Laboratory:_____ Date Implemented_____

Address:_____

_____

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BinaxNOW® Influenza A & B Laboratory Procedure

I. Test Principle

The BinaxNOW® Influenza A & B test is an immunochromatographic membrane assay that uses highly sensitive monoclonal antibodies to detect influenza type A and B nucleoprotein antigens in respiratory specimens. These antibodies and a control antibody are immobilized onto a membrane support as three distinct lines and combined with other reagents/pads to construct a test strip. This test strip is mounted inside a cardboard, book-shaped hinged test device.

Swab specimens require a sample preparation step, in which the sample is eluted or washed off the swab into elution solution, saline or transport media. Nasal wash/aspirate samples require no preparation. Sample is added to the top of the test strip and the test device is closed. Test results are interpreted at 15 minutes based on the presence or absence of pink-to-purple colored Sample Lines. The blue Control Line turns pink in a valid assay.

II. Specimen Collection/Treatment

A. Specimen:	Acceptable: Nasal wash/aspirate; Nasopharyngeal or Nasal swab. Unacceptable: Specimens collected from other sources.
B. Swabs:	Acceptable: Cotton, rayon, foam, or polyester flexible-shaft NP swabs. Use cotton, rayon, foam, or polyester solid shaft swabs to collect nasal swab samples. Unacceptable: Calcium alginate swabs.
C. Transport Media:	Acceptable: Amies Media, Binax Elution Solution, Hank's Balanced Salt Solution, M4, M4-RT, M5, Stuart's Media, Saline, Brain Heart Infusion Broth, Dulbecco Medium, Phosphate Buffer, Stuart's Media, Tryptose Phosphate Broth, UTM-RT Media, Veal Infusion Broth. Unacceptable: Any other type of media. It has been determined that Sucrose-Phosphate Buffer may not be suitable for use with this test.
D. Specimen Transport:	Transport samples in a leak proof container.
E. Specimen Storage:	Process samples as soon as possible after collection. Swabs: Elute samples within 1 hr of collection. Eluted swab samples may be stored refrigerated up to 24 hrs. Nasal wash/aspirate: May be stored refrigerated up to 24 hrs.
F. Handling Precautions:	Patient samples, controls and test devices should be handled as though they could transmit disease. Observe established precautions against microbial hazards.

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III Reagents and Equipment

A. Reagents and Materials Provided

Component	Content
Test Device	A cardboard, book-like, hinged test device containing the test strip. A/Texas/1/77 was the master influenza virus strain used to develop the monoclonal antibodies incorporated into the test device.
Transfer Pipettes	Fixed Volume (100ul) transfer pipettes used to transfer sample to the test device. Use only pipettes provided by Binax.
Positive Control Swab	Inactivated influenza A/ Beijing or influenza A/Texas 1/77 (H3N2) and inactivated influenza B/ Harbin or influenza B/ Hong Kong 5/72 dried onto swab. The influenza viruses are originally grown in embryonic eggs and are Formalin or gamma radiation inactivated. Formalin treated viruses are tested for inactivation and non-infectiousness by re-growing virus in embryonic eggs. Viruses are considered inactivated when no viral propagation is seen in eggs.
Negative Control Swab	Inactivated *Streptococcus* Group A dried onto swab. Organism used to inoculate the swab is heat inactivated, and then tested for inactivation and non-infectiousness by standard culture. The organisms are determined to be inactivated when no growth is present on the plate.
Elution Solution Vials for Control Swabs/ Swab Specimens	Vials containing elution solution used to prepare the Control Swabs/Swab Specimens for testing.
NP Swabs	Sterile, foam swabs for use in the BinaxNOW Influenza A & B Test
Package Insert	

B. Materials not Provided

A timer, watch, or clock
Nasal wash/aspirate collection containers

C. Storage and Stability

Store Test Kit at room temperature 15° - 30 °C (59° - 86°F).
The BinaxNOW Influenza A & B Test kit and reagents are stable until the expiration dates marked on their outer packaging and containers.

D. Quality Control

Internal Procedural Controls

The BinaxNOW Influenza A & B test has built-in (internal) procedural controls. For daily quality control, Inverness Medical suggests that you record results of these controls for each test run.

- An untested strip has a blue line at the “Control” position. If the test flows correctly and the reagents work, this blue line will always turn pink on the strip.
- The clearing of background color from the result window is a negative background control. The background color in the window should change from light pink to white within 15 minutes. Background color should not interfere with the reading of the test.

External Quality Control Testing

Good laboratory practice suggests the use of positive and negative controls to guarantee that test reagents are working and the test is correctly performed. The BinaxNOW Test Kits contain Positive and Negative Control Swabs. These swabs will verify the entire assay. Test these swabs once with each new shipment received. Other controls may be tested in order to conform with local, state and/or federal regulations, accrediting groups, and/or your lab's standard Quality Control procedures.

Remedial Actions

When correct control results are not obtained, do not report patient results. Contact Inverness Medical Technical Services at 1-800-637-3717.

E. Precautions

- For *in vitro* diagnostic use.
- Leave test sealed in its foil pouch until just before use.
- Do not use kit past its expiration date
- Do not mix components from different kit lots.
- The **white** sample pad at the top of the test strip contains reagents that extract the target antigen from the virus. To ensure best performance, add the sample **SLOWLY** (drop by drop) to the **MIDDLE** of this pad, without touching with the pipette, such that all of the sample absorbs into the pad. DO NOT add sample to the pink/purple colored pad.
- Solutions used to make the control swabs are inactivated using standard methods. However, patient samples, controls, and tests should be handled as though they could transmit disease. Observe established precautions against microbial hazards. The use of lab coats, gloves, and safety eye glasses is recommended.
- All transfer pipettes and test vials are single use items - do not use with more than one specimen.
- The ability of this test to detect avian influenza was determined using cultured avian influenza viruses; the performance characteristics of this test with specimens collected from humans infected with H5N1 or other avian influenzas is unknown.
- If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3 + facility is available to receive culture specimens.
- Performance characteristics for influenza A were established when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

IV. Test Procedure

Sample Preparation Procedure

Nasal Wash/Aspirate: Nasal wash/Aspirate samples do not need preparation. Go to Test Procedure.

Precaution: When testing nasal wash/aspirate samples, avoid thick areas of the sample when drawing it into the transfer pipette. If the pipette becomes clogged, and the lower part of the pipette is not full, put the sample back into container by squeezing the top bulb. Redraw the sample into the pipette. Use a new pipette if needed

Swab (Control & Patient) Elution using Binax Elution Solution:

1. Twist off the test vial cap.
2. Put the swab to be tested into test vial. Rotate the swab vigorously (without making a lot of bubbles) three (3) times in the liquid.
3. Press the swab against the side of the vial and turn as you remove it from the vial. This removes sample from the swab.
4. Discard the swab into a container intended for contagious material.
5. Test the liquid sample (from the test vial) in the BinaxNOW Test as soon as possible. Go to Test Procedure.

Swab Elution Using Transport Media:
Remove sample from swab in 0.5 to 3.0 ml of saline or transport media/fluid by vigorously rotating the swab in the liquid. Refer to Specimen Collection and Handling section for acceptable transport media. Go to Test Procedure.

Test Procedure:

WARNING: INVALID RESULTS can occur when too little sample is added to the test. Be sure that the lower part of the transfer pipette is full and does not have any air spaces before you add the sample to the Sample Pad. If there are air spaces, put the sample back into the container by squeezing the top bulb. Redraw the sample from the bottom of the container into the pipette. Use a new pipette if needed.

1. Remove device from the pouch just prior to testing and lay flat on work bench.

2. Fill pipette by firmly squeezing the top bulb and **then** placing pipette tip into sample. Slowly release bulb while tip is still in sample. This will pull liquid into the pipette. Make sure there are no air spaces in the lower part of the pipette.

3. See arrow on test to find **WHITE** Sample Pad at the top of the test strip. **SLOWLY** (drop by drop) add entire contents of pipette (100uL) to the **MIDDLE** of this pad by squeezing the top bulb, such that all of the sample volume absorbs into this pad. **DO NOT** add sample to the pink/purple colored pad.

4. Immediately peel off brown adhesive liner from the test device. Close and securely seal the device. Read result in window 15 minutes after closing the device.

   NOTE: When reading test results, tilt the device to reduce glare on the result window if necessary

V. Interpretation of Test Results

NOTE: Do not read test result before or after 15 minutes as they may not be correct. . Individuals with color impaired vision may not be able to adequately interpret test results.

Negative Result: The BLUE Control Line in the **BOTTOM THIRD** of the window turns a pink to purple color. No other line appears.

Flu A Positive Result: The BLUE Control Line turns a pink to purple color and a SECOND pink to purple Sample Line appears above it in the **MIDDLE THIRD** of the window. Any shade of a pink to purple Sample Line, even when very faint, indicates a positive result.

Flu B Positive Result: The BLUE Control Line turns a pink to purple color and a SECOND pink to purple Sample Line appears above it in the **TOP THIRD** of the window. Any shade of a pink to purple Sample Line, even when very faint, indicates a positive result.

Flu A and Flu B Positive Result: The BLUE Control Line turns a pink to purple color, AND two pink to purple Sample Lines appear above it in the **MIDDLE and TOP** thirds of the window. Any shade of pink to purple Sample Lines indicates positive results.

Invalid: A test is invalid if the Control Line remains BLUE or is not present at all, whether a Sample Line(s) is present or not.

Remedial Actions
Repeat Invalid tests with a new test device. Call Inverness Medical Technical Services at 1-800-637-3717, if problem persists.

VI. Interfering Substances

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity or nasopharynx, were evaluated in the BinaxNOW Influenza A & B Test at the concentrations listed and were found not to affect test performance. Whole blood (1%) did not interfere with the interpretation of negative BinaxNOW Test results, but did interfere with the interpretation of Flu A LOD positive samples. Therefore, visibly bloody samples may not be appropriate for use in this test.

Substance	Concentration
1 OTC mouthwashes	20%
3 OTC nasal sprays	15%
3 OTC throat drops	15%
2 OTC throat sprays	20%
4-acetamidophenol	10 mg/ml
Acetylsalicylic acid	15 mg/ml
Albuterol	20 mg/ml
Chlorpheniramine	5 mg/ml
Dextromethorphan	10 mg/ml
Diphenhydramine	5 mg/ml
Guaiacol glycerol ether	20 mg/ml
Oxymetazoline	0.05%
Phenylephrine	50 mg/ml
Phenylpropanolamine	20 mg/ml
Rebetol®	500 ng/ml
Relenza®	20 mg/ml
Rimantadine	500 ng/ml
Synagis®	0.1 mg/ml
Tamiflu®	50 mg/ml

Transport Media:

The following transport media were tested in the BinaxNOW Influenza A and/or B Test as negative samples (no virus present) and after inoculation with the LOD levels of Influenza A & B. Media did not impact BinaxNOW test performance, with the media alone testing negative in the BinaxNOW Test and media inoculated with LOD Influenza A & B testing positive on the appropriate test line in BinaxNOW test.

Amies Media	Hank's Balanced Salt Solution
M4 Media	M4-RT Media
M5 Media	Stuart's Media
Saline	Brain Heart Infusion Broth
Dulbecco Medium	Phosphate Buffer Solution
UTM-RT Media	Tryptose Phosphate Broth
Veal Infusion Broth	

It has been determined that Sucrose-Phosphate Buffer may not be suitable for use with this test.

VII. Limitations

- A negative test result does not exclude infection with influenza A and/or B. Therefore, the results obtained with the BinaxNOW Influenza A & B Test should be used in conjunction with clinical findings to make an accurate diagnosis. Additional testing is required to differentiate any specific influenza A and B subtypes of strains, in consultation with state or local public health departments.
- The BinaxNOW Influenza A & B Test detects both viable and non-viable influenza A and B. Test performance depends on the amount of virus (antigen) in the specimen and may or may not compare with cell culture results performed on the same specimen.
- Monoclonal antibodies may fail to detect , or detect with less sensitivity, influenza A and B viruses that have undergone minor amino acid changes in the target epitope region.
- Inadequate specimen collection or improper sample handling/transport may yield a false-negative result.
- Performance of the BinaxNOW Influenza A & B Test has not been established for monitoring antiviral treatment of influenza.
- Positive and negative predictive values of *in vitro* diagnostic tests are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive results are more likely during periods of low influenza activity when prevalence is moderate to low.
- Individuals who have received nasally administered influenza A vaccine may test positive in commercially available influenza rapid diagnostic tests for up to three days after vaccination.
- Children tend to shed virus more abundantly and for longer periods of time than adults. Therefore, *in vitro* diagnostic tests for influenza may have lower sensitivity in adults than in children.
- Use of visibly bloody samples is not recommended with the BinaxNOW Influenza A & B Test.

VIII. Expected Results

The prevalence of influenza varies from year to year, with outbreaks typically occurring during the fall and winter months.[1] The rate of positivity found in influenza testing is dependent on many factors including the method of specimen collection, the test method used, geographic location, and the disease prevalence in specific localities. Type A viruses are typically

associated with most serious influenza epidemics, while Type B are typically milder. In multi-center clinical studies conducted by Binax outside the U.S. during the 2004 respiratory season and in the U.S. during the 2004-2005 respiratory season, the average prevalence of influenza A (as determined by viral cell culture) was 18%. The average prevalence of influenza B was 3%.

IX. Analytical Specificity and Cross Reactivity

To determine the analytical specificity of the BinaxNOW® Influenza A & B Test, 36 commensal and pathogenic microorganisms (27 bacteria, 8 viruses and 1 yeast) that may be present in the nasal cavity or nasopharynx were tested. All of the following microorganisms were negative when tested at concentrations ranging from 10^4 to 10^8 $TCID_{50}$/mL (viruses), 10^7 to 10^8 organisms/mL (bacteria) and 10^6 organisms/mL (yeast).

Bacteria

Acinetobacter	*Neisseria subflava*
Bordetella pertussis	*Proteus vulgaris*
Enterococcus faecalis	*Pseudomonas aeruginosa*
Escherichia coli	*Serratia marcescens*
Gardnerella vaginalis	*Staphylococcus aureus*
Haemophilus influenzae	*Staphylococcus aureus* (Cowan protein A producing strain)
Klebsiella pneumoniae	*Staphylococcus epidermidis*
Lactobacillus casei	*Streptococcus,* Group A
Legionella pneumophila	*Streptococcus*, Group B
Listeria monocytogenes	*Streptococcus*, Group C
Moraxella catarrhalis	*Streptococcus*, Group F
Neisseria gonorrhoeae	*Streptococcus mutans*
Neisseria meningitidis	*Streptococcus pneumoniae*
Neisseria sicca	

Virus	Yeast
Adenovirus	*Candida albicans*
Coronavirus	
Coxsackie B4	
Cytomegalovirus (CMV)	
Parainfluenza 1	
Parainfluenza 2	
Parainfluenza 3	
Respiratory Syncytial Virus (RSV)	

X. Analytical Reactivity

The Influenza A and B strains listed tested positive in the BinaxNOW Influenza A & B Test at concentrations specified. Although the specific influenza strains causing infection in humans can vary year to year, all contain the conserved nucleoproteins targeted by the BinaxNOW test.[2] Performance characteristics of the BinaxNOW Influenza A & B Test for detecting influenza A virus from human specimens was established when H1 and H3 subtypes were prevalent. Performance characteristics of the test when other influenza A virus subtypes are emerging as human pathogens have not been established.

Influenza Strain	ATCC#	Concentration
Flu A/WS/33 (H1N1)	VR-825	10^2-10^6 $CEID_{50}$/ml
Flu A/NWS/33 (H1N1)	VR-219	10^2-10^6 $CEID_{50}$/ml
Flu A/Hong Kong/8/68 (H3N2)	VR-544	10^2-10^6 $CEID_{50}$/ml
Flu A/Aichi/2/68 (H3N2)	VR-547	10^2-10^6 $CEID_{50}$/ml
Flu A /New Jersey/8/76 (Hsw1N1)	VR-897	10^2-10^6 $CEID_{50}$/ml
Flu A/ Mal/302/54 (H1N1)	VR-98	10^2-10^6 $CEID_{50}$/ml
Flu A/Port Chalmers/1/73 (H3N2)	VR-810	10^2-10^6 $CEID_{50}$/ml
Flu A/ Hong Kong/156/97 (H5N1)	-	1.3 x 10^2 $TCID_{50}$/ml
Flu A/ Vietnam/1194/04 (H5N1)	-	1.0 x 10^4 $TCID_{50}$/ml
Flu A / Chicken/NY/117228-7/01 (H5N2)	-	1.0 x 10^4 EID_{50}/ml
Flu A/ Turkey/VA SEP-66/02 (H7N2)	-	1.0 x 10^5 EID_{50}/ml
Flu B/Lee/40	VR-101	10^2 -10^6 $CEID_{50}$/ml
Flu B/Brigit	VR-786	10^2 -10^6 $CEID_{50}$/ml
Flu B/Russia/69	VR790	10^2 -10^6 $CEID_{50}$/ml
Flu B/Hong Kong/5/72	VR-791	10^2 -10^6 $CEID_{50}$/ml
Flu B/R75	VR-789	10^2 -10^6 $CEID_{50}$/ml

XI. Performance Characteristics

The clinical performance of the BinaxNOW Influenza A & B Test was established in multi-center, prospective, clinical studies conducted at a central testing laboratory outside the US during the 2004 respiratory season and at three US trial sites during the 2005-2006 respiratory season. Additional performance testing was conducted on retrospective frozen clinical samples collected from symptomatic patients at multiple physician offices, clinics and hospitals located in the Southern, Northeastern and Midwestern regions of the United States and from one hospital in Sweden.

Clinical Studies:
BinaxNOW Influenza A & B Test Performance vs. Cell Culture/ DFA- Prospective Study

A total of 846 prospective specimens collected from children (less than 18 years of age) and adults (18 years or older) were evaluated in the BinaxNOW Influenza A & B Test and compared to culture/ DFA. Evaluated specimens include nasopharyngeal and nasal swabs collected from patients presenting with influenza-like symptoms. Forty-four percent (44%) of the population tested was male, 56% female, 54% pediatric (< 18 years), and 46% adult (≥18 years). No differences in test performance were observed based on patient age or gender. A/H3 and A/H1 were the predominant influenza subtypes observed during this time.

BinaxNOW Influenza A & B Test performance by sample type versus cell culture/DFA, including 95% confidence intervals, is listed below.

BinaxNOW Influenza A & B Test Performance vs. Cell Culture/DFA for Detection of Flu A

	Test Sensitivity				Test Specificity			
Sample	**+/+**	**- /+**	**%Sens**	**95% CI**	**- /-**	**+/-**	**%Spec**	**95% CI**
NP Swab	53	16	77%	65-86%	278	3	99%	97-100%
Nasal Swab	85	17	83%	74-90%	378	16	96%	93-98%
Overall	138	33	81%	74-86%	656	19	97%	96-98%

BinaxNOW Influenza A & B Test Performance vs. Cell Culture/DFA for Detection of Flu B

	Test Sensitivity				Test Specificity			
Sample	**+/+**	**- /+**	**%Sens**	**95% CI**	**- /-**	**+/-**	**%Spec**	**95% CI**
NP Swab	2	2	50%	9-91%	346	0	100%	99-100%
Nasal Swab	9	4	69%	39-90%	481	2	100%	98-100%
Overall	11	6	65%	39-85%	827	2	100%	99-100%

BinaxNOW Influenza A & B Test Performance vs. Cell Culture/ DFA- Retrospective Study

A total of 293 retrospective frozen clinical samples were evaluated in the BinaxNOW Influenza A & B Test and compared to culture/ DFA. All clinical samples were collected from symptomatic patients at multiple physician offices, clinics and hospitals located in the Southern, Northeastern and Midwestern regions of the United States and from one hospital in Sweden. Fifty-three percent (53%) of the population tested was male, 47% female, 62% pediatric (<18 years) and 38% adult (≥ 18 years). Nasal wash/aspirate specimens comprised approximately 61% of the samples tested, while NP swabs represented 39%. No differences in test performance were observed based on patient age and gender or based on sample type tested.

BinaxNOW A & B Test performance by sample type versus cell culture/ DFA, including 95% confidence intervals, is listed below.

BinaxNOW Influenza A & B Test Performance vs. Cell Culture/ DFA for Detection of Flu A

	Test Sensitivity				Test Specificity			
Sample	**+/+**	**- /+**	**%Sens**	**95% CI**	**- /-**	**+/-**	**%Spec**	**95% CI**
NP Swab	19	8	70%	50-86%	77	9	90%	81-95%
Wash/Aspirate	51	6	89%	78-96%	117	6	95%	89-98%
Overall	70	14	83%	73-90%	194	15	93%	88-96%

BinaxNOW Influenza A & B Test Performance vs. Cell Culture/ DFA for Detection of Flu B

	Test Sensitivity				Test Specificity			
Sample	**+/+**	**- /+**	**%Sens**	**95% CI**	**- /-**	**+/-**	**%Spec**	**95% CI**
NP Swab	0	0	N/A	N/A	111	2	98%	93-100%
Wash/Aspirate	8	7	53%	27-78%	155	10	94%	89-97%
Overall	8	7	53%	27-78%	266	12	96%	92-98%

Analytical Sensitivity

The BinaxNOW Test limit of detection (LOD), defined as the concentration of influenza virus that produces positive BinaxNOW test results approximately 95% of the time, was identified by evaluating different concentrations of inactivated Flu A/Beijing and inactivated Flu B/Harbin in the BinaxNOW Test.

Twelve (12) different operators each interpreted 2 tests run at each concentration for a total of 24 determinations per level. The following results identify a concentration of 1.03×10^2 ng/ml as the LOD for Flu A/Beijing and 6.05×10^1 ng/ml for Flu B/Harbin.

Flu A/Beijing		
Concentration (ng/ml)	# Detected	% Detected
1.03×10^2 (LOD)	23/24	96
5.60×10^1 (Cutoff)	*	50
3.27×10^1 (High Neg)	4/24	17
True Negative	0/24	0

Flu B/Harbin		
Concentration (ng/ml)	# Detected	% Detected
6.05×10^1 (LOD)	23/24	96
2.42×10^1 (Cutoff)	11/24	46
1.51×10^1 (High Neg)	6/24	25
True Negative	0/24	0

*Linear regression was used to calculate a line equation, which was then used to project the cutoff concentration of Flu A/Beijing.

XII. Reproducibility

A blind study of the BinaxNOW Influenza A & B Test was conducted at 3 separate sites using panels of blind coded specimens containing negative, low positive, and moderate positive samples. Participants tested each sample multiple times on 3 different days. There was 97% (242/250) agreement with expected test results, with no significant differences within run (replicates tested by one operator), between run (3 different days), between sites (3 sites), or between operators (6 operators).

XIII. Consumer Precision Studies:

Swab Specimen

The BinaxNOW Influenza A & B Test was evaluated by twelve (12) adults with no professional laboratory experience (intended user) at three (3) intended use sites. Each operator at each site tested thirty-five (35) samples from a randomly coded panel of true positive, true negative, limit of detection (LOD) and below LOD for both influenza A and influenza B. In order to demonstrate equivalent performance among intended users and trained laboratorians, twenty-four (24) trained laboratorians, ran blind coded panels of Flu A and Flu B samples containing the same true negative, true positive, below LOD, and LOD samples described above.

As indicated by the overlapping 95% confidence intervals in the tables below, no significant differences were observed between the intended users and the expected results established by the trained laboratorians. These results demonstrate that users with no formal laboratory training can read the package insert and perform the Binax test with the same precision as trained laboratorians.

Influenza A and Influenza B Sample Testing – Intended Users vs. Trained Laboratorians – Overall Results

	Participant Type	Negative - % Negative (95% CI)	Below LOD - % Detection (95% CI)	LOD - % Detection (95% CI)	True Positive % Detection (95% CI)	% Invalid Tests
Flu A Samples	Intended User	97% (57/59) (88-99)	79% (46/58) (67-88)	95% (59/62) (87-98)	100% (60/60) (94-100)	1.6% (4/243)
	Trained Laboratorian	99%(179/180) (97-100)	84%(102/121) (77-90)	99%(179/180) (97-100)	100%(120/120) (97-100)	0% (0/601)
Flu B Samples	Intended User		72% (43/60) (59-81)	97% (59/61) (89-99)	98% (58/59) (91-100)	.6% (1/181)
	Trained Laboratorian		78% (93/120) (69-84)	99% (179/180) (97-100)	100%(120/120) (97-100)	0% (0/420)

Influenza A Sample Testing by Site – Intended Users (IU) and Trained Laboratorians (TL)

	Site#	Negative % Negative (95% CI)	Below LOD % Detection (95% CI)	LOD % Detection (95% CI)	True Positive % Detection (95% CI)	% Invalid Tests
Intended User (IU) Results	1 IU	89% (17/19) (68-97)	63% (12/19) (41-84)	100% (20/20) (84-100)	100% (20/20) (84-100)	2.5% (2/80)
	2 IU	100% (20/20) (84-100)	79% (15/19) (56-91)	90% (19/21) (71-97)	100% (20/20) (84-100)	2.4% (2/82)
	3 IU	100% (20/20) (84-100)	95%(19/20) (76-99)	95% (20/21) (77-99)	100% (20/20) (84-100)	0% (0/81)
Trained Laboratorian (TL) Results	1 TL	100% (25/25) (87-100)	64% (16/25) (44-80)	100% (25/25) (87-100)	100% (25/25) (87-100)	0% (0/100)
	2 TL	100% (15/15) (79-100)	87% (13/15) (62-96)	100% (15/15) (79-100)	100% (15/15) (79-100)	0% (0/60)
	3 TL	100% (20/20) (84-100)	75% (15/20) (53-89)	100% (20/20) (84-100)	100% (20/20) (84-100)	0% (0/80)
	4 TL	99% (119/120) (95-100)	95% (58/61) (87-98)	99% (119-120) (95-100)	100% (60/60) (94-100)	0% (0/361)

Influenza B Sample Testing by Site – Intended Users (IU) and Trained Laboratorians (TL)

	Site#	Below LOD % Detection (95% CI)	LOD %Detection (95% CI)	True Positive % Detection (95% CI)	% Invalid Tests
Intended User (IU) Results	1 IU	70% (14/20) (48-85)	95% (19/20) (76-99)	100% (19/19) (83-100)	1.7% (1/60)
	2 IU	65% (13/20) (43-82)	95% (20/21) (77-99)	100% (20/20) (84-100)	0% (0/61)
	3 IU	80% (16/20) (58-92)	100% (20/20) (84-100)	95% (19/20) (76-99)	0% (0/60)
Trained Laboratorian (TL) Results	1 TL	60% (15/25) (41-77)	100% (25/25) (87-100)	100% (25/25) (87-100)	0% (0/75)
	2 TL	73% (11/15) (48-89)	100% (15/15) (79-100)	100% (15/15) (79-100)	0% (0/45)
	3 TL	65% (13/20) (43-82)	100% (20/20) (84-100)	100% (20/20) (84-100)	0% (0/60)
	4 TL	90% (54/60) (80-95)	99% (119/120) (95-100)	100% (60/60) (94-100)	0% (0/240)

Liquid Specimen

According to the 1995 CLIA Rule, Inverness Medical conducted their Consumer Precision testing of the BinaxNOW Influenza A & B Test with a total of 120 lay users at 6 sites. Participants tested proficiency panels consisting of high negative, assay cutoff, and limit of detection (LOD) samples for both influenza A and influenza B, as well as true negative samples. Expected results for each sample type were generated by trained laboratorians. Six percent (6%) of the total tests run by the lay users and 0.4% of the test run by the trained laboratorians resulted in invalid tests. The tables below detail the number of invalid tests generated by each site.

As indicated by the overlapping 95% confidence intervals in the tables below, no significant differences were observed between the lay users and the expected results established by the trained laboratorians. These results demonstrate that users with no formal laboratory training can read the package insert and perform the Inverness Medical tests with a relatively high level of precision.

Influenza A and Influenza B Sample Testing – Lay Users vs. Trained Laboratorians – Overall Results

	Participant Type	Negative: % Negative (95% CI)	High Negative*: % Detection (95% CI)	Assay Cutoff*: % Detection (95% CI)	LOD: % Detection (95% CI)	% Invalid Tests
Flu A Samples	Lay User	96% (54/56) (88-99)	32% (18/57) (21-44)	78% (46/59) (66-87)	95% (57/60) (86-98)	3.3% (8/240)
	Trained Laboratorian	100% (8/8) (66-100)	22%(8/36) (12-38)	64% (23/36) (47-77)	94% (34/36) (82-98)	0.9% (1/117)
Flu B Samples	Lay User	96% (53/55) (88-99)	4% (2/52) (1-13)	27% (15/56) (17-40)	82% (45/55) (70-90)	9.2% (22/240)
	Trained Laboratorian	100% (9/9) (69-100)	11% (4/36) (4-25)	49% (17/35) (33-64)	92% (33/36) (78-97)	0.0% (0/116)

* These levels are below the detection limit of the test.

Warning: Invalid test results can occur when an insufficient volume of sample is added to the test device due to misuse of the transfer pipette. Please see the Test Procedure section for detailed instructions on the proper use of the transfer pipette.

Influenza A Sample Testing by Site – Lay Users (LU) and Trained Laboratorians (TL)

	Site#	Negative: % Negative (95% CI)	High Negative: % Detection (95% CI)	Assay Cutoff: % Detection (95% CI)	LOD: % Detection (95% CI)	% Invalid Tests
Lay User (LU) Results	2 LU	100% (18/18) (82-100)	33% (6/18) (16-57)	70% (14/20) (48-85)	90% (18/20) (70-97)	5.0% (4/80)
	4 LU	90% (18/20) (70/97)	45% (9/20) (26-66)	85% (17/20) (64-94)	100% (20/20) (84-100)	0% (0/80)
	6 LU	100% (18/18) (82-100)	16%(3/19) (6-38)	79% (15/19) (56-91)	95% (19/20) (76-99)	5.0% (4/80)
Trained Laboratorian (TL) Results	1 TL	100% (3/3) (40-99)	0% (0/12) (0-25)	25% (3/12) (9-54)	100% (12/12) (75-100)	0% (0/39)
	2 TL	100% (2/2) (29-99)	33% (4/12) (14-61)	92% (11/12) (64-98)	83% (10/12) (54-95)	3% (1/39)
	3 TL	100% (3/3) (40-99)	33% (4/12) (14-61)	75% (9/12) (46-90)	100% (12/12) (75-100)	0% (0/39)

Influenza B Sample Testing by Site – Lay Users (LU) and Trained Laboratorians (TL)

	Site#	Negative: % Negative (95% CI)	High Negative: % Detection (95% CI)	Assay Cutoff: % Detection (95% CI)	LOD: % Detection (95% CI)	% Invalid Tests
Lay User (LU) Results	1 LU	100% (20/20) (84-100	5% (1/19) (1-25)	25% (5/20) (11-47)	85% (17/20) (64-94)	1% (1/80)
	3 LU	100% (19/19) (83/100)	0% (0/18) (0-18)	25% (5/20) (11-47)	79% (15/19) (56-91)	5% (4/80)
	5 LU	87% (14/16) (63-96)	7%(1/15) (2-30)	31% (5/16) (14-56)	81% (13/16) (57-93)	21% (17/80)
Trained Laboratorian (TL) Results	1 TL	100% (3/3) (40-99)	0% (0/12) (0-25)	9% (1/11) (21-38)	75% (9/12) (46-91)	0% (0/38)
	2 TL	100% (3/3) (40-99)	33% (4/12) (14-61)	67% (8/12) (39-86)	100% (12/12) (75-100)	0% (0/39)
	3 TL	100% (3/3) (40-99)	0% (0/12) (0-25)	67% (8/12) (39-86)	100% (12/12) (75-100)	0% (0/39)

XIV. References

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