# The development and validation of QC analytical techniques for the study of peptides and proteins.



Case Study – Optimization of an antibody in a colorimetric Western Blot and improvement of the blot transfer process.

David Jardine, Dr. Penny Davey, Laura Kelman and Niels Donnelly Tepnel Pharma Services, Hologic Ltd., Livingston, UK

# History

Historically Tepnel has performed Western Blot by transferring proteins to a blot membrane via wet transfer. A test involving primary antibodies using colorimetric Western Blot was not performing as expected. Tepnel was tasked with the investigation of the performance, optimisation, and validation of the analytical process together with a new antibody.

# We aimed to optimise one of the western blot procedures.

- This was achieved through good collaboration between Tepnel and our client
- The assay has been revalidated using the new antibody
- The traditional transfer method did not provide adequate transfer efficiency for the antibody previously used
- The new antibody has a much higher affinity for the target protein and performed exceedingly well

A novel method of dry transfer was developed, optimised, and validated.

## Benefits of new methodology

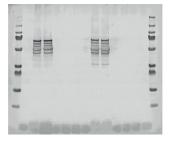
- Due to the use of our novel dryblot system the process has been improved dramatically
- Transferring the proteins from the gel to the blot can now be better standardised
- Transfer time reduced from 60 minutes to just 7 minutes (88 % decrease) resulting in time and cost savings

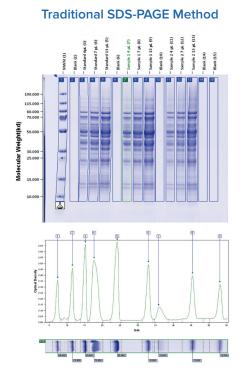
#### **Pre-Optimisation**

Western blot using the old antibody

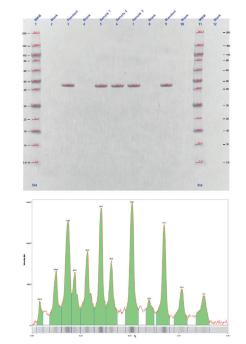
		-
-		-
-		-
-		-
3 (		-
-		=
-		
-		-
-	•	-

### **Post-Optimisation** Western blot using the new antibody









001371 @2016 Hologic, Inc. All rights reserved. Hologic, Science of Sure and associated logos are trademarks and/or registered trademarks of Hologic, Inc. and/or its subsidiaries in the United States and/ or other countries. All other trademarks, registered trademarks, and product names are the property of their respective owners. This information is intended for medical professionals and is not intended as a product solicitation or promotion where such activities are prohibited. Views and opinions expressed therein by third parties are theirs alone and do not necessarily reflect those of Hologic. Reccause Hologic materials are distributed through websites, eBroadcasts and tradeshows, it is not always possible to control where such materials appear. For specific information on what products are available for sale in a particular country, please contact you rolcal Hologic representative or write to pharma@Hologic.com.

