Comparison of immunofluorescence and culture for the detection of \textit{Actinomyces israelii} in wearers of intra-uterine contraceptive devices

D. E. LESLIE* and SUZANNE M. GARLAND†

Microbiology Department, The Royal Women's Hospital, Melbourne, Australia

\textbf{Summary.} A direct immunofluorescence (IF) method was compared with traditional culture methods for the detection of \textit{Actinomyces israelii} in endocervical and intra-uterine-device (IUD) smears from 124 IUD wearers. Of 11 specimens that gave positive results by IF, only one was positive by culture. Of the 10 patients with positive IF specimens, three (30\%) had signs and symptoms suggestive of pelvic infection and no other pathogen was detected. Direct IF of cervical smears offers a simple, relatively cheap method to screen IUD wearers for \textit{A. israelii}. Clinical management of such cases is discussed.

\textbf{Introduction}

In the past 20 years, over 400 cases of pelvic actinomycosis have been reported throughout the world, predominantly in association with the wearing of an intra-uterine contraceptive device (IUD). Genital actinomycosis is a non-contagious, chronic, suppurative disease which leads to fibrosis and may present as a “frozen pelvis”, tubo-ovarian abscess, or with uterine and endometrial involvement. The principal pathogen, \textit{Actinomyces israelii}, is a fastidious, slowly growing, filamentous, gram-positive anaerobe. Consequently, diagnosis by traditional culture methods from genital swabs is slow, tedious and relatively insensitive. Immunofluorescence (IF) with direct smears of the genital tract provides a rapid method for the identification of \textit{A. israelii} by morphological and immunological characteristics. We report the use of an IF stain of smears, from the endocervices of IUD wearers and from IUDs at removal, in the Royal Women's Hospital, Melbourne, over a 4-month period and discuss the clinical significance and management of patients with a positive smear.

\textbf{Materials and methods}

\textbf{Patients}

The study group consisted of 124 women with an IUD \textit{in situ} who attended the Family Planning Clinic (FPC), Gynaecology Clinic or Emergency Department of the Royal Women's Hospital, Melbourne, between Sept. 1988 and Jan. 1989. IUDs included plastic devices such as Lippes Loops and copper-containing devices. The reasons for presentation were: attendance for cessation of contraception, for change of IUD (recommended at the FPC for all copper IUDs after 2 years, or earlier if symptoms occur), for symptoms or signs suggestive of pelvic infection (such as lower abdominal pain, vaginal discharge, altered menses; and lower abdominal or cervical or adnexal tenderness on examination), or for clinical review after previous episodes of infection or abnormal test results, e.g., cervical cytology reports of the presence of actinomyces-like organisms.

\textbf{Specimens}

The attending medical practitioner placed a cotton-tipped swab in the endocervix of each patient who presented at the Hospital, and smeared the swab on to two glass slides for staining by Gram's method and IF. Horse-blood agar (HBA) was inoculated with a second endocervical swab, which was then placed into cooked-meat broth; two further swabs, one of them a charcoal swab, were placed into Amies transport medium.

It has been Hospital policy that, before IUD insertion, all patients are screened for \textit{Chlamydia trachomatis} by wiping away any remaining pus or mucus with a cotton swab, then sampling the endocervix with a cotton-tipped aluminium ENT swab (Medical Wire and Equipment Co., Wilts). Swabs were placed in a sucrose phosphate glutamate transport medium supplemented with vancomycin 10 \(\mu\)g/ml, gentamicin 20 \(\mu\)g/ml, and amphotericin B 10 \(\mu\)g/ml; they were transported to the laboratory on ice.
and were either processed the same day or stored at
-70°C.

After removal, IUDs were placed in sterile con-
tainers and sent to the laboratory immediately. On
arrival, a smear from the IUD was made on a glass
slide, for IF staining; it was heat-fixed and stored until
tested near the end of the study period.

Bacteriology

One of the cervical smears was stained by Gram's
method and examined for polymorphs and organisms.
The HBA that had been inoculated with a cervical
swab was incubated at 37°C in CO2 5-10% for 24 h;
and the cooked-meat broth was incubated at 37°C for
24 h before routine subculture for anaerobes. Actino-
myces agar (modified by the addition of metronida-
zole 5 µg/ml) was inoculated with the plain cotton
swab from Amies medium, and incubated anaero-
biically at 37°C for 4 weeks; plates were examined
weekly, and the identity of likely colonies was
confirmed by gram-stain morphology and by gas-
liquid chromatography. Thayer-Martin medium
was inoculated with the charcoal swab in Amies medium
for the detection of Neisseria gonorrhoeae.

Swabs from the IUDs were inoculated on to HBA
(37°C, CO2 5-10%, 24 h), on to supplemented HBA
for anaerobic organisms4 (37°C, anaerobically, 48 h)
and on to actinomycies agar (37°C, anaerobically, 4
weeks). Bacteria were identified by conventional
bacteriological methods;5 they included potential
pathogens such as Staphylococcus aureus, coliforms, β-
haemolytic streptococci, large numbers of anaerobes
and actinomycyes. Specimens for C. trachomatis
were cultured on HeLa-229 cells in 48-well cluster trays
as described previously.6

Immunofluorescence

The method of Pine et al.7 was used with minor
modifications. Briefly, heat-fixed smears were digested
with peptic solution (140 U/ml in 0-01 M HCl; Sigma)
for 3 h; they were then rinsed and dried, and 20 µl of
fluorescein-conjugated antibody to A. israelii types I
and II (Biological Products Division, Centers for
Disease Control, Atlanta, GA, USA) was spread over
an area 1-cm square. After incubation at 37°C for 20
min, the slides were thoroughly rinsed, and were
counterstained with aqueous Evans Blue 0·5% for 2
min. They were then rinsed, dried, and mounted in
phosphate-buffered saline with glycerol, and examined
by fluorescence microscopy (Leitz) with 450-490-nm
excitor and 515-nm suppressor filters.

A control slide, with each IF test, consisted of a
negative smear containing cellular material, normal
vaginal bacterial flora, and mucus from an actino-
myces-free IUD, and positive smears made by mixing
material from the same negative IUD with A. israelii
type I (ATCC 12103) and type 2 (ATCC 19322) grown
in Brewer's thioglycollate medium. Smears of orga-

Identification criteria

Slides were considered to be positive for A. israelii
if examination revealed more than two microcolonies
of filamentous bacteria with evidence of branching
and strong IF of the cell wall (grade 3-4). Older
positive specimens tended to have a more granular
appearance. In most cases, actinomycyes microcolonies
were embedded in faintly staining amorphous cellar
debris, often with large numbers of leucocytes in
the smear. Inadequate slides, with no visible cellula
material before digestion, were rejected.

Cytology

All patients reviewed at the Hospital clinics were
screened for dysplasia or neoplasia, by cervical
cytology. Ecto- and endo-cervices were sampled by
Ayre spatulae and cytobrushes respectively; smears
were made and fixed immediately, and stained by the
method of Papanicolaou.

Results

Specificity of the IF test

No more than a trace of fluorescence (less than
grade 1) was observed in smears of vaginal commensal
bacteria. Occasionally, fungal hyphae showed weak
fluorescence (grade 1-2), but these were readily
distinguished from actinomycyes by size and other
morphological features. Despite previously reported
low-level antigenic cross-reactions,1-7 a smear of the
actinomycyes-like organism, Ar. propionica, did not
even show grade 1-2 fluorescence.

Detection of actinomycyes

A total of 131 specimens (82 cervical and 49 IUD)
from 124 patients was examined. Nine (11%) of the
cervical smears and two (4%) of the IUD smears were
positive by IF for A. israelii (table I); these specimens
were from 10 patients. Only one specimen was positive
by culture, a cervical swab from a patient with an
IUD in situ for 18 months, who complained of lower
abdominal pain and vaginal discharge; this patient's
cervical smear was also positive by IF. The IUD was
removed from three of the 10 patients with a positive
cervical smear; in only two of these three was the IUD
smear positive by IF.
Table I. Results of specimens tested for A. israelii over a period of 4 months from Sept. 1988 to Jan. 1989

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Number tested</th>
<th>Number (%) positive by culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IF (A)</td>
</tr>
<tr>
<td>Cervical smear</td>
<td>82</td>
<td>9 (11)</td>
</tr>
<tr>
<td>IUD smear</td>
<td>49</td>
<td>2† (4)</td>
</tr>
<tr>
<td>Total</td>
<td>131*</td>
<td>11 (8)</td>
</tr>
</tbody>
</table>

* Both cervical smears and IUDs were received from seven patients.† The two positive IUDs were from patients with positive cervical smears, though one of the cervical specimens was received outside the 4-month study period.

Symptoms and management of patients

Details of the 10 patients positive for A. israelii are outlined in Table II. IUDs in all these patients had been present for at least 13 months (mean, 29 months). Clinically, three were asymptomatic; two had minor complaints (discharge with or without lower abdominal pain); three had symptoms and signs suggestive of pelvic inflammation, not in association with other recognised pathogens; two had other pathogens also (chlamydia or trichomonas). In the patient with chlamydia, symptoms were sufficiently severe to require admission to hospital for treatment of pelvic inflammatory disease (PID). Of the asymptomatic patients, two presented for cessation of contraception; the third (no. 9) came for a routine IUD change, but because of the presence of actinomyces the IUD was removed and an oral contraceptive was prescribed; all three were managed by IUD removal alone. Those with pelvic signs were managed with antibiotics.

Table II. Ten patients in whose specimens A. israelii was detected by immunofluorescence (IF)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Duration of IUD use (months)</th>
<th>Reason for attendance</th>
<th>IF result with smear from cervix</th>
<th>IF result with smear from IUD</th>
<th>Result of culture for A. israelii</th>
<th>Symptoms, signs and other pathogens</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>18</td>
<td>Unwell</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>LAP PV-discharge</td>
<td>IUD-removal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>metronidazole doxycycline</td>
<td>metronidazole Amoxycillin</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>20</td>
<td>Unwell</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>LAP PV-discharge fever</td>
<td>IUD-removal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chlamydia</td>
<td>metronidazole Amoxycillin</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>58</td>
<td>Cessation of contraception</td>
<td>+*</td>
<td>+</td>
<td>-</td>
<td>None</td>
<td>IUD-removal</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>17</td>
<td>Unwell</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>LAP PV-discharge Cx-excitation</td>
<td>Amoxycillin metronidazole</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>36</td>
<td>Unwell</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>LAP PV-discharge Cx-excitation</td>
<td>Amoxycillin metronidazole</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>24</td>
<td>Unwell</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>LAP PV-discharge Cx-excitation</td>
<td>IUD-removal Amoxycillin metronidazole</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>25</td>
<td>Unwell</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>LAP PV-discharge Trichomonas</td>
<td>IUD-removal Amoxycillin metronidazole</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>13</td>
<td>Cessation of contraception</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>None</td>
<td>IUD-removal Amoxycillin metronidazole</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>36</td>
<td>Routine change of IUD</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>None</td>
<td>IUD-removal</td>
</tr>
<tr>
<td>10</td>
<td>26</td>
<td>42</td>
<td>Unwell</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>PV-discharge</td>
<td>IUD-removal Amoxycillin metronidazole</td>
</tr>
</tbody>
</table>

ND, not done during the study period; LAP, lower abdominal pain; PV, per vaginum; PID, pelvic inflammatory disease; Cx, cervix.

* This specimen was collected outside the study period.
organisms, and difficulty in selecting it from other Actinomyces sensitive, fastidious and slow-growing nature of the techniques are insensitive because of the oxygen-monest of which is usage of an IUD.'59

Examination of Papanicolaou-stained in genital specimens'.

factor for genital actinomycosis has been long-term flora of the genital tract. This has been demonstrated in our study, in which only one patient was positive varied from 17% to 25%:13,14 the most significant risk factor for genital actinomycosis has been long-term usage of an IUD.15,16

The method of choice for the detection of A. israelii in genital specimens1,14,17,18 has been IF. Culture techniques are insensitive because of the oxygen-sensitive, fastidious and slow-growing nature of the organisms, and difficulty in selecting it from other faster-growing anaerobes18-20 found in the normal flora of the genital tract. This has been demonstrated in our study, in which only one patient was positive by culture. Examination of Papanicolaou-stained smears for the presence of actinomyces-like organisms has been shown to be more sensitive than culture, but it still lacks sensitivity and specificity even in the most highly skilled hands.16,17 Furthermore, by morphology alone, it is not possible to distinguish A. israelii from related Actinomyces, Arachnia and Nocardia. In contrast, IF can utilise both morphology and antigenic features. The anti-A. israelii conjugate used in this study, and other similar preparations, have been tested extensively by several authors7,16,18 who found this to be the most sensitive and specific method for the detection of A. israelii. Also, this method can be applied directly to histological sections in cases of invasive disease.

Actinomyces is detected frequently in genital smears of asymptomatic IUD wearers. The clinical significance is uncertain, and whether this represents a risk factor for subsequent development of PID is a pertinent question. Where IF has been used in both retrospective and prospective studies, there are two schools of thought. One believes that recognition of actinomyces in a genital smear is always related to a foreign body, most commonly an IUD.13,16 Although in the majority of such patients infection is confined to the superficial layers of the endometrium, there is potential for more serious disease if the device remains in situ. In contrast, others1,7,21,22 consider actinomyces colonisation in healthy non-IUD users to be part of the normal indigenous vaginal flora. Generally, colonisation rates reported by the latter group were low but the study populations were small (table III).

In our 4-month study, nine (11%) of 82 cervical smears from IUD users were positive for actinomyces; the rate for the whole of 1989 was 10% of 350 specimens. Three (30%) of our positive patients had significant symptoms, a higher proportion than expected; and three (30%) were asymptomatic. In all seven symptomatic patients, the IUD had been in situ for more than 13 months (mean, 29 months).

With such diversity of opinion, the management of IUD users with actinomycosis is somewhat arbitrary, and varies from centre to centre.1,7,13,14,21,22 For

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients with IUD</th>
<th>Patients without IUD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients</td>
<td>Number of patients</td>
</tr>
<tr>
<td></td>
<td>IF-positive</td>
<td>IF-negative</td>
</tr>
<tr>
<td>Pine et al. (1981)7</td>
<td>18</td>
<td>7 (39)</td>
</tr>
<tr>
<td>Persson et al. (1983)21</td>
<td>68</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Persson and Holmberg (1984)22</td>
<td>5</td>
<td>5 (100)*</td>
</tr>
<tr>
<td>Valicenti et al. (1982)16</td>
<td>6450</td>
<td>103 (2)†</td>
</tr>
<tr>
<td>Present study</td>
<td>82</td>
<td>9 (11)</td>
</tr>
</tbody>
</table>

ND, not done.
* Multiple genital and perineal sites from each patient were cultured over two menstrual cycles.
† Papanicolaou-stained smears were examined for actinomyces-like organisms. These were not detected in 63 250 smears from women without IUDs; but they were found in 212 of 6450 smears from IUD wearers and, when these 212 were re-examined by IF, 103 were positive.
IUD wearers, we propose that *A. israelii* be sought by IF of a cervical smear at 2-year intervals, or earlier if symptoms occur. An asymptomatic IUD user with actinomycosis should have the device removed, and should use other means of contraception. Alternatively, after 2–3 months, another cervical smear may be examined; if negative for *A. israelii*, as is usual in our experience, another IUD may be inserted.

If the IUD wearer presents with localised symptoms, we recommend antibiotic therapy (amoxycillin and metronidazole, or doxycycline, for 2–3 weeks) and removal of the IUD. Fortunately pelvic actinomycosis is uncommon; but it is a serious and potentially life-threatening infection. If a pelvic mass is evident, aggressive and prolonged antibiotic therapy with possible surgical intervention is required. In 6 years at the Royal Women’s Hospital, one of us (SMG) has found records of only two cases of tubo-ovarian abscess due to actinomycosis; both were associated with IUD usage, and both presented with pelvic fibrosis, ovarian carcinoma being the provisional diagnosis until histology of frozen sections suggested actinomycosis.

We thank Dr Gytha Betheras, FPC, Royal Women’s Hospital, for helpful discussion of this manuscript and Mrs Judy Jackson for typing it.

**Addendum.** Actinomycosis antisera are no longer available from the Centers for Disease Control, Atlanta, GA, in the large quantities that may be needed for routine diagnostic use.

### References