The quality of bacterial infection serology tests in Germany: Evaluation of the 2021 external quality assurance schemes – a report by the Quality Assurance Commission of the German Society for Hygiene and Microbiology

Qualität der bakteriologischen infektionsserologischen Verfahren in Deutschland: Auswertung der Ringversuche 2021 – ein Beitrag der Qualitätssicherungskommission der Deutschen Gesellschaft für Hygiene und Mikrobiologie

Abstract

High-quality patient care is contingent on the accuracy and reliability of diagnostic testing, which in turn relies on external quality assessment schemes. Participation in external quality assurance (EQA) schemes is a mandatory part of external quality assurance in many areas of laboratory medicine, however the frequency of participation depends on the parameters offered by each laboratory. The analyses are performed under routine conditions and the results are then submitted to a reference institute (Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen [guideline of the German Medical Association for the quality assurance of laboratory medical examinations] 2023). This paper provides a detailed analysis of the results of the 2021 INSTAND e.V. EQA surveys for bacteriological infection serology.

Keywords: EQA tests, quality assurance

Zusammenfassung

Die Qualität von diagnostischen Untersuchungsergebnissen muss verlässlich sein, um eine gute Patientenversorgung zu gewährleisten. Dies ist durch Ringversuche gegeben. Sie sind im Rahmen der externen Qualitätssicherung in vielen Bereichen der Labormedizin verpflichtend, wobei die vorgeschriebene Teilnahmehäufigkeit für die unterschiedlichen Parameter variiert. Die Ergebnisse der zuvor unter Routinebedingungen getätigten Analysen werden anschließend an ein Referenzinstitut übermittelt (Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen 2023). Die Ergebnisse der INSTAND e.V. Ringversuche in der bakteriologischen Infektionsserologie 2021 werden in der folgenden Arbeit genauer dargestellt.

Schlüsselwörter: Ringversuche, Qualitätssicherung

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1 Introduction

To ensure the quality of medical laboratory analyses, standardized and validated processes must undergo certification through external quality assurance (EQA) testing [1]. Regular participation in EQA testing serves to verify and compare the performance and suitability of testing methods [2]. Participating laboratories are provided with identical samples for testing. The sample material is usually serum or plasma, however, urine can also be used to directly detect pathogens. Results are submitted within a specified timeframe and are then evaluated by a reference institute and specialist laboratories and made available to the participants [3]. This evaluation helps to improve and further develop diagnostic methods. It also aids in characterizing infection status and in determining vaccine titers.
This report presents and discusses the results of the 2021 INSTAND e.V. EQA surveys for bacterial infection serology.

2 Methods

2.1 Sample collection and implementation

The EQA test samples are obtained from healthy donors or donors who have had a previous infection. Informed consent is obtained from the donors beforehand.
Every laboratory participating in INSTAND e.V. EQA schemes receives two serum samples per year which are used to detect specific antibodies against yersinia, bordetella pertussis, mycoplasma, campylobacter and coxiella respectively. All other parameters (including diagnostic inflammatory markers) are tested two to four times a year depending on the participant. For EQA schemes 313 and 316, participants receive two pre-fixed slides and two urine samples that have been spiked with the inactivated cell culture supernatant of a *Chlamydia trachomatis* culture. The antibody reactivity of the serum samples is not revealed to the individual participants. Furthermore, no detailed clinical information is disclosed in order to ensure maximum objectivity with regard to the testing and reporting of the laboratory results. The microbiological stability, sterility and homogeneity of the samples are ensured during production (in accordance with DIN ISO 13528 Annex B) [4], and the samples have tested negative for human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV).

2.2 Target values

The target values for the qualitative, semi-quantitative and quantitative test results (for the EQA) as well as the comprehensive diagnostic evaluation and the interpretative comments are based on the results of 3 to 10 designated reference laboratories. If no uniform target value can be determined for a particular quantitative parameter or analytical method, the robust mean (Algorithm A according to DIN ISO 13528, Annex C) of a collective is used as the target value for a specific test method [4].
With respect to the qualitative test results, the target value is 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. The laboratory receives a passing grade if its results correspond to those reported by the reference laboratories. A combination of results and comments is also accepted where applicable. Test results are only evaluated if a specific assay or test method is used by more than 8 participants (values obtained with the same method and/or reagent manufacturer combination). Evaluating smaller participant collectives based on a consensus value can, at times, lead to statistically invalid assessments. Therefore, a consensus value is not used to evaluate smaller cohorts of participants ($n \leq 8$). In such cases, participants only receive a participation certificate.

3 Results

3.1 Tetanus serology (310)

All samples were donated by healthy blood donors. The results for samples 31 and 61 showed that long-term protection was present and that titers should be rechecked after 5 to 10 years. Sufficient immune protection was also determined for samples 32 and 62. A booster shot is recommended after 10 years [5]. The pass rates ranged from 88–100%.

3.2 Syphilis serology (311)

Positive sample 32 was obtained from a patient years after a sufficiently treated syphilis infection (titer (modal): *Treponema pallidum* particle agglutination (TPPA): 640, venereal disease research laboratory (VDRL): <1; fluorescent treponemal antibody absorption immunoglobulin M (IgM-FTA-abs) test: <5; enzyme immunoassay (EIA); immunoblot: positive for immunoglobulin G (IgG) and negative for IgM). Even without clinical information, the results clearly point to a past infection. The donor, however, should not donate blood. Negative sample 31 originated from a healthy blood donor whose medical history provided no clinical or serological evidence of a syphilis infection. With regard to clinical comments, only comments or combinations of comments indicating a past infection without need for further treatment were accepted (pass rates: 78–93%). Overall, pass rates were more or less similar to those observed during recent surveys. New screening tests (chemiluminescent immunoassay (CLIA), chemiluminescent microparticle immunoassay (CMIA), etc.) could not be evaluated quantitatively this time due to a lack of standardization. Figure 1 shows

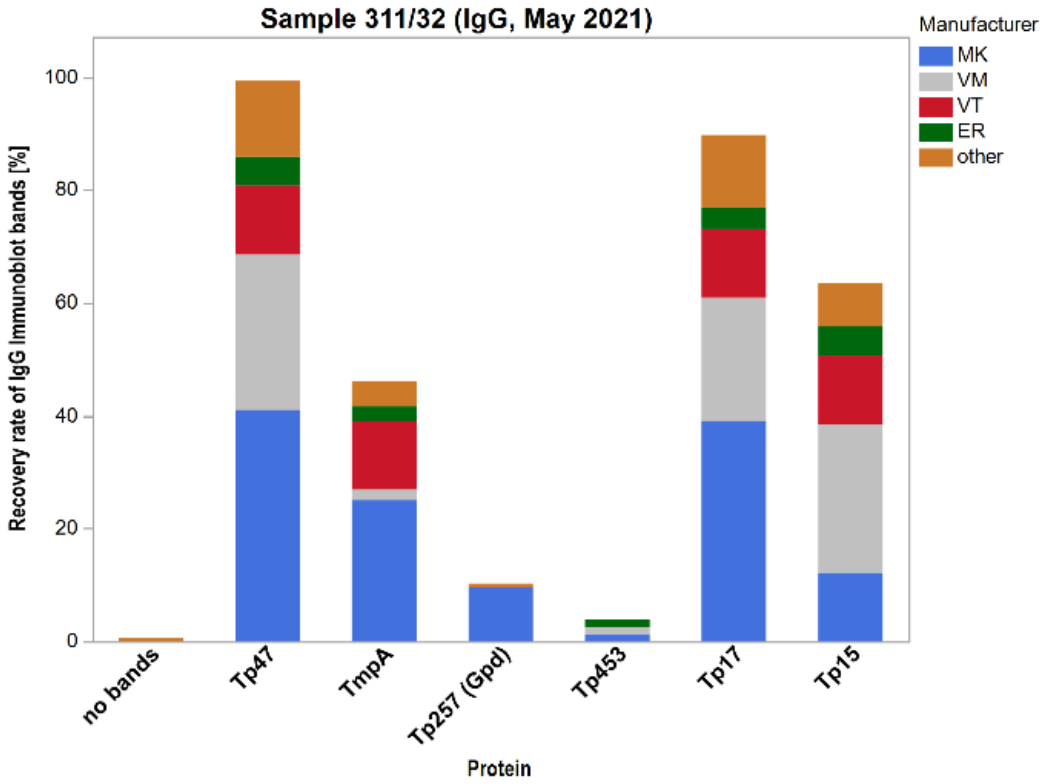


Figure 1: Manufacturer-specific distribution of immunoblot bands for sample 32 (IgG)

the distribution of the immunoblot bands for the different assays for sample 32.

Positive Sample 62 (target values: TPPA: 320, polyvalent enzyme linked immunosorbent assay (ELISA): borderline, positive, IgG-ELISA: borderline, positive, VDRL: 0, negative, FTA-ABS-IgG: 10, borderline, positive; FTA-ABS-IgM and IgM-ELISA: negative) was taken from a blood donor with a history of an adequately treated syphilis infection. Both the reference laboratories and the participants reported negative test results for sample 61, which was clearly negative and taken from a healthy blood donor (overall pass rates for both samples: 77–100%). The overall pass rate for the clinical assessment, which included a weak reactive sample, was lower than in the past (65%). Figure 2 shows the distribution of the immunoblot bands for the different assays for sample 62.

3.3 Chlamydia trachomatis serology (312)

All samples were taken from healthy blood donors with no history of a previous urogenital illness. The pass rates for the various methods were encouraging (clinical comment: 96.7%; overall pass rates: 68–100%).

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

Samples 31 and 61 were produced from sterile urine that tested negative for *Chlamydia trachomatis* by polymerase chain reaction (PCR). Samples 32 and 62 were prepared from sterile filtered urine spiked with *Chlamydia trachomatis* from an inactivated CT culture. The pass rates were 85–100%.

3.5 Chlamydia pneumonia serology (314)

Sample 61 was taken from a positive donor who had had a respiratory infection two months prior. Positive macrophage migration inhibitory factor (MIF) IgG (titer: 160), negative MIF IgM (titer: <20), and positive MIF IgA (titer: 160) reactivity were detected so that both responses "indication of an acute infection" and "indication of a past infection" were accepted. Negative sample 62 was taken from a healthy blood donor without a pre-existing respiratory illness. The pass rates for the different methods were, with a relatively clear constellation of findings, predominantly very encouraging (clinical comment: 94%; overall pass rates: 67–100%).

Negative sample 31 was taken from a healthy blood donor without a pre-existing respiratory illness. Sample 32 was a follow-up serum taken from a PCR-positive pneumonia patient in autumn 2020. The initial infection had occured in the late summer months of 2020. At the time, the clinical and radiological diagnosis was atypical pneumonia! Positive MIF IgG (titer: 160), negative/borderline MIF IgM (titer: 0–10), and borderline/positive MIF IgA (titer: 40) reactivity were detected. "Indication of an acute infection" or "indication of a recent (past) infection" were accepted as clinical comments. The pass rates for the different methods were, with a relatively clear constel-

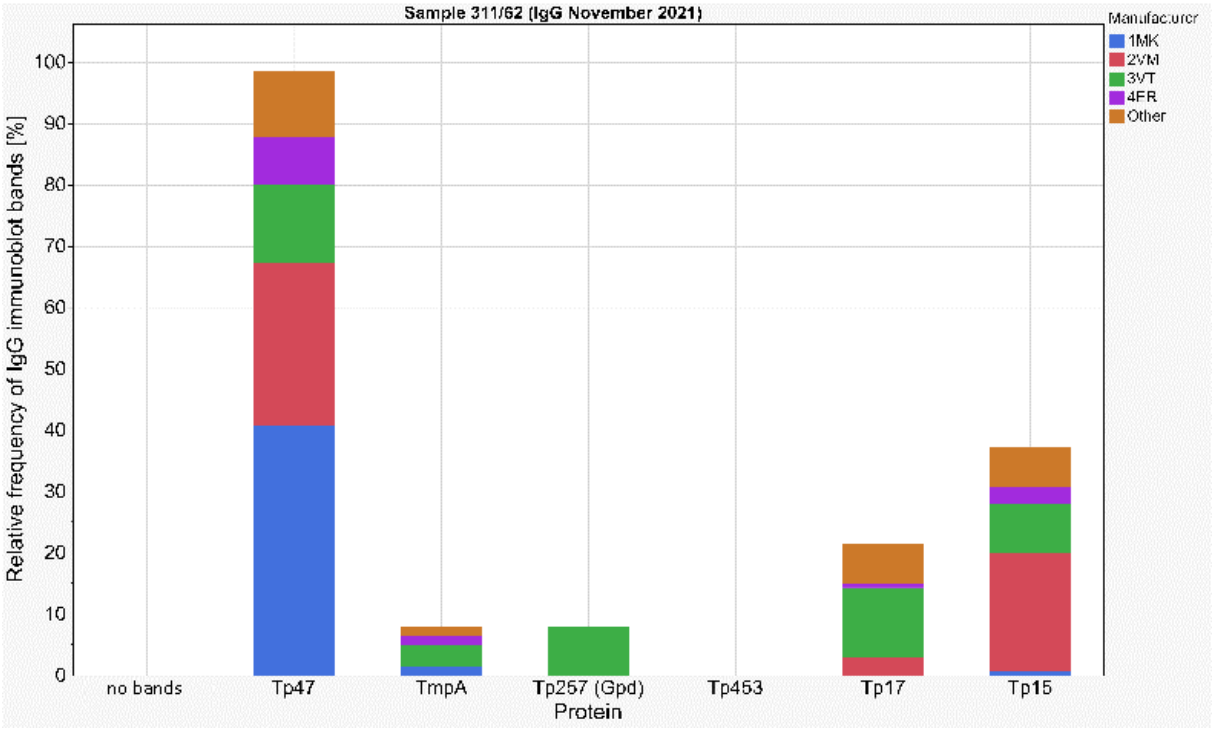


Figure 2: Manufacturer-specific distribution of immunoblot bands for sample 62 (IgG)

lation of findings, predominantly very encouraging (clinical comment: 97%; overall pass rates: 54–100%).

3.6 Yersinia serology (315)

Sample 32 originated from a healthy donor and was deemed negative by serological testing. Sample 31 was obtained from a patient with a past infection and was found to be negative by WIDAL testing. It clearly showed reactivity to the IgG immunoglobulin class and had a negative to weak reactivity to IgA, but tested negative for IgM in EIA and immunoblot. The constellation of test results points to a past infection and/or yersinia-associated post-infectious sequelae. Overall pass rates were again encouraging (pass rates: 73–92%).

3.7 Chlamydia trachomatis IFT (316)

Immune fluorescent testing (IFT) was used to directly detect *C. trachomatis* (CT). The negative and positive CT samples were fixed on slides before shipment. The slides of negative samples 31 and 61 were coated with non-infected squamous epithelial cells of a urine sediment. The slides of positive samples 32 and 62 were coated with squamous epithelial cells from a urine sediment to which *C. trachomatis* from a culture supernatant was added. The pass rate was 75% for both the analytics and the overall diagnostic assessment.

3.8 Bordetella pertussis-serology (317)

Samples 61 and 62 were donated by healthy blood donors without evidence of a respiratory infection in their recent medical history. Sample 62 (containing less than 40 IU/ml of specific anti PT-IgG and less than 12 IU/ml of specific anti PT-IgA) mainly showed negative or borderline reactivity for IgG and IgA in IgG- and IgA-ELISAs when only PT was used. Results were negative for specific IgM. This was a survey for *B. pertussis* antibody testing and not for *B. parapertussis*! As such, only negative and borderline results were accepted for sample 62. Sample 61 tested negative for specific antibodies against *B. pertussis* and showed no evidence of an active or recent infection. The test results for sample 62 indicated a past infection/immunization (if at all) or, better yet, no infection at all (overall pass rates: 61–83%).

3.9 Diphtheria serology (318)

All samples were obtained from healthy, vaccinated individuals. Many participants were not aware that, according to the WHO, anti-toxoid antibody concentrations between 0.01 and 0.099 IU/ml are regarded as quantitatively positive result, although this does not mean there is immune protection. This led to slightly lower qualitative pass rates for sample 31, although the quantitative determination and the clinical interpretation were excellent (pass rates: 69–96%).

3.10 Campylobacter serology (319)

Samples 31 and 32 originated from healthy blood donors and yielded negative test results with complement fixation testing (CFT), ELISA and immunoblot testing, leading to pass rates of 92–100%.

3.11 Procalcitonin (320)

Samples 31, 61 and 62 were obtained from clinically healthy blood donors. A systemic infection (sepsis) was deemed unlikely. Sample 32 was produced from pooled left-over sera from septic patients and pointed to a systemic infection (sepsis). The overall pass rates were between 83% and 98%. The pass rate for the interpretative comments was 77%.

3.12 Streptococcal serology (321)

Samples 31, 32 and 61 came from clinically healthy blood donors. Positive sample 62 was prepared by pooling positive left-over patient sera with serum from a healthy donor. The pass rates for the different analytical methods used to detect specific antibody concentrations against streptodornase and streptolysin-O ranged from 86–98%.

3.13 Rheumatoid factor (323)

Samples 32 and 61 were obtained from a clinically healthy blood donor. Positive samples 31 and 62 were produced by pooling patient sera that were positive for rheumatoid factor with serum from a healthy donor. The overall pass rates ranged between 78–93%.

3.14 Mycoplasma pneumonia serology (324)

Samples 61 and 62 originated from healthy blood donors with no evidence of a respiratory infection in their recent medical histories. The overall pass rates for the different test methods and the interpretative comments were mostly encouraging (overall pass rates: 80–99%; interpretative comment: 91%).

3.15 Coxiella burnetii serology (325)

Sample 61 was donated by a healthy blood donor without evidence of a recent infection and tested negative for *C. burnetii* antibodies. Sample 62 was donated by a clinically healthy patient several years after receiving effective treatment for a PCR-confirmed acute case of *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. Despite the passage of time, the constellation of test results was still consistent with the clinical indication of a relatively recent infection according to the semi-quantitative test results of the various assays. The clinical comments of most participants as well as the expert laboratories varied as to whether the test constellation should be interpreted as an acute, past, or chronic Coxiella-infection. Thus, there was a more generous grading of the clinical comments (pass rate: 96.5%).

3.16 Salmonella serology (331)

Sample 32 was obtained from a healthy blood donor without evidence of a salmonella infection. Sample 31 was prepared from *rabbit sera* containing moderate to high titers of antibodies against *Salmonella enterica* subsp. *enterica*, Serovar Typhi (9, 12, [Vi]: d, [–]) with anti-S. *Typhi* –(O) titers of 400 (100–1,600) and (O)H titers of 400 (100–1,600) upon direct agglutination by WIDAL test. In addition, EIA tests to detect *human* anti-salmonella IgG and IgA antibodies yielded negative results for this sample. Accordingly, test results and medical comments were accepted based on the individual test methods used (over all pass rates: 53–100%).
Samples 61 and 62 were taken from healthy blood donors with no indication of a salmonella infection (overall pass rates: 83–100%).

3.17 Borrelia burgdorferi (332)

Sample 31 was donated by a healthy blood donor whose medical history showed no evidence of a tick bite or a manifestation of Lyme disease. Sample 32 also originated from a healthy blood donor but demonstrated isolated reactivity for specific IgG antibodies and negative to weak reactivity for IgM antibodies against *B. burgdorferi* in all test systems used in the survey. Immunoblot results and banding patterns point to the late phase of an anti-*B. burgdorferi* immune response. The overall pass rates (89–100%) and the pass rates for the clinical comments were encouraging (93.8%). The distribution of immunoblot bands reported for positive sample 32 is depicted in Figure 3.
Sample 61 came from a healthy blood donor whose medical history indicated no tick bite or Lyme disease. Sample 62 was taken from a patient who had been successfully treated for erythema migrans approximately two years after treatment of the infection. The sample shows the lingering persistence of an immune response after a borrelia infection and – depending on the assays used – still exhibited a borderline reactivity to specific IgM and IgG, as well as a constellation of findings in the immunoblot that corresponded to either no infection or to the early stage of a *Borrelia burgdorferi*-specific immune response. Both the overall pass rates for the various tests (89–100%) and for the clinical assessment (95.3%) were relatively encouraging. The distribution of immunoblot bands reported for positive sample 62 is depicted in Figure 4.

3.18 Helicobacter pylori serology (334)

Negative sample 32 was obtained from a healthy blood donor. Sample 31 also originated from a healthy blood donor and exhibited specific IgG- and IgA-antibody reactivity upon EIA and immunoblot testing. Consequently, comments pointing to a possible infection/colonization with *H. pylori* were accepted for this sample (overall pass rates: 78–98%).

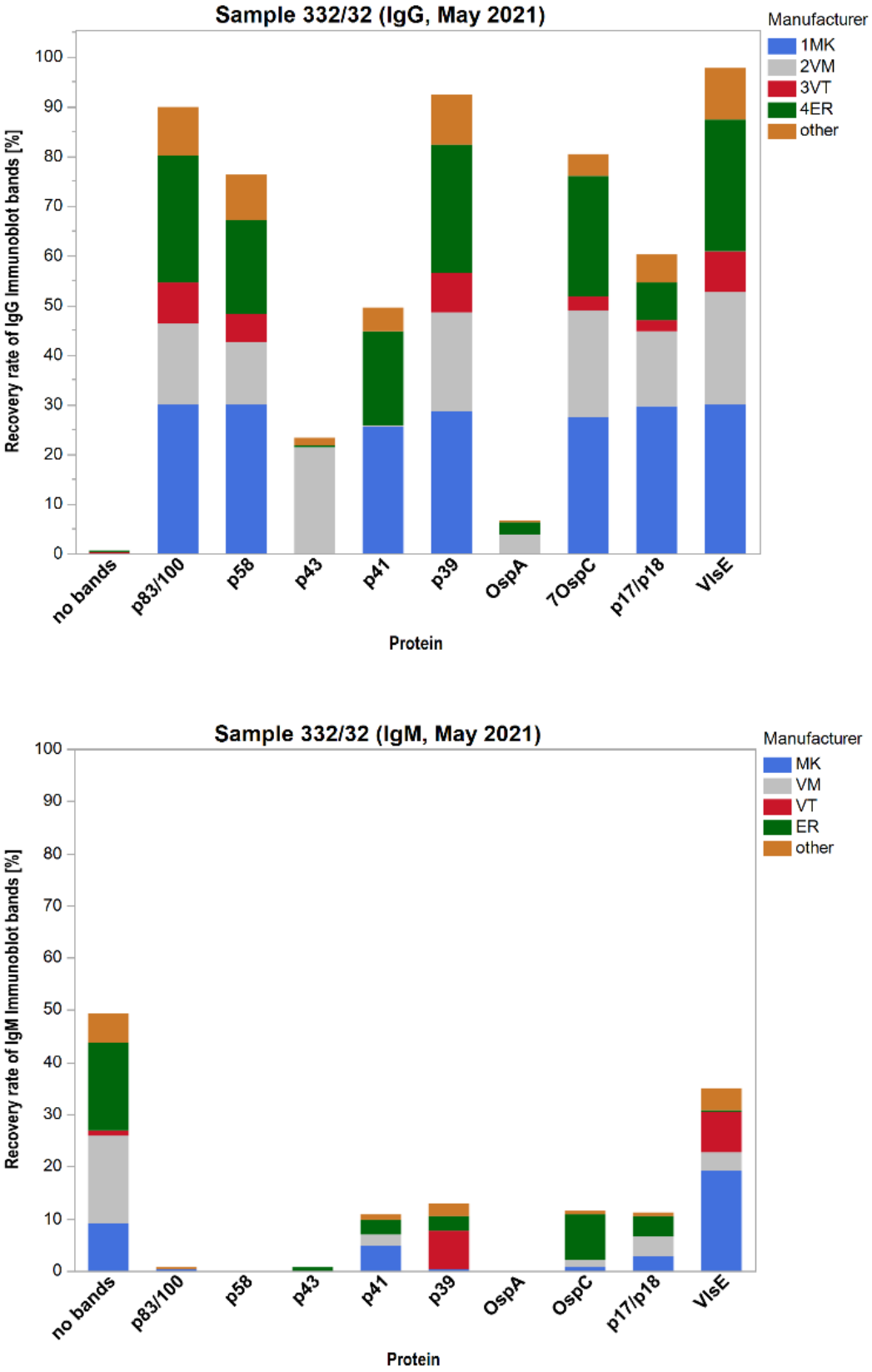


Figure 3: Manufacturer-specific distribution of immunoblot bands for sample 32 (IgG and IgM)

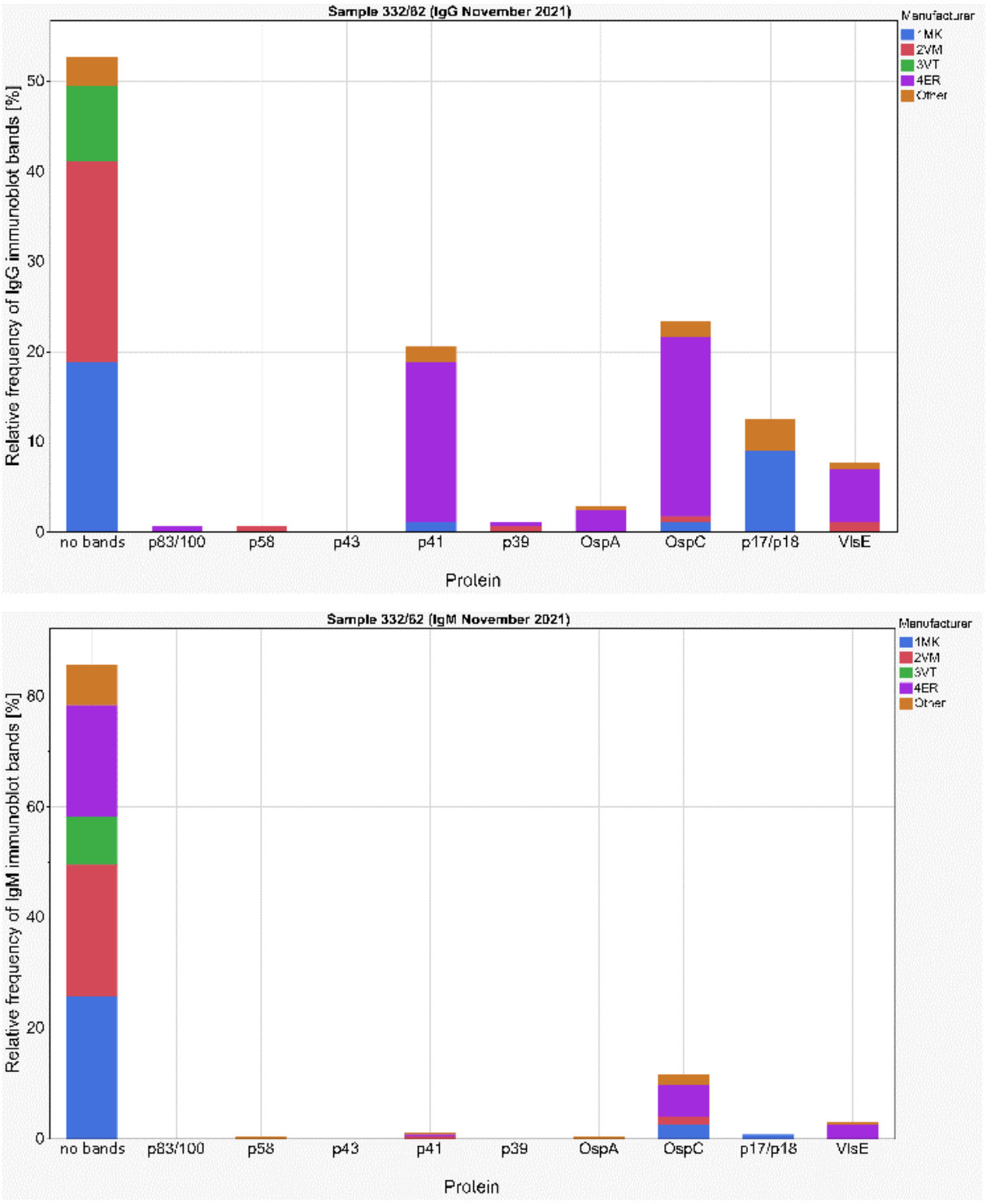


Figure 4: Manufacturer-specific distribution of immunoblot bands for sample 62 (IgG and IgM)

Negative Sample 61 was taken from a healthy blood donor. In contrast, positive sample 62 exhibited positive IgG results and weakly positive IgA results when ELISA and immunoblot were used. The sample was taken from a Helicobacter-positive patient approximately one year after completion of eradication treatment. The constellation of results for sample 62 indicated to an *H. pylori* infection/colonization (overall pass rates: 63–99%).

4 Discussion

The evaluation of the 2021 EQA program for bacterial infections revealed very few issues. Only the EQA test for diphtheria conducted in spring 2021 showed a low pass rate, similar to that of the previous year. Unfortunately, many participants did not consider anti-toxoid antibody test results of between 0.01 and 0.099 IU/ml to be positive. However, according to WHO guidance on anti-toxoid antibody concentrations, these values are considered positive even if there is insufficient immune protection.

The quantitative test results and clinical interpretations, on the other hand, were excellent, and the pass rates for most of the other bacterial infection serology schemes examined were excellent and mostly in line with the results of previous EQA schemes.

Notes

Competing interests

The authors declare that they have no competing interests.

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