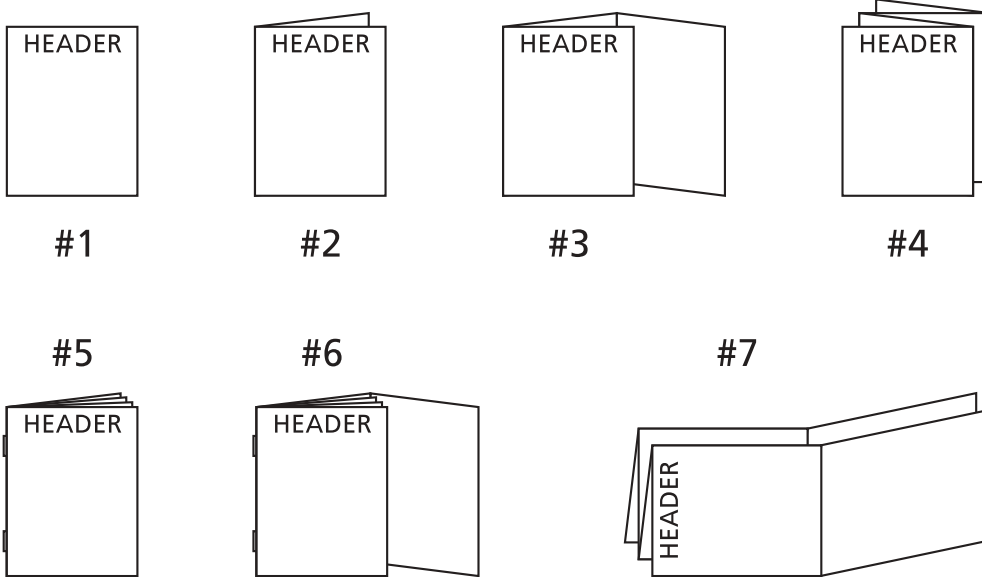



Rev From	Rev To	ECO #	Date	Appr.
0899	0703	2143-03		

Notes:

- BD Cat. No. 298610
- Blank (Sheet) Size : Length: Width:
 Number of Pages: 4 Number of Sheets: 1
 Page Size: Length 7.312" Width 3.625" Final Folded Size: 3.156" x 3.625"
- Style (see illustrations below): 2



- See Specification Control No. NA for Material Information
- Ink Colors: Printed two sides Yes No
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Part Number: S1191JAA		Category and Description Package Insert Difco KL Virulence Enrichment	Sheet: 1 of 5 Scale: 1 : 1	A

BD Difco™ KL Virulence Enrichment

S1191JAA
2003/07

INTENDED USE

Difco™ KL Virulence Enrichment is used with KL Virulence Agar and Tellurite Solution 1% in conjunction with a paper strip saturated with diphtheria antitoxin for differentiating virulent (toxigenic) from nonvirulent strains of *Corynebacterium diphtheriae*.

SUMMARY AND EXPLANATION

Elek¹ was the first to describe the agar plate diffusion technique for demonstrating the toxigenicity (virulence) of *C. diphtheriae*. King, Frobisher, and Parsons² expanded on Elek's technique and, by using a carefully standardized medium, obtained results in agreement with animal inoculation tests. The authors used rabbit, sheep and horse serum as enrichments, finding human serum to be unsatisfactory. To overcome irregularities encountered in previous formulations, Hermann, Moore, and Parsons³ developed a nonserous enrichment. The medium and enrichment described by these authors has been standardized for use in the KL Virulence Test.

PRINCIPLES OF THE PROCEDURE

KL Virulence Enrichment, composed of casamino acids, glycerol and polysorbate 80, provides a source of nonserous enrichment. Casamino acids is derived from acid-hydrolyzed casein that has low sodium chloride and iron concentrations. The low iron concentration is beneficial because iron is known to prevent the production of diphtheria toxin when present in more than minute amounts. Glycerol (glycerine) contains no heavy metals and is used by bacteria as a source of carbon. Polysorbate 80 improves growth of certain strains of *C. diphtheriae*. Toxin produced by bacteria and diffused into the medium is detected by precipitation with the antitoxin present on the strip.

REAGENTS

KL Virulence Enrichment

Approximate Formula* Per 100 mL

Casamino Acids1.0 g

Polysorbate 801.0 g

Glycerol1.0 mL

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions:

For Laboratory Use.

Follow established laboratory procedures in handling and disposing of infectious materials.

Storage: Store KL Virulence Enrichment at 2 - 8°C.

Product Deterioration: Do not use if product fails to meet specifications for performance.

PROCEDURE

Materials Provided: KL Virulence Enrichment.

Materials Required But Not Provided: Glassware, autoclave, water bath (55 - 60°C), incubator (35°C), Tellurite Solution 1%, KL Virulence Agar and antitoxin strips.

Method of Preparation:

1. Suspend 37.5 g of KL Virulence Agar in 1L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 min to completely dissolve the powder.
2. Autoclave at 121°C for 15 min.
3. Cool in a water bath to 55 - 60°C.
4. Aseptically dispense 10 mL of KL Virulence Agar into a Petri dish containing 2 mL KL Virulence Enrichment and 0.5 mL Tellurite Solution 1%; mix thoroughly by rotating the plate approximately 20 times to obtain a uniform mixture.
5. Using aseptic techniques, submerge a sterile filter paper strip (1 cm x 8 cm) saturated with diphtheria antitoxin or a **BBL Taxo** KL Antitoxin Strip.
6. Allow the medium to solidify.

Test Procedure

Inoculate the medium by streaking a loopful of a 24-h culture in a single line across the plate perpendicular to (right angle to) the antitoxin strip. (Do not touch the actual strip itself). As many as eight cultures may be tested on a single plate.⁴ Place test isolates about 1 cm apart. Also inoculate a toxigenic (positive control) and a nontoxigenic (negative control) *C. diphtheriae* strain approximately 1 cm on either side of the test isolates.⁴ Incubate the inverted plates under CO₂ at 37°C for 72 h. Examine at 24-, 48- and 72-h intervals.

USER QUALITY CONTROL

Test Strain	Expected Results
<i>Corynebacterium diphtheriae</i> biotype gravis ATCC™ 8028	+
<i>Corynebacterium diphtheriae</i> biotype intermedius ATCC 8032	+
<i>Staphylococcus aureus</i> ATCC 25923	-

+ = positive, line of precipitation at 45° angle to the strip

- = negative, no line of precipitation

RESULTS

Toxigenic (virulent) cultures of *C. diphtheriae* will show fine lines of precipitation at approximately 45° angles from the culture streak. This line forms where toxin (from the bacteria) combines with antitoxin from the strip. Primary precipitin lines form an arc of identity with the precipitin line produced by an adjacent positive control strain.⁵ Nontoxigenic strains of *C. diphtheriae* will show no lines of precipitation.

LIMITATIONS OF THE PROCEDURE

1. False-positive reactions may be seen after 24 h as weak bands near the antitoxin strip. These can be recognized when compared with the positive control.⁶
2. *Corynebacterium ulcerans* and *C. pseudotuberculosis* may also produce lines of toxin-antitoxin.⁷

AVAILABILITY**Cat. No. Description**

222192	Difco™ KL Virulence Agar, 500 g.
298610	Difco™ KL Virulence Enrichment, 12 x 20 mL.
231740	BBL™ Taxo™ KL Antitoxin Strips, 12 strips per vial.
211917	BBL™ Tellurite Solution 1%, 20 mL per tube.

REFERENCES

1. Elek, S.D. 1948. The recognition of toxicogenic bacterial strains in vitro. *Brit. Med. J.* 7:493.
2. King, E.O., M. Frobisher, Jr., and E.I. Parsons. 1949. The in vitro test for virulence of *Corynebacterium diphtheriae*. *Am. J. Public Health* 39:1314.
3. Hermann, G.J., M.S. Moore, and E.I. Parsons. 1958. A substitute for serum in the diphtheriae in vitro test. *Am. J. Clin. Pathol.* 29:181-183.
4. MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 410-414. Williams & Wilkins, Baltimore, MD.
5. Washington, J.A., Jr. 1981. Laboratory procedures in clinical microbiology. Springer-Verlag, New York, NY.
6. Lennette, E.H., A. Balows, W.J. Hausler, Jr., and J.P. Truant (eds.). 1980. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, DC.
7. Branson, D. 1972. Methods in clinical bacteriology. Charles C. Thomas, Springfield, IL.



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