Quality of bacteriological infection serological procedures in Germany: evaluation of the proficiency testing trials 2018 – contribution of the Quality Assurance Commission of the German Society for Hygiene and Microbiology (DGHM)

Qualität der bakteriologischen infektionsserologischen Verfahren in Deutschland: Auswertung der Ringversuche 2018 – Beitrag der Qualitätssicherungskommission der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM)

Abstract

In order to be allowed to carry out laboratory medical tests, it is mandatory for every laboratory to take part in proficiency testings. These are conducted under routine conditions in order to verify and ensure quality-assured processes. In order to be able to assess the measurement accuracy of the participating laboratories, the results of the samples to be tested, which are identical for all laboratories, are collected and statistically processed (Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen (guideline of the German Medical Association for the quality assurance of laboratory medical examinations)). In the following paper these results are now presented in more detail.

Keywords: proficiency test, quality-assured process

Zusammenfassung

Um labormedizinische Untersuchungen durchführen zu dürfen, ist es für jedes Labor Pflicht, an Ringversuchen teilzunehmen. Diese werden unter Routinebedingungen durchgeführt, um so die qualitätsgesicherten Prozesse nachzuweisen und sicherzustellen. Um die Messgenauigkeit der teilnehmenden Labore beurteilen zu können, werden die Ergebnisse der zu testenden und für alle Labore identischen Proben gesammelt und statistisch aufbereitet (Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen). Diese Ergebnisse werden in der folgenden Arbeit nun genauer dargestellt.

Schlüsselwörter: Ringversuch, qualitätsgesicherter Prozess

Klaus-Peter Hunfeld[1,2,3]
Ulyana Gräf[1,2]

1 Central Institute of Laboratory Medicine, Microbiology and Hospital Hygiene, Northwest Hospital, Frankfurt am Main, Germany

2 INSTAND e.V. Düsseldorf, Germany

3 German Society for Hygiene and Microbiology (DGHM), Quality Assurance Commission, Hannover, Germany

1 Introduction

Bacteriological infection serology is aimed at the indirect detection of pathogens by detecting specific antibodies in the patient's serum, which are produced as a result of a previous exposure to microorganisms [1]. Serum is usually used as the test material, but urine can also be applied for the detection of certain pathogens. In addition molecular biological test methods are increasingly used for the direct pathogen detection in the diagnostics of infectious diseases in the clinical laboratory.

Round robin trials are a powerful tool for external quality assurance in order to obtain a qualified overview on the quality and efficiency of the currently available various serological techniques [2]. Under such conditions the methods used can be evaluated independently of the manufacturer and assessed in more detail in regard to their diagnostic value. The external quality control surveys, which are mandatory for every laboratory, aim at compar-

ing the test quality of serological assays on the market and at continuously improving the quality of diagnostics and treatment [3]. Such surveys provide the laboratories with the opportunity of a transparent and verifiable external quality control approach allowing a better assessment of the diagnostic features of the available contemporary assay systems.
This report presents and discusses the results of the 2018 INSTAND bacteriological infection serology interlaboratory surveys.

2 Methods

2.1 Sample collection and implementation

The round robin test samples were obtained from healthy donors or donors who underwent an infection after attaining informed consent.
Each laboratory participating in the INSTAND e.V. interlaboratory tests was provided with two serum samples once a year for the detection of specific antibodies against yersinia, *Bordetella pertussis*, mycoplasma, campylobacter, and coxiella. All the other parameters (including diagnostic inflammatory markers) were sent out two to four times a year depending on the individual registration of participants. For the external quality assessment (EQA) schemes 313 and 316, two pre-fixed slides and two urine samples spiked with inactivated cell culture supernatant of a *Chlamydia trachomatis* culture were sent out. The antibody reactivity of the serum samples was blinded to the individual participants. Furthermore, no detailed clinical information was made available to guarantee a maximum objectivity to the testing and reporting of the laboratory results. The microbiological stability, sterility and homogeneity of the samples were tested and ensured during production, as well as a negative status of sera for human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV).

2.2 Target values

The target values for qualitative, semi-quantitative and quantitative test results (if applicable in the EQA schemes) as well as the comprehensive diagnostic evaluation and the clinical comment are based on the results of 3 to 10 assigned reference laboratories. In case uniform target values for a particular quantitative parameter or analytical method cannot be determined, the robust mean (Algorithm A according to DIN ISO 13528 [4], annex C) of a collective is stipulated as target value for a specific test method.
For qualitative test results either the mode of the results of the reference laboratories or – if a uniform target value for a particular parameter or analytical method cannot be determined – the mode of the results of the participants is set as target value. The clinical comments are graded “passed” if the individual results corresponded to the reports of the reference laboratories. Combinations of results or comments are also accepted where applicable.
A prerequisite for the evaluation of test results is that the number of participants using a certain assay or test method (values obtained with the same method and/or reagent manufacturer combination) is larger than 8 participants. The evaluation of smaller numbers of participants by consensus value may lead to statistically invalid assessments in some cases. Therefore, the evaluation of smaller numbers of participants ($n \leq 8$) by consensus value is not performed. In addition, only a certificate of participation is issued to such participants. To provide a comparison for the individual participant with the results of other sub-collectives, however, all parameters are calculated and specified as listed and displayed for the assessable collectives in the report.

3 Results

3.1 Tetanus serology (310)

All samples were donated by healthy blood donors. The results for samples 31 and 32 show that protective immunity is not present and a booster shot is strongly recommended in these donors. Sample 61 shows sufficient protective immunity. A booster shot leads to long term immune protection. The donor of sample 62 needs a booster shot in about 5 to 10 years because protective immunity as revealed from the sample is adequate [5]. The pass rates range between 68% and 95%.

3.2 Syphilis serology (311)

The positive sample 31 was obtained from a patient with a known sufficiently treated syphilis infection three months ago (target values [modal]: *Treponema pallidum* particle agglutination (TPPA): 5,120, venereal disease research laboratory (VDRL): 16; immunoglobulin M (IgM) fluorescent treponemal antibody absorption (FTA-ABS) test: 160; enzyme immunoassay (EIA) and immunoblot for immunoglobulin G (IgG) and IgM: positive). The results clearly point to an acute or recent infection that needs further treatment. The negative sample 32 originated from a healthy blood donor without clinical or serological evidence for syphilis infection in his medical history. Concerning the clinical statements, clinical comments or combinations of comments pointing to the correct diagnosis were accepted. Similar to our recent surveys overall pass rates for the different test systems and the clinical comments were encouraging (83%–100%). Due to the highly positive sample 31 the new screening tests (Clinical Laboratory Improvement Amendments (CLIA), chemiluminescent microparticle immunoassay (CMIA), etc.) could not be quantitatively evaluated. The distribution of immunoblot bands for the different assays is displayed in Figure 1. The positive sample 62 (target values: TPPA: 1,280 polyval. enzyme-linked immunoassay (ELISA):

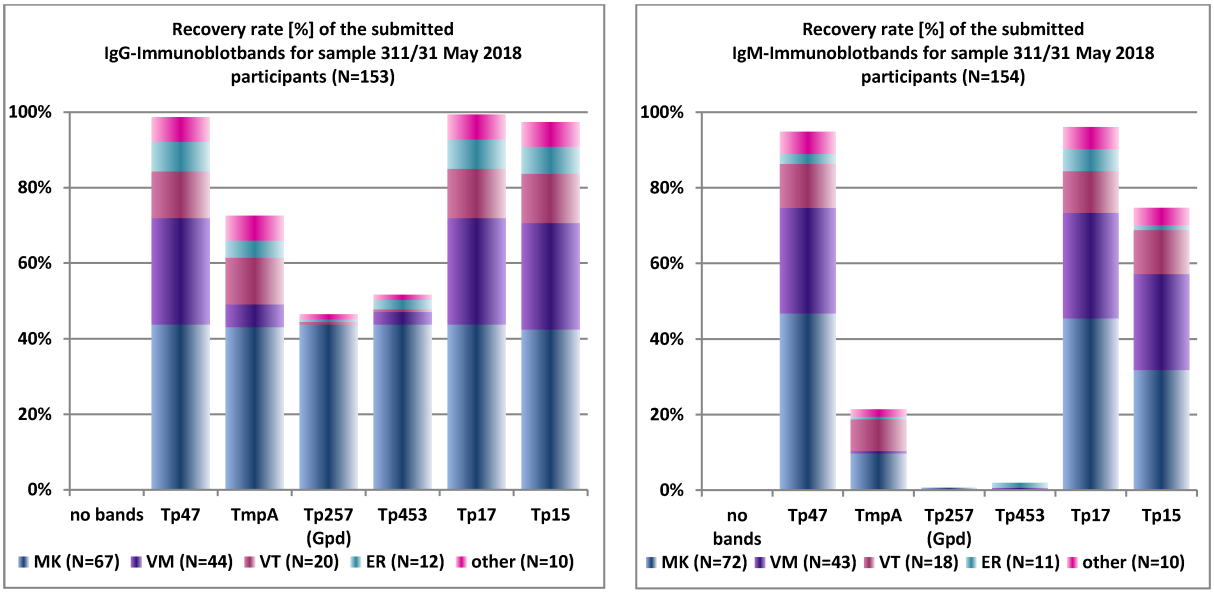

Figure 1: Distribution of immunoblot bands for different assays

positive, IgG-ELISA positive, VDRL: 0–2 positive, borderline, negative, FTA-ABS-IgG: 160, FTA-ABS-IgM and IgM-ELISA negative) was donated during blood donor screening by an individual treated for a syphilis infection several years ago. The other sample (sample 61) was donated by healthy blood donor and yielded negative test results for *T. pallidum*-specific antibodies as reported by most participants and the reference laboratories. The overall pass rates for most of the test methods (pass rates: 87%–100%) and the clinical comment (pass rate: 78%) turned out to be encouraging again.

3.3 Chlamydia trachomatis serology (312)

All samples were donated by healthy blood donors. The samples 32, 61 and 62 showed no serological evidence of an infection with *C. trachomatis*. Positive IgG and borderline immunoglobulin A (IgA) reactivity of sample 31 are consistent with both a past or a possibly active infection. The overall pass rate was between 82% and 100%. The pass rates for the clinical comment (97%–99%) were encouraging.

3.4 Chlamydia trachomatis (direct detection of chlamydia antigen (313))

Samples 31 and 62 were produced from sterile urine that tested negative for *Chlamydia trachomatis* by polymerase chain reaction (PCR). Samples 32 and 61 have been prepared from sterile filtered urine spiked with chlamydia from an inactivated *C. trachomatis* (CT) culture. The pass rate for the negative samples 31 and 62, with no evidence of infection, was 80%–100%. The results for positive samples 32 and 61 were also 80%–100%.

3.5 Chlamydia pneumonia serology (314)

Both the seronegative samples 32 and 61 and the seropositive samples 31 and 62 were donated by clinically healthy blood donors without respiratory infection symptoms in their recent medical history.
Diagnostically there was no serodiagnostic evidence for an infection in the samples 32 and 61. For sample 31 a positive specific IgG antibody reactivity was found corresponding to a past infection.
For sample 62, in addition to a positive IgG reactivity, also a positive/borderline specific IgA response could be detected, pointing to a recent or active *chlamydia pneumonia* (CP) infection. This result was also reported by most laboratories upon their diagnostic comments (pass rates: 81%–100%).

3.6 Yersinia serology (315)

Both reactive samples (samples 31 and sample 32) originated from healthy blood donors without evidence for yersinia infection. Both samples showed a predominant reactivity for specific IgG antibodies and a more weak reactivity for specific IgA antibodies. The results of WIDAL testing and for specific IgM antibodies clearly turned out negative in the reference labs. Accordingly, only clinical comments or combination of comments pointing to a passed yersinia infection and possible infection-associated sequelae were accepted. The overall pass rates for the different assays and the clinical comments were less encouraging than during our recent surveys, mainly due to questionable IgM reactivity in some assays (pass rates: 52%–100%).

3.7 Chlamydia trachomatis IFT (316)

For direct *C. trachomatis* (CT) detection by immune fluorescent testing (IFT), the CT positive and negative samples were fixed on slides before shipment. The slides of the negative samples 31 and 61 were coated with non-infected squamous epithelial cells of a urine sediment. The coating of the slides for the positive samples 32 and 62 consisted of squamous epithelial cells from a urine sediment to which *C. trachomatis* from a culture supernatant were added.
The pass rates ranged between 82% and 90% for both the analytics and the overall diagnostic assessment.

3.8 Bordetella pertussis serology (317)

Samples 61 and 62 have been donated by healthy blood donors without evidence for respiratory infections in their recent medical history. Sample 62 showed positive test results for IgG in pertussis toxin (PT)/filamentous hemagglutinin-(FHA-)ELISAs and also in IgG-ELISAs/imunoblots using PT only. In addition, results were positive for specific IgM and showed variable IgA reactivity upon EIA and immunoblot testing! Because of the relative variability of test results the survey was graded generously. Sample 61 tested negative for specific antibodies against *B. pertussis* and showed no evidence for an active or recent infection. The test results for sample 62 point to an acute or recent infection or immunization (overall pass rates: 79.7%–100%).

3.9 Diphtheria serology (318)

From a serological point of view, it can be assumed that the donor samples 31 and 61 show adequate immune protection. A booster would provide long-term protection. The donor of samples 32 and 62, on the other hand, do not show adequate immune protection and a booster should be recommended. The overall pass rates for the qualitative analysis were 77%–97%. The pass rate for the quantitative analysis was 78%.

3.10 Campylobacter serology (319)

Samples 31 and 32 originated from healthy blood donors without evidence for recent gastroenteritis. The positive sample 31 showed week anti-campylobacter reactivity. Complement fixation testing (CFT) remained negative and most assays showed negative results for specific IgM and IgA antibodies. IgG-ELISA and IgG immunoblot, however, showed week reactivity for specific IgG antibodies. Nevertheless, this reactivity remained undetected by some assay systems. These observations again point to the current limitations of serology in the diagnostics of acute campylobacter infection. Consequently, test results and the clinical comments have been graded more generously, leading to pass rates of 97.3% to 100%.

3.11 Procalcitonin (320)

Samples 32 and 61 were obtained from clinically healthy blood donors. Samples 31 and 62 were produced from pooled leftover sera of septic patient. The results for samples 31 and 62 pointed to a systemic infection (sepsis). However, a systemic infection (sepsis) is rather unlikely for samples 32 and 61. The overall pass rates were between 76% and 100%. The pass rates for the clinical comments ranged between 84% and 94%.

3.12 Streptoccocal serology (321)

Samples 31, 32 and 61 were derived from clinically healthy blood donors. To prepare the positive sample 62, positive leftover patient sera were pooled with the serum of a healthy donor. The pass rates for the different analytical methods for the detection of specific antibody concentrations against streptodornase and streptolysin-O were in the range of 76%–97%.

3.13 Rheumatoid factor (323)

Samples 31, 32 and 61 were obtained from clinically healthy blood donors. For the production of the positive sample 62 patient sera positive for rheumatoid factor were pooled with the serum of a healthy donor. The overall pass rates were ranged between 82% and 100%.

3.14 Mycoplasma pneumonia serology (324)

Samples 61 and 62 originated from healthy blood donors without evidence for respiratory infections in their recent medical history. For sample 61 most participants and the reference laboratories found only borderline or negative results for specific IgG antibodies corresponding to no infection or a past infection. For sample 62 positive reactivity for specific IgG, IgM, and IgA antibodies in the different test systems points to an acute or very recent infection. Pass rates for the different immunoassays and the clinical comment were 63%–100% and 89%, respectively.

3.15 Coxiella burnetii serology (325)

Sample 61 was donated by a healthy blood donor without evidence for a recent infection and tested negative for *C. burnetii* antibodies. Samples 62 was donated by a patient several months after PCR-confirmed acute *C. burnetii* pneumonia. The sample demonstrated IgG phase I IFT titers of 1,280 (median), IgG phase II IFT titers of 640 (median) as well as weakly reactive IgM and IgA results. This constellation of test results is consistent with the clinical information of a relatively recent pneumonia. Most participants and also the expert laboratories reported variable clinical comments as to whether the test constellation should be interpreted as an acute or

chronic Coxiella infection. Thus the grading of clinical comments was performed more generously (pass rate: 71.6%). The overall pass rates (80%–100%) of the immunoassays are encouraging.

3.16 Salmonella serology (331)

The positive sample 32 was obtained from a healthy blood donor without evidence for recent gastrointestinal infection (GI) and showed negative results upon serological testing. The positive sample 31 originated from a patient with acute *Salmonella enterica*, ssp. *enterica*, *Serovar Enteritidis* [1,9,12:gm:(1,7):-] infection about 3 weeks before sampling and showed variable reactivity for the different salmonella antigens upon WIDAL testing due to the well-known cross-reactivity between different salmonella serovars. The results of WIDAL testing are in accordance with a *Salmonella Enteritidis* infection but did not allow exact serotyping. Both evidence for acute and a more recent infection were accepted for clinical comment (pass rates: 75%–100%).
The negative sample 62 was donated by a healthy blood donor without evidence for a recent GI infection. The positive sample 61 originated from a patient with a systemic *Salmonella enterica subsp. enterica*, *Serovar Typhimurium* (1, 4, [5] , 12: i: 1,2) infection (blood culture confirmed!) and showed corresponding reactivity upon WIDAL and ELISA testing. Test results obtained for sample 61 by both the expert laboratories and most participants clearly point to an acute or recent salmonella infection (pass rates: 94%–99%).

3.17 Borrelia burgdorferi (332)

Both negative samples (samples 31 and 32) were obtained from clinically healthy blood donors without evidence for a tick bite or acute or recent Lyme disease in their medical history and tested negative in the reference laboratories. Not surprisingly, the pass rates including the clinical comments were encouraging (90%–99%). A graphic display of immunoblot banding distribution was left out this time because mainly p41 was recognized upon immunoblot testing.
Sample 61 was donated by a healthy blood donor without evidence for tick bites or known manifestation of Lyme disease in his medical history. Sample 62 originated from a patient with a successfully treated facial palsy apx. 1 month after the infection and showed pronounced IgM antibody titers together with weakly reactive IgG test results. The constellation of test results in the immunoblot corresponds well to an early phase of the borrelia-specific immune response. Both the test-specific over all pass rates (85%–99%) as well as the quality of the clinical comments (overall pass rates: 76%) have been encouraging again.

3.18 Helicobacter pylori serology (334)

Samples 31 and 32 were obtained from clinically healthy blood donors. Sample 31 revealed no evidence for immunological contact with *Helicobacter pylori*. However, specific IgG antibodies and additional IgA antibodies in the cutoff range were detectable in sample 32. The serological findings indicate an infection or colonization and further diagnostic clarification is recommended. The pass rates are in the range between 95% and 99%. The negative sample 61 was obtained from a healthy blood donor. The positive sample 62 showed positive IgG and IgA antibody reactivity upon ELISA and immunoblot testing. The sample originated from a helicobacter-positive patient and was obtained shortly after eradication therapy ended. The constellation of results was interpreted as corresponding to infection or colonization with helicobacter both by the reference laboratories and most of the participants (overall pass rates: 94%–99%).

4 Discussion

Overall, the evaluation of the current laboratory survey in bacteriologic infection serology was unproblematic and pass rates for most of the parameters examined were found within the range of previous surveys. As outlined above in the more detailed synopsis for the different parameters the actual proficiency testing survey of 2018 also clearly shows the advantages and limitations of the different analytes and diagnostic methods. Again, syphilis and borrelia serology turned out to be the most reproducible diagnostic methods, together with parameters such as procalcitonine or anti-streptococcal antibodies, in comparison to the other parts of the survey. In general, there has been a steady progress when it comes to the pass rates of diagnostics for the different pathogens and analytics over the years. Unfortunately, this does not hold through for parameters like the chlamydia, campylobacter, pertussis, and salmonella serology. Such parameters are clearly called into question when it comes to both their performance in external quality control surveys and their practical analytic value, and importance under routine diagnostic conditions. Consequently, such limitations should be transparently communicated in medical diagnostic guidelines and maybe lead to the propagation of alternative better suited assay formats, and technical approaches for the laboratory detection of such pathogens.

Notes

Competing interests

The authors declare that they have no competing interests.

References

1. Rüttger S, Müller I, Hunfeld KP. Zur Qualität bakteriologisch-infektionsserologischer Verfahren in Deutschland: Auswertung der infektionsserologischen Ringversuche 2015 – Beitrag der Qualitätssicherungskommission der DGHM. GMS Z Forder Qualitatssich Med Lab. 2018;9:Doc03. DOI: 10.3205/lab000031
2. Verordnung über das Errichten, Betreiben und Anwenden von Medizinprodukten (Medizinprodukte-Betreiberverordnung - MPBetreibV). § 9 Qualitätssicherungssystem für medizinische Laboratorien.
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 Beschlussfassungen des Vorstands der Bundesärztekammer am 14.04.2023. Dtsch Ärztebl. 2023 May 30;120(21-22). DOI: 10.3238/arztebl.2023.rili_baek_QS_Labor
4. DIN ISO 13528:2020-09: Statistische Verfahren für Eignungsprüfungen durch Ringversuche (ISO 13528:2015, korrigierte Fassung 2016-10-15). Berlin: Beuth; 2020. DOI: 10.31030/3116750
5. Kuhlmann WD. Tetanus. Impfung, Impftiter und Impfreaktion. Koblenz: Zentrales Institut des Sanitätsdienstes der Bundeswehr; 1991.

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. Quality of bacteriological infection serological procedures in Germany: evaluation of the proficiency testing trials 2018 – contribution of the Quality Assurance Commission of the German Society for Hygiene and Microbiology (DGHM). GMS Z Forder Qualitatssich Med Lab. 2023;14:Doc01.
DOI: 10.3205/lab000046, URN: urn:nbn:de:0183-lab0000467

This article is freely available from
https://doi.org/10.3205/lab000046

Published: *2023-12-27*