**Optimizing Respiratory Syncytial Virus** (RSV) Antibody Pair Selection for **Enhanced Diagnostic Accuracy and Speed** 

T. Sorsa-Leslie<sup>1</sup>, K. Grönholm<sup>1</sup>, A. Moore<sup>2</sup>, M. Mattila<sup>1</sup>, S. Suutari<sup>1</sup>, L. Vaahtoranta<sup>1</sup> <sup>1</sup>Medix Biochemica – Espoo, Finland <sup>2</sup>Medix Biochemica USA Inc. – Maryland Heights, United States

**Antibody Platform Tests in Modern Diagnostics Essential** for Reliable and Rapid Results

# Results

Multiple antibodies (Table 1) were tested in FIA (Figures 1 and 2), LF (Figures 3 and 4) and BLI (Table 2). The tested antibodies showed specificity for recombinant RSV and native RSV antigens and none of the antibodies cross-reacted with influenza A and B.

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Antibody	k <sub>on</sub> (1/Ms)	k <sub>off</sub> (1/s)	К <sub>D</sub> (М)			
HM433	2.6 x 10 <sup>5</sup>					
HM434	3.2 x 10⁵					
V0631	3.2 x 10⁵					
V0651	5.1 x 10 <sup>4</sup>					
140042	4.6 x 10 <sup>4</sup>	Does not dissociate under conditions used				
140044	9.5 x 10 <sup>3</sup>					

# Background

Respiratory syncytial virus (RSV) significantly contributes to acute respiratory infections, especially among children and older adults. Annually, RSV leads to over 3.6 million hospitalizations and approximately 100,000 deaths in children under the age of five. The majority of these pediatric deaths (97%) occur in low- and middle-income countries, where access to supportive medical care is limited<sup>1</sup>.

# **Challenge:**

Selecting optimal antibody pairs is time-consuming and costly

- WHO criteria for ideal point-of-care testing (POCT): Affordable, Sensitive, Specific, User-friendly, Rapid/Robust, Equipment-free, Deliverable (ASSURED).
- Antibody functionality varies across IVD applications, emphasizing pre-testing during method development.
- Medix Biochemica developed monoclonal antibodies (mAbs) against RSV, targeting nucleoprotein (NP)<sup>2</sup> and fusion protein (FP). Some of these were tested using fluorescence immunoassay (FIA) and lateral flow (LF) platforms, assessing recognition of native and recombinant RSV antigens and kinetics.

# **Materials & Methods**

Target RSV Protein	Catalog	Catalog	Manufacturer
Fusion	V0631	Anti-Respiratory Syncytial virus (fusion)	Medix Biochemica
Fusion	V0651	Anti-Respiratory Syncytial virus (fusion)	Medix Biochemica
Fusion	HM434	Respiratory Syncytial Virus (RSV) Antibody	Medix Biochemica
Fusion	HM433	Respiratory Syncytial Virus (RSV) Antibody	Medix Biochemica
NP	140042	Anti-RSV NP R13401 SPTN-5	Medix Biochemica
NP	140044	Anti-RSV NP R13403 SPTN-5	Medix Biochemica
NP	140045	Anti-RSV NP R13404 SPTN-5	Medix Biochemica
NP	V0671	Anti-Respiratory Syncytial virus (NP)	Medix Biochemica
Rec Antigen (fusion)	LA512	Respiratory Syncytial Virus (RSV-fusion) Antigen, Recombinant >90%	Medix Biochemica
Native Antigen	FC517	Respiratory Syncytial Virus (RSV) Antigen, Native	Medix Biochemica
Rec Antigen (NP) A	40821-V08E	Human respiratory syncytial virus (RSV) (strain A2, strain Long) Nucleoprotein Protein (His Tag)	Sino Biological
Rec Antigen (NP) B	40822-V07E	Human respiratory syncytial virus (RSV) (B, strain 18537) Nucleoprotein Protein (His Tag)	Sino Biological

#### **RSV** fusion sensitivities with best performing antibody pairs (FIA)



*Figure 1. RSV fusion sensitivities with best performing* antibody pairs using rec. RSV fusion LA512 antigen.



*Figure 2. RSV NP sensitivities with best performing antibody* pairs using native FC517 antigen.

40045	5.7 x 10 <sup>4</sup>
0671	3.2 x 10 <sup>4</sup>

**Table 2.** Kinetic parameters of the best performing RSV fusion and RSV NP antibodies using rec Antigens LA512 and 40822-V07, respectively.



*Figure 3. LF* sensitivity testing for an RSV fusion antibody pair (HM433 + HM433-latex) using rec. RSV fusion LA512 antigen.

#### **RSV** fusion sensitivities with best performing antibody pairs (LF)



#### **RSV NP** sensitivities with best performing antibody pairs (FIA)

**Table 1.** Selected tested RSV antibodies and antigens.

# Methods used

# • Fluorescence-based immunoassay (FIA)

Fluoroimmunoassays are based on labelling of the immunoreactants with fluorescent molecules (fluorophores) to detect and quantify specific antigen-antibody interaction. When the labelled antibody binds to its target antigen, the fluorescence emitted can be measured, providing information about the presence and concentration of the antigen/antibody.

## • Lateral flow (LF)

Lateral flow test format is a simple, rapid diagnostic device used to detect the presence (or absence) of a target substance (antibody or antigen) in a liquid sample. The detection can be visual or detected by reader.

Furthermore, the top functioning pairs were assessed in LF (Figures 3 and 4) and kinetics evaluated with Octet RED96e (Table 2). The kinetics play crucial role in the performance and accuracy of LF tests (binding and release kinetics affects the sensitivity and nonspecific binding).

Preliminary screening of antibody pairs in different platforms

speed up the development of desired test format for both RSV

Preliminary testing of an antibody pair on different platforms

underscores the need to evaluate antibody compatibility with

the chosen platform early in method development. This approach

can speed up development and offer accurate, early diagnostics

has produced the following recommendations (Table 3) which will

*Figure 4. LF* sensitivity testing for RSV fusion antibody pairs using rec. RSV fusion LA512 antigen.

Fusion		Detection			
		V0631	V0651	HM433	HM434
Capture	V0631	FIA, LF	FIA, LF	LF *	LF *
	V0651	FIA, LF	LF	LF *	LF *
	HM433	LF *	LF *	LF *	FIA, LF
	HM434	LF *	LF *	FIA, LF	LF *

NP		Detection			
		140042	140044	140045	V0671
Capture	140042	FIA	_	LF	LF
	140044	FIA	FIA	FIA	FIA
	140045	FIA	_	_	LF
	V0671	FIA	FIA	FIA, LF	FIA

\* = not determined in FIA. - = Does not perform as pair.

**Table 3.** Best performing RSV NP and RSV fusion antibody pairs in FIA and LF applications.

## • Biolayer interferometry (BLI) – kinetics

Biolayer interferometry (BLI) is an optical technique used to measure biomolecular interactions (Ab-Ag) in real-time without the need for labels.

# References

for end users.

Conclusion

nucleoprotein and RSV fusion protein.

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2. P-E Baurand, J Balland, E Galli, S Eklin, R Bruley, L Ringerbach. New Anti-RSV Nucleoprotein Monoclonal Antibody Pairs Discovered Using Rabbit Phage Display Technology. Antibodies 2023, 12(4), 73; https://doi.org/10.3390/antib12040073.

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Klovinpellontie 3, FI-02180 Espoo, Finland medix@medixbiochemica.com • www.medixbiochemica.com