

Immunochromatographic Strip Test for Rapid Detection of Diphtheria Toxin: Description and Multicenter Evaluation in Areas of Low and High Prevalence of Diphtheria

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An immunochromatographic strip (ICS) test was developed for the detection of diphtheria toxin by using an equine polyclonal antibody as the capture antibody and colloidal gold-labeled monoclonal antibodies specific for fragment A of the diphtheria toxin molecule as the detection antibody. The ICS test has been fully optimized for the detection of toxin from bacterial cultures; the limit of detection was approximately 0.5 ng of diphtheria toxin per ml within 10 min. In a comparative study with 915 pure clinical isolates of *Corynebacterium* spp., the results of the ICS test were in complete agreement with those of the conventional Elek test. The ICS test was also evaluated for its ability to detect toxigenicity from clinical specimens (throat swabs) in two field studies conducted within areas of the former USSR where diphtheria is epidemic. Eight hundred fifty throat swabs were examined by conventional culture and by use of directly inoculated broth cultures for the ICS test. The results showed 99% concordance (848 of 850 specimens), and the sensitivity and specificity of the ICS test were 98% (95% confidence interval, 91 to 99%) and 99% (95% confidence interval, 99 to 100%), respectively.

Diphtheria is an acute, infectious disease caused by toxin-producing *Corynebacterium diphtheriae* and *Corynebacterium ulcerans*. The disease is typically characterized by local infection of the upper respiratory tract and occasionally the skin, together with systemic manifestations that affect, in particular, the heart, kidneys, and peripheral nerves. The introduction of childhood immunization with diphtheria toxoid in the 1940s led to a dramatic decrease in the number of reported cases of diphtheria worldwide and resulted in the virtual elimination of the disease in many countries. The diphtheria epidemic in countries of the former Soviet Union during the 1990s highlighted that whenever there is a decrease in immunization coverage, epidemic diphtheria can reemerge (9). Recent studies in Europe have also shown that between 16 and 50% of adult populations in some European countries lack protective levels of diphtheria toxin antibodies (2); diphtheria could therefore potentially reemerge in countries where it has been well controlled for many years.

The detection of toxigenicity is the most important test for the microbiological diagnosis of diphtheria. Disadvantages with current methodologies have been documented (5–7). Reliable, specific, accurate, and affordable phenotypic methods for the detection of diphtheria toxin are urgently needed. Within areas where diphtheria is epidemic, the detection of

toxigenicity is essential to confirm the clinical diagnosis, which usually precedes microbiological diagnosis. In regions where diphtheria is not epidemic and clinical disease is uncommon, such as Europe and the United States, these methods are essential for the differentiation of sporadic toxigenic isolates from circulating nontoxigenic isolates. We have therefore developed and standardized an immunochromatographic strip (ICS) test for the detection of diphtheria toxin from pure isolates and clinical specimens and have undertaken a multicenter evaluation of the methodology.

MATERIALS AND METHODS

Bacterial isolates. Toxigenic *C. diphtheriae* biotype *gravis* (NCTC 10648 and NCTC 3984) and nontoxigenic *C. diphtheriae* biotype *belfanti* (NCTC 10356) were used as positive and negative control isolates, respectively, for all toxigenicity tests. Ten isolates each of *C. diphtheriae* and *C. ulcerans* were used for optimization and standardization of the ICS test. These isolates have been described previously (7) and are known to produce different amounts of diphtheria toxin in *in vitro* toxigenicity tests. A variety of other organisms were used to determine the specificity of the ICS test, by the optimized methodology describe below, and included *Corynebacterium pseudodiphtheriticum* (strain NCTC 11136 and four clinical isolates), *Corynebacterium imitans* (NCTC 13015), *Corynebacterium argentoratense* (CIP 104296), *Corynebacterium striatum* (strain NCTC 764 and four clinical isolates), *Corynebacterium minutissimum* (strain NCTC 10288 and four clinical isolates), *Corynebacterium amycolatum* (five clinical isolates), *Corynebacterium bovis* (NCTC 11398), *Corynebacterium propinquum* (clinical isolate), *Streptococcus pyogenes* (NCTC 12067), *Streptococcus agalactiae* (NCTC 12906), *Streptococcus pneumoniae* (NCTC 11894), *Streptococcus dysgalactiae* subsp. *equisimilis* (group C; ID 217/2000), *S. dysgalactiae* subsp. *equisimilis* (group G; ID/254/2000), *Streptococcus mitis* (ID/255/2000), *Streptococcus anginosus* (ID/294/2000), *Streptococcus sanguis* (ID/255/2000), *Streptococcus oralis* (ID/190/2000), *Streptococcus salivarius* (ID/287/2000), *Neisseria meningitidis*

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gittidis (QAL 3115), *Haemophilus influenzae* (QAL 491), *Haemophilus parainfluenzae* (QAL 3093), and *Staphylococcus epidermidis* (CN 3384).

Preparation of ICS test strips. ICS test strips were prepared by the Program for Appropriate Technology in Health, Seattle, Wash. Reagents previously used to develop the enzyme immunoassay (EIA) for diphtheria toxin were used in the preparation of the ICS test (7). These included equine polyclonal antitoxin coated onto a nitrocellulose membrane for use as the capture antibody and monoclonal antibodies specific for fragment A of the diphtheria toxin molecule and labeled with colloidal gold as the detection antibody. When they are packaged in foil pouches, the ICS strips are stable for at least 6 to 12 months at 4°C and ambient room temperatures (24 to 30°C); other, more extreme storage temperatures were not evaluated.

ICS test. (i) Optimization of ICS test for detection of diphtheria toxin. Isolates on Columbia blood agar (Oxoid, Basingstoke, United Kingdom) were suspended in 0.5 ml of serum-supplemented Elek broth (SSEB) at a cell density corresponding to a McFarland no. 1 standard (approximately 10^8 CFU/ml). SSEB consisted of Elek broth (7) supplemented with 16.6% newborn bovine serum. In order to determine the effect of incubation time on the detection of toxigenicity, cell suspensions were incubated for 1 to 6 h at 37°C in air, after which an ICS test strip was added to each tube and the result was read after 10 min at room temperature. Isolates were grown on various diagnostic culture media commonly used for the laboratory diagnosis of diphtheria prior to testing by the standardized ICS test to determine whether these media have any effects on the ICS test. The media evaluated included Hoyle's tellurite agar (Oxoid), Tinsdale agar (Beckton Dickinson, Oxford, United Kingdom), and Loeffler's agar (Oxoid), which are commonly used in Europe and the United States for laboratory diagnosis, as well as Corynebacter agar (NPO Nutrient Media, Obolensk, Russian Federation) and Pizu medium (prepared in-house for the detection of cystinase activity), which are used in the Russian Federation and the Newly Independent States of the former USSR (13).

The use of freeze-dried SSEB was compared with the use of liquid broth for the growth of isolates prior to evaluation of the ICS test. Aliquots of 15 ml of SSEB were freeze-dried by the Media Department of the Public Health Laboratory Service (PHLS; London, United Kingdom) and were transported to the field evaluation sites, where they were reconstituted; 0.5-ml aliquots of the reconstituted media were used for the growth of the isolates prior to testing by the ICS test.

(ii) Detection of toxigenicity from pure clinical isolates: standardized methodology. SSEB media (fresh and freeze-dried) were prepared by the PHLS Media Department and were distributed to the participating laboratories. Isolates on Columbia blood agar were suspended in 0.5 ml of fresh or reconstituted freeze-dried SSEB at a cell density corresponding to a McFarland no. 1 standard (10^8 CFU/ml). Cell suspensions were incubated for 3 h at 37°C in air. An ICS test strip was added to each tube, and the result was read after 10 min at room temperature.

(iii) Detection of toxigenicity from broth cultures directly inoculated with clinical specimens (throat swabs). The ICS test was compared with conventional culture for the detection of toxigenicity from broth cultures directly inoculated with clinical specimens (throat swabs) in field trials in Ukraine and Latvia. Swabs were inoculated onto Columbia blood agar and a tellurite agar (either Hoyle's tellurite or Corynebacter agar) and were then emulsified in 0.5 ml of SSEB (provided by PHLS). The broths were incubated for 16 h at 37°C. An ICS test strip was added to each tube, and the result was read after 10 min at room temperature. For conventional culture, any suspect black colonies on the tellurite-containing agar were screened (by Gram staining and tests for catalase, pyrazinamidase, and cystinase activities) to determine whether they were potentially toxigenic species, as detailed in the World Health Organization *Manual for the Laboratory Diagnosis of Diphtheria* (4). Biochemical identification of potentially toxigenic *Corynebacterium* spp. was performed with the API Coryne system (BioMérieux) and/or fermentation of Hiss serum water sugars (3, 4).

Elek immunoprecipitation test. All isolates were tested for the production of diphtheria toxin by conventional and modified Elek immunoprecipitation tests as described previously (4, 8).

PCR for detection of diphtheria toxin gene. Detection of fragment A of the diphtheria toxin gene (248 bp) was performed with some of the isolates which were nontoxigenic by the Elek test, as described previously (15).

RESULTS

Sensitivity of ICS test. Purified diphtheria toxin (Calbiochem-Novobiochem [United Kingdom] Ltd., Nottingham, United Kingdom) was used to determine the sensitivity of the

ICS test. In a dilution series, the limit of detection was found to be 0.5 ng/ml.

Specificity of ICS test. The specificity of the ICS test was determined by the optimized methodology and with various *Corynebacterium* spp. and other bacterial species commonly found as respiratory tract pathogens or as part of the normal upper respiratory tract flora. The ICS test was negative with the species tested, and no false-positive results were detected.

Optimization of ICS test for detection of diphtheria toxin. The ICS test was optimized for the detection of diphtheria toxin from pure clinical isolates by using 10 isolates each of *C. diphtheriae* and *C. ulcerans*, as described previously (7). A standard inoculum density corresponding to a McFarland no. 1 standard (approximately 10^8 CFU/ml) was used for all experiments. Toxigenicity could be detected from all isolates after a minimum of 3 h of incubation at 37°C. The effects of culture media commonly used for the laboratory diagnosis of diphtheria on the detection of toxigenicity by the ICS test were also evaluated. Isolates were grown on Hoyle's tellurite, Tinsdale, Loeffler's, Corynebacter, and Pizu agars prior to testing by the ICS test. A positive reaction occurred for all toxigenic isolates tested, irrespective of the medium on which they were grown prior to inoculation into SSEB. The use of freeze-dried SSEB also had no effect on the detection of toxigenicity by the ICS test.

Detection of toxigenicity among pure clinical isolates. The use of the ICS test for the detection of toxigenicity among pure clinical isolates was evaluated in the United Kingdom and countries of the former USSR where diphtheria is epidemic. Seven laboratories in six countries tested a total of 915 isolates (Table 1). There was 100% agreement between the results of the ICS test and those of the Elek test, with 538 toxigenic and 377 nontoxigenic isolates detected. The estimated sensitivity of the ICS test was 100% (95% confidence interval, 99 to 100%), and the specificity was also 100% (95% confidence interval, 99 to 100%).

A further 110 nontoxigenic, *tox* gene-bearing (NTTB) isolates were also tested in three of the laboratories by the ICS test, and the results were compared with those of the Elek test and PCR detection of fragment A of the *tox* gene (Table 2). The results of the Elek test and the ICS test were in agreement for 102 of 110 (93%) of the isolates, which were negative for toxigenicity (nontoxigenic) by both phenotypic tests. The remaining eight isolates were found to be positive for toxigenicity (toxigenic) by the ICS test. These isolates were retested for toxigenicity by the ICS test, EIA, Elek test, and PCR for detection of the *tox* gene in the originating laboratory and/or the PHLS Diphtheria Reference Laboratory. All eight isolates were subsequently found to be positive for toxigenicity (toxigenic) by all four methods. Therefore, after retesting, the results of the Elek test and the ICS test showed 100% correlation, with 102 nontoxigenic (NTTB) isolates and 8 toxigenic isolates detected.

Detection of toxigenicity from broth cultures directly inoculated with clinical specimens (throat swabs). Eight hundred fifty throat swab specimens were used to evaluate the detection of diphtheria toxin by the ICS test, and the results were compared with those of conventional culture. These included swabs taken from patients with suspected cases of diphtheria and contacts of the case patients and from patients with pharyngi-

TABLE 1. Evaluation of ICS test for detection of toxigenicity from pure cultures in countries of the former USSR and the United Kingdom by number, biotype and toxigenicity status of isolates tested in each center

Biotype	Toxigenicity ^a	No. of isolates							Total
		Riga, Latvia	Kiev, Ukraine	Moscow, Russia	St. Petersburg, Russia	Minsk, Belarus	Almaty, Kazakhstan	London, United Kingdom	
<i>C. diphtheriae</i> var. <i>gravis</i>	+	212	83	61	19	34	7	32	448
	-	37	31	12	11	11	4	45	151
<i>C. diphtheriae</i> var. <i>mitis</i>	+	5	3	12	2	1		28	51
	-	64	62	11	10	11	6	16	180
<i>C. diphtheriae</i> var. <i>belfanti</i>	-			4		2		15	21
<i>C. ulcerans</i>	+		2	5				32	39
	-		7	4				14	25
Total		318	188	109	42	59	17	182	915

^a +, toxigenic; -, nontoxigenic.

tis. The results of the ICS test showed 99% correlation (848 of 850 specimens) with the results of conventional culture, with 59 toxigenic and 789 nontoxigenic isolates detected. For the remaining two isolates, one was positive by conventional culture but negative by the ICS test, and the other one was negative by culture but positive by the ICS test. The sensitivity of the test was 98% (95% confidence interval, 91 to 99%), and the specificity was 99% (95% confidence interval, 0.99 to 100%).

DISCUSSION

The detection of toxigenicity is the most important test for the microbiological diagnosis of diphtheria. The phenotypic methods available for the detection of diphtheria toxin tend to be technically demanding, lacking in sensitivity, and relatively expensive or have not been fully evaluated against a large panel of isolates (5, 8, 10, 12, 14, 16, 17). We recently described an amplified EIA for the detection of diphtheria toxin (7)

which had a number of advantages over previously documented phenotypic methods for the detection of toxigenicity, including improved sensitivity, specificity, and speed. This enabled same-day phenotypic toxigenicity testing of clinical isolates of *C. diphtheriae*. However, the cost and labor intensity of the EIA, alongside the need for cold-chain storage of reagents such as the monoclonal antibody conjugate and the amplification reagent, are likely to hinder its use in resource-limited countries where diphtheria remains endemic or epidemic. ICS tests have been developed for the detection of a variety of bacterial antigens (1, 11) and have a number of advantages over EIAs: they are very simple to perform, inexpensive to manufacture, and have long-term stability over a wide range of climates. We therefore applied the reagents used in the EIA to develop an ICS test for the detection of diphtheria toxin.

The ICS test that we have developed is a simple, rapid method with a limit of detection of 0.5 ng/ml. This limit of detection is comparable to that of the amplified EIA that we described previously (limit of detection, 0.1 ng/ml) (7), is 20-fold more sensitive than other EIAs (limit of detection, 10 ng/ml) (14, 16), and is 10-fold more sensitive than agglutination assays (12, 17).

The standardization of conditions for diphtheria toxin production from culture supernatants was essential. The methodology was optimized by using 10 isolates each of *C. diphtheriae* and *C. ulcerans* described previously (7). These isolates produce variable and small amounts of diphtheria toxin. A standard inoculum density of approximately 10⁸ CFU/ml (corresponding to a McFarland no. 1 standard) was used for all experiments. This was determined to be the optimal cell density for the amplified EIA, from which the ICS test was developed, and is easily achieved with colonies from pure plate or slope cultures. By use of this inoculum density, a final, definitive toxigenicity result was available within 3.5 h of selection of colonies from pure clinical isolates, which is comparable to the time to detection by the amplified EIA (7). Toxigenicity could also be detected by the ICS test from isolates grown on a variety of media, including selective agars used for the isolation or screening of potentially toxigenic corynebacteria. These included media such as Hoyle's tellurite agar and Tinsdale agar,

TABLE 2. Detection of toxigenicity among NTTB isolates^a by ICS test

Center and biotype	No. of isolates tested	No. of isolates with toxigenicity result by:			
		ICS test		EIA	
		+	-	+	-
Kiev, Ukraine					
<i>C. diphtheriae</i> var. <i>gravis</i>	3	1 ^b	2	1 ^b	2
<i>C. diphtheriae</i> var. <i>mitis</i>	73	5 ^b	68	5 ^b	
<i>C. ulcerans</i>	1	1 ^b		1 ^b	
<i>C. pseudotuberculosis</i>	1	1 ^b		1 ^b	
Moscow, Russia, <i>C. diphtheriae</i> var. <i>mitis</i>	30		30		
St. Petersburg, Russia, <i>C. diphtheriae</i> var. <i>gravis</i>	2		2		
Total	110	8 ^b	102	8 ^b	2

^a NTTB isolates are negative by the Elek test and positive by PCR detection of the *tox* gene.

^b The isolates were retested by the Elek test and were found to be toxigenic.

used in the United Kingdom and Western Europe, and Corynebacterium and Pizua agars, used in Russia and the Newly Independent States of the former USSR. It was found that reconstituted freeze-dried SSEB can be used as an alternative to fresh SSEB for the growth of isolates prior to testing by the ICS test, and media for use in the ICS test can therefore be provided in a freeze-dried format. This is important for standardization and quality control of the test and may be essential for application of the ICS test in some countries where facilities and resources for production of Elek broth, which is complex (4), are limited.

The use of the ICS test for the detection of toxigenicity from pure clinical isolates was evaluated in the United Kingdom and in six laboratories in the Newly Independent States of the former USSR. The results of the assay showed an excellent correlation with those of the Elek test, and no false-positive or false-negative results were observed. The ICS test was also used to test a selection of NTTB isolates. These isolates possess the diphtheria toxin gene (and are PCR positive) but do not express a biologically or immunologically active form of the toxin molecule and are therefore negative for toxigenicity by phenotypic tests, such as the Elek test. The results of the ICS test were in agreement with those of the Elek test for 102 of 112 isolates; however, the ICS test identified the remaining 8 Elek test-negative isolates as toxigenic. When these isolates were retested for toxigenicity by the ICS test, EIA, the Elek test, and PCR for detection of the *tox* gene, all eight isolates were found to be positive (toxigenic) by all four methods. This highlights the fact that the Elek test can be technically demanding, that its results can be difficult to interpret, and that the sensitivity and ease of performance and interpretation of the ICS are superior to those of the Elek test.

The use of the ICS test to detect toxigenicity from broth cultures directly inoculated with clinical specimens (throat swabs) was evaluated in field trials in Latvia and Ukraine. Toxigenicity could not be detected directly from the swabs without incubation in SSEB. However, toxigenicity could be detected following overnight incubation (approximately 16 h) of the swabs in 0.5 ml of SSEB. Therefore, by use of the ICS test a definitive toxigenicity result can be obtained within 16 h of collection of a specimen from a patient with suspected diphtheria, whereas conventional culture requires 48 to 72 h.

In conclusion, the ICS test is a reliable, rapid method for the detection of diphtheria toxin. By use of the ICS test a definitive result on toxigenicity is available from pure isolates within 3.5 h and is available from clinical specimens, inoculated directly into broth, within 16 h. The ICS test is simple to perform, the results are easy to interpret, and the method does not require any specialized equipment. The assay is applicable for the rapid testing of sporadic isolates or for batch testing of larger numbers of isolates in areas where diphtheria is endemic or epidemic. The method is now in routine use in the PHLS Diphtheria Reference Laboratory.

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REFERENCES

1. Dominguez, J., N. Gali, S. Blanco, P. Pedrosa, C. Prat, L. Matas, and V. Ausina. 2001. Detection of *Streptococcus pneumoniae* antigen by a rapid immunochromatographic assay in urine samples. *Chest* **19**:243–249.
2. Edmunds, W. J., R. G. Pebody, H. Aggerback, S. Baron, G. Berbers, A. E. Conyn-Van Spaendonck, et al. 2000. The seroepidemiology of diphtheria in Western Europe. *Epidemiol. Infect.* **125**:113–135.
3. Efstratiou, A., and R. C. George. 2000. Laboratory guidelines for the diagnosis of infections caused by *Corynebacterium diphtheriae* and *C. ulcerans*. *Communicable Dis. Public Health* **2**:250–257.
4. Efstratiou, A., and P. A. C. Maple. 1994. Manual for the laboratory diagnosis of diphtheria. The Expanded Programme on Immunisation in the European Region of W.H.O. Report ICP/EPI038(C). World Health Organization, Copenhagen, Denmark.
5. Efstratiou, A., K. H. Engler, C. S. Dawes, and D. Sesardic. 1998. Comparison of phenotypic and genotypic methods for the detection of diphtheria toxin among isolates of pathogenic corynebacteria. *J. Clin. Microbiol.* **36**:3173–3177.
6. Efstratiou, A., K. H. Engler, I. K. Mazurova, T. Glushkevich, J. Vuopio-Varkila, and T. Popovic. 2000. Current approaches to the laboratory diagnosis of diphtheria. *J. Infect. Dis.* **181**(Suppl.):S138–S145.
7. Engler, K. H., and A. Efstratiou. 2000. A rapid enzyme immunoassay for the detection of diphtheria toxin among clinical isolates of *Corynebacterium* spp. *J. Clin. Microbiol.* **38**:1385–1389.
8. Engler, K. H., T. Glushkevich, I. K. Mazurova, R. C. George, and A. Efstratiou. 1997. A modified Elek test for the detection of toxigenic corynebacteria. *J. Clin. Microbiol.* **35**:495–498.
9. Galazka, A. M., S. E. Robertson, and G. P. Oblapenko. 1995. Resurgence of diphtheria. *J. Epidemiol.* **11**:95–105.
10. Hallas, G., T. G. Harrison, D. Samuel, and G. Colman. 1990. Detection of diphtheria toxin in culture supernates of *Corynebacterium diphtheriae* and *C. ulcerans* by immunoassay with monoclonal antibody. *J. Med. Microbiol.* **32**:247–253.
11. Heiter, B. J., and P. P. Bourbeau. 1995. Comparison of two rapid streptococcal antigen detection assays with culture for diagnosis of streptococcal pharyngitis. *J. Clin. Microbiol.* **33**:1408–1410.
12. Jalgaonkar, S. V., and A. M. Saoji. 1993. Coagglutination for rapid testing of toxin producing *Corynebacterium diphtheriae*. *Indian J. Med. Res.* **97**:35–36.
13. Mazurova, I. K., V. G. Melnikov, S. J. Kombarova, and O. J. Borisova. 1998. Textbook of methods for diphtheria laboratory diagnosis, p. 1–47. MU 4.2.698-98. Russian Federation Ministry of Health, Moscow, Russia.
14. Nielsen, P. B., C. Koch, H. Friss, I. Heron, J. Prag, and J. Schmidt. 1987. Double-sandwich enzyme-linked immunosorbent assay for rapid detection of toxin-producing *Corynebacterium diphtheriae*. *J. Clin. Microbiol.* **25**:1280–1284.
15. Pallen, M. J., A. J. Hay, L. H. Puckey, and A. Efstratiou. 1994. Polymerase chain reaction for screening clinical isolates of corynebacteria for the production of diphtheria toxin. *J. Clin. Pathol.* **47**:353–356.
16. Pietrzak, J., S. Muehlestein, and M. Gasser. 1990. Sandwich-dot immunobinding assay (sandwich-DIA), a new immunological method for the detection of diphtheria toxin. *Zentbl. Bakteriol. Parasitenkd. Infektkrankh. Hyg. Abt. 1 Orig.* **274**:61–69.
17. Toma, C., L. Sisvath, and M. Iwanaga. 1997. Reversed passive latex agglutination assay for detection of toxigenic *Corynebacterium diphtheriae*. *J. Clin. Microbiol.* **35**:3147–3149.