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# Use of the India-ink Immuno-reaction for the rapid detection of enteric pathogens in two areas of Nigeria

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## SUMMARY

The India-ink Immuno-reaction (IIR) was used as a simple, convenient procedure for the detection of carriers of enteric organisms in an unselected sample of patients attending a mission clinic at Kubacha in a remote area of Kaduna State, northern Nigeria. To assess the reliability of this procedure in difficult working conditions a similar population from the same clinic was subsequently examined by routine bacteriological culture techniques. Because of a temporary shortage of suitable anti-sera we were unable also to examine specimens from this second group by IIR.

A further group of patients attending the out-patients clinic of the General Hospital in Kaduna with symptoms of acute enteric disease was investigated using both IIR and routine culture.

Proportionally more positive results were obtained for *Salmonellae*, *Shigellae* and *Vibrios* with IIR than with routine culture. A larger scale controlled survey is desirable to evaluate the procedure further.

## INTRODUCTION

The India-ink Immuno-reaction (IIR) of Geck (1971) appeared to be an ideal method for the diagnosis of enteric (and other bacterial) disease in parts of the world where facilities for complex laboratory investigation are lacking. Such areas are often those in which intestinal bacterial disorders continue to be major causes of ill-health in the population generally and important contributory factors in the failure of infants to thrive. The technique, if proven to be as sensitive and specific

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in the field as Geck (1971) and Duc (1971) have found in Budapest, could offer scope for large scale epidemiological surveys with minimal expenditure of resources. One of us (G.T.) began employing the technique in a hospital to detect salmonella and other antigens in faecal specimens using materials kindly supplied from the National Institute of Public Health in Budapest. Subsequently the surveys reported here were undertaken.

## MATERIALS AND METHODS

### *Collection of specimens*

Rectal swabs were collected from a non-selected sample of 544 patients attending the St. Michael's Health Centre in Kubacha (an out-station of the St Louis Hospital, Zonkwa) in July and August, 1976. These were each used in the preparation of three smears for IIR testing (see below).

A further 341 patients were sampled from September to November 1976, two swabs being collected from each patient for routine culture for salmonellae, etc. and for *Vibrio cholerae*.

From January to May, 1978 rectal swabs for both investigations were collected from a total of 203 out-patients at Kaduna General Hospital presenting with symptoms of acute enteric disease.

### *India-ink Immuno-reaction*

The technique is essentially that described by Geck (1971). Well-cleaned glass microscope slides were used for the preparation of three thin smears from each of the freshly collected rectal swabs (six from the General Hospital specimens). These were allowed to dry in air at room temperature. The India-ink used in this study was 'Hollo', Politur es Vegyiternek Co., Budapest, but all kinds of india-ink producing 4+ positive reaction with *Staphylococcus epidermidis* smears without specific antisera can be used, Geck (1976). One drop was applied plus one drop of the working dilution of the specific anti-serum to be used. The two drops of fluid were mixed together carefully and spread evenly over the smear with a bacteriological loop. The smears were then left for 5 min in a moist chamber before washing off the reagents using a solution of pH 7 comprising two parts of physiological saline to three parts water and a broad orifice pipette to avoid excessive 'jetting'. Dry slides were examined with an oil immersion objective at × 1000.

A positive reaction shows ash-grey bacterial cells of typical morphology with cell walls surrounded by a thick, black contour. With monovalent sera, homologous cells show a particularly thick coating (of antibody + carbon particles + surface antigen) as in Fig. 1, + + + + positive. A somewhat thinner layer, graded + + + positive, is acceptable when polyvalent sera are employed. Much thinner layers (+ + and +) may be due to cross-reactions with organisms sharing some surface antigens and are therefore discounted. Smears showing no bacterial cells after thorough searching are also counted negative.

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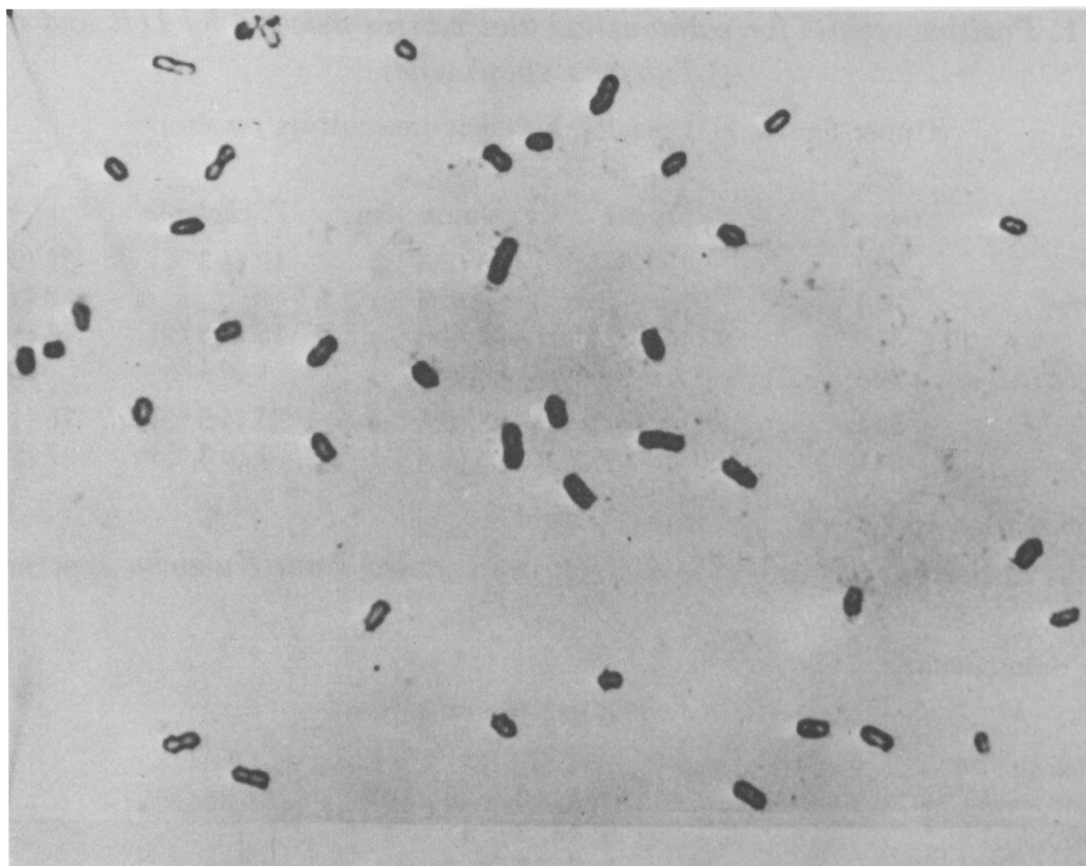

Fig. 1. Positive IIR reaction (graded 4+) using salmonella from an *in vitro* culture. (Magnification ×1000; photograph kindly supplied by P. Geck.)

*Anti-sera used*

The anti-sera were supplied at use dilution. They were more potent by a factor of three than the usual commercially prepared antisera employed for slide agglutination tests. The following antisera were used for the present work:

(i) *Salm. typhi* Vi
(ii) *Salmonella* '0' polyvalent (containing factors, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 19, 21, 46)
(iii) *Vibrio cholerae* polyvalent
(iv) *Shigellae dysenteriae* polyvalent
(v) *Sh. flexneri* polyvalent 1–6
(vi) *Sh. sonnei* (Phase I)

i–iii on all specimens; iv–vi on General Hospital specimens only.

*Routine isolation and identification*

One swab from each patient was broken off into approximately 15 $cm^3$ Selenite F broth, the other into alkaline peptone water (pH 8·6). After overnight incubation at 37 °C subcultures were made from Selenite onto Oxoid MacConkey Agar and D.C.A. plates and from alkaline peptone water to thiosulphate citrate bile salts sucrose agar (TCBS) plates. Isolates of salmonellae or shigellae and vibrios were identified by routine morphological, biochemical and serological tests. Those isolated at Kubacha were sent to the Salmonella & Shigella Reference Laboratory at Colindale, London, and the Public Health Laboratory, Maidstone, respectively.

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Table 1. *Positive results for salmonellae and vibrios detected by IIR and culture (Kubacha specimens)*

(Upper figures IIR results, lower figures culture results).

| | No. tested | *S. typhi* | Other salmonellae | *V. cholerae* | Total +ve |
|---|---|---|---|---|---|
| Children | 230 | 6 (2·6%) | 6 (2·6%) | 10 (4·3%) | 22 (9·6%) |
| < 12 yrs | 78 | 0 | 2 (2·6%) | 0 | 2 (2·6%) |
| Adults | 314 | 17 (5·4%) | 22 (7·0%) | 15 (4·8%) | 54 (17·2%) |
| | 263 | 0 | 4 (1·5%) | 1 (0·4%) | 5(1·6%) |
| Total | 544 | 23 (4·2%) | 28 (5·1%) | 25 (4·6%) | 76 (14·0%) |
| | 341 | 0 | 6 (1·8%) | 1 (0·3%) | 7 (1·3%) |

Table 2. *Salmonella and vibrio serotypes isolated from Kubacha specimens*

| Salmonellae: | | No. of isolates |
|---|---|---|
| Serotype | Antigenic structure | |
| *Salm. kubacha** | 1, 4, [5], 12, 27: l, $z_{13}$, $z_{28}$: 1, 7 | 1 |
| *Salm. saint-paul* | 4, 5, 12: e, h: 1, 2 | 2 |
| *Salm. garba* | 6, 14, 25: a: 1, 5 | 1 |
| *Salm. dublin* | 1, 9, 12: g, p: – | 1 |
| *Salm.* unnamed | o = rough: y: e, n, x | 1 |
| *Vibrio:* | | |
| *Vibrio cholerae* el tor ogawa (Mukerjee phage type 4; sensitive to phage beta) | | 1 |

* *Salm. kubacha* is a new serotype.

## RESULTS

Table 1 summarizes the results obtained with rectal swabs from Kubacha, 544 tested by IIR and 341 examined by routine culture. The identity of the six cultured salmonellae and single vibrio is given in Table 2. No shigella organisms were isolated.

Table 3 gives results of the 203 specimens from Kaduna tested by both IIR and culture. The 21 swabs positive by culture include 17 positive with compatible antisera in the IIR tests. The other four culture positive specimens yielded growths of *Shigella* spp. (two *Sh. dysenteriae*, and one each of *Sh. flexneri* and *Sh. sonnei*). These were not detected by the IIR test but one of the specimens reacted with the polyvalent Salmonella 0 antisera in IIR. A positive reaction to one or more IIR antisera was obtained with 44 specimens negative on culture. Sixty-eight per cent (138 specimens) proved negative in both procedures. In two cases cultures yielded growths of two pathogens each (*Sh. sonnei* + *Salm. typhi* and *Sh. sonnei* + another salmonella). In these cases only *Sh. sonnei* was detected by IIR. In six swabs which reacted with two antisera in IIR, only one (reacting with *Salm. typhi* Vi and *Sh. sonnei*) was positive on culture, when only *Sh. sonnei* was isolated.

Results of the two procedures are compared in summary form in Table 4.

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Table 3. *Positive results, Kaduna General Hospital specimens*

(Upper figures IIR results, lower figures culture results.)

| | No. tested | *Salm. typhi* | Other salmonella | *V. cholerae* | *Sh. dysenteriae* | *Sh. flexneri* | *Sh. sonnei* | Total |
|---|---|---|---|---|---|---|---|---|
| Children | | 4 | 18 | 2 | 7 | 11 | 8 | 50 |
| < 12 years | 139 | 0 | 3 | 2 | 3 | 4 | 2 | 14 |
| Adults | 64 | 7 | 4 | 1 | 1 | 1 | 4 | 18 |
| | | 3 | 1 | 1 | 0 | 0 | 4 | 9 |
| Total | 203 | 11 (5·4 %) | 22 (10·8 %) | 3 (1·5 %) | 8 (3·9 %) | 12 (5·9 %) | 12 (5·9 %) | 68*(33·5 %)* |
| | | 3 (1·5 %) | 4 (2·0 %) | 3 (1·5 %) | 3 (1·5 %) | 4 (2·0 %) | 6 (2·9 %) | 23†(11·3 %)† |

* Includes six swabs each reacting with two antisera.

† Includes two swabs each yielding growths of two pathogens. (Percentages calculated accordingly.)

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Table 4. *Comparison of results for Kaduna specimens in both procedures*

| Pos IIR/ Pos culture | Pos IIR/ Neg culture | Neg IIR/ Pos culture | Neg IIR/ Neg culture | Total |
|---|---|---|---|---|
| 17 (8·4%) | 44 (21·6%) | 3 (1·5%) | 138 (68·0%) | 202* (99·5%)* |

* One swab reacted with salmonella poly 'O' antiserum but grew *Sh. dysenteriae* on culture.

## DISCUSSION

The Kubacha samples used for culture, collected a few months after those tested by IIR and from different patients, provide inadequate control of the latter procedure. However, they do confirm the presence of both salmonellae and *Vibrio cholerae* in the local population and thus support to some extent the validity of the IIR results. The Kaduna Hospital samples, though fewer, provide a more effective comparison – and show a similar higher proportion of positives with the IIR technique. The higher incidence of positive findings in the Kaduna survey is possibly due to the more selected nature of this sample population.

Geck (1971) in laboratory experiments using faecal samples artificially seeded with $10^3$–$10^9$ cells of pathogenic *E. coli* strains, *Sh. flexneri*, *Sh. sonnei* or three salmonella serotypes, found slightly better sensitivity with IIR compared to routine culture for all but the salmonella specimens where results marginally favoured culture (of 350 specimens, 241 were proven positive by culture, 239 by IIR). Additionally, comparison of IIR and immuno-fluorescent staining of several thousand routine faecal specimens 'showed that IIR and immuno-fluorescent staining were practically equivalent'.

The examination of rectal swabs from groups of hospital out-patients at (i) Kubacha, a remote, rural area of Kaduna State, northern Nigeria, and (ii) the state capital, suggest that IIR is more sensitive than routine bacteriological culture in field conditions in detecting enteric pathogens. Alternatively, the slide technique may simply be less specific.

As with other systems of direct examination of faeces, doubts about specificity remain largely unresolved due to the sharing of surface antigens by both pathogenic and commensal enteric organisms. However, the simplicity of the procedure combined with modest requirements of apparatus and reagents (oil-immersion microscope, suitably prepared antisera, india-ink and washing fluid) make its introduction to remote areas where laboratory aids to diagnosis in general are limited seem very attractive. The work reported here was, necessarily, limited. Further studies of the IIR procedure under field conditions are needed and careful comparison with classical diagnostic methods to determine its true value as a diagnostic aid and/or epidemiological screen.

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