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Short communication: **Performance of a commercially available latex agglutination test kit for the identification of *Salmonella***

Kurzmitteilung:
Evaluierung eines kommerziell erhältlichen Latex-Agglutinations Tests zur Identifizierung von Salmonellen

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Summary

Immunological methods for the identification of *Salmonella* spp. allow rapid confirmation of presumptive-positive colonies. In this study, a commercially available *Salmonella* latex agglutination test kit was evaluated. Thirty *Salmonella* and 11 non-*Salmonella* strains were used to determine its inclusivity and exclusivity performance. The test kit yielded negative results for seven *Salmonella* strains and three false-positive results. The *Salmonella* strains with a negative result belonged to the serovars *Salmonella bongori, Salmonella arizonae, Salmonella diarizonae* and *Salmonella houtenae.* All strains belonging to groups B to E and G were correctly identified. The false-positive results were attributed to an *E. coli* and two *Citrobacter* strains. The evaluated *Salmonella* latex agglutination test kit is an easy-to-handle tool for the screening of colonies, but our results also demonstrate the limitations of such systems.

Keywords: *Salmonella,* latex agglutination test, inclusivity, exclusivity, performance

Zusammenfassung

Immunologische Methoden zum Nachweis von *Salmonella* spp. ermöglichen im Rahmen eines Screenings eine schnelle Identifikation verdächtiger Kolonien. In dieser Studie wurde ein kommerziell erhältlicher Latex-Agglutinations Tests anhand von 30 Salmonellen- und 11 nicht-Salmonellen-Stämmen auf seine Inklusivität und Exklusivität geprüft. Der Test ergab für sieben Salmonellen-Stämme negative Ergebnisse und drei falsch-positive Resultate. Negative Ergebnisse lagen für Stämme der Serovare *Salmonella bongori, Salmonella arizonae, Salmonella diarizonae* und *Salmonella houtenae* vor. Alle Stämme, die den Gruppen B bis E und G angehören, wurden korrekt nachgewiesen. Falsch-positive Ergebnisse wurden für einen *E. coli* und zwei *Citrobacter* spp. Stämme verzeichnet. Der evaluierte Latex-Agglutination Test ist ein einfach zu handhabendes Screening-Werkzeug für Kolonien. Unsere Ergebnisse zeigen aber auch die Grenzen solcher Systeme auf.

Schlüsselwörter: Salmonellen, Latex-Agglutinations Test, Inklusivität, Exklusivität

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Introduction

Non-typhoidal salmonellae (NTS) are important foodborne pathogens causing gastroenteritis and bacteraemia (Rabsch et al., 2001). It is estimated that *Salmonella* causes annually 93.8 million human infections and 155,000 deaths worldwide (Majowicz et al., 2010). Based on the lipopolysaccharide (O antigen) and the flagellar structures (H antigen), *Salmonella* are currently divided into 2610 serovars (Guibourdenche et al., 2010). About 60 % of NTS belong to *S. enterica* subsp. *enterica,* one of the two species within the genus *Salmonella* (Guibourdenche et al., 2010).

The detection of *Salmonella* in food and human samples traditionally relies on the use of a variety of cultural media and biochemical tests. The current ISO 6579:2002 method for *Salmonella* detection in food and feed includes a non-selective enrichment followed by a selective enrichment using two selective enrichment media. Clinical samples are usually cultured directly on selective solid media as Xylose-Lysine-Desoxycholate (XLD) agar. Confirmation of presumptive-positive colonies is done by biochemical, immunological, or molecular based methods. Besides, different rapid identification systems are currently commercially available. There are for example immunological identification and confirmation tests based on latex agglutination and enzyme-linked immunosorbent assays. *Salmonella* latex agglutination test kits thereby provide a useful tool for the rapid identification of presumptive-positive colonies grown on selective agars. These tests are based on latex particles coated with polyvalent antisera targeted against a wide range of *Salmonella* serovars. In the presence of *Salmonella,* the latex particles rapidly agglutinate and form visible lumps. In the present study, a commercially available *Salmonella* latex agglutination test kit was evaluated for its inclusivity and exclusivity by testing 30 target and eleven non-target strains.

Materials and Methods

Thirty *Salmonella* and 11 non-*Salmonella* strains obtained from the strain collection of the Swiss National Centre for Enteropathogenic Bacteria (NENT) were selected (Table 1). Strains were chosen to represent a variety of *Salmonella* serovars, including the top 10 serovars in Europe (EFSA/ECDC, 2012). For the test evaluation, strains were grown on triple sugar iron-agar (Oxoid, Basingstoke, United Kingdom) for 24 h at 37 °C. The SALMONELLA LATEX KIT (BioRad, Cressier, Switzerland) was performed according to the manufacturer's instructions by using an isolated colony.

Results and Discussion

Of the 30 target strains tested with the SALMONELLA LATEX KIT, seven (23.3 %) *Salmonella* strains were not detected (Table 1). The *Salmonella* strains with a negative result belonged to the serovars *Salmonella bongori, Salmonella arizonae, Salmonella diarizonae* and *Salmonella houtenae.* These serovars belonged to the O-groups Y, S, P and K, respectively. For the NordVal certification, the SALMONELLA LATEX KIT was validated as a confirmation test for colonies isolated from food products based on 47 *Salmonella* and 42 non-*Salmonella* strains (NordVal certificate 032). In that evaluation, the test also yielded 5 % false-negative results (after incubation in RVS broth for 24 h at 41.5 °C). *Salmonella* strains with negative results thereby belonged to the serovars *Salmonella arizonae, Salmonella bongori, Salmonella cerro, Salmonella houtenae* and *Salmonella veneziana adria.* Hence, the limitation of the method for the detection of groups B to E and G is mentioned in the validation certificate of the SAL-

TABLE 1: *Strains tested with the SALMONELLA LATEX KIT.*

Strains		Agglutination results
S. Enteritidis	O: 1, 9, 12 (D_1)	+++
S. Typhimurium	O: 1, 4, [5], 12 (B)	+++
S. enterica	O: 4, 12 (B)	+++
S. Infantis	O: 6, 7, 14 (C_1-C_4)	+++
S. Heidelberg	O: 1, 4, [5], 12 (B)	++
S. Virchow	O: 6, 7, 14 (C_1-C_4)	+++
S. Livingstone	O: 6, 7, 14 (C_1-C_4)	+++
S. Rissen	O: 6, 7, 14 (C_1-C_4)	+++
S. Agona	O: 1, 4, [5], 12 (B)	+++
S. Hadar	O: 6, 8 (C_2-C_3)	+++
S. Paratyphi A	O: 1, 2, 12 (A)	++
S. Paratyphi B	O: 1, 4, [5], 12 (B)	+++
S. Senftenberg	O: 1, 3, 19 (E_4)	+++
S. London	O: 3, {10}, {15} (E_1-E_2-E_3)	+++
S. Koketime	O: 44 (V)	++
S. enterica	O: 9,12 (D_2)	+++
S. enterica	O rough	++
S. salamae	O: 3 (N)	++
S. salamae	O: 58	+/–
S. salamae	O: 13, 23 (G_1-G_2)	+++
S. arizonae	O: 56	–
S. arizonae	O: 48 (Y)	–
S. arizonae	O: 41 (S)	–
S. diarizonae	O: 61	++
S. diarizonae	O: 61	–
S. diarizonae	O: 48 (Y)	–
S. houtenae	O: 38 (P)	–
S. houtenae	O: 18 (K)	+/–
S. indica	O: 6, 14, 25 (H)	++
S. bongori	O: 48 (Y)	–
Escherichia coli		+
Hafnia alvei		–
Hafnia sp.		–
Proteus mirabilis		–
Citrobacter freundii		–
Citrobacter sp.		–
Citrobacter sp.		+/–
Citrobacter sp.		–
Citrobacter sp.		–
Citrobacter sp.		+++
Citrobacter sp.		–

+++: strong agglutination, ++: agglutination, +/–: doubtful result, –: no agglutination

MONELLA LATEX KIT. In the manufacturer's test instructions, however, a note in view of the test limitations/restrictions is missing. Also based on our results, all strains belonging to groups B to E and G were correctly detected. The test even agglutinated with strains not belonging to the target groups, e. g. *Salmonella* Paratyphi A.

Of the 11 non-target strains tested with the SALMONELLA LATEX KIT, three strains incorrectly agglutinated with the latex beads. The false-positive results were attributed to an *E. coli* and two *Citrobacter* strains. One of those even gave a very strong positive result. The close relationship between the two genera of *Salmonella* and *Citrobacter* is known to be a challenge for cultural and biochemical identification methods (Bennett et al., 1999). No false-positive results were reported in the NordVal evaluation.

With regard to the *Salmonella* strains with negative results, it must be considered that involved *Salmonella* serovars are not common foodborne pathogens. *Salmonella enterica* ssp. *arizonae* infections have rarely been reported in immunocompromised patients (Starakis et al., 2007; Di Bella et al., 2011). Besides, *Salmonella houtenae* has been reported as cause of reptile-associated meningitis in infants (Wybo et al., 2004). *Salmonella enterica* ssp. *diarizonae* are mainly found in association with reptiles and healthy sheep (Zweifel et al., 2004; Bonke et al., 2012). On the other hand, false-positive results could be a great challenge for testing of ready-to-eat lettuce and sprouts for *Salmonella* with this test system, since *Citrobacter* spp. is part of the natural flora of theses matrices.

The results obtained by testing colonies directly from plate media indicate that the SALMONELLA LATEX KIT is a valuable and easy-to-handle tool for screening of presumptive-positive colonies. Nevertheless, the negative results for some *Salmonella* strains and false-positive results demonstrate the limitations of such test systems, especially when *Salmonella* testing will be performed in specific matrices. The limitation to certain *Salmonella* groups is regarded to be a clear restriction of the method. A note should be added to the manufacturer's test instructions to inform users in view of the mentioned application restrictions.

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