Syphilis-kodecytes - novel Function-Spacer-Lipid (FSL) modified red cells capable of sensitive and specific detection of syphilis antibodies

S Komarraju[1], S Chesla[2], N Bovin[3], S Henry[1*]

[1]Biotechnology Research Institute, AUT University, Auckland, New Zealand.
[2]Immucor Inc, Atlanta, USA,
[3]Shemyakin Institute of Bioorganic Chemistry RAS, Moscow, Russia
*email: kiwi@aut.ac.nz

AUCKLAND UNIVERSITY OF TECHNOLOGY
TE WĀNANGA ARONUI O TAMAKI MAKAU RAU

Background

The ability to detect infectious agents is a prerequisite to the treatment and containment of transmittable diseases. Many diagnostics assays are based upon the detection of antibodies in serum against the infectious agent and often rely on ELISA or agglutination-based assays to determine the infectious status. Using function-spacer-lipid (FSL) constructs[1-3] bearing peptides[4] representative of an infectious agent attached to human red cells, offers a potential alternative diagnostic approach to the detection of antibody-defined infections. Using the syphilis spirochaete as a model, and a peptide selection algorithm, a series of FSL constructs were created and attached to human red cells creating syphilis-kodecytes. These kodecytes were then tested in routine blood typing platforms against serum from panels and blood donors of known syphilis status. This approach has already been successfully used to detect ethnic-specific blood group antibodies[4].

FSL constructs are analogous in structure to a flower and consist of three components; a **F**unctional head group (like the flower head), a **S**pacer (like a stalk) and a diacyl **L**ipid tail (the anchoring roots). All FSLs are biocompatible and allow users to introduce novel **F**unctional moieties to the membrane surface of living cells.

Materials & Methods

Using a series of algorithms to predict biological activity three candidate FSL- syphilis constructs were synthesised. Two of these FSLs showed no biological activity but one, FSL-SYPH3 (figure 1) showed activity against syphilis positive samples. Two methods based on established microplate and gel card serological methods were established.

Method 1 - FSL-SYP3 Capture: FSL-SYPH3 kodecytes were created by incubating 40% packed blood group O red cells with an equal volume of a solution containing (10μg/ml) FSL-SYPH3 and incubation overnight at @ 4°C, followed by washing. Modification was confirmed by flow cytometry (figure 2). SYPH3-kodecytes were captured onto microplates, dried and used essentially as described[5].Method 1 was evaluated in parallel with the Capture S method on a Galileo automated blood bank instrument against panels of validated positive and negative samples. The negative panel was 32 syphilis serology negative donor plasma samples. The positive panel was 5 Syphilis Qualification Panel samples obtained from SeraCare Life Sciences and confirmed positive using the Olympus PK TP system, plus 20 Syphilis Validation Panel samples from Plasma Services Group, Inc. These samples were confirmed positive using the Arlington Scientific RPR and EuroimmunTP IgG and IgM ELISA assays.

Method 2 - FSL-SYPH3 gelcard:
SYPH3-kodecytes were created as above except incubation was for 2 hours @ 37°C. Serology was in Diamed anti-IgG LISS gel cards and used as per instructions. Method 2 was evaluated in parallel against 98 syphilis-positive clinical patient samples which were confirmed as positive by both TPPA & EIA.

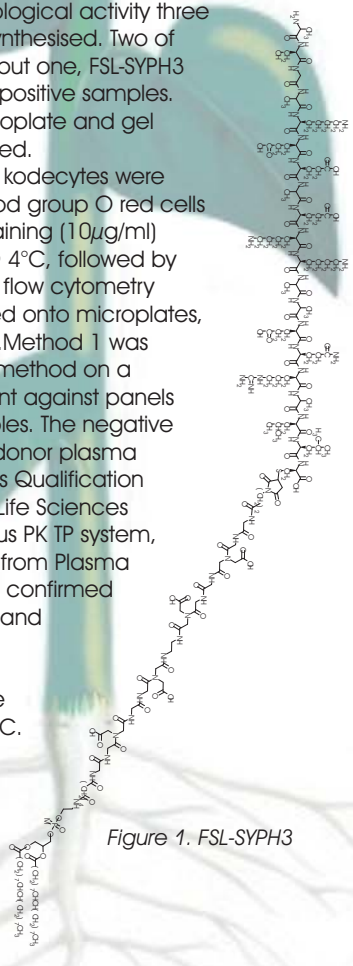

Figure 1. FSL-SYPH3

Results

The sensitivity of the FSL-SYPH3 kodecytes in the Capture microplate assay (Method 1) was found to be better than the Capture S method which uses modified VDRL antigen bound to the microplates (Table1). FSL-SYPH3 kodecytes, when used in blood typing anti-IgG gel cards (Method 2), produced good reactions (figure 3) and was found to be more sensitive than RPR (rapid plasma reagin) and TPHA (treponemal pallidum hemagglutination assay) syphilis assays and detected 96 of 98 (98%) of EIA+TPPA (enzyme immuno assay + treponemal pallidum particle agglutination) validated positive samples (Table 2).

Table 1. Comparison of Capture S method with new FSL-SYPH3 Capture method 1

	Method result	Known positive	Known negative	Sensitivity	Specificity
Method Capture S	Positive	20	0	80%	
	Negative	5	32		100%
Method 1 **FSL-SYPH3** Capture	Positive	24	0	96%	
	Negative	1	32		100%

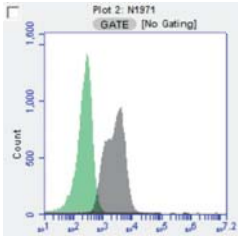

Figure 2. Flow cytometry of FSL-SYPH3 kodecytes. Grey = positive serum Green = negative serum

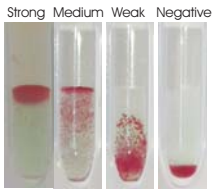

Figure 3. Examples of FSL-SYPH3 kodecyte gelcard reactions

Table 2. FSL-SYPH3 gelcard Method 2 comparison with TPHA and RPR of 98 EIA+TPPA positive samples

	Positives by method		
EIA + TPPA validated positive samples	RPR	TPHA	*Method 2* **FSL-SYPH3**
n = 98	56	90	96

Conclusions

It was found that syphilis-kodecytes used in blood grouping platforms are capable of the same sensitivity and specificity as validated assays. The ability to engineer the FSL construct and replace the functional peptide for other infectious markers allows for a highly flexible and rapid-development technique for the detection of antibody-defined infectious markers and may be an important low-technology tool for use in epidemics and pandemics, particularly in third-world environments.

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