TOTAL PROTEIN STAINING (TPS) AS AN ALTERNATIVE TO ROUTINE IMMUNODETECTION LOADING CONTROL

A. Sohail[1], I. Aleem[1], S. Saleem[1].

[1]Shaukat Khanum Memorial Cancer Hospital and Research Centre, Basic Sciences Research, Lahore, Pakistan.

OBJECTIVE

In Western blotting, immunodetection of housekeeping proteins is routinely performed to detect differences in protein amounts. In the present work, we show that it is possible to use a conventional Coomassie staining procedure after the immunodetection of proteins blotted onto polyvinylidene fluoride (PVDF) membranes to control the total protein load and see the blotting efficiency. In addition, the method can be used after immunodetection with superior linearity compared to antibody detection of house-keeping proteins. Protein staining method not only assesses protein transfer and but also weather a PVDF blot could be used for protein semi-quantification or not.

METHODS

Total protein from Tongue cancer and colon cancer cell lysate was separated by SDS-PAGE, blotted to a PVDF membrane, detected with a β-Actin and Tubulin antibody and then stained with Coomassie R-250 (Figure 1). The protein quantification was performed by chemiluminescent exposures captured on a Syngene (Cambridge, UK) GBOX at 600 dpi and staining density for each complete lane was analyzed in GeneTool software with an area outside the protein lanes defining the background. Membranes were blocked with Tris Buffered Saline (TBS) containing 5% nonfat dry milk with addition of 0%, 0.05% or 0.2% Tween-20.

RESULTS

The protein staining method offers quality control as protein transfer can be assessed by the appearance of the immunodetected band. Coomassie protein staining does not affect the antibody binding because it is performed after all detection steps. Blocking with fat-free dry milk and bovine serum albumin (BSA) show similar results. The resulting total protein staining density is much higher than the immunodetection linearity (Figure 2A-C).

Figure 1

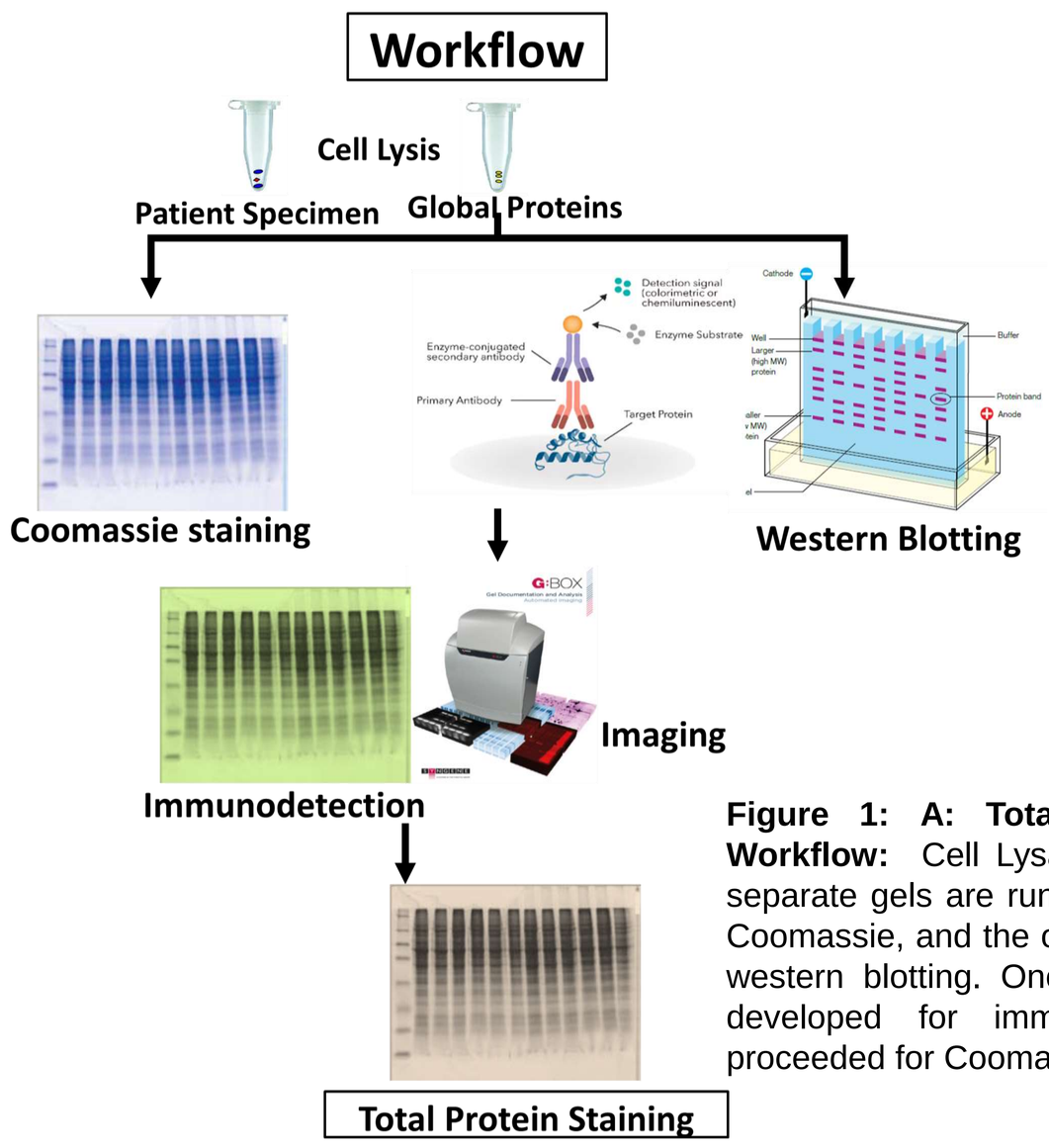

Figure 1: A: Total Protein Staining Workflow: Cell Lysate is prepared. Two separate gels are run. One is stained with Coomassie, and the other is proceeded for western blotting. Once the PVDF blot is developed for immunodetection, it is proceeded for Coomassie staining.

Figure 2

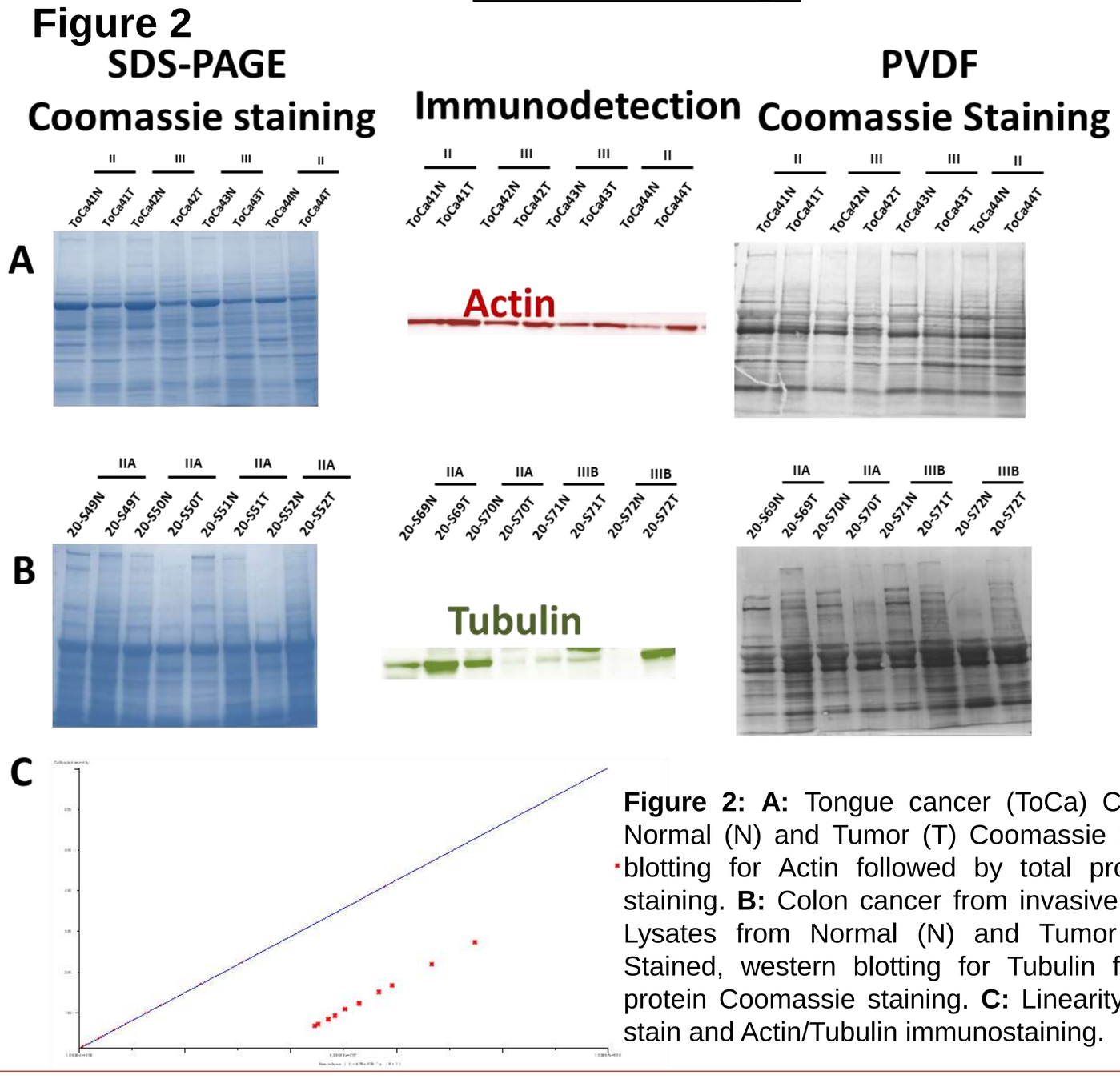

Figure 2: A: Tongue cancer (ToCa) Cell Lysates from Normal (N) and Tumor (T) Coomassie Stained, western blotting for Actin followed by total protein Coomassie staining. **B:** Colon cancer from invasive surgery (S) Cell Lysates from Normal (N) and Tumor (T) Coomassie Stained, western blotting for Tubulin followed by total protein Coomassie staining. **C:** Linearity of Total Protein stain and Actin/Tubulin immunostaining.

CONCLUSION

The use of immunodetection of house-keeping proteins for loading control might produce mistaken results. The presented Coomassie based method optimized on human cell lysates offers alternative approach to the routine use of immunodetection of house-keeping proteins as loading controls.