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SPECIAL ARTICLE

Screening for anti-rubella IgM *ad libitum*

There are few applications of virology to clinical medicine that have received more attention than the laboratory diagnosis of rubella. Many laboratories attempt it and most readers of this journal will be familiar with the techniques that have been used. It would therefore be superfluous to review the 'state of the art' had there not recently been an innovation that is likely to alter laboratory practice in this field.

Rubella cannot be diagnosed accurately at the bedside (though this should not detract from the importance given to clinical observation in the disease) and laboratory investigation is necessary to confirm the diagnosis in patients, as well as to identify infections that are not clinically apparent. Virus isolation, the original method, is now little used. Though it has led to a fuller understanding of the natural history of rubella and provided the tissue-culture adapted strains from which the large volumes of rubella antigen currently used in serological tests are made, it is a slow and exacting technique. There are only a few circumstances, for instance in deciding whether an affected infant is excreting virus, where the isolation of rubella virus in tissue culture is required.

Serological methods are the mainstay of rubella diagnosis. They are fully described in a recent monograph (Public Health Laboratory Service, 1982) on the subject. Rubella antibody may be measured by haemagglutination inhibition, radial haemolysis, immunofluorescence and complement fixation. If specimens of sera taken at appropriate times are available, any of these methods may be used to diagnose both acquired and congenital rubella infections. Unfortunately, difficulty in obtaining suitable paired specimens often robs these comparatively simple tests of diagnostic value and more complex tests for anti-rubella IgM are then required. Tests for serum anti-rubella IgM are considered valuable because it is generally thought that this class of immunoglobulin is only produced in detectable amounts for a short time during and immediately after a primary infection. Rarely, specific IgM has been found during so-called re-infections, where exposure of a previously immune patient to rubella evokes a rise in antibody titre; but the accuracy of these reports of 'secondary' IgM production, and their significance, remains open to question. For the present it is both sensible and diagnostically convenient to regard rubella-specific IgM exclusively as a mark of primary infection. Specific IgM in the serum of a pregnant woman is consistent with a risk of fetal infection and, in the serum of a neonate, is indicative of congenital infection. If a serum specimen has been collected at an appropriate time for the technique used, the simple diagnostic rule is that when IgM is detected fetal infection is possible and that when IgM is not detected fetal infection is most unlikely. Reports of unusual cases in which clinical and laboratory evidence do not conform to this rule go against a large body of experience and often seem flawed

in regard to clinical detail available or techniques used (Krugman, 1981). Although each such case deserves careful study, none have effectively called the conventional wisdom into question.

In rubella, an IgM response is the hallmark of the primary infection. How then to detect it? This is a technical challenge to which diagnostic laboratories have responded in various ways and with variable success. Many methods have been described and evaluated, but those that have proved generally reliable have all involved laborious and rather costly physical methods to separate IgM from IgG antibody. The fractions of serum rich in IgM are then assayed for haemagglutination-inhibiting antibody. Not all laboratories have perfected these procedures and inaccurate reporting has sometimes arisen from improper fractionation of specimens. Misgivings about test sensitivity and laboratory accuracy continue to cause uncertainty.

Against this background the introduction of a test for rubella IgM that is technically straightforward, precise and objective is significant. The test is the M-antibody capture assay (Mortimer *et al.* 1981*b*; Vejtorp, 1981) that has been developed along the lines of a similar assay recently introduced for hepatitis A (Duermyer, Weilaard & Van der Veen, 1979; Flehmig *et al.* 1979). The principle of these assays is that serum IgM will be bound selectively to polystyrene surfaces to which anti-human IgM has previously been fixed (Diment & Pepys, 1976). It can then be tested for anti-virus specificity by the addition of an antigen followed, finally, by a labelled anti-serum that reacts strongly with the antigen. Routine use over the past year in several English laboratories indicates that the rubella M-antibody capture assay will be as useful diagnostically as the hepatitis A M-antibody assay (Mortimer, Parry and Appleton, 1981) has already proved to be.

There are several reasons why M-antibody capture assay (MACRIA) will replace other anti-rubella IgM tests. First, it is an easy assay in which the reagents are simply incubated successively with a surface (bead, tube or well) to which the anti-human IgM preparation has previously been absorbed. Most of the components of the test are readily available commercially. Secondly, the method is more sensitive than any other, including the indirect immunoassays, and has the additional advantage over them of greater specificity and resistance to the effects of rheumatoid factor. Thirdly, providing a range of controls is assayed in the run, each reactive specimen may be ascribed a level of anti rubella IgM by reference to a curve derived from the standard sera so that earlier semi-quantitative assays can be replaced by a fully quantitative assay.

Initially, some difficulties may be encountered when using the rubella MACRIA. Most stem from unfamiliarity with this sort of assay: its sensitivity, for instance, is such that specific IgM may be detected at low levels for many months after infection. In most cases only evidence of very recent infection is required, and the extra sensitivity may be misleading. There are other drawbacks. Equipping laboratories with adequate washing systems, gamma counters or spectrophotometers is expensive and disposal of radioactive waste may be a problem.

The quality of the rubella MACRIA is greatly influenced by the potency of the anti-rubella reagent. The first assays, using ^{125}I-labelled IgG prepared from the serum of hyper-immunized rabbits and sheep, and from human sera, yielded ratios of test counts to negative counts (T:N) that were rarely higher than 20, and some

specimens taken from patients in the acute phase of rubella gave T:N ratios of less than 10. Recently, the use of ^{125}I-labelled mouse monoclonal anti-rubella IgG has been shown to increase greatly the robustness of the assay, and to reduce fivefold the amount of radioactivity required per test (Tedder, Yao & Anderson, 1982). The use of the monoclonal anti-rubella label in the MACRIA is now being assessed in a number of laboratories in the UK and Europe and the results are encouraging.

In order to detect bound rubella antigen the final, anti-rubella stage of an M-capture assay must at present incorporate a radioisotope or a colour-generating enzyme. When radio-labelled anti-rubella is used the proportion of the reagent bound can easily be measured. With the new monoclonal reagent used in an optimized assay this proportion ranges from 0·05 % for a negative serum to more than 15 % for a serum taken from a patient with acute rubella. The previous 'polyclonal' anti-rubella reagent contained too much redundant IgG to permit such good discrimination between rubella IgM positive and negative sera. The monoclonal label, prepared from pure specific anti-rubella IgG, avoids this problem. Its apparently high avidity also allows radioactivities to be reduced to a minimum (about 30 nCi per test, *c.* 30000 c.p.m.), a step that will go a long way to making these assays safer for the user.

Though radiolabels are easier to prepare, and more convenient to use than enzyme conjugates, some diagnostic laboratories will be reluctant to start radio-isotopic work. Anticipating this reluctance, difficulties in distributing isotopic reagents and the possibility of further controls being imposed on radioactive work in the future, several groups have developed alternative assays employing enzyme-linked anti-rubella (Vejtorp, 1981; Diment & Chantler, 1981). An enzyme-conjugate prepared from monoclonal IgG is the basis of an IgM capture assay readable by eye which could satisfy the needs of the small laboratories as well as those who wish to avoid using radio-isotopes. No spectrophotometry would be involved, the label would have a long shelf-life, and control sera could be made available to help distinguish between positive, negative and borderline reactions. In a few reference centres quantitative radio – or enzyme – immunoassays using measurements by gamma counter or spectrophotometer would be maintained.

The uses of a freely available test for rubella IgM are obvious enough. Unrestricted testing of pregnant women with, or in contact with, rubella-like illnesses will be feasible. Readily available rubella tests for sick and premature infants (requiring only a heel-prick specimen) will permit the rapid and accurate diagnosis of congenital rubella. The added sensitivity of the M-antibody capture assays will make it possible to demonstrate the aetiology in some infants when 'late' stigmata of congenital rubella, such as deafness, come to light (Chantler *et al.*, 1982).

It will also be possible to detect the IgM response in patients given the attenuated vaccines *Almevax* and *Cendevax*. Both these vaccines evoke an IgM response in the non-immune patient that is easily detectable by the M-antibody capture assay and that persists long enough to be a useful measure of pre-vaccine susceptibility. This may help to decide the management of women inadvertently immunized when they are pregnant (Mortimer *et al.* 1981*b*).

We expect the new IgM assays to supplant other serological tests for rubella, with the exception of two simple screening tests for antibody, radial haemolysis

and the haemagglutination-inhibition test. The M-antibody capture assay is not susceptible to interference by rheumatoid factor but there does seem to be a low incidence of false-positive reactivity among patients with glandular fever (Morgan-Capner & Tedder, unpublished observation). For the present, it would seem prudent to exclude the presence of Paul-Bunnell heterophil antibody in sera reactive in the assay. When a pregnant woman is involved, positive reactions also should, if possible, be confirmed by serum fractionation and the demonstration of IgM class haemagglutination-inhibiting antibody.

In epidemic years, when the number of rubella-infected babies born may increase several-fold, the M-capture assays will be especially helpful. Surveillance in the United Kingdom is good enough to recognize a new epidemic quickly and it should then be possible to identify affected pregnancies by the unrestricted use of the new and cheaper M-antibody tests. For the first time, questioning at antenatal clinics about recent illness and contacts with rubella can be backed up by a readily available anti-rubella IgM assay. This will help clinicians and patients decide whether to allow pregnancies to proceed, and it may lead to fewer not more pregnancies being terminated. In the past the fear of fetal rubella has been such that many terminations have been carried out on account of the risk alone of being infected.

The prospect of having the M-capture assays in fairly general use before the next rubella epidemic is most satisfactory. Routine use has already shown that M-antibody capture is probably the method of choice for diagnosis and, with its wider introduction to diagnostic laboratories, there will be little more to be done to improve the serology of rubella. Attention must then be focused on the relatively neglected problems of vaccine use and acceptance. Congenital rubella can only be eradicated by making sure that all women are immune or by using vaccine to interrupt the transmission of rubella between children and, thereby, from children to adult women. As the latter course has been rejected in the United Kingdom the former must be pursued all the more vigorously. Simple and accurate tests for rubella infection like the M-capture assays, useful though they will be, can only be a palliative. They are no substitute for an effective immunization programme that prevents pregnant women from catching rubella.

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