Collection and Handling of Nasal Lavage Specimens

The protocol for collecting nasal lavage fluid and preparing samples for analysis is described below.

Sample Collection:

Nasal lavage samples were collected prior to viral challenge on Day 0, and on each subsequent post-challenge day. Specimens collected on Day 0 were used for viral isolation, and those collected on the post-challenge days were used for viral isolation and quantification as well as for measurement of local cytokine production (see *Nasal Cytokine Assay*).

Nasal lavage was performed by asking participants to close the soft palate (by repeating the velar consonant [k,k,k,k,k…]) while instilling 5 ml of warmed saline solution into each nostril. Samples were then collected by having participants expel the lavage fluid into a waxed paper cup. Immediately after collection, lavage samples were transferred to sterile 15-m1 centrifuge tubes and kept on wet ice until they were delivered to the laboratory for further preparation.

Preparation:

Nasal lavage samples were centrifuged at 3000 rpm, 4°C for 15 minutes to remove mucus and debris. From each sample, 1.35 ml of lavage fluid was transferred to a cryovial containing 0.450 ml of 4X concentrated viral collecting broth (see below). The nasal lavage aliquots were then frozen and stored at -70°C until packed in dry ice and shipped to the laboratory for analysis.

Viral Collecting Broth for Nasal Lavage

A standard 4X concentration of viral collecting broth (VCB) was prepared by dissolving 10 g of Bovine Serum Albumin (BSA) in 500 ml of Hank's Balanced Salts Solution in addition to appropriate quantities of the following antibiotics in order to obtain the proper concentrations (in parentheses): Gentamicin (80 μg/ml); Vancomycin (80 μg/ml); Amphotericin B (8 μg/ml).