Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information for Lab on Paper: Assay of Beta-Lactam Pharmaceuticals by Redox Titration

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S1. Literature reports of quality of amoxicillin and ampicillin pharmaceutical products

Table S1. Summary of data from peer-reviewed literature

Leading author	Publishing year	Location	API	Sample size	Bad quality rate (%)
Fadeyi[63]	2015	Ghana	Amoxicillin	8	0
Fadeyi[63]	2015	Nigeria	Amoxicillin	4	25*
Yong[64]	2015	Cambodia	Amoxicillin	16	13+
Yong[64]	2015	Cambodia	Ampicillin	15	53+#
Yong[64]	2015	Laos	Amoxicillin	6	83+
Yong[64]	2015	Laos	Ampicillin	5	100+
Hetzel[65]	2014	Papua New Guinea	Amoxicillin	47	2
Baratta[66]	2012	Many African countries, Brazil, India	Amoxicillin	24	46
Hadi[67]	2010	Indonesia	Amoxicillin	20	20
Kyriacos[68]	2008	Lebanon, Jordan, Egypt, Saudi Arabia	Amoxicillin	111	56
Kayumba[69]	2004	Rwanda, Tanzania	Amoxicillin	7	0
Taylor[70]	2001	Nigeria	Amoxicillin	37	27
Taylor[70]	2001	Nigeria	Ampicillin	46	61
Wondemagegnehu[71]	1999	Myanmar	Amoxicillin	18	11
Wondemagegnehu[71]	1999	Myanmar	Ampicillin	13	15
Wondemagegnehu[71]	1999	Viet Nam	Amoxicillin	10	30
Wondemagegnehu[71]	1999	Viet Nam	Ampicillin	6	33

Note: *The one failure had no expiry date listed on the package. +The authors used 85-115% as the "good quality" criteria, which deviates from USP compendial standards. #In one failing pill, the ampicillin had been replaced with amoxicillin, and amoxicillin was not stated on the package.

Table S2. Summary of data from the USP Medicines Quality Database

Country	Year	Amoxicillin % Bad quality (n)	Ampicillin % Bad quality (n)
Peru	2010	13 (55)	NA
Cambodia	2010	0 (28)	4 (27)
Cambodia	2011	0 (30)	0 (22)
Mozambique	2012	0 (27)	NA
Viet Nam	2013	2 (143)	NA
Mozambique	2014	0 (40)	NA

S2. Fabrication of the aPAD

Fabrication is detailed in the Methods section. The chemicals listed in Table S3 were pipetted into the locations show in Figure S1.

Table S3. Chemicals deposited onto aPAD

All volumes are 2.0 µL. Locations refer to Figure S1

Location	Chemical
A	2% Starch
B	1.0 M p-toluenesulfonic acid
C	0.5 M KI/0.3 M $CdCl_2$
D	3.0 mM $Na_2S_2O_3$
E	16.5 mM $Na_2S_2O_3$
F	30.0 mM $Na_2S_2O_3$
G	43.5 mM $Na_2S_2O_3$

Figure S1. Spotting guide for aPAD fabrication

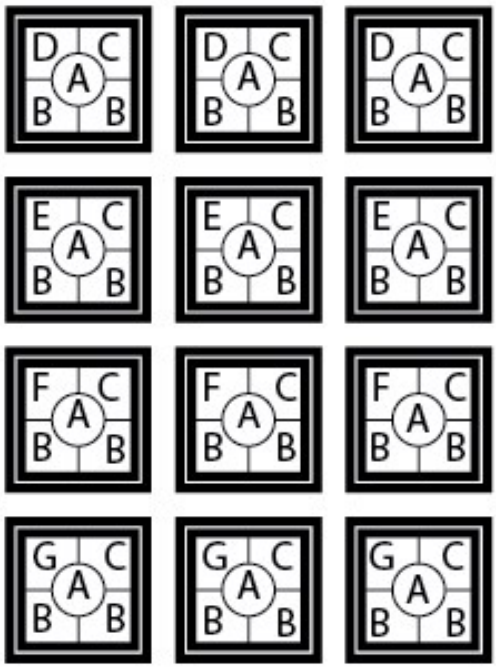

Table S4. Cost analysis of aPAD and HPLC Analysis

Expenditure	HPLC analysis ($USD)	Test kit ($USD)*
Ahlstrom 319 Paper	NA	0.05
Wax	NA	0.03
Chemicals (for test card)	NA	0.01
Plastic wrap to seal test cards	NA	0.05
0.0050 M I_3^-	NA	0.18
Glass scintillation vial for I_3^-	NA	0.10
1.0 M NaOH	NA	0.002
Polyethylene scintillation vial	NA	0.01
1.2 M HCl	NA	0.005
Polyethylene scintillation vial	NA	0.01
Disposable plastic pipets x 3**	NA	0.03
Weigh paper	0.02	0.02
Glass reaction vial x 3***	NA	0.04
Packaging box	NA	0.02
Column#	0.70	NA
UV Bulb##	0.06	NA
Syringe	0.22	NA
Filter	0.79	NA
Autosampler vial	0.27	NA
Autosampler vial lid	0.22	NA
Secondary standard###	0.23	NA
Mobile phase####	0.09	
Total	2.60	0.54

*Cost to analyze 1 sample in triplicate (only need one test card to do so), assuming 20 test cards per pack. **Three are included, one per solution. They should be washed if used for more than one day. ***Three are included so three analyses can happen simultaneously. These would have to be washed between analyses. #Assumes the column costs $700 and 1000 samples can be analyzed before it needs to be replaced. ## Assumes the bulb costs $600, has a 2000 hr life, and each sample has a 12 minute run time; 10,000 samples can be run before it needs to be replaced. ###250 mg of a secondary standard of ampicillin costs $37.10 from Sigma-Aldrich. Assumes 30 mg of the standard is massed for analysis everyday and that 20 unknown samples are analyzed against it. ####Assumes methanol consumed during 12 minute run flowing at 1 mL/min.

A single point measurement on a test card is ($0.54 USD / 3) = $0.18 USD.

S3. HPLC methodology for analyzing amoxicillin and ampicillin

HPLC Instrument: Waters 2695
Detector: Waters 2487 Dual λ Absorbance

Amoxicillin
Column: Symmetry C18 5µm, 4.6 x 100 mm column
Run time: 12 min
Peak retention time: 3.3-3.4 min
Wavelength: 220 nm
Injection volume: 18 µL
Flow rate: 1.00 mL/min
Nominal sample concentration: 0.5 mg/mL (water)
Mobile phase: Gradient method with methanol and 20 mM phosphate buffer, pH= 4.4±0.1.

Time (min)	Methanol (%)	Phosphate buffer (%)*	Flow (mL/min)	Change
0.0	5.0	95.0	1.00	hold
0.50	5.0	95.0	0.50	hold
5.00	30.0	70.0	0.50	linear
7.00	90.0	10.0	0.50	linear
8.00	90.0	10.0	0.50	hold
8.50	25.0	75.0	0.50	linear
10.00	10.0	90.0	0.50	linear
11.00	5.0	95.0	0.50	linear
12.00	5.0	95.0	0.50	hold

Ampicillin
Column: XBridge C18 5µm, 3.0 x 50 mm
Run time: 6 min
Peak retention time: 1.2-1.4 (void time = 0.6 min)
Wavelength: 230 nm
Injection volume: 40 µL
Flow rate: 1.00 mL/min
Nominal sample concentration: 0.5 mg/mL
Mobile phase: 20% Methanol, 80% 20 mM phosphate buffer pH 4.4±0.1 (isocratic)

S4. Comparison of inexpensive milligram balances

The accuracy and precision of three portable scales were assessed to see which would be the best to use in a test kit. 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 performance is acceptable for field assay (±2% repeatability) if at least 140 mg is weighed.

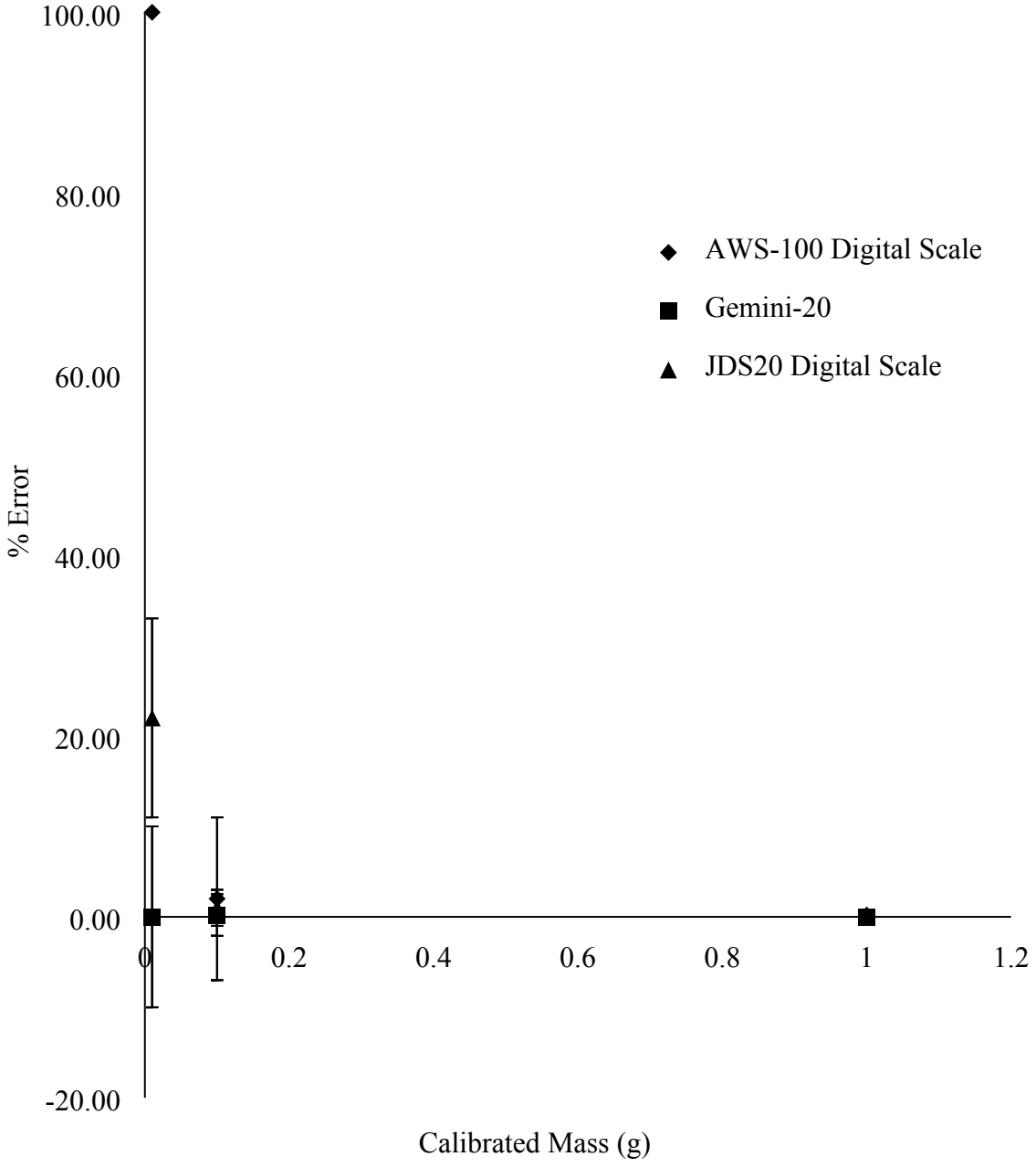

Figure S2. Accuracy of portable scales.

Calibration masses used: 0.01000 g, 0.10000 g, 1.00000 g. The Gemini-20 consistently produced the most accurate result, and it is a milligram scale. n=5 for 0.01000 g and 0.10000 g; n=10 for 1.00000 g.

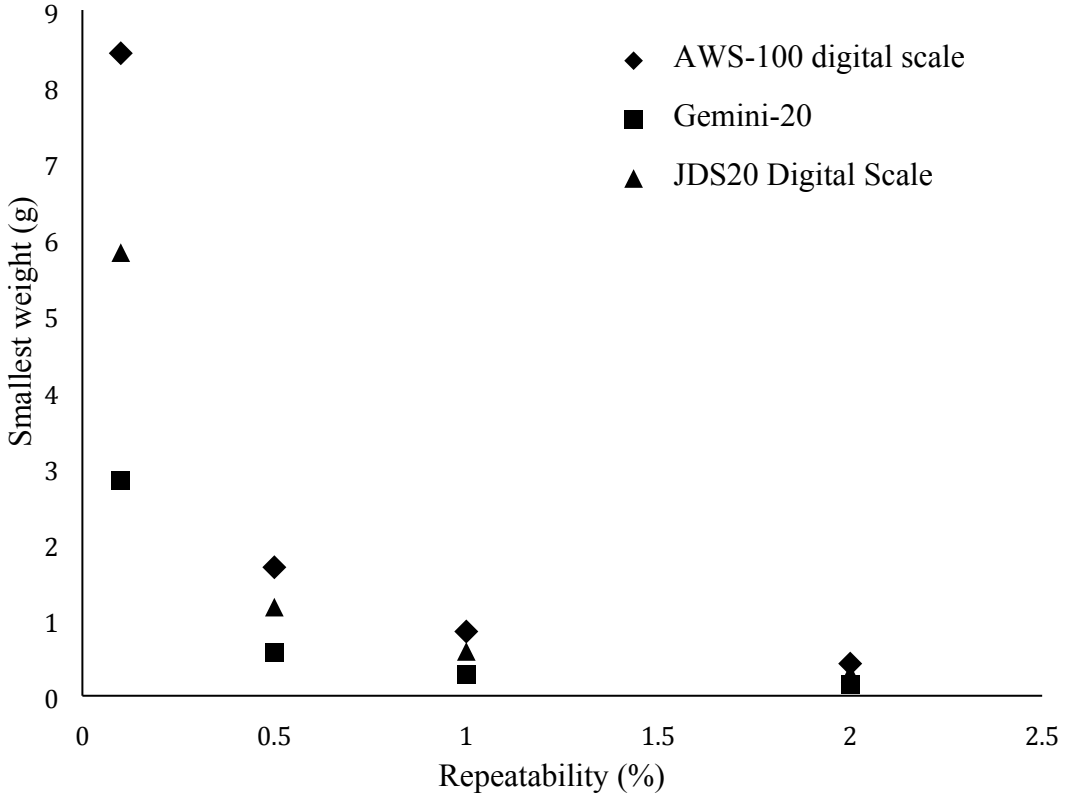

Figure S3. Repeatability of portable scales.

In order to prepare antibiotic pills for analysis that meet the USP requirement for repeatability (NMT 0.1%), at least 3 g would have to be weighed on the Gemini-20. Most pills weigh less than 1 g, so this is not possible. For field analysis, we target a 2% repeatability since the Gemini 20 can mass ~140 mg reliably at that point. This is the only level that allows 250 mg pills to be analyzed. (n=10 for each balance; the repeatability was set to different levels).

Table S5. Stoichiometric ratio between degraded antibiotic and triiodide

AB	$[AB]_i$ (mM)	$[I_3^-]_i$ (mM)	AB_i (mol)	$I_3^-{}_i$ (mol)	$I_3^-{}_f$ (mol)	$I_3^-{}_{i-f}$ (mol)	I_3^-: AB	AVG	SD
Amox	0.16	3.11	2.38E-06	4.66E-05	3.29E-05	1.37E-05	5.76	5.73	0.18
Amox	0.23	3.01	3.58E-06	4.66E-05	2.73E-05	1.94E-05	5.41		
Amox	0.30	2.91	4.77E-06	4.66E-05	1.95E-05	2.71E-05	5.68		
Amox	0.36	2.82	5.96E-06	4.66E-05	1.36E-05	3.31E-05	5.55		
Amox	0.10	3.73	2.38E-06	9.32E-05	7.95E-05	1.37E-05	5.74		
Amox	0.14	3.65	3.58E-06	9.32E-05	7.19E-05	2.13E-05	5.96		
Amox	0.18	3.58	4.77E-06	9.32E-05	6.50E-05	2.82E-05	5.91		
Amox	0.22	3.52	5.96E-06	9.32E-05	5.85E-05	3.47E-05	5.83		
Amp	0.19	3.33	2.86E-06	5.00E-05	3.38E-05	1.62E-05	5.65	5.67	0.65
Amp	0.28	3.23	4.29E-06	5.00E-05	2.66E-05	2.34E-05	5.45		
Amp	0.36	3.13	5.72E-06	5.00E-05	2.01E-05	2.99E-05	5.22		
Amp	0.43	3.03	7.16E-06	5.00E-05	1.59E-05	3.41E-05	4.76		
Amp	0.11	4.00	2.86E-06	1.00E-04	8.00E-05	2.00E-05	6.99		
Amp	0.17	3.92	4.29E-06	1.00E-04	7.45E-05	2.55E-05	5.93		
Amp	0.22	3.85	5.72E-06	1.00E-04	6.68E-05	3.32E-05	5.80		
Amp	0.27	3.77	7.16E-06	1.00E-04	6.02E-05	3.98E-05	5.56		
CA	0.33	3.11	4.88E-06	4.66E-05	4.64E-05	2.50E-07	0.05	0.27	0.15
CA	0.49	3.01	7.31E-06	4.66E-05	4.50E-05	1.60E-06	0.22		
CA	0.63	2.91	9.75E-06	4.66E-05	4.38E-05	2.82E-06	0.29		
CA	0.76	2.82	1.22E-05	4.66E-05	4.16E-05	5.03E-06	0.41		
CA	0.20	3.73	4.88E-06	9.32E-05	9.23E-05	9.25E-07	0.19		
CA	0.30	3.65	7.31E-06	9.32E-05	9.20E-05	1.23E-06	0.17		
CA	0.39	3.58	9.75E-06	9.32E-05	9.00E-05	3.18E-06	0.33		
CA	0.47	3.52	1.22E-05	9.32E-05	8.68E-05	6.38E-06	0.52		

Note: AB = antibiotic, CA = clavulanic acid

S5. Agreement of USP method <425> with HPLC

The USP assay requirement is that beta-lactam medicines must contain 90.0-120.0% of the labeled dosage. We checked to see if USP method <425> gave good agreement with HPLC for normal and thermally degraded beta lactam antibiotics.

Table S6. Good agreement between titration and HPLC for good quality ampicillin dosage forms

Sample	Titration		HPLC		Error	Dosage
	mg	% of labeled	mg	% of labeled	% of labeled	mg
1	238	95.2	225	90.0	5.2	250
2	528	105.6	467	93.4	12.2	500
3	540	108.0	510	102.0	6.0	500
4	460	92.0	510	102.2	-10.2	500
5	443	88.6	508	101.6	-13.0	500

Error (%) = 9.3 and bias (%) = 0.2.

Table S7. Poor agreement between titration and HPLC for thermally degraded amoxicillin dosage forms

Sample	Titration		HPLC		Absolute error (titration – HPLC)	Dosage
	mg	% of labeled	mg	% of labeled	% of labeled	mg
14-0676 P1	318	63.6	172	34.4	29.2	500
14-0670 P1	438	87.6	206	41.2	46.4	500
14-0670 P2	280	56.0	264	52.8	3.2	500
14-0664 P1	438	87.6	198	39.6	48.0	500
14-0651 P2	255	51.0	162	32.4	18.6	500
14-0652 P1	277	55.4	163	32.6	22.8	500

Error (%) = 28.0 and bias (%) = 28.0.

S6. Field Study of aPAD in Kenya

Training secret shoppers

The secret shoppers role-played purchasing medicines with and without a prescription for 1-1.5 hours during training and practiced filling out their portion of the sample information sheet. Secret shoppers were asked to dress casually and to speak in Kiswahili (national language) or a local dialect. Pharmacists educated them about co-purchasing medicines to treat both the main ailment and its symptoms (e.g., amoxicillin to treat an infection and acetaminophen to kill the pain) to simulate a typical patient encounter. The secret shopper asked for a 10% discount on the price (a common practice) and accepted a cheaper product if it was offered.

Selection of pharmacies and medicines

A list of 245 registered pharmacies in the study region was obtained from the Kenyan Pharmacy and Poisons Board's website. Our intent was to collect samples from every pharmacy on the list. During the sample collection, we found that about 10% of the shops listed had closed and new ones had opened. Due to the flux of pharmacy outlets, the sample collection strategy had to change. The secret shoppers used their discretion to select licensed and unlicensed pharmacies within commercial and residential areas. For example, if two pharmacies were in close proximity, medications were purchased from one but not the other, as we were concerned that the shopkeepers would remark upon a repeated purchase of the same products. The costs, drugs, date, and name of the outlet were indicated on the receipt. After leaving the shops, secret shoppers documented the outlets' locations using addresses or landmarks. The samples in their original packaging were put into a bag along with a paper form listing the sample's metadata. Samples were transported to Moi Teaching and Referral hospital within 1 week.

Use of aPAD at Moi Teaching and Referral Hospital, Eldoret, Kenya

The aPAD developer trained a new analyst how to use the aPAD. This new analyst then traveled to Moi Teaching and Referral Hospital in Eldoret, Kenya. Over the course of several days, the new analyst calibrated the aPAD and tested 8 samples of dosage forms purchased in Kenya.

Upon arrival in Eldoret, the analyst prepared solutions needed for the analysis. Elemental iodine and excess KI (10 times the stoichiometric amount) were dissolved in deionized water (triiodide concentration 0.050 M). It took 3 hours for dissolution to occur using a sonicator and manual shaking. The stock was accurately diluted 1:10 to create the 0.0050 M working triiodide solution for the aPAD analysis. A nominal 1.00 mg/mL solution of amoxicillin was prepared by placing the contents of a 500 mg dosage pill into a 500 mL water bottle, filling it with deionized water,

and shaking it on-and-off for about 20 minutes. All amoxicillin solutions were analyzed by the prescribed aPAD procedure. Reagents were massed on a Gemini 20 balance, instead of measured volumetrically. The 1.00 mg/mL solutions were also analyzed by another researcher, using the Waters 2695 HPLC system in the Eldoret laboratory to determine the true amoxicillin content of the pill. Different researchers performed the different analysis techniques using a blinded methodology. A Bland-Altman plot of the results is shown in Figure S4. The results show a 1.1% error and -0.3% bias. All samples analyzed were in the 95% - 110% API range. The limit of agreement for the analyses is ± 3.02%, and its 95% confidence interval is ±1.84%.

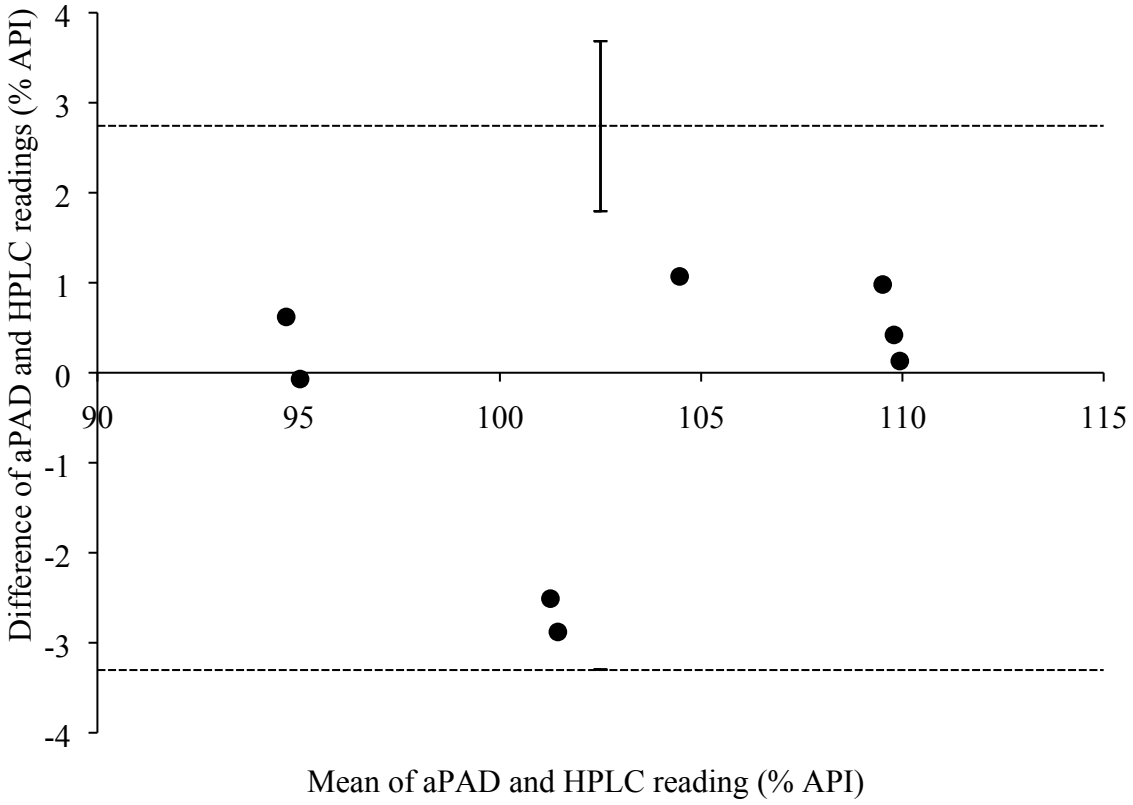

Figure S4. Bland-Altman plot for aPAD and HPLC assays conducted in Kenya.

The limits of agreement (± 2 SD) are plotted with a dashed line, and the error bar shows its 95% confidence interval.

S7. Characterization of insoluble filler material from bad quality pill

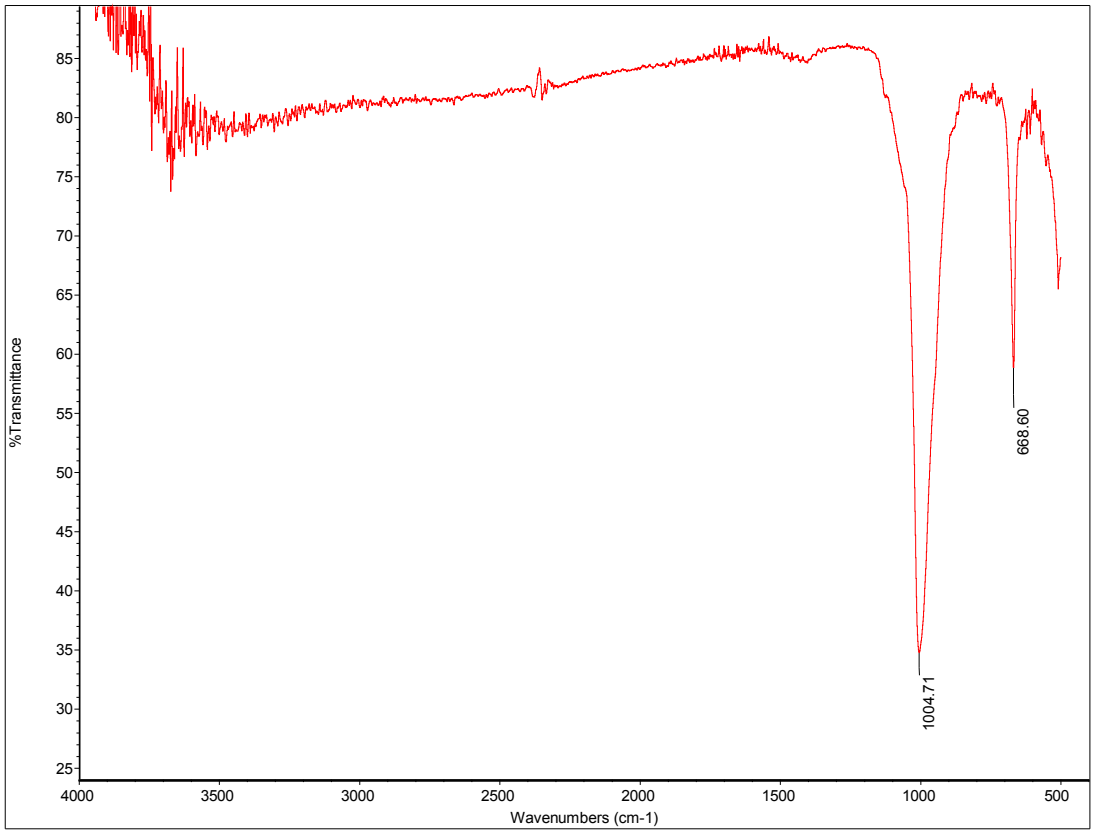

Figure S5. IR of insoluble material isolated from amoxicillin sample

Packaging indicated the brand as Caremox (Shandong Shenglu Co., Ltd.)

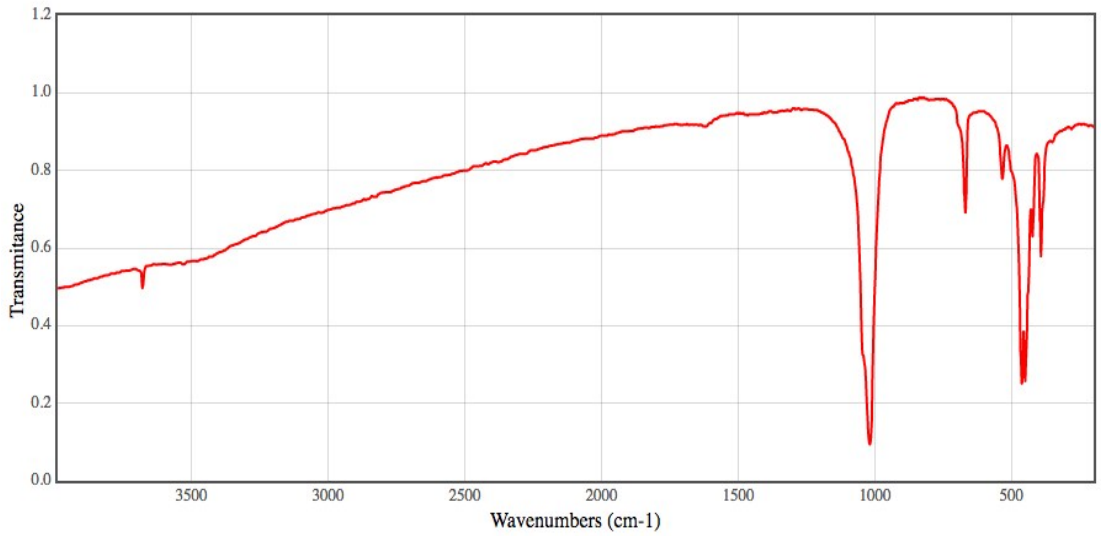

Figure S6. NIST reference spectrum of talc.

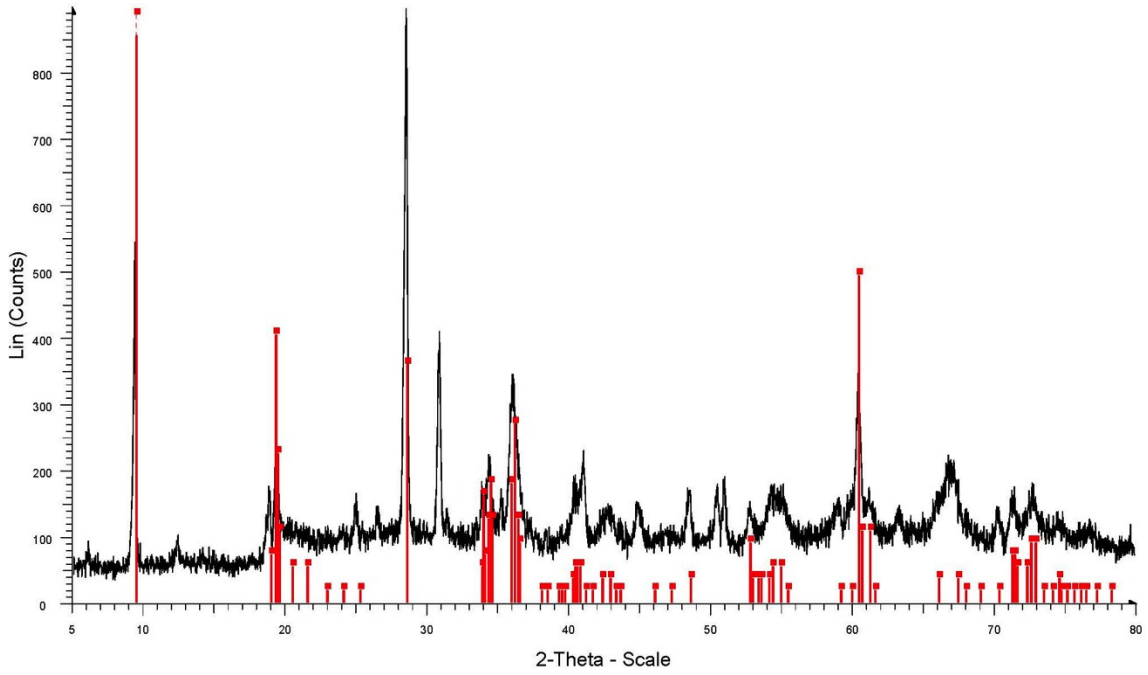

Figure S7. Powder X-ray diffraction of insoluble material isolated from suspect amoxicillin capsule.

The black trace of the diffraction pattern is overlaid by monoclinic and triclinic talc reference spectra (85% and 15% respective intensities) represented by red sticks.

S8. Thermal sensitivity of sample preparation for aPAD analysis

Samples corresponding to 100% amoxicillin content were prepared via 15 min base degradation followed by 15, 30, 45, or 60 min reaction with triiodide at the indicated temperature. The first two rows of dots must turn blue to read correctly (standard images shown on next page)

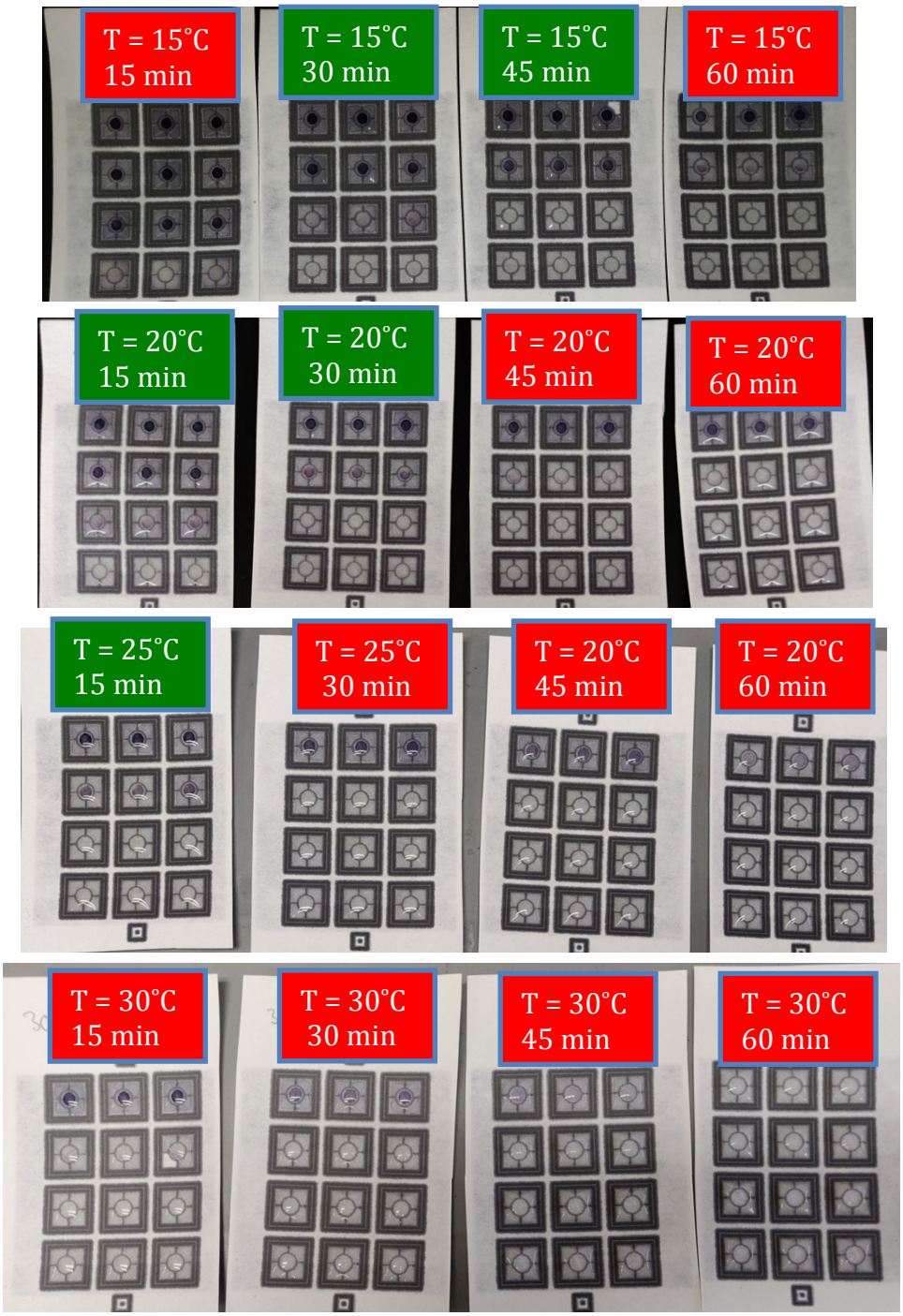

Figure S8. Thermal sensitivity of sample preparation for aPAD

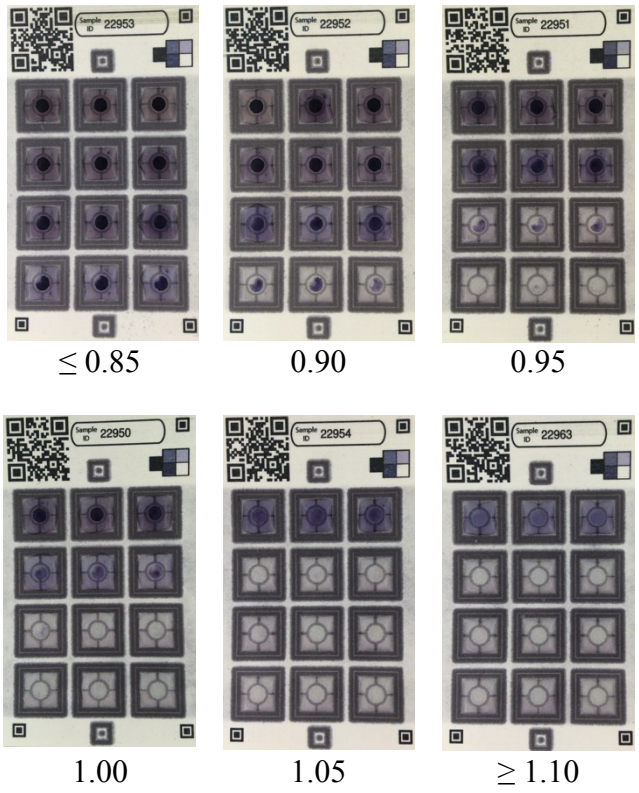

Figure S9: Amoxicillin standard images, figure 2 in mss.

Units are mg anhydrous amoxicillin per mL water. 1.00 mg/ml = 100% API content.