USP Technology Review: Global Pharma Health Fund–Minilab™

This report is one of an ongoing series of reports evaluating the capabilities of various screening technologies, performed under USP's established Technology Review program.

Report Date: August 2020

Disclaimer

This report is one of an ongoing series of reports evaluating the capabilities of various screening technologies, performed under USP's established Technology Review program (see Introduction for details). Although this evaluation was performed according to principles developed by the USP Review of Surveillance and Screening Technologies for the Quality Assurance of Medicines Expert Panel, the report does not represent certification or verification by USP that the equipment evaluated meets compendial standards.

Certain commercial equipment, instruments, vendors, or materials may be identified in this report to adequately specify the experimental procedure. Such identification does not imply approval, endorsement, or certification by USP of a particular brand or product, nor does it imply that the equipment, instrument, vendor, or material is necessarily the best available for the purpose or that any other brand or product was judged to be unsatisfactory or inadequate. All product names, logos, and brands are property of their respective owners.

Acknowledgments

The authors would like to acknowledge the following for their guidance, contribution, support, and feedback in the development of this report:

- USP Review of Surveillance and Screening Technologies for the Quality Assurance of Medicines Expert Panel
- National Agency of Drug and Food Control of Indonesia (BBPOM)
- Zambia Medicines Regulatory Authority (ZAMRA)

Table of Contents

List of Tables

List of Figures

Acronyms

API	active pharmaceutical ingredient
GPHF	Global Pharma Health Fund
LMIC	low- and middle-income country
PMS	post-marketing surveillance
Rf	retention factor
TLC	thin-layer chromatography
TTM	Technologie Transfer Marburg
UV	ultraviolet
WHO	World Health Organization

Executive Summary

A technology review was carried out on the Global Pharma Health Fund (GPHF) – Minilab, a portable, self-contained kit ready packed with glassware, secondary standards, and thin-layer chromatography (TLC) plates. The objective of the review was to determine whether Minilab can feasibly be used as a first-line screening technology to identify the presence of active pharmaceutical ingredients (APIs) in select drug products. The performance evaluation involved the analysis of four co-formulated tablet samples (artemether + lumefantrine, rifampicin + isoniazid + pyrazinamide + ethambutol, rifampicin + isoniazid + ethambutol, and rifampicin + isoniazid), single API capsules and tablets (amoxicillin), one co-formulated oral suspension sample (sulfamethoxazole and trimethoprim), and one gel formulation (chlorhexidine digluconate gel). All samples were analyzed as per the procedures indicated on the Minilab manuals that come with the kit, except for the additional sample concentrations that were prepared and spotted for comparative purposes.

Overall, the Minilab TLC procedures were able to screen and identify the API in each sample and perform semiquantitative estimates of the contents. The first step involved physical inspection of the dosage form and the packaging material. The products were then subjected to additional tests to evaluate other quality attributes. The additional tests included simple disintegration tests to assess whether solid dosage forms disintegrate within a predetermined timeframe. Disintegration is not a measure of active ingredient dissolution, but it is required for proper dissolution to occur. The second test involved easy-to-use TLC tests to provide a quick check of the identity of the API and a semiquantitative assessment of its content to verify label claims about identity and potency. The API content determination is limited to a visual assessment within an 80–100% range. The Minilab is a non-sophisticated, low-cost screening method assembled as a self-contained kit, ready packed, and able to detect substandard or falsified medicines (SF) containing the wrong, too high, too low, or zero levels of active ingredients. Personnel may need to have some knowledge of basic sciences and laboratory experience, and the analysis must be performed at a location where chemicals can be handled and disposed safely.

The field evaluation showed that most inspectors, chemists, and laboratory analysts with various levels of technical experience from the regulatory authorities of two countries, Zambia and Indonesia, could become advanced users of the technology in two weeks. In conclusion, Minilab can be deployed as a screening tool in many areas, including ports of entry, as part of a risk-based post-marketing surveillance (PMS).

1. Introduction

Assuring the quality of medicines along all points of the supply chain is vital for promoting positive health outcomes for patients around the world [1]. The importance of medicine quality screening technologies as part of this endeavor is becoming increasingly recognized [2]. USP launched the Technology Review program, an initiative guided by a technical expert panel established through the organization's collaborative and volunteer-driven governance. The Technology Review program works towards four objectives:

1. Develop standards and guidelines for evaluating medicine quality screening technologies.
2. Generate and disseminate tailored information on the capabilities of these technologies through a two-step review process: a laboratory-based technical performance evaluation and a collaborative field-based utility evaluation.
3. Build the knowledge of key stakeholders to appropriately procure and sustainably utilize screening technologies for the purposes of combating SF medicines.
4. Foster the development and enhancement of new and emerging screening technologies.

One of the major challenges in low- and middle-income countries (LMICs) is the great number of samples that have to be tested in order to maintain assurance of medicines quality and a high level of patient protection. Compendial analyses have become more and more expensive, and only a few centers of excellence in some countries are currently available to perform them in the quantities required.

To bridge the capacity gap in regular drug quality monitoring at the national level in LMICs and overcome limited access to regular drug quality testing facilities, GPHF developed an inexpensive field test kit with simple test methods for rapid drug quality verification and SF medicines detection.

Minilab contains essential laboratory ware and chemicals. Secondary standards for sample testing essential components include a full range of glassware for sample extraction, preparation, pipetting, and spotting; TLC plates; developing chambers; an electronic pocket balance; ultraviolet (UV) lamps with different wavelengths; a hot plate; and a caliper.

Minilab employs simple drug quality verification in a three-stage test plan that employs simple physical and chemical testing:

1. A physical inspection scheme of dosage forms and associated materials to provide grounds for early rejection based on physical characteristics, as well as failure to meet regulatory considerations, such as registration.
2. Simple disintegration tests to assess whether solid oral dosage forms disintegrate within predetermined timeframes.
3. Easy-to-use TLC tests to provide a quick check of the identity of the API and a semiquantitative assessment of its content to verify label claims about identity and potency.

Minilab has been used mostly in priority disease programs to monitor the quality of essential medicines to treat malaria, tuberculosis, and HIV/AIDS in countries where they are endemic. Its use has been adopted by several national regulatory authorities, including global partners like the U.S. Agency for International Development (USAID) and USP's Promoting the Quality of Medicines (PQM) program. It is estimated that, overall, more than 800 Minilabs have been

supplied across 97 countries. Cambodia, Laos, Myanmar, Tanzania, and Vietnam have adopted the technology for PMS of antimalarials. Because of its extensive use in many countries, technical capabilities, and cost, Minilab was selected for review.

2. Methodology

2.1. General Information

Table 1 provides general information on the Minilab: how it functions, its basic specifications, and the upfront and recurring costs of using the kit. All data in this section were obtained from the vendor's website in December 2018.

Table 1: General Information on Minilab

Short Description	Non-sophisticated, low-cost screening methods assembled as a self-contained kit, ready packed and able to detect SF medicines containing the wrong, too high, too low, or zero levels of active ingredients. It can be used outside of a laboratory environment by those with some understanding of analytical chemistry (e.g., medical or pharmaceutical technicians). It comes packed with manuals, basic laboratory procedures, a starter kit of chemicals, and a collection of reference standards for 90 medicines prevailing in developing countries for priority diseases.
Technology	Physical testing: Visual inspection guide and disintegration test Chemical testing: TLC
Specifications	*TLC kit equipment:* One protective case including manuals and collection of secondary reference standards (83 x 52 x 29 cm, 25 kg, black with preformed dividers/pockets, wheels, and extension handle) *Starter kit chemicals:* Capacity sufficient for about 1,000 TLC runs; comes in safety packs for air travel: 22 to 27 boxes (~25 kg) *Total weight:* ~50 kg *Shelf life:* ~Two years for authentic secondary reference standards (may be shorter for antiretrovirals; minimum five years for reagents and solvents in their original packaging)
Cost	*Upfront cost* • Minilab cost: €3422, includes manuals with method inventory on 90 drug compounds plus electronic pocket balance, other laboratory equipment, solvents, and other consumables for approximately 1,000 test runs • Reference standards cost: Ranges from €80 to €580, depending on the number of reference standards and class of medicines *Other costs* • *Shipment:* Between €700 and €900 for the carriage and insurance of a single Minilab; actual price depends on Minilab quantities, individual port, and country of destination • *Port clearance:* Estimated to be around €1000

Table 2: List of Samples Tested During Performance Evaluation

Symbol	Sample
AL	Artemether + lumefantrine co-formulated tablets
AMX	Amoxicillin tablets
AMX	Amoxicillin capsules
CHX	Chlorhexidine (4% w/w) digluconate gel
RH	Rifampicin + isoniazid tablets
RHE	Rifampicin + isoniazid + ethambutol tablets
RHZE	Rifampicin+ isoniazid + pyrazinamide + ethambutol tablets
ST	Sulfamethoxazole + trimethoprim (200/40 mg) oral suspension

Additional details for the samples used can be found in Annex 1.

2.2. Procedure Details

The procedures were performed as indicated in the Minilab manuals that are typically supplied with the kit, with the exception of the additional sample concentrations which were prepared and spotted for comparison purposes in the laboratory (protocol attached in Annex 2). The extra concentrations were compared with the original concentrations using the Minilab recommended method to determine if there was an intensity difference among the spots. All of the spots could not fit onto a single Minilab supplied 5×10 cm silica gel TLC plate, so the spotting plan listed below was used on all the procedures. The percentages are based on the individual concentrations specified in the procedure.

Table 3: Spotting Plan

Lane	Plate 1	Plate 2	Plate 3
1	100% standard	100% standard	100% standard
2	100% sample	120% sample	70% sample
3	100% sample	90% sample	50% sample
4	80% standard	80% standard	80% standard

The Minilab TLC procedures specify the visual procedure to be used for evaluating identity and content. The procedures do not require photos to be recorded. For the purposes of this report, photos of the TLC plates were recorded separately using a Camag TLC camera and VisioCats Software. A total of three analysts performed the procedures independently.

Figure 1: A TLC plate with samples being observed with UV light at 254 nm

2.3. Methodology Limitations

Certain limitations were encountered during this performance review, which were inevitable given the nature of the technology and the objectives of the review. They are identified as the following:

1. Only twelve different drug product samples were analyzed (Annex 1) as opposed to the over 100 products which can be screened using Minilab. Although most products are on

the World Health Organization's (WHO) Essential Medicines List [3], they represent only a small fraction of the list. Ideally, many more samples would be analyzed. However, these 12 samples represented a variety of therapeutic indications, dosage forms, and dosage strengths to enable broader conclusions about the utility of the Minilab. The samples selected also represent chemically diverse compounds and therefore able to demonstrate the most common detection methods of shortwave (254nm) UV light , longwave (365nm) UV, visible light, and other various detections that require exposure of the plate to specific detection reagents. The samples were also able to evaluate if the minilab disintegration method is applicable among different formulations (uncoated and film-coated tablets and capsules).

2. Procedural limitations
    a. A limited number of methods are available for screening. New methods are added each year, but many compounds and different formulations found on WHO's list of essential medicines are not yet addressed by the Minilab.
    b. In the Minilab manual section 5.5 Sample application, the instructions indicate that "up to five spots can be placed on a plate of 5 x 10 cm at a time." The use of four spots is recommended to reduce interpretation issues when reading the plates. The shape and distance moved by the spots near the sides may be slightly different from the spots in the center of the plates, hence affecting the interpretation of the results.
    c. In addition to specifying the detection procedure to be used for identification and quantification, the Minilab methods often contain additional detections. The use (or non-use) of these other detections may cause inconsistent reporting because some analysts will perform all of the detections specified in the methods while others many only perform the detections specified to be used for identification and quantification. The use of additional detections should be clarified.
    d. The methods often give information about the presence of additional spots other than the APIs, making it difficult to distinguish whether the spots are from the excipients or from impurity/degradation products.

3. General Information

3.1. Data

- Performance evaluation involved using nine Minilab TLC procedures to screen medicines to identify and perform semiquantitative estimates of content using supplies from the Minilab kit and from the secondary standards provided by GPHF. The analysts were able to visually compare the spots from the sample solutions to the spots obtained from the standard solutions using the procedure-required detection to identify and estimate content.
- In addition to preparing samples as per the procedure-required 100% level, additional sample solutions were prepared at 120%, 90%, 70%, and 50% levels and compared to the standard solutions by visual comparison. The results showed that the Minilab TLC methods are suitable for screening medicines for identification and for semiquantitative estimation of content.

3.2. Access, Handling, Maintenance, and Repair

Minilab is made by GPHF in Frankfurt, Germany, and can be purchased directly from Technologie Transfer Marburg (TTM) in Coelbe, Germany. TTM is able to arrange shipment of Minilab to an airport nearby the client's location. No major services and repairs are required for the kit. Replacement of consumables, glassware, and secondary reference standards are required.

3.3. Durability

Minilab is a portable kit that allows portability in the field, although at 50 kg, its total weight can exceed some peoples' carrying limits. The kit is tropics compatible, but direct sunlight should be avoided. No special storage area is required for the quantities of chemicals supplied. The kit is completely sealed, and though dropping the kit can break the glassware, each item in the kit has a dedicated storage area to avoid breakages (especially the glassware) during transportation.

3.4. Use

Minilab can indicate the presence or absence of an API in various drug formulations (e.g., tablets, capsules, solutions) and in gels. Additionally, Minilab can carry out semiquantitative estimates of the content of the API contained in those formulations.

Further details and the instrument brochure can be found at the manufacture website: https://www.gphf.org/en/index.htm.

4. Performance Evaluation

This involved review of the performance characteristics of Minilab in the laboratory where variables were controlled to evaluate the kit's analytical qualitative capabilities as per Application II of USP General Chapter <1850>: *Evaluation of Screening Technologies for Assessing Medicine Quality* [4], to ensure a structured, effective approach to performing a pragmatic review of the technology.

Application II involves Identification of Bulk Drug Substances or Active Pharmaceutical Ingredients in Finished Pharmaceutical Products. All data below were collected between March and October 2018.

Twelve products (Annex 1) were analyzed as per the TLC procedures provided in the manufacturer's manuals. The samples selected to evaluate the capabilities of the Minilab were all products from the WHO Essential Medicines List and represented different therapeutic indications, dosage forms, formulations, and dosage strengths from different manufacturers

4.1. Observations

The evaluation of Minilab identified some of the factors that could affect Minilab results for disintegration testing and TLC. Results of visual inspection depend on the observation skills of the user. The Minilab manual provides sufficient information for users to become adept at identifying missing or incorrect parts of the required elements of a label, but additional time to notice the often small omissions and errors of substandard medicines may be required.

For identification and semiquantitative analysis, testing and procedures showed that results obtained from all the sample formulations tested could be used for quick screening of SF samples.

The analysts were able to visually compare the spots from the sample solutions to the spots obtained from the standard solutions using the procedures specified in the manuals and to use the required detection to identity and estimate the API content in each sample. The analysts then calculated the Retention Factor (Rf) value for each sample and compared them to the reference standard. The average Rf and the average Rf sample error from the observations indicated that Minilab can be used for identification and quantitation. The Rf sample error is calculated between the 100% standard spot and the 100% sample spot using the formula given by the Minilab manual:

$$\text{Rf test solution} = \frac{\text{Distance moved by spot}}{\text{Distance moved by solvent front}}$$

$$\text{Rf sample error} = \frac{\text{Rf standard solution} - \text{Rf test solution}}{\text{Rf standard solution}} \times 100\%$$

Amoxicillin

The Minilab test procedure for amoxicillin by TLC tests was used to evaluate two lots of amoxicillin capsules and one lot of amoxicillin tablets. The procedure-specified detection to be used for identification and quantification of amoxicillin is observation of the dried chromatoplate under UV light at 254 nm before and after iodine staining. The 100% spots obtained from the sample solutions corresponded to the 100% standard solution in color intensity and size, and differences in the spots from the other concentrations (120%, 90%, 80%, 70% and 50%) could

be seen in the visual inspection. Additional detections in the procedure, daylight after iodine staining, and daylight after ninhydrin staining were also performed.

Table 4: Detection of Amoxicillin – UV Light at 254 nm Before and After Iodine Staining

Sample	Manufacturer and Lot #	Rf Reported by Minilab	Average Observed Rf	Average Rf Sample Error*
Amoxicillin capsules	Sandoz lot HG9361	0.32	0.33 ± 0.03	0.0%
Amoxicillin capsules	Sandoz lot GS0051	0.32	0.34 ± 0.05	0.8%
Amoxicillin tablets	Teva Pharmaceuticals Industries Ltd. lot 35442174A	0.32	0.33 ± 0.04	0.0%

** Average Rf sample error from 24 spots: Two analysts performed 12 applications each.*

Figure 2: UV 254 nm before iodine staining of amoxicillin capsules

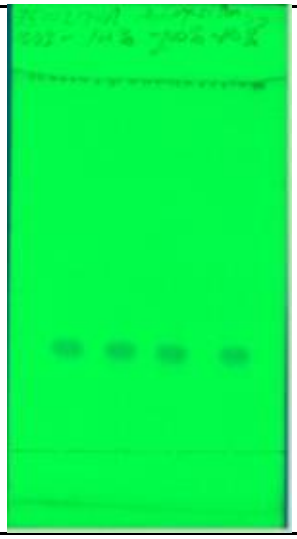
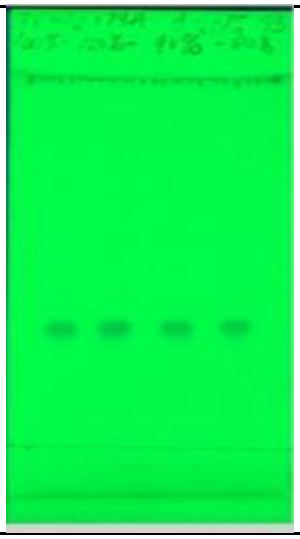
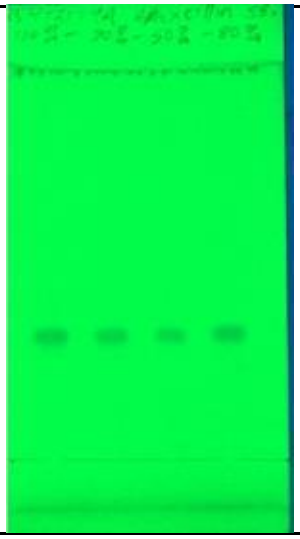
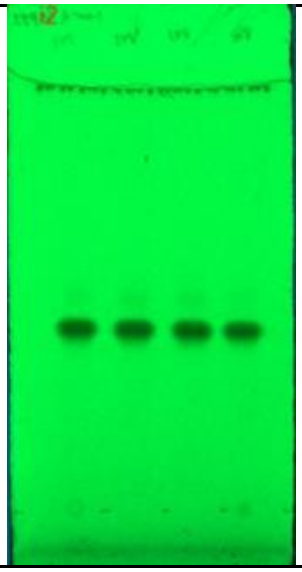
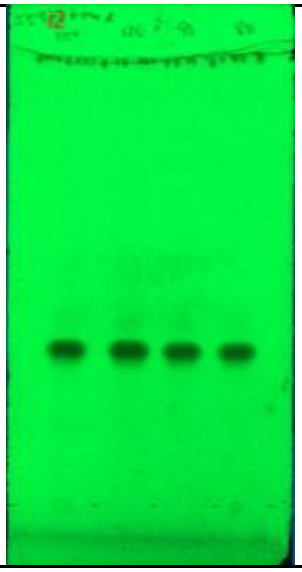
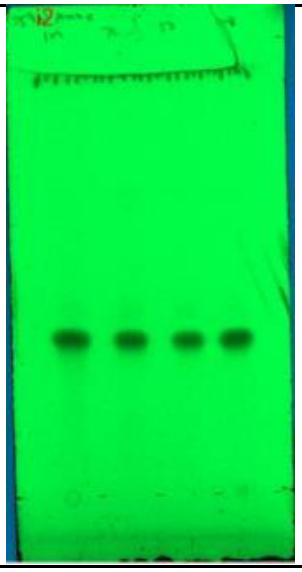
Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

Figure 3: UV 254 nm after iodine staining of amoxicillin capsules

Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

Artemether

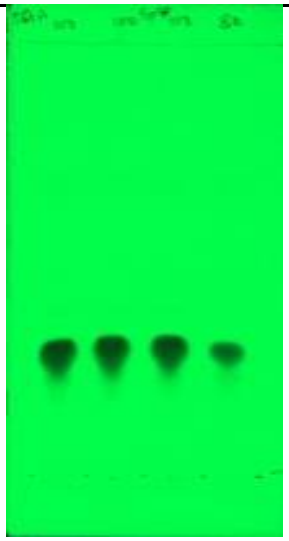
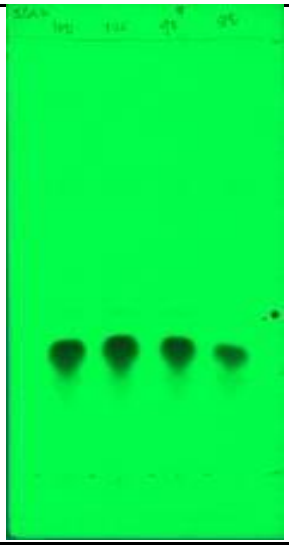
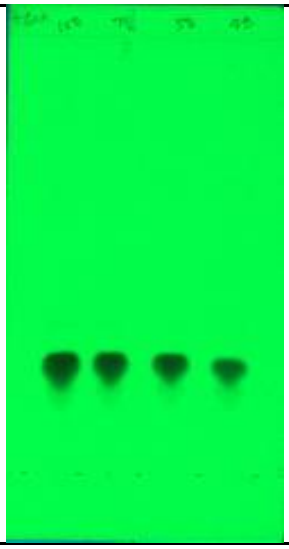
The Minilab test procedure for artemether (in lumefantrine tablets, dispersible tablets, and dry syrups) was used to evaluate artemether in two lots of artemether + lumefantrine tablets (AL). In the UV detection prior to acid staining, artemether was not visible, and only lumefantrine was visible. The daylight after acid staining was used for the identification and quantification of artemether as it was the procedure-specified detection. The spots obtained from the 100% sample solutions corresponded to the 100% standard solution by visual comparison, and differences in the spots from the other concentrations could be seen. Additional identification of artemether was done by observing the chromatoplate with UV light of 366 nm after acid staining (see Figure 5).

Table 5: Detection of AL – Daylight After Acid Staining

Sample	Manufacturer and Lot #	Rf Reported by Minilab	Average Observed Rf for Artemether	Average Rf Sample Error*
Artemether + lumefantrine tablets	Ipca Laboratories Ltd., lot DYI466166	0.59	0.61 ± 0.04	0.0%
Artemether + lumefantrine tablets	Novartis lot K0050	0.59	0.61 ± 0.05	0.3%

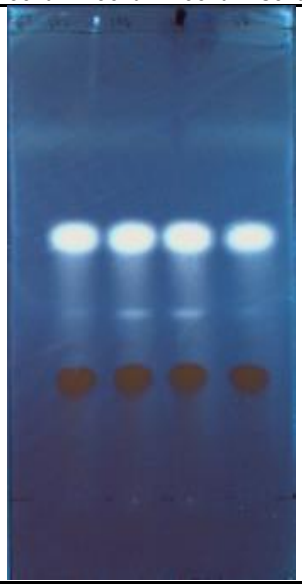
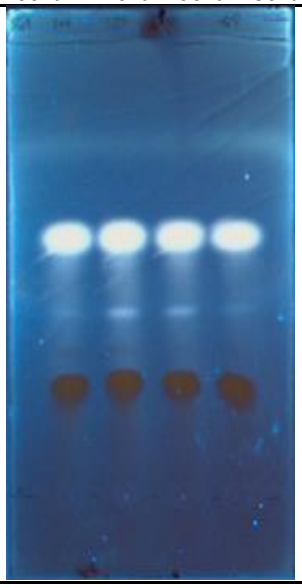
** Average Rf sample error from 24 spots: Two analysts performed 12 applications each.*

Figure 4: UV 254 nm before sulfuric acid staining for identification of artemether in AL tablets

Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

The visible spots seen in Figure 4 are from the lumefantrine content of the sample. Artemether is not visible in the UV light at 254 nm. Artemether is visible in the UV light at 366 nm detection and is seen in Figure 5.

Figure 5: UV 366 nm after sulfuric acid staining for identification of artemether in AL tablets

Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

The white spots that are visible in the chromatoplate are from artemether while the dark spots are from lumefantrine. The less visible spots located in between the white and dark spots may indicate a preservative.

Co-trimoxazole (sulfamethoxazole and trimethoprim)

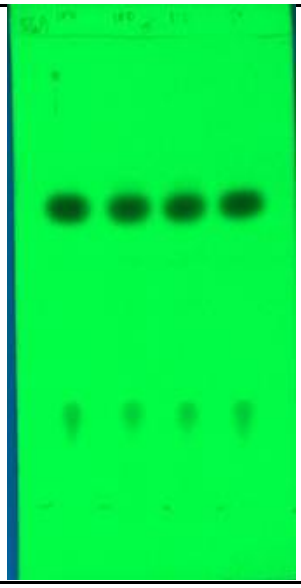
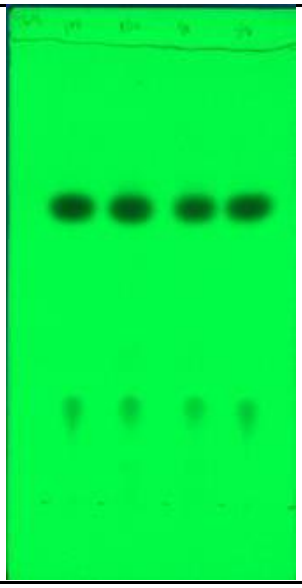
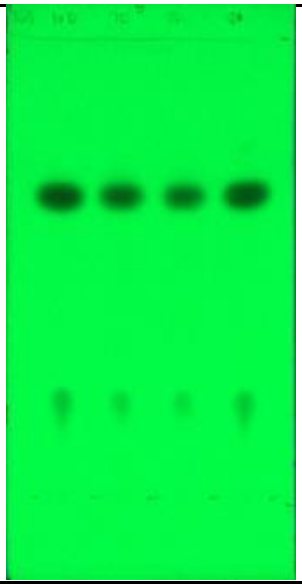
The Minilab test procedure for sulfamethoxazole (including co-trimoxazole formulations) was used to evaluate one lot of the sulfamethoxazole and trimethoprim (ST) oral suspension product. The procedure-specified detection used for identification and quantification of both sulfamethoxazole and trimethoprim involved exposing the dried chromatoplate to UV light at 254 nm. The spots obtained from the 100% sample solutions corresponded to the 100% standard solution by visual comparison, and differences in the spots from the other concentrations could be seen.

Table 6: Detection of ST – UV 254 nm Light for Primary Spot

Sample	Manufacturer and Lot #	Rf Reported by Minilab	Average Observed Rf	Average Rf Sample Error*
Sulfamethoxazole + trimethoprim for oral suspension	BDH Industries, Ltd., lot D-10217	0.65 (sulfamethoxazole)	0.66 ± 0.01	0.0%
Sulfamethoxazole + trimethoprim for oral suspension		0.24 (trimethoprim)	0.21 ± 0.01	0.0%

** Average Rf sample error from 24 spots: Two analysts performed 12 applications each.*

Figure 6: UV 254 nm light for ST

Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

Chlorhexidine

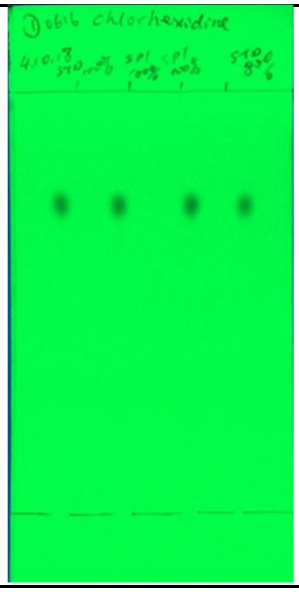
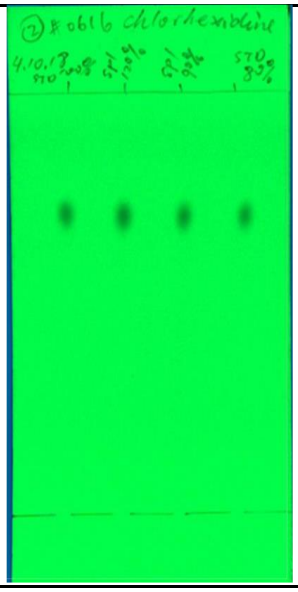
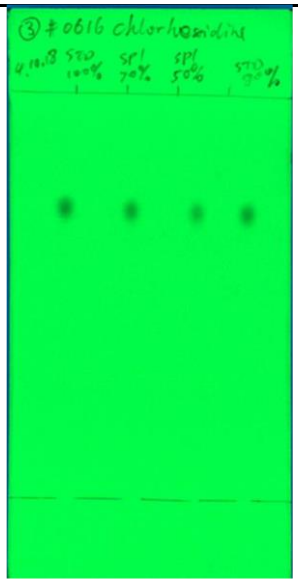
The Minilab test procedure for chlorhexidine was used to evaluate two lots of chlorhexidine digluconate (CHX) gel. The procedure-specified detection for identification and quantification of chlorhexidine is exposing the dried chromatoplate to UV light at 254 nm. The 100% spots obtained from the sample solutions corresponded to the 100% standard solution by visual comparison, and differences in the intensity spots from the other concentrations could be seen.

Table 7: Detection of CHX – UV light of 254 nm for Primary Spot

Sample	Manufacturer and Lot #	Rf Reported by Minilab	Average Observed Rf	Average Rf Sample Error*
Chlorhexidine 4% w/w digluconate gel, umbilical gel	Manufacturer: N/A, lot 326 L15	0.62	0.70 ± 0.01	1.4%
Chlorhexidine gel, 4% w/w digluconate gel, Kawach gel	Lomus Pharmaceuticals, Pvt. Ltd, lot KW1.0616	0.62	0.70 ± 0.02	−0.4%

** Average Rf sample error from 24 spots: Two analysts performed 12 applications each.*

Figure 7: UV 254 nm for detection of chlorhexidine

Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

Ethambutol

The Minilab test procedure for ethambutol was used to evaluate the ethambutol content in two different medicines (rifampicin + isoniazid + ethambutol tablets [RHE], rifampicin+ isoniazid + pyrazinamide + ethambutol tablets [RHZE]) containing ethambutol. As specified in the monograph, ethambutol was not visible in the initial detection at 254 nm of light, and the spots seen were from other components from the fixed-dose combinations of RHE and RHZE. The procedure-specified detection for identification and quantification of ethambutol is daylight after exposure to the ninhydrin staining solution. The 100% spots obtained from the sample solutions corresponded to the 100% standard solution by visual comparison, and differences in the spots from the other concentrations could be seen.

Table 8: Detection of RHE and RHZE – Daylight After Exposure to Ninhydrin Stain Primary Spot

Sample	Manufacturer and Lot #	Rf Reported by Minilab	Average Observed Rf	Average Rf Sample Error*
Ethambutol in rifampin + isoniazid + ethambutol hydrochloride tablets	Macleods Pharmaceuticals Ltd., lot ERD2706B	0.33	0.36 ± 0.05	0.6%
Ethambutol in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets	Lupin Pharmaceuticals, Inc., lot A603606	0.33	0.36 ± 0.04	−0.3%
Ethambutol in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets	Macleods Pharmaceuticals Ltd., lot ERC6690C	0.33	0.38 ± 0.03	−0.2%

** Average Rf sample error from 24 spots: Two analysts performed 12 applications each.*

Figure 8: Daylight ninhydrin staining for identification of ethambutol in RHE tablets

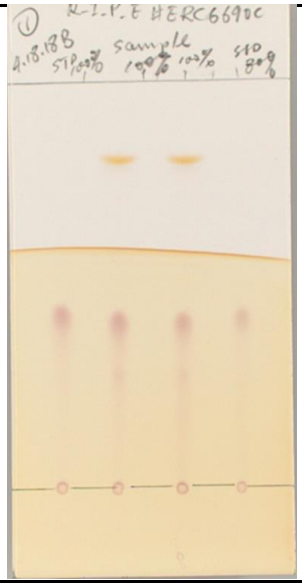
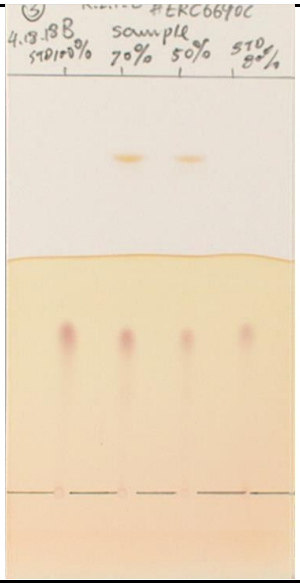
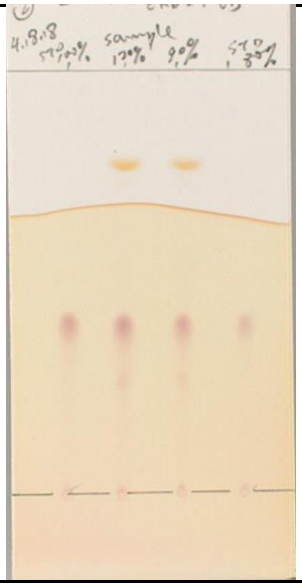
Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

Figure 9: Daylight ninhydrin staining for identification of ethambutol in RHZE tablets

Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

The additional yellow spot seen on top is from the rifampin content of the sample, which was not stained. The line is from the staining solution.

Isoniazid

The Minilab test procedure for isoniazid was used to evaluate the isoniazid content in three anti-tuberculosis medicines (rifampicin + isoniazid tablets [RH], RHE, and RHZE) containing isoniazid. The procedure-specified detection for identification and quantification of isoniazid is exposure of the dried chromatoplate to UV light at 254 nm. The spots obtained from the sample solutions corresponded to the 100% the standard solution by visual comparison, and differences in the spots from the other concentrations could be seen. The additional detection of observing the chromatoplate in daylight after exposure to iodine staining was also performed.

Table 9: Detection of Isoniazid – Exposure to 254 nm Light, Developing Solution A, Primary Spot

Sample	Manufacturer and Lot #	Rf Reported, Solution A	Average Observed Rf	Average Rf Sample Error*
Isoniazid in rifampin + isoniazid tablets	6159001	0.33	0.39 ± 0.05	0.0%

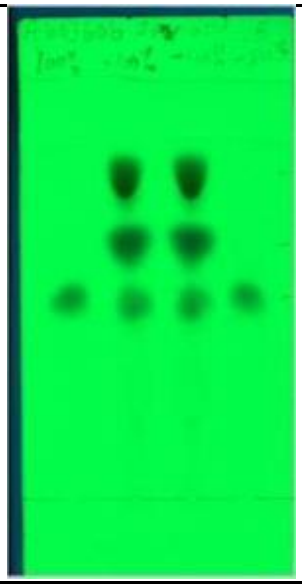
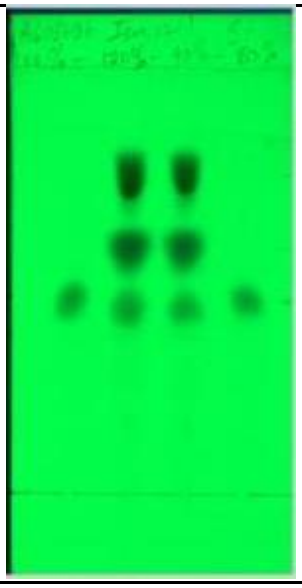
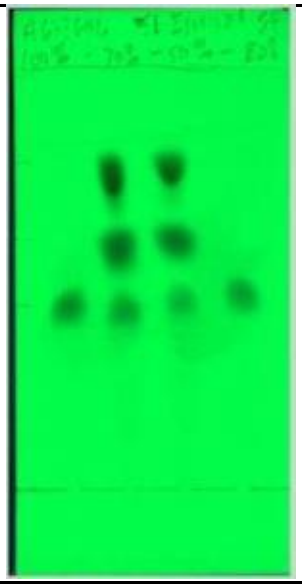
** Average Rf sample error from 24 spots: Two analysts 12 applications each.*

Table 10: Detection of Isoniazid – Exposure to 254 nm Light, Developing Solution B

Sample	Manufacturer and Lot #	Rf Reported, Solution B	Average Observed Rf	Average Rf Sample Error*
Isoniazid in rifampin + isoniazid + ethambutol hydrochloride tablets	Macleods Pharmaceuticals Ltd., lot ERD2706B	0.33	0.43 ± 0.02	3.7%
Isoniazid in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets	Lupin Pharmaceuticals, Inc., lot A603606	0.33	0.44 ± 0.02	0.0%
Isoniazid in rifampin+ isoniazid + pyrazinamide + ethambutol hydrochloride tablets	Macleods Pharmaceuticals Ltd., lot ERC6690C	0.33	0.45 ± 0.02	3.3%

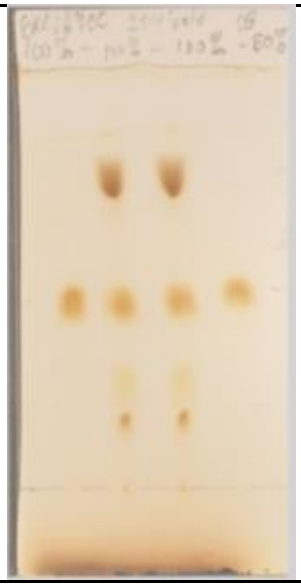
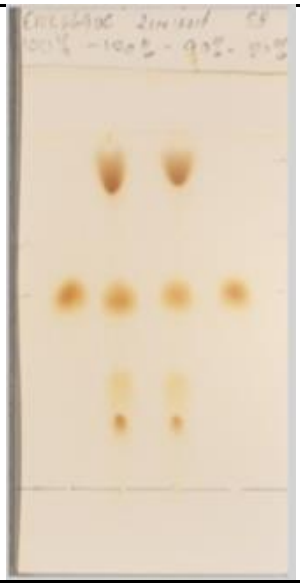
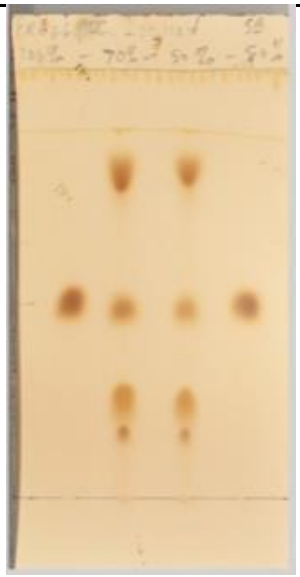
** Average Rf sample error from 24 spots: Two analysts performed 12 applications each.*

Figure 10: UV 254 nm for detection of isoniazid in RHZE

Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

The top, middle, and bottom spots are rifampin, pyrazinamide, and isoniazid, respectively.

Figure 11: Daylight after iodine staining for detection of isoniazid in RHZE tablets

Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

The top, middle and bottom spots are rifampin, pyrazinamide, and isoniazid, respectively.

Lumefantrine

The Minilab test procedure for lumefantrine (in artemether tablets, dispersible tablets, and dry syrups) was used to evaluate lumefantrine in two lots of AL tablets. The procedure-specified detection for identification and quantification of lumefantrine is exposing the dried chromatoplate to UV light at 254 nm. The 100% spots obtained from the sample solutions corresponded to the 100% standard solution by visual comparison, and differences in the spots from the other concentrations could be seen. The additional detection is observation in daylight after iodine staining.

Table 11: Detection of Lumefantrine – Exposure to 254 nm Light, Primary Spot

Sample	Manufacturer and Lot #	Rf Reported by Minilab	Average Observed Rf	Average Rf Sample Error*
Lumefantrine in artemether + lumefantrine tablets	Ipca Laboratories, Ltd., lot DYI466166	0.69	0.77 ± 0.05	0.0%
Lumefantrine in artemether + lumefantrine tablets	Novartis, lot K0050	0.69	0.77 ± 0.06	0.0%

** Average Rf sample error from 24 spots: Two analysts performed 12 applications each.*

Figure 12: UV 254 nm for detection of lumefantrine in AL tablets

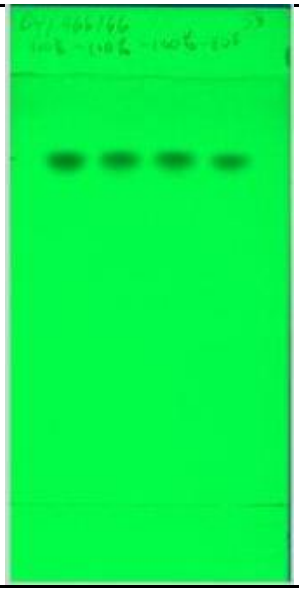
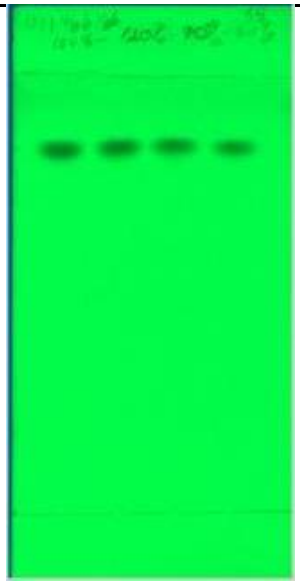
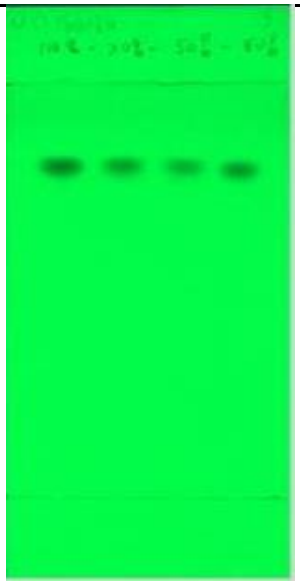
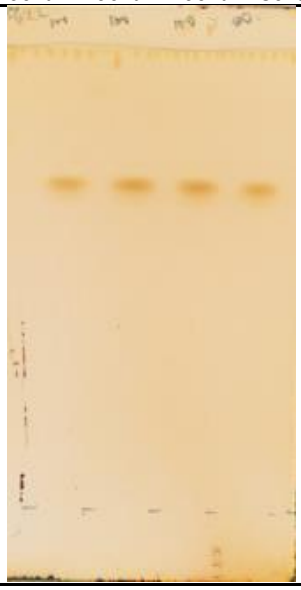
Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

Figure 13: Daylight after iodine staining for detection of lumefantrine in AL tablets

Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

Rifampicin

The Minilab test procedure for rifampicin (including fixed-dose combinations) was used to evaluate the rifampicin content in three anti-tuberculosis medicines (RH, RHE, and RHZE) containing rifampicin. The procedure-specified detection for identification and quantification of rifampin is observation of the dried chromatoplate in daylight, a method which was found to be suitable. The spots obtained from the 100% sample solutions corresponded to the 100%

standard solution by visual comparison, and differences in the spots from the other concentrations could be seen. Table 12 shows data for the specific detection of the RH sample, which used developing solution A, whereas Table 13 shows data for the specific detection of the RHE and RHZE samples, which used developing solution B.

Table 12: Detection of Rifampicin – Observation in Daylight, Developing Solution A, Primary Spot

Sample	Manufacturer and Lot #	Rf Reported, Solution A	Average Observed Rf	Average Rf Sample Error*
Rifampicin in rifampin + isoniazid tablets	Phapros, lot 6159001	0.43	0.51 ± 0.01	0.0%

** Average Rf sample error from 24 spots: Two analysts performed 12 applications each.*

Table 13: Detection of Rifampicin – Observation in Daylight, Developing Solution B

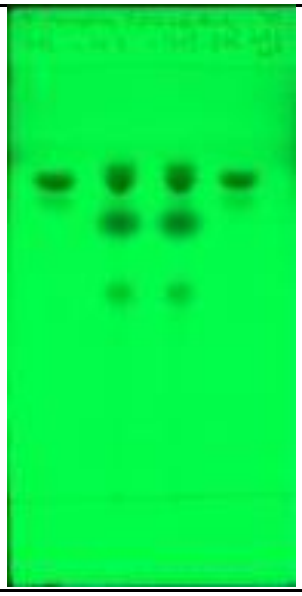
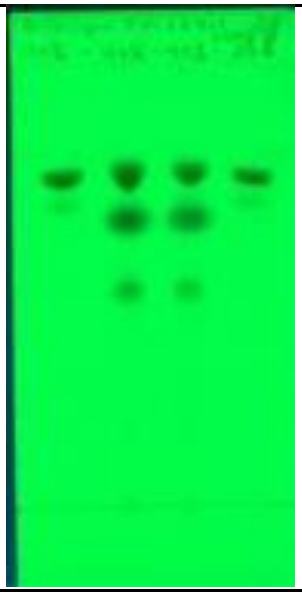
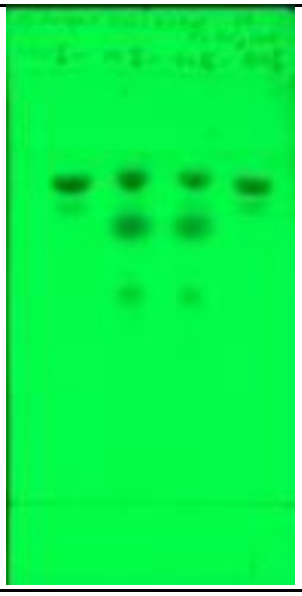
Sample	Manufacturer and Lot #	Rf Reported, Solution B	Average Observed Rf	Average Rf Sample Error*
Rifampicin in rifampin + isoniazid + ethambutol hydrochloride tablets	Macleods Pharmaceuticals Ltd., lot ERD2706B	0.71	0.74 ± 0.04	0.0%
Rifampicin in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets	Lupin Pharmaceuticals Inc., lot A603606	0.71	0.71 ± 0.01	0.4%
Rifampicin in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets	Macleods Pharmaceuticals Ltd., lot ERC6690C	0.71	0.74 ± 0.03	0.0%

** Average Rf sample error from 24 spots: Two analysts performed 12 applications each on three plates.*

Figure 14: UV daylight detection of rifampin in RHZE tablets

Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

Figure 15: UV 254 nm for detection of rifampin in RHZE tablets

Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

The top, middle, and bottom spots are rifampin, pyrazinamide, and isoniazid, respectively.

Pyrazinamide

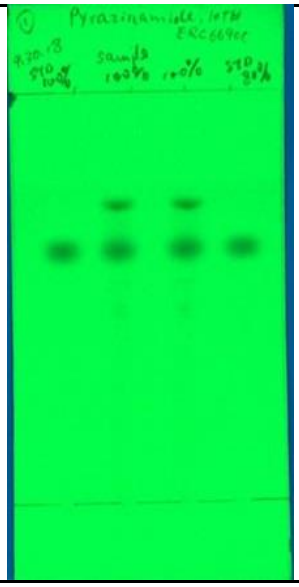
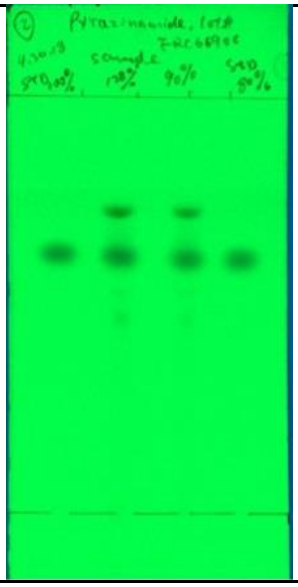
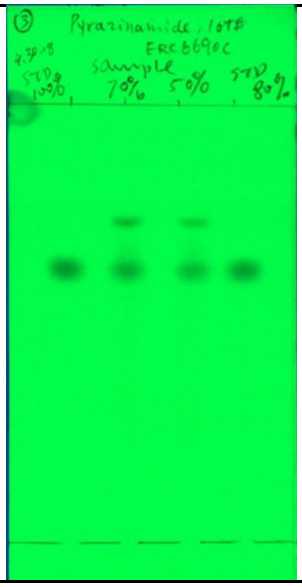
The Minilab test procedure for pyrazinamide (including fixed-dose combinations) was used to evaluate the pyrazinamide content in two lots of anti-tuberculosis RHZE medicines. The procedure-specified detection for identification and quantification of pyrazinamide is observation of the dried chromatoplate under UV light at 254 nm. The spots obtained from the 100% sample solutions corresponded to the 100% standard solution by visual comparison, and differences in the spots from the other concentrations could be seen.

Table 14: Detection of Pyrazinamide – Observation Under UV Light of 254 nm, Primary Spot

Sample	Manufacturer and Lot #	Rf Reported by Minilab	Average Observed Rf	Average Rf Sample Error*
Pyrazinamide in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets	Lupin Pharmaceuticals Inc., lot A603606	0.56	0.64 ± 0.05	0.0%
Pyrazinamide in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets	Macleods Pharmaceuticals, Ltd., lot ERC6690C	0.56	0.66 ± 0.05	0.6%

** Average Rf sample error from 24 spots: Two analysts performed 12 applications each.*

Figure 16: UV 254 nm for identification of pyrazinamide in RHZE tablets

Plate 1	Plate 2	Plate 3
Concentration 100% – 100% – 100% – 80%	Concentration 100% – 120% – 90% – 80%	Concentration 100% – 70% – 50% – 80%
		

The top and bottom spots are rifampin and pyrazinamide, respectively.

Table 15: Summary of TLC results

Sample	Manufacturer and Lot #	Detection method, Primary spot	Rf Reported by Minilab	Average Observed Rf	Average Rf Sample Error
Amoxicillin capsules	Sandoz lot HG9361	UV Light at 254 nm Before and After Iodine Staining	0.32	0.33 ± 0.03	0.0%
Amoxicillin capsules	Sandoz lot GS0051	UV Light at 254 nm Before and After Iodine Staining	0.32	0.34 ± 0.05	0.8%
Amoxicillin tablets	Teva Pharmaceuticals Industries Ltd. lot 35442174A	UV Light at 254 nm Before and After Iodine Staining	0.32	0.33 ± 0.04	0.0%`
Artemether in Artemether + lumefantrine tablets	Ipca Laboratories Ltd., lot DYI466166	Daylight After Acid Staining	0.59	0.61 ± 0.04	0.0%
Artemether in Artemether + lumefantrine tablets	Novartis lot K0050	Daylight After Acid Staining	0.59	0.61 ± 0.05	0.3%
Sulfamethoxazole in Sulfamethoxazole + trimethoprim for oral suspension	BDH Industries, Ltd., lot D-10217	UV 254 nm Light	0.65 (sulfamethoxazole)	0.66 ± 0.01	0.0%
Trimethoprim Sulfamethoxazole + trimethoprim for oral suspension	BDH Industries, Ltd., lot D-10217	UV 254 nm Light	0.24 (trimethoprim)	0.21 ± 0.01	0.0%
Chlorhexidine 4% w/w digluconate gel, umbilical gel	Manufacturer: N/A, lot 326 L15	UV light of 254 nm	0.62	0.70 ± 0.01	1.4%

Chlorhexidine gel, 4% w/w digluconate gel, Kawach gel	Lomus Pharmaceuticals, Pvt. Ltd, lot KW1.0616	UV light of 254 nm	0.62	0.70 ± 0.02	−0.4%
Ethambutol in rifampin + isoniazid + ethambutol hydrochloride tablets	Macleods Pharmaceuticals Ltd., lot ERD2706B	UV light of 254 nm	0.33	0.36 ± 0.05	0.6%
Ethambutol in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets	Lupin Pharmaceuticals, Inc., lot A603606	Daylight After Exposure to Ninhydrin Stain	0.33	0.36 ± 0.04	−0.3%
Ethambutol in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets Developing Solution A	Macleods Pharmaceuticals Ltd., lot ERC6690C	Daylight After Exposure to Ninhydrin Stain	0.33	0.38 ± 0.03	−0.2%
Isoniazid in rifampin + isoniazid tablets	Phapros, lot 6159001	Exposure to 254 nm Light	0.33	0.39 ± 0.05	0.0%
Isoniazid in rifampin + isoniazid + ethambutol hydrochloride tablets Developing Solution B	Macleods Pharmaceuticals Ltd., lot ERD2706B	Exposure to 254 nm Light	0.33	0.43 ± 0.02	3.7%
Isoniazid in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets; Developing Solution B	Lupin Pharmaceuticals, Inc., lot A603606	Exposure to 254 nm Light	0.33	0.44 ± 0.02	0.0%
Isoniazid in rifampin+ isoniazid + pyrazinamide + ethambutol hydrochloride tablets; Developing Solution B	Macleods Pharmaceuticals Ltd., lot ERC6690C	Exposure to 254 nm Light	0.33	0.45 ± 0.02	3.3%
Lumefantrine in artemether + lumefantrine tablets	Ipca Laboratories, Ltd., lot DYI466166	Exposure to 254 nm Light	0.69	0.77 ± 0.05	0.0%
Lumefantrine in artemether + lumefantrine tablets	Novartis, lot K0050	Exposure to 254 nm Light	0.69	0.77 ± 0.06	0.0%
Rifampicin in rifampin + isoniazid tablets; Developing Solution A	Phapros, lot 6159001	Observation in Daylight	0.43	0.51 ± 0.01	0.0%
Rifampicin in rifampin + isoniazid + ethambutol hydrochloride tablets; Developing Solution B	Macleods Pharmaceuticals Ltd., lot ERD2706B	Observation in Daylight	0.71	0.74 ± 0.04	0.0%
Rifampicin in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets; Developing Solution B	Lupin Pharmaceuticals Inc., lot A603606	Observation in Daylight	0.71	0.71 ± 0.01	0.4%

Rifampicin in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets; Developing Solution B	Macleods Pharmaceuticals Ltd., lot ERC6690C	Observation in Daylight	0.71	0.74 ± 0.03	0.0%
Pyrazinamide in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets	Lupin Pharmaceuticals Inc., lot A603606	Observation Under UV Light of 254 nm	0.56	0.64 ± 0.05	0.0%
Pyrazinamide in rifampin + isoniazid + pyrazinamide + ethambutol hydrochloride tablets	Macleods Pharmaceuticals, Ltd., lot ERC6690C	Observation Under UV Light of 254 nm	0.56	0.66 ± 0.05	0.6%

4.2. General Observations

1. If the user chooses to dry a test plate using an iron, the drying should be done in a consistent manner for all the plates in order to have uniform results. The Minilab manual indicates two potential advantages of using the hot plate. First, the use of the hot plate decreases the time needed for drying. Plates can still be air dried if no electricity is present, but this a more time-consuming procedure. Second, the hot plate is beneficial when drying low volatile solvents such as ethyl acetate, toluene, and water. These can also be air dried, but additional time can be saved if the hot plate is used.
2. The users were uncertain about how to open the locks upon initial receipt. No instructions are provided. The locks were initially set to the telephone access code for Germany, which is 049.
3. The users should apply all of the solution in one application because this eliminates the possibility of applying the subsequent application in a slightly different position, which may lead to noncircular or nonuniform spots. Repeated applications with the capillary to an absorbent layer may scratch or damage the adsorbent layer and cause the mobile phase to elute unevenly. Additionally, the user also can avoid making the mistake of applying the sample in different lanes, which can occur easily because in many procedures, the solvent will evaporate instantly and the spot will disappear.
4. Each TLC plate should be inspected with UV light at 254 nm to ensure the spots are uniform and circular before proceeding to the development stage.

4.3. Field Evaluation

The field evaluations for Minilab were performed in Zambia and Indonesia on May 28–June 8, 2018, for two major parameters: training requirements and field utility. Zambia and Indonesia were selected because they represent two countries with different regulatory environments, where Minilab technology has not been used extensively in the past but has the potential to be deployed effectively to combat SF medicines.

Training Requirements

The first component of the field evaluation involved working with and training local staff from both Zambian and Indonesian national medicines regulatory authorities, retail pharmacies, and customs to assess the amount of training required to enable staff to reliably and productively

utilize the Minilab. The training involved two full days of training, which included hands-on and theoretical work, followed by one day of testing samples in the laboratory where feedback was received from the trainees to assess if the required training period was sufficient in learning the technology. The field collection of samples took place at retail pharmacies, health facilities, etc., but the Minilab testing of the samples took place at the lab since the collection sites did not have the infrastructure to handle reagents and chemical safely. However, analysis can still be carried out inside a building, even in remote settings, by making minor modifications. Across both countries, 26 participants were trained, with 23 trainees being from the National Agency of Drug and Food Control of Republic of Indonesia (Badan POM) and Zambia Medicines Regulatory Authority (ZAMRA). These included 15 laboratory staff (either microbiologists or chemists), eight pharmaceutical inspectors, two retail pharmacists, and one customs officer. To evaluate the perceived training timeframes for the three levels of Minilab proficiency (basic, intermediate, and advanced), the following two data sources were used to develop a training timeframe requirements matrix: (1) a survey completed by trainees following the training and (2) the trainer observations. Two variables were used to develop the matrix:

1. User experience (prior to training):
    a. *Non-technical experience*: A trainee with no prior laboratory experience and no background in one of the physical sciences (e.g., chemistry, physics).
    b. *Technical experience*: A trainee with prior experience working in a laboratory and/or a background in one of the physical sciences.
    c. *Specialized experience*: A trainee with theoretical and practical experience utilizing the technology or the technique underpinning the technology.
2. User type[1] (following training):
    a. *Basic user*: A user with the ability to follow a standard operating procedure (SOP) or work instruction to set up and run the instrument and collect data.
    b. *Intermediate user*: A user with the ability to develop and modify methods and evaluate and interpret results.
    c. *Advanced user*: A user with the ability to train other staff and perform basic troubleshooting.

Table 16 provides recommended training timeframes for trainees to reach each user level depending on the user's experience. Recommendations are based on the performance evaluation, field evaluation, trainer observations, and surveys given to trainees and local staff.

Table 16: Training Timeframe Requirements

User Experience	User Type		
	Basic	Intermediate	Advanced
Non-Technical	1 to 2 days	1 week	2 weeks
Technical	1 day	1 day to 1 week	1 to 2 weeks
Specialized	1 to 2 hours	1 day	Less than 1 week

[1] The user type abilities build upon the previous level (e.g., an advanced user can perform the functions of an advanced user as well as a basic and intermediate user).

Field Utility

The second component of the field evaluation involved trying to run samples using the technology in field settings and determining the utility of the instrument in these environments. It also included identifying any challenges associated with traveling with the instrument.

No problems were encountered during routine international air transportation of the Minilab to the field evaluation countries. The instrument was shipped as a dangerous good delivery via cargo aircraft to its destination. Additionally, the following recommendations can help improve the field utility of Minilab:

1. The design of the developing chamber jar could be improved. The current developing chamber jar has a relatively narrow opening in relation to the TLC plate, and the bottom has a slight curve. An improved design would have a slightly larger opening and a flat bottom to allow accurate insertion and stable positioning of the TLC plate in minimal time to maintain saturation inside the vessel.
2. The kit contains limited numbers of key components; adding more units would contribute to better utilization. The kit contains two UV lamps (254 nm and 366 nm), and many of the TLC detection methods require use of one or both of the lamps. A lamp failure or a broken lamp will delay detections until a new lamp is ordered and received. Also, even though all the TLC procedures require many pipetting steps, the kit contains only one suction bulb for pipetting liquids. If the suction bulb becomes contaminated or damaged, additional suction bulbs in the kit would allow the user to continue working while the damaged unit is repaired and dried.
3. The current rack in the kit has limitations. The smallest 10 mL vials in the kit are relatively narrow, tall, and top heavy with their screwcap tops and can tip over easily; the rack in the kit do not hold them properly. A different type of tray or rack to hold these vials could contribute to less spillage and greater productivity. Additionally, if users do not carefully balance items placed in the rack, it may flip over. Another issue occurs when attempting to filter solutions using the kit-supplied funnels. The filter, filter paper, and liquid are very top heavy and may tip over in the current rack if not well-balanced. Different types of racks may be needed to address these problems.
4. Performing the simple disintegration test procedure requires the users to balance the use of supplies in the kit with time. The method specifies to test six units individually in 100 mL of water at 37°C. The kit provides five 100 mL bottles and two larger 500 mL bottles that can be used for this purpose; however, these bottles are often in use for other TLC preparations. Another concern is that it can take a relatively long time to perform simple disintegration, as each individual test can take up to 30 minutes—and, if a unit fails, then another six individual units must be tested. The hot plate provided in the kit has limited capacity (only a few bottles can be heated on it at a time), requiring users to move solutions back and forth or to use alternative means to heat the media to 37°C. To reduce the overall time to perform simple disintegration, a suggestion is to consider reducing the number of individual units tested, providing additional glassware, or combining disintegration of multiple units at the same time.
5. Emphasis should be increased on ensuring the applied solutions have dried completely before placing the TLC plate into the developing solution and when performing detection. While the procedure may contain cautionary statements such as “to completely dry off all extraction solvent before chromatoplate development using the hot plate supplied” or that use of the iron is “preferred for low volatile solvents, for example ethyl acetate, toluene, and water,” this information can be overlooked. For example, if the TLC plate is not completely dry and the user observes the plate with the UV lamp, the UV

fluorescence of the TLC plate will be masked, and no dark spot areas will appear. However, after the plate has dried and on subsequent inspection later, the dark spot areas will be visible. The users should be made aware that developing or interpreting a partially dried TLC plate can affect the results.

Figure 17: Minilab training during field evaluation in Indonesia

5. Review and Conclusions

5.1. Performance Evaluation

Results from the evaluation indicated that Minilab can identify APIs in drug products for various dosage forms. In addition, semiquantitative analysis can be done to determine how much of the API is present in the product. All drug products tested generated spots corresponding to their reference standards for the 100% sample. The 120% sample showed a more intense spot than the reference, indicating too much of the API. The 90%, 80%, 70%, and 50% showed less intense spots than the reference, indicating Minilab may be able to semi quantify the amount of API in a product. In analyzing the samples, there was good agreement between the product spots and reference spots of equal concentration. It is of critical importance to follow the sample preparation procedures closely, since an error in dilution or chemical use can lead to results that could inaccurately label a product as SF.

5.2. Field Evaluation

Based on the feedback survey from the trainees and the ongoing observations of the trainers, the training required to become a basic, intermediate, or advanced user of the Minilab kit was manageable. More specifically, most of the trainees (22 out of 26, or 84.6%) with both technical and non-technical backgrounds indicated that an individual could become an advanced user within two weeks of training. Trainees were able to use the items in the kit appropriately to prepare samples as per the procedures in the manual, perform the spotting, develop the TLC plates, perform simple disintegration, and enter the required data on sample forms (including visual observations made on the products and calculation of the Rf values). They were also able to follow laboratory safety precautions and handle chemicals appropriately, including disposal. The kit is portable, self-contained, and packaged in a rugged box, which makes it easy to carry and use in remote settings. It is necessary to routinely replace the used consumables, particularly the secondary standards upon expiry, chemicals, and TLC plates.

References

[1] PQM, *Annual Performance Report FY 2017*, Promoting the Quality of Medicines program, Rockville, MD, 2017.

[2] IOM, *Countering the Problem of Falsified and Substandard Drugs*, Institute of Medicine (now the National Academies of Sciences, Engineering, Medicine), Washington, D.C., 2013.

[3] WHO, *WHO Model List of Essential Medicines, 20th List (March 2017)*, March 2017b. [Online]. Available: http://www.who.int/medicines/publications/essentialmedicines/20th_EML2017.pdf?ua=1. [Accessed 10 December 2017].

[4] USP. USP 43–NF 38., USP Review of Surveillance and Screening Technology for the Quality Assurance of Medicines Expert Panel, <1850>: EVALUATION OF SCREENING TECHNOLOGIES FOR ASSESSING MEDICINE QUALITY. Rockville, MD, United States Pharmacopeial Convention; To be official November 2020. [Online] P. 8626. DocID: GUID-E43742F7-8522-4D65-91B3-B6E9D33461AC_2_en-U

Annex 1: Sample Materials Used During Performance Evaluation

Item	Acronym	Manufacturer/Source	Lot No.	Expiry
amoxicillin (250 mg) capsules	AMX	Sandoz	HG9361	March 2020
amoxicillin (500 mg) capsules	AMX	Sandoz	GS0051	March 2020
amoxicillin (500 mg) tablets	AMX	Teva Pharmaceutical Industries Ltd.	35442174A	March 2020
artemether (20 mg) + lumefantrine (120 mg) tablets*	AL	Ipca Laboratories Ltd.	DY1466166	April 2018
artemether (80 mg) + lumefantrine (480 mg) tablets	AL	Novartis	K0050	October 2018
chlorhexidine (4% w/w) digluconate gel	CHX	Lomus Pharmaceuticals, Pvt. Ltd	KW1.0616	February 2019
chlorhexidine (4% w/w) digluconate gel	CHX	NA	326L15	February 2023
rifampicin (150 mg) + isoniazid (75 mg) tablets	RH	Phapros	6159001	April 2020
rifampicin (150 mg) + isoniazid (150 mg) + ethambutol HCL (275 mg) tablets	RHE	Macleods Pharmaceuticals Ltd.	ERD2706B	May 2019
rifampicin (150 mg) + isoniazid (75 mg) + pyrazinamide (400 mg) + ethambutol HCl (275 mg) tablets	RHZE	Lupin Pharmaceuticals, Inc.	A603606	May 2019
rifampicin (150 mg) + isoniazid (75 mg) + pyrazinamide (400 mg) + ethambutol HCl (275 mg) tablets	RHZE	Macleods Pharmaceuticals Ltd.	ERC6690C	May 2018
sulfamethoxazole (200 mg) + trimethoprim (40 mg) oral suspension	ST1	BDH Industries Ltd.	D-10217	January 2020

**Sample had expired at the time of the analysis.*

Annex 2: Protocol for Evaluation of TLC Procedures

Subject: Minilab Technology Review

Objective: To evaluate selected Minilab TLC procedures.

Requested Work Summary

Analyze samples following Minilab TLC procedures with additional concentrations for comparison purposes.

Background

USP's Technical Review program is evaluating selected Global Pharma Health Fund Minilab procedures. The request included evaluation of the method by two analysts. An analyst from the Reference Standard Laboratory (RSL) has evaluated the procedures. The PQM Laboratory Services Group will provide the second analyst.

Sample Details

A. The following drug products[3] have been selected from the current WHO Essential Medicines List to represent a variety of active ingredients, dosage strengths, and dosage forms:

1. Amoxicillin capsules
2. Artemether + lumefantrine tablets
3. Chlorhexidine gluconate topical gel
4. Rifampicin + isoniazid + pyrazinamide + ethambutol tablets
    a. Rifampicin + isoniazid tablets
    b. Rifampicin + isoniazid + ethambutol tablets
5. Sulfamethoxazole + trimethoprim for oral suspension (cotrimoxazole)

[1]The sample list will be adjusted according to sample availability.

Sample	Manufacturer	Lot No.	Expiry
Amoxicillin capsules (250 mg)	Sandoz	HG9361	March 2020
Amoxicillin capsules (500 mg)	Sandoz	GS0051	March 2020
Amoxicillin tablets (500 mg)	Teva	35442174A	March 2020
Artemether (20 mg) + lumefantrine (120 mg)	Ipca Laboratories	DYI466166	April 2018
Artemether (80 mg) + lumefantrine (480 mg)	Novartis	K0050	October 2018
Chlorhexidine 4% w/w digluconate gel, Kawach gel	Lomus	KW1.0616	February 2019
Chlorhexidine 4% w/w digluconate gel, umbilical gel	N/A	326 L 15	February 2023
Rifampicin (150 mg) + isoniazid (75 mg) tablets	Phapros	6159001	April 2020
Rifampicin (150 mg) + isoniazid (75 mg) + ethambutol hydrochloride (275 mg) tablets	Macleods Pharma	ERD2706B	May 2019
Rifampicin (150 mg) + isoniazid (75 mg) + pyrazinamide (400 mg) + ethambutol hydrochloride tablets (275 mg)	Lupin LTD	A603606	May 2019
Rifampicin (150 mg) + isoniazid (75 mg)+ pyrazinamide (400 mg) + ethambutol hydrochloride tablets (275 mg)	Macleods Pharma	ERC6690C	May 2018
Sulfamethoxazole (200 mg) + trimethoprim (40 mg) for oral suspension	BDH Industries	D-10217	January 2020

B. Minilab semi-quantitative capabilities will be determined by:

1. Comparing the samples to standards using the Minilab methods.
   Standards to be prepared at concentrations from the Minilab methods.
   Samples prepared at varying concentrations (50%, 70%, 90%, and 120%)
   Method details
   - Perform test on the target formulations identified above in section A
   - Perform the test using the Minilab-supplied standard material
     - Prepare the standards at 100% and 80% as per the method
     - Prepare the samples at dilutions to obtain the other required concentrations
       - Use calibrated pipets to prepare
   - Follow procedures in Minilab method for developing solutions for the detection
     - Note: Will not be able to put all spots on one Minilab TLC 5 x 10 cm plate
       - Follow the Minilab Spotting procedure (100% in far-left lane, 80% in far-right lane) on all plates
       - Please prepare three plates
         - Plate 1:
           - Lane 1: 100% standard

- Lane 2: 100% sample
- Lane 3: 100% sample
- Lane 4: 80% standard

- Plate 2:
  - Lane 1: 100% standard
  - Lane 2: 120% sample
  - Lane 3: 90% sample
  - Lane 4: 80% standard

- Plate 3:
  - Lane 1: 100% standard
  - Lane 2: 70% sample
  - Lane 3: 50% sample
  - Lane 4: 80% standard

- Expected results:
  - Photos of TLC plates
  - Analyst to report if they can visually detect any differences between the spots

- See Table A. 1 below for the expected concentrations

Table A. 1: Varying Sample Concentrations

#	**Minilab Method[3]**	API	120% mg/mL	**100%* mg/mL**	90% mg/mL	**80%* mg/mL**	70%* mg/mL	50% mg/mL
1	Amoxicillin capsules	Amoxicillin	3.00	**2.5**	2.25	**2.00**	1.75	1.25
2a	Artemether + lumefantrine tablets	Artemether	2.4	**2.0**	1.80	**1.60**	1.4	1.0
2b	Artemether + lumefantrine tablets	Lumefantrine	0.96	**0.8**	0.7	**0.64**	0.56	0.40
3	Chlorhexidine gluconate gel	Chlorhexidine	0.6	**0.5**	0.45	**0.40**	0.35	0.25
4a	Rifampicin + isoniazid + ethambutol tablets	Rifampin	2.4	**2.0**	1.8	**1.6**	1.4	1.0
4a	Rifampicin + isoniazid + ethambutol tablets	Isoniazid	3.0	**2.5**	2.25	**2.0**	1.75	1.25

4b	Rifampicin + isoniazid + ethambutol tablets	Pyrazinamide	1.5	**1.25**	1.125	**1.0**	0.875	0.625
4c	Rifampicin + isoniazid + ethambutol tablets	Ethambutol	1.5	**1.25**	1.125	**1.0**	0.875	0.625
5	Sulfamethoxazole + trimethoprim for oral suspension	Sulfamethoxazole	6.0	**5.0**	4.5	**4.0**	3.5	2.5
		Trimethoprim	1.2	**1.0**	0.9	**0.8**	0.7	0.5

[3]Minilab methods source: "A Concise Quality Control Guide on Essential Drugs and Other Medicines, Volume II, Thin Layer Chromatographic Tests", and supplements

*The concentrations of the standards (100% and 80%) are the same as specified in the specific Minilab procedure for the sample .