method for *S. aureus*. However, some strains have been untypeable because of low amount of SC expressed from bacterial cells or presence of putative novel or variant types. Recently, gene sequences of SC defining SC serotypes I-X were determined and divergent portions in SC genes were clarified. We found also novel SC genotypes, XI and XII. In the present study, based on the findings of SC genes, a novel genetic typing of SC (gene) types by PCR assay was developed.

Methods: Two PCR reactions 1 and 2 were designed. In both reactions, a common primer which is complementary to 5′-end conserved region of SC gene is included. Reaction 1 contains SC serotypes I through VI-specific primers, and reaction 2 contains SC serotypes VII, VIII, and X-specific primers. SC serotype-specific primers were mostly complementary to D1 or D2 region sequences which are divergent among different SC serotypes. Depending on the SC serotype, only a single PCR product with the size specific to each SC serotype is amplified in either the reaction 1 or 2. When no product is amplified, an additional PCR reaction (reaction 3) is carried out to determine SC serotype IX or genotype XI. When type IV was assigned by reaction 1, an additional reaction (reaction 4) is performed to discriminate between SC serotype IV and genotype XII.

Results: With the established SC types I-XII *S. aureus* strains, a single PCR product with the specific size to each type was amplified. When 63 *S. aureus* strains isolated during a period between 1993 and 2003 which were untypeable serologically were tested by this genetic typing method, about 90% of the strains were successfully typed. When 117 *S.aureus* strains isolated in 2008 were examined, SC types were identified for 96% of the strains.

Conclusion: The PCR assay established in this study could assign SC types for *S. aureus* accurately with high determination rate, and was considered to be useful for epidemiologic characterization of *S. aureus* clinical isolates.

doi:10.1016/j.ijid.2010.02.416

75.017

Serological diagnosis of lyme disease in Valencia (Spain)

M.T. Fraile Fariñas[1,*], J.L. Ramos Marti[2], N. Tormo Palop[2], C. Gimeno Cardona[2]

[1] *Hospital General Universitario de Valencia, Valencia, Spain*
[2] *CHGUV, Valencia, Spain*

Lyme borreliosis (BL) is the tick-borne disease with the highest incidence worldwide. Its geographical distribution shows a pattern of ''endemic areas'', where infected **Ixodes ricinus** in enough density to have an epidemiological impact. In Spain, it is a low incidence process although the problems inherent in their clinical and laboratory diagnosis are probably due to an underdiagnosis. Studies are needed to supplement information on the distribution of the etiologic agent in different areas of our country, which are not considered endemic but where their presence is suspected.

Methods: From 1 January 2008 to August 31, 2009, 1422 samples were analyzed in patients from the influence area of the Valencia University General Hospital with clinical Sorin Borrelia IgG) for the quantitative determination of IgG antibodies. We used as a confirmatory test the Western blot (European Borrelia plus Virotech), considering positive findings, the presence of at least two bands of the following: p83/100, BmpA (p39), Ospc (p23), DbpA or vice-Mix -Mix. Indeterminate results were considered in the presence of a band; p83/100, BmpA (p39), OspC (p23), DbpA or vice-Mix-Mix. In all samples detection of antibodies to Treponema pallidum was performed. Positive results were found by the screening test in 137 samples (9.63%), corresponding to 105 patients. The Western blot showed positive results in 39 samples of 28 patients, indeterminate in 16 samples from 12 patients and negative in 82 samples. The 39 positive samples were from patients (28) from the services of Neurology $N = 16$, Internal Medicine $N = 13$, $N = 6$, $N = 4$ Dermatology and Psychiatry. Luetic serology were negative in all samples. In 8 patients administered ceftriaxone 2 mg/día for three weeks with good clinical and serological survey.

Conclusion: Our diagnostic methods, show a group of patients with specific characteristics and clinical outcome, and support the existence of probable cases of Lyme borreliosis. Screening methods have high sensitivity but are not very specific, because only 28 (26.6%) of 105 patients tested positive by Western blot.

doi:10.1016/j.ijid.2010.02.417

75.018

Multicenter evaluation of Ziehl-Neelsen bleach sedimentation method for diagnosis of smear negative tuberculosis in Kenya

S. Matu*, L. Muita, E. Juma

Kenya Medical Research Institute, Nairobi, Kenya

Background: World Health Organization (WHO) recommends direct smear microscopy using Ziehl-Neelsen (ZN) technique for tuberculosis (TB) case finding in resource poor settings. This method has a low sensitivity. Bleach methods have been shown to increase sensitivity in some settings. However, no evaluation has been carried out in settings where TB burden is high and improved diagnosis is desperately needed.

Objective: To evaluate the ZN bleach sedimentation technique for diagnosis of smear negative tuberculosis in peripheral canters.

Methods: 1122 direct ZN smear negative sputum specimens from new TB suspects attending three peripheral health centers in Nairobi were collected. At each center, sputum specimens were homogenized then divided into two equal portions. One portion was processed for culture. The other portion was treated with 3.5% bleach. The treated specimens were kept at room temperature and left overnight for at least 15 hours. Smears were prepared from the deposit and examined using ZN method.

Results: Of the 1112 smear negative specimens, 968 were analyzed. Of these, 84(8.7%) were culture positive**.** Of these, 23 (27.3%) were both culture and smear positive. The sensitivity was (27.4%) and specificity of (98.4%) with a PPV and