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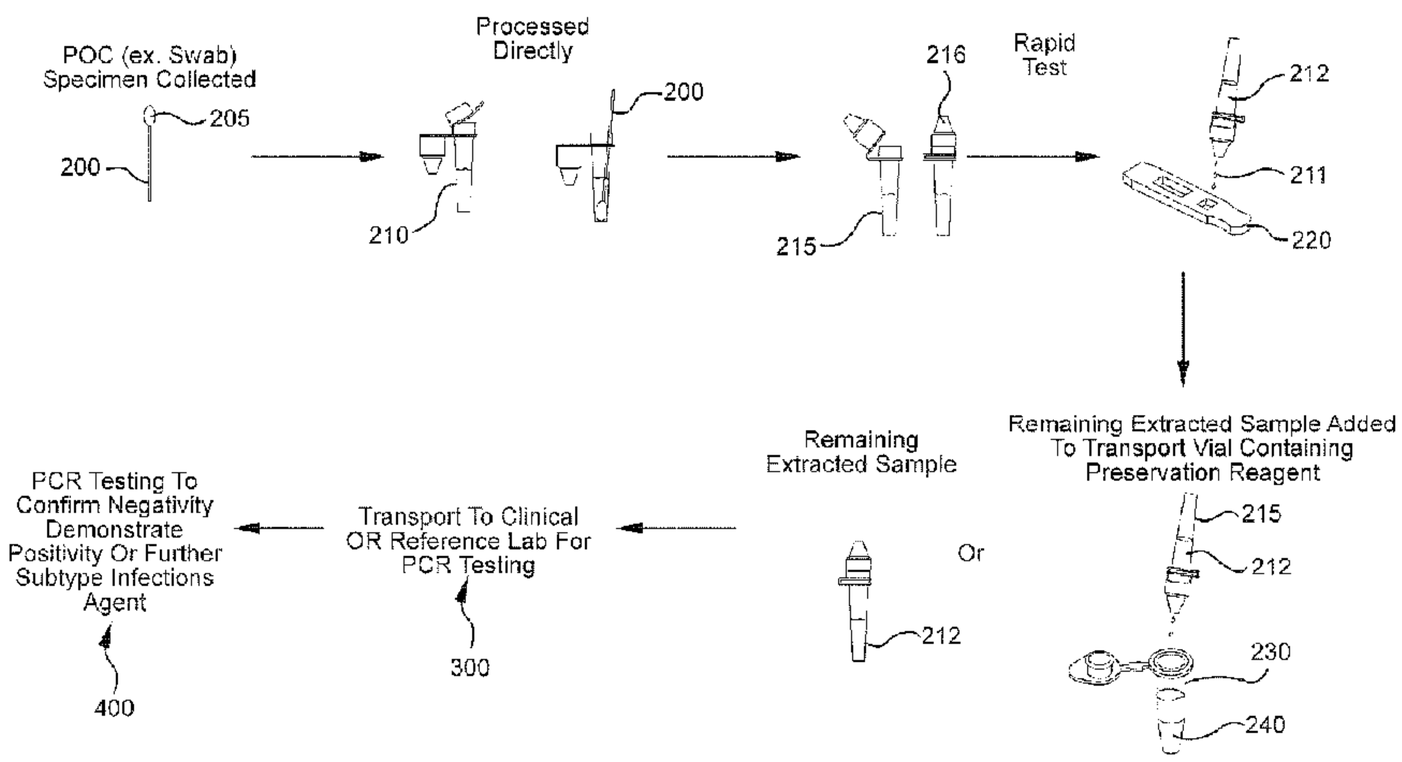
(54) Title: METHOD FOR LINKING POINT OF CARE RAPID DIAGNOSTIC TESTING RESULTS TO LABORATORY-BASED METHODS



(57) Abrégé/Abstract:
A method for using a single sample suspected of containing a microorganism for both a local rapid test immunoassay and a remote laboratory test. The sample is collected from a patient at a physician's office or from the environment to be tested. The sample is collected using a swab or other implement, combined with a rapid test processing reagent and a portion of the processed sample is used for the local rapid test. The rapid test processing reagent typically consists of a buffer, a salt, and a detergent and is compatible with the local rapid test immunoassay. Only a portion of the processed sample is used for the local rapid test, leaving a remaining portion of the processed sample to be used in a remote laboratory assay. At least some of the remaining portion of the processed sample is combined with a stabilization agent that preserves at least the nucleic acid in the processed sample for the remote laboratory assay.

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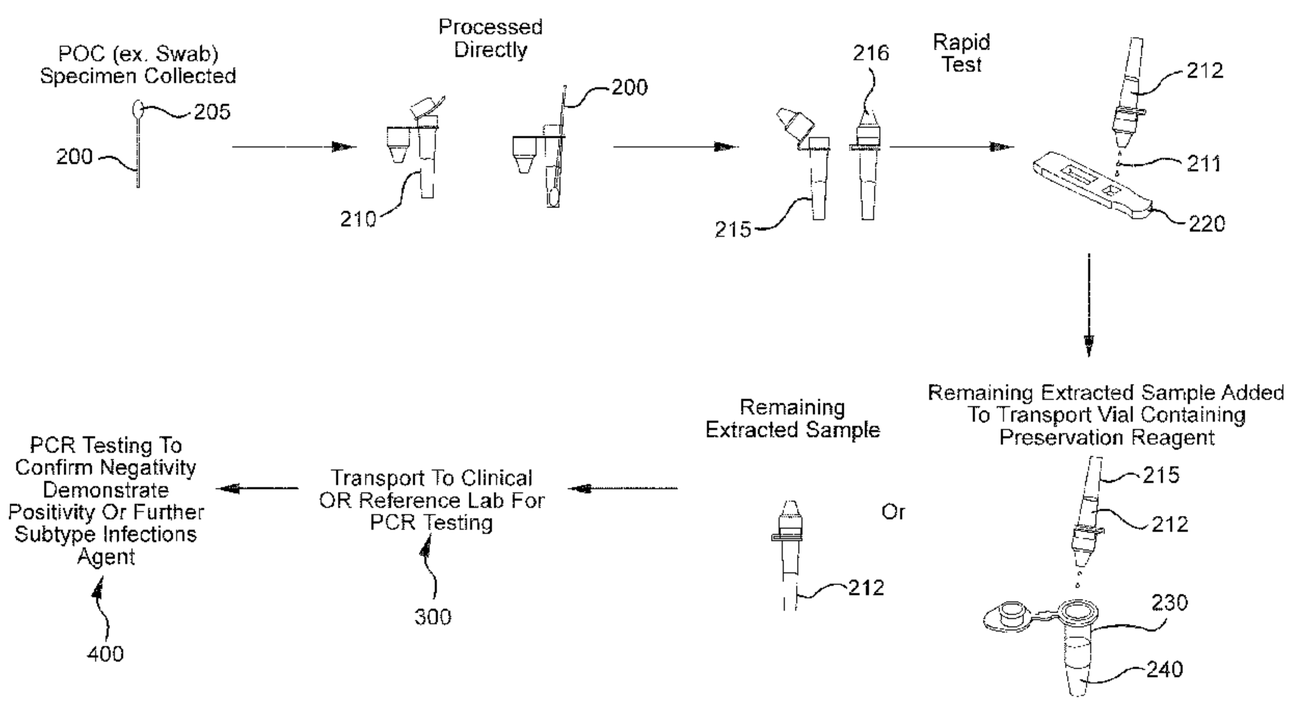
FIG. 1B



(57) **Abstract**: A method for using a single sample suspected of containing a microorganism for both a local rapid test immunoassay and a remote laboratory test. The sample is collected from a patient at a physician's office or from the environment to be tested. The sample is collected using a swab or other implement, combined with a rapid test processing reagent and a portion of the processed sample is used for the local rapid test. The rapid test processing reagent typically consists of a buffer, a salt, and a detergent and is compatible with the local rapid test immunoassay. Only a portion of the processed sample is used for the local rapid test, leaving a remaining portion of the processed sample to be used in a remote laboratory assay. At least some of the remaining portion of the processed sample is combined with a stabilization agent that preserves at least the nucleic acid in the processed sample for the remote laboratory assay.

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[0001] # METHOD FOR LINKING POINT OF CARE RAPID DIAGNOSTIC TESTING RESULTS TO LABORATORY-BASED METHODS

BACKGROUND OF THE INVENTION

[0002] The linkage between point of care (POC) rapid testing and laboratory-based testing has typically been addressed through preservation of samples to support culture-based laboratory testing methods. Currently, samples collected at the POC site are either processed and used directly in a rapid test (the portion of the processed sample not used in the rapid test being discarded), or diluted in liquid transport media to enable transfer for laboratory-based testing such as rapid immunoassay, culture and/or polymerase chain reaction (PCR). Specimens that generate negative results from a POC test are often reflex tested -the negative result is confirmed by lab-based testing methods such as PCR. In addition, specimens that generate positive POC test results are frequently tested for additional characterizations such as subtyping or other epidemiologic information.

[0003] Referring to FIG. 1, at physician office POC sites (and other non-laboratory sites where patients are seen or samples for rapid testing are collected), swab specimens 100 are used almost exclusively to deliver sample into solution 110 for the rapid test 120. Processing and testing the sample in the physician's office and other non-laboratory sample collection sites does not contemplate a means for enabling lab-based testing such as confirmatory and/or reflex testing or other tests the require lab-based analysis. With the current methods, a physician (or other administrator of a

POC rapid test) cannot perform both a POC rapid test and a lab-based test using the sample collected at the site (e.g. the physician's office). Therefore, an opportunity to perform lab-based testing on such samples is lost. Swab samples 130 collected at POC sites within a hospital or clinic are almost exclusively placed within a volume of liquid transport media 140 for transfer to the testing laboratory for remote testing. The diluted samples 150 may be further processed by adding them to a solution 160 for a rapid test 170. However, this method often results in a POC sample diluted 5 to 10-fold, or more, which can diminish performance of the rapid test due to sample dilution effects.

[0004] Collection and transport of a second swab at the POC site could be used to address the need to perform laboratory-based testing, although this is clearly not the standard of practice and doubles the number of samples to be taken. In addition, although collected from the same patient, variations in collection methods, organism load, etc. could lead to erroneous results when comparing the test results between two independently collected swab specimens. Accordingly, a system and method that addresses these problems is desired.

SUMMARY OF THE INVENTION

[0005] The various embodiments of the present invention enable linkage between POC rapid tests (e.g. immunoassays such as a test for flu virus) and laboratory-based testing (confirmatory testing or other laboratory tests such as diagnostic and identification testing) through the use of a single sample collected and subjected to a rapid test at the POC. The sample (which is any sample that is suspected of containing a target microorganism) is collected and processed under optimal conditions for the particular POC test utilized, ensuring the best possible clinical performance for the POC test (also referred to as a rapid test herein). The

sample can be a biological sample collected from a patient and can include any biological fluid or tissue sample including, but not limited to, blood, urine, saliva, and tissue scraped or swabbed from a patient. Samples can also include environmental samples collected in the conventional manner of wiping or swabbing a surface suspected of having the contaminating microorganism. Environmental samples can also include soil samples, air samples, water samples, food samples, etc. Typically, the sample is processed by combining it with a processing reagent compatible with the rapid test. The portion of the sample not used for the rapid test has heretofore been typically discarded. According to one embodiment of the present invention, the remainder of the processed sample is then preserved to allow transfer to a laboratory-based testing environment where confirmatory testing, such as nucleic acid-based testing, can be performed. In other embodiments the remainder is not further diluted, but is still subjected to a laboratory-test. For purposes of the present invention, the site of sample collection and rapid test is referred to as local or POC, while the site for laboratory-based testing is referred to as remote. In the context of the invention, remote simply means removed from the site of sample collection and rapid testing. Remote could vary from very close distances such as different locations in the same building to much larger distances.

[0006] The methods described herein can be applied to rapid immunoassays used at POC for rapid diagnosis leading to a critical treatment decision. Rapid immunoassays for use at a POC site are well known and commercially available. They are not described in detail herein. For example, rapid immunoassays are known to detect a wide array of infectious diseases from patient sample including but not limited to influenza testing (e.g. H1N1), RSV testing, Chlamydia trachomatis testing, Neisseria gonorrhea testing, etc.

[0007] In another embodiment, samples are collected and first processed directly for use in a rapid immunoassay. The processing step utilizes a rapid test processing reagent and is optimized for producing the maximum clinical performance for the particular immunoassay to be used. This typically involves a relatively gentle lysis treatment in the presence of various salts and detergents. Such lysis reagents are well known and used in conjunction with the commercially available rapid immunoassays and are not described in detail herein. One skilled in the art is aware of the need to select a rapid test processing reagent that will not degrade the sample and make it unsuitable for a contemplated laboratory test.

[0008] A portion of the processed sample is then delivered to the POC test device to generate a rapid diagnostic test result. The remainder (or a portion thereof) of the sample is preserved for transfer to a laboratory-based test environment for testing such as confirmatory testing using a molecular diagnostic method. In one embodiment, the molecular diagnostic test is nucleic acid-based. In a preferred embodiment, the nucleic acid-based diagnostic test is PCR.

[0009] In another embodiment of the invention, different stabilization transport diluents are utilized to increase stability of the sample. Various formulations are possible where possible constituents include but are not limited to buffers, salts, chelating agents, enzyme inhibitors, nucleic acid binding proteins, chaotropes, etc. One skilled in the art is aware of the suitable constituents and conditions (e.g. pH) for a stabilization transport diluent for a particular application. For example, if the target microorganism in a sample is susceptible to being degraded by a chaotrope, then the skilled person would know not to include chaotropes in the stabilization transport diluent.

In certain embodiments of the present invention, the remainder of the processed sample not used for the rapid test may be added to the stabilization transport diluent. In one embodiment, the stabilization transport diluent may already be present in the rapid test processing reagent used for the POC test. In another embodiment, the stabilization transport diluent may be added to the remainder of the processed sample after a portion of the sample has been removed and used for the rapid immunoassay. In a preferred embodiment, the stabilization transfer diluent stabilizes nucleic acids in the sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIG. 1A illustrates the prior art method for the standard protocol for POC and lab-based testing.

[0011] FIG. 1B illustrates the method for POC and lab-based testing of the present invention.

[0012] FIG. 2A/B demonstrates the results from RT-PCR for Influenza A from samples processed for POC testing under various dilution conditions.

[0013] FIG. 3 demonstrates the results from RT-PCR for Influenza A from samples processed for POC testing using two different rapid test processing reagents and various storage conditions using the method of the present invention.

[0014] FIG. 4 demonstrates the results from RT-PCR for Influenza A from samples processed for POC testing and then mixed with two different stabilization transport diluents and various storage conditions using the method of the present invention.

[0015] FIG. 5 demonstrates the results from RT-PCR for Influenza A from samples processed for POC testing and then mixed with a stabilization transport diluent and various storage conditions using the method of the present invention.

[0016] FIG. 6A-C demonstrate the results from RT-PCR for two strains of Influenza A and one strain of Influenza B

under various storage conditions using the method of the present invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0017] FIG. 1A illustrates the current standard protocol for POC and lab-based testing. A specimen 105 is collected, for example, using a swab 100. Other conventional implements for collecting biological samples are contemplated for use herein. Such implements, such as a scraper or spatula are not described in detail herein and are well known to those skilled in the art. Specimen 105 is then processed directly by placing swab 100 with sample 105 in solution 110 for POC rapid testing 120. In this situation, any remaining sample is discarded and a new sample must be collected for additional lab-based testing such as confirmatory testing or reflex testing. In the alternative standard protocol, specimen 105 on swab 130 is first diluted in transport media 140. A portion of the diluted transport media 140 containing specimen 105 is further processed in solution 160 for POC testing 170. In this situation, processed specimen 105 is diluted to a level that diminishes the results of POC testing 170 as illustrated in Example 1 below. The remaining portion in the diluent is used for laboratory testing 150, such as subtyping and reflex testing.

[0018] FIGS. 1B illustrates one embodiment of the method for POC and laboratory testing of the present invention. Specimen 205 is collected on swab 200 and processed directly using a rapid test processing reagent 210 that is optimized for producing the maximum clinical performance for the particular immunoassay. The swab 200 is removed and the rapid test container 215 is closed with dispenser lid 216. The capped test container 215 with dispenser lid 216 is used to dispense a portion of processed sample 211 onto rapid test strip 220. Rapid POC testing 220 is performed using a portion of the specimen 211 processed in the rapid test

processing reagent. The remaining portion 212 of the processed sample after POC testing is transported 300 to the clinical lab for laboratory testing 400. In the alternative, the remaining portion 212 of the processed sample after POC testing is added to transport vial 230 that contains stabilization transport diluent 240. The stabilization transport diluent is designed to help maintain the integrity of the sample. In this regard, various formulations are possible depending upon a variety of factors including the stability of the target microorganism, the type of laboratory test contemplated, and the constituents of the rapid test reagent. Considering these factors, the skilled person will select conditions (e.g. optimal pH conditions) and constituents (e.g. buffer types, salts, chelating agents, enzyme inhibitors, nucleic acid binding proteins, chaotropes, etc.) for the stabilization transport diluent. The stabilized sample is then transported to the clinical lab 300 for confirmatory or other laboratory testing 400. This embodiment illustrates how one sample, specimen 205, can be processed at the POC site for both POC testing and lab-based testing. This embodiment also demonstrates that a sample processed in conditions optimal for POC testing can be used for lab-based testing.

[0019] There are a variety of rapid tests that are currently commercially available. Such rapid tests are not described in detail herein, but are available from a variety of sources including Becton Dickinson, Alere, Quidel, Meridian, Genzyme, etc. The invention is not limited to use with a particular rapid test.

EXAMPLES

[0020] The following examples illustrate various embodiments of the invention and are not meant to limit the invention except in a manner consistent with the claims presented herein.

EXAMPLE 1

[0021] The ability to detect influenza viral RNA in samples processed for use in a POC rapid immunoassay was demonstrated using an H1N1 positive clinical specimen collected by upper nasal swab from an individual exhibiting positive flu symptoms. The swab was placed in 3 ml of commercially available transport media (BD™ Universal Viral Transport Media available from Becton Dickinson) and confirmation that the sample tested positive for H1N1 was obtained. For testing, certain 50 μl aliquots of that specimen were obtained. One aliquot was mixed directly with a rapid test processing reagent for the immunoassay and others were further diluted (5X, 25X, 125X or 625X) with stabilization transport diluent prior to mixing with the rapid test processing reagent. Each 50 μl aliquot of sample was combined with 25 μl of rapid test processing reagent. The rapid test processing reagent (tris buffer, NaCl, 6% detergent and pH adjusted to 8.0) was optimized to release and preserve the influenza nucleoprotein which is the target antigen for the rapid immunoassay. The immunoassay testing results on the various sample dilutions are shown in Table 1.

Table 1: Immunoassay Results

H1N1 Clinical Sample	Rapid Immunoassay Result
undiluted	Flu A positive/ Flu B negative
1:5 dilution	Flu A positive/ Flu B negative
1:25 dilution	Flu A negative/ Flu B negative
1:125 dilution	Flu A negative/ Flu B negative
1:625 dilution	Flu A negative/ Flu B negative

[0022] The immunoassay test results in Table 1 demonstrate the effect of specimen dilution on rapid test performance. Samples diluted greater than 1:5 resulted in a negative rapid

immunoassay test. In order to provide optimal POC clinical performance, specimen dilution should therefore be minimized or avoided. Dilution of the specimen (excluding the initial placement of the sample into solution) greater than 1:5 diminishes the possibility of detection with a rapid immunoassay test. The use of direct swab processing in the POC setting enhances the clinical performance of rapid immunoassays. However, standard POC testing methods using direct swab samples, as noted above, do not enable lab-based testing because of initial placement of such samples into a transport diluent.

EXAMPLE 2

[0023] Aliquots (50 μl) of each dilution prepared in Example 1 were mixed with 25 μl rapid test processing reagent. One set of processed samples was stored at room temperature (RT) for 5 minutes prior to RNA extraction using a Qiagen Viral RNA miniprep kit according to the manufacturer's instructions. Additional sets of processed samples were stored for 4 hours at either 4°C or RT prior to RNA extraction. A 5 μl portion of the extracted RNA samples was then used as target for reverse transcription-polymerase chain reaction (RT-PCR) with primers specific for the matrix gene of influenza A virus. The RT-PCR results are shown in FIGS. 2A and 2B. Tables 2 and 3 show the processing conditions corresponding to each lane of the agarose gel of the RT-PCR results shown in FIGS 2A and 2B.

Table 2: Lanes of Agarose Gel of FIG. 2A

Processing Method	Lane on Agarose Gel
Molecular Weight Marker	M
Sample diluted 1:5, processed for rapid test, RNA extraction immediately	1
Sample diluted 1:5, processed for rapid test, stored at 4°C for 4 hrs before RNA extraction	2

Sample diluted 1:5, processed for rapid test, stored at Room Temperature for 4 hrs before RNA extraction	3
Sample diluted 1:25, processed for rapid test, RNA extraction immediately	4
Sample diluted 1:25, processed for rapid test, stored at 4°C for 4 hrs before RNA extraction	5
Sample diluted 1:25, processed for rapid test, stored at Room Temperature for 4 hrs before RNA extraction	6
Sample diluted 1:5, RNA extraction (no rapid test/no rapid test reagent)	7
Sample diluted 1:25, RNA extraction (no rapid test/no rapid test reagent)	8
Sample diluted 1:125, RNA extraction(no rapid test/no rapid test reagent)	9
Sample diluted 1:625, RNA extraction (no rapid test/no rapid test reagent)	10

Table 3: Lanes of Agarose Gel of FIG. 2B

Processing Method	Lane on Agarose Gel
Molecular Weight Marker	M
Sample diluted 1:125, processed for rapid test, RNA extraction immediately	11
Sample diluted 1:125, processed for rapid test, stored at 4°C for 4 hrs before RNA extraction	12
Sample diluted 1:125, processed for rapid test, stored at Room Temperature for 4 hrs before RNA extraction	13
Sample diluted 1:625, processed for rapid test, RNA extraction immediately	14
Sample diluted 1:625,	15

processed for rapid test, stored at 4°C for 4 hrs before RNA extraction	
Sample diluted 1:625, processed for rapid test, stored at Room Temperature for 4 hrs before RNA extraction	16
Sample diluted 1:5, RNA extraction (no rapid test/no rapid test reagent)	17
Sample diluted 1:25, RNA extraction (no rapid test/no rapid test reagent)	18
Sample diluted 1:125, RNA extraction (no rapid test/no rapid test reagent)	19
Sample diluted 1:625, RNA extraction (no rapid test/no rapid test reagent)	20

[0024] Figures 2A and 2B demonstrate that samples processed for rapid immunoassay testing could also be used for RNA extraction, which enabled lab-based PCR testing to be performed. RNA was extracted from samples diluted well below the limit of detection for the rapid test, indicating that even small amounts of viral RNA remained stable in the processed sample. Storage of the processed samples at 4°C or room temperature for up to four hours before RNA extraction also indicates the viral nucleic acid remained stable after processing. Comparison of PCR test results using RNA isolated from the processed samples to RNA extracted directly from the sample dilutions (lanes 7-10, 17-20) demonstrated that the integrity of the viral RNA was minimally affected by the processing step for the rapid test.

EXAMPLE 3

[0025] The stability of viral RNA in processed samples was examined using two different rapid test processing reagents optimized for use in the rapid immunoassay for influenza A/B. Sample processing for rapid immunoassays typically involves

the use of a relatively gentle lysis treatment mediated by a reagent containing various salts and detergents. Two different formulations for the rapid test processing reagent were examined for compatibility with the described method. Formulation A contained Tris buffer, NaCl, 16% detergent at a pH of 7.8. Formulation B contained Tris buffer, NaCl, 6% detergent at a pH of 8.0. Aliquots of an H1N1 positive clinical specimen described in Example 1 were processed with both formulations, and the processed samples were used immediately for RNA extraction using the Qiagen Viral RNA miniprep kit, or stored at RT and 4°C for up to 24 hours prior to RNA extraction. A portion of the extracted RNA samples was then used as target for RT-PCR with primers specific for the matrix gene of the influenza A virus. The RT-PCR results are shown in FIG. 3. Table 4 shows the processing conditions corresponding to each lane of the agarose gel of the RT-PCR results shown in FIG. 3.

Table 4: Lanes of Agarose Gel of FIG. 3.

Processing Method	Lane on Agarose Gel
Molecular Weight Marker	M
Sample processed with rapid test processing reagent A, RNA extraction immediately	1
Sample processed with rapid test processing reagent A, stored at 4°C for 4 hrs before RNA extraction	2
Sample processed with rapid test processing reagent A, stored at 4°C for 24 hrs before RNA extraction	3
Sample processed with rapid test processing reagent B, RNA extraction immediately	4
Sample processed with rapid test processing reagent B, stored at 4°C for 4 hrs before RNA extraction	5
Sample processed with rapid test processing reagent B, stored at 4°C for 24 hrs before RNA extraction	6
Sample processed with rapid test processing reagent A, stored at room temperature for 4 hrs before RNA extraction	7
Sample processed with rapid test processing reagent A, stored at room temperature for 24 hrs before RNA extraction	8
Sample processed with rapid test processing reagent B, stored at room temperature for 4 hrs before RNA extraction	9
Sample processed with rapid test processing reagent B, stored at room temperature for 24 hrs before RNA extraction	10

[0026] FIG. 3 demonstrates both formulation A and formulation B are compatible with use of the processed sample for RNA extraction and PCR testing. Storage of the processed samples at 4°C for up to 24 hours prior to RNA extraction suggests little degradation of the viral RNA occurred in samples processed with either formulation. However, prolonged storage of the extracted samples at RT demonstrated decreased PCR performance, possibly due to viral RNA degradation over time (lanes 8, 10).

EXAMPLE 4

[0027] Two potential stabilization transport diluents were examined in an attempt to increase stability of viral RNA in samples processed for POC testing. The stabilization transport diluent was designed to help maintain the integrity of nucleic acids present in the sample. Various formulations are possible where optimal pH conditions, buffer types, salts, chelating agents, enzyme inhibitors, nucleic acid binding proteins, chaotropes, etc. may be employed. Stabilization transport diluent A contained Qiagen viral RNA lysis/binding buffer. Stabilization transport diluent B contained 6 M guanidine thiocyanate + 20 mM EDTA. Aliquots of an H1N1 positive clinical specimen were processed using rapid test processing reagent B. The processed samples were immediately used for RNA extraction or mixed with one of the two different stabilization transport diluents and stored at 4°C for up to six days prior to RNA extraction. A portion of the extracted RNA samples was then used as target for RT-PCR with primers specific for the matrix gene of the influenza A virus. The RT-PCR results are shown in FIG. 4. Table 5 shows the processing conditions corresponding to each lane of the agarose gel of the RT-PCR results shown in FIG. 4.

Table 5: Lanes of Agarose Gel of FIG. 4.

Processing Method	Lane on Agarose Gel
Molecular Weight Marker	M
Sample processed for rapid test, RNA extraction immediately	1
Sample processed for rapid test, mixed with stabilization transport diluent A, stored at 4°C for 3 days before RNA extraction	2
Sample processed for rapid test, mixed with stabilization transport diluent B, stored at 4°C for 3 days before RNA extraction	3
Sample processed for rapid test, mixed with stabilization transport diluent A, stored at 4°C for 6 days before RNA extraction	4
Sample processed for rapid test, mixed with stabilization transport diluent B, stored at 4°C for 6 days before RNA extraction	5

[0028] Using either formulation of the stabilization transport diluent, intact viral RNA was extracted from samples processed for POC testing that had been stored for up to 6 days at 4°C. Comparing the PCR results from the stored samples (lanes 2-5) to those obtained using RNA extracted immediately after processing (lane 1) suggest little, if any, degradation of the viral RNA occurred over time in the processed samples treated with either stabilization transport diluent.

EXAMPLE 5

[0029] A stabilization transport diluent was used in an attempt to increase stability of the viral RNA in samples processed for POC testing, particularly when samples are stored for extended periods of time at room temperature.

Aliquots of an H1N1 positive clinical specimen were processed using rapid test processing reagent formulation B described in Example 3, and the processed samples were immediately used for RNA extraction, or mixed with stabilization transport diluent A and stored at RT and 4°C for up to seven days prior to RNA extraction. A portion of the extracted RNA samples was then used as target for RT-PCR with primers specific for the matrix gene of the influenza A virus. The PCR results are shown in FIG. 5. Table 6 shows the processing conditions corresponding to each lane of the agarose gel of the RT-PCR results shown in FIG. 5.

Table 6: Lanes of Agarose Gel of FIG. 5.

Processing Method	Lane on Agarose Gel
Molecular Weight Marker	M
Sample processed for rapid test, RNA extraction immediately	1
Sample processed for rapid test, mixed with stabilization transport diluent, stored at 4°C for 3 days before RNA extraction	2
Sample processed for rapid test, mixed with stabilization transport diluent, stored at 4°C for 4 days before RNA extraction	3
Sample processed for rapid test, mixed with stabilization transport diluent, stored at 4°C for 5 days before RNA extraction	4
Sample processed for rapid test, mixed with stabilization transport diluent, stored at 4°C for 6 days before RNA extraction	5
Sample processed for rapid test, mixed with stabilization transport diluent, stored at 4°C for 7	6

days before RNA extraction	
Sample processed for rapid test, mixed with stabilization transport diluent, stored at room temperature for 1 day before RNA extraction	7
Sample processed for rapid test, mixed with stabilization transport diluent, stored at room temperature for 2 days before RNA extraction	8
Sample processed for rapid test, mixed with stabilization transport diluent, stored at room temperature for 3 days before RNA extraction	9
Sample processed for rapid test, mixed with stabilization transport diluent, stored at room temperature for 4 days before RNA extraction	10

[0030] Mixing the processed sample with a stabilization transport diluent increased the stability of the viral nucleic acid, and enabled longer-term storage and transport of the processed sample at various temperatures. FIG. 5 demonstrates that intact viral RNA can be extracted from processed samples mixed with the stabilization transport diluent after storage of the samples for up to 7 days at 4°C or up to 4 days at room temperature.

EXAMPLE 6

[0031] Stabilization transport diluent B was used to examine stabilization properties across different influenza strains: A: Influenza A strain A/Solomon Island/03/06 (H1N1); B: Influenza A strain A/Wisconsin/67/2005 (H3N2); and C: Influenza B strain B/Jiangsu/10/2003. Aliquots (50 μl) of cell culture supernatants from cultures into which virus had been introduced from nasal swabs of a patient exhibiting

symptoms of influenza and these aliquots were combined with rapid test processing reagent B (25 μl) and the processed samples were either immediately used for RNA extraction, or mixed with stabilization transport diluent B (75 μl) and stored at 4°C or -20°C for up to fourteen days prior to RNA extraction. A portion of the extracted RNA samples was then used as target for RT-PCR reactions with primers specific for the matrix gene of influenza A or the nucleoprotein gene of influenza B. The PCR results are shown in FIG. 6. Table 7 shows the processing conditions corresponding to each lane of the agarose gel of the RT-PCR results shown in FIG. 6.

Table 7: Table 6: Lanes of Agarose Gel of FIG. 6.

Processing Method	Lane on Agarose Gel
Sample processed for rapid test, RNA extraction immediately	1
Sample processed for rapid test, mixed with stabilization transport diluents B, stored at 4°C for 2 days before RNA extraction	2
Sample processed for rapid test, mixed with stabilization transport diluents B, stored at 4°C for 7 days before RNA extraction	3
Sample processed for rapid test, mixed with stabilization transport diluents B, stored at 4°C for 10 days before RNA extraction	4
Sample processed for rapid test, mixed with stabilization transport diluents B, stored at 4°C for 14 days before RNA extraction	5
Sample processed for rapid test, mixed with stabilization transport diluents B, stored at -20°C for 7 days before RNA extraction	6

[0032] Mixing the processed sample with a stabilization transport diluent increased the stability of the viral nucleic acid in all three strains of Influenza for up to 14 days at 4°C or up to 7 days at -20°C. FIG. 6 demonstrates that intact viral RNA from various strains can be extracted from processed samples mixed with the stabilization transport diluent after storage of the samples for up to 7 days at 4°C or up to 14 days at -20°C. For both the A and B strains, intact viral RNA was extracted after storage under all conditions.

EXAMPLE 7

[0033] Utility of one embodiment of the method of the present invention illustrated in FIG. 1B was demonstrated in a clinical trial performed during the 2010-2011 influenza season. Paired nasopharyngeal (NPS) or upper nasal swabs (NS) were collected from patients enrolled in the POC influenza study. One swab was processed directly for use in an investigational rapid immunoassay at the POC site and then a portion (3 to 5 drops) of the remaining sample was mixed with the stabilization transport diluent B (200 μl) and stored at either 2 to 8°C for up to 5 days or -20°C for up to two weeks prior to being sent to a laboratory for PCR analysis. The second swab was placed in 3 ml of viral transport media and sent directly to a clinical laboratory for PCR testing.

[0034] All PCR testing was performed using the Prodesse ProFlu+ assay available from GenProbe, Inc. (San Diego, CA). The Prodesse ProFlu+ test is FDA-cleared and is able to detect and differentiate Influenza A, Influenza B, and RSV in respiratory specimens. For the swab-in-transport media specimens, RNA was extracted using the NucliSENS easyMAG System (bioMérieux) according to the Prodesse ProFlu+ package insert. For the POC processed samples in the stabilization

transport diluent, RNA was extracted using the Qiagen Viral RNA miniprep kit according to the manufacturer. Five microliters of extracted RNA was used for PCR amplification using a Cepheid SmartCycler II instrument according to the assay procedure described in the Prodesse ProFlu+ package insert. Interpretation of PCR results for specimens and controls was determined using the Cepheid SmartCycler Dx software according to the protocols outlined in the Prodesse ProFlu+ package insert. The positive and negative percent agreement between results obtained from the POC stabilized sample (POC PCR) and the swab-in-transport media sample (Reference PCR) are shown below in Table 7.

Table 7: Comparison of PCR results from POC processed samples compared to Reference PCR

Influenza A

	Reference PCR		
POC PCR	P	N	
P	150	22	*172*
N	5	335	*340*
	155	*357*	*512*

Reference Method:
PCR from swab in transport media

Positive Percent Agreement: 96.8%
Negative Percent Agreement: 93.8%

Influenza B

	Reference PCR		
POC PCR	P	N	
P	91	12	*103*
N	8	401	*409*
	99	*413*	*512*

Reference Method:
PCR from swab in transport media

Positive Percent Agreement: 91.9%
Negative Percent Agreement: 97.1%

RSV

	Reference PCR		
POC PCR	P	N	
P	18	2	*20*
N	1	491	*492*
	19	*493*	*512*

Reference Method:
PCR from swab in transport media

Positive Percent Agreement: 94.7%
Negative Percent Agreement: 99.6%

[0035] Table 7 demonstrates that samples can be processed for rapid POC testing and a portion of that processed sample can be used for lab-based testing such as PCR. Greater than 91.9% agreement was obtained in various viral strains when comparing a sample that was directly processed for PCR to a sample that was first processed under conditions optimal for rapid POC testing and then subsequently processed for PCR.

[0036] Although the invention herein has been described with reference to particular embodiments, it is to be understood that these embodiments are merely illustrative of

the principles and applications of the present invention. It is therefore to be understood that numerous modifications may be made to the illustrative embodiments and that other arrangements may be devised without departing from the spirit and scope of the present invention as defined by the appended claims.

CLAIMS:

1. A method for using a biological sample initially collected at a point of care (POC) as source for a sample for use in a laboratory that is not at the point of care (POC) comprising:

a) collecting a sample suspected of containing a target microorganism for a POC test,

b) preparing the collected sample for use in the POC test by combining the collected sample with POC test processing reagents comprising at least one salt, at least one buffer and at least one detergent configured for use with a POC test thereby yielding a processed sample,

c) using only a first portion of the processed sample for the POC test leaving a remaining second portion not used for the POC test, and

d) using at least a portion of the remaining second portion of the processed sample for a laboratory test.

2. The method of claim 1, wherein an implement is used to present the biological sample and the implement is selected from the group consisting of a scraper and a swab.

3. The method of claim 1, wherein the location for the POC test is a physician's office.

4. The method of any one of claims 1 to 3, wherein the POC test is an immunoassay.

5. The method of claim 4, wherein the POC test processing reagent comprises a lysis reagent.

6. The method of claim 1, wherein, after the first portion of the processed sample has been removed for use in the POC test, the at least a portion of the remaining second portion of the processed sample and a stabilization transport diluent are combined together in a transport container for sample stabilization during transport to the laboratory test.

7. The method of claim 6, wherein the stabilization transport diluent stabilizes nucleic acids in the processed sample and comprises at least one buffer and at least one salt.

8. The method of claim 1, after the first portion of the processed sample has been removed

for use in the POC test, the at least a portion of the remaining second portion of the processed sample is not combined with a stabilization transport diluent for sample stabilization during transport to the remote laboratory test.

9. The method of claim 7, wherein the laboratory test is subtyping based upon a nucleic acid assay.

10. The method of claim 7, wherein the laboratory test is reflex testing.

11. The method of claim 7, wherein the laboratory test is a nucleic acid assay.

12. The method of claim 11, wherein the nucleic acid assay is PCR.

13. The method of claim 6 wherein at least a portion of the remaining second portion of the processed sample is added to a container containing the stabilization transport diluent.

14. The method of claim 1 wherein the biological sample suspected of containing a target microorganism is selected from the group consisting of a biological sample and an environmental sample.

15. A method for using a single sample suspected of containing a target microorganism initially collected at a point of care (POC) for both a POC test immunoassay and a laboratory test not conducted at the POC comprising:

a) collecting a sample for a POC test as the single sample,

b) processing the single sample for the POC test by combining the single sample with a POC test processing reagent comprising a buffer, a salt, and a detergent thereby yielding a processed sample,

c) selecting the POC test;

d) using only a first portion of the processed single sample for the POC test, leaving a remaining second portion of the processed single sample;

e) combining, in a container, at least some of the second portion of the processed single

sample and a stabilization transport diluent that stabilizes at least nucleic acids in the second portion of the processed single sample combined with the stabilization transport diluent;

f) transferring the container containing the combined remaining portion of the processed single sample and stabilization transport diluent to a laboratory not at the POC for laboratory testing, and

g) using the combined remaining portion of the processed single sample combined and stabilization transport diluent for reflex testing or subtyping using PCR.

16. The method of claim 15, wherein an implement is used to present the single sample and the implement is selected from the group consisting of a scraper and a swab.

17. The method of claim 15, wherein the location for the POC test is a physician's office.

18. The method of claim 15, wherein POC test processing reagent comprises a lysis reagent.

19. The method of claim 15, wherein the stabilization transport diluent stabilizes nucleic acids in the processed sample and comprises at least one buffer and at least one salt.

20. The method of claim 15 wherein the single sample suspected of containing a target microorganism is selected from the group consisting of a biological sample and an environmental sample.

21. A method for testing a single biological or environmental sample initially collected at the point of care (POC) using both a POC test and a laboratory test, wherein the laboratory test is not conducted at the POC, that indicate the presence or absence of microorganisms in the sample comprising:

a) collecting a biological or environmental sample for the POC test, wherein the POC test is a rapid immunoassay;

b) processing the sample for a rapid test by combining the presented sample with the POC test processing reagent configured for use with a POC test thereby yielding a processed sample the POC test processing reagent comprising at least one buffer, at least one salt and at

least one detergent;

c) using a first portion of the processed sample for the POC test leaving a remaining second portion not used for the POC test, and

d) using at least a portion of the remaining second portion of the processed sample for the laboratory test.

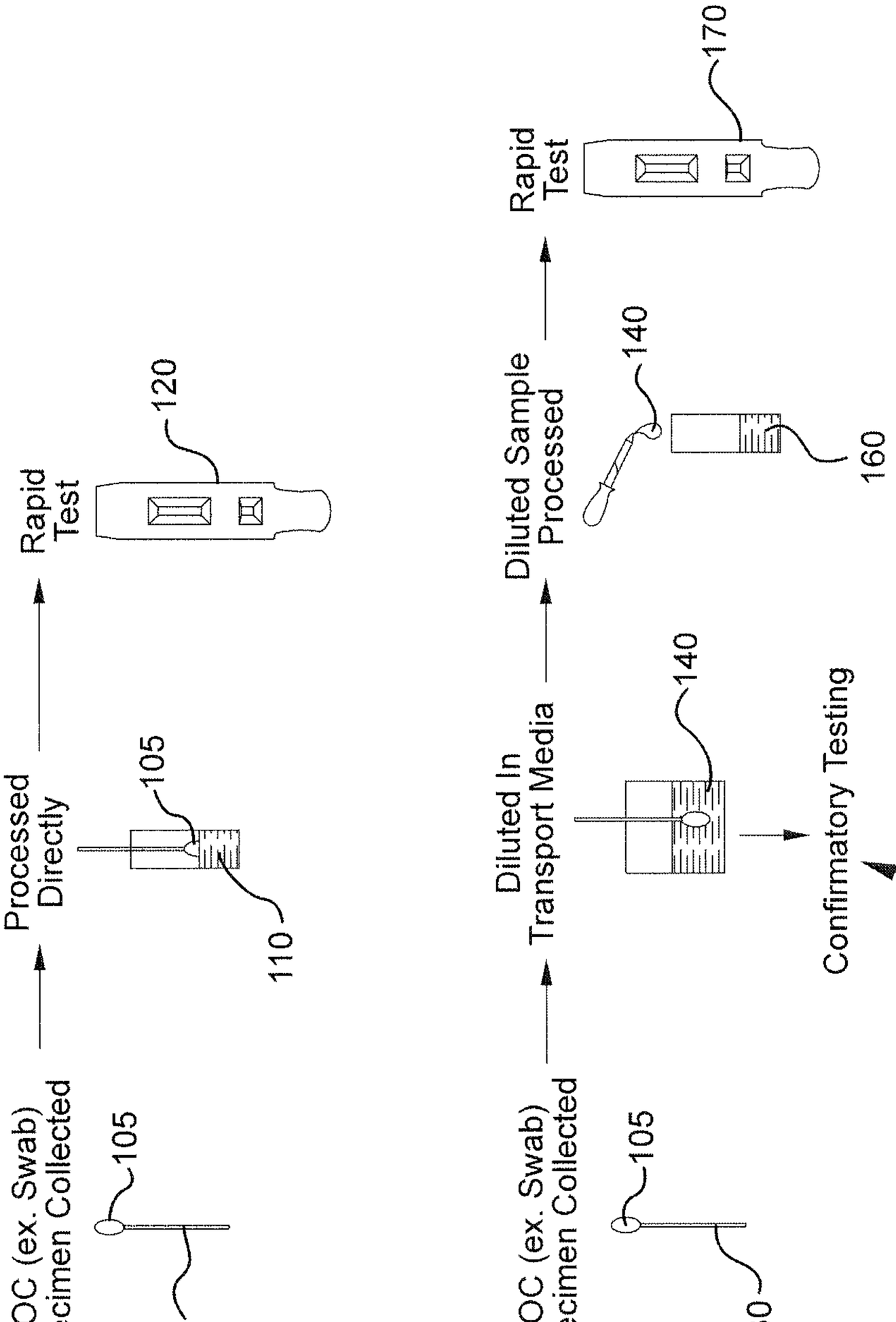

Standard POC Protocol
POC (ex. Swab)
Specimen Collected
105
100
Processed
Directly
105
110
Diluted In
Transport Media
105
140
130
Confirmatory Testing
150
Rapid
Test
120
iluted Sample
Processed
140
160
Rapid
Test
170

FIG. 1A

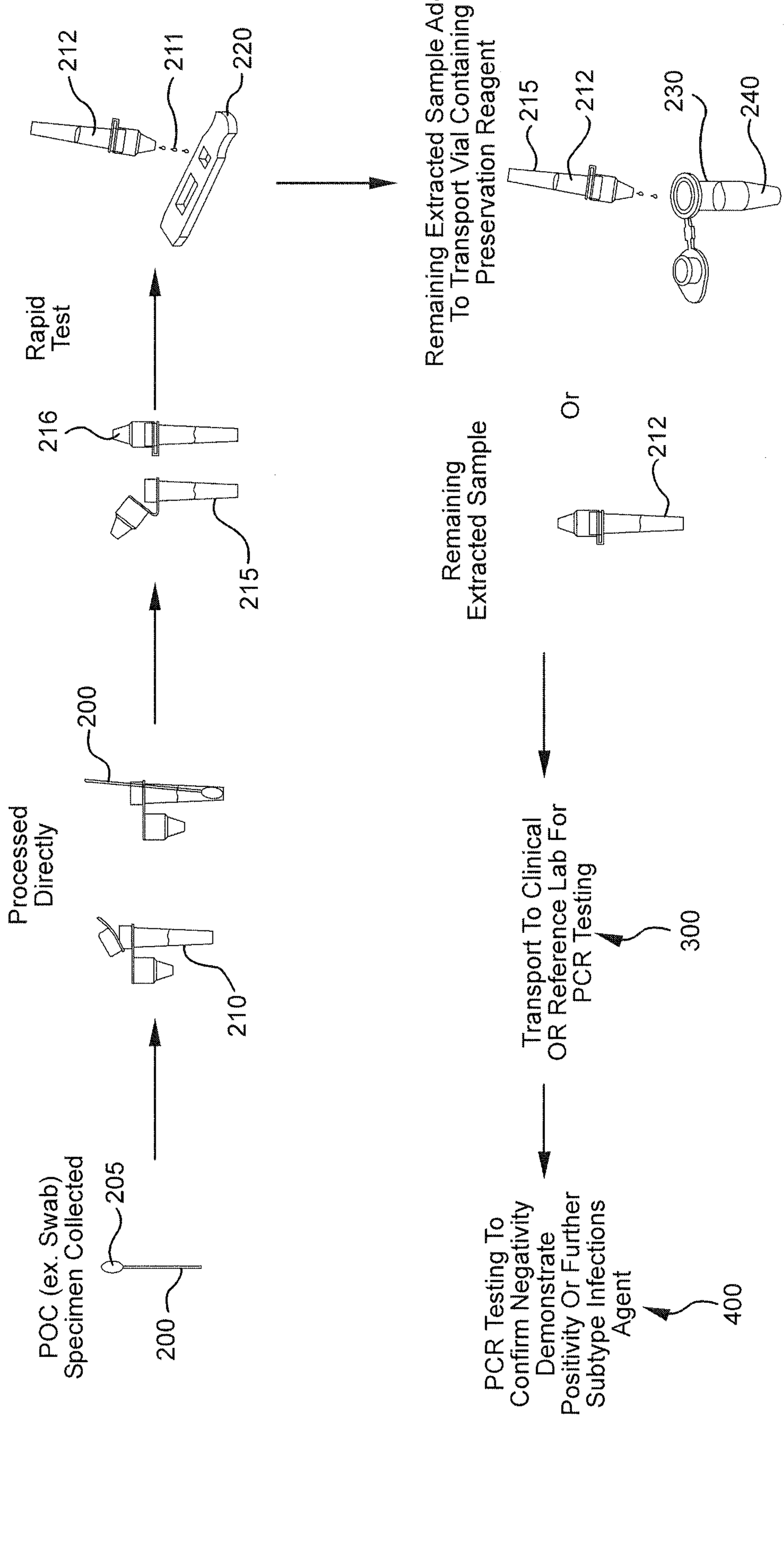

POC (ex. Swab) Specimen Collected
205
200
Processed Directly
200
210
Rapid Test
216
215
Remaining Extracted Sample Added To Transport Vial Containing Preservation Reagent
215
212
230
240
Or
Remaining Extracted Sample
212
Transport To Clinical OR Reference Lab For PCR Testing
300
PCR Testing To Confirm Negativity Demonstrate Positivity Or Further Subtype Infections Agent
400

FIG. 1B

FIG. 2A

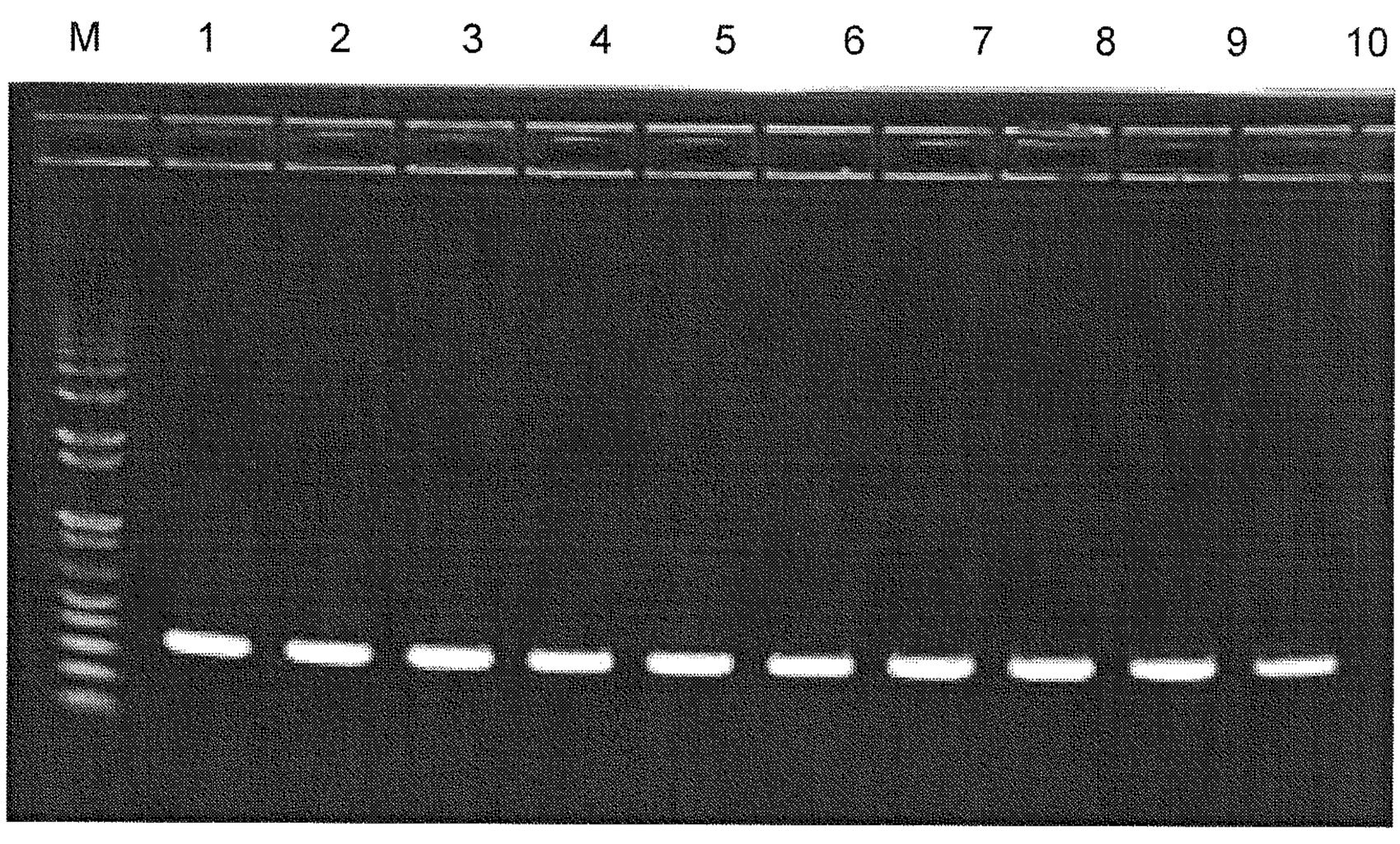


FIG. 2B

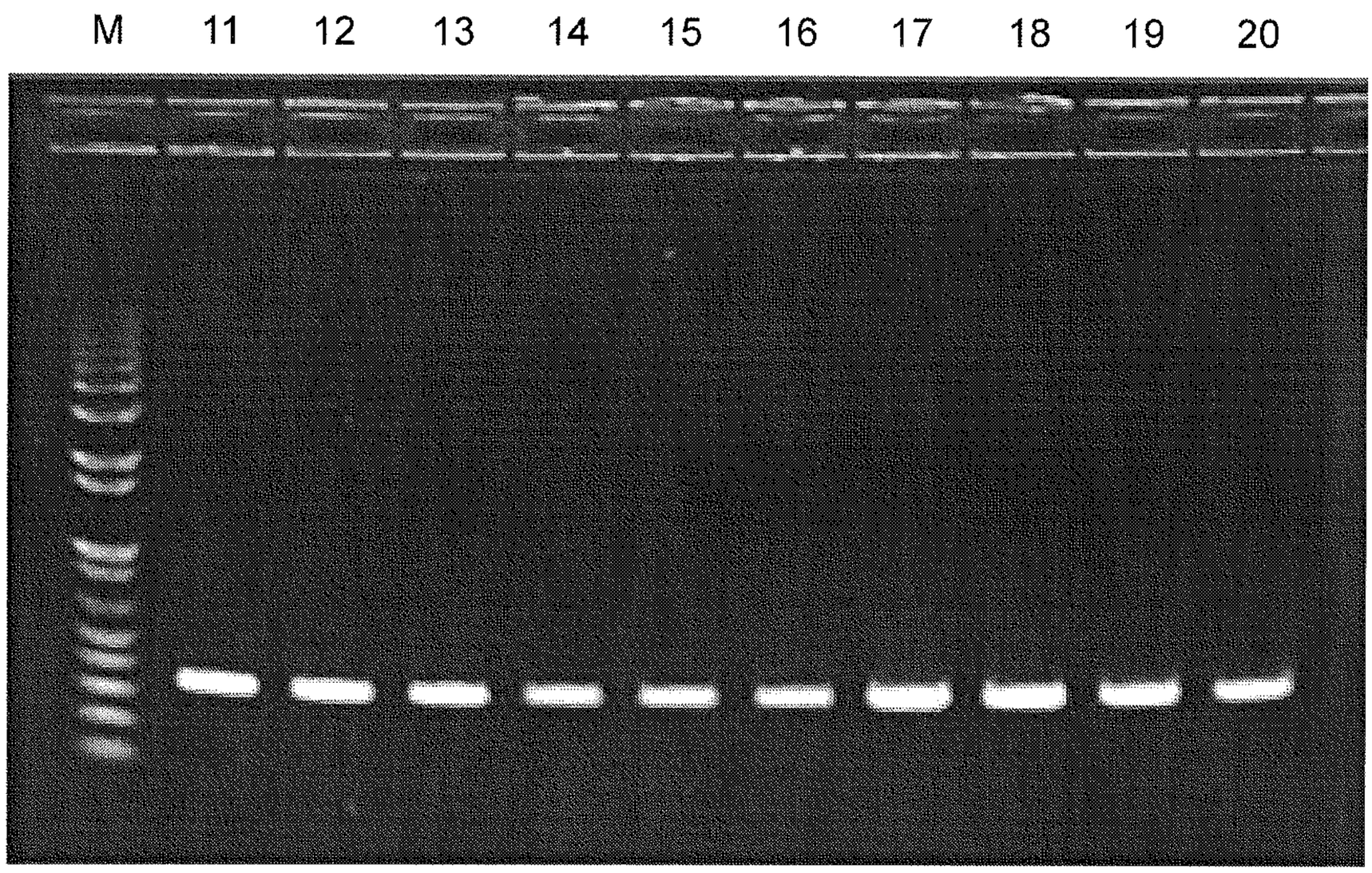


FIG. 3

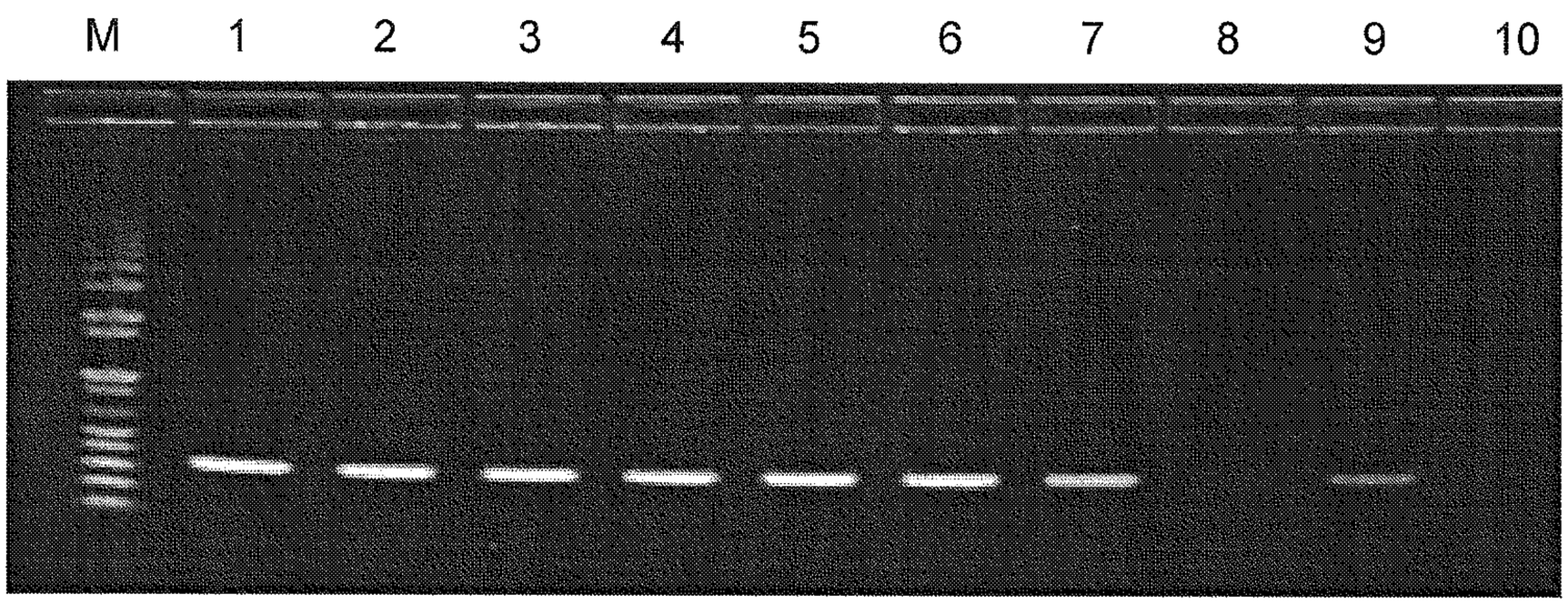


FIG. 4

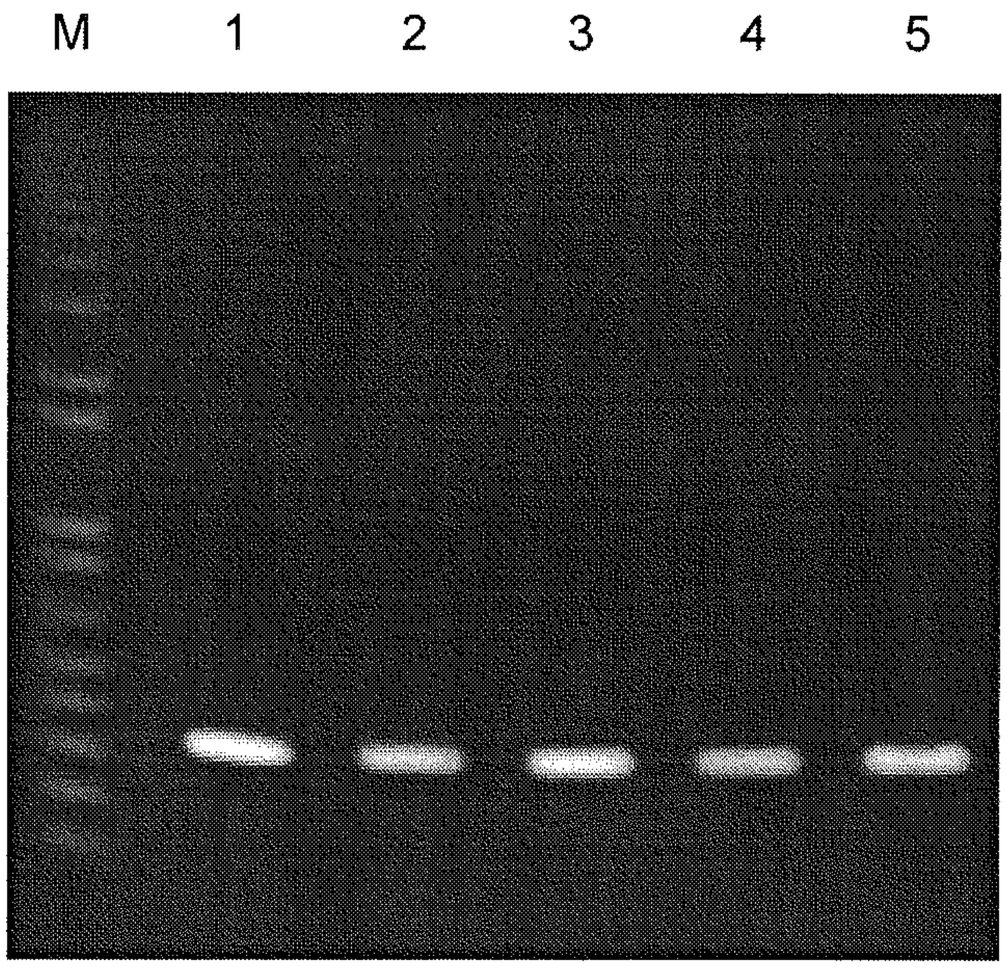


FIG. 5

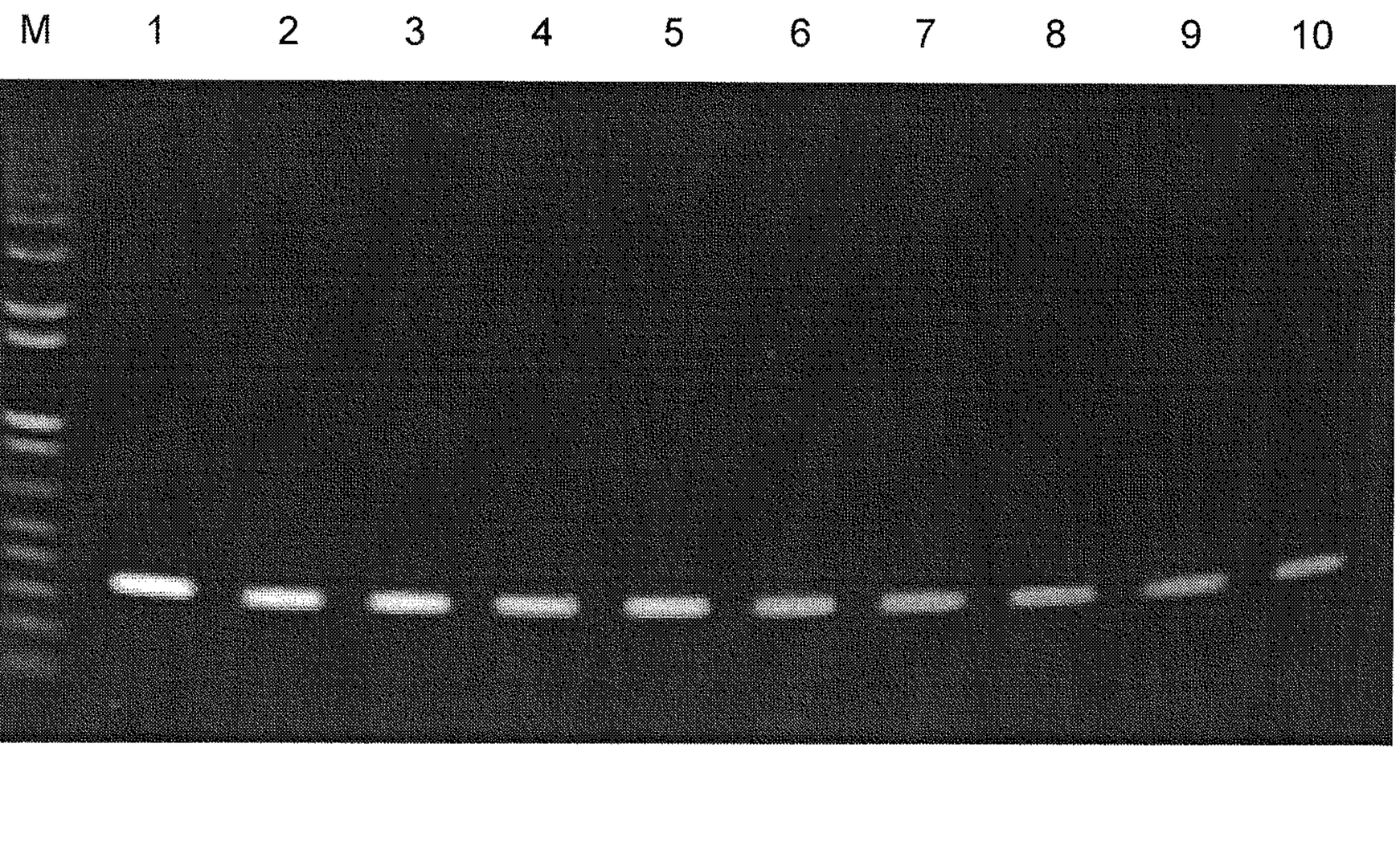


FIG. 6A

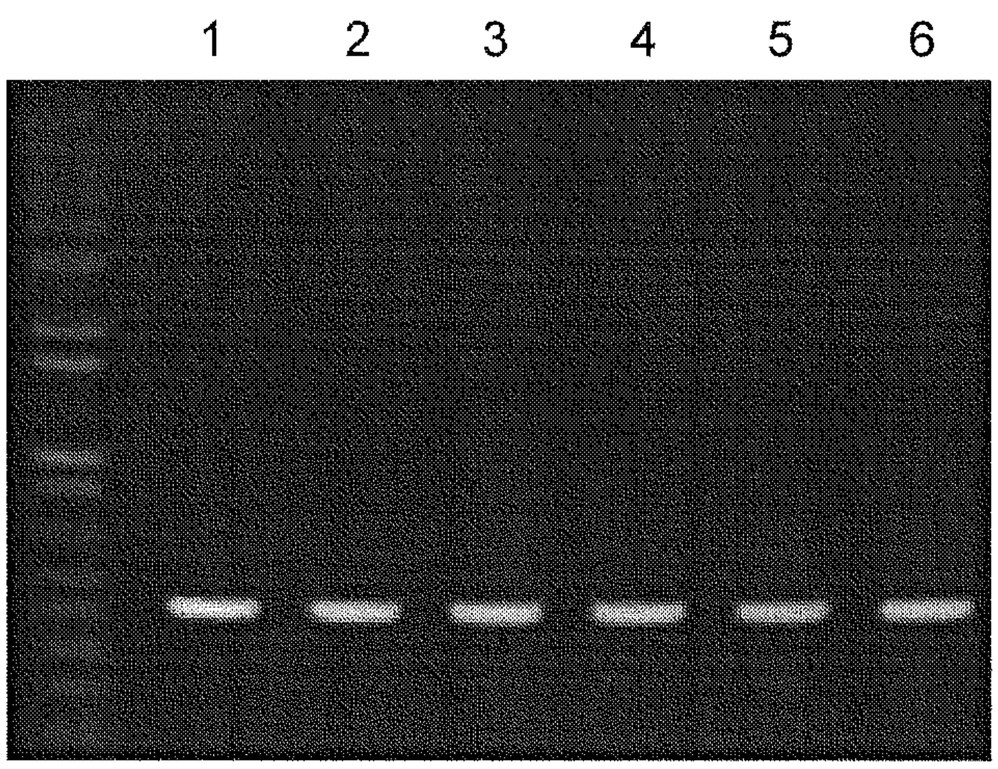


FIG. 6B

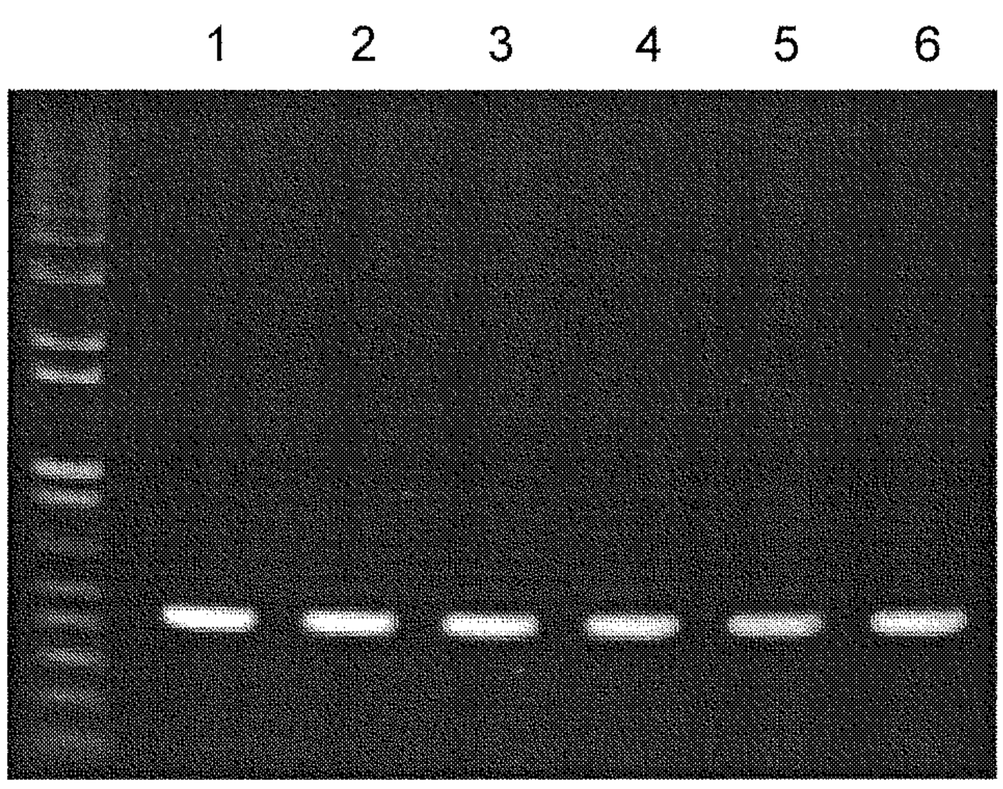
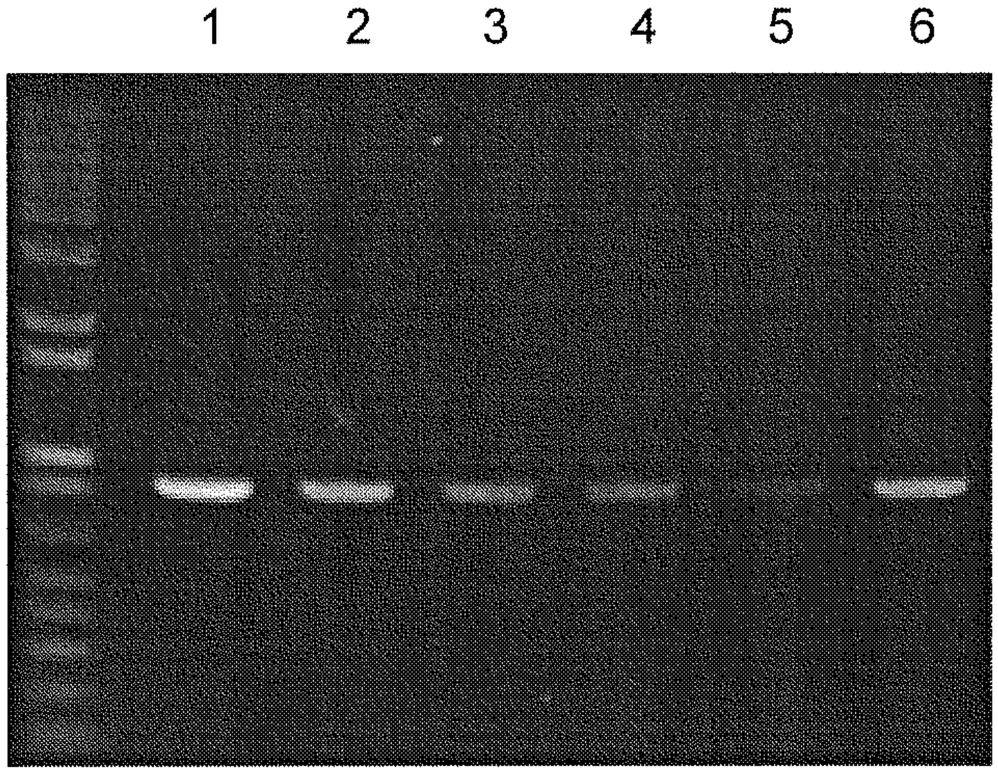
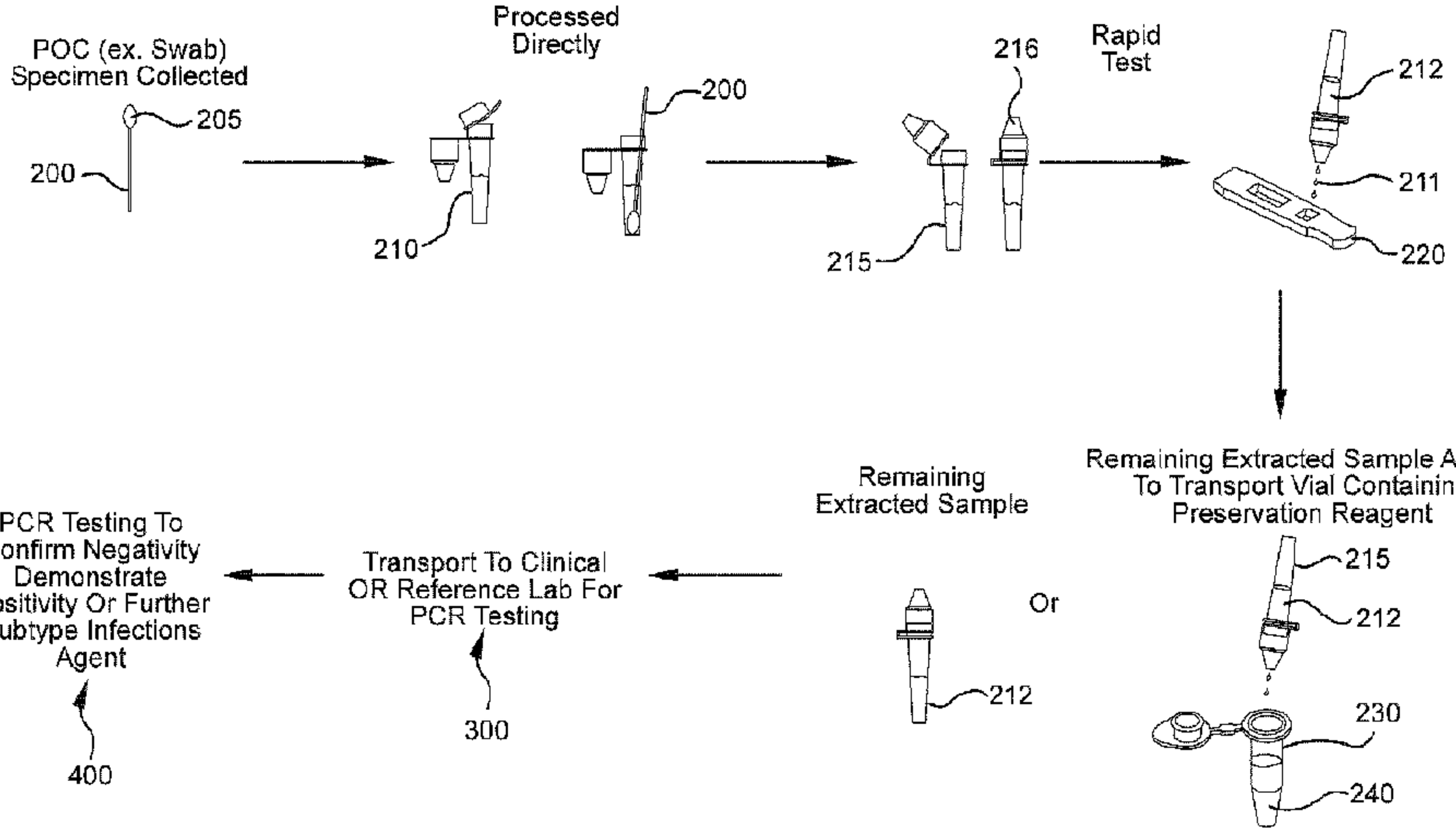


FIG. 6C




POC (ex. Swab)
Specimen Collected
205
200
Processed
Directly
210
200
216
215
Rapid
Test
212
211
220
Remaining Extracted Sample Added
To Transport Vial Containing
Preservation Reagent
215
212
230
240
Remaining
Extracted Sample
Or
212
Transport To Clinical
OR Reference Lab For
PCR Testing
300
PCR Testing To
Confirm Negativity
Demonstrate
Positivity Or Further
Subtype Infections
Agent
400