Benefits of *Haemophilus influenzae* type b (Hib) vaccine are primarily limited to children of the developed part of the world mainly for lack of convincing local disease burden data from the developing countries. A principal reason for the underestimation of Hib is the failure of most laboratories in developing countries, especially in Asia, to detect this fastidious micro organism.

Addition of isovitalex in chocolate agar for the isolation of *Haemophilus influenzae*


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**Background & objectives:** The reason for lack of data on burden of *Haemophilus influenzae* type b (Hib) in developing countries was mainly failure of detection of this fastidious organism in laboratories. Use of isovitalex (IVX) was suggested as an essential supplement for growing this organism. This study was carried out to investigate the impact of IVX supplementation to chocolate agar for detection of Hib.

**Methods:** Chocolate agar with and without supplementation of IVX was prepared. Clinical samples as well as reference strains of Hib were simultaneously cultured on both the media.

**Results:** *H. influenzae* isolates (N=194) were simultaneously grown on chocolate agar (CA) with and without isovitalex (IVX). Average colony size of *H. influenzae* on CA with IVX (CA-IVX) was larger only by 0.10 cm (range 0.05 to 0.16 cm) compared to CA alone. Addition of IVX to CA increased the cost of media by 2.1-fold.

**Interpretation & conclusion:** Isovitalex is not essential for the isolation and growth of *H. influenzae* almost halving the cost.

**Key words** Growth - *Haemophilus influenzae* - isovitalex

Hib has appeared as a predominant cause of meningitis and pneumonia whenever explored applying standard microbiological procedures and rigorous quality controls. However, use of special base media and/or supplement(s) is being recommended for investigating Hib burden, instead of emphasizing on good laboratory practices mentioned in the text books. In 1994, Gellert *et al* reported isovitalex (IVX) as an
essential nutrient for the growth of *H. influenzae* and that lack of this supplement in the culture medium led to failure of the Latvian National Bacteriology Laboratory to isolate the organism. They also reported that plain chocolate agar (CA) could not support growth of *H. influenzae* on subculture, unless supplemented with IVX. This suggestion has been well taken by the public health practitioners and policy makers, and the addition of IVX as an essential supplement for the isolation of *H. influenzae* has been included as a standard procedure in the laboratory manuals. It is now widely believed that isolation of *H. influenzae* is difficult and requires substantial resources, which has served to discourage laboratories of many developing countries from exploring the burden of Hib diseases.

We therefore used plain CA and IVX-supplemented (1%) CA (CA-IVX) simultaneously to isolate *H. influenzae* and other fastidious organisms, *Neisseria meningitidis* and *Streptococcus pneumoniae*, and compared for their colony size. Comparative cost of CA with and without IVX were also calculated and compared.

### Material & Methods

The study was conducted at the Department of Microbiology of Dhaka Shishu Hospital, Dhaka, Bangladesh; during the period of 1995-1998. CA was prepared by heating blood agar base (Oxoid Ltd. Hampshire, England) with 5 per cent sheep blood at 75-80°C for 15 min in a water bath. IVX (Becton Dickinson, Sparks, USA) was added to CA at 45°C, and equal amounts (10 ml) of CA or CA-IVX were dispensed into respective separate chambers of the same petriplate. Each batch of CA media with and without supplement was tested for adequate growth of reference strain *H. influenzae* ATCC 49247. Cerebrospinal fluid (CF) specimens were collected from the children with suspected meningitis and sent to the department of microbiology as part of their clinical care. The specimens were simultaneously cultured on both the media and 194 *H. influenzae* isolates were obtained. Of these, 189 were type b, 2 were type a and 3 were nontypable. All strains were isolated on both CA and CA-IVX after the same period of incubation, irrespective of their serotype and biotype. The ATCC strain 49247 always showed confluent growth on both the media. Although Oxoid blood agar base was mainly used in this study, we transiently switched to blood agar base of Remel (Lenexa, USA), and Mast Diagnostics (Merseyside, UK), and found that the CA made from any of these basal media, either with or without IVX supplementation, supported confluent growth of *H. influenzae*. All isolates of *H. influenzae* collected from Japan and the USA also grew well on CA and CA-IVX.

Average colony size of *H. influenzae* on CA-IVX was larger by 0.10 cm (range 0.05 to 0.16 cm) compared to CA. Calculation of cost showed that addition of isovitalex to CA increased its price by 2.1-fold.

One of the major reasons for the low usage of Hib vaccines particularly in developing countries, is the perception that Hib is not an important cause of disease. This perception is a result of low isolation rates of Hib in developing countries, in part due to poor laboratory facilities and limited resources. It is, therefore, important to use methods that are inexpensive as well as sensitive for the detection of Hib.

We have successfully grown all 194 *H. influenzae* isolates on plain CA, irrespective of their sero- or biotype, without the addition of IVX. This indicates that CA is sufficient to support the growth of this organism.
Failure of other laboratories in Bangladesh, India, and Guatemala (Dr Edwin Esturias, JHU, personal communication) to isolate this fastidious organism was possibly due to faulty preservation of specimens before culturing, use of poor quality base media or source of blood, faulty preparation of media or a combination of these factors. Our success in isolating all *H. influenzae* isolates further emphasizes that the most important factors in preparing media for isolation of this organism are to (i) use a good quality and appropriate basal medium; (ii) addition of blood from an animal source (not human blood); (iii) use of correct temperature and time to chocolatise the media; (iv) check the quality of each batch for its optimum ability to grow a set of test strains; and (v) use the prepared plates in a timely fashion. Minimal difference in colony diameter (0.10 cm) and growth of all isolates of *H. influenzae* indicates that IVX is not essential for primary isolation of this organism.

In conclusion, we recommend that isovitalex is not essential for the growth of *H. influenza*, and the resource-poor institutions, with good laboratory practice, can continue their effort to isolate Hib without wasting their resources for IVX.

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