Final report PT-10/2026

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

Design

Purpose	Assessment of the competence of participants in molecular identification of Plasmodium species	
Timetable	Invitation mail: 15/06/2026 Website updates: within 15/06/2026 Registration deadline: 31/08/2026 PT items production: 03/09/2026 Shipping: 16/09/2026 Results submission deadline: 02/10/2026 Publication of the Final PT Report: 13/11/2026	
Participants	Operational Contact Points (OCP) and National Focal Points (NFP) of the EVD-Net (European Emerging and Vector-borne Diseases Network) coordinated by European Centre for Disease Prevention and Control (ECDC)	
Number of participants	Depending on requests	
PT items	Matrix	Water
	Item	Genomic DNA
	Number of PT items	4 per each participant
	Panel composition	4 items: *P. falciparum* DNA (x2), *P. vivax* DNA, *T. gondii* DNA
	Number of surplus items	- NA
Activities provided by external providers	Shipping	
Provider name	DHL	
Results evaluation	Qualitative evaluation	

Implementation

PT staff: Anna Olivieri, Silvio Paone, Marianna Ascierto, Eleonora Bello, Francesco Silvestrini.

Compliance with planned timelines: YES X NO ☐

Participant number and type: 19 public institutions

0 private institutions

Acquisition of matrix and analyte: genomic DNA of (1) *P. falciparum*, (2) *P. vivax*, and (3) *T. gondii* are provided respectively by: (1, 2) Vector-borne Diseases and Emerging Viral Zoonoses Unit, Istituto Superiore di Sanità, (3) Foodborne and Neglected Parasitic Diseases Unit, Istituto Superiore di Sanità.

Production of PT items: DNA samples were extracted using the commercial kit QIAamp DNA mini kit (QIAGEN) from clinical samples previously diagnosed as positive to *P. falciparum* and *P. vivax* (1,2) and *T. gondii* tachyzoites cultivated *in vitro* (3). Two aliquots of *P. falciparum* DNA (10 µL each), one aliquot of *P. vivax* DNA

Proficiency Testing Provider
Unit of Foodborne and Neglected Parasitic Diseases
Director: Dr. Adriano Casulli (adriano.casulli@iss.it)
e-mail: eurl-ph-hp@iss.it

Person in charge of PT
Dr. Anna Olivieri
e-mail: anna.olivieri@iss.it
tel: +39 0649903794

Istituto Superiore di Sanità, Viale Regina Elena, 299 – 00161 Rome, Italy

(10 µL), and one aliquot (10 µL) of *T. gondii* DNA (10 µL) were transferred into separate tubes and sealed with parafilm.

Activities provided by external providers: Shipment provided by DHL.

Homogeneity and stability of PT items: PT items corresponding to the same species were prepared as aliquots from a single master DNA preparation to ensure homogeneity. Based on established literature, DNA remains stable for up to 10 years at a temperature ≤−15°C and up to six months if refrigerated (temperature between +4°C and +8°C). PT items were stored at ≤8°C until shipment and distributed under refrigerated conditions.

Distribution of PT items: PT items were shipped on 16/09/2026, and the deadline for submission of results was set for 02/10/2026. Each set of PT items consisted of a 50 ml vial marked with the participant's identification code and containing four 1.5 ml vials identified with the codes assigned by the PT provider (PTP) to the PT items. Packaging consisted of a polystyrene and cardboard container with enough refrigerant packs to ensure a temperature between 4 °C and 15 °C.

Instructions for participants: participants were informed of the shipment via email on 16/09/2026. The email also contained a link for submitting results, which was active from 16/09/2026 (coinciding with shipment). Instructions for participants were made available on the PTP website starting from 15/06/2026. These instructions also included procedures for submitting feedback information and results.

Data analysis: feedback information and participants' results were collected via the web app and transferred from the PTP to an Excel file used for data processing and further used to generate the tables with the participant results contained in this PT report.

Assigned value: An aliquot of each *Plasmodium* DNA preparation was tested, using a Nested PCR method developed and currently undergoing validation/accreditation at the EURL-PH-HP, to confirm species identity. An aliquot of the *T. gondii* DNA preparation was tested to confirm the absence of *Plasmodium* identification.

Criteria for results evaluation: The result for each PT item was considered "correct" if the participant correctly identified the *Plasmodium* species or correctly reported no *Plasmodium* species in the PT item containing *T. gondii* DNA. A result is considered "incorrect" if the *Plasmodium* species was incorrectly identified or unidentified, or a *Plasmodium* species was reported in the PT item containing *T. gondii* DNA.

Confidentiality of results: the confidentiality of the data contained in this report is ensured using a unique code guaranteeing participant anonymity. The identity of participants in a PT scheme is kept confidential and subject to official secrecy. The PT provider reserves the right to provide participant PT results to competent authority and ECDC, upon request. Participants will be notified in writing if a competent legislative authority requests access to their PT results.

Results provided by participants and performance EVALUATION

Participant code	Applied method	Item code	Assigned value	Result	Outcome	Final evaluation
MAL26_1	Real-time PCR	MAL01	P. falciparum	P. falciparum	correct	positive
		MAL02	P. vivax	P. vivax	correct	
		MAL03	Negative	Negative	correct	
		MAL04	P. falciparum	P. falciparum	correct	
MAL26_2	PCR	MAL05	P. falciparum	P. falciparum	correct	negative
		MAL06	P. vivax	P. ovale	incorrect	
		MAL07	Negative	Negative	correct	
		MAL08	P. falciparum	P. falciparum	correct	
MAL26_3	Real-time PCR	MAL09	P. falciparum	P. falciparum	correct	positive
		MAL10	P. vivax	P. vivax	correct	
		MAL11	Negative	Negative	correct	
		MAL12	P. falciparum	P. falciparum	correct	

Proficiency Testing Provider
Unit of Foodborne and Neglected Parasitic Diseases
Director: Dr. Adriano Casulli (adriano.casulli@iss.it)
e-mail: eurl-ph-hp@iss.it

Person in charge of PT
Dr. Anna Olivieri
e-mail: anna.olivieri@iss.it
tel: +39 0649903794

Istituto Superiore di Sanità, Viale Regina Elena, 299 – 00161 Rome, Italy

Participant code	Applied method	Item code	Assigned value	Result	Outcome	Final evaluation
MAL26_4	Real-time PCR	MAL13	P. falciparum	P. falciparum	correct	positive
		MAL14	P. vivax	P. vivax	correct	
		MAL15	Negative	Negative	correct	
		MAL16	P. falciparum	P. falciparum	correct	
MAL26_5	PCR+ Sequencing	MAL17	P. falciparum	P. falciparum	correct	negative
		MAL18	P. vivax	P. vivax	correct	
		MAL19	Negative	Plasmodium spp	incorrect	
		MAL20	P. falciparum	P. falciparum	correct	
MAL26_6	Real-time PCR	MAL21	P. falciparum	P. falciparum	correct	positive
		MAL22	P. vivax	P. vivax	correct	
		MAL23	Negative	Negative	correct	
		MAL24	P. falciparum	P. falciparum	correct	
MAL26_7	FRET Real-time PCR	MAL25	P. falciparum	P. falciparum	correct	positive
		MAL26	P. vivax	P. vivax	correct	
		MAL27	Negative	Negative	correct	
		MAL28	P. falciparum	P. falciparum	correct	
MAL26_8	Real-time PCR	MAL29	P. falciparum	P. falciparum	correct	positive
		MAL30	P. vivax	P. vivax	correct	
		MAL31	Negative	Negative	correct	
		MAL32	P. falciparum	P. falciparum	correct	
MAL26_9	Real-time PCR	MAL33	P. falciparum	Plasmodium spp	incorrect	negative
		MAL34	P. vivax	Plasmodium spp	incorrect	
		MAL35	Negative	Negative	correct	
		MAL36	P. falciparum	Plasmodium spp	incorrect	
MAL26_10	Real-time PCR	MAL37	P. falciparum	P. falciparum	correct	positive
		MAL38	P. vivax	P. vivax	correct	
		MAL39	Negative	Negative	correct	
		MAL40	P. falciparum	P. falciparum	correct	
MAL26_11	Real-time PCR	MAL41	P. falciparum	P. falciparum	correct	positive
		MAL42	P. vivax	P. vivax	correct	
		MAL43	Negative	Negative	correct	
		MAL44	P. falciparum	P. falciparum	correct	
MAL26_12	PCR	MAL45	P. falciparum	P. falciparum	correct	positive
		MAL46	P. vivax	P. vivax	correct	
		MAL47	Negative	Negative	correct	
		MAL48	P. falciparum	P. falciparum	correct	
MAL26_13	Real-time PCR	MAL49	P. falciparum	P. falciparum	correct	positive
		MAL50	P. vivax	P. vivax	correct	
		MAL51	Negative	Negative	correct	
		MAL52	P. falciparum	P. falciparum	correct	
MAL26_14	PCR	MAL53	P. falciparum	P. falciparum	correct	positive
		MAL54	P. vivax	P. vivax	correct	
		MAL55	Negative	Negative	correct	
		MAL56	P. falciparum	P. falciparum	correct	
MAL26_15	Real-time PCR	MAL57	P. falciparum	P. falciparum	correct	positive
		MAL58	P. vivax	P. vivax	correct	
		MAL59	Negative	Negative	correct	
		MAL60	P. falciparum	P. falciparum	correct	
MAL26_16	FRET Real-time PCR	MAL61	P. falciparum	Plasmodium spp	incorrect	negative
		MAL62	P. vivax	Plasmodium spp	incorrect	
		MAL63	Negative	Plasmodium spp (weak)	incorrect	
		MAL64	P. falciparum	Plasmodium spp	incorrect	

Proficiency Testing Provider
Unit of Foodborne and Neglected Parasitic Diseases
Director: Dr. Adriano Casulli (adriano.casulli@iss.it)
e-mail: eurl-ph-hp@iss.it

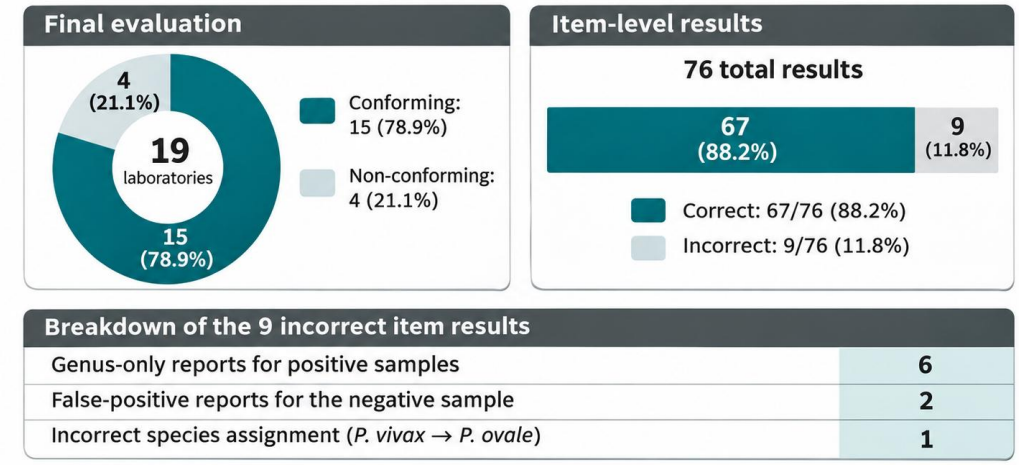
Person in charge of PT
Dr. Anna Olivieri
e-mail: anna.olivieri@iss.it
tel: +39 0649903794

Istituto Superiore di Sanità, Viale Regina Elena, 299 – 00161 Rome, Italy

Participant code	Applied method	Item code	Assigned value	Result	Outcome	Final evaluation
MAL26_17	Real-time PCR	MAL65	P. falciparum	P. falciparum	correct	positive
		MAL66	P. vivax	P. vivax	correct	
		MAL67	Negative	Negative	correct	
		MAL68	P. falciparum	P. falciparum	correct	
MAL26_18	Real-time PCR	MAL69	P. falciparum	P. falciparum	correct	positive
		MAL70	P. vivax	P. vivax	correct	
		MAL71	Negative	Negative	correct	
		MAL72	P. falciparum	P. falciparum	correct	
MAL26_19	Real-time PCR	MAL73	P. falciparum	P. falciparum	correct	positive
		MAL74	P. vivax	P. vivax	correct	
		MAL75	Negative	Negative	correct	
		MAL76	P. falciparum	P. falciparum	correct	

Incorrect items and laboratories that failed the PT are marked in red.

Summary of results:



Total number of PT panels	19
Number of participant	19
Number of participants that passed the PT	15
Number of participants that failed the PT	4
Total number of items	76
Number of correct items	67
Number of incorrect items	9

Proficiency Testing Provider
Unit of Foodborne and Neglected Parasitic Diseases
Director: Dr. Adriano Casulli (adriano.casulli@iss.it)
e-mail: eurl-ph-hp@iss.it

Person in charge of PT
Dr. Anna Olivieri
e-mail: anna.olivieri@iss.it
tel: +39 0649903794

Istituto Superiore di Sanità, Viale Regina Elena, 299 – 00161 Rome, Italy

Comments on participants' performance:

Overall, 15 of 19 participating laboratories (78.9%) achieved a satisfactory PT outcome, correctly identifying all four items. At the individual item level, 67 of 76 results (88.2%) met the acceptance criteria.

Four laboratories reported at least one non-conforming result. The nine non-conforming results comprised six genus-level identifications without species discrimination, two false-positive results for the negative item, and one incorrect species identification (*P. vivax* reported as *P. ovale*).

Importantly, genus-only results correctly indicated the presence of *Plasmodium* DNA but did not meet the PT requirement for species-level identification. Therefore, non-conformity did not necessarily indicate an analytical failure.

Performance evaluation if different methods are applied:

Participating laboratories used a wide variety of molecular diagnostic methods, including in-house assays and commercial kits, based on different amplification approaches and molecular targets.

Ten laboratories used in-house methods and nine used commercial assays. Of the four non-conforming laboratories, three used in-house methods. The fourth used a commercial genus-only assay, which correctly detected *Plasmodium* DNA but could not identify individual species.

All three genuinely incorrect analytical results (two false positives and one species misidentification) were obtained using in-house methods. No commercial assay produced an incorrect analytical result.

However, the heterogeneity of the assays does not allow reliable conclusions about the relative performance of different diagnostic methods.

Comments and recommendations based on the outcomes of PT:

Overall, participants demonstrated satisfactory performance, with 15 of 19 laboratories achieving a positive evaluation. The nine incorrect results can be grouped into three categories, each highlighting different potential issues.

Incomplete species identification (6 results)
Two laboratories reported *Plasmodium* at genus level without identifying the species. One laboratory used a commercial kit designed for genus-level detection only, while the other reported that the limited DNA volume allowed only genus-level testing. As species-level identification was required by the PT, laboratories are recommended to use species-specific assays. It should be noted that the DNA amount provided was sufficient for ten reactions by nested PCR method. Where compatible with the analytical method, dilution of the supplied DNA may be considered to obtain the volume required to complete species-specific testing. Only one laboratory reported to have diluted the supplied DNA.

False-positive results (2 results)

Two laboratories reported *Plasmodium*-positive results for the negative item containing *Toxoplasma gondii* DNA. Non-specific amplification due to sequence similarity between the 18S rRNA genes of *T. gondii* and *Plasmodium* represents a plausible explanation, although DNA contamination cannot be excluded. Participants are advised to verify the analytical specificity of their assays and review procedures aimed at preventing DNA contamination.

Incorrect species identification (1 result)
One laboratory identified *P. vivax* as *P. ovale* using a nested multiplex PCR targeting 18S rRNA, in which species identification relies on amplicon size. Possible causes include non-specific amplification, DNA

Proficiency Testing Provider
Unit of Foodborne and Neglected Parasitic Diseases
Director: Dr. Adriano Casulli (adriano.casulli@iss.it)
e-mail: eurl-ph-hp@iss.it

Person in charge of PT
Dr. Anna Olivieri
e-mail: anna.olivieri@iss.it
tel: +39 0649903794

Istituto Superiore di Sanità, Viale Regina Elena, 299 – 00161 Rome, Italy

contamination or incorrect interpretation of electrophoretic results. The laboratory is recommended to review the electrophoretic profile and repeat the analysis with appropriate controls.

In addition to the specific issues identified in this PT, other potential sources of error in molecular *Plasmodium* identification should be considered, as outlined below.

A failure to identify PT items may be due to:

- Inappropriate storage conditions or temperatures of the DNA items.
- Errors or inappropriate modifications introduced during PCR amplification procedures.
- Use of unsuitable or expired reagents during PCR amplification steps.

Incorrect species identification may be due to:

- Errors or inappropriate modifications introduced during PCR amplification procedures.
- Accidental exchange or mislabeling of PT items during the analytical process.
- DNA contamination occurred during sample handling or analysis.
- Incorrect interpretation of the results, including electrophoretic banding patterns, real-time PCR amplification data, and sequencing data.

Written and elaborated by
PTP person in charge

Dr Anna Olivieri

Verified and issued by
The Director

Dr. A. Casulli

Date 06/11/2026

Notes:

1. Personal data are processed in compliance with the regulatory provisions referred to in EU Regulation 2016/679 and Privacy Code, as reported in Legislative Decree no. 101/2018. The data controller of personal data is the Istituto Superiore di Sanità with registered office in Viale Regina Elena n. 299 - 00161 Rome, in the person of its President. In addition, the ISS has appointed its own Data Protection Officer (D.P.O.), e-mail address: responsabile.protezionedati@iss.it. Data are processed exclusively for carrying out the PT activities, for this purpose adequate physical, technical and organizational security measures have been set up to prevent and avoid their destruction and/or loss of integrity, as well as their illicit or incorrect use. Data is accessible only to authorized personnel who have their own credentials and their own operating station. The participant has the rights referred to in art. 15 GDPR et seq., more precisely right of access, right of rectification, right of treatment limitation, right to data portability, right of opposition, as well as the right to lodge a complaint with the Guarantor Authority (art. 77 GDPR and 141 Privacy Code, as reported by Legislative Decree 101/2018). The ISS, in its capacity as Data Controller, undertakes to keep the records of processing activities correctly pursuant to art. 30 GDPR.
2. The original raw data and a copy of Final PT Report are kept for 10 years at the PTP site.

End of the report

Proficiency Testing Provider
Unit of Foodborne and Neglected Parasitic Diseases
Director: Dr. Adriano Casulli (adriano.casulli@iss.it)
e-mail: eurl-ph-hp@iss.it

Person in charge of PT
Dr. Anna Olivieri
e-mail: anna.olivieri@iss.it
tel: +39 0649903794

Istituto Superiore di Sanità, Viale Regina Elena, 299 – 00161 Rome, Italy