Numero - number

Numeracy is a basic life skill so fundamental that we get our first introduction to numbers before going to primary school. Yet, as the saying goes, biologists don't do math. At least in this respect, clinical microbiologists and infectious disease physicians seem to fit the mould, their preoccupation being with the qualitative aspects of infection: the names of the disease causing agents (nouns) and their consequences (verbs).

But scratch the surface and you find a discipline teeming with numeric detail from the most basic statistical tests used to appreciate the strength of potential connections, through quantitative assessments of biological burdens to the sophisticated analyses that underly the burgeoning discipline of bioinformatics . Chemistry and physics have long exploited the boundaries of computational mathematics. It seems that biology is the waking giant, stretching its limbs before really learning how to play with numbers.

The problem with zero

Modern languages have an interesting variety of number conventions. Most are based on a decimal system, though there are often remnants of older, imperial currency systems such as the duodecimal. Much of our current numeracy leans on the infusion of the Arabic system, including the number zero. Zero, nothing, null is a difficult idea in the language of infection. There is far more interest in zero infection and particularly in the demonstration that zero infection is present, than is many other aspects of the biology of infection. You might say that the general view is a naive attachment to zero tolerance of a phenomenon that is widely misunderstood. But that is being a little harsh. This simple view of infection, and the presumed presence of microscopic life that is behind the infection process is actually a simple binary view: present or absent. Zero or One. In the popular imagination, there is no such thing as half an infection, let alone one tenth or one hundredth of an infection. Ironically, there is a whiff of science behind this simplification since the majority of single cell organisms multiply by division and thus increase on a binary scale; one becomes two, two become four, four become eight and so on. The potential for logarithmic expansion is wrapped up in their genes, and restricted only by external limits such as exhaustion of nutrients or attack by toxic substances like antibiotics.

The myth of one

Most microscopic life capable of causing the processes we collectively label 'infection' is conveniently described as unicellular i.e. having a single cell. The descriptive biology on which much of the classification of bacteria, yeasts and protozoa has been based relies on the single-cell concept. It is a useful idea, but has limited practical use. After all, what is a single staphylococcus? Infection with almost all priobes requires more than a single cell. The infective dose is usually multiple and often follows a threshold phenomenon. Agreed; some agents of infection are so potent that they can cause infection after encounter with a very small dose indeed. The single unit is also problematic because of the cell cycle in which each unit is either recovering from, or preparing for cell division. Maybe there are some forms of microscopic life that go through prolonged resting periods such as sessile bacteria in survival mode, but note that these are

usually found in aggregates or communities that resemble the cellular consortia described as tissues in multicellular organisms. The single unit is even harder to nail when you try to count microscopic life. Bacteria can be dispersed in suspension and cultivated on solid media which are then incubated in the right conditions to generate a colony count (colony forming units per mL or CFL/mL), but when a range of different methods are used to measure the number of bacteria present in a suspension, they very rarely tally due to microaggregates, cells at varying stages of division and cell death.

Making it count

The emergence of quantitative microbiology over the last two decades has caught some by surprise. Initially quantitative appreciation of bacterial load was the preserve of public health laboratories working to arbitrary safety standards for risk-prone food and drink such as seafood, dairy products and drinking water. Techniques such as the time-consuming most probable number method were used to determine the load of indicator organisms. These have been replaced by automated or semi-automated bacterial and viral counting methods. More recently a plethora of cell biology methods have come on line and have been quickly adapted for microbial counting tasks, though regulatory standards linked to traditional methods often slow down the adoption of promising new methods of quantitation. The other area in which quantitation has opened new analytic horizons is nucleic acid amplification and expression analysis. Quantitative PCR, in particular, provides rapid numeric insight into a huge range of clinically significant organisms. But there is a catch: PCR assays will only measure the known target i.e. dumb DNA (or if you hanker after certain viruses, dumb RNA). They do not necessarily measure the number of intact, living, metabolising and replicating cells. That requires at least some conventional, culture-based effort. From a contemporary position, the future for quantitative molecular and cellular microbiology is promising but has yet to show its full potential.

Another dimension

One the face of it, numbers allow us to take a measure of how many units of a given life form are present. They also give us a useful set of tools for other kinds of measurement. Size is probably one of the most significant since it determines what can and cannot be seen with the naked eye, and therefore what is by definition a microscopic form of life. Size has a bearing on what does and does not cause infection. Lower respiratory infections are usually caused by infective agents in a narrow size range capable of inhalation. Too large and they hit the back of the throat due to momentum during an inward breath. Too small and they are unlikely to contain any intact infective agent. There is more to this than a mere measurement of length, breadth and depth. These measurements combine to give volume, and with a measure of mass result in the derivative measure: density. Buoyant density , for instance, is likely to affect how long breathable particles can remain suspended in air. In the case of viruses, their small size dictates that they must be viewed with an electron microscope, whereas the larger size of typical bacteria and fungi allows viewing under a light microscope. The measured size of protozoa and helminths
is of use in their identification. Time is another dimension capable of measurement. A binary pattern of growth results in many microscopic life forms going through a period of logarithmic growth in which a key measurement is the doubling time; a figure that is reproducible for a given species in controlled growth conditions.
E.coli
has a doubling time of only a few minutes, while
Mycobacterium leprae
takes days to double its number. Viruses can be very fast, and this is reflected in the speed of onset of acute viral infections. Another measure of microbial time is their so-called genetic clock; the amount of genetic variation accumulated by their genomes since their presumed species origin. Although this concept can be a source of heated argument due to the assumptions and uncertainties on which the concept is based, it is a useful working hypothesis for developing ideas on the chronological time scale over which microbial life has developed.

Getting your ducks in a row

Time has another numeric significance. It is one of the key forms of setting priorities. What came first, second, third and so on until the last. Lining up a series of observations or events in temporal sequence is a way to impose a sense of order. It gives meaning to those events. An early example of combining bacterial count and time sequence in order to investigate a complex causation was a study we performed on how bacteria get from the stomach into the lungs of mechanically ventilated intensive care patients . Temporal priority or getting in first is so important in our culture, that we often give pre-eminence to the first. First has champion status. Second is the first among losers, at least in competitive sports. But in a cascade of biological events, the first one is significant because it is the beginning and this idea lies close to the heart of the priobe concept , which regards the minute infective agent as the principal priming factor in the pathway or process that leads to what we eventually recognise as an infectious disease. In this we have to be a bit careful to avoid the *post hoc ergo proper hoc* erro r of logic in which we argue that just because something happened first, it must be the cause of a subsequent event. Thus the fact that my grandfather was a good fly fisherman does not necessarily explain why I know how to barrel cast with a fly rod. It might have been due to lessons given me by my grandmother, my father or a family friend. So, returning to lining things up in proper order, there are obviously other aspects of prioritisation such as ordering by size, mass, or as clearly happens in biology, according to order of name under the Linnean classification . Whatever feature has been used to list items in order, they can be given a number which is called an ordinal. Some of these are given above (first, second etc). It has been a source of grief in recent years that news announcers and other professional media communicators have chosen to drop the use of ordinals from dates, either to dumb down their delivery or to make it sound more decisive. Biologists have avoided this silliness if for no other reason than getting the credit for a discovery requires publishing first in order to establish priority. Far more important than that, on grounds of the number of clinical cases and thus burden of disease is the trio of malaria, tuberculosis and HIV/AIDS. These are priority diseases. The numbers say it all.

Clinical reality

And so to the pointy end of the numbers - patients with infection. The numbers come into heavy use for the analysis of infection data. Epidemiology has its origins in the study of epidemics, and continues to demonstrate new methods of numeric analysis in the investigation of emerging infections. In the clinical microbiology laboratory, work is still largely based on a series of qualitative value judgements. Look closely, though, and you will find pockets of quantitative biology (biometrics). Cell counts are conducted on sterile fluid samples, bacterial counts are performed on urine samples for which an interpretive threshold has been described, viral load measurements are made in viral hepatitis and HIV/AIDS. In the molecular microbiology lab quantitative PCR assays are performed and their numeric output used for result determination. In some molecular epidemiology methods, amplified fragment size is measured to determine the specific genotype, and antibiotic susceptibility determination relies heavily on quantitative or semi-quantitative methods. Numbers are necessary, particularly where consistency of results are important. Measurement and an orderly sequence of events play an increasing role in the clinical microbiology laboratory in future, as it already does in other pathology disciplines such as haematology and clinical chemistry.

9th January, 2011.