Enigma® MiniLab Respiratory Viral Panel (RVP) Test Instructions for Use

For use with the Enigma MiniLab Platform

For *In Vitro* Diagnostic Use Only. Not for sale or use in the USA

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The Enigma MiniLab Platform is protected by patents listed at: www.enigmadiagnostics.com/products/patents

The purchase of this product grants the purchaser rights under one or more of U.S. Patent numbers 6,174,670, 7,160,998, 6,569,627, 5,804,375 AND 6,245,514 and their foreign counterparts to use this product solely for providing human and animal *in vitro* diagnostic services. No general patent or other license of any kind than this specific right of use from purchase is granted hereby.

Use the definitions in the table below as a guide to interpreting symbols used throughout the MiniLab RVP Test Instructions for Use and cartridge labelling.

	Manufacturer		Consult instructions
CE	European Union Conformity	IVD	In vitro diagnostic use
REF	Catalog number	LOT	Lot number
2	Do not use more than once		Sufficient for < n > uses
	Expiry date YYYY-MM-DD		Do not use if damaged
	Do not dispose of in the municipal waste system		

Intended Use

The Enigma MiniLab Respiratory Viral Panel (RVP) Test is an automated multiplex PCR test for the simultaneous in vitro diagnostic qualitative detection of Influenza A, Influenza B and Respiratory Syncytial Virus (RSV). The test uses nasopharyngeal swab (NPS) specimens collected from individuals exhibiting signs and symptoms of respiratory infection in conjunction with other clinical and epidemiological risk factors. The test is designed solely for use with the Enigma MiniLab as an aid in the diagnosis of respiratory infections.

Summary and Explanation

Respiratory tract infections can be caused by a range of viral and bacterial pathogens and are associated with a range of similar symptoms which include, but are not limited to fever, chills, headache, malaise, cough, sneezing and sinus congestion. While the majority of respiratory infections are mild and self-limiting, they are also a leading cause of morbidity and mortality, placing a substantial burden on healthcare systems.

Symptom severity and levels of infectivity can vary depending on a range of factors such as the infectious agent and health status of the infected individual. Certain population groups, such as the young and elderly, are more susceptible to infections. Viral pathogens are the most common agents in respiratory tract infections. Influenza and RSV are acute contagious viral infections of the respiratory tract that often occur seasonally, usually in the winter, and account for the majority of seasonal respiratory infections.

Influenza, or "Flu", is an acute respiratory illness caused by infection with the Influenza virus, primarily types A and B. Both Influenza A and B strains are constantly genetically evolving resulting in the replacement of older circulating influenza strains with new antigenic variant strains. Prior vaccination or infection in humans may not provide subsequent protection. Influenza A viruses can infect a variety of hosts including humans, birds, pigs and horses. The Influenza A viruses are further categorized into subtypes based on two major surface protein antigens, haemagglutinin (H) and neuraminidase (N). Of these subtypes, two are of particular importance to human infection: H1N1 and H3N2. Influenza A is the most common cause of influenza in human infections, causing seasonal flu epidemics and more rarely, pandemics. Influenza B infections usually only occur in humans and are generally less common than Influenza A infections. Influenza B virus generally causes less serious epidemics compared to Influenza A and has not caused any known pandemics.

Transmission of influenza is primarily via airborne droplets (coughing or sneezing). On average, symptoms arise 1 to 2 days post-exposure and can include fever, chills, headache, malaise, aching muscles and joints, cough, sneezing and sinus congestion. Gastrointestinal symptoms such as nausea, vomiting and diarrhoea can occur, primarily in young children. Complications instigated by influenza include conditions such as pneumonia, causing increased morbidity and mortality in paediatric, elderly and immunocompromised populations.

Human Respiratory Syncytial Virus (RSV) causes respiratory tract infections in patients of all ages, but its influence is more prevalent in paediatric, elderly and immunocompromised populations. It is the major cause of severe lower respiratory tract infection amongst children under 2 years old. Natural infection with RSV induces incomplete protective immunity, thus an individual can be infected multiple times. There are two types of RSV (A and B), categorised based on antigenic variations. It is not currently understood if there is any difference in clinical severity of infection between the two types. Most yearly epidemics contain a mix of type A and type B viruses, although one subgroup can dominate during a season

Principle of the MiniLab RVP Test on the MiniLab Platform

The MiniLab RVP Test is an automated in vitro diagnostic test for simultaneous qualitative detection of Influenza A, Influenza B and RSV (types A and B) using PCR and fluorescent probes, which is performed using dedicated cartridges on the MiniLab Platform.

The MiniLab Platform consists of a fully integrated Control Module (CM) and up to six Processing Modules (PM). The fully integrated Control Module has a touch-screen computer for user input along with the display of the operating status and results, barcode reader, printer, data transmission and can simultaneously run up to six completely independent random access Process Modules that automate sample processing, nucleic acid amplification and target sequence detection using single-use dedicated cartridges. The disposable cartridges contain all of the reagents required for sample processing and nucleic acid detection. These cartridges maintain stability at ambient temperature. The cartridge's self-contained format minimizes cross-contamination between samples and the sealing of the amplification reaction eliminates amplicon contamination.

The MiniLab RVP test cartridge contains reagents for the detection and differentiation of Influenza A, Influenza B and RSV, as well as an internal positive control to ensure efficient sample extraction and removal of PCR inhibitors.

Before Use

The MiniLab Platform should only be installed by an authorised technical representative of Enigma Diagnostics Ltd.

For all MiniLab Platform related operations please follow both the instructions detailed in the **Enigma MiniLab Operator Manual** and in the laminated **Enigma MiniLab Quick Reference Guide (QRG)** that accompanies each MiniLab Platform.

The MiniLab Platform

The MiniLab Platform is the world's first Point of Care (POC) highly multiplex Polymerase Chain Reaction (PCR) platform (Figure 1). The easy-to-use sample-to-result MiniLab provides clinicians 24/7 access to fast, comprehensive, laboratory-quality molecular diagnostic results in about an hour for the simultaneous in vitro diagnostic detection of targets using samples taken directly from the patient. Fast comprehensive results enable a quicker diagnosis with the ability to initiate the appropriate treatment protocol that improves clinical outcomes while reducing costs. The MiniLab is well suited in a range of clinical settings where fast laboratory-quality results are imperative from the Emergency Department through Critical Care. The fully automated sample-to-results MiniLab Platform does the sample preparation, nucleic acid amplification and target detection without requiring the operator to be familiar with PCR technology.

The fully integrated MiniLab's Control Module has a touch-screen computer, barcode reader, printer, data transmission connection and it can run up to six random access Processing Modules. The MiniLab Platform's modular architecture is easily scalable providing flexibility to increase the throughput as needed by simply adding up to six Processing Modules. The lowest throughput configuration is having a single Processing Module attached to the Control Module; however, increasing throughput only requires adding up to six Processing Modules. The random access Processing Modules run different types of tests at independent times. It exhibits zero operator-to-operator variance.

The MiniLab operates using single-use disposable cartridges, removing the costly and time-consuming logistical challenges and sample preparation procedures normally associated with PCR. The MiniLab cartridges can be stored at ambient temperature.

Figure 1: MiniLab Platform

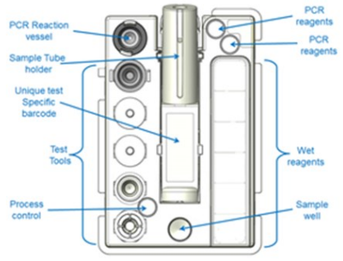

Figure 2: MiniLab Cartridge

RVP Test Cartridge

The MiniLab uses a novel single-use disposable cartridge (Figure 2) and a separately packaged single-use disposable nasopharyngeal sample collection kit containing a sterile nasopharyngeal swab and VTM partially filled sample collection tube with customised cap. Both of these components can be stored at ambient temperature. The rigid plastic cartridge has discrete sections that contain reagents in both freeze-dried and liquid form, as well as the sample preparation tools.

To process a patient's sample, collect a nasopharyngeal sample from the patient, break the swab off into the sample collection tube at the scored line, and screw on the customised cap. The sample collection tube, containing the sample, swab, and VTM, is placed into the sample tube holder of the cartridge. The cartridge is placed into a Processing Module where all of the steps are fully automated thus minimizing the risk of human error and sample-to-sample contamination. For a full description, please refer to the MiniLab Operator Manual.

MiniLab RVP Test Principle

The MiniLab RVP Test is based upon magnetic bead sample purification and concentration combined with fluorogenic reverse transcriptase (RT) polymerase chain reaction. Chemical and thermal lysis of viral particles is used to liberate ssRNA (single stranded ribonucleic acid) genomes from clinical samples. The addition of binding reagents to the ssRNA provides high affinity binding to magnetic beads. Beads are transferred through sequential washing steps to remove inhibitory substances using a magnetic wand system within the Platform. ssRNA is liberated from the magnetic beads into an elution fluid and transferred to a lyophilised multiplex RT-PCR formulation containing a complete reaction cocktail of RT and PCR enzymes blended with fluorescently labelled primers and probes. The target RNA and PCR mix are transferred to a thermal reaction vessel in the single-use disposable cartridge. This is sealed (to prevent cross contamination) before cDNA (complementary deoxyribonucleic acid) synthesis and PCR amplification start. The analysis is carried out in a post amplification melt analysis, in which fluorescently labelled probes dissociate from target amplicons at temperatures that are specific for each target virus (Influenza A, Influenza B, RSV (A&B) and MS2 control). These temperature dependent specific fluorescent responses are detected and measured by the MiniLab optics and an automated algorithmic process reports the presence or absence of each target.

Internal Process Control

Each MiniLab RVP Cartridge contains a freeze-dried cake of bacteriophage MS2 that is used as an "Internal Process Control" (IPC) to validate that both sample preparation and PCR amplification have occurred efficiently. At the start of the test process, the IPC is reconstituted and then mixed with the sample prior to the sample-processing step. Failure to detect MS2 in the post amplification melt analysis, in the absence of any target signals, indicates that the test has failed.

Table 1: Targets Detected by the MiniLab RVP Test

Organism	Assay Type	Number of PCR Targets	Target
Influenza A	RNA	1	Segment 8
Influenza B	RNA	1	Segment 8
RSV A/B	RNA	2	Fusion gene
MS2 Internal Control	RNA	1	Lys gene

Reagents Contained within the MiniLab RVP Cartridge

The MiniLab RVP Cartridge contains PCR primers for the qualitative detection of Influenza A, Influenza B and RSV.

Wet reagents:

Lysis buffer, Magnetic bead reagent, Binding buffer, Wash buffer 1, Wash buffer 2, Elution buffer (with Azide)

PCR reagents:

Freeze-dried cake consisting of Reverse Transcriptase enzyme, Taq Polymerase enzyme, Bovine Serum Albumin (BSA), dNTPs, Salts, Primers and Probes

Process control contains:

Freeze dried cake of Bacteriophage MS2

Storage and Handling

MiniLab A/B RSV cartridges should be stored at ambient temperature until their stated expiration date.

Do not open the foil pouch containing the cartridge until running the test

Once the foil pouch has been opened, the cartridge run must be initiated within 5 hours

Do not use a cartridge if the foil pouch or other packaging has been damaged

Do not use a cartridge if any of the 'Test Tools' are loose or missing or the 'reagents'' integrity has been breached

Do not use a cartridge which is past the 'Expiry Date' displayed on the packaging

When handling swabs and cartridges, follow your Institution's safety procedures for handling biological and chemical hazardous material

Materials required but not provided

Enigma Diagnostics Ltd. MiniLab Platform:

Control Module REF EML-IA-0002

Process Module REF EML-IA-0003

Enigma Diagnostics Ltd. Nasopharyngeal Swab Sample Collection Kit (Ref MWEN2)

Warnings and Precautions

Negative results do not preclude respiratory infection and should not be used as the sole basis for diagnosis, treatment, or other management decisions. Positive results do not rule out infection with other viruses, bacteria, or other organisms. The virus detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) and clinical presentation must be taken into consideration in order to obtain the final diagnosis of respiratory infection.

All specimens, including used cartridges and swabs, should be treated as capable of transmitting infectious agents regardless of any result displayed by the MiniLab Platform.

When handling used cartridges treat any open pot as a potential chemical and biological hazard and follow your institution's standard procedures for handling biological and chemical hazards.

BSA in this product is sourced in the United States from bovine plasma taken from animals that have not been fed any animal protein.

Do not substitute or add any reagents or chemicals to the cartridge.

Do not open any of the pots or reagent wells on the cartridge before or after test execution.

Do not re-use spent cartridges.

Specimen Collection, Handling and Storage

Nasopharyngeal swabs:

1. *Ensure gloves are worn for the entire operation.*
2. *Open the Enigma Diagnostics Ltd. Nasopharyngeal Swab Sample Collection Kit (manufactured by MWE Ref MWEN2) and remove the sample collection tube and nasopharyngeal swab.*
3. *Individually label or number the sample collection tube(s) according to your institution's standard procedure for clinical sample labelling (applying a barcode label if required) according to your institution's standard procedure.*
4. *Loosen the cap of the sample collection tube(s) containing 3 ml of Viral Transport Medium (VTM) and place the sample collection tube(s) in a suitable tube rack (not supplied).*
5. *Collect the nasopharyngeal sample using the nasopharyngeal swab following your institution's standard procedure.*
6. *After the sample has been taken from the patient, introduce the nasopharyngeal swab into its appropriately labelled sample collection tube*
7. *Once the swab tip is at the bottom of the sample collection tube, bend the shaft of the swab, on the edge of the sample collection tube, to snap it off (Figure 3).*

Figure 3: Breaking the Swab off in the Sample Collection Tube

8. *Replace the cap tightly and return the sample collection tube to the rack. Repeat as needed with any further samples.*
9. *The VTM in the sample collection tube is designed to stabilise the sample for up to 48 hours if kept at 2°C to 25°C by which time the sample must have been processed on the MiniLab Platform or a re-sampling will be necessary.*
10. *Alternatively, samples can be stored in -80°C after sample collection for use at a later time point (as a result of clinical evaluations etc.). NB once the samples are to be used they must be brought up to RT before they can be processed on the MiniLab.*

Reagent Handling and Storage

- Preparing the MiniLab RVP Cartridge:
- Wear gloves during the entire procedure.
- Use the correct test cartridge labelled "MiniLab RVP Test".

1. *Peel back the foiled lid and remove the cartridge.*
2. *Lift up the sample tube holder in the centre of the cartridge until vertical (Figure 4)*
3. *Insert the sample collection tube into the sample tube holder (Figure 4).*
4. *Fold the sample tube holder down into the cartridge body until it clicks into place (Figure 4).*
5. *The cartridge and sample are now ready for processing using the MiniLab Platform (Figure 4).*
6. *Consult the MiniLab Operator Manual for full details of operating the MiniLab and/or the MiniLab Quick Reference Guide (QRG)*

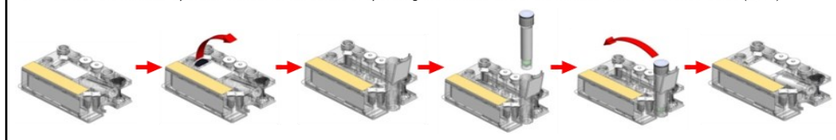

Figure 4: Sequence Required to Load the Sample Collection Tube into the Cartridge

Storage of Unused Cartridges

- Cartridges are stable up until the expiration date indicated on the cartridge package label.

Disposal of Cartridges

- Used cartridges must be handled and disposed of as clinical biohazardous waste material following your institution's standard procedure.
- Do not dispose of cartridge through municipal waste system
- Do no open any of the capped reaction pots on the cartridge prior to disposal

1. *When removing the spent cartridge from the tray, keep it upright. Do not dislodge the stopper tool that is capping the reaction vessel.*
2. *Place the used cartridge in the biohazard bags accompanying each box of 24 cartridges.*
3. *Dispose of used cartridges according to your institution's standard procedure governing the disposal of clinical biohazardous waste material*

Interpretation of Results

The results for each viral target are interpreted by the MiniLab and displayed on the touchscreen and printed at the end of each run as follows:

Influenza A - *"Positive or Negative"*
Influenza B - *"Positive or Negative"*
Respiratory Syncytial Virus (RSV) A/B - "Positive or Negative"
When the MS2 control and all target viruses, has not been detected, the result is a test failure and the test will need to be repeated. The following result combinations are possible with this test on the MiniLab Platform:

Table 2: MiniLab RVP Test Result Combinations

MiniLab RVP Result Possibilities	Result			Internal MS2 control result	Test Interpretation
	InfA	InfB	RSV A/B		
	-	-	-	+	Negative for all targets
	+	-	-	+	Positive for Influenza A
	+	-	-	-	Positive for Influenza A
	-	+	-	+	Positive for Influenza B
	-	+	-	-	Positive for Influenza B
	+	+	-	+	Positive for Influenza A & B
	+	+	-	-	Positive for Influenza A & B
	-	-	+	+	Positive for RSV A/B
	-	-	+	-	Positive for RSV A/B
	+	-	+	+	Positive for Influenza A & RSV A/B
	+	-	+	-	Positive for Influenza A & RSV A/B
	-	+	+	+	Positive for Influenza B & RSV A/B
	-	+	+	-	Positive for Influenza B & RSV A/B
	+	+	+	+	Positive for Influenza A & Influenza B & RSV A/B
	+	+	+	-	Positive for Influenza A & Influenza B & RSV A/B
	-	-	-	-	Test failure - repeat test

A **"Negative"** result indicates that the sample is unlikely to be positive for Influenza A, B, or RSV A/B; however, this result should not be used as the sole basis for diagnosis and treatment of the patient.

A **"Positive"** result indicates that sample is likely to be positive for Influenza A, B, RSV A/B or a combination of these targets; however, this result should not be used as the sole basis for diagnosis and treatment of the patient.

A **"Test Failure"** result indicates a test has not performed correctly and should be repeated.

Critical Failures Requiring Technical Support

On rare occasions, the MiniLab may suffer a critical failure that requires technical support. If this occurs, the MiniLab will display a system failure screen with an error code.

NOTE: Most critical errors are related to a single processing module and do not affect other modules. Where more than one processing module is available, the unaffected modules can still be used while waiting for technical support.

If the critical error screen persists, please contact Technical Support relaying any error code or information provided by the MiniLab Platform.

Failures Resolved by User

If the MiniLab Platform has been powered down with a cartridge still inside the Process Module, an error screen in will be displayed. Press the 'Eject Tray' button and remove the cartridge. Follow the on-screen instructions to start a new test.

If the MiniLab cannot detect the sample in a loaded cartridge an error screen will be displayed. Press the 'Eject Tray' button and remove the cartridge for inspection. If the sample has been released from the sample collection tube, the test will need to be repeated (see Re-test Procedure). If the sample has not been released and the sample collection tube is intact, carefully remove and insert it into a new cartridge and re-start the test.

Reasons to Repeat Test

If any of the results listed below occur, the test should be repeated.

If the MS2 control is not detected "Test Failure" will be reported

If the test is aborted before finishing (for example the operator aborts the test), "Test stopped" will be reported.

If the MiniLab cannot confirm that all of the cartridge components are correctly placed, an error will be displayed.

Quality Control

Each test includes an internal process control that ensures sample preparation and RNA extraction steps are correctly processed. It also detects any specimen associated inhibition of the RT-PCR reaction.

Limitations

Although an assay result can be positive for one, two, three or four components, the MiniLab RVP Test is a multivalent test and as such although multiple components can be detected, the sample may also be inhibiting the detection of other components contained in that sample. Therefore, co-infection cannot be accurately determined from this assay result.

Where extremely high levels of target virus (>10^7 $TCID_{50}$/ml) are present in the sample, the MS2 control may not be detected resulting in a test control being called negative.

Results from the MiniLab RVP Test should be interpreted in conjunction with other available laboratory, clinical data and epidemiological risk factors aids in respiratory infection diagnosis.

False test results may occur due to improper sample collection, failure to follow the recommended sample collection, handling and storage procedures, technical error, sample mix-up, infections below the limit of detection or infections above the upper threshold for MS2 control amplification. Careful compliance with this instruction insert is necessary to avoid erroneous test results.

Highly viscous samples may produce pipetting errors in the assay process, resulting in lower than expected sample volumes being tested. It has been shown that mucin up to 2.5% (w/v) has no effect on result reporting.

Performance Characteristics:

The diagnostic sensitivity and specificity of the RVP panel should perform in line with the comparator test - Luminex system / xTAGfast

A total of 598 swabs from patients showing symptoms of respiratory infection were tested on the MiniLab, and the Luminex Respiratory Viral Panel FAST v2 CE-IVD system. The study was carried out at the Lothian Health Board Department of Microbiology, in the Royal Infirmary of Edinburgh (RIE), UK.

There were 26 failed runs on the MiniLab and the remaining 572 tests gave a result on the MiniLab on the first attempt. There were 31 results from the MiniLab panel that were discordant with the Luminex test. 4 individual results were resolved by secondary PCR and sequencing in favour of the MiniLab and removed from the data set leaving 568 scorable results, except for influenza A where the failure of the comparator technology for 2 samples left 566 results.

Clinical Performance:

Influenza A		**Luminex RVP Fast v2**	
		Positive	Negative
Enigma® RVP test	Positive	78	0
	Negative	14	474

		95% Upper CI	**95% Lower CI**
NPV(%)	97.1	98.6	95.7
PPV(%)	100.0	100.0	100.0
PPA(%)	84.8	92.1	77.4
NPA(%)	100.0	100.0	100.0
Prevalence(%)	16.3	19.3	13.2
Diagnostic accuracy (%)	97.5	98.8	96.2

Influenza B		**Luminex RVP Fast v2**	
		Positive	Negative
Enigma® RVP test	Positive	103	0
	Negative	9	456

		95% Upper CI	**95% Lower CI**
NPV(%)	98.1	99.3	96.8
PPV(%)	100.0	100.0	100.0
PPA(%)	92.0	97.0	86.9
NPA(%)	100.0	100.0	100.0
Prevalence(%)	19.7	23.0	16.4
Diagnostic accuracy (%)	98.4	99.4	97.4

RSV		**Luminex RVP Fast v2**	
		Positive	Negative
Enigma® RVP test	Positive	103	0
	Negative	9	456

		95% Upper CI	95% Lower CI
NPV(%)	99.8	100.2	99.3
PPV(%)	100.0	100.0	100.0
PPA(%)	99.4	100.6	98.2
NPA(%)	100.0	100.0	100.0
Prevalence(%)	28.7	32.4	25.0
Diagnostic accuracy (%)	99.8	100.2	99.5

Analytical Sensitivity (Limit of Detection)

The limit of detection for a range of influenza and RSV viruses representative of currently circulating strains was undertaken by spiking known concentrations of virus into Vircult™ transport medium and running the sample on the MiniLab. The LoD was determined to be the lowest concentration of virus at which ≥95% gave a positive result (n=20).

Virus	**LoD ($TCID_{50}$/mL)**
Inf A/Cal/07/09 (vH1N1)	100.0
Inf A/Texas/50/12 (H3N2)	1.0
Inf B/Brisbane/60/08	0.5
Inf B/Phuket/3073/13	3.2
RSVA A2 operations stock	3.0
RSVA A2	3.0
RSV A Long	0.9
RSV B 18537	5.0
RSVB WV/14617/85	0.15

Analytical Reactivity (Inclusivity)

The inclusivity of the Enigma® RVP test was determined using 28 different viruses representative of currently circulating strains. The LoD for each strain was estimated by spiking known concentrations of virus into Virocult and testing in triplicate (or 20x where full LOD was estimated as described above) on the MiniLab. The LOD was determined as the lowest concentration of virus that produced 95% positive results (either 3/3 or 19/20).

Strain	LoD ($TCID_{50}$/mL)	Strain	LoD ($TCID_{50}$/mL)
Influenza A H1N1			
vH1N1 A/Cal/04/09	200	H1N1 A/Brisbane/59/07	200
vH1N1 A/Swine/NY/2/09	200	H1N1 A/Solomon Islands/03/06	200
H1N1A/ PR/8/34	200	H1N1 A/Texas/36/91	200
Inf A/Cal/07/09	100		
Influenza A H3N2			
A/Hong Kong/8/68	2.0	A/Perth/16/09	2.0
A/Wisconsin/67/05	2.0	A/South Australia/55/14	2.0
A/Wuhan/359/95	2.0	A/Switzerland/15293/13	10.0
A/Beijing/32/92	2.0	A/Texas/50/12	1.0
Influenza B			
B/Lee/40	6.4	B/Panama/45/90	12.8
B/Mass/2/12	6.4	B/Malaysia/2506/04	6.4
B/Florida/04/06	12.8	B/Wisconsin/1/10	6.4
B/Brisbane/60/08	0.5	B/Phuket/3073/13	3.2
RSV			
A A2	3.0	B 18537	5.0
A Long	0.9	B WV/14617/85	0.15
		B Birmingham/17134	10.0

Analytical Specificity (Cross Reactivity)

The exclusivity of the Enigma® RVP test was determined with a panel of 17 bacteria and 26 viruses representing common respiratory pathogens or commensal organisms typically found in the nasopharynx. Each target was tested in triplicate on three different MiniLab Platforms.

Virus	**$TCID_{50}$/mL**	**Inf A**	**Inf B**	**RSV**
Adenovirus type 1	$1.30x10^6$	-	-	-
Adenovirus type 3	$4.50x10^5$	-	-	-
Measles	$2.50x10^5$	-	-	-
Mumps	$7.80x10^5$	-	-	-
*Epstein Barr virus B95**	$4.77x10^7$	-	-	-
Human Parainfluenza virus 1	$1.50x10^5$	-	-	-
Human Parainfluenza virus 2	$4.10x10^6$	-	-	-
Human Parainfluenza virus 3	$2.60x10^5$	-	-	-
Human Parainfluenza virus 4A	$2.50x10^4$	-	-	-
Human Parainfluenza virus 4B	$1.30x10^4$	-	-	-
Herpes Simplex Virus Type 1 (McIntyre)	$1.00x10^5$	-	-	-
Herpes simplex virus type 2 (MS)	$1.30x10^4$	-	-	-
Human Metapneumovirus A1-Type 9	$1.00x10^5$	-	-	-
Human Metapneumovirus B1-Type 3	$7.70x10^5$	-	-	-
Cytomegalovirus AD-169	$3.60x10^4$	-	-	-
*Varicella Zoster (275)**	$1.53x10^7$	-	-	-
Human Coronavirus 229E	$7.50x10^4$	-	-	-
Human Coronavirus NL63	$7.00x10^3$	-	-	-
Human Coronavirus OC43	$1.10x10^6$	-	-	-
Echovirus 11	$5.50x10^6$	-	-	-
Enterovirus type 68	$1.00x10^5$	-	-	-
Enterovirus 71	$5.80x10^4$	-	-	-
Coxsackievirus A9	$1.10x10^5$	-	-	-
Coxsackievirus A21	$7.00x10^3$	-	-	-
Coxcaskievirus B4	$1.60x10^5$	-	-	-
Coxsackievirus B5	$1.00x10^5$	-	-	-

Bacterial strain	**Concentration (cfu/mL)**	**Inf A**	**Inf B**	**RSV**
Mycoplasma pneumoniae	$6x10^4$	-	-	-
Mycobacterium bovis	$5x10^4$	-	-	-
Candida albicans	$1x10^4$	-	-	-
Proteus mirabilis	$1x10^6$	-	-	-
Neisseria meningitidis DNA(1)	$4x10^6$	-	-	-
Bordetella pertussis DNA(1)	$2x10^6$	-	-	-
E. coli	$5x10^7$	-	-	-
Moraxella cattarhalis	$7x10^4$	-	-	-
Legionella pneumophila	$7x10^4$	-	-	+(2)
Streptococcus pneumoniae	$1x10^5$	-	-	-
Klebsiella pneumoniae	$2x10^6$	-	-	-
Haemophilus influenzae	$3x10^4$	-	-	-
Pseudomonas aeruginosa	$2x10^6$	-	-	-
Staphylococcus aureus(MSSA)	$7x10^6$	-	-	-
Staphylococcus aureus (MRSA)	$1x10^6$	-	-	-
Staphylococcus epidermidis	$4x10^6$	-	-	-
Streptococcus pyogenes	$1x10^5$	-	-	-

(1) DNA concentrations expressed as genomes/mL (2) 1/3 samples was positive for RSV

Interfering Substances

Blank analytical samples and analytical samples spiked with different concentrations of Influenza A, Influenza B or RSV were challenged in triplicate with high concentrations of potential interfering chemicals as recommended in FDA guidelines for verifying influenza diagnostic assays.

Substance	**Type of product**	**Active ingredient**	**Test concentration**
Mucin (10g)	Mucin (porcine type III stomach mucin)	Purified mucin protein	1.5% (w/v)
Blood	Blood (human)	N/A	2.0% (v/v)
Vicks Sinex (20ml)	Decongestant nasal spray	Oxymetazoline hydrochloride (0.05% w/v)	15.0% (v/v)
Vicks First Defence (15ml)	Cold preventer spray	Hydroxypropyl methylcellulose gel	5.0% (v/v)
Sudafed (12 x 12mg capsules)	Blocked nose capsules	Phenylephrine Hydrochloride (12mg)	5.0% (v/v)
Sterimar (50ml)	Sea water nasal spray	Sea water (100%)	15.0% (v/v)
Pirinase (60 x 50ug sprays)	Hayfever nasal spray	Fluticasone proprionate (0.05% w/v)	2.0% (v/v)
Nelson Sootha (150ml)	Homeopathic childrens cough syrup	Bryonia dioica 6c	5.0% (v/v)
Ultra Chloraseptic (15ml)	Anaesthetic throat spray	Benzocaine (0.71% w/v)	2.5% (v/v)
Menthol (5g)	Menthol 99%	Menthol	1.7mg/mL
Zanamivir (10mg)	Zanamivir >98%	Zanamivir	1.0mg/mL
Mupirocin (100mg)	Mupirocin	Mupirocin	1.0mg/mL
Tobramycin (100mg)	Tobramycin	Tobramycin	4µg/mL

No false results were obtained for any of the listed substances. Partial interference, affecting only one replicate in each case at LoD, was noted for:

Influenza A	**Influenza B**	**RSV B**
Mucin	Phenylephrine Hydrochloride	Hydroxypropyl methylcellulose
Bryonia dioica 6c	Sea water	
	Menthol	

Repeatability & Reproducibility

Two panels of two viruses each (influenza A and RSV A or influenza B and RSV B), were tested up to 40 times at low positive concentrations. Each concentration was tested at three times on 10 different MiniLab Platforms by three different operators using a single batch of Enigma® RVP test Cartridges.

Sample	**Strain**	**Concentration ($TCID_{50}$/mL)**	**Operator 1**	**Operator 2**	**Operator 3**	**%Total agreement by sample**
1	Influenza A Texas 50/12	10.0	100% (34/34)	100% (40/40)	98% (39/40)	99% (113/114)
	RSV A Long	9.0	100% (34/34)	100% (40/40)	95% (38/40)	98% (112/114)
2	Influenza B Brisbane 60/08	5.0	97% (33/34)	94% (31/33)	100% (37/37)	97% (101/104)
	RSV B WV 14617/85	1.5	97% (33/34)	97% (32/33)	100% (37/37)	98% (102/104)
% Total agreement by operator			99% (202/204)	98% (215/219)	99% (228/231)	99% (645/654)

Safety Data Sheet

The MiniLab RVP Test Safety Data Sheet (SDS) sheet is available from Enigma Diagnostics Ltd. by contacting the Technical Support department.

Technical Support

Enigma Diagnostics Ltd. strives to support its customers and collaborators to the very best of their ability. Should you have any inquires please contact the Technical Support department.

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