Content

1 Quality management system, document control

Issued/revised	14.06.2022, Dr. Sandra Appelt
Revised	22.06.2022, Dr. Daniela Jacob
Approved	24.06.2022, Dr. J. Kleymann-Hilmes

1 copy QMB-OE: ZBS2-PT

Note modifications:

This version replaces the version 3 of this document. As the lead management position in ZBS2 has changed this document had to be updated. Also, the requirements to be met to participate in the proficiency test were clarified in terms of PT sample import and export. The revision was also used, to transfer the document into the new format of QM documents at the RKI. Changes are highlighted in grey.

2 Objective and purpose

This manual guide is valid for ZBS2 (Center for Biological Threats and Special Pathogens, Highly Pathogenic Microorganisms) at the RKI. This document highlights the scope of proficiency tests (PT) offered to laboratories specialized in diagnosing highly pathogenic bacteria that are classified to belong to Risk Group (RG) 3 pathogens. The focus, but not exclusively, is on the following bacteria: *Bacillus anthracis, Francisella tularensis, Burkholderia mallei, Burkholderia pseudomallei, Yersinia pestis*, and *Brucella* species.

The PT guide gives an overview about the scope of the PT including requirements for participation, offered test items and procedures to follow based on the DIN EN ISO/IEC 17043. The offered PTs aim to allow laboratories to participate in External Quality Assurance Exercises (EQAEs) for accreditation and evaluation purposes, helping laboratories to perform a self-assessment in order to identify diagnostic gaps, and to also test new diagnostic procedures before final implementation. Individual recommendations for diagnostic improvements are provided.

3 Abbreviations and definitions

3.1 Abbreviations

BSL	Biosafety level
ELISA	Enzyme-Linked Immunosorbent Assay
EQAE	External Quality Assurance Exercises
RG	Risk Group
HPB	Highly Pathogenic Bacteria
IFA	Immunofluorescence Assay
MTA	Material Transfer Agreement
PCR	Polymerase Chain Reaction

PT Proficiency Test

3.2 Definitions

Target: refers to the bacteria that has to be identified or excluded in PT samples by participants. The target bacteria are announced in advance of a PT.

4 Responsibilities and general information about the PT provider

PT Coordinators: Dr. Daniela Jacob and PD Dr. Holger Scholz

The PT provider offers the following services to all participants.

Task ZBS 2 PT provider	Trained staff member
Announcement and planning of the PT	PT Coordinator, Manager & Scientist in charge
Reviewing applications for participation	PT Coordinator, Manager & Scientist in charge
Preparation, quality control, and pretesting of offered test items	PT Coordinator, Scientist in charge & Technical Assistants
Shipping of test items	PT Coordinator & external certified shipping Agency
Evaluation of provided results, statistical analyses and development of a summarizing PT reports	PT Coordinator, Manager & Scientist in charge (and upon need an agency specialized on statistics and analytical quality assurance*)
Preparation and provision of certificates of attendance including individual PT reports (restricted to participants)	PT Coordinator, Manager & Scientist in charge and upon need an Agency specialized on statistics and analytical quality assurance
Signature on certificates of attendance and performance, if required	PT Coordinator

* if for - complex statistical analysis, an external company is consulted, their analysis are not part of the PT provider quality management system (DIN EN ISO/IEC 17043)

5 Operating procedure

5.1 Information quality management system

For the quality management system of PTs, the most relevant guideline to follow is the DIN EN ISO/IEC 17043 on “Conformity assessment — General requirements for proficiency testing”, next to other directives (DIN EN ISO/IEC 17025 and DIN EN ISO/IEC 15189). Procedures used for the organization, performance and preparation of test items are standardized and in accordance with respective directives, controlled through the performance of internal audits and reviewed periodically by an external regulation body: DAkkS (Deutsche Akkreditierungsstelle GmbH). Activities defined in the quality management systems include quality planning, quality control, quality assurance and continuous efforts for further improvement.

5.2 Overall task of the PT

The tasks of the PT can vary dependent of the overall situations and requirements. PT could be provided for preparedness of laboratories or in outbreak situations for evaluation of specific approaches regarding the outbreak causing agent in order to ensure and improve the responsiveness of laboratories.

The most common approach will be PTs for preparedness. The overall task of the exercises consists in the identification of target bacteria that are spiked into the PT samples and to rule out targets in PT samples that are spiked with non-targets and/or in blank samples. For the identification, the participants are asked to use diagnostic procedures available in their laboratories if not otherwise agreed. When providing the results, the participants are asked to provide information about the identified target and about the method used for the identification. Depending on the requirements and description of the PT, qualitative and if needed quantitative results could be expected.

In concordance with the PT description and if not otherwise planned, the participants could be asked to provide for samples with viable targets (e.g. living test items) preliminary results equal to rapid diagnostics due to the reason that some of the HPB are very slow growing and specific measures must be taken promptly in case of a positive result. These time critical results need to be confirmed by final findings. For the identification of the viable targets in samples a time period of two weeks is typically given.

For samples with inactivated targets (e.g. inactivated test items), preliminary results are usually not required, final results need to be usually provided only. In concordance with the PT description the participants can be asked to provide additional quantitative information about the test items. For the analysis of these inactivated samples 3 to 4 weeks are typically given.

Optional, the PT can also include serological testing. Specific antibodies against defined targets should be tested and a quantitative or qualitative result can be expected.

Usually, a time frame of four week is given to participants for completing the PT and for providing final results.

The PT coordinator will finally decide about tasks of the exercises upon needs and requirements.

5.3 Offered PT items

The scope and nature of PT items can vary dependent on the requirements of the PT participants.

Typically, and exemplarily, samples with viable targets or inactivated targets (e.g. living and/or inactivated samples) are offered to participants of the EQAE performed by the PT provider. Each PT is composed of a number (e.g. 5) of living and/or a number (e.g. 5) of inactivated and/or a number (e.g. 5) of serological samples. The test items are labeled respectively. For both, living and inactivated samples: target(s) and/or non-target(s) are spiked into matrices to mimic food, environmental

or clinical samples. Thereby, the term “target” refers typically to the following bacteria species: *Bacillus anthracis*, *Francisella tularensis*, *Yersinia pestis, Burkholderia mallei, Burkholderia pseudomallei*, *Brucella abortus, Brucella melitensis*, *Brucella ovis, Brucella suis, Brucella canis, and Brucella neotomae*. The term “non-target” refers to all bacteria not listed as targets and classified mainly as Risk Group (RG) 2 such as *Escherichia coli* and *Staphylococcus aureus*. The final concentration of target(s) and/or non-target(s) in test items is predetermined to enable downstream application of standard laboratory procedures used to identify and quantify, if so requested, possible targets.

In living samples, target(s) and/or non-target(s) are still viable, infectious and can be cultured under appropriate conditions. Next to culture, the identification of targets inside living samples is also possible by for instance molecular (PCR, real-time PCR, Next-generation sequencing strategies), microscopy and immunological tests (ELISA, IFA). For the analysis of living samples, PT participants need to provide a signed official document / statement outlining their permission to handle RG3 pathogens under appropriate biosafety and biosecurity conditions in concordance with national and/or international regulations (e.g. EU, WHO).

In inactivated samples, target(s) and/or non-target(s) are inactivated for example by heat. Hence, the pathogen is uncultivable and the bacteria are not infectious any longer. Inactivation is performed and proven to best of knowledge and technical practices. For sterility testing, at least 10% of the bulk sample volume is checked by plating on appropriate culture media and/or in liquid broth media, and when no growing bacterium after appropriate incubation time and conditions is detected, the sterility of the sample is confirmed. The extrapolation of the result to the whole bulk sample is state of the art. Pathogens inside inactivated test items can be identified by different means including molecular biological tests (PCR, real-time PCR, Next-generation sequencing strategies) and immunological tests (ELISA, IFA).

The PT participant is entitled to ask either only to receive living or inactivated PT items, otherwise the entire sample set will be provided.

In addition, when agreed in advance, the detection of specific body fluid, usually serum antibodies against pathogens (serology) can be included in the PT. Therefore, original positive sera from patients or animals or pre-diluted sera typically diluted in negative human AB serum are provided. The sera should be handled by participants in accordance with their biosafety regulations for unknown clinical sera. Antibodies can be identified by the application of immunological tests (e.g. ELISA, Western blot, IFA).

5.4 PT scheme

The PT scheme includes following phases: Preparation of the PT, pre-analytical steps, analytical steps, and post-analytical steps. The overall PT scheme is briefly outlined below.

Preparation of the PT:

Definition of PT tasks > Development of the PT description > Announcement of the PT > Selection or confirmation, respectively, of participants > Dependent on the situation agreeing of the PT content and approach > Production and quality assurance of PT item(s); arrangement of sample shipment under appropriate conditions; If required: arrangement of export and import licenses, evaluation of participant´s permissions for handling of biological agents, exchange of MTA and end-user certificates, agreement on cost coverage.

Pre-analytical steps of the PT:

> Timely notice to participants that the items will be sent out, including PT instructions > Distribution of appropriate test item(s) to participants, including printed PT instructions

Analytical steps of the PT:

> Sample analyses by participants, typically in-process control by PT provider

Post-analytical steps of the PT:

> Transmission of result(s) and method information from participants to the provider **>** Circulation of PT item decoding > Determination of assigned value(s) by the PT provider and acceptable range of results depending on the consensus mean calculated from the results provided by all participants > Reviewing of result(s) and comparison of individual results with the overall performance of all participants (assessment of data) > Production of individual reports, including recommendations for improvement > Distribution of certificates of participation and reports > Possibility for participants to express objections against the evaluation and in case of acceptance by the PT provider corrections of certificates > Confirmation about the disposal of the remaining sample material if not otherwise agreed by the participants to the PT provider > Face-to-face meetings might be a further tool for analyzing diagnostic approaches, usually in framework of funded projects

5.5 PT schedule

PTs can be performed on a regular basis and dependent on the requirements of a specific project, typically once a year. After successful registration, participants of the PT will be informed about the exact date and deadlines of the exercise. In the event that the schedule may change, all participants will be informed at least 1 month in advance.

After test item reception, typically results for living test items should be provided within the first two weeks and results for inactivated test items are due after three or four weeks. Certificates including the report of the PT, individual test results, and recommendations for improvement are delivered by the PT provider latest three months later. Participant complaint is possible up to four weeks after delivery of the certificates and corrections when acceptable will be done in-between another two weeks.

5.6 Shipment of PT items

The PT provider and an external shipping agency seek to ensure a rapid and safe transportation of the PT items to the PT participants. The transportation time for test items should – in an ideal case - be limited to 96 hours. A sample reception during the weekend and on holidays will be avoided as far as possible.

Living test items are shipped according to IATA regulations for category A substances (UN2814) following the packing instruction PI620. As living test items contain bacteria that are still viable, the test items are shipped within security sealed and thermally stable boxes that are refrigerated (2-8°C). Temperature variations during the shipment are registered using temperature loggers. In case temperature has not been kept in the given range, the participant is requested to inform the provider. Dependent of a quality risk assessment, a second shipment should be conducted if not otherwise agreed.

Inactivated items or serological items are shipped according to IATA regulations for category B substances (UN3373) following the packing instruction PI650, PI650 light or as ”Exempt Human Specimen”.
In case of international PT export and import regulations have to be considered.

5.7 Instruction for PT item storage

All test items, regardless of their nature, are meant to be stored at 2-8°C. The stability of test items is guaranteed by the PT provider usually for a time period of 2 to 3 weeks for living test items and for 3 to 4 weeks for inactivated test items after reception if not otherwise reported. The stability has been tested in advance by the PT provider considering the typical period of time for preparation and shipment. Thereby, the PT provider does not take any responsibilities for possible degradation of test items if not stored in accordance with recommendations made.

5.8 Instruction for PT item disposal

The disposal of infectious and non-infectious test items should be performed after autoclave passage following standard procedures in accordance with state-of-the-art science and technology. The PT provider does not accept responsibilities for possible damage. If not otherwise agreed, all PT items have to be destroyed after the PT. This must be documented and information must be sent to the provider.

5.9 PT application

For the initial participation in the PTs, it is necessary to register 4 months in advance, if not otherwise agreed. The registration has to be filled out and sent in two copies to the PT provider by email or mail. The successful registration will be confirmed and the potential participant will be asked to provide all required documents (please see section 5.9) to finalize the application. The application will be reviewed by the PT provider who decides upon the participation. Once an institution/laboratory is admitted to participate in a PT, an update of all documents is required triennial.
Please note, special export and import licenses are required for PT participants outside the European Union. In addition, individual regulations in EU Member States have to be considered.

5.10 General prerequisites for participation

The institutions/laboratories who intend to participate in the PT need to meet criteria that are listed below and to provide relevant official documents confirming these criteria:

- Contact data of two persons responsible for the PT (Name of institution/laboratory, responsible persons, shipping address (street, country)) – English language skills are mandatory
- Official permission of the institution and/or the responsible researcher to handle bacterial agents classified as risk group 3, in case of receiving living bacterial specimens
- Functional BSL 3 containment, in case of receiving living bacterial specimens
- Arrangement of dangerous goods (RG 3 pathogens) import, in case of receiving living bacterial specimen
- Arrangement of Nagoya protocol regulations if applicable
- Material transfer agreements (MTAs) provided by the PT provider needs to be signed by both parties in advance
- For the analysis of living test items: Completion of Integrated European Checklist for Laboratory Biorisk Management in Handling of High Consequence Risk Group 3 and 4 Agents (ECL-Biorisk**,** http://www.emerge.rki.eu/Emerge/EN/Content/Topics/Rules/ECL_Biorisk.html)
- Registration form for the PT including the confirmation for taking over the responsibility for all import and custom regulations

The export of PT items is primarily arranged by the PT-provider in close cooperation with participants if required. The import of PT items is to be arranged by PT participants. The PT-provider offers support to participants.

If a laboratory does not fulfil all requirements listed above or the export/import of PT samples cannot be arranged, the PT provider decides about the participation either the participant cannot participate in the exercises or if decided only a part of the sample batch is offered as a compromise.

5.11 Information about fees

The PT might be conducted in framework of funded projects. In this case the regulations of the grant agreement will be applied. In case of not funded projects, PT might be charged to the participants according to the German administrative regulations, in principle participants can be charged for the provision of samples, analyses of results including reports, and shipment.

5.12 Follow up and complaints

For further questions, follow ups as for complaints the PT Coordinator can be contacted. Working hours are from Monday to Friday, 8.30h to 17.30h (UTC+01:00, Central European Time).

Contact persons:

Dr. Daniela Jacob (Coordinator)
Phone: +49 (0)30 - 18754-2934
Email: JacobD@rki.de

PD Dr. Holger Scholz (Co-coordinator)
Phone: +49 (0)30 - 18754-2100
Email: ScholzH@rki.de

Additional staff members can be contacted only upon agreements.

5.13 Objection procedure

All PT participants have the possibility to express objections against the evaluation of results within four weeks after receipt. In case of objections are accepted, the PT provider will perform corrections of certificates, reports within two weeks. Revisions are highlighted in the updated version of the certificate, reports. For objections the PT coordinator has to be contacted.

5.14 Declaration of confidentiality

The PT provider will keep confidential any data, documents or other material (in any form) that is identified as confidential at the time it is disclosed. The confidentiality obligations no longer apply if (a) the disclosing party (PT participant) agrees to release the other party (PT provider) (b) information becomes generally and publicly available without breaching any confidentiality obligation (c) the disclosure of the confidential information is required by national or international law. The disclosure can be agreed for partial or anonymized information or as otherwise agreed.

6 Quality control

The preparation of test items is performed in accordance with the quality assurance system at the RKI, ZBS 2. All samples are tested for achievability of expected results (diagnostic reference). Therefore, for diagnostic reference and stability testing of living and inactivated PT items validated assays are used only. For the stability testing of serological PT items assays specific for antibody detection of *Bacillus anthracis* and *Francisella tularensis* are validated. In the event non-validated assays are used as reference methods or for stability testing, PT participants are informed.

6.1 Internal quality control

As internal quality controls, negative and positive controls previously validated are introduced in all applied diagnostic assays. Growth media for bacteria are pre-tested with reference bacterial cultures. Internal extraction and amplification controls are used for validation of DNA based methods. Characterized positive and negative sera are used as controls for serological assays. As for the test items, the preparation as also pre- and post-shipping tests are performed and documented. The pre- and post-shipping tests are also meant to ensure homogeneity and stability of PT items by evaluating of appropriate spot sample checks.

6.2 External quality control

External quality controls are carried out through the participation in national or international PTs or inter-laboratory comparisons, if available.

7 Safety requirements

7.1 Safety and security instructions to follow when handling PT items

In case of living or otherwise security sensitive samples, the provider will check the biosafety and biosecurity conditions present in working environments of participants, e.g. by applying the Integrated European Checklist for Laboratory Biorisk Management (ECL-Biorisk)

(http://www.emerge.rki.eu/Emerge/EN/Content/Topics/Rules/ECL_Biorisk.html) or other relevant approaches for evaluation. The participants are fully responsible for providing relevant information and the provider will assess and confirm or not confirm on this basis if the participation in the PT or part of it is possible. A written, signed official document confirming the permission of the participant to handle RG 3 pathogens under appropriate biosafety and biosecurity conditions in concordance with national and/or international regulations (e.g. EU, WHO) must be provided.

The participants will be instructed in writing that highly pathogenic bacteria are to be handled under BSL3 conditions. Often, living samples are provided in liquid matrices; they need to be handled with care. The inner part of the lid screw might be covered with droplets and short spinning down of the samples is recommended.

The provider (ZBS2, RKI) will not take over any guarantee or assurance for handling, further transportation or storage of these PT samples. It is the only responsibility of the recipient to take care of all national and international regulations for handling, storage and further transportation. Moreover, protective measures to ensure the health of lab workers and to avoid contamination of the environment must be guaranteed by the recipient/participant. The recipient/participant is not allowed to pass these samples to any third party if not otherwise agreed with the provider.

8 Literature

Grunow R, Ippolito G, Jacob D, Sauer U, Rohleder A, Di Caro A, and Iacovino R. 2014. Benefits of a European project on diagnostics of highly pathogenic agents and assessment of potential "dual use" issues. Front Public Health 2:199.

9 Supporting documents

All supporting documents including standard operational procedures and process instruction are up to date and available in ZBS2, RKI. These documents can be viewed if required, upon consultation with the Quality management representative of the RKI (QMB-OL) and the Quality management officer of ZBS2 (QMB-OE).

10 Attachments

- Application Form PT HBP (HPB_ZBS2-PT_Services_FLT-Ax01_AppliForm).

The Application Form is made available by the PT provider to participants in advance of a PT.

11 Short instruction

None.

12 Signatures

Read and trained:

Date	Name	Signature