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TECHNICAL METHODS

A Quick Method of Identifying Salmonella H Antigens

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The identification of Salmonella H antigens by unspecialized laboratories has become a routine procedure in recent years, but the systematic detection of these antigens may be a tedious process when a comparatively uncommon type is encountered. Edwards and Kauffmann (1952) introduced a shorter version of the Kauffmann-White scheme giving sufficient detail in classification for the ordinary bacteriology department. The basis of this scheme was to amalgamate certain antigenic complexes under one heading as shown in Table I. Very uncommon and atypical organisms such as *S. salinatis* and *S. chittagong* were omitted.

TABLE I

POOLING OF ANTIGENIC COMPLEXES FOR SIMPLIFIED KAUFFMANN-WHITE SCHEME

G	f,g; f,g,t; g,m; g,m,s; g,m,t; g,p; g,p,u; g,q; g,s,t; ms,t
L	l,v; l,w; l,z_{13}; l,z_{28}
Z_4	z_4z_{23}; z_4z_{24}; z_4z_{32}
e,n	e,n,x; e,n,z_{15}
1	1,2; 1,5; 1,6; 1,7; z_6

The method put forward in this note is designed to identify the flagellar serotype, on the basis of this scheme, using a maximum of six agglutinations. All unusual or atypical organisms would in any case have to be confirmed by a Salmonella reference laboratory.

The total number of H antisera required for testing are reduced to the 16 given in Table II, to which must

TABLE II

REQUIRED SERA FOR ANTIGENS IN SIMPLIFIED KAUFFMANN-WHITE SCHEME

a	L
b	r
c	y
d	z
h	z_4
G	z_{10}
i	z_{29}
k	z_{36}

be added two polyvalent phase 2 sera for identifying the 1,2 1,5 . . . z_6, and e,n,x complexes. For routine flagellar identification four polyvalent sera are made up containing the mixtures given in Table III. In this table G would be a combined serum against the complexes fg, mt, gp, and gst, while L is represented by the l,w complex.

TABLE III

COMPOSITION OF POLYVALENT ANTISERA FOR RAPID IDENTIFICATION OF SALMONELLA SEROTYPES

Serum 1	Serum 2	Serum 3	Serum 4
a	a	a	a
b	b	b	c
c	c	h	h
d	d	G	i
h	L	L	L
G	r	r	y
i	y	z_4	z_4
k	z	z_{10}	z_{29}

When a motile salmonella in phase I is tested against all these sera it will give a standard pattern of agglutinations corresponding to the H antigen which it carries and can be immediately identified from Table IV.

TABLE IV

PATTERNS OF RESPONSE OF POLYVALENT SERA AND CORRESPONDING ANTIGENS

Corresponding Antigen	Serum (1)	Serum (2)	Serum (3)	Serum (4)
a	+	+	+	+
b	+	+	+	−
c	+	+	−	+
d	+	+	−	−
h	+	−	+	+
G	+	−	+	−
i	+	−	−	+
k	+	−	−	−
L	−	+	+	+
r	−	+	+	−
y	−	+	−	+
z	−	+	−	−
z_4	−	−	+	+
z_{10}	−	−	+	−
z_{29}	−	−	−	+

The actual procedure of agglutination with an unknown organism would be to test first against an overall polyvalent Salmonella H serum, and then against the four polyvalent identifying sera. If no reaction was obtained with the latter it would be assumed that the organism was in phase 2 and further investigation would be required to reveal the first phase. An organism which fails to react with the four polyvalent sera and is not in phase 2 must either have antigen z_{36} or be of some other very uncommon or undescribed type. The detailed investigation of organisms such as *S. san diego* can only be carried out in a highly specialized laboratory.

The method outlined above has been found to work well with standard laboratory strains and has so far proved practically useful. It is quite suited to slide

agglutination provided that the precautions mentioned by Bridges and Taylor (1944) are observed; it is obviously best to confirm the findings by tube agglutination if possible when an unusual organism has been found. Whether the method will be of universal application in the field can only be discovered by trial. There are no obvious pitfalls except that a minor antigen in the organism may cause cross reactions, but this seems unlikely if dilute sera are used. Although the concentration of each individual serum in any mixture is low the total quantity of serum is rather large, and it is possible that this may occasionally give rise to non-specific effects.

An objection that might be raised from the point of view of serum production is that there is some waste of sera against rare antigens since these are used almost as often as commoner ones.

This does not appear to be a very serious fault when dealing with H− antisera, which are usually of high titre and therefore economical to produce.

Extension of this method to Salmonella O antisera is not very practical owing to their low titre and to the overlapping of antigens between O groups.

I am very grateful to Dr. Joan Taylor for her co-operation in testing this method and to her and Dr. G. S. Wilson for their comments on the manuscript. I must also thank Mr. T. Nash for a suggestion that initiated the work.

The polyvalent sera mentioned in this paper are available in limited quantities from the Standards Laboratory, Central Public Health Laboratory, Colindale, London, N.W.9.

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Third International Congress of Clinical Pathologists

The third international congress of clinical pathologists will be held in Brussels from July 14 to 20, 1957, preceded by a symposium to be held at Liège under the auspices of the C.I.O.M.S. The subject of the symposium is "Sensitivity to Drugs." Those who wish to attend all or part of the conference are asked to register as soon as possible with Dr. W. H. McMenemey, Maida Vale Hospital for Nervous Diseases, London, W.9.

The Estimation of True Lipase in Small Intestinal Contents

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An estimation of lipolytic activity in specimens of duodenal juice obtained by intubation is a necessary step in the assessment of intestinal function in relation to fat absorption.

In this method conditions *in vitro* were made to resemble as closely as possible those found *in vivo* during normal fat absorption. Therefore, neither preformed emulsion (Palmer, 1922), short-chain glycerides (Lagerlöf, 1942), or polyoxyethylene esters (Archibald, 1946) were used as substrates, but one of the common dietary long-chain triglycerides. A similar substrate was used by Willstätter, Waldschmidt-Leitz, and Memmen (1923). The mixing properties of intestinal motility were reproduced by agitation on a mechanical shaker. Bile was added to give the concentration normally found in the intestine.

Experimental

Special Apparatus.—A microid flask shaker (Griffin and Tatlock Ltd.), which carries two flasks each held in clamps 9 cm. from the fulcrum, is used. It was calibrated by recording vibrations on a smoked drum. This can also be done roughly by clamping a pencil in one arm and counting the number of dots on a paper held near during a period of 15 seconds.

Reagents.—The following are required:

Pancreatin U.S.P. XI, Philip Harris Ltd., Birmingham, 5%, w/v in water.

Bile salts, Difco No. 3, 25% w/v in water.

Tetramethyl ammonium hydroxide, B.D.H., 25%, diluted with ethanol to give an N/20 solution.

Olive oil, B.P., rendered acid-free and almost colourless by passage through an alumina column.

Thymol blue, 0.1% in ethanol.

Alumina, Hopkin and Williams, "chromatographic grade."

Method.—Acid-free olive oil, 1 ml., was added by syringe to 5 ml. phosphate buffer, *p*H 7.8, containing 0.5% bile salts in a 50 ml. stoppered conical flask. Duodenal juice, 0.1 ml., or 1 ml. pancreatin solution,