EBERHARD KARLS
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# New Cheap and Reliable Detection Tool for the Forssman Antigen

## Technology Description

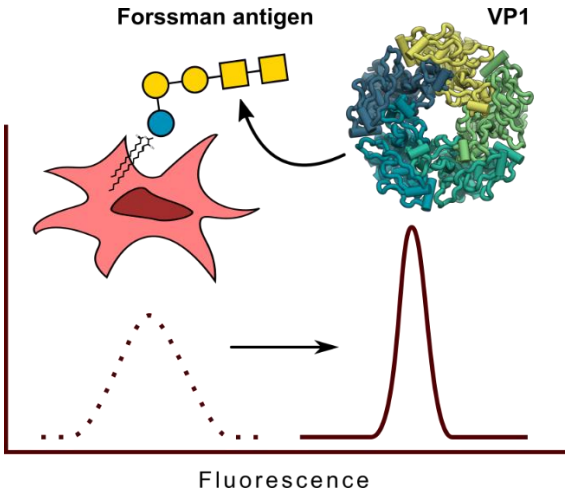


*Fig. 1: The pentameric VP1 specifically binds to the Forssman antigen on cellular surfaces, enabling detection by flow cytometry.*

The Forssman antigen (FA; after John Forssman, a Swedish physician who described it in 1911), is a glycolipid present in tissues of several mammals. In healthy humans the presence of FA is an exception, which defines the very rare FORS blood group[1]. Nevertheless, there is a growing number of reports about the *de novo* synthesis of FA in clinical disease, especially in oncological patients[2]. In some cases FA is also associated with bacterial pathology (e.g., pneumococcus infection)[3]. Taken together, FA is a potential target to assess disease and/or disease progression.

Current resources for the detection of the FA glycolipid, which is defined by the sequence GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Cer, are available in the form of commercial antibodies and α-GalNAc lectins such as the *Helix pomatia* agglutinin (HPA). However, these tools are relatively costly or bind FA only *inter alia*.

Here we present recombinant VP1, a non-hazardous protein of the sheep polyomavirus, as a potential means for the direct detection of FA in blood and tissues. With VP1, a precise and cheap detection of the important antigen FA comes into reach[4]. First data point to a high potential of excellent sensitivity and specificity of a future detection method based upon VP1.

## Innovation

**Up to now:** Specific detection of FA actually only possible via commercial antibodies.

**Now:** High-precision verification of FA status using a single viral protein produced via an inexpensive biotechnological route.

## Market Potential / IP Status

We are looking for an industrial partner interested in licensing and development of a potentially highly reliable test for the Forssman antigen.

**Priority-Application filed 2022-09-07, Application Number EP22194458.0**

## Applications

- New means for the diagnosis of Forssman antigen-related diseases such as human cancer and its progression
- Direct attestation of FORS blood group status due to excellent hemagglutination capabilities

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# PROOF OF CONCEPT

## High Specificity

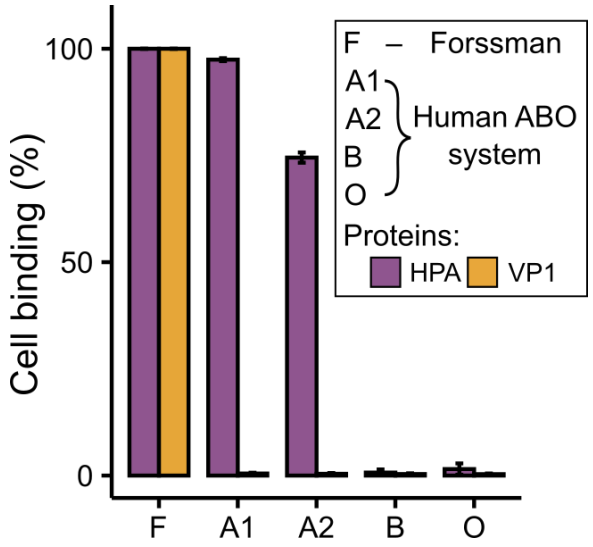


*Fig. 2: Binding of Forssman antigen-positive sheep blood cells (F – Forssman) and Forssman antigen-negative human ABO erythrocytes using VP1 and HPA. Figure adapted from [4].*

VP1 permits a specific detection of Forssman Antigen, which was assessed via broad spectrum glycan screening[4]. The data shown here in Figure 2 demonstrate that VP1 binds specifically to Forssman antigen on sheep blood cells but not to FA-negative human ABO erythrocytes. Sheep blood serves as a model for cellular Forssman expression, as erythrocytes from sheep present FA in huge amounts on their surfaces.

In sharp contrast to this, *Helix pomatia* agglutinin (HPA) not only binds FA but also the common blood group A antigens (Fig. 2). Thus, although HPA is an established research tool for the detection of α-GalNAc antigens as cellular cancer markers, its affinity towards antigens of FA-negative healthy individuals impedes its clinical deployment as a specific marker for FA.

## High Sensitivity

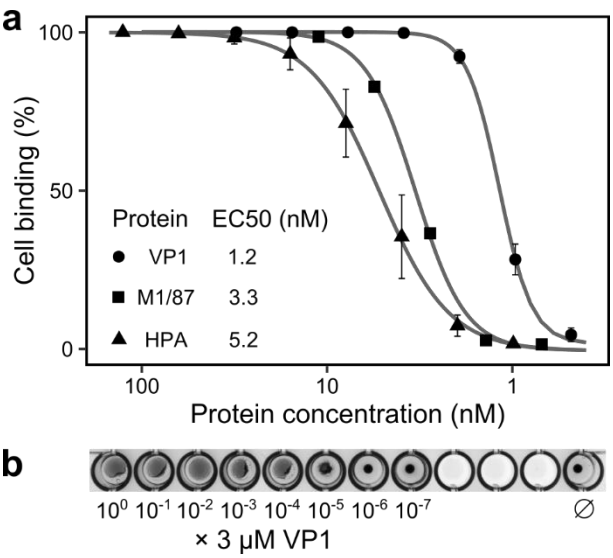


*Fig.3 a: Effective concentrations at 50% cell binding (EC50) for the detection of FA using commercial antibody (M1/87), HPA, and VP1.*
*Fig. 3 b: Hemagglutination assay of FA-positive blood cells (0.25 % sheep erythrocytes) and VP1.*
*Figure adapted from [4].*

Comparison of our VP1 to commercially available HPA and anti-FA antibody in the context of affinity towards the Forssman antigen on cellular surfaces. We incubated erythrocytes from sheep with our VP1, a formulation of the FA-specific antibody M1/87 (Santa Cruz Biotechnology) or HPA (Sigma-Aldrich). Subsequent flow cytometry analysis showed that VP1 yields the lowest effective concentration (EC50) to indicate the presence of FA among these proteins (Fig. 3a).

Complementary, we show that VP1 is able to hemagglutinate FA-postive erythrocytes in picomolar concentrations (Fig. 3b).

The data shown in Figure 3 demonstrate the exceptional premises for the development of a highly sensitive and, taken together with data of Figure 2, also specific detection kit for the Forssman antigen.

## REFERENCES


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2 Cid, E., Yamamoto, M., & Yamamoto, F. (2022). Mixed-Up Sugars: Glycosyltransferase Cross-Reactivity in Cancerous Tissues and Their Therapeutic Targeting. *ChemBioChem*, 23(5), e202100460.

3 Domenech, M., & García, E. (2017). Fluorescence imaging of *Streptococcus pneumoniae* with the *Helix pomatia* agglutinin (HPA) as a potential, rapid diagnostic tool. *Frontiers in Microbiology*, 8, 1333.

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