– Supplementary Material –

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

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Supplemental Methods. Additional details regarding methods.

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 I^2 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.[3] 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.[5,6] 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).

Supplemental References

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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
Citation	Citation.
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

	• FST = fludrocortisone suppression test • CCT = captopril challenge test Note: there may be variations for a particular test (e.g., SIT may be performed recumbent or seated).
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 receives 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.

	• 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.
Other comments	Additional notes.

Table S3. Summary of included studies.

Study author, year [ref.]	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 [7]	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 × 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 [8]	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 × 3 days; 24 h urinary aldosterone collected on 3rd day ≥18.9 mcg/d for diagnosis of PA	Paper chromato-graphy and liquid scintilla-tion spectro-metry	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 [9]	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	Unclear if participants with

		NR ARR	alternative diagnosis controls				with high-salt diet >300 mEq/d × 3 days; 24 h urinary aldosterone starting on 2nd day ≥5 mcg/d for diagnosis of PA		unspecified laboratory abnormalities +/- selected surgical pathology +/- BP response to spironolactone; EH based on normal pyelogram, renogram, and catecholamines; renal hypertension based on abnormal renal arteriogram, renal function, or anatomical disease; hypertension due to oral contraceptive pill based on history	hypertension from the oral contraceptive pill at baseline were the same as those who were evaluated after stopping the oral contraceptive pill (i.e., whether the total was 8 or 16 people); the 2×2 table was reconstructed assuming these were the same people
Kem, 1971a [10]	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 [11]	USA	NR age, NR	Multi-gate	Case-control	Prospective	5/32	SIT (recumbent):	Immuno-	Different	Participants

		sex, NR hypokalemia, NR ARR	with healthy and alternative diagnosis controls				discontinuation of all estrogen-containing drugs × 1 month and diuretics × 1 week; recumbent for 2 L of 0.9% NaCl IV beginning at 8 AM over 4 h; PAC >5 ng/dL after infusion for diagnosis of PA	assay	standards used: criteria for PA unclear apart from presence of hypokalemia (with some having resolution after surgery and one with improvement after dexamethasone); EH based on normal urinalysis, pyelogram, renogram, aortogram, vanillylmandelic acid, corticosteroids, aldosterone, and renin levels; normal control subjects had no history of hypertension or renal disease	in Kem 1971a and 1971b appear unique (i.e., different number of participants and different PA subtypes)
Espiner, 1971 [12]	USA	44.1 y, 50 M, NR hypokalemia, NR ARR	Multi-gate with healthy and alternative diagnosis controls	Case-control	Prospective	6/87	SIT (posture not specified): discontinuation of antihypertensives × 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	Chromato-graphy with liquid scintilla-tion spectro-metry	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 [13]	New Zealand	NR age, NR sex, 5 hypokalemia, NR ARR	Two-gate design with alternative	Case-control	Unclear	5/15	FST: discontinuation of antihypertensives × 2 weeks;	Immuno-assay	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 [14]	Denmark	NR age, NR sex, 34 hypokalemia, NR ARR	Multi-gate with healthy and alternative diagnosis controls	Case-control	Prospective	24/50	FST: discontinuation of all medications × 3 weeks; fludrocortisone 0.3 mg PO qid × 3 days with urine collection before and afterwards; reduction of 24 h urinary tetrahydro-aldosterone by less than 24% from baseline for diagnosis of PA	Immuno-assay	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-	—

									aldosterone, and hypokalemia; normal control subjects had normal BP, electrolytes, and 24 h urinary tetrahydro-aldosterone	
Streeten, 1982 [15,16]	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	Immuno-assay	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 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 [17]	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	Immuno-assay	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

									PRA with normal investigations for renal disease	
Bravo, 1983 [18]	USA	NR age, NR sex, NR hypokalemia, NR ARR	Two-gate design with alternative diagnosis controls	Case-control	Retrospective	80/150	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	The investigators described this as a salt loading test, but the actual intervention involved IV saline infusion
Lyons, 1983 [19]	USA	43.5 y, 18 M, 12 hypokalemia, NR ARR	Multi-gate with healthy and alternative diagnosis controls	Case-control	Prospective	12/31	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 [20]	USA	47.2 y, NR sex, NR hypokalemia, NR ARR	Two-gate design with alternative diagnosis controls	Case-control	Prospective	26/120	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-	2×2 table was reconstructed based on the assumption that those who did not receive FST did not have PA

									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	
Naomi, 1985 [21]	Japan	NR age, NR sex, NR hypokalemia, NR ARR	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	Immuno-assay	Different standards used: PA based 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 [22,23]	Japan	41.4 y, NR sex, NR hypokalemia, NR ARR	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	Immuno-assay	Complete verification: SLT as verification standard for PA vs. EH, but protocol and	—

							recumbent for captopril 25 mg PO × 1 at 10 AM; PAC collected 2 h after captopril ≥8.9 ng/dL for diagnosis of PA		diagnostic cut-offs not stated	
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 × 1 week; captopril 100 mg PO × 1 at 9 AM; PAC collected 2 h after captopril >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 with healthy and alternative diagnosis controls	Case-control	Retrospective	8/26	SIT (recumbent): continuation of usual antihypertensive drugs; recumbent × 30 min, then 1.5 L of 0.9% NaCl IV beginning at 9 AM over 2.5 h; PAC ≥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 × 2 weeks; unrestricted salt diet for 1 week, then recumbent for captopril 50 mg PO × 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

							collected 90 min after captopril >15 ng/dL for diagnosis of PA		and surgical confirmation; diagnostic criteria not given for EH	cases of bilateral PA were included in the study; it was assumed that subjects were unique from those reported in Naomi 1985, but it was not possible to confirm, though the reference standards were different and the subtypes of hypertension were also different between studies
Hambling, 1992 [27]	UK	NR age, NR sex, NR hypokalemia, NR ARR	Two-gate design with alternative diagnosis controls	Case-control	Prospective	10/22	CCT: discontinuation of all medications × 3 weeks; unrestricted salt diet for 1 week, then recumbent for captopril 50 mg PO × 1 at 9 AM; PAC collected 2 h after captopril >444 pmol/L for diagnosis of PA	Immuno-assay	Different standards used: 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 [28]	Japan	47.1 y, 85 M, NR hypokalemia, NR ARR	Two-gate design with alternative diagnosis controls	Case-control	Unclear	16/190	CCT: discontinuation of all medications × 2 weeks; unrestricted salt diet, then captopril 50 mg PO × 1 at 9:30 AM; interpretation based on PAC and	Immuno-assay	Different standards used: 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 [29]	Canada	52 y, NR sex, 49 hypokalemia, NR ARR	Single-gate	Consecutive patients	Prospective	44/49	CCT: discontinuation of spironolactone × 6 weeks, BB and clonidine × 1 week; use of alpha blockers and CCBs if needed; seated for captopril 25 mg PO × 1; PAC collected 2 h after captopril >240 pmol/L (8.65 ng/dL) for diagnosis of PA	Immuno-assay	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)
Castro, 2002 [30]	USA	52.1 y, 7 M, 6 hypokalemia, ARR less than 30 ng/dL per ng/mL/h	Single-gate	Unclear	Retrospective	6/7	CCT: discontinuation of spironolactone × 3 months, and all other potentially confounding medications (except clonidine) × 1 week; use of alpha blockers if needed; captopril 25 mg PO × 1; ARR collected 2 h after captopril ≥26 ng/dL per ng/mL/h or PAC >12 ng/dL for diagnosis of PA	Immuno-assay	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

Rossi, 2002 [31]	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	Immuno-assay	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 [32]	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	Immuno-assay	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 fludro-cortisone administratio n with a mandatory hospitaliza-tion

Giachetti, 2006 [33]	Italy	NR age, NR sex, NR hypokalemia, NR ARR	Single-gate	Consecutive	Retrospective	48/82	CCT: 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	Immuno-assay	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	2×2 table reconstructed using estimates of sens. and spec. from digitized version of figure 3
	Italy	NR age, NR sex, NR hypokalemia,	Single-gate	Consecutive	Retrospective	61/118	SIT (recumbent): preparation as above; recumbent	Immuno-assay	As above	2×2 table reconstructed using back-

		NR ARR					× 2 h, then 2 L of 0.9% NaCl IV beginning at 8 AM over 4 h; PAC ≥7.0 ng/dL after infusion for diagnosis of PA			calculation from table 3
Mulatero, 2006 [34]	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	Immuno-assay	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 [35]	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	Immuno-assay	Different standards used: PA based on repeatedly elevated ARR (>21 pg/mL per mIU/L), 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

									subjects had no hypertension or kidney disease, and did not use contraceptives	
Mulatero, 2007 [36]	Italy	NR age, NR sex, 2 hypokalemia, NR ARR	Single-gate	Unclear	Retrospective	6/11	CCT: discontinuation of diuretics × 6 weeks, spironolactone × 8 weeks, and all other antihypertensives × 3 weeks; use of alpha blockers and CCBs if needed; seated for captopril 50 mg PO × 1 between 8-10 AM; ARR collected 2 h after captopril >30 ng/dL per ng/mL/h or PAC ≥8.5 ng/dL for diagnosis of PA	Immuno-assay	Complete verification: concordant FST and SIT as verification standard for PA vs. EH	Participants were drawn from the same population as those in Mulatero 2006, but evaluating a different index test
Rossi, 2007a [37-39]	Italy	47 y, NR sex, NR hypokalemia, NR ARR	Two-gate design with alternative diagnosis controls	Consecutive	Prospective	46/243	CCT: discontinuation of mineralocorticoid receptor antagonists × 6 weeks and other antihypertensives × 2 weeks; use of doxazosin and CCBs if needed; seated for captopril 50 mg PO × 1; PAC collected 1 h after captopril >13.9 ng/dL for diagnosis of PA	Immuno-assay	Partial verification: clinical diagnosis as verification standard ("4 corners approach") for PA vs. EH; APA based on a combination of all the following: (1) positive screening test (i.e., ARR ≥40 ng/dL per ng/mL/h), or post-captopril ARR ≥30 ng/dL per ng/mL/h, or logistic discrimination function [a risk score that predicts probability of PA based on	Participants from the PAPY cohort [39] with main results for the CCT reported in 2007a article [37]; 2×2 table reconstructed for APA (but not possible for all PA); although the investigators described enrollment as consecutive, patients with idiopathic hyper-aldosteronism were excluded from the final analysis; this

									baseline PRA, post-captopril aldosterone, and baseline K^+] ≥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	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
Rossi, 2007b [39,40]	Italy	47.2 y, NR sex, NR hypokalemia, ARR ≥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 × 6 weeks and other antihypertensives × 2 weeks; use of doxazosin and CCBs if needed; recumbent for 2 L of 0.9% NaCl IV	Immuno-assay	Partial verification: clinical diagnosis as verification standard ("4 corners approach") for PA vs. EH; APA based on same criteria as Rossi 2007a study [37], as above;	Participants from the PAPY cohort [39] with the most complete reporting of the SIT in the 2007b article [40]

							beginning between 8-9:30 AM over 4 h; PAC ≥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 [41]	Taiwan	47.9, 69 M, NR hypokalemia, ARR >30 ng/dL per ng/mL/h	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 ≥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

									adenoma on CT, and post-SIT PAC >10 ng/dL or pathology-proven APA with surgical cure of hypertension)	
Wu, 2010 [42]	Taiwan	48.7, 54 M, NR hypokalemia, ARR >30 ng/dL per ng/mL/h	Single-gate	Consecutive	Prospective	51/114	CCT: discontinuation of antihypertensives × 3 weeks; use of diltiazem and doxazosin if needed; seated for captopril 50 mg PO × 1; ARR collected 1.5 h after captopril >35.5 pmol per ng for diagnosis of PA	Immuno-assay	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	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 [43]	Poland	53 y, 79 M, 4 hypokalemia, NR ARR	Single-gate	Consecutive	Retrospective	13/198	SIT (recumbent): discontinuation of diuretics and spironolactone × 4 weeks, and other antihypertensives × 2 weeks; recumbent for 2 L of 0.9% NaCl IV over 4 h; PAC >6.5 ng/dL after infusion for diagnosis of PA	Immuno-assay	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	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

									response in those with a positive confirmatory test, but, no verification in those with negative tests	using data from the abstract because these numbers were the most clearly reported
Willenberg, 2012 [44]	Germany	NR age, NR sex, NR hypokalemia, NR ARR	Single-gate	Consecutive	Unclear	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 × 4 days; PAC at 10 AM on 5th day >53.5 ng/L (5.35 ng/dL) for diagnosis of PA	Immuno-assay	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; 2×2 table was reconstructed using table 3
	Germany	NR age, NR sex, NR hypokalemia, NR ARR	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

							(3.15 ng/dL) after infusion for diagnosis of PA			
Ceral, 2014 [45]	Czech Republic	49.0 y, 30 M, NR hypokalemia, NR ARR	Single-gate	Consecutive	Prospective	33/49	SLT: high-salt diet (6 g/d) × 3 days with 24 h urinary Na^+ ≥200 mmol/d to verify salt intake; 24 h urinary aldosterone after salt loading ≥36 nmol/d for diagnosis of PA	Immuno-assay	Complete verification: SIT (recumbent) as verification standard for PA vs. non-PA; PA based on post-infusion PAC >100 pmol/L	—
Nakama, 2014 [46]	Japan	NR age, NR sex, NR hypokalemia, NR ARR	Single-gate	Consecutive	Retrospective	42/58	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	Immuno-assay	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	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
	Japan	NR age, NR sex, NR hypokalemia, NR ARR	Single-gate	Consecutive	Retrospective	40/57	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	Immuno-assay	As above	The SIT 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
Kuo, 2015 [47]	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)

									based on biochemical evidence of PA without lateralization; EH based on ARR <35 ng/dL per ng/mL/h and negative confirmatory test—patients with negative confirmatory testing did not receive remainder of verification process, including treatment	
Cornu, 2016 [48]	France	48 y, 125 M, NR hypokalemia, ARR >64 pmol/L per mIU/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
Li, 2016 [50]	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 mineralocorti-coid receptor antagonist; EH based on exclusion of secondary hypertension (but details not provided);	SIT (recumbent) was included both as the index test and part of the reference standard to verify PA; the reference standard was not equally applied to those who did not have PA

									normal control subjects had no hypertension	
Tsiavos, 2016 [51]	Greece	53.6 y, NR sex, 19 hypokalemia, NR ARR	Single-gate	Consecutive	Prospective	45/148	FST: discontinuation of all drugs affecting the renin-aldosterone axis × 3 weeks; use of CCBs if needed; received NaCl 4 g PO tid × 4 days, fludrocortisone 0.1 mg PO q6h × 4 days, and dexamethasone 2 mg × 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	Immuno-assay	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 [52]	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	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 >10 ng/dL after infusion for diagnosis of PA	Immuno-assay	Different standards used: PA based on either a positive FST (fludrocortisone 0.1 mg PO q6h × 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

	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	CCT: 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	Immuno-assay	As above	As above
Meng, 2018 [53]	China	47.0 y, 63 M, 86 hypokalemia, ARR >30 ng/dL per ng/mL/h	Single-gate	Consecutive	Prospective	115/ 164	CCT: 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)	Immuno-assay	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	SIT (posture not specified): discontinuation of spironolactone × 6	Immuno-assay	As above	Details about SIT protocol not given; details about

		ng/dL per ng/mL/h					weeks, other diuretics × 4 weeks, and other confounding 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)			biochemical testing for verification standard not given (i.e., unclear if confirmatory test used for diagnosis beyond screening ARR)
Stowasser, 2018 [54,55]	Australia	55.3 y, 62 M, NR 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	Single-gate	Consecutive	Prospective	77/108	SIT (seated): discontinuation of diuretics × 4 weeks, and ACEI, ARB, BB, and dihydropyridine CCB × 2 weeks; use of verapamil, hydralazine, prazosin, and moxonidine if needed; seated for 2 L of 0.9% NaCl IV over 4 h; PAC ≥162 pmol/L with DRC <8.4 mIU/L and plasma cortisol lower (compared to baseline) after infusion for diagnosis of PA	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 adrenalec-tomy to confirm cure); it was probable that the patients included in the Ahmed 2014 article [55] were also included here because of overlapping study period

										and the description of an "expanded patient cohort"; the Thuzar 2020 article [56] reports the same people, but using immuno-assay—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
	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

		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					weeks, and ACEI, ARB, BB, and dihydropyridine CCB × 2 weeks; use of verapamil, hydralazine, prazosin, and moxonidine if needed; recumbent for 2 L of 0.9% NaCl IV over 4 h; PAC ≥106 pmol/L with DRC <8.4 mIU/L and plasma cortisol lower (compared to baseline) after infusion for diagnosis of PA			the pooled meta-analysis of SIT in the present study, only the seated SIT was included from Stowasser 2018 [54]
Velema, 2018 [57]	Nether-lands	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	Immuno-assay	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	—

									were manually reviewed with the possibility of reclassification based on above criteria	
Kidoguchi, 2019 [58]	Japan	50.3 y, 49 M, NR hypokalemia, ARR >200 pg/mL per ng/mL/h	Single-gate	Unclear	Unclear	71/71	CCT: 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	NR	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 (2L 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 [1]
Okamoto, 2018 [59]	Japan	56 y, 48 M, NR hypokalemia, ARR >20 ng/dL per ng/mL/h	Single-gate	Consecutive	Prospective	75/102	CCT: 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	NR	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

										subtyping study; 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)
	Japan	56 y, 48 M, NR hypokalemia, ARR >20 ng/dL per ng/mL/h	Single-gate	Consecutive	Prospective	75/102	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 SIT in the actual study (but details not provided)
Zhu, 2019 [60]	China	48.2 y, 166 M, 97 hypokalemia, ARR ≥25 ng/dL per ng/mL/h	Multi-gate with healthy and alternative diagnosis controls	Case-control	Prospective	110/ 313	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	Immuno-assay	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:	CCT was included both as the index test and part of the reference standard

							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 [61]	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	Immuno-assay	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

									partial clinical success defined as same BP after surgery but needing less meds, or a reduction in BP with either same amount or less meds; absent clinical success defined by no change (or increase) in BP after surgery on same amount (or more) meds; those with complete/partial clinical success were defined to have verified unilateral PA	success = disease absent) with only APA included; 2×2 table reconstructed using table 4 and figure 3
Vivien, 2019 [62]	France	NR age, NR sex, NR hypokalemia, NR ARR	Single-gate	Consecutive	Prospective	44/120	SIT (recumbent): discontinuation of ACEI, ARB, central alpha agonists, direct renin inhibitors, and potassium-wasting diuretics, estrogen, and progesterone × 4 weeks, and potassium-sparing diuretics × 6 weeks; recumbent for 2 L of 0.9% NaCl IV over 4 h; PAC >160 pmol/L after infusion for diagnosis of PA	Immuno-assay	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 × 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 [63]	Germany	52.3 y, 37 M, 23 hypokalemia, NR ARR	Single-gate	Consecutive	Prospective	32/99	SIT (recumbent): discontinuation of mineralocorticoid receptor antagonists and potassium-sparing diuretics × 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 mineralocorti-coid 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	single-gate design, risk of bias for patient selection was rated high because people who were indeterminate were excluded (i.e., "Failure to establish an unequivocal diagnosis with the diagnostic criteria listed subsequently led to exclusion") and only patients with advanced features of PA were verified to have disease
Lin, 2020 [64]	China	48.3 y, 129 M, NR hypokalemia, ARR ≥3.7 ng/dL per mIU/L	Single-gate	Consecutive	Prospective	161/ 280	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	2×2 table reconstructed using figure 1 and pre-determined PAC cut-off ≥10 ng/dL for

							dihydropyridine CCBs if needed; recumbent for 2 L of 0.9% NaCl IV over 4 h; PAC ≥10 ng/dL after infusion for diagnosis of PA		(fludrocortisone 0.6 mg PO q6h × 4 days; 10 AM post-FST PAC ≥8 ng/dL and "suppressed" renin and no rise in cortisol between 7 AM to 10 AM on the last day)	diagnosis of PA (rather than using table 3 that uses a different PAC cut-off)
Zhang, 2020 [65]	China	48.5 y, 46 M, 49 hypokalemia, ARR ≥30 ng/dL per ng/mL/h, or ARR ≥20 ng/dL per ng/mL/h plus PRA <1 ng/mL/h plus aldosterone >15 ng/dL	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	Immuno-assay	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	SIT 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

									bilateral (idiopathic) PA based on biochemical evidence of PA without lateralization; EH based on absence of criteria for PA—patients with negative confirmatory testing did not receive remainder of verification process, including treatment	numbers were the most clearly reported (and the reported sens. and spec. were ignored)
	China	48.5 y, 46 M, 49 hypokalemia, ARR ≥30 ng/dL per ng/mL/h, or ARR ≥20 ng/dL per ng/mL/h plus PRA <1 ng/mL/h plus aldosterone >15 ng/dL	Single-gate	Consecutive	Prospective	93/113	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	Immuno-assay	As above	As above; the numbers cannot be replicated so 2×2 table was reconstructed using data from the text because these raw numbers were the most clearly reported
Liu, 2021 [66]	China	48.8 y, 88 M, NR hypokalemia, ARR ≥1.0 ng/dL per mIU/L	Single-gate	Consecutive	Prospective	196/269	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	Immuno-assay	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

							for diagnosis of PA		subsequent adrenalectomy with complete biochemical success; EH based on absence of the criteria required for PA	
	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	Immuno-assay	As above	As above
Fuss, 2021 [67]	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	high	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	high	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	low	high	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	low	high	high	high	low	high
Naomi, 1985 [21]	high	unclear	high	low	high	high	high
Muratani, 1986 [22,23]	high	high	high	low	high	low	high
Wu, 1986 [24]	high	high	high	high	high	high	high
Hamlet, 1987 [25]	high	high	high	high	high	low	unclear
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	high	unclear	high	high	high
Agharazii, 2001 [29]	high	unclear	high	low	high	low	high
Castro, 2002 [30]	unclear	low	high	high	high	high	unclear
Rossi, 2002 [31]	high	high	high	low	low	high	low
Juutilainen, 2005 [32]	low	high	unclear	unclear	low	high	unclear
Giachetti, 2006 [33]	low	high	high	low	low	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	high	low	high	high
Wu, 2010 [42]	low	high	high	low	low	high	low

Myśliwiec, 2012 [43]	low	high	high	high	low	high	high
Willenberg, 2012 [44]	low	high	high	high	low	unclear	high
Ceral, 2014 [45]	low	low	high	low	low	low	high
Nakama, 2014 [46]	low	low	high	high	low	high	high
Kuo, 2015 [47]	low	low	high	low	low	low	high
Cornu, 2016 [48]	low	low	high	low	low	low	low
Kim, 2016 [49]	low	high	high	low	low	high	low
Li, 2016 [50]	high	high	high	high	high	high	low
Tsiavos, 2016 [51]	low	high	high	high	unclear	high	high
Song, 2018 [52]	high	high	low	low	low	low for SIT; high for CCT	low
Meng, 2018 [53]	low	high	high	high	low	unclear	high
Stowasser, 2018 [54,55]	low	high	high	low	low	unclear	low
Velema, 2018 [57]	low	low	high	high	low	low	unclear
Kidoguchi, 2019 [58]	high	low	high	low	unclear	low	low
Okamoto, 2018 [59]	low	high	high	high	low	high	high
Zhu, 2019 [60]	high	high	high	high	high	high	high
Wu, 2019 [61]	low	high	low	low	low	high	low
Vivien, 2019 [62]	low	high	high	high	low	high	low
Fries, 2020 [63]	high	low	low	low	low	low	low
Lin, 2020 [64]	low	low	high	low	low	low	high
Zhang, 2020 [65]	low	high	high	high	low	high	high
Liu, 2021 [66]	low	high	high	low	low	high	low
Fuss, 2021 [67]	high	high	unclear	high	low	high	low

Table S5. Summary of reference standards used to verify disease status for primary aldosteronism.

Study author, year [ref.]	Criteria used for verification (presence vs. absence of disease)										Application of reference standard			
	Clinical factors (e.g., history of hypertension, hypokalemia)	Screening test results (e.g., elevated aldosterone, suppressed renin)	Confirmatory test results (e.g., saline infusion test, salt loading test, captopril challenge test, fludrocortisone suppression test)	Adrenal nodule (e.g., seen on cross-sectional imaging or surgery)	Anatomical pathology	Adrenal vein sampling or NP59 (e.g., lateralization)	Treatment response (e.g., improvement in BP following spironolactone; normalization of biochemistry and/or BP after adrenalectomy)	Exclusion of renovascular disease (e.g., tests of renal function, pyelography, renal arteriography)	Exclusion of other forms of endocrine hypertension (e.g., catecholamines)	Not stated (e.g., criteria for PA or healthy subjects not given)	Complete	Partial	Different reference standards	Unclear
Intravenous saline infusion test, recumbent (n=26)														
Kem, 1971a [10]	✓	✓						✓		✓			✓	
Kem, 1971b [11]	✓	✓					✓	✓	✓				✓	
Espiner, 1971 [12]	✓							✓	✓	✓			✓	
Streeten, 1982 [15,16]	✓		✓	✓						✓		✓		
Bravo, 1983 [18]										✓				✓
Holland, 1984 [20]			✓									✓		
Hamlet, 1987 [25]				✓						✓			✓	
Mulatero, 2006 [34]			✓								✓			
Schirpenbach, 2006 [35]	✓	✓	✓										✓	
Giachetti, 2006 [33]		✓	✓	✓									✓	
Rossi, 2007b [39,40]	✓	✓		✓		✓	✓			✓		✓		
Myśliwiec, 2012 [43]							✓		✓			✓		
Willenberg, 2012 [44]		✓	✓			✓		✓	✓		✓			
Nakama, 2014 [46]			✓									✓		
Cornu, 2016 [48]						✓					✓			
Li, 2016 [50]		✓	✓	✓	✓	✓	✓			✓			✓	
Song, 2018 [52]			✓			✓	✓						✓	
Meng, 2018 [53]		✓		✓		✓	✓	✓	✓	✓			✓	
Stowasser, 2018 [54,55]			✓			✓							✓	
Velema, 2018	✓		✓									✓		

[57]														
Okamoto, 2018 [59]			✓										✓	
Vivien, 2019 [62]		✓	✓										✓	
Fries, 2020 [63]	✓	✓	✓				✓							✓
Lin, 2020 [64]			✓								✓			
Zhang, 2020 [65]	✓	✓	✓	✓	✓	✓	✓						✓	
Fuss, 2021 [67]	✓		✓	✓	✓		✓							✓
Total	10	11	17	8	3	8	9	5	5	8	4	6	13	3
Intravenous saline infusion test, seated (n=4)														
Stowasser, 2018 [54,55]			✓			✓							✓	
Wu, 2019 [61]						✓	✓					✓		
Zhang, 2020 [65]	✓	✓	✓	✓	✓	✓	✓						✓	
Liu, 2021 [66]			✓				✓						✓	
Total	1	1	3	1	1	3	3	0	0	0	0	1	3	0
Oral salt loading test (n=2)														
Collins, 1970 [9]	✓	✓			✓		✓	✓	✓				✓	
Ceral, 2014 [45]			✓								✓			
Total	1	1	1	0	1	0	1	1	1	0	1	0	1	0
Fludrocortisone suppression test (n=7)														
Horton, 1969 [7]	✓						✓			✓			✓	
Biglieri, 1970 [8]	✓	✓			✓			✓					✓	
Dunn, 1976 [13]	✓	✓	✓				✓						✓	
Lund, 1980 [14]	✓	✓			✓		✓		✓				✓	
Juutilainen, 2005 [32]	✓	✓		✓			✓				✓			
Willenberg, 2012 [44]		✓	✓			✓		✓	✓		✓			
Tsiavos, 2016 [51]	✓		✓				✓						✓	
Total	6	5	3	1	2	1	5	2	2	1	2	0	5	0
Captopril challenge test (n=25)														
Thibonnier, 1982 [17]	✓	✓					✓	✓					✓	
Lyons, 1983 [19]			✓							✓		✓		
Naomi, 1985 [21]	✓	✓	✓			✓		✓					✓	
Muratani, 1986 [22,23]			✓								✓			
Wu, 1986 [24]	✓	✓	✓	✓	✓					✓			✓	
Naomi, 1987 [26]	✓	✓				✓	✓			✓			✓	
Hambling, 1992 [27]			✓							✓			✓	
Iwaoka, 1993 [28]	✓	✓					✓	✓		✓			✓	

Agharazii, 2001 [29]			✓								✓			
Castro, 2002 [30]			✓	✓		✓	✓						✓	
Rossi, 2002 [31]			✓								✓			
Giachetti, 2006 [33]		✓	✓	✓									✓	
Mulatero, 2007 [36]			✓								✓			
Rossi, 2007a [37-39]	✓	✓		✓		✓	✓			✓		✓		
Wu, 2009 [41]		✓	✓	✓	✓	✓	✓				✓			
Wu, 2010 [42]		✓	✓								✓			
Nakama, 2014 [46]			✓									✓		
Kuo, 2015 [47]	✓	✓	✓	✓		✓	✓						✓	
Kim, 2016 [49]			✓								✓			
Song, 2018 [52]			✓			✓	✓						✓	
Meng, 2018 [53]		✓		✓		✓	✓	✓	✓	✓			✓	
Kidoguchi, 2019 [58]			✓								✓			
Okamoto, 2018 [59]			✓										✓	
Zhu, 2019 [60]	✓	✓	✓					✓	✓				✓	
Liu, 2021 [66]			✓				✓						✓	
Total	8	12	20	7	2	8	10	5	2	7	8	3	14	0

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.

Table S6. Summary of interpretation criteria used for the confirmatory tests.

Test	Laboratory measure	Thresholds used for diagnosis [ref.]
Intravenous saline infusion test (recumbent)	Post-infusion PAC measured by immunoassay	3.15 ng/dL (87 pmol/L) [44] 5.0 ng/dL (139 pmol/L) [10,11,34] 5.8 ng/dL (160 pmol/L) [62] 6.0 ng/dL (166 pmol/L) [46] 6.5 ng/dL (180 pmol/L) [43] 6.8 ng/dL (189 pmol/L) [40] 7.0 ng/dL (194 pmol/L) [33] 8.5 ng/dL (236 pmol/L) [16] 8.65 ng/dL (240 pmol/L) [35] 9.0 ng/dL (250 pmol/L) [25] 10.0 ng/dL (280 pmol/L) [20,48,52,57,64] 11.2 ng/dL (311 pmol/L) [53] 11.45 ng/dL (318 pmol/L) [50] 12.04 ng/dL (334 pmol/L) [65] 15.2 ng/dL (422 pmol/L) [59]
	Post-infusion PAC measured by HPLC-MS/MS	3.8 ng/dL (106 pmol/L) [54] 5.1 ng/dL (140 pmol/L) [63] 14.0 ng/dL (388 pmol/L) [67]
	Post-infusion 24 hour urinary aldosterone	14 mcg/d [18] 300 mg/d [12]
Intravenous saline infusion test (seated)	Post-infusion PAC measured by immunoassay	12.0 ng/dL (333 pmol/L) [66] 12.94 ng/dL (359 pmol/L) [65] 25.0 ng/dL (694 pmol/L) [68]
	Post-infusion PAC measured by HPLC-MS/MS	5.8 ng/dL (162 pmol/L) [54]
Oral salt loading test	24 hour urinary aldosterone	5 mcg/d (13.9 nmol/d) starting on day 2 [9] 13 mcg/d (36.0 nmol/d) after 3 days [45]
Fludrocortisone suppression test	Post-fludrocortisone challenge PAC	3.0-3.1 ng/dL (83-86 pmol/L) [51] 5.35 ng/dL (148 pmol/L) [44] 7.5 ng/dL (208 pmol/L) [13] 12.6 ng/dL (350 pmol/L) [7]
	Post-fludrocortisone challenge 24 hour urinary aldosterone	Reduction of 24 hour urinary tetrahydroaldosterone by less than 24% compared to baseline [14] 13.2 mcg/d (36.6 nmol/d) [32] 18.9 mcg/d (52.4 nmol/d) [8]
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 [41,47] PAC 13.9 ng/dL (386 pmol/L) [37]
	60- to 90-min post-captopril (50 mg) PAC +/- ARR	PAC 13 ng/dL [49] ARR 20 ng/dL per ng/mL/h [46]
	90-min post-captopril (50 mg) PAC +/- PRA +/- ARR	Reduction of PAC by less than 30% compared to baseline [58] PAC 15 ng/dL (416 pmol/L) [21,26] ARR 35 ng/dL per ng/mL/h [31] ARR 35.5 pmol per ng [42] ARR 42.2 ng.dL per ng/mL/h [59] Formula (Q) with final value >0 for diagnosis: [28] $Q = -6.06 \times (PRA)^2 - 6.99 \times (PAC)^2 - 7.11 \times (PRA) \times (PAC) - 7.06 \times (PRA) + 39.89 \times (PAC) - 39.82$
	2-hour post-captopril (25 mg) PAC +/- ARR	PAC 8.65 ng/dL (240 pmol/L) [29] PAC 8.9 ng/dL (247 pmol/L) [22,23]

		PAC 12.0 ng/dL (333 pmol/L) or ARR 26 ng/dL per ng/mL/h [30] PAC 15.0 ng/dL (416 pmol/L) [19]
	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		

Yes	SIT recumbent	22	1,127 / 2,545	1.87 (0.28, 12.34)	0.517
No	SIT recumbent	4	562 / 1,109		
Yes	CCT	21	740 / 1,524	0.97 (0.14, 6.70)	0.973
No	CCT	4	487 / 1,061		
Frequency of primary aldosteronism among study participants less than 50%?					
Yes	All	31	840 / 3,115	5.38 (1.78, 16.22)	<0.001
No	All	33	2,738 / 4,242		
Yes	SIT recumbent	12	361 / 1,428	4.12 (0.96, 17.61)	0.056
No	SIT recumbent	14	1,328 / 2,226		
Yes	CCT	12	336 / 1,277	4.32 (0.95, 19.55)	0.058
No	CCT	13	891 / 1,308		
Frequency of unilateral disease among those with primary aldosteronism less than 50%?					
Yes	All	21	1,405 / 2,880	0.79 (0.21, 2.96)	0.726
No	All	39	1,892 / 4,005		
Yes	SIT recumbent	11	664 / 1,699	1.20 (0.24, 6.02)	0.823
No	SIT recumbent	13	824 / 1,618		
Yes	CCT	6	418 / 606	0.28 (0.04, 1.87)	0.190
No	CCT	18	767 / 1,921		
Frequency of hypokalemia among study participants less than 30%?					
Yes	All	15	582 / 1,426	2.55 (0.26, 25.06)	0.423
No	All	8	612 / 983		
Yes	CCT	6	198 / 490	1.97 (0.03, 126.50)	0.750
No	CCT	4	300 / 456		
Proportion of males among study participants less than 50%?					
Yes	All	22	1,764 / 3,147	1.06 (0.23, 4.92)	0.945
No	All	14	782 / 1,569		
Yes	SIT recumbent	7	621 / 1,189	5.70 (0.87, 37.28)	0.070
No	SIT recumbent	6	389 / 821		
Yes	CCT	10	704 / 1,306	0.33 (0.04, 2.43)	0.274
No	CCT	6	283 / 591		
Mean age less than 50 years old?					
Yes	All	28	2,121 / 4,347	0.63 (0.14, 2.80)	0.547
No	All	15	805 / 1,478		
Yes	SIT recumbent	10	856 / 1,753	0.67 (0.09, 4.74)	0.687
No	SIT recumbent	6	367 / 792		
Yes	CCT	14	836 / 2,020	2.11 (0.28, 15.97)	0.468
No	CCT	6	278 / 353		

The reference category for all comparisons was "No." [a] 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. [b] There were two studies that stated they enrolled consecutive patients even though they were based on a two-gate design [37,40]. **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.

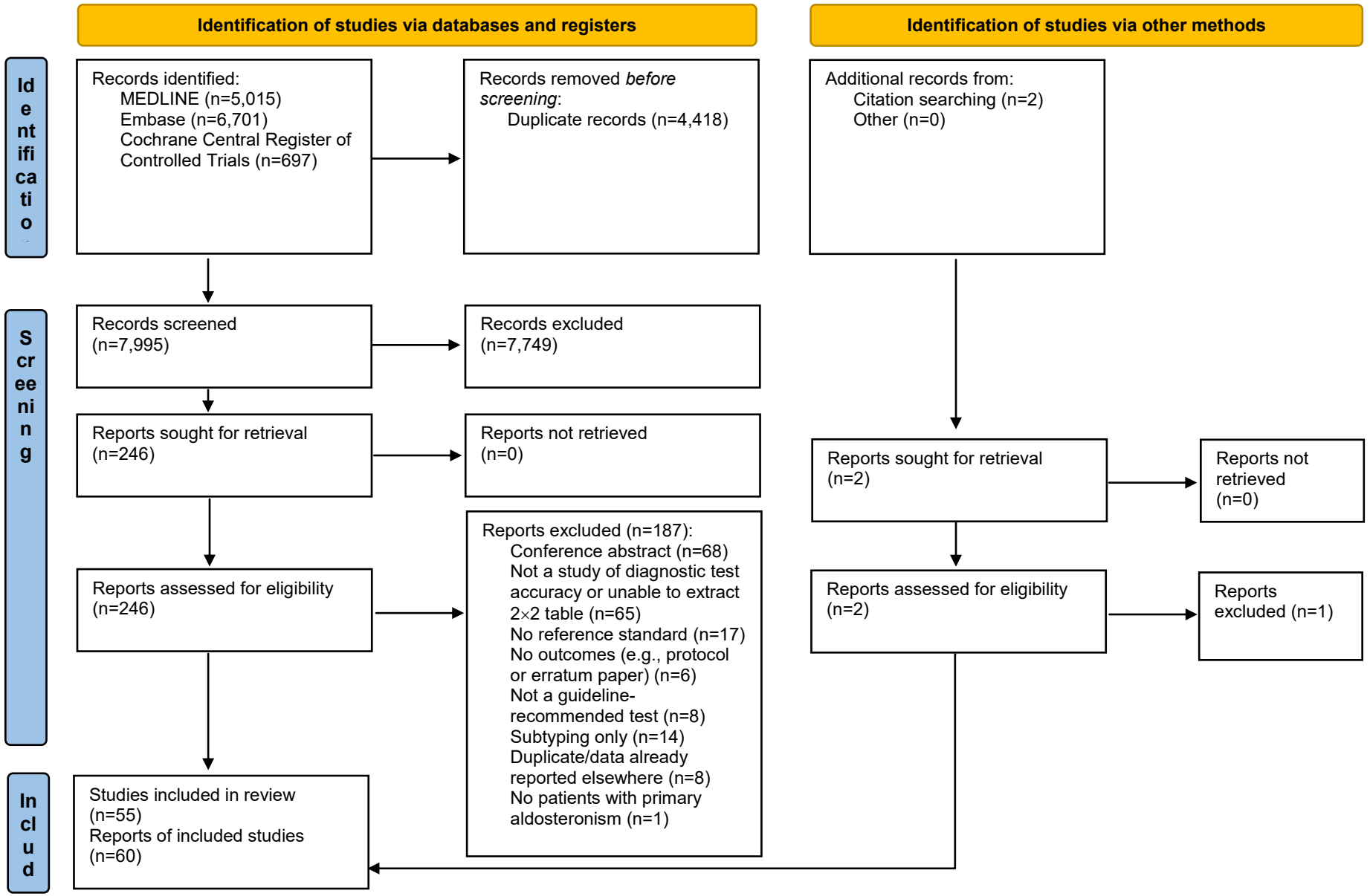


Figure S2. Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) plot.

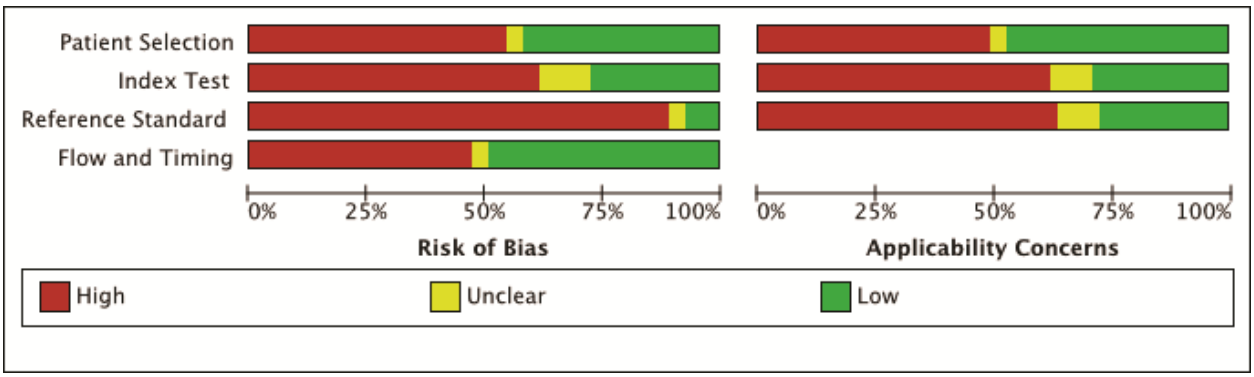
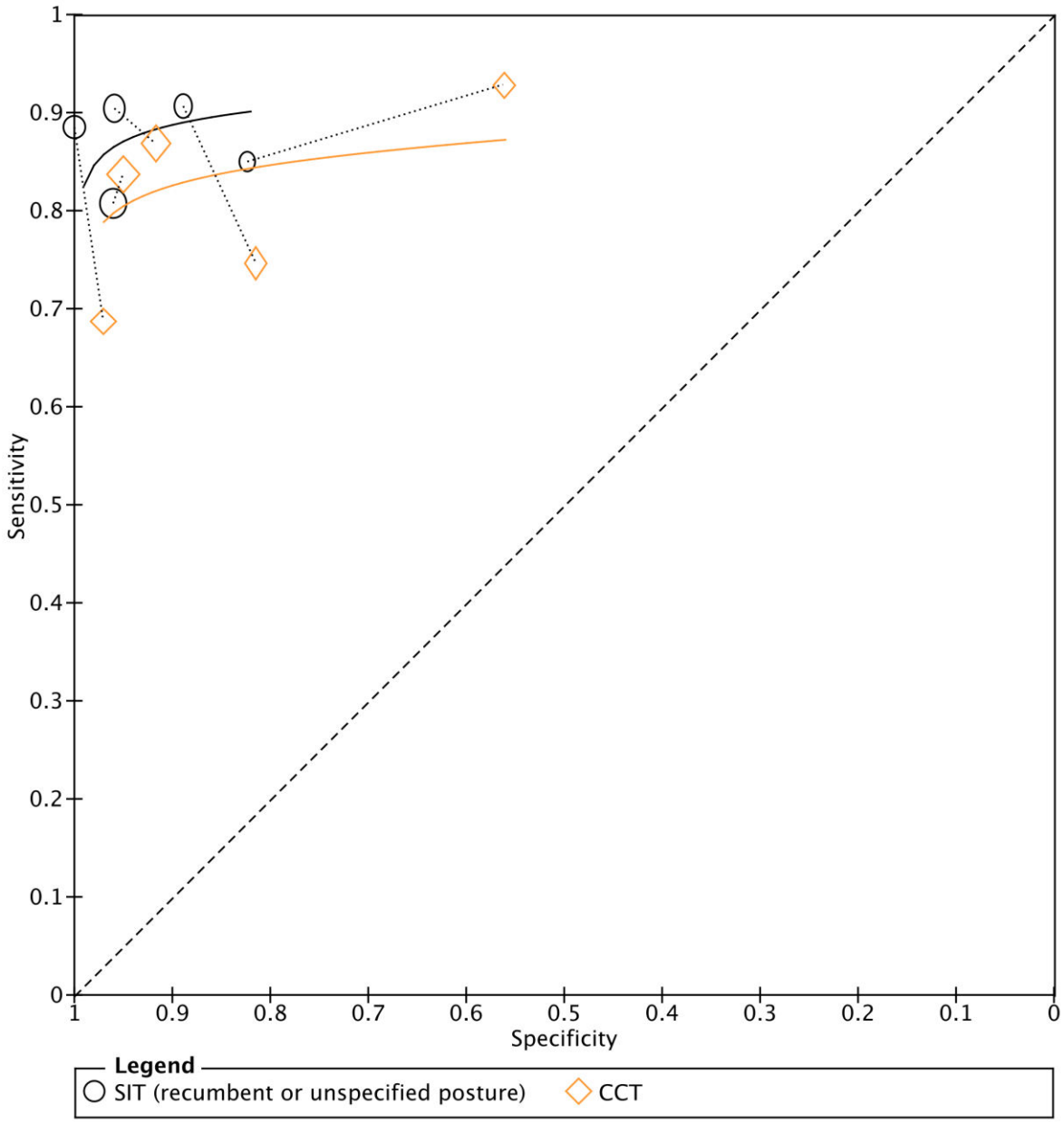
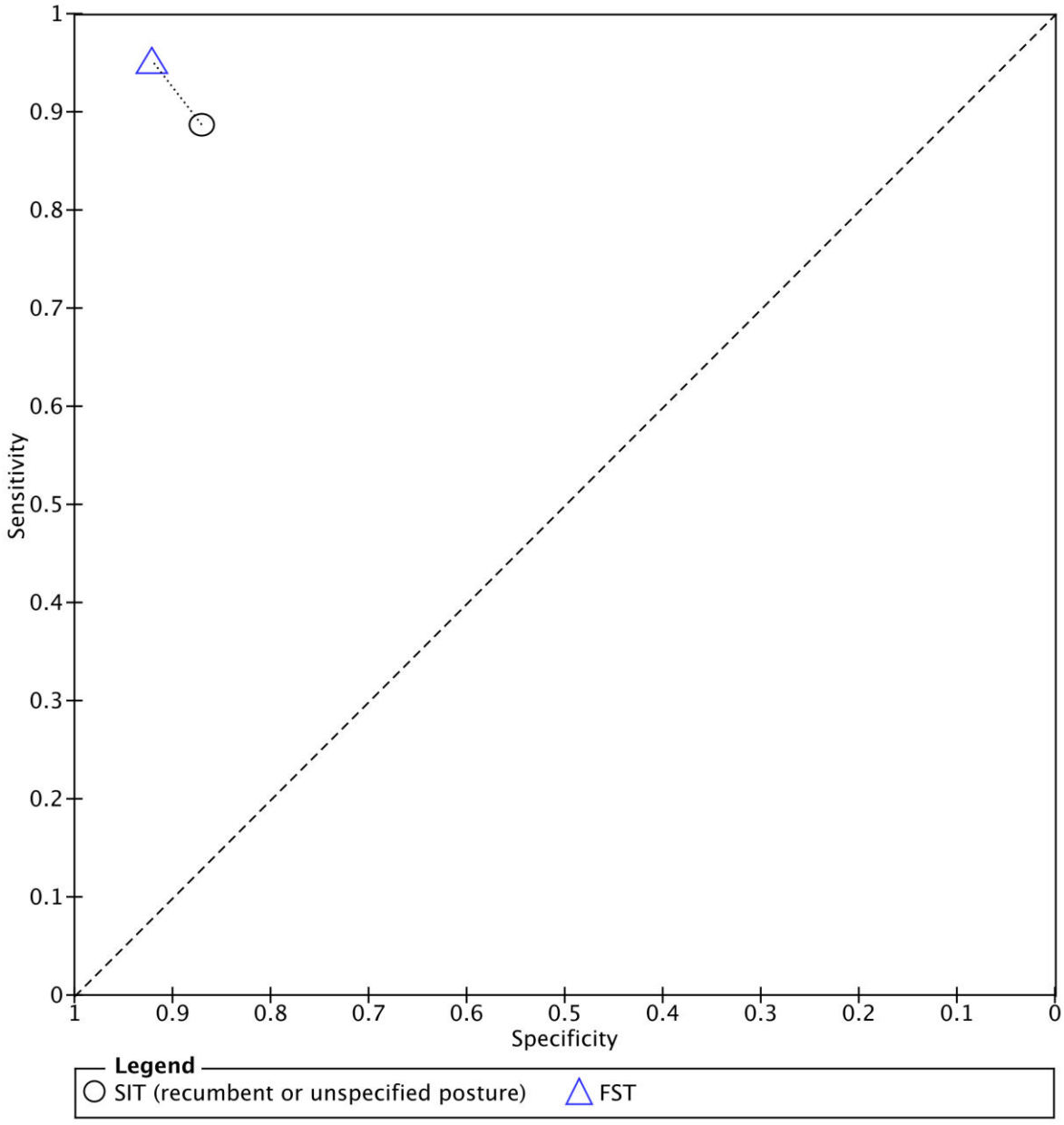


Figure S3. Summary receiver operating characteristics curves for studies that compared two confirmatory tests with a common reference standard (direct comparisons). There is a line joining tests that were compared. Curves were only plotted when there were more than 2 studies available. To avoid extrapolation 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.

2A)



2B)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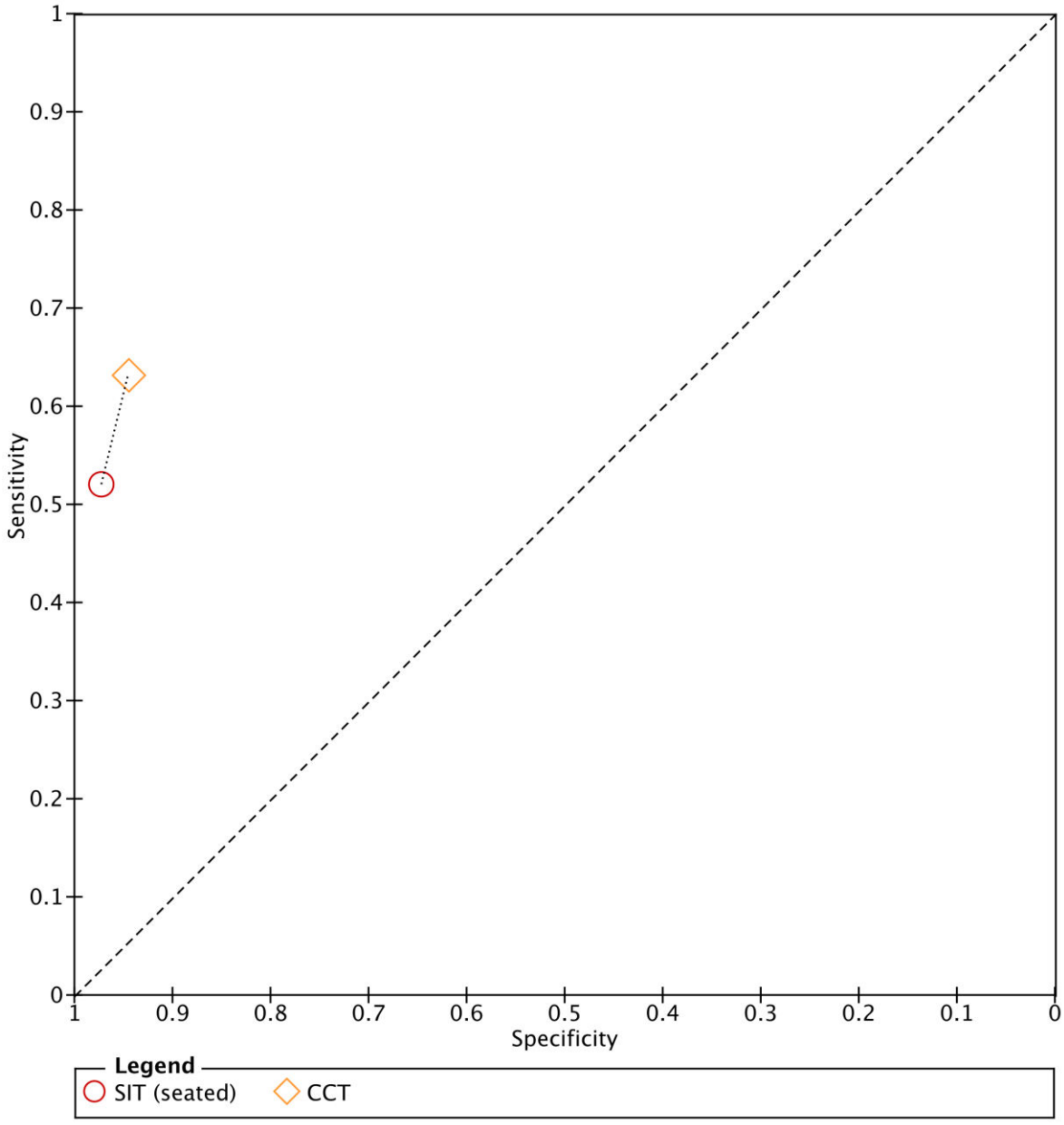
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Specificity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Legend
SIT (recumbent or unspecified posture)
FST

2C)


Sensitivity
1
0.9
0.8
0.7
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0.5
0.4
0.3
0.2
0.1
0
Specificity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Legend
SIT (seated)
CCT

2D)

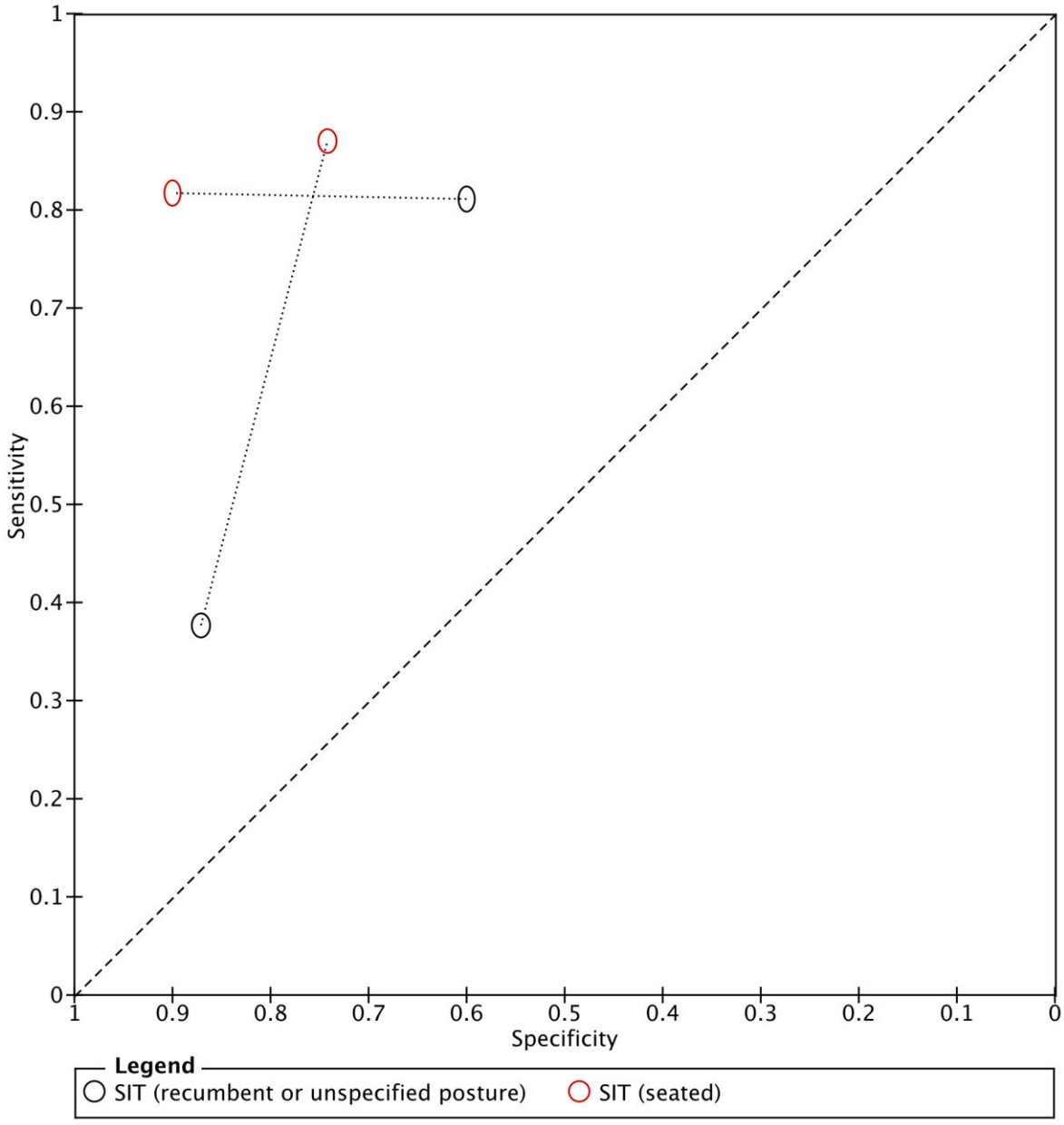
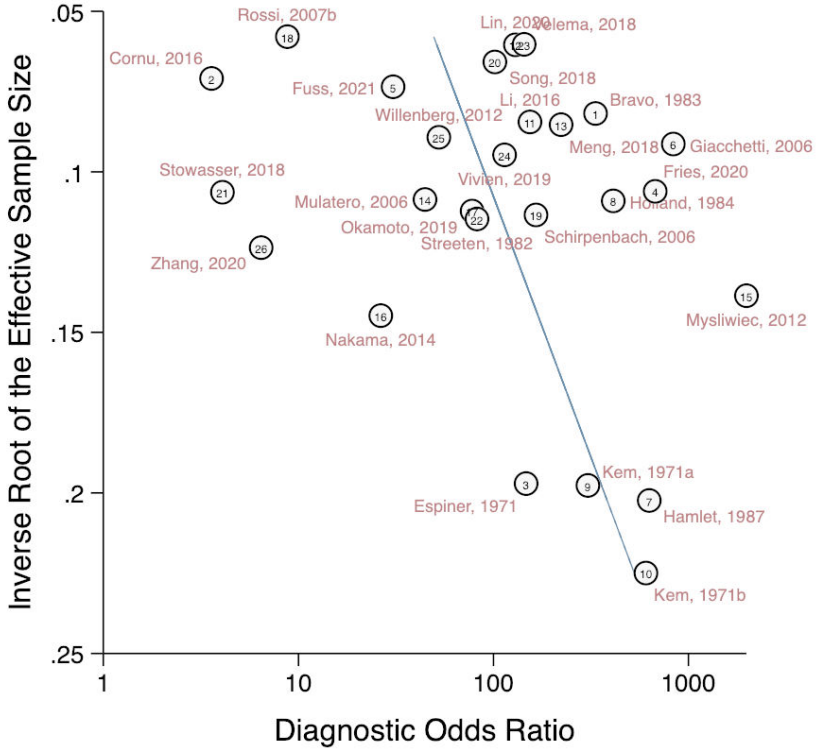
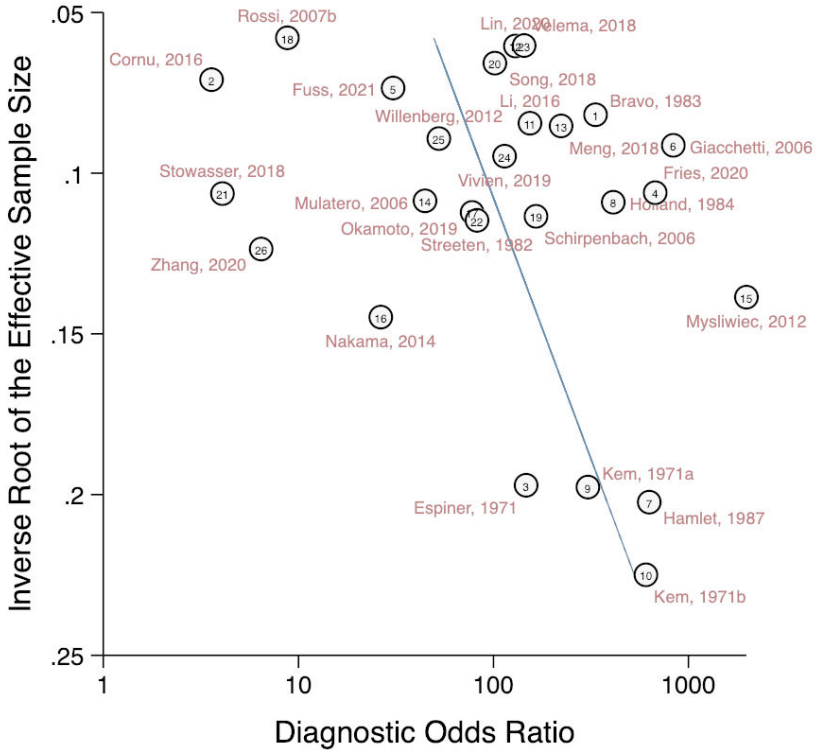

Sensitivity
Specificity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Legend
SIT (recumbent or unspecified posture)
SIT (seated)

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)



3B)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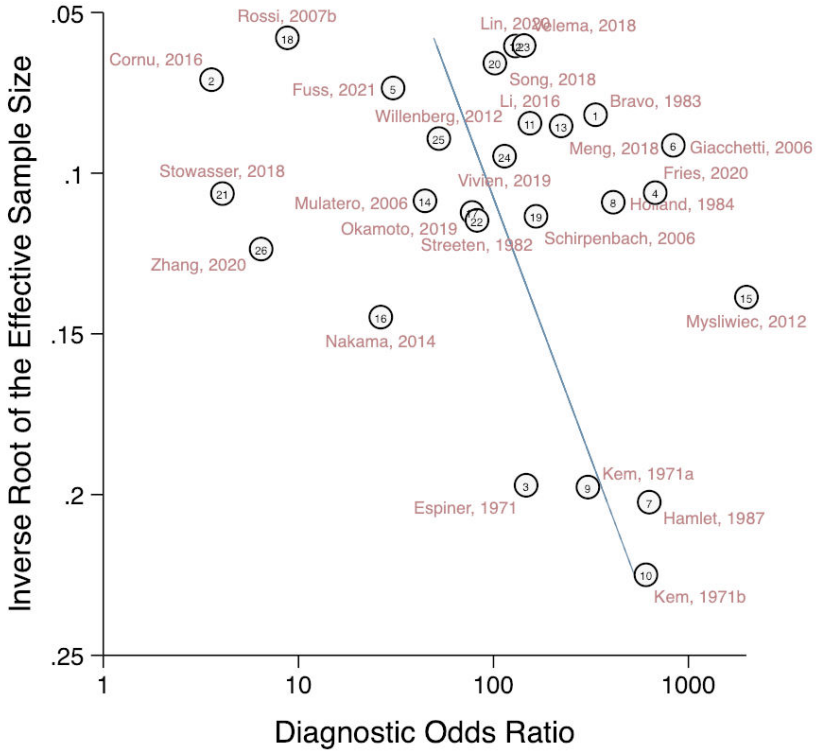
Lin, 2020
Nelema, 2018
Cornu, 2016
Song, 2018
Fuss, 2021
Li, 2016
Bravo, 1983
Willenberg, 2012
Meng, 2018
Giacchetti, 2006
Stowasser, 2018
Vivien, 2019
Fries, 2020
Mulatero, 2006
Holland, 1984
Okamoto, 2019
Schirpenbach, 2006
Streeten, 1982
Zhang, 2020
Mysliwiec, 2012
Nakama, 2014
Kem, 1971a
Espiner, 1971
Hamlet, 1987
Kem, 1971b
Inverse Root of the Effective Sample Size
.05
.1
.15
.2
.25
1
10
100
1000
Diagnostic Odds Ratio

3C)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Kem, 1971a
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Hamlet, 1987
Kem, 1971b
Inverse Root of the Effective Sample Size
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.15
.2
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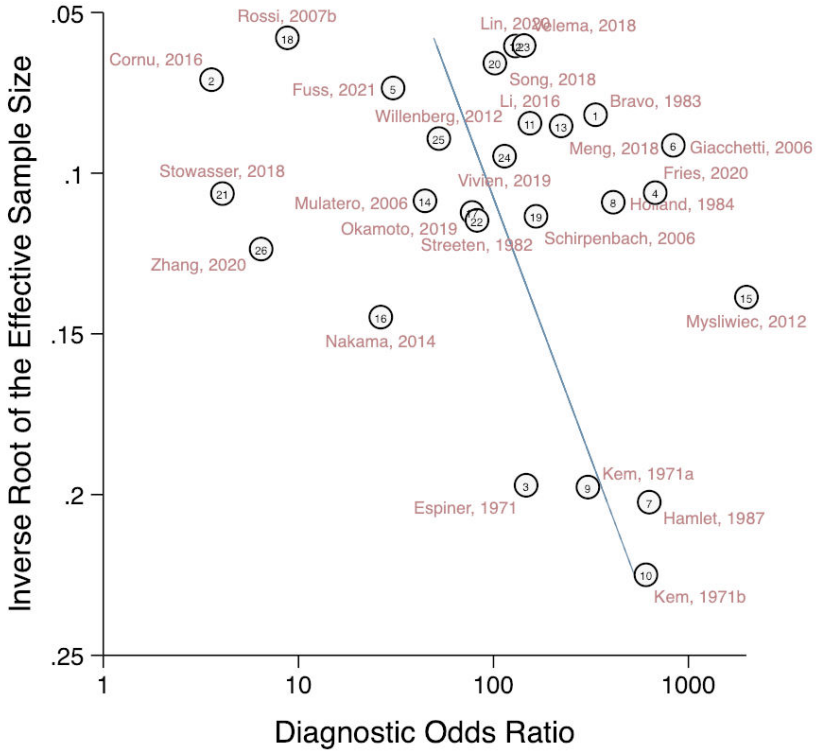
100
1000
Diagnostic Odds Ratio

3D)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Nakama, 2014
Kem, 1971a
Espiner, 1971
Hamlet, 1987
Kem, 1971b
Inverse Root of the Effective Sample Size
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.1
.15
.2
.25
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10
100
1000
Diagnostic Odds Ratio