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Evaluation of Silica Gel Packages for Transport of *Neisseria meningitidis*

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Eight *Neisseria meningitidis* reference strains, representing six different serogroups, were plated on 57 blood agar plates each. The growth was harvested and stored in silica gel packages at different temperatures for up to 90 days. When held at 4°C, all strains were recovered after 90 days of storage. Strains held at room temperature or alternately at 4°C and room temperature survived for at least 10 and 17 days, respectively.

Transportation of *Neisseria meningitidis* is often unsuccessful because strains tend to die out under less than optimal conditions. In the early 1900s, researchers overcame the obstacles encountered in transporting *N. meningitidis* by traveling with their cultures so that they could subculture them regularly to keep their strains alive during transport on ships (3). More recently, media such as chocolate agar slants, tubes of Transgrow or cystine tryptic agar, and Jembec plates have been used for transport of *N. meningitidis*, but they provided only limited survival times. Trans-Isolate medium (1) permits growth and survival of *N. meningitidis* strains for several weeks or longer, but contamination is a problem unless aseptic procedures for collecting specimens and inoculating the bottles are rigorously followed. Thus, a need exists for a simple, easy-to-use method of transport which can be used under field conditions and which is reasonably insensitive to temperature fluctuations. In this study, we tested the use of silica gel packages (SGPs) (Grace Davison, Baltimore, Md.) for the temporary storage and transport of *N. meningitidis*. SGPs are foil envelopes containing sterile silica gel, a desiccant. SGPs have been previously shown to be very effective for transporting *Corynebacterium diphtheriae*, *Staphylococcus aureus*, and *Streptococcus pyogenes* (4, 5, 7, 8).

Isolates. The following *N. meningitidis* strains from the Centers for Disease Control and Prevention culture collection were used in this study: 318, 2785, 2817 (serogroup A), ATCC 13090 (serogroup B), 323 (serogroup C), 327, (serogroup W135) 326 (serogroup Y), and 329 (serogroup Z). Strains were stored at −70°C in sterile, defibrinated sheep blood until needed. A 10-μl inoculum from each strain was streaked onto a blood agar plate (BAP) of BBL tryptic soy agar II with 5% sheep blood (Becton Dickinson Microbiology Systems, Cockeysville, Md.). Plates were incubated overnight at 37°C in a 5% CO_2 atmosphere. Fifty-seven BAPs were streaked for each strain and incubated overnight at 37°C in a 5% CO_2 atmosphere.

Storage in SGPs. The overnight growth from each BAP was harvested with a sterile polyester swab (Becton Dickinson Microbiology Systems). The top of the SGP was cut open, and the swab with the harvested growth was placed in the packet. The corners of the packet were folded and sealed with tape to ensure closure. For each strain, 19 swabs were then kept at 4°C, 19 swabs were kept on the bench in the laboratory at room temperature (generally considered to be between 22 and 25°C), and 19 were kept at 4°C during business hours (9 a.m. to 5 p.m.) and were left on the bench at night and over the weekend. The latter conditions were used to simulate the occurrence of power outages frequently encountered in some African countries. All swabs were kept in SGPs under these conditions for 90 days. Strains were recovered by removing the swab from the SGP, streaking it onto a BAP, and incubating overnight at 37°C in a CO_2 atmosphere. We designated the initial storage day as day 0. On day 4, for each of the eight tested strains, we opened three packages, each containing a swab held at a different storage condition, and streaked each swab onto a BAP. Subsequently, at days 5, 6, 10, 12, 14, 17, 19, 21, 24, 26, 31, 35, 42, 49, 61, 70, 77, and 90, the procedure was repeated with the remaining swabs. Growth of the strains was defined as heavy when more than 100 CFU were present on a single plate, moderate when 20 to 100 CFU were present, and light when fewer than 20 CFU were observed on a plate.

Confirmation of strains. Before storage and after recovery, the identity of each strain was confirmed by standard procedures: the oxidase test, utilization of carbohydrates (maltose and dextrose positive and lactose and sucrose negative) and Gram staining (6). Serogroups were identified by slide agglutination tests with specific antisera.

Statistical analysis. Differences in the numbers of days of survival among the three storage temperatures were tested with the Wilcoxon rank sum test (2).

Results and discussion. All strains held at 4°C were successfully recovered with various intensities of growth after 90 days (Table 1). The shortest period during which heavy growth was observed was 4 days for the *N. meningitidis* serogroup B strain, while on average the other seven strains produced heavy growth until day 46 (range, 31 to 61 days). For all strains, moderate growth was observed until day 49 (average, 76; range, 49 to 90). Five of eight *N. meningitidis* strains produced moderate growth even after 90 days. When held at room temperature, all strains exhibited heavy growth for at least 4 days (range, 4 to 6), but moderate growth on average persisted until day 9 (range, 4 to 17). All strains survived with light growth for at least 10 days (average, 28, range, 10 to 49). When the swabs were kept alternately at 4°C and at room temperature all strains were recovered with heavy, moderate, or light growth for at least 4, 5, and 17 days, respectively (averages, 7, 12, and 40 days, respectively).

Clearly, the best recovery was observed when SGPs were

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TABLE 1. Recovery of *N. meningitidis* serogroups A, B, C, W135, Y, and Z from SGPs held at different temperatures for up to 90 days

Strain (serogroup)	No. of days of survival[a]								
	4°C			4°C/room temperature			Room temperature		
	+++	++	+	+++	++	+	+++	++	+
318 (A)	42	49	90	5	6	42	6	6	26
2785 (A)	49	90	90	12	24	49	4	12	26
2817 (A)	49	90	90	6	21	42	4	14	35
ATCC 13090 (B)	4	61	90	4	5	17	4	4	10
323 (C)	42	49	90	4	5	26	4	4	26
327 (W135)	61	90	90	5	19	61	5	17	49
326 (Y)	49	90	90	14	14	42	4	6	31
329 (Z)	31	90	90	4	4	42	4	6	21
Avg	41	76	90	7	12	40	4	9	28
SE	6.07	6.90		1.40	2.91	4.75	0.26	1.76	3.97
Median	45.50	90	90	5	10	42	4	6	26
Range	4–61	49–90	90	4–14	5–19	17–61	4–6	4–17	10–49

[a] For consistency, the last day of survival was defined as the sampling day preceding the one on which no growth was first recorded. In several instances, strains were intermittently recovered for up to 9 days after no growth was initially recorded. +++, more than 100 CFU/plate; ++, 20 to 100 CFU/plate; +, fewer than 20 CFU/plate.

held at 4°C, and storage at 4°C provided significantly better survival rates than the other storage conditions, regardless of the intensity of the growth recorded: heavy ($P < 0.008$), moderate ($P < 0.0009$), and light ($P < 0.0005$). Interestingly, no significant differences in the survival rates and intensities of the recovered growth were observed when swabs were kept only at room temperature and when they were kept alternatingly at 4°C and room temperature: heavy growth ($P = 0.13$), moderate growth ($P = 0.49$), and light growth ($P = 0.10$).

It may be of significance that room temperature in Africa may be somewhat higher than that of laboratories in the United States. Furthermore, the eight strains used in the study were not fresh clinical isolates but stock culture strains. Such strains are often adapted to survival in the laboratory and may be more forgiving of unfriendly environments than fresh clinical isolates.

Contamination of swabs while they were stored in SGPs did not appear to present a significant problem. A total of 456 plates were inoculated, and *N. meningitidis* growth collected from them for storage was subsequently streaked on another 456 plates at the above-mentioned intervals. In over 95% of the plates, no contamination was noted. Most of the contaminants were present in heavy to moderate growth after 42 days and at 4°C.

While carrying out this study, we had the opportunity to field-test the use of SGPs for the transport of *N. meningitidis*. SGPs containing the strains used in this study were transported from Atlanta, Ga., to Lagos, Nigeria, where they were used for training purposes. The nine isolates were easily recovered after 8 days in SGPs. These strains were transported in a cooler with an ice pack, and all strains were recovered with heavy growth and no contamination. Several months later, another set of *N. meningitidis* isolates was sent from Atlanta to Lagos. *N. meningitidis* on six swabs in SGPs was recovered with heavy to moderate growth and no contamination after 11 days in transit at ambient temperature.

Conclusion. Transport and temporary storage of *N. meningitidis* strains have been difficult in the past. Chocolate agar slants and other media have been used, but survival time with these is limited. Trans-Isolate medium has been used for the transport of strains from Africa in surveillance and case control studies. However, contamination is often a problem with the use of this medium, particularly when cultures are transported by mail. In this study, we evaluated the use of SGPs as a possible alternative to chocolate agar slants and Trans-Isolate medium for the transport of *N. meningitidis*. The ability of strains to survive for a longer period in SGPs with little contamination should aid in carrying out field studies. SGPs also provide a simple and reliable means to transport isolates from remote areas to regional reference centers within developing countries.

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