USP Technology Review: Paper Analytical Device (PAD)

Report Date: October 2020

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Executive Summary

The paper analytical device (PAD) was developed as a cost-effective tool for field screening of a wide variety of pharmaceutical dosage forms in low-resource settings. The PAD is produced by wax printing on Ahlstrom 319 paper, which is a fast chromatography paper that creates separate reaction lanes and contains trace quantities of chemical reagents to create color changes in response to different pharmaceutical articles. Due to the COVID-19 pandemic, there has been continued upsurge of substandard and falsified (SF) medical products, especially those that claimed to be a possible treatment for the disease (e.g., azithromycin, chloroquine, and hydroxychloroquine), and which the PAD can be used to screen the products for quality. With the help of appropriate analytical techniques and methodologies, a preliminary laboratory study was conducted on all the samples used in the performance validation of the PAD to ascertain the quality status of each sample prior to the validation study on the PAD. The validation study was performed on the PAD by three different scientists using three different brands each of seven finished dosage forms (FDFs), their respective active pharmaceutical ingredients (APIs), SF formulations, and selected fillers (pharmaceuticals excipients). Upon evaluation of all data generated by the three scientists at the end of the validation study, the PAD was determined to be effective in identifying the active ingredients in all the samples collected for evaluation. The technology was also able to detect fillers such as corn starch present in some of the FDFs and formulations labeled to contain them. Results of the laboratory identification tests performed were consistent with those obtained with PAD by all three scientists. All SF formulations were prepared as per the study protocol, and all falsified formulations were correctly identified as falsified by the PAD. However, the PAD was incapable of distinguishing between substandard or degraded formulations (even those with 50% APIs) and good quality products and formulations and their respective pure APIs, making it impossible for the technology to be used to screen substandard products. Only falsified products were able to be identified correctly.

During field evaluation, the PAD was found to be easy to use, with little skill required for sample preparation and interpretation of results and new users only needed minor training. PAD was able to provide results within 5 minutes and requiring only a small working space. Also, no chemicals or reagents are required for sample preparation and development. However, the PAD is not able to sustain the color result for a long period, requiring users to read the result outcome immediately.

Acknowledgments

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- National Drug Authority (NDA), Uganda

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Acronyms

API	Active Pharmaceutical Ingredient
FDF	Finished Dosage Form
FPP	Finished Pharmaceutical Product
HPLC	High-Performance Liquid Chromatography
IH	In-House
PAD	Paper Analytical Device
Ph. Int.	International Pharmacopeia
RS	Reference Standards
SF	Substandard or Falsified
USP	United States Pharmacopeia

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's Technology Review Program, an initiative guided by a USP technical Expert Panel established through the organization's collaborative and volunteer-driven governance 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 substandard and falsified (SF) medicines.
4. Foster the development and enhancement of new and emerging screening technologies.

This report contributes directly to objectives 2, 3, and 4, and is part of an ongoing series evaluating the capabilities of various promising screening technologies. The paper analytical device (PAD) has been developed as a cost-effective tool for field screening a variety of pharmaceutical dosage forms in low-resource settings. It is a presumptive test that employs the concept of thin-layer chromatography to identify SF products that are at a high risk of causing harm to patients. The PAD can identify pharmaceutical products that do not contain the stated active pharmaceutical ingredients (APIs) or that contain substitute APIs. Since this is a screening test, it must be followed up by more accurate laboratory testing, such as high-performance liquid chromatography (HPLC) to confirm the result. The ability to detect substandard APIs with a color test requires that the test results lie in the linear range of the color test. For some APIs, the test results lie in the saturated range of the color tests. In these cases, the test can only be used for determining the presence or absence of the API. During the COVID-19 pandemic, some pharmaceutical products are being promoted as treatment for the disease (e.g., azithromycin, chloroquine, and hydroxychloroquine). This has fueled a surge in SF products for these medicines [3]. The PAD is one of the screening technologies that can be used to identify these SF products and was selected for review because of claims regarding its technical capabilities, simplicity of use, cost, and ability to be used in remote settings. USP's Technology Review Program decided to review PAD with input from the Expert Panel.

2. Methodology

2.1. General Information

Table 1 provides general information of the PAD and its functions, manufacturer, basic specifications, and upfront and recurring costs. All data in this section was collected between March 2020 and August 2020 through email exchange, virtual conversations, and review of the vendor's protocol for evaluating the technology.

Table 1: General Information

Technology	The PAD is developed as a cost-effective screening tool for field screening of a wide variety of pharmaceutical dosage forms in low-resource settings. The PAD is produced by wax printing on Ahlstrom 319 paper, a fast chromatography paper to create separate reaction lanes and that contains trace quantities of chemical reagents to create color changes in response to different pharmaceutical articles. PAD is currently available from the Lieberman research group, University of Notre Dame, Indiana, U.S. Information about the PAD is available on its project website https://padproject.nd.edu.
Specifications*	*Dimensions*: 7 cm x 11 cm x 400 µm *Weight*: 1.5 g *Power source*: No power required *Composition*: Cellulose paper, wax, trace quantities of chemical reagents *Language*: English *Operational temperature*: 15–40˚C *Disposal*: Safe to discard in trash *Security features*: Individually serialized and contains serial numbers that can be assigned to specific projects
Cost*	*Upfront costs* • $2 USD plus cost of mailing (Available in packs of 10 cards at $20/pack or 20 cards at $40/pack) • Training and certification pack: $40 USD (includes 14 PADs and 14 blinded samples) *Recurring costs* • No recurrent costs

*Source: Lieberman Research Group, University of Notre Dame

Data

The PAD performs twelve chemical tests on each sample and the results are displayed as a color bar code which is read by comparing it to pictures of known good samples. USP scientists and NDA Uganda staff also captured the results and sent them to the PAD developer through an app developed to compare the pictures of results obtained but this was not evaluated during the review. USP had no access to the pictures captured to evaluate them.

Access, Handling, Maintenance, and Repair

The PAD is currently available from the Lieberman research group, University of Notre Dame in Indiana, U.S. at a cost of $2 per PAD plus mailing costs. They are supplied in packs of 10 or 20 units that are heat-sealed in a metallized zip-top bag. No maintenance or repairs are required since the PAD is a single-use product.

Durability

PADs sealed in their packaging are stable for at least 12 months if stored in a refrigerator, and stable for at least 4 months under tropical conditions. Once the zip-top bag is opened, the PADs should be stored in the bag and used within 2 weeks.

Use

The PAD is able to identify the APIs in drug samples formulated as tablets, capsules, and powdered injections. The PAD is also able to detect fillers such as corn starch in some FDFs. However, it cannot identify slightly substandard or degraded products (e.g., if the API content is 50% in some formulations).

Table 2: List of Finished Dosage Forms (FDFs) Used in PAD Validation Study

Sample	Content and Strength	Batch /Lot	Manufacturer/ Source	Lab Code	Expiry Date
Amoxicillin capsules	Amoxicillin 500 mg	AXBBV0071	Brown and Burk	PAD/20/001	05/2022
Amoxicillin capsules	Amoxicillin 500 mg	1230239	Letap Pharmaceuticals	PAD/20/003	08/2021
Amoxicillin capsules (Exeter)	Amoxicillin 500 mg	1999013	Exeter Pharmaceuticals	PAD/20/002	06/2022
Azilex capsules	Azithromycin 250 mg	17	Luex	PAD/20/012	02/2022
Azitex capsules	Azithromycin 500 mg	BL90008	Exeter Pharmaceuticals	PAD/20/011	07/2022
Chloroquine tablets	Chloroquine phosphate 250 mg	0104W	Ernest Chemist	PAD/20/016	04/2023
Chlorquine tablets	Chloroquine phosphate 100 mg	02	Quantum Pharmacy	PAD/20/017	06/2022
Ciprolex tablets	Ciprofloxacin 500 mg	169	Luex	PAD/20/007	02/2022
Cipromax	Ciprofloxacin 500 mg	X03843	Phyto-Riker	PAD/20/008	02/2021
Doxycycline capsules	Doxycycline 100 mg	0810V	Ernest Chemist	PAD/20/013	10/2022
Doxycycline capsules	Doxycycline 100 mg	03	Eskay Therapeutic Ltd	PAD/20/014	02/2022
Doxycycline capsules	Doxycycline 100 mg	1360119	Letap Pharmaceuticals	PAD/20/015	10/2021
G-Ceftria (GPSC)	Ceftriaxone 1g	181207	Sinopharm Weiqida	PAD/20/004	05/2021
Inno-Ceft	Ceftriaxone 1g	10119248	O&J Pharmaceuticals	PAD/20/005	10/2021
Lextriax powder for injection	Ceftriaxone 1g	193051225	Luex	PAD/20/006	08/2022
Maxiquine	Chlorquine phosphate 250 mg	T29919	Vitabiotics	PAD/20/018	09/2024
Quinoric tablets	Hyrdroxychlorquine 200 mg	DET059028	Bristol	PAD/20/019	11/2023
Rhumatas tablets	Hyrdroxychlorquine 200 mg	M2006183	Intas	PAD/20/021	02/2022
Shalcip 500	Ciprofloxacin 500 mg	J9009	Shalina	PAD/20/009	11/2022
Zentiva (Hydroxychloroquine) tablets	Hyrdroxychlorquine 200 mg	9R878	Zentiva	PAD/20/020	03/2022
Zymax capsules	Azithromycin 500 mg	0103W0	Ernest Chemist	PAD/20/010	03/2023

Additional details about equipment, material, and all samples used can be found in the Annexes.

2.2 Procedure

In order to validate the PAD, three different brands each of seven finished pharmaceutical products, seven APIs, 14 falsified, and 14 substandard drug formulations were analyzed by three different scientists. Prior to the validation study, a preliminary study was performed to establish the quality status of all the FDFs, excipients (fillers), and APIs used in the PAD validation with the assistance of appropriate laboratory techniques and the summary of tests performed on each of them. The results obtained are provided in Annex 7.

2.3 Samples preparation

All sample preparations were carried out by each of the scientists as follows:

Amoxicillin

- API: Fifty milligrams of pure API was weighed in an aluminum weighing boat.
- Dosage forms: Three capsules of amoxicillin trihydrate (500 mg dosage) from each of the three different brands were obtained and emptied.
- Falsified formulation #1: Seven hundred milligrams of chalk (calcium carbonate) and 300 mg of corn starch were uniformly mixed, and 50 mg was weighed in an aluminum weighing boat.
- Falsified formulation #2: Fifty milligrams of paracetamol was weighed in an aluminum weighing boat.
- Substandard formulation #1: Five hundred milligrams of talcum powder and 500 mg of pure amoxicillin API were mixed. Fifty milligrams of the mixture were weighed in an aluminum weighing boat.
- Substandard formulation #2: Five hundred milligrams of corn starch and 500 mg of pure amoxicillin API were mixed, and 50 mg was weighed in an aluminum weighing boat.

Azithromycin

- API: Fifty milligrams of pure azithromycin API was weighed in an aluminum weighing boat.
- Dosage forms: Three capsules of azithromycin (250 mg dosage) from three different brands were obtained and emptied.

- Falsified formulation #1: Seven hundred milligrams of chalk (calcium carbonate) and 300 mg of corn starch were uniformly mixed. Fifty milligrams of the mixture were weighed and smeared across the PAD. Its base was immersed in water for 3 minutes.
- Falsified formulation #2: Fifty milligrams of paracetamol was weighed in an aluminum weighing boat.
- Substandard formulation #1: Five hundred milligrams of talcum powder and 500 mg of pure azithromycin API were mixed, and 50 milligrams was weighed in an aluminum weighing boat.
- Substandard formulation #2: Five hundred milligrams of corn starch and 500 mg of pure azithromycin API were mixed. Fifty milligrams of the mixture were weighed in an aluminum weighing boat.

Ceftriaxone

- API: Fifty milligrams of pure ceftriaxone API was weighed in an aluminum weighing boat.
- Dosage forms: Three different brands of ceftriaxone powder for injection were obtained. Fifty milligrams was weighed in an aluminum weighing boat.
- Falsified formulation #1: Five hundred milligrams of sodium chloride was weighed and powdered. Fifty milligrams of the sodium chloride was weighed in an aluminum weighing boat.
- Falsified formulation #2: Five hundred milligrams of sucrose was weighed and powdered. Fifty milligrams of sucrose were weighed in an aluminum weighing boat.
- Substandard formulation #1: Five hundred milligrams of powdered sodium chloride and 500 mg of pure ceftriaxone API were mixed. Fifty milligrams of the mixture were weighed in an aluminum weighing boat.
- Substandard formulation #2: Five hundred milligrams of powdered sucrose and 500 mg of pure ceftriaxone API were mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.

Chloroquine

- API: Fifty milligrams of pure chloroquine API was weighed in an aluminum weighing boat.
- Dosage forms: Three tablets of chloroquine (250 mg and 100 mg dosage) from each of the three different brands were obtained and powdered.
- Falsified formulation #1: Seven hundred milligrams of chalk (calcium carbonate) and 300 mg of corn starch were uniformly mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.
- Falsified formulation #2: Fifty milligrams of paracetamol was weighed in an aluminum weighing boat.
- Substandard formulation #1: Five hundred milligrams of talcum powder and 500 mg of pure chloroquine API were mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.
- Substandard formulation #2: Five hundred milligrams of corn starch and 500 mg of pure chloroquine API were mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.

Ciprofloxacin

- API: Fifty milligrams of pure ciprofloxacin API was weighed in an aluminum weighing boat.
- Dosage forms: Three tablets of ciprofloxacin tablets from each of the three different brands were obtained and powdered.
- Falsified formulation #1: Seven hundred milligrams of chalk (calcium carbonate) and 300 mg of corn starch were uniformly mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.
- Falsified formulation #2: Fifty milligrams of paracetamol was weighed in an aluminum weighing boat.
- Substandard formulation #1: Five hundred milligrams of talcum powder and 500 mg of pure ciprofloxacin API were mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.
- Substandard formulation #2: Five hundred milligrams of corn starch and 500 mg of pure ciprofloxacin API were mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.

Doxycycline

- API: Fifty milligrams of pure doxycycline API was weighed in an aluminum weighing boat.
- Dosage forms: Three capsules of doxycycline (500 mg dosage) from each of the three different brands were obtained and emptied.
- Falsified formulation #1: Seven hundred milligrams of chalk (calcium carbonate) and 300 mg of corn starch were uniformly mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.
- Falsified formulation #2: Fifty milligrams of paracetamol was weighed in an aluminum weighing boat.
- Substandard formulation #1: Five hundred milligrams of talcum powder and 500 mg of pure doxycycline API were mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.
- Substandard formulation #2: Five hundred milligrams of corn starch and 500 mg of pure doxycycline API were mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.

Hydroxychloroquine

- API: Fifty milligrams of pure hydroxychloroquine API was weighed in an aluminum weighing boat.
- Dosage forms: Three tablets of hydroxychloroquine (100 mg dosage) from each of the three different brands were obtained and powdered.
- Falsified formulation #1: Seven hundred milligrams of chalk (calcium carbonate) and 300 mg of corn starch were uniformly mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.
- Falsified formulation #2: Fifty milligrams of paracetamol was weighed in an aluminum weighing boat.
- Substandard formulation #1: Five hundred milligrams of talcum powder and 500 mg of pure hydroxychloroquine API were mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.
- Substandard formulation #2: Five hundred milligrams of corn starch and 500 mg of pure hydroxychloroquine API were mixed. Fifty milligrams of the mixture was weighed in an aluminum weighing boat.

2.4 Loading and Running of the PAD

The procedure for loading and running the sample was strictly followed by all three scientists. All PADs used by the scientists were coded for traceability. Using a spatula, each of the prepared samples listed above were placed on the PAD at the arrow mark and spread from one arrow mark to the other, across all 12 lanes of the PAD. The powder was pressed down and made to stick to the paper. The PAD was turned on its side to tap off excess powder, making sure that there was powder in each lane and the lines between the lanes were visible. The bottom edge of the PAD was then placed upright in 1 cm of water for about 3 minutes. The PAD was then removed from the developing solvent (water) when a red dot appeared at the top of lane A and laid flat on a clean piece of paper for another 3 minutes before being photographed. Results obtained were then compared with expected results and the data was interpreted appropriately. *See PAD validation study results under performance evaluation results section.*

Methodology Limitations

Certain limitations were encountered during this performance evaluation. They are identified below:

- Only seven different drug product samples were analyzed to target the specific COVID-19 products that were being promoted as possible treatments, including a few antibiotics. This represents a small fraction of the medicines in the World Health Organization's essential medicines list. More drug samples may need to be analyzed to validate the technology use for other drug products.
- No pure ceftriaxone APIs was included in the evaluation as per the protocol. This was unavailable locally and therefore one of the brands of ceftriaxone powder for injection was used in place of the pure ceftriaxone API.
- Ciprofloxacin and doxycycline APIs were used in their respective laboratory formulations as their assay results from the laboratory preliminary study did not meet pharmacopeial requirements.

3. Results

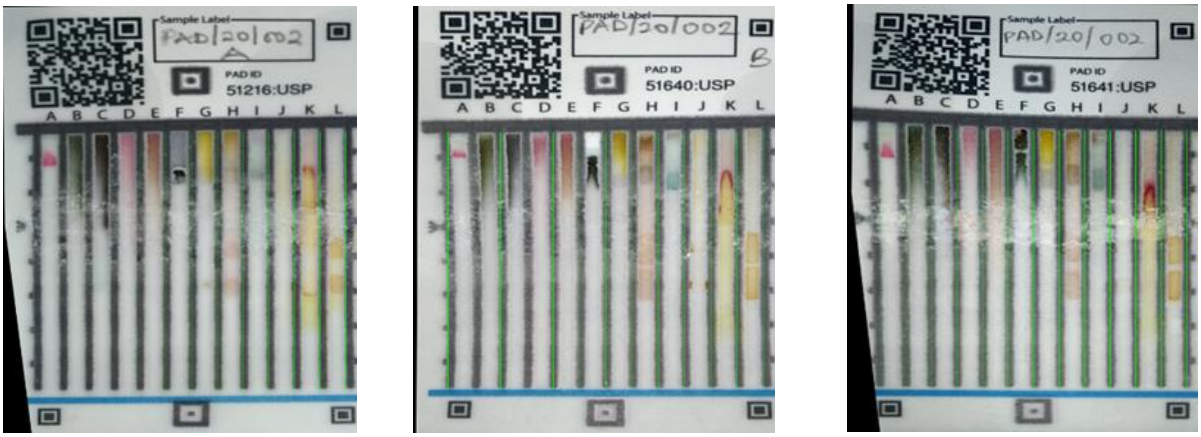
3.1. Performance Evaluation

Performance evaluation involved validation of the PAD characteristics in the laboratory. Variables were controlled to evaluate the technology 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 APIs in finished pharmaceutical products. All of the data below was collected between July 2020 and August 2020.

Amoxicillin

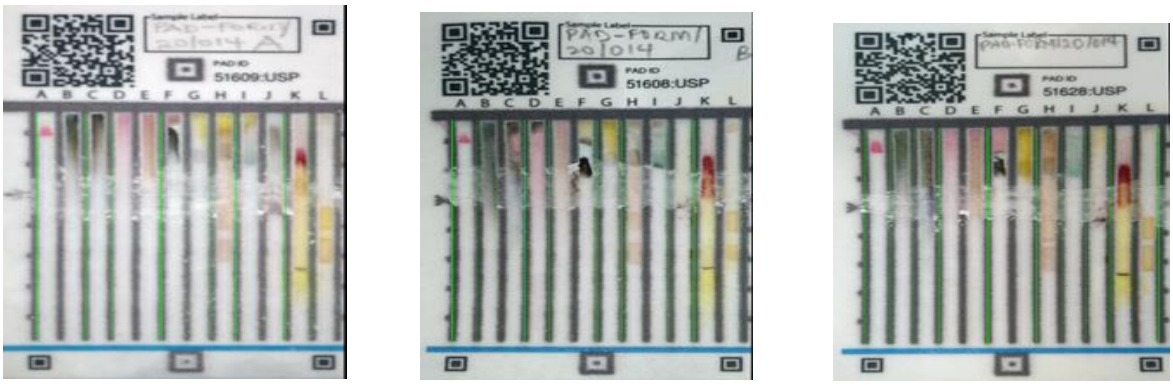
Eight samples were evaluated by three scientists. The scientists obtained similar results for all the samples tested. Samples containing the correct amount of amoxicillin and substandard samples produced the same colors (olive green in lane B, dark green black in lane F, cherry red in lane K, and no black in lane J). Falsified formulations produced different colors in the same lanes indicating the product had a different API

Figure 1a: Results of a quality amoxicillin product



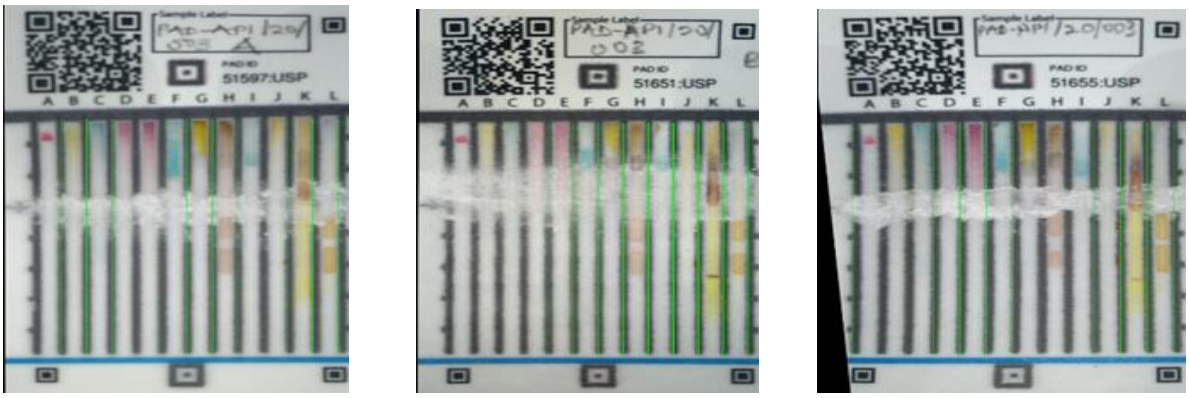
Samples containing the correct amount of amoxicillin produced olive green in lane B, dark green black in lane F, and cherry red in lane K

Figure 1b: Results of a substandard amoxicillin product



Substandard samples showing the same colors as quality samples (olive green in lane B, dark green black in lane F, and cherry red in lane K

Figure 1c: Result of a falsified amoxicillin formulation

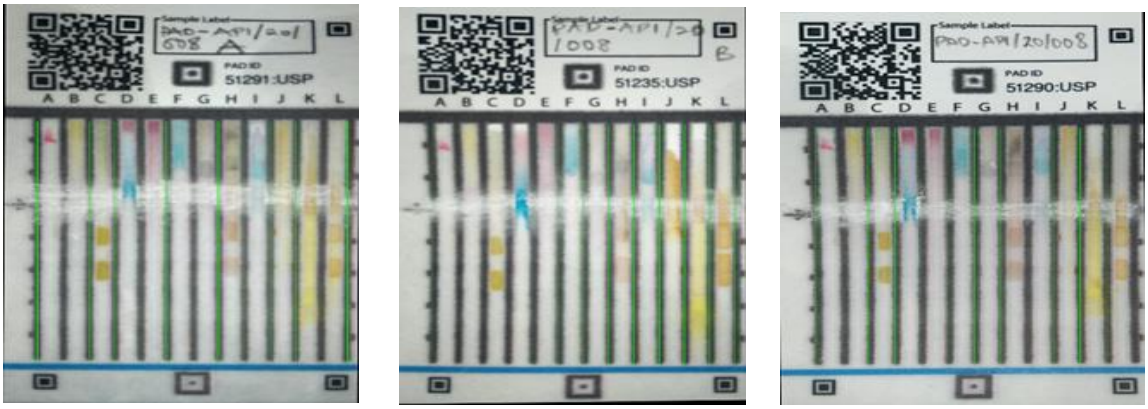


Falsified formulations showing different colors in lanes B, F, and K indicating the formulation had a different API

Azithromycin

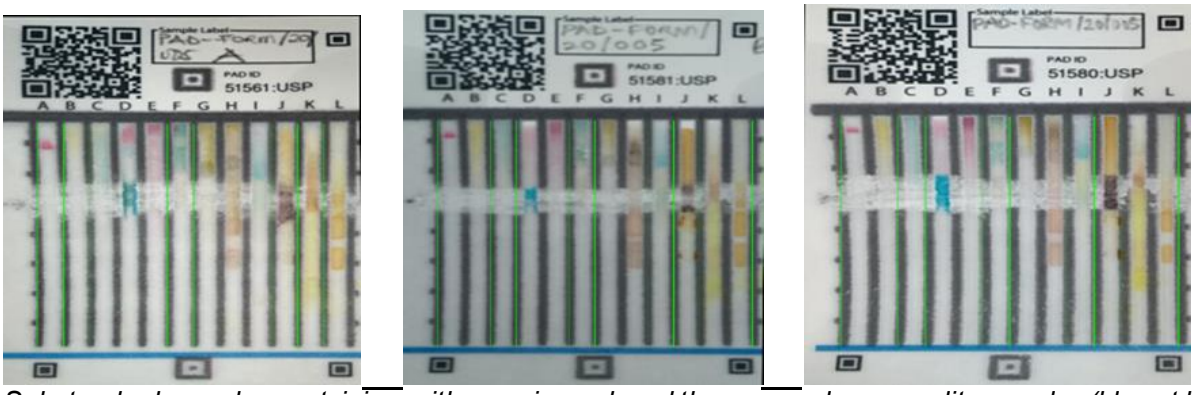
Eight samples were evaluated, and the scientists obtained similar results for all the samples tested. Samples containing the correct amount of azithromycin and substandard samples produced the same color (blue at lane D). Falsified samples produced a different color in lane D

Figure 2a: Results of a quality azithromycin formulation



Samples containing the correct amount of azithromycin produced blue color at lane D

Figure 2b: Results of a substandard azithromycin formulation



Substandard samples containing azithromycin produced the same color as quality samples (blue at lane D)

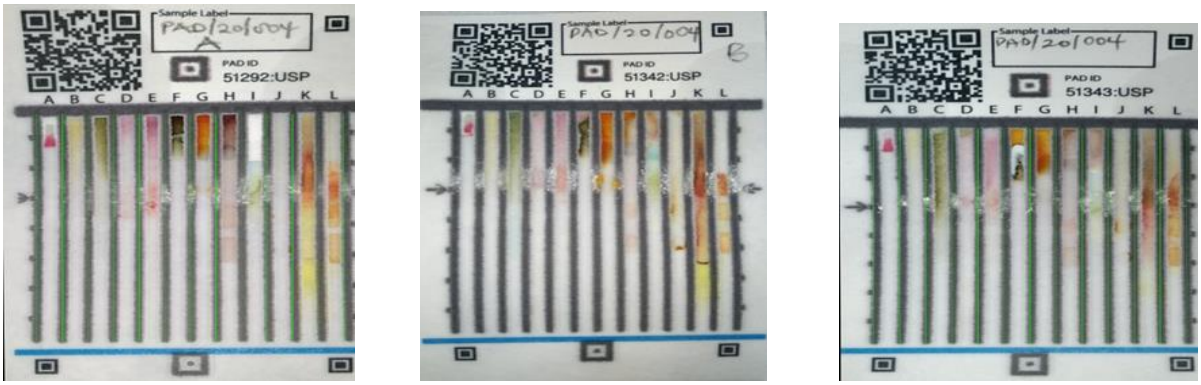
Figure 2c: Results of a falsified azithromycin formulation

Falsified samples showing a different color in lane D

Ceftriaxone

The USP scientists obtained similar results for all the samples tested. Samples containing the correct amount of ceftriaxone and substandard samples produced the same colors on all lanes (green on lane C, olive green on lane F, gold on lane G, purple brown on lane H, no black on lane J, a black streak on top of the red color on lane K, and orange/red on lane L). The falsified product did not show corresponding colors on those lanes

Figure 3a: Results of a quality ceftriaxone formulation



Samples containing the correct amount of ceftriaxone (green on lane C, olive green on lane F, gold on lane G, purple brown on lane H, no black on lane J, a black streak on top of the red color on lane K, and orange/red on lane L)

Figure 3b: Results of a substandard ceftriaxone formulation

Substandard samples indicating the same colors as quality samples (green on lane C, olive green on lane F, gold on lane G, purple brown on lane H, no black on lane J, a black streak on top of the red color on lane K, and orange/red on lane L)

Figure 3c: Results of a falsified ceftriaxone formulation

Falsified samples showing different colors on lanes C, F, G, H, J, K and L

Chloroquine

Eight samples were evaluated, and the scientists obtained similar results for all the samples tested. Samples containing the correct amount of chloroquine and substandard samples produced the same color on lanes D and E (deep blue on lane D and E). Falsified products produced a different color on the same lanes, an indication the product contained a different API.

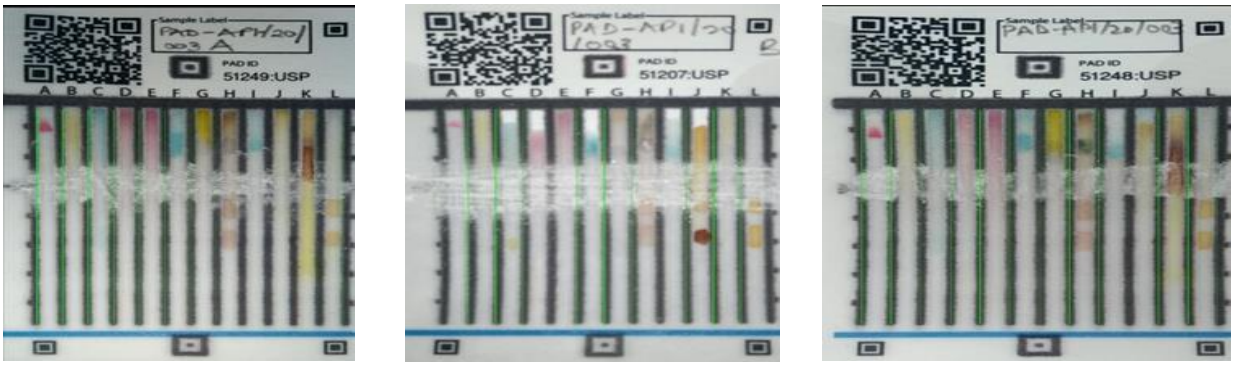
Figure 4a: Results of a quality chloroquine formulation

Samples containing the correct amount of chloroquine showing expected colors on lanes D and E (deep blue on lanes D and E)

Figure 4b: Results of a substandard chloroquine formulation

Substandard samples with the same colors as quality samples on lanes D and E (deep blue on lane D and E)

Figure 4c: Results of a falsified chloroquine formulation



Falsified samples showing different colors on lanes D and E

Ciprofloxacin

Eight samples were evaluated, and the scientists obtained the same results for all the samples tested. Samples containing the correct amount of ciprofloxacin and substandard samples produced the same color (blue at swipe line at lane D and orange at lane L). Falsified products produced different colors at those same lanes.

Figure 5a: Results of a quality ciprofloxacin formulation

Samples containing the correct amount of ciprofloxacin showing the correct colors at lanes D and L (blue at swipe line at lane D and orange at lane L)

Figure 5b: Results of a substandard ciprofloxacin formulation

Substandard samples showing the same colors as quality samples (blue at swipe line at lane D and orange at lane L)

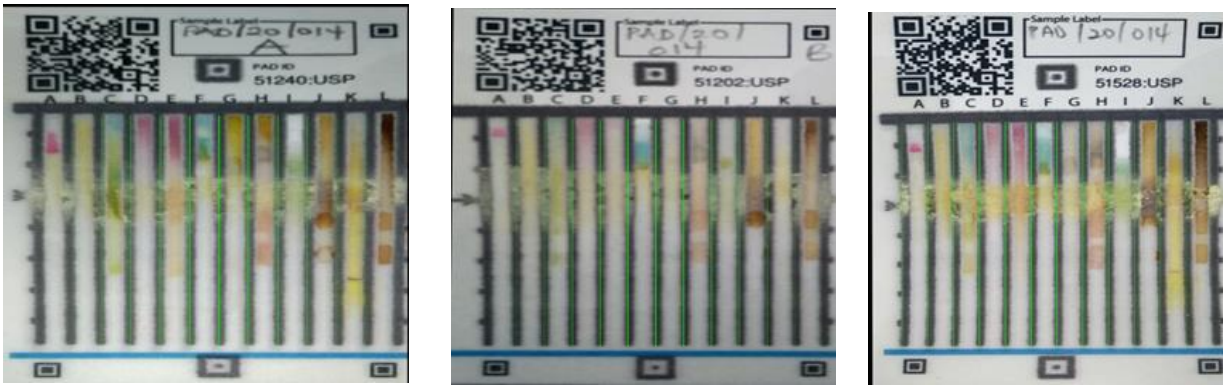
Figure 5c: Results of a falsified ciprofloxacin formulation

Falsified formulation of ciprofloxain showing different colors on lanes D and L

Doxycycline

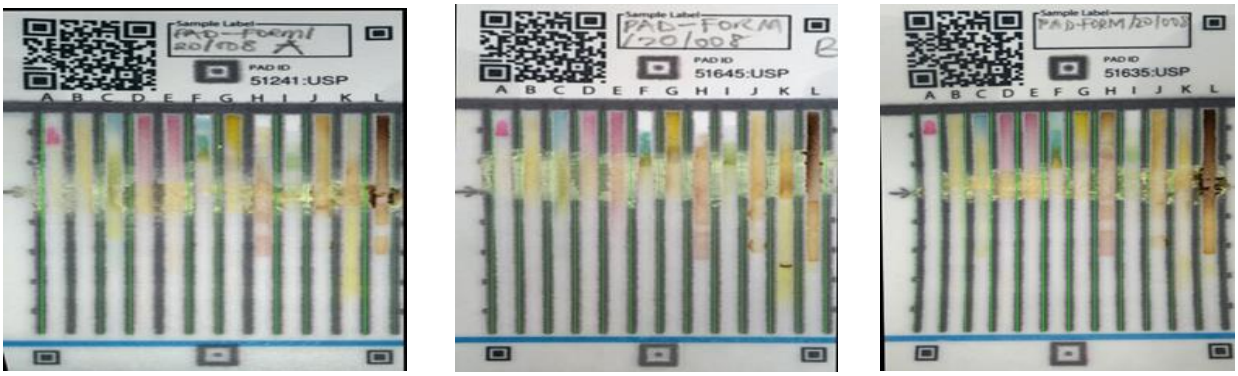
Eight samples were evaluated, and the scientists obtained the same results for all the samples tested. Samples containing the correct amount of doxycycline and substandard samples produced same color on lane L (brown). One of the substandard formulations produced a black color in lane J in addition to a brown color in lane L, indicating the product contained corn starch. Usually capsule formulations are not supposed to contain starch and therefore the PAD can be used to identify capsule formulations containing corn starch instead of the correct API.

Figure 6a: Results of a quality doxycycline formulation



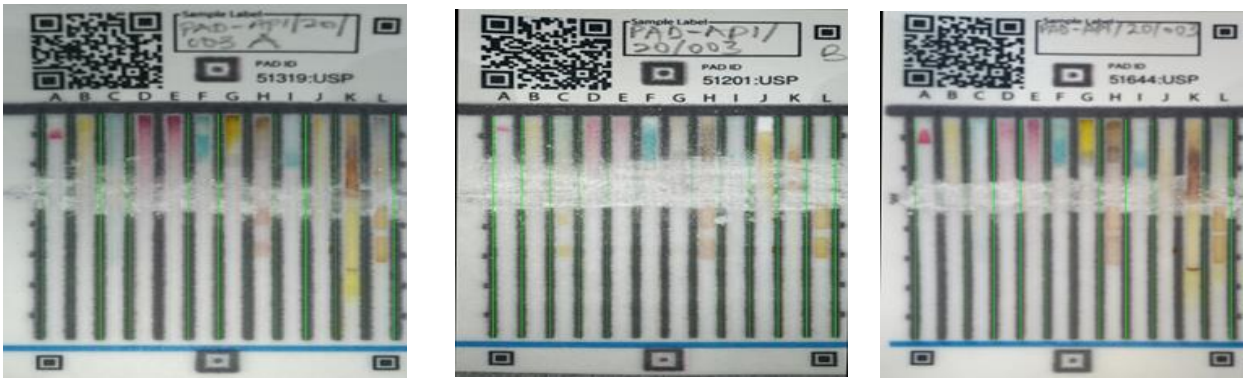
Samples containing the correct amount of doxycycline showing the correct color on lane L (brown).

Figure 6b: Results of a substandard doxycycline formulation



Substandard samples of doxycycline showing same color as quality samples on lane L (brown).

Figure 6c: Results of a falsified doxycycline formulation



Falsified samples of doxycycline with no brown color in lane L

Hydroxychloroquine

Eight samples were evaluated, and the scientists obtained the same results for all the samples tested. Commercial samples containing the correct amount of hydroxychloroquine and substandard samples produced the same colors (deep blue in lanes D and E and black in lane J). The pure API did not produce a black color at lane J since the API does not contain corn starch. The commercial formulations showed a black color at lane J because they contain starch as an ingredient. The falsified samples did not produce deep blue color on lanes D and E.

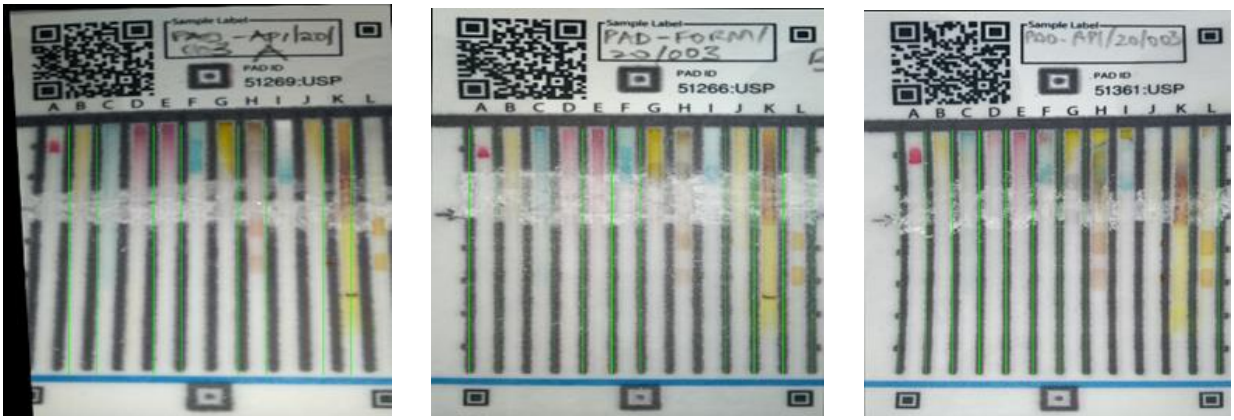
Figure 7a: Results of a quality hydroxychloroquine formulation

Samples containing the correct amount of hydroxychloroquine showing correct colors (deep blue in lanes D and E)

Figure 7b: Results of a substandard hydroxychloroquine formulation

Substandard samples showing same colors as quality samples (deep blue in lanes D and E, and black in lane J).

Figure 7c: Results of a falsified hydroxychloroquine formulation



Falsified samples without deep blue color on lanes D and E

3.2. Field Evaluation

The field evaluation was performed in Kampala, Uganda from June 29, 2020, to July 30, 2020, to evaluate two major parameters: training requirements and field utility. Uganda was selected because it represents a country where the screening technology has not been used in the past but has the potential to be deployed effectively to combat substandard and falsified medicines. In addition, Uganda's regulatory authority, the National Drug Authority (NDA), volunteered to participate in the field evaluation.

Training Requirements

This first component of the field evaluation involved working with and training NDA's staff in Uganda to assess the amount of training required to enable staff to reliably and productively utilize the PAD in the field. The training involved 4 days of virtual training, including hands-on work at the NDA Quality Control (QC) Laboratory. A total of eight staff were trained. The low number of trainees was because there was a limitation on the number of staff who could be at the laboratory due to COVID-19 restrictions, including social distancing. This was followed by several days in the field collecting and testing products for using the PAD. The eight trainees included three quality control specialists, three inspectors, one regulatory officer, and one principal officer.

To evaluate the perceived training timeframes for three levels of use of the technology (basic, intermediate, and advanced), a training timeframe requirements matrix was developed for trainees to complete as a survey following the training. 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 any of the physical sciences (e.g., chemistry, biology).
    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 (following training):
    a. *Basic user*: A user with the ability to follow a standard operating procedure 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.

Field Utility

The second component of the field evaluation involved collecting and testing samples in the field settings and determining the utility of the PAD in these environments. It was also to determine if the use of the PAD is affected by environmental conditions. Four groups of two individuals were formed and the PADs were used in a retail pharmacy, a national general hospital, and a central medical warehouse where samples were collected and analyzed onsite. The exercise was only carried out in the central district of Kampala (capital of Uganda) due to the COVID-19 travel restrictions. The scope of the medicines covered was restricted to seven molecules (APIs). The list of samples collected and tested is shared under Annex 8.

Staff from the National Drug Authority (NDA) during the PAD training at their QC lab

Staff from NDA Uganda carrying out a field evaluation of the PAD

4. Review and Conclusions

4.1. Performance Evaluation

The PAD was able to identify the active ingredients tested in all the brands of pharmaceutical FDFs and in their respective pure raw materials as they showed the appropriate colors in their respective lanes for all three of the scientists. The PAD was able to detect fillers such as corn starch in some tablet dosage forms, namely: azithromycin, chloroquine, and all three brands of hydroxychloroquine. Corn starch was easily seen in lane J as a black color and this agreed with the products information leaflets provided by the drug manufacturers. All falsified formulations tested were correctly identified by the PAD as results from all the scientists were reproducible and comparable.

The PAD was also able to identify all falsified drugs containing fillers such as corn starch or having no or wrong APIs. However, the PAD failed to identify substandard formulations (with up to 50% API) of all the FPP, indicating that the PAD may not differentiate between a quality product and a substandard one, even if the substandard product contained 50% of the API. All substandard formulations produced the appropriate color in their lanes with comparable color intensity as those of their respective APIs and FDFs. In order to increase detection of the colors, the developer has developed an app where the color images captured during analysis can be uploaded and compared with standard colors. However, this app comparison was not evaluated in this study.

4.2. Field Evaluation

Based on feedback from trainees, the training required to become a basic, intermediate, or advanced user of the PAD is reasonable. More specifically, most staff (seven out of eight) with either technical or non-technical backgrounds indicated one can become basic user of the technology within 5 days of training. All the trainees indicated that they could become advanced users of the technology within 7 days of training. Regarding the field utility, the PAD was easy to use in the field settings and all trainees were able to generate results and interpret them easily. The PAD provided results of the medicines tested in the field within 5 minutes, from sample preparation to PAD development. In addition, the teams only required a small working place to carry out the analysis, making it suitable for use in various locations within the field. The sample development procedure only requires water, no other chemicals or reagents, making the technology cheap and cost effective. Also, one dosage unit of a sample was enough to carry out the screening and generate results, making the cost of acquiring samples for testing minimal. The

users of the technology require little skill since sample preparation and interpretation of results is easy. However, some challenges were noted during the use of the PAD in the field. For example, it was not possible to determine whether lack of corn starch in a product meant a product passed or failed the quality test since the users did not have access to details of the authentic product formulation ingredients when in the field. It is therefore recommended for users carrying out the screening to have access to information of the registered authentic product ingredients to enable better conclusions on the product quality. Secondly, the PAD was not able to sustain the color results an hour after the sample development. Sample results need to be read immediately before the colors change. In a case where the app is being used, the pictures need to be captured immediately after development. Also, since this is a color-based detection, the technology may pose a challenge to color blind people who may not be able to interpret results correctly

5. References

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6. Annexes

Annex 1. Table of Reagents Used in Preliminary Laboratory Study

Reagent Name	Source	Lab Code	Expiration Date
Acetic acid glacial	USP Ghana	GAA/20/001	Feb 28, 2022.
Acetonitrile	USP Ghana	ACN/20/011	Aug 07, 2023
Acetonitrile	USP Ghana	ACN/20/013	Aug 13, 2023.
Ammonium hydroxide	USP Ghana	AMH/19/001	Nov 16, 2024
Ammonium oxalate	FDA Ghana	AMO/20/001	N/A
Citric acid	USP Ghana	CTA/20/001	Dec 2021.
Dibasic sodium phosphate	USP Ghana	DSP/19/001	May 29, 2024.
Disodium hydrogen phosphate anhydrous	USP Ghana	DHP/20/001	Aug 06, 2025
EDTA	USP Ghana	EDT/20/001	Dec 31, 2050.
Ethanol absolute	USP Ghana	ETH/20/001	Apr 27, 2025.
Hydrochloric acid	USP Ghana	HCl/18/001	May 17, 2022.
Iodine	USP Ghana	IDN/17/001	Dec 06, 2022
Iron (III) chloride	USP Ghana	IRC/20/001	Aug 03, 2021
Methanol HPLC grade	USP Ghana	MET/20/008	Aug 05, 2023
Methyl red	Danadams Pharmaceuticals	MER/20/001	N/A
pH 2 Standard buffer solution	USP Ghana	PHP/20/004	May 26, 2022.
pH 5 Standard buffer solution	USP Ghana	PHB/19/021	Nov 26, 2021
pH 6 Standard buffer solution	USP Ghana	PHP/20/001	Jan 28, 2022.
pH 8 Standard buffer solution	USP Ghana	PHB/19/022	Apr 2021
pH 11 Standard buffer solution	USP Ghana	PHB/20/005	Nov 2020
Phosphoric acid	USP Ghana	OPA/18/002	Apr 04, 2021
Potassium bromide	USP Ghana	KBR/19/003	Dec 19, 2024.
Potassium dihydrogen phosphate	USP Ghana	PDP/18/001	May 02, 2023
Potassium hydroxide	USP Ghana	PHD/19/001	Aug 06, 2023.
Potassium Iodide	USH Ghana	PID/20/001	Aug 03, 2025
Purified water	USP Ghana	N/A	N/A
Silver nitrate	USP Ghana	SIN/19/001	Mar 29, 2024.
Sodium hydroxide pellets	USP Ghana	SDH/19/001	Jun 20, 2024
Tert-butyl alcohol	USP Ghana	TBA/14/002	August,2025
Tetrabutylammonium hydrogen sulfate		TAS/18/001	Sep 23, 2023.
Tetradecylammonium bromide	USP Ghana	TDB/20/001	Aug 10, 2025
Tetraheptylammonium bromide	USP Ghana	ThAB/20/001	Aug 10, 2025
Triethylamine	USP Ghana	TEA/20/001	Jul 30, 2025.

Annex 2. Table of Equipment Used in Preliminary and PAD Validation Study

Equipment Name	Brand/Make	Lab Code	Calibration Due Date
Analytical balance	Mettler Toledo	BL/TSL/16/01	Jan 14, 2021
Analytical balance	Mettler Toledo	BL/TSL/16/02	Jan 14, 2021
Android mobile phone	Samsung	N/A	N/A
Aspirator Pump	Cole Palmer	AP/TRSL/13/01	N/A
Hotplate	Stuart	ST/TSL/13/02	N/A
HPLC	Agilent Technologies	LC/TSL/16/03	Dec 20,2020
HPLC	Agilent Technologies	LC/TSL/16/02	Dec 20,2020
Karl Fisher Titrator	Mettler Toledo	KF/TSL/13/01	Nov 20,2020
Microbalance	Mettler Toledo	BL/TSL/13/03	Jan 14, 2021
Microbalance	Mettler Toledo	BL/TSL/16/01	Jan 14, 2021
pH Meter	Agilent	PH/TSL/13/01	N/A
Sonicator	Elma	UB/TRL/13/01	N/A
Water Purification System	Merck	WS/TSL/13/02	Dec 20,2020

Annex 3. Table of Finished Dosage Forms (FDFs) Used in PAD Validation Study

Sample	Content and Strength	Batch /Lot	Manufacturer/ Source	Lab Code	Expiry Date
Amoxicillin capsules	Amoxicillin 500mg	AXBBV0 071	Brown and Burk	PAD/20/001	05/2022
Amoxicillin capsules	Amoxicillin 500mg	1230239	Letap Pharmaceuticals	PAD/20/003	08/2021
Amoxicillin capsules (Exeter)	Amoxicillin 500mg	1999013	Exeter Pharmaceuticals	PAD/20/002	06/2022
Azilex capsules	Azithromycin 250mg	17	Luex	PAD/20/012	02/2022
Azitex capsules	Azithromycin 500mg	BL90008	Exeter Pharmaceuticals	PAD/20/011	07/2022
Chloroquine tablets	Chloroquine phosphate 250mg	0104W	Ernest Chemist	PAD/20/016	04/2023
Chlorquine tablets	Chloroquine phosphate 100mg	02	Quantum Pharmacy	PAD/20/017	06/2022
Ciprolex tablets	Ciprofloxacin 500mg	169	Luex	PAD/20/007	02/22
Cipromax	Ciprofloxacin 500mg	X03843	Phyto-Riker	PAD/20/008	02/21
Doxycycline capsules	Doxycycline 100mg	0810V	Ernest Chemist	PAD/20/013	10/2022
Doxycycline capsules	Doxycycline 100mg	03	Eskay Therapeutic Ltd	PAD/20/014	02/2022
Doxycycline capsules	Doxycycline 100mg	1360119	Letap Pharmaceuticals	PAD/20/015	10/2021
G-Ceftria (GPSC)	Ceftriaxone 1g	181207	Sinopharm Weiqida	PAD/20/004	05/2021
Inno-Ceft	Ceftriaxone 1g	1011924 8	O&J Pharmaceuticals	PAD/20/005	10/2021

Sample	Content and Strength	Batch /Lot	Manufacturer/ Source	Lab Code	Expiry Date
Lextriax powder for injection	Ceftriaxone 1g	193051225	Luex	PAD/20/006	08/2022
Maxiquine	Chlorquine phosphate 250mg	T29919	Vitabiotics	PAD/20/018	9/2024
Quinoric tablets	Hyrdroxychlorquine 200mg	DET059028	Bristol	PAD/20/019	11/2023
Rhumatas tablets	Hyrdroxychlorquine 200mg	M2006183	Intas	PAD/20/021	02/2022
Shalcip 500	Ciprofloxacin 500mg	J9009	Shalina	PAD/20/009	11/2022
Zentiva (Hydroxychloroquine) tablets	Hyrdroxychlorquine 200mg	9R878	Zentiva	PAD/20/020	03/2022
Zymax capsules	Azithromycin 500mg	0103W0	Ernest Chemist	PAD/20/010	03/2023

Annex 4. Table of APIs and Fillers Used in PAD Validation Study

Sample	Source	Lab Code	Expiry/Retest Date
Amoxicillin	Letap Pharmaceuticals, Accra - Ghana	PAD-API/20/001	12/2023
Azithromycin	Letap Pharmaceuticals, Accra - Ghana	PAD-AP1/20/008	01/2024
Calcium Carbonate	Letap Pharmaceuticals, Accra - Ghana	PAD-API/20/004	03/2023
Ceftriaxone	Letap Pharmaceuticals, Accra - Ghana	PAD-API/20/011	05/2021
Chloroquine	Letap Pharmaceuticals, Accra - Ghana	PAD-API/20/009	01/2025
Ciprofloxacin	Phyto-Ryker Pharmaceuticals, Accra - Ghana	PAD-API/20/010	11/2021
Doxycyline	Letap Pharmaceuticals, Accra - Ghana	PAD-API/20/002	06/2022
Hydroxychloroquine	Entrance Pharmaceuticals, Accra - Ghana	PAD-API/20/013	05/2022
Maize Starch	Letap Pharmaceuticals, Accra - Ghana	PAD-AP1/20/005	02/2025
Paracetamol	Letap Pharmaceuticals, Accra - Ghana	PAD-API/20/003	01/2025
Sodium Chloride	Letap Pharmaceuticals, Accra - Ghana	PAD-API/20/007	01/2022
Sucrose	Letap Pharmaceuticals, Accra - Ghana	PAD-AP1/20/008	10/2021
Talcum Powder	Letap Pharmaceuticals, Accra - Ghana	PAD-API/20/006	08/2021

Annex 5. Table of Falsified and Substandard Formulations Used in PAD Study

Sample (Formulation)	Composition/Material Used	Lab Code
Amoxicillin falsified formulation #1	70% Calcium carbonate + 30% corn starch	PAD-FORM/20/001
Amoxicillin falsified formulation #2	Paracetamol	PAD-API/20/003
Amoxicillin substandard formulation #1	50% talcum powder + 50% amoxicillin API	PAD-FORM/20/010
Amoxicillin substandard formulation #2	50% corn starch + 50% amoxicillin API	PAD-FORM/20/014
Azithromycin falsified formulation #1	70% chalk (calcium carbonate) + 30% corn starch	PAD-FORM/20/001
Azithromycin falsified formulation #2	Paracetamol	PAD-API/20/003
Azithromycin substandard formulation #1	50% talcum powder and 50% pure azithromycin API	PAD-FORM/20/004
Azithromycin substandard formulation #2	50% corn starch and 50% pure azithromycin API	PAD-FORM/20/005
Ceftriaxone falsified formulation #1	Sodium chloride	PAD-API/20/007
Ceftriaxone falsified formulation #2	Sucrose	PAD-AP/20/012
Ceftriaxone substandard formulation #1	50% powdered NaCl + 50% pure ceftriaxone API	PAD-FORM/20/011
Ceftriaxone substandard formulation #2	50% ground sucrose and 50% pure ceftriaxone API	PAD-FORM/20/013
Chloroquine falsified formulation #1	70% chalk (calcium carbonate) + 30% corn starch	PAD-FORM/20/001
Chloroquine falsified formulation #2	Paracetamol	PAD-API/20/003
Chloroquine substandard formulation #1	50% talcum powder + 50% pure chloroquine API	PAD-FORM/20/006
Chloroquine substandard formulation #2	50% corn starch + 50% pure chloroquine API	PAD-FORM/20/007

Sample (Formulation)	Composition/Material Used	Lab Code
Ciprofloxacin falsified formulation #2	Paracetamol API	PAD-API/20/003
Ciprofloxacin substandard formulation #1	50% talcum powder + 50% ciprofloxacin API	PAD-FORM/20/003
Ciprofloxacin substandard formulation #2	50% corn starch + 50% pure ciprofloxacin API	PAD-FORM/20/002
Doxycycline falsified formulation #1	70% chalk (calcium carbonate) + 30% corn starch	PAD-FORM/20/001
Doxycycline falsified formulation #2	Paracetamol	PAD-API/20/003
Doxycycline substandard formulation #1	50% talcum powder + 50% pure doxycycline API	PAD-FORM/20/008
Doxycycline substandard formulation #2	50% corn starch + 50% pure doxycycline API	PAD-FORM/20/009
Hydroxychloroquine falsified formulation #1	70% chalk (calcium carbonate) + 30% corn starch	PAD-FORM/20/001
Hydroxychloroquine falsified formulation #2	Paracetamol	PAD-API/20/003
Hydroxychloroquine substandard formulation #1	50% hydroxychloroquine API + 50% talcum powder	PAD-FORM/20/015
Hydroxychloroquine substandard formulation #2	50% hydroxychloroquine API + 50% corn starch	PAD-FORM/20/016

Annex 6. Table of Reference Standards Used for Preliminary Study

USP Reference Standard (RS)	Lot Number	Manufacturer/ Source	Lab Code
Amodiaquine Hydrochloride	R078L0	USP	USPRS/20/034
Amoxicillin	R106H0	USP	USP/RS/20/025
Azithromycin	R103C0	USP	USPRS/20/028
Chloroquine Phosphate	R075S0	USP	USPRS/20/033
Ceftriaxone Sodium	R07420	USP	USPRS/20/030
Ceftriaxone Sodium E-isomer	R131A0	USP	USPRS/20/031
Ciprofloxacin hydrochloride	R05170	USP	USPRS/20/026
Ciprofloxacin Ethylenediamine Analog	R013T0	USP	USPRS/20/027
Ciprofloxacin	R12590	USP	USPRS/20/029
Doxycycline Hyclate	R065H0	USP	USPRS/20/032

Annex 7. Table of Results for Preliminary Study Performed on Samples Collected

Material/Product	Lab Code	Test Performed	Test Reference	Result
Amoxicillin API	PAD-API/20/001	Identification and Assay	USP/IH	Pass
Amoxicillin capsules	PAD/20/001	Identification and Assay	USP/IH	Pass
Amoxicillin capsules	PAD/20/002	Identification and Assay	USP/IH	Pass
Amoxicillin capsules	PAD/20/003	Identification and Assay	USP/IH	Pass
Azilex (Azithromycin) capsules	PAD/20/012	Identification and Assay	USP/IH	Pass
Azitex (Azithromycin) capsules	PAD/20/011	Identification and Assay	USP/IH	Pass
Azithromycin API	PAD-API/20/008	Identification and Assay	USP/IH	Pass
Calcium carbonate	PAD-API/20/004	Identification Test	USP/IH	Pass
Chloroquine API	PAD-API/20/009	Identification and Assay	USP	Pass
Chloroquine Phosphate tablets	PAD/20/017	Identification and Assay	USP/IH	Pass
Chloroquine tablets	PAD/20/016	Identification and Assay	USP/IH	Pass
Ciprofloxacin API	PAD-API/20/010	Identification	USP/IH	Pass
		Assay	USP/IH	Fail
Ciprolex (ciprofloxacin) tablets	PAD/20/007	Identification and Assay	USP/IH	Pass
Cipromax (ciprofloxacin) tablets	PAD/20/008	Identification and Assay	USP/IH	Pass
Corn starch	PAD - API/20/005	Identification	USP/IH	Pass
Doxycycline API	PAD-API/20/002	Identification	USP/IH	Pass
		Assay	USP/IH	Fail
Doxycycline capsules	PAD/20/013	Identification and Assay	USP/IH	Pass
Doxycycline capsules	PAD/20/014	Identification and Assay	USP/IH	Pass
G-Ceftria (Ceftriaxone for injection)	PAD/20/004	Identification and Assay	USP/IH	Pass
Hydroxychloroquine API	PAD-API/20/013	Identification and Assay	USP/IH	Pass
INNO-CEFT (Ceftriaxone for injection)	PAD/20/005	Identification and Assay	USP/IH	Pass

Lextriax (Ceftriaxone for injection)	PAD/20/006	Identification and Assay	USP/IH	Pass
Maxiquine (chloroquine) tablets	PAD/20/018	Identification and Assay	USP/IH	Pass
Paracetamol API	PAD-API/20/003	Identification Test	Ph. Int.	Pass
Quinoric tablets (Hydroxychloroquine) tablets	PAD/20/019	Identification and Assay	USP/IH	Pass
Rhumatas tablets (Hydroxychloroquine) tablets	PAD/20/021	Identification and Assay	USP/IH	Pass
Shalcip (ciprofloxacin) tablets	PAD/20/009	Identification and Assay	USP/IH	Pass
Sodium chloride	PAD-API/20/007	Identification Test	USP/IH	Pass
Sucrose	PAD-API/20/012	Identification Test	USP/IH	Pass
Zentiva (Hydroxychloroquine) tablets	PAD/20/020	Identification and Assay	USP/IH	Pass
Zymax (Azithromycin) capsules	PAD/20/010	Identification and Assay	USP/IH	Pass

Annex 8. Table of Products Sampled and Tested During Field Evaluation

Team Number	Sample Name	Brand	Results
Team 1	Amoxicillin capsules	Duramox 500 mg	Pass
		Moxileb 250 mg	Pass
		Amoxikid 250 mg	Pass
	Chloroquine tablets	Sugaquin 250mg	Pass
Team 2	Ciprofloxacin tablets	Cipro Denk 500 mg	Pass
		Ciprobid 500 mg	Pass
		Ciropharm 500mg	Pass
	Hydroxychloroquine tablets	Rhumatas 200mg	Pass
Team 3	Ceftriaxone injection	Nectram injection	Pass
		Zefone injection	Pass
		Epicephin injection	Pass
Team 4	Azithromycin tablets	Azithro-Denk 250 mg	Pass
		Ezecure 500 mg	Pass
	Doxycycline capsules	Remycin 100 mg	Pass
		Doxyren 100 mg	Pass