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Comparison of Nine Antigen Detection Kits for Diagnosis of Urogenital Infections Due to *Chlamydia psittaci* in Koalas

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***Chlamydia psittaci* is the major cause of infectious disease in the koala (*Phascolarctos cinereus*). It causes four disease syndromes in the koala, namely, conjunctivitis, rhinitis, cystitis, and infertility (females only). Diagnosis of chlamydial infections in koalas relies primarily on isolation of the organism in cell culture. Serology has generally not been useful, and little use has previously been made of the commercially available antigen detection kits. We examined the sensitivity, specificity, and usefulness of three direct fluorescent-antibody kits (Vet-IF [Cell Labs], IMAGEN [Celltech], Chlamydia-Direct IF [Bio Merieux]) and six antigen detection enzyme-linked immunosorbent assay (ELISA) kits (Clearview [Unipath], Surecell [Kodak], Pathfinder [Kallestad], Chlamydia-EIA [Pharmacia], Chlamydiazyme [Abbott], IDEIA [Celltech]) for the detection of urogenital infections in koalas. Laboratory studies showed that the direct fluorescent-antibody kits were the least sensitive in this case and did not detect fewer than 10^4 elementary bodies per ml, while most ELISA kits detected between 130 and 600 elementary bodies per ml. Field study results showed that the Clearview kit was the most sensitive (91%) compared with the IDEIA (88%) and the Surecell (73%) kits. All three kits were more sensitive than cell culture (36%), highlighting viability loss problems that occur during transport. This study showed that the Clearview kit is sensitive, specific, and easy to use for the detection of type II (urogenital) *C. psittaci* from koalas in the field and warrants further evaluation.**

Chlamydia psittaci causes a number of diseases in the koala (*Phascolarctos cinereus*) including keratoconjunctivitis (6), rhinitis and pneumonia (2), urinary tract disease (3), and reproductive tract disease leading to infertility (females only) (2, 18). There appear to be two genotypes of *C. psittaci* affecting the koala (9, 20). Type I is mainly isolated from the conjunctivae and respiratory mucosae of diseased koalas, while type II is associated with both ocular and urogenital tract disease. The presumptive diagnosis of chlamydial infection in koalas is often difficult to make because clinical signs of infection are often not present in the early stages of the disease (28). Disease therefore has been measured by isolation of the organism in cell culture (11) or by the presence of antichlamydial antibodies in serum (18). There are several disadvantages associated with both of these methods. The major disadvantage of cell culture is the requirement for viable organisms. This means transport and storage of samples must be at −70°C, a requirement which cannot always be met when collecting samples in the field. Koalas are slow to respond to many antigens and can take up to 3 months to produce complement-fixing antichlamydial antibodies. The complement fixation test therefore is not useful in detecting the early stages of chlamydial infection. New methods which detect chlamydial nucleic acid in specimens, such as DNA hybridization and polymerase chain reaction, have also been used for the detection of chlamydial infections in koalas (10). These methods were shown to be extremely sensitive, but their use is restricted to suitably equipped laboratories and experienced researchers.

Currently, antigen detection tests such as direct fluorescent-antibody (DFA) stains and enzyme-linked immunosorbent assays (ELISAs) are used extensively for the diagnosis of human chlamydial infections (1, 12–14, 16, 22, 24, 26). However, little use has been made of these tests for detecting chlamydial infections in koalas, and to date the results are conflicting. Weigler et al. (27) found the IDEIA kit to be insensitive (3% when compared with cell culture) for detecting chlamydial infections in a free-ranging koala population. In comparison, the Chlamydia-Cel Vet-IF test (DFA stain) was found to have a sensitivity of 89% and appears to be suitable for detecting *C. psittaci* in direct smears from infected sites (4). In human populations with high prevalences of infections, DFA staining and ELISAs are considered to be both sensitive and specific for the detection of chlamydiae, but they are not considered sufficiently reliable in human populations with low prevalences of infections. Because of the lack of a suitable test, the number of koalas with chlamydial infections can only be estimated. Therefore, new rapid techniques such as DFA staining and ELISAs warrant evaluation for their use in diagnosing chlamydial infections in koalas. In the study described here, three DFA stains (Vet-IF, IMAGEN, Chlamydia Direct-IF), six ELISAs (Clearview, Surecell, Pathfinder, Chlamydiazyme, IDEIA, Chlamydia-EIA), and cell culture were evaluated with either a laboratory-cultured chlamydial isolate or clinical specimens for their ability to detect chlamydiae in the urogenital tracts of koalas.

MATERIALS AND METHODS

Evaluation of chlamydial detection methods under laboratory conditions. (i) Isolation of *C. psittaci* in cell culture. Chlamydiae were isolated in coverslip cultures of African buffalo green monkey (BGM) kidney cells (Flow Laboratories, Inc., McLean, Va.) in Dulbecco's modification of Eagle's medium and were treated with cycloheximide and centrifugation as described by Rush and Timms (20). Briefly, chlamydial samples (stored at −70°C) were rapidly thawed at 37°C and vortexed for 15 s, and 0.1 ml was inoculated onto

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BGM cell monolayers. Inoculated monolayers were centrifuged for 1 h at 2,000 × *g* and 34°C and were equilibrated for a further 3 h at 37°C in 5% CO_2 before the medium was removed and replaced with Dulbecco's modification of Eagle's medium containing 1 μg of cycloheximide per ml. Cultures were incubated at 37°C in 5% CO_2 until they were harvested at days 3, 6, and 10 for staining with 5% Giemsa in phosphate buffer (pH 6.8) for the detection of chlamydial inclusions.

(ii) Commercial chlamydial detection kits tested. Three DFA kits—IMAGEN (Celltech), Chlamydia Direct-IF (Bio Merieux), and Vet-IF (Cell Labs)— and six ELISA kits—IDEIA (Celltech), Surecell (Kodak), Clearview (Unipath), Pathfinder (Kallestad), Chlamydia-EIA (Pharmacia), and Chlamydiazyme (Abbott)—were evaluated. All kits were used according to the manufacturers' instructions.

(iii) Comparison of sensitivity. Type II *C. psittaci* from a koala was grown in cell culture. Twenty infected culture vials were pooled, shaken vigorously with glass beads, and serially diluted in sucrose-phosphate-glutamate (SPG) transport buffer (220 mM sucrose, 7 mM K_2HPO_4, 5 mM glutamic acid, 10% fetal calf serum, 100 μl of gentamicin per ml, 100 μg of gentamicin per ml, 2 μg of amphotericin B [Fungizone] per ml). Appropriate dilutions were stained with the IMAGEN fluorescent-antibody stain, and the number of fluorescing elementary bodies (EBs) per milliliter was estimated. This starting inoculum was serially diluted in SPG buffer to give 10^7 to 10^0 EBs per ml, and aliquots were frozen at −70°C. The sensitivity of cell culture detection in this laboratory was evaluated by inoculating duplicate BGM cell cultures with 0.1-ml aliquots of each serial dilution.

The three DFA kits and the six ELISA kits were assessed for their sensitivities under laboratory conditions by using the same serial dilutions of type II *C. psittaci* from a koala. Each kit was tested with 0.1-ml aliquots of each dilution (10^7 to 10^0 EBs per ml) according to the manufacturers' instructions. When the lowest detectable 10-fold dilution of chlamydiae was determined, each kit was retested with a 2-fold dilution of the last positive result. All kits were tested at least twice, and each dilution was tested in duplicate during an assay.

(iv) Comparison of specificity. Nine bacteria (*Lactobacillus fermentum*, *Acinetobacter calcoaceticus*, *Streptococcus faecalis*, *Staphylococcus epidermidis*, *Peptostreptococcus anaerobius*, *Proteus vulgaris*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) were tested in the present study to determine the specificities of the monoclonal and polyclonal antibodies used in the diagnostic kits. The bacteria chosen were (i) common vaginal or rectal flora, (ii) opportunistic pathogens likely to be present in the event of an infection, or (iii) those bacteria previously shown to react with antibodies to the chlamydial lipopolysaccharide via a similar antigenic structure. Bacterial cultures from the mid-logarithmic phase were diluted to 10^7 to 10^8 CFU/ml, and 0.1-ml volumes were used.

Evaluation of chlamydial detection methods under field conditions. (i) Source of koalas. Samples were collected from a population of 74 free-ranging koalas at Mutdapilly, in southeastern Queensland, Australia. Many of these animals have collars that transmit radio waves and have been monitored for chlamydial infections at regular intervals over a 3-year period. Previously, infections have been diagnosed by both the presence of clinical signs and isolation of the organism in cell culture. Ethical considerations restrict the use of large numbers of koalas for experimental purposes, particularly those involving invasive procedures, such as swabbing. As a consequence, only 18 female koalas could be used to compare detection methods under field conditions.

(ii) Effect of multiple swab collection on chlamydial diagnosis. From human *Chlamydia trachomatis* studies it is known that the sampling procedure is critical to the performance of the diagnostic test (12, 21). We therefore conducted an experiment to determine the influence of swab sampling on the isolation of *C. psittaci* from the urogenital tracts of koalas. The effect of swab order was determined by taking four consecutive swabs from the urogenital tract of each of four koalas with *C. psittaci* infections. Cell culture and the Clearview kit were used to compare the number of chlamydiae or chlamydial antigen on each swab. Briefly, swabs were collected into 1 ml of SPG transport buffer and stored at −70°C prior to inoculation of 0.1-ml amounts of sample onto overnight coverslip cultures of BGM cells. Samples of 0.1 ml from the SPG transport buffer were also tested with the Clearview kit.

(iii) Specimen collection. Clinical specimens were collected at random from 18 of the 43 female koalas in the study population. Only urogenital specimens were used in the study, and these were collected from female koalas by vigorously swabbing the urogenital tract. Four swab samples were taken from each koala and were randomly assigned to the various tests in the following manner.

Swab 1 was always used for the isolation of chlamydiae in cell culture, because this had been the procedure used in previous studies (28). It was collected into 1 ml of SPG transport buffer by using a cotton tipped aluminum-shafted swab. Vials were stored on wet ice for transport to the laboratory, where they were stored at −70°C until they were processed. Subsamples of 0.1 ml were used to inoculate BGM cell culture monolayers.

Swabs 2, 3, and 4 were randomly assigned to the IDEIA, Clearview, or Surecell kits. Swab samples were collected with the swabs provided by the manufacturers and were placed into the accompanying transport vials. Surecell and IDEIA swabs were transported to the laboratory on wet ice and were stored at −20°C until they were processed. Clearview swabs were transported on wet ice and were stored at 4°C until they were processed. All samples were stored at the recommended temperature and were processed within the specified time outlined by the manufacturer.

(iv) Test sensitivity and specificity. The sensitivities and specificities of the tests (see Table 1) were determined as follows. Either a positive cell culture result or clinical signs of disease were used as a definitive diagnosis of chlamydial infection. However, a negative result by either of these tests does not imply that the disease is not present. Traditionally, new diagnostic methods have been evaluated against cell culture. This incorrectly assumes that the cell culture technique is 100% sensitive. To avoid this assumption, a positive infection in the present study was determined in the following manner: (i) the presence of characteristic clinical signs of disease, (ii) a positive cell culture result, or (iii) a positive result obtained by any two of the other diagnostic tests. Eleven of the 18 koalas tested were positive for *C. psittaci* by these criteria. Sensitivity, specificity, and false-positive and false-negative rates were determined by standard procedures.

RESULTS

Evaluation of chlamydial detection methods under laboratory conditions. (i) Cell culture. After 6 days in cell culture, characteristic Giemsa-stained inclusions were easily seen in

all dilutions (10^7 to 10^0 EBs per ml). This highlights the sensitivity of cell culture and shows that, under optimal conditions, one EB can be detected.

(ii) Antigen detection kits. Type II *C. psittaci* from koalas was easily visualized with all three DFA stains when high concentrations of antigen were present in the DFA sample (10^7 to 10^5 EBs per ml). However, they were difficult to see in the less concentrated samples (10^4 to 10^0 EBs per ml). The cutoff value for a positive result, as recommended by all three kit manufacturers, was 10 chlamydial particles per sample. The cutoff value in this series of experiments was increased to >10 chlamydial particles in 50 microscope fields. Therefore, the limits of sensitivity of the kits at this cutoff level were as follows: for IMAGEN and Vet-IF, 1.3×10^4 EBs per ml; for Chlamydia Direct-IF, 1×10^6 EBs per ml. The brightness of the fluorescent label varied between the kits, as did the amount of nonspecific staining. The IMAGEN kit had the brightest fluorescence, consistently giving a clear apple green color and no nonspecific staining.

When laboratory cultures of *C. psittaci* from koalas were used, all six ELISA kits tested were significantly more sensitive than the fluorescent-antibody stains. The performance of each kit tested is discussed below.

The Pathfinder kit was the least sensitive of the six ELISA kits evaluated, with a sensitivity limit of 6×10^3 EBs per ml. When high concentrations of chlamydiae were tested, a particulate suspension formed in the test solution. This resulted in a fluctuating absorbance reading, and it was necessary to let this settle before a definitive reading could be taken. The polyclonal antibody used in the Pathfinder assay has been shown to be both less sensitive and less specific than the monoclonal antibodies used in the other ELISAs (23), which may account for the lack of sensitivity seen in the present study.

The Chlamydia-EIA, IDEIA, and Chlamydiazyme kits were all reasonably sensitive, detecting *C. psittaci* from koalas at concentrations from 1.3×10^2 to 1.3×10^3 EBs per ml. The Chlamydia-EIA kit showed some variation in results when the sample contained low concentrations of EBs, giving a positive result on one occasion but a negative result with the same sample on another occasion. This variation was investigated by using a sample containing 10^3 EBs per ml, which was tested in triplicate. Possible concentration differences between samples were also assessed by testing three different samples, each of which was estimated to contain 10^3 EBs per ml. The A_{405} readings of the sample tested in triplicate were 0.158, 0.176, and 0.204 absorbance units, while the A_{405} readings of the three individual samples were 0.152, 0.171, and 0.376 absorbance units. In all instances the absorbance values of one of the samples was noticeably higher than the other two values, indicating some lack of repeatability. This variation in absorbance was also reported by Taylor-Robinson et al. (25) with *C. trachomatis* samples from humans tested by the Chlamydiazyme test.

The Surecell kit was one of the more sensitive of the ELISA kits compared, detecting 1.3×10^2 to 6×10^2 EBs per ml, although this was not entirely consistent on a day-to-day basis or with different batches of kits. The interpretation of a positive result obtained by this test relies on visual comparison between the color formed in the negative control well and that formed in the sample well. This end point is not always clear-cut, although the test was reported by Hammerschlag et al. (13) to have a clear and unambiguous end point when used to detect chlamydial conjunctivitis in infants. All infants tested in that study had signs of conjunctivitis indicating an active infection and probably had high numbers of chlamydiae. A clear end point was also seen in our study when samples containing high numbers of chlamydiae (10^7 to 10^4 EBs per ml) were tested, but the end point was less distinct in samples with low numbers of chlamydiae (10^2 EBs per ml).

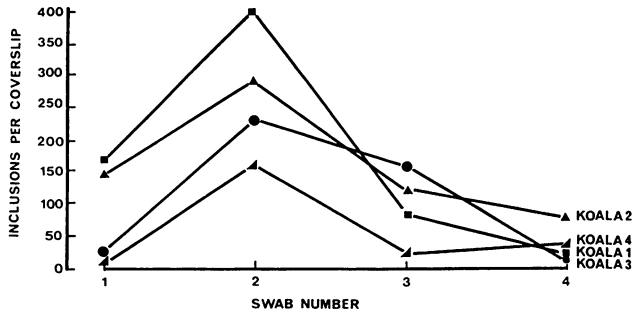

FIG. 1. Effect of swab order on the recovery of viable *C. psittaci* (in BGM cell culture) from koala urogenital tract specimens.

The Clearview test kit, like the Surecell test kit, also uses visual comparisons to determine a positive result. Whereas the enzyme reactions in the Surecell kit take place in a well, those in the Clearview kit occur behind a window, on a thin membrane support. A positive reaction is seen as a blue-purple line where the antibody-antigen complexes have reacted with the enzyme-labeled capture antibodies. Although the intensity of the color reaction varied depending on the amounts of antigen present, the end point was quite evident. The sensitivity of this test kit was estimated to be 1.3×10^1 to 1.3×10^2 EBs per ml.

Each diagnostic kit was used to test all nine bacterial isolates at a concentration of 10^8 organisms per ml. There was no false-positive reaction between any of the bacteria and the monoclonal antibodies used in the DFA kits at this concentration. Three of the ELISA kits—IDEIA, Clearview, and Chlamydiazyme—gave a positive reaction with some of the bacteria at a concentration of 10^8 colonies per ml. When these bacteria were diluted to 10^7 colonies per ml, all false-positive reactions were eliminated.

Effect of multiple swab collection on chlamydial diagnosis. The number of chlamydial inclusions present, as detected by cell culture, varied significantly depending on the order in which the swabs were collected (Fig. 1). This difference in numbers was consistent with samples from each of the four koalas. Higher numbers of chlamydiae were isolated from the second swab from all four koalas. The amount of chlamydiae isolated with the first swab may be directly related to the severity of the infection. Inclusions were not seen in the first swab taken from koala 4. This koala had antichlamydial antibodies but no clinical signs of disease at the time of sampling or during the previous year. Koalas 1, 2, and 3 were all diagnosed as having chlamydial infections in the previous 6 months, and both koalas 1 and 2 had clinical signs of dirty tail at the time of sampling.

The effect of swab number on the detection of chlamydial lipopolysaccharide antigen (rather than viable chlamydiae) was tested by using the Clearview kit. The intensity of the reaction for each swab tested was graded from 4+ (strong positive) to 1+ (weak positive). The result of the Clearview test with the swab from koala 1 was similar to the results obtained with cell culture for this animal. A 4+ positive reaction was seen with the second swab, while the remaining swabs were rated 2+ or less (Fig. 2). This was not observed

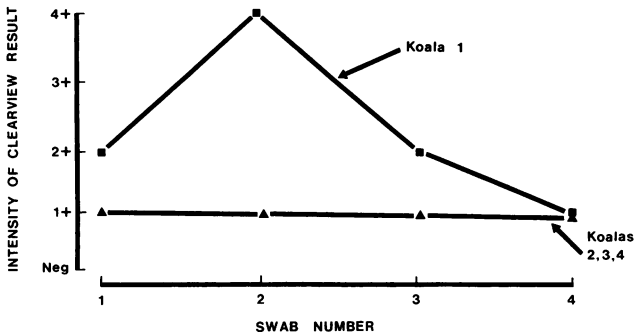

FIG. 2. Effect of swab order on the recovery of chlamydial lipopolysaccharide antigen from urogenital specimens (assayed by using the Clearview kit).

with samples from koalas 2, 3, and 4, for which all samples were rated as a 1+ positive result. The Clearview test detected chlamydial antigen in the first swab from koala 4 when there were no inclusions seen in cell culture for swab no. 1 from this animal.

Evaluation of chlamydial diagnostic methods under field conditions. Eighteen female koalas were tested for chlamydial infection of their urogenital tracts by using cell culture and three commercial kits (Table 1).

(i) Cell culture. In the present study, cell culture had a sensitivity of 36% and a specificity of 100%. After 6 days in culture, swabs from only 4 of the 18 koalas were positive by cell culture. Seven of the clinical specimens had contaminating bacteria, and in some situations the bacteria had destroyed sections of the cell monolayer. Four of the collection vials leaked during transport, leading to a decrease in sample size and, if the swab was exposed, a subsequent decrease in viability.

(ii) Clearview kit. Only one false-negative sample was seen on testing with the Clearview test kit, resulting in a sensitivity of 91% and a specificity of 100%.

(iii) Surecell kit. Three of the positive samples tested negative by the Surecell test kit, resulting in a sensitivity of 73% and a specificity of 100%. Two of the false-negative reactions were from specimens collected with the third and fourth swabs, which may have had significantly fewer organisms. The third false-negative sample, however, was tested with the optimal second swab.

(iv) IDEIA kit. The IDEIA test kit identified 10 of the 11 infected koalas, giving a sensitivity of 91% and a specificity of 86%. The one sample that gave a false-negative result was the fourth swab. The IDEIA kit was the only one of the four methods tested with actual field specimens (urogenital swabs) that recorded a false-positive result. This sample was contaminated with an unidentified rod-shaped bacterium which may have reacted with the monoclonal antibodies used in the IDEIA kit.

TABLE 1. Diagnostic characteristics of cell culture and three antigen detection ELISA kits used to detect urogenital infections caused by *C. psittaci* in koalas[a]

Diagnostic test	Sensitivity	Specificity	False-positive rate	False-negative rate
IDEIA	10/11	6/7	1/7	1/11
Surecell	8/11	7/7	0/7	3/11
Clearview	10/11	7/7	0/7	1/11
Cell culture	4/11	7/7	0/7	7/11

[a] Values are number of positive swabs/total number of swabs tested.

DISCUSSION

The isolation of *C. trachomatis* in cell culture, compared with direct antigen detection in clinical specimens, is still considered the most sensitive technique for the diagnosis of human chlamydial infections. However, cell culture is being replaced in some diagnostic laboratories with antigen detection tests such as DFA stains and ELISA kits. The value of cell culture compared with that of antigen detection tests for the diagnosis of *C. psittaci* in koalas, however, has not been thoroughly evaluated. In the present study, cell culture was 10 to 100 times more sensitive than the diagnostic kits for the detection *C. psittaci* infections in koalas under laboratory conditions. Cell culture was able to detect a single chlamydial EB per ml, whereas the best antigen detection kit (Clearview) detected 130 chlamydial EBs per ml. Even though cell culture was more sensitive, it does have several disadvantages. The procedure is time-consuming and takes 3 to 6 days before a result is available. There are a number of factors which affect the isolation of chlamydiae in cell culture, including specimen collection and storage, growth media, the incubation conditions used, and the possibility of microbial superinfection or inhibitory body fluids in the sample. Many workers now consider that, because of these factors, cell culture is not 100% sensitive when used with clinical specimens and that its sensitivity is perhaps closer to 70 to 80% (21).

Tests which detect chlamydial antigen, as opposed to viable chlamydiae, have several advantages over cell culture. Antigen detection systems detect both viable and nonviable EBs as well as soluble lipopolysaccharide antigen in secretions. Several of the diagnostic kits used in the present study have been evaluated for use in the detection of human chlamydial infections. The sensitivities and specificities of these kits have been shown to vary depending on the population samples. The Surecell kit was shown to have a sensitivity of 93% when used in the detection of chlamydial conjunctivitis in infants (13). The IDEIA, Chlamydia-EIA, and Chlamydiazyme kits have been thoroughly tested with clinical specimens (12, 14, 22). Researchers (12, 14, 22) have reported a range of sensitivities with the tests (60 to 90%), with the lower sensitivities seen more often in populations with a low prevalence of infection or asymptomatic patients.

The monoclonal antibodies used in several of the DFA tests and ELISAs have previously been reported to cross-react with certain bacterial species including *A. calcoaceticus* and group B streptococci (25) as well as *S. aureus* and *Peptostreptococcus productus* (5, 16), although no cross-reactions in any of the DFA tests evaluated were observed in the present study. In our hands, the three DFA tests which we evaluated (IMAGEN, Vet-IF, and Chlamydia Direct-IF) were not as sensitive as cell culture or the ELISAs. Experience with fluorescence microscopy is important when using DFA stains (8, 16), and lack of experience may account for the reduced sensitivity seen in the present study. In addition, the amplification effect seen with the cell culture and ELISA techniques does not occur with DFA stains, and this may also lead to reduced sensitivity. Microscopic evaluation of the DFA stains is both time-consuming and tiring, but it does allow sample quality to be assessed, something which is lacking in the other diagnostic tests.

The solid-phase ELISAs and the DFA stains were the quickest and simplest tests to perform, taking about 30 min

to complete. The Clearview kit was more sensitive than the Surecell kit, detecting 130 EBs per ml compared with 600 EBs per ml, respectively. The plate ELISA kits (Chlamydia-EIA, IDEIA, Chlamydiazyme, and Pathfinder) required up to 10 times more time to obtain a result and were slightly less sensitive than the solid-phase ELISA kits, detecting from 6,000 to 130 chlamydial EBs per ml. Results from the ELISAs, which were read with a spectrophotometer (IDEIA, Pathfinder, Chlamydiazyme, and Chlamydia-EIA), were sometimes difficult to interpret because sample absorbances were often close to the cutoff zone recommended by the manufacturer. The need for an equivocal zone around the cutoff value has been stressed by other workers (1, 14).

Regardless of the diagnostic test being used, the quality of the specimen is of the utmost importance. There is some disagreement in the literature as to the advantage of obtaining more than one swab specimen per site and as to which swab is the optimum one (i.e., which swab contains the maximum numbers of chlamydiae) when diagnosing *C. trachomatis* infections in humans. The manufacturers of chlamydial diagnostic kits recommend that the first swab should be used to remove cervical secretions and mucus, while the second swab should be retained for testing (8, 17, 19). Several groups (7, 15) actually reported that the sensitivity of *C. trachomatis* detection is increased by 10 to 36% when more than one swab is taken. There is no information concerning the collection of multiple swabs for the increased isolation of *C. psittaci* from the urogenital tracts of koalas. Our results support those found in the diagnosis of human chlamydial infections of the genital tract; the first swab collects mostly cervical secretions (perhaps some soluble lipopolysaccharide antigen) and should be used only to clean the cervix. Diagnosis of chlamydiae should be done by using the second or third swab, which removes infected epithelial cells. Swab specimens from the urogenital tract of the koala are collected from the urogenital sinus rather than the cervix, so it is even more important that either the second or third swab be used for the diagnosis of chlamydial infections in koalas. To ensure that the maximum number of chlamydiae are collected in a specimen, it is recommended that the second swab be combined with the first swab in one collection vial and that the specimen be processed as a single sample.

Four genus-specific chlamydia diagnostic kits were chosen for evaluation with clinical specimens: IDEIA, Clearview, Surecell, and Chlamydia-EIA. Only 11% of the animals in the free-range population examined in the present study had clinical signs of disease, although 62.5% were shown to be infected with chlamydiae. This highlights the shortcomings of using clinical signs alone as a means of diagnosing chlamydial infections in koalas. Cell culture performed very poorly in this study. This was partly due to the difficulty of transporting the samples from the field collection site back to the laboratory without the loss of sample or was due to inadequate cooling. Again, this highlights the advantage of the antigen detection methods, particularly for samples from koalas, which are often collected at remote sites. The two solid-phase ELISA kits evaluated in the present study proved to be reasonably sensitive. In particular, the Clearview kit gave a sensitivity of 91%, and we found its end point quite easy to read.

The IDEIA test has previously been used for the diagnosis of *C. psittaci* in a koala colony in the Redlands Shire in Queensland, Australia (27). Weigler et al. (27) reported a sensitivity of 10%, using specimens from the urogenital tract. The prevalence of infection in their study population, as determined by tissue culture isolation, was 47%, but it was only 11% by use of the IDEIA kit. In our study, the IDEIA kit had a sensitivity of 88% and appeared to be an adequate test for the diagnosis of infection in this high-prevalence (62.5% infection rate) population.

Results of the present study demonstrate that chlamydial cell culture has many limitations when used in a field situation, such as with koalas. Under these circumstances, a test which detects nonviable chlamydiae provides a rapid and reliable alternative. The results of this study have shown that the Clearview kit is a simple, rapid, and sensitive test for the detection of urogenital infections in koalas and, therefore, should provide a reliable alternative to cell culture, at least in high-prevalence populations.

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