example urine or sputum. However when an invasive procedure is used on a patient and only scanty material obtained, such as spinal aspirate or endometrium, the inclusion of an animal test may be a valuable additional diagnostic aid. In particular, GPI of cerebrospinal fluid can be especially helpful when acid fast bacilli are not found on direct microscopy but the clinical observations, as well as the biochemical and cytological findings on the fluid, favour a diagnosis of tuberculous meningitis. A positive test in these circumstances confirms the diagnosis but when culture and animal tests are both negative this will allow the diagnosis to be reconsidered after at worst six weeks of chemotherapy.

The advantage of GPI in these few patients has, we believe, usually depended more on the scanty nature of the sample than on inadequate culture technique. Experience with numerous other samples examined by microscopy and culture alone (mainly sputum or urine) has shown close correlation between clinical and laboratory findings; after decontamination material has been cultured on Löwenstein-Jensen medium, pyruvate egg medium and into Kirschner enrichment medium and strains of Mycobacterium tuberculosis of both human and bovine type have been isolated satisfactorily by this means. In addition, with very small samples it is also worthwhile adding Kirschner enrichment medium to an apparently empty or almost empty specimen container or washing through the aspiration needle with this medium to permit growth of very scanty bacilli adherent to the container or needle surface.

By use of the tuberculin test, knowledge of the BCG immunisation history, and by selection of "at risk" patients (as for example those with a history of contact, or with a past history of pneumonia or pleurisy around the menarche) many inessential animal inoculations can be avoided. Culture of samples from more than one site— for example, urine and endometrium, may also assist in establishing the diagnosis without need to resort to guinea-pig inoculation.

The answer to the question, "Is guinea-pig inoculation ever justified for the diagnosis of tuberculosis?" must be, for the United Kingdom, that it is rarely justified but is nevertheless valuable for the selected "problem" situation. The decision to perform a guinea-pig test can usually be determined by discussion between microbiologist and clinician. For this purpose few animal houses are now needed and those that remain will provide facilities for other tests (especially virus, toxicity, and some pathogenicity tests) for which animal inoculations still remain essential.

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References


In vitro stability of cyclosporin A

We would like to report some new data on the stability of cyclosporin A (CyA) in human serum which is relevant to our article on this subject in the January edition of the Journal. Investigation at the Sandoz Research Laboratories indicated that when tritiated CyA was mixed with normal blood, the percentage of whole blood CyA present in the plasma was dependent on the temperature at which the blood was stored prior to separation. When the blood was stored at 21°C before separation the plasma concentration of CyA was 50-70% of the plasma concentration when the blood was stored at 37°C before separation. The decrease in plasma CyA concentration on storage at room temperature (21°C) occurred within one hour of collection and was probably due to temperature dependent alterations in the cellular binding of CyA (Sandoz, unpublished data).

We have repeated in vitro temperature studies using the blood of patients treated with CyA. Measurements were by radioimmunoassay. Figures 1 and 2 show our results which confirm the Sandoz findings, using both plasma and serum samples. Our published work on in vitro stability of CyA in human serum was carried out on blood samples stored at room temperature for 2-3 hours before separation. The "toxic" and "therapeutic" ranges for serum CyA in our patients were based on samples handled in the same way. Using this method we have found a useful correlation between trough serum CyA concentrations and renal toxicity.

The data shown in Figs. 1 & 2 suggest that plasma and serum concentrations of CyA in vivo are consistently higher than our measurements indicate. However, at present we still recommend that trough serum concentrations of CyA should be used to monitor patients but that the blood

Fig. 1 Decrease of plasma concentration of cyclosporin A after storage of whole blood at room temperature (20°C) and 37°C. The initial plasma cyclosporin A concentration was 445 ng/ml
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![Graph of serum concentration over time](image)

**Fig. 2 Decrease of serum concentrations of cyclosporin A after storage of whole blood at room temperature (20°C) and 37°C. The initial serum cyclosporin A concentration was 235 ng/ml.**

A collection procedure should be standardised so that samples remain at room temperature (20°C) for 2–3 hours to allow equilibrium to occur before separation. Keeping blood samples strictly at 37°C before and during separation is not practical for routine monitoring. As yet no clear correlations between the whole blood CyA concentration, immunosuppressive effect and toxicity have been found, although in the future the measurement of whole blood CyA concentrations may eliminate variation due to changes in cellular binding of CyA in vitro.

**Recognition of Haemophilus species**

It is fairly general practice, and is also stated in some texts that in the clinical laboratory the recognition of *Haemophilus* species is primarily based on the observation of satellitism on a fresh blood agar plate, either in a mixed culture or when growing in pure culture, on such a plate streaked with a staphylococcus strain which provides V factor.

This useful procedure is inapplicable, however, when dealing with *Haemophilus* species that are X factor dependent only, such as *H. aphrophilus*, *H. haemoglobinophilus* (canis) or *H. ducreyi*, and with haemolytic species such as *H. haemolyticus*, *H. paraaeromonas*, *H. parahaemolyticus* and *H. pleuropneumoniae*. On fresh blood agar plates the haemolytic species obtain their optimum supply of V factor by virtue of their haemolytic property alone and, therefore, reach their maximum colony size without additional sources of V factor. Thus, the primary recognition of such species depends on cultural and microscopic appearances.

Using manufactured blotting paper discs impregnated with X, V and X plus V factors we have been aware for a long time that occasionally dependence on one or both of these factors could not be elicited when it was clearly expected. During checking the point discussed in the preceding paragraphs with a number of haemolytic strains we were surprised to observe that a proportion of discs were surrounded by an area of inhibition. The discs were clearly contaminated by some antibiotics inhibiting haemophili. On inquiry the manufacturers had to admit that through faulty production technique such contamination had occurred (in our experience this occasional contamination must have been taking place for years) but assured us that measures had been taken to avoid such unfortunate errors. In the experience of one of us this is not the first time that a manufactured laboratory product from a reputable firm contained a substance which was not expected to be present.

Testing for X and/or V factor requirements can be organised easily with a microbiological laboratory's own resources and is based on two simply prepared media. The scheme was first recommended in 1960 and in its modified form is perfectly adequate for first stage testing of X and/or V factor requirements.

Medium 1 is an autoclaved blood agar which, apart from adequate nutrients, contains X factor only. Medium 2 is either Difco Proteose Peptone No 3 agar or Oxoid Isosentest agar both of which do not contain X or V factors. More than one strain suspected of belonging to the genus *Haemophilus* can be tested on one plate each of these two media by first being streaked separately across the plates from edge to edge. Then a strain of *Staphylococcus aureus* is streaked at right angle to the previous streak(s) down the centre of the plates from edge to edge. After overnight incubation X dependent strains will grow uniformly from edge to edge on the autoclaved blood agar plate only. V dependent strains will grow on both plates for a distance of 0-5 to 1-0 cm from the staphylococcal streak whilst X plus V dependent strains will grow for a similar short distance on chocolate agar plates only.

Any further tests, if desired, such as ß-amino-laevulnic acid assimilation, sugar fermentations and tests for the presence of enzyme systems can be carried out as a second or third stage progression but for most practical clinical purposes the first stage described here will be found to be quite adequate. As one colleague remarked in 1960 ‘the method does not look very scientific and is a bit messy but it works.’

**References**

