Comparison of immunofluorescence and culture for the detection of *Actinomyces israelii* in wearers of intra-uterine contraceptive devices

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Summary. A direct immunofluorescence (IF) method was compared with traditional culture methods for the detection of *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 *A. israelii*. Clinical management of such cases is discussed.

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, supplicative disease which leads to fibrosis and may present as a "frozen pelvis", tubo-ovarian abscess, or with uterine and endometrial involvement. The principal pathogen, *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 *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.

Materials and methods

Patients

The study group consisted of 124 women with an IUD 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.

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 *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 μg/ml, gentamicin 20 μg/ml, and amphotericin B 10 μg/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 organ-
isms. 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 agar3 (modified by the addition of metronida-
zole 5 µg/ml) was inoculated with the plain cotton 
swab from Amies medium, and incubated anaerobi-
cally 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 actinomyces 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 actinomyces. 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 pepsin solution (140 U/ml in 0.01 N 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 1 (ATCC 12103) and type 2 (ATCC 19322) grown 
in Brewer's thioglycollate medium. Smears of organ-
isms found in the normal vagina (diphtheroids, 
lactobacilli, streptococci and coliforms), and of Arachi-
nea propionica, were also tested.

IF was graded subjectively from 0 (no detectable 
cell wall IF) to 4 (the intense fluorescence of the 
positive control).

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, actinomyces microcolonies 
were embedded in faintly staining amorphous cellular 
debris, often with large numbers of leucocytes in the 
smear. Inadequate slides, with no visible cellular 
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 Papamicolaou.

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 actinomyces by size and other 
morphological features. Despite previously reported 
low-level antigenic cross-reactions,1,7 a smear of the 
actinomyces-like organism, Ar. propionica, did not 
even show grade 1–2 fluorescence.

Detection of actinomyces
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</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IF</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>Result of culture for <em>A. israelii</em></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>ND</td>
<td>LAP PV-discharge</td>
<td>None</td>
<td>IUD-removal metronidazole, doxycycline, IUD-replacement</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>20</td>
<td>Unwell</td>
<td>ND</td>
<td>LAP PV-discharge, fever PID Chlamydia</td>
<td>IUD-removal metronidazole, doxycycline</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>58</td>
<td>Cessation of contraception</td>
<td>+[*] ND</td>
<td>ND</td>
<td>None</td>
<td>IUD-removal</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>17</td>
<td>Unwell</td>
<td>ND</td>
<td>LAP PV-discharge, Cx-excitation</td>
<td>Amoxycillin metronidazole, IUD-removal</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>36</td>
<td>Unwell</td>
<td>ND</td>
<td>LAP PV-discharge, Cx-excitation</td>
<td>Amoxycillin metronidazole, IUD-removal</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>24</td>
<td>Unwell</td>
<td>+</td>
<td>LAP PV-discharge, Cx-excitation</td>
<td>IUD-removal amoxycillin, metronidazole</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>25</td>
<td>Unwell</td>
<td>+</td>
<td>LAP PV-discharge, Trichomonas</td>
<td>IUD-removal metronidazole</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>13</td>
<td>Cessation of contraception</td>
<td>+</td>
<td>ND</td>
<td>None</td>
<td>IUD-removal</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>36</td>
<td>Routine change of IUD</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>PV-discharge</td>
<td>IUD-removal</td>
</tr>
</tbody>
</table>

ND, not done during the study period; LAP, lower abdominal pain; PV, per vaginam; PID, pelvic inflammatory disease; Cx, cervix.
* This specimen was collected outside the study period.
organisms, and difficulty in selecting it from other sensitive, fastidious and slow-growing nature of the techniques are insensitive because of the oxygen-monest of which is usage of an IUD.'59

by culture. 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 polymicrobial, with various aerobes and anaerobes in faster-growing anaerobes'

transmitted organisms are important factors in assess-

Discussion

The incidence of PID is reported to be higher in IUD users than in non-users, although the type of IUD, patient selection, and the presence of sexually transmitted organisms are important factors in assessing the real risk. In most instances PID is polymicrobial, with various aerobes and anaerobes in addition to sexually transmitted organisms, the commonest of which is C. trachomatis. Reports of Actinomyces spp. in cases of PID in IUD wearers have varied from 17% to 25%, the most significant risk factor for genital actinomycosis has been long-term usage of an IUD.

The method of choice for the detection of A. israelii in genital specimens 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 anaerobes 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. 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 authors 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. 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, others 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 actinomyces is somewhat arbitrary, and varies from centre to centre. For

### Table III. Comparison of present results with previous studies of A. israelii immunofluorescence (IF) in smears from patients with and without an IUD

<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 (%) IF-positive</td>
</tr>
<tr>
<td>Pine et al. (1981)</td>
<td>18</td>
<td>7 (39)</td>
</tr>
<tr>
<td>Persson et al. (1983)</td>
<td>68</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Persson and Holmberg (1984)</td>
<td>5</td>
<td>5 (100)*</td>
</tr>
<tr>
<td>Valicenti et al. (1982)</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 actinomyces 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 actinomyosis 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 actinomyces; both were associated with IUD usage, and both presented with pelvic fibrosis, ovarian carcinoma being the provisional diagnosis until histology of frozen sections suggested actinomyosis.

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

**Addendum.** Actinomyces 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**