AUSTRALASIAN ASSOCIATION FOR CLINICAL BIOCHEMISTRY AND LABORATORY MEDICINE INC
SYDNEY OFFICE: 5/85 Bourke Rd, Alexandria NSW, 2015
Telephone: +61 2 9669 6600 Email: office@aacb.asn.au

Guideline

Title	Aldosterone Renin Ratio sample processing and reporting: Recommendations from the Aldosterone Renin Ratio Harmonisation (ARRh) Working group
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Aldosterone Renin Ratio sample processing and reporting: Recommendations from the Aldosterone Renin Ratio Harmonisation (ARRh) Working group

Members	Site	Affiliations	email
Cherie Chiang (chair)	VIC	ESA, AACB, RCPA	cherie.chiang@mh.org.au
Jun Yang	VIC	ESA	Jun.Yang@hudson.org.au
Andrea Rita Horvath	NSW	AACB, RCPA	Andrea.Horvath@health.nsw.gov.au
Tina Yen	NSW	AACB, RCPA	Tina.Yen@health.nsw.gov.au
Zhong Lu	VIC	AACB, RCPA	Zhong.Lu@monashhealth.org
Greg Ward	QLD	AACB	Greg_Ward@snp.com.au
Peter Ward	NSW	AACB	Peter.Ward@health.nsw.gov.au
Julia Chang	QLD	AACB, RCPA	Julia.Chang@qml.com.au
Ee Mun Lim	WA	ESA, AACB, RCPA	EEmun.Lim@health.wa.gov.au
Penny Coates	SA	ESA, AACB, RCPA	penelope.coates@health.sa.gov.au
Ian Phillips	NZ	AACB	Ian.Phillips@sclabs.co.nz

Introduction:

Aldosterone Renin Ratio (ARR) is currently the recommended screening test for Primary Aldosteronism (PA), the most prevalent potentially curable form of hypertension. Although screening guidelines have been available since 2008, the uptake of ARR as a screening test remains poor. Patient preparation, assay variability and result interpretation have been flagged as significant barriers for primary care physicians to adopt evidence based ARR screening. [1]

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The Australasian Association of Clinical Biochemists (AACB) and Endocrine Society of Australia (ESA) endorsed the formation of a working group to address harmonisation issues in endocrine dynamic tests and a subgroup was formed in 2021 to address the harmonisation in ARR testing (ARRh working group). Recommendations were formulated according to current practices, evidence based guidelines and outcome studies. Patient selection for PA screening, patient preparation including medication changes, and the investigations following an elevated ARR have been published elsewhere, and are beyond the scope of this recommendation.

Target Group:

This guideline is primarily intended to be used by laboratories processing ARR samples. It is also relevant to general practitioners, endocrinologists, renal physicians and other specialists who request ARR testing.

Purpose and scope:

We address the following key questions:

1. What is the ideal time and posture for specimen collection for ARR testing?
2. What is the ideal procedure for handling renin samples?
3. What is the lowest reportable value for direct renin assay and how does this impact the ARR?
4. How should ARR values be reported?
5. How to comment on ARR reports?

Methods:

The Councils of the AACB and ESA endorsed the expert representatives of the societies within the ARRh working group. Additional authors with expertise in medical informatics, and pathologists from private and public hospital laboratories also contributed to the development of this guideline.

Recommendations:

1. What is the ideal time and posture for specimen collection for ARR testing?

1.1 Recommendation

ARR is optimally collected mid-morning (0900 – 1100) after patient has been up (sitting, standing, walking) for at least 2 hours and seated at least 5 minutes before collection.

The working group acknowledges that early morning fasting blood test is required for secondary hypertension screen including plasma metanephrines and dexamethasone suppression test. Therefore the above collection procedure is not mandatory. However, to avoid falsely low ARR and missed diagnosis of PA, ARR should be repeated using optimal specimen collection conditions if a normal ARR result is returned in a patient with high pre-test probability of primary aldosteronism.

For reproductive age females tested with Direct Renin Concentration (DRC), collection should take place during follicular phase of the menstrual cycle.

1.2 Evidence

Midmorning collection is recommended because early morning sampling might miss angiotensin II responsive forms of PA (Low renin essential hypertension / idiopathic hyperaldosteronism). Renin peaks at 6 am independent of posture and diet and persists in patients with PA. Midmorning collection also allows for patients to be at least 2 hours out of bed because supine posture is associated with significant decrease in aldosterone and to a lesser extent renin, therefore falsely lowering the ARR compared to the seated position.[2]

The current Endocrine Society Guideline recommends ARR collection mid-morning, after the patient has been up (sitting, standing, or walking) for at least 2 h and seated for 5–15 min.[3] Being in a seated position for at least 5 minutes avoids the effect of acute posture changes on the ARR.

When renin concentration is measured using a DRC assay, the ARR is higher in females than males for all phases of the menstrual cycle, but especially in the luteal phase. To reduce the risk of a false positive ARR in pre-menopausal women when DRC is measured, it is recommended to collect blood during the follicular phase.[4] Alternatively, plasma renin activity can be measured, but this assay is less readily available. Information is lacking on the impact of gender transition on renin and aldosterone levels.[5]

Patient preparation prior to the day of ARR testing, including normal/ non-restricted dietary salt intake, correction of hypokalaemia, and substitution of interfering medications, is beyond the scope of this guideline.

2. What is the ideal renin handling procedure to avoid cryoactivation?

2.1 Recommendation

For laboratory that can analyse renin within 5 days of collection

Collect blood in to EDTA tube for renin, centrifuge, aliquot and transport plasma at room temperature. Renin is stable at room temperature for at least 6 hours prior to centrifugation.[6] Send plasma aliquot at room temperature if it will reach the laboratory within 5 days.

For laboratory that cannot analyse renin within 5 days of collection

Collect blood in to EDTA tube for renin, centrifuge, aliquot and transport plasma at room temperature. The separated plasma should be immediately frozen at -70 to -80 ◦C. Please note that freezing at -20 ◦C is NOT sufficient and will cause cryoactivation of pro-renin leading to falsely high results. Once frozen at – 70 to - 80 ◦C, samples can be transported on dry ice.

For all laboratories

Frozen samples should be thawed at room temperature prior to immediate analysis. Repeated freeze-thaw cycle must be avoided.

2.2 Evidence

Cryoactivation of renin may occur more quickly in serum than in EDTA plasma, therefore the recommended sample type is EDTA plasma. Aldosterone can be analysed using either serum or EDTA plasma. The Endocrine Society recommends sample collection and processing at

room temperature, followed by rapid freezing of plasma. Snap freezing of individual plasma samples using alcohol bath (e.g. methanol, ethanol, acetone) and dry ice pose a safety risk and is not feasible in practice. Local and published data [7,8] have demonstrated that renin cryoactivation will significantly falsely elevate the renin concentration and thus lower the ARR (false negative ARR) in the following circumstances:

- Storage of EDTA blood or separated plasma specimen at 4 ◦C
- Gradual freezing from room temperature to -20 ◦C
- multiple freeze-thaw cycles
- gradual thawing at 4 ◦C prior to analysis

Storage of samples at room temperature beyond 5 days results in a significant decrease in renin concentration due to degradation and will falsely lower renin leading to false positive ARR. A reduction of direct renin concentration of 7.9% has been observed with room temperature storage at 7 days compared to a non-significant reduction of 3.4% at 3 days.[8]

3. What is the lowest reportable value for direct renin assay and how does this impact the ARR?

3.1 Recommendation

The Limit of Quantitation (CV up to 20%) of the direct renin assay should be used as the lowest reportable value.

3.2 Evidence

False positive ARR results can be due to imprecision of measuring low renin concentrations. Local data using the Diasorin renin assay noted a false positive ARR rate of 12% if the Limit of Detection (< 0.5 mIU/L) is used as the lowest reportable value rather than the Limit of Quantitation (< 2.0 mIU/L). When 6.0 mIU/L (Diasorin direct renin concentration assay) was used as the lowest reportable for ARR calculation, the clinical diagnosis did not alter with area under the curve analysis.[9] Other publications have set a minimum threshold of direct renin concentration at 5 mIU/L for the calculation of ARR due to concerns with imprecision. Therefore the lowest reportable renin should be where a CV of 20% is achieved (2.0 mIU/L for the Diasorin Liaison renin assay and 5.0 mIU/L for the IDS iSYS renin assay). [10,11]

4. How should ARR be reported?

4.1 Recommendation

ARR is reported with a unit. For example, ARR calculated from direct renin concentration will be reported as pmol/mIU.

For renin results below the lowest reportable value, ARR is calculated (aldosterone divided by lowest reportable renin value of the assay) and reported as a greater than value rather than reported as "unavailable".

4.2 Evidence

The ARR unit will indicate whether renin was analysed via the DRC mass assay or the plasma renin activity (PRA) assay. It is important for clinicians to observe the units and decision

limits reported by the laboratory as they may compare the patients' ARR values against clinical practice guidelines or other publications when interpreting results.

A suppressed renin result (below the lowest reportable value) can be due to PA. Although a final numerical value cannot be calculated for ARR, clinicians should be alerted to the high ARR when the measured aldosterone is not low. Please refer to the table below.

Test	Result reported as	ARR value	Rationale
Renin	< lowest reportable value	Calculated using renin lowest reportable value and reported as a greater than (calculated value)	*Example 1:* renin (Diasorin) < 2 mIU/L, aldosterone = 400 pmol/L, ARR > 200 pmol/mIU *Example 2:* renin (Diasorin) < 2 mIU/L, aldosterone = 120 pmol/L, ARR > 60 pmol/mIU.
Renin	> highest reportable value	unavailable	ARR is not calculated for high renin values because there is no clinical utility
Aldosterone	< lowest reportable value	unavailable	ARR is not calculated for low aldosterone values because there is no clinical utility
Aldosterone	> highest reportable value should be diluted to final value as per manufacturer's instructions	Calculated, generally renin will be < lowest reportable value in this instance	

5. How to comment on ARR reports?

5.1 Recommendation

A ratio of > "ARR cut off" is a positive screening test for Primary Aldosteronism if the aldosterone concentration is greater than * pmol/L. (* Refers to the local cut off aldosterone value for the seated saline suppression test. For example: Diasorin Liaison immunoassay = 170 pmol/L, LCMS aldosterone assay = 160 pmol/L, IDS (iSYS) immunoassay = 140 pmol/L.)

Falsely elevated ARR results can occur due to medication interference, oral contraceptive pill, older age, renal failure, and suboptimal collection condition. Consider referral to specialists. Please refer to the PTEX webpage for information on interpretation. (https://pathologytestsexplained.org.au/ptests-pro.php?q=Renin)

A ratio of < "ARR cut off" is a negative screening test for Primary Aldosteronism. Falsely lowered results can occur due to hypokalaemia, sodium loading, interfering antihypertensive medications and incorrect sample collection especially for renin. Consider medication adjustment supervised by a clinician, and recollecting specimens mid-morning (0900 – 1100) after patient has been up for at

least 2 hours and seated for at least 5 minutes. Please refer to the PTEX webpage for information on interpretation. https://pathologytestsexplained.org.au/learning/test-index/aldosterone

5.2 Evidence

There is no published consensus on the accompanying comment to assist ARR interpretation. The working group recommends a short comment with referral to the PTEX website for more details on patient preparation and test interpretation. In patients with high pre-test probability, a negative ARR should be repeated with special attention paid to recommended patient preparation and sample collection.[12]

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