Interpreting pathology tests
Results and reference intervals

PAT PHILLIPS MB BS, ma(Oxon), FRACP, MRACMA, GradDipHealthEcon(UNE)

Laboratory reference intervals are the basis for the interpretation of laboratory results, and are discussed in this first article of a series of four aiming to provide a framework for the reviewing of test results.

Interpreting laboratory results requires more than relying on the laboratory to tell us whether the test is positive or on scanning the results for the laboratory's asterisks indicating the abnormal results. Sometimes we want to know if a test reported as positive is a true positive for the problem being tested or a false positive in someone without the problem. We may also want to know how abnormal an abnormal result is and whether a difference between consecutive results indicates a real change (a 'signal') or background result variability (the 'noise'). The clinical context should, of course, be taken into account when test results are being considered, and the laboratory's interpretation should not be relied on completely.

MedicineToday 2014; 15(11): 64-68

Dr Phillips is a Consultant Endocrinologist at the QE Specialist Centre, Adelaide, SA

This article is the first in a series of four articles about interpreting laboratory results and focuses on the reference intervals for these results. (The term 'reference interval' is now preferred to 'reference range'.) The article discusses:

- when laboratory reference intervals may not apply to a particular patient or particular specimen
- the degree of abnormality of results that lie outside the reference interval
- how to compare two different tests assessing the same physiological process
- when a result within the reference interval may be physiologically abnormal for an individual and when a result outside the reference interval may be physiologically normal for an individual.

Future articles will discuss variability, sensitivity, specificity and predictive value, and a case study will be presented to illustrate the practical application of the framework.

WHEN THE REFERENCE INTERVAL MAY NOT APPLY

The reference interval implemented by the laboratory for a particular test may be provided by the manufacturer of the measuring kit, derived by the laboratory or developed from other sources.[1] In all cases, however, it applies to a population of people who are similar to those from whom samples were taken and to samples collected under the same conditions (usually fasting, seated and in the morning). Although the reference limits placed in laboratory reports generally reflect a population reference interval, they may represent clinical decision points (e.g. lipid levels, glucose levels) or therapeutic intervals (e.g. drug concentrations, INR).

Examples of when reference intervals may not apply

- Indigenous Australians often have high serum globulin values outside the reference interval of the testing laboratory because the reference interval was derived from a non-Indigenous population in whom globulin levels are lower.

- Prolactin values are higher after meals and both early in the morning and later in the day, and results for an individual may lie outside the reference interval because the sample was not collected under the appropriate conditions rather than because of pathology.
- A random urine albumin to creatinine ratio test may give a high result because the urine in the specimen was not produced when the person was fasting, supine and inactive (i.e. as applies to a first voided urine specimen). Food, erect posture and exercise can all increase glomerular pressure, albumin filtration and urine albumin excretion. A result collected under nonideal conditions that is within the reference interval can be accepted as normal; however, an elevated result should be repeated on the appropriate specimen.

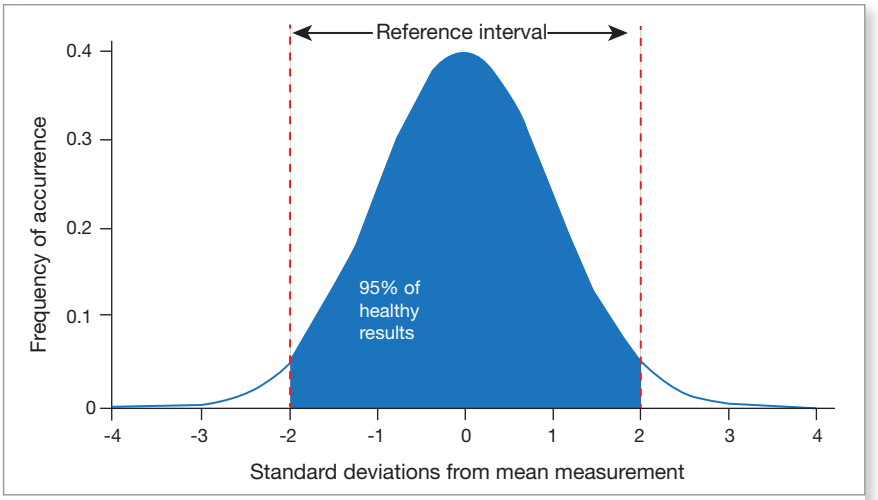

Figure 1. Reference interval and standard deviations. A reference interval for the results of a pathology test covers 95% of the apparently healthy population tested. Assuming the results have a normal distribution, it includes results within two standard deviations either side of the mean. One standard deviation is therefore one-quarter of the reference interval.

Key point

- If the person being tested is different from the reference population then the reference interval may not apply. Always check that the specimen was collected correctly if the result lies outside the reference interval; if there is any doubt, repeat the test under the correct conditions.

HOW ABNORMAL IS A RESULT OUTSIDE THE REFERENCE INTERVAL?

A population reference interval is usually based on results from 95% of the apparently healthy population tested (excluding the lowest 2.5% and highest 2.5%) and spans ± 1.96 standard deviations (SD) from the mean (midpoint) value (in practice, ± 2 SD), as shown in Figure 1. This means that the standard deviation of results from the population mean (i.e. 1 SD) is one-quarter of the reference interval. This information can be used to obtain a rough assessment of the degree of abnormality of a result outside the reference interval.

The probability of test results lying outside the reference interval and other ranges is discussed in Box 1. The table within the box shows approximately how likely it is that the result of a single test that is outside the reference interval is actually from a member of the healthy population. It also shows the same information about results from 10 independent tests.

As indicated in Box 1, if one test is performed, the chance of the result of that test lying within its respective reference interval is 95%, or 0.95, and 5% of results will lie outside. If 10 tests independent of each other are performed, the chance of all results lying within their respective reference intervals is 0.95 for the first test, 0.95 x 0.95 (0.9, or 90%) for the first two tests and 0.95^{10} (0.6 or 60%) for all 10 tests, the respective figures for results lying outside the reference intervals being 5%, 10% and 40%.

A common biochemical profile may have about 20 reported results but they are not all independent; for example, urea, creatinine and uric acid are all affected by renal function and all the liver enzymes are affected by liver damage. Even so, there are enough tests to explain why minor abnormalities in individual results are so common.

In practice results that appear to be abnormal can often be ignored unless they lie beyond about 2.5 SDs from the midpoint of the reference interval for the test (where an abnormal result from a healthy person is very unlikely, less than 2%). In patients with symptoms, and for some tests, smaller changes may have a greater likelihood of clinical importance.

An example of when a result outside the reference interval is abnormal

A serum sodium level of 133 mmol/L (reference interval 135 to 145 mmol/L) lies 7 mmol/L below the midpoint of the reference interval (140 mmol/L). Because 1 SD is one-quarter of the reference interval, in this case one-quarter of 10 mmol/L, or 2.5), a result of 133 mmol/L lies almost 3 SD below the midpoint of the reference interval.

As such a result or one more abnormal would be expected in a healthy population about 0.27% of the time, a serum sodium result of 133 mmol/L is very unlikely to be from a healthy patient.

Key points

- Comparing the deviation (in SDs) of a test result from the midpoint of the reference interval gives an estimate of the likelihood that a result outside the reference interval is significantly abnormal.
- An abnormal result for a single result is more likely to reflect pathology than one abnormal result from a battery of tests.

COMPARING DIFFERENT TESTS THAT ASSESS THE SAME PHYSIOLOGICAL PROCESS

Blood glucose monitoring and tests for glycated haemoglobin (formally known as haemoglobin A_{1c}; HbA_{1c}) and glycated serum protein (formally known as fructosamine) all assess the level of glycaemia but do so at different points in time (immediately or over the preceding six and two weeks, respectively).

There is a simplified rule for the association between the estimated average blood glucose level (eAG) and the HbA_{1c} level:

eAG (mmol/L) = 2 x HbA_{1c} (%) – 6.

Online calculators are available that use standard equations to determine eAG from HbA_{1c} in % or mmol/mol (http://www.diabetes.co.uk/hba1c-to-blood-sugar-level-converter.html).

The levels of HbA_{1c} and fructosamine can also be compared by assessing how far results lie from the midpoints of the respective reference intervals in terms of SDs.

An example of different tests comparing the same physiological process[2]

A truck driver with diabetes asked to be assessed to see if his diabetes satisfied the criteria for a heavy vehicle licence.[2]

- The blood glucose level (BGL) results in his record book ranged between 4 and 7 mmol/L with an average of 5.2 mmol/L.
- His HbA_{1c} was 8.2%, which was 3.2% (6.4 SDs) above the midpoint of the reference interval (4 to 6%; midpoint, 5%; SD [one-quarter of 2], 0.5). His eAG was calculated to be 10.4 mmol/L (eAG = 2 x HbA_{1c} – 6).
- His fructosamine was 386 µmol/L and was 141 µmol/L (6.3 SDs) above the midpoint of the reference interval (200 to 290 µmol/L; midpoint, 245 µmol/L; SD, 22.5).

The results of the two glycated protein tests were similarly high (6.4 and 6.3 SDs from the reference interval midpoints) and his predicted eAG level from his HbA_{1c} was high at 10.4 mmol/L.

1. PROBABILITY OF PATHOLOGY TEST RESULTS LYING OUTSIDE CERTAIN RANGES

For single tests

- The reference interval (the mean value ± 2 SD) covers 95% of results – i.e. 0.95 of them. So 5% of results lie outside the reference interval.
- The mean value ± 3 SD covers 99.73% of results – i.e. 0.9973 of them. So 0.27% of results lie outside.
- The mean value ± 4 SD covers 99.994% of results – i.e. 0.99994 of them. So 0.006% of results lie outside.

For multiple independent tests

- The probability that both results of two independent tests lie within the reference interval is:
  (0.95 x 0.95) or 0.95^2 = 0.9
  And so the probability that at least one of these results lies outside is:
  (1-0.9) i.e. 0.1, or 10%.
- The probability that all 10 results of 10 independent tests lie within the reference interval is:
  0.95^{10} = 0.6
  And so the probability that at least one of these results lies outside is:
  (1-0.6) i.e. 0.4, or 40%.
- The probabilities that all 10 results of 10 independent tests lie within ± 3 SD and ± 4 SD is calculated the same way as for the reference interval, i.e.
  1 - (0.9973^{10}) = 2.7% and 1 - (0.99994^{10}) = 0.06%, respectively.

The table shows the likelihood that test results will lie outside different ranges.

TABLE. LIKELIHOOD THAT TEST RESULTS WILL LIE OUTSIDE DIFFERENT RANGES

	Range		
	Reference interval (i.e. mean ± 2 SD)	**Mean ± 3 SD**	**Mean ± 4 SD**
Probability of single test result outside range	5%	0.27%	0.006%
Probability of one or more of 10 independent test results outside range	40%	2.7%	0.06%

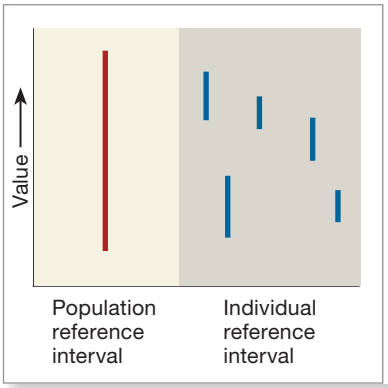

Figure 2. Population reference interval versus individual normal range for laboratory tests. Physiological ranges for individuals lie within the population reference interval but the mean values (the 'set-points') and the physiological ranges around the mean for individuals may be quite different from the population mean and reference interval and from each other.

The patient had a clear motivation in providing 'good' BGL results and when confronted with the results of the other tests he confirmed that his BGL results had really been much higher than those he recorded.

As a postscript, two months later his BGL and HbA_{1c} results gave similar results for overall glycaemia and his application to the licensing authorities was completed.

Key point

- Results from tests that reflect the same physiological process should be similar in terms of their distance in SDs from the midpoint of their respective relative intervals.

VARIABILITY AND THE REFERENCE INTERVAL

The laboratory's reference interval includes several sources of variability:

- biological variability between individuals
- biological variability within each individual
- variability introduced by specimen collection and transport
- variability introduced within the laboratory.

The collection system aims to eliminate the third component but variability between and within individuals and variability within the laboratory remain. The relationship between the laboratory (population) reference interval and the range within which results from particular individuals vary is shown in Figure 2. An individual's normal range around their individual physiological mean value (the 'set-point') is much narrower than the reference interval for a population. A result may lie inside the population reference interval but outside the physiologically normal range for that individual.

In many clinical situations an individual has multiple tests over a period of time. Information from previous results and an understanding that the variability within one individual is much less than the total variability within a population allows the clinical significance of a new result for that individual to be assessed.

Variability and the significance of changes in measurements over time are discussed in detail in the second article in this series, to be published in a forthcoming issue of *Medicine Today*.

Examples of results within/outside the reference interval that are abnormal/normal for the individual

- **'Subclinical' hypothyroidism: a normal free thyroxine (T4) but a high thyroid stimulating hormone (TSH).** The range of expected results for free T4 in a particular patient is smaller than the population reference interval. Thus when a patient develops a subclinical hypothyroidism, the free T4 level may be low for that patient (i.e. abnormal) but still within the population reference interval, and the elevated TSH level (above the population reference interval) is a marker that the level of thyroid hormone is identified as low by the feedback mechanism within the patient.
- **'Normocalcaemic hyperparathyroidism': a serum calcium within the reference interval, a high parathyroid hormone (PTH) result**

2. PRACTICE POINTS: PATHOLOGY TEST RESULTS AND REFERENCE INTERVALS

- If the person is different from the reference population then the reference interval may not apply. Always check that the specimen was collected correctly if the result lies outside the reference interval. If there is any doubt repeat the test under the correct conditions.
- From the reference interval the approximate SD of results from a normal population can be derived. Comparing the deviation of a result from the mid-point of the reference interval with the SD gives an estimate of the likelihood that a result outside the reference interval is from a healthy person.
- An abnormal result for a single result is more likely to reflect pathology than one abnormal result from a battery of tests.
- Results from tests that reflect the same physiological process should be similar in terms of their distance in SDs from the mid-point of their respective reference intervals.
- The biological variability within a single individual is much less than the total variability within the reference population. This means that a result within the reference interval can be physiologically abnormal for an individual and also that a result outside the reference interval may be physiologically normal for that individual.

outside the reference interval and a parathyroid adenoma or hyperplasia. The population reference interval for serum calcium is much wider than the normal range around the set-point for an individual. A high PTH level (above the population reference interval) in the presence of a physiologically high plasma calcium level in a patient signifies primary hyperparathyroidism (parathyroid adenoma or parathyroid hyperplasia) despite the plasma calcium result being within the population reference interval. Both analytes are deviated from the patient's set-points in the same direction.

- **Hyponatraemia.** The population reference interval for sodium is much wider than the individual's physiological range around the set-point. For an individual with a set-point at the lower end of the reference interval, a result outside the population reference interval may still lie within that individual's physiologically normal range.

Key point

- The biological variability within a single individual is much less than the total variability within the reference population. This means that a result within the reference interval can be physiologically abnormal for an individual and also that a result outside the reference interval may be physiologically normal for that individual.

CONCLUSION

The clinical presentation should be taken into account when considering the results of laboratory results, and the laboratory's interpretation should not be relied on completely. Laboratory reference intervals are but one of the factors involved in interpreting results.

Reference intervals may not apply to all patients and specimens. Results lying within the reference interval may be physiologically abnormal for some individuals and those lying outside may be physiologically normal for others; also, minor abnormalities in individual results are common. In practice, results lying outside the reference interval for a particular test can often be ignored unless they are beyond about 2.5 SDs from the midpoint of the reference interval for the test. Practice points are listed in Box 2. MT

REFERENCES

1. Phillips P. Pitfalls in interpreting laboratory results. Australian Prescriber 2009; 32: 43-46.

2. Phillips P. Referring to the reference range. Australian Diabetes Educator 2009; 12: 38-40.

FURTHER READING

Kellerman G, ed. Abnormal laboratory results. 3rd ed. Text © Commonwealth Government of Australia and National Prescribing Service; Illustrations and design © McGraw-Hill Australia; 2011.

COMPETING INTERESTS: Dr Phillips has received research and travel grants, acted on advisory boards and been involved with clinical trials and seminars sponsored by a range of pharmaceutical companies. He does not think these associations have influenced the content of this article.