Performance of Confirmatory Tests for Diagnosing Primary Aldosteronism: a Systematic Review and Meta-analysis

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Original studies evaluating any guideline-recommended confirmatory test for PA were eligible if they included comparison to a reference standard. Studies that required multiple sequential tests to establish a diagnosis were not included if the performance of any single test could not be determined. Conference abstracts, reviews, editorials, and protocols were excluded. When the same group of patients was likely reported across several publications for the same test, only the most complete publication was included to avoid double counting.

For each study included, the number of true positive, false positive, false negative, and true negative cases were extracted (or manually calculated from available data). When the necessary data were not reported in the text or tables, they were derived from published figures using WebPlotDigitizer version 4.4 (Ankit Rohatgi, Pacifica, CA, USA). When multiple sensitivity and specificity pairs (at different thresholds) were reported for the same individuals in a single study, we only considered the threshold associated with the highest specificity (aligning with the primary purpose of the test to rule-in disease) or the one designated as “optimal” by the original investigators to avoid double counting. If variations of the same confirmatory test were performed multiple times in the same patients, the set most closely aligning to the testing protocol described by guidelines was used.¹

Meta-analyses were conducted using hierarchical summary ROC (HSROC) models that included random-effects terms for variations in accuracy and thresholds between studies, and allowed for non-symmetrical ROC curves to be fitted.² The diagnostic accuracies of the different tests were compared between all studies (indirect comparisons) and, where possible, head-to-head from studies that evaluated more than one test against a common reference standard (direct comparisons).

We relied on visual inspection of the coupled forest plots and summary ROC plots to describe heterogeneity, rather than using the I² statistic, as the latter is univariate and does not account for threshold effects.³ We explored for potential sources of heterogeneity using meta-regression, considering differences in methodological quality and clinical characteristics between studies, and incorporated these separately as covariates in the HSROC model.³ The likelihood ratio (LR) test was used to compare models with and without the covariate terms to formally test for differences. To quantify differences, we calculated the relative diagnostic odds ratio (DOR), which is a summary measure of the relative accuracy between two tests, assuming the summary ROC curves were parallel.⁴ We assessed for publication bias using Deeks’ funnel plot, noting that the statistical test has low power to detect asymmetry when heterogeneity is large.³

Because summary statistics are only interpretable when studies share a similar threshold (but thresholds varied considerably in our current review), we estimated the sensitivities at discrete points on the summary ROC curve corresponding to the lower quartile, median, and upper quartile of the reported specificities to facilitate
comparisons. We calculated the number of missed cases and over-diagnosed cases per 1000 patients and presented these in a “summary of findings” table with evidence profiles adapted from the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework. Analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), Stata version 17.0 (StataCorp, College Station, TX, USA), and RevMan version 5.4.1 (The Cochrane Collaboration, Copenhagen).
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Table S1. Electronic search strategies.

A search strategy was developed with a health science librarian (DLL). Medical subject headings and author supplied keywords were combined using the Boolean operator “OR” and grouped into two themes: primary aldosteronism and confirmatory test. Both components were combined using the Boolean operator “AND.” References of included articles were also searched to identify other relevant studies.

| Database (Dates): Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily (1946 to June 01, 2021) |
|---|---|---|
| Line no. | Search | Results |
| 1 | exp hyperaldosteronism/ | 9000 |
| 2 | exp aldosterone/ | 24431 |
| 3 | (hyperaldosteron* or aldosteron*).tw,kf. | 40763 |
| 4 | 1 or 2 or 3 | 48494 |
| 5 | (saline or salt or captopril or fludrocortisone or confirm*).tw,kf. | 1647205 |
| 6 | 4 and 5 | 7692 |
| 7 | limit 6 to animals | 2737 |
| 8 | limit 6 to (animals and humans) | 738 |
| 9 | 7 not 8 | 1999 |
| 10 | 6 not 9 | 5693 |
| 11 | limit 10 to English language | 5015 |

| Database (Dates): Embase (1974 to 2021 June 01) |
|---|---|---|
| Line no. | Search | Results |
| 1 | exp primary hyperaldosteronism/ | 6582 |
| 2 | hyperaldosteronism.tw,kw. | 4367 |
| 3 | aldosteron*.tw,kw. | 48445 |
| 4 | 1 or 2 or 3 | 50999 |
| 5 | (saline or salt or captopril or fludrocortisone or confirm*).tw,kw. | 2237182 |
| 6 | 4 and 5 | 10075 |
| 7 | limit 6 to animals | 2595 |
| 8 | limit 6 to (animals and humans) | 0 |
| 9 | 7 not 8 | 2595 |
| 10 | 6 not 9 | 7480 |
| 11 | limit 10 to English language | 6701 |

| Database (Dates): EBM Reviews - Cochrane Central Register of Controlled Trials (April 2021) |
|---|---|---|
| Line no. | Search | Results |
| 1 | exp hyperaldosteronism/ | 74 |
| 2 | exp aldosterone/ | 1121 |
| 3 | (hyperaldosteron* or aldosteron*).tw,kw. | 4997 |
| 4 | 1 or 2 or 3 | 5213 |
| 5 | (saline or salt or captopril or fludrocortisone or confirm*).tw,kw. | 139256 |
| 6 | 4 and 5 | 882 |
| 7 | limit 6 to English language | 697 |
## Table S2. Summary of data extraction sheet.

| Variable             | Description                                                                                                                                                                                                 |
|----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Author               | Last name of the first author.                                                                                                                                                                              |
| Year                 | Year of publication. If the first author has published more than one article within the same year, enter the year using sequential letters (e.g., 2009a, 2009b, 2009c, etc.). |
| Country              | Country in which the study was conducted. For multi-site trials, list all countries separated by a comma (e.g., USA, Canada, UK, and Australia). If this is not reported, use the country of origin of the first author. |
| Design               | Select from the following options:                                                                                                                                                                          |
|                      | • “Single-gate design” (single set of criteria for inclusion; entire study sample drawn from clinical population suspected to have primary aldosteronism [PA]) |
|                      | • “Two-gate design with healthy controls” (cases and controls are sampled from 2 distinct source populations; cases are known or highly likely to have PA, and controls are healthy participants) |
|                      | • “Two-gate design with alternative diagnosis controls” (cases and controls are sampled from 2 distinct source populations; cases are known or highly likely to have PA, and controls have a specific alternative condition similar to PA [e.g., essential hypertension]) |
|                      | • “Multi-gate design with healthy controls and alternative diagnosis controls” (cases and controls sampled from multiple populations; cases are known or highly likely to have PA, and compared with multiple controls, including healthy people and those with essential hypertension). |
| Sampling             | Select from the following options:                                                                                                                                                                          |
|                      | • Consecutive patients                                                                                                                                                                                      |
|                      | • Random sample                                                                                                                                                                                            |
|                      | • Case-control (non-consecutive, non-random)                                                                                                                                                               |
|                      | • Unclear                                                                                                                                                                                                |
| Data collection      | Select from the following options:                                                                                                                                                                          |
|                      | • Prospective (e.g., consent was obtained prior to testing)                                                                                                                                                 |
|                      | • Retrospective (e.g., chart review)                                                                                                                                                                       |
|                      | • Unclear                                                                                                                                                                                                |
| N total              | Total number of participants in all groups.                                                                                                                                                                |
| N disease            | Total number of people with PA.                                                                                                                                                                             |
| N unilateral         | Total number of people with PA that were reported to have unilateral disease (either by presence of adrenal mass, lateralization, or surgery—as defined by study).                                           |
| TP                   | Number of true positive cases.                                                                                                                                                                             |
| FP                   | Number of false positive cases.                                                                                                                                                                            |
| FN                   | Number of false negative cases.                                                                                                                                                                            |
| TN                   | Number of true negative cases.                                                                                                                                                                            |
| Mean age             | Mean age of all participants                                                                                                                                                                              |
| Range age            | If mean age not reported (or cannot be estimated), report age range when available.                                                                                                                                 |
| Number male          | Number of males of all participants.                                                                                                                                                                       |
| Number hypokalemia   | Number of participants with hypokalemia.                                                                                                                                                                   |
| ARR threshold        | Minimum ARR required for inclusion in study.                                                                                                                                                               |
| Confirmatory test    | Select from the following options:                                                                                                                                                                          |
|                      | • SIT = intravenous saline infusion test                                                                                                                                                                   |
|                      | • SLT = oral salt loading test                                                                                                                                                                            |
| Confirmatory test protocol | Describe how confirmatory test was performed (including preparation, posture, time of day). |
|----------------------------|---------------------------------------------------------------------------------------------------|
| Confirmatory test interpretation | Describe how confirmatory test was interpreted. |
| Aldosterone units | Units for aldosterone (e.g., pmol/L) |
| Aldosterone assay | Type of laboratory assay for aldosterone |
| Renin units | Units for renin (e.g., mIU/L) |
| Renin assay | Type of laboratory assay for renin |
| Renin type | Plasma renin activity (PRA) vs. direct renin concentration (DRC) |
| Reference | Reference standard (“gold standard”) used for disease verification: |
| | • Clinical outcomes to targeted treatment |
| | • Adrenal vein sampling (AVS) |
| | • Histopathology |
| | • Another confirmatory test: FST |
| | • Another confirmatory test: SIT recumbent |
| | • Another confirmatory test: SIT seated |
| | • Another confirmatory test: SLT |
| | • Another confirmatory test: CCT |
| | • Different reference used (e.g., patients who had a positive confirmatory test result received targeted treatment, but those with a negative confirmatory test result received another confirmatory test) |
| Reference details | Details of reference standard. |
| Verification | How many people received the reference test: |
| | • Complete (everyone received the same reference test) |
| | • Partial (not everyone was subjected to the reference test) |
| | • Different reference tests |
| | For partial verification, it captures the situation where a reference test is not applied to all (e.g., abnormal confirmatory testing gets additional work-up or treatment and those with normal confirmatory test results get nothing at all). |
| | For different reference tests, it captures the situation where a different definition of PA is applied depending on the results of the confirmatory test (e.g., abnormal confirmatory testing gets AVS, but normal confirmatory results receive another confirmatory test). |
| Patient selection risk of bias | Risk of bias assessment for patient selection. |
| | • Low = “single-gate design,” enrolling patients suspected (but not proven) to have PA. |
| | • High = “two-gate design” or case-control studies at risk of spectrum bias (e.g., patients with florid disease were compared with those who were entirely normal). |
| | • Unclear = not enough data to make judgment. |
| Patient selection applicability | Concerns about applicability for patient selection. |
| | • Low = patients represent those that would likely receive a confirmatory test in clinical practice. |
| | • High = patients are highly selected and unlikely to reflect those who would receive a confirmatory test in clinical practice. |
| | • Unclear = not enough data to make judgment. |
| **Index test risk of bias** | Risk of bias assessment for index test. |
|-----------------------------|----------------------------------------|
| Low                         | confirmatory test was interpreted without knowledge of reference standard and/or the interpretation threshold was pre-specified. |
| High                        | there was potential of subjective interpretation of the confirmatory test (e.g., some patients were already deemed to have diagnosis of PA, then threshold for positive/negative test was determined afterwards). |
| Unclear                     | not enough data to make judgment. |

| **Index test applicability** | Concerns about applicability of index test. |
|-----------------------------|----------------------------------------|
| Low                         | confirmatory test similar to what is expected to be used in clinical practice (as per guidelines), or derived from objective standard. |
| High                        | confirmatory test significantly different than what is done in clinical practice. |
| Unclear                     | not enough data to make judgment. |

Note, confirmatory tests are commonly conducted and interpreted as follows, adapted from the Endocrine Society 2016 guidelines\(^1\):

- **SLT**: 3-7 d of salt loading (verified with urine sodium >200 mmol/d). Urine aldosterone >10-12 mcg/d (28-33 nmol/d) suggests PA.
- **SIT**: fast overnight, then give 2 L NS over 4 hours while recumbent. Plasma aldosterone >280 pmol/L (10 ng/dL) suggests PA and <140 pmol/L (5 ng/dL) is considered normal.
- **FST**: fludrocortisone 0.1 mg q6h (or 0.25 mg daily) for 4 days with NaCl supplementation. Plasma aldosterone ≥140-170 pmol/L (5-6 ng/dL) suggests PA.
- **CCT**: captopril 25-50 mg x1 after seated or standing for 1 hour. Plasma aldosterone reduction by <30% and/or ≥240 pmol/L (8.7 ng/dL) after 2 hours suggests PA.

| **Reference standard risk of bias** | Risk of bias assessment for reference standard. |
|-------------------------------------|----------------------------------------|
| Low                                 | classification of disease was most likely correct and interpreted independently of index test (e.g., clinical response to targeted treatment). It is reasonable to assume that any disagreements between the reference standard and index test is because of misclassification from the index test. |
| High                                | significant potential of misclassification of disease and/or inconsistent reference standard (e.g., AVS lateralization may miss bilateral forms of PA; histopathology may miss cases that did not undergo surgery and bilateral forms of PA that underwent surgery; another confirmatory test may be subject to false positive/negative results). |
| Unclear                             | not enough data to make judgment. |

| **Reference standard applicability** | Concerns about applicability of reference standard. |
|--------------------------------------|----------------------------------------|
| Low                                  | interpretation of the reference standard is similar to what is expected in clinical practice. |
| High                                 | interpretation of the reference standard is significantly different than usual clinical practice. |
| Unclear                              | not enough data to make judgment. |

| **Flow and timing risk of bias** | Risk of bias assessment for study flow and timing. |
|---------------------------------|----------------------------------------|
| Low                             | adequate time was provided for verification of disease status (e.g., clinical outcome following treatment); all patients received the same reference standard; all patients were accounted for in the analysis. |
| Other comments | Additional notes. |
|----------------|-------------------|
|                |                   |

- **High** = inadequate time was provided for verification of disease status; only some patients received a reference standard and/or inconsistent reference standards were used; some patients were unaccounted for in the analysis.
- **Unclear** = not enough data to make judgment.
Table S3. Summary of included studies.

| Study author, year | Country | Population tested: mean age (or range if mean not reported), number male, number with hypokalemia, ARR cut-off for inclusion | Study design | Sampling method | Data collection | No. with PA / total sample | Confirmatory test: abbreviated protocol; interpretation | Aldosterone assay | Verification reference standard: description | Comments |
|--------------------|---------|----------------------------------------------------------------------------------------------------------------------------------|-------------|-----------------|-----------------|-----------------------------|--------------------------------------------------------|-----------------|--------------------------------|----------|
| Horton, 1969 🇺🇸   | USA     | NR age, NR sex, 6 hypokalemia, NR ARR                                                                                           | Two-gate with healthy controls | Case-control | Unclear         | 6/12                        | FST: fludrocortisone 0.3 mg PO q6h x 3 days with blood test afterwards; PAC >12.6 ng/dL for diagnosis of PA | Double-isolate derivative assay | Different standards used: PA based on hypertension, retinopathy, hypokalemia, alkalosis, and improvement with spironolactone; criteria for healthy subjects not given | Only 6 of the 30 healthy volunteers (table 1) and 5 patients with PA (table 2) received the verification standard for a final study number of 11 people |
| Biglieri, 1970 🇺🇸 | USA     | NR age, NR sex, NR hypokalemia, NR ARR                                                                                           | Multi-gate with healthy and alternative diagnosis controls | Case-control | Prospective     | 13/26                       | FST: fludrocortisone 0.4 mg PO qd x 3 days; 24 h urinary aldosterone collected on 3rd day ≥18.9 mcg/d for diagnosis of PA | Paper chromatography and liquid scintillation spectrometry | Different standards used: PA based on hypertension, hypokalemia, reduced PRA, high PAC, absence of renovascular disease +/- surgical pathology; EH based on hypertension and occasional hypokalemia; normal control subjects had no history of cardiovascular or renal disease | 2×2 table reconstructed using figures 1-5; upper limit of normal for 24 h urinary aldosterone estimated using digitized version of figure 1 |
| Collins, 1970 🇺🇸  | USA     | NR age, 17 M, NR hypokalemia,                                                                                                     | Two-gate design with | Case-control | Unclear         | 5/50                        | SLT: discontinuation of all medications                  | Isotope dilution | Different standards used: PA based on hypertension, hypokalemia, reduced PRA, high PAC, absence of renovascular disease +/- surgical pathology; EH based on hypertension and occasional hypokalemia; normal control subjects had no history of cardiovascular or renal disease | Unclear if participants with |
| Study | Country | Age, Sex | Study Design | Recruitment Method | Screening Criteria | Diagnosis | Reporting Criteria |
|-------|---------|----------|--------------|--------------------|-------------------|-----------|-------------------|
| Kem, 1971a | USA | NR age, NR sex, NR hypokalemia, NR ARR | Multi-gate with healthy and alternative diagnosis controls | Case-control | Prospective | 7/38 | SIT (recumbent): discontinuation of all estrogen-containing drugs × 1 month and antihypertensives × 1 week; recumbent for 2 L of 0.9% NaCl IV beginning at 6 AM over 4 h; PAC >5 ng/dL after infusion for diagnosis of PA | Immuno-assay | Different standards used: PA based on hypertension, hypokalemia, elevated urinary aldosterone, and suppressed PRA; renovascular hypertension based on abnormalities with pyelography and renal arteriography; EH based on normal screening tests (unspecified); normal control subjects had no history of hypertension or renal disease |
| Kem, 1971b | USA | NR age, NR | Multi-gate | Case-control | Prospective | 5/32 | SIT (recumbent): | Immuno- | Different | Participants |
| Study          | Country | Age, Sex, Hypokalemia | Study Design | Posture | Protocol | Criteria | Standards Used | Comment |
|---------------|---------|-----------------------|--------------|---------|----------|----------|----------------|---------|
| Espiner, 1971 | USA     | 44.1 y, 50 M, NR     | Multi-gate  | SIT (posture not specified) | Discontinuation of antihypertensives x 2 weeks; 2 L of 0.9% NaCl IV beginning at 10 AM over 4 h repeated over 2 days; 24 h urinary aldosterone starting at 7 AM on final day >300 mcg/d for diagnosis of PA | Different standards used: criteria for PA not given; EH based on normal renal function, urinary steroids, vanillylmandelic acid, and pyelogram; renal hypertension diagnosed clinically; normal control subjects had no history of cardiovascular or endocrine disease | There were 2 people in the normal control group, 1 person in the renal hypertension group, and 1 person in the EH group that were missing outcomes |
| Dunn, 1976    | New Zealand | NR age, NR sex, 5 hypokalemia | Two-gate design with alternative | FST: discontinuation of antihypertensives x 2 weeks; | Immunoassay | Different standards used: PA based on spontaneous | — |
| Diagnosis | Controls |
|-----------|----------|
| fludrocortisone 0.4 mg PO qd × 3 days with blood test afterwards; PAC >7.5 ng/dL for diagnosis of PA | hypokalemia, low PRA on low-salt diet, and failure to suppress plasma and urine aldosterone with IV NaCl challenge, and normalization of biochemistry after surgical removal of adrenal adenoma; other forms of hypertension had normal electrolytes, but did not receive further biochemical testing or targeted treatment |

Lund, 1980 **

| Location | Study Design | Study Details | Immuno-assay |
|----------|--------------|---------------|--------------|
| Denmark | Case-control | Multi-gate with healthy and alternative diagnosis controls | Different standards used: PA based on hypertension, DRC <15 mIU/L, high aldosterone, and hypokalemia +/- surgical pathology +/- postoperative outcomes; EH based on normal serum potassium, normal 24 h urinary tetrahydro-aldosterone; hyperreninemic hyperaldosteronism based on DRC >15 mIU/L, high 24 h urinary tetrahydro- |
| Study Reference | Country | Age, Sex, Hypokalemia, ARR | Study Design | Study Population | Diagnostics | Follow-up Verification | Comments |
|-----------------|---------|-----------------------------|--------------|------------------|-------------|-----------------------|----------|
| Streeten, 1982  | USA     | NR age, NR sex, NR hypokalemia, NR ARR | Two-gate design with alternative diagnosis controls | Case-control | Unclear | 22/162 | SIT (recumbent): discontinuation of all antihypertensives × 3 days minimum; furosemide 40 mg IV × 1 dose, then supine × 1 h, then ambulation × 2 h, then saralasin, then 2 L of 0.9% NaCl IV beginning around 12:30 PM over 3.5 h; PAC >236 pmol/L after infusion for diagnosis of PA. | Immunoassay: Partial verification: only those with hypokalemia <3.5 mmol/L and (either PRA <1.7 ng/mL/h or PAC >236 pmol/L after saline infusion test) received follow-up verification with either (1) deoxycorticosterone one acetate 10 mg IM q12h ×3 days with failure to suppress PAC <236 pmol/L, or (2) presence of adrenal tumor on CT for diagnosis of PA; EH criteria not given. |
| Thibonnier, 1982 | Unclear | 43.9 y, NR sex, NR hypokalemia, NR ARR | Single-gate Consecutive patients | Prospective | 18/93 | CCT: discontinuation of all medications × 1 week; NaCl 6 g PO qd × 3-5 days, then captopril 1 mg/kg PO × 1 at 9 AM; PAC collected 3 h after captopril >676 pmol/L for diagnosis of PA. | Immunoassay: Different standards used: PA based on hypokalemia, low PRA, high basal aldosterone +/- surgery; renovascular and renal hypertension based on history, pyelography, and renal arteriography; EH based on non-suppressed. Unclear if study was conducted in France or USA; 2×2 table was reconstructed from figure 3. |
| Study   | Country | Age/Sex | Design | Study Type    | Outcome Measure                                      | Verification Standard          | Unclear:                                                                                                                  |
|---------|---------|---------|--------|---------------|------------------------------------------------------|--------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| Bravo, 1983 | USA     | NR age, NR sex, NR hypokalemia, NR ARR | Two-gate design with alternative diagnosis controls | Retrospective | SIT (recumbent): discontinuation of all medications × 2 weeks; recumbent × 30-45 min, then 25 mL/kg (e.g., 1.5 L for 60 kg person) of 0.9% NaCl IV beginning at 10 AM over 4 h repeated over 3 days; 24 h urinary aldosterone on final day >14 mcg/d for diagnosis of PA | Immuno-assay | Unclear: verification standard for differentiating PA from primary hypertension not stated; diagnostic criteria not given |
| Lyons, 1983 | USA     | 43.5 y, 18 M, 12 hypokalemia, NR ARR   | Multi-gate with healthy and alternative diagnosis controls | Prospective | CCT: discontinuation of spironolactone × 3 weeks and all other medications × 2 weeks; captopril 25 mg PO × 1 at 8 AM while seated; PAC collected 2 h after captopril >15 ng/dL for diagnosis of PA | Immuno-assay | Partial verification: SIT (recumbent) as verification standard for PA vs. EH, but diagnostic cut-offs not stated; normal control subjects did not have any tests |
| Holland, 1984 | USA     | 47.2 y, NR sex, NR hypokalemia, NR ARR | Two-gate design with alternative diagnosis controls | Prospective | SIT (recumbent): discontinuation of antihypertensives × 3 weeks; ambulatory × 2 h then recumbent to receive 2 L of 0.9% NaCl IV over 4 h; PAC ≥ 10 ng/dL after infusion for diagnosis of PA | Immuno-assay | Partial verification: participants selectively received FST with high salt diet and fludrocortisone 0.5 mg PO bid × 3 d with normal response considered as PAC < 6 ng/dL and/or 24 h urinary aldosterone < 6 mcg/d and/or 24 h urinary tetrahydro- |
| Study | Country | Age | Sex | Hypokalemia | Design | Control | Prospective | Protocol | Verification Standard | Hypertension Type |
|-------|---------|-----|-----|-------------|--------|---------|-------------|----------|---------------------|------------------|
| Naomi, 1985 | Japan | NR | NR | NR | Multi-gate with healthy and alternative diagnosis controls | Case-control | Prospective | 7/39 | CCT: captopril 50 mg PO × 1 in AM; PAC collected 90 min after captopril >15 ng/dL for diagnosis of PA | Immunoassay | Different standards used: PA based on elevated aldosterone and low PRA after furosemide injection with AVS lateralization; renovascular hypertension based on arteriography; renal parenchymal disease based on biopsy; EH based on normal response to SLT (but criteria not given); normal control subjects had no hypertension | No cases of bilateral PA included; it was assumed that subjects were unique from those reported in Naomi 1987, but it was not possible to confirm, though the reference standards were different and the subtypes of hypertension were also different between studies |
| Muratani, 1986 | Japan | 41.4 y | NR | NR | Two-gate design with alternative diagnosis controls | Case-control | Prospective | 19/91 | CCT: discontinuation of antihypertensives × 2 weeks; high-salt diet for 7-10 days, then | Immunoassay | Complete verification: SLT as verification standard for PA vs. EH, but protocol and |

Aldosterone <32 mcg/d. However, verification with FST was only performed in 26 of the 120 participants; those with positive SIT results were all assumed to have PA; otherwise, it was assumed that anyone who had a negative SIT as well as those who did not get FST did not have PA.
| Wu, 1986 24 | Taiwan | 38.2 y, 19 M, NR hypokalemia, NR ARR | Two-gate design with alternative diagnosis controls | Case-control | Unclear | 13/34 | CCT: discontinuation of all medications $\times$ 1 week; captopril 100 mg PO $\times$ 1 at 9 AM; PAC collected 2 h after captopril $\geq$6 ng/dL for diagnosis of PA | Immuno-assay | Different standards used: APA based on pathological examination; bilateral PA based on hypokalemia, low PRA, abnormal response to SIT (cut-off not stated), and abnormal CT of the adrenals; EH based on exclusion of secondary causes of hypertension, but process not stated |
| Hamlet, 1987 25 | Australia | NR age, NR sex, NR hypokalemia, NR ARR | Multi-gate design with healthy and alternative diagnosis controls | Case-control | Retrospective | 8/26 | SIT (recumbent): continuation of usual antihypertensive drugs; recumbent $\times$ 30 min, then 1.5 L of 0.9% NaCl IV beginning at 9 AM over 2.5 h; PAC $\geq$9.0 ng/dL after infusion for diagnosis of PA | Immuno-assay | Different standards used: APA based on surgically-proven adenoma; diagnostic criteria not given for EH and normal subjects |
| Naomi, 1987 26 | Japan | 45.8, 15 M, 12 hypokalemia, NR ARR | Two-gate design with alternative diagnosis controls | Case-control | Prospective | 12/32 | CCT: discontinuation of antihypertensives $\times$ 2 weeks; unrestricted salt diet for 1 week, then recumbent for captopril 50 mg PO $\times$ 1 at 9 AM; PAC | Immuno-assay | Different standards used: APA based on hypertension, hypokalemia, elevated PAC, suppressed PRA, AVS lateralization, Protocol with normal salt diet was included because CCT was performed in all patients in this group; no |
| Study                | Country | Age, Sex | Hypokalemia, | Design | Case-control | Prospective | Subjects | CCT | Discontinuation of all medications | Immuno-assay | Different standards used: PA based on | Renovascular |
|----------------------|---------|----------|---------------|--------|--------------|-------------|----------|-----|-----------------------------------|--------------|---------------------------------------| 2 cases      |
| Hambling, 1992       | UK      | NR age, NR sex, NR hypokalemia, NR ARR | Two-gate design with alternative diagnosis controls | Case-control | Prospective | 10/22        | 24       | PAC collected 90 min after captopril >15 ng/dL for diagnosis of PA and surgical confirmation; diagnostic criteria not given for EH | Immuno-assay | PA based on FST (i.e., fludrocortisone 0.5 mg PO daily with salt supplements) but diagnostic criteria for SLT not given; diagnostic criteria not given for secondary hyperaldosteronism and EH | —            |
| Iwaoka, 1993         | Japan   | 47.1 y, 85 M, NR hypokalemia, NR ARR | Two-gate design with alternative diagnosis controls | Case-control | Unclear      | 16/190       | 24       | PAC collected 2 h after captopril >444 pmol/L for diagnosis of PA | Immuno-assay | PA based on hypertension, hypokalemia, low PRA, and high PAC with confirmation by surgery; renovascular | 2×2 table reconstructed using table 3; patients with pheochromocytoma and Cushing syndrome included as comparators |
PRA collected 90 min after captopril, and using a formula (Q) with final value >0 for diagnosis of PA, where: 
\[
Q = -6.06 \times (PRA)^2 - 6.99 \times (PAC)^2 - 7.11 \times (PRA) \times (PAC) - 7.06 \times (PRA) + 39.89 \times (PAC) - 39.82
\]

hypertension based on >75% stenosis of renal artery by angiography; diagnosis criteria for other forms of hypertension not stated

| Agharazii, 2001 | Canada | 52 y, NR sex, 49 | Single- | Consecutive | Prospective | 44/49 | Immuno- | Complete verification: SLT as verification standard for PA vs. EH; everyone received 3 days of high sodium diet (300 mmol/d) with 24 h urine to confirm high sodium excretion; it was implied that the criterion for PA was a PAC >240 pmol/L (8.65 ng/dL) following oral salt loading | All participants had hypokalemia (i.e., severe disease) |
|----------------|--------|-----------------|--------|-------------|------------|-------|---------|-------------------------------------------------|--------------------------------------------------|
|                |        | hypokalemia, NR | gate   | patients    |            |       |         |                                                 |                                                  |
|                |        |                 |        |             |            |       |         |                                                 |                                                  |
|                |        |                 |        |             |            |       |         |                                                 |                                                  |
|                |        |                 |        |             |            |       |         |                                                 |                                                  |
|                |        |                 |        |             |            |       |         |                                                 |                                                  |
|                |        |                 |        |             |            |       |         |                                                 |                                                  |
|                |        |                 |        |             |            |       |         |                                                 |                                                  |

| Castro, 2002 | USA | 52.1 y, 7 M, 6 | Single- | Unclear | Retrospective | 6/7 | Immuno- | Different standards used: PA based on abnormal SIT (cut-off not stated), abnormal cross-sectional imaging, and lateralization with AVS or NP59 +/- surgical response; SIT was performed in 6 out of 7 people | Inclusion into the study required a screening ARR less than 30 ng/dL per ng/mL/h (i.e., under the typical threshold for case detection) and all participants were male with overt or borderline hypokalemia |
|--------------|-----|----------------|--------|---------|--------------|-----|---------|-------------------------------------------------|--------------------------------------------------|
|               |     | hypokalemia, ARR less than 30 ng/dL per ng/mL/h | gate   |         |              |     |         |                                                 |                                                  |
|               |     |                 |        |         |              |     |         |                                                 |                                                  |
|               |     |                 |        |         |              |     |         |                                                 |                                                  |
|               |     |                 |        |         |              |     |         |                                                 |                                                  |
|               |     |                 |        |         |              |     |         |                                                 |                                                  |
|               |     |                 |        |         |              |     |         |                                                 |                                                  |
|               |     |                 |        |         |              |     |         |                                                 |                                                  |
| Study | Country | Age | Gender | Hypokalemia | ARR | Study Design | Control | Setting | CCT Description | ARR Description | Other | Immuno-assay | Verification | Notes |
|-------|---------|-----|--------|-------------|-----|--------------|---------|---------|-----------------|----------------|-------|-------------|-------------|-------|
| Rossi, 2002 | Italy | 49.6 y, 32 M, NR | hypokalemia, NR | ARR | Two-gate design with alternative diagnosis controls | Case-control | Unclear | 22/75 | CCT: discontinuation of aldosterone antagonists × 8 weeks, and all other antihypertensives × 4 weeks; use of alpha blockers if needed; seated for captopril 50 mg PO × 1 between 7:30-10 AM; ARR collected 90 min after captopril >35 ng/dL per ng/mL/h for diagnosis of PA | Complete verification: SIT (recumbent) as verification standard for PA vs. EH; everyone received 2 L 0.9% NaCl over 4 h from 8 AM to 12 PM while recumbent on a different date than CCT; post-infusion PAC >7.5 ng/dL used as reference standard for PA | Classified as two-gate study because 75 patients were known beforehand to have PA vs. EH, and all these had CCT and follow-up SIT; there were also 1046 people screened with CCT, but only those with positive tests received SIT, and therefore a 2×2 table could not be reconstructed for the larger group |
| Juutilainen, 2005 | Finland | 53.5 y, 36 M, 63 | hypokalemia, NR | ARR | Single-gate | Unclear | Retrospective | 38/77 | FST: discontinuation of spironolactone and estrogen × 4 weeks, and diuretics, ACEI, ARB, and BB × 2 weeks; received high-salt diet (16 g/d) and fludrocortisone 0.5 mg PO daily × 3 days with potassium supplementation if needed during a 5-day hospitalization; 24 h urinary aldosterone following salt loading ≥36.6 nmol/d for diagnosis of PA | Complete verification: clinical diagnosis as verification standard for PA vs. EH; chart review was used to look at laboratory data (i.e., screening test and confirmatory test [posture test], but no cut-offs stated), imaging data, and response to targeted treatment (i.e., improvement in hypokalemia and reduction in BP, but exact criteria not given) | The investigators described this as a salt loading test, but the actual intervention involved fludrocortisone administration with a mandatory hospitalization |
| Study            | Country | Age, Sex, Hypokalemia | Study Design | Sample Size | Measurement Details                                                                 | Diagnosis Criteria                                                                 |
|------------------|---------|-----------------------|--------------|-------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Giachetti, 2006  | Italy   | NR age, NR sex, NR hypokalemia, NR ARR | Single-gate | Consecutive | Retrospective 48/82 | Discontinuation of antihypertensives × 4 weeks; use of alpha blockers and CCBs if needed; supine × 2 h, then upright × 2 h, then captopril 50 mg PO × 1, then seated × 2 h; ARR collected 2 h after captopril >30 ng/dL per ng/mL/h for diagnosis of PA |
|                  |         |                       |              |             | Immunoassay Different standards used: four possible ways to diagnose PA with 3 of the 4 requiring abnormal SIT and the fourth way requiring an adrenal mass: (1) baseline elevated aldosterone (plasma or urine) plus upright PRA ≤1.0 ng/mL/h plus abnormal SIT (i.e., PAC ≥10 ng/dL); (2) baseline elevated aldosterone (plasma or urine) plus normal upright PRA plus abnormal SIT (i.e., ≥10 ng/dL); (3) normal baseline aldosterone (plasma and urine) plus upright PRA ≤1.0 ng/mL/h plus abnormal SIT with plasma (i.e., ≥10 ng/dL); (4) baseline elevated aldosterone (plasma or urine) plus upright PRA ≤1.0 ng/mL/h plus adrenal mass, even if SIT normal |
| Italy            | NR age, NR sex, NR hypokalemia | Single-gate | Consecutive | Retrospective 61/118 | SIT (recumbent): preparation as above; recumbent |
|                  |         |                       |              |             | Immunoassay As above |

2×2 table reconstructed using estimates of sens. and spec. from digitized version of figure 3.
| Mulatero, 2006 | Italy, Chile | 50.6 y, NR sex, NR hypokalemia, variable ARR cut-offs (i.e., >40 ng/dL per ng/mL/h with PAC >15 ng/dL, or ARR >25 to >35 ng/dL per ng/mL/h, or >32 pg/mL) | Single-gate | Consecutive | Prospective | 67/98 | SIT (posture not specified): discontinuation of spironolactone × 8 weeks, other diuretics × 6 weeks, and all other antihypertensives × 3 weeks; use of alpha blockers or CCBs if needed; 2 L of 0.9% NaCl IV over 4 h; PAC ≥5 ng/dL after infusion for diagnosis of PA | Immunoassay | Complete verification: FST as verification standard for PA vs. EH; everyone received fludrocortisone 0.1 mg PO q6h × 4 days with sodium and potassium suppl.; 24 h urinary sodium ≥3 mmol/kg/d with 10 AM post-FST PAC >5 ng/dL used as reference standard for PA | Each center originally used different cut-offs for SIT, but this was standardized to >5 ng/dL for the final analysis; 2-2 table extracted from table 2, though there was a slight difference in the sensitivity compared to what was reported in the narrative text |
| Schirpenbach, 2006 | Germany | 39.5 y, 56 M, 11 hypokalemia, ARR >21 pg/mL per mIU/mL | Multi-gate with healthy and alternative diagnosis controls | Case-control | Prospective | 25/101 | SIT (recumbent): discontinuation of spironolactone × 6 weeks; recumbent for 2 L of 0.9% NaCl IV beginning between 8-9:30 AM over 4 h; PAC ≥8.65 ng/dL after infusion for diagnosis of PA | Immunoassay | Different standards used: PA based on repeatedly elevated ARR (>21 pg/mL per mIU/mL), elevated 24 urinary aldosterone (>15 mcg/d), and previous abnormal SIT (i.e., PAC >8 ng/dL after 4 h); EH based on normal ARR, normal potassium, and normal 24 h urinary aldosterone; normal control | Index test and reference standard both included SIT |
| Study                      | Country | Age, Sex, Hypokalemia, ARR | Study Design | Side Effects | Subjects | Verification Method | Participants |
|---------------------------|---------|-----------------------------|--------------|--------------|----------|---------------------|--------------|
| Mulatero, 2007            | Italy   | NR age, NR sex, 2 hypokalemia, NR ARR | Single-gate  | Unclear      | 6/11     | Immuno-assay        | Participants were drawn from the same population as those in Mulatero 2006, but evaluating a different index test |
| Rossi, 2007a              | Italy   | 47y, NR sex, NR hypokalemia, NR ARR | Two-gate design with alternative diagnosis controls | Consecutive | 46/243   | Immuno-assay        | Participants from the PAPY cohort with main results for the CCT reported in 2007a article; 2×2 table reconstructed for APA (but not possible for all PA); although the investigators described enrollment as consecutive, patients with idiopathic hyperaldosteronism were excluded from the final analysis; this |
baseline PRA, post-captopril aldosterone, and baseline K+ \( \geq 0.50 \), plus (2) lateralization with AVS or NP59, plus (3) adenoma seen with cross-sectional imaging, surgery, or pathology, plus (4) cure of hypokalemia and improvement/cure of hypertension after surgery; diagnostic criteria not explicitly given for EH, but likely based on failure to fulfill all 4 criteria for PA, as above—but unclear if all patients, even those who had negative confirmatory testing, received entire verification process, including treatment.

| Rossi, 2007b | Italy | 47.2 y, NR sex, NR hypokalemia, ARR \( \geq 40 \) ng/dL per ng/mL/h | Two-gate design with alternative diagnosis controls | Consecutive | Prospective | 120/317 | SIT (recumbent): discontinuation of mineralocorticoid receptor antagonists \( \times 6 \) weeks and other antihypertensives \( \times 2 \) weeks; use of doxazosin and CCBs if needed; recumbent for 2 L of 0.9% NaCl IV | Immunoassay | Participants from the PAPY cohort with the most complete reporting of the SIT in the 2007b article was a two-gate study design because people who had high probability features of PA as well as 1-in-4 patients who did not have features of PA were tested; CCT was included both as the index test and part of the reference standard |
beginning between 8-9:30 AM over 4 h; PAC $\geq$ 6.8 ng/dL after infusion for diagnosis of PA

bilateral (idiopathic) PA based on biochemical evidence of PA but without lateralization; diagnostic criteria not explicitly given for EH, but likely based on failure to fulfill criteria for APA or bilateral PA—but unclear if all patients, even those who had negative confirmatory testing, received entire verification process, including treatment

| Wu, 2009 ** | Taiwan | 47.9, 69 M, NR | Single-gate | Consecutive | Prospective | 71/135 | CCT: discontinuation of antihypertensives × 2 weeks; use of diltiazem and doxazosin if needed; high-salt diet (6 g/d) × 3 days then seated for captopril 50 mg PO × 1 at 9 AM; ARR collected 1 h after captopril >35 ng/dL per ng/mL/h plus PAC >10 ng/dL for diagnosis of PA | Immuno-assay | Complete verification: SIT (recumbent) as verification standard for PA vs. EH; everyone received 2 L 0.9% NaCl over 4 h while recumbent on a different date than CCT; post-infusion PAC $\geq$ 10 ng/dL used as reference standard for PA; subtype of APA based on modified “4 corners approach” (i.e., ARR $>$ 30 ng/dL per ng/mL/h, lateralization on AVS or NP59, 2×2 table reconstructed using table 2; it was assumed that subjects were unique from those reported in Wu 2010 because the CCT protocol, laboratory assay, and interpretation criteria were different between studies |
| Study | Country | Hypertension | Method | Study Design | Sample Size | Test Details | Verification | Notes |
|-------|---------|--------------|--------|-------------|-------------|--------------|--------------|-------|
| Wu, 2010 [4] | Taiwan | Hypokalemia, ARR >30 ng/dL per ng/mL/h | Single-gate | Consecutive | 51/114 | Complete verification: clinical diagnosis as verification standard for PA vs. EH; PA based on a combination of (1) ARR >30 ng/dL per ng/mL/h (using PRA) and (2) abnormal SIT test (post-infusion PAC >10 ng/dL) or 24 h urinary aldosterone ≥12 mcg/d; diagnostic criteria not explicitly given for EH, but likely based on failure to fulfill criteria for PA | Immunoassay | It was assumed that subjects were unique from those reported in Wu 2009 because the CCT protocol, laboratory assay, and interpretation criteria were different between studies |
| Myśliwiec, 2012 [4, 5] | Poland | Hypokalemia, NR ARR | Single-gate | Consecutive | 13/198 | Partial verification with different standards used: investigations to look for secondary causes of hypertension were variably performed (e.g., tests for cortisol and catecholamine excess); PA based on treatment | Immunoassay | Suspected error in the original report because sens. of 93% and spec. of 97% in narrative text do not match the data from table 1 (i.e., absence of false negatives); therefore, 2×2 table was reconstructed |
| Study                  | Country | Age, Sex, Hypokalemia, ARR | Study Design | Consecutive | Sample Size | Hypertension Protocol | Verification Criteria | Notes |
|------------------------|---------|---------------------------|-------------|-------------|-------------|----------------------|-----------------------|-------|
| Willenberg, 2012       | Germany | NR, NR, NR, NR            | Single-gate | Consecutive | 21/59       | FST: BP controlled with nifedipine, nitroglycerin, or alpha blockers; timing of discontinuation of other antihypertensives not stated; received fludrocortisone 0.1 mg PO qid x 4 days; PAC at 10 AM on 5th day >53.5 ng/L (5.35 ng/dL) for diagnosis of PA | Complete verification: APA based on hypertension, elevated ARR (value not stated), PAC >2.5 ng/dL after SIT or FST, AVS with lateralization index of >3:1, and CT evidence of ipsilateral adrenal nodule of >5 mm; other causes of hypertension investigated with Doppler ultrasound of renal arteries, plasma metanephrines, and tests of renal function; criteria not explicitly given for non-APA, but likely based on failure to fulfill criteria for APA | No cases of bilateral PA included; the FST was included both as the index test and part of the reference standard; 2x2 table was reconstructed using table 3 |
| Germany                | NR, NR, NR, NR, NR         | Single-gate | Consecutive | Unclear     | 53/130      | SIT (recumbent): medication preparation as above; recumbent for 2 L of 0.9% NaCl IV beginning between 8-9:30 AM over 4 h; PAC ≥31.5 ng/L | Immuno-assay          | As above | As above |
| Study | Country | Age, Sex, Hypokalemia, ARR | Study Design | Study Design | Method | Verification | Reference Standard for PA vs. non-PA | Notes |
|-------|---------|--------------------------|-------------|-------------|--------|--------------|-----------------------------------|-------|
| Ceral, 2014 | Czech Republic | 49.0 y, 30 M, NR hypokalemia, NR ARR | Single-gate | Consecutive | Prospective | SLT: high-salt diet (6 g/d) × 3 days with 24 h urinary \(\text{Na}^+\) ≥200 mmol/d to verify salt intake; 24 h urinary aldosterone after salt loading ≥36 nmol/d for diagnosis of PA | Immunoassay | Complete verification: SIT (recumbent) as verification standard for PA vs. non-PA; PA based on post-infusion PAC >100 pmol/L |
| Nakama, 2014 | Japan | NR age, NR sex, NR hypokalemia, NR ARR | Single-gate | Consecutive | Retrospective | CCT: discontinuation of antihypertensives × 2 weeks; use of alpha blockers and CCBs if needed; recumbent for captopril 50 mg PO × 1; ARR collected 60 min or 90 min after captopril ≥200 pg/mL per ng/mL/h (20 ng/dL per ng/mL/h) for diagnosis of PA | Immunoassay | Partial verification: PA based on having at least two positive confirmatory tests (CCT, SIT, and furosemide upright test)—but not everyone received all three confirmatory tests |
| | Japan | NR age, NR sex, NR hypokalemia, NR ARR | Single-gate | Consecutive | Retrospective | SIT (recumbent): discontinuation of antihypertensives × 2 weeks; use of alpha blockers and CCBs if needed; recumbent for 2 L of 0.9% NaCl IV over 4 h; PAC ≥6 ng/L after infusion for diagnosis of PA | Immunoassay | As above |

The CCT was included both as the index test and part of the reference standard; not everyone received all three confirmatory tests that were required for verification; not explained why some tests were given to some patients, but not others.
not explained why some tests were given to some patients, but not others

| Kuo, 2015 4th | Taiwan | 60.9 y, 29 M, NR hypokalemia, ARR >35 ng/dL per ng/mL/h | Single-gate | Consecutive | Retrospective | 31/60 | CCT: discontinuation of antihypertensives × 3 weeks and other interfering medications (e.g., glucocorticoids, sex hormones, licorice, non-steroidal anti-inflammatory drugs) × 6 weeks; seated for captopril 50 mg PO × 1 at 9 AM, then ambulation; ARR collected 1 h after captopril >35 ng/dL per ng/mL/h plus PAC >10 ng/dL for diagnosis of PA | Immuno-assay | Different standards used: only those with negative CCT were verified with independent reference standard; clinical diagnosis as verification standard (modified “4 corners approach”) for PA vs. EH; APA based on a combination of all the following: (1) positive screening test (i.e., ARR ≥35 ng/dL per ng/mL/h and post-confirmatory test PAC >10 ng/dL, plus (2) lateralization with AVS or NP59, plus (3) adenoma seen with cross-sectional imaging, plus (4) cure of hypokalemia and improvement/cure of hypertension after surgery; diagnosis of bilateral (idiopathic) PA | CCT was included both as the index test and part of the reference standard; only those with negative CCT were verified with independent reference standard; it was presumed everyone with positive CCT had PA (i.e., not allowing for possibility of false positive) |
| Comu, 2016 [46] | France | 48 y, 125 M, NR hypokalemia, ARR >64 pmol/L per mlU/L on at least two occasions | Single-gate | Consecutive | Retrospective | 102/199 | SIT (recumbent): discontinuation of mineralocorticoid receptor antagonists and renin antagonists × 6 weeks, and other interfering drugs × 2 weeks; use of peripheral alpha blockers, central alpha agonists, and CCBs if needed; recumbent for 2 L of 0.9% NaCl IV starting at 8 AM over 4 h; PAC >277 pmol/L (10 ng/dL) after infusion for diagnosis of PA | Immuno-assay | Complete verification: AVS as verification standard; AVS interpretation criteria included selectivity index >2.1 to verify cannulation, plus aldosterone: cortisol ratio of dominant side to non-dominant side of >4.1 to define lateralization | Disease defined by presence of lateralization on AVS

| Kim, 2016 [49] | South Korea | 50.9 y, 27 M, 4 hypokalemia, ARR >20 ng/dL per ng/mL/h | Single-gate | Consecutive | Prospective | 51/64 | CCT: discontinuation of ACEI, ARB, and BB × 4 weeks; use of alpha blockers and CCBs if needed; seated for captopril 50 mg PO | Immuno-assay | Complete verification: SIT (recumbent) as verification standard for PA vs. non-PA; PA based on post-infusion PAC | Suspected error in the original report because sens. of 98.0% and spec. of 78.6% in
× 1; PAC collected 60 min or 90 min after captopril ≥13 ng/dL for diagnosis of PA

≥10 ng/dL

narrative text and table 2 do not match the data when back-calculated; 2×2 table was reconstructed using data from table 2 with rounding

| Study | Country | Age, Gender, Diagnosis | Study Design | Number of Participants | Test | Standards Used | Diagnosis Criteria |
|-------|---------|------------------------|--------------|------------------------|------|---------------|-------------------|
| Li, 2016 | China | 43.3 y, 90 M, 55 hypokalemia, ARR >30 ng/dL per ng/mL/h with PAC >15 ng/dL | Multi-gate with healthy and alternative diagnosis controls | Case-control | Prospective | 76/141 | SIT (recumbent): discontinuation of diuretics × 4 weeks, and ACEI, ARB, and BB × 2 weeks; use of alpha blockers and CCBs if needed; recumbent for 2 L of 0.9% NaCl IV starting at 8 AM over 4 h; PAC >11.45 ng/dL after infusion for diagnosis of PA | Immuno-assay | Different standards used: PA based on a combination of (1) ARR >30 ng/dL per ng/mL/h plus aldosterone ≥15 ng/dL, (2) PAC after saline infusion of ≥10 ng/dL, and (3) adrenal nodularity or thickening on CT; subtype of APA based on lateralization on AVS and/or surgery with pathologically-proven adenoma; subtype of bilateral PA based on normokalemia and improved BP after treatment with a mineralocorticoid receptor antagonist; EH based on exclusion of secondary hypertension (but details not provided); |
| Reference | Country | Age | Gender | Design | Comparison | Sample Size | Study Details |
|-----------|---------|-----|--------|--------|------------|-------------|---------------|
| Tsiavos, 2016 | Greece | 53.6 y, NR | sex, 19 | Single-gate | Consecutive | 45/148 | FST: discontinuation of all drugs affecting the renin-aldosterone axis for 3 weeks; use of CCBs if needed; received NaCl 4 g PO tid for 4 days, fludrocortisone 0.1 mg PO q6h for 4 days, and dexamethasone 2 mg x 1 at midnight on 4th day; PAC between 8:30-9 AM on 5th day ≥3.0-3.1 ng/dL for diagnosis of PA. Immunoassay Different standards used: PA based on either a positive FST or, in the case of a negative FST, a combination of uncontrolled BP on ≥2 drugs, spontaneous hypokalemia, kaliuresis, and normalization of BP with spironolactone or eplerenone; EH was based on absence of all the criteria required for PA. It was presumed everyone with positive FST had PA (i.e., not allowing for possibility of false positive); cut-off for FST not clear (i.e., PAC 3.1 ng/dL on p. 24; PAC 3 ng/dL on pp. 23 and 26). |
| Song, 2018 | China | 47.9 y, 117 M | | Two-gate design with alternative diagnosis controls | Consecutive | 135/236 | SIT (recumbent): discontinuation of diuretics for 4 weeks, and ACEI, ARB, and BB for 2 weeks; use of alpha blockers and CCBs if needed; recumbent for 2 L of 0.9% NaCl IV starting at 8 AM over 4 h; PAC >10 ng/dL after infusion for diagnosis of PA. Immunoassay Different standards used: PA based on either a positive FST (fludrocortisone 0.1 mg PO q6h for 4 days; 24 h urinary sodium ≥3 mmol/kg/d with 10 AM post-FST PAC ≥8 ng/dL for diagnosis of PA) or, in the case of a negative FST, the presence of lateralization on AVS leading to biochemical cure after adrenalectomy; EH was based on absence of all the criteria required for PA. Patient selection applicability considered to be at low risk, even though there was a two-gate design, because all participants were considered to be at risk for PA before screening. |
| Country | Age | Gender | Hypokalemia | ARR Cut-off | Design | Controls | Study Population | CCT | Immunoassay | Notes |
|---------|-----|--------|-------------|-------------|--------|----------|-----------------|-----|-------------|-------|
| China   | 47.9 y, 117 M, 127 hypokalemia, ARR ≥3.7 ng/dL per mIU/L | Two-gate design with alternative diagnosis controls | Consecutive | Prospective | 135/236 | Discontinuation of diuretics × 4 weeks, and ACEI, ARB, and BB × 2 weeks; use of alpha blockers and CCBs if needed; seated for captopril 50 mg PO × 1 at 8-9 AM; PAC collected 2 h after captopril ≥13 ng/dL for diagnosis of PA | Immunoassay | As above | As above |
| Meng, 2018 | 47.0 y, 63 M, 86 hypokalemia, ARR >30 ng/dL per ng/mL/h | Single-gate | Consecutive | Prospective | 115/164 | Discontinuation of spironolactone × 6 weeks, other diuretics × 4 weeks, and other confounding antihypertensives × 2 weeks; use of alpha blockers and CCBs if needed; exact protocol for CCT not given (no dose of drug, body posture, or timing); PAC after captopril >16.7 ng/dL for diagnosis of PA (timing of collection not stated) | Immunoassay | Different standards used: PA based on “biochemical diagnosis” (criteria not stated) with screening ARR ≥30 ng/dL per ng/mL/h; APA subtype based on lateralization on AVS, CT/surgical evidence of adenoma, and normokalemia with improvement/cure of hypertension after surgery; EH based on ARR below 30 ng/dL per ng/mL/h, normal Doppler US of renal arteries, normal catecholamines, normal UFC, and normal renal function | Details about CCT protocol not given; details about biochemical testing for verification standard not given (i.e., unclear if confirmatory test used for diagnosis beyond screening ARR) |
| China   | 47.0 y, 63 M, 86 hypokalemia, ARR >30 | Single-gate | Consecutive | Prospective | 115/164 | Discontinuation of spironolactone × 6 | Immunoassay | As above | Details about SIT protocol not given; details about... |
| Country     | Age, Gender, Diagnosis | Test Description | Participants | Standards Used | Additional Notes |
|-------------|------------------------|------------------|--------------|----------------|------------------|
| Australia   | 55.3 y, 62 M, hypokalemia, ARR >70 pmol/L per mIU/L when PAC measured by immunoassay or >55 pmol/L per mIU/L when PAC measured by HPLC-MS/MS | SIT (seated): discontinuation of diuretics × 4 weeks, and other antihypertensives × 2 weeks; use of alpha blockers and CCBs if needed; exact protocol for SIT not given (no dose of drug, body posture, or timing); PAC after infusion >11.2 ng/dL for diagnosis of PA (timing of collection not stated) | 77/108 | HPLC-MS/MS | Different standards used: PA based on either a positive FST (fludrocortisone 0.6 mg PO q6h × 4 days; 10 AM post-FST PAC ≥165 pmol/L when measured using radioimmunoassay or ≥133 pmol/L when measured using HPLC-MS/MS after being upright for 2 hours plus DRC <8.4 mIU/L for diagnosis of PA) or, in the case of a negative FST (in 1 patient), the presence of lateralization on AVS; “non-PA” was based on absence of all the criteria required for PA |

The study double counts some patients (i.e., 100 participants with some having two tests for a total of 108 tests; specifically, 8 people had confirmatory testing before adrenalectomy for PA, and then again after adrenalectomy to confirm cure); it was probable that the patients included in the Ahmed 2014 article were also included here because of overlapping study period.
and the description of an "expanded patient cohort"; the Thuzar 2020 article reports the same people, but using immunoassay—and these were excluded to avoid double counting; verification with the same reference standard near-complete (i.e., only 1 person with PA did not have positive FST); 2×2 table reconstructed based on table 3 of Stowasser 2018 article, but the final specificity does not match the number reported in the article, possibly because of differences in how inconclusive results were handled.

| Country | Age, Sex, NR | Study Design | Approach | Sample Size | Results | Method | Notes |
|---------|--------------|--------------|----------|-------------|---------|--------|-------|
| Australia | 55.3 y, 62 M, NR hypokalemia, | Single-gate Consecutive Prospective 77/108 | SIT (recumbent); discontinuation of diuretics × 4 | HPLC-MS/MS | As above | As above; to avoid double counting in |
| Study | Country | Age, Sex | Study Design | Number of Participants | SIT (Recumbent): | Immunoassay | Notes |
|-------|---------|----------|--------------|------------------------|------------------|------------|-------|
| Velema, 2018 | Netherlands | NR age, NR sex, NR hypokalemia, NR ARR | Single-gate Consecutive Retrospective | 146/276 | SIT (recumbent): discontinuation of medications interfering with renin and aldosterone axis × 4-6 weeks; semi-recumbent for 2 L of 0.9% NaCl IV starting at 8-9:30 AM over 4 h; PAC ≥280 pmol/L after infusion for diagnosis of PA | Immunoassay | Partial verification: PA based on clinical assessment by experts (e.g., endocrinologists and vascular medicine specialist) who reviewed demographics and clinical data (e.g., results of SIT, potassium, BP, and age) with final decision reached by consensus; anyone with post-infusion PAC <140 pmol/L assumed to have no PA (i.e., not allowing for possibility of false negative), but all indeterminate or positive saline infusion tests |
Kidoguchi, 2019

| Country | Age | Gender | Hypokalemia | ARR | Methodology | CCT: | Diagnosis Criteria | Verificaion Method | Study Details |
|---------|-----|--------|-------------|-----|-------------|------|--------------------|-------------------|---------------|
| Japan   | 50.3 y, 49 M, NR | Hypokalemia, ARR >200 pg/mL per ng/mL/h | Single-gate | Unclear | Unclear | 71/71 | discontinuation of antihypertensives × 6 weeks; use of alpha blockers and CCBs if needed; supine for captopril 50 mg PO × 1 at 8 AM; reduction of PAC collected 90 min after captopril less than 30% from baseline for diagnosis of PA | Complete verification: PA based on positive result from at least one of two alternate confirmatory tests: (1) upright furosemide loading test (furosemide 40 mg IV × 1 with PRA <2.0 ng/mL/h after 2 h collected in seated position) or (2) SIT (2 L 0.9% NaCl IV × 1 with PAC >60 pg/mL [166 pmol/L] after 4 h collected in recumbent position) | In this study, everyone had PA and nobody was disease-free; the third interpretation criterion for CCT (i.e., reduction in PAC by less than 30% after captopril) was chosen for data extraction because it aligned closest with the Endocrine Society guidelines |

Okamoto, 2018

| Country | Age | Gender | Hypokalemia | ARR | Methodology | CCT: | Diagnosis Criteria | Verificaiton Method | Study Details |
|---------|-----|--------|-------------|-----|-------------|------|--------------------|-------------------|---------------|
| Japan   | 56 y, 48 M, NR | Hypokalemia, ARR >20 ng/dL per ng/mL/h | Single-gate | Consecutive | Prospective | 75/102 | discontinuation of antihypertensives (timing not stated); use of alpha blockers and CCBs if needed; captopril 50 mg PO × 1; ARR collected 90 min after captopril ≥42.2 ng/dL per ng/mL/h for diagnosis of APA | Different standards used: PA based on at least 1 positive confirmatory test where every participant received at least 2 of 3 tests: (1) SIT (PAC >6 ng/dL), (2) CCT (ARR >20 ng/dL per ng/mL/h), and (3) upright furosemide loading test (PRA <2.0 ng/mL/h) | CCT was included both as the index test and part of the reference standard; in this study, there was a comparison of APA vs. non-APA (a group that included people with EH) and therefore it was not considered to be a pure
| Country | Age | Gender | Hypokalemia | ARR Cut-off | Study Design | Setting | Normal SIT | CCT | Immunoassay | Reference Standard |
|---------|-----|--------|-------------|-------------|--------------|---------|-----------|-----|-------------|-------------------|
| Japan   | 56 y, 48 M, NR | Single-gate | Consecutive | Prospective | SIT (posture not specified): discontinuation of antihypertensives (timing not stated); use of alpha blockers and CCBs if needed; 2 L of 0.9% NaCl IV over 4 h; PAC >15.2 ng/dL after infusion for diagnosis of APA | NR | As above | As above; 2×2 table reconstructed based on reported sens. and spec., but the final numbers do not perfectly match because it is possible that not everybody received the CCT in the actual study (but details not provided) |
| China   | 48.2 y, 166 M, 97 | Multi-gate with healthy and alternative diagnosis controls | Case-control | Prospective | CCT: discontinuation of diuretics × 4 weeks, and ACEI, ARB, and BB × 2 weeks; use of alpha blockers and CCBs if needed; supine × 2 h, then | Immunoassay | Different standards used: PA based on ARR ≥25 ng/dL per ng/mL/h and PAC >12 ng/dL, plus at least one of the following abnormalities: | Different standards used: PA based on ARR ≥25 ng/dL per ng/mL/h and PAC >12 ng/dL, plus at least one of the following abnormalities: |
upright × 2 h for captopril 50 mg PO × 1 at 8-9 AM; ARR collected 2 h after captopril ≥20 ng/dL per ng/mL/h for diagnosis of PA

(1) upright PRA <1.0 ng/ml/h, (2) post-captopril ARR ≥20 ng/dL per ng/mL/h, or (3) post-captopril PAC reduced less than 30% compared to baseline; EH based on ruling-out of renal parenchymal hypertension, renovascular hypertension, endocrine hypertension, aortic dissection, sleep apnea, and contributing drugs

| Wu, 2019 | Taiwan | 47.8 y, 61 M, NR hypokalemia, NR ARR | Single-gate | Consecutive | Prospective | 107/143 | SIT (seated): discontinuation of antihypertensives × 3 weeks; use of diltiazem and doxazosin if needed; seated for 2 L of 0.9% NaCl IV starting at 8 AM over 4 h; PAC ≥25 ng/dL after infusion for diagnosis of PA | Immunoassay | Partial verification: patients with PAC ≥16 ng/dL after SIT received further tests for lateralization and consideration of surgery; clinical outcomes to targeted treatment as verification standard for surgically-amenable PA vs. other; Primary Aldosteronism Surgical Outcome (PASO) criteria used: complete clinical success defined as normal BP without needing medications; Post-SIT PAC ≥16 ng/dL was used in clinical practice for PA, but post-SIT PAC ≥25 ng/dL was used for the research study; SIT was index test and clinical outcomes to surgery was the gold standard for diagnosis (i.e., complete or partial success after surgery = disease present; absent |
| Study     | Country | Age | Sex | Hypokalemia | ARR | Study Design | Treatment | SIT (recumbent): | Immunoassay | Reference Standard |
|-----------|---------|-----|-----|-------------|-----|--------------|-----------|-----------------|-------------|-------------------|
| Vivien, 2019 | France | NR | NR | NR | NR | Single-gate | Consecutive | Discontinuation of ACEI, ARB, central renin inhibitors, and potassium-wasting diuretics, estrogen, and progesterone x 4 weeks, and potassium-sparing diuretics x 6 weeks; recumbent for 2 L of 0.9% NaCl IV over 4 h; PAC >160 pmol/L after infusion for diagnosis of PA | Different standards used: PA based on baseline ARR >64 pmol/L per mIU/L and positive confirmatory test by traditional criteria (i.e., post-SIT PAC >140 pmol/L, or CCT [captopril 50 mg x 1] with reduction in PAC by less than 30% after 2 hours) | SIT was included both as the index test and part of the reference standard |
| Fries, 2020 | Germany | 52.3 y, 37 M, 23 | | | NR | Single-gate | Consecutive | Discontinuation of mineralocorticoid receptor antagonists and potassium-sparing diuretics x 4 weeks, and ACEI, ARB, BB, and | HPLC-MS/MS | Unclear: clinical outcomes to targeted treatment as verification standard as adjudicated by panel of experienced | SIT was included both as the index test and part of the reference standard; even though this is a |
direct renin inhibitors × 2 weeks; use of alpha blockers, CCBs, and vasodilators if needed; recumbent for 2 L of 0.9% NaCl IV over 4 h; PAC ≥140 pmol/L after infusion for diagnosis of PA

endocrinologists; PA based on all of the following: (1) elevated ARR (cut-offs not stated), (2) baseline PAC >550 pmol/L, (3) spontaneous hypokalemia, (4) either a suppressed renin or positive confirmatory test (i.e., post-SIT PAC ≥140 pmol/L, or post-CCT PAC reduction of ≤20%), and (5) cure/improvement in BP and/or normalization of biochemistry after mineralocorticoid receptor antagonist or surgery; implied that all others were classified as non-PA—but unclear if all patients, even those who had negative confirmatory testing, received entire verification process, including treatment

Lin, 2020

| Country   | Study Design | Study Type | n | SIT (recumbent): discontinuation of ACEI, ARB, BB, and diuretics (details not stated); use of alpha blockers and non-Immuno-assay | Complete verification: FST as verification standard for PA vs. EH; PA based on positive FST |
|-----------|--------------|------------|---|----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| China     | Single-gate  | Consecutive | 161/280 | 2 × 2 table reconstructed using figure 1 and pre-determined PAC cut-off ≥10 ng/dL for
| Study                          | Country  | Age (y) | Gender | Race | Study Design | Protocol Duration | Test Used | Criteria for PA Diagnosis |
|-------------------------------|----------|---------|--------|------|--------------|-------------------|-----------|---------------------------|
| Zhang, 2020                   | China    | 48.5 y, 46 M, 49 | M, M | | Single-gate | Consecutive | Prospective | 90/110 |
|                               |          |         |        |      | SIT (recumbent): discontinuation of diuretics and spironolactone × 4 weeks, and ACEI, ARB, and BB × 2 weeks; use of alpha blockers and CCBs if needed; detailed protocol for SIT not stated (but assumed to be recumbent for 2 L of 0.9% NaCl IV over 4 h); PAC ≥12.04 ng/dL after infusion for diagnosis of PA |
|                               |          |         |        |      | Immunoassay | Different standards used: clinical diagnosis as verification standard (modified “4 corners approach”) for PA vs. EH; APA based on a combination of all the following: (1) biochemical evidence of PA (details not stated, but likely included elevated ARR and post-recumbent SIT aldosterone >11.2 ng/dL (page 893)), plus (2) lateralization with AVS or NP59, plus (3) adenoma seen with cross-sectional imaging, surgery, or pathology, plus (4) cure of hypokalemia and improvement/cure of hypertension after surgery; diagnosis of PA |

Note: Diagnosis of PA was included both as the index test and part of the reference standard; some patients were not accounted for (e.g., 3 patients with recumbent SIT); suspected error in the original report because sens. of 83.15% and spec. of 57% in figure 2 does not match the data from the text (i.e., true positives of 73 with false negatives of either 17 or 20); therefore, 2×2 table was reconstructed using data from the text because these raw
| Study | Country | Age, Gender | Hypokalemia, AR% | ARR cut-off | Gate Type | Sample Size | Protocol Details | Test Details | Immunodiagnostic Method | Numbers |
|-------|---------|-------------|------------------|-------------|------------|-------------|------------------|--------------|------------------------|---------|
| China | China   | 48.5 y, 46 M, 49 M | Hypokalemia, ARR 30 ng/dL per mg/mL/h, or ARR 20 ng/dL per mg/mL/h plus PRA <1 ng/mL/h plus aldosterone >15 ng/dL | SIT (seated): discontinuation of diuretics and spironolactone × 4 weeks, and ACEI, ARB, and BB × 2 weeks; use of alpha blockers and CCBs if needed; detailed protocol for SIT not stated (but assumed to be seated for 2 L of 0.9% NaCl IV over 4 h); PAC ≥12.94 ng/dL after infusion for diagnosis of PA | Immunoassay | As above |
| Liu, 2021 | China | 48.8 y, 88 M | Hypokalemia, ARR ≥1.0 ng/dL per mU/L | SIT (seated): discontinuation of diuretics × 4 weeks, and ACEI, ARB, and BB × 2 weeks; use of alpha blockers and CCBs if needed; seated for 2 L of 0.9% NaCl IV starting at 8 AM over 4 h; PAC ≥12 ng/dL after infusion | Immunoassay | Different standards used: PA based on either a positive FST (fludrocortisone 0.1 mg PO q6h × 4 days; 10 AM post-FST PAC ≥6 ng/dL) or, in the case of a negative FST (in 1 patient), Extracted for diagnostic threshold associated with highest specificity with 12 ng/dL for SIT and 13 ng/dL for CCT |
| Country     | Age (y), Gender, Hypokalemia, ARR | Study Type | Prospective/Retrospective | Test Method | Diagnosis Criteria                                                                 | SIT Method/Reference                                      | Verification Process                                                                 |
|-------------|----------------------------------|------------|---------------------------|-------------|-------------------------------------------------------------------------------------|----------------------------------------------------------|--------------------------------------------------------------------------------------|
| China       | 48.8 y, 88 M, NR hypokalemia, ARR ≥1.0 ng/dL per mIU/L | Single-gate Consecutive Prospective | 196/269 | CCT: discontinuation of diuretics × 4 weeks, and ACEI, ARB, and BB × 2 weeks; use of alpha blockers and CCBs if needed; captopril 50 mg PO × 1 at 8-9 AM; PAC collected 2 h after captopril ≥13 ng/dL for diagnosis of PA | Immunoassay | As above As above                                                                 |
| Germany     | 52.6 y, 94 M, NR hypokalemia, ARR >20 ng/L per ng/L | Single-gate Consecutive Retrospective | 103/187 | SIT (recumbent): discontinuation of mineralocorticoid receptor antagonists × 4 weeks, and other antihypertensives × 1 week; use of alpha blockers and CCBs if needed; recumbent for 2 L of 0.9% NaCl IV starting at 8-10 AM over 4 h; PAC ≥140 ng/L (14.0 ng/dL) after infusion for diagnosis of PA | HPLC-MS/MS | Unclear: PA based on retrospective review of clinical factors including history, results of SIT by immunoassay with aldosterone >50 ng/L, imaging, AVS, pathology, and clinical response to treatment (surgery or medicine); unclear if every individual went through every single step for verification (e.g., including definitive treatment) | SIT was included both as the index test and part of the reference standard; although it was a single-gate study, risk of selection bias was high because 49 patients were excluded, including some where it was difficult to determine if disease was present |

Data for the same subjects were sometimes reported across multiple articles. In these cases, the most recent or complete citation was used to avoid double counting the same subjects for the same test. **Abbreviations:** ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; APA, aldosterone-producing adenoma; ARR, aldosterone-to-renin ratio; AVS; adrenal vein sampling; BB, beta-blocker; BP, blood pressure; CCB, calcium channel blocker; CCT, captopril challenge test; CI, confidence interval; CT,
computed tomography; DRC, direct renin concentration; EH, essential hypertension; FST, fludrocortisone suppression test; HPLC-MS/MS, high-performance liquid chromatography with tandem mass spectrometry; IM, intramuscularly; IV, intravenously; NaCl, sodium chloride; NP59, norcholesterol scan; NR, not reported; PA, primary aldosteronism; PAC, plasma aldosterone concentration; PO, *per os*, orally; PRA, plasma renin activity; SIT, intravenous saline infusion test; SLT, oral salt loading test; USA, United States of America; UK, United Kingdom.
Table S4. Risk of bias of included studies.

| Study author, year ref. | Risk of bias | Applicability concerns |
|-------------------------|--------------|------------------------|
|                         | Patient selection | Index test | Reference standard | Flow and timing | Patient selection | Index Test | Reference standard |
| Horton, 1969 7           | high          | unclear        | low               | low             | high             | unclear       | low             |
| Biglieri, 1970 8         | high          | unclear        | high              | low             | high             | high          | high            |
| Collins, 1970 9          | high          | low            | high              | high            | high             | low           | high            |
| Kem, 1971a 10            | high          | high           | high              | low             | high             | low           | high            |
| Kem, 1971b 11            | high          | unclear        | high              | low             | high             | low           | high            |
| Espiner, 1971 12         | high          | high           | high              | high            | high             | high          | high            |
| Dunn, 1976 13            | high          | high           | high              | high            | high             | low           | high            |
| Lund, 1980 14            | high          | low            | high              | low             | high             | high          | high            |
| Streeten, 1982 15,16     | high          | high           | high              | high            | high             | low           | high            |
| Thibonnier, 1982 17      | low           | high           | high              | low             | high             | low           | high            |
| Bravo, 1983 18           | high          | low            | high              | high            | high             | high          | high            |
| Lyons, 1983 19           | high          | high           | high              | high            | high             | high          | high            |
| Holland, 1984 20         | high          | high           | low               | high            | high             | high          | low             |
| Naomi, 1985 21           | high          | unclear        | high              | low             | high             | low           | high            |
| Muratani, 1986 22,23     | high          | high           | low               | high            | low             | high          | high            |
| Wu, 1986 24              | high          | high           | high              | high            | high             | high          | high            |
| Hamlet, 1987 25          | high          | high           | high              | high            | low             | unclear       | high            |
| Naomi, 1987 26           | high          | unclear        | high              | high            | high             | high          | high            |
| Hambling, 1992 27        | high          | high           | high              | high            | high             | high          | high            |
| Iwaoka, 1993 28          | high          | high           | unclear           | high            | high             | high          | high            |
| Agharazii, 2001 29       | high          | unclear        | high              | low             | high             | low           | high            |
| Castro, 2002 30          | unclear       | low            | high              | high            | high             | high          | high            |
| Rossi, 2002 31           | high          | high           | high              | low             | high             | high          | low             |
| Juutilainen, 2005 32     | low           | high           | unclear           | low             | high             | high          | high            |
| Giachetti, 2006 33       | low           | high           | high              | low             | high             | high          | high            |
| Mulatero, 2006 34        | low           | high           | high              | low             | low             | low           | low             |
| Schirpenbach, 2006 35    | high          | high           | high              | high            | high             | high          | high            |
| Mulatero, 2007 36        | unclear       | low            | high              | low             | high             | low           | unclear         |
| Rossi, 2007a 37,39       | high          | high           | high              | low             | high             | high          | high            |
| Rossi, 2007b 39,40       | high          | high           | high              | low             | high             | high          | high            |
| Wu, 2009 41              | low           | low            | high              | low             | high             | high          | high            |
| Wu, 2010 42              | low           | high           | high              | low             | high             | high          | low             |
| Reference       | Condition 1 | Condition 2 | Condition 3 | Condition 4 | Condition 5 | Condition 6 | Condition 7 |
|-----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Myśliwiec, 2012 | low         | high        | high        | high        | low         | high        | high        |
| Willenberg, 2012| low         | high        | high        | high        | low         | unclear     | high        |
| Ceral, 2014     | low         | low         | high        | low         | low         | low         | high        |
| Nakama, 2014    | low         | low         | high        | high        | low         | low         | high        |
| Kuo, 2015       | low         | low         | high        | low         | low         | low         | high        |
| Cornu, 2016     | low         | low         | high        | low         | low         | low         | low         |
| Kim, 2016       | low         | high        | low         | low         | high        | low         | low         |
| Li, 2016        | high        | high        | high        | high        | high        | high        | low         |
| Tsiavos, 2016   | low         | high        | high        | low         | unclear     | high        | high        |
| Song, 2018      | high        | high        | low         | low         | low         | low         | low         |
| Meng, 2018      | high        | high        | high        | high        | low         | unclear     | high        |
| Stowasser, 2018 | low         | high        | high        | low         | unclear     | high        | low         |
| Velema, 2018    | low         | low         | high        | high        | low         | low         | unclear     |
| Kidoguchi, 2019 | high        | low         | high        | low         | unclear     | low         | low         |
| Okamoto, 2018   | low         | high        | high        | high        | low         | high        | low         |
| Zhu, 2019       | high        | high        | high        | high        | low         | high        | high        |
| Wu, 2019        | low         | high        | low         | low         | low         | high        | low         |
| Vivien, 2019    | low         | high        | high        | high        | low         | high        | low         |
| Fries, 2020     | high        | low         | low         | low         | low         | low         | low         |
| Lin, 2020       | low         | low         | high        | low         | low         | low         | high        |
| Zhang, 2020     | low         | high        | high        | low         | high        | high        | high        |
| Liu, 2021       | low         | high        | high        | low         | low         | high        | low         |
| Fuss, 2021      | high        | high        | unclear     | high        | low         | high        | low         |
Table S5. Summary of reference standards used to verify disease status for primary aldosteronism.

| Study author, year | Criteria used for verification (presence vs. absence of disease) | Application of reference standard |
|--------------------|---------------------------------------------------------------|---------------------------------|
| Kem, 1971a □ 10    | ✓ Screen test results (e.g., elevated aldosterone, suppressed renin) | ✓ Complete                      |
| Kem, 1971b □ 11    | ✓ Confomatory test results (e.g., saline infusion test, salt loading test, captopril challenge test, fludrocortisone suppression test) | ✓ Complete                      |
| Espiner, 1971      | ✓ Adrenal nodule (e.g., seen on cross-sectional imaging or surgery) | ✓ Complete                      |
| Streeten, 1982 15,16 | ✓ Clinical factors (e.g., history of hypertension, hypokalemia) | ✓ Complete                      |
| Bravo, 1983 18      | ✓ Screen test results (e.g., elevated aldosterone, suppressed renin) | ✓ Complete                      |
| Holland, 1984      | ✓ Confomatory test results (e.g., saline infusion test, salt loading test, captopril challenge test, fludrocortisone suppression test) | ✓ Complete                      |
| Hamlet, 1987       | ✓ Adrenal nodule (e.g., seen on cross-sectional imaging or surgery) | ✓ Complete                      |
| Mulatero, 2006 34   | ✓ Clinical factors (e.g., history of hypertension, hypokalemia) | ✓ Complete                      |
| Schirpenbach, 2006 33 | ✓ Confomatory test results (e.g., saline infusion test, salt loading test, captopril challenge test, fludrocortisone suppression test) | ✓ Complete                      |
| Giachetti, 2006 33  | ✓ Adrenal nodule (e.g., seen on cross-sectional imaging or surgery) | ✓ Complete                      |
| Rossi, 2007b 39,40  | ✓ Clinical factors (e.g., history of hypertension, hypokalemia) | ✓ Complete                      |
| Myśliwiec, 2012 43  | ✓ Confomatory test results (e.g., saline infusion test, salt loading test, captopril challenge test, fludrocortisone suppression test) | ✓ Complete                      |
| Willenberg, 2012 44 | ✓ Adrenal nodule (e.g., seen on cross-sectional imaging or surgery) | ✓ Complete                      |
| Nakama, 2014 46     | ✓ Clinical factors (e.g., history of hypertension, hypokalemia) | ✓ Complete                      |
| Comu, 2016 □ 48     | ✓ Confomatory test results (e.g., saline infusion test, salt loading test, captopril challenge test, fludrocortisone suppression test) | ✓ Complete                      |
| Li, 2016 □ 30       | ✓ Adrenal nodule (e.g., seen on cross-sectional imaging or surgery) | ✓ Complete                      |
| Song, 2018 □ 31     | ✓ Clinical factors (e.g., history of hypertension, hypokalemia) | ✓ Complete                      |
| Meng, 2018 □ 34     | ✓ Confomatory test results (e.g., saline infusion test, salt loading test, captopril challenge test, fludrocortisone suppression test) | ✓ Complete                      |
| Stowasser, 2018 54,55 | ✓ Adrenal nodule (e.g., seen on cross-sectional imaging or surgery) | ✓ Complete                      |
| Velema, 2018        | ✓ Clinical factors (e.g., history of hypertension, hypokalemia) | ✓ Complete                      |
| Test                                                                 | Authors          | Studies | Yes | No | Total |
|----------------------------------------------------------------------|------------------|---------|-----|----|-------|
| Intravenous saline infusion test, seated (n=4)                      | Okamoto, 2018    | ✓       | ✓   | ✓  | ✓     |
|                                                                     | Vivien, 2019     | ✓       | ✓   | ✓  | ✓     |
|                                                                     | Fries, 2020      | ✓ ✓     | ✓ ✓ | ✓ ✓| ✓     |
|                                                                     | Lin, 2020        | ✓ ✓     | ✓ ✓ | ✓ ✓| ✓ ✓   |
|                                                                     | Zhang, 2020      | ✓ ✓ ✓   | ✓ ✓ ✓| ✓ ✓ | ✓ ✓ ✓ |
|                                                                     | Fuss, 2021       | ✓ ✓ ✓   | ✓ ✓ ✓| ✓ ✓ | ✓ ✓ ✓ |
| Total                                                               |                  | 10      | 11  | 17 | 8 3 95 5 8 4 6 13 3 |
| Oral salt loading test (n=2)                                        | Collins, 1970    | ✓       | ✓   | ✓  | ✓     |
|                                                                     | Ceral, 2014      | ✓ ✓     | ✓ ✓ | ✓ ✓| ✓ ✓   |
| Total                                                               |                  | 1 1 3 1 1 3 3 0 0 0 0 1 3 0 |
| Fludrocortisone suppression test (n=7)                              | Horton, 1969     | ✓       | ✓   | ✓  | ✓     |
|                                                                     | Biglieri, 1970   | ✓ ✓     | ✓ ✓ | ✓ ✓| ✓ ✓ ✓ |
|                                                                     | Dunn, 1976       | ✓ ✓ ✓   | ✓ ✓ ✓| ✓ ✓ | ✓ ✓ ✓ |
|                                                                     | Lund, 1989       | ✓ ✓ ✓   | ✓ ✓ ✓| ✓ ✓ | ✓ ✓ ✓ |
|                                                                     | Juutilainen, 2005| ✓ ✓ ✓   | ✓ ✓ ✓| ✓ ✓ | ✓ ✓ ✓ |
|                                                                     | Willenberg, 2012 | ✓ ✓ ✓   | ✓ ✓ ✓| ✓ ✓ | ✓ ✓ ✓ |
|                                                                     | Tsiavos, 2016    | ✓ ✓ ✓   | ✓ ✓ ✓| ✓ ✓ | ✓ ✓ ✓ |
| Total                                                               |                  | 6 5 3 1 2 1 5 2 2 1 2 0 5 0 |
| Captopril challenge test (n=25)                                     | Thibonnier, 1982 | ✓ ✓     | ✓ ✓ | ✓ ✓| ✓ ✓   |
|                                                                     | Lyons, 1983      | ✓ ✓     | ✓ ✓ | ✓ ✓| ✓ ✓   |
|                                                                     | Naomi, 1985      | ✓ ✓ ✓   | ✓ ✓ ✓| ✓ ✓ | ✓ ✓ ✓ |
|                                                                     | Muratani, 1986   | ✓ ✓ ✓   | ✓ ✓ ✓| ✓ ✓ | ✓ ✓ ✓ |
|                                                                     | Wu, 1986         | ✓ ✓ ✓   | ✓ ✓ ✓| ✓ ✓ | ✓ ✓ ✓ |
|                                                                     | Naomi, 1987      | ✓ ✓ ✓   | ✓ ✓ ✓| ✓ ✓ | ✓ ✓ ✓ |
|                                                                     | Hamblin, 1992    | ✓ ✓     | ✓ ✓ | ✓ ✓| ✓ ✓   |
|                                                                     | Iwaoka, 1993     | ✓ ✓     | ✓ ✓ | ✓ ✓| ✓ ✓   |
For complete verification, all participants received the same reference test. For partial verification, a reference test was not applied to all participants. For different reference tests, different criteria are used to define participants. **Abbreviations:** BP, blood pressure; PA, primary aldosteronism.
| Test                                               | Laboratory measure                          | Thresholds used for diagnosis |
|---------------------------------------------------|---------------------------------------------|-------------------------------|
| **Intravenous saline infusion test (recumbent)**   | Post-infusion PAC measured by immunoassay   |                               |
|                                                   |                                             | 3.15 ng/dL (87 pmol/L)        |
|                                                   |                                             | 5.0 ng/dL (139 pmol/L)        |
|                                                   |                                             | 5.8 ng/dL (160 pmol/L)        |
|                                                   |                                             | 6.0 ng/dL (166 pmol/L)        |
|                                                   |                                             | 6.5 ng/dL (180 pmol/L)        |
|                                                   |                                             | 6.8 ng/dL (189 pmol/L)        |
|                                                   |                                             | 7.0 ng/dL (194 pmol/L)        |
|                                                   |                                             | 8.5 ng/dL (236 pmol/L)        |
|                                                   |                                             | 8.65 ng/dL (240 pmol/L)       |
|                                                   |                                             | 9.0 ng/dL (250 pmol/L)        |
|                                                   |                                             | 10.0 ng/dL (280 pmol/L)       |
|                                                   |                                             | 11.2 ng/dL (311 pmol/L)       |
|                                                   |                                             | 11.45 ng/dL (318 pmol/L)      |
|                                                   |                                             | 12.04 ng/dL (334 pmol/L)      |
|                                                   |                                             | 15.2 ng/dL (422 pmol/L)       |
|                                                   | Post-infusion PAC measured by HPLC-MS/MS    | 3.8 ng/dL (106 pmol/L)        |
|                                                   |                                             | 5.1 ng/dL (140 pmol/L)        |
|                                                   |                                             | 14.0 ng/dL (388 pmol/L)       |
| **Intravenous saline infusion test (seated)**      | Post-infusion PAC measured by immunoassay   | 12.0 ng/dL (333 pmol/L)       |
|                                                   |                                             | 12.94 ng/dL (359 pmol/L)      |
|                                                   |                                             | 25.0 ng/dL (694 pmol/L)       |
|                                                   | Post-infusion PAC measured by HPLC-MS/MS    | 5.8 ng/dL (162 pmol/L)        |
| **Oral salt loading test**                        | 24 hour urinary aldosterone                 | 5 mcg/d (13.9 nmol/d) starting on day 2  |
|                                                   |                                             | 13 mcg/d (36.0 nmol/d) after 3 days  |
| **Fludrocortisone suppression test**              | Post-fludrocortisone challenge PAC          | 3.0-3.1 ng/dL (83-86 pmol/L)  |
|                                                   |                                             | 5.35 ng/dL (148 pmol/L)       |
|                                                   |                                             | 7.5 ng/dL (208 pmol/L)        |
|                                                   |                                             | 12.6 ng/dL (350 pmol/L)       |
|                                                   | Post-fludrocortisone challenge 24 hour     | Reduction of 24 hour urinary tetrahydroaldosterone by less than 24% compared to baseline |
|                                                   | urinary aldosterone                         | 13.2 mcg/d (36.6 nmol/d)      |
|                                                   |                                             | 18.9 mcg/d (52.4 nmol/d)      |
| **Captopril suppression test**                    | 1-hour post-captopril (50 mg) PAC +/- ARR   | PAC 10 ng/dL (277 pmol/L) and ARR >35 ng/dL per ng/mL/h  |
|                                                   |                                             | PAC 13.9 ng/dL (386 pmol/L)   |
|                                                   |                                             | 60- to 90-min post-captopril   | PAC 13.9 ng/dL  |
|                                                   | (50 mg) PAC +/- ARR                         | ARR 20 ng/dL per ng/mL/h      |
|                                                   |                                             | 90-min post-captopril (50 mg)  | Reduction of PAC by less than 30% compared to baseline |
|                                                   | (50 mg) PAC +/- PRA +/− ARR                 | PAC 15 ng/dL (416 pmol/L)     |
|                                                   |                                             | ARR 35 ng/dL per ng/mL/h      |
|                                                   |                                             | ARR 35.5 pmol per ng          |
|                                                   |                                             | ARR 42.2 ng/dL per ng/mL/h    |
|                                                   |                                             | Formula (Q) with final value >0 for diagnosis:  |
|                                                   |                                             | Q = − 6.06 × (PRA)² − 6.99 × (PAC)² − 7.11 × (PRA) × (PAC) − 7.06 × (PRA) + 39.89 × (PAC) − 39.82 |
|                                                   | 2-hour post-captopril (25 mg) PAC +/- ARR   | PAC 8.65 ng/dL (240 pmol/L)   |
|                                                   |                                             | PAC 8.9 ng/dL (247 pmol/L)    |
| Time Point | PAC Measurements | ARR Measurements |
|------------|------------------|------------------|
| 2-hour post-captopril (50 mg) PAC +/- ARR | PAC 8.5 ng/dL (236 pmol/L) or ARR 30 ng/dL per ng/mL/h \(^{36}\) |  |
|  | PAC 13.0 ng/dL (361 pmol/L) \(^{52,66}\) |  |
|  | PAC 16.0 ng/dL (444 pmol/L) \(^{27}\) |  |
|  | ARR 20 ng/dL per ng/mL/h \(^{60}\) |  |
|  | ARR 30 ng/dL per ng/mL/h \(^{33}\) |  |
| 2-hour post-captopril (100 mg) PAC | PAC 6.0 ng/dL (166 pmol/L) \(^{24}\) |  |
| 3-hour post-captopril (1 mg/kg) PAC | PAC 24.4 ng/dL (676 pmol/L) \(^{17}\) |  |
| Unclear timing for test (unknown dosage of captopril) PAC | PAC 16.7 ng/dL \(^{53}\) |  |

**Abbreviations:** ARR, aldosterone-to-renin ratio; HPLC-MS/MS, high-performance liquid chromatography with tandem mass spectrometry; PAC, plasma aldosterone concentration; PRA, plasma renin activity.
### Table S7. Meta-regression analysis for potential sources of diagnostic test accuracy variability.

| Potential source of heterogeneity | Confirmatory test\(^a\) | No. of studies | No. of cases of PA / no. of participants | Relative diagnostic odds ratio (95% CI) | P-value |
|-----------------------------------|-------------------------|----------------|------------------------------------------|----------------------------------------|---------|
| **Case-control sampling?\(^b\)**  |                         |                |                                          |                                        |         |
| Yes                               | All                     | 25             | 798 / 2,306                              | 7.26 (2.46, 21.43)                     | <0.001  |
| No                                | All                     | 39             | 2,780 / 5,051                            |                                        |         |
| Yes                               | SIT recumbent           | 10             | 390 / 1,091                              | 5.08 (1.21, 21.34)                     | 0.027   |
| No                                | SIT recumbent           | 16             | 1,299 / 2,563                            |                                        |         |
| Yes                               | FST                     | 4              | 47 / 102                                 | 2.71 (0.14, 50.83)                     | 0.504   |
| No                                | FST                     | 3              | 104 / 284                                |                                        |         |
| Yes                               | CCT                     | 10             | 356 / 1,063                              | 10.28 (2.84, 37.26)                    | <0.001  |
| No                                | CCT                     | 15             | 871 / 1,522                              |                                        |         |
| **Two-gate or multi-gate study design?\(^b\)** |                         |                |                                          |                                        |         |
| Yes                               | All                     | 27             | 964 / 2,866                              | 3.92 (1.27, 12.05)                     | 0.017   |
| No                                | All                     | 37             | 2,614 / 4,491                            |                                        |         |
| Yes                               | SIT recumbent           | 11             | 510 / 1,408                              | 2.78 (0.64, 12.02)                     | 0.172   |
| No                                | SIT recumbent           | 15             | 1,179 / 2,246                            |                                        |         |
| Yes                               | FST                     | 4              | 47 / 102                                 | 2.71 (0.14, 50.83)                     | 0.504   |
| No                                | FST                     | 3              | 104 / 284                                |                                        |         |
| Yes                               | CCT                     | 11             | 402 / 1,306                              | 4.80 (1.11, 20.77)                     | 0.036   |
| No                                | CCT                     | 14             | 825 / 1,279                              |                                        |         |
| **Partial verification, different reference tests, or unclear verification?** |                         |                |                                          |                                        |         |
| Yes                               | All                     | 49             | 2,768 / 5,855                            | 5.12 (1.48, 17.77)                     | 0.010   |
| No                                | All                     | 15             | 810 / 1,502                              |                                        |         |
| Yes                               | SIT recumbent           | 22             | 1,306 / 2,947                            | 4.22 (0.70, 25.36)                     | 0.115   |
| No                                | SIT recumbent           | 4              | 383 / 707                                |                                        |         |
| Yes                               | CCT                     | 17             | 892 / 1,975                              | 3.70 (0.68, 20.09)                     | 0.130   |
| No                                | CCT                     | 8              | 335 / 610                                |                                        |         |
| **Index test interpreted without blinding (i.e., risk of bias assessment for index test high or unclear)?** |                         |                |                                          |                                        |         |
| Yes                               | All                     | 48             | 2,702 / 5,685                            | 3.32 (0.94, 11.79)                     | 0.063   |
| No                                | All                     | 16             | 876 / 1,672                              |                                        |         |
| Yes                               | SIT recumbent           | 19             | 1,102 / 2,473                            | 0.99 (0.19, 5.01)                      | 0.987   |
| No                                | SIT recumbent           | 7              | 587 / 1,181                              |                                        |         |
| Yes                               | CCT                     | 19             | 1,000 / 2,243                            | 8.57 (1.48, 49.71)                     | 0.017   |
| No                                | CCT                     | 6              | 227 / 342                                |                                        |         |
| **Retrospective or unclear timing of data collection?** |                         |                |                                          |                                        |         |
| Yes                               | All                     | 24             | 957 / 2,303                              | 0.74 (0.22, 2.45)                      | 0.621   |
| No                                | All                     | 40             | 2,621 / 5,054                            |                                        |         |
| Yes                               | SIT recumbent           | 10             | 628 / 1,503                              | 1.16 (0.26, 5.13)                      | 0.842   |
| No                                | SIT recumbent           | 16             | 1,061 / 2,151                            |                                        |         |
| Yes                               | CCT                     | 9              | 255 / 588                                | 0.58 (0.11, 3.23)                      | 0.537   |
| No                                | CCT                     | 16             | 972 / 1,997                              |                                        |         |
| **Significant risk of misclassification of disease (i.e., risk of bias assessment for reference standard high or unclear)?** |                         |                |                                          |                                        |         |
| Yes                               | All                     | 59             | 3,164 / 6,632                            | 0.76 (0.08, 7.35)                      | 0.815   |
| No                                | All                     | 5              | 414 / 725                                |                                        |         |
| **Study size less than 200 participants?** |                         |                |                                          |                                        |         |
| Yes                               | All                     | 55             | 2,333 / 4,918                            | 1.41 (0.29, 6.90)                      | 0.674   |
| No                                | All                     | 9              | 1,245 / 2,439                            |                                        |         |
The reference category for all comparisons was "No." Subgroup analysis was performed for each individual test provided that there were at least three studies in each stratum and the hierarchical summary receiver-operating characteristic meta-regression model could achieve successful convergence. Separate subgroup analyses were not performed for the seated SIT or oral SLT because there were only four studies and two studies, respectively, for each.

Abbreviations: ARR, aldosterone-to-renin ratio; CCT, captopril challenge test; CI, confidence interval; FST, fludrocortisone suppression test; PA, primary aldosteronism; PAC, plasma aldosterone concentration; SIT, intravenous saline infusion test.
Figure S1. Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) flow diagram.

Identification of studies via databases and registers

Records identified:
- MEDLINE (n=5,015)
- Embase (n=6,701)
- Cochrane Central Register of Controlled Trials (n=697)

Records removed before screening:
- Duplicate records (n=4,418)

Records screened (n=7,995)

Records excluded (n=7,749)

Reports sought for retrieval (n=246)

Reports assessed for eligibility (n=246)

Reports excluded (n=187):
- Conference abstract (n=68)
- Not a study of diagnostic test accuracy or unable to extract 2×2 table (n=65)
- No reference standard (n=17)
- No outcomes (e.g., protocol or erratum paper) (n=6)
- Not a guideline-recommended test (n=8)
- Subtyping only (n=14)
- Duplicate/data already reported elsewhere (n=8)
- No patients with primary aldosteronism (n=1)

Studies included in review (n=55)

Reports of included studies (n=60)

Identification of studies via other methods

Additional records from:
- Citation searching (n=2)
- Other (n=0)

Reports sought for retrieval (n=2)

Reports not retrieved (n=0)

Reports assessed for eligibility (n=2)

Reports excluded (n=1)
Figure S2. Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) plot.
**Figure S3.** Summary receiver operating characteristics curves for studies that compared two confirmatory tests with a common reference standard (direct comparisons). There is a line joining tests that were compared. Curves were only plotted when there were more than 2 studies available. To avoid extrapolation beyond the data, the curves were drawn within the range of observed specificities. Comparisons were made for the recumbent SIT vs. CCT in 5 studies (panel A); recumbent SIT vs. FST in 1 study (panel B); seated SIT vs. CCT in 1 study (panel C); and recumbent SIT vs. seated SIT in 2 studies (panel D). **Abbreviations:** CCT, captopril challenge test; FST, fludrocortisone suppression test; SIT, intravenous saline infusion test.
Figure S4. Deeks’ funnel plot and asymmetry test for publication bias for the intravenous recumbent saline infusion test, p=0.11 (panel A); seated saline infusion test, p=0.70 (panel B); fludrocortisone suppression test, p=0.38 (panel C); and captopril suppression test, p=0.42 (panel D). The oral salt loading test was not examined for publication bias because there were only two studies.

3A)
