

# Bacteriologic infection serology: old-fashioned or still important?

## Bakteriologische Infektionsserologie: Veraltet oder doch noch wichtig?

### Abstract

The discovery of the Gruber-Widal reaction is essential for the diagnosis of infections. Despite significant advances in modern molecular biological assays, the detection of pathogen-specific antibodies using immunological methods is still essential for the diagnosis of pathogens from blood and cerebrospinal fluid. The advantage of indirect diagnosis of bacterial pathogens by serological tests is that an immune reaction can also be detected against microorganisms that are difficult-to-cultivate or slow-growing, and can hardly be detected by conventional culture or molecular methods. However, the difficulty of assessment lies in the fact that no clear conclusions about the need for treatment or the success of the therapy can be derived from serological results alone. The limitations of these methods, however, also cannot be fully remedied by molecular biological methods, and therefore there is currently no perfect alternative to the use of infectious immunological detection methods. Ongoing problems with the quality of serological tests and the still inadequate regulatory efforts of the supervisory authorities make it absolutely necessary to carry out external quality control measures such as round robin tests.

**Keywords:** Gruber-Widal reaction, infection diagnostics, pathogen detection

### Zusammenfassung

Die Entdeckung der Gruber-Widal-Reaktion gilt als grundlegend für die Infektionsdiagnostik. Der Nachweis erregerspezifischer Antikörper durch immunologische Methoden ist, trotz bedeutender Fortschritte moderner molekularbiologischer Assays, als indirekter Erregernachweis weiterhin maßgeblich für die Diagnose von Krankheitserregern aus Blut und Liquor. Der Vorteil einer indirekten Diagnose bakterieller Krankheitserreger durch serologische Tests liegt darin, dass eine Immunreaktion auch gegen Mikroorganismen nachgewiesen werden kann, die schwer zu kultivieren oder langsam wachsend sind und mit kultur- oder molekularbiologischen Methoden kaum nachgewiesen werden können. Die Schwierigkeit der Beurteilung besteht allerdings darin, dass allein aus serologischen Ergebnisse nicht immer eindeutige Aussagen über die Behandlungsnotwendigkeit oder den Erfolg der Therapie abgeleitet werden können. Die Einschränkungen, die diese Verfahren liefern, sind aber auch durch molekularbiologische Ansätze nicht immer zu verbessern. Daher gibt es derzeit für den Einsatz infektionsimmunologischer Nachweismethoden keine echte Alternative. Derzeit fortbestehende Qualitätsdefizite der serologischen Diagnostik und die bislang unzureichenden Regulierungsbemühungen der Aufsichtsbehörden machen die Durchführung von externen Qualitätskontrollmaßnahmen wie z.B. Ringversuchen unbedingt notwendig.

**Schlüsselwörter:** Gruber-Widal-Reaktion, Infektionsdiagnostik, Erregernachweis

**Klaus-Peter Hunfeld<sup>1,2</sup>**  
**Ulyana Gräf<sup>1,2</sup>**

1 Institute of Laboratory  
Medicine, Microbiology &  
Infection Control, Northwest  
Medical Centre, Frankfurt am  
Main, Germany

2 INSTAND e.V. Düsseldorf,  
Germany

## Mini review

Since the introduction of Koch's postulates in microbiological diagnostics in 1882, the direct detection of pathogens has been considered the gold standard in the diagnosis of infectious diseases [1]. With the discovery of the Gruber-Widal reaction about 150 years ago (Figure 1), infection serological pathogen diagnostics were also established. Today, many different serological test methods for the indirect detection of bacteria and viruses are established in routine microbiological laboratories. The Viennese scientist Max von Gruber first used the serological detection of antibodies from sera of infected animals and patients by agglutination of *Salmonella typhi* and *Vibrio cholerae* bacteria for the diagnosis of infectious diseases. The same observation was independently described later by Fernand Widal in 1897 [2]. Since then, the detection of pathogen-specific antibodies by immunological methods has remained an essential pillar for diagnosing pathogens from blood and cerebrospinal fluid (CSF), despite the significant progress of modern molecular biological assays for direct pathogen detection.

In addition to the fundamental medical historical significance of this discovery, the so-called Gruber-Widal reaction is still used today in infection diagnostics, particularly for diagnosing secondary diseases, typhoid fever, and yersiniosis [1], [2]. Another groundbreaking discovery was the development of the complement fixation reaction by Bordé and Gengou in 1901. Although today complement fixation reactions are generally no longer considered creditable, they still represent a helpfully established method in many laboratories worldwide, especially for diagnosing infections caused by rare and difficult-to-cultivate pathogens by relatively simple means [1], [2]. The further development of this method by August Paul von Wassermann and its adaptation in 1906 as a rapid and widely available technique for the diagnosis of syphilis also counts among the milestones in establishing infection serological tests in the clinical laboratory [1].

Even if such techniques are primarily of medical-historical importance today, the partially or fully automated immunological test methods developed in their succession are still indispensable today for the detection of pathogen-specific antibodies and antigens in the medical laboratory. These methods use a variety of modifications of antigen and antibody reactions with the aid of a wide range of reagents and techniques, and their worldwide presence and availability represent a very lucrative market for the diagnostics industry. However, despite all efforts towards standardization, these methods are still of very different quality and reliability, especially regarding reliable diagnostics of infectious diseases. The clinical diagnostic requirement to prove or exclude specific infectious disease patterns using these diagnostic techniques remains a major challenge as far as a transparent information policy regarding the specificity and sensitivity of these test systems is concerned.

From the published international literature on the quality of bacteriological infectious serology, it is clear that, de-

spite many efforts, significant problems with test standardization persist, and there is, thus, still a need for better comparability of test results for a wide variety of pathogens [1], [2]. The indirect diagnosis of bacterial pathogens by serological assays has the advantage that an immune response can also be detected against microorganisms that are difficult to cultivate or slow-growing and can hardly be detected by culture or molecular methods. However, due to the variability of the test results, especially at the beginning of the symptoms or in the course of monitoring, it is difficult to achieve good comparable significant test value changes with the different test systems. Also, especially with these pathogens, the findings are still negative in the early phase of infection [1], [2]. A positive antibody detection clearly confirms that an immunological confrontation with the corresponding pathogen has occurred in the host organism. However, in many cases, it must remain open whether this is actually an acute infection or, as is frequently observed, a disease that has been passed through with a residual persisting antibody response (past infection). In the same way, clear statements on the need for treatment or the success of therapy can rarely be derived from serological results alone.

In the vital indication fields of bacteriological infectious serology, such as the diagnosis of syphilis or Lyme borreliosis, the limitations mentioned above cannot usually be resolved even by the application of modern culture and polymerase chain reaction (PCR) methods. This is because even for these methods, especially molecular biological approaches, there are still significant problems with the sensitivity of the detection methods, depending on the pathogen and the manifestation. This is even more true for many of the difficult-to-detect pathogens in parasitology, mycology, and virology, so in many routine diagnostic medical laboratories, the use of infection immunological detection methods is currently without alternative. The frequently reported poor quality and poor comparability of immunodiagnostic assays for microbiological pathogen detection results, not unexpectedly, from insufficient regulatory efforts by regulatory agencies in recent years. The newly issued IVDR attempts to counteract this, but overshoots the mark due to the now ambitious expectations and requirements for diagnostics approved for the European market. Many manufacturers of commercial assays fear the high regulatory burden and the not inconsiderable investments required for the approval of assays, especially for rare or difficult-to-diagnose pathogens. Under the pressure of the new regulatory requirements, a whole range of previously available diagnostics have been and are even being withdrawn from the market. This problem, including the poor or not transparently evaluated quality of infectious serological and, here, in particular, bacteriological-infectious serological test systems, underlines the urgent need and the great importance of external quality control within the framework of the regular interlaboratory comparisons and similar measures prescribed by the guideline of the German Medical Association (RiliBäk) [3]. This includes

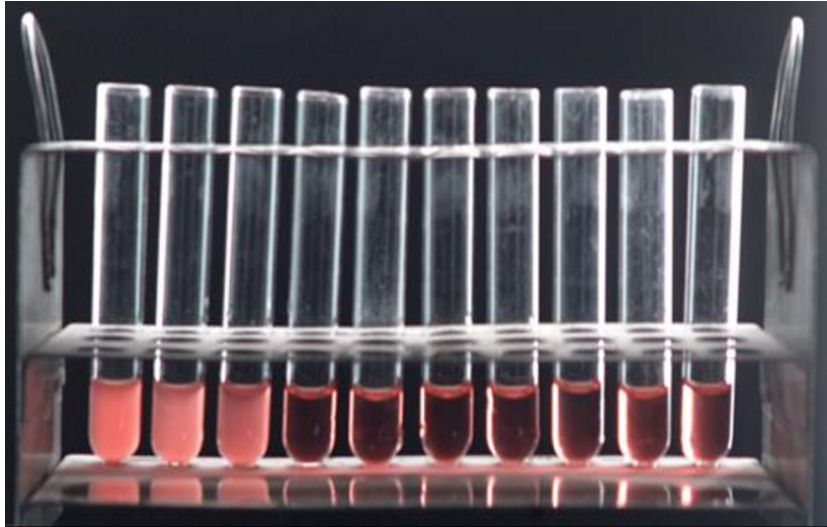


Figure 1: Gruber-Widal reaction in tubes (source: Department of Pathology, University of Cambridge: [http://old-www.path.cam.ac.uk/Immunology/I\\_07.jpg](http://old-www.path.cam.ac.uk/Immunology/I_07.jpg))

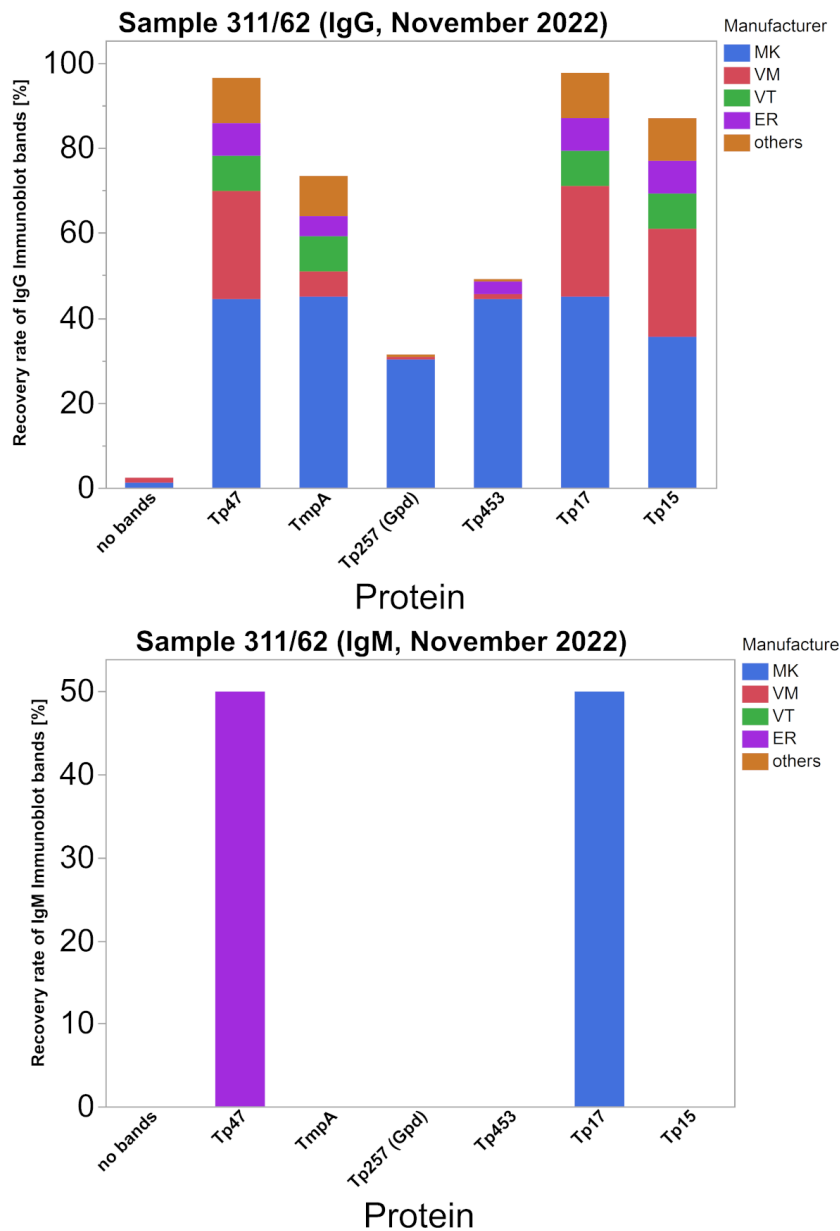


Figure 2: Interlaboratory comparisons of assays: syphilis

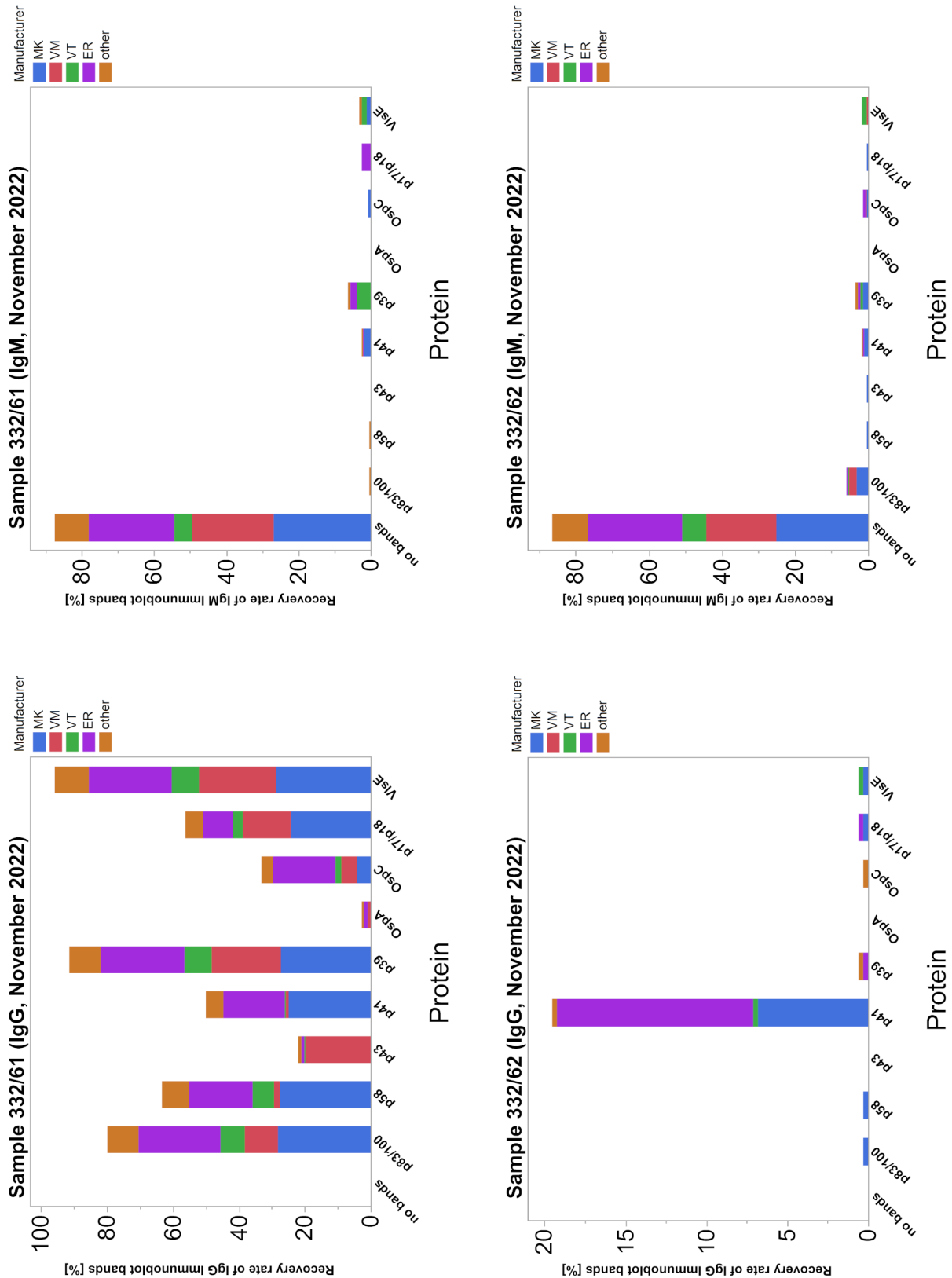


Figure 3: Interlaboratory comparisons of assays: borrelia

an independent evaluation of tests beyond the repertoire of parameters mapped in the RiliBäk in E2. Without appropriate interlaboratory comparisons and their transparent evaluation and publication (Figure 2 and Figure 3), a reasonable selection of assays in the routine laboratory and an assessment of their diagnostic quality is practically

impossible. Nevertheless, it is essential that, even under the influence of the new IVDR, assays approved in the future are better evaluated clinically and put through their paces in larger-scale pivotal studies before they are then launched on the European market. The coordinated and joint implementation of external quality assurance and

good clinical evaluation must go hand in hand to achieve significant improvements in diagnostic quality, the comparability of tests, and the assessment and significance of test results in everyday clinical practice in the future.

## Notes

## Competing interests

The authors declare that they have no competing interests.

## References

1. Wellinghausen N, Abele-Horn M, Donoso Mantke O, Enders M, Fingerle V, Gärtner B, Hagedorn J, Rabenau HF, Reiter-Owona I, Tintelnot K, Weig M, Zeichhardt H, Hunfeld KP. MIQ, Immunological Methods for the Detection of Infectious Diseases. Düsseldorf, Oberhaching: INSTAND e.V., Dustri-Verlag Dr. Karl Feistle; 2018.
2. Wellinghausen N, Abele-Horn M, Donoso Mantke O, Enders M, Fingerle V, Gärtner B, Hagedorn J, Rabenau HF, Reiter-Owona I, Tintelnot K, Weig M, Zeichhardt H, Hunfeld KP. MiQ 35, Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik: Infektionsimmunologische Methoden. München: Urban & Fischer; 2016.
3. Bundesärztekammer. Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen. Gemäß des Beschlusses des Vorstands der Bundesärztekammer in seiner Sitzung am 18.10.2019, zuletzt geändert durch Beschlussfassung des Vorstands der Bundesärztekammer am 14.04.2023. Dtsch Arztebl. 2023 May 30;120(21-22):1-37. DOI: 10.3238/arztebl.2023.rili\_baek\_QS\_Labor

## Corresponding author:

Ulyana Gräf  
Zentralinstitut für Labormedizin, Mikrobiologie und  
Krankenhaushygiene, Krankenhaus Nordwest,  
Steinbacher Hohl 2–26, 60488 Frankfurt am Main,  
Germany  
graef.ulyana@KHNW.de

## Please cite as

Hunfeld KP, Gräf U. Bacteriologic infection serology: old-fashioned or still important? GMS Z Forder Qualitatssich Med Lab. 2023;14:Doc02. DOI: 10.3205/lab000047, URN: urn:nbn:de:0183-lab0000471

## This article is freely available from

<https://doi.org/10.3205/lab000047>

**Published:** 2024-01-15

## Copyright

©2024 Hunfeld et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License. See license information at <http://creativecommons.org/licenses/by/4.0/>.