Physical Testing & Thin-Layer Chromatography

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Review and Extension 2020
- Completely revised and updated
- Combines and replaces all former issues
- First time covering 100 active agents
**Minilab Test Protocols Sorted by Therapeutic Classes***

### Transmissible Diseases

#### Antibacterials
- Amoxicillin
- Ampicillin
- Azithromycin
- Benzathine benzylpenicillin
- Benzylpenicillin
- Cefalexin
- Cefazolin
- Cefixime
- **Cefotaxime**
- Cefpodoxime
- Ceftriaxone
- Cefuroxime
- Chloramphenicol
- Chlorhexidine
- Ciprofloxacin
- Clindamycin
- Clavulanic acid
- Cloxacillin
- Doxycycline
- Erythromycin
- Gentamicin
- Levofloxacin
- Metronidazole
- Moxifloxacin
- Ofloxacin
- Phenoxyethylpenicillin
- Procaine benzylpenicillin
- Sulfamethoxazole/Trimethoprim
- Tetracycline

#### Antimalarials
- Amodiaquine
- Artemether
- Artesunate
- Atovaquone
- Chloroquine
- Dihydroartemisinin
- Doxycycline
- Halofantrine
- Lumefantrine
- Mefloquine
- Piperaquine
- Primaquine
- Proguanil
- Pyrimethamine
- Pyronaridine
- Quinine
- Sulfadoxine
- Sulfamethoxypyrazine

#### Anti(retro)virals
- **Aciclovir**
- Didanosine
- EFavirenz
- Indinavir
- Lamivudine
- Nevirapine
- Oseltamivir
- **Ritonavir**
- Stavudine
- Zidovudine

### Non-Transmissible Diseases

#### Analgesics
- Acetylsalicylic acid
- Diclofenac
- Mefenamic acid
- Naproxen
- Paracetamol

#### Antiallergics
- Cetirizine
- Chlorphenamine
- Prednisolone

#### Antiasthmathics
- Aminophylline
- Salbutamol

#### Cardiovasculars
- Amlodipine
- Atenolol
- Bisoprolol
- Captopril
- Furosemide
- Hydrochlorothiazide
- Lisinopril
- Nifedipine
- Simvastatin

#### Endocrines
- Clomifene
- Glibenclamide
- Metformin

#### Gastrointestinalals
- Metoclopramide
- Omeprazole
- Ranitidine

#### Anthelminths
- Albendazole
- Mebendazole
- Praziquantel

#### Antifungals
- **Fluconazole**
- Griseofulvin

*Usual fixed-dose combinations are included. For full detail on this, see alphabetical order in the table of contents.
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XI. OBSERVATIONS MADE AT 254 NM BEFORE STAINING

Artemether itself stays almost invisible and no other spots should be detected unless the medicine under investigation is presented as a co-formulated product. In the latter case, a strong violet spot at a travel distance of about 0.20 indicates the presence of lumefantrine and, in case of dry powders for oral suspensions, a second strong spot between a travel distance of 0.40 and 0.50 the presence of a preservative either from the benzoate or paraben family. Saccharin sodium as sweetener in dispersible tablets would settle at about 0.20 but stays below its limit of detection due to strong dilutions during sample preparation. For a better identification of the lumefantrine fraction go to 286 of this manual.

XII. OBSERVATIONS MADE AT DAYLIGHT AFTER SULPHURIC ACID STAINING

A dark brown spot at a travel distance of about 0.58 indicates the presence of artemether in the test solution. Auxiliary agents incorporated in the different tablet and powder formulations may cause further spots near or on the origin line. Beyond this, no other spots should be visible even if artemether is combined with lumefantrine. Additional strong spots generated by the test solution would point at other drugs or artemether degradation, the latter case being more likely when associated with a smaller principal spot. A smaller principal spot from the test solution may also indicate a poor artemether content and no spot at all a complete artemether absence.

XIII. OBSERVATIONS MADE AT 366 NM AFTER SULPHURIC ACID STAINING

When exposing the chromatoplate to UV light of 366 nm after heating with sulphuric acid, all brown artemether spots previously observed at daylight are now showing an off-white fluorescence.

XIV. RESULTS & ACTIONS TO BE TAKEN

The artemether spot in the chromatogram obtained with the test solution must correspond in terms of colour, size, intensity, shape and travel distance to that in the chromatogram obtained with the lower and higher standard solution. This result must be obtained for each method of detection. If this is not achieved, repeat the run from scratch with a second sample. Reject the batch if the drug content cannot be verified in a third run. For a second opinion, refer additional samples to a fully-fledged drug quality control laboratory. Retain some samples and put the batch on quarantine until a final decision on rejection or release has been taken. For documentation purposes, take pictures of all the readings with a digital camera turning off the flash first.
XI. OBSERVATIONS MADE AT 254 NM

Clavulanic acid stays invisible and spots at a travel distance of about 0.28 indicate the presence of amoxicillin in the test solution. Additional strong spots generated by the test solution would point at other drugs. For a further verification of amoxicillin identity and content follow the relevant protocol shown in this manual.

XII. OBSERVATIONS MADE AT DAY-LIGHT AFTER IODINE STAINING

A strong yellow-brown spot at a travel distance of about 0.38 indicates the presence of clavulanic acid in the test solution. Amoxicillin spots already observed at 254 nm are now turning yellowish brown, too. Additional strong spots generated by the test solution would point at other drugs or some degradation of clavulanic acid or amoxicillin, the latter case being more likely when each time associated with a smaller principal spot. A smaller principal spot from the test solution may also indicate a poor clavulanic acid content and no spot at all a complete absence of clavulanic acid. Still observe the plate when iodine evaporates. Spots reflecting poor quality products will disappear first gradually followed by the reference spots representing a drug content of an 80 and 100 percent, respectively. Auxiliary agents incorporated in different finished products might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line.

XIII. RESULTS & ACTIONS TO BE TAKEN

The spot for clavulanic acid in the chromatogram obtained with the test solution must correspond in terms of colour, size, intensity, shape and travel distance to that in the chromatogram obtained with the lower and higher standard solution. This result must be obtained for each method of detection. If this is not achieved, repeat the run from scratch with a second sample. Reject the batch if the drug content cannot be verified in a third run. For a second opinion, refer additional samples to a fully-fledged drug quality control laboratory. Retain samples and put the batch on quarantine until a final decision on rejection or release has been taken. For documentation purposes, take pictures of all the readings with a digital camera turning off the flash first.
XI. OBSERVATIONS MADE AT 254 NM

Ethambutol stays invisible and no other spots should be detected unless the medicine under investigation is presented as a fixed-dose combination product containing also other antituberculosis compounds. In the latter case, spots made of isoniazid will become visible at a travel distance of about 0.45 and spots made of pyrazinamide at a travel distance of about 0.57. Spots made of rifampicin will be visible at a travel distance of about 0.72 at daylight already. For all of this, consult also the picture shown on page 385.

XII. OBSERVATIONS MADE AT DAYLIGHT AFTER STAINING WITH NINHYDRIN

A red spot at a travel distance of about 0.34 indicates the presence of ethambutol in the test solution. Next to ethambutol, rifampicin and pyrazinamide will be become visible, too. Additional strong spots generated by the test solution would point at other drugs or ethambutol degradation, the latter case being more likely when associated with a smaller principal spot. A smaller principal spot from the test solution may also indicate a poor ethambutol content and no spot at all a complete ethambutol absence. Auxiliary agents incorporated in different finished products might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line.

XIII. RESULTS & ACTIONS TO BE TAKEN

The ethambutol spot in the chromatogram obtained with the test solution must correspond in terms of colour, size, intensity, shape and travel distance to that in the chromatogram obtained with the lower and higher standard solution. This result must be obtained for each method of detection. If this is not achieved, repeat the run from scratch with a second sample. Reject the batch if the drug content cannot be verified in a third run. For a second opinion, refer additional samples to a fully-fledged drug quality control laboratory. Retain samples and put the batch on quarantine until a final decision on rejection or release has been taken. For documentation purposes, take pictures of all the readings with a digital camera turning off the flash first.
X. DETECTION

Dry off all residual solvent and observe the chromatoplate under UV light of 254 nm using the battery-driven lamp supplied. Use this method of detection for both, identification and quantification purposes.

XI. OBSERVATIONS MADE AT 254 NM

A strong blue-violet spot at a travel distance of about 0.23 indicates the presence of lamivudine in the test solution. If combined with other antiretroviral medicines, a spot with a relative retention factor of about 0.42 would further indicate the presence of tenofovir disoproxil, a spot at about 0.62 the presence of nevirapine or zidovudine and a spot at about 0.72 the presence of efavirenz. Additional strong spots generated by the test solution would point at other drugs or lamivudine degradation, the latter case being more likely when associated with a smaller principal spot. A smaller principal spot could also be due to a poor lamivudine content and no spot at all due to a complete lamivudine absence. Auxiliary agents incorporated in different finished products might cause some fainter spots emerging near or on the origin line.

XII. RESULTS & ACTIONS TO BE TAKEN

The lamivudine spot in the chromatogram obtained with the test solution must correspond in terms of colour, size, intensity, shape and travel distance to that in the chromatogram obtained with the lower and higher standard solution. This result must be obtained for each method of detection. If this is not achieved, repeat the run from scratch with a second sample. Reject the batch if the drug content cannot be verified in a third run. For a second opinion, refer additional samples to a fully-fledged drug quality control laboratory. Retain samples and put the batch on quarantine until a final decision on rejection or release has been taken. For documentation purposes, take a picture of the reading with a digital camera turning off the flash first.
XI. OBSERVATIONS MADE AT 254 NM

A strong blue-violet spot at a travel distance of about 0.27 combined with a smaller satellite spot just above the principal spot indicates the presence of primaquine in the test solution. Additional strong spots generated by the test solution would point at other drugs or even primaquine degradation, the latter case being more likely when associated with a smaller principal spot. A smaller principal spot from the test solution may also indicate a poor primaquine content and no spot at all a complete primaquine absence.

XII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

When exposing the chromatoplate to iodine vapour, all spots already observed at 254 nm are now turning greenish black. Primaquine performs strong here and the colour stays stable. Auxiliary agents incorporated in different finished products might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line.

XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER NINHyDRIN STAINING

When exposing a second chromatoplate to ninhydrin and heat, then all primaquine spots previously observed at UV light of 254 nm are now turning lilac. This will facilitate further assay reading and interpretation.

XIV. RESULTS & ACTIONS TO BE TAKEN

The primaquine spot in the chromatogram obtained with the test solution must correspond in terms of colour, size, intensity, shape and travel distance to that in the chromatogram obtained with the lower and higher standard solution. This result must be obtained for each method of detection. If this is not achieved, repeat the run from scratch with a second sample. Reject the batch if the drug content cannot be verified in a third run. For a second opinion, refer additional samples to a fully-fledged drug quality control laboratory. Retain samples and put the batch on quarantine until a final decision on rejection or release has been taken. For documentation purposes, take pictures of all the readings with a digital camera turning off the flash first.
XI. OBSERVATIONS MADE AT 254 NM

A strong blue spot at a travel distance of about 0.59 indicates the presence of quinine in the test solution. Additional strong spots generated by the test solution would point at other drugs or quinine degradation, the latter case being more likely when associated with a smaller principal spot. A smaller principal spot from the test solution may also indicate a poor quinine content and no spot at all a complete quinine absence. Auxiliary agents incorporated in different finished products might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line.

XII. OBSERVATIONS MADE AT 366 NM

On exposure to 366 nm in a dark room, the blue fluorescence observed for the quinine spots at 254 nm will now turn into an intense white fluorescence. In addition, under ideal detection conditions, a minor satellite spot probably arriving from dihydroquine will now become visible just below each quinine spot. The latter observation will further emphasise the existence of quinine in the test solution.

XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

When exposing the chromatoplate to iodine vapour, all quinine spots already observed at 254 and 366 nm are now turning orange-brown. Still observe the plate when iodine evaporates. Spots reflecting poor quality products will disappear first gradually followed by the reference spots representing a drug content of an 80 and 100 percent, respectively.

XIV. RESULTS & ACTIONS TO BE TAKEN

The quinine spot in the chromatogram obtained with the test solution must correspond in terms of colour, size, intensity, shape and travel distance to that in the chromatogram obtained with the lower and higher standard solution. This result must be obtained for each method of detection. If this is not achieved, repeat the run from scratch with a second sample. Reject the batch if the drug content cannot be verified in a third run. For a second opinion, refer additional samples to a fully-fledged drug quality control laboratory. Retain samples and put the batch on quarantine until a final decision on rejection or release has been taken. For documentation purposes, take pictures of the readings with a digital camera turning off the flash first.
The GPHF-Minilab™
is a unique miniature laboratory which comes with affordable test methods for a rapid and easy detection of falsified and substandard medicines as entry-level technology for resource limited health settings in low- and middle-income countries.

In more than twenty years of project work, the GPHF-Minilab™ has proven its suitability in nearly 100 countries.

A comprehensive review of the Minilab’s general methods and operations and its test protocols drawn from the main manuals issued 1998 and 2008 and their many extensions issued each year until 2018.

Topped with test protocols for more active pharmaceutical ingredients usually found in priority medicines for transmissible and non-transmissible diseases, this new manual now provides first time test methods for 100 active agents to rapidly verify drug quality for a plethora of finished pharmaceutical products.