WHO
External Quality Assurance Assessment Scheme
Phase 9

INTRODUCTION

The participation of Pharmaceutical Quality Control Laboratories (PQCLs) in appropriate proficiency testing schemes is an internationally recognised requirement[1&2] as this enables the PQCL to demonstrate, monitor and improve the quality of the analytical services provided. Proficiency testing covers the overall performance of a laboratory, evaluating the process from the reception and storage of samples, the experimental work in the laboratory, the interpretation and the transcription of the data and the conclusions to the reporting sheets. Failure at any of these stages also reflects on the competence of the respective laboratory.

In support of PQCLs, the World Health Organization (WHO) offers proficiency testing through its External Quality Assurance Assessment Scheme (EQAAS) which offers a platform for PQCLs to measure their performance through a confidential system of blind testing. Since 2000, the EQAAS is organized by WHO with the assistance of the European Directorate for the Quality of Medicines and HealthCare (EDQM).

This proficiency testing scheme also serves to demonstrate the reliability of laboratory analytical results by objective means; thereby fostering the establishment of mutual confidence/recognition within collaborating networks, promoting work sharing based on reliance, especially in countries with limited or no quality control testing capabilities.

The EQAAS is facilitated in accordance with the International Organization for Standardization and International Electrotechnical Commission (ISO/IEC) standards for proficiency testing (i.e. ISO/IEC 17043:2010). This Scheme has entered its tenth phase period in 2020. Laboratories across WHO's six regions have participated in the past comparative external assessment studies and more than 1 100 studies involving 33 different tests were carried out.

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DESCRIPTION OF EQAAS PHASE 9

During EQAAS Phase 9, laboratories were provided with the opportunity to evaluate their performance with regards to three procedures using mebendazole chewable tablets as a common test sample (as depicted in Figure 1).
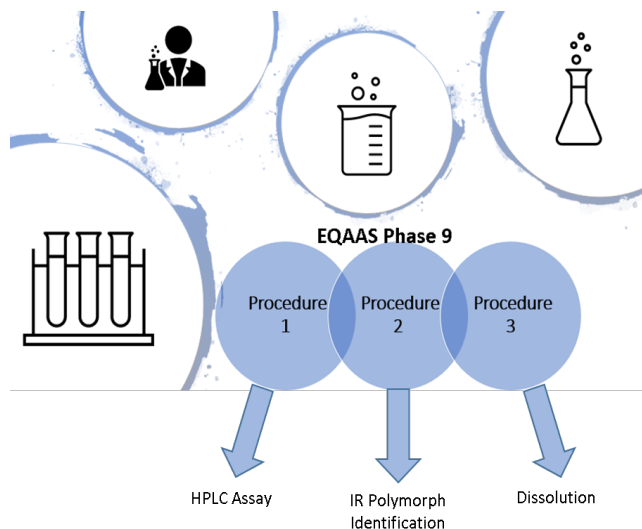

Figure 1: Schematic presentation of analytical procedure bouquet incorporated into EQAAS Phase 9.

- **Procedure 1:** the aim of this procedure was to assess the performance of the laboratory with regards to the determination of the assay by liquid chromatography. Laboratories were requested to determine (in triplicate) the percentage content of mebendazole in mebendazole chewable tables using the liquid chromatography method from the monograph on mebendazole chewable tablets of *The International Pharmacopoeia.*
- **Procedure 2:** the aim of this procedure was to assess the performance of the laboratories with regards to the identification by Infrared Absorption Spectrophotometry. Laboratories were requested to confirm the polymorphic form of mebendazole present in mebendazole chewable tablets through infrared absorption spectrophotometry; and
- **Procedure 3:** the aim of this procedure was to assess the performance of laboratories with regard to the performance of a dissolution test. Laboratories were requested to carry out the dissolution test and to determine the percentage of mebendazole released at 60 minutes from mebendazole chewable tablets, according to the monograph of *The International Pharmacopoeia* published by WHO.

STATISTICAL METHODS

For procedures 1 and 3, the following approaches applied: Different approaches may be adopted to assign the content of the analyte in the samples. The methods commonly applied in the WHO EQAAS operated in accordance with the Proficiency Testing Scheme developed by the EDQM are the use of a theoretical value or the addition of a known quantity of the analyte to the sample ("true" value) confirmed in the feasibility study or the use of a consensus value based on the results from the participants. To determine the consensus value, robust statistics are generally applied (e.g. the median value, mean interquartile range, Huber's robust mean) to avoid the influence of "outliers" on the overall mean.

The target standard deviation is set based on experience, or on the reported or expected precision of techniques, and according to fitness for purpose.

Assigned value

The assigned values used in this study are the consensus values obtained when calculating the Huber's robust mean. Table 1 provides a summary of the consensus values and the values obtained during the feasibility studies.

Table 1: Summary of consensus values and feasibility study values for procedures 1 and 3

	Consensus Value	Feasibility Value
Procedure 1: Mebendazole Assay	99.25%	99.2%
Procedure 3: Mebendazole Dissolution	69.0%	72.8%

Target standard deviation

The target values for the standard deviation (TSD) for procedures 1 and 3 are summarized in Table 2.

Table 2: The target values for the standard deviation (TSD) for procedures 1 and 3

	Target value for TSD
Procedure 1: Mebendazole Assay	0.8%
Procedure 3: Mebendazole Dissolution	3.5%

The target value for the TSD for the assay values took into account the variability between the mean results, calculated at the EDQM on the basis of the individual values reported by the participants.

The uncertainty of the assigned value was found to be negligible compared with the defined TSD and can be ignored in the interpretation of the performance scores.

Scoring

The z-score gives a bias estimate of the result. Absolute z-scores less than 2 are acceptable. A zone of doubtful performance exists for absolute z-scores between 2 and 3. Those do not necessarily have to be unacceptable since there is some uncertainty how close the consensus value is to the true value. An absolute z-score of 3 or more can be interpreted as an unacceptable performance.
Corrective actions should also be triggered when z-scores are frequently in the doubtful zone. For the purposes of this exercise, the calculation of a z-score has then been made for each laboratory according to:

$$z - score = \frac{\bar{x} - \hat{x}}{TSD}$$

Where $\bar{x}$ is the unrounded mean value calculated by EDQM based on the reported results of the individual laboratory,
$\hat{x}$ is the assigned value,

TSD is the target value for the standard deviation.

As a first step, a check for high standard deviations (Cochran's test) and for outlying means (Grubbs' test) was carried out. An outlier is a value that is so unlikely in the light of the overall distribution of results, that it would have an unreasonable impact on the calculation of certain statistics (e.g. the overall mean and the overall standard deviation). These tests do not necessarily detect values that are obviously unacceptable to a trained eye. Standard deviations or relative standard deviations printed on a black background are only to indicate that these values are high compared to the (R)SDs found in other laboratories, but they do not necessarily imply that they are unacceptable.

The purpose of (R)SDs is to provide participants with comparative material so that they can interpret their own data in the light of the performances of other laboratories and draw their own conclusions. It is also important to be aware that the SD for precision is not the same as the SD for accuracy (TSD) on which the z-scores are based.

Since only correct identification of the mebendazole polymorph was requested from the participants (yes / no), for procedure 2, no consensus value or z-score was determined, thus no statistical evaluation of data *sensu stricto* was carried out.

DISCUSSION OF THE RESULTS REPORTED FOR EQAAS PHASE 9

A total of 43 participants participated in Phase 9 of the EQAAS. The tests were well designed and the results obtained were subjected to sound statistical evaluation, as described above.

The z-scores of the participants in procedure 1 are depicted in Figure 2. The black dots indicate the respective z-scores. Thirty-six (36) of the laboratories, which equates to eighty-four per cent of the laboratories, reported satisfactory results ($|z\text{-score}| < 2$). Four (4) laboratories reported doubtful results ($2 < |z\text{-score}| > 3$). Three (3) laboratories reported unsatisfactory results ($|z\text{-score}| \geq 3$). Eight (8) laboratories showed a high variability between the individual results they reported and are therefore found to be outliers for the standard deviation according to Cochran's test. One laboratory expressed the results in mg instead of as a percentage of the declared content, which resulted in the high z-score. If reported in percentage as requested in the protocol, they would have obtained a result of 100.41%.
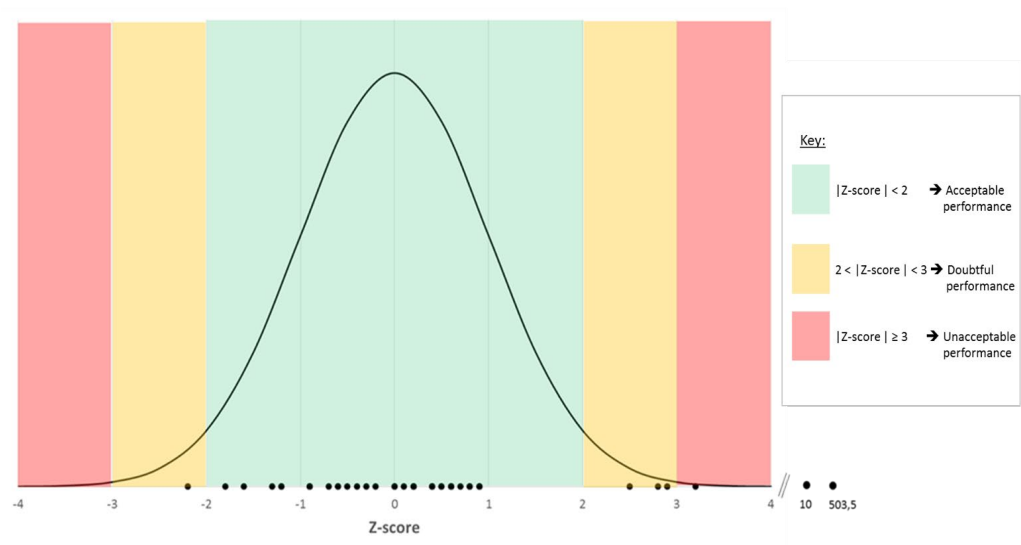
Figure 2: The z-scores of the participants in procedure 1.

The characteristic IR bands used for the identification of mebendazole polymorphs A, B and C are listed in Table 3.

TABLE 3: The characteristic IR stretching frequencies used for the identification of mebendazole polymorphs A, B and C[3]

Form	-NH	>C=O
A	3370 cm^{-1}	1730 cm^{-1}
B	3340 cm^{-1}	1700 cm^{-1}
C	3410 cm^{-1}	1720 cm^{-1}

Figure 3 depicts the changes in the characteristic stretching frequencies at 3370 cm^{-1} and 3410 cm^{-1} of polymorph A and polymorph C respectively in the DRIFT-IR spectra of a commercially available product containing mebendazole[3]. The conversion from polymorph C into the thermodynamically stable polymorph A is clearly detected by the decrease in the intensities of the 3404 cm^{-1} band and increase in the intensities of the 3369 cm^{-1} band.
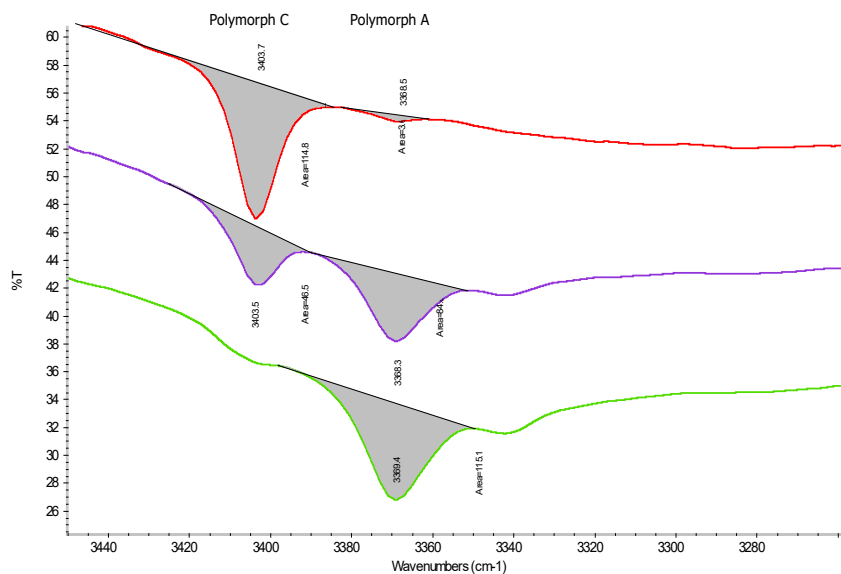
Figure 3: Characteristic stretching frequencies (cm^{-1}) and the areas thereof in the DRIFT-IR spectra of a commercially available product 3 at 0 (top), 3 (middle) and 6 (bottom) months respectively indicating the decreasing polymorph C and increasing polymorph A content[3].

Figure 4 depicts the IR spectra of mebendazole ICRS and the mebendazole extracted from the chewable tablets used during EQAAS Phase 9.
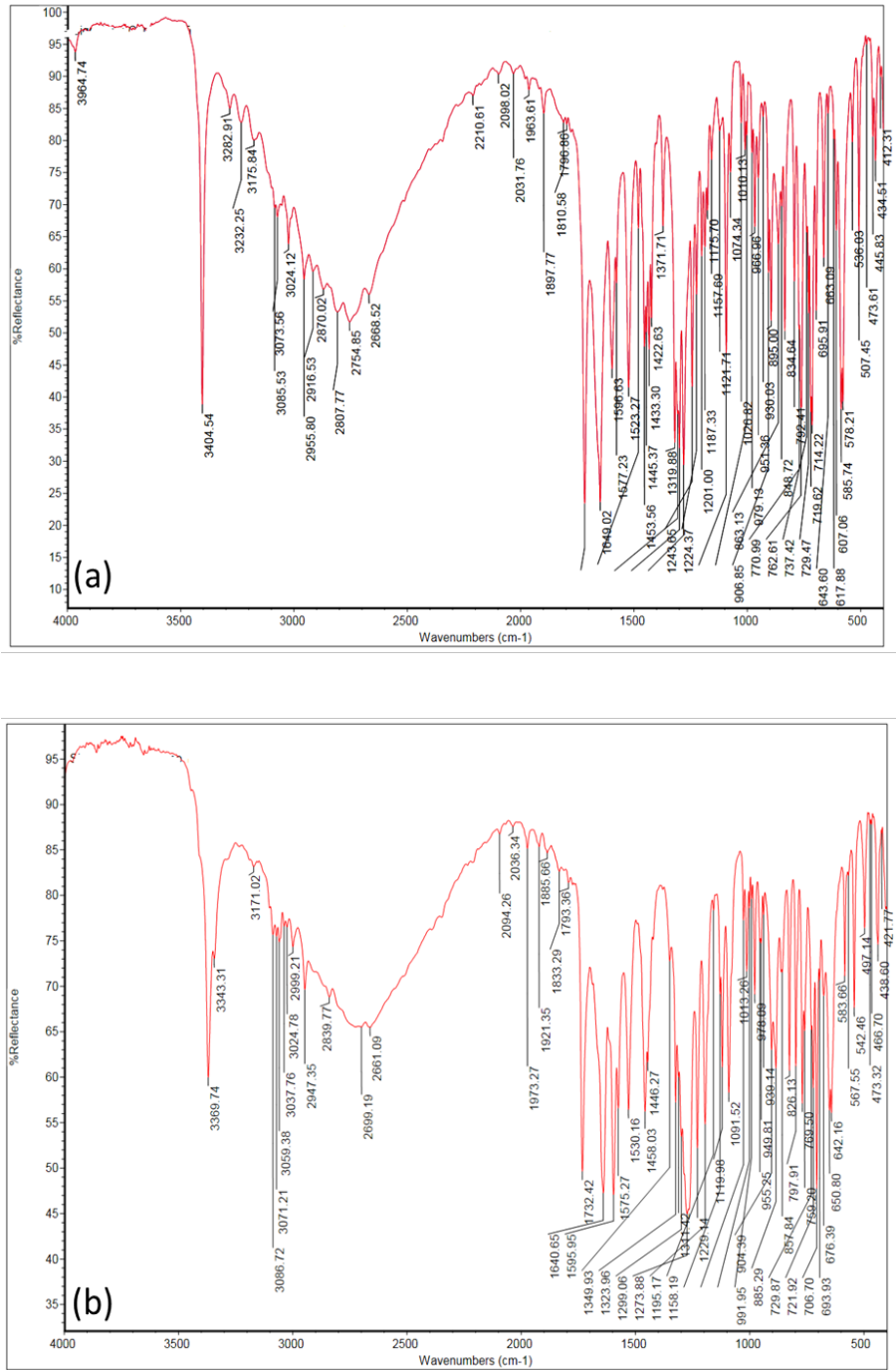
Figure 4: IR spectra of (a) mebendazole ICRS and (b) the mebendazole extracted from the chewable tablets used during EQAAS Phase 9.

The presence of the strong absorption bands at 3404 and 1720 cm^{-1} in the IR spectrum of mebendazole ICRS (Figure 4 (a)) are characteristic of polymorphic form C. From Figure 4 (b), it is clear that the polymorph predominantly present in the chewable tablets was polymorph A due to the presence of the strong absorption bands at 3370 & 1732 cm^{-1}. The outcomes of the results reported by the participants in procedure 2 are depicted in Figure 5.
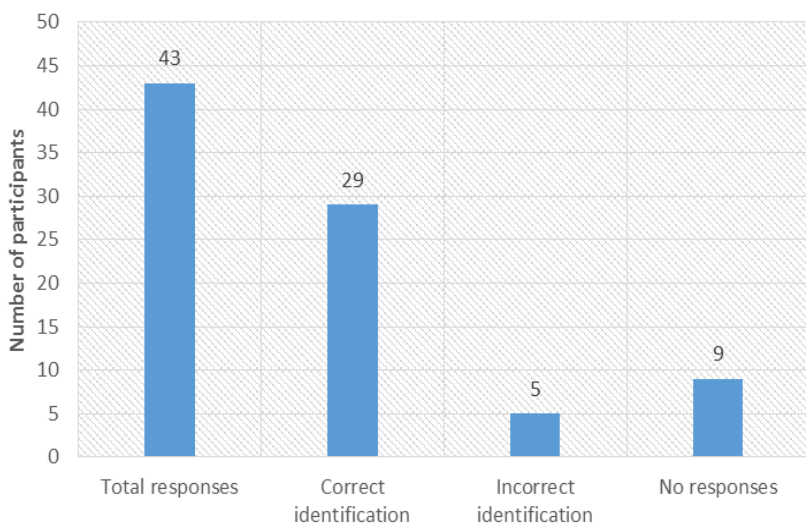

Figure 5:   Summary of responses received for the identification of the predominant mebendazole polymorphic form present in the mebendazole chewable tablets in procedure 2.

Twenty-nine (29) of the laboratories correctly identified that polymorphic form A was the predominant form present in the chewable tablets. Five (5) laboratories reported unsatisfactory results as they indicated that the predominant polymorphic form present was C. Nine (9) laboratories did not report results for this procedure. Six (6) of them explained that the IR equipment was lacking or out of working order.

The z-scores of the participants in procedure 3 are depicted in Figure 6. The black dots indicate the respective z-scores. Thirty-eight (38) of the forty-three (43) participants submitted results for procedure 3.

Thirty-one (31) of the laboratories, which equates to seventy-two (72) per cent of the laboratories, reported satisfactory results (|z-score| < 2). Seven (7) laboratories reported unsatisfactory results (|z-score| ≥ 3). Four (4) laboratories showed a high variability between the individual results they reported and are therefore found to be outliers for the standard deviation according to Cochran's test. Five (5) laboratories did not report any results for this procedure.
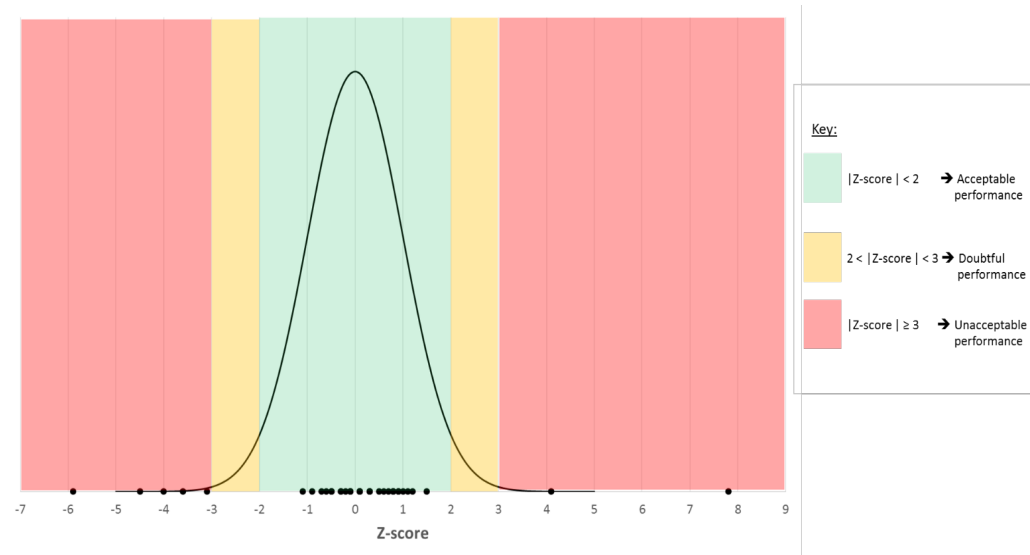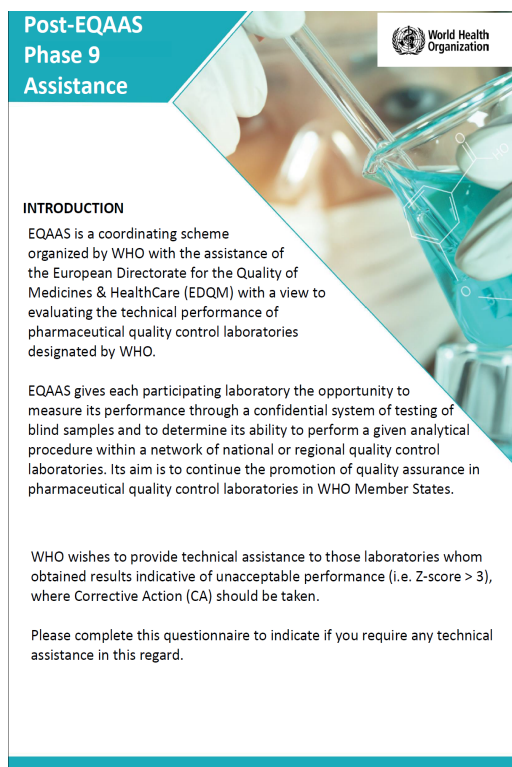

Figure 6: The z-scores of the participants in procedure 3.

POST-EQAAS PHASE 9 ASSISTANCE PROGRAM

Laboratories that produced acceptable results were encouraged to use the EQAAS as a stimulus for continuous improvement, whilst those laboratories that reported unacceptable results were requested to investigate their procedures. These laboratories are subject to a root cause investigation, the results of which they are invited to share and use as the basis for corrective and preventive action plans and targeted training, as and where necessary.
To assist such laboratories, WHO invited them to participate in a Post-EQAAS Phase 9 Assistance Program (PEP-9-AP). Participation in this PEP-9-AP was voluntary and free of cost – Figure 7.

Post-EQAAS Phase 9 Assistance

World Health Organization

INTRODUCTION

EQAAS is a coordinating scheme organized by WHO with the assistance of the European Directorate for the Quality of Medicines & HealthCare (EDQM) with a view to evaluating the technical performance of pharmaceutical quality control laboratories designated by WHO.

EQAAS gives each participating laboratory the opportunity to measure its performance through a confidential system of testing of blind samples and to determine its ability to perform a given analytical procedure within a network of national or regional quality control laboratories. Its aim is to continue the promotion of quality assurance in pharmaceutical quality control laboratories in WHO Member States.

WHO wishes to provide technical assistance to those laboratories whom obtained results indicative of unacceptable performance (i.e. Z-score > 3), where Corrective Action (CA) should be taken.

Please complete this questionnaire to indicate if you require any technical assistance in this regard.

Figure 7: Post-EQAAS Phase 9 Assistance Program (PEP-9-AP) flyer.

Laboratories from four (4) different countries responded to the invitation, and three (3) expressed interest in the PEP-9-AP. The PEP-9-AP consisted of four (4) parts, as depicted in the following flow diagram (Figure 8).
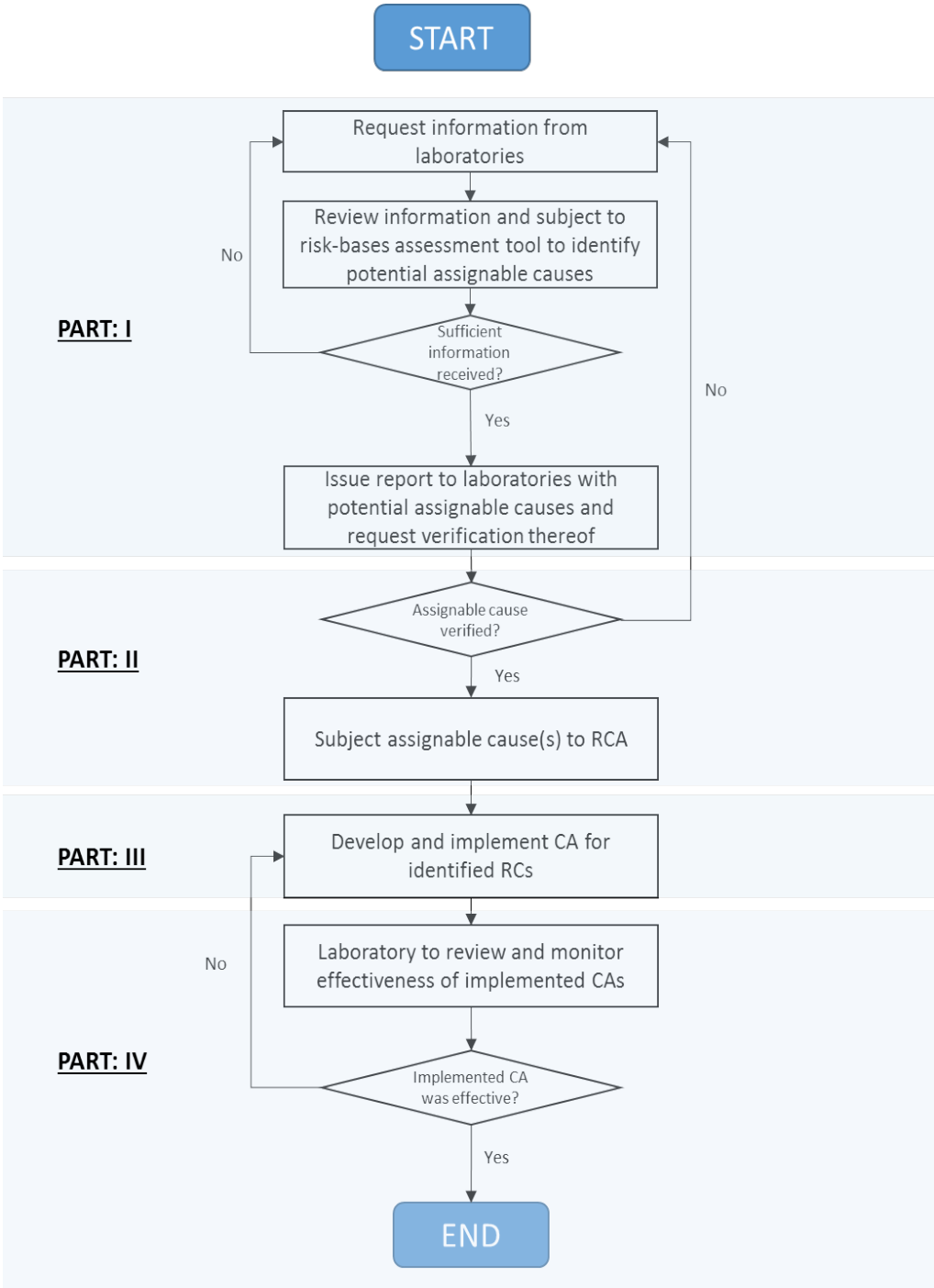
Figure 8: Flow diagram illustrating the rollout of the PEP-9-AP.

During Part I, the participating laboratories were requested to provide copies of their preliminary investigation report, raw data and processed results generated during the testing phase. A risk-based assessment tool was developed to facilitate identification of all potential assignable causes that might have led to the reporting of failing/unacceptable results. All information provided by the laboratories were then reviewed and subjected to the risk-based assessment tool. Comprehensive reports with feedback were compiled and issued to the respective laboratories. These reports detailed potential assignable causes for the delivery of failing/unacceptable results.

In Part II, the participating laboratories had to investigate and verify the assignable causes. Verified assignable causes were then subjected to a Root Cause Analysis (RCA) in an attempt to establish the Root Cause(s) (RC) of the failures. Thereafter the laboratories were assisted (during Part III) to develop and implement a corrective action plan with detailed Corrective Actions (CA) to address and prevent the potential reoccurrence of similar failures in future.

During Part IV, the laboratories were requested to review and monitor the effectiveness of the implemented CAs .

To conclude, the PEP-9-AP aimed to assist laboratories in the effective management of non-conforming results through the collection of information, analysing of information, identification and investigation of the quality problems, and assisting in taking the appropriate and effective corrective and/or preventive action in an attempt to prevent their recurrence, and ultimately building capacity within these PQCLs.

References

1.   WHO Good practices for pharmaceutical quality control laboratories. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations, forty-fourth report. Geneva: World Health Organization, 2010: Annex 1 (WHO Technical Report Series, No. 957).

2.   ISO/IEC 17025:2017 – General requirements for the competence of testing and calibration laboratories.

3.   BRITS, M. 2010. Characterization of polymorph transitions that decrease the stability of tablets containing the WHO essential drug Mebendazole. Journal of Pharmaceutical Sciences, 99(3): 1138-1151.

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