

The raw data from the plate, i.e., absorbance at 450/620 nm, were plotted into histograms and graphs. The relative specific binding was quantified by the signal to noise (S/N) ratio which was calculated by dividing the mean absorbance, i.e., absorbance at 450/620 nm, of test sera with the mean absorbance of negative sera, and plotted. The S/N ratio was directly proportional to the specific antibody reactivity in ELISA. A batch of n=22 endemic sera from North Africa were evaluated and resulted with 100% specificity and sensitivity, 99.99% PPV (positive predictive values) and 95.45% NPV (negative predictive values).

The LabSystem plate did not perform well in this study. There was no discrimination between the positive and negative sera. There was a clear distinction between sera in the Greiner plate. It was found that the PBS+methyl glyoxal, phosphate buffer and culture medium respectively showed higher reactivity with positive sera and relatively less reactivity with negative sera. A higher S/N ratio was seen using sera at a 1:500 sera dilution. Phosphate buffer, culture medium and PBS-glyoxal gave higher S/N ratios. Therefore, the Greiner plate and PBS+methyl glyoxal were selected as ELISA plate and coating buffer respectively.

Using the Greiner ELISA plate and PBS+methyl glyoxal as the coating buffer for the test, positive and negative sera were used at 1:500 dilution. Two anti-human IgG-HRP conjugates were evaluated at 4 different dilutions. The PAb conjugate was found to be highly reactive with the negative sera indicating a high level of non-specific reaction. On the other hand, the same level of reactivity was observed in the blank and negative sera sample wells with the MAb conjugate. The reactivity with MAb conjugate appeared to be more specific with positive sera as evidenced by higher S/N ratios.

Negative and positive sera were reacted at 1:500 dilution and washed with two different wash solutions. MAb conjugate was used at 1:8000 dilution. Plates were read at 450/620 nm. Reactivity of blanks and negative sera were lower in wells washed with PBS/T+0.2M NaCl. The data indicated that PBS/T+0.2M NaCl was more effective in removing non-specific binding (nearly 45% reduction of non-specific signal 0.283 v. 0.114) and increasing the S/N ratio from 3.7 to 7.2. Despite its higher S/N ratio, 0.2M NaCl was left out of the final wash buffer because it formed a precipitate on standing.

Having selected the Greiner plate and PBS+glyoxal as the coating buffer, optimum levels of antigen and appropriate blocking reagents were investigated. Wells were coated with a series of antigen concentration from 1.25 µg/ml to 40 µg/ml. Two blocking reagents, 0.4% gelatin and 0.5% casein prepared in distilled water were evaluated. Positive and negative sera, diluted 1:500 in PBS/T, reacted in the Ag-coated and blocked wells for 1 hour at room temperature. Two conjugates, PAb and MAb-conjugates, diluted 1:4000 in PBS/T were added to wells and incubated for 30 minutes at room temperature. The color was developed for 25 minutes by adding the substrate and immediately read after addition of stopping solution. The reactivity was higher in blank and negative sera wells with PAb conjugate thereby reducing the differences between samples that resulted with a low S/N ratio. With the MAb conjugate, the absorbance of blank and negative sera wells was almost equal. There was a pattern in the reactivity relative to the antigen concentration. Wells reacted with the positive sera showed a gradual rise in absorbance dependent upon the antigen concentration. On the whole, MAb conjugate reactivity was relatively lower in control sample wells. The S/N ratios were higher with MAb conjugate when casein used for blocking MAb conjugate with casein blocking generated excellent S/N ratios, at 20 and 40 µg/ml antigen levels.

Experiments involving the relative kinetics of antibody reactivity at different sera dilutions provided a good discrimination at 1:500 dilution. This formed the basis for future assays.

After optimizing assay steps, the following protocol was followed for evaluating test sera samples of subjects from endemic areas. In short, the wells were coated with 50 µg/ml soluble antigen mixed in PBS-methyl-glyoxal buffer overnight and after removing the antigen, the wells were blocked with 0.5% casein for 1 hour at room temperature. Test serum along with control sera diluted 1:500 in PBS/T, was reacted for 1 hour at room temperature. After washing the plate in PBS/T, the wells were reacted with MAb conjugate at 1:8000 dilution for 40 minutes at room temperature and after washing, the TMB substrate was added and color development was allowed to proceed for 25 min and then stopped with the stop solution.

Absorbance was read at dual filter (450/620 nm) and the results were analyzed. A total of n=22 test clinical sera obtained from the endemic areas of North Africa were evaluated along with n=5 reference control negative sera.

SDS-PAGE analysis was conducted. Coomassie staining showed several major bands with approximate molecular weights of 11, 30, 42, 50 and 161 kDa. In addition to these abundant bands, silver staining revealed more distinct protein bands of approximately 6, 15, 17, 22, 58, and 107 kDa. This illustrates that the test contained a variety of protein antigens.

The cut-off value in the current assay was mean +3 SD of negative (n=5) sera (Mean 0.1304, SD=0.042), i.e., 0.278 which is rounded off to 0.300. Using an absorbance OD450/620 nm of 0.300 as the cut off, n=22 test sera from field were categorized as either positive or negative. With the exception of one sample, all were positive. The sensitivity, specificity, PPV and NPV were calculated. Thus, the sensitivity and specificity were both 100%, the PPV was 99.9% and NPV was 95.45%. The resultant S/N ratio of these samples suggests that the assay is highly sensitive and specific.

Clearly, one of ordinary skill in the art may further optimize the assays of the invention by changing various assay conditions by methods standard in the art.

To the extent necessary to understand or complete the disclosure of the present invention, all publications, patents, and patent applications mentioned herein are expressly incorporated by reference therein to the same extent as though each were individually so incorporated.

I claim:

1. A method of obtaining a cellular product from a cell or a tissue comprising cultivating the cell or the tissue in a protein free medium containing (a) an oncotic agent that balances the oncotic pressure across a semi-permeable cell membrane, and (b) at least one of the following ingredients: Hepes buffer, L-glutamine and sodium bicarbonate without phenol red.
2. The method of claim 1, wherein the cellular product is secreted by the cell into the protein free medium.
3. The method of claim 1, wherein the cell is a hybridoma, a beta-islet cell, a lymphocyte, a monocyte, a macrophage, a fibroblast, a stem cell, or an endothelial cell.
4. The method of claim 1, wherein the oncotic agent is sucrose, polysucrose, sorbitol or D-xylose.
5. The method of claim 1, wherein the protein free medium further comprises RPMI Medium 1640, Hepes buffer, L-glutamine, and sodium bicarbonate without phenol red.
6. The method of claim 1, wherein the protein free medium is RPMI Medium 1640 comprising D-xylose at 0.076 mM, Hepes buffer at 25 mM, L-glutamine, and sodium bicarbonate at 30 mM without phenol red.