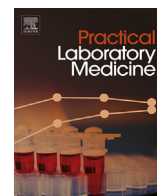


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Survey of renin and aldosterone testing practices by Ontario laboratories – Providing insight into best practices

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ABSTRACT

Objectives: Testing for renin and aldosterone in clinical laboratories is complicated by pre-analytical considerations such as the posture for blood collection and susceptibility to cryoactivation of renin. From an analytical perspective, there are both renin activity and renin mass or concentration assays available. There can also be variability in result reporting practices and the aldosterone-renin ratio (ARR) cut-off applied to screen for primary aldosteronism (PA). The Institute for Quality Management in Healthcare (IQMH) Centre for Proficiency Testing surveyed laboratories on their handling of renin and aldosterone testing to better understand current practices.

Design and methods: An online survey was prepared and sent to 134 Canadian laboratories enrolled in endocrinology proficiency testing with IQMH.

Results: One hundred twenty Ontario laboratories submitted responses. While only six (5%) laboratories perform testing for both renin and aldosterone, 108 (90%) collect and process specimens to be tested by reference laboratories. The survey revealed considerable variation in practices including the recommended state of patients prior to sample collection (for example, regarding medications or salt intake), the patient posture specifications for sample collection, the precautions taken against cryoactivation of renin, the choice of renin activity or mass assay, and the ARR cut-off used. The available literature on these factors was then reviewed.

Conclusions: Although there is no standardized procedure for specimen collection, analysis, or result reporting for renin or aldosterone testing, we have attempted to summarize the available literature to develop evidence-based recommendations. Where laboratory practice differs from peers and/or recommended protocols, laboratories should review their practices.

Abbreviations: ARR, aldosterone-renin ratio; IQMH, Institute for Quality Management in Healthcare; PA, primary aldosteronism.

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1. Introduction

Renin and aldosterone are commonly measured to screen hypertensive patients for primary aldosteronism (PA), a condition in which aldosterone production is inappropriately elevated and is autonomous rather than being driven by renin production. Calculation of an aldosterone-renin ratio (ARR) is currently considered the best screening test for PA [1], but the performance of the test depends not only on accurate measurement of renin and aldosterone, but also application of an appropriate ARR cut-off in distinguishing patients who require confirmatory testing for PA from those who do not.

Renin is secreted by the kidney. However, prorenin is secreted by the kidney, adrenal glands, gonads, and uteroplacental units and its level in circulation is about ten times higher than the renin concentration [2]. In patients with PA, the prorenin level can be up to 100 times higher than the renin concentration [3]. Conversion of secreted prorenin to renin is not thought to occur *in vivo*. However, cryoactivation is the cleavage of prorenin to renin by plasma proteases that occurs *in vitro* when blood or plasma in liquid form is exposed to temperatures of -5 to 4 °C for prolonged periods of time [4,5]. Cryoactivation may result in a false elevation of renin mass or activity.

Historically, renin was measured using renin activity assays and blood was collected in pre-chilled tubes and spun in refrigerated centrifuges. Plasma was either kept refrigerated or frozen prior to analysis and, when it was frozen, was thawed at 4 °C. This was all done to inhibit *in vitro* renin activity, which would cause depletion of the angiotensinogen substrate and formation of angiotensin I prior to analysis [2,6,7]. However, these protocols became questionable with the discovery of cryoactivation. It is now recognized that care must be taken to avoid pre-analytical cryoactivation of renin and provide accurate results when either renin activity or mass assays are used.

Accurate measurement of renin and aldosterone is complicated by pre-analytical considerations, the availability of renin activity and mass assays, and non-standardized reporting of renin and the ARR. These issues may impact result interpretation and treatment decisions by health care providers. For this reason, the Institute for Quality Management in Healthcare (IQMH), one of Canada's largest providers of medical laboratory proficiency testing accredited by the American Association for Laboratory Accreditation to International Organization for Standardization 17043:2010 *Conformity Assessment - General Requirements for Proficiency Testing*, surveyed practices of laboratories regarding these tests. While few of the laboratories participating in the IQMH proficiency testing program perform renin and aldosterone testing on-site, many laboratories are involved in the collection and processing of samples for these tests.

For many of the topics surveyed, no consensus exists on the optimal conditions. The Endocrine Society Clinical Practice Guidelines [1] represent one of the most well-developed consensus documents regarding renin and aldosterone testing. While this document is intended mainly for clinicians managing PA, it is a useful resource for laboratories as well. The key recommendations from this document that pertain to pre-analytical factors affecting renin and aldosterone measurement are summarized in Table 1 for laboratories to compare against their practices.

2. Materials and methods

In the Canadian province of Ontario, IQMH is mandated by the Ontario Ministry of Health to provide proficiency testing programs for diagnostic laboratories, with mandatory participation for all licensed Ontario laboratories. IQMH patterns-of-practice surveys are periodically prepared by discipline-specific voluntary expert scientific committees and are provided to laboratories participating in IQMH proficiency testing programs with the purpose of obtaining information on various current laboratory practices. The patterns-of-practice survey on current laboratory practices for aldosterone and renin testing (included in the Appendix) was prepared by the IQMH Endocrinology and Immunology Scientific Committee and distributed online to 134 Canadian laboratories that participate in the IQMH endocrinology proficiency testing surveys. Participation was expected to be mandatory for those laboratories, but only 128 (96%) responses were received. One hundred twenty (94%) responses were from Ontario laboratories and the other 8 (6%) were from laboratories in Newfoundland. The Newfoundland laboratories indicated they do not perform renin or aldosterone testing on-site and were excluded to allow the summary results to focus on practices in Ontario laboratories. Responses were collated and analyzed using

Table 1

Checklist of pre-analytical recommendations for laboratories based on Endocrine Society Clinical Practice Guidelines [1].

| |
|--|
| Patient Preparation |
| <input type="checkbox"/> Unrestricted dietary intake of salt |
| <input type="checkbox"/> Medications with a significant effect on the ARR (e.g., spironolactone, eplerenone, amiloride, triamterene, potassium-wasting diuretics) ideally discontinued at least 4 weeks prior to testing ^a |
| <input type="checkbox"/> Medications with a moderate effect on the ARR (e.g., β -adrenergic blockers, central α -2 agonists (e.g., clonidine, α -methyl dopa), non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, renin inhibitors, dihydropyridine calcium channel antagonists) potentially discontinued at least 2 weeks prior to testing if measurement of the ARR following withdrawal of medications listed above with significant effects on the ARR is not diagnostic ^a |
| Blood Collection Conditions |
| <input type="checkbox"/> Blood collected midmorning after the patient has been up (sitting, standing, or walking) for a minimum of 2 h and then seated for 5–15 min prior to collection |
| Sample Handling |
| <input type="checkbox"/> Blood kept at room temperature (not chilled) during transportation to the laboratory and before centrifugation |
| <input type="checkbox"/> Plasma frozen rapidly and maintained frozen prior to testing |

^a Medications with a minimal effect on the ARR (e.g., verapamil slow-release, hydralazine, prazosin, doxazosin, terazosin) may be implemented in place of anti-hypertensive medications with a significant or moderate effect on the ARR.

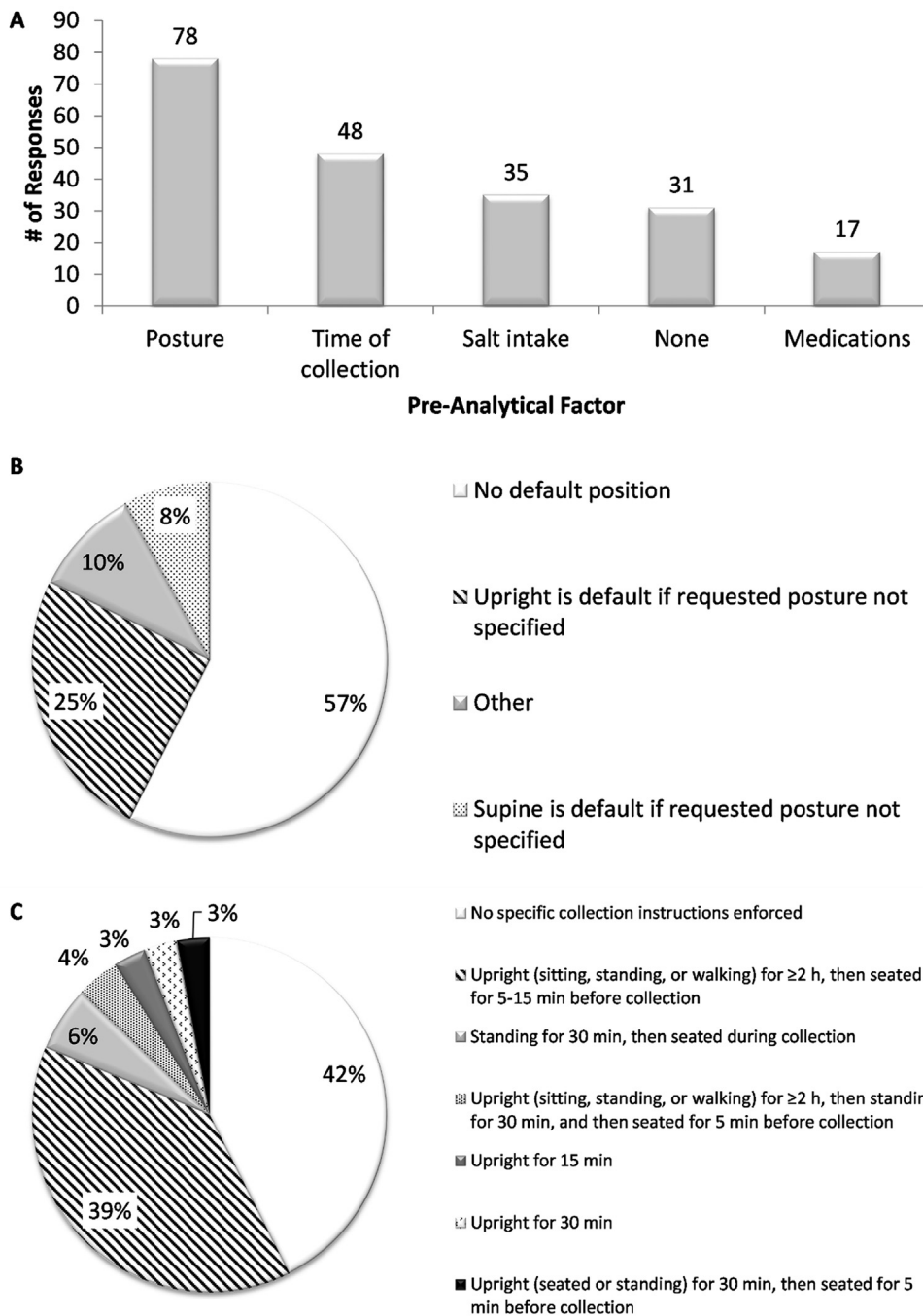


Fig. 1. A) Specimen collection recommendations regarding specific pre-analytical factors affecting renin and/or aldosterone testing. Multiple responses were allowed by each laboratory (n = 209 responses from 111 laboratories). B) Default patient position used prior to/during specimen collection for renin and/or aldosterone testing (n = 113). C) Protocol for upright specimen collection (n = 109).

Microsoft Excel software. A literature review was performed to try to ascertain best practices for each of the topics covered in the survey. The collated survey results were compiled with the results of the literature review and shared with participating laboratories in the form of committee comments, which were prepared by the IQMH Endocrinology and Immunology Scientific Committee members.

3. Results and discussion

3.1. Testing site for renin and aldosterone

Only six of the 120 (5%) laboratories perform testing for both renin and aldosterone on-site, one (1%) tests only renin on-site, one (1%) tests only aldosterone on-site, and four (3%) do not have testing for renin or aldosterone available even as referred-out tests. The majority (90%) collect samples on-site and then refer out the testing to reference laboratories. The six sites that test both renin and aldosterone on-site include two large community laboratories and four hospital-based laboratories located in large cities in Ontario. All six of these laboratories are believed to receive samples for renin and aldosterone testing from some of the Ontario laboratories that indicated they refer out these tests. However, it is suspected based on the survey results that referral sites outside of Ontario are also being utilized.

3.2. Pre-analytical considerations

3.2.1. Collection instructions

Fig. 1A details the most common pre-analytical factors that laboratories make recommendations about for these tests. Posture is most commonly addressed, followed by collection time. One laboratory that selected salt intake and posture (included in Fig. 1A), indicated that these recommendations are only made for renin and not for aldosterone.

3.2.1.1. Medications. The performance of the ARR will be affected by the pre-analytical conditions under which samples are collected and handled. The Endocrine Society recommends withdrawal of medications that have a significant effect on the ARR (spironolactone, eplerenone, amiloride, triamterene, K^+ -wasting diuretics, products derived from licorice root) at least four weeks prior to blood collection. If the ARR is not diagnostic following removal of those agents, if possible, it is suggested to withdraw additional agents that have a lesser effect on the ARR (β -adrenergic blockers, central α -2 agonists such as clonidine and α -methyl dopa, non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, angiotensin II type I receptor blockers, renin inhibitors, and dihydropyridine Ca^{2+} channel blockers) for at least two weeks, replace them with anti-hypertensive agents that have minimal interference with the ARR, and then repeat the ARR measurement [1,8].

3.2.1.2. Salt intake. Restricting salt intake has the effect of increasing renin and aldosterone levels and decreasing the ARR, which can result in a false negative with regard to PA diagnosis. Since the ARR is used as a screening test and sensitivity is desired over specificity, false negatives are to be avoided as much as possible. While salt loading has the effect of decreasing renin and aldosterone levels and increasing the ARR, which can result in a false positive and is not desirable either, it is preferable to a false negative at this stage of the investigation. Therefore, it is recommended that patients have unrestricted salt intake prior to testing. The patient should also be potassium replete, so supplementation may be required if the patient is hypokalemic [1].

3.2.1.3. Specimen collection time. Circadian variation has been observed particularly for aldosterone, with significantly higher levels in the morning compared to the afternoon or evening. Thus, the ARR is higher in the morning and lower in the afternoon or evening, and morning blood collection optimizes sensitivity with less chance of a false negative ARR [9].

3.2.1.4. Posture. Upright posture is known to increase renin and aldosterone levels, but the effect on the ARR is not clear. A study by Tiu et al. [10] compared 9:00 a.m. sampling after being supine overnight, 10:00 a.m. sampling after patients had been seated for 30 min, and 1:00 p.m. sampling after patients had been ambulatory for 4 h. The ARR from the supine position appeared to offer the best performance. However, the supine protocol is highly inconvenient and Barigou et al. [11] found the ARR values from supine, seated, and upright postures to be correlated. Yin et al. [12] found the ARR to be highest in the supine group (compared to upright for 1, 2, or 4 h) in patients with essential hypertension and there were no significant differences in ARR values between postures in patients with PA, so the upright for 1 h position was the most efficient at differentiating PA from essential hypertension. Overall, there is no clear consensus on the best posture to use. The Endocrine Society recommends mid-morning sampling after the patient has been up (sitting, standing, or walking) for 2 h or more and then seated for 5–15 min [1].

3.2.1.5. Other variables. A number (7%) of laboratories commented that they make recommendations regarding other factors such as chronic kidney disease, age, gender, menstrual phase, pregnancy, and use of oral contraceptives. The majority of these sites are using the same referral laboratory and, while that referral laboratory mentions that these factors can affect renin and/or aldosterone levels, it does not actually make any *recommendations* regarding these factors. Therefore, these additional factors were not included in the list of pre-analytical recommendations in Fig. 1A.

Evidence is available for some of these other factors affecting the ARR. Renal impairment can decrease renin activity and thereby increase the ARR and contribute to a false positive result [1].

Renin and aldosterone levels decrease with age [13], but in adults at least 65 years old, renin is decreased more than aldosterone, resulting in a higher ARR [1].

There are also sex-based differences in renin and aldosterone concentrations with females having lower renin concentrations than males [1]. During the follicular phase of the menstrual cycle, aldosterone levels are similar in males and females. However, during the

luteal phase, aldosterone levels become significantly higher in females [14,15]. Therefore, when renin is measured using a mass assay, the ARR is higher in females than males for all phases of the menstrual cycle, but especially the luteal phase. To reduce the risk of a false positive ARR in pre-menopausal women when renin mass is measured, it is recommended to collect blood during the follicular phase [1]. Information is lacking on the impact of gender transition on renin and aldosterone levels.

During pregnancy, both the aldosterone level and renin activity are increased, resulting in a lower ARR and increased possibility of a false negative [1,16].

Oral contraceptives and hormone replacement therapy may lower renin mass, but not activity, resulting in an increased likelihood of a false positive ARR when renin mass is measured [1,17].

Some of these pre-analytical factors may warrant recommendations from the laboratory. For other factors, the laboratory may wish to make clinicians aware of the possible impact on aldosterone, renin, and/or ARR results. In some cases, different ARR cut-offs would be ideal, however this is difficult to achieve and would require specifically designed clinical trials to identify cut-offs specific for every pre-analytical variable.

3.2.2. Patient posture prior to/during specimen collection

Thirty-nine percent of laboratories responded that they do not note the patient posture used for specimen collection for renin and/or aldosterone testing.

Laboratories were also asked about specimen collection practices when the ordering health care provider does not request a specific posture for renin and/or aldosterone testing. Perhaps because the literature does not consistently demonstrate an optimal posture, there appear to be multiple ways that laboratories handle this situation. As shown in Fig. 1B, most laboratories do not have a default patient position. Nine percent of laboratories (within the groups that responded “Other” or “No default position”) commented that health care

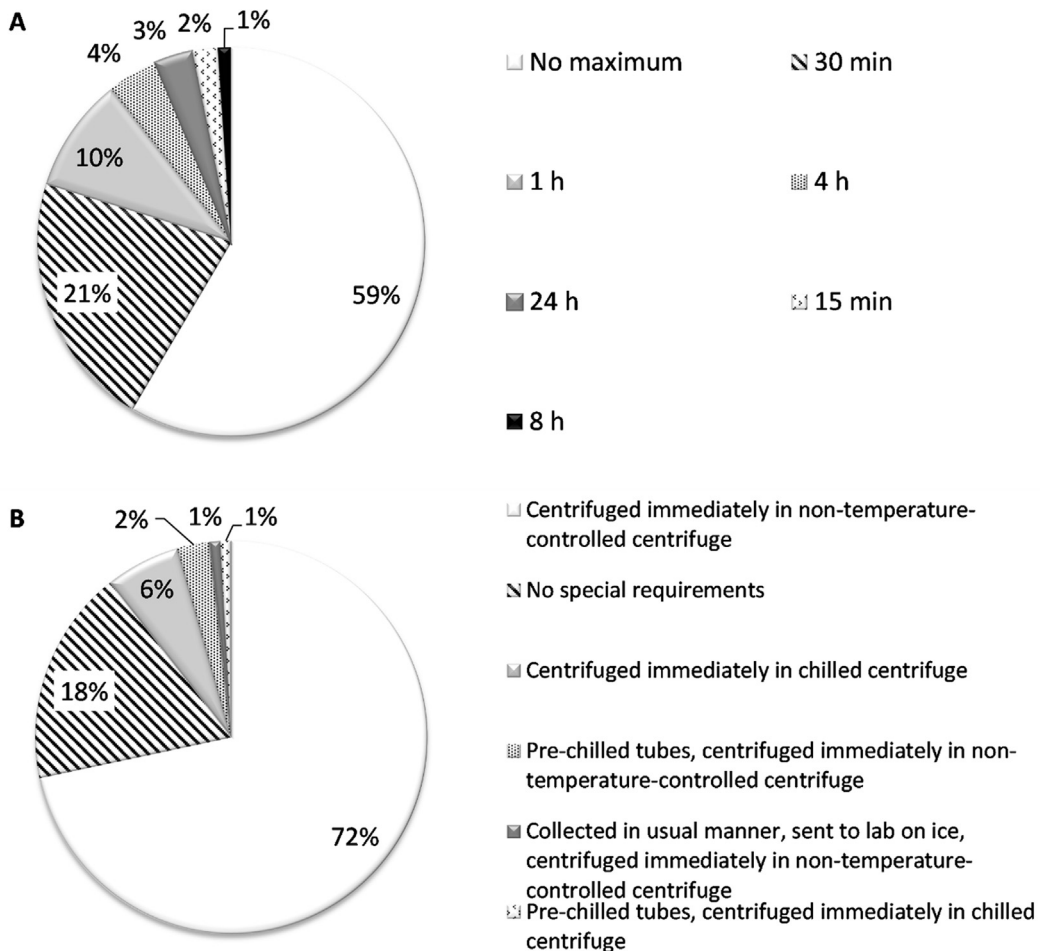


Fig. 2. A) Maximum post-collection time allowed for sample to be received in the laboratory for processing for renin and/or aldosterone testing. If the time period was different for renin and aldosterone, the shorter maximum allowable time was used in constructing the pie chart (n = 94). B) Processing conditions for blood collected for renin testing (n = 113).

providers must select either supine or upright when ordering at their institution so the laboratory does not need to have a default position. One quarter of laboratories responded that upright is their default position if posture is not specified, while approximately one-third of that amount said supine is their default position.

3.2.3. Protocol for upright specimen collection

There is a lot of variability in the protocols being followed for ambulatory or upright specimen collection. As shown in Fig. 1C, most commonly, laboratories do not follow a specific collection protocol. The next most common selection was that patients must be upright (sitting, standing, or walking) for a minimum of 2 h and then seated for 5–15 min prior to sample collection. The latter protocol is that recommended by the Endocrine Society [1].

3.2.4. Specimen type

All laboratories collect EDTA plasma for renin testing, although one laboratory responded that they would also accept serum for renin. For aldosterone, most (62%) laboratories use EDTA plasma as their primary sample type, although many (35%) laboratories collect serum for aldosterone testing, and some (3%) even use heparin plasma. Since the focus of the survey was tests that are most commonly used to screen for PA, laboratories were not asked about practices related to aldosterone testing in urine.

Cryoactivation of renin may occur more quickly in serum than in EDTA plasma [18], resulting in higher renin levels in serum specimens. Chakera et al. [19] even observed significantly lower renin mass in EDTA plasma compared to serum in samples that were centrifuged immediately and frozen at -80°C prior to analysis. Not all studies have observed this difference though, as de Bruin et al. [20] did not find any significant differences in renin mass between serum, EDTA plasma, or heparin plasma. However, due to the potential for sample type related differences, EDTA plasma would be the preferred sample type for renin testing.

Aldosterone concentrations were found to be significantly higher in EDTA plasma compared to serum in one study [21], but significantly lower in EDTA plasma compared to serum or heparin plasma in another study [22]. This suggests that different aldosterone reference intervals and ARR cut-offs may be required depending on the sample type.

3.2.5. Maximum duration of time allowed between sample collection and receipt in the laboratory for processing

As shown in Fig. 2A, most laboratories do not have a maximum allowable time from specimen collection to sample receipt in the laboratory for renin and/or aldosterone testing. For those laboratories that do, the period varied from 15 min to 24 h.

There is conflicting information regarding renin stability at room temperature in whole blood. Estimates range from less than 6 h [19] to greater than 72 h [20]. Due to the uncertainty concerning renin stability, it may be best to err on the side of caution and allow less time rather than more, particularly for renin activity assays. For either renin mass or activity, one should be wary of a significant time unspun if the samples were chilled during that time, due to the risk of cryoactivation. Aldosterone appears to be stable for at least 48 h at room temperature in unspun whole blood [19].

3.2.6. Handling of specimens collected for renin testing

As shown in Fig. 2B, 18% percent of participants responded that they are not following any special precautions in their handling of specimens for renin testing. Most (72%) respondents indicated that they do not follow any special precautions aside from immediate centrifugation in a non-temperature-controlled centrifuge. Ten percent of laboratories chill the specimens in some way, either by collecting blood in pre-chilled tubes, transporting the tubes to the laboratory on ice, and/or centrifuging in a temperature-controlled centrifuge. Of the laboratories that chill the samples, most use renin mass assays and the others report using both mass and activity. In other words, none of the laboratories chilling the samples report using only renin activity assays.

Glinicki et al. [21] observed no differences in renin mass or activity whether blood was collected in pre-chilled tubes and spun in a refrigerated centrifuge within 30 min or handled at room temperature over the same period of time. Therefore, such short-term exposure to refrigeration temperatures is unlikely to induce significant cryoactivation and it may not make much difference whether tubes are pre-chilled or temperature-controlled centrifuges are used. However, chilled temperatures during sample collection and processing seem to offer little advantage for renin mass and potential risk of cryoactivation if prolonged. For renin activity, chilled temperatures may help to inhibit pre-assay renin activity, but there is also risk of cryoactivation if the exposure is prolonged. For either mass or activity, the Endocrine Society recommends sample collection and processing at room temperature, followed by rapid freezing of plasma [1].

3.2.7. Storage and shipment conditions for specimens collected for renin testing

All but one laboratory indicated that samples for renin testing are frozen immediately and then stored frozen until testing or until they are shipped frozen. The other laboratory responded that they store specimens at room temperature prior to testing or shipping. This is a laboratory that does not perform renin testing on-site. As stated above, it is recommended to rapidly freeze plasma for renin testing [1].

3.2.8. Storage and shipment conditions for specimens collected for aldosterone testing

Similar to renin, most (87%) laboratories freeze samples for aldosterone testing immediately and keep them frozen until testing or until they are shipped frozen. However, for aldosterone, 10% of laboratories keep samples refrigerated until shipping if samples will arrive at the testing site within 24 h, 2% of laboratories keep specimens refrigerated until testing or shipping, and one (1%) laboratory keeps specimens at room temperature until shipping. The one laboratory storing samples for aldosterone testing at room temperature is the same one storing samples for renin testing at room temperature prior to shipping.

According to a study by Evans et al. [22], aldosterone was stable in plasma or serum stored at 4 °C or 30 °C for greater than 120 h as compared to frozen at -20 °C. Therefore, all of the conditions reported by IQMH survey participants for storage and shipment of samples for aldosterone testing are likely acceptable.

3.2.9. Awareness of and precautions against cryoactivation of renin

Fifty-five percent of laboratories responded that they are not aware of renin cryoactivation. Despite this, it appears that many laboratories are taking precautions against cryoactivation, perhaps due to instructions from referral laboratories. As shown in Fig. 3, the most common precautions are freezing samples immediately after centrifugation, centrifuging at room temperature, and shipping samples with dry ice or ice packs. One laboratory commented that they have validated that ice packs will keep their samples frozen for up to 2 h.

Requiring samples to be sent to the laboratory on ice or spinning samples in a refrigerated centrifuge are not precautions to prevent cryoactivation. These procedures might help to inhibit renin activity prior to assay [2,6,7], but most of the laboratories selecting these options use renin mass assays and only about one-third report using both mass and activity assays.

Rejecting samples sent to the laboratory on ice and spinning samples in a room temperature centrifuge are precautions meant to prevent cryoactivation. However, cryoactivation does not occur immediately, as demonstrated by one study that observed no cryoactivation if samples were collected in pre-chilled tubes and spun in a refrigerated centrifuge within 30 min [21] and another study that observed that cryoactivation occurred between six to 24 h at 4 °C [19]. This suggests that chilling the specimens before and/or during centrifugation may not cause cryoactivation as long as samples are processed in a timely manner and are not kept chilled for several hours. Nevertheless, it is still recommended by the Endocrine Society to keep samples at room temperature prior to and during centrifugation [1].

Freezing plasma at -20 °C or -80 °C immediately after centrifugation in a non-defrosting freezer is a way to protect against cryoactivation and is recommended by the Endocrine Society [1]. Similarly, ensuring that samples stay frozen during shipping either with ice packs or dry ice is protective against cryoactivation.

Prior to analysis at the testing laboratory, samples should be thawed at room temperature rather than in the refrigerator and they should be analyzed soon after thawing to reduce the risk of cryoactivation [16]. In addition, multiple freeze-thaw cycles are not recommended. Hillebrand et al. [23] observed increased renin activity after two or three freeze-thaw cycles, likely due to cryoactivation. Therefore, it is appropriate to request two frozen aliquots of each sample, to only thaw one for analysis, and to keep the second aliquot frozen in case any repeat analysis is required.

The laboratories that indicated they are not taking any precautions against cryoactivation were those that were also not aware of cryoactivation. If laboratories are not following the recommended procedures for renin testing outlined here, changes ought to be made to ensure the integrity of the samples and the validity of results.

3.3. Analytical considerations

3.3.1. Renin testing methodology

When asked about the methodology used for renin testing, 66% of the 93 valid responses indicated renin mass measurement

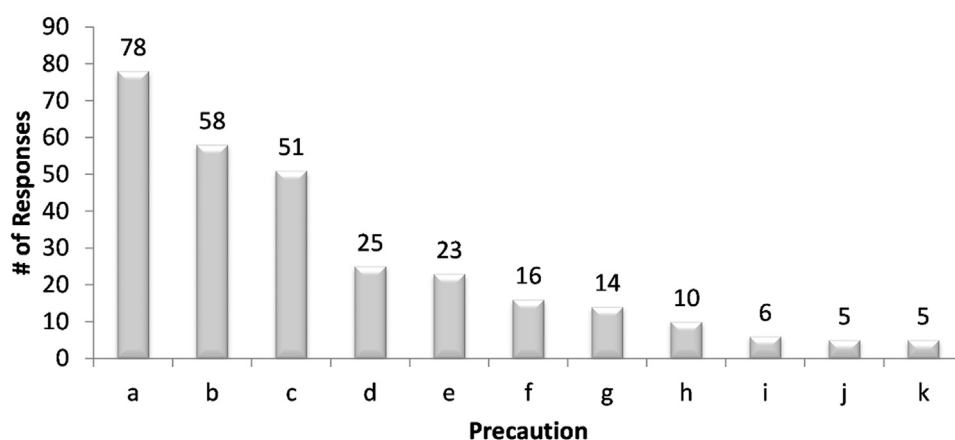


Fig. 3. Precautions taken against cryoactivation of renin. The precautions on the x-axis are: a) plasma frozen at -20 °C immediately after centrifugation, b) samples spun in centrifuge at room temperature, c) plasma shipped with dry ice, d) plasma shipped with ice pack, e) no precautions taken, f) two aliquots requested; any repeat analysis done on second aliquot kept frozen, g) samples thawed at room temperature and analyzed immediately, h) samples rejected if sent to laboratory on ice, i) samples spun in refrigerated centrifuge, j) plasma frozen at -80 °C immediately after centrifugation, k) samples must be sent to laboratory on ice. Laboratories were asked to select all applicable responses (n = 291 responses from 106 laboratories).

methods. Of the remaining one-third of laboratories, 18% use renin activity and the other 16% have access to both renin mass and renin activity assays. Seventeen laboratories responded that they use neither renin mass nor renin activity assays; however, those responses were excluded because they were from laboratories that responded that they send samples to another laboratory for renin testing. The interpretation of these responses is that the referring laboratory was unaware of the methodology used at the referral location.

Considering only the seven laboratories performing renin testing in Ontario, all use renin mass assays. Therefore, the 32 laboratories that report using renin activity assays through referral testing, either as their sole testing methodology or in addition to renin mass testing, are either incorrect or refer the test to a laboratory located outside of Ontario.

Table 2 compares renin mass and activity assays. For most patients, renin mass and activity assays give comparable information and either can be used [25]. However, there are several situations in which renin activity and mass assays may give conflicting results:

- i) *Patients taking direct renin inhibitors.* In this case, renin mass rises, but activity is suppressed, giving conflicting results [25].
- ii) *Patients with significantly elevated renin activity (> 40 ng/mL/h).* In this situation, the liver cannot make angiotensinogen quickly enough to keep up with its consumption by renin, renin production is accordingly upregulated, and the cycle repeats. The limited angiotensinogen may also impair measurement of renin activity (depending on the assay protocol), which would result in a falsely low activity result. In this case, renin mass would be more accurate and would be measured to be higher relative to the activity [25].
- iii) *Patients with high estrogen states like pregnancy.* The assumption of renin activity assays that angiotensinogen levels are relatively constant does not hold true in high estrogen states like pregnancy, which upregulate angiotensinogen levels. In response, renin production is lower, which is accurately measured by the mass assay; however, the increased angiotensinogen level may result in a falsely high renin activity measurement [25].
- iv) *Patients with congestive heart failure.* The assumption of fairly constant angiotensinogen levels also does not hold for these patients, who have lower than normal levels. The result is a discrepancy between renin activity, which is measured to be falsely low, compared to the more accurate renin mass [25].

3.4. Post-analytical considerations

3.4.1. Reporting patient posture

Forty-two percent of laboratories indicated that they do not report the posture used prior to/during collection of specimens for renin and/or aldosterone testing. This is similar to the percentage (39%) of laboratories that do not note the posture.

The Endocrine Society recommends that clinicians take factors such as the patient posture and the length of time in that posture prior to/during specimen collection into account when interpreting aldosterone, renin, and ARR results [1]. In order to allow clinicians to do so, laboratories should provide the patient posture information on the result reports.

3.4.2. Reference intervals

Most surveyed laboratories provide renin and aldosterone reference intervals for supine and upright postures (Fig. 4). However, the number of laboratories providing separate reference intervals for supine and upright postures is higher than the number of laboratories that responded that they note (3.2.2) and report (3.4.1) the posture used. It is not clear for those laboratories how clinicians know whether the supine or upright posture was used and, therefore, which reference interval to compare against.

It is less common for labs to have separate partitions based on salt intake or age for renin or aldosterone. It is interesting that a significant number of laboratories report having reference intervals for low salt diets given that patients are recommended not to restrict salt intake prior to aldosterone, renin, and ARR testing [1].

3.4.3. Units reported for renin assays

Of the laboratories using renin activity assays, 40% report in units of ng/mL/h (Fig. 5A). The remainder are evenly split between $\mu\text{IU/mL}$ and ng/L. The units of ng/L appear more likely to be units for renin concentration, but were included in the graph. One laboratory responded that they use units of pmol/ng. This response was excluded as it was thought to be a unit for the ARR rather than renin activity. The responses agree with another publication [24] that stated that units of ng/mL/h were most commonly used for renin

Table 2

Comparison of the properties of renin mass and activity assays.

| Factor | Renin Activity | Renin Mass |
|----------------------|--|--|
| Standardization | Not well standardized; thus, conversion factors exist to relate activity measurements from different assay manufacturers [18] | Can be calibrated against international reference standard, so better standardization between assays from different manufacturers [20] |
| Labour-intensiveness | Fairly manual and time consuming | Can be automated |
| Sensitivity | Can incubate for a prolonged period of time to generate a signal in low renin samples, providing better sensitivity [18] | May not be able to measure renin concentrations as low as renin activity assays |
| Reproducibility | Technical process dependent on many variables (pH, incubation time, choice of angiotensinase inhibition, purity of calibrators) [18, 24] | Better intra- and interassay reproducibility [20] |
| Acceptance | Have been used longer; greater amount of supporting literature [24] | In use for shorter period of time; less supporting literature |

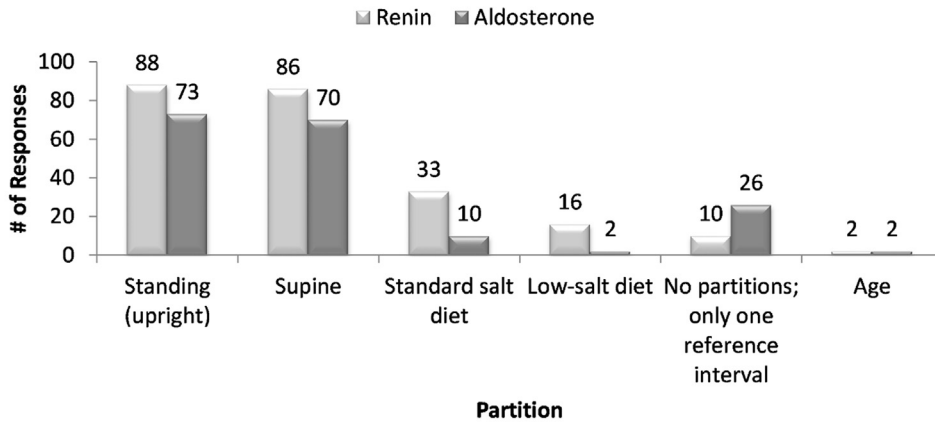


Fig. 4. The partitions by which renin (n = 235 responses from 102 laboratories) and aldosterone (n = 183 responses from 100 laboratories) reference intervals are divided. Multiple responses were allowed by each laboratory.

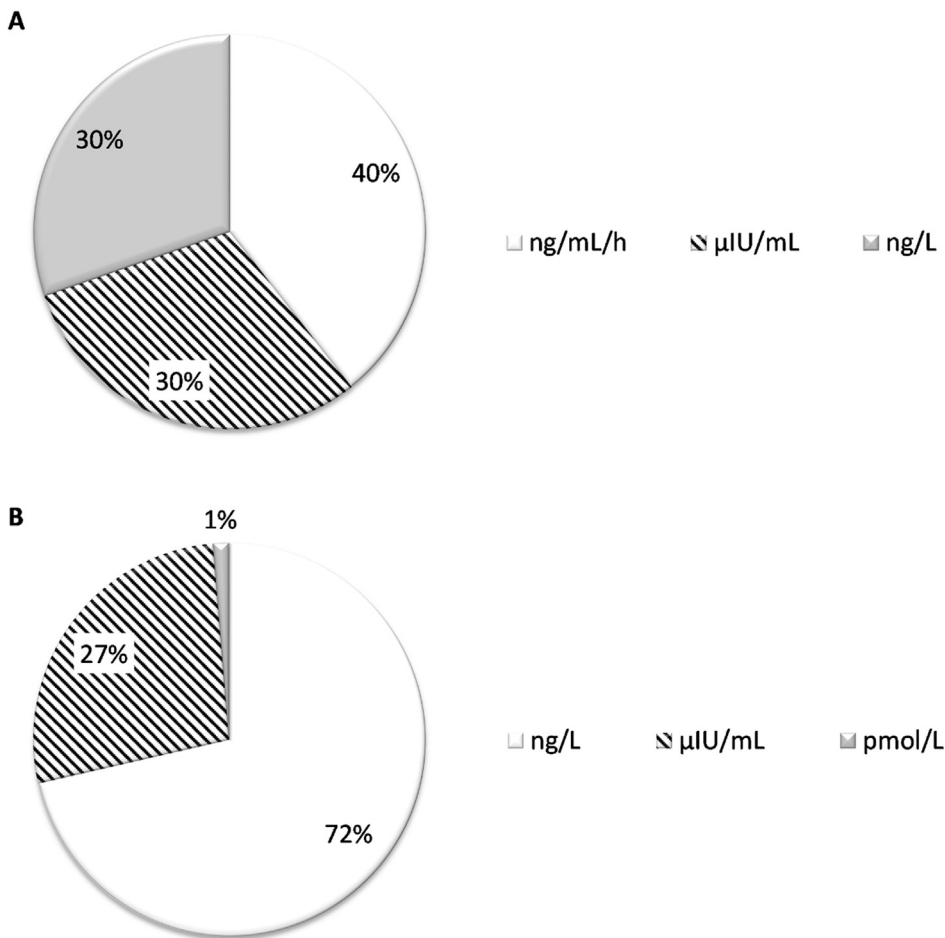


Fig. 5. Units in which renin activity (A; n = 33) and renin mass (B; n = 77) are reported.

activity assays in Canada.

Of the laboratories using renin mass assays, 72% report in units of ng/L (Fig. 5B). The next most common option is $\mu\text{IU}/\text{mL}$. Within the seven laboratories testing renin mass on-site, five report in units of ng/L and two use units of $\mu\text{IU}/\text{mL}$.

The total number of laboratories that provided units for the renin activity and mass assays was similar to the number of laboratories that indicated use of each type of assay.

3.4.4. ARR reporting

About two-thirds (65%) of the laboratories responded that they report an ARR. Fig. 6A shows the units used for ARR reporting. Of the laboratories that provided units for their ARR, most reported using pmol/ng or pmol/L/ng/L (which are equivalent) or pmol/L/ $\mu\text{IU}/\text{mL}$. Within the six laboratories that measure renin and aldosterone on-site, only four report an ARR, three in units of pmol/ng or pmol/L/ng/L and one in units of pmol/L/ $\mu\text{IU}/\text{mL}$.

The total number of laboratories that submitted ARR units was lower than the number of laboratories reporting an ARR. Several (10%) laboratories mistakenly commented that the aldosterone-renin ratio is a ratio and therefore does not have units. This is not correct. The units associated with the aldosterone and renin measurements are different from each other, so they do not cancel out; therefore, there are units associated with the ratio. As indicated, there are multiple options for the units, and the values for the ARR can vary considerably depending on the units used. It is important for clinicians to understand the units used as they may compare the patients' ARR values against clinical practice guidelines or other publications when interpreting results. At least one app has been created to help health care providers use electronic devices to calculate the ARR based on the units used at their sites to report aldosterone and renin results [26]. It is best if laboratory information systems are set up to calculate the ARR and apply a cut-off appropriate for the units used to reduce the burden on health care providers of calculating this themselves and to reduce the chance of errors in interpretation.

One important factor in calculating an ARR is that the specimens for renin and aldosterone testing should be collected at the same point in time for the ARR to be meaningful. The survey did not address whether the laboratories have policies related to paired collection

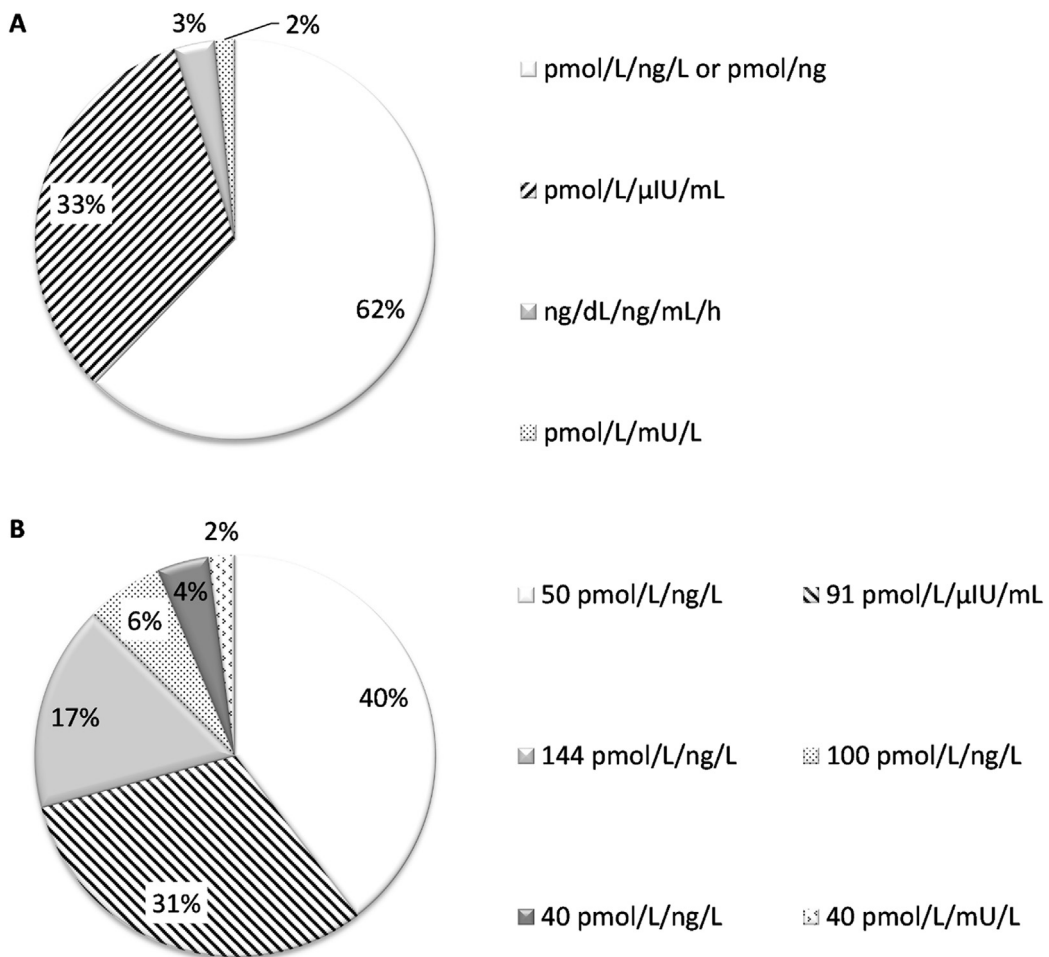


Fig. 6. A) Units in which the ARR is reported (n = 61). B) ARR cut-offs used by surveyed laboratories to screen for PA (n = 48).

and/or the time window allowed between collection of samples for renin and aldosterone testing.

3.4.5. ARR cut-off to screen for PA

Of the cut-offs provided by laboratories, 50 pmol/L/ng/L (40%) was the most common, followed by 91 pmol/L/ μ IU/mL (31%) and 144 pmol/L/ng/L (17%) (Fig. 6B). Some laboratories report in pmol/L/ng/L and some report in pmol/ng, which are mathematically equivalent; for the purposes of this analysis, both of these units were called pmol/L/ng/L to make the data more comparable. Of the four laboratories that perform testing for renin and aldosterone on-site and report an ARR, each had a different cut-off: 50 pmol/L/ng/L, 100 pmol/L/ng/L, 144 pmol/L/ng/L, and 91 pmol/L/ μ IU/mL.

The ARR cut-off used to screen for PA will have a significant impact on the sensitivity and specificity of the test. A lower cut-off will have a higher sensitivity at the cost of decreased specificity, while a higher cut-off will have lower sensitivity and increased specificity. Since the ARR is used as a screening test, it is desirable to detect as many patients with PA as possible. Confirmatory testing such as oral sodium loading, saline infusion, fludrocortisone suppression, and captopril challenge tests can then be used to distinguish patients who truly have PA from those who do not [1]. However, it is also desirable that the ARR cut-off not be set too low, which would generate unnecessary confirmatory testing. The optimal ARR cut-off is not well established and varies with factors such as patient posture and time of day at specimen collection [10]. While it is a very difficult task to complete, it would be ideal if institutions could establish or validate cut-offs based on the patient preparation protocols and assays used at their laboratories.

Although not addressed in this survey, some laboratories may have a policy to only calculate the ARR in patients with an aldosterone concentration above a certain cut-off. This is to avoid the situation of a patient with a very low renin level having an ARR above the PA screening cut-off despite not having a high aldosterone concentration. Including a minimum aldosterone cut-off would increase the positive predictive value of the ARR and reduce the likelihood of patients without PA undergoing confirmatory testing unnecessarily. Minimum aldosterone concentrations can vary considerably, e.g., 170 or 410 pmol/L [1]. Aldosterone thresholds at which to calculate an ARR can be as important as an ARR cut-off in determining the sensitivity and specificity of PA screening.

3.4.6. Source of ARR cut-off

Laboratories were asked to provide the source of their ARR cut-off. Where possible, we tried to identify the source of the ARR cut-off for laboratories that chose “Unknown/as per referral laboratory” if it was clear from their response which referral laboratory they use. The adjusted data (44 responses) reveal that there are two main sources of the ARR cut-offs being used by surveyed laboratories, the Endocrine Society clinical practice guidelines [1] (which suggest a cut-off of either 91 pmol/L/ μ IU/mL or 144 pmol/L/ng/L, depending on the units used) (54%) or an in-house determination confirmed with data from the German Conn’s database [27] and local patient data (42%). Of the four laboratories that measure renin and aldosterone on-site and report an ARR, two cut-offs were from the Endocrine Society clinical practice guidelines, one was a manufacturer’s recommendation, and one was an in-house determination confirmed with the German Conn’s database and local patient data.

3.4.7. Interpretation of renin, aldosterone, and/or ARR results

Laboratories were asked if they provide an interpretation of renin, aldosterone, and/or ARR results, and the majority (56%) responded that they do not. Similarly, 62% of laboratories that test for renin and/or aldosterone on-site responded that they do not provide an interpretation. Although the survey did not address in detail what laboratories are doing in this regard, it is possible that most laboratories provide only reference intervals for renin and aldosterone and a cut-off for the ARR, with flagging of results outside these limits, but leave individualized interpretations to the health care providers. The laboratories that do provide interpretations were not asked to provide their interpretive comments.

The survey did not include any questions about confirmatory testing for PA. Most confirmatory testing likely happens at tertiary care centres within clinical areas and it was felt that questions about confirmatory testing would be too specialized for most of the survey participants.

4. Conclusions

Only six of the 120 (5%) Ontario laboratories that responded to the survey perform renin and aldosterone testing on-site, with most laboratories collecting samples and sending them to referral laboratories for testing. Despite having a limited number of testing sites, the survey results reveal considerable variation in the pre-analytical, analytical, and post-analytical practices being used for these tests.

One of the limitations of the survey is that it did not specify which laboratory professional should complete the survey. Renin and aldosterone testing are specialized and it is possible that the laboratory staff completing the survey may not have had access to all the pre-analytical, analytical, and post-analytical information required. For instance, while the person completing the survey may not have been familiar with cryoactivation, others within the organization may have been and may have put procedures in place to protect against cryoactivation of renin. Similarly, the person completing the survey may not have known the source of the ARR cut-off used by the laboratory. Therefore, the responses may not accurately represent the laboratories’ processes in all instances. To the extent that it was possible to exclude or correct inaccurate data without contacting the responding laboratory for clarification, we have tried to do so. Despite this limitation of the data received, we believe the results provide an indication of the many differences and few similarities in laboratory practices concerning renin and aldosterone testing among surveyed laboratories. The survey results may help individual laboratories determine how their processes differ from their peers and how they can adjust processes to align with the current best laboratory practices for renin and aldosterone testing.

Several factors can impact the comparability of results between laboratories. International reference materials are available for renin

mass. In addition, there is a reference material for angiotensin I, the analyte commonly measured in renin activity assays. Such standards can aid assay manufacturers in achieving analytical agreement between their assays. To the best of our knowledge, no such reference material is available for aldosterone. However, there is more to screening for PA than the analytical phase of renin and aldosterone testing. Pre-analytical and post-analytical practices also affect result interpretation. The practices of Ontario laboratories with regard to renin and aldosterone testing are likely to be heavily influenced by the laboratories that perform these tests on-site and the instructions that they provide to referring laboratories. The significant variability in practices identified by this survey may reflect variability in the recommendations made by the testing sites themselves as well as a lack of comprehensiveness in the instructions provided to all the referring laboratories. A starting point to standardize pre-analytical procedures and result reporting for renin and aldosterone in Ontario could involve formation of a task force comprised of representatives from the testing sites. The information revealed by this survey could provide insight into the practices in need of standardization. Upon agreement of best practices, detailed instructions could be published and disseminated to referring laboratories with the goal of ultimately improving the quality of the renin and aldosterone results provided and the performance of the screen for PA.

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Author contributions

Angela C. Rutledge: Conceptualization, methodology, formal analysis, writing – original draft; **Dana Bailey, Ronald A. Booth, Pamela Edmond, Anna Johnston, Victor Leung, Kika Veljkovic:** Conceptualization, methodology, formal analysis, writing – review and editing

Declarations of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plabm.2021.e00229>.

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