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Comparison of haemagglutination-inhibition and single radial haemolysis techniques for detecting antibodies to influenza A and B viruses

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SUMMARY

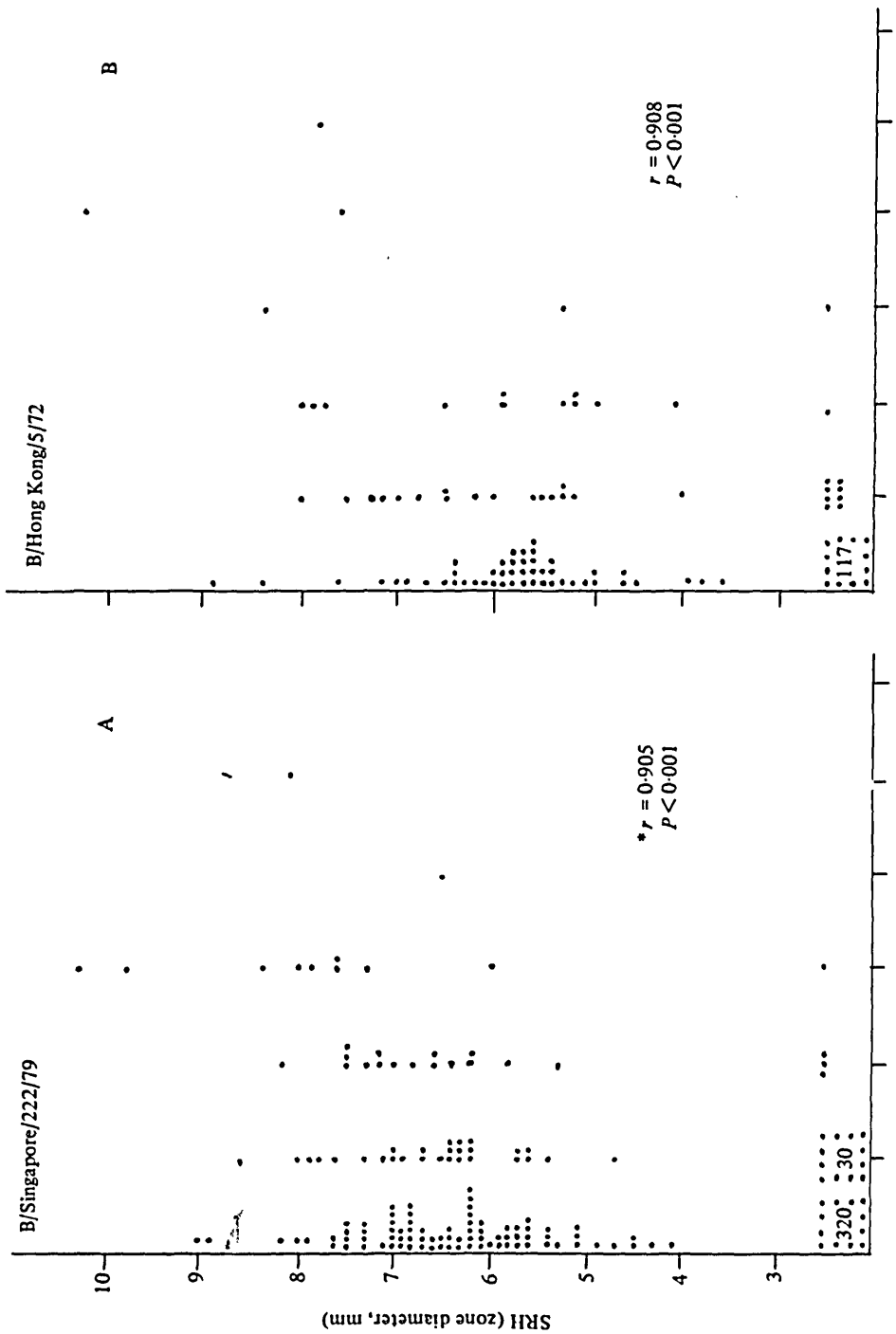
The sensitivities of haemagglutination-inhibition (HI) and single radial haemolysis (SRH) techiques in detecting antibodies against influenza A/Bangkok/1/79, A/Brazil/11/78, B/Singapore/222/79, B/Hong Kong/5/72 strains, in human sera were compared. For antibodies to influenza B viruses the HI tests employing ether-treated antigens were also evaluated. The SRH technique appears to be more sensitive for detecting protective titres of antibodies against influenza B strains and influenza A/Brazil/11/78.

The yearly serological evaluation of antibodies to influenza haemagglutinin (HA) is important in influenza surveillance. Information is obtained concerning: (i) the immunity level in the human population to current and newly isolated strains of virus, before and after the influenza season, (ii) the extent of virus infection, (iii) the response to vaccination. In this respect the available methods are the haemagglutination-inhibition (HI) test, which has been the standard method for many years, and the more recently developed single radial haemolysis (SRH) test.

However, serological data for influenza B virus obtained with the HI test have often revealed low percentages of sera with a titre of $\geqslant 40$, which is the titre commonly considered protective. Chakraverty (1980) and Oxford, Yetts & Schild (1982) have demonstrated that SRH is more sensitive than HI in detecting antibodies against influenza B virus. To overcome the low sensitivity of the HI for influenza B virus, Monto & Maassab (1981) modified this test by using ether-treated antigen which reportedly increases the antibody titres.

The present serological survey, carried out on 490 human sera collected from healthy persons of all ages, both males and females, in a post epidemic period of the 1981 influenza season, has been aimed at comparing the sensitivity of HI and SRH techniques for detecting antibodies against both influenza A and B viruses. For the latter virus, moreover, a further comparison has been made with the serological results obtained by an HI test employing the ether-treated antigen (Berlin *et al.* 1963).

The HI test was performed according to the WHO recommended method (Advanced Laboratory Techniques for Influenza Diagnosis, 1975); the SRH test



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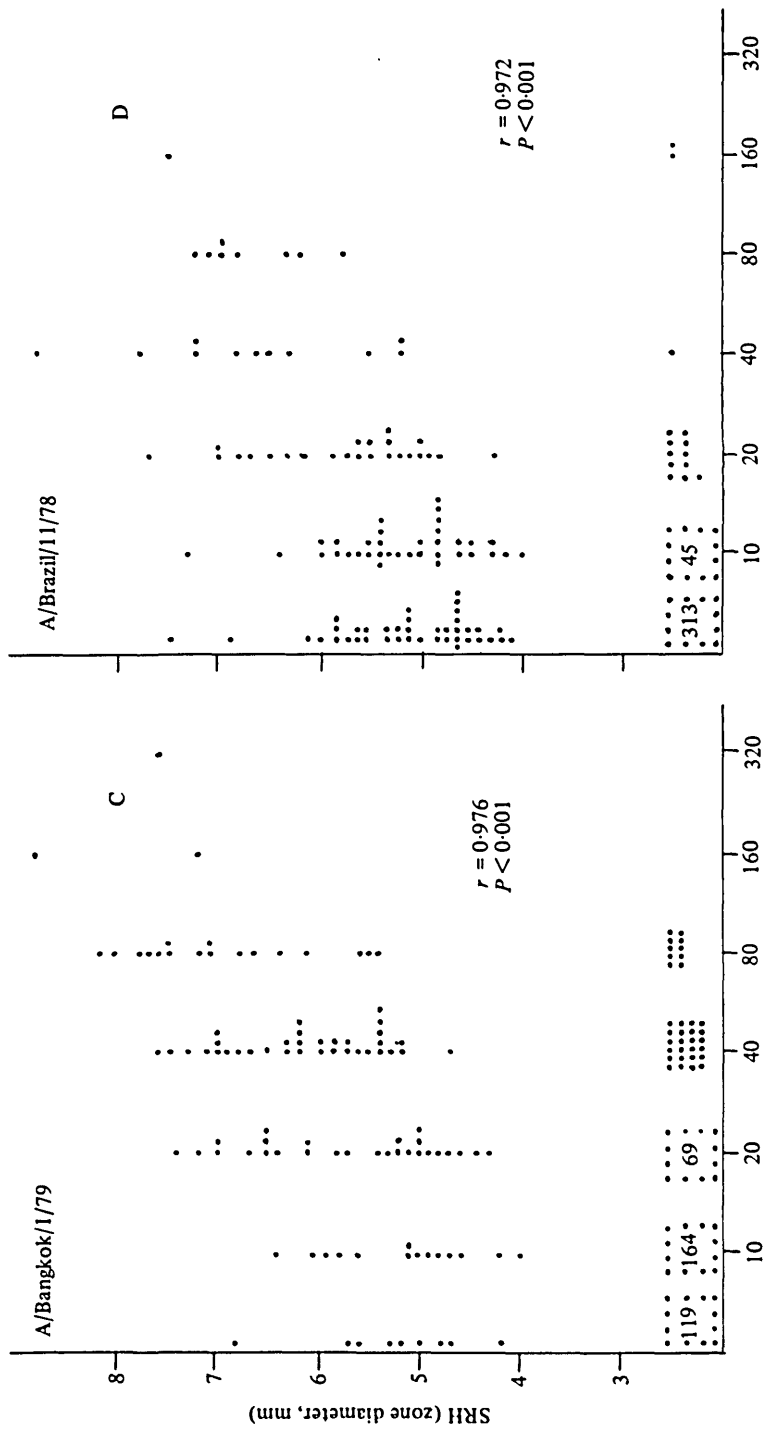

Fig. 1. Comparison between HI and SRH zone diameters obtained on 490 human sera against A/Bangkok/1/79, A/Brazil/11/78, B/Singapore/222/79 and on 208 sera against B/Hong Kong/7/72.

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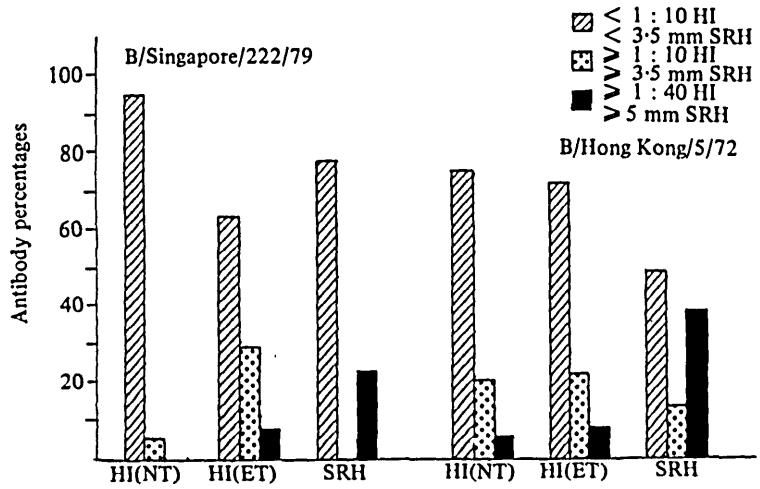

Fig. 2. Comparison of antibody levels to influenza B strains detected by HI using untreated (NT) or ether-treated (ET) antigen and SRH on 80 human sera.

Table 1. *Comparison of results obtained, on 80 human sera, by HI using an ether-treated (ET) and an untreated (NT) antigen and SRH in the detection of protective antibodies against B/Singapore/222/79 and B/Hong Kong/5/72*

	Number (percentage) of sera with stated antibody result					
	HI (NT)		HI (ET)		SRH	
Virus	< 10	⩾ 40	< 10	⩾ 40	<3·5 mm	⩾ 5 mm
B/Singapore/222/79	76 (95)	0*	51 (64)	6* (7·5)	62 (78)	18* (23)
B/Hong Kong/5/72	60 (75)	4** (5)	57 (71)	6** (8)	39 (49)	31** (39)

* Statistical significance: 0 versus 6 $P = < 0{\cdot}025$; 0 versus 18 $P = < 0{\cdot}001$; 6 versus 18 $P = < 0{\cdot}01$.

** Statistical significance: 4 versus 6 not significant; 4 versus 31 $P = < 0{\cdot}001$; 6 versus 31 $P = < 0{\cdot}001$.

was carried out as described by Schild, Pereira & Chakraverty (1975) and Oxford *et al.* (1982) using veronal buffer pH 7·3–7·4 and a well diameter of 3 mm. The antigens used were the influenza strains: A/Bangkok/1/79, A/Brazil/11/78, B/Singapore/222/79 and B/Hong Kong/5/72.

From results of preliminary studies we compared HI titres of ⩾ 10 and ⩾ 40 (the latter usually being taken to indicate protective levels of influenza HI antibody) with clear zones of haemolysis ⩾ 3·5 mm in diameter. As a consequence we decided arbitrarily to consider an SRH zone of ⩾ 5·0 mm as indicating the presence of protective levels of antibody. This is not to say that an HI titre of ⩾ 40 is equivalent to an SRH zone of 5·0 mm. More work is needed to warrant such an assumption and the results described below indicate that the relationship between HI titres and SRH zones will depend upon the particular influenza virus under study.

Using the above criteria, the two techniques do not reveal differences in the percentages (17·9%) of sera with protective antibody to A/Bangkok/1/79 strain (Fig. 1). However when sera were tested against A/Brazil/11/78 a higher

percentage was found with SRH (16·7%) than with the HI test (4·5%). Even greater differences were obtained with the influenza B strains (B/Singapore/222/79 and B/Hong Kong/5/72), the percentages of protective antibodies being respectively 26·7% and 34·1% with SRH ($\geqslant 5$ mm), and only 2·4% and 2·8% with HI ($\geqslant 40$). The differences between these percentages for A/Brazil and both B strains are statistically significant ($P < 0{\cdot}0001$).

It follows that more accurate epidemiological information can be inferred from the serological data obtained with the SRH test, and that this applies to the circulation of A/Brazil/11/78 and the two influenza B strains in the winter of 1981. Fig. 1 also shows the comparison between HI antibody titres and the corresponding SRH diameters for the four viruses. It is evident that a large proportion of sera have no antibodies detectable by either technique: B/Singapore 65·3% (panel A); B/Hong Kong 56·2% (panel B); A/Brazil 63·8% (panel D), less for A/Bangkok 24·3% (panel C). Many sera with low HI titres of 10 and 20 are negative by SRH, but there are also a few sera showing a zone of haemolysis $\geqslant 5$ mm. It must also be emphasized that a significant number of sera which are negative (< 10) by HI are clearly positive by SRH. The percentages of sera giving a detectable area of haemolysis, ranging from 3·5 up to 9 mm, among the sera negative by HI, are for: A/Bangkok, 7·3%; A/Brazil, 10·8%; B/Singapore, 20·2% and B/Hong Kong, 28·6%. These results clearly indicate that, in some respects, there is little correlation between the two techniques. Nevertheless, if one takes into account only the sera positive by either test, a good correlation (calculated by the weighted least squares method: Draper & Smith, 1966) is evident (see r factors in Fig. 1). However, in panel C there are 34 sera giving, by repeated tests, a negative result by SRH but an HI titre $\geqslant 40$. This discrepancy could be due to incomplete removal of non-specific inhibitors of haemagglutination in the HI test, as observed by Pettersson (1980) in the case of strain A/Victoria/3/75. It is known that different strains of influenza virus could have different sensitivity to non-specific inhibitors of HA (Schild & Dowdle, 1975).

Fig. 2 shows the percentages of sera with antibodies to influenza B virus strains detected in 80 human sera by SRH and HI tests employing in the latter test the ether-treated antigens. In Table 1 the total numbers and the percentages of negative, positive and protective sera are reported. It is apparent that ether-treatment of the antigens of both influenza B strains has only a slight effect on improving the detection of protective sera by HI. Again the SRH detection of sera with protective ($\geqslant 5$ mm) levels of antibody is clearly higher than that of HI.

We conclude that the choice of the SRH method, which is simple, rapid and easy to read, is essential for sero-epidemiological studies relying on the detection of antibodies to influenza B virus. Moreover, it also appears more suitable for some influenza A viruses as shown by the data obtained for A/Brazil/11/78. The use of ether-treated antigen in the HI test is of little value.

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