Milestones in the testing of surface disinfectants: from Robert Koch to CEN TC 216

Meilensteine in der Testung von Flächendesinfektionsmitteln: von Robert Koch bis zu CEN TC 216

Abstract

The first publication on testing disinfection procedures dates back to 1881 and was compiled by Robert Koch. Although his conclusions were erroneous, we continue to use the “germ carrier test” right up to the present day even if preference is now given to the suspension test first devised by Geppert. Over the years many conceptual milestones had to be reached, and much knowledge and many insights were needed to develop this procedure further to ensure reproducible results. Today, the disinfectant effect is calculated in terms of logarithmic reduction factors for the microbial count: a reduction of 5 log levels has been agreed, i.e. an inactivation kinetics of 99.999%.

Of paramount importance was, in particular, the insight that different methods had to be employed to test disinfectants, while doing so not only in the laboratory but also under everyday working conditions. This gave rise to a situation whereby each country developed its own test methods, producing significantly different results. It was only in 1970 that the parties concerned came together so as to reach a uniform solution in Europe. The achievements of the “International Colloquium” were later adopted and continued by the European Standardization Committee (CEN) in a special working group (TS 216). The most important accomplishment of this working group is, no doubt, the insight that it is not a test result but rather a test system that will reveal the truth.

The tests carried out in Phase 1 are quantitative suspension tests to elucidate the bactericidal, virucidal, tuberculocidal, fungicidal and sporicidal efficacy. Phase 2 defines the requisite concentration per exposure time.

CEN was founded and is sponsored by industry, since the latter needs reliable standards. Time will tell whether this was not perhaps the greatest milestone in the development of test methods. However, the successes scored by CEN are ultimately only the logical consequence of the accomplishments already achieved by the “International Colloquium”. In reality, most of the procedures that today have been accepted via TS 216 are based on the activities set in motion by the Colloquium. It is impossible to acknowledge just how pivotal was the role played by both legendary “International Colloquia” in 1970 and 1972 in Hamburg in the development of uniform test procedures.

Keywords: disinfectant, testing, history

Zusammenfassung

Der erste Artikel über die Testung von Desinfektionsverfahren stammt aus dem Jahr 1881 und wurde geschrieben von Robert Koch. Auch wenn seine Schlussfolgerungen auf einen Irrtum beruhten, den „Keimträgertest“ selbst gibt es bis heute, auch wenn man heute den von Geppert erstmalig entwickelten Suspensionsversuch vorzieht. Es hat über die Jahre viele gedankliche Meilensteine, viel Wissen und Erkenntnisse gebraucht, dieses Verfahren so weiter zu entwickeln, dass
die Ergebnisse reproduzierbar wurden. Heute berechnet man die desinfizierende Wirkung in der logarithmischen Keimreduktion: Man hat sich auf 5 Zehnerlogarithmen verständigt, d.h. auf eine Absterbekinetik von 99,999%.

Wichtig war vor allem die Erkenntnis, dass man Desinfektionsmittel auf unterschiedliche Weise testen müsse, nicht nur im Labor, sondern auch möglichst praxisnah. Das führte in der Folge dazu, dass jedes Land seine eigenen Testmethoden propagierte mit signifikant unterschiedlichen Ergebnissen. Erst 1970 setzte man sich an einen Tisch, um in Europa zu einer einheitlichen Lösung zu gelangen. Die Arbeit des „Internationalen Kolloquiums“ wurde später übernommen und weitergeführt in einer eigenen Arbeitsgruppe (TS 216) vom Europäischen Komitee für Standardisierung (CEN). Das wichtigste Ergebnis dieses Arbeitskreises ist wohl die Einsicht, dass nicht ein Testergebnis, sondern ein Testsystem die Wahrheit bringt. Die Tests in Phase 1 sind quantitative Suspensionstest, um die bakterizide, viruzide, tuberkulozide, fungizide und sporizide Wirkung festzuhalten. Phase 2 definiert die notwendige Konzentration pro Einwirkzeit.

CEN wurde gegründet und wird gesponsert von der Industrie, denn die braucht verlässliche Standards. Später wird man vielleicht einmal feststellen, dass diese Gründung der größte Meilenstein in der Entwicklung von Testmethoden war. Die CEN Erfolge sind aber letztlich nur die logische Konsequenz dessen, was das „Internationale Kolloquium“ begonnen hat. Tatsächlich stammt das allermeiste, das heute über TS 216 akzeptiert wurde aus den Arbeiten, initiiert durch das Kolloquium. Die Rolle der beiden legendären „Internationalen Kolloquien“ 1970 und 1972 in Hamburg für die Entwicklung einheitlicher Testverfahren kann nicht hoch genug eingeschätzt werden.

Text

The first article on the testing of disinfectants was written by Robert Koch and appeared in 1881 [1]. It was entitled über Desinfektion (on disinfection). He described the following testing method. A silk thread is contaminated by submersion in a liquid culture of the test organism Bacillus anthracis; after drying, this contaminated thread is immersed in the disinfectant solution for a given exposure time; thereafter the thread is cultured in a nutrient broth; no growth after incubation indicates that the product is active. Such a test is now considered as a carrier test. Koch compared several chemical substances; he found that, for the same concentration of the active substance, mercuric chloride was the most active product. It was, however, an erroneous result: there was a carry-over of residues of the disinfectant into the subculture medium, so that the bacteriostatic action of the mercuric chloride continued to act: it was indicated wrongly that there was no survival. The problem is now solved by the neutralization of the disinfectant at the end of the exposure.

The number of bacteria dried on a carrier is hard to standardize. The survival of the germs on the carrier during drying is not constant. Therefore the use of a suspension of bacteria is preferred to that of carriers. Geppert developed the first suspension tests ten years after Koch [2]. A sample of the bacterial culture, the inoculum, is suspended into the disinfectant solution and after exposure it is verified by the culture of this mixture whether the inoculum is killed or not. No growth means activity of the preparation. Samples of the bacterial suspension and of the disinfectant-bacteria mixture were taken by a loop as it was usual at that time. The results of these tests were not reproducible (the interlaboratory spread of the results is large), even not always repeatable (large intralaboratory spread of the results). The reason was not only that the quantity of bacteria supplied by a loop differed in the various experiments, but also that the resistance of the test organism could vary.

One of the most ingenious innovations in the testing of disinfectants by means of a suspension test was the introduction of a standard preparation, phenol, in 1903 by Rideal and Walker [3]. In their test qualitative results (growth or no growth) of the tested disinfectant for several exposure times are compared with those of an active dilution of phenol which is tested in the same experiment. The relation of the active dilution of the disinfectant to this of phenol is the so-called phenol coefficient. The determination of the phenol coefficient remained the standard test for more than half a century, as long as most disinfectants were phenolic compounds.

Such simple tests as the carrier tests or the suspension tests give a good picture of the activity of a preparation, but the results are not always related to the real value in practice, especially in the case of surface disinfection. Heicken described a new test for the disinfection of rooms, particularly of floors, walls and other surfaces in...
hand disinfection). The third phase comprises the field examination (e.g. surface disinfection, instrument disinfection, contact time the preparation is active for a specific application, which conditions and at which use-dilution after a given incubation time, the observed growth is estimated semi-quantitatively (from no growth till ++++). This test was the basis of the practical test of the German society for hygiene and microbiology (Deutsche Gesellschaft für Hygiene und Mikrobiologie).

The last kind of tests to be applied in the evaluation of surface disinfectants was the capacity test. Each time a mop is soaked into a bucket with disinfectant solution, a certain quantity of dirt and bacteria is added to this solution, which diminishes the activity of the solution. The ability to retain activity in the presence of an increasing load of organic matter and bacteria is the capacity of the disinfectant. Kelsey introduced the principle of capacity tests in the testing of surface disinfectants in the sixties of past century [5]. Such tests, however, give more information about the disinfection of the fluid in the bucket than that of the floor.

A further step in the standardization of disinfectant test was the introduction of quantitative tests after the Second World War. From the study of the kinetics of disinfection, it was clear that the end-point of no growth, or no-surviving organisms, is determined by the size of the inoculum, namely the number of organisms present in the bacterial suspension or on the carrier and brought in contact with the disinfectant. If the inoculum consists of only a few bacteria, then the end-point of no-growth will be reached after a much shorter exposure time than when more bacteria were present in the beginning. To overcome this difficulty, it is better to calculate the real reduction quantitatively, originally done as the percentage of cells killed. In a more scientific way the decimal log reduction or microbicidal effect is calculated by subtracting the logarithm of the survivors from the logarithm of the original inoculum. The most circulating requirement is a microbicidal effect that equals or is greater than 5, what means that at least 99.999% of the germs is killed.

A further milestone in the development of disinfection tests was the gradual awakening of the fact that the antimicrobial efficiency of a disinfectant should be examined at three stages of testing. The first stage concerns laboratory tests in which it is verified in which concentration-time relation a chemical compound or a disinfectant preparation possesses antimicrobial activity: for these preliminary tests, essentially quantitative suspension tests are considered. The second stage is still carried out in the laboratory, but in conditions simulating real-life conditions. Not disinfectants but disinfection procedures are examined. It is determined in the practical tests in which conditions and at which use-dilution after a given contact time the preparation is active for a specific application (e.g. surface disinfection, instrument disinfection, hand disinfection). The third phase comprises the field tests or pilot studies. This testing of disinfectants in several steps was firstly developed by the Deutsche Gesellschaft für Hygiene und Mikrobiologie as preliminary tests (Vorversuche) and main tests (Hauptversuche) in the famous guidelines on the testing of disinfectants, the first edition of which appeared in 1959 [6]. At the end of the sixties of past century four kinds of tests were used to determine the microbicidal activity of surface disinfectants: carrier tests, suspension tests, capacity tests and practice-mimicking tests. At that time surface disinfectants were evaluated in the United States of America by the so-called Use-Dilution Method of the Association of Official Analytical (formerly Agricultural) Chemists [7], which is a carrier test. In the United Kingdom the capacity test of Kelsey and Sykes was usual [8]. In other countries the sole test was a simple quantitative suspension test, as in the Netherlands the so-called 5-5-5-test of the Centraal Instituut voor Voedingsonderzoek [9]. In Germany the Deutsche Gesellschaft für Hygiene und Mikrobiologie followed its own scheme of Vorversuche and Hauptversuche. This led to the boring situation that a given disinfectant preparation was approved in one country but refused in another, or that the concentration considered as active could vary to a fivefold following the country [10].

In 1970 a scientific working group was founded and sponsored by the Rudolf Schülke Stiftung; the International Colloquium for the Evaluation of Disinfectants in Europe. It consisted of scientists from Austria, Belgium, (West-)Germany, the Netherlands, Sweden, Switzerland and the United Kingdom. The aim was to develop a general consensus on the requirements of all kinds of disinfectant procedures and on the methods to be followed in the evaluation of their microbicidal activity. The foundation organized not only workshops for the active members, but also three successful colloquia to discuss the progress of their work in front of an international public.

As the unification of disinfection tests is a priority for the European industry, the English, French, German and Swiss standard institutes asked the CEN (Comité Européen de Normalisation, European Committee for Standardization) to develop European Norms. In April 1990 the CEN started a new technical committee TC 216, named Chemical disinfectants and antiseptics, with as scope: the standardization of the terminology, requirements, test methods including potential efficacy under in use conditions, recommendations for use and labelling in the whole field of chemical disinfection and antisepsis; areas of activity include agriculture (but not crop protection chemicals), domestic service, food hygiene and other industrial fields, institutional, medical and veterinary applications.

The most relevant fact is that the testing scheme accepted by the CEN TC 216 follows the view that a test must be seen as a part of a complete testing scheme; the predicting value of one test in itself is relatively low, if the results are not interpreted in the framework of a testing strategy. It is the merit of the CEN TC 216 that this principle is applied in the testing of disinfectants and antisept-
tics. The phase 1 tests comprise the basic bactericidal, respectively fungicidal, tuberculocidal, virucidal, sporocidal tests; they are essentially quantitative suspension tests in which the biocidal activity is determined towards a limited number of test organisms: for the bactericidal tests they are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. From the results of the phase 2 tests, it should be deduced at which concentration and after which contact time the preparation tested is active for the proposed application (e.g., disinfection of floors in the food industry, handwashing in health care facilities, disinfection of swimming-pool water, prevention of growth by *Legionella* in cooling-tower water). The first step in phase 2 consists again in basic (bactericidal, fungicidal, etc.) tests, but the number of test organisms is extended, the influence of interfering substances as hard water, soap, detergents, blood etc. can be estimated. The phase 2/step 2 tests are essentially practical tests simulating real-life conditions. It remains an open question whether field tests or pilot studies can be standardized in such way that they can be accepted as a European standard for phase 3 at all.

The CEN is founded and sponsored by the industry as the industry needs standards. The CEN brings together people from different countries, with different insights, delegates from the industry, from the governments or official institutes, from the universities. The advantage is that a broad consensus is sought. The disadvantage is that it takes a long way with many meetings.

It can be supposed that in some decades the work of the CEN shall be regarded as the greatest progress in the evaluation of disinfectants since Rideal and Walker. The work and even the foundation of CEN TC 216, however, are the logical consequence of the International Colloquium for the Evaluation of Disinfectants in Europe and would perhaps not have occurred if the preparatory work was not done. In fact the great principles that are accepted now generally, were elaborated in the workshops of the Rudolf Schülke Stiftung. They are the detailed description and standardization of the tests, the expression of the results in a quantitative way with the use of appropriate statistics, and the conception that not disinfectants in itself but disinfectant procedures must be evaluated, which can be done only in successive steps: the totality of the results determines whether a preparation is considered as active or not for a given application.

The conclusion is that the CEN has much merit but that the original ideas and the establishment of the great principles of disinfectant testing originated in the past, many decades ago. The role of the International Colloquium for the Evaluation of Disinfectants in Europe cannot be overestimated.

---

**Curriculum Vitae**

**Prof. Dr. med. habil. Gerald Reybrouck**

*Emeritus Head of Department of Hospital Hygiene and Infection Control of the University Hospital Leuven.*

Gerald Reybrouck received his doctorate in 1962 at the Catholic University of Leuven with „summa cum laude“. Afterwards he qualifies as specialist for clinical pathology (clinical chemistry, hematology and micro-biology). He moves to the „Universitaire Ziekenhuizen“ for it. In 1977 he obtained his doctorate once again at the Catholic University of Leuven and became associate professor of the Medicinal Faculty of the Leuven University in 1985. His extraordinary expertise is documented by more than 150 scientific papers which deal primarily with the testing of disinfectants and antiseptics, with issues of hospital hygiene and infection control. He became emeritus professor in 2002.

---

**References**

1. Koch R. Über Desinfektion. Mitt kaiserl Gesundheitsamte. 1881;1:234-82.
2. Geppert J. Zur Desinfektionsfrage. Dtsch med Wschr. 1891;25:797,829,855.
3. Rideal S, Walker JTA. The standardisation of disinfectants. J roy sanit Instit. 1903;24:424-41.
4. Heicken K. Die Prüfung und Wertbestimmung chemischer Desinfektionsmittel für die Zimmerdesinfektion. Z Hyg. 1949;129:538-69.
5. Kelsey JC, Beeby MM, Whitehouse CW. A capacity use-dilution test for disinfectants. Mon Bull Ministry Hlth. 1965;24:152-60.
6. Deutsche Gesellschaft für Hygiene und Mikrobiologie. Richtlinien für die Prüfung chemischer Desinfektionsmittel. Stuttgart: Gustav Fischer Verlag; 1959.
7. Association of Official Agricultural Chemists. Official methods of analysis. 10th ed. Washington: AOAC; 1965.

8. Kelsey JC, Sykes G. A new test for the assessment of disinfectants with particular reference to their use in hospitals. Pharm J. 1969;202:607-9.

9. Centraal Instituut voor Voedingsonderzoek TNO. Methode van onderzoek voor de waardebepaling van desinfectiemiddelen en gecombineerde reinigings-desinfectiemiddelen. Werkrapport. 1970.

10. Reybrouck G. The assessment of the bactericidal activity of surface disinfectants. V Correlation of the tests with practice. Zentrbl Hyg Umweltmed. 1992;192:438-46.

Corresponding author:
Prof. Dr. med. Gerald Reybrouck
Kwadeschuurstraat 14, 3210 Lubbeek, Belgium
gerald@reybrouck.be

Please cite as
Reybrouck G. Milestones in the testing of surface disinfectants: from Robert Koch to CEN TC 216. GMS Krankenhaushyg Interdiszip. 2007;2(1):Doc08.

This article is freely available from
http://www.egms.de/en/journals/dgkh/2007-2/dgkh000041.shtml

Copyright ©2007 Reybrouck This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by-nc-nd/3.0/deed.en). You are free: to Share — to copy, distribute and transmit the work, provided the original author and source are credited.