

CF. They suggest that chronic lower airway infection with *P. aeruginosa* is associated with significant morbidity and mortality among CF patients. However, they also suggest that first acquisition of *P. aeruginosa* does not appear to cause an immediate and rapid decline in lung function, as early isolates are generally non-mucoid, antibiotic-sensitive and present at low densities. This suggests a possible window of opportunity for early intervention.

To date, there have been no reports on the bacterial composition of cosmetic products. Furthermore, the survival dynamics of problem Gram-negative pathogens in cosmetics are not known. Thus, further work is required to determine the survival and persistence of Gram-negative pathogens, including *P. aeruginosa* and *B. cepacia*, in these matrices. Until such studies report, it is recommended that patients with CF avoid sharing their cosmetic products among each other, as a precautionary measure, to help eliminate the potential for cross-infection with these pathogens.

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Potentially misleading Western blot results in Lyme disease diagnosis

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The laboratory diagnosis of Lyme disease is complex¹ and serology remains the technique of choice. Recommended practice is a two-step process involving a sensitive screening enzyme immunoassay (EIA) followed by a more specific confirmatory Western blot for all EIA-positive and equivocal samples and for EIA-negative samples with a high clinical suspicion of Lyme disease (e.g., tick bite and erythema migrans).^{1,2} However, Western blot results require careful interpretation.

The National Lyme Disease Testing Service Laboratory,

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Raigmore Hospital, Inverness, tests over 3000 samples annually from across Scotland, many of which are from complex clinical cases. It was noted recently that serum from a patient with confirmed parvovirus B19 infection cross-reacted with the in-house *Borrelia burgdorferi* IgG Western blot. This could lead to the wrong interpretation of Western blot results. This study aims to discover if other viral infections produce similar results with the *B. burgdorferi* IgG Western blot.

The study group consisted of six patients found to contain IgM antibodies to parvovirus B19 infection (parvovirus B19 IgM EIA, Biotrin International), six with cytomegalovirus (CMV) IgM (CMV IgM EIA, Microgen Bioproducts) and six with Epstein-Barr virus (EBV) IgM (EBV VCA IgM EIA, Diasorin). All patients had good clinical evidence of viral infection (Table 1).

The 18 serum samples were analysed by commercial *B. burgdorferi* IgG/IgM EIA (Zeus Scientific, New Jersey, USA) and an in-house *B. burgdorferi* IgG Western blot. The EIA was performed according to the manufacturer's instructions and the Western blot was performed as described previously.²

The blots used contained sodium dodecyl sulphate-extracted *B. burgdorferi* (i.e., antigen). A commercial positive control (Zeus Scientific) and in-house positive control, with defined band patterns, were included in each blot run. The number, intensity and molecular weight of bands observed with each sample were recorded, and a negative, equivocal, weak positive or positive result assigned according to predetermined criteria.³ The Western blot was repeated for all samples.

Previous or subsequent serum samples from five patients (serum samples 5, 8, 9, 11 and 12 [Table 1]) who reacted with the *B. burgdorferi* IgG Western blot were also tested by the *B. burgdorferi* IgG Western blot to determine *B. burgdorferi* status.

All samples tested were EIA negative (Table 1). However, as the infections studied may produce clinical symptoms similar to Lyme disease (such as a rash), a Western blot may be performed on EIA-negative samples, based on clinical suspicion.^{2,3} Western blot may also be performed on EIA-negative samples if they have been referred from another laboratory for confirmation.

Some of the polypeptides from a *B. burgdorferi* extract may react with patient antibodies that are not specific to *B. burgdorferi*.⁴ These include the 41 kDa flagellin polypeptide, which, although it must be detected for a positive diagnosis, is known to cross-react with antibodies to proteins from other bacteria.^{4,5} Other polypeptides in this category include those of 44, 56, 62, 64, 72 and 82 kDa. In contrast, bands with 18, 22, 32, 34, 39, 46, 58 and 92 kDa polypeptides are generally accepted as specific for *B. burgdorferi*.^{1,6}

Three of six parvovirus B19 IgM-positive serum samples reacted with specific *B. burgdorferi* antigens and the 41-kDa antigen (Table 1). The antibody response to parvovirus B19 is directed mainly against the two structural proteins of the viral capsid (VP1 [83 kDa] and VP2 [58 kDa]).^{7,8} Although the molecular weights of these polypeptides are different from the *B. burgdorferi*-specific bands detected here (Table 1), they may share sufficient homology at particular epitopes for parvovirus B19-specific antibody to bind with *B. burgdorferi*-specific polypeptides.

Five of six CMV-IgM positive sera produced equivocal or

Table 1. *Borrelia burgdorferi* IgG/IgM EIA and IgG Western blot results on serum samples from patients with good evidence of parvovirus B19 (n=6), CMV (n=6) and EBV (n=6) infection.

| Organism | Serum | Clinical information | Western blot | | |
|----------------|-------|---|--------------|----------------|-------------|
| | | | 41 kDa band | Specific bands | Result |
| Parvovirus B19 | 1 | Rash on body, muscle aches | Yes | 0 | Negative |
| | 2 | Arthropathy, abnormal LFT's | Yes | 92 | Equivocal |
| | 3 | Viral symptoms, reactive arthropathy | Yes | 0 | Negative |
| | 4 | Viral infection with arthralgia | Yes | 0 | Negative |
| | 5 | Generalised macular, confluent rash for 24 h | Yes | 34 kDa | Equivocal |
| | 6 | Inflammatory arthritis | Yes | 32 kDa | Equivocal |
| CMV | 7 | Pyrexia ?neutropenia | Yes | 0 | Negative |
| | 8 | ?CMV, epigastric discomfort | Yes | 34 kDa | Equivocal |
| | 9 | Unwell for past month, malaise | Yes | 39 kDa | Equivocal |
| | 10 | Viral illness, increased ALT, low WCC | Yes | 34 kDa | Equivocal |
| | 11 | Pneumonitis, respiratory failure, pericardial and pleural effusions | Yes | 58, 92 kDa | Wk positive |
| | 12 | Sore throat, recurrent glandular swelling, TATT | Yes | 92 kDa | Equivocal |
| EBV | 13 | ?Glandular fever | Yes | 0 | Negative |
| | 14 | Previous CFS. Persistently EBV+, now asymptomatic | Yes | 0 | Negative |
| | 15 | TATT. Previous infectious mononucleosis | Yes | 0 | Negative |
| | 16 | Recent glandular fever | No | 0 | Negative |
| | 17 | Ongoing pharyngitis | Yes | 0 | Negative |
| | 18 | Cervical lymphadenopathy | Yes | 0 | Negative |

weak positive *B. burgdorferi* Western blot results (Table 1). The individual structural proteins of CMV recognised by sera from IgG- and IgM-positive patients are 155, 149, 82.5, 74.5, 67, 57, 55, 38.5 and 28 kDa polypeptides.⁹ The 38.5 and 57 kDa polypeptides are of similar size to the 39 and 58 kDa polypeptides of *B. burgdorferi*, but there is no information about their homology. Other shared epitopes are suggested as the CMV IgM-positive sera also detect 34 and 92 kDa polypeptides on the *B. burgdorferi* blot.

In one case (sample 11, Table 1) the result was a weak positive. Although concurrent *B. burgdorferi* infection cannot be ruled out in the patients with parvovirus B19 and CMV infection whose sera cross-reacted, the *B. burgdorferi* IgG Western blot results from available previous or follow-up sera (samples 5, 8, 9, 11 and 12, Table 1) suggest that none of the patients had current or previous *B. burgdorferi* infection.

None of the EBV IgM-positive serum samples were equivocal or positive by *B. burgdorferi* IgG Western blot. This was surprising as there is excessive production of antibodies during EBV infection and cross-reactions are observed frequently in tests for other infections. However, it confirms the lack of cross-reactions observed when Engstrom *et al.* tested sera from EBV patients with their *B. burgdorferi* IgG Western blot.¹⁰

The findings presented here highlight the problems with a Western blot result. Western blot needs to be sensitive to avoid false-negative results, particularly in early Lyme disease. Most cross-reactions in this study produced equivocal results, which, although inconclusive, are beneficial as repeat samples are requested automatically in order to help confirm or refute the presence of infection. A repeat equivocal result means that the clinical picture would have to be closely considered. The weak positive result

obtained with CMV was more problematic. Knowledge of these cross-reactions enables us to better advise our users, and emphasises the need for good clinical information in the interpretation of laboratory results.

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