

Opinion paper: deriving harmonised reference intervals – global activities

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ABSTRACT

Harmonisation of reference intervals (RIs) refers to use of the same or common RI across different platforms and /or assays for a specified analyte. It occurs optimally for those analytes where there is sound calibration and traceability in place and evidence from a between-method comparison shows that bias would not prevent the use of a common RI. The selection of the RI will depend on various sources of information including local formal RI studies, published studies from the literature, laboratory surveys, manufacturer's product information, relevant guidelines, and mining of databases. Pre-analytical and partitioning issues, significant figures and flagging rates, are assessed for each analyte.

Several countries and regions including the Nordic countries, United Kingdom, Japan, Turkey, and Australasia are using common RIs that have been determined either by direct studies or by a consensus process. In Canada, the Canadian Society of Clinical Chemists Taskforce is assessing the feasibility of establishing common reference values using the CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) and CHMS (The Canadian Health Measures Survey) databases as the basis. Development of platform-specific common reference values for each

of the major analytical systems may be a more practical approach especially for the majority of analytes that are not standardised against a primary reference method and are not traceable to a primary or secondary reference material.

We encourage laboratories to consider adopting reference intervals consistent with those used by other laboratories in your region where it is possible and appropriate for your local population. Local validation of the adopted

reference interval is also recommended as per CLSI guidelines.



INTRODUCTION

Despite studies having shown that the variation in reference intervals (RIs) for chemistry analytes may be greater than the analytical inaccuracy of the measurement, differences in RIs persist between laboratories that use the same

Table 1 Sequence of events to derive and validate common reference intervals (RIs) through an evidence-based approach and extensive data analysis

Identify problem

Agree to address common RIs

Identify relevant groups

Seek formal co-operation (if external bodies involved)

Form working group

Describe problem in detail

Allocate a budget and determine sources of funding

Gather information (surveys, RI studies, data mining, bias study, calibration traceability, RI verification laboratory information, flagging rates)

Consider solutions

Produce discussion paper, etc.

Seek feedback from stakeholders

Revise recommendations

Obtain formal endorsement

Publish

Promote

Monitor introduction

platforms and the same reagents (1-3). This has implications for result interpretation and patient outcomes where the same values may be interpreted differently due to differences in RIs or decision limits hence leading to inappropriate over- or under-investigation or treatment of the patient.

One way to overcome this situation is to use the same interval. Harmonisation of RIs refers to use of the same or common RI across different platforms and /or assays for a specified analyte. Importantly, harmonisation of RIs occurs optimally for those analytes where there is sound calibration and traceability in place and evidence from a method comparison study shows that bias would not prevent the use of a common RI. The advantages of using a harmonised RI are less confusion and misinterpretation of results for both doctors and patients. Irrespective of the pathology provider or the method, provided the same RI, unit and

terminology are used, an individual patient's results can then be amalgamated.

An organisational plan is required before setting out on the sequence of practical processes that are required to achieve a major national change in pathology RIs. This is not a trivial matter and the importance of a structured approach cannot be overemphasised. Table 1 outlines the sequence of steps required to derive and validate common RIs that was used for the Australasian RIs study (3). The four key areas are: 1) seeking the evidence; 2) consultation; 3) verification; and 4) implementation (Fig.1 A and 1B). The Australasian Association of Clinical Biochemists (AACB) and the Royal College of Pathologists of Australasia (RCPA) invited pathologists and medical scientists to harmonise RIs at the same time as other RCPA initiatives for standardisation of pathology units, terminology, and report formatting and flagging were being undertaken (4). The input by main stakeholders, i.e. pathologists, scientists, clinical societies and government

Figure 1A Implementation plan for the introduction of adult common reference intervals

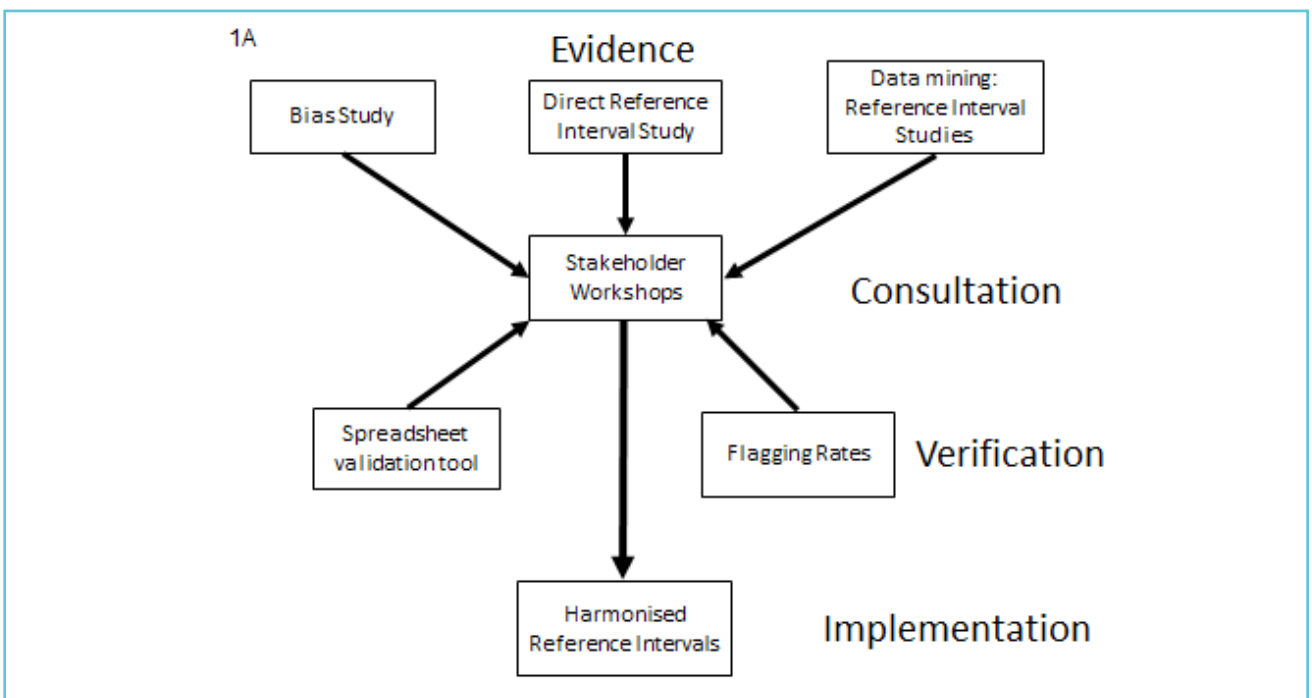
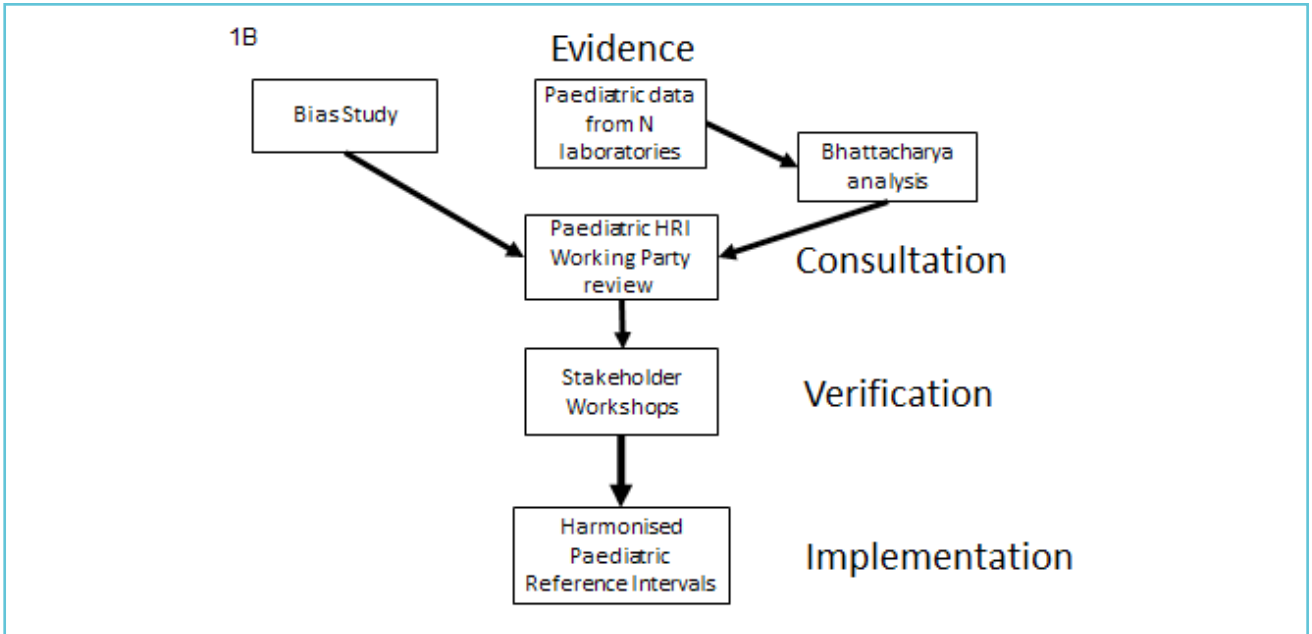


Figure 1B Implementation plan for the introduction of paediatric common reference intervals



bodies, is central to the success of any harmonisation project and can provide helpful advice and guidance as was the case for the UK Pathology Harmony RIs project (5,6).

REQUIREMENTS FOR USE OF HARMONISED REFERENCE INTERVALS

Seeking the evidence is paramount to the implementation of common RIs. One such approach used in Australasia to assess the feasibility of using common RIs was an evidence-based checklist approach. (7). It was based on the following criteria (8):

1. Define analyte (measurand)
2. Define assays used, accuracy base, analytical specificity, any method-based bias
3. Consider important pre-analytical differences, and actions in response to interference
4. Define the principle behind the RI (e.g. central 95%)
5. Describe evidence for selection of common RIs

- data sources (literature, lab surveys, local RI studies, manufacturers' product information)
 - data mining
 - bias goal as quality criterion for acceptance
6. Consider partitioning based on age, sex, etc.
 7. Define degree of rounding
 8. Consider the clinical implications of the RI
 9. Consider use of common RI
 10. Document and implement

An example of the checklist approach is shown for creatinine (Table 2).

Assessment of method differences

Bias study

Any significant method bias will result in misclassification of too many patients. The expected information derived from the combination of assay and RI must meet the appropriate clinical sensitivity and specificity required for each test. Hence a key requirement for the use

Table 2 Checklist reference interval (RI) approach for creatinine

Analyte	Creatinine (plasma and serum)
Population RI	Based on healthy subjects not hospital patients. eGFR used for decision making.
Units	µmol/L
JCTLM-listed traceability or preferred method and reference material	ID-GC/MS and ID-LC/MS (some methods require instrument factors). SRM 914 (pure creatinine). SRM 909, 967 (human serum).
Pre-analytics	
1. Serum/plasma	1. Interchangeable.
2. Sample collection	2. Increases with meat consumption.
3. Interferences	
Analytical differences	Analytically there are no differences.
Partitioning by	
1. Gender	1. Gender differences.
2. Age	2. Age-related increases above 60 years not agreed by Renal Physicians.
Reporting Interval	1 µmol/L

of common RIs is the effect of methodological differences on bias and if this would affect the sharing of a common RI. Method differences are best assessed for bias using commutable patient-based samples. In the case of the Australasian Harmonised RI study specified performance limits based on biological variation were applied to determine whether bias would prevent the use of a common RI by assessing if all results fell within the allowable limits of agreement and if regression lines

were all within allowable limits for the tested measurement procedures (10). The allowable limits of performance or allowable error specify that the imprecision and bias of a method must be within stated limits. Of 27 tested analytes among eight platforms/assays, 19 gave acceptable bias for a common RI (11). Note that where a RI is shared the analytical variation for more analysers in more laboratories using more methods will be larger than a singly-derived interval, resulting in a wider RI (12).

Calibration traceability

An initial assessment of methodology and calibration traceability of laboratory assays to be used to establish the common RI is required. Laboratories need to assess the traceability claims made by manufacturers including the reference material and reference measurement procedures used to assign values to master calibrators from which product calibrators are traceable in routine assays. Preliminary information can be gathered from the manufacturer, external quality assurance (EQA) programs and other published data. If a laboratory uses a method known to be biased compared with the method used to set the RI, a common RI cannot be used. Rather, for analytes with established traceability, traceable assays should be used to both set and to use the interval (13). Ideally, analytes should have a complete reference measurement system or a reference material and/or a reference measurement procedure listed on the Joint Committee for Traceability in Laboratory Medicine (JCTLM) website (14).

Selection of reference intervals

Various sources of information on RIs should be searched including local formal RI studies, published studies from the literature, laboratory surveys, manufacturer's product information, relevant guidelines, and mining of databases. Pre-analytical and partitioning issues, significant figures and flagging rates, which provide an indication of the clinical considerations of the RI, should also be assessed for each analyte.

Common laboratory usage

A survey of local laboratories ideally through the national EQA provider provides the opportunity for laboratories to compare their RIs with those from other laboratories using the same and different methods. By linking RIs to results from measurements on commutable samples, it is also possible to see the effect of

the intervals on between-laboratory differences. For the majority of common chemistry analytes the between-laboratory variation in RIs is usually greater than the variation in results (15). These types of data can be used to support the use of common RIs for many analytes.

Published studies

The Nordic Reference Interval Project (NORIP) established common RIs in apparently healthy adult populations from five Nordic countries for 25 of the most common clinical chemistry analytes (16). Results were traceable to higher-order reference measurement systems. More recently Nordic paediatric RIs have been determined for 21 common biochemistry analytes and intervals were suggested for combined age groups (17). In the United Kingdom, reference limits have been established by a survey of RIs in use followed by an assessment of analytical variability, any age and sex related variation, or other variances in populations where these were seen as relevant to the analyte (5,6). The aim was to remove unnecessary variation that was demonstrated to lack scientific validity prior to taking on new work to formally validate the consensus RIs (6).

Global formal reference interval studies

The CALIPER Initiative

The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) (18) was established by a Canadian team of investigators to develop a new database of biomarker reference values (stratified by age, sex and ethnicity) determined from a large, healthy population of community children and adolescents. The CALIPER project was initiated as a result of several detailed gap analyses evaluating the availability of pediatric RIs in four clinical subspecialties: bone markers (19), risk markers for cardiovascular disease and metabolic syndrome (19,20), hormones of the thyroid and growth

hormone axes (21), and markers of inborn errors of metabolism (22). These analyses revealed major gaps in data available to clinical laboratories and paediatricians and highlighted the critical need for new initiatives. Since its inception in 2009, the CALIPER program has made considerable strides in establishing and publishing a new RI database for biochemical markers (23-33), however, the reference values were initially established on a single analytical system, the Abbott Architect assay system. To address this limitation, a series of transference studies (34-37) have recently been completed by the CALIPER program, allowing transference of paediatric reference values from the Abbott database to four other major analytical systems including Beckman, Ortho, Roche, and Siemens. Additional transference studies are in progress to complete transference of the entire CALIPER RI database to all major chemistry assay systems allowing widespread application of CALIPER reference standards in clinical laboratories worldwide using any one of the five major biochemical assay systems.

Canadian Health Measures Survey (CHMS)

The Canadian Health Measures Survey (CHMS) is the most comprehensive, direct health measures survey ever conducted in Canada. The study was launched in 2007 by Statistics Canada, in partnership with Health Canada and the Public Health Agency of Canada, to collect population-representative health information from Canadians aged 3-79 years. An initial household interview collected information about general health including nutrition, smoking habits, alcohol use, medical history, physical activity, and socioeconomic variables. Respondents then visited a mobile examination centre, where direct physical measures of health were taken, such as height, weight and blood pressure, and blood specimens were collected and analysed for biomarkers of health and disease (25). Individuals

were selected in a systematic manner to be representative of 96.3% of the Canadian population. Data from CHMS samples were then weighted to ensure that the study population was truly representative of age, geographical distribution and ethnic origin of the Canadian population. In a recent collaboration between CALIPER and CHMS, laboratory data from approximately 12000 Canadian children and adults were used to establish a comprehensive database of paediatric and adult reference intervals for 24 chemistry (38), 13 endocrine/special chemistry (39), and 16 haematology markers (40). These reference intervals provide a valuable description of the changes in key biochemical parameters within the Canadian population. The use of common patient selection, pre-analytical, analytical and post-analytical methods allowed for assessment of fluctuations in 'normal' levels over time and prevalence of disease risk factors. Together, these studies provide a comprehensive description of the changes in important biomarkers within the Canadian population throughout the course of a lifetime, from childhood to adulthood to geriatrics.

The CALIPER and CHMS initiatives also provide a unique opportunity to strive towards establishment of common RIs across Canada. A taskforce has recently been developed by the Canadian Society of Clinical Chemists and discussions have begun among a number of opinion leaders across the country to assess the feasibility of establishing common reference values using the CALIPER and CHMS databases as the basis. The Canadian common reference interval initiative is also examining the potential development of platform-specific common reference values for each of the major analytical systems. This may be a more practical approach especially for the majority of analytes that are not standardised based on primary reference method and not traceable to a primary or secondary reference material.

Asian Studies

Although not attempting to define population reference intervals, Ichihara et al. (41) found unexpectedly large variations between the results obtained from samples sourced from 6 Asian cities (Hong Kong, Shanghai, Seoul, Kuala Lumpur, Taipei and Tokyo) for the 13 analytes tested suggesting that harmonised RIs would be difficult between these countries that these cities represent .

In contrast to the study by Ichihara, a Japanese multicentre study by Yamamoto et al. (42) involving 105 laboratories across Japan and using 4 different chemistry platforms demonstrated no regional differences and concluded that the RIs established in this study were also suitable for adoption nationwide (Table 3).

Turkish Study: Similar to the Japanese study, a multi-centre study by Ozarda et al. (43) determining RIs for 25 commonly tested analytes showed similar results between the seven Turkish geographical regions in 28 laboratories where the samples were sourced. They concluded that the intervals determined by this study using the same Architect 8000 analysers were suitable for use in all Turkish clinical chemistry laboratories that used the same platforms (Table 3).

Australian Study

The Aussie Normals study was a formal reference interval study of 1876 male and female healthy adult Australians in the age group 18 to 95 years (44). Up to 91 biochemistry analytes were measured by Abbott Architect analysers. Partitioning was done according to the effects of gender, age and body mass index (BMI) on these RIs. For the most part these differences were statistically small such as for lactate dehydrogenase and phosphate where they were less than day to day biological variation. As shown in Table 3, reference intervals for the Aussie

Normals formal RI study were in general similar to those for the Australasian common RIs study although somewhat tighter as they were determined using one platform only. However, γ -glutamyltransferase (GGT) upper reference limits were notably higher in the Aussie Normals study which demonstrated BMI differences with increasing age in men and women (44). For the 18-<45y age group and BMI <25 kg/m², GGT was 12-37 U/L for men and 9-38 U/L for women. However, it is difficult to adopt RIs in association with BMI at this stage as this parameter is not routinely provided to the laboratory.

Data mining

Expert groups can provide RI information through their data mining of millions of data points from primary care patients. This method has advantages over the direct RI validation process by providing large amounts of data on the local population being tested and reflects the actual analytical and pre-analytical conditions for the tested population. This approach is valid only if there is a majority of results from the primary care population such that the healthy distribution of values can be clearly identified in the midst of a smaller number of non-healthy values. Bhattacharya analysis to determine underlying distributions in the presence of outlier results can be used to assess proposed RIs. For example, in Australasia data mining of over 200,000 paediatric data points provided by 15 laboratories for the main general chemistry analytes from birth to 18 years of age was used for establishing partitioned paediatric RIs (3).

Final selection of the common reference interval

One approach to the setting of a common RI that was used in Australasia is described as follows. The starting point to develop a common RI was to do a national survey of laboratory RIs and determine the predominant RI in use. Then

a method comparison study across the major chemistry platforms using commutable samples from healthy subjects was used to assess if bias would prevent use of a common RI with acceptability based on the specified allowable limits of performance such as those based on biological variation for example (11). For analytes where bias may prevent use of a common RI for one or two main platforms, it may be possible for other platforms to share a common RI, e.g. lactate dehydrogenase methods that use pyruvate to lactate [P to L] rather than the IFCC-recommended [L to P] method cannot be combined.

The next step involves gathering supportive data for the proposed common RI using data from formal local RI studies, if available, and from data mining. In Australia values from the Aussie Normals adult RI study were used to confirm the common RIs recommended for use in Australia and New Zealand (44). Note that reference intervals are wider for the common RIs that have been established for eight platforms compared with those obtained using the one platform; inclusion of between-method variation results in wider intervals than for a singly-derived RI (Table 3). Further mining of hundreds of thousands of data points from primary care patients who are relatively healthy was then employed to show the biochemical physiology from childhood to adulthood through to geriatric age, according to age and gender (45). In order to compare partitioning according to the continuous variables of age and pregnancy, and whether merged or separate partitions will affect clinical outcomes, there must be an understanding of the physiological processes affecting an analyte. Without the knowledge of clinical outcomes and their association with partitioned RIs, the lesser approaches of clinical opinion, statistics or laboratory consensus are used to determine the suitability of partitioning (45).

Once RIs are agreed upon, the proposed reference limits should be supported by flagging rates which provide an indication of the clinical considerations of the RI. Excess flagging of results can lead to inappropriate testing due to decreased specificity of the RI. Horowitz suggests that laboratories should be mindful of excess partitioning which is due to minor changes in physiology not to pathology (46). Hence local laboratories should assess flagging rates to determine if a change to historical RIs will create higher flag rates. For example, the pre-analytical effect of delayed sample transport would impact on potassium levels and hence for pragmatic reasons laboratories may choose to have a higher upper reference limit (URL) of 5.5 mmol/L rather than 5.2 mmol/L (Fig. 2A) (3).

Final agreement by a majority of stakeholders is required to support the selected common RI and a laboratory's intention to implement it, as described in the next section. The consensus process for deriving common RIs is not perfect and there are limitations. As noted above, intervals are usually wider than for singly-derived RIs obtained on the same platform, pre-analytical issues can cause elevated flagging rates, and elevated BMI in the population is not factored into clinical interpretation by the routine laboratory of GGT for example. Traceable analytes with JCTLM-listed reference materials and reference measurement procedures are more likely to share common RIs. However, countries may not be using IFCC recommended methods for enzymes as is the case in Australia where non-pyridoxal-5'-phosphate (P5P) AST and ALT methods are predominantly in use (Table 3). A harmonised RI with non-P5P methods is better than no harmonised RI and a future goal is for Australian laboratories to use P5P methods for AST and ALT.

Table 3 Adult reference intervals (RIs) for chemistry analytes determined by direct RI studies or by consensus

Analyte	Unit	Australia ⁴⁴	Turkey ⁴³	Nordic countries ¹⁶	United Kingdom ⁵	Japan ⁴²	Canada ³⁸	Australasia ³
		Cat 2a Direct	Cat 2a Direct	Cat 2a Direct	Cat 4 Consensus	Cat 2a Direct	Cat 2a Direct	Cat 4 Consensus
		Architect	Architect	Multiple platforms	Multiple platforms	4 main platforms	Architect	8 main platforms
Sodium (M)	mmol/L	136-145	137-144	137-145	133-146	137-144	16-49y: 137-142	135-145
							50-79y: 136-143	
Sodium (F)	mmol/L	136-145	137-144	137-145	133-146	137-144	16-49y: 137-143	135-145
							50-79y: 136-143	
Potassium	mmol/L	3.7-4.9	3.7-4.9	3.6-4.6	3.5-5.3	3.6-4.8	3.8-4.9	3.5-5.2
Chloride	mmol/L	101-110	99-107	-	95-108	101-108	30-79y: 102-108	95-110
Bicarbonate	mmol/L	20-29*	-	-	22-29	-	19-26	22-32
Creatinine (M)	µmol/L	<75y: 65-103	59-92	60-100	60-100	57-94	16-79y: 63-102	60-110***
		75+y: 47-120						
Creatinine (F)	µmol/L	<75y: 54-83	50-71	50-90	60-100	41-69	17-79y: 49-85	45-90***
		75+y: 40-91						

Calcium (M)	mmol/L	2.19-2.56	2.15-2.47	2.15-2.51	2.2-2.6 (adjusted)**	2.2-2.5	20-39y: 2.28-2.60 40-79y: 2.24-2.56	2-10-2.60
Calcium (F)	mmol/L	2.19-2.56	2.15-2.47	2.15-2.51	2.2-2.6 (adjusted)**	2.2-2.5	20-39y: 2.24-2.53 40-79y: 2.24-2.56	2-10-2.60
Magnesium	mmol/L	0.77-1.04	0.77-1.06	0.71-0.94	0.7-1.0	0.7-1.0	-	0.7-1.1
Phosphate (M)	mmol/L	0.83-1.36	0.80-1.40	<50y: 0.75-1.65 50+y: 0.75-1.35	0.8-1.5	-	16-47y: 0.95-1.52 48-79y: 0.89-1.52	0.75-1.50
Phosphate (F)	mmol/L	0.88-1.44	0.80-1.40	0.85-1.50	0.8-1.5	-	16-47y: 0.95-1.52 48-79y: 0.99-1.54	0.75-1.50
LDH (M)	U/L	130-230	126-220	<70y: 105-205 70+y: 115-255	-	124-226 [JSCC]	-	120-250 (L-P [IFCC])
LDH (F)	U/L	122-232	126-220	<70y: 105-205 70+y: 115-255	-	124-226 [JSCC]	-	120-250 (L-P [IFCC])
CK (M)	U/L	<45y: 52-340 45-65y: 55-357 65+y: 49-207	48-227	<50y: 50-400 50+y: 40-280	40-320	61-257 [JSCC]	-	<60y: 45-250 60+y: 40-200

CK (F)	U/L	<45y: 37-247	34-131	35-210	25-200	43-157 [JSCC]	-	30-150
		45-65y: 39-230						
		65+y: 36-190						
ALP (M)	U/L	<75y: 43-112	43-116	35-105	30-130	122-330 [JSCC]	16-21y: 56-167	30-110
		75+y: 42-126					22-79y: 50-116	
ALP (F)	U/L	<45y: 32-96	<50y: 34-97	35-105	30-130	104-299 [JSCC]	16-29y: 44-107	30-110
		45-75y: 40-132	50+y: 47-133				30-79y: 46-122	
		75+y: 44-146						
ALT (M)	U/L	<75y: 11-41	9-57	10-70		10-42 [JSCC]	18-49y: 18-78	5-40 (no P5P)
		75+y: 9-48					50-79y: 20-62	
ALT (F)	U/L	<75y: 9-35	7-28	10-45		7-27 [JSCC]	12-49y: 14-41	5-35 (no P5P)
		75+y: 8-33					50-79y: 16-44	
AST (M)	U/L	<75y: 14-36	13-30	15-45		14-32 [JSCC]	18-54y: 18-54	5-35 (no P5P)
		75+y: 14-34					55-79y: 18-39	
AST (F)	U/L	<75y: 13-31	11-25	15-35		12-27 [JSCC]	20-54y: 18-34	5-30 (no P5P)
		75+y: 14-35					55-79y: 18-39	

GGT (M)	U/L	<45y: 9-63	11-69	<40y: 10-80		12-65 [JSCC]	20-35y: 12-62	5-50
		45-75y: 13-72		40+y: 15-115			36-79y: 13-109	
		75+y: 15-78						
GGT (F)	U/L	<45y: 9-49	7-33	<40y: 10-45		9-38 [JSCC]	18-35y: 12-38	5-35
		45-75y: 9-55		40+y: 10-75			36-79y: 10-54	
		75+y: 9-57						
Total Protein	g/L	62-79	66-82	62-78	60-80	66-80	20-29y: 65-83	60-80
							30-79y: 65-78	
Total Bilirubin (M)	µmol/L	5-20	3.8-24.1	5-25	<21	6.4-24.8	16-48y: 3-18	1-20
							49-79y: 2-20	
Total Bilirubin (F)	µmol/L	5-21	2.7-15.9	5-25	<22	6.4-24.8	16-48y: 1-16	1-20
							49-79y: 1-17	

* Bicarbonate measured prior to Abbott recalibration; ** Calcium is adjusted for albumin;
 *** Creatinine has harmonised RIs for adults up to the age of 60 y.

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Cat: category according to Stockholm Hierarchy; CK: creatine kinase; GGT: γ -glutamyltransferase; IFCC: International Federation of Clinical Chemistry and Laboratory Medicine; JSCC: Japan Society of Clinical Chemistry; LDH: lactate dehydrogenase; P5P: pyridoxal 5'-phosphate.

FINAL ACCEPTANCE, ADOPTION AND IMPLEMENTATION OF HARMONISED REFERENCE INTERVALS

Communication and discussion by all stakeholders

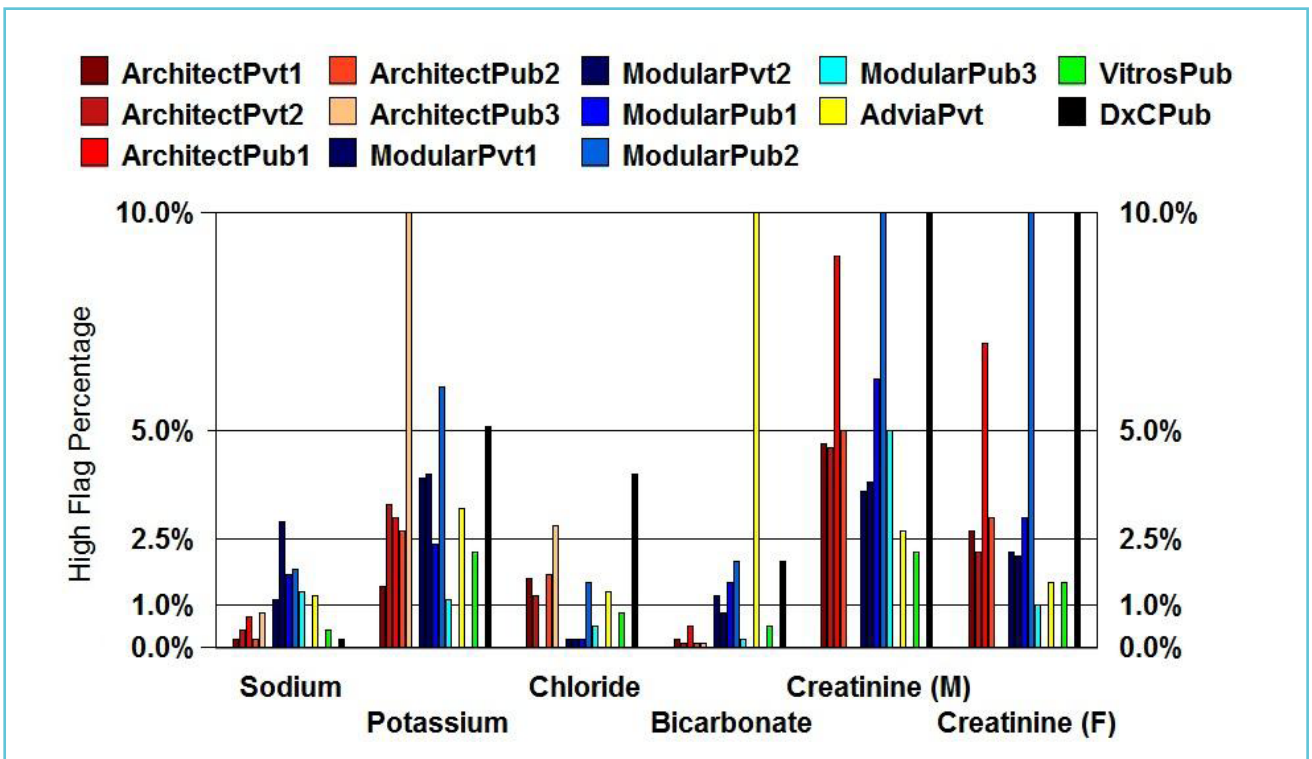
Laboratory acceptance should be sought at a national level prior to introduction of common RIs. Various approaches can be used to assess the likely adoption rates for the panel of RIs including a survey as to whether the laboratory is using the common RI already,

would accept the RI, or ask for comments and their reason if they do not accept the common RI. Representation is required from the whole nation and from public and private pathology, small and large laboratories and networks if harmonised RIs are to have any chance of being implemented. National acceptance of a change to pathology RIs requires that there is an on-going discussion by all involved stakeholders especially those at the highest management level who are responsible for patient pathology results and their interpretation. Harmonisation workshops provide a forum for presenting and discussing the evidence and reaching a consensus decision.

Validation of reference intervals by local laboratories

Responsibility for adoption of common RIs lies with each laboratory. Advice on how to do this is found in guidelines from the Clinical and Laboratory Standards Institute (CLSI) (47). Key questions are: 'Is this RI suitable for my method and for my population?' Validations of RIs may be by subjective assessment assuming the same method and the same population are used or by a simple validation using 20 normal subjects representing the local population (47,48). Alternatively, you can mine your laboratory's existing data. The most useful parameter is the midpoint of the extracted data, which can be used to assess

Figure 2A Typical high flagging rates for the first measurement in outpatient adults (18y – 60y) for sodium, potassium, chloride, bicarbonate, creatinine (M), creatinine (F)



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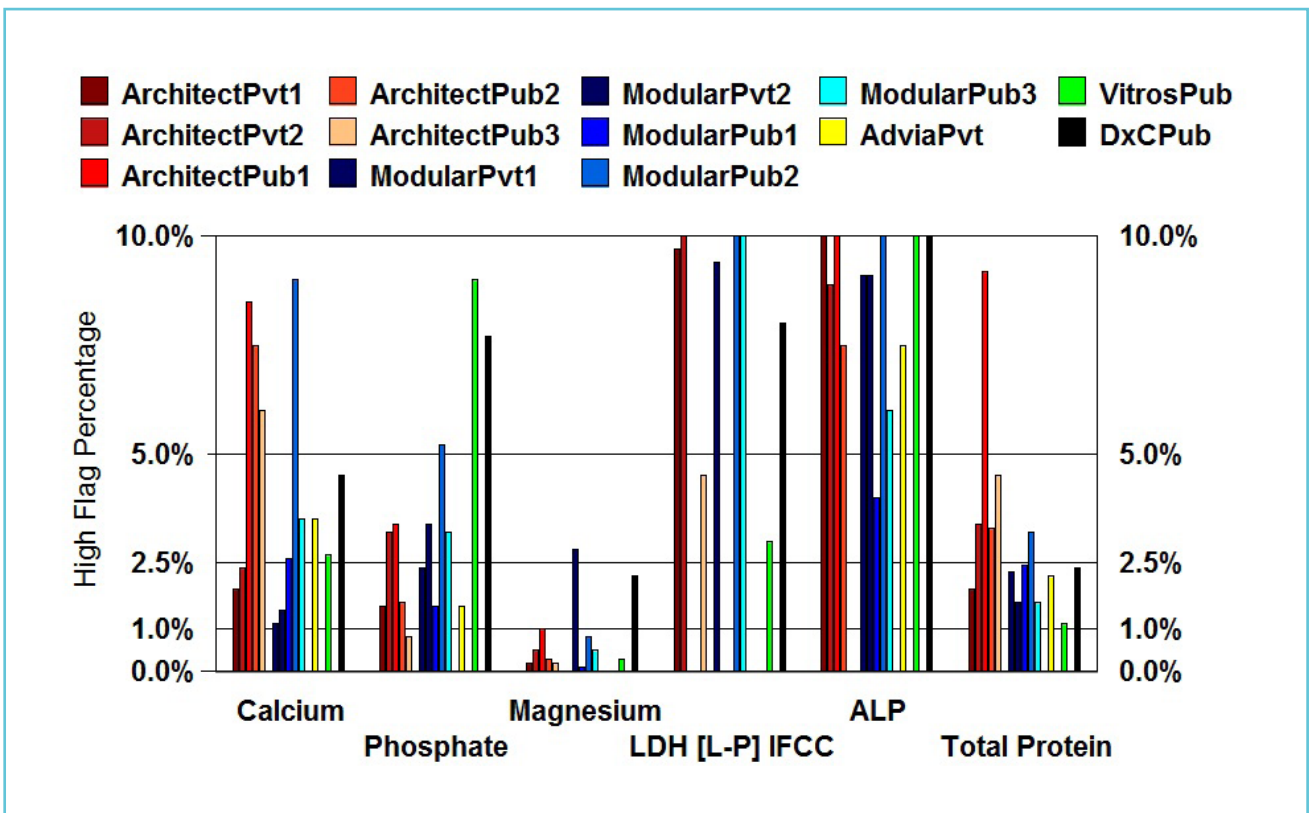
analytical or population bias by comparison with the corresponding midpoint of the data used to set the reference interval. Bhattacharya analysis can also be used to assess the proposed intervals (12).

Validation of flagging rates for local population

Based on the principle of minimum, desirable and optimal categories used to define allowable bias limits, flag rates may range from 1.0% to 1.8% for low flagging rates, and 5.7% to 3.3% for high flagging rates, respectively. Flag rates however, may be quite complex to interpret depending on the population used to derive them. For example in the

Australasian common RIs project, a URL of 110 U/L for alkaline phosphatase may result in a flag rate of 7-8% (3). However, the clinical benefit of using the URL of 110 U/L is to detect pathology in postmenopausal women. Increasing the URL to 115 U/L did not have any significant impact due to the logarithmic distribution of reference values. In contrast, the flag rate at the URL for sodium was 1% indicating that hypernatraemia is uncommon (Fig. 2A and 2B). Data mining of local population values also allows for an assessment of the expected number of results outside the RI (12). The laboratory can then compare the expected flagging rates with their current rates.

Figure 2B Typical high flagging rates for the first measurement in outpatient adults (18y – 60y) for calcium, phosphate, magnesium, lactate dehydrogenase, alkaline phosphatase, total protein



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CLOSING THE HARMONISED REFERENCE INTERVAL LOOP

Following the endorsement of common RIs by pathologists and scientists, formal endorsement by the profession is sought from the National Pathology College and National Clinical Chemistry, Biochemistry or Laboratory Medicine Society. Support by the National Testing Authorities for Laboratory Medicine, who should be included in meetings on harmonisation, is via formal recommendations to laboratories that they use these intervals, or if not, to provide supporting evidence for other references.

Continuing work is required to produce and validate common RIs, to manage ongoing issues, e.g. problems with implementation of RIs by the local laboratory Information Technology unit into the Laboratory Information System. These issues may be changes to reporting units, significant figures, rounding, report formatting, etc. Consultation with clinical societies and education of local clinicians are imperative if the new RIs are to be used. Other flow-on effects can be those regarding the reimbursement of pharmaceutical benefits according to national government benefit schemes that use specific RIs or decision limit values when assessing the provision of a treatment drug.

The level of uptake of common RIs can be readily surveyed through EQA programs. One such scheme that also surveys the bias of methods within the reference interval measuring range by using commutable samples from healthy subjects is the RCPA Quality Assurance Program Liquid Serum Chemistry program (15). The scheme allows assessment of the between-laboratory variation in results, RIs and the information transmitted by the combination of these factors. For most common chemistry analytes, use of common RIs has improved the variation seen in the information produced by different laboratories.

CONCLUSION

Consideration should be given by laboratories to adopting RIs consistent with those used by other laboratories in the region where it is possible and appropriate for the local population. These may be common RIs for use across several major platforms in the region, e.g. United Kingdom, Nordic countries, Japan, Australasia, or for use with one specific platform, e.g. Canada, Asia, Turkey. Scientific evidence supports the use of common RIs for many general chemistry analytes especially those with sound calibration and traceability in place. For other non-harmonised immunoassay analytes where either there is currently no secondary reference material or reference measurement procedure for value assignment, it seems logical to use platform-specific RIs and decision limits across regions, provided that laboratories have acceptable assay precision, until such time when methods become harmonised internationally.

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