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Communicable Diseases Intelligence

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Contents:

VIRUS REPORTING SCHEME: A total of 1,389 reports were processed for this period.

Three cases of Q fever were reported, two from New South Wales and one from Western Australia. Occupational exposure data were only available for the Western Australian case, a 31 year old male abattoir worker with pyrexia of unknown origin. None of these three patients was involved in the Q fever vaccine field trial conducted in South Australia.

Cytomegalovirus specific IgM antibody was detected in the serum of a 21 year old male with Guillain-Barré syndrome.

Hepatitis A specific IgM antibody was detected in the serum of a 12 year old female post-appendectomy patient who had abnormal liver function tests.

Cytomegalovirus was isolated from:

- the post-mortem tissues derived from the lungs, liver, heart and parotid, adrenal and prostate glands of a 25 year old HIV-antibody positive male who died of CMV retinitis. The patient had reported being unwell three weeks earlier when CMV was isolated from leucocytes. It was not known whether the patient had received any DHPG or AZT therapy.
- the saliva and urine of a 40 year old male with CMV colitis. The patient had DHPG therapy withdrawn one week earlier but was still being treated with AZT.

The Bulletin is compiled and distributed by the Public Health Section, Communicable Diseases Branch, Department of Community Services and Health.

AIDS UPDATE - INTERNATIONAL
(based on WER No 40, 2 October 1987)

Global data - AIDS cases reported to WHO, by country, as of 30 September 1987

Country/Area — Pays/Territoire	Date of report Date de notification	Number of cases Nombre de cas
Albania	31.08.87	—
Algeria	01.06.87	5
Angola	26.09.86	6
Anguilla	31.03.87	—
Antigua and Barbuda	31.03.87	2
Argentina	31.03.87	78
Australia	30.08.87	583
Austria	30.06.87	93
Bahamas	31.03.87	104
Bangladesh	14.04.87	—
Barbados	31.03.87	39
Belgium	30.06.87	255
Belize	31.03.87	2
Benin	18.05.87	3
Bermuda	30.06.87	60
Bhutan	14.04.87	—
Bolivia	30.06.86	1
Botswana	30.06.87	13
Brazil	30.04.87	1 695
British Virgin Islands	31.12.86	—
Brunei Darussalam	21.07.87	—
Bulgaria	12.08.87	2
Burkina Faso	13.11.86	—
Burma	14.04.87	—
Burundi	31.03.87	128
Cameroon	05.03.87	25
Canada	27.04.87	1 000
Cape Verde	30.04.87	4
Cayman Islands	31.12.86	2
Central African Republic	31.10.86	254
Chad	13.11.86	1
Chile	31.03.87	28
China	02.04.87	2
China (Province of Taiwan)	26.01.86	1
Colombia	31.03.87	57
Comoros	13.11.86	—
Congo	13.11.86	250
Cook Islands	08.09.87	—
Costa Rica	31.12.86	20
Côte d'Ivoire	13.11.86	118
Cuba	31.12.86	3
Cyprus	01.06.87	3
Czechoslovakia	31.03.87	7
Democratic People's Republic of Korea	09.05.87	—
Denmark	30.06.87	176
Dominica	31.03.87	3
Dominican Republic	31.03.87	200
Eastern Mediterranean Region	10.09.87	36
Ecuador	31.03.87	18
Egypt	06.07.87	1
El Salvador	31.03.87	9
Ethiopia	30.06.87	5
Fiji	08.09.87	—
Finland	30.06.87	19
France		
Metropolitan	30.06.87	1 980
Overseas:		
French Guiana	31.12.86	58
French Polynesia	01.04.87	1
Guadeloupe	31.12.86	40
Martinique	31.03.87	25
Reunion	10.06.87	1
Gabon	06.07.87	13
Gambia	16.03.87	14
German Democratic Republic	30.06.87	4
Germany, Federal Republic of	31.08.87	1 298
Ghana	25.05.87	145
Greece	30.06.87	49
Grenada	30.06.87	5
Guatemala	31.03.87	22
Guinea	30.06.87	9
Guinea-Bissau	30.06.87	2
Guyana	31.12.86	2
Haiti	31.03.87	851
Honduras	31.03.87	20
Hong Kong	31.12.86	4

Country/Area — Pays/Territoire	Date of report Date de notification	Number of cases Nombre de cas
Hungary	30.06.87	5
Iceland	30.06.87	4
India	09.05.87	9
Indonesia	21.04.87	1
Ireland	30.06.87	19
Israel	30.06.87	39
Italy	30.06.87	870
Jamaica	11.05.87	16
Japan	16.06.87	43
Kenya	30.07.87	625
Lebanon	03.06.87	3
Lesotho	13.11.86	1
Liberia	12.06.87	2
Luxembourg	31.03.87	7
Madagascar	25.04.87	—
Malawi	13.11.86	13
Malaysia	01.04.87	1
Maldives	30.06.87	—
Malta — Malte	30.06.87	6
Mariana Islands	05.08.87	—
Mauritania	3.11.86	—
Mauritius	15.09.87	1
Mexico	31.03.87	407
Montserrat	31.12.85	—
Mozambique	30.06.87	1
Nepal	09.05.87	—
Netherlands	30.06.87	308
New Zealand	31.08.87	51
Nicaragua	31.12.86	—
Nigeria	22.05.87	5
Norway	30.06.87	49
Panama	31.03.87	14
Papua New Guinea	31.03.87	—
Paraguay	31.03.87	10
Peru	30.06.86	9
Philippines	30.06.87	7
Poland	30.06.87	2
Portugal	30.06.87	67
Qatar	09.05.87	9
Republic of Korea	01.04.86	1
Romania	31.03.87	2
Rwanda	30.11.86	705
Saint Christopher and Nevis	31.12.86	1
Saint Lucia	31.12.86	3
Saint Vincent and the Grenadines	31.12.86	3
Samoa	08.09.87	—
Sao Tomé and Principe	01.12.86	—
Senegal	13.11.86	—
Seychelles	13.11.86	—
Singapore	30.06.87	2
Solomon Islands	08.09.87	—
South Africa	24.07.87	77
Spain	30.06.87	508
Sri Lanka	14.04.87	2
Sudan	23.08.87	12
Suriname	31.03.87	3
Swaziland	01.07.87	7
Sweden	28.08.87	136
Switzerland	30.06.87	266
Thailand	30.06.87	11
Togo	13.11.86	—
Tonga	08.09.87	—
Trinidad and Tobago	31.03.87	201
Tunisia	14.05.86	2
Turkey	30.06.87	21
Turks and Caicos Islands Turques et Caïques	31.12.86	2
Uganda	28.02.87	1 138
USSR	05.08.87	4
United Kingdom	31.07.87	935
United Republic of Tanzania	18.04.87	1 130
United States of America	14.09.87	41 825
Uruguay	31.12.86	8
Vanuatu	24.07.87	—
Venezuela	31.12.86	69
Viet Nam	08.09.87	—
Yugoslavia	30.06.87	11
Zaire	30.06.87	335
Zambia	30.06.87	395
Zimbabwe	28.08.87	380
Total		**60 653**

SCREENING FOR AIDS: TRANSFUSION ASPECTS
(Based on CDR 87/38, 25 September 1987)

Screening for specific antibody as evidence of past or current infection with HIV is currently the most appropriate laboratory method of excluding blood donations that may transmit that virus. Nevertheless, a small number of infectious donors may be seronegative and it is therefore important to use exclusion of "high risk" donors as an adjunct to screening:

- one such donor is known to have transmitted HIV in Colorado.
- a similar occurence has been reported in Glasgow, where a donor, previously negative for HIV-Ab, was found positive at the time of the next donation and the recipient of the preceding donation was found to have been infected. This was the first observed case of its kind in 3.5 million screened donations in the UK, indicating the extreme rarity of such events.

Since 80% of UK donors are "repeat" donors and will therefore be subjected to more than one HIV-Ab screening test, this low frequency rate need not be considered falsely optimistic. The possible need for HIV antigen assays, which would detect some individuals in the early stage of infection before antibody appeared, should be considered in the light of these facts.

It is only rarely that a donor could be expected to be found positive for HIV antigen but not antibody; donor education and promotion of "self-exclusion" by those at risk of contracting AIDS should be continued vigorously to minimise the chance of such donors attending. It should also be remembered that HIV-Ab is far longer lasting that HIV-Ag with currently available assays so that the latter could not be used as the sole screening test. Furthermore, an individual could be negative for both HIV-Ag and HIV-Ab very early in acute infection but still be infectious, especially in the context of transfusion where a large inoculum is involved.

Because of the profound impact of a positive HIV report on both donor and any previous recipient, specificity is an important prerequisite of any screening test, though its predictive value will depend on the prevalence of antibody:

- The antiglobulin type of assay has until now been confounded by repeatable false-positive results due largely to cross-reacting HLA antibodies present in some donors. This cross reactivity is not unexpected with a virus that buds through the cell membrane, carrying with it cellular components.
- The specificity of new antibody tests based on cloned or even synthetic antigens may well be higher.
- Another innovative and simple test that merits study in a transfusion context is the gelatin particle agglutination assay for HIV-Ab which will soon become commercially available, together with an automated plate reader for it.

Other important factors in deciding suitability of a test for screening of blood donations are sensitivity, simplicity, rapidity and low cost. In most of these respects the "competititve" type of assay for HIV-Ab has proved equal to or better than the "antiglobulin" format. Competitive assays do

not have the drawback of cross-reactivity and have the added advantage of using undiluted serum in only one incubation step, leading to a simpler and quicker test. The sensitivity of the competitive assay has been demonstrated at the North London Blood Transfusion Centre. Parallel testing of serial serum samples from a subject who underwent seroconversion showed that the competitive assay (in common with an antiglobulin assay) was reactive at the point of seroconversion as determined by the "Western blot" technique. The competitive assay (again in common with an antiglobulin assay) was also able to detect "passive" antibody in stored serum samples from a patient who received 50ml of plasma from a donor later shown to be HIV-Ab positive.

The results of the first 4.5 million blood donor screening tests performed in the UK have been collected at the Manchester RTC:

- the same overall rate of HIV-Ab positive blood donors is obtained whether competitive or antiglobulin assays are used. However, the rate of repeatedly reactive samples is currently
  - only one in 6,000 for the competitive assay, compared with
  - one in 800 for the antiglobulin assays, illustrating the greater specificity of the antiglobulin test assay.
- the low seropositive rate for HIV-Ab in British blood donors of about one in 55,000 is most likely to be due to donor education and the availability of alternative test sites. Regional variations do occur due to local higher prevalences but overall the UK approach at encouraging "self-exclusion" has been estimated as more than 95% efficient.
- the appearance of new, serologically distinct strains of the AIDS virus (HIV 2), that may not give rise to antibody detectable by existing tests, underlines the importance of this approach.

AIDS AND IMMUNISATION - WHO STATEMENT

(Based on WER (1987) 62:297-99, 2 October 1987)

The following items which are part of the WHO joint statement resulting from the consultation on human immunodeficiency virus (HIV) and routine childhood immunisation, are of relevance to Australia.

Concern has been raised that children infected with the human immunodeficiency virus (HIV) who receive routine childhood immunisations may have decreased immune responses and be at increased risk for adverse effects or acceleration of HIV-induced immunosuppression. Limited experience suggests that the likelihood of successful immunisation is reduced in some HIV-infected individuals but that the risk of serious adverse effects remains low. The theoretical risk of accelerating HIV infection by simultaneous administration of

multiple antigens is not supported by limited clinical information and is likely to be negligible in contrast to other natural sources of antigenic stimulation.

After reviewing the available information, the WHO informal consultation on HIV and routine childhood immunisation

- notes that, in accordance with the Global Advisory Group, live vaccines are not usually given to immunocompromised individuals, but agrees that, in areas where the risk of exposure to measles and poliovirus is high, the benefits of immunisation outweigh the apparently low risk of adverse effects from these vaccines, even in the presence of symptomatic HIV infection. Inactivated poliomyelitis vaccine (IPV) is an alternative to OPV for immunisation of children with symptomatic HIV infection who may be at increased risk of OPV-associated paralytic poliomyelitis.

- Strongly encourages further investigations in the following areas:

  (a) Safety of immunisations in HIV-infected children:
    (i) surveillance of HIV-infected children to permit rapid identification of any unexpectedly frequent adverse events following immunisation;
    (ii) establishment or modification of population-based surveillance systems to detect rare serious adverse events associated with immunisation of HIV-infected children;
    (iii) comparison of the rates of frequent and less severe adverse events which occur in HIV-infected and uninfected children following immunisation.

  (b) The natural history of vaccine-preventable diseases in HIV-infected children:
    (i) determination of the rates of serious complications of vaccine-preventable diseases in HIV-infected children in health care facilities and in the community and correlation of such complication with the state of HIV infection and degree of immunosuppression;
    (ii) establishment or modification of population-based surveillance systems to detect serious complications of vaccine-preventable diseases in HIV-infected children;
    (iii) assessment of the role of immune globulin in protection of HIV-infected children against vaccine-preventable diseases.

  (c) Immunogenicity and efficacy of immunisations in HIV infected children:
    (i) determination of the serological response to immunisation in HIV-infected children compared to uninfected children and correlation of vaccine response to stage of HIV infection and degree of immunosuppression;
    (ii) development of methods to improve vaccine responses of HIV-infected children, if these are found to be decreased;

(iii) determination of the persistence of vaccine-induced antibody;
(iv) prospective follow-up of immunised HIV-infected children and retrospective evaluation of cases of vaccine-preventable diseases to determine rates of vaccine failure in HIV-infected children.

(d) Possible activation or acceleration of HIV-infection by repeated antigenic stimulation with immunisations, including simultaneous administration of multiple antigens:
(i) detection of increased HIV replication following immunisation of HIV-infected children;
(ii) detection of immunological abnormalities following immunisation of HIV-infected children;
(iii) retrospective studies of the relationship between total number of immunisations received and/or number of antigens received simultaneously by HIV-infected children and the onset of symptomatic HIV infection, progression of clinical HIV disease and/or fatal out-come of HIV infection; the informal consultation agreed that prospective placebo-controlled double-blind studies in which some HIV-infected children would not receive recommended immunisations are not appropriate.

(e) The immunogenicity and efficacy of tetanus toxoid immunisation of HIV-infected pregnant women in the prevention of neonatal tetanus.

SYPHILIS - MAJOR UPSURGE POSSIBLY ASSOCIATED WITH SUBSTANCE ABUSE - CALIFORNIA (U.S.A)

(Based on California Morbidity # 31, 14 August 1987)

The incidence of primary and secondary syphilis in Calfornia, as a whole, did not reflect the successive yearly decreases that were reported for the United States from 1982 to 1986[1]. During this period, the State reported:
. an increase of 194 (+4%) cases in 1983,
. a decrease of 787 (-15%) cases in 1984, and
. a decrease of 113 (-3%) cases in 1985, but
. a major increase of 1,150 (+26%) cases in 1986.
In addition, in the first six months of 1987, California reported 3,641 cases of primary and secondary syphilis, a 21% increase over the 2,412 cases reported in the first six months of 1986.

Eight California health jurisdictions reported significant increases of over 20 cases from the first six months of 1986 to 1987:
. Contra Costa 68,
. Fresno 57,
. Long Beach 133,

. Los Angeles 996,
. Sacramento 26,
. San Bernadino 53,
. San Diego 30, and
. Tulare 43.

Despite the State's dramatic upsurge in primary and secondary syphilis morbidity during the first half of 1987, four major jurisdictions reported decreases:

. Orange 61 (-21%),
. San Joaquin 38 (-45%),
. Santa Clara 18 (-32%) and
. San Francisco 96 (-47%).

These reductions were achieved predominantly in high-risk populations : homosexuals and/or Spanish-speaking undocumented single males whose infections usually result from sex with prostitutes. San Francisco's successive yearly decreases from 1982 to 1986 can probably be attributed to extensive, AIDS-related, education compaigns directed at safer sex practices.

With the exception of White males, in which the incidence of primary and secondary syphilis declined by 1,147 (-55%) cases from 1983 to 1986, diseases were reported in all other major population groups during this period:

. White females, 90 (+52%);
. Black males, 519 (+52%);
. Black females, 544 (+33%);
. Hispanic males, 435 (+33%); and
. Hispanic females 190 (+105%).

Case investigations have revealed an ever-increasing association between substance abuse and the incidence of primary and secondary syphilis throughout the State. More and more cases are being identified in young females who engage in sex in exchange for drugs, particularly cocaine/"crack".

In 1986, there was also a sudden unprecedented increase in the number of cases of congenital syphilis in Blacks. There were 38 newborns with definite or probable congenital syphilis as compared to 15 in 1985. The California Health Data and Statistics Branch of the State Department of Health Services reported that from 1981 to 1985 there was a gradual increase in the number of pregnant White and Black females who did not seek prenatal care or who received it late in their pregnancies. This trend continued into 1986.

The Sexually Transmitted Disease Section of the State Department of Health Services (California) reiterates its on-going recommendation that health providers be alert to lesions in their patients that reflect syphilis, and to perform serologic tests in pregnant women, preferably at each trimester. In the absence of prenatal care, the VDRL or RPR testing of cord blood at birth is critical.

The increase in primary and secondary syphilis prompts two major concerns. This trend is likely to have adverse consequences on :

1. the control of congenital syphilis, and
2. the control of AIDS (since a history of sexually transmitted disease is associated with increased risk of

AIDS and human immunodeficiency virus (HIV) infections in both homosexuals and heterosexuals).

The increase of primary and secondary syphilis may result from:
1. diversion of resources from syphilis control to other sexually transmitted diseases (especially penicillin-resistant gonorrhoea) and prevention of HIV transmission, and
2. prostitution-related drug abuse, particularly cocaine, since its relatively short-lived effect requires the user to engage in sex more frequently.

REFERENCE

1. CDI (1987) 16 : 7-13

LYME BORRELIOSIS - A CASE REPORT FROM QUEENSLAND
(Contributed by Neville Stallman, State Health Laboratory, Queensland)

A 34 year old Australian female who resides in Connecticut (USA) presented soon after her arrival in Mooloolaba (Queensland - Australia), with severe headaches, stiff neck, myalgia, sore eyes and ears and a flitting polyarthralgia. An itchy rash was also present in an area surrounding what resembled a boil. The patient reported to have been bitten by a tick one week prior to disease onset whilst still in Connecticut.

Serologic evidence of Borrelia burgdorferi infection was demonstrated by indirect fluorescent antibody test and a rising titre of specific IgG and IgM in two serum samples collected 7 and 18 days after onset.

Comment

In 1983 Lyme borreliosis was first diagnosed in Australia in a patient with the classical skin lesion (erythema chromicum migrans - ECM), and a history of insect bite. There was no serological confirmation of diagnosis.

Following the introduction of an indirect fluorescent antibody (IFA) test using B.burgdorferi as antigen, 30 cases have been serologically detected with specific IgG titres ranging from 1:64 to 1:2048:

. Several sera were
  - tested by the Western blot technique to confirm antibody response to B.burgdorferi,
  - Submitted to the Centers for Disease Control (CDC), Atlanta to confirm IFA results with ELISA.

. Arthritis was a common symptom, reported by these patients
. 2 of the patients recalled having been bitten by a flying insect,
. none had a history of tick bite, however only 4 had specific IgM titre 1:64.

Although IXODES ticks are considered to be the principal vector of Lyme borreliosis, the above serological survey suggests:

. that other insect vectors may be implicated in the transmission of B.burgdorferi,
. That Lyme borreliosis be considered in the differential diagnosis of patients with recent onset arthritis even in the absence of a history of tick or insect bite.

SCHISTOSOMIASIS IN AUSTRALIAN TRAVELLERS

(Contributed by Dr P. Jones, Department of Infectious Diseases, The Prince Henry Hospital, Sydney).

In late June 1987, two Australians, a 25 year old male and a 26 year old female presented with persistent cough and lethargy:

A. Disease Diagnosis
   . physical examination in both subjects was unremarkable,
   . laboratory investigation identified:
      - full blood count normal - apart from a marked oesinophilia in both subjects (5 x 10^9/L),
      - stool specimens from the female subject identified occasional ova of Schistosoma mansoni,
      - repeated stool specimens and a rectal mucosal biopsy specimen from the male subject were negative for ova, and
      - serology for schistosomiasis was positive by ELISA in both patients.

B. Travel history revealed that:
   . both patients had participated in a touring party accross Africa between February and June 1987.
   . both patients and several other members of the party had in May developed intense puritis after swimming in the Ouham River in the Central African Republic.

C. Medical history showed that in late June, one month following their return to Australia:
   - the male developed an acute febrile illness consistent with Katayama fever and characterised by headache, myalgia, dry cough and an urticarial rash - the acute illness largely subsided over the following two weeks, apart from persisting dry cough and lethargy.
   - the female subject developed, at approximately the same time, a shorter febrile illness lasting only 2 days but subsequently developed cough and lethargy.

D. Disease treatment
   Both patients were treated with Praziquantel (approved by the Department of Health) in a dose of 60 mg/kg body weight administered in three divided doses over one day. There were no adverse effects to therapy in either patient. When reviewed one month later both patients were asymptomatic and felt full recovered. Stool specimens were negative for ova.

RAPID DIAGNOSIS OF CYTOMEGALOVIRUS INFECTION IN IMMUNOCOMPROMISED PATIENTS

(Based on CDS 87/31, 1 August 1987)

As cytomegalovirus (CMV) causes severe infection in immunocompromised patients there is an increasing need for a rapid, sensitive and economical method of laboratory diagnosis of CMV infection. CMV detection by conventional tube cell-culture (CTC) technique depends on the development of a characteristic cytopathic effect (CPE) which may appear within a few days of, or up to six weeks after, inoculation. Rapid techniques in which monoclonal antibodies are used to detect early antigen in cells; in some of these, a centrifugation step drives the CMV on to the cells[1]. The detection system that has been used most often is immunofluorescence, however increased sensitivity of antigen detection gained by amplification of both immunofluorescence and immunoperoxidase systems is hereby reported.

Materials

A. Cells and Medium

Human Embryo Fibroblast (HEF) cells were grown in Eagle's minimum essential medium (Gibco) with the addition of

- 10% tryptose phosphate broth,
- glutamine (200 mM),
- bicarbonate (0.08%),
- penicillin (100 IU/mL),
- streptomycin (100 µg/mL),
- gentamicin (1 µg/mL) and
- fungizone (2 µg/mL).

- for outgrowth of HEF cells the medium (GM) was supplemented with inactivated foetal calf serum (FCS) 7% and
- for maintenance medium (MM), FCS 2% was added.

B. Specimens

- Urine from kidney-transplant recipients, bone-marrow transplant patients and other immunologically suppressed patients attending the Nuffield Transplant Surgical Unit (NTSU) and Haematology Clinics were included in this study.

- Specimens of urine (10mL) were collected in sterile containers containing neomycin (500 µg/mL) and stored at 4°C until transported to the laboratory where they were kept at 4°C and processed within two hours of arrival.

Methods and results

A. Cell-culture technique

1. Method

- CTC cultures were prepared in 100 x 12mm glass tubes seeded with 5 x 10^5 HEF cells in 1mL GM.

- the following volumes of inoculum were added to the various HEF tubes:
  . 0.1 mL of urine,
  . 0.1 mL of 1:100 diluted urine, and
  . 1.0 mL of urine (in an attempt to increase the volume of the inoculum)

- one hour is allowed for absorption, following which the inoculum is replaced with 1mL GM.
- all HEF-cultures were placed in roller drums, incubated at 36°C and examined microscopically for typical CPE three times a week for a total of six weeks.

2. Results

The results of attempting to isolate CMV in culture are shown in Table 1 which indicates that 1 mL of urine was not satisfactory due to contamination and toxicity.

TABLE 1 Comparison of the cell culture results using varying inocula

Inoculum	Number Inoculated	Contaminated	Positive at 6 weeks
1 mL urine	113	27	*
0.1 mL urine	177	20	17
0.1 mL 1:100 urine	177	6	5

* Cells did not last 6 weeks

Contamination was still a problem with an inoculum of 0.1 mL although 17 of the 177 specimens (9.6%) did yield the virus. In an attempt to overcome the problem of contamination the urine was diluted o1:100 prior to inoculation. While this resulted in fewer contaminated tubes, and two previously contaminated specimens yielded the virus, the overall isolation rate fell from 9.6% to 2.8%, a decrease of 71%. The average time for CPE to develop was 18 days.

B. Shell vials (Coverslips) technique

I) Method

1. Growth of CMV

- Shell vials (SVs) were prepared by placing a sterile 13mm No. 3 glass coverslip in a 7 mL plastic bijou bottle
- 1 mL GM with 5 x 10^4 HEF cells was added and the SVs incubated at 36°C in 10% CO_2 in air
- after 48 hours of incubation the GM was aspirated and 1mL of undiluted urine was added to each of two shell vials

- the vials were then centrifuged at 700 g for one hour at room temperature (RT), the inoculum removed, the monolayer washed twice with Dulbecco's basal salt solution A
- then 1 mL MM was added and the vials were returned to the CO_2 incubator for a further 16 hours incubation
- thereafter, coverslips were removed from the shell vials, fixed in acetone for 15 minutes and air dried.
- the dried preparations were mounted on slides with DPX, allowed to harden and stored at -20°C until stained.

2. Staining technique

- all sera were diluted in 2% bovine serum albumin in phosphate buffered saline pH 7.2 (PBS). The optimal dilution of the reagents was determined by checkerboard titration using HEF cells inoculated with AD 169 strain of CMV as antigen.
- the coverslips were washed by immersion in PBS for 15 minutes with two changes of buffer during that time.

II) Detection techniques and results

1. Detection by immunofluorescence (IF)

- adopting the technique of Alpert et.al[2], fixed HEF monolayers on coverslips were covered with 30 µL of a dilution of anti-CMV monoclonal antibody (Mab), incubated and then washed
- thereafter they are reacted with 30 µL of a 1:50 dilution of biotinylated anti-mouse immunoglobulin (whole antibody) for one hour at room temperature and washed again, before being reacted with 30 µL of a 1:100 dilution of fluorescein avidin D (FITC) for 30 minutes at room temperature
- further washing is needed before the coverslips were mounted in a non-fading fluorescent mountant[3] and viewed on an incident UV microscope.

. Results

One hundred and seventy seven (177) urine specimens from 104 patients were examined by this IF technique and the results are shown in Table 2 below.

TABLE 2 Detection of CMV growth by CTC and IF in shell vials.

		CTC technique +	CTC technique -	Contaminated
Shell Vials	+	18	4	1
IF technique	-	1	136	17
TOTAL		19	140	18

Of the 177 specimens in CTC, 18 were contaminated (either with undiluted or diluted urine) and have had to be disregarded. The sensitivity of the IF reaction was 94.7% with a specificity of 97%.

2. Detection by immunoperoxidase (IP)

- this technique used the first antibody and antispecies antibody as above.
- after washing, 30 µL streptavidin biotinylated peroxidase were added and incubated for 30 minutes at room temperature before the preparation was washed
- 2 to 3 drops of substrate * was filtered onto the coverslip for five minutes before the latter was rinsed in tap water
  (* a freshly prepared solution of 5mg 3, 3'-diamobenzidine tetrachloride in 100 mL 0.1 M imidazole Tris HCl buffer pH 7.6 with 100 µL of 30% hydrogen peroxide)
- then counterstain with Harris' Haematoxylin was applied for 30 seconds and the coverslip dipped twice in 64% then 74% and then absolute alcohol.
- finally, the preparation was cleared by dipping twice in xylene before it was mounted in one drop of Histomount and examined at x 100 magnification by light microscopy.

. Results

One hundred and seventy seven (177) urine specimens from 104patients were examined by this IP technique and the results are shown in Table 3 below:

TABLE 3 Detection of CMV growth by CTC and IP in shell vials

		CTC technique +	CTC technique -	Contaminated
Shell Vials	+	19	2	1
IF technique	-	0	138	17
TOTAL		19	140	18

Of the 177 specimens in CTC, 18 were contaminated (either with undiluted or diluted urine) and have had to be disregarded. The sensitivity of the IP reaction was 100% with a specificity of 98.5%.

3. Comparative results

- of the 19 specimens positive for CMV by CTC, all were detected by IP but only 18 were positive by IF possibly indicating the greater sensitivity of the IP technique,

- of the 140 specimens negative for CMV by CTC, 4 were found positive by IF, 2 of which were confirmed by IP, possibly indicating the greater specificity of the IP technique.
- it has been reported that IF revealed rather dull, greenish inclusions.
- none of the reagents used for the IF or IP tests reacted non-specifically with either control or inoculated cells.
- the optimal dilution of the first antibody (anti-CMV monoclonal antibody - MAb) was
  . 1 in 5 when incubated for one hour, but was
  . 1 in 20 when the incubation time was extended to 18 hours at 4°C.

- the sensitivity of both the IF and IP detection system was examined by inoculation of a series of ten-fold dilutions of the AD 169 strain of CMV into shell vials:
  . when the inoculum size was 0.1 ml the viral antigen was demonstrated in cells inoculated with a 1:100 dilution of the stock,
  . when the inoculum size was 1.0 ml the viral antigen was demonstrated in cells inoculated with a 1:1000 dilution of the stock.

The IF and IP technique gave individual results.

- The specificity of the reaction was examined following inoculation of shell vials with
  . herpes simplex viruses types 1 and 2,
  . varicella gaster virus, and
  . adenovirus.

  All these viruses grew in the shell vials and could be detected by the homologous antibody. However, in no case did the MAb (anti- CMV monoclonal antibody) react with these viruses. Thus there was no cross-reaction between CMV and other members of herpes group or adenoviruses.

Discussion

The problem of contamination and toxicity has prevented most laboratories from using larger inocula of urine in cell culture:
- however, Alpert et.al[2] who used 25 cm^2 flasks of cells were able to use 1 ml of urine as inoculum and observed the cultures for 6 weeks. They did not lose any cell cultures due to contamination but were not so successful with shell vials.
- on the other hand, Morris et.al[1] reported that shell vials were significantly more likely to give a result than cell-culture. The present findings confirm this observation and in addition one specimen which contaminated the cell culture yielded CMV in shell vials and was detected by both IF and IP.

The MAb (anti-CMV monoclonal antibody) can be used to give a result in about 20 hours if it is used in a 1:5 dilution. However the test is equally effective if the MAb is diluted 1:20 and the incubation time for the MAb extended from one hour to 18 hours.

This is a convenient period for overnight incubation, so results can be available within 48 hours. Considering that this method only needs 2 days to produce results compared to an average of 18 days needed for cell-culture, the reduction in cost of the MAb by using the greater dilution would seem justified.

The apparent sensitivity in cell-culture is reported to be very much influenced by the length of time the cell-culture is observed (Table 4).

- Morris et al[1] pointed out that their results for the non-centrifuged cultures compared well with the 93% sensitivity of Swenson and Kaplan[4] if the cell-cultures were observed for only 14 days.
- however, if examination was continued for 28 days the sensitivity fell to 74%.

Comparison of the studies in which the cell-cultures were examined for at least 4 weeks suggests that:

- while Morris et al[1] had almost 100% specificity, the sensitivity they recorded ranged from 66% to 78% (Table 4 - Category C), unlike a sensitivity of 97% reported by Alpert etal[2] (Table 4 - Category E) which fell within the range of 94-100% for sensitivity observed in this study (Table 4 - Category D)
- in this study the inclusion of the avidin step in the IF test almost doubled the rate of virus antigen detection in shell vials. There was however, a possible problem with the IF test. Two specimens produced dull inclusions and subsequent specimens from these two patients were negative. As the inclusions were not defected by IP they may have been false positives.

Because the IP technique results in sharply defined, brown nuclear inclusions, the CMV easily detected. The sensitivity and specificity of the IP test was slightly better than that of IF. This characteristic of the IP together with the possibility that preparations can be retained for comparison and can be viewed by light microscopy makes this method currently the technique of choice for the rapid detection of CMV in culture.

CDI Editorial Note

The present study reported rapid diagnostic procedures using indirect immunofluorescence and immunoperoxidase for the detection of CMV in suspected specimens. Both techniques have been accepted in principle to provide rapid detection of CMV in specialised laboratories. However, for

TABLE 4 Comparison of the isolation and detection of CMV antigen from clinical specimens.

Category	Inoculum (ml)	Time Observed	Results Positive/ Total	Inoculum (ml)	Time Incubated (hours)	Centrifugal force (g)	Detection Method	Sensitivity	Specificity	Reference
A	0.2	14 days	30/190 (15.8%)	0.2	48	-	IP/EA	93%	96%	(4)
B	0.2	21 days	128/846 (15.1%)	0.2	24	700	IF/EA	96.9%	90.4%	(5)
	unknown	21 days	15/385 (3.9%)	0.2	24	-	IF/EA	80%	98.9%	(6)
C	0.2	4 weeks	42/304 (13.8%)	0.2	24	2 000	IF/EA	66%	99.6%	(1)
	0.2	4 weeks	42/304 (13.8%)	0.2	48	2 000	IF/EA	78%	99.2%	(1)
D	0.2	6 weeks	19/159 (12.0%)	1.0	16	700	BA/IF/EA	94.7%	97%	Present
	0.2	6 weeks	19/159 (12.0%)	1.0	16	700	BA/IP/EA	100%	98.5%	study
E	1.0	6 weeks	31/88 (35.2%)	1.0	24	4 000	BA/IF/EA	97%	90.7%	(2)

IP = indirect immunoperoxidase
IF = indirect immunofluorescence
EA = MAb to early Ag
IEA = MAb to immediate early Ag.
BA = Biotin avidin

consistent reproducibility of results these techniques should be performed and the results read by experienced technologists who are in the position to authoritatively distinguish between false positives and true positive specimens because of the possibility of high background readings in both techniques. The problem of false negatives cannot be adequately addressed by these limited studies.

It needs to be pointed out that in this study, the sensitivity (reported to be between 94% and 100%) and the specificity (reported to approximately 98%) are relative to the conventional tube cell-culture (CTC) and do not represent both techniques' absolute sensitivity and specificity relative to an acceptable gold standard. The sensitivity and specificity of the CTC technique itself depends on the type of cell line used, the cells viability and the cells susceptibility/resistance to CMV infection and the subsequent cytopathic effect as a function of time.

These tests would of value to clinicians if their respective predictive values can be calculated to indicate a ranking of their effectiveness as a diagnostic tool, taking into account the possible trade-off in terms of diagnostic time needed for the unequivocal detection of CMV positive specimens. Such time lag has reportedly been reduced to between 16 and 36 hours[7] for direct immunofluorescence assay using fluorescein-conjugated murine monoclonal antibody (MAb) reactive with CMV for the detection of CMV in centrifugation culture.

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AUSTRALIA - COMMUNICABLE DISEASES INTELLIGENCE

REPORTING PERIOD 5-10-87 TO 18-10-87 BULLETIN NUMBER 87/21
VIRAL IDENTIFICATIONS FROM CONTRIBUTING LABORATORIES

VIRUS OR VIRAL ANTIGEN	ICPMR (NSW)/ WVH (ACT)	RAHC (NSW)	PHH/ POW (NSW)	FAIR-FIELD (VIC)	RCH (VIC)	IMVS (SA)	STATE LAB (QLD)	STATE LAB (WA)	Total
0100 ADENOVIRUS NOT TYPED	5		3	2	9	1	7		27
0101 ADENOVIRUS TYPE 1				1					1
0102 ADENOVIRUS TYPE 2		1	1	1	1	3			7
0103 ADENOVIRUS TYPE 3				4				1	5
0105 ADENOVIRUS TYPE 5						1			1
0111 ADENOVIRUS TYPE 11				1					1
0126 ADENOVIRUS TYPE 26	2								2
0199 ADENOVIRUS TYPING PENDING					7				7
0201 INFLUENZA A VIRUS	4		1	6	2	16		10	39
0202 INFLUENZA A VIRUS SUBTYPE H3N2	5			4	7	1			17
0203 INFLUENZA B VIRUS	20		9	4	6	13	13	2	67
0206 INFLUENZA A VIRUS SUBTYPE H1N1					1		5		6
0301 PARAINFLUENZA VIRUS TYPE 1				1	1	2			4
0302 PARAINFLUENZA VIRUS TYPE 2						1	3		4
0303 PARAINFLUENZA VIRUS TYPE 3	2				6	8	17	5	38
0400 RESPIRATORY SYNCYTIAL VIRUS (RS)	4	3	5	11	22	4	9	2	60
0500 RHINOVIRUS (ALL TYPES)	2	1		1	5	3	11		23
0600 MYCOPLASMA PNEUMONIAE	15	1	7	11	4	7		4	49
0700 ORNITHOSIS-PSITTACOSIS			3					1	4
0816 COXSACKIEVIRUS A16	4				1				5
0902 COXSACKIEVIRUS B2					5	1			6
0903 COXSACKIEVIRUS B3							1		1
0905 COXSACKIEVIRUS B5				2					2
1015 ECHOVIRUS TYPE 15					1				1
1022 ECHOVIRUS TYPE 22	1				1	1			3
1100 POLIOVIRUS NOT TYPED			2		5				7
1101 POLIOVIRUS TYPE 1								1	1
1103 POLIOVIRUS TYPE 3								1	1
1200 MUMPS VIRUS				3	1				4
1300 HERPES VIRUS GROUP-NOT TYPED	10		4	4				4	22
1301 HERPES SIMPLEX VIRUS NOT-TYPED		2							2
1302 EPSTEIN-BARR VIRUS (EB VIRUS)	3		4	2	1	4		4	18
1303 VARICELLA-ZOSTER VIRUS	2		8	1		2	1		14
1306 HERPES SIMPLEX TYPE 1	13		11	40		19	54	26	163
1307 HERPES SIMPLEX TYPE 2	42		25	49		38	60	65	279
1399 HERPES VIRUS TYPING PENDING					6				6
1401 COXIELLA BURNETI	2							1	3
1502 PICORNA VIRUS-NOT TYPED	12		8				6		26
1521 MEASLES VIRUS			4	9	1				14
1522 RUBELLA VIRUS	6			5		1			12
1531 HEPATITIS B VIRUS								1	1
1532 HEPATITIS B ANTIGEN	22	3	10	21		15	21	14	106
1535 HEPATITIS A ANTIBODY	2		2	1		3	2	3	13
1541 CHLAMYDIA A - C TRACHOMATIS	16		1		1	60	21	37	136
1543 CHLAMYDIA A - LGV TYPE	2								2
1556 CMV - CYTOMEGALOVIRUS	3	1	7	33	4	4	14	12	78
1564 ROTAVIRUS	17	1	8	3	11	25	16	2	83
1565 CALICI VIRUS	2								2
1566 NORWALK AGENT		1			1				2
1599 ENTEROVIRUS TYPING PENDING		4	2		2				8
9992 ROSS RIVER VIRUS				1				4	5
9994 SMALL VIRUS (LIKE) PARTICLE				1					1
Total	218	18	125	222	112	233	261	200	1,389

AUSTRALIA - COMMUNICABLE DISEASES INTELLIGENCE

PERIOD : 5-10-87 TO 18-10-87 BULLETIN NO 87/21
Viral Identifications by Clinical Information Table 1.
Code 00,99 -No ill or data; 01,02,11,12 -Respiratory; E3 -Encephalitis; M3 -Meningitis; 04 -Paralysis; 05,13 -CNS other unspec.; 07,49 -GI; 17,47 -Hepatic; 19 -CVS; 89 -Urinary; 06 -Skin/mucous.

VIRUS OR VIRAL ANTIGEN	No-ill or data	Respiratory	Encephalitis	Meningitis	Paralysis	CNS other unspec	GI	Hepatic	CVS	Urinary	Skin/mucs memb
0101 ADENOVIRUS TYPE 1.....		1									
0102 ADENOVIRUS TYPE 2.....		3									
0103 ADENOVIRUS TYPE 3.....		2					1				
0105 ADENOVIRUS TYPE 5.....		1									
0111 ADENOVIRUS TYPE 11.....										1	
0126 ADENOVIRUS TYPE 26.....							1				1
0201 INFLUENZA A VIRUS.....		27				3	1				2
0202 INFLUENZA A VIRUS SUBTYPE H3N2		13									
0203 INFLUENZA B VIRUS.....	4	48							3		
0206 INFLUENZA A VIRUS SUBTYPE H1N1		6					1				
0301 PARAINFLUENZA VIRUS TYPE 1....		4									
0302 PARAINFLUENZA VIRUS TYPE 2....		4									
0303 PARAINFLUENZA VIRUS TYPE 3....		35		1				1			
0400 RESPIRATORY SYNCYTIAL VIRUS (RS).....	1	56									
0500 RHINOVIRUS (ALL TYPES).....		1									
0600 MYCOPLASMA PNEUMONIAE.....	11	34				1					1
0816 COXSACKIEVIRUS A16.....	1										4
0902 COXSACKIEVIRUS B2.....		4		2							
0903 COXSACKIEVIRUS B3.....		1									
0905 COXSACKIEVIRUS B5.....	1								1		
1015 ECHOVIRUS TYPE 15.....		1									
1022 ECHOVIRUS TYPE 22.....		1					2				
1200 MUMPS VIRUS.....	1										
1300 HERPES VIRUS GROUP-NOT TYPED..											1
1301 HERPES SIMPLEX VIRUS NOT-TYPED											1
1302 EPSTEIN-BARR VIRUS (EB VIRUS).	6	5				1					
1303 VARICELLA-ZOSTER VIRUS.....	4	1									6
1306 HERPES SIMPLEX TYPE 1.....	6	8	1					1		2	117
1307 HERPES SIMPLEX TYPE 2.....	6										86
1401 COXIELLA BURNETI.....	1	1									
1502 PICORNA VIRUS-NOT TYPED.....	1										
1521 MEASLES VIRUS.....	5	1					1				4
1522 RUBELLA VIRUS.....	1										3
1532 HEPATITIS B ANTIGEN.....	43			1				56		1	
1535 HEPATITIS A ANTIBODY.....	2							10			
1541 CHLAMYDIA A - C.TRACHOMATIS...	7										
1543 CHLAMYDIA A - LGV TYPE.....		1									
1556 CMV - CYTOMEGALOVIRUS.....	6	21			1	2	2	1		4	1
1564 ROTAVIRUS.....							83				
1565 CALICI VIRUS.....							2				
1566 NORWALK AGENT.....		1									
9992 ROSS RIVER VIRUS.....	1										
9994 SMALL VIRUS (LIKE) PARTICLE...							1				
Total.....	108	281	1	4	1	7	95	69	4	8	227

AUSTRALIA - COMMUNICABLE DISEASES INTELLIGENCE

PERIOD : 5-10-87 TO 18-10-87 BULLETIN NO 87/21

Viral Identifications by Clinical Information Table 2.
Code 10 -Eye; 59 -Genital; 39 -Endo/sal gland;
38 -RES; 29 -Muscle/joint; 69 -Congenital; P8 -PUO;
G8 -Fever/malaise; 09 -Other; A1 -SIDS ...

VIRUS OR VIRAL ANTIGEN	Eye	Genital	Endo/sal gland	RES	Muscle/joint	Congenital	PUO	Fever/malaise	Other	SIDS
0101 ADENOVIRUS TYPE 1.....								1		
0102 ADENOVIRUS TYPE 2.....			1				1	1		1
0103 ADENOVIRUS TYPE 3.....	3									
0201 INFLUENZA A VIRUS.....				2			1	5	4	
0202 INFLUENZA A VIRUS SUBTYPE H3N2					1		2	1		
0203 INFLUENZA B VIRUS.....				2	2		5	5	1	
0303 PARAINFLUENZA VIRUS TYPE 3....								1		1
0400 RESPIRATORY SYNCYTIAL VIRUS (RS).....								5	1	
0600 MYCOPLASMA PNEUMONIAE.....						1			2	
0700 ORNITHOSIS-PSITTACOSIS.....		1						1	2	
1101 POLIOVIRUS TYPE 1.....										1
1103 POLIOVIRUS TYPE 3.....										1
1200 MUMPS VIRUS.....			2							
1301 HERPES SIMPLEX VIRUS NOT-TYPED									1	
1302 EPSTEIN-BARR VIRUS (EB VIRUS).			4				1	1	1	
1303 VARICELLA-ZOSTER VIRUS.....			2						1	
1306 HERPES SIMPLEX TYPE 1.....	4	22						1	3	
1307 HERPES SIMPLEX TYPE 2.....		186						1	1	
1401 COXIELLA BURNETI.....							1			
1502 PICORNA VIRUS-NOT TYPED.....							1		1	
1521 MEASLES VIRUS.....							2	2	1	
1522 RUBELLA VIRUS.....			1		2				5	
1532 HEPATITIS B ANTIGEN.....		1							4	
1535 HEPATITIS A ANTIBODY.....									1	
1541 CHLAMYDIA A - C.TRACHOMATIS...	1	126			1	1				
1543 CHLAMYDIA A - LGV TYPE.....							1			
1556 CMV - CYTOMEGALOVIRUS.....	4	2	1			11	3	5	22	2
1566 NORWALK AGENT.....									1	
9992 ROSS RIVER VIRUS.....					4					
Total.....	12	338	11	4	10	13	18	30	52	6