

NOTE: When using Selenite F broth the background colour shown above may be masked and appear as a pink or red/brown colour. In such a case identification is made using the colour of the agglutinated latex particles only.

If Vi antigen is detected (red agglutination with Latex Reagent 2), take a fresh sample of Selenite F broth or make a fresh suspension of the culture in saline containing 0.5% formalin in a suitable tube or bottle to kill the bacteria. Immerse in a boiling water bath for 5 minutes, cool and retest with Latex Reagent 1. Alternatively, make at least 400 µl of a fresh suspension of the culture in saline or distilled water. Immerse in a boiling water bath for 30 minutes, cool and re-test with Latex Reagent 1. Agglutination will identify the serogroup of a Salmonella; if there is no agglutination with Latex Reagent 1 the culture is not Salmonella. Note that boiled Vi antigen will still react with Latex Reagent 2.

A negative result indicates that the sample under test does not contain antigens belonging to the Salmonella serogroups covered by the reagents. Mixed growths of non-lactose fermenters are frequently present in stool cultures, and if a negative result is obtained when testing colonies it may be necessary to repeat the test on other selected colonies before discarding the culture as negative.

If a non-specific reaction is obtained the result is not interpretable, and conventional procedures for identification should be followed^{2,3,4}.

In enrichment broths, non-specific reactions may be caused by heavily mucoid faecal samples. Transfer 0.5 ml or more of the broth to a suitable glass tube or bottle, loosen the cap and place in a boiling water bath for 5 minutes. Cool to room temperature (18 to 30°C) and repeat the test procedure. A sample of the enrichment broth should be processed in the same way for use as a negative control.

12. LIMITATIONS OF THE PROCEDURE

Wellcolex Colour Salmonella is designed as a screening procedure for salmonellae from Selenite F broths and from solid media as a culture identification test. The test will identify Salmonella isolates to serogroup level, which is satisfactory for most purposes when full identification can be performed by a reference laboratory⁴. Definitive identification requires conventional biochemical and serological procedures^{2,3,4}; selection of appropriate antisera may be guided by the results of Wellcolex Colour Salmonella.

Occasional false-positive reactions might be encountered due to the presence of shared antigens in heterologous species or genera such as Citrobacter; these may be differentiated using conventional biochemical test procedures. Also Vi antigen may be found on bacteria other than Salmonella²; these may be differentiated by retesting a broth sample or colony suspension after boiling to remove the Vi antigen; if no agglutination is seen other than with red latex in Latex Reagent 2 the organism is not Salmonella. Particular caution should be exercised in case one of these organisms is present in an enrichment broth as well as Salmonella; it is wise to confirm the result on a subculture of the broth before reporting.

A negative result does not confirm the absence of Salmonella. For example, the test does not cover the full range of Salmonella serogroups, but will, for example, detect the serogroup present in over 99% of strains encountered in human infection in the UK⁶ and over 98% in the USA⁷. In addition the level of Salmonella in some broth cultures may be insufficient to give a positive result. For example this may occur with broths which have not been incubated for a full 18 to 24 hours.

Some sub-optimal preparations of Selenite F broth contain a brick-red precipitate and caution should be exercised in interpreting results obtained in tests with this medium.

13. EXPECTED RESULTS

Strains belonging to serogroups A, B, C, D, E or G, or which possess Vi antigen will give a red, blue or green agglutination with the corresponding component of Latex Reagent 1 or 2.

SPECIFIC PERFORMANCE CHARACTERISTICS

External Evaluation

Two studies have been performed to evaluate Wellcolex Colour Salmonella:

(a) A multicentre study involving five Public Health Laboratories in the UK and three hospital laboratories in the USA on routine cultures for Salmonella.

Each laboratory performed tests on one or more of the following samples from each faecal specimen:

- 1) Selenite enrichment broths (Selenite F).
- 2) Lactose negative colonies direct from enrichment broth subcultures on selective-differential agar plates.
- 3) Lactose negative colonies direct from primary selective-differential agar plates (MacConkey, XLD, DCA, SS and Hektoen).
- 4) Pure subcultures of lactose negative colonies on nutrient agar.

A rotator was used throughout.

The results are shown in Tables 1 and 2.

The performance of Wellcolex Colour Salmonella was determined by comparison with results of traditional bacteriological methods on the samples.

In this study the sensitivity and specificity of Wellcolex Colour Salmonella (see Tables 1 and 2) were:

	Sensitivity		Specificity	
Primary plate cultures	100%	(65/65)	99.2%	(127/128)
Enrichment broth subcultures	100%	(176/176)	100%	(147/147)
Pure cultures	99.5%	(191/192)	98.0%	(100/102)
Selenite broths	94.2%	(114/121)	99.7%	(305/306)

Nine non-Salmonella cultures were encountered which carried Vi antigen: none of these reacted with group-specific components in the test.

The predictive value of a positive result was 99.3% (432/435) from plate culture, and 99.1% (114/115) from Selenite broth. Negative predictive values were 99.7% (374/375) and 97.8% (305/312) respectively.

The prevalence of Salmonella in the samples studied was 44.8% (554/1237).

The occurrence of non-interpretable reactions with Wellcolex Colour Salmonella was 4.5% (9/202) for primary plate cultures, 6.1% (21/344) for enrichment broth subcultures, 3.6% (11/305) for pure cultures and 2.1% (9/436) from Selenite broth. These samples have been excluded from Tables 1 and 2.

(b) An independent study on fresh isolates and reference cultures.

In this study⁸, Wellcolex Colour Salmonella correctly identified 267 of 268 fresh isolates and reference cultures of Salmonellae. These included : ten isolates from each of the ten most common Salmonella serotypes, (*S. typhimurium*, *S. enteritidis*, *S. virchow*, *S. stanley*, *S. hadar*, *S. agona*, *S. heidelberg*, *S. infantis*, *S. newport* and *S. braenderup*), ten strains of *S. typhi* Vi+ and ten Vi-, ten different phagetypes each of *S. paratyphi* A and *S. paratyphi* B and seventy five additional strains from the groups C, D, E and G. No cross reaction was found with Groups F and H. Among the other groups up to and including O-67 tested, a weak reaction with the Group D latex in Reagent 1 was seen with an O-52 (*S. utrecht*) culture; this reaction was not seen with another O-52 (*S. flottbek*). *S. uclee* (O-3, 54) reacted as expected with the group E reagent.

NOTE: Further details of serological and biochemical test procedures for the identification of Salmonella may be obtained from Ewing (1986)².

Table 1
Identification of Salmonella from Plate Cultures

	Wellcolex Colour Salmonella	Routine Result		
		Test Result	POSITIVE ^a	NEGATIVE
PRIMARY CULTURE	POSITIVE	65	1 ^b	66
	NEGATIVE	0	127	127
SUBCULTURE FROM BROTH	POSITIVE	176	0	176
	NEGATIVE	0	147	147
PURE SUBCULTURE	POSITIVE	191	2 ^{b,c}	193
	NEGATIVE	1 ^d	100 ^e	101
TOTAL		433	377	810

^a 199 group B, 116 group C, 91 group D, 23 group E, 2 group G and 2 group D / Vi.

^b unidentified non-lactose fermenter (group E / G reaction).

^c *Citrobacter freundii* (group E / G reaction).

^d Group D which had been identified correctly by Wellcolex Colour Salmonella from the broth subculture.

^e Includes *Escherichia coli*, *Proteus mirabilis*, *Campylobacter jejuni*, *Citrobacter freundii*, *Enterobacter spp.*, *Klebsiella aerogenes* and unspecified coliforms.

Table 2
Identification of Salmonella from Selenite broth
Routine Culture Method

		POSITIVE	NEGATIVE	TOTAL
Wellcolex Colour Salmonella	POSITIVE	114 ^a	1 ^c	115
	NEGATIVE	7 ^b	305 ^d	312
TOTAL		121	306	427

^a 57 group B, 27 group C, 19 group D, 8 group E, 1 group G, 1 group D/Vi and 1 mixed C/E from which both were isolated.

^b 1 group B, 2 group C and 4 group D, all identified by Wellcolex Colour Salmonella after further subculture.

^c *Citrobacter freundii* (group E/G reaction); same reaction seen in plate culture.

^d Includes *Proteus spp.*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella aerogenes*, *Enterobacter spp.*, *Shigella sonnei* and unspecified coliforms.

14. BIBLIOGRAPHY

¹ **Advisory Committee on Dangerous Pathogens.** (1984). Categorisation of Pathogens According to Hazard and Categories of Containment, *H.M.S.O.*, London.

² **Ewing, W.H.** (1986). Edwards and Ewing's Identification of Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc. New York.

³ **Miller, J.M. and Holmes, H.T.** (1995). Specimen Collection, Transport and Storage. *Manual of Clinical Microbiology*, 6th Ed., Edited by Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Tenover, R.H. American Society of Microbiology, Washington, D.C. Pages 19-32.

⁴ **Farmer, J.J.** (1995). Enterobacteriaceae: Introduction and Identification. Manual of Clinical Microbiology, 6th Ed., Edited by Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Tenover, R.H. American Society of Microbiology, Washington, D.C. Pages 438-449.

⁵ **MacFaddin, J.F.** (1985). Media for Isolation – Cultivation – Identification – Maintenance of Medical Bacteria, Volume 1, Williams and Wilkins, Baltimore and London, Pages 701-705.


⁶ Communicable Disease Reports, Weekly Editions 1-52, 1986, Public Health Laboratory Service, Colindale, London.


⁷ Salmonella Surveillance Report (1984), U.S. Department of Health and Human Services, Atlanta, Georgia.

⁸ Data on file









⁹ **Margull, A., Schulz, P., et al** (1993). Laboratoriumsmedizin, 17:295 Evaluation of a Coloured Latex Test for Rapid Diagnosis of Salmonella in Stool Specimens.

PACKAGING

 ZC50/R30858301.....▽ 50

 ZC52/R30858302.....▽ 200

Symbol legend

	Catalogue Number
	<i>In Vitro</i> Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
	Contains sufficient for <N> tests
	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured by



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