AUBIQUITOUS PIECE OF KIT

How did the Petri dish first come into being and was Richard Julius Petri really the man responsible for its creation? **Stephen Mortlock** delves into the annals of microbiology.

Scientists use them every day and discard them without a second thought. They are ubiquitous, disposable and often disregarded. They are, of course, Petri dishes. These well-known shallow and lidded plastic plates haves been used to grow bacterial cultures for many years, serving a variety of uses, such as testing the virulence of bacterial cultures or examining the efficacy of antibiotic drugs in development.

When the scarlet fever bacteria, Streptococcus pyogenes, was found to grow in milk in the 1920s, studies were performed using Petri dishes to show the importance of keeping milk refrigerated [1]. Other studies showed how Petri dishes, once inoculated with bacteria, could be incubated in aerobic, micro-aerophilic and anaerobic conditions to grow a variety of organisms or by adding a metal cover to prevent moisture loss and the dehydration of the media [2-4].

The plates, originally made of glass, are now made of disposable plastics and used by labs around the globe for various studies in diverse fields. However, the Petri dish we know today did not start out this way. Enter Richard Julius Petri, a German microbiologist, who in the late 19th century worked as an assistant to the celebrated German physician and pioneering microbiologist, Robert Koch (1843-1910). At that time Koch, was considered to be, along with Pasteur and Lister, one of the late nineteenth century's "fathers of microbiology" [5].

Richard Julius Petri

Richard Julius Petri was born in the German city of Barmen near Wuppertal in 1852. He came from a distinguished family of scholars and he was the eldest son of Philipp Ulrich Martin Petri (1817-1864) a professor in Berlin and Louise Petri (his father's cousin). His paternal grandfather was Viktor Friedrich Leberecht Petri (1782-1857), who was the professor of classical literature and Oriental languages and Director of the

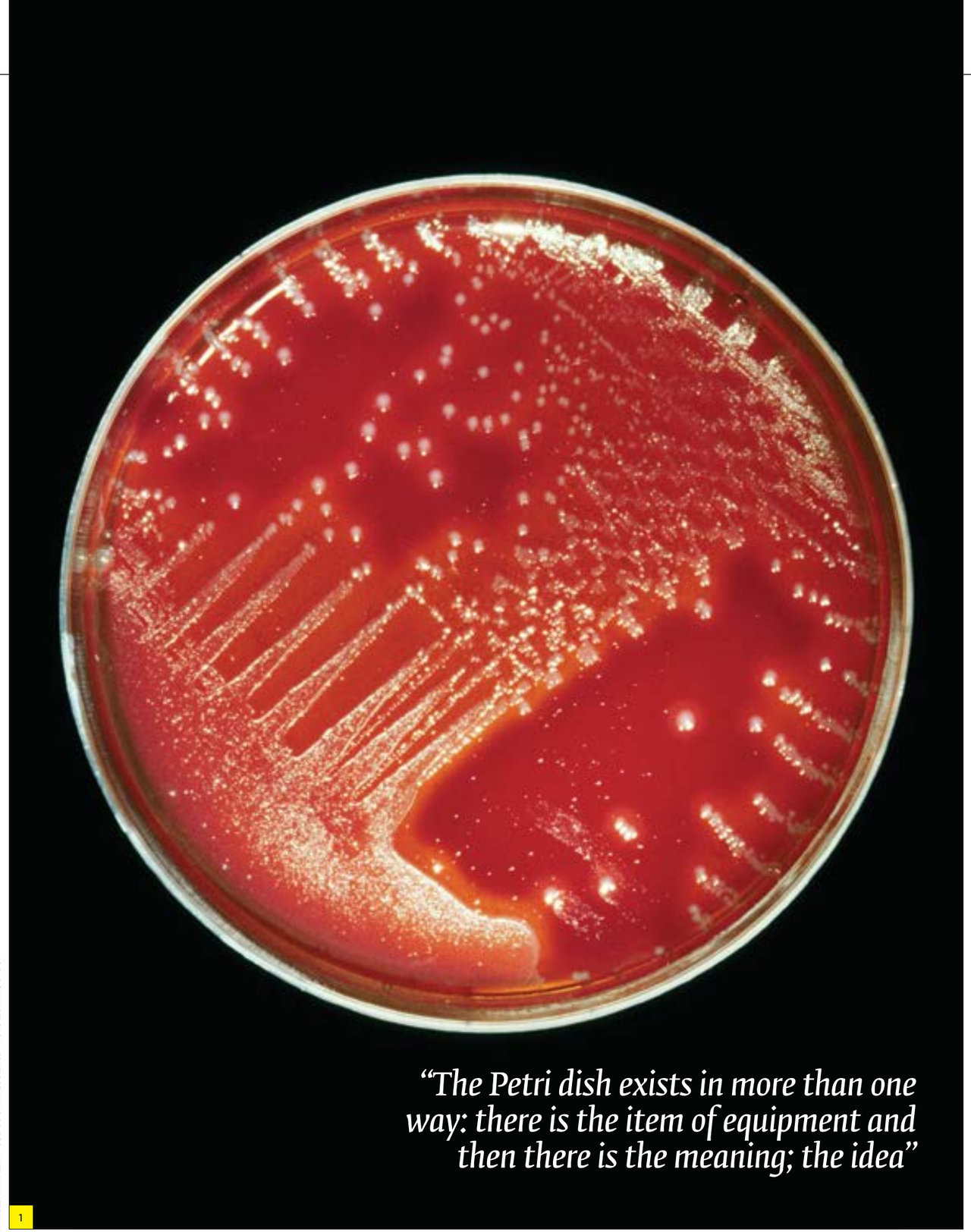

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Fig. 1. Colonies of Streptococcus pyogenes, a gram-positive bacterium, growing on blood agar in a Petri dish.

AN IDEA, A DESIGN AND A METAPHOR

Andrea Sella, an award-winning chemist, broadcaster and classic science kit and equipment enthusiast, says:

"The Petri dish has pretty much always stayed the same – there aren't a lot of differences that you can make. I think the only real thing that has changed over the years is standardisation. These days Petri dishes are very integrated pieces of equipment, with their standard sizes and their sterilised packs.

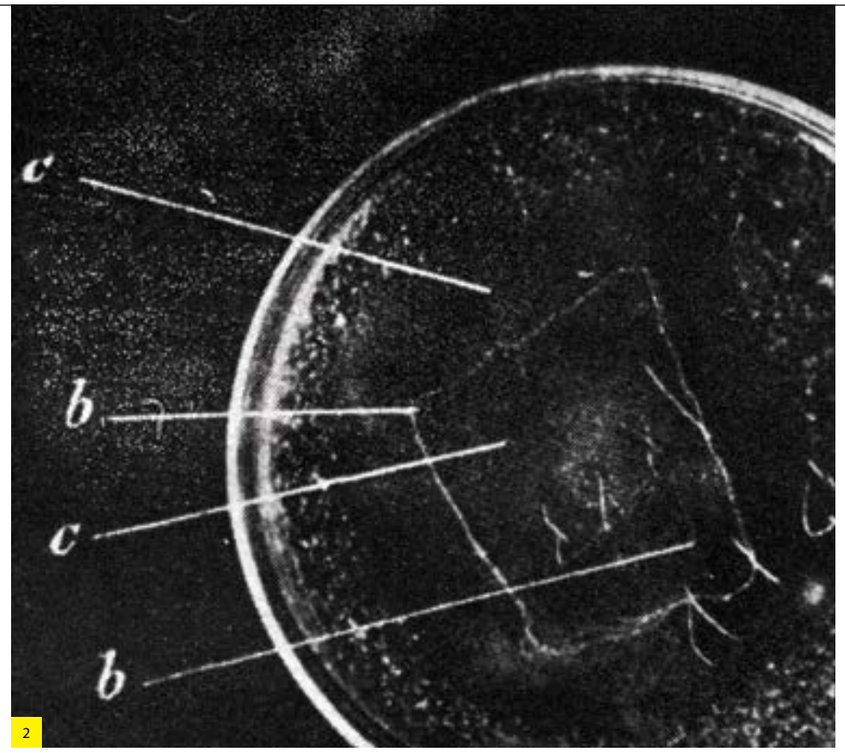
"This evolution – the idea of the one-time, one-use, throw away – is the only real change to what is essentially a 2D surface on which to grow a culture. It is a design classic – there is an elegant simplicity to the Petri dish and I don't think it can be improved upon.

"But the Petri dish exists in more than one way: there is the item of equipment and then there is the meaning; the idea of a Petri dish – it communicates the notion of life sciences in the same way that a bubbling test tube communicates chemistry.

"In the literal sense, it will remain, as we are always thinking about the next pathogen and you've got to grow that pathogen somewhere. But I also think that the Petri dish has longevity in the metaphoric sense.

"It means 'living laboratory' and is used to signify any interesting experiments that are taking place. I don't think there's an equivalent. The Petri dish, as both equipment and idea, will be around for a long time."

Fig. 2. Image taken in 1889, showing the action of an antibiotic compound on Bacillus anthracis bacteria cultured in a Petri dish.
Fig. 3. Dr Robert Koch
Fig. 4. Fanny Hesse



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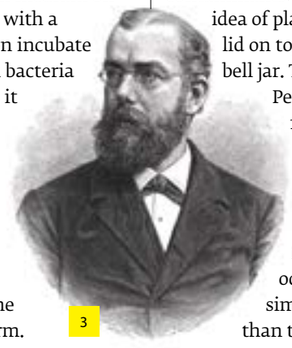
Collegium Carolinum in Braunschweig.

Richard Petri enrolled at the Kaiser Wilhelm-Akademie for military physicians from 1871 to 1875. He then undertook doctoral training as a subordinate physician at the Berlin Charité and received his doctorate in medicine in 1876 for his thesis "Versuche zur Chemie des Eiweissharns" (The Chemistry of Protein Urine Tests).

From 1876 until 1882 Petri practised as a military physician, serving as a volunteer with the 2nd Foot Guard Regiment. While serving as a military physician, he was assigned to a research facility in Berlin, the Imperial Health Office, working with Robert Koch. Not surprisingly, it was during his tenure here that Petri acquired his interest in bacteriology. It is also where pioneering work on agar was undertaken (see box).

When Petri arrived at the lab, Koch's assistants were already growing bacteria in liquid broth, but Koch had realised the benefits of developing a solid culture media. He began by observing fungal "colonies" growing on slices of potato. Each colony was "pure", containing similar organisms. But this had limitations. By placing liquid cultures in beef broth solidified with gelatin and then cooling the solution, he was able to produce a clear, smooth, homogeneous culture medium. In it, the bacteria would multiply to form visible colonies. The lab assistants would pour the bacteria mixed with the gelatin cultivation medium on top of a glass slide covered with a large glass bell jar, and then incubate the whole thing. Although bacteria grew well in this medium, it had a tendency to turn cloudy and many bacteria made enzymes that broke down the gelatin and turned it back into liquid as they grew. Even when this wasn't the case, the gelatin often liquefied if the laboratory became too warm.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Agar jelly

Another of Koch's assistants, Fanny Hesse (1850-1934), introduced the use of agar jelly poured into a shallow glass dish kept under a bell jar, as a medium on which to culture the bacteria. This jelly-like yet solid substance provided a rich substrate for bacteria growth and because it was clear and the bacteria remained on the surface, counting and identification became much easier.

But to observe the dish's contents under a microscope, the cover had to be removed, exposing the studied bacteria to uncontrolled conditions and contaminants. So, Petri came up with the idea of placing a slightly larger glass lid on top of the dish instead of a bell jar. The Petri dish was born. As Petri explained, in his short 1887 paper: "A minor modification of the plating technique of Koch... under these conditions, contamination from airborne germs rarely occurs" [8]. The invention was simple and more compact than the bell jar and allowed for more efficient experiments. The cover of these new "Petri dishes" could stay on at all times to protect experiments from contamination. Similarly, the cover was transparent and as close as possible without touching the media or the experimental bacteria, ensuring one could easily observe the dish.

The controversy

But was it really Petri's idea? As with all great scientific discoveries there is often controversy about who should be accredited for the invention. Petri's paper published in 1887 described a modification of the plating technique of Koch. Some have argued that credit should go to two other scientists, Victor André Cornil (1837-1908) and Victor Babes (1854-1926), who published a bacteriology textbook in 1885 *Bacteria and their Role in Pathological Anatomy and Histology of Infectious Diseases,* who not only described a similar dish for bacterial growth but included an illustration, something Petri had not done.

Also, the third edition of the textbook *Micro-Organisms and Disease* written by Emanuel Klein (1844-1925) in the same year, describes the dish, but here it is referred to as Koch's plate method. The English researcher, Percy Frankland (1858-1946) also published a paper in 1886 in the proceedings of the royal society that described a flat-lidded dish [9]. The general consensus now is that that it had been in use for some time before Petri published his article, but he standardised the idea of a double dish with a larger lid. So effective was his idea that it had spread rapidly across Europe and once his paper appeared, the scientific community started naming it after him and the name stuck.

Moving on

After leaving Koch's laboratory, Petri continued to be involved in bacteriology and from 1882 to 1885 he became the head of the Brehmerschen Göbersdorf sanatorium, administered by the Imperial Board of Health. He was a rather vain disciplinarian who ran the tuberculosis sanatorium on strict terms for staff and patients. In 1886 he became the Director of the Museum of Hygiene in Berlin, and in 1889 he returned to the Kaiserliches Gesundheitsamt as a Director from which he retired in 1900 with the title Geheimer Regierungsrat (Privy Councellor).

In addition to his inventions and innovations, Petri published almost 150 papers on hygiene and bacteriology. These included "Ueber die Methoden der modernen Bakterienforschung" (On the methods of modern bacterial research) in 1887, "Das Mikroskop von seinen Anfängen bis zur jetzigen Vervollkommnung für alle Freunde dieses Instruments" (The microscope from its beginnings up to the present perfection for all lovers of this instrument) in 1896, and "Aetiologie und Therapie der chronischen Lungenschwindsucht" (Etiology and therapy of chronic pulmonary tuberculosis) in 1902 as co-author with Hermann Brehmar (1826-1889).

Petri was also a freemason and a lodge member of the "to death's head and Phoenix lodge" in Königsberg with Ernst von Leyden (1832-1910) of Charcot-Leyden crystals and Leyden-Möbius syndrome fame. Petri was married twice, his first wife, Anna Riesch died during childbirth in 1894 and he remarried in 1897 to Elizabeth Turk, a marriage that remained childless. Petri died in the German city of Zeitz in 1921 at the age of 69.

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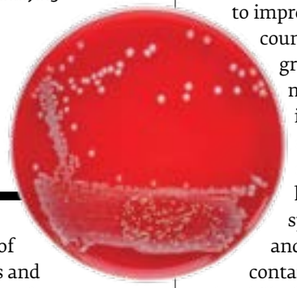
JAMS AND JELLIES

While working at Koch's laboratory, Walther and Fanny Hesse (nee Eilshemius) made their most famous contribution to microbiology – one that owed as much to Fanny's skill with jams and jellies as Walther's expertise with bacteria. Walther Hesse (1846-1911) had trouble getting pure cultures, so he confided his frustrations to his wife Fanny and she devised a solution. She recalled that when she was growing up in New York, they had a neighbour who had lived for a time in Java. The neighbour taught her family about agar-agar, a seaweed extract used in Asia to solidify jellies and thicken soups. Fanny had been using agar-agar in her jams for years.

Walther found that agar-agar was perfect to solidify the beef broth. At 100°C, it could be melted, mixed with the liquid broth and poured into dishes. The new medium was solid at room temperature and above. Bacteria grew well on it but they could not break it down. Agar-agar was even translucent, which made identifying bacterial colonies and their characteristics much easier. Though it's simply called agar now, the substance used in microbiology labs is the same one that originated in Fanny Hesse's kitchen.



Routine use

The routine cultivation of bacteria and fungi has hardly changed in centuries, with cultures in Petri dishes and methods used that would still be recognised by Robert Koch. The Petri dish remained almost unmodified, apart from the use of plastic, and became the most commonly used piece of microbiological equipment. Recent microbial cultivation technologies, however are starting to include highly subdivided multi-well plates and capillaries, as well as methods of sorting and counting encapsulated micro-colonies [10-11]. By incorporating a printed, disposable colorimetric sensor array into the Petri dish headspace, which is responsive to the volatile substances emitted by microorganisms during growth, it is possible to detect the presence of bacteria growing in the plate before colonies are visible [12].

Studies have also shown that nanospray desorption electrospray ionization imaging mass spectrometry can be used for in vivo metabolic profiling of living bacterial colonies directly from the Petri dish [13]. Both of these can provide a fast method for detection and identification to improve existing bacterial colony counting algorithms. And now groups of researchers are making replacement organs in Petri dishes [14-15]. But, too often these systems require expensive, sophisticated hardware that are often specialised in application and it is the simple Petri dish containing a solid jelly disc (and the skill of the scientist, of course) that continues to be used in routine microbiology laboratories. BMS

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He would like to thank: Dr Aleksandra Pawliczek, Head Archivist at the Humboldt-Universität in Berlin; Udo Garweg, Librarian at the Von der Heydt-Museum in Wuppertal; Sabine Tolksdorf, at the Staatsbibliothek in Berlin; all the pathology staff at Guildford Hospital, Nuffield Health.

References can be found at: bit.ly_BMSrefs_April