostly written in her own hand. Her bibliography comprises more than 50 publications over 60 years.

Dr. Lancefield's accolades include, (but are not limited to), President of the Society of American Bacteriologists, President of the American Association of Immunologists, recipient of the American Heart Association Achievement Award, recipient of the New York Academy of Medicine Medal, election to the National Academy of Sciences, recipient of honorary degrees from Rockefeller University and Wellesley College. National and International organizations of streptococcal study have renamed themselves "The Lancefield Society" in Dr. Lancefield's honor.

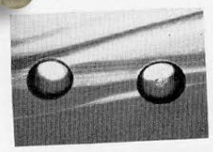
At 86 years of age, Dr. Lancefield died in March of 1981 at her home in New York, leaving a significant legacy of scientific findings. She was well known for her helpful attitude towards colleagues and coworkers. She freely shared the fruits of her lab's work and it is said that "a visitor with an interest in streptococcal problems would leave with a thorough indoctrination and with most of their questions answered—as well as with a collection of cultures of reference streptococcal strains and samples of the relevant antisera." She has been a role model for young women considering a career in science. Due to her success in the lab, she advanced the role of women as leaders in science.

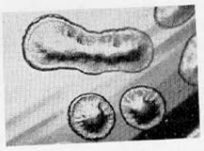
Colony Forms
Group A

Mucoid

Matt

Glossy





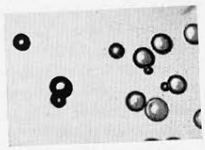
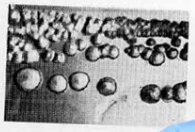
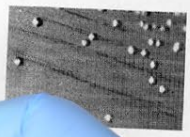


Fig. 3.

Group	Diagnosis	Number	Remarks
B	Puerperal Sepsis	14	Dr. J. H. Brown al
	Endocarditis	2	reported 45 strai
	Terminal pyelonephritis	1	isolated from 2,0 autopsies. He c
	Terminal diabetes	1	sidered few of et
	Meningitis	1	logical significan
	Pneumonia	1	
		—	
		20	
D	Endocarditis	2	Non-hemolytic
Proba-bly D	Ludwig's angina	1	Hemolytic
	Endocarditis	1	Hemolytic
	Endocarditis	6	Non-hemolytic
		—	(numerous others in literature)
F	Pansinusitis		
	Pneumonia with empyema		
G	Puerperal Sepsis		
	Agranulocytosis		
	Endocarditis		

Group G

Human and animal strains	Human strains
	
Matt	

Serological group	Streptolysins cha of Group	
	Streptolysin O	
A	Present	Pres
C (Matt colony)	Present	.....
G (Matt colony)	Present	.....
All other groups	.....	.....

ORANGE IS THE NEW BREAKTHROUGH LIFE-SAVER

Protecting the lives of newborns

Group B Streptococcus (GBS) is a Gram-positive bacteria which are normally found in 25% of all healthy, adult women. For the overwhelming majority of women, there are no symptoms of carrying GBS bacteria so there are no health changes that would alert a woman of her infection.

While Group B Streptococcus rarely affects the mother, it can cause devastating health issues for newborn babies. GBS affects one in every 2,000 babies in the United States alone. Babies born to GBS positive mothers can experience both early and late onset GBS infections with varying symptoms. Those affected by early onset GBS show signs within hours of delivery. These signs and symptoms include sepsis, pneumonia, and meningitis. These are the most common complications but trouble breathing, heart and blood pressure instability, and kidney failure are all symptoms. Late onset GBS infections usually begin between one week to a few months after birth and are most commonly associated with meningitis. Late onset GBS infections can actually be acquired through contact with another child or even an adult with a GBS infection regardless of whether or not they show the symptoms. However, due to the serious nature of the infection for newborn babies, it has become common practice with neonatal physicians to test women for GBS at 35 to 37 weeks of every pregnancy. They test during every pregnancy as a woman can colonize GBS at any time, even if she was GBS negative during an earlier pregnancy.

If a woman tests positive for Group B Streptococcus at any time during her pregnancy, then during active labor she will be immediately put on intravenous antimicrobials. Upon the breaking of the amniotic sac, an unborn child is immediately susceptible to infection, so even if a woman is not in active labor, she should be admitted to the hospital if her water breaks, especially if she is GBS positive.

*Hardy Diagnostics patented **Carrot Broth™ One-Step** turns orange when positive for beta-hemolytic GBS. Cat. no. Z40, 13x100mm tubes, pack of 20.*

LIM broth was the gold standard for the detection of Group B Streptococcus in pregnant women. However, in 2005, Hardy Diagnostics introduced the Carrot Broth Kit for the detection of GBS which decreased the turnaround time for labs in presenting positive results. Then in 2017, Hardy Diagnostics created **Carrot Broth™ One Step**, which eliminates the need for the addition of the tile to the broth. Development of any orange color in deemed a positive and this can happen in as little as six hours of incubation.

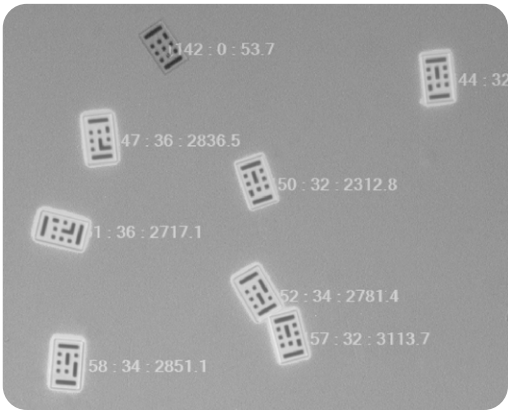
Hardy Diagnostics manufacturing technician does a visual quality check on CryoSavers™. CryoSavers™ cryogenic vials are for the long term storage of your QC strains.

HARDY GOES MULTIPLEX MOLECULAR

While it's important that Hardy Diagnostics remains true to our roots manufacturing and supplying culture media products to detect and diagnose disease, it is also important that Hardy Diagnostics evolve with the changing laboratory landscape. In 2022, Hardy Diagnostics began flexing its multiplexing molecular muscle by entering into a partnership with Applied BioCode as its exclusive distributor of the BioCode® MDx-3000 to the United States and its territories. The MDx-3000 is an automated, high throughput, multiplex, molecular diagnostic system. The MDx-3000 Molecular System utilizes a 96-well microplate format to carry out multiplex PCR amplification, hybridization/target capture, and detection steps of molecular testing. Laboratories can process up to 188 patient samples in an 8-hour shift. Additionally, up to three different assay panels, which have the same protocols, can be run on the system at the same time. Along with a number of other advantages designed to optimize laboratory workflow. The system's data management automation includes Laboratory Information System (LIS) connectivity.

The MDx-3000 Molecular System offers:

- Patented Barcoded Magnetic Bead Technology with up to 4,096 different digital codes
- FDA 510(k) Cleared Gastrointestinal Pathogen Panel*
- FDA 510(k) Cleared Respiratory Pathogen Panel*
- EUA authorized SARS-CoV-2 Assay** for automation of up to 564 individual samples in a day and 2,820 samples a day by pooled testing
- EUA authorized SARS-CoV-2 Flu Plus Assay***
- User Defined Mode supports the use of Laboratory Developed Tests



Pathogens Detected with BioCode® Gastrointestinal Pathogen Panel

Bacterial Gastroenteritis/colitis
- *Campylobacter* (*C. jejuni/C. coli*)
- *Clostridium difficile* toxins A and B
- *Escherichia coli* O157
- *Enterotoxigenic E. coli* LT/ST (ETEC)
- *Enteroaggregative E. coli* (EAEC)
- *Salmonella* spp.
- Shiga-like toxin producing *E. coli* stx1/stx2 (STEC)
- *Shigella/* Enteroinvasive *E. coli* (EIEC)
- *Vibrio* spp. (*V. cholerae/ V. parahaemolyticus/ V. vulnificus*)
- *Vibrio parahemolyticus*
- *Yersinia enterocolitica*

Viral Gastroenteritis
- Adenovirus 40/41
- Norovirus GI/GII
- Rotavirus A

Parasites
- *Cryptosporidium* (*C. hominis/ C. parvum*)
- *Entamoeba histolytica*
- *Giardia lamblia*

Pathogens Detected with BioCode® Respiratory Pathogen Panel

Viruses
- Adenovirus
- Coronavirus (229E, OC43, HKU1, and NL63)
- Human Metapneumovirus A/B
- Influenza A (subtypes H1 seasonal, H1 pdm09, and H3)
- Influenza B
- Parainfluenza 1
- Parainfluenza 2
- Parainfluenza 3
- Parainfluenza 4
- Respiratory Syncytial Virus A/B
- Rhinovirus/Enterovirus

Bacteria
- *Bordetella pertussis*
- *Chlamydia pneumoniae*
- *Mycoplasma pneumoniae*

For more information, go to **HardyDiagnostics.com/Biocode-MDx-3000/**

* *U.S. FDA 510(k) Cleared, EU: CE*

** *The BioCode® SARS-CoV-2 Assay has not been FDA cleared or approved; the test has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests. The BioCode® SARS-CoV-2 Assay has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens. Emergency use of the BioCode® SARS-CoV-2 Assay is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. §360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.*

*** *This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; This product has been authorized only for the detection and differentiation of nucleic acid from SARS-CoV-2, Influenza A (with H1 pdm09, H1 seasonal, H3 subtypes), Influenza B and/or Respiratory Syncytial Virus (RSV), not for any other viruses or pathogens; and the emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb3(b)(1), unless the declaration is terminated or authorization is revoked sooner.*

Products to detect and control COVID-19

When the pandemic emerged, Hardy Diagnostics immediately responded to the increased demand for Viral Transport Media and PPE to help meet our nation's need for supplies to combat COVID-19. Since then, we increased our manufacturing throughput, often making 1.5 million VTMs in one month, all to ensure that our laboratory partners had the tools to detect and diagnose disease.

CLINICAL PRODUCTS AT-A-GLANCE DIRECTORY

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Viral Transport Medium (VTM)
365-day shelf life at room temperature from date of manufacture.
20/pk **R99**

Viral Transport Medium (VTM)
Swabs sold separately, 3mL fill.
100/pk **R64BX**

Nasopharyngeal (NP) and Anterior Nares (AN) swabs

Flexible, Mini-tip Swab
100/pk **972012**

Standard Tip, Flocked Swab
100/pk **972029**

Mid-Turbinate Adult Flocked Swab
with stopper. 100/pk **972014**

Mid-Turbinate Pediatric Flocked Swab
with stopper. 100/pk **972015**

Test Disks, Strips and Reagents

PYR Test Kit
20ml chromogenic developer, on paper disks,
100 tests **Z175**

PYR Test Kit
15ml chromogenic solution and test cards,
75 tests/kit **Z275**

PYR Test Kit
5ml chromogenic developer, on paper disks,
25 tests **Z75**

PPE

Disposable Face Mask
50/pk **91106**

N95 Mask (V-Fold, NIOSH)
10/bx **20180016**

Disposable Full Face Shield
10/pk **MD30331**

ComfortPro Lab Coats
Medium, 70/pk **NEGU6073M**
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XLarge, 70/pk **NEGU6073XL**
XXLarge, 70/pk **NEGU6073XXL**

Disposable Exam Gloves
Small, 10x100/case **4614605027**
Medium, 10x100/case **4614602058**
Large, 10x100/case **4614605029**
XLarge, 10x90/case **4614605030**

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Gram Stain Kit Advanced™
With stabilized iodine, producing brighter colors for both Gram-positive and Gram-negatives. 4 x 8 oz. bottles.
Each **GK400A**

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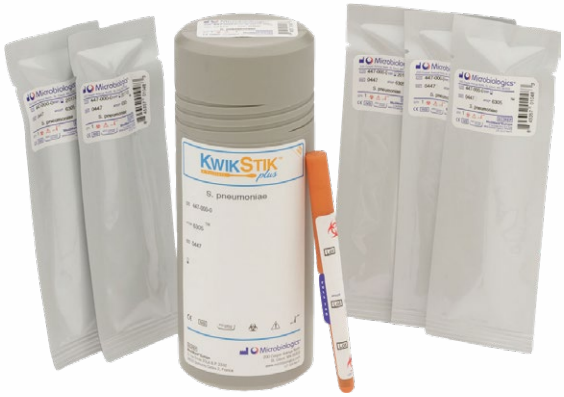
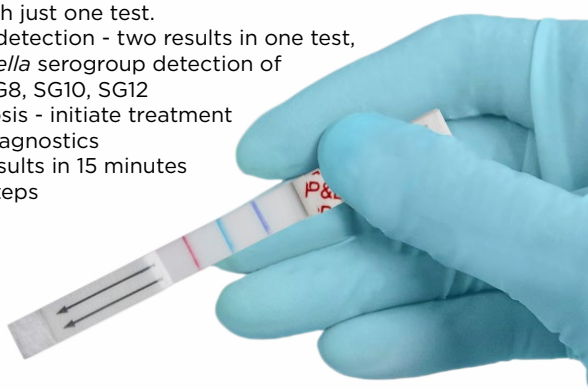
When it comes to patient safety, there's no room for error. We make it quick and easy for laboratories to confirm the accuracy of their test procedures and meet regulatory requirements. View our QC sets and panels for diagnostic instruments and test kits at HardyDiagnostics.com.



Diagnostic Test Kit

ImmuView® S. pneumoniae and L. pneumophila Urinary Antigen Test (ImmuView® P&L)
The **only** Urinary Antigen Test capable of identifying both *Streptococcus pneumoniae* and *Legionella pneumophila* at the same time - with just one test.

- Broad spectrum detection - two results in one test, as well as *Legionella* serogroup detection of SG1, SG3, SG6, SG8, SG10, SG12
- Confident diagnosis - initiate treatment based on clear diagnostics
- Quick results - results in 15 minutes with only three steps



22 tests per kit **2451**

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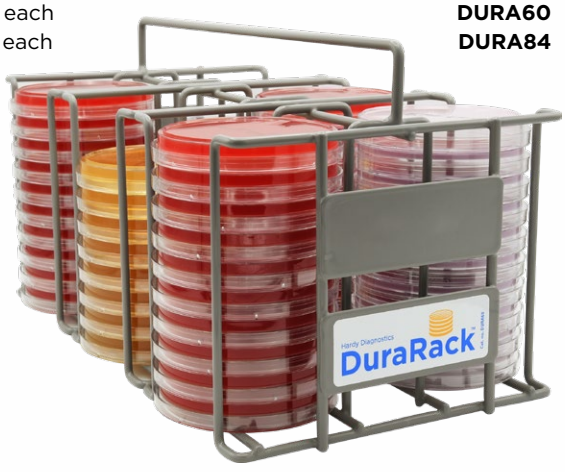
DuraRack®
Our durable, autoclavable, coated heavy gauge steel wire rack that will stand the test of time. The DuraRack® helps streamline bench top space and is ideal for refrigeration, transporting and incubating 100mm culture plates. It's the last rack you will ever have to buy!
60 Plate, each **DURA60**
84 Plate, each **DURA84**



HardyCHROM™ Chromogenic Media

In 1996, Hardy Diagnostics introduced chromogenic media to the United States and has pioneered new formulations ever since. As antimicrobial resistance grows into a greater threat, Hardy Diagnostics is consistently developing new media to quickly detect and differentiate these organisms.
View our complete line of 13 chromogenic media at **HardyDiagnostics.com/Hardy-Chrom**.

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15x100mm plate, reduced stacking ring, for use in automated sample processing system, 10/pk **GA337**

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HardyCHROM™ Sakazakii
15x100mm plate, 10/pk **G315**

*HardyCHROM™ Candida + auris can be used in conjunction with Rapid Trehalose Broth (Cat. no. Z205) or GlabrataQuick™ (Cat. no. Z298) to aid in the identification of *C. glabrata*. When HardyCHROM™ Candida + auris is used as the primary plating medium, only colonies that morphologically resemble *C. glabrata* should be tested for trehalose assimilation.

AnaeroGRO™ Anaerobic Pre-Reduced Culture Media

AnaeroGRO™ is our industry-leading pre-reduced anaerobic culture media. AnaeroGRO™ provides superior growth of anaerobes when compared to other brands. Our formulations promote robust and visibly larger colonies, thus producing faster and superior results.

Product features:

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View our complete line at **HardyDiagnostics.com/Anaerogro**

Visual inspection at plate line

At Hardy Diagnostics, you're not just a number. You're not a figure on a graph in a quarterly report. At Hardy Diagnostics, you're a partner. From laboratories that utilize our tests to diagnose illness, to our employee owners who ensure that every lot meets your rigorous expectations, Hardy Diagnostics is about a group of people coming together to better the world, one test at a time.

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