



PANSPERMIA MICROBIOLOGY

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To:

Everbuild Building Products
Site 41, Knowsthorpe Way
Cross Green Industrial Estate
Leeds
LS9 0SW

30th September, 2009

Tests carried out to the agreed protocol. Samples received on 13.8.09.

Samples received for testing are as follows:

TEST WIPES: EVERBUILD WONDER WIPES PW5006AB
CONTROL WIPE: TOUGH WIPES CRC

1. Preparation of sample:

Before testing the wipes, let them stand on a steady surface for at least 48 hours without piling them.

2. Contact times:

1 and 5 minutes

3. Inoculation of wipes:

Inoculation is carried out in sterile petri dishes, one dish per contact time.

One quarter of a wipe is placed into a petri dish, avoiding folding.

Dilute leptospiral culture, *Leptospira interrogans* strain RGA, (nominally at 10^6 leptospores per ml - 100ul culture in 10ml sterile PBS.

Inoculate the wipe with 0.25ml of diluted culture using a sterile 1ml pipette with a zigzag motion, and up and down to ensure uniform distribution.

Allow to soak for the required contact times in the closed petri dishes.

4. Testing:

At nominated times (contact time of 1 and 5 minutes) remove the wipe and immerse in 9ml of sterile EMJH (Ellinghausen and McCulloch medium modified by Johnson and Harris) medium in a wide mouthed Schott bottle, mixing in the EMJH five times in a plunging motion. Use sterile forceps.

Remove 1ml aliquots and inoculate two each of the following media:

EMJH + 2% rabbit serum + 0.1% Agar

A licensed contract laboratory

EMJH + 2% rabbit serum + 0.1% Agar + 200ug/ml 5FU (5 Fluoracil)

Leptospiral isolation medium

Incubate all media at $29^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 6 weeks examining weekly for the presence of leptospira.

Control: Above media uninoculated

Leptospiral culture inoculated into above media.

EMJH + wipe without leptospiral culture left in contact for 5 minutes.

5. Results:

TEST - EVERBUILD WONDER WIPES PW5006AB

Leptospira interrogans serovar Icterohaemorrhagiae strain RGA *not* isolated after six weeks incubation at 29°C .

CONTROL - TOUGH WIPES CRC

Leptospira interrogans serovar Icterohaemorrhagiae strain RGA *not* isolated after six weeks incubation at 29°C .

Yours sincerely,



Signed on behalf of:
Dr. Venkat Sivaprakasam
Consultant Microbiologist