CHAPTER 3

RESULTS

"Reality provides us with facts so romantic that imagination itself could add nothing to them"

Jules Verne
3.0 RESULTS

3.1 Quality control

![Graph showing OD at 405nm for control pins](image)

**Figure 3.1 Control pins.** This compares the peptides synthesized manually (label “1”) and those supplied by the manufacturer (label “2”) when reacted with the monoclonal antibody directed towards the peptides PLAQGGGG. The white bars indicate the positive control pins (PLAQ) and the black bars indicate the negative control pins (GLAQ).

3.2 Optimization of the Modified ELISA Assay

3.2.1 Effect of incubation time of pins in substrate on assay.

3.2.2 Effect of removal of the bound antibody (“Disruption”) on assay
Figure 3.2 Effect of incubation time of the pins in the chromogenic substrate on the Optical Density (O.D.). There is a definite increase in O.D. when the incubation time is increased from 10 minutes, to 20 minutes, then to 30 and subsequently to 40 minutes.
Figure 3.3 Effect of Disruption on Optical Density of peptides.
There is a marked decrease in O.D. values after the first cycle of disruption, and also after the second cycle of disruption. These two cycles are important because they remove antibodies to the peptides (on pins) before a fresh assay is done with a new serum.
3.3 Reactivities of individual sera

The pins on peptides were screened with individual sera, with 2 cycles of disruption in between the screenings to ensure minimal residual antibody binding to the pins after each assay.

The sera screened include sera from culture-confirmed cases of typhoid fever, sera from healthy blood donors and one commercially synthesized monoclonal antibody to GroEL (StressGen, Vancouver, British Columbia). The typhoid sera include those labelled “sera 016, sera019, sera 020, sera 022, sera 024, sera 025, sera 031, sera 034, sera 041, sera 049, sera 69159, sera 84650, sera 84933, sera M148, sera M265 and sera M353”. The normal sera (healthy blood donors) carry the labels “SD28/2, Norm LKY19/2, Norm-18/2 and 26552”. The monoclonal antibody is labelled as it is. Appendix 2 details the above sera.

The reactivities of each of these sera are shown in Figures 3.4 - 3.11. In each of the individual graphs, the Optical Density is plotted for each of the 9-mer peptides synthesized, which are shown numbered from the N-terminal residue (Appendix 3 lists the peptides synthesized). In each figure, reactivities of 2/3 typhoid sera are displayed in comparison to that of a normal serum.
Figure 3.4 Sera 16 and 19 (typhoid positive) compared to a normal serum (26552)
Figure 3.5 Sera 20 and 22 (typhoid sera) compared to “NormLKY-18-2” (normal)
Figure 3.6 Sera 24 and 025 (typhoid sera) compared with sera “norm-SD” (normal)
Figure 3.7 Sera 031 and 034 (typhoid) compared with “LKY19-2” (normal)
Figure 3.8 Sera 041 and 043 compared with the monoclonal antibody to GroEL
Figure 3.9 Sera 049, 69159 and 84650 (all typhoid sera)
Figure 3.10  Sera 84993, M148 (typhoid sera) compared with sera “norm-18/2” (normal).
Figure 3.11  Sera M265 and sera M353 (both typhoid sera).
Interpretation of Figs 3.4-3.11

Figures 3.4 -3.11 show the reactivities of the peptides are generally higher (O.D.s more than 1.0) in the typhoid sera when compared to that of the normal sera. This is shown quite clearly in Figures 3.4- 3.7 and Figure 3.10. There is also a generally high "background" and this complicates the identification of definite peaks. When analyzed carefully, there appears to be a common peak in the absorbance of the typhoid sera in 3 regions, at N-terminal residues 105, 190 and 200, respectively, which is not present in the normal sera. The monoclonal antibody gave high reactivities from N-terminal residue 205-. To identify with certainty the peptides which gave significantly higher signals above the background reactivities, a consensus plot algorithm was used.
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3.4 Consensus Plot

Figure 3.12 Consensus plot of binding of synthethic peptides to human sera and to monoclonal antibody against GroEL. The plot covers N-terminal residues 1-151 among the synthesized peptides. All sera tested have been plotted and the values taken are the average of the duplicate assays. The titer (top) is defined as the inverse dilution which would give an absorbance of > 1 above the background. Each square represents an individual serum sample. The bars shown in the titer output show the range between the sera values. The middle plot shows the number of sera, where the empty columns indicate indefinite values above the background and filled columns indicate statistically significant values above the background. The region covering the immunodominant peptides (EGQDRGYSY, YSYNKETGE and GKGTEEKEK) is indicated by the solid line below the N-terminal residue number.

SD28/2, Norm LKY19/2, Norm18-2 and 26552 represent normal sera; M-Ab represents monoclonal antibody against GroEL, all other sera are sera from typhoid patients.
Figure 3.13 Consensus plot of binding of synthetic peptides to human sera and to monoclonal antibody against GroEL. The plot covers N-terminal residues 151-245 among the synthesized peptides. All sera tested have been plotted and the values taken are the average of the duplicate assays. The titer (top) is defined as the inverse dilution which would give an absorbance of > 1 above the background. Each square represents an individual serum sample. The bars shown in the titer output show the range between the sera values. The middle plot shows the number of sera, where the empty columns indicate indefinite values above the background and filled columns indicate statistically significant values above the background. The region covering the immunodominant peptides (EGQDRGYSY, YSYNKETGE and GKGTEEKEK) is indicated by the solid line below the N-terminal residue number.

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3.5 ELISA with purified GroEL protein

Figure 3.14 O.D. readings at 405nm with GroEL as antigen in a simple ELISA assay. S1, S2, S3, S4, and S5 are sera from culture-confirmed cases of typhoid fever. N1, N2, N3 and N4 are sera from healthy blood donors. PC (positive control) refers to the reactivity seen with the commercially available monoclonal antibody to GroEL of *E.coli*. From the bar chart above, it is clear that the P/N ratio of the typhoid sera as compared to the normal sera is greater than 2. It should be noted that the positive control gives a very high reactivity with GroEL.