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-ml 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).