Development of a Colloidal Gold-Based Immunochromatographic Strip for Rapid Detection of *Klebsiella pneumoniae* Serotypes K1 and K2

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In this study, a novel colloidal gold-based immunochromatographic strip (ICS) containing anti-*Klebsiella pneumoniae* capsular polysaccharide polyclonal antibodies was developed to specifically detect *K. pneumoniae* serotypes K1 and K2. Capsular polysaccharide K1 and K2 antigens were first used to produce polyclonal anti-K1 and anti-K2 antibodies. Reference strains with different serotypes, nontypeable *K. pneumoniae* strains, and other bacterial species were then used to assess the sensitivity and specificity of these test strips. The detection limit was found to be 10^5 CFU, and the ICSs were stable for 6 months when stored at room temperature. No false-positive or false-negative results were observed, and equivalent results were obtained compared to those of more conventional test methods, such as PCR or serum agglutination. In conclusion, the ICS developed here requires no technical expertise and allows for the specific, rapid, and simultaneous detection of *K. pneumoniae* serotypes K1 and K2.

Klebsiella pneumoniae is a Gram-negative, rod-shaped bacterium that is found in the normal flora of the mouth, skin, and intestines. Recently, a new type of invasive infection caused by *K. pneumoniae* has emerged as a global disease (1). There are 77 different serotypes of the capsular polysaccharides (CPSs) of *K. pneumoniae* (2), with K2 and K21 being the most prevalent in western countries (3). Recently, by genotyping capsular polysaccharide genes, 79 capsular types were distinguished (4, 5). In contrast, the K1 and K2 serotypes are most often associated with bacteremia, liver abscess, and community-acquired pneumonia in Asia (3). However, reports of this new invasive syndrome have recently been on the rise in western countries (6, 7). In addition to causing human disease, *K. pneumoniae* serotypes K1 and K2 have also been detected in contaminated animal milk (8). Capsular serotypes have typically been identified by using either a capsular swelling test or counterimmunoelectrophoresis (3, 9). However, these conventional methods are time-consuming, require extensive materials and manpower, and often produce false-positive or false-negative results that are caused by cross-reactions. Another option is PCR, but this analysis requires a long time for preparation, expensive equipment, and skilled technicians, and previous studies have recommended that rapid detection is ideal for early clinical diagnosis and treatment (1).

To address these concerns, this study involved the development of a colloidal gold-based immunochromatographic strip (ICS) that contained anti-*K. pneumoniae* capsular polysaccharide polyclonal antibodies (pAbs) and was able to detect *K. pneumoniae* serotypes K1 and K2. This testing kit provides a sensitive, rapid, and simultaneous method to detect *K. pneumoniae* serotypes K1 and K2.

MATERIALS AND METHODS

Bacterial strains. The various serotypes of *K. pneumoniae* that were used here were obtained from our previous studies in Taiwan and at the Serum Institute in Denmark. The panel of bacterial strains contained all 77 sero-

types of *K. pneumoniae* along with other nontypeable strains. Additional, unrelated bacteria were also selected to assess the specificity of the ICS (Table 1). In total, 100 clinical isolates that had been collected from previous studies were used to assess the ICS (3, 10–12), including 30, 20, and 50 isolates of *K. pneumoniae* serotypes K1, K2, and non-K1/K2, respectively.

Preparation of *K. pneumoniae* serotype K1 and K2 antigens. Capsular polysaccharide K1 and K2 antigens that were isolated from *K. pneumoniae* bacterial cells were used to produce polyclonal anti-K1 and anti-K2 antibodies. Briefly, *K. pneumoniae* serotype K1 or K2 bacterial cells were grown overnight in brain heart infusion (BHI) broth, which was then centrifuged at 2,000 rpm for 5 min. The pellet containing *K. pneumoniae* was washed 3 times in sterile phosphate-buffered saline (PBS) (Sigma-Aldrich, St. Louis, MO, USA), and the cells were heat killed at 65°C for 10 min.

Production of polyclonal antibodies. To obtain antibodies that would bind to *K. pneumoniae* serotypes K1 and K2, a polyclonal antibody (pAb) against each serotype was produced by injecting female, pathogen-free New Zealand White rabbits (~1.8 kg) with *K. pneumoniae* serotype K1 or K2. The rabbits were immunized every week for 6 weeks with 0.1 ml of the whole-cell antigen solution that contained heat-killed *K. pneumoniae* serotype K1 or K2 along with incomplete Freund's adjuvant (Becton Dickinson, USA). After the 6 weekly immunizations, blood was taken from the rabbit and the serum was purified using a protein A column (GE Healthcare Life Science, USA). Next, the concentrations of the purified

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TABLE 1 Bacterial strains used in this study

Bacterial strain (no. of strains)	Source or reference ^a
Gram-negative bacteria (218)	
<i>K. pneumoniae</i> serotype K1 (12)	SSI, this study, and 3
<i>K. pneumoniae</i> serotype K2 (8)	SSI, this study, and 3
<i>K. pneumoniae</i> serotypes K1-K74 and K80-K82 (75)	SSI and 3
<i>K. pneumoniae</i> clinical isolates (100)	3, 10–12
<i>Acinetobacter baumannii</i> ATCC 19606	ATCC
<i>Acinetobacter calcoaceticus</i> ATCC 14987	ATCC
<i>Acinetobacter haemolyticus</i> ATCC 17906	ATCC
<i>Acinetobacter johnsonii</i> ATCC 17909	ATCC
<i>Acinetobacter lwoffii</i> ATCC 15309	ATCC
<i>Acinetobacter radioresistens</i> ATCC 43998	ATCC
<i>Acinetobacter junii</i> ATCC 17908	ATCC
<i>Bordetella bronchiseptica</i> ATCC 10580	ATCC
<i>Burkholderia cepacia</i> ATCC 25608	ATCC
<i>Campylobacter coli</i> ATCC 33559	ATCC
<i>Campylobacter jejuni</i> ATCC 33560	ATCC
<i>Escherichia coli</i> ATCC 25922	ATCC
<i>Haemophilus influenza</i> ATCC 16028	ATCC
<i>Leclercia adcarboxylata</i> ATCC 23216	ATCC
<i>Neisseria gonorrhoeae</i> ATCC 43069	ATCC
<i>Plesiomonas shigelloides</i> ATCC 51903	ATCC
<i>Proteus mirabilis</i> ATCC 7002	ATCC
<i>Providencia alcalifaciens</i> ATCC 51902	ATCC
<i>Pseudomonas aeruginosa</i> ATCC 27853	ATCC
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> ATCC 14028	ATCC
<i>Salmonella enterica</i> serotype <i>Infantis</i> ATCC 51741	ATCC
<i>Serratia liquefaciens</i> ATCC 27592	ATCC
<i>Yersinia enterocolitica</i> ATCC 23715	ATCC
Gram-positive bacteria (11)	
<i>Enterococcus avium</i> ATCC 14025	ATCC
<i>Enterococcus casseliflavus</i> ATCC 12755	ATCC
<i>Enterococcus durans</i> ATCC 19432	ATCC
<i>Enterococcus faecalis</i> ATCC 29212	ATCC
<i>Enterococcus faecium</i> ATCC 19434	ATCC
<i>Enterococcus gallinarum</i> ATCC 49573	ATCC
<i>Listeria innocua</i> ATCC 51742	ATCC
<i>Staphylococcus aureus</i> ATCC 51740	ATCC
<i>Staphylococcus aureus</i> ATCC 43300 (MRSA) ^b	ATCC
<i>Staphylococcus saprophyticus</i> ATCC 35552	ATCC
<i>Streptococcus pneumoniae</i> ATCC 27336	ATCC

^a SSI: Statens Serum Institut; ATCC: American Type Culture Collection.

^b MRSA, methicillin-resistant *Staphylococcus aureus*.

anti-K1 and anti-K2 pAbs were measured by a protein assay using a 280-nm spectrophotometer, and their affinity was confirmed by Western blotting. Finally, the ability of the antibodies to bind to the CPS was assessed by an enzyme-linked immunosorbent assay (ELISA).

Preparation of colloidal gold serotype K1 and K2 antibody conjugates. Antibodies against *K. pneumoniae* serotypes K1 or K2 were added to 10 ml of colloidal gold solution and stirred for 30 min. An aqueous solution containing bovine serum albumin (BSA) (5%, wt/vol; 2.5 ml) was added to block endogenous colloidal gold reactivity. The mixture was then centrifuged at 12,000 rpm and 4°C for 15 min to remove any unbound antibody. The pellet was resuspended in 1 ml of 0.01 M Tris-HCl (pH 8.0), and the extent of conjugation between the colloidal gold and the antibodies was assessed by UV-visible (UV-Vis) spectroscopy.

Preparation of the immunochromatographic strip. The ICSs were prepared by KeMyth Biotech (Taipei, Taiwan) according to the following protocol (Fig. 1). First, the sample pad was saturated with a PBS solution (pH 8.5) containing Tween 20 and 1% (wt/vol) BSA and also anti-K1 and anti-K2 conjugated antibodies. The sample pad was then dried at 37°C for 1 h and stored in a desiccator at 4°C before use. The diluted pAbs and anti-rabbit IgG (1 mg/ml) were transferred onto a nitrocellulose (NC) membrane with PBS containing 1% BSA and dried for 2 h at 37°C before being stored in a desiccator at 4°C prior to use. Pure cellulose fiber was used as an absorbent pad. pAbs against serotype K1 or K2 were microsprayed onto the NC membrane at a concentration of 1 µl/cm and were localized to specific positions on the strip that were designated capture test lines 1 and 2 (Fig. 1). An anti-IgG antibody was also microsprayed onto the same NC membrane at a concentration of 1 µl/cm and localized to a specific position, which was designated the capture control line (Fig. 1).

Sample preparation and testing procedure. A single bacterial colony was picked up from an agar plate and placed into a 1.5-ml centrifugation tube or any sterile tube with 500 µl of saline (0.85% NaCl). The testing sample was mixed until the suspension was slightly turbid or reached an optical density at 600 nm (OD₆₀₀) of ~0.01 to ~0.5. A total of 120 µl of the mixture was added into the sample loading zone of the testing cassette. We waited for 1 to 3 min to read the results. Results were read within 5 min and used as a cutting point to determine the result. A result obtained beyond 5 min was considered invalid.

Reaction time, sensitivity, and specificity. Various serotypes and concentrations of *K. pneumoniae* and other Gram-negative bacteria were used to assess the reaction time, sensitivity, and specificity of the test strips. To evaluate the sensitivity of the ICS, various concentrations of bacteria (measured by CFU per milliliter) were suspended in 100 µl of PBS. Specificity was determined by exposing the strips to all 77 serotypes of *K. pneumoniae*. Additionally, the ICSs were stored at room temperature for 6 months to evaluate their stability. *K. pneumoniae* serotypes K1 and K2 were used as positive controls.

RESULTS AND DISCUSSION

Optimization of antibody colloidal gold conjugation. To ensure a sufficient concentration of antibodies for colloidal gold conjugation, >5 µg of purified antibodies against serotypes K1 and K2 was used for conjugation. UV-Vis spectral analysis of colloidal gold alone and colloidal gold conjugated to each antibody revealed that the peaks shifted when the antibody was conjugated. While the colloidal gold alone had a peak of 523 nm, adding the antibodies shifted the surface resonance band (Fig. 2). The interaction between the antibodies and the colloidal gold particles re-

FIG 1 Schematic illustration of ICS with labeled materials.

FIG 2 UV-Vis spectra of colloidal gold alone and the K1/K2 antibody gold conjugate.

sulted in a plasmon resonance band shift from 523 to 528 nm for anti-K1 antibodies and to 529 nm for anti-K2 antibodies.

Reaction time, specificity, and sensitivity of the ICS. Various serotypes of *K. pneumoniae* along with other bacterial species were used to assess the specificity of the ICS. Among all of the isolates listed in Table 1, no false-positive or false-negative results were ever seen with the ICS, and the results were comparable to PCR and serum agglutination (Table 2). Bands for both the control and the test lines were seen within 30 s to 3 min for isolates of both serotypes K1 and K2 (Fig. 3). Inconspicuous or faint bands were observed at the K1 and/or K2 test lines with serotypes K17, K40, and K69 only after 6 min had elapsed from sample loading. Previous studies have shown that different antisera of K antigens may cross-react with other serotypes (9, 13). Dilutions were chosen to improve and distinguish the specificity of the antiserum with strong positive results (13). In the present study, we optimized the working concentration of our antiserum and only false positives with very faint bands for K17, K40, and K69 were observed after 6

FIG 3 Colloidal gold immunochromatographic strips showing positive serotypes K1 (A) and K2 (B) and negative results (C).

min. Determination of the result within 5 min can be used as a cutting point for detection. No cross-reaction was observed with any other bacterial species (Table 2). In comparing the ICS to other conventional test methods, such as PCR and serum agglutination, the results were equally specific, indicating that the ICS exhibits a high degree of specificity.

Stability of the ICS. Several ICSs were stored at room temperature for 6 months to evaluate their stability under general conditions. These strips were tested monthly to determine their specificity and sensitivity in detecting serotypes K1 and K2 along with non-K1/K2 serotypes (Table 3). After 6 months of storage, the ICSs still had a detection limit of 10⁵ CFU for the serotype K1 and K2 control strains, which is the same sensitivity as freshly produced strips. The specificity of these 6-month-old strips was also maintained. These results suggest that ICSs can be stored at room temperature for at least 6 months without losing their sensitivity or specificity (Table 3).

TABLE 2 A comparison of results among various testing methods (colloidal gold immunochromatographic strips, PCR, and serum agglutination) for both *K. pneumoniae* and other bacterial species

Bacterial strain (no. of strains)	No. of strains								
	ICS test ^d			PCR ^e			Agglutination ^f		
	K1 ^g	K2 ^g	Non-K1/K2 ^g	K1	K2	Non-K1/K2	K1	K2	Non-K1/K2
K1 (12)	12	0	0	12	0	0	12	0	0
K2 (8)	0	8	0	0	8	0	0	8	0
K3 to K74 and K80 to K82 (75)	0	0	75	0	0	75	ND	ND	ND
<i>K. pneumoniae</i> clinical isolates (100) ^a	30	20	50	30	20	50	ND	ND	ND
Gram-negative bacteria (23) ^b	0	0	23	0	0	23	ND ^h	ND	ND
Gram-positive bacteria (11) ^c	0	0	11	0	0	11	ND	ND	ND

^a Clinical isolates included 30, 20, and 50 isolates of serotypes K1, K2, and non-K1/K2, respectively.

^b In addition to *K. pneumoniae*, the other species of Gram-negative bacteria tested are shown in Table 1.

^c The Gram-positive bacteria tested are also shown in Table 1.

^d Strip tests were conducted with 120 μl of bacterial suspension with a McFarland Standard of 0.5 in 0.85% NaCl, and the results were obtained within 5 min after loading the samples onto the strips.

^e The PCR primer pairs used to detect *K. pneumoniae* serotypes K1 and K2 have been previously described (18).

^f The anti-K1 and anti-K2 antibodies against *K. pneumoniae* were used in this test.

^g K1, *K. pneumoniae* serotype K1; K2, *K. pneumoniae* serotype K2; non-K1/K2, serotypes other than K1 or K2.

^h ND, not determined.

TABLE 3 Specificity and sensitivity of the colloidal gold immunochromatographic strips after storage at room temperature for 1 to 6 months

Storage time (mo) ^a	Detection limit (CFU/strip)	Positive sample ^b	Negative sample ^b
0	10 ⁵	+	—
1	10 ⁵	+	—
2	10 ⁵	+	—
3	10 ⁵	+	—
4	10 ⁵	+	—
5	10 ⁵	+	—
6	10 ⁵	+	—

^a Strip tests were performed by simultaneously analyzing 5 positive and 5 negative samples.

^b +, positive result; —, negative result.

The most virulent *K. pneumoniae* isolates are mostly from serotypes K1 and K2, which are known to cause community-acquired infections, such as liver abscesses and pneumonia (1, 11, 14, 15). The need for a rapid test to detect these two serotypes has already been established (1). Although a report has shown that sequence type 14 (ST14) in serotype K2 isolates was not associated with virulence (16), this should not decrease the need to detect serotypes K1 and K2 in patients with clinical illness. Previous studies have shown that *K. pneumoniae* serotype K1 and/or K2 contamination can be detected in milk from cows with mastitis (8), and intestinal colonization of serotype K1/K2 isolates is a predisposing factor for the development of human disease as well (14, 17). Therefore, the ability to specifically detect serotypes K1 and K2 may help to prevent future complications in infected patients (10). Previous serotyping methods have included PCR, serum agglutination, and counterimmunoelectrophoresis. However, these methods all require specific materials, such as primers, chemical mixes, and gel preparations, along with skilled technicians and equipment, which makes the results slow and complicated to obtain. Although PCR is a more sensitive test with a very low detection limit (i.e., a small amount of DNA can be enough for detection), the ICS is both rapid and simple. Unlike conventional detection methods, the ICS does not require skilled technicians or special equipment; in fact, it is extremely easy to use because it only requires a simple visual determination (Fig. 3). Importantly, the specificity of the ICS is comparable to the other methods. The advantages of the ICS that are described here include the lack of preparation before testing and the ease of storage.

In summary, this study describes the development of an ICS that is highly specific and sensitive in detecting *K. pneumoniae* serotypes K1 and K2. This ICS can be utilized as a rapid tool for screening large numbers of samples, such as those in an epidemiological study. This ICS may also be used to diagnose hypervirulent *K. pneumoniae* strains that cause liver abscesses, community-acquired pneumonia, or mastitis resulting in animal milk contamination.

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REFERENCES

1. Siu LK, Yeh KM, Lin JC, Fung CP, Chang FY. 2012. *Klebsiella pneumoniae* liver abscess: a new invasive syndrome. *Lancet Infect Dis* 12:881–887. [http://dx.doi.org/10.1016/S1473-3099\(12\)70205-0](http://dx.doi.org/10.1016/S1473-3099(12)70205-0).
2. Sahly H, Kekow J, Podschun R, Schaff M, Gross WL, Ullmann U. 1994. Comparison of the antibody responses to the 77 *Klebsiella* capsular types in ankylosing spondylitis and various rheumatic diseases. *Infect Immun* 62:4838–4843.
3. Fung CP, Hu BS, Chang FY, Lee SC, Kuo BI, Ho M, Siu LK, Liu CY. 2000. A 5-year study of the seroepidemiology of *Klebsiella pneumoniae*: high prevalence of capsular serotype K1 in Taiwan and implication for vaccine efficacy. *J Infect Dis* 181:2075–2079. <http://dx.doi.org/10.1086/315488>.
4. Brisse S, Passet V, Haugaard AB, Babosan A, Kassis-Chikhani N, Struve C, Decre D. 2013. *wzi* gene sequencing, a rapid method for determination of capsular type for *Klebsiella* strains. *J Clin Microbiol* 51:4073–4078. <http://dx.doi.org/10.1128/JCM.01924-13>.
5. Pan YJ, Lin TL, Chen CT, Chen YY, Hsieh PF, Hsu CR, Wu MC, Wang JT. 2015. Genetic analysis of capsular polysaccharide synthesis gene clusters in 79 capsular types of *Klebsiella* spp. *Sci Rep* 5:15573. <http://dx.doi.org/10.1038/srep15573>.
6. Decre D, Verdet C, Emirian A, Le Gourrierec T, Petit JC, Offenstadt G, Maury E, Brisse S, Arlet G. 2011. Emerging severe and fatal infections due to *Klebsiella pneumoniae* in two university hospitals in France. *J Clin Microbiol* 49:3012–3014. <http://dx.doi.org/10.1128/JCM.00676-11>.
7. Fazili T, Sharngoe C, Endy T, Kiska D, Javadi W, Polhemus M. 2016. *Klebsiella pneumoniae* liver abscess: an emerging disease. *Am J Med Sci* 351:297–304. <http://dx.doi.org/10.1016/j.amjms.2015.12.018>.
8. Osman KM, Hassan HM, Orabi A, Abdelhazef AS. 2014. Phenotypic, antimicrobial susceptibility profile and virulence factors of *Klebsiella pneumoniae* isolated from buffalo and cow mastitic milk. *Pathog Glob Health* 108:191–199. <http://dx.doi.org/10.1179/204773214Y.0000000141>.
9. Onokodi JK, Wauters G. 1981. Capsular typing of klebsiellae by coagglutination and latex agglutination. *J Clin Microbiol* 13:609–612.
10. Fung CP, Chang FY, Lee SC, Hu BS, Kuo BI, Liu CY, Ho M, Siu LK. 2002. A global emerging disease of *Klebsiella pneumoniae* liver abscess: is serotype K1 an important factor for complicated endophthalmitis? *Gut* 50:420–424. <http://dx.doi.org/10.1136/gut.50.3.420>.
11. Lin JC, Koh TH, Lee N, Fung CP, Chang FY, Tsai YK, Ip M, Siu LK. 2014. Genotypes and virulence in serotype K2 *Klebsiella pneumoniae* from liver abscess and non-infectious carriers in Hong Kong, Singapore and Taiwan. *Gut Pathog* 6:21. <http://dx.doi.org/10.1186/1757-4749-6-21>.
12. Siu LK, Fung CP, Chang FY, Lee N, Yeh KM, Koh TH, Ip M. 2011. Molecular typing and virulence analysis of serotype K1 *Klebsiella pneumoniae* strains isolated from liver abscess patients and stool samples from noninfectious subjects in Hong Kong, Singapore, and Taiwan. *J Clin Microbiol* 49:3761–3765. <http://dx.doi.org/10.1128/JCM.00977-11>.
13. Riser E, Noone P, Bonnet ML. 1976. A new serotyping method for *Klebsiella* species: evaluation of the technique. *J Clin Pathol* 29:305–308. <http://dx.doi.org/10.1136/jcp.29.4.305>.
14. Lin YT, Siu LK, Lin JC, Chen TL, Tseng CP, Yeh KM, Chang FY, Fung CP. 2012. Seroepidemiology of *Klebsiella pneumoniae* colonizing the intestinal tract of healthy Chinese and overseas Chinese adults in Asian countries. *BMC Microbiol* 12:13. <http://dx.doi.org/10.1186/1471-2180-12-13>.
15. Tsay RW, Siu LK, Fung CP, Chang FY. 2002. Characteristics of bacteraemia between community-acquired and nosocomial *Klebsiella pneumoniae* infection: risk factor for mortality and the impact of capsular serotypes as a herald for community-acquired infection. *Arch Intern Med* 162:1021–1027. <http://dx.doi.org/10.1001/archinte.162.9.1021>.
16. Bialek-Davenet S, Criscuolo A, Ailloud F, Passet V, Nicolas-Chanoine MH, Decre D, Brisse S. 2014. Development of a multiplex PCR assay for identification of *Klebsiella pneumoniae* hypervirulent clones of capsular serotype K2. *J Med Microbiol* 63:1608–1614. <http://dx.doi.org/10.1099/jmm.0.081448-0>.
17. Fung CP, Lin YT, Lin JC, Chen TL, Yeh KM, Chang FY, Chuang HC, Wu HS, Tseng CP, Siu LK. 2012. *Klebsiella pneumoniae* in gastrointestinal tract and pyogenic liver abscess. *Emerg Infect Dis* 18:1322–1325.
18. Turtton JF, Baklan H, Siu LK, Kaufmann ME, Pitt TL. 2008. Evaluation of a multiplex PCR for detection of serotypes K1, K2 and K5 in *Klebsiella* sp. and comparison of isolates within these serotypes. *FEMS Microbiol Lett* 284:247–252. <http://dx.doi.org/10.1111/j.1574-6968.2008.01208.x>.