ME-13 Testing what you made: simple assays and quality checks without a lab

HEALING after the FALL, research library, ME-13

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1. The three questions, and why only one of them can be answered

A dispensary holding a jar, a bottle or a salvaged blister strip asks three questions, and most of the confusion here comes from running them together. **Identity:** is it what it says it is? Answered by botany, the senses and the microscope, almost never by chemistry at village level. **Purity:** is it spoiled, adulterated or diluted? Answered by the senses, the microscope, simple physical constants and a short list of colour reactions. **Potency:** how strong is it? This a village cannot answer in absolute terms. ME-02 established the scale, with wild leaf varying tenfold and *Artemisia annua* a hundredfold, and no spot test, density reading or taste panel converts those into milligrams (THRIVING library, ME-02).

The honest summary of this report is that a dispensary can answer identity and purity well, and potency only as a ratio against something it has kept. That is what standardisation meant before chemistry arrived.

The most important procedural rule is also the cheapest. WHO's 1998 manual prints it beside the bitterness test: the test "should not be carried out until the identity of the plant material has been confirmed". Every check here is a check on material already identified. None is an identification test, and a healer who uses taste, foam or colour to decide what a plant is has inverted the method and will eventually poison somebody. Hemlock tastes of nothing much, and the mushroom folk tests are graded X in the library and have killed (THRIVING library, PH-10).

The brief names ME-04, ME-07 and OR-04 for cross-reference. None exists in the library at the time of writing.

2. The senses, used as a procedure

Organoleptic examination is the oldest routine in pharmacognosy and the only one that costs nothing. Its value depends on whether it is run as a procedure against a reference or as an impression. WHO sets out the macroscopic routine in a form a village can copy, and Protocol 1 reproduces it (WHO 1998). Two details are worth pulling forward. For dangerous material WHO crushes mechanically rather than by hand and pours boiling water over the sample so the odour comes off the steam. And taste it restricts to one line: applied only if specifically required. That excludes tasting to find out what something is, and tasting anything narrow-margin.

Three limits. The characters drift, a shade-dried and a sun-dried batch differing in colour. Judgement varies between people and from day to day, WHO's own caveat. And macroscopic identity can be imitated: substitutes and adulterants may closely resemble the genuine material, so macroscopy often needs microscopy behind it.

Microscopy is where the senses stop and the evidence starts. WHO calls microscopic inspection indispensable for broken or powdered materials, and the preparation suits a salvaged microscope: a drop of water or of glycerol

and ethanol on a slide, a little powder on a moistened needle tip, a cover glass and a light press. Villani and colleagues (2015) set microscopy against chemical analysis for bilberry fruit and found the chemistry insufficient while microscopy readily detected contaminants, chromatography being limited against dirt, insects, wood and sand (abstract and secondary summary).

The skullcap case is the one to tell at the bedside. A 2011 USDA study of thirteen skullcap supplements found only five with a measurable amount of true *Scutellaria lateriflora*, and four containing hepatotoxic American germander, *Teucrium canadense* (reported through the American Botanical Council) [VERIFY]. A microscope and a reference specimen separate those two plants. A taste test does not.

Quantitative microscopy is within a district's reach. The **lycopodium spore method** mixes *Lycopodium clavatum* spores, uniform at about 94,000 per milligram, into the powdered drug and counts the drug's own structures against the spore count for a percentage purity [VERIFY]. I could not confirm the brief's attribution to Wallis.

3. Physical checks, and the two that lie

Loss on drying is the most useful physical constant a village can measure, because water is what spoils stock. WHO dries a thin-cut sample at 100 to 105 degrees C until two consecutive weighings differ by not more than 5 mg. A village cannot hold that temperature, but a stone shelf beside a fire sits above boiling and below scorching, and the convergence criterion does the rest. The accurate azeotropic method is out of reach.

Ash tests for adulteration with earth and sand. Total ash is ignited at 500 to 600 degrees C until white, which a forge reaches. Acid-insoluble ash, the test that actually measures sand, needs dilute hydrochloric acid and ashless filter paper and is a district test. The village version is crude and honest: ignite a weighed sample on a clean tile in the hottest part of the fire and weigh the residue. Twice the reference batch's ash means something was added.

Extractable matter is WHO's own fallback for materials for which, it says, no suitable chemical or biological assay yet exists. It measures how much a given solvent pulls out of a given weight, not the active substance, and a batch rich in sugars and salts out-performs a batch rich in the thing you want. As a ratio against a reference batch dried the same way, it is the best purely physical potency proxy below a laboratory.

The two that lie are both hydrometers. ME-01 lists alcohol strength and specific gravity by hydrometer among the district's gains (THRIVING library, ME-01). I agree for spirits and syrups and disagree for tinctures. Dissolved solids raise density and make a hydrometer read a lower alcohol strength than the truth. The United States gauging regulations put numbers on it: above 400 mg of dissolved solids per 100 ml a hydrometer gives only apparent proof, which the regulation says is not a defined measure of alcohol content, and above 600 mg per 100 ml distillation becomes obligatory (27 CFR Part 30, TTB). A tincture is a solution whose whole purpose is dissolved solids, so the pharmacopoeial method is distillation first and density of the clear distillate afterwards (Medicina 2003 abstract).

The rule for the book: **a hydrometer tells the truth about a clear spirit and a sugar syrup, and lies about a tincture unless the tincture is distilled first.** The still a hamlet already owns makes the correction possible. Temperature is the second trap, since instruments are calibrated at a stated temperature and other readings need a table [VERIFY on any rule of thumb]. ME-01 gives the British syrup standard as specific gravity 1.330 at 15.5 degrees for 66.7 per cent sucrose, and a syrup that comes in low will ferment.

pH by vegetable indicator is real but coarse. Red cabbage juice tells you whether something is acid or base, not its pH, and must be calibrated against litmus before any number is read off it [VERIFY]. Enough to tell vinegar from water and to watch a syrup turn sour. Not enough to control a buffer.

Disintegration has the best cost-to-value ratio for salvaged tablets. The official method needs a basket-rack assembly raised and lowered 29 to 32 times a minute at about 37 degrees C (USP <701>). The GPHF Minilab's field version throws away the apparatus and keeps the question, as Protocol 2 gives it. A tablet that will not break up in half an hour in warm water will not break up in a stomach either.

4. Chemical spot tests: what a positive is worth

The answer divides sharply by reagent. **Available with salvage or a forge:** iodine in potassium iodide, which is Wagner's reagent and a cousin of Lugol's solution, an iron salt for phenols and tannins, copper in alkali for reducing sugars. **Not available:** Mayer's reagent is potassium mercuric iodide and Dragendorff's a bismuth iodide complex. Mercury and bismuth salts are not village chemistry. A district may have one salvaged bottle, never a second. I give no preparation quantities for either, and the book should not: the two most-used alkaloid reagents in the world's pharmacognosy teaching are the two a village loses first.

What an alkaloid spot test proves is very little, and the literature is blunt about it. Anderson, Doggett and Ross (1977) reported in *Planta Medica* that the range of compounds producing false positives with Dragendorff's reagent is far greater than generally realised, and that common plant sterols and triterpenes, containing no nitrogen at all, are readily detected by the spray (abstract and secondary summary only, full text blocked). A 1975 study tabulated both directions of error, with lidocaine and methadone giving positive alkaloidal tests and caffeine and morphine giving negative ones [VERIFY]. Surveys show the same: 137 of 417 Sri Lankan species were positive to Dragendorff's and 89 of 214 to iodoplatinate, with no report of confirmation by any other method (*Journal of Natural Products* 1978, search extract) [VERIFY], and in 130 species Mayer's and Wagner's found 40 positives against Dragendorff's 63 [VERIFY]. When reagents aimed at one class disagree by a factor of 1.6 on the same plants, the class membership is not being measured.

The rule the book needs is negative. **A positive alkaloid test says the extract contains something that precipitates with iodine, which may be an alkaloid, a protein, a sterol or a triterpene. It says nothing about which alkaloid and nothing about how much.** The one defensible use is as a negative screen on a batch that should contain alkaloids, run beside an extract of a kept reference batch in the same session. If the reference gives a dense precipitate and the new batch gives none, something is wrong with the new batch.

Iron for phenols and tannins is better, because the reaction is bulk and visible. Hydrolysable tannins give a blue-black precipitate, condensed tannins a brownish-green one. The brief proposes making ferric chloride from rust and vinegar. I disagree with the chemistry and agree with the result. Rust with vinegar gives an iron acetate, not ferric chloride, and the reaction exploited is the iron-tannate reaction that makes iron gall ink: tannin plus any iron salt gives a pale ferrous complex which atmospheric oxygen converts to a near-insoluble black ferric compound (irongallink.org, and the West Dean account). Any iron salt a village can make serves. This is a genuine village test, quantitative by eye against a reference, with a second use: the same reaction on a crushed aspirin tablet reveals free salicylic acid.

The goldbeater's skin test separates true tannins from the pseudotannins that merely precipitate protein, which the gelatin test cannot do. A membrane of ox caecum, behaving like an untanned hide, is soaked in acid, exposed to the test solution, rinsed and transferred to dilute ferrous sulphate, brown or black read as positive [VERIFY on the acid strength]. WHO's tannin assay needs purchased hide powder. The goldbeater's skin is an abattoir by-product.

Keller-Kiliani for cardiac glycosides is the test the brief names and the one I would leave out of the book. It colours the 2-deoxysugar portion, not the steroid nucleus, and manuals disagree on what a positive looks like [VERIFY]. For foxglove, a test needing concentrated sulfuric acid, a prior extraction and an ambiguous endpoint adds no safety to Withering's titration, only the illusion of measurement, which on a drug of therapeutic index about 2 is worse than nothing (THRIVING library, ME-02).

Benedict's and Fehling's for reducing sugars are the most tractable, the endpoint a bulk precipitate grading green to brick red, though sources disagree on where the bands fall. The reagent must be fresh and sucrose does not react [VERIFY].

Chromatography. WHO calls thin-layer chromatography effective, easy and inexpensive, which is right by laboratory standards and wrong by village ones: it needs glass plates, a spreader for a uniform 0.25 mm layer, a saturated

chamber, a fine spray, activation at 110 degrees C and usually ultraviolet at 254 and 365 nm. Paper chromatography needs only paper, a jar, a solvent and a pencil, but the alkaloid visualisation reagents are the bismuth and mercury ones a village lacks. Its best use is comparing a new batch against a kept reference on one sheet, asking "do the spots match" rather than "what is this".

5. The dilution-series assays, and what ME-02 got right and wrong

ME-02 concluded that the bitterness value is "the one pharmacopoeial assay that needs no equipment" and "the only assay here a village of 300 can run" (THRIVING library, ME-02). Having read the method in the WHO manual and in the British Pharmacopoeia's reproduction of Ph. Eur. 2.8.15, I find that half right, and wrong in a way the book must not inherit. Protocol 5 gives the method.

Where ME-02 is right. The measuring instrument is the tongue, which needs no manufacture and no electricity. The method handles inter-person variation honestly, excluding tasters who cannot detect the weakest quinine reference and giving each remaining one a correction factor. And it is a real acceptance criterion: gentian root must reach a bitterness value of 10,000, wormwood 15,000 and centaury herb 2,000 (secondary pharmacopoeial summaries) [VERIFY]. ME-02's warning that it cannot be used on narrow-margin plants is correct.

Where ME-02 is wrong, in three ways. First, **it is not equipment-free, it is reference-standard-dependent.** The number is a ratio to quinine hydrochloride weighed to the milligram, the scale anchored by setting quinine at 200,000. Without pure quinine there is no 200,000 and therefore no bitterness value, only a dilution series. A village out of salvaged standard, the normal case within a generation, can still run the series against a kept reference batch and say "this batch is bitter to half the dilution of the reference", which is not what the pharmacopoeia means. Second, **it needs more glassware than ME-02 implies**: nineteen tubes, dilutions accurate at the 0.2 ml level, a water bath holding boiling for 30 minutes, filtration, and all solutions at 20 to 25 degrees. A hamlet with a still and a scale can do this. A household cannot. Third, **it is not the only one.** WHO's manual holds four assays on the same architecture, and three need no purchased reference chemical when run as a comparison.

Assay	Endpoint	Equipment beyond jars and water	Reference needed
Bitterness value	Taste, after 30 s	19 tubes, water bath, panel of 6	Quinine hydrochloride, weighed
Foaming index	1 cm of foam	10 tubes 16 cm by 16 mm, a clock, a rule	None
Swelling index	Volume in ml	A 25 ml stoppered cylinder, 16 mm bore, 0.2 ml divisions	None
Haemolytic index	Clear red solution, no sediment	Tubes, citrate, buffer, ox blood	Saponin standard, or a kept reference batch

The foaming index is the discovery of this report. A decoction of about 1 g of the drug is set out in ten stoppered tubes in portions from 1 to 10 ml, each topped to 10 ml, shaken in a defined way, stood 15 minutes, and the foam measured. The index is 1000 divided by the millilitres of decoction in the first tube showing 1 cm of foam (WHO 1998, and Protocol 3). Read what that needs. Ten identical tubes, boiling water, a clock and a rule. No reference chemical, no panel, no tasting and therefore no poisoning risk. And it gives a number on a defined scale comparable between batches, years and villages, which is exactly what ME-02 says the village has lost. It applies only to saponin-bearing plants, but that family is large and medicinally important.

The swelling index is simpler: a weighed quantity in a graduated stoppered cylinder with 25 ml of water, shaken on a

schedule, stood 3 hours, and the volume of the swollen material including its mucilage read off, three determinations at once (Protocol 4). For the seed laxatives and the demulcents it measures the property being used.

The haemolytic index is a true bioassay runnable at hamlet level. Blood drawn fresh from a healthy ox into sodium citrate is diluted with phosphate buffer at pH 7.4 to a 2 per cent suspension, usable while the supernatant stays clear. Thirteen tubes of graded extract volume each take 1 ml of suspension and are read at 24 hours. The endpoint is total haemolysis, which WHO defines by eye as a clear red solution without any deposit of erythrocytes. The result is expressed against saponin standard, but the comparison that matters to a village is against its own reference batch. The blood is an abattoir by-product, and only the citrate and buffer are obstacles.

My reconciliation with ME-02. ME-02's three keeps are the comparison, the bitterness value and the logbook. I would rewrite the second. What the village keeps is not the bitterness value but **the dilution-series method itself**, of which bitterness is one instance and foam, swelling and haemolysis are three more. The pharmacopoeias' real gift to a post-collapse dispensary is not a reference chemical it cannot replace. It is the discovery that a biological property can be measured without measuring a chemical, by diluting until the property disappears and reporting the dilution. The architecture survives the loss of a laboratory. The reference standard does not, and the village substitutes its own sealed batch, exactly as ME-02's first keep says.

6. The bioassays of the record, and whether a district should revive any

The record is less flattering than the romance. The frog method for digitalis was official through the tenth United States Pharmacopoeia, using *Rana pipiens* of 20 to 30 grams, the standard fixed so a dose per gram of frog weight caused systolic arrest within an hour [VERIFY]. The cat method of Hatcher and Brody defined a unit as the amount stopping a cat's heart intravenously. Their 1910 paper could not be found, confirming ME-02's failure on the same point.

The numbers are damning. A 1935 study got reasonably accurate measurements only with groups of twenty to thirty frogs, and a 1935 comparison gave frog-based relative potencies of 50.1, 94.3 and 100 for three leaf samples against cat-based figures of 60.5, 116.7 and 100 [VERIFY]. An Indiana Academy of Science paper of 1932 called the method variable and inaccurate. The pigeon emesis assay carries the most quotable figure: with three birds the error may approach 300 per cent, with 25 birds about 30 per cent [VERIFY, through a search extract whose primary source I could not reach].

Set that beside ME-02's therapeutic index of about 2 for digitalis. **An assay with a 30 per cent error, needing 25 pigeons or 30 frogs per batch, cannot safely standardise a drug whose toxic dose is twice its working dose.** A district killing thirty frogs to learn a batch lay between 70 and 130 per cent of reference has learned nothing it can dose on. Keep Withering's patient titration for foxglove, with the stop signs written in advance.

The penicillin cylinder-plate assay is the exception, and IN-06 has written the protocol. Its endpoint is a measured zone diameter rather than a dead animal, and it survives as the modern disc-diffusion test standardised by WHO since 1976 (THRIVING library, IN-06). Heatley's 1944 paper remains unread by this library. I can add a precision figure from the same family: Sabath, Estey and Finland, assaying ampicillin and cloxacillin by cylinder-plate in 1967, reported error of 4 per cent or less run in quadruplicate, rising to 12 per cent when one drug was present at ten or more times the other (Sabath 1967). So the diffusion assay is the one historical bioassay worth reviving, with IN-06's rule of discarding both plates when duplicates differ by more than about 15 per cent. Its binding constraint is the reference preparation, not measurable after about eight days in solution even cold. What an agar bench gains the district is not potency measurement of plant drugs, for which there is no organism to inhibit, but the testing of salvaged antibiotics.

7. Salvaged drugs: expiry, counterfeits and degradation

Most expired drugs are still drugs. The United States Shelf Life Extension Program tested 122 drug products across 3,005 lots. Eighty-eight per cent of lots qualified for extension of at least one year beyond the original date, mean extension 66 months, and 18 per cent failed. Variation was large, ciprofloxacin extending a mean 55 months and amoxicillin 23. The caveats matter: stability can only be assured by periodic testing of each lot, and the results relate only to products carefully stored in their original sealed container closures (Lyon 2006, abstract and secondary summaries) [VERIFY against the full paper].

A very small number of degraded drugs become poisons. The documented case is tetracycline. Heat, moisture and low pH convert it to anhydrotetracycline and its epimers, and ingestion of badly stored capsules has caused a Fanconi-type syndrome of proximal renal tubular damage, reported by Frimpter and colleagues in *JAMA* and by Gross in the *Annals of Internal Medicine* in 1963 (citations located, papers not read). The rule is unambiguous: **discard tetracyclines that are discoloured, sticky or stored damp, whatever the date.**

A tablet that smells of vinegar has already told you. Acetylsalicylic acid hydrolyses to salicylic acid and acetic acid, driven by humidity, and the vinegar smell is the acetic acid leaving. Old aspirin also gives a colour with iron salts, the reaction of section 4 applied to free salicylic acid. A Syrian comparison found expired market samples shiny and sticky, with low aspirin content and high salicylic acid [VERIFY], which is itself gastric-damaging, the reason for acetylating it.

The district model for salvaged stock is the GPHF Minilab, and its performance is documented. The kit combines a visual inspection guide, a weight check, a simplified disintegration test and thin-layer chromatography against reference standards, at a material cost per test not above 2 euros (GPHF fact sheet). Gnegel and colleagues (2022) reported the largest field evaluation: of 1,919 samples across 13 countries, 88 (4.6 per cent) were deficient, 34 probably falsified and 54 probably substandard, with compendial confirmation in 25 cases upholding 19 of the 34. External cost was 25.85 euros per sample against about 618 euros for full compendial analysis.

The authors' own limitation is the sentence a village must memorise: the Minilab is highly sensitive and specific for products lacking the stated active ingredient and **less sensitive for products with insufficient content or dissolution**, so substandard products are missed. Visual inspection alone flagged 22 of the 34 probable falsifications, the most cost-effective finding in the quality-control literature. The TLC criterion is worth copying even where TLC cannot be run, because it states the standard: the sample spot must match the reference in travel distance, colour, size and shape, must not be weaker than an 80 per cent reference spot, and must show no extra spots.

8. Self-experiment: when it is defensible, and how it kills

Hahnemann, 1790. Disbelieving Cullen's explanation that cinchona worked by a tonic effect on the stomach, Hahnemann took by his own account four drams of good china daily for several days, which Edzard Ernst estimates at about 400 to 500 mg of quinine. He reported palpitations, pulsation in the head, redness of the cheeks, prostration, thirst and cold trembling extremities, and read them as the symptoms of intermittent fever. Ernst interprets the same list as an allergic reaction with hypotension, and reports that rigorous replications have failed to produce malarial symptoms in healthy people (Ernst 2015). A therapeutic system was founded on a sample of one, with no control and an investigator who knew what he expected to see (THRIVING library, FO-03).

Sertürner, 1817. Having isolated morphine, Sertürner gave it in alcohol to himself and three boys, none older than seventeen, in three gradual doses of half a grain each. After the third the intoxication became, in his description, nearly fatal: pain in the region of the stomach, exhaustion, severe narcosis close to fainting, and twitching in the extremities keeping time with the pulse. Alarmed and not fully conscious himself, he drank six to eight ounces of strong vinegar and had the boys do the same, inducing extreme vomiting (Wikipedia, summarising his own account). Three half-grains is about 97 mg, against the 5 mg orally four-hourly ME-02 gives as the naive adult dose. He gave adolescent boys roughly twenty times a starting dose, and only emesis saved them.

The modern frame. The Nuremberg Code's Article 5 bars experiments with a prior reason to expect death or disabling injury, then excepts those where the experimenting physicians serve as subjects. George Annas argues the exception has never been persuasive, since the researcher's own participation adds nothing to whether the research is justifiable at all. Altman, writing the only full history of the practice, concluded that researchers should not exempt themselves from risks they ask volunteers to take (book not read, secondary summaries). The record holds few suicides and many miscalculations: Carrión inoculated himself with blood from a fourteen-year-old patient on 27 August 1885 and died on 5 October.

The one modern case that worked is instructive about why. Barry Marshall, lacking an animal model, drank a broth of *Helicobacter pylori* in 1984, having first established by endoscopy that he was uninfected and confirmed the strain was sensitive to metronidazole. The defensible features are all preconditions, not outcomes: a baseline measurement, a known antidote, a short observable time course, and a question no other design could answer.

The tenth-dose rule. I could not find a historical source for taking one tenth of an expected dose first, and the brief presents it as a lead. The modern analogue is real: the Food and Drug Administration's 2005 guidance takes the no-observed-adverse-effect level in the most sensitive animal species, converts it to a human equivalent dose by body-surface scaling, then divides by a default safety factor of 10 [VERIFY on the exact wording]. Note the structure. The tenth is applied to a dose already shown harmless in animals, not to the dose you hope will work. A village applying a tenth to a guessed therapeutic dose is doing something much weaker.

What the book should say. Self-experiment on a new batch of a known plant, by a healthy adult who is not the only trained person in the village, with a written stop rule, a second person present and a plant of wide margin, is a defensible last resort. Self-experiment to find out what an unknown plant does is Hahnemann's error with Carrión's consequences. Self-experiment on a narrow-margin plant is Sertürner's experiment, and the book should print what it cost. ME-02's judgement that the healer should not be the test subject is correct for an operational reason: a village of 300 has one person who knows how to do this. FO-03's N-of-1 design adapts cleanly, running old batch against new in place of remedy against dummy, as Protocol 6 sets out (THRIVING library, FO-03).

Evidence grades

Check	Tradition says	Record shows	Grade
Organoleptic routine against a reference	The senses know the drug	WHO's first step, but adulterants imitate	**B** purity, **C** identity
Microscopy of powdered drugs	Not traditional	Indispensable for powders, beat chemistry on bilberry	**B**
Tasting to identify an unknown plant	Folk practice	Fatal with hemlock and aconite, forbidden by WHO	**X**
Bitterness value, Ph. Eur. 2.8.15	Bitters aid digestion	Monographed, reproducible in a corrected panel, criticised as subjective	**B** comparative, **D** absolute without quinine
Foaming index	Saponins foam	Defined endpoint, no reference chemical, monographed	**B**
Swelling index	Mucilage swells	Monographed, measures the property used	**B**
Haemolytic index against ox blood	Saponins break blood	Monographed, endpoint readable by eye	**B**

Check	Tradition says	Record shows	Grade
Loss on drying, ash, extractable matter	Not traditional	Pharmacopoeial, partly feasible, proxies not assays	**B** purity, **C** potency
Hydrometer on a clear spirit or syrup	The float tells the strength	Correct, with temperature correction	**A**
Hydrometer on an undistilled tincture	As above	Dissolved solids give apparent, not true, strength	**X** as potency
Wagner, Mayer, Dragendorff	Precipitate means alkaloid	Sterols and triterpenes positive, caffeine and morphine negative	**C** comparative, **X** as potency
Iron salt for phenols and tannins	Iron blackens galls	Bulk visible reaction, grades against a reference	**B**
Goldbeater's skin for true tannins	Tanning is the proof	Separates true tannins from pseudotannins	**C**
Keller-Kiliani	Colour means glycoside	Protocols disagree, prior extraction, shortfall	**D** at village level
Benedict's or Fehling's	Not traditional	Graded colour, false positives from several drugs	**B** presence, **C** amount
Disintegration in warm water	Not traditional	Minilab version, 6 units, 100 ml, 37 degrees, 30 min	**B**
Visual inspection of salvaged packaging	Fakes look wrong	Caught 22 of 34 probable falsifications in 1,919 samples	**B**
Thin-layer chromatography	Simple and cheap	Needs plates, chamber, spray and UV	**B** district, **D** below
Cylinder-plate antibiotic assay	Not traditional	4 per cent error in quadruplicate, WHO standard since 1976	**B**
Frog, cat, pigeon assays for digitalis	The animal tells the dose	30 frogs or 25 pigeons a batch, 30 per cent error, methods disagree	**X** village, **D** district
Self-experiment on a narrow-margin drug	The maker tests it first	Sertürner's poisoning of three boys, Carrión's death	**X**
N-of-1, old batch against new	Not traditional	50 of 57 definite answers in Guyatt's series (THRIVING library, FO-03)	**B**

Means ledger

Rung	What it can test	What it cannot	What changes
Hands and knowledge	Look, smell, break, feel. Reject mouldy, insect-damaged, discoloured, sticky or wrongly packaged material, against a kept whole specimen	Anything about strength. Identity beyond what the eye knew	Removes the worst material. The largest single safety gain in the ledger, at no cost
Hearth	Disintegration of a tablet in warm water. Vinegar smell on aspirin. Foam on shaking a decoction against last year's. Red cabbage for sour or alkaline. Boiling-water odour test	Numbers. Dilution series need matched tubes	A household can condemn a batch. It cannot grade one
Garden and still	Loss on drying. Crude ash against a reference. Foaming and swelling indices with ten matched tubes. Extractable matter. Hydrometer on spirits and syrups, and on tinctures after distillation. Iron for tannins. Bitterness series against a kept reference	Bitterness value in pharmacopoeial units without quinine. Reagents needing bismuth or mercury	The first rung with repeatable numbers. A batch can be called half as strong as last year's, which is the whole of potency control here
Village dispensary, 300	All the above, plus microscopy of powders for adulteration and foreign matter, the lycopodium count, a sealed reference sample of every batch, a logbook, condemnation records, N-of-1 of old against new	Chromatography. Antibiotic assay without agar and a culture. Any absolute potency figure	Adulteration becomes detectable and errors traceable. The microscope is the highest-value salvaged instrument
District infirmary, 3,000	Agar plates and the cylinder-plate assay for salvaged antibiotics. Haemolytic index with citrated ox blood. Acid-insoluble ash. Hand-coated plates run as batch against reference. Bitterness value in true units while quinine survives	Compendial assay. Content uniformity. Dissolution. Anything needing ultraviolet or purchased standards once stock is gone	The salvaged pharmacy becomes usable rather than guessed at. The power to destroy stock is worth more than the power to grade it

Rung	What it can test	What it cannot	What changes
Hospital before the Fall	Liquid chromatography, mass spectrometry, dissolution apparatus, validated reference standards, content uniformity, microbial limits	Nothing relevant	Potency known to a few per cent. Everything above approximates this

Harm test

Tasting kills by two routes. Directly, when the material is toxic, with hemlock, aconite and *Colchicum* all plausible objects of a tasting test and all having killed people who took a small amount to find out what they were (THRIVING library, PH-10). And indirectly, when a negative taste is read as a negative identification. The aconite case is sharpest. Traditional Chinese descriptions use the numbing sensation as a marker of processing, properly prepared *Fuzi* giving none, and a 2018 case report records numbness after a reheated *Fuzi* decoction spreading to four limbs within 30 minutes and reaching paraplegia by two hours. Anyone tasting aconite to judge its strength has already taken the dose.

Dose and error. The bitterness panel's safety margin is the dilution: 10 ml at a dilution factor of 100 or more, held 30 seconds and spat. For a plant whose lethal dose is milligrams that is not enough. **No narrow-margin plant goes to a taste panel, ever.** The list is ME-02's: foxglove, opium poppy, cinchona, belladonna, henbane, thorn apple, autumn crocus, monkshood.

Who must never taste. Children. Pregnant women. Anyone dehydrated, because the kidney has no margin. Anyone fasting, because absorption is faster. The village's only trained healer. WHO excludes anyone who cannot detect 0.058 mg of quinine hydrochloride in 10 ml, and a village should add anyone who has just smoked or eaten strongly flavoured food.

Contamination. The haemolytic index uses fresh ox blood, a biological hazard handled by people without gloves: keep it off broken skin, use a vessel kept only for it, and never run it where food is prepared. The spot-test reagents a district may salvage include mercury and bismuth salts, poisons that look like reagents. Everything in the testing corner is stored apart from anything that goes into a patient, and labelled so it cannot be misread in dim light.

Signs it is going wrong. A panel whose thresholds drift apart across a session is fatigued and must stop. A dilution series bitter at the most dilute tube was made wrongly or the glassware was not rinsed. Foam over 1 cm in every tube means dilute and repeat. Tablets that soften but do not break up are equivocal, and the stock is suspect.

Historical harm. Sertürner's three boys. Carrión's death on 5 October 1885. The tetracycline Fanconi cases of 1963, where the harm came from a drug kept rather than made. And the quieter harm with no case reports: batches passed because a spot test went positive, dosed as though a positive meant a potency.

Red flags

Signs that testing is beside the point and the patient needs what the village cannot give. A patient needing a narrow-margin drug at unknown potency, in a dose that cannot be titrated because the illness moves faster than the titration interval: acute heart failure needing digitalis now, severe malaria needing a quinine dose close to the fatal one, sepsis needing an antibiotic of unknown activity. The right answer is not to test harder but to accept that the dose is a gamble and say so to the family.

Signs that a preparation must be destroyed rather than tested. Visible mould, insect damage, or any abnormal odour, discoloration, slime or sign of deterioration, which is WHO's own condemnation list. A sticky or shiny tablet. A tetracycline of the wrong colour. A tincture with a layer, a film or new cloudiness. A syrup with gas. An unlabelled jar, which the library names as the commonest fatal error in the record (THRIVING library, ME-01).

Signs that the test itself has failed. No reference sample survives. The panel cannot taste the quinine threshold. The reference antibiotic preparation has been in solution more than about eight days (THRIVING library, IN-06). Duplicate plates differ by more than about 15 per cent. In each case record the failure and report no result, because a fabricated result from a broken assay will be dosed on.

What to do anyway. Comfort needs no assay. Water, warmth, food, pain relief titrated in the patient, honest words about what is and is not known, and a record so the next case starts better informed. A village that cannot measure its medicines can still measure its outcomes, and over years the register is the assay (THRIVING library, FO-03).

The case against

The strongest case is that none of this measures potency, and pretending otherwise is the danger. Every check here answers identity, purity or spoilage. Those touching strength do so as a ratio against something kept, and the keeping is the weak link: reference samples degrade, with echinacea alkamides falling over 80 per cent in 64 weeks at 24 degrees C (THRIVING library, ME-02). A ratio against a decayed reference flatters a decayed batch. The critic's version: your assays drift together, and you will never know.

The second is the romance of the Victorian analyst, which the brief names and the numbers support. The animal assays of 1900 to 1950 looked like science and performed like augury: thirty frogs or twenty-five pigeons per batch, an error of 30 per cent at best, methods disagreeing by 20 per cent on the same leaf. Reviving them would consume a district's animals and labour to produce numbers too coarse to dose a narrow-margin drug on. Biological standardisation did not solve the digitalis problem. Chemistry did, and chemistry is gone.

The third is the false reassurance of the spot test, the one most likely to kill. A positive Dragendorff on a sterol is a positive. A negative on caffeine or morphine is a negative. Nothing since 1977 has made the reagents specific. A dispensary that tests and passes is more dangerous than one that does not, because it has acquired confidence without information.

The fourth is against the bitterness panel in particular, made by the method's own users. A comparative analysis of European and German pharmacopoeial requirements describes it as resting on subjective assessment and the sensitivity of experts' taste receptors, with poor convergence between assessors, and notes that different pharmacopoeias use different reference standards, so values do not compare across them.

The answer to all four. None of this argues for testing less. It argues for testing within the limit of what the test proves, writing the limit beside the result, and never letting a passed test raise a dose. The checks surviving intact are those with physical endpoints and no chemistry: visual inspection, microscopy, disintegration, foam height, swelling volume, loss on drying. They are also the cheapest.

10-line summary

1. A dispensary can answer identity and purity well and can answer potency only as a ratio against a sample it has kept.
2. No test here is an identification test, and WHO forbids running its taste assay before identity is confirmed.
3. Visual inspection alone caught 22 of 34 probable falsifications among 1,919 samples, the best return on effort in the literature.
4. Microscopy of powders beat chemical analysis on bilberry contaminants and separates skullcap from hepatotoxic germander.

5. WHO's manual holds four dilution-series assays, not one: bitterness, foaming, swelling and haemolytic index, and three need no purchased reference chemical.
6. The foaming index needs ten matched tubes, boiling water, a clock and a rule, and yields a number on a defined scale.
7. A hydrometer reads true in a clear spirit and a sugar syrup and false in a tincture, where dissolved solids give apparent and not true strength.
8. Alkaloid precipitation tests give positives on sterols and triterpenes and negatives on caffeine and morphine, so a positive proves nothing about potency.
9. The cylinder-plate assay is the one historical bioassay worth reviving, good to about 4 per cent in quadruplicate, while the frog, cat and pigeon assays erred by 30 per cent or worse.
10. Sertürner gave three boys under seventeen about 97 mg of morphine in 1817 and saved them with vinegar, which is the whole argument against self-experiment on narrow-margin drugs.

Open questions

- The reproducibility of the bitterness value between panels in different places. The criticisms are qualitative and I found no inter-laboratory study with numbers.
- Whether the lycopodium spore count can be run by a lay counter with acceptable error, and what reference figures must be established locally.
- Whether the goldbeater's skin test can substitute for WHO's hide-powder tannin assay quantitatively, or only qualitatively.
- A kept reference batch's real decay rate for the plants a village depends on most, which sets how often the reference is remade and how a new one is tied to the old.
- Whether a pigment-based pH indicator can be calibrated to half a unit reliably enough to control anything.
- Whether Heatley's 1944 paper holds error figures that would change IN-06's 15 per cent tolerance. It is unread by this library.
- Whether any pre-1950 pharmacopoeia printed a rule of starting at one tenth of an expected dose. I could not find one.

Sources to read in full

1. **WHO, *Quality control methods for medicinal plant materials*, 1998.** The most valuable document for this chapter: the macroscopic routine, the microscopy preparations, loss on drying, ash, extractable matter and the four dilution-series assays with full quantities.
2. **British Pharmacopoeia Appendix reproducing Ph. Eur. 2.8.15.** Read beside WHO's version, which differs in the dilution scheme and the correction factor.
3. **Gnegel and others, 2022, *Scientific Reports*.** The best field data on what simple screening catches and misses, with costs.
4. **GPHF Minilab manual.** The district model, and the source of the simplified disintegration test.
5. **Trease and Evans, *Pharmacognosy*, 16th edition 2009.** The organoleptic and microscopical chapters, unreached, a gap in this report.
6. **Altman, *Who Goes First?*, 1998 reissue.** For the ethics and the case histories. Not read.
7. **Lyon and others, 2006, on the Shelf Life Extension Program.** The only large dataset on what expiry means.
8. **Heatley, 1944, *Biochemical Journal* 38:61-65.** Still unread by this library after two attempts.

At the bedside after the Fall

For a household. Two checks and one habit. The checks are look-and-smell before every use, condemning anything mouldy, discoloured, sticky, sour or unlabelled, and the cup test for any salvaged tablet. The habit is keeping a sealed

sample of every batch that worked, labelled with plant, part, place and month. *Needs:* jars with lids, a way to write a label, water, a fire. *First failure to expect:* the reference samples are used up in a hard winter, and the comparison is lost.

For a village of 300. Add a salvaged microscope, a balance, ten matched tubes, a rule, a clock and a bound logbook. Run loss on drying on every batch of dried herb before it goes into store, crude ash on anything suspected of being bulked out with earth, the foaming index on the saponin plants and the swelling index on the mucilage plants. Keep reference samples sealed, cool and dark in a cupboard nobody dispenses from. Give one named person the testing corner, the same person who prepares the narrow-margin plants under ME-02's one-in-a-village rule, and have that person keep the condemnation book, because the pattern of failures is how the dispensary learns. *Needs:* matched glassware, a balance, a microscope, reference specimens of every plant used, a bound book. *Skills:* weighing, timing, reading a meniscus, counting under a microscope, writing a record the same way every time. *First failure:* the logbook lapses within a month, and with it every comparison.

For a district of 3,000. Add agar, a maintained bacterial culture and the cylinder-plate assay for salvaged antibiotics, the highest-value test at this rung and the only one making salvaged stock dosable rather than guessable (THRIVING library, IN-06). Add the haemolytic index if citrate and buffer can be had, and hand-coated chromatography plates run as batch against reference rather than as identification. Hold the district's remaining quinine hydrochloride as a reference standard and nothing else, so bitterness values in true units survive a generation longer. Most importantly, hold the power to destroy stock and use it, because a district that cannot condemn cannot control. *Needs:* a bench, a flame, agar, cultures, a reference stock kept dry, cold, dark and sealed, and a person whose job this is. *First failure:* the reference preparation degrades silently and the assay goes on producing numbers that mean nothing.

Protocols

Protocol 1. The quality-check sheet, one per batch

- ☐ Batch mark, plant to Latin binomial, part, place gathered, date gathered, who gathered it.
- ☐ Size with a millimetre rule, ten small seeds in a row on paper ruled at 1 mm, divided by ten. Colour under diffuse daylight, untreated, against the reference sample.
- ☐ Surface, texture and fracture by touch and by breaking, with a 6x to 10x lens, the fracture plane recorded as fibrous, smooth, rough or granular.
- ☐ Odour: hold a small portion in the palm and inhale slowly and repeatedly, crushing between the fingers if nothing is perceptible. Record strength as none, weak, distinct or strong, then character as aromatic, fruity, musty, mouldy or rancid. **For anything dangerous, crush mechanically, pour boiling water over it and smell the steam.**
- ☐ Foreign matter: spread thin, sort by eye with the lens, weigh each group to 0.05 g, record as g per 100 g.
- ☐ Loss on drying if a balance exists: 2 to 5 g in pieces under 3 mm thick, dried hot but below scorching until two weighings agree within 5 mg.
- ☐ One class test from Protocol 3 or 4, beside the reference batch in the same session.
- ☐ Verdict: pass, pass with a note, or condemn. Condemnations get a reason.
- ☐ Seal and store a reference sample of about 10 g from every batch that passes.

Protocol 2. The cup test for a salvaged tablet (household, 35 minutes)

- ☐ Read the packet first. Wrong spelling, blurred or doubled print, a font unlike the rest of the strip, a missing or wrong batch mark, or foil opened and reclosed means discard without testing.
- ☐ Look at the tablets and smell them. Discoloured, mottled, sticky, shiny, crumbling or powdery means discard, and so does vinegar on an aspirin.
- ☐ Put six tablets in 100 ml of water at about 37 degrees, as near blood heat as a hand can judge.

- ☐ After 30 minutes every immediate-release tablet should have broken up completely. One only softened is suspect. One still whole is discarded.
- ☐ A coated or slow-release tablet should **not** have broken up before 30 minutes. One that falls apart in five is not what its coat says it is.
- ☐ Do not run this on injectables, dry syrups or chewable tablets, and record the result against the batch mark.

Protocol 3. Foaming index (hamlet or village, about 1 hour 15)

- ☐ Reduce about 1 g to a coarse powder and weigh it as accurately as the balance allows. Add to 100 ml of boiling water in a flask of about 500 ml and hold at a moderate boil 30 minutes.
- ☐ Cool, filter, and make the filtrate back to 100 ml through the filter.
- ☐ Set out ten identical stoppered tubes, about 16 cm tall and 16 mm across. Put 1 ml in the first, 2 ml in the second and so on to 10 ml. Top each to 10 ml with water.
- ☐ Stopper and shake lengthwise 15 seconds at two shakes a second. Shake all ten the same way.
- ☐ Stand 15 minutes exactly. Measure foam height in each tube with a rule.
- ☐ If no tube reaches 1 cm, the index is under 100. If every tube exceeds 1 cm, dilute the decoction and start again.
- ☐ Otherwise find the first tube with 1 cm of foam and divide 1000 by its millilitres of decoction.
- ☐ Run the reference batch the same day the same way, and record both. **The comparison is the result.**

Protocol 4. Swelling index (hamlet or village, 4 hours mostly waiting)

- ☐ Use a 25 ml stoppered measuring cylinder of about 16 mm internal bore, graduated in 0.2 ml.
- ☐ Weigh the quantity your written method states, at the stated fineness. Add 25 ml of water.
- ☐ Shake thoroughly every 10 minutes for one hour, then stand 3 hours at room temperature.
- ☐ Read the volume in ml occupied by the material including its mucilage.
- ☐ Run **three** determinations at once and take the mean, related to 1 g. If they disagree by more than about a fifth, the material was not evenly wetted, so repeat.

Protocol 5. The bitterness comparison (village, half a day, never for narrow-margin plants)

- ☐ **Stop here if the plant is on the narrow-margin list, or if its identity is not certain. Both are absolute.**
- ☐ Panel of at least six adults. Nobody who is the only trained healer. No pregnant woman, no child, nobody fasting, unwell, or lately smoking or eating strongly flavoured food.
- ☐ If quinine hydrochloride survives in salvaged stock, screen the panel: dissolve 0.100 g in water to 100 ml, dilute 5 ml of that to 500 ml, and make nine tubes from 4.2 to 5.8 ml of the diluted stock, each topped to 10 ml. Anyone who cannot taste bitterness at the strongest tube, holding 0.058 mg in 10 ml, is excluded.
- ☐ Test extract: 1.0 g in 100 ml of boiling water, held on a water bath 30 minutes with continuous stirring, cooled, made to 100 ml, shaken, filtered, first 2 ml discarded. Prepare the **reference batch** extract the same way, the same day.
- ☐ Make ten tubes from each, 1.00 ml to 10.0 ml, topped to 10 ml. All solutions and the rinse water at 20 to 25 degrees.
- ☐ Rinse the mouth. Take 10 ml of the most dilute tube, swirl near the base of the tongue 30 seconds, **spit it out**, wait 1 minute for a delayed sensation, rinse. Wait at least 10 minutes before the next tube, and always start at the most dilute end.
- ☐ The threshold is the lowest concentration still provoking bitterness after 30 seconds. Record each taster's threshold tube for test and for reference. The ratio is the result, and it goes on the batch label.
- ☐ Wait at least 10 minutes between the two series. A fatigued panel gives a flattering answer.

Protocol 6. Testing a new batch against the old in a patient (village)

- ☐ Only for a chronic, stable condition where the symptom returns when treatment stops, and only for a plant graded C or better and safe at the dose (THRIVING library, FO-03).
- ☐ Agree one daily measure, for example pain 0 to 10 each evening.
- ☐ Put old batch and new batch in identical jars labelled 1 and 2. A third person holds the key.
- ☐ At least three pairs of periods, order within each pair decided by a coin, with a gap between periods. The patient records daily without knowing which is which.
- ☐ Open the key at the end. The new batch is accepted if it matches or beats the old in all three pairs.
- ☐ Stop at once for serious worsening or any listed side effect, and enter the result in the register and on the label.

Protocol 7. Condemning stock

- ☐ Condemn: any mould, insect damage, slime, abnormal odour, discoloration or sign of deterioration. Any unlabelled or part-labelled container, whatever it looks like. Any tetracycline discoloured, sticky or stored damp, whatever the date. Any aspirin smelling of vinegar. Any tablet failing the cup test.
- ☐ Any batch whose reference sample is gone and whose preparation cannot be traced: downgrade to lowest confidence, never for a narrow-margin indication.
- ☐ Write the batch mark, date, reason and quantity destroyed in the book. Destroy it where it cannot be retrieved.

Numbers to carry

1. **0.058 mg in 10 ml.** The quinine hydrochloride threshold. A taster who cannot detect it may not taste for the dispensary (WHO 1998).
2. **200,000.** The bitterness value of quinine hydrochloride, the anchor of the scale. Gentian root must reach 10,000, wormwood 15,000, centaury herb 2,000.
3. **1 cm, 15 seconds, 15 minutes.** The foaming index: shake 15 seconds, stand 15 minutes, find the first tube with 1 cm of foam, divide 1000 by its millilitres.
4. **Six units, 100 ml, 37 degrees, 30 minutes.** The field disintegration test. An immediate-release tablet that has not broken up is condemned.
5. **22 of 34.** Probable falsifications caught by looking at the packaging alone. Looking is the cheapest test there is.
6. **88 per cent and 66 months.** The proportion of 3,005 drug lots qualifying for extension past expiry, and the mean extension, in sealed original containers only.
7. **A factor of 1.6.** The disagreement between Mayer's and Dragendorff's reagents on the same 130 plants.
8. **30 per cent with 25 pigeons, 300 per cent with three.** The error of the pigeon emesis assay, against a drug of therapeutic index about 2. This is why the animal assays are not revived.
9. **4 per cent in quadruplicate, 15 per cent between duplicate plates.** The precision of the cylinder-plate assay, and the tolerance beyond which both plates go (THRIVING library, IN-06).
10. **Three half-grains, about 97 mg.** What Sertürner gave each boy in 1817, roughly twenty times a naive adult starting dose of morphine. Say it aloud before anyone proposes a self-experiment.

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