Screening medicine quality with the Paper Analytical Device (PAD)

20 Nov 2020

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Technology overview

Introduction

The World Health Organization estimates that 1 out of 10 pharmaceutical products sold in low- and middle-income countries (LMICs) is substandard or falsified (SF). These products harm patients, increase the prevalence of drug-resistant pathogens, reduce trust in the medical system, and waste scarce resources. SF products that pose particularly large risks to patients may contain unstated substitute APIs or other bioactive materials,[1,2] or may contain an inert matrix with little or none of the stated active pharmaceutical ingredient (API).[3]

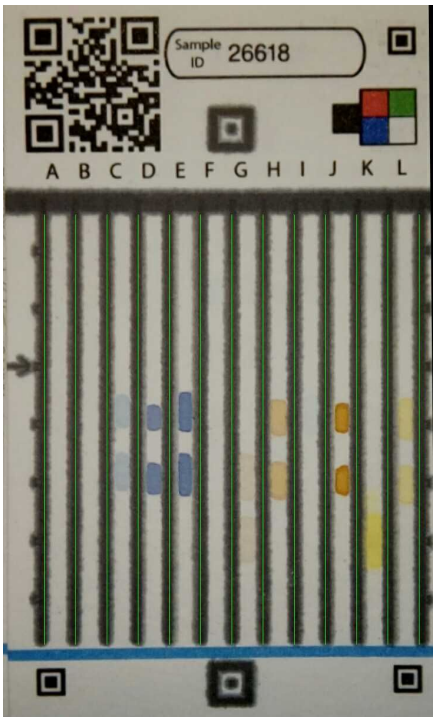
There are well-established methods for detection of SF products using laboratory instrumentation such as high-performance liquid chromatography (HPLC). However, there are 343 medicines on the WHO Essential Drugs List, and in many LMICs, dozens or even hundreds of manufacturers have received permission to sell their brand of products. In addition, products may enter the country from private importation by NGOs or through illicit supply networks. All of these products ought to be monitored throughout the supply chain to make sure that they meet quality standards when they reach the patient. In many LMICs, there is not enough HPLC capacity to meet all the testing needs.

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.[4] It

[1] Batch J093: Pathology of Negligence. Report of the Judicial Inquiry Tribunal, 2011-2012.
[2] An epidemic of dystonic reactions in central Africa. N. Payraud et al, 2017, The Lancet: Global Health, 5(2):e137-e138, DOI:https://doi.org/10.1016/S2214-109X(16)30287-X; see also WHO. Medical product alert No. 4/2015: adverse reactions caused by falsified diazepam in Central Africa. http://www.who.int/medicines/publications/drugalerts/ Alert4_2015DiazepamEN.pdf
[3] Manslaughter by fake artesunate in Asia—Will Africa be next? Newton PN, McGready R, Fernandez F, Green MD, Sunjio M, et al. (2006) PLos Med 3(6): e197. DOI: 10.1371/journal.pmed.0030197; see also WHO, Alert No. 127: Falsified batches of Coartem recently circulating in Western and Central Africa (2013)
[4] Paper analytical devices for fast field screening of beta lactam antibiotics and anti-tuberculosis pharmaceuticals, A. Weaver, H. Reiser, T. Barstis, M. Benvenuti, D. Ghosh, M. Hunkler, B. Joy, L. Koenig, K. Raddell, M.Lieberman, 2013, Anal. Chem., 85 (13), 6453–6460 http://dx.doi.org/10.1021/ac400989p

is a presumptive test to identify products that are at high risk of causing harm to patients. As a presumptive test, it must be followed up by more accurate laboratory testing, such as HPLC. By screening out the bulk of medicines that are of good quality, it enables labs to focus their HPLC resources on products that pose greatest risk to patients.

Specifications



Dimensions: 7 cm x 11 cm x 400 µm
Weight: 1.5 g
Composition: Cellulose paper, wax, trace quantities of chemical reagents
Power source: No power required
Operational temperature: 15-40˚C
Disposal: Safe to discard in trash
Security features: Individually serialized, serial numbers can be assigned to specific projects.

Cost: $2 USD/PAD plus cost of mailing
(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)

Analytical strengths and limitations of the PAD

The PAD is a reliable tool to identify SF pharmaceutical products that do not contain the stated active pharmaceutical ingredients (APIs) or that contain substitute APIs.

The PAD cannot identify slightly substandard or degraded products (eg, if the API content is 85%).

For some medicines, the strength or weakness of colors on the PAD can identify products that may be seriously substandard (API content <50%) or have undergone significant degradation (API content <50%). Confirmatory testing (eg, HPLC) is necessary to confirm a product is substandard.

The ability to detect substandard API with a color test requires that the test results lie in the linear range of the color test. For some APIs and some tests,the test results lie in the saturated range of the color tests. In these cases, the test can only be used for presence/absence of the API.

The PAD can detect fillers such as starch. Starch should not be present in most capsule formulations, so presence of starch indicates a failure of Good Manufacturing Practice. In other formulations, such as tablets, starch is one of the permissible excipients, so presence of starch in tablets must be followed up by reviewing the manufacturer's stated ingredients list.

The PAD cannot identify medicines that are substandard because they are underweight. An inexpensive milligram balance[5] is recommended for checking pill weights in a field setting.

[5] The accuracy and precision of three portable scales were assessed to see which would be the best to use in a field setting. None of the balances achieved the 0.1% repeatability specifications in USP method <41>, which would be expected of a certified lab, but the Gemini 20 (Smart Weigh Gem20, $19.99) performance is acceptable for field assay (±2% repeatability) if at least 140 mg is weighed.

Availability of the PAD

PADs are currently available from the Lieberman research group at a cost of $2 per PAD + mailing costs. They are supplied in packs of 10 or 20 units that are heat-sealed in a metallized zip-top bag.

Training packs are available from the Lieberman research group at a cost of $40 per unit + mailing costs. The training packs contain 14 PADs, 14 training and certification samples, and PAD reading guides and logbooks.

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.

Fabrication process

PADs are printed eight to a page using commercial printers. The substrate is Ahlstrom 319 paper, which is a fast chromatography paper. Each PAD is serialized and serial numbers can be assigned to specific projects or sites. The fabrication process has 4 main steps:

1. Wax printing and baking to define the hydrophobic lane barriers. The Lieberman lab has six Xerox ColorQube wax printers and considerable experience in maintaining and repairing them. Baking occurs in a laboratory oven at 100˚C for 10 minutes to allow the wax to spread and seal.
    - ➔ The pages are spot-tested with water drops to ensure that the PADs are properly baked.
2. Laser printing adds lane markers, fiducial marks, and other features.
    - ➔ Each card is individually serialized and has a unique QR code.
3. Reagent deposition is performed with a stamping tool and the PADs are allowed to air-dry.
    - ➔ 5% of the PADs from each reagent deposition run undergo QC testing to make sure that every lane is operating correctly.
4. PADs are cut from the sheets, packaged, and stored in a cold room.
    - ➔ Fabrication date is recorded on each pack to track the age of the PADs.

Commercialization of the PAD

The Lieberman group has the capacity to make up to 50,000 PADs per year. This activity is seen as a strong mission fit by the University of Notre Dame. If demand for PADs is larger than 50,000 units/year, fabrication must be transferred to a roll-to-roll manufacturing process and commercialized. Preliminary technical discussions with Serim Research and Hach Analytical have been held and an NSF-funded commercialization project with Veripad LLC is underway through the Partnerships for Innovation (PFI) program. Dr. Lieberman would be happy to talk with potential commercialization or distribution partners.

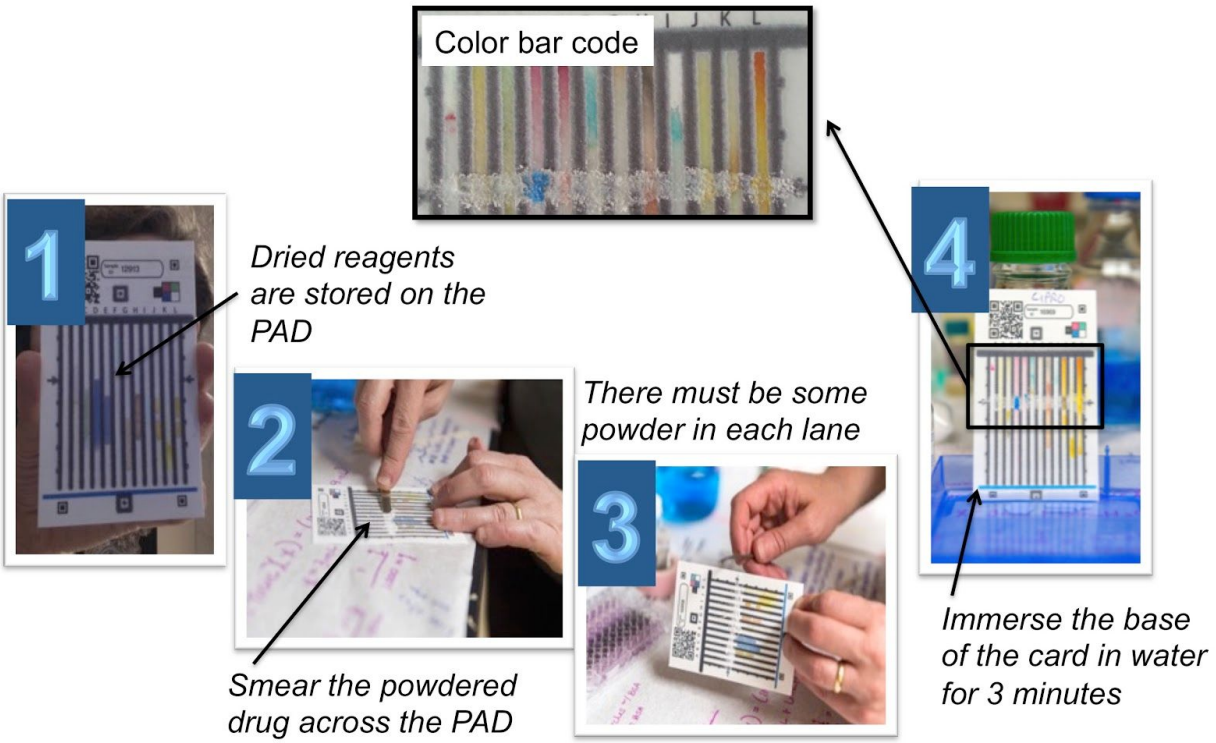
The PAD technology is patented by the University of Notre Dame (#US9354181). The University is the patent holder, but would support licensing the PAD patent to a manufacturer for use in screening of products on the WHO Essential Drugs list.

The US patent does not prevent others from making or using PADs outside the US, and the Lieberman group has already begun to transfer the PAD technology to several partners in low- and middle-income countries.

1. International Center for Diarrheal Disease Research, Bangladesh--wax printer and supplies set up at icddr,b. Contact: Dr. Wasif Ali Khan.
2. University of Malawi, Blantyre--wax printer and supplies set up at College of Medicine Pharmacy department, in use making PADs. Contact: Prof. Ibrahim Chikowe.
3. Addis Ababa University, Ethiopia--technology transfer was funded by the Ethiopian Ministry of Science and Technology--wax printer and PAD supplies set up at AAU. Contact: Prof. Ayenew Ashenef.

Currently the technology is used for academic research projects. Standard operating procedures for manufacture and QA/QC have been developed, and the Lieberman group is setting up a proficiency testing program to ensure that the sites are all producing comparable PADs.

How the PAD works



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. The PAD uses some of the laboratory color tests that were introduced by WHO in the 1990's[6] and later were incorporated into the original GMHB Minilab.[7] The chemical reagents are pre-loaded on the PAD, and all of the procedures that would normally have to be performed by the lab worker--sequential reagent additions, pH neutralizations, and mixing--are performed automatically in the lanes of the PAD when it is activated. From sample preparation to evaluating the test results takes about 7 min, and multiple PADs can be prepared and run at once to make efficient use of testing time.

Click here to view a 2 min video demonstration of PAD use.

[6] Basic Tests for Pharmaceutical Dosage Forms, WHO, Geneva, 1991.
[7] https://www.gphf.org/en/minilab/index.htm

The reactions in the twelve lanes consist of:

A) **Timer lane** This lane contains a chelating reagent at the bottom of the lane and a spot of metal cation at the top of the lane. When the water completely wets out the lane, the metal binds the chelating reagent and forms a pink spot at the top of the lane.

B) **Ninhydrin** The ninhydrin test for primary amines is implemented with spots of ninhydrin and potassium carbonate stored in lane B. While the classical ninhydrin test in the lab is heated to drive formation of Ruhrman's Purple, our test is done at room temperature. This reaction condition results in formation of yellow, orange, red, or green Schiff base intermediates. Drugs such as ampicillin, amoxicillin, and isoniazid give different colors in this lane.

C) **Biuret Reagent** The biuret reagent consists of basic copper tartrate and is usually used to detect polyamides in proteins. It yields colors ranging from yellow to blue when binding to polyfunctional amides found in many antibiotics.

D) **Acidic cobalt thiocyanate** Cobalt thiocyanate forms colored ion pairs with protonated tertiary amines and some bulky secondary amines. A strong acid at the bottom of the lane ensures that amines in the pharmaceutical are protonated. The ion pairs displace water from the octahedral pink cobalt species, giving blue or green tetrahedral cobalt salts with poor water solubility. Many pharmaceuticals with tertiary amines give blue or green colors at the "swipe line", and bulky secondary amines give blue or green colors that migrate up the lane or "wash out" of the center of the lane. This different behavior is due to the greater water solubility of the secondary amine ion pairs and the flow pattern in the lane.

E) **Neutral cobalt thiocyanate** This lane functions the same way as lane D, but instead of a strong acid, it contains a pH 8 buffer. Only the more basic amines (eg, chloroquine) react.

F) **Beta lactam test** Lane F contains the classic basic cupric sulfate test for the beta lactam functional group. Antibiotics that contain beta lactams, such as benzyl penicillin, form a dark green precipitate. The precipitate usually clogs up the lane partway, forming a green or black spot.

G) **Sodium nitroprusside** Sodium nitroprusside contains an electrophilic nitrosyl group which can react directly with nucleophiles or undergo substitution reactions on the iron. Strong base is used to help activate the nucleophilic groups on the pharmaceuticals. This lane detects a wide range of nucleophiles, giving strong red, orange, or yellow colors.

H) **Naphthaquinone sulfonate** Naphthaquinone sulfonate is a good substrate for Michael addition/elimination reactions with nucleophiles. Strong base is used to

help activate the nucleophilic groups on the pharmaceuticals. This lane detects a wide range of nucleophiles, giving strong red, orange, or yellow colors.

I) **Ethambutol test** This lane was specifically designed to recognize the TB drug ethambutol. It contains hydroxide below the swipe line and copper sulfate above the swipe line. Ethambutol forms a square planar copper complex related to ethylenediamine copper (II) with a beautiful royal blue color.

J) **Tri-iodide** This lane detects starch via the well known starch-iodine test. The tri-iodide anion is encapsulated in a polymer matrix that prevents evaporative loss of iodine during storage.

K) **Phenol test** As water moves up lane K, it sequentially dissolves portions of a strong acid and sodium nitrite (forming HONO), then it reaches an indicator species, para-nitroaniline, which is diazotized by the nitrous acid. The diazotized indicator and the analyte are then reacted with strong base, which deprotonates any phenols present in the analyte and enables them to act as nucleophiles. They react with the diazonium group on the indicator to form azo dyes with strong brown or red colors.

L) **Ferric chloride** This lane uses ferric chloride to test for the presence of chelating functional groups, such as catechols (epinephrine), 1,3-dicarbonyls (ciprofloxacin), and polyphenols (doxycycline).

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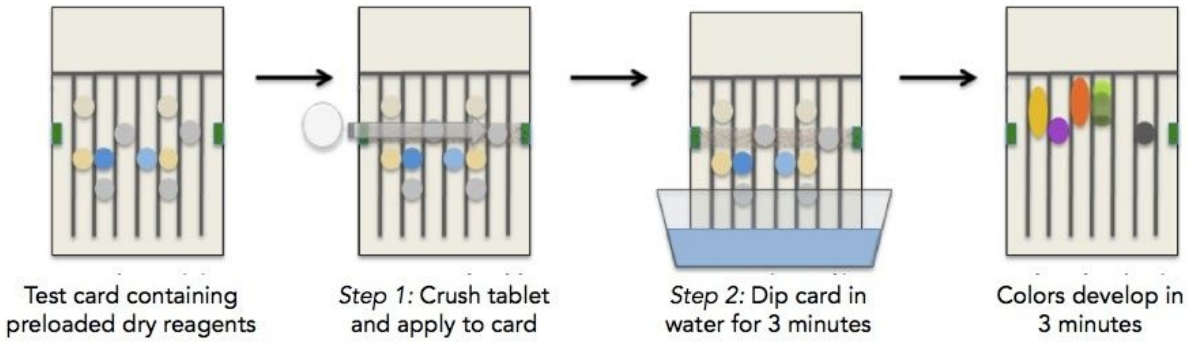
http://dx.doi.org/10.1021/ac400989p

Testing directions



Loading USP Data Capture on an Android phone

From an email

1. Request the Data Capture app by sending an email to Marya Lieberman (mlieberm@nd.edu). The files will be sent to you in an email.
2. The three .apk files in the email (PAD Analyze, USP Data Capture, and PADupload) must be installed on an Android phone. It does not matter what order you install the files, and you can install updates on top of current versions of the files.
3. Open the email in your email application, click on one apk file and tap on the Package Installer icon to install the application. Tap on “done”.
    a. Install the other two files in the same way.
    b. Three new app names and icons will appear on your phone: PAD Analyze, USP Data Capture, and PADupload.
4. If your phone can load a web page, it can upload the PAD data.
    a. You can run PADs and use USP Data Capture even if you don’t have an internet connection--the data will be saved on your phone and you can upload it later with PADupload.

Using USP Data Capture on an Android phone

1. Click on **USP Data Capture** to start imaging PADs.
    a. Load and run the PAD as directed.
    b. Place the PAD on a plain white surface and make sure there are no other PADs in the camera's view.
    c. The camera screen should show a pattern of square marks that match the squares at the corners of the PAD. The flashlight will turn on.
    d. Hold the camera about 6" above the PAD and move it slowly up or down until the app captures the image.
    e. The **USP Data Capture** screen will show the processed PAD image.
        Make sure you can see the whole PAD in the picture.
        The PAD serial number and the date-time stamp will appear at the top of the screen.
    f. Enter sample data:
        Stated Drug (select from pull-down menu or choose "unknown"),
        Brand Name (type in N/A or type "none"),
        Batch Number (type in N/A or type "none"),
        Notes (type in your sample number and any other comments).
        To enter each menu item, tap the arrow at the very bottom of the app window.
    g. Tap "save and continue" to save the PAD image and metadata on the phone.
    h. Read the PAD (refer to the "read the test result" section of the manual for the API) and record the results in the written log book.
    i. Image other PADs if desired.
2. Exit the USP Data Capture app by tapping the circle at the very bottom of the app window. Exiting from the app, updating it, or uninstalling the app will not harm your data--it is stored on your phone.
3. Run PAD Upload to send the stored data to the PAD database.

Uploading PAD data to the database

1. Upload the PAD data when you have a good Internet connection.
2. Tap the **PADupload** app.
    a. You will see a list of all the PAD files stored on your phone.
    b. Tap the “hide uploaded” switch to make the files that have already been uploaded to the database vanish.
    c. Tap the files you want to upload. They will turn grey and then turn green when they are uploaded.
    d. Tap “upload all” to send batches of five files.
3. Once data has been uploaded to the PAD database, you can delete the image from your phone.

Amoxicillin

Types of this drug that can be tested

Amoxicillin trihydrate formulated as capsules (250 mg or 500 mg)

Preparation of lab samples for evaluation of the PAD

- Amoxicillin API: Place ~50 mg pure API in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Amoxicillin dosage forms: Obtain three capsules of amoxicillin trihydrate (either 250 or 500 mg dosage) from each of three different brands known to be of good quality. Give one sample of each brand to each analyst.
- Amoxicillin falsified formulation #1: Mix 70% chalk (calcium carbonate) and 30% corn starch. Place 50 mg powder in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts. [Notre Dame has an "authentic" falsified product with this composition]
- Amoxicillin falsified formulation #2: Place ~50 mg paracetamol in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Amoxicillin substandard formulation #1: Mix 50% talcum powder and 50% pure amoxicillin API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts. [Notre Dame has an "authentic" substandard product with this composition]
- Amoxicillin substandard formulation #2: Heat 500 mg amoxicillin trihydrate at 70˚C and 100% relative humidity for 5 days.[8] Confirm loss of API by HPLC or TLC if possible. Weigh residue and mix 1:1 with pure amoxicillin API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Alternative amoxicillin substandard formulation #2: Mix 50% corn starch and 50% pure amoxicillin API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.

Examine the packaging (for dosage forms only)

1. Record the type of product, stated dosage, brand, manufacturer, manufacturing date, expiration date, and lot or batch number.

[8] WHO PHARM 86.529. Accelerated Stability Studies of Widely Used Pharmaceutical Substances under Tropical Conditions: under these conditions, the closely related drug ampicillin trihydrate is completely degraded.

2. Record the type of packaging: blister pack and box, blister pack, capsules in secondary packaging. Record the color of the capsule and any printing on the capsule.
3. Note any defects in the product. Defects might include torn or wet box, glue coming apart, torn or dented blister pack, faded printing, capsules broken or dented.
4. If a scale is available, weigh the powder in one gel capsule.
   ❌If the mass is less than 225 mg for a 250 mg dose product or less than 450 mg for a 500 mg product, the product fails.

Prepare the sample

1. Write the sample ID on the PAD
2. Enter the PAD number and sample ID in your logbook
3. Open the gel capsule by pulling and twisting the two halves apart.

Load and run the PAD

1. Use the wooden paddle to place a heap of powder as big as a grain of rice on the PAD at the arrow mark.
2. Spread the powder from one arrow mark to the other, across all 12 lanes of the PAD.
3. Press down on the paddle and pull it across the powder to make it stick to the paper.
4. Turn the PAD on its side and gently tap off excess powder.
5. You should see powder in each lane, and you should see the lines between the lanes. If you can't see the black lines under the powder, there is too much powder.
6. Place the bottom edge of the PAD (below the blue line) into 1 cm of water. Keep the PAD nearly upright.
7. Hold the PAD upright or lean the PAD against a support (a water bottle, beaker, or wall) for about 3 minutes. You will see water moving up the lanes.
8. When a red dot appears at the top of lane A, remove the PAD and lay it flat on a clean piece of paper.
9. After every 5 PADs, replace the water in the dish with clean water.

Photograph the PAD

1. Let the PAD lie flat for 3 minutes, then photograph the PAD.
2. **If you are using the PADapp,**

a. Hold the phone over the PAD until the app has taken the photo. You may need to move it up or down until the app has found all the square marks.
b. Select “amoxicillin” from the pull down menu.
c. Enter the brand (or “n/a”), lot or batch number (or “n/a”), and enter the sample number in the notes field.
d. To store the image and data, tap “save and continue” .

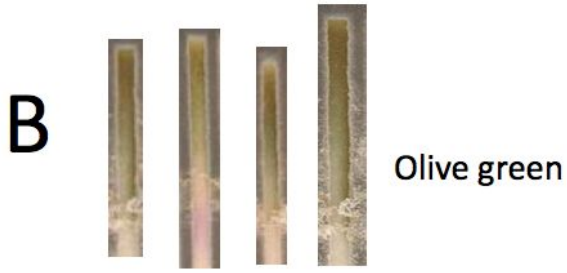
Read the test result

1. Compare your image to the sample image below and fill out the log book to record your results.

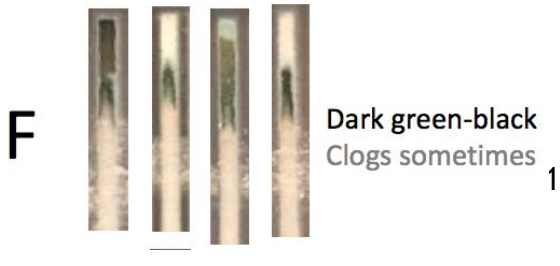
2. Amoxicillin makes special colors in three lanes: **B, F, and K**. All three special colors must be present.

3. **Lane B**

If Lane B looks like one of these examples, check the box marked “This lane passed with appropriate color” for Lane B, otherwise check the box marked “Product Fail”.



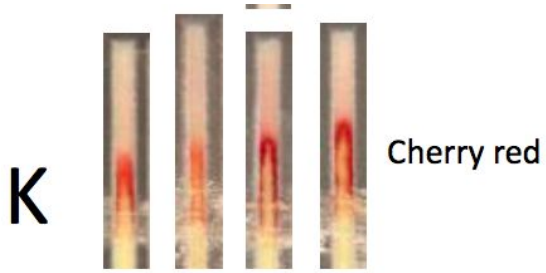
Lane F



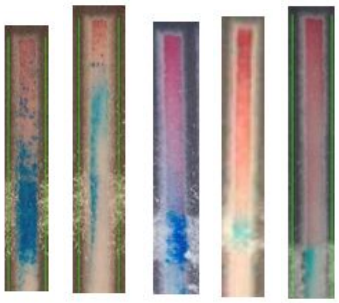
If Lane F looks like one of these examples, check the box marked “This lane passed with appropriate color” for Lane F, otherwise check the box marked “Product Fail”.

Lane K

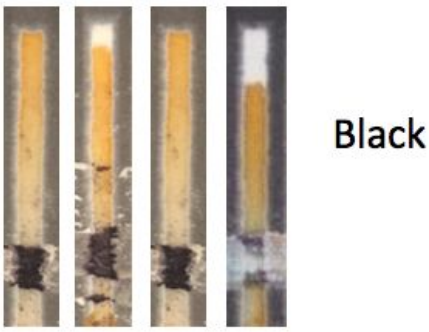
If Lane K looks like one of these examples, check the box marked “This lane passed with appropriate color” for Lane K, otherwise check the box marked “Product Fail”.



4. Check for unexpected filler materials.
    a. If Lane D has blue or green color like one of these examples, write “tertiary amine” in the notes section. ❌ If a tertiary amine is present, the product fails.



    b. If Lane J shows a black color like one of these examples, check the “Product Contains Starch” box for lane J. ❌ If starch is present in a capsule formulation, the product fails.



5. Evaluate the quality of the API.

a. ❌If any of the colors expected in Lanes B, F, and K are missing, the product fails denoted by the ‘Product Fail” box being checked.
b. If all Lanes reacted as expected, mark the “Product Pass” box.
c. If the colors in Lanes B and F are present but they are weak, the product may be substandard. Write “weak colors” in the notes section. If available, run another sample of the product on a fresh PAD.

If you are not sure of the result:

1. If you are not sure of the result, repeat the analysis on two fresh PADs.
2. Compare the three PADs. Base your final results on the two that look most similar to each other.

Azithromycin

Types of this drug that can be tested

Gel capsules (250 or 500 mg doses)
Tablets (250 or 500 mg doses)
Coated tablets may show unexpected colors from dyes in the coating

Preparation of lab samples for evaluation of the PAD

- Azithromycin API: Place ~50 mg pure API in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Azithromycin dosage forms: Obtain three capsules or tablets of azithromycin (either 250 or 500 mg dosage) from each of three different brands known to be of good quality. Give one sample of each brand to each analyst.
- Azithromycin falsified formulation #1: Mix 70% chalk (calcium carbonate) and 30% corn starch. Place 50 mg powder in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Azithromycin falsified formulation #2: Place ~50 mg paracetamol in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Azithromycin substandard formulation #1: Mix 50% talcum powder and 50% pure azithromycin API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Azithromycin substandard formulation #2: Mix 50% corn starch and 50% pure azithromcyin API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.

Examine the packaging (for dosage forms only)

1. Record the type of product, stated dosage, brand, manufacturer, manufacturing date, expiration date, and lot or batch number.
2. Record the type of packaging: blister pack and box, blister pack, tablets or capsules in secondary packaging. Record the color of the capsule or tablet and any printing on the capsule or imprint on the tablets.
3. Note any defects in the product. Defects might include torn or wet box, glue coming apart, torn or dented blister pack, faded printing, capsules broken or dented, tablets broken, moldy, or crumbling to powder.

4. If a scale is available, weigh the powder in one gel capsule or a single tablet. ❌If the mass is less than 225 mg for a 250 mg dose product or less than 450 mg for a 500 mg product, the product fails.

Prepare the sample

1. Write the sample ID on the PAD
2. Enter the PAD number and sample ID in your logbook
3. Open 1 gel capsule by pulling and twisting the two halves apart OR
4. Crush 1 tablet in a mortar and pestle, or by wrapping the tablet in a clean piece of paper and then using a blunt object

Load and run the PAD

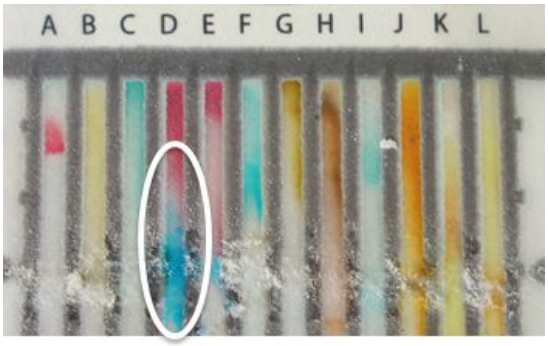
1. Use the wooden paddle to place a heap of powder as big as a grain of rice on the PAD at the arrow mark.
2. Spread the powder from one arrow mark to the other, across all 12 lanes of the PAD.
3. Press down on the paddle and pull it across the powder to make it stick to the paper.
4. Turn the PAD on its side and gently tap off excess powder.
5. You should see powder in each lane, and you should see the lines between the lanes. If you can't see the black lines under the powder, there is too much powder.
6. Place the bottom edge of the PAD (below the blue line) into 1 cm of water. Keep the PAD nearly upright.
7. Hold the PAD upright or lean the PAD against a support (a water bottle, beaker, or wall) for about 3 minutes. You will see water moving up the lanes.
8. When a red dot appears at the top of lane A, remove the PAD and lay it flat on a clean piece of paper.
9. After every 5 PADs, replace the water in the dish with clean water.

Photograph the PAD

1. Let the PAD lie flat for 3 minutes, then photograph the PAD.
2. **If you are using the PADapp,**
    a. Hold the phone over the PAD until the app has taken the photo. You may need to move it up or down until the app has found all the square marks.
    b. Select “azithromycin” from the pull down menu.
    c. Enter the brand (or “n/a”), lot or batch number (or “n/a”), and enter the sample number in the notes field.
    d. To store the image and data, tap “save and continue” .

Read the test result

1. Compare your image to the sample image below and fill out the log book to record your results.



2. Azithromycin makes a special color at the swipe line in lane **D**.

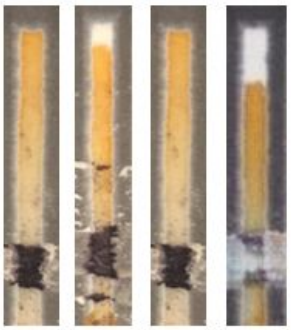
3. **Lane D**

If Lane D looks like one of these examples, check the box marked “This lane passed with appropriate color” for Lane D, otherwise check the box marked “Product Fail”.
If the color is weaker than the photos, write “weak D” in the notes section.

D

Blue at swipe line

5. Check for unexpected filler materials.
    a. alf Lane J shows a black color like one of these examples, check the “Product Contains Starch” box for Lane J. ❌ If starch is present in a capsule formulation, the product fails.



6. Evaluate the quality of the API.
    a. ❌If the color expected in Lane D is missing, the product fails. Check the ‘Product Fail” box .
    b. If all lanes reacted as expected, mark the “Product Pass” box.

If you are not sure of the result:

1. If you are not sure of the result, repeat the analysis on two fresh PADs.
2. Compare the three PADs. Base your final results on the two that look most similar to each other.

Ceftriaxone

Types of this drug that can be tested

Ceftriaxone sodium formulated for injection (1000 mg, dry powder)

Preparation of lab samples for evaluation of the PAD

- Ceftriaxone API: Place ~50 mg pure API in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Ceftriaxone dosage forms: Obtain three vials of ceftriaxone from each of three different brands known to be of good quality. Give one sample of each brand to each analyst.
- Ceftriaxone falsified formulation #1: Grind 500 mg NaCl in a mortar until it has a fine powdery consistency. Place 50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Ceftriaxone falsified formulation #2: Grind 500 mg sugar (sucrose) in a mortar until it has a fine powdery consistency. Place 50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Ceftriaxone substandard formulation #1: Mix 50% powdered NaCl and 50% pure ceftriaxone API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Ceftriaxone substandard formulation #2: Mix 50% ground sucrose and 50% pure ceftriaxone API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.

Examine the packaging (for dosage forms)

1. Record the type of product, stated dosage, brand, manufacturer, manufacturing date, expiration date, and lot or batch number.
2. Record the type of packaging: vial and box or vials in secondary packaging.
3. Note any defects in the product. Defects might include torn or wet box, glue coming apart, faded printing, loose cap.
4. If a scale is available, weigh the vial before and after removing the powder. ❌If the mass difference is less than 900 mg the product fails.

Prepare the sample

1. Write the sample ID on the PAD
2. Enter the PAD number and sample ID in your logbook

3. Use a de-capping tool if available to open the vial, or pry off the cap using a pair of needle-nosed pliers or forceps--be careful not to cut yourself on the sharp metal of the vial cap.

Load and run the PAD

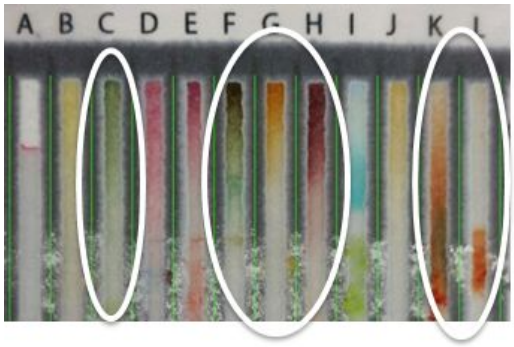
1. Use the wooden paddle to place a heap of powder as big as a grain of rice on the PAD at the arrow mark.
2. Spread the powder from one arrow mark to the other, across all 12 lanes of the PAD.
3. Press down on the paddle and pull it across the powder to make it stick to the paper.
4. Turn the PAD on its side and gently tap off excess powder.
5. You should see powder in each lane, and you should see the lines between the lanes. If you can't see the black lines under the powder, there is too much powder.
6. Place the bottom edge of the PAD (below the blue line) into 1 cm of water. Keep the PAD nearly upright.
7. Hold the PAD upright or lean the PAD against a support (a water bottle, beaker, or wall) for about 3 minutes. You will see water moving up the lanes.
8. When a red dot appears at the top of lane A, remove the PAD and lay it flat on a clean piece of paper.
9. After every 5 PADs, replace the water in the dish with clean water.

Photograph the PAD

1. Let the PAD lie flat for 3 minutes, then photograph the PAD.
2. **If you are using the PADapp,**
    a. Hold the phone over the PAD until the app has taken the photo. You may need to move it up or down until the app has found all the square marks.
    b. Select "ceftriazone" from the pull down menu.
    c. Enter the brand (or "n/a"), lot or batch number (or "n/a"), and enter the sample number in the notes field.
    d. To store the image and data, tap "save and continue" .

Read the test result

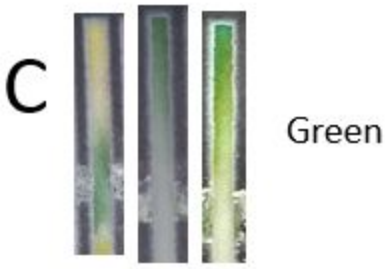
1. Compare your image to the sample image below and fill out the log book to record your results.



2. Ceftriaxone makes special colors in six lanes: **C, F, G, H, K, and L**. All six special colors must be present.

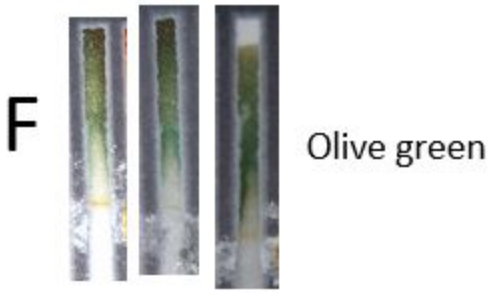
3. **Lane C**

If Lane C looks like one of these examples, check the box marked "This lane passed with appropriate color" for Lane C, otherwise check the box marked "Product Fail".



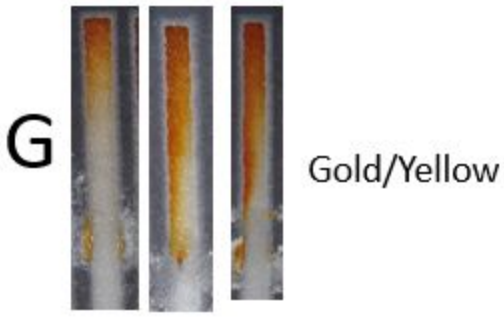
Lane F

If Lane F looks like one of these examples, check the box marked "This lane passed with appropriate color" for Lane F, otherwise check the box marked "Product Fail".



Lane G

If Lane G looks like one of these examples, check the box marked “This lane passed with appropriate color” for Lane G, otherwise check the box marked “Product Fail”.

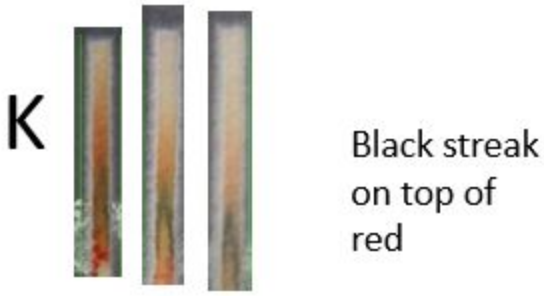


Lane H

If Lane H looks like one of these examples, check the box marked “This lane passed with appropriate color” for Lane H, otherwise check the box marked “Product Fail”.

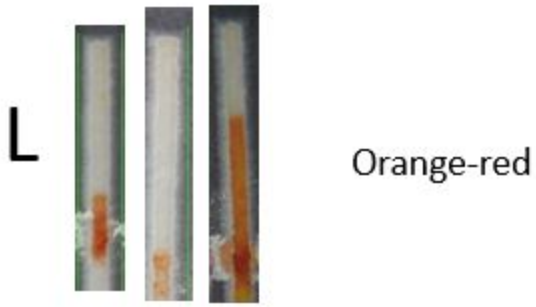
Lane K

If Lane K looks like one of these examples, check the box marked “This lane passed with appropriate color” for Lane K, otherwise check the box marked “Product Fail”.

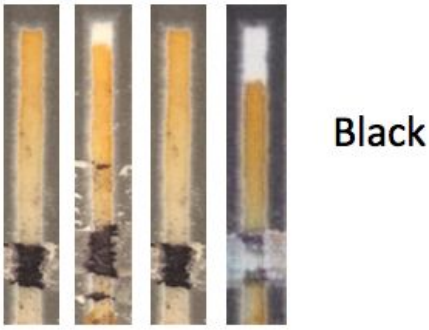


Lane L

If Lane L looks like one of these examples, check the box marked “This lane passed with appropriate color” for Lane L, otherwise check the box marked “Product Fail”.



4. Check for unexpected filler materials.
    a. If Lane J shows a black color like one of these examples, check the “Product Contains Starch” box for Lane J. ❌ If starch is present in a capsule formulation, the product fails.



5. Evaluate the quality of the API.
    a. ❌If any of the colors expected in lanes C, F, G, H, K, and L are missing, the product fails. Check the “Product Fail” box.
    b. If all Lanes reacted as expected, mark the “Product Pass” box.
    c. If the colors in lanes C, G, or H are present but they are weak, the product may be substandard. Write “weak color lane C (or G or H)” in the notes section. If available, run another sample of the product on a fresh PAD.

If you are not sure of the result:

1. If you are not sure of the result, repeat the analysis on two fresh PADs.
2. Compare the three PADs. Base your final results on the two that look most similar to each other.

Chloroquine

Types of this drug that can be tested

Tablet (500 mg dose or 2 x 250 mg dose)
Coated tablets may show unexpected colors from dyes in the coating

Preparation of lab samples for evaluation of the PAD

- Chloroquine API: Place ~50 mg pure API in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Chloroquine dosage forms: Obtain three tablets of chloroquine (500 mg dosage) from each of three different brands known to be of good quality. Give one sample of each brand to each analyst.
- Chloroquine falsified formulation #1: Mix 70% chalk (calcium carbonate) and 30% corn starch. Place 50 mg powder in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Chloroquine falsified formulation #2: Place ~50 mg paracetamol in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Chloroquine substandard formulation #1: Mix 50% talcum powder and 50% pure chloroquine API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Chloroquine substandard formulation #2: Mix 50% corn starch and 50% pure chloroquine API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.

Examine the packaging (for dosage forms)

1. Record the type of product, stated dosage, brand, manufacturer, manufacturing date, expiration date, and lot or batch number.
2. Record the type of packaging: blister pack and box, blister pack, tablets in secondary packaging. Record the color of the tablet and any imprint on the tablets.
3. Note any defects in the product. Defects might include torn or wet box, glue coming apart, torn or dented blister pack, faded printing, tablets broken or crumbling to powder.
4. If a scale is available, weigh a single tablet. ❌If the mass of a 500 mg tablet is less than 450 mg, or the mass of a 250 mg tablet is less than 225 mg, the product fails.

Prepare the sample

1. Write the sample ID on the PAD
2. Enter the PAD number and sample ID in your logbook
3. Crush one tablet in a mortar and pestle, or by wrapping the tablet in a clean piece of paper and then using a blunt object

Load and run the PAD

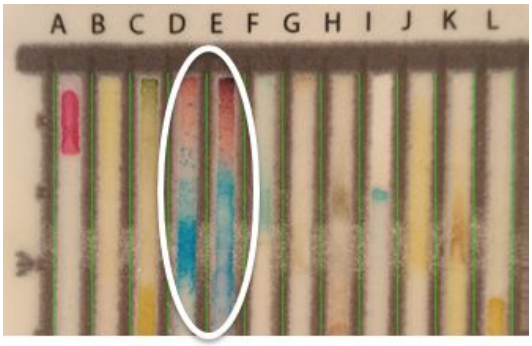
1. Use the wooden paddle to place a heap of powder as big as a grain of rice on the PAD at the arrow mark.
2. Spread the powder from one arrow mark to the other, across all 12 lanes of the PAD.
3. Press down on the paddle and pull it across the powder to make it stick to the paper.
4. Turn the PAD on its side and gently tap off excess powder.
5. You should see powder in each lane, and you should see the lines between the lanes. If you can't see the black lines under the powder, there is too much powder.
6. Place the bottom edge of the PAD (below the blue line) into 1 cm of water. Keep the PAD nearly upright.
7. Hold the PAD upright or lean the PAD against a support (a water bottle, beaker, or wall) for about 3 minutes. You will see water moving up the lanes.
8. When a red dot appears at the top of lane A, remove the PAD and lay it flat on a clean piece of paper.
9. After every 5 PADs, replace the water in the dish with clean water.

Photograph the PAD

1. Let the PAD lie flat for 3 minutes, then photograph the PAD.
2. **If you are using the PADapp,**
    a. Hold the phone over the PAD until the app has taken the photo. You may need to move it up or down until the app has found all the square marks.
    b. Select "chloroquine" from the pull down menu.
    c. Enter the brand (or "n/a"), lot or batch number (or "n/a"), and enter the sample number in the notes field.
    d. To store the image and data, tap "save and continue" .

Read the test result

1. Compare your image to the sample image below and fill out the log book to record your results.



2. Chloroquine makes special colors in Lanes **D and E**, both must be present.

3. **Lane D**

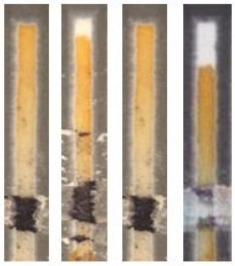
If Lane D looks like one of these examples, check the box marked "This lane passed with appropriate color" for Lane D, otherwise check the box marked "Product Fail".

If the color is significantly weaker than shown, write "weak D" in the notes box.

4. **Lane E.**

If Lane E looks like one of these examples, check the box marked "This lane passed with appropriate color" for Lane E, otherwise check the box marked "Product Fail". If the color is significantly weaker than shown, write "weak E" in the notes box.

5. Check for unexpected filler materials.
    a. If Lane J shows a black color like one of these examples, check the “Product Contains Starch” box for Lane J. ❌ If starch is present in a capsule formulation, the product fails.



Black

6. Evaluate the quality of the API.
    a. ❌If the colors in Lanes D and/or E are missing, the product fails, denoted by the ‘Product Fail” box being checked.
    b. If all Lanes reacted as expected, mark the “Product Pass” box.
    c. If the color in Lanes D or E are present but they are weak, the product may be substandard. Write “weak colors” in the notes section. If available, run another sample of the product on a fresh PAD.

If you are not sure of the result:

1. If you are not sure of the result, repeat the analysis on two fresh PADs.
2. Compare the three PADs. Base your final results on the two that look most similar to each other.

Ciprofloxacin

Types of this drug that can be tested

Tablets (250 and 500 mg doses)

Coated tablets may show unexpected colors from dyes in the coating

Preparation of lab samples for evaluation of the PAD

- Ciprofloxacin API: Place ~50 mg pure API in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Ciprofloxacin dosage forms: Obtain three tablets of ciprofloxacin (250 mg or 500 mg dosage) from each of three different brands known to be of good quality. Give one sample of each brand to each analyst.
- Ciprofloxacin falsified formulation #1: Mix 70% chalk (calcium carbonate) and 30% corn starch. Place 50 mg powder in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Ciprofloxacin falsified formulation #2: Place ~50 mg paracetamol in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Ciprofloxacin substandard formulation #1: Mix 50% talcum powder and 50% pure ciprofloxacin API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Ciprofloxacin substandard formulation #2: Mix 50% corn starch and 50% pure ciprofloxacin API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.

Examine the packaging (for dosage forms)

1. Record the type of product, stated dosage, brand, manufacturer, manufacturing date, expiration date, and lot or batch number.
2. Record the type of packaging: blister pack and box, blister pack, tablets in secondary packaging. Record the color of the tablet and any imprint on the tablets.
3. Note any defects in the product. Defects might include torn or wet box, glue coming apart, torn or dented blister pack, faded printing, tablets broken, moldy, or crumbling to powder.

4. If a scale is available, weigh a single tablet. ❌If the mass is less than 225 mg for a 250 mg dose product or less than 450 mg for a 500 mg product, the product fails.

Prepare the sample

1. Write the sample ID on the PAD
2. Enter the PAD number and sample ID in your logbook
3. Crush one tablet in a mortar and pestle, or by wrapping the tablet in a clean piece of paper and then using a blunt object to crush it to fine powder.

Load and run the PAD

1. Use the wooden paddle to place a heap of powder as big as a grain of rice on the PAD at the arrow mark.
2. Spread the powder from one arrow mark to the other, across all 12 lanes of the PAD.
3. Press down on the paddle and pull it across the powder to make it stick to the paper.
4. Turn the PAD on its side and gently tap off excess powder.
5. You should see powder in each lane, and you should see the lines between the lanes. If you can't see the black lines under the powder, there is too much powder.
6. Place the bottom edge of the PAD (below the blue line) into 1 cm of water. Keep the PAD nearly upright.
7. Hold the PAD upright or lean the PAD against a support (a water bottle, beaker, or wall) for about 3 minutes. You will see water moving up the lanes.
8. When a red dot appears at the top of lane A, remove the PAD and lay it flat on a clean piece of paper.
9. After every 5 PADs, replace the water in the dish with clean water.

Photograph the PAD

1. Let the PAD lie flat for 3 minutes, then photograph the PAD.
2. **If you are using the PADapp,**
    a. Hold the phone over the PAD until the app has taken the photo. You may need to move it up or down until the app has found all the square marks.
    b. Select "ciprofloxacin" from the pull down menu.
    c. Enter the brand (or "n/a"), lot or batch number (or "n/a"), and enter the sample number in the notes field.
    d. To store the image and data, tap "save and continue" .

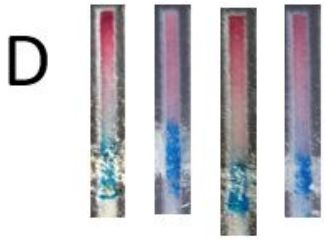
Read the test result

1. Compare your image to the sample image below and fill out the log book to record your results.

2. Ciprofloxacin makes special colors in Lanes **D and L,** both must be present.

3. **Lane D**

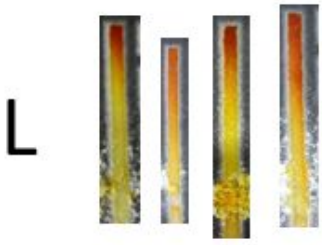
If Lane D looks like one of these examples, check the box marked “This lane passed with appropriate color” for Lane D, otherwise check the box marked “Product Fail”.



Blue at swipe line, fades to pink over time

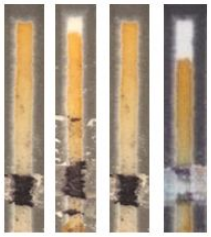
Lane L

If Lane L looks like one of these examples, check the box marked “This lane passed with appropriate color” for Lane L, otherwise check the box marked “Product Fail”.



Orange

4. Check for filler materials.
    a. If Lane J shows a black color like one of these examples, check the “Product Contains Starch” box for Lane J. ❌ If starch is present in a capsule formulation, the product fails.



Black

5. Evaluate the quality of the API.
    a. ❌If the colors in Lanes D and/or L are missing, the product fails, denoted by the ‘Product Fail” box being checked.
    b. If all Lanes reacted as expected, mark the “Product Pass” box.

If you are not sure of the result:

1. If you are not sure of the result, repeat the analysis on two fresh PADs.
2. Compare the three PADs. Base your final results on the two that look most similar to each other.

Doxycycline

Types of this drug that can be tested

Doxycycline Hyclate (HCl salt of doxycycline) gel capsule (100 mg dose)

Preparation of lab samples for evaluation of the PAD

- Doxycycline API: Place ~50 mg pure API in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Doxycycline dosage forms: Obtain three capsules of doxycycline (100 mg dosage) from each of three different brands known to be of good quality. Give one sample of each brand to each analyst.
- Doxycycline falsified formulation #1: Mix 70% chalk (calcium carbonate) and 30% corn starch. Place 50 mg powder in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Doxycycline falsified formulation #2: Place ~50 mg paracetamol in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Doxycycline substandard formulation #1: Mix 50% talcum powder and 50% pure doxycycline API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts. [Notre Dame has an “authentic” substandard product with this composition]
- Doxycycline substandard formulation #2: Mix 50% corn starch and 50% pure doxycycline API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.

Examine the packaging (for dosage forms)

1. Record the type of product, stated dosage, brand, manufacturer, manufacturing date, expiration date, and lot or batch number.
2. Record the type of packaging: blister pack and box, blister pack, capsules in secondary packaging. Record the color of the capsule and any printing on the capsules.
3. Note any defects in the product. Defects might include torn or wet box, glue coming apart, torn or dented blister pack, faded printing, capsules broken or dented.
4. If a scale is available, weigh the powder in one gel capsule. ❌If the mass is less than 90 mg, the product fails.

Prepare the sample

1. Write the sample ID on the PAD
2. Enter the PAD number and sample ID in your logbook
3. Open one gel capsule by pulling and twisting the two halves apart

Load and run the PAD

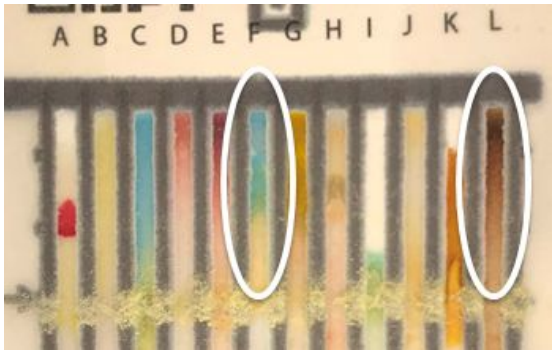
1. Use the wooden paddle to place a heap of powder as big as a grain of rice on the PAD at the arrow mark.
2. Spread the powder from one arrow mark to the other, across all 12 lanes of the PAD.
3. Press down on the paddle and pull it across the powder to make it stick to the paper.
4. Turn the PAD on its side and gently tap off excess powder.
5. You should see powder in each lane, and you should see the lines between the lanes. If you can't see the black lines under the powder, there is too much powder.
6. Place the bottom edge of the PAD (below the blue line) into 1 cm of water. Keep the PAD nearly upright.
7. Hold the PAD upright or lean the PAD against a support (a water bottle, beaker, or wall) for about 3 minutes. You will see water moving up the lanes.
8. When a red dot appears at the top of lane A, remove the PAD and lay it flat on a clean piece of paper.
9. After every 5 PADs, replace the water in the dish with clean water.

Photograph the PAD

1. Let the PAD lie flat for 3 minutes, then photograph the PAD.
2. **If you are using the PADapp,**
    a. Hold the phone over the PAD until the app has taken the photo. You may need to move it up or down until the app has found all the square marks.
    b. Select "doxycycline" from the pull down menu.
    c. Enter the brand (or "n/a"), lot or batch number (or "n/a"), and enter the sample number in the notes field.
    d. To store the image and data, tap "save and continue" .

Read the test result

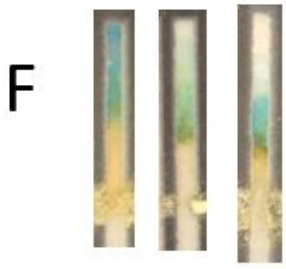
1. Compare your image to the sample image below and fill out the log book to record your results.



2. Doxycycline makes special colors in Lanes **F and L,** both must be present.

3. **Lane F**

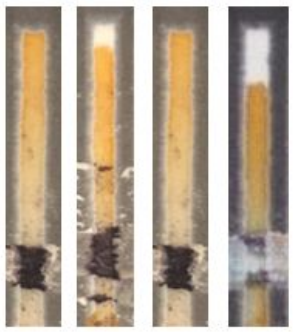
If Lane F looks like one of these examples, check the box marked “This lane passed with appropriate color” for Lane F, otherwise check the box marked “Product Fail”.



Lane L

If Lane L looks like one of these examples, check the box marked “This lane passed with appropriate color” for Lane L, otherwise check the box marked “Product Fail”.

4. Check for unexpected filler materials.
    a. If Lane J shows a black color like one of these examples, check the “Product Contains Starch” box for Lane J. ❌ If starch is present in a capsule formulation, the product fails.



5. Evaluate the quality of the API.
    a. ❌If the colors in Lanes F and/or L are missing, the product fails, denoted by the ‘Product Fail” box being checked.
    b. If all Lanes reacted as expected, mark the “Product Pass” box.

If you are not sure of the result:

1. If you are not sure of the result, repeat the analysis on two fresh PADs.
2. Compare the three PADs. Base your final results on the two that look most similar to each other.

Hydroxychloroquine

Types of this drug that can be tested

Hydroxychloroquine sulfate, typically in 100, 200, or 400 mg tablets
Coated tablets may show unexpected colors from dyes in the coating

Preparation of lab samples for evaluation of the PAD

- Hydroxychloroquine sulfate API: Place ~50 mg pure API in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Hydroxychloroquine dosage forms: Obtain three tablets of hydroxychloroquine (100 mg dosage) from each of three different brands known to be of good quality. Give one sample of each brand to each analyst.
- Hydroxychloroquine falsified formulation #1: Mix 70% chalk (calcium carbonate) and 30% corn starch. Place 50 mg powder in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Hydroxychloroquine falsified formulation #2: Place ~50 mg paracetamol in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Hydroxychloroquine substandard formulation #1: Mix 50% talcum powder and 50% pure hydroxychloroquine API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.
- Hydroxychloroquine substandard formulation #2: Mix 50% corn starch and 50% pure hydroxychloroquine API. Place ~50 mg in Eppendorf tube or gel-cap. Make 3 samples for the 3 analysts.

Examine the packaging (for dosage forms)

1. Record the type of product, stated dosage, brand, manufacturer, manufacturing date, expiration date, and lot or batch number.
2. Record the type of packaging: blister pack and box, blister pack, tablets in secondary packaging. Record the color of the tablet and any imprint on the tablets.
3. Note any defects in the product. Defects might include torn or wet box, glue coming apart, torn or dented blister pack, faded printing, tablets broken or crumbling to powder.

4. If a scale is available, weigh the powder of a single tablet. ❌If the mass is less than 90 mg for a 100 mg product, 180 mg for a 200 mg product, or 360 mg for a 400 mg product, the product fails.

Prepare the sample

1. Write the sample ID on the PAD
2. Enter the PAD number and sample ID in your logbook
3. Crush one tablet in a mortar and pestle, or by wrapping the tablet in a clean piece of paper and then using a blunt object

Load and run the PAD

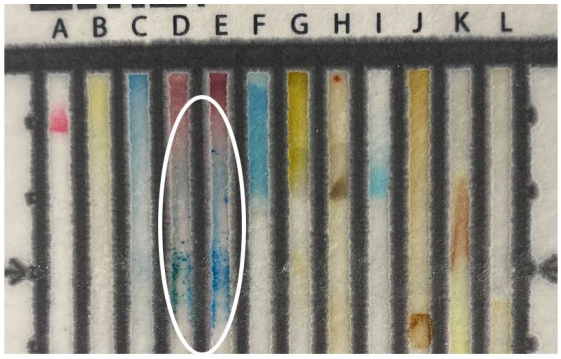
1. Use the wooden paddle to place a heap of powder as big as a grain of rice on the PAD at the arrow mark.
2. Spread the powder from one arrow mark to the other, across all 12 lanes of the PAD.
3. Press down on the paddle and pull it across the powder to make it stick to the paper.
4. Turn the PAD on its side and gently tap off excess powder.
5. You should see powder in each lane, and you should see the lines between the lanes. If you can't see the black lines under the powder, there is too much powder.
6. Place the bottom edge of the PAD (below the blue line) into 1 cm of water. Keep the PAD nearly upright.
7. Hold the PAD upright or lean the PAD against a support (a water bottle, beaker, or wall) for about 3 minutes. You will see water moving up the lanes.
8. When a red dot appears at the top of lane A, remove the PAD and lay it flat on a clean piece of paper.
9. After every 5 PADs, replace the water in the dish with clean water.

Photograph the PAD

1. Let the PAD lie flat for 3 minutes, then photograph the PAD.
2. **If you are using the PADapp,**
    a. Hold the phone over the PAD until the app has taken the photo. You may need to move it up or down until the app has found all the square marks.
    b. Select "hydroxychloroquine" from the pull down menu.
    c. Enter the brand (or "n/a"), lot or batch number (or "n/a"), and enter the sample number in the notes field.
    d. To store the image and data, tap "save and continue" .

Read the test result

1. Compare your image to the sample image below and fill out the log book to record your results.



2. Hydroxychloroquine makes special colors in Lanes **D and E,** both must be present.

3. **Lane D**

If Lane looks like one of these examples, check the box marked "This lane passed with appropriate color" for Lane E, otherwise check the box marked "Product Fail". If the color is significantly weaker than shown, write "weak E" in the notes box.

D

Deep blue/green
Spreads up lane/spotty

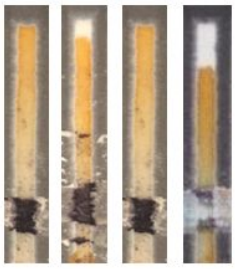
Lane E

If Lane E looks like one of these examples, check the box marked "This lane passed with appropriate color" for Lane E, otherwise check the box marked "Product Fail". If the color is significantly weaker than shown, write "weak E" in the notes box.

E

Deep blue
Spreads up lane/spotty

4. Check for unexpected filler materials.
   If Lane J shows a black color like one of these examples, write “starch” in the notes section. ❌ If starch is present in a capsule formulation, the product fails.



Black

5. Evaluate the quality of the API.
   a. ❌If the colors in Lanes D and/or E are missing, the product fails, denoted by the ‘Product Fail” box being checked.
   b. If all Lanes reacted as expected, mark the “Product Pass” box.
   c. If the color in Lanes D or E are present but they are weak, the product may be substandard. Write “weak colors” in the notes section. If available, run another sample of the product on a fresh PAD.

If you are not sure of the result:

1. If you are not sure of the result, repeat the analysis on two fresh PADs.
2. Compare the three PADs. Base your final results on the two that look most similar to each other.

Implementing PAD analysis

Setting up the workspace

Requirements:

- ❏ Clean, flat surface at least 25 cm square
- ❏ Daylight or other lighting
- ❏ Temperature between 15C and 40C
- ❏ Mortar and pestle or clean paper and bottle or mug
- ❏ Android cell phone with camera
- ❏ Logbook (provided during training)
- ❏ PADs
- ❏ Container with 1 cm depth water (any drinkable water can be used)
- ❏ Pen or pencil
- ❏ Wooden paddles or lab spatula

Training

Training covers preparing samples, running PADs, keeping records, and recommended practices for following up on samples that fail PAD testing.

In the era of COVID-19, training is provided as a set of short video seminars. Trainers with the PAD project at the University of Notre Dame can train participants in English. A train-the-trainers system could be arranged with implementation partners.

Step 1: Schedule the video training session. Contact mlieberm@nd.edu to arrange a 1 hour Zoom or Skype meeting. Participants do not need to be in the same room. Be prepared to discuss the following topics:

- Your goals for PAD use
- Point of contact at the national drug regulatory agency (DRA)
- Mailing address for shipping the training packs
- Possible issues related to shipping permissions, tariffs, etc.
- Number of participants who will be trained (list their names and roles)

- Do you prefer Skype, Zoom, or another video conferencing option?
- Local options for sending PAD images to UND archive (eg, email/DropBox/WhatsApp)
- Methods for participants to report suspect products.
- Plans for transporting suspect products to a lab for confirmatory testing

Step 2: Obtain a training pack for each participant. Our current training pack covers the following seven pharmaceuticals: Amoxicillin, azithromycin, ceftriaxone (injectable), chloroquine, ciprofloxacin, doxycycline, hydroxychloroquine.

The training pack consists of

- 14 PADs,
- 14 blinded samples
- PAD reading guideline
- logbook
- 14 wooden paddles
- 2 plastic weighing boats

Allow 3 weeks to order and ship the training materials; shipping times will vary depending on your location.

Obtaining PADs

Packs of 10 or 20 PADs can be ordered from the PAD project, email mlieberm@nd.edu. A pack of 10 PADs costs $20 + shipping; a pack of 20 PADs costs $40 + shipping. A training pack contains 14 PADs and 14 blinded proficiency samples and is used to train and certify new PAD users.

PADs are not yet a commercial product; they are being made in the Lieberman lab and a few other labs and are provided to collaborators for research purposes. These costs are on a cost-recovery basis.

Testing lab water supply

PADs were designed to run with deionized or demineralized water, but even in locations with very hard water, the tap water will also work at lower cost. Here is how to evaluate your local tap water.

Supplies needed:

6 PADs
Test substance (ceftriaxone is a good general test substance, or you could use any particular drug you plan to test)
DI or distilled water
tap water

Procedure:

1. Swipe the substance on all 6 PADs as directed. Try to get about the same quantity on each PAD. Label 3 as "tap" and 3 as "DI"

2. Run the PADs.
    Run 3 PADs in deionized or distilled water
    Run 3 PADs in tap water

3. Evaluate each PAD to see if the expected special colors are present. Note any missing colors or extra colors that occur in all three tap water cards and do not appear in the three DI water cards. If you can't reliably distinguish the 3 tap water cards from the 3 DI water cards, it is safe to use tap water in the field tests.

4. Record the test results in your log book.

Obtaining samples

- Samples can be obtained from different points in the supply chain, including customs posts, distribution warehouses, clinics, and medicine shops or pharmacies.
- Store each sample in a separate small bag.
- Store samples at temperatures consistent with the label directions (typically, at cool room temperature).
- Assign each sample a unique sample number. This should be written on the blister pack or vial, the box, and on the sample bag.

Keeping records

Critical records include:

1. sample number,
2. brand,
3. manufacturer,
4. stated API, dose, and dosage form (eg, 500 mg amoxicillin capsules)
5. Packaging (eg, blister pack inside box; vial but no box, capsules in paper envelope)
6. Date and location for sample collection
7. Manufacture date
8. Expiration date

Follow up for suspicious products

The PAD is a presumptive test, which indicates that a product MIGHT be falsified or substandard. Products that fail the PAD test must be followed up with more accurate laboratory tests. Before PAD testing is begun, there should be an arrangement in place to provide confirmatory testing for suspicious samples.

If a product fails a PAD test:

1. Repeat the PAD test with another sample of the same product.
    a. If multiple samples of a product fail multiple PAD tests, treat the product as possibly falsified. It should be reported with urgency. Consider

switching to another brand until results of confirmatory testing are received.

   b. If samples from some boxes of a product pass the PAD test and samples from other boxes fail, check the product packaging carefully. There may be a deceptive counterfeit in the market. Look for spelling mistakes or different package types, colors, fonts, or types of paper.

2. Find out if the product is widely available.
   a. A falsified product that is common in the market is more likely to harm people than a falsified product that is rare. If the product fails multiple PAD tests and can be bought in many locations, it should be reported with urgency.
3. Report the suspect product to authorities and to the PAD project.
   a. For each site, reporting procedures will be made available to participants as part of the initial PAD training.
   b. For each site, there must be a plan in place to enable participants to submit samples for confirmatory testing. The plan should include paying the cost of transporting the sample to the laboratory.

Log book sheets for printing or use in computer notebooks (OK to copy)

--Under construction--