Equipment and a method for rapidly as-
saying solid surfaces for contamination by
bacterial spores are undergoing develop-
ment. The method would yield a total (non-
viable plus viable) spore count of a surface
within minutes and a viable-spore count in
about one hour. In this method, spores
would be collected from a surface by use of
a transparent polymeric tape coated on one
side with a polymeric adhesive that would
be permeated with one or more reagent(s)
for detection of spores by use of visible lumi-
nescence. The sticky side of the tape
would be pressed against a surface to be assayed,
then the tape with captured spores would
be placed in a reader that illuminates the
sample with ultraviolet light and counts the
green luminescence spots under a micro-
scope to quantify the number of bacterial
spores per unit area. The visible lumines-
cence spots seen through the microscope
would be counted to determine the con-
centration of spores on the surface.

This method is based on the chemical
and physical principles of methods de-
scribed in several prior NASA Tech Briefs arti-
cles, including “Live/Dead Spore Assay
Using DPA-Triggered Tb Luminescence”
(NPO-30444), Vol. 27, No. 3 (March 2003),
page 7a. To recapitulate: The basic idea is to
exploit the observations that (1) dipicolinic
acid (DPA) is present naturally only in bac-
terial spores; and (2) when bound to Tb
ions, DPA triggers intense green lumines-
cence of the ions under ultraviolet excita-
tion; (3) DPA can be released from the vi-
able spores by using Lalanine to make
them germinate; and (4) by autoclaving, mi-
crowaving, or sonicating the sample, one
can cause all the spores (non-viable as well as
viable) to release their DPA.

One candidate material for use as the ad-
hesive in the present method is polydimeth-
ylsiloxane (PDMS). In one variant of
the method — for obtaining counts of all
(viable and nonviable) spores — the PDMS
would be doped with TbCl₃. After collection
of a sample, the spores immobilized on the
sticky tape surface would be lysed by heating
or microwaving to release their DPA. Tb³⁺
ions from the TbCl₃ would become bound
to the released DPA. The tape would then
be irradiated with ultraviolet and examined
as described above. In another variant of the
method — for obtaining counts of viable
spores only — the PDMS would be doped
with Lalanine in addition to TbCl₃.

As now envisioned, a fully developed
apparatus for implementing this method
would include a pulsed source of ultraviolet light and a time-gated elec-
tronic camera to record the images seen
through the microscope during a pre-
scribed exposure interval at a prescribed
short time after an ultraviolet pulse. As
in the method of the second-mentioned
prior article, the pulsing and time-gating
would be used to discriminate between
the longer-lived Tb³⁺/DPA lumines-
cence and the shorter-lived background
luminescence in the same wavelength
range. In a time-gated image, the bright
luminescence from bacterial spores
could easily be seen against a dark back-
ground.

This work was done by Adrian Ponce of
Caltech for NASA’s Jet Propulsion Labo-
atory. Further information is contained in a
TSP (see page 1).

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Innovative Technology Assets Management
JPL,
Mail Stop 202-233
4800 Oak Grove Drive
Pasadena, CA 91109-8099
(818) 354-2240
E-mail: iaoffice@jpl.nasa.gov
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