PT-10: "Molecular identification of *Plasmodium* species"

Instructions

PT (Proficiency Testing) ITEMS

Description. Each PT item consists of a 1.5 ml tube containing DNA from different *Plasmodium* species and a negative item. The PT panel consists of four tubes either containing DNA from different *Plasmodium* species or containing DNA from a non-*Plasmodium* species (negative item).

Production. DNA samples are extracted from infected patients' blood using a commercial kit. For the negative item, DNA extracted from organisms other than *Plasmodium* spp. is used.

Distribution. An aliquot (10 µL) of each DNA, either from Plasmodium species, or a non-Plasmodium species is transferred into each tube. The distribution is made from 5 to 90 days before the shipping date and PT items are stored at a temperature ≤ +8°C until shipment.

Labeling. Each PT item is plugged and sealed with plastic film, labeled with a unique code not attributable to its content.

Homogeneity check. Homogeneity is ensured by providing participants with aliquots of the same DNA preparations.

Packaging. Each PT panel (consisting of four PT items) is then placed in a larger container (e.g. 50ml tube) to ensure that the PT items remain grouped and protected during handling and transport. The larger container is labelled with participant code. The PT panel is placed inside a polystyrene carton, ready for shipment. Several ice packs are arranged in the package to maintain a temperature between 4°C and 15°C inside the package. A sheet indicating the participant code is included inside the package. The participant code changes annually.

Stability and quality control. Based on established literature, DNA remains stable for up to 10 years at a temperature ≤−15°C and up to 6 months if refrigerated (temperature between +4°C and +8°C).
Quality control requires that PT items have been analysed before distribution. An aliquot of each DNA is tested to confirm its identity. Species identification is performed using a Nested PCR method developed and currently undergoing validation/accreditation at the EURL-PH-HP.

SUBMIT A PARTICIPATION REQUEST

The PT provider (EURL-PH-HP) announces the PT via e-mail addressed to potential participants. The email provides information on:

- ✓ PT items shipping date
- ✓ PT scheme scheduled
- ✓ the link to the participation form and the deadline
- ✓ a summary of all deadline dates for the different phases of the PT scheme
- ✓ the link to the PT provider's website for further information on PT scheme.

Participation requests must be submitted by completing the participation form (PT request form) within the due date indicated in the form and in the email.

At the time of PT items shipment, the PT provider sends the link to the on-line form where participants are requested to provide the following information:

- Package content and its condition of preservation.
- Timing of analysis.
- Materials and Methods used to analyze PT items.
- Results.

PT ITEMS STORAGE

- Store PT items refrigerated (temperature between +4°C e +8°C) or frozen (temperature ≤ -15°C) until the analysis is performed.
- Check the packaging and its contents for correctness and completeness: three PT items properly labeled and sealed.
- Record the verification of the packaging upon arrival in the on-line form.
- In case of discrepancy, fill in the claim form available at PTP website: https://www.iss.it/en/phhp-eu-reference-laboratory or contact via e-mail the person in charge of the PT.

PT ITEMS ANALYSIS

1. The code assigned to participant is reported both on a paper sheet contained in the package and on the secondary envelope containing the PT panel.
2. PT items treatment:

- Treat PT items as routine samples.
- Record the codes reported on the larger container (participant code) and on each PT item (PT item identification code).

3. Analysis: each participant is requested to apply any molecular method suitable for the purpose of the scheme, preferably the one used routinely.
4. Results: each participant is requested to indicate the assigned code, the PT item codes and using a free text field, the *Plasmodium* species of each PT item or the negative result. It is not allowed to submit results after the due date.

   Results must be reported on the online form, sent to participants by the PT provider on the day the PT items were shipped. In the on-line form, participant must indicate full reference if a published method is applied and any relevant deviation from the chosen method.
5. Deadlines:
   - PT items should be received within 48-72 hours from shipment. In case of delay, please contact the person in charge of the PT by email.
   - The deadline for results submission is indicated in the annual timetable available on the EURL-PH-HP website (https://www.iss.it/en/phhp-eu-reference-laboratory), in the PT request form and in results form.
   - The individual PT report is sent, as .pdf file, via e-mail to the participant only, according to the annual timetable available online (https://www.iss.it/en/phhp-eu-reference-laboratory).
   - The final PT report is published on the EURL-PH-HP website according to the annual timetable available online (https://www.iss.it/en/phhp-eu-reference-laboratory)
6. PT participation certificate: EURL-PH-HP, as PT Provider, issues a certificate for participants that have completed the PT scheme within the indicated timeframe. The document is sent as .pdf file via e-mail.
7. Perform evaluation: result evaluation is qualitative. Result is considered “correct” if DNAs of the PT item are properly identified at species level or if the negative sample is identified, and “incorrect” in case of wrong identification. Results must be expressed reporting the species assigned to each PT item or the negative identification. Due to the nature of PT items, no statistical parameters are applied for result evaluation. Each result is evaluated against the species assigned by the PT provider. The final evaluation is “positive” if all PT items are correctly identified, otherwise it is “negative”.
8. Report:

   Individual PT report: the individual PT report is sent as .pdf file via e-mail to the participant only. The document includes the following information: i) species expected; ii) species identified by the participant; iii) final evaluation and iv) recommendation based on the participants’ performance. PTP reserves the right to provide, upon specific request, the participant’s results to ECDC or Competent Authorities. If a competent legislative authority requests disclosure of PT results, the participants will be formally informed in writing.

   Final PT Report: the final PT report contains data on design and implementation of the PT scheme and, for each PT item, the result (species assigned by participant), the assigned value (species stated by PTP), the outcome and the final evaluation. In the document there are also reported a summary of results, an overtime comparison of results, comments on participant’s performance and recommendations based on the outcomes of PT round. To guarantee confidentiality, in the final report participants are identified by alphanumeric codes. The Final PT Report is presented and discussed during the annual workshop of laboratories belonging to EVD network and published on the EURL-PH-HP website (https://www.iss.it/en/phhp-eu-reference-laboratory).
9. Follow up: in case of negative result, the PT provider contacts the participant who fails the PT and sends a link to a form where the participant is asked to analyze the causes of failure and indicate the planned or implemented corrective actions.

10. Satisfaction questionnaire: annually PT provider sends, via e-mail, to each participant a survey to monitor the satisfaction of the service and to improve the scheme according to participant's suggestions.
11. Confidentiality: EURL-PH-HP retains the individual and final PT Reports for 10 years. PT provider staff signs a confidential agreement regarding acquired data. In the final PT report, each participant is identified by alphanumeric code. The PT provider reserves the right to provide participant PT results to ECDC, competent authority and accreditation body, upon request. If a competent legislative authority requests disclosure of PT results, the participants will be formally informed in writing.

For any information or problem related to the web site access, please address:

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