

Veterinary Laboratory Medicine

CLINICAL BIOCHEMISTRY AND HAEMATOLOGY

Veterinary Laboratory Medicine covers all aspects of basic clinical biochemistry and haematology, and includes test-by-test interpretation of laboratory results. Information is provided on sampling techniques, the selection and use of an external laboratory, as well as near-patient testing and the practice laboratory. Also included are step-by-step instructions for most commonly used point-of-care tests, a guide to the evaluation of instruments for in-practice use, and a detailed explanation of the principles of impedance counting and photometric analysis. The book will be ideal for practitioners who require a guide to laboratory work, and for veterinary students studying laboratory medicine and clinical pathology.

The second edition has been fully updated to reflect advances in diagnostic techniques, and includes new chapters on diagnostic endocrinology and feline virus testing as well as a much expanded chapter on diagnostic profiling and pattern recognition.

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REVIEWS OF THE FIRST EDITION:

'Veterinary surgeons who require a handy guide to laboratory work and students studying laboratory medicine and clinical pathology should find this book invaluable.' *Veterinary Practice Management*

'This is a clinician's book written by a first rate clinical pathologist.' *Journal of Small Animal Practice*



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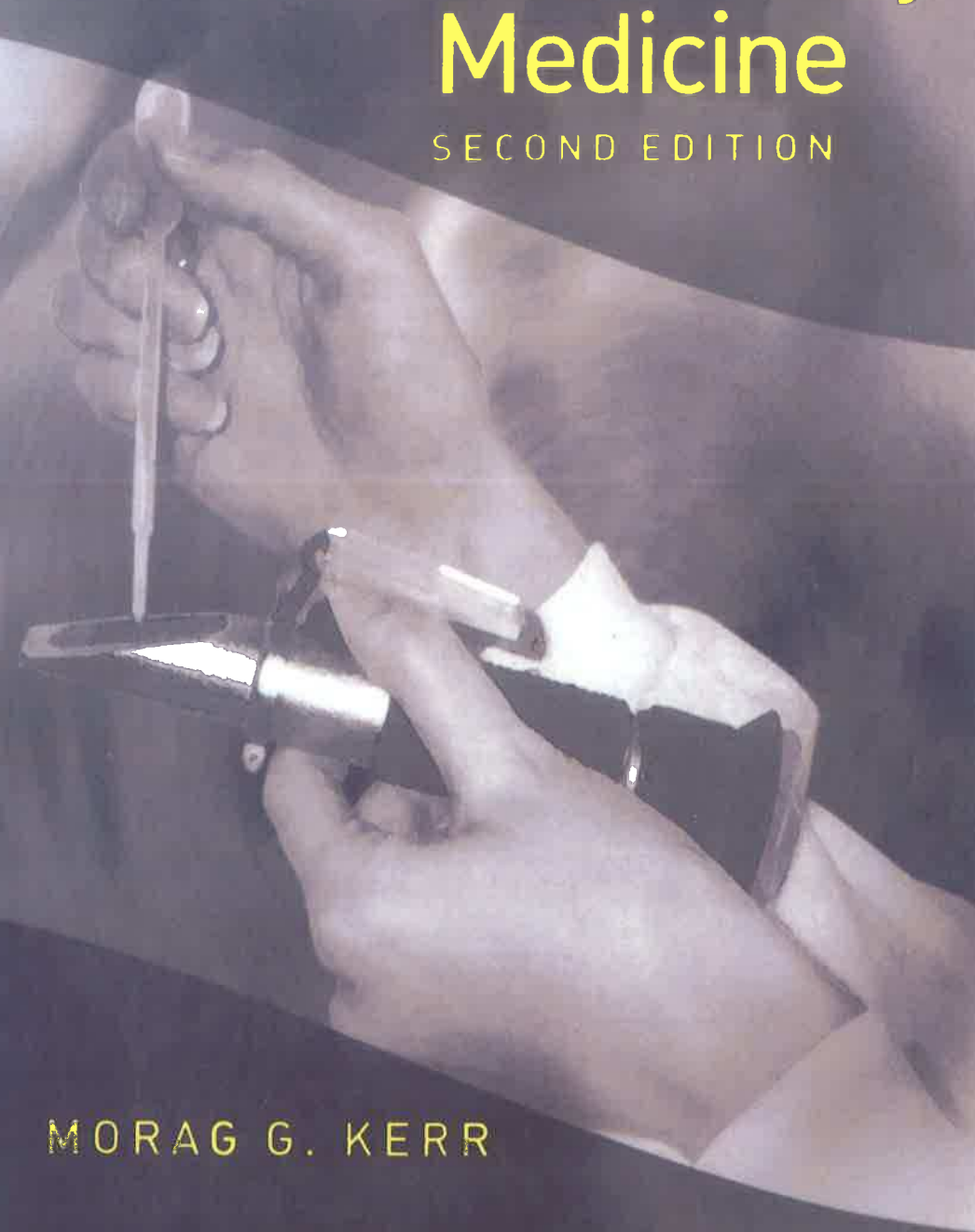
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Veterinary Laboratory Medicine

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SECOND EDITION



MORAG G. KERR

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*To my mother:
in gratitude for the winter of the millennium*

Veterinary Laboratory Medicine

CLINICAL BIOCHEMISTRY
AND HAEMATOLOGY

Second Edition

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Laboratory medicine and the veterinary surgeon

Since the first edition of this book was published in 1989, there have been many changes in veterinary laboratory practice – some very much for the better, others less so.

The most striking change is the much greater volume of biochemistry and haematology investigation being carried out. To a large extent this is a good thing, though a note of caution has to be sounded against using blood tests as a substitute for thorough clinical examination and history-taking, and anyone who finds themselves paralysed to act in an emergency because blood results are unavailable really ought to be reconsidering their priorities. In general, however, the more relevant information which is available to the clinician the more likely it is that the correct diagnosis will be arrived at, and so long as the laboratory data is *in addition to* the clinical data then more widespread use of laboratory investigation is to be welcomed. Indeed, the much greater readiness of practitioners to embark on laboratory investigation of the more challenging cases and to seek laboratory confirmation of the presumptive diagnosis in the more straightforward ones has made laboratory medicine a very rewarding discipline.

Following on from that, a more recent development has been the emergence of more veterinary surgeons specializing in clinical pathology/laboratory medicine at postgraduate level. Twelve years ago only a minority of commercial veterinary laboratories were under professional veterinary direction, with the majority run by technicians (often trained only in analysis of human samples) providing a results-only service without any professional interpretation. Now only a few laboratories remain in the latter category, and practitioners have a good choice of professionally-run laboratories offering not simply a string of numbers but a full range of advice covering selection of tests, interpretation of results and recommendations regarding treatment. Practitioners now recognize the laboratory as a second-opinion referral service, made extremely convenient and accessible by the fact that only the blood (or other) samples have to be referred rather than the entire patient.

In parallel with this there has also been an enormous increase in the amount of laboratory work carried out within veterinary practices. This is a bit of a mixed blessing. A near-patient facility designed to complement the professional laboratory and enable quick (if sometimes approximate) results of appropriate tests to be obtained as an interim measure in emergencies and out-of-hours, and to allow simple monitoring of already-diagnosed patients on treatment is invaluable. Certain items (e.g. the pocket glucose meter, the refractometer, the microhaematocrit centrifuge and, of course, the microscope) are so easy for the non-technician to use, so cheap and so useful, that it really is a case of 'every home should have one'. On the other hand, what is sometimes not appreciated is the enormous gulf between this type of side-room facility and a professional laboratory. However conscientiously those concerned with teaching the subject at undergraduate level try to instil a few of the principles of analytical procedure into veterinary students, a veterinary course is far removed from the sort of training a laboratory technician receives, and although some laboratory component is included in the veterinary nursing syllabus, this again should be regarded as helping equip nurses to perform the near-patient type of testing competently rather than expecting them to run a full laboratory service in between setting up drips and monitoring anaesthetics.

The main driving force of the 'practice lab' has been, as expected, the dry-reagent biochemistry analyser. Twelve years ago these machines were just emerging, having been developed for near-patient testing of human samples. It was clear that there were substantial problems when non-human samples were analysed by these methods, apparently due to what is termed the 'plasma matrix effect', but the optimistic view was that these problems would be solved and that there was good cause to hope that a wide range of reliable biochemistry results might be available in the practice side-room. Unfortunately this hope proved to be unfounded. There have been very few published studies comparing results of dry-chemistry methods to standard wet-chemistry methods for animal samples (and most of those are, for some reason, in German), but it is quite clear that for most of the methods the correlation is far poorer than would be required for professional laboratory application. Thus, although some practices owning these machines still do rely on them for routine work-up of non-emergency cases, many now realize that their place, if they are used at all, is in the near-patient emergency testing category, confining their use to the tests which are less poor performers (such as urea), concentrating on gross deviations from normal and not trying to read subtleties into smaller abnormalities which the accuracy of the methods is not really good enough to support.

Thus the thrust of this edition, contrary to expectations of twelve years ago, is much more towards the practitioner in partnership with the professional laboratory, performing relevant side-room tests where appropriate, but relying on the referral laboratory for the bulk of the routine testing and non-emergency case work-up.

So, does that mean that the clinical student or the practitioner can put this

book down, sit back, and wait for the clinical pathologist to tell him or her what is wrong with the patient and what to do about it? Well, no. Two heads are always better than one: the person who has actually seen the patient has an insight into the case which cannot be replicated simply by reading even the best-expressed clinical history, even the smartest clinical pathologist occasionally misses the blindingly obvious, and really successful use of the laboratory relies on an intelligent dialogue between the clinical pathologist and a well-informed and interested practitioner.

The format of the book remains based on the lecture notes approach. Some sections of comparatively basic science have been included, but the rule has been to cover only those areas which are genuinely relevant to clinical use. The information is initially organized on a test-by-test basis as this is still the essential way into the subject for the student, and it is important to have some way of assessing all the possible clinical implications of a single result. However, the systematic reassembly of the data has been expanded to give more emphasis to the pattern recognition approach to interpretation of laboratory reports. Detailed information regarding treatment and case management is given for a few specific conditions, but in general, information which is easily available in other basic texts has not been duplicated. Very unusual and rare conditions have also been omitted, as have tests which are not likely to be available to the general practitioner, and for information on these subjects the reader is referred to more advanced textbooks such as those listed on p. 355.

Laboratory medicine in case management

The most common use of laboratory work in veterinary practice is as an ancillary diagnostic aid. Other applications such as assessment of severity of the disease, prognosis and response to treatment tend to be secondary to this. It is therefore useful to consider where this type of procedure fits into the general management of a case.

The first rule of laboratory medicine is, *first catch your differential diagnosis*. This is something which must be arrived at, at least to a first approximation, on clinical grounds, for the very simple reason that only when you have at least some theory about what is going on can you begin to decide which tests to carry out to prove it.

At the most basic level, one first has to decide whether laboratory investigation (blood analysis or microbiological investigation), or radiography or other diagnostic imaging, or electrocardiography or whatever, is the most promising initial route to pursue.

The second step is to try to *ask the lab a specific question*. The clearer you are in your own mind just what question you want answered the easier it will be to decide which tests to ask for, to interpret the results when you get them back, and to realize when your question is, in fact, not one which a laboratory can really answer. For example, to consider a dog with severe acute vomiting, you

may decide to ask 'Does this dog have acute pancreatitis or is it in renal failure?', which leads straightforwardly to one set of test requests (amylase, lipase and urea and creatinine), or you may want to know 'How dehydrated is this dog and which i/v fluid should I be giving?', which leads to a different set of requests (total protein, albumin and electrolytes). Both questions are quite valid, both questions can be answered by the laboratory, but only you can decide which one you want to ask or whether you want to ask both. Or to consider a different point, 'Is this cow hypocalcaemic?' is obviously a realistic question, but 'Does this cow have a fractured pelvis, or obdurator paralysis?' is not really something which a laboratory is going to be able to answer with any real certainty. Here the formulation of the question, as opposed to just writing 'downer cow', can help clarify both the extent and the limitations of the information which the laboratory can be expected to provide. It is important in this context to realize that while laboratory data can be highly revealing in a large number of areas, there are certain areas of medicine where general 'routine' blood tests are usually not particularly informative, at least in a diagnostic sense. These include respiratory disease, most orthopaedic conditions and the majority of neurological cases.

Next, translate your question into a *request for specific tests* to be done. In order to do this it is necessary to know what information can be gained from each of the available tests and what is its likely applicability to the situation under consideration. This aspect occupies the bulk of the scope of this book. However, in spite of this, it is probably the actual formulation of the question which requires the most clinical skill, and turning this into a specific request soon follows on naturally. A single result is seldom pathognomonic for a particular disease, however, and the judicious selection of the most appropriate range of tests for each case is very important. It is necessary to strike a balance between requesting dozens of tests (which can be very expensive and may even lead to the relevant information being overlooked in the deluge of results), and the often false economy of restricting requests to one or two tests per sample. As one becomes more familiar with the extent and limitations of the information available from each test this process of acquiring maximum information from a reasonably small number of tests becomes easier and easier (the approach to this is outlined in Chapter 15). In addition, many laboratories have now adopted the approach to profiling first outlined in the previous edition of this book, where profiles are designed around common major presenting signs rather than on an organ-by-organ basis. Profiles designed in this way provide a short-cut to the most rational selection of tests by ensuring that all the differential diagnoses are covered which should realistically be considered when that presenting sign is present – for example, the polydipsia profile for dogs will include calcium, as hypercalcaemia is an extremely important but uncommon cause of polydipsia which might otherwise be forgotten when selecting tests. Nevertheless, it is still good practice to 'engage brain before ticking boxes', as sometimes an extra test or two might be needed to cover particular circumstances, or you might be confident enough that

certain conditions are *not* on the cards to allow a less extensive range of tests to be requested. Once you have decided on what information you require from the laboratory and which tests you need to acquire it, you are ready to collect and submit your sample.

The fourth step is to *consider the results in the context of the whole clinical picture*. The conscious act of formulating your original question will make this step much easier, in that when you ask a specific question you tend to have some idea in mind of the answers you are likely to receive, and of your probable response to these answers. However, this stage is definitely the time for some lateral thinking. Even in cases where the answer to the original question seems fairly straightforward, it is well worth asking 'Is there any other explanation which could fit all the facts of this case?', and in cases where unexpected or even apparently inconsistent results appear then it is essential to consider the situation in some depth. There is a sort of laboratory 'cringe' which says 'where the clinical picture and the lab results disagree then you should always believe the clinical picture', but this view is misleading. Results from a *reliable* laboratory should never be ignored just because they don't fit your cosy little theory – and if you can't rely on your laboratory, you shouldn't be using it. When arriving at a diagnosis it is essential to look every single fact straight in the eye and to come to a conclusion which can be reconciled with *all* of them. A laboratory result, normal or abnormal, is a fact just like any other piece of clinical information and should be given its *due consideration*. Obviously in each case some facts will weigh more heavily than others, and the decision as to just how much importance to give to each item involves a great deal of clinical skill which takes time and experience to acquire. Unfortunately there are no easy generalizations like 'clinical facts are always more important than lab facts' (or vice versa!) to help here, and there is really no substitute for a *thorough* knowledge of the significance and implications of *all* your findings.

The final maxim to bear in mind is *sample before treatment whenever possible*. The rather desperate approach to laboratory medicine which views lab investigations as a last resort when all attempts at 'diagnosis' by response to treatment have failed causes some veterinary surgeons to come unstuck at this point. It is true that antibiotic treatment is not often a direct cause of trouble with haematology or biochemistry tests (though it can play havoc with any bacteriology you may subsequently decide to do) but the ubiquitous corticosteroids have a wide range of haematological and biochemical effects which can mask vital information of diagnostic significance. Other culprits are fluid therapy (especially when the fluid contains glucose) and mineral preparations such as calcium borogluconate. Clearly, it is difficult to avoid the situation where a farmer has administered every nostrum in his cupboard before you arrive, but it is good practice, whenever treatment is about to be instituted, to consider 'Am I likely to want any laboratory work done on this case, and if so, am I going to regret not having a pre-treatment sample?' Even in circumstances where treatment must be started before any results will be received – a fairly

frequent occurrence – a pre-treatment sample can be invaluable and can save a lot of time and trouble in the long run.

Basic principles of haematology and biochemistry

Haematology is the study of the cellular elements of the blood and the associated clotting factors, and can be extended to include cytology of non-blood fluids such as cerebro-spinal fluid (CSF). It is a subject which can provide a great deal of useful information, but, like all diagnostic tests, intelligent assessment of the results is vital. In some ways haematology can be easier to cope with than biochemistry, if only because the easy option of a 'full blood count' or 'general series' examination is available on all lab request forms. This means that it is actually quite easy to bypass the mental disciplines outlined above which lead up to the selection of individual tests. However, if you omit this prior consideration of *why* you are taking this sample and what conclusions you might expect to derive from the results, you must expect to compensate by a particularly thorough assessment of the findings once you receive the results. Remember also that haematology can only tell you what is happening, directly or indirectly, to a fairly small number of circulating cell types, and that the actual number of tests available is quite limited. For general metabolic investigations the wider range of tests and the more direct nature of the information offered by clinical biochemistry is at least as helpful, possibly more so, and normal practice should be to consider *both* disciplines side by side when deciding on the range of tests required for each case.

Clinical biochemistry is a very different subject from pure biochemistry and an antipathy to the latter acquired in early student days should not deter anyone from tackling the former. Basically, clinical biochemistry involves the analysis of samples of body fluids, principally plasma (though occasionally other samples are used such as urine, faeces, CSF and pleural and peritoneal fluids), and the use of the results to clarify the clinical picture. The nature of the subject and the much larger number of 'routine' tests on offer mean that, in general, a wider range of specific information is available from biochemistry than from haematology, but also that a single group of tests cannot be regarded as a basic 'profile' applicable to all (or nearly all) situations. Judicious selection of the appropriate tests for each individual case is therefore of particular importance in clinical biochemistry.

'Normal values'

Many publications quote apparently rigid 'normal values' for biochemical and haematological measurements, sometimes to an extraordinary number of significant figures. The fact that it is extremely rare to find two publications in absolute agreement on these numbers demonstrates clearly the artificiality of this situation.

The spread of values from 'normal' individuals for most constituents (excluding some enzymes) takes the form of a normal distribution curve (see Fig. A.1). If the limits of this curve are defined as the mean ± 2 standard deviations then very rigid values to any number of significant figures can be derived. However, these limits will of necessity exclude 2.5% of all *normal* individuals on each side of the curve – how can you know that your individual patient is not one of this 5%? In addition, it is important to realize that a value *within* these limits is not necessarily 'normal' for every individual animal – one which was towards the lower part of the range when healthy may have a genuinely pathologically evaluated value when ill, which is still within the statistically 'normal' limits. Thus on either side of every 'normal range' there is a grey area where a result may be normal or may be abnormal, and only statistical probabilities of its being one or the other can be quoted. In dealing with individual results in these grey areas it is particularly important to take other factors into consideration, both clinical signs and other laboratory results.

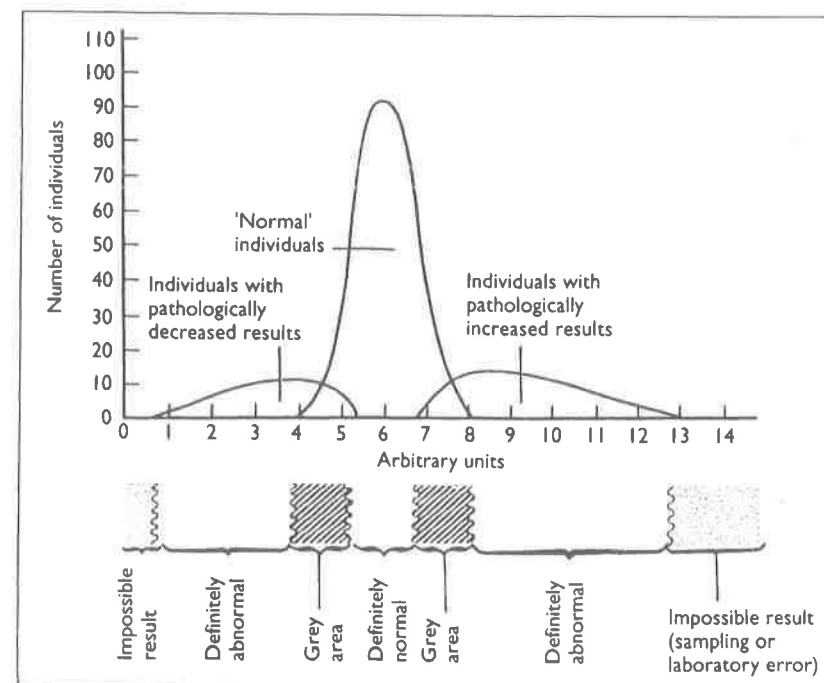


Fig. A.1 Schematic representation of the distribution of results for a figurative laboratory test showing overlaps of 'normal' and pathological ranges.

As a consequence of this, only approximate guideline values are given in this book for each constituent, and when interpreting actual results the modifying effects of species (only the very major species differences are highlighted), breed, sex, age, diet and management systems must be taken into account. It is this multiplicity of species, breeds and patient 'lifestyle' differences which make

veterinary laboratory medicine a bit of an art as well as a science, and there is no doubt that the best way to become proficient in interpreting laboratory data is to examine numerical results for as many actual cases as possible. In particular, remember that it is much more important to know what degree of weight to attach to a particular level of deviation from normal (e.g. insignificant-ill-dying) than to be able to quote glibly memorized 'normals'.

There is also the question of methodological variation. Since the advent of external quality assessment in NHS laboratories in the 1960s, great attention has been paid to uniformity of reference ranges and results between laboratories. This 'inter-laboratory precision' ensures that patients with chronic illnesses who move from one part of the country to another do not run into serious problems when their new consultant is faced with results from an unfamiliar laboratory with unfamiliar reference ranges. University, state and commercial veterinary laboratories have also benefited from these schemes and participated in them, and nowadays any discrepancies between laboratories' reference ranges should be minor and insignificant (with perhaps a few specific exceptions such as alkaline phosphatase (ALP), where method differences can still have an appreciable effect). Thus it is possible to quote general guideline values which are fairly universally applicable, and it should not be necessary either to completely relearn the subject when changing laboratories, or to be constantly enquiring 'what is your reference range for this analyte?'

Units

The changeover from the old 'conventional' (mostly gravimetric in biochemistry) units to the modern 'SI' (mostly molar in biochemistry) units has created some considerable confusion, particularly among clinical users who just want to know what is wrong with the patient and don't want to be bothered with technicalities. This was probably inevitable at the time, but now that it is at least 25 years since the actual changeover it is about time things settled down.

In haematology there has been comparatively little trouble, in that the adoption of the litre as the standard volume of measurement has usually involved either a simple change in the name of the units (or in the power of 10 included in it) while leaving the actual number unaffected, or at the most there has been a shift in the position of the decimal point. So, mean corpuscular volume (MCV) has moved from cubic microns (μ^3 or $\text{cu.}\mu$) to femtolitres (fl) with no change in the number (as they are actually the same thing), while packed cell volume (PCV) has changed from a percentage to a decimal fraction, which in effect moves the decimal point two places to the left (the decimal fraction is sometimes labelled 'l/l', but this is a non-unit in which the top and bottom cancel out – gallons/gallon would be equally valid, as PCV is in fact a v/v ratio). One place where care is required is where a unit of ' $\times 10^3/\text{mm}^3$ ' or 'thousands/cu.mm' has been replaced by ' $\times 10^9/\text{l}$ ', as with white cell and platelet counts. The numerical result has not in fact changed, but as some people were in the habit of quoting the figure as so many *thousand*, it is possible

to fall into the (sometimes potentially dangerous) trap of reporting a result as several thousand $\times 10^9/\text{l}$, which is of course out by three orders of magnitude.

Biochemistry unit changes have been more complex because the actual numbers involved have been affected. Historically, plasma constituents were measured by weight (usually mg/100 ml), but subsequently all branches of chemistry and pure biochemistry adopted molar concentration units as the only realistic way to describe reaction processes. In the early 1970s clinical biochemists also changed to molar (SI) units to describe concentrations of plasma constituents, as these are obviously much more meaningful in real terms. However, a few countries have lagged behind in this and the USA in particular has still failed to address the situation even at the beginning of the twenty-first century. This means that the old gravimetric units are still to be found not only in pre-1975 books and journals, but in modern American publications, and the table of conversion factors given below (Table A.1) should be used to convert these figures to the SI equivalents whenever they are

Table A.1 Conversion from old 'gravimetric' biochemistry units to SI units

Constituent	Gravimetric unit	SI unit	Conversion factor
Total protein, albumin, globulin	g/100 ml	g/l	10
Sodium	mg/100 ml*	mmol/l	0.435
Potassium	mg/100 ml*	mmol/l	no change
Chloride	mg/100 ml*	mmol/l	0.26
Calcium	mg/100 ml	mmol/l	no change
Magnesium	mg/100 ml	mmol/l	0.28
Phosphate	mg phosphorus/100 ml	mmol/l	no change
Copper	$\mu\text{g}/100\text{ ml}$	$\mu\text{mol/l}$	0.25
Urea	mg nitrogen/100 ml (BUN)	mmol/l	0.5
Creatinine	mg urea/100 ml	mmol/l	0.41
Ammonia	mg/100 ml	$\mu\text{mol/l}$	0.5
Glucose	$\mu\text{g}/100\text{ ml}$	$\mu\text{mol/l}$	0.32
Bilirubin	mg/100 ml	$\mu\text{mol/l}$	0.16
Cholesterol	mg/100 ml	mmol/l	0.36
Triglycerides	mg/100 ml	mmol/l	0.17
Tri-iodothyronine (T_3)	$\mu\text{g}/100\text{ ml}$	nmol/l	88.4
Thyroxine (T_4)	$\mu\text{g}/100\text{ ml}$	nmol/l	0.59
Cortisol	$\mu\text{g}/100\text{ ml}$	nmol/l	0.056
Urine protein/creatinine ratio	g/g	g/nmol	17.1
			0.026
			0.011
			15.4
			12.9
			27.6
			0.113

*Less commonly encountered units.

- Mayne, P. D. (1994) *Clinical Chemistry in Diagnosis and Treatment*, 6th edn. Arnold, London. ISBN 0340576472
- Piccoli, G., Varese, D. & Rotunno, M. (1984) *Atlas of Urinary Sediments: Diagnosis and Clinical Correlations in Nephrology*. Raven Press, New York. ISBN 0890005070
- Smith, A. F., Beckett, G. J., Walker, S. W. & Rae, P. W. H. (1998) *Lecture Notes on Clinical Biochemistry*, 6th edn. Blackwell Science, Oxford. ISBN 0632048344

It is unwise to adopt procedures and recommendations from human medicine without checking on their applicability to veterinary species; nevertheless, it is advantageous to acquire some familiarity with the field. These human texts have the advantage over major veterinary reference works of being UK publications, thus using more familiar language and SI units. They are aimed at the medical undergraduate/house physician, and contain some very practical common sense.

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