Conclusion
The global incidence of tuberculosis is increasing as indicated by the present scenario of the newly appearing cases with multidrug resistant strains and dominant cases reappearing with the resurgence of AIDS/HIV infection. Chemotherapy alone is unable to control tuberculosis, therefore development of a vaccine is of paramount importance since status of BCG has become all the more controversial in tuberculosis control. Live integral vaccines have there own drawbacks hence some purified molecules need to be identified for vaccination and for diagnosis of tuberculosis.

Recently, secretory proteins of mycobacteria- *M. tuberculosis* and BCG have been studied for developing them as vaccine and much hope has focused on these proteins.

CDRI has identified one strain of mycobacterium- *M. habana*, which at present has been given the status of a Vaccine candidate for leprosy and tuberculosis. In the present investigation, Secretory Proteins of the same strain have been studied for their immune, antibody and cell-mediated responses with a prelude to develop them as a vaccine for tuberculosis.

The secretory proteins of this mycobacterium was prepared from the liquid medium culture filtrate employing modified Sauton's medium. Mid-logarithmic phase growth filtrate has been selected for all the studies since late-log growth filtrate is contaminated with autolytic products and early-log growth filtrate is scanty in protein content.
Conclusion

Mid-log culture filtrate of *M. habana* of 10th day was selected on the basis of several parameters and this filtrate was used for all studies described here. The filtrate was concentrated 10 fold and was precipitated with TCA, ammonium sulphate and acetone. In SDS-PAGE, the resolution of proteins of these preparations indicated similar migration patterns. There was no perceptible difference and, for ease and rapidity, 10th day concentrated culture filtrate was used for protection and cell-mediated immune responses and diagnostic studies.

Secretory proteins (SP), SP+FIA and *M. habana* whole cell vaccines were tested in mice separately as vaccine preparations alongwith a fourth group as unvaccinated control. *M. tuberculosis* H37RV was used as a challenge organism. Eight parameters were included to study the protective efficacy. They were comprised of general appearance, weekly body weights, postmortem lesions, weight of visceral organs, histopathological observations of lungs, viable count of AFB in visceral organs, percent survival time and mean survival time. The study led for 60 day observation period.

On the basis of results obtained by these parameters it is evident that all the three preparations - the SP, the SP+FIA and *M. habana* whole cell vaccines have afforded protection against experimental tuberculosis of mice. Since they have increased the survival time and have reduced number of live AFB in autopsied organs and have healed lesions in tissues of vaccinated mice than control. The *M. habana* vaccine had an edge over SP and SP+FIA.

The cell-mediated immune parameters of SP were also studied alongwith heat killed *M. tuberculosis* and live BCG in guinea pigs. Two sensitins were used, the PPD and SP. The CMI response was tested in both *in vitro* and *in vivo* conditions. In the *in vitro* test the primed lymphocytes had acquired sufficient lymphoproliferative property against these sensitins. The homologous and heterologous responses have indicated common antigens in these mycobacteria.

In the *in vivo* tests, the delayed-type hypersensitivity response of these antigens have been studied. SP has given almost similar level of response as that of PPD. Here also generation of homologous and heterologous responses have indicated presence of common antigens in these mycobacteria.

SDS-PAGE and Western blot analysis of the immunodominant proteins have been probed with TB patients' sera and hyperimmune sera against *M. tuberculosis* H37Rv, H37Ra and BCG. Several immunodominant antigens of SP i.e., 72, 70, 65, 36, 21 and 14 kDa identified by patient's sera. Reactivity of SP with hyperimmune sera indicates antigenic similarity of SP with respective antigens. Antigens of 36
kDa and 14 kDa of SP recognized by various antisera used indicate the presence of common epitopes among these antigens.

To detect tuberculosis cases as a prelude to develop diagnostic antigen in SP, sera from TB patients of pulmonary, renal and meningitis origin along with serum from several related or remote diseases have been probed with SP, *M. habona* and PPD antigens. SP and PPD had almost equal potentiality of detecting antibodies in TB patients' sera and was just next to *M. habana* antigens.

Thus, it may be safely concluded that SP has great potential for developing it as a vaccine and/or diagnostic antigen for tuberculosis. The isolated protein may form a part of further studies.

A paragraph on future research needs in the field.